

Supplemental Materials

RNA binding protein motifs predict microRNA secretion and cellular retention in hypothalamic and other cell types.

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Extended Figure 4

RBP motifs statistically enriched in sEVs or cells. Annotations indicate motifs identified from homologous RNA-binding proteins (RNABPs) in other species: **Homo sapiens*, ^g*Gallus gallus*, ^t*Tetraodon nigroviridis*, ^x*Xenopus tropicalis*, [#]*Drosophila melanogaster*.

3T3-L1				
Cell-enriched RBP motifs				
RNABP	Binding Motif	E-value	Enrichment ratio	Enrichment ratio
RBMS1*		1.0e-3	35.7%	14.4%
KHDBS2*		1.3e-3	42.0%	19.6%
PABPC3*		1.0e-3	51.0%	27.5%
RBK3*		1.8e-3	75.8%	52.9%
PABPC1*		1.0e-3	47.1%	24.8%
SMT3*		1.5e-3	40.8%	19.6%
PABPC1*		1.5e-3	40.8%	19.6%
HMNRP1*		4.1e-3	31.2%	12.4%
KHDBS1*		1.0e-3	46.6%	24.2%
RBH42*		3.8e-3	29.9%	13.7%
MEK3C*		4.7e-3	74.5%	56.2%
sEV-enriched RBP motifs				
RNABP	Binding Motif	E-value	Enrichment ratio	Enrichment ratio
SRSP4*		2.4e-3	54.9%	25.5%
FUS*		4.8e-3	52.9%	28.7%

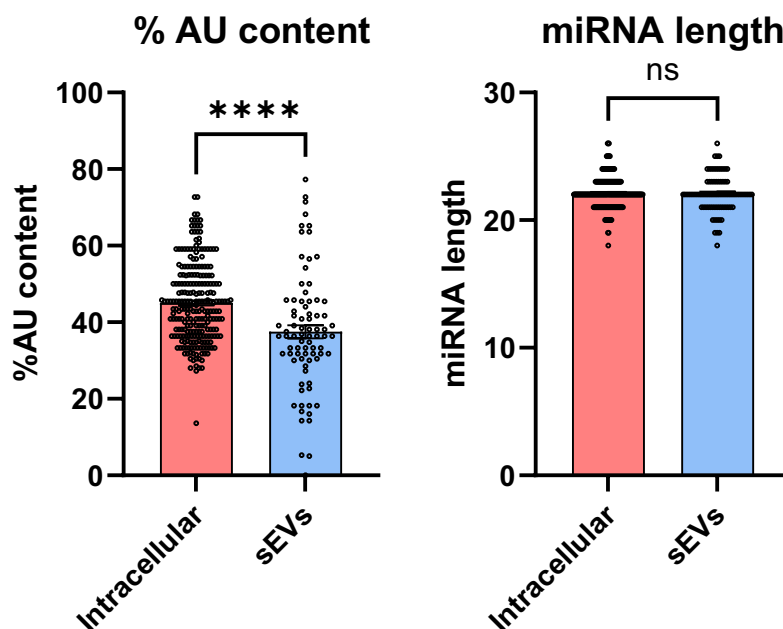
BAT				
Cell-enriched RBP motifs				
RNABP	Binding Motif	E-value	Enrichment ratio	Enrichment ratio
KHDBS2*		4.4e-3	46.5%	14.1%
PTSP1*		9.3e-3	31.9%	8.3%
HMNRP1*		9.8e-3	58.4%	24.4%
KHDBS1*		1.0e-3	33.0%	9.0%
RBH42*		2.0e-3	25.9%	5.8%
CSDA*		3.1e-3	46.5%	17.9%
PCBP3*		7.1e-3	20.5%	3.8%
YBX1*		6.2e-3	54.6%	24.4%
ZC3H1*		1.0e-3	27.6%	7.7%
SPTQ*		1.8e-3	54.1%	25.0%
SNRPB2*		1.8e-3	65.9%	33.3%
HMNRP1*		3.0e-3	59.5%	28.5%
SYNCRP*		3.9e-3	53.5%	25.6%
TUT1*		3.9e-3	64.3%	33.3%
TIAL1*		3.5e-3	48.1%	23.1%
HMNRP1*		1.1e-3	56.8%	29.5%
KHDBS1*		1.3e-3	43.8%	20.5%
SMT3*		1.4e-3	35.1%	14.7%
RBH3*		1.5e-3	24.9%	8.3%
FMR1*		2.7e-3	31.4%	12.8%
PCBP2*		2.7e-3	59.5%	32.7%
PABPC1*		2.4e-3	35.1%	15.4%
YBX2*		2.5e-3	56.8%	30.8%
HMNRP1*		2.8e-3	43.2%	21.2%
RBH11*		2.9e-3	63.2%	35.9%
PABPC3*		2.9e-3	50.3%	24.3%
PCBP1*		3.2e-3	23.8%	8.3%
HMNRP1*		3.0e-3	56.8%	31.4%
TIAL1*		3.6e-3	42.7%	21.2%
ALCP1*		3.9e-3	47.0%	24.4%
DAZAP1*		4.9e-3	49.2%	26.3%
sEV-enriched RBP motifs				
RNABP	Binding Motif	E-value	Enrichment ratio	Enrichment ratio
CDUT3*		2.3e-3	2.7%	16.0%
RBH42*		6.9e-3	3.2%	16.0%

AML12				
Cell-enriched RBP motifs				
RNABP	Binding Motif	E-value	Enrichment ratio	Enrichment ratio
HMNRP1*		3.47e-3	49.7%	11.7%
RBH41*		1.1e-3	75.2%	30.6%
SYNCRP*		7.4e-3	58.2%	22.5%
KHDBS1*		7.5e-3	46.4%	15.5%
HMNRP1*		1.2e-3	54.2%	20.7%
TUT1*		3.77e-3	70.6%	33.3%
HMNRP1*		3.8e-3	43.1%	15.3%
PABPC3*		6.1e-3	36.8%	10.8%
YBX2*		1.3e-3	40.5%	15.3%
TUT1*		1.3e-3	34.6%	11.7%
DAZAP1*		1.4e-3	75.8%	30.6%
Gm10101*		2.1e-3	20.9%	4.5%
SRSP3*		2.6e-3	41.8%	17.1%
ALCP1*		2.9e-3	30.1%	9.9%
TIAL1*		3.7e-3	49.0%	22.5%
YBX1*		3.7e-3	49.0%	22.5%
sEV-enriched RBP motifs				
RNABP	Binding Motif	E-value	Enrichment ratio	Enrichment ratio
Pp121*		1.0e-3	48.6%	15.7%
RBH4*		1.6e-3	25.2%	3.9%
HMNRP1*		1.7e-3	55.0%	20.9%
HMNRP1*		1.5e-3	40.5%	13.7%
Sand4*		4.1e-3	29.7%	8.5%
Lin28B*		2.1e-3	39.6%	16.3%
SRSP4*		4.3e-3	55.1%	14.4%

C2C12				
Cell-enriched RBP motifs				
RNABP	Binding Motif	E-value	Enrichment ratio	Enrichment ratio
ZC3H1*		1.01e-3	57.1%	16.2%
KHDBS1*		6.2e-3	55.0%	19.1%
Smt*		8.1e-3	18.6%	2.1%
Daazap1*		8.5e-3	27.3%	6.4%
HMNRP1*		8.7e-3	40.7%	13.5%
SYNCRP*		1.03e-3	65.0%	29.1%
ALCP1*		1.04e-3	58.6%	24.8%
YBX1*		1.09e-3	27.1%	6.4%
HMNRP1*		1.8e-3	67.1%	31.2%
KHDBS1*		2.3e-3	51.4%	21.3%
Pabpc3*		2.4e-3	57.9%	25.5%
Rbm3*		2.4e-3	55.8%	22.7%
Pabpc1*		3.8e-3	47.1%	19.1%
Tut1*		5.3e-3	44.3%	17.7%
Rbm3*		7.2e-3	63.6%	31.2%
Rbm1*		1.09e-3	45.4%	17.0%
Rbm1*		1.3e-3	29.0%	7.1%
Ela2*		1.3e-3	27.9%	8.5%
Rbm3*		2.2e-3	33.6%	12.8%
Gm10101*		3.2e-3	25.8%	7.1%
SRSP4*		3.9e-3	61.4%	32.6%
Cpb2*		4.0e-3	51.4%	25.5%
Sart3*		4.2e-3	40.0%	17.7%
Pabpc1*		4.2e-3	40.0%	17.7%
sEV-enriched RBP motifs				
RNABP	Binding Motif	E-value	Enrichment ratio	Enrichment ratio
RBH4*		6.0e-3	53.2%	27.1%
HMNRP1*		1.1e-3	13.5%	0.7%
FUS*		1.4e-3	41.1%	17.9%
EP251*		1.6e-3	58.2%	22.9%
HMNRP1*		6.2e-3	42.6%	20.7%
SRSP4*		1.4e-3	26.4%	10.7%
RBH4*		1.4e-3	36.9%	17.1%
CELF4*		1.7e-3	41.8%	21.4%
LMNB1*		1.2e-3	17.7%	4.3%
SAND4*		3.9e-3	39.7%	20.7%
RBH4*		3.9e-3	32.6%	15.0%

SVEC				
Cell-enriched RBP motifs				
RNABP	Binding Motif	E-value	Enrichment ratio	Enrichment ratio
ALCP1*		1.7e-3	58.4%	13.4%
HMNRP1*		1.52e-3	68.3%	22.4%
CYBB*		4.8e-3	46.5%	11.9%
SYNCRP*		1.12e-3	65.4%	20.9%
ZC3H1*		6.9e-3	53.5%	16.4%
RBH41*		7.67e-3	77.2%	31.3%
CYBB*		1.14e-3	55.6%	7.5%
YBX1*		1.45e-3	65.4%	23.9%
YBX2*		3.40e-3	44.6%	13.4%
PABPC3*		2.40e-3	44.6%	13.4%
KHDBS2*		2.62e-3	63.4%	25.4%
ROD1*		3.40e-3	66.4%	23.9%
KHDBS1*		3.91e-3	50.5%	17.0%
sEV-enriched RBP motifs				
RNABP	Binding Motif	E-value	Enrichment ratio	Enrichment ratio
SAND4*		5.6e-3	38.8%	6.9%
RBH4*		2.9e-3	43.3%	10.9%
SRSP4*		4.2e-3	47.8%	12.9%
SRSP4*		4.7e-3	64.2%	23.8%
LMNB1*		4.7e-3	29.9%	5.0%
RBH4*		1.0e-3	26.9%	5.0%

Supplemental Figure S1: AU% content and miRNA length of sEV or intracellularly enriched miRNAs. The percentage of AU versus GC composition was examined in sEV-enriched miRNAs versus miRNAs enriched intracellularly in mHypoE-46 neurons. Consistent with observations made by Garcia et al., 2021, sEV-enriched miRNAs, on average, contained a significantly lower AU%. This difference in AU% composition across multiple cell models is also reflected in the enriched RBP motifs, where sEV-enriched miRNAs typically have RBP motifs that are more CG-enriched than those enriched intracellularly. The average length of the sEV-enriched miRNAs is no different from that of the intracellularly enriched miRNAs.



Supplemental Table S1: Complete pathway analysis.

See excel document – He and Belsham Supplemental Table S1.

Supplemental Table S2: RNA-seq levels of the RBPs in mHypoE-46 neurons. Comparative mRNA expression levels of identified RBPs in hypothalamic neurons as determined by RNA-sequencing, with Rpl7 serving as a reference gene. Number represents fragments per kilobase of transcript per million mapped reads.

Pcbp3	7.3		Samd4	14.4
Ptbp1	243.5		Rbm4b	2
Pcbp1	257.4		Hnrnph2	52.9
Pcbp2	263.1		Srsf9	84.6
Tia1	11.9		Rbm4	8.7
U2af2	155.5		Srsf4	35.1
RbmX	13.6		Hnrnpa2b	434.6
Matr3	48.2		Rbm8a	55.2
Snrpb2	47.1		Nono	229.3
Tut1	21.8		Fxr2	55.4
Mbnl1	45.1		Pprc1	40.4
Hnrnpc	152.5		Fus	127.3
Cpeb2	2.1			
Hnrnpk	273.3			
Tiall	18.2			
Raly1	0		Rpl7	530.9

Supplemental Table S3: Detailed machine learning model statistics.

Model/Class	TP	FP	Precision	Recall	F-Measure	MCC	ROC Area	PRC Area
N46 - RF								
Intracellular	0.929	0.456	0.86	0.929	0.893	0.523	0.82	0.908
sEV	0.544	0.071	0.717	0.544	0.619	0.523	0.82	0.678
Weighted	0.833	0.36	0.825	0.833	0.825	0.523	0.82	0.851
N46 - NB								
Intracellular	0.703	0.177	0.923	0.703	0.798	0.459	0.837	0.918
sEV	0.823	0.297	0.478	0.823	0.605	0.459	0.835	0.669
Weighted	0.733	0.207	0.812	0.733	0.75	0.459	0.837	0.856
3T3-L1 - RF								
Intracellular	0.724	0.341	0.685	0.724	0.704	0.384	0.724	0.713
sEV	0.659	0.276	0.7	0.659	0.679	0.384	0.724	0.702
Weighted	0.692	0.309	0.692	0.692	0.691	0.384	0.724	0.708
3T3-L1 - NB								
Intracellular	0.546	0.2	0.736	0.546	0.627	0.357	0.718	0.661
sEV	0.8	0.454	0.633	0.8	0.706	0.357	0.718	0.702
Weighted	0.672	0.326	0.685	0.672	0.666	0.357	0.718	0.681
BAT - RF								
Intracellular	0.78	0.329	0.737	0.78	0.758	0.454	0.794	0.795
sEV	0.671	0.22	0.72	0.671	0.695	0.454	0.794	0.757
Weighted	0.73	0.279	0.73	0.73	0.729	0.454	0.794	0.778
BAT - NB								
Intracellular	0.605	0.231	0.756	0.605	0.672	0.376	0.766	0.778
sEV	0.769	0.395	0.621	0.769	0.687	0.376	0.766	0.731
Weighted	0.68	0.306	0.694	0.68	0.679	0.376	0.766	0.757
AML12 - RF								
Intracellular	0.822	0.382	0.747	0.822	0.783	0.452	0.825	0.852
sEV	0.618	0.178	0.717	0.618	0.664	0.452	0.825	0.754
Weighted	0.736	0.296	0.735	0.736	0.733	0.452	0.825	0.811
AML12 - NB								
Intracellular	0.734	0.268	0.79	0.734	0.761	0.461	0.792	0.816
sEV	0.732	0.266	0.667	0.732	0.698	0.461	0.791	0.719
Weighted	0.733	0.267	0.738	0.733	0.734	0.461	0.792	0.775
C2C12 - RF								
Intracellular	0.8	0.295	0.729	0.8	0.763	0.507	0.826	0.799
sEV	0.705	0.2	0.78	0.705	0.741	0.507	0.826	0.832

Weighted	0.752	0.247	0.755	0.752	0.752	0.507	0.826	0.816
C2C12 - NB								
Intracellular	0.703	0.244	0.741	0.703	0.722	0.46	0.81	0.784
sEV	0.756	0.297	0.72	0.756	0.738	0.46	0.811	0.798
Weighted	0.73	0.27	0.73	0.73	0.73	0.46	0.811	0.791
SVEC - RF								
Intracellular	0.866	0.324	0.802	0.866	0.833	0.556	0.829	0.857
sEV	0.676	0.134	0.769	0.676	0.719	0.556	0.829	0.802
Weighted	0.79	0.249	0.789	0.79	0.788	0.556	0.829	0.835
SVEC - NB								
Intracellular	0.768	0.284	0.804	0.768	0.785	0.479	0.802	0.813
sEV	0.716	0.232	0.671	0.716	0.693	0.479	0.801	0.762
Weighted	0.747	0.263	0.751	0.747	0.749	0.479	0.802	0.793