

Supplementary Figures

Supplementary Table S1. Detailed classification of patients as HLA DEF or PRO based on immunohistochemistry of HLA class I, and LOH in chromosomes 6 and 15, as well as a summary of the techniques performed on each sample. IHC; Immunohistochemistry; LOH, Loss of Heterozygosity; HLA PRO, HLA-proficient; HLA DEF, HLA-deficient; TMB, Tumor Mutational Burden; CPR, complete pathological response.

Supplementary Figure S1. *Identification of HLA-proficient or HLA-deficient patients.*

A) Immunohistology analysis of HLA class I expression in lung adenocarcinoma and lung squamous cell carcinoma tissues. Loss of HLA class I expression is detected in the upper part, while the opposite is detected in the lower part. **B)** Illustration of LOH assay using SNP arrays and the Illumina Infinium SNP ImmunoChip. The genomic plots show patterns of B-allele frequency (BAF) (top of each panel) and corresponding log R ratios (bottom of each panel) for chromosomes 6 and 15. LOH, Loss of Heterozygosity; HLA-I, HLA class I. Tumors with loss of HLA class I expression and/or LOH were classified as HLA-deficient, while those without any defects on HLA were categorized as HLA-proficient tumors.

Supplementary Figure S2. *Combination of HLA class I status and TMB in a single parameter*

seemed to improve the prognostic value of HLA class I status. Kaplan-Meier survival curves of progression free survival (PFS, on the left) and overall survival (OS, on the right) for HLA-PRO+TMB high (n=5) and other cases (HLA-DEF+TMB high/low, n=17) patients (p=0.114 and p=0.255). An adjusted p value of <0.05 was considered statistically significant. HLA PRO, HLA-proficient; TMB, Tumor Mutational Burden; HR, Hazard Ratio; CI, confidence interval; NE, not estimable.

Supplementary Figure S3. *Similar differences in cell densities were observed considering both*

the tumor area and stroma. Density of total, tumor area and stroma T-cells (CD-3+), PD-1+ T-

cells (CD3+PD-1+), cytotoxic T-cells (CD3+CD8+), PD-L1+ T-cells (CD3+PD-L1+), macrophages (CD68+) and PD-L1+ macrophages (CD68+PD-L1+) (n=11 for Multiplex IF data). Nonparametric Mann–Whitney test was used for comparisons. An adjusted p value of <0.05 was considered statistically significant. HLA PRO, HLA-proficient; HLA DEF, HLA-deficient.

Supplementary Figure S4. DESeq2 analysis identified 4 differential-expressed genes (DEGs) that were upregulated in HLA-deficient tumors. Volcano plot of DEGs in pretreatment samples between HLA-proficient tumors (n=7) and HLA-deficient tumors (n=5). Red dots indicate upregulated genes, while blue dots indicate downregulated genes in HLA-deficient tumors. An adjusted p value of <0.05 was considered statistically significant. HLA PRO, HLA-proficient; HLA DEF, HLA-deficient; DEGs, Differentially Expressed Genes.

Supplementary Figure S5. Same treatment induced-changes both in the tumor area and stroma. A) Percentage of total, tumor and stroma PD-L1+ tumor cells (CK PD-L1+). Density of total, tumor and stroma **B)** PD-L1+ T-cells (CD3+PD-L1+); **C)** PD-L1+ cytotoxic T-cells (CD3+CD8+PD-L1+); **D)** PD-L1+ macrophages (CD68+PD-L1+); **E)** cytotoxic T-cells (CD3+CD8+); **F)** PD-1+ T-cells (CD3+PD-1+); and **G)** PD-1+ cytotoxic T-cells (CD3+CD8+PD-1+). n=11 and n=12 for pretreatment and posttreatment Multiplex IF data, respectively. Nonparametric Mann–Whitney test was used for comparisons. An adjusted p value of <0.05 was considered statistically significant. HLA PRO pre, HLA-proficient pretreatment; HLA PRO post, HLA-proficient posttreatment; HLA DEF pre, HLA-deficient pretreatment; HLA DEF post, HLA-deficient posttreatment.

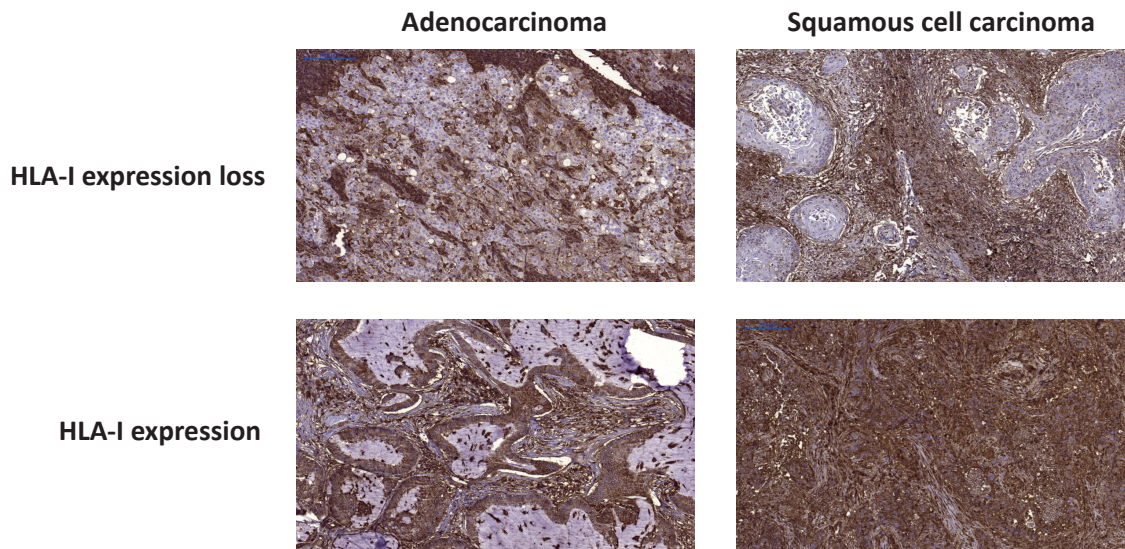
Supplementary Figure S6. HLA class II genes expression is higher in the HLA-deficient tumor with complete pathological response. HLA class II genes expression (HLA-DR, -DP and -DQ) in both CPR and non-CPR HLA-deficient tumors. Dots in purple express the corresponding gene. Marked in purple the spots in which at least one reading of the gene indicated in each case is detected. CPR, complete pathological response; HLA DEF, HLA-deficient; HLA-II, HLA class II.

Supplementary Table S1

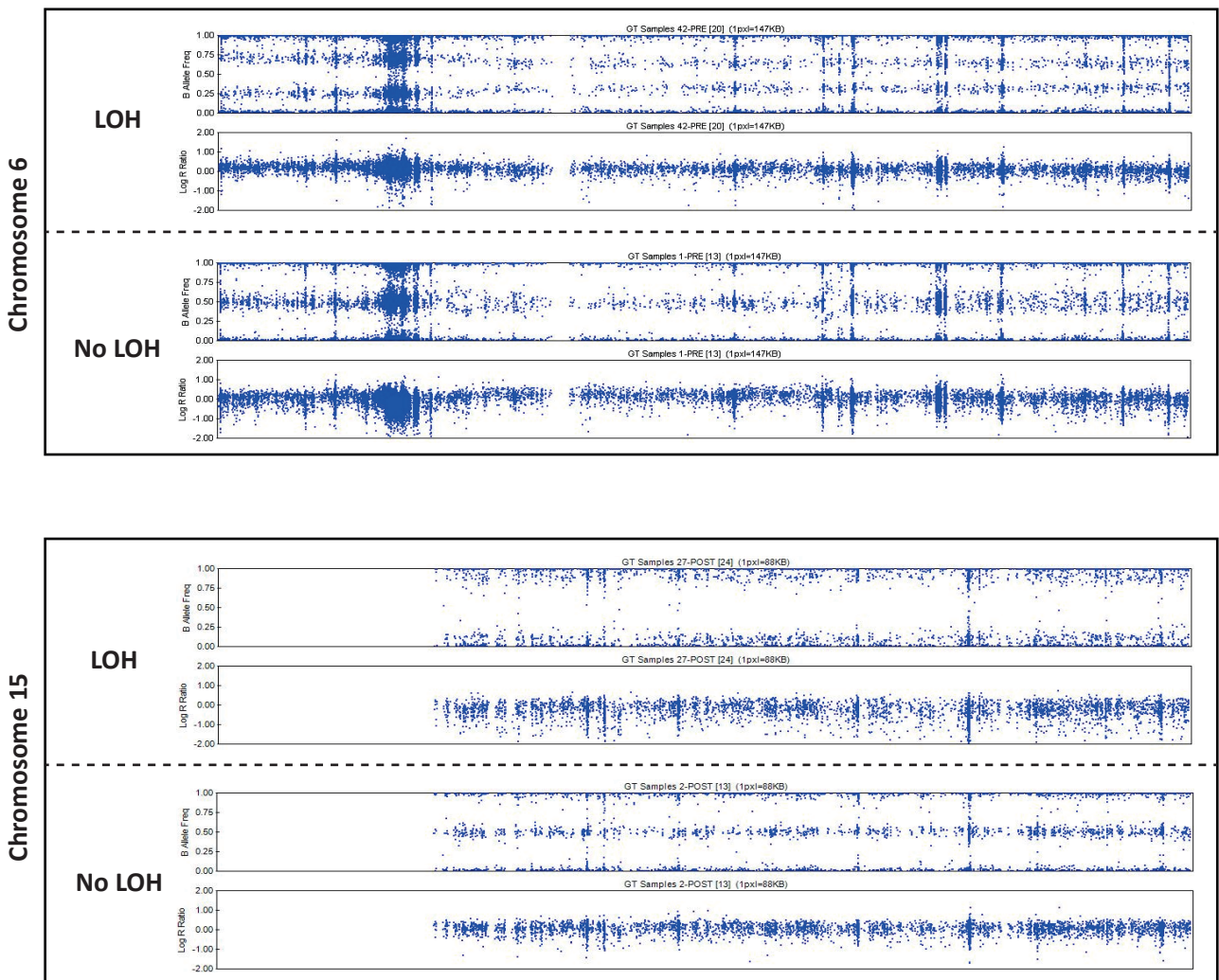
Patient	IHC	LOH Chr6	LOH Chr15	HLA Status	Pathological Response	Pretreatment					Posttreatment	
						TMB	PD-L1	TCR	Multiplex IF	RNASeq	Multiplex IF	Spatial Transcriptomics
1		No	No	PRO		x	x	x		x		
2		No	No	PRO	CPR	x	x	x	x	x	x	
3		No	No	PRO	CPR							
4		No	Yes	DEF	CPR	x	x	x	x			
5		Yes	Yes	DEF	Non-CPR	x	x	x	x		x	
6		No	No	PRO	CPR	x	x	x	x			
7		No	No	PRO	CPR	x	x	x	x	x	x	
8		No	No	PRO	CPR	x	x			x		
9	Negative	No	No	DEF	Non-CPR	x	x	x			x	
10		No	No	PRO	CPR	x	x	x				
11		Yes	No	DEF	Non-CPR	x	x	x	x	x		
12		Yes	No	DEF	CPR	x	x	x	x	x	x	x
13		Yes	Yes	DEF	Non-CPR	x	x	x	x	x	x	x
14		No	No	PRO	Non-CPR	x	x	x				
15	Negative	No	No	DEF	Non-CPR	x	x	x	x		x	
16		No	No	PRO		x	x	x		x		
17		Yes	Yes	DEF	CPR	x	x			x	x	
18		Yes	Yes	DEF	CPR	x	x	x	x		x	
19		Yes	No	DEF	CPR	x	x			x		
20	Positive	No	No	PRO	Non-CPR	x	x	x		x	x	
21	Positive	No	No	PRO	Non-CPR			x			x	
22	Positive	No	NA	PRO	Non-CPR	x	x		x		x	
23		No	No	PRO	CPR	x	x	x		x		
24		No	No	PRO		x	x	x				

Supplementary Figure S1

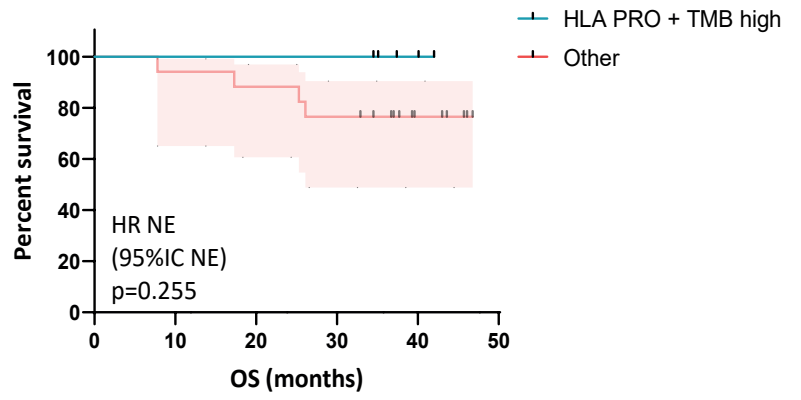
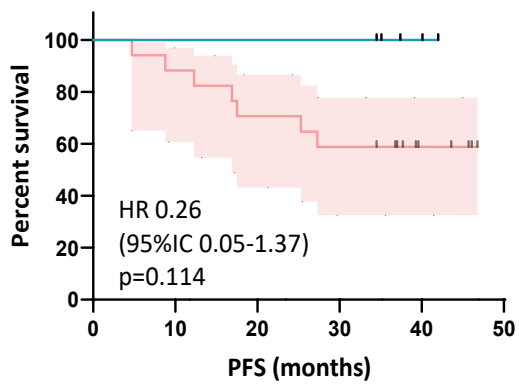
A)



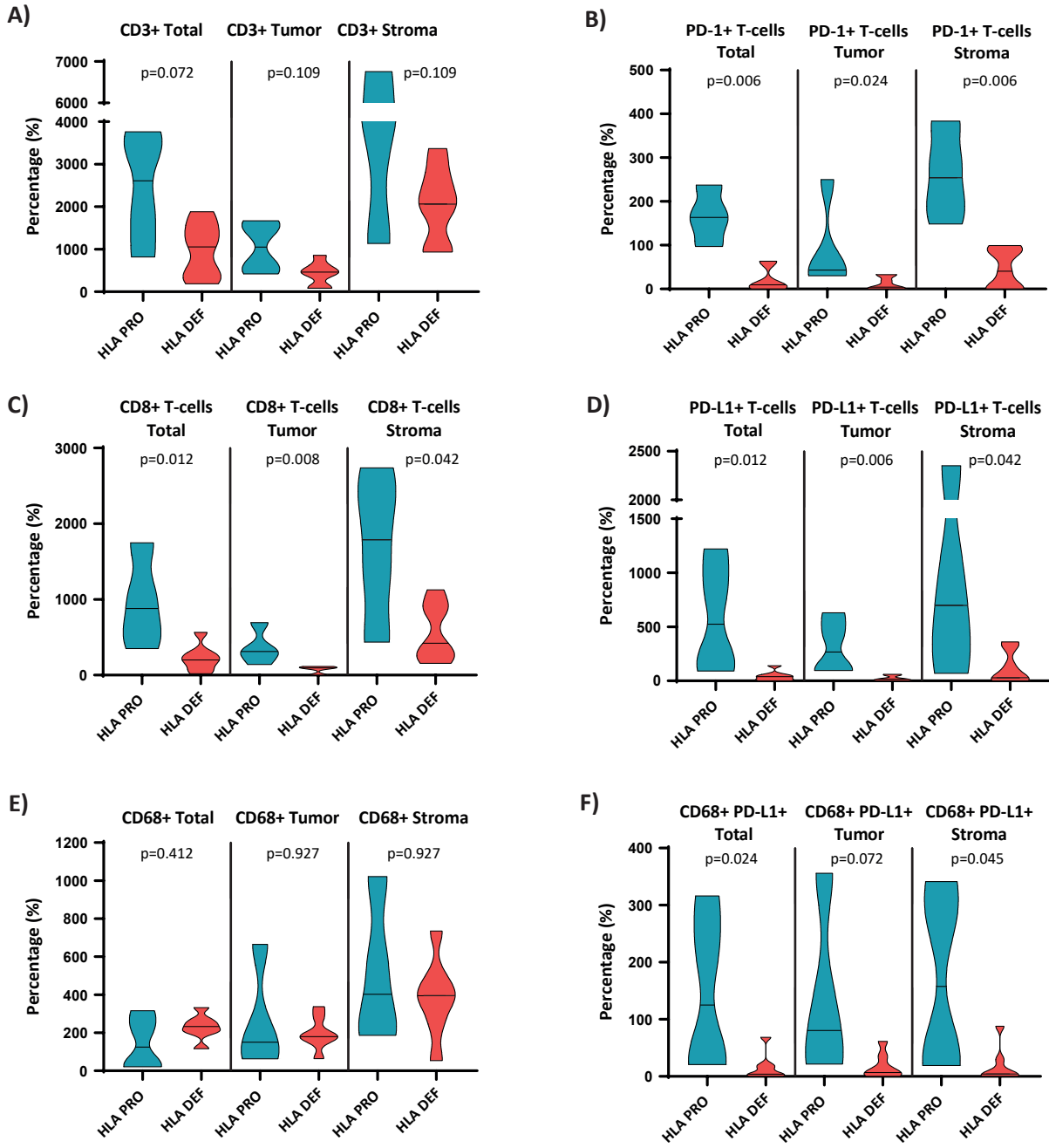
B)



Supplementary Figure S2

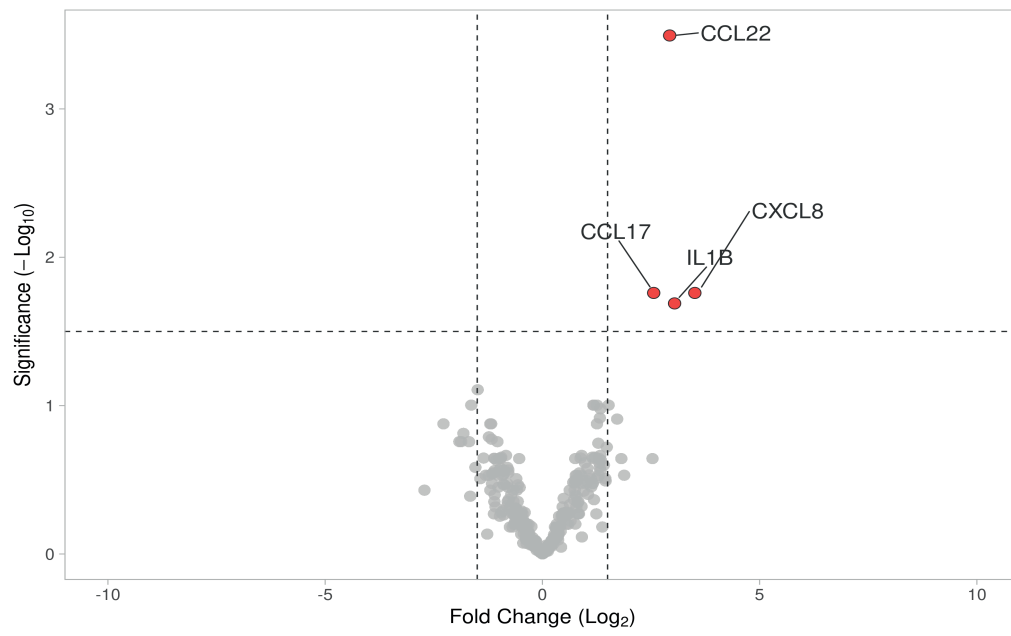


Supplementary Figure S3



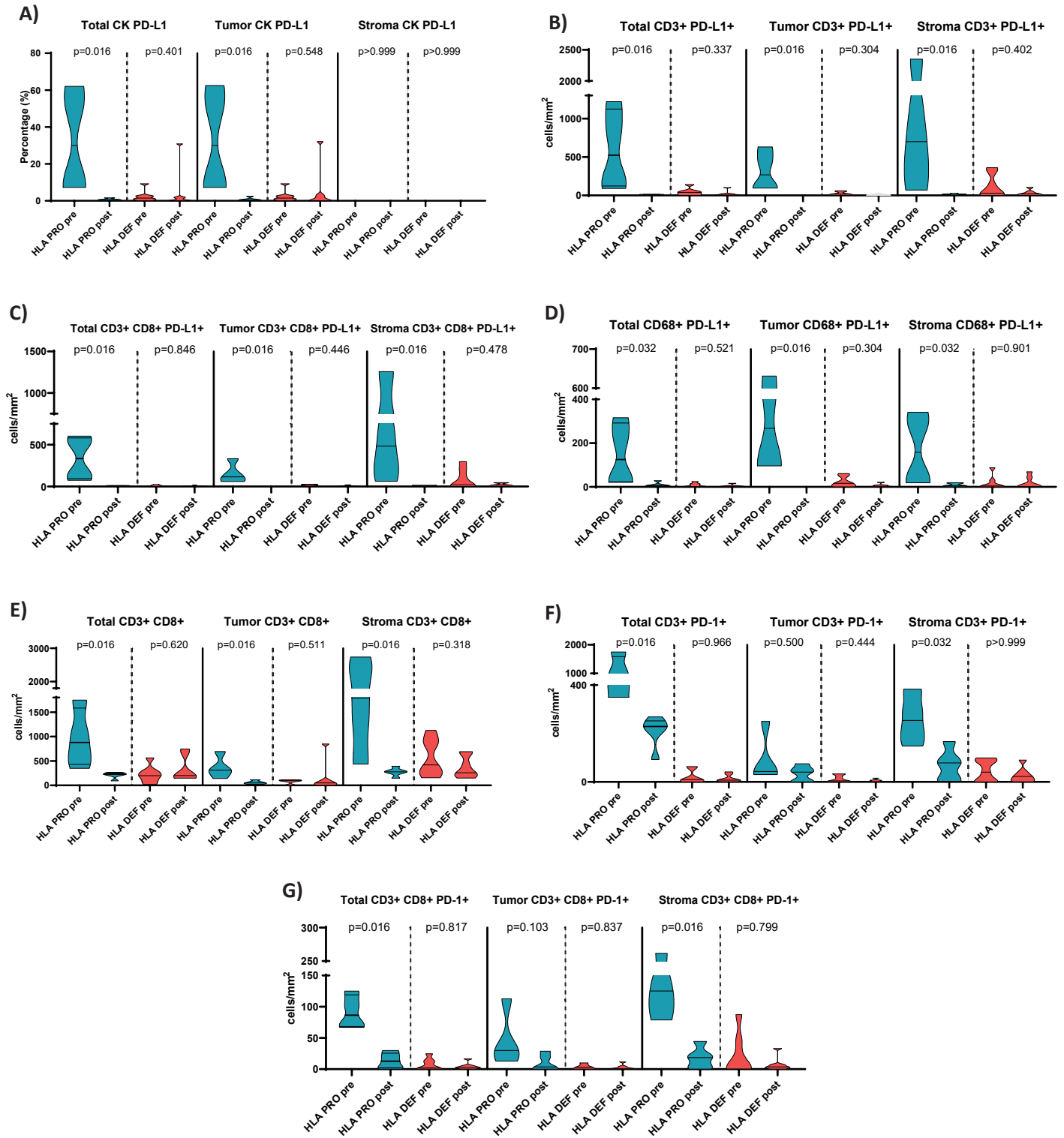
Supplementary Figure S4

UP-REGULATED GENES IN HLA DEF TUMORS



HLA DEF (n=5)
HLA PRO (n=7)

Supplementary Figure S5



Supplementary Figure S6

