Supplementary information

miR126-mediated vascular integrity dysfunction in Rett syndrome

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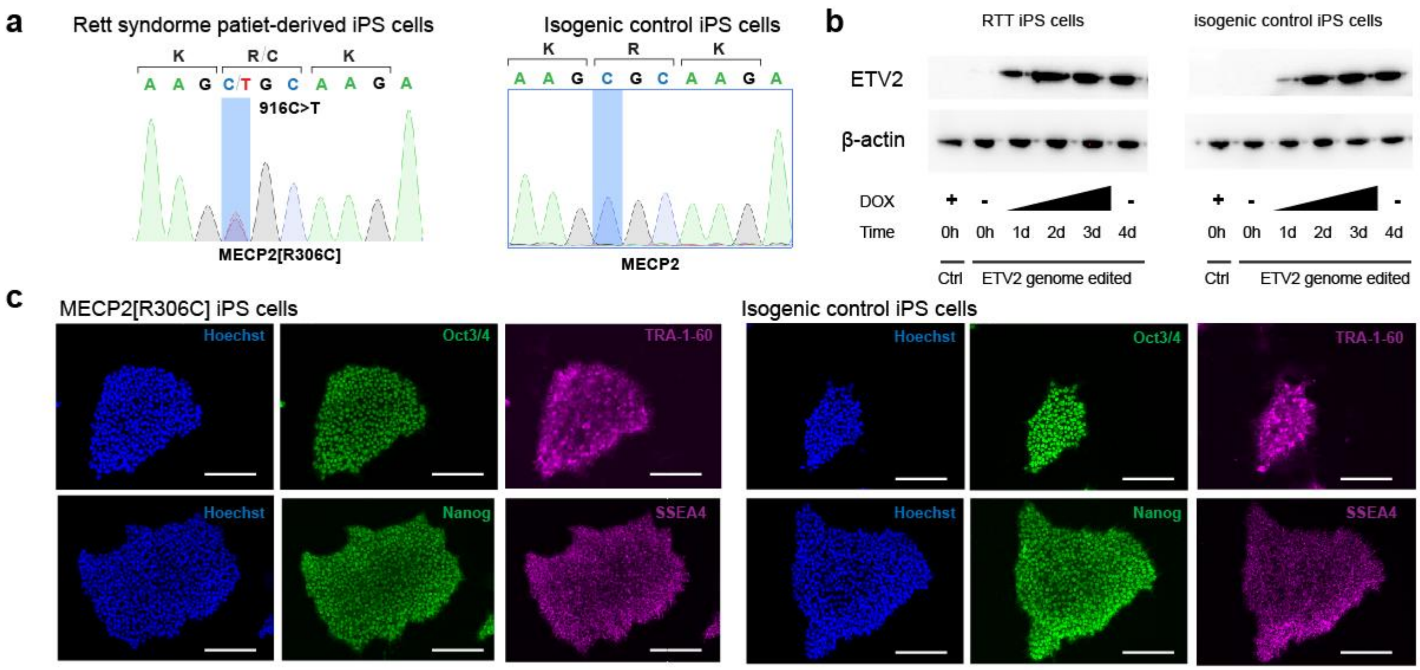
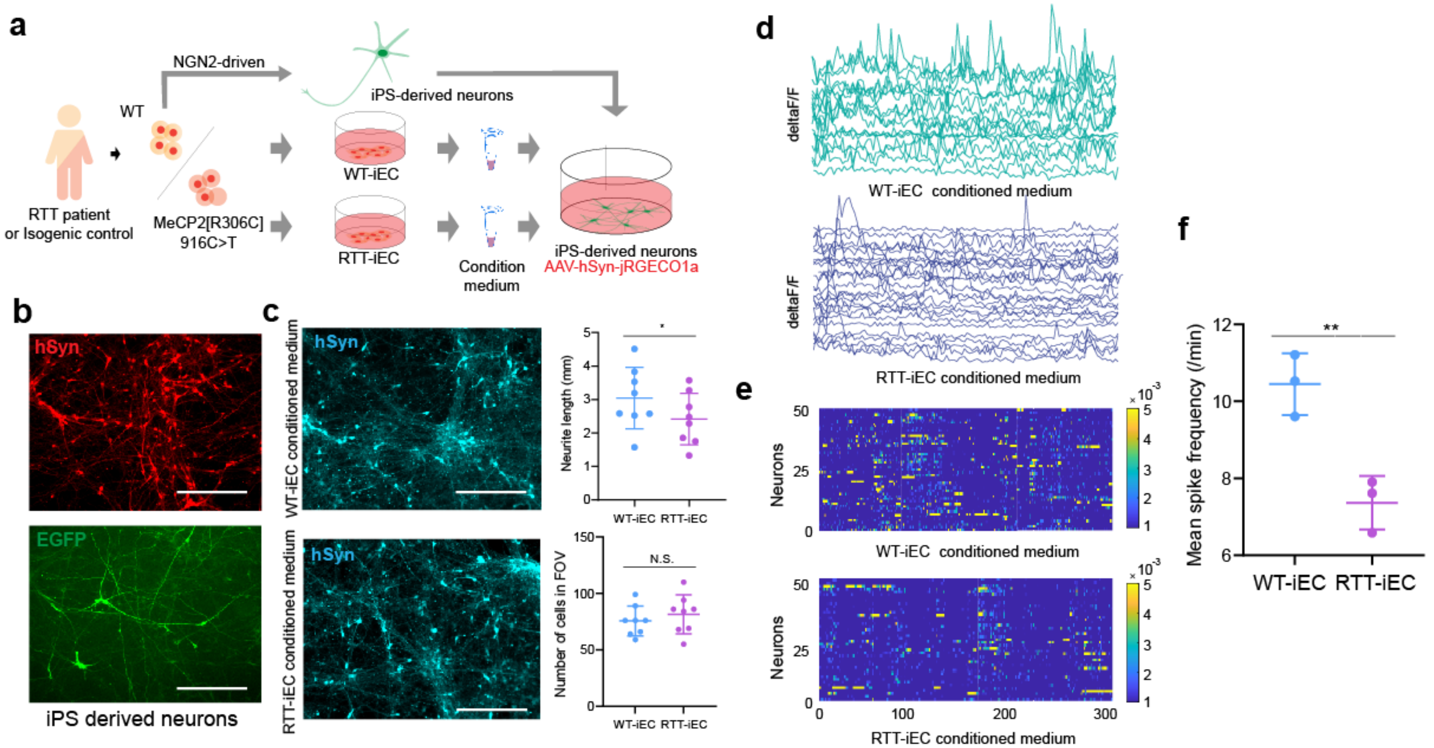
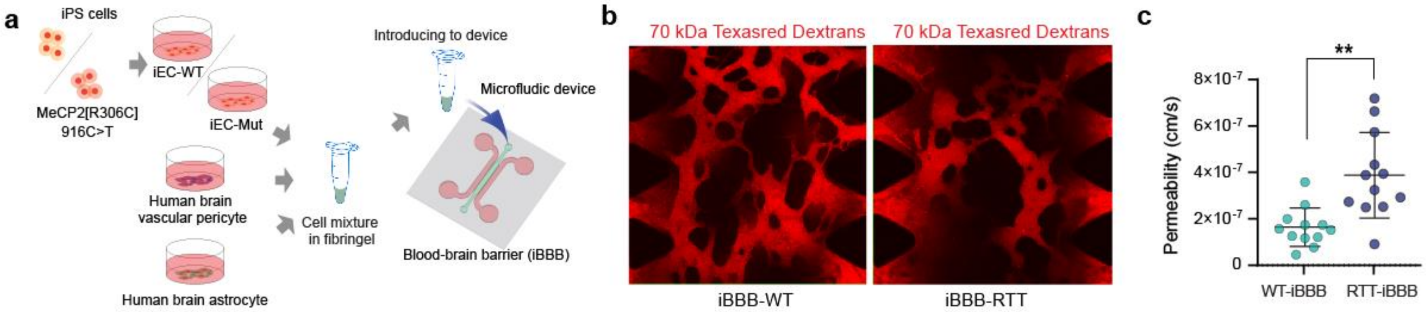


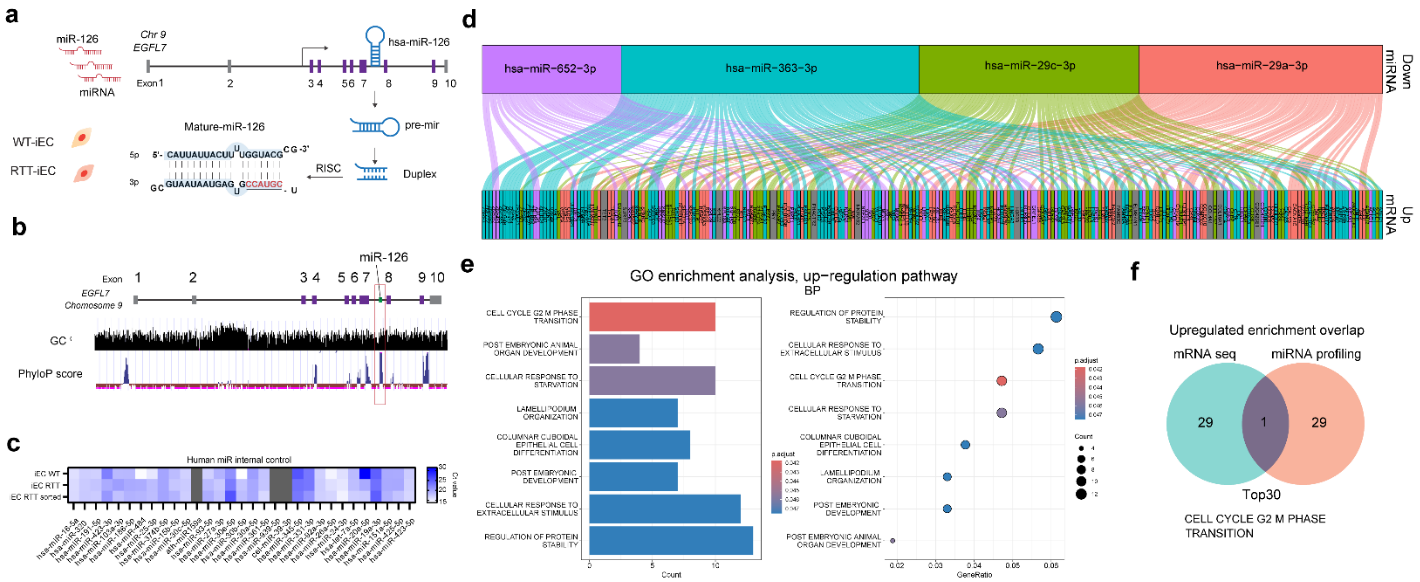
Supplemental figure. 1 Characterization of Rett syndrome patient-derived iPS cells. (**a**) Genotype to confirm MECP2 916c>T mutation in MeCP2[R306C] and isogenic control (**b**) ETV2 expression in the presence of DOX. (**c**) Immunostaining of OCT3/4, TRA-1-60 to show the pluripotency in iPS cells.



Supplemental Fig. 2 RTT-iEC condition medium negatively impacted neuronal activity. (**a**) Schematic illustration of treatment of WT-iNeurons with condition media from WT-iEC or RTT-iEC. (**b**) Immunostaining of hSyn to confirm differentiation to neurons from WT-iPS cells which express dox dependent NGN2. n =6. (**c**) Condition medium treatment did not affect morphological change with respect to neurite length and number of cells. (**d**) Calcium imaging was performed with AAV-hSyn-jRGECO1a. (**e, f**) Condition medium from RTT-iEC down reulgated neuronal activity. n =3. Student's t-test, **, p<0.01, * p<0.05.



Supplemental Fig. 3 Engieering BBB in vitro with iEC, pericyte and astrocyte. (**a**) Schematic illustration of formation of BBB by co-cultureing, endothelial cells, pericytes, and astrocytes in fibrin gel. (**b**) BBB formation from Rett syndrome patient-derived iEC and isogenic control iEC. (**c**) RTT-iBBB displayed higher permeability compared to control BBB. n =12. Student's t-test, **, p<0.01, * p<0.05.



Supplemental Fig. 4 miR profiling and downregulated miR in RTT-iEC. (**a, b**) miR126-3p is endothelial specific microRNA, which is located in intron of EGFL7 and has higher PhyloP score. (**c**) microRNA internal control that used for normalization of target miR expression. (**d**) four down-regulated miR in RTT-iEC and target gene predicted by miRDB. (**e**) GO enrichment analysis by upregulated genes obtained from (**d**). (**f**) miRNA-mRNA integrated analysis revealed that overwrapped sinaling pathway (Cell cycle G2 M phase transition).

Supplemental table S1. Key resources

Reagent and resource	Source	Identifier
Cell line		
Rett syndrome patient derived iPS cells (female, 8Y, missence)	Coriell Institute	WIC05i-127-325(MT)
Isogenic control pair, wild type	Coriell Institute	WIC04i-127-33(WT)
AAVpro 293T	Takara	N.A.
Plasmid		
pUCM-AAVS1-TO-hNGN2	Addgene	# 105840
pUCM-AAVS1-TO-hETV2	This study	N.A.
PX458-AAVS1	Addgene	#113194
pRSV-Rev	Addgene	#12253
pMDLg/pRRE	Addgene	#12251
pMD2.G	Addgene	#12259
miRZip-126-3p anti-miR-126-3p microRNA construct	SBI	MZIP126-3p-PA-1
miRZip™ & pGreenPuro™ shRNA Scramble Hairpin Negative Control	SBI	MZIP000-PA-1

Supplemental table S2.

Gene	Forward primer 5'-3'	Reverse primer 5'-3'
CD31	AACAGTGTTGACATGAAGAGCC	TGTAAAACAGCACGTCATCCTT
ZO-1	CAACATACAGTGACGCTTCACA	CACTATTGACGTTTCCCCACTC
OCLDN	ACAAGCGGTTTTATCCAGAGTC	GTCATCCACAGGCGAAGTTAAT
CLDN5	CTCTGCTGGTTCGCCAACAT	CAGCTCGTACTTCTGCGACA
PGP	TGACCCGCACTTCAGCTAC	GGGCTTCCCGATGATGTCG
LRP1	AGCCAGCTATGCACCAACAC	CCTTGCAGGAGCGGTTATC
LAT1	CCGTGAACTGCTACAGCGT	CTTCCCGATCTGGACGAAGC
hTfR	GGCTACTTGGGCTATTGTAAAGG	CAGTTTCTCCGACAACTTTCTCT
GLUT1	TCTGGCATCAACGCTGTCTTC	CGATACCGGAGCCAATGGT
ABCA	ACCCACCCTATGAACAACATGA	GAGTCGGGTAACGGAAACAGG
CAT1	ATCATCGGTACTTCAAGCGTAGC	GGCGTTCAGAGTCATGTGTGT
MRP1	AAGGAGGTACTAGGTGGGCTT	CCAGTAGGACCCTTCGAGC
MARP4	AGCTGAGAATGACGCACAGAA	ATATGGGCTGGATTACTTTGGC
MCT1	AGGTCCAGTTGGATACACCCC	GCATAAGAGAAGCCGATGGAAAT
EGFL7	TGCAGACGGTACACTCTGTGTG	TGCAGCCTCTGCACTTCTTCCT
IGFBP2	CGAGGGCACTTGTGAGAAGCG	TGTTCATGGTGCTGTCCACGTG
VEGFA	AGTCTGTCGTCCAGTAGCACCA	ACTGGAGCCATACTCATCCGAG
NFKBIA	TCCACTCCATCCTGAAGGCTAC	CAAGGACACCAAAAGCTCCACG
HIF1a	TATGAGCCAGAAGAACTTTTAGGC	CACCTCTTTTGGCAAGCATCCTG
ADAM9	CTTGCTGCGAAGGAAGTACCTG	CACTCACTGGTTTTTCCTCGGC
PIK3R2	CAGTACAACGCCAAGCTGGACA	TGCTGGTGGTAGACCTTGAGCT
SPRED1	CAGCCAGGCTTGGACATTCA	TGGGACTTTAGGCTTCCACAT
TEK	TTAGCCAGCTTAGTTCTCTGTGG	AGCATCAGATACAAGAGGTAGGG
EDN1	AGAGTGTGTCTACTTCTGCCA	CTTCCAAGTCCATACGGAACAA
FOXO1	TGATAACTGGAGTACATTTCGCC	CGGTCATAATGGGTGAGAGTCT
ANGPT2	CTCGAATACGATGACTCGGTG	TCATTAGCCACTGAGTGTTGTTT
GAPDH	TGT GGG CAT CAA TGG ATT TGG	ACA CCA TGT ATT CCG GGT CAA T