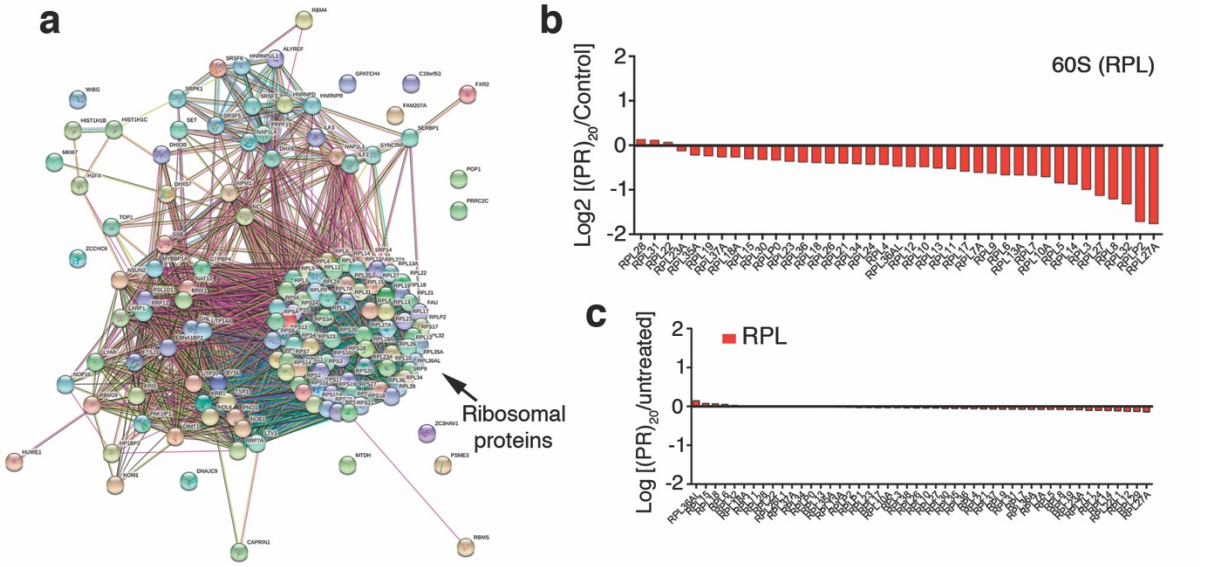

Fig. S1. Changes in ribosome composition by exposure to $(PR)_{20}$ peptides. a, STRING analysis (https://string-db.org) of the protein interaction networks found between the proteins identified in the purification of ribosomes from $RPS9^{SBP}$-expressing HeLa cells. The node containing 60S and 40S factors (RPLs and RPSs) is indicated (arrow). The panel illustrates the ribosome composition from $(PR)_{20}$-untreated cells. **b**, Protein levels of RPL factors in ribosomes purified from Hela $RPS9^{SBP}$ cells exposed to 10 μM of $(PR)_{20}$ for 16h, as identified by LC-MS/MS. **c**, Protein levels of RPL factors in the input extracts used for ribosome purification from Hela $RPS9^{SBP}$ cells exposed to 10 μM of $(PR)_{20}$ for 16h, as identified by LC-MS/MS.

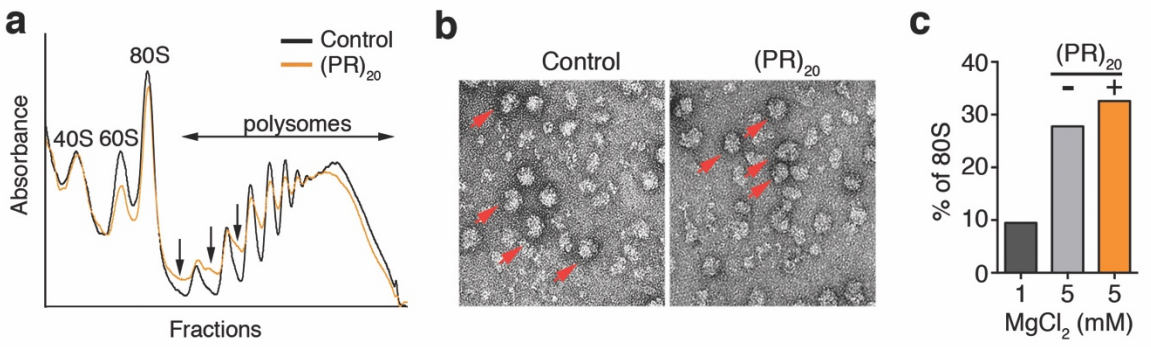

Fig. S2. $(PR)_{20}$ peptides impair the assembly of 80S ribosome particles on mRNA. a, Representative polysome profiles obtained from HeLa cells untreated or treated with 10 μM of (PR)20 for 16h. The presence of halfmers is indicated (arrows). **b,** Electron microscopy images from purified 40S and 60S ribosomal complexes (1 pmol each) assembled *in vitro* in the presence of $MgCl_2$, and in the presence or absence of 5 pmol of $(PR)_{20}$. Assembled 80S particles are indicated (red arrows). **c,** Quantification of 80S particles identified in (**d**) (n=1000) in non-assembly (1 mM $MgCl_2$) or assembly (5 mM $MgCl_2$) conditions.

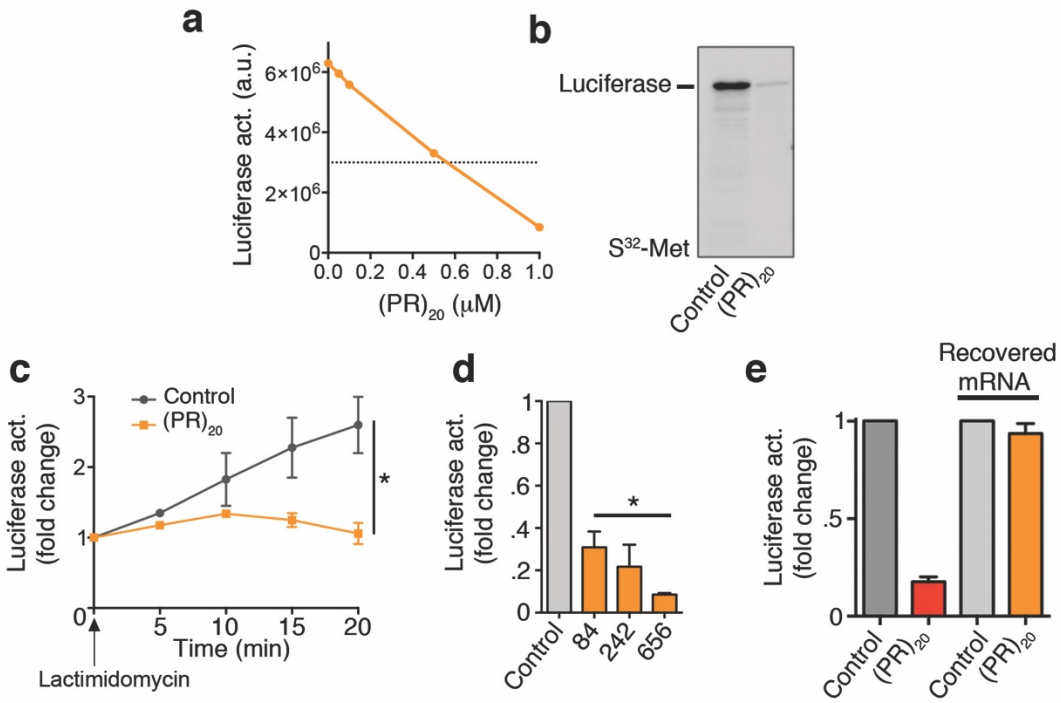

Fig. S3. Effects of $(PR)_{20}$ on mRNA translation. a, *In vitro* translation of 100 ng of luciferase mRNA (quantified by luciferase activity) in the presence of increasing doses of $(PR)_{20}$. **b,** *In vitro* translation of 100 ng of luciferase mRNA in the presence or absence of 0.5 μM $(PR)_{20}$. Translation products were labeled with [^{35}S]-Met/Cys and analyzed by SDS-PAGE and autorradiography. **c,** *In vitro* translation of 50 ng of luciferase mRNA was allowed for 15´and stopped in ice. After inhibition of new translation initiation with lactimidomycin for 10´, translation was allowed in the presence or absence 0.5 μM $(PR)_{20}$ for the indicated times. **d,** *In vitro* translation of 100 ng of luciferase mRNA with different 5' UTR lengths in the presence or absence of 0.5 μM $(PR)_{20}$. **e,** *In vitro* translation of 100 ng of luciferase mRNA in the presence or absence of 0.5 μM $(PR)_{20}$. 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 $(PR)_{20}$. *, $p<0.05$.

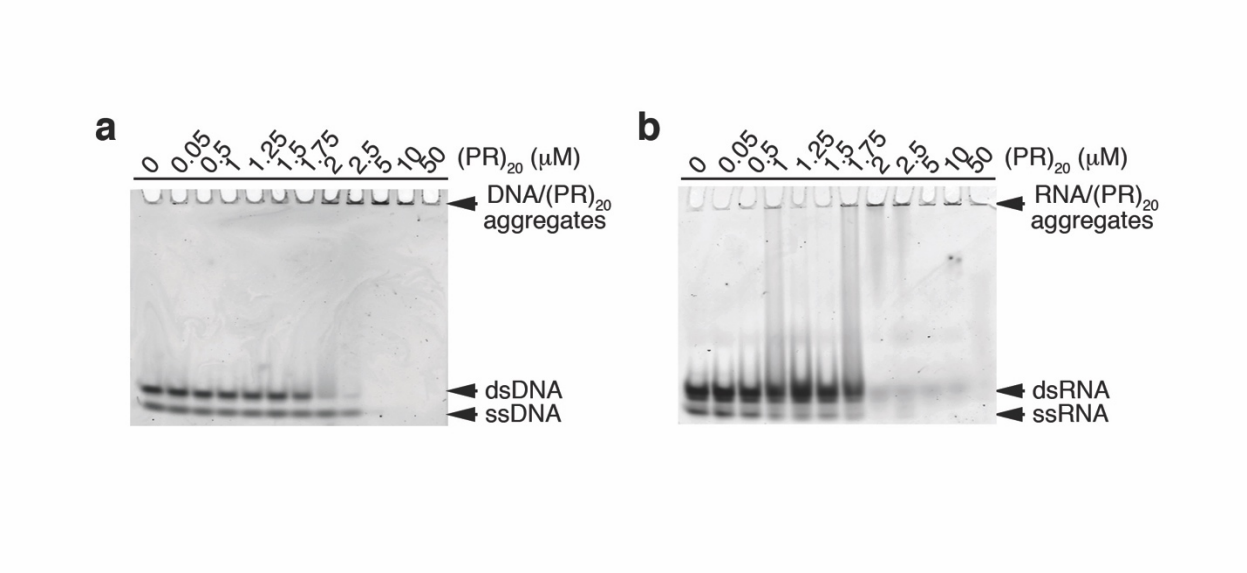

Fig. S4. DNA- and RNA-binding by $(PR)_{20}$ peptides. (**a, b**) Binding of $(PR)_{20}$ to DNA (**a**) or RNA (**b**) as examined by EMSA. Previously annealed 19 bp Cy3-dsDNA or Cy3-RNA oligonucleotides (0.2 μM) were incubated with increased doses of PR_{20} for 10′. The images show the disappearance of free dsDNA/dsRNA and non-annealed ssDNA/ssRNA oligonucleotides, which occurs concomitant with the accumulation of DNA- or RNA-$(PR)_{20}$ complexes on the loading well. The sharp transition from unbound to unbound fractions is indicative of cooperative binding in all cases. A quantification from these assays is shown in **Fig. a**.

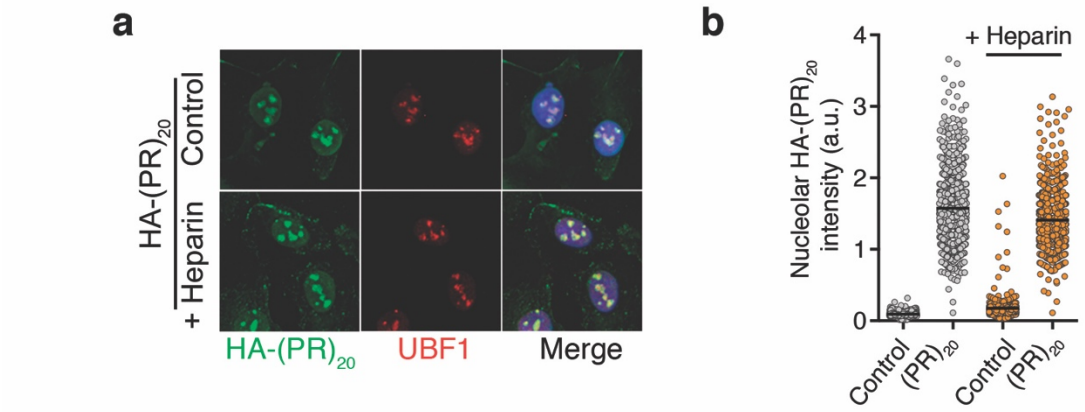

Fig. S5. Effect of heparin on the nucleolar accumulation of $(PR)_{20}$. a, Immunofluorescence of HA-$(PR)_{20}$ (green) and the nucleolar factor UBF1 (red) in U2OS cells treated with 7.5 μM HA-$(PR)_{20}$ alone or together with 0.5 μM heparin for 8 h. **b,** HTM-mediated quantification of the nucleolar HA-$(PR)_{20}$ intensity from U2OS cells treated as in (**a**).

Table S1. Proteins with reduced levels on ribosomal fractions purified from $(PR)_{20}$-treated HeLa-RPS9^{SBP} cells.

Gene Name	Log_2 ($(PR)_{20}$/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 $(PR)_{20}$ (20 μM) or PROTAMINE (30 μM).

Gen Symbol	$Log_2((PR)_{20}/control)$	$Log_2(Protamine/control)$
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
H1F0	-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