Lnk Controls Hematopoietic Stem Cell Self-renewal and Quiescence through Direct Interactions with JAK2

Alexey Bersenev [2], Chao Wu [2], Joanna Balcerek [2], and Wei Tong [1,2,3]

Supplemental Data:

Figure 1S. Lnk controls survival under myelosuppressive stress through interaction with TPO/Mpl. WT, $Mpl^{-/-}$, $Lnk^{-/-}$ and $Mpl^{-/-}Lnk^{-/-}$ mice were given weekly 5-FU injection, and the first 5-FU treatment was indicated as day 0. Percentages of mice survived after the first 5-FU injection is plotted. 5 – 8 mice per genotype was used in each experiment and representative of three independent experiments is shown here.

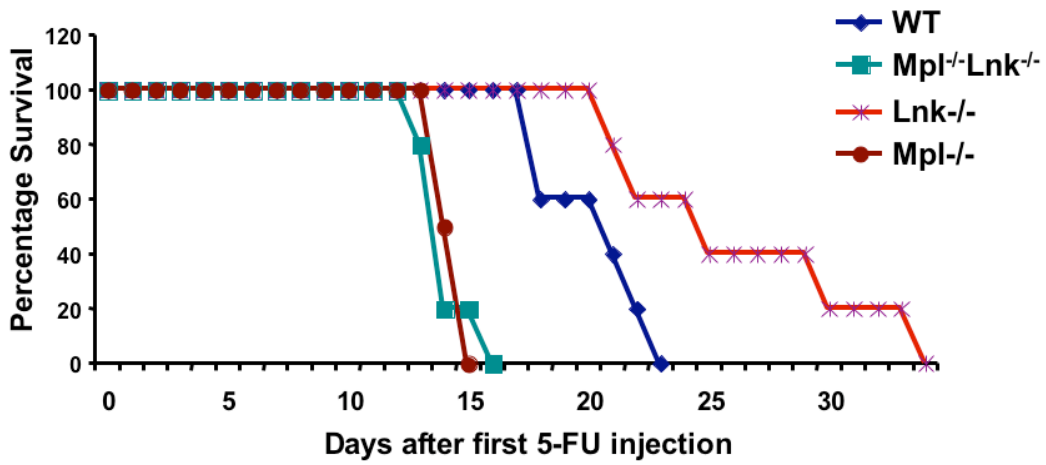

Figure 2S: Flow cytometric sorting of HSCs from WT, Mpl$^{-/-}$, Lnk$^{-/-}$ and Mpl$^{-/-}$Lnk$^{-/-}$ mice. Representative plots are shown below for flow cytometric sorting of LSK HSCs from BM.

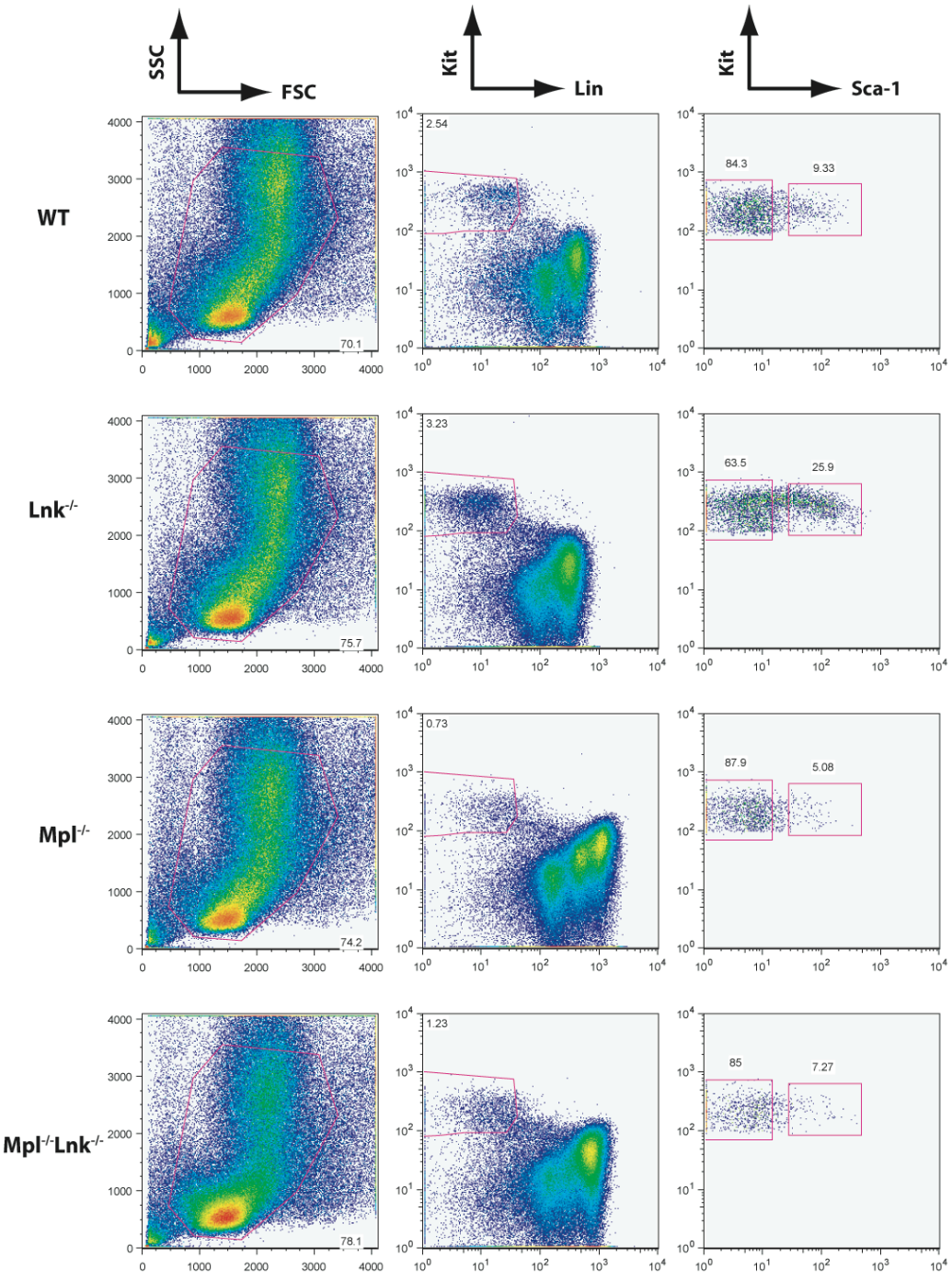

Figure 3S: Cells expressing JAK2Y813F show increased sensitivity to TPO in supporting cell growth, and are less sensitive to Lnk growth inhibition.
(a) Parental 32D cells and cells stably expressing Mpl in combination with wt JAK2, JAK2Y813F or JAK2 Y1007/8F mutant were established and cultured in IL-3 or different concentrations of TPO. Live cell numbers in the presence of TPO relative to that in IL-3 after three days' culture were determined by MTT (3-(4,5-dimethylthiazole-2-yl)-2, 5-diphenyl tetrazolium bromide) absorbance. Triplicates were plates in each condition and results shown are representative of two independent experiments. ∗: $p<0.01$. (b) 32D/Mpl cells expressing wt JAK2, JAK2Y813F or JAK2 Y1007/8F mutant were established. We introduced either vector alone (MIG) or MIG- Lnk into these cells and determined the proportion of infected cells as those that express GFP two days later (set as Day 0). We then cultured them in 1 ng/ml TPO, measured the GFP^+ fraction every day, and the percentage of GFP^+ cells relative to the infection rate (Day 0) was plotted. Results shown are representative of three independent experiments.

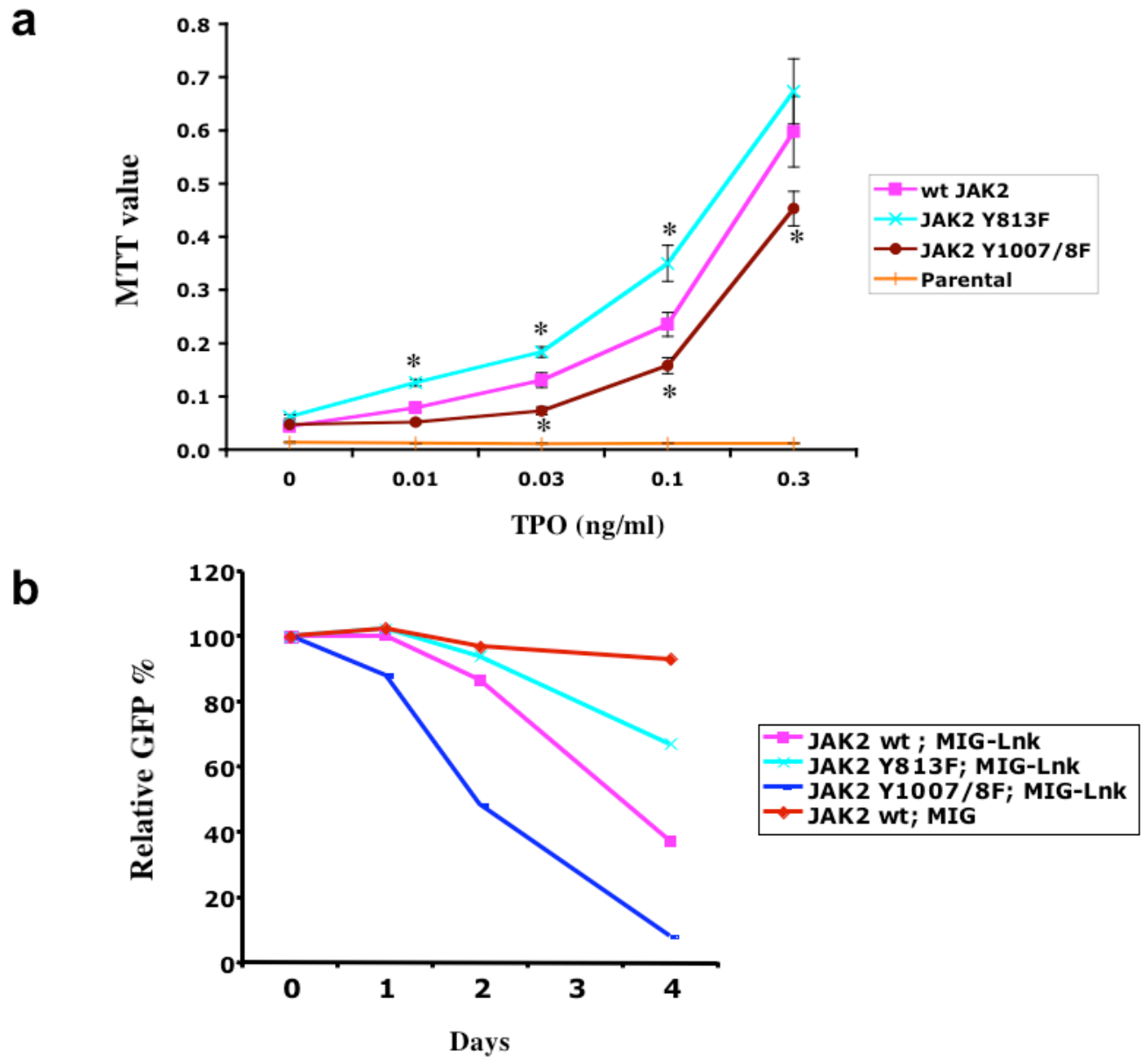

Table 1 S : Summary of experimental parameters used to calculate HSC frequency by quantifying competitive repopulating unit (CRU).

Cell dose injected	Number Positive	Number Tested
WT		
1000	1	11
3000	2	9
10 000	9	24
30 000	12	15
100 000	7	7
CRU frequency	1/18 570 (95% CI: 1/28 119 - 1/12 264)	

Cell dose injected	Number Positive	Number Tested
Lnk $^{-/-}$		
300	1	8
1 000	6	12
3 000	9	12
10 000	9	9
30 000	7	7
100 000	5	5
CRU frequency	1/1 848 (95% CI: 1/3 064 - 1/1 115)	

Cell dose injected	Number Positive	Number Tested
Mpl $^{-/-}$		
10 000	0	4
30 000	2	8
100 000	12	22
300 000	11	11
CRU frequency	1/109 380 (95% CI: 1/169 078-1/70 761)	

Cell dose injected	Number Positive	Number Tested
Mpl$^{-/-}$Lnk$^{-/-}$		
3000	1	11
10 000	4	14
30 000	8	13
100 000	3	3
CRU frequency	1/29 970 (95% CI: 1/50 813-1/17 677)	

BM cells were isolated from WT, Lnk$^{-/-}$, Mpl$^{-/-}$, or Mpl$^{-/-}$Lnk$^{-/-}$ mice, and different cell doses as indicated were mixed with 200,000 competitor BM cells and injected into lethally- irradiated recipient mice. Mice with over 1% donor-descent cells in the peripheral blood were counted as "positive reconstitution", and CRU frequencies were calculated using L-Calc software (StemCell Technologies). The data are pooled from three independent experiments. 95% confident internal (CI) is indicated.

Table 2 S : Summary of experimental parameters used to calculate HSC frequency after one and two times 5-FU treatments by quantifying competitive repopulating unit (CRU).

One time 5-FU treated	Cell dose injected	Number Positive	Number Tested
	WT		
	3 000	0	6
	6 000	0	4
	10 000	2	11
	15 000	4	6
	30 000	7	7
	60 000	3	3
	100 000	5	5
	CRU frequency	1/20 308 (95% CI: 1/33 216 - 1/12 417)	
	Lnk$^{-/-}$		
	1 000	1	10
	3 000	6	11
	6 000	7	9
	10 000	8	8
	30 000	5	5
	100 000	4	4
	CRU frequency	1/3 831 (95% CI: 1/6 063 - 1/2 421)	

Two times 5-FU treated	Cell dose injected	Number Positive	Number Tested
	WT		
	10 000	0	4
	30 000	0	5
	60 000	1	4
	100 000	9	12
	300 000	9	9
	600 000	6	6
	1 000 000	5	5
	CRU frequency	1/94 477 (95% CI: 1/156 434 - 1/57 058)	
	Lnk$^{-/-}$		
	1 000	1	9
	3 000	10	15
	6 000	9	16
	10 000	20	22
	30 000	9	9
	1 000 000	9	9
	CRU frequency	1/4 664 (95% CI: 1/6 569 - 1/3 311)	

HSC frequencies of WT and Lnk$^{-/-}$ mice after one or two 5-FU treatments were determined by quantifying CRUs. WT or Lnk$^{-/-}$ mice administered intravenously with 150 mg/ml 5-FU two days before BMT, or given two 5-FU treatments four days apart and BM isolated the day after the second 5-FU injection. Limiting dilution assays were performed as described in Table 1S. The data are pooled from three independent experiments. 95% confident internal (CI) is indicated.