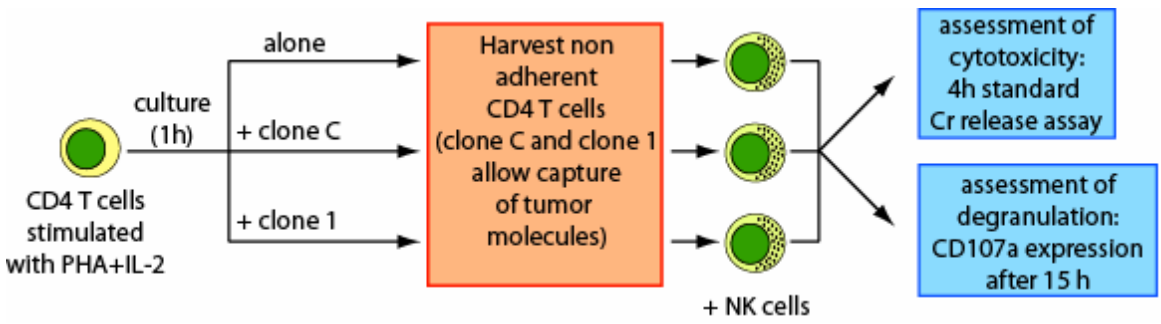
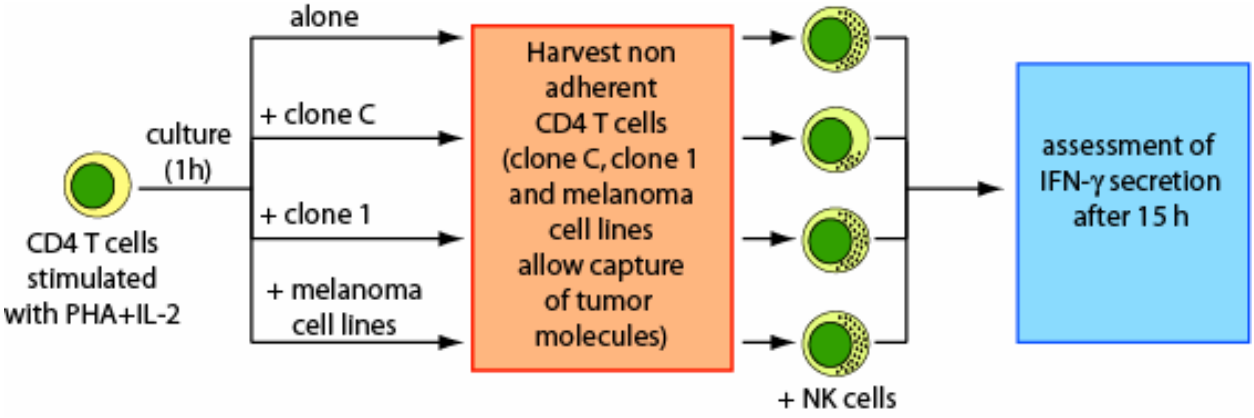
Supplementary Fig 1

Experimental approach used to evaluate NK cell-mediated cytotoxicity, degranulation and IFN-γ secretion by NK cells, triggered by activated "tumor experienced" $CD4^+$ T lymphocytes. A) $CD4^+$ T cells were stimulated with PHA and IL-2 for 72 h and cultured for 1 h either alone or with the tumor clone C or the tumor clone 1. Next, $CD4^+$ T lymphocytes were harvested, washed and co-cultured with NK cells at a ratio of 10:1 or 20:1 (for ^{51}Cr release assays) or 1:1 (for degranulation assays). After 4 h, cytotoxicity was evaluated performing standard ^{51}Cr release assays. ^{51}Cr release assays were performed using activated ^{51}Cr-labeled $CD4^+$ T lymphocytes, the tumor clone C or the tumor clone 1 as target cells. Alternatively, after 15 h degranulation was evaluated as the percentage of $CD107a^+$ cells in the $CD3^-CD56^+$ cells population. **B)** $CD4^+$ T cells were stimulated with PHA and IL-2 for 72 h and cultured for 1 h either alone or with tumor clone C, tumor clone 1 or other cell lines, as indicated in Fig 2. Next, $CD4^+$ T lymphocytes were harvested, washed and co-cultured with NK cells at a ratio of 1:1 for 15 h, and the amount of IFN-γ secreted was evaluated in cell culture supernatants by ELISA. This experimental setting allows engagement of NK cell receptors by ligands captured from the tumor cell surface now present on the cell surface of the activated tumor-experienced $CD4^+$ T lymphocytes, in the absence of direct NK cell-tumor cell contact. For degranulation assays, purity of $CD4^+$ T cells used for co-culture with NK cells was 98.98±0.53% (*n*=3), 99.02±0.21% (*n*=3) and 99.62±0.48% (*n*=2) for $CD4^+$ T cells cultured alone, exposed to tumor clone C or to tumor clone 1, respectively. For assessment of IFN-γ secretion, purity of $CD4^+$ T cells was 99.73±0.04% (*n*=3), 99.67±0.01% (*n*=4) and 99.73±0.19% (*n*=3) for T cells cultured alone, exposed to tumor clone C or to tumor clone 1, respectively. These values rule out the possibility of degranulation and IFN-γ secretion induced by contaminating tumor cells.

A)



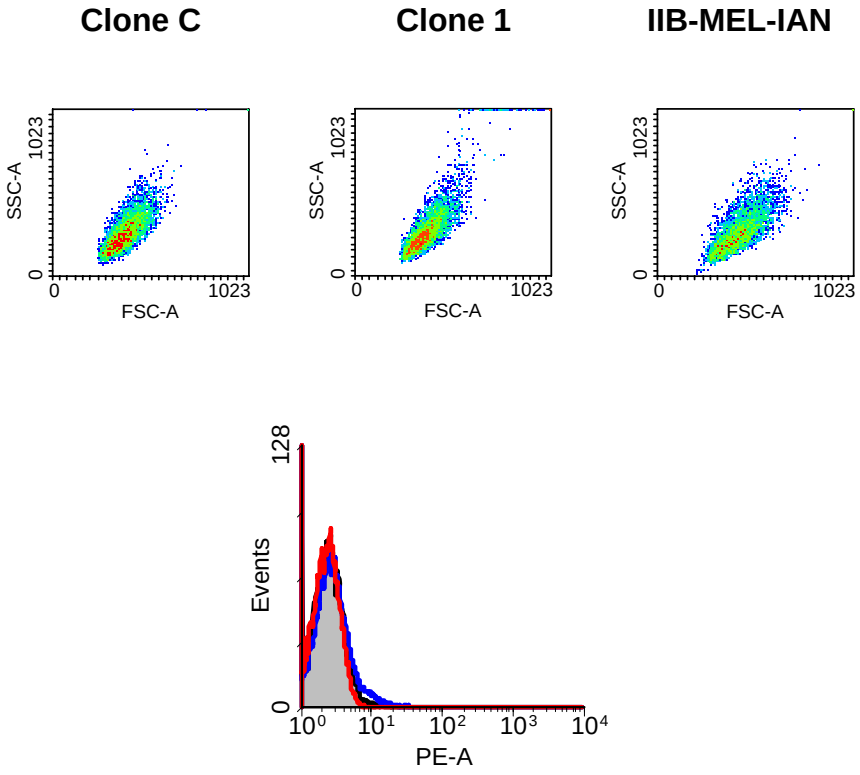
B)


alone
+ clone C
culture
(1h)
+ clone 1
+ melanoma
cell lines
CD4 T cells
stimulated
with PHA+IL-2
Harvest non
adherent
CD4 T cells
(clone C, clone 1
and melanoma
cell lines
allow capture
of tumor
molecules)
+ NK cells
assessment of
IFN-γ secretion
after 15 h

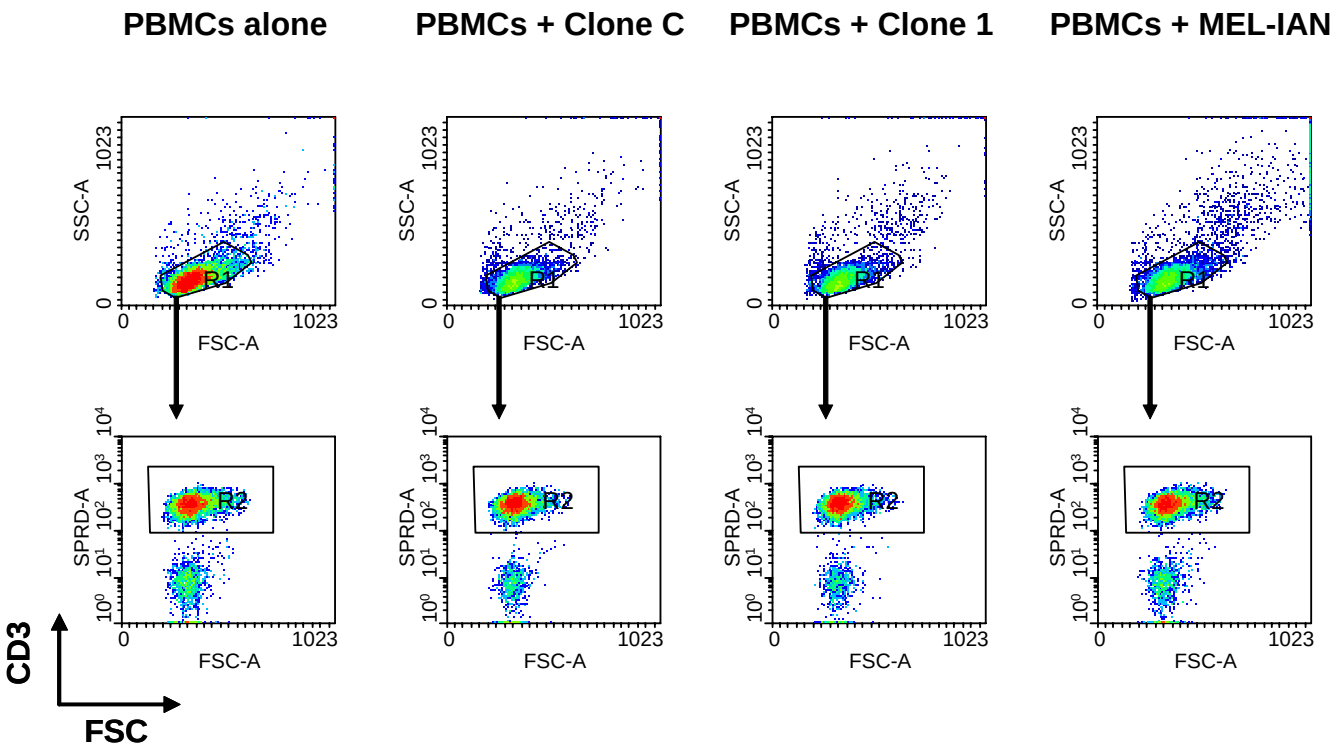
Supplementary Fig 2

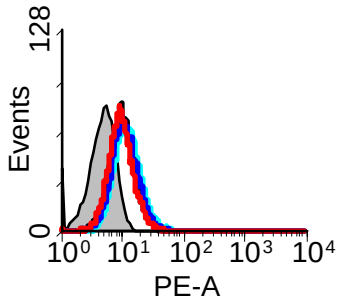
Expression of HLA-E by tumor and T cells. A) Tumor cells (clone C, clone 1 and the cell lines IIB-MEL-IAN) were harvested and HLA-E expression was assessed by flow cytometry with the MEM-E08 mAb. The first three density plots show the forward and side scatter properties of the tumor cells. The histogram shows the staining for HLA-E (***gray histogram: IC; black: clone C; blue: clone 1; red: IIB-MEL-IAN***). **B)** PBMCs were stimulated with PHA and IL-2 for 72 h, harvested and cultured alone or in the presence of the tumor clone C, the tumor clone 1 or the IIB-MEL-IAN cell line for 1 h. Thereafter, non-adherent cells were harvested and HLA-E expression was assessed by flow cytometry with the MEM-E08 mAb on T cells ($CD3^+$ cells). For the analysis, lymphoid cells were gated according to their forward and side scatter properties (indicated as R1 in upper line of density plots) and T cells (indicated as R2 in the lower line of density plots) were then gated as $CD3^+$ cells from R1. HLA-E expression (histograms) was depicted for $CD3^+$ cells (R2 region)). ***Gray histogram: IC; light blue: PBMCs cultured alone; black: PBMCs co-cultured with tumor clone C; blue: PBMCs co-cultured with tumor clone 1; red: PBMCs co-cultured with tumor IIB-MEL-IAN***. Results are representative of three independent experiments with similar results.

A)



B)

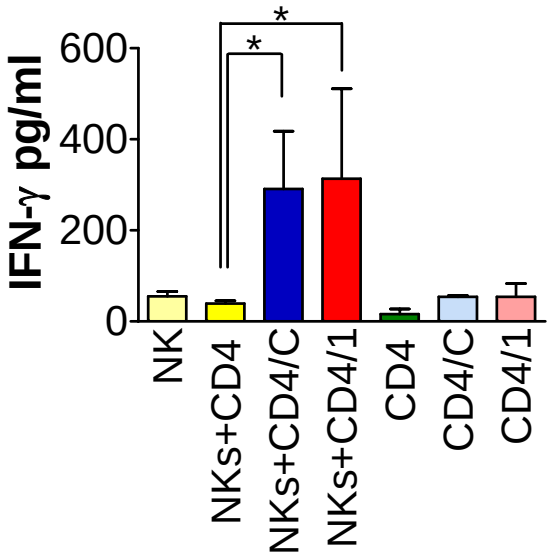




HLA-E in CD3⁺ cells (R2 region)

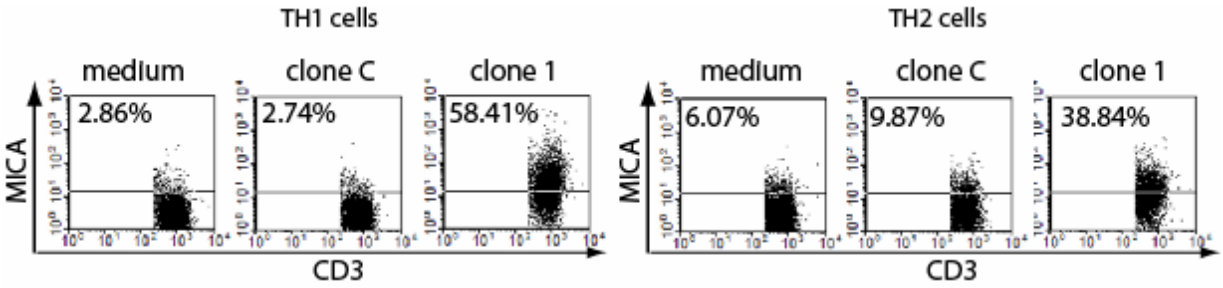
Supplementary Fig 3

IFN-γ secretion of NK cells upon co-culture with syngeneic tumor-experienced CD4$^+$ T lymphocytes. Experimental approach is detailed in **Suppl Fig 1B** and the experiments were performed as indicated for the experiments shown in **Fig 2A**. NK: NK cells alone; NK+CD4: NK cells co-cultured with CD4 T cells that did not contact tumor cells; NK+CD4/C and NK+CD4/1: NK cells co-cultured with CD4 T cells previously co-cultured with tumor clones C or 1, respectively. CD4, CD4/C and CD4/1: CD4$^+$ T cells cultured in the absence or in the presence of tumor clones C or 1, respectively. Data shown correspond to results obtained in 2 independent experiments performed with two different donors of cells.



Supplementary Fig 4

Transfer of MICA from tumor clone 1 to activated in vitro differentiated T_H1 and T_H2 cells. T_H1 and T_H2 cells were differentiated in vitro by culture of PBMCs with PHA, IL-12 and anti-IL-4 mAb or PHA, IL-4 and anti-IL-12 mAb, as described in Molinero LL et al., *Hum. Immunol*. 67:170-182, 2006. T_H1 and T_H2 cells were next co-cultured for 1 h without ("medium") or with the tumor clones C or 1. Thereafter, non-adherent cells were harvested and MICA expression was assessed on T lymphocytes (labeled with an anti-CD3-SPRD mAb). Numbers in dot-plots represent the percentage of MICA-positive T cells. Results are representative of three independent experiments with similar results. Increase in cell surface MICA on T_H1 and T_H2 cells co-cultured with tumor clone 1 was statistically significant, compared to T_H1 and T_H2 cells cultured alone ($p<0.05$).

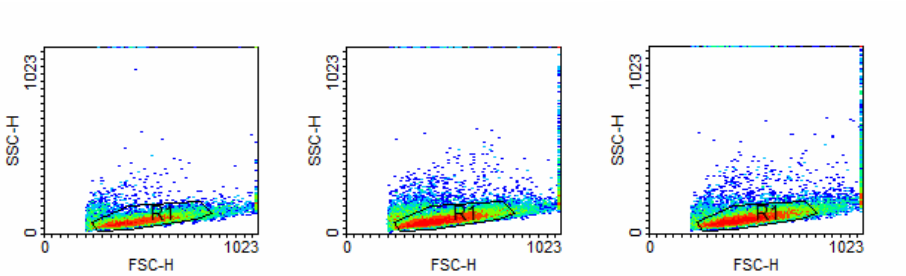


Supplementary Fig 5

Forward and side scatter properties of resting and activated T and NK cells after co-culture with tumor cells. PBMCs were stimulated as indicated in Figs 3 and 4, and co-cultured for 1 h without ("medium") or with the tumor clones C or the tumor clone 1 of the melanoma cell line IIB-MEL-LES, or with the melanoma cell lines IIB-MEL-LES, IIB-MEL-IAN, M8 or Mel-888. Then, non-adherent cells were harvested and labeled with an anti-CD3-SPRD mAb (or an anti-CD4-FITC mAb for results shown in Fig 3H). Also, similar data from co-culture experiments performed with *in vitro* differentiated T_H1 or T_H2 cells (in these two cases, transfer of MICA is depicted in Suppl Fig 4) and with cells labeled with CFSE or $DiOC_{18}$ (in these cases, transfer of MICA is depicted in Fig 4) are shown. In all cases, FSC, SSC and fluorescence intensity were acquired as detailed in Methods. The gate used to plot the results shown in Figs 3, and Suppl Fig 4 is indicated as R1. The gate used to plot the results shown in Figs 4 is indicated as R2. Results are representative of three independent experiments with similar results.

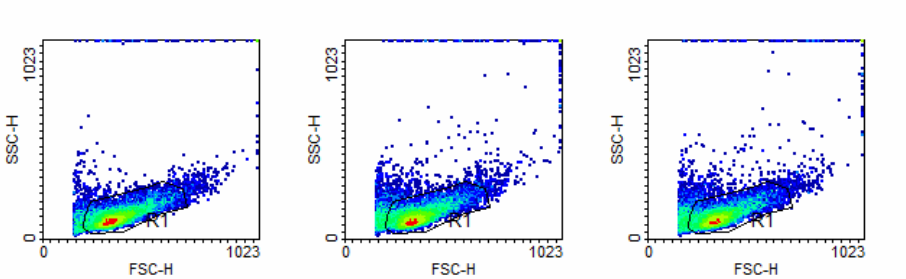
FSC vs SSC plots of cells used for experiments shown in Fig 3A

Medium clone C clone 1



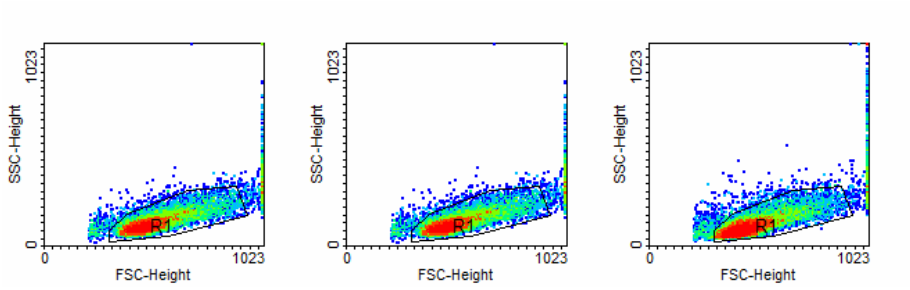
FSC vs SSC plots of cells used for experiments shown in Fig 3B

Medium clone C clone 1



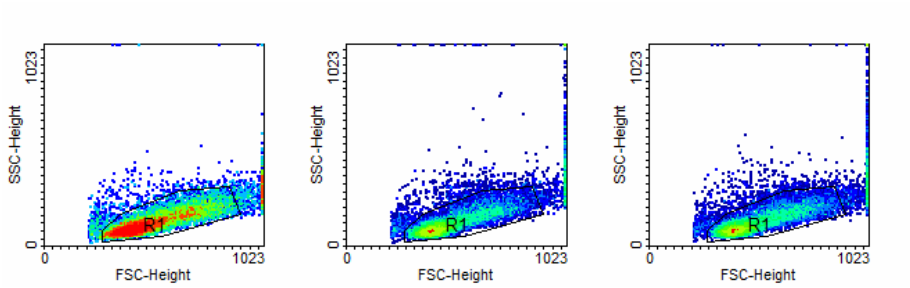
FSC vs SSC plots of cells used for experiments shown in Fig 3C

Medium clone C clone 1



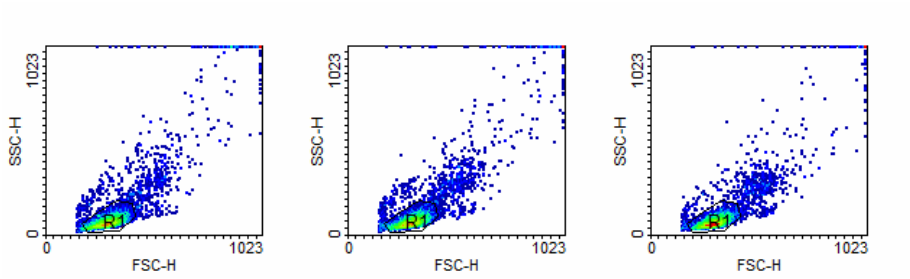
FSC vs SSC plots of cells used for experiments shown in Fig 3D

Medium clone C clone 1



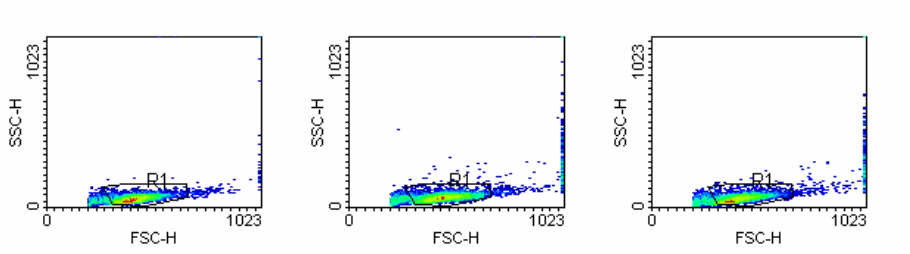
FSC vs SSC plots of cells used for experiments shown in Fig 3E

Medium clone C clone 1



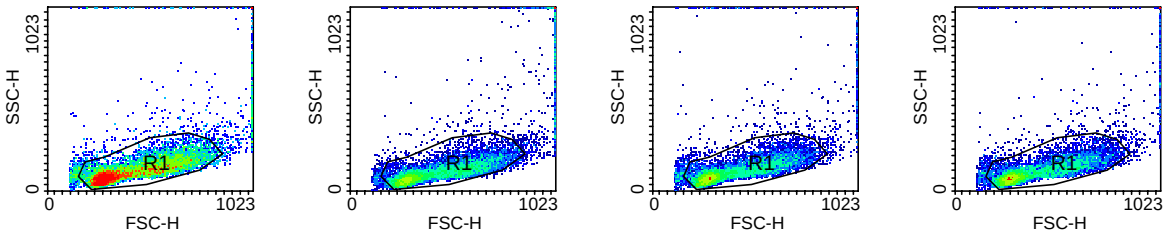
FSC vs SSC plots of cells used for experiments shown in Fig 3F

Medium clone C clone 1



FSC vs SSC plots of cells used for experiments shown in Fig 3H

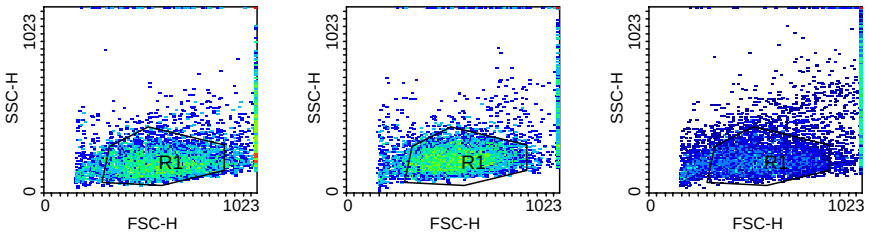
IIB-MEL-LES IIB-MEL-IAN M8 Mel-888



FSC vs SSC plots of cells used for experiments shown in Suppl Fig 4

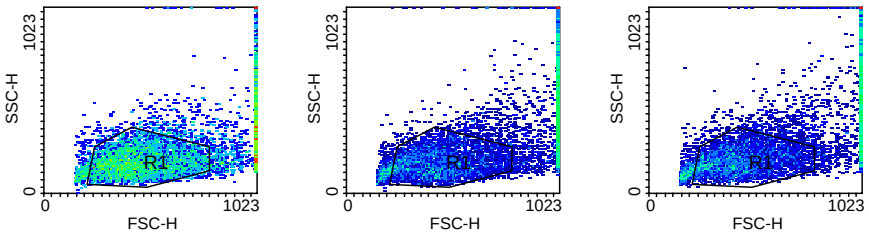
T_H1 cells

Medium clone C clone 1



T_H2 cells

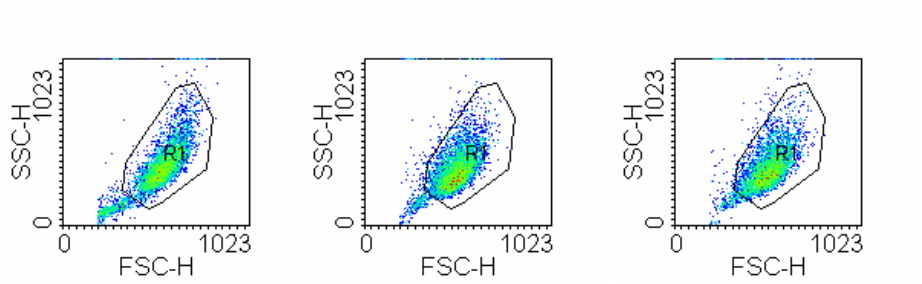
Medium clone C clone 1



FSC vs SSC plots of cells used for experiments shown in Fig 4

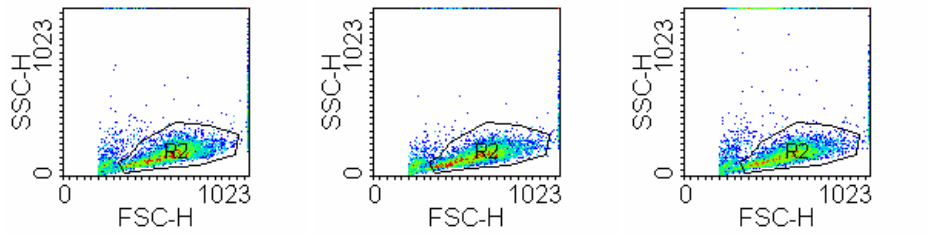
Donor cells: clone 1

clone 1 Unlabeled labeled with CFSE labeled with $DiOC_{18}$



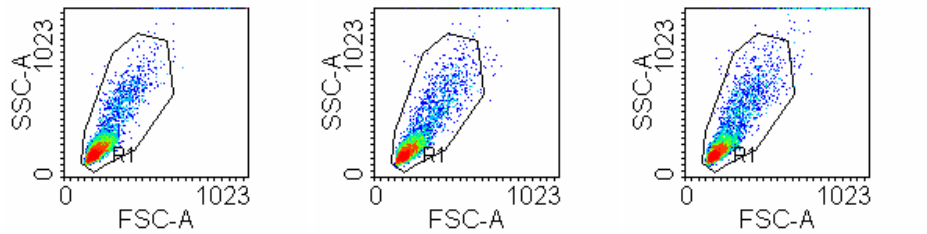
Acceptor cells (stimulated PBMCs)

For unlabeled Clone 1 | For clone 1 labeled with CFSE | For clone 1 labeled with $DiOC_{18}$



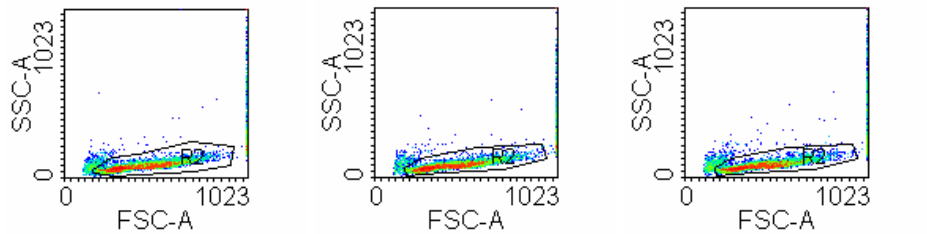
Donor cells: M8

Unlabeled labeled with CFSE labeled with $DiOC_{18}$



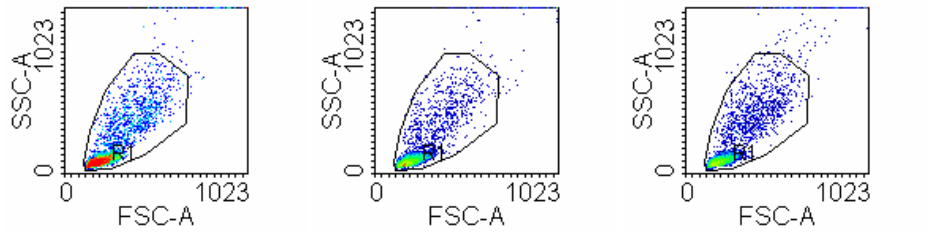
Acceptor cells (stimulated PBMCs)

For unlabeled M8 cells | For M8 cells labeled with CFSE | For M8 cells labeled with $DiOC_{18}$



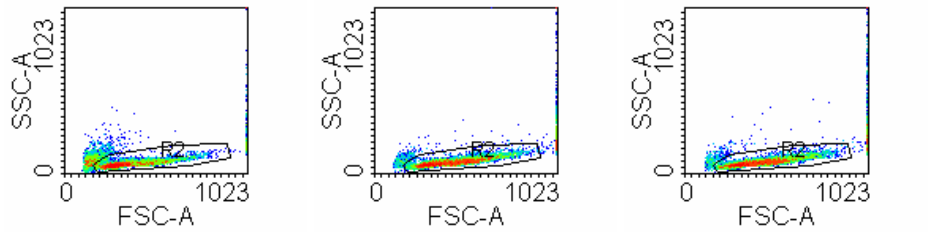
Donor cells: Mel-888

Unlabeled | labeled with CFSE | labeled with $DiOC_{18}$



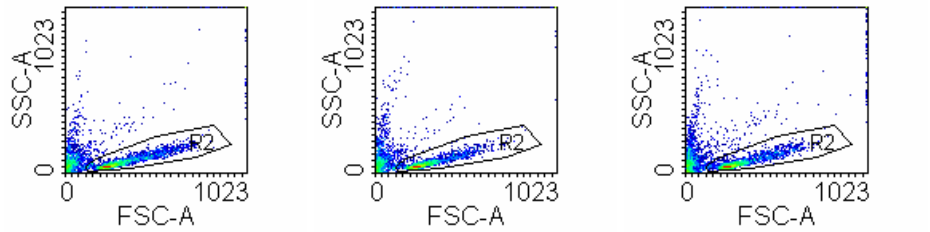
Acceptor cells (stimulated PBMCs)

For unlabeled Mel-888 cells | For Mel-888 cells labeled with CFSE | For Mel-888 cells labeled with $DiOC_{18}$



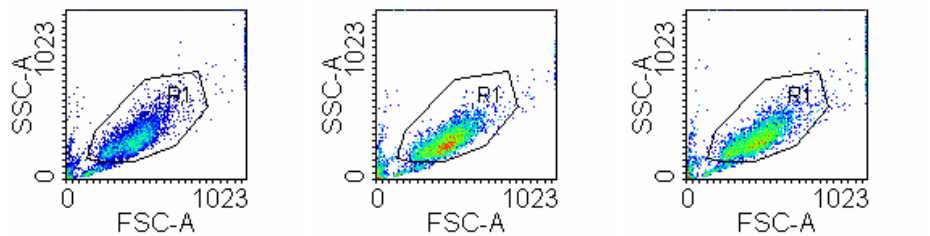
Donor cells: stimulated PBMCs

Unlabeled | labeled with CFSE | labeled with $DiOC_{18}$



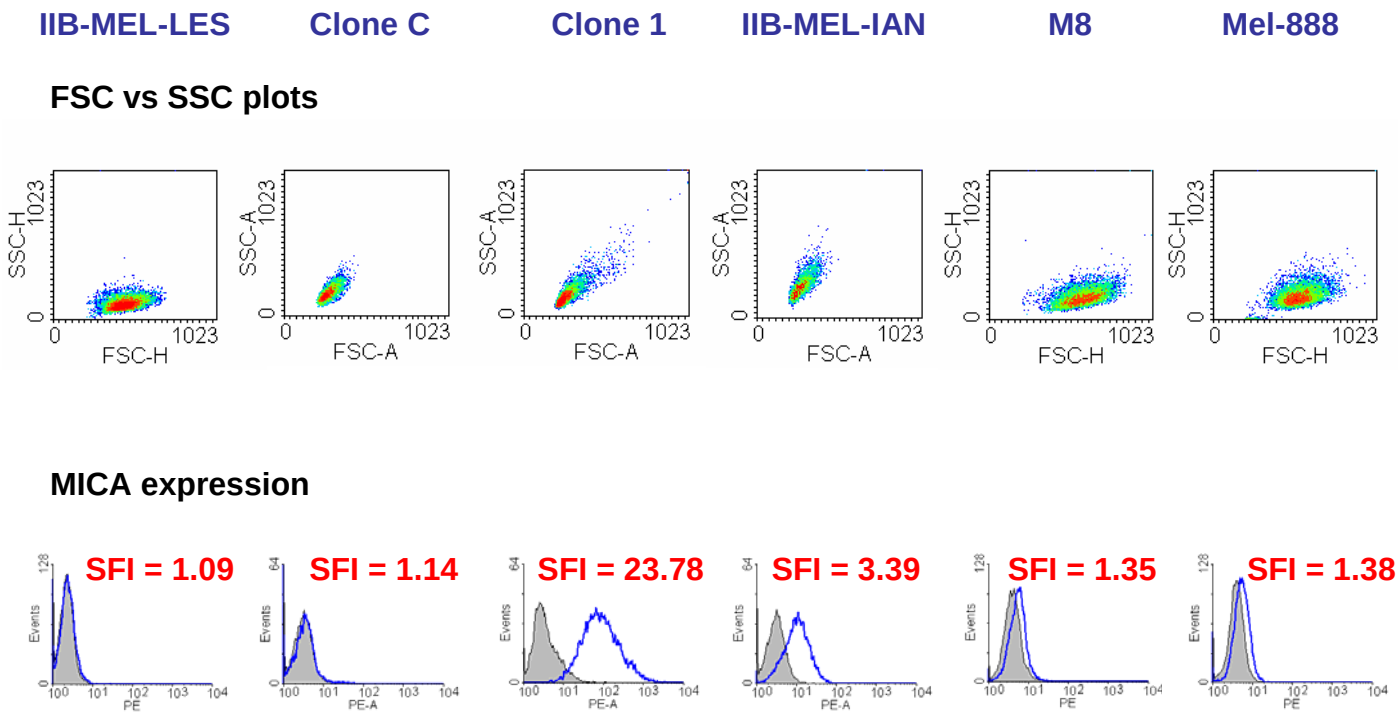
Acceptor cells: clone 1

For unlabeled PBMCs | For PBMCs labeled with CFSE | For PBMCs labeled with $DiOC_{18}$



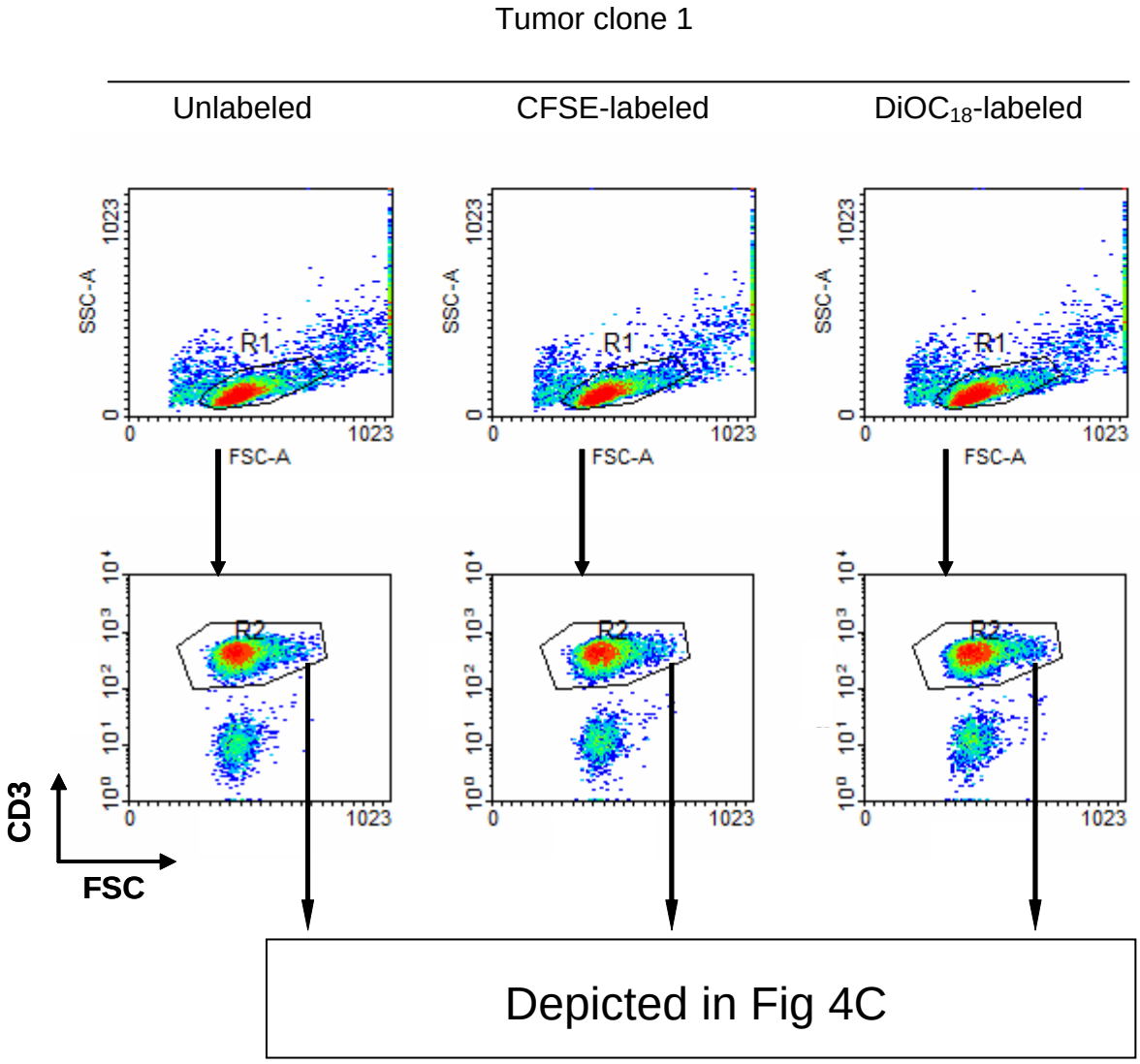
Supplementary Fig 6

MICA/B expression on the cell surface of the different human melanoma cell lines used in this study. Expression of MICA/B by human melanoma cell lines and clones (blue histograms) was analyzed by flow cytometry with the D7 mAb, as previously published by our group (Fuertes MB et al, *J. Immunol.* 180:4606-4614, 2008). Filled histograms: IC mAb. Top panel shows the forward and side scatter properties of these cells. Specific fluorescence index (SFI) for each cell line is indicated. Results are representative of 3 independent experiments.



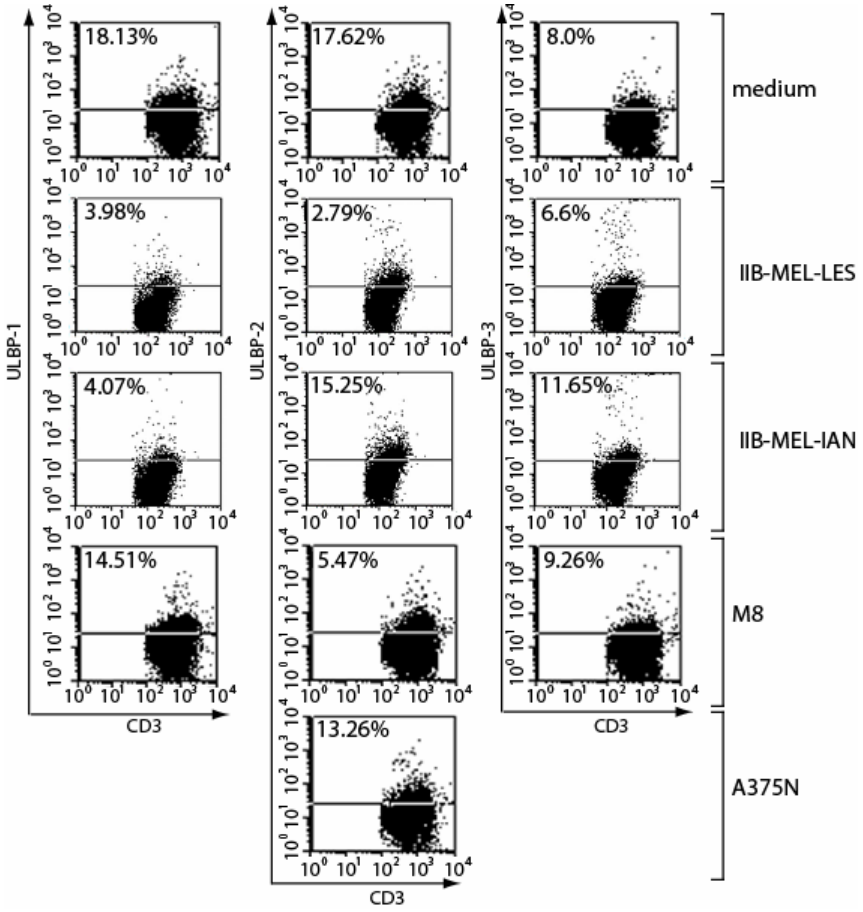
Supplementary Fig 7

Forward and side scatter properties, and gating procedure for co-transfer experiments shown in Fig 4C. PBMCs were stimulated as indicated in Fig 4 and co-cultured for 1 h with unlabeled (*left*), CFSE-labeled (*middle*) or $DiOC_{18}$-labeled tumor clone 1 (*right*). Thereafter, non-adherent cells were harvested, labeled for MICA with the D7 mAb and anti-mouse IgG conjugated to PE, and then counter-labeled with an anti-CD3-SPRD mAb. FSC, SSC and fluorescence intensity were acquired as detailed in Methods. To assess co-transfer of the fluorescent probe and MICA to T lymphocytes, T cells were gated as $CD3^+$ cells (R2) gated from lymphoid cells (R1 region). Results are representative of three independent experiments with similar results.



Supplementary Fig 8

Transfer of ULBPs from different tumors cells to activated CD4$^+$ T lymphocytes. PBMCs stimulated with PHA and IL-2 were co-cultured for 1 h without ("medium") or with the melanoma cell lines IIB-MEL-LES, IIB-MEL-IAN, M8 or A375N. Thereafter, non-adherent cells were harvested and ULBP-1, ULBP-2 and ULBP-3 expression was assessed on T lymphocytes (labeled with an anti-CD3-SPRD mAb). Numbers in dot-plots represent the percentage of ULBP-positive T cells. Results are representative of three independent experiments with similar results.



Cell line	ULBP-1[a]	ULBP-2	ULBP-3
IIB-MEL-LES	82.04±3.74	633.91±11.16	307.73±32.52
IIB-MEL-IAN	94.04±34.83	3062.10±645.40	1070.43±66.85
M8	1359.90±137.26	13449.29±9.80	741.53±84.57
A375N	15.66±8.04	2269.28±35.93	163.32±44.37

[a]Values shown correspond to the ABC (antibody molecules bound per cell) ± SEM calculated as number of Ab molecules bound per cell calibrating the flow cytometer with Quantibrite particles (BD). Expression above background staining of different ULBPs by these cell lines is underlined.

Supplementary Fig 9

Experimental approach used to confirm transfer of MICA by confocal microscopy. For results shown in Fig 5B and C, the clone 1 of IIB-MEL-LES was fixed with pfa, and thereafter stained for cell surface MICA using an anti-MICA mAb (D7) and donkey anti-mouse IgG labeled with Cy3 in order to avoid any Ab-mediated capping effect. Also, $CD4^+$ T cells were isolated from PBMCs, stimulated with PHA and IL-2, and stained for cell surface CD4 with a FITC-labeled anti-human CD4 mAb. Fixed and Cy3-labeled tumor cells (clone 1, red) were co-cultured with these FITC-labeled $CD4^+$ T cells (green) for 1 h. Subsequently, non adherent cells ($CD4^+$ cells) were harvested, seeded on poly-lysine-coated coverslips and analyzed by confocal microscopy.