

SUPPLEMENTARY TABLE 1. gRNA target and donor vector sequence for 1st editing.

1st edit gRNA target: CGGAGCGCGCGTAGTGCACC

Sequence of GSX2 1st donor vector (6778 bp):

TATATATGAGTAAACTTGGTCTGACAGTTA**CCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTTCGTTTCCATCATAGTTGCCCTGACTCCCCGT**
CGTGTAGATAACTACGATACGGGAGGGGCTTACCATCTGGCCCCAGTGTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATAGCAATAAAAC
CAGCCAGCCGGGAAGGGCCGAGCGCAGAAGTGGTCTGCAACTTTATCCCGCTCAATCCAGTCTATTAATTTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCA
GTTAATAGTTTGGCAACGCTTGTGCCATTGCTACAGGCATCGTGGTGTACCGCTCGTGGTTGGTATGGCTTCAATCAGCTCCGGTCCCAACGATCAAGGC
GAGTTACATGATCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCTCCGATCGTGTGCAGAAGTAAGTTGGCCGCAAGTGTATCACTCATGGTTATG
GCAGACTGCCATAAATCTCTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCCGGCAGCC
GAGTTGCTCTTGGCCGGGCTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAGGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACCTCA
AGATCTTACCGCTGTTAGATGTCAGTTCGATGTAAACCCTCGTGCACCCACTGTACTTCAGCATCTTTTACTTTCCAGCAGCTTTCTGGGTGAGCAAAAAC
AGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGAAATGTTGAATACTCATACTCTTCTTTTCAATATTATTGAAGCATTATCAGGGTTATTG
TCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAACAATAAGGGGTTCCGCGCACATTTCCCGGAAAAGTGCCACCTGACGTTAACTCGAGCACA
CGCACCTGTGCACCGCCACCTACAACCTGGCGGACCCCGGAGATTCACCTGCCATGCCATGGGTAGGGCGGGGCTTTGGGACCTCCGCTCCG
CGCTTTCCGCGCTCCTGGAGCAAACTTCCACCTCCAGTGGAGGAAGTGGGAGCCCGGGACAGGGTGAAGAGAGAGGACGGGCTTTAGTGTAACTGTAG
AGTCAGCCACAGAATCCACGAAAGTGAAGTGTAGTTGCCAGCTTCCACTCAGCCTGGTGCCTTTACTCTAGTAGTAGGTGCTCGAGTATTGCCTTAT
TAATCTGGGCGCTTAGGGCTCGAAGCTCACGGCGGAGAAAACCGAGTTAACTGCTTGGGATTTGGGTTGGTGTGTTTGAACCTGTGGGCATCCCG
GCTCGGGGCGGGTAAAGCGCTTACGCTTTTGGCTAAGCTGCGCGCTTCCGCTTGGCTAAAGGGAGGGCGATCAGATAGTGCAGCCCCCTCCCTCT
TTAATCTGTGACTTCGCATCCGATCGAGCCTTTATTTGCTTGCCTGCTCTTTTCTTCCCGCTAAGCAACCCAGTGTGCTTAAATGGGAAGCGGA
TACAGATGTTCCGGTGGGCTTCCCGGGTGGTCCCTGAAATGCGTCTGGTTAGCACATGGGGTGGGAGCACCTTCCCGAGCCTTACCTCTTACCCTCT
CTTCCGCGGTCGCGAGGAGGCTGTGACGCGCAGCCAGGTACCCAAATGGCAAGAGGATGAGGACGGCGTTCACTAGCAGCCAACTCTGGAGCTGGAGAGAGA
ATTCTCTTCAACATGTACCTGTCTCGACTCCGGAGGATTAATCGCCACTTACCTGAACCTGTCGGAGAAGCAGGTGAAAATCTGGTTTCCAGAACCCCGGA
GTGAAGCACAAGAAGGAGGGGAAGGGCAGCGCAGAGGAACAGTACGCGGGCTGCAAGTGCCTCGGGAGCCAGGTCATCCATGAGCGGCAGTGCCTTCC
CGCGCGGGTTTTGGCGCTCCCGGGGCGCCCCCTCTCACGGCGAGCGCTGCCACGTGACGCAAGGGCGCAGCGAGCGTCTGATCCTTCCGCCCCG
ACGCTCAGGACAGCGGCCCGCTGCTCATAAGACTCGGCCCTTGAACCCAGTATCAGCAGAAGGACATTTTAGGACGGGACTTTGGGTGACCTAGGCGACTG
GTTTTCTTCCAGAGAGCGGAACAGCGGAGGAAAAGTAGTCCCTTCCGGGACTTCCGCGGAGGATCTCCGTTGGGCGGGTGAACCGCGATGATTATAAAG
GACGCGCCGGGTGTGGCAGCAGTAGTTCGCTCGCAGCCGGATTTGGGTCCGGTCTTGTGTTGTGGATCGCTGTGATCGTGGTGGTGGTGGTGGTGGT
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GTGGAACCTGAAGTGGCAGGTCCTGCTGTCTAATCTACTTCTGGCTGTAGTGGCTGTTCCAGCCAGGAGGAGCAGCAGCTAGTCCCACCTTTCTGCTGTACC
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GGGCTGGACTTCCGCTACATTTGGCACTTGGCTGGAACTTGGCTGCGTGTGCTGTGAGCCTGGTCATCACACTGTATTGCAACCACTTGA
GGCGCGCCCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTTGGCCCTCCCGCTGCCCTTCTTACCCCTGGAGGTGCCACTCCC
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TGCCCCCTCCCAATGTCTAGAGAAGGCAATGTCAGGAAAAGGCTGTCTGAGGAAATTCGAGGAAAATGTTGAGTAAGAAGTGGTAATGTGGGTCTCTTGA
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AAAATAGGCAACCTCTCACAGTGTAGAGGACAACTACCTCATTGGAATGACACTGACCATTAACGACAGGTAAACCTTTATTTAGCAAGGAAGGTAAAAAT
CCCCATCTAGTTTTTGTCTTCTTCTACTTCTCATTCTCTCAGTACGAAAACCTGAATTATGTAAGGAATTTGTAGAGTCAAGATGTTTATGGAAGAG
CAACAATCCATCAACAGGTCAGAAGCAACAGTGAAGAACTAATAACGATGCTACAAGCAGAAATAGCGGGTTCCCTCAATGTTAAGAAAACAAAAAGTCAG
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CCGGTAAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAAACAGGATTAGCAGAGCAGGATGTAGGCGGTGCTACAGAGTCTTGAAGTGGTGGCC
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CCGCTGTTAGCGGTGGTTTTTTTTGTTTGAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAATCCTTTGATCTTTTACGGGGTCTGACGCTCAG
TGGAACGAAAACCTACGTTAAGGGATTTTGGTGTGAGATTACAAAAAGGATCTTACCTAGATCTTTTAAATTAATAAATGAAGTTTTAA

- AmpR (orange)
- GSX2 Homology arm (blue)
- Human Ubc promoter (red)
- EGFP (green)
- tCD8 (purple)
- bGH-polyA (magenta)

SUPPLEMENTARY TABLE 2. gRNA target and donor vector sequence for 2nd editing.

2nd edit gRNA target: AGCGCACTGCCGCTCATCGA

Sequence of GSX2 2nd donor vector (4818 bp):

TACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTTCGTTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGG
CTTACCATCTGGCCCCAGTGTCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACAGCCAGCCGGAAGGGCCGAGCGCAG
AAGTGTCTCTGCAACTTTATCCGCTCCATCCAGTCTATAAATGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCCAACGTTGTTGCCA
TTGCTACAGGCATCGTGGTCTCAGCTCGTCTTTGGTATGGCTTTCATTAGCTCCGGTCCCAACGATCAAGGCCAGTTACATGATCCCCCATGTTGTGCAA
AAAAGCGGTTAGCTCTTCGGTCTCCGATCGTTGTCAAGAAAGTGGCCGAGTGTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTACTGTCA
TGCCATCCGTAAGATGCTTTTCTGTGACTGGTACTCAACCAAGTCTTCTGAGAATAGTGTATCGGGCCAGCCGAGTTGCTCTTGGCCGGCTCAATAACG
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CGATGTAACCCACTCGTGCACCAACTGATCTTACGATCTTTTACTTTACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAGGG
AATAAGGGCGACACGAAATGTTGAATCACTCACTCTTCCCTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGT
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CCACCTCCAGTGGGAAGTGGGAGCCCGGGACAGGGTGAAGAGAGAGAGGAGGCTTTAGTGTAACTGTAGAGTCAAGGCAAGGTTCCAGCAAAAGT
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TCGAAGACCTTTATTTGCTTTGACGCTCTTTTCTTCCCGCTAAGCAACCACGTGCCTTGAATGGGAAAGGGATACAGATGTTGCGCTGGGCTTCCCGG
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CAGCAGAGGAACAGTACGCGGGCTGCAAAGTGTGCTGGGAGCCAGGTGCACCTACGCGCTCCGAGGATGAGGACTCCCTGTCCGCGCTCAGCCAGC
GATGACAAGGAGATTTCCCCCTTAGGATCCGGAAGAGGGGAGAGGCAGTCTGTTGACATGTGGAGACGTTGACGAAAAATCCAGGCCCTATGGTGTAGCAAGGG
CGAGGAGGATAACATGGCCATCATCAAGGAGTTTATGCGCTTCAAGGTGCACATGGAGGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCG
AGGGCCGCCCTACGAGGGCACCCAGACCGCAAGCTGAAGGTGACCAAGGGTGGCCCTGCCCTTCCCTGGGACATCCTGTCCCTCAGTTCACTGTAC
GGCTCAAGGCCCTACGTGAAGCACCCCGCGACATCCCGACTACTTGAAGCTGTCTTCCCGAGGGCTTCAAGTGGGAGCGCGTGTAACTTCGAGGA
CGCGCGCTGGTGACCGTGACCCAGGACTCCTCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCTCCGACGGCCCC
GTAATGCAGAAGAAGCCATGGGCTGGGAGGCCCTCCTCCGAGCGGATGTACCCCGAGGACGGCGCTGAAAGGGCGAGATCAAGCAGAGGCTGAAGCTGA
AGGACCGCGCCACTACGACGCTGAGGTCAAGACCACCTACAAGGCCAAGAGCCGCTGCAGCTGCCCGCGCTACACGCTCAACATCAAGTTGGACATC
ACCTCCCAACAGGACTACACCATCGTGGAAACAGTACGAACCGGGCCGCACTCCACCGGGGCGCATGGAGGAGCTGTACAAGTGAGGGAGGG
CCTCCTCCCTCACATCCCCGCTCCTGGCAGACCAGGCAACGCCAAGGCGTGGGGCACCCAGGGGCCAGAATCCTTGCTCATTGCAGTGGTCCCATCTGGA
GAAGAAACGAACCTGGAAGTTCTGGAATGGACAAGCCGGGACCTGACCCTTCCGCTCTCCTTTGACCTGTTTATCTAGGACTCCAACCTGAAAGTTACAGATT
TTTTAAAAAATGTAATAACCTATAAATCAACTCATTCTGTGCTATAACAGAGGAAAAACAGGGTTGGTTAAGTTAACAATGTATGGGGTTTTGAAAGCAC
ATGTCAGTTCCCATCCCTACTCTCTTTTCCAGAACTCGATAAAGACACAGGATTTATTGATATTTTCTGTTGCTGTTTTCAAAACAAAAACCCCAAGTTGAAAAA
ATTTAGAAAATAGACCTTGTAGGCTTGTCTTTTCAAATTTCCGCTGGGGAGAAATTTAAATCTAAGTCCGCTGGAAGTCCCTTTGTATGTGAATAGGTTTATATA
AAATTTATATTTATTTATTTAAATAAATGAAACAAAAACAGAAATTTAAATCTGTGGTGTGTTGCTCCTCCTATCTTCCCTGCCCCCTCCTCCAATGTCTAGAG
AAAGGCATATGCAGGAAAAAGCTGTCTGAGGAATTCGAGGAAAAATGTTGAGTAAGAACTTGGTAATGTGGGTCTCTTACTGAGGGAGTTTGTGGCTCAATGG
GTTTTCTATGTAACAGAACTTTCCGCAAGGAGACACCTTTAGTCAATGATGCACTACACAGGAGTGCCTTCCATCCTTAAATAGGCAACCTCCTCACAGTT
GATAGGACAAATCACTCCATTTGGAATGACACTGACCATTACGACAGGTAACCTTTATTTAGCAAGGAAGGGTAAAAATCCCCATCTAGTTTTTGTCTTCT
CTTTCTACTTCCCTCATTCTCTCAGTACGAAAACTTGAATATGTGAAGGAAATTTAGAGTCAAGAAATGTTTAGGAAAGAGCAACAATCCATCAAAACAGGTCCAG
AAGCAAAACAGTGGAACTTAATAACAGATGCTACAAGCAGAATTAGCGGGTTTCCCTCAATGTAAAGAAAAACAAAAAGTCAAGGACAGGAAGTATTCTGTTCAAA
GAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCTGTGCTGGCTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAATAATCGACGCTCAAGTCAAGAGG
TGGCGAAACCCGACAGGACTATAAAGATACAGGGCTTTCCCTGGAAGCTCCCTCGTGGCTCTCCTGTTCCGACCTGCCGCTTACCGGATACCTGTCC
GCCCTTCCCTTCCGGAAGCGTGGCGCTTCTCATAGCTCACGCTGTAGGTATCTCAGTTTCGGTGTAGGTCGTTCCGCTCAAGCTGGGCTGTGTGCACGAAAC
CCCCGTTTACGCCGACCGCTGCGCTTATCCGGTAACTATCGTCTTGTAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACA
GGATTAGCAGAGCGAGGATGTAGGCGGTGCTACAGAGTTTCTGAAGTGGTGGCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCT
GAAGCCAGTTACCTTCGAAAAAGAGTTGGTAGCTTGTATCCGGCAAAACAAACCACCGCTGGTAGCGGTGTTTTTTTGTGTTGCAAGCAGCAGATTACCGC
AGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGAACAGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAA
AAGGATCTTACATGATCCTTTTAAATAAAAATGAAGTTTTAAATCAATCTAAAGTA

- AmpR (orange)
- GSX2 Homology arm (blue)
- T2A (green)
- mCherry (magenta)

SUPPLEMENTARY TABLE 3. hiPS cell lines used in various experiments.

Experiment / Line	2242-1	8858-3	1205-4	0524-1	0410-1	1927-1	2631-3	0502-3
qPCR characterization	X	X	X	X	X	X	X	X
2D neuron culture	X	X	X	X				
Single-cell RNAseq	X	X	X					
Immunocytochemistry	X	X	X	X	X	X	X	X
Dendritic spine analysis	X		X	X				
Axon projection	X	X	X	X				
Retrograde viral tracing	X		X	X				
Optogenetics + calcium imaging	X	X	X		X			
Optogenetics & electrophysiology	X		X					
Electrophysiology	X		X	X				
Calcium imaging	X	X	X		X	X	X	X

SUPPLEMENTARY TABLE 4. Primers used for qPCR experiments.

Gene	Forward Primer	Reverse Primer
<i>GAPDH</i>	CATGAGAAGTATGACAACAGCCT	AGTCCTTCCACGATACCAAAGT
<i>FOXG1</i>	AACCTGTGTTGCGCAAATGC	AAACACGGGCATATGACCAC
<i>DLX5</i>	TTCCAAGCTCCGTTCCAGAC	GAATCGGTAGCTGAAGACTCG
<i>GSX2</i>	ATGTCGCGCTCCTTCTATGTC	ATGCCAAGCGGGATGAAGAAA
<i>BCL11B</i>	GGTGCCTGCTATGACAAGG	GGCTCGGACACTTTCCTGAG
<i>MEIS2</i>	ACAGCTGGAGTGGCAAAAAG	AAATTGTCAAGCCCCCGAAC
<i>RAX</i>	GGCCATCCTGGGGTTTACC	GGTCGAGGGGCTTCGTACT
<i>HOXB4</i>	TCCTCGTTTTTCAGCTTTGGC	TCATTTGTTAGCGGGGTGTCG
<i>NKCC1</i>	TAAAGGAGTCGTGAAGTTTGGC	CTTGACCCACAATCCATGACA
<i>KCC2</i>	AGGAAAGCAGTCCCTTCATCA	GCCTCTTCATGCTCCCTACTT

SUPPLEMENTARY TABLE 5. Comparison of protocols for striatal neuron differentiation from human pluripotent stem cells.

	Ma et al.,²⁰ 2012	Carri et al.,²¹ 2013	Arber et al.,²² 2015	hStrS Miura et al.
Type of culture	2D	2D	2D	3D
Patterning	SHH	SHHC-25II DKK1	Activin A	Activin A IWP-2 SR11237
Cell lines	2 hES cell lines	1 hiPS and 2 hES cell lines	2 hiPS and 3 hES cell lines	5 control hiPS cell lines
Cell diversity	Progenitors Neurons	Progenitors Neurons	Progenitors Neurons	Progenitors Neurons Astrocytes Oligodendrocytes
Proportion of GABAergic neurons	80%	78.4% (of MAP2 ⁺ cells)	Not shown	56.9%
Proportion of CTIP2 ⁺ cells	Not shown	60%	50%	50%
Proportion of DARPP32 ⁺ neurons	80%	20% (of MAP2 ⁺ cells)	20-50%	30% (of NeuN ⁺ cells)
Dendritic spine	Not shown	Not shown	Present	Present (0.02 spines/ μ m at day 120-130)
Resting membrane potential	-46.9 ± 2.9 mV	-43.0 ± 4.9 mV	-44.0 ± 1.7 mV	-75.9 ± 1.8 mV
Slow-ramp depolarization	Not shown	Present	Not shown	Present (at day 160)
Inward rectification	Not shown	Not shown	Not shown	Present (at day 110-120)

\pm , indicated s.e.m.