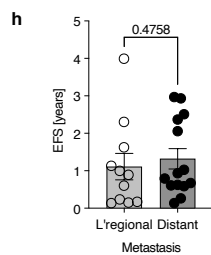
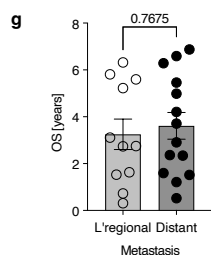
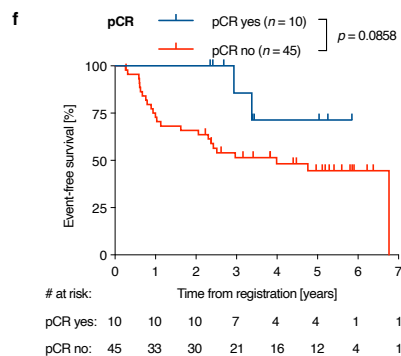
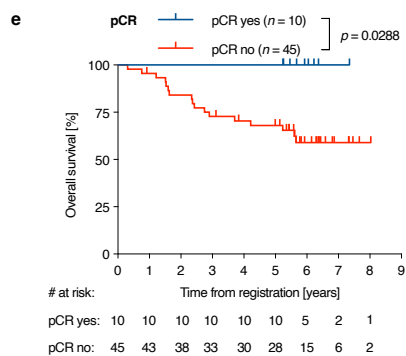
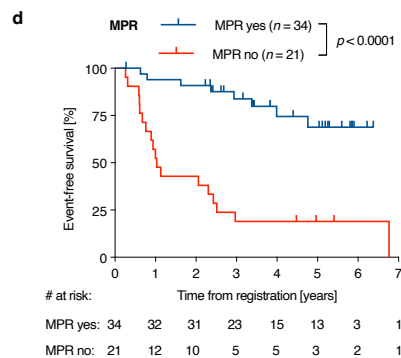
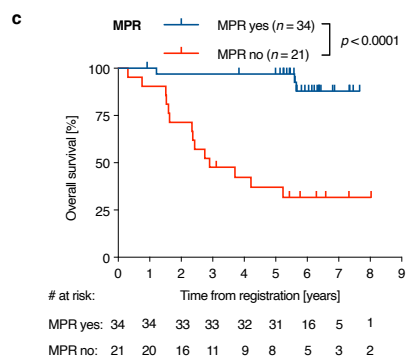
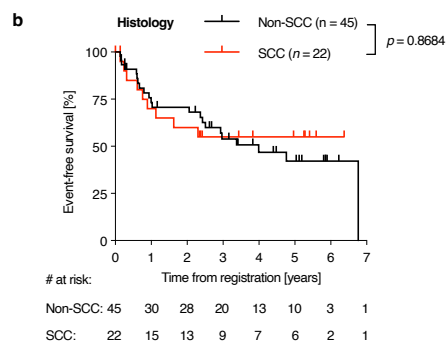
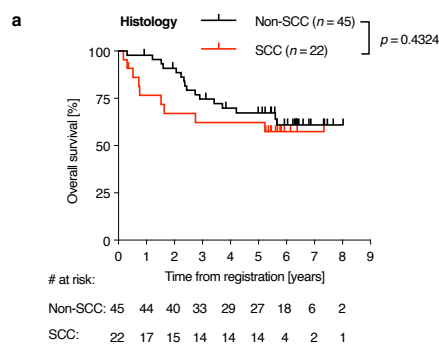


**Tumor immune dynamics and long-term clinical outcome of stage IIIA
NSCLC patients treated with neoadjuvant chemoimmunotherapy**

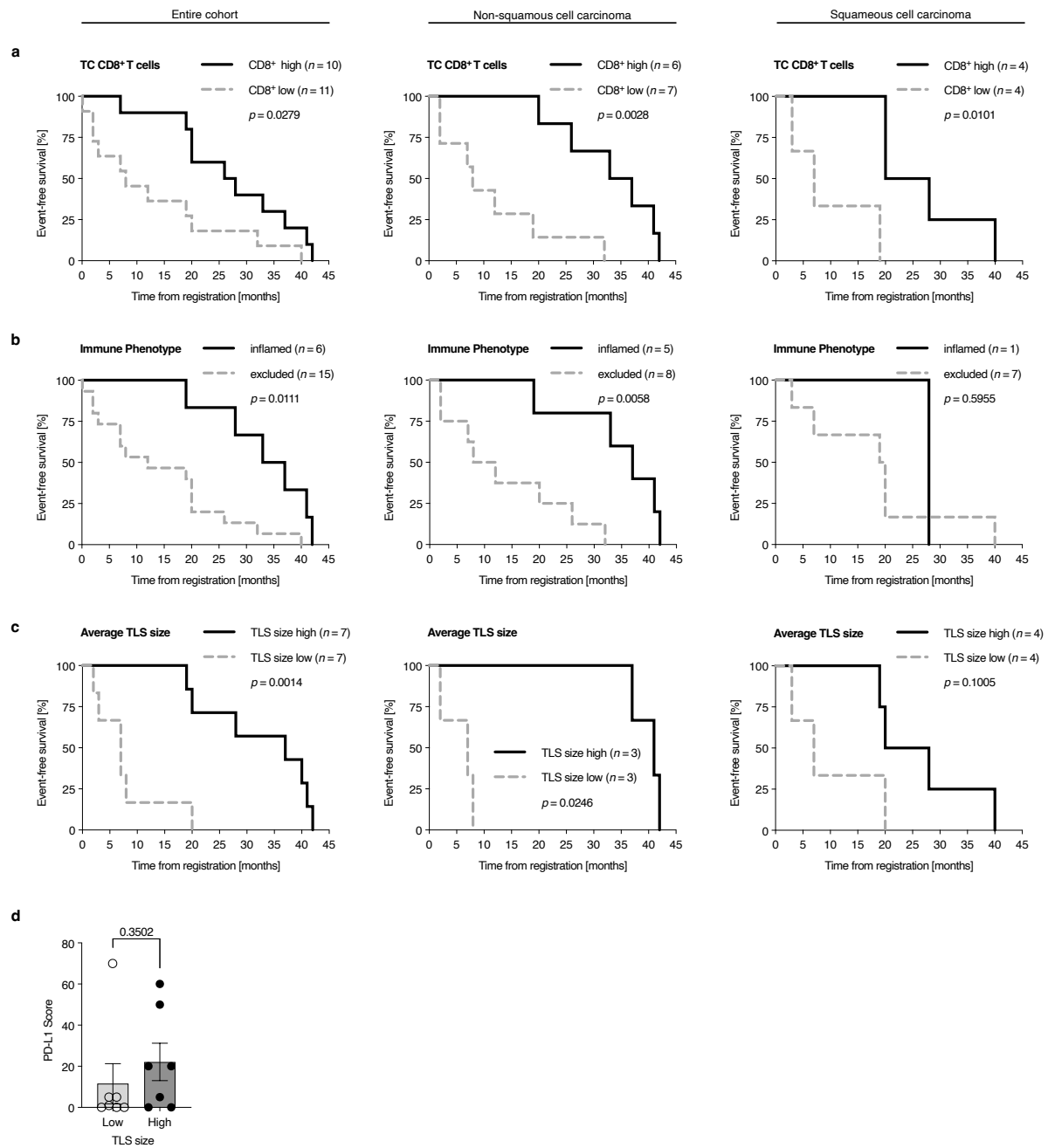
Schmid, Sobottka, Manzo et al.

Supplementary Information



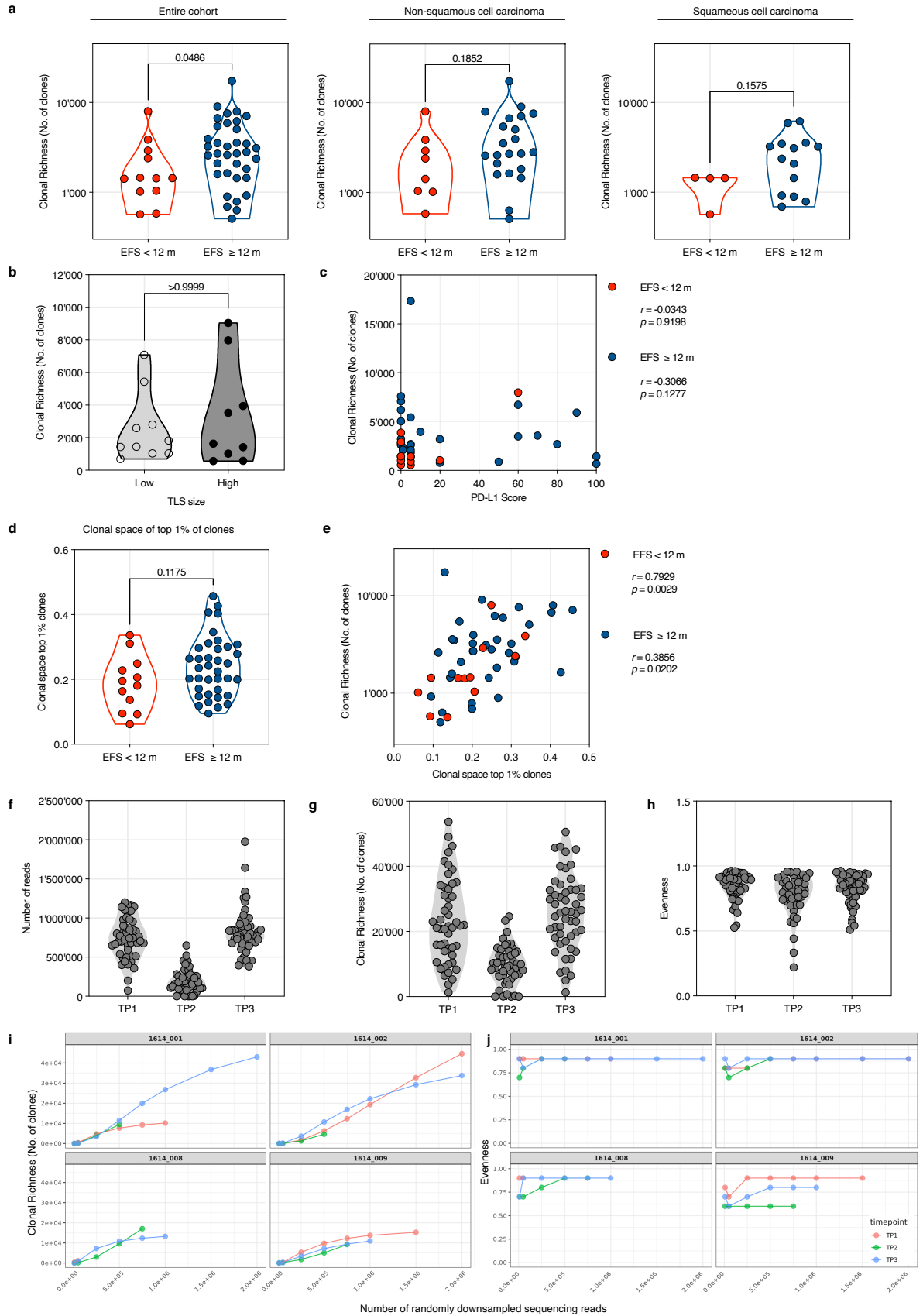
Supplementary Fig. 1. Clinical characteristics of the SAKK 16/14 cohort.

a-b Overall (OS) (**a**) and event-free (EFS) (**b**) survival of SAKK 16/14 trial participants stratified by tumor histology: squamous cell carcinoma (SCC, $n = 22$) and non-SCC (comprising adenocarcinoma, large cell carcinoma and not otherwise specified, $n = 45$). **c-f** Overall (**c**, **e**) and event-free (**d**, **f**) survival of SAKK 16/14 trials participants stratified by achievement of major pathological response (MPR: yes $n = 34$, no $n = 21$, **c**, **d**) or by complete pathological response (pCR: yes $n = 10$, no $n = 45$, **e**, **f**). **g-h** Overall (**g**) and event-free (**h**) survival between patients with locoregional (L'regional, $n = 11$) and distant metastasis ($n = 14$). Bars show means $\pm$ SEM. Statistical testing with log rank Mantel-Cox test (**a-f**) and two-sided Mann-Whitney test (**g-h**). Source data are provided as a Source Data file.



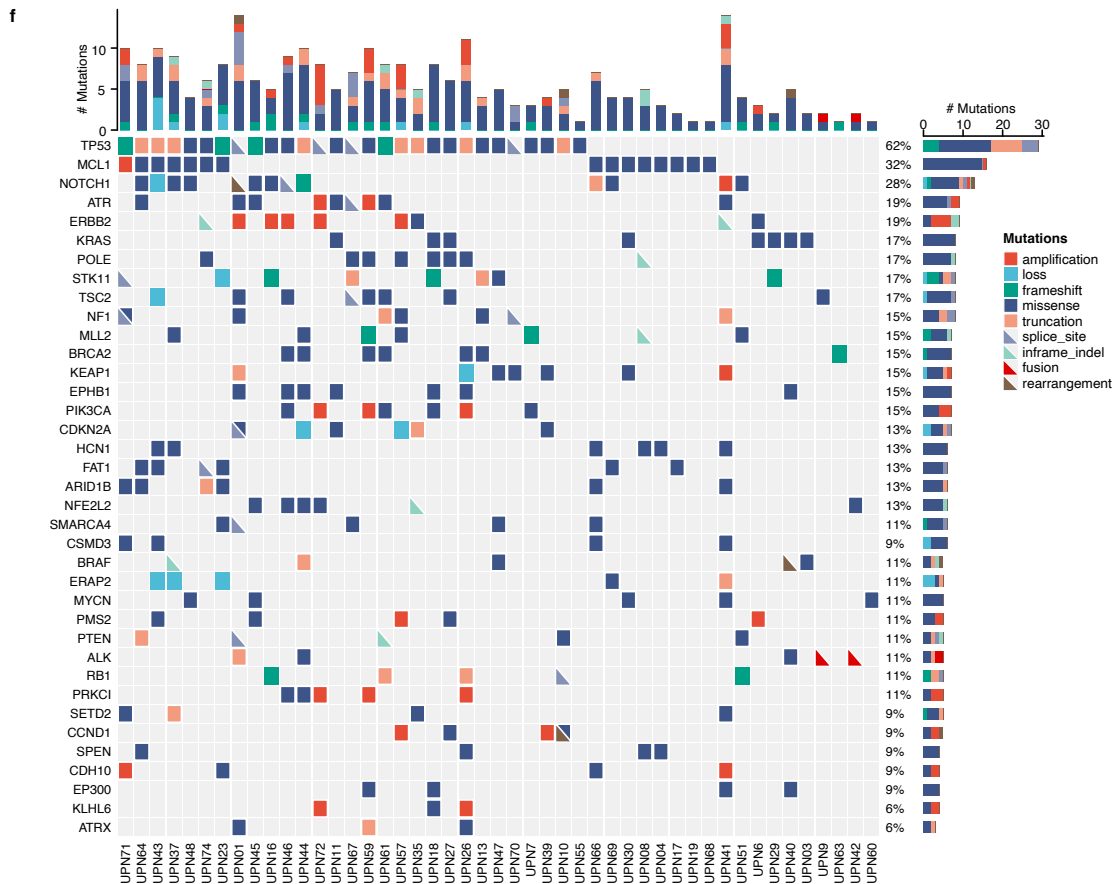
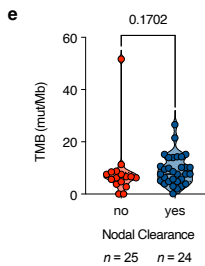
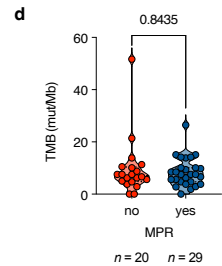
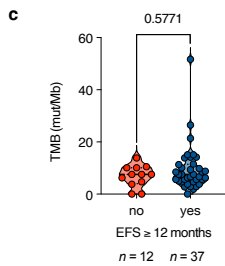
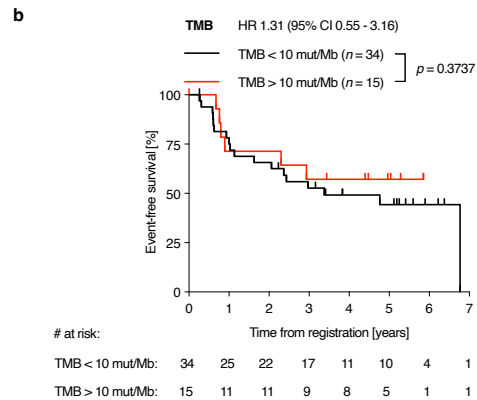
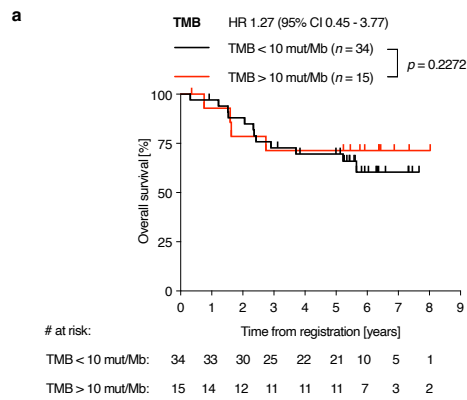
Supplementary Fig. 2. Digital pathology metrics in tumors with different histology and PD-L1 score.

a-c Event-free survival of patients with CD8 tumor compartment infiltration above or below the median (**a**), inflamed or excluded immunophenotype (**b**) or with above or below median TLS size (**c**) in the entire SAKK 16/14 cohort ($n = 21$), and in patients with non-squamous cell carcinoma (non-SCC, $n = 13$) and SCC tumors ($n = 8$). **d** PD-L1 score in initial biopsies (TP1) with TLS size above ($n = 7$) or below ($n = 7$) the median. Bars show means $\pm$ SEM. Statistical testing with log rank Mantel-Cox test (**a-c**) and two-sided Mann-Whitney test (**d**). Source data are provided as a Source Data file.



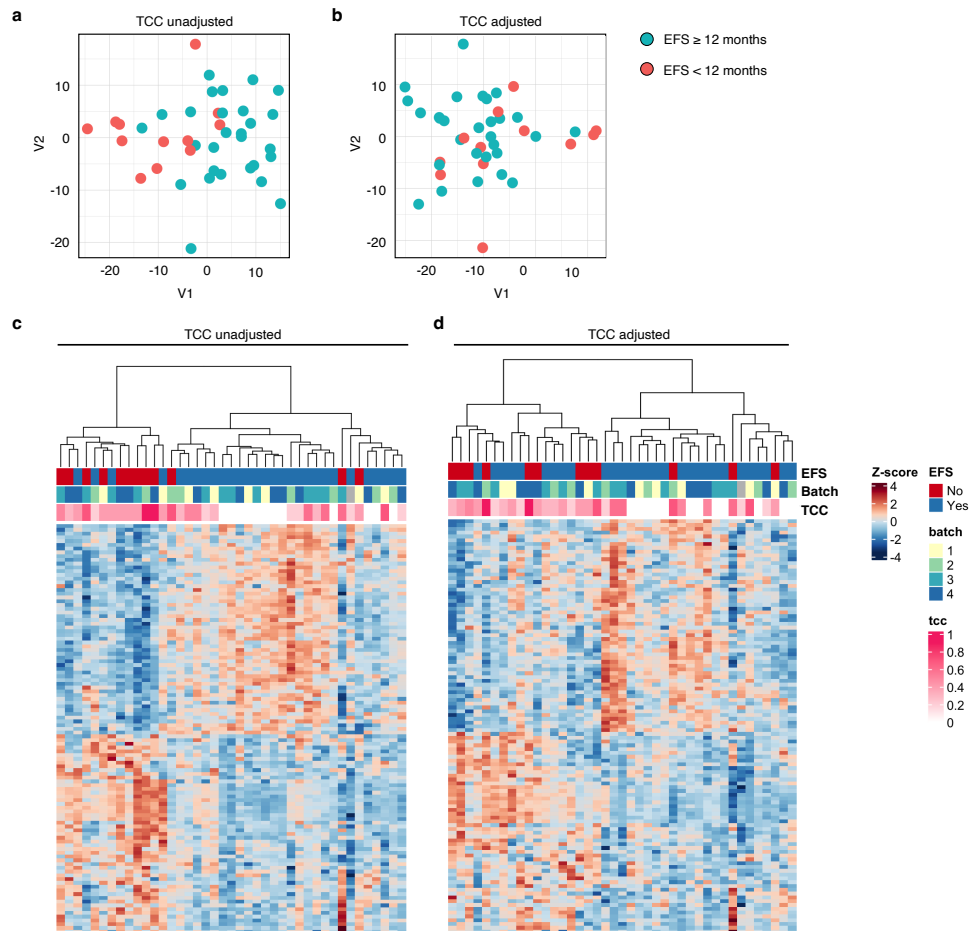
Supplementary Fig. 3. TCR clonality metrics.

a Number of TCR clones observed in tumor resection samples comparing patients achieving and not achieving a 12-months EFS in the entire SAKK 16/14 cohort ($n = 36/12$), in patients with non-squamous cell carcinoma (non-SCC, $n = 22/8$) and in patients with SCC ($n = 14/4$). **b** Number of TCR clones observed in tumor resection samples with TLS size below ($n = 10$) and above ($n = 9$) the median. **c** PD-L1 score plotted against number of observed TCR clones in tumor resection samples. Color indicates samples from patients achieving (blue, $n = 26$) and not achieving (red, $n = 12$) 12-months event-free survival. **d** Clonal space occupied by the top 1% TCR clones of the total repertoire in tumor samples post-surgery (TP3), as analyzed in the NADIM trial¹. Comparison by EFS ≥ 12 months (blue, $n = 36$) and < 12 months (red, $n = 12$). **e** Number of clones versus clonal space occupied by top 1 % clones in patients with EFS ≥ 12 months (blue, $n = 36$) and in patients with EFS < 12 months (red, $n = 12$). **f-h** Number of sequencing reads (**f**), number of TCR clones (**g**) and TCR evenness (**h**) from PBMC samples from TP1 ($n = 49$), TP2 ($n = 48$) and TP3 ($n = 52$). **i, j** Number of TCR clones (**i**) and TCR evenness (**j**) depending on the number of randomly downsampled sequencing reads in four illustrative patients. Statistical testing was performed with two-sided Mann-Whitney test (**a, b, d**), correlation was assessed with Spearman coefficient r and p value was calculated with two-sided t test (**c, e**). Source data are provided as a Source Data file.



Supplementary Fig. 4. Tumor mutational burden and OncoPrint.

a, b Kaplan-Meier event-free survival (EFS) (**a**) and overall survival (OS) (**b**) curves of patients in the SAKK 16/14 trial according to tumor mutational burden (TMB, TMB < 10 mutations/Mb, $n = 34$ vs TMB > 10 mutations/Mb, $n = 15$). *HR*, hazard ratio. **c** TMB of patients achieving and not achieving a 12-months EFS ($n = 37/12$), **d** major pathological response (MPR, $n = 29/20$), and **e** nodal clearance ($n = 24/25$). **f** OncoPrint showing the distribution of genomic alterations (rows) in individual participants (columns) of the SAKK 16/14 trial. The mutational frequency of each gene is labelled on the right. The relative TMB of each patient is shown on top ($n = 47$). Source data are provided as a Source Data file.

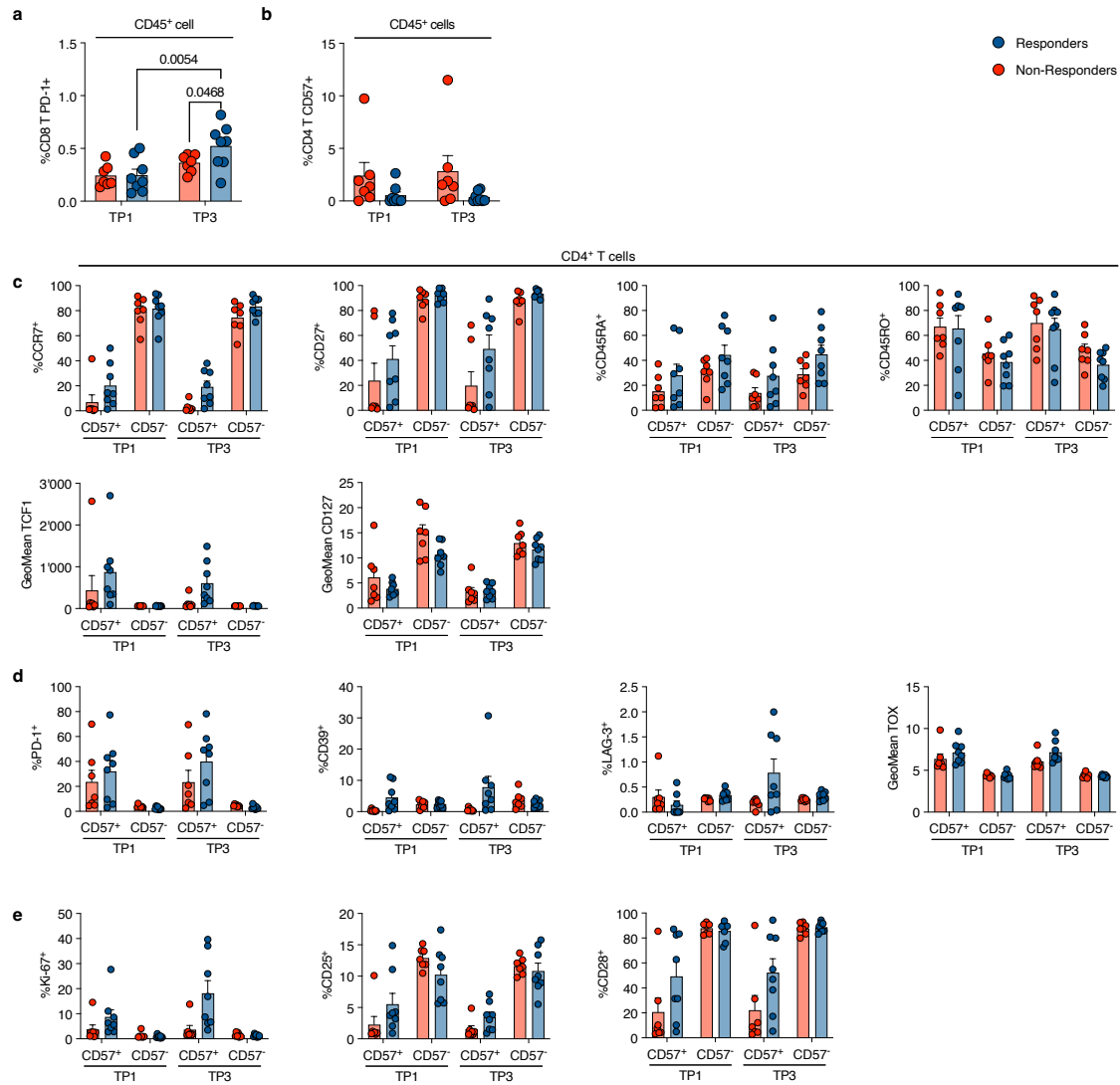


Supplementary Fig. 5. Differential gene expression of post-treatment tumor tissue.

a Multidimensional scaling plot (MDS) plot of overall transcriptomic profiles from post-treatment tumor samples (TP3) based on the expression levels of the 395 genes of the Oncomine Immune Response Research Assay. Color-coded by response, turquoise: EFS ≥ 12 months ($n = 31$), red: EFS < 12 months ($n = 12$). **b** Heatmap for $n = 109$ differentially expressed genes (rows) between $n = 43$ patients (columns) with EFS ≥ 12 months and EFS < 12 months displayed in z-score. Top annotations depict EFS, in which batch specific samples were processed and the tumor cell content (TCC). **c** MDS plot and **d** heatmap after normalization by tumor cell content.

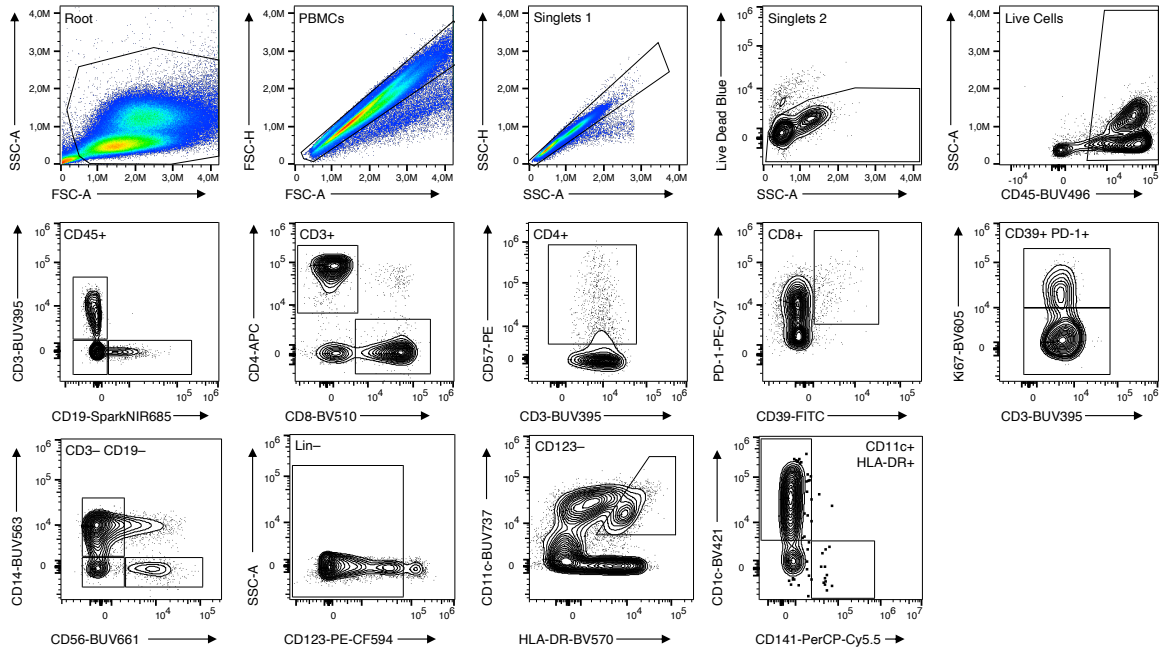
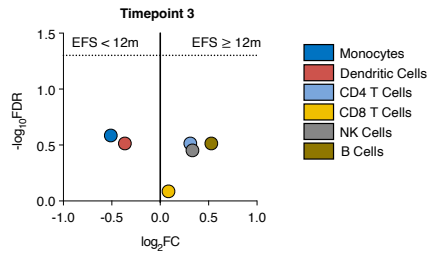
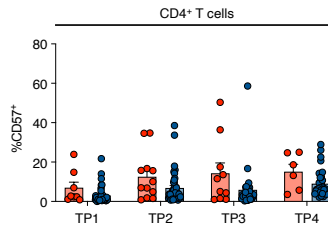
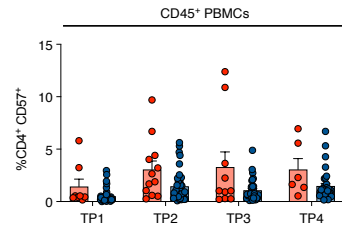
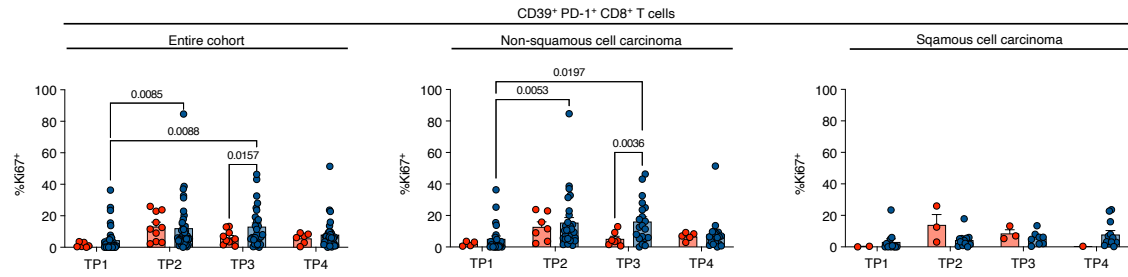
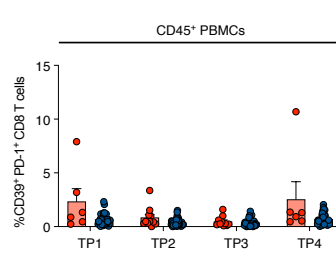
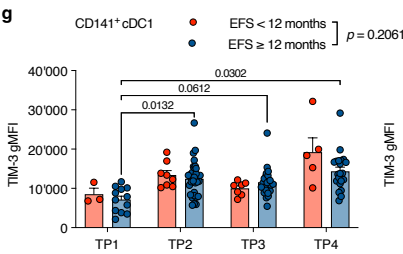
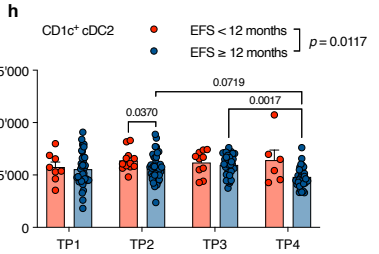
Supplementary Fig. 6. CyTOF of PBMCs pre- and post-neoadjuvant chemoimmunotherapy.

a Pre-gating strategy to exclude EQ normalization beads, identify single (uniform Gaussian parameters) and living (103Rh negative) cells. **b** Marker (columns) expression across clusters (rows) identified by FlowSOM as dual mean counts. **c** tSNE contour plot of mass cytometry data ($n = 5'596'279$ cells), color-coded by cell cluster. **d** UMAP of PBMCs at TP1 of responders (EFS ≥ 12 months, MPR, $n = 8$) and non-responders (EFS < 12 months, no MPR, $n = 7$). Each dot represents a single cell, color-coded by cell cluster, $n = 50'000$ randomly selected cells. **e** Proportion of FlowSOM clusters (rows) within all cells of individual pre-selected patients (columns) at baseline (TP1). Clusters were arranged by their FDRs (right bar graph). Source data are provided as a Source Data file.



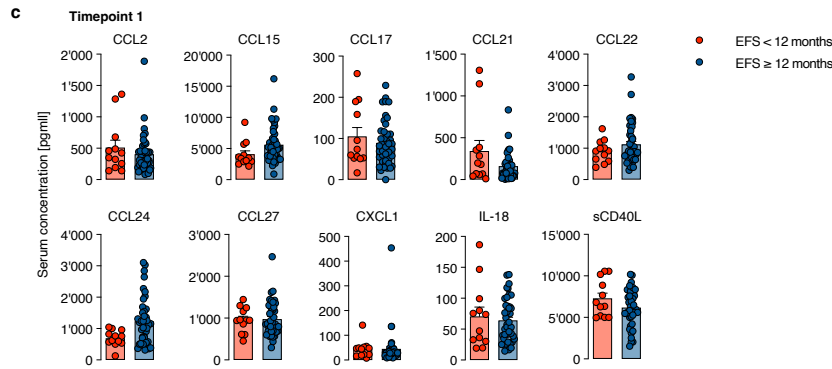
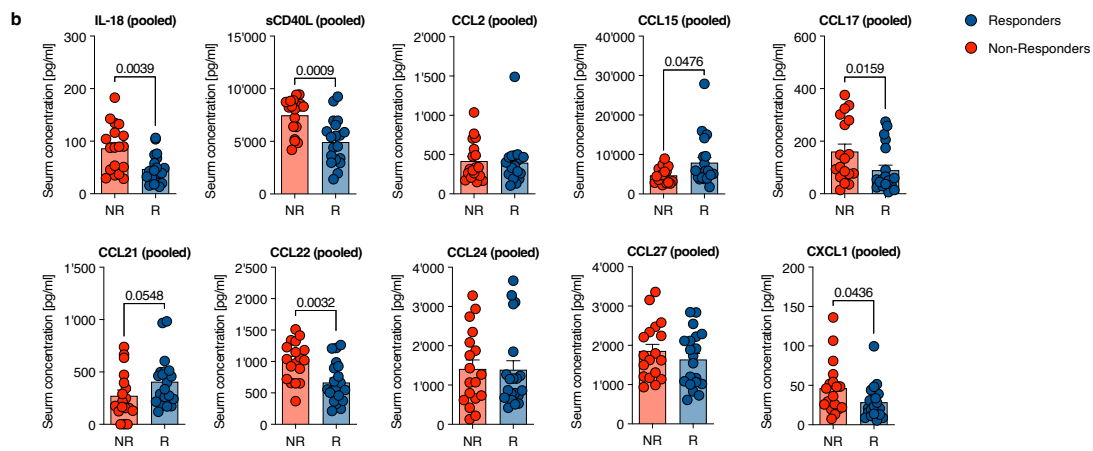
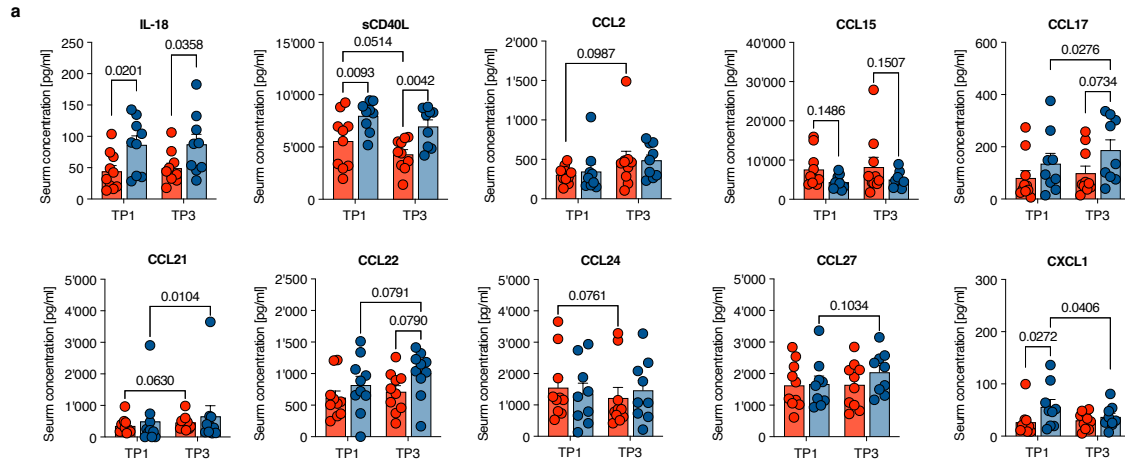
Supplementary Fig. 7. Characterization of senescent, CD57⁺ CD4⁺ T cells.

a-b CD8 T PD-1⁺ (**a**) and CD4 T CD57⁺ (**b**) clusters among total PBMCs in responders ($n = 8$, blue) and non-responders ($n = 7$, red) assessed via mass spectrometry. **c-e** Expression of memory markers (**c**, CCR7, CD27, CD45RA, TCF1, CD127), inhibitory receptors (PD1, CD39, LAG3) and TOX (**d**) and activation markers (**e**, Ki67, CD25, CD28) in CD57⁺ and CD57⁻ CD4⁺ T cells, stratified into responders ($n = 8$, blue) and non-responders ($n = 7$, red). Bars show means +SEM (**a-e**). Statistical testing with two-way ANOVA and uncorrected Fisher's least significant difference (LSD) test (**a, b**). Source data are provided as a Source Data file.

a**b****e****f****c****d****g****h**

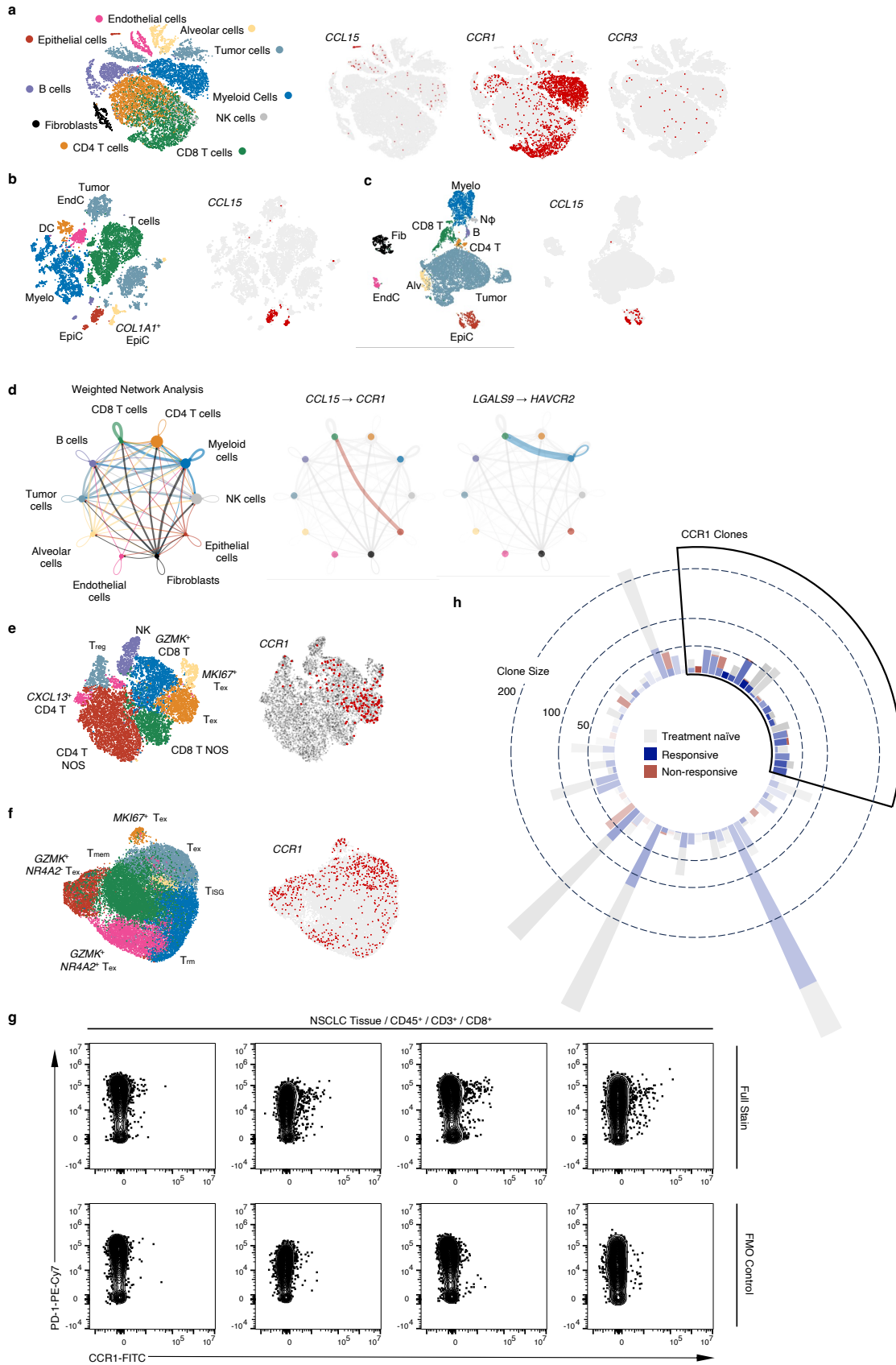
Supplementary Fig. 8. Spectral flow cytometry of PBMCs pre- and post-neoadjuvant chemoimmunotherapy.

a Gating strategy to identify immune cell subsets in spectral flow cytometry data. Parent gate is indicated in top left corner. **b** Volcano plot representing the false discovery rate ($-\log_{10}\text{FDR}$) as calculated by edgeR as a function of fold change (FC, $\log_2\text{FC}$) of major immune lineages identified in spectral flow cytometry analyses at TP3. Each dot denotes a lineage. **c** Expression of Ki67 by CD39⁺ PD-1⁺ CD8⁺ T cells stratified by EFS $\geq$ 12 months (blue) and $<$ 12 months (red) in the entire SAKK 16/14 cohort ($n = 36/6$ (TP1), 40/10 (TP2), 28/10 (TP3) and 32/6 (TP4)), in patients with non-squamous cell carcinoma (non-SCC, $n = 24/4$ (TP1), 28/7 (TP2), 20/7 (TP3), 20/5 (TP4)) and in patients with SCC ($n = 12/2$ (TP1), 12/3 (TP2), 8/3 (TP3), 12/1 (TP4)), **d** CD39⁺ PD-1⁺ CD8⁺ T cells in total PBMCs stratified by EFS $\geq$ 12 months and $<$ 12 months, $n = 37/6$ (TP1), 41/11 (TP2), 30/10 (TP3), 32/6 (TP4). **e-f** CD57⁺ cells in CD4⁺ T cells (**e**) and CD57⁺ CD4⁺ T cells in total PBMCs (**f**) of patients stratified by EFS $\geq$ 12 months and $<$ 12 months, $n = 39/8$ (TP1), 42/12 (TP2), 32/10 (TP3) and 33/6 (TP4). **g** TIM-3 geometric mean fluorescence intensity (gMFI) within CD141⁺ cDC1 stratified by EFS $\geq$ 12 months and $<$ 12 months, $n = 12/3$ (TP1), 31/8 (TP2), 22/7 (TP3) and 21/5 (TP4). **h** TIM-3 gMFI within CD1c⁺ cDC2 stratified by EFS $\geq$ 12 months and $<$ 12 months, $n = 37/8$ (TP1), 42/12 (TP2), 32/10 (TP3) and 33/6 (TP4). **c-g** Patients with EFS $\geq$ 12 months in blue, patients with EFS $<$ 12 months in red. Bars represent means $\pm$ SEM, statistical testing with mixed-effects model (restricted maximum likelihood, REML) with Tukey's multiple comparisons test. p values below 0.1 are shown. Source data are provided as a Source Data file.



Supplementary Fig. 9. Analysis of serum cytokines and chemokines in SAKK 16/14 participants.

a Cytokines with differences between responders (EFS $\geq$ 12 months, MPR, $n = 10$, blue) and non-responders (EFS < 12 months, no MPR, $n = 9$, red) and between timepoints. **b** Cytokines with differences between responders (EFS $\geq$ 12 months, MPR, $n = 10$, blue) and non-responders (EFS < 12 months, no MPR, $n = 9$, red), pooled for timepoints. **c** Pre-treatment (TP1) serum concentrations of 10 cytokines correlated with response in patients achieving 12 months EFS ($n = 42$ at TP1, $n = 36$ at TP3) or not ($n = 12$ at TP1 and TP3). Bars represent means $\pm$ SEM (**a-c**). Statistical testing with two-way ANOVA and uncorrected Fisher's least significant difference (LSD) test (**a**), two-sided Mann-Whitney test (**b**) or two-sided unpaired t test (**c**). p values below 0.2 are shown. Source data are provided as a Source Data file.



Supplementary Fig. 10. Analysis of serum cytokines and chemokines in SAKK 16/14 participants.

a tSNE plot of $n = 10,000$ randomly selected cells from lung tumors reported in Lambrechts et al, 2018². Left, plot is color coded for cluster annotation. Right, expression of indicated genes across all cells. **b** tSNE of $n = 10,000$ randomly selected cells isolated from lung cancer tissue, annotated by cluster (right) and with *CCL15* expression (left) from Wu et al, 2021³. **c**, tSNE of $n = 10,147$ cells isolated from NSCLC tissue, annotated with clusters (right) and with *CCL15* expression (left) from Song et al, 2019⁴. **d** Right, weighted network analysis showing relative signaling intensity between cell populations from Lambrecht et al, 2018². Right, depiction of two representative signaling axes, namely *CCL15* from epithelial cells to *CCR1* on CD8⁺ T cells and from *LGALS9* (encoding for Galectin-9) on myeloid cells to *HAVCR2* (encoding for TIM-3) on CD8⁺ T cells. **e** tSNE plots of $n = 24,911$ T cells, color-coded by their original cluster annotation (right) or *CCR1* expression (left) according to Lambrechts et al, 2018². **f** tSNE plot of $n = 12,851$ T cells, color-coded by consensus clusters (right) and with *CCR1* expression (left) from Liu et al, 2022⁵. **g** Flow cytometry analysis of *CCR1* expression by intratumoral T cells from a different NSCLC patient cohort ($n = 4$). **h** Every bar shows the population size of a single TCR clone from Liu et al, 2022⁵, stratified by the type of lesions it was isolated from: treatment naïve samples (grey), responsive post-therapy samples (blue) and non-responsive post-therapy samples (red). Transparency indicates *CCR1* expression.

Supplementary References

1. Provencio-Pulla, M. *et al.* Nivolumab+ chemotherapy versus chemotherapy as neoadjuvant treatment for resectable stage IIIA NSCLC: Primary endpoint results of pathological complete response (pCR) from phase II NADIM II trial. Preprint at (2022).
2. Lambrechts, D. *et al.* Phenotype molding of stromal cells in the lung tumor microenvironment. *Nat Med* 24, 1277–1289 (2018).
3. Wu, F. *et al.* Single-cell profiling of tumor heterogeneity and the microenvironment in advanced non-small cell lung cancer. *Nat Commun* 12, (2021).
4. Song, Q. *et al.* Dissecting intratumoral myeloid cell plasticity by single cell RNA-seq. *Cancer Med* 8, 3072–3085 (2019).
5. Liu, B. *et al.* Temporal single-cell tracing reveals clonal revival and expansion of precursor exhausted T cells during anti-PD-1 therapy in lung cancer. *Nat Cancer* 3, 108–121 (2022).