**Supplemental information**

# Neuropilin-1 cooperates with PD-1

# in CD8^{+} T cells predicting outcomes

# in melanoma patients treated with anti-PD1

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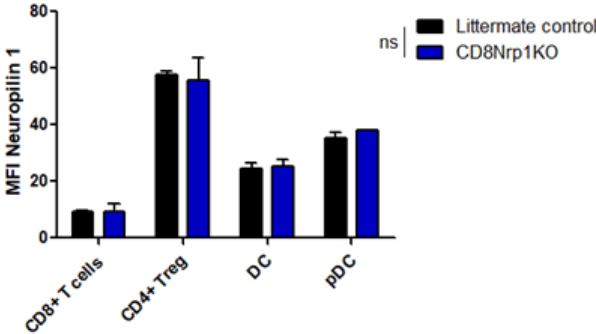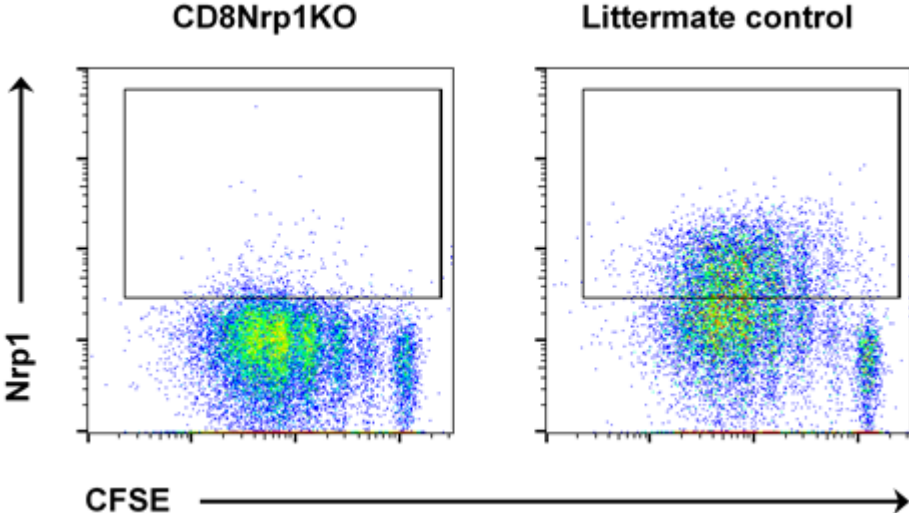
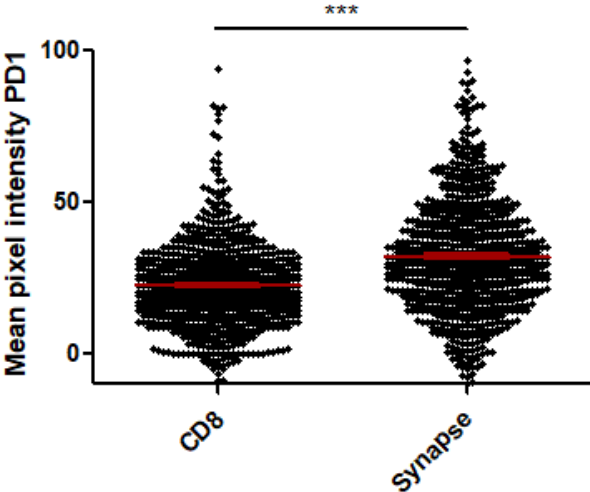**Figure S1**

**a**



**b**

**Figure S2**



CD8Nrp1KO          Littermate control

Nrp1

CFSE
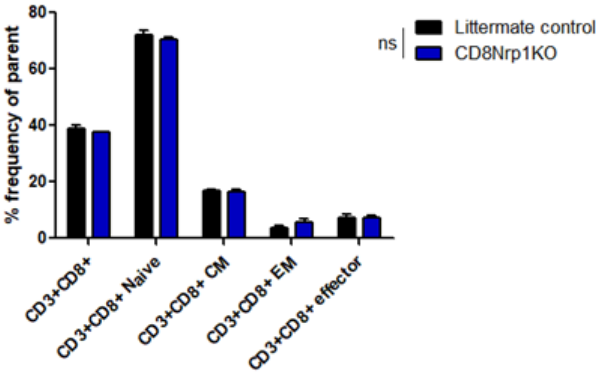
**Figure S3**

**Figure S4**

**Figure S5**



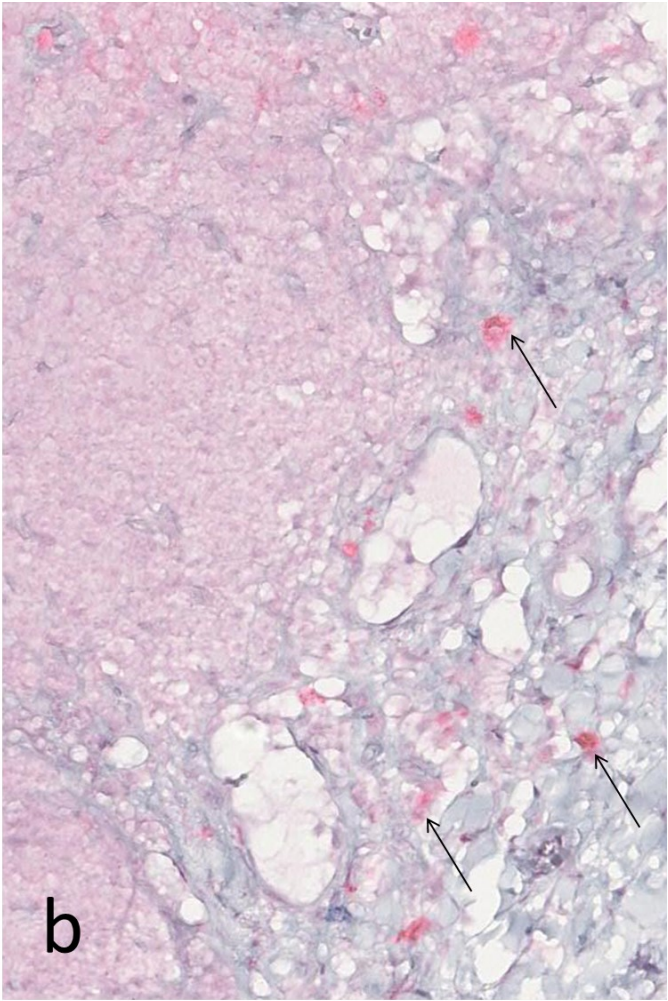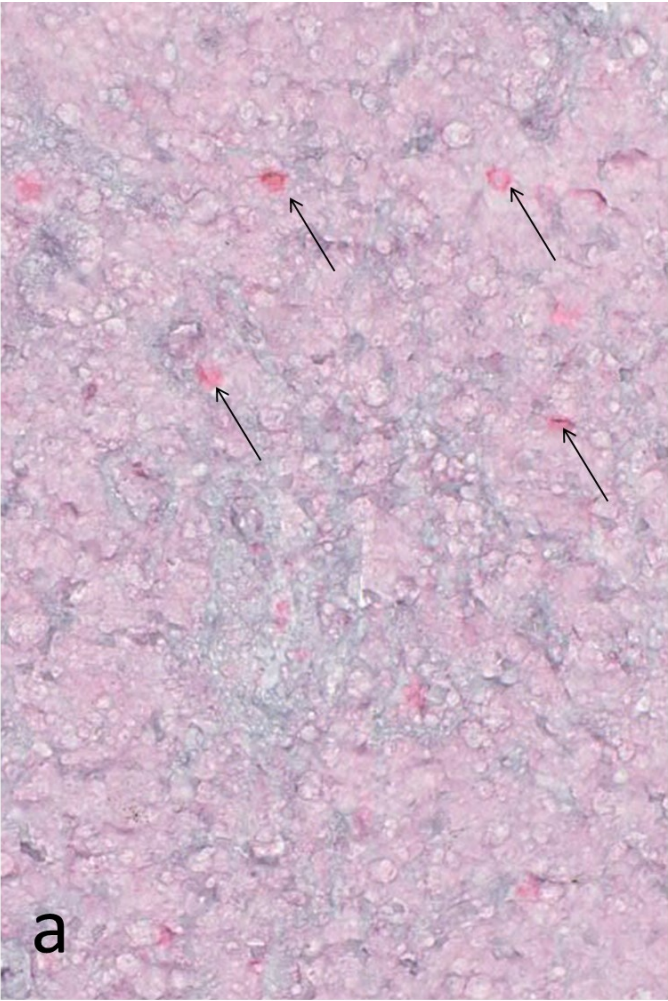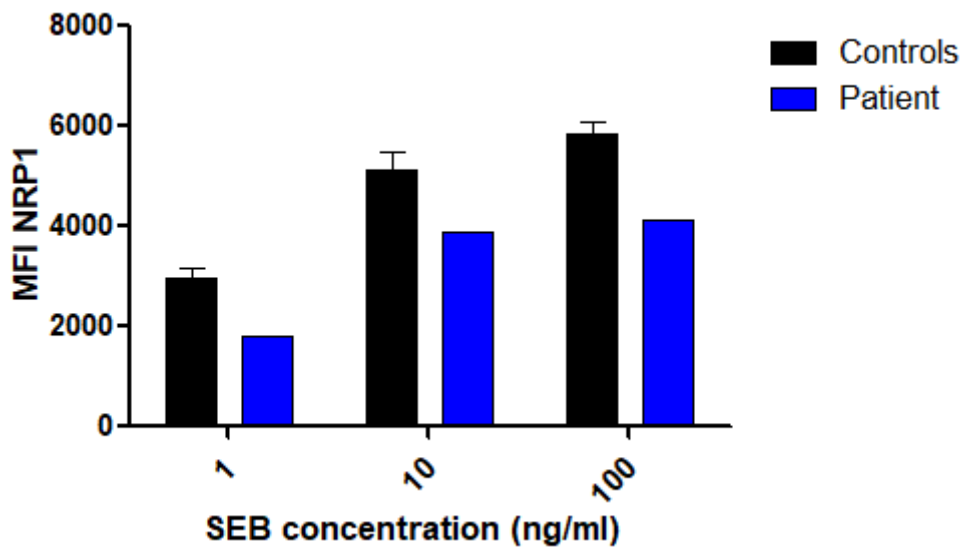**Supplementary Materials (Figures)**

**Figure S1.** [Spleen T-cells subsets in CD8Nrp1KO (KO) and littermate (WT) mice] Related to Figure 1.
**Figure S2.** [Expression of NRP1 on activated spleen CD8+ T-cells from CD8Nrp1KO (KO) and littermate (WT) mice] Related to Figure 1.
**Figure S3.** [PD1 is recruited within the synapse between CD8+ T-cells and tumor cells] Related to Figure 2.
**Figure S4.** [NRP1 expression in Tumor infiltrating CD8+ T-cells among human melanoma tumor] Related to Figure 3.
**Figure S5.** [NRP1 expression in activated CD8+ T-cells from one patient bearing a *NRP1* haploinsufficiency and from controls] Related to Figure 3.

**Figure S1. Frequency of spleen CD8+ T-cells subsets and NRP1 expression in spleen immune cells from CD8Nrp1KO and littermate controls mice.**
**(a)** Flow cytometry analysis of the subset composition (CM=central memory, EM=effector memory) of steady state spleen CD8+ T-cells from CD8Nrp1KO and littermate controls mice. Data are expressed in percentage of cells and presented as mean ± SEM. P values were determined by student T test, NS: non significant (n=3). **(b)** Flow cytometry analysis of NRP1 expression on spleen immune cells (Treg=Regulatory T-cells, DC=Dendritic Cells, pDC= plasmacytoid Dendritic Cells) from CD8Nrp1KO and littermate controls mice. Data are expressed in mean fluorescence intensity and presented as mean ± SEM. P values were determined by student T test, NS: non significant. Data are representative of 2 mice per group.


**Figure S2. Expression of NRP1 on activated spleen CD8+ T-cells from CD8Nrp1KO (KO) and littermate (WT) mice.**
Flow cytometry analysis of NRP1 expression according to cell tracer intensity, on spleen CD8+ T-cells from KO and WT mice, activated *in vitro* with coated anti-CD3 (5µg/ml) and soluble anti-CD28 antibodies (5µg/ml) for 72h. Data are representative of >10 experiments.


**Figure S3. PD1 is recruited within the synapse between activated CD8+ T-cells and tumor cells.**
Analysis by Imagestream of PD1 expression (mean pixel intensity/MPI) in a synapse model between activated CD8+ T-cells and allogeneic A20 tumor cells: PD1 expression was analysed in phalloidine high area between activated CD8+ T-cells and tumor cells (A20). Data are presented as mean MPI ± SEM. P value was determined by student T test ***$p<0.001$. Data are representative of 5 experiments.


**Figure S4. NRP1 expression in Tumor infiltrating CD8+ T-cells among human melanoma tumor.**
Immuno-alkaline phosphatase labeling of scattered NRP1+CD8+ T-cells (a) among melanoma tumor cells and (b) at the periphery of tumor masses, in the adjacent stroma. Arrows indicate NRP1+CD8+ T-cells. Original magnifications: x450. Data are representative of N=25 patients.


**Figure S5. NRP1 expression in activated CD8+ T-cells from one patient bearing a *NRP1* haploinsufficiency and from controls.**
Flow cytometry analysis of NRP1 expression (mean fluorescence intensity/MFI) on CD8+ T-cells from one patient bearing a *NRP1* haploinsufficiency (Patient) or from 5 controls, respective to SEB superantigen concentration (1, 10 or 100 ng/mL). Activation was performed with SEB superantigen during 72 hours. Data are presented as mean MFI ± SEM. Data are representative of 1 experiment with 5 different controls.