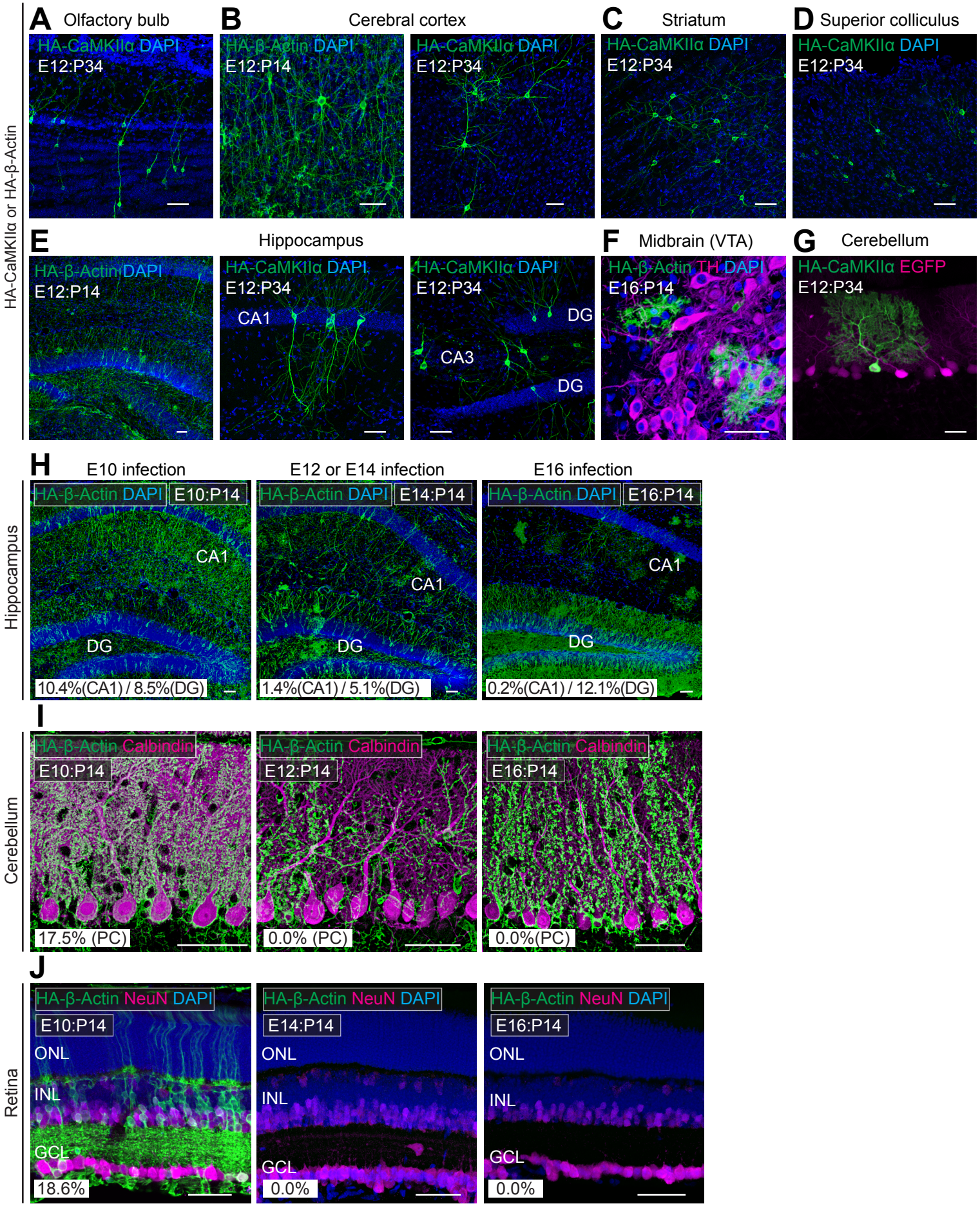
HA-CaMKIIα or HA-β-Actin
A Olfactory bulb
HA-CaMKIIα DAPI
E12:P34
B Cerebral cortex
HA-β-Actin DAPI
E12:P14
HA-CaMKIIα DAPI
E12:P34
C Striatum
HA-CaMKIIα DAPI
E12:P34
D Superior colliculus
HA-CaMKIIα DAPI
E12:P34
E Hippocampus
HA-β-Actin DAPI
E12:P14
HA-CaMKIIα DAPI
E12:P34
CA1
HA-CaMKIIα DAPI
E12:P34
CA3
DG
DG
F Midbrain (VTA)
HA-β-Actin TH DAPI
E16:P14
G Cerebellum
HA-CaMKIIα EGFP
E12:P34
H
Hippocampus
E10 infection
E12 or E14 infection
E16 infection
HA-β-Actin DAPI
E10:P14
CA1
DG
10.4%(CA1) / 8.5%(DG)
HA-β-Actin DAPI
E14:P14
CA1
DG
1.4%(CA1) / 5.1%(DG)
HA-β-Actin DAPI
E16:P14
CA1
DG
0.2%(CA1) / 12.1%(DG)
I
Cerebellum
HA-β-Actin Calbindin
E10:P14
17.5% (PC)
HA-β-Actin Calbindin
E12:P14
0.0% (PC)
HA-β-Actin Calbindin
E16:P14
0.0%(PC)
J
Retina
HA-β-Actin NeuN DAPI
E10:P14
ONL
INL
GCL
18.6%
HA-β-Actin NeuN DAPI
E14:P14
ONL
INL
GCL
0.0%
HA-β-Actin NeuN DAPI
E16:P14
ONL
INL
GCL
0.0%

Figure S1

Figure S1. HA Tag Sequence Insertion by HDR-Mediated Genome Editing, Related to Figure 1

(A–J) Confocal microscopic images of brain sections of Cas9 mice showing DAPI signal (blue), EGFP fluorescence (magenta), or immunoreactivities for TH, NeuN or calbindin (magenta) and the HA tag (green) fused to the N-terminus of endogenous CaMKIIα (A, B; right, C, D, E; middle and right, and G) or β-Actin (B; left, E; left, F, and H–J). Representative images of brain sections of hippocampus (H), cerebellum (I), and retina (J) transduced with AAV-HDR at E10 (left), E12 or E14 (middle), and E16 (right) are shown.
Scale bars, 50 μm.
See also Table S1.

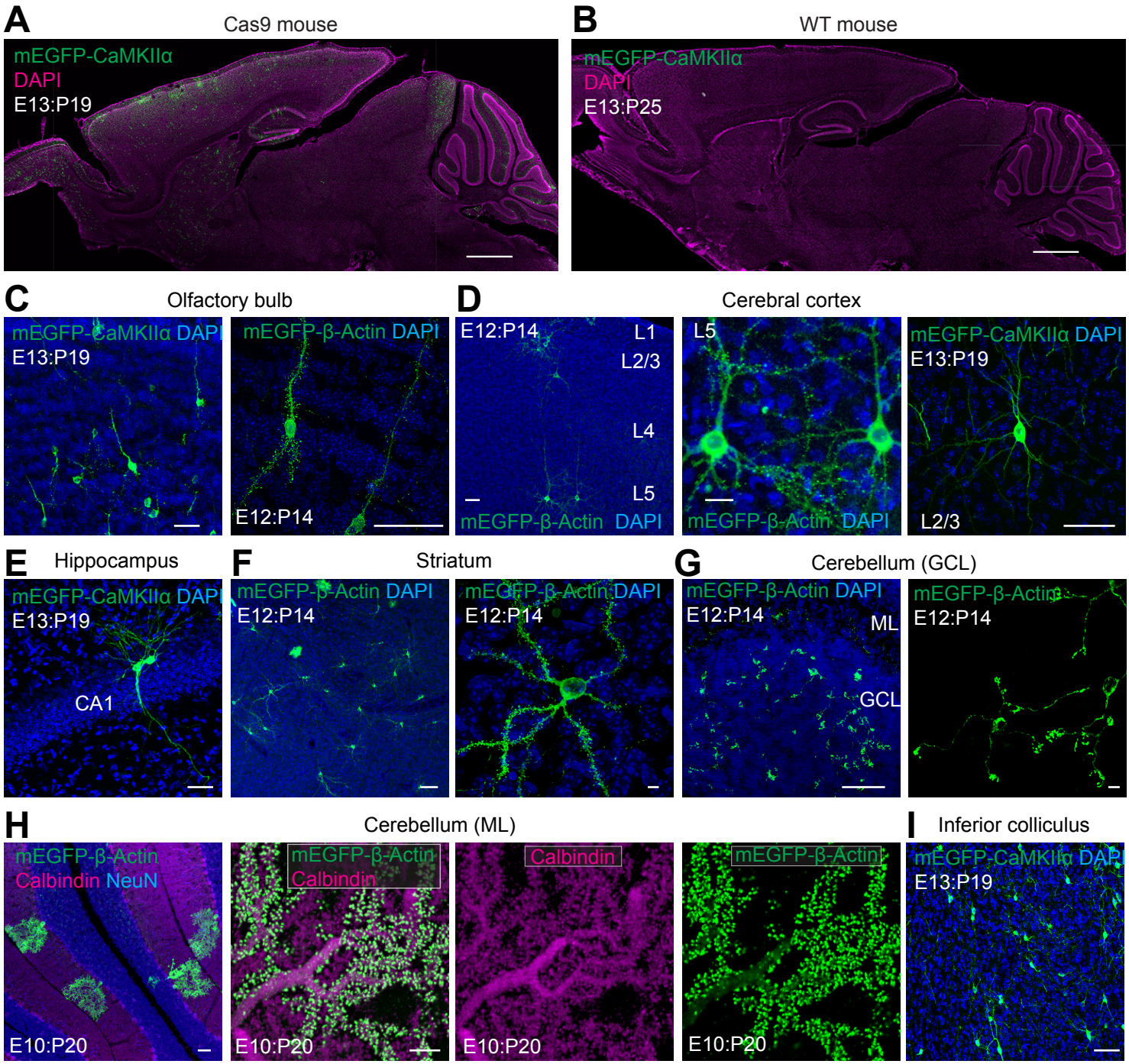
A
Cas9 mouse
mEGFP-CaMKIIα
DAPI
E13:P19
B
WT mouse
mEGFP-CaMKIIα
DAPI
E13:P25
C
Olfactory bulb
mEGFP-CaMKIIα DAPI
E13:P19
mEGFP-β-Actin DAPI
E12:P14
D
Cerebral cortex
E12:P14
L1
L2/3
L4
L5
mEGFP-β-Actin DAPI
L5
mEGFP-β-Actin DAPI
mEGFP-CaMKIIα DAPI
E13:P19
L2/3
mEGFP-CaMKIIα or mEGFP-β-Actin
E
Hippocampus
mEGFP-CaMKIIα DAPI
E13:P19
CA1
F
Striatum
mEGFP-β-Actin DAPI
E12:P14
mEGFP-β-Actin DAPI
E12:P14
G
Cerebellum (GCL)
mEGFP-β-Actin DAPI
E12:P14
ML
GCL
mEGFP-β-Actin
E12:P14
H
Cerebellum (ML)
mEGFP-β-Actin
Calbindin NeuN
E10:P20
mEGFP-β-Actin
Calbindin
E10:P20
Calbindin
E10:P20
mEGFP-β-Actin
E10:P20
I
Inferior colliculus
mEGFP-CaMKIIα DAPI
E13:P19

Figure S2

Figure S2. mEGFP Sequence Insertion by HDR-Mediated Genome Editing, Related to Figure 1

(A) Confocal microscopic images of whole sagittal brain sections of Cas9 (A) and wild type (B) mice showing mEGFP fluorescence (green) fused to the N-terminus of endogenous CaMKIIα and immunoreactivity for NeuN (magenta).
(C–I) Confocal microscopic images of brain sections of Cas9 mice showing DAPI signal (blue), mEGFP fluorescence (green) fused to the N-terminus of endogenous CaMKIIα (C; left, D; right, E, and I) or β-Actin (C; right, D; left and middle, F, G, and H), and immunoreactivities for NeuN and calbindin (magenta). L, cortical layer (D); ML, molecular layer; GCL, granule cell layer (G and H).
Scale bars, 1 mm (A); 50 μm (C, D; left and right, E, F; left, G; left, H; left, and I); 5 μm (D; middle, F; right, G; right, and H; middle and right).

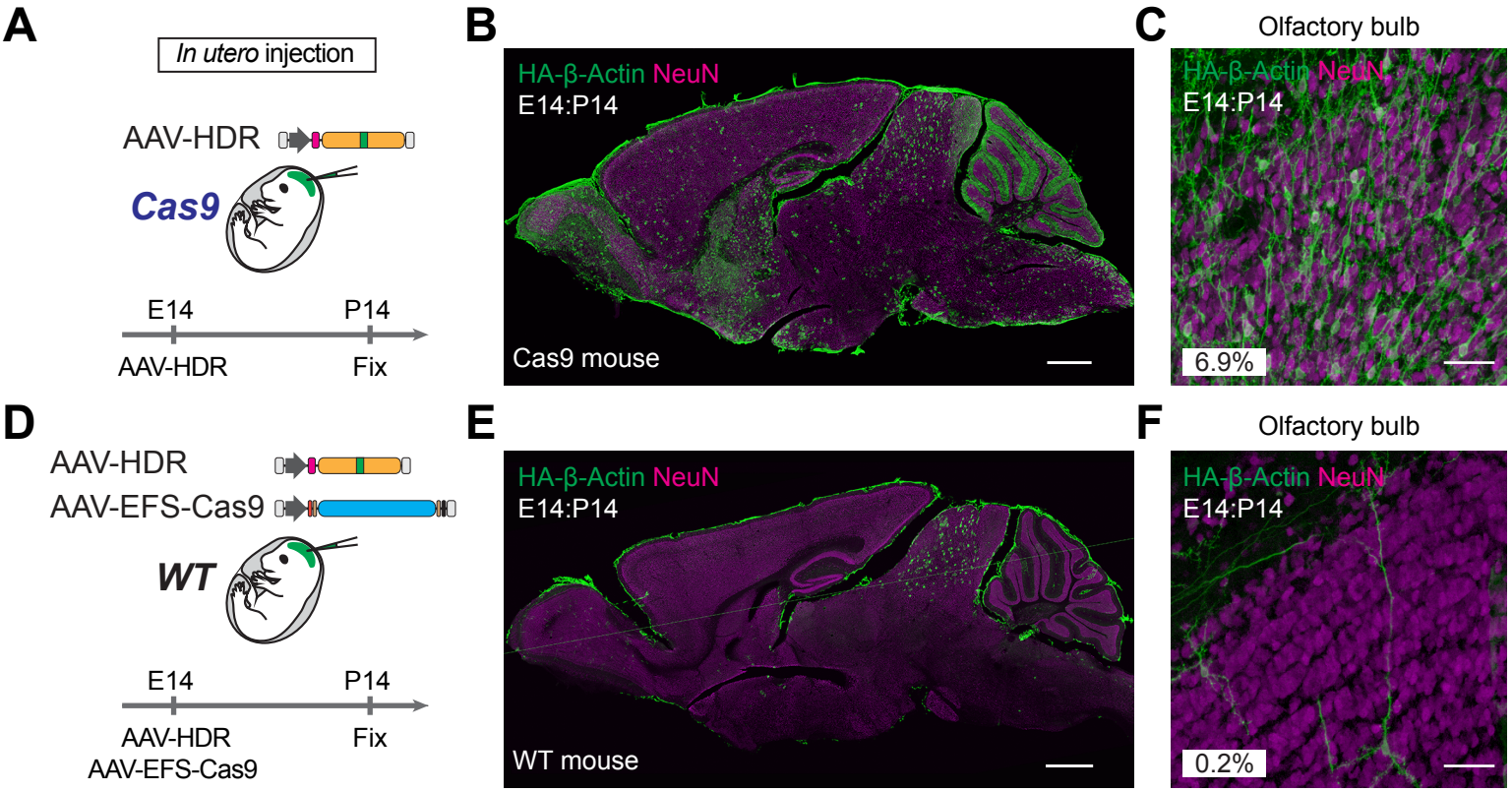
A
In utero injection
AAV-HDR
Cas9
E14
P14
AAV-HDR
Fix
B
HA-β-Actin NeuN
E14:P14
Cas9 mouse
C
Olfactory bulb
HA-β-Actin NeuN
E14:P14
6.9%
D
AAV-HDR
AAV-EFS-Cas9
WT
E14
P14
AAV-HDR
AAV-EFS-Cas9
Fix
E
HA-β-Actin NeuN
E14:P14
WT mouse
F
Olfactory bulb
HA-β-Actin NeuN
E14:P14
0.2%

Figure S3

Figure S3. Comparison of HDR Efficiency between Single and Dual AAV System in the Embryonic Mice Brain, Related to Figure 1 and 4

(A and D) Schematic illustrations of experiments.
(B, C, E, and F) Confocal microscopic images of brain sections of Cas9 mice injected with AAV-HDR (B and C) and wild type mice injected with AAV-HDR and AAV-EFS-Cas9 (E and F) showing immunoreactivities for NeuN (magenta) and HA tag (green) fused to the N-terminus of endogenous β-Actin. Representative images and knockin efficiency in the granule cell layer of the olfactory bulb are shown (C and F) ([olfactory bulb] single AAV system, HA/NeuN: 6.9±1.7%, n = 1910 cells / 3 slices; dual AAV system, HA/NeuN: 0.2±0.1%, n = 2861 cells / 3 slices).
Scale bars, 1 mm (B and E); 50 μm (C and F).

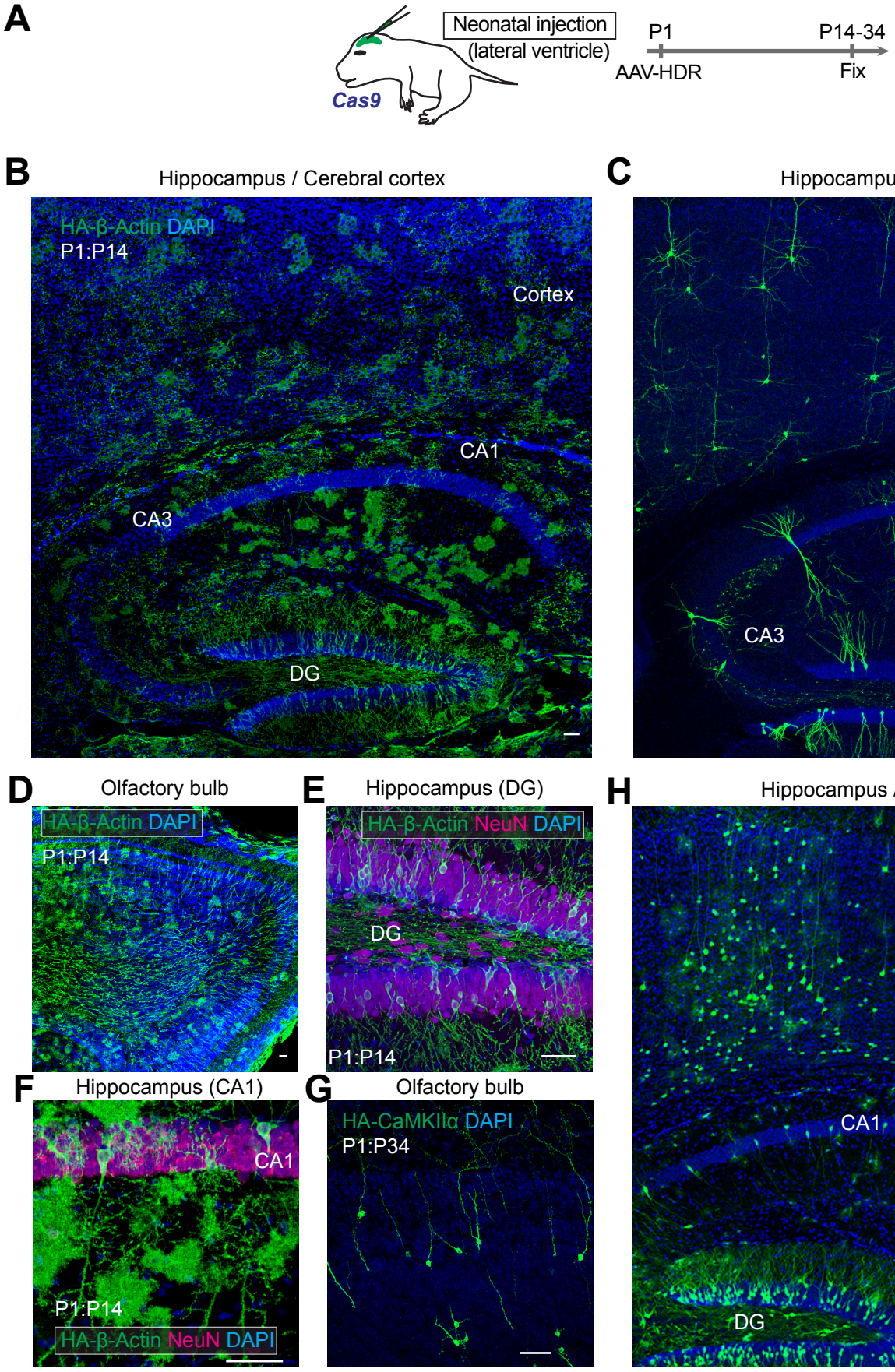
A
Neonatal injection
(lateral ventricle)
Cas9
P1
P14-34
AAV-HDR
Fix
B
Hippocampus / Cerebral cortex
HA-β-Actin DAPI
P1:P14
Cortex
CA1
CA3
DG
C
Hippocampus / Cerebral cortex
HA-CaMKIIα DAPI
P1:P34
Cortex
CA1
CA3
DG
D
Olfactory bulb
HA-β-Actin DAPI
P1:P14
E
Hippocampus (DG)
HA-β-Actin NeuN DAPI
DG
P1:P14
F
Hippocampus (CA1)
CA1
P1:P14
HA-β-Actin NeuN DAPI
G
Olfactory bulb
HA-CaMKIIα DAPI
P1:P34
H
Hippocampus / Cerebral cortex
HA-ERK2 DAPI
P1:P18
Cortex
CA1
DG

Figure S4

Figure S4. HDR-Mediated Genome Editing in Mitotic and Postmitotic Cells by Neonatal Intraventricular Injection, Related to Figures 1 and 2

(A) Schematic illustration of experiments.
(B–H) Confocal microscopic images of brain sections of the cortico-hippocampal regions (B, C, and H), olfactory bulb (D and G), and dentate gyrus (E) and CA1 (F) in the hippocampus showing DAPI signal (blue) and immunoreactivities for NeuN (magenta) and the HA tag (green) fused to the N-terminus of endogenous β-Actin (B, D, E, and F), CaMKIIα (C and G), and ERK2 (H).
Scale bars, 50 μm.

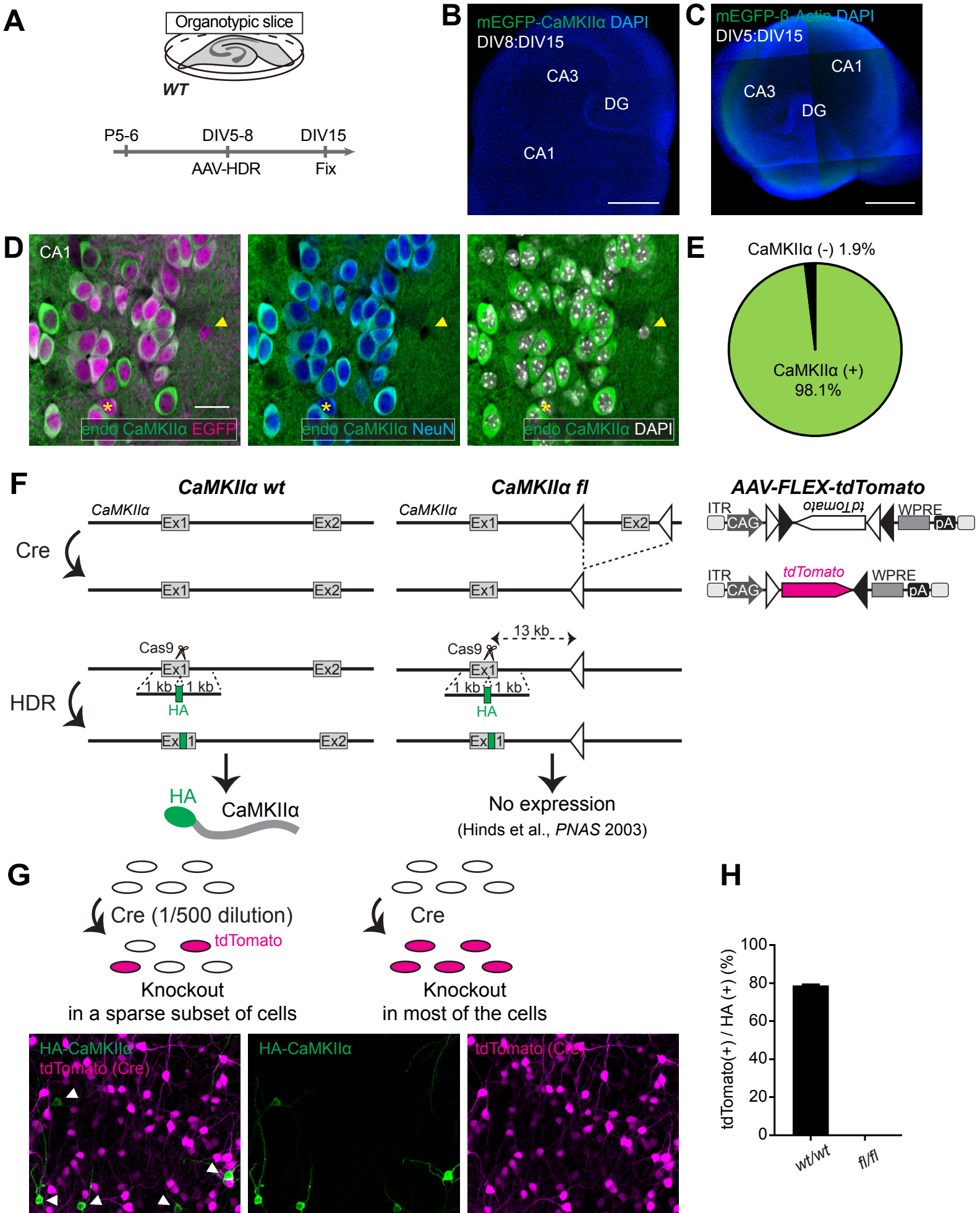
A
Organotypic slice
WT
P5-6
DIV5-8
AAV-HDR
DIV15
Fix
B
mEGFP-CaMKIIα DAPI
DIV8:DIV15
CA3
DG
CA1
C
mEGFP-β-Actin DAPI
DIV5:DIV15
CA1
CA3
DG
D
CA1
endo CaMKIIα EGFP
endo CaMKIIα NeuN
endo CaMKIIα DAPI
E
CaMKIIα (-) 1.9%
CaMKIIα (+)
98.1%
F
CaMKIIα wt
CaMKIIα fl
AAV-FLEX-tdTomato
CaMKIIα
Ex1
Ex2
Cre
ITR
CAG
tdTomato
WPRE
pA
Cas9
1 kb
HA
13 kb
HDR
HA
CaMKIIα
No expression
(Hinds et al., PNAS 2003)
G
Cre (1/500 dilution)
tdTomato
Knockout
in a sparse subset of cells
Cre
Knockout
in most of the cells
HA-CaMKIIα
tdTomato (Cre)
H
tdTomato(+) / HA (+) (%)
100
80
60
40
20
0
wt/wt
fl/fl

Figure S5

Figure S5. Verification of Specificity of AAV-Mediated Genome Editing, Related to Figures 2 and 4

(A) Schematic illustration of experiments.
(B and C) Confocal microscopic images of organotypic hippocampal slice cultures of wild type mice transduced with AAV-HDR for mEGFP-CaMKIIα (B) and mEGFP-β-Actin (C), showing the DAPI signal (blue) and mEGFP fluorescence (green) fused to the N-terminus of endogenous CaMKIIα and β-Actin.
(D) Confocal microscopic images of organotypic hippocampal slice cultures of Cas9 mice transduced with AAV-HDR for HA-CaMKIIα, showing the EGFP fluorescence (magenta), DAPI signal (white), and immunoreactivities for the endogenous CaMKIIα (green) and NeuN (blue). Yellow asterisk, CaMKIIα deleted cell (EGFP-positive, CaMKIIα-negative, NeuN-positive cell); yellow arrowhead, non-neuronal cell (EGFP-positive, CaMKIIα-negative, NeuN-negative cells).
(E) The ratio of CaMKIIα positive and negative cells in all infected neurons is shown (n = 53 cells).
(F) Graphical representation of the mouse genomic loci of *CaMKIIα* in wild type (left) and floxed (middle) mice. A sequence of 4 kb containing exon 2 is excised out by Cre recombinase only in floxed mice (upper). Thus, although the HA sequence is inserted by HDR in both genotypes (lower), CaMKIIα protein is expressed only in wild type mice. Schematic of AAV-FLEX-tdTomato (right). The inverted tdTomato sequence is re-inverted by Cre recombinase so that tdTomato can serve as a Cre reporter. Open triangle, loxP; filled triangle, lox2272.
(G) AAV-Cre is diluted to 1/500 so that CaMKIIα is knocked out in a sparse subset of cells, while CaMKIIα is deleted in most of the cells with the original concentration of AAV-Cre. Confocal microscopic images of organotypic hippocampal slice cultures prepared from CaMKIIα conditional knockout mice showing the tdTomato fluorescence (magenta) and immunoreactivity for the HA tag (green) fused to the N-terminus of endogenous CaMKIIα. Because CaMKIIα is deleted in a sparse subset of cells (tdTomato-positive), HA-positive neurons can be found (white arrowheads), although the HA-positive neurons are all tdTomato-negative.
(H) Summary data on the ratio of the number of tdTomato and HA double-positive cells to HA-positive cells in wild-type (n = 95 cells, 3 slices) and floxed (n = 16 cells, 3 slices) mice.
Scale bar, 50 μm (B–D and G).

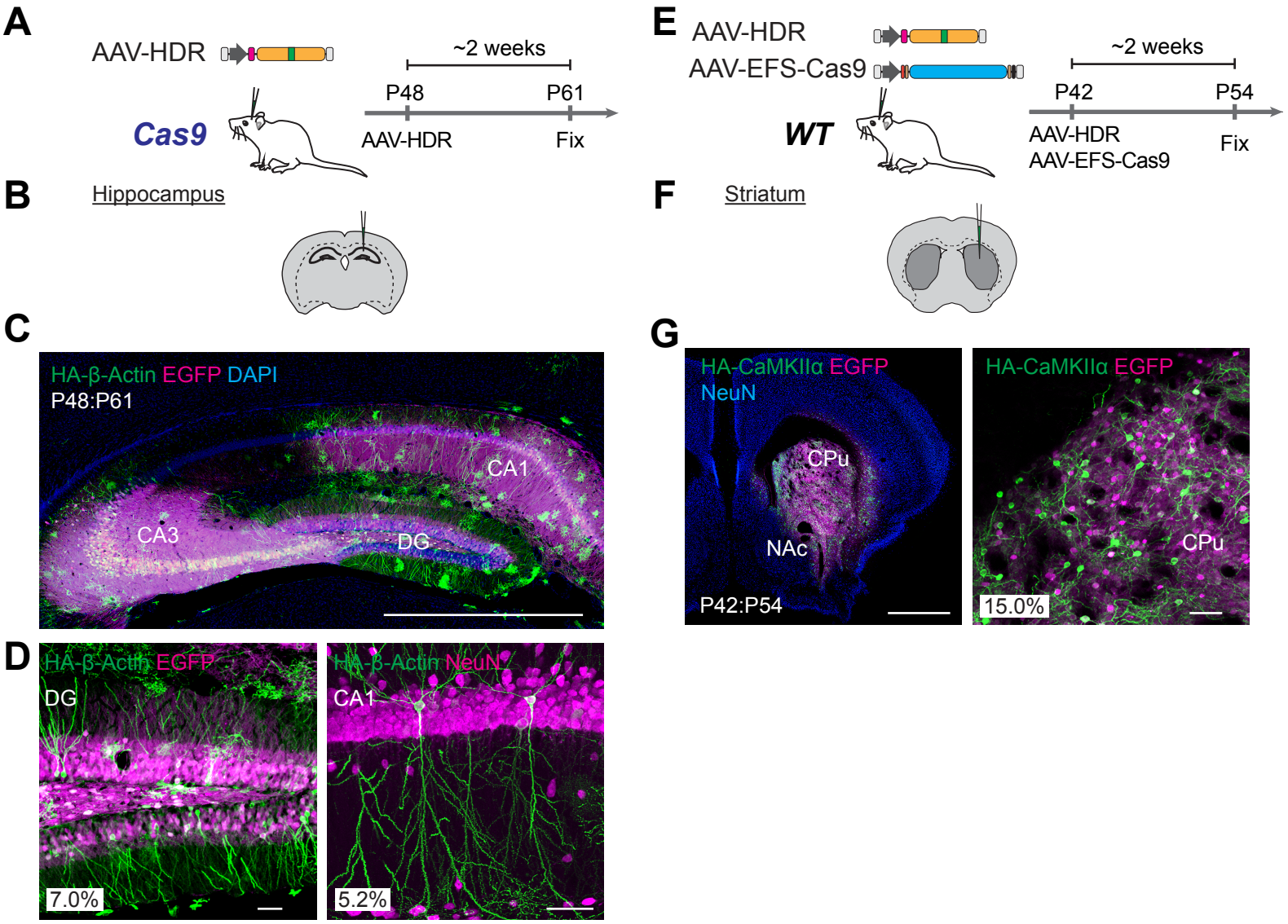
A
AAV-HDR
~2 weeks
Cas9
P48
P61
AAV-HDR
Fix
E
AAV-HDR
AAV-EFS-Cas9
~2 weeks
WT
P42
P54
AAV-HDR
AAV-EFS-Cas9
Fix
B
Hippocampus
F
Striatum
C
HA-β-Actin EGFP DAPI
P48:P61
CA1
CA3
DG
G
HA-CaMKIIα EGFP
NeuN
CPu
NAc
P42:P54
HA-CaMKIIα EGFP
CPu
15.0%
D
HA-β-Actin EGFP
DG
7.0%
HA-β-Actin NeuN
CA1
5.2%

Figure S6

Figure S6. HDR-Mediated Genome Editing *In Vivo*, Related to Figure 5

(A and E) Schematic illustrations of experiments.
(B and F) Schematic illustrations of AAV injection sites.
(C, D, and G) Confocal microscopic images of coronal brain sections of the hippocampus of Cas9 mice injected with AAV-HDR (C and D) and striatum of wild type mice injected with AAV-HDR and AAV-EFS-Cas9 (G) showing the EGFP fluorescence (magenta) and immunoreactivities for the HA tag (green) fused to the N-terminus of endogenous β-Actin (C and D) and CaMKIIα (G).
Scale bars, 50 μm (D and G; right); 1 mm (C and G).

	E10	E12 or E14	E16
Hippocampus : HA/NeuN (%)			
CA1 pyramidal neurons	10.4 ± 1.6 (496)	1.4 ± 0.1 (657)	0.2 ± 0.2 (647)
DG granule cells	8.5 ± 0.4 (1828)	5.1 ± 0.7 (1685)	12.1± 1.8 (1788)
Cerebellum: HA/Calbindin (%)			
Purkinje cells	17.5 ± 1.8 (238)	0.0 ± 0.0 (243)	0.0 ± 0.0 (238)
Retina : HA/NeuN (%)			
Ganglion cells	18.6 ± 2.6 (149)	0.0 ± 0.0 (257)	0.0 ± 0.0 (182)

Table S1. Spatiotemporal Regulation of Genome Editing Efficiency in Distinct Cell-types Regulated by the Timing of AAV-HDR Administration, Related to Figures S1.

The numbers of cells analyzed are indicated in parentheses.

HDR template	Sequences (5'-3'), bold lower case: start codon; bold underlined updper case: HA tag or mEGFP sequence ; bold upper case: linker
HA-β-Actin	tgcagctccttcgttgccggtccacacccgccaccaggtaagcagggacgccgggcccagcgggccttcgctctctcgtggctagtacctcactgcagggtcctgaggatcactcagaacggacaccatgggc gggtggagggtggtgccgggccgcggagcggacactggcacagccaactttacgcctagcgtgtagactctttgcagccacattcccgcggtgtagacactcgtgggcccgctcccgctcggtgcgtggggct tggggacacactagggtcgcggtgtgggcatttgatgagccggtgcggcttgcgggtgttaaaagccgtattaggtccatcttgagagtacacagtattgggaaccagacgctacgatcacgcctcaatggcctct gggtctttgtccaaaccggtttgcctattcggcttgccgggcgggcgggcgggcgggcgggcgcggcagggccggctcggccgggtgggggctgggatgccactgcgcgtgcgctctctatcactgggcatc gaggcgcgtgtgcgctagggagggagctcttcctctcccccctcttcctagttagctgcgcgtgcgtattgaggctgggagcgcggctgcccggggttgggcgagggcggggccgttgtccggaaggggcggg gtcacagtggcacgggcgccttgtttgcgcttcctgctgggtgtggtcgcctcccgcgcgcgcacaagccgcccgtcggcgcagtgtaggcggagcttgcgcccgtttggggagggggcggaggtctggcttc ctgccctaggtccgcctccgggccagcgtttgccttttatggtaataatgcggccggtctgcgcttcctttgtccctgagcttgggcgcgcgccccctggcggctcgagcccgcggcttgccggaagtgggcag ggcggcagcggctgctcttggcggccccgaggtgactatagccttctttttgtgtcttgatagttcgcc**atg**cat**TACCCATACGATGTTCCAGATTACGCT**gatgacgatatcgctgcg ctggtcgtcgacaacggctccggcatgtgcaaagccggcttcgcgggcgacgatgctccccgggctgttatcccctccatcgtgggccgccctaggcaccaggtaagtgacctgttacttttgggagtggcaagc ctggggttttcttggggatcgatgccggtgctaagaaggctgttcccttccacagggtgtgatggtgggaatgggtcagaaggactcctatgtgggtgacgaggcccagagcaagagaggtatcctgaccctgaa gtaccccattgaacatggcattgttaccaactgggacgacatggagaagatctggcaccacaccttctacaatgagctgcgtgtggcccctgaggagcaccctgtgctgctcaccgaggccccccctgaaccctaa ggccaaccgtgaaaagatgacccaggtcagtatcccgggtaacccttctctttggccagcttctcagccacgccctttctcaattgtctttcttctgccgttctcccataggactcccttctatgagctgagtctcccttg gatctttgcagtttctgctctttcccagacgaggtctttttttctctcaattgcctttctgactaggtgtttaaaccctacagtgctgtgggtttaggtactaacaatggctcgtgtgacaaagctaatgaggctggtgataag tggccttggagtgtgtattgagtagatgcacagtaggtctaagtggagcccctgtcctgagactcccagcacactgaacttagctgtgttcttgcactccttgcatgtctcagatctatccatacagtttcacctgccctg agtgtttcttgtggctttctgaacttgacaacattatttatttttctctacagatcatgtttgagaccttcaacaccccagccatgtacgtagccatccaggctgtgctgtccctgtatgcctctggtcgtaccacaggcattg tgatggac
mEGFP-β-Actin	tgcagctccttcgttgccggtccacacccgccaccaggtaagcagggacgccgggcccagcgggccttcgctctctcgtggctagtacctcactgcagggtcctgaggatcactcagaacggacaccatgggc gggtggagggtggtgccgggccgcggagcggacactggcacagccaactttacgcctagcgtgtagactctttgcagccacattcccgcggtgtagacactcgtgggcccgctcccgctcggtgcgtggggct tggggacacactagggtcgcggtgtgggcatttgatgagccggtgcggcttgcgggtgttaaaagccgtattaggtccatcttgagagtacacagtattgggaaccagacgctacgatcacgcctcaatggcctct gggtctttgtccaaaccggtttgcctattcggcttgccgggcgggcgggcgggcgggcgggcgcggcagggccggctcggccgggtgggggctgggatgccactgcgcgtgcgctctctatcactgggcatc gaggcgcgtgtgcgctagggagggagctcttcctctcccccctcttcctagttagctgcgcgtgcgtattgaggctgggagcgcggctgcccggggttgggcgagggcggggccgttgtccggaaggggcggg gtcacagtggcacgggcgccttgtttgcgcttcctgctgggtgtggtcgcctcccgcgcgcgcacaagccgcccgtcggcgcagtgtaggcggagcttgcgcccgtttggggagggggcggaggtctggcttc ctgccctaggtccgcctccgggccagcgtttgccttttatggtaataatgcggccggtctgcgcttcctttgtccctgagcttgggcgcgcgccccctggcggctcgagcccgcggcttgccggaagtgggcag ggcggcagcggctgctcttggcggccccgaggtgactatagccttctttttgtgtcttgatagttcgcc**atg**cat**GTGAGCAAGGGCGAGGAGCTCTTCACCGGGGTGGTG** **CCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCT** **ACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACC** **TACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTA** **CGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACC** **CTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACT** **ACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATC** **GAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACA** **ACCACTACCTGAGCACCCAGTCCAAGCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTG** **ACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAG**gatgacgatatcgctgcgctggtcgtcgacaacggctccggcatgtgcaaagccggcttcgcg ggcgacgatgctccccgggctgtattccccctccatcgtgggccgccctaggcaccaggtaagtgacctgttacttttgggagtggcaagcctggggttttcttggggatcgatgccggtgctaagaaggctgttcccttccacagggtgtgatggtgggaatgggtcagaaggactcctatgtgggtgacgaggcccagagcaagagaggtatcctgaccctgaagtaccccattgaacatggcattgttaccaactgggacgacatggaga agatctggcaccacaccttctacaatgagctgcgtgtggcccctgaggagcaccctgtgctgctcaccgaggcccccctgaaccctaaggccaaccgtgaaaagatgacccaggtcagtatcccgggtaaccct tctctttggccagcttctcagccacgccctttctcaattgtctttcttctgccgttctcccataggactcccttctatgagctgagtctcccttggatctttgcagtttctgctctttcccagacgaggtctttttttctctcaattg cctttctgactaggtgtttaaaccctacagtgctgtgggtttaggtactaacaatggctcgtgtgacaaagctaatgaggctggtgataagtggccttggagtgtgtattgagtagatgcacagtaggtctaagtggag ccccctgtcctgagactcccagcacactgaacttagctgtgttcttgcactccttgcatgtctcagatctatccatacagtttcacctgccctgagtgtttcttgtggctttctgaacttgacaacattatttatttttctctacag atcatgtttgagaccttcaacaccccagccatgtacgtagccatccaggctgtgctgtccctgtatgcctctggtcgtaccacaggcattgtgatggac
HA-CaMKIIα	gctgtggcagaggcctcaggaggggccctgctgctcagtggtgacagataggggtgagaaagcagacagagtcattccgtcagcattctgggtctgtttggtacttcttctcacgataaggtggcggtgtgatatg cacaatggctaaaaagcagggagagctggaaagaaacaaggacagagacagaggccaagtcaaccagaccaattcccagaggaagcaaagaaaccattacagagactacaaggggggaagggaaggaga gatgaattagcttcccctgtaaaccttagaacccagctgttgccagggcaacggggcaatacctgtctcttcagaggagatgaagttgccagggtaactacatcctgtctttctcaaggaccatcccagaatgtggc acccactagccgttaccatagcaactgcctctttgccccacttaatcccatcccgtctgttaaaagggccctatagttggaggtgggggaggtaggaagagcgatgatcacttgtggactaagtttgttcgcatcccc ttctccaacccccctcagtacatcaccctgggggaacagggtccacttgctcctgggcccacacagtcctgcagtattgtgtatataaggccagggcaaagaggagcaggttttaaagtgaaaggcaggcaggtgt tggggaggcagttaccggggcaacgggaacagggcgtttcggaggtggttgccatggggacctggatgctgacgaaggctcgcgaggctgtgagcagccacagtgccctgctcagaagccccaagctcgtc agtcaagccggttctccgtttgcactcaggagcacgggcaggcgagtggcccctagttctgggggcagcgcttcagcatcccagccctagttcccagcctaaagcctcgcctgcctgcccagtgccagg **atgT** **ACCCATACGATGTTCCAGATTACGCT**gctaccatcacctgcacccgattcacagaagagtaccagctctttgaggaactgggaaagtgagtcacacctcagggagtcctagagagca tggggatcagtctccttgggcaggggagggggcgtgtgcctatgtggccagagctgtcccgtgcatgcagagggaggtgtgcagcatgatttggaagcactgtgtatgcatagggtgtccagagttaggcgct gacacatttatgagtccagtcagcctggtgtgtgtgtgtgtgtgtgtgtgtgtgatggaaaggaaacagattggtggtgtatgcacacacctatgcatataaatacacatgacctgggttcacaaagatatctagaaac ttggggtttgaaagggattttcccacttggctggatctgagctggaatctatttttgtagttgtacttagaaacctccaatgacaaagaaagtttgccacccccctgacctggagatcttgtctttagacactgtgcagagtg gctagctttctcctagcacatcccctccggcttccccagataccttccgggagcaacagcttcagtacagaaggagttaatataaagcaatcacagtgttgggaggaggggaaggacagtcagccagccgggggg gtgccactgtggcactgactatacaaaacaactgtggtcctttggcaaagttgcccatcgtgttatatctcaggaggctgagaggagaaaggttccctccagaagagcagcaggctacagatataagagaaggttt ctagatgttcccagctaggttatggggtcctgggttggatcaggagttgggggagcagtttggcagagtgccccacggatgcatacgtgctcctaccagtcctgtttggttcctgctgtagcgcagtaacatgagac

mEGFP-CaMKIIα	gctgtggcagaggcctcaggaggggccctgctgctcagtggtgacagataggggtgagaaagcagacagagtcattccgtcagcattctgggtctgtttggtacttcttctcacgataaggtggcggtgtgatatg cacaatggctaaaaagcagggagagctggaaagaaacaaggacagagacagaggccaagtcaaccagaccaattcccagaggaagcaaagaaaccattacagagactacaagggggaagggaaggaga gatgaattagcttcccctgtaaaccttagaacccagctgttgccagggcaacggggcaatacctgtctcttcagaggagatgaagttgccagggtaactacatcctgtctttctcaaggaccatcccagaatgtggc acccactagccgttaccatagcaactgcctctttgccccacttaatcccatcccgtctgttaaaagggccctatagttggaggtgggggaggtaggaagagcgatgatcacttgtggactaagtttgttcgcatcccc ttctccaacccccctcagtacatcaccctgggggaacagggtccacttgctcctgggcccacacagtcctgcagtattgtgtatataaggccagggcaaagaggagcaggttttaaagtgaaaggcaggcaggtgt tggggaggcagttaccggggcaacgggaacagggcgtttcggaggtggttgccatggggacctggatgctgacgaaggctcgcgaggctgtgagcagccacagtgccctgctcagaagccccaagctcgtc agtcaagccggttctccgtttgcactcaggagcacgggcaggcgagtggcccctagttctgggggcagcgcttcagcatcccagccctagttcccagcctaaagcctcgcctgcctgcccagtgccagg **atg**tc c**GTGAGCAAGGGCGAGGAGCTCTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAG** **TTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGC** **TGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAG** **CAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTA** **CAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAG** **GACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGA** **ACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAA** **CACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCAAGCTGAGCAAAGACCCC** **AACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACA** **AG**gctaccatcacctgcacccgattcacagaagagtaccagctctttgaggaactgggaaagtgagtcacacctcagggagtcctagagagcatggggatcagtctccttgggcaggggaggggggcgtgtg cctatgtggccagagctgtcccgtgcatgcagagggaggtgtgcagcatgatttggaagcactgtgtatgcatagggtgtccagagttaggcgctgacacatttatgagtccagtcagcctggtgtgtgtgtgtgt gtgtgtgtgtgatggaaaggaaacagattggtggtgtatgcacacacctatgcatataaatacacatgacctgggttcacaaagatatctagaaacttggggtttgaaagggatttttcccacttggctggatctgagct ggaatctatttttgtagttgtacttagaaacctccaatgacaaagaaagtttgccacccccctgacctggagatcttgtctttagacactgtgcagagtggctagctttctcctagcacatcccctccggcttccccagata ccttccgggagcaacagcttcagtacagaaggagttaatataaagcaatcacagtgttgggaggaggggaaggacagtcagccagccgggggtgccactgtggcactgactatacaaaacaactgtggtcctt tggcaaagttgcccatcgtgttatatctcaggaggctgagaggagaaaggttccctccagaagagcagcaggctacagatataagagaaggtttctagatgttcccagctaggttatggggtcctgggttggatca ggagttgggggaggcagtttggcagagtgccccacggatgcatacgtgctcctaccagtcctgtttggttcctgctgtagcgcagtaacatgagac
mEGFP-CaMKIIα (Rat)	aggtaggggtgagaaagcagacagagccgttccttcagagttctgggtttgctctgtacttttcctcccccctaaggtgtcagtgtgatacacacagtgggtaaaaagcacacagagagagagagagagagagaga gagagagagagagagagagcaggagagaaatagggacagaggctgaggccaagtcaaccagaccaattcccagagggagcagagaaaccattacagagactacaagggggaagggaaggagaaagat gaattagccatccttcccccgtaaaccttagaacccagctgttgccagggcaacggggcaatacctgtctctccagaggagatgaagttgccagggtaactacatcctgtctttctcaaggaccatcccagaatgt ggcacccactagctgttaccatagcaactgcctctttgccccacttaatcccatcccgtctgttaaaagggccctatagttggaggtgggggaggtaggaagagaagatcacttgtggactaagtttgttcacatccc cttctccaacccccctcagtacatcaccctgggagaacaaggtccacttgcttctgggcccacacagtcctgcagtattgtgtatataaggccagggcaacggaggagcaggttttgaagtgaaaggcaggcaggt gttggggaggcagttaccggggcaacgggaacagggcgtttcggaggtggttgccatggggacctggatgctgacgaaggctcgcgaggctgtgagcagccacagtgccctgctcagaagccccaagctcg tcaatcaagctggttctccatttgcactcaggagcacgggcaggcgagtggcccctagttctgggagcagagtatcagcatcccagtcctagtcccgagcctaaagcctcgcctgcctgcccagtgccagg **atg**t cc**GTGAGCAAGGGCGAGGAGCTCTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAA** **GTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAG** **CTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAA** **GCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACT** **ACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGA** **GGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAG** **AACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGA** **ACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCAAGCTGAGCAAAGACCC** **CAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTAC** **AAG**gctaccatcacctgcacccgattcacggaagagtaccagctcttcgaggaactgggaaagtgagtcacacctcgggcagtcctagagggcatggggatcagcctcttgggcaggggggcgtgtgcctа tgtgactaaagctctcccgtgcatgcagacggaggcgtgcagcatgatttggaagcactgtgtatgcataggctgtccagagttaggcgttgacacatttatgtgcccagtcagcctggttgtgtgtgtgtgtgtgt gtgtgtgtgtgtgtgtgtgtgttggaaaggaagcagattgatattgatggtatttgcacacacctatgcatacaagtacacacgacctgggttcacaaagattttcagaaacttggggtttgaaagggattttcccact tggctgggtctgagctggaatcttttctgtagttgtacttagaaacctccagtgacggagagacttcacccccgcccctccccccttccattcccccttacttggagatcctgactttagactctatgcagggcagctag cttttctctgacacattcctttctgctttcccagcttccttccaggagcgagagcttcagtgctgaaggagttaacgtaaagcaatcgaggtgttgggaggaggggaaggacagtcagccagctgggggtgccactg tggctgtgtgcacaaaaactgtggtcctttggcaaagttgcctttggtgttatacctctatacctttgggaggctgcgaggagagaggttccctccagcagagcagcaggcaacagacataagaggtttctagatgttc ccagctgggttgtgggggtcctgggatggatcaggagttggggagcagtttggcagagtgccccacagatgcataagtgatcctaccagcc
HA-ERK2	agtggattctctagccacagagcagatcaaggtgtatctctccagatgtgtagagcaaatcactgcttttggtctctgcttatattctgtccagtgggcatcatggggttctaggactgtatctggttatcttgcgtgagtg atgtcactgagctctggatcaggagcagccttggatgctgtgggattcctgcttaatctagttttatatgtctaaaaggcatttttttaaaattatgctcattacagggtgaaagttttttttctgggtctgggttatgctttttggtt tctgtcccactctgatggtctaagttcatcagagtgcacaatgtgggccacgtattcttagaggtctgtctcaggggtcccgtggttggtgtgcagtgagtcctctggtctcaagccaagtccctggtcccgtgtgagtt cgttctggaccaggcacagcaccaggacagtgggtgggactctgtggggtccttatgcctaaattccaacctagctctctagagctacccagcgcttgggtctccgagcgcacatgacgtccctcccccgcagcc gcagtgctcctgcctcacctcgctgcttccgccgacccgcgagactccgcccctctctaccaagagaagccaatcaacacgcacgcccttgactccgccctagcgcccttcgacgcgaaccccttccctcctccca ctcgtagcccgcccgtcaggcaggaaggctggcagtggttctaccggcggttaattctctcctctgtgttgtcctccttcctcgttcccgatcgccggcgggggcggctacacgggcggcagcgcggttcctgcg ggaagcgcagcataagtcgagcggcagccgcgaagcgtcgaaccgaacgcggcggcggcggcggcggcggcggctgtgcagccaac**atg**TACCCATACGATGTTCCAGATTAC GCTTTGTACAAggcggcggcggcggcggcgggcccggagatggtccgcgggcaggtgttcgacgtagggccgcgctacaccaacctctcgtacatcggagaaggcgcctacggcatggtttggt gagtatccgcgctggatttcaggcccggcctcacgcggtcaccctgtgcccccgcctgtggctgatctggacgcacccacggcccttcccgcgccttggcctcgcgttctgcggcgccccggaccccggctt cttggccttcactcctggagccctggggcggggtcctggagccggtcgaggcttaggtgttgctcctcgtcttcctctgctgcttctcgtttgcggttggaggggcaaccccgattgaggccaggactcgaagactc cggggcttggtcttaaagcgcgtactttcgaggcttctccctcgggacgggaacggaagtacactgtgtctcctaatttatagcattatagccaccgcctcttgacattagattaaagccaggaattgtattagatgctt gtccgtggtggcggcggccatggttttgcaagacggaataataggcttgtgtgctgagcaccaaaatatttttcttctcttctgtgtacgtgaacagcattcacaaaatatcttaagtagcgctaatgatgctttctgcctc gggcttaaccgttaatgacacagcgttggttgtggacatattgaaatcttgaagtttcaaaatggatttaaatataccgattgtgtttcagaaaggataagccagcatcactctagaattattaagaagtatttggagttag tcctaatttgactttaaattatcccgtcgttaccctcaagagtactctcttaacagtgtgaatgtatccatatgttttagcccgattactgtgatcgtttccccataacgtttcagtgttcaagaatagtttttgcacgatcactt gcaggatttcctgcatgtggaatgttgaagtgtattgttttaactaa

Table S2. HDR donor template DNAs , Related to Figures 1, 2 and 4

	Name	Sequence (5'>3')	Source
sgRNA target sequence	β-Actin	tgtgtcttgatagttcgcca	Mikuni et al., 2016
	CaMKIIα	ctgcctgcccagtgccagga	Mikuni et al., 2016
	ERK2	cggcggctgtgcagccaaca	This study
Genomic PCR primer	HA-F	cccatacgatgttccagatt	Mikuni et al., 2016
	HA-R	gcgtaatctggaacatcgtatg	Mikuni et al., 2016
	GFP-F	catggtcctgctggagttcgtg	This study
	GFP-R	gctgaacttgtggccgtttac	This study
	β-Actin-F	ttcacctgccctgagtgtttc	This study
	β-Actin-R	gggagagcatagccctcgta	This study
	CaMKIIα-F	agcacaagcagaaactggga	This study
	CaMKIIα-R	tgaatcgggtgcaggtgatg	This study
	ERK2-F	tgtgttgtcctccttcctcg	This study
	ERK2-R	tcgccctgtaaaaggacttc	This study
DNA sequencing primer	β-Actin-S	gaacagccttcttagcaccg	Mikuni et al., 2016
	CaMKIIα-S	agccctagttcccagcctaa	This study
	ERK2-S	aggagtgaaggccaagaag	This study

Table S3. Oligonucleotide Sequences , Related to Figures 1 and 2