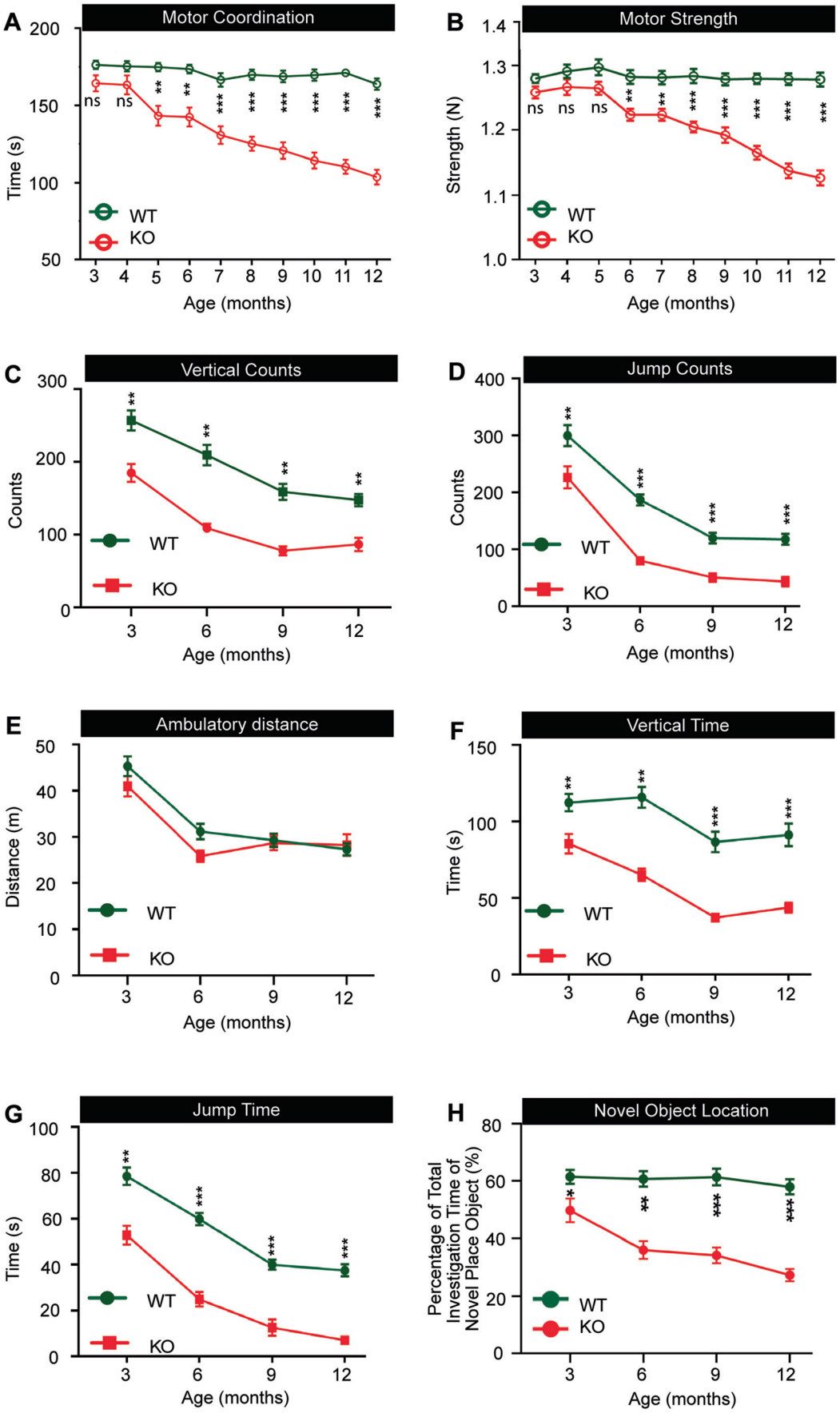
# Expanded View Figure

**Figure EV1. Longitudinal assessment of motor function, spatial memory, and explorative behaviours in *DDHD2*$^{+/+}$ vs *DDHD2*$^{-/-}$ mice.**

(**A**) Line graphs showing monthly recordings of a motor coordination, assessed using a rotarod device by determining the latency period from when the mouse is placed on the accelerating rotating rod device to the initial fall (s), and (**B**) motor strength (N), assessed using a grip strength device. Longitudinal monitoring of mouse activity with line graphs showing (**C**) vertical counts, (**D**) jump counts, (**E**) ambulatory distance (m), (**F**), vertical time (s), and (**G**) jump time (s). (**H**) Longitudinal assessment of spatial memory performance in mice, using the Novel Object Location (NOL) paradigm, represented as a line graph. Data information: In (**A–H**), the significance of the difference between each group as determined by one-way ANOVA ($n = 20$) is indicated by asterisks $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, ns = not significant. Error bars represent the cumulative standard error of the mean (SEM) for all groups and parameters.


A
Motor Coordination
Time (s)
Age (months)
WT
KO
ns
B
Motor Strength
Strength (N)
C
Vertical Counts
Counts
D
Jump Counts
E
Ambulatory distance
Distance (m)
F
Vertical Time
G
Jump Time
H
Novel Object Location
Percentage of Total Investigation Time of Novel Place Object (%)

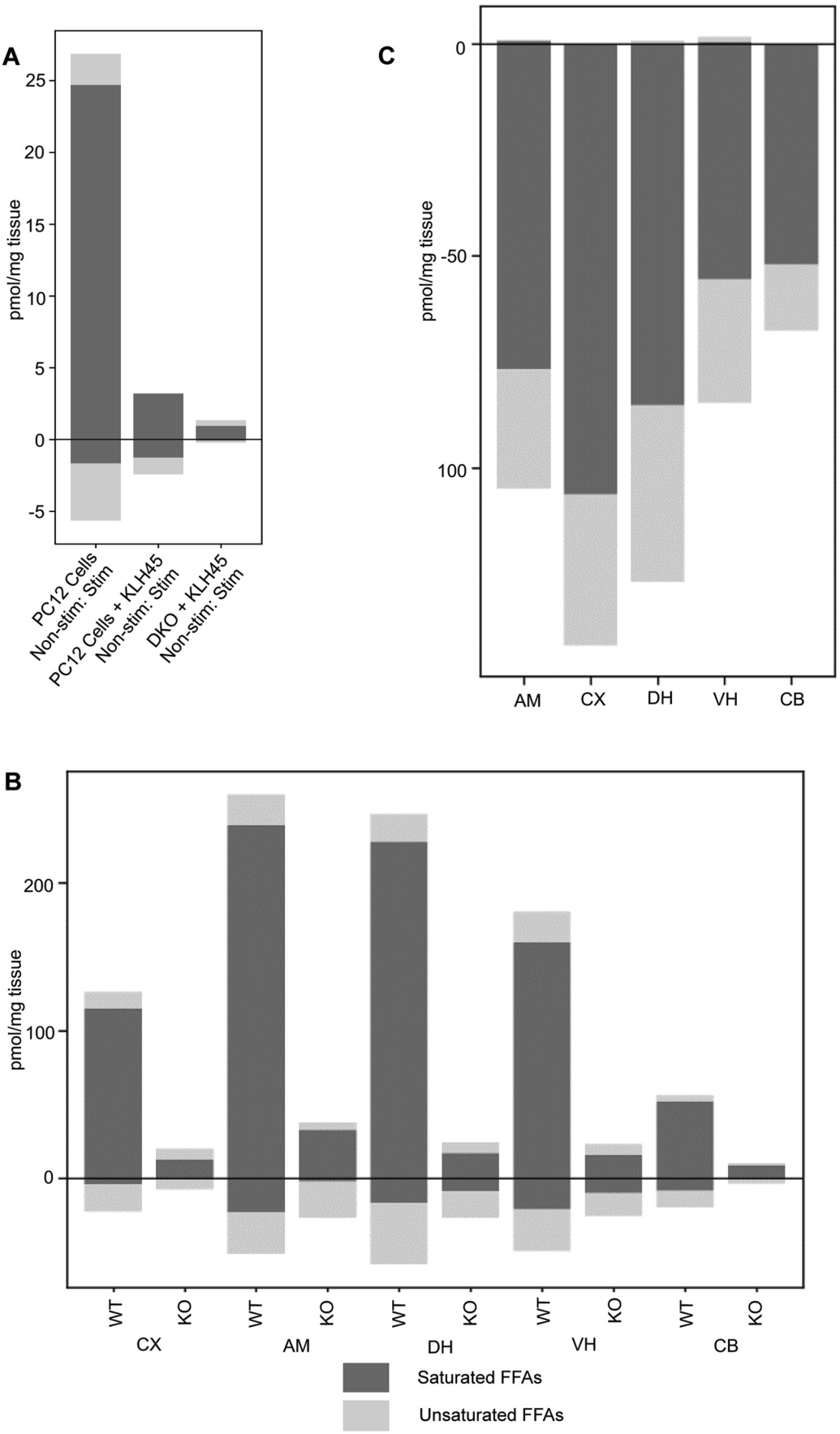
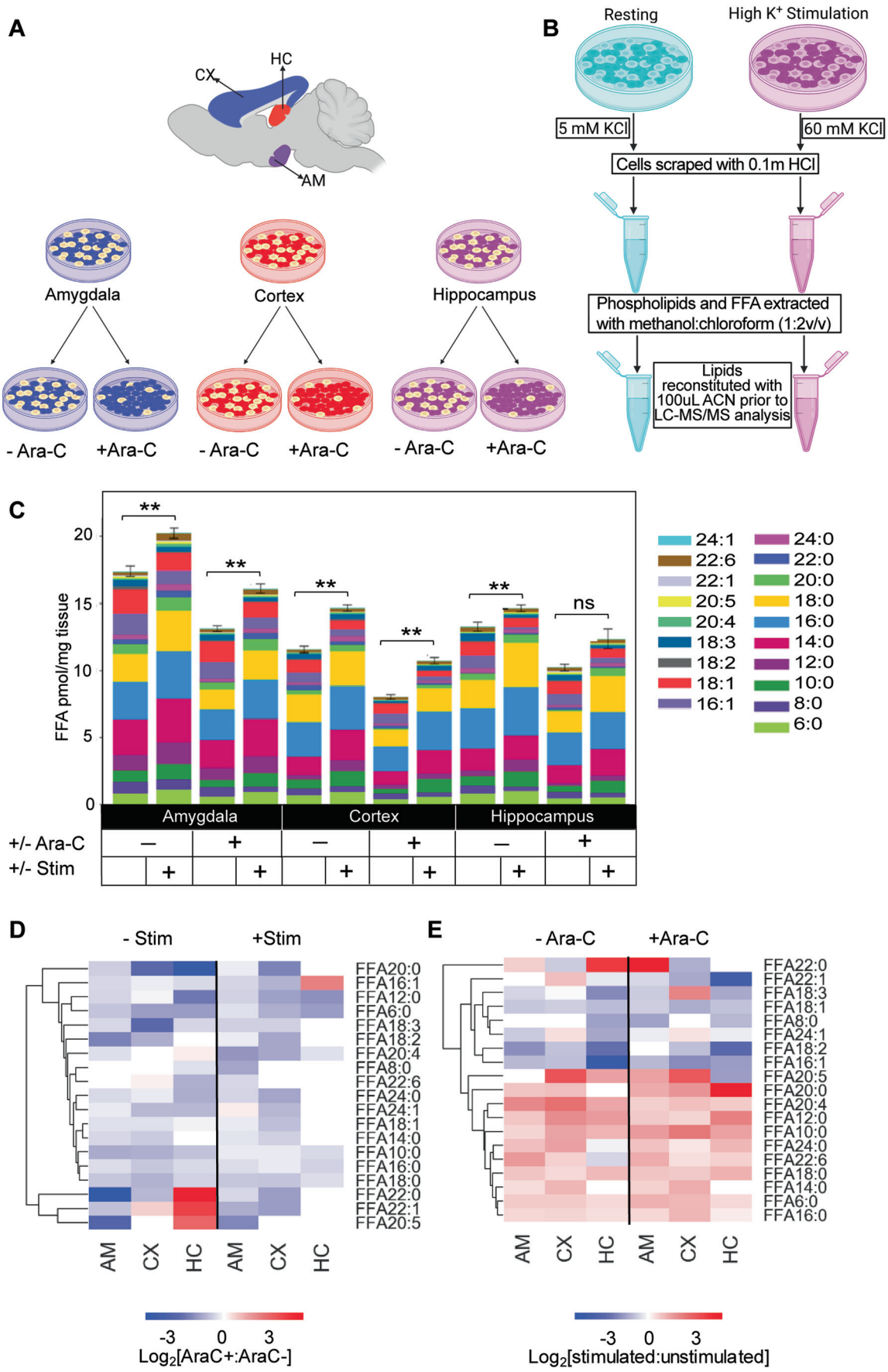

A
pmol/mg tissue
25
20
15
10
5
0
-5
PC12 Cells Non-stim: Stim
PC12 Cells + KLH45 Non-stim: Stim
DKO + KLH45 Non-stim: Stim
C
pmol/mg tissue
0
-50
100
AM
CX
DH
VH
CB
B
pmol/mg tissue
200
100
0
WT
KO
CX
AM
DH
VH
CB
Saturated FFAs
Unsaturated FFAs

**Figure EV2. Change in saturated (dark grey) vs unsaturated (light grey) FFAs in response to different conditions.**

Bar plots show the change of saturated vs unsaturated FFAs in in response to (**A**) secretagogue stimulation in PC12 cells; (**B**) instrumental conditioning across the brain of WT and DDHD2 KO mice. (**C**) STXBP1 heterozygote across the brain versus WT.


A
HC
CX
AM
Amygdala
Cortex
Hippocampus
- Ara-C
+Ara-C
- Ara-C
+Ara-C
- Ara-C
+Ara-C
B
Resting
High K+ Stimulation
5 mM KCl
60 mM KCl
Cells scraped with 0.1m HCl
Phospholipids and FFA extracted with methanol:chloroform (1:2v/v)
Lipids reconstituted with 100uL ACN prior to LC-MS/MS analysis
C
FFA pmol/mg tissue
0
5
10
15
20
**
**
**
**
**
ns
Amygdala
Cortex
Hippocampus
+/- Ara-C
+/- Stim
24:1
22:6
22:1
20:5
20:4
18:3
18:2
18:1
16:1
24:0
22:0
20:0
18:0
16:0
14:0
12:0
10:0
8:0
6:0
D
- Stim
+Stim
FFA20:0
FFA16:1
FFA12:0
FFA6:0
FFA18:3
FFA18:2
FFA20:4
FFA8:0
FFA22:6
FFA24:0
FFA24:1
FFA18:1
FFA14:0
FFA10:0
FFA16:0
FFA18:0
FFA22:0
FFA22:1
FFA20:5
AM
CX
HC
AM
CX
HC
-3
0
3
Log2[AraC+:AraC-]
E
- Ara-C
+Ara-C
FFA22:0
FFA22:1
FFA18:3
FFA18:1
FFA8:0
FFA24:1
FFA18:2
FFA16:1
FFA20:5
FFA20:0
FFA20:4
FFA12:0
FFA10:0
FFA24:0
FFA22:6
FFA18:0
FFA14:0
FFA6:0
FFA16:0
AM
CX
HC
AM
CX
HC
-3
0
3
Log2[stimulated:unstimulated]

◀ **Figure EV3. Profile of the effect of ara-C on FFAs response to stimulated neuroexocytosis.**

(**A**) Inhibition of glia proliferation in neuronal cultures using Ara-C. Cells from the cortex (CX), amygdala (AM), and hippocampus (HC) were isolated from embryonic-day-18 (E18) Sprague-Dawley rat embryos and seeded in separate wells from the indicated brain regions. (**B**) Potassium stimulation of neuroexocytosis and extraction of lipids. Cultured neurons were treated for 15 min using high potassium buffer; 60 mM $K^+$ (depolarised) or 2 mM $K^+$ (resting control). FFA and phospholipids were extracted in using methanol:chloroform using the liquid–liquid extraction protocol of Bligh and Dyer (Bligh and Dyer, 1959). (**C**) Bar graph showing quantification of FFA in Amygdala (AM), Cortex (CX), and Hippocampal (HC) neurons with and without ara-C treatment. (**D**) Hierarchical clustering heatmap showing FFA in response to ara-C treatment in stimulated versus non-stimulated cultures. (**E**) Hierarchical clustering heatmap showing FFA responses to stimulated neuroexocytosis with and without ara-C treatment. Data information: In (**C**), the significance of the difference between each group ($n = 3$ biological replicates) as determined by unpaired *t*-test with Holm–Sidak post hoc correction is indicated by asterisks $^{**}p < 0.01$, ns = not significant. Error bars represent the cumulative standard error of the mean (SEM) for all groups and parameters.