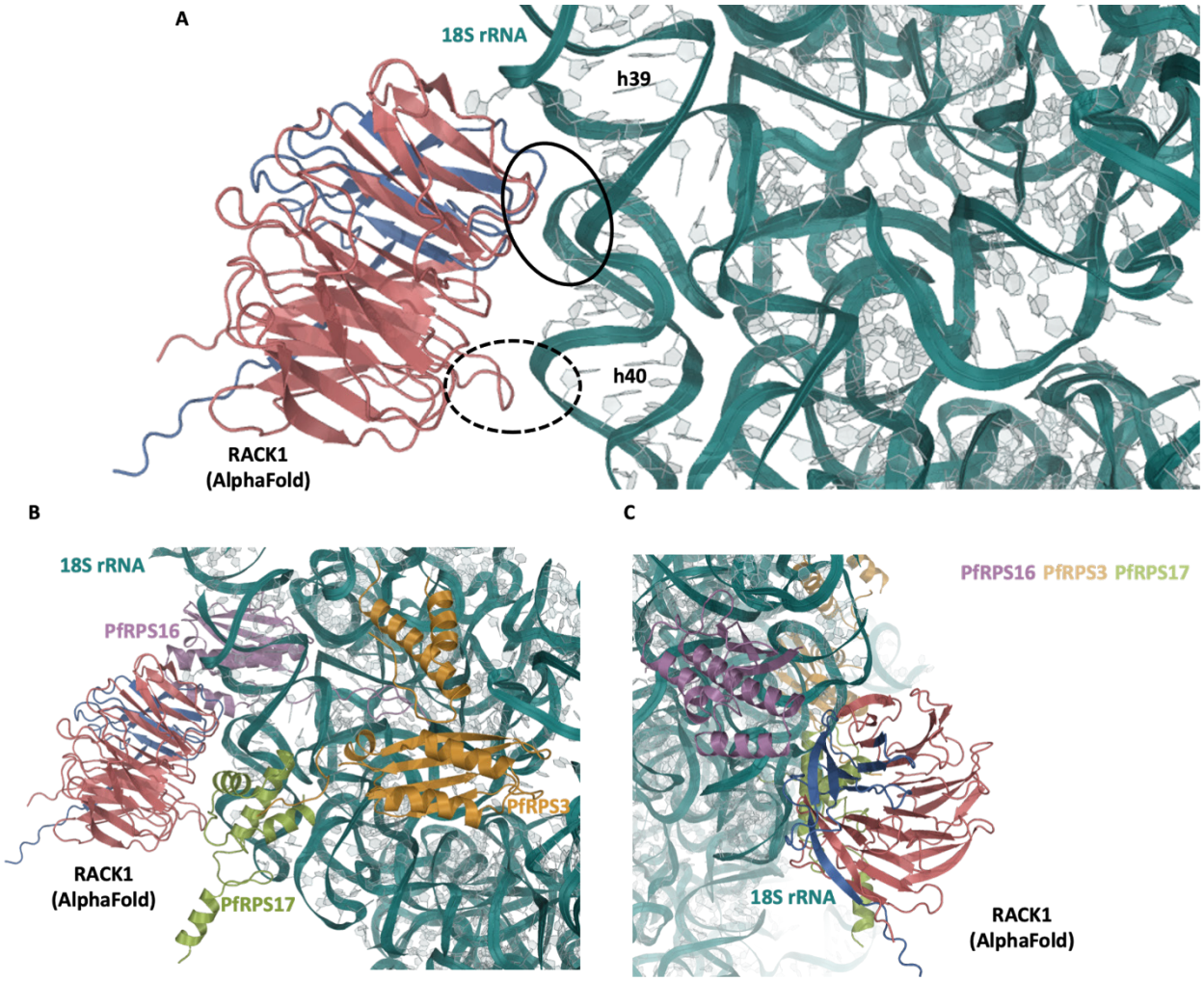


SUPPLEMENTAL FIGURE 1. STRUCTURAL ANALYSIS OF THE PFRACK1 ALPHAFOLD PROTEIN MODEL BINDING TO *P. FALCIPARUM* 40S RIBOSOME SUBUNIT. All structures displayed are P falciparum proteins/RNA. **(A)** The structure of the *P. falciparum* 80S ribosome (RCSB PDB ID: 3jbo) was structurally aligned with the human 80S ribosome (RCSB PDB ID: 3jag). The model of PfRACK1 was obtain from the AlphaFold protein database and structurally aligned with that of the human 80S bound RACK1. The view shown is that which been previously published arguing for the importance of the regions noted in RACK1 binding to the ribosome. Solid circle: region vital for HsRACK1:Hs80S binding. Dotted circle: Loop region that varies highly species to species, and while not required, may influence binding. **(B)** The same view as above, however ribosomal binding proteins with potential interactions are shown. **(C)** A 180-degree flipped view to show additional RPS16 (uS9) and RACK1 interactions. Note: Region in blue is that when exchanged between human and P. falciparum enabled binding of parasite RACK1 to the human 40S ribosome.

RPS3 (uS3) Alignment

```
HsRPS3      MAVQISKKRKFVADGIFKAELNEFLTRELAEDGYSGVEVRVTPTRTEIIILATRTQNVLG   60
PfRPS3      MSAPISKKRKFINDGVFQAELNEFLARILAEDGYSGVEVRVTPIRTEVIIRATRTREVLG   60
            *:. *******: **:*:*******:* *************** ***:** ****::***

HsRPS3      EKGRRIRELTAVVQKRF-GFPEGSVELYAEKVATRGLCAIAQAESLRYKLLGGLAVRRAC   119
PfRPS3      DKGRRIRELTSLVQKRFFNKSTNSVELFAERVEHRGLCAMAQAESLRYKLLKGLAVRRAC   120
            :*********::***** .   .****:**:*  *****:*********** ********

HsRPS3      YGVLRFIMESGAKGCEVVVSGKLRGQRAKSMKFVDGLMIHSGDPVNYYVDTAVRHVLLRQ   179
PfRPS3      YGVLRHIMESGAKGCEVIVSGKLRAQRAKSMKFRDGYLISTGEPSKRFVNTATRSAQLKQ   180
            *****.***********:******.******** ** :* :*:* : :*:**.* . *:*

HsRPS3      GVLGIKVKIMLPWDPTGKIGPKKPLPDHVSIVEPKDEILPTTPISEQKGGKPEPPAMPQP   239
PfRPS3      GVLGIKVKIMLPTAIDTRTGLTSILPDNISVLEPKTDTVDL------------------   221
            ************      : * .. ***::*::*** : :

HsRPS3      VPTA        243
PfRPS3      ----        221
```

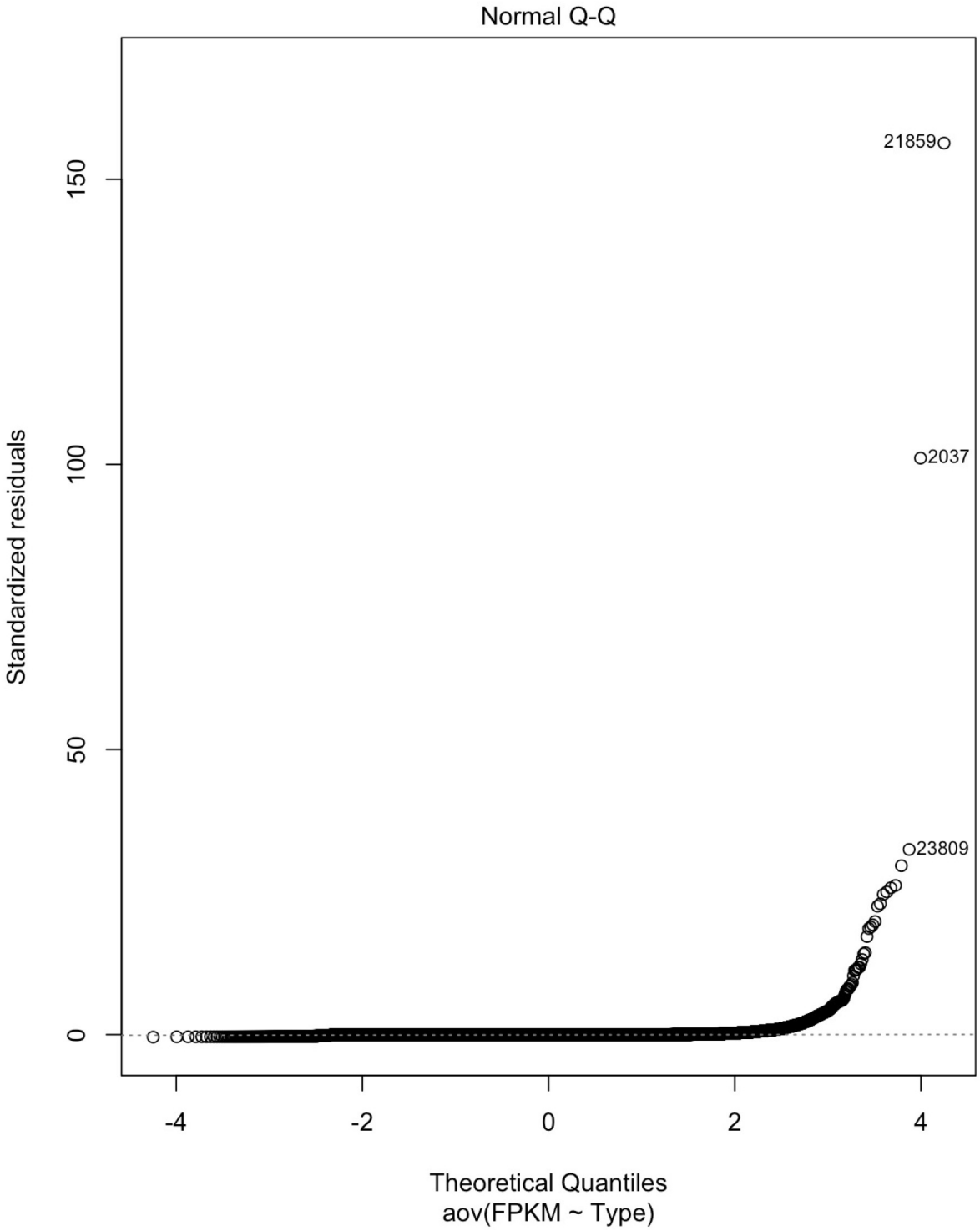
RPS16 (uS9) Alignment

```
HsRPS16     MPSKGPLQSVQVFGRKKTATAVAHCKRGNGLIKVNGRPLEMIEPRTLQYKLLEPVLLLGK   60
PfRPS16     --MTTKVKRVQTFGKKKTAVAVATVTNGKGLIKLNGKNLDLVEPYILKTKVYEPLWLIGS   58
               .  :: **.**:****.***  ..*:****:**: *:::**  *: *: **: *:*.

HsRPS16     ERFAGVDIRVRVKGGGHVAQIYAIRQSISKALVAYYQKYVDEASKKEIKDILIQYDRTLL   120
PfRPS16     GKLKNLDIRIRVKGGGQTSQIYAIRQAIGKGIISYYQKYVDESTKKELKDVLLRYDRSLL   118
             :: .:***:******:.:*******:*.*.:::********:***:**:*::***:**

HsRPS16     VADPRRCESKKFGGPGARARYQKSYR 146
PfRPS16     VGDTRRCEPKKFGGKGARARYQKSYR 144
            *.* **** ***** **********
```

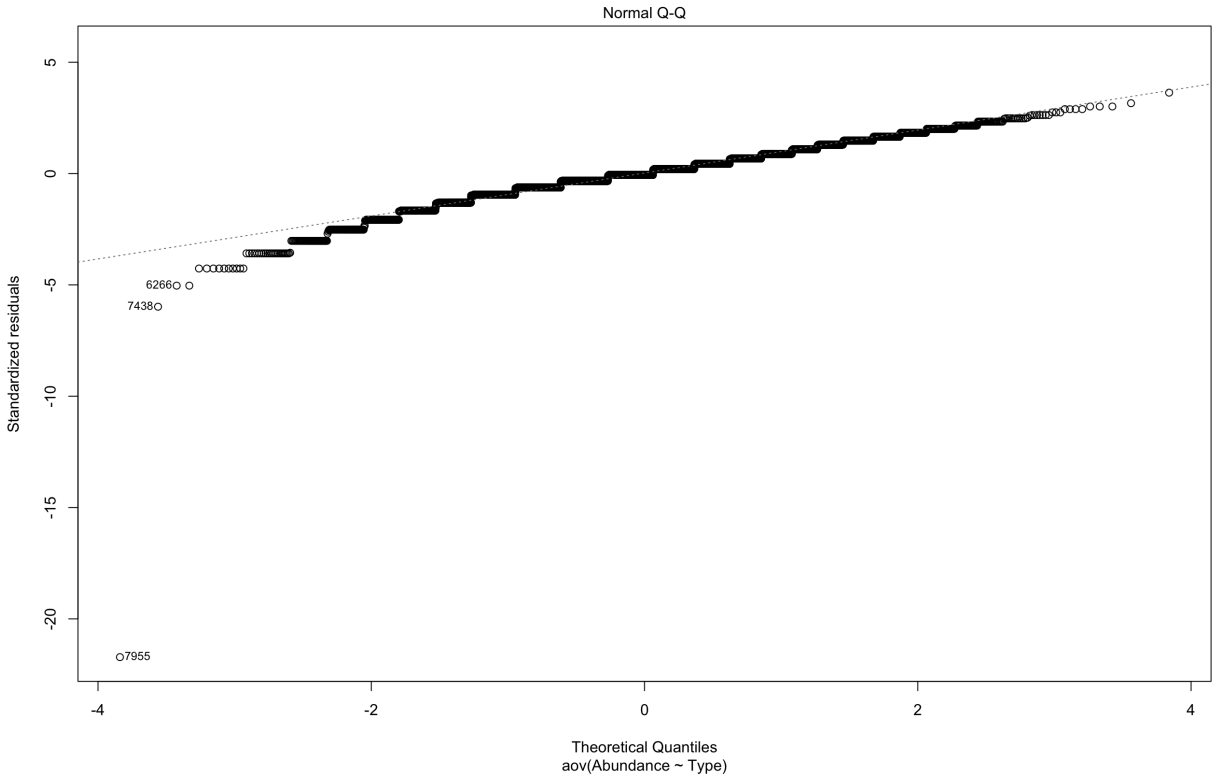
RPS17 (eS17) Alignment

```
HsRPS17     MGRVRTKTVKKAARVIIEKYYTRLGNDFHTNKRVCEEIAIIPSKKLRNKIAGYVTHLMKR   60
PfRPS17     MGRVRTKTIKRAARQIVEKYYAKLTLDFQINKKITEEVAIIPSKRMKNKVAGFVTHLMKR   60
            ********:*:*** *:*****::*  **: **:: **:*******:::**:**:*******

HsRPS17     IQRGPVRGISIKLQEEERERRDNYVPEVSALDQEIIEVDPDTKEMLKLLDFGSLSNLQVT   120
PfRPS17     IQKGPVRGISLKLQEEERERRLDFVPEKSQIDVSVIYVEPDTLRMIKSLGIN-ISNMKVH   119
            **:*******:********** ::*** * :* .:* *:*** .*:* *.:. :**::*

HsRPS17     QPTVGMNFKTPRGPV--- 135
PfRPS17     NPMINTNQQKQNRMNNQF 137
            :* :. * :. .
```

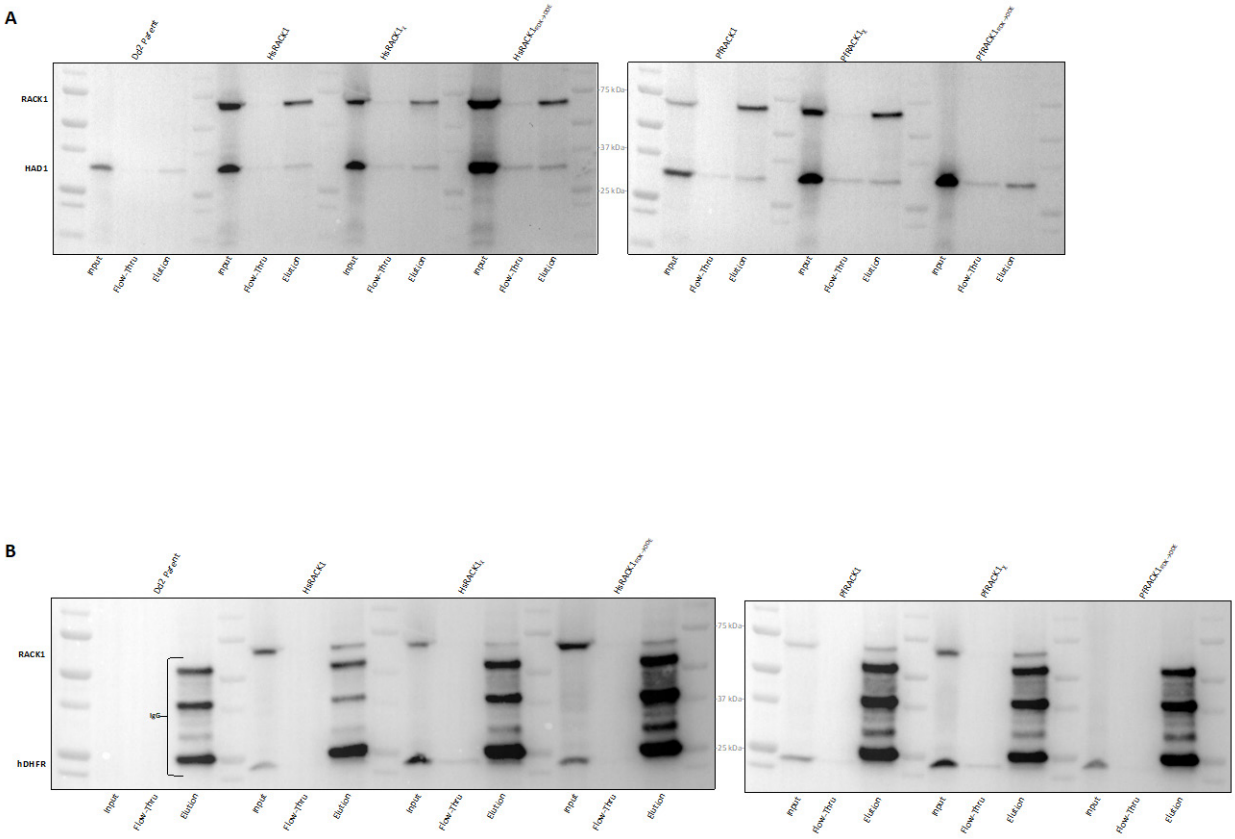
SUPPLEMENTAL FIGURE 2. MULTIPLE SEQUENCE ALIGNMENT OF *H. SAPIENS* AND *P. FALCIPARUM* RPS3 (US3), RPS16 (US9), RPS17 (ES17) PROTEINS. The *H. sapiens* and *P. falciparum* sequences were collected from the UniProt database[52] and PlasmoDB[31], respectively, and aligned using the Clustal Ω MSA tool[53]. Residues in the 3-5 Å range were determined using PyMol.



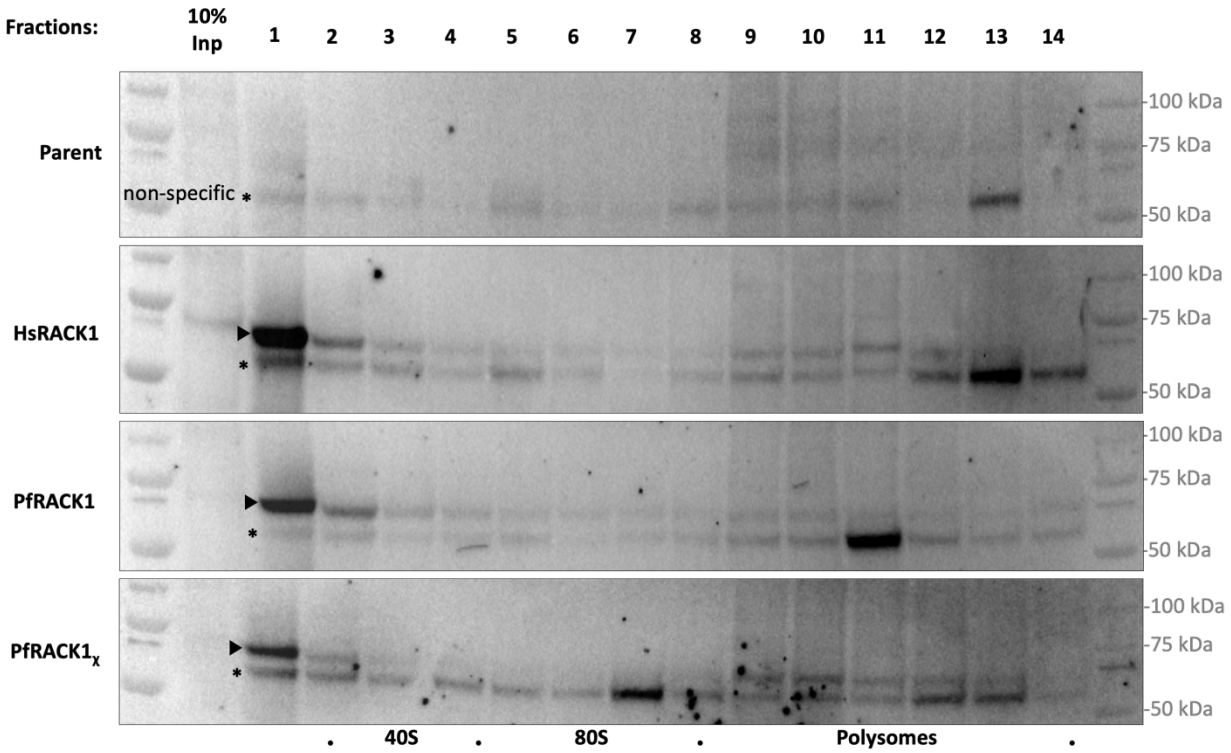
SUPPLEMENTAL FIGURE 3. PLOT OF RNASEQ DATA NORMALITY.



SUPPLEMENTAL FIGURE 4. PLOT OF PROTEIN MASS SPECTROMETRY DATA NORMALITY.

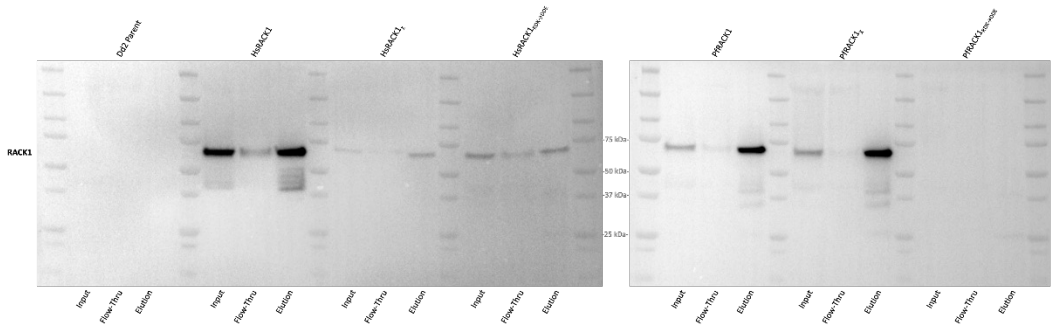


SUPPLEMENTAL FIGURE5. HA-IMMUNOPRECIPITATION OF RACK1 VARIANTS EXPRESSED IN *P. FALCIPARUM* DD2. **(A)** Samples were blotted with mouse anti-HA-HRP antibody and rabbit anti-PfHAD1/anti-rabbit-HRP. **(B)** Samples were blotted with mouse anti-HA and mouse anti-hDHFR antibodies and subsequently blotted with anti-mouse-HRP antibody demonstrating presence of plasmid resistance containing PfRACK1$_{RDK\rightarrow DDE}$ mutant.

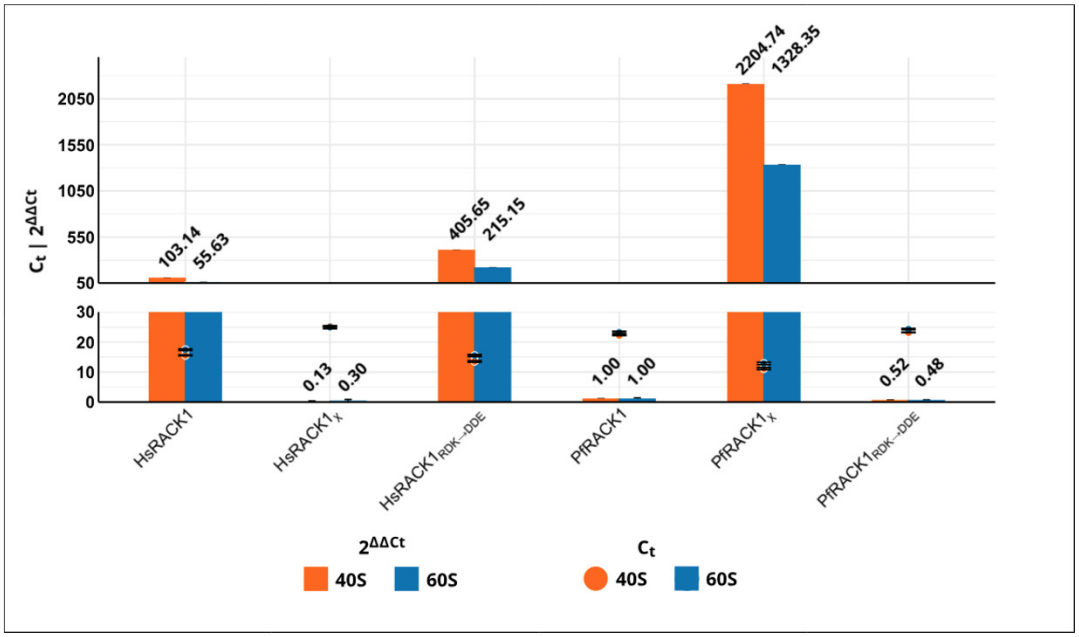


SUPPLEMENTAL FIGURE 6. POLYSOME PROFILING OF HSRACK1, PFRACK1, AND PFRACK1$_X$ VARIANTS IN *P. FALCIPARUM* DD2 PARASITES. Polysome profiling fractions generated from *P. falciparum* parasites expressing HsRACK1, PfRACK1, and PfRACK1$_\chi$ variants were analyzed. Western blot shows RACK1 variant polysome fraction localization. RT-qPCR analysis shows localization of 18S and 28S rRNAs associated with the 40S and 80S subunits, respectively. Asterisk indicates non-specific band found in parent line not correlating to RACK1 variants. Arrowhead indicates RACK1 variant band.

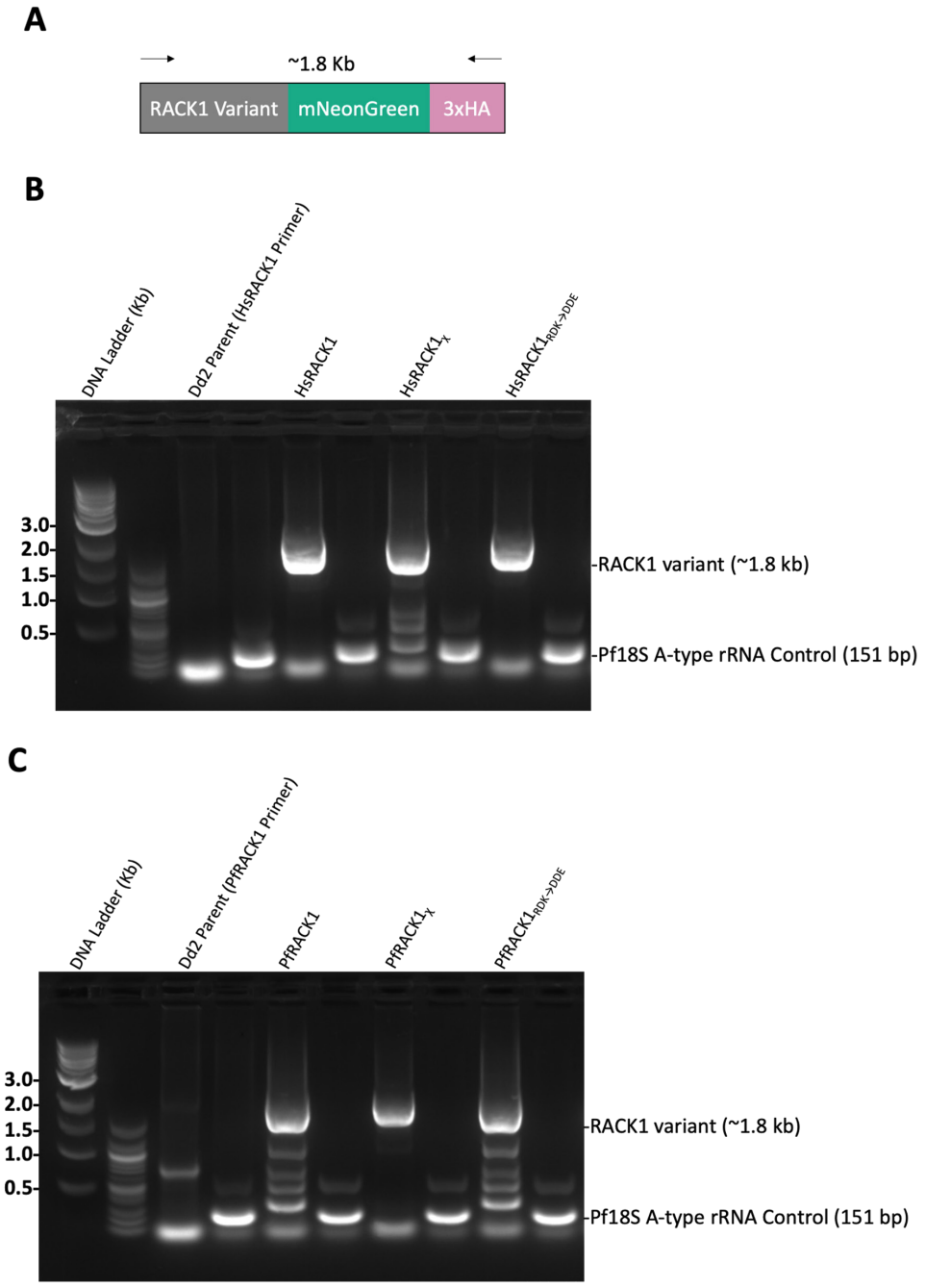
A



B



SUPPLEMENTAL FIGURE 7. HA-IMMUNOPRECIPITATION OF RACK1 VARIANTS BOUND TO RRNA EXPRESSED IN *P. FALCIPARUM* DD2. (A) Samples were blotted with mouse anti-HA-HRP antibody to show enrichment of HA-tagged RACK1 variants. **(B)** RT-qPCR was performed on RNA isolated from HA-immunoprecipitations of parasites expressing the RACK1 variants to examine rRNA binding. Samples were normalized to Dd2 parent line and then to the PfRACK1 wild type variant. The Ct (scatter plot) is displayed for each variant and the $2^{-\Delta\Delta Ct}$ (bar graph) was calculated and plotted for each RACK1 variant.



Supplemental Figure 8. Genotyping PCR of RACK1 variants expressed in *P. falciparum* Dd2. Total DNA was isolated from *P. falciparum* Dd2 parent line and lines expressing RACK1 variants. Genotyping PCRs generating a product schematized in (**A**) were performed on *P. falciparum* Dd2 parent and **(B)** HsRACK1 lines and **(C)** PfRACK1 lines. Note: PfRACK1 variants are cDNAs recoded for expression in mammalian cells whose sequences differ from the endogenous wild-type. Lanes following RACK1 variant PCR product (~1.8 Kb) is a PCR using 18S rRNA qPCR primers as a control (151 bp).

List of RACK1 sequences used in this manuscript. All sequences are cDNA version without introns.

>recoded P. falciparum RACK1
ATGATGGACAACATCAAAGAGGCCGAGATCAGCCTGCGGGGAGTTCTGGAAGGCGGACACTCTGATTG
GGTCACCAGCGTGTCCACACCTACAGACCCCAAGCTGAAAACCATCGTGTCCGCCAGCCGGGACAAGAA
ACTGATCGTGTGGAACATCAACACCGACGACGACAGCGGCGAGATCGGCACAGCCAAGAAGTCTCTGA
CAGGCCACAGCCAGGCCATCAACGACGTGTCCATCTCTAGCGACGGCCTGTTTGCCCTGAGCGGCTCTT
GGGATCACAGCGTCAGACTGTGGGATCTGAGCCTGGGCGAGACAATCAGAAGCTTCATCGGCCACACC
AGCGACGTGTTCTCCGTGTCTTTCAGCCCCGACAACCGGCAGATCGTGTCTGCCTCCAGAGACAAGACC
ATCAAGCTGTGGAACACACTGGCCCAGTGCAAGTACACCATCACCGACCAGCAGCACACCGACTGGATC
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AAGTGTGGAACCTGAAGAACTGCGACCTGAACAAGAACCTGGAAGGCCACACCGGCGTGCTGAACACC
GTGACAATTAGCCCTGATGGCAGCCTGTGTGCCAGCGGCGGAAAAGATGGCGTGGCAAAGCTGTGGGA
CGTGAAAGAGGGCAAGCACCTGTACAGCCTGGAAACCGGCAGCACCATCAACAGCCTGTGCTTCAGCCC
CTGCGACTACTGGCTGTGTGCCGCCACCGACCGGTTCATCAGAATCTGGAACCTCGAGAGCAAGCTGAT
CATCAGCGAGATCTACCCCGTGAAGCAGAGCAAGATCGGCGTGCCATGGTGTACCAGCCTGACTTGGA
GCGCCAATGGCCAGCTGCTGTACTGTGGCAGCACCGACGGCAACATCTACGTGTACGAAGTGAAGAAG
CACAGCGTC

>recoded P. falciparum RACK1 RDK->DDE mutation
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GGTCACCAGCGTGTCCACACCTACAGACCCCAAGCTGAAAACCATCGTGTCCGCCAGCGACGACGAGAA
ACTGATCGTGTGGAACATCAACACCGACGACGACAGCGGCGAGATCGGCACAGCCAAGAAGTCTCTGA
CAGGCCACAGCCAGGCCATCAACGACGTGTCCATCTCTAGCGACGGCCTGTTTGCCCTGAGCGGCTCTT
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CGTGAAAGAGGGCAAGCACCTGTACAGCCTGGAAACCGGCAGCACCATCAACAGCCTGTGCTTCAGCCC
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CATCAGCGAGATCTACCCCGTGAAGCAGAGCAAGATCGGCGTGCCATGGTGTACCAGCCTGACTTGGA
GCGCCAATGGCCAGCTGCTGTACTGTGGCAGCACCGACGGCAACATCTACGTGTACGAAGTGAAGAAG
CACAGCGTC

>recoded P. falciparum chimera (Human N-terminus, P. falciparum C-terminus)
ATGACTGAGCAGATGACCCTTCGTGGCACCCTCAAGGGCCACAACGGCTGGGTAACCCAGATCGCTACT
ACCCCGCAGTTCCCGGACATGATCCTCTCCGCCTCTCGAGATAAGACCATCATCATGTGGAAACTGACCA
GGGATGAGACCAACTATGGAATTCCACAGCGTGCTCTGCGGGGTCACTCCCACTTTGTTAGTGATGTGG
TTATCTCCTCAGATGGCCAGTTTGCCCTCAGCGGCTCTTGGGATCACAGCGTCAGACTGTGGGATCTGAG
CCTGGGCGAGACAATCAGAAGCTTCATCGGCCACACCAGCGACGTGTTCTCCGTGTCTTTCAGCCCCGAC
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TACACCATCACCGACCAGCAGCACACCGACTGGATCACCTGTGTGCGGTTCAGCCCATCTCCTAACCAGG
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AAACCGGCAGCACCATCAACAGCCTGTGCTTCAGCCCCTGCGACTACTGGCTGTGTGCCGCCACCGACC
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AGATCGGCGTGCCATGGTGTACCAGCCTGACTTGGAGCGCCAATGGCCAGCTGCTGTACTGTGGCAGCA
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>Human RACK1
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>Human RACK1 RDK->DDE mutation
ATGACTGAGCAGATGACCCTTCGTGGCACCCTCAAGGGCCACAACGGCTGGGTAACCCAGATCGCTACT
ACCCCGCAGTTCCCGGACATGATCCTCTCCGCCTCTGACGATGAGACCATCATCATGTGGAAACTGACCA
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TTATCTCCTCAGATGGCCAGTTTGCCCTCTCAGGCTCCTGGGATGGAACCCTGCGCCTCTGGGATCTCAC
AACGGGCACCACCACGAGGCGATTTGTGGGCCATACCAAGGATGTGCTGAGTGTGGCCTTCTCCTCTGA
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AGCAGCAAGGCAGAACCACCCCAGTGCACCTCCCTGGCCTGGTCTGCTGATGGCCAGACTCTGTTTGCT
GGCTACACGGACAACCTGGTGCGAGTGTGGCAGGTGACCATTGGCACACGC

>Human RACK1 chimera (P. falciparum N-terminus, Human C-terminus)
ATGATGGACAACATCAAAGAGGCCGAGATCAGCCTGCGGGGAGTTCTGGAAGGCGGACACTCTGATTG
GGTCACCAGCGTGTCCACACCTACAGACCCCAAGCTGAAAACCATCGTGTCCGCCAGCCGGGACAAGAA

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