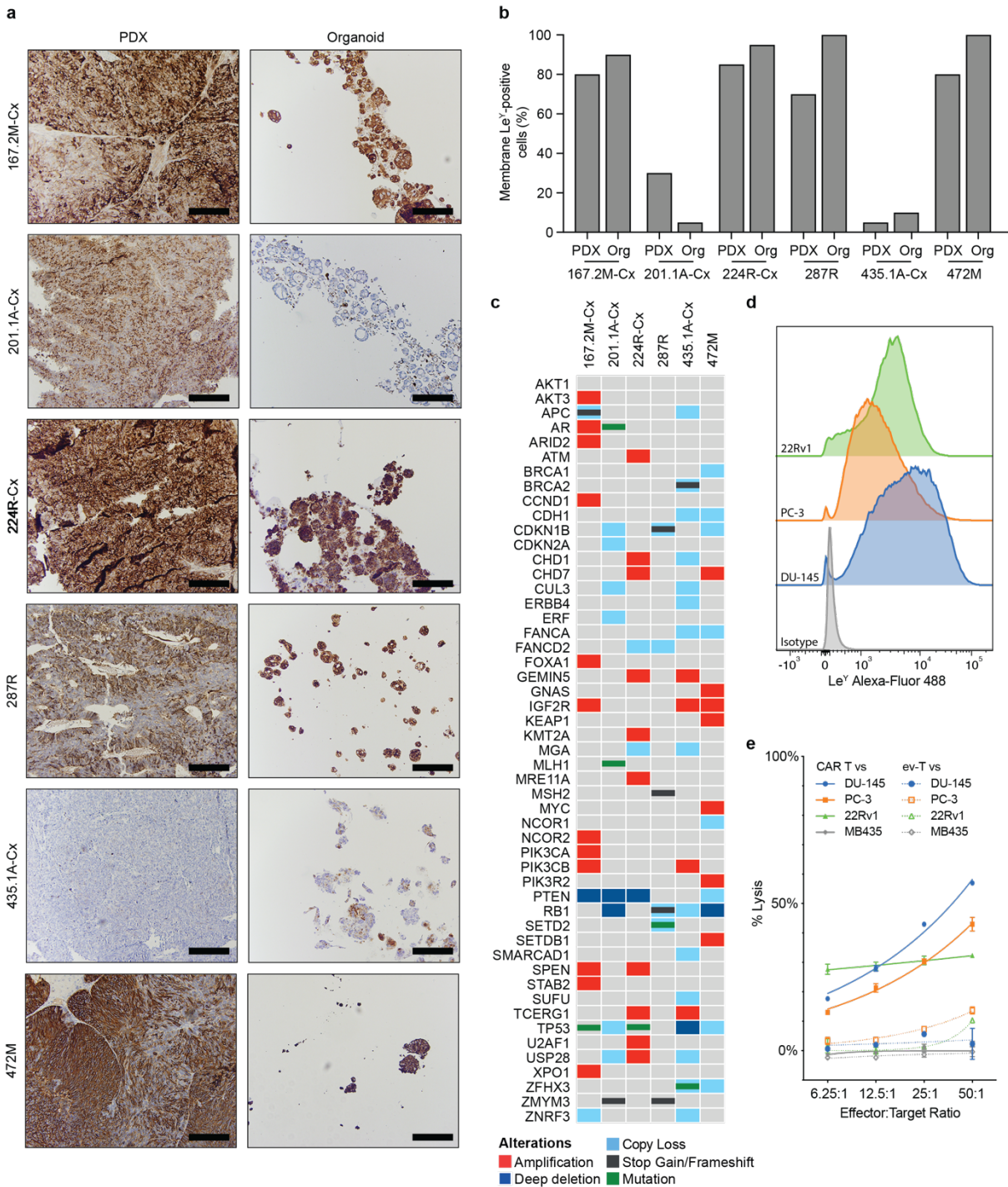
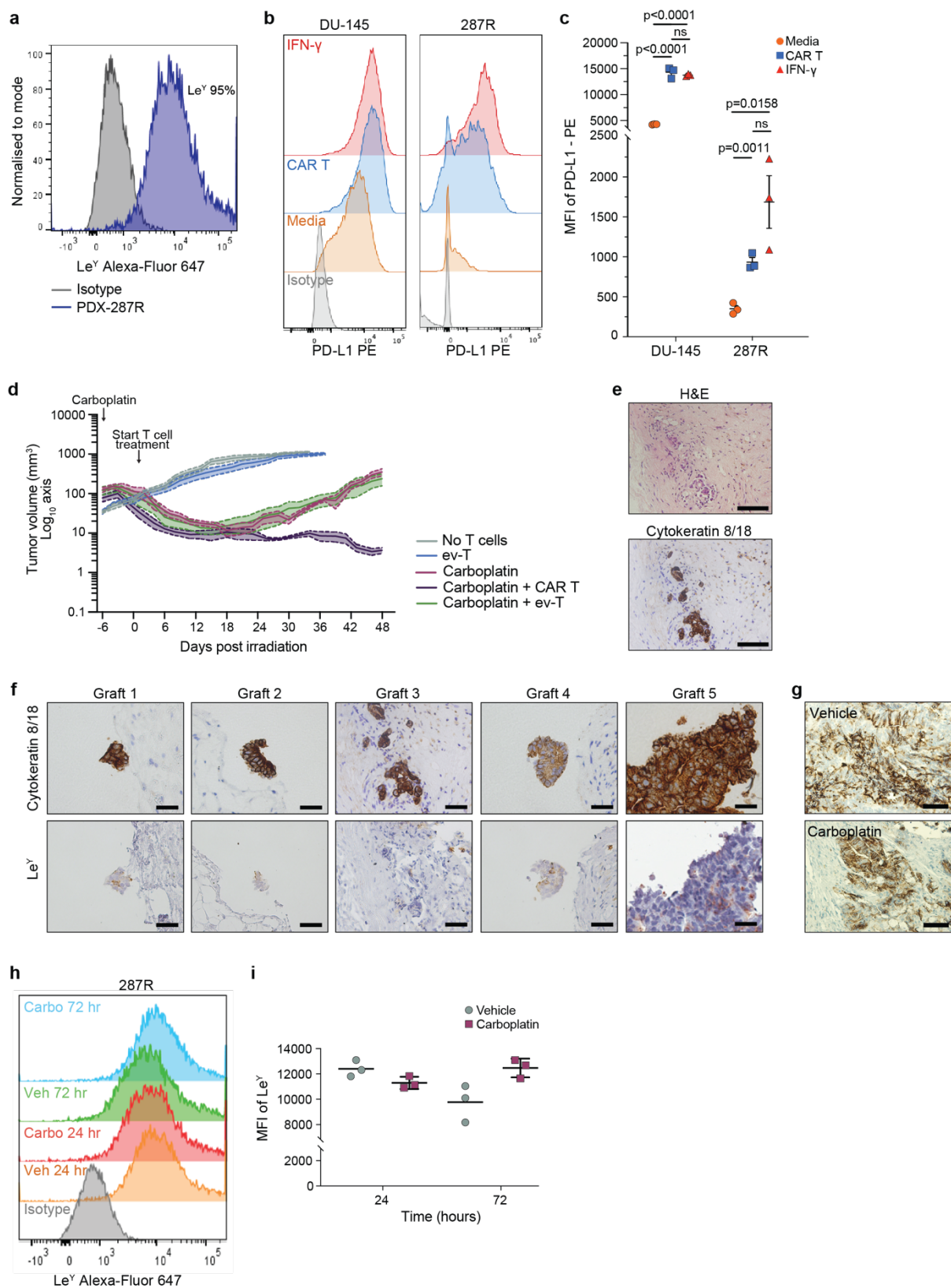


Supplementary Figures



Supplementary Figure 1. On-target killing of Le^Y-positive cells in organoids and cell lines in vitro. **a** Immunohistochemical staining of Le^Y in PDXs and organoids (scale bars = 200 μ m). PDX-167.2M-Cx, PDX-224R-Cx, PDX-287R and PDX-472M have high membrane staining compared to PDX-201.1A-Cx, which has less

membrane staining, but high cytoplasmic staining. **b** The percentage of cells positive for membrane Le^Y expression in PDXs and organoids. **a-b** Staining was repeated on three generations of PDX tissue, and results from the latest generation are shown. **c** Genomic alterations in MURAL PDXs based on targeted DNA sequencing. Somatic nucleotide variations with 0.75 or greater allelic frequency are reported (amplification with 3 or more copies – red; deep deletion – dark blue; copy loss – light blue; stop gains and frameshift mutations – black; missense mutation – green). **d** Representative FACS histograms (representative of n=3 samples/cell line) for the expression of Le^Y on prostate cancer cell lines DU-145 (blue), PC-3 (orange), 22Rv1 (green) and isotype control (grey). **e** ⁵¹Chromium release assays showing the percentage lysis of Le^Y⁺ DU-145 (blue), Le^Y⁺ PC-3 (orange), Le^Y⁺ 22Rv1 (green) and Le^Y⁻ MDA-MB435 (grey) after four hours in co-culture with Le^Y CAR T cells (solid lines) or ev-T cells (dot lines) at different effector to target ratios (n=3 wells/cell line). Data represent the mean ± SEM of triplicate cultures. Source data are provided as a Source Data file.

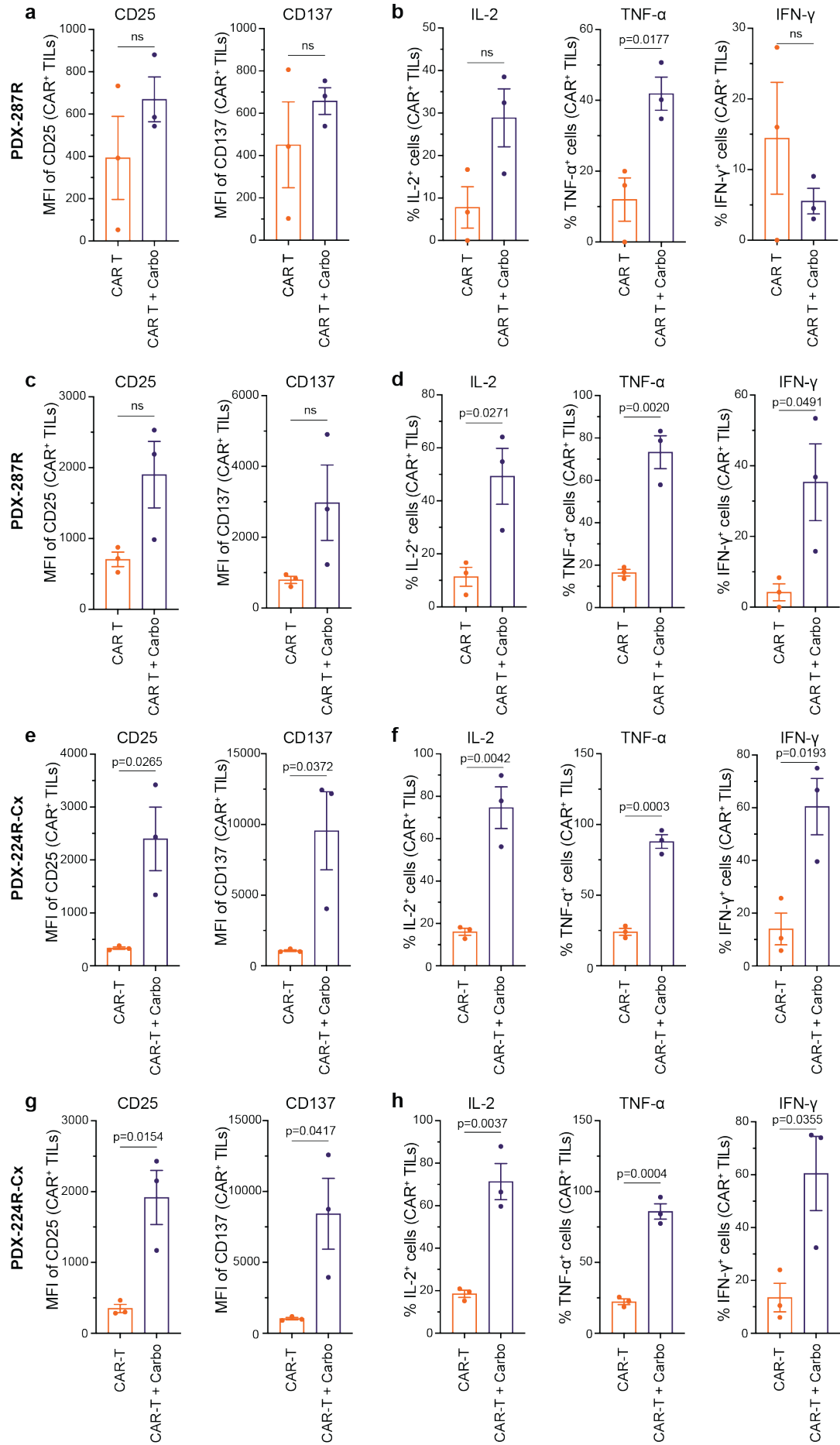


Supplementary Figure 2. A single dose of carboplatin improves the response to Le^Y CAR T cells without altering Le^Y expression.

a Representative FACS histogram for the expression of Le^Y on cells from PDX-287R. Expression of Le^Y was 94.5 ± 0.62 % from $n = 6$ independent samples. **b** Representative FACS histograms ($n=3$ samples) for the expression of PD-L1 on prostate cancer cell line DU-145 and prostate cancer organoid 287R, treated with CAR T conditioned media (CAR T, in blue), media containing 150 ng/ml IFN- γ (IFN- γ , in red) or fresh media only (Media, in orange). **c** Collective analysis of geometric mean fluorescence intensity (MFI) of PD-L1 on DU-145 cells and 287R organoids ($n=3$ wells). Data represents the mean $\pm$ SEM. Significance was determined by two-tailed unpaired t test. ns, not significant. **d** Tumor volume (mean $\pm$ SEM) of PDX-287R grafts following treatment with no T cell control ($n=12$ grafts), empty vector (ev) T cells ($n=6$ grafts), carboplatin (50 mg/kg, 1 dose; $n=6$), carboplatin and CAR T cells ($n=5$ grafts) and carboplatin and ev-T cells ($n=5$ grafts). Independent experiment from data presented in Fig. 3d including carboplatin + ev-T cell control group. **e** Representative images of residual tumour cells in PDX grafts ($n=5$ grafts) following 5-6 weeks of treatment with carboplatin plus Le^Y CAR T cells. Hematoxylin and eosin staining and human-specific epithelial cell marker cytokeratin 8/18 indicate large stromal infiltration with limited cancer foci. **f** Immunohistochemical staining of cytokeratin 8/18, showing small cancer foci, and Le^Y in matched cancer foci in grafts treated with carboplatin and Le^Y CAR T cells ($n=5$ grafts). **g** Immunohistochemical staining of Le^Y in PDX-287R grafts treated with vehicle control ($n=3$ grafts) or carboplatin (50 mg/kg, single dose; $n=3$ grafts) three weeks after treatment. **h-i** Representative FACS histograms for the expression of Le^Y (**h**) and geometric MFI of Le^Y (mean $\pm$ SEM), gated to isotype control (**i**), on 287R organoids following treatment with vehicle control or carboplatin (10 μ M) for 24 or 72

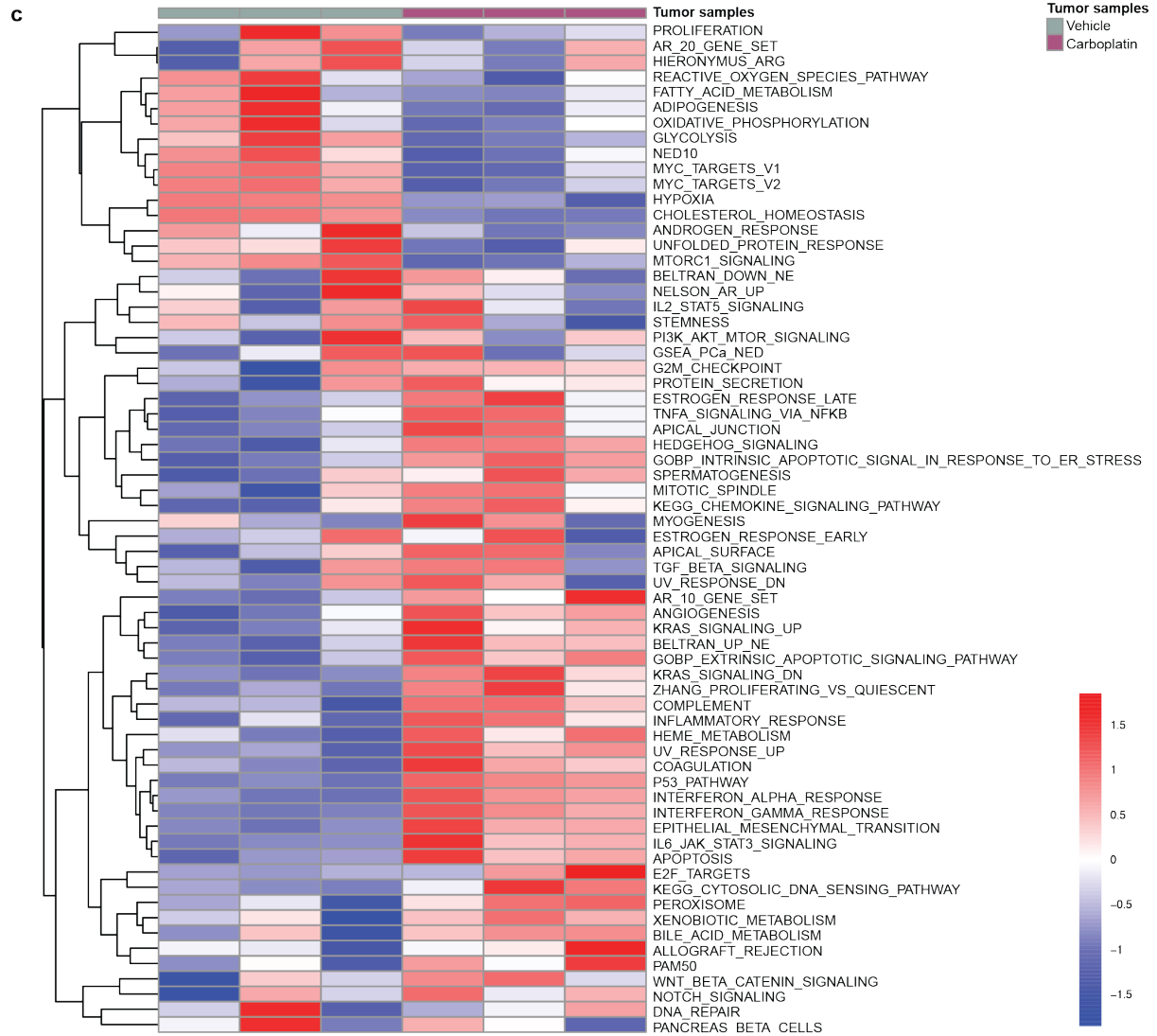
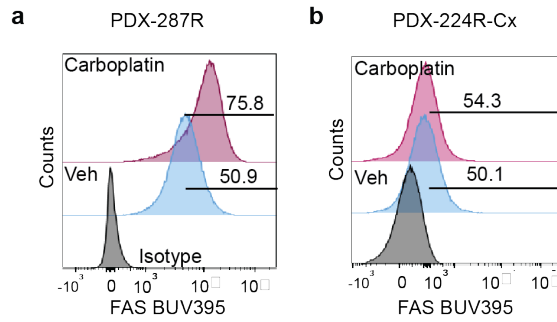
hours (n = 3 samples/treatment/time point). Scale bars = 50 μm (**e**) and 25 μm (**f-g**).

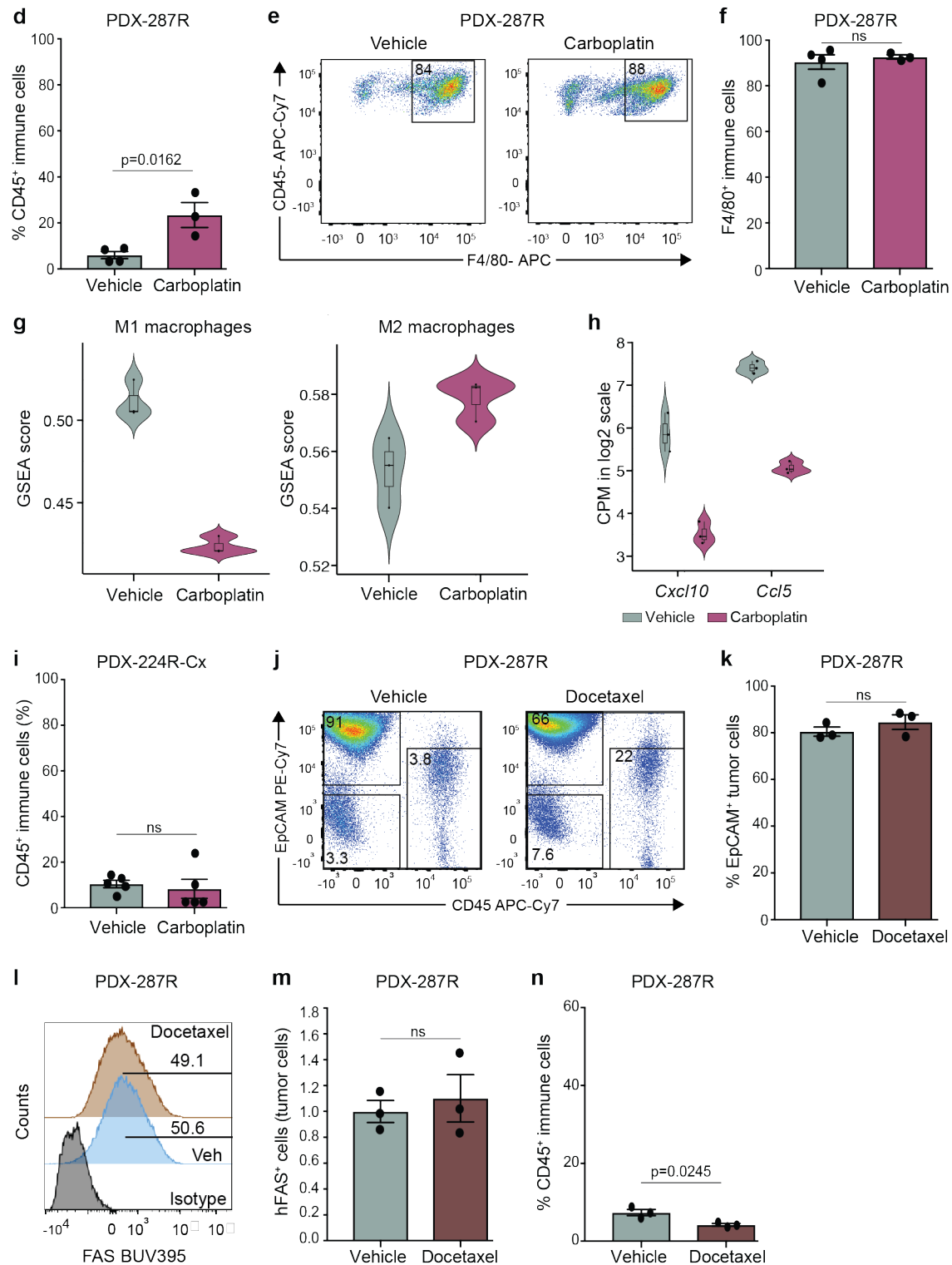
Source data are provided as a Source Data file.



Supplementary Figure 3. Carboplatin increases T cell activation in PDX tumors.

a-d Analysis of CAR T cells extracted from tumors (TILs) for PDX-287R without stimulation (**a-b**; cultured with fresh media only) and with TCR stimulation (**c-d**; activation by CD3 mAb clone OKT3) 48 hours post adoptive transfer of CAR T cells by flow cytometry (n = 3 tumors/treatment). **e-h** Analysis of CAR T cells extracted from tumors (TILs) for PDX-224R-Cx without stimulation (**e-f**; cultured with fresh media only) and with TCR stimulation (**g-h**; activation by CD3 mAb clone OKT3) 48 hours post adoptive transfer of CAR T cells by flow cytometry (n = 3 tumors/treatment). Compared to PDX-287R, PDX-224R-Cx had decreased sensitivity to carboplatin treatment alone, and a reduced response to carboplatin-CAR T cell combination treatment. **a,c,e,g** Geometric mean fluorescence intensity (MFI) of CD25 and CD137 without activation (**a,e**; baseline) or with activation by CD3 mAb (**c,g**) on TILs. **b,d,f,h** Percentage of IL-2-, TNF- α -, and IFN- γ -positive TILs without activation (**b,f**; baseline) or with activation by CD3 mAb (**d,h**) using intracellular staining. Data represents the mean $\pm$ SEM. Significance was determined by two-tailed unpaired t test. ns, not significant. Source data are provided as a Source Data file.

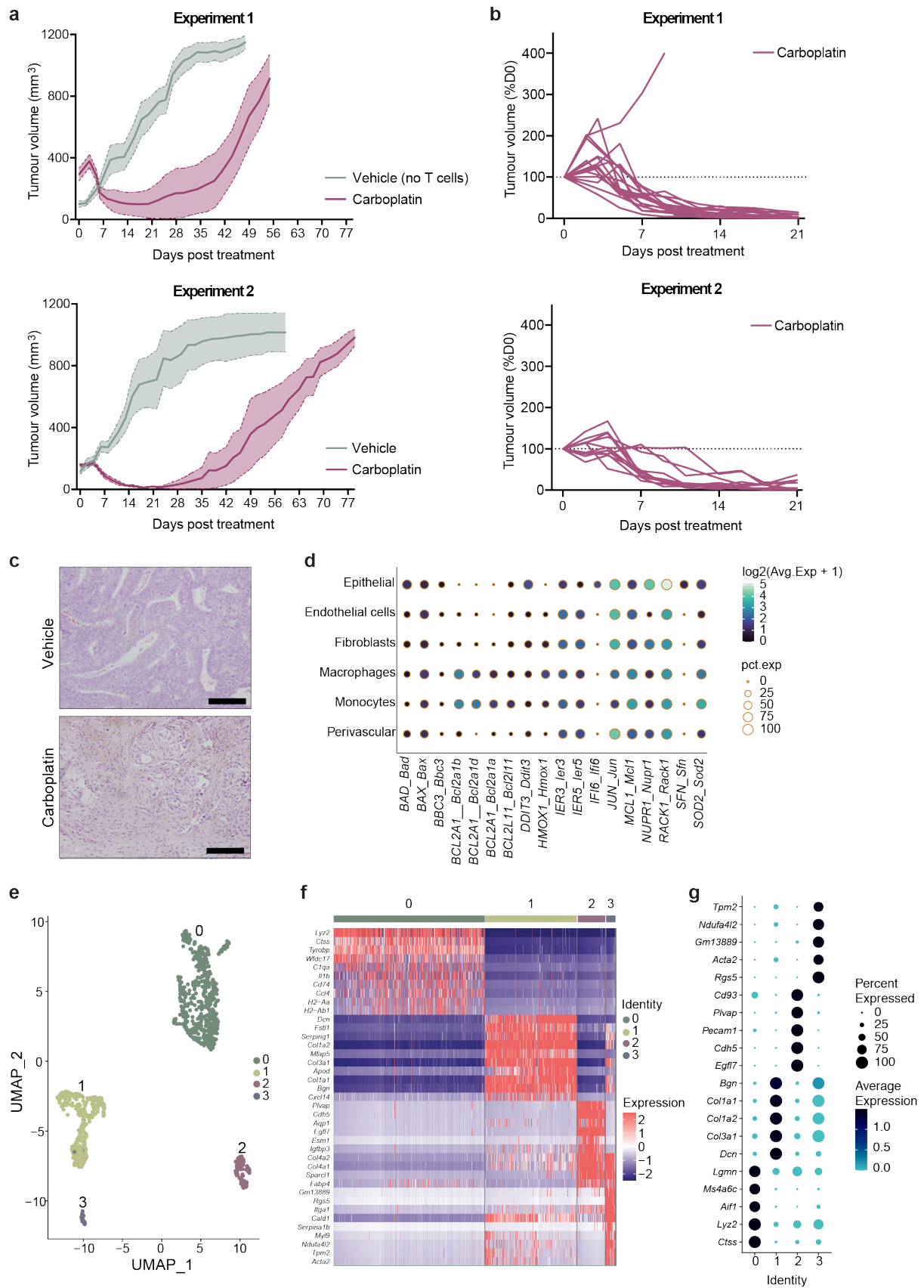




Supplementary Figure 4. Response of PDX-287R and PDX-224R-Cx to carboplatin or docetaxel one week post treatment. a-b Representative FACS histograms for FAS expression on EpCAM⁺ tumour cells in PDX-287R (a; n = 4 grafts for vehicle and 3 grafts for carboplatin) and PDX-224R-Cx (b; n = 5 grafts/treatment)

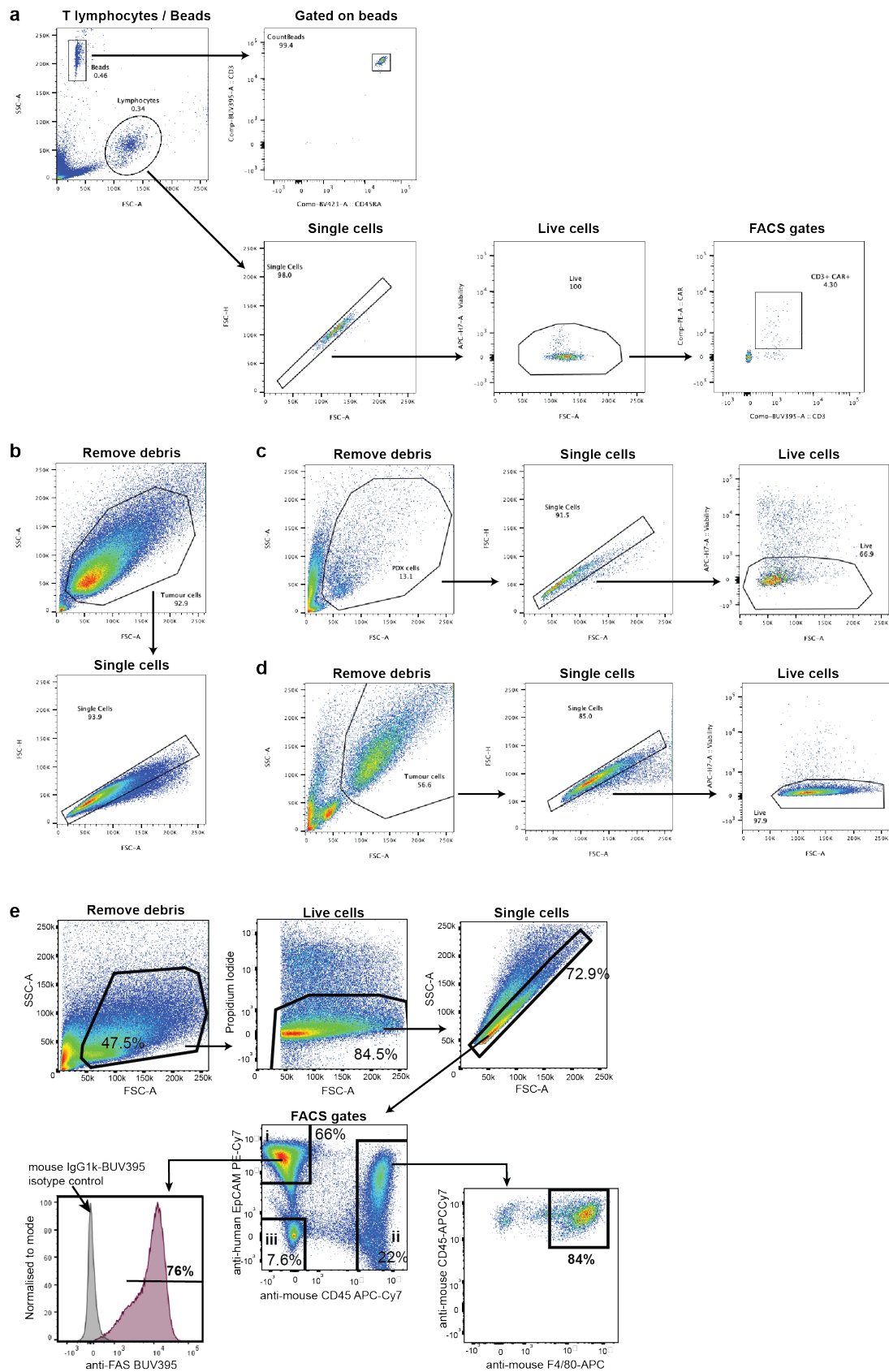
one week after treatment with vehicle or carboplatin (50mg/kg, single dose). Compared to PDX-287R, PDX-224R-Cx had decreased sensitivity to carboplatin treatment alone, and a reduced response to carboplatin-CAR T cell combination treatment. **c** Hieratically clustering and heatmap of single-sample gene set enrichment analyses (ssGSEA) based on RNA sequencing for the 50 MSigDB hallmark gene sets plus select KEGG and GOBP gene sets for PDX-287R tumors one week after treatment with vehicle control or carboplatin (n = 3 grafts/treatment). The enrichment of each gene set is shown. **d** The proportion of CD45⁺ immune cells in PDX-287R grafts after one week treatment with vehicle (n=4 grafts) or carboplatin (n=3 grafts) by flow cytometry. **e** Representative flow cytometry plot showing F4/80 expression in CD45⁺ immune cells in PDX-287R grafts one week after treatment with vehicle (n=4 grafts) or carboplatin (n=3 grafts). **f** The proportion of F4/80⁺ immune cells of the myeloid monocytic lineage out of CD45⁺ immune cells in PDX-287R grafts one week after treatment with vehicle (n=4 grafts) or carboplatin (n=3 grafts). **g-h** Violin plots showing GSEA score enriched proportions of M1 and M2 macrophages (**g**) and the expression of *Ccl5* and *Cxcl10* in immune cells (**h**) within carboplatin-treated and vehicle PDX-287R grafts (n=3 grafts/treatment). GSEA statistical and empirical evaluation were used in **g** to yield a normalized enrichment score (NES; significance level of 5%), and significance was determined by Welch's T-test. Box plots in **f-g** show the first to third quartile with median, whiskers show the minimum and maximum. **i** The proportion of CD45⁺ immune cells in PDX-224R-Cx grafts after one week treatment with vehicle or carboplatin by flow cytometry (n = 5 grafts/treatment). **j** Representative flow cytometry plot (n = 3 grafts/treatment) showing proportions of EpCAM⁺ epithelial tumor cells, CD45⁺ immune cells and EpCAM⁻CD45⁻ stromal cells in PDX-287R grafts one week after treatment with vehicle or docetaxel (10 mg/kg, single dose). **k** The

proportion of EpCAM⁺ epithelial tumor cells in PDX-287R grafts after one week treatment with vehicle or docetaxel (n = 3 grafts/treatment). **l** Representative FAC histograms for FAS expression on EpCAM⁺ tumour cells in PDX-287R grafts one week after treatment with vehicle or docetaxel (n=3 grafts/treatment). **m-n** hFAS⁺ tumor cells (**m**; normalized to average vehicle) and CD45⁺ immune cells (**n**) in PDX-287R grafts one week after treatment with vehicle or docetaxel (n=3 grafts/treatment). Data in **d,f,i,k,m,n** represents the mean $\pm$ SEM, and the significance was determined by two-tailed unpaired t test. ns, not significant. Source data are provided as a Source Data file.



Supplementary Figure 5. Single cell RNA sequencing of PDX-287R three weeks post carboplatin treatment demonstrates a shift to a pro-inflammatory

phenotype. a Tumour volume (mean $\pm$ SEM) of PDX-287R grafts following treatment with vehicle or carboplatin (single 50 mg/kg dose at day 0). Experiment 1 is data from Fig. 3d (n=15 grafts for vehicle, n=17 grafts for carboplatin; data from 2 independent experiments). Experiment 2 is tumor changes across time for treatment groups in Fig. 5a (n = 7 grafts for vehicle, n=12 grafts for carboplatin; data from one independent experiment). A subset of grafts from vehicle and carboplatin treatment groups in experiment 2 were harvested three weeks post treatment for ssRNA-seq. **b** Percent change in tumour volume from day 0 of carboplatin treatment (%D0) for individual grafts in experiment 1 (n = 17 grafts) and experiment 2 (n = 12 grafts) for three weeks following treatment. **c** Representative histological hematoxylin and eosin staining of PDX tissue one week post-carboplatin treatment (n=3 grafts), compared to vehicle control (n=2 grafts). Scale bar = 100 μ m. **d** Relative gene expression of pro- and anti-apoptotic agents by human epithelial cells and murine stroma following carboplatin-treatment when compared to control tissue. **e** UMAP clustering of cellular populations isolated from PDX tissue (0-4). **f** Heatmap illustrating the top 10 differentially expressed (DE) genes defining cellular clusters. **g** Primary genetic markers utilised to define cell type populations utilising a ROC test. Source data are provided as a Source Data file.



Supplementary Figure 6. Gating strategies for flow cytometry analysis and cell sorting. **a** Gating strategy for Fig. 4g-I and Supplementary Fig. 3a-h. Counting

beads were gated by size and positive fluorescence activity for BUV395 and BV421. CAR-T cells were gated by size, single cells, viability and positive fluorescence activity for CD3-BUV395 and CAR-PE. **b** Gating strategy for **Supplementary Fig. 1d**. Tumor cells were pre-tested for 100% viability by Trypan Blue staining (0.4%). Tumor cells were gated by size and single cells. **c** Gating strategy for **Supplementary Fig. 2a,h-l** and PDX-287R cells in **Supplementary Fig. 2b-c**. PDX tumors were digested and resuspended in a single cell suspension. PDX tumor cells were gated by size, single cells and viability. **d** Gating strategy for DU-145 tumor cells in **Supplementary Fig. 2b-c**. Tumor cells were gated by size, single cells and viability. **e** Gating strategy for **Fig. 5a-f** and **Supplementary Fig. 4a,b,d-f,i-n**. Cells were gated on SSC/FSC to exclude debris. Cells were then gated based on exclusion of propidium iodine to gate live cells. Single cells were then gated based on FSC-H and FSC-A properties. Final sort fates for i. Human prostate tumor cells; ii. Murine immune cells, and; iii. Murine stromal cells. EpCAM⁺ human tumor cells were sorted for FAS and CD45⁺ murine immune cells were sorted for F4/80.