Mall et al., http://www.jcb.org/cgi/content/full/jcb.201205103/DC1

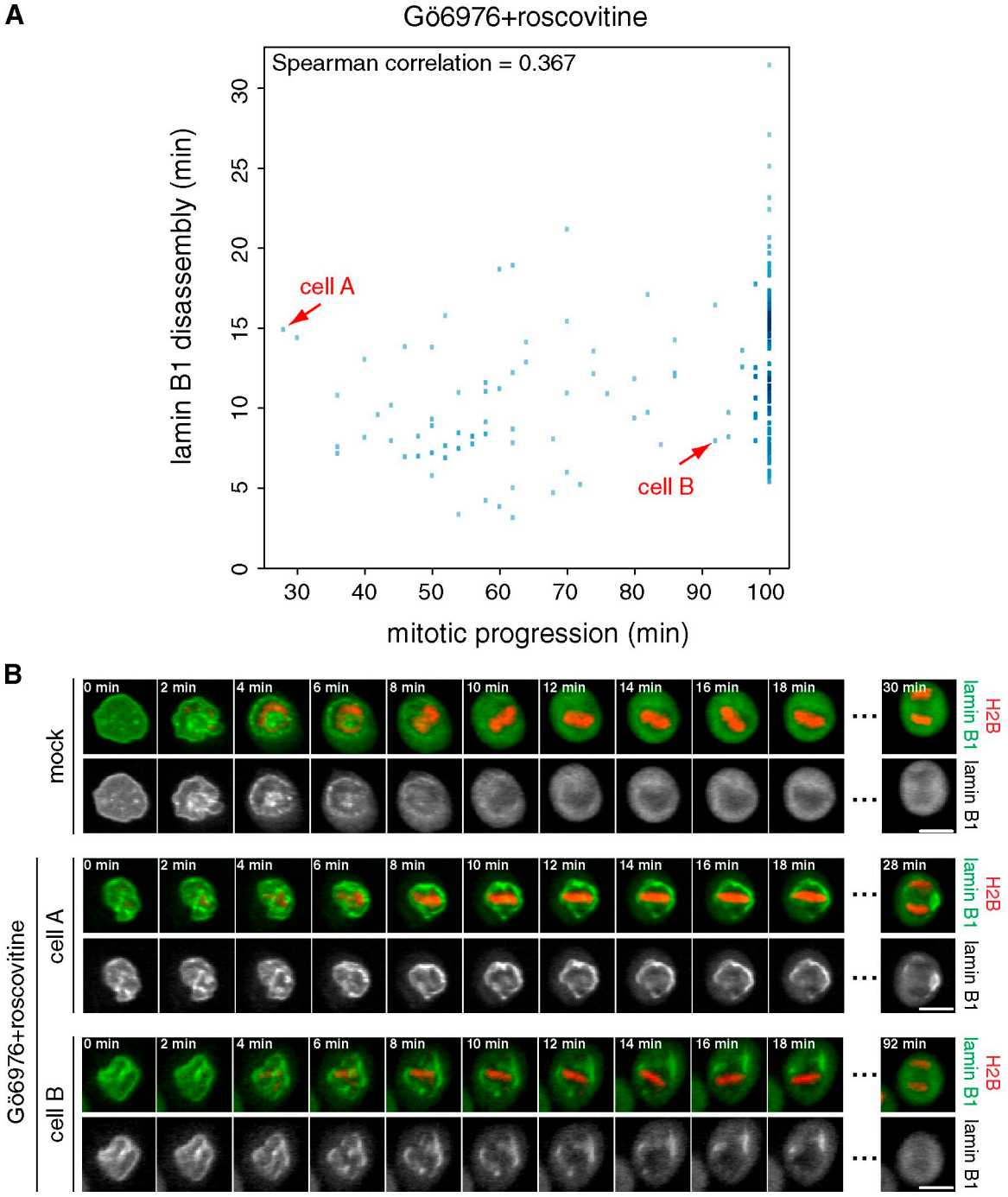


Figure S1. **Delay of lamin B1 disassembly and mitotic progression are uncoupled events in single cells.** (A) Lamin B1 disassembly duration and mitotic progression in individual cells from the cell population in Fig. 3 (C and D), synchronized by double thymidine arrest and treated with a combination of 2 µM Gö6976 and 50 µM roscovitine ~30–60 min before mitotic entry, were plotted, and their Spearman correlation was determined. Cells exceeding the quantitation cutoff for mitotic progression delay (>100 min) are plotted at 100 min. The data represent mitotic events from four replicate experiments. (B) Representative time-lapse microscopy images of mitotic HeLa cells stably coexpressing H2B-mCherry and GFP–lamin B1 (green and gray) after DMSO treatment (mock) and time-lapse microscopy images of indicated cells treated with a combination of 2 µM Gö6976 and 50 µM roscovitine. Mitotic entry as well as anaphase is shown relative to the onset of prophase, scored automatically by chromatin condensation, as time 0. Cell A shows an example of delayed lamin B1 disassembly but no delay in mitotic progression, and cell B shows the opposite phenotype. Bars, 10 µm.

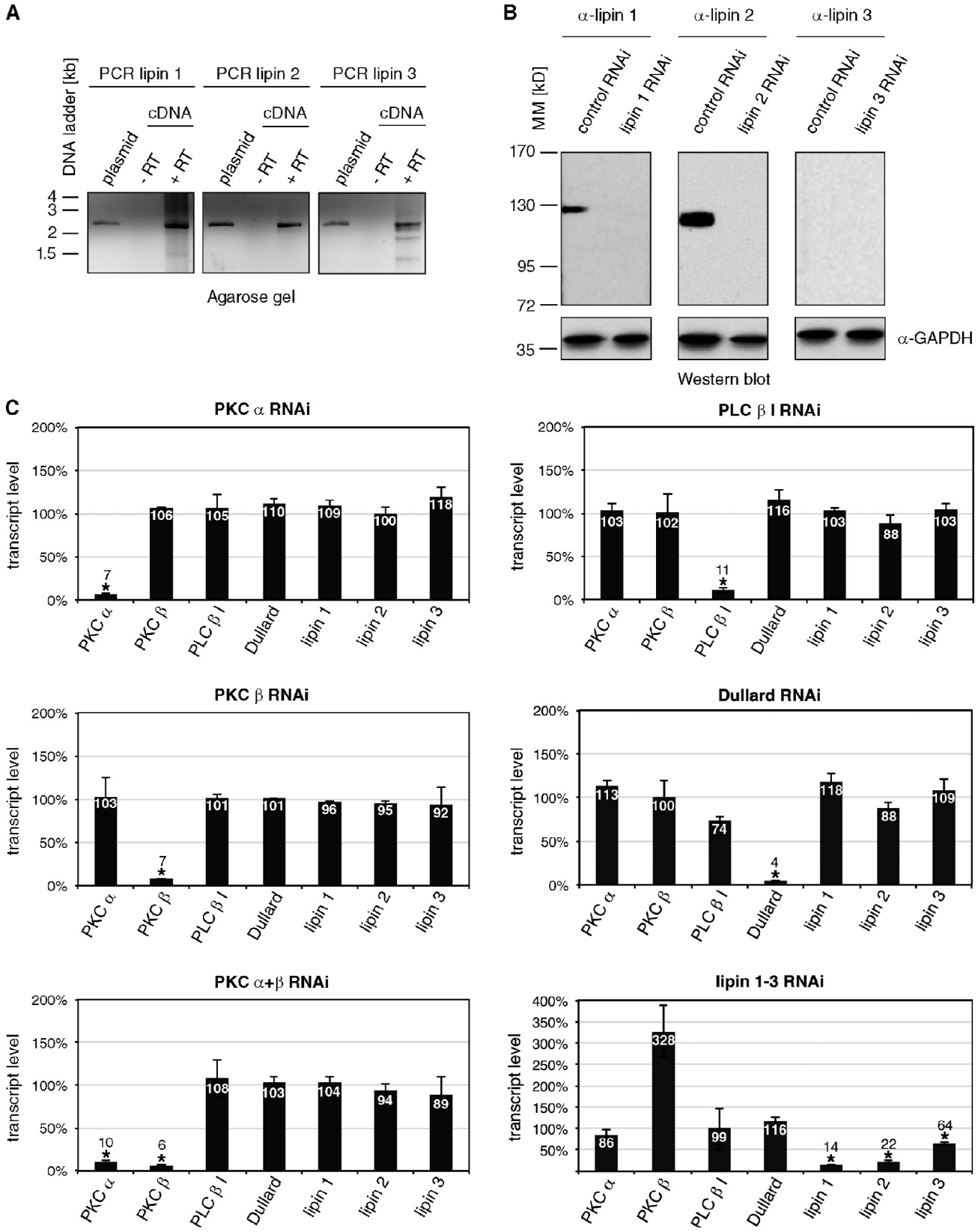


Figure S2. **Lipin 1–3 expression in HeLa Kyoto cells and validation of RNAi efficiency.** (A) PCR amplification of the denoted lipin isoforms from plasmids encoding the corresponding lipin cDNA or HeLa cDNA preparations generated with (+) or without (−) reverse transcription (RT). Sequencing of the PCR products confirmed expression of all three lipin isoforms (not depicted). (B) Western blot of HeLa whole-cell lysate after RNAi treatment using control oligos or oligos against the indicated lipin isoforms. Western blot detection was performed using antibodies raised against the denoted lipin isoforms, and GAPDH served as a loading control. (C) Expression levels of denoted transcripts in HeLa cells after the indicated RNAi treatment were determined by quantitative real-time PCR. Expression levels were normalized to control RNAi treatment and GAPDH expression. The results represent mean transcript levels from three replicate experiments. *, $P < 0.05$ (*t* test). Error bars indicate standard error of the mean. MM, molecular mass.

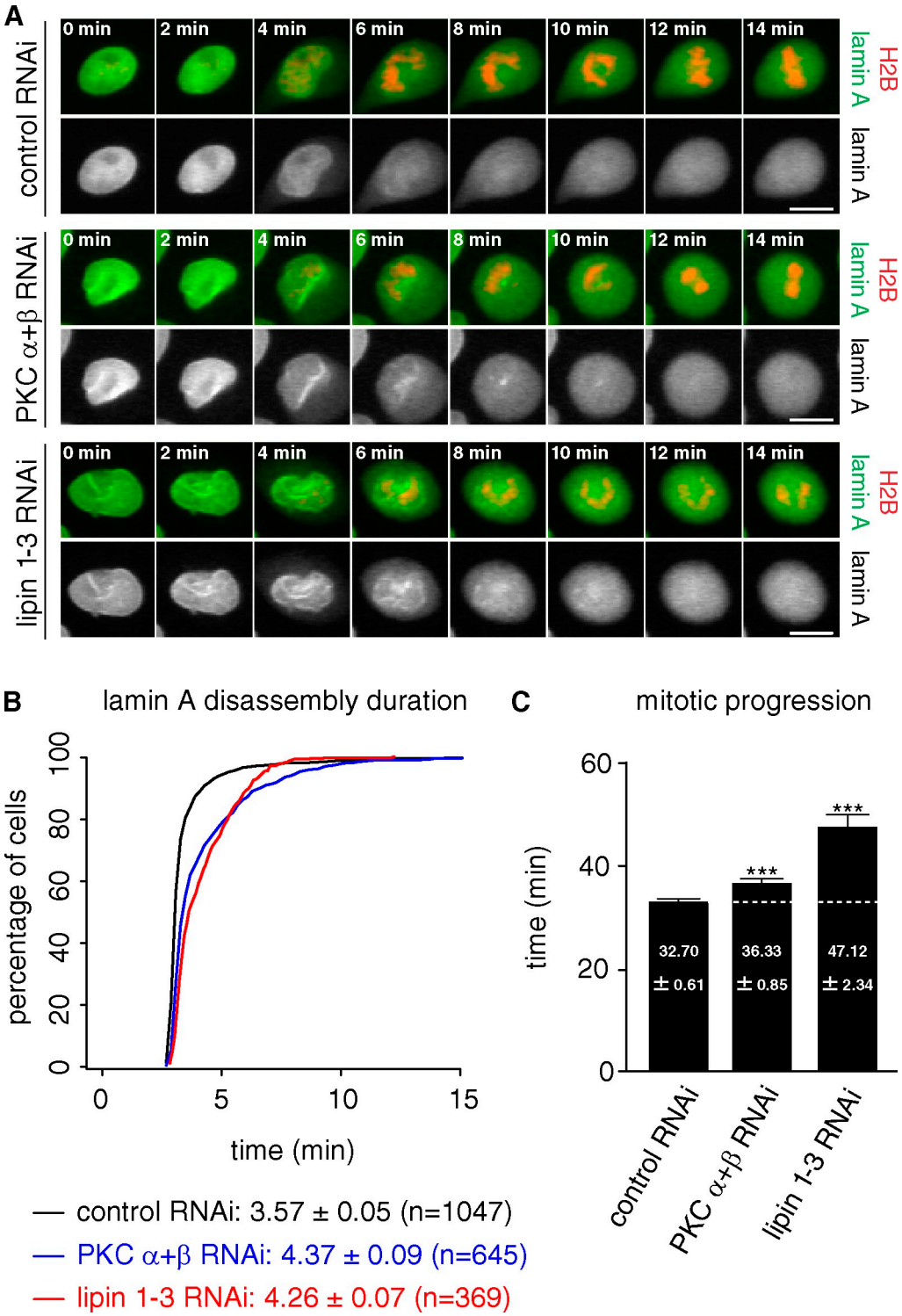
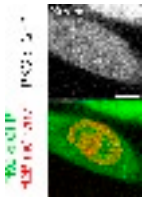
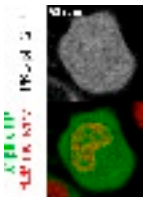


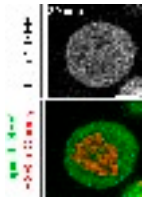
Figure S3. **cPKCs and lipins affect mitotic lamin A disassembly.** (A) Representative time-lapse microscopy images of mitotic HeLa cells stably coexpressing H2B-mCherry and GFP–lamin A (green and gray) after transfection with siRNA oligos against the indicated transcripts or control oligos. Progression through mitosis was followed, and time relative to chromatin condensation in prophase is shown. Bars, 10 µm. (B) Cumulative histograms of lamin A disassembly durations after the indicated RNAi treatment quantified as described in Fig. 2. $P < 0.0001$ (*U* test) in which PKC-α and -β or lipin 1–3 RNAi were compared with control RNAi. (C) Mean mitosis durations of cells quantified in B. The data represent the mitotic events from two replicate experiments, with ***, $P < 0.0001$ (*U* test). Dotted line indicates mean control timing. Error bars indicate 95% confidence intervals.



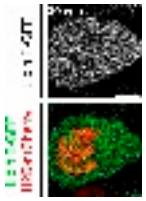
Video 1. **Localization of PKC-α during NEBD in mammalian cells.** HeLa cells stably expressing H2B-mCherry and transiently expressing PKC-α tagged with GFP (green and gray) at the C terminus during mitotic entry. Progression into mitosis was analyzed by time-lapse microscopy using a laser-scanning confocal microscope (LSM 510). Frames were taken every 30 s for 3 min, and time relative to NEBD is shown. Bar, 10 μm.



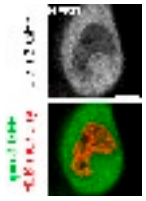
Video 2. **Localization of PKC-βII during NEBD in mammalian cells.** HeLa cells stably expressing H2B-mCherry and transiently expressing PKC-βII tagged with GFP (green and gray) at the C terminus during mitotic entry. Progression into mitosis was analyzed by time-lapse microscopy using a laser-scanning confocal microscope (LSM 510). Frames were taken every 30 s for 3 min, and time relative to NEBD is shown. Bar, 10 μm.



Video 3. **Localization of lipin 1 during NEBD in mammalian cells.** HeLa cells stably expressing H2B-mCherry and transiently expressing lipin 1 tagged with GFP (green and gray) at the C terminus during mitotic entry. Progression into mitosis was analyzed by time-lapse microscopy using a laser-scanning confocal microscope (LSM 510). Frames were taken every 30 s for 3 min, and time relative to NEBD is shown. Bar, 10 μm.



Video 4. **Localization of lipin 2 during NEBD in mammalian cells.** HeLa cells stably expressing H2B-mCherry and transiently expressing lipin 2 tagged with GFP (green and gray) at the C terminus during mitotic entry. Progression into mitosis was analyzed by time-lapse microscopy using a laser-scanning confocal microscope (LSM 510). Frames were taken every 30 s for 3 min, and time relative to NEBD is shown. Bar, 10 μm.



Video 5. **Localization of lipin 3 during NEBD in mammalian cells.** HeLa cells stably expressing H2B-mCherry and transiently expressing lipin 3 tagged with GFP (green and gray) at the C terminus during mitotic entry. Progression into mitosis was analyzed by time-lapse microscopy using a laser-scanning confocal microscope (LSM 510). Frames were taken every 30 s for 3 min, and time relative to NEBD is shown. Bar, 10 μm.

Table S1. **Quantification of lamin B1 disassembly and mitotic progression in cells stably expressing H2B-mCherry transfected with GFP–lamin B1**

Treatment	Lamin disassembly	P (*U* test)	Mitotic progression	P (*U* test)	Chromosome congression	P (*U* test)	*n*
	min ± CI		*min ± CI*		*min ± CI*		
Wild type	7.51 ± 0.87	NA	43.94 ± 2.44	NA	36.76 ± 2.44	NA	192
PKC mutant	7.17 ± 0.83	0.7106	45.20 ± 2.61	0.0491	35.76 ± 2.35	0.4964	157
CDK1 mutant	8.24 ± 0.79	0.0146	44.06 ± 2.38	0.1627	35.63 ± 2.23	0.5464	164
CDK1 + PKC mutant	8.86 ± 0.80	<0.0000	44.64 ± 2.59	0.1995	34.04 ± 1.92	0.9329	163

Lamin disassembly shows mean duration. Mitotic progression is mean duration from prophase to anaphase. Chromosome congression is mean duration of prometa- and metaphase. CI, confidence interval; NA, not available.

Table S2. **Quantification of lamin B1 disassembly and mitotic progression in cells stably coexpressing GFP–lamin B1 and H2B-mCherry**

Treatment	Lamin disassembly	P (*U* test)	Mitotic progression	P (*U* test)	Chromosome congression	P (*U* test)	*n*
	min ± CI		*min ± CI*		*min ± CI*		
Mock	6.24 ± 0.16	NA	27.33 ± 0.75	NA	24.96 ± 0.71	NA	829
Gö6976	8.10 ± 0.21	<0.0000	56.21 ± 1.82	<0.0000	51.92 ± 1.78	<0.0000	683
Roscovitine	8.07 ± 0.36	<0.0000	70.33 ± 2.49	<0.0000	63.43 ± 2.44	<0.0000	410
Gö6976 + roscovitine	11.95 ± 0.79	<0.0000	82.29 ± 3.62	<0.0000	77.66 ± 3.54	<0.0000	147
Mock	5.79 ± 0.19	NA	27.27 ± 1.04	NA	25.26 ± 1.00	NA	463
Propranolol	7.93 ± 0.66	<0.0000	49.32 ± 3.10	<0.0000	47.15 ± 3.00	<0.0000	228
U73122	5.81 ± 0.27	0.3955	33.06 ± 2.17	<0.0000	30.16 ± 2.04	<0.0000	213
Control RNAi	7.12 ± 0.15	NA	27.97 ± 0.61	NA	25.02 ± 0.56	NA	1,072
PKC-α RNAI	8.44 ± 0.20	<0.0000	39.44 ± 1.14	<0.0000	35.85 ± 1.11	<0.0000	808
PKC-β RNAi	8.46 ± 0.26	<0.0000	33.69 ± 0.99	<0.0000	29.93 ± 0.92	<0.0000	715
PKC-α + -β RNAI	7.93 ± 0.18	<0.0000	34.10 ± 0.99	<0.0000	29.79 ± 0.89	<0.0000	708
PLC-βI RNAI	7.27 ± 0.18	0.0784	30.39 ± 0.70	<0.0000	27.07 ± 0.65	<0.0000	925
Lipin 1–3 RNAi	9.14 ± 0.43	<0.0000	40.74 ± 2.41	<0.0000	38.39 ± 2.39	<0.0000	301
Dullard RNAi	8.02 ± 0.17	<0.0000	29.49 ± 0.78	<0.0000	27.11 ± 0.76	<0.0000	766
Control RNAi + mock	7.37 ± 0.27	NA	27.98 ± 1.17	NA	24.71 ± 1.09	NA	394
Control RNAi + DAG	6.93 ± 0.21	0.9993	28.47 ± 0.99	0.527	26.34 ± 0.94	0.0012	666
Lipin 1-3 RNAi + mock	9.02 ± 0.47	<0.0000	34.75 ± 2.85	<0.0000	32.21 ± 2.75	<0.0000	149
Lipin 1-3 RNAi + DAG	7.40 ± 0.30	0.2626	29.01 ± 1.46	0.0372	27.05 ± 1.35	<0.0000	268

Lamin disassembly shows mean duration. Mitotic progression is mean duration from prophase to anaphase. Chromosome congression is mean duration of prometa- and metaphase. CI, confidence interval; NA, not available.

Table S3. **DNA primers used for quantitative PCR, cloning, and mutagenesis**

PCR target	Forward primer 5′ → 3′	Reverse primer 5′ → 3′
GAPDH	CATGAGAAGTATGACAACAGCCT	AGTCCTTCCACGATACCAAAGT
Lipin 1	GCTGCCAAGCCATCAAAC	GGGGCCATTCTTCAACTTC
Lipin 2	ACCTTTTCACGTTCGGTTTG	TGCACTGCCGTTGATTTCTA
Lipin 3	GTCTTCAGCGTGACCACTCA	ACAGGTAGATGGTGGCCTTG
Dullard	CTCTGGAGCTTCTTCATTTACCTT	CACAGGAGATAAGGGGAGGA
PKC-α	TCGACTGGGAAAAACTGGAG	CTCTGCTCCTTTGCCACAC
PKC-β	GGCGAAATGCTGAAAACTTC	AGCTCTTGACTTCGGGTTTT
PLC-βI	TCGCACAACACCTACCTCAC	GCACTTGGCGATACATCTCA
pET28a-lipin 1 (aa 128–377)	GCGCGCTAGCATGAGAGGCCTGGAC	GCGCCTCGAGTTACTTGTTTGCTGTCTGG
pET28a-lipin 2 (aa 259–381)	GCGCGCTAGCGAGTCTCACATGGAG	GCGCCTCGAGTTATTTAGCTGCCGGTTTGG
pET28a-lipin 3 (aa 133–377)	GCGCGCTAGCATGGCAGGCACGGCC	GCGCCTCGAGTTACTGCCCGGTGGGAAC
pEFGP-N3-lipin 1 (aa 1–890)	GCGCAGATCTATGAATTACGTGGGG	GCGCGCCCGCGGTCGCTGAGGCAGAATGAATG
pEFGP-N3-lipin 2 (aa 1–896)	GCGCAGATCTATGAATTATGTGGGA	GCGCGCCCGCGGTAGACAGGTCATCCAG
pEFGP-N3-lipin 3 (aa 1–851)	GCGCAGATCTATGAACTACGTGGGG	GCGCGCCCGCGGTGTCCAGGGTATCAAGGTC
pEGFP-N3–PCK-α (aa 1–672)	GCGCGCTAGCATGGCTGACGTTTTC	GCGCCTCGAGATACTGCACTCTGTAAGATG
pEGFP-N3–PCK-βII (aa 1–673)	GCGCGCTAGCATGGCTGACCCGGCT	GCGCCTCGAGAGCTCTTGACTTCGGG
pEGFP-N3–lamin B1 (S23A)	CCACGCCGCTGGCCCCCACGCGCC	–
pEGFP-N3–lamin B1 (S391A,S393A)	AGAGAGGTTGAAGCTGGCTCCAGCCCCTTC TTCCCGTGTG	–
pEGFP-N3–lamin B1 (S395A)	CTGTCTCCAAGCCCTGCTTCCCGTGTGACAG	–
pEGFP-N3–lamin B1 (S405A)	AGTATCCCGAGCATCCGCAAGTCGTAGTG TACG	–

No reverse primers were used for the mutagenesis, indicated by dashes.