Supplemental information for

The BRISC De-ubiquitinating Enzyme Complex Limits Hematopoietic Stem Cell Expansion by Regulating JAK2 K63-ubiquitination

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Supplemental Figure 1. The LNK SH2 domain binds to BRISC through an interaction with pY377 in KIAA0157. (A) The LNK SH2 domain binds to BRISC. HEL-B/A cells stably expressing Flag-WT LNK or constructs containing mutant LNK were generated. IP-WBs were performed as in Fig. 1. The bottom illustrates the LNK structure and indicates the mutated regions. S150A is the corresponding mutant in human LNK that disrupts its interaction with 14-3-3, which is used here as a control. **(B) LNK binds to pY377 in KIAA0157.** 32D cells expressing Flag-KIAA0157 or mutants, along with Myc-Lnk, were lysed and precipitated with anti-Flag antibodies followed by WB with the indicated antibodies. Y: tyrosine; F: phenylalanine.

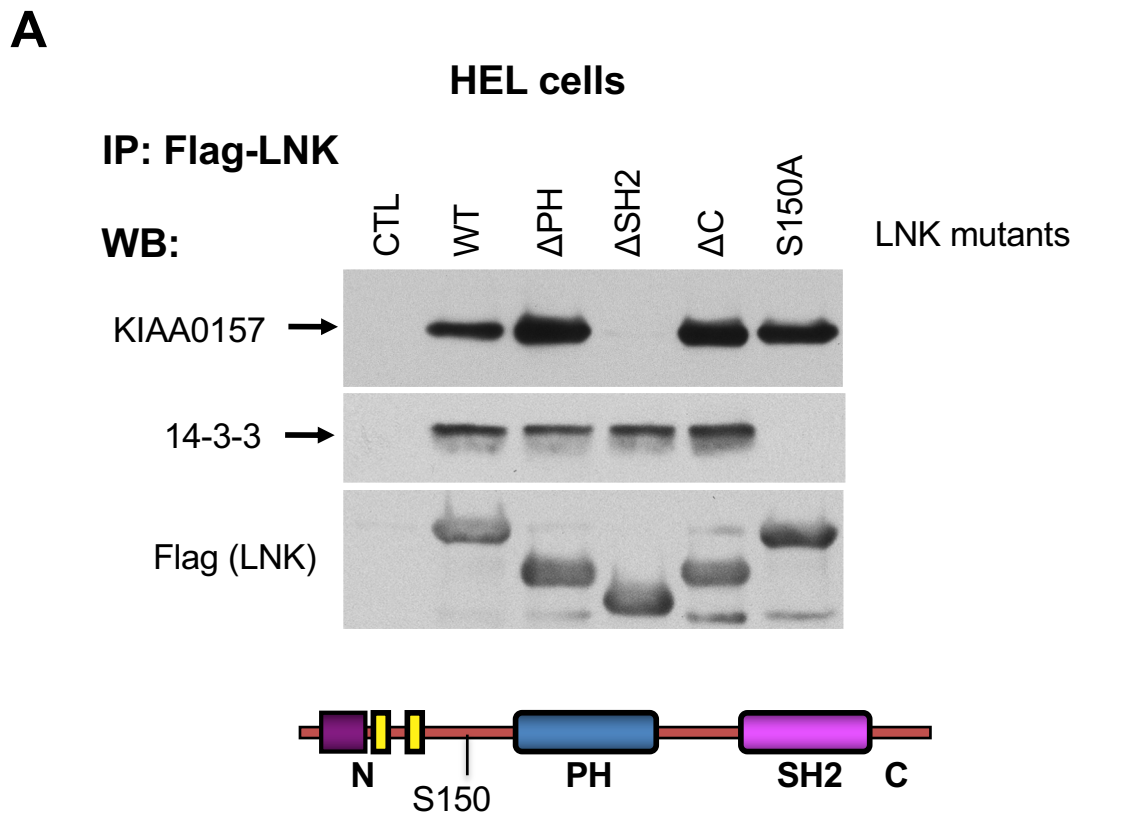
Supplemental Figure 2. *Kiaa0157* deficiency does not compromise DNA damage repair. Representative immunofluorescence images of BRCA1 and γH2AX foci after Irradiation in MEFs derived from WT, *M40*$^{-/-}$, and *Kiaa*$^{-/-}$ mice. The scale bar indicates 10um.

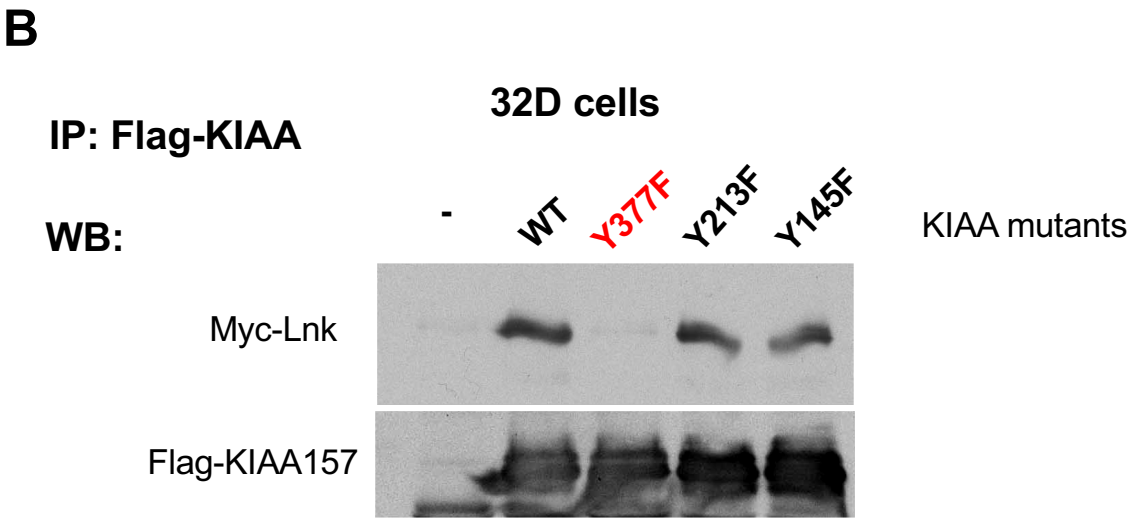
Supplemental Figure 3. JAK2 is JAK2 K63-ubiquitinated. Myc-JAK2 along with HA-Ub or Ub mutants were transfected into 293T cells. Lysates were denatured to prevent non-specific or indirect bindings, then renatured and precipitated with HA antibodies, followed by WB with Myc antibodies. K0 indicates the ubiquitin mutant that has all 7 lysines mutated to R. K48 only or K63 only indicates Ub mutants with all lysines mutated except K48 or K63, respectively.

Supplemental Figure 4. LNK does not affect BRISC DUB activities. *In vitro* reconstitution of BRISC DUB activity was performed using the baculovirus expression system. The Flag-tagged BRISC complex or the one containing an enzymatic inactive Brcc36 QSQ mutant were purified along with Myc-Lnk through IP with anti-Flag antibodies. The IP eluates were visualized by

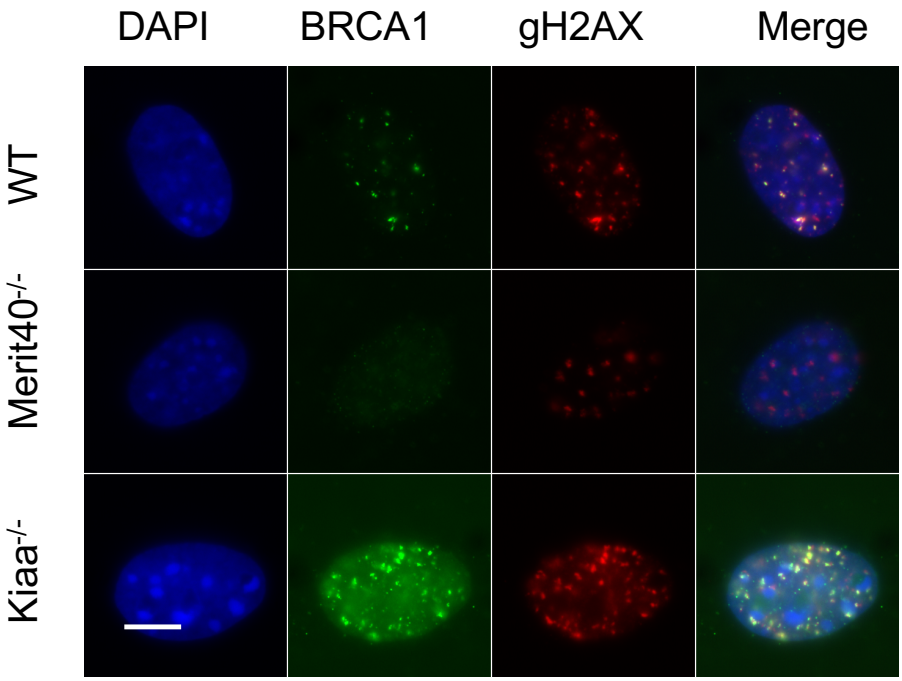
coomassie staining to show interaction with different amount of Lnk proteins **(left)**. The IP eluates were incubated with K63- hexa-Ub (Ub_6) substrates and the Ub cleavage products were detected by WB with antibodies to Ub **(right)**. BRISC containing WT BRCC36 but not QSQ mutant show *in vitro* DUB activity.

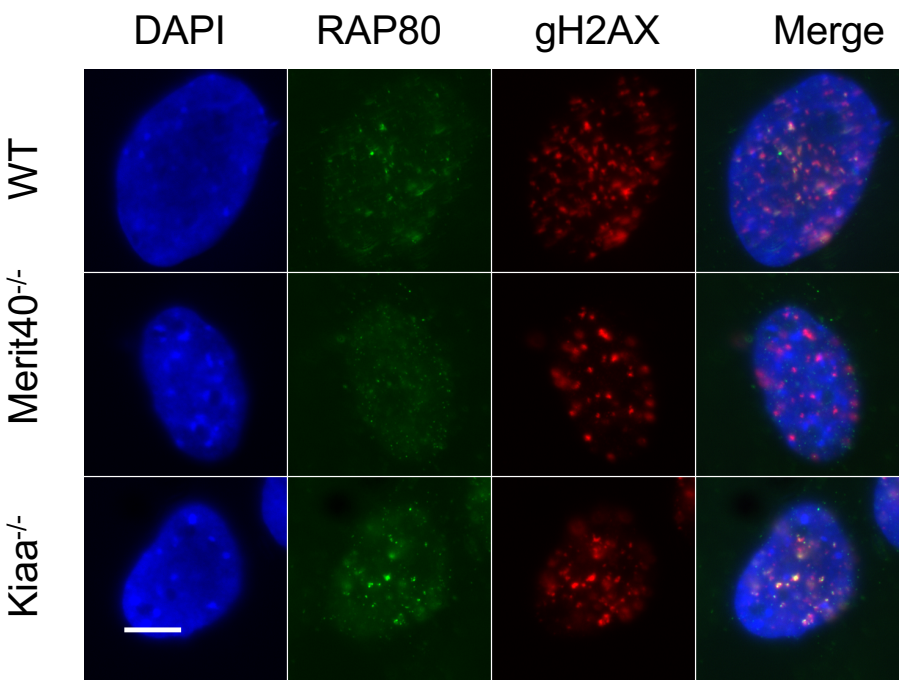
Supplemental Figure 1, Donaghy et al



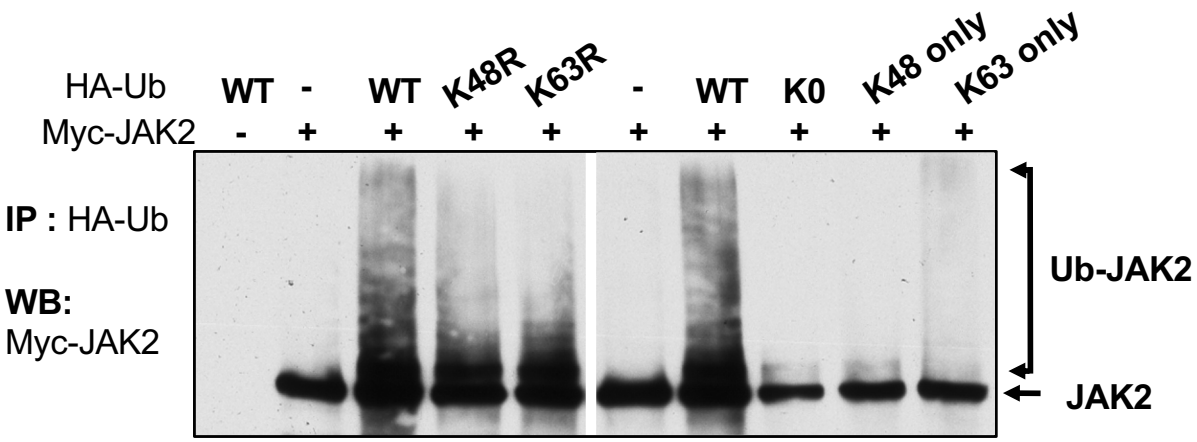


Supplemental Figure 2, Donaghy et al





Supplemental Figure 3, Donaghy et al



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