

Figure S1. TGF- β 1 upregulates EGFR expression in breast cancer cells, but EGFR overexpression does not alter tumor proliferation. **a** Western Blot analysis of EGFR expression in MCF-7, MDA-MB-231 and 4T1 cell lines. **b** Western blot results showing upregulation of EGFR expression in MDA-MB-231 cells after TGF- β 1 treatment. **c** Violin plot showing upregulation of *EGFR* mRNA levels in 4T1 cells treated by TGF- β 1 for 48 hours or 14 days. Data was derived from unpublished bulk RNA sequencing. Y-axis indicates log counts per million (log-CPM). **d** Results from qPCR analysis of EGFR mRNA levels in 4T1 cells engineered to overexpress EGFR (4T1-EGFR) compared to control cells (4T1-vector). **e** Western Blot analysis of stable EGFR overexpression in 4T1 cells compared to control cells (vector). **f** Real-time proliferation assay using IncuCyte live-cell imaging. Cell growth of 4T1-vector and 4T1-EGFR cells were monitored and compared over time.

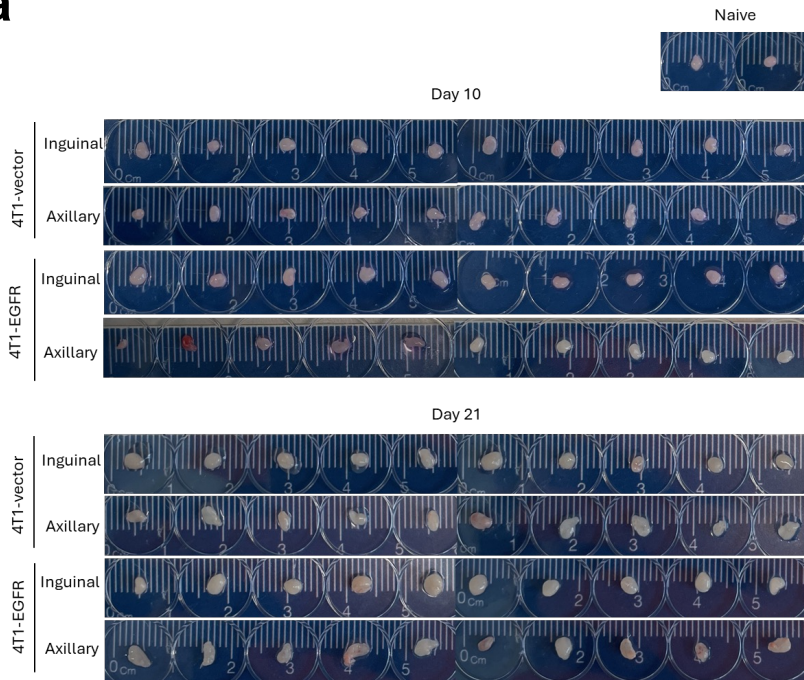
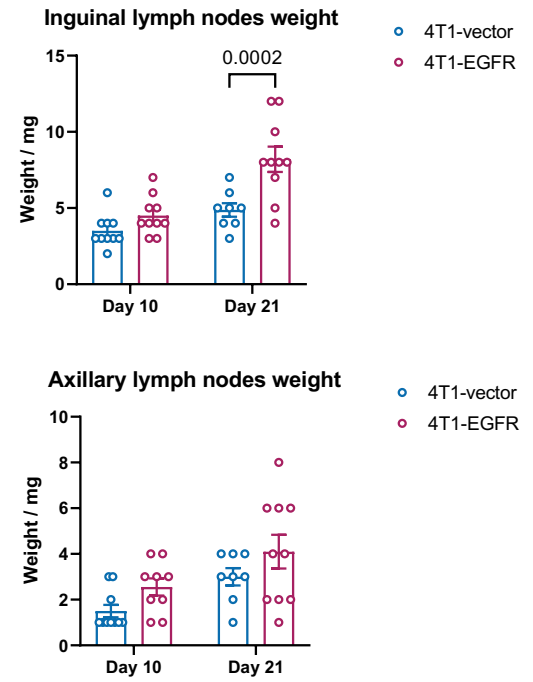
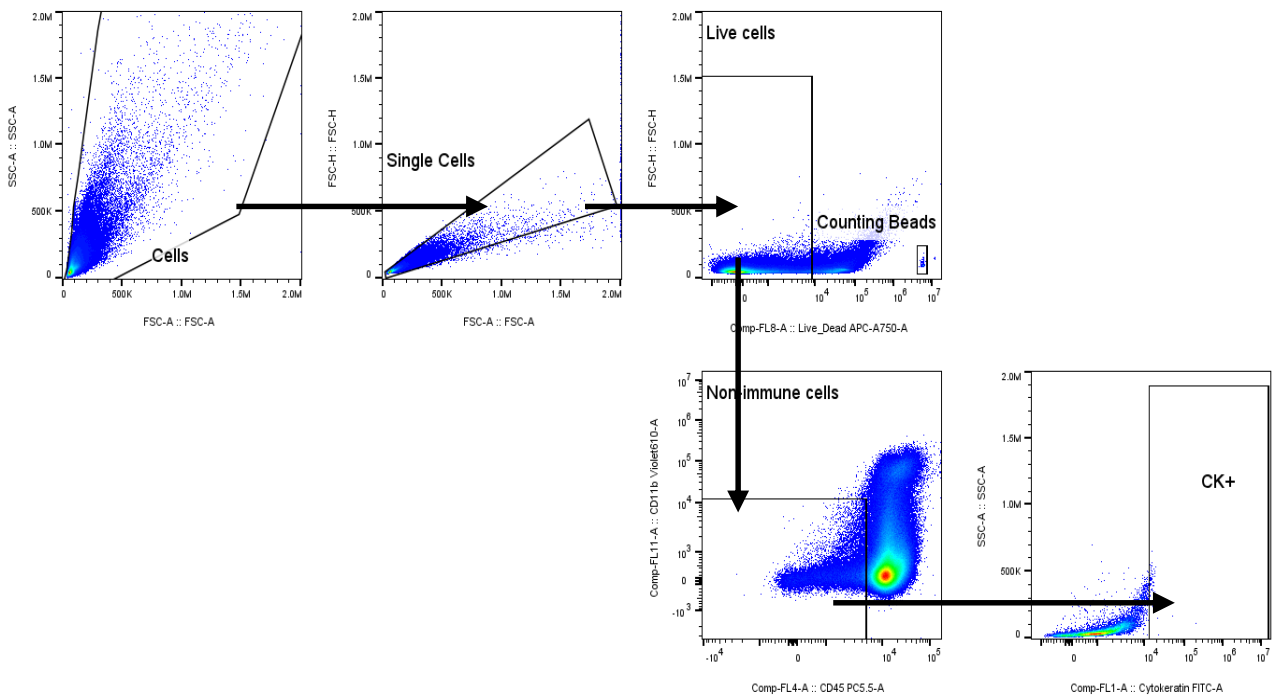
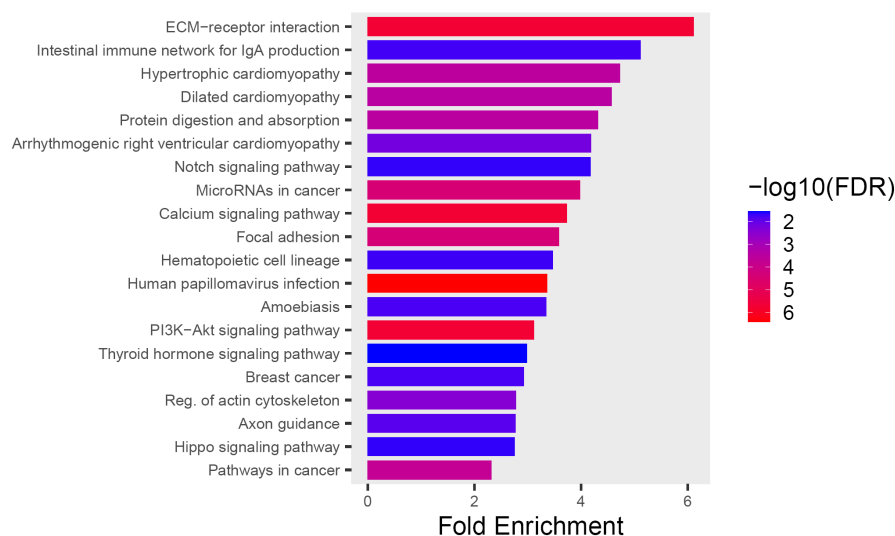
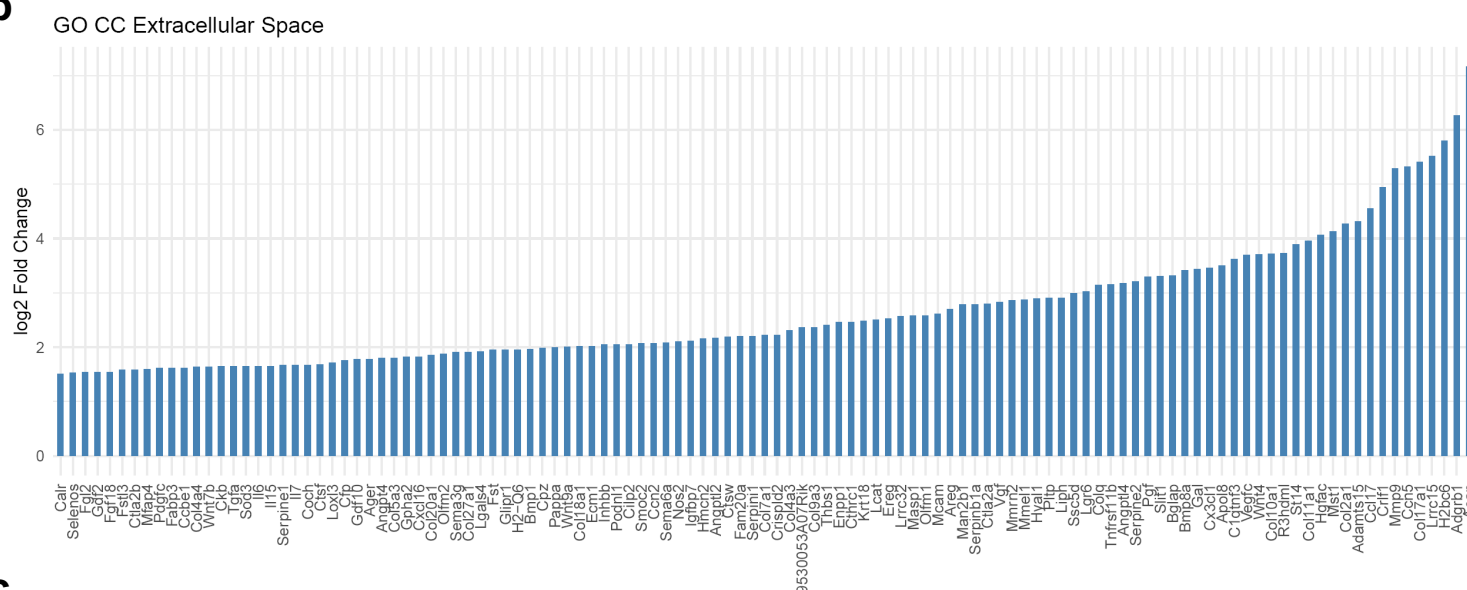
a**b****c**

Figure S2. Strategy for single cell quantification of lymph nodes. **a** Representative images of inguinal and axillary lymph nodes harvested at different time points after tumor implantation. The surrounding connective tissue was removed prior to imaging. The naïve lymph node (upper right) was collected from the contralateral inguinal region of a non-injected control mouse. **b** Weights of tumor-draining lymph nodes were measured using a microbalance. Statistical significance among groups was assessed using one-way ANOVA. Data are presented as mean \pm SEM. **c** Gating strategy for tumor cell quantification in draining lymph nodes. Immune cells were excluded by gating on CD45⁻CD11b⁻ cells to avoid signal contamination from cytokeratin uptake by antigen-presenting cells. Tumor cells were identified as pan-cytokeratin⁺ (CK⁺) within the CD45⁻CD11b⁻ population.

a



b



C

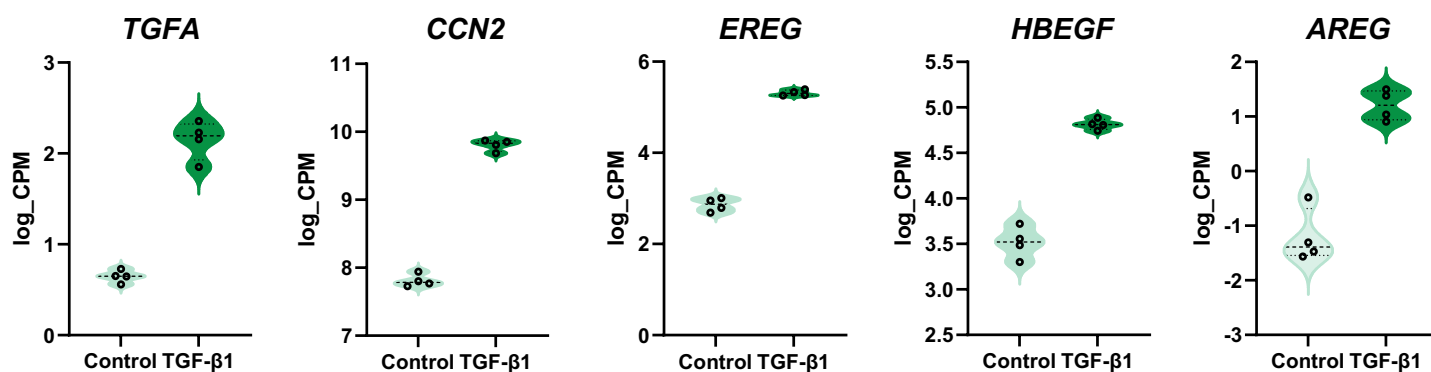


Figure S3. Identification of EGFR ligands in the secretome of TGF- β 1-activated lymphatic endothelial cells. **a** Gene enrichment analysis of KEGG pathways was performed to compare transcriptomic changes before and after TGF- β 1 treatment. The top 20 enriched pathways are shown, ranked by fold enrichment. **b** Genes encoding proteins in extracellular space (GO cellular component (GO:CC)) that were upregulated upon TGF- β 1 treatment in svLEC cells. **c** Violin plots show the upregulation of EGFR ligands at the mRNA level in svLEC cells before (control, n = 4) and after (TGF- β 1 n = 4) TGF- β 1 treatment. Y-axis indicates log counts per million (log-CPM). Differential expression analysis was performed, and all shown genes exhibited statistically significant increases with FDR < 0.05.

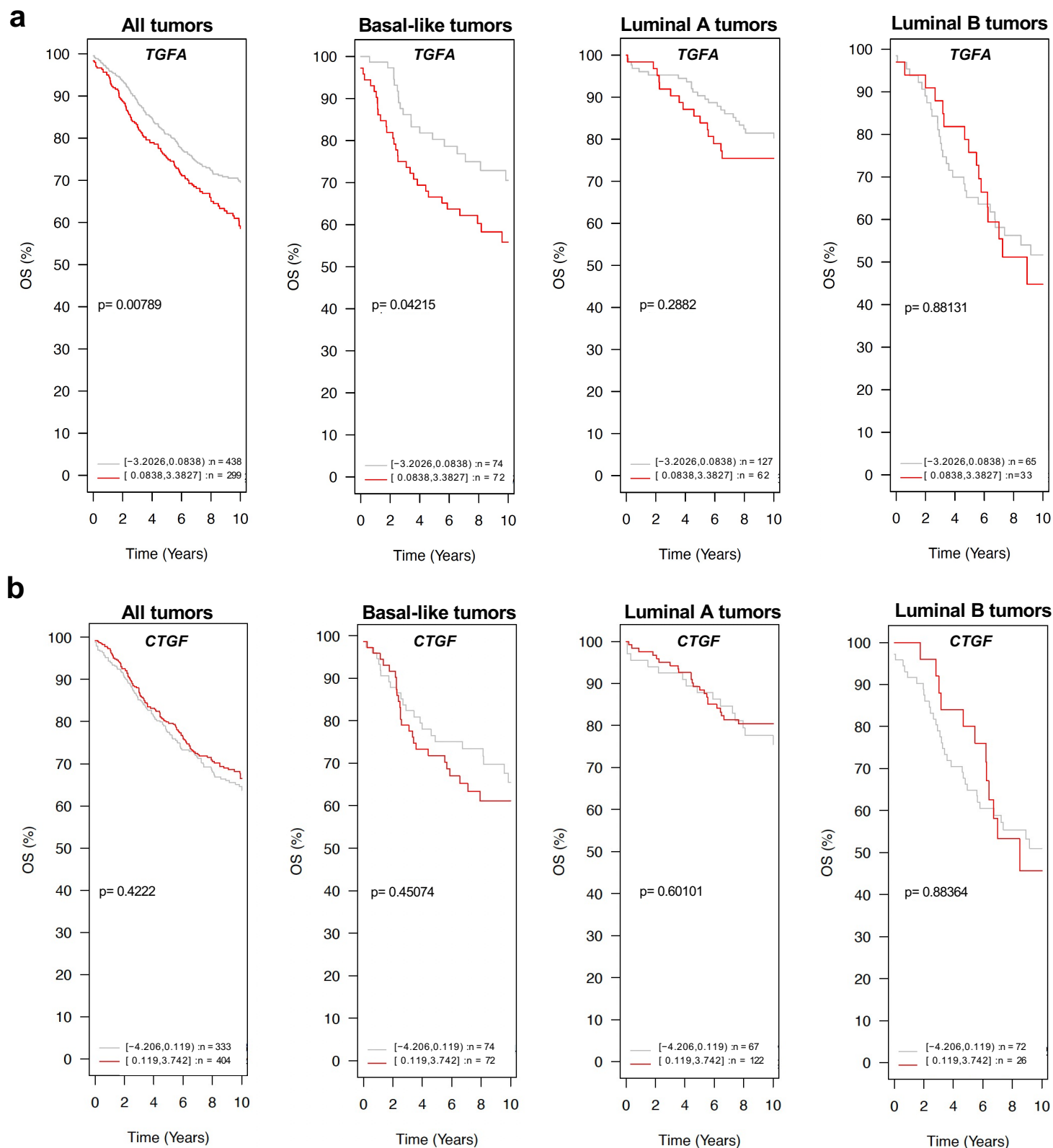


Figure S4. High *TGFA* expression correlates with poor survival in breast cancer patients. Kaplan-Meier survival analysis of breast cancer patients stratified by tumor expression levels of *TGFA* (a) and *CTGF* (b) using the GOBO (Gene expression-based Outcome for Breast cancer Online) database (Lund University, Sweden). Survival curves are shown for all breast cancer subtypes (All tumors), as well as for major intrinsic subtypes: basal-like, luminal A, and luminal B tumors. High *TGFA* expression is associated with significantly worse prognosis in all tumors, specifically in basal-like tumors, whereas *CTGF* expression shows no prognostic impact.

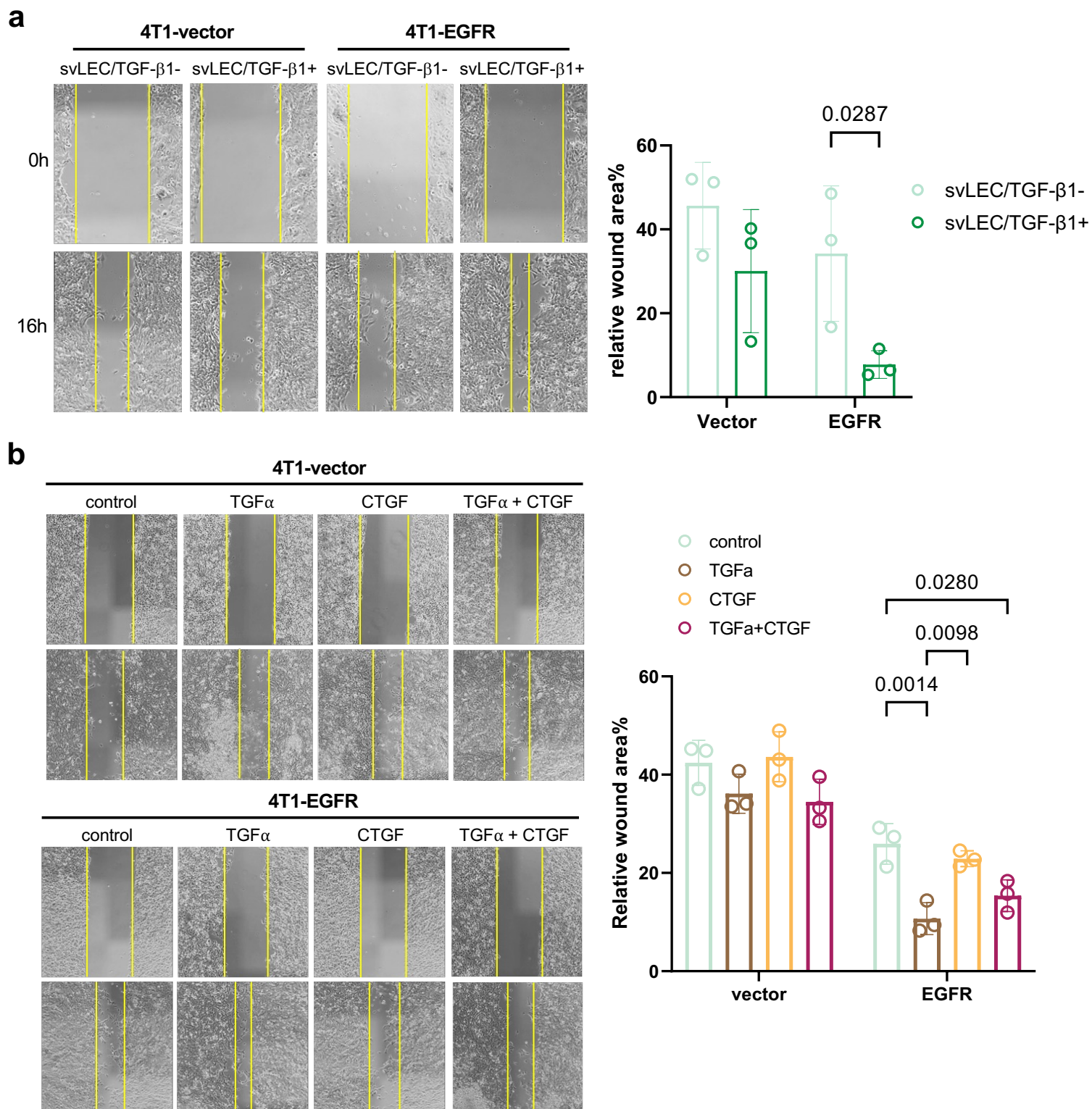


Figure S5. Migration of 4T1-EGFR cells toward lymphatic signals. a-b Results from wound healing assays testing the capacity of 4T1-vector and 4T1-EGFR cells to migrate toward conditioned medium (CondM) from svLECs, with or without TGF- β 1 stimulation (a), and the EGFR ligands TGF α and CTGF, alone or in combination (b). Wound closure was monitored by live-cell imaging every 15 minutes. The endpoint wound closure is shown as a bar graph. Statistical analysis was performed using two-way ANOVA.

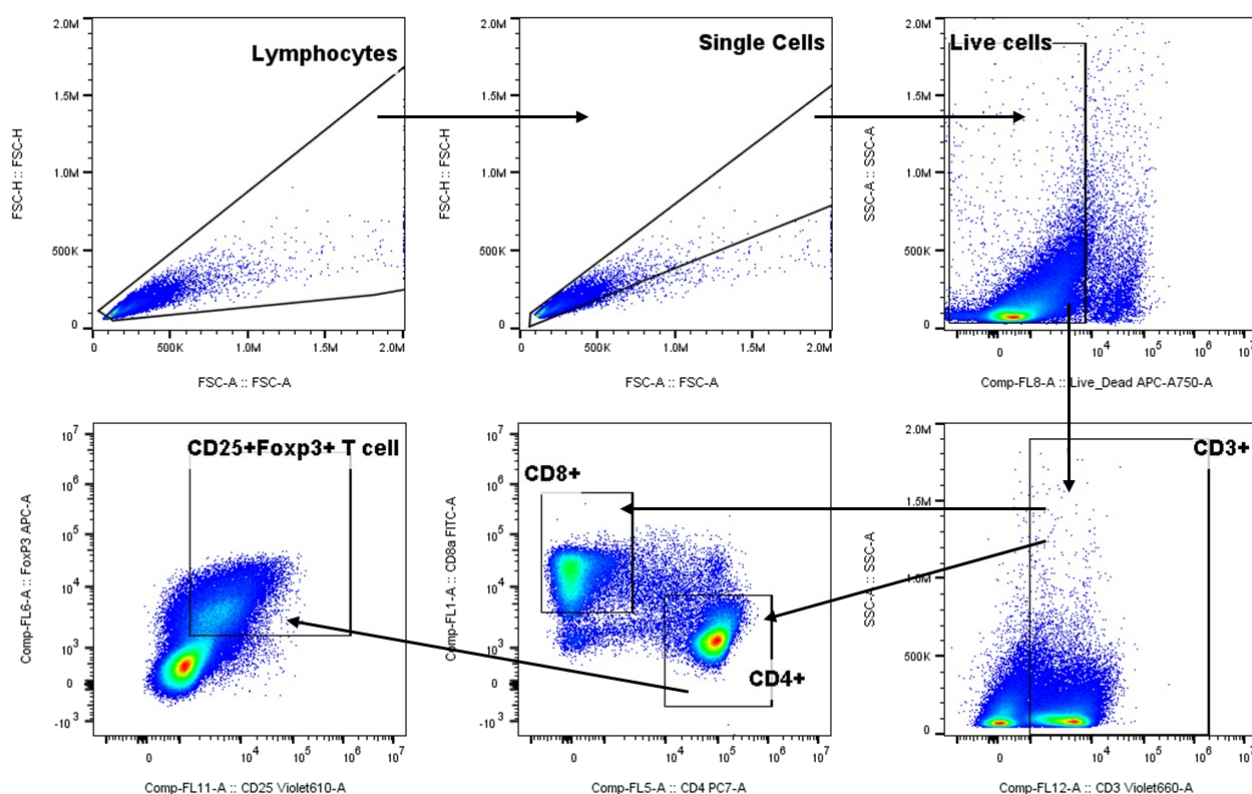


Figure S6. Gating strategy for T cell profiling in tumor-draining lymph nodes. Flow cytometry gating strategy used to identify CD3⁺ T cells, CD4⁺ and CD8⁺ subsets, and regulatory T cells (Tregs) in single-cell suspensions from lymph nodes. Live lymph node cells were first gated based on forward and side scatter, followed by singlet discrimination. CD3⁺ T cells were identified and subsequently gated for CD4⁺ and CD8a⁺ expression. Regulatory T cells (Tregs) were defined as CD4⁺CD25⁺FoxP3⁺ cells after intracellular staining using the FoxP3 Fix/Perm buffer set. Data acquisition was performed using a CytoFLEX flow cytometer and analyzed with FlowJo software.

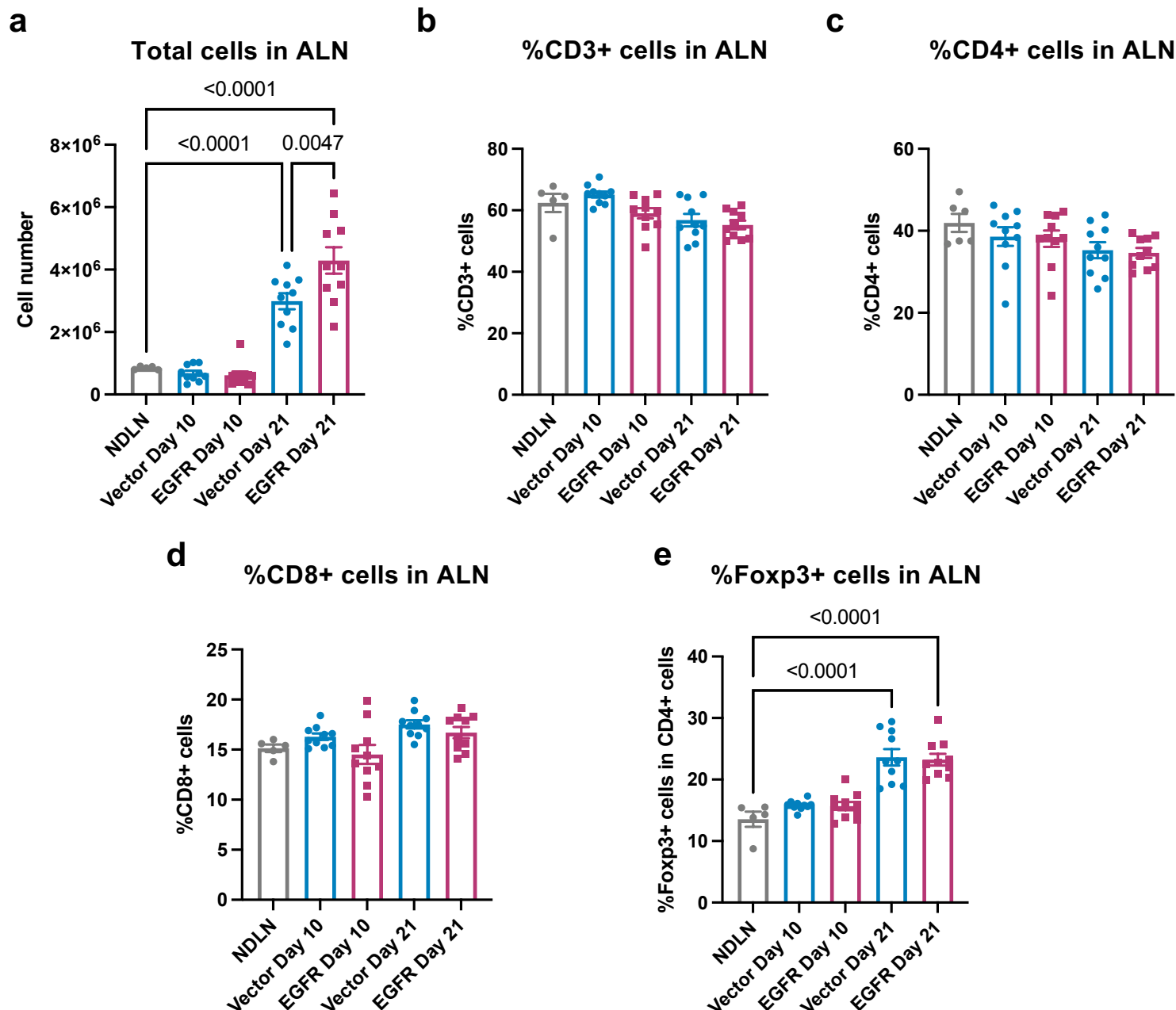


Figure S7. EGFR overexpression accelerates immune suppression in tumor-draining axillary lymph nodes. **a** Total number of cells in axillary lymph nodes (ALN) compared to non-draining lymph node (NDLN) at day 10, and day 21 following orthotopic implantation of 4T1-vector or 4T1-EGFR cells. **b-e** Percentage of CD3⁺ T cells (**b**), CD4⁺ T cells (**c**), CD8⁺ T cells (**d**) and Foxp3⁺ regulatory T cells (**e**) in ALN compared to NDLN at day 10, and day 21 following orthotopic implantation of 4T1-vector or 4T1-EGFR cells. Each dot represents one lymph node; group means \pm SEM shown. Statistical analysis was performed by two-way ANOVA with post hoc test.