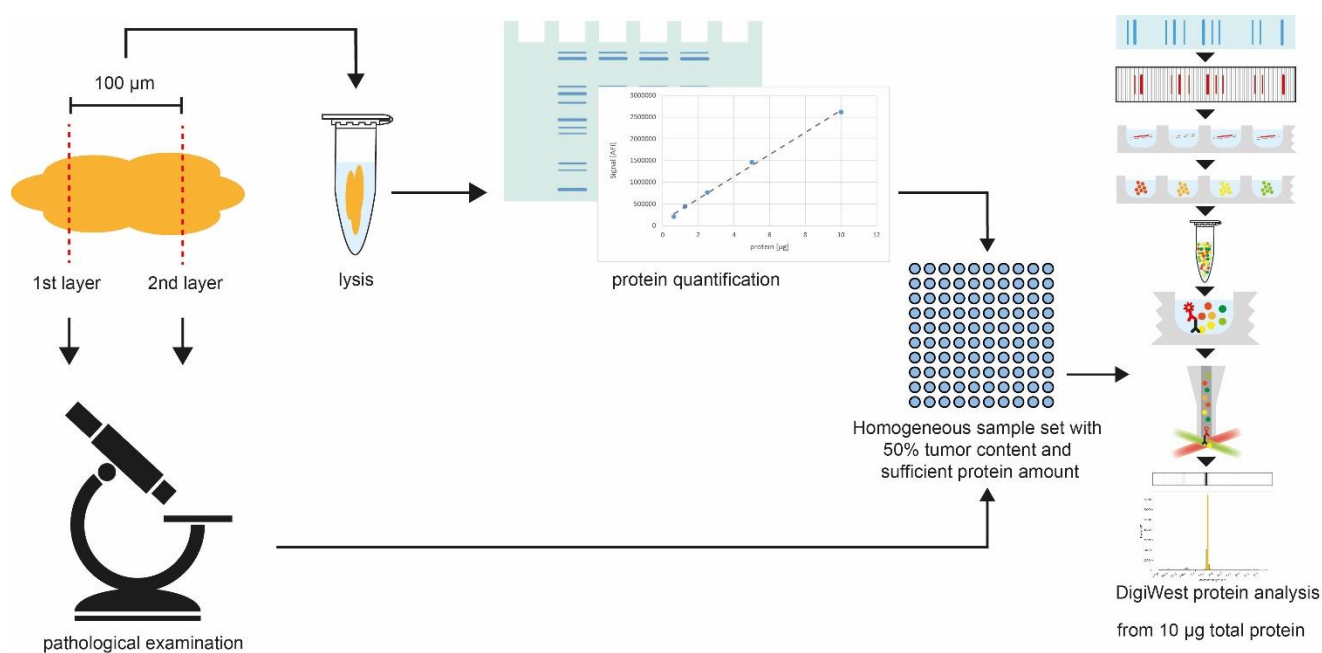
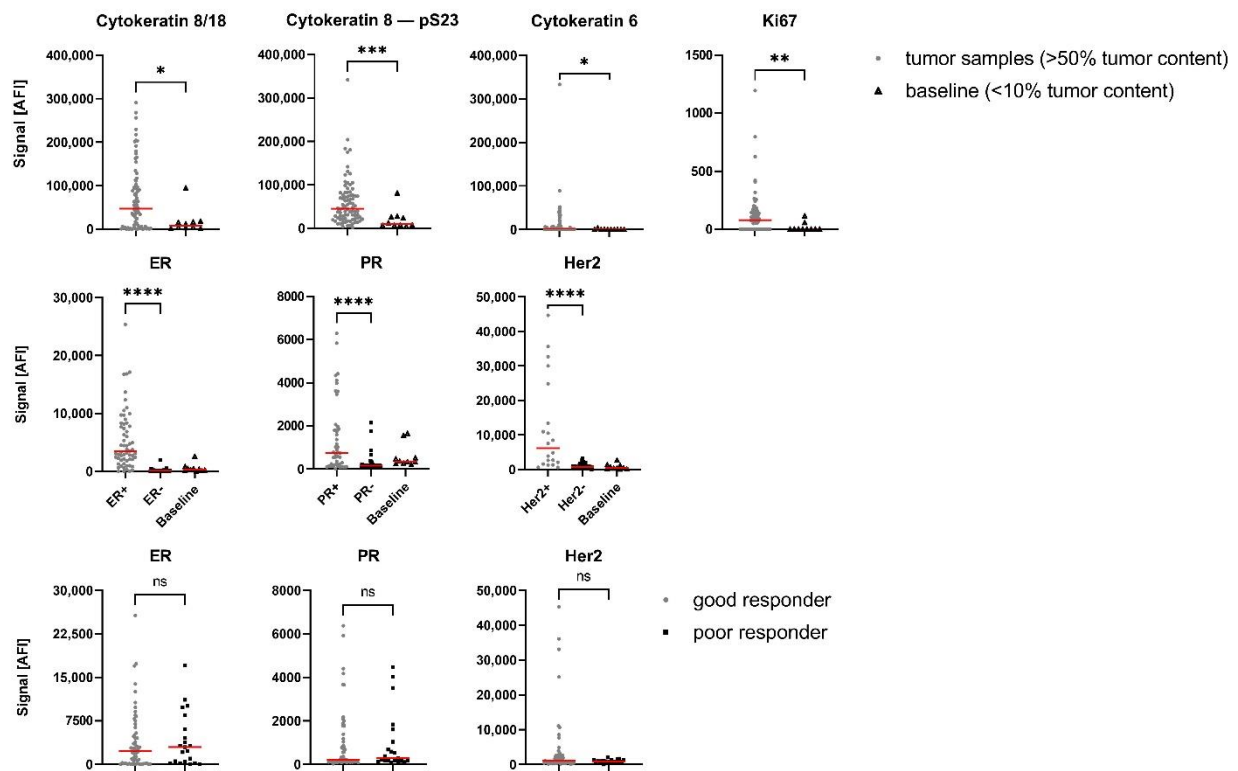


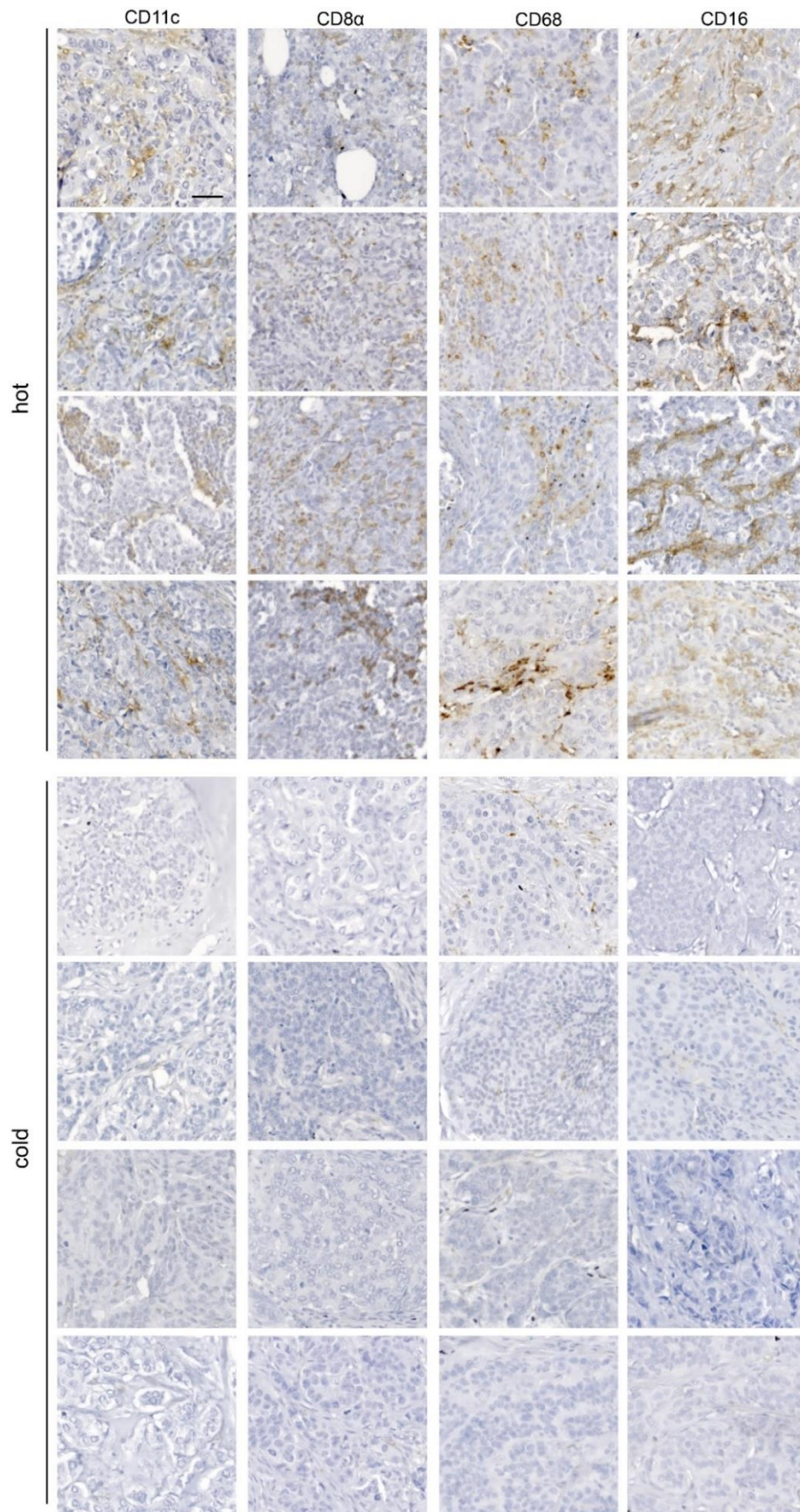
**Supplementary Figure S1. Study flowchart.**



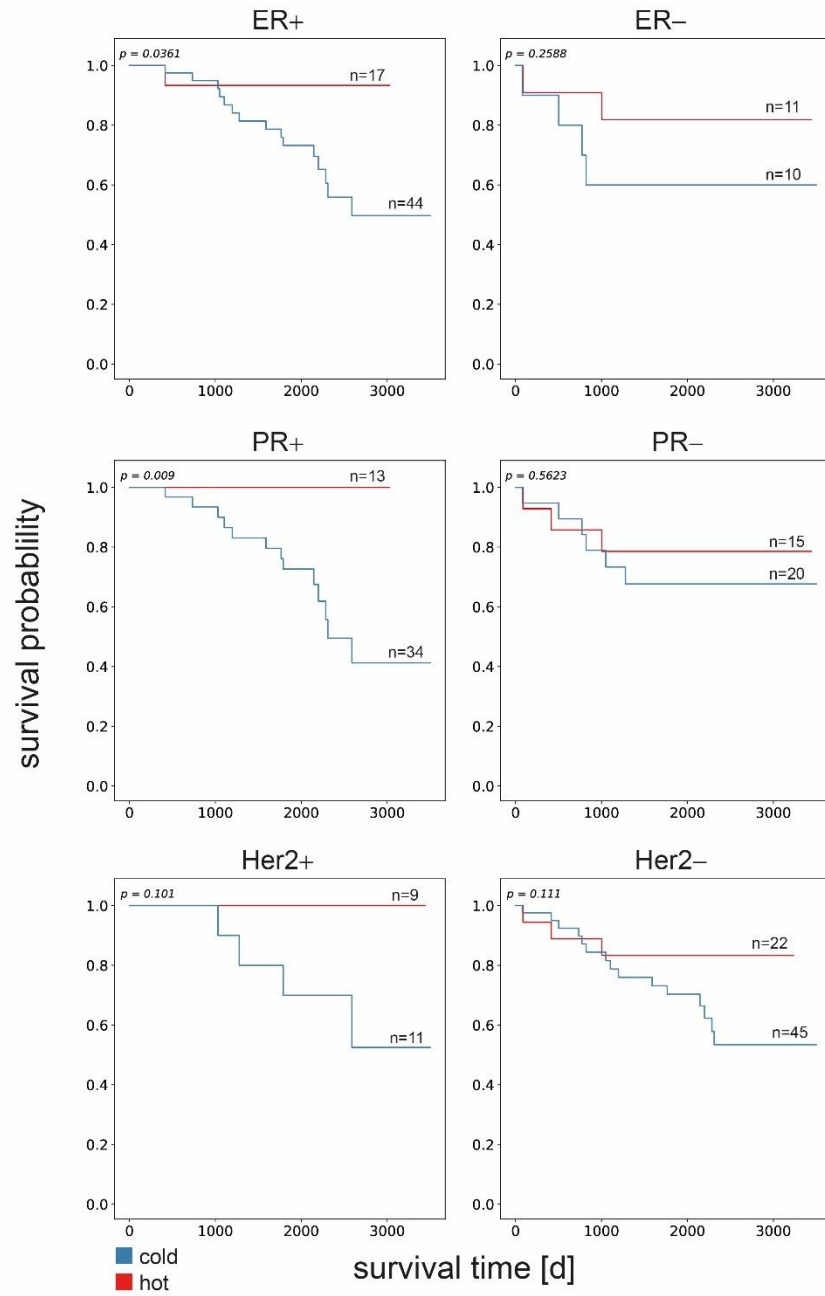
**Supplementary Figure S2. Schematic depiction of study workflow.** Layered cuts of tumor biopsies were generated, and intermediate tissue (100 µm) was collected. A pathologist assessed tumor content in first and second layer. Collected sample material was lysed and protein quantification was performed. Samples with tumor content of  $\geq 50\%$  tumor content and sufficient protein amount were selected for DigiWest analysis.



**Supplementary Figure S3. Tumor marker and receptor expression in baseline samples versus tumor samples.** (A) Cytokeratin 8/18, Cytokeratin 8 – pS23, Cytokeratin 6 and Ki67 expression as scatter plots in samples with  $\geq 50\%$  tumor content (Tumor samples,  $n=84$ ) and  $\leq 10\%$  tumor content (baseline,  $n=10$ ). (B) Scatter plots showing protein expression of ER, PR and Her2 in respective receptor-positive or negative and baseline subgroup (ER+  $n=60$ ; ER-  $n=24$ ; PR+  $n=46$ ; PR-  $n=38$ ; Her2+  $n=20$ ; Her2-  $n=63$ ) as well as (C) in good ( $n=58$ ) and poor responders ( $n=21$ ). In A, B, C Mann-Whitney-U test, \*\*\*\* $P<0.0001$ ; \*\*\* $P<0.001$ ; \*\* $P<0.01$ ; \* $P<0.05$ ; ns indicates no significant difference.

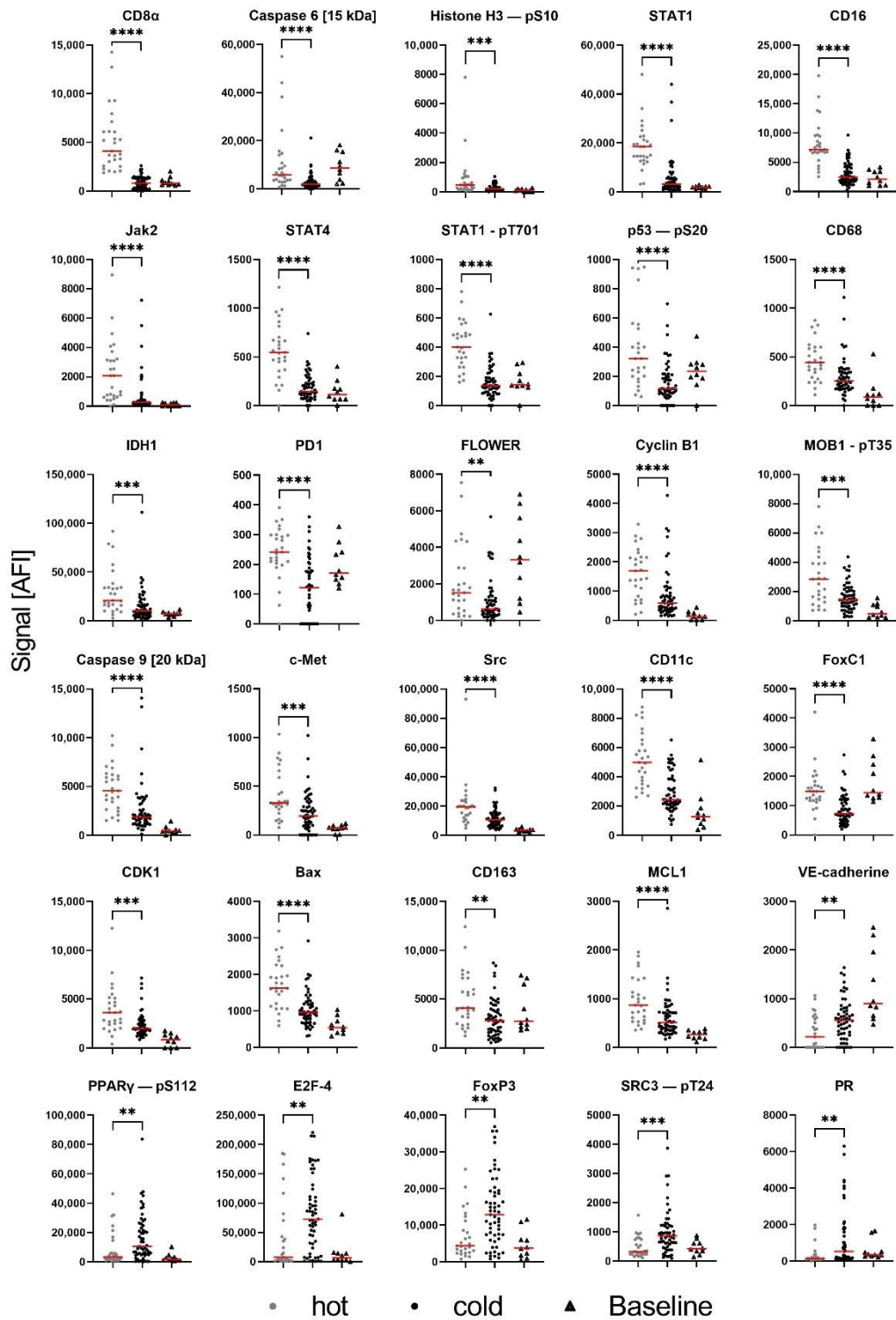


**Supplementary Figure S4.** Overview of IHC staining of immune cells. Representative images of CD11c, CD8, CD68 and CD16 immuno-histochemical staining in hot and cold samples. n=4. Scale bar, 50  $\mu$ m.



**Supplementary Figure S5. Influence of infiltrating immune cells on event-free survival in hormone receptor and Her2 positive/negative tumors.** Kaplan-Meier analysis of event-free survival between hot and cold carcinoma samples in ER, PR and Her2 positive and negative subgroups. A significant difference in EFS was found in ER+ and PR+ subgroup ( $P < 0.05$ ). Log-rank test.





**Supplementary Figure S6. Differences in protein expression between hot and cold tumors.** Protein expression of 30 analytes for hot (n=27), cold (n=57) and baseline (n=10) subgroup which revealed significant differences in protein expression and a fold change of at least 2/3 between hot and cold samples. Mann-Whitney-U test, \*\*\*\*P<0.0001, \*\*\*P<0.001; \*\*P<0.01.

**Table S1.** Correlation values for all measured immune cell markers. Spearman's correlation.

	CD4	CD25	CD56	CD163	CD11c	CD68	CD16	CD8a
CD4	1	-0.2	0.4	-0.4	0.1	0.2	0	0
CD25	-0.2	1	0.4	0.2	0.1	0.2	0.5	0.5
CD56	0.4	0.4	1	0	0.4	0.5	0.4	0.5
CD163	-0.4	0.2	0	1	0.5	0.4	0.3	0.4
CD11c	0.1	0.1	0.4	0.5	1	0.7	0.6	0.6
CD68	0.2	0.2	0.5	0.4	0.7	1	0.7	0.6
CD16	0	0.5	0.4	0.3	0.6	0.7	1	0.7
CD8a	0	0.5	0.5	0.4	0.6	0.6	0.7	1