Supplemental Table 1

A From 130kD

Sample	Antibody	Age	Raw tags	BLAT (mm8)	% align	Unique tags
Brain A	2A8	P13	5,057,657	4,168,244	**82.4**	237,824
Brain B	2A8	P13	4,291,392	3,504,911	**81.7**	359,405
Brain C	2A8	P13	3,843,297	3,073,034	**80**	328,916
Brain D	7G1-1	P13	6,259,297	5,118,928	**81.8**	420,627
Brain E	7G1-1	P13	6,394,071	5,188,918	**81.2**	423,024

B From 110kD

Sample	Antibody	Age	Raw tags	BLAST (miRBASE)	% align
Brain A	2A8	P13	4,918,586	3,717,702	**75.6**
Brain B	2A8	P13	4,160,480	3,004,104	**72.2**
Brain C	2A8	P13	3,490,474	2,576,516	**73.8**
Brain D	7G1-1	P13	4,853,688	4,275,690	**88.1**
Brain E	7G1-1	P13	5,409,367	4,765,957	**88.1**

Supplemental Table 2

A

AGO-mRNA CLIP				AGO-miRNA CLIP			
Top ranking motif	Regression coefficient	Number of tags	P-value	miRNA	Position of motif	Percentage in Ago-miRNA CLIP tags	Rank
TGCCTT	0.521	9153	8.3×10^{-58}	mir-124	2-7	2.97%	8
TACCTC	0.512	6403	1.6×10^{-40}	let-7	2-7	6.24%	5
CCAAAG	0.393	6878	3.1×10^{-32}	mir-9	2-7	9.33%	2
CTGTGA	0.388	4205	5.0×10^{-23}	mir-27	2-7	4.80%	6
TGTTTA	0.436	3206	7.9×10^{-21}	mir-30	3-8	34.01%	1
CTTGAA	0.356	2977	1.5×10^{-14}	mir-26	1-6	6.39%	4

B

AGO-miRNA CLIP					AGO-mRNA CLIP		
Rank	7mer seed motif	miRNA	Number of Ago-miRNA CLIP tags (Total)	%	7mer seed obs/exp	Number of mRNA tags with seed (obs)	Number of clusters with seed
1	TGTTTAC	miR-30	8,417,151	34.29	3.304	1521	172
2	ACCAAAG	miR-9	2,309,955	9.41	2.175	1676	211
3	TGAATGT	miR-181	2,050,154	8.35	1.549	862	132
4	TACTTGA	miR-26	1,581,514	6.44	2.436	687	68
5	CTACCTC	let-7	1,549,064	6.31	4.000	2636	268
6	ACTGTGA	miR-27	1,188,538	4.84	1.190	969	137
7	AGCTCCT	miR-708	1,172,287	4.78	0.202	83	12
8	GTGCCTT	miR-124	735,430	3.00	5.187	3362	294
9	CACTGCC	miR-34	333,089	1.36	0.312	147	30
10	CTCAGGG	miR-125	330,186	1.35	1.187	506	65

Supplemental Table 3

				All seeds				Conserved seeds				
Rank	Frequency in Ago miRNA CLIP	miRNA family	Seed motif	Obs/Exp	P-value	Observed Frequeny in Ago footprint	Expected frequency	Obs/Exp	P-value	Observed Frequency in Ago Footprint	Expected frequency	miRNAs
1	8417151	miR-30	TGTTTACA	7.002	1.99E-175	155	22	13.332	3.78E-297	119	9	miR-30c:miR-30b:miR-30a:miR-30e:miR-30d:miR-384-5p
1	8417151	miR-30	TGTTTAC	4.931	6.21E-167	242	49	10.458	5.25E-307	164	16	miR-30c:miR-30b:miR-30a:miR-30e:miR-30d:miR-384-5p
1	8417151	miR-30	GTTTACA	4.273	3.10E-113	204	48	8.735	2.10E-228	152	17	miR-30c:miR-30b:miR-30a:miR-30e:miR-30d:miR-384-5p
1	8417151	miR-30	GTTTAC	3.085	5.55E-109	349	113	6.889	7.48E-255	231	34	miR-30c:miR-30b:miR-30a:miR-30e:miR-30d:miR-384-5p
1	8417151	miR-30	TGTTTA	2.055	6.92E-52	424	206	4.772	7.35E-161	245	51	miR-30c:miR-30b:miR-30a:miR-30e:miR-30d:miR-384-5p
1	8417151	miR-30	TTTACA	1.905	3.52E-39	399	209	4.237	2.16E-128	235	55	miR-30c:miR-30b:miR-30a:miR-30e:miR-30d:miR-384-5p
1		miR-30	with all 6mers	2.216	4.53E-172	1172	529	5.066	0.00E+00	711	140	
2	2309955	miR-9	ACCAAAGA	6.381	8.87E-170	170	27	11.190	4.66E-234	115	10	miR-9
2	2309955	miR-9	CCAAAGA	4.317	1.58E-252	452	105	8.223	0.00E+00	299	36	miR-9
2	2309955	miR-9	ACCAAAG	4.107	1.23E-166	322	78	7.773	8.79E-264	204	26	miR-9
2	2325768	miR-9	ACCAAA	3.006	2.70E-209	712	237	5.833	0.00E+00	398	68	miR-9:miR-133a:miR-133b
2	2309955	miR-9	CCAAAG	2.847	8.84E-243	924	325	5.604	0.00E+00	548	98	miR-9:miR-1897-5p
2	2309955	miR-9	CAAAGA	2.013	5.44E-85	749	372	4.167	7.66E-233	441	106	miR-9
2		miR-9	with all 6mers	2.555	0.00E+00	2385	934	5.102	0.00E+00	1387	272	
3	2050154	miR-181	TGAATGTT	3.044	3.84E-15	45	15	8.177	4.00E-36	25	3	miR-181d:miR-181a:miR-181c:miR-181b
3	2050281	miR-181	GAATGTT	1.879	9.63E-10	91	48	4.105	3.26E-22	40	10	miR-181d:miR-181a:miR-181c:miR-181b:miR-543
3	2050154	miR-181	TGAATGT	2.966	1.86E-65	224	76	6.383	1.82E-121	121	19	miR-181d:miR-181a:miR-181c:miR-181b
3	2050281	miR-181	GAATGT	1.903	7.36E-41	418	220	3.688	3.21E-87	200	54	miR-181d:miR-181a:miR-181c:miR-181b:miR-543
3	2050281	miR-181	AATGTT	1.541	1.62E-15	334	217	2.803	3.56E-36	136	49	miR-181d:miR-181a:miR-181c:miR-181b:miR-543
3	2050154	miR-181	TGAATG	1.783	1.28E-34	438	246	3.388	1.79E-85	228	67	miR-181d:miR-181a:miR-181c:miR-181b
3		miR-181	with all 6mers	1.745	2.77E-84	1190	682	3.317	1.59E-200	564	170	
4	1581514	miR-26	TACTTGAA	6.130	2.42E-81	85	14	9.901	5.21E-89	50	5	miR-26a:miR-26b
4	1581514	miR-26	TACTTGA	3.600	8.61E-54	127	35	6.661	6.26E-69	64	10	miR-26a:miR-26b
4	1581514	miR-26	ACTTGAA	2.754	6.80E-46	181	66	6.260	8.00E-106	108	17	miR-26a:miR-26b
4	1581514	miR-26	ACTTGA	1.920	4.13E-35	347	181	4.351	4.96E-94	164	38	miR-26a:miR-26b
4	1581514	miR-26	CTTGAA	1.811	3.12E-34	410	226	4.152	3.65E-108	204	49	miR-26a:miR-26b
4	1581514	miR-26	TACTTG	1.959	1.85E-28	261	133	3.876	5.40E-46	95	25	miR-26a:miR-26b
4		miR-26	with all 6mers	1.884	9.23E-94	1018	540	4.158	1.61E-243	463	111	
5	1530176	let-7	CTACCTCA	9.250	5.66E-306	190	21	14.866	0.00E+00	140	9	miR-98:let-7c:let-7b:let-7a:let-7g:let-7f:let-7e:let-7i
5	1549064	let-7	CTACCTC	6.118	0.00E+00	364	59	11.358	0.00E+00	255	22	miR-98:let-7c:let-7b:let-7a:let-7g:let-7f:let-7e:let-7d:let-7i
5	1530176	let-7	TACCTCA	6.627	0.00E+00	370	56	12.152	0.00E+00	273	22	miR-98:let-7c:let-7b:let-7a:let-7g:let-7f:let-7e:let-7i
5	1551121	let-7	CTACCT	2.501	5.76E-108	541	216	4.987	9.10E-222	317	64	miR-98:let-7c:let-7b:let-7a:let-7g:let-7f:let-7e:let-7d:let-7i:miR-196a:miR-196b
5	1549232	let-7	TACCTC	4.479	0.00E+00	706	158	9.295	0.00E+00	467	50	miR-98:let-7c:let-7b:let-7a:let-7g:let-7f:let-7e:let-7d:miR-202-3p:let-7i
5	1533600	let-7	ACCTCA	2.755	6.59E-191	777	282	5.674	0.00E+00	453	80	miR-98:let-7c:let-7b:let-7a:let-7g:let-7f:let-7e:let-7i:miR-672
5		let-7	with all 6mers	3.085	0.00E+00	2024	656	6.388	0.00E+00	1237	194	
6	1188538	miR-27	ACTGTGAA	2.990	2.69E-26	85	28	6.255	5.76E-49	49	8	miR-27b:miR-27a
6	1211720	miR-27	ACTGTGA	2.525	1.31E-48	233	92	4.613	9.24E-81	128	28	miR-128:miR-27b:miR-27a
6	1188538	miR-27	CTGTGAA	1.753	2.78E-15	193	110	3.622	4.22E-41	95	26	miR-27b:miR-27a
6	1219457	miR-27	CTGTGA	1.559	1.21E-28	615	394	2.850	1.98E-77	289	101	miR-673-5p:miR-128:miR-27b:miR-27a
6	1211720	miR-27	ACTGTG	1.879	8.57E-61	658	350	3.865	1.94E-136	291	75	miR-128:miR-27b:miR-27a
6	1189726	miR-27	TGTGAA	1.480	1.74E-18	495	335	2.553	2.14E-50	236	92	miR-27b:miR-27a:miR-350
6		miR-27	with all 6mers	1.638	1.31E-97	1768	1079	3.032	1.35E-243	816	269	
7	1172287	miR-708	AGCTCCTT	0.839	4.68E-01	17	20	1.391	5.66E-01	3	2	miR-708:miR-28
7	1172287	miR-708	GCTCCTT	0.718	1.99E-02	49	68	1.287	3.80E-01	12	9	miR-708:miR-28
7	1172287	miR-708	AGCTCCT	0.562	1.44E-05	55	98	0.713	2.82E-01	10	14	miR-708:miR-28
7	1172287	miR-708	CTCCTT	0.934	2.55E-01	275	295	1.497	7.46E-04	69	46	miR-708:miR-28
7	1172287	miR-708	AGCTCC	0.582	2.19E-13	179	308	0.925	5.85E-01	49	53	miR-708:miR-28
7	1172287	miR-708	GCTCCT	0.574	1.52E-14	187	326	0.813	1.68E-01	44	54	miR-708:miR-28
7		miR-708	with all 6mers	0.691	4.55E-21	641	928	1.057	4.78E-01	162	153	
8	735430	miR-124	GTGCCTTA	12.685	1.48E-294	125	10	20.229	0.00E+00	82	4	miR-124
8	735430	miR-124	TGCCTTA	7.277	0.00E+00	324	45	13.758	0.00E+00	212	15	miR-124
8	735430	miR-124	GTGCCTT	6.299	0.00E+00	415	66	12.725	0.00E+00	281	22	miR-124
8	735430	miR-124	GCCTTA	4.358	2.02E-302	534	123	9.744	0.00E+00	335	34	miR-124
8	735430	miR-124	TGCCTT	3.155	0.00E+00	1020	323	7.489	0.00E+00	605	81	miR-124
8	735430	miR-124	GTGCCT	2.877	1.32E-198	738	256	6.866	0.00E+00	453	66	miR-124
8		miR-124	with all 6mers	3.263	0.00E+00	2292	702	7.690	0.00E+00	1393	181	
9	270706	miR-34	CACTGCCA	0.615	2.81E-02	20	33	1.344	4.01E-01	8	6	miR-34a:miR-449a
9	333089	miR-34	CACTGCC	0.879	2.23E-01	89	101	1.807	1.23E-03	29	16	miR-699:miR-34c:miR-34a:miR-34b-5p:miR-449b:miR-449c:miR-449a
9	270706	miR-34	ACTGCCA	0.948	6.36E-01	77	81	1.931	9.35E-05	34	18	miR-34a:miR-449a
9	333089	miR-34	ACTGCC	1.024	7.03E-01	259	253	2.062	3.50E-14	105	51	miR-699:miR-34c:miR-34a:miR-34b-5p:miR-449b:miR-449c:miR-449a
9	333089	miR-34	CACTGC	0.857	1.34E-02	257	300	1.595	3.13E-05	78	49	miR-699:miR-34c:miR-34a:miR-34b-5p:miR-449b:miR-449c:miR-449a
9	270706	miR-34	CTGCCA	0.930	1.58E-01	379	408	1.602	1.00E-08	145	90	miR-34a:miR-449a
9		miR-34	with all 6mers	0.932	3.51E-02	895	960	1.724	1.83E-23	328	190	
10	330186	miR-125	CTCAGGGA	2.365	2.79E-12	62	26	7.292	1.60E-51	42	6	miR-125b-5p:miR-351:miR-125a-5p
10	332795	miR-125	TCAGGGA	1.459	7.81E-05	108	74	4.251	1.75E-36	64	15	miR-125b-5p:miR-351:miR-125a-5p:miR-670
10	330186	miR-125	CTCAGGG	1.415	1.72E-04	116	82	4.476	4.74E-40	65	15	miR-125b-5p:miR-351:miR-125a-5p
10	333749	miR-125	CAGGGA	0.738	2.06E-06	242	328	2.025	9.18E-14	107	53	miR-125b-5p:miR-351:miR-339-5p:miR-125a-5p:miR-670
10	332795	miR-125	TCAGGG	0.951	4.61E-01	219	230	2.807	1.21E-28	106	38	miR-125b-5p:miR-351:miR-125a-5p:miR-670
10	330186	miR-125	CTCAGG	1.055	3.17E-01	351	333	2.745	3.38E-42	167	61	miR-125b-5p:miR-351:miR-125a-5p
10		miR-125	with all 6mers	0.911	8.20E-03	812	891	2.509	5.17E-77	380	151	

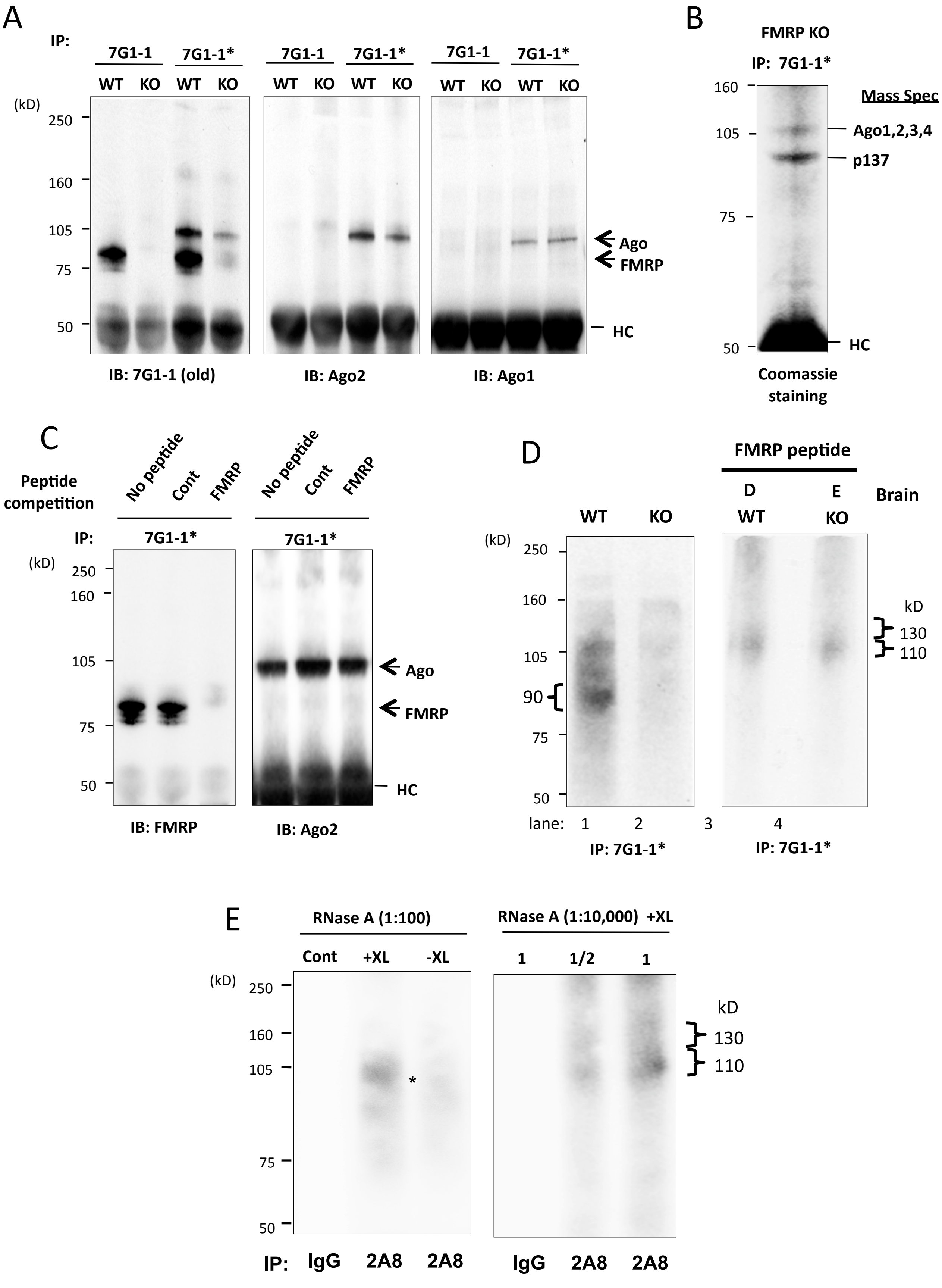
Supplemental Table 3 (continued)

				All seeds				Conserved seeds				
Rank	**Frequency in Ago miRNA CLIP**	**miRNA family**	**Seed motif**	**Obs/Exp**	**P-value**	**Observed Frequeny in Ago footprint**	**Expected frequency**	**Obs/Exp**	**P-value**	**Observed Frequency in Ago Footprint**	**Expected frequency**	**miRNAs**
11	307064	miR-193	GGCCAGTT	0.894	6.99E-01	12	13	1.242	7.07E-01	3	2	miR-193:miR-193b
11	307064	miR-193	GGCCAGT	0.669	6.62E-03	45	67	1.561	5.04E-02	19	12	miR-193:miR-193b
11	307064	miR-193	GCCAGTT	0.906	5.12E-01	44	49	0.830	5.98E-01	8	10	miR-193:miR-193b
11	330471	miR-193	GGCCAG	0.468	4.85E-25	177	378	0.819	9.66E-02	69	84	miR-328:miR-193:miR-193b
11	307064	miR-193	GCCAGT	0.814	3.64E-03	198	243	1.298	3.67E-02	64	49	miR-193:miR-193b
11	307064	miR-193	CCAGTT	0.893	1.06E-01	203	227	1.114	4.27E-01	54	48	miR-193:miR-193b
11		miR-193	with all 6mers	0.681	1.51E-20	578	849	1.027	7.13E-01	187	182	
12	138054	miR-17	GCACTTTG	1.311	2.14E-01	21	16	2.760	4.42E-04	11	4	miR-17:miR-20b:miR-93:miR-106a
12	297690	miR-17	GCACTTT	1.815	4.51E-09	94	52	3.806	1.22E-30	64	17	miR-17:miR-292-3p:miR-291b-3p:miR-295:miR-294:miR-20b:miR-20a:miR-93:miR-106a:miR-106b:miR-290-3p:miR-291a-3p
12	138054	miR-17	CACTTTG	1.001	9.91E-01	66	66	1.845	2.30E-03	24	13	miR-17:miR-20b:miR-93:miR-106a
12	306438	miR-17	GCACTT	1.306	7.00E-05	221	169	2.784	3.44E-30	114	41	miR-17:miR-292-3p:miR-291b-3p:miR-295:miR-294:miR-467b:miR-467c:miR-20b:miR-20a:miR-93:miR-105:miR-302b:miR-302c:miR-302a:miR-302d:miR-467d:miR-106a:miR-106b:miR-467a:miR-290-3p:miR-291a-3p
12	297690	miR-17	CACTTT	1.233	6.16E-04	267	217	2.834	3.62E-35	129	46	miR-17:miR-292-3p:miR-291b-3p:miR-295:miR-294:miR-20b:miR-20a:miR-93:miR-106a:miR-106b:miR-290-3p:miR-291a-3p
12	138054	miR-17	ACTTTG	1.022	7.14E-01	282	276	1.628	7.01E-08	120	74	miR-17:miR-20b:miR-93:miR-106a
12		miR-17	with all 6mers	1.164	2.59E-05	770	662	2.266	8.46E-58	363	160	
13	277918	miR-344	CTAGATCA	1.522	2.63E-01	7	5	1.928	5.04E-01	1	1	miR-344
13	277918	miR-344	CTAGATC	1.312	2.02E-01	22	17	2.873	2.71E-02	4	1	miR-344
13	277918	miR-344	TAGATCA	1.452	4.72E-02	28	19	2.201	3.22E-02	7	3	miR-344
13	278382	miR-344	CTAGAT	1.323	2.83E-03	113	85	2.714	3.46E-07	24	9	miR-878-5p:miR-344
13	277918	miR-344	TAGATC	1.308	1.36E-02	84	64	2.456	5.33E-06	24	10	miR-344
13	277918	miR-344	AGATCA	0.856	2.99E-02	194	227	1.041	7.41E-01	69	66	miR-344
13		miR-344	with all 6mers	1.039	4.49E-01	391	376	1.378	4.99E-04	117	85	
14	273527	miR-138	CACCAGCT	0.745	1.48E-01	24	32	1.954	1.07E-02	14	7	miR-138
14	273527	miR-138	CACCAGC	0.807	3.51E-02	96	119	1.837	3.84E-06	56	30	miR-138
14	273527	miR-138	ACCAGCT	0.821	7.92E-02	79	96	1.439	2.41E-02	38	26	miR-138
14	274185	miR-138	CCAGCT	0.660	6.00E-13	295	447	1.022	8.17E-01	111	109	miR-763:miR-138
14	273527	miR-138	ACCAGC	0.870	1.91E-02	282	324	1.621	1.41E-09	154	95	miR-138
14	273527	miR-138	CACCAG	0.699	1.66E-08	245	351	1.166	1.27E-01	99	85	miR-138
14		miR-138	with all 6mers	0.732	3.21E-19	822	1122	1.262	8.82E-06	364	289	
15	213825	miR-15	TGCTGCTA	3.649	4.53E-34	77	21	8.520	1.10E-59	40	5	miR-15b:miR-15a:miR-16:miR-195
15	250252	miR-15	TGCTGCT	1.724	1.77E-24	343	199	2.862	2.38E-40	146	51	miR-15b:miR-15a:miR-16:miR-322:miR-195:miR-497:miR-1907:miR-103:miR-107
15	214894	miR-15	GCTGCTA	2.072	4.41E-16	119	57	4.666	1.37E-37	57	12	miR-15b:miR-15a:miR-16:miR-195:miR-503
15	251321	miR-15	GCTGCT	1.080	6.14E-02	597	553	1.807	2.19E-22	263	146	miR-15b:miR-15a:miR-16:miR-322:miR-195:miR-497:miR-1907:miR-503:miR-103:miR-107
15	250938	miR-15	TGCTGC	1.275	6.23E-10	644	505	2.060	2.15E-36	291	141	miR-15b:miR-15a:miR-16:miR-322:miR-195:miR-497:miR-1907:miR-1906:miR-761:miR-103:miR-107
15	214894	miR-15	CTGCTA	1.485	3.03E-11	279	188	2.939	1.17E-29	100	34	miR-15b:miR-15a:miR-16:miR-195:miR-503
15		miR-15	with all 6mers	1.220	8.23E-15	1520	1246	2.038	3.33E-77	654	321	
16	179104	miR-101	GTACTGTA	3.500	6.15E-13	29	8	8.574	7.12E-34	22	3	miR-101a:miR-101b
16	195599	miR-101	TACTGTA	2.869	1.40E-32	116	40	6.778	1.86E-69	63	9	miR-101a:miR-101b:miR-144
16	179104	miR-101	GTACTGT	1.948	6.54E-09	73	37	4.637	1.64E-23	35	8	miR-101a:miR-101b
16	208225	miR-101	ACTGTA	1.901	3.54E-33	337	177	4.463	2.77E-80	134	30	miR-101a:miR-101b:miR-139-5p:miR-144:miR-582-5p
16	198109	miR-101	TACTGT	2.136	2.67E-53	391	183	4.269	4.21E-89	160	37	miR-101a:miR-101b:miR-144:miR-199b:miR-199a-3p
16	179104	miR-101	GTACTG	1.363	1.57E-05	193	142	2.809	5.25E-20	72	26	miR-101a:miR-101b
16		miR-101	with all 6mers	1.835	4.42E-78	921	502	3.930	7.24E-176	366	93	
17	207067	miR-153	CTATGCAA	3.011	1.72E-09	27	9	6.289	4.02E-21	20	3	miR-153
17	208220	miR-153	TATGCAA	2.421	7.07E-18	89	37	4.775	1.57E-39	58	12	miR-153:miR-448
17	207067	miR-153	CTATGCA	1.789	4.60E-07	73	41	5.233	2.23E-38	49	9	miR-153
17	208220	miR-153	TATGCA	1.604	4.23E-13	231	144	3.146	2.05E-40	121	38	miR-153:miR-448:miR-669j:miR-669k:miR-669i:miR-669h-3p
17	208220	miR-153	ATGCAA	1.521	1.99E-11	252	166	3.043	5.62E-41	131	43	miR-153:miR-448
17	207067	miR-153	CTATGC	1.407	2.01E-06	192	136	3.171	3.08E-32	94	30	miR-153
17		miR-153	with all 6mers	1.513	2.41E-27	675	446	3.113	6.39E-110	346	111	
18	153999	miR-19	TTTGCACA	1.969	2.67E-05	37	19	4.159	2.81E-15	26	6	miR-19b:miR-19a
18	153999	miR-19	TTTGCAC	1.977	1.63E-11	94	48	4.052	7.39E-33	62	15	miR-19b:miR-19a
18	153999	miR-19	TTGCACA	1.542	1.43E-04	76	49	3.869	1.52E-23	47	12	miR-19b:miR-19a
18	201896	miR-19	TTGCAC	1.669	2.02E-14	218	131	3.937	5.46E-62	126	32	miR-301b:miR-301a:miR-19b:miR-19a:miR-130a:miR-130b:miR-721
18	153999	miR-19	TTTGCA	1.161	1.25E-02	278	239	2.051	2.86E-18	141	69	miR-19b:miR-19a
18	155631	miR-19	TGCACA	0.929	2.52E-01	242	260	1.801	6.66E-10	107	59	miR-466h:miR-466j:miR-19b:miR-19a
18		miR-19	with all 6mers	1.171	1.84E-05	738	630	2.335	4.62E-64	374	160	
19	194366	miR-21	ATAAGCTA	1.956	2.34E-02	11	6	3.096	3.91E-02	3	1	miR-21
19	194811	miR-21	ATAAGCT	1.429	2.51E-02	39	27	3.144	1.31E-05	13	4	miR-590-5p:miR-21
19	194366	miR-21	TAAGCTA	1.559	1.79E-02	28	18	4.211	7.50E-07	10	2	miR-21
19	194811	miR-21	ATAAGC	1.291	5.62E-03	117	91	2.907	2.86E-12	39	13	miR-590-5p:miR-21
19	194811	miR-21	TAAGCT	1.349	3.78E-04	140	104	2.695	1.95E-10	38	14	miR-590-5p:miR-21
19	194366	miR-21	AAGCTA	1.136	9.14E-02	175	154	2.485	1.09E-11	52	21	miR-21
19		miR-21	with all 6mers	1.240	7.65E-06	432	348	2.663	5.49E-31	129	48	
20	155769	miR-221	ATGTAGCT	1.317	3.02E-01	14	11	3.856	3.63E-03	4	1	miR-221:miR-222
20	155769	miR-221	TGTAGCT	1.257	8.33E-02	57	45	2.913	5.32E-05	13	4	miR-221:miR-222
20	155769	miR-221	ATGTAGC	1.153	3.92E-01	36	31	2.729	1.61E-04	13	5	miR-221:miR-222
20	155769	miR-221	ATGTAG	1.182	4.42E-02	145	123	2.583	1.44E-11	47	18	miR-221:miR-222
20	155769	miR-221	TGTAGC	1.143	9.79E-02	153	134	2.424	2.02E-09	43	18	miR-221:miR-222
20	155769	miR-221	GTAGCT	1.000	9.99E-01	116	116	2.311	1.58E-06	31	13	miR-221:miR-222
20		miR-221	with all 6mers	1.111	3.19E-02	414	373	2.452	2.01E-24	121	49	

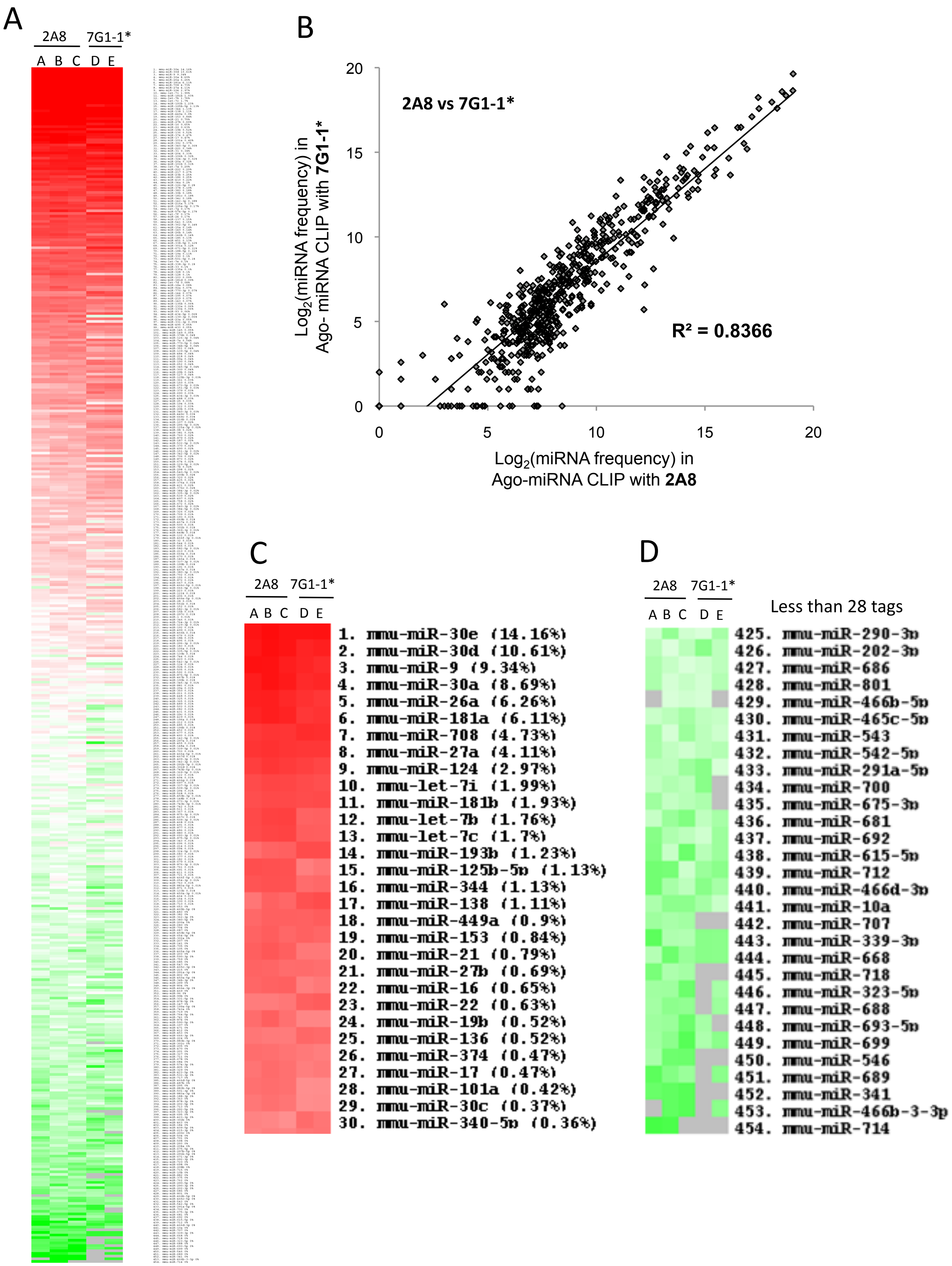
Supplemental Table 3 (continued)

				All seeds				Conserved seeds				
Rank	Frequency in Ago miRNA CLIP	miRNA family	Seed motif	Obs/Exp	P-value	Observed Frequeny in Ago footprint	Expected frequency	Obs/Exp	P-value	Observed Frequency in Ago Footprint	Expected frequency	miRNAs
21	116912	miR-374	TATTATAT	1.071	8.04E-01	13	12	2.220	6.71E-02	5	2	miR-374
21	116912	miR-374	TATTATA	1.195	2.48E-01	42	35	3.489	1.65E-10	23	7	miR-374
21	116912	miR-374	ATTATAT	0.830	2.75E-01	34	41	1.518	1.63E-01	11	7	miR-374
21	171240	miR-374	TTATAT	1.058	4.53E-01	179	169	1.780	1.01E-06	70	39	miR-410:miR-374
21	116912	miR-374	ATTATA	1.216	1.61E-02	151	124	2.223	4.70E-09	51	23	miR-374
21	119877	miR-374	TATTAT	1.135	1.03E-01	165	145	1.928	3.57E-07	58	30	miR-369-3p:miR-374
21		miR-374	with all 6mers	1.128	7.26E-03	495	439	1.938	1.96E-19	179	92	
22	154156	miR-22	GGCAGCTT	0.764	2.65E-01	17	22	1.018	9.72E-01	4	4	miR-22
22	154156	miR-22	GCAGCTT	0.800	7.21E-02	65	81	1.590	2.49E-02	23	14	miR-22
22	154156	miR-22	GGCAGCT	0.626	5.50E-05	73	117	0.957	8.24E-01	26	27	miR-22
22	157986	miR-22	CAGCTT	0.884	3.83E-02	283	320	1.652	1.55E-07	107	65	miR-320:miR-22
22	154156	miR-22	GCAGCT	0.623	1.49E-15	279	448	0.906	2.98E-01	111	123	miR-22
22	154156	miR-22	GGCAGC	0.452	1.73E-29	191	423	0.652	3.48E-04	69	106	miR-22
22		miR-22	with all 6mers	0.632	7.09E-37	753	1191	0.979	7.22E-01	287	293	
23	156668	miR-20	GCACTTTA	3.574	1.10E-18	42	12	6.309	5.66E-32	31	5	miR-20a:miR-106b
23	156668	miR-20	CACTTTA	2.116	2.01E-12	84	40	4.810	4.60E-30	43	9	miR-20a:miR-106b
23	297690	miR-20 or miR-17	GCACTTT	1.815	4.51E-09	94	52	3.806	1.22E-30	64	17	miR-17:miR-292-3p:miR-291b-3p:miR-295:miR-294:miR-20b:miR-20a:miR-93:miR-106a:miR-106b:miR-290-3p:miR-291a-3p
23	157636	miR-20	ACTTTA	1.332	2.41E-05	215	161	2.897	3.92E-26	90	31	miR-20a:miR-142-5p:miR-106b
23	306438	miR-20 or miR-17	GCACTT	1.306	7.00E-05	221	169	2.784	3.44E-30	114	41	miR-17:miR-292-3p:miR-291b-3p:miR-295:miR-294:miR-467b:miR-467c:miR-20b:miR-20a:miR-93:miR-105:miR-302b:miR-302c:miR-302a:miR-302d:miR-467d:miR-106a:miR-106b:miR-467a:miR-290-3p:miR-291a-3p
23	297690	miR-20 or miR-17	CACTTT	1.233	6.16E-04	267	217	2.834	3.62E-35	129	46	miR-17:miR-292-3p:miR-291b-3p:miR-295:miR-294:miR-20b:miR-20a:miR-93:miR-106a:miR-106b:miR-290-3p:miR-291a-3p
23		miR-20	with all 6mers	1.285	2.75E-11	703	547	2.833	6.58E-88	333	118	
24	127192	miR-136	AATGGAGT	1.228	3.96E-01	17	14	2.290	5.67E-02	5	2	miR-136
24	127946	miR-136	ATGGAGT	0.803	1.29E-01	48	60	0.991	9.72E-01	15	15	miR-880:miR-136
24	127192	miR-136	AATGGAG	0.880	2.90E-01	69	78	1.341	1.58E-01	23	17	miR-136
24	128805	miR-136	TGGAGT	0.690	2.26E-07	193	280	0.851	2.11E-01	60	71	miR-880:miR-136:miR-509-5p
24	127946	miR-136	ATGGAG	0.685	2.34E-09	247	360	0.851	1.08E-01	99	116	miR-880:miR-136
24	127192	miR-136	AATGGA	1.127	3.96E-02	297	264	1.679	3.52E-09	127	76	miR-136
24		miR-136	with all 6mers	0.816	3.03E-08	737	904	1.090	1.46E-01	286	262	
25	82032	miR-204	AAAGGGAA	0.534	1.35E-02	15	28	1.026	9.54E-01	5	5	miR-204:miR-211
25	82032	miR-204	AAAGGGA	0.562	1.53E-04	42	75	1.659	3.00E-02	18	11	miR-204:miR-211
25	82032	miR-204	AAGGGAA	0.683	3.73E-03	57	84	1.204	4.72E-01	15	12	miR-204:miR-211
25	109152	miR-204	AAGGGA	0.664	1.47E-07	163	245	1.387	2.03E-02	50	36	miR-204:miR-343:miR-211:miR-188-5p
25	82032	miR-204	AGGGAA	0.807	1.31E-03	224	278	1.923	5.22E-09	77	40	miR-204:miR-211
25	82032	miR-204	AAAGGG	0.610	9.55E-09	132	216	1.395	2.84E-02	43	31	miR-204:miR-211
25		miR-204	with all 6mers	0.702	5.43E-16	519	739	1.590	1.07E-09	170	107	
26	87290	miR-340	CTTTATAA	1.778	4.26E-03	24	13	2.914	3.01E-03	7	2	miR-340-5p
26	87290	miR-340	CTTTATA	1.358	2.16E-02	56	41	2.903	3.08E-07	21	7	miR-340-5p
26	87290	miR-340	TTTATAA	1.191	1.03E-01	87	73	1.765	4.61E-04	37	21	miR-340-5p
26	88258	miR-340	CTTTAT	1.329	7.51E-06	246	185	2.673	9.79E-23	92	34	miR-340-5p:miR-142-5p
26	87290	miR-340	TTATAA	1.184	1.93E-02	192	162	1.999	1.21E-09	74	37	miR-340-5p
26	87290	miR-340	TTTATA	1.100	1.29E-01	252	229	1.889	1.35E-13	131	69	miR-340-5p:miR-466l
26		miR-340	with all 6mers	1.197	2.18E-06	690	576	2.109	1.41E-39	297	141	
27	82568	miR-31	TCTTGCCT	1.838	3.11E-04	34	18	4.579	2.24E-06	8	2	miR-31
27	82568	miR-31	TCTTGCC	1.202	1.51E-01	61	51	3.179	4.63E-08	20	6	miR-31
27	82568	miR-31	CTTGCCT	1.506	2.00E-05	107	71	3.075	1.39E-08	23	7	miR-31
27	82568	miR-31	TTGCCT	2.030	6.53E-56	475	234	4.430	2.72E-108	184	42	miR-31
27	82568	miR-31	TCTTGC	1.177	2.06E-02	202	172	2.550	5.12E-15	65	25	miR-31
27	82568	miR-31	CTTGCC	1.129	6.19E-02	236	209	2.481	8.89E-15	68	27	miR-31
27		miR-31	with all 6mers	1.485	2.42E-33	913	615	3.357	4.30E-116	317	94	
28	78086	miR-324-3p	GGCAGTGG	0.202	2.58E-06	7	35	0.416	2.01E-01	2	5	miR-324-3p
28	78086	miR-324-3p	GGCAGTG	0.373	1.36E-10	39	105	0.897	6.85E-01	14	16	miR-324-3p
28	78086	miR-324-3p	GCAGTGG	0.392	2.93E-10	42	107	0.455	1.54E-02	9	20	miR-324-3p
28	78086	miR-324-3p	GCAGTG	0.618	1.33E-12	213	345	1.048	6.96E-01	69	66	miR-324-3p
28	78086	miR-324-3p	CAGTGG	0.673	1.16E-10	262	389	1.040	7.06E-01	91	87	miR-324-3p
28	78086	miR-324-3p	GGCAGT	0.535	1.67E-12	123	230	1.005	9.75E-01	38	38	miR-324-3p
28		miR-324-3p	with all 6mers	0.620	4.73E-32	598	964	1.036	6.18E-01	198	191	
29	74880	miR-23	AATGTGAT	1.541	2.94E-02	25	16	2.516	6.87E-03	8	3	miR-23b:miR-23a
29	74880	miR-23	AATGTGA	1.667	5.02E-09	128	77	3.300	4.12E-24	64	19	miR-23b:miR-23a
29	74880	miR-23	ATGTGAT	1.106	4.35E-01	60	54	1.440	1.55E-01	15	10	miR-23b:miR-23a
29	75593	miR-23	TGTGAT	1.284	1.76E-05	293	228	2.240	6.30E-19	115	51	miR-23b:miR-23a:miR-377
29	75148	miR-23	AATGTG	1.571	1.85E-20	414	264	2.873	1.62E-46	168	58	miR-323-3p:miR-23b:miR-23a
29	74880	miR-23	ATGTGA	1.380	4.61E-09	329	238	2.482	1.27E-27	134	54	miR-23b:miR-23a
29		miR-23	with all 6mers	1.419	1.06E-29	1036	730	2.546	4.05E-87	417	164	
30	65491	miR-217	ATGCAGTA	1.324	2.93E-01	14	11	2.648	1.31E-02	6	2	miR-217
30	65491	miR-217	TGCAGTA	1.140	3.40E-01	53	46	1.827	7.66E-03	19	10	miR-217
30	65491	miR-217	ATGCAGT	1.024	8.54E-01	60	59	1.452	9.33E-02	20	14	miR-217
30	66220	miR-217	TGCAGT	0.890	6.56E-02	247	278	1.407	1.29E-03	88	63	miR-883a-3p:miR-883b-3p:miR-217
30	65491	miR-217	GCAGTA	0.962	6.42E-01	141	147	1.911	3.35E-07	60	31	miR-217
30	65491	miR-217	ATGCAG	0.865	2.08E-02	252	291	1.085	4.29E-01	95	88	miR-217
30		miR-217	with all 6mers	0.894	4.62E-03	640	716	1.338	5.10E-06	243	182	

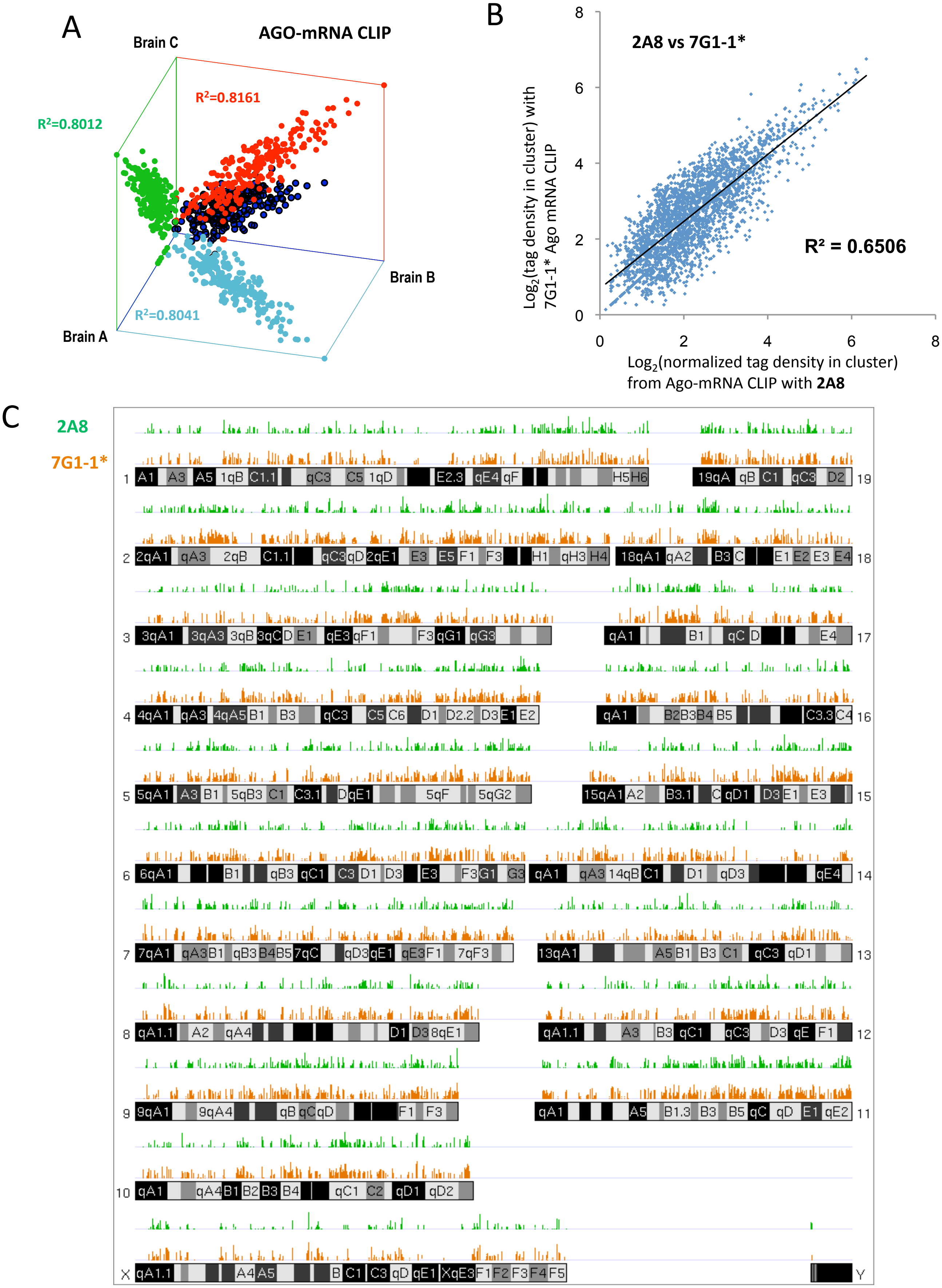
Supplemental Figure 1



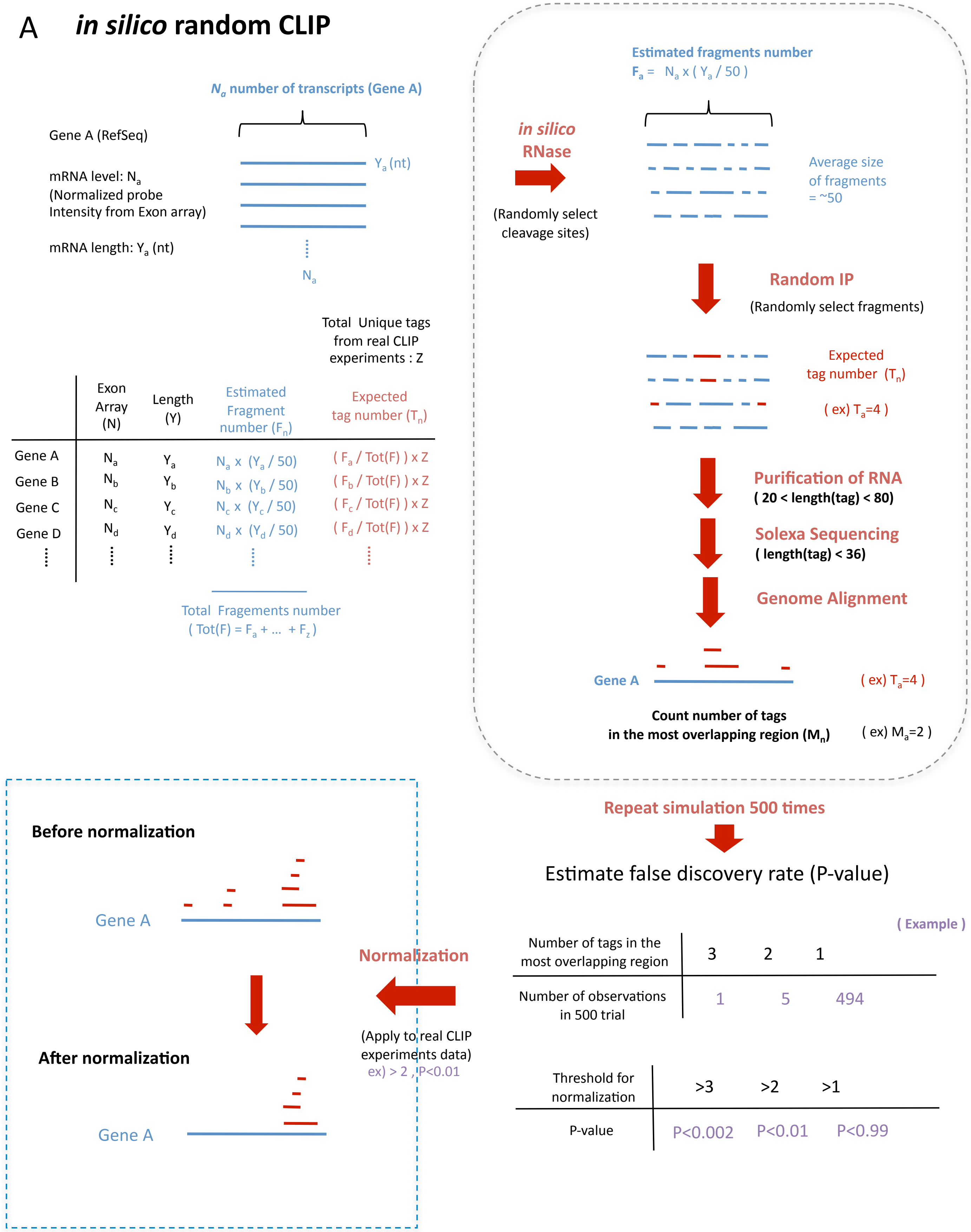
Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4



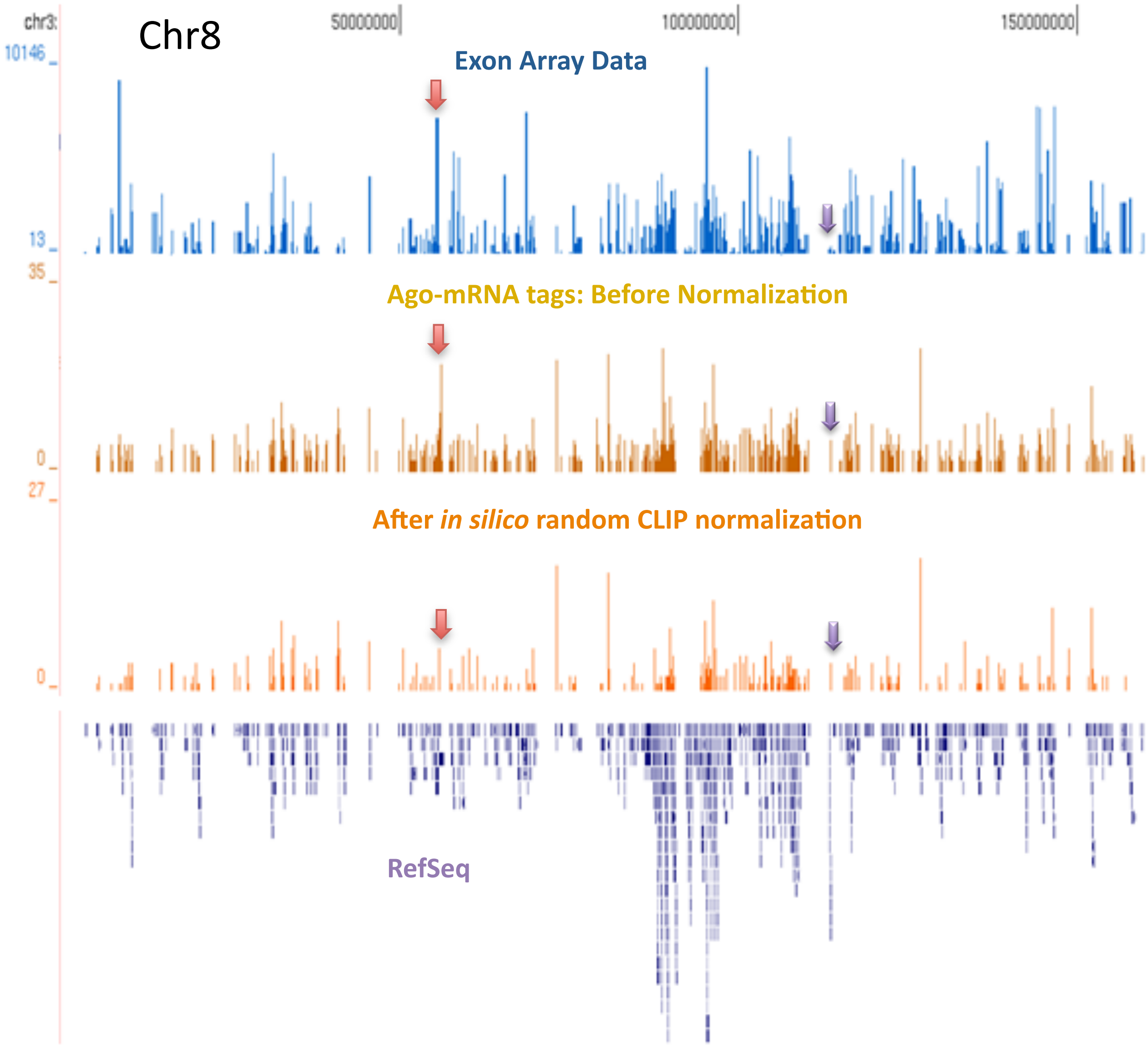
	Exon Array (N)	Length (Y)	Estimated Fragment number (F_n)	Expected tag number (T_n)
Gene A	N_a	Y_a	$N_a \times (Y_a / 50)$	$(F_a / Tot(F)) \times Z$
Gene B	N_b	Y_b	$N_b \times (Y_b / 50)$	$(F_b / Tot(F)) \times Z$
Gene C	N_c	Y_c	$N_c \times (Y_c / 50)$	$(F_c / Tot(F)) \times Z$
Gene D	N_d	Y_d	$N_d \times (Y_d / 50)$	$(F_d / Tot(F)) \times Z$
⋮	⋮	⋮	⋮	⋮

Number of tags in the most overlapping region	3	2	1
Number of observations in 500 trial	1	5	494

Threshold for normalization	>3	>2	>1
P-value	P<0.002	P<0.01	P<0.99

Supplemental Figure 4

B



Supplemental Figure 4

C

Chad7 (NM 001081417)	(N_n) Exon array value	Length (Y_n)	Expected number of tags (T_n)
1x	100	9444	5
2x	200	9444	5
4x	400	9444	5
8x	800	9444	5
16x	1600	9444	5
32x	3200	9444	5
72x	7200	9444	5
100x	10000	9444	5
500x	50000	9444	5

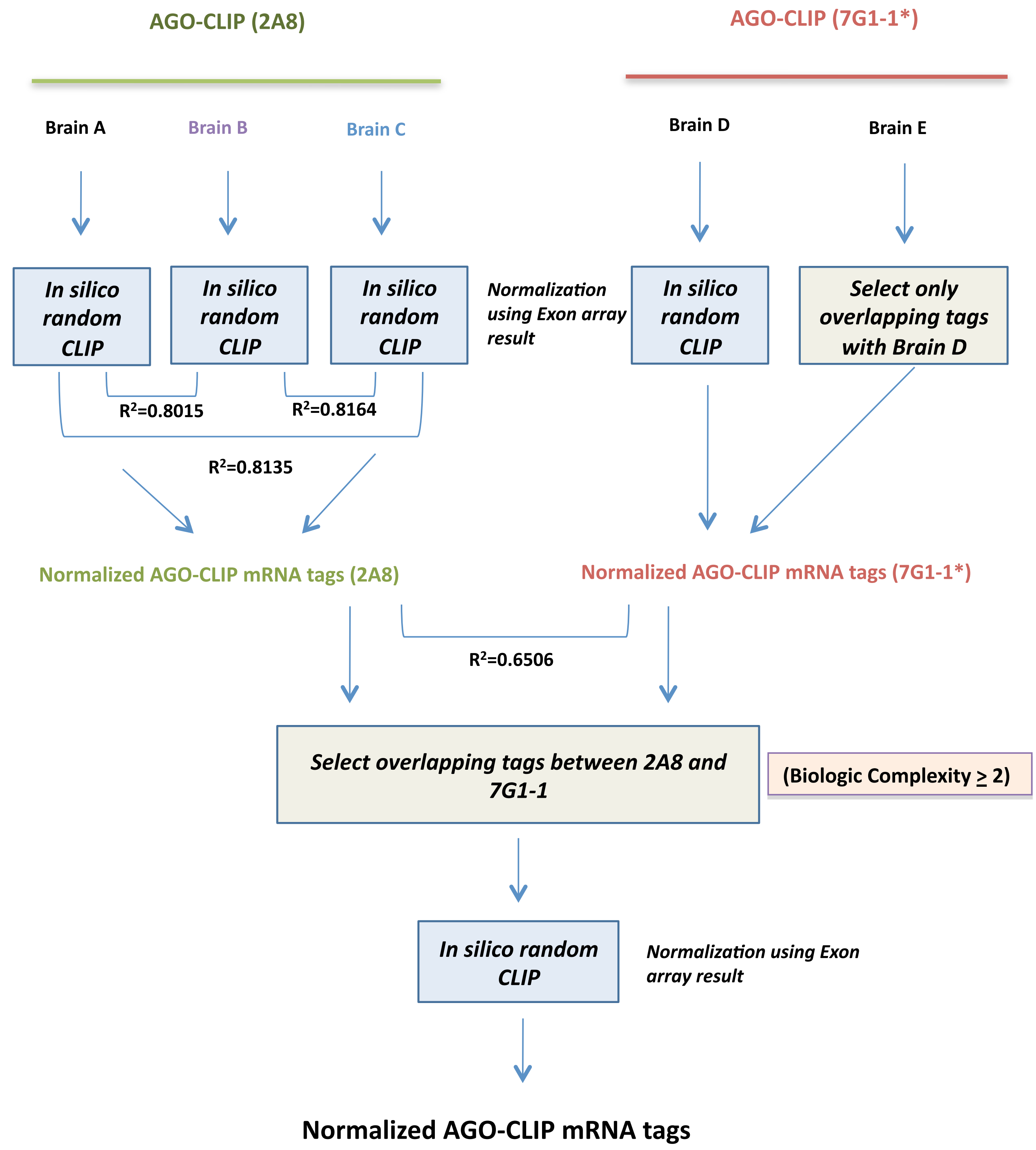
Number of tags in the most overlapping region (Mn)

Simulation	3	2	1
1x	0	12	488
2x	0	52	448
4x	0	21	479
8x	0	57	443
16x	0	43	457
32x	0	20	480
72x	0	41	459
100x	0	61	439
500x	0	35	465

False discovery rate base on *in silico* CLIP simulation result

Simulation	3	2	1
1x	<0.002	0.024	0.976
2x	<0.002	0.104	0.896
4x	<0.002	0.042	0.958
8x	<0.002	0.114	0.886
16x	<0.002	0.086	0.914
32x	<0.002	0.04	0.96
72x	<0.002	0.082	0.918
100x	<0.002	0.122	0.878
500x	<0.002	0.07	0.93

Supplemental Figure 5

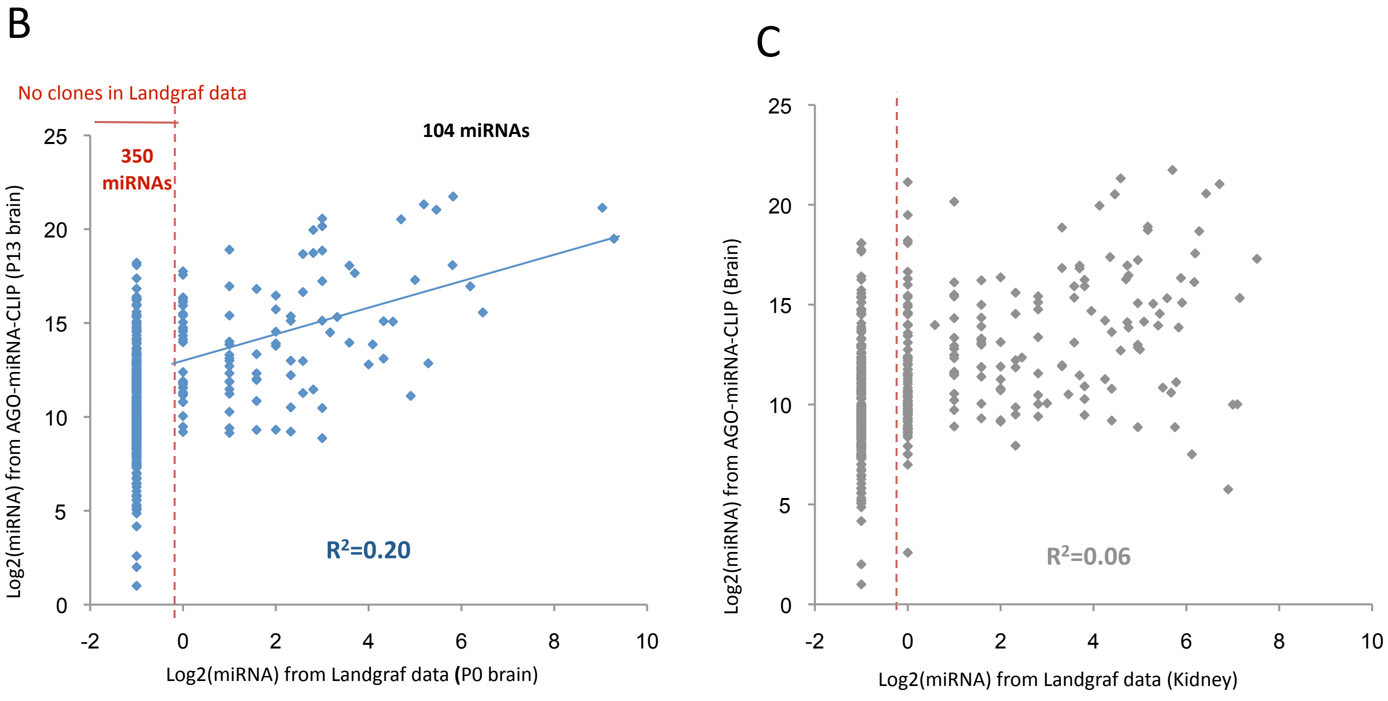


Supplemental Figure 6

A

Landgraf et al | AGO miRNA CLIP

miRNA	TOTAL CLONES NODE	Total clones in AGO-miRNA-CLIP	Rank in AGO-miRNA CLIP
mmu-miR-124	740	735430	9
mmu-miR-9	547	2309955	3
mmu-miR-126-3p	131	10238	103
mmu-miR-16	105	160843	22
mmu-miR-140	96	34537	62
mmu-miR-125b-5p	91	278206	15
mmu-miR-27a	84	1017631	8
mmu-miR-30e	82.5	3504225	1
mmu-miR-136	75	127192	25
mmu-miR-143	71	15878	89
mmu-miR-872	64	2224	195
mmu-miR-30a	60	2150543	4
mmu-miR-30d	47.5	2624768	2
mmu-miR-15a	41	35050	61
mmu-miR-379	39	7411	123
mmu-let-7c	35	419429	13
mmu-miR-29b	32	8154	116
mmu-miR-24	32	41024	57
mmu-miR-22	32	154156	23
mmu-miR-130a	28	14884	92

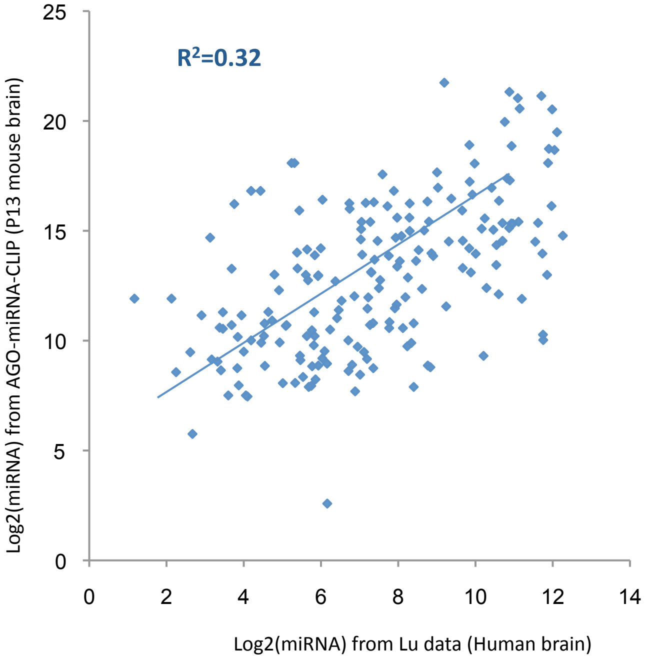


Supplemental Figure 6

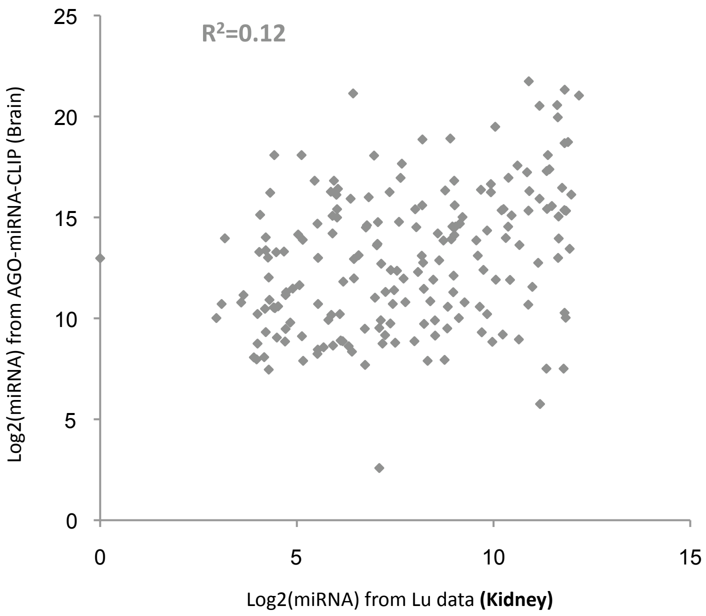
D

	Lu et al	AGO miRNA CLIP	
miRNA	Expression level	Total clones in AGO-miRNA CLIP	Rank in AGO-miRNA CLIP
miR-338-5p	4913	28123	67
miR-124	4425	735430	9
let-7c	4229	419429	13
miR-181a	4054	1510285	6
let-7a	4009	71661	38
let-7b	3824	435438	12
miR-125b-5p	3767	278206	15
miR-29b	3701	8154	116
miR-29c	3455	1045	246
miR-29a	3439	1238	236
miR-219	3403	15966	88
miR-9	3339	2309955	3
miR-125a-5p	3148	42068	53
miR-128	2993	23182	79
miR-320	2353	3830	156
miR-26a	2262	1547706	5
miR-181c	2217	43566	49
miR-30a	2190	2150543	4
miR-24	2005	41024	57
miR-181b	1958	475794	11
let-7f	1949	41306	56
miR-16	1897	160843	22
miR-30d	1882	2624768	2
miR-137	1874	35776	58
miR-27b	1803	170907	21
miR-27a	1733	1017631	8

E



F



Supplemental Figure 7

A

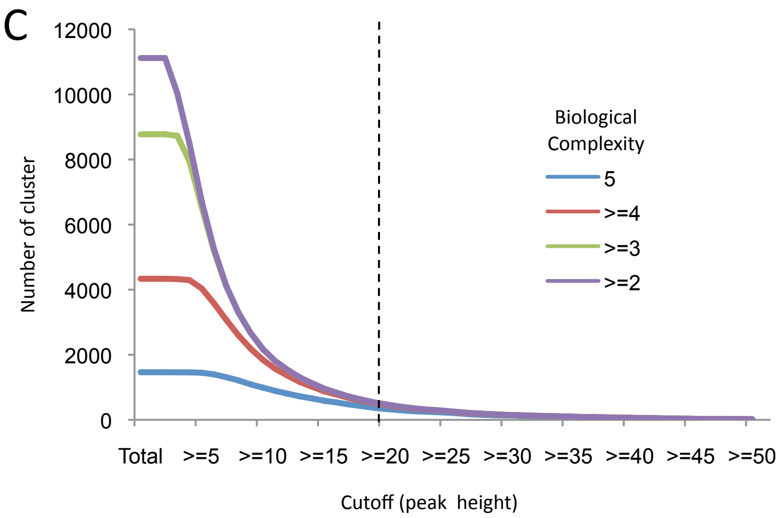
Number of Ago-mRNA clusters

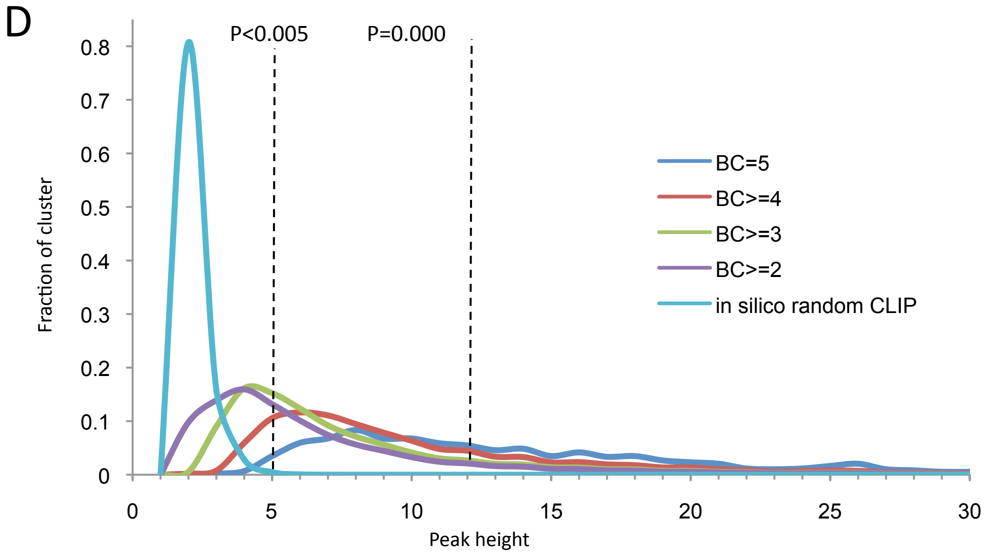
Cutoff (peak height)	Biological Complexity 5	>=4	>=3	>=2
Total	**1463**	**4335**	**8775**	**11118**
>=5	**1448**	**4036**	**6521**	**6710**
>=10	**990**	**1844**	**2172**	**2173**
>=15	**587**	**878**	**959**	**959**
>=20	**336**	**456**	**479**	**479**
>=25	**223**	**265**	**272**	**272**
>=30	**134**	**151**	**151**	**151**
>=35	**94**	**102**	**102**	**102**
>=40	**60**	**63**	**63**	**63**
>=45	**31**	**32**	**32**	**32**
>=50	**20**	**20**	**20**	**20**

B

complexity	>=5	>=4	>=3	>=2
Number of cluster	**1463**	**4335**	**8775**	**11118**
Number of genes	**829**	**2081**	**3563**	**4280**
Clusters / genes	**1.77**	**2.08**	**2.46**	**2.60**

P13 brain expressed gene: 10743 genes
(DABG : p<0.05 , Value > 100)





Supplemental Figure 7

E

miR-124 6mers

BC	Obs/Exp	p-value	True positive rate(%)	Obs	Exp
>=2	3.2649573	0.00 x 100	76.5439348	2292	702.3587
>=3	3.7799929	0.00 x 100	79.07946684	2032	537.5671
>=4	4.071868	0.00 x 100	80.28339873	1180	289.7933
=5	5.0520888	0.00 x 100	83.47677917	453	89.66588

miR-124 6mers

peak	Obs/Exp	p-value	True positive Rate(%)	Obs	Exp
>=2	3.2649573	0.00 x 100	76.55310621	2292	702
>=10	5.3136153	0.00 x 100	84.16121417	832	156.5789
>=20	6.4373708	1.32 x 10-290	86.55438834	289	44.8941
>=30	6.6297288	1.08 x 10-124	86.89337425	118	17.79862
>=40	5.5610835	1.4E-42	84.7586149	50	8.991054
>=50	5.0924938	2.68E-15	83.58636006	19	3.730981

miR-19 6mers

BC	Obs/Exp	p-value	True positive rate (%)	Obs	Exp
>=2	1.1714286	1.69E-05	53.94736842	738	630
>=3	1.2227305	9.95E-07	55.01029036	590	482.5266
>=4	1.1302395	0.035681	53.05692112	294	260.1219
=5	1.428835	0.000119	58.82799722	115	80.48515

miR-19 6mers

peak	Obs/Exp	p-value	True positive rate(%)	Obs	Exp
>=2	1.1714286	1.69E-05	53.94736842	738	630
>=10	1.1384087	0.100824	53.23625524	160	140.5471
>=20	1.0174334	0.91188	50.43206914	41	40.29748
>=30	1.0640796	0.797852	51.55225689	17	15.97625
>=40	1.2390843	0.497008	55.33888625	10	8.070476
>=50	0.597198	0.46104	37.39035571	2	3.348973

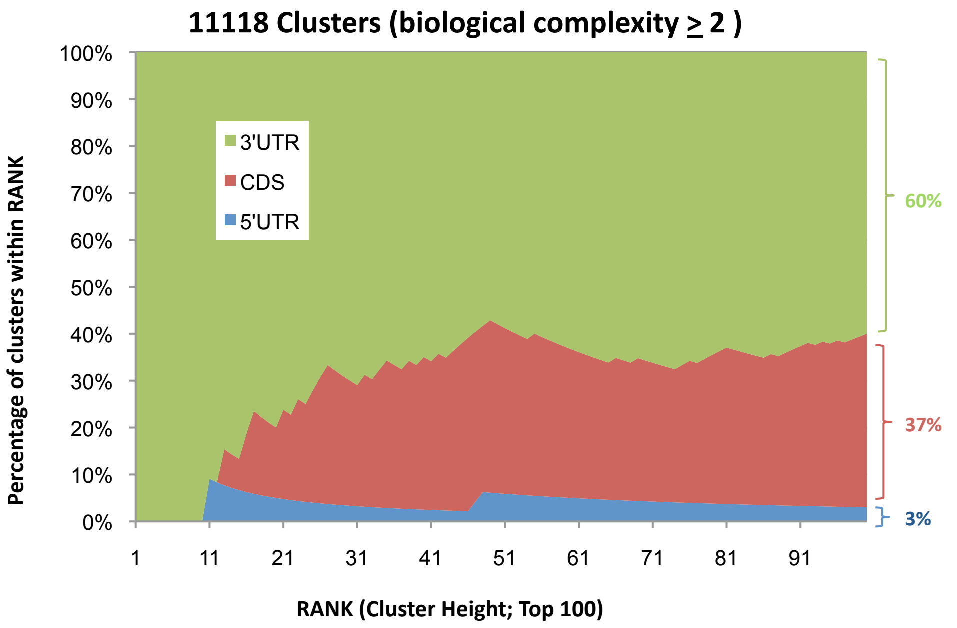
Supplemental Figure 8

A

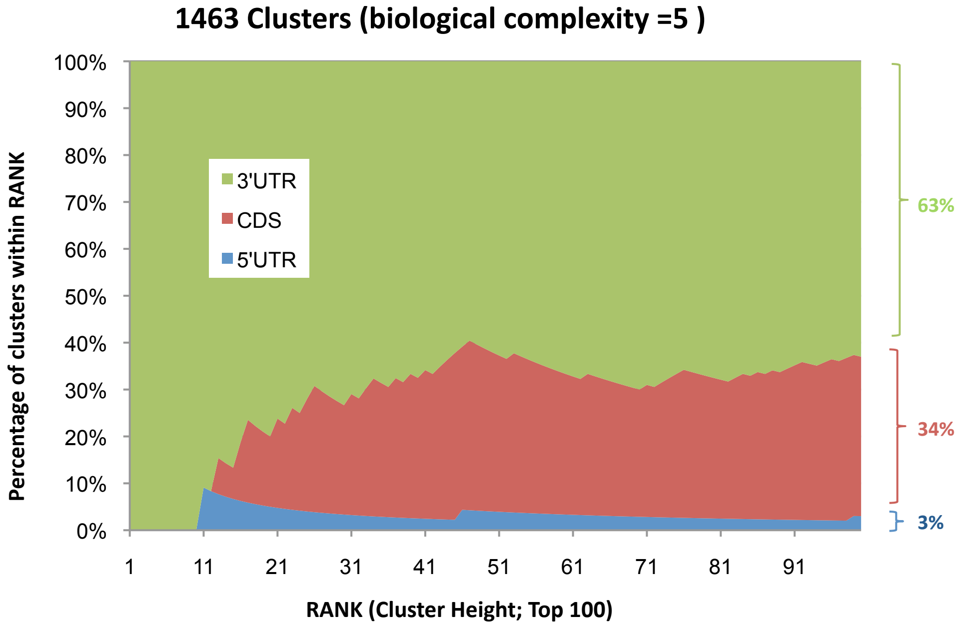
Enrichment of AGO-CLIP mRNA clustered tags in 3' UTR

	Number of clusters (BC ≥ 2)	% Clusters	Number of tags	% tags	Total length (brain expressed transcripts)	% Total Length	Obs/Exp (number of clusters/ total length)	P-value	Obs/Exp (number of tags/ total length)	P-value
5'UTR	211	1.9	1,742	1.7	3,686,859	6.8	0.28	$1.92x10^{-87}$	0.25	$0.00x10^{0}$
CDS	4992	44.9	42,553	41.8	31,287,950	57.4	0.78	$8.77x10^{-68}$	0.73	$0.00x10^{0}$
3'UTR	5915	53.2	57,589	56.5	19,571,210	35.9	**1.48**	$1.26x10^{-203}$	**1.58**	$0.00x10^{0}$
Total	11,118	100	101,884	100	54,546,019	100				

B



C



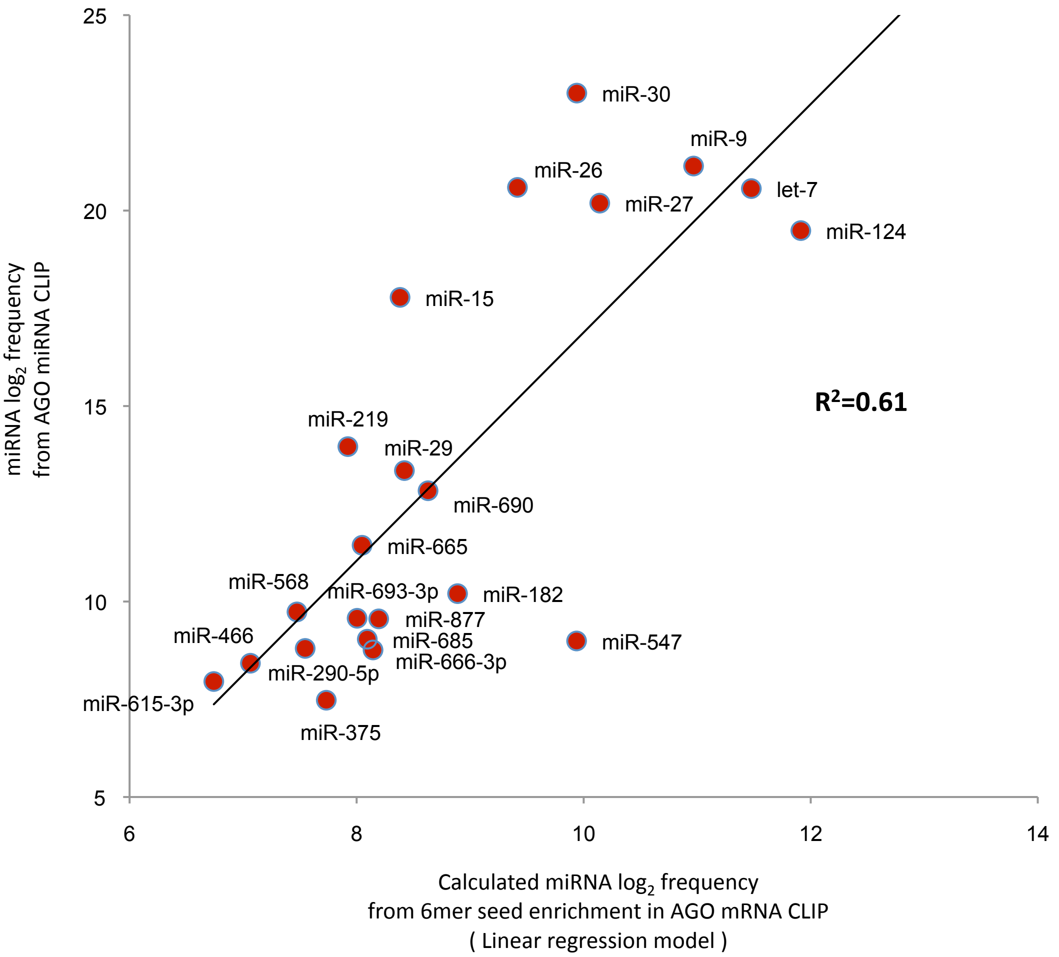
Supplemental Figure 9

A

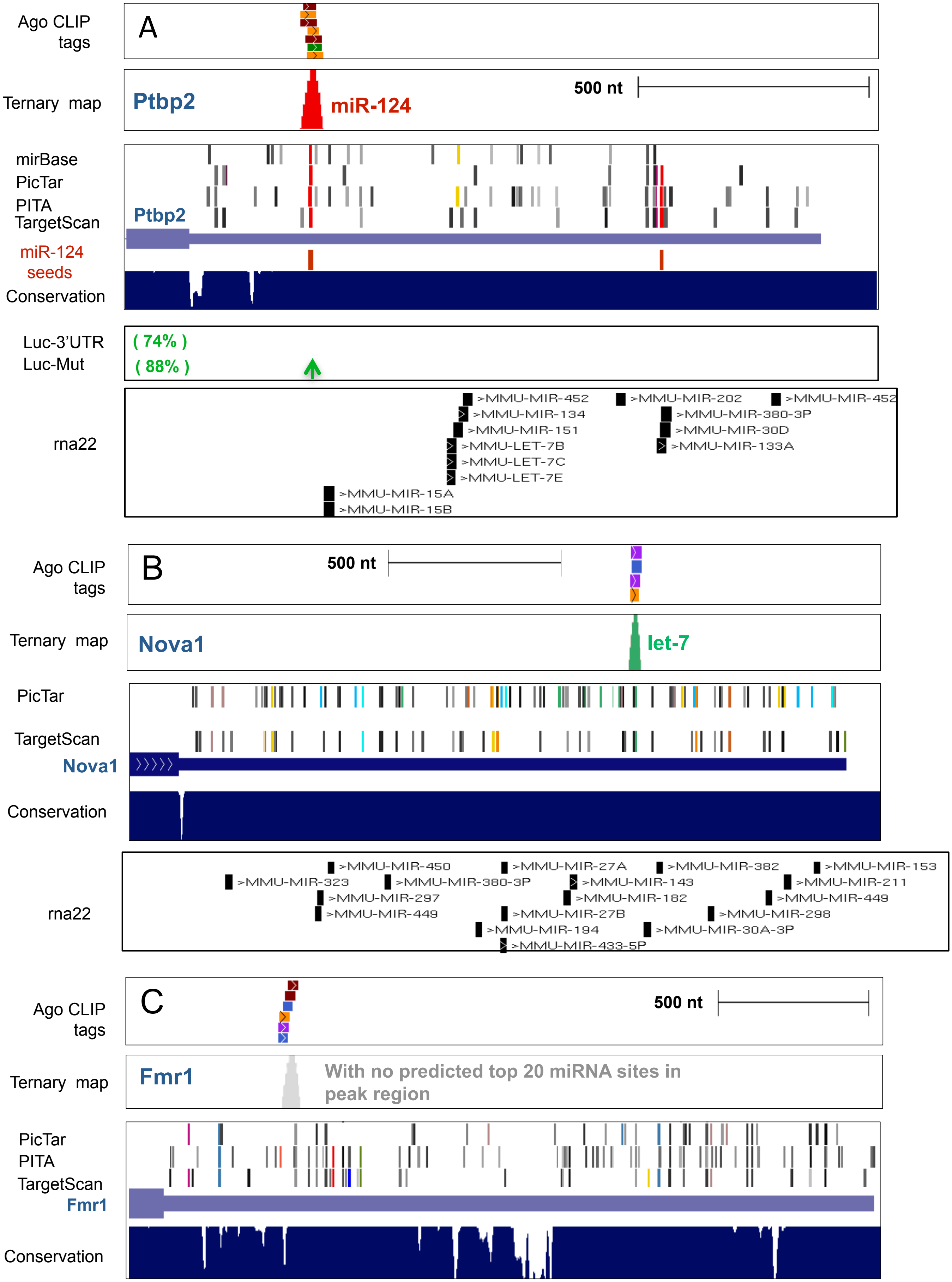
AGO-mRNA CLIP				AGO-miRNA CLIP			
Top ranking motif	**Regression coefficient**	**Number of unique tags**	**P-value**	**miRNA**	**Matched seed position**	**Fraction in AGO-miRNA CLIP**	**Rank in AGO-miRNA CLIP**
GTGCCTT	0.655	4581	9.01 x 10^{-31}	mir-124	2-8	2.97%	8
TGCCTT	0.421	9153	3.74 x 10^{-30}		2-7		
GTGCCT	0.247	6915	8.45 x 10^{-9}		3-8		
TACCTC	0.452	6403	2.13 x 10^{-25}	let-7	2-7	6.24%	5
TACCTCA	0.529	3558	1.82 x 10^{-18}		2-8		
CCAAAG	0.287	6878	9.22 x 10^{-14}	mir-9	2-7	9.33%	2
CCAAAGA	0.376	3549	2.06 x 10^{-12}		1-7		
TGTTTAC	0.404	2136	3.29 x 10^{-8}	mir-30	2-8	34.01%	1
GTTTAC	0.331	2964	7.19 x 10^{-8}		2-7		
CTGTGA	0.274	4205	1.47 x 10^{-9}	mir-27	2-7	4.80%	6
ACTGTGA	0.394	1665	3.01 x 10^{-7}		2-8		
TACTTGAA	0.676	877	3.89 x 10^{-8}	mir-26	1-8	6.39%	4
ACTTGA	0.290	2353	3.48 x 10^{-6}		2-7		

BC>=2 , -24 ~ +22 region

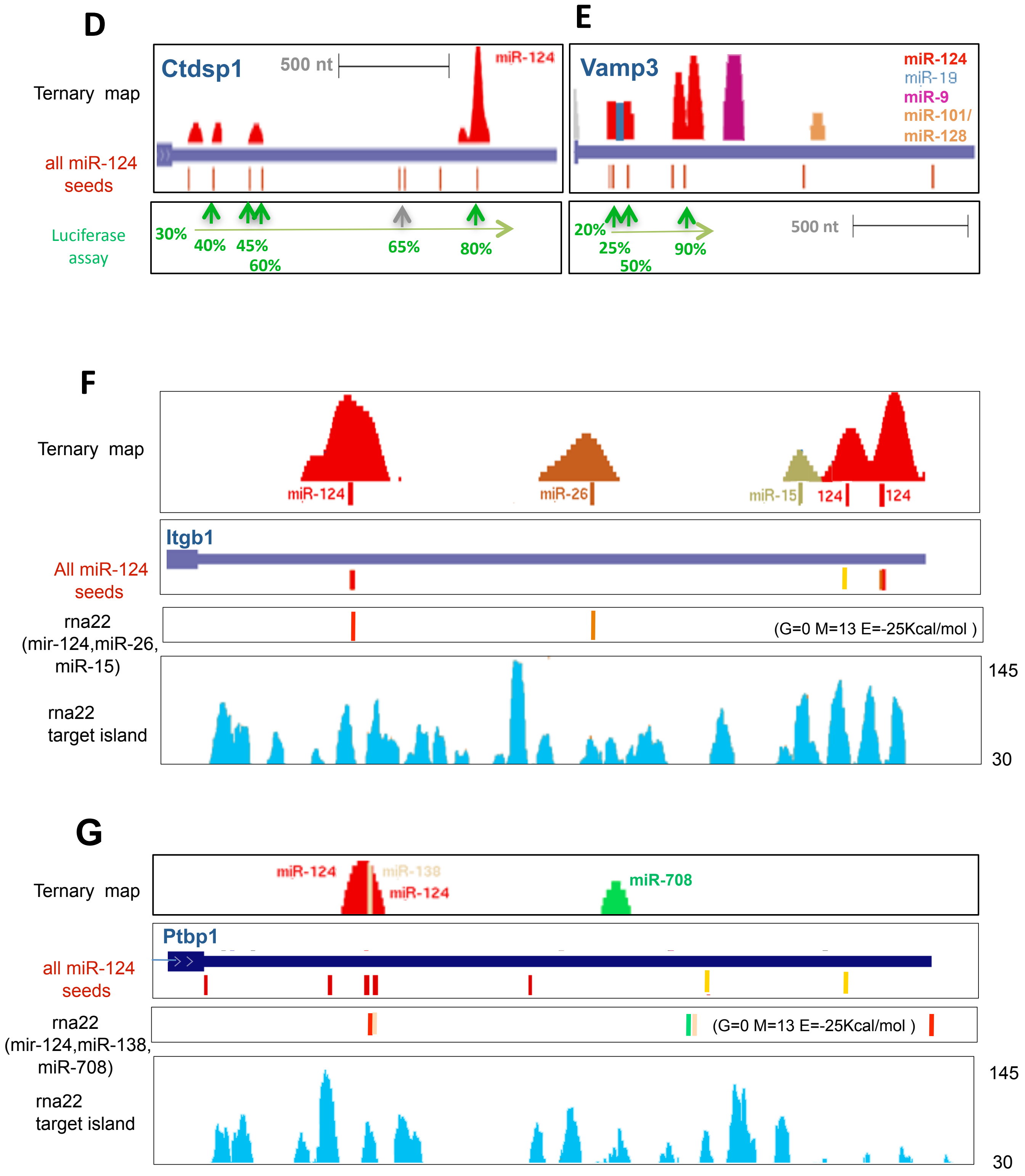
B



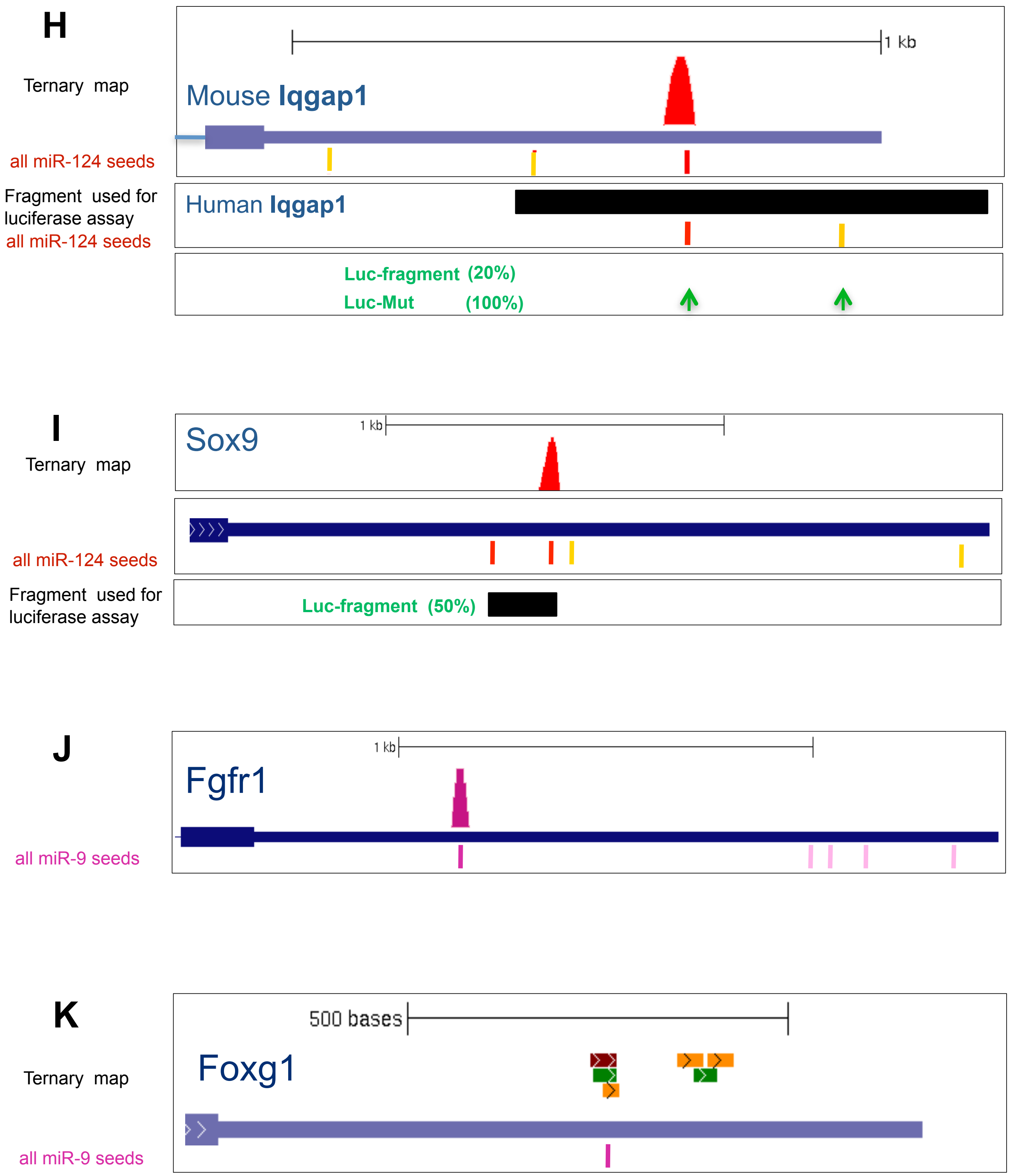
Supplemental Figure 10



Supplemental Figure 10



Supplemental Figure 10



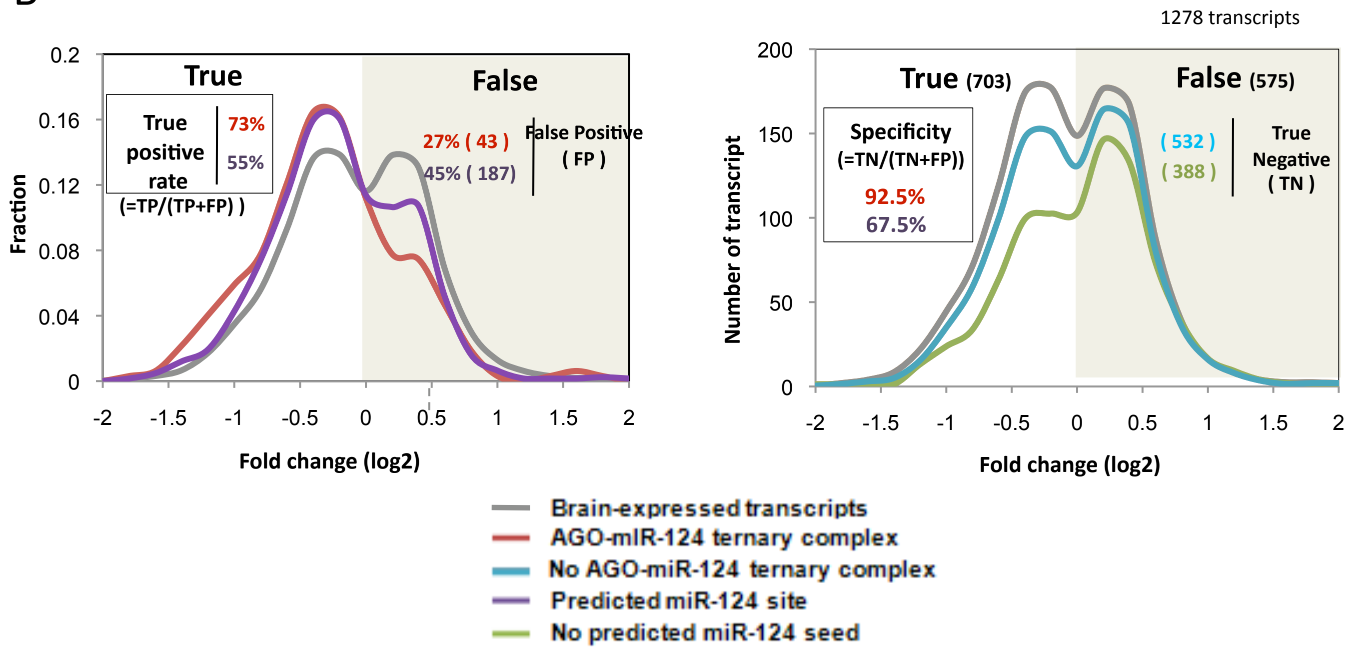
Supplemental Figure 11

A

GEO	experiments	Cell line	species	measure	Reference
GSE8498	overexpression	neuroblastoma CAD	mouse	microarray	Makeyev EV, Zhang J, Carrasco MA, Maniatis T. The MicroRNA miR-124 promotes neuronal differentiation by triggering brain-specific alternative pre-mRNA splicing. Mol Cell 2007 Aug 3;27(3): 435-48.
GSE6207	overexpression	HepG2	human	microarray	Wang X, Wang X. Systematic identification of microRNA functions by combining target prediction and expression profiling. Nucleic Acids Res 2006;34(5):1646-52
GSE2075	overexpression	Hela	human	microarray	Lim LP, Lau NC, Garrett-Engele P, Grimson A et al. Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. Nature 2005 Feb 17;433(7027):769-73
GSE11968	overexpression	Hela	human	SILAC-MS microarray	Baek D, Villén J, Shin C, Camargo FD et al. The impact of microRNAs on protein output. Nature 2008 Sep 4;455(7209):64-71
GSE11080	overexpression	HEK293T	human	microarray	David G. Hendrickson, Daniel J. Hogan, Daniel Herschlag, James E. Ferrell and Patrick O. Brown, Systematic Identification of mRNAs Recruited to Argonaute 2 by Specific microRNAs and Corresponding Changes in Transcript Abundance PLoS ONE, 2008 May 7;3(5):e2126

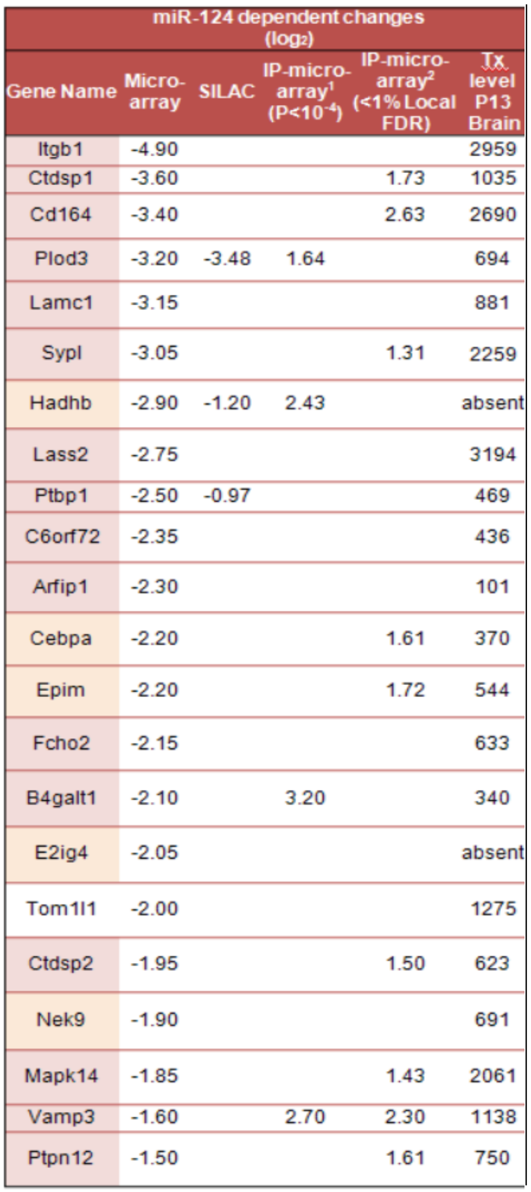
5 microarray (meta-analysis) : 1278 genes (more than two exp : p<0.05, brain expressed)

B



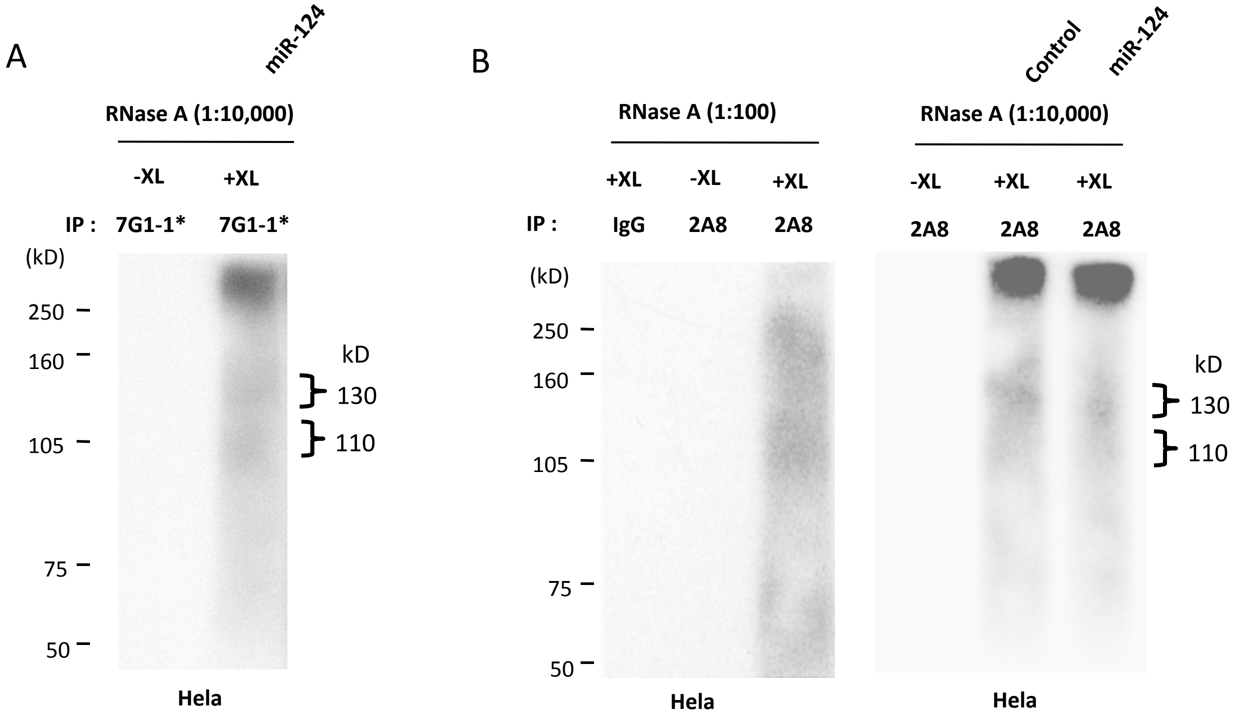
Supplemental Figure 11

C



	miR-124 dependent changes (log$_2$)				
Gene Name	Micro-array	SILAC	IP-micro-array^1 ($P<10^{-4}$)	IP-micro-array^2 (<1% Local FDR)	Tx level P13 Brain
Itgb1	-4.90				2959
Ctdsp1	-3.60			1.73	1035
Cd164	-3.40			2.63	2690
Plod3	-3.20	-3.48	1.64		694
Lamc1	-3.15				881
Sypl	-3.05			1.31	2259
Hadhb	-2.90	-1.20	2.43		absent
Lass2	-2.75				3194
Ptbp1	-2.50	-0.97			469
C6orf72	-2.35				436
Arfip1	-2.30				101
Cebpa	-2.20			1.61	370
Epim	-2.20			1.72	544
Fcho2	-2.15				633
B4galt1	-2.10		3.20		340
E2ig4	-2.05				absent
Tom1l1	-2.00				1275
Ctdsp2	-1.95			1.50	623
Nek9	-1.90				691
Mapk14	-1.85			1.43	2061
Vamp3	-1.60		2.70	2.30	1138
Ptpn12	-1.50			1.61	750

Supplemental Figure 12



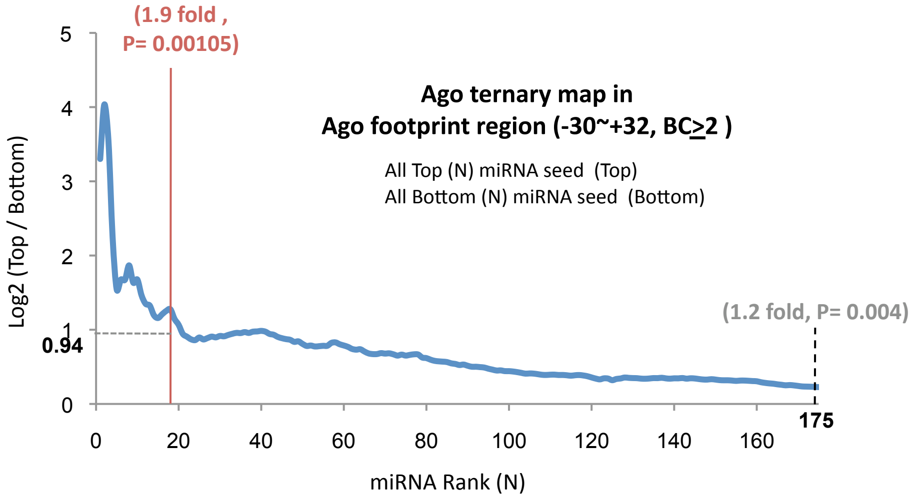
C

Sample	Transfected miRNA	Antibody	Raw tags (prefiltered)	Aligned tags (hg18)	% align	Unique tags
HeLa 1	miR-124	2A8	11,563,568	6,082,765	52.6	1,271,461
HeLa 2	miR-124	2A8	12,681,689	5,715,019	45.1	1,435,945
HeLa 3	miR-124	7G1-1	12,484,953	5,816,073	46.6	1,459,849
HeLa 4	miR-124	7G1-1	12,441,955	5,419,481	43.6	1,603,967
HeLa 5	control miRNA	2A8	10,802,378	5,951,833	55.1	1,184,079
HeLa 6	control miRNA	2A8	11,104,169	5,813,633	52.4	1,247,774
HeLa 7	control miRNA	7G1-1	12,204,108	6,916,662	56.7	1,272,437
HeLa 8	control miRNA	7G1-1	12,957,786	3,396,298	26.2	1,127,326

Supplemental Figure 13

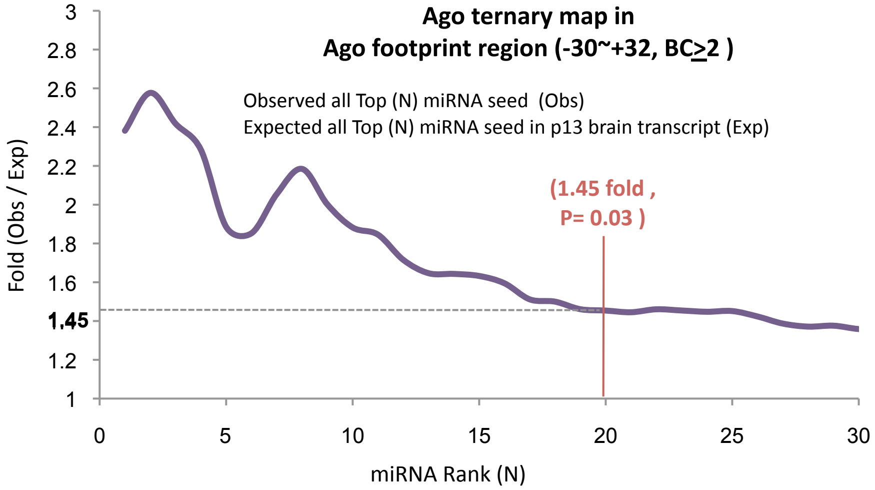
Without conservation

A



Without conservation

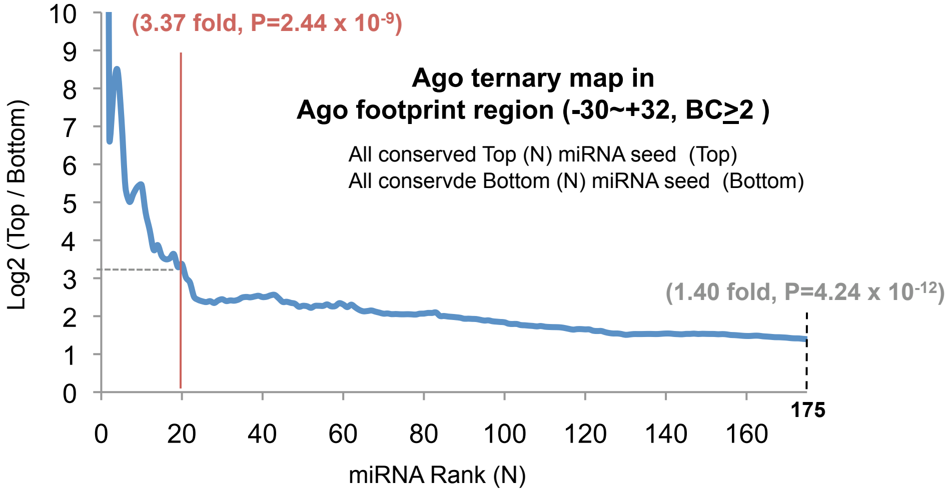
B



Supplemental Figure 13

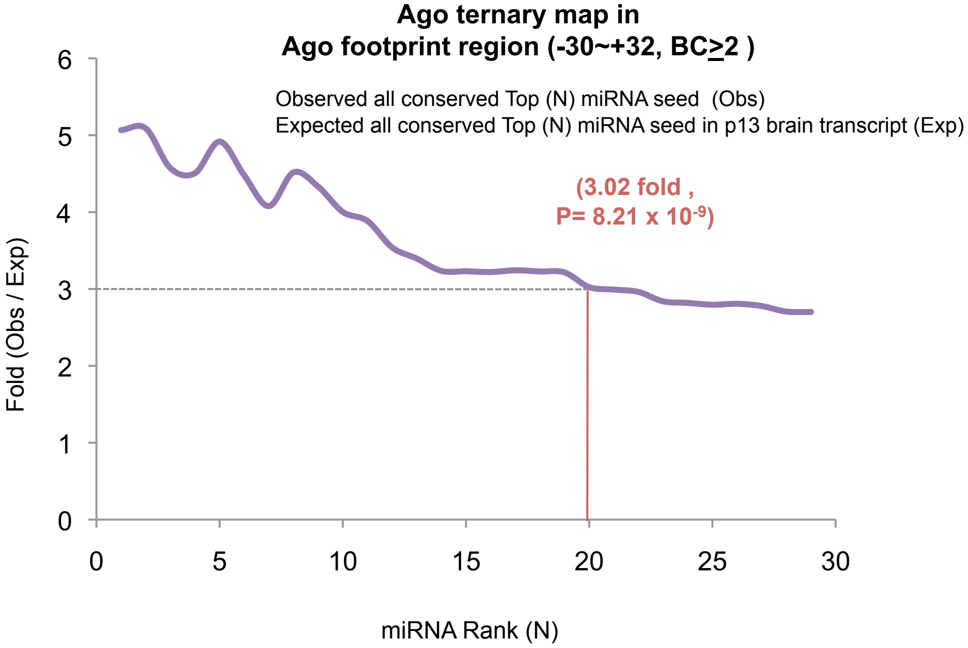
C

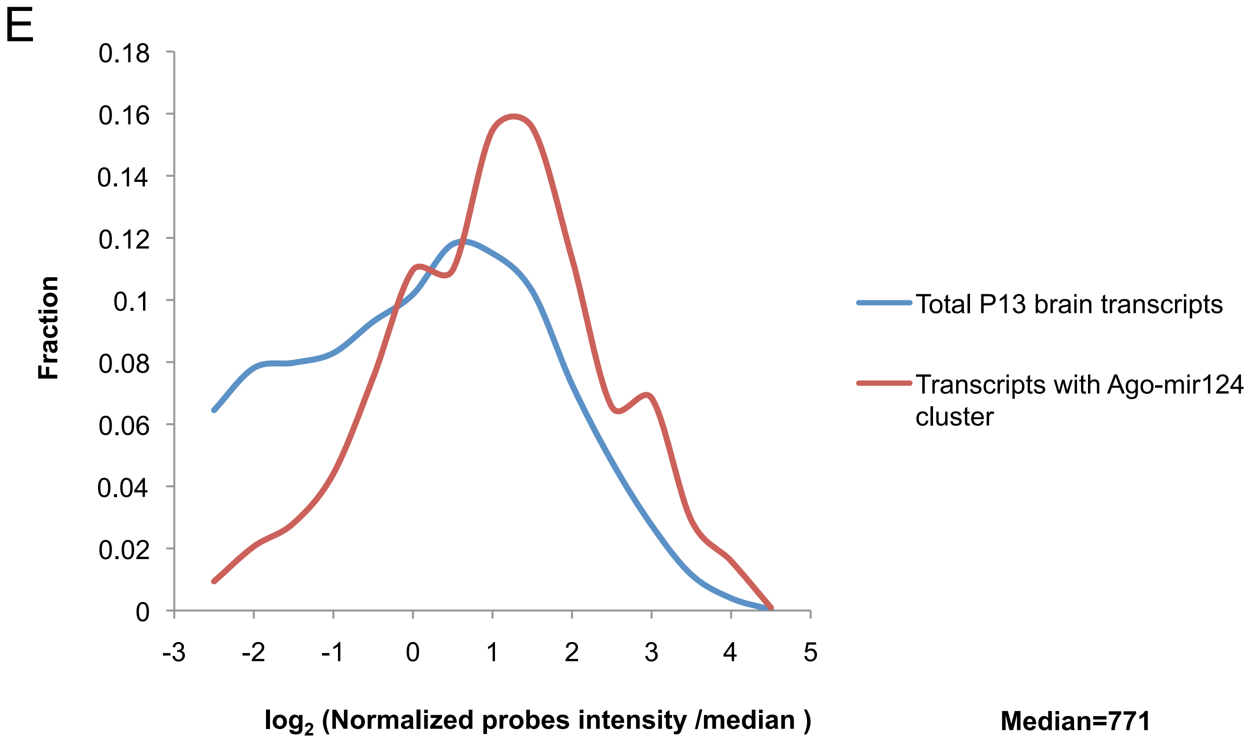
With conservation



With conservation

D




E
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Fraction
-3
-2
-1
0
1
2
3
4
5
log2 (Normalized probes intensity /median)
Total P13 brain transcripts
Transcripts with Ago-mir124 cluster
Median=771

Supplemental Figure 14

A

BC>=2

Number of Ago-mRNA clusters in 3'UTR	Number of 3'UTRs
1	1665
2	584
3	262
4	130
5	87
6	49
7	34
8	27
9	17
10	9
>10	43

Averages:
1.03 clusters/transcript in brain
2.60 clusters/Ago regulated transcript
2.12 clusters /3'UTR of Ago regulated transcript

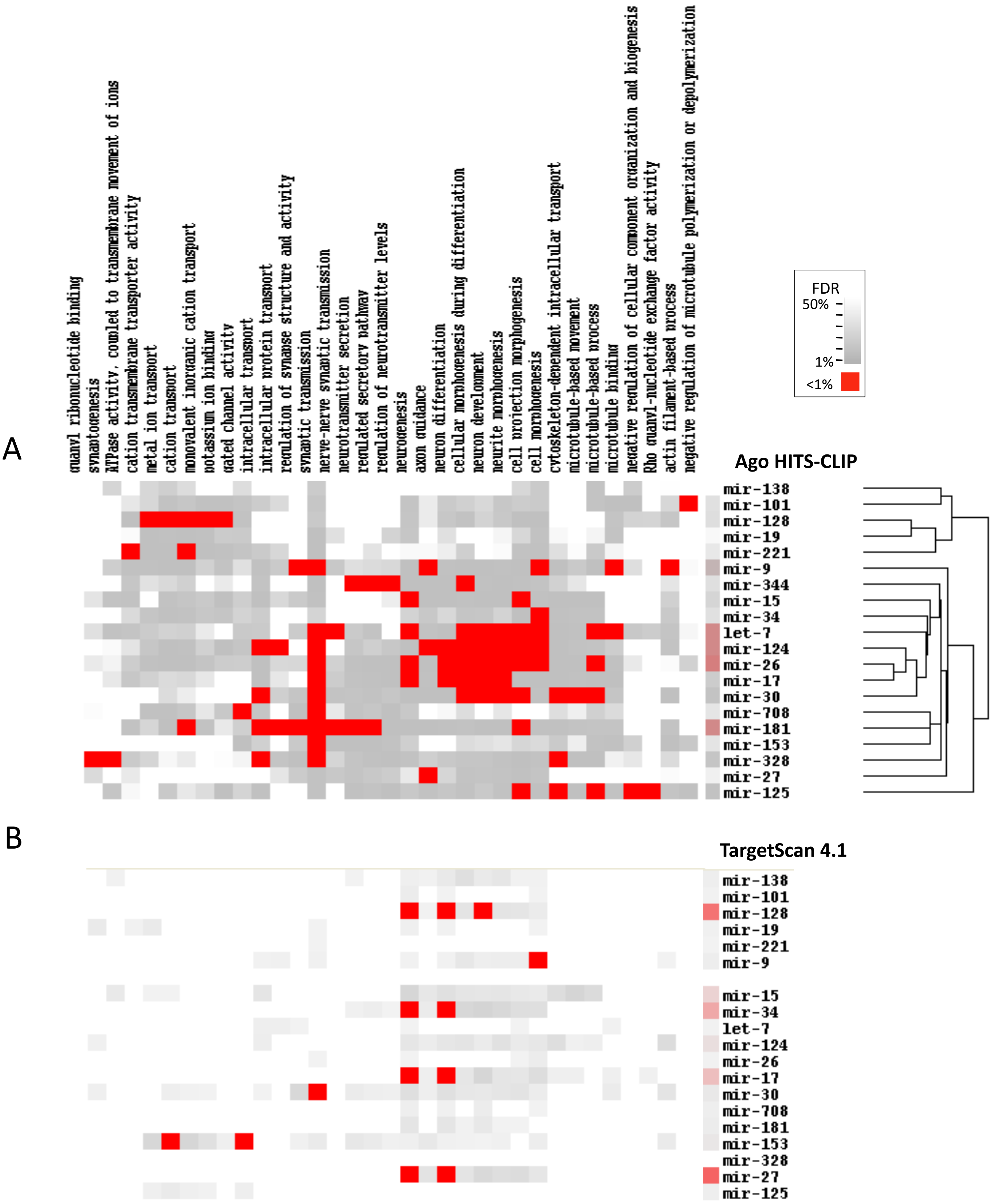
B

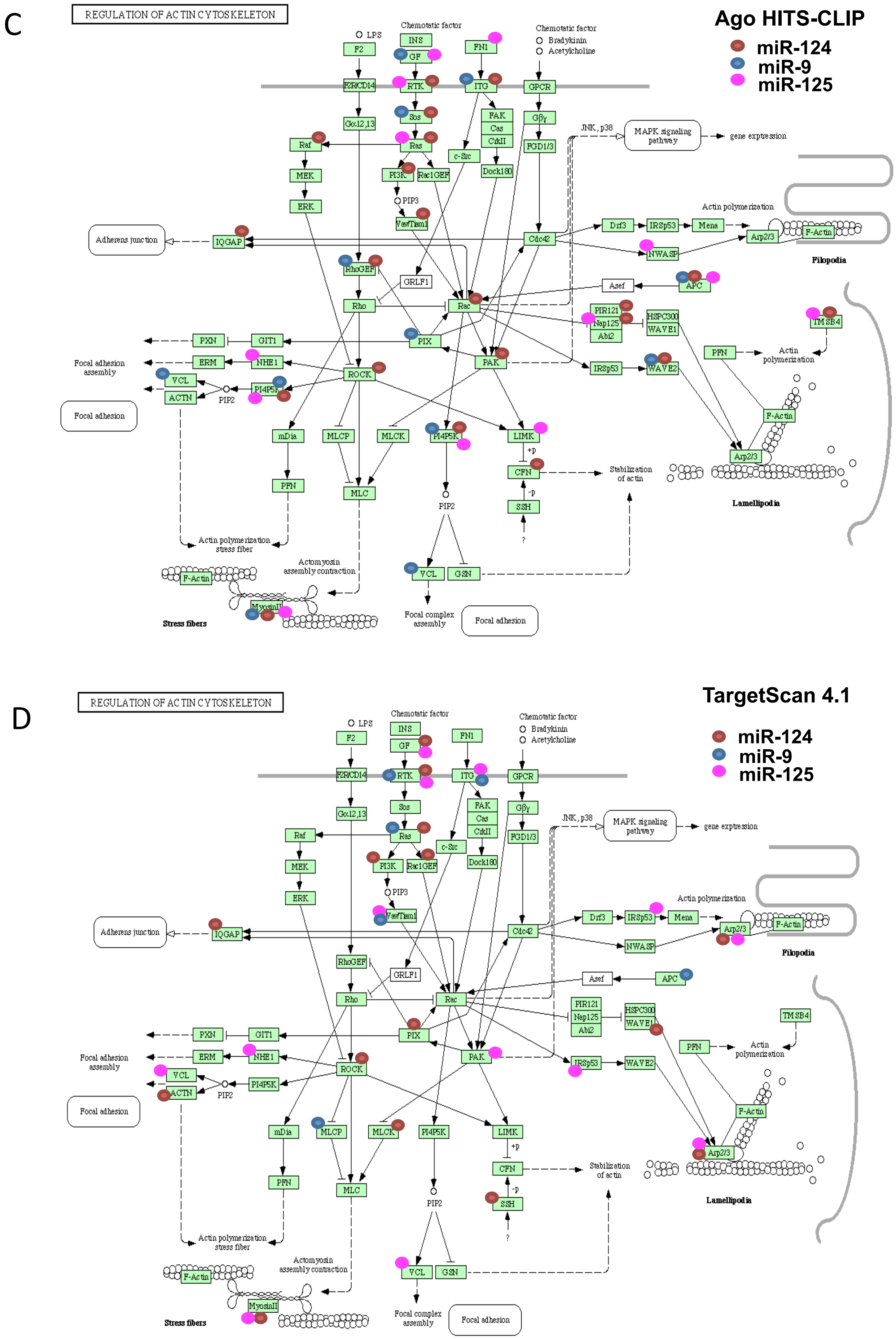
	miRNA family	**Number of target transcripts**
🟥	**miR-124**	**1254**
🟪	**miR-9**	**1207**
🟦	**miR-27**	**1080**
🟩	**let-7**	**1060**
🟨	**miR-15**	**787**
🟨	**miR-181**	**740**
🟧	**miR-101**	**631**
🟥	**miR-34**	**626**
🟦	**miR-30**	**610**
🟧	**miR-26**	**602**
🟨	**miR-138**	**573**
🟪	**miR-17**	**536**
🟦	**miR-125**	**517**
🟩	**miR-708**	**498**
🟦	**miR-19**	**486**
🟫	**miR-153**	**455**
🟧	**miR-193**	**448**
🟪	**miR-21**	**358**
🟥	**miR-344**	**320**
🟩	**miR-221**	**315**

Average 655 targets / miRNA

C

Supplemental Figure 15




C
REGULATION OF ACTIN CYTOSKELETON
Ago HITS-CLIP
● miR-124
● miR-9
● miR-125
LPS
F2
F2R/CD14
Gα12,13
Chemotactic factor
INS
GF
RTK
Sos
Ras
Raf
MEK
ERK
PI3K
Rac1GEF
PIP3
Vav/Tiam1
FN1
ITG
FAK
Cas
CrkII
c-Src
Dock180
Chemotactic factor
Bradykinin
Acetylcholine
GPCR
Gβγ
FGD1/3
JNK, p38
MAPK signaling pathway
gene exptression
Adherens junction
IQGAP
Cdc42
Drf3
IRSp53
Mena
Actin polymerization
Arp2/3
F-Actin
N-WASP
Filopodia
RhoGEF
GRLF1
Asef
APC
Rho
Rac
PIR121
Nap125
Abi2
HSPC300
WAVE1
TMSB4
PXN
GIT1
PIX
Focal adhesion assembly
ERM
NHE1
ROCK
PAK
IRSp53
WAVE2
PFN
Actin polymerization
VCL
ACTN
PIP2
PI4P5K
Focal adhesion
F-Actin
mDia
MLCP
MLCK
PI4P5K
LIMK
+p
CFN
Stabilization of actin
Arp2/3
PFN
MLC
PIP2
SSH
-p
?
Lamellipodia
Actin polymerization stress fiber
Actomyosin assembly contraction
F-Actin
MyosinII
Stress fibers
VCL
GSN
Focal complex assembly
Focal adhesion
D
REGULATION OF ACTIN CYTOSKELETON
TargetScan 4.1
● miR-124
● miR-9
● miR-125
LPS
F2
F2R/CD14
Gα12,13
Chemotactic factor
INS
GF
RTK
Sos
Ras
Raf
MEK
ERK
PI3K
Rac1GEF
PIP3
Vav/Tiam1
FN1
ITG
FAK
Cas
CrkII
c-Src
Dock180
Chemotactic factor
Bradykinin
Acetylcholine
GPCR
Gβγ
FGD1/3
JNK, p38
MAPK signaling pathway
gene exptression
Adherens junction
IQGAP
Cdc42
Drf3
IRSp53
Mena
Actin polymerization
Arp2/3
F-Actin
N-WASP
Filopodia
RhoGEF
GRLF1
Asef
APC
Rho
Rac
PIR121
Nap125
Abi2
HSPC300
WAVE1
TMSB4
PXN
GIT1
PIX
Focal adhesion assembly
ERM
NHE1
ROCK
PAK
IRSp53
WAVE2
PFN
Actin polymerization
VCL
ACTN
PIP2
PI4P5K
Focal adhesion
F-Actin
mDia
MLCP
MLCK
PI4P5K
LIMK
+p
CFN
Stabilization of actin
Arp2/3
PFN
MLC
PIP2
SSH
-p
?
Lamellipodia
Actin polymerization stress fiber
Actomyosin assembly contraction
F-Actin
MyosinII
Stress fibers
VCL
GSN
Focal complex assembly
Focal adhesion

Supplemental Figure 16

A

	Number of Ago mir-124 clusters	Number of Ago Top20 miRNA clusters
Ago-CLIP	1561	15665
Average # of clusters in 10 simulations	1487	14410.7
Standard deviation	2.05	16.57
simulation #1	1484	14401
simulation #2	1490	14417
simulation #3	1489	14401
simulation #4	1488	14440
simulation #5	1484	14413
simulation #6	1487	14408
simulation #7	1487	14388
simulation #8	1488	14412
simulation #9	1488	14393
simulation #10	1485	14434
recovery rate(%)	95	92