Supplemental Information

Ras and Rap signal bidirectional synaptic plasticity via distinct subcellular microdomains

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Figure S1

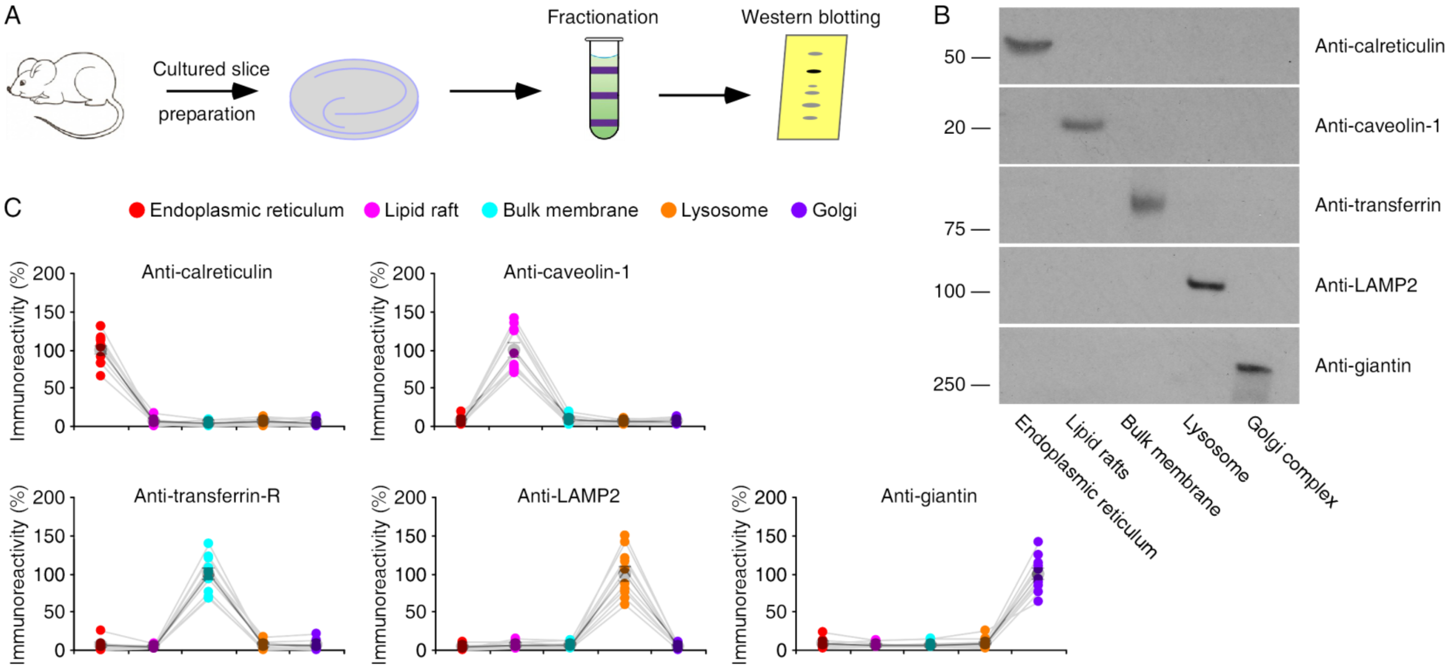


Figure S1. Blotting of microdomain specific markers authenticates the micro-fractionation method, related to figure 1.

(A) Schematic drawing outlines micro-fractionation experimental design.

(B) Blots of calreticulin, caveolin-1, transferrin receptor, LAMP2 and giantin in the endoplasmic reticulum, lipid rafts, bulk membrane, lysosome and Golgi complex fractionated from cultured rat hippocampal slices. Each lane was loaded with 5% of endoplasmic reticulum, lipid rafts, bulk membrane, lysosome or Golgi complex fractions isolated from 48 cultured hippocampal slices.

(C) Upper left, relative levels of calreticulin in all the other microdomains (Lipid rafts: 5.3±1.3%; Bulk membrane: 4.3±1.0%; Lysosome: 6.3±1.3%; Golgi complex: 4.8±1.3%) compared to that in the endoplasmic reticulum (100.0±6.1%; n=10 from 144-240 slices prepared from 6-10 animals; Z=-2.803; p<0.01; Wilcoxon tests). Upper right, relative levels of caveolin-1 in all the other microdomains (Endoplasmic reticulum: 6.5±1.5%; Bulk membrane: 7.9±1.8%; Lysosome: 6.3±0.8%; Golgi complex: 6.5±1.2%) compared to that in the lipid rafts (100.0±8.9%; n=10 from 144-240 slices prepared from 6-10 animals; Z=-2.803; p<0.01; Wilcoxon tests). Lower left, relative levels of transferrin receptor in all the other microdomains (Endoplasmic reticulum: 5.4±2.4%; Lipid rafts: 3.9±0.8%; Lysosome: 6.3±1.8%; Golgi complex: 5.5±2.0%) compared to that in the bulk membrane (100.0±7.5%; n=10 from 144-240 slices prepared from 6-10 animals; Z=-2.803; p<0.01; Wilcoxon tests). Lower middle, relative levels of LAMP2 in all the other microdomains (Endoplasmic reticulum: 4.2±0.9%; Lipid rafts: 6.0±1.4%; Bulk membrane: 6.2±1.2%; Golgi complex: 5.0±0.9%) compared to that in the lysosome (100.0±9.8%; n=10 from 144-240 slices prepared from 6-10 animals; Z=-2.803; p<0.01; Wilcoxon tests). Lower left, relative levels of giantin in all the other microdomains (Endoplasmic reticulum: 8.1±2.0%; Lipid rafts: 6.7±1.1%; Bulk membrane: 7.3±1.3%; Lysosome: 8.8±2.1%) compared to that in the Golgi complex (100.0±7.4%; n=10 from 144-240 slices prepared from 6-10 animals; Z=-2.803; p<0.01; Wilcoxon tests). The relative values and standard errors were normalized to average amounts of calreticulin, caveolin-1, transferrin receptor, LAMP2 and giantin in the endoplasmic reticulum, lipid rafts, bulk membrane, lysosome and Golgi complex fractionated from cultured rat hippocampal slices, respectively.

Figure S2

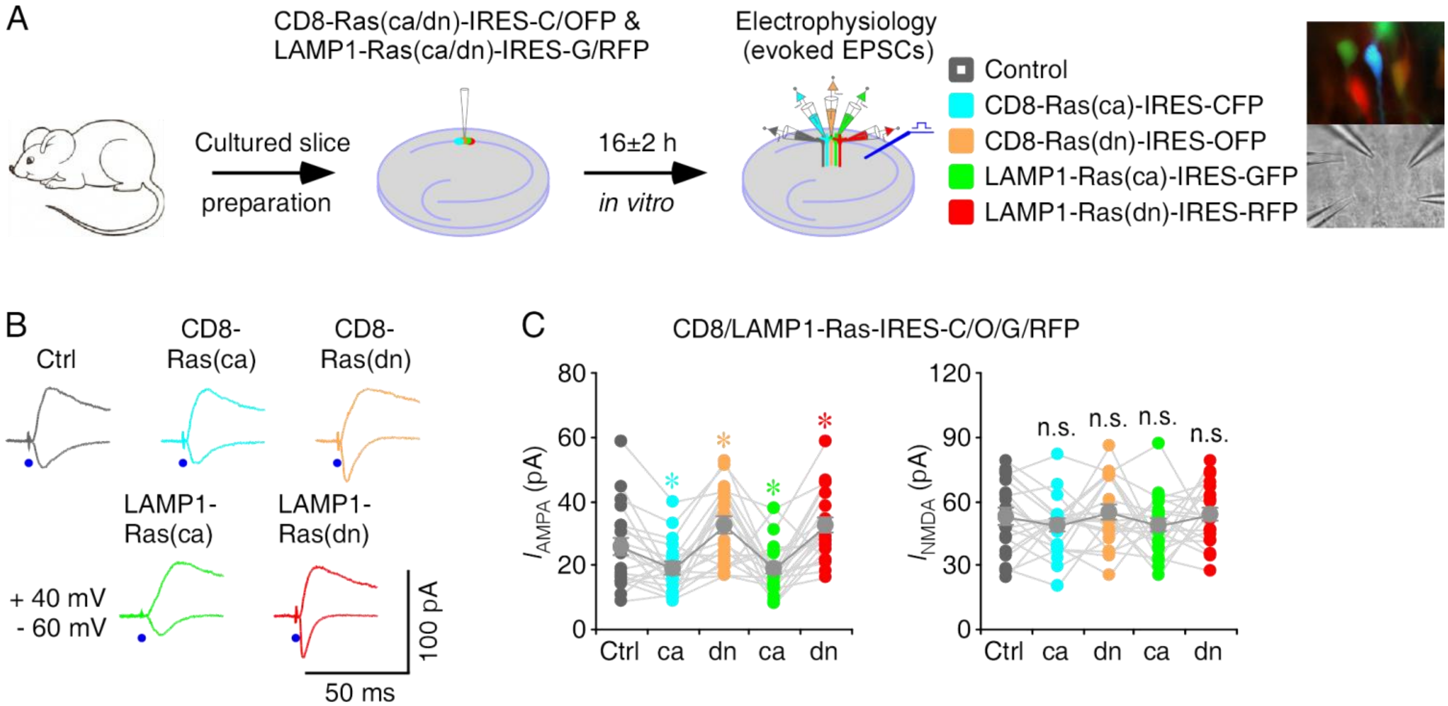


Figure S2. Target-delivered Ras in the bulk membrane and lysosome depresses transmission, related to figure 2.

(A) Schematic drawing outlines *in vitro* experimental design. The right images show simultaneous whole-cell recordings from LAMP1-Ras(dn)-IRES-RFP, LAMP1-Ras(ca)-IRES-GFP, CD8-Ras(ca)-IRES-CFP, CD8-Ras(dn)-IRES-OFP expressing and control non-expressing (in clockwise order) CA1 pyramidal neuron quintuplets under fluorescence (upper) and transmitted light (lower) microscopy.

(B) Evoked AMPA-R- (-60 mV) and NMDA-R- (+40 mV) mediated responses recorded from neighboring control non-expressing (Ctrl), CD8-Ras(ca)-IRES-CFP, CD8-Ras(dn)-IRES-OFP, LAMP1-Ras(ca)-IRES-GFP and LAMP1-Ras(dn)-IRES-RFP expressing CA1 cells after 16±2 h expression.

(C) AMPA and NMDA responses in CD8-Ras(ca), CD8-Ras(dn), LAMP1-Ras(ca) and LAMP1-Ras(dn) expressing neurons relative to neighboring non-expressing control cells. See **Table S5** for values of AMPA and NMDA responses. Asterisks indicate $p<0.05$ (Wilcoxon tests).

Figure S3

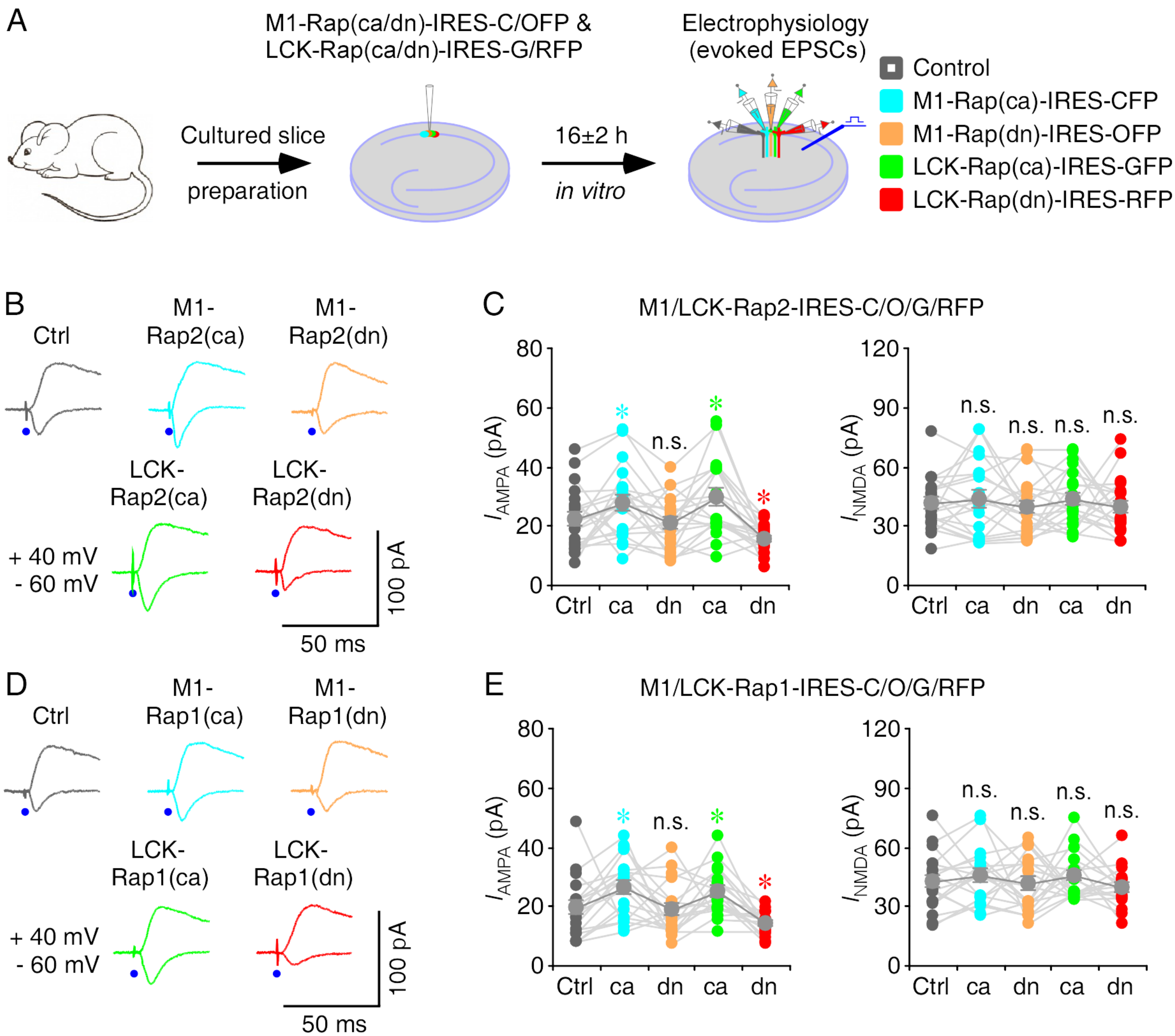


Figure S3. Target-delivered Rap in the endoplasmic reticulum and lipid rafts potentiates transmission, related to figures 3-4.

(A) Schematic drawing outlines *in vitro* experimental design.

(B) Evoked AMPA-R- (-60 mV) and NMDA-R- (+40 mV) mediated responses recorded from neighboring control non-expressing (Ctrl), M1-Rap2(ca)-IRES-CFP, M1-Rap2(dn)-IRES-OFP, LCK-Rap2(ca)-IRES-GFP and LCK-Rap2(dn)-IRES-RFP expressing CA1 cells after 16±2 h expression.

(C) AMPA and NMDA responses in M1-Rap2(ca), M1-Rap2(dn), LCK-Rap2(ca) and LCK-Rap2(dn) expressing neurons relative to neighboring non-expressing control cells. See **Table S5** for values of AMPA and NMDA responses. Asterisks indicate $p<0.05$ (Wilcoxon tests).

(D) Evoked AMPA-R- (-60 mV) and NMDA-R- (+40 mV) mediated responses recorded from neighboring control non-expressing (Ctrl), M1-Rap1(ca)-IRES-CFP, M1-Rap1(dn)-IRES-OFP, LCK-Rap1(ca)-IRES-GFP and LCK-Rap1(dn)-IRES-RFP expressing CA1 cells after 16±2 h expression.

(E) AMPA and NMDA responses in M1-Rap1(ca), M1-Rap1(dn), LCK-Rap1(ca) and LCK-Rap1(dn) expressing neurons relative to neighboring non-expressing control cells. See **Table S5** for values of AMPA and NMDA responses. Asterisks indicate $p<0.05$ (Wilcoxon tests).

Figure S4

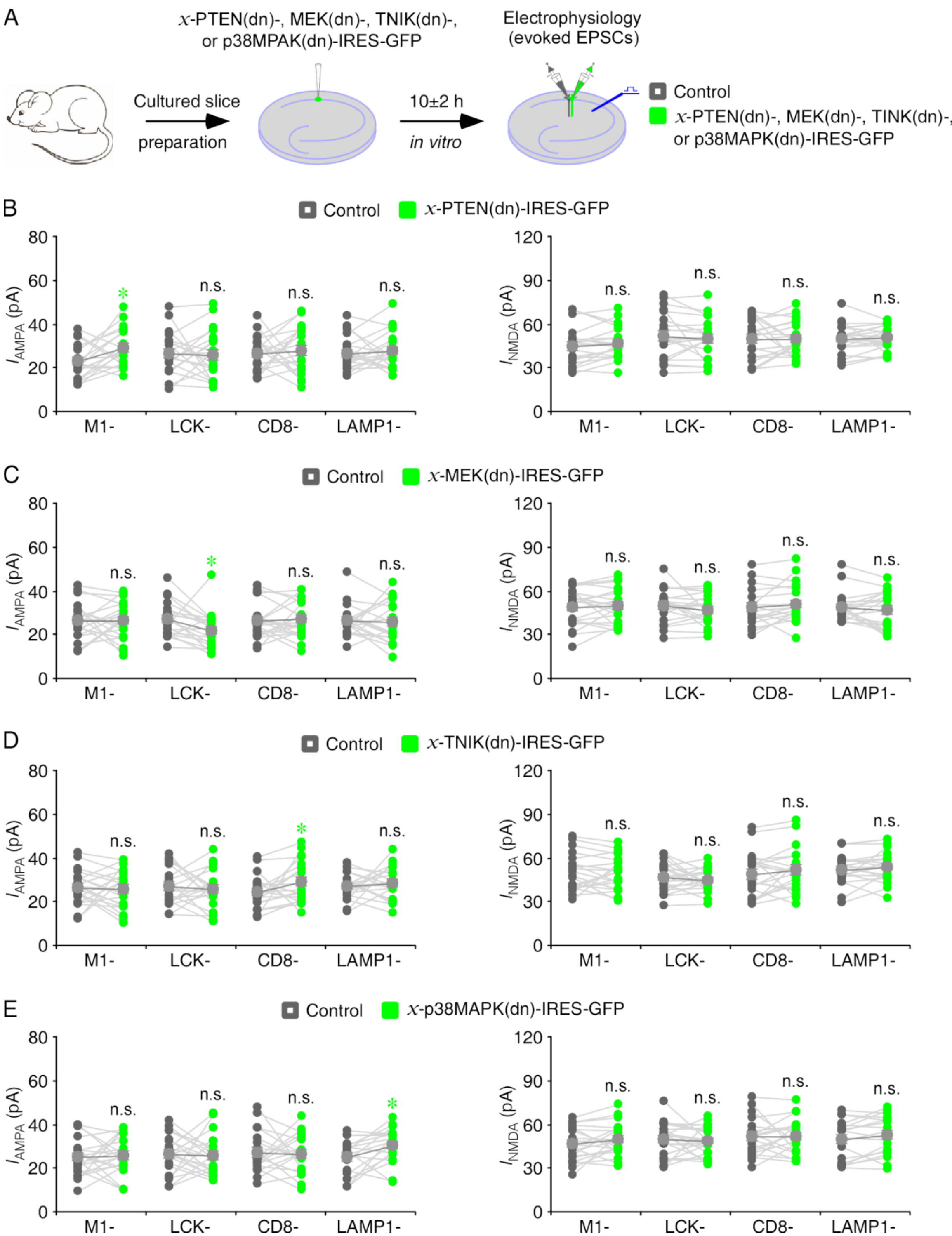

A
x-PTEN(dn)-, MEK(dn)-, TNIK(dn)-, or p38MPAK(dn)-IRES-GFP
Electrophysiology (evoked EPSCs)
Cultured slice preparation
10±2 h in vitro
Control
x-PTEN(dn)-, MEK(dn)-, TINK(dn)-, or p38MAPK(dn)-IRES-GFP
B
Control
x-PTEN(dn)-IRES-GFP
C
Control
x-MEK(dn)-IRES-GFP
D
Control
x-TNIK(dn)-IRES-GFP
E
Control
x-p38MAPK(dn)-IRES-GFP
I AMPA (pA)
I NMDA (pA)
M1-
LCK-
CD8-
LAMP1-
n.s.
*

Figure S4. Targeted expressions of PTEN, MEK, TNIK and p38MAPK alter transmission, related to figures 5-6.

(A) Schematic drawing outlines *in vitro* experimental design.

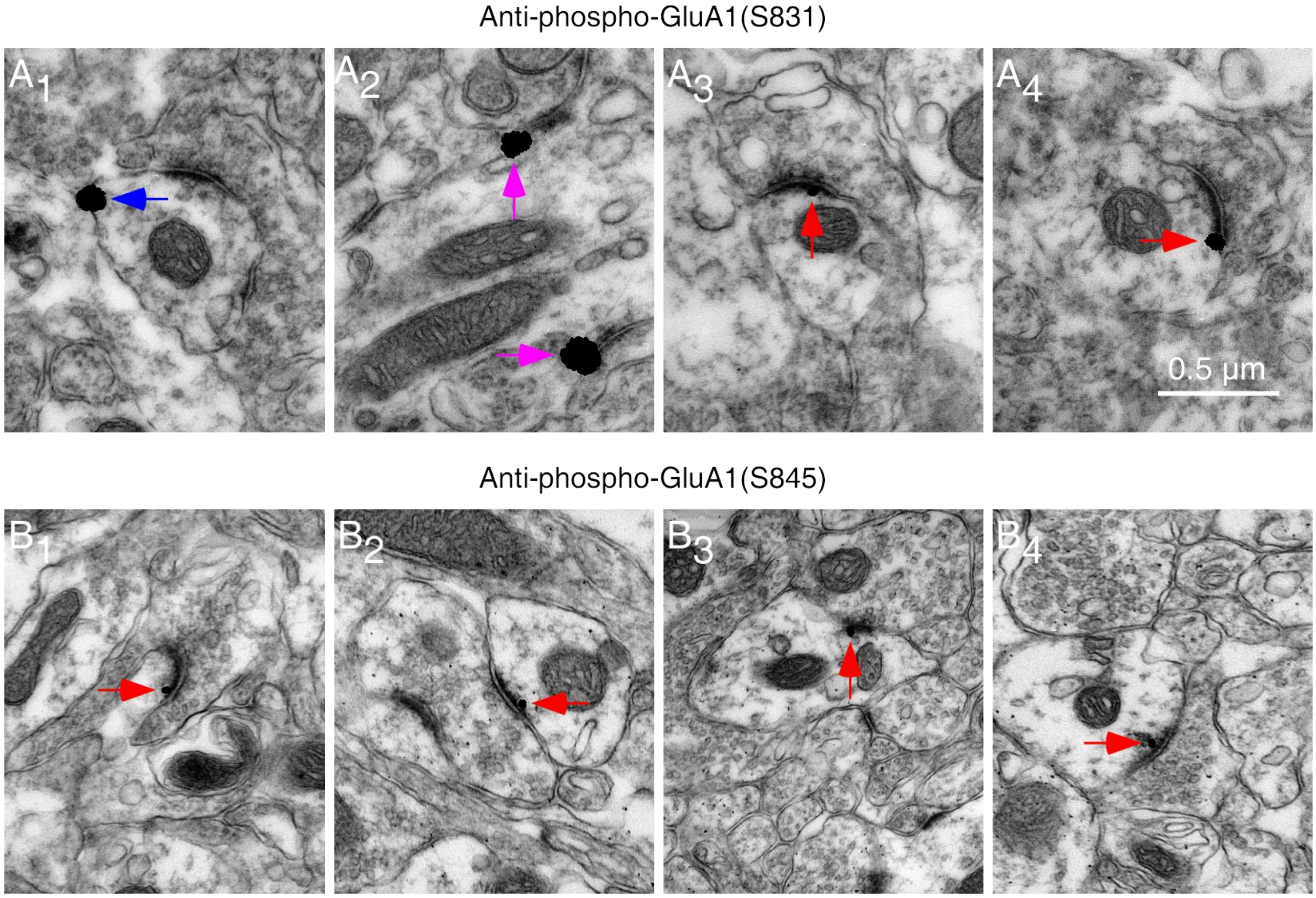
(B) AMPA (left) and NMDA (right) responses in M1-PTEN(dn), LCK-PTEN(dn), CD8-PTEN(dn) and LAMP1-PTEN(dn) expressing neurons relative to neighboring non-expressing control cells. See **Table S8** for values of AMPA and NMDA responses.

(C) AMPA (left) and NMDA (right) responses in M1-MEK(dn), LCK-MEK(dn), CD8-MEK(dn) and LAMP1-MEK(dn) expressing neurons relative to neighboring non-expressing control cells. See **Table S8** for values of AMPA and NMDA responses.

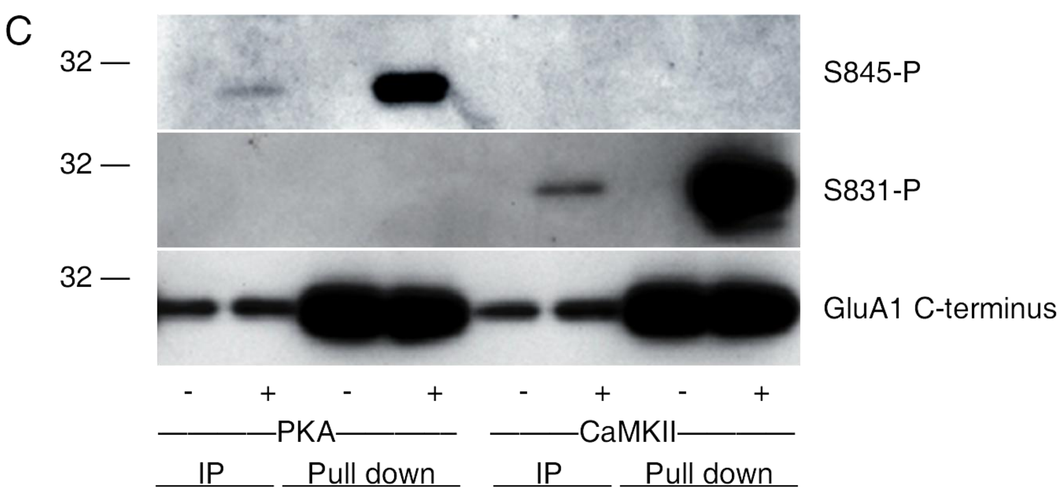
(D) AMPA (left) and NMDA (right) responses in M1-TNIK(dn), LCK-TNIK(dn), CD8-TNIK(dn) and LAMP1-TNIK(dn) expressing neurons relative to neighboring non-expressing control cells. See **Table S8** for values of AMPA and NMDA responses.

(E) AMPA (left) and NMDA (right) responses in M1-p38MAPK(dn), LCK-p38MAPK(dn), CD8-p38MAPK(dn) and LAMP1-p38MAPK(dn) expressing neurons relative to neighboring non-expressing control cells. See **Table S8** for values of AMPA and NMDA responses. Asterisks indicate $p<0.05$ (Wilcoxon tests).

Figure S5



Anti-phospho-GluA1(S831)
A1
A2
A3
A4
0.5 µm
Anti-phospho-GluA1(S845)
B1
B2
B3
B4



C
32 —
32 —
32 —
S845-P
S831-P
GluA1 C-terminus
- + - + - + - +
PKA
CaMKII
IP
Pull down
IP
Pull down

Figure S5. Phosphorylation at S845 and S831 regulates GluA1 subcellular distribution at synapses, related to figure 7.

($A_{1\text{-}4}$) Immunoelectron microscopic images show immunogold labeling of GluA1 with phosphorylation at S831 at mouse geniculate synapses (see (Kielland et al., 2009) for the methods). Note S831-phosphorylated GluA1 silver-gold particles located at the membrane within (red arrows), close to (pink arrows) and faraway from (blue arrow) the postsynaptic density (PSD)**.**

($B_{1\text{-}4}$) Immunoelectron microscopic images show immunogold labeling of GluA1 with phosphorylation at S845 at mouse geniculate synapses. Note S845-phosphorylated GluA1 silver-gold particles located predominantly at the membrane within PSD (red arrows). Also note that authentication of anti-phospho-GluA1(S831) and anti-phospho-GluA1(S845) antibodies made by IP and pull down assays in (**C**), and absence of S831- and S845-phosphorylated GluA1 silver-gold particles at synapses of *GluA1(S831A/S845A)* transgenic mice (not shown).

(**C**) GST fusion protein of GluA1 C-terminus (residues 833-907) was either immuneprecipitated with the anti-GluA1 C-terminus antibody (IP) or pulled down with glutathione Sepharose before incubation with PKA for S845 phosphorylation (lanes 2,4,6,8) or CaMKII for S831 phosphorylation (lanes 1,3,5,7). Samples were immunoblotted with anti-phospho-GluA1(S845), stripped and reprobed with anti-phospho-GluA1(S831), and ultimately with anti-GluA1 C-terminus. The anti-phospho-antibodies are highly specific for the respective phosphorylation sites.

Table S1

Relative protein expression levels in distinct microdomains of hippocampal cells (related to figure 1)

Protein	Endoplasmic reticulum	Lipid rafts	Bulk membrane	Lysosome	Golgi complex	Sample No. Z & p values
Ras	100.0±8.2%	117.1±10.1%	113.3±10.3%	110.5±10.8%	103.2±10.1%	n=11; Z<1.800; p>0.05
Rap2	100.0±6.8%	102.3±7.3%	110.3±8.3%	103.0±8.4%	106.7±9.3%	n=11; Z<1.800; p>0.05
Rap1	100.0±6.7%	94.1±5.1%	98.7±5.2%	89.7±4.4%	89.7±5.2%	n=11; Z<1.800; p>0.05
M1-Ras-YFP	100.0±7.7%	1.9±0.3%*	3.0±0.5%*	2.0±0.4%*	2.2±0.3%*	n=10; Z=-2.803; p<0.01
LCK-Ras-YFP	2.9±0.7%*	100.0±5.2%	7.3±1.7%*	2.7±0.4%*	2.6±0.4%*	n=10; Z=-2.803; p<0.01
CD8-Ras-YFP	2.7±0.5%*	18.3±2.3%*	100.0±7.3%	1.8±0.3%*	1.7±0.3%*	n=10; Z=-2.803; p<0.01
LAMP1-Ras-YFP	6.2±0.9%*	8.0±0.9%*	18.3±2.3%*	100.0±5.1%	7.6±0.9%*	n=10; Z=-2.803; p<0.01
KDELr-Ras-YFP	4.0±0.6%*	4.3±0.6%*	8.1±1.2%*	4.5±0.8%*	100.0±9.2%	n=10; Z=-2.803; p<0.01
Rap2	100.0±11.3%	99.4±10.0%	102.8±11.7%	99.8±10.4%	100.5±10.0%	n=10; Z<0.800; p>0.15
calreticulin	100.0±6.1%	5.3±1.3%*	4.3±1.0%*	6.3±1.3%*	4.8±1.3%*	n=10; Z=-2.803; p<0.01
caveolin-1	6.5±1.5%*	100.0±8.9%	7.9±1.8%*	6.3±0.8%*	6.5±1.2%*	n=10; Z=-2.803; p<0.01
transferrin-R	5.4±2.4%*	3.9±0.8%*	100.0±7.5%	6.3±1.8%*	5.5±2.0%*	n=10; Z=-2.803; p<0.01
LAMP1-Ras-YFP	4.2±0.9%*	6.0±1.4%*	6.2±1.2%*	100.0±9.8%	5.0±0.9%*	n=10; Z=-2.803; p<0.01
KDELr-Ras-YFP	8.1±2.0%*	6.7±1.1%*	7.3±1.3%*	8.8±2.1%*	100.0±7.4%	n=10; Z=-2.803; p<0.01

The relative values and standard errors were normalized to average amounts of the control groups and set to be 100%. In each row comparisons were made between any two groups if unmarked with asterisk, or between the unmarked control group and groups marked with asterisks. Note samples collected from 144-288 slices prepared from 6-12 animals.

Table S2

Synaptic effects of target-delivered Ras mutants in CA1 neurons (related to figure 2)

Constructs	AMPA currents (Ctrl vs. Exp; pA)	Sample No. *Z* & *p* values	NMDA currents (Ctrl vs. Exp; pA)	Sample No. *Z* & *p* values	Animal No.
M1-Ras(ca)	26.6±2.4 vs. 35.5±3.3	*n*=26; *Z*=2.212; *p*<0.05	51.1±4.6 vs. 52.4±4.4	*n*=26; *Z*=0.192; *p*=0.77	*n*=11
M1-Ras(dd)	26.6±2.4 vs. 27.9±2.4	*n*=26; *Z*=0.982; *p*=0.33	51.1±4.6 vs. 52.6±4.0	*n*=26; *Z*=0.749; *p*=0.45	*n*=11
M1-Ras(dn)	26.6±2.4 vs. 26.0±2.0	*n*=26; *Z*=-0.419; *p*=0.68	51.1±4.6 vs. 51.5±4.5	*n*=26; *Z*=-0.051; *p*=0.96	*n*=11
LCK-Ras(ca)	23.1±2.6 vs. 34.3±3.4	*n*=22; *Z*=2.841; *p*<0.01	39.3±4.3 vs. 40.8±4.1	*n*=22; *Z*=0.243; *p*=0.81	*n*=10
LCK-Ras(dd)	23.1±2.6 vs. 23.7±3.7	*n*=22; *Z*=0.016; *p*=0.99	39.3±4.3 vs. 38.8±4.1	*n*=22; *Z*=-0.146; *p*=0.88	*n*=10
LCK-Ras(dn)	23.1±2.6 vs. 16.2±2.0	*n*=22; *Z*=-2.468; *p*<0.05	39.3±4.3 vs. 38.5±4.8	*n*=22; *Z*=-0.485; *p*=0.70	*n*=10
CD8-Ras(ca)	25.1±2.6 vs. 21.6±2.8	*n*=20; *Z*=1.681; *p*=0.10	51.6±5.1 vs. 49.9±4.0	*n*=20; *Z*=-0.709; *p*=0.48	*n*=10
CD8-Ras(dd)	25.1±2.6 vs. 24.5±2.8	*n*=20; *Z*=0.112; *p*=0.91	51.6±5.1 vs. 51.2±4.9	*n*=20; *Z*=0.104; *p*=0.88	*n*=10
CD8-Ras(dn)	25.1±2.6 vs. 28.7±2.9	*n*=20; *Z*=1.605; *p*=0.11	51.6±5.1 vs. 53.1±5.2	*n*=20; *Z*=0.411; *p*=0.68	*n*=10
LAMP1-Ras(ca)	20.9±2.0 vs. 18.1±1.7	*n*=22; *Z*=-1.380; *p*=0.17	45.6±4.6 vs. 46.9±3.6	*n*=22; *Z*=0.568; *p*=0.57	*n*=11
LAMP1-Ras(dd)	20.9±2.0 vs. 21.2±2.0	*n*=22; *Z*=-0.306; *p*=0.76	45.6±4.6 vs. 47.3±3.7	*n*=22; *Z*=0.276; *p*=0.78	*n*=11
LAMP1-Ras(dn)	20.9±2.0 vs. 23.7±1.8	*n*=22; *Z*=1.899; *p*=0.06	45.6±4.6 vs. 48.0±4.1	*n*=22; *Z*=0.503; *p*=0.62	*n*=11
KDELr-Ras(ca)	26.4±3.0 vs. 28.9±3.1	*n*=20; *Z*=-1.369; *p*=0.17	43.2±2.7 vs. 43.0±4.0	*n*=20; *Z*=-0.448; *p*=0.65	*n*=10
KDELr-Ras(dd)	26.4±3.0 vs. 27.6±2.1	*n*=20; *Z*=0.724; *p*=0.47	43.2±2.7 vs. 41.6±2.4	*n*=20; *Z*=-0.635; *p*=0.53	*n*=10
KDELr-Ras(dn)	26.4±3.0 vs. 26.6±2.7	*n*=20; *Z*=0.261; *p*=0.79	43.2±2.7 vs. 44.2±3.0	*n*=20; *Z*=0.523; *p*=0.60	*n*=10

Table S3

Effects of target-delivered Rap2 mutants in CA1 neurons (related to figure 3)

Constructs	AMPA currents (Ctrl vs. Exp; pA)	Sample No. *Z* & *p* values	NMDA currents (Ctrl vs. Exp; pA)	Sample No. *Z* & *p* values	Animal No.
M1-Rap2(ca)	22.5±2.5 vs. 25.8±1.9	*n*=20; *Z*=1.307; *p*=0.19	48.3±4.9 vs. 47.6±5.1	*n*=20; *Z*=-0.859; *p*=0.39	*n*=9
M1-Rap2(dd)	22.5±2.5 vs. 21.3±2.3	*n*=20; *Z*=-0.653; *p*=0.51	48.3±4.9 vs. 46.7±4.2	*n*=20; *Z*=-0.355; *p*=0.72	*n*=9
M1-Rap2(dn)	22.5±2.5 vs. 22.1±2.3	*n*=20; *Z*=-0.261; *p*=0.79	48.3±4.9 vs. 46.3±4.6	*n*=20; *Z*=-0.933; *p*=0.35	*n*=9
LCK-Rap2(ca)	21.4±2.2 vs. 23.8±1.9	*n*=24; *Z*=1.572; *p*=0.12	41.9±3.5 vs. 42.6±2.5	*n*=24; *Z*=0.400; *p*=0.69	*n*=10
LCK-Rap2(dd)	21.4±2.2 vs. 21.3±2.4	*n*=24; *Z*=-0.429; *p*=0.67	41.9±3.5 vs. 41.6±3.0	*n*=24; *Z*=-0.429; *p*=0.67	*n*=10
LCK-Rap2(dn)	21.4±2.2 vs. 18.1±1.7	*n*=24; *Z*=-1.857; *p*=0.06	41.9±3.5 vs. 40.1±2.7	*n*=24; *Z*=0.086; *p*=0.93	*n*=10
CD8-Rap2(ca)	23.6±2.2 vs. 16.9±1.6	*n*=24; *Z*=-2.243; *p*<0.05	40.3±2.8 vs. 41.6±3.0	*n*=24; *Z*=0.157; *p*=0.88	*n*=11
CD8-Rap2(dd)	23.6±2.2 vs. 22.5±2.6	*n*=24; *Z*=2.329; *p*=0.74	40.3±2.8 vs. 39.5±3.4	*n*=24; *Z*=-0.057; *p*=0.95	*n*=11
CD8-Rap2(dn)	23.6±2.2 vs. 29.8±3.0	*n*=24; *Z*=2.171; *p*<0.05	40.3±2.8 vs. 41.9±2.8	*n*=24; *Z*=0.296; *p*=0.78	*n*=11
LAMP1-Rap2(ca)	24.5±2.3 vs. 21.2±1.5	*n*=22; *Z*=1.347; *p*=0.18	50.7±3.7 vs. 52.4±2.9	*n*=22; *Z*=0.417; *p*=0.68	*n*=11
LAMP1-Rap2(dd)	24.5±2.3 vs. 25.3±2.0	*n*=22; *Z*=-0.081; *p*=0.94	50.7±3.7 vs. 48.9±3.0	*n*=22; *Z*=-0.373; *p*=0.71	*n*=11
LAMP1-Rap2(dn)	24.5±2.3 vs. 27.8±1.9	*n*=22; *Z*=1.623; *p*=0.11	50.7±3.7 vs. 51.0±2.9	*n*=22; *Z*=0.114; *p*=0.91	*n*=11
KDELr-Rap2(ca)	21.4±2.1 vs. 21.7±1.8	*n*=22; *Z*=0.292; *p*=0.77	40.1±2.1 vs. 42.0±3.2	*n*=22; *Z*=-0.146; *p*=0.88	*n*=10
KDELr-Rap2(dd)	21.4±2.1 vs. 20.9±2.1	*n*=22; *Z*=-0.365; *p*=0.72	40.1±2.1 vs. 40.8±2.9	*n*=22; *Z*=-0.276; *p*=0.78	*n*=10
KDELr-Rap2(dn)	21.4±2.1 vs. 20.7±2.3	*n*=22; *Z*=-0.406; *p*=0.69	40.1±2.1 vs. 41.8±3.4	*n*=22; *Z*=-0.406; *p*=0.69	*n*=10

Table S4

Synaptic effects of target-delivered Rap1 mutants in CA1 neurons (related to figure 4)

Constructs	AMPA currents (Ctrl vs. Exp; pA)	Sample No. *Z* & *p* values	NMDA currents (Ctrl vs. Exp; pA)	Sample No. *Z* & *p* values	Animal No.
M1-Rap1(ca)	21.1±2.1 vs. 24.8±2.3	*n*=22; *Z*=1.607; *p*=0.11	48.2±2.3 vs. 49.0±2.3	*n*=22; *Z*=0.503; *p*=0.62	*n*=11
M1-Rap1(dd)	21.1±2.1 vs. 20.4±2.0	*n*=22; *Z*=-0.601; *p*=0.55	48.2±2.3 vs. 49.3±4.3	*n*=22; *Z*=0.243; *p*=0.81	*n*=11
M1-Rap1(dn)	21.1±2.1 vs. 20.6±1.5	*n*=22; *Z*=-0.179; *p*=0.86	48.2±2.3 vs. 49.7±3.5	*n*=22; *Z*=0.471; *p*=0.64	*n*=11
LCK-Rap1(ca)	23.4±2.2 vs. 25.5±1.7	*n*=20; *Z*=1.419; *p*=0.16	44.8±2.1 vs. 42.9±2.8	*n*=20; *Z*=-0.784; *p*=0.43	*n*=10
LCK-Rap1(dd)	23.4±2.2 vs. 22.9±2.2	*n*=20; *Z*=0.411; *p*=0.68	44.8±2.1 vs. 47.2±4.3	*n*=20; *Z*=0.635; *p*=0.53	*n*=10
LCK-Rap1(dn)	23.4±2.2 vs. 22.3±2.4	*n*=20; *Z*=-1.827; *p*=0.07	44.8±2.1 vs. 45.7±4.5	*n*=20; *Z*=0.075; *p*=0.94	*n*=10
CD8-Rap1(ca)	19.8±2.2 vs. 17.6±1.7	*n*=20; *Z*=-1.690; *p*=0.09	42.0±4.1 vs. 41.5±2.8	*n*=20; *Z*=0.075; *p*=0.94	*n*=9
CD8-Rap1(dd)	19.8±2.2 vs. 20.3±1.5	*n*=20; *Z*=0.411; *p*=0.68	42.0±4.1 vs. 43.7±2.8	*n*=20; *Z*=0.336; *p*=0.74	*n*=9
CD8-Rap1(dn)	19.8±2.2 vs. 21.6±1.9	*n*=20; *Z*=1.419; *p*=0.16	42.0±4.1 vs. 41.7±3.7	*n*=20; *Z*=-0.161; *p*=0.87	*n*=9
LAMP1-Rap1(ca)	26.7±2.3 vs. 19.2±1.6	*n*=22; *Z*=-2.368; *p*<0.05	50.5±3.6 vs. 51.5±2.8	*n*=22; *Z*=0.341; *p*=0.73	*n*=10
LAMP1-Rap1(dd)	26.7±2.3 vs. 26.6±1.7	*n*=22; *Z*=0.343; *p*=0.81	50.5±3.6 vs. 51.8±3.3	*n*=22; *Z*=0.146; *p*=0.88	*n*=10
LAMP1-Rap1(dn)	26.7±2.3 vs. 35.4±2.2	*n*=22; *Z*=2.873; *p*<0.005	50.5±3.6 vs. 52.2±3.2	*n*=22; *Z*=0.341; *p*=0.77	*n*=10
KDELr-Rap1(ca)	27.8±2.7 vs. 27.5±3.7	*n*=20; *Z*=-0.327; *p*=0.74	45.5±4.0 vs. 44.1±4.8	*n*=20; *Z*=-0.597; *p*=0.55	*n*=10
KDELr-Rap1(dd)	27.8±2.7 vs. 27.2±3.1	*n*=20; *Z*=0.075; *p*=0.94	45.5±4.0 vs. 44.7±4.3	*n*=20; *Z*=0.075; *p*=0.94	*n*=10
KDELr-Rap1(dn)	27.8±2.7 vs. 27.0±3.4	*n*=20; *Z*=-0.187; *p*=0.85	45.5±4.0 vs. 43.5±5.5	*n*=20; *Z*=-0.261; *p*=0.79	*n*=10

Table S5

Effects of Ras and Rap after prolonged expression in undesired microdomains (related to figures 2-4)

Constructs	AMPA currents (Ctrl vs. Exp; pA)	Sample No. *Z* & *p* values	NMDA currents (Ctrl vs. Exp; pA)	Sample No. *Z* & *p* values	Animal No.
CD8-Ras(ca)	25.7±2.8 vs. 19.0±1.8	n=20; Z=-2.613; p<0.01	52.5±3.8 vs. 48.0±3.1	n=20; Z=-1.344; p=0.18	n=10
CD8-Ras(dn)	25.7±2.8 vs. 32.3±2.7	n=20; Z=2.613; p<0.01	52.5±3.8 vs. 54.1±3.6	n=20; Z=0.448; p=0.65	n=10
LAMP1-Ras(ca)	25.7±2.8 vs. 19.1±1.9	n=20; Z=-1.979; p<0.05	52.5±3.8 vs. 48.3±3.3	n=20; Z=-1.045; p=0.30	n=10
LAMP1-Ras(dn)	25.7±2.8 vs. 32.0±2.5	n=20; Z=2.315; p<0.05	52.5±3.8 vs. 53.5±3.2	n=20; Z=0.187; p=0.85	n=10
M1-Rap2(ca)	19.3±2.5 vs. 26.2±2.3	n=18; Z=2.737; p<0.05	42.4±3.5 vs. 45.0±3.4	n=18; Z=1.002; p=0.32	n=9
M-Rap2(dn)	19.3±2.5 vs. 18.7±2.1	n=18; Z=0.414; p=0.68	42.4±3.5 vs. 41.0±3.3	n=18; Z=-0.260; p=0.80	n=9
LCK-Rap2(ca)	19.3±2.5 vs. 24.8±1.9	n=18; Z=2.025; p<0.05	42.4±3.5 vs. 45.0±2.8	n=18; Z=0.852; p=0.39	n=9
LCK-Rap2(dn)	19.3±2.5 vs. 13.9±0.9	n=18; Z=-2.504; p<0.05	42.4±3.5 vs. 39.5±2.4	n=18; Z=-0.806; p=0.42	n=9
M1-Rap1(ca)	21.9±2.4 vs. 27.9±2.7	n=20; Z=2.240; p<0.05	41.2±3.0 vs. 43.6±4.3	n=20; Z=0.095; p=0.94	n=10
M-Rap1(dn)	21.9±2.4 vs. 20.9±2.0	n=20; Z=0.261; p=0.79	41.2±3.0 vs. 39.5±3.2	n=20; Z=-0.672; p=0.50	n=10
LCK-Rap1(ca)	21.9±2.4 vs. 29.2±2.8	n=20; Z=2.901; p<0.05	41.2±3.0 vs. 43.7±3.2	n=20; Z=0.691; p=0.49	n=10
LCK-Rap1(dn)	21.9±2.4 vs. 15.7±1.0	n=20; Z=-2.446; p<0.05	41.2±3.0 vs. 39.3±3.2	n=20; Z=-1.176; p=0.24	n=10

Table S6

Effects of target-delivered Ras and Rap mutants in the presence of inhibitors (related to figures 5-6)

Constructs/ inhibitors	AMPA currents (Ctrl vs. Exp; pA)	Sample No. *Z* & *p* values	NMDA currents (Ctrl vs. Exp; pA)	Sample No. *Z* & *p* values	Animal No.
LY294002					
M1-Ras(ca)	19.2±1.7 vs. 19.8±1.9	*n*=24; *Z*=-0.129; *p*=0.90	46.0±3.7 vs. 47.2±4.4	*n*=24; *Z*=0.114; *p*=0.91	*n*=8
LCK-Ras(ca)	19.2±1.7 vs. 26.5±2.4	*n*=24; *Z*=-2.829; *p*<0.001	46.0±3.7 vs. 47.6±3.7	*n*=24; *Z*=0.557; *p*=0.58	*n*=8
M1-Ras(dn)	28.9±1.9 vs. 27.5±2.4	*n*=22; *Z*=-1.023; *p*=0.31	51.3±3.1 vs. 52.5±3.6	*n*=22; *Z*=-0.276; *p*=0.78	*n*=10
LCK-Ras(dn)	28.9±1.9 vs. 21.4±1.6	*n*=22; *Z*=-3.231; *p*<0.01	51.3±3.1 vs. 48.0±2.8	*n*=22; *Z*=-1.477; *p*=0.14	*n*=10
PD98059					
M1-Ras(ca)	23.7±1.6 vs. 23.1±2.0	*n*=24; *Z*=-0.271; *p*=0.79	53.4±3.3 vs. 51.6±2.5	*n*=24; *Z*=-0.343; *p*=0.73	*n*=7
LCK-Ras(ca)	23.7±1.6 vs. 25.1±1.9	*n*=24; *Z*=-0.271; *p*=0.79	53.4±3.3 vs. 53.9±2.5	*n*=24; *Z*=0.186; *p*=0.85	*n*=7
M1-Ras(dn)	27.4±2.3 vs. 28.9±2.7	*n*=21; *Z*=0.191; *p*=0.85	53.4±3.2 vs. 55.3±3.2	*n*=21; *Z*=0.382; *p*=0.70	*n*=9
LCK-Ras(dn)	27.4±2.3 vs. 27.9±2.4	*n*=21; *Z*=0.037; *p*=0.97	53.4±3.2 vs. 55.0±2.9	*n*=21; *Z*=0.087; *p*=0.93	*n*=9
SP600125					
CD8-Rap2(ca)	27.2±1.9 vs. 26.7±2.0	*n*=25; *Z*=-0.143; *p*=0.89	50.8±2.9 vs. 51.7±2.6	*n*=25; *Z*=0.457; *p*=0.65	*n*=13
LAMP1-Rap1(ca)	27.2±1.9 vs. 20.2±1.1	*n*=25; *Z*=-3.646; *p*<0.005	50.8±2.9 vs. 48.1±2.7	*n*=25; *Z*=-1.009; *p*=0.31	*n*=13
CD8-Rap2(dn)	23.3±1.9 vs. 25.2±1.9	*n*=20; *Z*=0.821; *p*=0.41	45.8±3.0 vs. 46.4±3.0	*n*=20; *Z*=0.747; *p*=0.46	*n*=9
LAMP1-Rap1(dn)	23.3±1.9 vs. 25.6±1.6	*n*=20; *Z*=2.464; *p*<0.05	45.8±3.0 vs. 48.9±2.8	*n*=20; *Z*=0.784; *p*=0.43	*n*=9
SB203580					
CD8-Rap2(ca)	29.5±2.3 vs. 21.8±1.9	*n*=24; *Z*=-4.215; *p*<0.005	52.7±2.9 vs. 50.6±2.8	*n*=24; *Z*=1.472; *p*=0.14	*n*=12
LAMP1-Rap1(ca)	29.5±2.3 vs. 29.2±2.2	*n*=24; *Z*=-0.029; *p*=0.98	52.7±2.9 vs. 52.6±2.7	*n*=24; *Z*=0.571; *p*=0.57	*n*=12
CD8-Rap2(dn)	24.8±1.8 vs. 30.9±2.6	*n*=21; *Z*=2.227; *p*<0.05	45.9±3.0 vs. 50.0±3.0	*n*=21; *Z*=1.703; *p*=0.09	*n*=10

LAMP1-Rap1(dn)	24.8±1.8 vs. 23.5±1.9	*n*=21; *Z*=-0.539; *p*=0.59	45.9±3.0 vs. 45.8±3.2	*n*=21; *Z*=-0.295; *p*=0.77	*n*=10

Table S7

Effects of target-delivered Ras mutants in knockout and transgenic mice (related to figure 5)

Constructs/ mouse lines	AMPA currents (Ctrl vs. Exp; pA)	Sample No. *Z* & *p* values	NMDA currents (Ctrl vs. Exp; pA)	Sample No. *Z* & *p* values	Animal No.
GluA1 KO					
M1-Ras(ca)	21.9±2.6 vs. 21.2±1.6	n=24; Z=0.200; p=0.84	46.3±2.5 vs. 47.1±2.2	n=24; Z=0.429; p=0.67	n=7
LCK-Ras(ca)	21.9±2.6 vs. 33.3±2.5	n=24; Z=3.143; p<0.005	46.3±2.5 vs. 48.4±2.4	n=24; Z=0.914; p=0.36	n=7
M1-Ras(dn)	28.5±2.2 vs. 30.1±2.6	n=22; Z=-1.023; p=0.31	52.9±2.9 vs.52.3±3.6	n=22; Z=0.539; p=0.59	n=10
LCK-Ras(dn)	28.5±2.2 vs. 20.7±1.5	n=22; Z=-3.231; p<0.005	52.9±2.9 vs.48.8±3.2	n=22; Z=-1.360; p=0.17	n=10
GluA1(S831A/ S831A)					
M1-Ras(ca)	26.1±2.4 vs. 27.1±2.2	n=24; Z=0.568; p=0.57	47.1±3.3 vs. 48.9±2.3	n=24; Z=0.925; p=0.36	n=5
LCK-Ras(ca)	26.1±2.4 vs. 33.7±2.1	n=24; Z=3.393; p<0.005	47.1±3.3 vs. 49.3±2.5	n=24; Z=0.552; p=0.58	n=5
GluA2 KO					
M1-Ras(ca)	21.0±1.6 vs. 29.1±2.4	n=24; Z=-0.271; p=0.79	46.9±3.0 vs. 48.1±3.2	n=24; Z=3.057; p<0.005	n=6
LCK-Ras(ca)	21.0±1.6 vs. 21.5±1.5	n=24; Z=0.143; p=0.89	46.9±3.0 vs. 48.7±2.6	n=24; Z=0.800; p=0.42	n=6
M1-Ras(dn)	25.5±2.6 vs. 25.1±1.8	n=22; Z=-0.341; p=0.73	45.5±3.7 vs. 46.9±2.9	n=22; Z=0.114; p=0.98	n=9
LCK-Ras(dn)	25.5±2.6 vs. 26.1±2.5	n=22; Z=-0.016; p=0.99	45.5±3.7 vs. 46.6±3.4	n=22; Z=0.211; p=0.83	n=9

Table S8

Effects of target-delivered PTEN, MEK, TNIK and p38MAPK (related to figures 5-6)

Constructs/ Inhibitors	AMPA currents (Ctrl vs. Exp; pA)	Sample No. *Z* & *p* values	NMDA currents (Ctrl vs. Exp; pA)	Sample No. *Z* & *p* values	Animal No.
X-PTEN(dn)					
M1-PTEN(dn)	22.6±1.7 vs. 29.0±1.9	n=20; Z=2.352; p<0.05	44.8±2.9 vs. 46.8±2.5	n=20; Z=1.232; p=0.22	n=8
LCK-PTEN(dn)	26.1±2.2 vs. 25.7±2.4	n=21; Z=0.156; p=0.88	51.8±3.5 vs. 49.2±3.1	n=21; Z=0.852; p=0.39	n=9
CD8-PTEN(dn)	26.5±1.7 vs. 27.9±2.3	n=21; Z=0.608; p=0.54	49.4±2.9 vs. 49.3±2.5	n=21; Z=0.643; p=0.52	n=8
LAMP1-PTEN(dn)	26.4±1.5 vs. 27.3±1.8	n=22; Z=0.552; p=0.58	49.4±2.1 vs. 49.9±1.7	n=22; Z=0.942; p=0.35	n=10
X-MEK(dn)					
M1-MEK(dn)	26.3±1.8 vs. 26.0±1.9	n=21; Z=0.017; p=0.99	48.8±2.8 vs. 49.2±2.6	n=21; Z=0.434; p=0.66	n=10
LCK-MEK(dn)	27.0±1.8 vs. 21.8±1.8	n=20; Z=-2.613; p<0.01	49.6±2.6 vs. 46.6±2.3	n=20; Z=-0.821; p=0.41	n=7
CD8-MEK(dn)	26.0±1.9 vs. 26.6±1.9	n=20; Z=0.073; p=0.97	48.2±2.8 vs. 50.8±2.9	n=20; Z=1.269; p=0.24	n=9
LAMP1-MEK(dn)	26.1±1.6 vs. 25.7±2.1	n=20; Z=-0.149; p=0.88	48.1±2.2 vs. 46.3±2.9	n=20; Z=-0.691; p=0.49	n=8
X-TNIK(dn)					
M1-TNIK(dn)	26.4±1.7 vs. 25.5±1.9	n=21; Z=0.643; p=0.52	49.7±2.8 vs. 49.7±2.4	n=21; Z=-0.365; p=0.72	n=9
LCK-TNIK(dn)	26.8±1.9 vs. 25.3±2.0	n=20; Z=-0.597; p=0.55	46.3±2.2 vs. 44.1±1.8	n=20; Z=-0.299; p=0.77	n=8
CD8-TNIK(dn)	24.2±1.8 vs. 28.9±2.1	n=20; Z=2.184; p<0.05	48.7±3.4 vs. 51.3±3.4	n=20; Z=1.083; p=0.28	n=7
LAMP1-TNIK(dn)	27.8±1.3 vs. 28.4±1.7	n=20; Z=0.448; p=0.65	51.1±2.3 vs. 53.3±2.3	n=20; Z=1.120; p=0.26	n=8
X-p38(dn)					
M1-p38(dn)	24.6±1.8 vs. 25.5±1.9	n=21; Z=0.297; p=0.77	45.9±2.5 vs. 49.3±2.4	n=21; Z=1.442; p=0.15	n=10
LCK-p38(dn)	26.2±1.9 vs. 25.4±2.0	n=21; Z=-0.365; p=0.72	49.7±2.5 vs. 48.3±2.1	n=21; Z=-0.365; p=0.72	n=10
CD8-p38(dn)	27.2±2.2 vs. 26.4±2.1	n=19; Z=-0.121; p=0.90	51.2±3.0 vs. 51.8±2.8	n=19; Z=0.463; p=0.64	n=8
LAMP1-p38(dn)	24.8±1.9 vs. 30.5±1.9	n=18; Z=2.243; p<0.05	49.2±3.3 vs. 52.4±3.2	n=18; Z=1.720; p=0.09	n=7

Table S9

Effects of target-delivered Rap mutants in knockout and transgenic mice (related to figure 6)

Constructs/ mouse lines	AMPA currents (Ctrl vs. Exp; pA)	Sample No. *Z* & *p* values	NMDA currents (Ctrl vs. Exp; pA)	Sample No. *Z* & *p* values	Animal No.
GluA1 KO					
CD8-Rap2(ca)	32.3±2.4 vs. 23.4±1.8	*n*=22; *Z*=-3.912; *p*<0.005	54.7±4.3 vs. 52.1±3.9	*n*=22; *Z*=-1.443; *p*=0.12	*n*=10
LAMP1-Rap1(ca)	32.3±2.4 vs. 24.1±1.9	*n*=22; *Z*=-3.717; *p*<0.005	54.7±4.3 vs. 51.3±3.7	*n*=22; *Z*=-1.510; *p*=0.13	*n*=10
CD8-Rap2(dn)	24.2±1.3 vs. 28.7±2.0	*n*=23; *Z*=2.433; *p*<0.05	46.2±2.4 vs. 48.7±2.3	*n*=23; *Z*=0.386; *p*=0.87	*n*=9
LAMP1-Rap1(dn)	24.2±1.3 vs. 29.5±2.1	*n*=23; *Z*=2.281; *p*<0.05	46.2±2.4 vs. 48.4±2.4	*n*=23; *Z*=0.354; *p*=0.93	*n*=9
GluA2 KO					
CD8-Rap2(ca)	25.0±2.3 vs. 25.5±2.0	*n*=24; *Z*=0.629; *p*=0.53	46.4±2.6 vs. 44.7±2.3	*n*=24; *Z*=-0.343; *p*=0.73	*n*=12
LAMP1-Rap1(ca)	25.0±2.3 vs. 24.5±2.1	*n*=24; *Z*=-0.086; *p*=0.93	46.4±2.6 vs. 45.1±2.5	*n*=24; *Z*=-0.200; *p*=0.84	*n*=12
CD8-Rap2(dn)	25.5±1.4 vs. 26.9±2.1	*n*=20; *Z*=0.261; *p*=0.79	48.9±3.0 vs. 50.6±3.0	*n*=20; *Z*=0.261; *p*=0.79	*n*=8
LAMP1-Rap1(dn)	25.5±1.4 vs. 26.2±1.8	*n*=20; *Z*=0.131; *p*=0.90	48.9±3.0 vs. 49.6±2.7	*n*=20; *Z*=0.168; *p*=0.87	*n*=8
GluA2(K882A)					
CD8-Rap2(ca)	29.4±1.4 vs. 22.1±1.7	*n*=22; *Z*=-3.880; *p*<0.005	55.8±2.9 vs. 52.5±3.1	*n*=22; *Z*=-1.380; *p*=0.17	*n*=6
LAMP1-Rap1(ca)	29.4±1.4 vs. 27.8±1.6	*n*=22; *Z*=-1.591; *p*=0.11	55.8±2.9 vs. 55.2±3.4	*n*=22; *Z*=-0.224; *p*=0.81	*n*=6

Table S10

Effects of target-delivered Ras(dn) and Rap(dn) on synaptic plasticity (related to figure 7)

Constructs	Exp Pathway (Ctrl vs. Exp; %)	Sample No. *Z* & *p* values	Control Pathway (Ctrl vs. Exp; %)	Sample No. *Z* & *p* values	Animal No.
LTP					
M1-Ras(dn)	195.0±12.0 vs. 146.4±11.3	*n*=14; *Z*=-3.296; *p*<0.005	89.9±8.7 vs. 91.1±3.7	*n*=14; *Z*=-0.157; *p*=0.88	*n*=14
LCK-Ras(dn)	198.7±12.3 vs. 100.9±6.0	*n*=14; *Z*=-3.296; *p*<0.005	94.4±7.9 vs. 94.3±4.1	*n*=14; *Z*=0.596; *p*=0.55	*n*=14
CD8-Rap2(dn)	195.7±7.9 vs. 212.0±10.0	*n*=14; *Z*=-1.287; *p*=0.10	93.2±7.0 vs. 99.9±4.9	*n*=14; *Z*=0.943; *p*=0.35	*n*=14
LAMP1-Rap1(dn)	198.4±6.6 vs. 199.3±6.6	*n*=14; *Z*=0.220; *p*=0.83	91.7±6.9 vs. 102.4±4.7	*n*=14; *Z*=1.726; *p*=0.08	*n*=14
Depotentiation					
M1-Ras(dn)	101.3±6.2 vs. 91.9±4.3	*n*=14; *Z*=-1.412; *p*=0.16	94.5±5.6 vs. 96.5±6.3	*n*=14; *Z*=-0.345; *p*=0.73	*n*=14
LCK-Ras(dn)	95.0±2.6 vs. 98.9±3.1	*n*=14; *Z*=0.910; *p*=0.36	99.3±3.5 vs. 94.7±2.8	*n*=14; *Z*=-1.412; *p*=0.16	*n*=14
CD8-Rap2(dn)	90.5±6.1 vs. 189.2±8.9	*n*=14; *Z*=3.296; *p*<0.005	89.8±5.1 vs. 97.9±3.7	*n*=14; *Z*=1.162; *p*=0.65	*n*=14
LAMP1-Rap1(dn)	93.4±6.1 vs. 98.8±4.3	*n*=20; *Z*=0.722; *p*=0.47	95.1±4.3 vs. 97.4±4.6	*n*=14; *Z*=0.157; *p*=0.88	*n*=14
LTD					
M1-Ras(dn)	56.5±6.2 vs. 54.5±3.8	*n*=16; *Z*=-0.103; *p*=0.92	96.0±3.5 vs. 98.7±3.4	*n*=16; *Z*=1.193; *p*=0.23	*n*=16
LCK-Ras(dn)	57.7±6.6 vs. 54.7±4.9	*n*=14; *Z*=-0.471; *p*=0.64	102.8±3.4 vs. 99.3±4.3	*n*=14; *Z*=-0.659; *p*=0.51	*n*=14
CD8-Rap2(ca)	53.3±4.6 vs. 55.6±6.3	*n*=15; *Z*=0.534; *p*=0.59	96.0±3.5 vs. 97.3±5.5	*n*=15; *Z*=0.284; *p*=0.78	*n*=15
LAMP1-Rap1(ca)	65.2±3.1 vs. 96.4±3.5	*n*=16; *Z*=3.561; *p*<0.001	98.1±3.4 vs. 97.5±2.1	*n*=16; *Z*=0.052; *p*=0.96	*n*=16

Table S11

Effects of target-delivered Ras(dn) and Rap(dn) expressed in CA1 neurons in intact brains (related to figure 8)

Constructs	AMPA currents (Ctrl vs. Exp; pA)	Sample No. *Z* & *p* values	NMDA currents (Ctrl vs. Exp; pA)	Sample No. *Z* & *p* values	Animal No.
Exp without TTX					
M1-Ras(dn)	25.7±3.1 vs. 18.6±1.9	n=18; Z=-2.853; p<0.005	38.3±3.6 vs. 35.2±2.7	n=18; Z=-1.546; p=0.12	n=9
LCK-Ras(dn)	25.7±3.1 vs. 13.7±1.1	n=18; Z=-3.114; p<0.005	38.3±3.6 vs. 34.4±2.7	n=18; Z=-1.720; p=0.09	n=9
Exp with TTX					
M1-Ras(dn)	23.3±2.1 vs. 21.6±2.4	n=21; Z=-1.269; p=0.20	36.8±2.6 vs. 35.3±2.4	n=21; Z=-1.164; p=0.24	n=11
LCK-Ras(dn)	23.3±2.1 vs. 21.5±1.9	n=21; Z=-1.616; p=0.11	36.8±2.6 vs. 35.7±2.2	n=21; Z=-0.191; p=0.85	n=11
Exp without TTX					
M1-Ras(ca)	23.1±2.1 vs. 28.6±2.6	n=23; Z=2.768; p<0.01	35.7±2.8 vs. 37.1±3.1	n=23; Z=1.632 p=0.10	n=14
LCK-Ras(ca)	23.1±2.1 vs. 27.8±2.1	n=23; Z=3.407; p<0.005	35.7±2.8 vs. 37.0±2.7	n=23; Z=1.400 p=0.16	n=14
Exp without TTX					
CD8-Rap2(dn)	24.7±3.3 vs. 35.2±3.2	n=16; Z=3.413; p<0.005	36.6±3.5 vs. 38.1±2.3	n=16; Z=0.569; p=0.57	n=8
LAMP1-Rap1(dn)	24.7±3.3 vs. 32.2±3.3	n=16; Z=2.585; p<0.05	36.6±3.5 vs. 37.3±2.3	n=16; Z=0.052; p=0.96	n=8
Exp with TTX					
CD8-Rap2(dn)	23.3±2.2 vs. 23.6±2.0	n=20; Z=-0.149; p=0.88	36.8±2.7 vs. 37.7±2.6	n=20; Z=0.336; p=0.74	n=10
LAMP1-Rap1(dn)	23.3±2.2 vs. 22.0±2.2	n=20; Z=-0.448; p=0.65	36.8±2.7 vs. 33.6±3.0	n=20; Z=-1.120; p=0.26	n=10
Exp without TTX					
CD8-Rap2(ca)	31.3±1.9 vs. 25.7±2.1	n=21; Z=-2.485; p<0.05	43.4±2.3 vs. 41.6±3.1	n=21; Z=-0.400; p=0.69	n=12
LAMP1-Rap1(ca)	31.3±1.9 vs. 25.3±2.2	n=21; Z=-2.798; p<0.01	43.4±2.3 vs. 40.6±3.0	n=20; Z=-0.539; p=0.59	n=12