**Supplementary Materials**

Figure S1. Genetic knockout of *Plxna4* in the stroma or in the hematopoietic lineage only, increases CD8+ T cell infiltration but does not affect other stromal cell populations. Related to Figure 1.

Figure S2. Additional evidence of *Plxna4* expression in circulating CD8+ T cells and CD8+ T cell subset representation in different tissues. Related to Figure 2.

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Table S1. Antibodies used in flow cytometry experiments.

Table S2. Probes used for quantitative RT-PCR analyses.

Table S3. Clinicopathologic features of melanoma cancer patients and cancer-free controls. Related to Figure 6.

Table S4. Clinicopathologic features of cancer patients and cancer-free controls for monocyte isolation. Related to Figure S6.

Table S5. Hematological parameters in WT and *Plxna4* KO mice. Related to Figure 1.

Table S6. Hematological parameters in WT🡪WT and *Plxna4* KO🡪WT mice. Related to Figure 1.

**Supplementary legends**

**Figure S1. Genetic knockout of *Plxna4* in the stroma or in the hematopoietic lineage only, increases CD8+ T cell infiltration but does not affect other stromal cell populations. Related to Figure 1.**

(A) Representative images of PlexinA4 staining on peripheral blood leukocytes isolated from WT and *Plxna4* KO LLC tumor-bearing mice. (B-D) Histological quantifications of tumor vessels on thin sections of LLC tumors growing in WT and *Plxna4* KO mice showing CD31+ vessel density (B), percentage of lectin-FITC+ perfused vessels over total number of CD34+ vessels (C) and percentage of NG2+ pericyte-covered vessels over the total number of CD31+ vessels (D). (E-H) Histological analysis (E-G) and micrographs (H) of LLC tumor sections stained for F4/80 and pimonidazole (PIMO), showing tumor hypoxia (E), total TAM infiltration (F) and TAM infiltration in hypoxic tumor regions (G) in WT and *Plxna4* KO mice. (I) Expression of M1-like (*Il-12, Cxcl10, Tnf, Cd80*) and M2-like (*Ccl17, Il-10, Mrc1, Cxcl12*) markers in TAMs sorted from subcutaneous LLC tumors growing in WT and *Plxna4* KO mice. (J) Flow cytometric analysis of naïve T cells (Naïve; CD44LoCD62LHi), central memory T cells (CM; CD44HiCD62LHi) and effector/memory T cells (EM; CD44HiCD62LLo) in the tumor-draining LN (TdLN) from B16F10 tumor-bearing WT and *Plxna4* KO mice. (K) Flow cytometric analysis of central memory T cells (CM; CD44HiCD62LHi), effector/memory T cells (EM; CD44HiCD62LLo), and terminally exhausted T cells (EXH; PD-1HiTIM-3Hi) in B16F10 orthotopic tumors from WT and *Plxna4* KO mice. (L) Flow cytometric analysis of CD4+ T cells in the TdLN from WT and *Plxna4* KO mice bearing subcutaneous LLC tumors. (M) Histological quantifications of CD4+ T cell infiltration in the inner tumor bed. (N and O) Flow cytometric analysis of CD4+ T cells in the TdLN (N) and orthotopic B16F10 tumors (O) from WT and *Plxna4* KO tumor-bearing mice. (P and Q) Representative micrographs (P) and paired histological analysis of CD4+ T cells in the inner and outer tumor areas (Q) of LLC tumor sections from WT and *Plxna4* KO tumor bearing mice. (R and S) FACS analysis of CD4+ T cells in the TdLN (R) and primary tumor (S) from WT🡪WT and *Plxna4* KO🡪WT chimeras bearing E0771 tumors. For the *in vivo* experiments, n= 4-8 mice per group. \*\*\*p <0.001 and \*\*\*\*p < 0.0001 versus CD4+ T cells in the tumor border (N). All graphs show mean ± SEM. Scale bars: 50 μm (A and P) and 100 μm (H).

**Figure S2. Additional evidence of *Plxna4* expression in circulating CD8+ T cells and CD8+ T cell subset representation in different tissues. Related to Figure 2.**

(A) *Plxna4* expression in circulating CD8+ T cells sorted from healthy mice and circulating OT-I+ T cells sorted from LCMV-OVA infected mice. (B) Flow cytometric analysis of naïve T cells (CD44LoCD62LHi), effector/memory T cells (CD44HiCD62LLo) and central memory T cells (CD44HiCD62LHi) in the circulation and lymph nodes (LNs) from healthy mice. (C) Flow cytometric analysis of naïve T cells (CD44LoCD62LHi), effector/memory T cells (CD44HiCD62LLo), central memory T cells (CD44HiCD62LHi) and terminally exhausted T cells (PD-1HiTIM-3Hi) in the circulation, TdLN and B16F10 orthotopic tumors from tumor-bearing mice. For the *in vivo* experiments, n= 3-4 mice per group. \*\*\*p < 0.001 versus circulating CD8+ T cells in healthy mice (A). Graph show mean ± SEM.

**Figure S3. Genetic knockout of *Plxna4* in CD8+ T cells increases their proliferation and migratory capacity but does not affect other effector T cell functions. Related to Figure 3.**

(A) *In vitro* proliferation of WT and *Plxna4* KO CD8+ T cells upon CD3/CD28 activation showing proliferation index. (B) Apoptosis of activated WT and *Plxna4* KO CD8+ T cells assessed by Annexin/PI staining. (C-F) Flow cytometric analysis of activated WT and *Plxna4* KO CD8+ T cells showing IFN (C and D) and GrzmB expression (E and F) and representative histograms of median fluorescence intensity (D and F). (G) *In vitro* proliferation of WT and *Plxna4* KO CD4+ T cells upon CD3/CD28 activation showing absolute cell numbers. (H) Transmigration assay of activated WT and *Plxna4* KO CD4+ T cells towards CXCL9 and CXCL10 chemokines. (I) Flow cytometric analysis of naïve splenocytes showing CCR7 expression in WT and *Plxna4* KO CD8+ T cells. (J) FACS analysis of peripheral blood leukocytes, isolated from WT and *Plxna4* KO LLC tumor-bearing mice, showing CXCR3 expression on activated CD8+ T cells. (K and L) GTP-bound Rap1 pulldown assay on activated WT and *Plxna4* KO CD8+ T cells. Quantification by densitometry, paired analysis on each individual experiment (K) and a representative image of the western blot (L). *In vitro* results were performed in triplicates and are representative of at least two independent experiments (A-H). For the *in vivo* experiments, n= 7-8 mice per group (I and J). Pull-down assay is represented as the pooled data from four independent experiments (K) and the western blot is representative of at least three independent experiments (L). \*\*\*\*p <0.0001 versus WT CD8+ T cells (A). All graphs show mean ± SEM. MFI, Median Fluorescent Intensity of the sample minus median fluorescent intensity of the FMO; FMO, Fluorescence Minus One.

**Figure S4. Additional evidence of the increased homing and proliferative capacities of *Plxna4*-deficient CD8+ T cells. Related to Figure 4.**

(A and B) Gating strategy (A) related to the homing of naïve WT and *Plxna4* KO CD8+ T cells to the LNs of healthy WT mice assessed by FACS (B). (C) Tumor homing of activated WT and *Plxna4* KO OT-I T cells to LLC-OVA tumor-bearing WT mice, 24 hours after tail vein injection. (D) Ratio of *Plxna4* KO to WT OT-I T cells infiltrated in B16F10-OVA tumors (related to tumor homing in Figure 4E), 24 hours and 48 hours after tail vein injection. (E) Dilution of the respective fluorescent cell tracer of activated WT (Violet Cell Tracer) and *Plxna4* KO OT-I T cells (CFSE Cell Tracer) in the tumor bed, 24 hours after tail vein injection in B16F10-OVA tumor-bearing mice. (F) Expression of the ovalbumin antigen in liver tissue of WT mice hydrodynamically injected with empty vector (EV) or ovalbumin vector (OVA), examined by western blot. Each lane represents an individual mouse in each group. (G) Ratio of *Plxna4* KO to WT OT-I T cells infiltrated in hydrodynamically injected livers with empty vector (EV) or ovalbumin vector (OVA) (related to liver homing in Figure 4G). (H-J) Expression of *Sema3a* (H), *Sema6a* (I) and *Sema6b* (J) in B16F10-OVA cancer cells transduced with empty vector (EV) or overexpression vector (OE) of the corresponding semaphorin. (K) Transmigration assay of activated WT and *Sema6a* KO CD8+ T cells towards CXCL10 chemokines. *In vitro* results were performed in triplicates (I and K) or duplicates (H and J). For the *in vivo* experiments, n= 4-5 mice per group (B-E and G). \*\*p < 0.01 and \*\*\*p < 0.001 versus WT CD8+ T cells (B), WT OT-I T cells (C and E), the ratio of tumor homing 24 hours after tail vein injection (D). All graphs show mean ± SEM.

**Figure S5. Additional evidence of improved anti-tumor immunity by adoptively transferred *Plxna4*-deficient CD8+ T cells. Related to Figure 5.**

(A-B) Tumor growth of LLC-OVA (A) and B16F10-OVA (B) tumor-bearing WT mice receiving ACT of naïve WT and *Plxna4* KO OT-I T cells. (C-D) Flow cytometric analysis of IFN (C) and GrzmB expression (D) on intratumoral OT-I+ T cells in B16F10-OVA tumors 4 days after ACT with activated WT and *Plxna4* KO OT-I T cells. For the *in vivo* experiments, n= 6-8 mice per group (A-D). \*p < 0.05 versus WT OT-I T cells (A and B). All graphs show mean ± SEM. MFI, Median Fluorescent Intensity of the sample minus median fluorescent intensity of the FMO.

**Figure S6. Other type-A plexins are not up-regulated in circulating CD8+ T cells but *PLXNA4* expression is also up-regulated in circulating CD4+ T cells of metastatic melanoma patients. Related to Figure 6.**

(A-C) Expression of *PLXNA1* (A), *PLXNA2* (B) and *PLXNA3* (C) in isolated CD8+ T cells from the circulation of treatment-naïve melanoma patients and healthy controls. (D) *PLXNA4* expression in isolated CD4+ T cells from the circulation of melanoma patients and healthy controls. (E) *PLXNA4* expression in isolated monocytes from the circulation of cancer patients and healthy controls. For the melanoma patient data (A-D), n= 8 healthy controls and n= 13 melanoma patients (see Table S3). For the cancer patient data (E), n= 13 healthy controls and n= 24 cancer patients (see Table S4). All graphs show mean ± SEM.