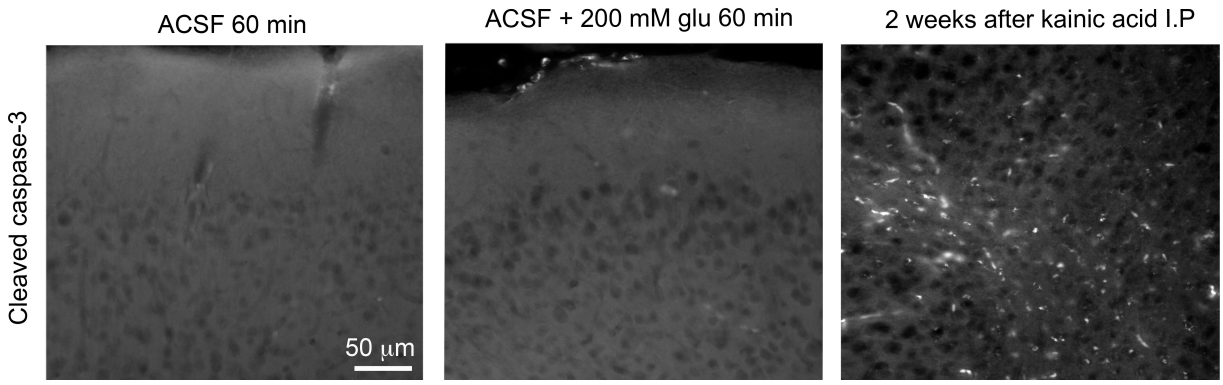
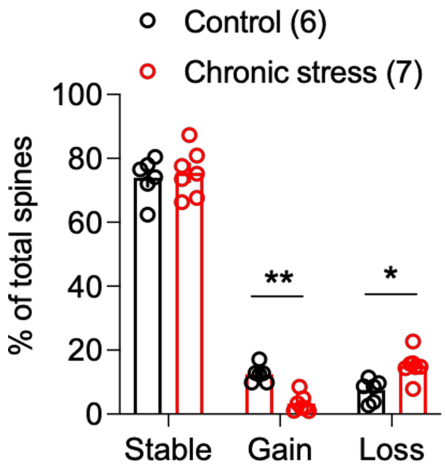
Dromard et al. Dual imaging of dendritic spines and mitochondria in vivo reveals hotspots of plasticity and metabolic adaptation to stress

Supplementary Figures



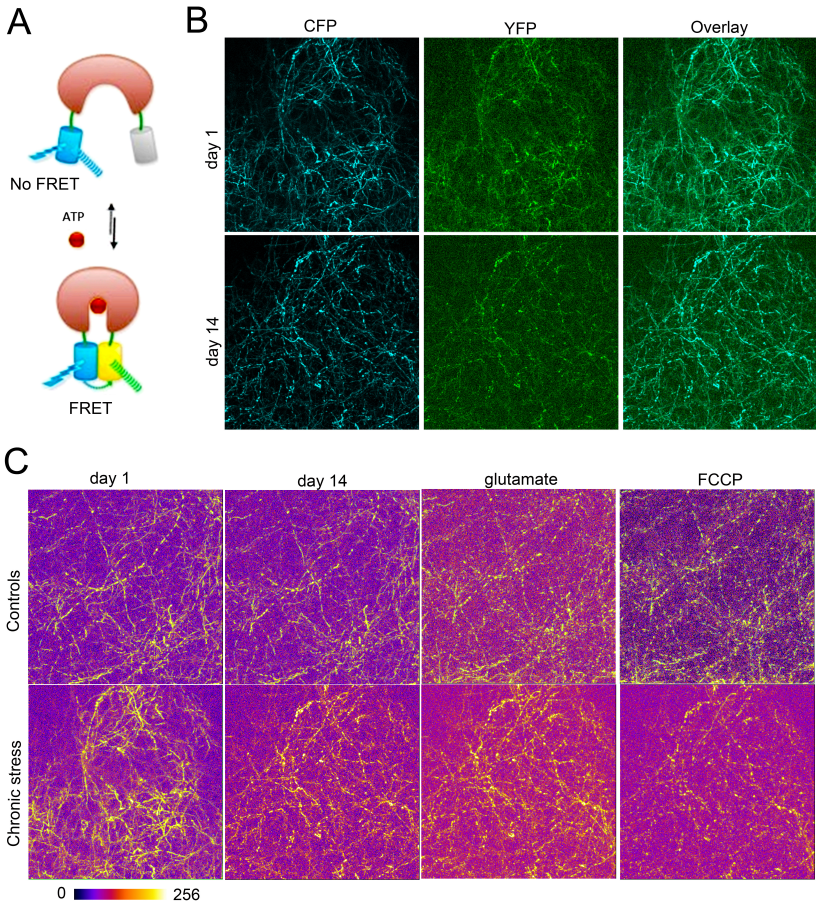
Supplementary Figure 1. No cell death upon focal application of glutamate on top of the cortex.

Cleaved caspase-3 immunolabeling in sections of somatosensory cortex pial prior exposed to ACSF with or without 200 mM glutamate. Drug application was made possible via a craniotomy but through an agarose bed that prevents motion artifacts due to heartbeats. The final concentration of glutamate reaching the cortex is diluted out. Positive control of cell death labeled with cleaved caspase-3 antibodies are shown in a section of somatosensory cortex from a mouse injected I.P. with 25 mg/kg of kainic acid that resulted in tonic-clonic epileptic seizures.



Supplementary Figure 2. Effect of chronic stress on dendritic spine dynamics.

Data expressed as means±SEM of N=6 controls, 7 chronic stress. Two-way ANOVA: effect of spine type $F(2,33)=777$ $p<0.0001$; interaction of spine type with stress $F(2,33)=10$ $p=0.0004$, post-hoc Sidak test comparing control and stress groups *$p=0.023$, **$p=0.005$.

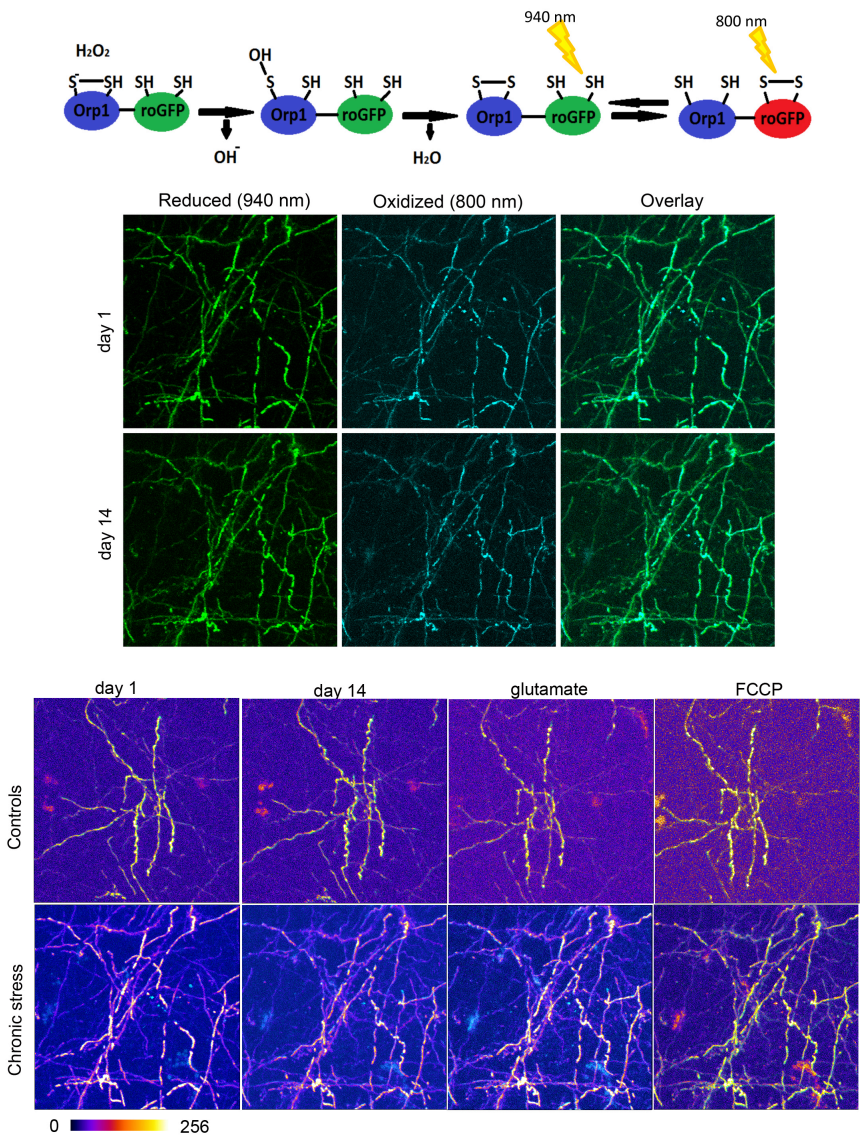


Supplementary Figure 3. Measuring relative ATP levels with Mito-ATeam probe.

A. Mito-ATeam is a reversible FRET probe to detect ATP levels (1).

B. Changes in fluorescence signal both of the CFP and YFP subunits of Mito-ATeam.

C. Time lapse images of CFP/YFP ratio relative to pre-stress values (day 1).

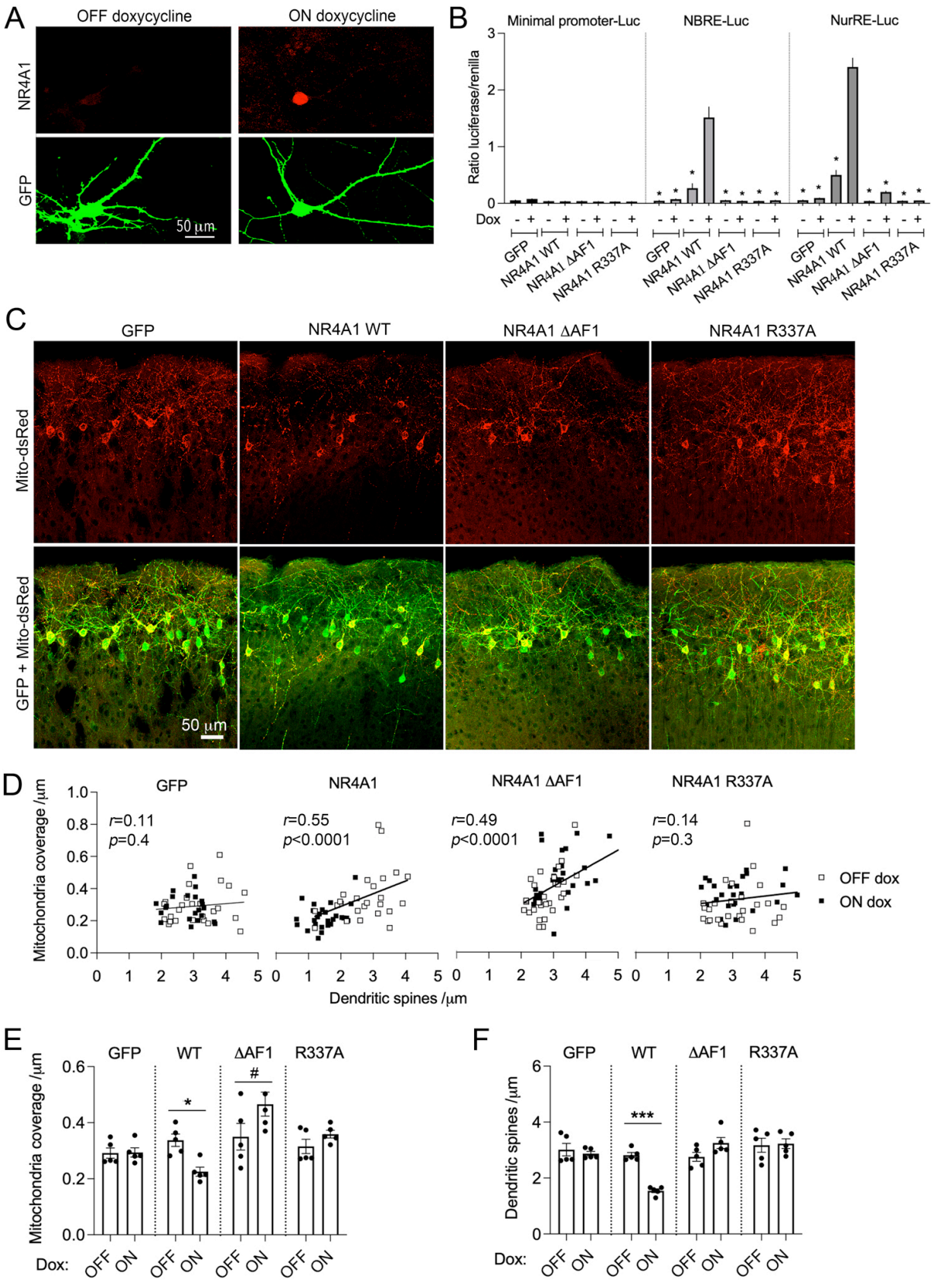


Supplementary Figure 4. Measuring relative H2O2 levels with Mito-roGFP-Orp1 probe.

A. Mito-roGFP-Orp1 is a reversible probe to detect H2O2 levels (2).

B. Changes in fluorescence signal both of the oxidized (800 nm) and reduced (940 nm) forms of Mito-roGFP-Orp1.

C. Time lapse images of oxidized/reduced ratio relative to pre-stress values (day 1).


A
OFF doxycycline
ON doxycycline
NR4A1
GFP
50 µm
B
Minimal promoter-Luc
NBRE-Luc
NurRE-Luc
Ratio luciferase/renilla
Dox
GFP
NR4A1 WT
NR4A1 ΔAF1
NR4A1 R337A
C
GFP
NR4A1 WT
NR4A1 ΔAF1
NR4A1 R337A
Mito-dsRed
GFP + Mito-dsRed
50 µm
D
GFP
NR4A1
NR4A1 ΔAF1
NR4A1 R337A
r=0.11
p=0.4
r=0.55
p<0.0001
r=0.49
p<0.0001
r=0.14
p=0.3
Mitochondria coverage /µm
Dendritic spines /µm
OFF dox
ON dox
E
GFP
WT
ΔAF1
R337A
Mitochondria coverage /µm
Dox:
OFF
ON
F
Dendritic spines /µm

Supplementary Figure 5. Doxycycline-dependent induction of NR4A1 constructs in vitro and in vivo.

A. Doxycycline-dependent expression of recombinant NR4A1 and constitutive GFP reporter in transfected primary cortical neurons (10 ng/ml, 3 days).

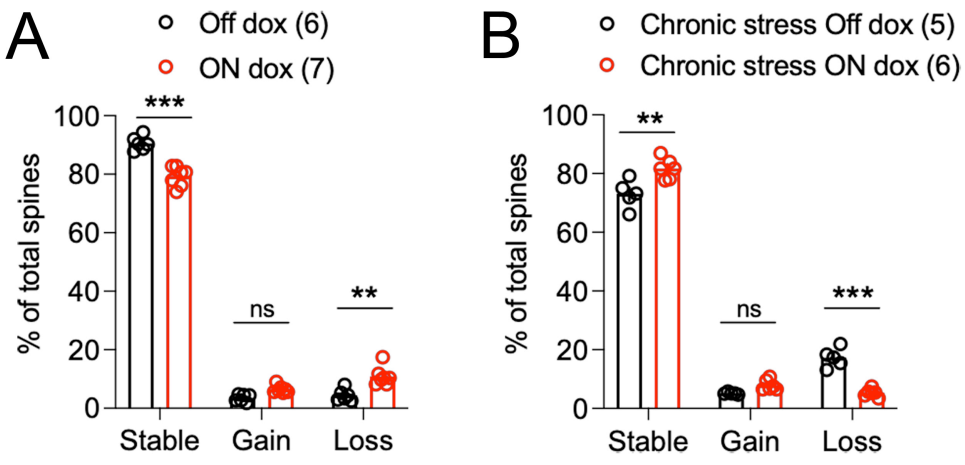
B. Ratio of luciferase/renilla emitted luminescence from lysates of cortical neurons electroporated with a TetON system to express GFP, NR4A1, ΔAF1 or R337A mutants together with a 1 to 10 proportion of renilla and Luciferase constructs (POMC as minimal promoter, NBRE-POMC and NurRE-POMC responsive elements for NR4A1 binding). Doxycycline was added to the cultures (10 ng/ml for 1 day) to induce expression of transgenes. Two-way ANOVA: interaction of promoter-Luc and NR4A1 constructs $F_{14,120}$=37.16, p<0.0001 post-hoc Dunnett test comparing NR4A1+dox and other groups *p<0.0001, N=4 independent experiments at least of replicate measures.

C. Pyramidal neurons of somatosensory cortex electroporated with the indicated construct.

D. Pearson correlation between dendritic mitochondrial coverage and spine density upon doxycycline intake in drinking water for 2 weeks. N=49 GFP dendrites, 52 NR4A1 dendrites, 60 ΔAF1 dendrites and 51 R337A dendrites from 5 mice/ group.

E. Mitochondrial coverage with the indicated constructs. Doxycycline had no effects in neurons expressing GFP whereas it reduced mitochondrial coverage in neurons expressing NR4A1 (means±SEM of N=5 mice/ group). Two-way ANOVA: Interaction of doxycycline with NR4A1 $F_{3,32}$=5.81 p=0.0027 post-hoc Sidak test *p=0.03, #p=0.02.

F. Spine density with the indicated constructs. Doxycycline had no effects in neurons expressing GFP whereas it reduced spine density in neurons expressing NR4A1 (means±SEM of N=5 mice/ group). Two-way ANOVA: Interaction of doxycycline with NR4A1 constructs $F_{3,32}$=10.22 p<0.0001 post-hoc Sidak test ***p<0.0001.



Supplementary Figure 6. Effect of dox-dependent NR4A1 contruct expression on the overall dendritic spine dynamics.

A. Effect of NR4A1-WT on dendritic spine dynamics (means±SEM of N=6 OFF dox, 7 ON dox). Two-way ANOVA: effect of spine type $F(2,33)=4508$ $p<0.0001$; interaction of spine type with dox treatment $F(2,33)=48.2$ $p<0.0001$, post-hoc Sidak test comparing control and stress groups $^{**}p=0.0001$, $^{***}p<0.0001$, ns: not significant.

B. Effect of NR4A1ΔAF1 on dendritic spine dynamics (means±SEM of N=5 chronic stress OFF dox, 7 chronic stress ON dox). Two-way ANOVA: effect of spine type $F(2,27)=2086$ $p<0.0001$; interaction of spine type with dox treatment $F(2,27)=37.3$ $p<0.0001$, post-hoc Sidak test comparing control and stress groups $^{**}p=0.001$, $^{***}p<0.0001$, ns: not significant.