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Supplemental Information

Gαo is a major determinant of cAMP signaling in the pathophysiology of movement disorders

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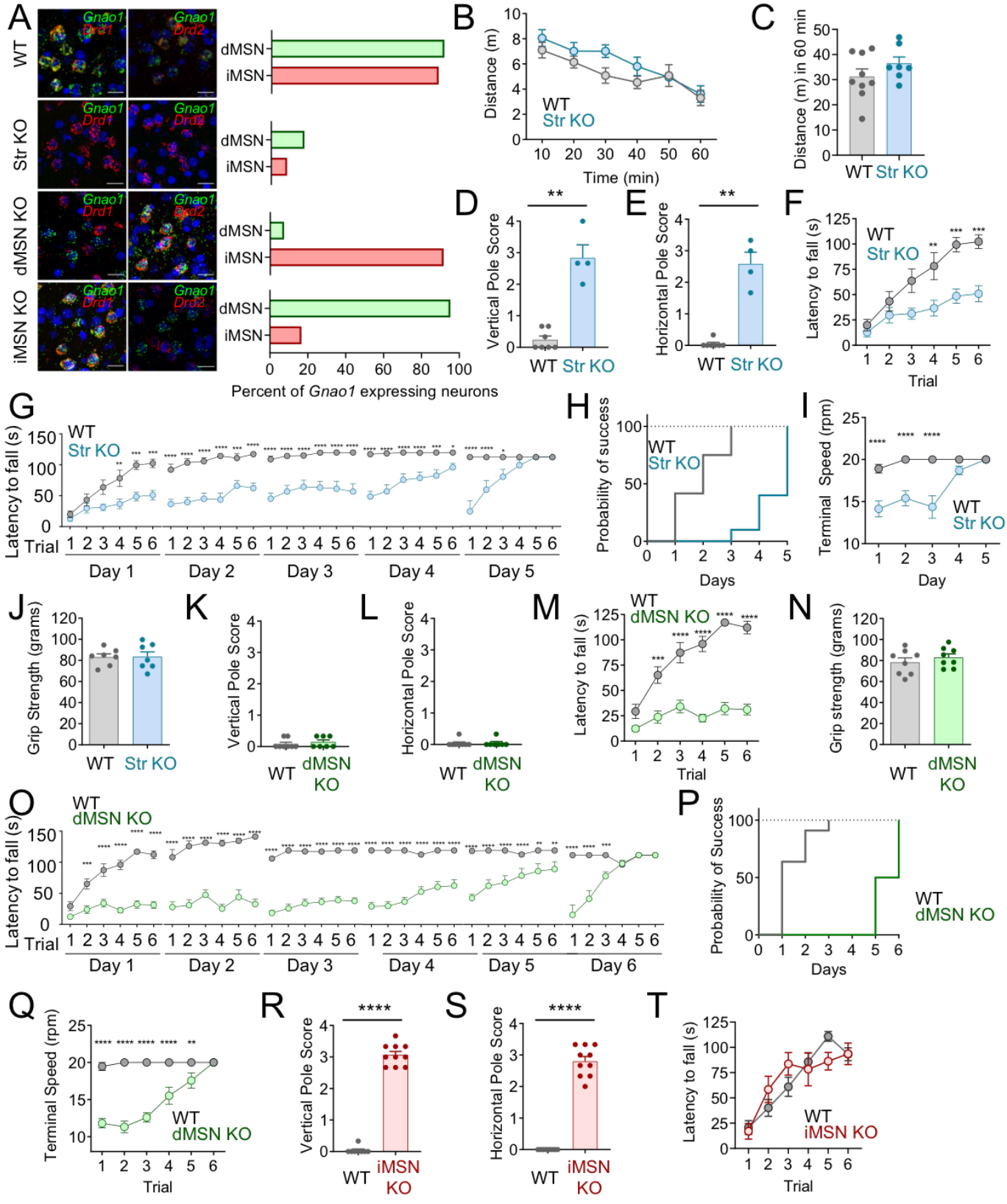
A
WT
Str KO
dMSN KO
iMSN KO
Gnao1
Drd1
Drd2
dMSN
iMSN
Percent of Gnao1 expressing neurons
B
Distance (m)
Time (min)
WT
Str KO
C
Distance (m) in 60 min
D
Vertical Pole Score
E
Horizontal Pole Score
F
Latency to fall (s)
Trial
G
Day 1
Day 2
Day 3
Day 4
Day 5
H
Probability of success
Days
I
Terminal Speed (rpm)
Day
J
Grip Strength (grams)
K
L
M
WT
dMSN KO
N
Grip strength (grams)
O
Day 6
P
Probability of Success
Q
R
iMSN KO
S
T

Figure S1. Related to Figure 1. Characterization of motor behavior in mice with striatal specific elimination of Gαo.

A) *In situ* hybridization showing expression of *Gnao1* mRNA in dMSN (*Drd1* mRNA) and iMSN (*Drd2* mRNA) in the dorsal striatum of adult WT (*Gnao1*$^{flox/flox}$*;n=4 mice*), Str KO (*Gnao1*$^{flox/flox}$*:RGS9*^{Cre}*;n=2 mice*), dMSN KO (*Gnao1*$^{flox/flox}$*:Drd1a*^{Cre}*;n=2 mice*), and iMSN KO (*Gnao1*$^{flox/flox}$*:Drd2*^{Cre}*;n=2 mice*). Quantification of percent of *Gnao1* expressing dMSNs or iMSNs. **B)** Time course in the open field assay. *Gnao1*$^{flox/flox}$ (WT; n=9) and *Gnao1*$^{flox/flox}$*:RGS9*^{Cre} (Str KO; n=8). **C)** Total distance traveled in the open field assay. *Gnao1*$^{flox/flox}$ (WT; n=9) and *Gnao1*$^{flox/flox}$*:RGS9*^{Cre} (Str KO; n=8), nonparametric t-test p=0.4302, Kolmogorov-Smirnov D=0.4127. **D)** Vertical pole assay pathology score for *Gnao1*$^{flox/flox}$ (WT; n=7) and *Gnao1*$^{flox/flox}$*:RGS9*^{Cre} (Str KO; n=4) mice. Nonparametric t-test p=0.0061, Kolmogorov-Smirnov D=1.000. **E)** Horizontal pole assay pathology score for *Gnao1*$^{flox/flox}$ (WT; n=7) and *Gnao1*$^{flox/flox}$*:RGS9*^{Cre} (Str KO; n=4) mice. Nonparametric t-test p=0.0030, Kolmogorov-Smirnov D=1.000. **F)** Performance of *Gnao1*$^{flox/flox}$ (WT; n=12) and *Gnao1*$^{flox/flox}$*:RGS9*^{Cre} (Str KO; n=10) littermates on intraday intervals on the accelerating rotarod. Two-way ANOVA, Bonferroni's multiple comparisons test. **G)** Daily performance of *Gnao1*$^{flox/flox}$ (WT; n=12) and *Gnao1*$^{flox/flox}$*:RGS9*^{Cre} (Str KO; n=10) mice on the accelerating rotarod over 5 days (6 trials/day). Two-way ANOVA, Bonferroni's multiple comparisons test. **H)** Probability of reaching maximal speed over 5 days of training on the accelerating rotarod for *Gnao1*$^{flox/flox}$ (WT; n=12) and *Gnao1*$^{flox/flox}$*:RGS9*^{Cre} (Str KO; n=10) mice. **I)** Time course of the terminal speed over 5 consecutive training days on the accelerating rotarod for *Gnao1*$^{flox/flox}$ (WT; n=12) and *Gnao1*$^{flox/flox}$*:RGS9*^{Cre} (Str KO; n=10) mice. Two-way ANOVA, Bonferroni's multiple comparisons test. **J)** Grip strength for *Gnao1*$^{flox/flox}$ (WT; n=7) and *Gnao1*$^{flox/flox}$*:RGS9*^{Cre} (Str KO; n=7) mice, nonparametric t-test p=0.9627, Kolmogorov-Smirnov D=0.2857. **K)** Vertical pole assay pathology score for *Gnao1*$^{flox/flox}$ (WT; n=8) and *Gnao1*$^{flox/flox}$*:Drd1a*^{Cre} (dMSN KO; n=7) mice. Nonparametric t-test p=0.6084, Kolmogorov-Smirnov D=0.1786. **L)** Horizontal pole assay pathology score for *Gnao1*$^{flox/flox}$ (WT; n=8) and *Gnao1*$^{flox/flox}$*:Drd1a*^{Cre} (dMSN KO; n=7) mice. Nonparametric t-test p>0.9999, Kolmogorov-Smirnov D=0.01786. **M)** Performance of *Gnao1*$^{flox/flox}$ (WT; n=11) and *Gnao1*$^{flox/flox}$*:Drd1a*^{Cre} (dMSN KO; n=8) littermates on intraday intervals on the accelerating rotarod. Two-way ANOVA, Bonferroni's multiple comparisons test. **N)** Grip strength for *Gnao1*$^{flox/flox}$ (WT; n=8) and *Gnao1*$^{flox/flox}$*:Drd1a*^{Cre} (dMSN KO; n=8) mice, nonparametric t-test p=0.6601, Kolmogorov-Smirnov D=0.3750. **O)** Daily performance of *Gnao1*$^{flox/flox}$ (WT; n=11) and *Gnao1*$^{flox/flox}$*:Drd1a*^{Cre} (dMSN KO; n=8) mice on the accelerating rotarod over 6 days (6 trials/day). Two-way ANOVA, Bonferroni's multiple comparisons test. **P)** Probability of reaching maximal speed over 6 days of training on the accelerating rotarod for *Gnao1*$^{flox/flox}$ (WT; n=11) and *Gnao1*$^{flox/flox}$*:Drd1a*^{Cre} (dMSN KO; n=8) mice. **Q)** Time course of the terminal speed over 6 consecutive training days on the accelerating rotarod for *Gnao1*$^{flox/flox}$ (WT; n=11) and *Gnao1*$^{flox/flox}$*:Drd1a*^{Cre} (dMSN KO; n=8) mice. Two-way ANOVA, Bonferroni's multiple comparisons test. **R)** Vertical pole assay pathology score for *Gnao1*$^{flox/flox}$ (WT; n=8) and *Gnao1*$^{flox/flox}$*:Drd2*^{Cre} (iMSN KO; n=10) mice. Nonparametric t-test p<0.0001, Kolmogorov-Smirnov D=1.000. **S)** Horizontal pole assay pathology score for *Gnao1*$^{flox/flox}$ (WT; n=8) and *Gnao1*$^{flox/flox}$*:Drd2*^{Cre} (iMSN KO; n=10) mice. Nonparametric t-test p<0.0001, Kolmogorov-Smirnov D=1.000. **T)** Performance of *Gnao1*$^{flox/flox}$ (WT; n=8) and *Gnao1*$^{flox/flox}$*:Drd2*^{Cre} (iMSN KO; n=7) littermates on intraday intervals on the accelerating rotarod. Two-way ANOVA, Bonferroni's multiple comparisons test. All data are represented as mean ± SEM; *p < 0.05; **p < 0.01; *** p < 0.001; **** p < 0.0001.

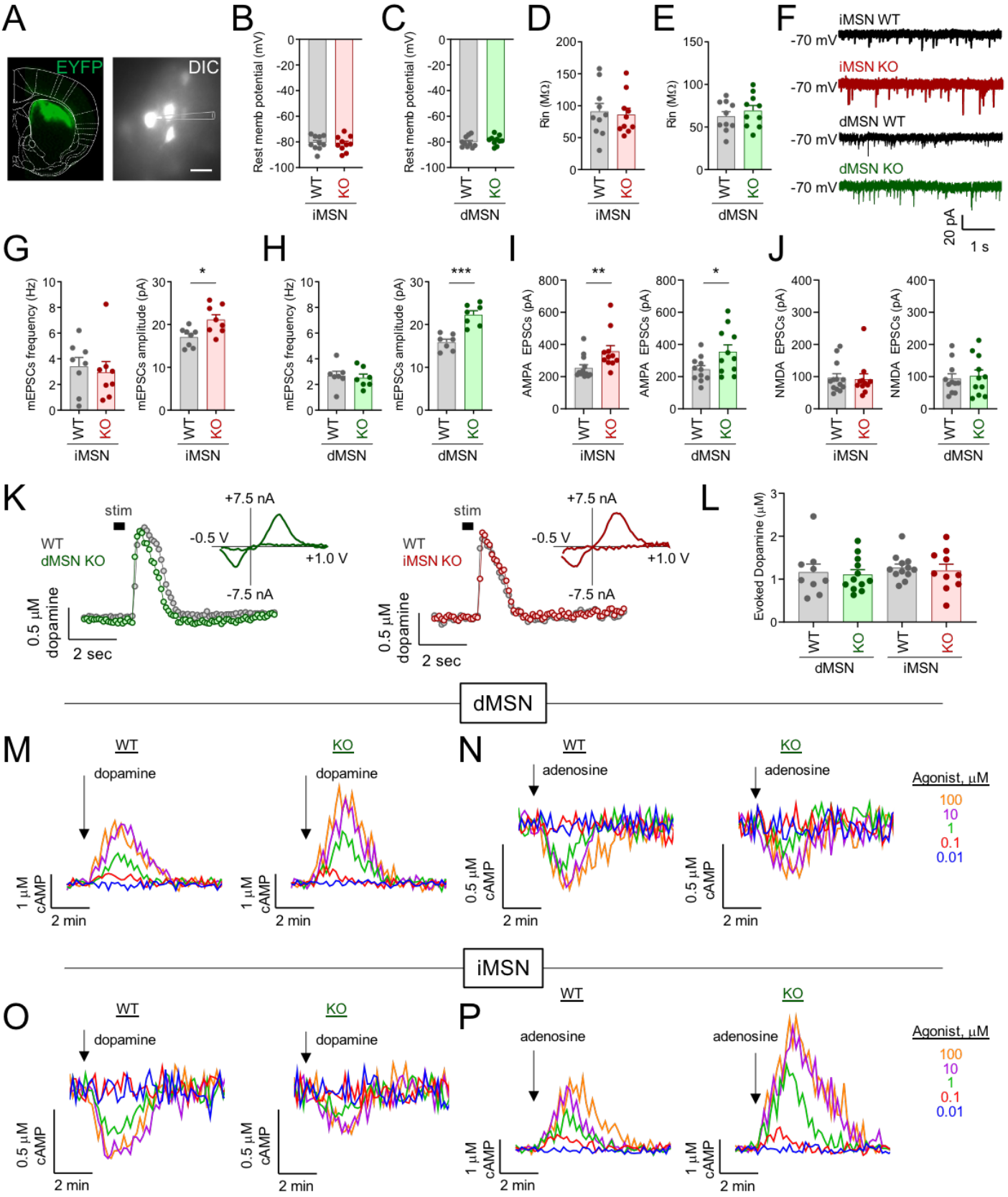
A
EYFP
DIC
B
Rest memb potential (mV)
WT
KO
iMSN
C
Rest memb potential (mV)
WT
KO
dMSN
D
Rin (MΩ)
WT
KO
iMSN
E
Rin (MΩ)
WT
KO
dMSN
F
iMSN WT
-70 mV
iMSN KO
-70 mV
dMSN WT
-70 mV
dMSN KO
-70 mV
20 pA
1 s
G
mEPSCs frequency (Hz)
mEPSCs amplitude (pA)
*
WT
KO
iMSN
iMSN
H
mEPSCs frequency (Hz)
mEPSCs amplitude (pA)
***
WT
KO
dMSN
dMSN
I
AMPA EPSCs (pA)
**
WT
KO
iMSN
AMPA EPSCs (pA)
*
WT
KO
dMSN
J
NMDA EPSCs (pA)
WT
KO
iMSN
NMDA EPSCs (pA)
WT
KO
dMSN
K
stim
WT
dMSN KO
+7.5 nA
-0.5 V
+1.0 V
-7.5 nA
0.5 µM
dopamine
2 sec
stim
WT
iMSN KO
+7.5 nA
-0.5 V
+1.0 V
-7.5 nA
0.5 µM
dopamine
2 sec
L
Evoked Dopamine (µM)
WT
KO
WT
KO
dMSN
iMSN
dMSN
M
WT
dopamine
KO
dopamine
1 µM
cAMP
2 min
1 µM
cAMP
2 min
N
WT
adenosine
KO
adenosine
Agonist, µM
100
10
1
0.1
0.01
0.5 µM
cAMP
2 min
0.5 µM
cAMP
2 min
iMSN
O
WT
dopamine
KO
dopamine
0.5 µM
cAMP
2 min
0.5 µM
cAMP
2 min
P
WT
adenosine
KO
adenosine
Agonist, µM
100
10
1
0.1
0.01
1 µM
cAMP
2 min
1 µM
cAMP
2 min

Figure S2. Related to Figure 2. Characterization *Gnao1* inactivation in striatal neurons. A) Coronal striatal section (100 µm) showing viral expression of EYFP targeted to dorsal striatum (left) and DIC image showing identification of EFYP MSNs in a coronal slice (300 µm) for electrophysiological recordings (right). **B)** Resting membrane potential of acute brain slice iMSNs from $Drd2^{Cre}$ (WT; n=7 mice/10 neurons) and $Gnao1^{flox/flox}$:$Drd2^{Cre}$ (iMSN KO; n=5 mice/10 neurons). Nonparametric t-test; Mann-Whitney test, p=0.7394. **C)** Resting membrane potential of acute brain slice dMSNs from $Drd1a^{Cre}$ (WT; n=7 mice/10 neurons) and $Gnao1^{flox/flox}$:$Drd1a^{Cre}$ (dMSN KO; n=6 mice/10 neurons). Nonparametric t-test; Mann-Whitney test, p=0.3930. **D)** Input resistance of acute brain slice iMSNs from $Drd2^{Cre}$ (WT; n=7 mice/10 neurons) and $Gnao1^{flox/flox}$:$Drd2^{Cre}$ (iMSN KO; n=5 mice/10 neurons). Nonparametric t-test; Mann-Whitney test, p=0.7959. **E)** Input resistance of acute brain slice dMSNs from $Drd1a^{Cre}$ (WT; n=7 mice/10 neurons) and $Gnao1^{flox/flox}$:$Drd1a^{Cre}$ (dMSN KO; n=6 mice/10 neurons). Nonparametric t-test; Mann-Whitney test, p=0.6305. **F)** Representative mEPSCs traces from the dorsal striatum of acute coronal brain slices. **G)** Quantification of iMSN mEPSCs frequency (nonparametric t-test;Mann-Whitney test, p=0.4418) and amplitude (nonparametric t-test;Mann-Whitney test, p=0.0148). (WT; n=4 mice/8 neurons; iMSN KO; n=3 mice/8 neurons). **H)** Quantification of dMSN mEPSCs frequency (nonparametric t-test;Mann-Whitney test, p=0.4557) and amplitude (nonparametric t-test;Mann-Whitney test, p =0.0006). (WT; n=3 mice/7 neurons; dMSN KO; n=3 mice/7 neurons). **I)** Quantification of AMPA EPSCs amplitude from $Drd2^{Cre}$ (WT; n=6 mice/13 neurons) and $Gnao1^{flox/flox}$:$Drd2^{Cre}$ (iMSN KO; n=6 mice/11 neurons). Nonparametric t-test; Mann-Whitney test, p=0.0048. Quantification of AMPA EPSCs amplitude from $Drd1a^{Cre}$ (WT; n=6 mice/11 neurons) and $Gnao1^{flox/flox}$:$Drd1a^{Cre}$ (dMSN KO; n=5 mice/11 neurons). Nonparametric t-test; Mann-Whitney test, p=0.0473. **J)** Quantification of NMDA EPSCs amplitude from $Drd2^{Cre}$ (WT; n=6 mice/13 neurons) and $Gnao1^{flox/flox}$:$Drd2^{Cre}$ (iMSN KO; n=6 mice/11 neurons). Nonparametric t-test; Mann-Whitney test, p=0.6905. Quantification of NMDA EPSCs amplitude from $Drd1a^{Cre}$ (WT; n=6 mice/11 neurons) and $Gnao1^{flox/flox}$:$Drd1a^{Cre}$ (dMSN KO; n=5 mice/11 neurons). Nonparametric t-test; Mann-Whitney test, p=0.9487. **K)** Representative traces of fast scan cyclic voltammetry (FSCV) and triangular waveform measuring evoked dopamine in response to electrical stimulation from the dorsal striatum of acute coronal brain slices. **L)** Quantification of evoked dopamine between dMSN WT (2 animals, 9 slices), dMSN KO (2 animals, 12 slices), iMSN WT (2 animals, 12 slices), and iMSN KO (2 animals, 9 slices). **M)** Representative traces of dopamine-induced cAMP responses in dMSNs from $Gnao1^{flox/flox}$ (WT) and $Gnao1^{flox/flox}$:$RGS9^{Cre}$ (dMSN KO). **N)** Representative traces of adenosine-induced cAMP responses in dMSNs from $Gnao1^{flox/flox}$ (WT) and $Gnao1^{flox/flox}$:$RGS9^{Cre}$ (dMSN KO). **O)** Representative traces of dopamine-induced cAMP responses in iMSNs from $Gnao1^{flox/flox}$ (WT) and $Gnao1^{flox/flox}$:$RGS9^{Cre}$ (iMSN KO). **P)** Representative traces of adenosine-induced cAMP responses in iMSNs from $Gnao1^{flox/flox}$ (WT) and $Gnao1^{flox/flox}$:$RGS9^{Cre}$ (iMSN KO). All data are represented as mean ± SEM; *p < 0.05; **p < 0.01; *** p < 0.001; **** p < 0.0001.

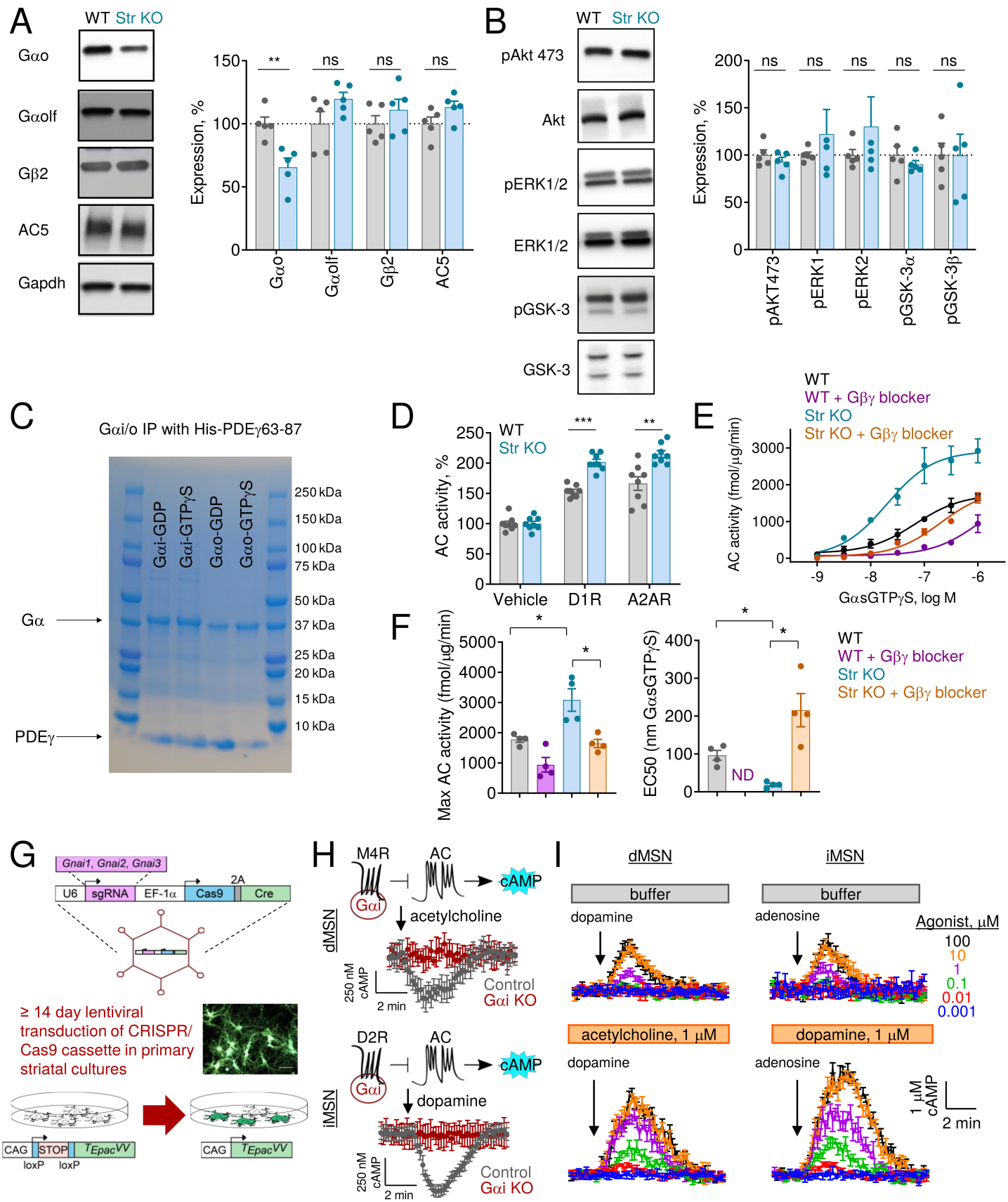
A
WT Str KO
Gαo
Gαolf
Gβ2
AC5
Gapdh
Expression, %
150
100
50
0
**
ns
ns
ns
Gαo
Gαolf
Gβ2
AC5
B
WT Str KO
pAkt 473
Akt
pERK1/2
ERK1/2
pGSK-3
GSK-3
Expression, %
200
150
100
50
0
ns
ns
ns
ns
ns
pAKT473
pERK1
pERK2
pGSK-3α
pGSK-3β
C
Gαi/o IP with His-PDEγ63-87
Gαi-GDP
Gαi-GTPγS
Gαo-GDP
Gαo-GTPγS
250 kDa
150 kDa
100 kDa
75 kDa
50 kDa
37 kDa
25 kDa
20 kDa
15 kDa
10 kDa
Gα
PDEγ
D
WT
Str KO
AC activity, %
250
200
150
100
50
0
***
**
Vehicle
D1R
A2AR
E
WT
WT + Gβγ blocker
Str KO
Str KO + Gβγ blocker
AC activity (fmol/µg/min)
3000
2000
1000
0
-9
-8
-7
-6
GαsGTPγS, log M
F
Max AC activity (fmol/µg/min)
5000
4000
3000
2000
1000
0
*
*
EC50 (nm GαsGTPγS)
400
300
200
100
0
*
*
ND
WT
WT + Gβγ blocker
Str KO
Str KO + Gβγ blocker
G
Gnai1, Gnai2, Gnai3
U6
sgRNA
EF-1α
Cas9
2A
Cre
≥ 14 day lentiviral transduction of CRISPR/ Cas9 cassette in primary striatal cultures
CAG
STOP
TEpacVV
loxP
loxP
CAG
TEpacVV
H
M4R
AC
cAMP
Gαi
acetylcholine
dMSN
250 nM cAMP
2 min
Control
Gαi KO
D2R
AC
cAMP
Gαi
dopamine
iMSN
250 nM cAMP
2 min
Control
Gαi KO
I
dMSN
iMSN
buffer
buffer
dopamine
adenosine
Agonist, µM
100
10
1
0.1
0.01
0.001
acetylcholine, 1 µM
dopamine, 1 µM
dopamine
adenosine
1 µM cAMP
2 min

Figure S3. Related to Figure 3. Biochemical characterization of striatal tissues lacking *Gαo*.
A) Western blot analysis of striatal tissue punches from *Gnao1*$^{flox/flox}$ (WT; n=5) and *Gnao1*$^{flox/flox}$*:RGS9*^{Cre} (Str KO; n=5) mice to examine Gαo (nonparametric t-test; Mann-Whitney test, p=0.0079), Gαolf (nonparametric t-test; Mann-Whitney test, p=0.2222), Gβ2 (nonparametric t-test; Mann-Whitney test, p=0.2698), and AC5 (nonparametric t-test; Mann-Whitney test, p=0.0952). **B)** Western blot analysis of striatal tissue punches from *Gnao1*$^{flox/flox}$ (WT; n=5) and *Gnao1*$^{flox/flox}$*:RGS9*^{Cre} (Str KO; n=5) mice to examine pAKT 473 (nonparametric t-test; Mann-Whitney test, p=0.5476), pERK1 (nonparametric t-test; Mann-Whitney test, p=0.8413), pERK2 (nonparametric t-test; Mann-Whitney test, p=0.6905), pGSK-3α (nonparametric t-test; Mann-Whitney test, p=0.3095), and pGSK-3β (nonparametric t-test; Mann-Whitney test, p>0.9999). **C)** Representative coomassie gel of *in vitro* immunoprecipitation between recombinant fragment of PDEγ with Gαi-GDP, Gαi-GTPγs, Gαo-GDP, or Gαo-GTPγS (n=3). **D)** Efficacy of vehicle (p>0.9999), D1R agonist (10 μM SKF81297; p=0.0002) and A2AR agonist (10 μM CGS21680; p=0.0031) on striatal AC activity (n=8 experiments), nonparametric t-test; Mann-Whitney test. **E)** Gαs-GTPγS dose response on striatal membranes in presence of a Gβγ scavenger (Grk2i peptide). n=4 experiments. **F)** Efficacy of Gαs-GTPγS, WT vs Str KO (nonparametric t-test; Mann-Whitney test, p=0.0286); Str KO vs Str KO + Gβγ scavenger (nonparametric t-test; Mann-Whitney test, p=0.0286). EC50 to Gαs-GTPγS, WT vs Str KO (nonparametric t-test; Mann-Whitney test, p=0.0286); Str KO vs Str KO + Gβγ scavenger (nonparametric t-test; Mann-Whitney test, p=0.0286). n=4 experiments. **G)** Schematic of Gαi KO utilizing CRISPR/Cas9 in cAMP Encoded Reporter (CAMPER) primary striatal neurons to simultaneously express the ^{T}Epac^{VV} biosensor. **H)** Average traces of Gαi KO (n=9 neurons) or control (n=10 neurons) dMSN response to acetylcholine (top) and iMSN response to dopamine (n= 30 neurons/control; 26 neurons Gαi KO) (bottom). **I)** Average traces of dopamine-induced cAMP response in Gαi KO dMSNs in the presence (n≥6 neurons/dose) or absence of acetylcholine (n=10 neurons/dose) (left). Average traces of adenosine-induced cAMP response in Gαi KO iMSNs in the presence (n≥5 neurons/dose) or absence of dopamine (n=10 neurons/dose) (right). All data are represented as mean ± SEM; *p < 0.05; **p < 0.01; *** p < 0.001; **** p < 0.0001.

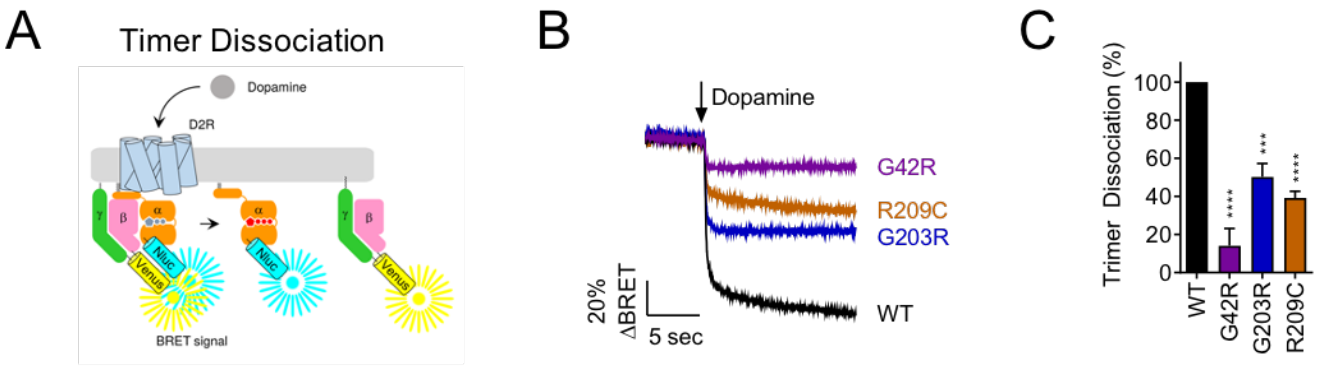

Figure S4. Related to Figure 4. Direct dissociation of Gαo and Gβγ subunits.

A) Assay design to monitor direct dissociation of Gαo from Gβγ. HEK293T/17 cells were transfected with plasmids encoding Flag-D2R, Gαo-Nluc, and Venus-Gβ1γ2. Dopamine application to the transfected cells induces dissociation of Gαo from Venus-Gβ1γ2, which decreases BRET ratio through loss of interaction with Venus-Gβ1γ2. **B)** Timecourse of agonist-mediated dissociation of Gαo and Venus-Gβ1γ2. **C)** Effect of mutations on trimer dissociated measured by change in BRET ratio. The ratio obtained with wild-type Gαo is designated as 100% trimer dissociation. The mean ± SEM of three independent experiments is shown in the bar graph. One-way ANOVA followed by Dunnett's multiple comparisons tests was conducted to determine the effect of genetic variants on the functions of Gαo with WT. *$p < 0.05$; **$p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

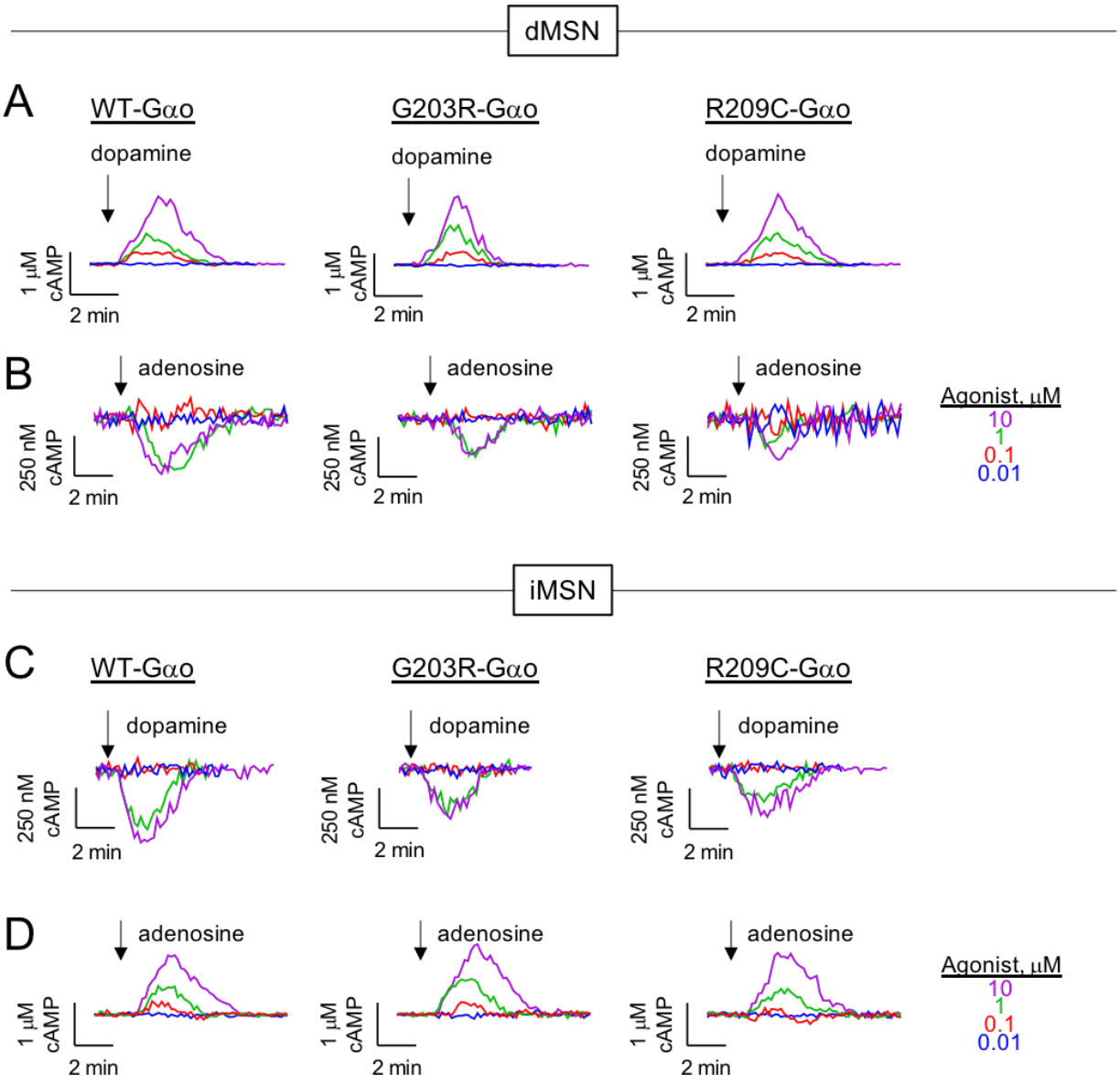

Figure S5. Related to Figure 5. *GNAO1* influence on dopamine and adenosine-mediated cAMP signaling in primary striatal neurons.

A) Average cAMP responses to dopamine dose response from *Gnao1*$^{flox/flox}$ dMSNs transfected with the indicated Gαo construct. n (# neurons)= No Gα (13), WT Gαo (10), G203R (8), R209C (10). **B)** Average cAMP responses to adenosine dose response from *Gnao1*$^{flox/flox}$ dMSNs transfected with the indicated Gαo construct. n (# neurons)= No Gα (13), WT Gαo (12), G203R (13), R209C (14). **C)** Average cAMP responses to dopamine dose response from *Gnao1*$^{flox/flox}$ iMSNs transfected with the indicated Gαo construct. n (# neurons)= No Gα (12), WT Gαo (6), G203R (8), R209C (7). **D)** Average cAMP responses to adenosine dose response from *Gnao1*$^{flox/flox}$ iMSNs transfected with the indicated Gαo construct. n (# neurons)= No Gα (12), WT Gαo (13), G203R (12), R209C (13).

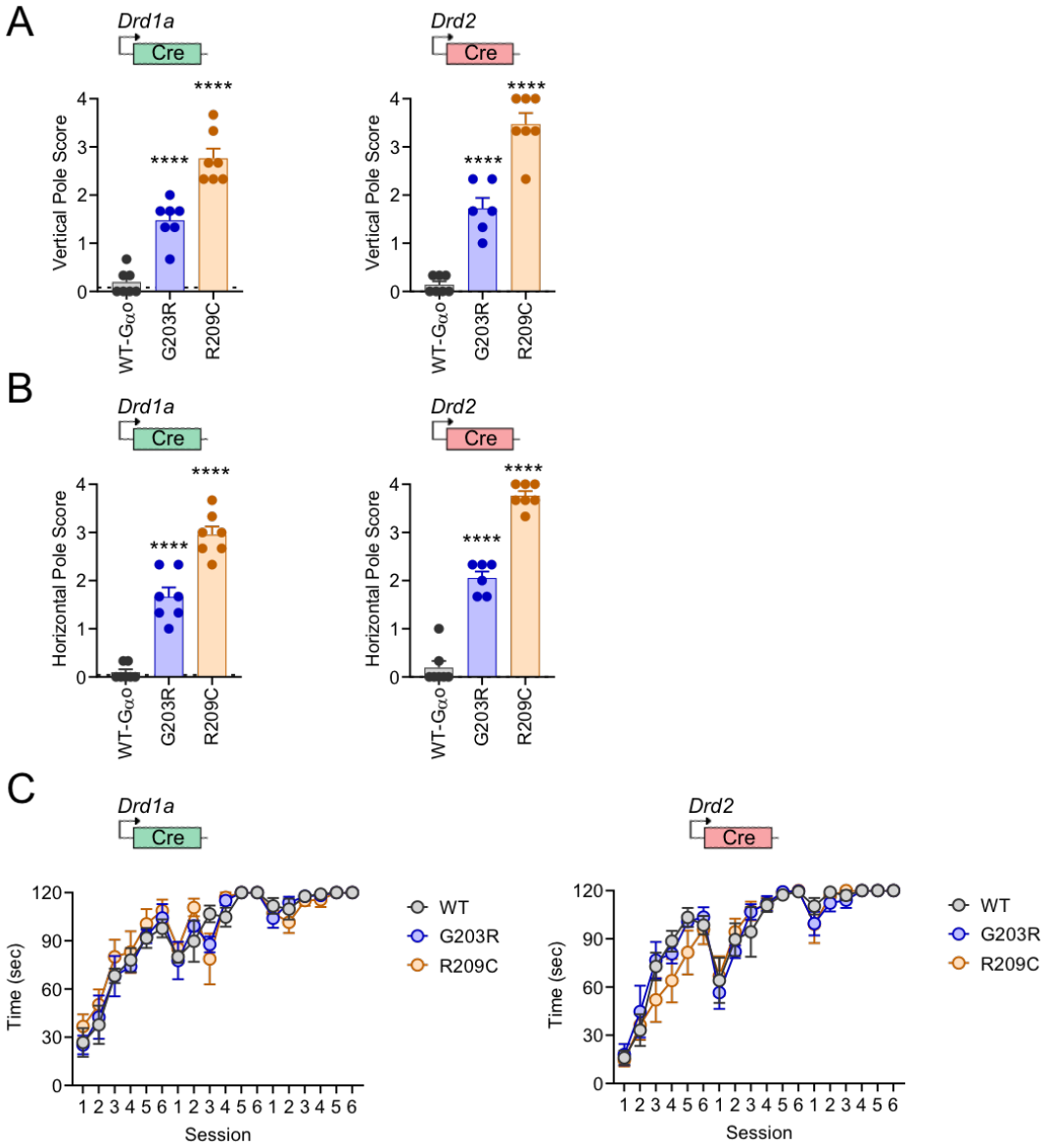

Figure S6. Related to Figure 6. Characterization of motor behavior in mice with striatal specific expression of GNAO1 clinical variants.

A) Vertical pole assay pathology score for *Drd1a*^{Cre} mice expressing WT Gαo (n=7), G203R Gαo (n=7), or R209C Gαo (n=7). One-way ANOVA, Dunnett's multiple comparisons test; WT vs G203R p<0.0001, WT vs R209C p<0.0001 (left). Vertical pole assay pathology score for *Drd2*^{Cre} mice expressing WT Gαo (n=7), G203R Gαo (n=6), or R209C Gαo (n=7). One-way ANOVA, Dunnett's multiple comparisons test; WT vs G203R p<0.0001, WT vs R209C p<0.0001 (right). **B)** Horizontal pole assay pathology score for *Drd1a*^{Cre} mice expressing WT Gαo (n=7), G203R Gαo (n=7), or R209C Gαo (n=7). One-way ANOVA, Dunnett's multiple comparisons test; WT vs G203R p<0.0001, WT vs R209C p<0.0001 (left). Horizontal pole assay pathology score for *Drd2*^{Cre} mice expressing WT Gαo (n=7), G203R Gαo (n=6), or R209C Gαo (n=7). One-way ANOVA, Dunnett's multiple comparisons test; WT vs G203R p<0.0001, WT vs R209C p<0.0001 (right). **C)** Daily performance on the accelerating rotarod over 3 days (6 trials/day) of *Drd1a*^{Cre} mice expressing WT Gαo (n=4), G203R Gαo (n=4), or R209C Gαo (n=4) and *Drd2*^{Cre} mice expressing WT Gαo (n=4), G203R Gαo (n=4), or R209C Gαo (n=4).

Table S1. Oligonucleotide information. Related to STAR Methods.

Name	**Sequence**
Gnai1 sgRNA 1 FW	5’-CACCGACGCTGCCGAATCGTTCAGC
Gnai1 sgRNA 1 RV	5’-AAACGCTGAACGATTCGGCAGCGTC
Gnai1 sgRNA 2 FW	5’-CACCGGGTAGGATTATCCACGAAGC
Gnai1 sgRNA 2 RV	5’-AAACGCTTCGTGGATAATCCTACCC
Gnai1 sgRNA 3 FW	5’-CACCGCTCCCGGGATCTGTTGAAGC
Gnai1 sgRNA 3 RV	5’-AAACGCTTCAACAGATCCCGGGAGC
Gnai2 sgRNA 1 FW	5’-CACCGGTGCCGGCAGTACCGTGCCG
Gnai2 sgRNA 1 RV	5’-AAACCGGCACGGTACTGCCGGCACC
Gnai2 sgRNA 2 FW	5’-CACCGAGGGCACGCACCGCACGCTG
Gnai2 sgRNA 2 RV	5’-AAACCAGCGTGCGGTGCGTGCCCTC
Gnai2 sgRNA 3 FW	5’-CACCGTTCTCGTGAGCGGCCAAAGC
Gnai2 sgRNA 3 RV	5’-AAACGCTTTGGCCGCTCACGAGAAC
Gnai3 sgRNA 1 FW	5’-CACCGCATTGCAATCATACGAGCCA
Gnai3 sgRNA 1 RV	5’-AAACTGGCTCGTATGATTGCAATGC
Gnai3 sgRNA 2 FW	5’-CACCGATCAATCTTCAACCGTCCCA
Gnai3 sgRNA 2 RV	5’-AAACTGGGACGGTTGAAGATTGATC
Gnai3 sgRNA 3 FW	5’-CACCGATTAAACGTTTATGGCGAGA
Gnai3 sgRNA 3 RV	5’-AAACTCTCGCCATAAACGTTTAATC

Table S2. F-test parameters for dose-response experiments.

Figure	Comparison	F (DFn, Dfd)	p value
2I	WT vs dMSN KO	4.321 (1, 132)	0.0396
2J	WT vs dMSN KO	0.1574 (1, 134)	0.6922
2K	WT vs IMSN KO	0.02787 (1, 117)	0.8677
2L	WT vs IMSN KO	2.038 (1, 117)	0.1561
3C	-Gbg vs +Gbg	1.613 (1, 113)	0.2067
3F	WT vs Str KO	18.41 (1, 138)	<0.0001
3L	buffer vs acetylcholine	27.10 (1, 95)	<0.0001
3Q	buffer vs dopamine	4.333 (1, 91)	0.0402
S3E	WT vs Str KO	2.672 (1, 50)	0.1084
S3E	Str KO vs Str KO +Gbg blocker	7.561 (1, 50)	0.0083
5B	WT Gao vs No Gao	0.1057 (1, 86)	0.7459
5B	WT Gao vs G203R Gao	8.968 (1, 66)	0.0039
5B	WT Gao vs R209C Gao	0.2986 (1, 74)	0.5864
5D	WT Gao vs No Gao	1.611 (1, 94)	0.2075
5D	WT Gao vs G203R Gao	0.02029 (1, 94)	0.887
5D	WT Gao vs R209C Gao	1.219 (1, 98)	0.2723
5F	WT Gao vs No Gao	1.091 (1, 66)	0.3001
5F	WT Gao vs G203R Gao	0.3727 (1, 50)	0.5443
5F	WT Gao vs R209C Gao	0.3380 (1, 46)	0.5638
5H	WT Gao vs No Gao	0.004690 (1, 94)	0.9455
5H	WT Gao vs G203R Gao	8.968 (1, 66)	0.0039
5H	WT Gao vs R209C Gao	0.006707 (1, 98)	0.9349