Supplementary material

Deregulation of tumor suppressive ASXL1−PTEN/AKT axis in myeloid malignancies

Lei Cao[1], Xianyou Xia[1], Yu Kong[1], Fengqin Jia[2], Bo Yuan[2], Rui Li[1], Qian Li[1], Yuxin Wang[1], Mingrui Cui[1], Zhongye Dai[1], Huimin Zheng[3], Jesper Christensen[4], Yuan Zhou[5], and Xudong Wu[1, 5, *]

[1] Collaborative Innovation Center of Tianjin for Medical Epigenetics, Tianjin Key Laboratory of Medical Epigenetics, Key Laboratory of Immune Microenvironment and Disease (Ministry of Education), Department of Cell Biology, Tianjin Medical University, Tianjin 300070, China

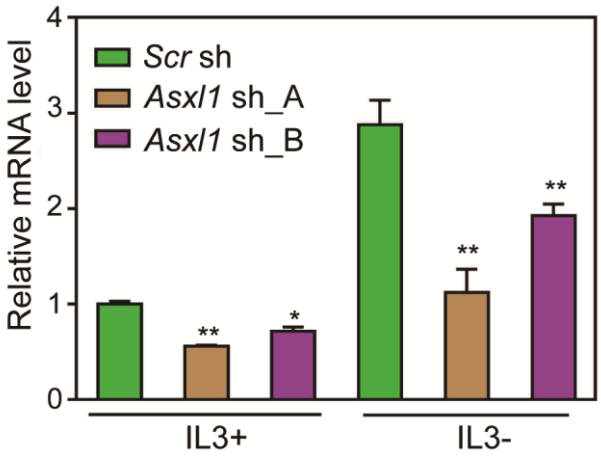
[2] National Demonstration Center for Experimental Basic Medical Science Education, Tianjin Medical University, Tianjin 300070, China

[3] Department of Prosthodontics, School and Hospital of Stomatology, Tianjin Medical University, Tianjin 300070, China

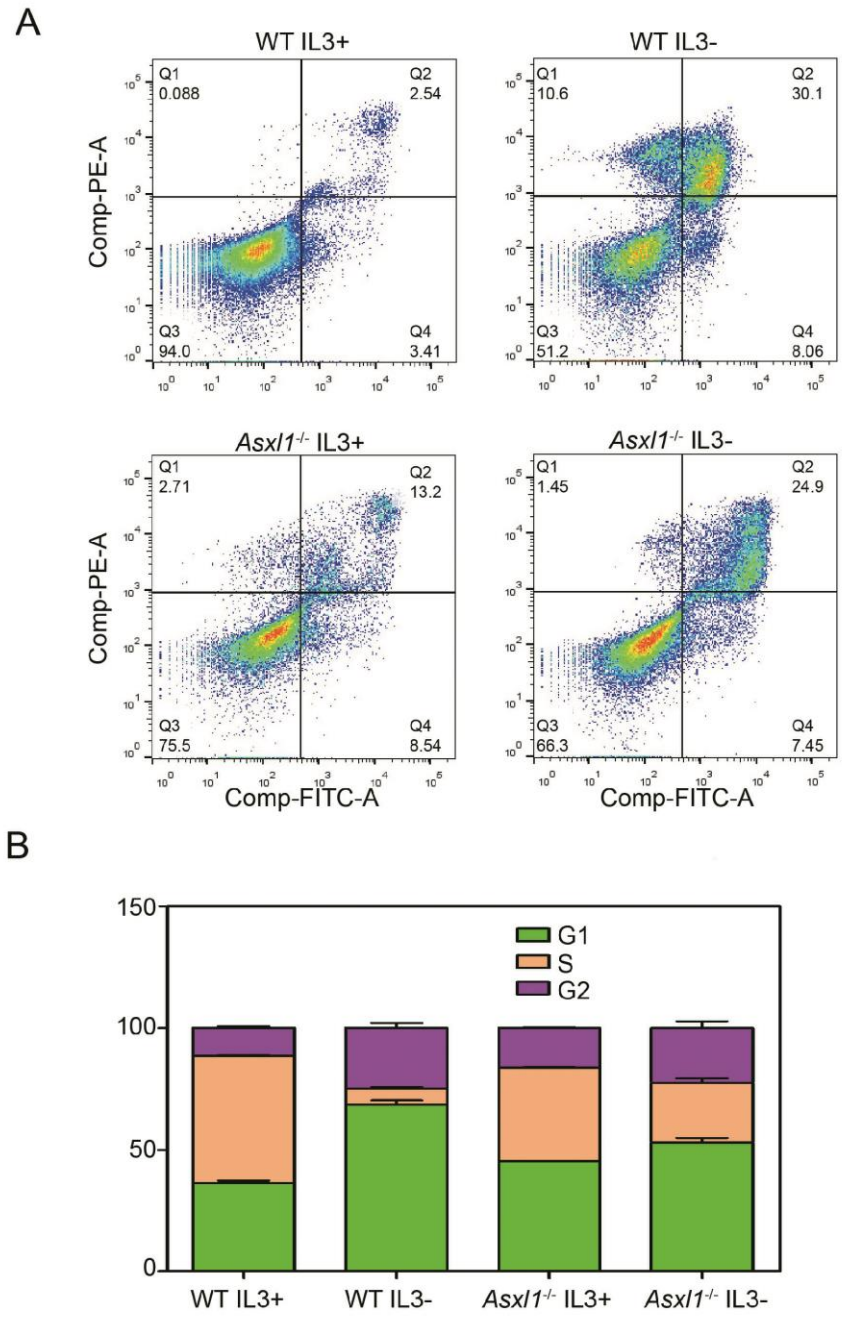
[4] Biotech Research and Innovation Centre and Centre for Epigenetics, University of Copenhagen, Ole Maaløes Vej 5, 2200 Copenhagen, Denmark

[5] State Key Laboratory of Experimental Hematology, Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Tianjin 300020, China

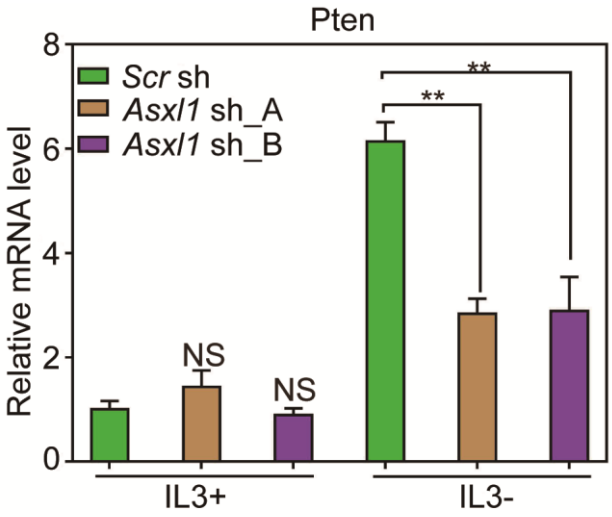
* Correspondence to: Xudong Wu, Tel: +86 8333 6825; Fax: +86 8333 6519; E-mail: wuxudong@tmu.edu.cn



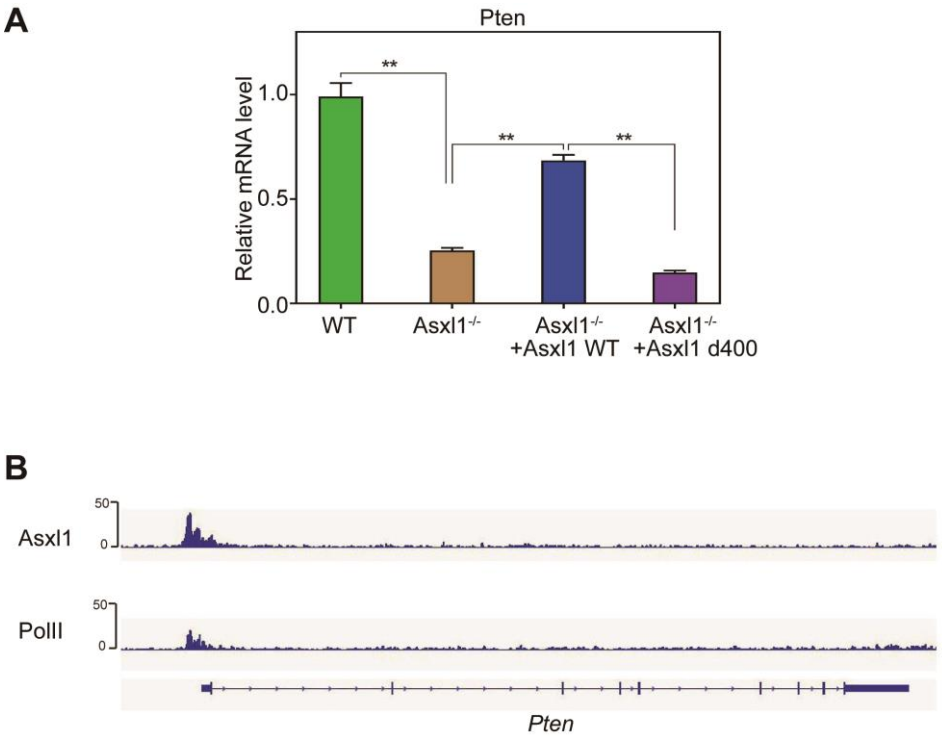
Supplementary Figure 1 Efficient knockdown of Asxl1 in 32D cells. RT-qPCR analysis of Asxl1 expression levels in control (*Scr* sh) and Asxl1-KD 32D cells (*Asxl1* sh_A and *Asxl1* sh_B) in the presence or absence of IL3. The error bars denote SD, n = 3.



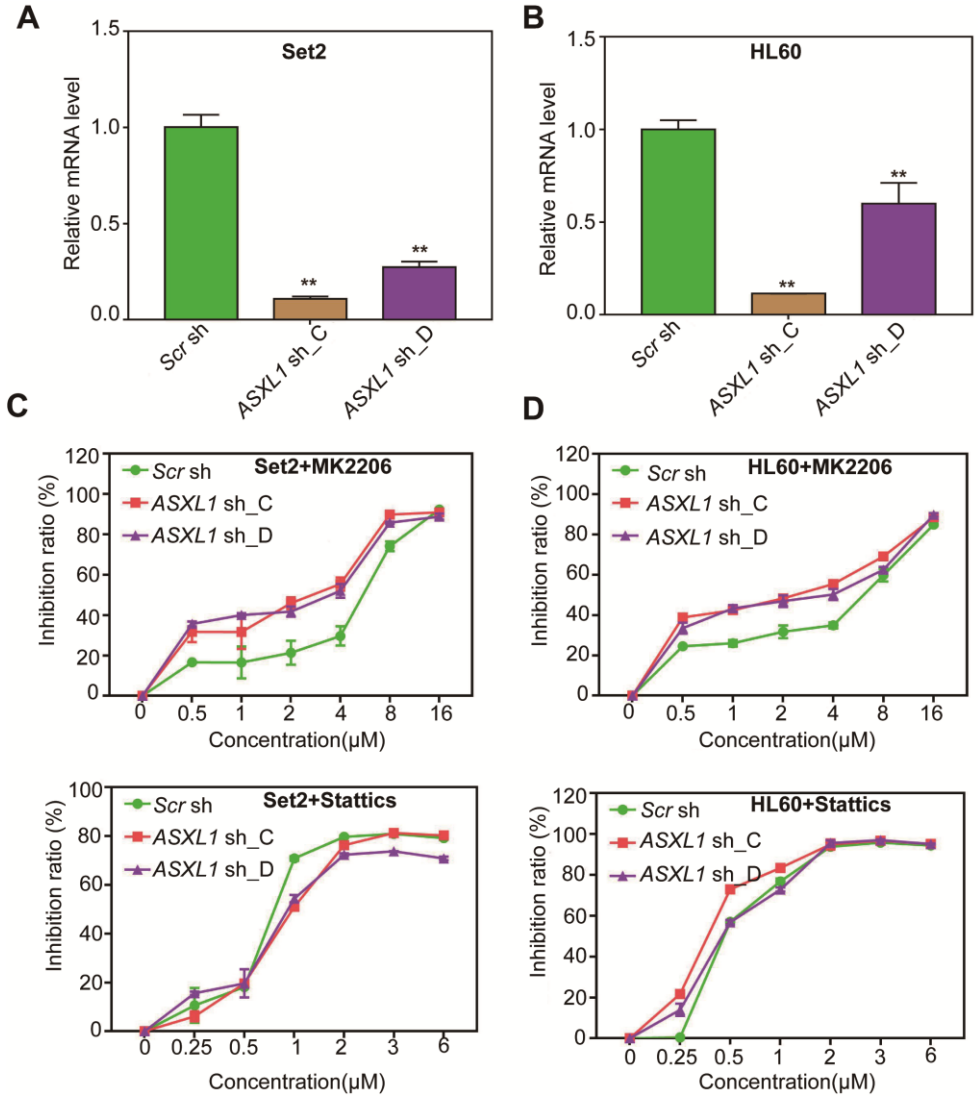
Supplementary Figure 2 Effects of Asxl1 loss on cell apoptosis and cell cycle. 32D cells were cultured with or without IL3 for 24 h. Cell apoptosis (**A**) and cell cycle (**B**) were tested by flow cytometry. The cell cycle distributions of four cell lines are presented as percentages. WT IL3+: G1 (36.47), S (52.02), G2 (11.52); WT IL3-: G1 (68.63), S (6.48), G2 (24.90); Asxl1$^{-/-}$ IL3+: G1 (45.31), S (38.56), G2 (16.13); Asxl1$^{-/-}$ IL3-: G1 (53.10), S (24.43), G2 (22.47).



Supplementary Figure 3 *Pten* fails to be activated with depletion of Asxl1. RT-qPCR analysis to determine the *Pten* expression levels in control (*Scr* sh) and Asxl1-KD 32D cells (*Asxl1* sh_A and *Asxl1* sh_B). The error bars denote SD, n = 3.



Supplementary Figure 4 Asx1 regulates Pten activation through binding to its promoter. (**A**) WT and Asxl1 d400 (deletion of 1-400 aa at the N-terminus) were overexpressed in Asxl KO 32D cells. RT-qPCR analysis to determine the Pten expression levels by withdrawal of IL3 for 24 h. (B) Asxl1 binds to the *Pten* promoter. Genomic snapshots of ASXL1 (Top) and RNA pol II (bottom) ChIP-seq analyses in haematopoietic stem cells (GSE99103) at the *Pten* locus.



Supplementary Figure 5 ASXL1 knockdown confers cells AKT sensitivity. (**A** and **B**) Efficient knockdown of ASXL1 in Set2 cells (**A**) and HL60 cells (**B**). (**C** and **D**) The inhibition ratio of each concentration in Set2 cells (**C**) and HL60 cells (**D**) using MK2206 and Stattics. Data are presented as sigmoid curves.

Supplementary Table S1 Specific oligonucleotides for shRNA and sgRNA cloning.

Name	Sequence (5'-3')
Asxl1 sgRNA	GAGGACTCCGCTGATGTGGA
Asxl1 sh_A	GTGGAAAGCTGTGGGTCTA
Asxl1 sh_B	GCTGATGGTGAATTCACTCAT
ASXL1 sh_C	GCTTCTGTAAGTATGCTCTAT
ASXL1 sh_D	CCAGGAGAATCAGTGCGTATA

Supplementary Table S2 Primers used in this study.

Primer name	Sequence (5'-3')	Notes
Asxl-qRT-F	GAAATGCAGCTACAGTGGATG	Forward primer for RT-qPCR of mouse *Asxl1*
Asxl-qRT-R	TGTCCCTGGGATTGGAGAG	Reverse primer for RT-qPCR of mouse *Asxl1*
Bap1-qRT-F	CCACAAATCTCAAGAGTCACAGC	Forward primer for RT-qPCR of mouse *Bap1*
Bap1-qRT-R	CTTCACCACCAGCTTACATTTG	Reverse primer for RT-qPCR of mouse *Bap1*
Pten-qRT-F	GAAAGGGACGGACTGGTGTA	Forward primer for RT-qPCR of mouse *Pten*
Pten-qRT-R	AGTGCCACGGGTCTGTAATC	Reverse primer for RT-qPCR of mouse *Pten*
ASXLl-qRT-F	GATGTGGAGAGCTGTGGGTCTAATG	Forward primer for RT-qPCR of human *ASXL1*
ASXLl-qRT-R	TTACCTTCAGAGGAGTCAGGACAAC	Reverse primer for RT-qPCR of human *ASXL1*
PTEN-qRT-F	TGGATTCGACTTAGACTTGACCT	Forward primer for RT-qPCR of human *PTEN*
PTEN-qRT-R	GGTGGGTTATGGTCTTCAAAAGG	Reverse primer for RT-qPCR of human *PTEN*
MYC-PtenEcoRI-F	GGAATTCATGGAGCAAAAGCTCATTTCTG AAGAGGACTTGAATGAAACAGCCATCATC AAAGAGATCG	Forward primer for cloning human *PTEN*
PTEN-BamHI-R	CGGGATCCTTAGACTTTTGTAATTTGTGAA TGCTG	Reverse primer for cloning human *PTEN*
PTEN C124S-F	CATGTTGCAGCAATTCACAGTAAAGCTGG	Forward primer for cloning human *PTEN C124S*
PTEN C124S-R	TGTGAATTGCTGCAACATGATTGTCATCTT C	Reverse primer for cloning human *PTEN C124S*
Pten TSS-F	GATGTGGCGGGACTCTTTGT	Forward primer for ChIP-qPCR of mouse *Pten*
Pten TSS-R	ACAGCGGCTCAACTCTCAAA	Reverse primer for ChIP-qPCR of mouse *Pten*

Supplementary Table S3 Clinical information of the MDS/CMML patients.

Patient samples	**Diagnosis***	**Sex**	**Age (yrs)**	**chromosome karyotype**	***ASXL1* genotyping**
1	MDS (RCMD)	M	48	ND	WT
5	CMML	M	54	46,xy[11]	c.1934dupG
9	MDS (RAEB1)	M	69	46,xy[13]	WT
10	CMML	F	34	46,xx[3]	WT
11	CMML	M	37	46,xy,del(7)(p12)[11]	c.1934dupG
12	MDS (RCMD)	F	49	ND	c.1934dupG
M30	PMF	M	33	ND	WT
M60	PMF	F	67	ND	WT
M251	CMML	M	78	47，XY,+8[9]/46,XY[2]	c.1934dupG p.Gly646TrpfsX12
M252	PMF	M	59	46,XY[7]	c.2468 delT, p.Leu823X
M319	MDS	M	42	46,XY[2]	c.1772dupA p.Thr591X
M689	MDS	M	68	46,XY[20]	WT
	*According to the WHO classification	M: male		ND: non-determined	WT: wild type
	RAEB1: Refractory anemia with excess blasts, type 1	F: female			Mut: mutations
	RCMD: Refractory cytopenias with multilineage dysplasia				