

Supplementary Materials for  
**CARM1-mediated methylation of ASXL2 impairs tumor-suppressive function  
of MLL3/COMPASS**

Zibo Zhao *et al.*

Corresponding author: Lu Wang, [lu.wang1@northwestern.edu](mailto:lu.wang1@northwestern.edu);  
Ali Shilatifard, [ash@northwestern.edu](mailto:ash@northwestern.edu)

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Figs. S1 to S5

## Supplementary Figures

### Figure S1. ASXL1 and ASXL2 depletion in CAL51 cells

A) Schematic of two BAP1 complexes, which is defined by two different additional sex-comb like proteins, ASXL1 and ASXL2. B) Design of a pair of CRISPR gRNA targeting exon 1 of ASXL1 and exons 1 and 2 of ASXL2. C) RNA-seq track examples that show the complete knockout of ASXL1, ASXL2, or both by CRISPR. D) The protein levels of BAP1 was determined by western blot in ASXL1/2 KO CAL51 cells. E) Heat maps generated from ChIP-seq data showing the occupancy of H3K27ac, H3K4me1, H3K4me3, H2AK119ub, as well as BAP1, MLL3, and UTX in BAP1-WT/KO CAL51 cells. All rows are centered on the BAP1 peaks and are further divided into two clusters based on K-means clustering (14). Group 1 peaks, which contain cluster 1–2, are enriched with enhancer marks, and group 2 peaks, which contain cluster 3–5, are enriched with promoter marks. F) The average plot shows the global occupancy of MLL3 in ASXL1-KO, ASXL2-KO, and BAP1-KO vs. wild type CAL51 cells.

### Figure S2. Identification of LOCAP (linker of COMPASS and PR-DUB) domain in ASXL2.

A) Alignment by CLUSTALW shows the similarities between ASXL2-LOCAP domains of different species, as well as human ASXL1. B) Flag-LSD2 was expressed in HEK293T cells, and then subjected to Flag tag-purification from nuclear extracts followed by mass spectrometry analysis. The peptide number from interactors are shown. C) The distribution of all truncating mutations that occur within the ASXL2 protein coding region from cBioPortal. D) ASXL2 truncating mutation frequencies across various human cancer types as obtained from cBioPortal.

### Figure S3. Validation of the specificity of me-ASXL2 antibody

A) Specificity of anti-methyl-ASXL2 was determined against non-methylated antigen peptide and di-methylated peptide via ELISA experiment. B) Endogenous immunoprecipitations with ASXL2 antibody from CARM1-WT and -KO CAL51 cells were subjected to western blotting with ASXL2 and methyl-ASXL2, n=2.

**Figure S4. CARM1 antagonizes MLL3 function in transcription regulation.**

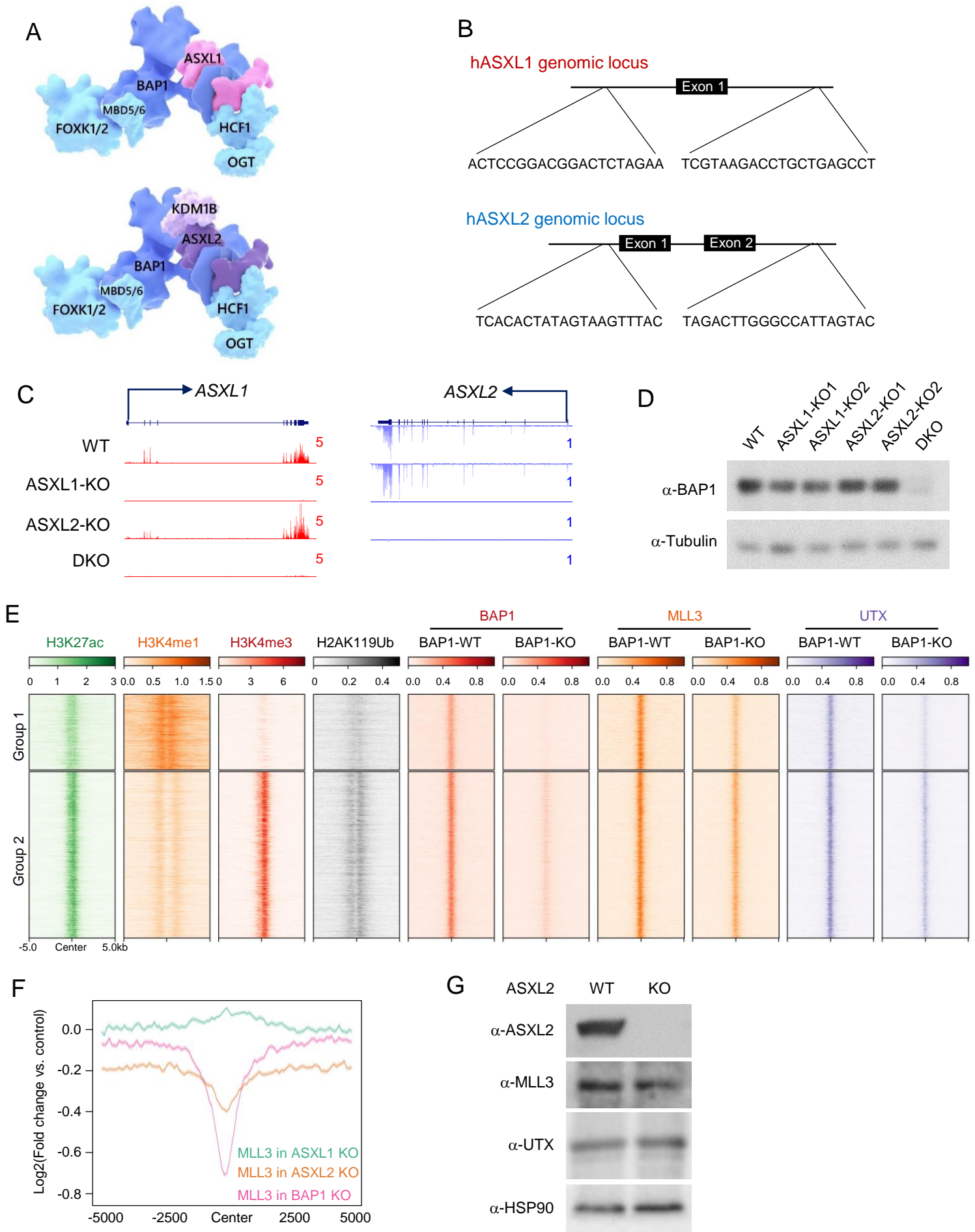
A) The protein levels of MLL3, NCOA6, PTIP, UTX, and RBBP5 were determined by western blot in CARM1-WT/KO cells by western blot, n=3. B) The Venn-diagram shows the overlap of up-regulated genes in CARM1-KO cells and down-regulated genes in MLL3-KO cells. C) Pathway analysis was performed with gene list from (A) by metaspape software (<http://metaspape.org/>). D) Both ChIP-Seq and RNA-Seq track examples showing MLL3 occupancy in CARM1-WT and CARM1-KO cells, as well as the expression of corresponding genes.

**Figure S5. Arginine methylation at ASXL2 impairs MLL3 function.**

A) The genomic DNA (gDNA) of ASXL2-WT gene that was chosen for targeting with CRISPR-Cas9. The sequence of donor ssDNA is shown with R639K/R641K (non-methylation mimic) and R639F/R641F (methylation mimic) mutations, respectively. B) ChIP-seq analysis of MLL3 binding in ASXL2-R639K/R641K and ASXL2-R639F/R641F CAL51 cells. Rows in the heat maps are centered on BAP1 peaks and show the log<sub>2</sub> (fold change) occupancy of MLL3 at BAP1 binding regions (left). RNA-seq (*n* = 2) was performed for ASXL2-R639K/R641K and ASXL2-R639F/R641F CAL51 cells. The heat maps show the log<sub>2</sub> (expression fold changes for the nearest gene of the indicated peaks) by comparing those in ASXL2-R639F/R641F with ASXL2-

R639K/R641K CAL51 cells (right). C) RNA-seq track examples comparing the enhancer binding regions of MLL3 in ASXL2-R639F/R641F with ASXL2-R639K/R641K CAL51 cells from corresponding genes. D) Pathway analysis was performed by Metascape using the commonly up/down-regulated genes upon both CARM1 inhibitor EZH2302 and EZH2 inhibitor GSK126 treatment.

# Figure S1. ASXL1 and ASXL2 depletion in CAL51 cells



# Figure S2. Identification of LOCAP (linker of COMPASS and PR-DUB) domain in ASXL2

**A**

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mASXL2 ATSSWEKRPRITENRQHQQPFQVSPQPFLLNR-----
rASXL2 ATSSWEKRPRITENRQHQQPFQASQPFLLNR-----
hASXL2 APVSWEKRPRTENRQHQQPFQVSPQPFLLNR-----
hASXL3 DESSATAKPLGENLTSQQKNLSNTPPEIIMSSSSIAPEAFPSDELHNKTLSSQQTCKSHVCTEKYPASIPELASTEMIKVKNHNSVLQRTEKKVLPSPLELSVSEGTDNKGNELPSAKLQ
hASXL1 -----SVHTEKQPQTKEEP-----
          .. : . *

mASXL2 -----GDRVQVR-----KVPPLKIPVSRISPML
rASXL2 -----GDRAQVR-----KVPPLKIPVSRISPML
hASXL2 -----GDRIQVR-----KVPPLKIPVSRISPMP
hASXL3 DKQYISSVDKAPFSEGSRNKTHKQGSTQSRLETSHTKSSEPSKSPDGIRNESRDSEISKRTAEQHSFGICKEKRARIEDDQSTRNISSSSPPEKEQPPEEPKVPPLKIQLSKIGPPF
hASXL1 -----KVPPIRIQLSRIKPPW-----
          :***:* :*:*

mASXL2 FSTSQVSP---RARFPISITSPYRTGARTLADIKAKAQLVEAQKAAAAAAAAAAAAASVGGTIPGPGPGGGGQ-----
rASXL2 FSTSQVSP---RARFPVSIITSPYRTGARTLADIKAKAQLVKAQKAAAAAAAAAAAAASVGGTIPGPGPGGGGQ-----
hASXL2 FHPSQVSP---RARFPVSIITSPNRTGARTLADIKAKAQLVKAQRAAAAAAAAAAAAAASVGGTIPGPGPGGGGQ-----
hASXL3 IIKSQVSPKPEASTSSTVSGGRNTGARTLADIKARAQAQRAQREAAAAAVAAASIVSGAMGSPGEGGKRTLAIHQETKAKLFAKHQARAHLFQTSKETRLPPLSSKEGPPNLE
hASXL1 VVKGQPTYQICPRIIPTTESSCRGTGARTLADIKARALQVRGARGHCHREAAATTAIGGGGGPGGGGGGATD-----
          . . *      *      :      *****.*      . . . :      . : * *      *      * *

mASXL2 -----
rASXL2 -----
hASXL2 -----
hASXL3 VSSTPETKMEGSTGVIIVNPNCRSPSNKSAHLRETTTVLQQSLNPSKLPETATDLSVHSSDENIPVSHLSEKIVSSTSESSVPMFLNKNVSVVSVCAISGAIKEHPFVSVDKSSV
hASXL1 -----

mASXL2 -----SPREGGERKIAGGGSAGSDPVSTNGKGPTEL LAGTGSRG--
rASXL2 -----SPREGGERKTAGGGSAGSDPVSTNGKGPAL ELAGTGSRG--
hASXL2 -----GPGEQGGQTARGGSPGSDRVSETGKGPTEL LAGTGSRG--
hASXL3 LMSVDSANTTISACNISMLKTIQGTDPICIAIIPKCIESTPISATTEGSSISSMDDKQLLISSSASNLVSTQYTSVPTPSIGNNLPNLSTSSVLIPPMGINNRFSEKIAIPGSEEQA
hASXL1 -----EGGGRGSSSGDGGEACGHPPEPRGGPSTPGKCTSDLQR-----
          *      . .      * :      . .

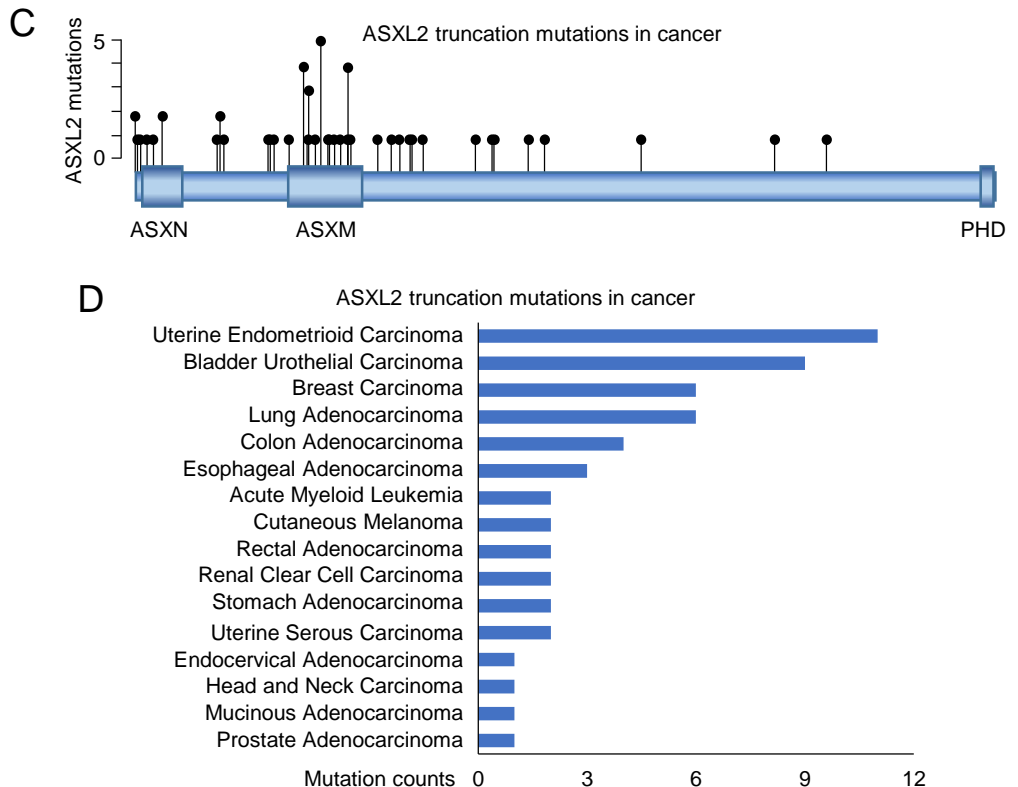
mASXL2 -----GTRELLPCGPQPETN-----MPGQAQPPG-----ISGA
rASXL2 -----GTRELLPCDPQPETN-----TPGQAQPPG-----VSGA
hASXL2 -----GTRELLPCGPETQPOSETKTPPSQAQPHS-----VSGA
hASXL3 TVSMGTTVRAALSCSDSVAVTDSLVAHPTVAMFTGNMLTINSYDSPKLSAESLDKNSGPNRDNNSGKPPQPPGGFAPAAINRSIPCKVIVDHSTLTSSLSLTVSVESSEASLDLQGR
hASXL1 -----TQLLPPYPLNGEHTQAGTAMSRARRED-----LPSLRKEES
          *      *

mASXL2 QLQQTSSVPTGLASSGACTSVPLPAHIEISNSEKPNLHKATATAASPCHLQDPRSCRLE-----
rASXL2 QLQQTSSVPAGLAASGTCTSVPLPAHIEIEMNREKPNPHRATATAASPCHSQEPRSCRLE-----
hASXL2 QLQQTTPVPPTPAVSGACTSVSPAHIEKLDNEKLNPTREATATVASVSHQGPSSCRQE-----
hASXL3 PVRTEASVQPVACQVSVISRPEPVANEGIDHSSTFIAASAQKQSKTLPATCTSLRELPLVPDKLNE
hASXL1 CLLQRATVGLTDGLGASQLPVAPTGDQPCQALPLLSSQTSVAERLVEQPQLHPDVRTECESGTTTSE
          : . . *      :      * . : :      : : . *
    
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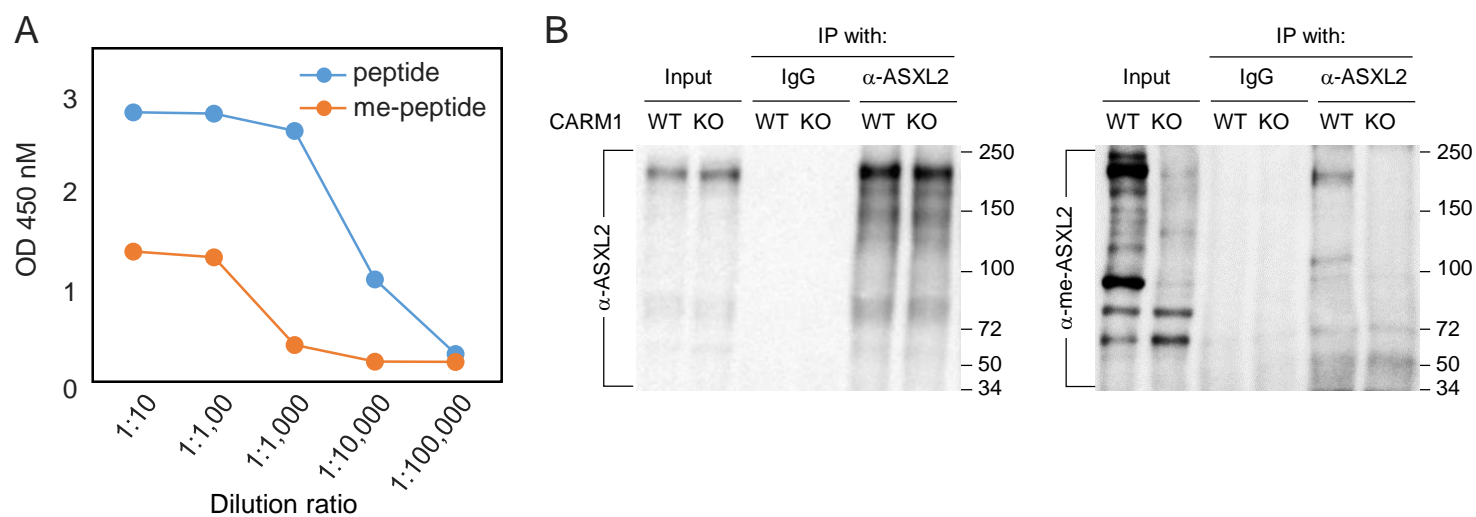
**B**

Mass spectrometry

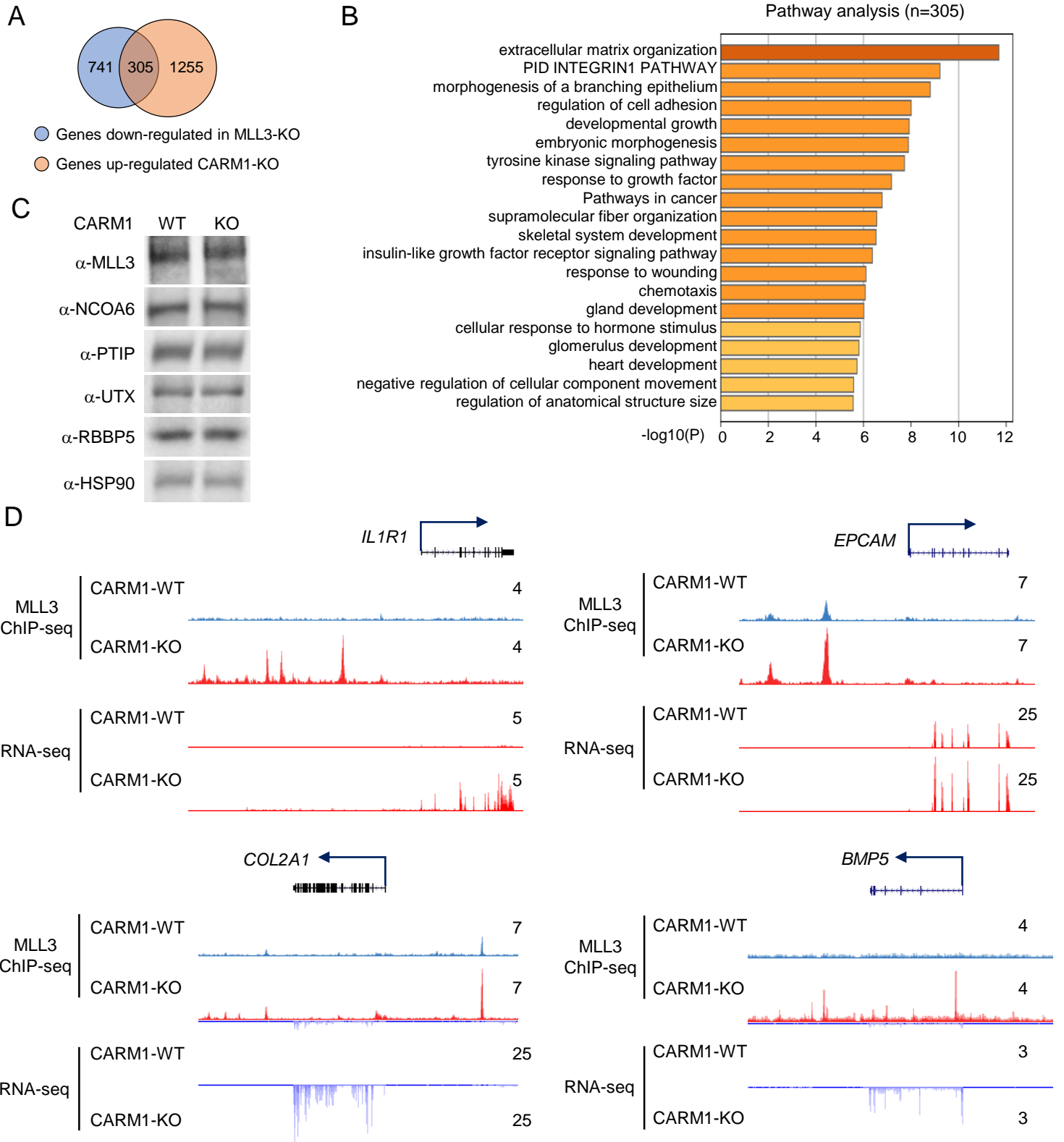
Protein	GFP	Flag-LSD2
KDM1B	0	136
ASXL2	0	31
HCFC1	0	12
WHSC1L1	0	10
MEPCE	0	9
BAP1	0	6
C1QBP	0	6
IARS	0	4
KEAP1	0	4
MBD6	0	4
MBD5	0	4
PKP3	0	4
HNRNPF	0	3
FOXK1	0	3
TMPO	0	3
MLL3	0	0



**Figure S3. The methylation level of ASXL2 correlates with breast cancer malignancy**



# Figure S4. CARM1 antagonizes MLL3 function in transcription regulation.





# Figure S5. Arginine methylation at ASXL2 impairs MLL3 function

