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Supplementary Materials for

LEDGF/p75 promotes transcriptional pausing through preventing SPT5 phosphorylation

Chenghao Guo et al.

Corresponding author: Zhuojuan Luo, zjluo@seu.edu.cn; Chengqi Lin, cqlin@seu.edu.cn; Zheng Ge, zhengge@seu.edu.cn

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This PDF file includes:

Figs. S1 to S9 Tables S1 and S2 References



Figure S1. SPT5-CTR is conserved in metazoan.

(A) Phylogenetic tree comparing the evolution of the modular domains of SPT5. FASTA sequences were obtained for 36 representative organisms for. Organisms were grouped using the National Center for Biotechnology Information (NCBI) Taxonomy Common Tree algorithm, and the generated tree was visualized using the European Molecular Biology Laboratory (EMBL) Interactive Tree of Life (iTOL) tool. Sequences were aligned using the NCBI Cobalt algorithm using default settings, and color coding was assigned based on frequency-based differences, where red indicates highly variable regions with high frequency of mutations and gray indicates highly conserved regions with low frequency of mutations. Sequence gaps are indicated by unbroken gray lines. The violet lines indicate the PLAAC prion propensity scores. (B) The conservation analysis of PRD or PLD in SPT5-CTR. (C) Schematic representation of the positions of the sgRNAs and homologous templates used to deletion of SPT5-PRD or -PLD.



Figure S2. Adjacent PRD and PLD in SPT5 exhibit different phase separation property.

(A) Confocal images showing co-localization of Pol II-pSer5 with SPT5 in nuclear puncta in WT, SPT5_{Δ PRD}, and SPT5_{Δ PLD} HCT 116 cells after serum starvation. (B) Violin plot (top and mid panel) showing the number of SPT5 and Pol II-pSer5 nuclear puncta per cell in Figure S2A. Box plots (bottom panel) showing the mean values of the Pearson correlation coefficient of co-localization ratios of SPT5 and Pol II-pSer5 in Figure S2A. (C) Confocal images showing co-localization of SPT5 with NELFE in nuclear puncta in WT, SPT5_{Δ PRD}, and SPT5_{Δ PLD} HCT 116 cells. (D) Violin plot (top and mid panel) showing the number of SPT5 and NELFE nuclear puncta per cell in Figure S2C. Box plots (bottom panel) showing the mean values of the Pearson correlation coefficient of SPT5 and NELFE nuclear puncta per cell in Figure S2C. Box plots (bottom panel) showing the mean values of the Pearson correlation coefficient of SPT5 and NELFE in Figure S2C. (E) Confocal images showing co-localization ratios of SPT5 in Figure S2C. (E) Confocal images showing co-localization ratios of SPT5 and NELFE in Figure S2C. (E) Confocal images showing the mean values of the Pearson correlation coefficient of co-localization ratios of SPT5 in Figure S2C. (E) Confocal images showing co-localization of AFF4 with SPT5 in nuclear puncta in WT, SPT5_{Δ PRD},

and SPT5_{Δ PLD} HCT 116 cells after serum induction. (F) Violin plot (top and mid panel) showing the number of SPT5 and AFF4 nuclear puncta per cell in Figure S2E. Box plots (bottom panel) showing the mean values of the Pearson correlation coefficient of co-localization ratios of SPT5 and AFF4 in Figure S2E. (G) Confocal images showing that SPT5, Pol II-S5P or NELFE formed nuclear puncta in WT, SPT5_{Δ PRD}, and SPT5_{Δ PLD} HCT 116 cells before and after treated with 1% 1,6-hexanediol (abbreviation as 1,6-hex) for 30 minutes in serum starvation condition. (H) Violin plot showing the number of cells containing nuclear puncta in Figure S2G. Results in Figure B, D, F, H are representative of three biological replicates, each n > 20. Two-tailed, unpaired Student's *t* test was performed. *p<0.05, **p<0.01, ***p<0.001.

Figure S3.



Figure S3. Adjacent PLD and PRD in SPT5 are required for Pol II pausing and elongation respectively.

(A) The recombinant BFP-AFF4-IDR, mCherry-SPT5-PRD-PLD, mCherry-SPT5-PRD, and mCherry-SPT5-PLD proteins were purified, and analyzed by SDS-PAGE followed by Coomassie blue staining. The line represents the purified protein. (B) Confocal imaging of nascent RNA labeled by EU in WT, SPT5_{Δ PRD}, and SPT5_{Δ PLD} HCT 116 cells. (C) Violin plot showing the intensity of cells in Figure S3B. Results are representative of three biological replicates, each n > 50. Two-tailed, unpaired Student's *t* test was performed. ***p < 0.001. (D) Box plots showing the correlation of log2 FC of Pol II pause index (PI) in four equal groups based on PI of SPT5_{Δ PRD} (left panel) or SPT5_{Δ PLD} (right panel) versus WT cells.



Figure S4. SPT5-PLD inhibits SEC mediated PRD phosphorylation.

(A) Representative gene examples of SPT5-pPRD and AFF4 ChIP-seq in WT and SPT5_{Δ PLD} HCT 116 cells. Y-axis representing RPM. (B) Western blot analysis of SPT5-pPRD, SPT5, Pol II-pSer2, and AFF4 in HCT 116 cells treated with 3.5 hours DRB (100 μ M) or not. (C) Western blot analysis of SPT5-pPRD, SPT5, Pol II-pSer2, AFF4, and AFF1 in HCT 116 cells treated with 6 hours KL-1 (20 μ M) or not. (D) Western blot analysis of SPT5-pPRD, SPT5-pPRD, SPT5-pPRD, SPT5, Pol II-pSer2, AFF4, and AFF1 in WT and AFF4 KO HCT 116 cells. (E) Western blot analysis of SPT5-pPRD, SPT5, Pol II-pSer2, and BRD4 in HCT 116 cells treated with 3 hours JQ1 (1 μ M) or not. (F) Western blot analysis of SPT5-pPRD, SPT5, Pol II-pSer2, and BRD4 in HCT 116 cells with knockdown of BRD4. α -Tubulin was used as a loading control. (G) The recombinant SPT5-PRD and SPT5-PRD-PLD proteins were purified, and analyzed by SDS-PAGE followed by Coomassie blue staining. The line represents the purified protein.

Figure S5.



Figure S5. LEDGF interacts with SPT5 at PLD.

(A) Generation of BioID tagged proximity labelling FLAG-BioID-SPT5 or -SPT5_{APLD} cell lines. (B) Endogenous IPs showing the interaction among Pol II and SPT5, LEDGF, PPP2CA, NELFE, AFF4 or Pol II-pSer2 in WT, SPT5_{APRD}, and SPT5_{APLD} HCT 116 cells. α -Tubulin was used as a control. (C) Western blot analysis of SPT5-pPRD in HCT 116 cells with knockdown of PPP2CA (left-top panel), TCERG1 (right-top panel), SCAF11 (left-bottom panel), or BCOR (right-bottom panel) by independent shRNAs. NonT represents non-target. α -Tubulin was used as a loading control. (D) Endogenous IPs showing the interaction among LEDGF, PPP2CA and SPT5 in LEDGF-dTAG HCT 116 cells treated with dTAG for 3 hours or not. (E) Peptide sequence and motifs associated with LEDGF were analyzed in mass spectrometry.



Figure S6. Rapid degradation of LEDGF leads to increased initiating and elongating Pol II.

(A) Confocal imaging of nascent RNA labeled by EU in LEDGF-dTAG HCT 116 cells treated with 3 hours dTAG or not. (B) Violin plot showing the intensity of cells in Figure S6A. Results are representative of three biological replicates, each n > 50. Two-tailed, unpaired Student's *t* test was performed. ***p < 0.001. (C) Scatter plots comparing LEDGF CUT&Tag using two different antibodies to recognize LEDGF/p75 CTD (Bethyl) or IBD (generated-in-house) in HCT 116 cells with Pearson correlation coefficients. (D) LEDGF CUT&Tag-qPCR analysis of example genes in in LEDGF-dTAG HCT 116 cells treated with 3 hours dTAG or not. The *HEMO* gene serves as a negative control. Two-tailed, unpaired Student's *t* test was performed. ***p < 0.001. (E) Heatmaps of LEDGF occupancy showing RPM and log2 FC on scaled genes ranked by decreasing occupancy in LEDGF-dTAG HCT 116 cells treated with 3 hours dTAG or

not. N= 6732 genes. (F) Representative gene examples of NELFE, AFF4 ChIP-seq in HCT 116 cells, and SPT5, Pol II ChIP-seq, or LEDGF CUT&Tag in LEDGF-dTAG HCT 116 cells treated with 3 hours dTAG or not were analyzed. Y-axis representing RPM.

Figure S7.





Figure S8.



Figure S8. LEDGF-IBD prevents SPT5-PRD phosphorylation by SEC.

(A) The recombinant SPT5-PRD, SPT5-PRD-PLD, and LEDGF-IBD proteins were purified, and analyzed by SDS-PAGE followed by Coomassie blue staining. The line represents the purified protein. (B) Quantification of relative density of SPT5-pPRD compared to SPT5-PRD or SPT5-PRD-PLD in Figure 7A, n = 4. Two-tailed, unpaired Student's *t* test was performed. *p < 0.05, ***p < 0.001. (C) The LEDGF IBD-binding motifs (IBMs) can be characterized by a region with α -helical propensity followed by an acidic stretch and contain an FxGF motif or not. (D) *In vitro* pull-down assays using purified SPT5-PRD or SPT5-PRD-PLD incubated with GST or GST-LEDGF-IBD first, followed by GST pull down, and analyzed by SDS-PAGE followed by Western blotting or Coomassie blue staining. (E) *In vitro* pull-down assays using purified SPT5-PRD-PLD (PLD-S/T-E) incubated with GST or GST-LEDGF-IBD first, followed by GST pull down, and analyzed by SDS-PAGE followed by Coomassie blue staining. (F) The recombinant mCherry-SPT5-PRD, mCherry-SPT5-PLD, eGFP-LEDGF-IBD, and BFP-AFF4-IDR proteins were purified, and analyzed by SDS-PAGE followed by Coomassie blue staining. The line represents the purified protein.



Figure S9. LEDGF-IBD and SEC form distinct condensates with SPT5.

(A) Fluorescence microscopy images showing individual- and co-phase-separated droplets formed in the presence of 10% PEG-8000 with 150 mM NaCl containing buffer and 2 μ M eGFP-LEDGF-IBD and BFP-AFF4-IDR along with mCherry, mCherry-SPT5-PRD, mCherry-SPT5-PLD, or mCherry-SPT5-PRD-PLD. (B) Dot plot showing the partition ration in Figure S9A. Fields per condition n = 5. Two-tailed, unpaired Student's *t* test was performed. ***p < 0.001. (C) Confocal images (left panel) showing co-localization of AFF4 with LEDGF in nuclear puncta in WT and LEDGF_{ΔIBD} HCT 116 cells. Box plots (right panel) showing the mean values of the Pearson correlation coefficient of co-localization ratios of LEDGF with AFF4. Results are representative of three biological replicates, each n > 20. Two-tailed, unpaired Student's *t* test was performed. *p < 0.05.

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
AFF4	Lin et al., 2010 (49)	N/A
	Santa Cruz	Cat.#sc-390310; RRID:
АГГ4	Biotechnology	AB_2924396
AFF1	Che et al., 2024 (57)	N/A
BRD4	Francisco et al., 2017	N/A
	(52)	
CDK9	Lin et al., 2010 (49)	N/A
SPT5	Santa Cruz	Cat.#sc-28678; RRID:
	Biotechnology	AB_668824
SPT5	Santa Cruz	Cat.#sc-133217; RRID:
	Biotechnology	AB_2196394
SPT5	Santa Cruz	Cat.#sc-133097; RRID:
	Biotechnology	AB_1568824
SPT5	Abelonal	Cat.#A9193; RRID:
5115	Abelonal	AB_2772472
SPT5	Guo et al., 2023 (23)	N/A
SPT5-pPRD	Hu et al., 2021 (20)	N/A
SPT5-pS666	Hu et al., 2021 (20)	N/A
NELFE	Santa Cruz	Cat.#sc-32912; RRID:
	Biotechnology	AB_2177858
NELEE	Santa Cruz	Cat.#sc-377052; RRID:
NELFE	Biotechnology	AB_2847957
NELFE	Guo et al., 2023 (23)	N/A
D 1 H	Santa Cruz	NI20
Pol II	Biotechnology	N20
PAF1	Abcam	Cat.#ab20662; RRID:
		AB_2159769
DD2 A	A1 1 1	Cat.#A6702; RRID:
PP2A	Abcional	AB_2767286
LEDGE		Cat.#A18101; RRID:
LEDGF	Abcional	AB_2861895
LEDGF	Abcam	Cat.#ab177159; RRID: N/A
LEDGE		Cat.#A300-848A; RRID:
LEDGF	Bethyl Laboratories	AB_2171223
LEDGF	This Paper	N/A
TCEDC1	Drotaintaah	Cat.#21858-1-AP; RRID:
ICEKUI	Proteintech	AB 11183756

Table S1. List of key resources used in the study.

RNA polymerase II CTD repeat YSPTSPS (phospho S5)	Abcam	Cat.#ab232852; RRID: N/A	
Phospho-POLR2A-S5 Rabbit pAb	Abclonal	Cat.#AP0828; RRID: AB_2771442	
RNApolymeraseIICTDrepeatYSPTSPS(phosphoS2)	Abcam	Cat.#ab238146; RRID: N/A	
α-Tubulin	Abcam	Cat.#ab7291; RRID: AB_2241126	
FLAG	Sigma-Aldrich	Cat.#F1804; RRID: AB 262044	
MYC-TAG	Abclonal	Cat.#AE010; RRID: AB 2770408	
HRP Anti-Streptavidin	Abcam	Cat.#ab191338; RRID: N/A	
Goat anti-Rabbit IgG Alexa Fluor 488	Life Technologies	Cat.#A11070; RRID: AB_2534114	
Goat anti-Mouse IgG Alexa Fluor 488	Life Technologies	Cat.#A10684; RRID: AB 2534064	
Goat anti-Rabbit IgG Alexa Fluor 647	Abcam	Cat.#ab150083; RRID: AB 2714032	
Bacterial strain			
BL21		N/A	
Chemicals, peptides, and reco	ombinant proteins		
Proteinase K	Thermo Fisher Scientific	Cat.#25530049	
Ribonuclease A	Sigma-Aldrich	Cat.#R6513	
Pierce Universal Nuclease	Sigma-Aldrich Vazyme	Cat.#R6513 Cat.#DD4301-PC	
Pierce Universal Nuclease 1,6-hexanediol	Sigma-Aldrich Vazyme Sigma-Aldrich	Cat.#R6513 Cat.#DD4301-PC Cat.#240117	
Ribonuclease APierce Universal Nuclease1,6-hexanediolPEG-8000	Sigma-Aldrich Vazyme Sigma-Aldrich Sangon Biotech	Cat.#R6513 Cat.#DD4301-PC Cat.#240117 Cat.#A100159	
Ribonuclease APierce Universal Nuclease1,6-hexanediolPEG-8000JQ1	Sigma-Aldrich Vazyme Sigma-Aldrich Sangon Biotech Sigma-Aldrich	Cat.#R6513 Cat.#DD4301-PC Cat.#240117 Cat.#A100159 Cat.#SML0974	
Ribonuclease APierce Universal Nuclease1,6-hexanediolPEG-8000JQ1Flavopiridol	Sigma-Aldrich Vazyme Sigma-Aldrich Sangon Biotech Sigma-Aldrich MedChemExpress	Cat.#R6513 Cat.#DD4301-PC Cat.#240117 Cat.#A100159 Cat.#SML0974 Cat.#HY-10005	
Ribonuclease APierce Universal Nuclease1,6-hexanediolPEG-8000JQ1FlavopiridolDRB	Sigma-Aldrich Vazyme Sigma-Aldrich Sangon Biotech Sigma-Aldrich MedChemExpress Sigma-Aldrich	Cat.#R6513 Cat.#DD4301-PC Cat.#240117 Cat.#A100159 Cat.#SML0974 Cat.#HY-10005 Cat.#D1916	
Ribonuclease APierce Universal Nuclease1,6-hexanediolPEG-8000JQ1FlavopiridolDRBSEC inhibitor KL-1	Sigma-Aldrich Vazyme Sigma-Aldrich Sangon Biotech Sigma-Aldrich MedChemExpress Sigma-Aldrich MedChemExpress	Cat.#R6513 Cat.#DD4301-PC Cat.#240117 Cat.#A100159 Cat.#SML0974 Cat.#HY-10005 Cat.#D1916 Cat.#HY-122720	
Ribonuclease APierce Universal Nuclease1,6-hexanediolPEG-8000JQ1FlavopiridolDRBSEC inhibitor KL-1Critical commercial reagents	Sigma-Aldrich Vazyme Sigma-Aldrich Sangon Biotech Sigma-Aldrich MedChemExpress Sigma-Aldrich MedChemExpress	Cat.#R6513 Cat.#DD4301-PC Cat.#240117 Cat.#A100159 Cat.#SML0974 Cat.#HY-10005 Cat.#D1916 Cat.#HY-122720	
Ribonuclease A Pierce Universal Nuclease 1,6-hexanediol PEG-8000 JQ1 Flavopiridol DRB SEC inhibitor KL-1 Critical commercial reagents Ni-NTA Agarose	Sigma-Aldrich Vazyme Sigma-Aldrich Sangon Biotech Sigma-Aldrich MedChemExpress Sigma-Aldrich MedChemExpress	Cat.#R6513 Cat.#DD4301-PC Cat.#240117 Cat.#A100159 Cat.#SML0974 Cat.#HY-10005 Cat.#D1916 Cat.#HY-122720 Cat.#30210	
Ribonuclease APierce Universal Nuclease1,6-hexanediolPEG-8000JQ1FlavopiridolDRBSEC inhibitor KL-1Critical commercial reagentsNi-NTA AgaroseProtein A Agarose	Sigma-Aldrich Vazyme Sigma-Aldrich Sangon Biotech Sigma-Aldrich MedChemExpress Sigma-Aldrich MedChemExpress QIAGEN Santa Cruz	Cat.#R6513 Cat.#DD4301-PC Cat.#240117 Cat.#A100159 Cat.#SML0974 Cat.#HY-10005 Cat.#D1916 Cat.#HY-122720 Cat.#30210 Cat.#sc-2001	
Ribonuclease APierce Universal Nuclease1,6-hexanediolPEG-8000JQ1FlavopiridolDRBSEC inhibitor KL-1Critical commercial reagentsNi-NTA AgaroseProtein A AgaroseGlutathione Beads	Sigma-Aldrich Vazyme Sigma-Aldrich Sangon Biotech Sigma-Aldrich MedChemExpress Sigma-Aldrich MedChemExpress QIAGEN Santa Cruz Smart-Lifesciences	Cat.#R6513 Cat.#DD4301-PC Cat.#240117 Cat.#A100159 Cat.#SML0974 Cat.#HY-10005 Cat.#D1916 Cat.#HY-122720 Cat.#30210 Cat.#30210 Cat.#sc-2001 Cat.#SEA008010	
Ribonuclease APierce Universal Nuclease1,6-hexanediolPEG-8000JQ1FlavopiridolDRBSEC inhibitor KL-1Critical commercial reagentsNi-NTA AgaroseProtein A AgaroseGlutathione BeadsGelatin Sepharose Beads	Sigma-Aldrich Vazyme Sigma-Aldrich Sangon Biotech Sigma-Aldrich MedChemExpress Sigma-Aldrich MedChemExpress QIAGEN Santa Cruz Smart-Lifesciences Smart-Lifesciences	Cat.#R6513 Cat.#DD4301-PC Cat.#240117 Cat.#A100159 Cat.#SML0974 Cat.#HY-10005 Cat.#D1916 Cat.#HY-122720 Cat.#30210 Cat.#sc-2001 Cat.#SEA008010 Cat.#SEC0061	
Ribonuclease APierce Universal Nuclease1,6-hexanediolPEG-8000JQ1FlavopiridolDRBSEC inhibitor KL-1Critical commercial reagentsNi-NTA AgaroseProtein A AgaroseGlutathione BeadsGelatin Sepharose BeadsFOS DNA FISH probe	Sigma-Aldrich Vazyme Sigma-Aldrich Sangon Biotech Sigma-Aldrich MedChemExpress Sigma-Aldrich MedChemExpress QIAGEN Santa Cruz Smart-Lifesciences Smart-Lifesciences Empire Genomics	Cat.#R6513 Cat.#DD4301-PC Cat.#240117 Cat.#A100159 Cat.#SML0974 Cat.#HY-10005 Cat.#D1916 Cat.#D1916 Cat.#HY-122720 Cat.#30210 Cat.#SEA008010 Cat.#SEA008010 Cat.#SEC0061 N/A	

VAHTS Universal DNA Library Prep Kit for Illumina	Vazyme	Cat.#ND607-02
V3		
VAHTS DNA Adapters set1 for Illumina	Vazyme	Cat.#N801-02
Hyperactive Universal		
CUT&Tag Assay Kit for	Vazyme	Cat.#TD904
True Prop. Index. Kit. V2 for		
Illumina	Vazyme	Cat.#TD202
Deposited data	L	
Raw and analyzed data	This paper	GEO: GSE250380
Experimental models: Cell lin	nes	
HCT 116	ATCC	ATCC [®] CCL-247 [™]
HCT 116 AFF4 KO	Che et al., 2024 (57)	N/A
HCT 116 SPT5 _{ΔPRD}	This Paper	N/A
HCT 116 SPT5 _{APLD}	This Paper	N/A
HCT 116 LEDGF-dTAG	This Paper	N/A
HCT 116 LEDGF $_{\Delta IBD}$	This Paper	N/A
HEK293 Flp-In TRex	Invitrogen	Cat.#R78007
HEK293 FLAG-BioID-SPT5	This Paper	N/A
HEK293 FLAG-BioID- SPT5 _{ΔPLD}	This Paper	N/A
Recombinant DNA		
pET16b-SPT5-PRD	This Paper	N/A
pET16b-SPT5-RPD-PLD	This Paper	N/A
pET16b-SPT5-PRD-PLD (PLD-S/T-E)	This Paper	N/A
pET16b-LEDGF-IBD	This Paper	N/A
pET16b-mCherry-SPT5- PRD-PLD	Guo et al., 2023 (23)	N/A
pET16b-mCherry-SPT5-PRD	This Paper	N/A
pET16b-mCherry-SPT5-PLD	This Paper	N/A
pET16b-BFP-AFF4-IDR	Guo et al., 2023 (23)	N/A
pET16b-eGFP-LEDGF-IBD	This Paper	N/A
pGEX-5X-1-LEDGF-IBD	This Paper	N/A
pcDNA5-FLAG-AFF4	Lin et al., 2010 (49)	N/A
pcDNA5-FLAG-SPT5	This Paper	N/A
pcDNA5-MYC-LEDGF	This Paper	N/A

pcDNA5-MYC- LEDGF _{ΔPWWP}	This Paper	N/A
pcDNA5-MYC-LEDGF _{AIBD}	This Paper	N/A
lentiCRISPR v2-SPT5- sgRNA-1	This Paper	N/A
lentiCRISPR v2-SPT5- sgRNA-2	This Paper	N/A
lentiCRISPR v2-SPT5- sgRNA-3	This Paper	N/A
lentiCRISPR v2-SPT5- sgRNA-4	This Paper	N/A
pX459-LEDGF-sgRNA	This Paper	N/A
pX459-LEDGF-sgRNA1	This Paper	N/A
pX459-LEDGF-sgRNA2	This Paper	N/A
pX459 PITCh sgRNA	Hu et al., 2021 (20)	N/A
pUC57 PITCh SPT5-HR1	This Paper	N/A
pUC57 PITCh SPT5-HR2	This Paper	N/A
pUC57 PITCh LEDGF-HR	This Paper	N/A
Software and Algorithms		
FIJI	Schindelin et al., 2012 (50)	https://imagej.net/Fiji
SAMtools v1.15.1	Li et al., 2009 (58)	http://samtools.sourceforge. net
Bedtools v2.30.0	Quinlan and Hall, 2010 (59)	https://bedtools.readthedocs .io/en/latest/
Trim Galore v0.6.7	N/A	https://www.bioinformatics .babraham.ac.uk/projects/tri m_galore/
Bowtie v2.4.5	Langmead and Salzberg, 2012 (53)	http://bowtie- bio.sourceforge.net/bowtie2 /index.shtml
deepTools v 3.5.1	Ramírez et al., 2016 (54)	https://deeptools.readthedo cs.io/en/develop/
Picard 2.27.4	N/A	https://broadinstitute.github .io/picard/
MACS 2.2.7.1	Zhang et al., 2008 (60)	https://github.com/macs3- project/MACS
ChIPseeker R package v1.30.3	Yu et al., 2015 (55)	https://bioconductor.org/pac kages/release/bioc/html/ChI Pseeker.html

Oligonucleotides used for sgRNAs		
Name	Description	Sequence (5'-3')
SPT5 sgRNA-1	guide RNA targeting SPT5 locus	GGACCGTCAGCGGCTCACCA
SPT5 sgRNA-2	guide RNA targeting SPT5 locus	ACAACCCCAACACGCCGTCA
SPT5 sgRNA-3	guide RNA targeting SPT5 locus	TATTCTTCCTCAGCCCTGTT
SPT5 sgRNA-4	guide RNA targeting SPT5 locus	GTTTAGGGGGGTACCAAGGAG
LEDGF sgRNA	guide RNA targeting LEDGF locus	TGAAATCGCGAGTCATGTTT
LEDGF sgRNA1	guide RNA targeting LEDGF locus	CAATGGATTCTCGACTTCAA
LEDGF sgRNA2	guide RNA targeting LEDGF locus	CAAAGTTAGTCAGGTAATCA
Primers used for get	notyping	
Name	Description	Sequence (5'-3')
SPT5 $_{\Delta PRD}$ -F	Genotyping	AGTGACATTCTCCCCAATCCC
SPT5 _{APRD} -R1	Genotyping	AGAGAACTGGTCTGTGTGTGTA CC
SPT5 $_{\Delta PRD}$ -R2	Genotyping	CCATACATGGGCGTCCTCC
$SPT5_{\Delta PLD}$ -F	Genotyping	AGTGACATTCTCCCCAATCCC
SPT5 $_{\Delta PLD}$ -R1	Genotyping	GGGACAGGTCTGTCTCCAGTT TA
SPT5 $_{\Delta PLD}$ -R2	Genotyping	AGAGAACTGGTCTGTGTGTGTA CC
LEDGF-dTAG-F	Genotyping	TTCGCTTTAACCGCCCTC
LEDGF-dTAG-R	Genotyping	TCTGTCCAAGTCTGCCAATA
$LEDGF_{\Delta IBD}$ -F1	Genotyping	GACGCCAAGTGGTGGTTTTG
$LEDGF_{\Delta IBD}$ -F2	Genotyping	AGGCCTTGGATGAACTTGCT
$LEDGF_{\Delta IBD}$ -R	Genotyping	GCAGTCCTGGCAAATGGTTT
Oligonucleotides used for HR arm		
Name	Description	Sequence (5'-3')
SPT5-PITCh-HR1	HR for SPT5 _{APRD}	GGAACCCTGGACTCACCGTG ACGGGAGCCGCTGACGGTCC ACAGAGAT
SPT5-PITCh-HR2	HR for SPT5 _{APLD}	TGTCTCCAGTTTAGGGGGGTAC CAATGTTGAAAAGAAAA

Table S2. List of oligonucleotides used in the study.

LEDGF-PITCh- HR1 dTAG	HR for LEDGF-	CCCGGTCTCGCCCCCGAAAC-	
		Puro-P2A-Flag-Fkbp12 ^{F36V} -	
	ACTCGCGATTTCAAACCTGG		
I EDGE-HR	HR for I EDGE AND	AAACATCAATGGATTCTCGAG	
		GTGAAGGAGATTCCGTGAT	
Oligonucleotides use	ed for shRNAs		
Name	Description	Sequence (5'-3')	
I EDGE shRNA 1	RNAi mediated	GCAGCAACTAAACAATCAAA	
	LEDGF knockdown	Т	
LEDGE shRNA2	RNAi mediated	GCAGCTACAGAAGTCAAGAT	
	LEDGF knockdown	Т	
	RNAi mediated		
PPP2CA shRNA1	PPP2CA	TGGAACTTGACGATACTCTAA	
	knockdown		
	RNAi mediated		
PPP2CA shRNA2	PPP2CA	CCCATGTTGTTCTTTGTTATT	
	knockdown		
	RNAi mediated	CCACGTTACAAAGCAGTAGA	
TCERG1 shRNA1	TCERG1	Т	
	knockdown		
	RNAi mediated		
TCERG1 shRNA2	TCERG1	CCTCCTATCGTACCCATGATA	
	knockdown		
BCOR shRNA1	RNAi mediated	CCACGAAACTTATACTTTCAA	
	BCOR knockdown		
BCOR shRNA2	RNAi mediated	GCTCTATATTTCTGTCTCCAA	
	BCOR knockdown		
	RNA1 mediated		
SCAFII shRNAI	SCAFII	GCATITATICIGICAGGITAA	
	Knockdown		
SCAELL -LDNAO	RNA1 mediated		
SCAFII SIKINA2	SCAFII	CATCIGIAAAIGCIGAICIIA	
knockdown			
CERTAD2 promoto			
<i>SERIAD2_</i> promote	CUT&Tag-qPCK	TGGGAACTAACGCATCAGGA	
I-F	CUT & Tax a DCD		
<i>SERTAD2_</i> promote	CUT&Tag-qPCK	AGAGAACGGCGGAGTCTTAG	
I-K	CUT & Tog a DCD		
KLF5_promoter-F	CUT&Tag-qPCK	GCCTCTCTCCCTGCTCATAG	
	CUT & Tog aDCD		
KLF5_promoter-R	nrimer	CGACTACTGACACTTGACGC	
	CUT&Tag aPCP		
EZR_promoter-F	nrimer	GCAACTTACTGGTTTCGGCA	
1	Princi		

EZR_promoter-R	CUT&Tag-qPCR	AAGGGTTCTGCTCTGACTCC
	primer	moderreibereibneree
LDLR_promoter-F	CUT&Tag-qPCR	TCCCCTTGTCCAATGTGAGG
	primer	TEEEETTOTEEAATOTOAOO
LDLR_promoter-R	CUT&Tag-qPCR	
	primer	AAUUTUAUUCTCAUACTTEE
SRSF1_promoter-F	CUT&Tag-qPCR	TCGTAGGGTGCGTCTAATCC
	primer	TEGTAGOOTGEOTETAATEE
SRSF1_promoter-R	CUT&Tag-qPCR	
	primer	UICIAAUCAUCCACOUICIA

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