Supplementary Information

RNA structure drives interaction with proteins

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Sequential alignment between HBG1 and HBG2

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HBG1    1    ACACTCGCTTCTGGAACGTCTGAGGTTATCAATAAGCTCCTAGTCCAGACGCCATGGGTC  60
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
HGB2    1    ACACTCGCTTCTGGAACGTCTGAGGTTATCAATAAGCTCCTAGTCCAGACGCCATGGGTC  60

HBG1    61   ATTTCACAGAGGAGGACAAGGCTACTATCACAAGCCTGTGGGGCAAGGTGAATGTGGAAG  120
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
HGB2    61   ATTTCACAGAGGAGGACAAGGCTACTATCACAAGCCTGTGGGGCAAGGTGAATGTGGAAG  120

HBG1    121  ATGCTGGAGGAGAAACCCTGGGAAGGCTCCTGGTTGTCTACCCATGGACCCAGAGGTTCT  180
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
HGB2    121  ATGCTGGAGGAGAAACCCTGGGAAGGCTCCTGGTTGTCTACCCATGGACCCAGAGGTTCT  180

HBG1    181  TTGACAGCTTTGGCAACCTGTCCTCTGCCTCTGCCATCATGGGCAACCCCAAAGTCAAGG  240
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
HGB2    181  TTGACAGCTTTGGCAACCTGTCCTCTGCCTCTGCCATCATGGGCAACCCCAAAGTCAAGG  240

HBG1    241  CACATGGCAAGAAGGTGCTGACTTCCTTGGGAGATGCCACAAAGCACCTGGATGATCTCA  300
             |||||||||||||||||||||||||||||||||||||||||| ||||||||||||||||||
HGB2    241  CACATGGCAAGAAGGTGCTGACTTCCTTGGGAGATGCCATTAAAGCACCTGGATGATCTCA  300

HBG1    301  AGGGCACCTTTGCCCAGCTGAGTGAACTGCACTGTGACAAGCTGCATGTGGATCCTGAGA  360
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
HGB2    301  AGGGCACCTTTGCCCAGCTGAGTGAACTGCACTGTGACAAGCTGCATGTGGATCCTGAGA  360

HBG1    361  ACTTCAAGCTCCTGGGAAATGTGCTGGTGACCGTTTTGGCAATCCATTTCGGCAAAGAAT  420
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
HGB2    361  ACTTCAAGCTCCTGGGAAATGTGCTGGTGACCGTTTTGGCAATCCATTTCGGCAAAGAAT  420

HBG1    421  TCACCCCTGAGGTGCAGGCTTCCTGGCAGAAGATGGTGACTGCAGTGGCCAGTGCCCTGT  480
             |||||||||||||||||||||||||||||||||||||||||| ||||||||||||||||||
HGB2    421  TCACCCCTGAGGTGCAGGCTTCCTGGCAGAAGATGGTGACTGGAGTGGCCAGTGCCCTGT  480

HBG1    481  CCTCCAGATACCACTGAGCTCACTGCCCATGATTCAGAGCTTTCAAGGATAGGCTTTATT  540
             |||||||||||||||||||||||||||||||||| ||||||||||||||||||||||||||
HGB2    481  CCTCCAGATACCACTGAGCTCACTGCCCATGATGCAGAGCTTTCAAGGATAGGCTTTATT  540

HBG1    541  CTGCAAGCAATACAAATAATAAATCTATTCTGCTGAGAGATCAC  584
             |||||||||||| |||||||||||||||||||||| |||||||||
HGB2    541  CTGCAAGCAAT-CAAATAATAAATCTATTCTGCTAAGAGATCAC  583
```

Sequences of *BRaf* (low structured RNA) and *HSP70* (high structured RNA)

>BRaf

GCGCTGAGCGGTGGCGGTGGTGGCGGCGCGGAGCCGGGCCAGGCTCTGTTCAACGGGGACATGGAGCCCG
AGGCCGGCGCCGGCGCCGGCGCCGCGGCCTCTTCGGCTGCGGACCCTGCCATTCCGGAGGAGGTGTGGAA
TATCAAACAAATGATTAAGTTGACACAGGAACATATAGAGGCCCTATTGGACAAATTTGGTGGGGAGCAT
AATCCACCATCAATATATCTGGAGGCCTATGAAGAATACACCAGCAAGCTAGATGCACTCCAACAAAGAG
AACAACAGTTATTGGAATCTCTGGGGAACGGAACTGATTTTTCTGTTTCTAGCTCTGCATCAATGGATAC
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GATATTTCCTGGCTTACTGGAGAAGAATTGCATGTGGAAGTGTTGGAGAATGTTCCACTTACAACACACA
ACTTTGTACGAAAAACGTTTTTCACCTTAGCATTTTGTGACTTTTGTCGAAAGCTGCTTTTCCAGGGTTT
CCGCTGTCAAACATGTGGTTATAAATTTCACCAGCGTTGTAGTACAGAAGTTCCACTGATGTGTGTTAAT
TATGACCAACTTGATTTGCTGTTTGTCTCCAAGTTCTTTGAACACCACCCAATACCACAGGAAGAGGCGT
CCTTAGCAGAGACTGCCCTAACATCTGGATCATCCCCTTCCGCACCCGCCTCGGACTCTATTGGGCCCCA
AATTCTCACCAGTCCGTCTCCTTCAAAATCCATTCCAATTCCACAGCCCTTCCGACCAGCAGATGAAGAT
CATCGAAATCAATTTGGGCAACGAGACCGATCCTCATCAGCTCCCAATGTGCATATAAACACAATAGAAC
CTGTCAATATTGATGACTTGATTAGAGACCAAGGATTTCGTGGTGATGGAGGATCAACCACAGGTTTGTC
TGCTACCCCCCCTGCCTCATTACCTGGCTCACTAACTAACGTGAAAGCCTTACAGAAATCTCCAGGACCT
CAGCGAGAAAGGAAGTCATCTTCATCCTCAGAAGACAGGAATCGAATGAAAACACTTGGTAGACGGGACT
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CCATCTCCATATCATTGAGACCAAATTTGAGATGATCAAACTTATAGATATTGCACGACAGACTGCACAG
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>HSP70

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GCAAGGTGGAGATCATCGCCAACGACCAGGGCAACCGCACCACCCCCAGCTACGTGGCCTTCACGGACAC
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CGGTGCAGGCGGCCATCCTGATGGGGGACAAGTCCGAGAACGTGCAGGACCTGCTGCTGCTGGACGTGGC
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CCCACCAAGCAGACGCAGATCTTCACCACCTACTCCGACAACCAACCCGGGGTGCTGATCCAGGTGTACG
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CCCCAGGGGCGTGCCCCAGATCGAGGTGACCTTCGACATCGATGCCAACGGCATCCTGAACGTCACGGCC
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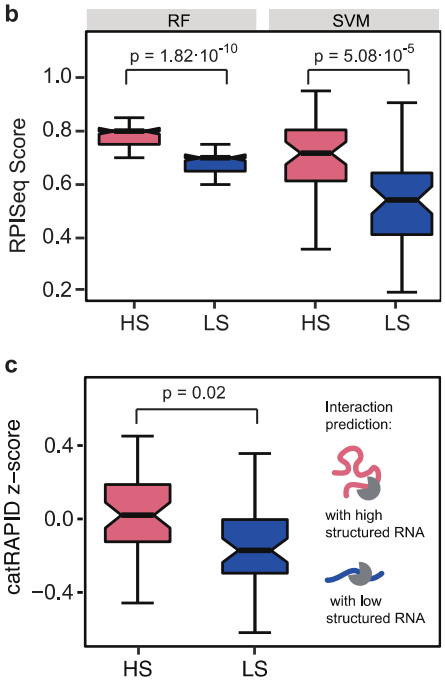
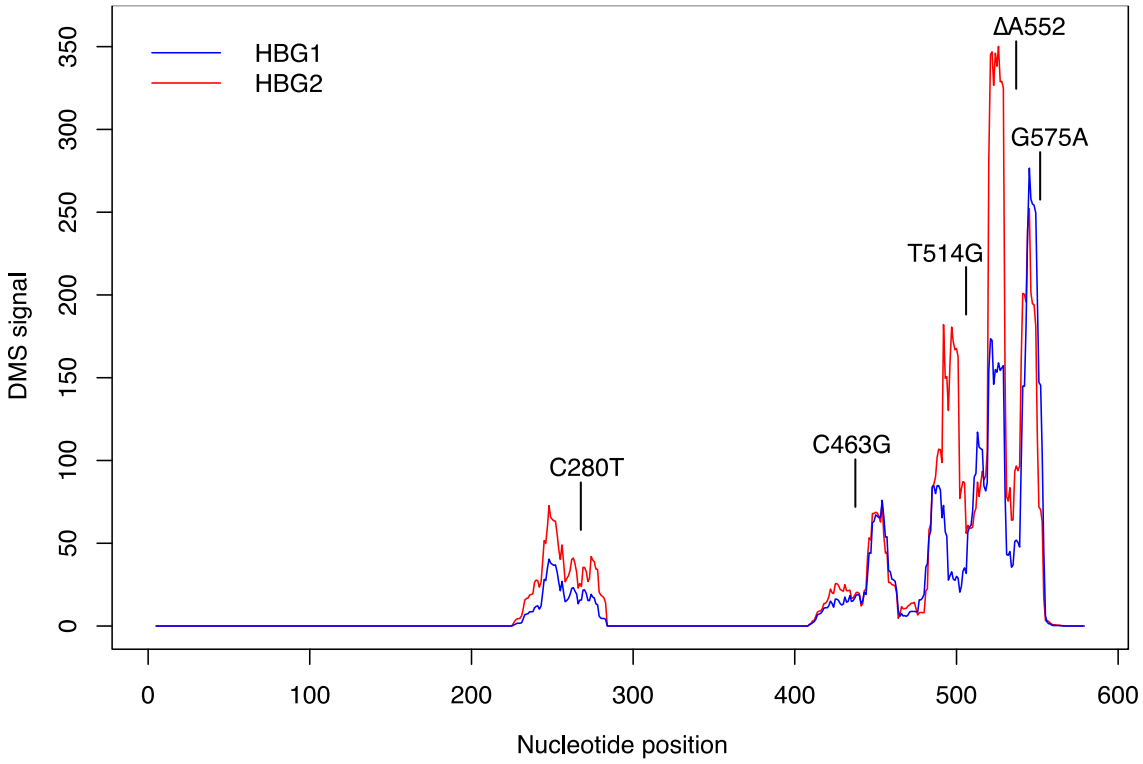
AAGATCAGCGAGGCCGACAAGAAGAAGGTGCTGGACAAGTGTCAAGAGGTCATCTCGTGGCTGGACGCCA
ACACCTTGGCCGAGAAGGACGAGTTTGAGCACAAGAGGAAGGAGCTGGAGCAGGTGTGTAACCCCATCAT
CAGCGGACTGTACCAGGGTGCCGGTGGTCCCGGGCCTGGGGGCTTCGGGGCTCAGGGTCCCAAGGGAGGG
TCTGGGTCAGGCCCCACCATTGAGGAGGTAGATTAG

a

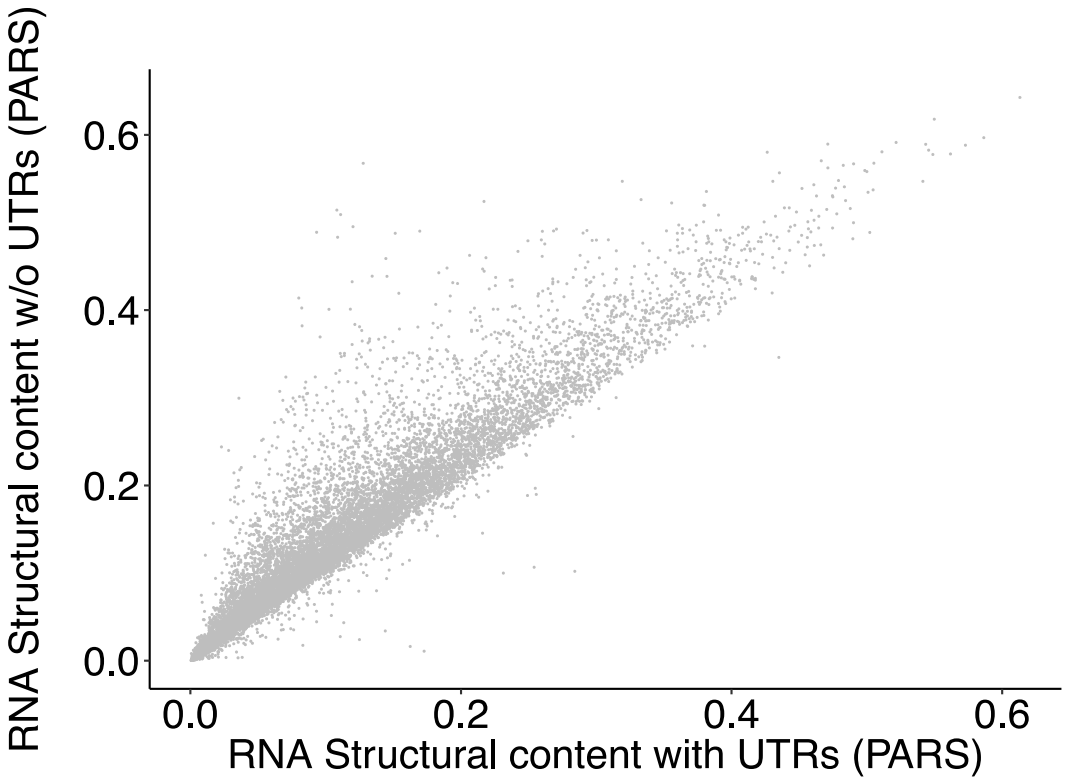
10%	3289	101178
15%	4933	143112
20%	6577	184902
25%	8222	224682
30%	9866	263598
35%	11510	303435
40%	13155	341111
45%	14799	378714
50%	16444	414929



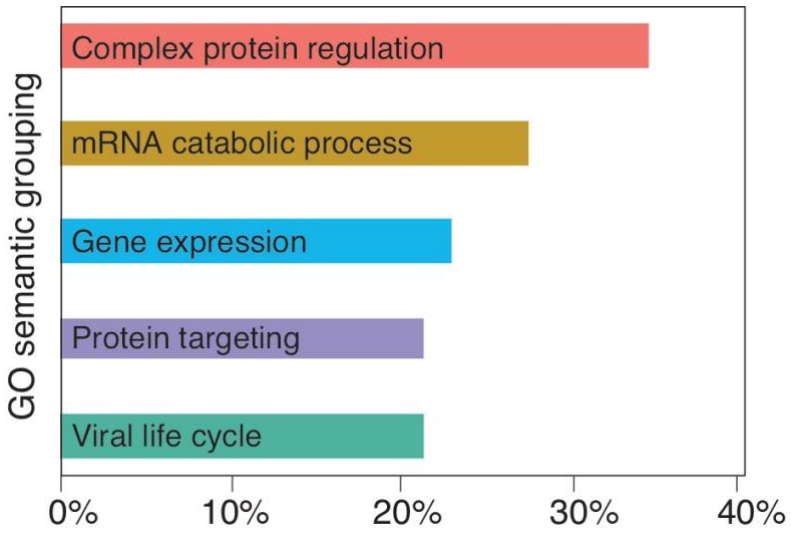
Supplementary Figure 1. RPIseq and catRAPID performances. a) Fractions (percentiles), RNAs selected for the highest-structural (HS) and lowest-structural (LS) content (equal HS and LS groups) and protein-RNA pairs reported in **Fig. 1b**. **b-c)** Interaction predictions for 50 proteins and 100 HS and LS transcripts reported in **Fig. 1b**. The boxplots show that **b)** RPIseq and **c)** catRAPID have the same trend.



Supplementary Figure 2. HBG1 and HBG2 sequential and structural differences. Comparison between the transcripts coding for HBG1 and HBG2 gamma globins.

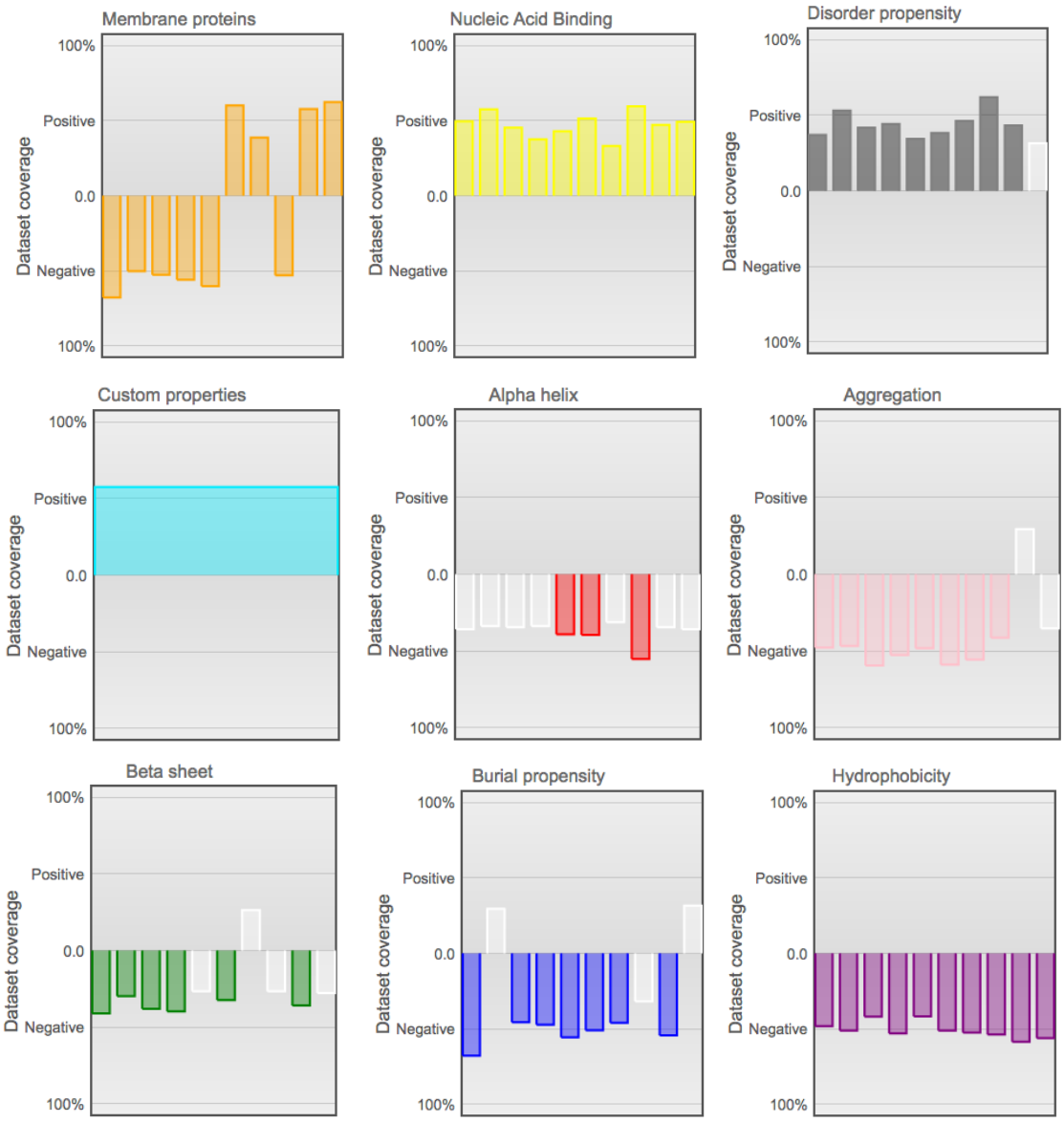


Supplementary Figure 3. Structural content per transcript with and without UTRs. We observe a transcriptome-wide correlation of 0.94 indicating that, in addition to UTRs, other regions of the RNAs are structured.



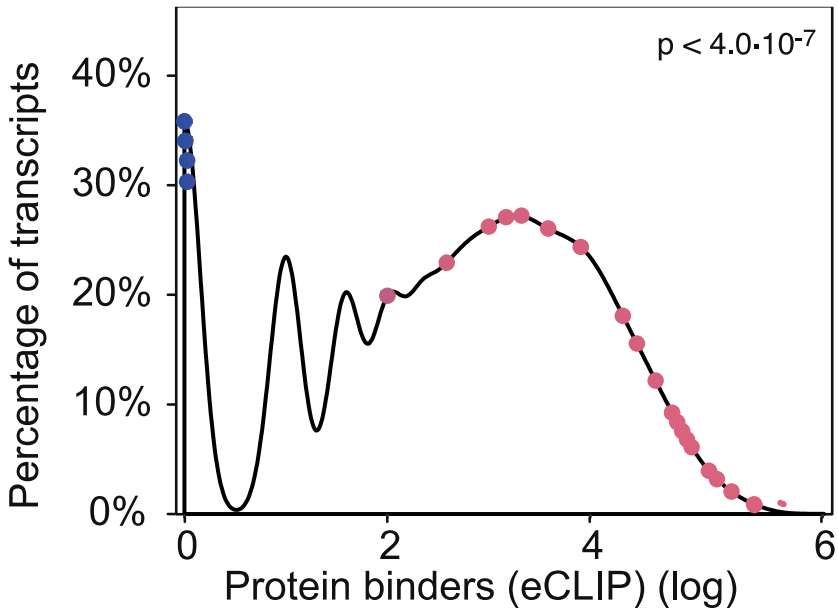
Supplementary Figure 4. Gene ontology for highly expressed RNAs. GO semantic grouping for HS against the 25% more expressed transcripts.

The data to build this image were extracted from our predictions[1] available at the webpage http://www.tartaglialab.com/GO_analyser/render_GO_universal/2500/b1c58f4a18/

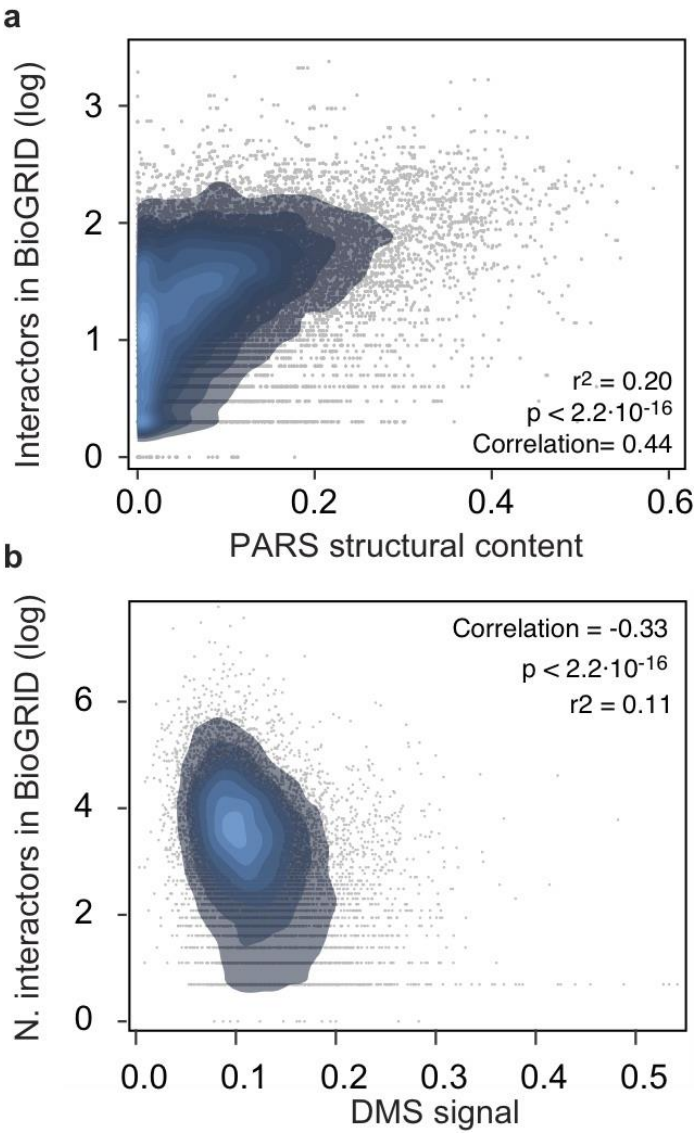


Supplementary Figure 5. Properties significantly different between ssRNA and dsRNA binders. Comparison made with cleverMachine[3]. The parameters for the plot using "custom properties" are taken from the polarity scale from *Chou* et al [2].

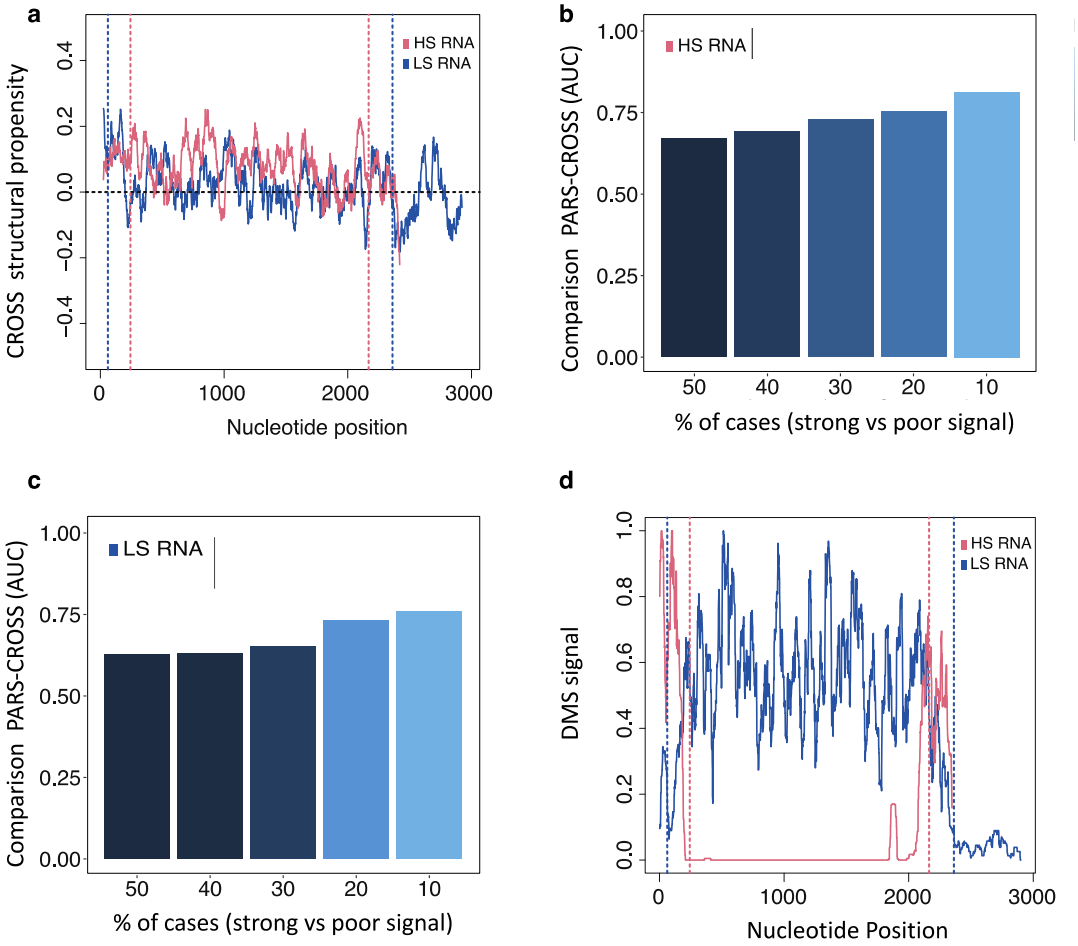
The data to build this image were extracted from our predictions[3] available at the webpage http://crg-webservice.s3.amazonaws.com/submissions/2018-04/124818/output/index.html?unlock=a798e0e111.



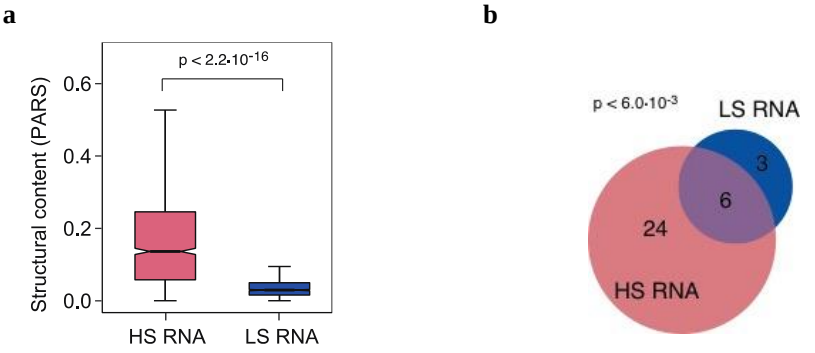
Supplementary Figure 6. RNAs coding for chaperones and RBP interactions. Fraction of proteins binding to RNA coding for chaperones as measured by eCLIP. The transcripts are represented as blue or pink dots according to their structural content (blue = low structural content and pink = high structural content; $p = 4x10^{-7}$; Kolmogorov-Smirnov).



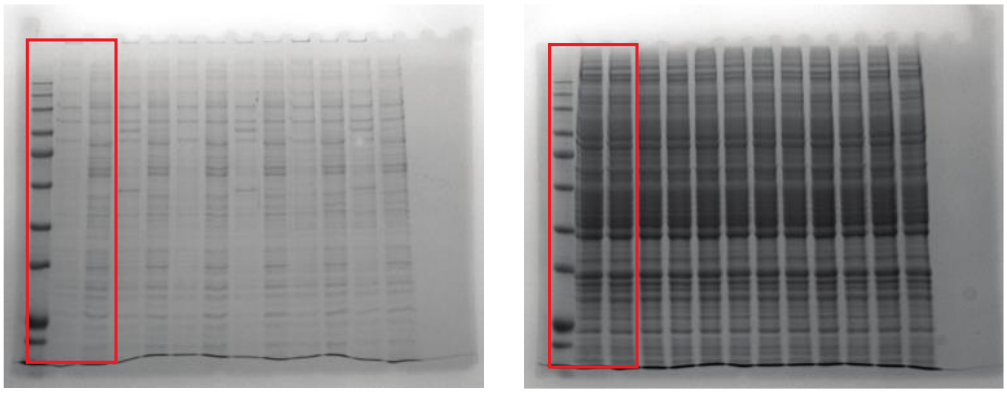
Supplementary Figure 7. Relationship between RNA secondary structure and the BioGRID reported interactors of the encoded proteins. a) Correlation between transcriptome PARS structural content and protein-protein interactions of the encoded product (BioGRID)**. b**) Correlation between transcriptome DMS structural content and protein-protein interactions of the encoded product (BioGRID)**.**



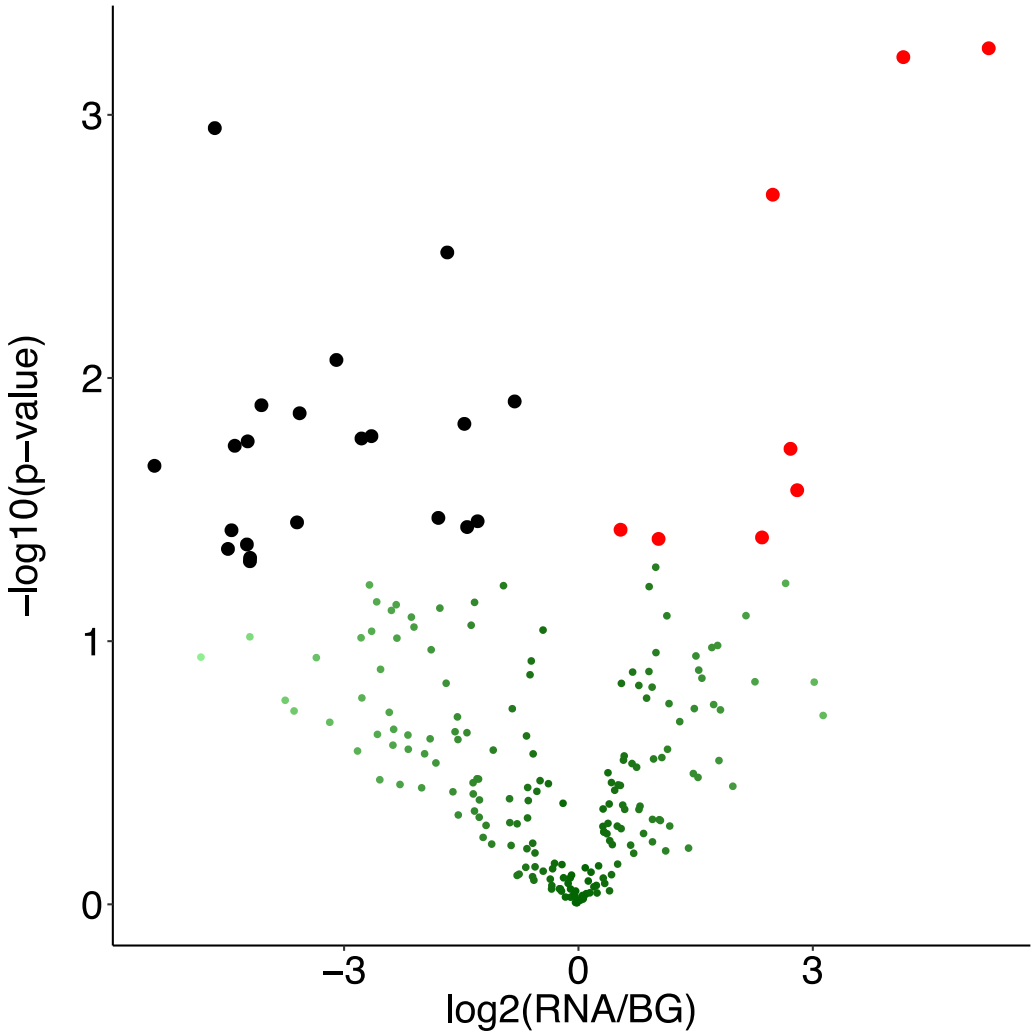
Supplementary Figure 8. CROSS and DMS structural content of HS and LS RNA. a) CROSS icSHAPE predicts that HS (*HSP70*) is more structured than LS (*BRaf*) RNA, in agreement with PARS experiments. Vertical dashed lines indicate UTRs; **b-c**) Comparison between PARS and CROSS icSHAPE for both **b**) HS and **c**) LS structures indicate strong agreement between predictions and experiments. From poor (50%) to strong (10%) PARS signals, the Area Under the ROC Curve (AUC) is used to assess CROSS performances on equal-size groups of nucleotides ranked by PARS scores. **d**) DMS signal analysis indicates that HS (*HSP70*) is more structured than LS (*BRaf*), in agreement with PARS experiments and CROSS predictions.



Supplementary Figure 9. ***cat*RAPID and eCLIP data including UTRS. a**) Boxplot distribution of the PARS the secondary structure content. **b**) Venn diagram showing the overlap (empirical p-value $p < 6 \cdot 10^{-3}$ computed on all the 100 eCLIP RBPs as background) between protein interactions of *HSP70* (HS, pink) and *BRaf* RNA (LS, blue).



Supplementary Figure 10. Uncropped gels from Figure 4a. Coomassie blue-stained gels showing the protein separation upon the b-isox-driven aggregation. The red square marks the cropped section presented in Figure 4a.



Supplementary Figure 11. Stringency of *HSP70* experiments. By reducing the RNA/Background ratio the number of false negatives decreases (see **Methods**). At low RNA/Background ratio we observe an increase in catRAPID predictive power (**Figure 5b**) (Area under the ROC curve) supporting our observation that released proteins (black dots) are direct binders of HSP70. In the plot, red dots represent proteins with increased abundance upon HSP70 incubation.

	Median DMS	Median PARS	Binds mainly to	Location	Ref.
coding	0.113	0.049	Proteins	Cytoplasm	4, 5
snRNA	0.112	0.032	Proteins	Nucleus	6, 7, 8
snoRNA	0.195	0.035	Proteins	Nucleolus	9, 10
lincRNA	0.150	0.001	Proteins and nucleotides	Cytoplasm/Nucleus	11
scaRNA	0.308	0.026	Proteins and nucleotides	Nucleus	12
antisense	0.172	0.002	Nucleotides	Cytoplasm	13, 14, 15
miRNA	0.360	0.010	Nucleotides	Cytoplasm	16, 17

Supplementary Table 1. Structural content by RNA type. secondary structure content measured by DMS and PARS arranged by the sum of the means max/min normalised.

Property	Correlation	Ref.
Hydrophobicity	-0.94	Fauchere *et al.*[18]
Hydrophobicity	-0.92	Roseman [19]
Hydrophobicity	-0.90	Black & Mould [20]
Hydrophobicity	-0.90	Sweet & Eisenberg [21]
Hydrophobicity	-0.86	Kyte & Doolittle [22]
Hydrophobicity	-0.83	Abraham & Leo [23]
Hydrophobicity	-0.82	Eisenberg *et al.*[24]
Hydrophobicity	-0.82	Bull & Breese [25]
Hydrophobicity	-0.82	Rao & Argos [26]
Hydrophobicity	-0.77	Janin [27]
Disorder	0.69	Campen *et al.* [28]

Supplementary Table 2. Correlation between polarity[29] and hydrophobicity or disorder.

Domain	Topology	Structure involved in the recognition	Chain recognised	Ref.
RRM	αβ	beta	ssRNA	30
KH	αβ	beta and loop	ssRNA	31, 32
dsRBD	αβ	alpha and loop	dsRNA	33
ZnF-CCHH	αβ	alpha	dsRNA	34
S1	β	beta	ssDNA	35, 36
PAZ	αβ	beta-barrel and alpha-beta	ssRNA	37, 38, 39
PIWI	αβ	alpha main pocket	dsRNA	39, 40
TRAP	β	beta	ssDNA	41
Pumilio	α	alpha	Structured RNA	42, 43
SAM	α	alpha	RNA hairpins	44

Supplementary Table 3. Common RNA-binding domains. Common RNA-binding domains, their structural elements and the type of RNA that they bind. The classification of proteins was taken from Lunde *at al*.[45] NA = Nucleic Acids. The table was adapted by permission from Springer Nature Customer Service Centre GmbH: Springer Nature, Nature Reviews Molecular Cell Biology, RNA-binding proteins: modular design for efficient function, Bradley M. Lunde, Claire Moore, Gabriele Varani, Copyright © 2007, Springer Nature (2007).

Supplementary References

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