Expanded View Figures

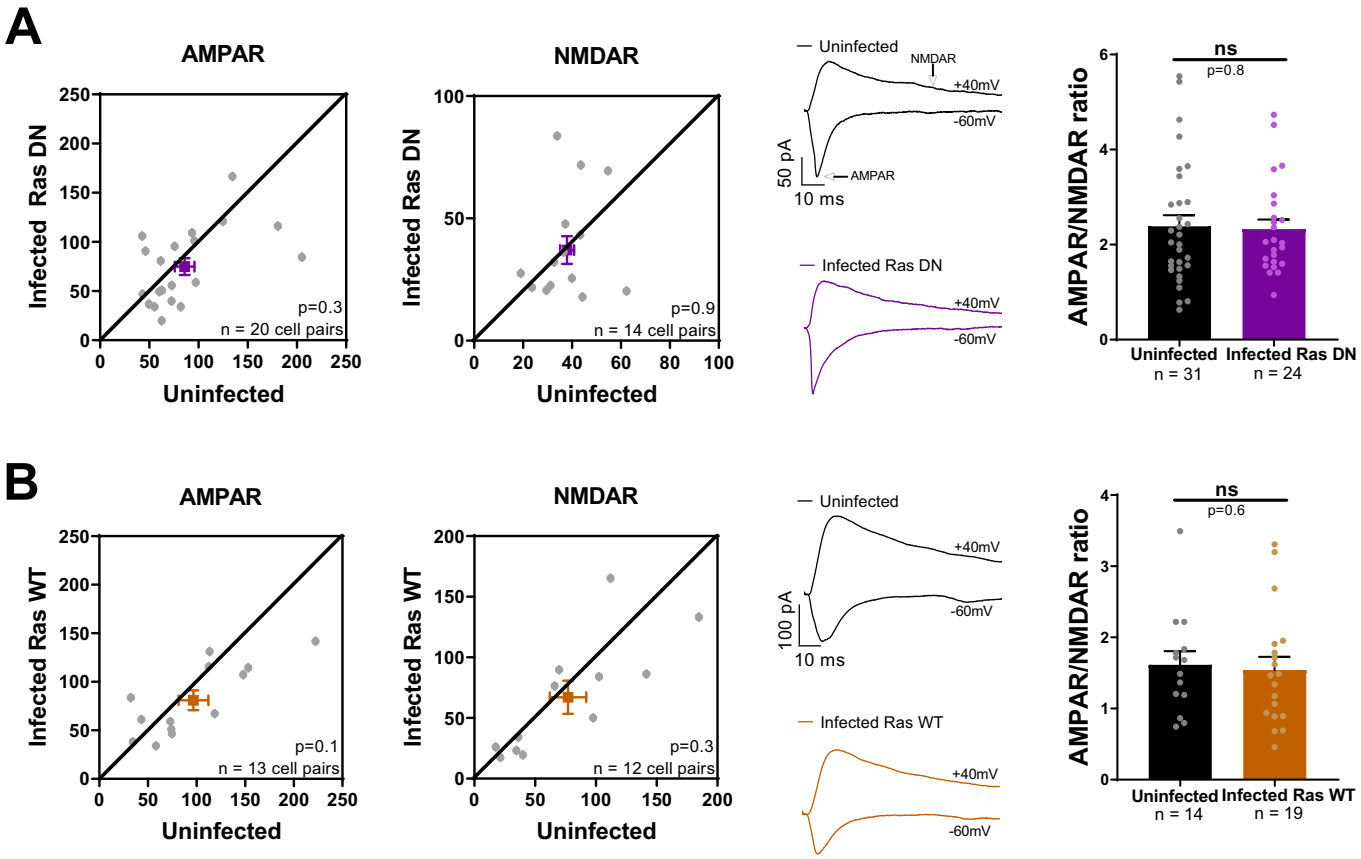


Figure EV1. Effect of Ras dominant negative on basal synaptic transmission.

Scatter plot of simultaneous recordings of AMPAR- or NMDAR-mediated responses from uninfected and Ras-DN (**A**) or Ras WT (**B**) infected neurons (left). Representative traces (middle). Quantification of AMPA/NMDA ratio (right) represented as mean ± SEM; individual values are also plotted. Mann-Whitney test was used to evaluate significant differences. ns, non-significant.

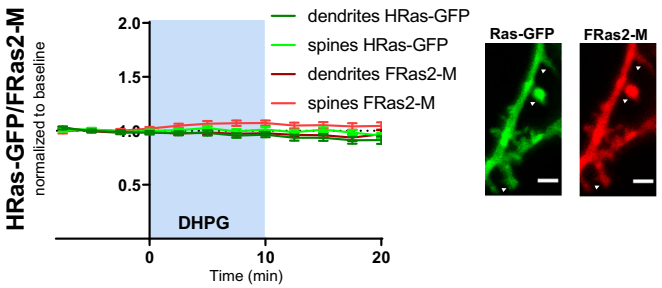
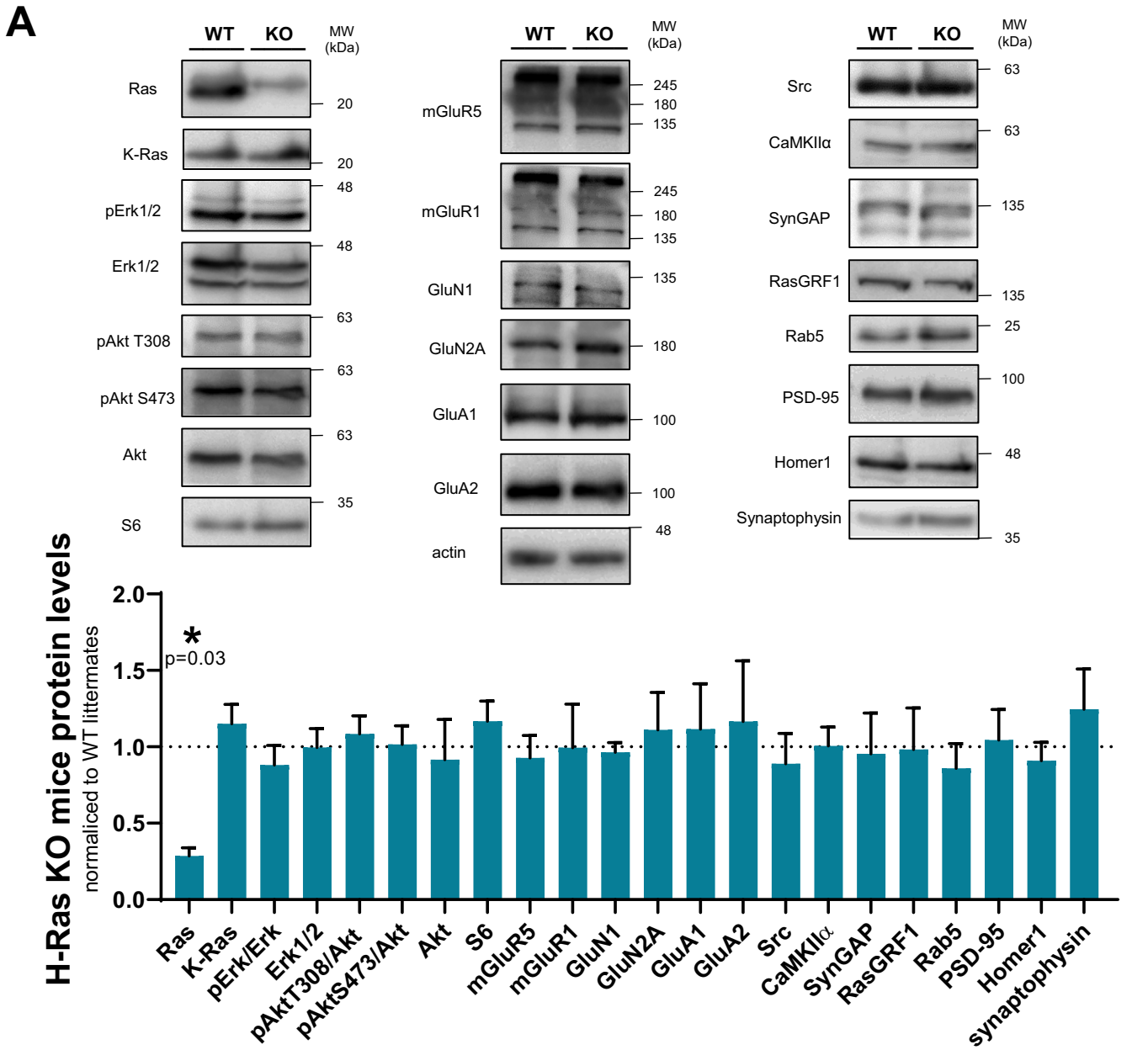


Figure EV2. H-Ras-GFP and FRas2-M fluorescence during DHPG treatment.

Left. Time course of H-Ras-GFP and FRas2-M signal in spines ($n = 50$) and dendrites ($n = 15$) of CA1 pyramidal cells ($n = 10$) upon DHPG stimulation. Results are represented as mean ± SEM. Kruskal–Wallis test was used to evaluate differences across time. [$H(11,571) = 15.1$, $p = 0.2$ for FRas2-M and $H(11,571) = 18.8$, $p = 0.06$ for Ras-GFP]. Right. Representative images of dendritic branches of CA1 neurons coexpressing H-Ras-GFP (green channel) and FRas2-M (red channel) constructs. Scale bars 2 µm. White arrows point dendritic spines.


A
WT
KO
MW (kDa)
Ras
20
K-Ras
20
pErk1/2
48
Erk1/2
48
pAkt T308
63
pAkt S473
63
Akt
63
S6
35
mGluR5
245
180
135
mGluR1
245
180
135
GluN1
135
GluN2A
180
GluA1
100
GluA2
100
actin
48
Src
63
CaMKIIα
63
SynGAP
135
RasGRF1
135
Rab5
25
PSD-95
100
Homer1
48
Synaptophysin
35
H-Ras KO mice protein levels
normaliced to WT littermates
*
p=0.03
2.0
1.5
1.0
0.5
0.0
Ras
K-Ras
pErk/Erk
Erk1/2
pAktT308/Akt
pAktS473/Akt
Akt
S6
mGluR5
mGluR1
GluN1
GluN2A
GluA1
GluA2
Src
CaMKIIα
SynGAP
RasGRF1
Rab5
PSD-95
Homer1
synaptophysin
B
Open field
Distance (m)
80
60
40
20
0
Total
Periphery
Inner
Center
C
Open field
Mean speed (cm/s)
7
6
5
4
3
2
1
0
WT
H-Ras KO
D
Fear conditioning: Training
% Time freezing
60
40
20
0
Initial T1
ITI1
T2
ITI2
T3
ITI3
WT
H-Ras KO

◀ **Figure EV3. Hippocampal levels of different synaptic and signaling proteins and animal behavior in H-Ras KO mice.**

(**A**) Representative Western blots (upper) and quantification (lower) of Ras proteins and different Ras effectors, signaling molecules and glutamate receptors. Some blots have been reused from Fig. 5 (inputs of K-Ras, Rab5, PSD-95, synaptophysin), as they come from the same experiment/animal. Unless otherwise indicated, each sample is normalized to actin levels and results are referred to WT animals and represented as mean ± SEM, $n = 6$ animals per condition. Wilcoxon signed-rank test (*) was used to evaluate changes; non-significant differences were found except for total Ras levels. (**B**) Total distance traveled in the open field test and in the different subsections of the arena. Mean ± SEM; individual values are also represented. Mixed effects analysis was used to evaluate differences between genotypes [$F(1,27) = 0.2$, $p = 0.7$]. (**C**) Mean speed in the open field test. Mean ± SEM; individual values are also represented. Mann-Whitney test was used to evaluate differences between genotypes ($p = 0.9$). (**B**, **C**) $n = 15$ mice for WT and 14 mice for H-Ras KO. (**D**) Percentage of time spent freezing during fear conditioning training session. T, tone; ITI, inter-tone interval. Mean ± SEM is represented. Mixed effects analysis was used to evaluate differences between genotypes [$F(1,45) = 0.1$, $p = 0.8$]. $n = 25$ mice for WT and 22 mice for H-Ras KO.

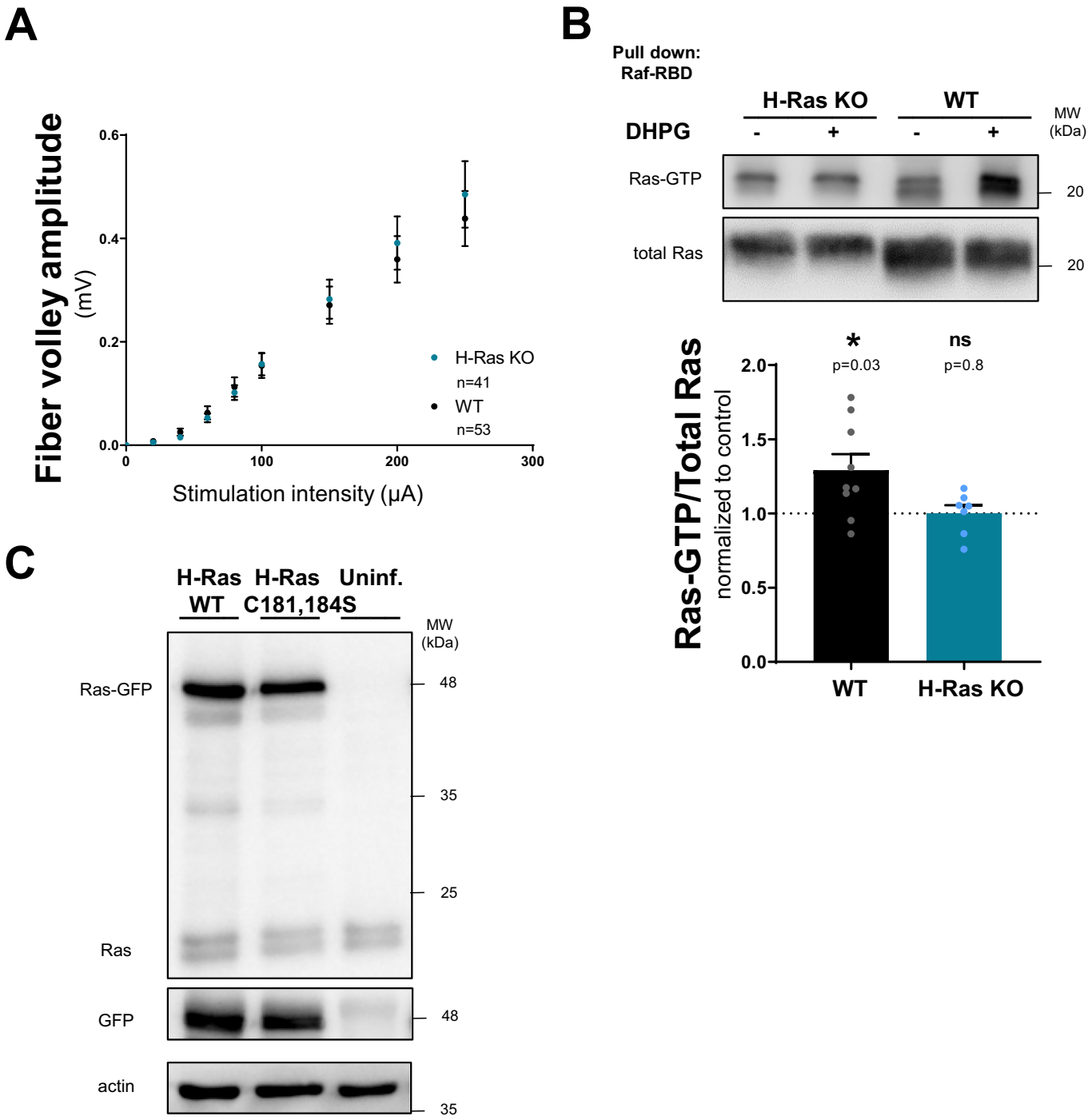


Figure EV4. Analysis of basal transmission and Ras activity in H-Ras KO mice.

(**A**) Fiber volley responses over increasing stimulation intensities during input/output curve measurements shown in Fig. 4A. Mean ± SEM; Mixed effects analysis [$F(1,92) = 0.1$, $p = 0.8$]. (**B**) Quantification (lower panel) and representative blot (upper panel) of active Ras pulled-down vs total Ras from acute hippocampal slices of WT and H-Ras KO mice 10 min after DHPG treatment. Results are normalized to control non-stimulated slices (in WT or KO mice) and expressed as mean ± SEM, $n = 7$–9 independent slices/mice. Wilcoxon signed-rank test (*) was used to assess statistically the effect of DHPG. ns, non-significant. (**C**) Representative blot of Ras and GFP in organotypic hippocampal slices infected with H-Ras WT and H-Ras C181,184S, or uninfected (uninf.). Note that both endogenous Ras (~21 KDa) and recombinant Ras-GFP (~48 KDa) were detected at their appropriate sizes. Actin was used a protein loading control.

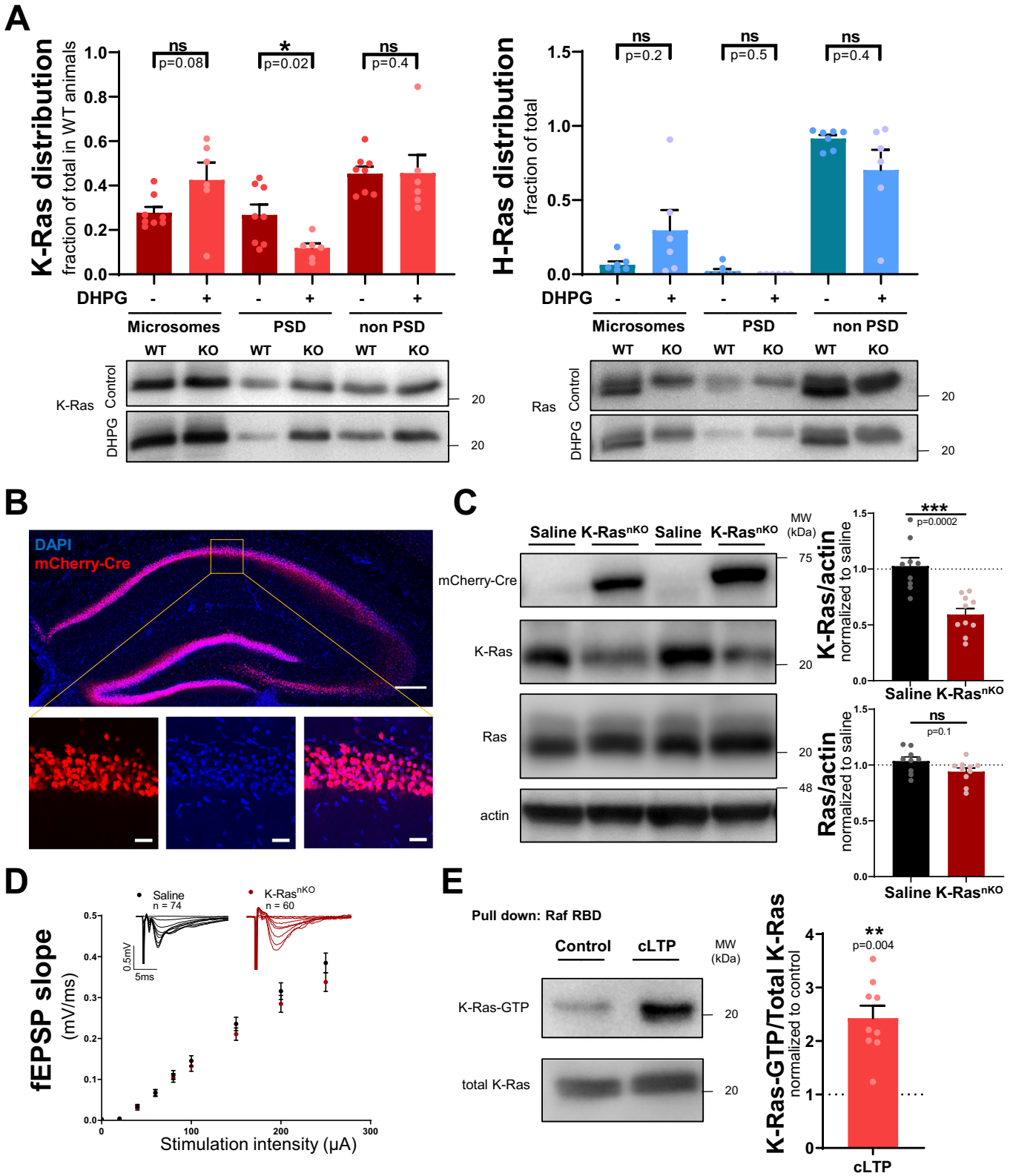

A
K-Ras distribution
fraction of total in WT animals
ns p=0.08
* p=0.02
ns p=0.4
DHPG
Microsomes
PSD
non PSD
WT KO WT KO WT KO
K-Ras
Control
DHPG
20
20
H-Ras distribution
fraction of total
ns p=0.2
ns p=0.5
ns p=0.4
Ras
B
DAPI
mCherry-Cre
C
Saline K-RasnKO Saline K-RasnKO
MW (kDa)
mCherry-Cre
K-Ras
Ras
actin
75
20
20
48
K-Ras/actin normalized to saline
*** p=0.0002
Saline K-RasnKO
Ras/actin normalized to saline
ns p=0.1
D
fEPSP slope (mV/ms)
Saline n = 74
K-RasnKO n = 60
0.5mV
5ms
Stimulation intensity (μA)
E
Pull down: Raf RBD
Control cLTP
MW (kDa)
K-Ras-GTP
total K-Ras
20
20
K-Ras-GTP/Total K-Ras normalized to control
** p=0.004
cLTP

Figure EV5. Analysis of Ras distribution, expression, and activity in H-Ras and neuronal K-Ras KO mice.

(**A**) Representative blots (lower pannel) of total Ras and K-Ras in different subcellular compartments following DHPG stimulation in hippocampal slices from WT and H-Ras KO mice. H- and K-Ras distribution in microsomes, PSD and non-PSD fractions was quantified (upper panel) as percentage of total protein (from inputs) for each isoform. H-Ras signal was obtained by subtracting total Ras signal of H-Ras KO mice from that of WT mice. Results expressed as mean ± SEM, $n = 6$–8 animals per condition (controls from Fig. 5A were included in the quantification); individual values are also represented. Mann-Whitney test was used to evaluate DHPG effect in each fraction compared to control, non-stimulated slices. (**B**) Representative image of mCherry fluorescence (red) and DAPI staining (blue) of a hippocampal slice from AAV-CaMKII-mCherry-Cre-infected K-Ras$^{flox/flox}$ mice (lower panels: zoom-in images of CA1 region). Scale bars 200 (upper) and 20 (lower) μm. (**C**) Representative Western blots (left) and quantification (right) of K-Ras and total Ras hippocampal levels. Results are normalized to saline average levels and expressed as mean ± SEM; individual values are also represented. Mann-Whitney test (*) was used to evaluate statistical differences. $n = 9$ (WT) and 10 (K-Ras nKO) mice. (**D**) Input/output curves of fEPSP slopes vs stimulation intensities. Representative traces are shown in the upper part. Mean ± SEM; Mixed effects analysis was used to assess statistical differences between genotypes [$F(1,132) = 1.0$, $p = 0.3$]. (**E**) Quantification (left) and representative blot (right) of active K-Ras pulled-down vs total K-Ras from mouse organotypic hippocampal slices 10 min after cLTP treatment. Results are normalized to control non-stimulated slices and expressed as mean ± SEM, $n = 9$. Wilcoxon signed-rank test (*) was used to assess statistically the effect of cLTP induction. ns, non-significant.

