**Supplementary data**

**Supplementary Fig. S1**

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**Supplementary Fig. S1. Gating strategies for flow cytometric analysis or sorting.** (A) The gating strategy for Fig. 1A-F, Fig. 2D-F, Fig. 3A-F, Fig. 4A-H, Fig. 5A-G, Fig. 6A, D-I, and Supplementary Fig. S2A, Fig. S3C, Fig. S4A, B, Fig. S5A, B, Fig. S6A-C, S7A-C. The last panel was not applied in some experiments (A). (B) The gating strategy for Supplementary Fig. S3D, E. (C) The gating strategy for Fig. 2A. (D) The gating strategy for Fig. 2B, C, and Supplementary Fig. S3F, Fig. S4C.

**Supplementary Fig. S2**

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**Supplementary Fig. S2. SRC-3 displays increased nuclear translocation in NK cells upon inflammatory cytokines stimulation.** (A) Western blotting analysis of SRC-3 expression in T, B and NK cells in the spleen of normal WT C57 mice. Representative images are shown. (B) SRC-3 expression profile from the BioGPS dataset (http://biogps.org/). (C-E) Human NK-92 cells were starved in complete DMEM Alpha medium without IL-2 overnight. At indicated time after stimulated with (C) IL-2 (200 IU/ml), (D) IL-12 (10 ng/ml) or (E) IL-15 (100 ng/ml), the expression of total or nuclear SRC-3 protein in NK-92 cells was analyzed by western blot. Nuclear or total SRC-3 protein was normalized to Lamin B1 or β-actin, respectively. Representative images are shown in the left, and densitometry quantified data from three independent experiments are shown in the right (All data was compared with 0 min group). Data are shown as mean ± SD (C-E) \*\*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001. one-way ANOVA (C-E).

**Supplementary Fig. S3**

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**Supplementary Fig. S3. SRC-3 deficiency has no effect on the early development of NK cells.** (A) The strategy for conditional deletion of SRC-3 (*Ncoa3* is the gene name of SRC-3). The floxed *Ncoa3* exon loci (exon 6) before and after Cre-mediated excision are indicated. Primers P1 and P2 were used to amplify wild-type (WT) band (913 bp), floxed band (1023 bp) and deleted band (217 bp). (B) Genotyping analysis for conditional SRC-3 deletion in the BM cells from SRC-3+/+, SRC-3fl/+, SRC-3fl/fl and SRC-3∆/∆ mice. (C) Western blotting analysis of SRC-3 expression in total BM cells from Control and SRC-3∆/∆(hematopoietic-specific) mice. (D, E) Flow cytometric analysis of the percentages and numbers of CLP (Lin- 2B4+ CD27+ CD127+ CD135+ CD122-), pre-NKP (Lin- 2B4+ CD27+ CD127+ CD135- CD122-) and rNKP (Lin- 2B4+ CD27+ CD127+ CD135-CD122+) in the BM of Control and SRC-3∆/∆ (hematopoietic-specific) mice (n = 8 mice per group). Lineage (Lin) includes CD3, B220, Ter119, NK1.1, Gr-1 and CD11b. (F) The number of CD3- CD19- NK1.1+ NK cells in the lungs and liver of Control and SRC-3∆/∆(hematopoietic-specific) mice (n = 8 mice per group). Data are shown as mean ± SD (E, F). \*P < 0.05, \*\*P < 0.01. Student’s t-test (E, F).

**Supplementary Fig. S4**

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**Supplementary Fig. S4. The generation of mice with NK-specific SRC-3 deletion.** (A) PCR-based analysis of genomic DNA in CD3+ T cells and CD3- CD19- NK1.1+ NK cells sorted from the spleen of SRC-3fl/fl and SRC-3NK∆/∆(NK-specific) mice. (B) Western blotting analysis of SRC-3 expression in total NK cells (CD3- CD19- NK1.1+ Nkp46+) sorted from the spleen of SRC-3fl/fl and SRC-3NK∆/∆(NK-specific) mice. (C) The number of CD3- CD19- NK1.1+ NK cells in the lungs and liver of SRC-3fl/fl and SRC-3NK∆/∆(NK-specific) mice (n = 8 mice per group). Data are shown as mean ± SD (C). \*\*P < 0.01. Student’s t-test (C).

**Supplementary Fig. S5**

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**Supplementary Fig. S5. Loss of SRC-3 compromises NK cell maturation.** (A) Flow cytometric analysis of the percentages of CD27+ CD11b-, CD27+ CD11b+ and CD27- CD11b+ cells in total NK cells (CD3- CD19- NK1.1+ NKp46+) from the BM, spleen and pLNs of Control and SRC-3∆/∆mice (n = 8 mice per group). (B) Flow cytometric analysis of the percentage of CD43+ KLRG1+ cells in total NK cells (CD3- CD19- NK1.1+ NKp46+) from the BM, spleen and pLNs of Control and SRC-3∆/∆mice (n = 8 mice per group). Data are shown as mean ± SD (A, B). \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001. Student’s t-test (A, B).

**Supplementary Fig. S6**

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**Supplementary Fig. S6. Loss of SRC-3 promotes the proliferation and inhibits the survival of NK cells.** (A) Ki67 staining of total NK cells (CD3- CD19- NK1.1+ NKp46+) from the BM and spleen of SRC-3fl/fl and SRC-3NK∆/∆mice (n = 8 mice per group). (B) SRC-3fl/fl and SRC-3NK∆/∆mice were administrated with BrdU (1 mg/6 g mouse weight) via intraperitoneal injection. Twelve hours later, mice were sacrificed and the BrdU incorporation in total NK cells (CD3- CD19- NK1.1+ NKp46+) from their BM and spleen was analyzed by flow cytometry (n = 8 mice per group). (C) Flow cytometric analysis of the apoptosis of NK cells from the BM and spleen of SRC-3fl/fl and SRC-3NK∆/∆mice (n = 8 mice per group). Data are shown as mean ± SD (A-C). \*\*\*P < 0.001. Student’s t-test (A-C).

**Supplementary Fig. S7**

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**Supplementary Fig. S7. SRC-3 deficiency does not significantly affect the phosphorylation of STATs in NK cells after stimulation with cytokines.** (A-C)Flow cytometric analysis of the MFI of p-STAT3Tyr705, p-STAT4Tyr693, or p-STAT5Tyr694 in total NK cells (CD3- CD19- NK1.1+ NKp46+) from the spleen of SRC-3fl/fl and SRC-3NK∆/∆mice after stimulation with (A) IL-2 (200 IU/ml), (B) IL-12 (10 ng/ml) or (C) IL-15 (100 ng/ml) *in vitro* at the indicated time (n = 5 mice per group). Data are shown as mean ± SD (A-C). Student’s t-test (A-C).

**Supplementary Table S1**

|  |  |  |
| --- | --- | --- |
| **Gene** | **Forward primer (5’-3’)** | **Reverse primer (5’-3’)** |
| *Ncoa3* | GCCTGGCTTTGAAGACATAATCCG | TCTTGATAGTGACGCTTCTGGGAC |
| *Ets1* | TGGAATGTGCAGATGTCCCG | TGAGCATGCTCGATACCGTA |
| *Tox* | CTACTGCAGTAGTCGGTACTCC | CTGAAGCAGCTAGCAGCATAC |
| *Nfil3* | GAACTCTGCCTTAGCTGAGGT | ATTCCCGTTTTCTCCGACACG |
| *Gata3* | TCGGCCATTCGTACATGGAA | GAGAGCCGTGGTGGATGGAC |
| *Irf2* | CGATTATTCAACTGACGGGCTTTC | GCATTCGCATCCGTTCCAC |
| *Eomes* | GATGTACGTTCACCCAGAAT | ATCGTAGTTGTCCCGGAAGC |
| *Id2* | ATGAAAGCCTTCAGTCCGGTG | AGCAGACTCATCGGGTCGT |
| *Stat5a* | GTTTGAGTCTCAGTTCAGCGT | CATGGACGATAACGACCACAG |
| *Stat5b* | TGTCCCTGAAACGAATCAAGAG | CTGAACTGTGAGTCAAACAGGAT |
| *Tbx21* | AACCGCTTATATGTCCACCCA | CTTGTTGTTGGTGAGCTTTAGC |
| *Elf4* | AACGTGTCATCCACTGAAGTC | TCAGGGGTAGAGAGCAGGAAG |
| *Smad4* | ACACCAACAAGTAACGATGCC | GCAAAGGTTTCACTTTCCCCA |
| *Foxo1* | CCCAGGCCGGAGTTTAACC | GTTGCTCATAAAGTCGGTGCT |
| *S1pr5* | TGGCTAACTCGCTGCTGAATC | TCGCTGCAAGCTGTTGGAG |
| *Gzmb* | TCTCGACCCTACATGGCCTTA | TCCTGTTCTTTGATGTTGTGGG |
| *Prf1* | CTGCCACTCGGTCAGAATG | CGGAGGGTAGTCACATCCAT |
| *Prdm1* | CTTCTCTTGGAAAAACGTGTGGG | TCATATCAGCGTCCTCCATGT |
| *Zeb2* | CCACGCAGTGAGCATCGAA | CAGGTGGCAGGTCATTTTCTT |
| *Kit* | GCCTGACGTGCATTGATCC | AGTGGCCTCGGCTTTTTCC |
| *GAPDH* | CCTCGTCCCGTAGACAAAATG | TCTCCACTTTGCCACTGCAA |

**Primers for mRNA expression analysis**

**Supplementary Table S2**

|  |  |  |
| --- | --- | --- |
| **Gene** | **Forward primer (5’-3’)** | **Reverse primer (5’-3’)** |
| *Zeb2*  (-1.65kb) | AAGAGGAGAGTGTGTAACCAACTG | GAGCTACAGGGCTTTGTATTTTGC |
| *Zeb2*  (TSS) | CTCTGCAGGATTTAGTGATGAGGA | CCAAGTTTCTCTCTGGGAAAGGATC |
| *Prdm1*  (-0.1kb) | GGAAGTAAGAAGATTCCCAGTCCT | CCGCAGACTTCTTTACCAATACTG |
| *S1pr5*  (-1.85kb) | GAAGAGTCCCACCGTGCTTG | CAGTAGGCGCTTGACAAATGG |

**Primers for qRT-PCR analysis after ChIP**

**Supplementary Table S3**

The RNA-Seq data from SRC-3fl/fl and SRC-3NK∆/∆ NK cells (uploaded as separate excel file).