Cell Genomics, Volume 3

## **Supplemental information**

## exRNA-eCLIP intersection analysis reveals a map of

### extracellular RNA binding proteins and associated

## **RNAs** across major human biofluids and carriers

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Kolmogorov-Smirnov tests identify RBPs with significantly correlated loci, Related to Figure 1. A) An example of RBP intersections – intersecting two RBPs leads to 4 regions, 3 unique and 1 shared. B) Taking the distribution of a single locus and correlating it to all other loci from the same RBP. That distribution is compared to the correlations from a random locus with similar coverage to the same set of loci from the original RBP. Kolmogorov-Smirnov tests determine if the locus is significantly correlated. C) P values from all loci of a given RBP created using the method from Figure S1B are compared to p values generated by comparing correlation distributions of all the random loci (left 3 plots). The distribution of the random locus correlated to the RBP loci is also compared against the distribution of a random locus correlated to random loci to create the second distribution of p values (right 3 plots). Kolmogorov-Smirnov tests determine if the p value of all loci in an RBP distribution are significantly different than random to determine an RBP level metric. D) Kolmogorov-Smirnov test Bonferroni adjusted p values for RBPs significant in both cell media and human plasma. E) Linear regression between the number of RBPs with Kolmogorov-Smirnov test Bonferroni adjusted p values < 0.05 in both cell media and human plasma and the total number of RPBs in that category. Labeled categories fall outside the regression line. F) Gencode annotations that intersect with regions bound by the 34 RBPs. 1 box = 50 overlaps.







D

RBP	Correlation Footprint Unique RBP Loci	Correlation Footprint All RBP Loci	Detected in Condition Media Western Blot?
AATE	Yes	No	Not tested
BUD13	No	No	No
DGCR8	Yes	No	Yes
DHX30	Yes	No	Not tested
DROSHA	Yes	No	Not tested
EFTUD2	No	No	Yes
EXOSC5	No	Yes	Not tested
FASTKD2	Yes	No	Not tested
FTO	Yes	No	Yes
FUS	No	Yes	Not tested
FXR2	Yes	No	Not tested
GEMIN5	Yes	No	Not tested
GRSF1	No	Yes	Not tested
HNRNPK	No	Yes	Not tested
HNRNPM	No	Yes	Not tested
IGF2BP1	No	No	Not tested
IGF2BP3	No	Yes	Not tested
ILF3	Yes	No	Yes
KHSRP	No	Yes	Not tested
LARP4	No	No	Not tested
LARP7	Yes	Yes	Not tested
LIN28B	Yes	No	Yes
MATR3	No	Yes	Not tested
NCBP2	Yes	No	Yes
NKRF	Yes	No	Not tested
NOLC1	Yes	No	Not tested
NSUN2	Yes	Yes	Not tested
PCBP1	Yes	No	Not tested
PPIG	Yes	Yes	No
PRPF4	No	No	Not tested
PRPF8	No	No	Yes
PTBP1	Yes	No	Not tested
PUM2	No	No	Not tested
QKI	Yes	No	Not tested
RBFOX2	No	No	Not tested
RPS3	Yes	No	Yes
SF3A3	No	No	Yes
SF3B4	Yes	No	Yes
SLTM	Yes	No	Not tested
SMNDC1	Yes	No	Not tested
SND1	No	No	Not tested
SSB	Yes	Yes	Yes
SUB1	No	No	Not tested
TBRG4	Yes	Yes	Not tested
TRA2A	No	No	Not tested
TROVE2	Yes	No	Not tested
U2AF2	No	No	Not tested
UPF1	No	Yes	Not tested
XPO5	Yes	No	Yes
XRCC6	Yes	No	Not tested
XRN2	Yes	No	Not tested
ZNF622	Yes	No	Yes
ZNF800	Yes	No	Not tested

В

**Results of exRBP Correlation Footprinting Analysis, Related to Figure 2. A)** The RBPs that were detected by correlation footprinting in each analyzed biofluid. **B)** Example Integrative Genomic Viewer trace of a BUD13 eCLIP binding locus used for correlation footprinting, here exRNA samples show expression across all biofluids. **C)** Example Integrative Genomic Viewer trace of a BUD13 eCLIP binding locus used for correlation footprinting, here exRNA samples show biofluid specific expression only in plasma. **D)** Comparison between correlation footprinting methods in CSF across 53 RBPs. Showing results for when either filtering to only loci unique to an RBP before footprinting (column 2) or including all loci of an RBP when footprinting (column 3). And how those results compare to the western blot validation results for conditioned media (column 4).



С



	D					
	Н	_	_			
ROVE2	ł				ľ	
YBX3	E				ł	
DDX6	ł			ŀ		
QKI	ſ			]		
RA2A	ŧ	ŀ				
	0.001	0.01	0.1	1		10
		dete (extrace)	ection ra ellular / c	atio cellula	ar)	

WB WB WB spectrometry AQR Detected Not expressed Detected Detected BCLAF1 Not detected No detected Detected Not expressed BUD13 No detected Not detected CSTF2T Not detected DDX3X Not detected Not detected No detected Detected DGCR8 Detected Not detected Detected DHX30 Not detected DROSHA Detected EFTUD2 Detected Detected Detected Detected EXOSC5 Detected FASTKD2 Not detected FTO Detected Detected Detected GEMIN5 Detected GRWD1 Detected Detected Detected Detected HNRNPL Detected ILF3 Detected Detected Detected KHSRP Detected LARP7 Not detected LIN28B Detected Not detected Not expressed NCBP2 Detected Not detected Detected Detected NKRF Detected PPIG Not detected Not expressed Not expressed Detected PRPF4 Detected PRPF8 Not detected Detected Detected Detected RPS3 Detected Not detected Detected Detected SF3A3 Detected Detected Detected Detected SF3B4 Detected Detected No detected Detected SLBP Detected SSB Detected Detected Not detected TBRG4 No detected Not detected UPF1 Detected XPO5 Detected Detected Detected Not detected ZNF622 Detected Detected Not expressed No detected ZNF800 Not detected 14/18 24/34 9/18 8/15 27/34

В

RBP

293T

cond. media

hMSC

cond. media

DiFi

cond. media

Plasma

mass

**RBP** detection in DiFi and HEK293T cell media, Related to Figure 3. A) Western blots performed on DiFi cell lysate, DiFi cell media, and HEK293T lysate with a media negative control. B) Summary table of the RBPs tested in the three cell types and mass spectrometry with corresponding detection success. C) Western blots performed in triplicate on HEK293T cell lysate and conditioned media for RBPs candidates computationally predicted in cell conditioned media. D) Ratio of detected signal between HEK293T conditioned medium and corresponding cell lysate blots. A value greater than one indicates a stronger detection of the RBP in the extracellular space, while below one shows a higher quantification intracellularly. Data are represented as mean + SD.



![](_page_7_Figure_1.jpeg)

**XDec deconvolution of human biofluids allows assignment of RBP cargo to exRNA carrier types, Related to Figure 4. A)** Heatmap of RBP CT enrichments with rows labeled. **B)**. Upset plot depicts how many RBPs are enriched for 1 or 2 different cargo types in deconvoluted profiles (regardless of enrichment in reference fractionated plasma/serum). **C)** Subset of Figure S4B for the 34 RBPs detected in bulk plasma and cell media (Figure S2C). **D)** Percent of biotype represented in exRNA Atlas with a coverage of 5 reads in at least one sample, vs those touched by at least 1 RBP site with a coverage of 5 reads in at least one sample in the studies used for deconvolution.

	CT1	CT2	CT3A	CT3B	CT3C	CT4
AARS	1	0	0	0	0	0
AATF	0	0	0	0	1	0
ABCF1	0	0	0	0	1	0
AGGF1	0	1	0	0	0	1
AKAP1	1	1	0	0	0	0
AKAP8L	1	0	0	0	1	1
APOBEC3C	1	1	0	0	0	0
AQR	0	1	0	0	1	0
BCCIP	0	1	0	0	1	0
BCLAF1	0	0	0	0	1	1
BUD13	0	1	0	0	0	0
CDC40	0	1	0	0	1	0
CPEB4	1	0	0	0	0	1
CPSF6	0	1	0	0	1	0
CSTF2T	0	0	0	1	0	1
CSTF2	1	0	0	0	0	0
DDX21	0	0	0	0	0	1
DDX24	1	0	0	0	1	0
DDX3X	1	0	0	0	0	0
DDX42	0	1	0	0	0	0
DDX51	1	0	0	0	0	0
DDX52	1	0	0	0	0	1
DDX55	0	1	0	0	0	0
DDX59	0	0	0	1	0	1
DDX6	1	0	0	0	1	0
DGCR8	0	0	0	1	1	0
DHX30	1	0	0	0	1	1
DKC1	0	0	0	0	1	0
DROSHA	0	0	0	1	1	0
EFTUD2	1	0	0	0	0	0
EIF3D	0	0	0	0	0	1
EIF3G	1	0	0	0	0	1
EIF3H	0	1	0	0	0	0
EIF4G2	0	0	0	0	0	1
EWSR1	0	1	0	0	0	1
EXOSC5	0	0	0	1	0	1
FAM120A	0	1	0	0	1	0
FASTKD2	1	0	0	0	1	0
FKBP4	0	0	0	0	1	1
FMR1	1	0	0	0	0	0
FTO	0	0	0	0	1	1
FUBP3	0	1	0	0	0	0
FUS	0	0	0	0	1	1
FXR1	1	0	0	0	0	1
FXR2	1	0	0	0	0	1
G3BP1	0	1	0	0	0	0

 Table S1 – CT enrichments of all 150 RBPs, Related to Figure 4

GEMIN5	0	1	0	0	0	0
GNL3	1	0	0	0	0	0
GPKOW	0	0	0	0	1	0
GRSF1	1	0	0	0	1	1
GRWD1	0	1	0	0	1	0
GTF2F1	0	1	0	0	0	0
HLTF	0	0	0	0	0	1
HNRNPA1	0	1	0	0	0	0
HNRNPC	0	0	0	0	1	0
HNRNPK	0	0	0	0	0	1
HNRNPL	1	0	0	0	1	1
HNRNPM	1	1	0	0	0	1
HNRNPUL1	1	0	0	0	0	0
HNRNPU	0	0	0	0	0	1
IGF2BP1	0	1	0	0	1	0
IGF2BP2	0	1	0	0	0	0
IGE2BP3	0	0	0	0	0	1
II F3	1	0	0	0	0	0
KHDRBS1	0	0	0	0	0	1
KHSRP	0	0	0	0	0	1
LARP4	0	1	0	0	0	0
LARP7	0	1	0	0	1	0
LIN28B	1	0	0	0	0	0
LSM11	1	0	0	0	1	0
MATR3	0	1	1	0	0	1
METAP2	0	1	0	0	0	0
MTPAP	1	0	0	0	0	1
NCBP2	1	0	0	0	0	0
NIP7	0	0	0	0	1	0
NIPBI	1	0	0	0	0	0
NKRF	1	0	0	0	0	0
NOL 12	0	0	0	0	1	0
NOL C1	0	0	0	0	1	0
NONO	0	1	0	0	0	1
NPM1	0	1	0	1	0	0
NSUN2	1	0	0	0	1	0
PABPC4	1	0	0	0	0	0
PARPN1	0	0	0	0	1	1
PCBP1	1	0	0	0	0	0
PCBP2	1	0	0	0	1	0
PHF6	0	0	0	0	1	1
POLR2G	0	1	0	0	0	1
PPIG	0	1	0	0	0	0
	0	0	0	0	0	1
PRPF4	0	1	0	0	0	1
PRPF8	0	1	0	0	0	0
PTRP1	0	0	0	0	1	0
	0		0	0	1	1
PLIM2	0	1	0	0	0	0
PUS1	1	0	0	0	0	0
	0		0	1	0	0
	0	1	0		0	0
		'				

RBM15	0	1	0	0	0	0
RBM22	0	1	0	0	0	0
RBM5	1	0	0	1	0	0
RPS11	1	0	0	0	0	1
RPS3	1	0	0	0	0	0
SAFB2	0	0	0	0	0	1
SAFB	0	1	0	0	0	1
SBDS	0	1	0	0	0	0
SDAD1	0	0	0	0	1	0
SERBP1	0	1	0	1	0	0
SF3A3	0	0	0	0	1	0
SF3B1	0	1	0	0	0	0
SF3B4	0	0	0	0	1	0
SFPQ	0	1	0	0	0	1
SLBP	0	0	0	0	0	1
SLTM	0	0	0	0	0	1
SMNDC1	0	0	0	0	1	0
SND1	1	0	0	0	0	0
SRSF1	0	1	0	0	0	0
SRSF7	0	0	0	0	0	1
SRSF9	0	0	0	0	1	0
SSB	1	0	0	0	0	0
STAU2	0	1	0	0	0	0
SUB1	1	1	0	0	0	0
SUGP2	1	1	1	0	0	0
SUPV3L1	1	0	0	0	1	1
TAF15	0	1	0	0	0	0
TARDBP	0	1	0	0	0	1
TBRG4	1	0	0	0	0	0
TIA1	1	0	0	0	1	0
TIAL1	0	1	0	0	0	0
TRA2A	0	0	0	0	1	0
TROVE2	0	0	0	1	0	0
U2AF1	0	1	0	0	0	0
U2AF2	0	1	0	0	0	0
UCHL5	1	1	0	0	0	0
UPF1	0	1	0	0	1	0
UTP18	0	0	0	0	1	0
UTP3	1	0	0	0	1	0
WDR3	0	0	0	0	1	0
WDR43	1	0	0	0	1	0
WRN	1	0	0	0	0	0
XPO5	1	0	0	0	0	0
XRCC6	1	0	0	0	0	0
XRN2	0	1	0	0	0	0
YBX3	1	0	0	0	1	0
YWHAG	0	1	0	0	0	0
ZC3H11A	1	0	0	0	1	0
ZC3H8	0	0	0	0	1	0
ZNF622	0	1	0	0	0	1
ZNF800	0	0	0	0	1	1
ZRANB2	0	0	0	0	1	0

	CT1	CT2	CT3A	СТ3В	CT3C	CT4
APOBEC3C	1	0	0	0	0	0
CSTF2	1	0	0	0	0	0
FASTKD2	1	0	0	0	0	0
FMR1	1	0	0	0	0	0
GNL3	1	0	0	0	0	0
HNRNPM	1	0	0	0	0	0
LIN28B	1	0	0	0	0	0
SSB	1	0	0	0	0	0
SUB1	1	0	0	0	0	0
TIA1	1	0	0	0	1	0
YBX3	1	0	0	0	0	0
AKAP1	0	1	0	0	0	0
GEMIN5	0	1	0	0	0	0
HNRNPA1	0	1	0	0	0	0
RBM22	0	1	0	0	0	0
SERBP1	0	1	0	0	0	0
TAF15	0	1	0	0	0	0
U2AF2	0	1	0	0	0	0
AGGF1	0	0	0	0	0	1
POLR2G	0	0	0	0	0	1
PPIL4	0	0	0	0	0	1
SFPQ	0	0	0	0	0	1
DDX59	0	0	0	1	0	0
EXOSC5	0	0	0	1	0	0
QKI	0	0	0	1	0	0
TROVE2	0	0	0	1	0	0
ABCF1	0	0	0	0	1	0
AQR	0	0	0	0	1	0
CDC40	0	0	0	0	1	0
CPSF6	0	0	0	0	1	0
DKC1	0	0	0	0	1	0
FAM120A	0	0	0	0	1	0
LARP7	0	0	0	0	1	0
NIP7	0	0	0	0	1	0
NOL12	0	0	0	0	1	0
NOLC1	0	0	0	0	1	0
PABPN1	0	0	0	0	1	0
PTBP1	0	0	0	0	1	0
PUM1	0	0	0	0	1	0
SMNDC1	0	0	0	0	1	0
TRA2A	0	0	0	0	1	0
UPF1	0	0	0	0	1	0
UTP18	0	0	0	0	1	0
WDR3	0	0	0	0	1	0
WDR43	0	0	0	0	1	0
ZC3H8	0	0	0	0	1	0
ZRANB2	0	0	0	0	1	0

# Table S2 – CT enrichments of 47 consistently enriched RBPs, Related to Figure 4

Accession	Biofluids	Accession	Biofluids			
EXR-DERLE1Dj6liR-AN	Plasma	EXR-DGALA1ECRXVs-AN	CSF			
		EXR-DGALA1mUDpAe-A				
EXR-DGALA1V5h5va-AN	Plasma	Ν	CSF			
EXR-KVICK1olp40e-AN	Plasma	EXR-DGALA1QDi9GG-AN	CSF			
EXR-JJONE1wAX8tF-AN	Plasma	EXR-KJENS1sPlvS2-AN	CSF, Serum			
EXR-KJENS17CZMbP-AN	Plasma	EXR-ANACC1S6IJ1C-AN	Plasma, Stool, Urine			
EXR-SADAS1EXER1-AN	Plasma	EXR-JJONE1HC7DHb-AN	Urine			
EXR-MTEWA1ZR3Xg6-AN	Plasma, Serum	EXR-JJONE1tHRzcU-AN	Urine			
EXR-KJENS10IPCIY-AN	Plasma	EXR-DWONG1qf3tcS-AN	Saliva			
EXR-MTEWA1cHYLo6-AN	Plasma, Serum	EXR-IGHIR1HnDH6K-AN	Plasma, Serum			
EXR-MTEWA1vczugX-AN	Plasma	EXR-JJONE1AcUuui-AN	Serum			
EXR-KJENS1RID1-AN	Plasma, Urine, Saliva	EXR-LLAUR1s0A1mX-AN	Serum			
EXR-AWEAV1lyeWbT-AN	Condition Medium	EXR-LLAUR1SFszrF-AN	Plasma, Serum			
EXR-AKRIC157ITEI-AN	Condition Medium	EXR-TTUSC1gCrGDH-AN	Plasma, Serum			
EXR-JFRAN1cDpMBU-AN	Condition Medium	EXR-KJENS12WGutU-AN	CSF			
EXR-JFRAN16VDql8-AN	Condition Medium	EXR-KJENS1WBaSro-AN	CSF, Plasma			
EXR-DGALA17UKOTF-AN	CSF	EXR-TPATE1OqELFf-AN	Plasma			
EXR-DGALA1adMfMp-AN	CSF	EXR-MBITZ12SHVIr-AN	Urine			
EXR-DGALA1B2gzMc-AN	CSF	EXR-SADAS1UJ0CzW-AN	Plasma			
EXR-DERLE1PHASE1PROT-AN	Saliva, Sputum, Ovarian Follicle Fluid, Seminal Fluid, Amniotic Fluid, N Plasma, Serum, CSF, BALF, Urine					

Table S3 – exRNA-atlas.org accessions of studies used in analysis, related to STAR Methods

# Table S4 – Detection of off strand reads, related to STAR Methods

	-			-	
	Av Wrong Strand		Av Wrong Strand		Av Wrong Strand
DDX42	0%	WDR43	0%	GTF2F1	1%
CDC40	0%	PUM2	0%	TBRG4	1%
NSUN2	0%	SLBP	0%	SERBP1	1%
GPKOW	0%	SF3B1	0%	HNRNPC	1%
SF3A3	0%	DROSHA	0%	EXOSC5	1%
GEMIN5	0%	RPS3	0%	PTBP1	1%
SMNDC1	0%	LIN28B	0%	AGGF1	1%
SSB	0%	ZNF622	0%	EIF4G2	1%
TAF15	0%	SND1	0%	SAFB2	1%

0E2D4	00/		0.0%		10/
	0%		0%		170
	0%		0%		10/
	0%		0%		170
	0%	EIF3G	0%		1%
FKBP4	0%	QKI	0%	GNL3	1%
NPM1	0%	NIPBL	0%	ZC3H11A	2%
WRN	0%	NCBP2	0%	ABCF1	2%
DKC1	0%	UTP18	0%	FXR1	2%
HNRNPUL1	0%	NIP7	0%	FUS	2%
DDX21	0%	NKRF	0%	PRPF4	2%
NOLC1	0%	FAM120A	0%	PABPC4	2%
BCCIP	0%	CPSF6	0%	RBFOX2	2%
PUM1	0%	PPIG	0%	IGF2BP3	2%
EIF3D	0%	BUD13	0%	RPS11	2%
SUPV3L1	0%	PHF6	0%	HLTF	3%
WDR3	0%	XRN2	0%	RBM5	3%
EFTUD2	0%	GRWD1	0%	FMR1	3%
XPO5	0%	SRSF1	0%	LARP4	3%
DDX51	0%	ZNF800	0%	PABPN1	3%
RBM22	0%	TRA2A	0%	FUBP3	3%
APOBEC3C	0%	DDX24	0%	KHSRP	3%
ZRANB2	0%	BCLAF1	0%	HNRNPU	5%
DDX6	0%	SRSF9	0%	SUGP2	6%
NOL12	0%	YWHAG	0%	SLTM	9%
SRSF7	0%	IGF2BP1	0%	AKAP1	9%
LARP7	0%	FXR2	0%	SFPQ	14%
SBDS	0%	AARS	0%	TIAL1	15%
IGF2BP2	0%	HNRNPM	0%	EWSR1	16%
AKAP8L	0%	STAU2	0%	CPEB4	16%
METAP2	0%	FASTKD2	0%	ILF3	23%
FTO	0%	PCBP1	0%	HNRNPA1	23%
EIF3H	0%	TARDBP	0%	CSTF2	24%
PRPF8	0%	YBX3	0%	KHDRBS1	37%
ZC3H8	0%	UCHL5	0%	U2AF2	40%
DGCR8	0%	UPF1	0%	NONO	45%
SDAD1	0%	PUS1	0%	CSTF2T	45%
U2AF1	0%	GRSF1	0%	DDX59	48%
DDX55	0%	PPIL4	0%	HNRNPK	54%
MTPAP	0%	DDX3X	0%	HNRNPL	61%
AATE	0%	LSM11	0%	PCBP2	63%
G3BP1	0%	RBM15	1%	MATR3	88%