Generation of human striatal organoids and cortico-striatal assembloids from human pluripotent stem cells

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Cortico-striatal projections are critical components of forebrain circuitry that regulate motivated behaviors. To enable the study of the human cortico-striatal pathway and how its dysfunction leads to neuropsychiatric disease, we developed a method to convert human pluripotent stem cells into region-specific brain organoids that resemble the developing human striatum and include electrically active medium spiny neurons. We then assembled these organoids with cerebral cortical organoids in three-dimensional cultures to form cortico-striatal assembloids. Using viral tracing and functional assays in intact or sliced assembloids, we show that cortical neurons send axonal projections into striatal organoids and form synaptic connections. Medium spiny neurons mature electrophysiologically following assembly and display calcium activity after optogenetic stimulation of cortical neurons. Moreover, we derive cortico-striatal assembloids from patients with a neurodevelopmental disorder caused by a deletion on chromosome 22q13.3 and capture disease-associated defects in calcium activity, showing that this approach will allow investigation of the development and functional assembly of cortico-striatal connectivity using patient-derived cells.

Neural activity in cortico-striatal circuits of the forebrain coordinates motivated behaviors and movement. In this pathway, glutamatergic neurons in the cerebral cortex project to the striatum, where they connect primarily to GABAergic medium spiny neurons that subsequently connect to downstream circuits in the basal ganglia (Fig. 1a). Dysfunctions in neural circuits of the cortico-striatal pathway are thought to contribute to neurodevelopmental disorders, such as autism spectrum disorder (ASD), schizophrenia and obsessive–compulsive disorder. However, how these circuits are assembled during human development and how functional defects arise in disease in human patients is still poorly understood. Therefore, new in vitro approaches to image and functionally manipulate human cortico-striatal circuits will facilitate research on their roles in neuropsychiatric disorders.

Human pluripotent stem (hPS) cells, including human induced pluripotent stem (hiPS) cells, have the ability to differentiate into any of the germ layers and, with the advent of three-dimensional (3D) culture methods, to self-organize in brain organoids and generate diverse cell types in the central nervous system. These cultures can, in principle, be derived from any individual and can be employed to study cell specification, model cell–cell interactions and investigate disease. We previously developed an approach to model cell–cell interactions during human brain development by generating region-specific brain organoids, also known as spheroids, and combining them in vitro to generate 3D cellular structures called assembloids. Using this approach, we modeled human interneuron migration into the cerebral cortex and identified phenotypes associated with genetic neurodevelopmental disease. Although hPS cells have been differentiated into striatal neurons in two-dimensional (2D) cultures, to our knowledge, they have not been used to generate striatal 3D organoids or cortico-striatal assembloids.

Here, we first developed a protocol to generate human 3D brain organoids resembling the lateral ganglionic eminence (LGE), which gives rise to the striatum during development. We leveraged transcriptomic trajectories of the developing human striatum and identified a small molecule that modulates the retinoic acid receptor pathway and that, in combination with WNT and activin signaling, generates 3D human striatal spheroids (hStrSs). As illustrated by single-cell RNA-sequencing (scRNA-seq) analyses and morphological analysis of cells, this approach yielded a diverse group of striatal cells, including medium spiny neurons that developed dendritic spines without a need for in vivo transplantation. Next, we assembled hStrSs with organoids resembling the cerebral cortex to form cortico-striatal assembloids (Fig. 1b). Using morphological analyses, retrograde viral tracing, live calcium imaging and patch-clamp experiments, we showed that cortical neurons in assembloids send axonal projections into hStrS and functionally connect to medium spiny neurons. Lastly, we identified functional defects in striatal neurons in assembloids derived from a cohort of patients with the 22q13.3 deletion syndrome.

Results

Generation of 3D hStrSs. To identify differentiation conditions that favor an LGE fate, we used two-step homology-directed repair-based genome editing to derive a reporter hiPS cell line that expresses a fluorescent protein under the promoter of the LGE-related transcription factor GSX2 (ref. ) (GSX2-mCherry; Extended Data Fig. 1). Similar to an approach we previously used to derive 3D spheroids resembling the medial ganglionic eminence (MGE), we applied several small molecules at early stages of 3D neural differentiation and used the GSX2 reporter cell line in combination with immunostaining for the early striatal marker CTIP2.
to verify the presence of LGE cells. We generated uniform 3D spheroids by aggregating ~10,000 dissociated hiPS cells in AggreWell 800 plates. To induce neural differentiation, we applied modulators of the SMAD and WNT pathways, as well as activin A, which has been shown to promote striatal differentiation27 (Extended Data Fig. 2a). To identify other pathways that we could modulate for LGE patterning, we inspected the BrainSpan transcriptome dataset of the developing human striatum27. The gene encoding retinoid X receptor gamma (RXRG) is one of most highly differentially expressed genes in the early developing human striatum (PCW 20–25). STr, striatum; NCX, neocortex; HIP, hippocampus; DTH, dorsal thalamus; CB, cerebellum; AMY, amygdala.

Fig. 1 | Generation of 3D hStrSs. a, Schematic diagram describing cortico-striatal projections in the developing human forebrain at mid-gestation. b, In vitro modeling of cortico-striatal projections using assembloids derived from hiPS cells. c, Differentiation conditions for hCSs and hStrSs. DM, dorsomorphin; SB, SB-431542; FGF2, fibroblast growth factor 2; EGF, epidermal growth factor. d, UMAP visualization of single-cell RNA expression in hStrSs at days 80–83 of in vitro differentiation (n = 25,772 cells from three hiPS cell lines). e, Dot plot shows percentage of selected markers for each cell cluster. f, VoxHunt spatial brain mapping of the GABAergic neuron cluster in hStrSs onto data from e13.5 mouse brains from the Allen Brain Institute. g, Correlation with the BrainSpan dataset of the developing human brain (PCW 20–25). STR, striatum; NCX, neocortex; HIP, hippocampus; DTH, dorsal thalamus; CB, cerebellum; AMY, amygdala.
major group of GABAergic neurons (STMN2, SYT1) expressing the GABA-synthesizing enzyme genes GAD1 and GAD2 (56.99%), a cluster of dividing progenitors expressing TOP2A (8.67%) and one cluster of ventral forebrain progenitors expressing GSX2, ASCL1 and HOPX (13.82%), as well astrocytes expressing AQP4 and GFAP (11.58%). We also observed a small group of glutamatergic neurons expressing the glutamate transporters encoded by SLC17A7 and SLC17A6 (6.79%) and a cluster of oligodendrocytes expressing SOX10 and OLIG2 (2.12%) (Fig. 1a–e and Supplementary Tables 6 and 7). Overall, the forebrain marker FOXG1 and the ventral forebrain markers DLX1, DLX2, DLX5 and DLX6 were broadly expressed, while the MGE marker NKX2-1 was only present in 0.002% of cells (Fig. 1a and Extended Data Fig. 3c), which suggests that hSTRs mostly include LGE-related cells. Cell proportions were similar across the three analyzed hiPS cell lines (with GABAergic neurons ranging from 50.12% to 66.25%), and gene expression correlations between pairs of lines were high (2242-1 versus 1205-4, r = 0.97, ***P < 0.001; 1205-4 versus 8858-3, r = 0.98, ***P < 0.001; 2242-1 versus 8858-3, r = 0.98, ***P < 0.001) (Extended Data Fig. 3f–h). To verify the regional identity of cells in hSTRs in a less biased fashion, we also mapped the scRNA-seq data onto 3D in situ hybridization data from the Allen Brain Atlas using the VoxHunt algorithm. We found that the GABAergic neuron cluster in hSTRs mapped onto the ventral forebrain of the embryonic day (E) 13.5 mouse brain (Fig. 1f). In addition, we mapped data from hSTRs onto the BrainSpan human transcriptomic dataset. We found that the GABAergic neuronal cluster in hSTRs showed the highest scaled correlation with ganglionic eminences (GE) when compared to primary brain samples at early stages (postconceptional weeks (PCW) 5–10) and with the striatum when compared to samples at PCW 10–25 (Fig. 1a and Extended Data Fig. 4a). We also found a correlation with the developing amygdala, which is consistent with previous reports of an LGE origin for some neurons in the amygdala. In contrast, the glutamatergic neuron cluster in hSTRs was highly correlated with the neocortex and the amygdala in BrainSpan, and these cells appeared to be either dorsal forebrain- (EMX1) or amygdala-related (expressing the early developing amygdala marker TFAP2D) (Extended Data Figs. 3a–c and 4b,c). We next further analyzed the GABAergic cluster in hSTRs and found neurons expressing SST, CALB1, CALB2, TH, NO15 and NPY and only very few PVALB- and CHAT-expressing cells (Extended Data Fig. 4d,e). Immunostaining in hSTRs confirmed the expression of calbindin (CALB1) and CALB2 (Extended Data Fig. 4f). We also compared hSTRs to our previously derived human subpallium spheroids (hSS)19 (Extended Data Fig. 5a–e). UMAP visualization showed that hSSs and hSTRs shared several clusters, including cluster 2 (enriched for cell proliferation markers, including TOP2A), cluster 4 (enriched for progenitors, including HOPX+ cells) and clusters 6, 8 and 16 (enriched for glial lineage-related markers and expressing S100B and MBP). Clusters 10 and 13 were mostly hSS specific and expressed LHX6 and SST, which are suggestive of an MGE fate30,31. These results suggest that hSSs (MGE-like) and hSTRs (LGE-like) display some cluster similarities, but overall, they resemble different domains of the ventral forebrain. Lastly, immunostaining of hSTRs at days 80–85 confirmed the presence of microtubule associated protein 2 (MAP2+) neurons, glial fibrillary acidic protein (GFAP+) astrocytes, myelin basic protein (MBP)+ oligodendrocytes (Extended Data Fig. 5f) and achaete-scute family bHLH transcription factor 1 (ASCL1)+ progenitors (Extended Data Fig. 6b) and showed cells coexpressing forkhead box P2 (FOXP2) and glutamate decarboxylase (GAD)2 (GAD65) (Extended Data Fig. 6c), GAD67 and Coup-Tf-interacting protein 2 (Ctip2) and dopamine- and cAMP-regulated phosphoprotein 32 (DARP32) and Ctip2 (Fig. 2a and Extended Data Fig. 6d), which are indicative of striatal medium spiny neurons. Overall, we found that ~50% of cells expressed Ctip2 and 8.5% expressed DARP32 in plated hSTR cultures at day 65 (Extended Data Fig. 6e–i). Up to 30% of neuronal nuclei (NeuN)+ cells expressed DARPP32 at days 80–90 in hSTRs (Fig. 2b,c; the patterns of expression of Ctip2 and DARPP32 in the E18.5 mouse striatum are shown in Extended Data Fig. 6a). We also found expression of both DRD1 and DRD2 in hSTRs, as well as expression of the striosome marker TAC1 and the matrix marker PANK (Extended Data Fig. 6j). In addition, immunocytochemistry confirmed the expression of the D2 receptors in hSTRs at day 124 (Extended Data Fig. 6k).

Medium spiny neurons in the striatum form abundant dendritic spines during development32. To inspect the morphology of hSTRs, we labeled GABAergic cells with an adeno-associated virus (AAV) driving enhanced (e)GFP expression from a DLX5 and DLX6 enhancer (AAV-mDLX:eGFP33). In 2D-plated hSTRs, we found dendritic spines with thick spine heads as early as day 65 of differentiation (Fig. 2d and Extended Data Fig. 6e,f). In intact 3D hSTRs, the number of spines increased significantly from days 80–90 to days 120–130 (***P < 0.0001; Fig. 2g–m and Extended Data Fig. 6l,m). The number of dendritic spines did not appear to be related to differences in the proportion of glutamatergic neurons in hSTRs derived from two hiPS cell lines (Extended Data Fig. 6n).

We next examined neural activity in hSTRs, using live imaging of the genetically encoded calcium indicator GCaMP6b. To restrict expression to GABAergic medium spiny neurons in hSTRs, we used an AAV expressing improved Cre (iCre) under a MiniPromoter for the striatal gene GPR88 (AAV-Ple94-iCre) (Extended Data Fig. 6o). The majority of hSTR cells labeled by AAV-Ple94-iCre and AAV-EF1a:DIO-eYFP were GABAergic cells (85% GAD65+, 23% GAD67+) (Extended Data Fig. 6p–q). Co-infection with AAV-Ple94-iCre and AAV-EF1a:DIO-GCaMP6b, followed by live imaging of intact hSTRs, showed spontaneous calcium events that were often synchronized in an imaging field (n = 39 cells; Fig. 2h and Supplementary Video 1). The spontaneous and synchronized calcium signals were blocked by application of the glutamate receptor antagonists NBQX and APV but not the GABA_A receptor antagonist bicuculline (Extended Data Fig. 7a–c). This suggests that spontaneous activity may be related to intrinsic glutamatergic transmission in hSTRs. We also verified the gene expression of SLC12A2 (also known as NKCC1) and SLC12A5 (also known as KCC2), which encode cation–chloride cotransporters that regulate the developmental transition in GABAergic transmission44. scRNA-seq analysis at days 80–83 showed that the majority of GABAergic neurons express KCC2, although NKCC1 was also expressed in some cells (Extended Data Fig. 7f). RT–qPCR experiments also showed that NKCC1 expression decreased slightly from day 15 to day 170 (Extended Data Fig. 7g). In contrast, levels of KCC2 expression were relatively low at early stages and increased by day 93 (Extended Data Fig. 7h).

To further examine whether hSTRs neurons display characteristic electrophysiological features of striatal medium spiny neurons, we analyzed the intrinsic membrane properties of hSTRs neurons. We found that 70% of hSTR neurons (2 of 13 cells) displayed slow-ramp depolarization (Fig. 2k) and hyperpolarization of the motivation with delayed first spike (Fig. 2l). At later stages of differentiation (days 160–170), but not at early stages (days 110–120), we found that 15% of hSTR neurons (2 of 13 cells) displayed slow-ramp depolarization with delayed first spike (Fig. 2k) and hyperpolarization of the resting membrane potential (RMP) (−78.2 ± 2.4 mV; Fig. 2l). These properties are reminiscent of observations in postnatal medium spiny neurons in rodents47.

Generation of cortico-striatal assembloids. To assemble hSTRs and hSSs, we first labeled hSSs with AAV-hSyn1::eYFP and, separately, labeled hSTRs with AAV-hSyn1::mCherry. We then placed hSSs and hSTRs in contact with each other in conical tubes, as we previously described for generating forebrain assembloids. After 72 h, the two region-specific spheroids were fused to form a
Fig. 2 | Characterization of hStrSs. a, Immunostaining for DARPP32 (yellow), GAD67 (magenta) and CTIP2 (cyan) in hStrSs at day 80. n = 5 hiPS cell lines. Scale bar, 100 μm. b, c, Immunostaining for DARPP32 (green), CTIP2 (magenta) and NeuN (blue) and quantification of DARPP32+ cells in hStrSs at day 85. n = 8 neural differentiation experiments of three hiPS cell lines. d, Representative image showing dissociated hStrS cells labeled with the AAV-mDlx:eGFP reporter and eGFP (green), CTIP2 (magenta) and Hoechst (blue). Scale bar, 50 μm (left) and 5 μm (right). Immunostainings were repeated in dissociated hStrS cultures from four independent differentiation experiments with similar results. e–g, Dendritic spine morphology in the hStrS neurons labeled with AAV-mDlx:eGFP and quantification of the number of dendritic spines (days 80–90, n = 40 neurons from three neural differentiation experiments of two hiPS cell lines; days 120–130, n = 38 neurons from four differentiation experiments of three iP5 cell lines; two-tailed Mann–Whitney test, ****P < 0.0001). h, Calcium imaging of hStrS neurons expressing the genetically encoded calcium indicator GCaMP6s signal from 39 cells. Imaging was repeated in dissociated hStrS neurons at day 160. The black arrow indicates slow-ramp depolarization; the red arrow indicates delayed first spike. i, RMP (n = 13 cells from hStrSs at days 160–170). Data show mean ± s.e.m. Box plots show maximum, third quartile, median, first quartile and minimum values.
of the rabies virus\textsuperscript{49}. Separately, we infected hCSs with AAV-DIO-mCherry, which drives mCherry expression following Cre recombination. Two days after viral delivery, we assembled hCSs and hStrSs and cultured them as assemblies for another 28 d. We observed extensive expression of GFP in hStrSs and cells coexpressing GFP and mCherry on the cortical side of the assembled hCS-mCherry expression following Cre recombination. Two days after viral delivery, we assembled hCSs and hStrSs and cultured them as assemblies for another 28 d. We observed extensive expression of GFP in hStrSs and cells coexpressing GFP and mCherry on the cortical side of the assembled hCSs (Extended Data Fig. 8c). We found that the majority of GFP\textsuperscript{+}mCherry\textsuperscript{+} cells in hCSs coexpressed the neuronal marker MAP2 (85.0%), and only 7.92% expressed the glial lineage marker GFAP (Fig. 3i). A major population of neurons projecting into the striatum is composed of cortical intratelencephalic projection neurons expressing the transcription factor SATB2. Deep-layer CTIP2\textsuperscript{+} cortical neurons of the pyramidal tract also send important collaterals into the striatum\textsuperscript{40,41}. We found a 3.4\% enrichment in the proportion of SATB2\textsuperscript{+} cells in the GFP\textsuperscript{+}mCherry\textsuperscript{+} population compared to all other cells in the same section of hCSs (\(P = 0.01\)), and, overall, \(\sim 41\%\) of the retrogradely labeled hCS neurons were SATB2\textsuperscript{+} (Fig. 3j). CTIP2\textsuperscript{+} cells accounted for \(\sim 34\%\) of GFP\textsuperscript{+}mCherry\textsuperscript{+} cells. We note that some cells in hCSs expressed GFP in the absence of mCherry, and this may be due to differences in viral transduction in hCSs (AAV-based) and hStrSs (rabies-based). Although there are still technical challenges with tracing connectivity\textsuperscript{42}, including ascertaining trans-synaptic tracing with rabies virus, these data suggest that projection neurons in assemblies are biased toward certain neuronal populations.

**Functional neural circuits in cortico-striatal assemblies.** To determine whether neurons projecting from hCSs can form functional synaptic connections with hStrS neurons, we implemented optogenetics with simultaneous calcium imaging in cortico-striatal assemblies. To detect optically evoked calcium responses, we first virally expressed hSyn\textsubscript{1}:ChrimsonR–tdTomato in hCSs, an opsin that excites neurons in response to red-shifted light\textsuperscript{41}, and separately in hStrSs, we delivered Ple94-iCre and DIO-GaMP6s (Fig. 4a). After 2 d of viral infection, we assembled hCSs and hStrSs and imaged them around day 90 of differentiation (Fig. 4b). We found that the application of 625-nm light reliably elicited calcium responses in hStrS cells (Fig. 4c,d). The median \(\Delta F/F\) of GaMP6s signals was significantly higher following light
Fig. 4 | Functional neural circuits in cortico-strial assembloids. a, Optogenetics coupled with calcium imaging of the cortico-strial assembloid. hCSs expressing AAV-ChrimsonR-tdTomato and hStrSs expressing AAV-Pie94-iCre and AAV-EPF1α::DIO-GCaMP6s were assembled. GCaMP6-expressing neurons in hStrSs were imaged by stimulating with 625-nm LED light (68 ms) at days 90–145 of differentiation. b, Axon projection of ChrimsonR-tdTomato-expressing neurons from hCSs to hStrSs of cortico-strial assembloids at day 90. Scale bar, 100 μm. Imaging was repeated in assembloids from tdTomato-expressing neurons from hCSs to hStrSs of cortico-strial assembloids at day 90. Scale bar, 100 μm. Imaging was repeated in assembloids from five independent differentiation experiments with similar results. Representative traces of GCaMP6s imaging (d) and median amplitudes of ΔF/F per cell before and during NBQX (20 μM) and APV (50 μM) treatment (e). n = 36 cells for before treatment with NBQX and APV and n = 17 cells for during treatment with NBQX and APV; two-tailed Mann-Whitney test, ****P < 0.0001. f, Schematic diagram illustrating the method for whole-cell patch-clamp recording with optogenetic activation in cortico-strial assembloids. g, Representative traces of oEPSCs, oEPSPs and neuronal firing of hStrS neurons stimulated by LED light (5-ms duration of 550-nm whole-field LED illumination). Percentage of responsive cells (11 of 35 cells) (h), peak oEPSC amplitudes (i) and onset delays (n = 6 cells) (j) of hStrS neurons in cortico-strial assembloids. k, Schematic showing whole-cell patch-clamp recording in hStrSs or cortico-strial assembloids in slices. l, Representative electrophysiological traces of neurons in hStrSs and hCS–hStrS. m, Frequency–current (F–I) curves showing spike frequency versus current injected in hStrSs and hCS–hStrS neurons (n = 17 cells in hStrSs, n = 25 cells in hCS–hStrS from three hiPS cell lines; two-way ANOVA, F{sub}_1,183 = 131.6, ***P < 0.0001 for current injection). n, Maximum spike frequency (n = 17 cells in hStrSs, n = 25 cells in hCS–hStrS from three hiPS cell lines; two-tailed unpaired t-test, **P = 0.005). Data show mean ± s.e.m.

stimulation as compared to the median ΔF/F at randomly selected time points (n = 180 cells, ***P = 0.0002, Extended Data Fig. 8d,e). Responding cells were also able to respond to different frequencies of light in sequential stimulation experiments (Extended Data Fig. 8f). We also observed, however, non-responding cells or cells that fired spontaneously during optogenetic stimulation (Fig. 4d and Extended Data Fig. 8e). Lastly, light-induced calcium responses were blocked by application of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonist NBQX (20 μM) and the NMDA (N-methyl-D-aspartate) receptor antagonist APV (50 μM) (****P < 0.0001; Fig. 4d,e), and this effect was reversible (Extended Data Fig. 8g), suggesting that some of these calcium signals were mediated by glutamatergic transmission from hCSs to hStrSs. In addition, light-emitting diode (LED) exposure, in
the absence of an opsin in hCSs, did not induce calcium responses in cortico-striatal assembloids (Extended Data Fig. 8h,i). We also quantified the median amplitude of ΔF/F GCaMP6 signals over time in hStrSs and found an increase in the amplitude of optically evoked GCaMP6 signals at days 100–120 and at days 140–150 as compared to hStrSs before day 100 (Extended Data Fig. 8). This result suggests that there may be a time-dependent increase in the strength or frequency of synaptic connections in cortico-striatal assembloids.

To further characterize connectivity in cortico-striatal assembloids, we combined optogenetic stimulation with electrophysiological whole-cell recording in slices (Fig. 4f). We found that delivery of 550-nm light stimulation could induce optically evoked excitatory postsynaptic currents (oEPSCs) and optically evoked excitatory postsynaptic potentials (oEPSPs), as well as firing of neurons in hStrSs (Fig. 4g). The success rate was moderately high, with 11 of 35 recorded cells (Fig. 4h) responding with approximately 40 pA of average oEPSC amplitude (Fig. 4i) and 6ms of onset delay (Fig. 4j). This indicates that neurons in hStrSs can synaptically connect with hCS projecting neurons in cortico-striatal assembloids.

Glutamatergic projections from the cerebral cortex into the striatum are thought to play a key role in the developmental maturation of medium spiny neurons (Fig. 5a). To examine whether the assembly of hCSs and hStrSs impacts the electrophysiological properties of neurons in hStrSs, we performed whole-cell patch-clamp recordings in sliced cortico-striatal assembloids (Fig. 4k). We found increased intrinsic excitability of hSyn1::eYFP-labeled neurons on the hStrS side of hCS–hStrS, as compared to neurons in hStrSs at the same in vitro stage (P < 0.0001, **P = 0.005; Fig. 4l–n). We also analyzed individual action potentials and found shorter spike half-widths in hCS–hStrS neurons without a change in the amplitude or spike threshold (Extended Data Fig. 9a–h), which is consistent with findings in the developing murine striatum (Fig. 5a). Neither capacitance nor input resistance was changed (P = 0.68 for Extended Data Fig. 9i, P = 0.82 for Extended Data Fig. 9j; Extended Data Fig. 9i,j), suggesting that the physiological change was not due to differences in the cell size or surface area of patched cells. Moreover, the RMPs were also not changed before or after assembly (P = 0.87; Extended Data Fig. 9k). We also observed a higher frequency of spontaneous EPSCs (sEPSCs) in hStrS neurons following assembly (***P = 0.0006; Extended Data Fig. 9l,m), while the number of dendritic spines in hStrS neurons was not changed following assembly (Extended Data Fig. 9n), suggesting that assembly with hCSs may not affect the morphology of hStrS neurons. In sum, these results indicate that functional connectivity can be assessed in cortico-striatal assembloids derived from hiPS cells in vitro and that this assembly results in changes of intrinsic firing properties in hStrSs.

**Modeling disease-related cellular phenotypes in cortico-striatal assembloids.** Lastly, we studied whether cortico-striatal assembloids could be used to model cellular defects associated with genetic neurodevelopmental disease. Phelan–McDermid syndrome, also known as 22q13.3DS, is a severe disorder characterized by global developmental delay, severe intellectual impairment, delayed speech and ASD. The syndrome is caused by a deletion on chromosome 22q13.3 that includes the SHANK3 gene, which encodes a key postsynaptic scaffold protein (Fig. 5a). SHANK3 is highly expressed in the striatum and considered to be essential for cortico-striatal circuit development and function, and its loss is considered to be a key player in the pathogenesis of 22q13.3DS (37–48). Defects in cortico-striatal connectivity were previously suggested in Shank3-knockout mice (39). However, whether cellular abnormalities are present in heterozygous human cells in cortico-striatal circuits from patients is not known. To investigate whether we could model cortico-striatal defects in 22q13.3DS, we first differentiated hiPS cell lines from three patients with 22q13.3DS into hCSs and, separately, into hStrSs (Fig. 5a–c). In these cases, two-tailed unpaired t-test, *P = 0.001; **P = 0.004 (Fig. 5a). It is possible that patient-derived hiPS cells could efficiently aggregate and differentiate in 3D cultures to form hStrSs (Fig. 5b and Extended Data Fig. 10a–c). We found that patient-derived hiPS cells could efficiently aggregate and differentiate in 3D cultures to form hStrSs (Fig. 5a–c) and, as expected, expressed the forebrain marker FOXG1 and the LGE markers GSX2, MEIS2 and BCL11B (CTIP2), but not the spinal cord marker HOXB4 (Extended Data Fig. 10e). The area of hCSs and hStrSs derived from patients with 22q13.3DS was different from that of controls (Fig. 5c), and all 22q13.3DS lines could efficiently generate DARPP32–NeuN+ neurons (Fig. 5d and Extended Data Fig. 10f). We next generated cortico-striatal assembloids from three patients with 22q13.3DS and three control individuals and performed calcium imaging (Fig. 5e–g). We found that 22q13.3DS-derived neurons labeled with AAV-mDiX::GCaMP6 in the hStrS side of cortico-striatal assembloids displayed increased number of calcium spike events (*P = 0.01; Fig. 5e–g). Notably, when tested at the same stage of in vitro differentiation, this defect was not present in hStrSs derived from patients with 22q13.3DS (P = 0.54; Fig. 5b–f). To follow up on these changes in global calcium events in 22q13.3DS-derived hStrSs and cortico-striatal assembloids, we used GCaMP6 signals to calculate mean correlation coefficients between cells. We found reduced network synchronization in both hStrSs and cortico-striatal...
assembloids derived from patients with 22q13.3DS (**p < 0.0001 in Fig. 5l, *p < 0.01 in Fig. 5m; Fig. 5k–m). In sum, these results indicate that cortico-striatal assembloids can be used to recapitulate the altered neural activity of human cells in disease.

**Discussion**

In this study, we generated human 3D striatal cultures that include morphologically and functionally mature striatal neurons that can be assembled with cortical glutamatergic projection neurons to model human cortico-striatal circuits in vitro. Classical ex vivo explant experiments\(^4\) and, more recently, in vivo functional studies in behaving animals\(^5\) serve as critical experimental models to functionally interrogate cortico-striatal pathways and to model disease in knockout animals. To date, however, there are no cellular models that allow these cell–cell interactions to be validated in a functional human preparation while also taking into account the complex genetic architecture of basal ganglia disorders. Previous methods of deriving striatal neurons from hPSC cells have yielded relatively immature cells, which has limited their application to disease modeling\(^6,7\). Although the proportion of neurons in hStrSs is not as high as that in conventional striatal 2D cultures\(^8,9\), we found that neurons in hStrSs form abundant dendritic spines and recapitulate intrinsic electrophysiological properties described in rodent medium spiny neurons. Moreover, assembly of hStrSs with cortical neurons accelerates the intrinsic functional maturation of striatal neurons, which could be due to glutamate release following assembly, mediated through NMDA receptor activation and subsequent modulation of potassium channel function\(^1\). Future studies could investigate whether assembly with midbrain organoids producing dopamine in three-part assembloids could even further advance their electrophysiological properties\(^5\).

The main advantage of our approach is its modularity. Cortical neurons, but not neurons in striatal 3D cultures, project into the counterpart spheroid, which is reminiscent of the directionality of this pathway in vivo. To probe the functionality of this 3D human cellular model, we implemented rabies virus tracing and optogenetic stimulation coupled with live imaging of genetically encoded calcium indicators delivered to specific cell types. Notably, we found that projecting cortical neurons were biased to express a marker for intratelencephalic projecting neurons, although a considerable fraction of these expressed a marker of corticofugal neurons, which send collaterals toward the basal ganglia\(^5\). Moreover, optogenetic stimulation of cortical neurons can reliably trigger calcium activity or even action potentials in medium spiny neurons in cortico-striatal assembloids, which are mediated by glutamate transmission. Although more studies using orthogonal mapping methods are required, these experiments suggest that connectivity with some level of specificity is possible in vitro. Future experiments should also explore whether selective cortical connectivity through dopamine receptor D1 or D2 pathways can be intrinsically achieved in vitro or if activity through the cortico-striatal-thalamic loop is required for this selectivity.

Modeling cytoarchitectural features of specific brain regions in vitro is a central goal of brain organoid research. Although we did not directly observe striosomes or other matrix structures that are present in the striatum\(^4\), it remains to be seen whether these features can organize at later stages of maturation in vitro, following midbrain assembly or, perhaps, as a consequence of broader circuit input after in vivo transplantation in rodents. Alternatively, it could be that embedding organizer-like structures, such as organoids secreting sonic hedgehog\(^5\), may induce spatial organization in hStrSs.

Our system has a number of potential applications for the study of human striatal development and the pathophysiology of diseases of cortico-striatal circuits. Here we used this system to capture cellular defects associated with 22q13.3DS, in which the postsynaptic, striatum-enriched SHANK3 gene is hemizygenously deleted. Previous work in homozygous knockout rodent models suggested potential functional defects in the cortico-striatal pathway. Indeed, we found that while hStrSs displayed similar spontaneous calcium activity in the 22q13.3DS background as compared to controls, following assembly with hCSs, medium spiny neurons become hyperactive in patient-derived cultures. This increase in intrinsic neuronal activity is in line with previously reported defects in mouse and human cortical neurons\(^10,11\), although a functional cellular defect in human striatal cells in the 22q13.3DS background has not been reported. Future studies should use more patients to test these defects in the context of various 22q13.3 chromosomal deletions and identify the role of SHANK3, establish whether this phenotype is cell-intrinsic (using hybrid assembloids as we previously showed for forebrain assembloids\(^5\)) and discover the specific contribution of cortical glutamatergic neurons to dysfunction in assembloids derived from patients with disease. We found network synchronization changes in 22q13.3DS cells in assembloids as well as in hStrSs. Future studies should investigate how this phenotype is related to cell composition in hStrSs. Notably, Shank3-\(^/-\) mice display a reduction in the density of dendritic spines in the striatum as well as changes in glutamate receptor expression, which are related to an increase in dendritic length\(^1\). Therefore, it is possible that changes in cell morphology and synaptic function in patient-derived medium spiny neurons could also contribute to network alterations in 22q13.3DS cells.

In addition to uncovering the molecular mechanism underlying the assembly-dependent cellular defect following the loss of SHANK3, our system should be useful for modeling other neuropsychiatric disorders of the cortico-striatal circuit, including Tourette syndrome, ASD and obsessive–compulsive disorder\(^1\). Moreover, it may allow the investigation of trans-neuronal cortico-striatal spreading of huntingtin in Huntington’s disease\(^5\). Ultimately, circuits built in assembloids generated by combining various region-specific brain organoids may advance our understanding of neuronal connectivity in disease and accelerate the search for novel therapeutics for neuropsychiatric disorders.

**Online content**

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41587-020-00763-w.

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Methods
Characterization and maintenance of hiPSCs. hiPSC cell lines used in this study were validated using standardized methods as previously described. Cultures were frequently tested for mycoplasma and maintained free of mycoplasma. A total of five hiPSC cell lines were derived from fibroblasts collected from five healthy individuals and three hiPSC cell lines derived from fibroblasts collected from three patients with 2q13.3D5 were used for experiments (Supplementary Table 3). Approval for this study was obtained from the Stanford IRB panel, and informed consent was obtained from all participants.

Generation of hiPSC mCherry hiPSC cells expressing CRISPR-Cas9 genome editing.

The GSX2-mCherry hiPSC cell reporter line was generated using a scarless genome editing method as previously described. Briefly, guide RNA (gRNA)–Cas9 expression vectors were constructed by ligation of BbsI-digested pCS1-U6-Chimeric_BB-CBh-hspCas9 (Addgene, plasmid 42230) with annealed oligonucleotides using T4 ligase (NEB). Editing design (position of homology arms, gRNA targets and primers for genotyping) and sequences of donor vectors are included in Extended Data Fig. 1 and Supplementary Tables 1 and 2. For gRNA design, no off-target candidates of any gRNAs used in this study were found by the CRISPR-CK tool (http://crisprcka.stanford.edu/default_search) and the generation of 1:1 (donor/DNA) doublets. Donor DNA molecules were constructed using NEBuilder HiFi DNA Assembly (NEB, E2621S). The left and right homology arms for donor plasmids were amplified by nested PCR using genomic DNA extracted from K562 cells (ATCC, ATCC CCL-243), and other fragments were obtained by PCR amplification from previously reported vectors. hiPSCs (2×10^5 cells) were electroporated using the P3 Primary Cell 4D Nucleofector X kit (V43002) and the 4D-Nucleofector system (Lonza, AAFP-10028) with 5ug of the pX330 plasmid and 5ug of donor plasmids, according to the manufacturer’s protocol (program, CA-137). Cells were then cultured for 3 d in a well of a Matrigel-coated 6-well plate in mTeSR1 medium (Stemcell Technologies, 85850) supplemented with 10% FBS (Thermo Fisher Scientific, 10010-049), and 1X non-essential amino acids (NEAA) (Thermo Fisher Scientific, 13300-104) in addition to BDNF, NT-3, AA, cAMP and DHA. From day 46, only neural medium containing B-27 Plus Supplement (Thermo Fisher Scientific, A19985-01) was used for medium changes every 4 d. hiPSCs were generated as previously described. From day 22, the neural medium was supplemented with BDNF, NT-3, AA, cAMP and DHA. From day 46, only neural medium containing B-27 Plus Supplement was used for medium changes every 4 d.

Dissociation of neural spheroids for 2D culture. Three to four randomly selected hiStrSs were collected in a 1.5-ml Eppendorf tube in a solution containing 10 μM l-papain (Workington Biochemical, L500319), 1 mg/ml EDTA (Thermo Fisher, 40100-038), 100 μM dNTPs, 50 μg/ml glycogen (Sigma-Aldrich, G8890), 1 μg/ml RNase A (Roche, 05415), 1X gibson buffer (1% human albumin, 0.5 μM Y27632 (Tocris, 1254) and then dissociated overnight at 4°C with primary antibodies diluted in PBS containing 2% NDS and 0.1% Triton X-100. hiPSCs were used to wash away excess primary antibodies, and the cryosections were incubated with secondary antibodies in PBS containing 2% NDS and 0.1% Triton X-100 for 1 h at room temperature. The sections were then incubated overnight at 4°C with primary antibodies diluted in PBS containing 2% NDS and 0.1% Triton X-100. hiPSCs were used to wash away excess primary antibodies, and the cryosections were incubated with secondary antibodies in PBS containing 2% NDS and 0.1% Triton X-100 for 1 h at room temperature.

Dissociated cultures on glass coverslips were fixed in 4% PFA in PBS for 20 min at 37°C and then rinsed twice for 5 min with PBS. hiPSC cell cultures on glass coverslips were fixed in 4% PFA in PBS for 20 min at room temperature and rinsed twice with PBS for 5 min. Coverslips were blocked in 10% NDS, 0.3% Triton X-100, 1% BSA in PBS for 1 h at room temperature and then incubated overnight at 4°C with primary antibodies diluted in PBS containing 2% NDS and 0.1% Triton X-100. hiPSCs were used to wash away excess primary antibodies, and the coverslips were incubated with secondary antibodies in PBS containing 2% NDS and 0.1% Triton X-100.

The following primary antibodies were used for staining: anti-calbindin (Synaptic Systems, 214011, 1:200), anti-calretinin (Swant, CR7697, 1:500), anti-GAD67 (Millipore, MAB5406, 1:200), anti-ASCL1 (BD, 556604, 1:200), anti-MAP2 (guinea pig, Synaptic Systems, 188004, 1:200), anti-calretinin (rabbit, Swant, CR7697, 1:200, 214011/5), anti-PSD95 (mouse, Chemicon, AB51502, 1:50 dilution, GR178263-4), anti-OCT4 (C30A3) (rabbit, Cell Signaling Technology, 5276S, 1:50 dilution, 2159373), anti-DARP32 (rabbit, Cell Signaling, 2301S, 1:200, 2159373), anti-DARP32 (rabbit, abcam10801, 1:200, GR232312-3), anti-NeuN (mouse, abcam104224, 1:200, GR3431933-1), anti-GAD67 (mouse, MilliporeSigma, MAB4056, 1:200, 2676521), anti-GFAP (mouse, GeneTex, GTX13970, 1:200, 821950332, 821704840, 821805508), anti-DXR2 (rabbit, MBL, MC-1405, 1:200, 107522), anti-cFos (rat, Millipore, MAB3340, 1:200, 180300), anti-Graded95 (guinea pig, Invitrogen, MA1-045, 1:200, SC294697), anti-SATB2 (mouse, abcam15102, 1:50 dilution, GR178264-4), anti-GTC4 (C30A3) (rabbit, Cell Signaling Technology, 2840, 1:200, 2159373), anti-SSEA4 (MC813) (mouse, Cell Signaling Technology, 4755, 1:200, 2159373). Alexa Fluor dye donkeys, anti-rabbit IgG (H+L) highly cross-adsorbed secondary antibody, Alexa Fluor 488 (Thermo Fisher Scientific, A-11008), anti-mouse IgG (H+L) highly cross-adsorbed secondary antibody, Alexa Fluor 568 (Thermo Fisher Scientific, A-11007), anti-goat IgG (H+L) highly cross-adsorbed secondary antibody, Alexa Fluor 647 (Thermo Fisher Scientific, A-11012).
AffiniPure donkey anti-rat IgG (H&L) antibody (Jackson Immunoresearch, 712-605-153), Alexa Fluor 647 AffiniPure donkey anti-rabbit IgG (H&L) antibody (Jackson Immunoresearch, 712-605-152), Alexa Fluor 488 AffiniPure donkey anti-rat IgG (H&L) antibody (Jackson Immunoresearch, 712-605-153) and Alexa Fluor 488 AffiniPure donkey anti-guinea pig IgG (H&L) (Jackson Immunoresearch, 706-605-148) were used at a 1:10,000 dilution, and nuclei were visualized with Hoechst 33258 (Thermo Fisher Scientific, H3569, 1:10,000 dilution, 1829927). Cyrosections and cover slips were mounted for microscopy on glass slides using Aquamount (Polysciences, 18606) and imaged on a Keyence BZ-X710, a Zeiss M1 Axioscope or a Leica TCS SP8 confocal microscope. Images were processed with Fiji (Image, version 2.1.0), National Institute of Health (NIH).

Real-time qPCR. mRNA from hCs and hStrs was isolated at days 15 and 22 was isolated using the RNeasy Mini kit (Qiagen, 74106) with DNase I, Amplification Grade (Thermo Fisher Scientific, EURS001575). PCR was performed using the SuperScript III First-Strand Synthesis SuperMix for qRT–PCR (Thermo Fisher Scientific, 17252250). qPCR was performed using the SYBR Green PCR Master Mix (Thermo Fisher Scientific, 4312704) on a ViIA 7 Real-Time PCR System (Thermo Fisher Scientific, 4453545). Primers used in this study are listed in Supplementary Table 4.

Single-cell RNA-sequencing library preparation and data analysis. To obtain a single-cell suspension, three to four hStrs were randomly selected from each hiPS cell line at days 80 or 83 and collected in a 1.5-ml Eppendorf tube containing 10U of RNaseOUT (Thermo Fisher, 10008002) and 500U of RNasin (Promega, Roche, 1031459001). The samples were then incubated in a 37 °C incubator for 15 min (with gentle shaking every 5 min). Papan was inactivated with 10% FBS in neurobasal medium, and hStrs were gently triturated with a P1000 pipette. Samples were centrifuged at 1,300 rpm for 7 min, filtered with a 70-µm Flowmi Cell Strainer (Bel-Art, H13680-0070) and suspended in 0.1% BSA in PBS (PipemideSigma, B6917-25M) at a concentration of 10,000, and the 2,000 most variable genes were selected (selection method, 'vst') using the 'FindVariableFeatures' function. Anchors were identified using the 'FindIntegrationAnchors' function with a dimensionality of 20. Biases in clustering due to the sex of the hiPS cell line donors. Cells with more than 15% were also excluded. Genes that were not expressed in at least three cells were removed from the count matrix to avoid biases in clustering due to the sex of the hiPS cell line donors. Cells with more than 10,000 or less than 100 detected genes or with mitochondrial content higher than 15% were also excluded. Genes that were not expressed in at least three cells were not included in the analysis. Gene expression was normalized using a global-scaling normalization method (normalization method, LogNormalize; scale factor, 20,000), using the 'count' and 'aggr' functions in Cell Ranger software (version 3.1.0). Further downstream analyses were performed using the R package Seurat (version 3.1.4).

For retrograde neural tracing experiments in cortico-striatal assembloids, the applied the hydrophilic chemical cocktail-based CUBIC protocol. Cortico-striatal assembloids were transferred to a 1.5-ml Eppendorf tube containing 200 µl neurobasal medium and incubated with virus overnight at 37 °C with 5% CO2. The next day, fresh culture medium (800 µl) was added. The following day, neural spheroids were transferred into fresh culture medium in ultra-low-attachment plates (Corning, 3741, 3261). For live-cell imaging, labeled hCs, hStrs or assembloids were transferred into one well of a Corning 96-well microplate (Corning, 4580) in 150 µl neural medium or onto a 20-mm glass coverslip in a 35-mm glass-bottom well (Cellvis, DCS-20-0-0-N) and incubated in an environmentally controlled chamber for 15–30 min before imaging on a Leica TCS SP8 confocal microscope.

The viruses used were AAV-DJ-mDxs-GFP-Fishell-1 (ref. 33) (Addgene, 83900), AAV-DJ-mDxs-GCaMP6F-Fishell-2 (ref. 33) (Addgene, 83899), AAV-DJ-iSyn:mCherry (Stanford University Neuroscience Gene Vector and Virus Core, GVVC-AAV-16), AAV-DJ-CaMKIIα-eYFP (Stanford University Neuroscience Gene Vector and Virus Core, GVVC-AAV-8), AAV-DJ-iSyn:mCherry (Stanford University Neuroscience Gene Vector and Virus Core, GVVC-AAV-17), rabies-ΔG-Cre-eGFP (Salk Institute Viral Vector Core), AAV-DJ-EF1a-CVS-G-WPRE-pGhA+ (Addgene, 67538), AAV-DJ-EFI-DIO-mCherry (Stanford University Neuroscience Gene Vector and Virus Core, GVVC-AAV-14), AAV1-Syn:ChrinmosRtGtD+ (Addgene, 59171-AAV1), AAV-DJ-Pl-eGFP (PKR88)+c (Addgene, 49123), AAV-DJ-EFI-DIO-eYFP (Stanford University Neuroscience Gene Vector and Virus Core, GVVC-AAV-13), AAV-DJ-EF1a-DIO-GCaMP6s (Stanford University Neuroscience Gene Vector and Virus Core, GVVC-AAV-9).

For imaging and quantification of dendritic spines in hStrs, mLx-Dxs::GFP cells were imaged using ×10 and ×20 objectives on a Leica TCS SP8 confocal microscope, and images were analyzed with Fiji (Image, version 2.1.0, NIH). The number of dendritic spines on a primary and a secondary dendrite within 200 µm of soma were counted.

For calcium imaging, hStrs or assembloids were labeled with AAV-DJ-Pl-eGFP: iCre and AAV-DJ-EFI-eLac: DIO-GCaMP6s or mDxs::GCaMP6-Fishell-2 and placed in a well of a Corning 96-well microplate (Corning, 4580) or on a 20-mm coverslip glass in a 35-mm glass-bottom plate in neural medium and imaged using a ×10 objective on a Leica TCS SP8 confocal microscope. GaMP6s was imaged at a frame rate of 14.7 frames per second, and results were analyzed with Fiji (Image, version 2.1.0, NIH) and MATLAB (version R2018a, 9.4.0, MathWorks).

Generation of cortico-striatal assembloids. To generate cortico-striatal assembloids, we hydrophilic chemical cocktail-based CUBIC protocol. Briefly, cortico-striatal assembloids at day 83 were fixed with 4% PFA–4% sucrose–PBS solution at 37 °C for 20 min and incubated at 4 °C overnight. The next day, assembloids were washed twice with PBS and incubated in Tissue-Clearing Reagent CUBIC-1 (TCI, T3740) at 37 °C for 2 d. Assembloids were washed three times with PBS for 2 h and then stained with anti-GFP (1:1,000 dilution) and anti-mCherry (1:1,000 dilution) antibodies in PBS containing 0.2% Triton X-100 and 3% NDS at 37 °C for 2 d. Assembloids were subsequently washed three times with PBS for 2 h and then incubated with secondary antibodies (Alexa Fluor, 1:1,000 dilution) in PBS containing 0.2% Triton X-100 and 3% NDS at 37 °C for 2 h. For refractive index matching, assembloids were incubated with Tissue-Clearing Reagent CUBIC-2 (TCI, T3741) at room temperature for 2.5 d. CUBIC-cleared assembloids were then transferred into a well of a Corning 96-well microplate (Corning, 4580) in 150 µl of CUBIC-R+ solution and imaged using a ×20 objective on a Leica TCS SP8 confocal microscope.

Axon projection imaging in cortico-striatal assembloids. AAV-DJ-iSyn:mCherry cells projecting into hCs from hStrs or AAV-DJ-iSyn:mCherry cells projecting into hCs were labeled with AAV-DJ-EFI-DIO-mCherry and AAV-DJ-EFI-eLac: DIO-GCaMP6s or mDxs::GCaMP6-Fishell-2 and placed in a well of a Corning 96-well microplate (Corning, 4580) at a concentration of 10,000 cells per well. After incubation in an environmentally controlled chamber for 15–30 min before imaging. Images were taken using a ×10 objective lens at a depth of 0–50 µm. The percentage of EFYP coverage in hStrs and of mCherry coverage in hCs were quantified with Fiji (Image, version 2.1.0, NIH).

Retrograde rabies tracing in cortico-striatal assembloids. For retrograde neural tracing experiments in cortico-striatal assembloids, hCs were labeled with AAV-DJ-EFI-DIO-mCherry and hStrs were labeled with rabies-ΔG-Cre-eGFP and AAV-DJ-EFIa-CVS-G-WPRE-pGhpA+ (Addgene, 83900). After viral infection, hCs and hStrs were assembled and maintained in culture with medium changes every 4 d. At 28 d after the cortico-striatal assembloids were fixed overnight in 4% PFA at 4 °C and processed for immunostaining. Images were taken with a Leica TCS SP8 confocal microscope and analyzed in Fiji (Image, version 2.1.0, NIH).

Optogenetic stimulation and calcium imaging. Cortico-striatal assembloids expressing AAV1-Syn::ChrinmosRtGtD in hCs or AAV-DJ-Pl-eGFP and Cre and
Whole-cell recordings in neural spheroid slices were performed in hStrSs and cortico-striatal assembloids were analyzed in MATLAB. Containing 126 mM NaCl, 2.5 mM KCl, 1.25 mM NaH2PO4, 1 mM MgSO4, 2 mM CaCl2, 26 mM NaHCO3, and 10 mM d- (+) glucose. Slices were cut at 200 µm at room temperature using a Leica VT1200 vibratome and maintained in ACSF at room temperature.

Whole-cell patch-clamp recordings from hStrS slices were performed under an upright SliceScope microscope (Scientifica). Slices were perfused with ACSF (bubbled with 95% O2 and 5% CO2) and signals from cells were recorded at 30°C. hSyn1::eYFP neurons were patched with a borosilicate glass pipette filled with an internal solution containing 127 mM potassium glutamate, 8 mM NaCl, 4 mM magnesium ATP, 0.3 mM sodium GTP, 10 mM HEPES and 0.6 mM EGTA, pH 7.2, adjusted with KOH (290 µmol/L). Data were acquired with a MultiClamp 700B Amplifier (Molecular Devices) and a Digidata 1550B Digitizer (Molecular Devices). The liquid junction potential was calculated using IPcalc, and recordings were corrected with an estimated −15 mV liquid junction potential.

For using eEPSCs were recorded at −70 mV in voltage-clamp mode and analyzed with Mini Analysis software (version 6.0.3, Synaptosoft). F/I curves, cells were current-clamped at −60 mV; current steps (1 s duration) were given with an increment of 10 pA. Animal brains from E18.5 mouse embryos were fixed with cold 4% PFA–PBS, and samples were prepared following the staining method described above. Array analysis was performed from the Stanford University Administrative Panel on Laboratory Animal Care (APLAC).

Data availability
Gene expression data were deposited in the Gene Expression Omnibus under accession number GSE149931. The Human Brain Transcriptome (https://hbatlas.org/) was used to explore transcriptomic data of the developing and adult human brain. The data in this study are available from the corresponding author. Source data are provided with this paper.

Code availability
The codes used for calcium imaging and electrophysiology analyses in this study are available on request from the corresponding author.

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Author contributions
Y.M. and F.B. conceived the project and designed experiments. Y.M. and F.B performed differentiation experiments and characterized spheroids. Y.M. carried out scRNA-seq experiments and related analyses and performed functional imaging assays. M.Y.L. conducted and analyzed the electrophysiological characterization. K.I. and M.H.P. performed histology and characterized spheroids. M.Y.L. performed differentiation experiments and characterized spheroids. J.Y.P. contributed to the characterization of spheroids and the quantification of retrograde tracing. A.P. contributed to differentiation experiments. S.H.L. contributed to the characterization of spheroids from 2q21q3.3DS and control hiPSC cells. Y.M. and S.P.P. wrote the manuscript with input from all authors.

Competing interests
Stanford University has filed a provisional patent application covering the protocol and methods for the generation of human striatal organoids and cortico-striatal assembloids. M.H.P. is a consultant for and has equity interest in CRISPRX. Throughout the duration of this study, K.I. was an employee of Daiichi Sankyo Co., Ltd, although the company had no input in the design or execution of the study or the interpretation or publication of data.

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Correspondence and requests for materials should be addressed to S.P.P.
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Extended Data Fig. 1 | Generation of the GSX2 gene reporter hiPS cell line. a, Schematic showing the 1st step of the HDR-based genome editing and b, the design of the 1st donor plasmids. The genomic sequence around the stop codon of GSX2 gene was replaced by eGFP and the truncated CD8 (tCD8) expression cassette. Arrows indicate the position of primer sets used for the genotyping PCR. Details on genome editing design and the sequence of 1st donor vector are available in Supplementary Table 1. c, Genotyping and detection of random integration. Primer set 1 (P1) shows targeted integration of the selection marker expression cassette in the GSX2 locus. Primer set 2 (P2) and set 3 (P3) show random integration. For hiPS cell lines #2 and #6, a single 5.5 kb band was detected by P1 and no band was detected by P2 or P3. Subsequent ddPCR showed that 2 copies of exogenous UbC promoter were integrated in #2 and #6. Line #2 was used in the next step of genome engineering. Pa: parental hiPS cell line. d, Schematic showing the 2nd editing step, and e, the design of the 2nd donor plasmid. The selection marker expression cassette was replaced by mCherry. Arrows show the primer sets used in the genotyping PCR. The size of amplicons is indicated by arrows. Information on the 2nd donor is available in Supplementary Table 2. f, Genotyping of the negatively selected lines by PCR. The single 3.5 kb band indicate biallelic editing. Line #1 from the 2nd editing step was used for further experiments. Full-length, unprocessed gel images for c and f are included in Source Data 1.
Extended Data Fig. 2 | See next page for caption.
Extended Data Fig. 2 | Generation of hStrS. a, Schematic illustrating the differentiation conditions for striatal differentiation as compared to the protocol for generating hCS. Ac (Activin), AcI (Activin+IWP-2), AcSr (Activin+SR11237), AcISr (Activin+IWP-2+SR11237) b, Immunostaining of neural spheroids at day 15 differentiation in the reporter GSX2::mCherry line. Scale bars: 200 μm. c–d, Percentage of (c) GSX2::mCherry + cells and (d) CTIP2 + cells in neural spheroids at day 15 (n=3 differentiation experiments with GSX2::mCherry line; one-way ANOVA, F_{4,10} = 5.7, P = 0.01, following Tukey’s multiple comparison test: *P = 0.01 for hCS vs AcISr and *P = 0.01 for A vs AcISr in c, one-way ANOVA F_{4,10} = 5.03, P = 0.01, following Tukey’s multiple comparison test for d; *** = 0.009 for hCS vs AcISr). e, Gene expression pattern of RXRG in developing striatum (Str), amygdala (Amy), neocortex (Ncx), hippocampus (Hip), mediodorsal nucleus thalamus (mdt) and cerebellar cortex (Cbc) in the BrainSpan transcriptome dataset (https://hbatlas.org/). f, Schematic illustrating differentiation conditions and different Sr11237 concentrations as compared to the protocol for generating hCS, and g, level of gene expression (rT-qPCr) for DLX5, GSX2, BCL11B (CTIP2), and MEIS2. n = 3 neural spheroids from 3 hiPS cell lines; one-way ANOVA, F_{5,12} = 4.6, P = 0.01, following Tukey’s multiple comparison test: *P = 0.01 for hCS vs 100 nM for DLX5, one-way ANOVA, F_{5,12} = 4.6, P = 0.01, following Tukey’s multiple comparison test: *P = 0.03 for hCS vs 25 nM, *P = 0.01 for hCS vs 100 nM, *P = 0.01 for hCS vs long 100 nM for GSX2, one-way ANOVA, F_{5,12} = 9.07, P = 0.0009, following Tukey’s multiple comparison test: **P = 0.001 for hCS vs 100 nM, ***P = 0.001 for hCS vs 25 nM, ***P = 0.0005 for hCS vs 50 nM, ***P = 0.001 for hCS vs 100 nM, ***P = 0.001 for hCS vs long 100 nM for BCL11B, one-way ANOVA, F_{5,12} = 11.69, P = 0.0003, following Tukey’s multiple comparison test: *P = 0.02 for hCS vs 25 nM, ***P = 0.0005 for hCS vs 100 nM, ***P = 0.0003 for hCS vs long 100 nM for MEIS2. h, Gene expression (by RT-qPCR) of FOXG1, DLX5, GSX2, BCL11B (CTIP2), MEIS2, RAX, and HOXB4. For FOXG1, GSX2, BCL11B (CTIP2), MEIS2, RAX and HOXB4: n = 9 neural spheroids (hCS or hStrS) from 3 differentiation experiments of 4 hiPS cell lines. For DLX5: n = 7 hCS from 2 differentiation experiments of 4 hiPS cell lines, n = 4 hStrS from 2 differentiation experiments of 2 hiPS cell lines. Two-tailed unpaired t-test **P = 0.002 for FOXG1, two-tailed Mann-Whitney test **P = 0.006 for DLX5, two-tailed unpaired t-test **P = 0.004 for GSX2, two-tailed unpaired t-test ***P = 0.0007 for BCL11B, two-tailed unpaired t-test **P = 0.002 for MEIS2, two-tailed Mann-Whitney test P = 0.67 for RAX, two-tailed unpaired t-test P = 0.88 for HOXB4. The 2242-1 hiPS cell line is shown in orchid blue, 8858-3 in blueberry blue, 1205-4 in midnight blue, and 0524-1 in ocean blue. Data shown are mean ± s.e.m.
Extended Data Fig. 3 | Characterization of hStrS. a, UMAP visualization of expression of selected genes in the hStrS single cell RNA-seq data at day 80-83 of in vitro differentiation (n = 25,772 cells from 3 hiPS cell lines). b, Percentage of major cell type clusters in hStrS. c, Expression of forebrain (FOXG1), midbrain, hindbrain (EN1), dorsal forebrain (EMX1), LGE and MGE markers in hStrS. d, e UMAP visualization of the resolved single cell RNA-seq data of hStrS, and (e) heat map for the top 10 genes in each cluster. f, UMAP visualization of the single cell RNA-seq data color coded by the hiPS cell lines: 2242-1 (red), 1205-4 (green), 8858-3 (blue). g, Plots showing the Pearson correlation of the normalized average gene expression between each of the three hiPS cell lines used. h, Graph showing the percentage of cells in each of the three hiPS cell lines belonging to each cluster in hStrS.
Extended Data Fig. 4 | Characterization of hStrS. a, VoxHunt mapping of the hStrS GABAergic neuron cluster to the BrainSpan human brain dataset (PCW 5–10, 10–15, and 15–20). b, VoxHunt mapping of the glutamatergic neuronal cluster in hStrS. c, Expression of early amygdala marker TFAP2D in hStrS. d, UMAP visualization of single cell RNA expression in GABAergic neurons subcluster of hStrS at day 80–83 of in vitro differentiation. e, Expression of SST, PVALB, CALB1, CALB2, CHAT, TH, NOS1, NPY in the hStrS GABAergic subcluster. f, Left, immunostaining for MAP2 (green), CALB1 (magenta), Hoechst (blue) in hStrS at day 85. Right, immunostaining for MAP2 (green), CALB2 (magenta), Hoechst (blue) in hStrS at day 85. n = 4 hiPS cell lines. Scale bar: 50 μm.
Extended Data Fig. 5 | See next page for caption.
Extended Data Fig. 5 | Comparison of hStrS to hSS. **a,b,** UMAP visualization of scRNA-seq data from hStrS and hSS. **c,** Percentage of cells from hStrS and hSS in each cluster. **d,** Heat map for the top 10 genes in each cluster. **e,** Expression of SP8, LHX6, HTR3A, SST in hStrS (top) and hSS (bottom). **f,** Representative immunocytochemistry images of hCS and hStrS (day 80) for the neuron marker MAP2 (magenta), astrocyte marker GFAP (cyan) and oligodendrocyte marker MBP (yellow). Scale bar: 100 μm for left and middle images, and 50 μm for right image. Immunostainings were repeated in spheroids from 2 independent differentiation experiments with similar results.
Extended Data Fig. 6 | See next page for caption.
Extended Data Fig. 6 | Characterization of hStrS neurons. a, Representative image of an E18.5 mouse brain section immunostained with antibodies against CTIP2 (BCL11B) (green), DARPP32 (magenta) and Hoechst (blue). Scale bar: 500 μm. b–d, Immunostaining for ASCL1 (green), CTIP2 (magenta), Hoechst (blue) in b, FOXP2 (green), GAD65 (magenta) and Hoechst (blue) in c, DARPP32 (yellow), GAD67 (magenta) and CTIP2 (cyan) in d, hStrS at day 85. n = 3 hiPS cell lines. Scale bar: 100 μm in b, c, and 50 μm in d. e–i, Representative immunocytochemistry images of dissociated hStrS neurons that have been labeled with an AAV-mDlx::eGFP at day 65 or at day 104. Scale bar: 50 μm for the left and middle image, and 5 μm for the right image (i). Quantification of CTIP2+ and DARPP32+ cells in mDlx::eGFP infected and 2D-plated hStrS cells; n = 3 neural spheroids from 2 hiPS cell lines in f, n = 2 neural spheroids from 2 hiPS cell lines in g, h, j, Expression of DRD1, DRD2, TAC1, PENK in hStrS. k, Immunostaining for GFP (green), DRD2 (magenta), Hoechst (blue), hStrS at day 124. Scale bar: 10 μm. Immunostainings were repeated in spheroids from 3 independent differentiation experiments with similar results. l, m, Dendritic spine morphology of hCS neurons labelled with CaMKII::eYFP and of hStrS neurons labelled with AAV-mDlx::eGFP. Quantification of the number of dendritic spines (day 100–110; n = 26 neurons for hCS, n = 27 neurons for hStrS; from 2 hiPS cell lines; two-tailed, Mann-Whitney test, P = 0.17). n, Quantification of number of dendritic spines (day 120–130; n = 8 neurons from the 2242-1 line, n = 22 neurons from the 1205-4 line; two-tailed, Mann-Whitney test, P = 0.44). o, Gene expression of GPR88 in developing human brain in the BrainSpan transcriptome dataset (https://hbatlas.org/). p, Representative image of dissociated hStrS neurons labeled with Ple94-iCre and DIO-eYFP at day 100. Expression of eYFP is induced by iCre expression under a mini-promoter including regulatory region of striatal gene GPR88 (Ple94). Arrow heads indicate eYFP+/GAD65+ cells. Scale bar: 100 μm. q, Quantitative results showing percentage of GAD65+ and GAD67+ cells out of eYFP+ cells following recombination with the Ple94 reporter; n = 3 hiPS cell lines. r, Percentage of GAD65+ and GAD67+ cells out of Hoechst+ cells (n = 2 differentiation with 2 iPS cell lines). s, Representative confocal live image of hStrS labeled with Ple94-iCre and DIO-eYFP at day 120. Scale bar: 100 μm. Data shown are mean ± s.e.m. Imaging were repeated in spheroids from 2 independent differentiation experiments with similar results.
Extended Data Fig. 7 | Functional characterization of hStrS. (a–e) Effect of bicuculine (50 μM) (a–c) and NBQX (20 μM) + APV (50 μM) (d,e) on calcium signals (GCaMP6s) in hStrS neurons at day 104. GCaMP6s was induced by iCre expression under a minipromoter that includes the regulatory region of the striatal gene GPR88 (Ple94). Heatmap showing ΔF/F of GCaMP6s signals. n = 74 cells before and n = 55 cells after bicuculine treatment in b; two-tailed, Mann-Whitney test, P = 0.09. n = 87 cells before and n = 56 cells after bicuculine exposure in c; two-tailed, unpaired t-test, P = 0.10. n = 25 cells before and n = 21 cells after NBQX+APV exposure in e. Data show mean ± s.e.m. (f) Expression of SLC12A2 and SLC12A5 in hStrS. (g,h) RT-qPCR for NKCC1 and KCC2. n = 4 neural spheroids from 4 hiPS cell lines at day 15, n = 3 neural spheroids from 3 hiPS cell lines at day 93, n = 6 neural spheroids from 3 hiPS cell lines at day 170; Kruskal-Wallis test, *P = 0.01, Dunn’s multiple comparisons test: *P = 0.02 for day 15 vs day 170 in NKCC1, one-way ANOVA, F3,12 = 4.38, P = 0.02, following Tukey’s multiple comparison test: *P = 0.04 for day 15 vs day 170 in KCC2. (i) Representative recording of spontaneous IPSC in hSyn1::eYFP expressing hStrS neurons at day 160. The 2242-1 hiPS cell line is shown in orchid blue, 8858-3 in blueberry blue, 1205-4 in midnight blue, 0524-1 in ocean blue, 0410-1 in aqua blue. Data show mean ± s.e.m.
Extended Data Fig. 8 | See next page for caption.
Extended Data Fig. 8 | Characterization of cortico-striatal assembloids. (a) Representative images of cortico-striatal assembloids at 8, 14, 21 days after assembly (daf). Scale bar: 500 μm. Imaging was repeated in assembloids from 2 independent differentiation experiments with similar results. (b) hCS neurons (Syn1::YFP+) projecting towards PSD95+ puncta on dendrites of mCherry+ hStrS neurons at day 96. Immunostainings were repeated in assembloids from 2 independent differentiation experiments with similar results. (c) mCherry+ projections from hCS in cortico-striatal assembloids (hCS was infected with a AAV-EF1a::DIO-mCherry; hStrS was infected with ΔG-Rabies virus-Cre-GFP and a AAV-EF1a::G). Scale bar: 100 μm. (d,e) Comparison of stimulus-triggered change in amplitude of ΔF/F of GCaMP6 signals to random time-locked ΔF/F in the same cell; (d) Representative traces of ΔF/F from real stimulation (top) and randomly selected time-points (bottom), and (e) quantitative results. n = 180 cells from 10 assembloids with 3 hiPS cell lines; two-tailed Wilcoxon test ***P = 0.0002. (f) Representative trace of ΔF/F of GCaMP6 signal at LED / 150 frame (top) and at LED / 300 frame (bottom). (g) Representative traces of GCaMP6s imaging and median amplitude of ΔF/F per cell before, during NBQX (20 μM) and APV (50 μM) treatment after wash. Data show mean ± s.e.m. (h,i) Schematics of a control optogenetics coupled with calcium imaging experiment in cortico-striatal assembloids. Quantitative results of ΔF/F from a real stimulation and a randomly selected time-point at day 108. n = 68 cells from 3 assembloids derived from 2 hiPS cell lines; two-tailed Wilcoxon test, P = 0.33. (j) Quantitative results of ΔF/F from day 90 to day 145 cortico-striatal assembloids. n = 68 cells from 3 assembloids derived from 2 hiPS cell lines; Kruskal-Wallis test, ****P < 0.0001, Dunn’s multiple comparisons test: ****P < 0.0001 for day < 100 vs day 100-120, **P = 0.004 for day < 100 vs day 140-150. Data show mean ± s.e.m. Box plots show maximum, third quartile, median, first quartile, and minimum values.
Extended Data Fig. 9 | Electrophysiological characterization of cortico-striatal assembloids. (a) Analyses on individual action potential traces in hStrS and hCS-hStrS neurons. (b) Quantification of spike amplitude, (c) spike threshold, (d) dV/dt max, (e) dV/dt min, (f) spike half width, (g) AHP, (h) time of AHP, (i) capacitance, (j) input resistance and (k) resting membrane potential in hCS, hStrS and hCS-hStrS neurons; n = 17 cells from hStrS, n = 25 cells from hCS-hStrS derived from 3 hiPS cell lines; two-tailed unpaired t-test P = 0.64 for b, two-tailed unpaired t-test P = 0.60 for c, two-tailed unpaired t-test P = 0.94 for d, two-tailed unpaired t-test P = 0.37 for e, two-tailed unpaired t-test *P = 0.02 for f, two-tailed unpaired t-test P = 0.53 for g, two-tailed unpaired t-test P = 0.87 for h, two-tailed unpaired t-test P = 0.68 for i, Mann-Whitney test P = 0.82 for j, Kruskal-Wallis test. ****P < 0.0001, Dunn’s multiple comparisons test: ****P < 0.0001 for hCS vs hStrS, ****P < 0.0001 for hCS vs hCS-hStrS, P = 0.80 for hStrS vs hCS-hStrS for k. (l) Representative traces of spontaneous EPSC (sEPSC) and (m) frequency of sEPSCs in hStrS and hCS-hStrS (n = 17 cells in hStrS, n = 10 cells in hCS-hStrS from 3 hiPS cell lines; two-tailed Mann-Whitney test **P = 0.0006. (n) Quantification of the number of dendritic spines (day 100–110: n = 9 neurons for hStrS, n = 27 neurons for hCS-hStrS from one hiPS cell line; two-tailed, Mann-Whitney test, P = 0.17). Data shown are mean ± s.e.m. Box plots show maximum, third quartile, median, first quartile, and minimum values.
Extended Data Fig. 10 | See next page for caption.
Extended Data Fig. 10 | Characterization of hiPS cells derived from subjects with 22q13.3 deletion syndrome. (a) Representative images showing morphology of hiPS cells from control and 22q13.3DS patients. Scale bars: 1 mm. Imaging was repeated in 2 independent differentiation experiments with similar results. (b) SNP array of hiPS cells showing the 22q13.3 deletion locus. Upper shows B Allele Frequency, and bottom Log R Ratio for chromosome 22q region. (c) Immunostaining for pluripotency stem cell markers OCT4 (green) and SSEA4 (magenta). Scale bars: 200 μm. (d) Representative images of 3D neural spheroids at day 5 of differentiation from control and 22q13.3DS hiPS cell lines. Scale bars: 1 mm. Imaging was repeated in spheroids from 3 independent differentiation experiments with similar results. (e) Gene expression (RT-qPCR) for the forebrain marker FOXG1, the LGE markers GSX2, MEIS2, BCL11B (CTIP2), and the spinal cord marker HOXB4 at day 22 of differentiation in hCS and hStrS derived from 22q13.3DS hiPS cells (n = 9 neural spheroids from 3 hiPS cell lines; two-tailed Mann-Whitney test ***P < 0.0001 for GSX2, two-tailed Mann-Whitney test ****P < 0.0001 for HOXB4. (f) Immunostaining for DARPP32 (green), CTIP2 (magenta) and NeuN (blue) in day 85 hStrS. Scale bar: 50 μm. Data shown are mean ± s.e.m. Immunostainings were repeated in spheroids from 2 independent differentiation experiments with similar results.
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Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection
Image Lab (Ver. 5.2.1) was used for gel image acquisition. The ZEN (Zeiss), BZ-X Analyzer (KEYENCE) and LAS-X (Leica) softwares were used for acquiring the microscopy data. Electrophysiology data were collected using a 1550A digitizer (Molecular Devices), a 700B patch-clamp amplifier (Molecular Devices) with the pClamp 10.6 software (Molecular Devices).

Data analysis
The Illumina GenomeStudio software (version 2.0.4) was used to generate 3-allele frequency and probe level log R ratio. COSMID tool was used for searching off-target candidates of gRNAs. The GraphPad Prism (Version 8.4.2) was used for statistical analyses. Fiji (Image), version 2.1.0, NIH was used for image quantification and processing. pClamp software (version 10.6, Molecular Devices) and MiniAnalysis software (version 6.0.3, Synaptosoft) were used for the analysis of electrophysiology data. R version 3.6.3 and Seurat version 3.0 were used for single cell RNA-seq analysis.

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- A description of any restrictions on data availability

Gene expression data is available in the Gene Expression Omnibus (GEO) under accession number GSE149931. The Human Brain Transcriptome database (https://
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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | Sample sizes were estimated empirically, based on previous studies (Birey et al., Nature 2017; Marton et al., Nature Neuroscience 2019; Pasca et al., Nature Medicine, 2019; Khan et al., Nature Medicine 2020). |
|-------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Data exclusions | No data was excluded. |
| Replication | Most experiments were performed using 2-4 hiPS cell lines. For disease modeling, 3 control and 3 patients hiPSC cell lines were used. For each assay, multiple hStR5 or cortico-striatal assemblies from more than one differentiation were used. Replicates for each type of experiment are indicated in the figure legends. The experiment in Extended Data Figure 1c,f performed once, but the insertion of gene of interest was confirmed in a separate experiment (expression of mCherry). The immunohistochemistry experiment in Extended Data Figure 6a was performed in multiple pups in one experiment. The validation of pluripotency by immunocytochemistry in Extended Data Figure 10c was performed once. |
| Randomization | The 22q13.3DS patients and control hiPS cell lines used in each experiments are summarized in Supplementary Table 3. Spheroids or assemblies were randomly picked for specific assays. At least 2-4 hiPSC lines were used for each experiment, except for data in Extended Data Figure 2b, c, d, which was performed in one GSX2 reporter cell line. |
| Blinding | Investigators were not blinded, but image quantifications were often performed by investigators not familiar with the details of the experimental conditions. |

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

| Materials & experimental systems | Methods |
|----------------------------------|---------|
| n/a | n/a |
| ☑ Antibodies | ☑ ChIP-seq |
| ☑ Eukaryotic cell lines | ☑ Flow cytometry |
| ☑ Palaeontology and archaeology | ☑ MRI-based neuroimaging |
| ☑ Animals and other organisms | | |
| ☑ Human research participants | | |
| ☑ Clinical data | | |
| ☑ Dual use research of concern | | |

Antibodies

| Antibodies used | |
|-----------------|---|
| anti-MAP2 (guinea pig, Synaptic Systems 188 004, 1:200 dilution, Lot#2-26) | |
| anti-GFAP (rabbit, DAKO, 70334, 1:3000 dilution, Lot#20035993) | |
| anti-MLP (rat, Millipore, MA386, 1:300 dilution) | |
| anti-Calretinin (rabbit, SWANT, CR7697, 1:200) | |
| anti-Calbindin (Synaptic Systems, #214 011, 1:200, Lot# 214011/5) | |
| anti-Calretinin (rabbit, SWANT, CR7697, 1:200) | |
| anti-ASCL1 (mouse, BD, 56604, 1:200 dilution, Lot#6063825) | |
| anti-FOX2 (mouse, Santa Cruz Biotechnology, sc-517261, 1:100 dilution, Lot#1517) | |
| anti-GAD65 (goat, R&D systems, AF2247, 1:100 dilution, Lot#UK6218101) | |
| anti-CRP2 (rat, Abcam, ab18465 1:300 dilution, Lot#GR322373-6, Lot#GR3272266-4) | |
| anti-DARPP32 (rabbit, Cell Signaling, 2306, 1:200 dilution, Lot# 7) | |
| anti-DARPP32 (rabbit, Abcam, #ab40801, 1:200, Lot#GR3213291-3) | |
| anti-NeuN (mouse, Abcam, #ab104224, 1:200, Lot#GR341933-3) | |
Validation

We used and/or validated some of the antibodies in our previous studies [Pasca et al., Nature Methods 2015; Birey et al., Nature 2017; Sloan et al., Neuron 2017; Yoon et al., Nature Methods 2018; Marton et al., Nature Neuroscience 2019, Khan et al., Nature Medicine 2020; anti-MAP2, GFAP, MBP, GAD67, GFP, SATB2 and CTIP2]. Most of these antibodies have also been used and validated in other studies, as follows:

anti-MAP2 (guinea pig, Synaptic Systems 188 004) has been used and validated with human cells in the Khan et al., Nature Medicine 2020.

anti-GFAP (rabbit, DAKO, Z0334) has been used in 8 studies according manufacturer’s website, and confirmed with human cells in the Pasca et al., Nature Methods 2015 and Sloan et al, Neuron 2017.

anti-MBP [rat, Millipore, MA3836] has been used and validated with human cells in the Marton et al., Nature Neuroscience 2019 anti-Calbindin (Synaptic Systems) has been used and in 11 studies according manufacturer’s website, and validated with human cells in the Birey et al., Nature 2017.

anti-Calretinin (rabbit, SWANT, CR7697) has been used in 9 studies according manufacturer’s website, and validated with human cells in the Birey et al., Nature 2017.

anti-ASCL1 (mouse, BD, 556604, 1:200 dilution, Lot#0603925) has been used in 6 studies according manufacturer’s website, and confirmed with human cells in the Drouin-Ouellet et al., EMBO Mol Med. for ICC.

anti-FOX2 (mouse, Santa Cruz Biotechnology, sc-517261) has been used in 1 study according manufacturer’s website, and confirmed with human cells in Tu et al., Stem Cells 2019.

anti-GAD65 (goat, R&D systems, AF2247) has been used in 1 study according manufacturer’s website, and confirmed with human cells in Lu et al., J Clin Invest 2017 for ICC.

anti-CTIP2 [rat, Abcam, AB18465] has been used in 240 studies according manufacturer’s website, and validated with human cells in Pasca et al., Nature Methods 2015.

anti-DARPP32 (rabbit, Cell Signaling, 2306) has been used in 46 studies according manufacturer's website, and confirmed with human cells in Maxan et al., Ann Neurol. 2018 for ICC.

anti-DARPP32 [rabbit, abcam, #ab40801] has been used in 56 studies according manufacturer’s website, and confirmed with human cells in Arber et al., Development 2015 for ICC.

anti-NeuN (mouse, abcam, ab104224) has been used in 190 studies according manufacturer’s website.

anti-GAD67 (mouse, Millipore Sigma, MA35406) has been used in 185 studies according manufacturer’s website, and validated with human cells in the Birey et al., Nature 2017.

anti-DR2 (rabbit MBL, #MC-1405) has been validated with human cells for IHC according manufacturer’s website.

anti-GFAP [chicken, GeneTex, GTX13970] has been used in 22 studies according manufacturer’s website.

anti-mCherry (rabbit, GeneTex, GTX128508) has been used in 19 studies according manufacturer’s website.

anti-PSD95 (guinea pig, Invitrogen, #MA1-045) has been used in 99 studies according manufacturer’s website, and confirmed with human cells in Huang et al., Front Neuroanat. 2019 for ICC.

anti-SATB2 [mouse, Abcam, AB51502] has been used in 87 studies according manufacturer’s website, and validated with human cells in Pasca et al., Nature Methods 2015.

anti-OCT4 (C30A3) (rabbit, Cell Signaling Technology, #28400) has been used in 82 studies according manufacturer’s website, and validated with human IPS cells in the Khan et al., Nature Medicine 2020 for ICC.

anti-SSEA4 (MC813) (mouse, Cell Signaling Technology, #4755) has been used in 29 studies according manufacturer’s website, and validated with human IPS cells in the Khan et al., Nature Medicine 2020 for ICC.

anti-human CD8 (Miltenyi Biotech, 130-045-201) has been used in 9 studies according manufacturer’s website, and validated in the Ikeda et al., Nature Methods 2018 for MICS.

anti-human CD8 (Miltenyi Biotech, 130-045-201) has been used in 9 studies according manufacturer’s website, and validated in the Ikeda et al., Nature Methods 2018 for MICS.

Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488 [Thermo Fisher SCIENTIFIC, A-21206, 1:1000 dilution]

Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 658 [Thermo Fisher SCIENTIFIC, A-10037, 1:1000 dilution, Lot#2110843]

Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 647 [Thermo Fisher SCIENTIFIC, A-10042, 1:1000 dilution, Lot#2136776]

Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 647 [Thermo Fisher SCIENTIFIC, A-31571, 1:1000 dilution, Lot#1839634]

Alexa Fluor® 488 AffiniPure Donkey Anti-Chicken IgY (goat, H+L) [Jackson ImmunoResearch Inc, 706-545-148, 1:1000 dilution]

Alexa Fluor® 488 AffiniPure Donkey Anti-Guinea Pig IgG (H+L) [Jackson ImmunoResearch Inc, 706-545-148, 1:1000 dilution]

Alexa Fluor® 647 AffiniPure Donkey Anti-Rat IgG (H+L) [Jackson ImmunoResearch Inc, 712-605-153, 1:1000 dilution]

Alexa Fluor® 647 AffiniPure Donkey Anti-Rabbit IgG (H+L) [Jackson ImmunoResearch Inc, 712-605-153, 1:1000 dilution]

Alexa Fluor® 647 AffiniPure Donkey Anti-Rat IgG (H+L) [Jackson ImmunoResearch Inc, 712-605-153, 1:1000 dilution]
Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)  
hiPSC cell lines (Supplementary Table 3) were reprogrammed at Stanford University from fibroblasts harvested with IRB approval and under written consent. K562 cells (ATCC® CCL-243™) were obtained from ATCC.

Authentication  
hiPSC cell lines were authenticated by SNP arrays. The karyotype of K562 cells was confirmed by ATCC.

Mycoplasma contamination  
All hiPSC cell lines were regularly tested and maintained Mycoplasma free.

Commonly misidentified lines (See CLAC register)  
No commonly misidentified cell lines were used.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals  
E18.5 pups (ICR/CD1 mice, Charles River) were used for immunohistochemistry.

Wild animals  
No wild animals were used in the study.

Field-collected samples  
No field-collected animals were used in the study.

Ethics oversight  
Approval for mouse experiments was obtained from the Administrative Panel on Laboratory Animal Care (APLAC) at Stanford University.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Human research participants

Policy information about studies involving human research participants

Population characteristics  
n/a (de-identified hiPSC cell lines).

Recruitment  
Participants carrying the 22q13.3 deletion or unaffected controls were recruited at Stanford University.

Ethics oversight  
Approval for this study was obtained from the Stanford University Institutional Review Board (IRB) panel.

Note that full information on the approval of the study protocol must also be provided in the manuscript.