SUPPLEMENTAL FIGURES

Preferential Homing of Tumor-specific and Functional $CD8^+$ Stem Cell-like Memory T Cells to the Bone Marrow

Running title: Homing of T_{SCM}s to the bone marrow

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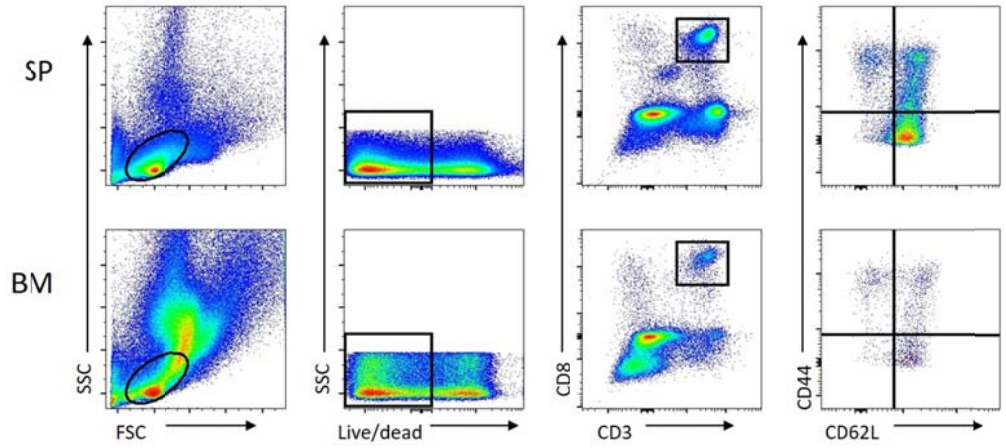

Figure S1 . Gating strategy for flow cytometric analysis of $CD8^+$ T_{SCM}s

The expression levels of CD122 and Sca-1 were detected on the basis of the phenotype of naive $CD8^+$ T cells to analyze $CD8^+$ T_{SCM}s. The $CD3^+$ $CD8^+$ $CD62^+$ $CD44^-$ cells in C57BL/6 mice spleen (A) and bone marrow (B) were analyzed by flow cytometry. The forward scatter (FSC) and side scatter (SSC) show the properties of the cell size and density.

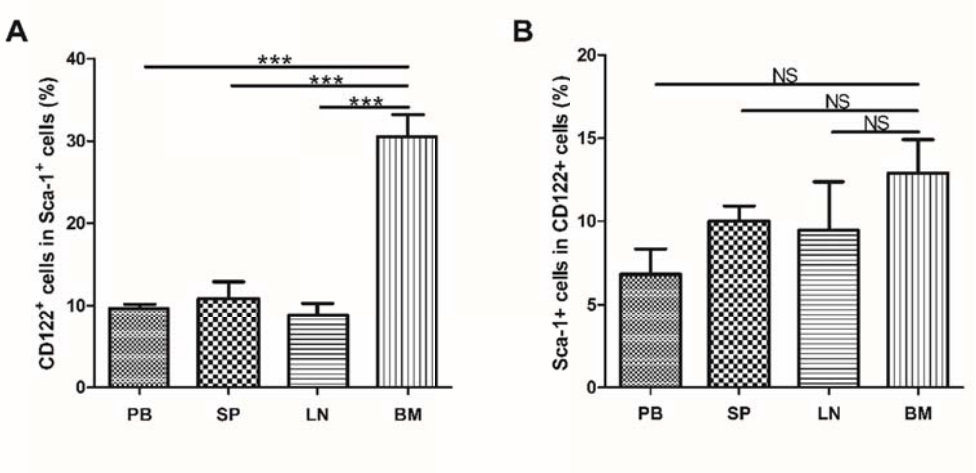

Figure S2. (A) The percentage of $CD122^+$ cells in Sca-1^+ cells and (B) the percentage of Sca-1^+ cells in $CD122^+$ cells in the organs, respectively.

The frequencies of $CD122^+$ cells in $CD3^+$ $CD8^+$ $CD4^-$ $CD62L^+$ $CD44^-$ Sca-1^+ T cell

compartment (A), and the frequencies of Sca-1$^+$ cells in CD3$^+$ CD8$^+$ CD4$^-$ CD62L$^+$ CD44$^-$ CD122$^+$ T cells (B) formulas as follow: CD122$^+$ cells in Sca-1$^+$ cells (%) = CD3$^+$ CD8$^+$ CD4$^-$ CD62L$^+$ CD44$^-$ CD122$^+$ Sca-1$^+$ cells /CD3$^+$ CD8$^+$ CD4$^-$ CD62L$^+$ CD44$^-$ Sca-1$^+$ cells × 100%. Sca-1$^+$ cells in CD122$^+$ cells (%) = CD3$^+$ CD8$^+$ CD4$^-$ CD62L$^+$ CD44$^-$ CD122$^+$ Sca-1$^+$ cells /CD3$^+$ CD8$^+$ CD4$^-$ CD62L$^+$ CD44$^-$ CD122$^+$ × 100%. Data are representative for three independent experiments (n=5). The data were shown as means ± SD, one-way ANOVA. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

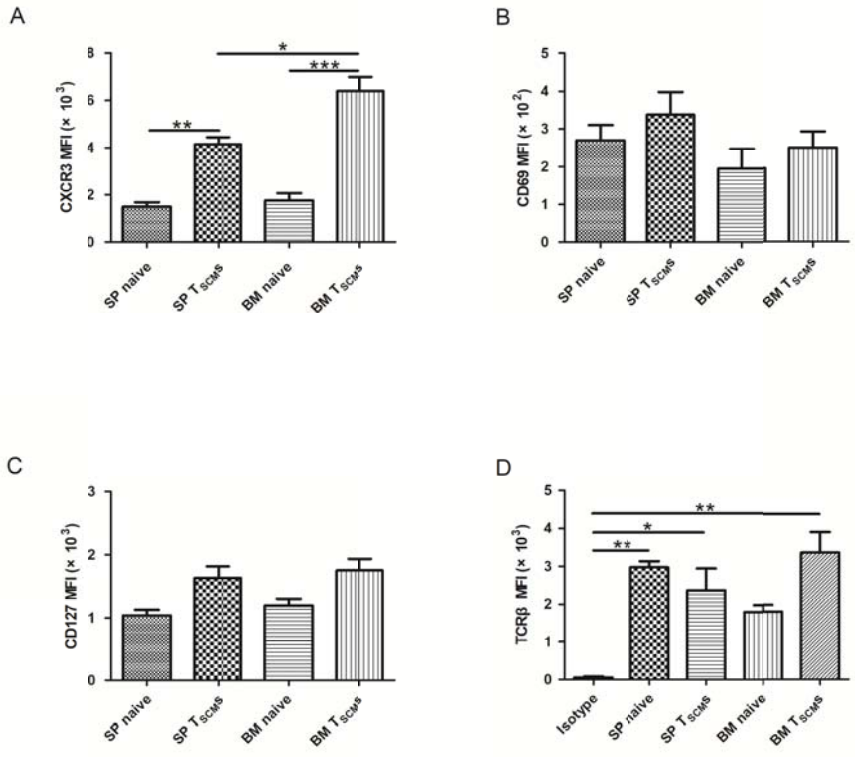

Figure S3.Flow cytometric analysis of SP naïve T cells, SP T_{SCM}s, BM naïve T cells and BM T_{SCM}s.

The MFI of a given molecule in different CD8$^+$ T cell subsets was analyzed by flow cytometry. CD8$^+$ T cell subsets were defined as follows: BM-resident T_{SCM}s, CD3$^+$ CD8$^+$CD4$^-$CD44^{low} CD62L^{high}CD122^{high} Sca-1^{high}; BM- and SP-resident naïve T cells,

$CD3^+$ $CD8^+$ $CD4^-$ $CD44^{low}CD62L^{high}$ $CD122^{low}Sca\text{-}1^{low}$. Data are representative for five independent experiments (n=7), one-way ANOVA, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$..

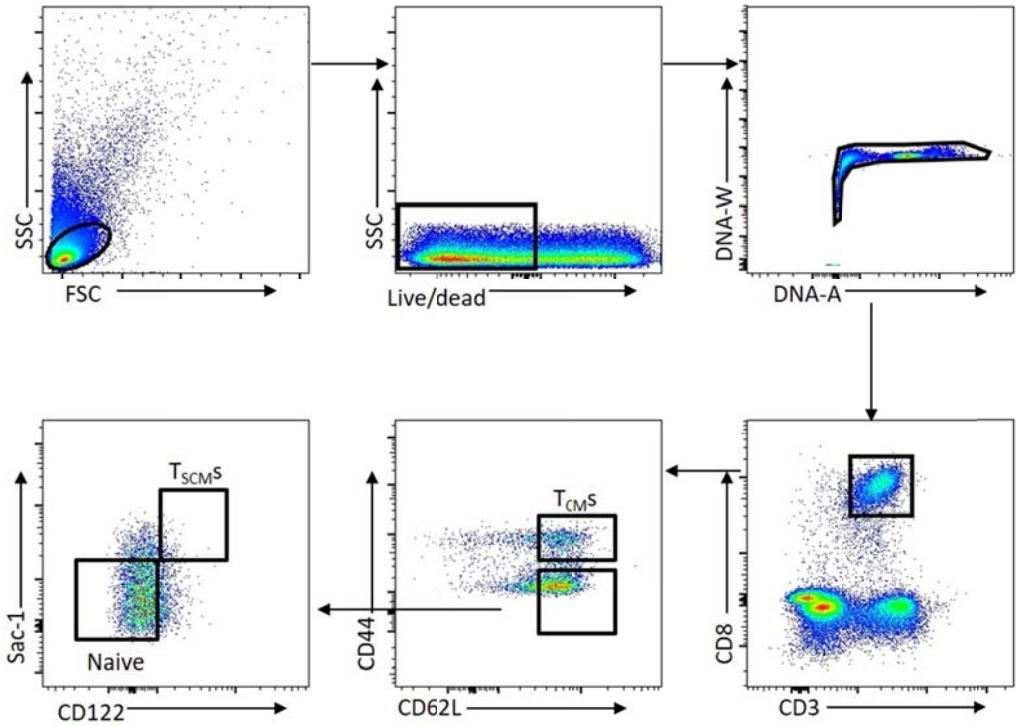

Figure S4. Gating strategy for flow cytometric analysis of DNA content in T-cell subsets.

The DNA content was tested by staining propidium iodide. The dot plots show the gating strategy of naïve T cells ($CD3^+$ $CD8^+$ $CD44^-$ $CD62L^+$ $CD122^-$ $Sca\text{-}1^-$) and T_{SCM}s ($CD3^+$ $CD8^+$ $CD44^-$ $CD62L^+$ $CD122^+$ $Sca\text{-}1^+$). The T_{CM}s were gated as $CD3^+$ $CD8^+$ $CD44^+$ $CD62L^+$ cells.

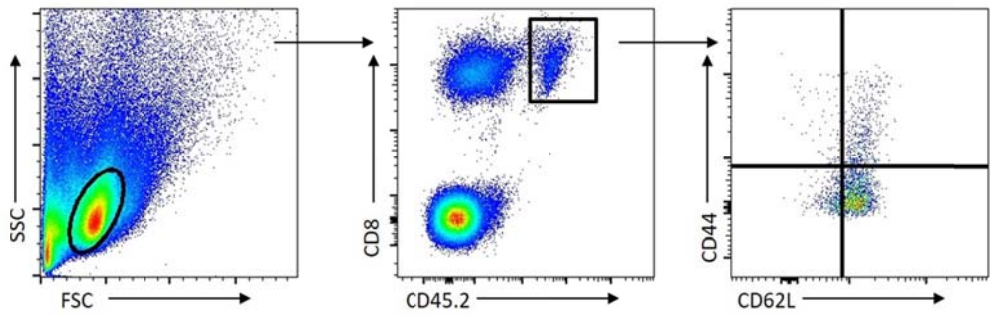

Figure S5.Gating strategy for the analysis of the in vivo activation of adoptive transfer OT Ⅰ T cells.

The OT Ⅰ mice $CD3^+$ $CD4^-$ $CD8^+$ $CD44^{low}$ $CD62L^{high}$ cells were sorted by flow cytometry and then the frequencies of $CD122^{high}$ $Sca\text{-}1^{high}$ in $CD44^{low}$ $CD62L^{high}$ T cells compartment was analyzed. Dot plots represent the strategy of flow cytometric analysis of adoptive transfer.

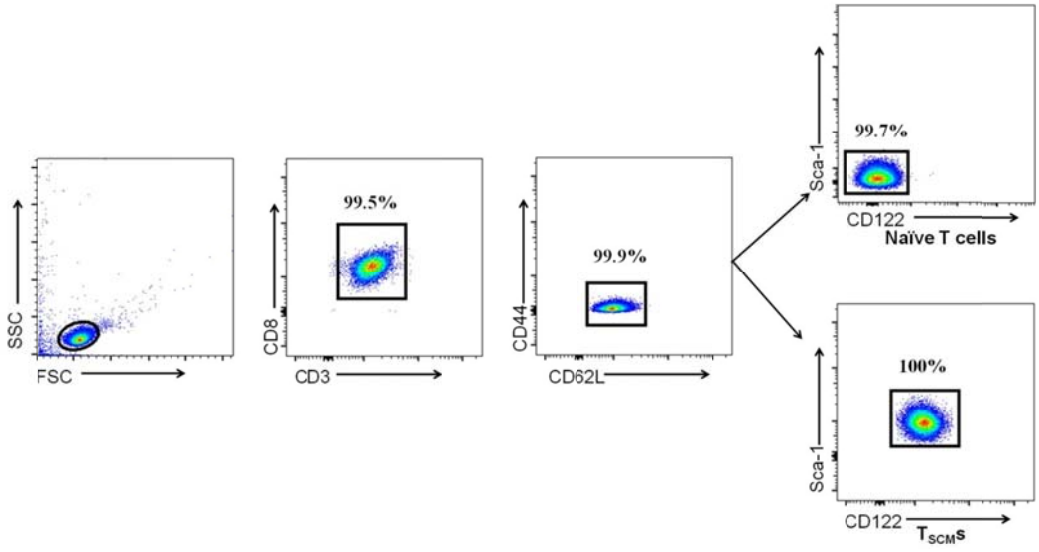

Figure S6. **The purity of naïve T cells ($CD3^+$ $CD8^+$ $CD44^{low}$ $CD62L^{high}$ $Sca\text{-}1^{low}$ $CD122^{low}$) and T_{SCM}s ($CD3^+$ $CD8^+$ $CD44^{low}$ $CD62L^{high}$ $Sca\text{-}1^{high}$ $CD122^{high}$) after sorted by flow cytometry.**

The $CD3^+$ $CD8^+$ $CD62L^+$ $CD44^-$ $Sca\text{-}1^{high}$ $CD122^{high}$ cells and $CD3^+$ $CD8^+$ $CD44^{low}$ $CD62L^{high}$ $Sca\text{-}1^{low}$ $CD122^{low}$ cells were sorted. Numbers in dot plots show the purities of $CD3^+$ $CD8^+$ $CD44^{low}$ $CD62L^{high}$ $Sca\text{-}1^{high}$ $CD122^{high}$ cells and $CD3^+$ $CD8^+$ $CD44^{low}$ $CD62L^{high}$ $Sca\text{-}1^{low}$ $CD122^{low}$ cells which were sorted by flow cytometry. Data are representative for three independent experiments (n=5).

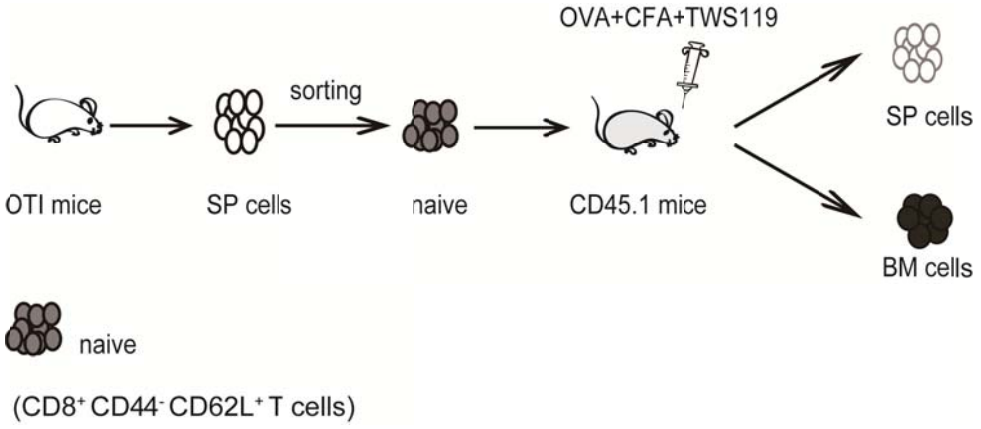

Fig S7.The strategy for testing frequency of in-vivo generated $CD8^+$ T_{SCM}s with TWS119.

The 2×10^6 OT-Ⅰ naïve $CD8^+$ T cells were adoptively transferred into congenic CD45.1 mice and then injected i.p. 500 μg per mice ovalbumin (OVA) (Sigma) with complete Freund's adjuvant (CFA) (Sigma). Mice received four doses per day of TWS119 at 40 mg/kg from day 0 to day 3. Six days after injection, mice with or without the treatment of TWS119 were sacrificed for further analysis.

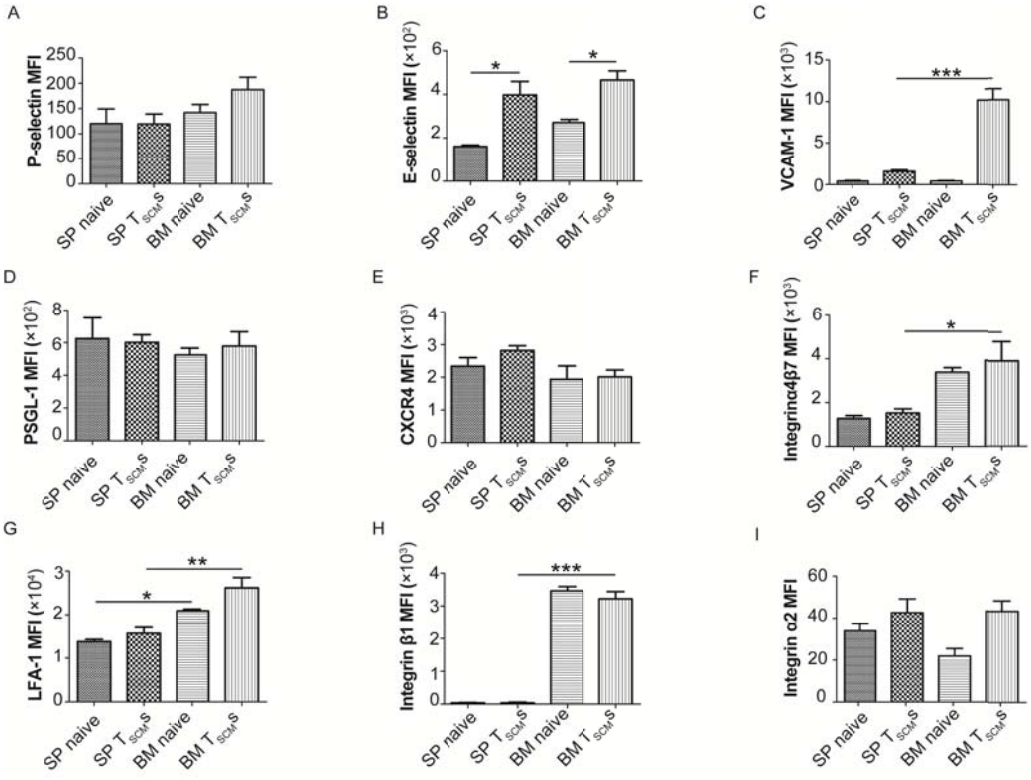

Figure S8. Expression levels of adhesion molecules on the surface of T_{SCM}s.

The MFI of a given adhesion molecule in different $CD8^+$ T cell subsets was analyzed by flow cytometry. $CD8^+$ T cell subsets were defined as follows: BM-resident T_{SCM}s, $CD3^+$ $CD8^+CD4^-CD44^{low}$ $CD62L^{high}CD122^{high}$ $Sca\text{-}1^{high}$; BM- and SP-resident naïve T cells, $CD3^+$ $CD8^+$ $CD4^-$ $CD44^{low}CD62L^{high}$ $CD122^{low}Sca\text{-}1^{low}$.Data are representative for three independent experiments (n=6), one-way ANOVA, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

Table I

Primer sequences for qRT-PCR.

Ccr2 forward	ATGGAAGACAATAATATGTTACC
Ccr2 reverse	ATGACAAGGCTCACCATC
Tcf 7 forward	GTACATGGAGAAGCCGAGGG
Tcf 7 reverse	ACTCTGGAAGTTTGTCCGGG
Tcf 7 RT	ACCTCCTAGGCCAATAGGGAGGTCCAGCCA
Klf7 forward	CGTTGAAACTGGTGGCCAAG
Klf7 reverse	ATAAACTTTCCGGCACCCGT
Lef1 forward	AGCACGGAAAGAGAGACAGC
Lef1 reverse	GCTGTCATTCTGGGACCTGT
Mouse beta catenin forward	CGCCGCTTATAAATCGCTCC
Mouse beta catenin reverse	TTCACAGGACACGAGCTGAC
Ccr5 forward	GTTGTTTTGGAGAACGCCCC
Ccr5 reverse	CAACACTGCTCCGAAACTGC
CD122 forward	TTGTCTGCTACCTAAGCTGCG
CD122 reverse	GGCTCCAGGGAAGAGCTATG
CD44 forward	AAAAAGCCATGCAGCAGCTC
CD44 reverse	TTGCCTCTTGGGTGGTGTTT
Ly6a forward	CCACATCTGACAGAACTTGCC
Ly6a reverse	GCTGCACAGATAAAACCTAGCAG
Mouse *GAPDH* forward	GGACCTCATGGCCTACATGG
Mouse *GAPDH* reverse	TAGGGCCTCTCTTGCTCAGT