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Supplemental Information

A Genetically Encoded Fluorescent Sensor for Rapid and Specific *In Vivo* Detection of Norepinephrine

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Fig S1

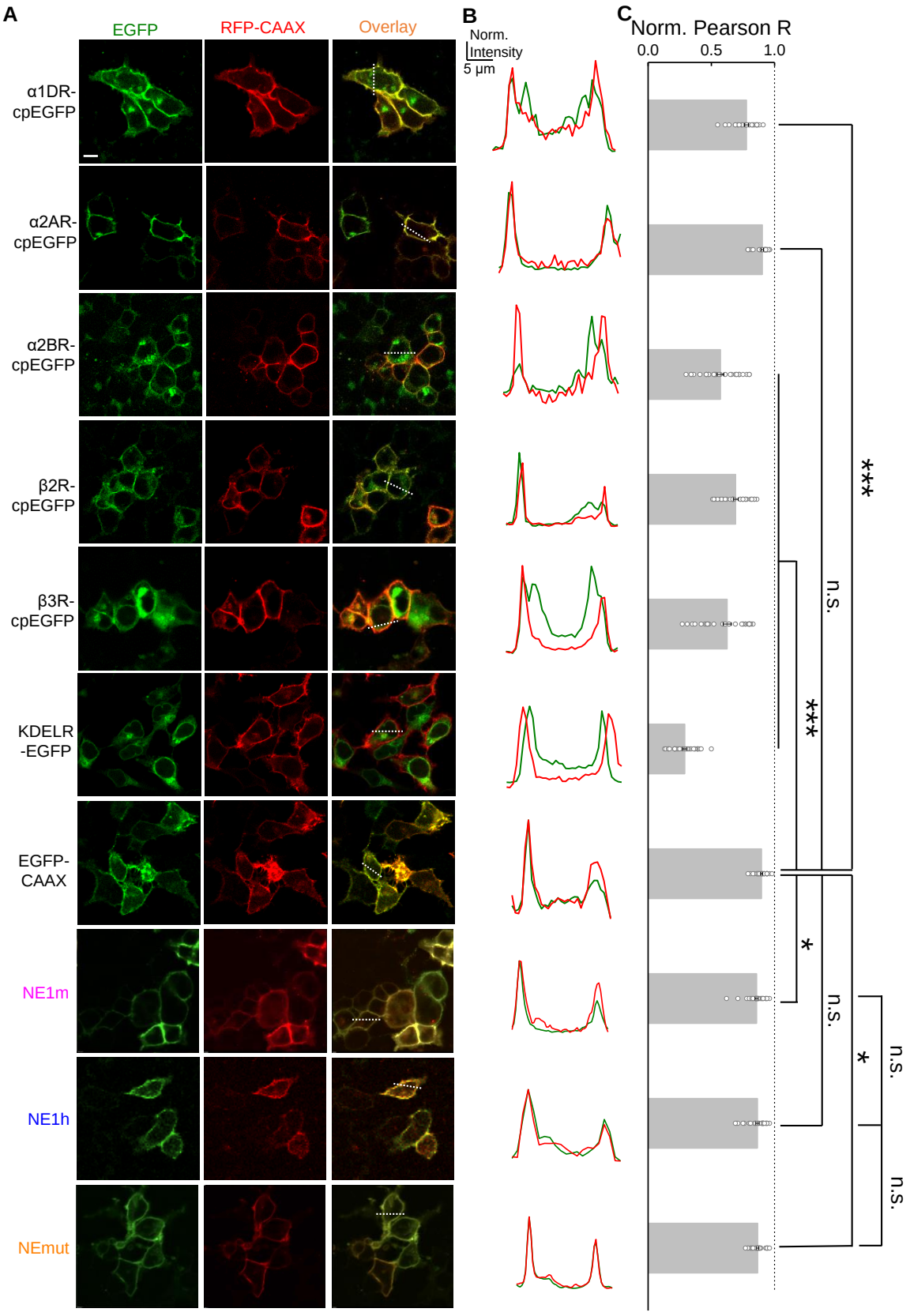
A
EGFP
RFP-CAAX
Overlay
α1DR-cpEGFP
α2AR-cpEGFP
α2BR-cpEGFP
β2R-cpEGFP
β3R-cpEGFP
KDELR-EGFP
EGFP-CAAX
NE1m
NE1h
NEmut
B
Norm. Intensity
5 µm
C
Norm. Pearson R
0.0
0.5
1.0
***
n.s.
***
*
n.s.
*
n.s.
n.s.

Fig S2

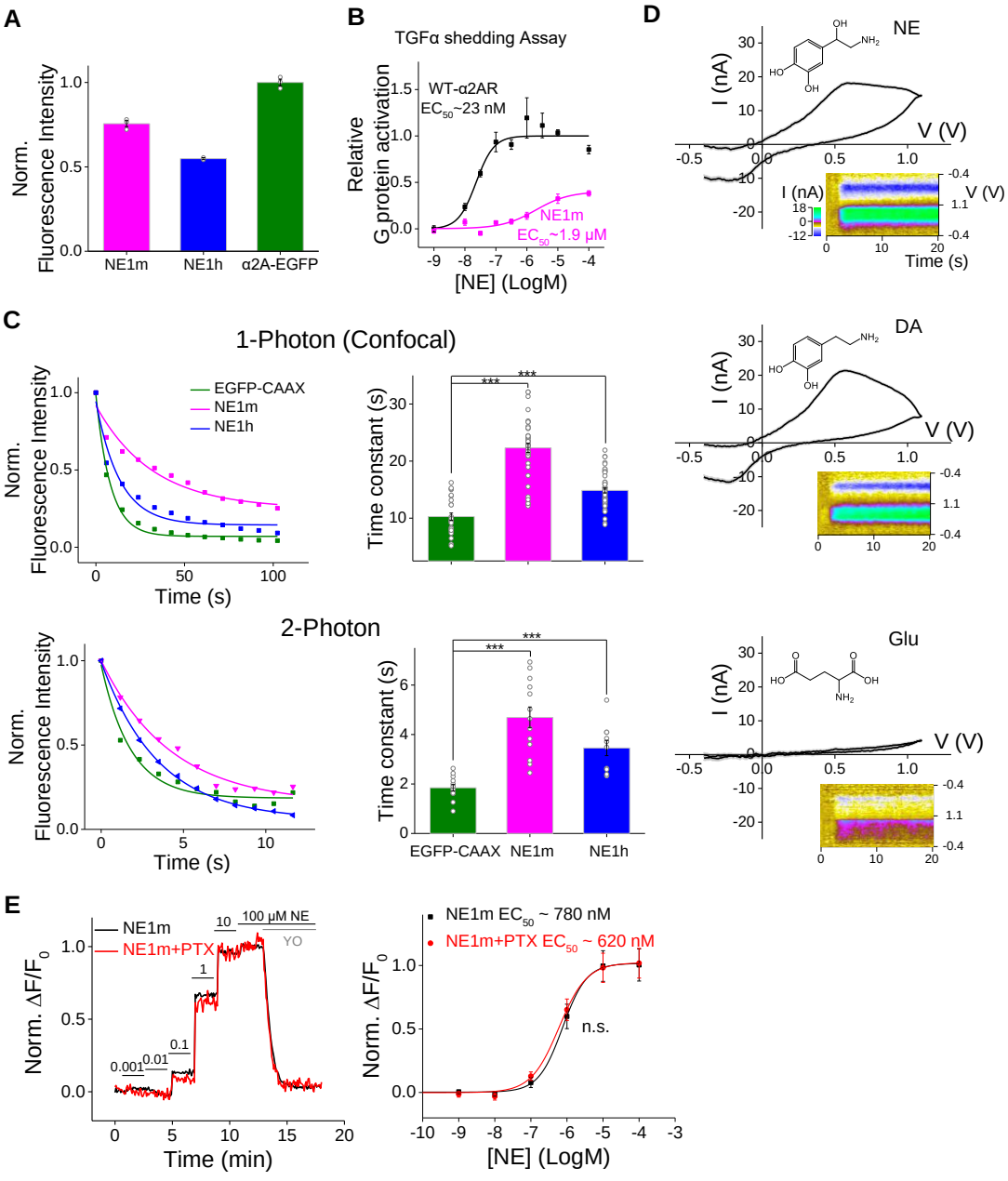
A
Norm. Fluorescence Intensity
NE1m
NE1h
α2A-EGFP
B
TGFα shedding Assay
Relative G protein activation
WT-α2AR
EC50~23 nM
NE1m
EC50~1.9 μM
[NE] (LogM)
D
NE
I (nA)
V (V)
Time (s)
DA
Glu
C
1-Photon (Confocal)
Norm. Fluorescence Intensity
EGFP-CAAX
NE1m
NE1h
Time (s)
Time constant (s)
2-Photon
E
NE1m
NE1m+PTX
100 μM NE
YO
Norm. ΔF/F0
Time (min)
NE1m EC50 ~ 780 nM
NE1m+PTX EC50 ~ 620 nM
n.s.
[NE] (LogM)

Fig S3

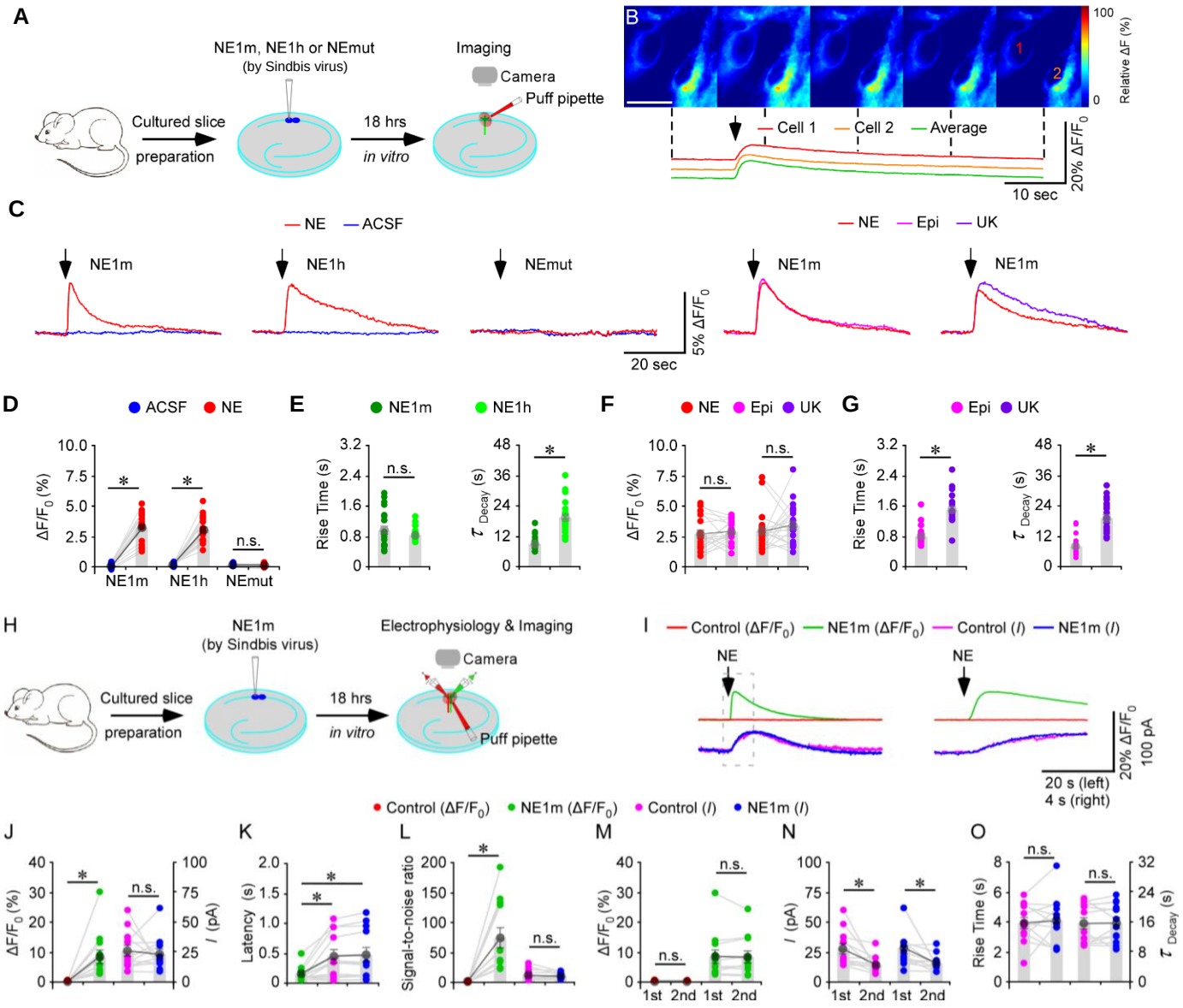
A
NE1m, NE1h or NEmut
(by Sindbis virus)
Imaging
Camera
Puff pipette
Cultured slice
preparation
18 hrs
in vitro
B
Relative ΔF (%)
Cell 1
Cell 2
Average
20% ΔF/F0
10 sec
C
NE
ACSF
NE1m
NE1h
NEmut
5% ΔF/F0
20 sec
NE
Epi
UK
D
ACSF
NE
ΔF/F0 (%)
n.s.
E
NE1m
NE1h
Rise Time (s)
τ Decay (s)
F
G
H
Electrophysiology & Imaging
I
Control (ΔF/F0)
NE1m (ΔF/F0)
Control (I)
NE1m (I)
20% ΔF/F0
100 pA
20 s (left)
4 s (right)
J
K
Latency (s)
L
Signal-to-noise ratio
M
N
I (pA)
O
1st
2nd

Fig S4

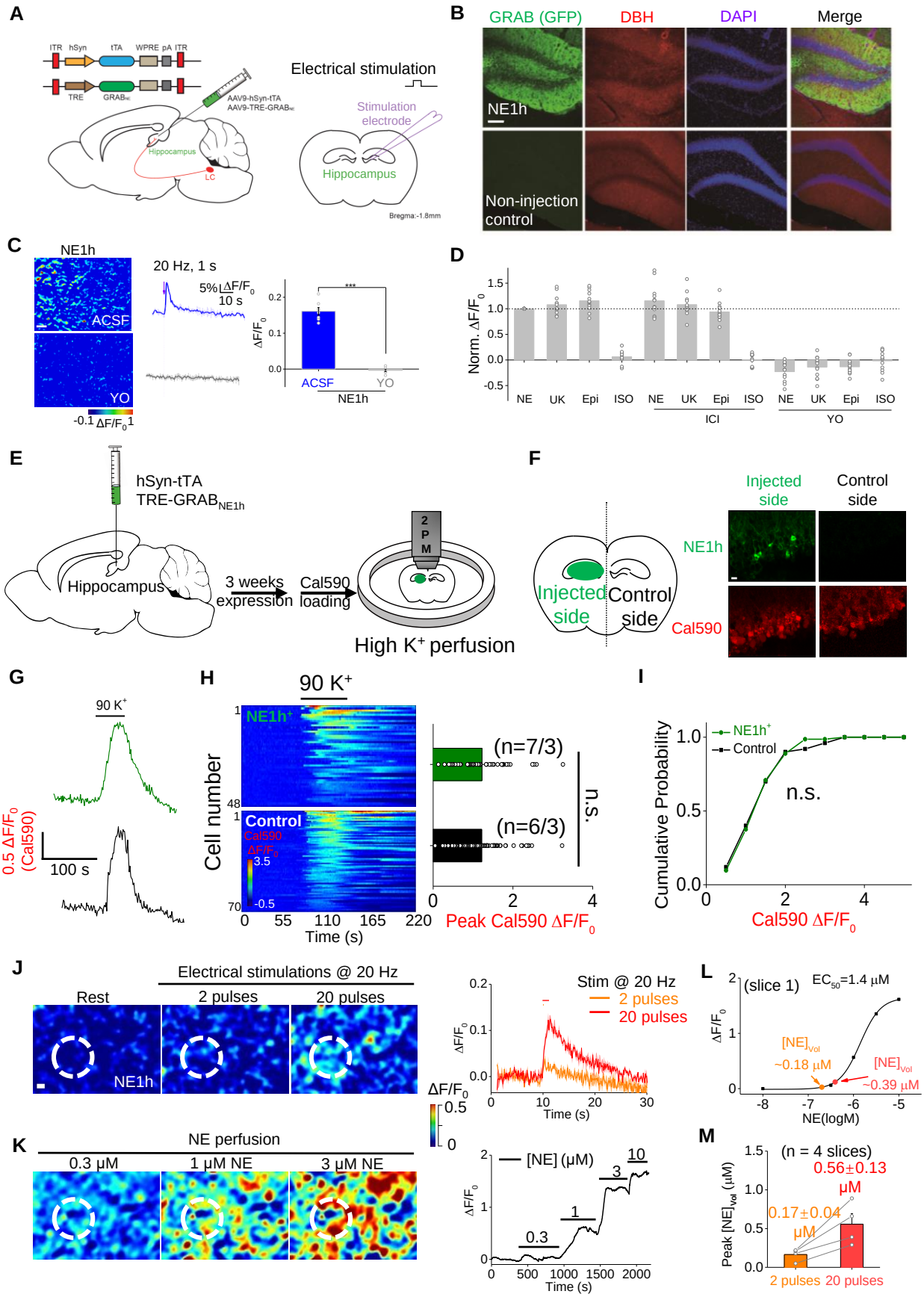
A
ITR hSyn tTA WPRE pA ITR
TRE GRAB
AAV9-hSyn-tTA
AAV9-TRE-GRAB
Hippocampus
LC
Electrical stimulation
Stimulation electrode
Hippocampus
Bregma:-1.8mm
B
GRAB (GFP)
DBH
DAPI
Merge
NE1h
Non-injection control
C
NE1h
ACSF
YO
-0.1 ΔF/F0 1
20 Hz, 1 s
5% ΔF/F0
10 s
ΔF/F0
***
ACSF
YO
NE1h
D
Norm. ΔF/F0
NE UK Epi ISO NE UK Epi ISO NE UK Epi ISO
ICI
YO
E
hSyn-tTA
TRE-GRAB NE1h
Hippocampus
3 weeks expression
Cal590 loading
2 P M
High K+ perfusion
F
Injected side
Control side
NE1h
Cal590
G
90 K+
0.5 ΔF/F0 (Cal590)
100 s
H
90 K+
NE1h+
Control
Cal590 ΔF/F0
3.5
-0.5
Cell number
Time (s)
(n=7/3)
(n=6/3)
n.s.
Peak Cal590 ΔF/F0
I
NE1h+
Control
n.s.
Cumulative Probability
Cal590 ΔF/F0
J
Electrical stimulations @ 20 Hz
Rest
2 pulses
20 pulses
NE1h
Stim @ 20 Hz
2 pulses
20 pulses
ΔF/F0
Time (s)
K
NE perfusion
0.3 μM
1 μM NE
3 μM NE
[NE] (μM)
0.3
1
3
10
Time (s)
L
(slice 1)
EC50=1.4 μM
[NE]Vol ~0.18 μM
[NE]Vol ~0.39 μM
ΔF/F0
NE(logM)
M
(n = 4 slices)
0.56±0.13 μM
0.17±0.04 μM
Peak [NE]Vol (μM)
2 pulses 20 pulses

Fig S5

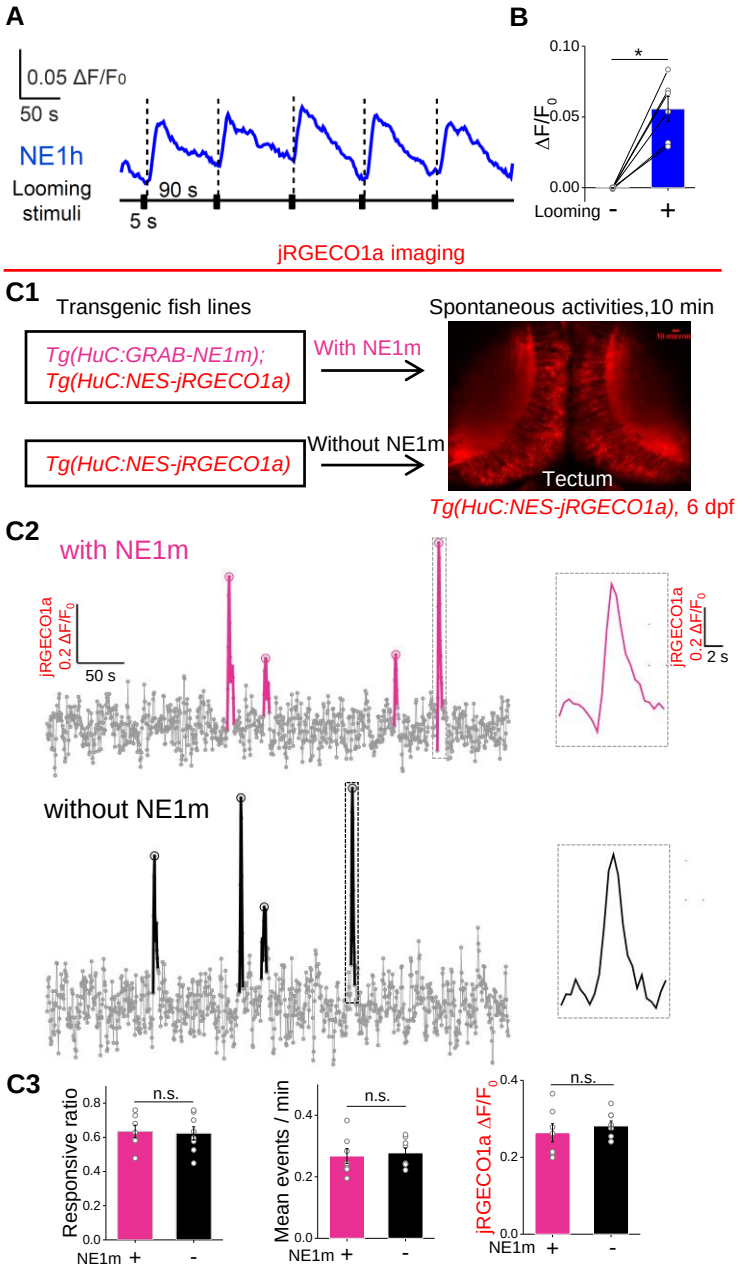
A
0.05 ΔF/F0
50 s
NE1h
Looming stimuli
90 s
5 s
B
0.10
0.05
0.00
ΔF/F0
*
Looming - +
jRGECO1a imaging
C1
Transgenic fish lines
Tg(HuC:GRAB-NE1m); Tg(HuC:NES-jRGECO1a)
With NE1m
Tg(HuC:NES-jRGECO1a)
Without NE1m
Spontaneous activities,10 min
Tectum
Tg(HuC:NES-jRGECO1a), 6 dpf
C2
with NE1m
jRGECO1a 0.2 ΔF/F0
50 s
2 s
without NE1m
C3
Responsive ratio
n.s.
NE1m + -
Mean events / min
jRGECO1a ΔF/F0

Fig S6

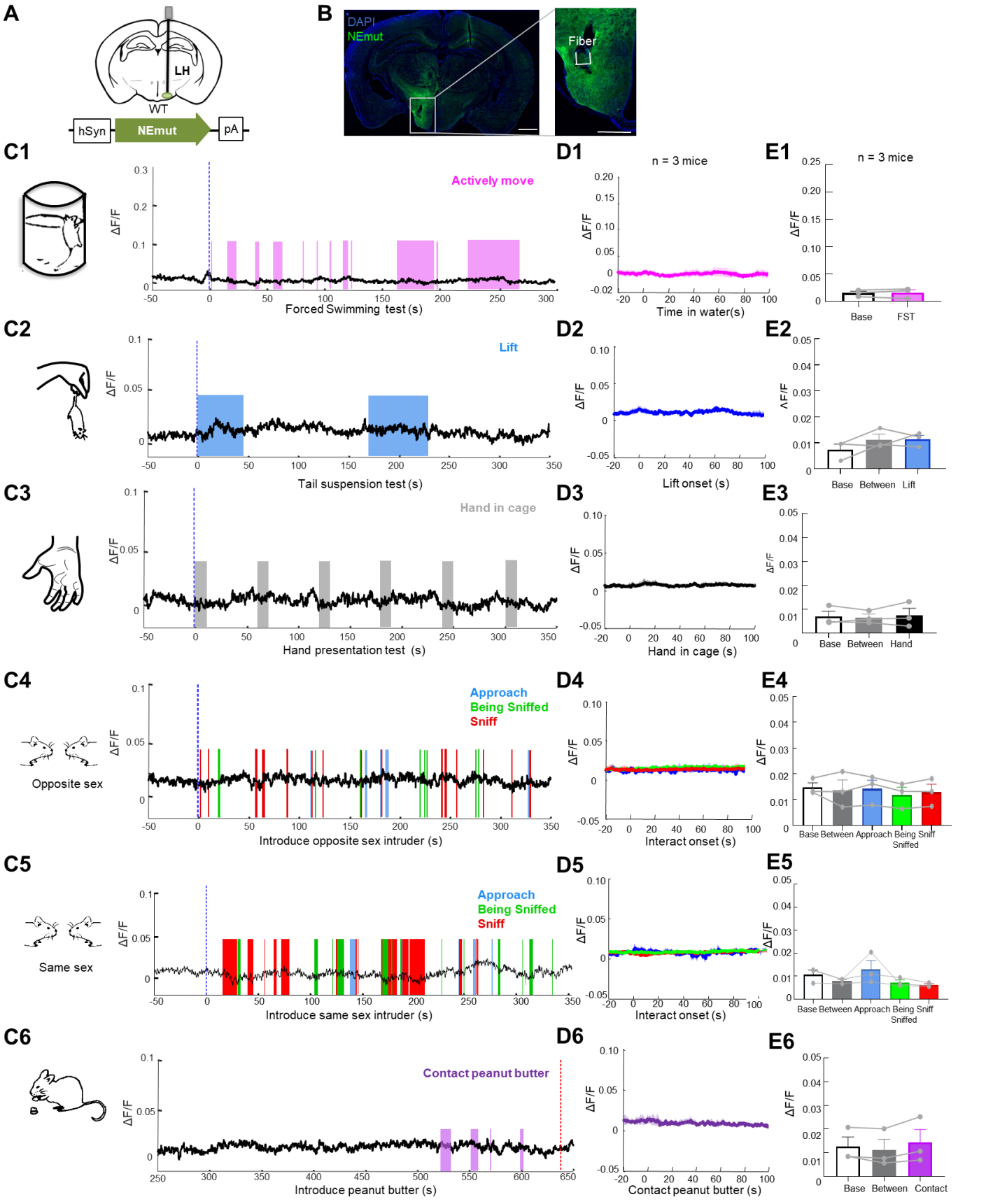
A
LH
WT
hSyn
NEmut
pA
B
DAPI
NEmut
Fiber
C1
Actively move
ΔF/F
Forced Swimming test (s)
D1
n = 3 mice
ΔF/F
Time in water(s)
E1
n = 3 mice
ΔF/F
Base
FST
C2
Lift
ΔF/F
Tail suspension test (s)
D2
ΔF/F
Lift onset (s)
E2
ΔF/F
Base
Between
Lift
C3
Hand in cage
ΔF/F
Hand presentation test (s)
D3
ΔF/F
Hand in cage (s)
E3
ΔF/F
Base
Between
Hand
C4
Approach
Being Sniffed
Sniff
Opposite sex
ΔF/F
Introduce opposite sex intruder (s)
D4
ΔF/F
Interact onset (s)
E4
ΔF/F
Base
Between
Approach
Being Sniff
Sniffed
C5
Approach
Being Sniffed
Sniff
Same sex
ΔF/F
Introduce same sex intruder (s)
D5
ΔF/F
Interact onset (s)
E5
ΔF/F
Base
Between
Approach
Being Sniff
Sniffed
C6
Contact peanut butter
ΔF/F
Introduce peanut butter (s)
D6
ΔF/F
Contact peanut butter (s)
E6
ΔF/F
Base
Between
Contact

Figure S1. Characterization of the membrane trafficking of a panel of screening candidates, related to Figure 1.

Representative images (**A**) of HEK293T cells co-transfected with the indicated screening candidates (green) together with RFP-CAAX (red) to label the plasma membrane. KDELR-EGFP was used as an ER marker. The dashed white lines indicate the line used for the line-scanning data shown in (**B**) and summarized in

(**C**) n = 30 cells from 4-5 cultures.

The scale bars in (**A**) represent 10 μm.

**p* < 0.05 and ****p* < 0.001; n.s., not significant (Student's *t*-test).

Figure S2. Further characterization of $GRAB_{NE}$ sensors, related to Figure 2 and Figure 3.

(**A**) Fluorescence intensity of $GRAB_{NE1m}$ and $GRAB_{NE1h}$ expressed relative to EGFP-α2AR. n ≥ 2 wells with 300-500 cells per well.

(**B**) G protein activation mediated by $GRAB_{NE1m}$ and wild-type α2AR was measured using the TGFα shedding assay and is expressed relative to α2AR. n = 4 wells with $\geq 10^5$ cells per well.

(**C**) Exemplar (**left**) and summary data (**right**) showing the photostability of $GRAB_{NE}$ sensors and EGFP-CAAX using confocal (**top**) and 2-photon (**bottom**) microscopy. n > 10 cells from at least 3 cultures.

(**D**) Exemplar cyclic voltammograms for 10 μM NE (**top**), 10 μM DA (**middle**), and 10 μM Glu (**bottom**) measured using FSCV are shown. The traces were averaged from separate 200 trials.

(**E**) Disrupting of G protein activation with pertussis toxin does not affect the NE-induced fluorescence change in $GRAB_{NE1m}$-expressing neurons. n = 27 neurons from 3 cultures.

***$p < 0.001$ (Student's *t*-test).

Figure S3. $GRAB_{NE}$ sensors respond selectively to noradrenergic agonists in brain slices without affecting endogenous NE receptor functions, related to Figure 4.

(**A**) Schematic drawing showing the experimental design for measuring CA1 pyramidal neurons in cultured rat hippocampal slices.

(**B**) Heat-map images of the change in fluorescence in $GRAB_{NE1m}$-expressing CA1 neurons in response to a 10-ms local application of NE (20 μM). The red and orange traces show the fluorescence responses of two neurons, and the green trace shows the average response of all neurons in the field.

(**C**) Fluorescence responses measured in $GRAB_{NE1m}$-, $GRAB_{NE1h}$-, and $GRAB_{NEmut}$-expressing CA1 neurons following a 10-ms puff (arrow) of ACSF, NE (20 μM), Epi (100 μM), or brimonidine (UK, 20 μM).

(**D**) Maximum $\Delta F/F_0$ responses measured in $GRAB_{NE1m}$-, $GRAB_{NE1h}$-, and $GRAB_{NEmut}$-expressing CA1 neurons following a 10-ms puff of ACSF or NE. n = 20-21 cells from 8 animals per group.

(**E**) Rise times and decay time constants measured in CA1 neurons expressing $GRAB_{NE1m}$- and $GRAB_{NE1h}$- expressing CA1 neurons in response to a puff of NE. n = 21 cells from 8 animals.

(**F**) Maximum $\Delta F/F_0$ responses measured in $GRAB_{NE1m}$-expressing CA1 neurons following a puff of NE, Epi, or brimonidine (UK). n = 20-21 cells from 8 animals per group.

(**G**) Rise times and decay time constants measured in $GRAB_{NE1m}$-expressing CA1 neurons following a puff of Epi or brimonidine (UK).

(**H**) Schematic drawing outlines the design of simultaneous imaging and electrophysiological recording experiments in rat cultured hippocampal slices.

(**I**) Left, simultaneous fluorescence and current responses of a pair of $GRAB_{NE1m}$ expressing and neighboring control non-expressing CA1 neurons to a 10-ms puff application of 0.2 mM norepinephrine (NE). Right, the responses in the left rectangle box are shown again in an expanded time scale. Note the different latencies of fluorescence and current responses.

(**J**) Values for the amplitude of noradrenergic fluorescence ($GRAB_{NE1m}$: 8.52 ± 2.26%; Ctrl: 0.14 ± 0.02%; $Z = 3.059$; $p = 0.002$; $n = 12$ from 12 animals) and current ($GRAB_{NE1m}$: 23.4 ± 4.2 pA; Ctrl: 26.4 ± 4.7 pA; $Z = 0.078$; $p = 0.937$; $n = 12$ from 12 animals) responses of $GRAB_{NE1m}$ expressing CA1 neurons compared to non-expressing neurons.

(**K**) Values for the latency of noradrenergic current responses in $GRAB_{NE1m}$ expressing ($GRAB_{NE1m}$: 462.0 ± 124.2 ms; $Z = 2.578$; $p = 0.01$) and non-expressing CA1 neurons (Ctrl: 440.6 ± 113.1 ms; $Z = 2.432$; $p = 0.015$) compared to those of fluorescence responses of

63 $GRAB_{NE1m}$ expressing neurons ($GRAB_{NE1m}$: 145.8 ± 36.4 ms; *n* = 12 from 12 animals).

64 (**L**) Values for the signal-to-noise ratio (SNR) of noradrenergic fluorescence responses of
65 $GRAB_{NE1m}$ expressing CA1 neurons compared to non-expressing neurons ($GRAB_{NE1m}$:
66 75.9 ± 17.1; Ctrl: 2.5 ± 0.3; *Z* = 3.509; *p* = 0.002; *n* = 12 from 12 animals) and noradrenergic
67 current responses of $GRAB_{NE1m}$ expressing CA1 neurons compared to non-expressing
68 neurons ($GRAB_{NE1m}$: 9.6 ± 1.6; Ctrl: 11.3 ± 2.8; *Z*=-0.235; *p*=0.814; *n* = 12 from 12 animals).
69 Note the larger SNR of noradrenergic fluorescence responses of $GRAB_{NE1m}$ expressing
70 CA1 neurons compared to current responses of $GRAB_{NE1m}$ expressing and non-expressing
71 CA1 neurons ($GRAB_{NE1m}$: *Z* = -3.509; *p* = 0.002; Ctrl: *Z* = -2.981; *p* = 0.002).

72 (**M**) Values for the two consecutive fluorescence responses of $GRAB_{NE1m}$ expressing (1^{st}:
73 8.56 ± 0.02%; 2^{nd}: 8.43 ± 0.02%; *Z* = 0; *p* = 1; *n* = 12 from 12 animals) and control non-
74 expressing (1^{st}: 0.14 ± 0.02%; 2^{nd}: 0.11 ± 0.01%; *Z* = -1.832; *p*=0.067; *n* = 12 from 12
75 animals) CA1 neurons.

76 (**N**) Values for the two consecutive noradrenergic current responses in $GRAB_{NE1m}$
77 expressing (1^{st}: 28.6 ± 5.0 pA; 2^{nd}: 15.6 ± 2.0 pA; *Z* = -2.51; *p*=0.012; *n* = 12 from 12
78 animals) and control non-expressing (1^{st}: 27.7 ± 4.4 pA; 2^{nd}: 14.9 ± 2.0 pA; *Z* = -3.059;
79 *p*=0.02; *n* = 12 from 12 animals) CA1 neurons.

80 (**O**) Values for the rise time ($GRAB_{NE1m}$: 4.14 ± 0.46 s; Ctrl: 3.98 ± 0.38 s; *Z* = 0.314; *p* =
81 0.754; *n* = 12 from 12 animals) and decay time constant ($GRAB_{NE1m}$: 15.81 ± 1.50 s; Ctrl:
82 15.70 ± 1.53 s; *Z* = 0.784; *p* = 0.433; *n* = 12 from 12 animals) of noradrenergic current
83 responses in $GRAB_{NE1m}$ expressing neurons compared to control non-expressing CA1
84 neurons. Large gray dots indicate average responses and asterisks indicate *p*<0.05
85 (Wilcoxon tests).
86
87

Figure S4. $GRAB_{NE}$ sensors respond selectively to noradrenergic agonists in brain slices without disturbing neuronal activities, related to Figure 4.

(**A**) Schematic illustration depicting AAV-mediated delivery of $GRAB_{NE1h}$ in the mouse hippocampus and bath application of various agonists in the dentate gyrus.

(**B**) Example images showing $GRAB_{NE1h}$ (green) expression and dopamine beta hydroxylase (DBH) immunostaining (red) in the dentate gyrus of AAV-$GRAB_{NE1h}$- and control-injected hippocampus. The nuclei were counterstained with DAPI. The scale bar represents 100 μm.

(**C**) Electrical stimulation evokes NE release in the hippocampus measured as a change in $GRAB_{NE1h}$ fluorescence. The response was blocked by batch application of yohimbine (YO). Exemplar images (**left**), representative traces (**middle**), and the summary data (**right**) are shown.

(**D**) Normalized change in $GRAB_{NE1h}$ fluorescence in response to bath application of the indicated noradrenergic agonists in the presence or absence of ICI 118,551 or yohimbine.

(**E**) Schematic illustration of the calcium imaging experiments in acute brain slices. The AAVs expressing $GRAB_{NE1h}$ were injected unilaterally into the dentate gyrus and acute brain slices were prepared after 3 weeks and loaded with Cal590 red calcium dye for imaging.

(**F**) The fluorescent signal of $GRAB_{NE1h}$ sensor (green) and calcium dye Cal590 (red) in acute brain slices. Scale bar, 20 μm.

(**G**) Representative traces of the Cal590 fluorescent response in either a $GRAB_{NE1h}$-expressing neuron (**upper**) or a non-expressing control neuron (**lower**) to the perfusion of high potassium solution (90mM K^+).

(**H**) The group data of the Cal590 fluorescence responses in $GRAB_{NE1h}$-expressing neurons or non-expressing control neurons to the perfusion of high potassium solution (n=48 neurons from 7 slices of 3 mice for $GRAB_{NE1h}$, n=70 neurons from 6 slices of 3 mice for control, p=0.95, student-t test)

(**I**) Cumulative plot of the Cal590 fluorescence response. (P=0.93, Kolmogorov-Smirnov test)

(J,K) Fluorescence responses of $GRAB_{NE1m}$-expressing cells in an acute LC slice to electrical stimulation of different pulses at 20 Hz in (**J**)**,** or to the exogenous perfusion of different concentrations of NE in (**K**). Left, pseudocolor snapshots of $GRAB_{NE1m}$ fluorescence responses. The white dash circles indicate ROI (50 μm in diameter) used for the fluorescence analysis. Right, corresponding fluorescence responses of left.

(L) Dose-dependent curve of fluorescence response to different concentrations of NE. Response data were fitted by the Boltzmann equation, and the evoked volume-averaged

NE concentration ($[NE]_{Vol}$) was estimated based on the calibration curve from the same slice.

(M) Group data of the evoked $[NE]_{Vol}$ during electrical stimulations (n = 4 slices from 3 mice). Error bars indicate S.E.M.

The scale bar shown in (**B**) represents 100 μm. The scale bar shown in (**C**) and (**J**) represent 10 μm.

***$p < 0.001$ (Student's *t*-test).

Figure S5. GRAB$_{NE1h}$ can sense endogenous NE release and optic tectal neurons with or without HuC:GRAB$_{NE1m}$ overexpression show no difference in spontaneous calcium responses, related to Figure 5.

(**A,B**) Detection of endogenous NE release in the midbrain of GRAB$_{NE1h}$ zebrafish evoked by visual looming stimuli. Quantification data is shown in (**B**). n = 6 fish.
(**C**) Spontaneous calcium activities of optic tectal neurons revealed by jREGCO1a fluorescent signals show no difference with- or without HuC:GRAB$_{NE1m}$ expression. Experimental diagram is shown in (**C1**). Traces for representative calcium responses are shown in (**C2**). Group data are shown in (**C3**). n = 7 for transgenic HuC:GRAB$_{NE1m}$ zebrafish, and n = 8 for fish without expressing GRAB$_{NE1m}$.

*p< 0.05 and ***p< 0.001; n.s., not significant (Student's *t*-test, Wilcoxon test, or Mann-Whitney rank sum test).

Figure S6. No detectable changes in noradrenergic activity are observed in freely moving mice after expression of the GRAB$_{NEmut}$ sensor during stress, social interactions and food related behaviors, related to Figure 7.

(**A**) Schematic diagrams depicting the AAV virus injection, and recording sites.

(**B**) Histology showing the expression of GRAB$_{NEmut}$ (green) and placement of the recording; the nuclei were counterstained with DAPI (blue). Scale bar: 1 mm (left), 500μm (right).

(**C1-E5**) Representative traces (**C1-C5**), average per-stimulus histograms (**D1-D5**), and summary data (**E1-E5**) showing normalized GRAB$_{NE1m}$ fluorescence (ΔF/F) before and during the forced swim test (**1**), and before, between and during the tail suspension test (**2**), the hand presentation test (**3**), social interaction with an intruder of the opposite sex (**4**) , an intruder of the same sex (**5**) and presentation of peanut butter (**6**). n = 3 animals each.

The Shapiro-Wilk normality test was performed; if the test revealed it followed a normal distribution, a paired Student's *t*-test or one-way repeated measures ANOVA followed by Tukey's multiple comparisons was performed. If the values did not follow a normal distribution, a non-parametric ANOVA (Friedman's test) was performed followed by Dunn's multiple comparisons test. In (**C**) and (**D**), the blue dotted lines represent the start of the stimulus, and the red dotted lines represent the end of the trial.

*$p < 0.05$ and **$p < 0.01$.