Supplemental Figure 1

A

B

C

% expression

WT
SLP-76 KO

* WT
* SLP-76 KO

Ly49D+  Ly49H+  Ly49G2+  Ly49A+  Ly49C/I+

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Supplemental Figure 1. SLP-76 KO mice express normal NK cell maturation markers but decreased proportions of Ly49-expressing NK cells. The expression of NK cell-associated receptors on splenic NK cells from WT and SLP-76 KO mice was examined by flow cytometry. (A) CD3⁻CD122⁺ lymphocytes from WT (top plots) and SLP-76 KO mice (bottom plots) were analyzed for the expression of DX5, NK1.1, CD27, and CD11b. (B) CD3⁻NK1.1⁺ lymphocytes from WT (top plots) and SLP-76 KO mice (bottom plots) were analyzed for the expression of Ly49A, Ly49C/I, Ly49G2, Ly49D, Ly49H, and NKG2A. One representative of 3-8 independent experiments is shown. (C) Splenic NK cells (CD3⁻NK1.1⁺ lymphocytes) from WT and SLP-76 KO mice were analyzed for the expression of Ly49A, Ly49C/I, Ly49G2, Ly49D, and Ly49H and represented as mean % positive ± SEM of 3-8 independent experiments.

Supplemental movies: Bone marrow chimeric mice were generated with WT or LAT/LAT2 DKO murine splenic bone marrow retrovirally transduced with WT SLP-76, a Gads binding mutant of SLP-76 (G2), a SH2 mutant of SLP-76 (RK), or a double G2/RK mutant GFP fusion protein. WT cell with WT.SLP-76 (video 1), LAT1/LAT2 DKO cell with WT.SLP-76 (video 2), WT cell with Gads-binding mutant (G2.SLP-76) (video 3), WT cell with SH2 mutant (RK.SLP-76) (video 4), ADAP KO cell with WT.SLP-76 (video 5), WT cell with G2.RK.SLP-76 (video 6), LAT1/LAT2 DKO cell with RK.SLP-76 (video 7), WT cell with Y3F.G2.RK.SLP-76 (video 8). 8 weeks later, splenic NK cells were harvested, expanded in IL-2, then stimulated on an anti-Ly49D Ab coated surface. Clustering of SLP-76 at the plasma membrane was imaged by TIRF microscopy. Frames collected at 1 frame/second. Representative movie shown, still
images in Figure 6. Scale bar 50 μm. Representative of 3-8 independent experiments with 27-78 total cells analyzed.