

**Figure S1. Related to Figure 1. Ventral forebrain organoids derived from OLIG2-GFP hPSCs and OLIG2<sup>+</sup> ventral forebrain NPCs mainly give rise to CR<sup>+</sup> GABAergic neurons under 2D culture conditions.**

(A) Representative bright-field and fluorescence images of OLIG2-GFP hPSC-derived ventral forebrain organoids at different stages. Scale bar: 200  $\mu$ m.

(B) Representatives of SOX2,  $\beta$ IIIIT and GFP immunostaining, showing ventricular zone (VZ)-like areas in 6-week-old OLIG2-GFP hPSC-derived ventral forebrain organoids. Scale bars: 50  $\mu$ m.

(C) Representatives of MBP-expressing mature oligodendrocytes in 8-week-old OLIG2-GFP hPSC-derived ventral forebrain organoids cultured under glial differentiation condition. Scale bars: 10  $\mu$ m.

(D) Representatives of OLIG2-, Nestin-, NKX2.1-, and OTX2-expressing cells among the GFP<sup>+</sup> cells after FACS purification. Scale bars: 50  $\mu$ m.

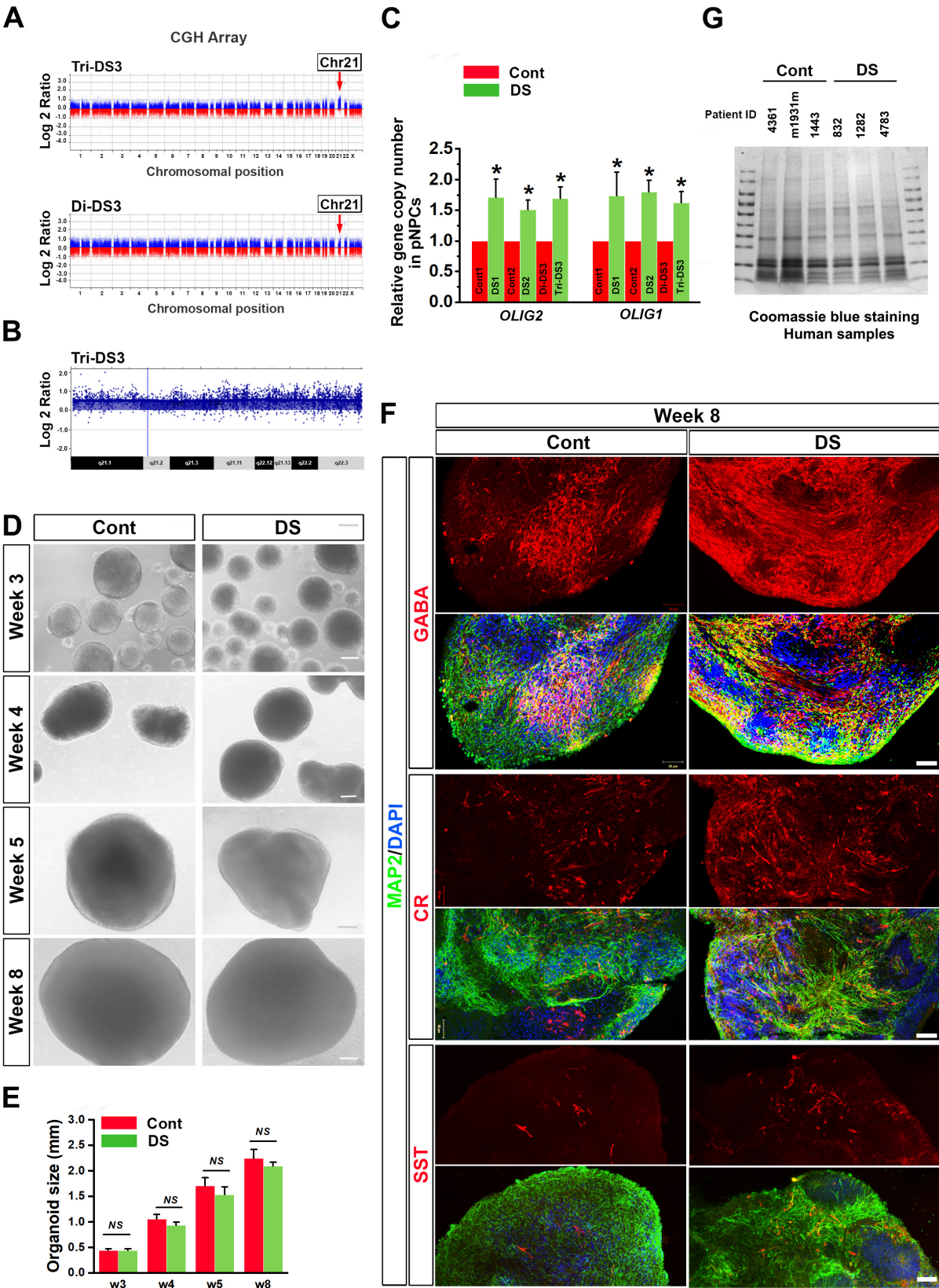
(E) Representative bright-field and fluorescence images showing OLIG2<sup>+</sup>/GFP<sup>+</sup> cell-derived organoids at different time points. Scale bar: 500  $\mu$ m.

(F) Representatives of NeuN-, S100 $\beta$ -, NG2-, and PDGFR $\alpha$ -expressing cells in 8-week-old OLIG2<sup>+</sup>/GFP<sup>+</sup> cell-derived organoids cultured under neuronal differentiation condition. Scale bars: 20  $\mu$ m.

(G) Quantification of pooled data from OLIG2-GFP hiPSCs and hESCs showing the percentage of NeuN<sup>+</sup>, S100 $\beta$ <sup>+</sup>, NG2<sup>+</sup>, and PDGFR $\alpha$ <sup>+</sup> in 8-week-old GFP<sup>+</sup> cell-derived organoids (n = 3 and for each experiment, 4 to 6 organoids from each line are used). Data are presented as mean  $\pm$  s.e.m.

(H) Representatives of GABA-, CR-, and MAP2-expressing neurons differentiated from OLIG2<sup>+</sup> ventral forebrain NPCs under neuronal differentiation condition in 2D cultures. Scale bar: 50  $\mu$ m.

(I) Quantification of pooled data from OLIG2-GFP hiPSCs and hESCs showing the percentage of GABA<sup>+</sup>, CR<sup>+</sup>, PV<sup>+</sup>, and SST<sup>+</sup> cells among total MAP2<sup>+</sup> neurons (n = 4). Data are presented as mean  $\pm$  s.e.m.





**Figure S2. Related to Figure 2 and 3. Overabundance of GABAergic neurons in DS organoids.**

(A) Comparative genomic hybridization (CGH) array of isogenic pair of Di-DS3 and Tri-DS3 demonstrates a gain of one chromosome 21 (red arrow) in Tri-DS3. No significant deletions or insertions were detected between the isogenic pair.

(B) Close-up of Chr21 CGH of Tri-DS3 shows full chromosome 21 trisomy with no deletions or duplications.

(C) qPCR results from genomic DNA showing the relative *OLIG1* and *OLIG2* DNA copy numbers in control (Cont1, Cont2, and Di-DS3) and DS (DS1, DS2, and Tri-DS3) pNPCs.  $*P < 0.05$ , comparison between Cont1 vs. DS1, Cont-2 vs. DS2, or Di-DS3 vs. Tri-DS3 pNPCs.

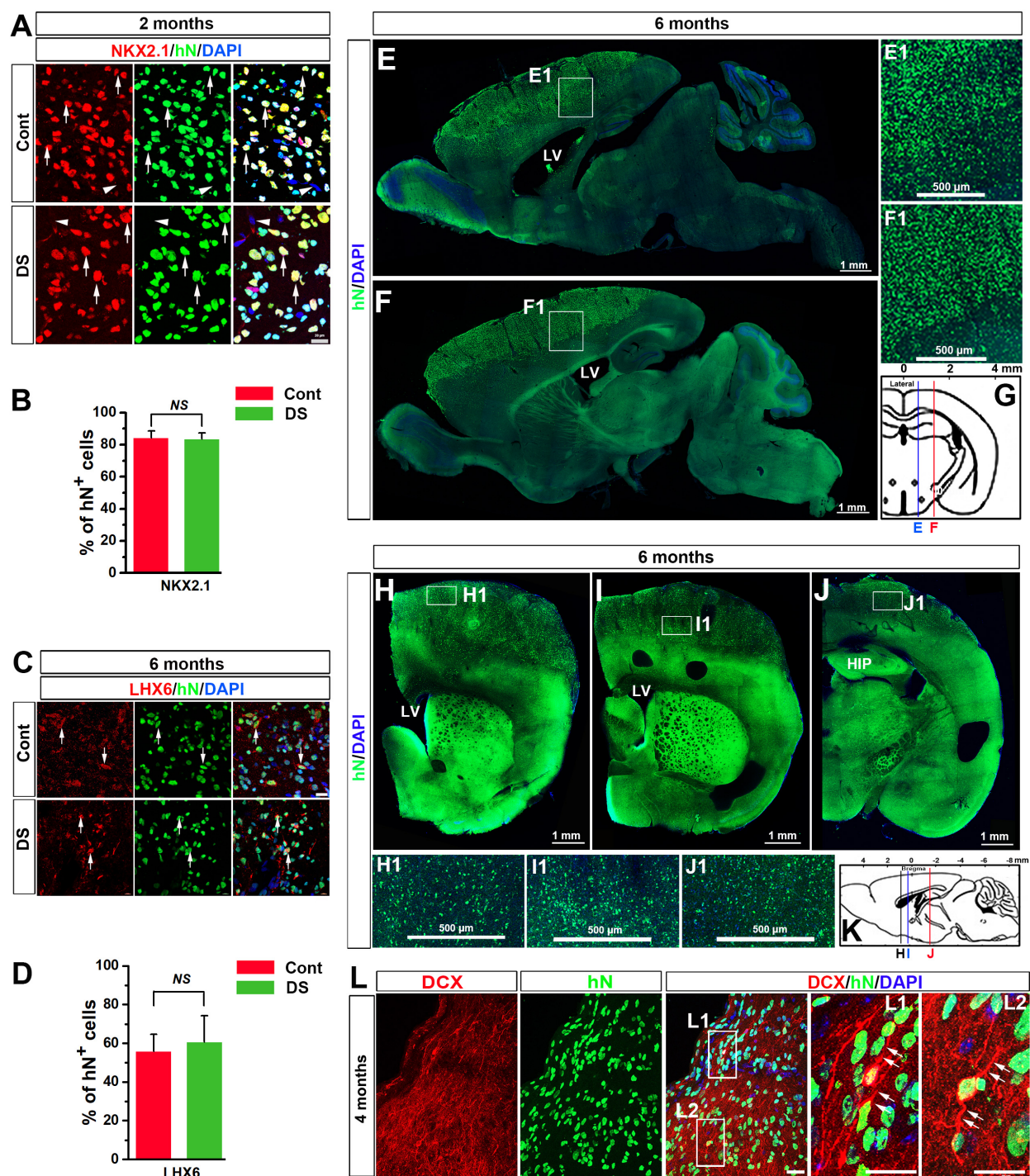
(D) Representative bright-field images of control (Cont) and DS hiPSC-derived ventral forebrain organoids at different stages. Scale bars: 200  $\mu\text{m}$ .

(E) Quantification of size of control (Cont) and DS hiPSC-derived ventral forebrain organoids at different stages. Scale bars: 200  $\mu\text{m}$ . Student's *t* test. *NS* represents no significance. Data are presented as mean  $\pm$  s.e.m.

(F) Representative low magnification images of organoids showing GABA-, CR- and SST-expressing neurons in 8-week-old control and DS organoids. Scale bar: 50  $\mu\text{m}$ .

(G) Coomassie blue staining of the human samples after running denatured SDS-PAGE showing high sharpness and resolution of protein bands and the absence of smear (little or no degradation) of protein bands.





**Figure S3. Related to Figure 4. Distribution and characterization of donor-derived cells after transplantation to the sites overlying lateral ventricles.**

(A) Representatives of NKX2.1-expressing donor-derived hN<sup>+</sup> cells in the cerebral cortex at 2 months post-transplantation of control or DS NPCs. Arrows indicate the donor-derived hN<sup>+</sup> cells expressing NKX2.1 and arrowhead indicates the NKX2.1-negative endogenous mouse cell. Scale bars, 20  $\mu$ m.

(B) Quantification of pooled data from control (Cont1 and Di-DS3) and DS (DS1 and Tri-DS3) hiPSCs showing the percentage of NKX2.1<sup>+</sup> cells among the total hN<sup>+</sup> cells in the cerebral cortex at 2 months post-transplantation (n = 7 mice for each group). Student's *t* test. *NS* represents no significance. Data are presented as mean  $\pm$  s.e.m.

(C) Representatives of LHX6-expressing donor-derived hN<sup>+</sup> cells in the cerebral cortex at 6 months post-transplantation of control or DS NPCs. Arrows indicate the donor-derived hN<sup>+</sup> cells expressing LHX6. Scale bars, 20  $\mu$ m.

(D) Quantification of pooled data from control (Cont1 and Di-DS3) and DS (DS1 and Tri-DS3) groups showing the percentage of LHX6<sup>+</sup> cells among the total hN<sup>+</sup> cells in the cerebral cortex at 6 months post-transplantation (n = 7 mice for each group). Student's *t* test. *NS* represents no significance. Data are presented as mean  $\pm$  s.e.m.

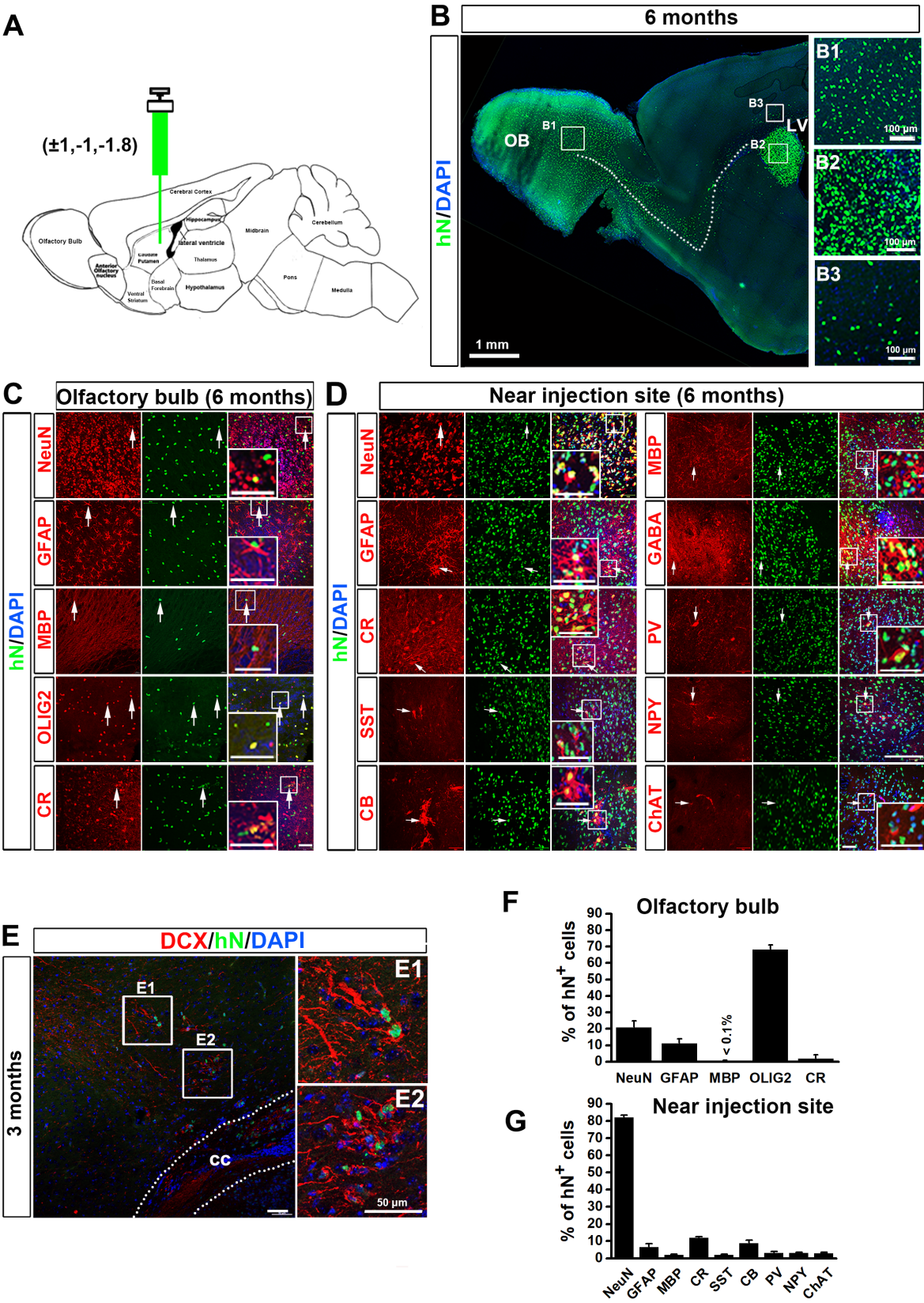
(E and F) Representative images from sagittal brain sections showing the wide distribution of hN<sup>+</sup> donor-derived cells in the cerebral cortex at 6 months post-transplantation. Areas in E1 and F1 are enlarged. The images were from confocal stitched tile scan. Scale bars: 1mm or 500  $\mu$ m as indicated.

(G) A schematic diagram showing the relative positions of the sagittal sections shown in (E) and (F).

(H-J) Representative images from coronal brain sections showing the wide distribution of hN<sup>+</sup> donor-derived cells in the cerebral cortex at 6 months post-transplantation. The sections were collected after whole-cell patch-clamp recording and visualized by post hoc labeling with hN staining. Areas in H1, I1 and J1 are enlarged. The images were from confocal stitched tile scan. Scale bars: 1mm or 500  $\mu$ m as indicated.

(K) A schematic diagram showing that relative positions of coronal sections shown in (H), (I), and (J).

(L) Representatives of DCX-expressing donor-derived hN<sup>+</sup> cells in the cerebral cortex at 4 months post-transplantation. Arrows indicate the bipolar processes of DCX<sup>+</sup>/hN<sup>+</sup> cells. Scale bars, 20  $\mu$ m.





**Figure S4. Related to Figure 4. Characterization of donor-derived cells after transplantation to the sites near subventricular zone.**

(A) A schematic diagram showing that control hiPSC-derived ventral forebrain NPCs are engrafted into the brains of P0 *rag1*<sup>-/-</sup> mice into the sites near the subventricular zone (SVZ).

(B) Representatives showing that donor-derived hN<sup>+</sup> cells have migrated along the rostral migratory stream to the olfactory bulb at 6 months after transplantation. Scale bar, 1 mm or 100  $\mu$ m as indicated.

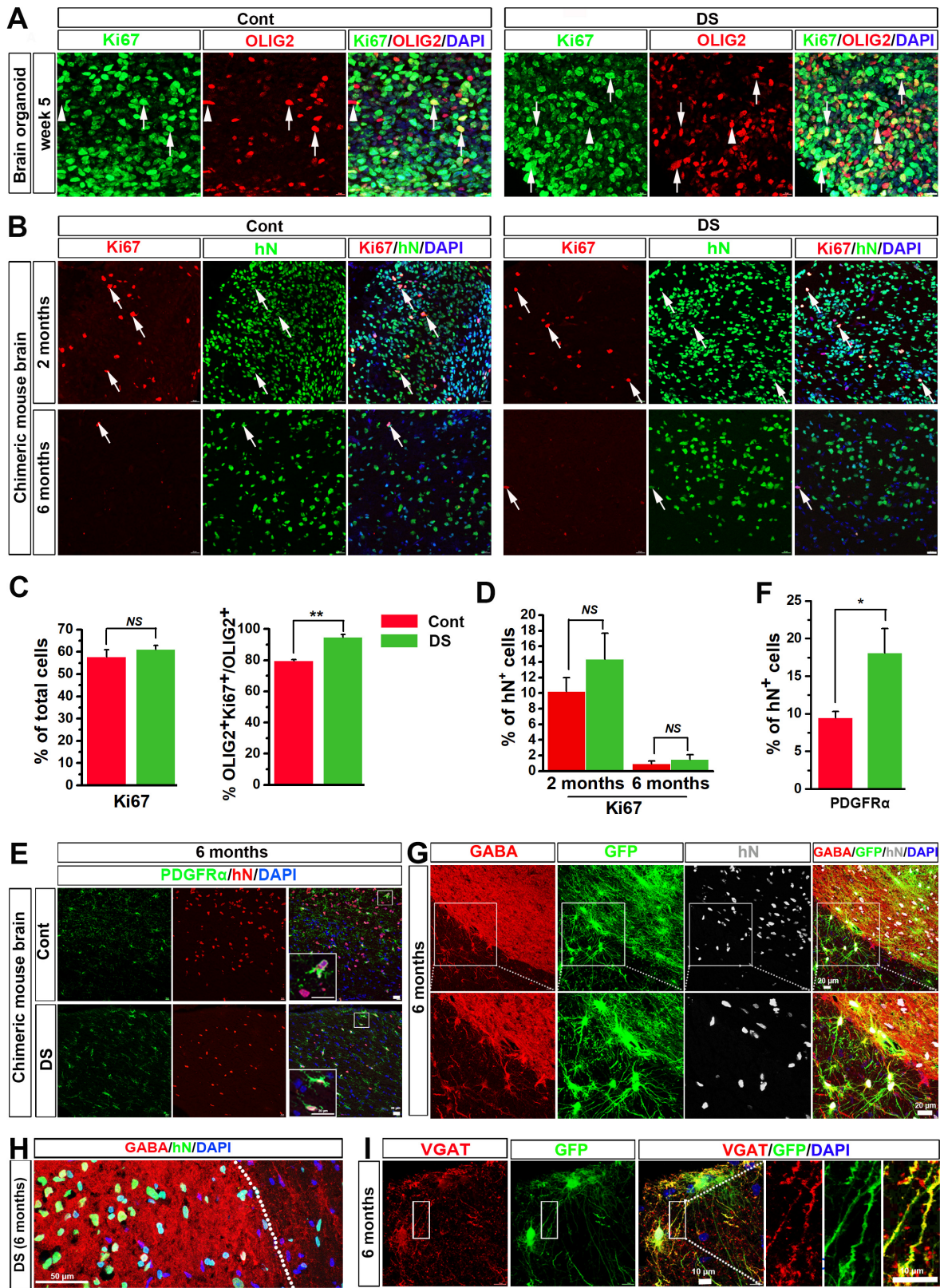
(C) Representatives of NeuN-, GFAP-, MBP-, OLIG2- and CR-expressing donor-derived hN<sup>+</sup> cells in the olfactory bulb at 6 months post-transplantation. Arrows indicate the donor-derived hN<sup>+</sup> cells expressing the indicated markers. Scale bars, 50  $\mu$ m.

(D) Representatives of NeuN-, GFAP-, MBP-, CR-, SST-, CB-, PV-, NPY-, and ChAT-expressing donor-derived hN<sup>+</sup> cells in the regions near injection sites at 6 months post-transplantation. Arrows indicate the donor-derived hN<sup>+</sup> cells expressing the indicated markers. Scale bars, 50  $\mu$ m.

(E) Representatives of DCX-expressing donor-derived hN<sup>+</sup> cells at 3 months post-transplantation in the cerebral cortex. CC, corpus callosum. Scale bars, 50  $\mu$ m.

(F) Quantification of pooled data from control (Cont1 and Di-DS3) hiPSCs showing NeuN-, GFAP-, MBP-, OLIG2- and CR-expressing donor-derived hN<sup>+</sup> cells among the total hN<sup>+</sup> cells in the olfactory bulb at 6 months post-transplantation (n = 7 mice for each group). Data are presented as mean  $\pm$  s.e.m.

(G) Quantification of pooled data from control (Cont1 and Di-DS3) hiPSCs showing NeuN-, GFAP-, MBP-, CR-, SST-, CB-, PV-, NPY-, and ChAT-expressing donor-derived hN<sup>+</sup> cells in the regions near injection sites among the total hN<sup>+</sup> cells at 6 months post-transplantation (n = 7 mice for each group). Data are presented as mean  $\pm$  s.e.m.



**Figure S5. Related to Figure 2 and Figure 4. Proliferation of the cells before and after transplantation and interneuron identity after transplantation.**

(A) Representatives of Ki67-expressing proliferating cells in control and DS 5-week-old ventral forebrain organoids. Arrows indicate OLIG2<sup>+</sup>/Ki67<sup>+</sup> cells and arrowheads indicate OLIG2<sup>+</sup>/Ki67<sup>-</sup> cells. Scale bars: 10  $\mu$ m.

(B) Representatives of Ki67-expressing donor-derived hN<sup>+</sup> cells in the cerebral cortex at 2 and 6 months post-transplantation. Arrows indicate the donor-derived hN<sup>+</sup> cells expressing Ki67. The images were from confocal stitched tile scan. Scale bars, 20  $\mu$ m.

(C) Quantification of pooled data from three pairs of control and DS hiPSCs showing the percentage of Ki67<sup>+</sup> cells in the total cells, as well as the percentage of Ki67<sup>+</sup>/OLIG2<sup>+</sup> in total OLIG2<sup>+</sup> cells in 5-week-old control and DS ventral forebrain organoids ( $n = 4$  and for each experiment, 4 to 6 organoids from each cell line are used). Student's  $t$  test.  $**P < 0.01$  and  $NS$  represents no significance. Data are presented as mean  $\pm$  s.e.m

(D) Quantification of pooled data from control (Cont1 and Di-DS3) and DS (DS1 and TriDS3) hiPSCs showing the percentage of Ki67<sup>+</sup> cells among the total hN<sup>+</sup> cells in the cerebral cortex at 2 and 6 months post-transplantation ( $n = 7$  mice for each group). Student's  $t$  test.  $NS$  represents no significance. Data are presented as mean  $\pm$  s.e.m.

(E) Representatives of PDGFR $\alpha$ -expressing donor-derived hN<sup>+</sup> cells in the cerebral cortex at 6 months post-transplantation. Scale bars, 20  $\mu$ m.

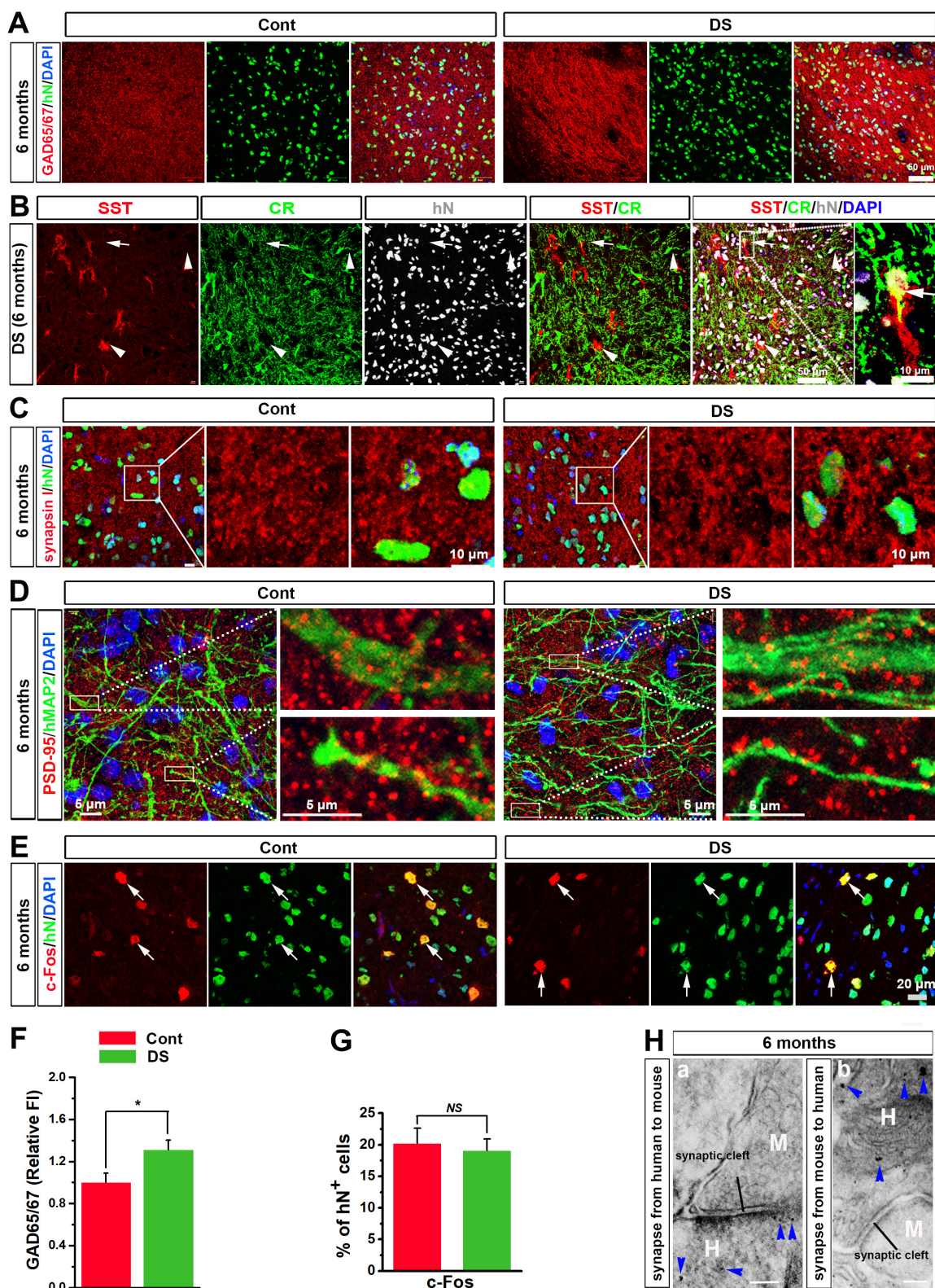
(F) Quantification of pooled data from control (Cont1 and Di-DS3) and DS (DS1 and Tri-DS3) hiPSCs showing the percentage of PDGFR $\alpha$ <sup>+</sup> cells among the total hN<sup>+</sup> cells in the cerebral cortex at 6 months post-transplantation ( $n = 7$  mice for each group). Student's  $t$  test.  $*P < 0.05$ . Data are presented as mean  $\pm$  s.e.m.

(G) Representative images showing strong GABA staining in areas enriched of donor-derived hN<sup>+</sup> cells in the cerebral cortex at 6 months after transplantation. The cells are labeled by lenti-CMV-GFP, prior to transplantation. Scale bar, 20  $\mu$ m.

(H) A representative image from DS chimeric mice showing high fluorescence intensity of GABA staining in areas enriched of donor-derived hN<sup>+</sup> cells in the cerebral cortex at 6 months after transplantation. Scale bar, 50  $\mu$ m.

(I) Representative images showing GFP<sup>+</sup> donor-derived cells also express VGAT in the cerebral cortex at 6 months after transplantation. The cells are labeled by lenti-CMV-GFP, prior to transplantation. Scale bar, 10 $\mu$ m.





**Figure S6. Related to Figure 4. Overabundance of GABAergic neurons in DS chimeric mouse brains and integration of donor cell-derived neurons into the brain.**

(A) Representatives of GAD65/67 staining in the cerebral cortex at 6 months after transplantation of control or DS hiPSC-derived ventral forebrain NPCs. Scale bar, 50  $\mu$ m.

(B) Representatives of SST, CR, and hN triple-staining in the cerebral cortex at 6 months after transplantation of DS hiPSC-derived ventral forebrain NPCs. Notably, few hN<sup>+</sup> human cells co-express SST and CR. Arrows indicate the donor-derived hN<sup>+</sup> cells co-expressing SST and CR. Arrowheads indicate the donor-derived hN<sup>+</sup> cells only expressing SST or CR. Scale bar, 50  $\mu$ m or 10  $\mu$ m in the original or enlarged images, respectively.

(C) Representatives of the hN<sup>+</sup> cell nuclei surrounded by the staining of presynaptic marker synapsin I in the cerebral cortex at 6 months after transplantation of control or DS hiPSC-derived ventral forebrain NPCs. Scale bar, 10  $\mu$ m.

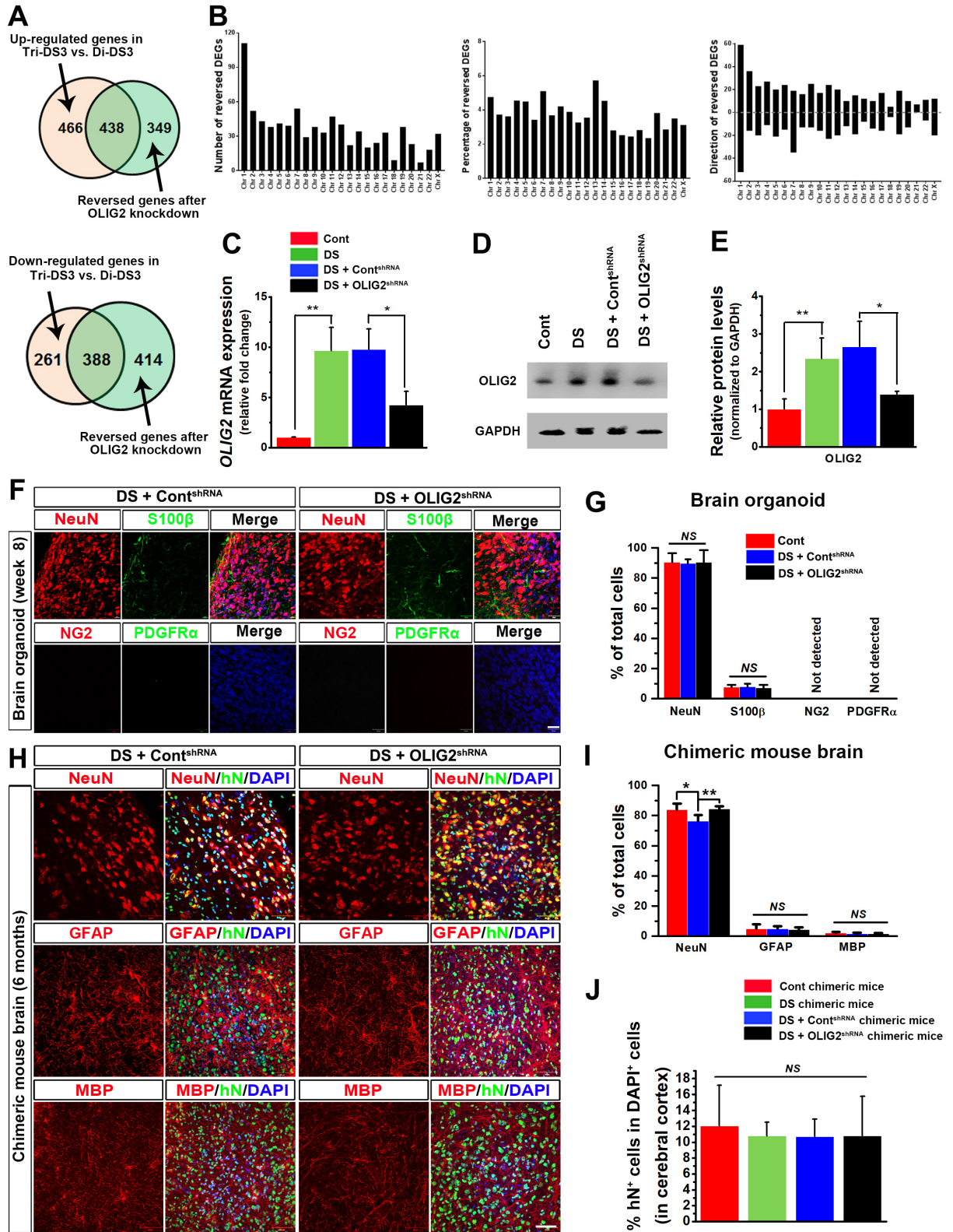
(D) Representatives of human-specific MAP2 (hMAP2)-expressing dendrites that co-localize with puncta of PSD-95 staining, a postsynaptic marker, in the cerebral cortex at 6 months after transplantation of control or DS hiPSC-derived ventral forebrain NPCs. Scale bar, 5  $\mu$ m.

(E) Representatives of c-Fos-expressing donor-derived hN<sup>+</sup> cells in the cerebral cortex at 6 months post-transplantation of control or DS NPCs. Arrows show engrafted hN<sup>+</sup> cells expressing c-Fos. Scale bars, 20  $\mu$ m.

(F) Quantification of pooled data from control (Cont1 and Di-DS3) and DS (DS1 and Tri-DS3) showing the fluorescence intensity (FI) of GAD65/67 staining in the cerebral cortex at 6 months after cell transplantation ( $n = 7$  mice for each group). Student's  $t$  test. \* $P < 0.05$ . Data are presented as mean  $\pm$  s.e.m.

(G) Quantification of pooled data from control (Cont1 and Di-DS3) and DS (DS1 and Tri-DS3) hiPSCs showing the percentage of c-Fos<sup>+</sup> cells among the total hN<sup>+</sup> cells in the cerebral cortex at 6 months post-transplantation ( $n = 7$  mice for each group). Student's  $t$  test. NS represents no significance. Data are presented as mean  $\pm$  s.e.m.

(H) Representative electron microscopy images of synaptic terminals formed between human neurons labeled by DAB staining against a human-specific marker STEM121 and mouse neurons that were not labeled by the DAB staining. (a) synaptic contacts from human transplant to mouse host tissue and (b) synapse from mouse neurons to human neurons at 6 months post-transplantation. Synaptic cleft structure was seen in the synaptic terminals. "H" denotes human neurons, "M" denotes mouse neurons, and arrowheads indicate DAB electron-dense precipitates. Scale bar represents 100 nm.





**Figure S7. Related to Figure 5 and Figure 6. Reversed expression of differentially expressed genes (DEGs) in Tri-DS3 organoids by OLIG2 knockdown and differentiation of DS ventral forebrain NPCs after OLIG2 knockdown in the organoids and chimeric mouse brains.**

(A) Venn diagrams showing 438 upregulated and 388 downregulated DEGs are reversed after *OLIG2* knockdown (KD) in 5-week-old Di-DS3 and Tri-DS3 organoids.

(B) The total number of reversed DEGs, percentage of reversed DEGs, and number of up-regulated or down-regulated of DEGs that are reversed after *OLIG2* knockdown on each chromosome in 5-week-old Di-DS3 and Tri-DS3 organoids.

(C) qPCR analysis of *OLIG2* mRNA expression in 5-week-old control, DS, DS + Cont<sup>shRNA</sup> and DS + *OLIG2*<sup>shRNA</sup> organoids. The data are pooled from control (Cont1 and Di-DS3) hiPSCs, DS (DS1 and Tri-DS3) hiPSCs, and the two lines of DS hiPSCs infected with lentiviruses that carry control shRNA or *OLIG2* shRNA. The experiments are repeated for three times ( $n = 3$ ) and for each experiment, 20 to 30 organoids from each line are used. Student's  $t$  test.  $**P < 0.01$ ,  $*P < 0.05$ . Data are presented as mean  $\pm$  s.e.m.

(D) Western blotting analysis of *OLIG2* expression in 5-week-old control, DS, DS + Cont<sup>shRNA</sup> and DS + *OLIG2*<sup>shRNA</sup> organoids.

(E) Quantification data showing *OLIG2* expression by western blot in 5-week-old control, DS, DS + Cont<sup>shRNA</sup> and DS + *OLIG2*<sup>shRNA</sup> organoids. The data are pooled from control (Cont1 and Di-DS3) hiPSCs, DS (DS1 and Tri-DS3) hiPSCs, and the two lines of DS hiPSCs infected with lentiviruses that carry control shRNA or *OLIG2* shRNA. The experiments are repeated for three times ( $n = 3$ ) and for each experiment, 30 to 40 organoids from each line are used. Student's  $t$  test.  $**P < 0.01$ ,  $*P < 0.05$ . Data are presented as mean  $\pm$  s.e.m.

(F) Representatives of NeuN-, S100 $\beta$ -, NG2-, and PDGFR $\alpha$ -expressing cells in 8-week-old DS + Cont<sup>shRNA</sup> and DS + *OLIG2*<sup>shRNA</sup> organoids. Scale bars: 20  $\mu$ m.

(G) Representatives of NeuN-, GFAP-, and MBP-expressing donor-derived hN<sup>+</sup> cells in the cerebral cortex at 6 months after transplantation of DS + Cont<sup>shRNA</sup> and DS + *OLIG2*<sup>shRNA</sup> ventral forebrain NPCs. Scale bars, 50  $\mu$ m.

(H) Quantification of percentage of NeuN<sup>+</sup>, S100 $\beta$ <sup>+</sup>, NG2<sup>+</sup>, and PDGFR $\alpha$ <sup>+</sup> in 8-week-old control (Cont), DS + Cont<sup>shRNA</sup>, and DS + *OLIG2*<sup>shRNA</sup> organoids. The data are pooled from control (Cont1 and Di-DS3) hiPSCs, DS (DS1 and Tri-DS3) hiPSCs, and the two lines of DS hiPSCs infected with lentiviruses that carry control shRNA or *OLIG2* shRNA. The experiments are repeated for three times ( $n = 3$ ) and for each experiment, 8 to 10 organoids from each line are used. Data are presented as mean  $\pm$  s.e.m.

(I) Quantification of the percentage of NeuN<sup>+</sup>, GFAP<sup>+</sup>, and MBP<sup>+</sup> expressing cells among total hN<sup>+</sup> cells in the cerebral cortex at 6 months after transplantation of control (Cont), DS + Cont<sup>shRNA</sup>, or DS + *OLIG2*<sup>shRNA</sup> ventral forebrain NPCs ( $n = 7$  mice for each group). The data are pooled from mice received transplantation of the NPCs derived from control (Cont1 and Di-DS3) hiPSCs, DS (DS1 and Tri-DS3) hiPSCs, and the two lines of DS hiPSCs infected with lentiviruses that carry control shRNA or *OLIG2* shRNA. One-way ANOVA test,  $*P < 0.05$ ,  $**P < 0.01$ , and *NS* represents no significance. Data are presented as mean  $\pm$  s.e.m.

(J) Quantification of the percentage of hN<sup>+</sup> cells in total DAPI<sup>+</sup> cells in the cerebral cortex at 6 months post-transplantation in control (Cont), DS + Cont<sup>shRNA</sup>, or DS + *OLIG2*<sup>shRNA</sup> chimeric mouse groups ( $n = 7$ -8 mice for each group). The data are pooled from mice received transplantation of the NPCs derived from control (Cont1 and Di-DS3) hiPSCs, DS (DS1 and Tri-DS3) hiPSCs, and the two lines of DS hiPSCs infected with lentiviruses that carry control shRNA or *OLIG2* shRNA. One-way ANOVA test. *NS* represents no significance. Data are presented as mean  $\pm$  s.e.m.

**Table S1. Related to Figure 2. Expression of Olig1 and 2 and major physiological phenotypes in iTg-Nes transgenic mice with mis-expression of Olig2 and mouse genetic models of DS.**

Mouse models	Genotype and phenotypic expression of <i>Olig</i> genes	Changes in the number of GABAergic neurons	Synaptic plasticity	Behavioral phenotypes
<b>iTg-Nes</b>	Olig2 is overexpressed and mis-expressed in cortical neural stem cells (Liu et al., 2015)	Increased but observed abnormally massive neuronal cell death (Liu et al., 2015)	Not examined	Locomotion abnormalities (Liu et al., 2015)
<b>Ts65Dn</b>	Trisomic for <i>Olig1</i> and 2; Olig1 and 2 are overexpressed (Aziz et al., 2018; Chakrabarti et al., 2010)	Increased (Chakrabarti et al., 2010; Hernandez-Gonzalez et al., 2015; Perez-Cremades et al., 2010)	Deficient long-term potentiation (LTP) in CA1 and DG (Costa and Grybko, 2005; Fernandez et al., 2007; Garcia-Cerro et al., 2014; Kleschevnikov et al., 2012; Kleschevnikov et al., 2004; Lysenko et al., 2014; Siarey et al., 1997)	Locomotion abnormalities and Learning and memory deficits (Braudeau et al., 2011; Escorihuela et al., 1995; Escorihuela et al., 1998; Faizi et al., 2011; Hyde et al., 2001; Kleschevnikov et al., 2012; Lysenko et al., 2014; Olson et al., 2007; Rueda et al., 2010; Smith et al., 2014; Stasko and Costa, 2004)
<b>Ts1Cje</b>	Trisomic for <i>Olig1</i> and 2; Olig2 expression is normal (may be related to chromatin state) (Aziz et al., 2018)	Not examined, but observed Increase GAD65 <sup>+</sup> inhibitory input in the hippocampus (Belichenko et al., 2007).	Deficient LTP in CA1 and DG (Belichenko et al., 2007; Siarey et al., 2005)	Learning and memory deficits (less severe than Ts65Dn)(Belichenko et al., 2007; Fernandez and Garner, 2007; Sago et al., 1998; Sago et al., 2000)
<b>Tc1</b>	Trisomic for <i>Olig1</i> and 2; expression of Olig1 and 2 is not examined	Not examined	Deficient LTP in DG (Morice et al., 2008; O'Doherty et al., 2005)	Locomotion abnormalities and mild learning impairment(Galante et al., 2009; Morice et al., 2008; O'Doherty et al., 2005)
<b>TTS</b>	Trisomic for <i>Olig1</i> and 2; expression of Olig1 and 2 is not examined	Not examined	Deficient LTP in CA1 and DG (Belichenko et al., 2015; Yu et al., 2010a)	Locomotion abnormalities and Learning and memory deficits (Belichenko et al., 2015)
<b>Dp(16)1Yey</b>	Trisomic for <i>Olig1</i> and 2, but Olig2 expression is normal (may be related to chromatin state)(Olmos-Serrano et al., 2016)	Decreased (Olmos-Serrano et al., 2016)	Deficient LTP in CA1 (Yu et al., 2010b)	Locomotion abnormalities and Learning and memory deficits (Olmos-Serrano et al., 2016; Yu et al., 2010b)
<b>Dp(10)1Yey</b>	Disomic for <i>Olig1</i> and 2	Not examined	No change (Yu et al., 2010b)	No change(Yu et al., 2010b)
<b>Dp(17)1Yey</b>	Disomic for <i>Olig1</i> and 2	Not examined	Increased LTP in CA1(Yu et al., 2010b)	No Change(Yu et al., 2010b)
<b>Ts1Rhr</b>	Disomic for <i>Olig1</i> and 2	Not examined	Deficient LTP in fascia dentata (Belichenko et al., 2009; Olson et al., 2007)	Locomotion abnormalities and learning impairment (less severe than Ts65Dn)(Belichenko et al., 2009)
<b>Ts1Yah</b>	Disomic for <i>Olig1</i> and 2	Not examined	Increased LTP in CA1(Pereira et al., 2009)	Defects in working memory; Improvement in spatial memory(Pereira et al., 2009)

**Table S2. Related to Figure 2. Basic information of Down syndrome patient fibroblasts from Coriell Institute for Medical Research.**

Coriell's cat. no.	Sex	Age	Race	Cell type	Karyotype	Generated iPSC lines
GM04616	Female	3 Days	Caucasian	Fibroblast	47,XX,+21	DS1 iPSCs
AG08942	Male	21 Years	Caucasian	Fibroblast	47,XY,+21	DS2 iPSCs
AG06872	Female	1 Year	Caucasian	Fibroblast	47,XX,+21	Isogenic Di-DS and Tri-DS iPSCs

**Table S3. Related to Figure 3. Basic information of the human brain tissues obtained from the University of Maryland Brain and Tissue Bank (UMB).**

UMB no.	Disorder	Sex	Age (Days)	Cell type	Race	Post mortem interval (hours)
832	Trisomy 21	Male	339	Cerebral cortex	African American	23
1282	Trisomy 21	Female	186	Cerebral cortex	Caucasian	28
4783	Trisomy 21	Female	261	Cerebral cortex	Caucasian	37
M1931M	Control	Male	332	Cerebral cortex	Caucasian	10
4361	Control	Female	236	Cerebral cortex	African American	27
1443	Control	Female	332	Cerebral cortex	African American	10

**Table S4. Related to Figure 5. Taqman Primers.**

Gene	Gene expression assay catalog number
<i>OLIG2</i>	Hs00300164_s1
<i>OLIG1</i>	Hs00744293_s1
<i>DLX1</i>	Hs00698288_m1
<i>DLX2</i>	Hs00269993_m1
<i>DLX5</i>	Hs00193291_m1
<i>DLX6</i>	Hs00231999_m1
<i>LHX6</i>	Hs01030943_m1
<i>LHX8</i>	Hs00418293_m1
<i>SOX6</i>	Hs00264525_m1
<i>GAPDH</i>	Hs02758991_g1

**Table S5. Related to Figure 5. ChIP-PCR Primers.**

Human LHX promotor	Forward	Reverse
-162 to -345	5'- CCTCTGCTACTGCACACAAT-3'	5'- ATCTGCAAGGAAAAGGAGAGA-3'
-479 to -696	5'- GCTTGAAAGATGCAGGAACT-3'	5'- ATCCAGGAAGCTTGTAGAGC-3'
-860 to -1643	5'- CTTAGGCACTCAGAGTAGGT-3'	5'- AGCCCTCCGTGTCGGATGTC-3'
-1149 to -1321	5'- TTCTAGGCCAGAGAGGAT-3'	5'- GTCCAGAGTCTGTCTATGGG-3'
-1384 to -1521	5'- GACAAGGGCCTGTTTCTGCT-3'	5'- ATGAAGACTCCATCTACTG-3'
-1557 to -1701	5'- GCTGAGTTCGCAAATCTTCT-3'	5'- GCTGGTAAAACCATTCAGT-3'
-1756 to -1890	5'- TTAGCCACATCAGGGTGTAT-3'	5'- GGTATTTGAGGGAATAAATGGG-3'
Human DLX1 promotor	Forward	Reverse
-25 to -186	5'- CGCTCCAGAGGCAGAGGT -3'	5'- AGCCGCTGGTTGGTTCCTG -3'
-328 to -452	5'- GCACTGCTTTAATCAGACC -3'	5'- GAGTGAGTGAGTTCGCAGG -3'
-642 to -830	5'- AGGTGAGGAAGATCCTGGGT -3'	5'- AGATCTGAGCCCCTAG -3'
-1126 to -1251	5'- CATCAGTGTTATGCTAATGG -3'	5'- ACAAGATCATTTCCCCGTT-3'
-1467 to -1621	5'- TTGCTCCAACCACTCTGCCT -3'	5'- AACGACAGCACCGATGTAAT -3'
-1670 to -1921	5'- AGGGCACGCGCGAGAGACCT -3'	5'- AGCGGCTCCCGAGTTGCCTG -3'

**Table S6. Related to Figure 5. Primers for luciferase reporter assay.**

	Forward	Reverse
LHX8 (-1 to -2000)	5'-GTAGCTAGCCCTTAAAAAGGCATCGTATG -3'	5'-GTAAGATCTGCCCCTTCCGCTGAGCAGC-3'
DLX1 (-10 to -1250)	5'- GTAGCTAGCATCAGTGTTATGCTAATGG -3'	5'- TACAAGCTTGCCCTCCCTCGCGCGCTTTCC -3'