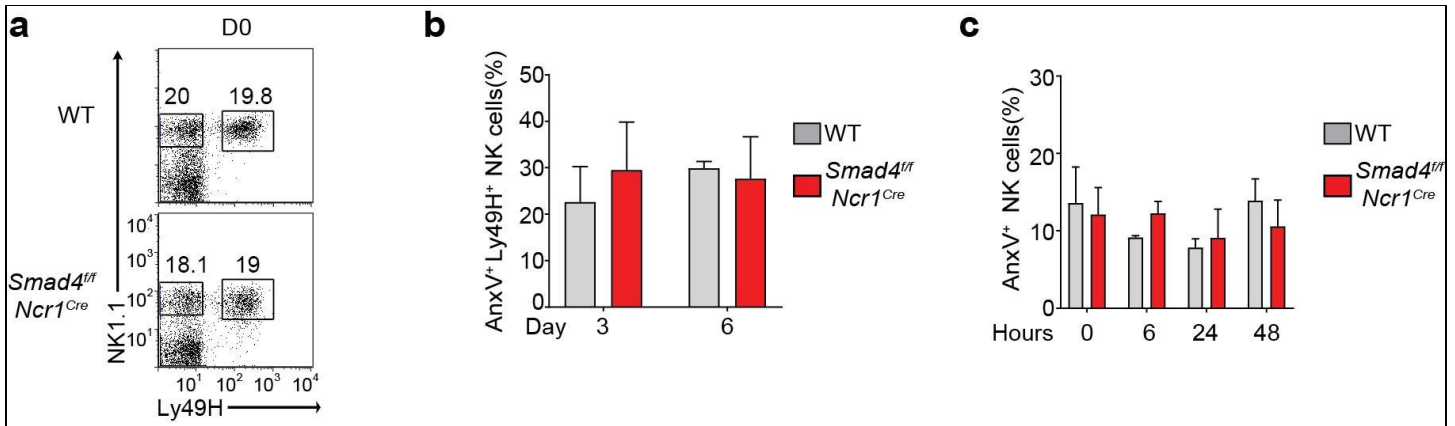


Supplementary Figure 1

Marker expression by NK cells and from WT and *Smad4^{fl} x Ncr1^{Cre}* mice.

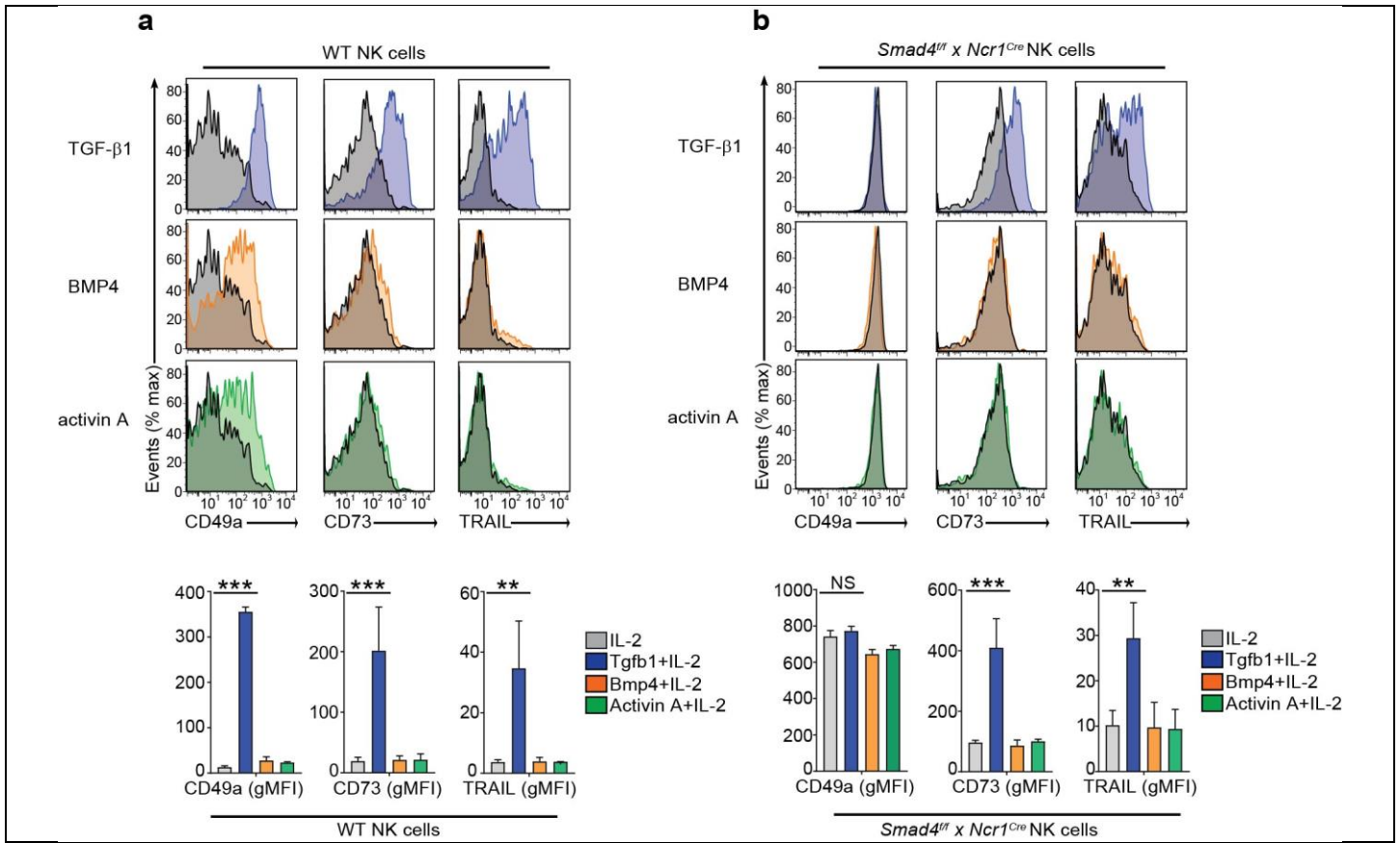
(a) Representative plots displaying pre-sort and post-sort purity of splenic NK cells from WT and SMAD4-deficient mice. (b) Quantification of surface expression of the indicated markers by splenic NK cells from WT and SMAD4-deficient mice. (c) Representative histograms showing expression of the indicated proteins by splenic NK cells from WT and SMAD4-deficient mice. (d) Representative plots and quantification of siLP ILC3 populations in WT and SMAD4-deficient mice. (e) Representative plots and quantification of intracellular IL-22 by siLP NKp46⁺ ILC3 after stimulation by IL-23. (f) Representative histograms of cell surface expression of CD49a in NKp46⁻ or NKp46⁺ NK cells from the bone marrow of WT and *Smad4^{fl/fl} × Il7r^{Cre}* mice. ***P* < 0.01, ****P* < 0.001; (unpaired Student's *t*-test). Data are pooled from four (b) or two (d,e,f) independent experiments with 1-2 mice of each genotype for each independent experiment (error bars represent mean ± s.d. in b,d,e).



Supplementary Figure 2

Apoptosis of WT and SMAD4-deficient NK cells during MCMV infection or *in vitro* culture.

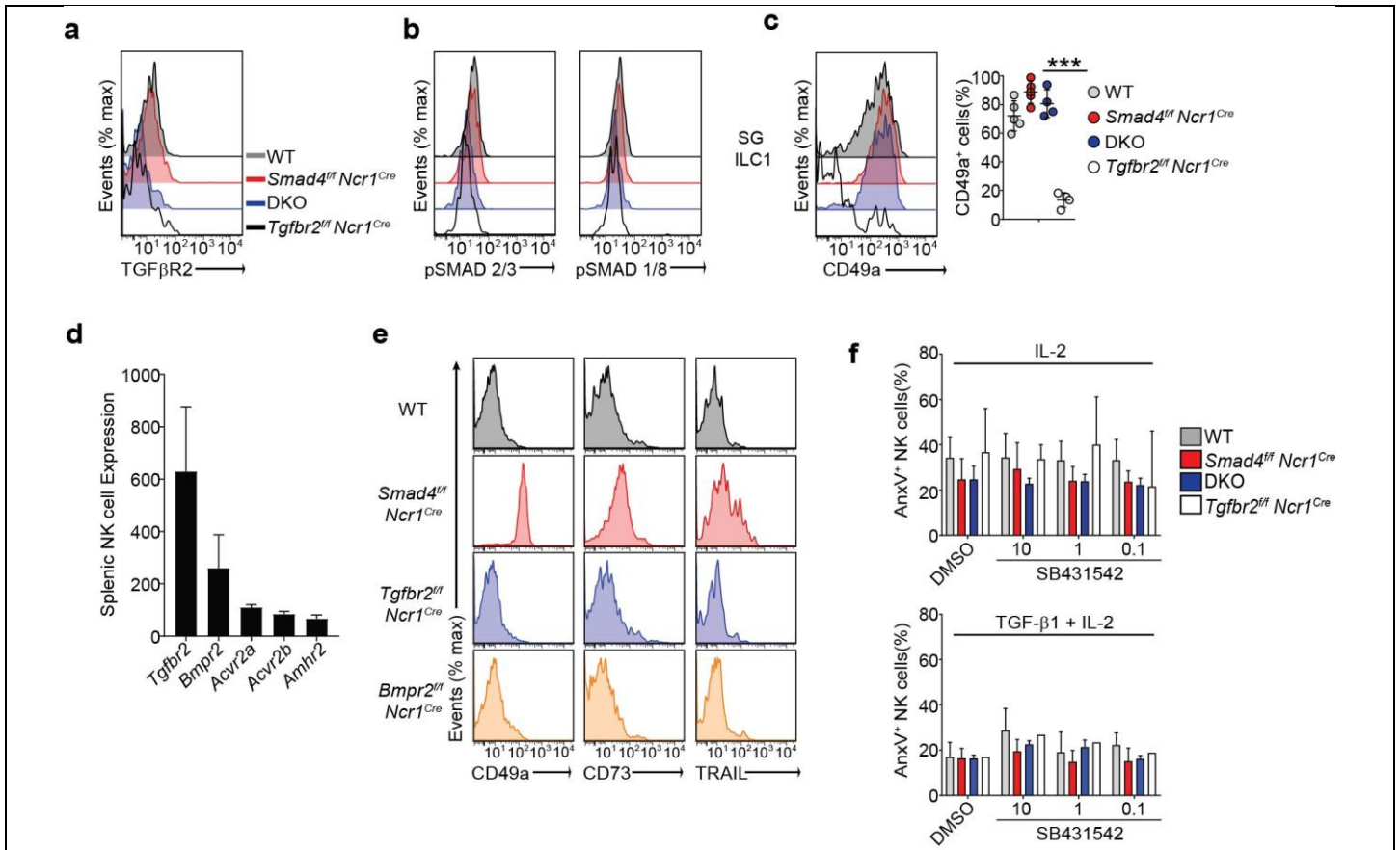
(a) Representative plots of Ly49H⁺ and Ly49H⁻ splenic NK cells (gated on CD3⁺CD19⁻) from naive WT and *Smad4^{fl/fl} x Ncr1^{Cre}* mice. (b) Ly49H⁺ splenic NK cells from WT and *Smad4^{fl/fl} x Ncr1^{Cre}* mice positive for Annexin V at days 3 and 6 of MCMV infection. (c) Splenic NK cells from WT and *Smad4^{fl/fl} x Ncr1^{Cre}* mice positive for Annexin V ex vivo or after 24 and 48 h of culture with IL-2. Data are pooled (b, c) from two independent experiments with at least 1-2 mice of each genotype for each independent experiment (error bars represent mean \pm s.d. in b, c).



Supplementary Figure 3

Culture of WT and SMAD4-deficient NK cells with TGF- β -family cytokines

Representative histograms and quantification of CD49a, CD73, and TRAIL surface expression by (a) WT and (b) SMAD4-deficient NK cells after 48 h of culture with TGF- β 1 + IL-2, BMP4 + IL-2, activin A + IL-2, or IL-2 alone. ** $P < 0.01$, *** $P < 0.001$ (unpaired Student's t -test). Data are pooled from three independent experiments with 1-2 mice of each genotype for each independent experiment (error bars represent mean \pm s.d.).



Supplementary Figure 4

Impact of SMAD4, DKO, TGFβ2, and BMP2 deficiency on conventional NK cells and SG ILC1.

(a) Representative histograms of TGFβ2 expression by splenic NK cells from WT, *Smad4^{fl/fl} x Ncr1^{Cre}*, DKO, and *Tgfb2^{fl/fl} x Ncr1^{Cre}* mice *ex vivo*. (b) Representative histograms of intracellular phospho-SMAD2/3 and phospho-SMAD1/8 in splenic NK cells after 60 min of culture with TGF-β1 and IL-2. (c) Representative histograms and quantification of CD49a expression by SG ILC1 from WT, *Smad4^{fl/fl} x Ncr1^{Cre}*, DKO, and *Tgfb2^{fl/fl} x Ncr1^{Cre}* mice. (d) Relative gene expression of type II receptors by splenic NK cells of WT mice (Immgen.org). (e) Representative histograms of cell surface expression of CD49a, CD73, and TRAIL (after 48 h of culture with IL-2) by splenic NK cells from WT, *Smad4^{fl/fl} x Ncr1^{Cre}*, *Tgfb2^{fl/fl} x Ncr1^{Cre}*, and *Bmpr2^{fl/fl} x Ncr1^{Cre}* mice. (f) Quantification of Annexin V⁺ NK cells from WT, *Smad4^{fl/fl} x Ncr1^{Cre}*, DKO, and *Tgfb2^{fl/fl} x Ncr1^{Cre}* mice after 48 h of culture with IL-2 or TGF-β1 plus IL-2 and DMSO or SB431542. ****P* < 0.001 (unpaired Student's *t*-test). Data are pooled from at least two independent experiments with 1-2 mice of each genotype for each independent experiment (error bars represent mean ± s.d.).