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Supplementary Materials for

ASXL1 interacts with the cohesin complex to maintain chromatid separation and gene expression for normal hematopoiesis

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:
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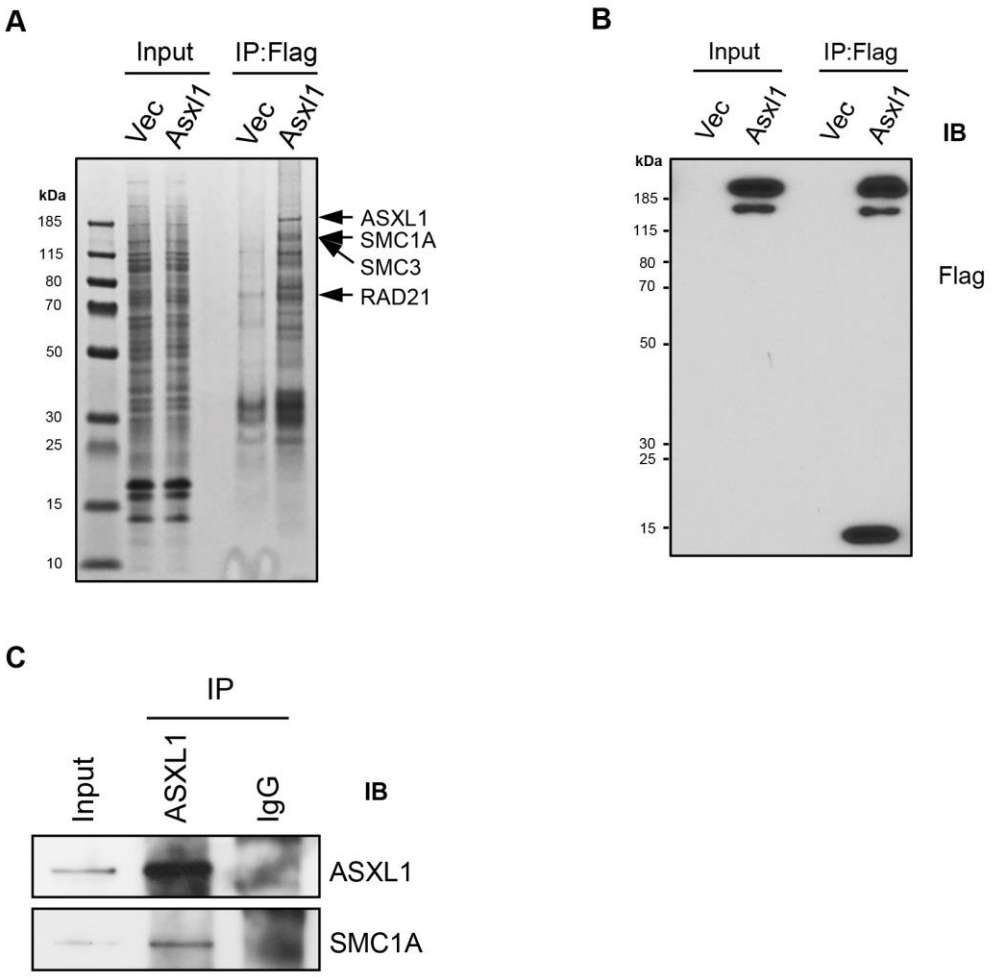

fig. S1. ASXL1 forms a complex with the cohesin complex. (**A**) Affinity purification of ASXL1 binding proteins. FLAG-tagged ASXL1 and its binding proteins were purified with M2 beads from nuclear fraction of either pcDNA3.1^{+} (Vec) or Flag-tagged ASXL1 (ASXL1) transfected HEK293T cells and were subjected to NuPAGE 4-12% Bis-Tris Gel analysis (Coomassie blue staining). The arrows show the positions of ASXL1, SMC1A, SMC3 and RAD21 based on their molecular weights. (**B**) Western blot analysis shows the expression of FLAG-tagged ASXL1 in FLAG-ASXL1 overexpressing HEK293T cell nuclear extraction and the immunoprecipitates with anti-FLAG antibody conjugated beads. (**C**) Western blot shows the endogenous interaction between ASXL1 and SMC1A in BM cells of WT mice.

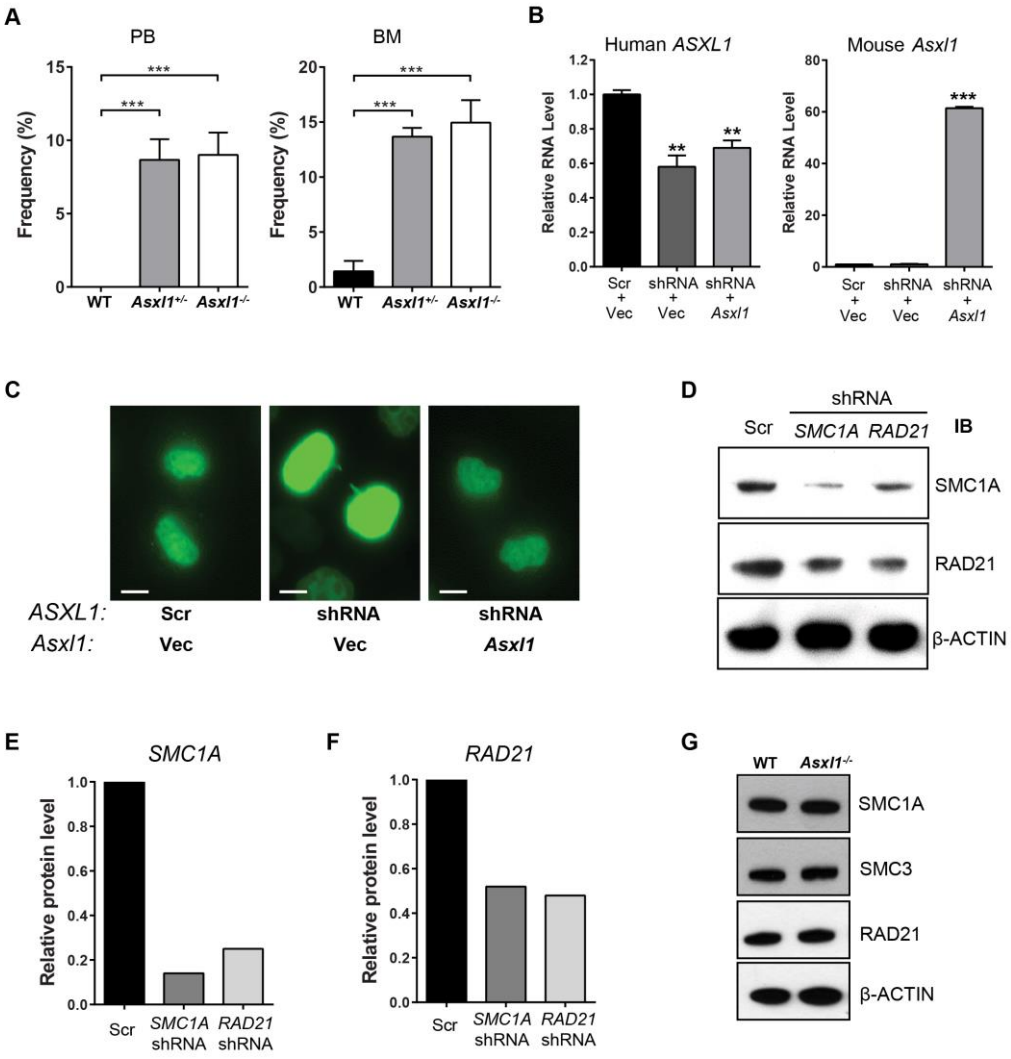

fig. S2. Reintroducing *mAsxl1* rescued the premature sister chromatid separation in HeLa cells with ASXL1 KD. (**A**) The frequencies of myeloid cells with premature sister chromatid separation in PB (left) and BM (right) of *Asxl1*$^{+/-}$ and *Asxl1*$^{-/-}$ mice are shown. Data are represented as means ± SEM from six independent experiments. ****P*<0.001. (**B**) The mRNA expression of *hASXL1* (left) or *mAsxl1* (right) in HeLa$^{GFP\text{-}H2B}$ cells transduced with (1) Scramble (Scr) shRNA + vector (vec) control; (2) shRNA-*hASXL1* + Vec; and (3) shRNA-*hASXL1* + *mAsxl1.* Data are represented as means ± SEM from three independent experiments. ****P*<0.001, ***P*<0.01. (**C**) Representative photomicrographs of the HeLa$^{GFP\text{-}H2B}$ cells with *hASXL1* KD and *hASXL1* KD plus mASXL1 rescues. (**D**-**F**) Western blot assays show the protein expression levels of

SMC1A or RAD21 in HeLa$^{GFP\text{-}H2B}$ cells transfected with shRNA-*SMC1A* or shRNA-*RAD21*. (**G**) Representative western blotting analysis of the protein levels of SMC1A, SMC3, and RAD21 in BM LK cells of WT and *Asxl1*$^{-/-}$ mice. β-Actin was used as a loading control.

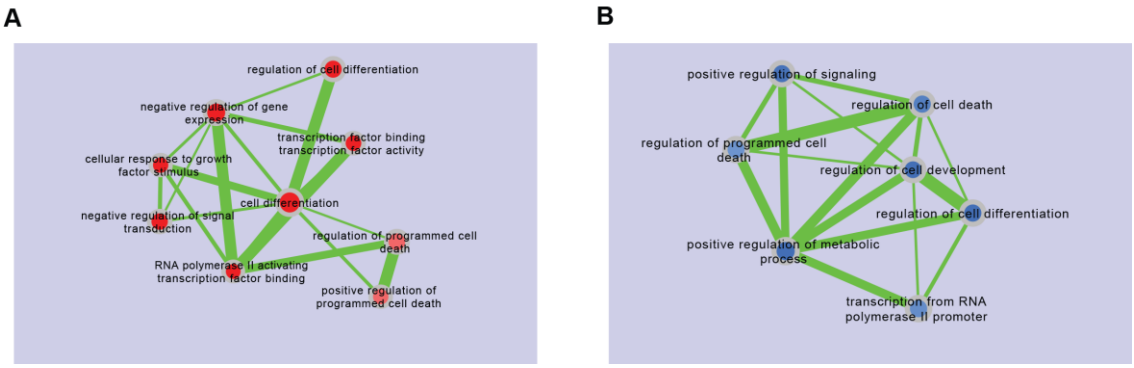

fig. S3. Enrichment map was used for visualizing the network of selected GO terms enriched with up-regulated and down-regulated genes in *Asxl1*$^{-/-}$ LK cells. Nodes indicate the enriched functional gene sets. Node size represents the frequencies of genes in the gene-set. Red and blue node colors represent up- and down-regulated gene sets, respectively. Enrichment significance (p-value) is conveyed as node color intensity. Edges represent gene overlap between sets.

table S1 (Microsoft Excel format). List of ASXL1 interaction proteins identified by MS in HEK293T cells transfected with FLAG-ASXL1.

table S2. qPCR primer sequences.

Gene	strand	Sequence	Exon
Asxl1	mAsxl1-F	TCTACAGAGTCTCAGAGCCG	6
	mAsxl1-R	AGCATAACCCCAGTCCTTTTC	7
ASXL1	hASXL1-F	AGGATGCAAAATCTGTGGCCT	13
	hASXL1-R	GTGCTGCAGAGGATGTGC	13
hACTB	hACTB-F	GCACAGAGCCTCGCCTT	1
	hACTB-R	CCTTGCACATGCCGGAG	2
Cbfb	mCbfb-F	CTTGAAGGCTCCCATGATTCT	4
	mCbfb-R	AAACTCCAGGCAACCCATAC	4
Fus	mFus-F	GAAGCAGTGGTGGCTATGAA	6
	mFus-R	CCCGAGGACCACCAAATTTAT	8
Stat3	mStat3-F	CTCAGCCCCGGAGACAGT	1
	mStat3-R	CTGCTCCAGGTAGCGTGTGT	2
mActb	mActb-F	CGGCCAGGTCATCACTATT	4
	mActb-R	GATGCCACAGGATTCCATAC	5

table S3. Statistical evidence for binding between SMC1A, RAD21, and ASXL1.

Binging index	SMC1A	RAD21	ASXL1	p-value
1	0	+	+	2.143216e-124
2	+	0	+	5.74251e-108
3	+	+	0	1.1861239e-155

“+” indicates the co-occurrence for two proteins in binding.