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Supplementary materials

Tumor Microenvironment, HLA Class I and APM Expression in HPV-Negative Oral Squamous Cell Carcinoma

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Table S1. Concordant membranous HLA-I HC and β2-m expression with HLA-I surface expression in FFPE vs. frozen OSCC lesions.

cases	FFPE specimen		fresh frozen specimen
	HC: IRS	β2-m: IRS	HLA-A/B/C: IRS
high membranous HLA expression			
Case # 47	9	6	8
Case # 53	9	6	6
Case # 64	8	6	8
Case # 100	8	8	8
Case # 263	8	8	8
low / negative membranous HLA expression			
Case # 136	0	0	1
Case # 148	0	1	0
Case # 151	0	1	0
Case # 182	0	2	3
Case # 202	0	2	2

IRS: immune reactive score.Staining patterns of HLA-I HC and β2-m on FFPE specimen with known HLA expression status (concordant membranous positivity) were compared to HLA-I surface expression on fresh frozen tissues using the W6/32 antibody recognizing the trimeric HLA-I complex.

Table S2. Primers used for analyses of HLA-I and APM component expression.

primer	sequence	Tm
TAP1 FWD*	GGA ATC TCT GGC AAA GTC CA	60° C
TAP1 REV**	TGG GTG AAC TGC ATC TGG TA	60° C
TAP2 FWD	CCA AGA CGT CTC CTT TGC AT	60° C
TAP2 REV	TTC ATC CAG CAG CAC CTG TC	60° C
β2-m FWD	CTC GCG CTA CTC TCT CTT	60° C
β2-m REV	AAG ACC AGT CCT TGC TGA	60° C
HLA-A, -B, -C FWD	GCC TAC CAC GGC AAG GAT TAC	60° C
HLA-A, -B, -C REV	GGT GGC CTC ATG GTC AGA GA	60° C
LMP2 FWD	TGC TGC ATC CAC ATA ACC AT	60° C
LMP2 REV	TGT GCA CTC TCT GGT TCA GC	60° C
LMP10 FWD	GGG CTT CTC CTT CGA GAA CT	60° C
LMP10 REV	CAG CCC CAC AGC AGT AGA TT	60° C

*fwd = forward; **rev = reverse.

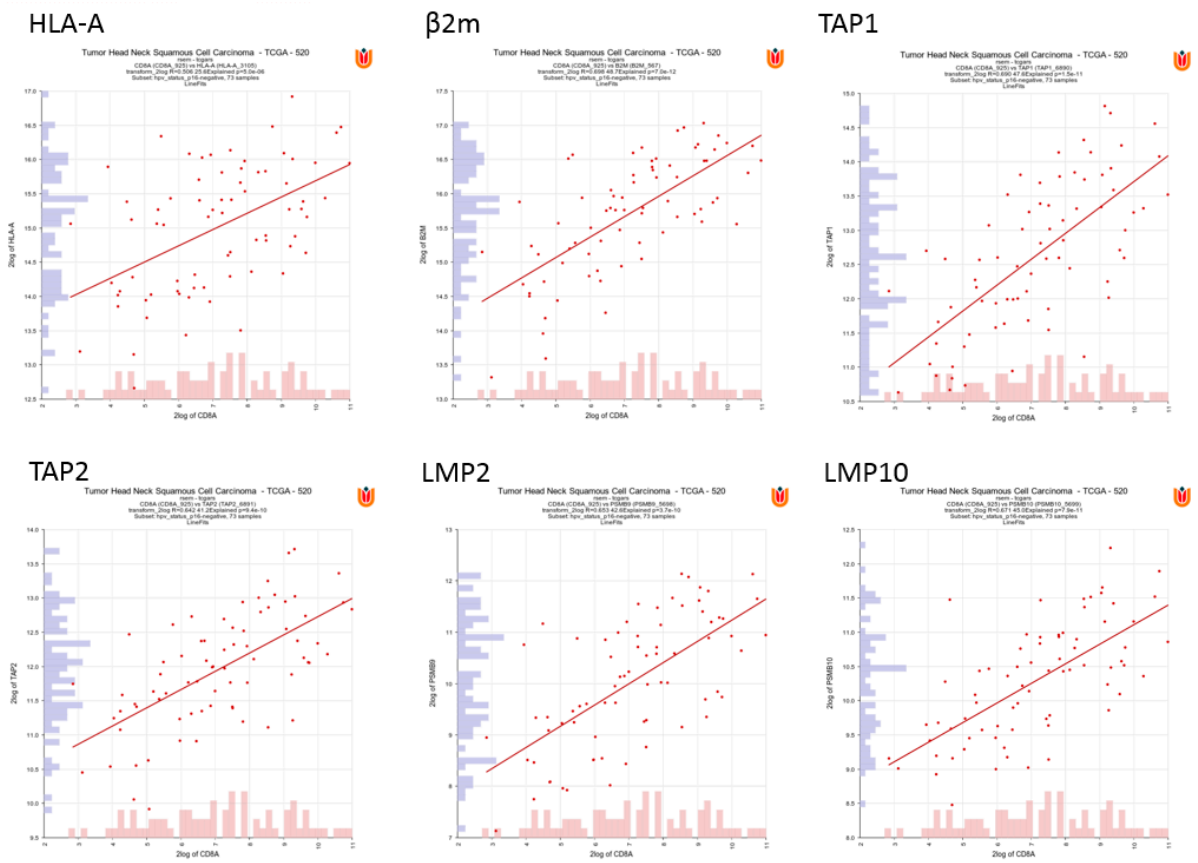
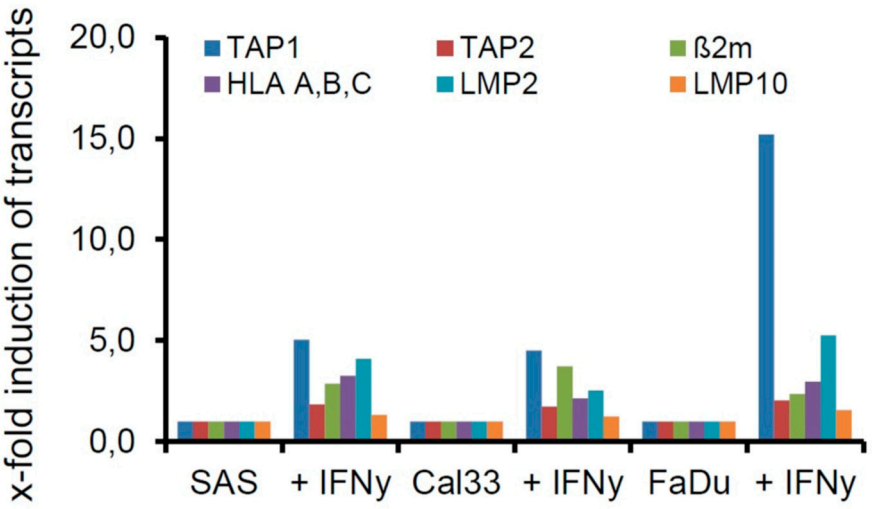


Figure S1. ***In silico*** **correlation of CD8 mRNA expression with HLA-A, β2-m and APM components.**mRNA expression of CD8 was correlated with the mRNA expression of HLA-A, β2-m, TAP1, TAP2, LMP2 and LMP10 in tumor material of HVP- HNSCC patients of the TCGA cohort (n=73). The histograms show the distribution of the patients as a xy-plot with the histogram and linear fit options. The p-value is given in the header of every histogram.

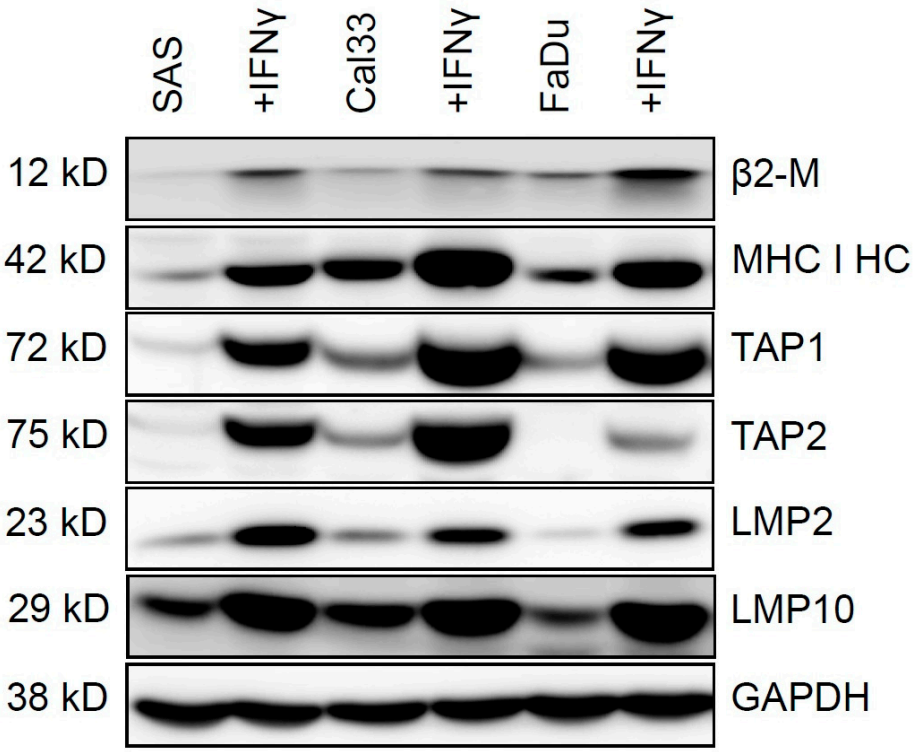


Ct value of APM component transcription

	SAS	SAS+IFNy	Cal33	Cal33+IFNy	FaDu	FaDu+IFNy
ß2m	8	9	9	9	8	9
HLA A,B,C	5	7	7	8	7	8
TAP1	6	7	7	8	6	8
TAP2	5	5	5	6	4	5
LMP2	7	8	8	9	7	9
LMP10	5	5	5	6	4	6

Ct value	10-12	12-14	14-16	16-18	18-20	20-22	22-24	24-26	>26
	9	8	7	6	5	4	3	2	1

(A)



MHC class I surface expression as mean specific fluorescence intensity

Cell line	MFI (constitutive)	average deviation	MFI (IFN-γ)	average deviation
SAS	2.2	± 0.4	3.8	± 0.4
Cal33	3.5	± 0.8	6.9	± 1.7
FaDu	3.2	± 0.9	10.9	± 1.9

* mean of three independent replicates

(B)

Figure S2. Constitutive and IFN-γ mediated upregulation of HLA-I APM component expression in HNSCC cell lines.A Effect of IFN- treatment on mRNA expression of HNSCC cell lines.A: HNSCC cell lines were left untreated or treated for 48 h with IFN-γ (200 U/ml) as described in Materials and Methods before qPCR was performed. The data represent the relative mRNA expression levels of the different APM components normalized to that of the housekeeping gene ALAS1. The transcription levels of untreated HNSCC cell lines were set to one, and respective expression levels of IFN-γ-treated cells were calculated. Ct values of HLA-/APM component transcription and their classification are given. B Effect of IFN- on protein expression of HNSCC cell lines.B: Western blot analysis using 50 μg protein lysate/lane of three different HNSCC cell lines

(SAS, Cal33, FaDu) left untreated or treated for 48 h with IFN-γ (200 U/ml) was performed as described in Materials and Methods. The detection of GAPDH served as loading control. HLA-I surface expression of untreated and IFN-γ-treated HNSCC cell lines was performed as described and data expressed as MFI.