

SUPPLEMENTARY TABLES:

Supplementary Table 1: Compositions of media used in the study

	CDM (Cell Differentiation Medium)	NIM (Neural Induction Medium)	NDM (Neural Differentiation Medium)
DMEM/F12 [11330-032; Gibco]	50%	97.5%	-
Neural basal medium [21103-049; Gibco-Invitrogen, Waltham, MA]	47%	1% N2	96%
N2 [17502-048, Life Technologies, Carlsbad, CA]	1%	-	1%
B27 [12587-010; Life Technologies]	1%	-	1%
l-glutamine [G7513, Sigma, St. Louis, MO]	1%	-	1%
MEM NEAA [11140-050; Fisher]	-	1%	-
2 µg/ml Heparin [H3149; Sigma]	-	1%	-
glucose [G7021; Sigma]	-	-	0.3%
cyclic adenosine 3',5'-monophosphate [cAMP; NC0526368; Fisher]	-	-	1 µM
ascorbic acid [A8960; Sigma]	-	-	200 µM
Penicillin-Streptomycin [1514012; Gibco]	-	-	1%
brain-derived neurotrophic factor [BDNF; 10 ng/mL, 450-02; PeproTech, Rocky Hill, NJ]	-	-	10 ng/mL
glial-derived neurotrophic factor [GDNF; 450-10; PeproTech]	-	-	10 ng/mL
SB431542 [1614; Tocris, Bristol, UK]	10µM	-	-
LDN-193189[NC0225637; Fisher]	100nM	-	-

Supplementary Table 2: RT-PCR Primers used in this study

Gene	Forward Primer	Reverse Primer
KP	GCCATTAGAAAAGGTGGCCTC	TTGTAGTTCGGCAGGTCTTC
sgRNA Offtarget analysis Primers		
GnRH sglA OT 1	GCATGGCTTTCCTTCCAGTTT	GAAGTGGGCGCCAGCTTAAA
GnRH sglA OT 2	GAAGGGGCCTTGACCCAGTAA	GAAGGGGCCTTGACCCAGTAA
GnRH sglA OT 3	GGGCCAACTGGGTATCGTC	TCCATCTCATCCAGCCCAGA
GnRH sglA OT 4	GACCAGGGATTCTGGTCATCTC	TCCAGACGTTACTTCCACCG
GnRH sglA OT 5	CACCACTCCTGCATGGCTTA	TGAAGCAAATCGGATGCTTTGA
GnRH sglB OT1	GTTTCATCATGGACACAGGTAGG	ACAGTCTTGCTGTGTGAGGGC
GnRH sglB OT2	ACACCTTCGTTGAGTGGTTGG	CTGCTTCAATACTAAATAAACGGTGAG
GnRH sglB OT3	AGAAGGTTCCACATGCAGGG	GACAGCATTGGCTTTGTGGG
GnRH sglB OT4	GCGGGGAGGATATTGGACTC	TAACCCGGGTCAAACGACAC
GnRH sglB OT5	TGTCATAGCAGAATGGAGTAACC	GGGGTTTCACCATTTTGGCC
Genotyping Primers		
mCherry middle up R		TCACCTTGTAGATGAACTCGCC
mCherry N-term up R		CTTGGTCACCTTCAGCTTGG
mCherry C-term up R		GCGCGTTCGTACTGTTCCAC
GnRH 5' close 2F		AGGTGGAGGTTGCCATGAGC
GnRH 5' geno 2F		TATCTGCAGCAATCAGAAGGC
GnRH 3' geno R		GGACAAAATAACCCAGCCTCC
GnRH 5' close 3F		GCCTGGGTGACAGAGTGAGG
mCherry middle down F		AAGCTGTCCTTCCCCGAGGG
GnRH 3' close 2R		GTTCTGCTTGTGGGTAGATGC
P2A downstream		GAGATGTCTGAAGAGAATCCTGG

Supplementary Table 3: Antibodies used in this study

Antigen (Antibody ID)	Supplier	Catalog #	species	Dilution
GnRH (AB_2490075)	Gift from Min Kyun Park (University of Tokyo, Tokyo, Japan)	LRH13	mouse	1:2500
GnRH (AB_2629221)	Gift from Susan Wray (NINDS, NIH, Bethesda, MD, USA)	SW1	rabbit	1:10000
GnRH (AB_2818954)	Gift from Nancy Sherwood (University of Victoria, Victoria, Canada)	GF6	rabbit	1:6000
β -tubulin III (AB_477590)	Sigma	T8660	mouse	1:1000
mCherry (AB_2536611)	Fisher Scientific	M11217	rat	1:1000
MAP2 (AB_2800501)	EMD Millipore	AB5622MI	rabbit	1:1000
GFAP (AB_10013382)	DAKO	Z0334	rabbit	1:1000
TH (AB_572268)	Immunostar	22941	mouse	1:1000
NPY (AB_1566510)	ABCAM	ab30914	rabbit	1:1000
GABA (AB_477652)	Sigma	A2052	rabbit	1:1000
GnRH for RIA (AB_2686919)	Gift from Terry M. Nett (Colorado State University, Fort Collins, CO, USA)	R42	rabbit	1:20000
Secondary Antibodies				
anti-rabbit IgG Alexa Fluor 488	Invitrogen	A11008	goat	1:1000
anti-rabbit IgG Alexa Fluor 594	Invitrogen	A11037	goat	1:1000
anti-mouse IgG Alexa Fluor 488	Invitrogen	A11029	goat	1:1000
anti-rat IgG Alexa Fluor 594	Invitrogen	A11007	goat	1:1000

Supplementary Table 4: The time course of GnRH neurogeneration by FGF8 treatment: Timelapse total live cell count (n=4/group)

Days	NDM alone	NDM + FGF8
0	0	0
10	0	0
12	0	20.5 ± 20.5
14	0	546.5 ± 319.8
16	0.8 ± 0.5	1309.3 ± 607.9
18	5.3 ± 2.6	2756.5 ± 1261.5
20	60.0 ± 51.6	3978.0 ± 1895.2*
22	88.5 ± 74.7	4695.7 ± 2144.4**
24	98.3 ± 84.4	5264.5 ± 2256.8**
26	127.0 ± 113.0	5312.2 ± 2238.3**
28	156.7 ± 136.5	5330.0 ± 2244.3**

Significant interactions ($p < 0.05$), treatment effects ($p < 0.0001$), and time effects ($p < 0.05$).
Post hoc analysis: * $p < 0.05$ and ** $p < 0.01$ vs. Day 0.

Supplementary Tables 5 (A-J), 6 (A-H), 7 (A-D) and 8 (A-E) are attached separately. Descriptions are as follows.

Supplementary Table 5 (A-J): Result tables for differential expression analysis of Day 8, Day 15, and Day 25 H9 cell line FGF-treated samples.

(A) Day 8 and Day 15 samples DESeq2 analysis result table containing base means across samples, log2 fold changes, standard errors, test statistics, p-value and adj. p-value. (B) GO enrichment terms associated with up-regulated genes from Day 8 to Day 15 samples. (C) KEGG pathway terms associated with up-regulated genes from Day 8 to Day 15 samples. (D) Day 15 and Day 25 samples DESeq2 analysis result table containing base means across samples, log2 fold changes, standard errors, test statistics, p-value and adj. p-value. (E) GO enrichment terms associated with up-regulated genes from Day 15 to Day 25 samples. (F) KEGG pathways associated with up-regulated genes from Day 15 to Day 25 samples. (G) KEGG pathways associated with down-regulated genes from Day 15 to Day 8 samples. (H) GO enrichment terms associated with down-regulated genes from Day 15 to Day 8 samples. (I) KEGG pathways associated with down-regulated genes from Day 25 to Day 15 samples. (J) GO enrichment terms associated with down-regulated genes from Day 25 to Day 15 samples.

Supplementary Table 6 (A-H): Result tables for differences in the differential expressed genes between Day 8 and Day 15 and between Day 15 and Day 25.

(A) List of genes corresponding to 1) the overlapping up-regulated genes, 2) genes that are up-regulated between Day 8 and Day 15, but not between Day 15 and Day 25, 3) genes that are up-regulated between Day 15 and Day 25, but not between Day 8 and Day 15, 4) the overlapping down-regulated genes, 5) genes that are down-regulated between Day 8 and Day 15, but not between Day 15 and Day 25, 6) genes that are down-regulated between Day 15 and Day 25, but not between Day 8 and Day 15. (B) GO enrichment terms associated with the overlapping up-regulated genes. (C) KEGG pathway terms associated with the overlapping up-regulated genes. (D) GO enrichment terms associated with genes that are up-regulated between Day 8 and Day 15, but not between Day 15 and Day 25. (E) GO enrichment

terms associated with genes that are up-regulated between Day 15 and Day 25, but not between Day 8 and Day 15. **(F)** KEGG pathway terms associated with genes that are up-regulated between Day 8 and Day 15, but not between Day 15 and Day 25. **(G)** KEGG pathway terms associated with genes that are up-regulated between Day 15 and Day 25, but not between Day 8 and Day 15. **(H)** KEGG pathway terms associated with genes that are down-regulated between Day 15 and Day 25, but not between Day 8 and Day 15.

Supplementary Table 7 (A-D): Time-course differential expression analysis of Day 0 to Day 25 in H9 cell line FGF-treated samples.

(A) List of genes following the time course treatment and can be divided into 1) 439 up-regulated genes from Day 0 to Day 25, 2) 1403 down-regulated genes from Day 25 to Day 0. **(B)** GO enrichment terms associated with up-regulated genes from Day 0 to Day 25. **(C)** GO enrichment terms associated with the down-regulated genes from Day 25 to Day 0. **(D)** KEGG pathway terms associated with the up-regulated genes from Day 0 to Day 25.

Supplementary Table 8 (A-E): Comparison of GnRH protocol to the induced pluripotent stem cell (iPSC) to iPSC-derived induced neurons (iN) maturation.

(A) List of differentially expressed genes from iPSC to iN maturation. **(B)** List of overlapping set of genes between differentially expressed genes from GnRH neuron Day 0 to Day 25 and differentially expressed genes from iPSC to iN maturation. **(C)** KEGG pathway terms associated with the overlapping up-regulated genes between GnRH protocol and iPSC and iN maturation. **(D)** GO enrichment terms associated with the overlapping up-regulated genes between GnRH protocol and iPSC and iN maturation. **(E)** GO enrichment terms associated with the overlapping down-regulated genes between GnRH protocol and iPSC and iN maturation.