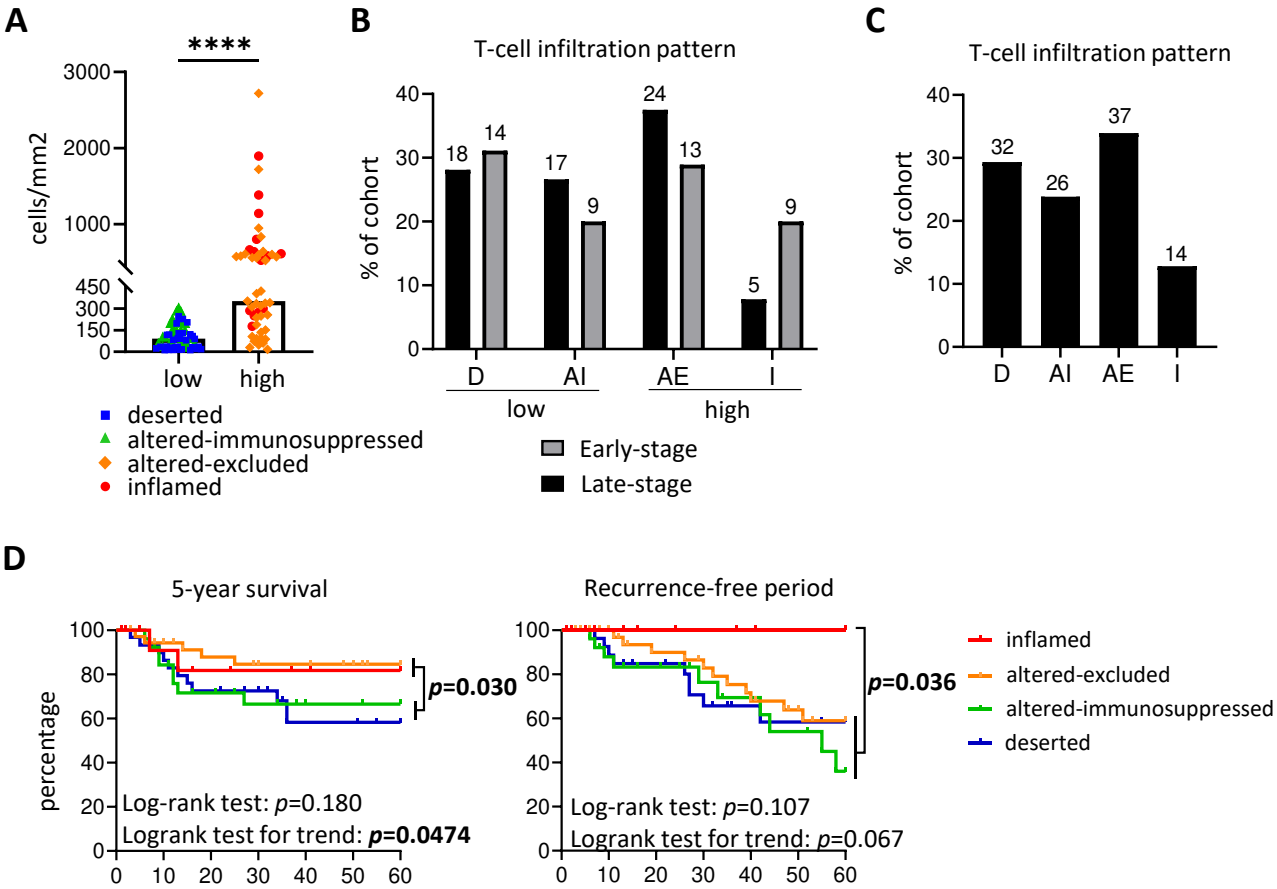
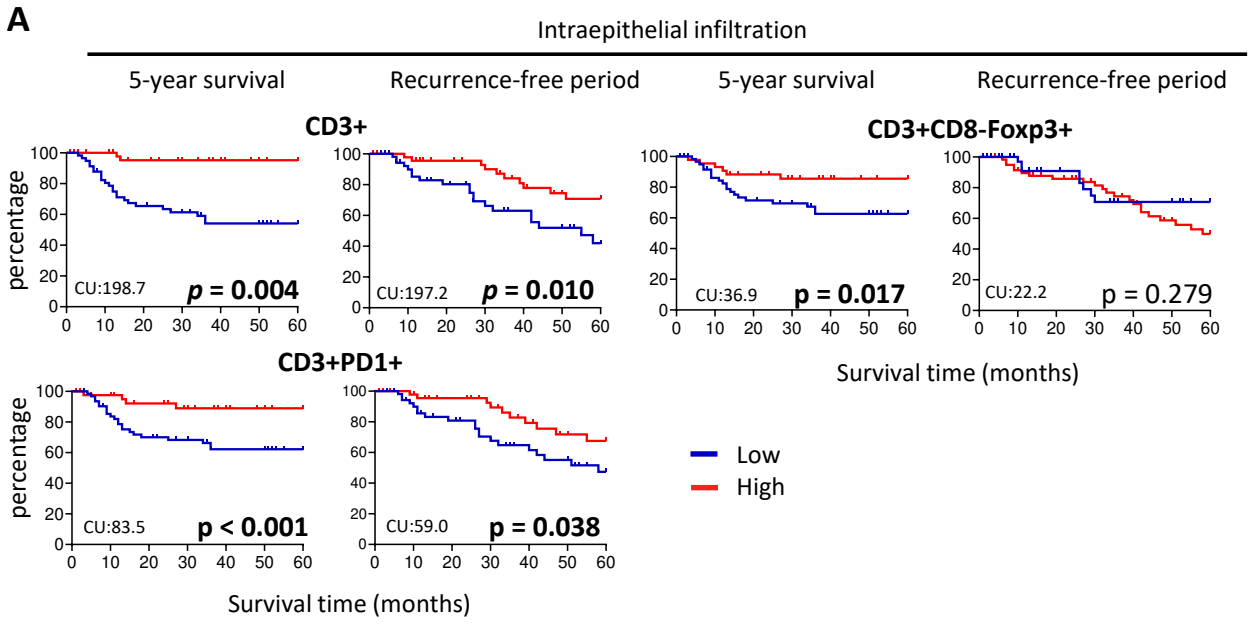
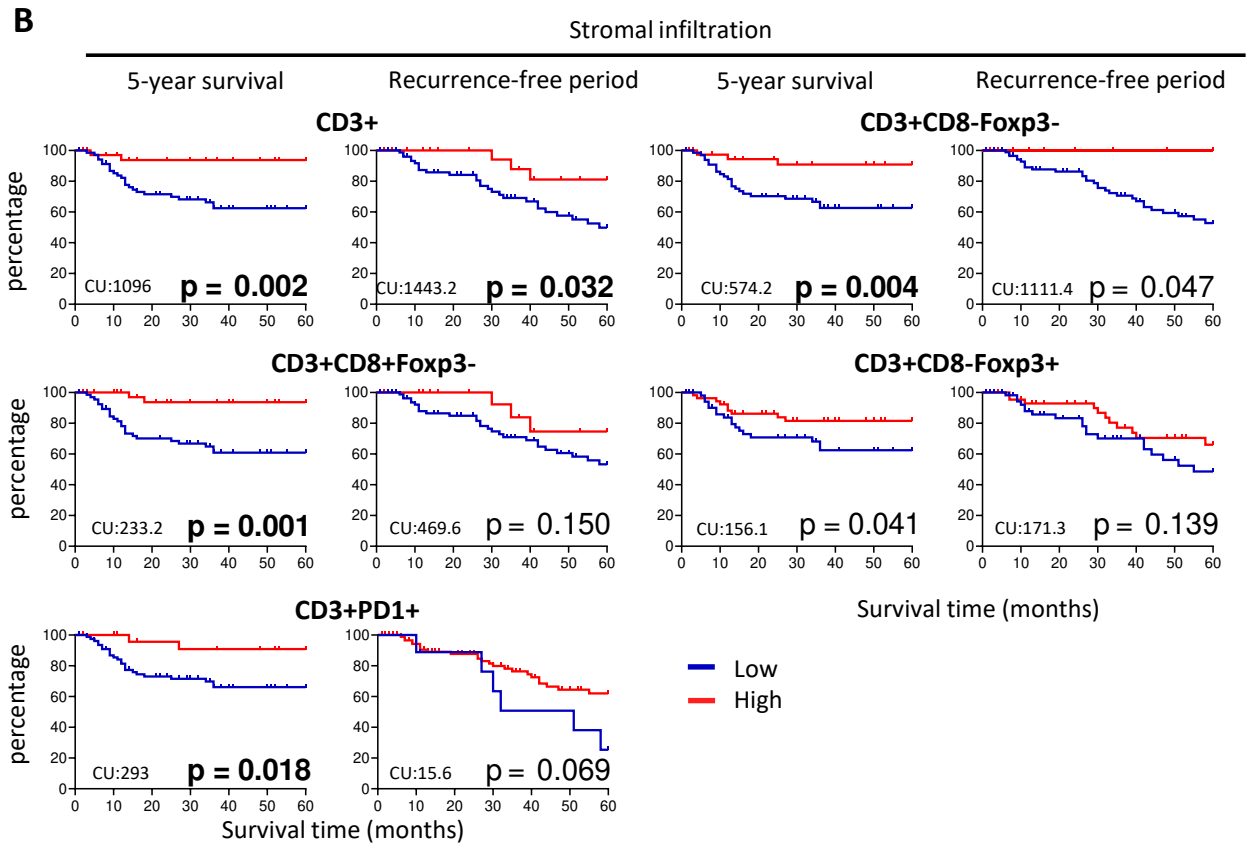
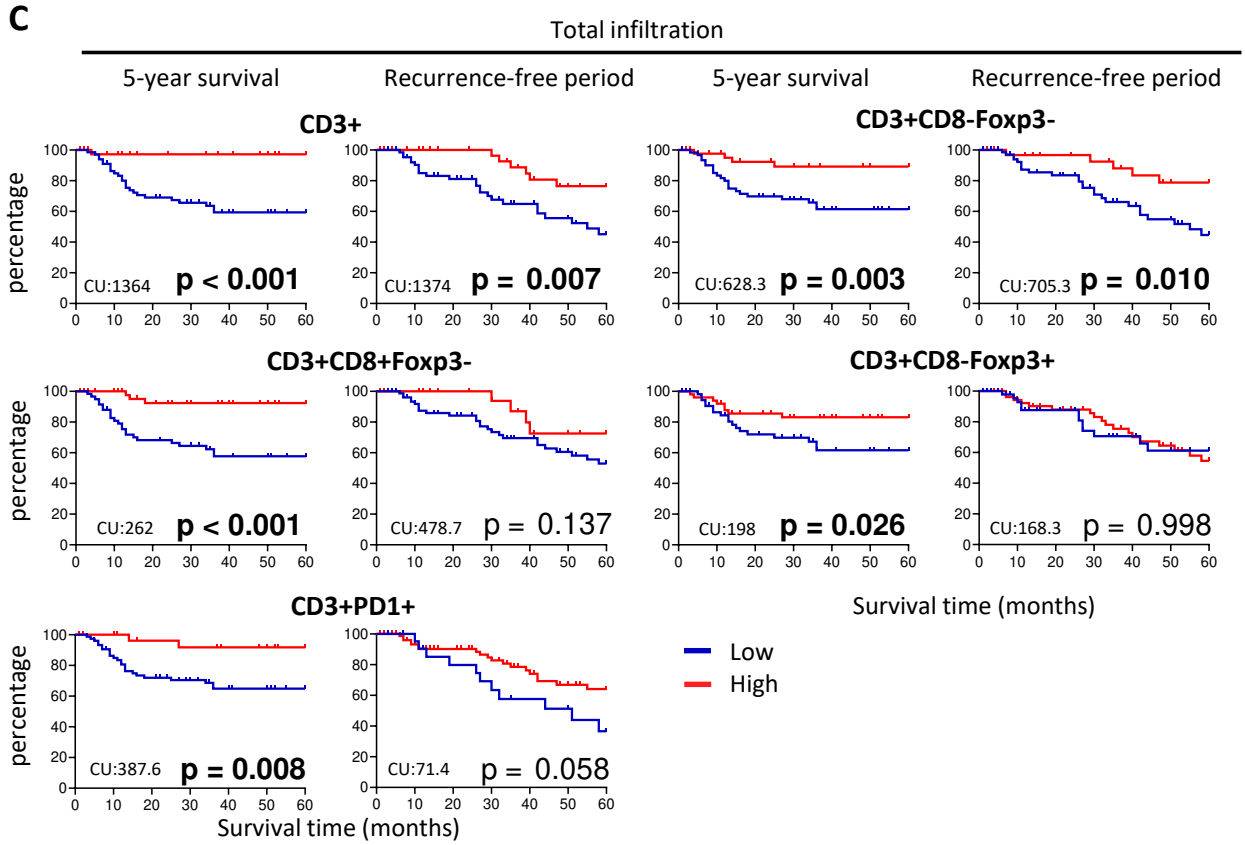


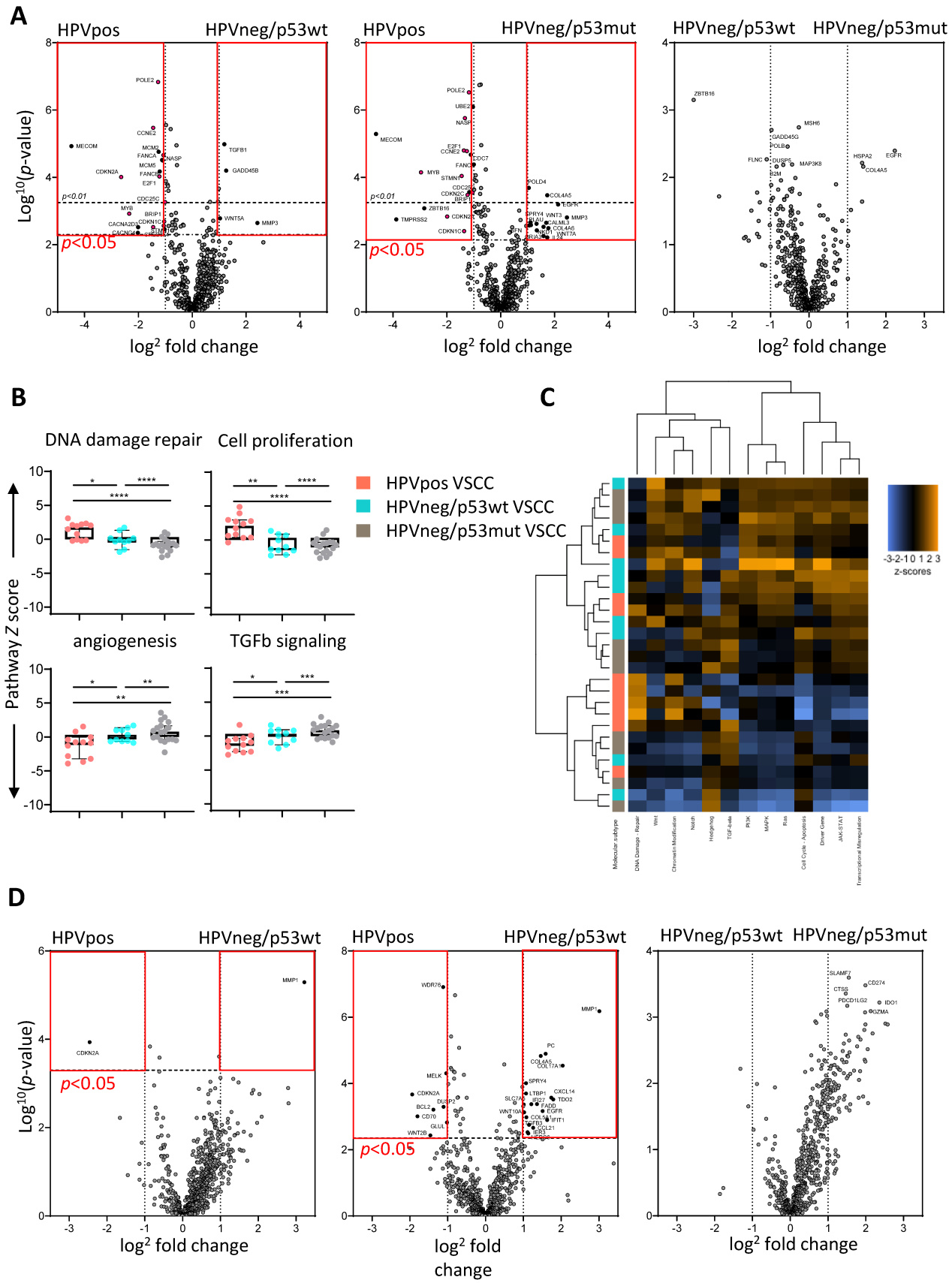
Supplemental figure 1. Low and high infiltrated tumors were categorized based on their intraepithelial CD3⁺ cell count. Paraffin-embedded tumor tissue of VSCC patients was analyzed by multiplex immunofluorescent VECTRA analysis with antibodies directed against CD3, CD8, Foxp3, PD-1, pan-cytokeratin and DAPI as described in reference 9. **A)** Scatter plot displaying the number of intraepithelial CD3+ T cell counts/mm2 for patients with low infiltration (i.e. deserted (D; blue) and altered-immunosuppressed (AI, green)) or high infiltration (i.e. altered-excluded (AE, orange) and inflamed (I; red)) VSCC for the 109 FIGO I-III VSCC. **B, C)** Bar graphs displaying the observed T cell infiltration patterns for early-stage (black) and late-stage (grey) VSCC (**B**) and total cohort of VSCC (**C**)**. D)** Kaplan-Meier curves showing 5-year survival (left) and the recurrence free period (RFP; right) for 109 FIGO I-III VSCC patients with inflamed (red; n=14), altered-excluded (orange; n=37), altered-immunosuppressed (green; n=26) and deserted (blue; n=32) T-cell infiltration patterns. Statistical significance of the survival distribution was analyzed by log-rank testing (Mantel-Cox and trend), and differences were considered significant when $p<0.05$.


A
Intraepithelial infiltration
5-year survival
Recurrence-free period
5-year survival
Recurrence-free period
CD3+
CD3+CD8-Foxp3+
CD3+PD1+
percentage
CU:198.7
p = 0.004
CU:197.2
p = 0.010
CU:36.9
p = 0.017
CU:22.2
p = 0.279
CU:83.5
p < 0.001
CU:59.0
p = 0.038
Survival time (months)
Low
High

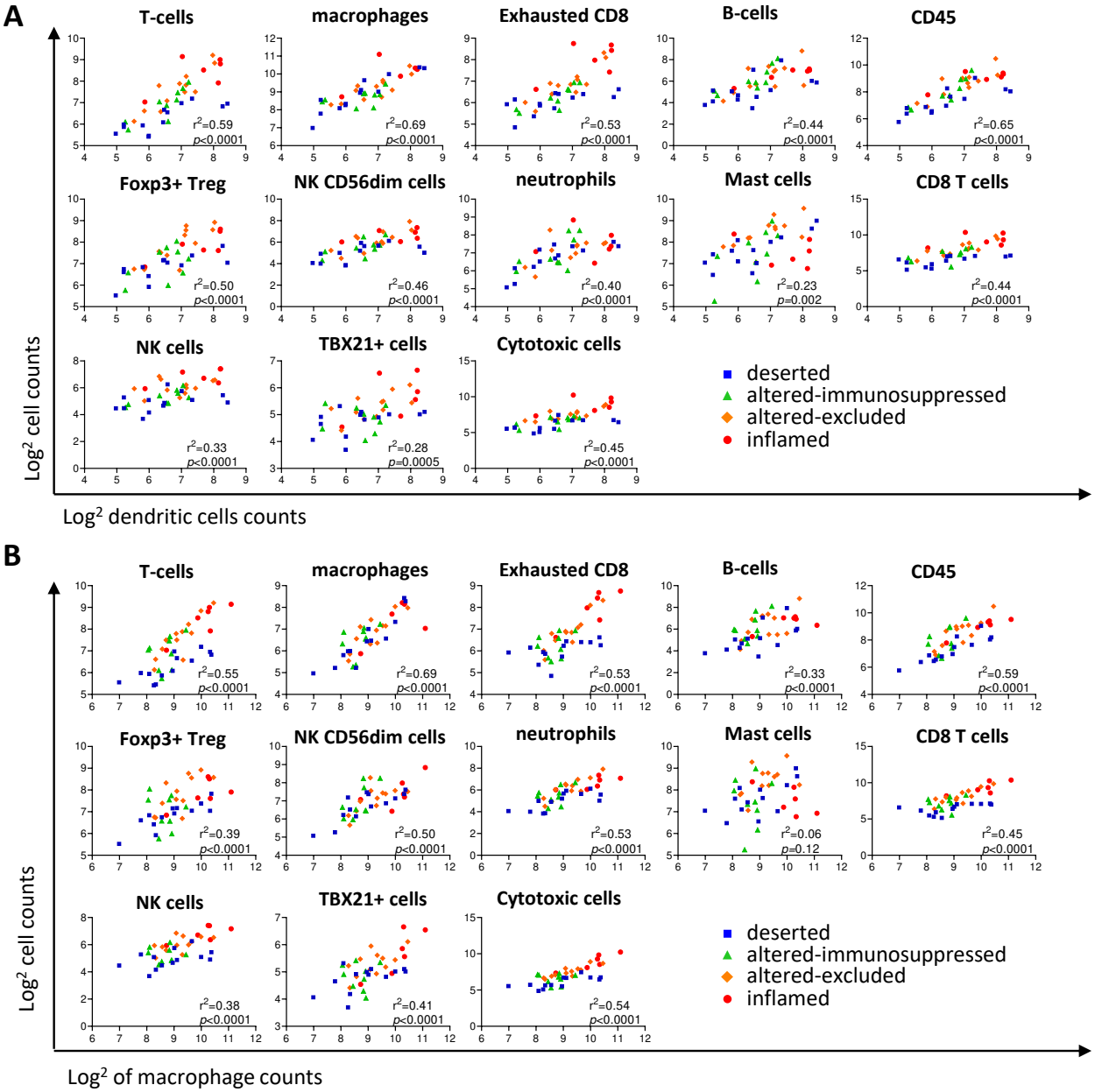

B
Stromal infiltration
5-year survival
Recurrence-free period
5-year survival
Recurrence-free period
CD3+
CD3+CD8-Foxp3-
CD3+CD8+Foxp3-
CD3+CD8-Foxp3+
CD3+PD1+
percentage
CU:1096
p = 0.002
CU:1443.2
p = 0.032
CU:574.2
p = 0.004
CU:1111.4
p = 0.047
CU:233.2
p = 0.001
CU:469.6
p = 0.150
CU:156.1
p = 0.041
CU:171.3
p = 0.139
CU:293
p = 0.018
CU:15.6
p = 0.069
Survival time (months)
Low
High



Supplemental Figure 2. High immune infiltration is associated with better clinical outcome. Kaplan-Meier curves showing the 5-years overall survival (first and third column) and recurrence-free period (second and fourth column) for early- and late-stage VSCC patients with low (blue) or high (red) numbers of **A)** intraepithelial CD3+, CD3+CD8-Foxp3+ and CD3+PD1+ cells/mm2 and of stromal **(B)** and total **(C)** CD3+, CD3+CD8-Foxp3-, CD3+CD8+Foxp3-, CD3+CD8-Foxp3+, and CD3+PD1+ cells/mm2. Patients were grouped into the low or high groups based on the best cut-off value (CU) for each subset as determined by receiver operating characteristics (ROC) curve analysis. Patients with a T cell count below the cut-off value were classified as low, and visa versa. CU values are given in the low left corner of each Kaplan-Meier curve. Statistical significance of the survival distribution was analyzed by log-rank testing. Significant differences $p<0.05$ were shown in bold.

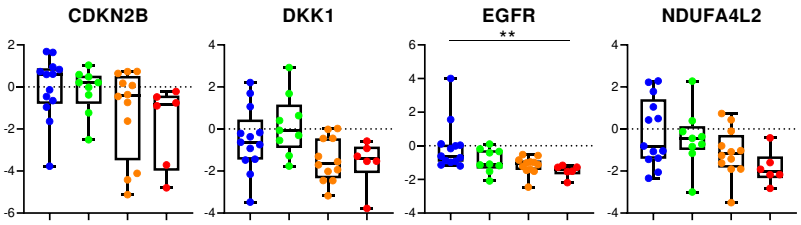

A
HPVpos
HPVneg/p53wt
HPVneg/p53mut
Log10(p-value)
log2 fold change
p<0.01
p<0.05
B
DNA damage repair
Cell proliferation
angiogenesis
TGFb signaling
Pathway Z score
HPVpos VSCC
HPVneg/p53wt VSCC
HPVneg/p53mut VSCC
C
z-scores
D

Supplemental figure 3. Minor differences observed between molecular subtypes of VSCC. Early-stage (n=29) and late-stage (n=11) VSCC of all three molecular subtypes (HPVpos (n=12), HPVneg/p53wt (n=10), and HPVneg/p53mut (n=18) VSCC) were analyzed by the PanCancer oncogenic pathway (early-stage only) and PanCancer IO360 (early- and late-stage) panels. The data were analyzed using nCounter Advanced Analysis module 2.0 software. **A)** Volcano plots depicting the differentially expressed genes (DEGs) between HPVpos and HPVneg/p53wt (left), HPVpos and HPVneg/p53mut (middle) and HPVneg/p53wt and HPVneg/p53mut (right) early-stage VSCC based on a $\log^2$ fold change of >1 or <-1 in combination with Benjamini-Hochberg (BH) adjusted $p<0.05$. Significant (BH p-values) genes are indicated by the red lines. Overlapping DEGs that are up in HPVpos vs HPVneg/p53wt or HPVneg/p53mt are indicated in pink. **B**) Box plots showing the differences in the cancer-associated canonical signaling pathways Z scores for HPVpos (red), HPVneg/p53wt (blue) and HPVneg/p53mut (grey) VSCC. **C)** Heatmap plot displaying pathway scores of 13 cancer-associated canonical signaling pathways within the PanCancer Pathway panel. Pathway scores were calculated and displayed as Z-transformed values where orange represents high scores, and blue represents low scores. **D)** Volcano plots depicting the differentially expressed genes (DEGs) between HPVpos and HPVneg/p53wt (left), HPVpos and HPVneg/p53mut (middle) and HPVneg/p53wt and HPVneg/p53mut (right) early-stage and late-stage VSCC based on a $\log^2$ fold change of >1 or <-1 in combination with Benjamini-Hochberg (BH) adjusted $p<0.05$. Significant (BH p-values) genes are indicated by the red lines. *$p<0.05$, **$p<0.01$, ***$p<0.001$, and ****$p<0.0001$.

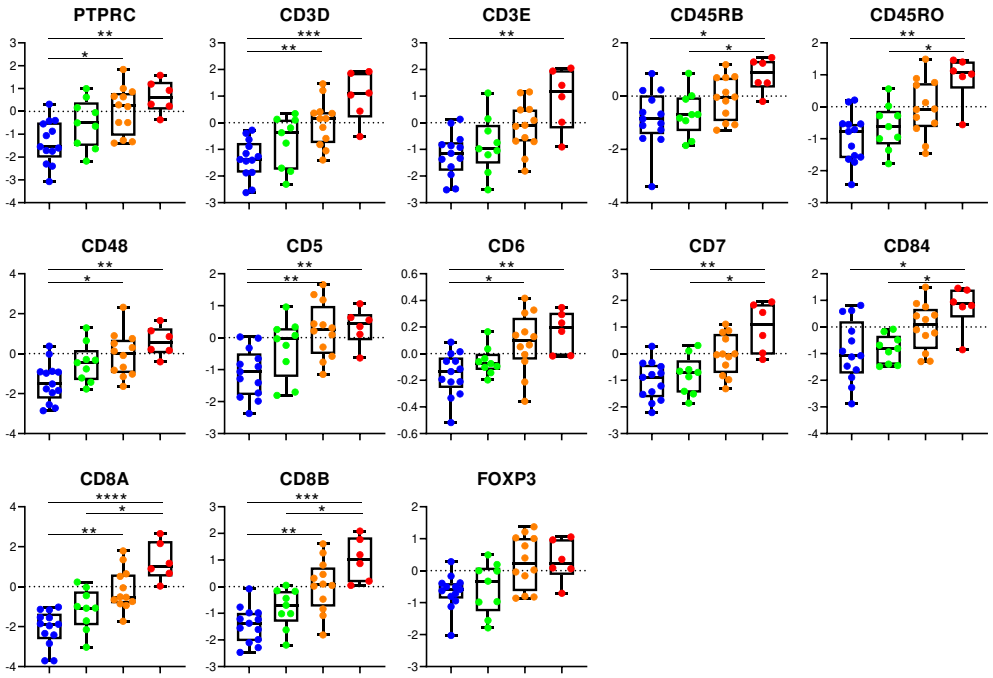


Supplemental figure 4. A coordinated immune response between immune cell types. Automated cell type profiling was performed by using nCounter Advanced Analysis module 2.0 software and was based on the expression of pre-defined genes (Supplemental Table 4). Linear regression analyses of indicated deconvoluted cell types (Y-axis) versus **A)** dendritic cells (DCs, X-axis) and **B)** macrophages (X-axis). Data is given as log2 transformed cell counts. The different T-cell infiltration patterns are depicted in color code. **p*<0.05, ***p*<0.01, ****p*<0.001, and *****p*<0.0001.

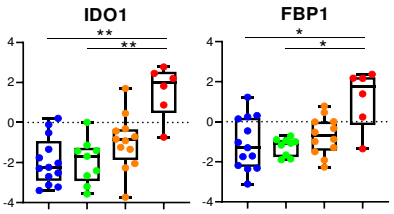
Upregulated in cold



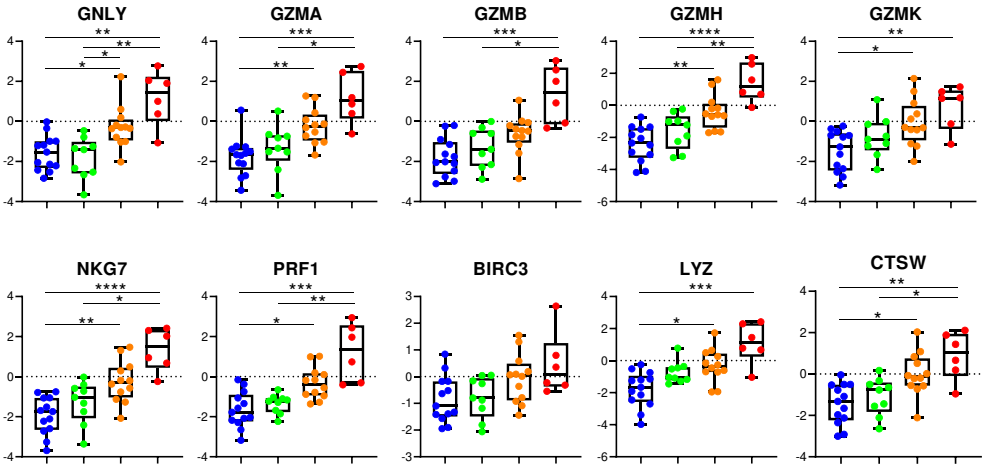
Lymphoid compartment



Myeloid compartment

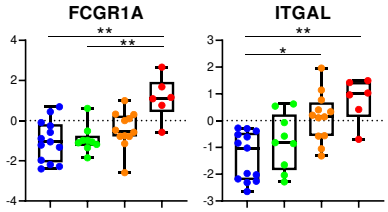


cytotoxicity

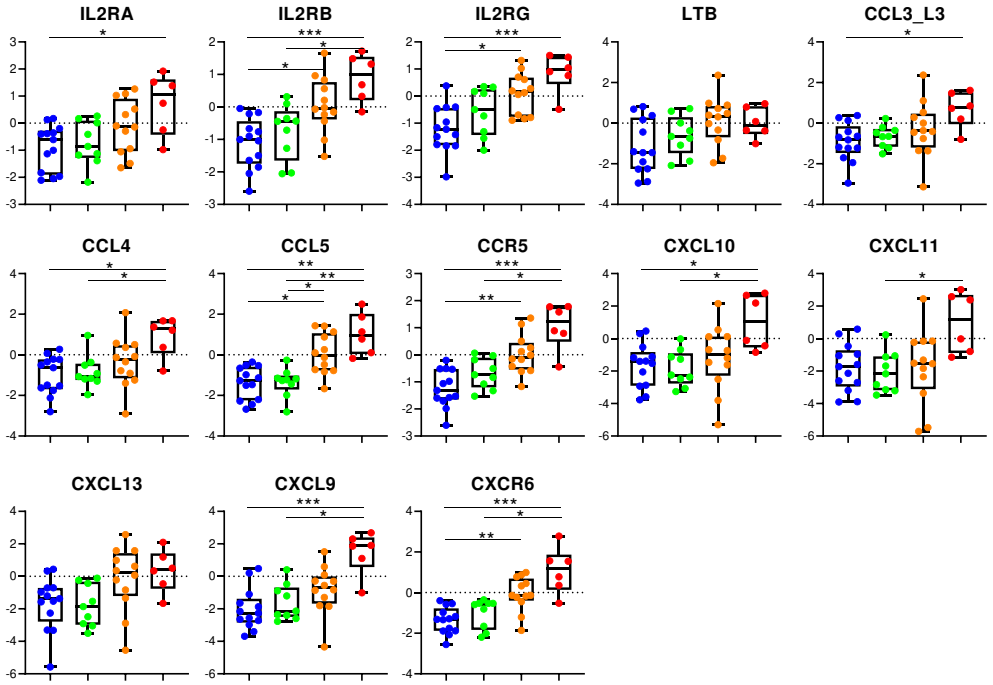


Supplemental figure 5

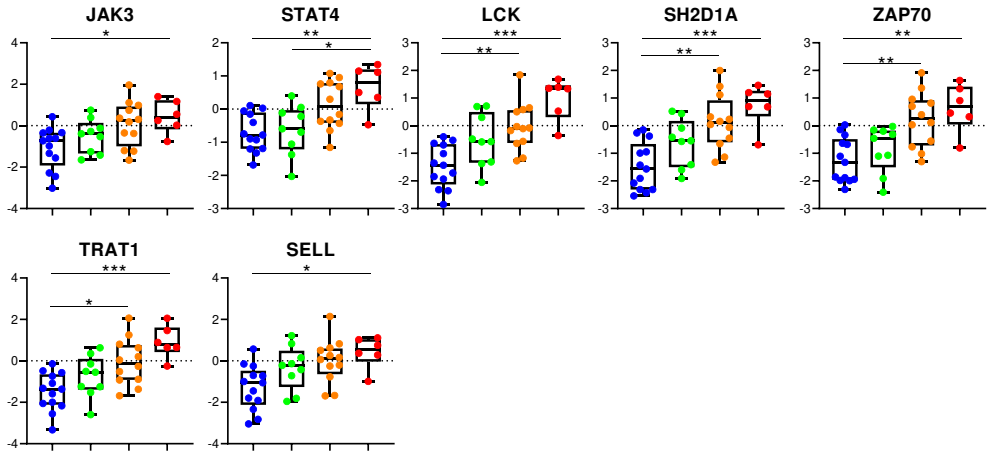
Adhesion and migration



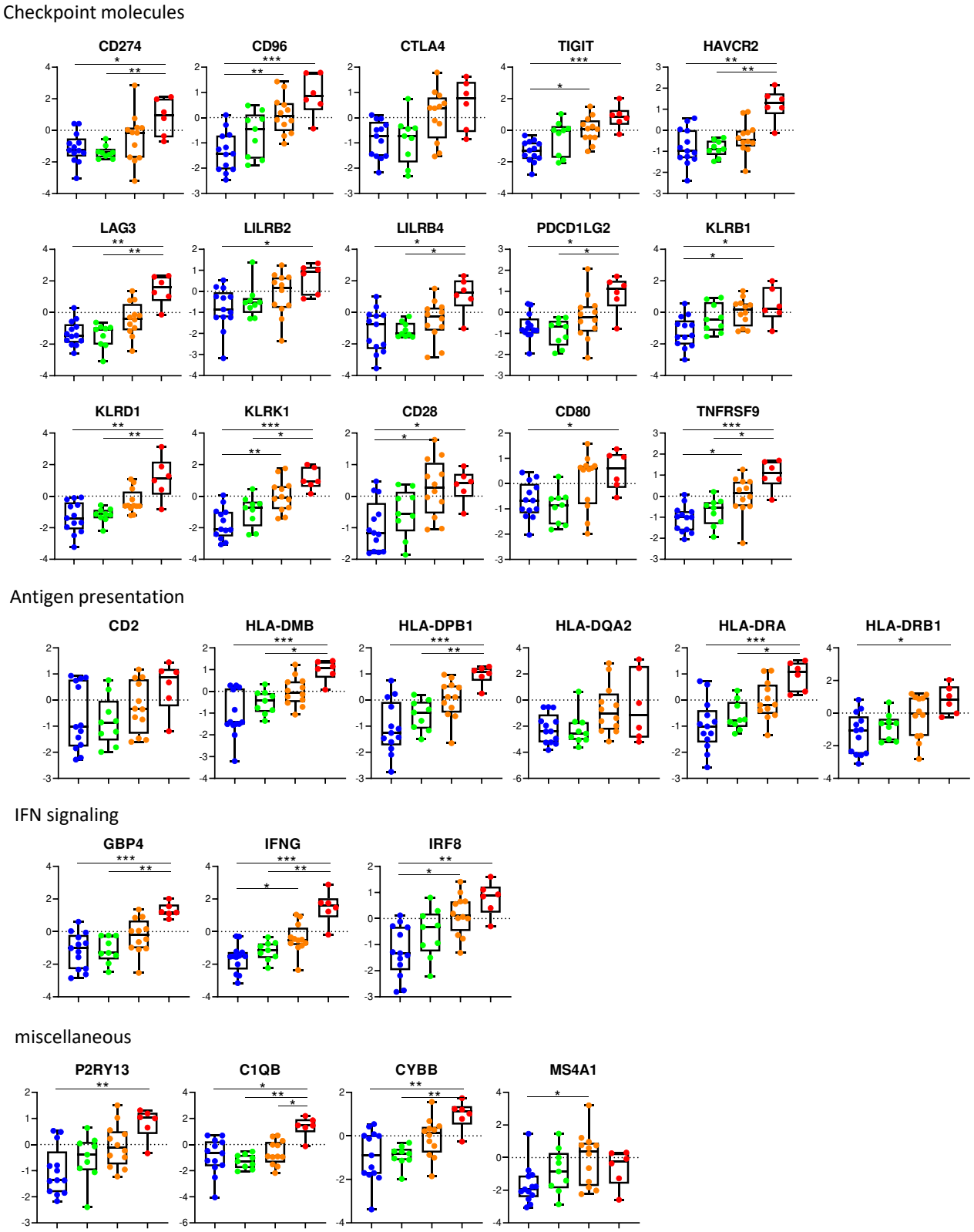
Cytokine and chemokine signaling



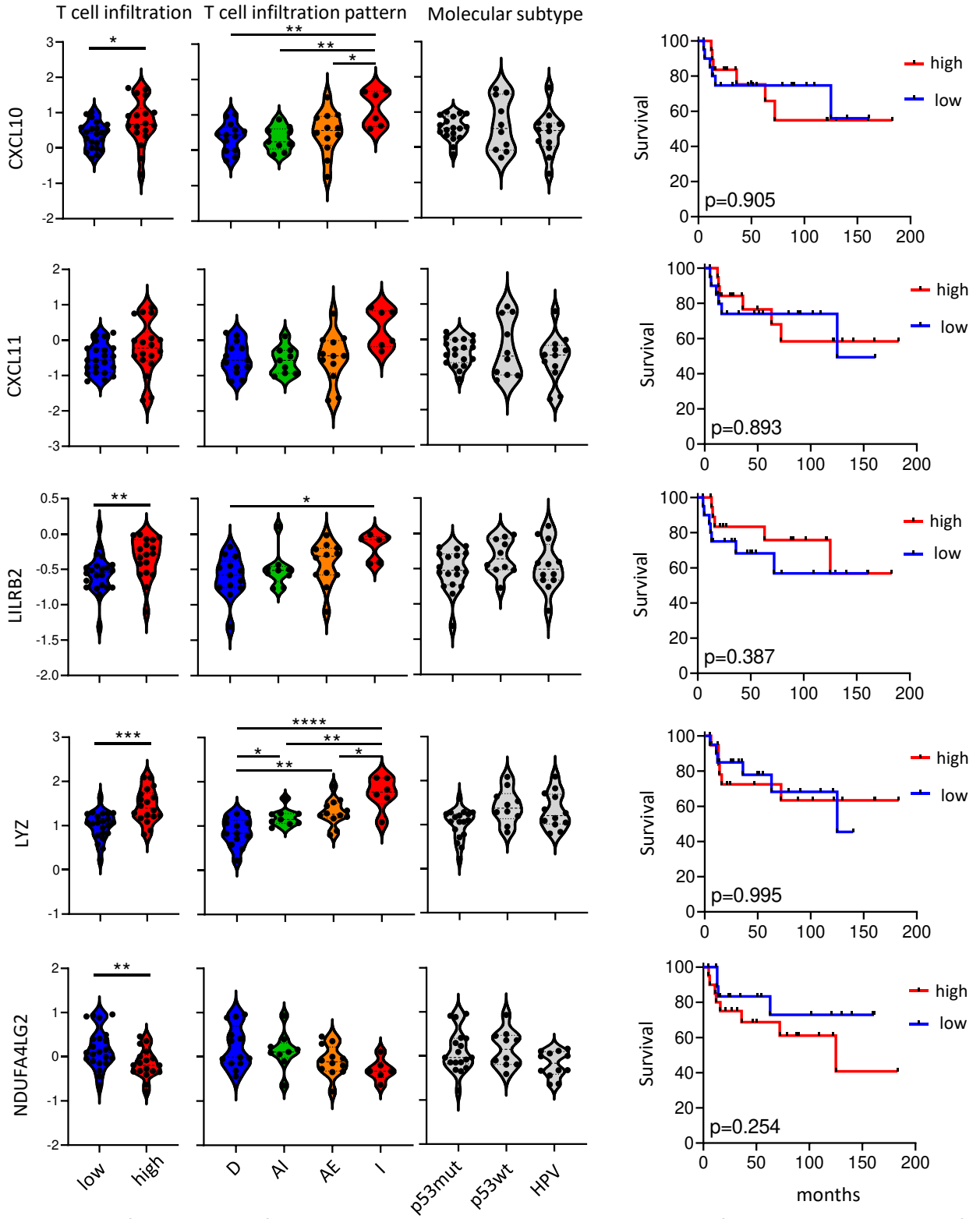
T cell signaling



Supplemental figure 5 (continued)



Supplemental figure 5. All differentially expressed single genes that were different between high and low infiltrated tumors were depicted in respect to their T cell infiltration pattern. Box plots displaying all significant differentially expressed genes (corrected *p*-value <0.05 and $\log^2$ fold change >1 or <-1) between high and low infiltrated tumors. Expression of the selected single genes in the heatmap were given as $\log^2$ transformed values relative to the average value of that particular gene. VSCC patients are categorized based on the T-cell pattern: deserted (blue), altered-immunosuppressed (green), altered-excluded (orange) and inflamed (red). **p*<0.05, ***p*<0.01, ****p*<0.001, and *****p*<0.0001.



Supplemental figure 6. High infiltrated tumors are associated with a higher VSCC-inflamed gene expression profile (GEP) score. Forty VSCC samples of all three molecular subtypes (HPVpos, HPVneg/p53wt and HPVneg/p53mut VSCC) were analyzed by the Nanostring IO360 Pancancer panel, and log10 transformed expression values for *CXCL10, CXCL11, LILRB2, LYZ* and *NDUFA4LG2* were determined. Graphs depict violin plots and Kaplan-Meier survival curves for the individual genes. Violin plots display the log10-transformed expression values for T cell infiltration (low versus high tumors), T cell infiltration pattern (deserted (D), altered-immunosuppressed (AI), altered-excluded (AE) and Inflamed (I) VSCC) and tumor molecular subtype (HPVneg/p53mut, HPVneg/p53wt and HPVpos VSCC). Kaplan-Meier survival curves showing the survival of 40 VSCC patients with high (red; above the median) and low (blue; below the median) expression of the indicated genes. **p*<0.05, ***p*<0.01, ****p*<0.001, and *****p*<0.0001.