SUPPLEMENTARY DATA

SLIRP stabilizes LRPPRC via an RRM-PPR protein interface

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Table S1

	Gene	**mtDNA position**	**Number of reads**	**3′ to 5′ CLIP read sequence**
1	H-strand	269-294	>600	ACACAGACATCATAACAAAAAATTT
2	12S	1561-1594	20	CGTAACATGGTAAGTGTACTGGAAAGTGCACTTG
3	16S	2218-2249	11	CCACTACCTAAAAAATCCCAAACATATAACTG
4	MTND1	4020-4048	12	CACTACAATCTTCCTAGGAACAACATATG
5	MTND2	5222-5262	~87	AGGAGGCCTGCCCCCGCTAACCGGCTTTTTGCCCAAATGG
6	MTCO1	5959-5988	~30	ACCTATTATTCGGCGCATGAGCTGGAGTCC
7	MTCO1	6122-6152	40	CATAATCGGAGGCTTTGGCAACTGACTAGTT
8	MTCO1	6551-6582	~160	CACCACCTTCTTCGACCCCGCCGGAGGAGGAG
9	MTCO1	6848-6871	20	CACCGGCGTCAAAGTATTTAGCTG
10	MTCO1	7026-7065	>20	GCCCACTTCCACTATGTCCTATCAATAGGAGCTGTATTTG
11	MTCO2	7873-7900	>40	CATCAAATCAATTGGCCACCAATGGTAC
12	MTCO2	7976-8005	15	GGCGACCTGCGACTCCTTGACGTTGACAAT
13	MTCO2	8135-8177	>30	TTCACCGCTACACGACCGGGGGTATACTACGGTCAATGCTCTG
14	MTATP8	8372-8394	29	CAACTAAATACTACCGTATGGCC
15	MTATP8/6	8562-8592	152	CACAATCCTAGGCCTACCCGCCGCAGTACTG
16	MTATP6	8657-8685	~680	CCACCCAACAATGACTAATCAAACTAACC
17	MTATP6	8974-9002	>600	CTCATTCAACCAATAGCCCTGGCCGTACGC
18	MTCO3	9279-9322	~30	CTCCTAATGACCTCCGGCCTAGCCATGTGATTTCACTTCCACTC
19	MTCO3	9563-9597	~26	AGGCATCACCCCGCTAAATCCCCTAGAAGTCCCAC
20	MTCO3	9917-9948	~20	CGCCGCCTGATACTGGCATTTTGTAGATGTGG
21	MTND3	10161-10186	>60	ACCCCTTACGAGTGCGGCTTCGACCC
22	MTND4L	10644-10675	16	GTGCCTATTGCCATACTAGTCTTTGCCGCCTG
23	MTND4	10993-11029	~35	GGCAAGCCAACGCCACTTATCCAGTGAACCACTATCA
24	MTND4	11872-11896	>200	CACTATTAACCTACTGGGAGAACTC
25	MTND5	13682-13712	~26	CCCTACTAAACCCCATTAAACGCCTGGCAGC
26	MTCYB	14814-14842	37	CCCCATCCAACATCTCCGCATGATGAAAC
27	MTCYB	14956-14985	28	TCGAGACGTAAATTATGGCTGAATCATCCG
28	tRNA^{Pro}	15957-15991	22	CAGAGAAAAAGTCTTTAACTCCACCATTAGCACCC

Table S1. Sequences of the RNA fragments identified after CLIP performed on HEK293-FlpIn TRex cells expressing FLAG-tagged SLIRP. Positions of the RNA fragments along mtDNA and numbers of reads are indicated.

Table S2

LRPPRC-SLIRP

	Amount (ng)	Protein score	Molecular weight (Da)	Sequence coverage (%)	Amount (fmol)
Sample1					
LRPPRC	26.42	9705.80	153037.62	94.16	172.64
SLIRP	1.46	15022.65	10737.11	89.13	135.98
Ratio					1.27
Sample2					
LRPPRC	27.32	8905.34	153037.62	94.61	178.52
SLIRP	1.52	14860.53	10737.11	88.04	141.57
Ratio					1.26
Sample3					
LRPPRC	23.69	7920.79	153037.62	94.68	154.80
SLIRP	1.32	14470.08	10737.11	89.13	122.94
Ratio					1.26
Average ratio					**1.26**
LRPPRC-SLIRP-ATP6_8657-8685					
Sample1					
LRPPRC	35.91	10720.66	153037.62	94.53	234.65
SLIRP	1.82	14747.49	10737.11	79.35	169.51
Ratio					1.38
Sample2					
LRPPRC	34.47	9993.46	153037.62	92.58	225.24
SLIRP	1.80	14239.73	10737.11	90.22	167.64
Ratio					1.34
Sample3					
LRPPRC	32.96	9619.88	153037.62	93.18	215.37
SLIRP	1.80	15464.74	10737.11	90.22	167.64
Ratio					1.28
Average ratio					**1.33**

Table S2. Stoichiometry analysis of LRPPRC-SLIRP with and without RNA. The amounts were determined by absolute quantification MS in triplicates.

Figure S1

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                                                       TPRpred
Protein       PPR sequence                             P-value
---------------------------------------------------------------------
LRPPRC 128   RSCGSLLPELKLEERTEFAHRIWDTLQKLGAVYDV 162  1.9e-05
LRPPRC 163   SHYNALLKVYLQNEYKFSPTDFLAKMEEANIQPNR 197  1.6e-07
LRPPRC 198   VTYQRLIASYCNVGDIEGASKILGFMKTKDLPVTE 232  8.2e-11
LRPPRC 233   AVFSALVTGHARAGDMENAENILTVMRDAGIEPGP 267  7.9e-12
LRPPRC 268   DTYLALLNAYAEKGDIDHVKQTLEKVEKSELHLMD 302  2.6e-09
LRPPRC 303   RDLLQIIFSFSKAGYPQYVSEILEKVTCERRYIPD 337  9.4e-04
LRPPRC 405   FPLQFTLHCALLANKTDLAKALMKAVKEEGFPIRP 439  4.1e-04
LRPPRC 440   HYFWPLLVGRRKEKNVQGIIEILKGMQELGVHPDQ 474  4.2e-06
LRPPRC 712   GGYAALINLCCRHDKVEDALNLKEEFDRLDSSAVL 746  3.1e-07
LRPPRC 749   GKYVGLVRVLAKHGKLQDAINILKEMKEKDVLIKD 783  2.0e-09
LRPPRC 787   LSFFHMLNGAALRGEIETVKQLHEAIVTLGLAEPS 821  1.1e-06
LRPPRC 823   NISFPLVTVHLEKGDLSTALEVAIDCYEKYKVLPR 857  8.3e-03
LRPPRC 956   QMYYNLLKLYKINGDWQRADAVWNKIQEENVIPRE 990  1.2e-07
LRPPRC 993   LRLLAEILREGNQEVPFDVPELWYEDEKHSLNSSS 1027 3.9e-01
LRPPRC 1033 PDFQKDILIACRLNQKKGAYDIFLNAKEQNIVFNA 1067 8.3e-04
LRPPRC 1068 ETYSNLIKLLMSEDYFTQAMEVKAFAETHIKGFTL 1102 7.7e-04
LRPPRC 1105 AANSRLIITQVRRDYLKEAVTTLKTVLDQQQTPSR 1139 1.8e-03
LRPPRC 1140 LAVTRVIQALAMKGDVENIEVVQKMLNGLEDSIGL 1174 5.6e-03
LRPPRC 1178 VFINNIALAQIKNNNIDAAIENIENMLTSENKVIE 1212 4.3e-04
LRPPRC 1215 YFGLAYLFRKVIEEQLEPAVEKISIMAERLANQFA 1249 5.2e-01
LRPPRC 1252 KPVTDFFLQLVDAGKVDDARALLQRCGAIAEQTPI 1286 3.0e-03
LRPPRC 1319 EAYNSLMKSYVSEKDVTSAKALYEHLTAKNTKLDD 1353 9.6e-07
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Figure S1. Predicted PPRs in LRPPRC using TPRpred. PPR sequences flanked by amino acid numbers are indicated.

Figure S2

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                                                          Predicted
Feature     Sequence                                   recognition code
---------------------------------------------------------------------
MTS   1    MAALLRSARWLLRAGAAPRLPLSLRLLPGGPGRLHAASYLPAARAGPVAGGLLSPARLYA
Flank 61   IAAKEKDIQEESTFSSRKISNQFDWALMRLDLSVRRTGRIPKKLLQKVFNDTCRSGGLGGSHALLLL
PPR1  128  RSCGSLLPELKLEERTEFAHRIWDTLQKLGAVYDV         G
PPR2  163  SHYNALLKVYLQNEYKFSPTDFLAKMEEANIQPNR         C
PPR3  198  VTYQRLIASYCNVGDIEGASKILGFMKTKDLPVTE         c/u
PPR4  233  AVFSALVTGHARAGDMENAENILTVMRDAGIEPGP         a/u
PPR5  268  DTYLALLNAYAEKGDIDHVKQTLEKVEKSELHLMD         c/u
PPR6  303  RDLLQIIFSFSKAGYPQYVSEILEKVTCERRYIPDA        g
PPR7  339  MNLILLLVTEKLEDVALQILLACPVSKEDGPSV           c
PPR8  372  FGSFFLQHCVTMNTPVEKLTDYCKKLKEVQMHS           a
PPR9  405  FPLQFTLHCALLANKTDLAKALMKAVKEEGFPIRP         c
PPR10 440  HYFWPLLVGRRKEKNVQGIIEILKGMQELGVHPDQ         c/u/g/?
PPR11 475  ETYTDYVIPCFDSVNSARAILQENGCLSDSDM            G
PPR12 507  FSQAGLRSEAANGNLDFVLSFLKSNTLPIS              ?
PPR13 537  LQSIRSSLLLGFRRSMNINLWSEITELLYKDGRYCQEPRGPTE ?
PPR14 580  AVGYFLYNLIDSMSDSEVQAKEEHLRQYFHQLEKMNVKIPEN  ?
PPR15 622  NIYRGIRNLLESYHVPELIKDAHLLVESKNLDFQKTVQLTSSELESTLETLKAENQPIR ?
PPR16 679  DVLKQLILVLCSEENMQKALELKAKYESDMVT            ?
PPR17 712  GGYAALINLCCRHDKVEDALNLKEEFDRLDSSAVLDT       g/u
PPR18 749  GKYVGLVRVLAKHGKLQDAINILKEMKEKDVLIKDTTA      ?
PPR19 787  LSFFHMLNGAALRGEIETVKQLHEAIVTLGLAEPST        a/c
PPR20 823  NISFPLVTVHLEKGDLSTALEVAIDCYEKYKVLPRI        c/g
PPR21 859  HDVLCKLVEKGETDLIQKAMDFVSQEQGEMVMLYDLFFAFLQTGNYKEAKKIIETPGI  ?
PPR22 917  RARSARLQWFCDRCVANNQVETLEKLVELTQKLFECDRD     G
PPR23 956  QMYYNLLKLYKINGDWQRADAVWNKIQEENVIPRE         c/g
PPR24 991  KTLRLLAEILREGNQEVPFDVPELWYEDEKHSLNSSSASTTE  g/u
PPR25 1033 PDFQKDILIACRLNQKKGAYDIFLNAKEQNIVFNA         c
PPR26 1068 ETYSNLIKLLMSEDYFTQAMEVKAFAETHIKGFTLND       A
PPR27 1105 AANSRLIITQVRRDYLKEAVTTLKTVLDQQQTPSR         A
PPR28 1140 LAVTRVIQALAMKGDVENIEVVQKMLNGLEDSIGLSKM      a/g
PPR29 1178 VFINNIALAQIKNNNIDAAIENIENMLTSENKVIEPQY      C/u
PPR30 1216 FGLAYLFRKVIEEQLEPAVEKISIMAERLANQFAIY        a/c
PPR31 1252 KPVTDFFLQLVDAGKVDDARALLQRCGAIAEQTP          G
PPR32 1286 ILLLFLLRNSRKQGKASTVKSVLELIPELNEKE           ?
PPR33 1319 EAYNSLMKSYVSEKDVTSAKALYEHLTAKNTKLDD         U/c
Flank 1353 LFLKRYASLLKYAGEPVPFIEPPESFEFYAQQLRKLRENSS
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Figure S2. *In silico* analysis of the PPR motifs of LRPPRC. Key:
X = PPR identified by TPRpred
X = highly degenerate PPR/pair of PPRs, hard to infer function/structure
X = possible RNA specificity determining residue
UPPER CASE = good prediction
lower case = speculative prediction
? = can't predict

Figure S3

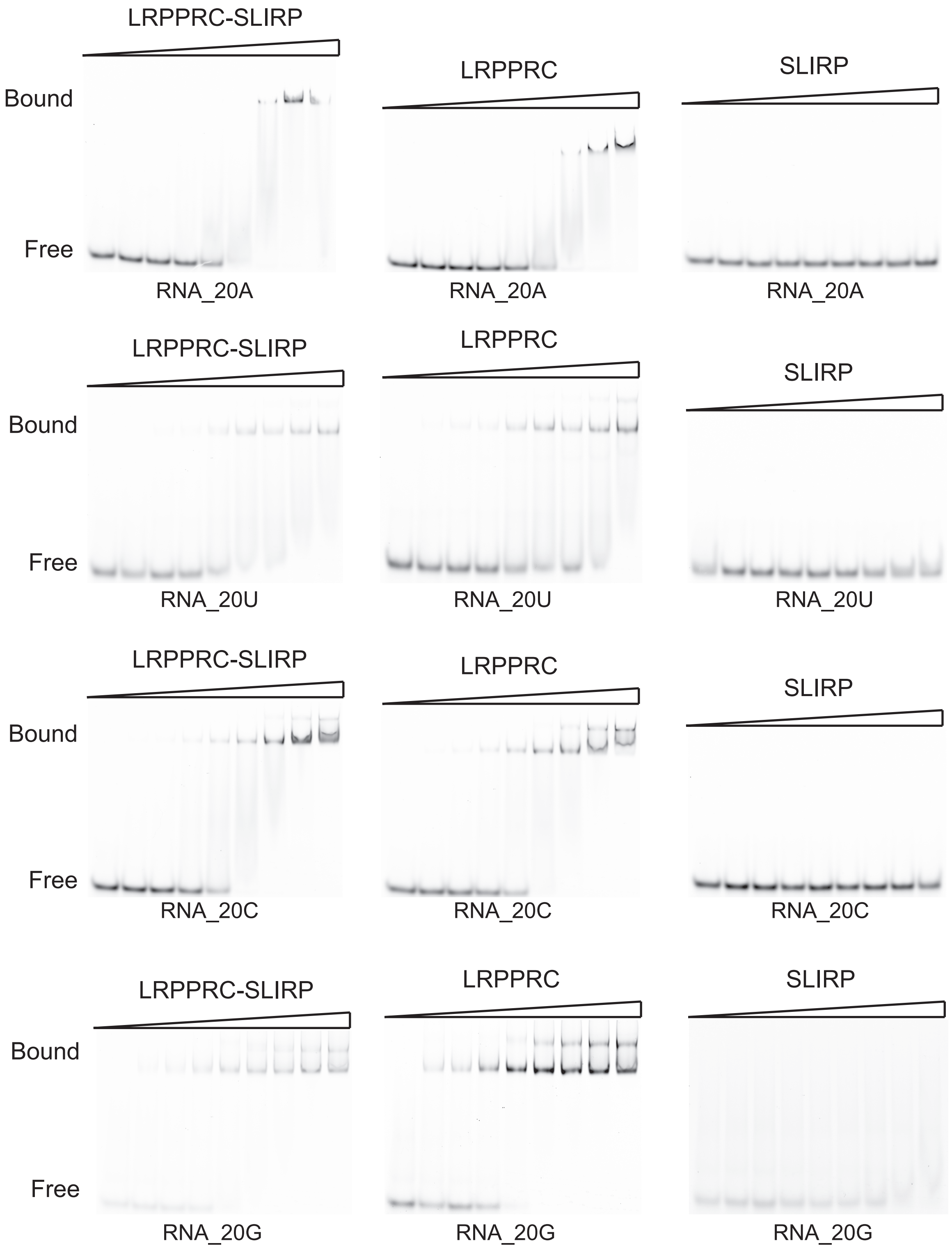

Figure S3. LRPPRC-SLIRP and LRPPRC have strong non-specific RNA binding capacity. RNA EMSA of LRPPRC-SLIRP, LRPPRC or SLIRP. RNA templates used are indicated. Protein concentrations used were 0, 0.02, 0.04, 0.08, 0.16, 0.36, 0.64, 1.28, 2.56 μM, respectively.

Figure S4

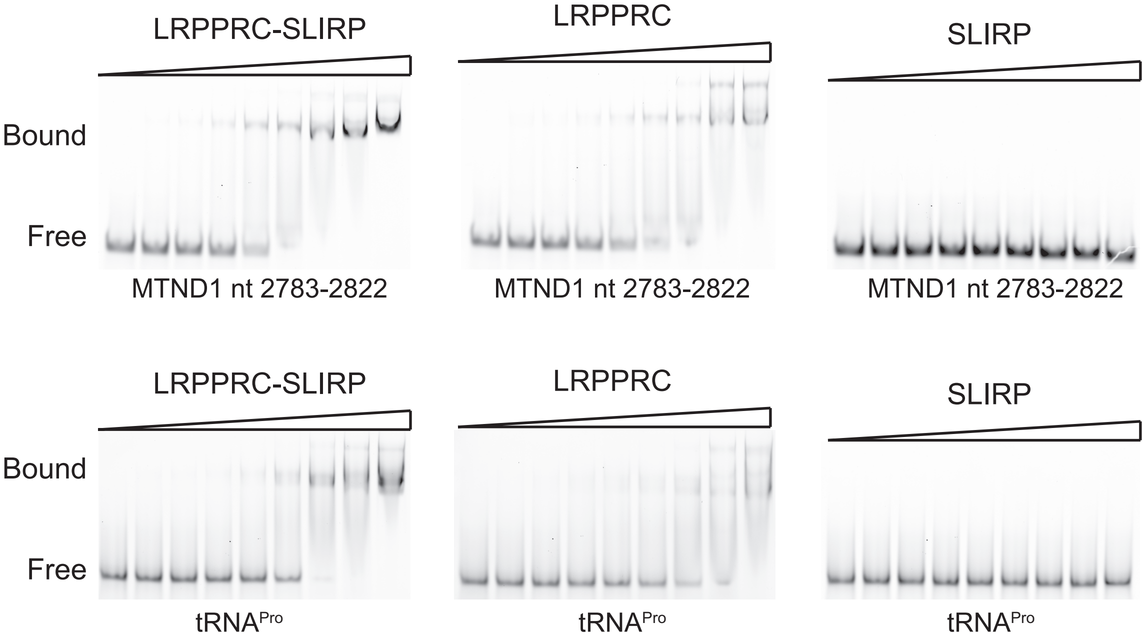

Figure S4. LRPPRC-SLIRP or LRPPRC bind both mRNA and tRNA. RNA EMSA of LRPPRC-SLIRP, LRPPRC or SLIRP. RNA templates used are indicated. Protein concentrations used were 0, 0.02, 0.04, 0.08, 0.16, 0.36, 0.64, 1.28, 2.56 μM, respectively.

Figure S5

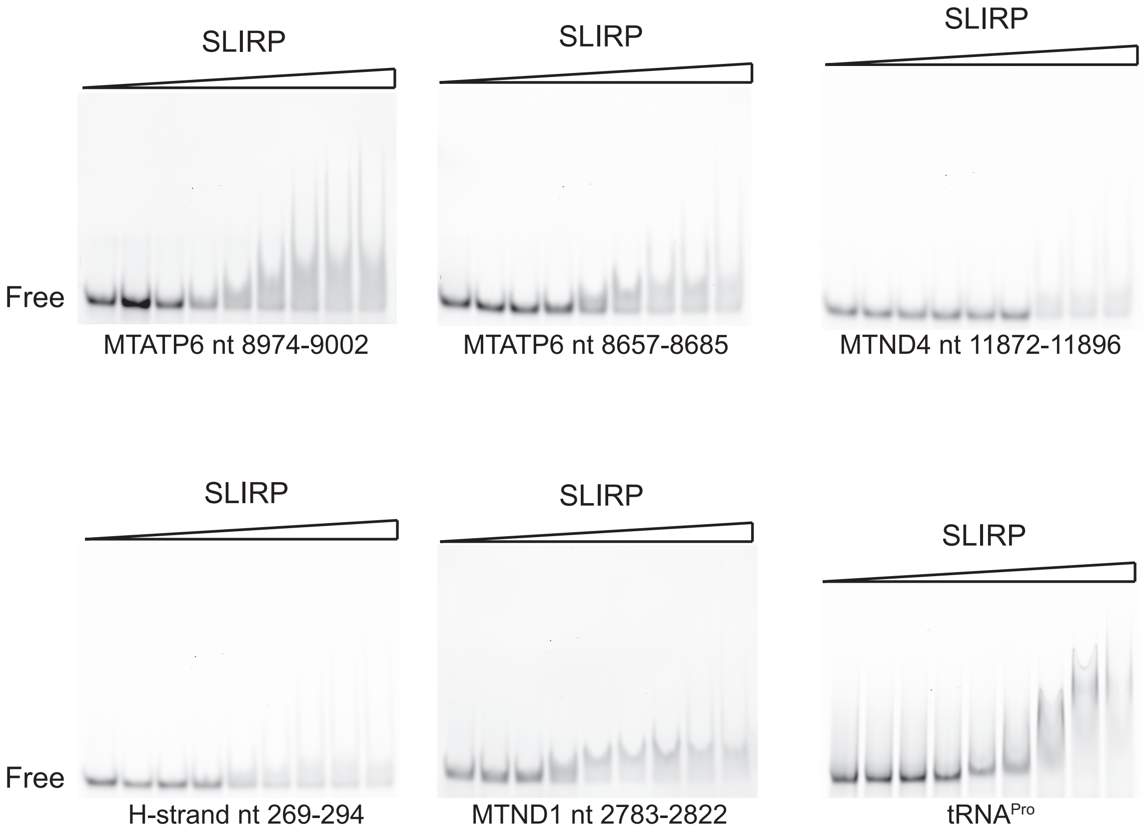

Figure S5. SLIRP binds RNA with low affinity. RNA EMSA of SLIRP with RNA templates used indicated. Protein concentrations used were 0, 0.2, 0.4, 0.8, 1.6, 3.6, 6.4, 12.8, 25.6 μM, respectively.