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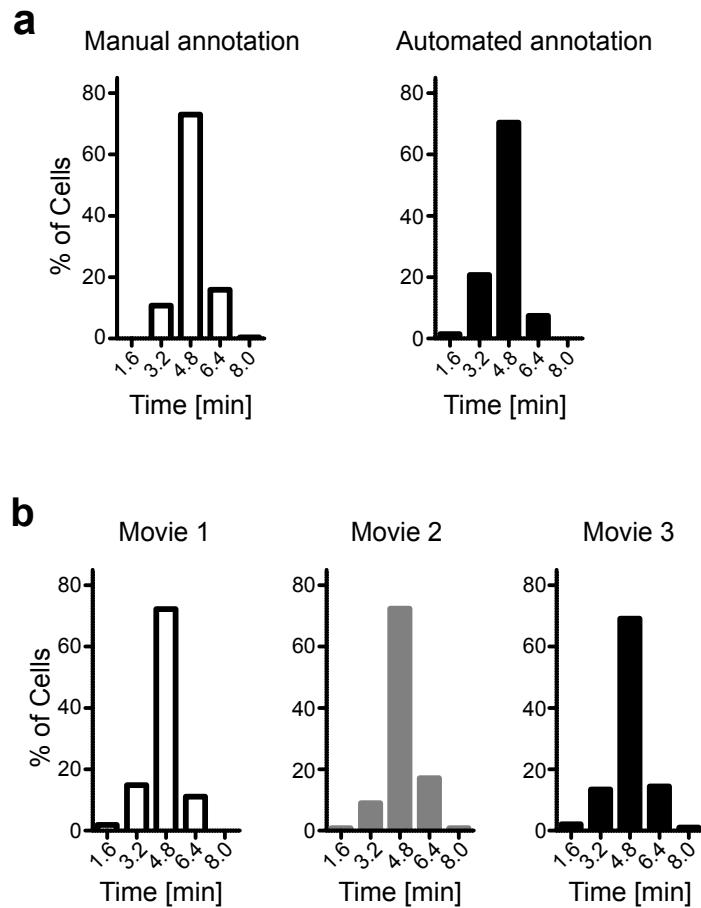


Figure S1 Validation of automated image analysis. **(a)** Histograms of mitotic exit timing based on IBB-import after anaphase onset ($t = 0$) of the same dataset analysed manually (left, white bars), or automatically (right, black bars). **(b)** Automated annotation is highly reproducibly between movies (three independent movies shown).

SUPPLEMENTARY INFORMATION

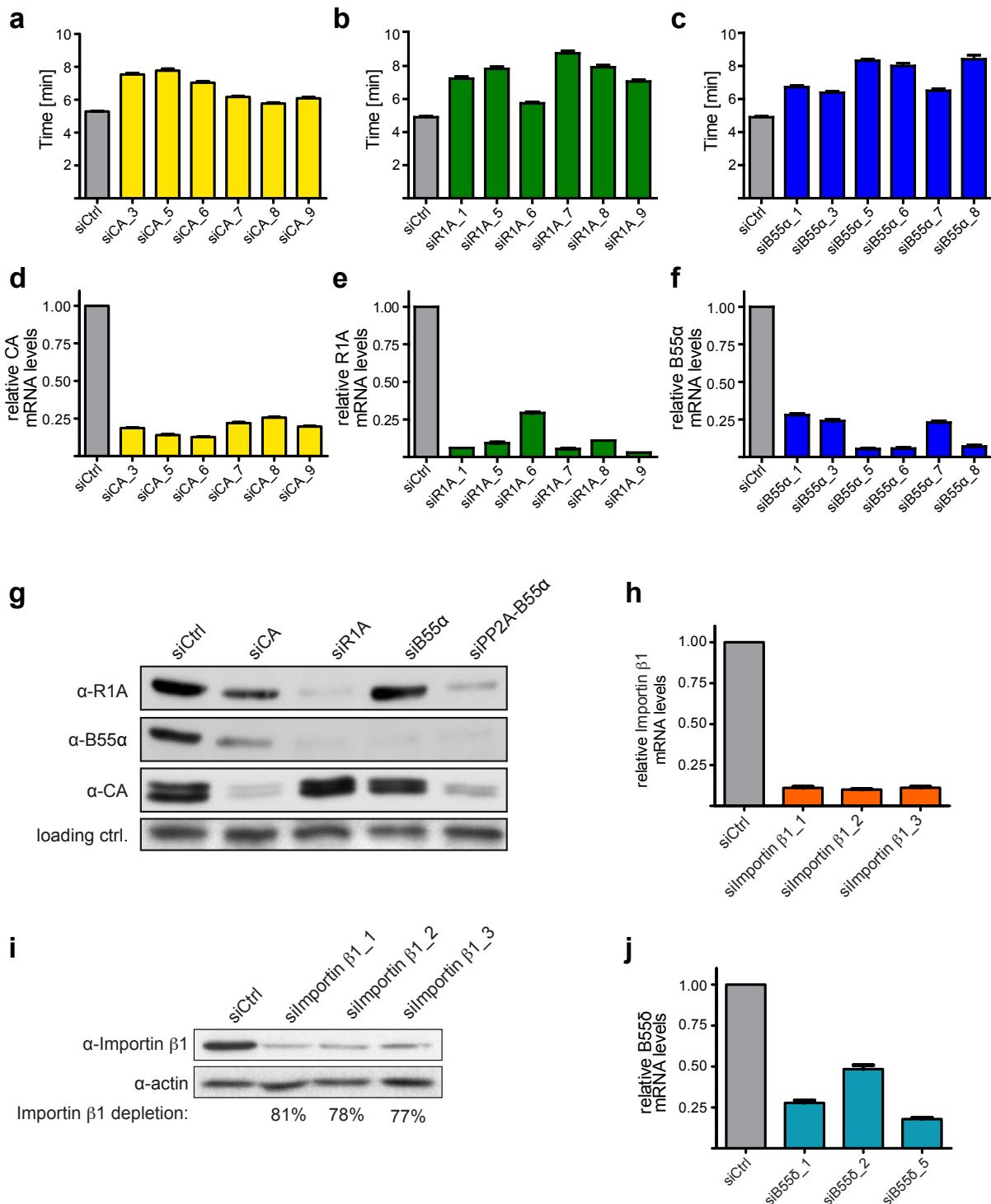


Figure S2 Validation of RNAi efficiency. **(a-c)** Mitotic exit timing measured as in Figure 1, for six different siRNAs targeting CA ($n \geq 382$ for each siRNA condition; mean \pm s.d.) **(a)**, R1A ($n \geq 175$ for each siRNA condition) **(b)**, or B55 α ($n \geq 108$ for each siRNA condition) **(c)**. **(d-f)** Quantification of mRNA knockdown 40 h post transfection by real-time PCR for the same siRNAs as in **(a-c)**, targeting CA **(d)**, R1A **(e)**, or B55 α **(f)**, normalized against GAPDH ($n = 3$ for each condition; mean \pm s.d.). See Supplementary Information, Table 2 for

siRNA sequences. **(g)** Protein depletion levels of CA, R1A, and B55 α , detected by Western blotting in cells depleted for the indicated siRNAs 60 h post-transfection. Note that depletion of CA or R1A co-depletes other subunits of the PP2A-B55 α complex, consistent with previous reports^{39, 40}. **(h)** Quantification of Importin β 1 mRNA knockdown 48 h post transfection. **(i)** Importin β 1 protein levels, detected by Western blotting 64 h after siRNA transfection. **(j)** Quantification of B55 δ mRNA knockdown 48 h post transfection.

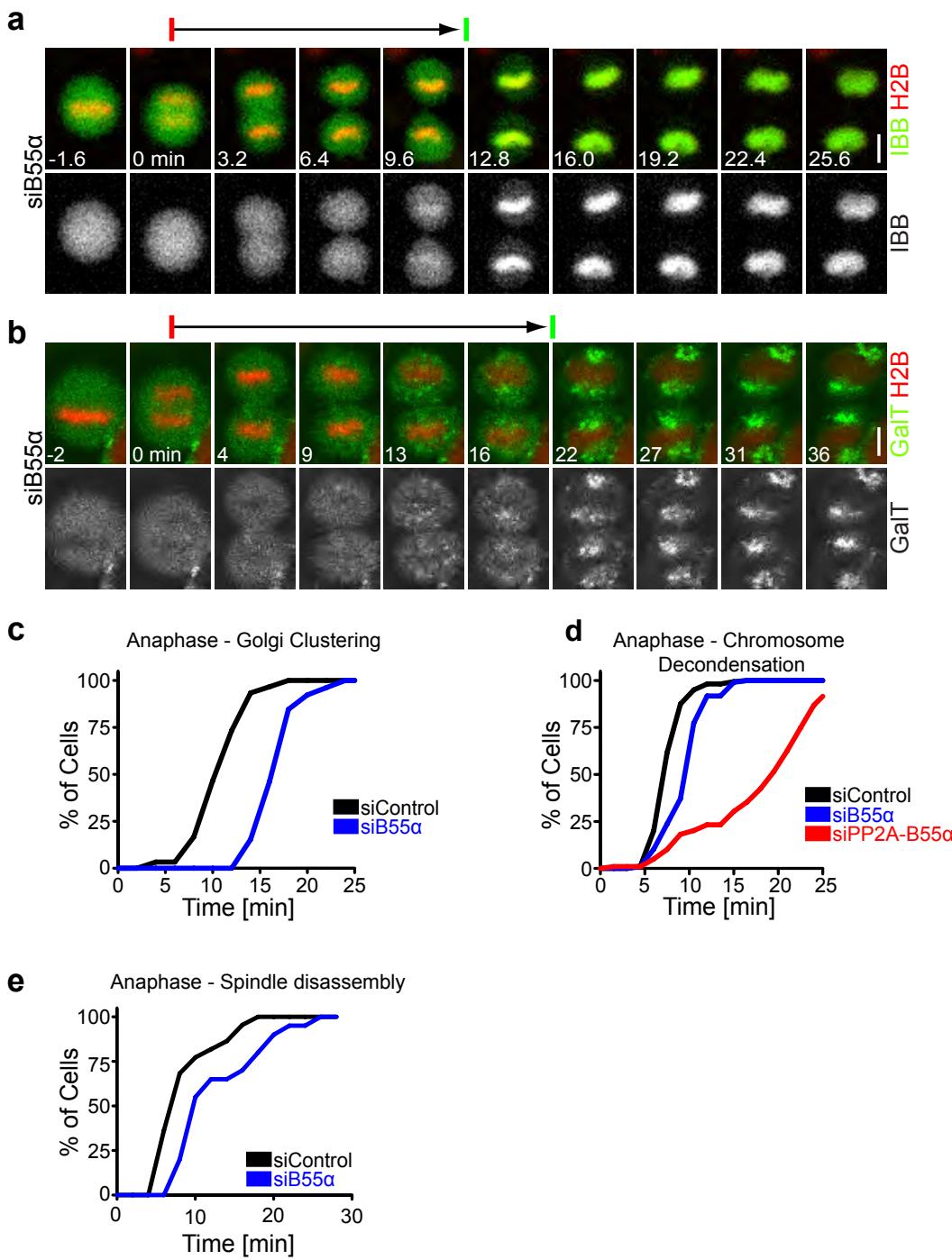


Figure S3 Phenotypes of B55 α single depletions. **(a)** Nuclear reassembly timing in a B55 α -depleted cell, see also Supplementary Information, Movie S11. **(b)** Golgi reassembly timing in a B55 α -depleted cell, see also

Supplementary Information, Movie S12. **(c-e)** Cumulative histograms of postmitotic Golgi clustering **(c)**, chromosome decondensation **(d)**, and spindle disassembly **(e)** relative to anaphase onset (0 min). Scale bars: 10 μ m.

SUPPLEMENTARY INFORMATION

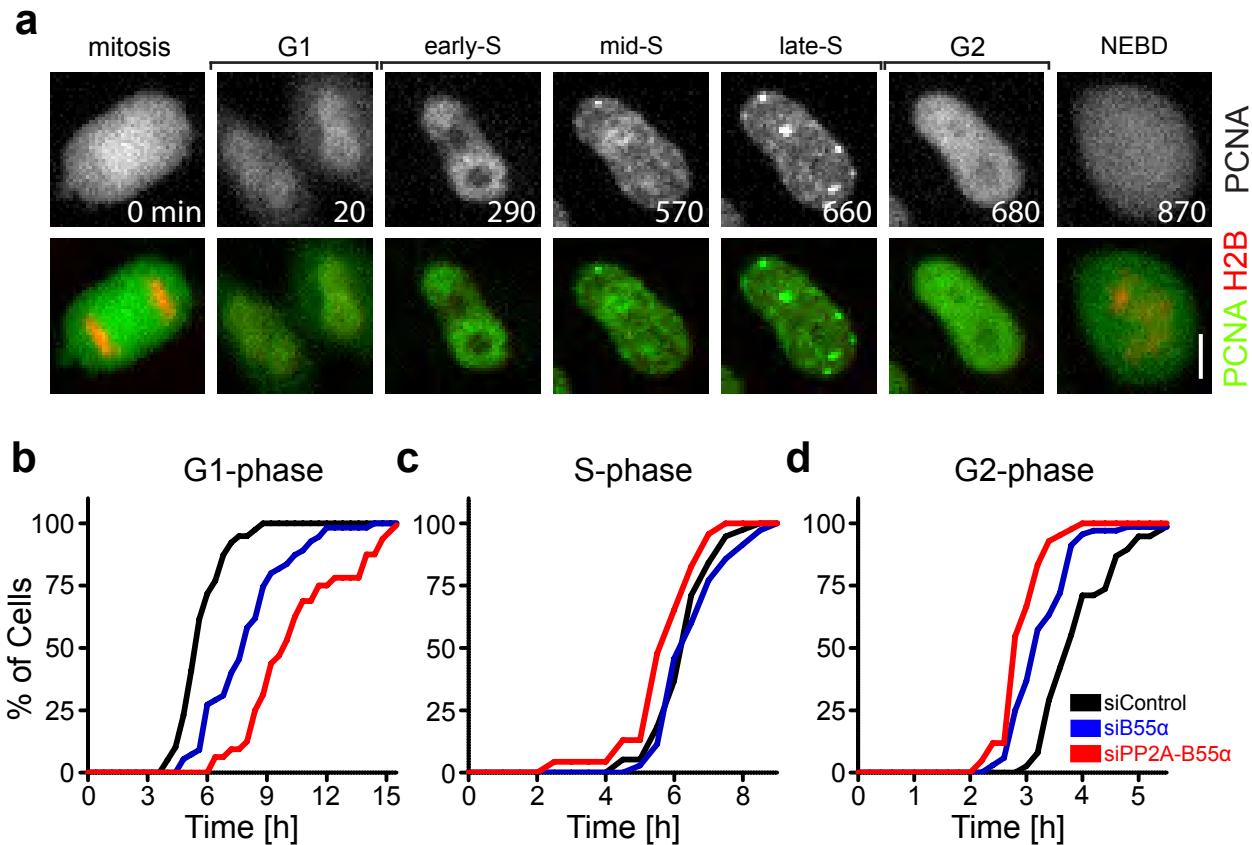


Figure S4 Effect of PP2A-B55 α -depletion on cell cycle progression. (a) Cell cycle staging by DNA replication factor pattern of EGFP-PCNA. (b, c,

(d) Cumulative histograms of G1-phase (b), S-phase (c), and G2-phase (d) duration. Scale bars: 10 μ m.

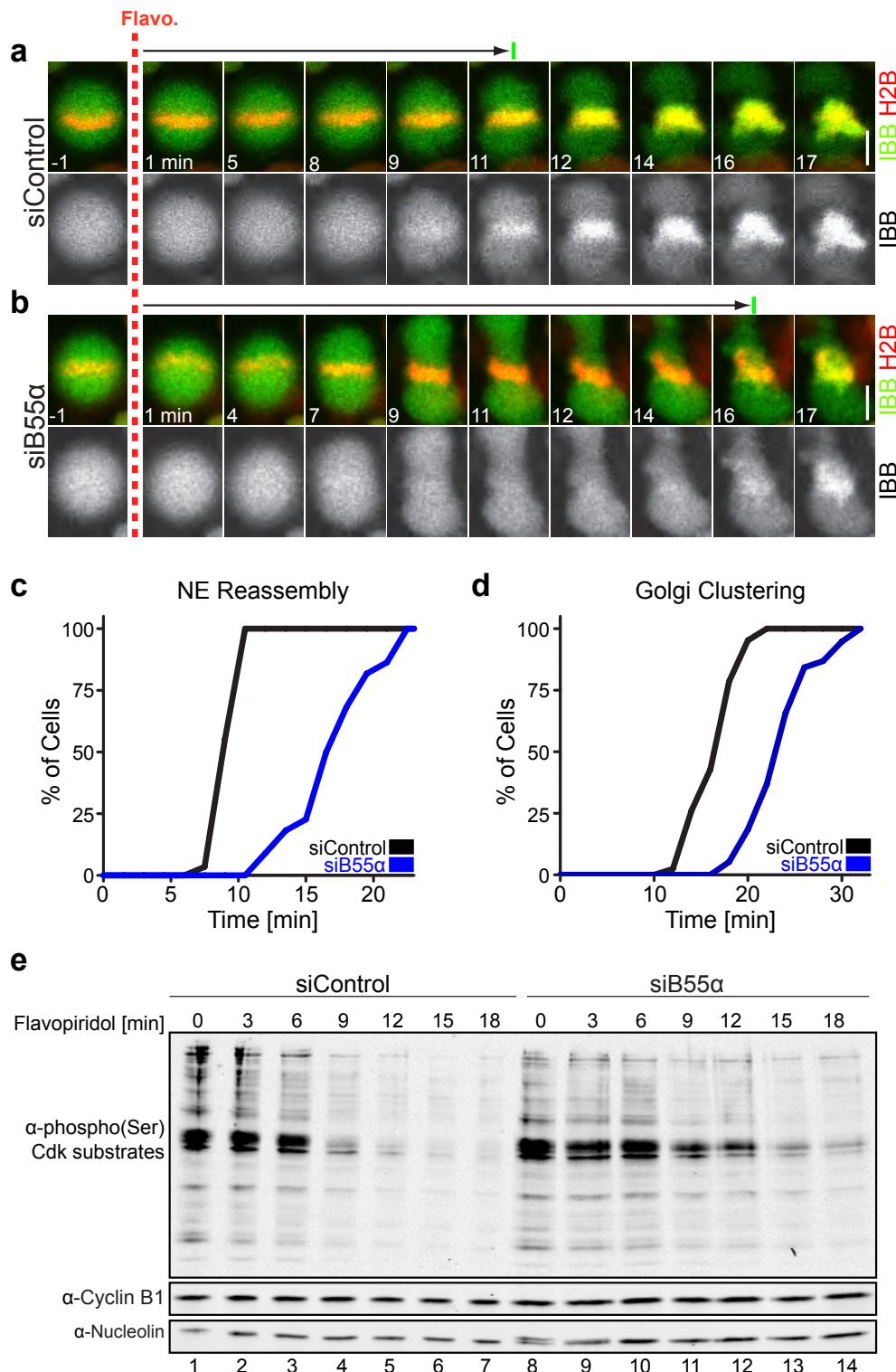


Figure S5 B55 α functions downstream of Cdk1-cyclin B inactivation.
(a) Movie of a cell transfected with non-silencing control siRNA, following the protocol indicated in Figure 4a. Arrowhead indicates onset of nuclear import of IBB-EGFP. **(b)** Time-lapse images of a cell transfected with siRNA targeting B55 α , see also Supplementary Information, Movie S13. **(c, d)** Cumulative histograms of nuclear envelope assembly

(c) and Golgi clustering (d) timing based on the data shown in (a-b, and data not shown). **(e)** Chemical induction of mitotic exit in presence of proteasome inhibitor in nocodazole arrested mitotic cells results in rapid dephosphorylation of Cdk substrates (siControl, lanes 1-7). Cells depleted for B55 α show delayed dephosphorylation (siB55 α , lanes 8-14). Scale bars: 10 μ m.

SUPPLEMENTARY INFORMATION

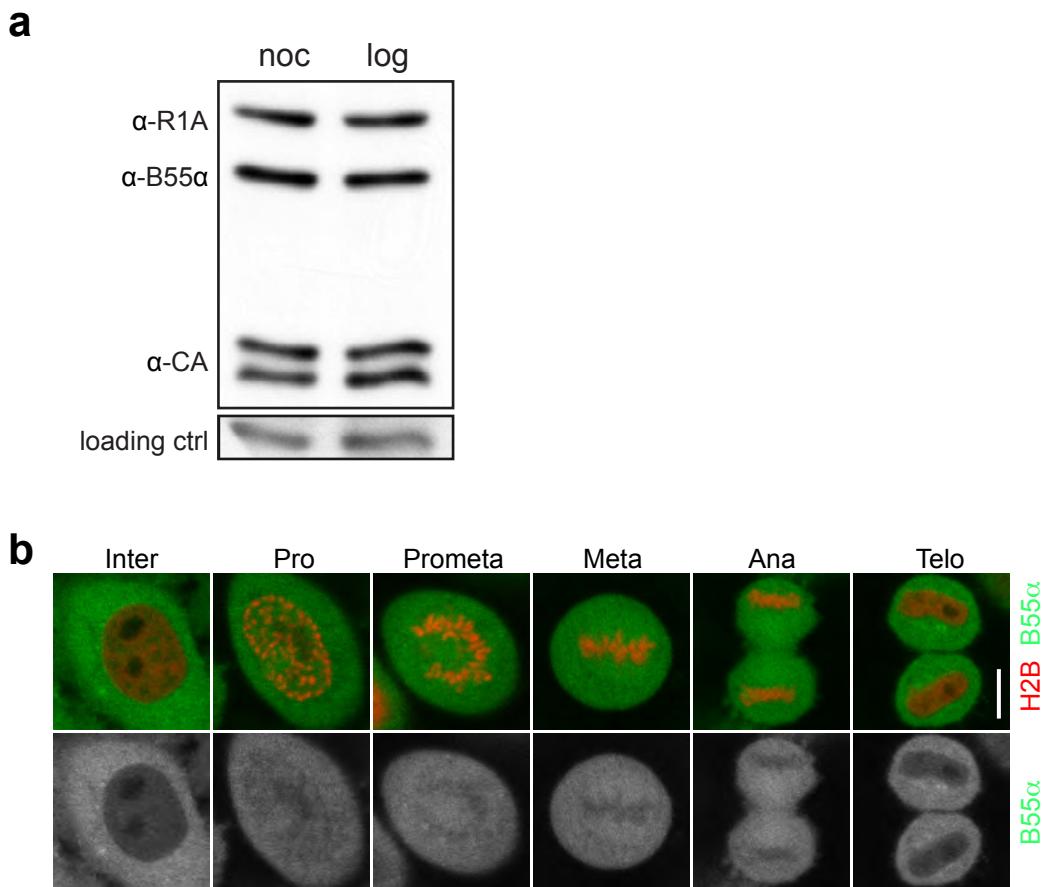


Figure S6 Expression levels and localization of PP2A-B55 α . **(a)** PP2A-B55 α subunits are expressed at constant levels during the cell cycle. Whole cell lysates were probed by Western Blotting using the antibodies against CA, R1A, and B55 α . Left lane: 17 h nocodazole arrested cells (noc), right lane:

unsynchronized interphase cells (log). Note that CA migrates as a doublet band (see also Supplementary Information, Figure S2g). **(b)** Localization of B55 α . Confocal live images of cells expressing EGFP-mouse-B55 α from endogenous promotor (see Fig. 2b). Scale bar: 10 μ m.

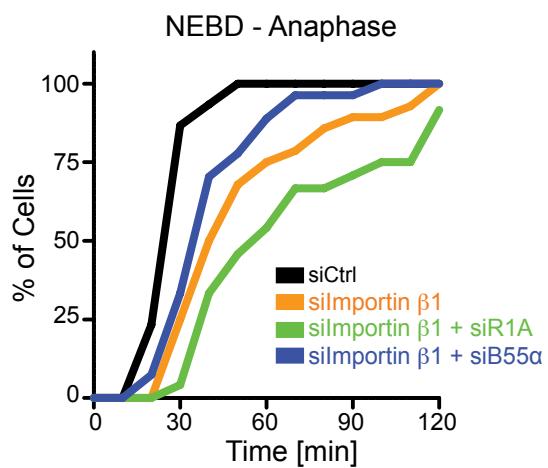


Figure S7 Early mitotic progression in Importin $\beta 1$ -depleted cells. Timing from prometaphase until anaphase was measured for the same cells shown in Fig. 5e.

SUPPLEMENTARY INFORMATION

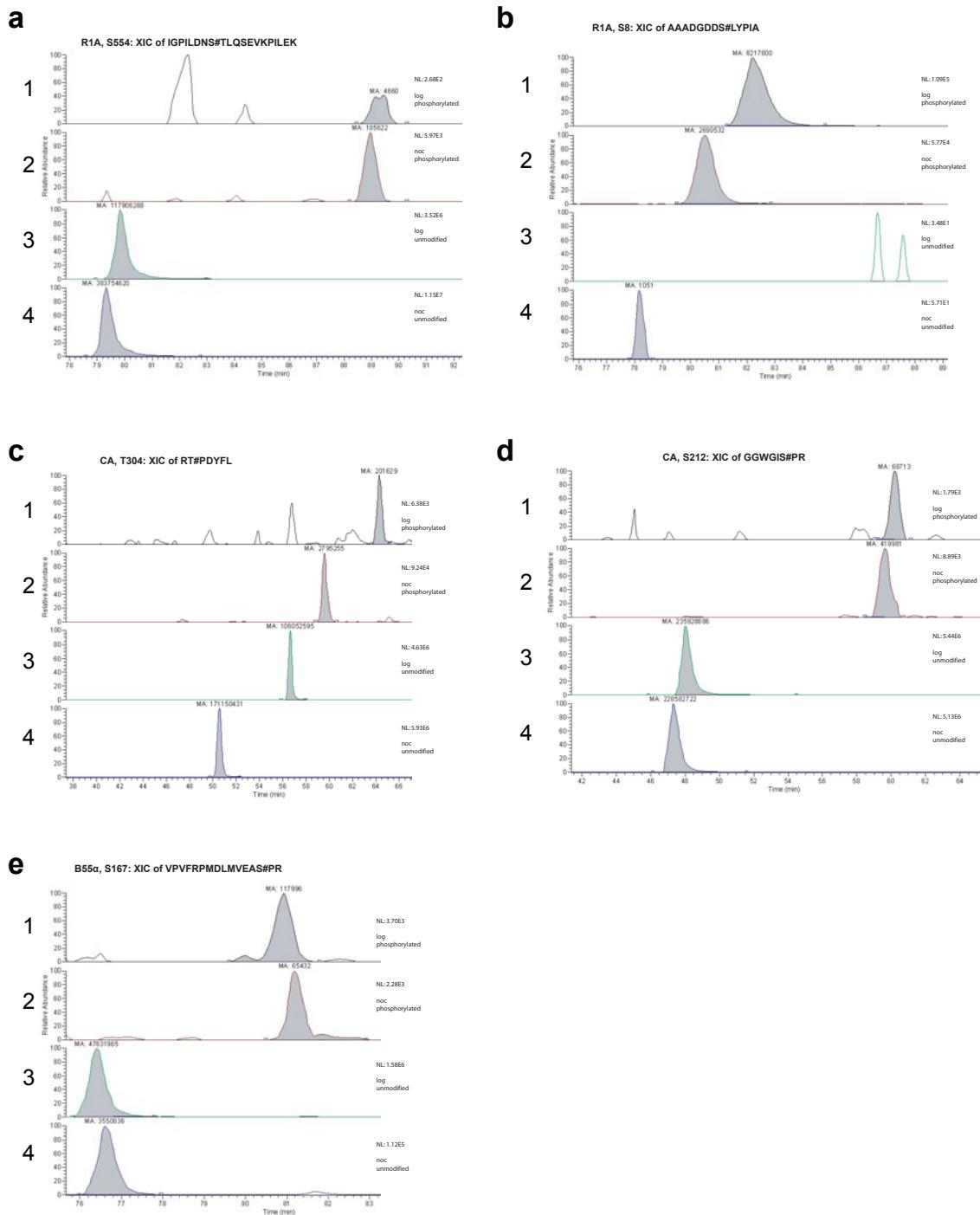


Figure S8 Quantification of phosphorylation levels by extracted ion chromatograms. Extracted ion chromatograms (XICs) of phosphopeptides identified (hash indicates the phosphorylated amino acid), and their unmodified counterparts, from purified PP2A complexes. Peak-area values for the phosphopeptides were used for quantification of the mitotic increase in phosphorylation abundance, whereas those of the unmodified peptides were used for normalization, as described in the legend for Fig. 5f. The panels show: 1. XIC featuring the phosphorylated

peptide identified from the PP2A complex purified from interphase (log) cells. 2. XIC featuring the same phosphorylated peptide identified from the mitotic (noc) cell state. 3. XIC featuring the unmodified counterpart peptide from interphase. 4. XIC featuring the unmodified counterpart peptide from the mitotic (noc) cell state. **(a)** Quantitation of S554 phosphorylation on R1A. **(b)** S8 phosphorylation on R1A. **(c)** T304 phosphorylation on CA. **(d)** S212 phosphorylation on CA. **(e)** S167 phosphorylation on B55 α .

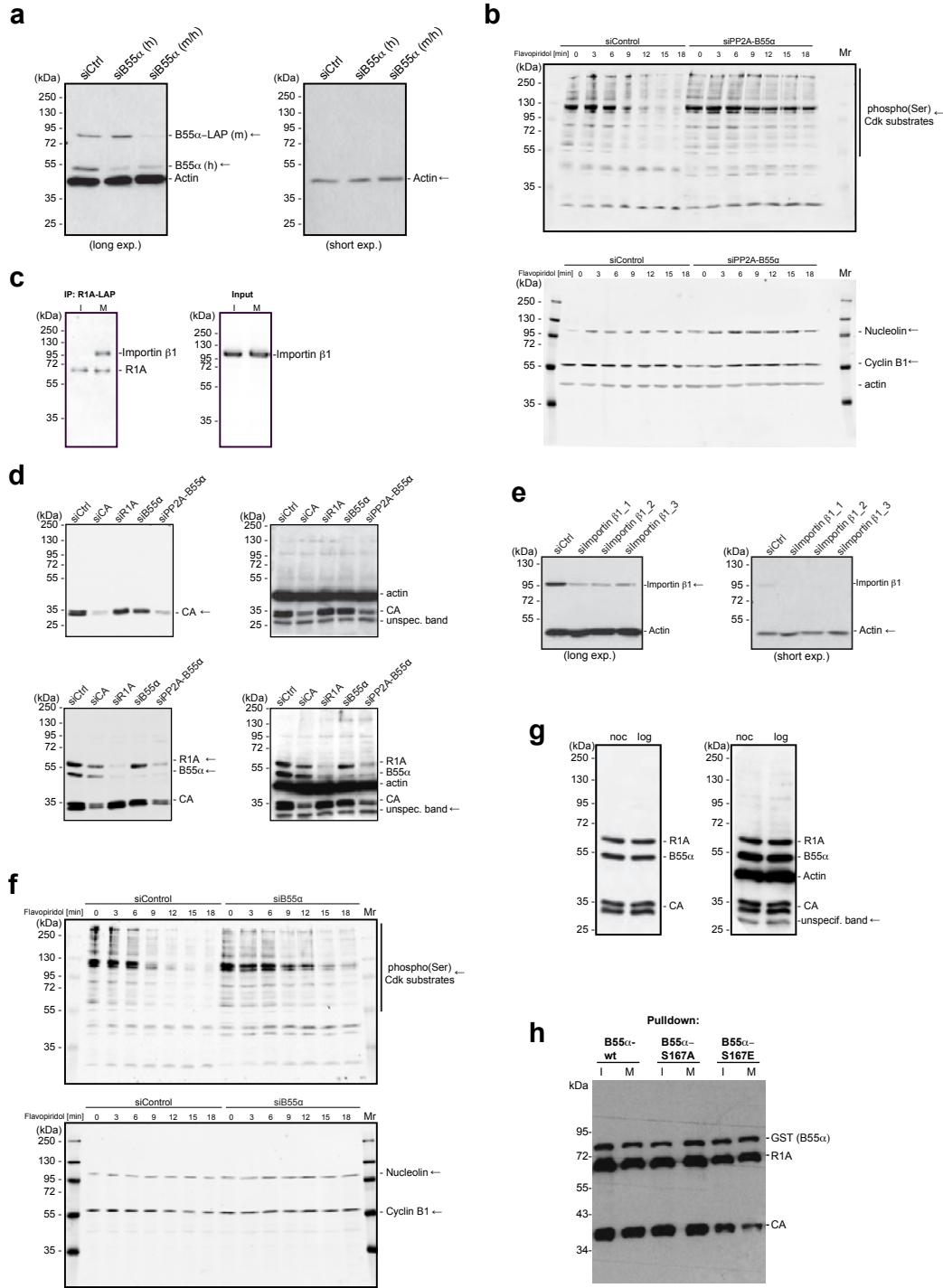


Figure S9 Full scans of blots. **(a)** Full scan for B55 α and Actin immunoblot of Figure 2b. **(b)** Full scan for phospho(Ser)Cdk substrates (upper) and Cyclin B1, Nucleolin, and Actin (lower) immunoblot of Figure 4f. **(c)** Full scan for Importin β 1 and R1A IP-blot (left) and Importin β 1 Input-blot (right) of Figure 5d. **(d)** Full scan for CA (upper left), R1A and B55 α (lower left), and loading control blots (right panels) of Figure S2g. **(e)** Full scan

for Importin β 1 and Actin immunoblot of Figure S2i. **(f)** Full scan for phospho(Ser)Cdk substrates (upper) and Cyclin B1, and Nucleolin (lower) immunoblot of Figure S5e. **(g)** Full scan for CA, R1A, and B55 α (right) and Actin (left) immunoblot of Figure S6a. (Sliced bands are indicated with arrows). **(h)** Relevance of B55 α -S167 phosphorylation for PP2A complex assembly. Independent replica of the experiment shown in Fig. 5g.

SUPPLEMENTARY INFORMATION

Supplementary Movie Legends

Movie S1 Monoclonal fluorescent HeLa cell line expressing chromatin marker H2B-mCherry and the nuclear import substrate IBB-EGFP. The movie shows 13% of the total image size and represent a typical overnight screening movie. Time-lapse covers a period of ~21 hours.

Movie S2 H2B-mCherry / IBB-EGFP reporter cell that has been automatically detected, tracked, and segmented over time. Movie shows original images (left); mitotic stage classification with color label as in Fig. 1c (middle), and regions of IBB measurements (right). Time-lapse covers a period of 216 minutes.

Movie S3 H2B-mCherry / IBB-EGFP cell treated with siControl. This movie shows mitotic progression and nuclear envelope reassembly in a control cell. Time-lapse covers a period of 192 minutes.

Movie S4 H2B-mCherry / IBB-EGFP cell treated with siPP2A-B55 α . This movie shows mitotic progression and delayed nuclear envelope reassembly in a cell depleted for PP2A-B55 α . Time-lapse covers a period of 192 minutes.

Movie S5 H2B-mCherry / GaIT-EGFP cell treated with siControl. This movie shows mitotic progression and Golgi reassembly in a control cell. Time-lapse covers a period of 132 minutes.

Movie S6 H2B-mCherry / GaIT-EGFP cell treated with siPP2A-B55 α . This movie shows mitotic progression and delayed Golgi reassembly in a cell depleted for PP2A-B55 α . Time-lapse covers a period of 161 minutes.

Movie S7 H2B-mCherry / mEGFP- α -tubulin cell treated with siControl. This movie shows mitotic progression and mitotic spindle dynamics in a control cell. Time-lapse covers a period of 108 minutes.

Movie S8 H2B-mCherry / mEGFP- α -tubulin cell treated with siPP2A-B55 α . This movie shows mitotic progression and delayed mitotic spindle disassembly in a cell depleted for PP2A-B55 α . Time-lapse covers a period of 108 minutes.

Movie S9 H2B-mCherry / IBB-EGFP cell treated with siControl. This movie shows forced mitotic exit in presence of MG132 by addition of the Cdk inhibitor flavopiridol in a control cell. Time-lapse covers a period of 47 minutes.

Movie S10 H2B-mCherry / IBB-EGFP cell treated with siPP2A-B55 α . This movie shows delayed mitotic exit in presence of MG132 by addition of the Cdk inhibitor flavopiridol in a cell depleted for PP2A-B55 α . Time-lapse covers a period of 47 minutes.

Supplementary Information Table 1. Library of siRNA oligos

54704	PPM2C	Hs	PPM2C_5	NM_018444	SI02759372	protein phosphatase 2C, magnesium-dependent, catalytic subunit	ATCGTACTTCITTTATTAGTA	UUACUAAAUAAGAACUACGat	CGUACUUCUAAUUAAGAUAA
54705	PPM2C	Hs	PPM2C_4	NM_018444	SI02759371	protein phosphatase 2C, magnesium-dependent, catalytic subunit	GGAAAGCTTCTTCAAGGAA	GUAAAGGGGAAUAGAUAA	GGAAAGCAACUACCUUAGAUAA
54778	PPPI1B12C	Hs	PPPI1B12C_5	NM_017667	SI02776922	protein phosphatase, 1, regulatory (inhibitor) subunit 12C	CACAGGGACCTTCGGAACCAA	GUACUUGGAAUAGGGCUCCU	CGAACAGGGACUCCGGAA
54778	PPPI1B12C	Hs	PPPI1B12C_6	NM_017667	SI0303750	protein phosphatase, 1, regulatory (inhibitor) subunit 12C	ACCGGGAGGAGTCAGCTTACAGA	UCUUGGAGGACUCCGGCGCG	CGAACGGGGAGGACUCCGGAA
54778	PPPI1B12C	Hs	PPPI1B12C_7	NM_017667	SI0308776	protein phosphatase, 1, regulatory (inhibitor) subunit 12C	CAGGGAGCTTGTGAGCCGCTGA	UACAGGGCUCUAAAGGCCG	GAGGGGGAGGACUCCGGAA
54866	PPPI1B14D	Hs	PPPI1B14D_1	NM_017726	SI03011943	protein phosphatase, 1, regulatory (inhibitor) subunit 14D	CCGCGCTGACAGTGATGATGA	UACAUACUUCAGUCCAGCG	GGCGUACAGAGUAGUAUAG
54866	PPPI1B14D	Hs	PPPI1B14D_2	NM_017726	SI030119441	protein phosphatase, 1, regulatory (inhibitor) subunit 14D	CAGGGAGCTTGTGAGCCGCTGA	UACUUGGAGGAGAACUCCG	GGAGGAGCUCCGGAA
54866	PPPI1B14D	Hs	PPPI1B14D_5	NM_017726	SI03045546	protein phosphatase, 1, regulatory (inhibitor) subunit 14D	AGGGACATTTGCATCTGC	UAGAGAGAUAGGAAUAUAGCt	GGAGGAGAUUUAUAGAUAA
54935	DUSP23	Hs	DUSP23	NM_017623	SI0274885	dual specificity phosphatase 23	CGATTCATCCAGGACGAA	UUACUUGGAAUAGGGCUCC	GGUACUCCGGAA
54935	DUSP23	Hs	DUSP23	NM_017623	SI0274886	dual specificity phosphatase 23	CGATTCATCCAGGACGAA	UUACUUGGAAUAGGGCUCC	GGUACUCCGGAA
54935	DUSP23	Hs	DUSP23	NM_017623	SI0274887	dual specificity phosphatase 23	CGATTCATCCAGGACGAA	UUACUUGGAAUAGGGCUCC	GGUACUCCGGAA
55069	PDPR	Hs	PDPR_5	NM_017990_XM_001134215	SI02642983	pyruvate dehydrogenase phosphatase regulatory subunit	ATCTCTATCTCCGTGATA	UAUACUAGGGAGUAUAGGt	CGUUCUACUUCUCCAUUAG
55069	PDPR	Hs	PDPR_6	NM_017990_XM_001134215	SI03053655	pyruvate dehydrogenase phosphatase regulatory subunit	CAAGGGGTGTCAGCTTGTGATA	UUACUUGGAGUACCCUCCUtg	AAGGGGUAGUACUCCAUUAG
55069	PDPR	Hs	PDPR_7	NM_017990	SI03063846	pyruvate dehydrogenase phosphatase regulatory subunit	CAGCATGACAGCAGCTAACAA	UUUUCUAGGUGUCCAUUAGCt	CGAUGACAGCAGUACAA
55183	RIF1	Hs	RIF1_1	NM_018151	SI07052227	RAF1 interacting factor homolog (yeast)	CTCATGAGAAATTTGAGTTAA	UUUACAUUUAUAGAUUAGCt	CGAUGAUUUAUAGAUAA
55183	RIF1	Hs	RIF1_1	NM_018151	SI07052234	RAF1 interacting factor homolog (yeast)	AGGAAGACATTTGAGTTAA	UUUACAUUUAUAGAUUAGCt	CGAUGAUUUAUAGAUAA
55183	RIF1	Hs	RIF1_3	NM_018151	SI07052234	RAF1 interacting factor homolog (yeast)	AGGAAGACATTTGAGTTAA	UUUACAUUUAUAGAUUAGCt	CGAUGAUUUAUAGAUAA
55291	SAP33	Hs	C11orf23_3	NM_018312	SI0317331	SAPS domain family, member 3	CGAACAGCTTCAATGTTT	UAUACAUUAGGAGUCCU	GAAGAACGAGUACUCCAU
55370	PPP4R1L	Hs	PPP4R1L_1	NM_018498_NR_003505	SI00917597	protein phosphatase 4, regulatory subunit 1-like	CGGGACTCTGGTACAAAGTA	UUUACUUGUACGAGAUCC	GGAGUACUUCGACUAA
55370	PPP4R1L	Hs	PPP4R1L_2	NM_018498	SI00917598	protein phosphatase 4, regulatory subunit 1-like	AGGCAGATTTTGAACCAAA	UUUACUUGUACAAUAGAUUAGCt	GGAGAUAUUAUAGAUAA
55370	PPP4R1L	Hs	PPP4R1L_3	NM_018498	SI00917599	protein phosphatase 4, regulatory subunit 1-like	AGGATAATACAGGATTTCAA	UUUACUUGUACUUAUAGAUAA	GGAGAUAUUAUAGAUAA
55607	PPPI1B5A	Hs	PPPI1B5A_2	NM_017650_XM_374853_XM_374857	SI0251474	protein phosphatase, 1, regulatory (inhibitor) subunit 5A	GGGGGGGGGGGGGGGGGGGG	UAGGGGGGGGGGGGGGGGGGG	GGGGGGGGGGGGGGGGGGGG
55607	PPPI1B5A	Hs	PPPI1B5A_3	NM_017650_XM_374853_XM_374857	SI0251475	protein phosphatase, 1, regulatory (inhibitor) subunit 5A	GGGGGGGGGGGGGGGGGGGG	UAGGGGGGGGGGGGGGGGGGG	GGGGGGGGGGGGGGGGGGGG
55607	PPPI1B5A	Hs	PPPI1B5A_6	NM_017650_XM_374853_XM_374857	SI0251476	protein phosphatase, 1, regulatory (inhibitor) subunit 5A	GGGGGGGGGGGGGGGGGGGG	UAGGGGGGGGGGGGGGGGGGG	GGGGGGGGGGGGGGGGGGGG
55613	MTMR8	Hs	MTMR8_3	NM_017677	SI02659223	myotubularin related protein 8	CGGGGGAGTCTGGAGCTATCA	UAUACUUCUUAUAGAUUAGCt	GGAAAGUJCAGUAUAGAUAA
55613	MTMR8	Hs	MTMR8_6	NM_017677	SI02659230	myotubularin related protein 8	GGGGGGGGGGGGGGGGGGGG	UAGGGGGGGGGGGGGGGGGGG	GGGGGGGGGGGGGGGGGGGG
55625	YLP1M	Hs	YLP1M_1	NM_019589_XM_085151_XM_930487_XM_930487	SI02764491	VLP motif containing 1	GGGGGGGGGGGGGGGGGGGG	UAGGGGGGGGGGGGGGGGGGG	GGGGGGGGGGGGGGGGGGGG
55625	YLP1M	Hs	YLP1M_2	NM_019589_XM_085151_XM_930487_XM_930487	SI02764495	VLP motif containing 1	GGGGGGGGGGGGGGGGGGGG	UAGGGGGGGGGGGGGGGGGGG	GGGGGGGGGGGGGGGGGGGG
55625	YLP1M	Hs	YLP1M_3	NM_019589_XM_085151_XM_930487_XM_930487	SI02764499	VLP motif containing 1	GGGGGGGGGGGGGGGGGGGG	UAGGGGGGGGGGGGGGGGGGG	GGGGGGGGGGGGGGGGGGGG
55625	YLP1M	Hs	YLP1M_4	NM_019589_XM_085151_XM_930487_XM_930487	SI02764505	VLP motif containing 1	GGGGGGGGGGGGGGGGGGGG	UAGGGGGGGGGGGGGGGGGGG	GGGGGGGGGGGGGGGGGGGG
55625	YLP1M	Hs	YLP1M_5	NM_019589_XM_085151_XM_930487_XM_930487	SI02764509	VLP motif containing 1	GGGGGGGGGGGGGGGGGGGG	UAGGGGGGGGGGGGGGGGGGG	GGGGGGGGGGGGGGGGGGGG
55625	YLP1M	Hs	YLP1M_6	NM_019589_XM_085151_XM_930487_XM_930487	SI02764513	VLP motif containing 1	GGGGGGGGGGGGGGGGGGGG	UAGGGGGGGGGGGGGGGGGGG	GGGGGGGGGGGGGGGGGGGG
55625	YLP1M	Hs	YLP1M_7	NM_019589_XM_085151_XM_930487_XM_930487	SI02764517	VLP motif containing 1	GGGGGGGGGGGGGGGGGGGG	UAGGGGGGGGGGGGGGGGGGG	GGGGGGGGGGGGGGGGGGGG
55625	YLP1M	Hs	YLP1M_8	NM_019589_XM_085151_XM_930487_XM_930487	SI02764521	VLP motif containing 1	GGGGGGGGGGGGGGGGGGGG	UAGGGGGGGGGGGGGGGGGGG	GGGGGGGGGGGGGGGGGGGG
55625	YLP1M	Hs	YLP1M_9	NM_019589_XM_085151_XM_930487_XM_930487	SI02764525	VLP motif containing 1	GGGGGGGGGGGGGGGGGGGG	UAGGGGGGGGGGGGGGGGGGG	GGGGGGGGGGGGGGGGGGGG
55625	YLP1M	Hs	YLP1M_10	NM_019589_XM_085151_XM_930487_XM_930487	SI02764529	VLP motif containing 1	GGGGGGGGGGGGGGGGGGGG	UAGGGGGGGGGGGGGGGGGGG	GGGGGGGGGGGGGGGGGGGG
55625	YLP1M	Hs	YLP1M_11	NM_019589_XM_085151_XM_930487_XM_930487	SI02764533	VLP motif containing 1	GGGGGGGGGGGGGGGGGGGG	UAGGGGGGGGGGGGGGGGGGG	GGGGGGGGGGGGGGGGGGGG
55625	YLP1M	Hs	YLP1M_12	NM_019589_XM_085151_XM_930487_XM_930487	SI02764537	VLP motif containing 1	GGGGGGGGGGGGGGGGGGGG	UAGGGGGGGGGGGGGGGGGGG	GGGGGGGGGGGGGGGGGGGG
55625	YLP1M	Hs	YLP1M_13	NM_019589_XM_085151_XM_930487_XM_930487	SI02764541	VLP motif containing 1	GGGGGGGGGGGGGGGGGGGG	UAGGGGGGGGGGGGGGGGGGG	GGGGGGGGGGGGGGGGGGGG
55625	YLP1M	Hs	YLP1M_14	NM_019589_XM_085151_XM_930487_XM_930487	SI02764545	VLP motif containing 1	GGGGGGGGGGGGGGGGGGGG	UAGGGGGGGGGGGGGGGGGGG	GGGGGGGGGGGGGGGGGGGG
55625	YLP1M	Hs	YLP1M_15	NM_019589_XM_085151_XM_930487_XM_930487	SI02764549	VLP motif containing 1	GGGGGGGGGGGGGGGGGGGG	UAGGGGGGGGGGGGGGGGGGG	GGGGGGGGGGGGGGGGGGGG
55625	YLP1M	Hs	YLP1M_16	NM_019589_XM_085151_XM_930487_XM_930487	SI02764553	VLP motif containing 1	GGGGGGGGGGGGGGGGGGGG	UAGGGGGGGGGGGGGGGGGGG	GGGGGGGGGGGGGGGGGGGG
55625	YLP1M	Hs	YLP1M_17	NM_019589_XM_085151_XM_930487_XM_930487	SI02764557	VLP motif containing 1	GGGGGGGGGGGGGGGGGGGG	UAGGGGGGGGGGGGGGGGGGG	GGGGGGGGGGGGGGGGGGGG
55625	YLP1M	Hs	YLP1M_18	NM_019589_XM_085151_XM_930487_XM_930487	SI02764561	VLP motif containing 1	GGGGGGGGGGGGGGGGGGGG	UAGGGGGGGGGGGGGGGGGGG	GGGGGGGGGGGGGGGGGGGG
55625	YLP1M	Hs	YLP1M_19	NM_019589_XM_085151_XM_930487_XM_930487	SI02764565	VLP motif containing 1	GGGGGGGGGGGGGGGGGGGG	UAGGGGGGGGGGGGGGGGGGG	GGGGGGGGGGGGGGGGGGGG
55625	YLP1M	Hs	YLP1M_20	NM_019589_XM_085151_XM_930487_XM_930487	SI02764569	VLP motif containing 1	GGGGGGGGGGGGGGGGGGGG	UAGGGGGGGGGGGGGGGGGGG	GGGGGGGGGGGGGGGGGGGG
55625	YLP1M	Hs	YLP1M_21	NM_019589_XM_085151_XM_930487_XM_930487	SI02764573	VLP motif containing 1	GGGGGGGGGGGGGGGGGGGG	UAGGGGGGGGGGGGGGGGGGG	GGGGGGGGGGGGGGGGGGGG
55625	YLP1M	Hs	YLP1M_22	NM_019589_XM_085151_XM_930487_XM_930487	SI02764577	VLP motif containing 1	GGGGGGGGGGGGGGGGGGGG	UAGGGGGGGGGGGGGGGGGGG	GGGGGGGGGGGGGGGGGGGG
55625	YLP1M	Hs	YLP1M_23	NM_019589_XM_085151_XM_930487_XM_930487	SI02764581	VLP motif containing 1	GGGGGGGGGGGGGGGGGGGG	UAGGGGGGGGGGGGGGGGGGG	GGGGGGGGGGGGGGGGGGGG
55625	YLP1M	Hs	YLP1M_24	NM_019589_XM_085151_XM_930487_XM_930487	SI02764585	VLP motif containing 1	GGGGGGGGGGGGGGGGGGGG	UAGGGGGGGGGGGGGGGGGGG	GGGGGGGGGGGGGGGGGGGG
55625	YLP1M	Hs	YLP1M_25	NM_019589_XM_085151_XM_930487_XM_930487	SI02764589	VLP motif containing 1	GGGGGGGGGGGGGGGGGGGG	UAGGGGGGGGGGGGGGGGGGG	GGGGGGGGGGGGGGGGGGGG
55625	YLP1M	Hs	YLP1M_26	NM_019589_XM_085151_XM_930487_XM_930487	SI02764593	VLP motif containing 1	GGGGGGGGGGGGGGGGGGGG	UAGGGGGGGGGGGGGGGGGGG	GGGGGGGGGGGGGGGGGGGG
55625	YLP1M	Hs	YLP1M_27	NM_019589_XM_085151_XM_930487_XM_930487	SI02764597	VLP motif containing 1	GGGGGGGGGGGGGGGGGGGG	UAGGGGGGGGGGGGGGGGGGG	GGGGGGGGGGGGGGGGGGGG
55625	YLP1M	Hs	YLP1M_28	NM_019589_XM_085151_XM_930487_XM_930487	SI02764601	VLP motif containing 1	GGGGGGGGGGGGGGGGGGGG	UAGGGGGGGGGGGGGGGGGGG	GGGGGGGGGGGGGGGGGGGG
55625	YLP1M	Hs	YLP1M_29	NM_019589_XM_085151_XM_930487_XM_930487	SI02764605	VLP motif containing 1	GGGGGGGGGGGGGGGGGGGG	UAGGGGGGGGGGGGGGGGGGG	GGGGGGGGGGGGGGGGGGGG
55625	YLP1M	Hs	YLP1M_30	NM_019589_XM_085151_XM_930487_XM_930487	SI02764609	VLP motif containing 1	GGGGGGGGGGGGGGGGGGGG	UAGGGGGGGGGGGGGGGGGGG	GGGGGGGGGGGGGGGGGGGG
55625	YLP1M	Hs	YLP1M_31	NM_019589_XM_085151_XM_930487_XM_930487	SI02764613	VLP motif containing 1	GGGGGGGGGGGGGGGGGGGG	UAGGGGGGGGGGGGGGGGGGG	GGGGGGGGGGGGGGGGGGGG
55625	YLP1M	Hs	YLP1M_32	NM_019589_XM_085151_XM_930487_XM_930487	SI02764617	VLP motif containing 1	GGGGGGGGGGGGGGGGGGGG	UAGGGGGGGGGGGGGGGGGGG	GGGGGGGGGGGGGGGGGGGG
55625	YLP1M	Hs	YLP1M_33	NM_019589_XM_085151_XM_930487_XM_930487	SI02764621	VLP motif containing 1	GGGGGGGGGGGGGGGGGGGG	UAGGGGGGGGGGGGGGGGGGG	GGGGGGGGGGGGGGGGGGGG
55625	YLP1M	Hs	YLP1M_34	NM_019589_XM_085151_XM_930487_XM_930487	SI02764625	VLP motif containing 1	GGGGGGGGGGGGGGGGGGGG	UAGGGGGGGGGGGGGGGGGGG	GGGGGGGGGGGGGGGGGGGG
55625	YLP1M	Hs	YLP1M_35	NM_019589_XM_085151_XM_930487_XM_930487	SI02764629	VLP motif containing 1	GGGGGGGGGGGGGGGGGGGG	UAGGGGGGGGGGGGGGGGGGG	GGGGGGGGGGGGGGGGGGGG
55625	YLP1M	Hs	YLP1M_36	NM_019589_XM_085151_XM_930487_XM_930487	SI02764633	VLP motif containing 1	GGGGGGGGGGGGGGGGGGGG	UAGGGGGGGGGGGGGGGGGGG	GGGGGGGGGGGGGGGGGGGG
55625	YLP1M	Hs	YLP1M_37	NM_019589_XM_085151_XM_930487_XM_930487	SI02764637	VLP motif containing 1	GGGGGGGGGGGGGGGGGGGG	UAGGGGGGGGGGGGGGGGGGG	GGGGGGGGGGGGGGGGGGGG
55625	YLP1M	Hs	YLP1M_38	NM_019589_XM_085151_XM_930487_XM_930487	SI02764641	VLP motif containing 1	GGGGGGGGGGGGGGGGGGGG	UAGGGGGGGGGGGGGGGGGGG	GGGGGGGGGGGGGGGGGGGG
55625	YLP1M	Hs	YLP1M_39	NM_019589_XM_085151_XM_930487_XM_930487	SI02764645	VLP motif containing 1	GGGGGGGGGGGGGGGGGGGG	UAGGGGGGGGGGGGGGGGGGG	GGGGGGGGGGGGGGGGGGGG
55625	YLP1M	Hs	YLP1M_40	NM_019589_XM_085151_XM_930487_XM_930487	SI02764649	VLP motif containing 1	GGGGGGGGGGGGGGGGGGGG	UAGGGGGGGGGGGGGGGGGGG	GGGGGGGGGGGGGGGGGGGG
55625	YLP1M	Hs	YLP1M_41	NM_019589_XM_085151_XM_930487_XM_930487	SI02764653	VLP motif containing 1	GGGGGGGGGGGGGGGGGGGG	UAGGGGGGGGGGGGGGGGGGG	GGGGGGGGGGGGGGGGGGGG
55625	YLP1M	Hs	YLP1M_42	NM_019589_XM_085151_XM_930487_XM_930487	SI02764657	VLP motif containing 1	GGGGGGGGGGGGGGGGGGGG	UAGGGGGGGGGGGGGGGGGGG	GGGGGGGGGGGGGGGGGGGG
55625	YLP1M	Hs	YLP1M_43	NM_019589_XM_085151_XM_930487_XM_930487	SI02764661	VLP motif containing 1	GGGGGGGGGGGGGGGGGGGG	UAGGGGGGGGGGGGGGGGGGG	GGGGGGGGGGGGGGGGGGGG
55625	YLP1M	Hs	YLP1M_44	NM_019589_XM_085151_XM_930487_XM_930487	SI02764665	VLP motif containing 1	GGGGGGGGGGGGGGGGGGGG	UAGGGGGGGGGGGGGGGGGGG	GGGGGGGGGGGGGGGGGGGG
55625	YLP1M	Hs	YLP1M_45	NM_019589_XM_085151_XM_930487_XM_930487	SI02764669	VLP motif containing 1	GGGGGGGGGGGGGGGGGGGG	UAGGGGGGGGGGGGGGGGGGG	GGGGGGGGGGGGGGGGGGGG
55625	YLP1M	Hs	YLP1M_46	NM_019589_XM_085151_XM_930487_XM_930487	SI02764673	VLP motif containing 1	GGGGGGGGGGGGGGGGGGGG	UAGGGGGGGGGGGGGGGGGGG	GGGGGGGGGGGGGGGGGGGG
55625	YLP1M	Hs	YLP1M_47	NM_019589_XM_085151_XM_930487_XM_930487	SI02764677	VLP motif containing 1	GGGGGGGGGGGGGGGGGGGG	UAGGGGGGGGGGGGGGGGGGG	GGGGGGGGGGGGGGGGGGGG
55625	YLP1M	Hs	YLP1M_48	NM_019589_XM_085151_XM_930487_XM_930487	SI02764681	VLP motif containing 1	GGGGGGGGGGGGGGGGGGGG	UAGGGGGGGGGGGGGGGGGGG	GGGGGGGGGGGGGGGGGGGG
55625									

170954	KIAA1949	Hs	KIAA1949_4	NM_133471 XM_929887 XM_934035 XM	Si02824423	KIAA1949	TCCGGTCAGGCCGAGAGAAA	UUUCUCUCGGCCUCGAACGaa	CGUUCGAGCCGAGAGAAAH
201562	PTPLB	Hs	PTPLB_5	NM_198402	Si02659454	protein tyrosine phosphatase-like (proline instead of catalytic arginine), member b	CAAGGGCTACGGGGTCATCTTA	UAGAUUCAGUUCACCGGGaa	CGGGGACCUUGUGCAUCUAA
201562	PTPLB	Hs	PTPLB_4	NM_198402	Si02659454	protein tyrosine phosphatase-like (proline instead of catalytic arginine), member b	CGGGGGGGGGGGGGGGGGGGGG	CGGGGGGGGGGGGGGGGGGGGG	CGGGGGGGGGGGGGGGGGGGGG
201562	PTPLB	Hs	PTPLB_3	NM_198402	Si02659454	protein tyrosine phosphatase-like (proline instead of catalytic arginine), member b	UUCGGCTTAACTCTCTATGGAT	AUCGUACUAGAAAGUAGGGaa	CGGUUAUCUUCUUCUGCAUR
286262	Clnorf75	Hs	Clnorf75_1	NM_173691	Si00339695	chromosome 9 open reading frame 75	TCTGCCTAAAGAAAAGATGAA	UUCUACUULCUCUUCUAGGGaa	CGUCUAAAGAACAGAUAAAH
286262	Clnorf75	Hs	Clnorf75_2	NM_173691	Si0033972	chromosome 9 open reading frame 75	CACAGCTGGTCCCCAAAGAGAA	UUCUUCUUCUUCGGCCACCUUgg	CAGUGGGUGCCCAAAGGGAAH
286262	Clnorf75	Hs	Clnorf75_3	NM_173691	Si0033979	chromosome 9 open reading frame 75	TGCGAAATTCTTCATGTGTCAT	AUGACCAUCUAGAGAUUUUcsa	GAAGAUUCUUCUCAUGUGCAUR
391025	LOC391025	Hs	LOC391025_5	NM_00131226 XM_372775	Si0436859	similar to protein tyrosine phosphatase, receptor type, U isoform 2 precursor	CCCGCAGGGAAAGCCGATGAACGA	UCGUUCAUCCGGCUUCUCCGg	GCAGGAAGCCGAUAAAGAA
391025	LOC391025	Hs	LOC391025_6	NM_00131226 XM_372775	Si0436866	similar to protein tyrosine phosphatase, receptor type, U isoform 2 precursor	CCCATGATGAGCACATGGAA	UCCAUUGUUCUCAUCUAGUUsa	ACAUAGUAGGACCAUUGAA
391025	LOC391025	Hs	LOC391025_7	NM_00131226 XM_372775	Si0436873	similar to protein tyrosine phosphatase, receptor type, U isoform 2 precursor	CCCTGTGCTTATAGACGCTCA	UGAGGUUCUUAUAGACGAGaa	UGUCUCUUAUAGACGCUAA
400927	LOC400927	Hs	LOC400927_1	NM_001034843 NR_002821 XM_378010	Si00548667	TPTE and PTEN homologous inositol lipid phosphatase pseudogene	TCCTTGTTTCTTCTTCTTCTT	UUCUUCUUCUUCUUCUUCUUCUUC	CGGUUCUUCUUCUUCUUCUUCUUC
400927	LOC400927	Hs	LOC400927_2	NM_001034843 NR_002821 XM_378010	Si00548674	TPTE and PTEN homologous inositol lipid phosphatase pseudogene	ATGGATGTTCTCTTGGAGTA	UACUCGAAAGAAACAUUccs	CGAUUGUUCUUCUUCAGUAAH
400927	LOC400927	Hs	LOC400927_3	NM_001034843 NR_002821 XM_378010	Si00548674	TPTE and PTEN homologous inositol lipid phosphatase pseudogene	TCCACAGACAAACGAATTAA	UUAAALUUCUUCUUCUUCUUCUUC	CACAGACAAACGAUUAH

Supplementary Information Table 2. SiRNA oligos targeting PP2A-B55 α , B55 δ , and Importin β 1.

Entrez Gene ID	NCBI gene symbol	Symbol used this study	Gene Description	mRNA Accessions	siRNA Target Sequence	Qiagen Product ID	Product Name	mRNA knockdown this study
5515	PPP2CA	CA_3	protein phosphatase 2 (formerly 2A), catalytic subunit, alpha isoform	NM_002715	ACACCTCGTGAATACAATTAA	SI00041853	HS PPP2CA_3	81%
5515	PPP2CA	CA_5	protein phosphatase 2 (formerly 2A), catalytic subunit, alpha isoform	NM_002715	ATGGAACITGAGATACTCTAA	SI02225763	HS PPP2CA_5	86%
5515	PPP2CA	CA_6	protein phosphatase 2 (formerly 2A), catalytic subunit, alpha isoform	NM_002715	CAAACAACTATTGGAGCTTAA	SI02225790	HS PPP2CA_6	87%
5515	PPP2CA	CA_7	protein phosphatase 2 (formerly 2A), catalytic subunit, alpha isoform	NM_002715	TAAGACGATGTGACTGCACAAA	SI04436453	HS PPP2CA_7	78%
5515	PPP2CA	CA_8	protein phosphatase 2 (formerly 2A), catalytic subunit, alpha isoform	NM_002715	ATGGTGTCTCTGCCATCTAA	SI04436460	HS PPP2CA_8	74%
5515	PPP2CA	CA_9	protein phosphatase 2 (formerly 2A), catalytic subunit, alpha isoform	NM_002715	TACAAAGCCTCTGTCTCAA	SI04436467	HS PPP2CA_9	80%
5518	PPP2R1A	R1A_1	protein phosphatase 2 (formerly 2A), regulatory subunit A, alpha isoform	NM_014225	TCCCCATCTGGGCAAAGACAA	SI00103733	Hs PPP2R1A_1	94%
5518	PPP2R1A	R1A_5	protein phosphatase 2 (formerly 2A), regulatory subunit A, alpha isoform	NM_014225	CTGGTGTCCGATGCCAACAA	SI02225811	Hs PPP2R1A_5	91%
5518	PPP2R1A	R1A_6	protein phosphatase 2 (formerly 2A), regulatory subunit A, alpha isoform	NM_014225	ACGGCTGAACATCATCTCTAA	SI02225818	Hs PPP2R1A_6	71%
5518	PPP2R1A	R1A_7	protein phosphatase 2 (formerly 2A), regulatory subunit A, alpha isoform	NM_014225	GACCAGGATGTGGACGTCAAA	SI04436495	Hs PPP2R1A_7	95%
5518	PPP2R1A	R1A_8	protein phosphatase 2 (formerly 2A), regulatory subunit A, alpha isoform	NM_014225	ATGCCGGTGTCTAGAGCAGAA	SI04436502	Hs PPP2R1A_8	89%
5518	PPP2R1A	R1A_9	protein phosphatase 2 (formerly 2A), regulatory subunit A, alpha isoform	NM_014225	CACCTTGACAGAGTGAAGTCAA	SI04436509	Hs PPP2R1A_9	97%
5520	PPP2R2A	R2A_1	protein phosphatase 2 (formerly 2A), regulatory subunit B, alpha isoform	NM_002717	CTCGCCGTGCTCGCACTGAA	SI00041895	Hs PPP2R2A_1	72%
5520	PPP2R2A	R2A_3	protein phosphatase 2 (formerly 2A), regulatory subunit B, alpha isoform	NM_002717	AAGCGAGACATAACCCCTAGAA	SI00041909	Hs PPP2R2A_3	76%
5520	PPP2R2A	R2A_5	protein phosphatase 2 (formerly 2A), regulatory subunit B, alpha isoform	NM_002717	CTCGAGATGTTGGGATTA	SI02225825	Hs PPP2R2A_5	95%
5520	PPP2R2A	R2A_6	protein phosphatase 2 (formerly 2A), regulatory subunit B, alpha isoform	NM_002717	ATGGAAGGTATAGAGATCTCA	SI02225832	Hs PPP2R2A_6	94%
5520	PPP2R2A	R2A_7	protein phosphatase 2 (formerly 2A), regulatory subunit B, alpha isoform	NM_002717	CCCGGCTTTGTTGTTGATA	SI04436516	Hs PPP2R2A_7	77%
5520	PPP2R2A	R2A_8	protein phosphatase 2 (formerly 2A), regulatory subunit B, alpha isoform	NM_002717	CAGTCTCATAGCAGAGGAGAA	SI04436523	Hs PPP2R2A_8	93%
55844	PPP2R2D	B55 δ	protein phosphatase 2, regulatory subunit B, delta isoform	NM_001003656 NM_018461	CAGAGACTACCTGTCGGTGA	SI00691523	Hs PPP2R2D_1	72%
55844	PPP2R2D	B55 δ	protein phosphatase 2, regulatory subunit B, delta isoform	NM_001003656 NM_018461	CCGCTCCTTAAAGAACAGTGA	SI00691530	Hs PPP2R2D_2	52%
55844	PPP2R2D	B55 δ	protein phosphatase 2, regulatory subunit B, delta isoform	NM_001003656 NM_018461	TTCATCCATTCGGATGTAAA	SI02759148	Hs PPP2R2D_5	82%
3837	KPNB1	Importin β 1	karyopherin (importin) beta 1	NM_002265	TCGGTTATTTGCCAAGATA	SI00035490	Hs_KPNB1_1	89%
3837	KPNB1	Importin β 1	karyopherin (importin) beta 1	NM_002265	CAAGAACTCTTGACATCTAA	SI00035497	Hs_KPNB1_2	90%
3837	KPNB1	Importin β 1	karyopherin (importin) beta 1	NM_002265	AAAGGGCGGAGATCGAAGACTA	SI00035504	Hs_KPNB1_3	89%

red = used for single or triple knockdown, this study.

Supplementary Information Table 3. Plasmids generated for this study

For efficient generation of cell lines stably expressing fluorescently tagged marker proteins, the genes were subcloned into pIRES-puro2 and pIRES-neo3 vectors (Clontech) that allow expression of resistance genes and tagged proteins from a single transcript.

Table 3. Generated plasmids

Plasmid name	Tag	Source plasmids	Vector backbone	Backbone cloning sites
pIBB-mEGFP-IRES-puro2b	mEGFP ¹	pIBB-mEGFP ²	pIRES-puro2b	EcoRI/NotI
pGalT-GFP-IRES-puro2b	GFP	pGalT-GFP (MluI blunted/EcoRI) ³	pIRES-puro2b	(NotI blunted/EcoRI)
pEGFP-PCNA-IRES-puro2b	mEGFP ¹	pNLS-EGFP-PCNA ⁴	pIRES-puro2b	(NdeI, XbaI)

mEGFP indicates monomeric EGFP.

Supplementary Information Table 4. Monoclonal fluorescent reporter cell lines

Stable cell lines were generated as described in ⁵.

Table 4. HeLa Kyoto monoclonal cell lines

Background	Cell line name	Plasmid 1	Plasmid 2
HeLa ‘Kyoto’	H2B-mCherry	pH2B-mCherry-IRES-neo3	-
HeLa ‘Kyoto’	H2B-mCherry and IBB-mEGFP	pH2B-mCherry-IRES-neo3	pIBB-mEGFP-IRES-puro2b
HeLa ‘Kyoto’	H2B-mCherry and GalT-GFP	pH2B-mCherry-IRES-neo3	pGalT-GFP-IRES-puro2b
HeLa ‘Kyoto’	H2B-mCherry and PCNA-mEGFP	pH2B-mCherry-IRES-neo3	pPCNA-mEGFP-IRES-puro2b
HeLa ‘Kyoto’	H2B-mCherry and mEGFP-α-tubulin ⁶	pH2B-mCherry-IRES-neo3	pmEGFP-α-tubulin-IRES-puro2b

Supplementary references

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