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Supplemental information

Severe deficiency of the voltage-gated sodium channel $Na_V1.2$ elevates neuronal excitability in adult mice

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Supplementary Figures:

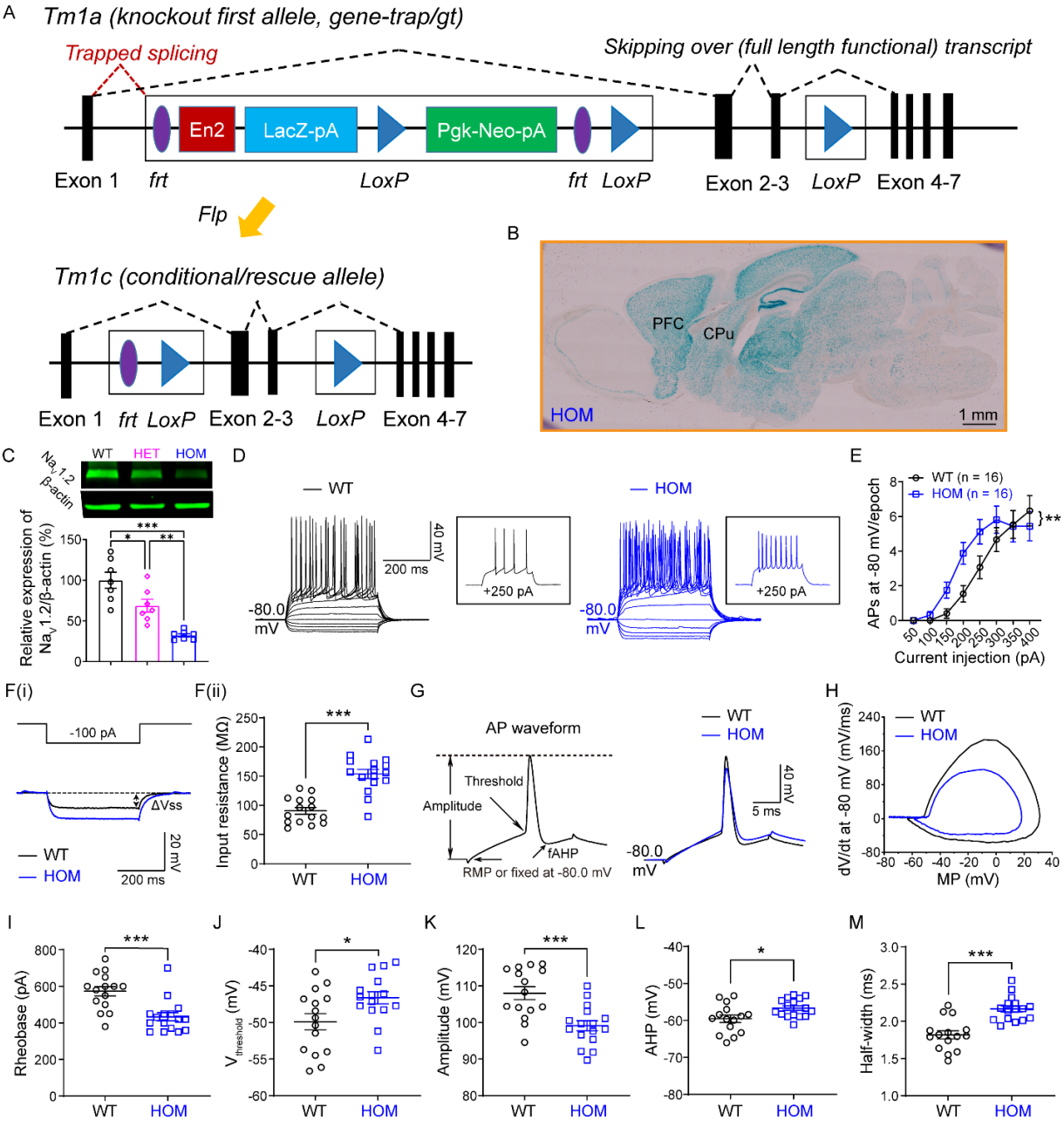

Figure S1. Elevated neuronal firings of striatal MSNs at a fixed membrane potential of -80 mV in adult $Na_V1.2$-deficient mice. Related to Figure 1.

(**A**) Gene trap (gt) allele has an inserted tm1a trapping cassette between the Exon 1 and Exon 2 of *Scn2a* gene in the genome, which traps the transcription from Exon 1 to tm1a cassette, resulting in a deficiency of *Scn2a*. In the presence of Flp recombinase, frt sites flanked trapping cassette will be removed, producing conditional ("rescue") allele that allows the expression of

Scn2a like the WT. *frt*, *Flp* recognition target (purple); *En2*, engrailed-2 splice acceptor (red); *LacZ*, *lacZ* β-galactosidase (light blue); *LoxP*, locus of X-over P1 (dark blue); and *Neo*, neomycin (green).

(**B**) gt cassette contains a *LacZ* element and is driven by the native *Scn2a* promoter. Thus, the *LacZ* expression can be used as a surrogate of *Scn2a* expression. Representative *LacZ* staining of a sagittal slice from a *Scn2a*$^{gt/gt}$ (HOM) mouse showing a strong blue signal across the brain including the prefrontal cortex (PFC) and dorsal striatum (CPu, caudate nucleus and the putamen). Scale bar, 1 mm.

(**C**) Upper: Representative Western blots of striatal tissues from WT (black circle), HET (magenta diamond), and HOM (blue square) mice. Lower: associated quantification of $Na_V1.2$ protein. One-way ANOVA followed by Tukey's multiple-comparison test: *p < 0.05; **p < 0.01; ***p < 0.001.

(**D**) Representative current-clamp recordings of MSNs from WT (black) and HOM (blue) mice were obtained at a fixed membrane potential of -80 mV. A series of 400-ms hyperpolarizing and depolarizing steps in 50-pA increments were applied to produce the traces. Inset: representative trace in response to +250 pA current injection.

(**E**) The average number of APs generated in response to depolarizing current pulses at -80 mV. Unpaired two-tailed non-parametric Mann-Whitney *U*-test for each current pulse: **p < 0.01.

(**Fi**) Representative traces in response to -100 pA current injection. $V_{steady\text{-}state}$ (V_{ss}) is the voltage recorded at 0-10 ms before the end of the stimulus.

(**Fii**) Individuals and average input resistance values at -80 mV. Unpaired two-tailed Student's *t*-test: ***p < 0.001.

(**G**) Left: plot of a typical AP showed its various phases. Right: typical spikes of MSNs from WT (black) and HOM (blue) mice were obtained at a fixed membrane potential of -80 mV.

(**H**) Associated phase-plane plots.

(**I-M**) Individuals and average spike rheobase, voltage threshold, amplitude, fast after-hyperpolarization (AHP), and half-width values. unpaired two-tailed Student's *t*-test: *p < 0.05; ***p < 0.001. Data were shown as mean ± SEM.

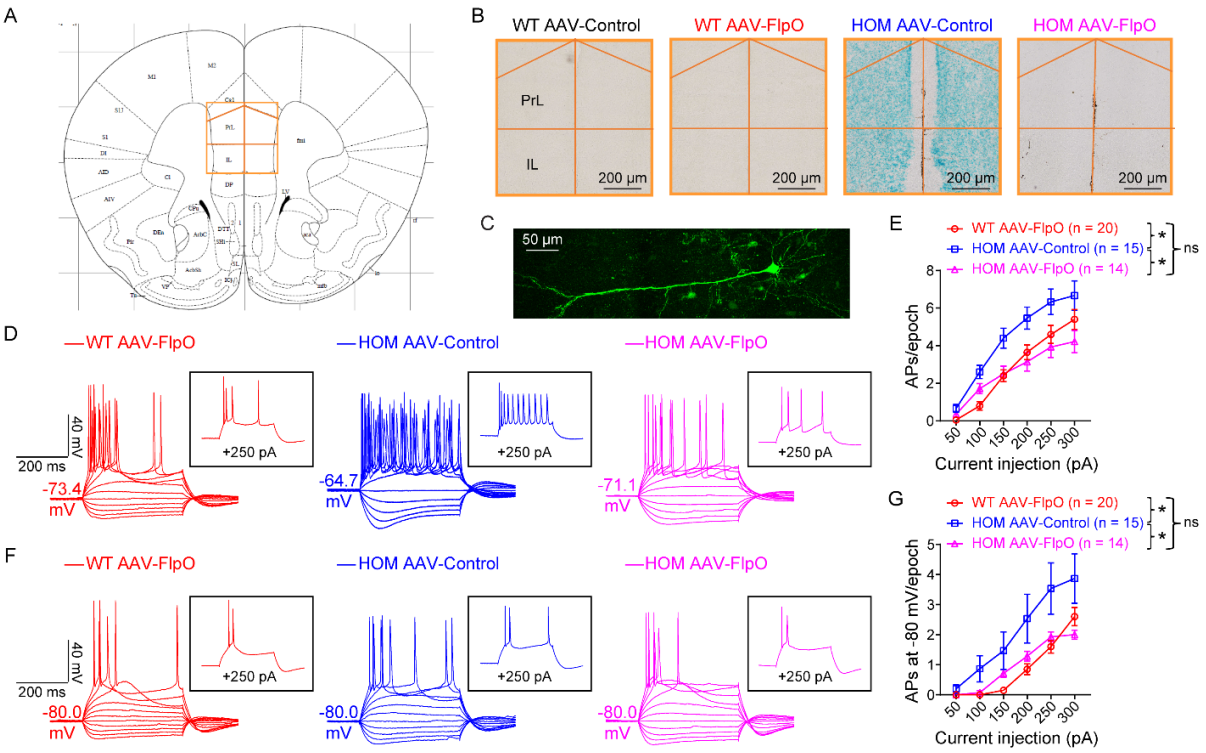

Figure S2. Elevated neuronal firings of layer V pyramidal cells in the mPFC are reversible by FlpO-mediated rescue in adult $Na_V1.2$-deficient mice. Related to Figure 2.

(**A-B**) *LacZ* staining of coronal brain slices containing mPFC from WT and *Scn2a*$^{gt/gt}$ (HOM) mice, which were systemically administered with AAV-Control or AAV-FlpO. PrL, prelimbic cortex; IL, infralimbic cortex.

(**C**) A typical layer V pyramidal neuron in the mPFC was labeled by neurobiotin. Scale bar, 50 μm.

(**D**) Representative current-clamp recordings of pyramidal cells from WT mice transduced with AAV-FlpO (red), HOM mice transduced with AAV-Control (blue), and HOM mice transduced with AAV-Control (magenta) at the RMP. A series of 400-ms hyperpolarizing and depolarizing steps in 50-pA increments were applied to produce the traces. Inset: representative trace in response to +250 pA current injection.

(**E**) The average number of APs generated in response to depolarizing current pulses at the RMP. Unpaired two-tailed non-parametric Mann-Whitney *U*-test for each current pulse: ns, no significance, $p > 0.05$; *$p < 0.05$.

(**F**) Representative current-clamp recordings of layer V pyramidal cells in the mPFC from WT transduced with AAV-FlpO (red), HOM transduced with AAV-Control (blue) and HOM transduced with AAV-Control (magenta) at a fixed membrane potential of -80 mV. A series of 400-ms hyperpolarizing and depolarizing steps in 50-pA increments were applied to produce the traces. Inset: representative trace in response to +250 pA current injection.

(**G**) The average number of APs generated in response to depolarizing current pulses at -80 mV. Unpaired two-tailed non-parametric Mann-Whitney *U*-test for each current pulse: ns, no significance, $p > 0.05$; *$p < 0.05$. Data were shown as mean ± SEM.

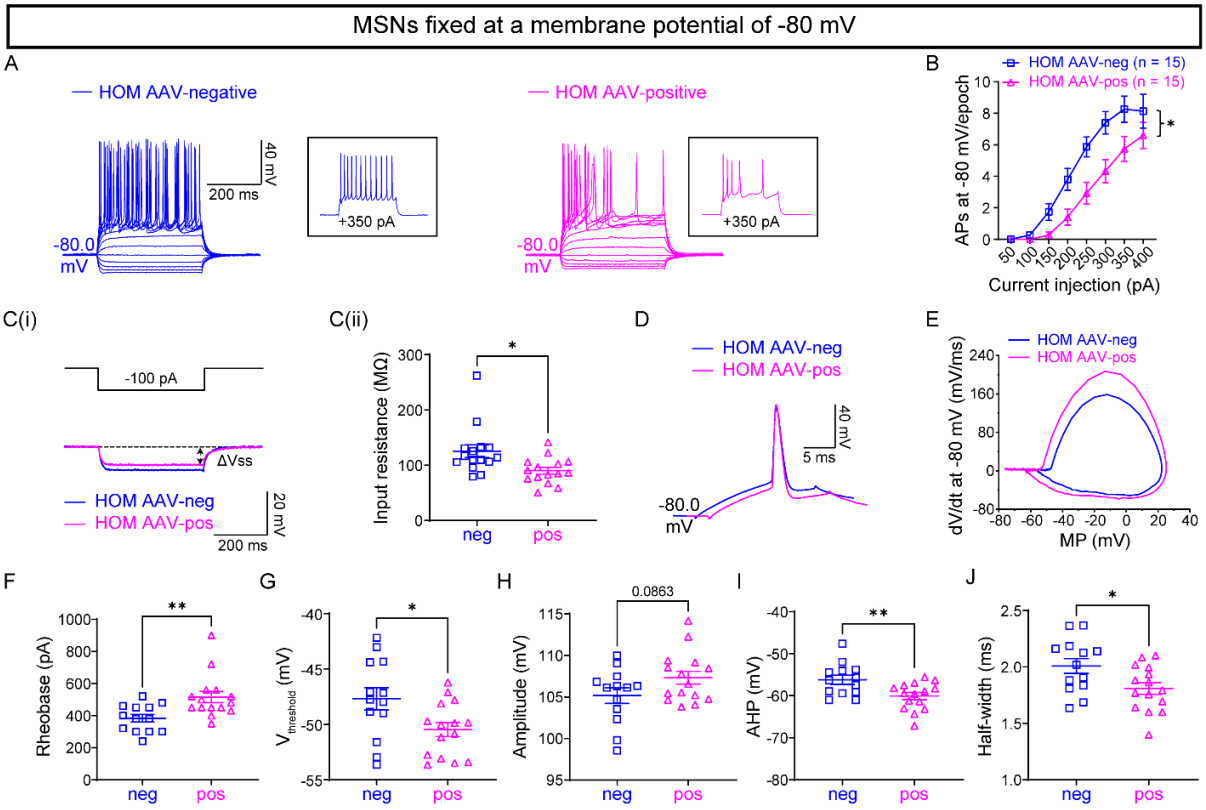

Figure S3. *Ex vivo* recordings of MSNs at a fixed membrane potential of -80 mV in adult $Na_V1.2$-deficient mice with a dilute AAV-FlpO-mCherry injection. Related to Figure 3.

(**A**) Representative current-clamp recordings of MSNs with AAV-negative (blue) or with AAV-FlpO-positive (magenta) in *Scn2a*$^{gt/gt}$ (HOM) mice were obtained at a fixed membrane potential of -80 mV. A series of 400-ms hyperpolarizing and depolarizing steps in 50-pA increments were applied to produce the traces. Inset: representative trace in response to +350 pA current injection.

(**B**) The average number of APs generated in response to depolarizing current pulses. Unpaired two-tailed non-parametric Mann-Whitney *U*-test for each current pulse: *p < 0.05.

(**Ci**) Representative traces in response to 100 pA negative current injection. $V_{steady\text{-}state}$ (V_{ss}) is the voltage recorded at 0-10 ms before the end of the stimulus.

(**Cii**) Individuals and average input resistance values at -80 mV. Unpaired two-tailed Student's *t*-test: *p < 0.05.

(**D**) Typical spikes of MSNs with AAV-negative (blue) or AAV-FlpO-positive (magenta) in HOM mice were obtained at a fixed membrane potential of -80 mV.

(**E**) Associated phase-plane plots at -80 mV.

(**F-J**) Individuals and average spike rheobase, voltage threshold, amplitude, AHP, and half-width values. Unpaired two-tailed Student's *t*-test: ns, no significance, p > 0.05; *p < 0.05; **p < 0.01. Data were shown as mean ± SEM.

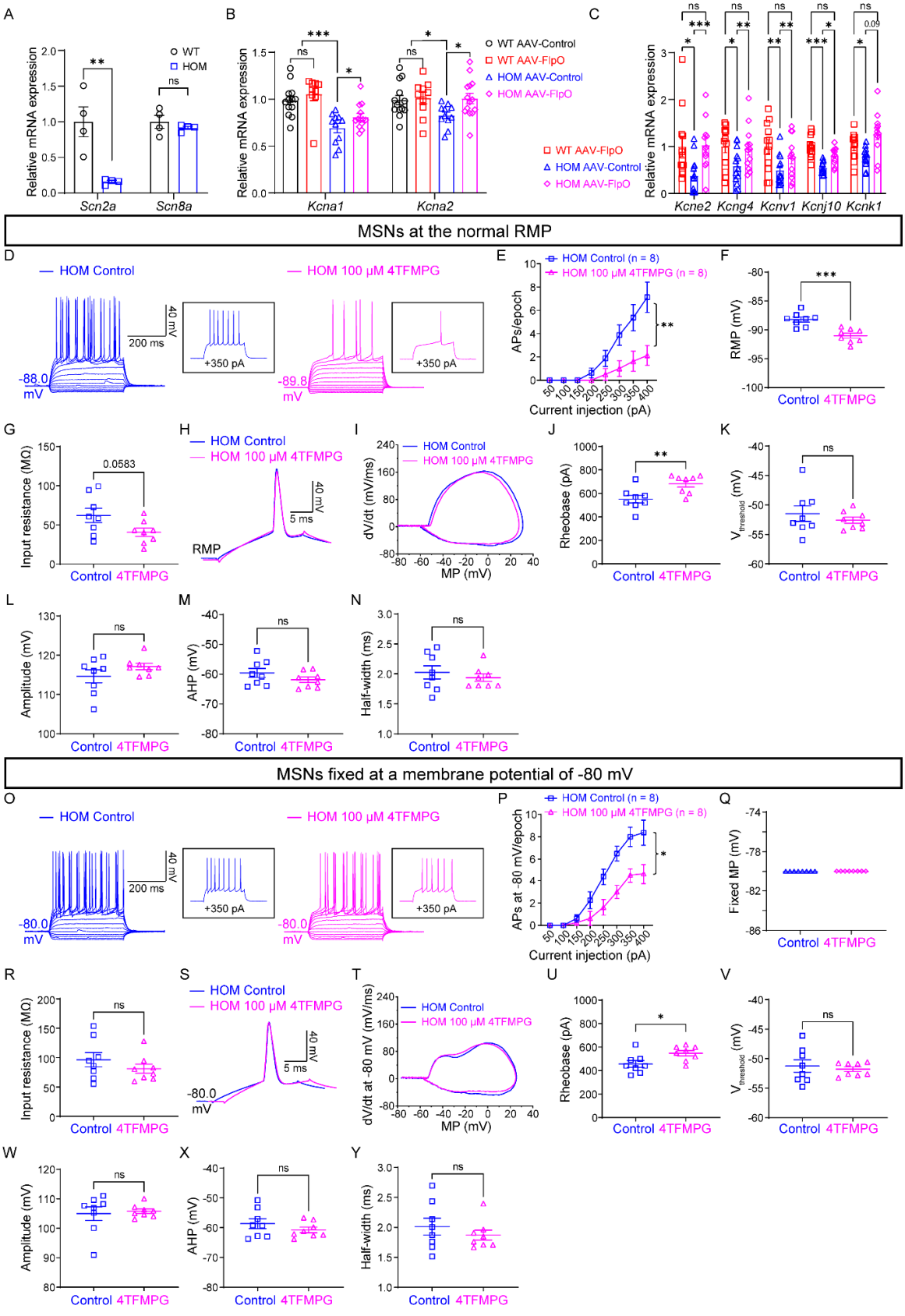
A
B
C
Relative mRNA expression
WT
HOM
Scn2a
Scn8a
WT AAV-Control
WT AAV-FlpO
HOM AAV-Control
HOM AAV-FlpO
Kcna1
Kcna2
Kcne2
Kcng4
Kcnv1
Kcnj10
Kcnk1
MSNs at the normal RMP
D
HOM Control
HOM 100 μM 4TFMPG
200 ms
40 mV
+350 pA
-88.0 mV
-89.8 mV
E
HOM Control (n = 8)
HOM 100 μM 4TFMPG (n = 8)
APs/epoch
Current injection (pA)
F
RMP (mV)
Control 4TFMPG
G
Input resistance (MΩ)
0.0583
H
5 ms
RMP
I
dV/dt (mV/ms)
MP (mV)
J
Rheobase (pA)
K
V threshold (mV)
L
Amplitude (mV)
M
AHP (mV)
N
Half-width (ms)
MSNs fixed at a membrane potential of -80 mV
O
-80.0 mV
P
APs at -80 mV/epoch
Q
Fixed MP (mV)
R
S
T
U
V
W
X
Y
ns

Figure S4. Specific activation of $K_V1.1$ channel by 4TFMPG reverses the elevated neuronal firings in adult $Na_V1.2$-deficient mice. Related to Figure 4.

(**A**) Quantitative (q)PCR analysis of *Scn2a* and *Scn8a* mRNA in the striatum samples from WT and *Scn2a*$^{gt/gt}$ mice. Unpaired two-tailed Student's *t*-test for each group: ns, no significance, $p > 0.05$; $^{**}p < 0.01$.

(**B**) qPCR analysis of *Kcna1* and *Kcna2* mRNA in the striatum samples from WT and HOM mice transduced with AAV-Control or AAV-FlpO, showing that the downregulated mRNA levels of $K_V1.1$ and $K_V1.2$ were partially reversible by FlpO-mediated restoration of $Na_V1.2$ expression in adult $Na_V1.2$-deficient mice. Unpaired two-tailed Student's *t*-test: ns, no significance, $p > 0.05$; $^{*}p < 0.05$; $^{***}p < 0.001$.

(**C**) qPCR analysis of *Kcne2*, *Kcng4*, *Kcnv1*, *Kcnj10,* and *Kcnk1* mRNA in the striatum samples from WT and HOM mice transduced with AAV-Control or AAV-FlpO, showing that the downregulated mRNA levels of *Kcne2*, *Kcng4*, *Kcnv1*, *Kcnj10,* and *Kcnk1* were partially reversible by FlpO-mediated restoration of $Na_V1.2$ expression in adult $Na_V1.2$-deficient mice. Unpaired two-tailed Student's *t*-test: ns, no significance, $p > 0.05$; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

(**D**) Representative current-clamp recordings of MSNs from HOM slices perfused with aCSF (HOM Control, blue) and HOM slices perfused with aCSF containing 4TFMPG (HOM 100 μM 4TFMPG, magenta) at the RMP. A series of 400-ms hyperpolarizing and depolarizing steps in 50-pA increments were applied to produce the traces. Inset: representative trace in response to +350 pA current injection.

(**E**) The average number of APs generated in response to depolarizing current pulses at the RMP. Unpaired two-tailed non-parametric Mann-Whitney *U*-test for each current pulse: $^{**}p < 0.01$.

(**F**) Individuals and average spike RMP values. Unpaired two-tailed Student's *t*-test: $^{***}p < 0.001$.

(**G**) Individuals and average input resistance values at the RMP. Unpaired *t*-test.

(**H**) Typical spikes of MSNs from HOM slices perfused with aCSF (HOM Control, blue) and HOM slices perfused with aCSF containing 4TFMPG (HOM 100 μM 4TFMPG, magenta) were obtained at the RMP.

(**I**) Associated phase-plane plots.

(**J-N**) Individuals and average AP rheobase, voltage threshold, amplitude, AHP, and half-width values.

(**O**) Representative current-clamp recordings of MSNs from HOM slices perfused with aCSF (HOM Control, blue) and HOM slices perfused with aCSF containing 4TFMPG (HOM 100 μM 4TFMPG, magenta) at a fixed membrane potential of -80 mV. A series of 400-ms hyperpolarizing and depolarizing steps in 50-pA increments were applied to produce the traces. Inset: representative trace in response to +350 pA current injection.

(**P**) The average number of APs generated in response to depolarizing current pulses at -80 mV. Unpaired two-tailed non-parametric Mann-Whitney *U*-test for each current pulse: $^{*}p < 0.05$.

(**Q**) Fixed MP values for recording.

(**R**) Individuals and average input resistance values at -80 mV. Unpaired two-tailed Student's *t*-test: ns, no significance, $p > 0.05$.

(**S**) Typical spikes of MSNs from *Scn2a*$^{gt/gt}$ slices perfused with aCSF (HOM Control, blue) and *Scn2a*$^{gt/gt}$ slices perfused with aCSF containing 4TFMPG (HOM 100 μM 4TFMPG, magenta) were obtained at a fixed membrane potential of -80 mV.

(**T**) Associated phase-plane plots.

(**U-Y**) Individuals and average AP rheobase, voltage threshold, amplitude, AHP, and half-width values. Unpaired two-tailed Student's *t*-test: ns, no significance, $p > 0.05$; *$p < 0.05$. Data were shown as mean ± SEM.

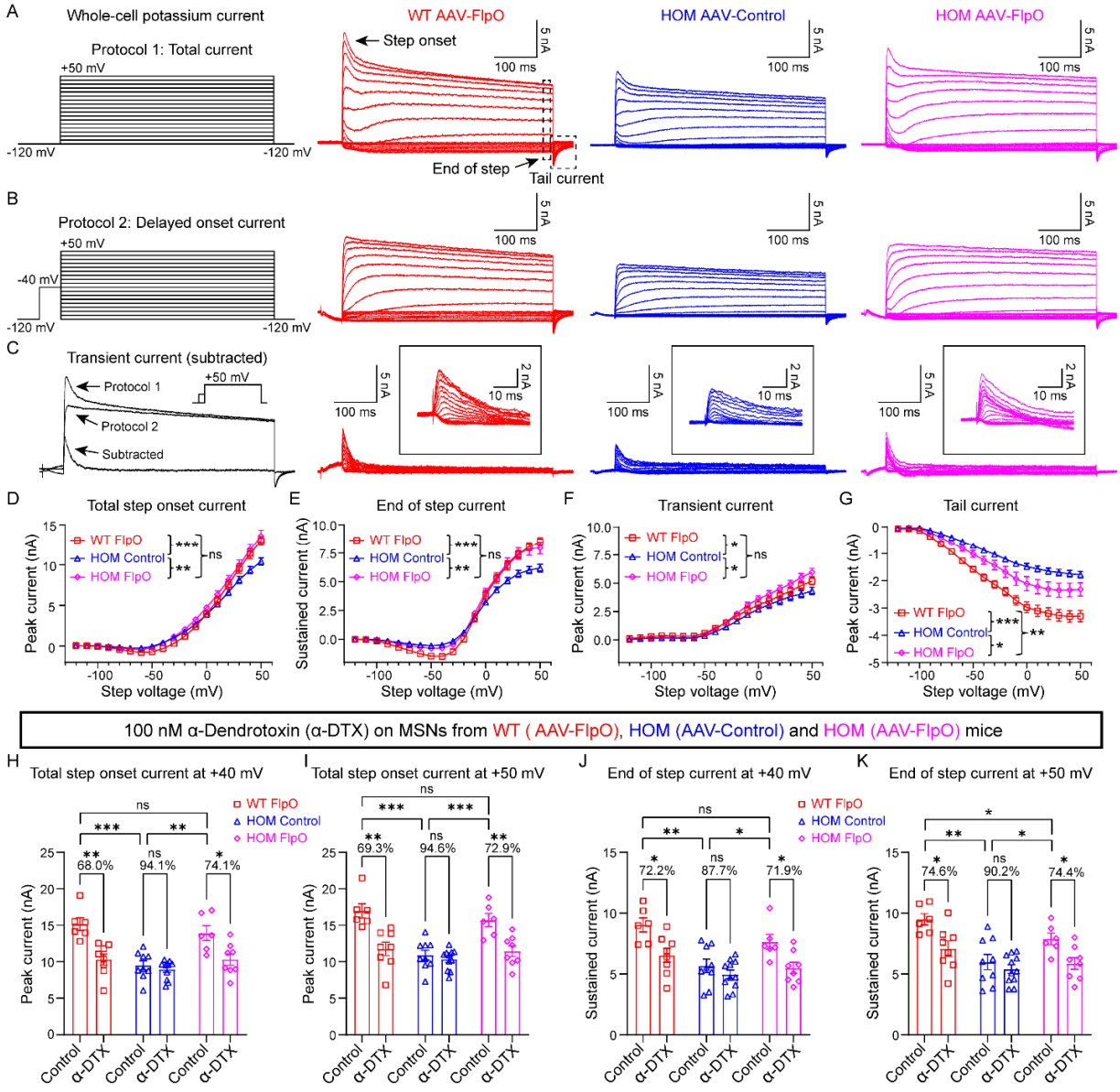

Figure S5. Reduced potassium currents in striatal MSNs are reversible by AAV-FlpO-mediated rescue in adult $Na_V1.2$-deficient mice. Related to Figure 4.

(**A-C**) Representative whole-cell potassium currents of MSNs in slices from WT mice transduced with AAV-FlpO (red), HOM mice transduced with AAV-Control (blue), and HOM mice transduced with AAV-FlpO (magenta). Voltage steps were from -120 mV to +50 mV. Total currents were obtained from 500-ms steps followed by 100-ms pulse back to -120 mV. Step onset, end of step, and tail are shown. Delayed onset currents were measured following 50-ms prepulse to -40 mV. Transient currents were calculated by subtracting delayed onset currents from the total currents. Voltage-gated Ca^{2+} channels were not blocked to allow for activation of Ca^{2+}-dependent K^+ channels.

(**D-G**) Summary for total step onset current, end of step current, transient current, and tail current, respectively. n = 17 neurons in the group of WT mice transduced with AAV-FlpO (red), n = 23

neurons in the group of HOM mice transduced with AAV-Control (blue), and n = 15 neurons in the group of HOM mice transduced with AAV-FlpO (magenta). Two-way ANOVA with repeated measures: ns, no significance, $p > 0.05$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

(**H-K**) 100 nM α-Dendrotoxin (α-DTX, blocker of K_V1) reduces ~30% whole-cell total potassium currents of MSNs in slices from WT mice transduced with AAV-FlpO (red), ~10% total currents from HOM mice transduced with AAV-Control (blue), and ~30% total currents from HOM mice transduced with AAV-Control (magenta). Summaries for total step onset currents (**H**, at +40 mV; **I**, at +50 mV) and end of step current (**J**, at +40 mV; **K**, at +50 mV) were shown respectively. Values are the percentage for peak currents recorded in slices perfused 100 nM α-DTX compared to each corresponding control group. Two-way ANOVA with repeated measures: ns, no significance, $p > 0.05$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. Data were shown as mean ± SEM.

Table S1. Oligonucleotides used in this study. Related to STAR Methods.

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Oligonucleotides		
Scn2a-Forward Sequence (5' -> 3') ATTTTCGGCTCATTCTTCACACT	https://pga.mgh.harvard.edu/primerbank/	PrimerBank ID: ID840654a1
Scn2a-Reverse Sequence (5' -> 3') GGGCGAGGTATCGGTTTTTGT	https://pga.mgh.harvard.edu/primerbank/	PrimerBank ID: 840654a1
Scn8a-Forward Sequence (5' -> 3') GCAAGCTCAAGAAACCACCC	https://pga.mgh.harvard.edu/primerbank/	PrimerBank ID: 951126a1
Scn8a-Reverse Sequence (5' -> 3') CCGTAGATGAAAGGCAAACTCT	https://pga.mgh.harvard.edu/primerbank/	PrimerBank ID: 951126a1
Kcna1-Forward Sequence (5' -> 3') CCCTACCCGAGAAGGAGTACC	https://pga.mgh.harvard.edu/primerbank/	PrimerBank ID: 119395751c2
Kcna1-Reverse Sequence (5' -> 3') GGATGACCATGACCGACACAA	https://pga.mgh.harvard.edu/primerbank/	PrimerBank ID: 119395751c2
Kcna2-Forward Sequence (5' -> 3') GCACCCACAAGACACCTATGA	https://pga.mgh.harvard.edu/primerbank/	PrimerBank ID: 31543024a1
Kcna2-Reverse Sequence (5' -> 3') GTCTCTGGGAACTGGGCTAAG	https://pga.mgh.harvard.edu/primerbank/	PrimerBank ID: 31543024a1
Kcne2-Forward Sequence (5' -> 3') CACATTAGCCAATTTGACCCAGA	https://pga.mgh.harvard.edu/primerbank/	PrimerBank ID: 19882205a1
Kcne2-Resverse Sequence (5' -> 3') GAACATGCCGATCATCACCAT	https://pga.mgh.harvard.edu/primerbank/	PrimerBank ID: 19882205a1
Kcng4-Forward Sequence (5' -> 3') CGCAGCCATGAGGAGATCAC	https://pga.mgh.harvard.edu/primerbank/	PrimerBank ID: 28076887a1
Kcng4-Resverse Sequence (5' -> 3') GCCAGGAAGCTCACGATCAC	https://pga.mgh.harvard.edu/primerbank/	PrimerBank ID: 28076887a1
Kcnv1-Forward Sequence (5' -> 3') ATTGCGCTCACTTGGGATGAC	https://pga.mgh.harvard.edu/primerbank/	PrimerBank ID: 28460685a1
Kcnv1-Resverse Sequence (5' -> 3') GTGAAGGTTGTATCAGGAATGCT	https://pga.mgh.harvard.edu/primerbank/	PrimerBank ID: 28460685a1
Kcnj10-Forward Sequence (5' -> 3') ACCTCAAGGATCTATGGACGAC	https://pga.mgh.harvard.edu/primerbank/	PrimerBank ID: 87116685c2
Kcnj10-Resverse Sequence (5' -> 3') AGCTACCAGATACCACACCAC	https://pga.mgh.harvard.edu/primerbank/	PrimerBank ID: 87116685c2
Kcnk1-Forward Sequence (5' -> 3') GAGGAGCTGCCTTATGAGGAC	https://pga.mgh.harvard.edu/primerbank/	PrimerBank ID: 6680538a1

Kcnk1-Resverse Sequence (5' -> 3') TCCCAATTCCAATTTCCCGAG	https://pga.mgh.harvard.edu/primerbank/	PrimerBank ID: 6680538a1
Actb-Forward Sequence (5' -> 3') GGCTGTATTCCCCTCCATCG	https://pga.mgh.harvard.edu/primerbank/	PrimerBank ID: 6671509a1
Actb-Reverse Sequence (5' -> 3') GGCTGTATTCCCCTCCATCG	https://pga.mgh.harvard.edu/primerbank/	PrimerBank ID: 6671509a1
Gapdh-Forward Sequence (5' -> 3') AGGTCGGTGTGAACGGATTTG	https://pga.mgh.harvard.edu/primerbank/	PrimerBank ID: 6679937a1
Gapdh-Reverse Sequence (5' -> 3') TGTAGACCATGTAGTTGAGGTCA	https://pga.mgh.harvard.edu/primerbank/	PrimerBank ID: 6679937a1
Scn2a1L-Forward Sequence (5' -> 3') GAGGCAAAGAATCTGTACTGTGGGG	https://www.nlac.narl.org.tw/RMRC/upload/deposit_file/201704241012510.pdf	RMRC13300
Scn2a1L-Reverse Sequence (5' -> 3') GACGCCTGTGAATAAAACCAAGGAA	https://www.nlac.narl.org.tw/RMRC/upload/deposit_file/201704241012510.pdf	RMRC13300