Lnk Constrains Myeloproliferative Diseases in Mice

Alexey Bersenev [2], Chao Wu [2], Joanna Balcerek [2], Jiang Jing [2], Mondira Kundu [3], Gerd A. Blobel[1, 2], Kudakwashe R. Chikwava [4], and Wei Tong [1, 2, 5]

Supplemental Figures:

Figure 1S. Viral infection rates of Lin$^-$ progenitor cells. GFP expression was examined two days after viral infection of Lin$^-$ progenitors. WT and Lnk$^{-/-}$ cells infected with retroviruses encoding either MIG vector alone or Tel/JAK2 show similar infection frequencies.

Figure 2S: Lnk deficiency leads to an expansion of immature myeloid cells in the transplanted mice expressing Tel/JAK2. A) shows Wrights-Giemsa staining of BM cytospins from WT;Tel/JAK2 and Lnk$^{-/-}$;Tel/JAK2 mice. As reference, **B)** shows Wrights-Giemsa staining of cytospins from purified WT BM cells. Majority of Gr-1$^+$Mac-1$^+$ cells assume mature granulocytic morphology, while Gr-1$^{-/low}$Mac-1$^+$ cells appear to be immature myeloid cells. Magnification: 600x.

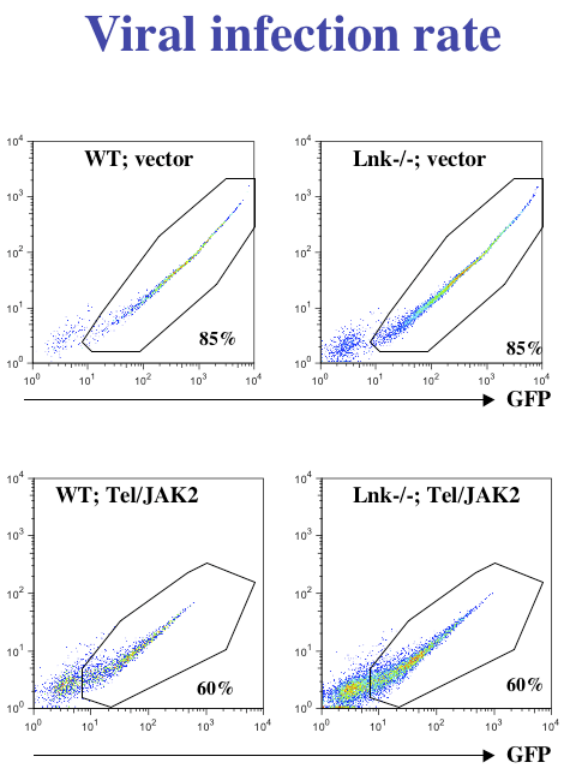
Figure 3S: WT Lnk ameliorates CML development in Lnk$^{-/-}$ mice expressing Tel/JAK2. Lnk$^{-/-}$ progenitor cells were infected with retroviruses encoding either Tel/JAK2 or Tel/JAK2-Lnk, and transplanted into irradiated recipients. **A)** Viral infection rates two days post transduction are shown on the left panels. Myeloid chimerisms of

reconstituted mice at indicated time points post transplantation are shown on the right panels. **B)** Survival curves of reconstituted mice.

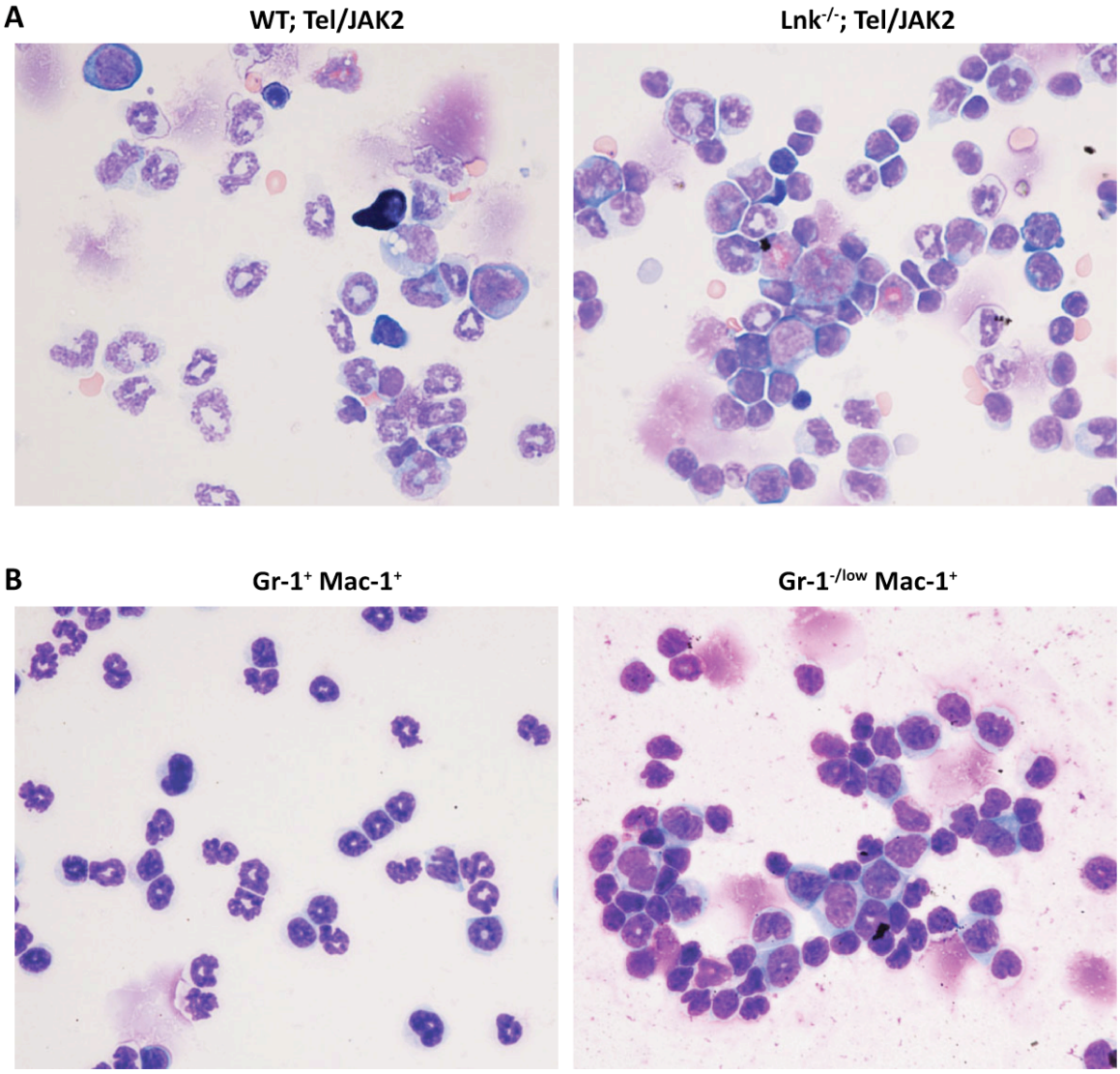
Figure 4S: The addition of Y813F mutation in JAK2V617F ablates its Lnk binding ability. 293T cells were transfected with Flag-tagged Lnk and various JAK2 constructs. Cell lysates were immunoprecipiated (IP) with anti-Flag antibodies followed with Western blot (WB) with anti-JAK2 and Lnk antibodies sequentially. Total cell lysates (TCL) were also directly subjected to WB analysis with anti-JAK2 antibodies. wt: wild type; VF: JAK2V617F; VF/YF: JAK2V617F/Y813F.

Figure 5S. Lnk deficiency exacerbates CML development initiated by Bcr/Abl in mice. WT and $Lnk^{-/-}$ BM cells were infected with retroviruses encoding either MIG vector alone or Bcr/Abl, and transplanted to irradiated host animals. Kaplan-Meier survival analysis (left) and blood count of transplanted mice (right) are shown. The box plot shows total neutrophil counts measured 2 weeks after the transplant. The ends of the boxes define the 25^{th} and 75^{th} percentiles, a line indicates the median, and bars define the 5^{th} and 95^{th} percentiles. N=5. $p<0.01$ comparing WT;Bcr/Abl and $Lnk^{-/-}$;Bcr/Abl groups.

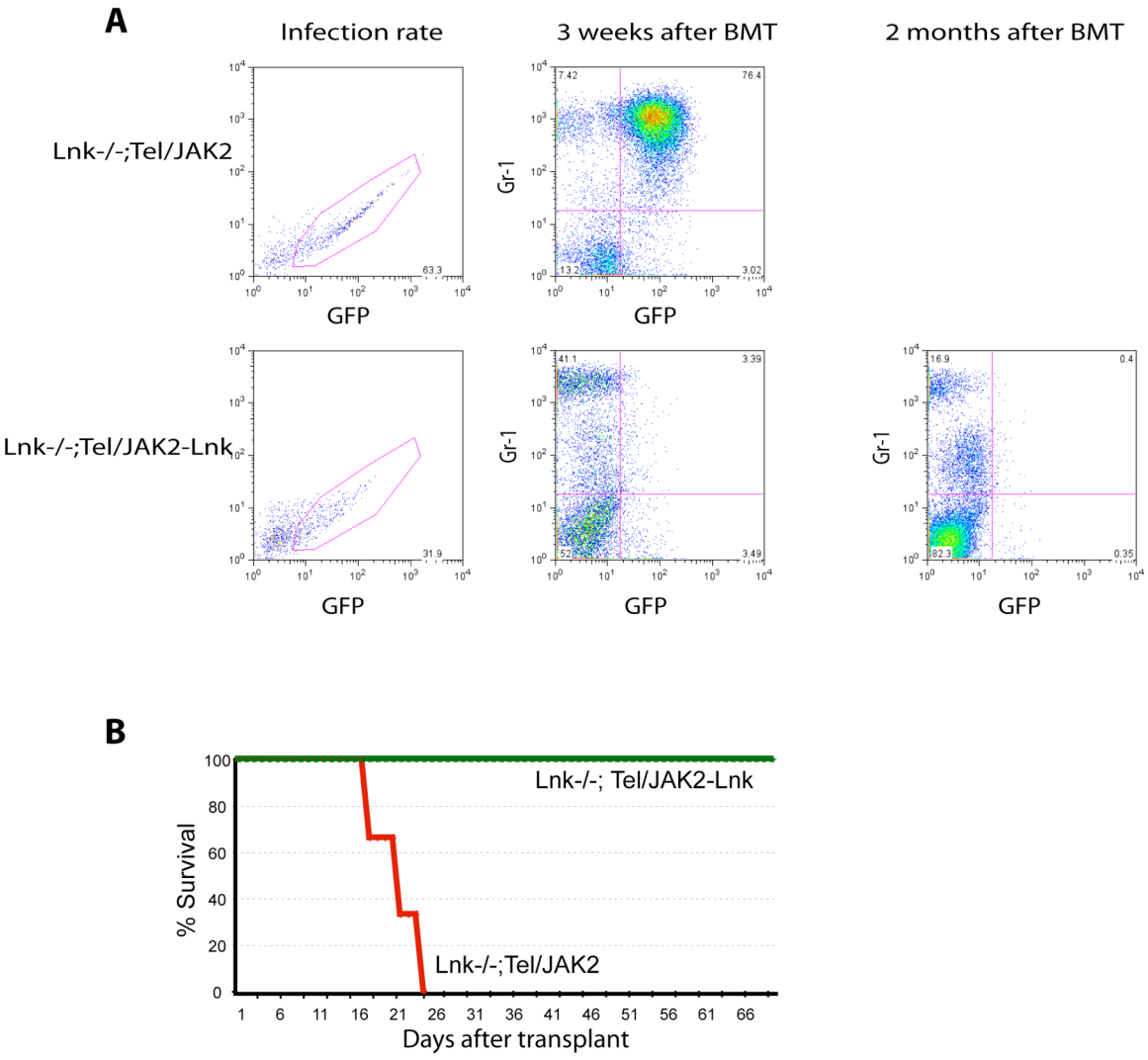
Supplemental Figure 1S:



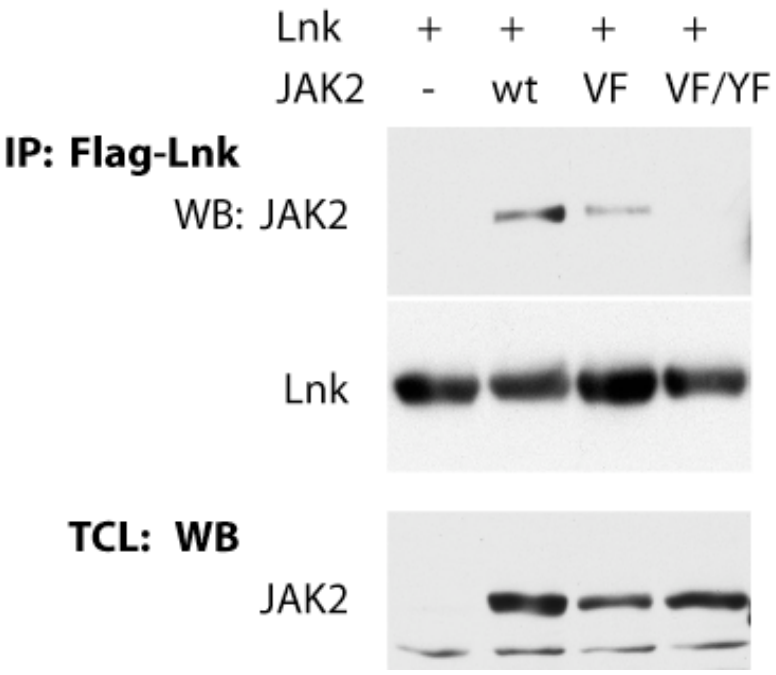
Supplemental Figure 2S:



Supplemental Figure 3S:



Supplemental Figure 4S:



Supplemental Figure 5S: