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Supplementary information

Biomolecular condensation of NUP98 fusion proteins drives leukemogenic gene expression

In the format provided by the authors and unedited

Supplementary Table 3: Summary of all analyses to assess correlation and co-occurrence in co-localization studies of NUP98-KDM5A with RAE1 or DDX24 (related to Figure 2e and Extended Data Figure 2c)

staining:	NUP98-KDM5A RAE1	mock RAE1	p-value	NUP98-KDM5A DDX24	mock DDX24	p-value
Spearmans Rank	0,155	0,071	<0.0001	0,355	0,049	<0.0001
Kendalls Tau	0,131	0,061	<0.0001	0,285	0,04	<0.0001

n=6

staining	NUP98-KDM5A RAE1	NUP98-KDM5A DDX24
Costes p (200 Randomizations)	1	1

Supplementary Note

RT-qPCR primer

qPCR primer	sequence
Gapdh_mouse_fw	AGAAGGTGGTGAAGCAGGCAT
Gapdh_mouse_rev	CGGCATCGAAGGTGGAAGAGT
GAPDH_human_fw	TGCACCACCAACTGCTTAGC
GAPDH_human_rev	GGCATGGACTGTGGTCATGAG
NUP98-KDM5A_fw	GTTCTCCAGCAGCACATCAA
NUP98-KDM5A_rev	CCGTTTCCGTTTCTTCTCTG

Nucleotide sequences for artAA-KDM5A and artFG-KDM5A

>cDNA|artAA-KDM5A

CCAAAAAAGAAGAGAAAGGTAGAAGACCCCGCTGCAGCCGCGGCCGCAACTAAGCTTCAG

GCCGGCACCGGCAACACCGGCAACAGCCCCGCCAGCACATCCGCAGCCGCTGCCGCTGC

TACCGGTACCAAGAGCCAGACCAACAGCGGCAACGGCCTGACCCCCGCCGCCAGCGCTG

CTGCTGCAGCAGCTCAGAACAAGAGCTCGGGCGCCGCCACCAACACCCCCCTTGGCGGA

AGCACTACGGCTGCGGCCGCCGCTGCGAAGCTTAACAGCAACCAGGGCGCCACCGGATC

CACTGGCACCCCCGCCACCAGTGCCGCGGCTGCAGCTGCAACCCAGAACAAGCCCACCA

GCGGCACCAGCGCCGCTAACGGCCTGAGCACCGGCGCTGCCGCTGCTGCTGCAAACGGC

AGCGGCACCAAGAGCGCCCAGCTGAACGCCACCGGCAGCACCCCCACCGCTGCAGCTGC

CGCCGCTACCGCTAGCACCCCCCAGACCAGCAACGCCGGCGGCAGCAAGACCGGCCTGA

ACGCGGCTGCTGCAGCTGCGCTGACCCAGAAGGGCCCCGCCAGCACCAACGGCAGCAAC

GGCACCACCTCCGCTGCTGCAGCTGCGGCTGCTGCCAAGAGCAACGGCGGATCCACCCT

GCCCGGTACCACCGCCAGCACCAACCAGGCTGCCGCTGCAGCTGCTCTGAACACCAGCA

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CCTGCAAGGACAAGGTAGACTGGGTACAATGTGATGGTGGCTGTGATGAGTGGTTTCATCA

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CAAACTACCAATGGAGGATCTTAAAGAGACCAGTTA

>cDNA|artFG-KDM5A

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TTGGATTCGGTCAGAACAAGAGCTCGGGCGCCGCCACCAACACCCCCCTGGGCGGCAGC

ACTACGTTTGGGTTCGGCTTTGGGAAGCTTAACAGCAACCAGGGCGCCACCGGATCCACT

GGCACCCCCGCCACCAGTTTCGGGTTTGGATTTGGAACCCAGAACAAGCCCACCAGCGGC

ACCAGCGCCGCCAACGGCCTGAGCACCGGCTTTGGCTTTGGTTTTGGAAACGGCAGCGGC

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CTCCGCTTTTGGATTTGGGTTTGGTGCCAAGAGCAACGGCGGATCCACCCTGCCCGGTAC

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GTGTATCTCCAGAAATGGCTGAAAATGAAGATTACATCTGTATAAACTGTGCAAAGAAGCAG

GGGCCAGTTAGCCCAGGTCCAGCACCACCTCCTTCCTTCATAATGAGCTACAAACTACCAA

TGGAGGATCTTAAAGAGACCAGTTAG

Primary antibodies

anti-HA.11 (BioLegend, 901513; 1:2000, RRID: AB_2565335), anti-alpha Tubulin (Abcam, ab7291; 1:5000, RRID: AB_2241126), anti-beta Actin (Cell Signaling, 4967S; 1:5000, RRID: AB_330288), anti-HSC70 (Santa Cruz, sc-7298; 1:10000, RRID: AB_627761), anti-NUP98 (Cell Signaling, #2288; 1:1000, RRID: AB_561204), anti-RAE1 (Cell Signaling, sc-374261; 1:1000, RRID: AB_11008069)

Secondary antibodies

sheep anti-mouse HRP (GE Healthcare Austria GmbH & Co OG, NA931V; 1:10000, RRID: AB_772210), goat anti-rabbit HRP (Cell Signaling, 7074; 1:10000, RRID: AB_2099233) and imaged on a Vilber Fusion FX. For detection on the LI-COR Odyssey platform the following secondary antibodies (Thermo Fisher Scientific) were used: goat anti-mouse 800 (Cat# SA5-35521,1:5000, RRID:AB_2556774), goat anti-mouse 680 (Cat# A32729, 1:5000, RRID:AB_2633278), goat anti-rabbit 800 (Cat# SA5-35571, 1:5000, RRID:AB_2556775) or goat anti-rabbit 680 (Cat# A32734, 1:5000, RRID:AB_2633283)

Protein network analysis: Figure 1 and S1

The endogenous NUP98 interactome of mock-transduced HL-60 cells was background corrected by subtracting the CRaPome[1] using a cutoff of an average spectrum count (Ave SC) smaller than 10. The NUP98-KDM5A interactome was filtered for proteins that are enriched in Strep-Tactin pulldowns from mock-transduced cells. Specific interactors were identified based on a log2 fold change greater than 0.5. Protein hubs were clustered based on String db interactions using K means clustering.

Protein network analysis: Figure 2

The NUP98-fusion protein interactome was filtered for proteins that are enriched in Strep-Tactin pulldowns from mock-transduced cells. Specific interactors were identified based on a log2 fold change greater than 0.5. The network was generated using Cytoscape 3.6.1[2] using the yFiles organic layout.

Protein network analysis: Figure 4

biCon-MS data of mock-transduced HL-60 cells were filtered for proteins that are more abundant in 33 µM precipitates than in 11 µM precipitates. These proteins were subjected to ClueGO analysis with the following settings: Evidence codes used: All_Experimental_(EXP,IDA,IPI,IMP,IGI,IEP); Ontology used: GO_BiologicalProcess-EBI-UniProt-GOA_27.02.2019_00h00; Merge redundant groups with >50.0% overlap; Statistical Test = Enrichment/Depletion (Two-sided hypergeometric test); Correction Method = Bonferroni step down. The protein list of the most significant hub ("gene expression") was extracted and subjected to String db interaction analysis (cutoff 0.4) and clustered using Reactome FI based on String interactions.

Protein network analysis: Figure 5

biCon-MS data of HL-60 cells expressing NUP98-KDM5A or NUP98-NSD1 were analyzed for proteins that are more abundant in 33 µM precipitates than in 11 µM precipitates. In a next step, these lists were compared to proteins that were precipitated in mock-transduced cells. All proteins that were enriched with a log2 fold change greater than 1 were subjected to String db (cutoff 0.4) and Reactome FI analysis. The three most significantly enriched clusters are shown.

Protein network analysis: Figure 6

Dose-dependent proteins were identified by significant enrichment of 33 µM precipitates compared to 11 µM precipitates and only proteins with a log2 fold change > 1 and a p-value < 0.05 were used for further comparisons. Proteins uniquely enriched in artFG-KDM5A precipitates compared to artAA-KDM5A were subjected to String db (cutoff 0.4) and Reactome FI analysis. The three most significantly enriched nuclear clusters are shown.

Supplementary Figure 1. Full, uncropped scans of Western blots

Figure 2a

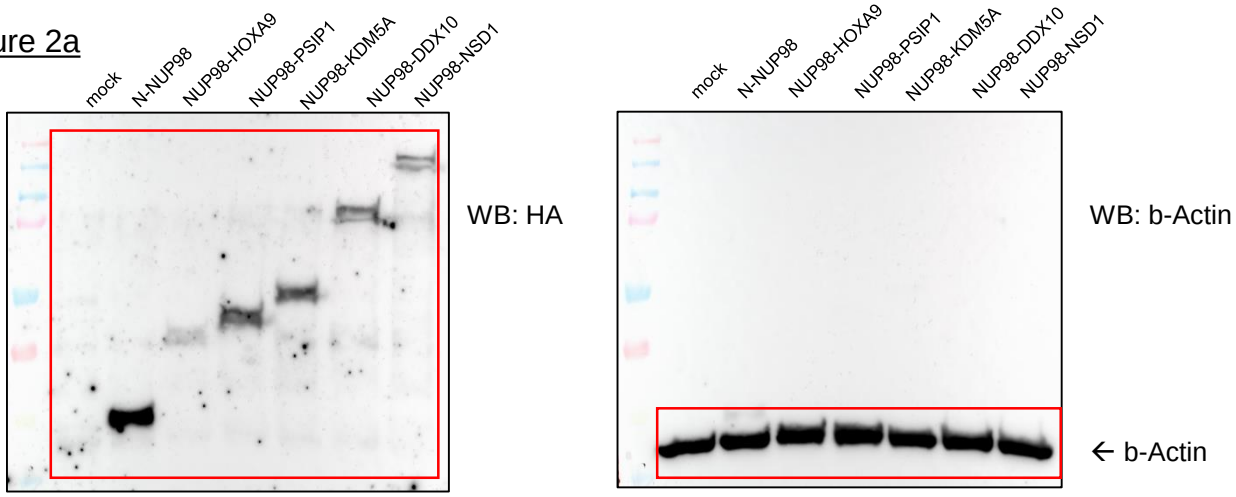


Figure 3d

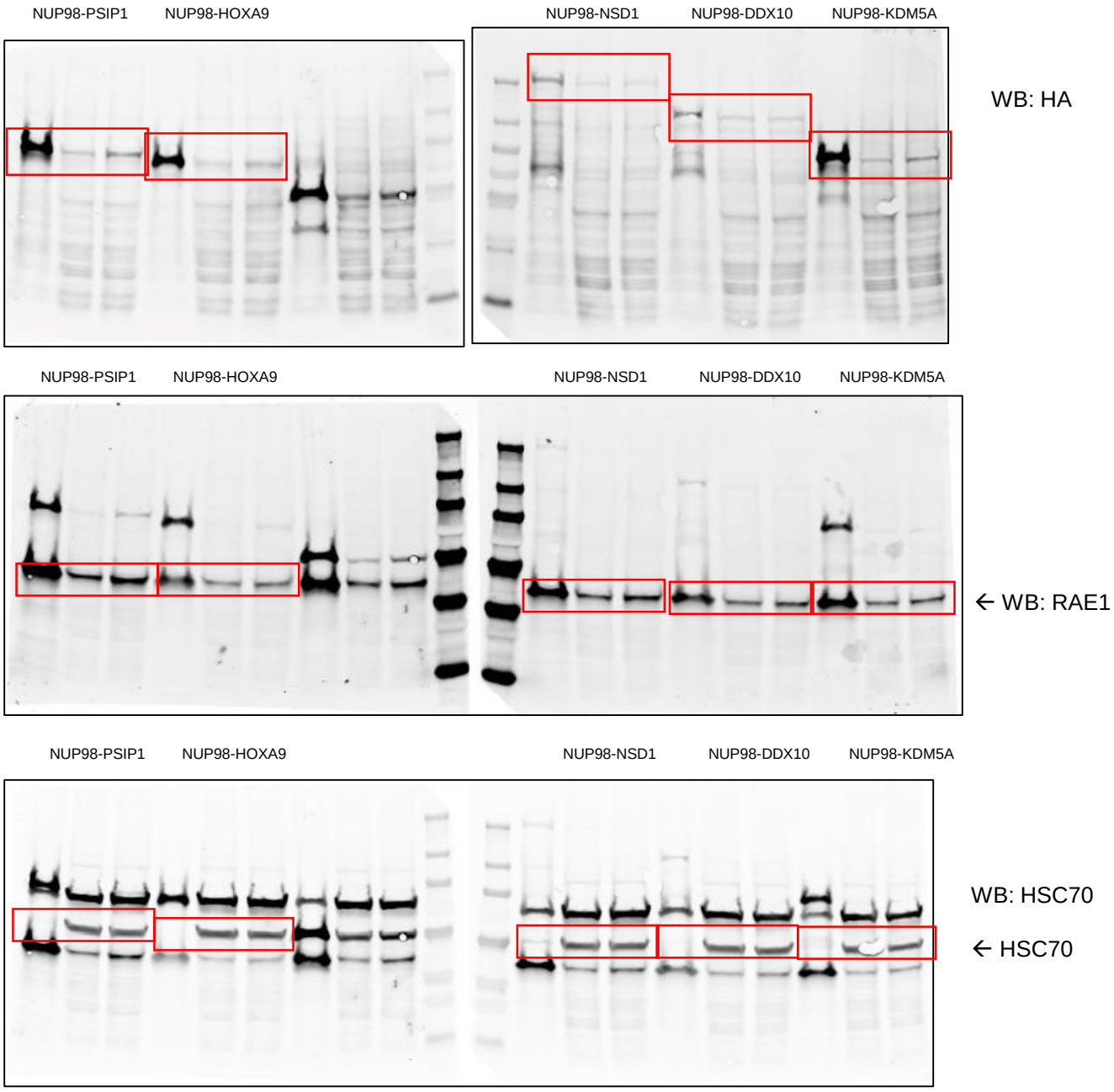


Figure 4b

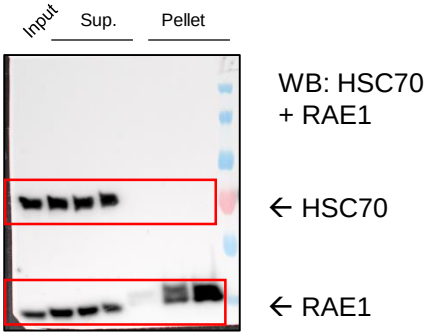
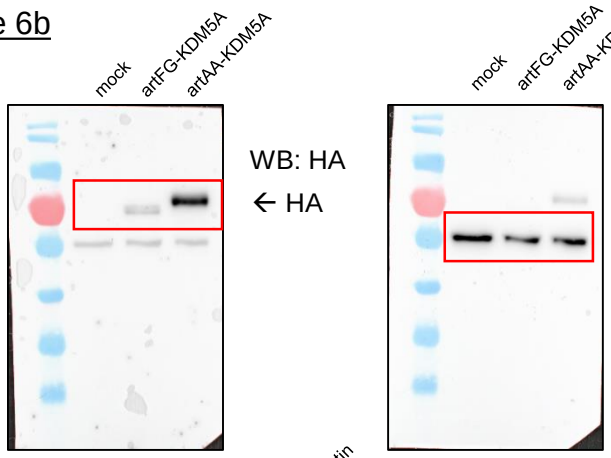
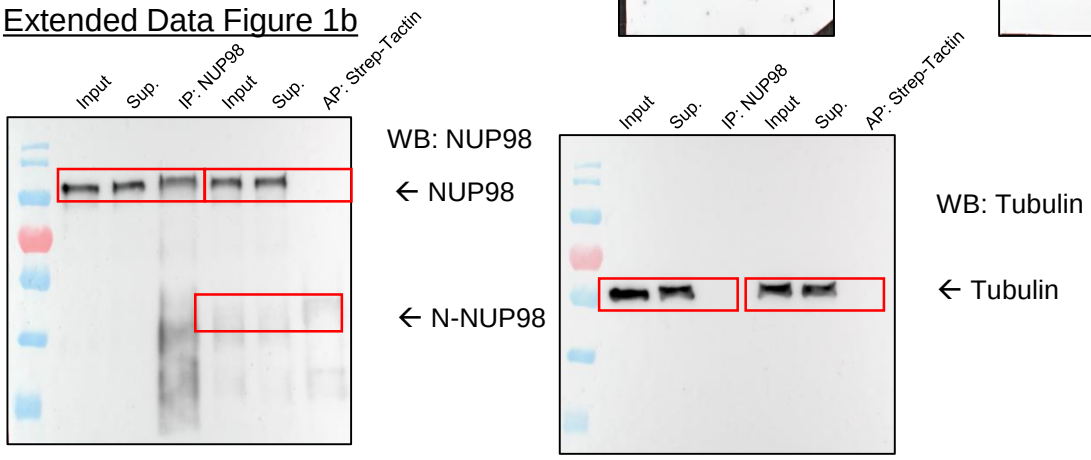


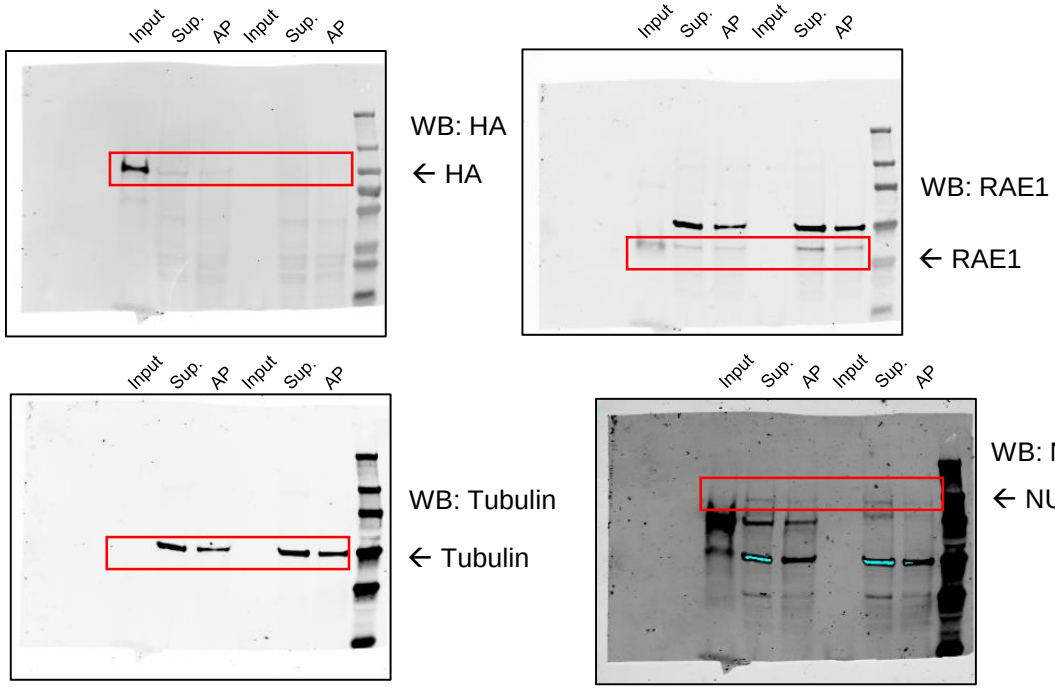
Figure 6b



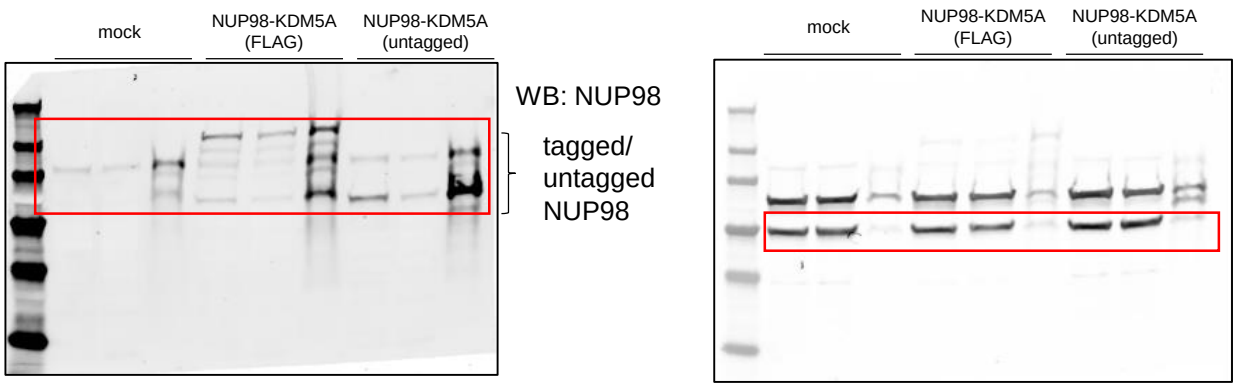
Extended Data Figure 1b



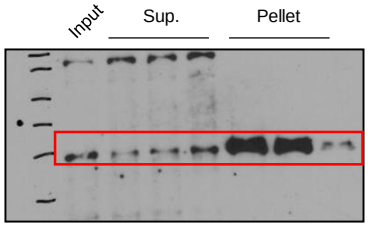
Extended Data Figure 2d



Extended Data Figure 3c



Extended Data Figure 4a



WB: HA

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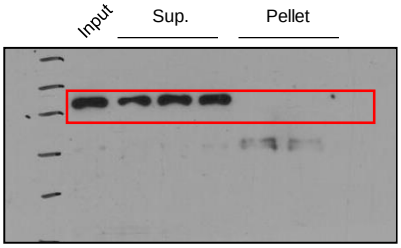

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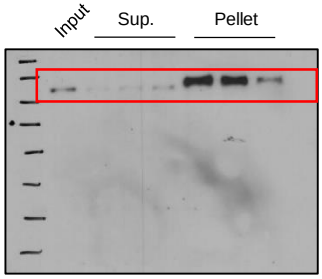
Extended Data Figure 5c



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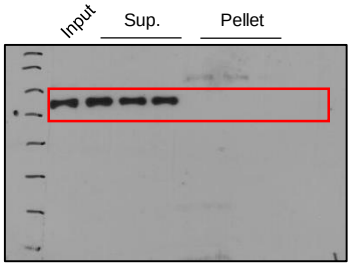

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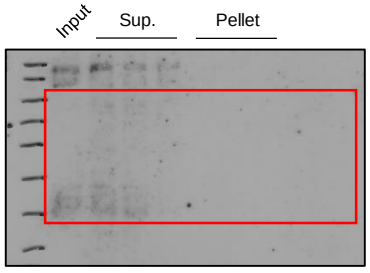
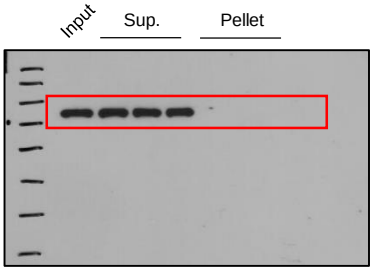
WB: HSC70

Extended Data Figure 5d



WB: HA



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