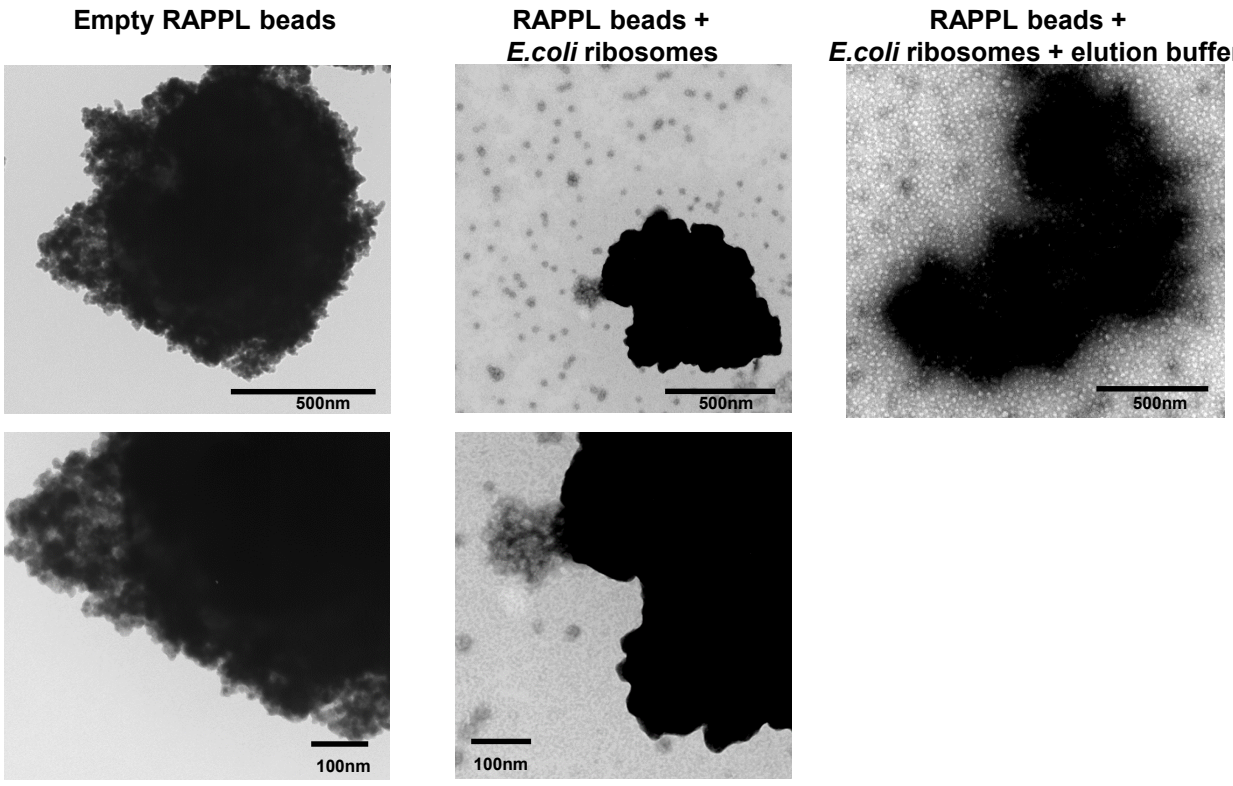
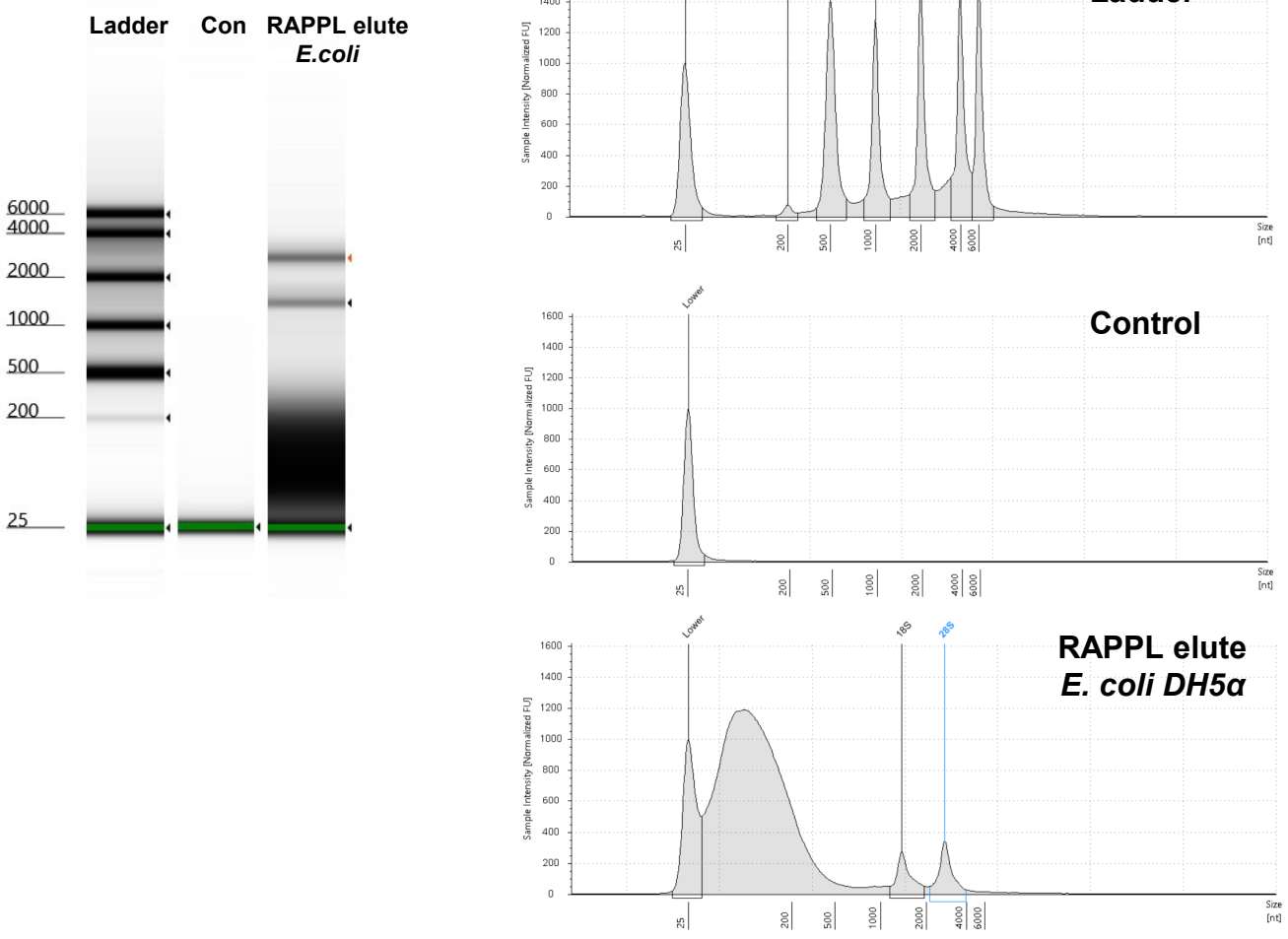
Supplementary Figure 1



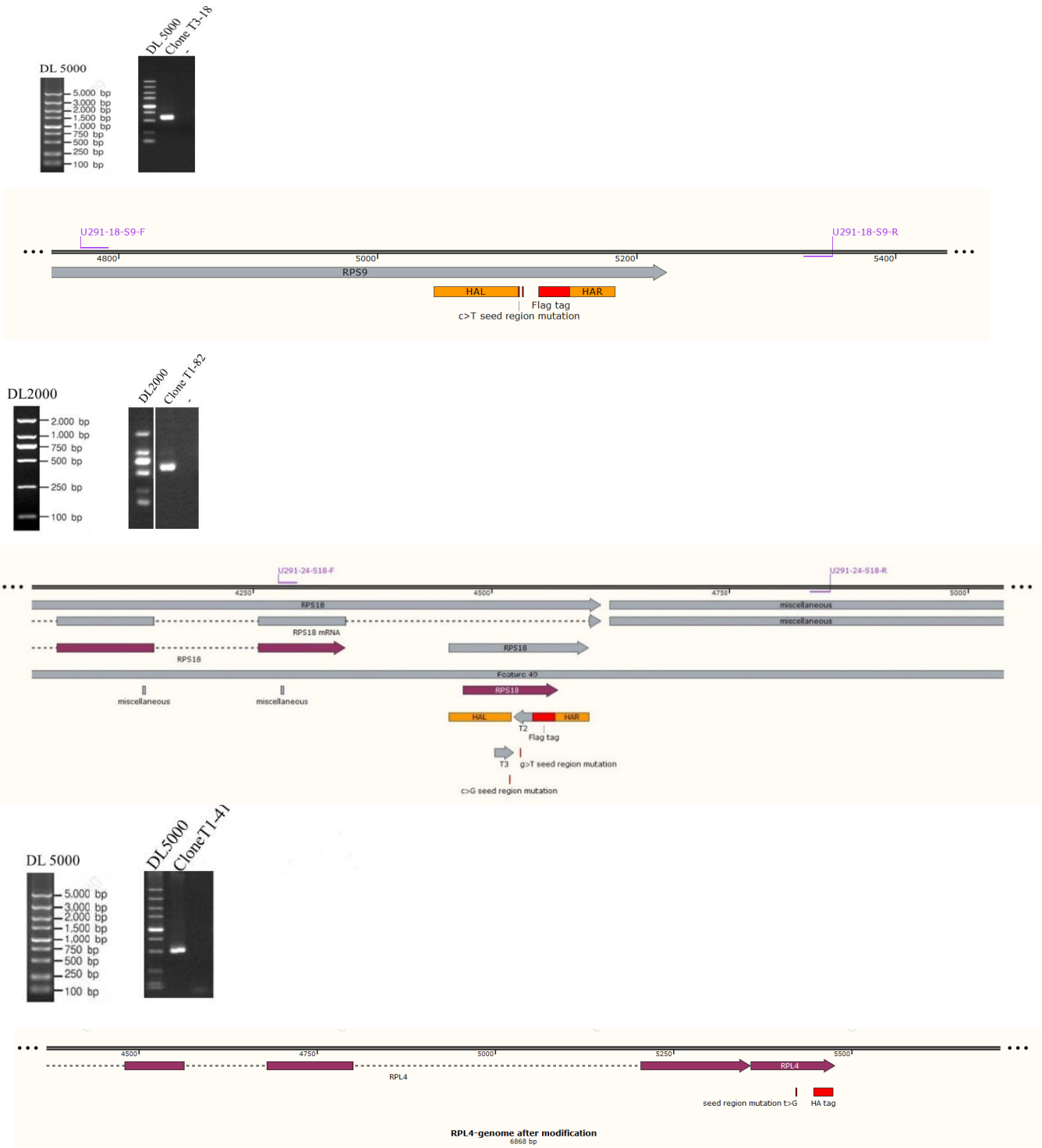
Supplementary Figure 1. Binding of E.coli ribosomes (NEB PURExpress® *in vitro* kit) to poly-L-lysine beads (Puramag™, MCLAB). Poly-L-lysine magnetic beads were incubated with wash buffer only or with *E. coli* ribosomes in absence or presence of elution buffer.

Supplementary Figure 2



Supplementary Figure 2. Bioanalyzer analysis of RNA species associated with RAPPL purification from *E.coli* DH5α cells. Image of the ladder, control (elution buffer) and diluted RAPPL elution from *E. coli* lysate purification. Electropherogram indicating ribosomal subunits as well as smaller and partially degraded RNA species.

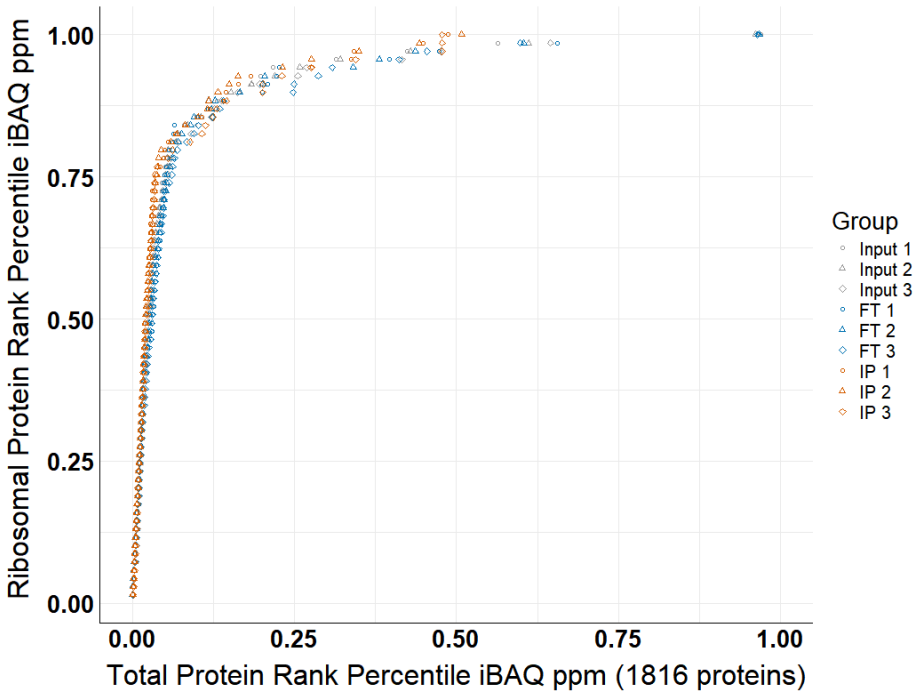
Supplementary Figure 3



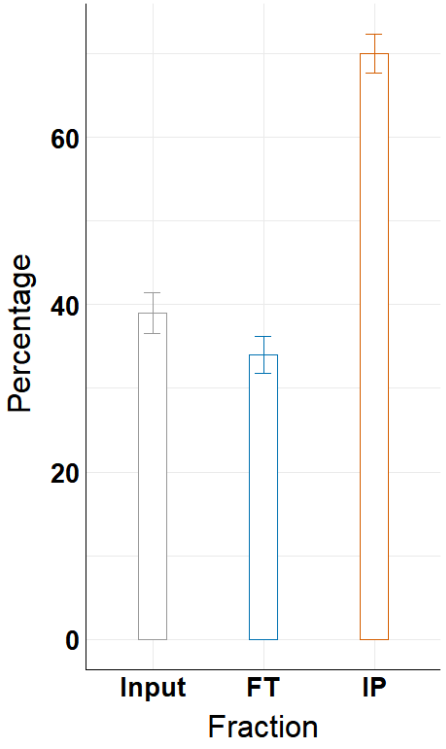
Supplementary Figure 2. CRISPR/Cas9 engineered Hek293 cell lines. Images of the DNA isolated from engineered uS4, uS13 and uL4 as well as map of primers for modified single clone validation are indicated. Targeted insertion of Flag- and HA-tags in ribosomal proteins is indicated in maps as well. Cell lines and images were generated by Genscript.

Supplementary Figure 4

A.

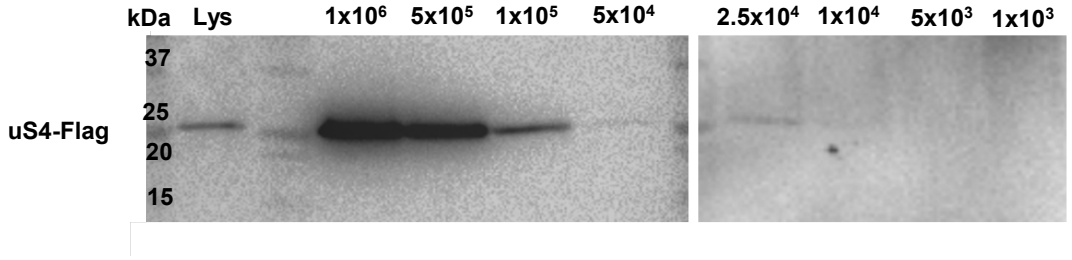


B.



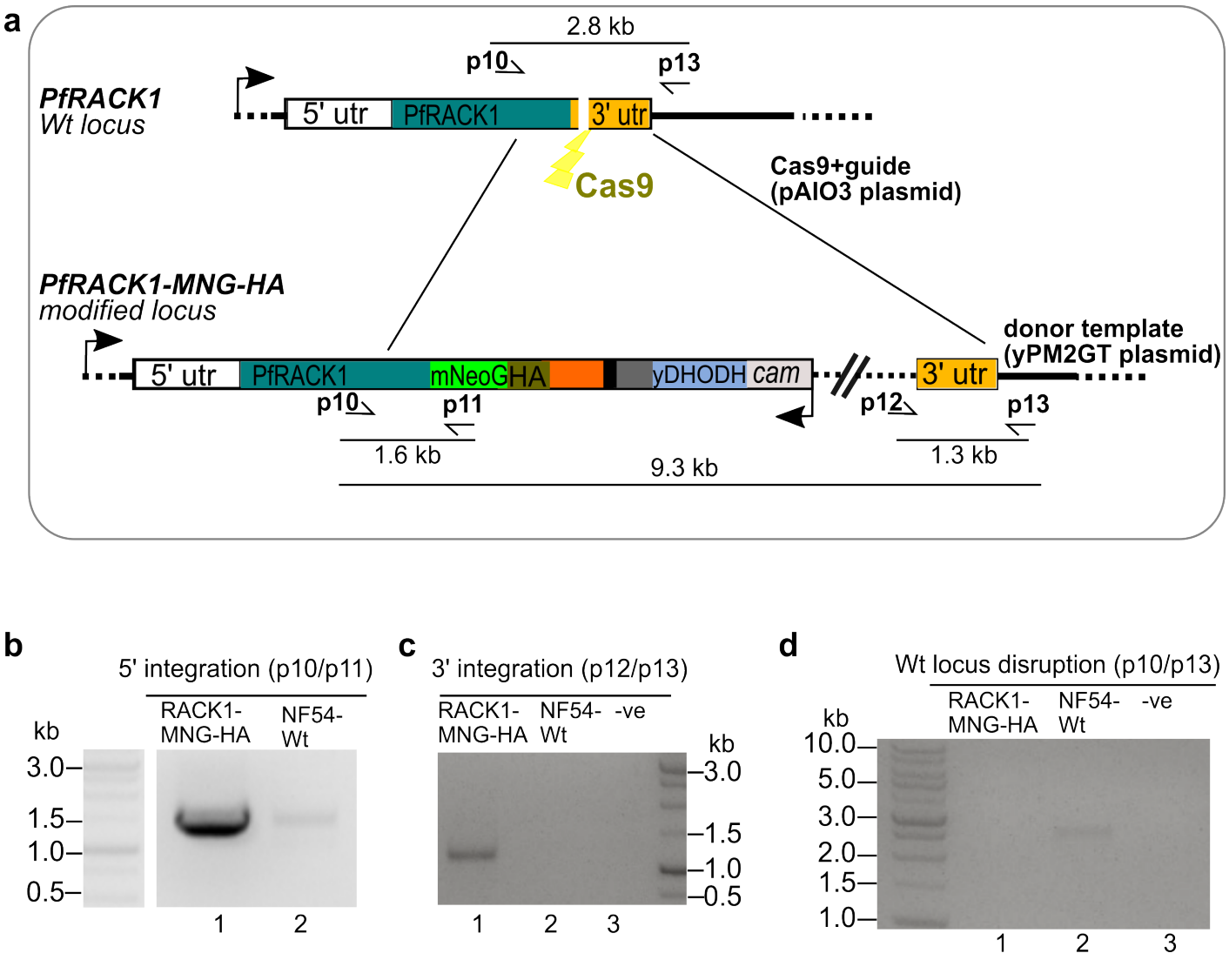
Supplementary Figure 4. Mass spectrometry analysis of *E. coli* RAPPL purifications. a, Plot of the of each ribosomal protein's rank percentile in relation to total protein rank percentile for each replicate of input, flow-through (FT), and bead-bound (IP) fractions. **b,** Graph representing percentage of ribosomal proteins in total protein associated with input, flow-through (FT), and bead-bound (IP) fractions. Error bars represent standard deviation of triplicate averages.

Supplementary Figure 5



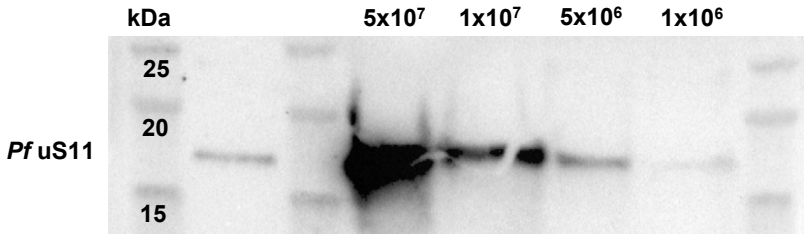
Supplementary Figure 5. RAPPL can purify ribosomes from limited HEK-293 cell sample. Western blot analysis using αFlag antibody on RAPPL eluates of HEK-293 cells in which uS4 was Flag-tagged by CRISPR/Cas9 whereby the starting cells were diluted to $1x10^6$, $5x10^5$, $1x10^5$, $2.5x10^4$, $1x10^4$, $5x10^4$ and $1x10^3$ cells prior to lysis. Molecular weight markers indicate size of tagged uS4 protein.

Supplementary Figure 6



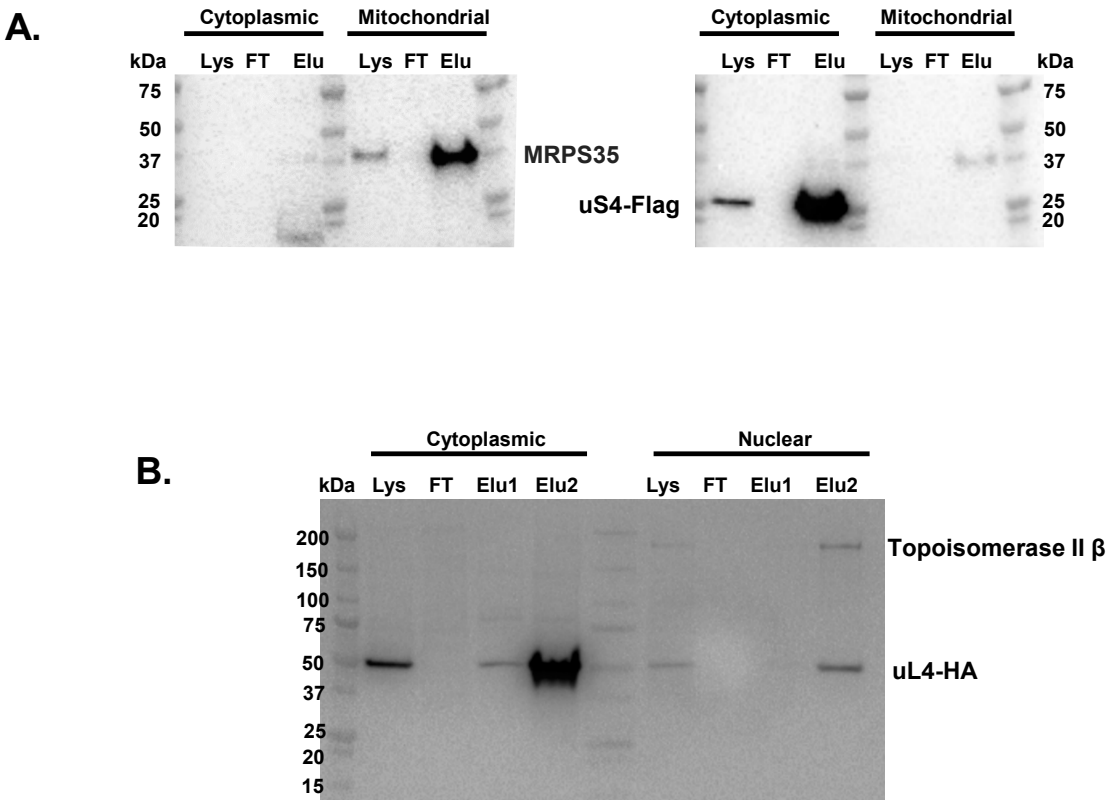
Supplementary Figure 6: Endogenous C-terminal tagging of *Plasmodium* PfRACK1 with mNeonGreen-3xHA tag. **(a)** Schematic of CRISPR/Cas9 strategy used to edit *PfRACK1* locus. **(b-d)** Agarose gels showing PCR products from genomic DNA of the cell line with the modified locus, demonstrating PCR amplicons across the 5' **(b)** and 3' **(c)** integration junction as well as the absence of the original locus **(d)**. Primers used are as indicated in **(a)** and are listed in material section. MNG: mNeonGreen; HA: hemagglutinin epitope tag; utr: untranslated region; yDHODH: yeast dihydroorotate dehydrogenase selectable marker; cam: calmodulin promoter; NF54-Wt: wild type parental line; -ve: no template negative control.

Supplementary Figure 7



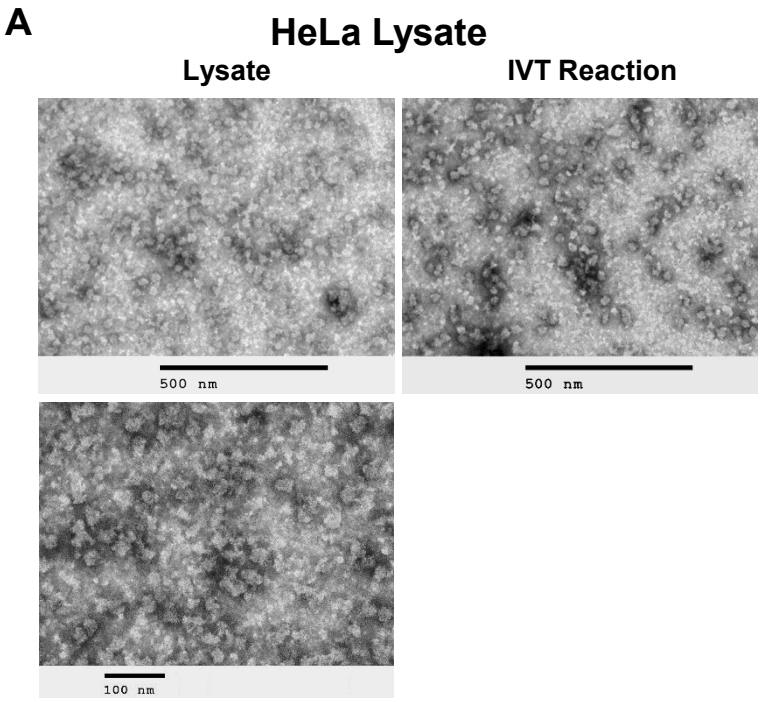
Supplementary Figure 7. RAPPL can purify ribosomes from limited P. falciparum. Western blot analysis using α*Pf* uS11 antibody on RAPPL eluates of P. falciparum NF54 cells. Starting cells were diluted to $5x10^7$, $1x10^7$, $5x10^6$, and $1x10^6$ cells before RAPPL procedure was executed. 20% of total bound fraction was used for analysis. Molecular weight markers indicate size of tagged Pf uS11 protein.

Supplementary Figure 8



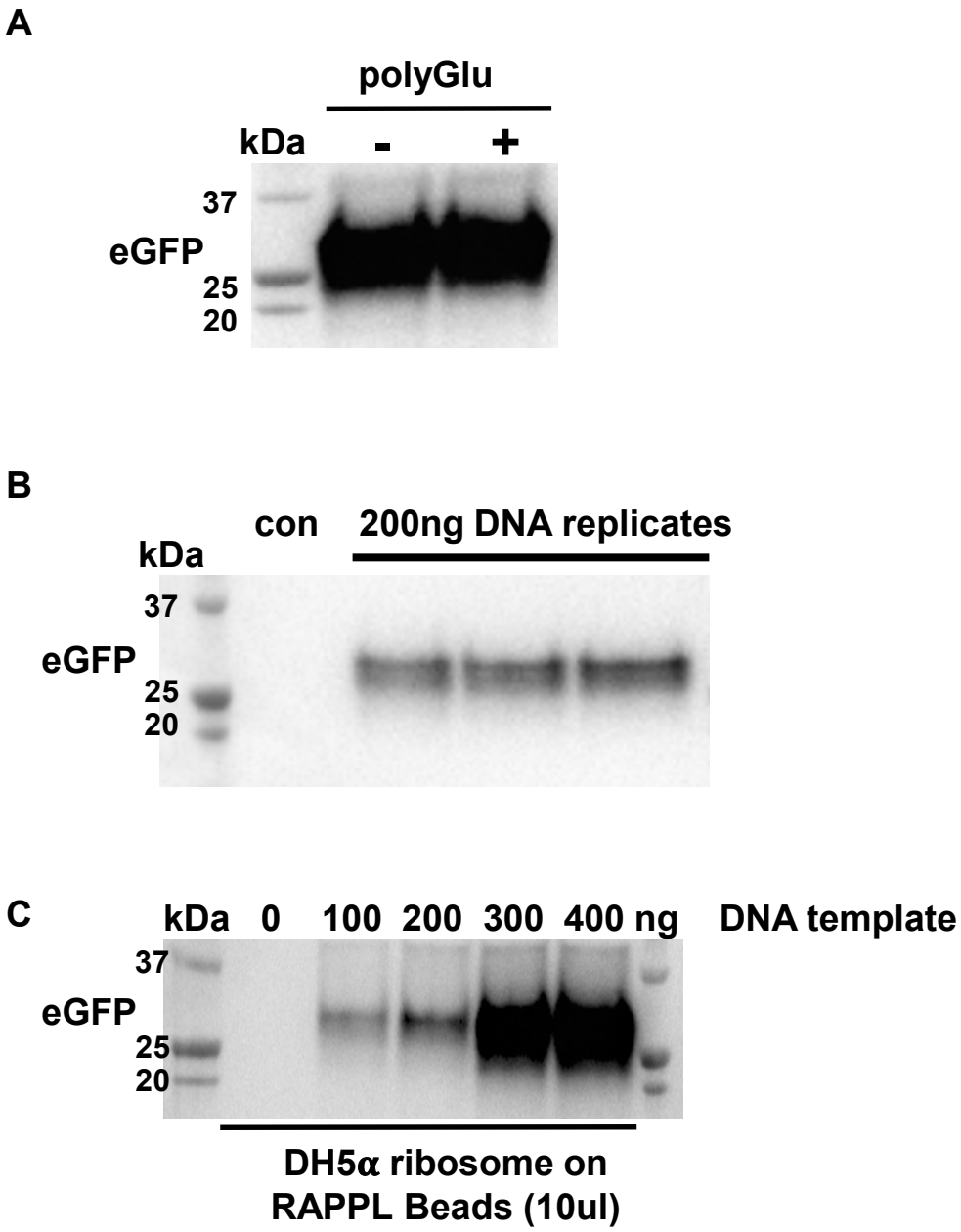
Supplementary Figure 8. RAPPL can purify and enrich ribosomes from lysates of different organelles. A. Western blot analysis using αMRPS35 and αFlag antibody on RAPPL eluates from mitochondrial and cytoplasmic fractions isolated from HEK-293 cells in which uS4 was Flag-tagged by CRISPR/Cas9. **B.** Western blot analysis using αTopoisomerase II β and αHA antibody on RAPPL eluates from cytoplasmic and nuclear fractions isolated from HEK-293 cells in which uL4 was HA-tagged by CRISPR/Cas9. Lysate (Lys), flow-through (FT), and RAPPL elution (Elu) are indicated in the figures Molecular weight markers indicate size of tagged uS-4. MRPS25, uL4 and Topoiosmerase II β protein.

Supplementary Figure 9



Supplementary Figure 9. RAPPL can isolate non-translating and translating ribosomes from commercial cell lysates. A. Thermo Scientific 1-Step IVT HeLa lysates in absence and presence of *in vitro* synthesized mCherry mRNA (IVT reaction). **B.** Promega TnT® Coupled Wheat Germ Lysate in absence and presence of *in vitro* synthesized mCherry mRNA (IVT reaction). All samples were subject to RAPPL and the eluates visualized by TEM. The scale bar represents 100 or 500 nm.

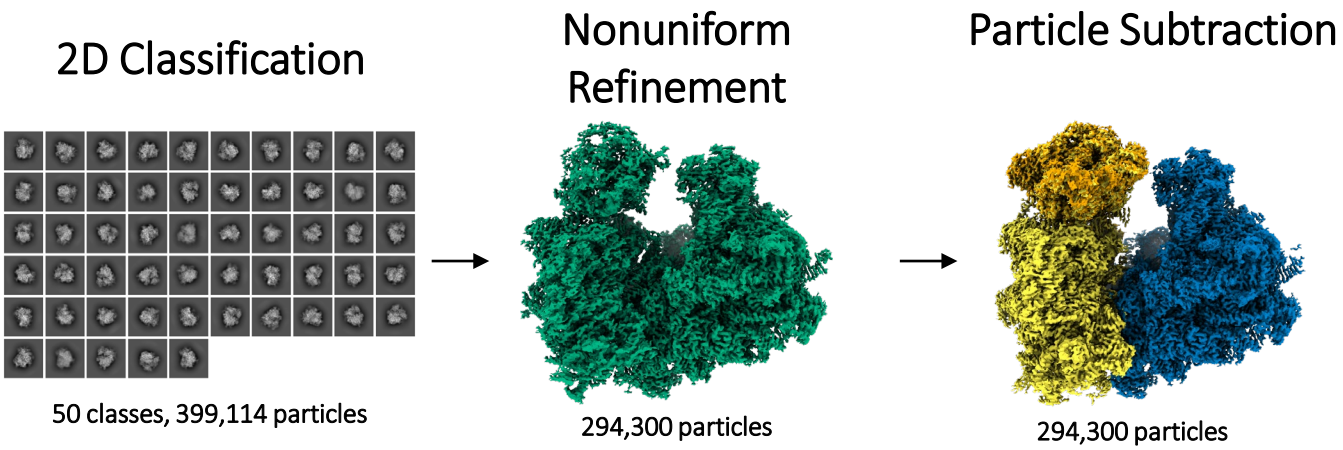
Supplementary Figure 10



Supplementary Figure 10. RAPPL purified *E. coli* ribosomes are translationally active. A. Western blot analysis of NEB PURExpress *in vitro* reaction using eGFP DNA template. Reactions were executed in the absence (-) and presence (+) of poly-D-glutamate used in RAPPL elution buffer. **B.** Western blot analysis of eGFP protein synthesized in *in vitro* reactions using RAPPL isolated *E. coli Dh5α* ribosomes (1.5µg / µL). Western blot samples correspond to fluorescence reading shown in Figure 5B. **C.** PURExpress reactions in the presence of increasing amounts of eGFP DNA template and 10µL of poly-lysine (RAPPL) beads used to purify *E. coli Dh5α* ribosomes. αeGFP antibody was used to visualize synthesis of eGFP protein. Molecular weight markers indicate size of eGFP protein.

Supplementary Figure 11

STATE	Partially Rotated
Data collection statistics	
Microscope	Titan Krios G3 300kV Cryo-TEM
Camera	Gatan K2 Summit on GIF filter
Voltage (kV)	300
Magnification	59kx
Total dose (e-/Å^2)	55
Defocus range (μm)	-0.6 to -2.0μm
Exposure Time (s)	11.63
Dose Rate (e/px/s)	5.95
Fractions	50
Calibrated pixel size (Å)	1.122
Micrographs collected	2,498
Initial number of particles	646683
Refined particles	294300
Symmetry	C1
Map resolution (Å)	2.7



Supplementary Figure 11. CryoEM processing of RAPPL purified and structurally characterized *C. neoformans* ribosomes. Table representing cryoEM dataset collection parameters. Images indicate 2D classification, nonuniform refinement, and particle subtraction particle numbers.