

MATERIALS AND REAGENTS

Reagents, antibodies, and plasmids used in this study are summarized below:

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Mouse anti-AnkG (IgG2a)	NeuroMab	Cat# 75-146; RRID: AB_10673030
Mouse anti-Gephyrin (IgG1)	Synaptic Systems	Cat# 147011; RRID: AB_887717
Guinea pig anti-VGAT	Synaptic Systems	Cat# 131004; RRID: AB_887873
Rabbit monoclonal anti-HA	Cell Signaling Technology	Cat# 3724; RRID: AB_1549585
Mouse anti-Myc	MBL International Corporation	Cat# M192 PRID: AB_11160947
Mouse anti-PSD95	Antibodies Incorporated	Cat# 75-028 PRID: AB_2292909
Mouse anti-Tenascin-R	R&D Systems	Cat# MAB1624 RRID: AB_2207001
Mouse anti-Tuj1	BioLegend	Cat# 801202 RRID: AB_10063408
Mouse anti-V5	Invitrogen	Cat# R960CUS RRID: AB_159298
Rabbit anti- β IV-Spectrin	Rasband lab	RRID: AB_2315634
Rabbit anti-Kv1.2	Gift of Dr. James Trimmer	PRID: 2756300
Rabbit anti-NrCAM	Abcam	Cat# ab24344 RRID: AB_448024
Chicken anti-MAP2	Encor	Cat# CPCA-MAP2
Chicken anti-Neurofascin	R&D Systems	Cat# AF3235 RRID: AB_10890736
Goat anti-Cntn1	R&D Systems	Cat# AF904 RRID: AB_2292070
Rat anti-HA	Millipore Sigma	Cat# 11867423001 RRID: AB_390918

HRP-conjugated goat anti-chicken IgY	Aves Labs	Cat# H-1004 RRID: AB_2313517
HRP-conjugated goat anti-rabbit IgG	Jackson ImmunoResearch Labs	Cat# 111-035-003 PRID: AB_2313567
Alexa Fluor 594 conjugated streptavidin	Thermo Fisher Scientific	Cat# S11227
Aminomethylcoumarin (AMCA) conjugated goat anti-chicken IgY	Jackson ImmunoResearch Labs	Cat# 103-155-155 RRID: AB_2337385
Aminomethylcoumarin (AMCA) conjugated goat anti-rat IgG	Thermo Fisher Scientific	Cat# A21093 PRID: AB_2535748
Alexa Fluor 488 conjugated goat anti-chicken IgY	Jackson ImmunoResearch Labs	Cat# 103-545-155 RRID: AB_2337390
Alexa Fluor 488 conjugated goat anti-mouse IgG	Thermo Fisher Scientific	Cat# A11029 RRID: AB_2534088
Alexa Fluor 488 conjugated goat anti-rabbit IgG	Thermo Fisher Scientific	Cat# A11034 RRID: AB_2758380
Alexa Fluor Plus 594 conjugated goat anti-mouse IgG	Thermo Fisher Scientific	Cat# A32742 RRID: AB_2762825
Alexa Fluor 594 conjugated donkey anti-goat IgG	Thermo Fisher Scientific	Cat# A11058 RRID: AB_2758385
Alexa Fluor 488 conjugated goat anti-rat	Thermo Fisher Scientific	Cat# A-11006; RRID: AB_2534074
Alexa Fluor Plus 555 conjugated goat anti-rabbit	Thermo Fisher Scientific	Cat# A32732; RRID: AB_2633281
Alexa Fluor 647 conjugated goat anti-rabbit	Thermo Fisher Scientific	Cat# A-21244; RRID: AB_2535812
Alexa Fluor 555 conjugated goat anti-guinea pig	Thermo Fisher Scientific	Cat# A-21435; RRID: AB_2535856
Alexa Fluor 647 conjugated goat anti-guinea pig	Thermo Fisher Scientific	Cat# A-21450; RRID: AB_2735091
Alexa Fluor 647 conjugated goat anti-mouse IgG1	Thermo Fisher Scientific	Cat# A-21240; RRID: AB_2535809
Alexa Fluor 488 conjugated goat anti-mouse IgG2a	Thermo Fisher Scientific	Cat# A-21131; RRID: AB_2535771
Bacterial and Virus Strains		
Stellar competent cells	Takara	Cat# 636763

NEB® Stable Competent E. coli (High Efficiency)	New England Biolabs	Cat# C3040H
NEB® 5-alpha Competent E. coli (High Efficiency)	New England Biolabs	Cat# C2987H
AAV PHP.S encapsulated vectors	This paper	N/A
AAV PHP.eB encapsulated vectors	This paper	N/A
Chemicals, Peptides, and Recombinant Proteins		
Biotin tyramide	Perkin Elmer	Cat# NEL749A001KT
Neurobasal Medium	Gibco	Cat# 21103-049
B-27 supplement	Gibco	Cat# 17504-044
GlutaMax-I	Gibco	Cat# 35050-061
Penicillin-Streptomycin	Gibco	Cat# 15140122
DMEM, high glucose, no glutamine	Gibco	Cat# 11960-044
Fetal bovine serum	Cytiva	Cat# SH30109.03
0.5% Trypsin-EDTA	Gibco	Cat# 15400054
2.5% trypsin	Gibco	Cat# 15090046
Hank's buffered salt solution (HBSS)	Gibco	Cat# 14175-095
Poly-D-lysine	Gibco	Cat# A3890401
Poly-L-lysine	Sigma	Cat# P4832-50ML
Laminin	Gibco	Cat# 23017015
Phosphate buffer saline (PBS)	Corning	Cat# 21-031-CV
Opti-MEM	Thermo Fisher Scientific	Cat# 31985070
Hoechst 33258	Thermo Fisher Scientific	Cat# H3569 RRID: AB_2651133
Polyethylenimine (PEI)	Polysciences	Cat# 24765-1
Normal goat serum	Gibco	Cat# 16210-072
Gelatin from bovine skin	Sigma	G9382-100G
FastGreen	Sigma	Cat# F7258
Streptavidin Mag Sepharose	GE Healthcare	Cat# 28-9857-38
Vectashield plus anti-fade mounting media	Vector labs	Cat# H-1900
Sequencing Grade Trypsin	Promega	V5111
Tamoxifen	Sigma-Aldrich	Cat# T5648; CAS: 10540-29-1
Fluoromount-G	Southern Biotech	0100-01
Critical Commercial Assays		
Q5 High-Fidelity DNA Polymerase	New England Biolabs	Cat# M0491S

CloneAmp HiFi PCR Premix	Takara	Cat# 639298
NucleoSpin Plasmid Transfection-grade	Takara	Cat# 740490.250
In-Fusion Snap Assembly Master Mix	Takara	Cat# 638947
Gel purification	Cytiva	28903470
Amicon Ultra-15 Centrifugal Filter Unit 100 kDa	Millipore	Cat# UFC910008
C18 ZipTips	Millipore	Cat# ZTC18S096
BamHI restriction enzyme	New England Biolabs	Cat# R3136T
EcoRI restriction enzyme	New England Biolabs	Cat# R3101T
NotI restriction enzyme	New England Biolabs	Cat# R3189L
XhoI restriction enzyme	New England Biolabs	Cat# R0146M
Experimental models: Cell Lines		
HEK293T cells	Provided from Baylor College of Medicine Neuroconnectivity Core	N/A
Experimental models: Organisms/ Strains		
Rat: Sprague-Dawley rat embryos	Charles River Laboratories	SAS-SD
Mouse: C57BL/6	Baylor College of Medicine Center for Comparative Medicine	N/A
Mouse: CFW (Swiss Webster)	Charles River	Cat# CRL:24; RRID: IMSR_CRL:24
Mouse: Nkx2-1 ^{tm1.1(Cre/ERT2)Zjh/J}	Gift from Z.J. Huang; (Taniguchi et al., 2013)	JAX: 014552; RRID: IMSR_JAX:014552
Mouse: B6;129S6-Gt(Rosa)26Sortm9(CAG-tdTomato)Hze/J	Gift from Z.J. Huang; (Taniguchi et al., 2013)	JAX: 007905; RRID: IMSR_JAX:007905
Mouse: Igs2tm1.1(CAG-cas9*)Mmw/J (also known as H11Cas9 mice)	The Jackson Laboratory	Cat# JAX: 027650 RRID: IMSR_JAX:027650
Mouse: Cntn1 knockout	Boyle et al., 2001	Cat# JAX: q034216, RRID: IMSR_JAX:034216
Mouse: ICR	Baylor College of Medicine Center for Comparative Medicine	N/A

Oligonucleotides		
Target for knock-in	Sequence (5'->3')	
rat Adgr1 #1	TTGAGGGGCCAGGGCCCGAT	
rat Adgr1 #2	GTTGGTCACTAGTCTCTGAG	
rat Cacna2d1 #1	ATACTCCTTTGGCTGGTATC	
rat Cacna2d1 #2	CTGGCAGCAGACTATCTA	
rat Celsr2 #1	CGCCGGCCAGGGTTAATGC	
rat Celsr2 #2	ATCGCCATGAGCATCAAGGC	
rat Cntn1 #1	GGTGTGTCCACGCTGTCATC	
rat Cntn1 #2	GAGGAGACCCGATGACAGCG	
rat Dcc #1	GTTTGGCAATGCATGTCAA	
rat Dcc #2	AAGCAACTCAATGCCATCAC	
rat Fat3 #1	CTACGGGTGTCCTCTACACC	
rat Fat3 #2	GGAAACGCAGCACCAGACCC	
rat Kctd12 #1	ACACCGAGTACGCTTCTGC	
rat Kctd12 #2	CTCCCTGCAGAAGACGTACT	
rat L1Cam #1	GCAGTAGCCCTAGAATAGCA	
rat L1Cam #2	GACTGGACCTTGCTATTCTA	
rat Lrp1 #1	ATAGGGGATCCCTTAGCATA	
rat Lrp1 #2	TGGGGCAGGGCCCTATGCTA	
rat Lrrc4b #1	GCTCTCAAGAGCGGCTCCA	
rat Lrrc4b #2	GAGCCGCTCTTGAAGAGCAG	
rat Ncam1_v1,2,5 #1	GGGACCGTCTTGACTTCAGT	
rat Ncam1_v1,2,5 #2	CTCATTCTTTTCGTTTGTG	
rat Ncam1_v3,4 #1	AGGGTGGCTGGGATGGCCGT	
rat Ncam1_v3,4 #2	CAATGAGACCAAGGTGTAGG	
rat Neo1 #1	AGGTCATCAAGCCGTTGTGA	
rat Neo1 #2	GGACCTAAATGCCATCACAA	
rat Nfasc #1	AGTCAATGCCATCTATTCCC	
rat Nfasc #2	CCATCTATTCCCTGGCCTGA	
rat Nlgn3 #1	ATAGAGCTATACACGGGTAG	
rat Nlgn3 #2	TCTGGGATAGAGCTATACAC	
rat NrCAM #1	AAGGAGTTCATTGCGTTGAC	
rat NrCAM #2	GAGCTTAAAGACTTAAACAA	

rat Nrnx1 #1	GTCCAATGGGGCTGTGGTCA		
rat Nrnx1 #2	CTCCTTGACCACAGCCCAT		
rat Plxna2 #1	GCAACGACTGGCCTACAAGG		
rat Plxna2 #2	CATGTCCATAGAGAGCTGAA		
rat Plxna4 #1	CACACTGGTCATCATGGTCC		
rat Plxna4 #2	TCAGCTGTCTAAGCTCATGA		
rat Plxnb2 #1	AACCTTATTCTCGAGCGCAG		
rat Plxnb2 #2	TGCCGCTGCGCTCGAGAATA		
rat Ptprs #1	CCAGGTATTCCAACGCCGCC		
rat Ptprs #2	GTTCTGCTCCAGGCGGCGT		
rat Tenm4 #1	GACAGAGCGAGATGGGCCGA		
rat Tenm4 #2	TTCATGAGACAGAGCGAGAT		
rat Cntn2 #1	GGAGAACGCAGGTCCGGGAT		
rat Cntn2 #2	ATGGTGATATTGATGCTCGC		
mouse Cntn1 #1	GAGGAGACTCGAAGACAGCG		
mouse Cntn1 #2	CCTTGCTTTCTGTCTACT		
mouse Cntn1 #3	CGAGTAGACAAGAAAGCCAA		
Target for knock-out	Sequence (5'->3')	Sequence (5'->3')	Sequence (5'->3')
Control	CTGTCTTCATCATGGCCGAC	GTTTCGCATTATCCGAACCAT	TAAGCGTCGCAAGAAGACGG
Nfasc	ACGATCTCGGTGAGAGTAAA	CATGTCCTGCAGCATGACGT	ACTGCGGAAGTCGATCACCA
Cntn1	GCATAGCAGAAAACGCGTAT	CTACGTGATTGACTTTAACA	TCGGATGAGCAGTCCCCAT
NrCAM	ATGTGTTCCGTTACGAGTCC	GCCGGTGGTAATCCAATTGG	GGATTACAATATTCTCCCGT
Recombinant DNA plasmids			
PX551 (AAV-SpCas9)	Gift of Dr. Feng Zhang		Addgene #60957
PX552 (pAAV-U6sgRNA(SapI)_hSyn-GFP-KASH-bGH)	Gift of Dr. Feng Zhang		Addgene #60958
pCAG-1BP-NLS-Cas9-1BP-NLS	Gift of Dr. Juan Belmonte		Addgene #87108
pCAG_smFP HA	Gift of Dr. Loren Looger		Addgene #59759
pCAG_smFP V5	Gift of Dr. Loren Looger		Addgene #59758
pMJ114	Gift of Dr. Jonathan Weissman		Addgene #85995
pMJ117	Gift of Dr. Jonathan Weissman		Addgene #85997
pMJ179	Gift of Dr. Jonathan Weissman		Addgene #85996
pUCmini-iCAP-PHP.S	Gift of Dr. Viviana Gradinaru		Addgene #103002
pUCmini-iCAP-PHP.eB	Gift of Dr. Viviana Gradinaru		Addgene #103005

pHelper	Agilent Technologies	Cat #240071
Knock-in sgRNA and donor plasmid	This paper	N/A
pAAV-3x-gRNA-smFP	This paper	N/A
rat contactin-myc	Gift of Dr. Elior Peles	N/A
pAAV-hSyn-EGFP	Gift of Dr. Bryan Roth	Addgene # 5046
pcDNA3	Invitrogen	N/A
hSYNp-Cntn1-Myc full-length	This paper	N/A
hSYNp-Cntn1-Myc truncates	This paper	N/A
Software and Algorithms		
Fiji	NIH	https://fiji.sc/
Zeiss Zen (Blue Edition) Imaging Software	Zeiss	http://www.zeiss.com/microscopy/en_us/products/microscope-software/zen.html#introduction ; RRID: SCR_013672
NIS Elements Imaging Software	Nikon	https://www.microscope.healthcare.nikon.com/products/software/nis-elements
CRISPOR	Haeussler et al., 2016	http://crispor.tefor.net/crispor.py
Adobe Photoshop (version 23.5.1)	Adobe	https://www.adobe.com/products/photoshop.html
Adobe Illustrator (version 26.5)	Adobe	https://www.adobe.com/products/illustrator.html
GraphPad Prism 9	GraphPad	https://www.graphpad.com/
MATLAB	Mathworks	https://www.mathworks.com/products/matlab.html
Python	Python Software Foundation	https://www.python.org/

Script for calculation of extracellular tyrosines	Rasband lab	https://github.com/jrasband/extracellular-tyrosines
BioRender	BioRender	https://biorender.com/
Other		
Axiomager Z2 with Apotome	Zeiss	
Ni2 upright fluorescence microscope	Nikon	
Microtome HM450	Thermo Scientific	
Cryostat CryoStar NX70	Thermo Scientific	
QExactive Plus	Thermo Scientific	
NanoAcquity Ultra Performance UPLC system	Waters	
15-cm EasySpray C18 column	Thermo Scientific	