Runx3 Regulates Interleukin-15 Dependent Natural Killer Cell Activation

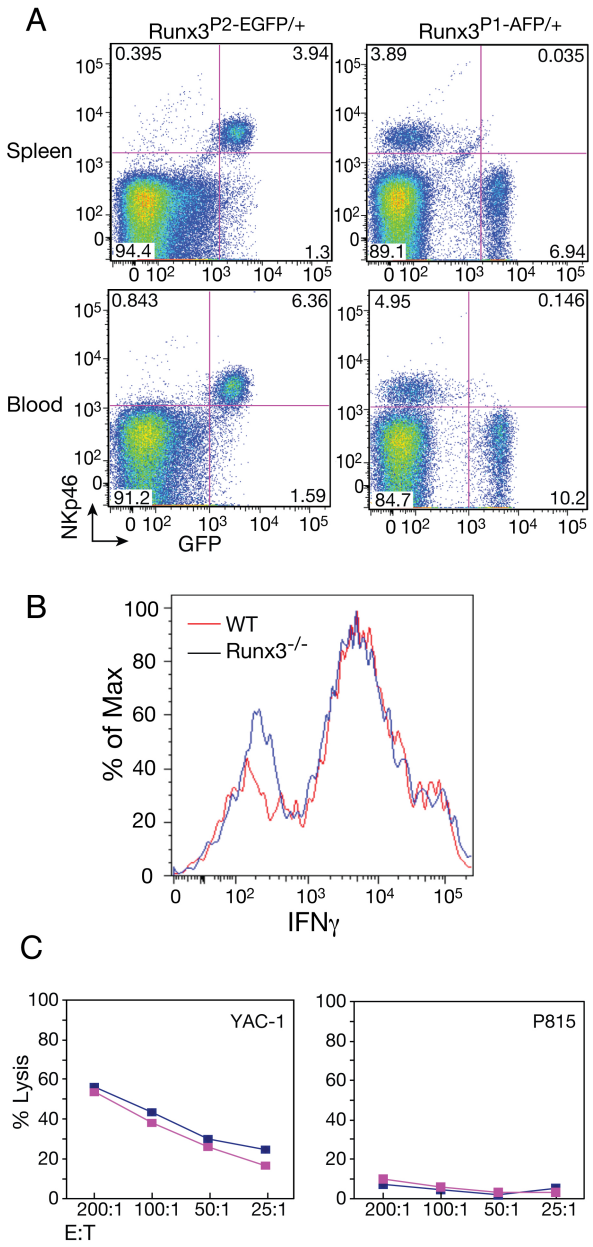
Supplemental material

Files in this data supplement:

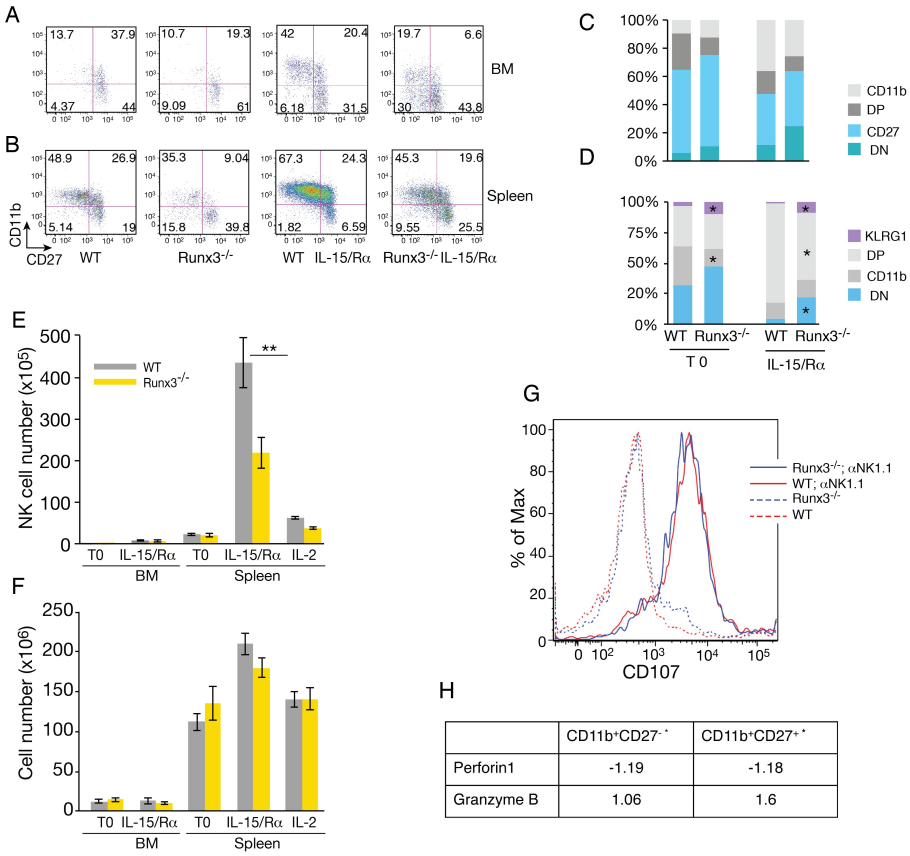
Supplemental file 1

Figures S1-S6

Tables S1-S6 (Tables S4 and S6 are appended as separate Excel files)



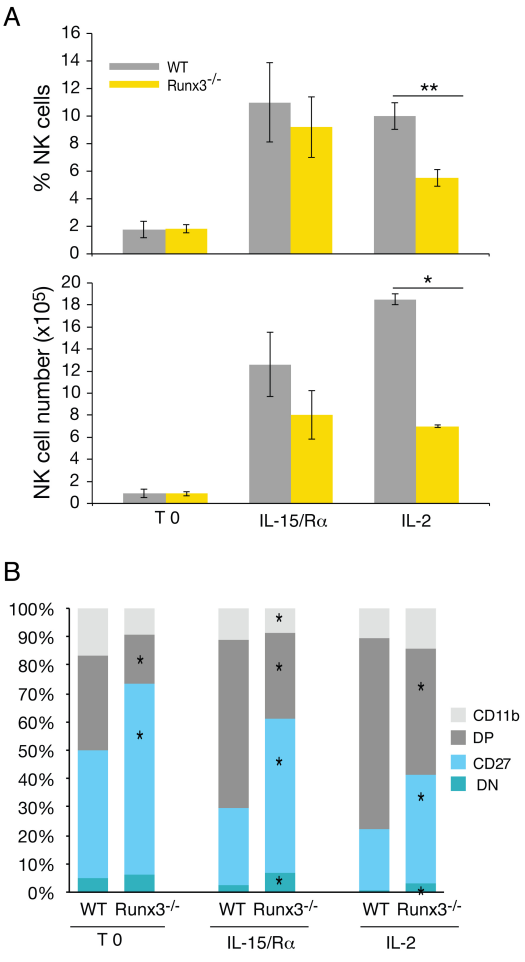
Supplementary Figure S1 Runx3 expression and function in NKCs under resting conditions. (A) Analysis of Runx3$^{P1\text{-}AFP/+}$ and Runx3$^{P2\text{-}EGFP/+}$ expression in spleen and blood. Lymphocytes were analyzed for co-expression of NKp46 and either P1, or P2-derived GFP. (B) IFNγ production by WT and Runx3-/- spleen NKCs. Splenocytes were analyzed for intracellular IFNγ production. (C) PolyI:C treated WT and Runx3$^{-/-}$ splenocytes were analyzed for cytotoxicity against the YAC-1 and P815 cell lines.



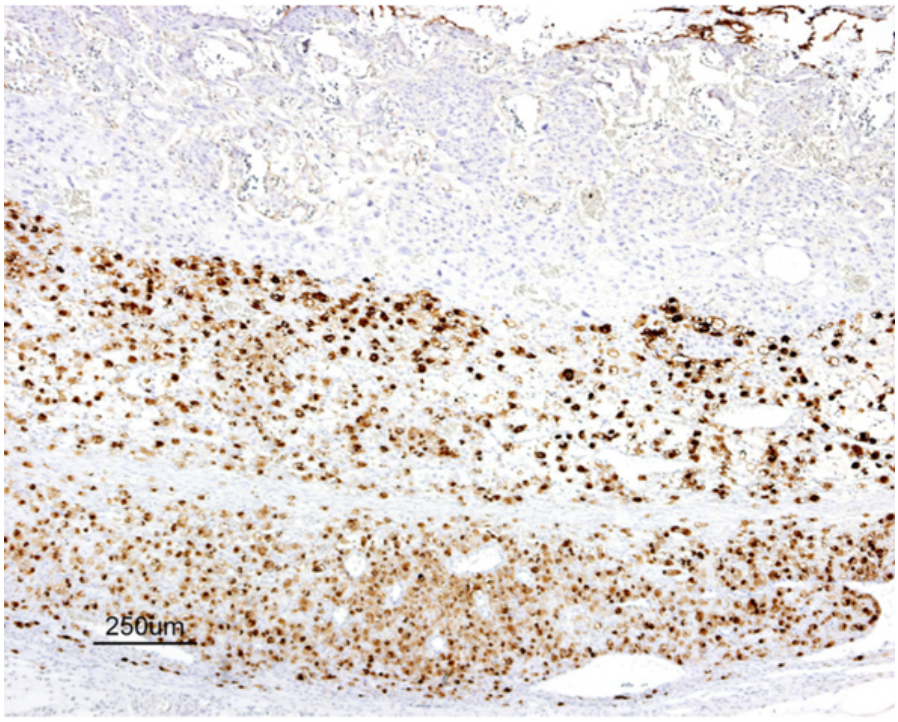
	CD11b⁺CD27⁻	CD11b⁺CD27⁺
Perforin1	-1.19	-1.18
Granzyme B	1.06	1.6

Supplementary Figure S2 Accumulation and maturation of WT and Runx3$^{-/-}$ NKCs following IL-2/15 *in vivo* activation. (A) Representative dot plots comparing maturation stages of WT and Runx3$^{-/-}$ BM NKCs under resting and *in-vivo* IL-15/Rα activation based on expression of CD11b and CD27. (B) Representative dot plots comparing maturation stages of WT and Runx3$^{-/-}$ spleen NKCs under resting and *in vivo* IL-15/Rα activation based on expression of CD11b and CD27. (C) Bar graphs showing the frequency of WT and Runx3$^{-/-}$ BM NKC subsets under resting conditions (left) and following IL-15/Rα activation (right) based on expression of CD11b and CD27. Mean values are shown for the four NKC sub-populations (n=3). (D) Bar graphs showing the frequency WT and Runx3$^{-/-}$ spleen NKC sub-populations under resting conditions (T0) or following IL-15/Rα activation, based on CD11b and KLRG1. Mean values are shown for the four populations (n=5). Significance: WT vs Runx3$^{-/-}$ NKC- *p<0.01. KLRG1=KLRG1^{+}CD11b^{-}; DP=KLRG1^{+}CD11b^{+}; CD11b=KLRG1^{-}CD11b^{+}; DN=KLRG1^{-}CD11b^{-}. (E) Number of NKp46^{+} NKCs in WT and Runx3$^{-/-}$ BM and spleen under resting conditions (T0) and following

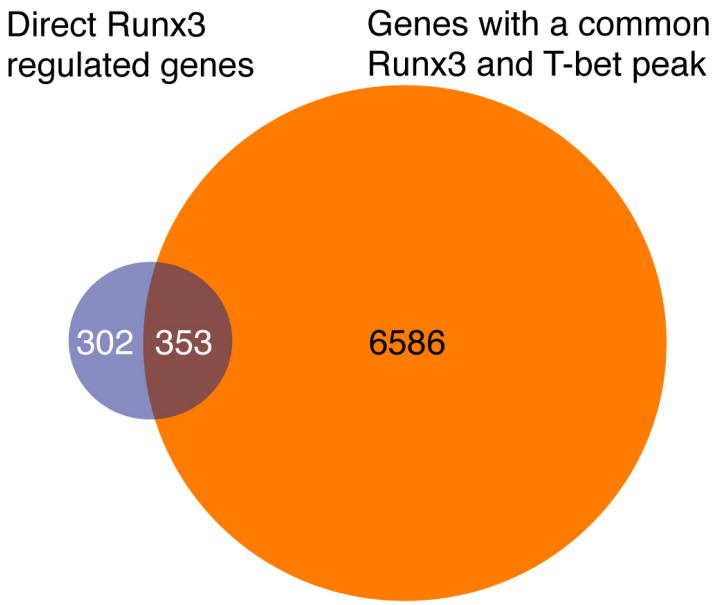
administration of IL-15/Rα or IL-2. Significance: **p<0.001. (F) Total number of cells in WT and Runx3$^{-/-}$ BM and spleen under resting conditions (T0) and following administration of IL-15/Rα or IL-2. (G) Degranulation of IL-15/Rα treated NKCs. Forty eight hours after IL-15/Rα administration splenocytes were stimulated with anti-NK1.1 for 4 hours and stained with anti-CD107. (H) Fold change of cytotoxic mediators in mature IL-15/Rα activated NKC (* marks the ratio between Runx3$^{-/-}$ and WT expression level in data from microarray analysis).



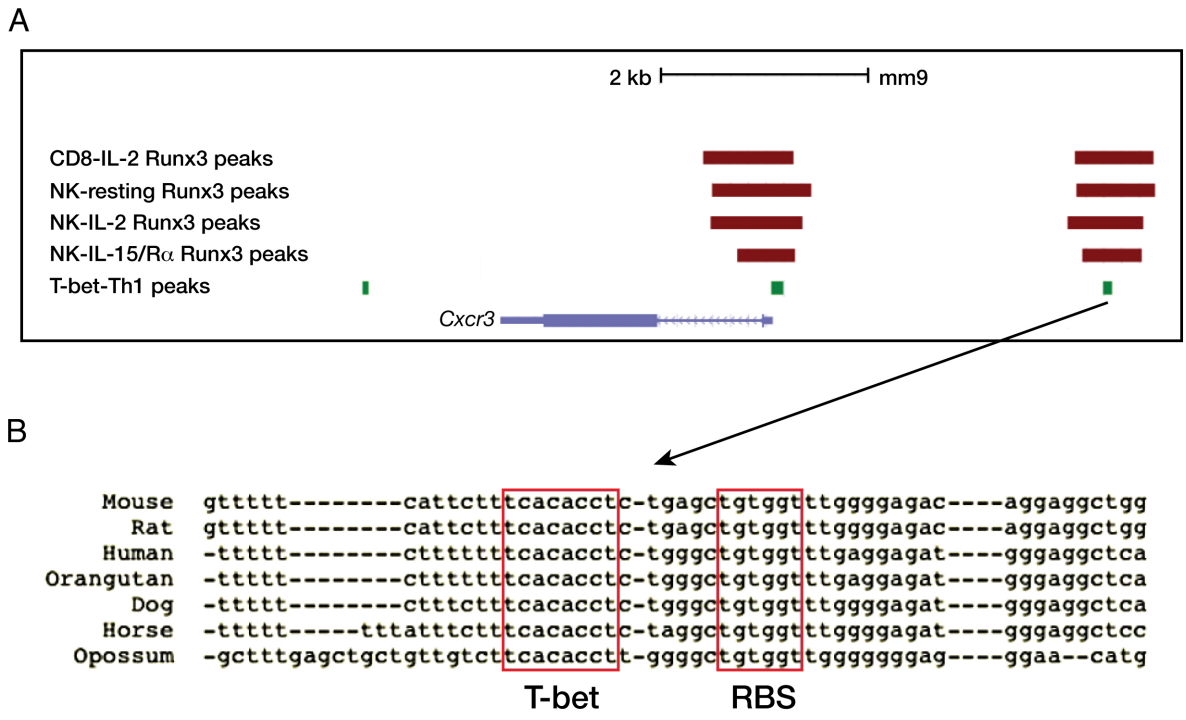
Supplementary Figure S3. Accumulation and maturation of peritoneal NKCs following administration of IL-15/Rα or IL-2 is severely affected by loss of Runx3. (A) Percentage (upper panel) and cell number (lower panel) of peritoneal NKp46+ NKCs in WT and Runx3$^{-/-}$ mice under resting conditions (T0) (n=5) and after injection of IL-15/Rα (n=4) or IL-2 (n=3). Significance: **p<0.01, *p< 0.05. (B) Bar graphs showing the frequency of WT and Runx3$^{-/-}$ peritoneal NKC sub-populations under resting conditions or following administration of IL-15/Rα or IL-2. Mean values are shown for the four sub-populations based on expression of CD11b and CD27. Significance: WT vs Runx3-/- NK cells **p<0.01, *p<0.05.



Supplementary Figure S4 uNKCs are present in deciduae of mice ablated of Runx3 in DCs. DBA staining of a gd10.5 decidua of Runx3$^{fl/fl}$/CD11c::Cre female.



Supplementary Figure S5 Runx3-regulated genes that harbor Runx3 peaks overlapping T-bet peaks in Th1 cells. Intersection between the list of Runx3-regulated genes from *in vivo* IL-15/Rα-activated NKCs and the list of genes harboring both Runx3 peaks in NKCs and T-bet peaks in Th1 cells.



Supplementary Figure S6 Runx3 and T-bet co-binding to the *Cxcr3* promoter and enhancer. (A) The genomic region of *Cxcr3* with location of Runx3 peaks (RBS) in resting and *in-vivo* IL-15/Rα-activated NKC and the location of T-bet peaks [2] in Th1 CD4+ T cells. (B) The conserved RUNX and T-bet binding sites in the *Cxcr3* enhancer.

Supplementary Table S1 Sequence of primers used for qPCR

Gene name	Sequence - 5' - 3'
Actb	F-ggctgtattcccctccatcg R-ccagttggtaacaatgccatgt
Prdm1	F-ttctcttggaaaaacgtgtggg R-ggagccggagctagacttg
Tnfrsf9	F-cgtgcagaactcctgtgataac R-gtccacctatgctggagaagg
Styk1	F-ggatcaccctaagccaaaaagt R-tgtaccaggttcttgtgtttcc
Crtam	F-ccttttcatcatcgttcagctct R-ggagcctggctgctattctc
CD96	F-tgggaagagctattcaatgttgg R-agaggccatattggggatgataa
Tigit	F-gaatggaacctgaggagtctct R-agcaatgaagctctctaggct
Tspan32	F-tgcgctattgggccttcttatg R-caccaggatgcagaatgacag
Cx3cr1	F-cggccatcttagtggcgtc R-ggatgttgacttccgagttgc
Itgb7	F-acctgagctactcaatgaagga R-caccgttttgtccacgaagg

Supplementary Table S2 Presence of uNKCs at implantation sites in pregnant mice

WT	$DC^{Runx3-/-}$	$Runx3^{-/-}$	$Runx3^{-/-}$ plus progesterone
+	+	-	-

*Progesterone (0.2mg) was injected daily between gd2.5-5.5

Supplementary Table S3 Enriched ontology terms identified by GREAT in Runx3 peaks of *in-vivo* IL-15/Rα-activated NKCs.

	Term name	**Binom FDR q-value**	**Binom Fold enrichment**	**Hyper FDR q-value**	**Hyper Fold-enrichment**
Mouse phenotype					
	Abnormal T cell physiology	$4.13e\text{-}^{179}$	2.022	$6.7e^{-37}$	1.418
	Increased T cell number	$4.20e\text{-}^{116}$	2.075	$1.1e^{-17}$	1.377
	Abnormal T cell activation	$1.8e\text{-}^{102}$	2.001	$1.53e^{-23}$	1.433
	Abnormal T cell proliferation	$1.2e\text{-}^{96}$	2.031	$5.07e^{-21}$	1.429
Panther pathway					
	Apoptosis signaling	$9.33e^{-43}$	2.338	$2.28e^{-7}$	1.422
	T cell activation	$6.95e^{-12}$	2.222	$1.69e^{-3}$	1.406
	JAK/STAT signaling	$1.93e^{-14}$	3.722	$2.16e^{-3}$	1.652
Pathway commons					
	IL-2 mediated signaling events	$1.92e^{-64}$	2.538	$3.13e^{-14}$	1.568
MSigDB pathway					
	Natural killer cell mediated cytotoxicity	$3.84e^{-78}$	2.912	$1.45e^{-7}$	1.360

Supplementary Table S4 Lists of differentially expressed genes in CD11b, DP and CD27 subpopulations of Runx3$^{-/-}$ vs WT NKC and the list of 655 Runx3-regulated genes in IL-15/Rα *in-vivo* activated NKC, including down-regulated and up-regulated genes in Runx3$^{-/-}$ (see a separate Excel file).

Supplementary Table S5 Enriched ontology terms identified by Ingenuity[@] for Runx3-regulated genes of *in vivo* IL-15-activated NKCs.

Category	**Count**	**Percent**	**P-value**
Cellular growth and proliferation	169	30	$6.11E^{-18}$-$5.98E^{-04}$
Cell death	182	33	$1.59E^{-17}$-$6.79E^{-04}$
Cell movement	126	23	$8.65E^{-12}$-$5.44E^{-04}$

Supplementary Table S6 List of Runx3 candidate target genes in IL-15 cultured NK cells.

Transcriptome comparisons between WT and Runx3$^{-/-}$ NKCs that were cultured for 7 days in the presence of IL-15 revealed 1222 differentially expressed; Runx3-responsive genes (fold change $\geq$1.5, $p < 0.05$). To delineate the candidate Runx3-regulated gene subset, the group of 1222 differentially expressed genes in IL-15-activated NKC was intersected with the list of Runx3 occupied genes that contain bound RUNX motifs (in *in-vivo* IL-15 activated NKC). This analysis revealed 791 Runx3-regulated genes, of which 359 genes were up-regulated and 432 genes were down-regulated in Runx3$^{-/-}$ NKCs compared to WT (see a separate Excel file).