Expanded View Figures

Figure EV1. Validation and phenotypic characterization of HEK293T cells stably expressing V5-BirA*-AURKA.

A Representative immunofluorescence images of HEK293T::V5-BirA*-AURKA fixed and stained for gamma-tubulin, alpha-tubulin, and DAPI. Centrosome number higher than 2 was quantified as "centrosome amplification". Cells with more than one nucleus were quantified as "multinucleated"). Scale bar, 5 μm

B, C Quantification for (B) centrosome amplification and (C) multinucleation for A. Data represent mean value from two experiments per condition ± SEM (ns: non-significant, unpaired Student's *t*-test). $n > 100$ cells per experiment.

D Representative immunofluorescence images of mitotic HEK293T::V5-BirA*-AURKA fixed and stained for centrin 2 (centrioles), alpha-tubulin, and DAPI. Scale bar, 5 μm.

E, F Quantification for (E) mitotic index (F) percentage of multipolar spindles for D. Data represent mean value from two experiments per condition ± SEM (ns: non-significant, unpaired Student's *t*-test). $n > 100$ cells per experiment.

G Cell cycle analysis of asynchronous HEK293T::V5-BirA*-AURKA cells. Cells were fixed and stained with propidium iodide (PI) and in flow cytometer. Data represent mean value from two experiments per condition ± SEM (ns: non-significant, unpaired Student's *t*-test).

H Relative expression of Caspase3 in control cells andHEK293T::V5-BirA*-AURKA cells. Cells were lysed and immunoblotted with antibodies against Caspase3 and vinculin (loading control).

I Representative images of HEK293T::V5-BirA*AURKA cells stained for V5, AURKA, and gamma-tubulin. DNA was stained with DAPI. Scale bar is 10 μm.

J Expression and biotinylation of BirA* (control) and BirA*-AURKA in the absence and the presence of biotin (50 μM biotin, 18 h). Cells were lysed, run on an SDS–PAGE gel, and immunoblotted with streptavidin. Arrows indicate the bait proteins V5-BirA* and V5-BirA*-AURKA.

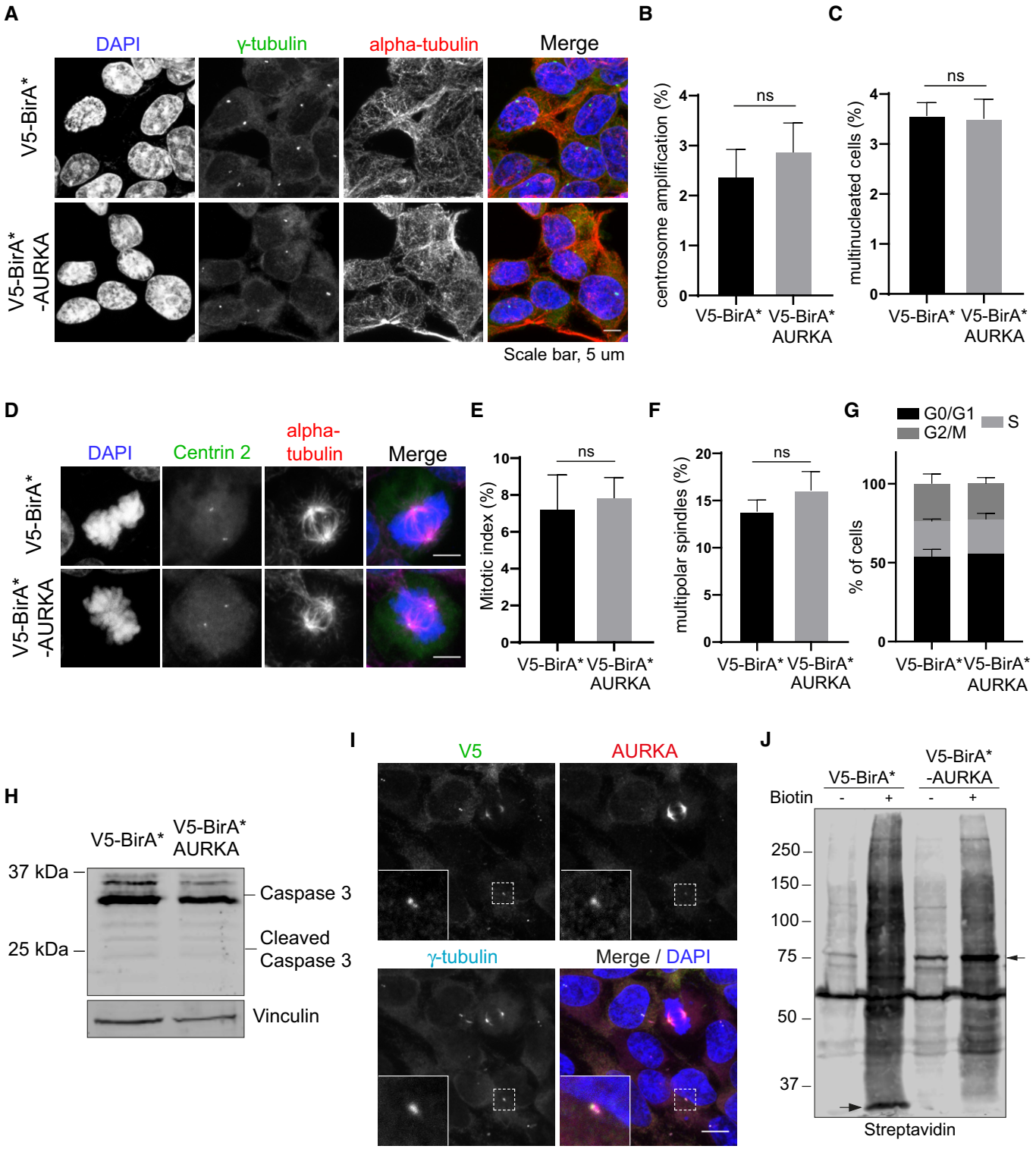

Figure EV1.

Figure EV2. Networking analysis of the AURKA proximity interactome.

A Comparison of AURKA proximity interactome with Aurora Kinase B (AURKB) interactors derived from BioGRID. Pink circle indicates the AURKA proximity interactome, and blue circle indicates AURKB interactome.

B High-confidence proximity interactors of AURKA were determined using SAINT analysis. AURKA proximity interactors were ranked by their fold change in the V5-BirA*-AURKA dataset relative to V5-BirA*-dataset. The first 200 proteins were visualized in CytoScape, and the functional clusters were determined by combining STRING analysis with the ClusterONE plug-in. Five functional clusters were enriched in the interactome, which include cell cycle (green), RNA processing (pink), ribosome biogenesis (blue), translation regulation (yellow) metabolic processes (purple). Uncategorized interactors are shown in gray, and the published interactors are shown in red circles.

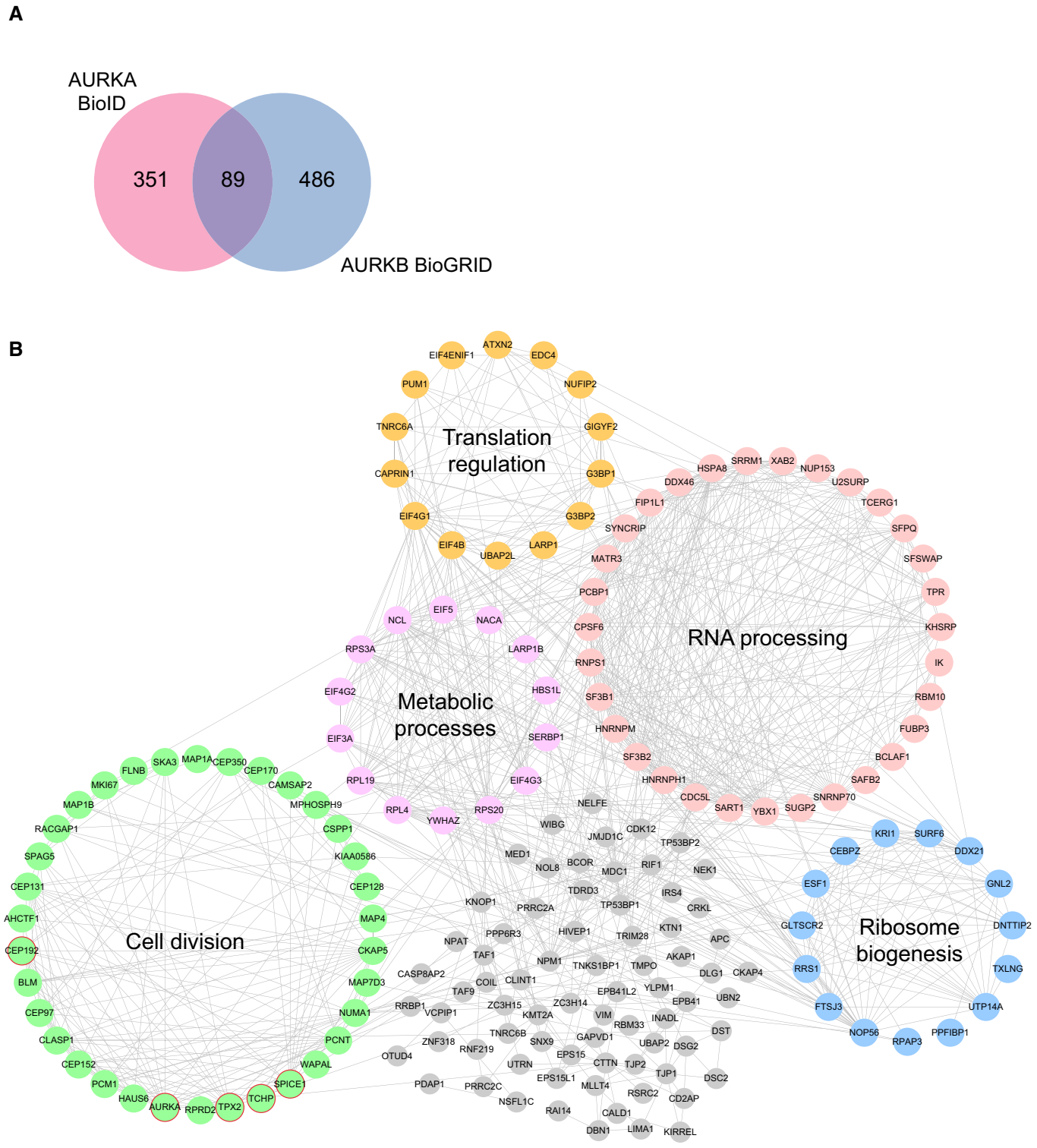
A
AURKA BioID
351
89
486
AURKB BioGRID
B
Translation regulation
RNA processing
Metabolic processes
Cell division
Ribosome biogenesis
ATXN2
EIF4ENIF1
EDC4
PUM1
NUFIP2
TNRC6A
GIGYF2
CAPRIN1
G3BP1
EIF4G1
G3BP2
EIF4B
UBAP2L
LARP1
SYNCRIP
MATR3
HSPA8
SRRM1
XAB2
NUP153
DDX46
U2SURP
FIP1L1
TCERG1
SFPQ
SFSWAP
TPR
KHSRP
IK
RBM10
FUBP3
BCLAF1
SAFB2
SNRNP70
SUGP2
YBX1
SART1
CDC5L
HNRNPH1
SF3B2
HNRNPM
SF3B1
HBS1L
SERBP1
EIF4G3
NELFE
WIBG
JMJD1C
CDK12
TP53BP2
RIF1
NEK1
MDC1
NOL8
BCOR
MED1
TDRD3
IRS4
CRKL
TP53BP1
PRRC2A
KNOP1
HIVEP1
TRIM28
KTN1
APC
PPP6R3
NPAT
TAF1
NPM1
TNKS1BP1
TMPO
AKAP1
DLG1
CKAP4
CASP8AP2
COIL
CLINT1
TAF9
EPB41L2
YLPM1
EPB41
UBN2
RRBP1
VCPIP1
ZC3H15
ZC3H14
KMT2A
VIM
RBM33
INADL
DST
TNRC6B
GAPVD1
SNX9
UBAP2
DSG2
ZNF318
RNF219
EPS15
OTUD4
UTRN
CTTN
TJP2
TJP1
DSC2
EPS15L1
MLLT4
RSRC2
CD2AP
PDAP1
PRRC2C
NSFL1C
RAI14
CALD1
DBN1
LIMA1
KIRREL
EIF5
NCL
NACA
RPS3A
LARP1B
EIF4G2
EIF3A
RPL19
RPL4
RPS20
YWHAZ
PCBP1
CPSF6
RNPS1
KRI1
SURF6
CEBPZ
DDX21
ESF1
GNL2
GLTSCR2
DNTTIP2
RRS1
TXLNG
FTSJ3
UTP14A
NOP56
RPAP3
PPFIBP1
SKA3
MAP1A
CEP350
CEP170
FLNB
MKI67
CAMSAP2
MAP1B
MPHOSPH9
RACGAP1
CSPP1
SPAG5
KIAA0586
CEP131
CEP128
AHCTF1
MAP4
CEP192
CKAP5
BLM
MAP7D3
CEP97
NUMA1
CLASP1
PCNT
CEP152
WAPAL
PCM1
SPICE1
HAUS6
AURKA
RPRD2
TPX2
TCHP

Figure EV2.

Figure EV3. AURKA interacts with centriolar satellites.

A AURKA activity is required for its interaction with PCM1. HEK293T cells were transfected with FLAG-BirA*-AURKA. 24 h post-transfection, cells were treated with DMSO+biotin or MLN8237+biotin, lysed, and biotinylated proteins were precipitated by streptavidin beads. The initial sample and precipitated proteins were run on an SDS–PAGE gel and immunoblotted for streptavidin, FLAG, and PCM1.

B Localization of V5-BirA*-AURKA relative to markers of the centrosome and centriolar satellites. HEK293T::V5-BirA*-AURKA cells were incubated with 50 μM biotin for 18 h and immunostained with fluorescent streptavidin and antibodies against CEP63, CEP152, CEP131, and PCM1. DNA was stained with DAPI. Scale bar, 5 μm.

C Experimental workflow for quantitative phosphoproteomics. HEK293T cells were transfected with FLAG-PCM1 (4 × 15 cm plates), treated either DMSO (vehicle control) or 1 μM MLN8237. Following cell lysis and FLAG pulldown, FLAG-PCM1 was excised, digested with trypsin, and analyzed by mass spectrometry.

D Validation of AURKA inhibition upon MLN8237 treatment. Cells were treated with DMSO, 0.5 μM MLN8237 and 1 μM MLN8237, lysed, run on an SDS–PAGE, and immunoblotted with antibodies against AURKA, p-AURKA, and vinculin (loading control).

E Coomassie gel image of FLAG-PCM1 pulldown. The rectangle indicates the corresponding band excised for mass spectrometry.

F Phos-tag and non-phos-tag immunoblots of cells were treated with DMSO (vehicle) or 1 μM MLN8237. Cells were lysed and immunoblotted for PCM1 and GAPDH.

G Representative images and quantification of pericentrosomal levels of PCM1. RPE1 cells were treated with 0.5 μM MLN8237, fixed, and immunostained with PCM1, acetylated tubulin, and gamma-tubulin. Centrosomal AURKA and p-AURKA fluorescence intensities were measured from maximum projections, and average means of the levels in control cells were normalized to 1. Data represent mean value from two experiments per condition ± SEM (ns: non-significant, unpaired Student's *t*-test). $n > 100$ cells per experiment Scale bar, 5 μm.

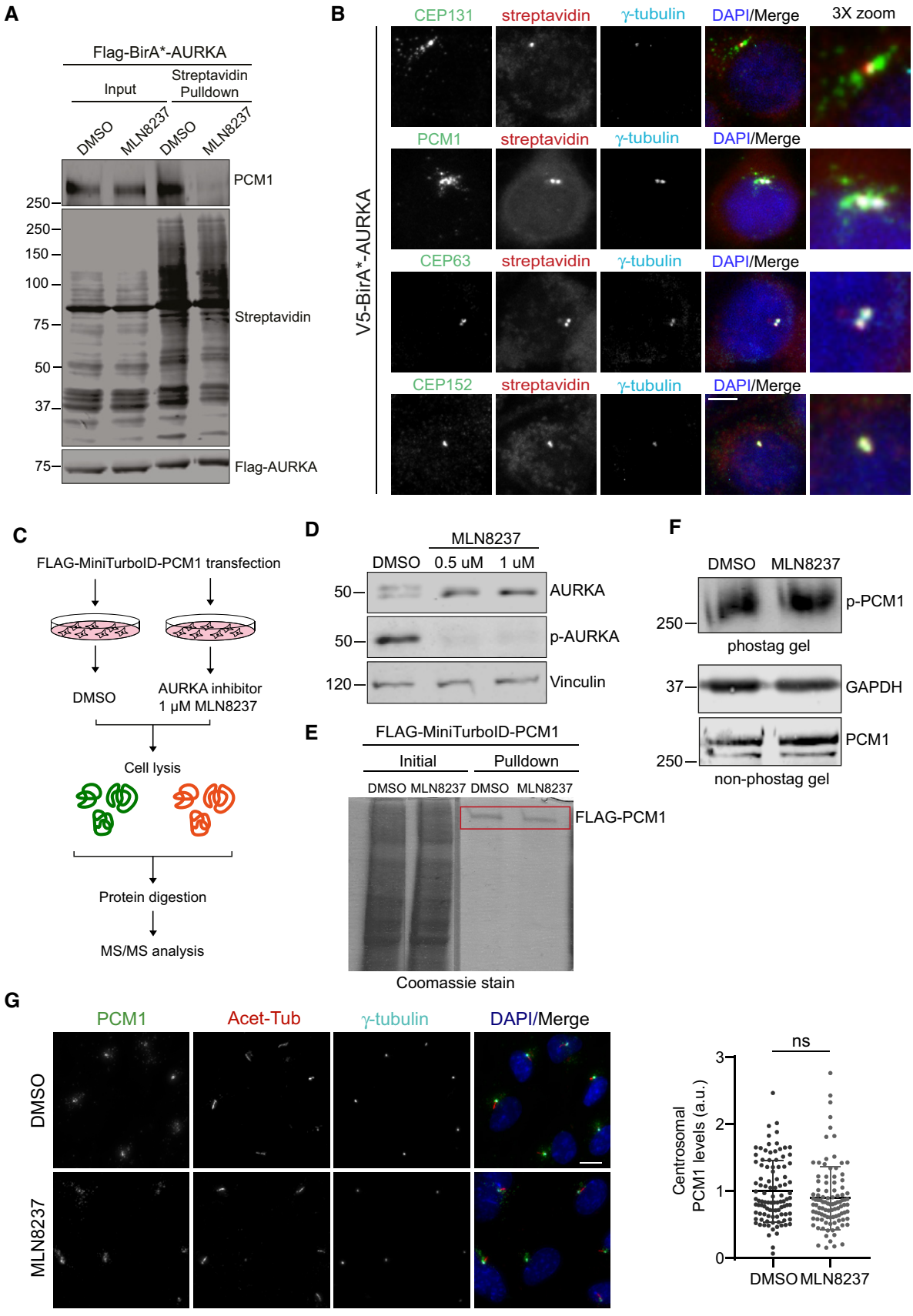
A
Flag-BirA*-AURKA
Input
Streptavidin Pulldown
DMSO
MLN8237
DMSO
MLN8237
PCM1
250
250
150
100
75
50
37
Streptavidin
75
Flag-AURKA
B
CEP131
streptavidin
γ-tubulin
DAPI/Merge
3X zoom
PCM1
streptavidin
γ-tubulin
DAPI/Merge
CEP63
streptavidin
γ-tubulin
DAPI/Merge
CEP152
streptavidin
γ-tubulin
DAPI/Merge
V5-BirA*-AURKA
C
FLAG-MiniTurboID-PCM1 transfection
DMSO
AURKA inhibitor
1 μM MLN8237
Cell lysis
Protein digestion
MS/MS analysis
D
MLN8237
DMSO
0.5 uM
1 uM
50
AURKA
50
p-AURKA
120
Vinculin
E
FLAG-MiniTurboID-PCM1
Initial
Pulldown
DMSO MLN8237 DMSO MLN8237
FLAG-PCM1
Coomassie stain
F
DMSO
MLN8237
250
p-PCM1
phostag gel
37
GAPDH
250
PCM1
non-phostag gel
G
PCM1
Acet-Tub
γ-tubulin
DAPI/Merge
DMSO
MLN8237
ns
Centrosomal PCM1 levels (a.u.)
3
2
1
0
DMSO
MLN8237

Figure EV3.

Figure EV4. Validation of PCM1 depletion and regulation of AURKA abundance by satellites.

A Immunoblotting analysis of PCM1 depletion by RNAi. RPE1 cells were transfected with control or PCM1 siRNA #1 and #2 for 72 h. Cells were lysed and immunoblotted with PCM1 and Actin (loading control). Band intensities for immunoblots were quantified from two experimental replicates. Scale bar, 5 μm.

B Immunofluorescence analysis of PCM1 depletion and loss of satellites by RNAi. RPE1 cells were transfected with control or PCM1 siRNA #1 and #2 for 72 h. Cells were fixed and stained with PCM1, gamma-tubulin, and DNA. DNA was stained with DAPI. Scale bar, 10 μm.

C Confirmation of CCDC66 depletion by RNAi and quantification of AURKA and p-AURKA levels upon CCDC66 depletion. RPE1 cells were transfected with control or CCDC66 siRNA for 72 h. Cells were lysed and immunoblotted for AURKA, p-AURKA, CCDC66, vinculin (loading control), and alpha-tubulin (loading control).

D Depletion of PCM1 in RPE1 ^{GFP}CCDC66 line. RPE1 cells were transfected with control or PCM1 siRNA 72 h. Cells were fixed and immunostained with GFP, PCM1, and centrin3. DNA was stained with DAPI. Scale bar, 10 μm.

E Total AURKA in control and RPE1 PCM1 KO cells. RPE1 WT and RPE1 PCM1 KO cells were serum-starved for 24 h, cell lysates were prepared and run on an SDS–PAGE gel. Proteins were detected by immunoblotting with antibodies against AURKA and vinculin (loading control). The band intensities were measured from two replicates (unpaired Student's *t*-test).

F Representative images and quantification of basal body levels of AURKA and phospho-AURKA (p-AURKA) in control and RPE1 PCM1 KO cells. Following 24 h serum starvation, cells were fixed and stained with the indicated antibodies and DAPI. Scale bar, 10 μm.

G Quantification of AURKA and p-AURKA levels in RPE1 WT and PCM1 KO cells for G. Centrosomal AURKA and p-AURKA fluorescence intensities were measured from maximum projections, and average means of the levels in control cells were normalized to 1. $n > 100$ cells per experiment. Data represent mean value from two experiments per condition ± SEM (**$P < 0.01$, ns: non-significant, unpaired Student's *t*-test).

Data information: (B, D, F) Sizes of the scale bars are indicated in the figure legends.

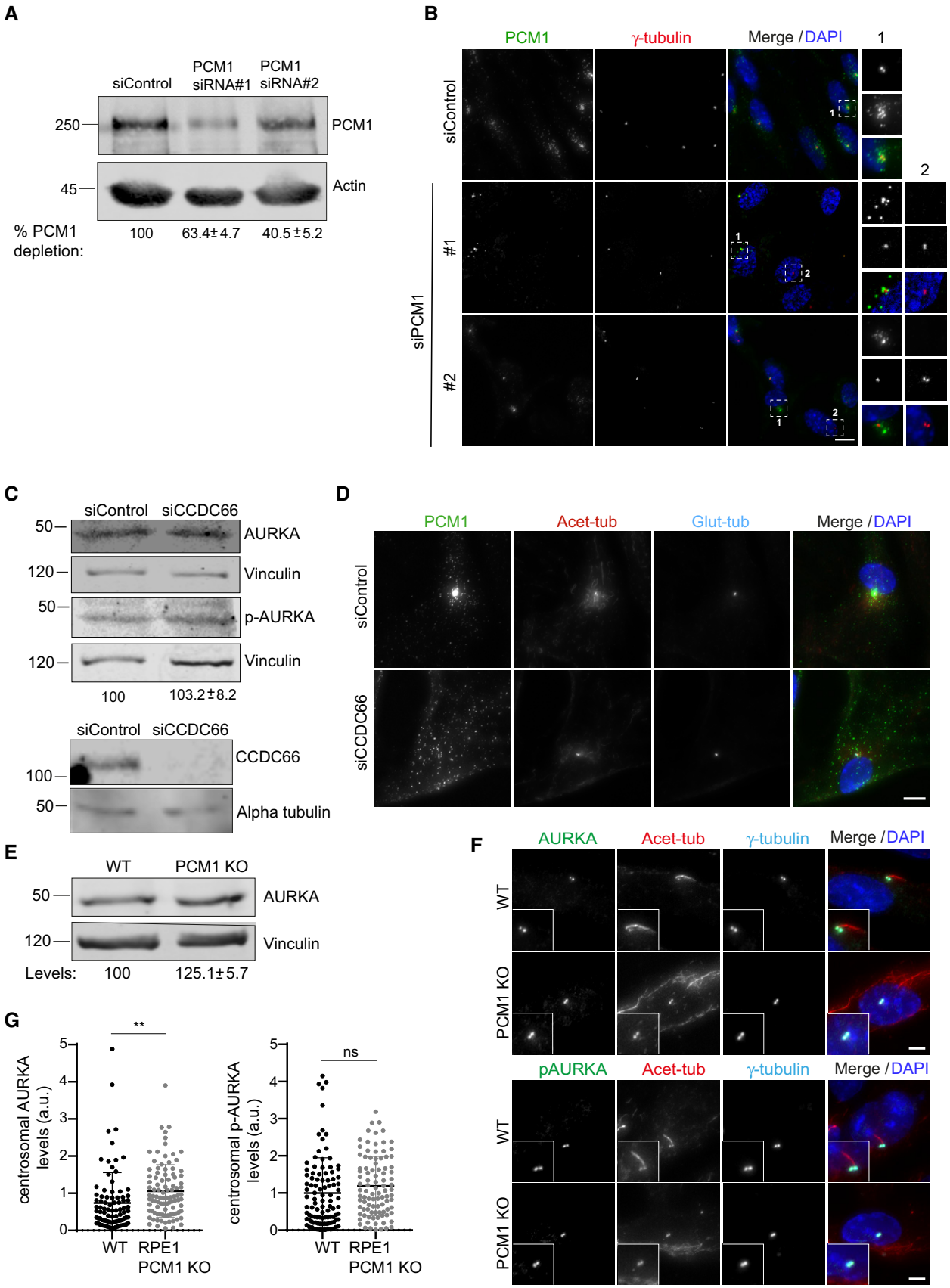
A
siControl
PCM1 siRNA#1
PCM1 siRNA#2
250
PCM1
45
Actin
% PCM1 depletion:
100
63.4±4.7
40.5±5.2
B
PCM1
γ-tubulin
Merge /DAPI
1
2
siControl
siPCM1
#1
#2
C
siControl
siCCDC66
50
AURKA
120
Vinculin
50
p-AURKA
120
Vinculin
100
103.2±8.2
siControl
siCCDC66
100
CCDC66
50
Alpha tubulin
D
PCM1
Acet-tub
Glut-tub
Merge /DAPI
siControl
siCCDC66
E
WT
PCM1 KO
50
AURKA
120
Vinculin
Levels:
100
125.1±5.7
F
AURKA
Acet-tub
γ-tubulin
Merge /DAPI
WT
PCM1 KO
pAURKA
Acet-tub
γ-tubulin
Merge /DAPI
WT
PCM1 KO
G
centrosomal AURKA levels (a.u.)
**
WT
RPE1 PCM1 KO
centrosomal p-AURKA levels (a.u.)
ns
WT
RPE1 PCM1 KO

Figure EV4.

Figure EV5. Characterization of ciliogenesis and cell cycle phenotypes in cilium assembly and disassembly experiments.

A Quantification of ciliogenesis efficiency and cilium length in control and PCM1-depleted RPE1 cells treated with DMSO or MLN8237. Cells were transfected with control or PCM1 siRNA#2 for 48 h and treated with DMSO (vehicle control) or 0.5 μM MLN8237 in serum starvation medium for 24 h. Cells were fixed and immunostained for the primary cilium with acetylated tubulin antibody (Acet-tub) and the centrosome with gamma-tubulin antibody. $n > 100$ cells per experiment. Data represent mean value from two experiments per condition ± SEM (***$P < 0.001$; *$P < 0.1$; ns, non-significant, unpaired Student's *t*-test).

B Quantification of ciliogenesis percentage in RPE1 WT and PCM1 KO cells treated with DMSO (control) and 0.5 μM MLN8237. $n > 100$ cells per experiment. Data represent mean value from two experiments per condition ± SEM (***$P < 0.001$, ns: non-significant, unpaired Student's *t*-test).

C Representative images and quantification of proliferating cells upon serum starvation. RPE1 cells were transfected with control or PCM1 siRNA for 48 h, serum-starved for 24 h, and treated with DMSO or 0.5 μM MLN8237. Cells were fixed and immunostained with Ki67 to mark proliferating cells and acetylated tubulin to mark the primary cilium. DNA was stained with DAPI. Scale bar, 10 μm. Ciliated Ki67- cells and Ki67- cells were quantified. Data represent mean value from two experiments per condition ± SEM (**$P < 0.01$, ns: non-significant, unpaired Student's *t*-test).

D Representative immunofluorescence images for cilium disassembly experiments. RPE1 cells were transfected with control or PCM1 siRNA for 48 h, serum-starved for 24 h, and treated with DMSO (vehicle control) or 0.5 μM MLN8237 in serum stimulation medium for 2 and 24 h. Cells were fixed and immunostained with antibodies against acetylated tubulin antibody, gamma-tubulin antibody to mark the centrosome. DNA was stained with DAPI. Scale bar, 10 μm.

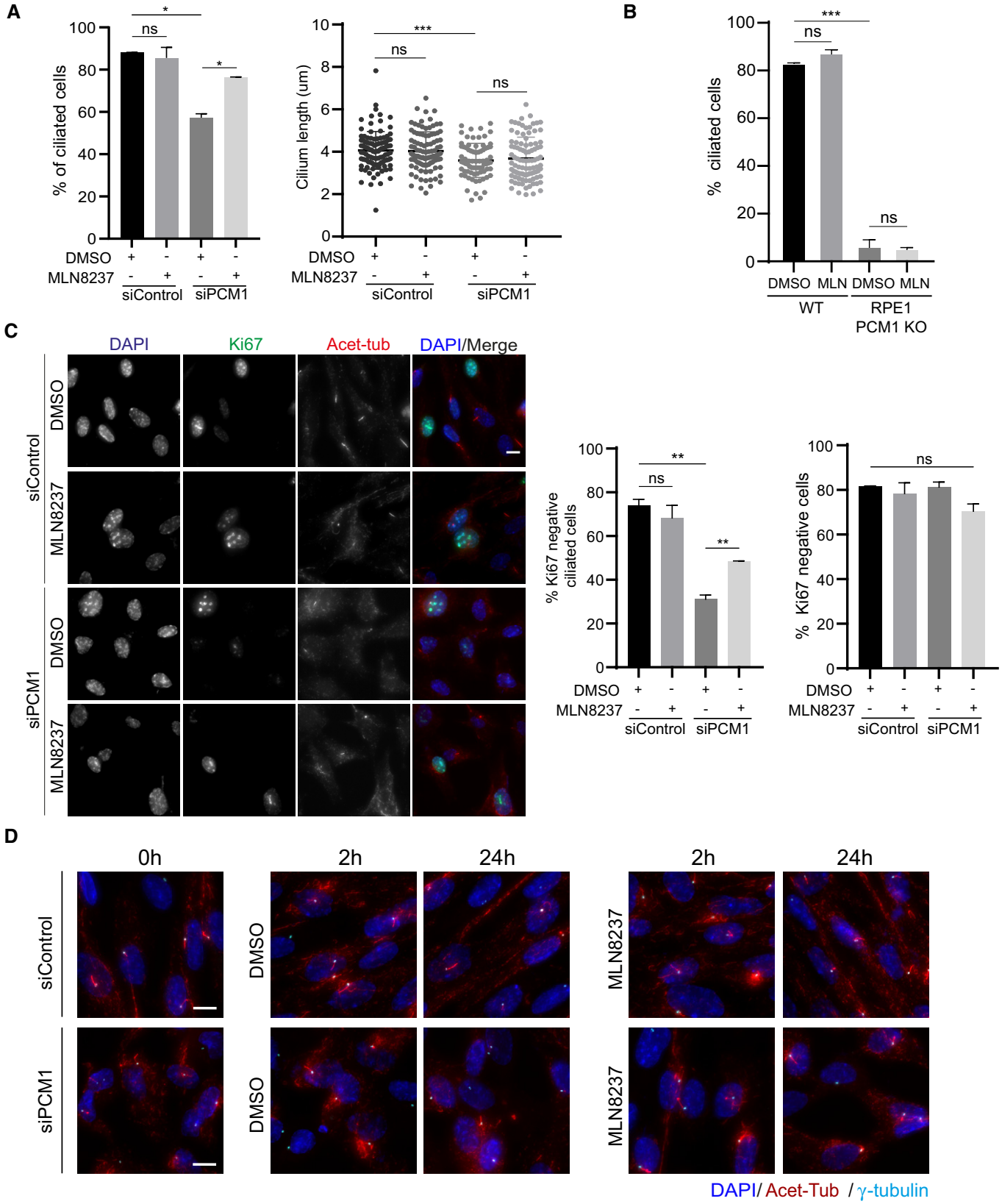
A
% of ciliated cells
Cilium length (um)
DMSO
MLN8237
siControl
siPCM1
ns
*
***
B
% ciliated cells
DMSO MLN DMSO MLN
WT
RPE1 PCM1 KO
C
DAPI
Ki67
Acet-tub
DAPI/Merge
siControl
siPCM1
DMSO
MLN8237
% Ki67 negative ciliated cells
% Ki67 negative cells
**
D
0h
2h
24h
DAPI/ Acet-Tub / γ-tubulin

Figure EV5.