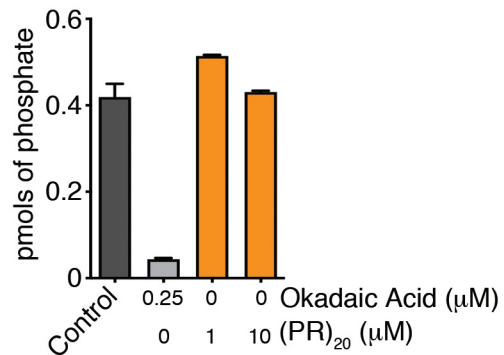
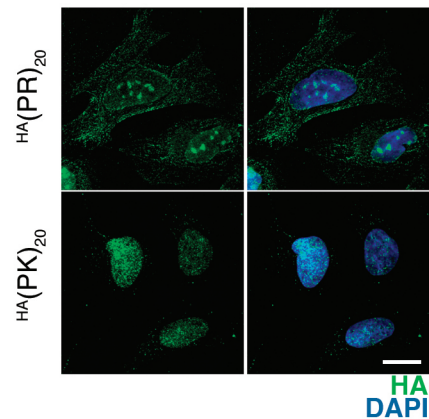


**Fig. S1.** (Related to **Fig. 1**) **(A)** STRING analysis (<https://string-db.org>) of the protein interaction networks found among the proteins identified in the purification of ribosomes from RPS9<sup>SBP</sup>-expressing HeLa cells. The node containing 60S and 40S factors (RPLs and RPSs) is indicated (arrow). The panel illustrates the ribosome composition from (PR)<sub>20</sub>-untreated cells. **(B)** Protein levels of RPL factors in ribosomes purified from HeLa RPS9<sup>SBP</sup> cells exposed to 10  $\mu$ M of (PR)<sub>20</sub> for 16h, as identified by LC-MS/MS. **(C)** Protein levels of RPL factors in the input extracts used for ribosome purification from HeLa RPS9<sup>SBP</sup> cells exposed to 10  $\mu$ M of (PR)<sub>20</sub> for 16h, as identified by LC-MS/MS. **(D)** Representative polysome profiles obtained from HeLa cells untreated or treated with 10  $\mu$ M of (PR)<sub>20</sub> for 16h. The presence of halfmers is indicated (arrows). **(E)** Electron microscopy images from purified 40S and 60S ribosomal complexes (1 pmol each) assembled *in vitro* in the presence of MgCl<sub>2</sub>, and in the presence or absence of 5 pmol of (PR)<sub>20</sub>. Assembled 80S particles are indicated (red arrows). **(F)** Quantification of 80S particles identified in **(E)** (n=1000) in non-assembly (1 mM MgCl<sub>2</sub>) or assembly (5 mM MgCl<sub>2</sub>) conditions. **(G)** *In vitro* translation of 100 ng of luciferase mRNA (quantified by Relative Luciferase Units (RLU)) in the presence of increasing doses of (PR)<sub>20</sub>. **(H)** *In vitro* translation of 100 ng of luciferase mRNA in the presence or absence of 0.5  $\mu$ M (PR)<sub>20</sub>. Translation products were labeled with [<sup>35</sup>S]-Met/Cys and analyzed by SDS-PAGE and autoradiography. **(I)** *In vitro* translation of 100 ng of luciferase mRNA with different 5' UTR lengths in the presence or absence of 0.5  $\mu$ M (PR)<sub>20</sub>. **(J)** *In vitro* translation of 100 ng of luciferase mRNA in the presence or absence of 0.5  $\mu$ M (PR)<sub>20</sub>. In the right two columns, the mRNA was extracted from a translation reaction done in the presence of the DPR, and subsequently used in a new translation reaction performed in the absence of (PR)<sub>20</sub>. \*, p<0.05.

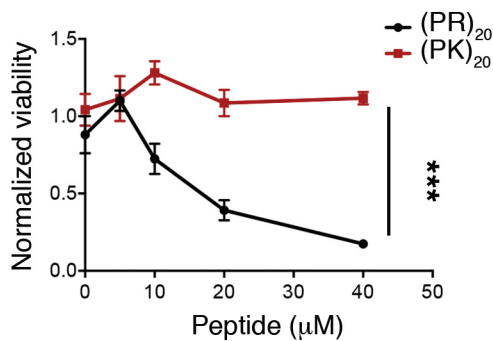
**A**



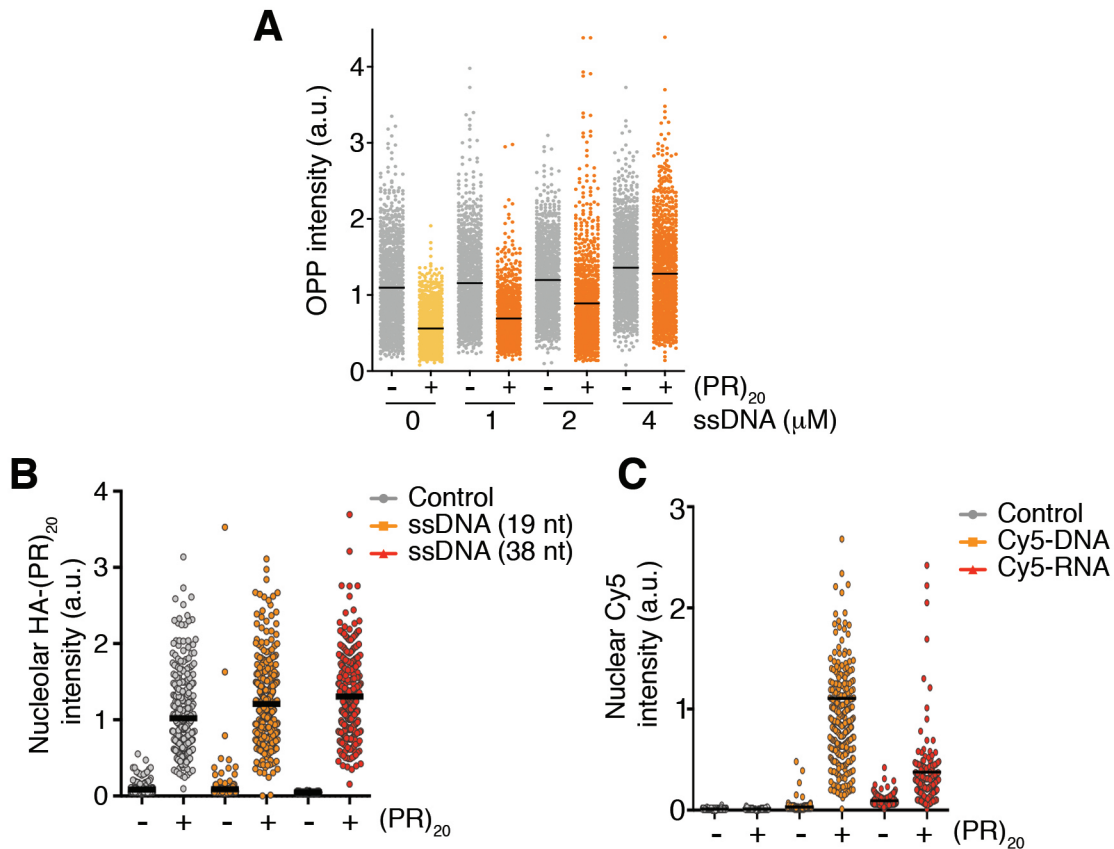
**B**



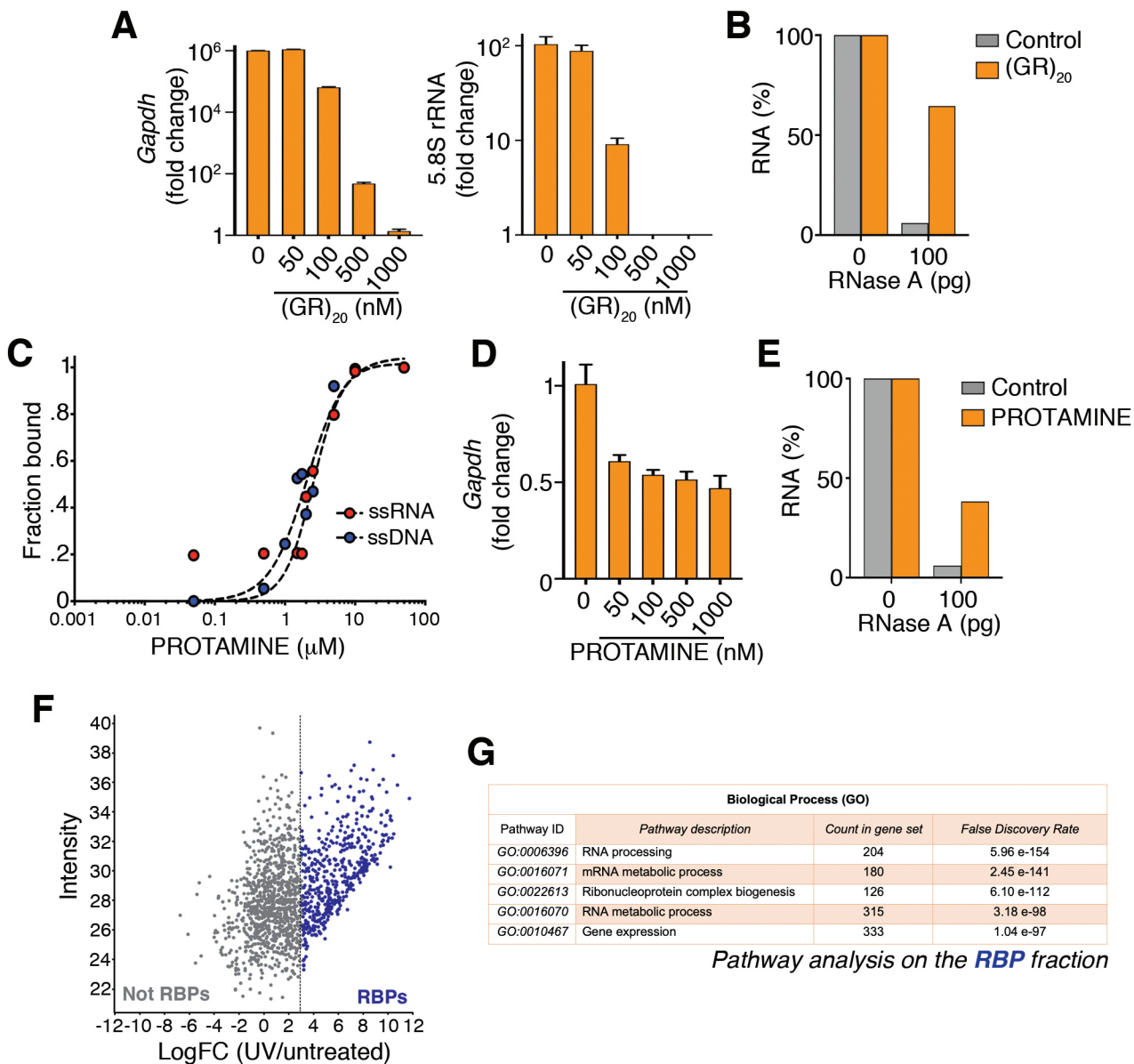
**C**



**Figure S2** (related to [Fig. 4](#)). **(A)** A PP2A in vitro phosphatase assay performed in the presence or absence of (PR)<sub>20</sub> or Okadaic acid (a phosphatase inhibitor). PP2A activity is measured through the liberation of phosphates from a KRpTIRR peptide. **(B)** Immunofluorescence of HA-(PR)<sub>20</sub> or (PK)<sub>20</sub> (green) in U2OS cells treated with 7.5 μM of each peptide for 1 h. Scale bar (white) indicates 2.5 μm. DAPI was used to stain DNA. **(C)** Percentage of viable cells as evaluated with a CellTiter-Glo luminescent assay in U2OS cells treated with increasing doses of (PR)<sub>20</sub> or (PK)<sub>20</sub> peptides at the indicated doses. \*\*\*\*, p<0.0001



**Figure S3** (*related to Fig. 3*). **(A)** HTM-mediated quantification of OPP levels per cell (PR)<sub>20</sub> (10 μM, 16h) alone or together with a 38 nt ssDNA oligonucleotide at the indicated doses. **(B)** HTM-mediated quantification of the nucleolar levels of a HA-(PR)<sub>20</sub> peptide in U2OS cells treated with 7.5 μM HA-(PR)<sub>20</sub> alone or together with 2 μM of 19 or 38 nt ssDNA oligonucleotides for 8 h. **(C)** HTM-mediated quantification of the nuclear levels of Cy5-labeled ssDNA or ssRNA 19 nt oligonucleotides in U2OS cells treated with 7.5 μM HA-(PR)<sub>20</sub> alone or together with 4 μM of the oligonucleotides for 8 h. Black lines indicate mean values. Examples of the images used for the analysis shown in **(B, C)** are shown in **Fig. 3D, E**.



**Figure S4** (related to [Fig. 4](#)). **(A)** Percentage of *GAPDH* or 5.8 rRNA levels quantified by qPCR in reactions containing increasing doses of (GR)<sub>20</sub> (n=3). **(B)** Percentage of RNA (1 μg) remaining after a 15' digestion with 100 pg of RNase A in the presence or absence of (PR)<sub>20</sub> (5 μM). **(C)** Quantification of EMSA assays evaluating the binding of PROTAMINE to 19 nt-long ssDNA and ssRNA molecules. Each probe (0.2 μM) was incubated with increasing concentrations of (PR)<sub>20</sub> for 10'. Curve-fitting was performed using non-linear regression with the Hill equation. **(D)** Percentage of *GAPDH* levels quantified by qPCR in reactions containing increasing doses of PROTAMINE (n=3). **(E)** Percentage of RNA (1 μg) remaining after a 15' digestion with 100 pg of RNase A in the presence or absence of PROTAMINE (5 μM). **(F)** Distribution of the proteins and their levels from the proteomic analysis following isolation of mRNA-binding proteins. Proteins that showed significant enrichment on the oligo(dT)-captured fractions following UV crosslinking were identified as RBPs for subsequent analyses. **(G)** Gene Ontology (GO) analysis illustrating the biological pathways that were most significantly enriched among the proteins defined as RBPs in the proteomic experiment shown in **(F)**. Note that, indeed, all biological pathways are related to RNA metabolism.



**Table S1.** Proteins with significantly reduced levels on ribosomal fractions purified from (PR)<sub>20</sub>-treated HeLa-RPS9<sup>SBP</sup> cells. *Related to Fig 1.*

Gene Name	Log <sub>2</sub> ((PR) <sub>20</sub> /control)
PSME3	-3.61
H1FX	-1.94
RPL27A	-1.76
RPLP2	-1.71
BRIX1	-1.52
GTPBP4	-1.35
RPL32	-1.32
KRR1	-1.22
NAP1L4	-1.21
HP1BP3	-1.20
RPL3	-0.99
RPL14	-0.87
RPL10A	-0.70
RPL6	-0.66
UTP14A	-0.65
RPL9	-0.63

**Table S2.** Proteins that show statistically significant reduced levels on chromatin after treatment of U2OS cells with (PR)<sub>20</sub> (20 μM) or PROTAMINE (30 μM). Related to **Figure 4**.

Gen Symbol	Log <sub>2</sub> ((PR) <sub>20</sub> /control)	Log <sub>2</sub> (PROTAMINE/control)
FURIN	-2.45	-3.61
POTEJ	-2.05	-2.15
POTEKP	-1.72	-1.63
TMEM126B	-1.40	-1.19
POTEF	-1.38	-1.20
HIST1H1C	-1.33	-0.61
FARS2	-1.28	-0.75
HIST1H1A	-1.27	-0.46
SNX5	-1.06	-0.82
HIF0	-1.05	-0.39
PITPNA	-1.02	-0.60
PDK2	-1.01	-0.64
GLTSCR2	-1.01	-0.42
EEF1B2	-1.01	-0.86
EEF1G	-0.98	-0.75
GAPDH	-0.97	-0.80
NRF1	-0.96	-0.90
PSMF1	-0.93	-0.42
AURKC	-0.92	-0.87
CORO1B	-0.88	-0.47
EBAG9	-0.87	-0.64
APEX1	-0.86	-0.70
EEF1D	-0.83	-0.69
ALDOC	-0.82	-0.45
REPIN1	-0.82	-0.54
H2AFY	-0.82	-0.43
RXRB	-0.75	-0.50
HMGA1	-0.75	-0.68
TPT1	-0.73	-0.65
RPUSD4	-0.72	-0.87
DNAJC15	-0.70	-0.40
RING1	-0.70	-0.57
PCBP3	-0.67	-0.41
HMGA2	-0.66	-0.51
SLC25A40	-0.65	-0.41
HN1L	-0.65	-0.40
MEN1	-0.65	-0.40
MSN	-0.64	-0.58
HDGF	-0.62	-0.44
HCFC1	-0.62	-0.52
TGIF2LX	-0.62	-0.52
HMGB1;HMGB1P1	-0.62	-0.50
LRRC57	-0.60	-0.49
FLNA	-0.60	-0.50
RANBP1	-0.60	-0.59
NCKIPSD	-0.59	-0.61

EZR	-0.58	-0.51
CMSS1	-0.58	-0.35
LANCL2	-0.58	-0.58
CSRP2	-0.58	-0.48
TMF1	-0.58	-0.44
EEF1A2	-0.56	-0.54
TARS	-0.56	-0.45
CDYL	-0.56	-0.75
DIDO1	-0.55	-0.44
AHNAK	-0.55	-0.48
ACAP2	-0.55	-0.39
SYNGR3	-0.55	-0.60
PALM2	-0.55	-0.48
ARHGAP17	-0.55	-0.57
ID1	-0.55	-0.39
CCAR1	-0.54	-0.40
RPL18	-0.54	-0.38
COPS7A	-0.54	-0.51
CCDC50	-0.53	-0.53
ZBTB10	-0.53	-0.88
CCNC	-0.53	-0.38
SFSWAP	-0.52	-0.60
SLC43A3	-0.52	-0.45
WASF2	-0.52	-0.61
SPR	-0.52	-0.74
GAP43	-0.51	-0.97
FSCN1	-0.51	-0.40
VPS39	-0.51	-0.57
FUS	-0.50	-0.46
SARS	-0.50	-0.46
FIP1L1	-0.49	-0.38
DIAPH2	-0.49	-0.67
CSTF2	-0.49	-0.40
SCAF4	-0.49	-0.36
CBX8	-0.48	-0.47
PRPF38B	-0.48	-0.35
DCUN1D1	-0.47	-0.61
DDX31	-0.47	-0.45
PDLIM7	-0.47	-0.41
RECQL	-0.47	-0.36
FNBP4	-0.47	-0.40
EHD3	-0.46	-0.38
SCAF1	-0.46	-0.37
MRGBP	-0.46	-0.54
MAVS	-0.45	-0.38
CORO1C	-0.45	-0.55
MINA	-0.45	-0.48
COPS8	-0.45	-0.74
TCERG1	-0.45	-0.42
NEDD1	-0.44	-0.59
SEPT9	-0.44	-0.43
HDGFRP3	-0.44	-0.36
FAHD1	-0.44	-0.73

FKBP3	-0.43	-0.42
PA2G4	-0.43	-0.42
DDX42	-0.43	-0.40
CASK	-0.42	-0.69
GABPA	-0.41	-0.36
MAD2L1BP	-0.40	-0.40
ITPRIP	-0.40	-0.67
EHD4	-0.40	-0.36
NARS	-0.39	-0.41
EEF1A1;EEF1A1P5	-0.39	-0.47
WDR33	-0.39	-0.42
DEK	-0.39	-0.44
TBP	-0.39	-0.36
DOK1	-0.39	-0.35
IFIT2	-0.39	-0.72
UBAP2L	-0.38	-0.49
SEPT11	-0.38	-0.36
RSU1	-0.37	-0.38
NACA	-0.37	-0.51
ORC1	-0.37	-0.42
CCNL1	-0.37	-0.37
CEP97	-0.37	-0.45
UBE2I	-0.36	-0.42
VPS13C	-0.36	-0.41
PRDX1	-0.36	-0.40
DR1	-0.36	-0.64
BCL7B	-0.35	-0.36
KDM2A	-0.35	-0.41