Supplementary information

Wnt signaling arrests effector T cell differentiation and generates CD8⁺ memory stem cells

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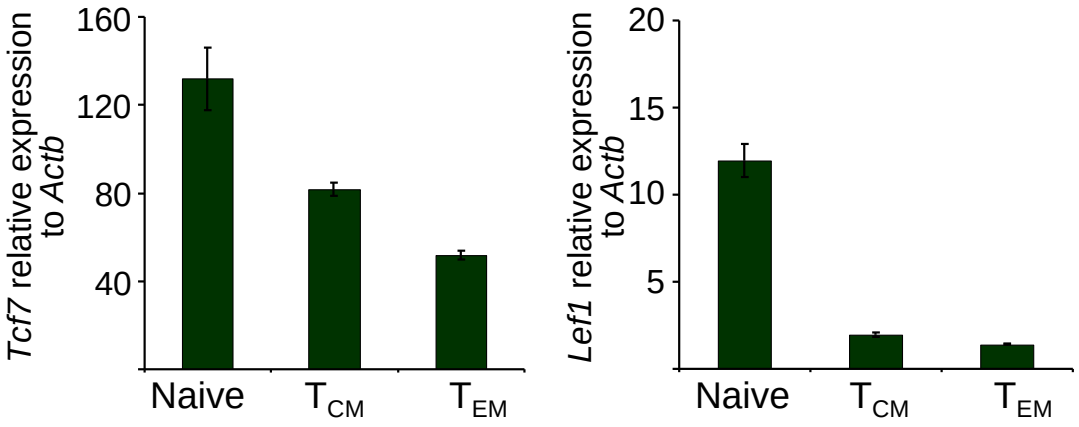
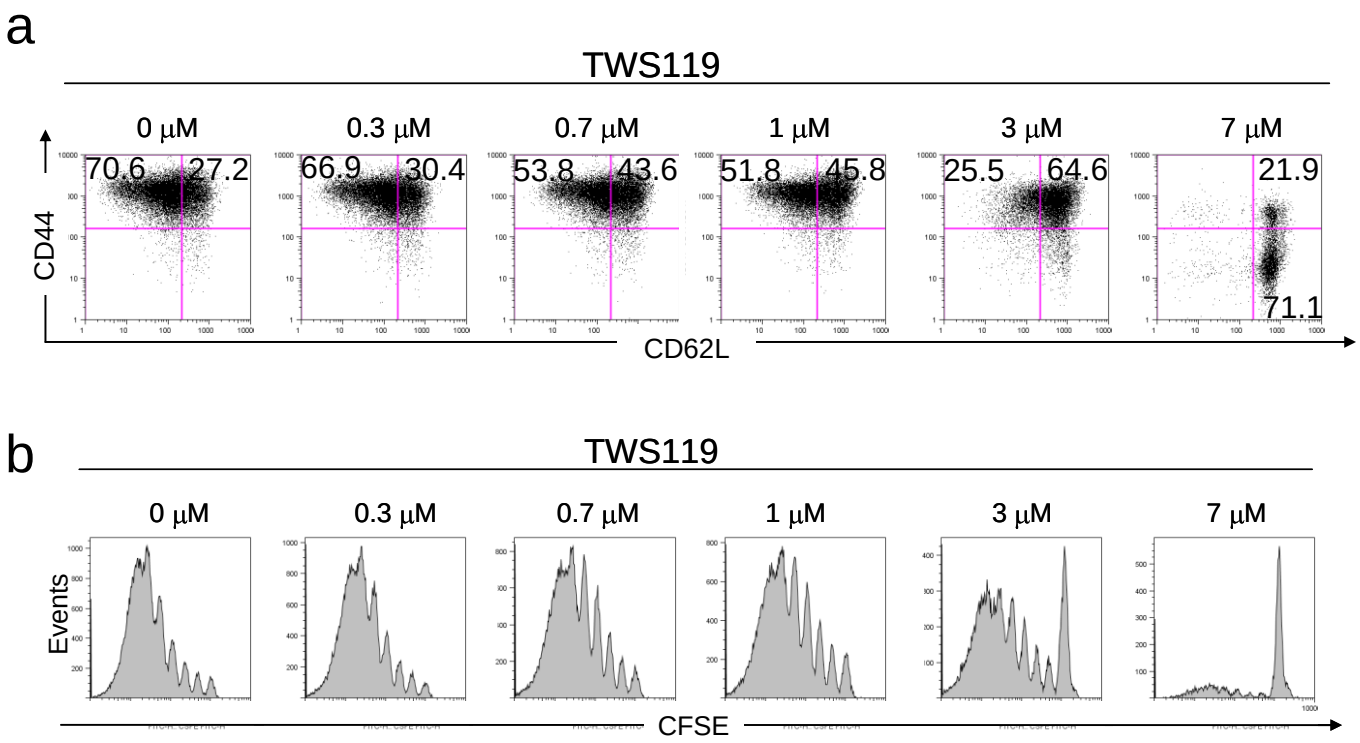
Supplementary Fig.1



Supplementary Figure 1. Expression of *Tcf7* and *Lef1* is down-regulated with progressive CD8⁺ T-cell differentiation. Mice receiving 10^6 ly5.1⁺ naïve, pmel-1 CD8⁺ T cells were vaccinated with a recombinant vaccinia virus encoding hgp100. Three weeks after transfer, mice received a boost immunization with s.c injection of human $gp100_{25–33}$ peptide and incomplete Freund's adjuvant. Sixty days after transfer, ly5.1⁺ T_{CM} ($CD44^{high}$, $CD62L^{high}$) and T_{EM} ($CD44^{high}$, $CD62L^{low}$ cells) were sorted. T_N ($CD44^{low}$, $CD62L^{high}$) were sorted from pmel-1 mice splenocytes. Expression of *Tcf7* and *Lef1* was determined by RT-PCR. Data are represented as mean +/– SEM

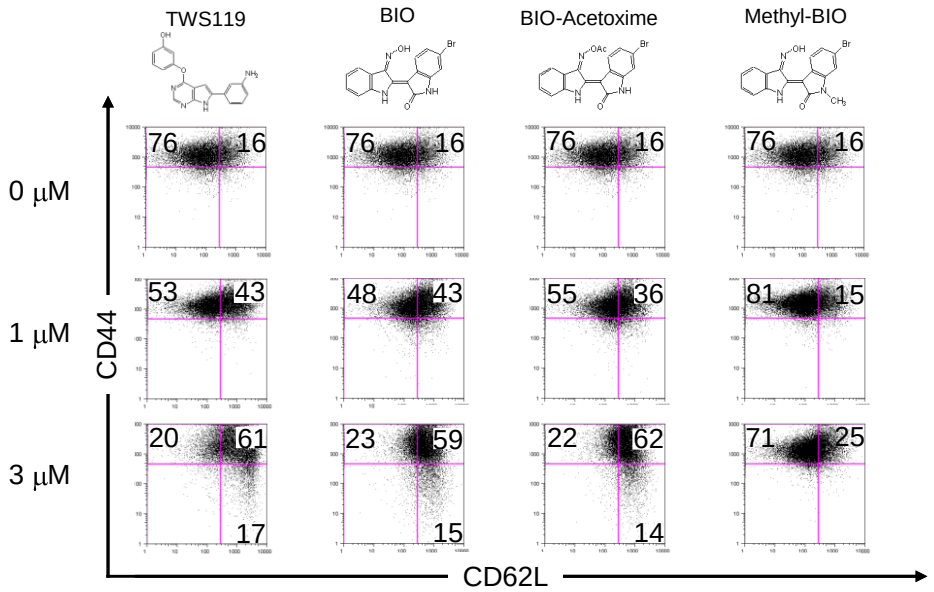
Supplementary Fig.2



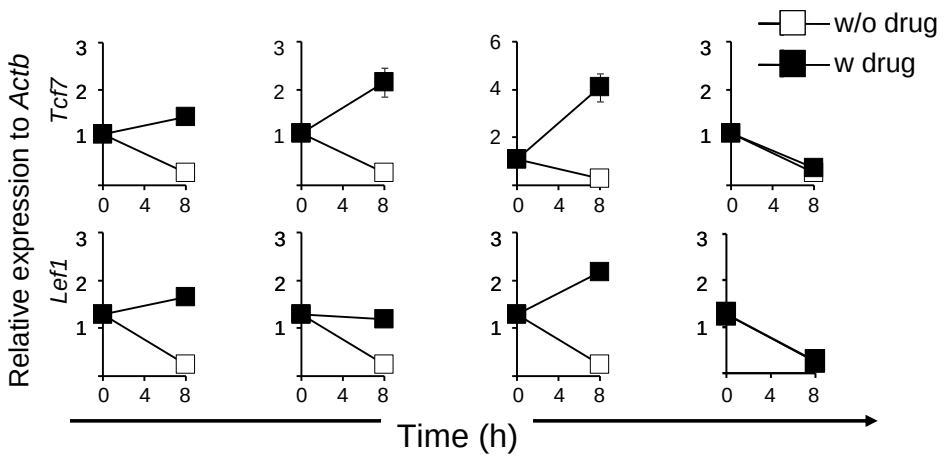
Supplementary Figure 2. Direct inhibition of CD8⁺ T cell proliferation and effector differentiation by activation of Wnt signaling. a,b, CFSE-labeled, naive CD8⁺ T cells were primed *in vitro* with anti-CD3 (2 μg ml^{-1}) and anti-CD28 (1 μg ml^{-1}) specific antibodies in conjunction with 10 ng ml^{-1} IL-2 and titrated doses of TWS119. Four days following T cell activation, T cell phenotype **(a)** and CFSE dilution (**b)** were evaluated

Supplementary Fig.3

a

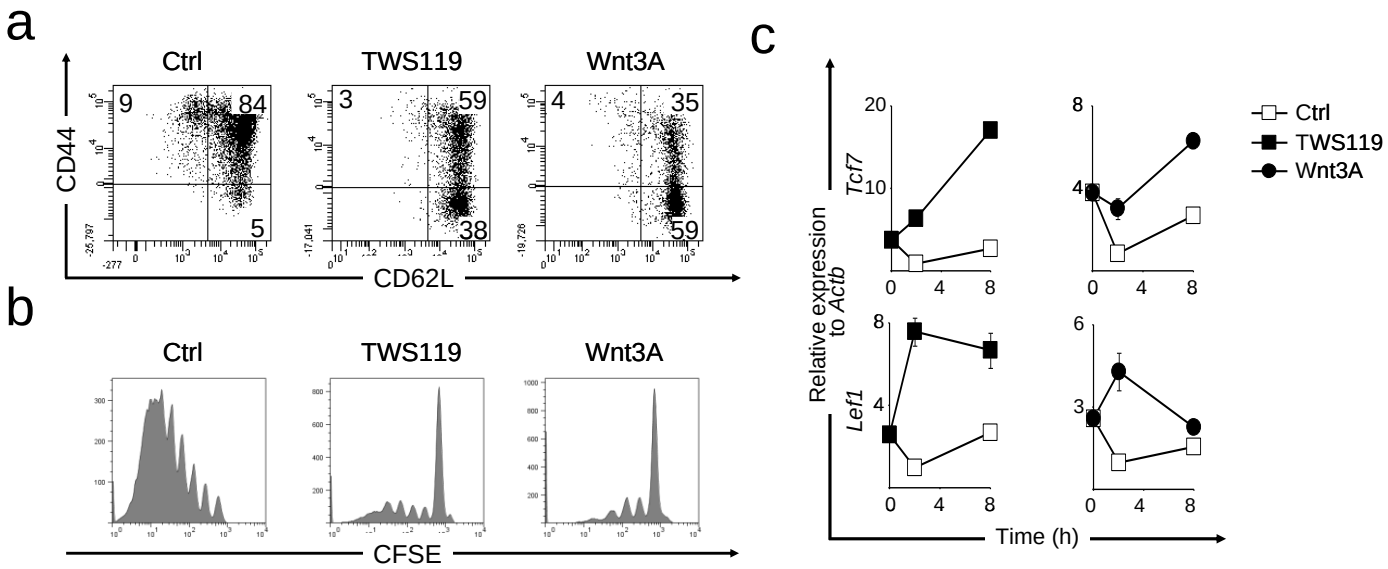


b



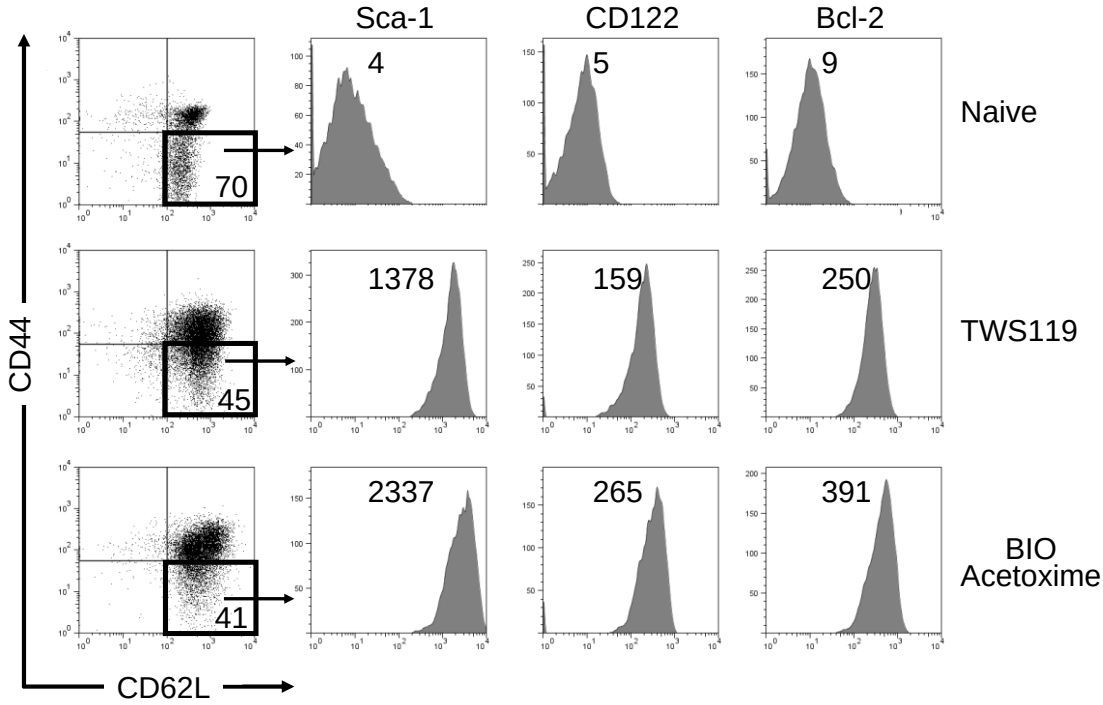
Supplementary Figure 3. BIO and BIO-acetoxime induce Wnt signaling and inhibit CD8⁺ T cell effector differentiation. a, BIO and BIO-acetoxime inhibit the acquisition of the phenotypic traits of effector T cells. Pmel-1 splenocytes were primed *in vitro* with 1 μM $hgp100_{25–33}$, in conjunction with 10 ng ml^{-1} IL-2 and titrated doses of TWS119, BIO and BIO-acetoxime. Methyl-BIO, a N-methylated analog of BIO was used as a relevant kinase inactive control. Four days following T cell activation, phenotypic analyses were performed. **b,** BIO and BIO-acetoxime promote the expression of *Tcf7* and *Lef1*. Naive $CD8^+$ T cells were primed *in vitro* with anti-CD3 and anti-CD28 specific antibodies with or without 3 μM of indicated compounds. Expression of *Tcf7* and *Lef1* was determined by RT-PCR. Data are represented as mean +/– SEM

Supplementary Fig.4



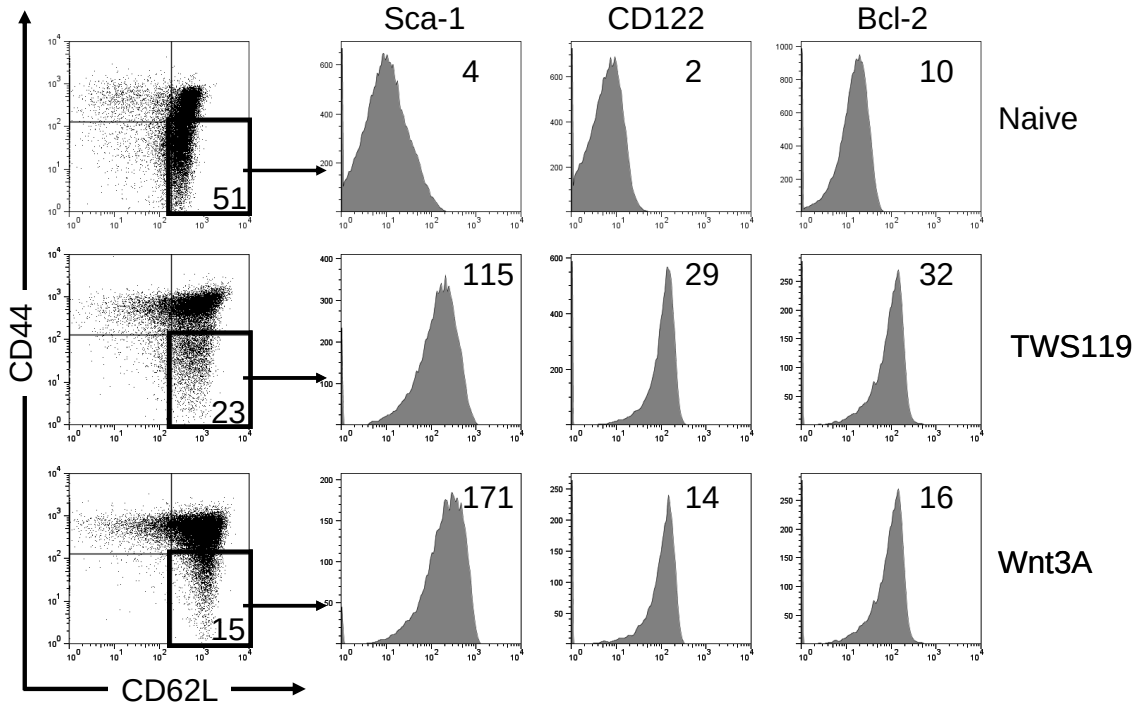
Supplementary Figure 4. Wnt3A inhibits CD8⁺ T cell proliferation and effector differentiation. Pmel-1 naive CD8⁺ T cells were primed *in vitro* with anti-CD3 (2 μg ml^{-1}) and anti-CD28 (1 μg ml^{-1}) specific antibodies in conjunction with 10 ng ml^{-1} IL-2 with or without 1 μg ml^{-1} of Wnt3A or 3 μM TWS119. Four days following T cell activation, phenotypic (**a**) and CFSE dilution assays (**b**) were performed. **c**, Wnt3A promotes the expression of *Tcf7* and *Lef1*. Naive CD8⁺ T cells were primed *in vitro* with anti-CD3 (2 μg ml^{-1}) and anti-CD28 (1 μg ml^{-1}) specific antibodies with or without 3 μg ml^{-1} of Wnt3A or 3 μM TWS119. Expression of *Tcf7* and *Lef1* was determined by RT-PCR. Data are represented as mean +/– SEM.

Supplementary Fig.5



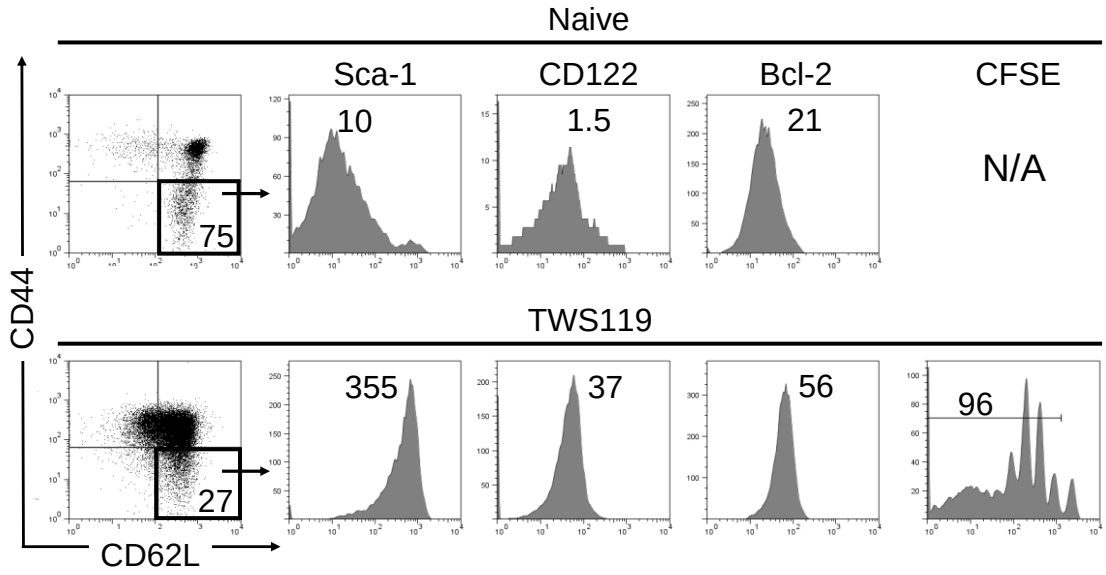
Supplementary Figure 5. BIO-acetoxime promotes the generation of T_{SCM}. Pmel-1 naive $CD8^+$ T cells were primed *in vitro* with 1 μM $hgp100_{25–33}$, in conjunction with 10 ng ml^{-1} IL-2 and 3 μM TWS119, or BIO-acetoxime. Four days following T cell activation, TWS119-treated, BIO-acetoxime-treated and naive pmel-1 cells were evaluated by flow cytometry for the expression of CD62L, CD44, Sca-1, CD122 and Bcl-2 in $CD8^+$ lymphocytes

Supplementary Fig.6



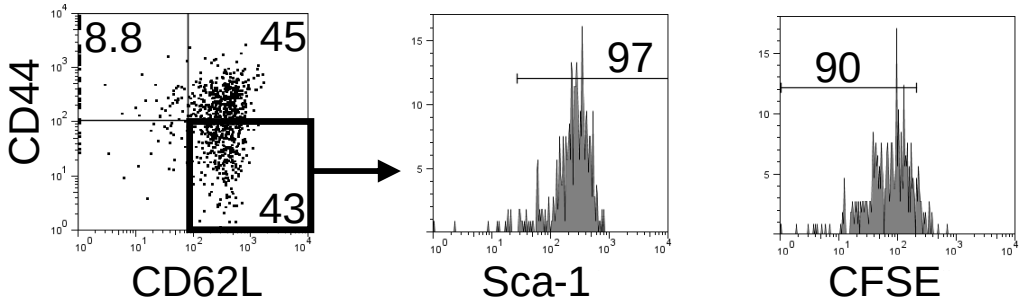
Supplementary Figure 6. Wnt3A promotes the generation of T_{SCM}. Pmel-1 naive $CD8^+$ T cells were primed *in vitro* with anti-CD3 (50 ng ml^{-1}) and anti-CD28 (1 μg ml^{-1}) specific antibodies in conjunction with 10 ng ml^{-1} IL-2 and 1 μg ml^{-1} of Wnt3A or 1 μM TWS119. Five days following T cell activation, Wnt3A-treated, TWS119-generated and naive pmel-1 cells were evaluated by flow cytometry for the expression of CD62L, CD44, Sca-1, CD122 and Bcl-2 in $CD8^+$ lymphocytes

Supplementary Fig.7



Supplementary Figure 7. Wnt signaling promotes the generation of T_{SCM} *in vivo*. C57BL/6 mice received adoptive transfer of 1.5 × 10^6 CFSE-labeled naive pmel-1 thy1.1$^+$ CD8$^+$ T cells in conjunction with recombinant fowlpox-based hgp100 vaccine. Mice received four daily doses of TWS119 (at 30 mg kg^{-1}) from day 0 to day 3. Six days after treatment, treated mice and unmanipulated pmel-1 mice were sacrificed. Splenocytes from two pooled mice were analyzed by flow cytometry

Supplementary Fig.8



Supplementary Figure 8. TWS119-generated T_{SCM} are multipotent and capable of long-term self-renewal. Four weeks after primary transfer CD44^{low} CD62L^{high}, Sca-1^{high} thy1.1^{+} pmel-1 CD8^{+} T cells were re-isolated and re-labeled with CFSE prior to secondary transfer into sublethally-irradiated C57BL/6 mice. Four weeks later, pooled cells from spleen and lymph nodes were analyzed by flow cytometry for the expression of CD62L, CD44, Sca-1 and CFSE in ly5.1^{+}, CD8^{+} lymphocytes