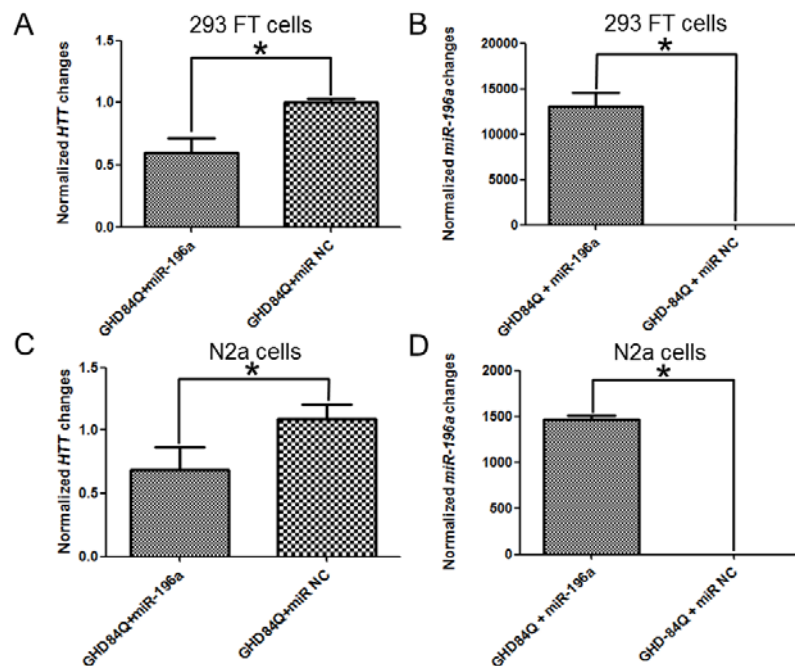


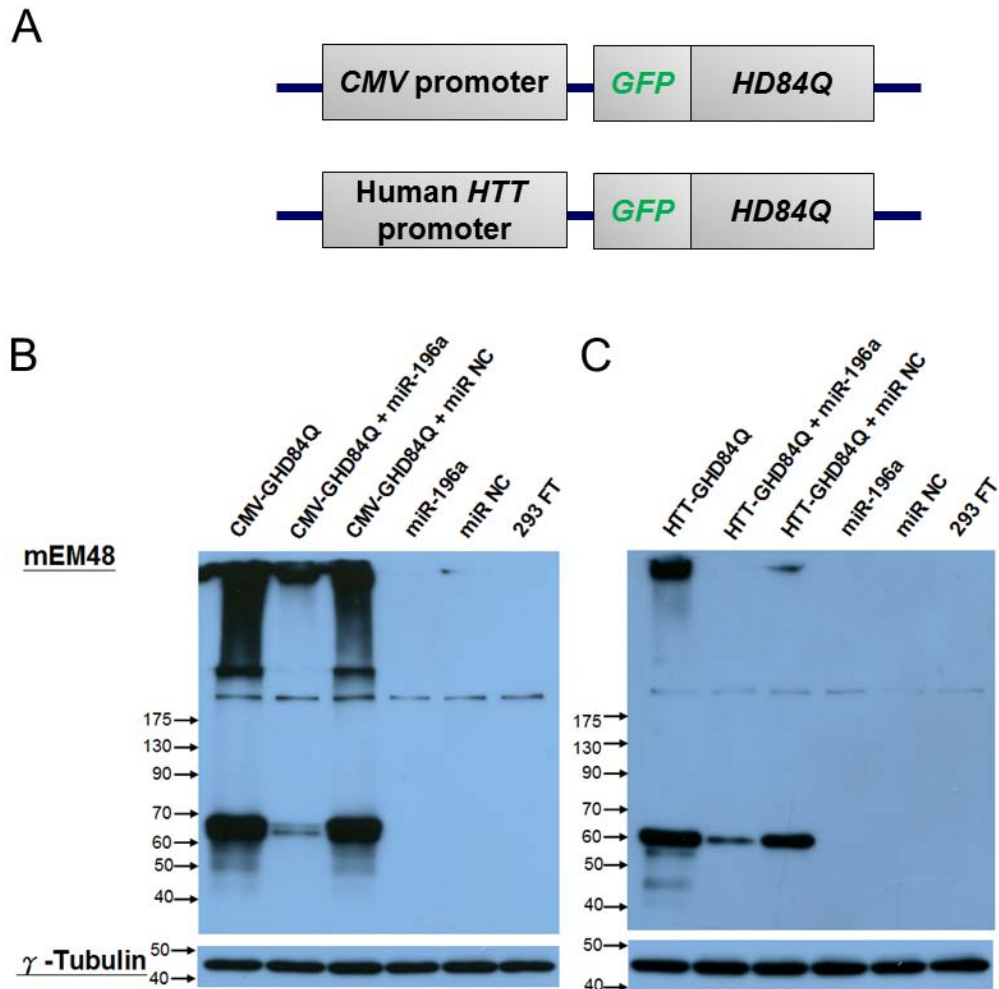
## Supplemental Data

### miR-196a Ameliorates Phenotypes of Huntington Disease in Cell, Transgenic Mouse, and Induced Pluripotent Stem Cell Models

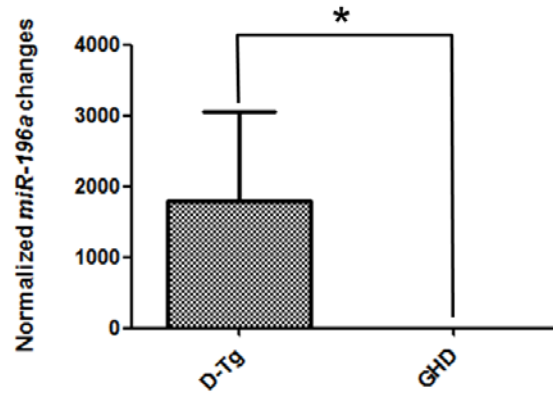
Pei-Hsun Cheng, Chia-Ling Li, Yu-Fan Chang, Shaw-Jeng Tsai, Yen-Yu Lai, Anthony W. S. Chan, Chuan-Mu Chen, and Shang-Hsun Yang



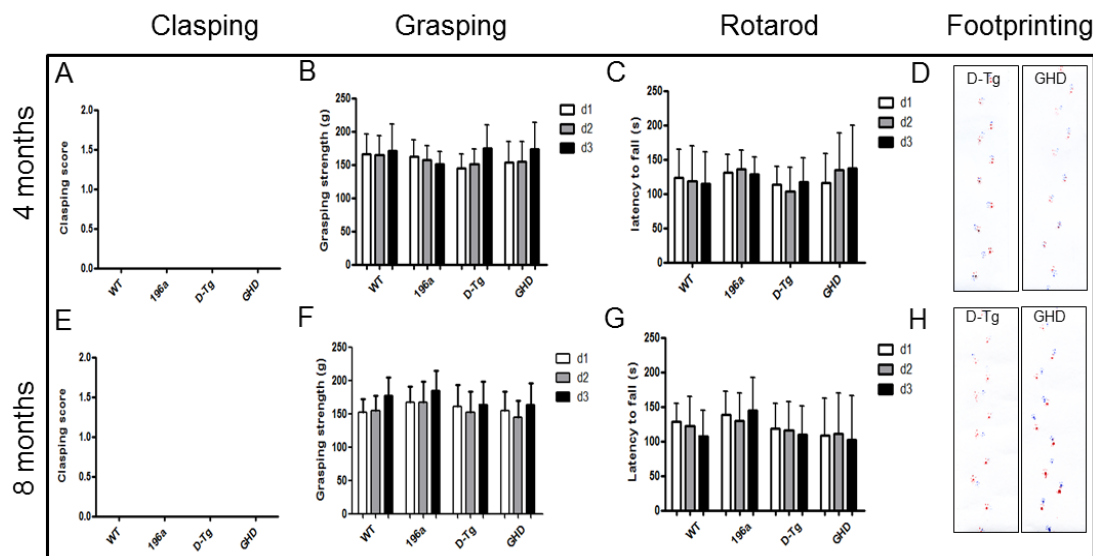
**Figure S1. RNA expression profiling of miR-196a and mutant *HTT* in 293 FT and N2a cells after the treatments of miR-196a mimics.** 293 FT (A and B) and N2a (C and D) cells were transfected with mutant *HTT* (GHD84Q), miR-196a mimics or miRNA unrelated control (miR NC), and then subjected for Q-RT-PCR (Applied Biosystems). (A and C) show the mRNA level of mutant *HTT* in 293 FT and N2a cells, respectively. (B and D) show the expression level of miR-196a in 293 FT and N2a cells, respectively. n=3, data represent mean  $\pm$  SD, and \* represents significant difference between two groups.



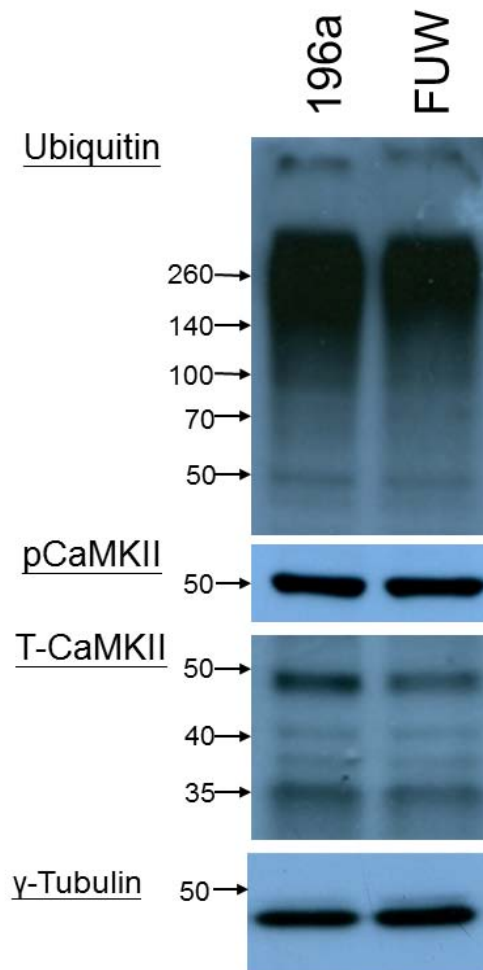
**Figure S2. miR-196a suppressed the expression of mutant *HTT* driven by different promoters *in vitro*.** (A) Top panel shows the mutant *HTT* construct containing the cytomegalovirus (CMV) promoter and mutant *HTT* with 84 CAG repeats (HD84Q) and GFP fusion protein. Bottom panel shows the mutant *HTT* construct containing the human endogenous *HTT* promoter, HD84Q and GFP fusion protein. (B) miR-196a mimics, unrelated controls (miR-NC) and mutant *HTT* driven by the CMV promoter (CMV-GHD84Q) were transfected into 293 FT cells, and then analyzed via Western blotting using mEM48 and  $\gamma$ -tubulin antibodies. Cells transfected with CMV-GHD84Q and miR-196a show lower expression level of mutant *HTT* compared to that of cells transfected with CMV-GHD84Q and miR-NC. (C) miR-196a mimics, unrelated controls (miR-NC) and mutant *HTT* driven by the human *HTT* promoter (HTT-GHD84Q) were used to perform the same experiment as described in (B). Cells transfected with HTT-GHD84Q and miR-196a also show lower expression level of mutant *HTT* compared to that of cells transfected with HTT-GHD84Q and miR-NC.



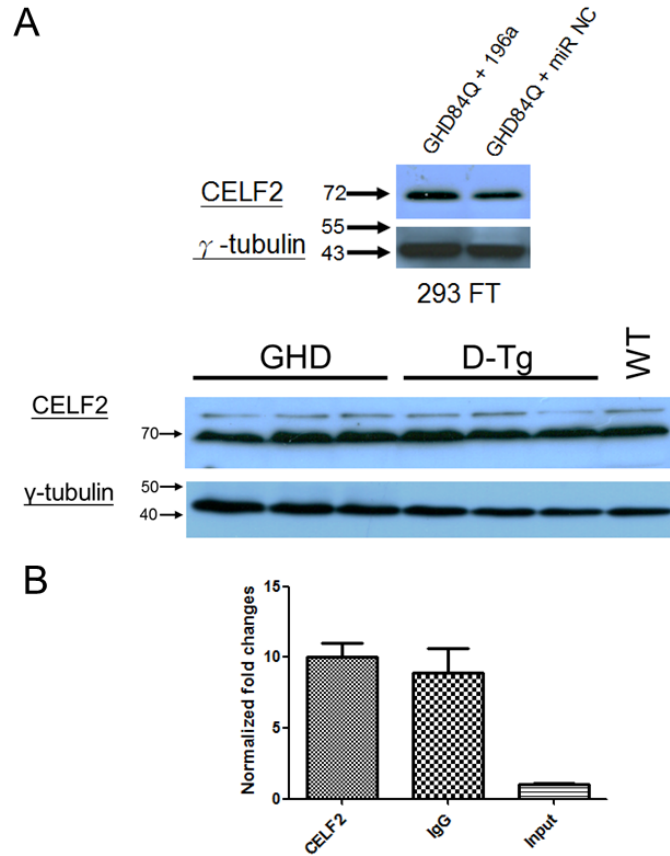
**Figure S3. Expression of miR-196a in the brain of D-Tg and GHD transgenic mice.** Brain tissues of different transgenic mice at one month of age (n=5-6 for each group) were subjected for Q-RT-PCR to detect the expression of miR-196a. Data represent mean  $\pm$  SD, \* represents significant difference between two groups.



**Figure S4. Behavioral phenotypes in four groups of mice at four and eight months of ages.** Claspings (A, E), Grasping (B, F), Rotarod (C, G) and Footprinting (D, H) tests were performed at 4 (A-D) and 8 (E-H) months of age in four groups (n=6-9 mice for each group). Data represent mean  $\pm$  SD.



**Figure S5. Expression of Ubiquitin and CaMKII in HD-iPSCs.** Western blotting using the same membrane in Figure 4E immunoblotted with ubiquitin, pCaMKII and total CaMKII antibodies, showing stronger ubiquitin and total CaMKII in miR-196a infected HD-iPSCs, whereas similar signal of pCaMKII is observed between these two groups.  $\gamma$ -tubulin is used as an internal control.



**Figure S6. CELF2 does not involve in the mechanism of miR-196a on HD.** (A) Top panel shows the Western blotting result revealing similar expression level of CELF2 between HD 293 FT cells transfected with miR-196a mimics and unrelated (NC) controls. Bottom panel is the Western blotting result revealing similar level of CELF2 in the brain samples of GHD and D-Tg transgenic mice.  $\gamma$ -tubulin was used as the internal control. (B) Real-time RT-PCR performed after RNA immunoprecipitation using a CELF2 antibody in 293 FT cells showed similar binding ability of a CELF2 antibody compared to that of IgG control. Data represent mean  $\pm$  SD (n=3).

**Table S1. Candidate genes involved in CaMKII-CREB, inflammatory response in brain and ubiquitin-dependent protein catabolic process pathways. miR-196a TargetScan genes and miR-196a microarray genes were imported into the MetaCore online software, and candidate genes related to three pathways are listed.**

CaMK II-CREB pathway				Pathway of inflammatory response in brain				Pathway of ubiquitin-dependent protein catabolic process				
miR-196a targetscan genes		miR-196a microarray		miR-196a targetscan genes		miR-196a microarray		miR-196a targetscan genes		miR-196a microarray		
Input IDs	Input IDs	Fold change (D-1g /GHD)	p-value	Input IDs	Input IDs	Fold change (D-1g /GHD)	p-value	Input IDs	Input IDs	Fold change (D-1g /GHD)	p-value	
CALM3;CALM1	0610040J01Rik	-1.29	0.0131	EPHA3		Ahey	-1.204	0.012	NEDD4L	Neadd4;Rrp1	-1.30	0.0369
COL24A1	1700026L06Rik	-1.19	0.0067			Almp1	-1.180	0.006	RNF5	Rnf5	-1.18	0.0254
HABP4	5730577I03Rik	-1.15	0.0221			Akt1	-1.240	0.047	USP15	Usp15	-1.23	0.0256
	Acdy3	-1.21	0.0031			Bgaln1	-1.169	0.003	CDC34	A1314180	-1.33	0.0115
	Aldh6a1	-1.23	0.0055			Bmp2	-1.198	0.023	ERLIN2	Amir	-1.35	0.0415
	Atp2a2	-1.21	0.0443			Blk	-1.069	0.031	MAP3K1	Anapc2	-1.15	0.0414
	Bag3	-1.25	0.0469			Ccl5	-3.236	0.021	RAD23B	Birc3	-1.21	0.0194
	Casp2	-1.23	0.0078			Cd24a	-1.295	0.001	SMURF1	Birc	-1.36	0.0332
	Ctnc3	-1.31	0.0336			Ce1pa	-1.221	0.042	UBE2G2	Cdzap	-1.47	0.0051
	Ctla	-1.43	0.0444			Cela1	-1.512	0.040	USP31	Cul1	-1.27	0.0032
	Cnn1	-2.43	0.0420			Cnr2	-1.388	0.004	YOD1	Cul3	-1.41	0.0167
	Cnr2	-1.39	0.0040			Crcp	-1.166	0.029		Derl2	-1.21	0.0112
	D230025D16Rik	-1.26	0.0067			Csf1	-1.165	0.036		DnaIb9	-1.31	0.0014
	Dpysl2	-1.25	0.0600			Csf1r	-1.231	0.007		Edem3	-1.31	0.0263
	Gria1	-1.14	0.0340			Cyba	-1.185	0.002		Fbxw11	-1.28	0.0011
	Hctc1r1	-1.19	0.0338			F12	-1.315	0.002		Herpud1	-1.22	0.0256
	Map3k7	-1.26	0.0069			F8	-1.126	0.008		Huwe1	-1.23	0.0404
	Mbd31	-1.26	0.0355			Fcer1g	-1.194	0.045		Itch	-1.19	0.0403
	Ncor2	-1.29	0.0105			Hif1a	-1.382	0.031		Katd10	-1.43	0.0140
	Nf5dcl1	-1.25	0.0205			Lias	-1.326	0.011		Mdm2	-1.32	0.0162
	Rnf146	1.24	0.0325			Lipa	-1.317	0.018		Pebp2	-1.46	0.0367
	Sic39a1	-1.34	0.0287			Map2k3	-1.405	0.046		C6Psmat7	-1.34	0.0120
	Smap4	-1.32	0.0230			Mecom	-1.166	0.034		Psmc2	-1.27	0.0369
	Sympo	-1.35	0.0203			Myd88	-1.278	0.013		Psmc5	-1.16	0.0373
	Sympo	-1.35	0.0203			Naf1	-1.300	0.015		Rbbp6	-1.23	0.0074
	Tpad52	-1.39	0.0393			Ncf1	-1.286	0.033		Rnf20	-1.18	0.0187
	Ubxn7	-1.23	0.0057			Notch1	-1.398	0.040		Sirt2	-1.14	0.0005
	Usp13	-1.27	0.0117			Park7	-1.280	0.034		Tnfrap1	-1.32	0.0203
	Xrcc1	-1.19	0.0132			Pgd	-1.275	0.003		Trim13	-1.27	0.0227
	Yipf2	-1.22	0.0481			Plser1	-1.170	0.032		Trpc4ap	-1.25	0.0359
						Ptrf	-1.105	0.024		Ube2a3	-1.21	0.0419
						Pxx	-1.148	0.036		Ube2w	-1.26	0.0075
						Reg3a	-1.123	0.025		Ube3a	-1.30	0.0284
						Scyl1	-1.354	0.024		Usp14	-1.39	0.0019
						Serpina3n	-1.428	0.040		Usp18	-2.05	0.0469
						Serpinp9	-1.237	0.049		Usp22	-1.32	0.0096
						Serpint2	-1.113	0.036		Usp39	-1.26	0.0085
						Smad1	-1.324	0.047		Usp4	-1.17	0.0362
						Tfrc	-1.214	0.044				
						Thbs1	-1.638	0.037				
						Tir7	-1.255	0.035				
						Tnf	-1.459	0.020				
						Tnfrsf1a	-1.259	0.011				