

SUPPLEMENTAL MATERIAL

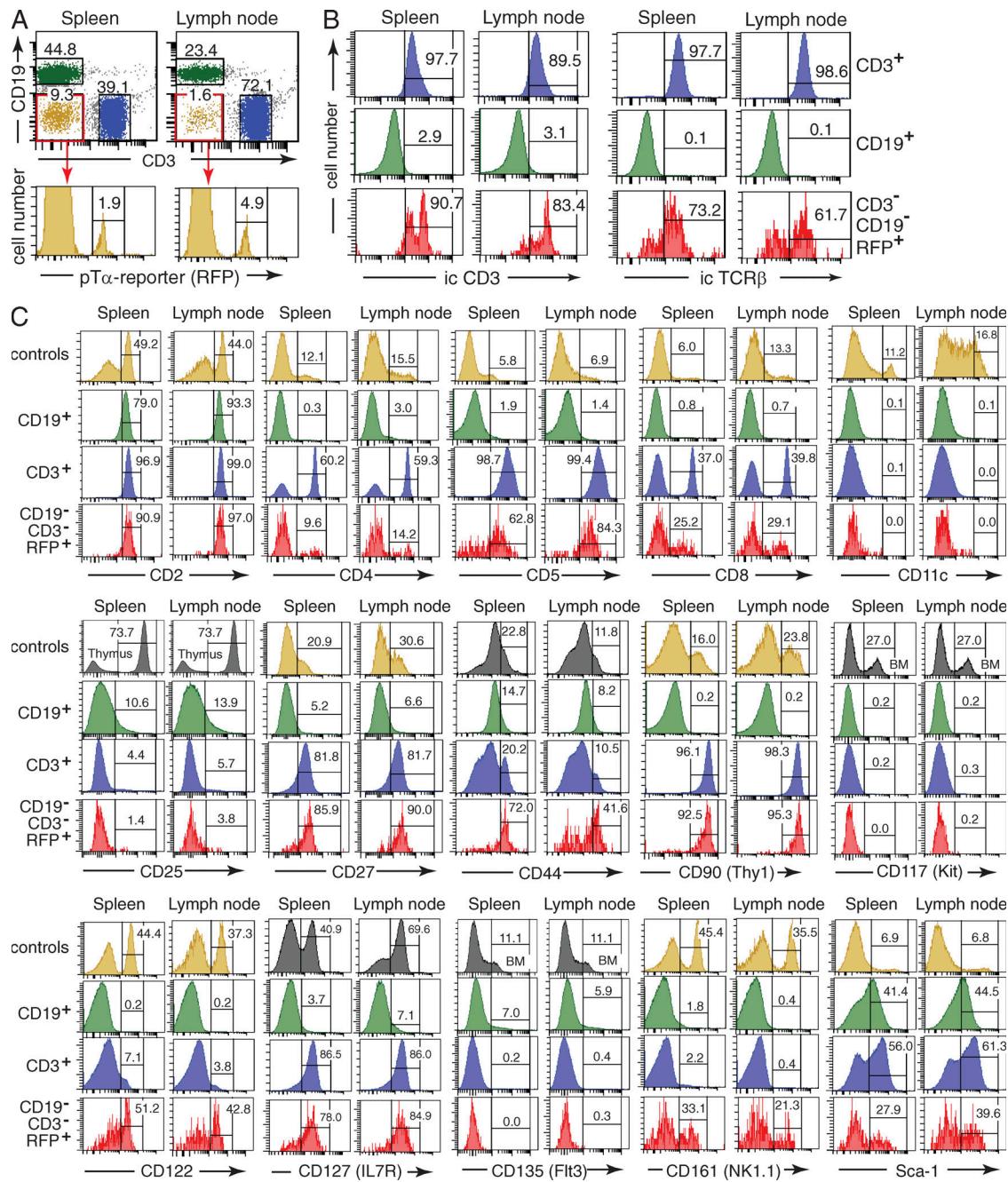
Luche et al., <http://www.jem.org/cgi/content/full/jem.20122609/DC1>

Figure S1. Comprehensive cytofluorometric characterization of pT α ^{iCre}-labeled cells in the CD3-CD19- compartments of lymph node and spleen.
 (A) Gating scheme to identify pT α ^{iCre}-labeled splenocytes and lymph node cells within the CD3-CD19- compartments of pT α ^{iCre/WT} × Rosa^{RFP/WT} mice. (B) Intracellular (ic) staining for CD3 ϵ and TCR β . 40–65% of reporter-positive CD3-CD19- cells (red histograms) express TCR β , and ~90% express CD3 ϵ intracellularly. Gating on B cells (green histograms) and T cells (blue histograms) provided negative and positive staining controls, respectively. RFP+CD3-CD19- cells were entirely negative for intracellular TCR $\gamma\delta$ expression (not depicted). (C) Analysis of surface marker expression on CD3-CD19-RFP+ cells (red histograms). Expression patterns of respective markers on B and T cells (green and blue histograms, respectively) are shown for comparison. The panels labeled "controls" refer to marker expression on total CD3-CD19- cells (yellow histograms) or total splenocytes and lymph node cells (gray histograms), respectively. For CD25 stainings, thymocytes from a *Rag2*^{-/-} mouse and for CD117 and CD135 stainings, BM cells were used in the same experiment as staining controls (gray histograms labeled "Thyus" or "BM"). Numbers indicate percentages of cells in each gate. Each histogram is representative of at least three independent staining experiments.

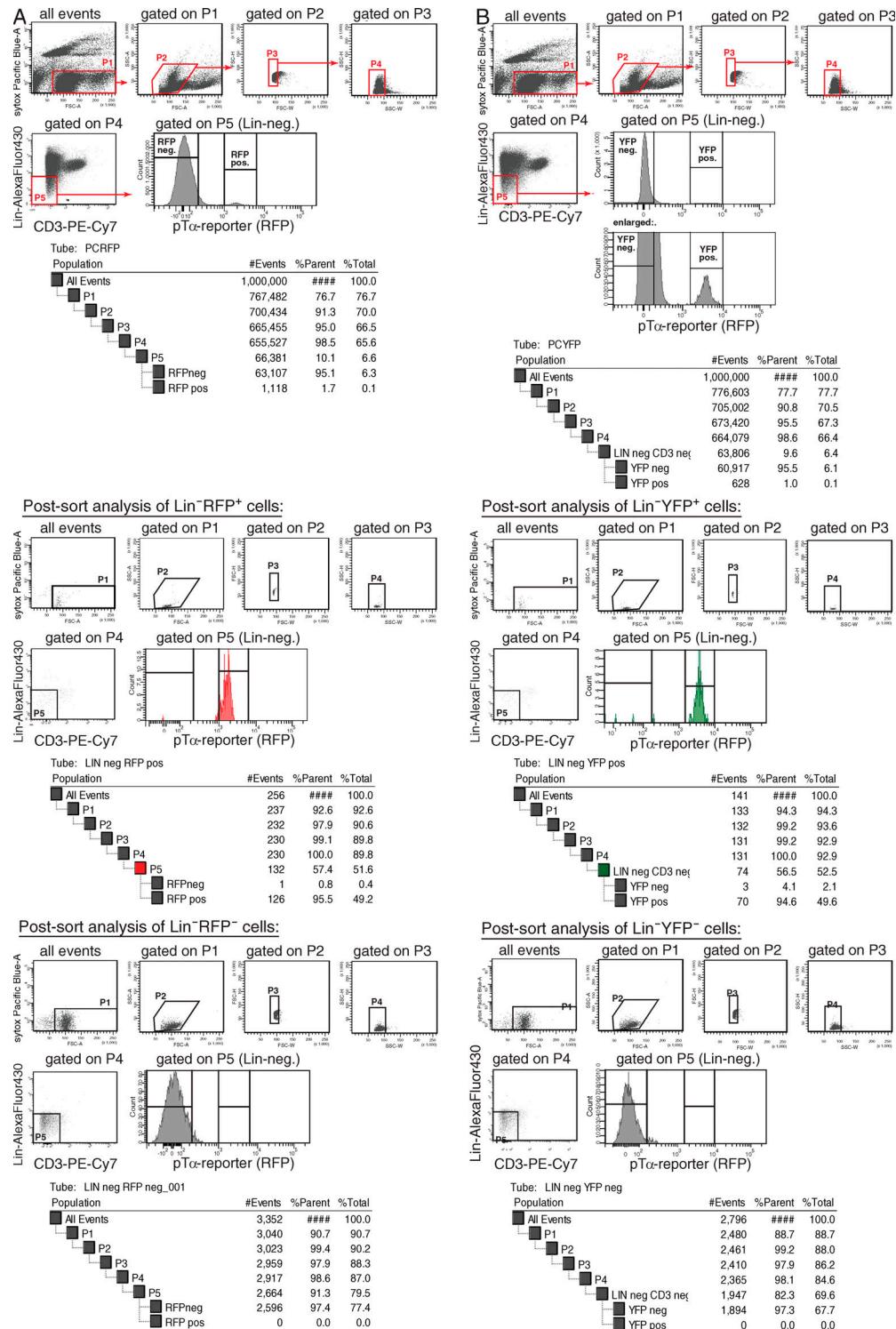


Figure S2. Isolation of tester and competitor BM donor cell populations for competitive complementation assay. (A) Gating scheme and post-sort analysis of Lin⁻RFP⁺ (tester) and Lin⁻RFP⁻ (competitor) BM cells from pT α ^{iCre/WT} × Rosa^{RFP/WT} mice. BM cells from eight female pT α ^{iCre/WT} × Rosa^{RFP/WT} mice were stained with biotinylated, lineage-specific rat anti-mouse antibodies and bead depleted (for details, see Materials and methods). Remaining cells were pooled and stained with Streptavidin-QDot605 and CD3-PECy7 in the presence of Cytox. True Lin⁻ cells (gate P5) were sorted for RFP⁻ and RFP⁺ subsets on a FACSCanto II after gating out FSC^{low} and Cytox⁺ (dead) cells (gate P1), followed by gating on cells with an FSC/SSC pattern typical for lymphocytes (gate P2) and followed by doublet exclusion (gates P3 and P4). Reanalysis of a small fraction of sorted cells confirmed their purity (panels labeled postsort analysis). (B) Gating scheme and postsort analysis of Lin⁻YFP⁺ (tester) and Lin⁻YFP⁻ (competitor) BM cells from pT α ^{iCre/WT} × Rosa^{YFP/WT} mice. The sorting experiment was performed in analogy to the procedure described in A.

Table S1. Antibodies used for flow cytometry

Antigen	Clone	Conjugate	Source	Figure in article
CD2	RM2-5	FITC	BD	Fig. 6 F and Fig. S1 C
	RM2-5	PE	BD	Fig. 6, D and E; and Fig. 7, A and B
CD3ε	500A2	Biotin	BD	Fig. 5 C–E; Fig. 6 F; Fig. 7, A–C; and Fig. S2
	145-2C11	APC-Cy7	eBioscience	Figs. 3 A and 7 B
	145-2C11	PECy7	BD/eBioscience	Figs. 1 D, 2 D, and 6 F; and Figs. S1 B and S2
	145-2C11	APC	BD	Figs. 3 D, 5 D, and 7, A–C; and Fig. S1 A
	17A2	Pacific Blue	eBioscience	Fig. 5, C and E; and Fig. 6 E
CD4	RM4-5	APC	BD	Figs. 1 B, 5 A, and 8 B
	H129.19	FITC	BD	Fig. S1 C
CD5	53-7.3	PE-Cy7	eBioscience	Figs. 6 D and 7 B and Fig. S1 C
CD8	LY-2	PE	BD	Fig. 5 A
		FITC		Fig. 1 B
	53-6.7	Biotin	BD/eBioscience	Fig. 5, C–E; Fig. 7, A–C; Fig. 8 B; and Fig. S1 C
CD11b	M1/70	Biotin	BD	Fig. 5, C–E; Figs. 6 F and 7, A–C; and Fig. S2
	M1/70	PECy7	eBioscience	Fig. 4 A
CD11c	HL3	Biotin	BD	Fig. 5, C–E; Figs. 6 F and 7, A–C; and Fig. S2
	N418	APC-eFluor780	eBioscience	Fig. S1 C
	N418	APC-Cy7	eBioscience	Fig. 4 A
CD16	93	PE-Cy5.5	eBioscience	Figs. 6 D and 7 B
CD19	1D3	Biotin	BD	Fig. 5, C–E; Figs. 6 F and 7, A–C; and Fig. S2
	1D3	PerCP-Cy5.5	eBioscience	Fig. 3, A, B, and D; and Fig. S1 A
CD24	30-F1	FITC	eBioscience	Fig. 5 C and Fig. S1 C
CD25	PC61	PECy7	BD	Figs. 1 B and 5 D
	7D4	FITC	BD	Fig. S1 C
CD27	LG.3A10	Biotin	BD	Fig. S1 C
	LG.3A10	PE	eBioscience	Figs. 6 D and 7 B
CD44	IM7	PerCP-Cy5.5	eBioscience	Figs. 1 B and 5, C–E; and Fig. 7 B
	Pgp.1	FITC	BD	Fig. S1 C
CD45	30-F11	Biotin		Fig. 2 B
CD117 (Kit)	2B8	APC	BD	Fig. 5, C and E; Fig. 6, D and E; and Fig. 7 B
	2B8	APC-eFluor780	eBioscience	Fig. S1 C
CD122	TM-B1	FITC	BD	Fig. S1 C
CD127 (IL-7Rα)	A7R34	FITC	eBioscience	Fig. S1 C
	A7R34	PE-Cy7	eBioscience	Figs. 6 D and 7 B
CD135 (Flt3)	A2F10	Biotin	eBioscience	Fig. S1 C
	A2F10	PE	eBioscience	Fig. 7 B
CD161 (NK1.1)	PK136	Biotin	eBioscience	Fig. 5, C–E; Figs. 6 F and 7, A–C; and Figs. S1 C and S2
B220	RA-6B2	Biotin	BD	Fig. 5, C–E
	RA-6B2	PE-Cy5.5	eBioscience	Fig. 4 A
	RA-6B2	APC	BD	Fig. 7 B
	RA-6B2	PerCP-Cy5.5	eBioscience	Fig. 6 D
CCR7	4B12	PE-Cy7	eBioscience	Fig. 6 D
CCR9	eBioCW-1.2	PE	eBioscience	Figs. 6 D and 7 B
GR1	RB6-8C5	Biotin	eBioscience	Fig. 5, C–E; Figs. 6 F and 7, A–C; and Fig. S2
IgM	1B4B1	FITC		Fig. 3 B
MHC class II	M5/114.15.2	APC	eBioscience	Fig. 4 A
PDCA-1	eBio-927	FITC	eBioscience	Fig. 4 B
Sca1	D7	PE-Cy7	BD/eBioscience	Fig. 6 E and Fig. S1 C
	D7	PerCP-Cy5.5	BD	Fig. 7 B
TCRβ	H57-597	Biotin	eBioscience	Fig. 5, C–E; Fig. 7, A–C; and Fig. S1 B
	H57-597	FITC		Fig. 1 D
TCRγδ	GL3	Biotin	BD	Fig. 5, C–E; Figs. 6 F and 7, A–C; and Fig. S2

Table S1. Antibodies used for flow cytometry (*Continued*)

Antigen	Clone	Conjugate	Source	Figure in article
TER119	GL3	APC	eBioscience	Fig. 2, A and D
		Biotin	BD	Fig. 5, C-E; Figs. 6 F and 7, A-C; and Fig. S2
Thy1.2	53-2.1	APC	eBioscience	Fig. 6, D and F; and Fig. 7 B
	53-2.1	PE-Cy7	eBioscience	Figs. 6 E and 7, A and C
V γ 5	F536	FITC	BD	Fig. 2 B
Streptavidin		eFluor-450	eBioscience	Fig. 4 A
		FITC		Fig. 1 B
		APC-Cy7	BD	Figs. 5 C and 6 E; and Fig. S1 C
		PE-Cy7	eBioscience	Fig. 8 B
		QDot605	Invitrogen	Figs. 5 D, 6 F, and 7, A-C; and Fig. S2