

A glucocorticoid–FAS axis controls immune evasion during metastatic seeding

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Metastasis is the major cause of death for patients with triple-negative breast cancer and other solid malignancies. Metastases arise from cancer cells that disseminate from the original tumour, survive systemic immune surveillance and colonize new organs¹. Little is known about how initial disseminated tumour cells (DTCs) overcome anti-tumour immunity after seeding a new organ. Here we use a visible antigen in a model of triple-negative breast cancer with cognate CD8⁺ T cells to study the mechanisms of immune evasion in early metastatic seeding. Analysis of surviving DTCs revealed glucocorticoid receptor (GR) activation as a key driver of resistance to both CD8⁺ T cells and natural killer cells. Niche profiling using an optimized labelling tool identified FAS–FASL as a key pan-cytotoxic pathway against DTCs, which is repressed by GR activation. Pharmacological inhibition of GR in combination with immunotherapy reduced metastatic burden and expanded lifespan in mice. Thus, we identified a mechanism of immune evasion that operates specifically in DTCs, illustrating the unique immune–cancer interactions at this stage in the metastatic cascade. Our findings suggest that there are therapeutic opportunities to eliminate DTCs, separately from treatments aimed at primary tumours, and GR inhibition is one promising target.

Triple-negative breast cancer (TNBC) is characterized by early recurrence, predominantly within 5 years of initial diagnosis^{2,3}. While stage I TNBC has a disease-free survival rate of over 90%, metastatic TNBC is considered an incurable disease². Metastatic disease develops from cancer cells that escape the primary tumour mass, reach new organs and survive initial therapy¹. Increasing evidence suggests that these cells disseminate early, often before treatment^{4–6}. Complete eradication of these DTCs in patients with early-stage disease would therefore prevent metastasis and improve overall survival rates.

As DTCs often resist conventional therapies, such as chemotherapy, immunotherapy represents an attractive approach to eliminate them^{7–9}. However, little is known about how DTCs resist immunity during early metastatic seeding. The investigation of cancer immune evasion has largely focused on the tumour mass, where multiple immune suppressive populations cooperate with cancer cells to halt immune responses^{9–11}. By contrast, DTCs persist outside of this protective tumour microenvironment and therefore need to evolve added mechanisms of resistance^{1,12–15}. Understanding how DTCs avoid immune

clearance requires targeted studies of tumour cells during seeding in the new host organs. Reports in this context remain limited, largely due to the technical challenge of studying the interactions between surveillant anti-tumour T cells and the rare DTCs that resist their attack.

To address this question, we used green fluorescent protein (GFP) as a visible fluorescent tumour antigen in combination with our GFP-specific CD8⁺ Jedi T cell platform¹⁶. This enabled precise isolation of antigen-expressing DTCs that escaped T cell attack. Our studies revealed that GR is a key driver of immune evasion in DTCs. Notably, GR has been associated with a worse outcome in patients^{17–21} and metastatic disease in preclinical models^{22,23}. Here our data revealed that activation of GR in DTCs suppressed the death receptor FAS to protect from FAS ligand (FASL)-mediated killing by cytotoxic lymphocytes. Pharmacological inhibition of GR in combination with immunotherapy sensitized DTCs to immune clearance and reduced metastatic burden. Thus, our work revealed GR as a unique program that protects cancer cells from immune killing after metastatic seeding, and GR repression as a promising strategy to eliminate DTCs and prevent metastasis in patients with TNBC.

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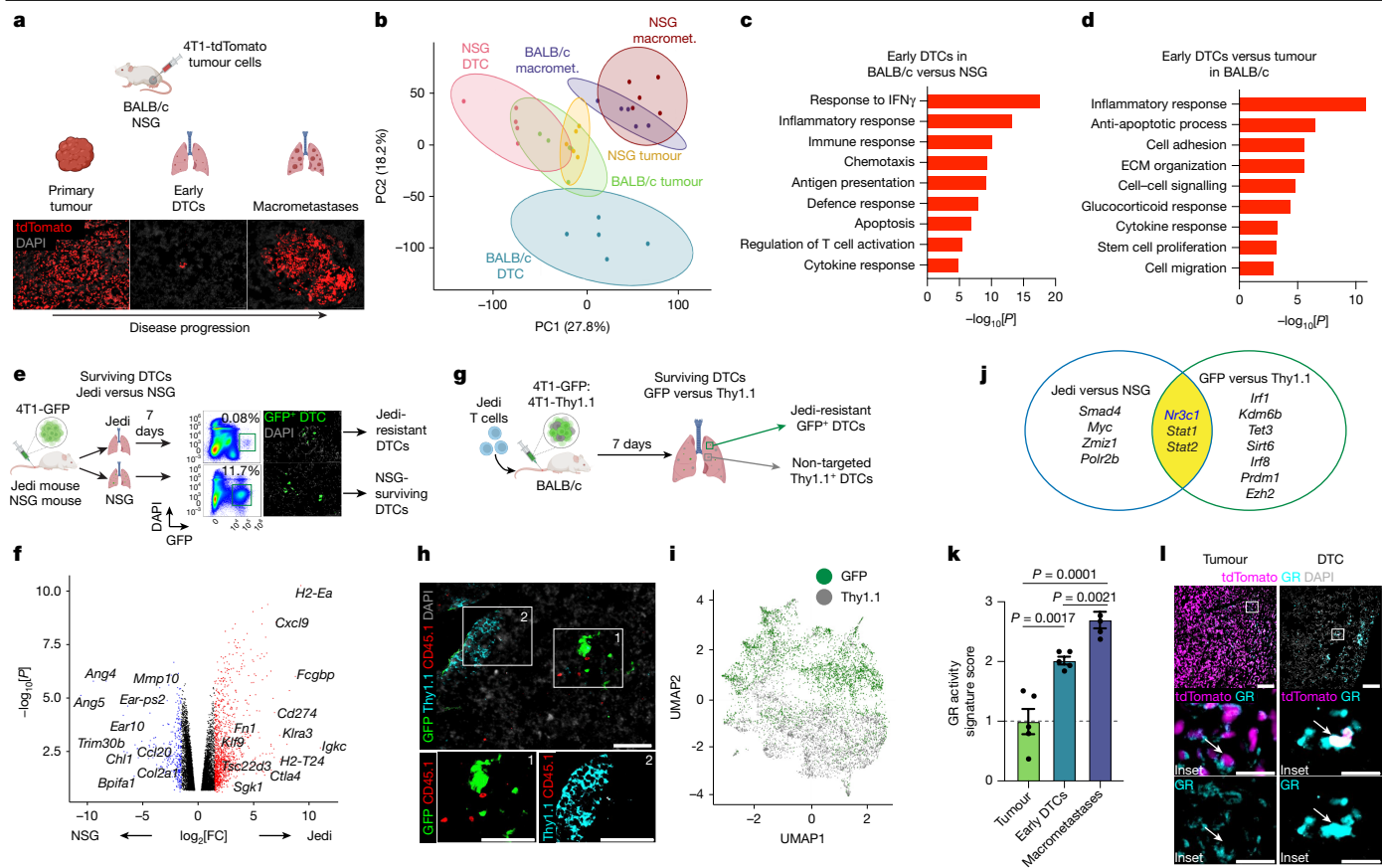


Fig. 1 | DTCs that escape from T cell killing express an *Nr3c1* activation program. **a, b**, Schematic and representative images (**a**) and PCA (**b**) of the RNA-seq experiment to profile tumour cells from immune-competent BALB/c and immune-deficient NSG mice across disease stages: primary tumour, lung DTCs (day 7) and overt lung macrometastases (terminal end point). **c, d**, GO pathway analysis of DEGs upregulated in DTCs from BALB/c mice compared with DTCs from NSG mice (**c**) and DTCs from BALB/c mice compared with BALB/c primary tumours (**d**). For **a–d**, $n = 5$ mice per group. Fold change (FC) > 1.5 and FDR-adjusted $P < 0.05$ was considered to be significant. **e, f**, Schematic of the RNA-seq experiment (**e**) and volcano plot (**f**) of surviving GFP⁺ DTCs from Jedi and NSG mice. Selected DEGs are labelled. $n = 3$ (NSG) and 4 (Jedi) mice. FC > 1.5 and FDR-adjusted $P < 0.05$ was considered to be significant. **g–i**, scRNA-seq analysis of surviving GFP⁺ and Thy1.1⁺ DTCs after Jedi CD8⁺ T cell adoptive transfer. **g, h**, Schematic (**g**) and representative image (**h**) of targeted anti-GFP DTC

DTCs activate GR to resist immunity

Immune evasion in cancer has largely been studied in established tumours and far less is known about how metastatic seeds escape immune attack. To address this question, we transcriptionally profiled tumour cells at different stages of the metastatic cascade using bulk RNA-sequencing (RNA-seq) analysis. We used the mouse TNBC cell line 4T1, and focused on lung metastasis, as this is a common site of metastasis in TNBC³. Tumour cells expressing the fluorescent reporter tdTomato were isolated from primary orthotopic tumours, early lung DTCs and established lung macrometastases from immune-competent BALB/c mice. As a comparison, the same samples were isolated from immune-deficient *NOD-scid-IL2rg^{null}* (NSG) mice (Fig. 1a and Supplementary Fig. 1a).

Principal component analysis (PCA) of their transcriptomes revealed that primary tumours and established macrometastases clustered predominantly based on stage rather than by mouse origin (NSG or BALB/c) (Fig. 1b). However, notably, early DTCs displayed a profound separation in PCA space between immune-competent and immune-deficient

killing by CD45.1⁺ Jedi T cells. **i**, Merged uniform manifold approximation and projection (UMAP) visualization of DTCs. For **g–i**, $n = 5$ mice pooled per group. **j**, Comparison of the top expressed upstream regulators in Jedi-resistant DTCs predicted by LISA ranking (left, GFP⁺ DTCs from Jedi versus NSG (**e, f**); right, GFP⁺ versus Thy1.1⁺ Jedi-surviving DTCs (**g–i**)). *Nr3c1* is a top-ranked transcriptional regulator in the RNA-seq dataset in **e** and **f** and **g–i**. **k**, Relative gene expression signature of GR activity in tdTomato⁺ tumour cells purified from BALB/c mice. Statistical analysis was performed using two-tailed unpaired *t*-tests. $n = 5$ mice per group; data are plotted as the fold change relative to tumour. **l**, Immunofluorescence analysis of GR in paired primary tumour and lung DTCs in BALB/c mice. tdTomato fluorescence is fused to H2B nuclear localization signal (representative of $n = 4$ mice). Data are mean $\pm$ s.e.m. Scale bars, 100 μ m (**a, e, h** and **l** (main)) and 25 μ m (**l** (insets)). The diagrams in **a**, **e** and **g** were created using BioRender; Agudo-Cantero, J. <https://BioRender.com/7lerlqz> (2026).

mice (Fig. 1b). Differentially expressed genes (DEGs) and Gene Ontology (GO) pathway analysis of DTCs showed increased immune response programs as the top changes in BALB/c-derived DTCs compared with both NSG DTCs and BALB/c-derived primary tumours (Fig. 1c,d). This highlights the metastatic seeding stage as a key node of immune surveillance.

Having established DTCs as a bottleneck for immunity, we next aimed to identify mechanisms that allow DTCs to escape T cell killing. To this end we took advantage of our GFP-specific Jedi CD8⁺ T cells¹⁶. As a visible antigen, GFP enables the isolation of the rare DTCs that unequivocally retain expression of the targeted antigen and have resisted T cell killing (Extended Data Fig. 1a). We injected GFP-expressing 4T1 tumour cells (4T1-GFP cells) into Jedi mice and isolated surviving GFP⁺ cells to perform transcriptional analysis (Fig. 1e). We injected the same 4T1-GFP cells into NSG mice for comparison (Fig. 1e). Jedi-resistant DTCs upregulated genes previously implicated in immune evasion (such as PD-L1, encoded by *Cd274*) and pathways related to immune responses, confirming that they were under T cell attack^{24,25} (Fig. 1f and Extended Data Fig. 1b).

We sought to exclude any transcriptional differences that could be caused by the minimal cytokine milieu in NSG mice. To this end, we

next compared T cell-resistant DTCs with non-targeted DTCs from the same lungs. A mix of GFP⁺ and non-GFP 4T1 cells (with the tag Thy1.1) was injected into BALB/c mice with adoptively transferred anti-GFP Jedi T cells, such that only GFP⁺ DTCs were attacked by T cells (Fig. 1g,h). We sorted the surviving GFP⁺ and untargeted Thy1.1⁺ DTCs from the same lung using fluorescence-activated cell sorting (FACS) 7 days later and performed profiling using single-cell RNA-seq (scRNA-seq) (Fig. 1g). Uniform manifold approximation and projection (UMAP) visualization showed that GFP⁺ and Thy1.1⁺ tumour cells clustered separately despite sharing the same lung environment (Fig. 1i), indicating that Jedi pressure drives substantial changes (Extended Data Fig. 1c,d).

Both experimental settings revealed over 1,000 DEGs in Jedi-resistant DTCs, suggesting that survival could be the result of the cooperative action of multiple genes. We reasoned that an upstream regulator could orchestrate these programs and elicit the most robust protection from immune attack. To investigate this, we used epigenetic landscape in silico deletion analysis (LISA)²⁶ to infer transcriptional drivers of the genes enriched in each Jedi-resistant dataset (Extended Data Fig. 1e,f) and then identified the overlapping upstream regulators (Fig. 1j). Importantly, LISA analysis revealed *Nr3c1* (encoding GR) as a top ranked regulator enriched in Jedi-resistant DTCs from both experiments (Fig. 1j). We confirmed that *Nr3c1* is expressed at detectable levels in DTCs (Extended Data Fig. 1g) and that a GR activity signature of canonical *Nr3c1*-induced genes^{21,22} was also increased in Jedi-resistant DTCs (Extended Data Fig. 1h).

To confirm our prediction that GR is a key regulator in immune-resistant DTCs, we returned to our RNA-seq dataset of tumour cells across disease progression (Fig. 1a). In BALB/c mice, LISA analysis predicted an increase in *Nr3c1* transcriptional activity in both early DTCs and established macrometastases compared with in primary tumours (Extended Data Fig. 1i). Consistently, tumour cells from BALB/c mice showed progressively higher GR transcriptional activity along metastatic progression (Fig. 1k). Higher GR activity in DTCs was corroborated by immunofluorescence (IF), which showed a localized nuclear GR signal (suggesting GR transcriptional activity) in contrast to the diffuse cytoplasmic signal in paired primary tumours (Fig. 1l). By contrast, in NSG mice, GR transcriptional activity remained unchanged in early DTCs, but increased later in overt metastases, in agreement with previous reports²² (Extended Data Fig. 1j,k). Taken together, the higher GR activity in DTCs from BALB/c mice, but not from NSG mice, supports a role for GR in immune evasion during initial metastatic seeding.

GR activity supports metastatic seeding

To determine the function of GR in cancer cell immune resistance, we engineered tdTomato⁺ 4T1 breast carcinoma cells to express an *Nr3c1*-targeted short-hairpin RNA (shRNA) (4T1-shGR cells). A non-targeted shRNA cell line was also engineered as a control (4T1-shControl cells). We first validated downregulation of *Nr3c1* and downstream GR activity in shGR cells (Extended Data Fig. 2a–c and Supplementary Fig. 2), and confirmed that their baseline viability, proliferation and migration were unchanged (Extended Data Fig. 2d–i). We next assessed the role of GR in spontaneous dissemination and DTC survival in the lung. shGR and shControl tumour cells were orthotopically implanted into the mammary fat pad of BALB/c mice, and tumours were allowed to grow and metastasize (Fig. 2a). No difference was observed in primary tumours between shGR and shControl (Fig. 2b and Extended Data Fig. 2j). However, GR deficiency (shGR) reduced the number of micrometastatic foci in the lung (Fig. 2c). To confirm that the effect of GR in DTCs was immune mediated, we implanted shControl and shGR tumour cells into NSG mice. This revealed no difference in either primary tumour growth or in the number of lung micrometastases (Fig. 2a,d and Extended Data Fig. 2k), supporting a role in immune evasion.

To exclude the possibility that our results were due to any reduced ability of shGR cells to leave the primary tumour, we developed a

complementary tumour-bearing model of metastasis. To this end, we inoculated tdTomato-shGR and tdTomato-shControl 4T1 cells intravenously, which ensured equal numbers of tumour cells in circulation. To generate a pre-existing anti-tumour T cell response when tumour cells seeded the lungs, we pre-implanted wild-type breast carcinomas (4T1-Thy1.1) into these mice (Extended Data Fig. 3a–d). We used tdTomato to specifically detect the shGR or shControl intravenously-injected DTCs (Extended Data Fig. 3a–c). This model confirmed that shGR cells exhibited reduced survival in the lungs compared with shControl cells 1 week after intravenous injection (Fig. 2e). We orthogonally confirmed this effect in a second TNBC model using shGR and shControl E0771 cells injected in syngeneic C57BL/6 mice (Extended Data Fig. 3e). Moreover, mice with shGR DTCs showed prolonged survival compared with shControl-injected mice (Fig. 2f). Consistently, activation of GR in 4T1 cells by pretreatment with the synthetic glucocorticoid dexamethasone (Dex) increased the number of DTCs (Extended Data Fig. 3f). Notably, GR activation also conferred enhanced survival in CT26 colon cancer and B16 melanoma, suggesting a possible pro-metastatic role for GR in solid cancers beyond TNBC (Extended Data Fig. 3g,h).

To assess whether the protective effect of GR occurs after seeding in the lungs, we engineered 4T1 cells with a doxycycline-inducible shGR to control the timing of GR inhibition. shGR induction by doxycycline 1 day after intravenous injection (when tumour cells have left circulation; Extended Data Fig. 3i) reduced DTC numbers to a similar level as constitutive shGR cells (Fig. 2g). Inducing shGR at day 3 after injection also provided protection, albeit milder than at day 1 (Fig. 2g). This indicates that GR protects DTCs once they have seeded the lungs.

To confirm that GR increases DTC survival through protection from anti-tumour immunity, we intravenously injected shGR and shControl cells into NSG mice and observed no difference in the number of foci or total DTCs after 7 days (Extended Data Fig. 3j). As we initially identified GR activation through our Jedi T cell platform, we hypothesized that GR activity protected DTCs from CD8⁺ T cell killing. To test this, we depleted CD8⁺ cells in our tumour-bearing metastasis model. While IgG-treated control mice showed a 19.2-fold decrease in the number of metastatic foci in the shGR group compared with the shControl group, depletion of CD8⁺ T cells resulted in only a fourfold decrease (Fig. 2h). However, this was an incomplete rescue, indicating that another immune population was also eliminating shGR DTCs. Both CD8⁺ T cells and natural killer (NK) cells are known to kill DTCs^{9,11,12}. Indeed, depletion of NK cells resulted in only a 0.7-fold difference, affording a greater rescue in shGR focus number (Fig. 2h). Both co-depletion of CD8⁺ T cells and NK cells and injection into NSG mice completely rescued the number of foci (Fig. 2h). Taken together, these studies revealed that GR activation protects early DTCs from killing by both CD8⁺ T cells and NK cells.

GR worsens outcome in patients with TNBC

We next examined whether GR activation provided analogous protection from lymphocyte killing in human breast tumour cells. To this end, we co-cultured MDA-MB-231 TNBC cells with human NK and CD8⁺ T cells. GR activation by Dex pretreatment reduced tumour cell killing by both NK-92 cells and CD8⁺ T cells (Fig. 2i,j), confirming that GR-mediated protection from cytotoxic lymphocytes is conserved in a human setting.

Several studies have established a correlation between GR and worse relapse-free or overall survival in patients with TNBC^{18–21}. In agreement with these previous reports, we confirmed that tumours with elevated *Nr3c1* expression or increased GR protein associated with shortened survival in patients with ER-negative breast cancer (Extended Data Fig. 3k,l). However, as we identified GR activation specifically in DTCs, we aimed to extend this reported connection into a metastasis setting. We analysed RNA-seq data of patients with metastatic TNBC from the AURORA US dataset²⁷ and adapted a published human GR-activity signature derived from TNBC cells²¹ to find that metastatic lesions

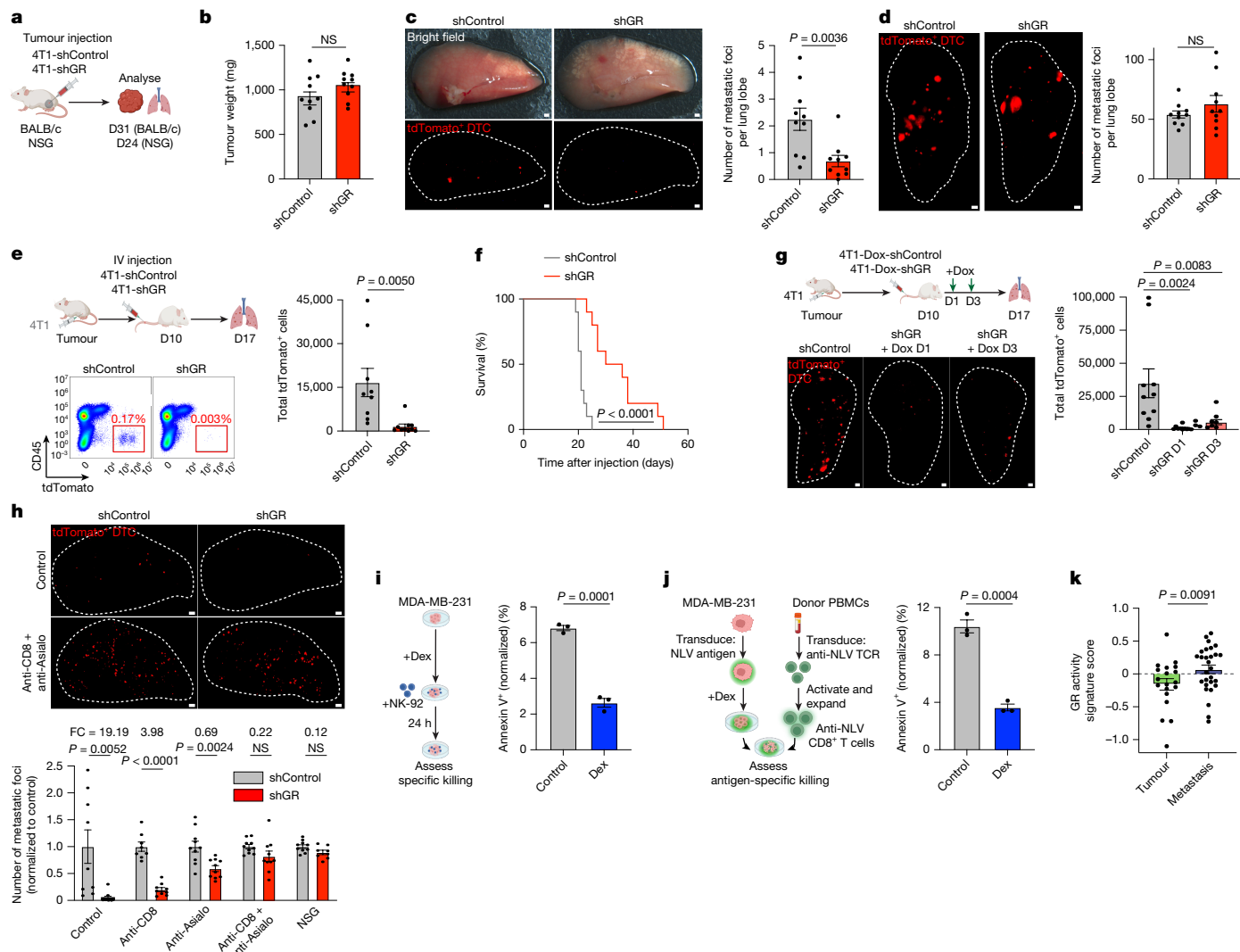


Fig. 2 | GR in tumour cells promotes lung metastasis by enabling cytotoxic lymphocyte resistance. **a, b**, Schematic (**a**) and end-point primary tumour weights (**b**) after orthotopic injection of shControl or shGR 4T1-tdTomato tumour cells in the mammary fat pad of BALB/c mice. **c**, Representative stereoscope images of the lung lobe (left) and corresponding quantification of tdTomato⁺ spontaneous micrometastatic foci (right) in immune-competent BALB/c mice (**c**) or immune-deficient NSG mice (**d**). For **a–d**, statistical analysis was performed using two-tailed unpaired *t*-tests; $n = 10$ mice per group. **e**, Schematic and representative flow cytometry plots (left), and quantification (right) of DTC lung micrometastases after intravenous (IV) injection into tumour-bearing BALB/c mice. Statistical analysis was performed using two-tailed unpaired *t*-tests. $n = 9$ (shControl) and 10 (shGR) mice. **f**, Mouse survival after intravenous injection of shGR and shControl 4T1 DTCs. Statistical analysis was performed using the log-rank test. $n = 10$ mice per group. **g**, Doxycycline (Dox)-inducible shGR or shControl 4T1-tdTomato DTCs after intravenous injection into tumour-bearing mice. Doxycycline administration started 1 day or 3 days after DTC injection. Statistical analysis was performed using ordinary one-way analysis of variance (ANOVA). $n = 10$ mice per group. **h**, Representative

images and quantification of the fold change in tdTomato⁺ shGR versus tdTomato⁺ shControl DTC lung foci, 1 week after intravenous injection into tumour-bearing BALB/c mice treated with the indicated depleting antibodies. Statistical analysis was performed using two-tailed unpaired *t*-tests. $n = 10$ (shGR, shControl + anti-Asialo, shGR + anti-Asialo, shControl + anti-CD8/anti-Asialo, shGR + anti-CD8/anti-Asialo, shControl in NSG), 9 (shControl, shGR + anti-CD8) and 8 (shControl + anti-CD8, shGR in NSG) mice. **i, j**, Killing of MDA-MB-231 human TNBC cells by human NK-92 cells (**i**) or primary antigen-specific CD8⁺ T cells (**j**). Tumour cells were pretreated with Dex to activate GR. The proportion of annexin V⁺ cells determined using flow cytometry after 24 h lymphocyte co-culture was normalized to tumour cells cultured alone. Statistical analysis was performed using two-tailed unpaired *t*-tests; $n = 3$ biological replicates per group. **k**, GR activity signature in paired primary tumours and metastases from patients with TNBC in the AURORA US dataset. Statistical analysis was performed using two-tailed paired *t*-tests. $n = 19$ patients. Data are mean $\pm$ s.e.m. Scale bars, 500 μ m. The diagrams in **a, e, g, i** and **j** were created using BioRender; Agudo-Cantero, J. <https://BioRender.com/7lrlqz> (2026).

displayed higher GR transcriptional activity compared with paired primary tumours (Fig. 2k). Lastly, to further confirm that GR activation correlates with risk of metastatic disease, we took advantage of transcriptional data from TBCRC-030—a recent chemotherapy clinical trial for patients with TNBC at the Dana-Farber Cancer Institute^{28,29} (Extended Data Fig. 4a). This study uniquely provided RNA-seq of pre-treatment primary tumours from early-stage patients with long-term metastatic recurrence outcome information, albeit from a small

number of patients. Indeed, out of the 15 patients from whom we had RNA-seq data, four patients developed metastasis within 3 years (defined as progressors). Six patients, defined as responders, had no recurrence at 5 years and were analysed as controls. Progressors exhibited heightened pro-metastatic programs (Extended Data Fig. 4b,c) and, importantly, expressed higher levels of *NR3C1* (GR) and an increased GR-activity signature²¹ compared with the responders (Extended Data Fig. 4d–g). Collectively, these results support a role for GR activation in

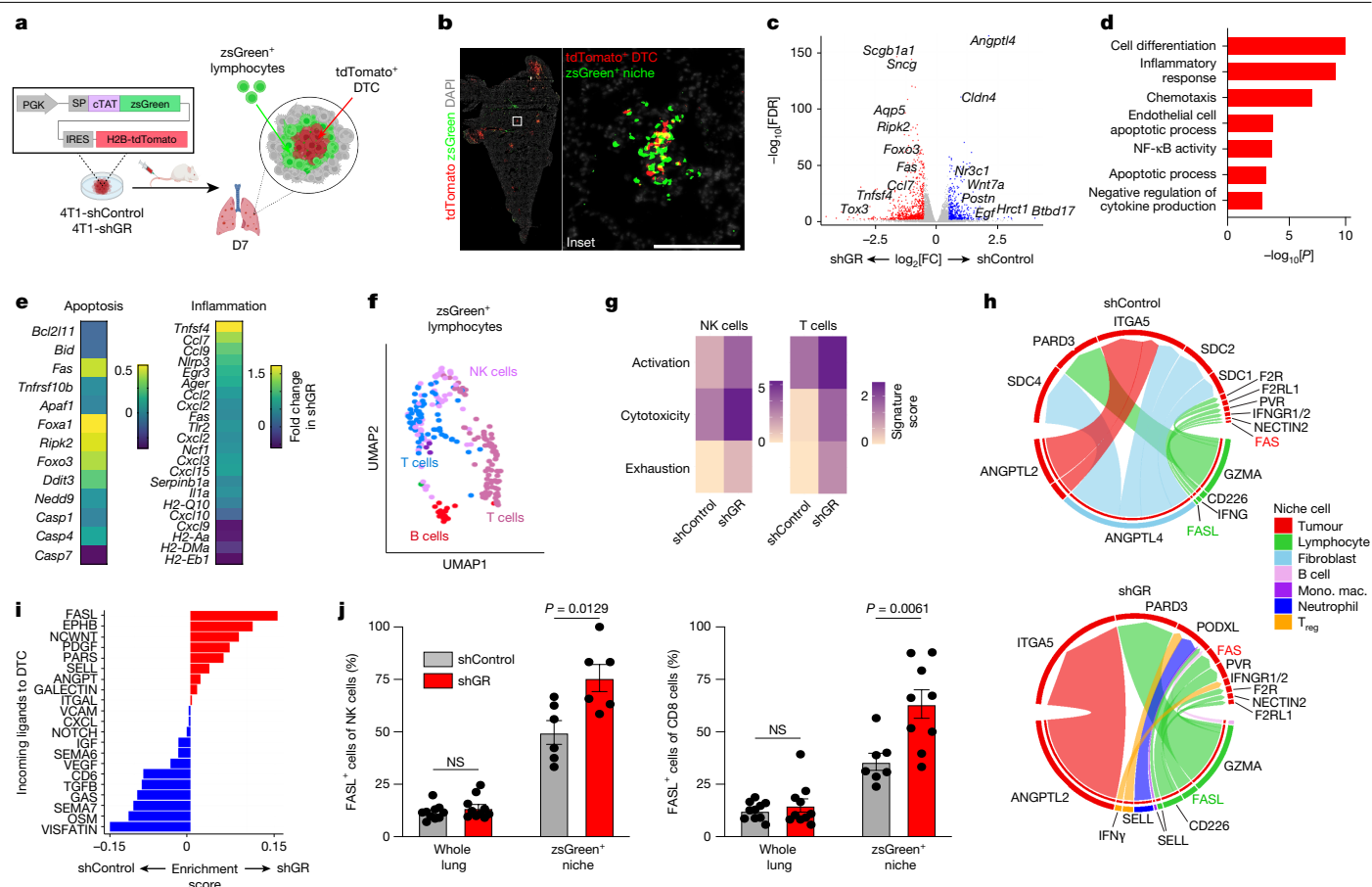


Fig. 3 | GR activation in DTCs modulates the cytotoxicity of interacting lymphocytes. **a–i**, cTAT–zsGreen enables optimized labelling of lymphocytes that surround DTCs in the lung. **a**, Experiment schematic. shControl or shGR 4T1–tdTomato tumour cells expressing cTAT–zsGreen were intravenously injected in BALB/c mice. tdTomato⁺ DTCs and zsGreen-tagged lymphocytes were FACS-sorted from the lung 7 days later for scRNA-seq analysis. *n* = 4 mice pooled per group. **b**, Representative image of tdTomato⁺ DTCs and zsGreen⁺ niches. Representative of *n* = 4 mice. **c**, DEGs between shControl and shGR tdTomato⁺ DTCs. Selected DEGs are labelled. **d**, GO pathway analysis of DEGs upregulated in shGR DTCs. For **c** and **d**, fold change > 1.5 and FDR-adjusted *P* < 0.01 was considered significant. **e**, The relative expression of genes related to apoptosis and inflammation in shGR DTCs compared to shControl DTCs (FDR adjusted *P* < 0.05). **f**, UMAP visualization of zsGreen⁺ niche lymphocytes. **g**, Activation, cytotoxicity and exhaustion signatures in the zsGreen⁺ NK cells (left) and T cells (right) from shControl- or shGR-injected mice. **h, i**, CellChat

analysis of predicted receptor–ligand interactions that are differentially active between DTC niches. **h**, Cell–cell communication networks from the top differentially active immune-related signalling pathways between shControl (top) and shGR (bottom) niches. The outer ring denotes ligand signals from niche cells that are incoming to DTCs (red). Signalling through FASL–FAS is highlighted. Mono. mac., monocyte-derived macrophage. **i**, Analysis of the incoming signals to tumour cells that are enriched in shGR (right, red) or shControl (left, blue) DTCs. Scored by the difference in predicted activity in each niche. **j**, Flow cytometry analysis of FASL expression on NK cells (left) and CD8⁺ T cells (right) in the whole lung and in the zsGreen⁺ labelled niche. Statistical analysis was performed using two-tailed unpaired *t*-tests; *n* = 10 (whole lung), 9 (zsGreen⁺ CD8 shGR), 7 (zsGreen⁺ CD8 shControl) and 6 (zsGreen⁺ NK) mice. Data are mean $\pm$ s.e.m. For **b**, scale bar, 100 μ m. The diagram in **a** was created using BioRender; Agudo-Cantero, J. <https://BioRender.com/71erlqz> (2026).

promoting metastatic progression in patients with TNBC, with resistance to immune clearance as an important mechanism.

DTC–lymphocyte interactions in vivo

We next aimed to determine how GR activation in DTCs blocks lymphocyte killing in vivo. We observed no differences in MHCI and PD-L1 levels (Extended Data Fig. 5a,b) or in recruitment of lymphocytes to the niche (Extended Data Fig. 5c,d and Supplementary Fig. 1b) between shGR and shControl DTCs. Having excluded these mechanisms of immune evasion, we sought to characterize the interactions between DTCs and responding lymphocytes in the lung. To this end, based on a previously reported approach³⁰, we developed a labelling system to isolate lymphocytes that surround DTCs. Previous systems did not achieve sufficient tagging of these small, mobile immune populations. Thus, to expand this tool set, we tested a battery of cell-penetrating peptides (CPPs), fused to the stable fluorescent protein zsGreen, which

allows zsGreen to be released by the expressing cell and taken up by nearby cells in the niche (Extended Data Fig. 6a–e). We identified CPPs that demonstrated improved labelling of lymphocytes, and selected cTAT–zsGreen for further use (Extended Data Fig. 6f).

We exploited this niche-labelling tool to profile T and NK cells near shControl and shGR DTCs in vivo. tdTomato⁺ DTCs (shGR and shControl) and their corresponding zsGreen⁺ infiltrating niche cells were FACS-sorted to perform scRNA-seq (Fig. 3a,b and Extended Data Fig. 7a). First, we investigated the shControl and shGR DTCs (Extended Data Fig. 7b). In total, 1,242 genes were differentially expressed, with 813 upregulated and 429 downregulated in shGR DTCs (Fig. 3c). Pathways including inflammatory response, apoptotic process and NF- κ B activity were increased in shGR DTCs, in agreement with higher immunogenicity (Fig. 3d). Gene set enrichment analysis (GSEA) confirmed differences in inflammatory response and apoptosis and downregulation of epithelial-to-mesenchymal transition in shGR cells, consistent with previous reports^{17,20,22} (Extended Data Fig. 7c). We further investigated

the apoptosis and inflammatory pathways, and found upregulation of several pro-apoptotic and immune-related genes in shGR DTCs (Fig. 3e), which was confirmed by bulk RNA-seq analysis of shGR and shControl tumour cells *ex vivo* (Extended Data Fig. 7d). Within this analysis, the death receptor *Fas* was a consistent significantly enriched gene, based on false discovery rate (FDR)-adjusted *P* value, in shGR DTCs across datasets (in vivo and in vitro) and across both apoptotic and inflammatory pathways (Fig. 3e and Extended Data Fig. 7d).

Next, we analysed the corresponding labelled T and NK cells in the DTC niche (zsGreen⁺ lymphocytes) (Fig. 3f). Both NK and T cells surrounding shGR DTCs displayed enhanced signatures of activation and cytotoxic function (such as *Cd28*, *Nkg7* and *FasI*) (Fig. 3g). To identify how GR activity in DTCs was altering the interacting lymphocytes, we used CellChat³¹—a computational tool to infer receptor–ligand signalling (Extended Data Fig. 7e). shGR DTCs showed an expanded communication with cytotoxic lymphocytes through FASL and GZMA (Fig. 3h). Importantly, signalling through FAS–FASL was the top communication node enriched in the shGR DTC niche (Fig. 3i and Extended Data Fig. 7e) and FASL is highly expressed by lymphocytes in the lung (Extended Data Fig. 7f,g).

To confirm our *in silico* predictions, we used the cTAT–zsGreen niche labelling tool for protein quantification of activation (for example, CD69) and FASL in lymphocytes. A higher proportion of both NK and CD8⁺ T cells expressed FASL and CD69 in the DTC niche compared with the rest of the lung, supporting their anti-tumour recognition (Fig. 3j, Extended Data Fig. 7h and Supplementary Fig. 1b,c). Importantly, within their respective zsGreen⁺ niches, shGR-injected mice showed increased proportions of FASL⁺ and CD69⁺ NK cells compared with shControl-injected mice (Fig. 3j and Extended Data Fig. 7h). Increased FASL⁺ CD8⁺ T cells were similarly observed in the niche surrounding shGR DTCs (Fig. 3j). Orthogonal validation in a second breast cancer model of E0771-shGR tumour cells also showed higher percentages of FASL⁺ lymphocytes in the lung compared with E0771-shControl DTCs (Extended Data Fig. 7i). Collectively, this analysis revealed that lymphocytes recruited to the DTC niche are highly activated, including induction of FASL, and this activation is dampened by GR activity in DTCs.

GR protects DTCs by suppressing FAS

Having identified changes in FASL in responding lymphocytes, we next assessed FAS in the corresponding DTCs. Our scRNA-seq analysis showed higher *Fas* expression in shGR DTCs compared with shControl DTCs (Fig. 3e). We confirmed elevated FAS protein on the cell surface in both 4T1 and E0771 metastasis models *in vivo* (Fig. 4a and Extended Data Fig. 8a). Immunofluorescence analysis of FAS in lung DTCs further confirmed increased FAS in shGR DTCs compared with in shControl DTCs (Fig. 4b). To test GR regulation of FAS, we activated GR by treating shControl and shGR 4T1 tumour cells *in vitro* with Dex and observed a decrease in FAS protein only in shControl cells (Fig. 4c,d). Human breast cancer cell lines MDA-MB-231 and SKBR3 similarly reduced FAS expression after Dex treatment (Fig. 4e,f).

The repression of FAS by GR activation suggested that GR protects cancer cells from FAS–FASL cell death during metastatic seeding. Consistently, *Fas* mRNA expression progressively decreased during disease progression from primary to metastasis in BALB/c mice (Extended Data Fig. 8b), concurrent with rising GR activity (Fig. 1k). Importantly, reduced FAS activity was also observed in samples of patients with metastatic TNBC from the AURORA US cohort²⁷ compared with their matched primary tumours (Fig. 4g and Extended Data Fig. 8c) and lower *FAS* expression correlated with shorter time to recurrence in patients with ER-negative breast cancer and TNBC (Extended Data Fig. 8d,e).

To test the role of FAS repression in TNBC cells, we assessed the ability of FAS to induce cell death with soluble FASL *in vitro*. Apoptosis was higher in shGR cells compared with in shControl cells (Extended Data Fig. 8f,g). Next, to investigate the role of the GR–FAS axis in metastatic

seeding, we generated shRNA-guided knockdown of *Fas* in 4T1 cells to achieve FAS downregulation in shGR DTCs that matched levels in shControl DTCs (Extended Data Fig. 8h). We then injected tumour-bearing BALB/c mice with shControl, shControl-shFas, shGR or shGR-shFas tumour cells intravenously and assessed early DTC metastatic seeding (Fig. 4h). Reducing FAS levels in shGR cells to shControl baseline levels (shGR-shFas) rescued the number of metastatic foci (Fig. 4h,i). This suggests that GR activity in DTCs can repress FAS to promote resistance to killing by cytotoxic lymphocytes.

GR controls FAS through NF-κB

We next examined how GR regulates FAS. We performed cleavage under targets and release using nuclease (CUT&RUN) for GR on 4T1 tumour cells after Dex treatment to activate GR. We identified 4,072 unique binding sites across two replicates of Dex-treated tumour cells compared with 73 unique sites after control vehicle treatment, consistent with increased GR binding after Dex treatment (Extended Data Fig. 9a). We confirmed enrichment at well-established GR binding sites (*Sgk1* and *Vim*) in Dex-treated samples (Extended Data Fig. 9b,c). Reactome analysis revealed apoptosis among the top enriched pathways in GR-activated cells (Extended Data Fig. 9d,e), consistent with our scRNA-seq data (Fig. 3d) and previous reports²⁰. Importantly, we identified a unique GR peak within the *Fas* promoter of Dex-treated cells (Fig. 4j). This site did not contain a known glucocorticoid-response element but, instead, occurred at a predicted binding site for p65 (encoded by *Rela*)—a component of the NF-κB complex (Fig. 4j (inset)). GR repression frequently occurs through cooperative binding to other TFs, including NF-κB^{32,33}, and GR is known to interact with p65 at genomic NF-κB response elements for transrepression in TNBC cells²⁰.

Furthermore, the NF-κB pathway is known to promote *Fas* expression^{34,35}. We confirmed that NF-κB activation increased FAS and found that GR activation (Dex treatment) suppressed this induction (Extended Data Fig. 9f,g). Accordingly, knockout of *Rela* in both mouse 4T1 and human MDA-MB-231 cells reduced FAS to match levels in Dex-treated control cells (Fig. 4k,l). To assess whether GR was acting by preventing p65 function at the identified binding site in the *Fas* promoter, we deleted the region containing this *Rela*-binding sequence by CRISPR–Cas9 (Fig. 4j,m). This deletion reduced FAS to a comparable level to that observed in *Rela*-knockout cells (Fig. 4k,l) as well as to levels in GR-activated (Dex treated) cells (without promoter deletion) (Fig. 4m). Taken together, our data revealed that active GR transrepresses *Fas* expression by blocking NF-κB transcriptional activity at the *Fas* promoter.

Activation of FAS induces cell death through intrinsic mitochondrial apoptosis or extrinsic caspase-directed apoptosis^{36,37}. We functionally tested whether GR activation reduced vulnerability to mitochondrial apoptosis by performing BH3 profiling. Dex treatment showed reduced apoptotic priming in 4T1 cells, while shGR cells showed increased susceptibility (Fig. 4n and Extended Data Fig. 9h). Furthermore, BH3 profiling revealed that the largest differences in apoptotic sensitivity were in response to the BH3 peptide BID (Fig. 4n), while sensitivity was unchanged in response to other key pro-apoptotic factors (MCL-1, BCL-2 and BCL-X_L) (Extended Data Fig. 9h). This is consistent with the mechanism of FAS-induced cell death, which requires cleavage of BID³⁸ (Extended Data Fig. 9i). Consistently, knockout of *Bid* in 4T1 cells and *BID* in human MBA-MD-231 cells reduced FAS-induced apoptosis in a GR-dependent manner (Extended Data Fig. 9j,k). These findings indicate that the GR–FAS axis suppresses BID-mediated intrinsic apoptosis to protect from FASL-induced lymphocyte killing.

GR blockade enhances elimination of DTCs

Given that GR activation protects DTCs from lymphocyte killing, we next tested whether GR inhibition could synergize with anti-PD-1

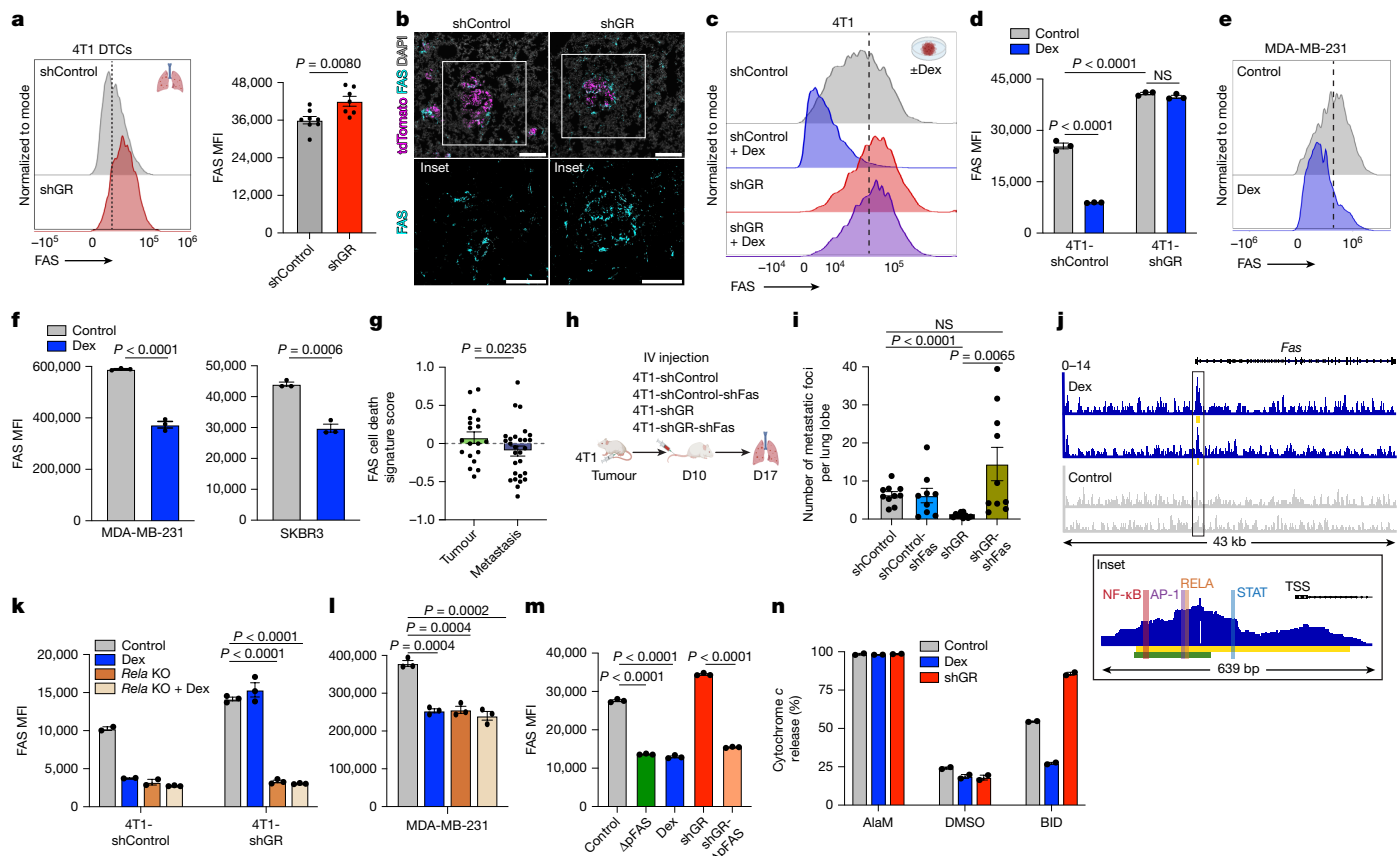


Fig. 4 | GR activation directly represses FAS to protect DTCs from immune-mediated killing. **a, b**, Representative flow cytometry histogram (left) and median fluorescence intensity (MFI) quantification (right) (**a**) and representative immunofluorescence images (**b**) of FAS in shControl and shGR DTCs after intravenous injection. Statistical analysis was performed using two-tailed unpaired *t*-tests. *n* = 8 (shControl) and 7 (shGR) mice. **c–f**, Representative flow cytometry histograms (**c, e**) and MFI quantification (**d, f**) of FAS levels in shControl and shGR 4T1 cells (**c, d**) and MDA-MB-231 and SKBR3 human breast tumour cells (**e, f**) after GR activation by Dex treatment in vitro in IFN γ -supplemented medium. For **d** and **f**, statistical analysis was performed using two-tailed unpaired *t*-tests; *n* = 3 biological replicates per group. **g**, The relative FAS-induced cell death signature in paired primary tumours and metastases from patients with TNBC. Statistical analysis was performed using two-tailed paired *t*-tests. *n* = 19 patients; AURORA US dataset. **h, i**, Schematic (**h**) and lung micrometastases (**i**) of *Fas* knockdown (shFas) in shControl or shGR 4T1-tdTomato tumour cells after intravenous injection into tumour-bearing BALB/c mice. Statistical analysis was performed using two-tailed unpaired *t*-tests. *n* = 10 (shControl, shGR,

shGR-shFas) and 9 (shControl-shFas) mice. **j**, CUT&RUN analysis of GR, showing the tracks of GR binding at the *Fas* promoter in Dex-treated tumour cells (yellow, FDR *q* < 0.05; *n* = 2 biological replicates per condition). Inset: identified binding sequences within the *Fas* peak (average of *n* = 2 Dex-treated replicates). **k, l**, FAS MFI after *Rela* knockout in shControl and shGR 4T1 cells (**k**) and MDA-MB-231 cells (**l**). Statistical analysis was performed using two-way ANOVA. *n* = 2 (shControl) and 3 (shGR, MDA-MB-231) biological replicates per group. **m**, FAS levels after deletion of the GR occupancy region in the *Fas* promoter from **j** (green) in 4T1 cells in IFN γ medium. Statistical analysis was performed using two-tailed unpaired *t*-tests. *n* = 3 biological replicates per group. **n**, Quantification of cytochrome c release as a measure of apoptotic priming in 4T1 cells after treatment with pro-apoptotic BH3 activator peptide BID. *n* = 2 biological replicates per group. AlaM and DMSO are positive and negative controls, respectively. Data are mean $\pm$ s.e.m. Scale bars, 100 μ m. The diagrams in **a, c** and **h** were created using BioRender; Agudo-Cantero, J. <https://BioRender.com/7lerlqz> (2026).

immunotherapy to eliminate DTCs. We intravenously injected shControl or shGR 4T1 tumour cells into tumour-bearing mice and treated these mice with anti-PD-1 (Fig. 5a). No difference was observed in primary tumours in any treatment group (Extended Data Fig. 10a). However, shControl DTCs were reduced after anti-PD-1 treatment, indicating modest response to immunotherapy in the dissemination setting. This anti-PD-1 response was enhanced in mice with shGR DTCs (Fig. 5a). Consistently, shGR DTCs possessed higher levels of FAS, and lymphocytes in lungs with shGR DTCs showed increased FASL (Extended Data Fig. 10b,c). Next, to extend this into a pre-clinical setting, we investigated pharmacological blockade of GR in combination with immunotherapy. Mifepristone (RU486) is an FDA-approved drug that inhibits GR function^{39,40}. Tumour-bearing mice were intravenously injected with 4T1 tumour cells and treated with anti-PD-1 and mifepristone, anti-PD-1 alone, mifepristone alone or vehicle (Fig. 5b). While no treatment reduced primary tumours (Extended Data Fig. 10d), pharmacological blockade of GR decreased

the number of metastatic foci (Fig. 5b). Combination treatment of mifepristone and anti-PD-1 further reduced metastatic burden and increased the proportion of FASL⁺ NK cells and CD8⁺ T cells (Fig. 5b and Extended Data Fig. 10e). Consistently, micrometastases in the lungs of mice treated with combination mifepristone and anti-PD-1 showed higher FAS expression in DTCs, and increased FASL in their surrounding niche (Fig. 5c,d). Treatment with mifepristone did not alter number or activation of lymphocytes and did not affect the survival of shGR DTCs (Extended Data Fig. 10f–j), proving its effect on cancer cell GR and excluding an effect through immune modulation. Finally, mifepristone and anti-PD-1 combination therapy extended mouse survival in both intravenous and orthotopic models of metastasis (Fig. 5e,f).

Thus, our study highlights an important role for GR activation in protecting DTCs from pan-cytotoxic immune clearance, enabling metastatic recurrence (Fig. 5g). Pharmacological GR inhibition in combination with immunotherapy could represent a promising strategy to

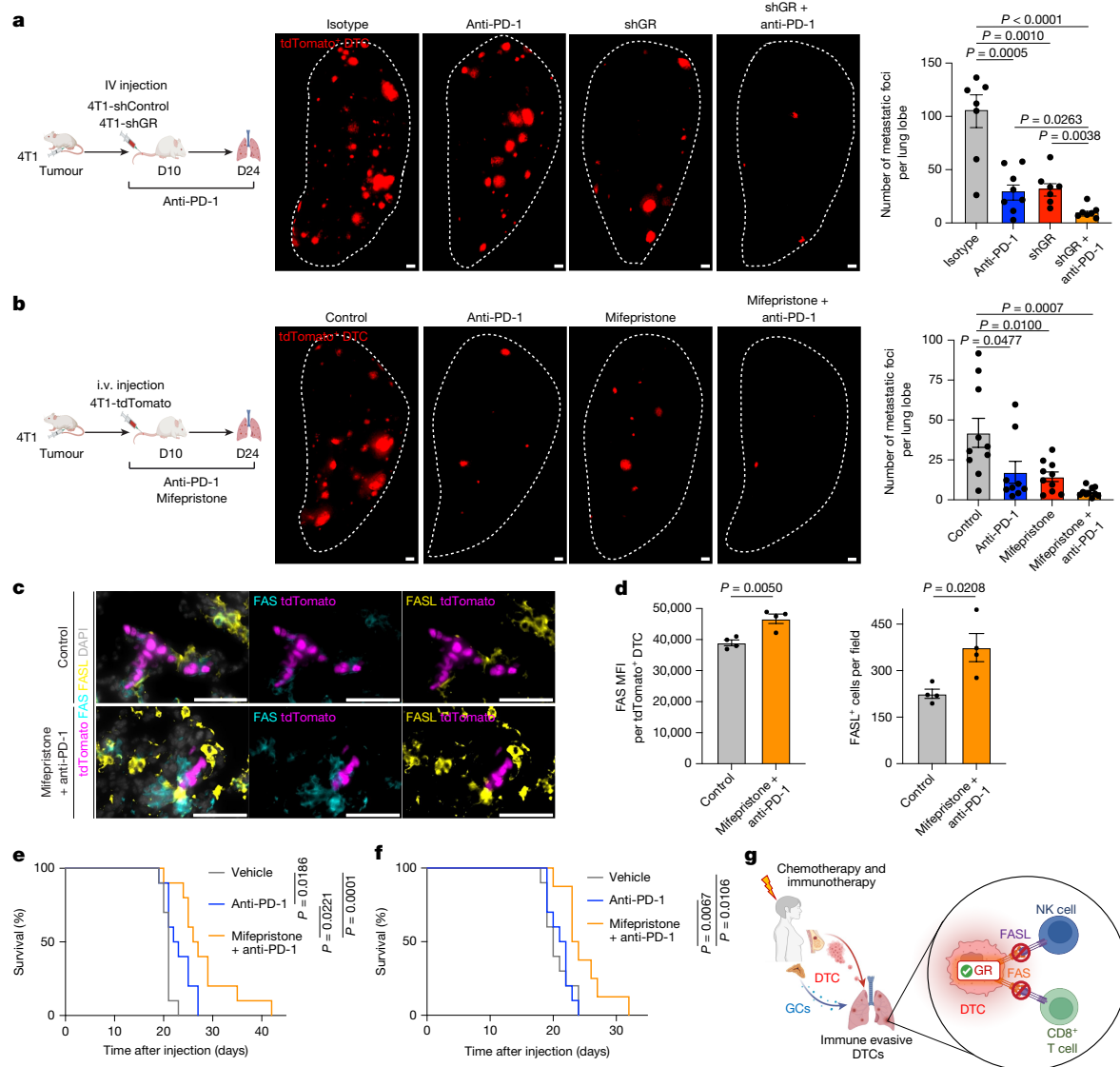


Fig. 5 | Blocking GR activity increases elimination of DTCs by anti-PD-1 immunotherapy. a, b, Schematic, representative lung images (left) and quantification (right) of DTC micrometastases in tumour-bearing BALB/c mice. **a**, shControl or shGR 4T1-tdTomato tumour cells were injected into tumour-bearing BALB/c mice. Mice were treated with anti-PD-1 therapy. $n = 8$ (isotype, anti-PD-1, shGR + anti-PD-1) and 7 (shGR) mice. **b**, Mice received intravenous injection of tdTomato-expressing 4T1 tumour cells and were treated with control vehicle, anti-PD-1, mifepristone or anti-PD-1 + mifepristone combination therapy. $n = 10$ (control, mifepristone, mifepristone + anti-PD-1) and 9 (anti-PD-1) mice. Statistical analysis was performed using two-tailed unpaired t -tests. **c, d**, Representative immunofluorescence (c) and quantification (d) of FAS and FASL in the lungs of mice with tdTomato⁺ DTC micrometastases after treatment

with control vehicle or combination anti-PD-1 and mifepristone. Statistical analysis was performed using two-tailed unpaired t -tests. $n = 4$ mice per group. **e, f**, Survival analysis of mice treated with mifepristone and anti-PD-1 after DTC intravenous injection (e; $n = 10$ mice per group) or after resection of orthotopically implanted mammary tumours (f; $n = 10$ (vehicle, anti-PD-1) and 8 (mifepristone + anti-PD-1) mice). Statistical analysis was performed using the log-rank test. **g**, Model showing the role of glucocorticoids (GCs) and GR activity in facilitating metastatic seeding by preventing FAS–FASL cytotoxic immune surveillance. Data are mean $\pm$ s.e.m. Scale bars, 500 μ m (a and b) and 50 μ m (c). The diagrams in a, b and g were created using BioRender; Agudo-Cantero, J. <https://BioRender.com/7lerlqz> (2026).

eliminate DTCs and micrometastases. This illustrates how approaches specifically tailored to target DTCs, including through blockade of GR, may be effective to prevent metastatic recurrence.

Discussion

While there are extensive studies describing mechanisms of immune evasion in established tumours (either primary or metastatic)^{10,11,24,41}, fewer reports have focused on how initial cancer cells that seed the metastatic organ resist immune responses. To identify the unique adaptations that enable DTCs to overcome systemic immunosurveillance, we exploited a visible antigen to isolate DTCs that unequivocally

escaped killing by anti-tumour T cells. We found that immune-resistant DTCs displayed increased GR transcriptional activity. We found that GR represses FAS-mediated cell death, allowing escape from FAS–FASL killing by both CD8⁺ T cells and NK cells. Previous reports have shown that NK cells can use FASL to kill liver metastases⁴² and that astrocyte-derived FASL can eliminate brain DTCs⁴³. These studies, together with our work, suggest that FAS–FASL may be a widespread mechanism of immune clearance after metastatic seeding. We report GR as a critical repressor of FAS-induced cell death, implicating the GR–FAS axis as a key node of control in metastasis.

Our findings additionally link chronic stress with metastasis, a phenomenon for which there is incomplete mechanistic understanding^{17,44}.

Activation of GR in cancer cells induces stemness, angiogenesis and epithelial-to-mesenchymal transition, enhancing the metastatic potential of human TNBC in immune-deficient mice^{19–23}. Our finding here that GR repression of FAS drives immune resistance, collectively with previous reports^{19–23}, suggests that GR represents a singular regulator capable of controlling multiple aspects of the metastatic cascade. Given this, we tested pharmacological inhibition of GR with mifepristone as a strategy to target DTCs, which demonstrated therapeutic benefit and improved mouse survival. This work, taken together with previous studies elucidating the effects of GR on immune cell suppression^{40,45–47}, provides a thorough understanding of the diverse effects of glucocorticoids in cancer progression.

In summary, our findings describe how GR activation drives a broad resistance state in DTCs despite negligible effect in the primary tumour. This illustrates that DTCs, the solitary seeds for metastasis, overcome anti-tumour immunity distinctly from a tumour mass. Thus, we posit that the immune system should be leveraged differently to target established tumours and DTCs. Our approach of combining mifepristone with immunotherapy constitutes a proof-of-concept for developing therapies that specifically eliminate DTCs. Such strategies could reduce the probability of recurrence and the risk of metastatic disease in patients with early-stage cancer.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41586-026-10222-2>.

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Methods

Animal studies

All animal procedures were performed in accordance with the Institutional Animal Care and Use Committee (IACUC) under the approved protocol 17-017 at Dana-Farber Cancer Institute (DFCI). Mice were under the husbandry care of the animal research facility at DFCI. Female BALB/c (aged 8–12 weeks, 000651), C57BL/6 (000664) and *NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ* (NSG, 005557) mice were purchased from Jackson Laboratories. Just eGFP death inducing (Jedi) mice¹⁶ (*Ptprc^aTcrb^{ln1Bdb}Tcrd^{ln1Bdb}H2d^d/J*) in the BALB/c background were obtained from B. Brown. Mice were age and litter matched and randomized between groups for each experiment. Mice were housed in no more than five mice per cage. Cages contained consistent bedding, enrichment, food, and water and cages were refreshed regularly. Mice were housed under the following conditions: controlled room temperature of 64–79 °F (18–26), 30–70% humidity and 12 h–12 h light–dark cycles (lights on from 06:00–18:00).

Mammary tumours were generated by orthotopic intramammary fat pad injection. The surgical site was shaved and a 2 mm incision was made under isoflurane anaesthesia to expose the fourth mammary fat pad. 250,000 4T1 cells in 50 µl PBS were injected. Local analgesic was used for pain relief. Tumours were monitored using callipers and tumours were not permitted to exceed 2 cm in any direction in accordance with IACUC guidelines. Tumours were also monitored for skin lesions. The humane end points as outlined in the IACUC protocol (skin lesions; 2 cm length or width tumour growth) were not exceeded in any experiment. Tumour volume was calculated as $l \times w^2/2$. For survival experiments, tumours were resected when they reached approximately 200 mm³ (days 12 and 13 after injection). Mice were sutured and treated with subcutaneous Meloxicam to relieve pain for 3 days after surgery and monitored for metastasis in accordance with IACUC guidelines.

For intravenous tumour cell injection, 250,000–500,000 cells were injected through the tail vein in 200 µl PBS. Mice were placed under a heat lamp for 1–5 min and immobilized using a restraining device. Mice were euthanized at the indicated timepoints after tail-vein injection (day 7 or day 14) or after reaching the IACUC protocol end point (body condition score 2 or 15% bodyweight loss).

Treatment with anti-mouse PD-1 (BioXCell, BE0146, 100 µg every 2 or 3 days), rat IgG2b isotype (BioXCell, BE0090, 100 µg every 2 or 3 days), anti-mouse CD8a (BioXCell, BE0117, 100 µg every 2 or 3 days), anti-Asialo GM1 (Invitrogen, 16-6507-39, 50 µg every 2 or 3 days) and mifepristone (Selleckchem, S2606, 200 mg per kg every 24 h) were performed by intraperitoneal injection. 5-ethynyl-2'-deoxyuridine (EdU) (10 mg per kg) (BaseClick) was injected intraperitoneally one day before collection.

For adoptive transfer of Jedi T cells, pre-activated CD8⁺ T cells were purified from the spleens of Jedi mice using the CD8⁺ T cell isolation kit (StemCell, 19853) according to the manufacturer's instructions. In total, 25,000 T cells were injected through the tail vein per mouse in 200 µl PBS. T cells were injected 1 day before intravenous tumour cell injection.

Cell lines

4T1, E0771, MDA-MB-231, SKBR3, CT26 and B16F10 cancer cell lines were purchased from ATCC. The NK-92 human NK cell line was purchased from ATCC. All cell lines were authenticated by ATCC and further validated by flow cytometry of known markers, assessment of morphology and growth kinetics, or bulk RNA-seq gene expression analysis. NK-92 cells were cultured in Roswell Park Memorial Institute (RPMI) with 10% heat-inactivated FBS, 10 mM HEPES, 1% penicillin–streptomycin, 10 µg ml⁻¹ insulin (Sigma-Aldrich, I0516) and 100 ng ml⁻¹ hIL-2 (Peprotech, 200-02). 4T1 cells were cultured in Iscove's modified Dulbecco's medium (IMDM) with 10% heat-inactivated FBS, 1% penicillin–streptomycin and 1% GlutaMAX (Gibco). E0771 cells were

maintained in Dulbecco's modified Eagle's medium (DMEM) with 10% heat-inactivated FBS, 10 mM HEPES, 1% penicillin–streptomycin and 1% GlutaMAX. CT26 were cultured in RPMI with 10% heat-inactivated FBS and 1% penicillin–streptomycin. B16 cells were cultured in DMEM with 10% heat-inactivated FBS and 1% penicillin–streptomycin. MDA-MB-231 and SKBR3 human cell lines were cultured in RPMI with 10% heat-inactivated FBS, 20 mM HEPES, 1% penicillin–streptomycin and 10 µg ml⁻¹ insulin (Sigma-Aldrich, I0516). To mimic immune responses, in vitro assays with tumour cells were performed in medium containing IFNγ (100 ng ml⁻¹ for 3 days) (Peprotech, 315-05 (for mice), 300-02 (for human)). All cancer cells were collected from culture by treatment with 0.25% trypsin-EDTA (Gibco) for 5 min at 37 °C. Cells were washed with PBS before and after trypsin treatment before downstream use. Cells were regularly tested for mycoplasma.

For GR activation, cells were treated with 700 nM Dex (Sigma-Aldrich, D2915) for 7 days. Treatment was refreshed daily. Treatment with soluble FASL (Peprotech, 310-03H) was performed at the indicated doses for 16 h. For NF-κB treatments, TNF (20 ng ml⁻¹, Peprotech, 315-01A), LCL161 (500 nM, Selleckchem, S7009) and DHMEQ (10 µg ml⁻¹, Med Chem Express, HY-14645) were added to the medium for 3 days and treatment was refreshed daily. All of the experiments were performed with 80–100% confluent cells.

Human tumour–lymphocyte co-culture assays

To perform co-culture killing assays, MDA-MB-231 tumour cells were pretreated with Dex for 7 days. Dex was thoroughly washed off before lymphocyte co-culture. NK-92 cells and MDA-MB-231 cells were co-cultured overnight in a U-bottom 96-well plate at a 5:1 effector:target (E:T) ratio. An antigen-specific CD8⁺ T cell co-culture system was engineered as previously described⁴⁸ using the model antigen NLV. Plasmids for tumour and T cells were received as a gift from M. Birnbaum and S. Dougan. We confirmed that MDA-MB-231 cells express the HLA haplotype HLA-A2.1 by flow cytometry, and then we transduced these cells to express NLV. Peripheral blood mononuclear cells from a healthy human donor were transduced to express the TCR that recognizes the NLV antigen presented on HLA-A2.1. Peripheral blood mononuclear cells were stimulated with the NLV peptide for 3 days to activate and expand anti-NLV CD8⁺ T cells. Cells were then expanded for 14 days in RPMI medium with 10% heat-inactivated FBS, 1% penicillin–streptomycin and 50 U ml⁻¹ hIL-2 (Peprotech, 200-02) before co-culture. Antigen-specific T cell expansion was confirmed by flow cytometry using a fluorescently labelled dextramer for NLV before co-culture. Anti-NLV CD8⁺ T cells and MDA-MB-231-NLV tumour cells (after Dex or vehicle pretreatment) were co-cultured overnight in a U-bottom 96-well plate at a 4:1 E:T ratio.

Apoptosis was measured using the flow cytometry annexin V⁺ quantification kit (BioLegend, 640932). Lymphocyte-specific killing was assessed by normalizing annexin V⁺ apoptosis to MDA-MB-231 culture alone (without NK or CD8⁺ T cells) and the fold change compared with these control conditions was graphed.

Cloning, lentiviral production and transduction

Tumour cells were engineered to express reporter proteins by stable transduction with lentiviral vectors for H2B-tdTomato (Addgene, 58101), eGFP⁴⁹ (received from B. Brown) or Thy1.1⁴¹ (received from K. Wucherpfennig). All of the transgenes were expressed under the ubiquitous promoter *PGK*.

A third-generation lentiviral packaging system was used to produce lentivirus in HEK293T cells. Tumour cells were transduced with filtered lentivirus in the presence of polybrene (1 µg ml⁻¹; MilliporeSigma) for 16 h and FACS-sorted (BD Biosciences Aria III) based on reporter expression at least 72 h after infection.

Nr3c1-knockdown tumour cells were generated by transduction with shRNA targeting *Nr3c1* (encoding GR) (Sigma-Aldrich, TRCN0000026186) according to the manufacturer's instructions.

The target sequence for the shGR construct was: CCCAGAGATGTT AGCTGAAAT. shControl cells were generated in parallel using a non-targeted control vector (Sigma-Aldrich, SHC002). *Fas*-knockdown cells (shFas) were generated using the target sequence CCCGAGAATTG CTGAAGACAT (Sigma-Aldrich, TRCN0000012331) according to the manufacturer's instructions. Cells were selected with two rounds of 48–72 h in puromycin (10 µg ml⁻¹) (Invitrogen). Genetic knockdown was confirmed by qPCR, immunofluorescence (Invitrogen GR antibody, PA1-511A, used 1:200), western blotting (GeneTex GR antibody, GTX101120, used 1:1,000) or flow cytometry. Doxycycline-inducible knockdown of GR was generated using a previously designed backbone construct⁵⁰ with the same *Nr3c1* and non-targeted sequence as the constitutive shGR and shControl cell lines, respectively (described above).

Generation of optimized lymphocyte niche labelling tool

Generation, testing and optimization of CPPs for fluorescent niche labelling were developed and cloned in-house. Plasmids were designed using published sequences for several CPPs of different classes, including amphipathic and cyclic peptides: TATk³⁰, cTAT⁵¹, transportan⁵¹ and AP⁵². In brief, the different CPPs were synthesized (Twist Biosciences) and digested with HindIII and AgeI (NEB) and cloned into a shuttle construct containing a signal peptide. The insert was then cloned into a lentiviral backbone under the *PGK* promoter, the fluorescent reporter zsGreen (Addgene, 138463), an Internal Ribosome Entry Sites (IRES) element and H2B-tdTomato. The signal peptide-CPP fragments were cloned into the vector using Golden Gate assembly with BsmBI (NEB, R0739S) and a ligase mix (NEB, M1100S) and transformation into chemically competent *Escherichia coli* (NEB, C3040H). CPP construct variants were cloned and transduced into tumour cells as described above (see the 'Cloning, lentiviral production and transduction' section).

Generation of knockout cell lines

Genetic knockout in tumour cells was generated by CRISPR–Cas9 genome editing. Cas9–gRNA ribonucleoprotein (RNP) was made by combining trRNA, gRNA and recombinant CAS9 (IDT), and RNP was then delivered into 200,000 tumour cells using the SF Cell Line 4D-Nucleofector TM X Kit S (Lonza). Cells were electroporated using the A549 program in a Amaxa 4D-Nucleofector X Unit device (Lonza). Cells were plated to recover for several days and then subjected to a second round of electroporation. The following guide RNA sequences were used: *Fas*: GGAATTAACAAAACAAGGA; mouse *Rela*: ATCGAAC AGCCGAAGCAACG and GATTCGCTATAAATGCGAG; human *RELA*: CCTTCTCTACAAGCTCGTGG; mouse *Bid*: GCCTGTGCAAGCTTACTGGG; human *BID*: GGAGGAAGCCAAACACCAGT. To remove the GR-binding region in the *Fas* promoter we used two guides: upstream (GTGTGACTG TGAAGCGCCC); downstream (CAATCTTCGTAAAGCCCCAA). Knockouts were validated by flow cytometry or Sanger sequencing and TIDE sequencing analysis.

Flow cytometry and cell sorting

Mice were euthanized and the heart was perfused with PBS. Lungs were extracted into digestion buffer (400 U ml⁻¹ collagenase IV, 20 µg ml⁻¹ DNase I, 10 µM Y-27632 compound, 10% FBS in HBSS). Lungs were mechanically dissociated with scissors and incubated at 37 °C for 30 min on a rotator. The samples were subjected to a second round of mechanical dissociation using a syringe and maintained on ice in FACS buffer (0.1% BSA, 2 mM EDTA in PBS) for all of the subsequent steps. Cells were filtered through a 100 µm cell strainer and red blood cell lysis was performed for 5 min at room temperature. Single-cell suspensions were blocked with TruStain FcX (BioLegend, 101319) at 1:100 for 15 min at 4 °C. The samples were stained with the following rat anti-mouse or mouse anti-human antibodies (BioLegend) at the indicated concentrations for 30 min at 4 °C: CD45-AF700 (1:200), CD45-PE/Cy7 (1:200), CD11c-PE/Cy7 (1:200), CD3e-APC (1:200), CD8a-APC/Fire750 (1:100), CD8a-APC (1:100), CD8a-BV510 (1:100), CD49b-Pacific Blue (1:150), CD49b-APC

(1:150), CD56-APC/Cy7 (1:100), FAS-FITC (1:100), FAS-APC (1:100), FAS-PE (1:100), FASL-APC (1:50), FASL-PE (1:100), FASL-PE/Cy7 (1:100), FASL-Biotin (1:50), CD69-PE/Cy7 (1:100), H2-Kd-Pacific Blue (1:200), PD-L1-APC (1:200), Thy1.1-APC (1:200), PD-1-FITC (1:200), CD366-PE (1:200), streptavidin-APC (1:200). DAPI (1:10,000) was used to assess viability. For apoptosis studies, cells were collected from culture as normal and stained with annexin V and propidium iodide according to the manufacturer's protocol (BioLegend, 640932). The samples were analysed for flow cytometry using a CytoFLEX instrument (Beckman) using CytExpert (Beckman) (v.2.4) software. The samples were FACS-sorted with the Aria III cell sorter (BD Biosciences) using FACSDiva software (BD Biosciences) (v.9.0.1) and cells were collected into 15 ml tubes with 5 ml of culture medium. Data were analysed using FlowJo (v.10) software. All cells were gated on FSC-A versus SSC-A to eliminate debris, FSC-A versus FSC-H to eliminate doublets and DAPI⁻ to select live cells. Gating strategies are shown in Supplementary Fig. 1.

Immunofluorescence analysis

Lungs were collected after PBS perfusion through the heart and fixed in 4% paraformaldehyde (Electron Microscopy Sciences) overnight at 4 °C. Lungs were then washed with PBS, transferred to 30% sucrose (Sigma-Aldrich) to rock overnight at 4 °C, and embedded in Tissue-Tek OCT (VWR). Tissue sections were cut at a thickness of 8 µm onto Superfrost Plus microscope slides (Thermo Fisher Scientific) and stored at –80 °C. The sections were blocked with 10% FBS and 2% normal mouse serum in 0.1% Tween-20 TBS for at least 1 h at room temperature. The samples were then incubated in primary antibodies overnight at 4 °C, washed, then incubated in secondary antibodies (if needed) for at least 1 h at room temperature. The following antibodies were used at the indicated concentrations: rat anti-mouse CD45.1-AF647 (BioLegend, 110720, used 1:200), rat anti-mouse Thy1.1-AF594 (BioLegend, 202545, used 1:100), rat anti-mouse CD3-AF594 (BioLegend, 100240, used 1:200), rat anti-mouse CD3-AF647 (BioLegend, 100209, used 1:200), rat anti-mouse CD8a-AF647 (BioLegend, 100727, used 1:200), mouse anti-human FASL (Santa Cruz, sc19681, used 1:50), goat anti-mouse Nkp46 (R&D, AF2225, used 1:50), rabbit anti-mouse FAS (Abcam, ab216991, used 1:100), rabbit anti-mouse GR (Invitrogen, PA1-511A, used 1:200), donkey anti-rabbit IgG AF647 (Invitrogen, A31573, used 1:200 with FAS and GR), donkey anti-goat IgG AF647 (Invitrogen, A32849, used 1:200 with Nkp46), donkey anti-mouse IgG AF647 (Invitrogen, A31571, used 1:200 with FASL). EdU staining using BaseClick-647 (Sigma-Aldrich, BCK-EDU647) was performed according to the manufacturer's instructions. Nuclear counterstaining with DAPI (1:10,000) was performed and slides were mounted with ProLong Diamond Antifade Mountant (Invitrogen) and covered with a #1.5 coverslip. Images were acquired on a Leica THUNDER microscope imaging system. Image processing was performed using LAS X (Leica) (v.3.7.4.23463) software. Image analysis was performed using Fiji (ImageJ) (v.2.1.0/1.53c) software.

Image quantification of metastatic foci

For image quantification of fluorescent micrometastases, lungs were collected at the indicated timepoints and each lobe was separately imaged under the Leica M205 stereoscope (×0.78 magnification) before fixation. The number of metastatic foci per lung lobe was manually counted in Fiji (ImageJ) (v.2.1.0/1.53c) using the Cell Counter plug-in based on expression of tdTomato fluorescence. The number of foci was normalized to the area of the lung lobe calculated in Fiji from the corresponding bright-field lobe images. The right and left lung lobes were separately quantified for each mouse and averaged to determine the focus number per lobe. For studies using depletion antibodies, the number of metastatic foci was normalized per treatment condition and quantification of shGR was plotted relative to shControl per treatment. The FC in shGR compared with shControl per treatment condition was calculated as the percentage change: (shControl-shGR)/(shGR). Calculations were performed using Microsoft Excel (v.16.91).

To measure the size and MFI of metastatic foci, images were set to a consistent threshold and the Analyze Particles function in Fiji was used to analyse each individual tdTomato⁺ metastatic focus. The area of all individual foci per lung lobe was averaged and the two lung lobes were averaged per mouse to determine the average size.

Bulk RNA-seq analysis

To study disease progression and immune responses, 4T1-tdTomato tumour cells from BALB/c and NSG mice were isolated from orthotopic mammary tumours (day 20 post-tumour in BALB/c; day 10 in NSG), early DTCs (day 7 after tail-vein injection) or macrometastases (day 19–23 after tail-vein injection; when lungs approached the end point). Tumour cells were collected and processed from the mouse tumour or lung according to standard flow cytometry single-cell preparation, and FACS-sorted into culture medium. Tumour cells were sorted as DAPI⁺CD45⁺tdTomato⁺ in high purity using the Aria III cell sorter (BD Biosciences). The same number of cells (20,000 cells per mouse) was sorted from all of the samples for downstream RNA analysis. DTCs from Jedi or NSG mice were similarly processed and DTCs were sorted as DAPI⁺GFP⁺. shGR and shControl 4T1 tumour cells were collected from in vitro culture according to a standard procedure. RNA was extracted using the RNeasy Mini Kit (Qiagen, 74106) according to the manufacturer's instructions. RNA samples were submitted to GENEWIZ for low-input (for Jedi and NSG GFP⁺ DTCs) or standard (for tdTomato⁺ tumour cells) bulk RNA-seq. GENEWIZ performed basic computational analysis including quality control and gene matrix construction. Further analysis was performed in RStudio (v.6.1.0). Gene matrices were normalized and filtered using Bioconductor EdgeR (v.3). Volcano plots and PCA plots were generated using Bioconductor packages. DEGs were calculated in EdgeR by fitting a negative binomial generalized linear model (for multiple comparisons) or a negative binomial exact test (for pairwise comparisons) and using a quasi-likelihood *F*-test. FDR-adjusted *P* values were calculated using the Benjamini–Hochberg method. FDR-adjusted *P* ≤ 0.05 and fold change ≥ 1.5 or ≤ −1.5 were considered to be significant. DEGs were used for GO Biological Processes database term enrichment analysis (<https://david.ncifcrf.gov/tools.jsp>) and LISA²⁶ (<https://lisa.cistrome.org/>) analysis. The resulting fold enrichment, inferred rankings and *P* values (no FDR adjustment) from these analyses are reported.

Z scores were calculated across all compared samples per gene after RPM normalization and log transformation. The GR activity signature score was calculated as the average of gene expression z scores across all GR activity signature genes per sample. The mouse GR activity signature gene set consisted of the following genes: *Abhd5*, *Ankrd1*, *Fn1*, *Id3*, *Klf6*, *Klf9*, *Mcl1*, *Mt2*, *Per1*, *Sgk1*, *Snail*, *Tsc22d3*, *Tsen2*, *Vim* and *Wnt5a*.

scRNA-seq preparation and analysis

DTCs and niche cells were collected and processed from the mouse lung according to standard flow cytometry single-cell preparation, and FACS-sorted using the Aria III cell sorter (BD Biosciences) into culture medium. For scRNA-seq analysis of Jedi-treated DTCs (Fig. 1g), tumour cells were identified as DAPI⁺ and GFP⁺ or Thy1.1⁺. For niche labelling scRNA-seq using 4T1-tdTomato-cTAT-zsGreen (Fig. 3), tumour cells were identified as DAPI⁺tdTomato⁺ and niche cells were identified as DAPI⁺tdTomato[−]zsGreen⁺. The samples were submitted to the DFCI Translational Immunogenomics Lab for 10x Genomics analysis. Libraries were prepared using the 3' v3.1 HT kit from 10x Genomics according to the manufacturer's instructions with 10,000 cell recovery and sequenced at 50,000 reads per cell. To avoid batch effects, the samples were sorted, prepared and sequenced at the same time. Reads were mapped to the mouse reference genome (assembly mm10; configured to include introns) and quantified using Cell Ranger (v.8.0.1). Further analyses were performed on filtered count matrices in RStudio (v.4.3.2) with the Bioconductor SingleCellExperiment⁵³ (v.1.24.0) framework. The scuttle⁵⁴ (v.1.12.0) package was used first to compute

log-normalized counts, and then to identify and filter out low-quality cells based on mitochondrial reads to eliminate dead cells (>10–15% mitochondrial genes), low read depth (<800 reads) and low gene detection (<500–800 genes). Doublets were identified for removal using scDbtFinder⁵⁵ (v.1.16.0). For analysis of Jedi-resistant DTCs, after discarding doublets and cells with low mRNA quality, we analysed 4,109 GFP⁺ cells and 3,649 Thy1.1⁺ cells in total. Dimension reduction by correspondence analysis with Freeman–Tukey residuals was performed using the corral⁵⁶ (v.1.12.0) package. Clustering was performed using the Louvain algorithm as implemented in igraph (v.2.1.1) using the bluster (v.1.12.0) package. For the cTAT-zsGreen scRNA-seq dataset, cell types were annotated by iteratively performing dimension reduction, clustering and manual curation to identify cell type subsets. UMAP embeddings for visualization were computed using the uwot (v.0.1.16) package.

Differential expression analysis was performed using scan⁵⁷ (v.1.30.2). Genes with FDR-adjusted *P* ≤ 0.01 and FC ≥ 1.5 or ≤ −1.5 were considered to be significant and used for GO Biological Processes pathway analysis (<https://david.ncifcrf.gov/tools.jsp>). The top 500 genes with an FDR-adjusted *P* ≤ 0.05 and FC ≥ 1.5 were used for LISA (<http://lisa.cistrome.org/>) analysis. The resulting fold enrichment, inferred rankings and *P* values (no FDR adjustment) from GO and LISA analyses are reported. For analysis of Thy1.1 and GFP DTCs, GSEA analysis^{58,59} was performed using the fgsea package (v.1.28.0). To analyse tdTomato DTCs from the cTAT-zsGreen dataset, signed FDR values (−1 × log[FDR] × sign(log[FC])) were used to perform Preranked Gene Set Enrichment Analysis (v.4.3.3) using the MSigDB Mouse Collections annotated gene sets.

Gene signature scores for zsGreen⁺ lymphocytes were calculated by first scaling the normalized log expression values to the 99th percentile value per gene, then computed as an unweighted linear combination of the scaled values. The scaling adjusts for differences in magnitude of expression between different genes, such that each gene in the signature contributes equally to the assigned score. The following genes were used to form each signature: NK activation: *Slamf7*, *Eomes*, *Il2rb*, *Cd69*, *Tnfrsf9*, *Cd28*, *Klrg1*, *Cd27*; T cell activation: *Slamf7*, *Eomes*, *Il2rb*, *Cd69*, *Tnfrsf9*, *Cd28*, *Tnfrsf4*, *Cd40lg*; cytotoxicity: *Gzma*, *Gzmb*, *Gzmk*, *FasL*, *Prf1*, *Nkg7*, *Lamp1*, *Runx1*, *Runx3*; exhaustion: *Pdcd1*, *Ctla4*, *Havcr2*, *Klrc1*, *Klrd1*, *Tox*, *Slamf6*, *Tigit*.

Cell–cell signalling analysis was performed using CellChat³¹ (v.1.6.1). To identify changes in signalling pathway activity between conditions, a signalling enrichment score was computed by taking the difference between normalized network centrality scores for each condition. Chord plots were generated with CellChat, and all other figures were created using ggplot2 (v.3.5.1) and ComplexHeatmap (v.2.18.0).

CUT&RUN

4T1 tumour cells were treated with Dex or vehicle control for 1 week and cells were collected as normal. Two independent replicates of Dex- and vehicle control-treated tumour cells each were collected for CUT&RUN analysis. CUT&RUN⁶⁰ was performed using the EpiCypher (V4) kit according to the manufacturer's instructions from 500,000 cells. Permeabilization was performed using 0.01% digitonin and 0.5 µg of GR antibody (CST, 12041) was used for overnight immunoprecipitation. *E. coli* Spike-in DNA was added after chromatin digestion. The resulting DNA was made into sequencing libraries and sequenced using NovaSeq 2 × 150bp chemistry by Genewiz.

For CUT&RUN analysis, reads were filtered for quality and adapters were removed using TrimGalore (v.0.6.10). Filtered reads were aligned to GRChM39 or the *E. coli* genome using bowtie2 (v.2.5.4)⁶¹ with the following flags: --no-mixed --no-discordant -X 2000. Samtools (v.1.21)⁶² was used to remove PCR duplicates (samtools markdup -r). MACS3 (v.3.0.3)⁶³ was used to call peaks with the following settings: callpeak -g mm --keep-dup all -B -q 0.05 --nolambda. For visualization, deepTools2 (v.3.5.6)⁶⁴ was used. BAM files were scaled to 10 million reads in 1 bp bins (bamcoverage --scaleFactor -bs 1 --effectiveGenomeSize 2654621783

--exactScaling) and replicates were averaged using bigwigAverage (-bs 1). The resulting tracks were visualized using IGV⁶⁵. For motif discovery within the *Fas* peak, the JASPAR Transcription Factor Binding Site Database (2024), was used, considering enriched motifs with a $P < 10^{-4}$. For determining overlapping peaks and Reactome enrichment, R (v.4.4.2) was used. After removing peaks in blacklisted regions, peaks were determined to overlap when they shared at least a single base pair and overlapping peaks were merged into a single continuous region (Granges 1.58). Peaks were only considered for analysis if they had FDR $q < 0.05$, had overlapping regions in both Dex-treated replicates that were absent or significantly diminished in both control samples, and were not part of blacklisted regions. For binding enrichment in GR response loci and Reactome pathway analysis, only shared peaks within or 5 kb proximal to gene bodies were considered. Peaks were annotated to the nearest gene using ChIPSeeker (v.1.42) and ReactomePA (v.1.50) and enrichplot (v.1.26.6) were used for Reactome enrichment and visualization, respectively. Overlapping Venn diagrams were plotted using ChIPpeakAnno (v.3.4) and Reactome enrichment was plotted with enrichplot (v.1.26.6).

BH3 profiling

BH3 profiling was performed as previously described^{66,67}. In brief, 4T1 tumour cells were pretreated with Dex or vehicle and 1.5×10^6 cells per condition were used for BH3 profiling. Cells were permeabilized with digitonin and treated with titrated doses of pro-apoptotic BH3 peptides for 60 min at 28 °C. Cells were then fixed with 8% paraformaldehyde for 15 min, and then neutralized with N2 buffer (1.7 M Tris base, 1.25 M glycine, pH 9.1) for another 15 min, and intracellular antibody staining for cytochrome c (BioLegend, 612310) and DAPI (Abcam, ab228549) was performed overnight in a Tween20-based intracellular stain buffer (0.2% Tween-20, 1% BSA). Loss of cytochrome c was analysed by flow cytometry to identify cells that underwent MOMP in response to each peptide treatment.

TBCRC-030 patient tumour RNA-seq analysis

All research involving human samples was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice. TBCRC-030 (NCT01982448) was approved by the Dana-Farber Cancer Institute and Harvard Cancer Center Institutional Review Board (IRB). All patients provided informed written consent before study initiation. TBCRC-030 was an investigator-initiated, prospective, randomized, phase 2 trial of chemotherapy in patients with TNBC. Patient enrolment and clinical trial regimens were previously described²⁸. In brief, patients with confirmed TNBC (ER ≤ 5%, PR ≤ 5%, HER2 IHC = 0/1+, clinical stage I–III) received neoadjuvant, preoperative, single-agent cisplatin or paclitaxel chemotherapy for 12 weeks. After treatment, pathologic response was assessed by residual cancer burden (RCB). Patients with an RCB score of 0/1 were considered responders and patients with RCB 2/3 were defined as non-responders. Tumour tissue was collected for sequencing concurrently with treatment.

As a post hoc study amendment, a subset of patients was followed for up to 5 years to track event-free survival or disease recurrence. Of the 68 patients enrolled and sequenced in this study, 15 patients with known RCB status were followed for event-free survival. We identified 4 non-responder patients with diagnosed disease recurrence, RCB 2/3, and sufficient tumour tissue for sequencing. These patients were defined as progressors and were analysed with 6 case–control matched responders with a confirmed pathologic response, RCB 0/1, and without known recurrence up to 5 years after treatment. Individual patient metrics are indicated in Extended Data Fig. 4. Notably, this analysis was blinded to individual patient demographics (age, race and so on) and we were therefore unable to account for these potential covariates in our subsequent analysis.

Gene counts were filtered and normalized to transcripts per million mapped reads (TPM). Only protein-coding genes found in two or more

samples and with a minimal number of ten counts were considered for analysis. DEGs between responders and progressors were computed using DESeq2 package⁶⁸ in RStudio. DEGs with $P \leq 0.05$ and fold change ≥ 1.5 or ≤ -1.5 were analysed using the GO (<https://david.ncicfcrf.gov/tools.jsp>) Biological Processes database. DEGs were ranked by test statistic (DESeq2 “Stat”) to perform preranked GSEA (v4.3.3) (<https://www.gsea-msigdb.org/gsea/index.jsp>) using human C5 Ontology Gene Sets from MSigDB. Gene expression changes were assessed by calculating z-scores across all patient samples per gene after TPM normalization and log transformation. The GR activity signature score was calculated as the average of gene expression z-scores across all GR activity signature genes per patient. The GR activity signature gene set was adapted from an independently developed published signature of human TNBC cells²¹. To test for potential confounding factors, we performed Pearson correlation analysis for expression of clinically relevant genes with known roles in breast cancer metastasis (such as *TP53*, *MKI67*, *HLA-A*, *CD274* and *SERBINB9*). No consistent correlation was observed in any tested covariate with *NR3C1* expression and GR activity signature.

Analysis of published patient cohorts

To investigate the correlation between GR, FAS and patient outcomes, we performed meta-analysis of published datasets of patients with breast cancer using the Kaplan–Meier Plotter (<https://kmplot.com/analysis>) breast mRNA⁶⁹ database. We also used the UCSC XenaBrowser (<https://xenabrowser.net/>) and GEPIA (<http://gepia2.cancer-pku.cn/>) browsers for breast TCGA patient analysis. Correlations between GR protein and patient outcome were analysed from a published proteomics dataset of patients with breast cancer⁷⁰ using the Kaplan–Meier Plotter (<https://kmplot.com/analysis>) breast protein database⁷¹. Breast tumour samples were filtered for ER, PR and HER2 negativity as indicated and then assessed for the expression of *NR3C1* or *FAS*. Patients were stratified by quartile expression and correlated with survival. Log-rank tests were used to determine statistical differences. Proportional hazard ratios were determined by performing proportional hazard assumption tests using a Cox regression model. For meta-analyses, we performed multivariate and covariate testing on relevant available parameters (*HER2*, *MKI67* and *PR* expression, *TP53* mutation status, lymph node status, patient treatment regimen) to account for any associations with *NR3C1* or *FAS* in the survival analysis. We were unable to perform multivariate analysis of single cohort survival correlations with *NR3C1* or *FAS* due to small sample size.

To compare expression between paired primary and metastatic breast tumour samples, we analysed matched patient samples from the AURORA US cohort²⁷. Normalized gene expression data (mRNA z-scores log₂ transformed from RNA-seq data) were downloaded from the cBio Portal (<https://cbioportal.org>). Patient samples were filtered for ER, PR and HER2 negativity. Gene expression analysis was then performed in Microsoft Excel (v.16.91). GR activity signature and FAS-induced cell death signature scores were calculated as the mean z-score expression per sample across all signature genes. The GR activity signature score gene set consisted of the following genes: *ABHD5*, *ACSL3*, *ANKRD1*, *APIAR*, *ATP2B1*, *BBS10*, *BCOR*, *CCT6A*, *CDK7*, *CHMP2B*, *CRY1*, *DDX18*, *DLAT*, *ETF1*, *F2R*, *FN1*, *HEATR3*, *HPSS*, *HSPA9*, *ID3*, *IMPACT*, *IPO7*, *KCTD3*, *KLF6*, *KLF9*, *LYPLA1*, *MCL1*, *MT2A*, *NAP1L1*, *NOL11*, *PER1*, *PEX3*, *PLCB4*, *PRPF39*, *RABGGTB*, *RMND1*, *SEH1L*, *SERP1*, *SGK1*, *SNAI1*, *SNAI2*, *SPATA5L1*, *SSB*, *TSC22D3*, *TSEN2*, *USE1*, *UTPI4A*, *VIM*, *WDR43* and *WNT5A*. The FAS-induced cell death signature consisted of the following genes: *FAS*, *BID*, *BAD*, *BCL2*, *BCL2L1*, *NFKB1*, *NFKB2*, *CHUK*, *DIRAS3*, *HLA-G*, *APAF1*, *CASP9*, *CASP8*, *CASP10*, *CASP3*, *CASP6*, *CASP7*, *GSK3B*, *ARL11*, *WWOX*, *PEG3*, *TGFB1*, *TGFBRI* and *TGFBRI2*.

Statistical analysis

Exact replicates for each experiment are listed in the figure legend as *n* values. All experiments in this study were successfully repeated at least

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twice. All data represented are the result of an independent experiment; no separate experiments were pooled or combined for analysis. For all in vivo studies, each dot in the plot represents an individual mouse. Statistical methods were not used to predetermine sample size. Samples were randomized into experimental groups for in vitro and in vivo experiments. Researchers were blinded when performing immunofluorescence staining, image acquisition, flow cytometry sample processing, staining and acquisition, and FACS sorting. Immunofluorescence quantification was performed in a blinded manner using Fiji batch functions. For sequencing experiments, separate researchers performed sample collection, sample processing and data analysis. Researchers were not blinded during in vitro or in vivo experiments involving treatment regimens, as knowledge of this information was necessary to conduct the experiment. Microsoft Excel (v.16.91) was used for data processing. All statistical analyses were performed in PRISM (GraphPad) (v.10) software using the indicated test stated in the figure legend. All statistical tests were two-sided and $P < 0.05$ was considered significant. All data are represented as mean $\pm$ s.e.m.

Schematics

Figures were prepared using Adobe Illustrator (v.30.0) and schematics were generated using BioRender (<https://biorender.com>) (Figs. 1a,e,g, 2a,e,g,i,j, 3a, 4a,h and 5a,b,g and Extended Data Figs. 2e, 3a,i, 4a, 6b,c, 8a,f, 9i and 10j) with permission to publish under an Academic License (Agudo-Cantero, J. <https://BioRender.com/7lerlqz> (2026)).

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The raw data corresponding to bulk RNA-seq datasets generated in this study have been deposited in the Gene Expression Omnibus (GEO) repository under accession numbers GSE313249 (tdTomato⁺ 4T1 tumour cells from NSG versus BALB/c mice across disease stages) and GSE313241 (GFP⁺ 4T1 DTCs from Jedi versus NSG mice). scRNA-seq raw data have been deposited in the GEO under accession numbers GSE313245 (GFP⁺ versus Thy1.1⁺ 4T1 DTCs after Jedi adoptive transfer) and GSE313243 (tdTomato-cTAT-zsGreen niche labelling of shGR versus shControl 4T1 DTCs). CUT&RUN raw data have been deposited in the GEO under accession number GSE313250. Clinical trial data from TBCRC-030 are available on reasonable request. Any additional data reported in this study are available on request. All other datasets reanalysed in this study are publicly available⁷⁰ (AURORA US, <https://cbiportal.org>; TCGA, <https://xenabrowser.net/> and <http://gepia2.cancer-pku.cn/>; meta-analysis of patients with TNBC, <https://kmplot.com/analysis>; Tabula Muris, <https://tabula-muris.sf.czbiohub.org/>). Source data are provided with this paper.

Code availability

No custom code was used in the generation of the data in this study. Data analysis using public software packages is described in the Methods.

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Author contributions M.C. and J.A. conceived the study. M.C., X.S., L.H., Y.H., A.J.G., D.G., P.B., I.C., J.C., B.E.S., E.M., M.S. and X.Q. designed and performed the experiments. L.H. performed scRNA-seq analysis of mouse studies. A.J.G. performed chromatin immunoprecipitation experiments and CUT&RUN analysis. E.E.M., H.A.P. and E.L.M. performed analysis of DFCI TBCRC-030 clinical trial. S.K.D., E.A.M. and S.S.M. provided expertise in breast cancer, immunology and current clinical practices. X.Q. and K.A.S. assisted with expertise in BH3 profiling and cell death. Y.-C.H. provided expertise in GR biology. M.C. and J.A. wrote the paper. J.A. designed and supervised the study. All of the authors assisted in the interpretation of the experiments and editing the paper.

Competing interests H.A.P. receives funding from Pfizer and Merck; and is the recipient of honoraria or consultation fees from Daichi-Sankyo, AZ, Caris, SAGA, Natera, Illumina, Guardant, Sermonix and Lilly. E.L.M. is the recipient of honoraria or consultation fees from Lilly, AstraZeneca, Novartis and Arvinas. S.K.D. received research funding from Novartis, BMS, and Takeda unrelated to the current project and consulting fees from Revolution Medicines. The other authors declare no competing interests.

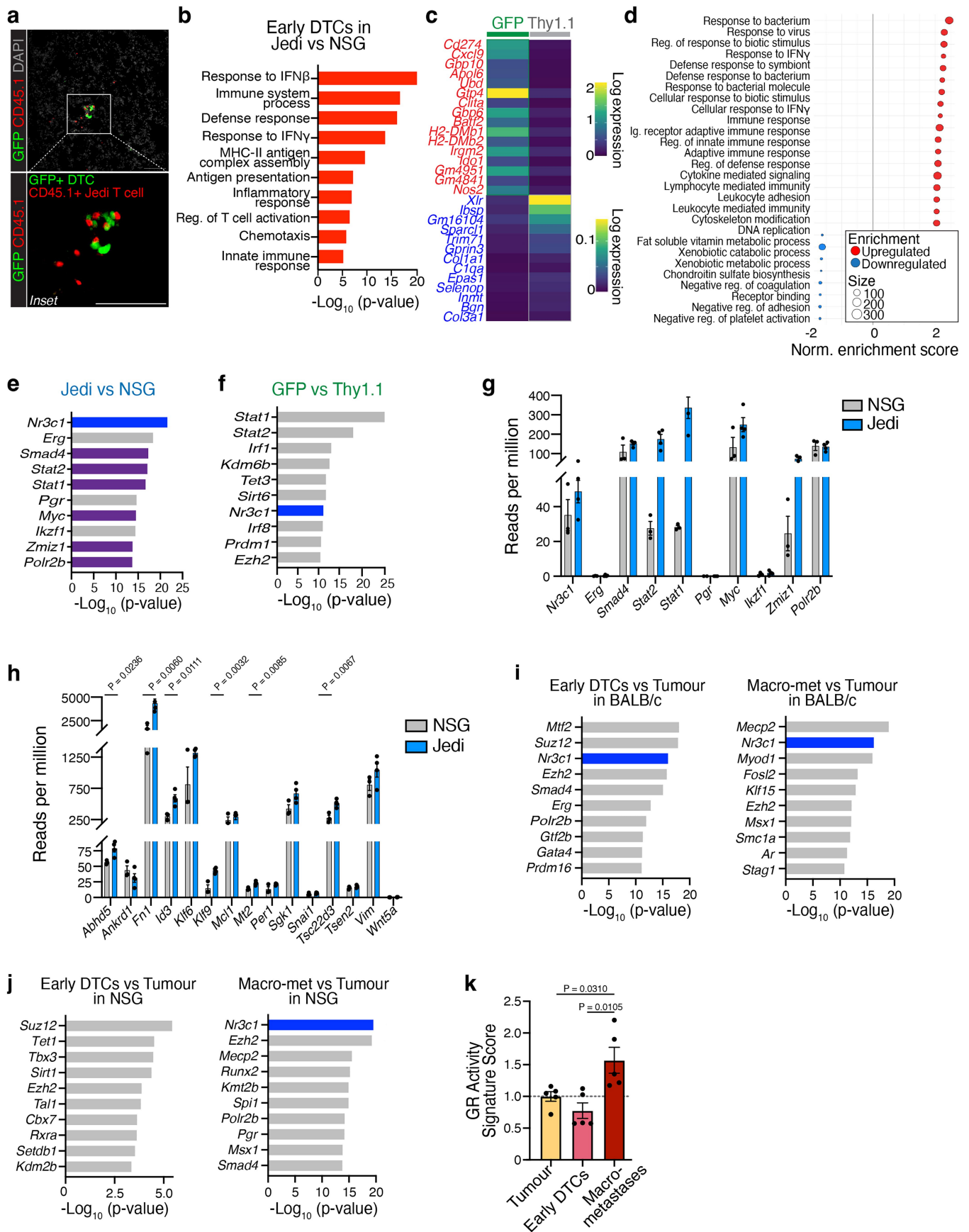
Additional information

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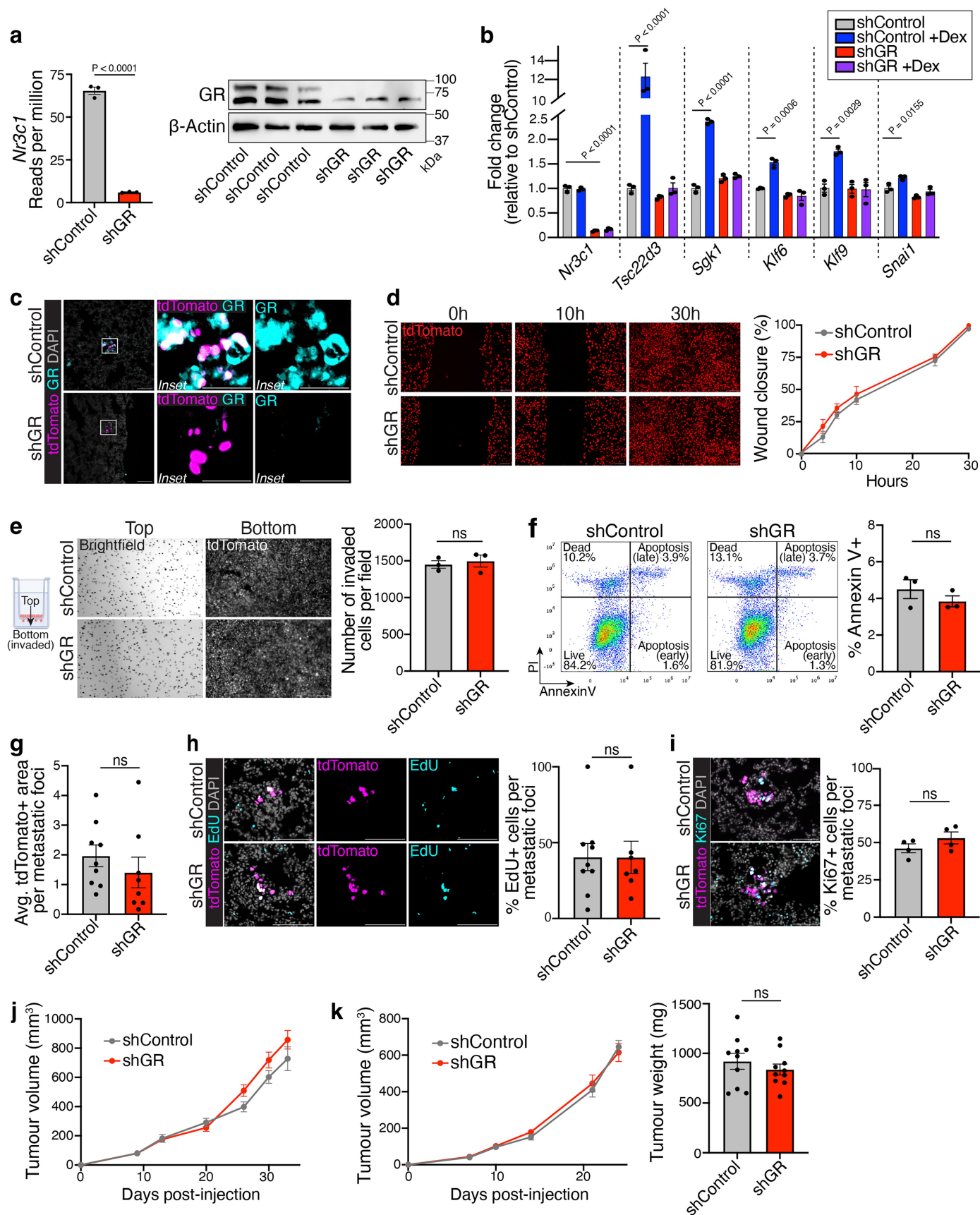
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Extended Data Fig. 1 | See next page for caption.

Extended Data Fig. 1 | RNA sequencing of surviving Jedi-resistant DTCs identifies mechanisms of immune evasion. **a**, Immunofluorescence of DTCs in the lung. Adoptively transferred Jedi T cells (CD45.1 +) in BALB/c mice localize near GFP+ DTCs that resist killing (inset) (representative of n = 4 mice). **b**, Gene Ontology (GO) pathway analysis of differentially expressed genes (DEGs) upregulated in Jedi-resistant DTCs compared to NSG-surviving DTCs (n = 3 (NSG), 4 (Jedi) mice; Fold change (FC) > 1.5, FDR adjusted P-value < 0.05). **c**, Top DEGs upregulated (red) and downregulated (blue) in GFP+ Jedi-resistant DTCs compared to Thy1.1+ DTCs by scRNA-seq. **d**, Unsupervised gene set enrichment analysis (GSEA) of DEGs between GFP+ and Thy1.1+ DTCs (**c,d**, n = 5 mice pooled per group; FC > 1.5, FDR P-value < 0.01). **e,f**, Top ranked transcriptional regulators from the DEGs enriched in Jedi-resistant DTCs by LISA analysis. **e**, Jedi-resistant DTCs compared to NSG-surviving DTCs.

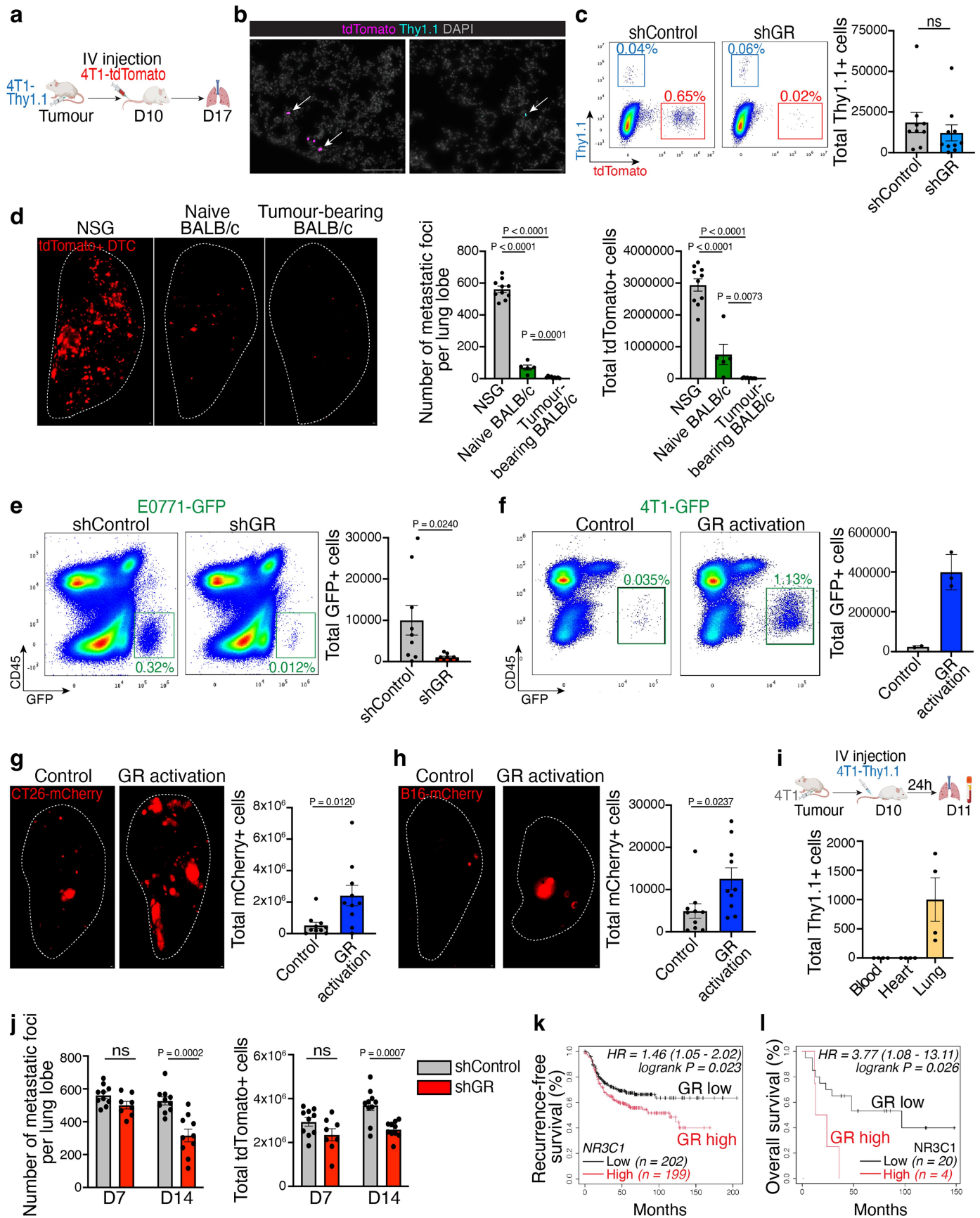
Transcription factors with detectible expression in DTCs are labelled, as assessed in (**g**). **f**, Resistant GFP+ DTCs compared to non-targeted Thy1.1+ DTCs from Jedi-treated mice. *Nr3c1* (GR) is highlighted (**e,f**, FC > 1.5, FDR adjusted P-value < 0.05, top 500 genes by FC). **g**, Expression of the top 10 transcriptional regulators predicted by LISA in RNA-seq of surviving GFP+ DTCs from Jedi and NSG mice. **h**, Expression of GR Activity Signature genes in Jedi-resistant DTCs compared to NSG-surviving DTCs (**g,h**, n = 3 (NSG), 4 (Jedi) mice; **h**, two-tailed unpaired t-test). **i,j**, LISA ranking of the top 10 transcriptional regulators enriched in the indicated comparison of tumour cells from BALB/c mice (**i**) or NSG mice (**j**) by RNA-seq. **k**, Relative GR Activity gene expression signature in purified 4T1 tumour cells from NSG mice (**i-k**, n = 5 mice per group; **i,j**, FC > 1.5, FDR adjusted P-value < 0.05, top 500 genes by FC; **k**, two-tailed unpaired t-test; plotted as FC to primary tumour). Data are mean ± s.e.m. Scale bar, 100 µm.



Extended Data Fig. 2 | See next page for caption.

Extended Data Fig. 2 | GR function in cancer cells does not alter primary tumour growth or proliferation of DTCs. **a**, *Nr3c1* (GR) expression in shControl and shGR 4T1 tumour cells by bulk RNA-seq (left) and Western blot (right) (n = 3 technical replicates per group). For gel source data, see Supplementary Fig. 2. **b**, Expression of GR Activity genes by qPCR following GR activation with Dexamethasone (Dex) treatment (n = 3 biological replicates per group). **c**, Immunofluorescence of GR in shControl and shGR lung DTCs (representative of n = 4 mice). **d-f**, Migration (**d**, scratch-wound assay, n = 4 technical replicates per group), invasion (**e**, transwell assay, n = 3 technical replicates per group), and apoptosis (**f**, proportion of Annexin V+ cells by flow

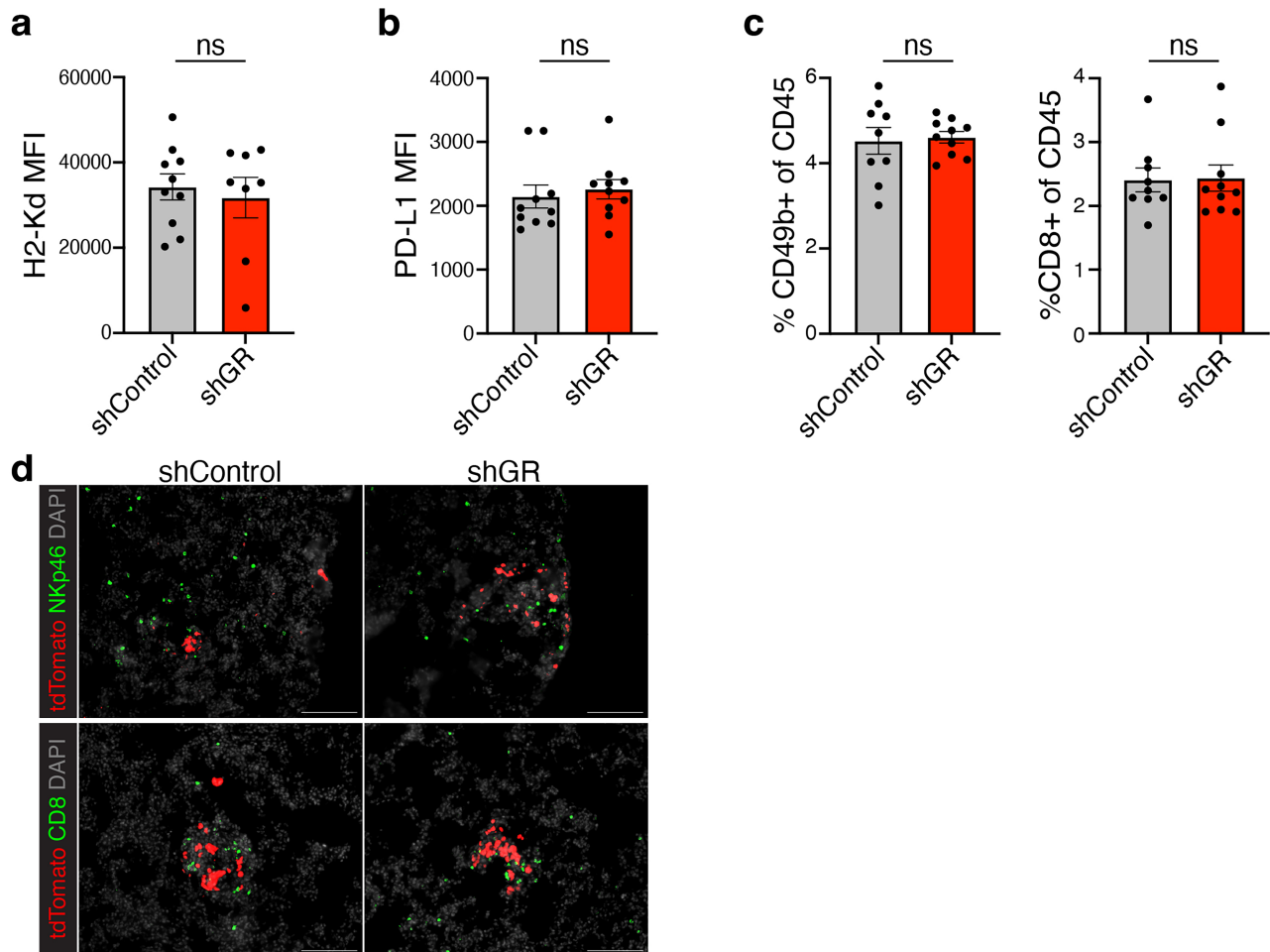
cytometry, n = 3 technical replicates per group) of shControl and shGR 4T1 cells in vitro. **g-i**, Average size per metastatic foci (**g**) (n = 9 (shControl), 8 (shGR) mice) and percentage of EdU+ (**h**) (n = 9 (shControl), 7 (shGR) mice) or Ki67+ (**i**) (n = 4 mice per group) proliferating DTCs per metastatic foci in vivo. **j,k**, Primary tumour growth of shControl or shGR 4T1-tdTomato tumour cells following orthotopic injection into BALB/c mice (**j**) (n = 15 mice per group) or NSG mice (**k**) (n = 10 mice per group). Two-tailed unpaired t-test. Data are mean ± s.e.m. Scale bar, 100 µm (c-e,h,i), 50 µm (c, inset). The diagram in **e** was created using BioRender; Agudo-Cantero, J. <https://BioRender.com/71erlqz> (2026).



Extended Data Fig. 3 | See next page for caption.

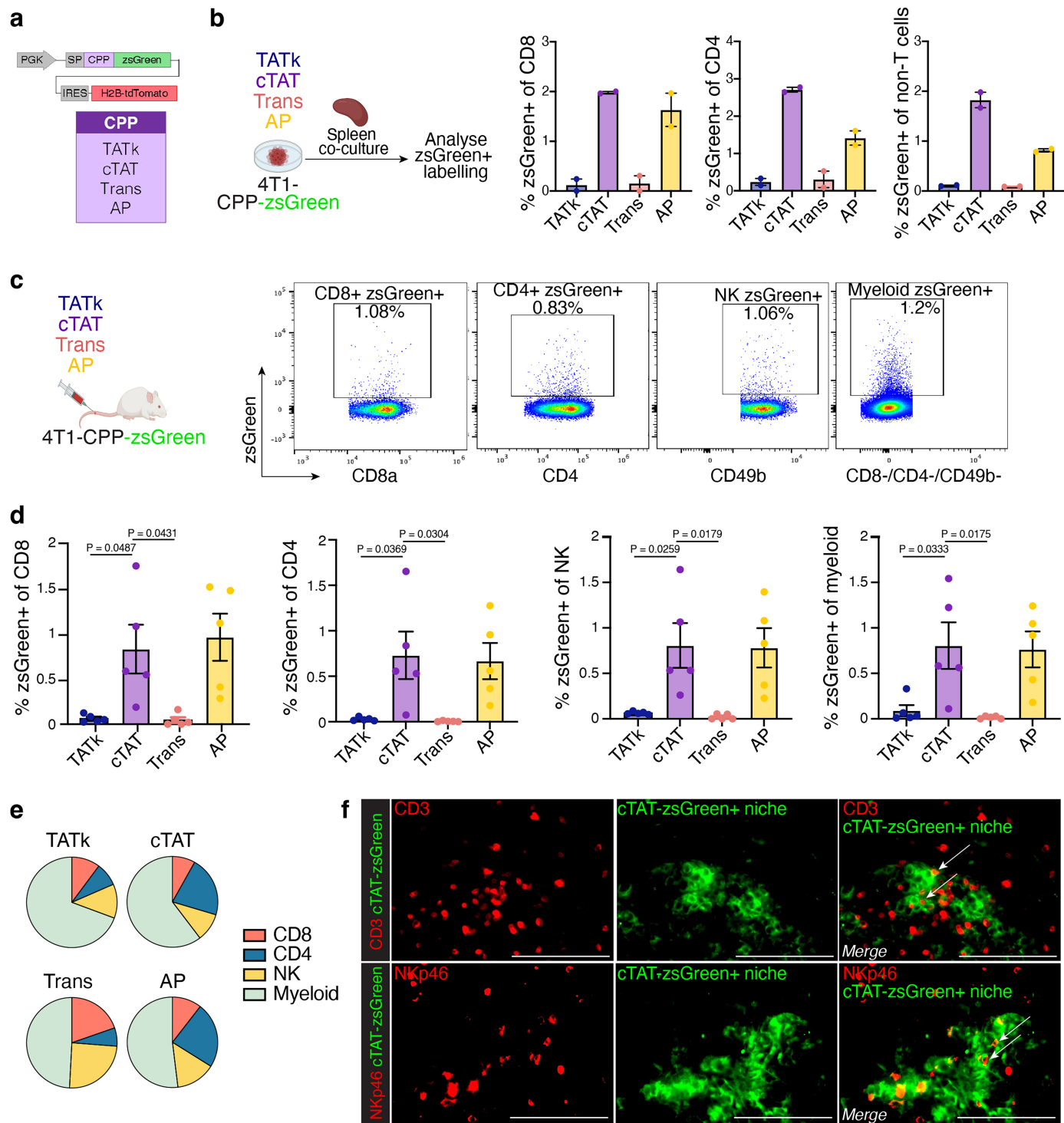
Extended Data Fig. 3 | Activation of GR in cancer cells promotes lung metastasis. **a-d**, A tumour-bearing BALB/c mouse model recapitulates anti-tumour immune responses and reduces DTC survival. Schematic (**a**) and representative images (**b**) of intravenous tdTomato+ DTCs and Thy1.1+ cells from the primary tumour in the same lung of a tumour-bearing BALB/c mouse (representative of $n = 4$ mice). (**c**) Quantification of Thy1.1+ cells in the lung. 4T1-Thy1.1 were implanted in the mammary gland 10 days prior to intravenous injection of tdTomato+ shControl or shGR DTCs ($n = 9$ (shControl), 10 (shGR) mice). **d**, Number of 4T1-tdTomato+ metastatic foci by image quantification (left) and total DTCs by flow cytometry (right) 7 days after intravenous injection into NSG, naïve BALB/c (no primary tumour), or tumour-bearing BALB/c mice ($n = 10$ (NSG), 5 (naïve BALB/c), 9 (tumour-bearing BALB/c) mice). **e**, shControl or shGRE0771-GFP lung DTCs in immune-competent C57BL/6 mice following intravenous injection ($n = 9$ mice per group). **f-h**, Number of lung DTCs following

intravenous injection of 4T1-GFP breast (**f**) ($n = 2$ (control), 3 (GR activation) mice), CT26-mCherry colorectal (**g**), and B16-mCherry melanoma cells (**h**) (**g,h**, $n = 10$ mice per group). Tumour cells were pre-treated with Dexamethasone or vehicle control in vitro to activate GR and injected into syngeneic immune-competent BALB/c (**f,g**) or C57BL/6 (**h**) mice. **i**, Tumour cell number in blood, heart, and lung one day after intravenous injection ($n = 4$ mice per group). **j**, Lung metastasis of shControl or shGR 4T1-tdTomato tumour cells in NSG mice following intravenous injection by number of metastatic foci (left) and total DTCs (right) ($n = 10$ (shControl D7, shControl D14, shGR D14), 8 (shGR D7) mice). **k, l**, Kaplan-Meier survival analysis of Estrogen Receptor-negative breast cancer patients based on expression of NR3C1 (GR) by RNA (**k**, meta-analysis of published cohorts) or protein (**l**, Tang. et al., 2018). Two-tailed unpaired t-test. Data are mean $\pm$ s.e.m. Scale bar, 100 μ m. The diagrams in **a** and **i** were created using BioRender; Agudo-Cantero, J. <https://BioRender.com/7lerlqz> (2026).



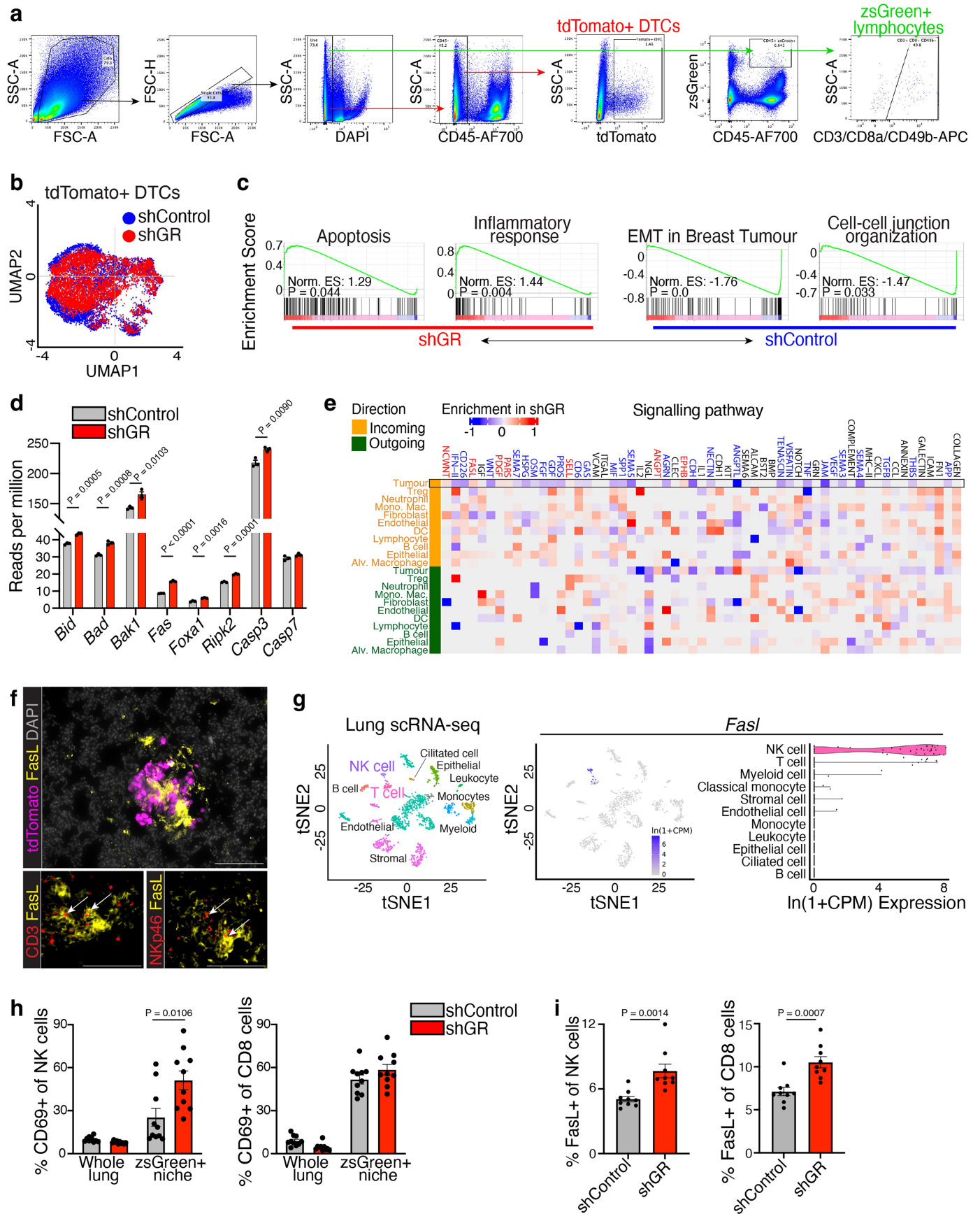
Extended Data Fig. 5 | GR activation in DTCs does not alter frequent mechanisms of immune evasion. **a, b**, Flow cytometry analysis of H2-Kd (MHC I) (**a**) ($n = 10$ (shControl), 8 (shGR) mice) and PD-L1 (**b**) ($n = 10$ mice per group) levels in DTCs by Median Fluorescent Intensity (MFI) in tumour-bearing BALB/c mice. **c**, Flow cytometry quantification of NK cells (CD49b+, left) and CD8 + T cells

(right) in the lung of mice with shControl or shGR 4T1 DTCs ($n = 10$ mice per group). **d**, Representative immunofluorescence images of NK cells (NKp46+, top) and CD8 + T cells (CD8+, bottom) surrounding shControl or shGR 4T1-tdTomato DTCs in the lung (representative of $n = 4$ mice). Two-tailed unpaired t-test. Data are mean $\pm$ s.e.m. Scale bar, 100 μ m.



Extended Data Fig. 6 | Generation of cTAT-zsGreen niche labelling tool to study lymphocytes interacting with DTCs. **a**, Construct design and summary of all tested cell-penetrating peptides (CPP) fused to zsGreen. **b**, Percentage of zsGreen+ cell populations following in vitro co-culture with 4T1 tumour cells expressing zsGreen with the indicated CPP construct as measured by flow cytometry ($n = 2$ biological replicates per group). **c-e**, In vivo analysis of CPP constructs following tail vein injection of 4T1 tumour cells engineered with the indicated CPP fusion to zsGreen. **c**, Representative flow cytometry plots of zsGreen+ niche labelling of CD8+, CD4+, NK (CD49b+), and myeloid (CD45+ CD8- CD4- CD49b- CD11c-) immune cells in the lung with the cTAT CPP (representative

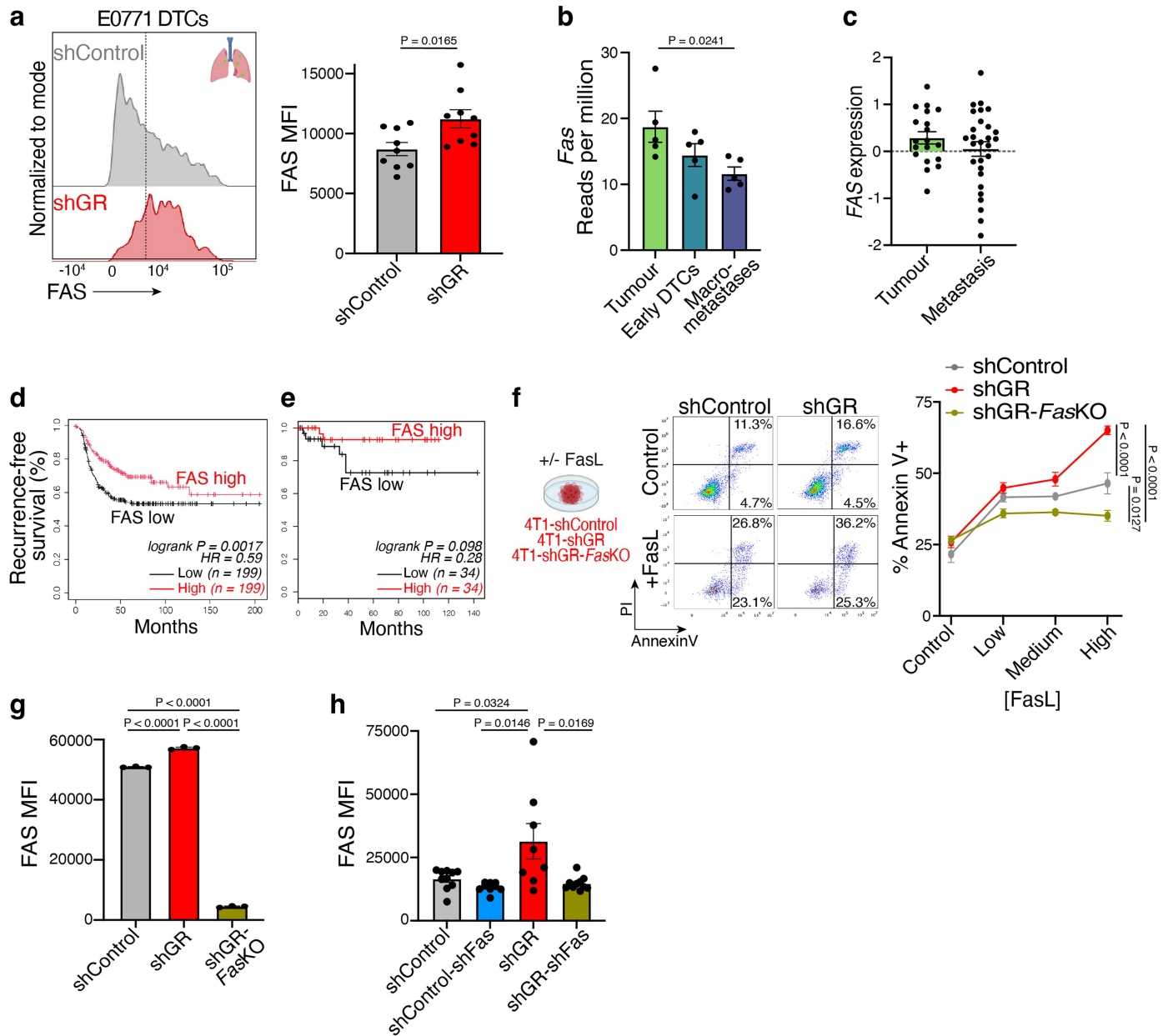
of $n = 5$ mice). **d**, Labelled niche cells are quantified as the percentage zsGreen+ of total indicated immune cell population in the lung (FDR P-adjusted one-way ANOVA; $n = 5$ mice per group). **e**, Proportions of in vivo immune cell labelling within the zsGreen+ compartment for the indicated CPP. Immune populations are quantified as percentage of total zsGreen+ CD45+ cells (average of $n = 5$ mice per group). **f**, Immunofluorescence analysis of CD3 (top) and NKp46 (bottom) demonstrate zsGreen+ labelling of T cells and NK cells, respectively, in the niche using the cTAT CPP (representative of $n = 10$ mice). Data are mean $\pm$ s.e.m. Scale bar, 100 μ m. The diagrams in **b** and **c** were created using BioRender; Agudo-Cantero, J. <https://BioRender.com/7erlqz> (2026).



Extended Data Fig. 7 | See next page for caption.

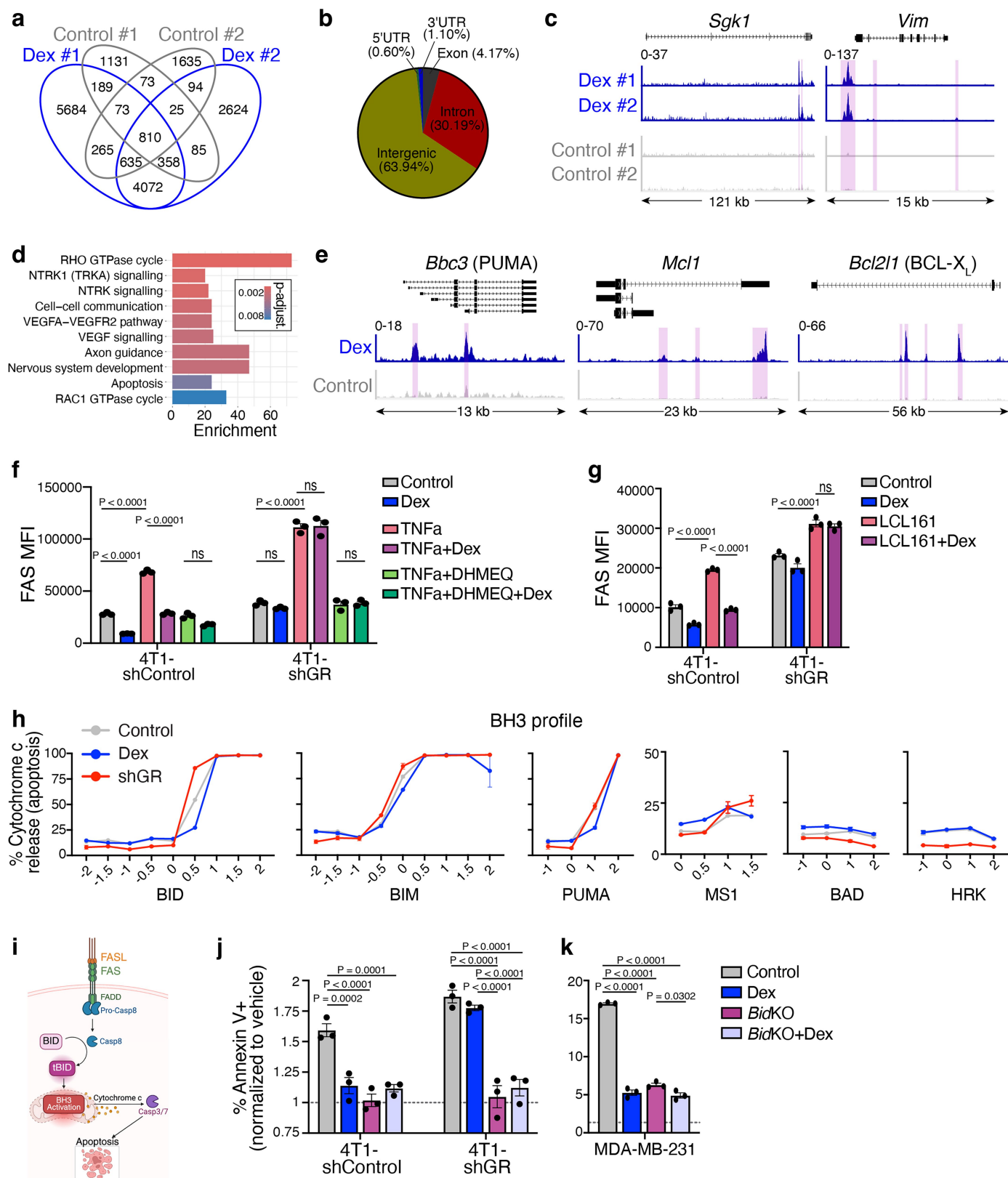
Extended Data Fig. 7 | Analysis of DTCs and interacting cytotoxic lymphocytes reveals Fas-Fas-Ligand as dominant mechanism of GR-mediated immune evasion. **a**, Representative flow cytometry gating strategy for isolating tdTomato+ DTCs and zsGreen+ niche lymphocytes. **b**, Merged UMAP of shControl and shGR tdTomato+ DTCs. **c**, GSEA of the differentially expressed genes (DEGs) between shControl and shGR DTCs by scRNA-seq. **d**, Expression of pro-apoptotic genes from RNA-seq of shGR and shControl tumour cells in vitro (FDR-adjusted multiple two-tailed unpaired t-test; n = 3 technical replicates per group). **e**, CellChat analysis of all differentially active receptor-ligand signalling pathways in shControl and shGR DTC niches. The predicted incoming (orange) and outgoing (green) signalling activities are visualised by pathway (column) and by cell type in the niche (row). Scored by the

predicted enrichment in signalling activity in shGR niche (red) compared to shControl niche (blue). Box: incoming signals to DTCs from the niche. **f**, Representative immunofluorescence of FasL in the niche surrounding 4T1-tdTomato DTCs (representative of n = 4 mice). **g**, Expression of *FasI* in the mouse lung by scRNA-seq (from *Tabula Muris* database). tSNE (t-distributed stochastic neighbour embedding) (centre) and violin plot (right) of *FasI* expression (in counts per million, CPM) in lung populations identified by tSNE (left). **h**, Proportion of NK cells (left) and CD8 + T cells (right) expressing CD69 in the whole lung and within the zsGreen+ population (n = 10 mice per group). **i**, Proportion of FasL+ NK cells (left) and CD8 + T cells (right) in the lung containing shControl or shGR E0771 DTCs (n = 9 mice per group) (**h,i**, two-tailed unpaired t-test). Data are mean $\pm$ s.e.m. Scale bar, 100 μ m.



Extended Data Fig. 8 | FAS-mediated cell death of tumour cells is suppressed by GR activation. **a**, Flow cytometry analysis of FAS levels in shControl or shGR E0771 DTCs in the lung by Median Fluorescent Intensity (MFI) (two-tailed unpaired t-test; $n = 9$ mice per group). **b**, FAS expression in tumour cells purified from primary tumours, early lung DTCs, and established lung macrometastases in BALB/c mice (RNA-seq samples from Fig. 1a) (two-tailed unpaired t-test; $n = 5$ mice per group). **c**, Relative gene expression of FAS in paired primary tumours and matched metastases from patients with TNBC in the AURORA US dataset ($n = 19$ patients). **d**, **e**, Kaplan-Meier survival analysis based on expression of FAS in patients with Estrogen Receptor-negative breast cancer (**d**, meta-analysis of

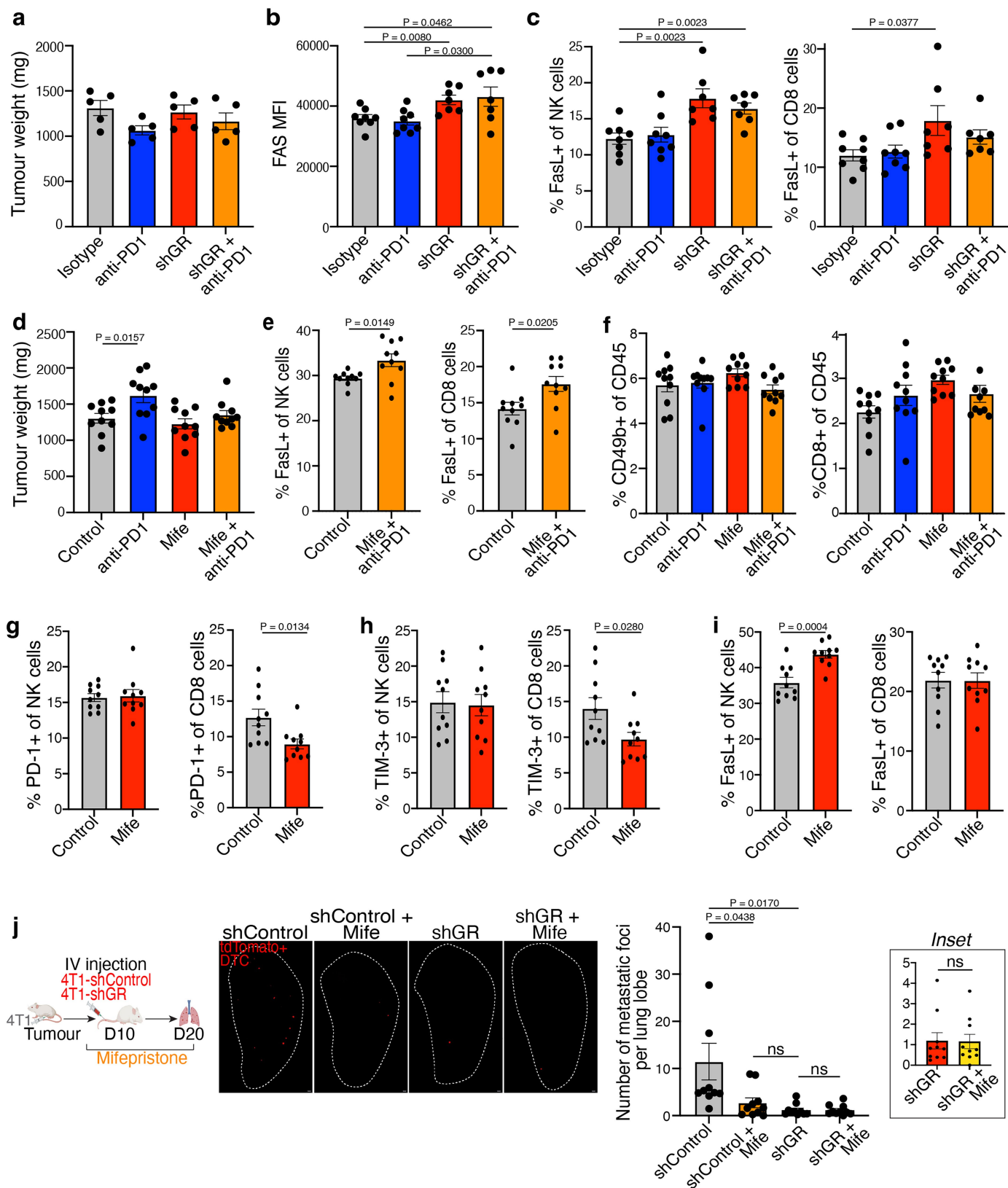
published cohorts) or Triple Negative Breast Cancer (**e**, TCGA). **f**, FAS-induced apoptosis (Annexin V+) following exposure to low (0.5 $\mu\text{g/mL}$), medium (1 $\mu\text{g/mL}$), or high (50 $\mu\text{g/mL}$) concentrations of soluble Fas-Ligand (FasL). Representative flow plots (left) show treatment with vehicle control and high FasL (two-way ANOVA; $n = 3$ biological replicates per group). **g**, FAS levels in 4T1 tumour cell lines with Fas knockout (FasKO) (two-tailed unpaired t-test; $n = 3$ technical replicates per group). **h**, FAS MFI of DTCs with Fas knockdown (shFas) in vivo (two-tailed unpaired t-test; $n = 10$ (shControl, shGR-shFas), 9 (shControl-shFas), 8 (shGR) mice). Data are mean $\pm$ s.e.m. The diagrams in **a** and **f** were created using BioRender; Agudo-Cantero, J. <https://BioRender.com/71erlqz> (2026).



Extended Data Fig. 9 | See next page for caption.

Extended Data Fig. 9 | Analysis of GR genomic binding reveals regulation of FAS-induced cell death pathway. **a-e**, Immunoprecipitation of GR and CUT&RUN analysis. 4T1 tumour cells were treated with Dexamethasone (Dex) for 7 days to match the chronic stimulation of GR in DTCs (analysed at day 7). **a**, Venn diagram of GR binding sites in each sample. **b**, Proportional genome-wide binding locations of Dex-specific GR peaks conserved across both Dex-treated replicates (n = 4,072 peaks total). **c**, GR occupancy at canonical GR-induced sites. **d**, Reactome Pathway Analysis of GR bound genes in Dexamethasone-treated cells. **e**, GR binding at BCL-2 family apoptotic loci (**a-e**, n = 2 biological replicates per treatment condition; **c,e**, GR peak regions are highlighted in purple, FDR $q < 0.05$; **e**, average of n = 2 per condition). **f,g**, FAS MFI following NF- κ B activation by treatment with TNF α (**f**) or LCL161 (**g**). Treatment with the NF- κ B inhibitor DHMEQ neutralizes the effect of TNF α activation (n = 3 biological replicates per group; cultured in IFN γ media). **h**, BH3 profiling of shControl and shGR 4T1

tumour cells following Dexamethasone (Dex) treatment to activate GR. Cells were permeabilized and treated with pro-apoptotic BH3 peptides. Mitochondrial cytochrome c release was measured by flow cytometry. Treatment with BID, BIM, and PUMA assess overall apoptotic priming. MSI, BAD, and HRK measure cellular dependence on specific pro-survival proteins (MCL-1, BCL-2/BCL-X_L, and BCL-X_L, respectively) (peptide concentrations indicated as log[] in μ M; n = 2 biological replicates per group). **i**, Schematic of FAS-induced intrinsic cell death pathway via BID and BH3 activation. **j,k**, FAS-induced apoptosis of *Bid* knockout cell lines in shGR or shControl 4T1 mouse (**j**) and *BID* knockout in MDA-MB-231 human (**k**) TNBC cells following treatment with soluble Fas-Ligand (FasL) (normalized to vehicle control treatment) in IFN γ media (n = 3 biological replicates per group). Two-way ANOVA. Data are mean $\pm$ s.e.m. The diagram in **i** was created using BioRender; Agudo-Cantero, J. <https://BioRender.com/7lerlqz> (2026).



Extended Data Fig. 10 | See next page for caption.

Article

Extended Data Fig. 10 | Blocking GR function in DTCs correlates with higher FasL levels in cytotoxic lymphocytes in the lung. **a**, Primary breast tumour weight after the indicated treatments (n = 5 mice per group). **b**, FAS expression in DTCs in the lung following indicated treatments. **c**, Proportion of FasL+ NK cells (left) and CD8 + T cells (right) in the lung (**b,c**, n = 8 (Isotype, anti-PD1), 7 (shGR, shGR+anti-PD1) mice). **d**, Primary tumour weight following anti-PD1 and mifepristone (Mife) treatments. **e**, Proportion of FasL+ NK cells (left) and CD8 + T cells (right) in the metastatic lung following combination treatment. **f**, Flow cytometry quantification of NK cells (CD49b+, left) and CD8 + T cells (right) in the lung of mice following anti-PD1 and mifepristone treatments.

g-i, Flow cytometry analysis of PD-1 (**g**), TIM-3 (**h**), FasL (**i**) proportions of NK (left) and CD8 + T cells (right) in the metastatic lung after mifepristone treatment (**d-i**, n = 10 mice per group). **j**, Schematic, representative lung images, and quantification of DTC micrometastases following intravenous injection of shControl or shGR 4T1-tdTomato tumour cells into tumour-bearing BALB/c mice and treatment with mifepristone (inset, shGR only) (n = 10 mice per group). Two-tailed unpaired t-test. Data are mean $\pm$ s.e.m. Scale bar, 500 μ m. The diagram in **j** was created using BioRender; Agudo-Cantero, J. <https://BioRender.com/7lerlqz> (2026).

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Software and code

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Data collection

Flow cytometry data was collected using FACSDiva (BD Biosciences) (v9.0.1) or CytExpert (Beckman) (v2.4) software.

Immunofluorescence images were acquired on a Leica THUNDER microscope imaging system. Fluorescent images of mouse lung lobes were acquired on a Leica M205 stereoscope.

Data analysis

Flow cytometry data was analyzed using FlowJo (v10) software.

Image processing was performed using LAS X (Leica) (v3.7.4.23463) software. Image analysis and metastatic foci quantification was performed using Fiji (ImageJ) (v2.1.0/1.53c) software.

Bulk RNA-seq analysis was performed in RStudio (v6.1.0) using Bioconductor EdgeR (v3) and Preranked Gene Set Enrichment Analysis (GSEA) (v4.3.3).

Single cell RNA-seq analysis was performed in RStudio (v4.3.2). The following open source algorithms were used: Cell Ranger (v8.0.1), Bioconductor SingleCellExperiment (v1.24.0), scuttle (v1.12.0), scDbfFinder (v1.16.0), corral (v1.12.0), igraph (v2.1.1), bluster (v1.12.0), uwot (v0.1.16), scan (v1.30.2), Gene Ontology (GO) pathway analysis (<https://david.ncifcrf.gov/tools.jsp>), LISA (<http://lisa.cistrome.org/>), fgsea (v1.28.0), Preranked Gene Set Enrichment Analysis (v4.3.3), CellChat (v1.6.1), ggplot2 (v3.5.1), ComplexHeatmap (v2.18.0). CUT&RUN analysis was performed in RStudio (v4.4.2). The following open source algorithms were used: TrimGalore (v0.6.10), bowtie2 (2.5.4), Samtools (v1.21), MACS3 (v3.0.3), deepTools2 (v3.5.6), ChIPSeeker (v1.42), ReactomePA (v1.50), enrichplot (v1.26.6), ChIPpeakAnno (v3.4), enrichplot (v1.26.6).

Microsoft Excel (v16.91) was used for data processing. Statistical analysis was performed using PRISM (GraphPad) (v10) software.

Figures were prepared using Adobe Illustrator (v30.0).

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- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

The raw data corresponding to bulk RNA-seq datasets generated in this study are deposited in the Gene Expression Omnibus (GEO) repository under accession numbers GSE313249 (tdTomato+ 4T1 tumour cells from NSG vs BALB/c mice across disease stages) and GSE313241 (GFP+ 4T1 DTCs from Jedi vs NSG mice). Single cell RNA-seq raw data is deposited in GEO under accession numbers GSE313245 (GFP+ vs Thy1.1+ DTCs following Jedi adoptive transfer) and GSE313243 (tdTomato-cTAT-zsGreen niche labelling of shGR vs shControl DTCs). CUT&RUN raw data is deposited in GEO under accession number GSE313250.

Clinical trial data from TBCRC-030 is available upon reasonable request. This data is restricted due to ongoing clinical trial and patient privacy and confidentiality requirements. Relevant raw data for the analysis in this study is provided in the source data for ED Fig. 4.

Any additional data reported in this study is available upon request. All other datasets re-analysed in this study are publicly available (Tang et al., Genome Med, 2018; AURORA US via cbiportal.org; TCGA via xenabrowser.net/ and <http://gepia2.cancer-pku.cn/>; Meta-analysis of TNBC patients via kmplot.com/analysis; Tabula Muris via tabula-muris.sf.czbiohub.org/).

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender

Patient samples analyzed in this study were from female breast cancer patients (>99% of total breast cancer cases).

Reporting on race, ethnicity, or other socially relevant groupings

No socially relevant variables were analyzed in this study.

Population characteristics

Population characteristics for the TBCRC-030 clinical trial were previously reported (Mayer et al., Ann Oncol, 2020). All patients were early-stage with clinical stage I and histologic grade II or III. All patients were pathologically defined as TNBC (ER≤5%, PR≤5%, HER2 IHC 0/1+ or FISH ratio <2.0) and patients with BRCA1/2 mutations were not eligible. All patients were untreated at the time of tumour removal and RNA-seq tissue analysis. All patients were female.

Recruitment

Recruitment for TBCRC-030 was previously reported (Mayer et al., Ann Oncol, 2020).

Ethics oversight

TBCRC-030 (NCT01982448) was approved by the Dana-Farber Cancer Institute and Harvard Cancer Center Institutional Review Board.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences

☐ Behavioural & social sciences

☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size

For in vivo experiments, no statistical methods were used to predetermine sample size. Sample sizes were determined based on preliminary studies in our lab assessing biological variation and literature review of previous published studies (Hu et al, Nature, 2023; Goto et al., Nature, 2024; Spiegel et al., Cancer Discovery, 2016; Agudo et al., Nat Biotechnol, 2015). For in vitro experiments, no statistical methods were used to predetermine sample size. Sample size was determined based on previous experience with specific assays (Baldominos et al., Cell, 2022; Spetz et al., Sci Adv, 2022; Agudo et al., Nat Biotechnol, 2015), preliminary experiments to assess expected variation between samples and statistically significant differences, and prior literature using similar systems and assays (West et al., Clin Canc Res, 2018; Fraser et al., Methods Mol Biol, 2019; Meers et al., eLife, 2019; Pan et al., Cancer Res, 2011).

Data exclusions

No data was excluded. For scRNA-seq analysis, doublets and low quality cells were excluded as described in the methods.

Replication	All experiments in this study were successfully repeated at least twice. Number of biological replicates are listed in figure legends.
Randomization	Mice were randomized into experimental groups. All mice were female and were age and litter matched between groups. For in vitro experiments, samples were randomly allocated for culture and treatment. For analysis of patients in TBCRC-030 clinical trial, patients were stratified based on outcome information (progressor or responder) as this was necessary for the analysis. Covariates were controlled based on initial patient selection criteria (see above) and additional covariates were tested by correlation analysis (see Methods for list of tested covariates).
Blinding	Researchers were blinded when performing IF staining and image acquisition for IF images and metastatic foci quantification. Researchers were blinded during flow cytometry sample processing, staining, and acquisition and FACS-sorting. IF quantification was blindly performed using Fiji batch functions. For sequencing experiments, separate researchers performed sample collection, sample processing, and data analysis. Researchers were not blinded during in vitro or in vivo experiments involving treatment regimens, as knowledge of this information was necessary to conduct the experiment.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☐	☒ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☐	☒ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

Antibodies used in this study are as follows:

In vivo:

Anti-mouse PD-1 (BioXCell #BE0146, 100µg every 2-3 days), rat IgG2b isotype (BioXCell #BE0090, 100µg every 2-3 days), anti-mouse CD8a (BioXCell #BE0117, 100µg every 2-3 days), anti-Asialo GM1 (Invitrogen #16-6507-39, 50µg every 2-3 days).

Flow cytometry:

TruStain FcX (Biolegend #101319), CD45-AF700 (1:200), CD45-PE/Cy7 (1:200), CD11c-PE/Cy7 (1:200), CD3e-APC (1:200), CD8a-APC/Fire750 (1:100), CD8a-APC (1:100), CD8a-BV510 (1:100), CD49b-Pacific Blue (1:150), CD49b-APC (1:150), CD56-APC/Cy7 (1:100), CD95(Fas)-FITC (1:100), CD95(Fas)-APC (1:100), CD95(Fas)-PE (1:100), CD178(FasL)-APC (1:50), CD178(FasL)-PE (1:100), CD178(FasL)-PE/Cy7 (1:100), FasL-Biotin (1:50), CD69-PE/Cy7 (1:100), H2-Kd-Pacific Blue (1:200), CD274(PD-L1)-APC (1:200), Thy1.1-APC (1:200), CD279(PD-1)-FITC (1:200), CD366(Tim-3)-PE (1:200), Streptavidin-APC (1:200). All antibodies are rat anti-mouse or mouse anti-human (Biolegend).

Immunofluorescence:

Rat anti-mouse CD45.1-AF647 (Biolegend #110720, used 1:200), rat anti-mouse Thy1.1-AF594 (Biolegend #202545, used 1:100), rat anti-mouse CD3-AF594 (Biolegend #100240, used 1:200), rat anti-mouse CD3-AF647 (Biolegend #100209, used 1:200), rat anti-mouse CD8a-AF647 (Biolegend #100727, used 1:200), mouse anti-human FasL (Santa Cruz #sc19681, used 1:50), goat anti-mouse Nkp46 (R&D #AF2225, used 1:50), rabbit anti-mouse FAS (abcam #ab216991, used 1:100), rabbit anti-mouse GR (Invitrogen #PA1-511A, used 1:200), donkey anti-rabbit IgG AF647 (Invitrogen #A31573, used 1:200 with FAS and GR), donkey anti-goat IgG AF647 (Invitrogen #A32849, used 1:200 with Nkp46), donkey anti-mouse IgG AF647 (Invitrogen #A31571, used 1:200 with FasL).

Validation

All antibodies are from common clones and were validated by the manufacturer according to the data provided on their website (see below). Negative control samples without primary antibodies or FMOs were used for gating and thresholding.

In vivo:

Anti-mouse PD-1: <https://bioxccl.com/invivomab-anti-mouse-pd-1-cd279-be0146>

Rat IgG2b isotype: https://bioxccl.com/invivomab-rat-igg2b-isotype-control-anti-keyhole-limpet-hemocyanin-be0090?queryID=e02ce8eef06a5f8ef42bbe780cba241&objectID=30561&indexName=bioxccl_live_default_products

Anti-mouse CD8a: https://bioxccl.com/invivomab-anti-mouse-cd8-alpha-be0061?queryID=cdb1b775dce8cd1f11841fe9f5a4a60a&objectID=30539&indexName=bioxccl_live_default_products

Anti-Asialo GM1: <https://www.thermofisher.com/antibody/product/Asialo-GM1-Antibody-Polyclonal/16-6507-39>

Flow cytometry:

Mouse:

TruStain FcX: <https://www.biolegend.com/fr-fr/products/trustain-fcx-anti-mouse-cd16-32-antibody-5683?GroupID=BLG9237>
 CD45-AF700: <https://www.biolegend.com/ja-jp/products/alexa-fluor-700-anti-mouse-cd45-antibody-16471>
 CD45-PE/Cy7: <https://www.biolegend.com/en-ie/products/pe-cyanine7-anti-mouse-cd45-antibody-9794?GroupID=BLG6834>
 CD11c-PE/Cy7: <https://www.biolegend.com/en-gb/products/pe-cyanine7-anti-mouse-cd11c-antibody-3086>
 CD3e-APC: <https://www.biolegend.com/de-de/products/apc-anti-mouse-cd3epsilon-antibody-21>
 CD8a-APC/Fire750: <https://www.biolegend.com/nl-nl/products/apc-fire-750-anti-mouse-cd8a-antibody-13048>
 CD8a-APC: <https://www.biolegend.com/nl-nl/products/apc-anti-mouse-cd8a-antibody-150?GroupID=BLG6765>
 CD8a-BV510: <https://www.biolegend.com/nl-nl/products/brilliant-violet-510-anti-mouse-cd8a-antibody-7992>
 CD49b-Pacific Blue: <https://www.biolegend.com/en-ie/products/pacific-blue-anti-mouse-cd49b-pan-nk-cells-antibody-6571?GroupID=BLG4896>
 CD49b-APC: <https://www.biolegend.com/en-ie/products/apc-anti-mouse-cd49b-pan-nk-cells-antibody-231>
 CD95(Fas)-FITC: <https://punchout.biolegend.com/fr-ch/search-results/fic-anti-mouse-cd95-fas-antibody-13897>
 CD95(Fas)-APC: <https://punchout.biolegend.com/fr-ch/products/apc-anti-mouse-cd95-fas-antibody-13906>
 CD178(FasL)-APC: <https://www.biolegend.com/de-at/clone-search/apc-anti-mouse-cd178-fas-antibody-18262>
 CD178(FasL)-PE: <https://www.biolegend.com/de-at/products/pe-anti-mouse-cd178-fas-antibody-391>
 FasL-Biotin: <https://www.biolegend.com/de-at/products/biotin-anti-mouse-cd178-fas-antibody-389>
 CD69-PE/Cy7: <https://www.biolegend.com/en-ie/products/pe-cyanine7-anti-mouse-cd69-antibody-3168?GroupID=BLG10515>
 H2-Kd-Pacific Blue: <https://www.biolegend.com/fr-lu/products/pacific-blue-anti-mouse-h-2kd-antibody-6186>
 CD274(PD-L1)-APC: <https://www.biolegend.com/en-ie/products/apc-anti-mouse-cd274-b7-h1-pd-l1-antibody-6655?GroupID=BLG5396>
 Thy1.1-APC: <https://www.biolegend.com/en-gb/products/apc-anti-rat-cd90-mouse-cd901-thy11-antibody-5621?GroupID=BLG10566>
 CD279(PD-1)-FITC: <https://www.biolegend.com/en-ie/products/fic-anti-mouse-cd279-pd-1-antibody-7004?GroupID=BLG7930>
 CD366(Tim-3)-PE: <https://www.biolegend.com/ja-jp/products/pe-anti-mouse-cd366-tim-3-antibody-5908>
 Streptavidin-APC: <https://www.biolegend.com/de-de/products/apc-streptavidin-1470?GroupID=GROUP23>

Human:

CD95(Fas)-PE: <https://www.biolegend.com/en-gb/products/pe-anti-human-cd95-fas-antibody-643?GroupID=BLG4463>
 CD56-APC/Cy7: <https://www.biolegend.com/de-at/products/apc-cyanine7-anti-human-cd56-ncam-antibody-10212>

Immunofluorescence:

Rat anti-mouse CD45.1-AF647: <https://www.biolegend.com/en-gb/products/alexa-fluor-647-anti-mouse-cd45-1-antibody-3104?GroupID=BLG1933>
 Rat anti-mouse Thy1.1-AF594: <https://www.biolegend.com/ja-jp/products/alexa-fluor-594-anti-rat-cd90-mouse-cd901-thy11-antibody-16658>
 Rat anti-mouse CD3-AF594: <https://www.biolegend.com/en-us/products/alexa-fluor-594-anti-mouse-cd3-antibody-9735?pdf=true&displayInline=true&leftRightMargin=15&topBottomMargin=15&filename=Alexa%20Fluor%C2%AE%20594%20anti-mouse%20CD3%20Antibody.pdf&v=20231129073014>
 Rat anti-mouse CD3-AF647: <https://www.biolegend.com/de-de/products/alexa-fluor-647-anti-mouse-cd3-antibody-2693?GroupID=BLG242>
 Rat anti-mouse CD8a-AF647: <https://www.biolegend.com/en-us/products/alexa-fluor-647-anti-mouse-cd8a-antibody-2699?GroupID=BLG2559>
 Mouse anti-human FasL: https://www.scbt.com/p/fas-l-antibody-nok-1?srsltid=AfmBOopjEV9MVosAXBdfN10dlf3mpbilQcifZXxcml_hJhsW41fmjwQ
 Goat anti-mouse NKp46: https://www.rndsystems.com/products/mouse-nkp46-ncr1-antibody_af2225
 Rabbit anti-mouse FAS: <https://www.abcam.com/en-us/products/primary-antibodies/fas-antibody-epr21088-ab216991>
 Rabbit anti-mouse GR: <https://www.thermofisher.com/antibody/product/Glucocorticoid-Receptor-Antibody-Polyclonal/PA1-511A>
 Donkey anti-rabbit IgG AF647: <https://www.thermofisher.com/antibody/product/Donkey-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-31573>
 Donkey anti-goat IgG AF647: <https://www.thermofisher.com/antibody/product/Donkey-anti-Goat-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A32849TR>
 Donkey anti-mouse IgG AF647: <https://www.thermofisher.com/antibody/product/Donkey-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-31571>

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	4T1, E0771, MDA-MB-231, SKBR3, CT26, and B16F10 cancer cell lines were purchased from ATCC. The NK-92 human natural killer cell line was purchased from ATCC.
Authentication	Initial authentication was performed by ATCC. MDA-MB-231, SKBR3, NK-92 were authenticated via STR profiling. 4T1, E0771, CT26, B16F10 murine cancer cell lines were authenticated by assessing morphology and growth kinetics (doubling time and passage density). We further validated NK-92 cells by flow cytometry analysis of CD56 expression. We further validated E0771 and 4T1 cells by bulk RNA-sequencing to confirm stable expression of housekeeping genes and expected expression of relevant markers (high Mki67 proliferation gene expression, low expression of hormone receptors). We further validated MHC-I expression levels in 4T1, E0771, CT26, B16F10 by flow cytometry analysis of H2-Kd and H2-Kb.
Mycoplasma contamination	Cells were regularly tested for mycoplasma. All cells used in this study were confirmed to be negative for mycoplasma.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this study.

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals	Mouse strains used in this study: Just EGFP Death Inducing (Jedi) (Ptprca TcrbLn1Bdb TcrLn1Bdb H2d/J), BALB/c (JAX stock #000651), C57BL/6 (JAX stock #000664), NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (NSG, JAX stock #005557). All mice used in this study were female and used at 8-12 weeks of age. Mice were housed in no more than 5 mice per cage. Cages contained consistent bedding, enrichment, food, and water and cages were refreshed regularly. Mice were housed under the following conditions: controlled room temperature (64-79 F), 30-70% humidity, and 12:12 hour light-dark cycles (lights on from 6:00-18:00).
Wild animals	No wild animals were used in this study.
Reporting on sex	Only female mice were used in this study.
Field-collected samples	No field-collected samples were used in this study.
Ethics oversight	All animal procedures were performed in accordance with the Institutional Animal Care and Use Committee (IACUC) under the approved protocol #17-017 at Dana-Farber Cancer Institute (DFCI).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about [clinical studies](#)

All manuscripts should comply with the ICMJE [guidelines for publication of clinical research](#) and a completed [CONSORT checklist](#) must be included with all submissions.

Clinical trial registration	TBCRC-030 is registered under NCT01982448.
Study protocol	Study protocol for TBCRC-030 was previously reported (Mayer et al., Ann Oncol, 2020).
Data collection	Data collection for TBCRC-030 was previously reported (Mayer et al., Ann Oncol, 2020).
Outcomes	Outcome assessment for TBCRC-030 was previously reported (Mayer et al., Ann Oncol, 2020).

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A

ChIP-seq

Data deposition

- ☒ Confirm that both raw and final processed data have been deposited in a public database such as [GEO](#).
- ☒ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links <i>May remain private before publication.</i>	CUT&RUN raw and processed data is deposited on GEO prior to publication.
Files in database submission	Raw files: c1_R1_001.fastq.gz c1_R2_001.fastq.gz c1_R1_001_v1.fastq.gz c1_R2_001_v1.fastq.gz c2_R1_001.fastq.gz c2_R2_001.fastq.gz

c2_R1_001_v1.fastq.gz
 c2_R2_001_v1.fastq.gz
 d1_R1_001.fastq.gz
 d1_R2_001.fastq.gz
 d1_R1_001_v1.fastq.gz
 d1_R2_001_v1.fastq.gz
 d2_R1_001.fastq.gz
 d2_R2_001.fastq.gz
 d2_R1_001_v1.fastq.gz
 d2_R2_001_v1.fastq.gz

Processed files:
 C1_1bp.bw
 C2_1bp.bw
 D1_1bp.bw
 D2_1bp.bw

Genome browser session
 (e.g. [UCSC](#))

No longer applicable.

Methodology

Replicates	N=2 replicates per condition (Dex-treated or vehicle control treated 4T1 tumour cells)
Sequencing depth	Each replicate was sequenced to a depth of at least 50 million reads and between 40 and 45% of all reads aligned uniquely to GRCh38. The majority of non GRCh38 reads aligned to spike-in controls. After removal of PCR duplicates, approximately 10-15 million reads per sample were utilized for peak calling. 150bp paired end chemistry was used.
Antibodies	0.5ug of Glucocorticoid Receptor antibody (CST #12041)
Peak calling parameters	MACS3 (v3.0.3) was used to call peaks with the following settings: callpeak --g mm --keep-dup all -B -q 0.05 --nolambda.
Data quality	Quality of I.P.s was confirmed via ChIP-qPCR targeting known GR binding regions in FKBP5 after dexamethasone treatment (Data not shown). Further GR specific binding was confirmed via enrichment of GR signal at known dexamethasone target genes (shown in Extended data) and at the FKBP5 GR binding locus (Data not shown). Peaks for analysis were considered if they had an FDR <0.05, had overlapping regions in both replicates, and were not part of blacklisted regions. Specifically each replicate had the following number of peaks over FDR 0.05 and FC 5: Dexamethasone Rep1: 9,540; Dexamethasone Rep2: 6,971; Control Rep1: 2,487; Control Rep2: 2,395.
Software	DNA was sequenced using Nova-Seq 2x150bp chemistry by Genewiz. Reads were filtered for quality and adapters were removed using TrimGalore (v0.6.10). JASPAR Transcription Factor Binding Site Database (2024) was used for motif discovery.

Flow Cytometry

Plots

Confirm that:

- ☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- ☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- ☒ All plots are contour plots with outliers or pseudocolor plots.
- ☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Mice were euthanized and the heart was perfused with PBS. Lungs were extracted into digestion buffer (400U/mL Collagenase IV, 20µg/mL DNase I, 10µM Y-27632 compound, 10% FBS in HBSS). Lungs were mechanically dissociated with scissors and incubated at 37C for 30 minutes on a rotator. Samples were subjected to a second round of mechanical dissociation using a syringe and maintained on ice in FACS buffer (0.1% BSA, 2mM EDTA in PBS) for all subsequent steps. Cells were filtered through a 100µm cell strainer and red blood cell lysis was performed for 5 minutes at room temperature. Single cell suspensions were blocked with TruStain FcX (Biolegend #101319) at 1:100 for 15 minutes. Samples were stained with rat anti-mouse antibodies (Biolegend) at the indicated concentrations for 30 minutes. DAPI (1:10,000) was used to assess viability.
Instrument	Samples were analysed for flow cytometry using a CytoFLEX instrument (Beckman). Samples were FACS-sorted using an Aria III cell sorter (BD Biosciences).
Software	Data were analyzed using FlowJo (v10) software.
Cell population abundance	Tumor cells for bulk RNA-seq were sorted on high purity and confirmed by expression of known tumor markers. Purity of single cell RNA-seq sorting was confirmed by expression of known markers.

Gating strategy

All cells were gated on FSC-A vs SSC-A to eliminate debris, FSC-A vs FSC-H to eliminate doublets, and DAPI- to select live cells. Tumor cells were gated as CD45- and GFP+ or tdTomato+ (depending on experiment and fluorescent reporter). Lymphocytes were gated as CD45+ and CD8a+ (CD8+ T cells) or CD49b+ (NK cells). For cTAT-zsGreen niche labelling experiments, lymphocytes were additionally gated as zsGreen+. Gating strategies are shown in Supplementary Figure 1.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.