Figure S1. Membrane cholesterol content in hippocampal neurons, pre-treated or not for 30 minutes with 1 μM tetrodotoxin (TTX), and stimulated with 55 mM KCl. Blockage of pre-synaptic activity with TTX did not affect KCl-induced cholesterol loss; * $p < 0.05$ vs. 5 mM KCl, Student's t-test.

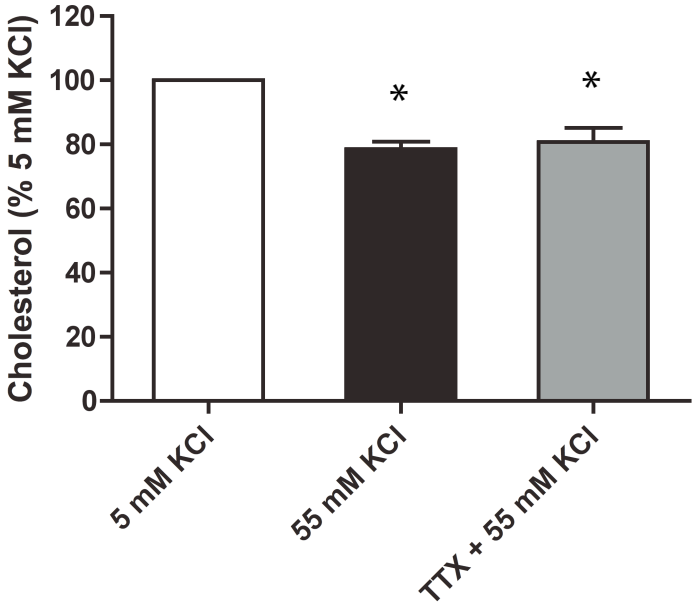
Figure S2. (A) Representative field images of cultured hippocampal neurons treated with vehicle or 10 μM NMDA for 30 minutes. Neither morphological changes nor cell loss were observed after the NMDA treatment. Scale bar: 30 μm. (B) Representative western blots of total membrane extracts obtained from cultured hippocampal neurons. Treatment with 10 μM NMDA for 30 minutes did not alter the expression levels of several synaptic proteins. Syp: Synaptophysin.

Figure S3. (A) 24S-hydroxycholesterol to phospholipids ratio assessed by liquid chromatography/mass spectrometry (LC/MS) in the medium of cultured hippocampal neurons exposed for 30 minutes to vehicle or 10 μM NMDA (* $p < 0.05$ vs VEH, Student's t-test). (B) Cultured hippocampal neurons were incubated with a lentivirus encoding a shRNA sequence against CYP46A1 at 3 days *in vitro* (DIV), and maintained in culture until 14 DIV. Comparison of the CYP46A1 levels by western immunoblotting revealed a knockdown efficiency of 97%. (B) Lentivirus-incubated and non-incubated cells were exposed for 30 minutes to 55 mM KCl. Membrane fractions were purified and cholesterol levels were assessed by fluorimetric detection (see Materials and Methods for details). Stimulation of neurons in the absence of the shRNA induced a 19% reduction in the levels of membrane cholesterol. Incubation with the shRNA did not affect cholesterol levels but prevented the cholesterol reduction induced by 55 mM KCl.

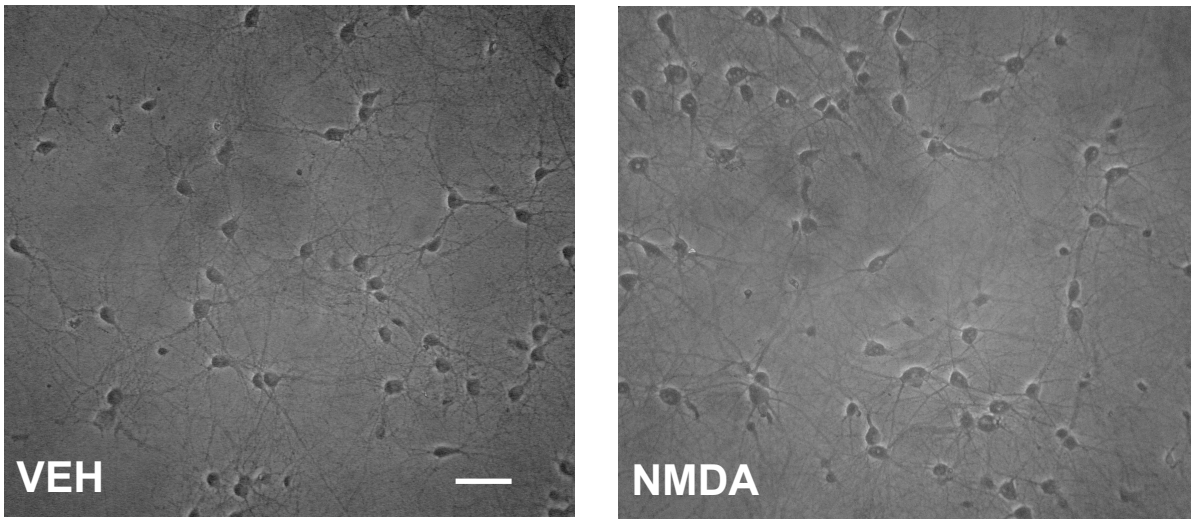
Supplementary movie S1: Mobilization of GFP-CYP46A1 to the plasma membrane in HEK293T cells, as studied using Total Internal Reflection Fluorescence (TIRF) microscopy. Ionomycin (0.5 μM) and Ca^{2+} (2 mM) were added at the indicated time points.

Supplementary movie S2: Mobilization of GFP-CYP46A1 to the plasma membrane in 7 *div* hippocamapal neurons, as studied using TIRF microscopy. High potassium buffer (50 mM) was added at the indicated time point.

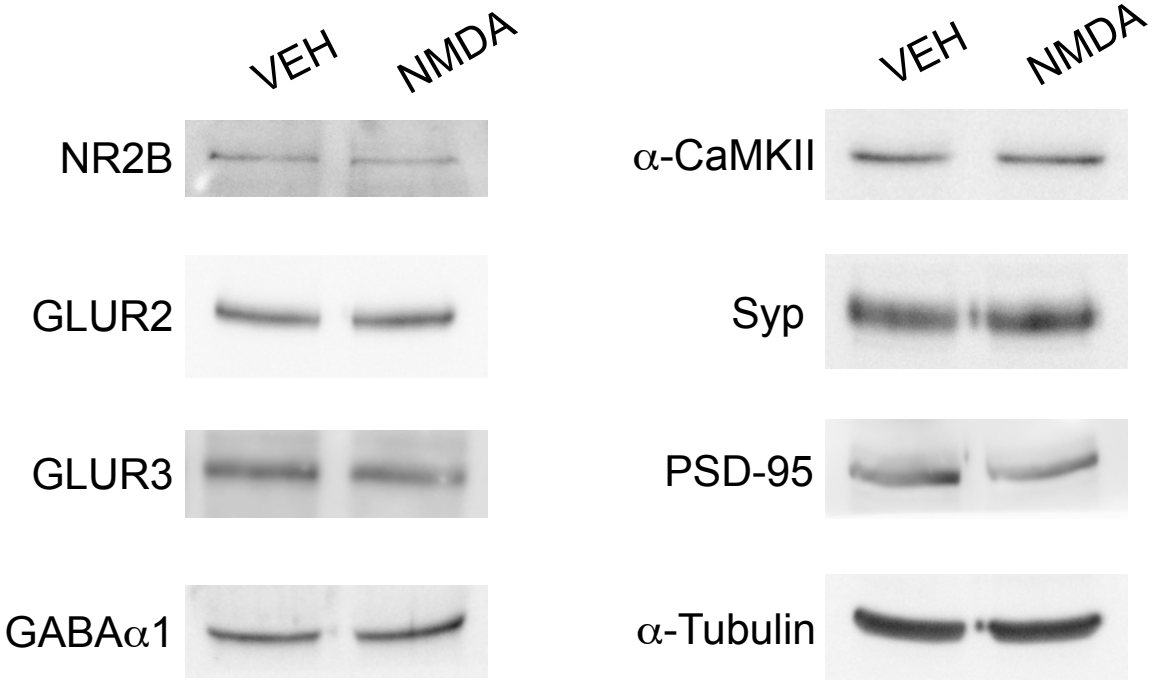
Supplementary movie S3: Mobilization of GFP-CYP46A1 to the plasma membrane in 14 *div* hippocamapal neurons, as studied using TIRF microscopy. High potassium buffer (50 mM) was added at the indicated time point.


Cholesterol (% 5 mM KCl)
120
100
80
60
40
20
0
*
*
5 mM KCl
55 mM KCl
TTX + 55 mM KCl

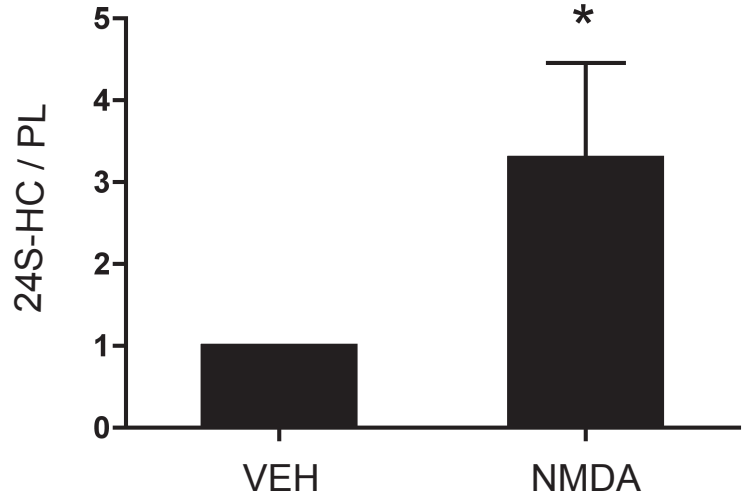
A


VEH
NMDA

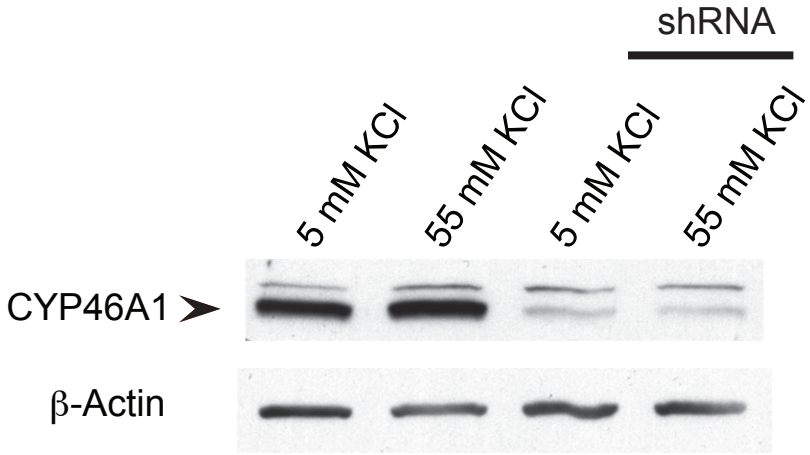
B


VEH
NMDA
NR2B
GLUR2
GLUR3
GABAα1
VEH
NMDA
α-CaMKII
Syp
PSD-95
α-Tubulin

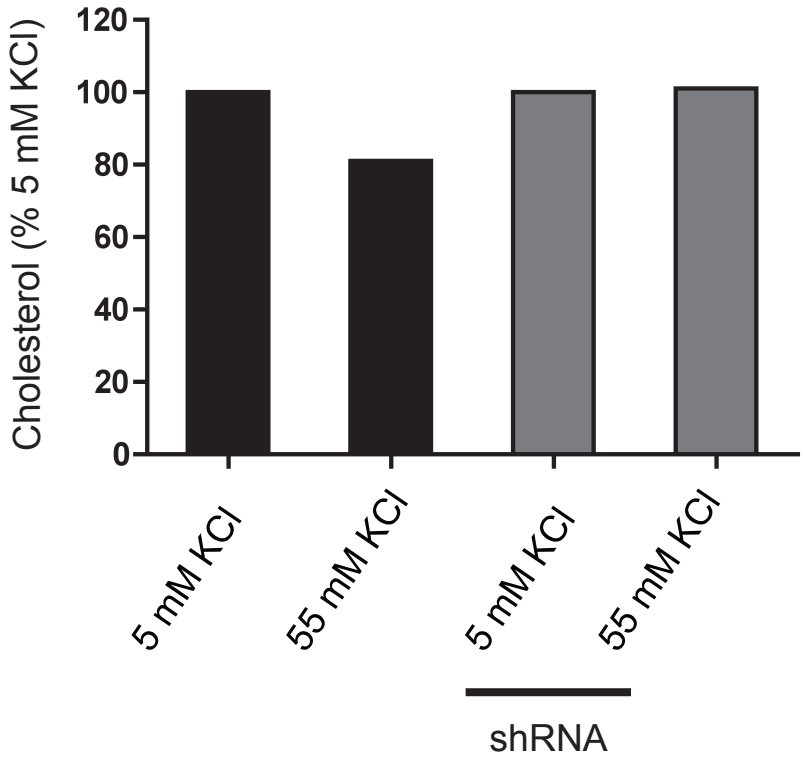
A


24S-HC / PL
5
4
3
2
1
0
*
VEH
NMDA

B


shRNA
5 mM KCl
55 mM KCl
5 mM KCl
55 mM KCl
CYP46A1
β-Actin

C


Cholesterol (% 5 mM KCl)
120
100
80
60
40
20
0
5 mM KCl
55 mM KCl
5 mM KCl
55 mM KCl
shRNA