

SUPPLEMENTARY MATERIAL

Enhanced mGluR₅ intracellular activity causes psychiatric alterations in Niemann Pick type C disease

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FIGURE S1

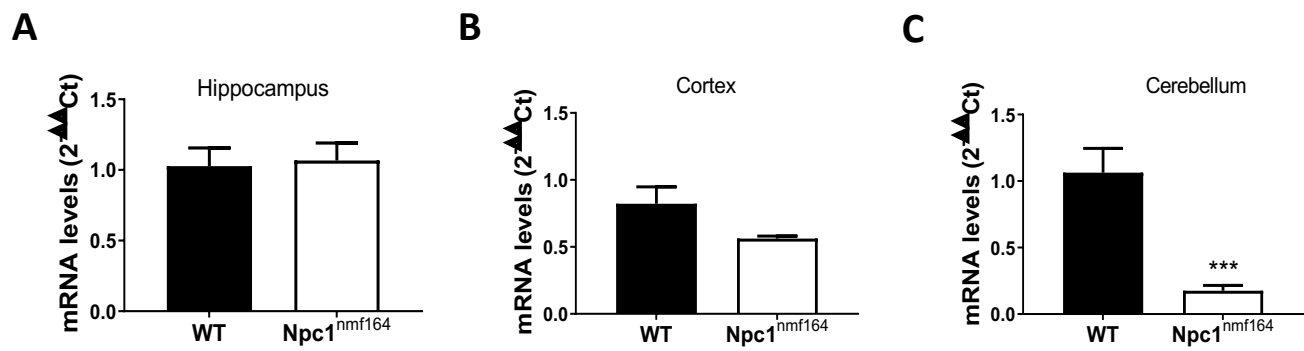


Figure S1. mGluR5 mRNA levels in the brain of wt and Npc1^{nmf164} mice

Graph showing mean \pm SEM levels of mGluR₅ mRNA quantified by qRT-PCR in the hippocampus (a), cortex (b) and cerebellum (c) of 10 week-old wt or Npc1^{nmf164} mice ($P_{\text{cerebellum}} = 0.0002$; $n = 4-7$; Student's *t*-test).

FIGURE S2

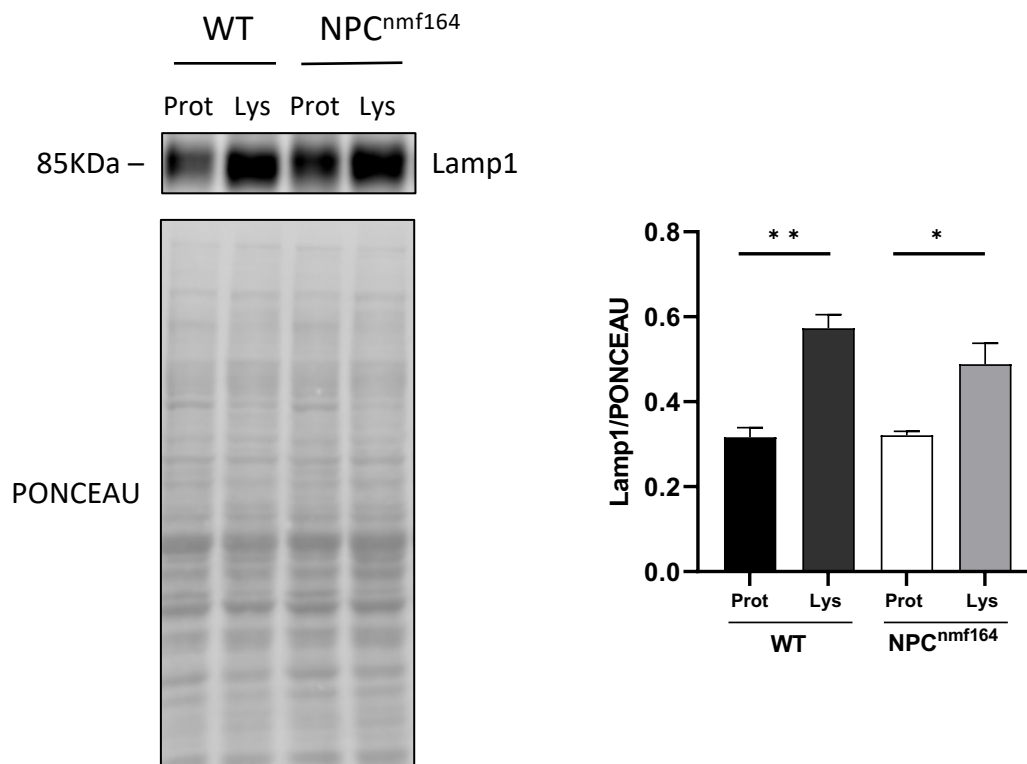


Figure S2. Isolation of lysosomal-enriched fractions from the brain of wt and *Npc1^{nmf164}* mice

Western blot analysis of the lysosomal marker Lamp1 in the total protein extract, which served as starting fraction of the isolation protocol (Prot), and in the fraction corresponding to the interface 24-26% nycodenz gradient in which lysosomes are recovered (Lys). Lower panel shows Ponceau staining confirming similar amount of protein loaded in each lane. Graph shows mean \pm SEM levels of Lamp1 in each fraction normalized to the ponceau staining in wt and *Npc1^{nmf164}* mice ($P_{WT} = 0.0027$; $P_{NPC^{nmf164}} = 0.0293$); $n = 3$ independent isolations using two brains in each of them; Paired T-test).

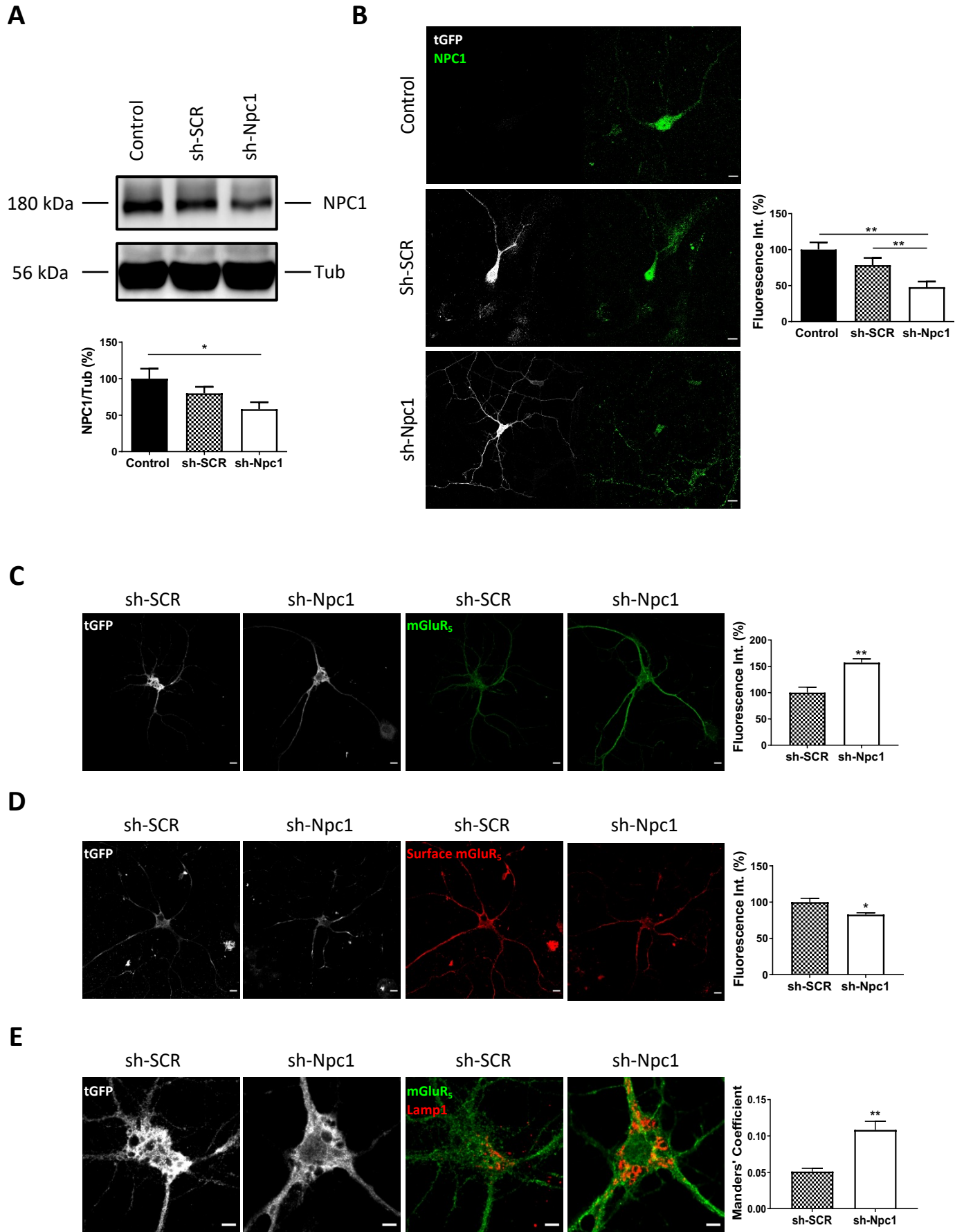
FIGURE S3

Figure S3. mGluR5 alterations in genetically NPC1-silenced cultured neurons

- A. Western blot showing NPC1 and tubulin (Tub) in cultured neurons from wt mice incubated with vehicle (Veh) or with lentiviruses encoding sh-RNA specific for Npc1 (sh-Npc1) or scrambled control (sh-SCR). Graph shows mean \pm SEM levels of NPC1 normalized to Tub as a percentage of the values obtained in the Veh-incubated neurons ($P = 0.0354$; $n = 6$; grouped one-way ANOVA Bonferroni *post hoc*).
- B. Immunofluorescence analysis (IFA) of NPC1 and tGFP, which identifies transfected neurons, in cultured neurons from wt mice incubated with Veh or with lentiviruses encoding sh-SCR or sh-Npc1. Graph shows NPC1 associated intensity as a percentage of the values obtained in the Veh- incubated neurons ($P_{control-shNPC1} = 0.0084$; $P_{shSCR-shNPC1} = 0.0093$; $n = 6$; grouped one-way ANOVA Bonferroni *post hoc*). Scale bar 10 μ m.
- C.D. IFA of total (c) and surface (d) mGluR₅ in cultured neurons transfected with either sh-SCR or sh-Npc1 RNA as indicated by the tGFP positive signals. Graphs shows mean \pm SEM fluorescence intensity associated with mGluR₅ as a percentage of the values obtained in the sh-SCR transfected neurons ($P_C = 0.009$; $P_D = 0.0304$ $n = 3-4$; paired Student's *t*-test). Scale bar 10 μ m.
- E. IFA of mGluR₅ and Lamp1 (endolysosomal marker) in cultured neurons transfected with either sh-SCR or sh-NPC1 RNA as indicated by the tGFP positive signals. Graph shows mean \pm SEM Manders' coefficient of co-localization between mGluR₅ and Lamp1 ($P = 0.0039$; $n = 3-4$; paired Student's *t*-test). Scale bar 5 μ m.

FIGURE S4

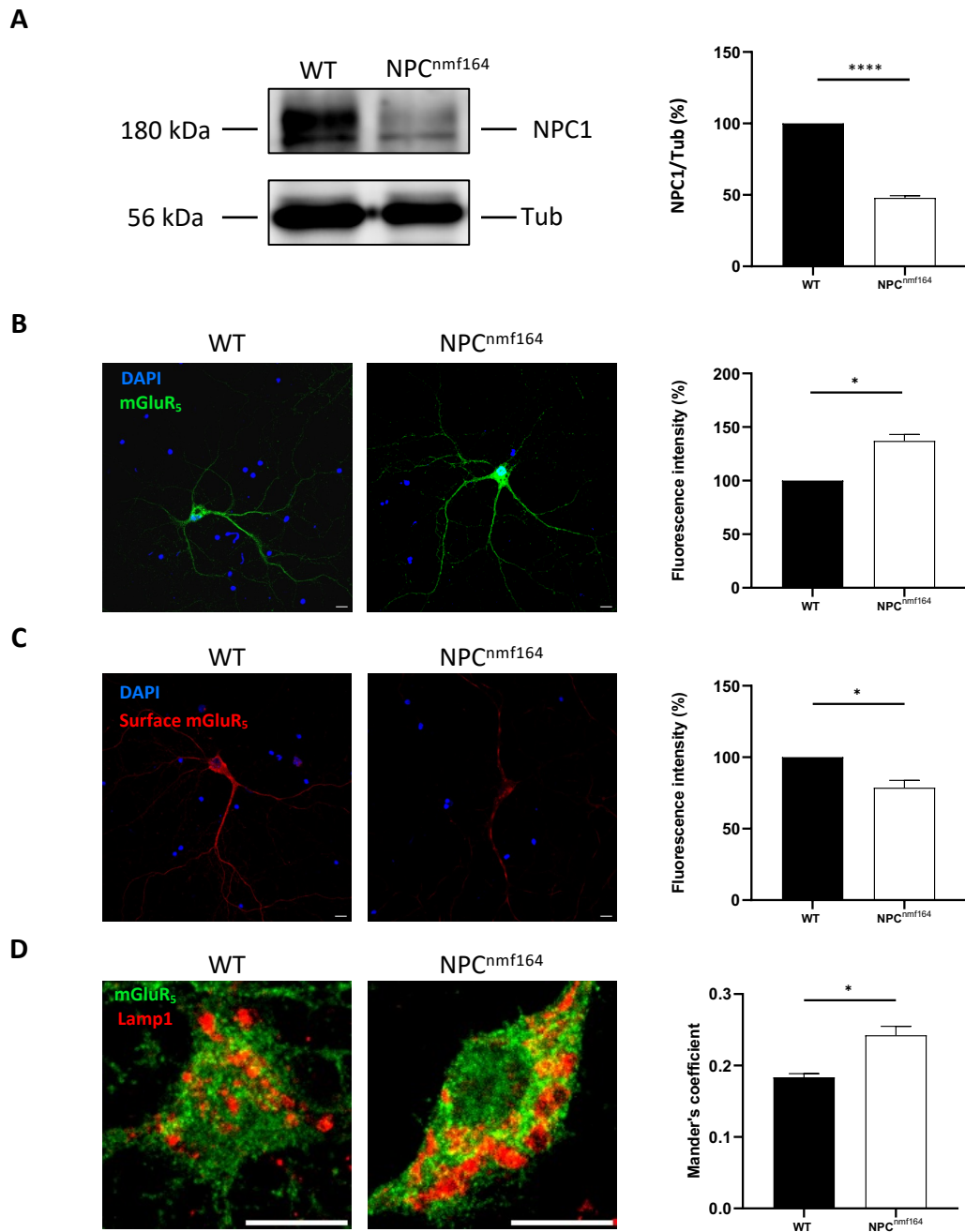


Figure S4. mGluR5 alterations in primary cultured neurons derived from *Npc1*^{nmf164} mice

A. Western blot showing NPC1 and tubulin (Tub) levels in cultured neurons from wt and *Npc1*^{nmf164} mice. Graph shows mean ± SEM levels of NPC1 normalized to Tub as a percentage of the values obtained in the wt neurons ($P < 0.0001$); $n = 3$; Unpaired two-tailed t-test).

B.C. IFA of total (B) and surface (C) mGluR₅ in cultured neurons from wt and *Npc1*^{nmf164} mice. Graphs show mean ± SEM fluorescence intensity associated with mGluR₅ as a percentage of the values obtained in the wt neurons ($P_B = 0.0432$; $P_C = 0.0483$ $n = 3$; Paired two-tailed t-tests). Scale bar 10 μm .

D. IFA of mGluR₅ and Lamp1 in cultured neurons from wt and *Npc1*^{nmf164} mice. Graph shows mean ± SEM Manders' coefficient of co-localization between mGluR₅ and Lamp1 ($P = 0.0203$; $n = 3$; Paired two-tailed t-tests). Scale bar 5 μm .

FIGURE S5

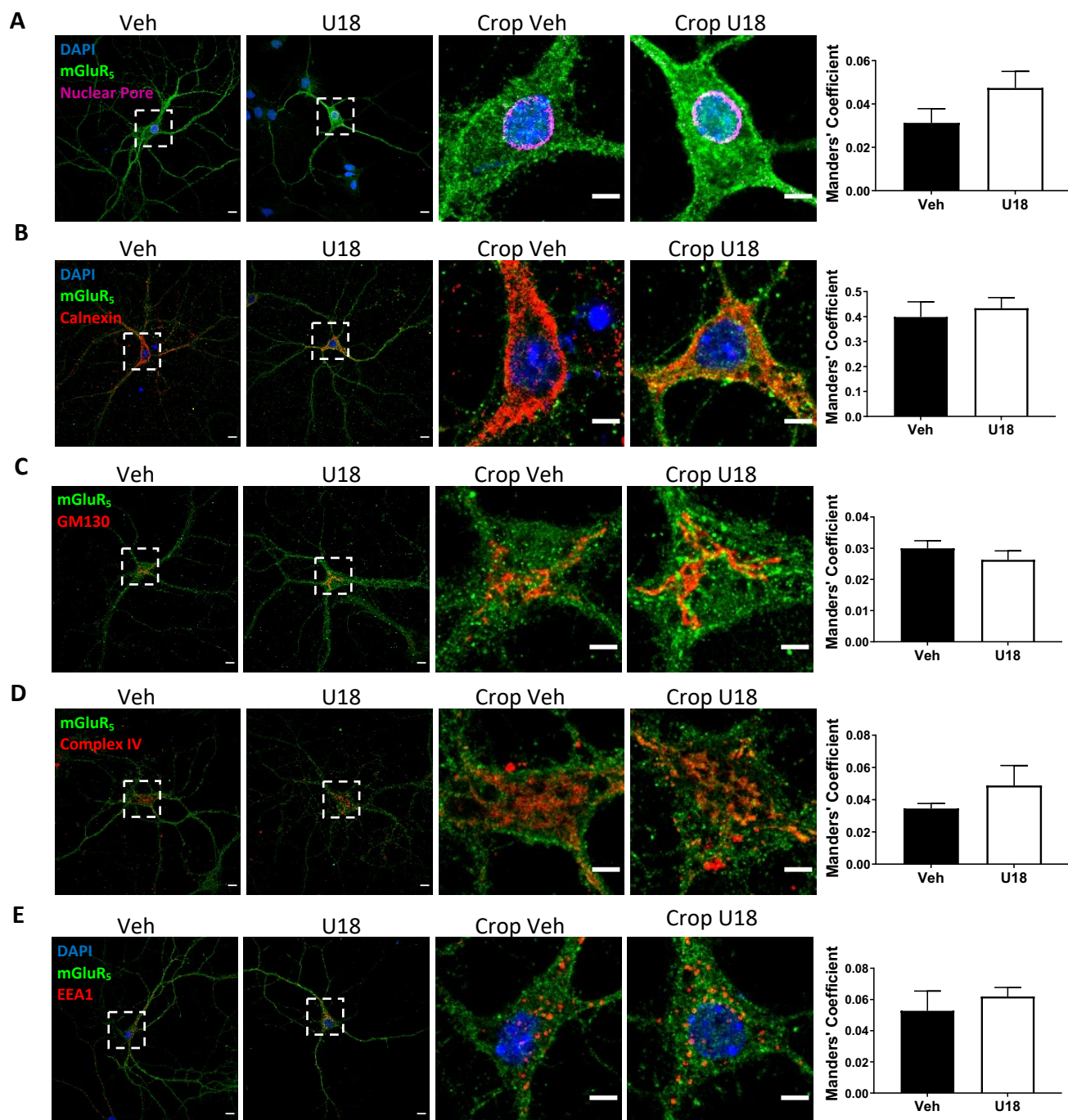


Figure S5. mGluR5 subcellular distribution in wt and NPC1-deficient cultured neurons

Immunofluorescence analysis of mGluR₅ co-stained with markers for nucleus (nuclear pore, **A**), endoplasmic reticulum (calnexin, **B**), Golgi apparatus (GM130, **C**), mitochondria (complex IV, **D**) and early endosomes (EEA1, **E**) in cultured neurons from wt mice treated with vehicle (Veh) or with U18 (NPC1 inhibitor). Graphs show mean \pm SEM Manders' coefficient of co-localization between mGluR₅ and each of the markers for the indicated subcellular compartments ($n = 5-6$; paired Student's *t*-test). Scale bar 10 μ m (5 μ m in cropped images).

FIGURE S6

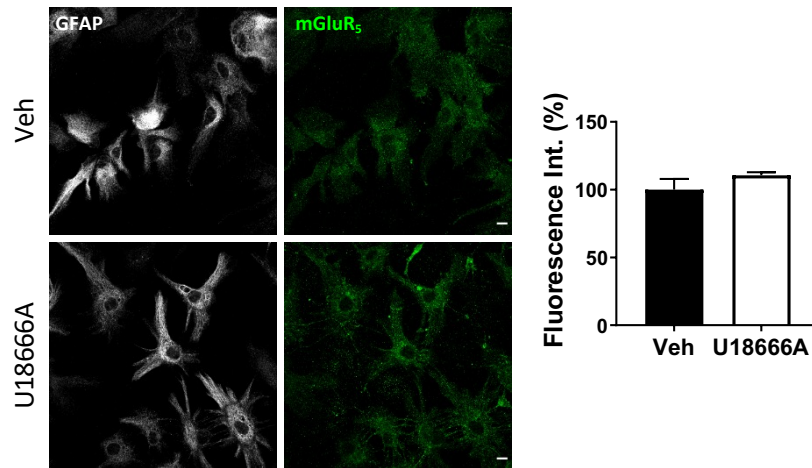


Figure S6. mGluR5 levels in wt and NPC1-deficient cultured astrocytes

Immunofluorescence analysis of mGluR₅ (green) and GFAP (white, marker of astrocytes) in cortical cultures from wt mice treated with vehicle (Veh) or with U18 (NPC1 inhibitor). Graph shows mean \pm SEM fluorescence intensity associated with mGluR₅ as a percentage of the values obtained in the Veh-treated astrocytes (n = 3; paired Student's *t*-test). Scale bar 10 μ m.

FIGURE S7

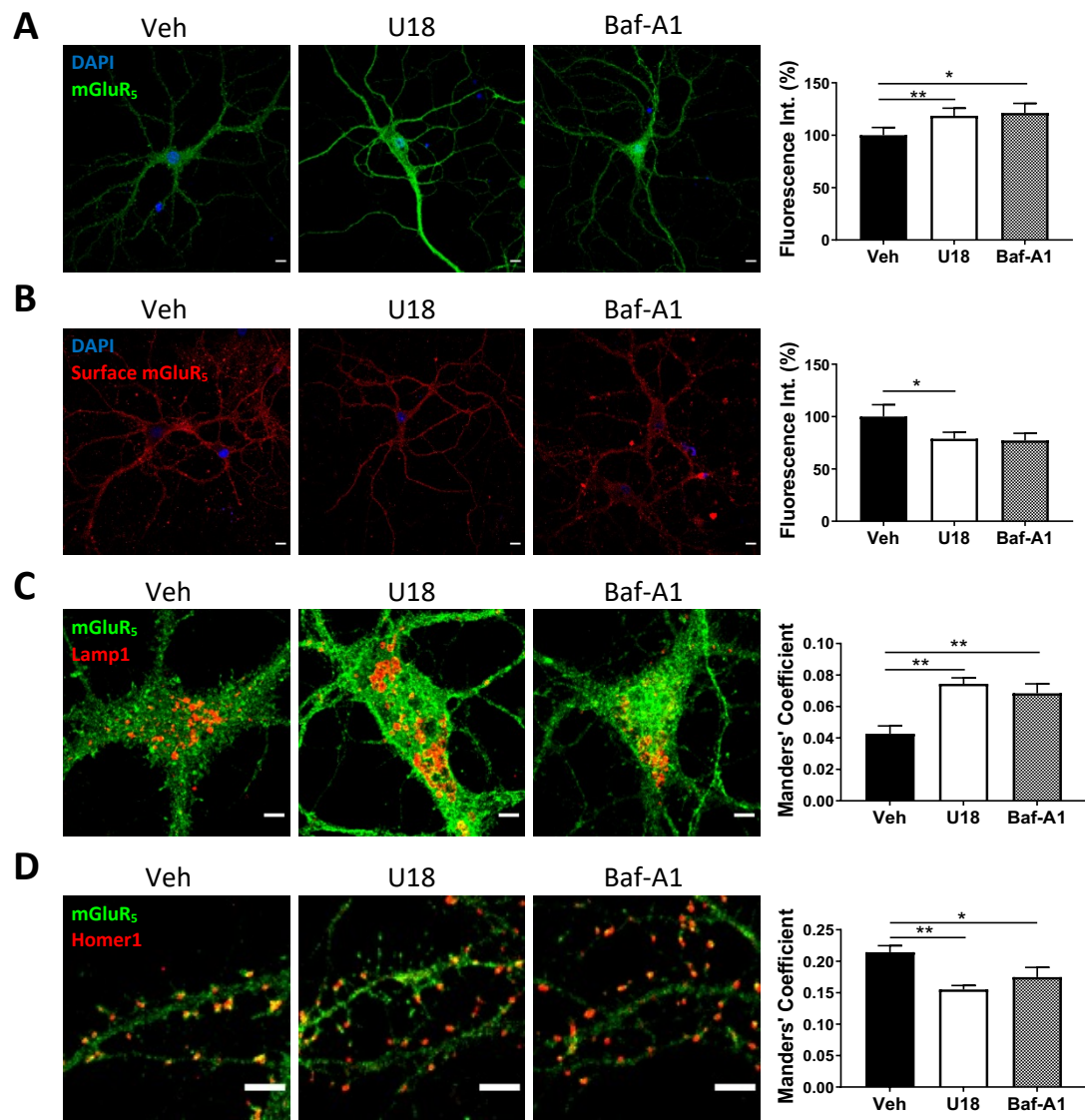


Figure S7. mGluR5 distribution after lysosomal inhibition

A,B. Immunofluorescence analysis (IFA) of total (a) and surface (b) mGluR₅ in cultured neurons from wt mice treated with vehicle (Veh), U18 (NPC1 inhibitor) or with bafilomycin A1 (Baf-A1; inhibitor of lysosomal function). Graphs shows mean ± SEM fluorescence intensity associated with mGluR₅ as a percentage of the values obtained in Veh-treated neurons ($P_{A, Veh-U18} = 0.0093$; $P_{A, Veh-Baf-A1} = 0.0435$; $P_{B, Veh-U18} = 0.0265$; $n = 5$; grouped one-way ANOVA Bonferroni *post hoc*). Scale bar 10 μm.

C,D. IFA showing co-staining of mGluR₅ with either Lamp1 (c) or Homer1 (d) in cultured neurons from wt mice treated with Veh, U18, or Baf-A1. Graphs show mean ± SEM Manders' coefficient of co-localization between mGluR₅ and Lamp1 or Homer1 ($P_{C, Veh-U18} = 0.0034$; $P_{C, Veh-Baf-A1} = 0.0017$; $P_{D, Veh-U18} = 0.0029$; $P_{D, Veh-Baf-A1} = 0.0291$; $n = 5$; grouped one-way ANOVA Bonferroni *post hoc*). Scale bar 5 μm.

FIGURE S8

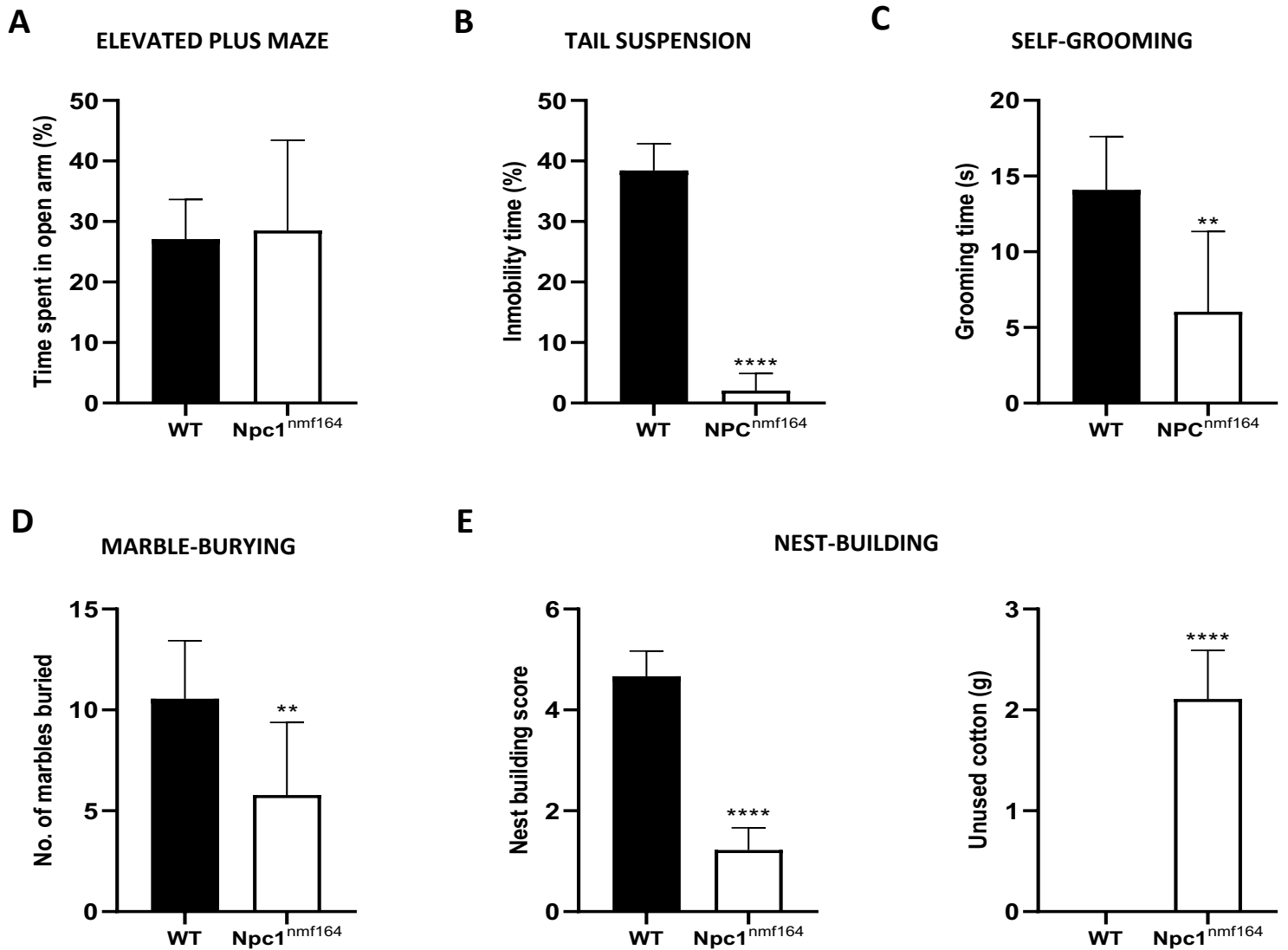


Figure S8. Behavioural alterations in Npc1^{nmf164} mice at 2.5 months of age

A. Graph showing mean \pm SEM time spent in the open arm of the elevated plus maze as a percentage of the total exploring time by wt or Npc1^{nmf164} mice ($P < 0.8201$; $n = 7-8$; Student's t -test).

B. Graph showing mean \pm SEM immobility time spent in the tail suspension test as a percentage of the total time by wt or Npc1^{nmf164} mice ($P < 0.0001$; $n = 8-9$; Student's t -test).

C. Graph showing mean \pm SEM of time spent self-grooming by wt or Npc1^{nmf164} mice ($P < 0.0030$; $n = 8$; Student's t -test).

D. Graph showing mean \pm SEM of number of marbles buried by wt or Npc1^{nmf164} mice ($P < 0.0067$; $n = 9$; Student's t -test).

E. Graphs show mean \pm SEM of nest-building test score (left) and unused cotton material in grams (right) by wt or Npc1^{nmf164} mice in the nest-building test ($P_{left} < 0.0001$; $P_{right} < 0.0001$ $n = 9$; Student's t -test).

FIGURE S9

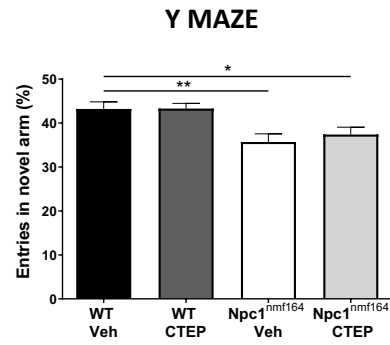


Figure S9. CTEP treatment does not ameliorate memory deficits in Npc1^{nmf164} mice

Graph showing mean \pm SEM number of entries in the novel arm of the Y maze as a percentage of the total entries in all arms in wt or Npc1^{nmf164} mice treated with vehicle (Veh) or with CTEP (membrane-permeable mGluR₅ antagonist) ($P = 0.0046$; $n = 8-13$; grouped one-way ANOVA Bonferroni *post hoc*).