Acetylation Drives Nonhistone Chromatin Protein PC4-mediated Nucleolar Organization and Function

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Supplemental Information

Table S1

Name	Forward primer (5'-3')	Reverse primer (5'-3')	Purpose
EGFP-N1-PC4 K26A-R27A	GAAAAAAAGTTAGCGGCGAAAAAGCAAGCG	CGCTTGCTTTTTCGCCGCTAACTTTTTTTC	Cloning Primers
EGFP-N1-PC4 K23A-K24A	CAGTGATTCGGACAGCGAAGTTGAAGCAGCGTTAAAGAGGAAAAAGCAAGCGGTT	AACCGCTTGCTTTTTCCTCTTTAACGCTGCTTCAACTTCGCTGTCCGAATCACTG	Cloning Primers
EGFP-N1-PC4 K35R	AAGCAAGCGGTTCCAGAGAGGCCCGTGAAG	CTTCACGGGCCTCTCTGGAACCGCTTGCTT	Cloning Primers
mouse actin	CGTTGACATCCGTAAAGACCTC	AGCCACCGATCCACACAGA	RT-qPCR
mouse 47S	GCTGTTTTGCTTGTCCAGCC	CTCTCCGGAATCGAACCCTGA	RT-qPCR
human actin	ATTTGCGGTGGACGATGGAG	AGAGATGGCCACGGCTGCTT	RT-qPCR
human 47S	TGTCAGGCGTTCTCGTCTC	AGCACGACGTCACCACATC	RT-qPCR
Promoter	GGTATATCTTTCGCTCCGAG	AGCGACAGGTCGCCAGAGGA	ChIP-qPCR
18S	CGACGACCCATTCGAACGTCT	CTCTCCGGAATCGAACCCTGA	ChIP-qPCR
5.8S	AGTCGGGTTGCTTGGGAATGC	CCCTTACGGTACTTGTTGACT	ChIP-qPCR

28S	GAGCTCAGGGAGGACAGAAA	AGGTCAGAAGGATCGTGAGG	ChIP-qPCR
IGS	GTTGACGTACAGGGTGGACTG	GGAAGTTGTCTTCACGCCTGA	ChIP-qPCR

Table S1: List of primers used in this study.

Table S2:

	Nucleolar proteins interacting with PC4	Nucleolar localization	Function
1	Tcof1	FC	RNA polymerase I transcription
2	Smarca5	FC	
3	Supt16h	FC	
4	Mybbp1a	Nucleoli	
5	Top1	FC	rDNA topology, rDNA transcription
6	Top2a	Nucleoli	
7	Fbl	FC, DFC	rRNA processing
8	Nop56	FC	
9	Nop58	FC, Nucleoli	
10	Ftsj3	Nucleoli rim, Nucleoli	
11	Rrp5	Nucleoli rim	
12	Hmgb2	Nucleoli	Chromatin organization
13	Lbr	Nucleoli rim	
14	Ddx21	Nucleoli rim, Nucleoli	rDNA transcription, rRNA processing
15	Ddx5	Nucleoli	
16	Ddx18	Nucleoli rim, Nucleoli	rRNA processing, ribosome biogenesis
17	Npm1	Nucleoli rim, Nucleoli	
18	Ncl	Nucleoli rim, Nucleoli	
19	Rpl13	Nucleoli	Nucleolar structure, ribosome biogenesis
20	Rpl13a	Nucleoli	
21	Rpl4	Nucleoli	
22	Rpl40	Nucleoli	
23	Rpl7	Nucleoli	
24	Rpl7a	Nucleoli	
25	Rpl18a	Nucleoli	
26	Rpl26	Nucleoli	

27	Rpl27	Nucleoli	
28	Rpl22	Nucleoli	
29	Rpl5	Nucleoli rim, Nucleoli	
30	Rps6	Nucleoli	Ribosome biogenesis
31	Rps25	Nucleoli	
32	Rps27a	Nucleoli	
33	Ddx18	Nucleoli rim, Nucleoli	rRNA processing, nucleolar structure
34	Ddx56	Nucleoli	
35	Nono	FC, Nucleoli	DNA damage response at rDNA

Table S2: List of nucleolar proteins interacting with PC4.

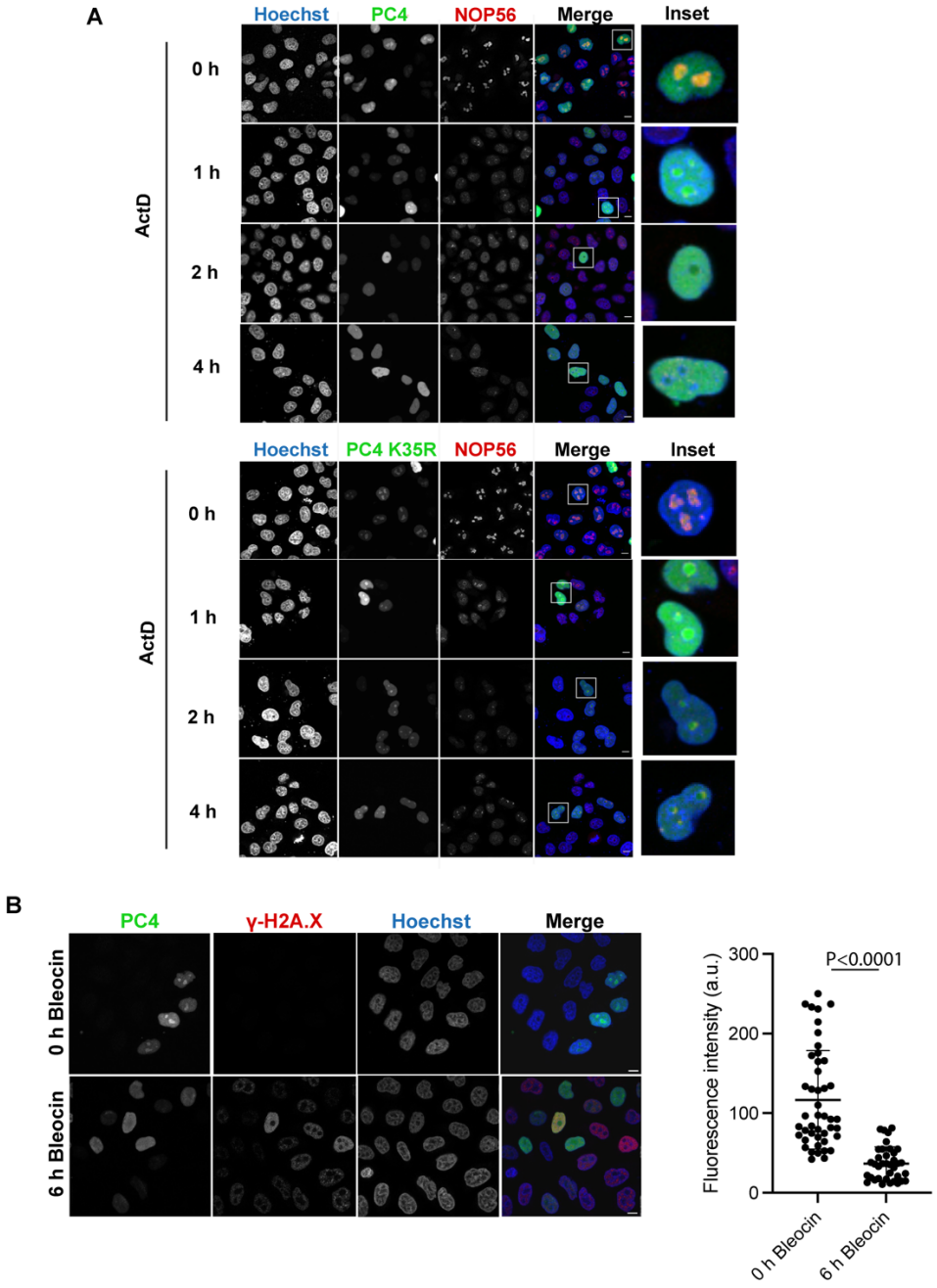


Figure S1. Time-dependent dynamics of PC4 and NOP56 under ActD treatment. A. Sub-cellular localization of WT PC4 (green) and NOP65 (red) under ActD treatment of 0, 1, 2, and 4 hours (h) duration. N=1. B. PC4-GFP (green) and γ-H2A (red) localization upon 5 μM Bleocin treatment for 6 h. Nucleolar fluorescence intensities were plotted in the graph alongside. DMSO control, n=45, Bleocin, n=37. Unpaired t-test was performed. N=3. Scale bar = 10 μm.

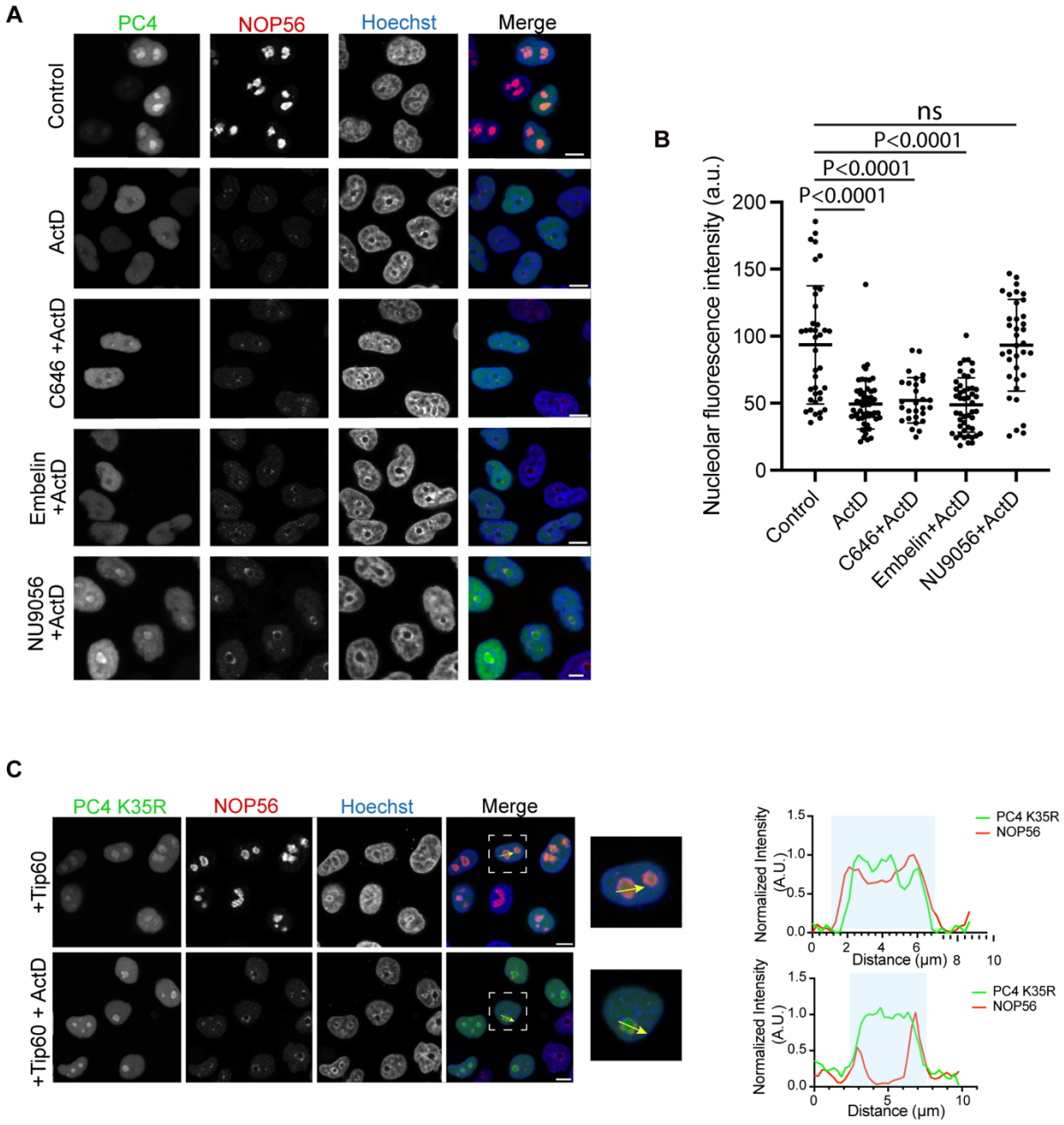


Figure S2. Tip60-mediated acetylation of PC4 on K35R determines its subnuclear localization. A. Subcellular localization of PC4 (green) and Nop56 (red) in HeLa cells treated with10 μM C646 for 2h, 20 μM Embelin for 24 h, or 10 μM NU9056 for 6 h in addition to ActD treatment. Nuclei was counterstained with Hoechst (blue). B. Graphical representation of the nucleolar intensities of control cells (n=39 nucleoli in 22 cells) or cells treated with ActD (n=55 nucleoli in 31 cells), C646+ ActD (n=28 in 20 cells), Embelin+ActD (n=48 nucleoli in 33 cells), or ActD+NU9056 (n=36 nucleoli in 26 cells).

N=2. PC4 K35R (green) and Nop56 (red) subnuclear localization in cells overexpressing Tip60 with or without ActD. Nuclei was counterstained with Hoechst (blue). Line plots across the yellow arrows were plotted to show the colocalization between PC4 and Nop56 nucleolar protein. The nucleolar regions are shaded in blue. N=1. Scale bar = 10 μm.

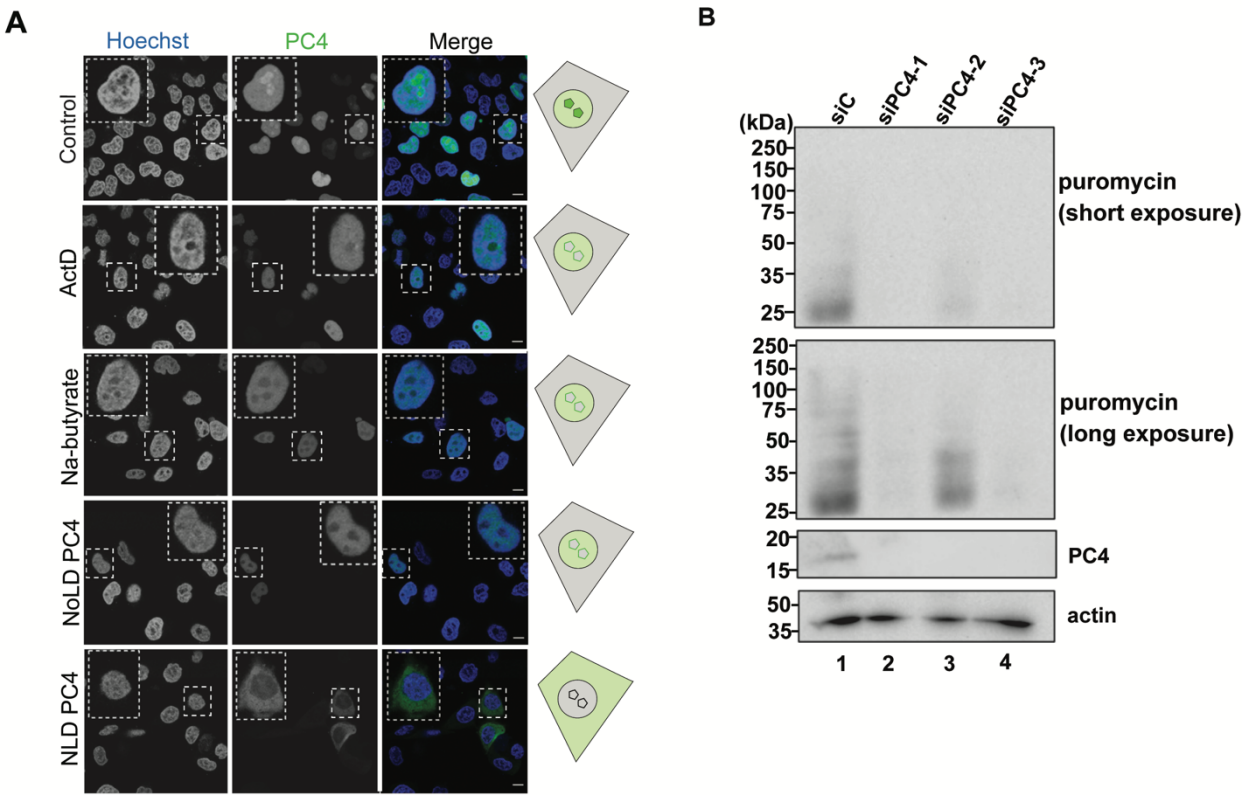


Figure S3: Loss of nucleolar PC4 leads to abrogation of protein synthesis. A. Sub-cellular localization of WT PC4 and the PC4 localization mutants (nucleolar localization defective mutant (NoLD), and nuclear localization defective mutant (NLD)). PC4 tagged with GFP and DNA were stained green and blue respectively in the merged images. Scale bar = 10 μm. N=3. Scale bar = 10 μm. B. Western blotting analysis of puromycin labelling assay performed on Hela cells following siRNA knockdown of PC4. siRNA against a scrambled sequence was used a control. N=2.

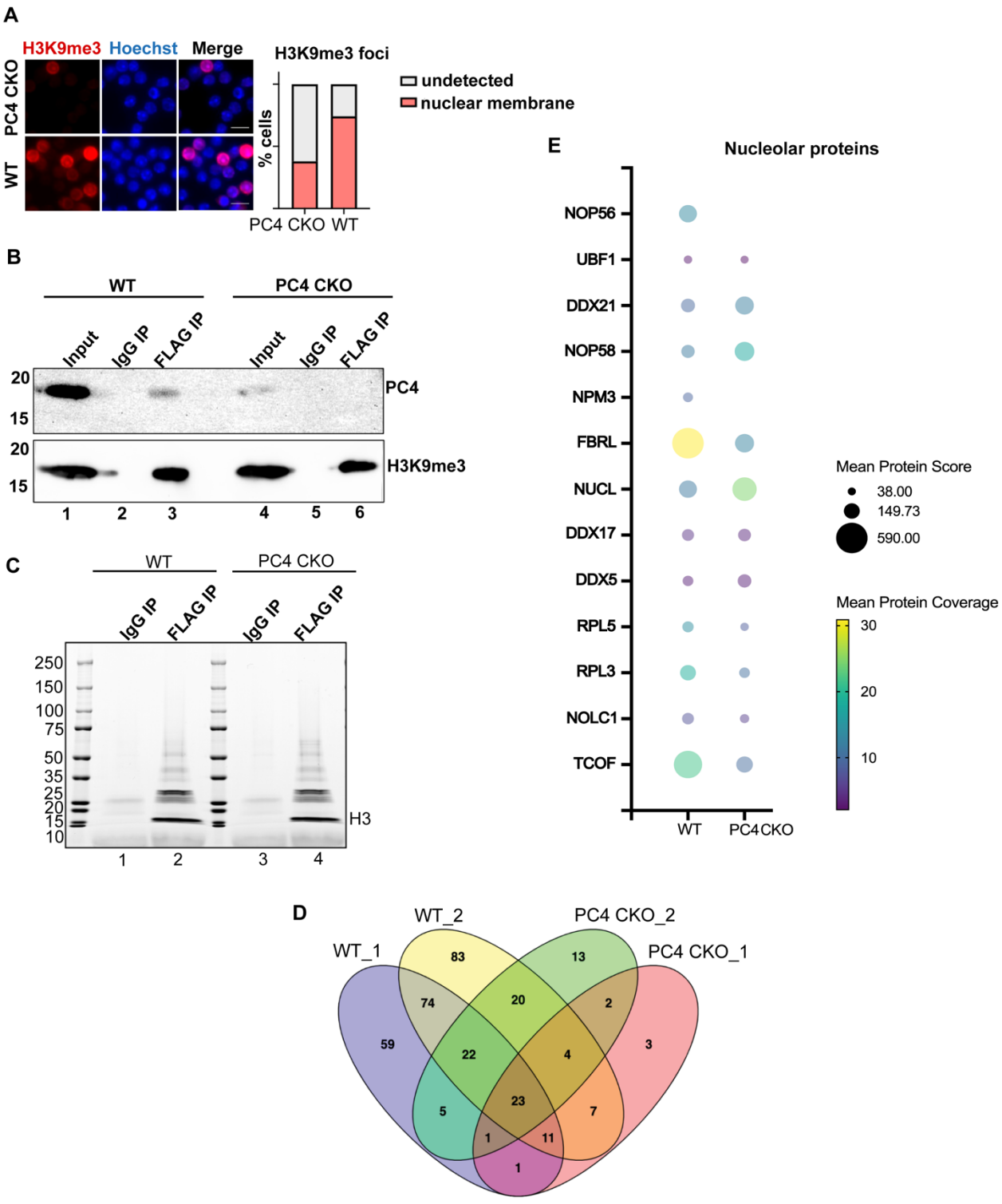
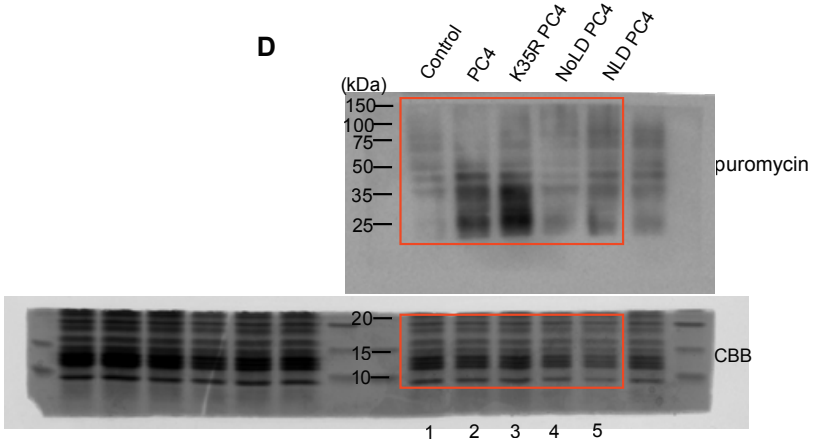
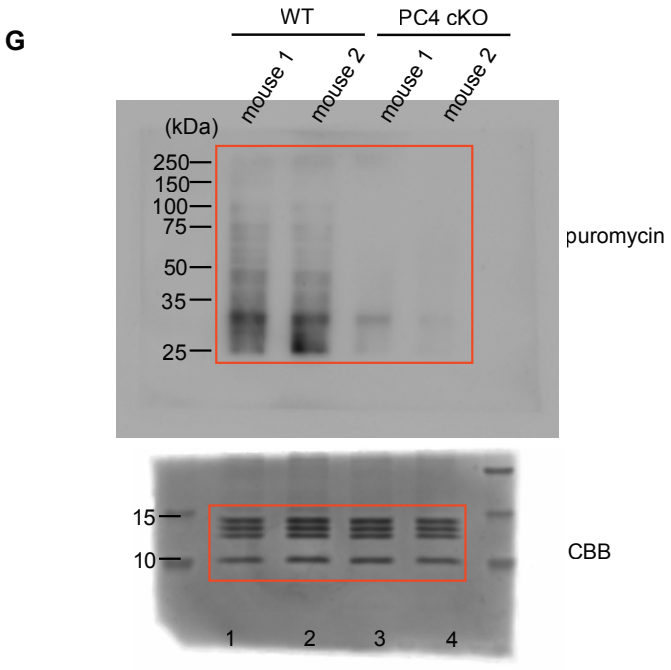


Figure S4. Loss of H3K9me3 foci in PC4 cKO B cells. A. Immunofluorescence showing H3K9me3 foci (red) in WT and PC4 cKO B cells. The nuclei were counterstained with Hoechst (blue). B. Western blotting analysis of H3K9me3 IP in WT and PC4 cKO B cells. C. Protein profile of the H3K9me3 IP in WT and cKO B cells. D. Venn diagram

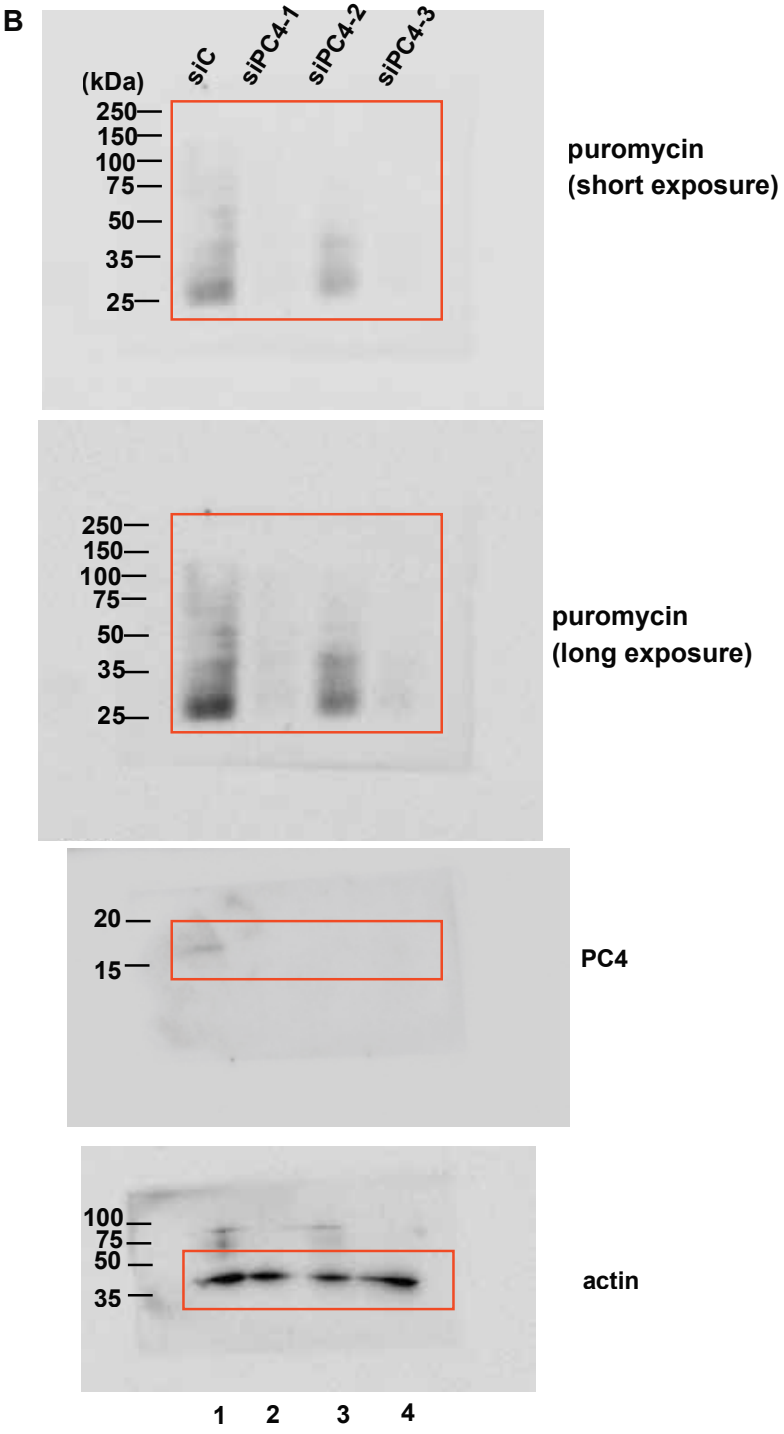
representing the proteins identified in the H3K9me3 IP. E. Graphical representation of the mean protein score and protein coverage of nucleolar proteins present in the H3K9me3 IP from WT and PC4 cKO B cells. Data from N=3 independent biological repeats.

Uncropped blots and gel Figure 3





Uncropped blots Figure S3



Uncropped blots and gel Figure S4