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Supplemental Information

Targeted Transgene Activation in the Brain Tissue by Systemic Delivery of Engineered AAV1 Expressing CRISPRa

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SUPPLEMENTAL FIGURES

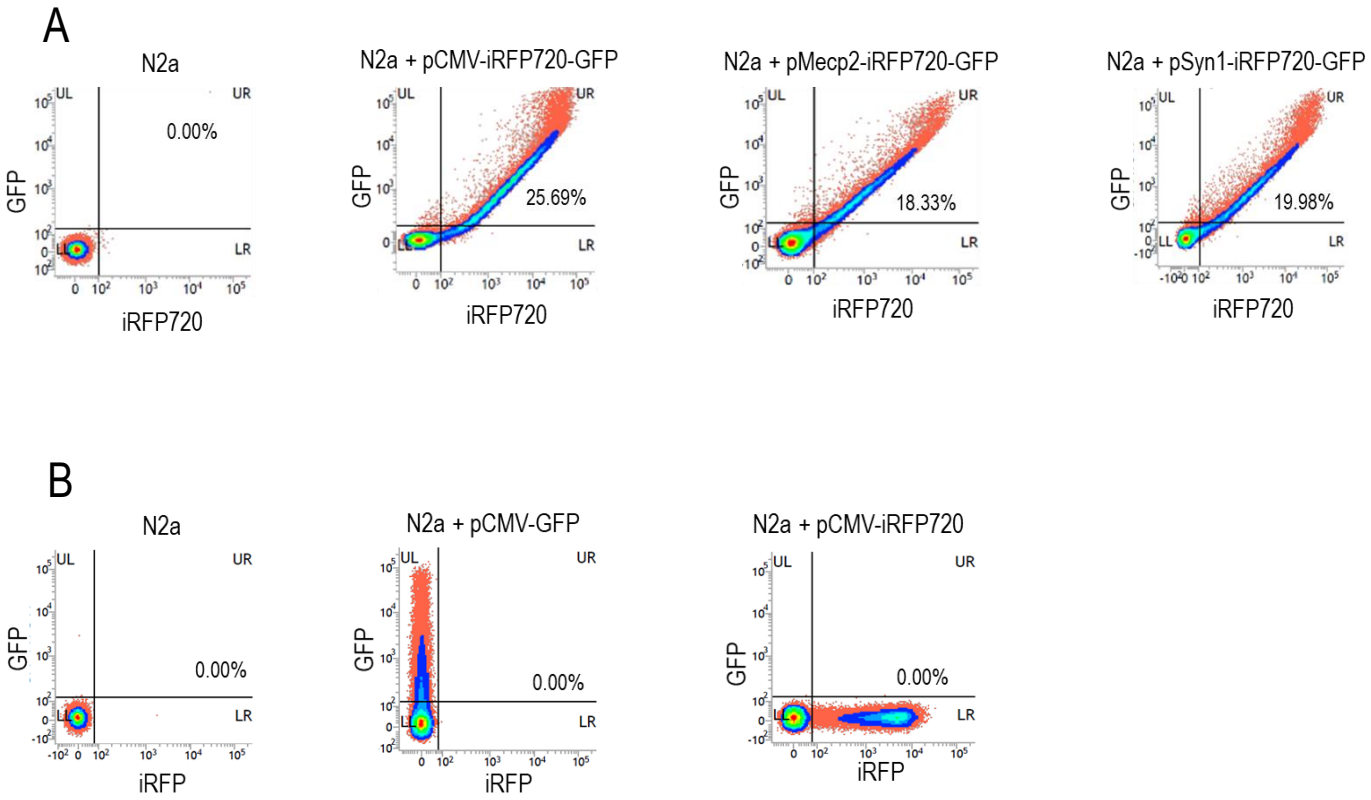

Figure S1. Flow Cytometry Analysis to Quantify iRFP720 and GFP Expressing Cells. (A) Flow cytometry analysis of mouse N2a cells after transfected with iRFP720-GFP fusion vector driven by different promoters. **(B)** Flow cytometry analysis of mouse N2a cells after transfected with iRFP or GFP alone. Two days after plasmids transfection, the iRFP720- and GFP-expressing cells were analysed with flow cytometry. The fluorescent channels APC-H7 and FITC were used to detect the iRFP720- and GFP-expressing cells, respectively.

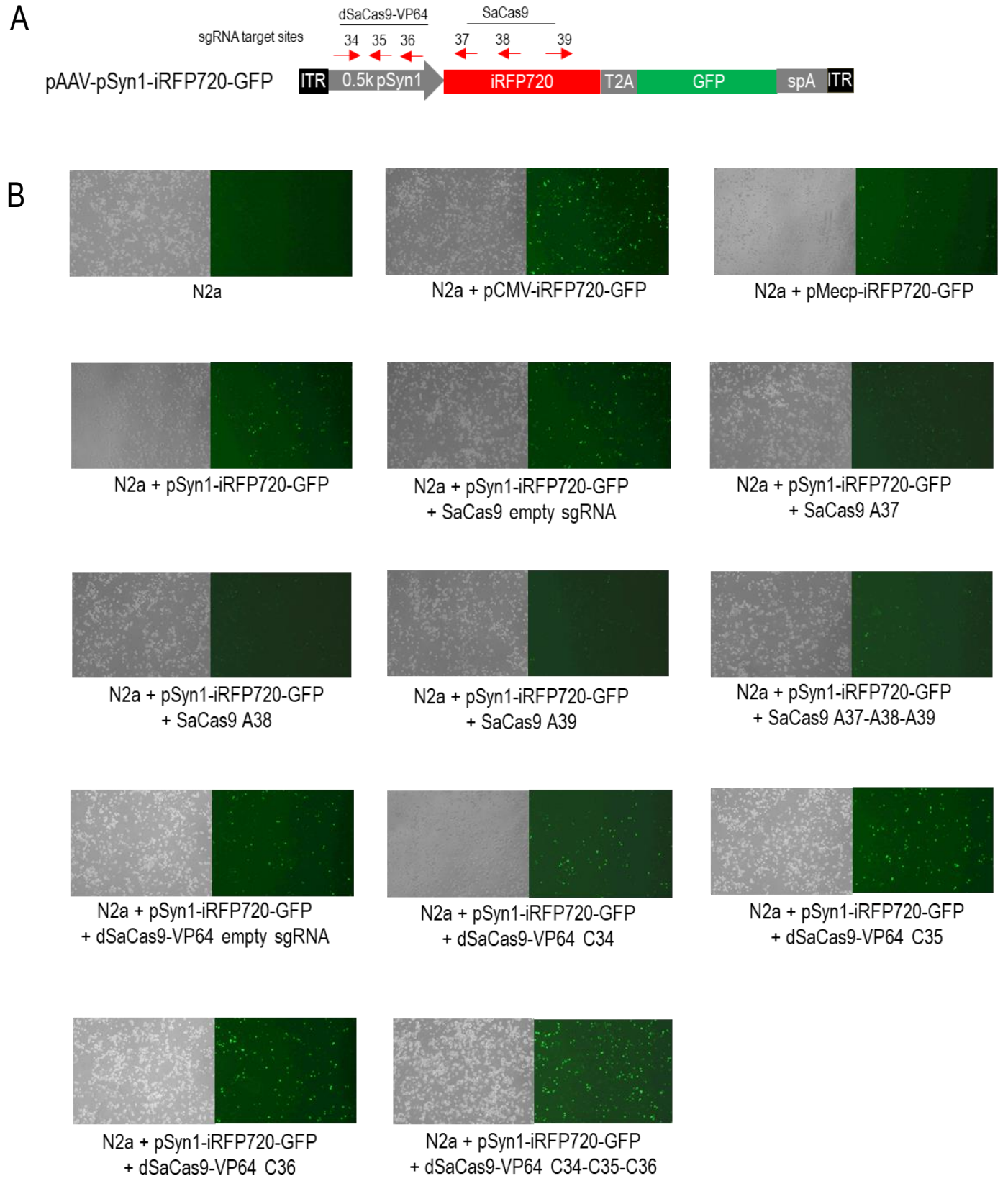

Figure S2. CRISPR-mediated Modulation of Bicistronic Infrared-GFP Fluorescent Reporter Genes Expression in Mouse N2a Cells. (A) sgRNA target sites on hSyn1 promoter and iRFP720. The location of three different sgRNA target sites of dSaCas9-VP64 designed to target the hSyn1 promoter, and three different sgRNA target sites of SaCas9 designed to target the iRFP720 open reading frame are shown. Red arrows indicate the sense or antisense orientation of sgRNAs designed to recognize target DNA sequences. **(B)** Cellular images of GFP protein expression in mouse N2a cells. Two days after plasmids co-transfection of various CRISPR and iRFP720-GFP reporter genes, cellular images of GFP protein expression were taken under fluorescent microscope at 4X magnification. The left and right panels are phase contrast and GFP fluorescent images, respectively.

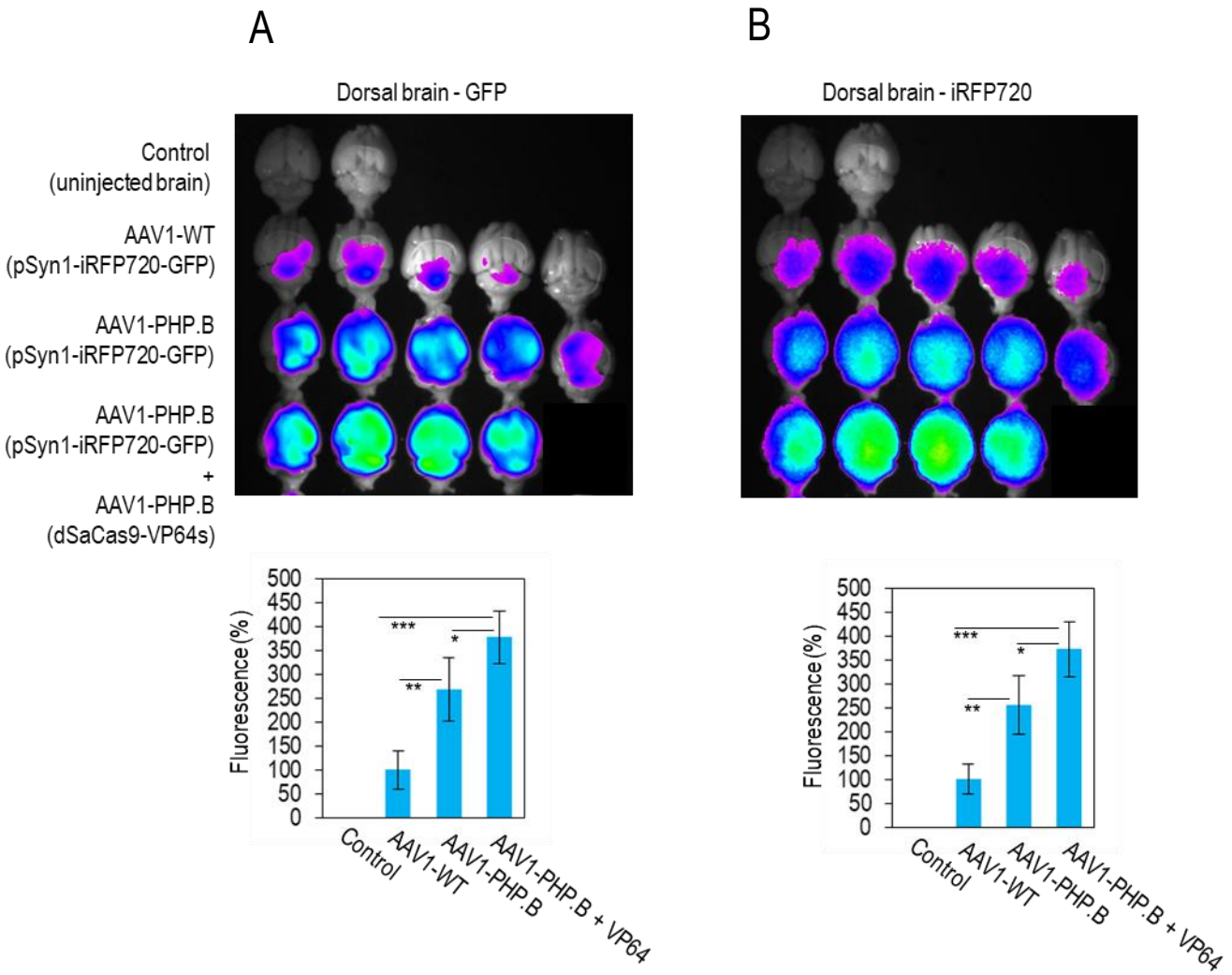

Figure S3. Ex Vivo Imaging of the Dissected Dorsal Brain Tissues. Ex vivo imaging was carried out to detect and quantify the **(A)** GFP and **(B)** iRFP720 fluorescent signals on the mouse dorsal brains. AAV1-PHP.B expressing dSaCas9-VP64 was injected together with AAV1-PHP.B expressing iRFP720 and GFP fluorescent proteins. The statistical significance levels are indicate as *P<0.05, **P<0.01 and ***P<0.001. All data are presented as mean ± standard deviation.

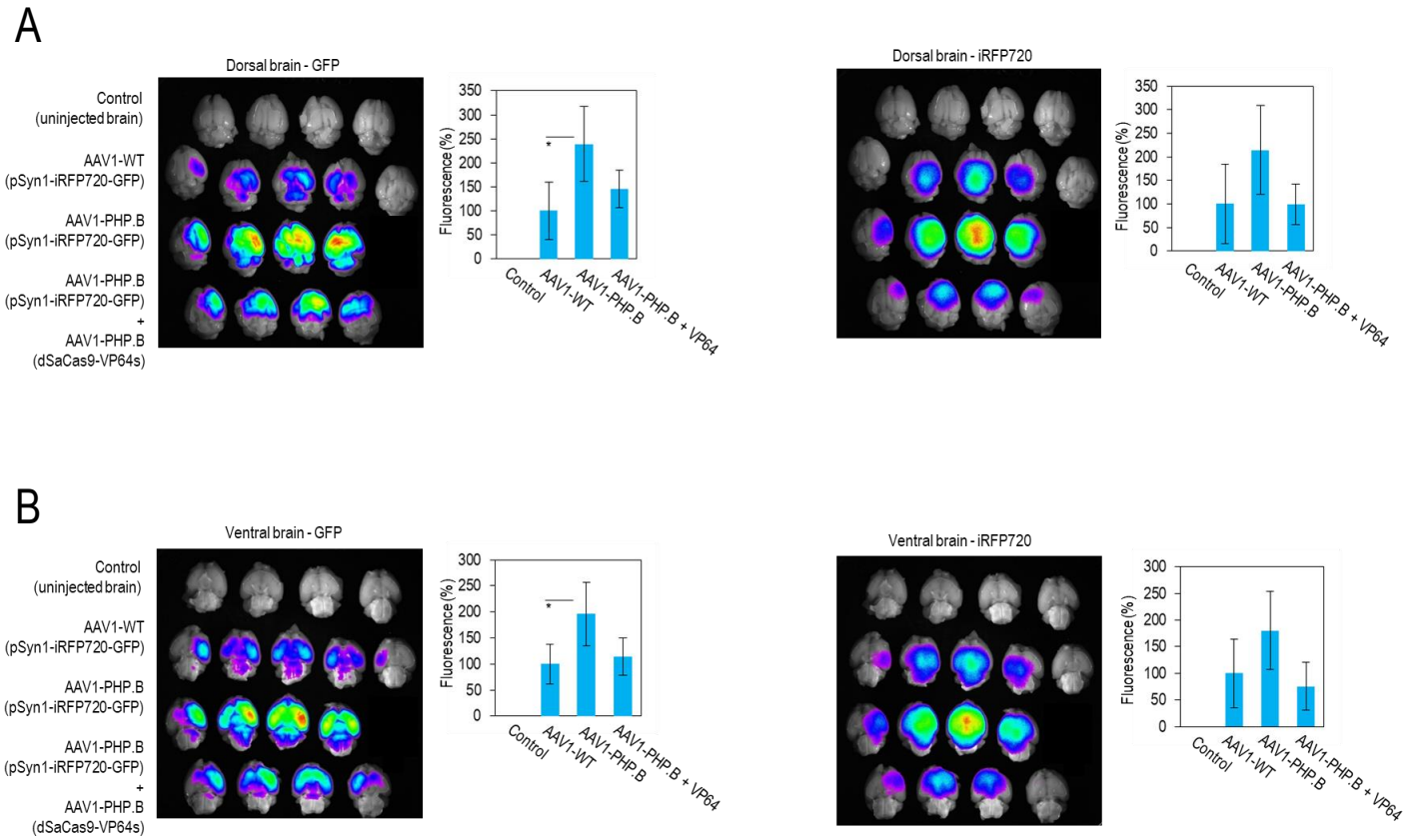

Figure S4. Immune Responses Inhibit CRISPRa-mediated Transgene Activation in the Mouse Brains. AAV1-PHP.B expressing dSaCas9-VP64 was introduced into the mice only after 24 hours tail-vein injection of AAV1-PHP.B expressing iRFP720 and GFP fluorescent proteins. Ex vivo imaging was carried out to detect and quantify the GFP (left) and iRFP720 (right) fluorescent signals on the mouse **(A)** dorsal and **(B)** ventral brains. The statistical significance levels are indicate as *P<0.05, **P<0.01 and ***P<0.001. All data are presented as mean ± standard deviation.

SUPPLEMENTAL TABLES

Table S1. Primers used for SaCas9s and dSaCas9-based CRISPR Backbone Construction

Primer Sequence (5' to 3') (Forward, FP; Reverse, RP)	Amplicon Size (bp)	Description
FP: GGGG GAATTC AATAAAAGATCTTTATTTTCATTAGA TCTGTGTGTTGGTTTTTTGTGT GGTACC GGGG RP: CCCC GGTACC ACACAAAAAACCAACACACAGATCTA ATGAAAATAAAGATCTTTTATT GAATTC CCCC	-	68 bp DNA fragment bearing EcoRI-spA-KpnI for CRISPR backbone construction
FP: CTGCGGCCTCTAGAAAGCTTAGC RP: GGTACATGGTGGCACCGGT	268	PCR pMecp2 with added XbaI PCR pMecp2 with added AgeI
FP: AAAA GGATCC GGACGGGCTGACGCATTGGACG RP: AAAA GAATTC TTA GTTAATCAGCATGTCCAGGTCGA	177	PCR VP64 with added BamHI PCR VP64 with added EcoRI-Stop
FP: AAAA GGATCC GGGCGCGCCGACGCGCTGGACGAT RP: AAAA GAATTC TTAATCGATATACAACATATCCAAATCGAAGTCATCG	414	PCR VP160 with added BamHI PCR VP160 with added EcoRI-Stop
FP: AAAA GGATCC ATGGACGCGAAATCACTTACGGCA RP: AAAA GAATTC TTA TACCAGCCAAGGTTCTTCCCCCT	237	PCR KRAB with added BamHI PCR KRAB with added EcoRI-Stop
FP: AAAA GGATCC AGCCCCAAGAAAAAGAGGAAGGTGG RP: AAAA GAATTC TTA GGGCAGCATAGAGGCATAGCCA	429	PCR SID4X with added BamHI PCR SID4X with added EcoRI-Stop
FP: CAGCACAGACATTCTGGGCAACCT	-	Sequencing BamHI-domain-spA
FP: GATCGAGGAAATCATCCGGACCAC	-	Sequencing dSaCas9 (mutation N580A)
FP: GCAGGTTGTAGTCGAACAGCAGCT	-	Sequencing pMecp2-dSaCas9 (mutation D10A)

Table S2. Primers used for Single Guide RNAs Designed

Primer Sequence (5' to 3') (Forward, FP; Reverse, RP)	Target sequence (bp)	Description
FP: CACC GGGCGAGCAGCAGTCCATGCGG RP: AAAC CCGCATGGACTGCTGCTCGCCC	22	SaCas9-37 construction for exonic knockout of iRFP720
FP: CACC GGTTTCGGCGGCCTGCAGGCG RP: AAAC CGCCTGCAGGCCGCCGAAACC	21	SaCas9-38 construction for exonic knockout of iRFP720
FP: CACC GGCTCTATACCATCAACCCGGT RP: AAAC ACCGGGTTGATGGTATAGAGCC	22	SaCas9-39 construction for exonic knockout of iRFP720
FP: CACC GGGCCCTGCGTATGAGTGCAA RP: AAAC TTGCACTCATACGCAGGGCCC	21	dSaCas9-34 construction for modulating promoter activity of hSyn1
FP: CACC GTTGGGTGCTTGTCCAGTGGGT RP: AAAC ACCCACTGGACAAGCACCCAAC	22	dSaCas9-35 construction for modulating promoter activity of hSyn1
FP: CACC GCGACCAAGGTGGCCGGGAAG RP: AAAC CTTCCCGGCCACCTTGGTCGC	21	dSaCas9-36 construction for modulating promoter activity of hSyn1
FP: CACC GTGTGAAGGTGCTGGCTGGTC RP: AAAC GACCAGCCAGCACCTTCACAC	21	SaCas9-40 construction for exonic knockout of mouse alpha CaMKII
FP: CACC GATACCCAACCAGCAAGATATA RP: AAAC TATATCTTGCTGGTTGGGTATC	22	SaCas9-41 construction for exonic knockout of mouse alpha CaMKII
FP: CACC GGACACCGTCACCCCAGAAGCC RP: AAAC GGCTTCTGGGGTGACGGTGTCC	22	SaCas9-42 construction for exonic knockout of mouse alpha CaMKII
FP: CACC GACTCGTCAGCTTGTGGATGAG RP: AAAC CTCATCCACAAGCTGACGAGTC	22	dSaCas9-9 construction for modulating distal super-enhancer activity of mouse alpha CaMKII
FP: CACC GCGTAGGTTGTGTATTTGTGT RP: AAAC ACACAAATACACAACCTACGC	21	dSaCas9-8 construction for modulating distal super-enhancer activity of mouse alpha CaMKII
FP: CACC GCCAGGGTGGCAAGCCAGCAAG RP: AAAC CTTGCTGGCTTGCCACCCTGGC	22	dSaCas9-7 construction for modulating distal super-enhancer activity of mouse alpha CaMKII
FP: CACC GGCATCAAGGAGTCAAGCATGC RP: AAAC GCATGCTTGACTCCTTGATGCC	22	dSaCas9-6 construction for modulating proximal super-enhancer activity of mouse alpha CaMKII
FP: CACC GCCTTTGGTAGACACCTGCATG RP: AAAC CATGCAGGTGTCTACCAAAGGC	22	dSaCas9-5 construction for modulating proximal super-enhancer activity of mouse alpha CaMKII
FP: CACC GGGCTAAGGGATAGGCAGGTCC RP: AAAC GGACCTGCCTATCCCTTAGCCC	22	dSaCas9-4 construction for modulating proximal super-enhancer activity of mouse alpha CaMKII
FP: CACC GAGCAAGTGGACCCTGTTCCCC RP: AAAC GGGGAACAGGGTCCACTTGCTC	22	dSaCas9-1 construction for modulating promoter activity of mouse alpha CaMKII
FP: CACC GCAGTTGCTATGGTAACGGCTA RP: AAAC TAGCCGTTACCATAGCAACTGC	22	dSaCas9-2 construction for modulating promoter activity of mouse alpha CaMKII
FP: CACC GAGAAGAAGTACCAAACAGACC RP: AAAC GGTCTGTTTGGTACTTCTTCTC	22	dSaCas9-3 construction for modulating promoter activity of mouse alpha CaMKII
FP: CACC GAGAAGCAGACCAGATGGGATG RP: AAAC CATCCCATCTGGTCTGCTTCTC	22	dSaCas9-30 or dSaCas9-31 construction for modulating promoter activity of human *PDGFRA*
FP: CACC GAGGGCCCTATTTCTCGTTGGG RP: AAAC CCCAACGAGAAATAGGGCCCTC	22	dSaCas9-29 construction for modulating promoter activity of human *PDGFRA*
FP: CACC GTTGAGTCCAATATGACAATG RP: AAAC CATTGTCATATTGGACTCAAC	21	dSaCas9-28 construction for modulating promoter activity of human *PDGFRA*
FP: CACC GTTACTTCGCTTTCTCCAGTCC RP: AAAC GGACTGGAGAAAGCGAAGTAAC	22	dSaCas9-32 construction for modulating expression level of human *PDGFRA*
FP: CACC GGCCTACAGCACAGGGAGCCGG RP: AAAC CCGGCTCCCTGTGCTGTAGGCC	22	dSaCas9-33 construction for modulating expression level of human *PDGFRA*
FP: CACC GATCTCGAAGGAAGGCGACAC RP: AAAC GTGTCGCCTTCCTTCGAGATC	21	dSaCas9-10 construction for modulating promoter activity of mouse *Mycn*
FP: CACC GGAGTGCAGCGGGTGCAAGCCA RP: AAAC TGGCTTGCACCCGCTGCACTCC	22	dSaCas9-11 construction for modulating promoter activity of mouse *Mycn*
FP: CACC GACAGTCATCTGTCTGGACGCG RP: AAAC CGCGTCCAGACAGATGACTGTC	22	dSaCas9-12 or dSaCas9-13 construction for modulating promoter activity of mouse *Mycn*
FP: CACC GGATCCGGAGGCGACTCGGGGC RP: AAAC GCCCCGAGTCGCCTCCGGATCC	22	dSaCas9-14 construction for modulating expression level of mouse *Mycn*
FP: CACC GTCTCTTCCAGCCAGGGTGCCT RP: AAAC AGGCACCCTGGCTGGAAGAGAC	22	dSaCas9-15 construction for modulating expression level of mouse *Mycn*
FP: CACC GCCCGAGGGCGGGGCATGGAC RP: AAAC GTCCATGCCCCGCCCTCGGGC	21	dSaCas9-18 construction for modulating promoter activity of mouse *Nrf2*
FP: CACC GCGAGAGGAGGATCAACAGTG RP: AAAC CACTGTTGATCCTCCTCTCGC	21	dSaCas9-17 construction for modulating promoter activity of mouse *Nrf2*

FP: CACC GGCAGTTGGCCTCTTGCAAAGT RP: AAAC ACTTTGCAAGAGGCCAACTGCC	22	dSaCas9-16 construction for modulating promoter activity of mouse *Nrf2*
FP: CACC GGCAGGACAAGGGCATGGAGG RP: AAAC CCTCCATGCCCTTGTCCTGCC	21	dSaCas9-19 construction for modulating expression level of mouse *Nrf2*
FP: CACC GGAGGATGTTGGGGCCGCGAC RP: AAAC GTCGCGGCCCCAACATCCTCC	21	dSaCas9-20 construction for modulating expression level of mouse *Nrf2*
FP: CACC GGCAGAGACACCACCACCTCG RP: AAAC CGAGGTGGTGGTGTCTCTGCC	21	dSaCas9-21 construction for modulating expression level of mouse *Nrf2*
FP: CACC GTTGGACCGTGCAGGCTGTGG RP: AAAC CCACAGCCTGCACGGTCCAAC	21	dSaCas9-24 construction for modulating promoter activity of mouse *Keap1*
FP: CACC GATAAATATCGCAACCAGGTAG RP: AAAC CTACCTGGTTGCGATATTTATC	22	dSaCas9-23 construction for modulating promoter activity of mouse *Keap1*
FP: CACC GTGGAGCCTGCAAAGTGCAGC RP: AAAC GCTGCACTTTGCAGGCTCCAC	21	dSaCas9-22 construction for modulating promoter activity of mouse *Keap1*
FP: CACC GCGGGAGGGCGGAAACGGGCG RP: AAAC CGCCCGTTTCCGCCCTCCCGC	21	dSaCas9-25 construction for modulating expression level of mouse *Keap1*
FP: CACC GGCACCTACAGAGACACCCGG RP: AAAC CCGGGTGTCTCTGTAGGTGCC	21	dSaCas9-26 construction for modulating expression level of mouse *Keap1*
FP: CACC GGTGGCCGCGGCGAGTAGAGGT RP: AAAC ACCTCTACTCGCCGCGGCCACC	22	dSaCas9-27 construction for modulating expression level of mouse *Keap1*
FP: GCATATACGATACAAGGCTGTTAGAGAG	-	Sequencing pU6-sgRNA for successful of target sequence insertion

Table S3. Primers used for Luciferase Reporter Vectors Construction

Primer Sequence (5' to 3') (Forward, FP; Reverse, RP)	Amplicon Size (bp)	Description
FP: AAAA GGTACC GAACCCCATTATGGCCTTAGGTCAC RP: AAAA AAGCTT CTAGGGCTGGGATGCTGAAGC	1316	To insert amplified 1316bp mouse alpha CaMKII promoter into pGL4.10[luc2] with KpnI and HindIII
FP: GTCCTGCCACAGGCTTACCATG RP: GTGATGGTAGCCATCCTGGCACT	7kb	Nested PCR (outer primers) to amplify entire promoter and super-enhancer regions of mouse alpha CaMKII
FP: AAAA GAGCTC GGGGTGGTTGTAGAGCCTGCTAG RP: AAAA AAGCTT CTAGGGCTGGGATGCTGAAGC	6.8kb	Nested PCR (inner primers) to amplify entire promoter and super-enhancer regions of mouse alpha CaMKII for inserting into pGL4.10[luc2] with SacI and HindIII
FP: TAGCAAAATAGGCTGTCCCCAGTG RP: CATGGTGGCTTTACCAACAGTACC	-	Sequencing enhancer or promoter that has been cloned into pGL4.10[luc2] firefly luciferase vector

Table S4. Primers used for PCR and Quantitative RT-PCR

Primer Sequence (5' to 3') (Forward, FP; Reverse, RP)	Amplicon Size (bp)	Description
FP: ACCATCTTCCAGGAGCGAGA RP: TGGCATGGACTGTGGTCATG	319	qPCR mRNA expression level of human and mouse *Gapdh*
FP: GCTCAGCCCTGTGAGAAGAC RP: ATTGCGGAATAACATCGGAG	95	qPCR mRNA expression level of human *PDGFRA*
FP: TGATGCCAGCCACTGTATCC RP: CTGCGAACCCAAACCATGC	194	qPCR mRNA expression level of mouse alpha CaMKII
FP: CCTCCGGAGAGGATACCTTG RP: TCTCTACGGTGACCACATCG	90	qPCR mRNA expression level of mouse *Mycn*
FP: GATCCGCCAGCTACTCCCAGGTTG RP: CAGGGCAAGCGACTCATGGTCATC	122	qPCR mRNA expression level of mouse *Nrf2*
FP: CATTGGCATCGCCAACTTCG RP: GGAACACCTCGGACTCGCA	188	qPCR mRNA expression level of mouse *Keap1*

Table S5. Primers used for Construction of iRFP720-GFP Fusion Transgenes

Primer Sequence (5' to 3') (Forward, FP; Reverse, RP)	Amplicon Size (bp)	Description
FP: AAAA ACCGGT ATGGCGGAAGGATCCGTCGC RP: AAAA GAATTC CTCTTCCATCACGCCGATCTGC	948	To insert amplified 948bp iRFP720 into pAAV-pMecp2-SpCas9-spA by replacing SpCas9 with AgeI and EcoRI
FP: AAAA GAATTC GAGGGCAGAGGATCCCTGCTA RP: AAAA GAATTC TTACTTGTACAGCTCGTCCATGCC	774	To insert amplified 774bp T2A-GFP into pAAV-pMecp2-iRFP720-spA with EcoRI
FP: AAAA TCTAGA GTGGATAACCGTATTACCGCCATGC RP: AAAA ACCGGT GCTAGCGGATCTGACGGTTCAC	553	To insert amplified 553bp CMV promoter into pAAV-pMecp2-iRFP720-T2A-GFP-spA by replacing pMecp2 with XbaI and AgeI
FP: AAAA TCTAGA CGCGTGTGTCTAGACTGCAGAG RP: AAAA ACCGGT GTACCTTCTCGACTGCGCTCTCA	476	To insert amplified 476bp hSyn1 promoter into pAAV-pMecp2-iRFP720-T2A-GFP-spA by replacing pMecp2 with XbaI and AgeI
FP: GCCATCACCGAACGCCGT	-	Sequencing T2A-GFP that has been cloned into pAAV-pSyn1-iRFP720-T2A-GFP-spA vector
FP: TTCATCGGCTCCTGGCATC	-	Sequencing iRFP720-T2A that has been cloned into pAAV-pSyn1-iRFP720-T2A-GFP-spA vector
RP: AGATCATCACCCGATCGAAGC	-	Sequencing pSyn1-iRFP720 that has been cloned into pAAV-pSyn1-iRFP720-T2A-GFP-spA vector
RP: ACCGCACAGATGCGTAAGGAG	-	Sequencing GFP-spA that has been cloned into pAAV-pSyn1-iRFP720-T2A-GFP-spA vector

Table S6. Primers used for Modification of AAV1 Capsid

Primer Sequence (5' to 3') (Forward, FP; Reverse, RP)	Amplicon Size (bp)	Description
FP: AAAATGGCCACCGAAAGATTTGGGACCGTGGCAGTCAATTTCCAGAGCAGCA GCACTTTGGCGGTGCCTTTTAAGACAGACCCTGCGACCGGAGATGTGCATGCAAAA RP: TTTTGCATGCACATCTCCGGTCGCAGGGTCTGTCTTAAAAGGCACCGCCAAA GTGCTGCTGCTCTGGAAATTGACTGCCACGGTCCCAAATCTTTCGGTGGCCATTTT	-	108 bp DNA fragment bearing MscI-PHP.B-SphI for modification of AAV1 capsid
FP: TCCATCATCAACCCTGGCACTG	-	sequencing AAV1 capsid for validating 21bp of PHP.B sequence insertion

Table S7. Primers used for TaqMan qPCR

Primer Sequence (5' to 3') (Forward, FP; Reverse, RP)	Amplicon Size (bp)	Description
6-FAM-AGCGCCATCCGCCGCCTGCA-ZEN/Iowa	-	probe specific to iRFP720
FP: TTCATCGGCTCCTGGCATC RP: AGATCATCACCCGATCGAAGC	204	quantify the copies number of iRFP720 DNA
6-FAM-CACCACGCCGAGGACGCCCTGA-ZEN/Iowa	-	probe specific to dSaCas9
FP: TCCATCAATGGCGGCTTCA RP: GGCCTTGTCCAGTTTCTTCCA	150	quantify the copies number of dSaCas9 DNA