with <i>Notch2</i> deleted BM cells in homeostasis			
Positive mice / Total mice injected with BM cell number from primary transplant	Notch2 <sup>fl/fl</sup> Cre <sup>-</sup>	Notch2 <sup>fl/fl</sup> Cre <sup>+</sup>	
$5.0 \ge 10^5$	8 / 8	7 / 8	
$3.0 \ge 10^5$	3/3	3/3	
$1.0 \ge 10^5$	4 / 7	5 / 8	
$5.0 \ge 10^4$	5 / 7	2 / 7	
$3.3 \times 10^4$	1 / 5	1 / 5	
$1.1 \ge 10^4$	0 / 4	0 / 4	
CRU frequency / 1 x $10^6$ BM cells	12.0	6.5	
P value	0.1		

Supplemental Table 1 HSC generation following transplant

Lethally irradiated recipients were transplanted with decreasing numbers of BM cells from Notch<sup>fl/fl</sup> 2 Cre<sup>-</sup> or *Notch2<sup>fl/fl</sup> Cre*<sup>+</sup> mice. Peripheral blood was analyzed 15 weeks after the transplant for the percentage of donor derived cells. Animals were considered positive if engraftment in peripheral blood was more than 2.0% donor derived cells with both lymphoid and myeloid contribution. Poisson statistics were used to calculate HSC frequency.

Supplemental Table 2 HSC generation following transplant				
with Notch1 deleted BM cells in homeostasis				
Positive mice / Total mice injected with BM cellnumber from primary transplant	Notch1 <sup>fl/fl</sup> Cre <sup>-</sup>	Notch1 <sup>fl/fl</sup> Cre <sup>+</sup>		
$4.0 \ge 10^5$	4 / 5	5 / 5		
$2.0 \ge 10^5$	5 / 6	4 / 6		
$1.0 \ge 10^5$	3 / 6	4 / 6		
$5.0 \ge 10^4$	1 / 5	0 / 5		
$2.5 \times 10^4$	1 / 5	0 / 5		
CRU frequency / 1 x $10^6$ BM cells	6.1	6.2		
P value	0.9			

Lethally irradiated recipients were transplanted with

decreasing numbers of BM cells from  $Notch1^{fl/fl}$   $Cre^-$  or  $Notch1^{fl/fl}$   $Cre^+$  mice. Peripheral blood was analyzed 13 weeks after the transplant for the percentage of donor derived cells. Animals were considered positive if engraftment in peripheral blood was more than 2.0% donor derived cells with both lymphoid and myeloid contribution. Poisson statistics were used to calculate HSC frequency.

transplanted 40 weeks previously with <i>Notch2</i> deleted BM cells				
Positive mice / Total mice injected with BM cell number from primary transplant	Notch2 <sup>fl/fl</sup> Cre <sup>-</sup>	Notch2 <sup>fl/fl</sup> Cre <sup>+</sup>		
$4.0 \ge 10^6$	6 / 6	6 / 6		
$2.0 \ge 10^6$	5 / 6	3 / 5		
$1.0 \ge 10^{6}$	6 / 6	4 / 6		
$5.0 \ge 10^5$	2 / 6	2 / 6		
$2.5 \ge 10^5$	3 / 6	0 / 6		
CRU frequency / 1 x 10 <sup>6</sup> BM cells	1.5	0.8		
P value	0.06			

Supplemental Table 3 HSC generation following transplant with BM from mice

BM from 5 mice previously transplanted with Notch2<sup>fl/fl</sup> Cre<sup>-</sup> or Notch2<sup>fl/fl</sup> Cre<sup>+</sup>

cells was pooled and decreasing numbers injected into lethally irradiated recipients. The mean percent donor was similar between the two groups (41.5 +/- 12.2% vs 43.3 +/- 13.3%: mean percent donor +/- S.E.M.). To verify Notch2 was still deleted in primary transplants, donor cells were sorted from PBL prior to sacrifice and quantitative genomic PCR performed to measure amount of deletion. Sorted donor cells from  $Notch2^{fl/fl} Cre^+$  mice were more than 95% deleted. Following the secondary transplant peripheral blood was analyzed 15 weeks after the transplant for the percentage of donor derived cells. Animals were considered positive if engraftment in peripheral blood was more than 2.0% donor derived cells with both lymphoid and myeloid contribution. Poisson statistics were used to calculate HSC frequency.



**Supplemental Figure 1. Fringe shRNA reduces expression of Lunatic, Manic and Radical Fringe mRNA levels.** Respective Fringe mRNA levels were measured 10 days following culture with either Delta1<sup>ext-IgG</sup>, Jagged1<sup>ext-IgG</sup> or Human-IgG. mRNA levels were measured using quantitative RT-PCR. Each bar represents the mean fold reduced expression of duplicates (+/- range) incubated with Fringe shRNA compared to control shRNA.



Supplemental Figure 2. Notch2 enhances the tempo of HSC and MPP recovery after transplant into irradiated recipients. Survival outcome after transplant into lethally irradiated recipients of  $1 \times 10^6$  cells from primary recipients transplanted 13 days previously with  $2 \times 10^6$  Notch2<sup>*fl/fl*</sup> Cre<sup>-</sup> (solid line) or  $2 \times 10^6$  Notch2<sup>*fl/fl*</sup> Cre<sup>+</sup> (short dashed line). Results were analyzed with a log-rank nonparametric test and expressed as Kaplan-Meier Survival curves (n=4, p=0.04).