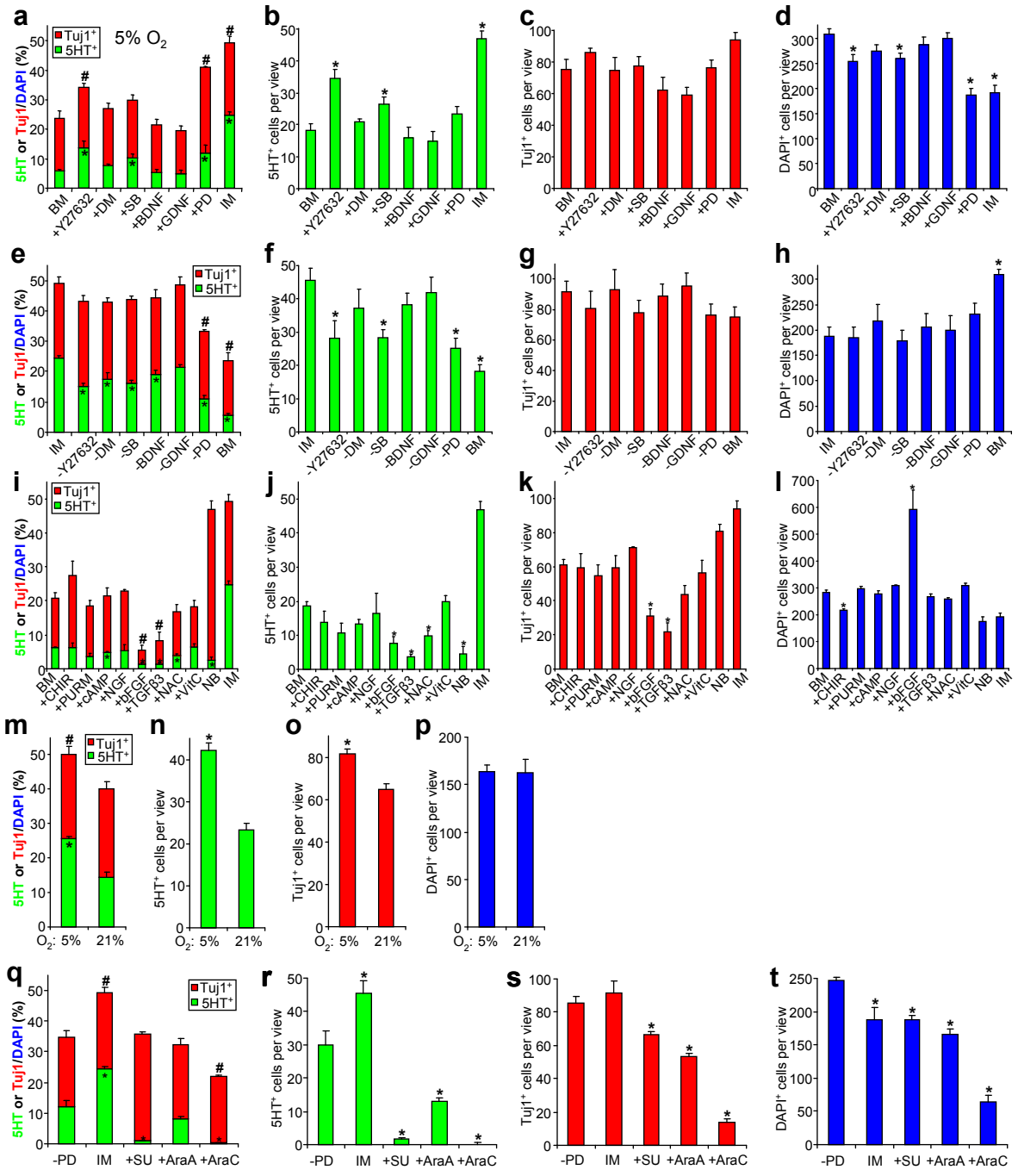

Figure S1. **Media additives and oxygen conditions.** MRC5 cells were converted to i5HT neurons in various media and oxygen conditions. (**a**-**d**) Basal Medium (BM) without or with (+) the indicated additive, or all of the additives (IM, Induction Medium). Y27632, Rock inhibitor (10 μM), DM, dorsomorphin (0.5 mM), SB, SB431542 (5 μM), PD, PD-0332991 (1 μM). (**e**-**h**) Induction Medium minus the indicated additive, or minus all additive (i.e. BM, Basal Medium). (**i**-**l**) Basal Medium without or with the indicated additive that was found to be ineffective or detrimental. CHIR, CHIR99021; PURM, Purmorphamine; NAC, N-Acetyl-L-cysteine; VitC, ascorbic acid; NB, DMEM/F12 in Induction Medium (IM) was replaced with Neurobasal. (**m**-**p**) Reprogramming in Induction Medium with 5% or 21% O_2. *, #, $p < 0.05$, vs. 21% oxygen for $5HT^+$ or $Tuj1^+$ cells respectively. (**q**-**t**) The effects of various mitotic inhibitors replacing PD (PD-0332991) in Induction Medium. +SU (SU9516 replacing PD in IM), +AraA (AraA replacing PD in IM), +AraC (AraA replacing PD in IM). *, #, $p < 0.05$ vs. IM without PD for $5HT^+$ or $Tuj1^+$ respectively.

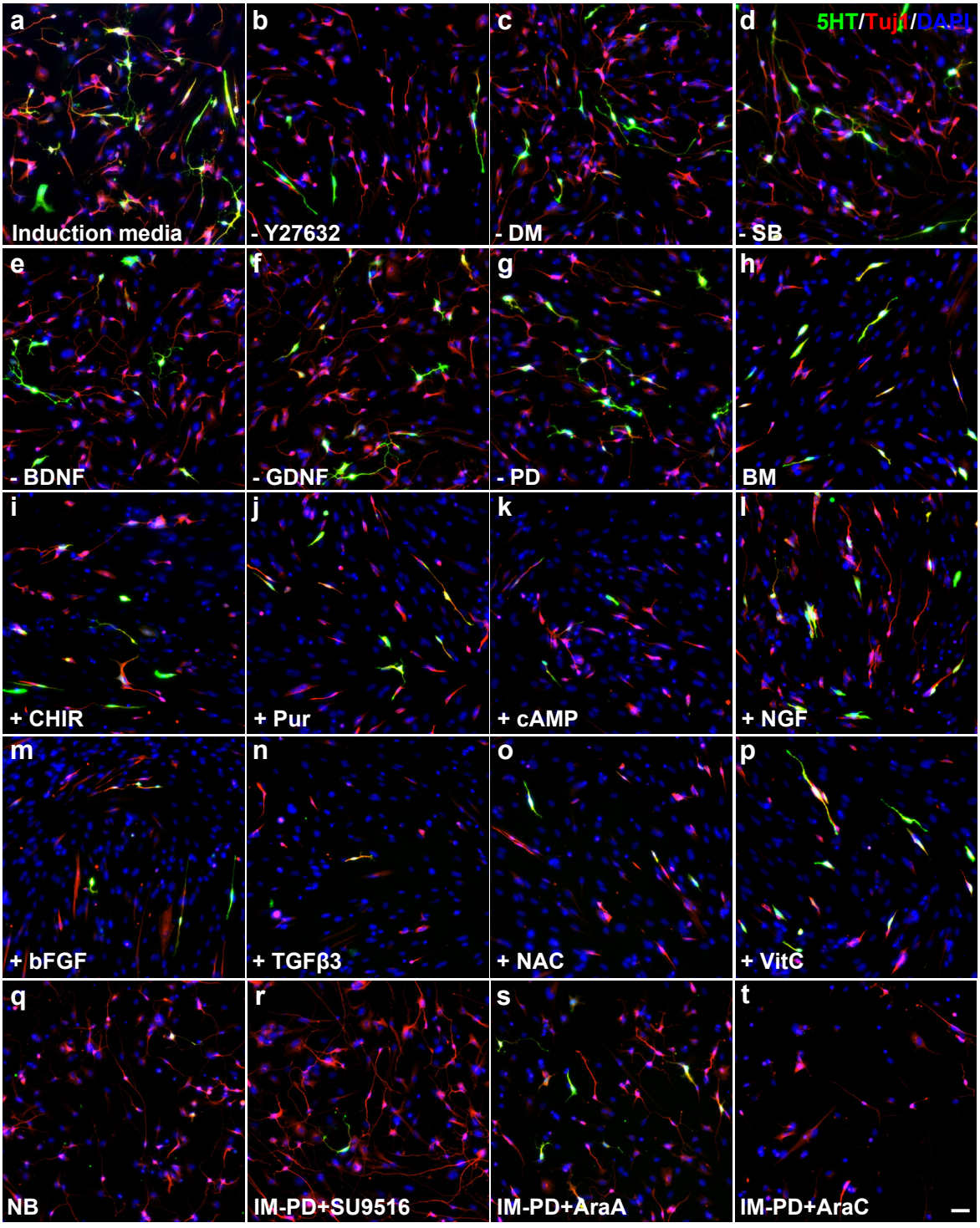

Figure S2. **Representative images of i5HT neurons generated with different media conditions.** MRC5 cells were converted to i5HT neurons in Induction Medium minus or plus the indicated additive. Quantification of the data was shown in Figure S1. bar, 50 μm.

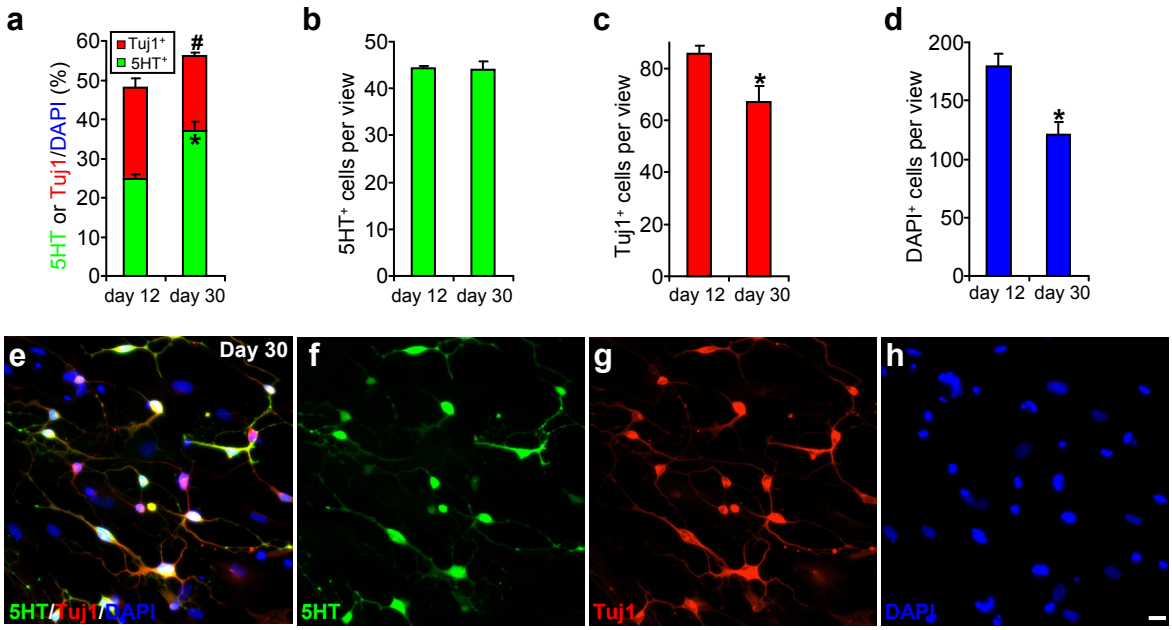

Figure S3. **Long-term survival of i5HT neurons.** Conversion efficiency (**a**) and yield (**b-d**) at 12 or 30 days after MRC5 cells were transduced with lentiviruses expressing AFLVp. *, # $p < 0.05$, vs. $5HT^+$ or $TuJ1^+$ at day 12, respectively, n= 3 independent experiments. Scale bar, 20 μm.

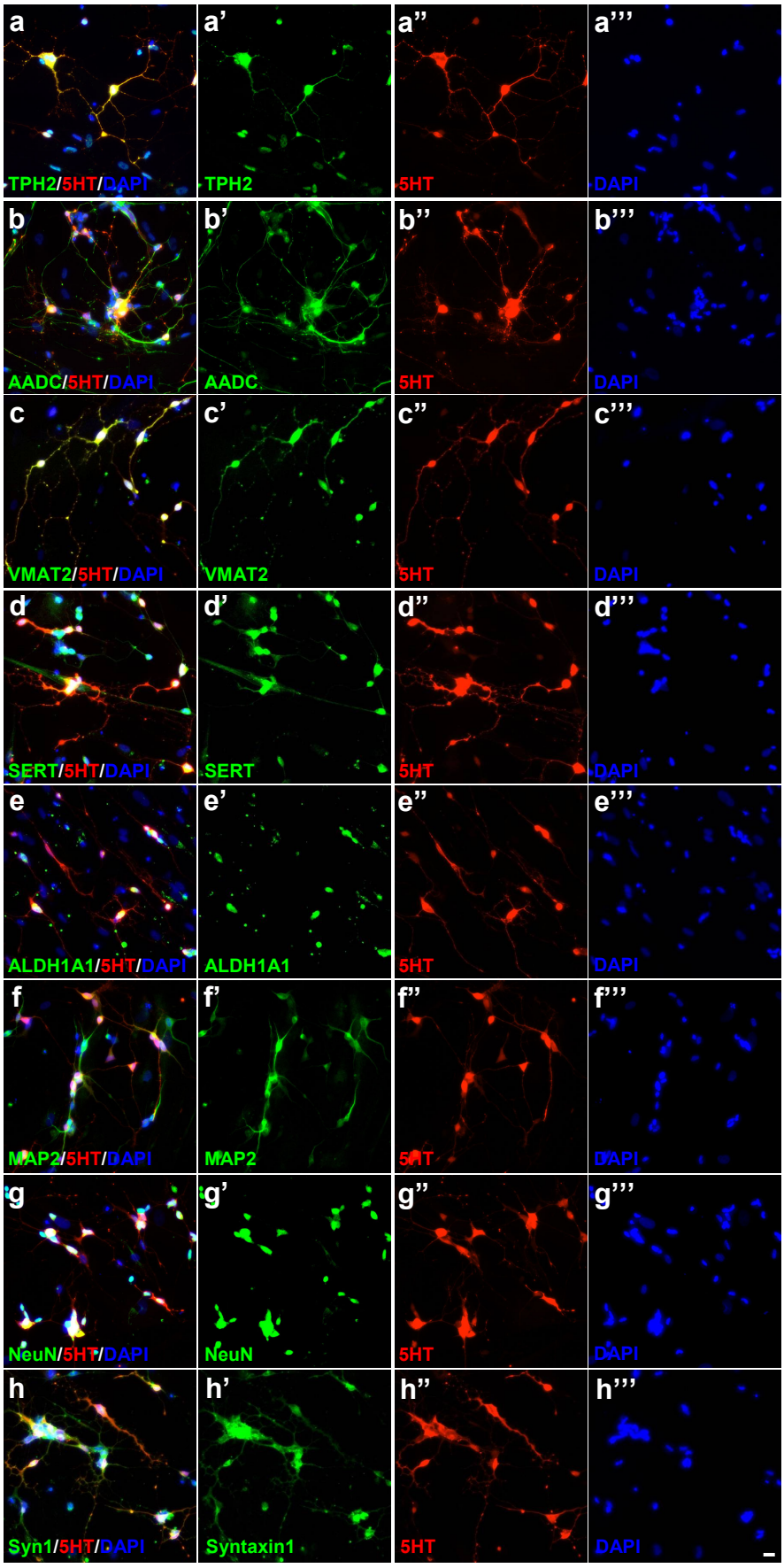

Figure S4. **Separate channels of Fig. 4a-h.** Scale bar, 20 μm

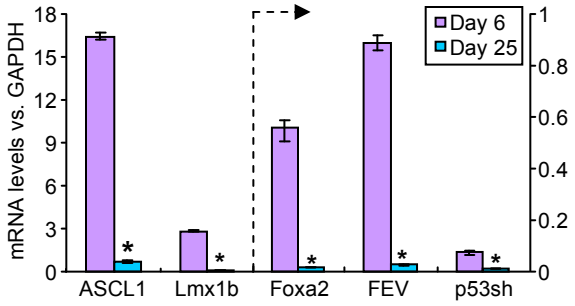

Figure S5. **Silencing of viral transgenes.** Expression levels of viral transgenes in MRC5 cells transduced with AFLVp at the indicated days. Arrow, values were according to the Y-axis on the right, n = 4 from 3 independent experiments.

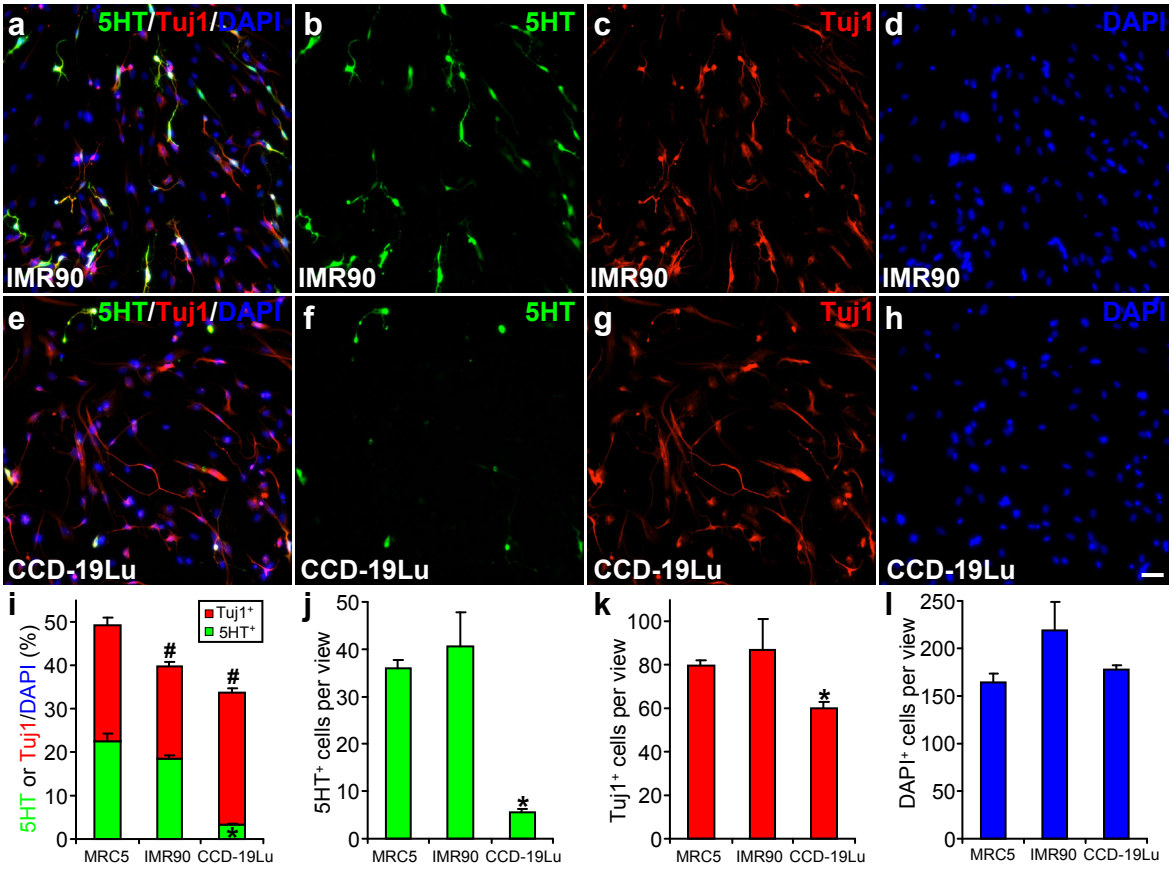

Figure S6. **Conversion of additional human fibroblast lines to i5HT neurons.** (**a**-**h**) Representative images of i5HT neurons derived from human fetal fibroblasts IMR90 cells (**a**-**d**) and human adult fibroblasts CCD-19Lu cells (**e**-**h**). Bar, 50 μm. (**i**-**l**) Quantification of data on IMR90 and CCD-19Lu cells, in comparison to the data on the human fetal fibroblasts MRC5 cells. *, #, $p<0.05$ vs. MRC5 for $5HT^+$ or $Tuj1^+$ neurons, respectively, n = 3 independent experiments.

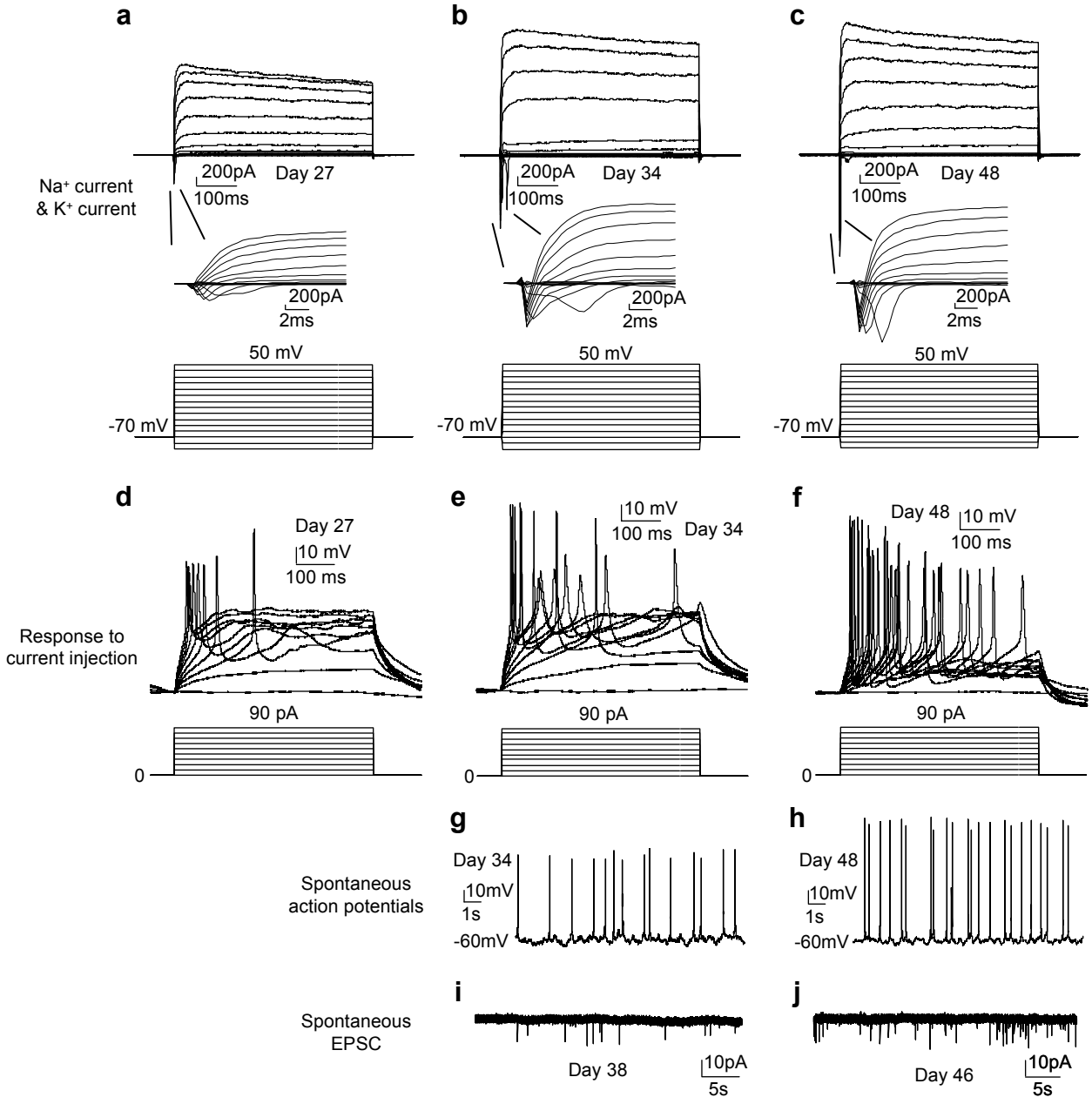

Figure S7. **Maturation of electrophysiological properties.** Electrophysiological recordings of i5HT neurons at the indicated days since Dox addition. Voltage dependent Na^+ currents and K^+ currents increased from small (**a**) to significant levels (**b, c**). Response to current injections changed from one (**d**) to multiple action potentials (**e, f**). Spontaneous action potentials were detected at day 34 (**g**) and increased in amplitude and frequency at day 48 (**h**). Spontaneous Excitatory Postsynaptic Currents (EPSCs) were observed at day 38 (**i**) and increased frequency at day 46 (**j**). Detailed quantifications of these electrophysiological parameters are in Supplementary Table 1.

Supplementary Table 1: Maturation of Electrophysiological Properties

	Day 27	**Day 34**	**Day 48**
Resting Membrane Potential (mV)	-20.2 ± 1.8 (n=3)	-33.3 ± 2.1 (n=10)	-42.6 ± 2.3 (n=13)
Na+ Currents (pA) at -20mV	534 ± 24 (n=3)	916 ± 34 (n=8)	1425 ± 86 (n=8)
K+ Currents (pA) at 50mV	1264 ± 112 (n=3)	1648 ± 123 (n=8)	2176 ± 124 (n=8)
Evoked Action Potentials	2/4	8/8	8/8
Spontaneous Action Potentials	n.d.	3/8	3/8
Spontaneous AP Frequency (Hz)	n.d.	0.7 ± 0.1	1.1 ± 0.2
Spontaneous AP Amplitude (mV)	n.d.	61.7 ± 3.3	78.3 ± 6.0
Spontaneous EPSC	n.d.	2/7 (Day 38)	2/6 (Day 46)
Spontaneous EPSC Frequency (Hz)	n.d.	0.3 ± 0.1	1.3 ± 0.2
Spontenous EPSC Amplitude (pA)	n.d.	8.3 ± 1.2	7.6 ± 1.4

Supplementary Table 2: Antibodies Used in the Study

Protein	Species	Company(Catalog Number)	Dilution
5-HT	Rabbit	Sigma-Aldrich (S5545)	1:2000
5-HT	Rat	Millipore (MAB352)	1:500
TPH2	Rabbit	Novus Biological Inc. (NB100-74555)	1:1000
SERT	Mouse	MAb technologies (ST51-2)	1:1000
TuJ1	Mouse	Civabce(MMS-435P-250)	1:1000
MAP2	Mouse	Santa Cruz (sc-74421)	1:500
NeuroN	Mouse	Millipore (MAB377)	1:500
AADC	Rabbit	Millipore (AB1569)	1:1000
ALDH1A1	Rabbit	Abcam (ab23375)	1:2000
VMAT2	Rabbit	Millipore (AB1598p)	1:500
Syntaxin-1	Mouse	Life Technologies (P21943)	1:1000
AlexaFluor 488	Anti-Rabbit IgG	Life Technologies (A11008)	1:2000
AlexaFluor 594	Anti-Rabbit IgG	Life Technologies (A11012)	1:2000
AlexaFluor 488	Anti-mouse IgG	Life Technologies (A21202)	1:2000
AlexaFluor 594	Anti-mouse IgG2a	Life Technologies (A21135)	1:2000
AlexaFluor594	Anti-Rat IgG	Life Technologies (A11007)	1:2000

Supplementary Table 3: Sequence of PCR Primers

Gene	Accession	Primer Sequences
hASCL1 transgenic	NM_004316	Forward: TACTCGTCGGACGAGGGCTCTTA Reverse: AAAGCAGCGTATCCACATAGCGTA
hFOXA2 transgenic	NM_021784.4	Forward: GTCACGAACAAAACGGGCCT Reverse: AAAGCAGCGTATCCACATAGCGTA
hLMX1b transgenic	NM_002316.3	Forward: CTCTACTCCATGCAGAGTTCCT Reverse: AAAGCAGCGTATCCACATAGCGTA
hFEV transgenic	NM_017521.2	Forward: TCTACCCCAGTCCCAGCTTGCA Reverse: AAAGCAGCGTATCCACATAGCGTA
hp53shRNA transgenic	NM_000546	Forward: TACAGGGACAGCAGAGATCCACTT Reverse: AACCGCAAGGAACCTTCCCGACTT
hASCL1 endogenous	NM_004316	Forward: TACTCGTCGGACGAGGGCTCTTA Reverse: GCACTAAAGATGCAGGTTGTGCGA
hFOXA2 endogenous	NM_021784.4	Forward: CTACGAACAGGTGATGCACTAC Reverse: TTGCTCTCTCACTTGTCCTCGA
hLMX1b endogenous	NM_002316.3	Forward: GGCCTCACGCCGCCCCAAAT Reverse: TGGCTGGCTCTCAGGAGGCGAA
hFEV endogenous	NM_017521.2	Forward: TCTACCCCAGTCCCAGCTTGCA Reverse: CTCTGGATTAGAGGACGGTTGAC
hMAP2 endogenous	NM_031845	Forward: CAGGTGGCGGACGTGTGAAAATTGAGAGTG Reverse: CACGCTGGATCTGCCTGGGGACTGTG
hTUBB3 endogenous	NM_001197181	Forward: TTTGGACATCTCTTCAGGCCTGACA Reverse: AGCGAGTGGGTCAGCTGGAAGC
hSYN1 endogenous	NM_006950	Forward: TGAAGCCGGATTTTGTGCTGA Reverse: GACCAAACTGCGGTAGTCTCC
hPCLO endogenous	NM_033026	Forward: CAGACACTTTCAGGTCAGAGC Reverse: AGGCATCATACTAGACTTGTGCT
hALDH1A1 endogenous	NM_000689.4	Forward: GAATTTCCCGTTGGTTATGCT Reverse: TGTAGGCCCATAACCAGGAA
hAADC endogenous	NM_001082971	Forward: AGAACAGACTTAACGGGAGCCT Reverse: CTGGACATGCTTGCGGATATAA
hVMAT2 endogenous	NM_003054	Forward: CTTTGGAGTTGGTTTTGC Reverse: GCAGTTGTGATCCATGAG
hGAPDH endogenous	NM_002046.3	Forward: GACAACAGCCTCAAGATCATCAG Reverse: ATGGCATGGACTGTGGTCATGAG
hCACNA1C endogenous	NM_001167625	Forward: TCCGCTGCTTCTGAAGATGA Reverse: GGCCGTCGCTTTGGTAGTA
hSCN1A endogenous	NM_001165963	Forward: TGGGGAGTGGATAGAGACCA Reverse: GAAAGAGATTCAGGACCACTAGG
hTPH1 endogenous	NM_004179.2	Forward: CCCTTTGATCCCAAGATTAC Reverse: CATTCATGGCACTGGTTATG
hTPH2 endogenous	NM_173353.3	Forward: GGTTCCCTCGGAAGATCTCTGAGTTAGACA Reverse: AGAGCTCCCGGAATACAACACCCCAAGT
hSERT endogenous	NM_001045.4	Forward: CAGCGTGTGAAGATGGAGAAG Reverse: TGGGATAGAGTGCCGTGTGT