Science Advances
AAAS

advances.sciencemag.org/cgi/content/full/6/18/eaaz7001/DC1

Supplementary Materials for

RasGRP1 is a causal factor in the development of L-DOPA–induced dyskinesia in Parkinson's disease

Mehdi Eshraghi, Uri Nimrod Ramírez-Jarquín, Neelam Shahani, Tommaso Nuzzo, Arianna De Rosa, Supriya Swarnkar, Nicole Galli, Oscar Rivera, George Tsaprailis, Catherina Scharager-Tapia, Gogce Crynen, Qin Li, Marie-Laure Thiolat, Erwan Bezard, Alessandro Usiello*, Srinivasa Subramaniam*

*Corresponding author. Email: usiello@ceinge.unina.it (A.U.); ssubrama@scripps.edu (S.S.)

Published 1 May 2020, *Sci. Adv.* **6**, eaaz7001 (2020)
DOI: 10.1126/sciadv.aaz7001

The PDF file includes:

Figs. S1 to S5

Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/6/18/eaaz7001/DC1)

Data files S1 to S6

Supplementary Figure legends:

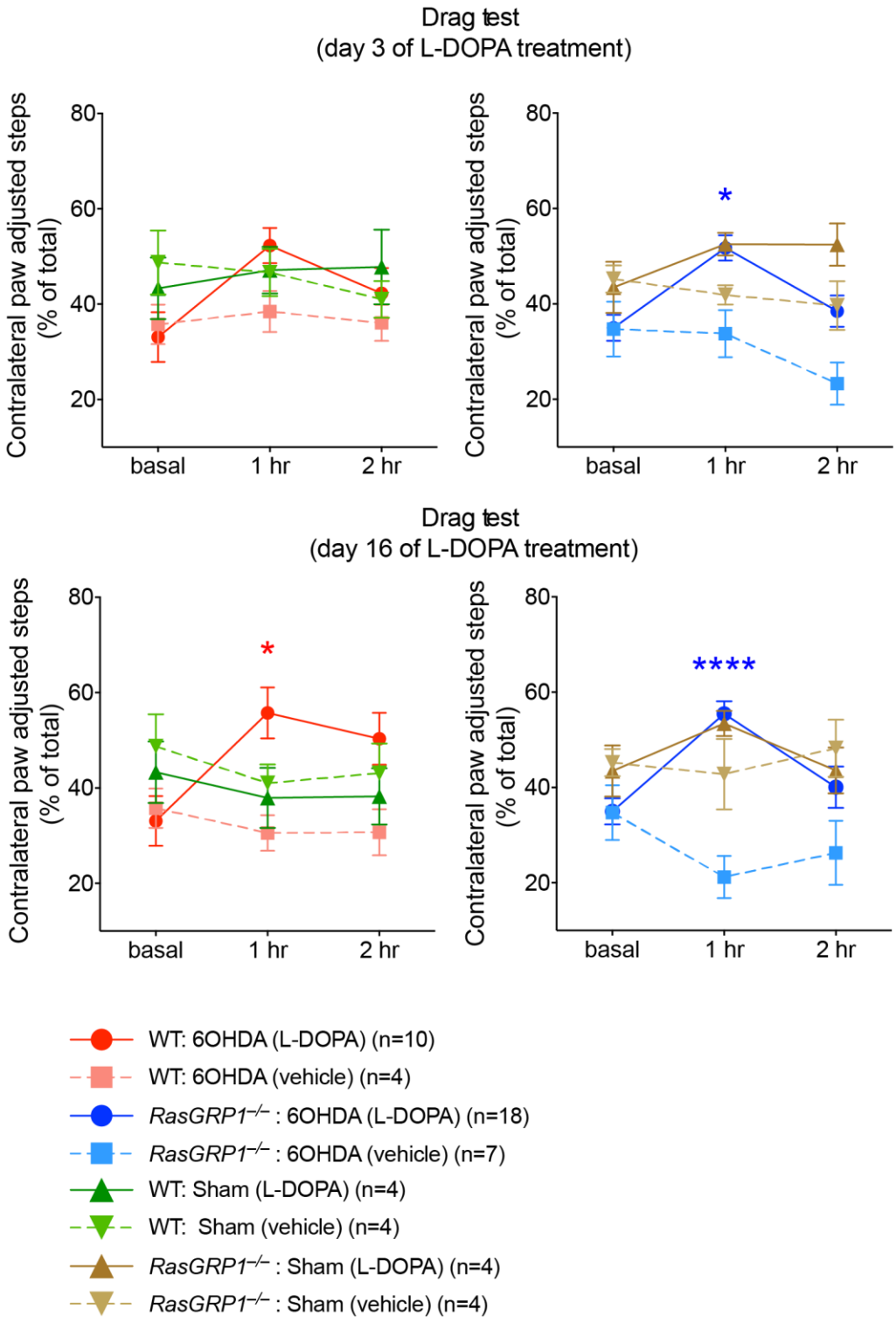
figure S1. **Drag test comparison between WT and *RasGRP1*$^{-/-}$ mice.** Data shows drag test comparing the sham and 6-OHDA lesioned WT and *RasGRP1*$^{-/-}$ mice on day 3 and day 16 of vehicle or L-DOPA treatment. Error bars illustrate the means ± SEM (n = 4-18), **P*<0.05; *****P* <0.0001 by repeated measures Two-Way ANOVA followed by Bonferroni post hoc test.

figure S2. **Open field test comparison between WT and *RasGRP1*$^{-/-}$ mice**. Data shows open field test comparing the sham and 6-OHDA lesioned WT and *RasGRP1*$^{-/-}$ on day 5 of vehicle and L-DOPA treatment. Error bars illustrate the means ± SEM (n = 4-18), n. s; not significant by Two-Way ANOVA followed by Bonferroni post hoc test.

figure S3. **Rotarod test comparison between WT and *RasGRP1*$^{-/-}$ mice**. Data shows rotarod test after 2 or 15 day of vehicle or L-DOPA treatment in the sham and 6-OHDA lesioned WT and *RasGRP1*$^{-/-}$ mice. Error bars illustrate the means ± SEM (n = 4-18), n. s; not significant by repeated measures Two-Way ANOVA followed by Bonferroni post hoc test.

figure S4. **RasGRP1 levels are upregulated by L-DOPA in 6-OHDA-lesioned animals.** Data shows Western blotting of indicated proteins from the intact and 6-OHDA-lesioned striatum of WT mice injected with vehicle or L-DOPA. Error bars illustrate the means ± SEM (n = 3). ***P* < 0.01; *****P* < 0.0001 by One-Way ANOVA followed by Tukey's multiple comparison test.

figure S5. **Depletion of RasGRP1 decreases mTORC1 activity**. Data shows Western blotting of the indicated proteins (A, B) and quantification (C) from HEK293 cells transfected with control or different RasGRP1 shRNA. Error bars illustrate the means ± SEM (n = 3/independent experiments). ****P* < 0.001 by Student's *t* test. Arrow indicate the endogenous RasGRP1 band. Jurkat cell lysates were used as positive control.



(basal levels were measured 5 days before starting L-DOPA treatment)

figure S1

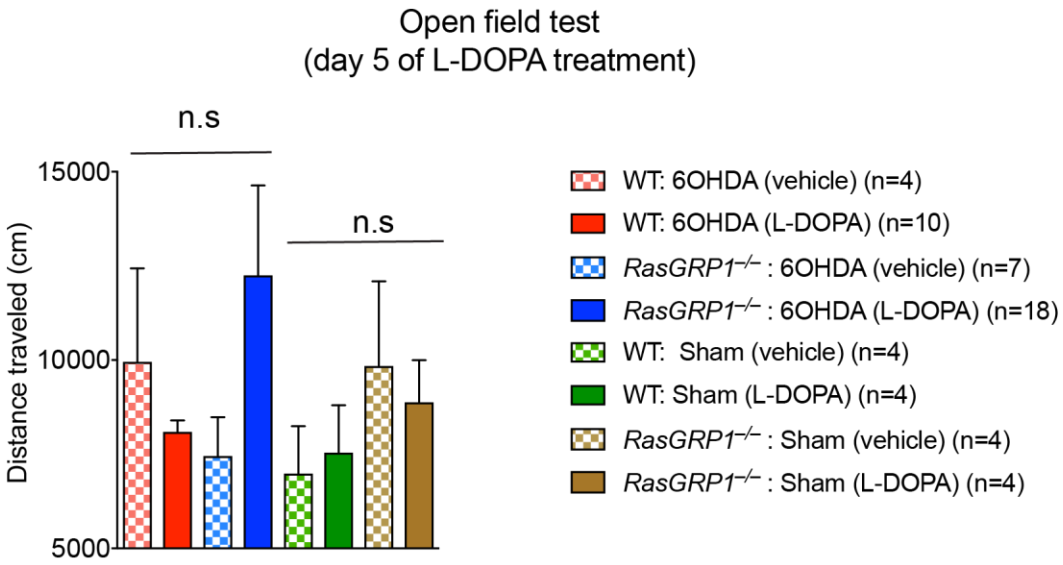

Open field test
(day 5 of L-DOPA treatment)
n.s
n.s
15000
10000
5000
Distance traveled (cm)
WT: 6OHDA (vehicle) (n=4)
WT: 6OHDA (L-DOPA) (n=10)
RasGRP1−/− : 6OHDA (vehicle) (n=7)
RasGRP1−/− : 6OHDA (L-DOPA) (n=18)
WT: Sham (vehicle) (n=4)
WT: Sham (L-DOPA) (n=4)
RasGRP1−/− : Sham (vehicle) (n=4)
RasGRP1−/− : Sham (L-DOPA) (n=4)

figure S2

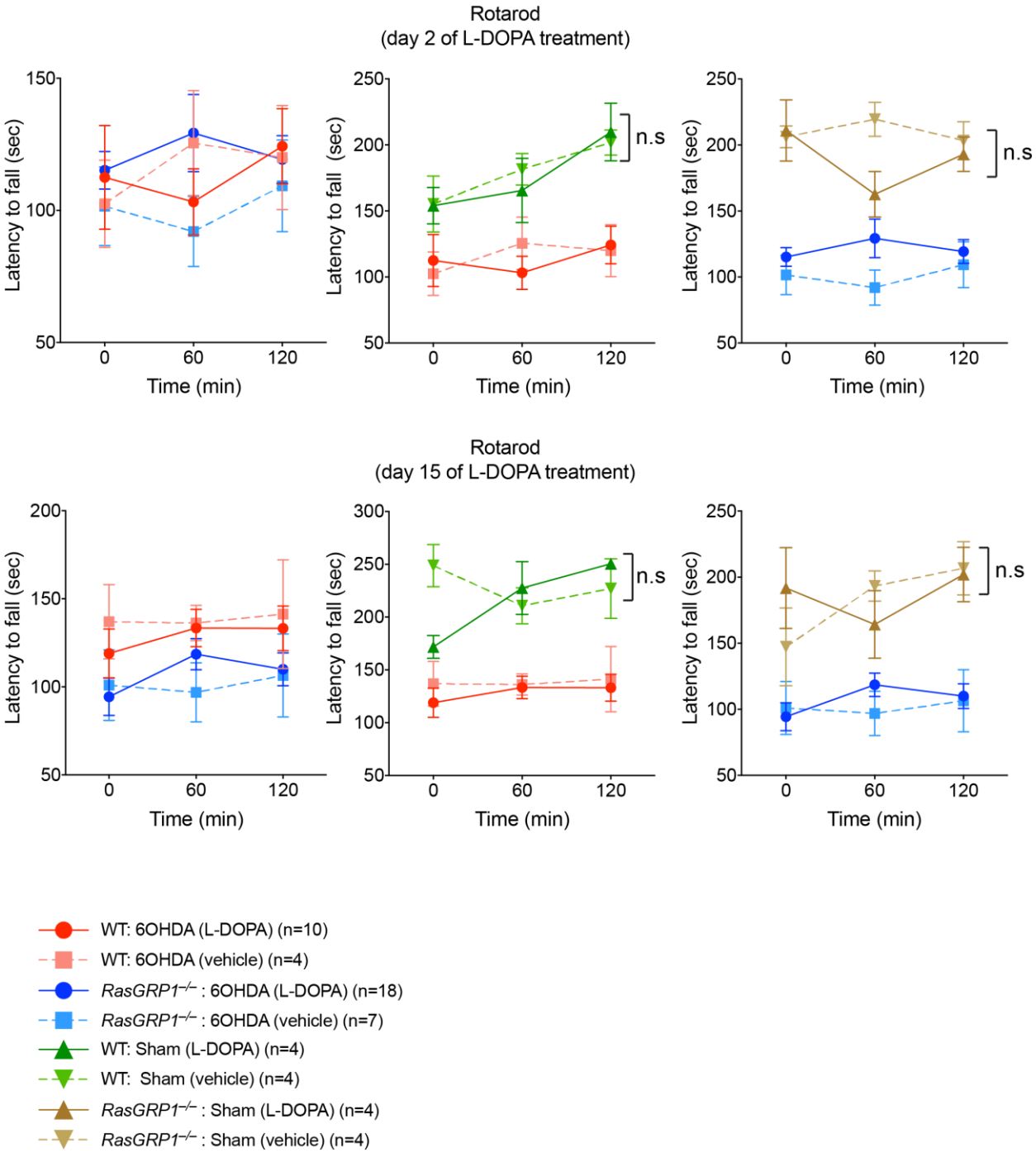

Rotarod
(day 2 of L-DOPA treatment)
Latency to fall (sec)
Time (min)
n.s
Rotarod
(day 15 of L-DOPA treatment)
WT: 6OHDA (L-DOPA) (n=10)
WT: 6OHDA (vehicle) (n=4)
RasGRP1−/− : 6OHDA (L-DOPA) (n=18)
RasGRP1−/− : 6OHDA (vehicle) (n=7)
WT: Sham (L-DOPA) (n=4)
WT: Sham (vehicle) (n=4)
RasGRP1−/− : Sham (L-DOPA) (n=4)
RasGRP1−/− : Sham (vehicle) (n=4)

figure S3

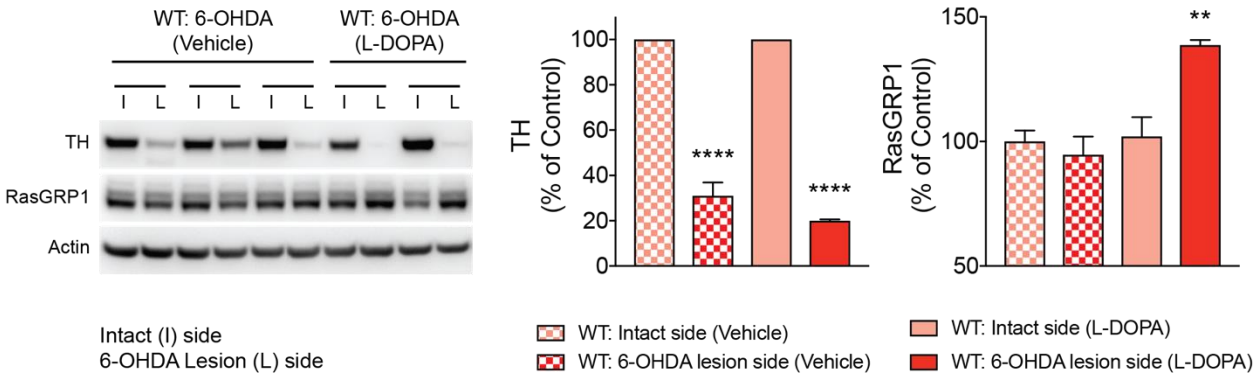

WT: 6-OHDA (Vehicle)
WT: 6-OHDA (L-DOPA)
I L I L I L I L I L
TH
RasGRP1
Actin
Intact (I) side
6-OHDA Lesion (L) side
TH (% of Control)
100
80
60
40
20
0
****
****
RasGRP1 (% of Control)
150
100
50
**
WT: Intact side (Vehicle)
WT: 6-OHDA lesion side (Vehicle)
WT: Intact side (L-DOPA)
WT: 6-OHDA lesion side (L-DOPA)

figure S4

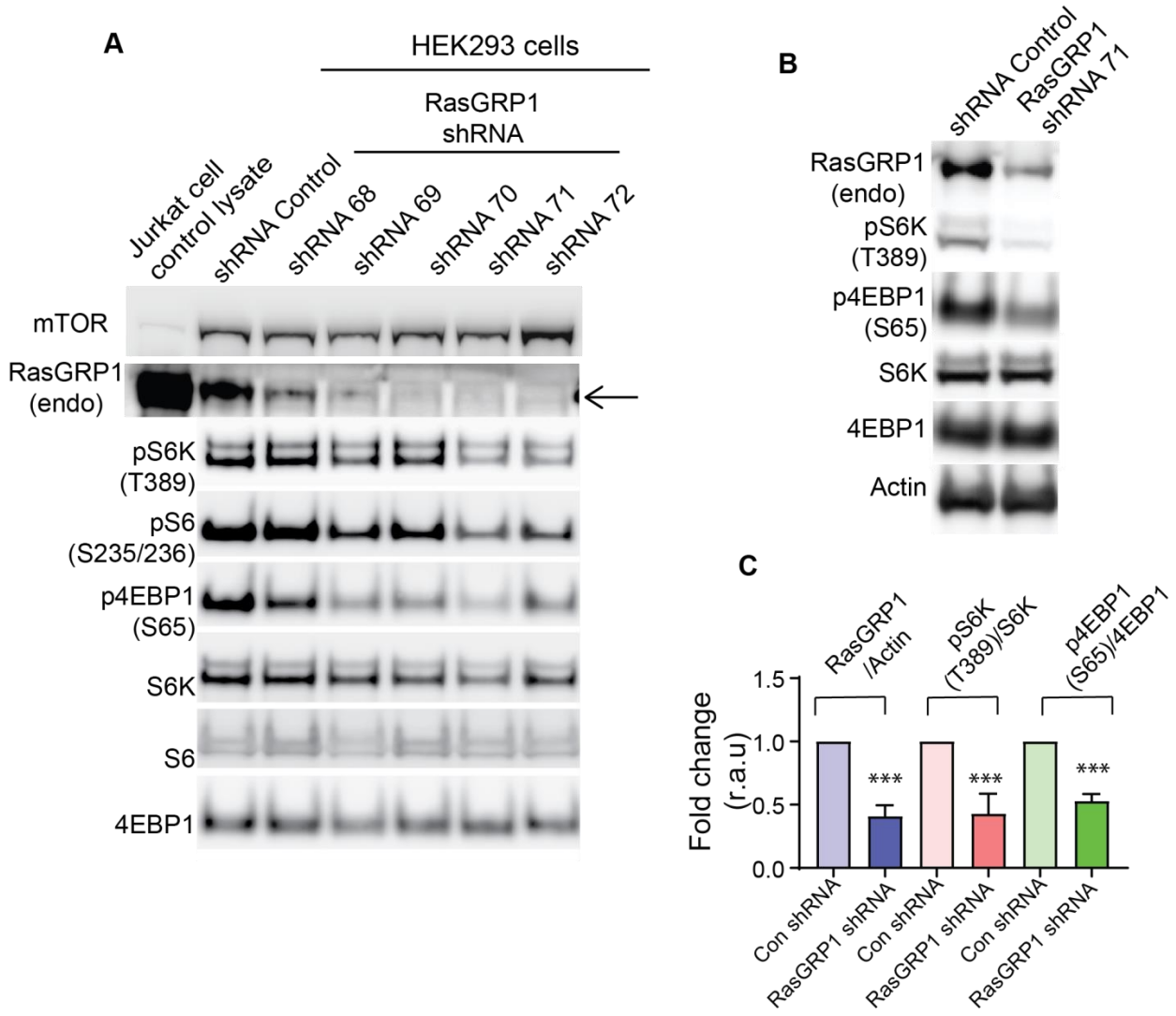

A
HEK293 cells
RasGRP1 shRNA
Jurkat cell control lysate
shRNA Control
shRNA 68
shRNA 69
shRNA 70
shRNA 71
shRNA 72
mTOR
RasGRP1 (endo)
pS6K (T389)
pS6 (S235/236)
p4EBP1 (S65)
S6K
S6
4EBP1
B
shRNA Control
RasGRP1 shRNA 71
RasGRP1 (endo)
pS6K (T389)
p4EBP1 (S65)
S6K
4EBP1
Actin
C
RasGRP1 /Actin
pS6K (T389)/S6K
p4EBP1 (S65)/4EBP1
Fold change (r.a.u)
1.5
1.0
0.5
0.0
***
***
***
Con shRNA
RasGRP1 shRNA
Con shRNA
RasGRP1 shRNA
Con shRNA
RasGRP1 shRNA

figure S5