**SUPPLEMENTARY FIGURE LEGENDS**

**Supplementary Figure S1. Representative dot plots of *in vitro* inhibition of T cell activation by TPIN-SCs.** (A-C) Splenocytes were activated alone or in the presence of irradiated TPIN-SCs (1:10 ratio) as described in Figure 1, and analysed by FACS after 5 or 3 days, for CD4+ (left panels) or CD8+ (right panels) respectively. Representative dot plots are gated on CD4+ or CD8+ cells. (D) Representative dot plots, gated on CD3+ cells, depicting proliferation of PBMCs as measured by CFSE dilution at day 6 of stimulation with ConA alone (Nihil) or in the presence of irradiated TPIN-SCs or TNE-SCs (1:10 ratio).

**Supplementary Figure S2. Non-irradiated TPIN-SCs arrest *in vitro* T cell activation.** (A) Left panels: Representative dot plots of *in vitro* proliferation of CD4+ (upper panels) and CD8+ splenocytes (lower panels) measured by FACS as CFSE dilution after 5 or 3 days, respectively, of stimulation with anti-CD3/CD28 beads alone (Nihil) or in the presence of non irradiated TPIN-SCs or TNE-SCs. CSCs (1:10 ratio) were added in co-culture (coc), or in the upper chamber of a 0.4 mm transwell (tw). Right panel: Percentage of proliferating CD8+ (black bars) and CD4+ (white bars) T cells. (B) Expression [mean fluorescence intensity (MFI)] of surface CD44 and percentage of cells producing IFN in cultured CD8+ (black bars) and CD4+ (white bars) T cells. (C) Representative dot plots of histograms reported in panel B. Each panel of the figure is representative of 2 independent experiments. Data are reported as average ± SD. Anova followed by Tukey’s test and Student’s T test: \* p< 0.05; \*\* p<0.01; \*\*\* p<0.0001.

**Supplementary Figure S3. TPIN-SCs inhibit restimulation of antigen-experienced T cells but do not affect fully activated T cells.** (A) Left panel: FACS analysis of splenocytes from Tag-presensitized mice after a 5-day *in vitro* antigen restimulation alone (Nihil) or in the presence of irradiated CSCs (co-culture, coc or transwell, tw at 1:10 ratio). Columns depict the absolute number ± SD of CD8+ T cells. Right panel: Percentage (average ± SD) of CD44+IFN+ cells within the gate of CD8+ T cells upon challenge with RMA cells pulsed with Tag (values are subtracted of background IFNγ release against unpulsed RMA cells). (B) Left panels: Blasts were also challenged for their killing activity against Tag-pulsed (circles) or unpulsed RMA cells (squares) in a 51Cr cytotoxic assay. Right panel: Quantification of specific lysis as Lytic units values (average ± SD). (C) Left panels: OTII (CD4+; upper panels) and OTI (CD8+; lower panels) cells were primed *in vitro* with OVA peptides. At day 5 or day 3, respectively, primed OTII and OTI cells were labeled with CFSE and seeded with IL-2, alone (Nihil) or in co-culture with irradiated TPIN-SCs (1:10), and assessed by FACS for CFSE dilution and IFN production against OVA expressing cells after additional 48 h. Right panel: Percentage ± SD of IFNγ producing cells that have diluted CFSE within the gate of CD4+ (white columns) and CD8+ T cells (black columns). Representative of at least 3 experiments with at least 3 mice per group. Anova followed by Tukey’s test: \* p< 0.05; \*\*\* p<0.0001.

**Supplementary Figure S4. TPIN-SC-conditioned T cells are hyporesponsive**. (A) Phosphorylation of Zap70 (pZAP70), ERK (pERK) and STAT5 (pSTAT5) expressed as MFI (average ± SD) in CFSE-labeled splenocytes after 3 (CD8+; black columns) or 5 days (CD4+; white columns) of *in vitro* stimulation with anti-CD3/CD28 beads alone (Nihil) or in the presence of irradiated TPIN-SCs (1:10 ratio). (B) SILAC analysis on protein extracts from cultures of OTI (CD8+) cells that were primed *in vitro* with OVApeptides in the presence of TPIN-SCs or TNE-SCs and in SILAC medium containing light (12C and 14N) or heavy (13C 15N) labeled L-lysine and L-arginine, respectively. At day 7, CD8+ cells were sorted for CD62Lhigh (i.e. inhibited by TPIN-SCs) or CD62Llow (i.e. activated in presence of TNE-SCs). Differential expressed proteins are reported as the log2 of protein ratio plotted against the log10 of total intensity calculated for of each protein both in heavy and light conditions. Data points are colored by their ‘significance B’, with blue squares having values >0.05, red squares between 0.05 and 0.01, yellow squares between 0.01 and 0.001 and green squares <0.001. (C) Naïve splenocytes were stimulated *in vitro* with anti-CD3/CD28 beads alone (Nihil) or in the presence of irradiated TPIN-SCs. At day 3, purified CD8+ T cells were labeled with CFSE and stimulated again with anti-CD3/CD28 beads. Proliferation and activation were tested 3 days later. Representative of at least 3 independent experiments performed with at least 3 mice per group. Student’s T test: \* p< 0.05; \*\* p<0.01; \*\*\* p<0.0001.

**Supplementary Figure S5. Silencing of TNC modulates the inhibitory activity of TPIN-SCs.** (A-C) Splenocytes were activated alone or in the presence of irradiated TPIN-SC shRNA-TNC or TPIN-SC shRNA-ctr (1:10 ratio) as described in Figure 5, and analysed by FACS for phosphorylation of Zap70 (pZAP70), ERK (pERK) and STAT5 (pSTAT5). The figure reports representative histograms.

**Supplementary Figure S6. CSCs migration is mediated by the CXCR4/CXCL12 axis.** (A) FACS analysis of TPIN-SCs and TLN-SCs for CXCR4 expression. Black profile: specific staining; white profile: isotype control. (B-C) Number of *in vitro* migrated TPIN-SCs towards CXCL12 (2.5nM) as quantified by FACS. Migration was inhibited by the addition of the CXCR4 inhibitor AMD3100 (10M) or the CXCR4 antagonist peptide R (10M), as indicated. Negative control was TPIN-SCs in normal medium. (D) Quantification (average ± SD) of CXCL12 expression by real-time PCR in PDLNs or NDLNs of TRAMP and WT mice. Values were normalized to the positive control (a prostate of a TRAMP mouse) using the ΔΔCT method. (E) FACS analysis of TPIN-SCs for CCR7, CCR6 and CXCR3 expression. Red profile: specific staining; black profile: isotype control. Each panel of the figure is representative of at least 3 independent experiments. Anova followed by Tukey’s test and T test: \* p< 0.05; \*\* p<0.01; \*\*\* p<0.0001.