Supplemental Tables:

Vector Backbone	RM mutant constructs	Insert length (bp)	Insert length (aa)	Predicted MW without tag (kD)
pEGFP-C1	RAXA	3069	1023	113
pEGFP-C1	1-135	405	135	15
pEGFP-/mCh-C1	1-305	915	305	33
pEGFP-/mCh-C1	1-496	1488	496	54
pEGFP-C1	1-591	1773	591	65
pEGFP-C1	135-305	510	170	19
pEGFP-C1	305-496	573	191	21
pEGFP-/mCh-C1	305-713	1224	408	46
pEGFP-/mCh-C1	496-591	285	95	11
pEGFP-/mCh-C1	496-713 (stop codon inserted)	1581	217	24
pEGFP-/mCh-C1	496-1023	1581	527	59
pEGFP-C1	591-1023	1296	432	48
pEGFP-C1	713-946	699	233	26
pEGFP-/mCh-C1	713-1023	930	310	35

Supplemental Table S1. RepoMan truncation mutants generated

Antigen	Source		Clonality	Species	Dilution (WB)
GFP	Roche	11814460001	2 monoclonals	mouse	1:1000
RFP	chromotek	5f8	monoclonal	rat	1:1000
RepoMan	Abcam	ab45129	polyclonal	rabbit	1:1000
PP1γ	Santa Cruz	sc-6108	polyclonal	goat	1:1000
pan-PP1 (sold as α-PP1β)	Millipore	07-1217	polyclonal	rabbit	1:1000
KPNA2	Abcam	ab6036	polyclonal	goat	1:1000
KPNB1	Thermo Scientific	MA3-070	monoclonal	mouse	1:1000
KPNA4	Bethyl Laboratories	A301-627A	polyclonal	rabbit	1:1000
IPO7	Abnova	H00010527-M07	monoclonal	mouse	1:1000
NUP153	Bethyl Laboratories	A301-789A	polyclonal	rabbit	1:1000
MAb414	Covance	MMS-120P	monoclonal	mouse	1:1000
PP2A/R1A	Cell Signaling	#2260	monoclonal	rat	1:250
PP2A	Cell Signaling	#2259	monoclonal	rabbit	1:250
PP2A/R5C (B56γ)	Santa Cruz	sc-67038	polyclonal	rabbit	1:250
RPL7	Abcam	ab72550	polyclonal	rabbit	1:1000
RPS6	Lifespan Biosciences	LS-C117710/31360	polyclonal	rabbit	1:1000
Nucleolin	Abcam	ab22758	polyclonal	rabbit	1:1000
14-3-3ζ	Cell Signaling	#9639	polyclonal	rabbit	1:250
pan-14-3-3	Santa Cruz	sc-629	polyclonal	rabbit	1:250
PRKDC	Proteintech group	19983-1-AP	polyclonal	rabbit	1:500
Histone H3	Abcam	ab1791	polyclonal	rabbit	1:1000
Caprin1	Proteintech group	15112-1-AP	polyclonal	rabbit	1:500
G3BP2	Abcam	ab86135	polyclonal	rabbit	1:500
hn RNP K/J	Sigma	R8903	monoclonal	mouse	1:499
hn RNP A1	Abcam	ab5832	monoclonal	mouse	1:500
Nucleolin	Abcam	ab22758	polyclonal	rabbit	1:1000
Lamin A/C	Santa Cruz	sc-7292	monoclonal	mouse	1:500
Tubulin	Sigma	T 6199	monoclonal	mouse	1:1000
Nucleophosmin/B23	Sigma	B 0556	monoclonal	mouse	1:1000
A190	J. Zomerdijk	gift	polyclonal	sheep	1:250
PRMT1	Sigma	P1620	monoclonal	mouse	1:1000
PRMT5	Sigma	P0493	monoclonal	mouse	1:1000
SmB	Abcam	ab3138	monoclonal	mouse	1:1000
CKIIa	Abcam	ab75309	polyclonal	rabbit	1:250
NSEP1	Aviva Systems Biology	ARP34395_P050	polyclonal	rabbit	1:1000
Histone H1.X	Abcam	ab31972	polyclonal	rabbit	1:500
XPOT/Crm1	Santa Cruz	sc-74454	monoclonal	mouse	1:100
P16INK4a/CDKN2A	R&D Systems	AF5779	polyclonal	goat	1:250
PARP	Cell Signaling	#9542	polyclonal	rabbit	1:1000

Supplemental Table S2. Antibodies used for immunoblotting and immunoprecipitation

A

	Run 1				Run 2			
Protein	# peps	Ratio M:L	Sig B, p<0.05	Ratio H:L	# peps	Ratio M:L	Sig B, p<0.05	Ratio H:L
RepoMan(1-496)	22	32.4	1.78E-71	0.77	25	21.27	2.43E-57	0.77
PP1β	6	27.77	9.02E-46	0.71	6	10.67	2.14E-37	0.49
KPNA2	14	13.86	1.08E-44	0.65	12	14.93	2.83E-47	0.64
PP1γ	2	19.06	1.17E-44	0.79	2	17.09	1.12E-36	0.32
KPNB1	23	13.76	1.67E-44	0.66	20	8.98	1.13E-35	0.6
PP1α	4	10.37	1.51E-37	0.74	4	8.22	1.12E-36	0.54
KPNA4	2	8.64	4.93E-33	0.9	1	8.89	2.25E-35	NQ
KPNA1	3	5.71	2.69E-24	0.92	ND			
NUP153	7	4.53	5.23E-20	0.71	3	3.14	7.05E-15	0.26
IPO7	2	1.61	2.94E-06	2.01	2	1.98	1.11E-18	2.22
NUP50	1	NQ		NQ	3	5.55	4.55E-06	0.4

B

	Run 1				Run 2			
Protein	# peps	Ratio M:L	Ratio H:L	Sig B, p<0.05	# peps	Ratio M:L	Ratio H:L	Sig B, p<0.05
RepoMan(496-1023)	12	0.66	36.50	7.14E-07	15	0.66	17.91	1.59E-05
PPP2R5C / B56γ	2	0.37	9.15	0.00151	2	0.65	5.65	0.00177
PPP2R1A	10	1.07	6.39	0.00687	9	0.84	6.13	0.00113
RPL10A	5	0.51	4.36	0.02770	3	0.50	3.94	0.00860
RPL12	2	0.54	4.28	0.02937	3	0.56	3.99	0.01616
RPLP0	2	0.38	4.08	0.03447	3	0.41	4.00	0.00959
RPL6	2	0.60	4.02	0.03615	3	0.39	3.20	0.00610
RPL27	2	0.54	4.01	0.03625	2	0.66	4.09	0.00885
RPL7A	4	0.59	3.90	0.03975	6	0.54	3.81	0.01015
RPL8	2	0.59	3.86	0.04087	3	0.61	3.66	0.01459
RPS8	2	0.38	3.75	0.04505	3	0.44	4.19	0.01122
RPLP2	2	0.59	3.64	0.04903	3	0.58	4.18	0.00677
RPL5	4	0.34	3.61		3	0.46	3.48	0.01616
RPS19	2	0.39	3.58		3	0.33	3.70	0.01381
RPL28	3	0.56	3.51		1	NQ	NQ	
RPL7	2	0.59	3.48		6	0.55	4.26	0.00729
RPL13A	3	0.63	3.40		3	0.47	4.10	
RPS18	3	0.38	3.39		5	0.34	3.18	0.03252
RPS10L	2	0.26	3.38		2	0.35	3.25	0.03379
RPS24	3	0.29	3.21		3	0.43	3.77	0.01281
RPL13	2	1.09	3.05		2	0.60	3.09	0.02924
NSEP1/YBX1	3	0.40	2.92		5	NQ	NQ	
PPP2CA	4	0.90	2.90		3	0.87	2.66	
RPL10	2	0.60	2.88		ND			
RPS3	5	0.47	2.78		7	0.50	2.46	
NCL	7	0.62	2.74		7	0.64	3.02	0.03209
RPL11	2	0.47	2.74		2	0.59	2.79	0.04368
RPS16	7	0.47	2.69		8	0.40	2.64	
RPL26	3	0.36	2.54		1	0.35	2.46	
RPS20	2	0.61	2.39		3	0.50	2.01	
RPS12	2	0.48	2.38		2	0.48	2.46	
RPS2	4	0.51	2.37		4	0.41	2.17	
NEXN	3	0.22	2.23		2	0.19	1.81	
IPO7	2	1.62	2.02		2	1.98	2.22	
DDX21	2	0.01	2.00		4	0.55	1.79	
RPL19	2	0.69	2.00		ND			
CDKN2	2	0.76	1.98		ND			
ACTN4	43	0.15	1.92		39	0.15	1.88	
PRMT5	3	0.70	1.75		ND			
RPS11	2	0.43	1.74		ND			
RPL31	3	0.54	1.73		ND			
C19orf21	4	0.16	1.68		2	0.15	1.47	
DBN1	8	0.25	1.66		5	0.14	1.60	
NPM	4	0.50	1.59		4	0.45	1.40	
PAB1	7	0.42	1.56		9	0.39	1.51	
ACTN1	27	0.17	1.56		24	0.16	1.52	

Supplemental Table S3. N- (1-496, M) and C- (496-1023, H) terminal-specific RepoMan interactors. Proteins identified in the quantitative mass spectrometry experiment by 2 or more peptides and with SILAC ratios > 1.5 are shown here (full datasets provided as Supplemental Datasets). A.Proteins enriched with GFP-RepoMan/1-496. B. Proteins enriched with GFP-RepoMan/496-1023. Runs 1 and 2 indicate technical replicates of the SILAC IP experiment. Significance B values indicate variance from mean log ratios for a chosen confidence threshold value ($p < 0.05$). Proteins in bold were validated as RepoMan interactors by co-IP/WB analysis. Protein classes highlighted: RepoMan (dark green), PP1 (light green), import factors (yellow), PP2A complex members (orange), RPLs (dark pink) and RPSs (light pink). ND: not detected in this run. NQ: not quantified in this run.

	Run 1				Run 2			
Protein	**# peps**	**Ratio M:L**	**Sig B, p<0.05**	**Ratio H:L**	**# peps**	**Ratio M:L**	**Sig B, p<0.05**	**Ratio H:L**
RepoMan (496-713)	**10**	**21.89**	**0.049**	0.85	**11**	**23.56**	**0.041**	1.62
PPP2R5E/ B56ε	8	17.66		0.35	10	10.88		0.47
PPP2R1A	22	16.32		0.51	25	15.27		0.59
PPP2R5C/ B56γ	**14**	**14.65**		**0.37**	**16**	**8.85**		**0.51**
CK2A1	4	14.61		0.57	5	9.70		0.59
CK2A2	7	11.71		0.45	7	5.59		0.69
PPP2CA	**10**	**10.96**		**0.59**	**7**	**14.43**		**0.49**
PPM1C	3	10.31		0.62	8	7.51		0.75
IPO7	**14**	**9.97**		**1.13**	**16**	**9.14**		**0.90**
RPS10	5	9.94		0.68	6	7.64		0.56
MEP50	2	9.92		2.38	1	NQ		NQ
RPS18	5	9.29		0.57	5	7.35		0.54
RPL1	7	9.05		0.88	11	7.65		0.85
MRPS23	2	8.94		NQ	ND			
RPLP2	6	8.78		0.78	6	8.05		0.81
RPS25	4	8.52		0.56	3	8.35		0.56
UBE20	7	8.48		0.49	8	4.51		0.61
RPL7	**10**	**8.26**		**0.83**	**14**	**8.55**		**0.78**
RPL6	5	8.24		0.70	7	7.80		0.81
RPL14	4	8.20		0.79	4	7.55		0.85
RPL13A	2	7.88		0.56	2	3.00		0.46
RPS29	1	7.87		0.53	2	5.47		0.51
NCL	**21**	**7.82**		**0.82**	**21**	**7.29**		**0.82**
RPL10A	6	7.77		0.86	7	6.65		0.82
RPS19	3	7.68		0.67	5	7.60		0.60
NAP1L1	4	7.63		0.53	4	6.41		0.70
RPLP0	8	7.58		0.82	8	6.70		0.70
RPL30	3	7.50		0.77	3	7.98		1.04
SIR2L1	7	7.48		0.49	9	4.36		0.39
RPS16	7	7.40		0.61	9	7.70		0.59
RPL11	2	7.25		0.98	2	6.62		0.86
RPS12	5	7.24		0.63	6	6.43		0.68
RPL12	6	7.22		0.74	6	7.39		0.73
RPL18	5	7.15		0.66	6	7.43		0.80
RPL27	**4**	**7.15**		**0.70**	**7**	**8.00**		**0.77**
KPNB2	6	7.05		1.80	5	4.56		2.22
RPL7A	8	6.94		0.83	11	7.83		0.92
C18orf25	2	6.92		0.20	1	NQ		NQ
PPP2R5A/ B56α	3	6.87		0.89	6	4.82		0.50
RPL31	2	6.87		1.24	2	NQ		NQ
RPL8	3	6.84		0.65	3	9.84		0.83
RPS20	4	6.81		0.55	3	7.06		0.54
RPS3	17	6.80		0.68	13	6.41		0.59
PRMT5	5	6.74		1.37	7	6.23		1.29
RPL13	6	6.74		0.76	5	7.02		0.74
RPL36	3	6.66		0.28	4	5.07		0.66
RPS5	3	6.58		0.50	4	4.88		0.65
RPL21	4	6.58		0.59	5	8.77		0.81
RPL3	6	6.57		0.69	8	6.17		0.80
RPL26	2	6.36		0.87	3	5.67		0.74
NSEP1	4	6.29		0.66	6	5.79		0.66
RPS3A	5	6.26		0.89	6	6.00		0.79
RPS9	8	6.23		0.74	8	5.72		0.79
RPS8	4	6.23		0.67	4	6.73		0.61
RPS6	**6**	**6.08**		**0.89**	**3**	**5.49**		**0.79**
RPS24	2	6.04		0.72	2	6.11		0.70
RPL15	3	5.90		0.13	4	5.39		0.58
PPP2R5D/ B56δ	7	5.87		0.98	9	5.14		0.57
CSDA	3	5.84		0.75	5	NQ		NQ
RPL23A	2	5.82		0.83	6	5.65		0.86
RPL35	2	5.80		0.83	4	5.41		0.77
H1FX	2	5.80		0.75	1	NQ		NQ
NPM	**6**	**5.75**		0.93	**4**	**5.77**		0.98
RPS2	6	5.60		0.62	7	5.76		0.64
RPL9	8	5.51		0.78	6	5.59		0.79
C1QBP	2	5.39		1.07	2	4.97		0.93
RPL5	**6**	**5.19**		**0.83**	**6**	**6.32**		0.88
AHCYL2	2	5.14		1.09	ND			
RPSA	7	5.07		0.58	9	4.39		0.45

Supplemental Table S4. RepoMan interactors specific to the 496-713 domain. Proteins identified in the quantitative mass spectrometry experiment by 2 or more peptides and with SILAC ratios > 5 are shown here (full datasets provided as Supplemental Datasets). Significance B values indicate variance from mean log ratios for a chosen confidence threshold value ($p < 0.05$). The shift of such a larger number of proteins from the mean affects significance calculations, with only the RepoMan fragment itself appearing to be significantly enriched above background. Runs 1 and 2 indicate technical replicates of the SILAC IP experiment. Proteins in bold were validated as RepoMan interactors by co-IP/WB analysis. Protein classes highlighted: RepoMan (green), import factors (yellow), PP2A complex members (orange), RPLs (dark pink), RPSs (light pink) and histones (turquoise). ND: not detected in this run. NQ: not quantified in this run.

Protein	Run 1				Run 2			
	# peps	Ratio M:L	Ratio H:L	Sig B, p<0.05	# peps	Ratio M:L	Ratio H:L	Sig B, p<0.05
RepoMan (713-1023)	**21**	2.05	**38.98**	**1.50E-08**	**21**	1.48	**28.58**	**3.21E-12**
YWHAG / 14-3-3γ	6	0.71	10.46	0.0003	6	0.64	10.28	1.49E-08
YWHAE / 14-3-3ε	11	0.76	10.27	0.0003	10	0.81	9.55	1.43E-07
YWHAZ / 14-3-3ζ	**8**	0.83	**9.36**	**0.0005**	**7**	0.79	**8.52**	**4.47E-07**
YWHAQ / 14-3-3θ	5	0.86	8.19	0.0011	4	0.75	8.66	3.86E-07
MSH6	4	1.12	5.81	0.0060	ND			
TUBB2C	23	1.17	5.79	0.0061	24	1.13	5.66	4.09E+05
COPB	2	1.01	5.48	0.0079	1	0.31	3.54	2.09E-03
TUBA1	15	1.05	5.44	0.0082	16	0.86	5.14	1.06E-03
TRIP13	4	0.81	5.33	0.0090	5	0.83	4.40	3.62E-03
TUBB	22	1.06	5.26	0.0095	24	1.15	5.63	3.80E-05
GLUT1	3	0.94	4.62	0.0167	1	NQ	NQ	
TUBB4	18	1.15	4.60	0.0169	20	1.11	3.56	7.54E-04
MCT1	2	1.16	4.54	0.0179	ND			
TUBA1B	17	0.88	4.41	0.0201	16	0.92	4.40	3.90E-04
DFFRX	2	1.93	4.25	0.0234	ND			
SLC25A3	2	1.30	4.23	0.0238	1	NQ	NQ	
DNAJ2	2	1.00	3.98	0.0302	2	0.76	4.20	0.0003
SURF4	2	1.97	3.91	0.0325	1	NQ	NQ	
XPOT	2	1.32	3.76	0.0374	ND			
PRKDC	**56**	1.23	**3.73**	0.0387	**37**	1.06	**3.85**	0.0010
SLC25A5	6	0.98	3.71	0.0397	8	0.94	3.51	0.0022
CAND1	8	4.14	3.66	0.0416	4	2.88	2.04	
TUBB2	19	0.82	3.54	0.0471	ND			
DHC1	11	0.85	3.52	0.0479	4	0.63	2.63	0.0091
MCM6	2	1.43	3.44		1	NQ	NQ	
CTPS	3	1.23	3.44		2	NQ	NQ	
ATAD3A	5	0.75	3.42		1	NQ		
C17orf27	3	0.24	3.36		3	NQ	3.66	
NUP160	6	1.24	3.35		ND			
CPS1	8	1.10	3.34		2	0.90	3.34	
PFKF	4	0.95	3.24		1	NQ	NQ	
ACAC	8	0.83	3.20		1	NQ	NQ	
SLC25A13	5	0.95	3.20		2	0.88	3.45	
SLC25A6	6	0.95	2.98		6	1.13	3.32	
IARS	6	0.96	2.92		3	0.92	2.99	
COPG	3	0.79	2.91		1	NQ	NQ	
CAD	17	0.69	2.87		9	0.68	2.95	
NSUN2	2	1.25	2.79		1	NQ	NQ	
SLC3A2	2	1.08	2.70		4	0.98	2.48	
ATP5A	6	0.89	2.59		5	1.01	2.71	
RPL23	3	1.80	2.59		2	1.81	2.96	
TKT	9	0.94	2.57		7	0.93	2.48	
XPO1	2	0.05	2.53		4	0.94	2.92	
GCN1L1	5	0.83	2.49		1	NQ	NQ	
EIF3B	2	1.42	2.44		1	NQ	NQ	
HKMT1069	2	9.92	2.38		1	NQ	NQ	
CCT1	3	1.02	2.36		2	0.89	1.81	
PGDH3	3	0.96	2.24		3	0.89	2.16	
RPS15A	5	1.87	2.22		4	2.01	2.01	
SLC20A4	3	0.74	2.19		1	0.56	1.77	
CDC47	5	0.81	2.15		5	0.66	1.99	
BAP	5	1.12	2.09		1	0.56	1.77	
PHB	4	1.13	2.07		3	0.86	1.64	
CBP1	2	0.91	2.01		1	NQ	NQ	
PPP2R2A	3	4.19	2.00		2	3.59	1.36	

Supplemental Table S5. RepoMan interactors specific to the 713-1023 domain. Proteins identified in the quantitative mass spectrometry experiment by 2 or more peptides and with

SILAC ratios > 2 are shown here (full datasets provided as Supplemental Datasets). Significance B values indicate variance from mean log ratios for a chosen confidence threshold value ($p < 0.05$). Runs 1 and 2 indicate technical replicates of the SILAC IP experiment. Proteins in bold were validated as RepoMan interactors by co-IP/WB analysis. Protein classes highlighted: RepoMan (green), import factors (yellow), PP2A complex members (orange), RPLs (dark pink), RPSs (light pink) and DNA damage response-related proteins (blue). ND: not detected in this run. NQ: not quantified in this run.

Supplemental Figure Legends

Supplementary Figure 1. Truncation mutant dynamics. A. FLIP (Fluorescence Loss In Photobleaching) experiment demonstrating that cleavage of RepoMan at residue 591 (into 1-591 and 591-1023 fragments) results in a C-terminal fragment (591-1023) that is not retained efficiently in the nucleus. A cytoplasmic ROI was continually photobleached every 1 second for a total of 126 seconds, with images taken after each bleach event. The intensity over time of a nuclear region of interest is normalized for any photobleaching due to image acquisition and plotted. Data are mean ± SE (n = 4-7, 3 separate experiments). GFP alone (shuttles freely) and full-length GFP-tagged RepoMan (does not shuttle) are shown for comparison. B. FRAP (Fluorescence Recovery After Photobleaching) experiment comparing the turnover rate of GFP-tagged fragments 1-591 (green) and 591-1023 (purple) to that of full-length GFP-tagged RepoMan (red) and free GFP (blue). A pre-bleach image was acquired, a nucleoplasmic ROI photobleached at the 0 time point and the GFP signal within that ROI monitored over time. C. FRAP experiment, carried out as described above, comparing the turnover rate of the 1-305 (green) and 305-713 (purple) and 713-1023 (turquoise) GFP-RepoMan fragments to that of the full-length protein (red) and free GFP (blue). D. FRAP experiment comparing the turnover rate of the N-terminal 1-305 (green), 1-496 (purple) and 1-591 (turquoise) GFP-RepoMan fragments to that of the full-length protein (red) and free GFP (blue), focusing on the early post-bleach time points. For all FRAP experiments shown here, data are mean ± SE (n = 7-31, 3 separate experiments).

Supplementary Figure 2. Transient overexpression of GFP-RepoMan (or fragments used for interactome study) has no effect on cell cycle distribution. A. FACS analysis of HeLa

cells 24 hours after Effectene-based transfection with pEGFP, pEGFP-RepoMan, pEGFP-RepoMan/RAXA or the 4 RepoMan truncation mutants used in the fragmentome experiments (1-496, 496-1023, 496-713 and 713-1023). Non-transfected cells are shown for comparison. B. FACS analysis of HeLa cells 24 hours after Polyethylenimine (PEI)-based transfection with pEGFP, pEGFP-RepoMan, pEGFP-RepoMan/RAXA or the 4 RepoMan truncation mutants used for the fragmentome experiments (1-496, 496-1023, 496-713 and 713-1023). Non-transfected cells are shown for comparison. C. Table summarizing the cell cycle distributions (between G1, S and G2/M) for each condition. D. Equivalent total protein amounts of cell lysates were analyzed by Western blot, using anti-GFP antibodies, to demonstrate the relative amounts of fusion protein expressed. Transfection efficiencies ranged from 40-80%.

Supplementary Figure 3. Fragmentome approach increases sensitivity of detection of interactors. A. Peptides detected in the SILAC IP experiment comparing mCh-RepoMan to the non-PP1 binding mutant mCh-RepoMan/RAXA are highlighted in purple, with sequence coverage and normalized intensity (summed peptide intensity/MW of RepoMan * $1e^6$) noted below. B. Peptides detected in the fragmentome experiment comparing the N-terminal (1-496, orange) and C-terminal (496-1023, green) halves of RepoMan. Sequence coverage and normalized intensity (summed intensity for all RepoMan peptides detected/MW of RepoMan * $1e^6$) noted below. C. Distribution of protein classes between N- and C-terminal halves of RepoMan. Relative abundance (summed peptide intensities for each protein normalized to molecular weight) vs. log H:M (enrichment with FP-RepoMan/1-496 vs. FP-RepoMan/496-1023) was plotted and the main protein classes of RepoMan interactors identified in our interactome screens highlighted, as indicated in the legend.

Supplementary Figure 4. Subcellular distribution and additional validation experiments. A. Fractionation of HeLa cells and analysis of cell equivalent volumes of CP (cytoplasmic), NP (nucleoplasmic) and No (nucleolar) extracts by WB using anti-RepoMan (RM) antibodies and antibodies raised against cell compartment-specific proteins (CP, alpha-tubulin; NP, Lamin A/C; No, RNA Pol I subunit A190). B. Fractionation of HeLa cells into cytoplasmic (CP) and nuclear (NP) extracts for analysis of RepoMan and PP2Ac/R1A/B56γ complex member distribution between these cellular compartments. In the top right panel, HeLa cells transiently expressing FP-RepoMan were fractionated and analyzed by WB using anti-RepoMan antibodies, to compare cellular distribution of FP-tagged and endogenous protein. C. Fractionation of HeLa cells transiently expressing either FP-tagged RepoMan fragment 1-496 or 496-1023 and analysis of cell equivalent volumes of cytoplasmic (CP) and nuclear (NP) extracts using anti-FP antibodies to detect the fusion proteins (and the indicated cell compartment-specific markers). D. Validation of the co-purification of endogenous RepoMan with the FP-tagged importins KPNA2 and KPNB1. E. Validation of the co-purification of both endogenous RepoMan and G3BP2 with FP-tagged Caprin. F. Demonstration of the C-terminal domain-specificity of the interaction between RepoMan and Caprin (496-713), histone H3 (713-1023) and hnRNP A1 (496-713). An equal enrichment of hnRNP K was observed with both fragments (496-713 and 713-1023). G. Co-IP/WB experiment testing whether the phosphorylation status of amino acid S591 in RepoMan affects interaction with the PP2Ac/R1A/B56γ complex. Western blot analysis was carried out using the antibodies indicated. Anti-FP antibodies were used to demonstrate the amount of fusion protein (or free FP) recovered in each IP.

Supplementary Figure 5. Additional validation and domain-specific mapping of ribosomal protein and histone H3 interaction with RepoMan. A. Western blot demonstrating mapping of the interaction of the 496-591 domain of RepoMan with RPL7 and nucleolin (NCL). Control IPs of FP alone and FP fused to a nuclear localization signal (FP-NLS) are shown for comparison. B. Summary of the co-purification of RPL7, RPS6 and NCL with FP-RepoMan vs. FP alone, as measured by quantitative Western blot analysis (n = 4 independent experiments). The bars indicate enrichment of each protein normalized to the amount of fusion protein recovered. Asterisks indicate significant enrichment of these interactors above the control FP IP ($p < 0.05$ in an unpaired Student's t-test). C. The 496-591 fragment of RM is predominantly nuclear and shows additional accumulation in nucleoli, as shown here by fluorescence imaging of PFA-fixed HeLa cells transiently expressing GFP-RepoMan/496-591 and stained with antibodies raised against NCL, a marker for the nucleolus (arrow). Scale bar is 5 μM. WB analysis of nuclei purified from these cells and fractionated into separate nucleoplasmic (NP) and nucleolar (No) extracts demonstrates the distribution of the 496-591 fragment between the nucleoplasm and nucleoli. In the left panel, anti-Lamin A/C (nuclear envelope marker) and anti-nucleophosmin (NPM, nucleolar marker) antibodies demonstrate the purity of the fractions. Anti-FP detects FP-RepoMan/496-591 in both, albeit with the majority of the fusion protein found in the nucleoplasm. The panel on the right shows that endogenous RPL7 co-purifies predominantly with the nucleoplasmic pool of FP-RepoMan/496-591. A control IP of FP alone from nuclear extracts is shown for comparison. D. In the FLIP experiment shown here, the same pool of nucleolar GFP-RM was repeatedly photobleached every second (arrows) for a total of 126 seconds, with images taken after each bleach event. The intensity over time for three different nuclear regions of interest (dark gray squares, neighbouring nucleoplasm; gray circles, other

nucleolus; pale gray triangles, remote area of nucleoplasm) was normalized for photobleaching due to image acquisition and plotted. Data are mean ± SE (n = 4-7, 2 separate experiments). Decreasing fluorescence indicates loss of GFP-RepoMan signal as it continuously shuttles through the nucleolus and is photobleached. E. Western blot demonstrating the domain-specific enrichment (496-1023 and 713-1023) of histone H3 with RepoMan. F. Summary of the co-purification of histone H3 with RepoMan and RepoMan fragments as measured by quantitative Western blot analysis (n = 3-4 independent experiments). The bars indicate enrichment of H3 with the GFP fusion proteins relative to the amount enriched in a control GFP-alone IP, with the dashed line at 1 indicating the same enrichment with both. Asterisks indicate significant enrichment of histone H3 above the control IP ($p < 0.05$ in an unpaired Student's t-test).

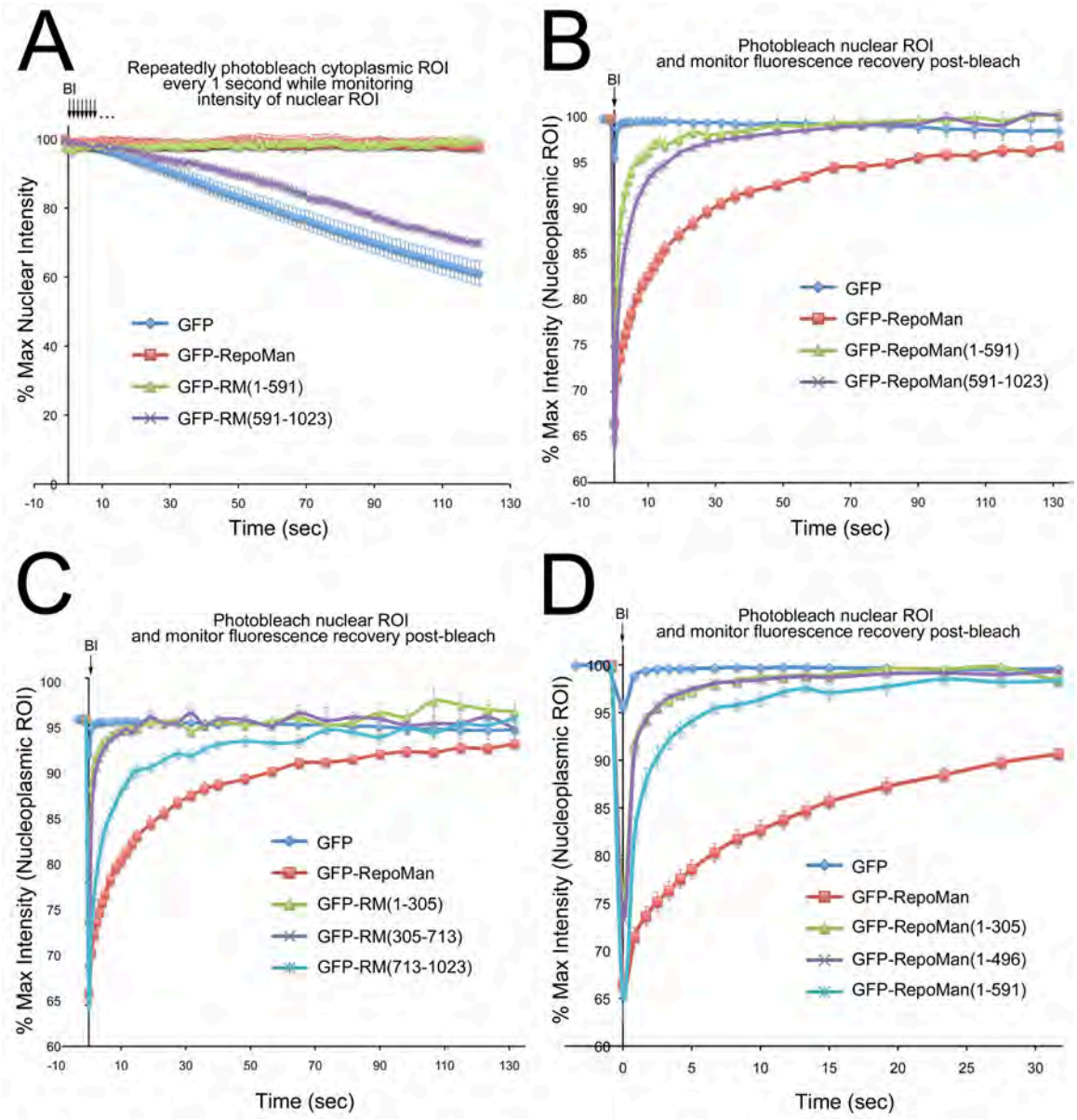
Supplementary Figure 6. Full Western blots for data shown in Figures 1 and 4. Lines indicate where lanes not presented in the figure were omitted, while dashed lines indicate where a Western blot was cut so that different regions could be probed with different antibodies, and then reassembled for chemiluminescence imaging. Dashed arrows indicate where sections of a cut Western blot were reassembled for probing of the entire blot with a single antibody. All antibodies used are indicated below the blots. Molecular weight standards are shown to the left of each blot. Lanes are as indicated. "Data not shown" indicates regions of a blot that were probed with different antibodies, with the data obtained not presented in this study. "RM" indicates wild type RepoMan and "RAXA" the non PP1-binding mutant.

Supplementary Figure 7. Full Western blots for data shown in Figures 5 and 7. Lines indicate where lanes not presented in the figure were omitted, while dashed lines indicate where

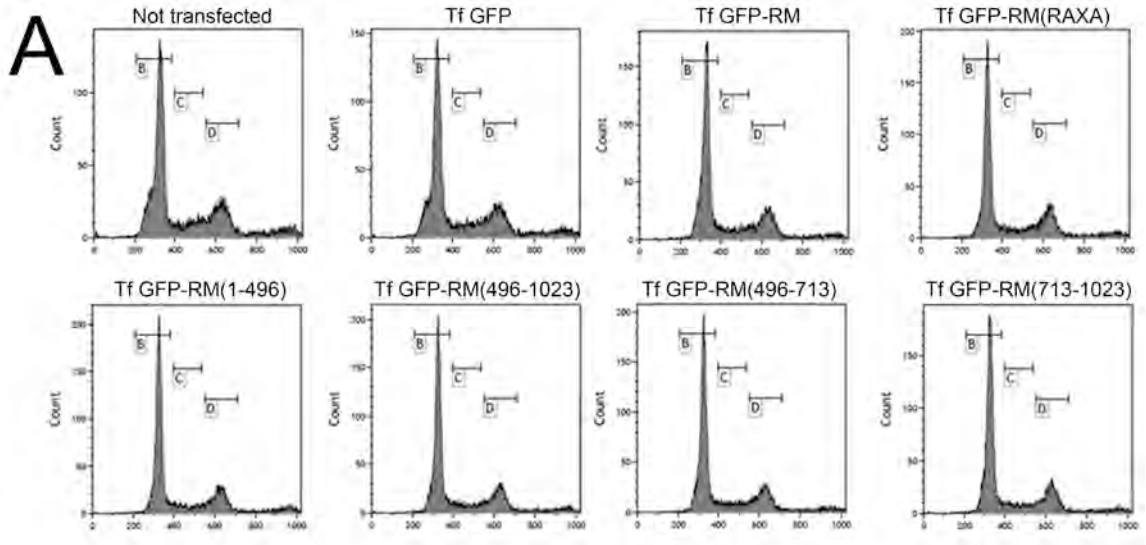
a Western blot was cut so that different regions could be probed with different antibodies, and then reassembled for chemiluminescence imaging. Dashed arrows indicate where sections of a cut Western blot were reassembled for probing of the entire blot with a single antibody. All antibodies used are indicated below the blots. Molecular weight standards are shown to the left of each blot. Lanes are as indicated. "Data not shown" indicates regions of a blot that were probed with different antibodies, with the data obtained not presented in this study. Abbreviations used here include Async (asynchronous), Noc (nocodazole), Thy (thymidine), HU (hydroxyurea), Mim (L-mimosine) and Tax (taxol). "WT" indicates wild type RepoMan and "RAXA" the non PP1-binding mutant.

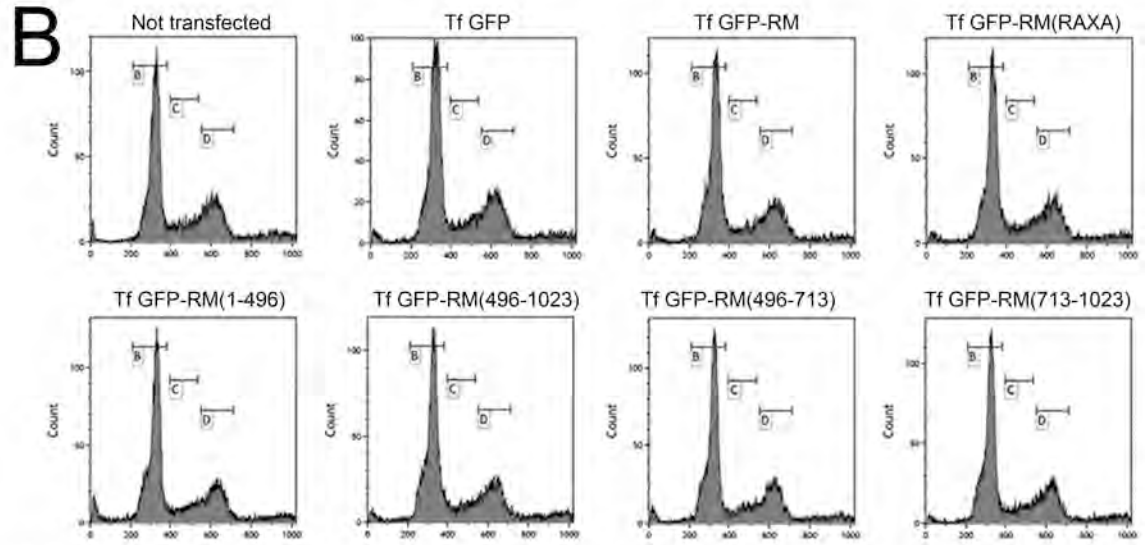
Supplementary Figure 8. Full Western blots for data shown in Figure 6. Lines indicate where lanes not presented in the figure were omitted, while dashed lines indicate where a Western blot was cut so that different regions could be probed with different antibodies, and then reassembled for chemiluminescence imaging. Dashed arrows indicate where sections of a cut Western blot were reassembled for probing of the entire blot with a single antibody. All antibodies used are indicated below the blots. Molecular weight standards are shown to the left of each blot. Lanes are as indicated. "Data not shown" indicates regions of a blot that were probed with different antibodies, with the data obtained not presented in this study. "WT" indicates wild type RepoMan and "RAXA" the non PP1-binding mutant.

Supplemental Figure 9. Mascot search results for RepoMan phosphopeptide mapping experiment.



Supplemental Figure 1





C

Effectene	% G1	% S	% G2/M
not Tf	65.4	12.0	22.6
GFP	66.7	11.8	21.5
RM	67.0	10.7	22.3
RM RAXA	66.1	10.2	23.7
1-496	67.6	10.0	22.5
496-1023	67.0	10.4	22.6
496-713	67.1	11.1	21.9
713-1023	67.7	10.6	21.7

PEI	% G1	% S	% G2/M
not Tf	60.4	13.6	26.0
GFP	61.7	12.8	25.5
RM	63.6	11.0	25.5
RM RAXA	61.2	12.4	26.4
1-496	63.5	11.9	24.7
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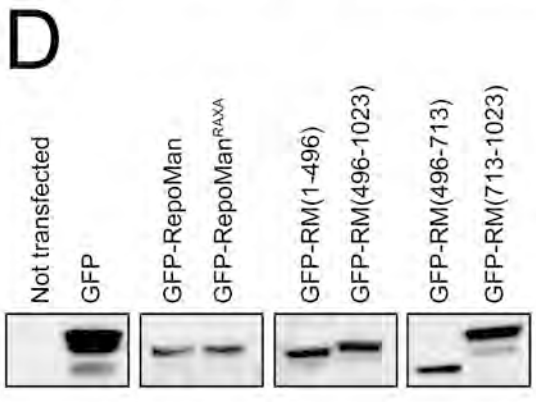
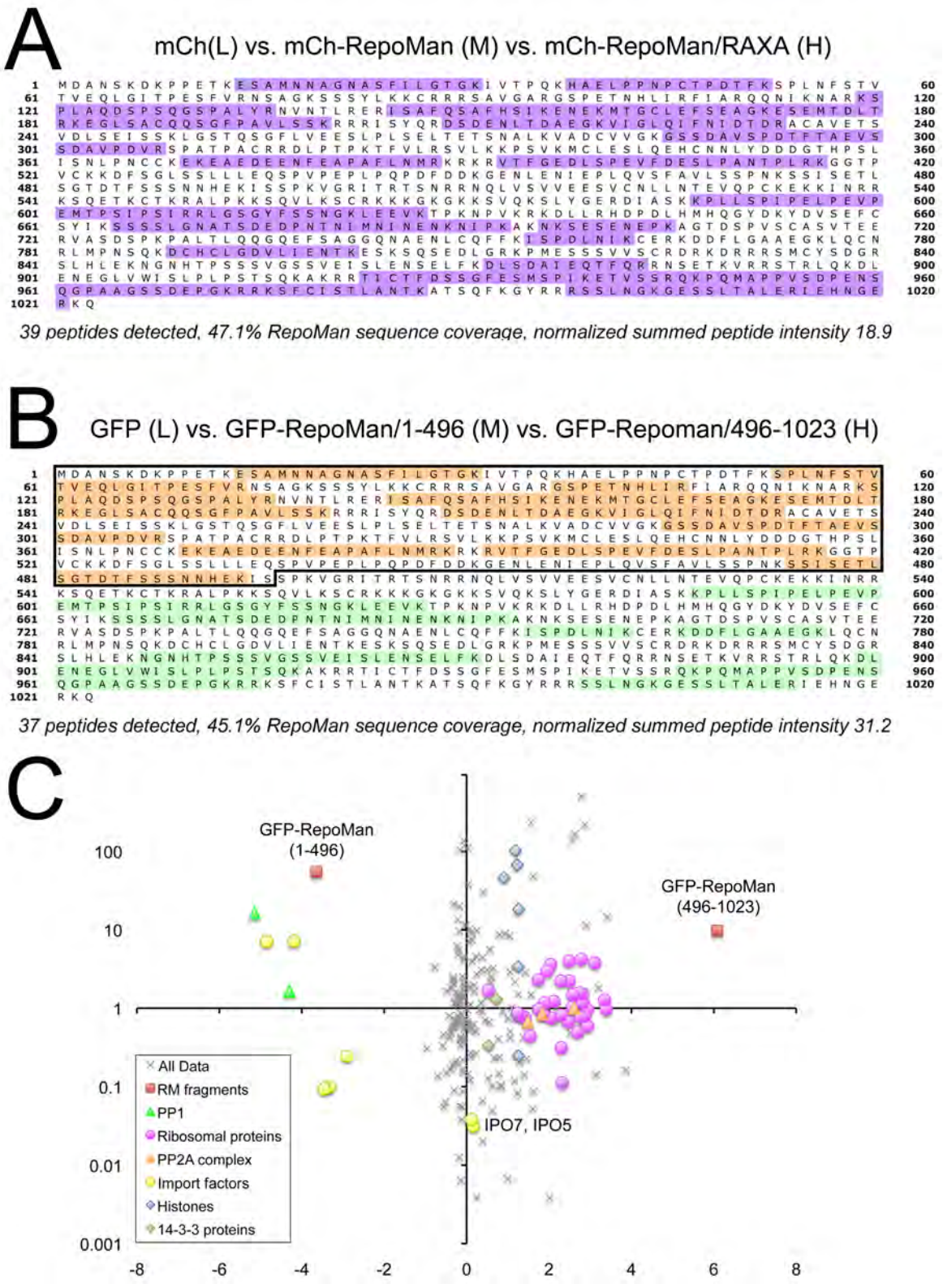
496-713	63.3	11.7	25.0
713-1023	63.5	12.1	24.4



Supplemental Figure 2



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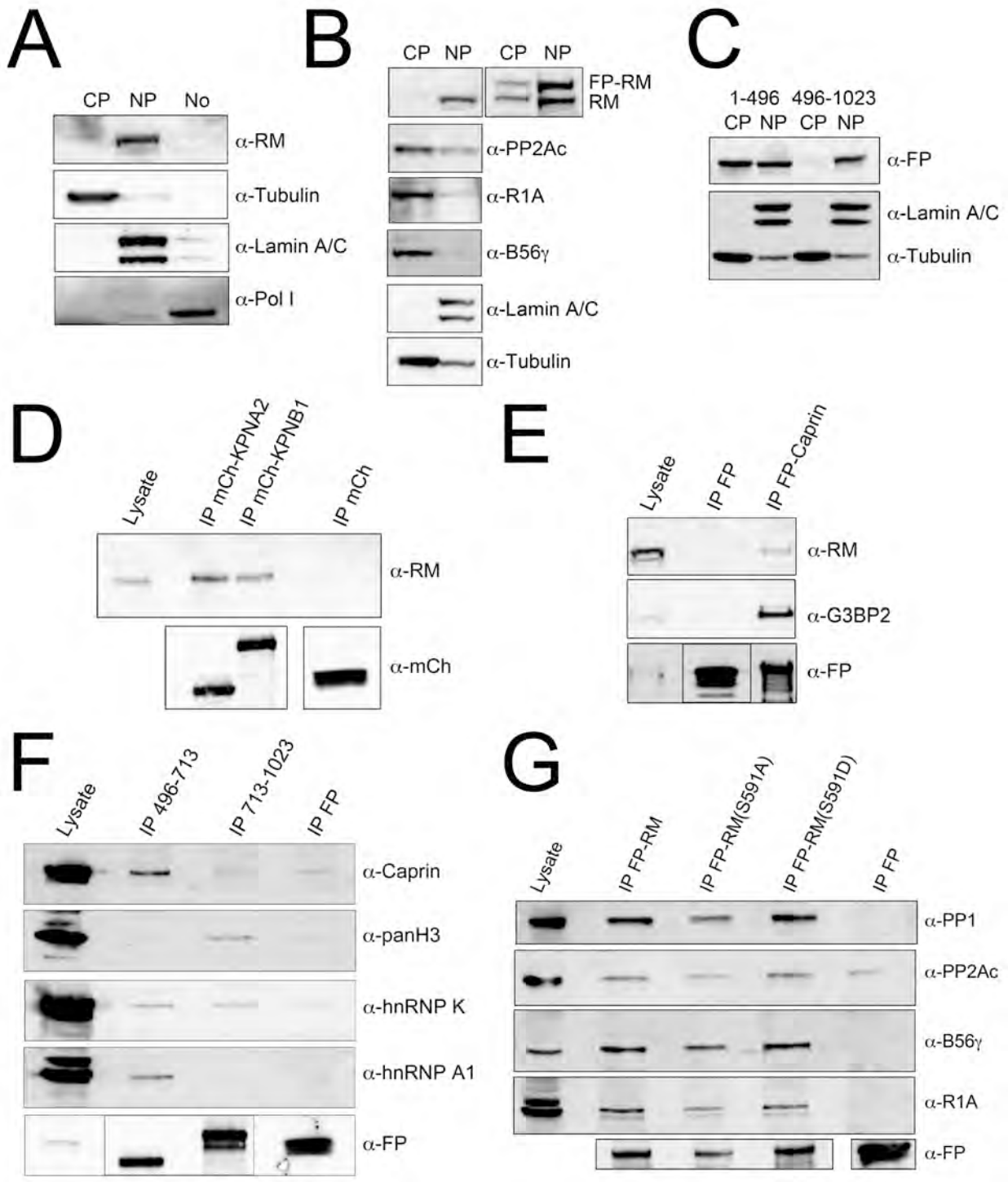

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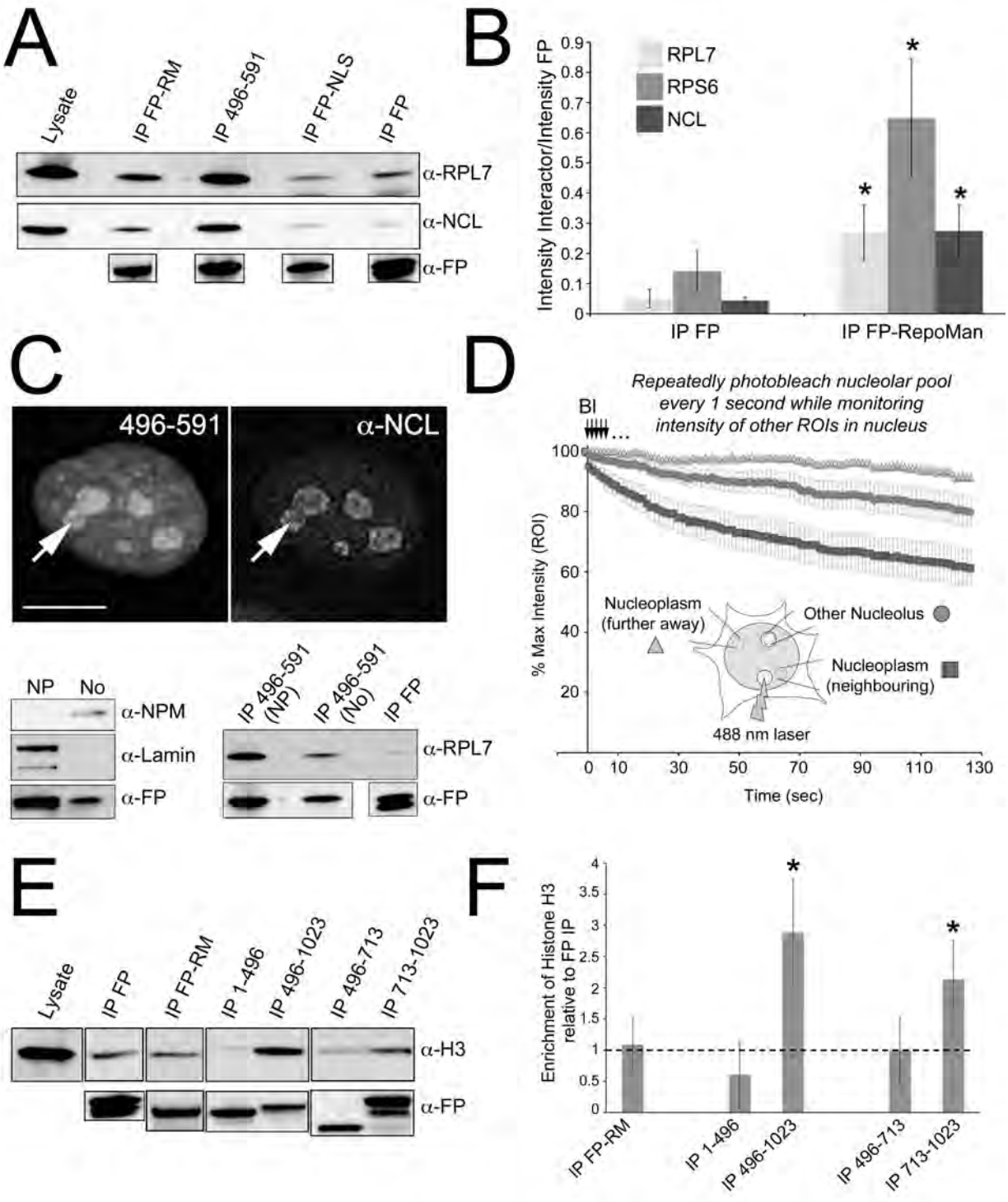

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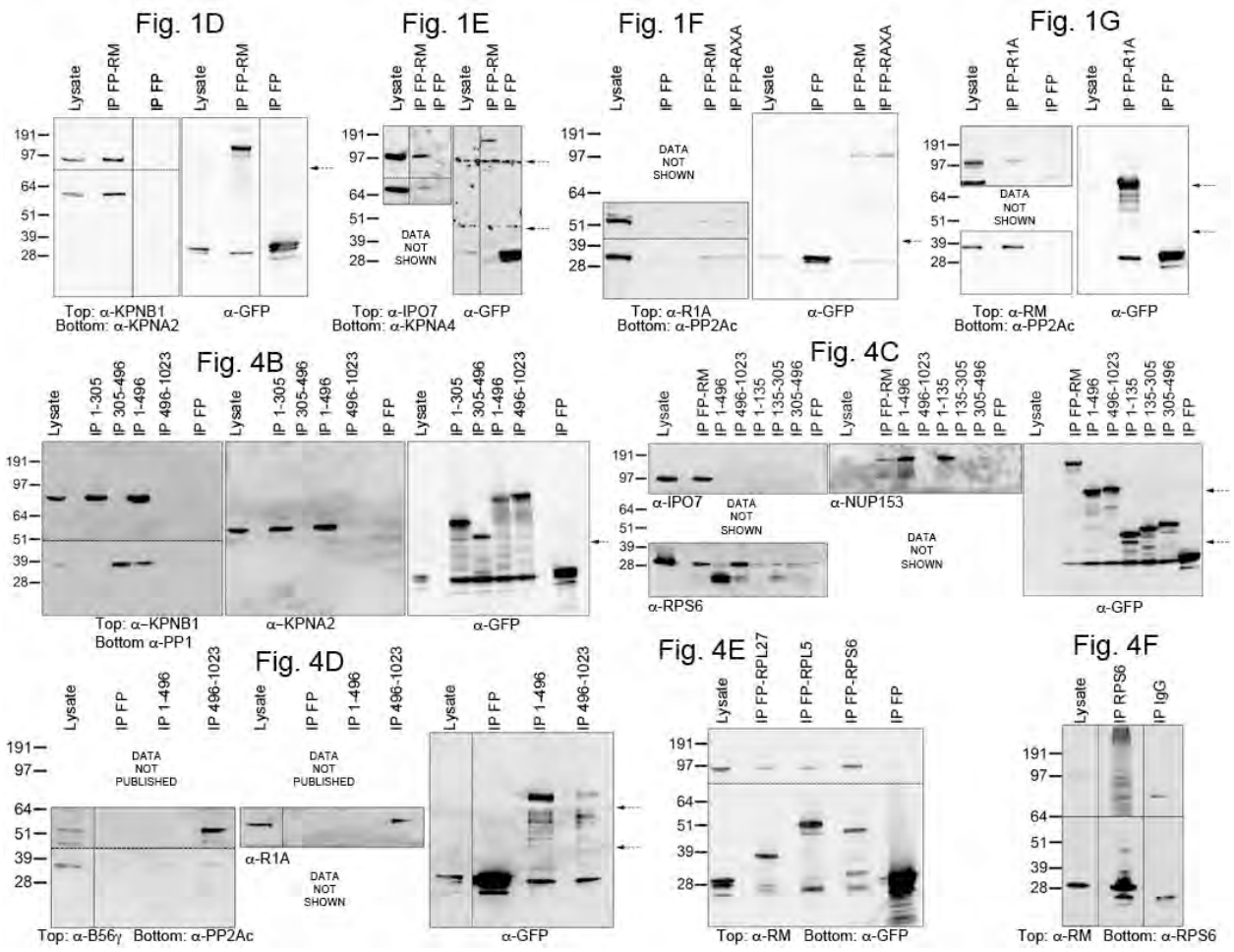

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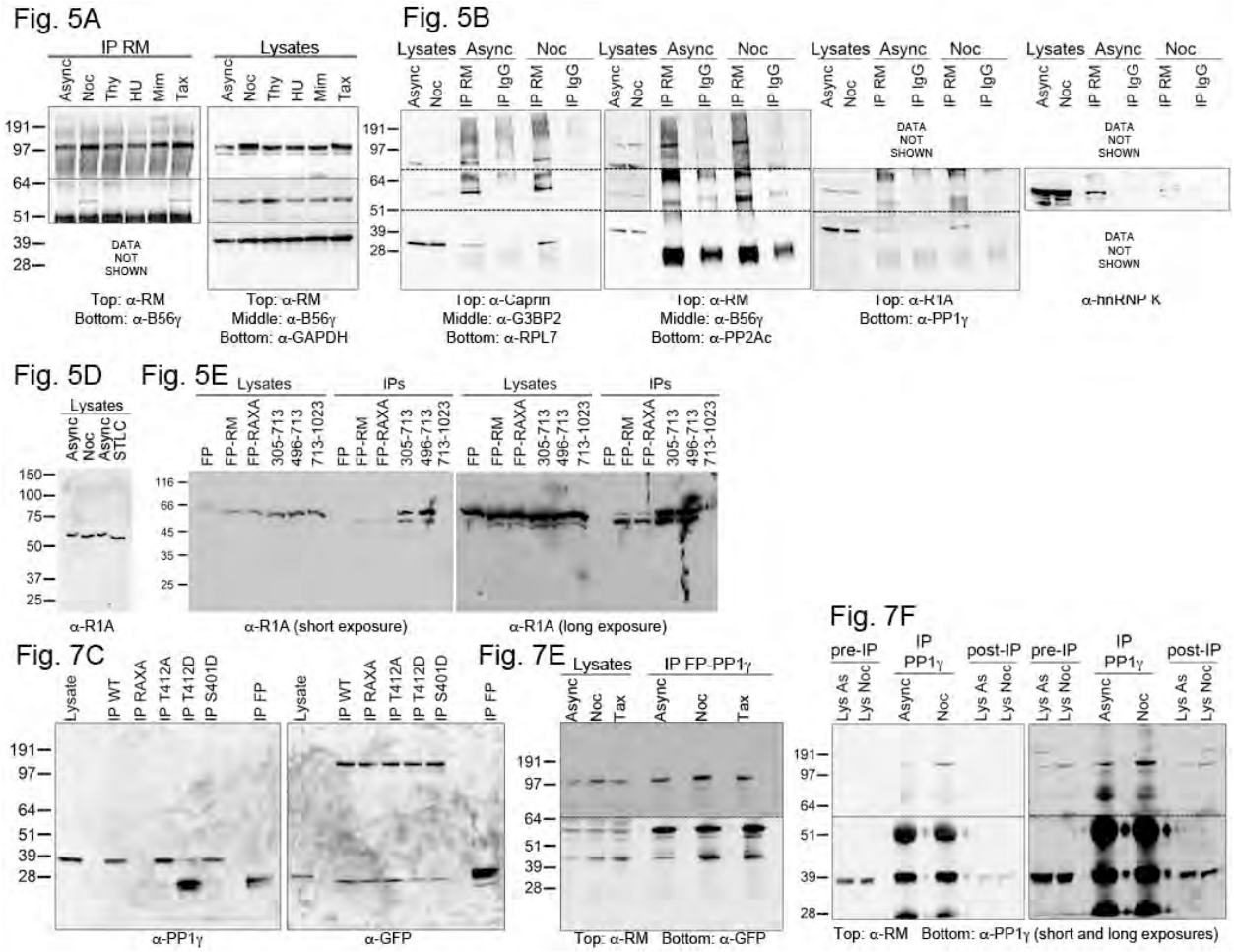
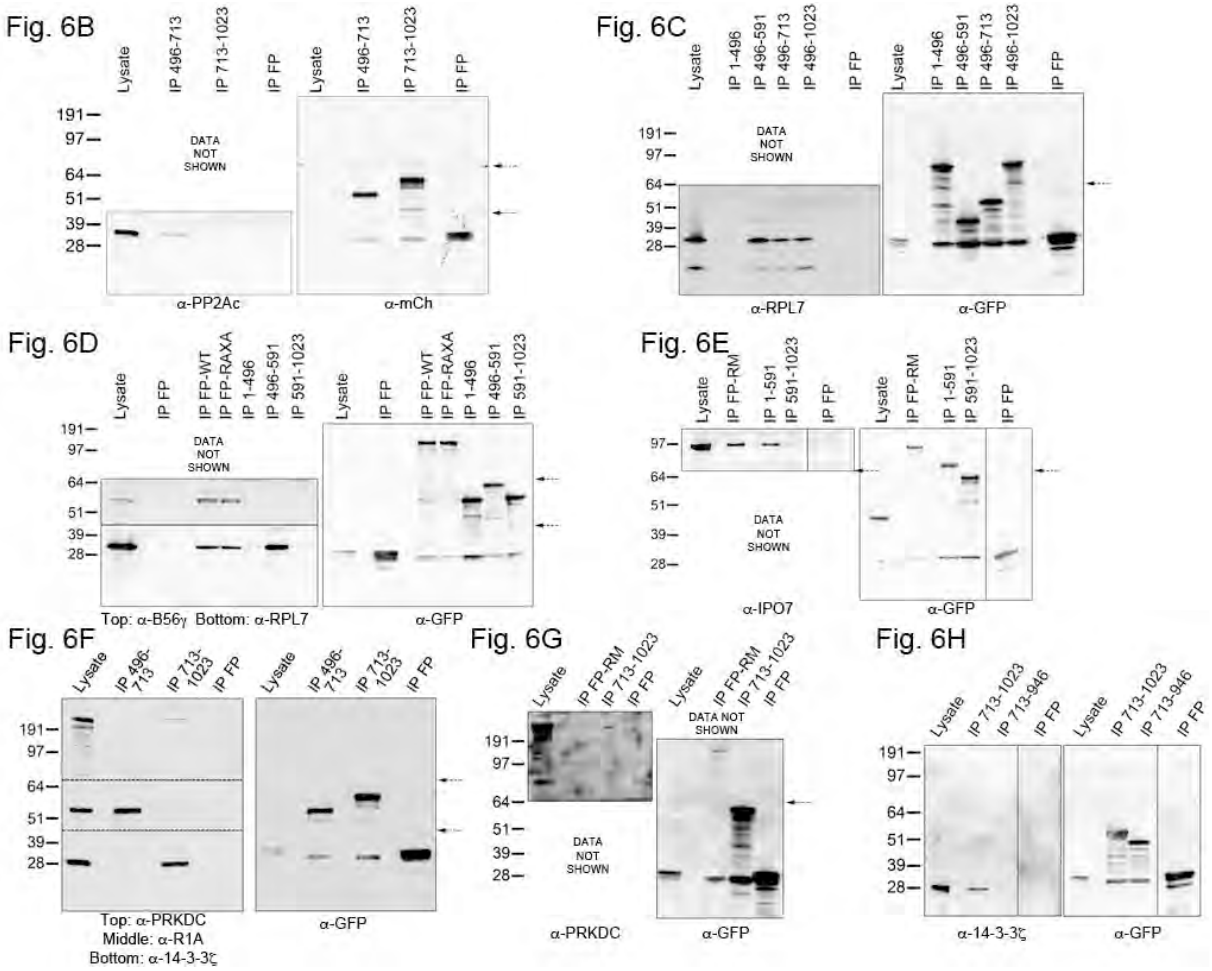


Supplemental Figure 7



Supplemental Figure 8