Supplementary results

Phenotypic analysis of cutaneous and ocular melanoma cell lines

The characterization of the immune profile of metastatic cutaneous (# 1061, 1067, 2710, 49318, 4478D, 0342, 7 and 25368 mel) and primary or metastatic ocular (#2130, 4330, 4022, 1141, 37165, 48409 and 15765 mel) melanoma cell lines has been carried out by analyzing the expression of MHC class I and II molecules and NKG2D ligands (NKG2DLs; MICA/B, ULBP1-4). MHC class I expression was detected in all the melanoma cell lines (though to a different extent, 2-8 of MRFI), while low levels or lack of MHC class II and NKG2DLs, with the exception for the 4478D mel. While ULBP-3 and -4 were detected at high levels in the 1061 and the 2130 mel lines (representative results are shown in Table 1S of Supplementary data).

The presence of a panel of TAAs (gp100, MART-1, NY-ESO-1, SVV, SOX-2, COA-1, IL-13Rα2), known to be expressed by cutaneous melanoma was evaluated by IF and cytofluorimetric analysis. These molecules were detected (Table 2S), though with heterogeneous levels, in all the cell lines. IL13-Rα2 was expressed at high level (MRFI 29) on the cell surface only by primary ocular melanoma cells. However, cytoplasmic expression of this molecule was commonly found in all the melanoma lines. These results indicate that similar immune profile was observed in cutaneous and ocular melanoma lines and, furthermore, in ocular (N=10) and cutaneous (N=10) melanoma tissues (data not shown). We conclude that ocular melanoma cells can display a potential immunogenic role similar to that of cutaneous melanoma counterparts.

Table 1S. Expression of MHC molecules and NKG2DLs by cutaneous and ocular melanoma lines.

Cell line		Antigen*							
		MHC I	MHC II	MICA	MICB	ULBP-1	ULBP-2	ULBP-3	ULBP-4
Cutaneous Melanoma	1061 mel	**6**	**4**	**2**	1	1	**2**	**6**	**5**
	4478D mel	**3**	**20**	**2**	1	1	**2**	1	**2**
	JOFR-IA mel	**8**	**2**	1	1	1	1	1	**2**
Ocular Melanoma	2130 mel	**2**	**2**	**2**	**2**	**2**	**2**	**8**	**4**
	15765 mel	**9**	**2**	1	1	**2**	**2**	**2**	**2**

*: The expression of MHC and of NKG2DL molecules by the melanoma lines was evaluated by IF and cytofluorimetric analysis. Data are expressed as MRFI, the ratio between the mean of fluorescence intensity of cells stained with the defined mAbs and the one of the negative control. Significant MRFI values (≥ 2) are indicated in bold. The 1061 mel and 4478D mel cell lines have been isolated from metastatic cutaneous melanoma patients; the 2130 mel and 15765 mel have been isolate from one primary and one metastatic ocular melanoma patients, respectively. n.d. : not done.

Table 2S. Expression of TAAs by cutaneous and ocular melanoma cell lines.

Cell line		Antigen*							
		Gp100	Melan-A/ MART-1	SVV-1	NY-ESO1	SOX2	COA-1	IL13-Rα2[#]	IL13-Rα2[§]
Cutaneous Melanoma	1061 mel	**55**	**2**	**16**	**4**	**8**	**2**	**2**	**8**
	4478D mel	**62**	**5**	**7**	**3**	**30**	n.d.	1	**11**
	JOFR-IA mel	**4**	1	**15**	1	**18**	n.d.	1	1
Ocular Melanoma	2130 mel	**23**	**2**	**5**	**4**	**2**	**2**	**29**	**3**
	15765 mel	**341**	**10**	**72**	**8**	**14**	**5**	n.d.	**39**

*: The expression of the indicated TAAs by the melanoma lines was evaluated by IF and cytofluorimetric analysis. Data are expressed as MRFI, the ratio between the mean of fluorescence intensity of cells stained with the defined mAbs and the one of the negative control. Significant MRFI values (≥ 2) are indicated in bold. The IL13-Rα2 was detected either as surface expression (#) or at the cytoplasmic level onto permeabilized cells (§) (see Materials and methods). The 1061 mel and 4478D mel cell lines have been isolated from metastatic cutaneous melanoma patients; the 2130 mel and 15765 mel have been isolated from one primary and one metastatic ocular melanoma patients, respectively. n.d. : not done.

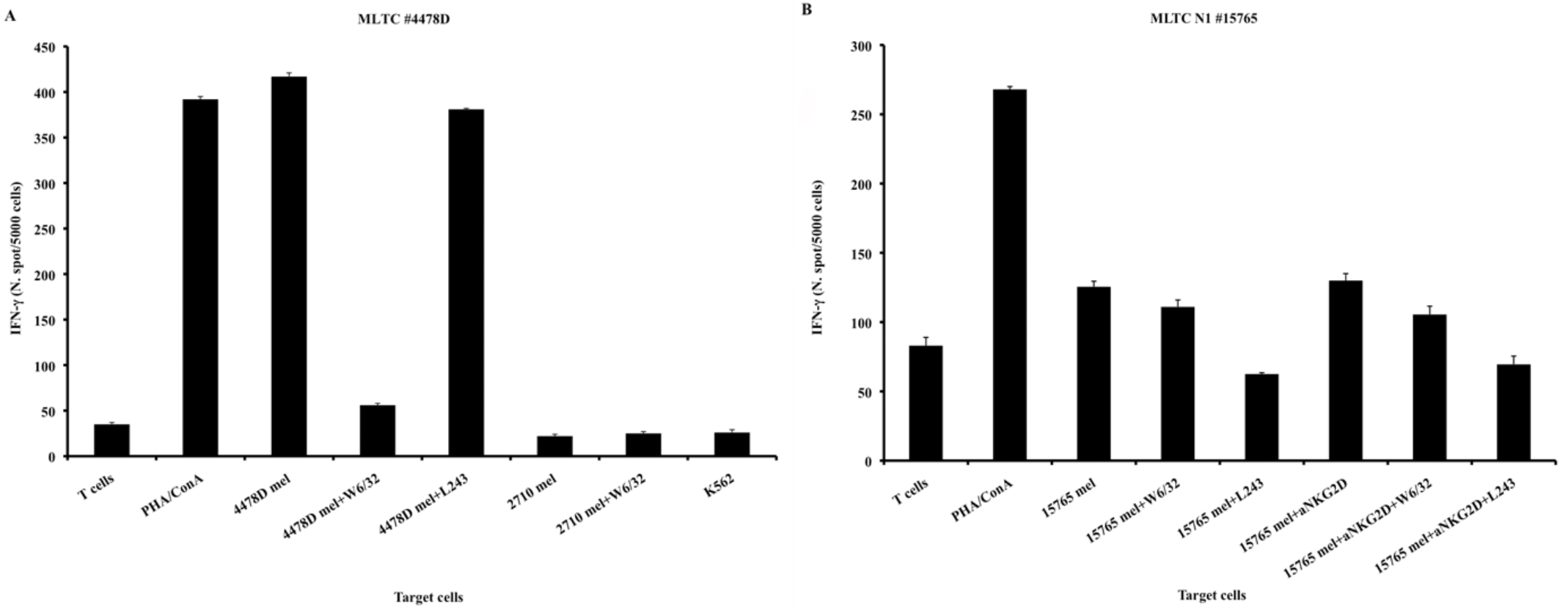

Figure 1S. The MLTC protocol can efficiently isolate both CD4+ and CD8+ anti-tumor T lymphocytes from the peripheral blood of cutaneous and ocular melanoma patients. **PBMCs from the metastatic cutaneous (#4478D, Panel A) and ocular (#15765 panel B) melanoma patients were stimulated in vitro with irradiated autologous tumor cells at 1:5 tumor cell lymphocyte ratio in the presence of IL-2+IL-15. Following 2 weekly stimulations the tumor recognition by these T cells was assessed by measuring IFN-γ secretion (ELISPOT assay) after the incubation with the autologous melanoma cells (# 4478D or 15765 mel, Panels A and B, respectively) pre-treated or not with anti-HLA class I (W6/32) or anti-HLA class II (L243) mAbs. Moreover, T cells were also pre-incubated or not with the anti-NKG2D mAb. PHA/Con-A were used as positive control for IFN-γ release. Statistical analysis of differences between means of IFN-γ released by T cells was done by two-tailed t test ($p \leq 0,01$).**

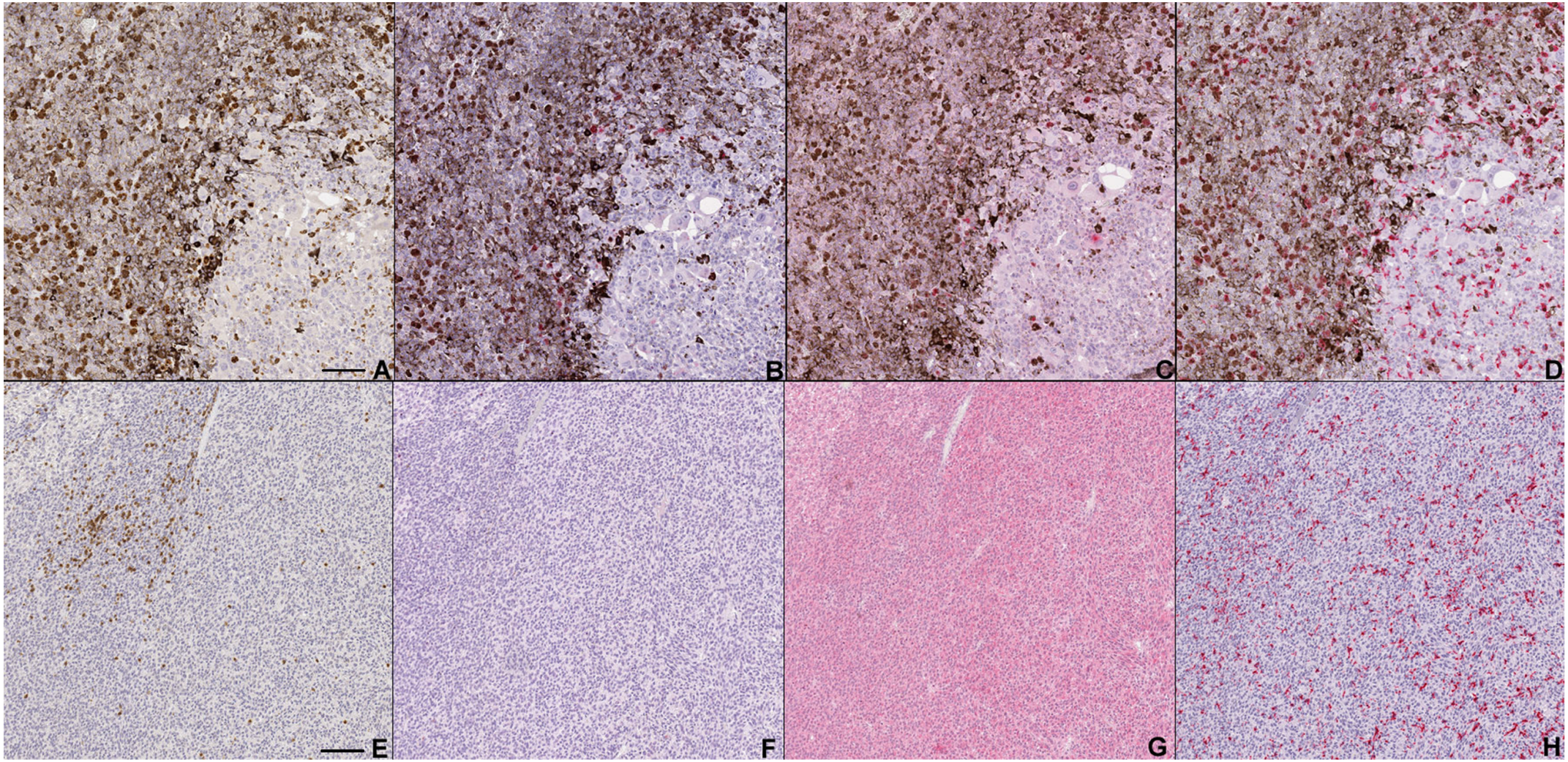

Figure 2S. IHC analysis of the immune infiltrate in ocular melanoma tissues. **Representative staining of CD3 (brown staining), GATA3 (red), T-Bet (red) and CD163 (red) in two primary ocular melanoma tissues (# 050306324 - 0503161250). The scale bars represent 100 µm. Magnification 10X.**

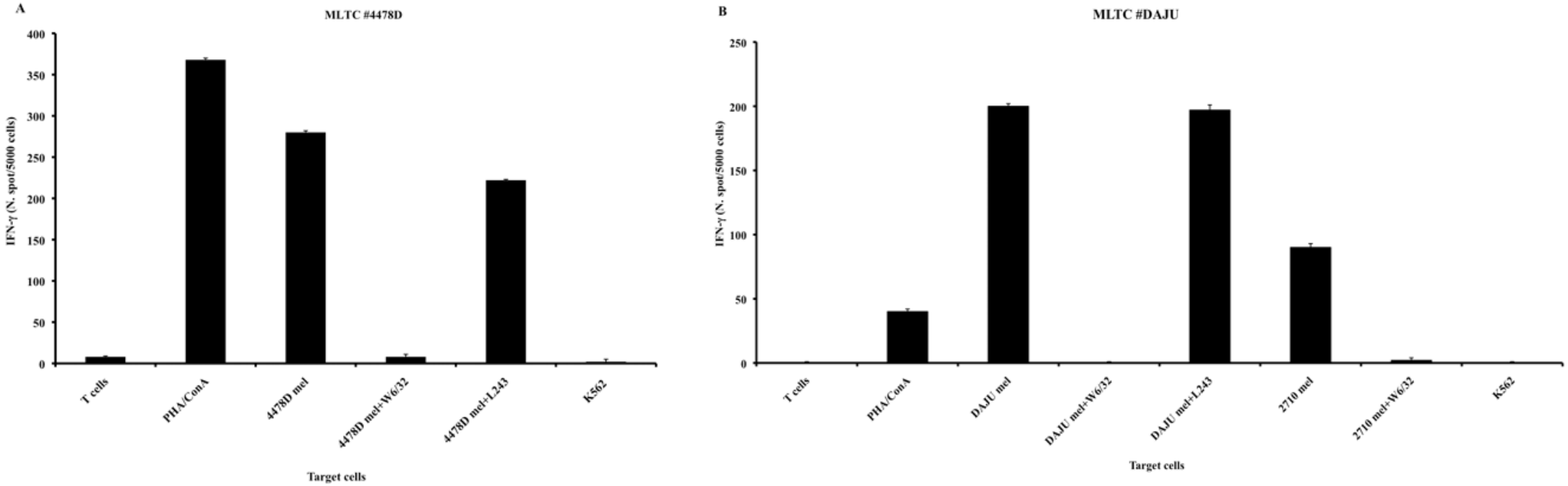

Figure 3S. Tumor specific reactivity of T lymphocytes following REP of MLTCs. MLTCs from 4478D (Panel A) and DAJU (Panel B) cutaneous melanoma patients were expanded in vitro stimulated by REP protocol, based on the usage of irradiated (50 Gy) allogeneic PBMC from 3 healthy donors plus OKT3 (30 ng/ml) and, at day 4, 6000 IU/ml of rh-IL-2 were added. At day +15, tumor recognition of these T cells was assessed by measuring IFN-γ release (ELISPOT assay) after the incubation with the autologous melanoma cells (#4478D mel and #DAJU mel, Panels A and B, respectively) pretreated or not with anti-HLA class I (W6/32) or anti-HLA class II (L243) mAbs. The recognition of the allogeneic HLA-matched (HLA-A*0201+ 2710 mel, Panel A) melanoma line and of the NK target cell line K562 was also determined. PHA/Con-A were used as positive control for IFN-γ release. Statistical analysis of differences between means of IFN-γ released by T cells was done by two-tailed t test ($p \leq 0{,}01$).

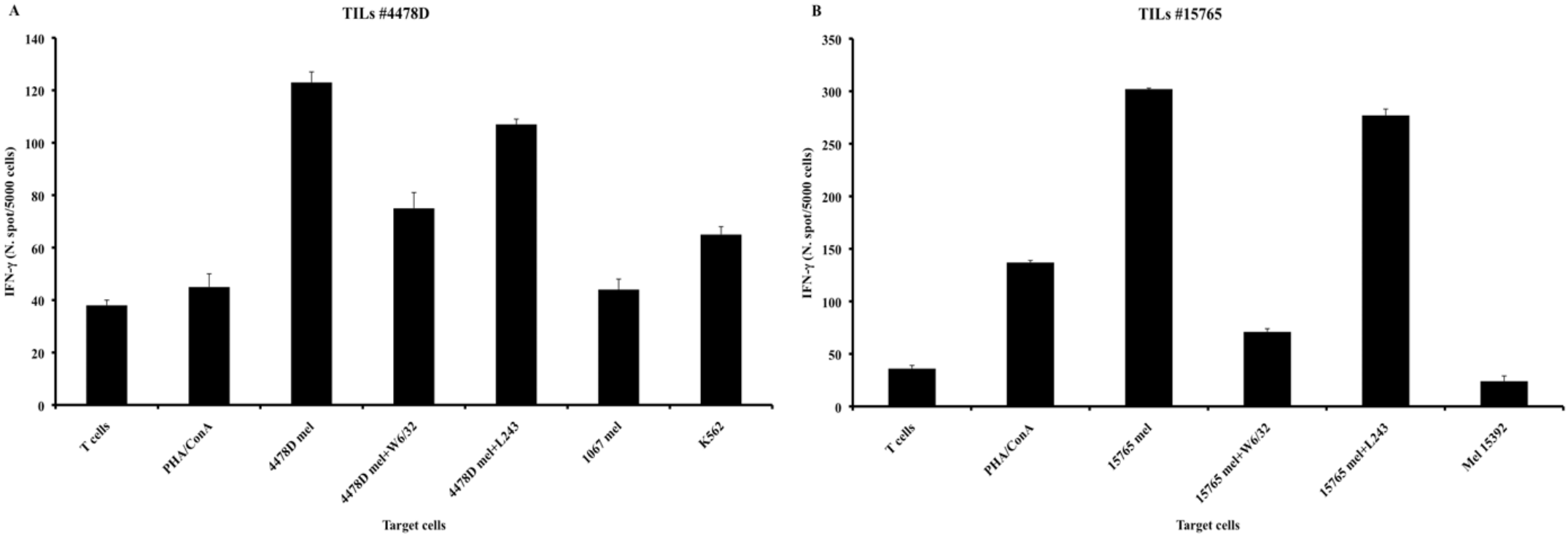

Figure 4S. Tumor specific reactivity of TILs following REP. TILs from #4478D (Panel A) and #15765 (Panel B) cutaneous and ocular melanoma patients, respectively were expanded in vitro stimulated by REP protocol, based on the usage of irradiated (50 Gy) allogeneic PBMC from 3 healthy donors plus OKT3 (30 ng/ml) and, at day 4, 6000 IU/ml of rh-IL-2 were added. At day +15, tumor recognition of these T cells was assessed by measuring IFN-γ release (ELISPOT assay) after the incubation with the autologous melanoma cells (#4478D mel and #15765 mel, Panels A and B, respectively) pretreated or not with anti-HLA class I (W6/32) or anti-HLA class II (L243) mAbs. The recognition of the NK target cell line K562 was also determined. PHA/Con-A were used as positive control for IFN-γ release. Statistical analysis of differences between means of IFN-γ released by T cells was done by two-tailed t test ($p \leq 0{,}01$).