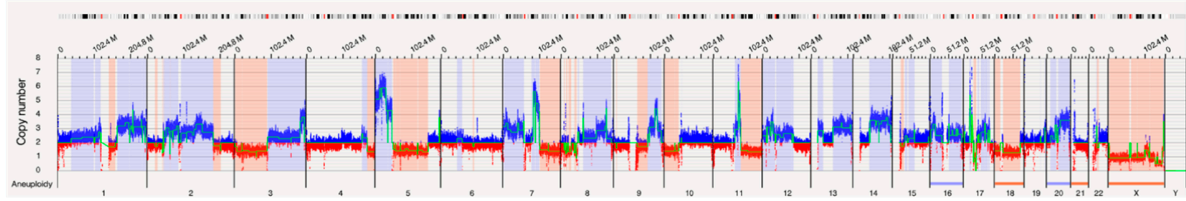
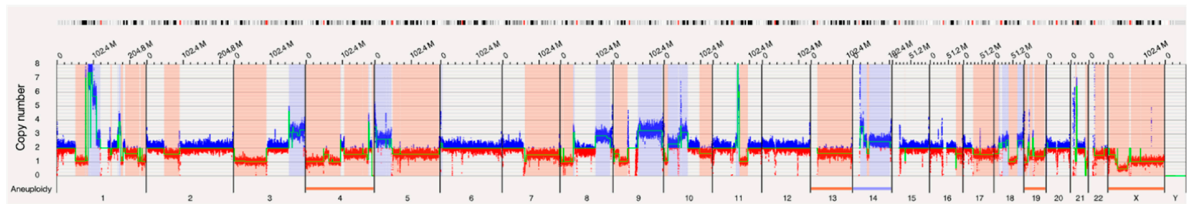


## 1. Supplementary figures

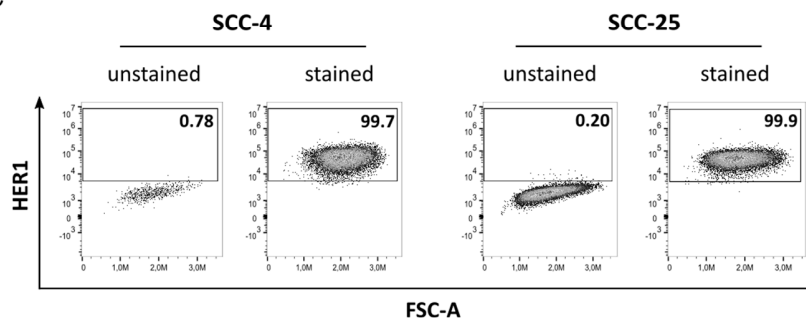
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B

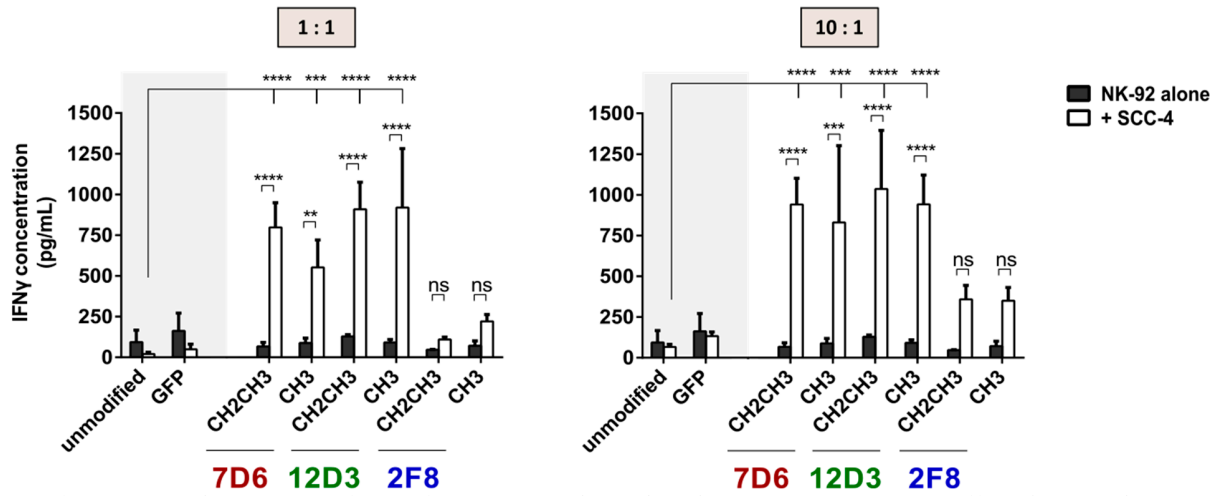


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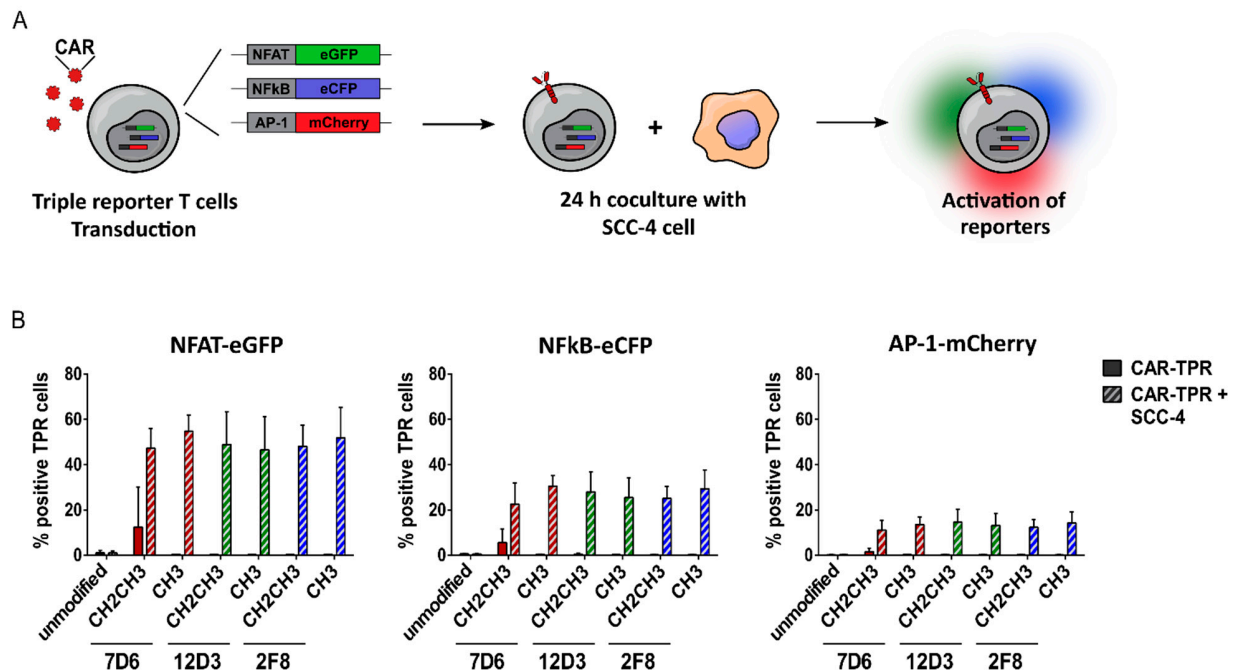


**Supplementary Figure S1. Characterization of SCC-4 and SCC-25 cell lines.** Optical genome mapping of (A) SCC-4 cells and (B) SCC-25 cells. Depicted copy number gains (●) and losses (●) for each chromosome (>500 kbp). (C) HER1/EGFR expression on SCC-4 and SCC-25 cell lines assessed via flow cytometry.

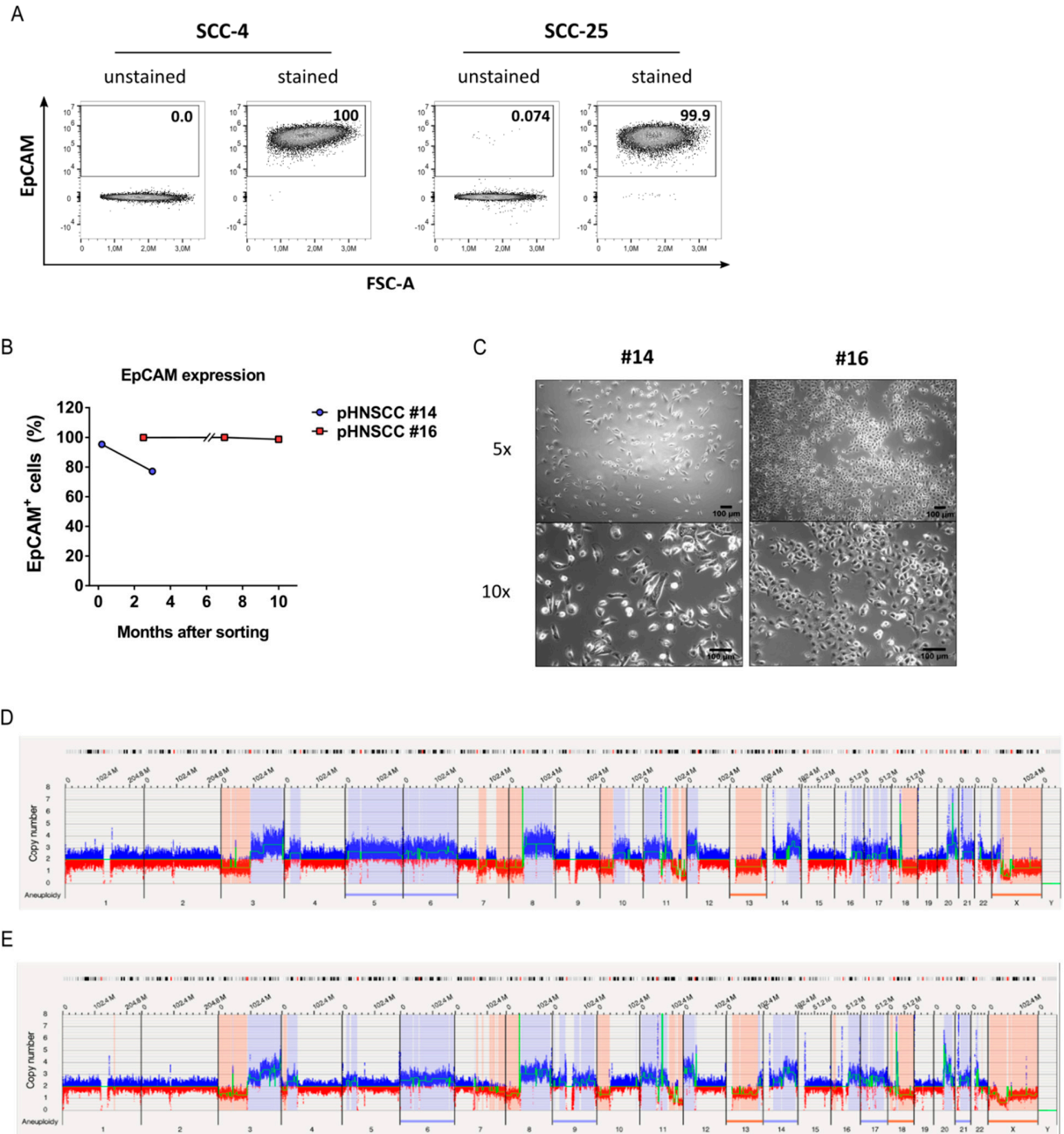
A



**Supplementary Figure S2. Enhanced IFN $\gamma$  secretion of anti-HER1 CAR-NK-92 cell variants using E:T ratios of 1:1 and 10:1. (A)** Supernatants from 48 h co-cultures with and without SCC-4 cells at E:T of 1:1 and 10:1 were collected and analyzed for IFN $\gamma$  secretion via ELISA. E:T = effector:target cell ratio. Mean with standard deviation shown. Statistical analysis was performed using two-way ANOVA with Bonferroni's post-hoc test for comparison to unmodified NK-92 cells within the same time points (e.g. 24 h to 24 h), n = 3.

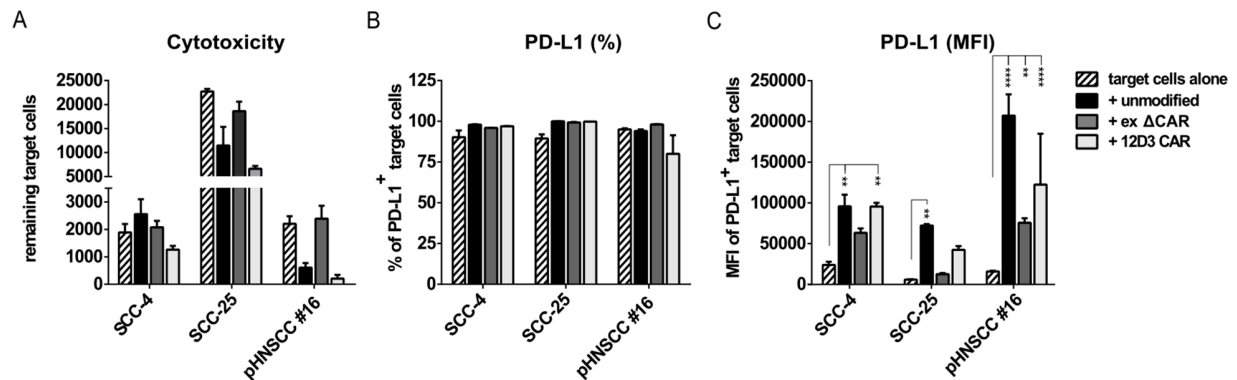


**Supplementary Figure S3. Tonic signaling of anti-HER1 CAR-TPR cell line. (A)** Experimental design of triple parameter Jurkat cells (NFAT-eGFP, NFkB-eCFP, AP1-mCherry) modified with six anti-HER1 CAR variants (E:T ratio = 10:1). Activation of reporters analysed by flow cytometry. **(B)** Activation of reporters of CAR-TRP cells in monoculture or co-culture with SCC-4 cells. E:T = effector:target cell ratio. Mean with standard deviation shown, n = 3-5.



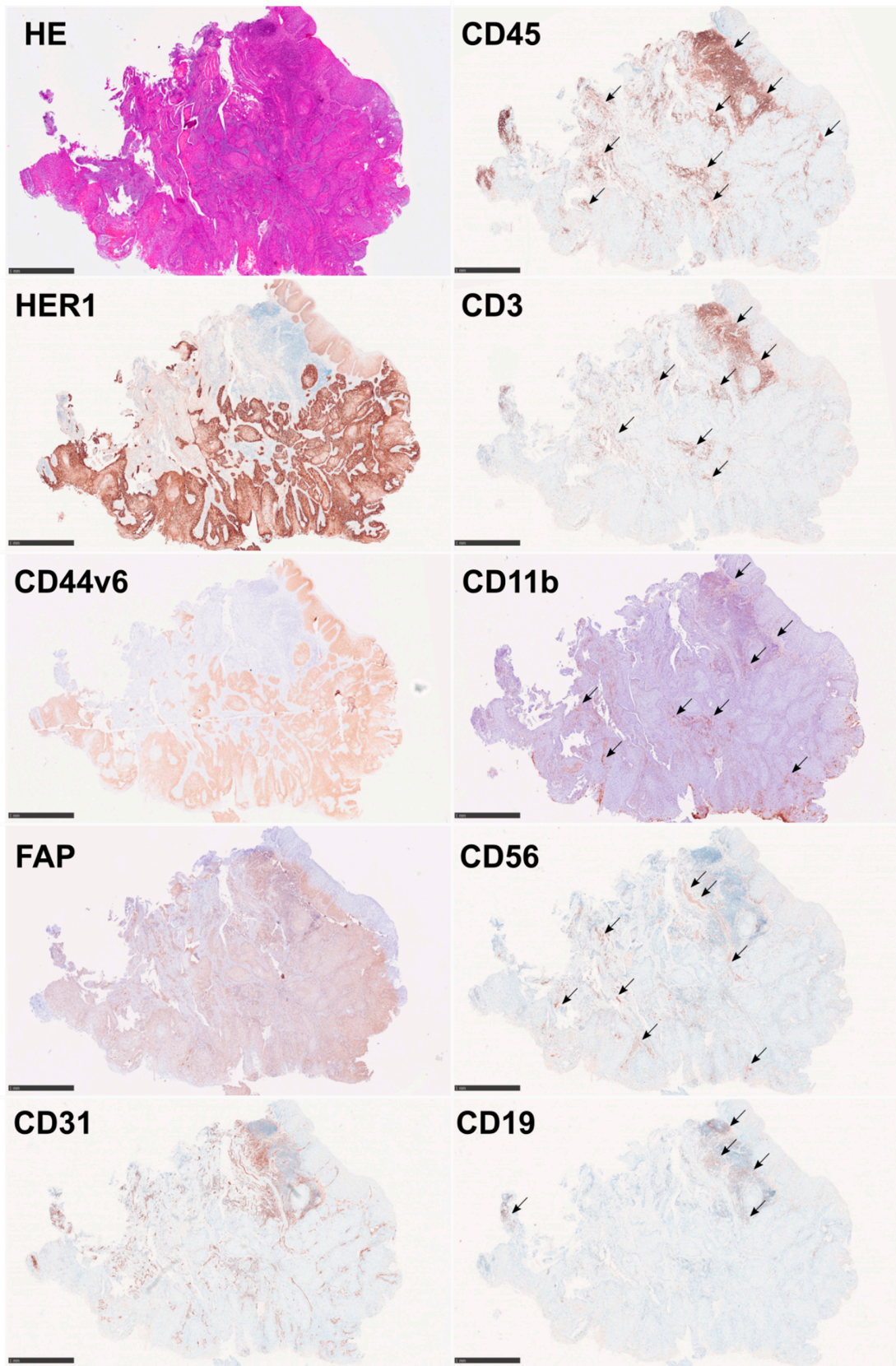
**Supplementary Figure S4. EpCAM expression on target cell lines.** (A) EpCAM expression on SCC-4 and SCC-25 cells. (B) EpCAM expression on EpCAM-sorted and expanded pHNSCC cells #14 and #16 over time. Timeline disruption (//) indicates freezing/thawing cycle. (C) Morphology of EpCAM sorted pHNSCC cells #14 and #16 in 5x and 10x magnification (Scale bar indicates 100  $\mu$ m). Optical genome mapping of EpCAM sorted pHNSCC samples (D) #14 and (E) #16. Depicted copy number gains (●) and losses (●) for each chromosome (>500 kbp).





**Supplementary Figure S5. Upregulation of PD-L1 on target cells upon NK-92 co-culture.** (A) Percentage of remaining target cells 24 h after monoculture (target cells alone) or co-culture with unmodified, ex ΔCAR or full length anti-HER1 12D3 CH3 CAR-NK-92 cells (SCC-4 cells = 1:1 E:T ratio; SCC-25 and pHNSCC #16 = 0.5:1 E:T ratio). Expression of PD-L1 on remaining target cells after 24 h monoculture or co-culture in (B) percentages and (C) MFI. E:T = effector:target cell ratio. Mean with standard deviation shown. Statistical analysis performed using two-way ANOVA with Bonferroni's post-hoc test,  $n = 3$ .

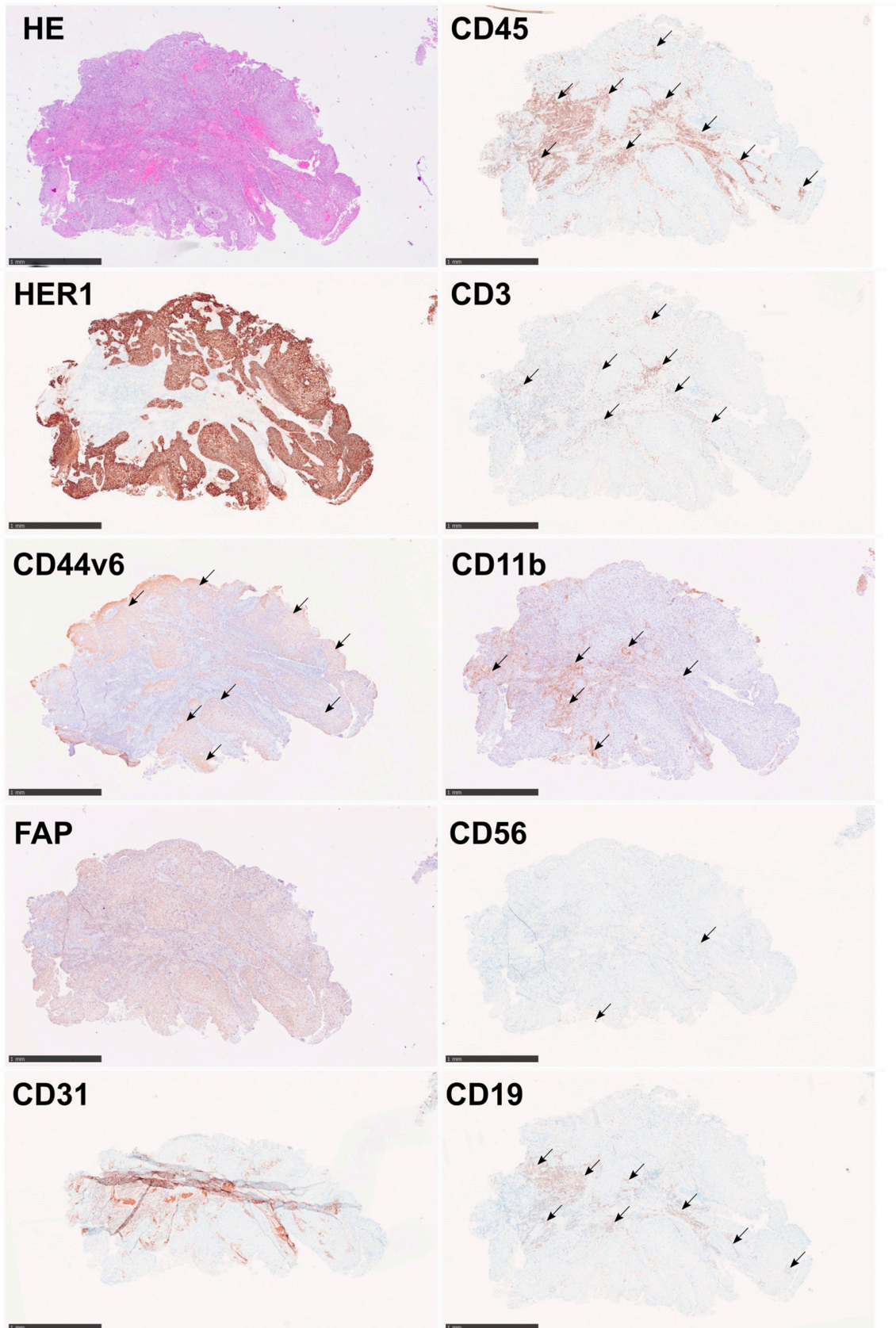
A



**Supplementary Figure S6. Immunohistochemistry staining of patient #16.** (A) Different immunohistochemistry stainings were performed on paraffin sections. HE staining showed pink nodules confirming squamous cell carcinoma, which also demonstrated strong HER1 expression. HER1 expression was co-localized with CD44v6 expression. CD45<sup>+</sup> cells mainly localized to regions lacking HER1<sup>+</sup> and CD44v6<sup>+</sup> cells. CD45<sup>+</sup> regions mainly contained CD3<sup>+</sup> cells and CD11b<sup>+</sup> cells, and to a lower extent CD56<sup>+</sup> cells and CD19<sup>+</sup> cells. Immunohistochemistry (IHC) confirms presence of FAP<sup>+</sup> and CD31<sup>+</sup> cells. HE = hematoxylin and eosin, IHC = immunohistochemistry.



A



**Supplementary Figure S7. Immunohistochemistry staining of patient #15.** (A) Different immunohistochemistry stainings were performed on paraffin sections. HE staining showed pink nodules confirming squamous cell carcinoma, which also demonstrated strong HER1 expression. HER1 expression was co-localized with CD44v6 expression. CD45<sup>+</sup> cells mainly localized to regions lacking HER1<sup>+</sup> and CD44v6<sup>+</sup> cells. CD45<sup>+</sup> regions mainly contained CD3<sup>+</sup> cells and CD11b<sup>+</sup> cells, and to a lower extent CD56<sup>+</sup> cells and CD19<sup>+</sup> cells. Immunohistochemistry (IHC) confirms presence of FAP<sup>+</sup> and CD31<sup>+</sup> cells. HE = hematoxylin and eosin, IHC = immunohistochemistry.

## 2. Supplementary materials and methods

### 2.1. Flow cytometry: surface marker expression

The following anti-human antibodies were used for flow cytometry analysis using CytoFLEX S (Beckman Coulter, Brea, CA, USA): DYKDDDDK-tag-APC (flag-tag) (BioLegend, San Diego, CA, USA), HER1/EGFR-APC (BioLegend, San Diego, CA, USA), HER1/EGFR-BV605 (BioLegend, San Diego, CA, USA), CD44v6-PE (R&D Systems, MN, USA), CD133-APC (BioLegend, San Diego, CA, USA), CD45-BV650 (BD Biosciences, Franklin Lakes, NJ, USA), CD56-PC7 (BD Biosciences, Franklin Lakes, NJ, USA), CD3-BV785 (BioLegend, San Diego, CA, USA), CD19-FITC (Miltenyi Biotec, Bergisch Gladbach, Germany), CD11b-PE (Miltenyi Biotec, Bergisch Gladbach, Germany), EpCAM-PE (BioLegend, San Diego, CA, USA), FAB-FITC (BioLegend, San Diego, CA, USA), CD31-APC (Miltenyi Biotec, Bergisch Gladbach, Germany), CD140a-PerCP-Cy5.5 (Miltenyi Biotec, Bergisch Gladbach, Germany). Analysis was performed using FlowJo10 (BD Biosciences, Franklin Lakes, NJ, USA).

### 2.2. Immunohistochemistry staining and analysis

Stainings were performed using the antibodies listed in supplementary table 1.

**Supplementary Table S1. Antibodies used for immunohistochemistry**

Target of antibody	Product name	Host species	Pretreatment	Dilution	Manufacturer
<b>CD11b</b>	Recombinant Anti-CD11b antibody [EPR1344] (ab133357)	Rabbit	Tris-EDTA buffer	1:2000	Abcam
<b>CD19</b>	CD19 antibody (NCL-L-CD19-163)	Mouse	Tris-EDTA buffer	1:50	Leica
<b>CD3</b>	CONFIRM anti-CD3 (2GV6) Rabbit Monoclonal Primary Antibody	Rabbit	Tris-EDTA buffer	RTU	Roche
<b>CD31</b>	CD31 antibody (JC70)	Mouse	Tris-EDTA buffer	RTU	Roche
<b>CD44v6</b>	CD44var (v6) Monoclonal Antibody (VFF-18) (BMS125)	Mouse	Tris-EDTA buffer	1:200	eBioScience

<b>CD45</b>	CD45 (LCA) (2B11 & PD7/26)	Mouse	Tris-EDTA buffer	RTU	Roche
<b>CD56</b>	CD56 antibody (MRQ-42)	Rabbit	Tris-EDTA buffer	RTU	Roche
<b>EGFR</b>	CONFIRM anti-Epidermal Growth Factor Receptor (3C6) Primary Antibody	Mouse	Protease 1 pretreatment (8 minutes)	RTU	Roche
<b>EpCAM</b>	Recombinant Anti-EpCAM antibody [VU-1D9] (ab187372)	Mouse	Protease 1 pretreatment (4 minutes)	1:100	Leica
<b>FAP</b>	Anti-Fibroblast activation protein, alpha antibody (ab53066)	Rabbit	Tris-EDTA buffer	1:100	Abcam
<b>PD-1</b>	PD-1 (NAT105) Mouse Monoclonal Antibody	Mouse	Tris-EDTA buffer	1:400	Cell Marque

CD11b/19/3/31/44v6/45/56, cluster of differentiation 11b/19/3/31/44v6/45/56; EGFR, epidermal growth factor receptor; EpCAM, epithelial cell adhesion molecule; FAP, fibroblast activated protein; PD-1, programmed cell death 1. EDTA, ethylenediamine tetraacetic acid; RTU, ready to use concentration provided by manufacturer.