SI figure legends

Table S1. Phenotypes of the melanoma TIL lines included in the study as analyzed by flow cytometry.

Figure S1. Tumor specificity of the TIL lines included in the study. TILs derived from stage IV melanoma patients were evaluated by IFNγ ELISpot for the ability to recognize autologous tumor cells in a HLA-restricted fashion. Targets were pre-treated or not with anti-HLA class I and anti-HLA class II mAbs to assess T cell receptor involvement. *$.05<p<.01$; **$.01<p<.001$;***$p<.001$ (Student's t-test) with respect to recognition of the same target in the absence of anti-HLA class I or anti-HLA class II mAbs.

Figure S2. Effect of low pH culture conditions on the viability and proliferation of different types of T cells. (A) PBMCs from healthy donors (N=5) or short-term (14 days) MelanA/MART-1-specific T cell cultures (N=5) generated from melanoma patients' PBMCs by in vitro peptide sensitization and 60 IU/ml IL2 were cultured for 3 days in complete medium at the indicated pH values (*horizontal line*). Cells were then evaluated for viability by a PI/AnnexinV assay. (B) Cells from the same cases were also tested for their proliferative activity in the presence of CD3/CD28 T Cell Expander beads (T cells:beads ratio = 20:1) using the intracellular fluorescent dye CFSE. Data represent the mean±SD of the tested samples. * $p<.05$; **$.01<p<.001$ (Student's t-test).

Figure S3. Down-modulation of the ζchain and CD3 expression in TILs cultured at low pH. Melanoma TILs were cultured for 3 days in complete medium at the

indicated pH values and then evaluated for their expression of ζchain and CD3 by flow cytometry. TILs restored to pH 7.4 for 24h before the assay were also included in the analysis (6.5/7.4). *Bars*, box-and-whisker diagrams (N=5). *Horizontal line*, median. *MFI*, mean fluorescence intensity.

Figure S4. Short-term culture of melanoma cell lines at low pH does not affect specific immune recognition by autologous TILs. (A) Melanoma cell lines were cultured in complete medium for 24h at the indicated pH values and then tested for their expression of HLA class I and MelanA/MART-1 antigens by flow cytometry. *MFI*, mean fluorescence intensity. In the *right panel*, histogram plots of two representative cases are shown (#6348, #4189). (B) TILs from stage IV melanoma patients were evaluated for the ability to secrete IFNγ (as assessed by Elispot assay) in the presence of autologous tumor cells previously cultured either at physiological pH (7.4) or at low (6.5) pH conditions. Targets were pre-treated or not with anti-HLA class I mAb to assess T cell receptor involvement. *.05<p<.01;**.01<p<.001 (Student's t-test) with respect to recognition of the same target in the absence of anti-HLA class I mAb.

Figure S5. Low pH impairs *in vitro* restimulation of *in vivo*-activated T cells. Mice were immunized by one i.d. injection of DC-STEAP$_{186\text{-}193}$. One week later, splenocytes from vaccinated animals were restimulated *in vitro* in the presence of STEAP$_{186\text{-}193}$. Five-day blasts were either assessed for viability by trypan blue exclusion (A) or for IFNγ production against STEAP by flow cytometry (B). Data are reported as the mean percentage±SD of trypan blue^{+} cells (A) or as the number of IFNγ^{+} cells within the CD8^{+} T cell population (B). Data are representative of at least

two independent experiments. **.001<p<.01 (Student's t-test).

Figure S6. PPI treatment increases the level of activated ERK in TIL. Mice were challenged s.c. with B16-OVA cells, and 12 days later, they were infused with activated and CFSE-labelled OTI cells (3 x 10^7). One day after, animals were treated i.p. with esomeprazole (PPI) or PBS (PBS), and after an additional day, tumor cell suspensions were evaluated by flow cytometry for ERK activation (pERK) in response to PMA (200 ng/ml). Representative histograms of pERK expression on unstimulated (gray) or PMA stimulated (200 ng/ml) $CD44^+CFSE^+$ cells from mice treated with PPI (red; A) or PBS (blue; B).

Figure S7. PPI treatment favors high-functional avidity of $CD8^+$ T cells. (A) Schematic representation of the experiment. Mice were adoptively transferred with 12×10^6 naïve OTI cells. One day later, they were challenged s.c. with B16-OVA cells. Seven days later (day 8), mice were vaccinated with DC-$OVA_{257-264}$, and after 3 additional days (day 11) mice were treated i.p. with PBS (*black squares*) or PPI (*open circles*). Animals were sacrificed 24h after the last treatment. (B) $CD8^+$ T cells (OTI and endogenous) were magnetic-bead sorted from tumor cell suspensions and assessed for intracellular cytokine production in the presence of RMA cells either pulsed or not with increasing concentrations of $OVA_{257-264}$ peptide. Data are representative of two independent experiments.

Figure S8. Time dependent effect of the combination between adoptive immunotherapy and PPI on melanoma growth. Mice bearing an 8-day old B16-OVA melanoma (A-F) were treated with PBS (A) or OTI cells (6×10^6/mouse) alone

(B) or in combination with a single infusion of PPI at day 8 (C), 11 (D), 14 (E) or 17 (F). Tumor growth was monitored for individual mice (5 animals/group). See Materials and Methods sections for more experimental details.

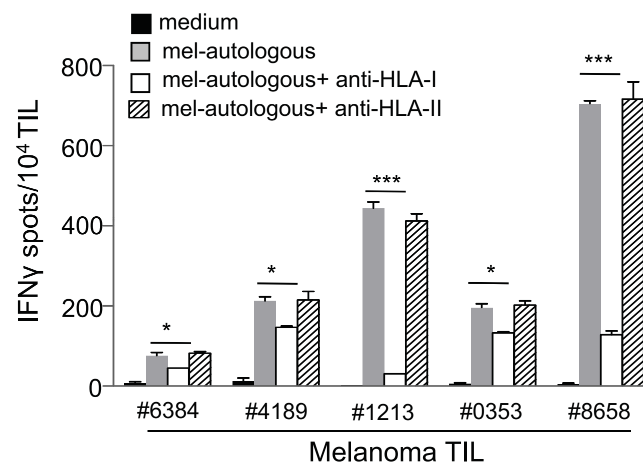
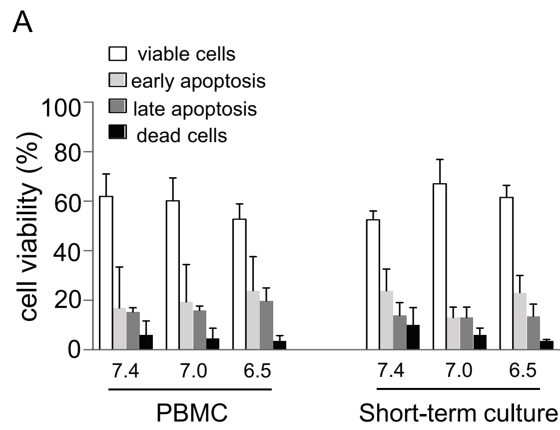
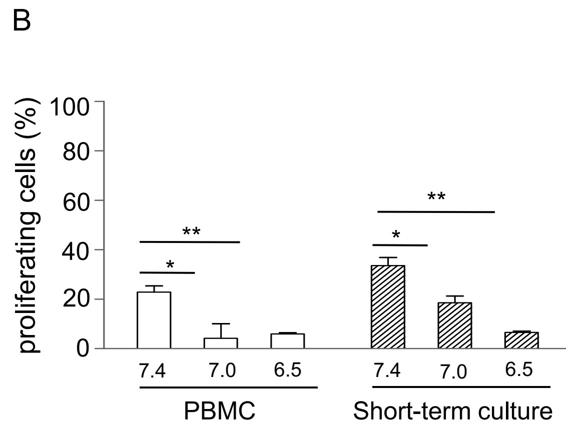


Figure 1-supplemental data





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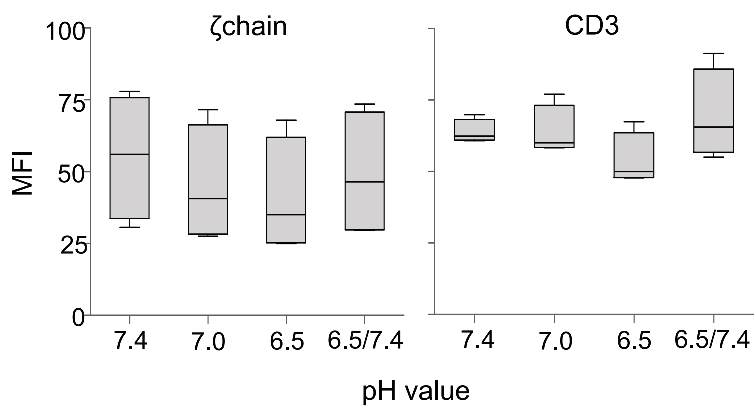

Figure 3-supplemental data

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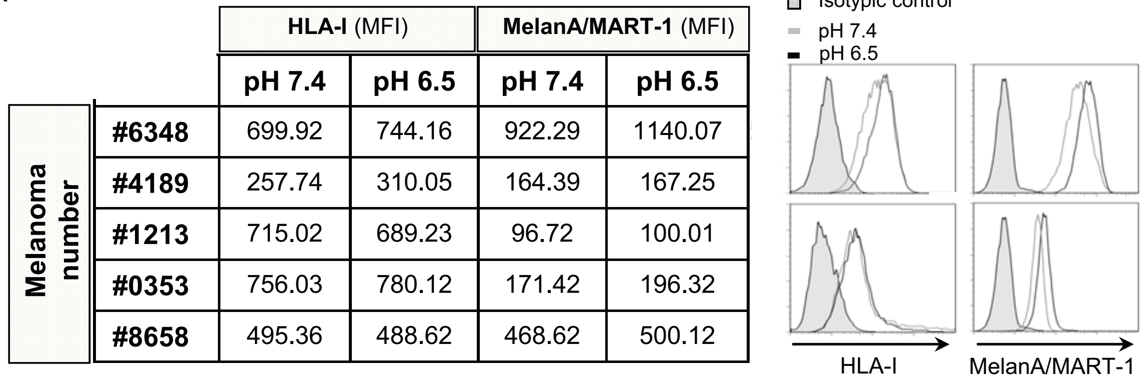
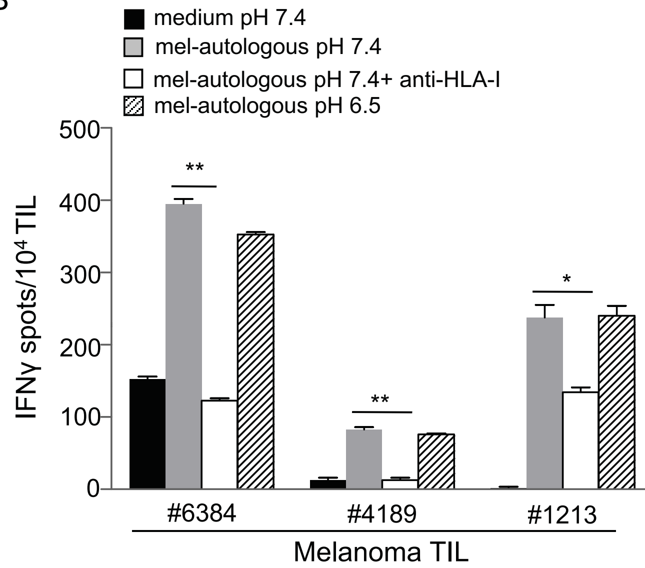


Melanoma number	HLA-I (MFI)		MelanA/MART-1 (MFI)	
	pH 7.4	pH 6.5	pH 7.4	pH 6.5
#6348	699.92	744.16	922.29	1140.07
#4189	257.74	310.05	164.39	167.25
#1213	715.02	689.23	96.72	100.01
#0353	756.03	780.12	171.42	196.32
#8658	495.36	488.62	468.62	500.12

B



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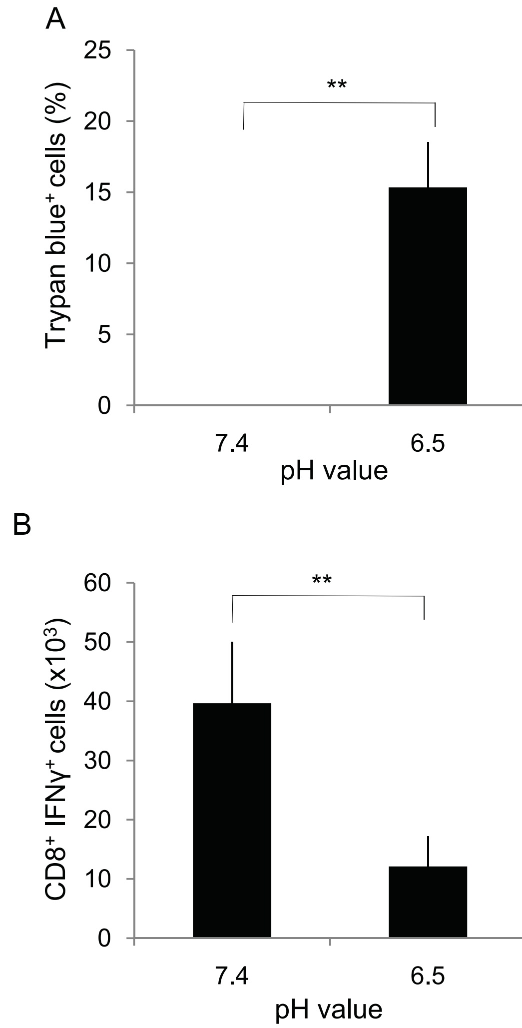

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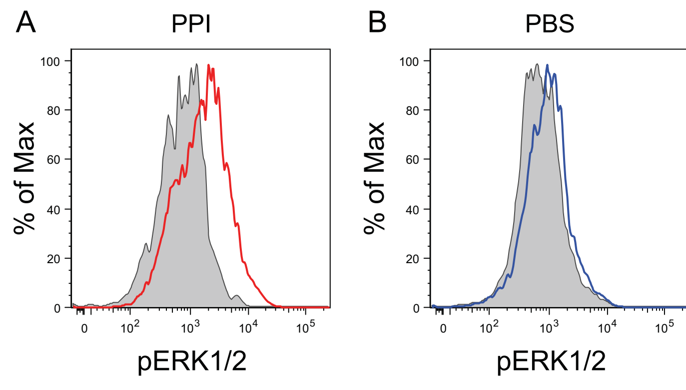
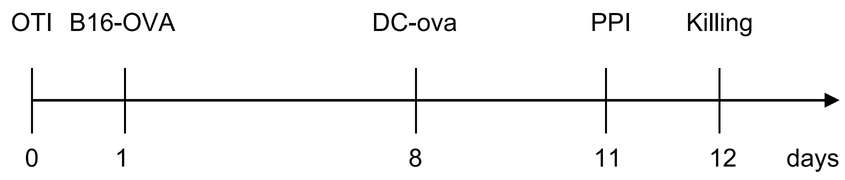
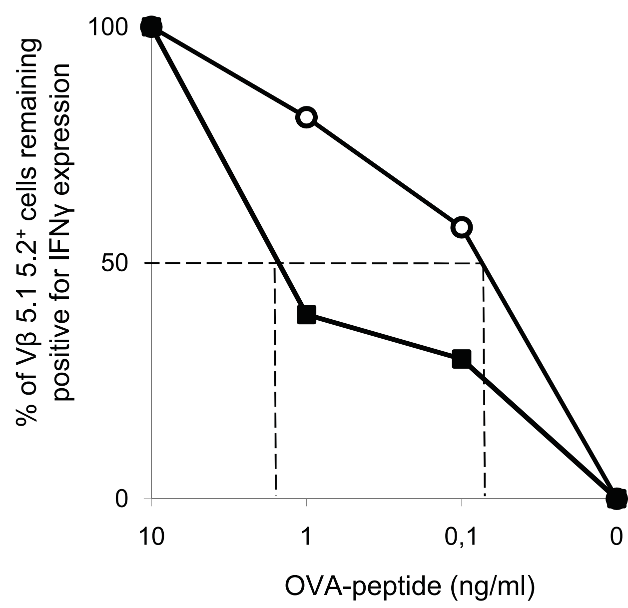


Figure 6-supplemental data

A



B



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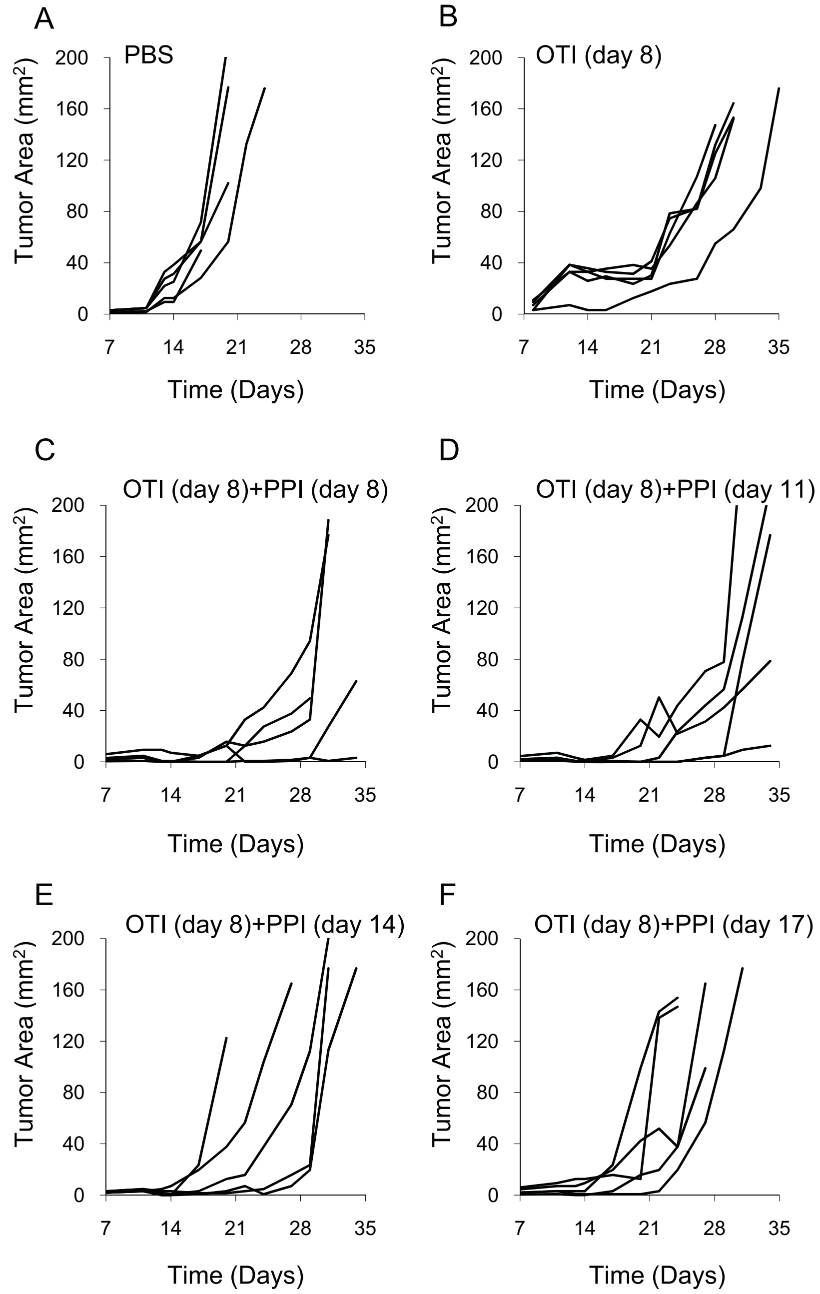

Figure 8-supplemental data

TIL number		Percentage		
		CD8	CD4	CD56
	#6348	87.58	9.51	2.91
	#4189	82.86	16.80	0.34
	#1213	96.72	1.23	2.05
	#0353	71.98	19.83	8.19
	#8658	85	10.8	4.2

Table 1-supplemental data