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Supplemental information

Rheb-mTOR activation rescues Aβ-induced cognitive impairment and memory function by restoring miR-146 activity in glial cells

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Supplementary Figures and Tables

Supplemental Table S1 Differentially expressed miRNAs in the pre-frontal cortex with their respective fold change and p-value

	miRNA ID	log2FoldChange	P-value
1	hsa-miR-100-5p	1.43	1.00E-02
2	hsa-miR-141-3p	1.69	9.81E-03
3	hsa-miR-142-5p	1.82	1.94E-05
4	hsa-miR-146a-5p	1.29	2.25E-02
5	hsa-miR-148b-3p	2.07	1.04E-03
6	hsa-miR-152	1.78	4.14E-06
7	hsa-miR-153	1.67	1.99E-07
8	hsa-miR-18a-5p	1.58	5.09E-05
9	hsa-miR-19a-5p	1.46	1.75E-02
10	hsa-miR-208b	1.65	6.30E-08
11	hsa-miR-20a-3p	1.52	4.01E-04
12	hsa-miR-26a-1-3p	2.03	3.34E-03
13	hsa-miR-302a-3p	2.41	3.06E-02
14	hsa-miR-302b-3p	2.35	4.78E-03
15	hsa-miR-32-5p	1.27	6.25E-07
16	hsa-miR-338-3p	1.32	1.84E-06
17	hsa-miR-3613-3p	1.56	3.82E-05
18	hsa-miR-3676-5p	1.58	4.55E-03
19	hsa-miR-374a-5p	2.15	2.18E-04
20	hsa-miR-374b-5p	1.47	1.69E-04
21	hsa-miR-488-3p	1.31	2.96E-08
22	hsa-miR-5000-3p	1.58	1.02E-02
23	hsa-miR-500a-5p	1.44	3.68E-02
24	hsa-miR-516a-5p	1.69	3.03E-05
25	hsa-miR-519a-3p	1.29	1.94E-03
26	hsa-miR-590-3p	1.27	2.15E-06
27	hsa-miR-675-3p	2.11	4.54E-02
28	hsa-miR-7-1-3p	1.52	9.75E-10
29	hsa-miR-941	2.20	3.75E-03
30	hsa-miR-99b-5p	1.46	3.69E-03
31	hsa-miR-1225-3p	-2.30	5.66E-10
32	hsa-miR-1225-5p	-1.78	1.39E-04
33	hsa-miR-1276	-1.66	1.49E-03
34	hsa-miR-132-3p	-2.02	2.77E-14
35	hsa-miR-132-5p	-1.89	3.19E-10
36	hsa-miR-212-3p	-1.70	4.76E-08
37	hsa-miR-212-5p	-1.87	2.07E-06
38	hsa-miR-25-5p	-1.36	8.88E-03
39	hsa-miR-323a-3p	-1.30	3.66E-07
40	hsa-miR-323a-5p	-1.53	9.33E-06
41	hsa-miR-3607-3p	-2.76	2.69E-10
42	hsa-miR-3651	-1.95	4.09E-02
43	hsa-miR-3653	-2.08	4.96E-07
44	hsa-miR-376a-5p	-1.49	1.97E-05

45	hsa-miR-4284	-1.43	2.11E-02
46	hsa-miR-4520a-3p	-1.71	3.28E-03
47	hsa-miR-505-5p	-1.43	1.92E-02
48	hsa-miR-511	-1.85	1.51E-02
49	hsa-miR-5701	-2.23	1.97E-08
50	hsa-miR-6087	-2.56	1.54E-03
51	hsa-miR-6721-5p	-2.39	9.94E-03
52	hsa-miR-877-5p	-1.52	1.94E-07
53	hsa-miR-885-3p	-2.15	9.54E-15

Supplemental Table S2: Expression profile of miR-146a-5p target genes in brain cortical regions in Alzheimer's disease patients. Fold change and p-values of differentially expressed miR-146a-5p targets within cortical regions of Alzheimer's disease based on differential expression analysis of mRNA profiling data, is represented here.

Frontal cortex			Superior frontal gyrus			Dorsolateral pre-frontal cortex		
Target gene name	Fold change	p-value	Target gene name	Fold change	p-value	Target gene name	Fold change	p-value
BRCA2	1.27	8.96E-03	CXCR4	4.45	5.39E-05	TLR4	-2.34	5.96E-03
CDKN1A	1.27	3.93E-09	EGFR	1.89	5.03E-03	WASF2	-1.44	2.92E-02
EGFR	1.48	1.36E-03	ERBB4	2.76	4.37E-07	NOTCH2	-1.58	2.12E-02
TLR4	1.27	3.03E-08	TLR4	1.33	2.16E-03	LFNG	-1.75	2.13E-02
SLPI	1.64	1.71E-03	STAT1	-1.42	2.39E-02	TGFB1	-2.57	2.32E-02
CARD10	1.45	6.02E-11	CARD10	1.39	4.72E-02	KLF4	-3.34	2.67E-03
ICAM1	1.40	2.87E-02	COPS8	-1.26	4.64E-02	TCF7	-1.92	1.15E-02
CCL5	1.62	5.28E-06	ELAVL1	2.11	3.86E-03	COL18A1	-2.04	3.82E-02
CNOT6L	1.62	1.25E-02	CASP7	1.69	3.86E-03	PRKACB	1.66	4.21E-02
CPM	1.71	1.59E-13	NOTCH2	2.26	1.48E-03	IGF1	2.91	4.39E-03
TGFB1	1.72	5.13E-03	SOX2	2.11	9.91E-04	SLC3A1	2.20	2.66E-02
FANCM	2.94	2.61E-02	LFNG	1.74	2.26E-03	CPT1A	-1.32	3.16E-02
NOTCH1	1.34	1.29E-19	MIF	-1.81	4.21E-02	LRP5	-2.88	1.22E-02
NOS2	1.49	4.48E-03	NOTCH1	1.33	1.20E-03	RASSF3	-1.67	3.20E-02
EGR1	-2.05	9.95E-13	NFAT5	1.55	3.48E-02	BCAT2	-1.94	2.45E-02
TGIF1	1.40	5.36E-04	MED1	1.39	1.10E-02	NECAB1	2.11	1.77E-02
SNAP25	-3.55	7.86E-26	EGR1	-1.95	7.87E-03	ZNRF3	-1.32	1.50E-02
ST6GAL2	1.46	1.39E-02	CAMK2D	-1.47	9.12E-03	MAFF	-2.54	3.95E-02
TMPRSS5	1.78	4.29E-11	SNAP25	-4.50	1.35E-04	GPR146	-1.88	4.60E-02
SHCBP1	1.28	6.20E-11	KLF4	2.49	1.28E-02	GPR116	-1.57	5.62E-03
RHOBTB3	1.42	2.96E-16	METTL7A	1.31	3.75E-04	EID1	1.75	1.63E-02
MICAL2	-1.62	9.70E-24	KCTD15	1.85	1.54E-02	PIGB	1.41	3.53E-02
PLEKHG5	2.38	2.25E-02	RHOBTB3	2.34	9.71E-03	TPD52	1.56	1.38E-02
LBR	1.50	1.31E-03	PPP1R11	-1.46	3.47E-03	SOX4	-1.63	3.19E-02
CD93	1.43	8.11E-09	MICAL2	-1.52	4.29E-02	RHPN2	-2.20	2.83E-03
SGK3	1.53	5.53E-12	COPA	-1.33	4.03E-02	BTBD3	1.29	3.77E-02
HORMAD2	2.32	1.20E-03	PACS2	1.28	1.40E-03	PLA1A	-4.08	3.65E-03
ALG10B	1.90	4.30E-03	CYBRD1	1.78	1.31E-02	FLI1	-1.86	3.39E-02
TCF7	1.45	2.54E-05	BRWD1	1.54	7.17E-05	SYNJ1	1.58	4.35E-02
MID1IP1	1.37	3.61E-15	KAT2B	1.68	7.78E-03	MIDN	-1.89	2.07E-02
KIF11	-3.94	2.75E-02	SEP-07	-2.00	2.73E-03	ZNF768	-1.61	2.73E-02
CD274	1.88	4.86E-04	BRAF	-1.33	3.25E-02	METRNL	-2.40	3.26E-02
HNF4A	1.38	2.52E-02	CSE1L	-2.28	4.27E-03	AEN	-2.01	4.39E-03
YPEL2	1.43	8.66E-03	CROT	-1.30	1.05E-02	STK40	-1.80	1.09E-02
PLK2	-2.95	5.39E-21	INSIG2	-1.29	4.06E-02	ITPRIPL2	-1.99	2.38E-02
IL6ST	1.38	1.42E-02	HLF	-1.72	1.38E-02	PCDHGB6	-1.89	3.11E-02
C6	2.40	1.54E-03	SMURF2	1.31	1.97E-04			

ZBTB20	1.48	2.66E-20	UBE2B	-1.62	1.93E-02			
CPT1A	1.46	8.40E-04	XBP1	2.01	3.44E-04			
LRP5	1.40	5.50E-14	PLK2	-2.49	1.42E-02			
ABLIM1	1.31	1.02E-02	SERINC5	1.29	4.75E-03			
METTL7B	1.57	4.35E-13	ARF4	-1.58	3.46E-05			
CREB1	1.32	4.52E-11	SAMM50	-1.65	1.01E-03			
CDC73	-1.38	6.84E-03	ZBTB20	2.92	2.29E-04			
FAM107B	1.38	2.65E-12	USP25	-1.40	4.23E-02			
HSD17B7	1.34	4.82E-10	SLC3A1	-1.46	3.17E-02			
ATRN	-1.36	9.68E-17	ABLIM1	1.56	9.72E-03			
RBL1	1.41	9.21E-05	ATP5B	-2.15	3.13E-02			
CLTA	-2.97	6.19E-17	TM9SF2	-1.84	8.74E-03			
SLC6A13	1.96	1.66E-13	MRPL30	-1.50	1.96E-03			
PSMA1	-1.39	1.08E-02	GLUL	2.14	1.77E-02			
TIRAP	1.53	1.17E-03	FAM107B	1.46	3.43E-02			
DDX6	2.49	6.30E-05	CAMSAP1	-1.41	2.77E-02			
PANK1	1.52	3.67E-03	CERS6	-2.30	8.53E-05			
ATP6V1H	-1.56	1.79E-23	SP3	2.05	4.70E-04			
HSD17B13	1.63	1.08E-07	NDRG3	-1.57	6.69E-03			
ROR1	1.30	6.14E-14	RALGAPB	-1.29	1.84E-02			
AGO2	1.70	1.91E-14	DHX36	-1.50	1.63E-02			
RCOR1	2.01	4.13E-02	PNISR	1.74	3.90E-02			
NECAB1	-2.80	2.37E-23	CLTA	-1.44	3.60E-02			
MAFF	2.20	8.69E-04	AKR1A1	-1.54	7.02E-03			
SERPINB9	1.91	1.28E-04	PSMA1	-2.40	6.68E-04			
TNRC6A	1.33	8.46E-08	NEK1	1.97	1.47E-03			
DDX17	1.79	7.05E-08	ATP6V1H	-2.39	2.59E-02			
CFHR1	1.31	7.76E-03	DHCR24	-2.39	3.87E-04			
RBM47	1.46	9.45E-07	SRRM2	2.02	4.06E-02			
CCR5	1.28	2.98E-07	NECAB1	-1.67	2.51E-02			
SLC5A3	2.54	1.15E-09	ZNRF3	2.15	3.77E-04			
NDC1	1.29	7.13E-08	MAFF	1.47	1.72E-03			
CD84	1.26	1.52E-03	MAML1	1.52	5.06E-03			
RORA	1.40	9.37E-05	HNRNPC	-1.39	6.10E-03			
STXBP2	1.36	4.81E-09	DLGAP4	1.50	7.99E-03			
ITCH	1.39	6.78E-09	RAB18	-1.59	1.90E-03			
ZNF264	1.36	1.97E-12	SLC5A3	1.56	1.79E-02			
DDHD1	-1.86	4.45E-03	EID1	-1.91	1.41E-02			
HM13	1.58	9.86E-05	REV3L	1.47	2.22E-02			
AKT2	3.32	1.28E-03	GOPC	-1.86	2.22E-03			
SLC1A5	1.50	9.02E-14	KMT2C	1.29	2.54E-03			
FBXO3	-1.67	2.07E-10	RORA	1.53	3.23E-02			
DNPH1	2.00	1.25E-04	TPD52	-2.47	1.36E-03			
SRSF11	1.48	1.04E-08	GSK3B	-1.32	2.44E-02			
MKLN1	1.30	8.40E-19	PAPOLA	1.27	3.14E-02			
GTPBP3	1.43	1.45E-04	SUPT16H	-1.28	1.18E-03			

BTBD3	1.32	6.22E-03	CECR2	2.34	5.76E-04			
CCNB1	-1.67	9.49E-14	CHMP4B	-1.39	3.30E-02			
GLTP	2.12	1.29E-18	MID1	1.65	1.16E-02			
PLA1A	1.63	9.92E-04	AKT2	1.35	1.02E-02			
TNFAIP8	1.81	4.37E-10	CARD8	1.64	4.37E-04			
NACC2	1.44	3.42E-02	GRPEL1	-1.66	7.79E-03			
HNRNPU	1.31	1.47E-04	FBXO3	-1.44	1.51E-02			
SLC26A2	2.69	3.00E-03	SLC38A1	-1.39	3.76E-03			
ELK4	1.44	3.37E-02	AAK1	-2.10	9.46E-03			
SYNJ1	2.16	7.42E-04	PDS5A	-1.34	1.14E-02			
SQSTM1	1.68	3.98E-16	MKLN1	1.36	1.48E-02			
HIPK1	1.80	5.20E-06	ARPP19	-2.82	5.90E-05			
SLC4A1AP	-1.60	9.21E-15	PHF20L1	-1.28	2.74E-03			
TP53INP1	1.35	3.14E-18	CLIP1	1.56	1.94E-02			
ZNF367	1.73	3.19E-03	RHPN2	2.45	5.04E-05			
MIDN	1.55	5.59E-15	ANXA7	-1.68	3.39E-03			
ZDHHC16	1.38	5.82E-03	GLTP	1.29	2.57E-02			
KLHL15	1.46	2.18E-04	RFX5	-1.51	2.62E-02			
ZBTB33	1.64	1.38E-03	ABRACL	-2.02	2.17E-03			
ZNF620	1.56	2.98E-06	NACC2	2.64	3.87E-03			
EFNA5	2.00	2.52E-02	EIF4EBP2	1.44	2.45E-04			
BAZ1A	1.69	2.63E-06	ALDOA	-1.26	3.53E-02			
LTB4R2	1.36	9.44E-04	NEK7	1.48	1.78E-02			
TXNIP	1.78	1.79E-15	FLI1	1.33	1.17E-03			
RASSF5	1.72	2.93E-05	UHMK1	-2.06	3.05E-03			
			CARHSP1	2.17	2.57E-03			
			AZIN1	-1.54	4.60E-02			
			RER1	-1.95	4.14E-06			
			SYNJ1	-1.60	4.91E-02			
			SQSTM1	2.22	8.84E-04			
			SLC4A1AP	-1.47	8.42E-03			
			TP53INP1	2.36	9.87E-04			
			KBTBD6	-1.30	3.83E-02			
			USP54	1.53	4.09E-03			
			TMEM41B	-1.45	3.51E-03			
			ZNF597	-1.44	3.56E-03			
			CLIC4	1.34	2.14E-02			
			JUN	1.58	4.47E-02			
			PTAR1	1.53	1.07E-02			
			PELI1	1.30	4.59E-02			
			YTHDF2	-1.45	2.15E-02			
			BAZ1A	1.73	4.95E-04			
			ITPRIPL2	2.21	3.60E-03			
			SFT2D2	2.07	1.26E-03			

Supplemental Table S3 Details of Antibodies used for western blot (WB), Imunohistochemistry (IHC) and Immunocytochemistry (ICC)

Antibody Name	Raised In	Dilution	Source
GFAP	Mouse	IHC/ICC-1:50	Sigma Aldrich
$A\beta_{1-42}$	Rabbit	IHC-1:100, ICC/IHC-1:100	Abcam
Ago2 (eIF2C2)	Mouse	WB-1:1000	Abnova
HA	Rat	WB-1:1000	Roche
Dcp1	Mouse	WB-1:1000, ICC/IHC-1:100	Novus
Rck/p54	Rabbit	WB-1:10000, ICC/IHC-1:1000	Bethyl
Alix	Mouse	WB-1:200	Santa cruz
Calnexin	Rabbit	WB-1:10000	Bethyl
HRS	Rabbit	WB-1:1000	Bethyl
β-Actin	Mouse Monoclonal (HRP Conjugated)	WB-1:10000	Sigma Aldrich
Rab7	Rabbit	WB-1:1000, ICC-1:100	Cell Signalling
DICER	Rabbit	WB-1:5000	Bethyl
Rab5	Rabbit	WB-1:1000	Cell Signalling
RILP	Goat	WB-1:250	Santa cruz
p-mTOR	Rabbit	WB-1:1000	Cell Signalling
p70-S6K	Rabbit	WB-1:1000	Cell Signalling
mTOR	Rabbit	WB-1:1000, ICC-1:100	Cell Signalling
4G-10	Mouse	WB-1:1000	Millipore
Myc	Mouse	WB-1:1000	Cell Signalling

Supplemental Table S4 mRNA Primers used for quantification

Target	5'Forward Primer3'	5'Reverse Primer3'
RL	CCAAGCAAGATCATGC	GCTCTTGATGTACTTACCC
Pre-miR-122	CCTTAGCAGAGCTGTGGAG	GCCTAGCAGTAGCTATTTAG
18SrRNA	TGACTCTAGATAACCTCGGG	GACTCATTCCAATTACAGGG
IL-1β	GTGGATCCCAAACAATACCC	AACTATGTCCCGACCATTGC
IL-6	TACCCCAACTTCCAATGCTC	ACCACAGTGAGGAATGTCCA
GAPDH	CAGGGGGGAGCCAAAAGGG	CTTGGCCAGGGGTGCTAAGC

Supplemental TableS5 Details of miRNA primers used for Taqman based quantification

miRNA Name	ASSAY ID
Let-7a	000377
miR-9	001089
miR-16	000391
miR-21	000397
miR-24	000402
miR-29a	000412
miR-33-5p	002135
miR-101	000438
miR-122	000445
miR-125b	000449
miR-128a	002216
miR-142-3p	000464
miR-145	002278
miR-146a	000468
miR-155	002571
miR-181c	000482
miR-184	000485
U6 SnRNA	001973

Supplementary Figure S1

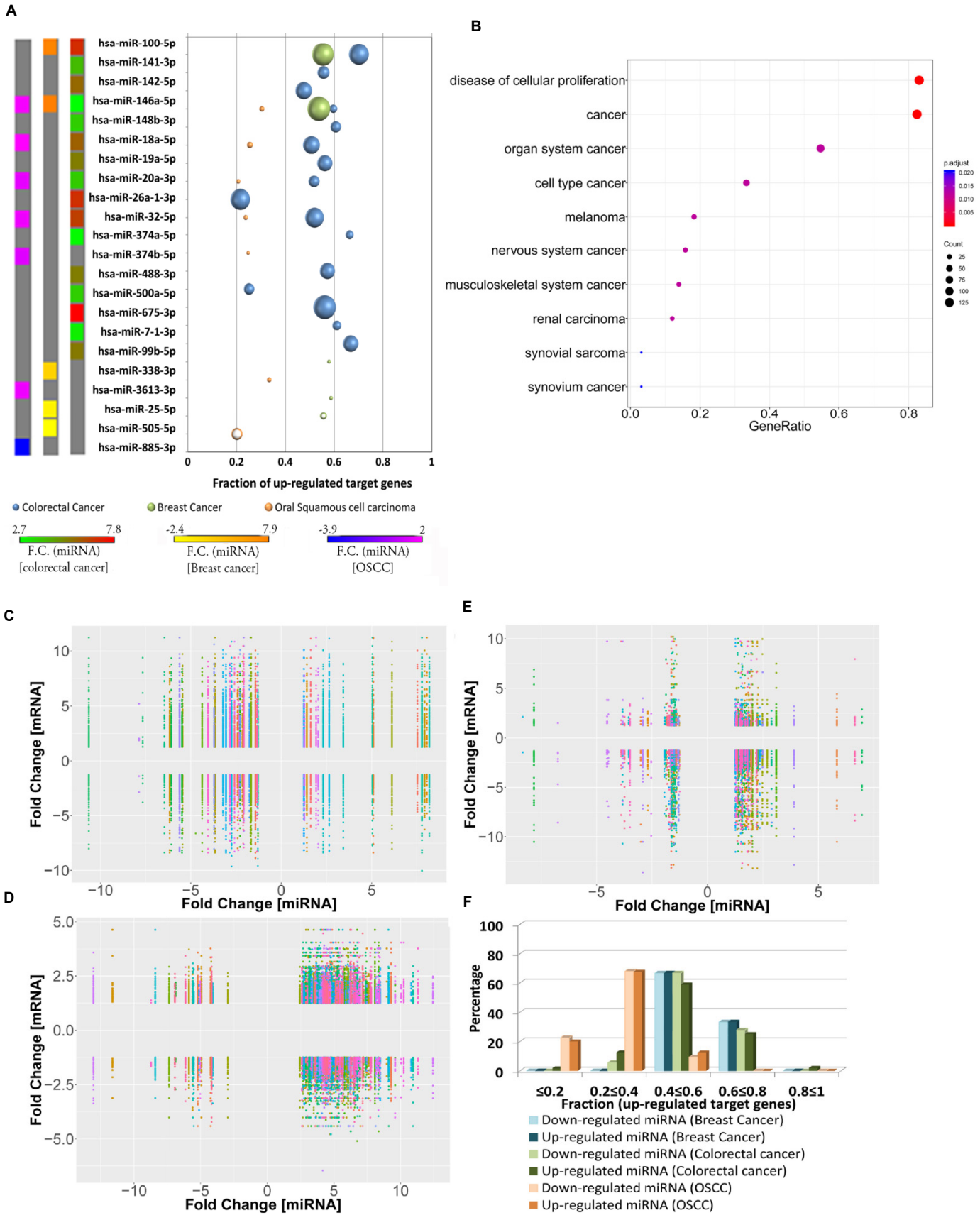

Figure S1 Probable regulator(s) to target(s) [miRNA:mRNA] expression relationships in Cancer.

A The possible relationship between miRNA and their target mRNA in different cancer tissues was studied considering commonly differentially expressed miRNA in AD and cancer. The fraction of up-regulated target mRNA of these differentially expressed miRNA in colorectal cancer, breast cancer and oral squamous cell carcinoma (OSCC) is shown here.

B Disease associations of genes up-regulated among multiple brain cortical regions in Alzheimer's patients. P-value and gene ratio of diseases (top 10) obtained based on enrichment analysis of the common up-regulated genes among different cortical regions in Alzheimer's disease (AD) patients is shown here.

C-E Probable miRNA:mRNA expression relationships in cancer patient samples**.** Fold changes in differentially expressed miRNA and their corresponding differentially expressed target mRNA in oral squamous cell carcinoma (C), colorectal (D) and breast cancer (E) are depicted here.

F Comparison among percentage of differentially expressed miRNA that have different subsets or fractions of their target mRNA as up-regulated in highly proliferative tissues (different cancer datasets).

Supplementary Figure S2

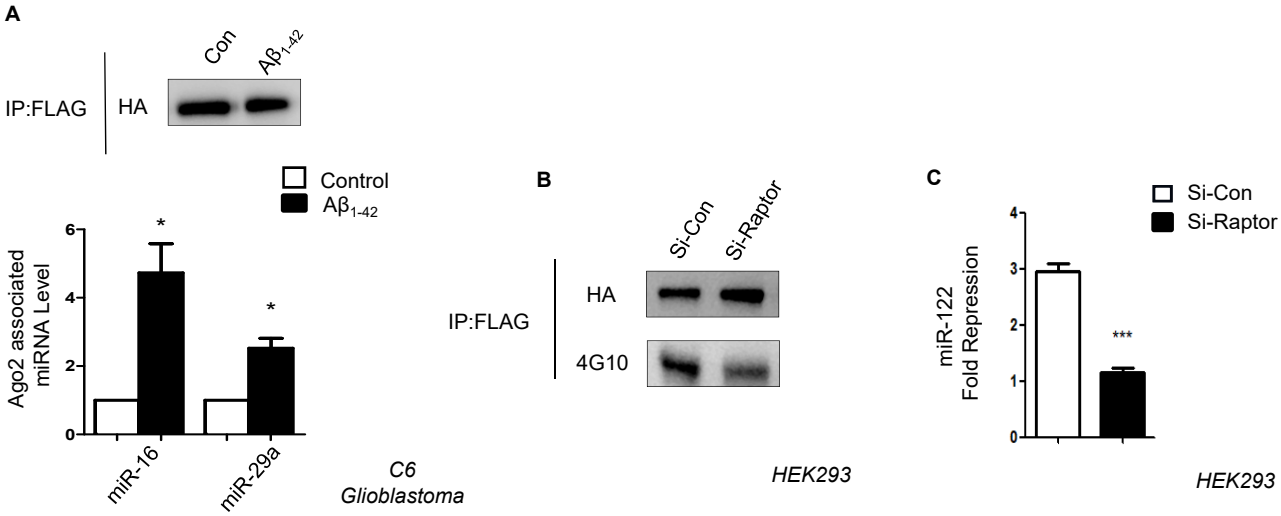

Figure S2 RAPTOR knockdown decreases miRNA activity and Ago2 phosphorylation

A Graphs showing level of Ago2 associated miR-16 and miR-29a level upon $A\beta_{1-42}$ treatment in C6 glioblastoma cells. qPCR data was normalized with the amount of Ago2 pulled down from IP reaction.

B Western blot data showing amount of Ago2 phosphorylation and the amount of Ago2 pulled down from Si-con and Si-Raptor transfected HEK293 cells stably expressing F-HA-Ago2.

C Effect of Raptor knockdown on miR-122 activity in HEK293 cells. Dual Luciferase assay showing fold repression of exogenously expressed miR-122 in both control and Raptor knockdown cell.

For statistical significance, minimum three independent experiments were considered in each case unless otherwise mentioned and error bars are represented as mean ± S.E.M. P-values were calculated by utilizing Student's t-test. ns: non-significant, *P < 0.05, ***P < 0.0001.

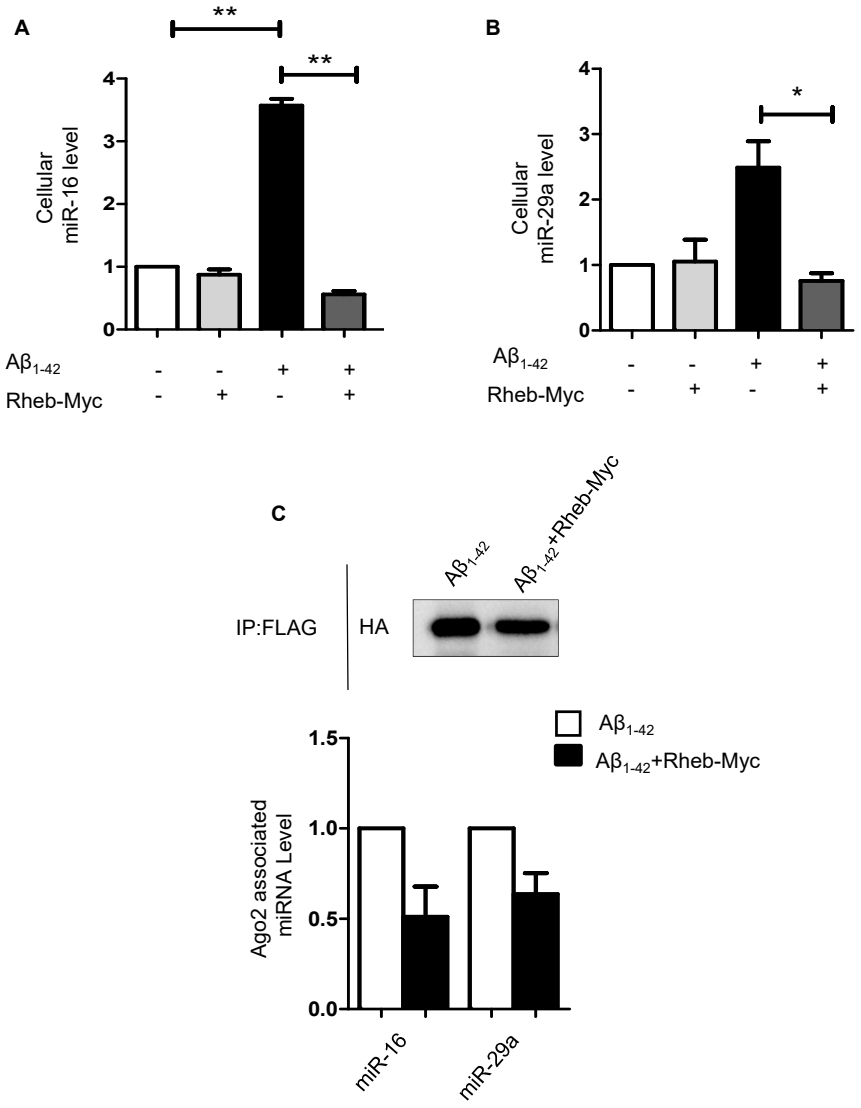

Figure S3 Rheb-myc expression decreases miRNP formation in $A\beta_{1-42}$ treated cells

A-B Graphs showing levels of miR-16 and miR-29a miRNA in both control vector and Rheb-myc expressing cells exposed with $A\beta_{1-42}$. qPCR data was normalized with U6 snRNA.

C Graphs depicting Ago2 associated miR-16 and miR-29a level in both control vector and Rheb-myc expressing cells exposed with $A\beta_{1-42.}$ qPCR data was normalize with the amount of Ago2 pulled down from IP reaction.

For statistical significance, minimum three independent experiments were considered in each case unless otherwise mentioned and error bars are represented as mean ± S.E.M. P-values were calculated by utilizing Student's t-test. ns: non-significant, *P < 0.05, **P < 0.01.

Supplementary Figure S4

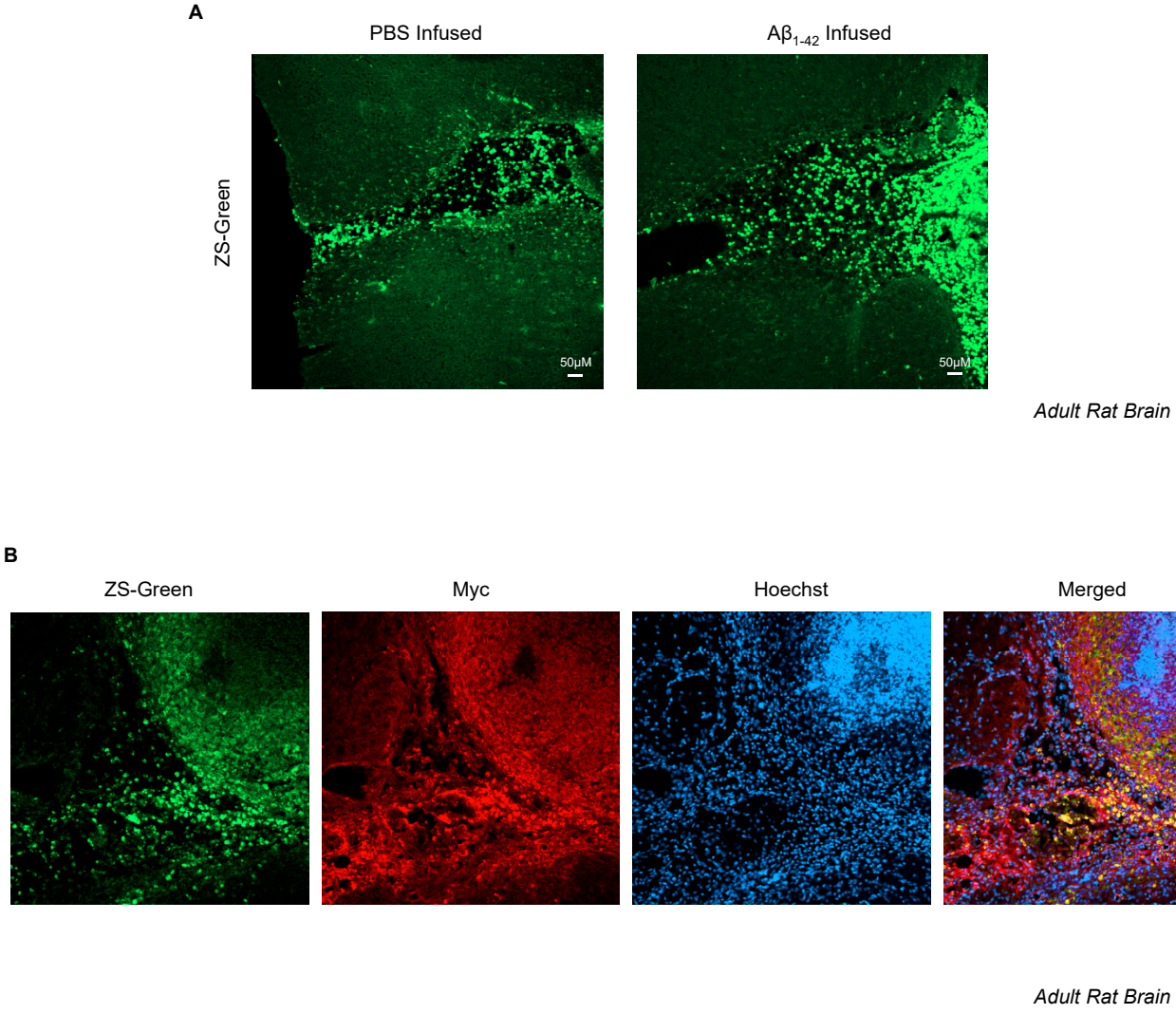

Figure S4 Confirmation of Rheb-Myc expression in adult rat brain. The protein expression plasmid was co-infused along with $A\beta_{1-42}$ oligomer in adult rat brain.

A Immunohistochemistry panels are showing brain sections that were infused either with PBS or $A\beta_{1-42}$ oligo along with ZS-Green expression vector.

B Confocal images showing immunohistochemistry sections with ZS-Greenand Rheb-Myc expression vector. The green color positive cells that are depicting ZS-Green expression were also stained for Rheb-Myc and dual coloured cells were visualized as yellow.