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Supplemental information

A genetically encoded fluorescent sensor for monitoring spatiotemporal prostaglandin E2 dynamics *in vivo*

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Inventory of Supplemental Information:

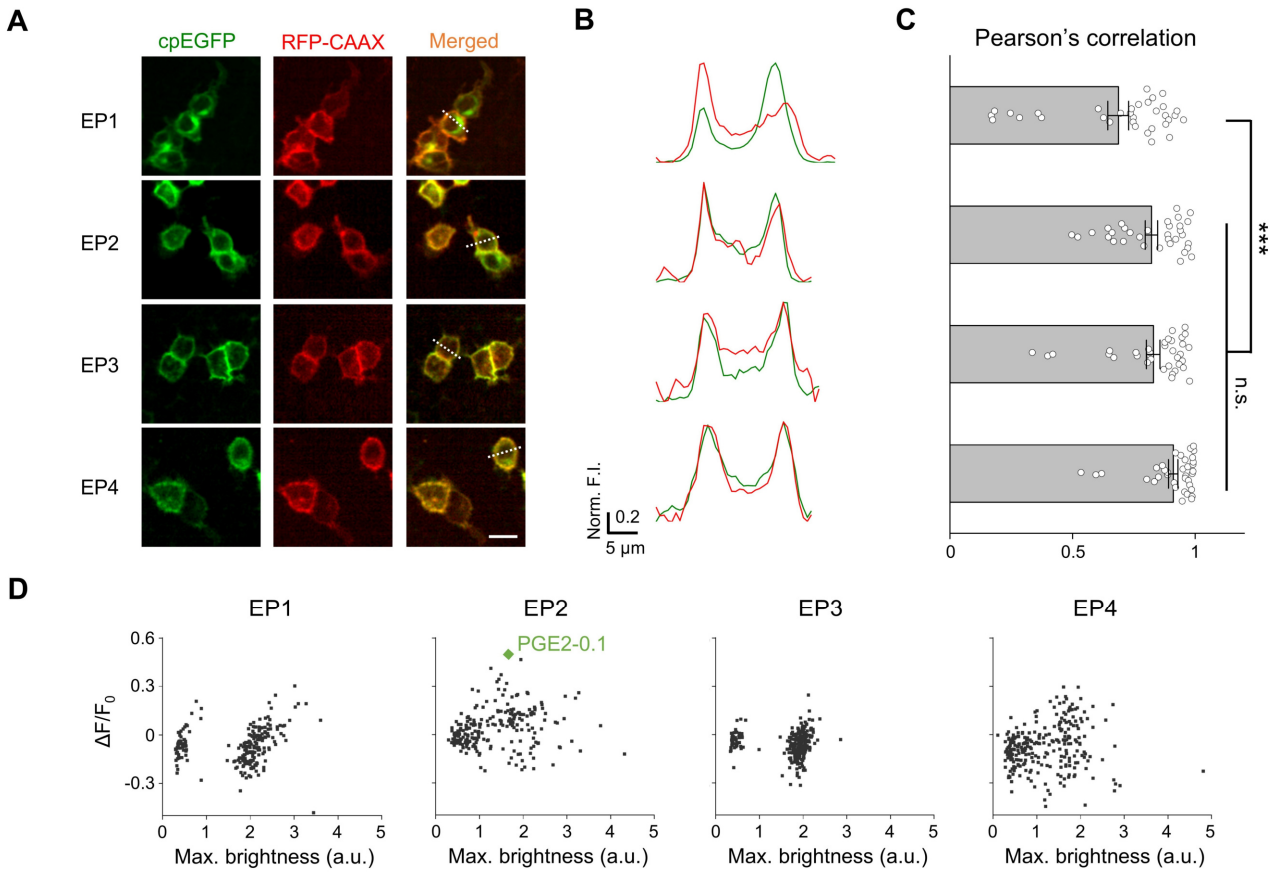
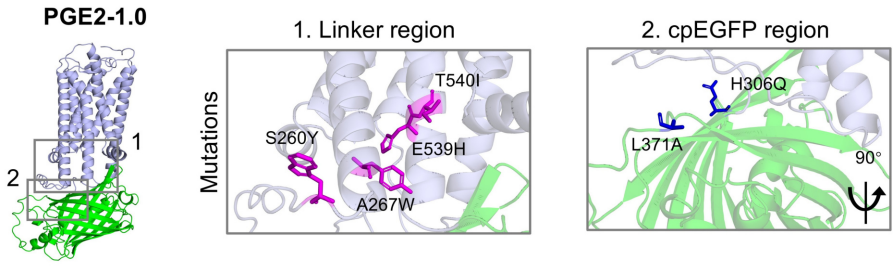

A
cpEGFP
RFP-CAAX
Merged
EP1
EP2
EP3
EP4
B
Norm. F.I.
0.2
5 μm
C
Pearson's correlation
***
n.s.
0
0.5
1
D
EP1
EP2
EP3
EP4
PGE2-0.1
ΔF/F0
0.6
0.3
0
-0.3
0
1
2
3
4
5
Max. brightness (a.u.)

Figure S1. Screening of the $GRAB_{PGE2}$ sensor GPCR scaffold and insertion sites, related to Figure 1.

(A) Representative images of HEK293T cells co-expressing red fluorescent RFP-CAAX (to label the plasma membrane) and prototype $GRAB_{PGE2}$ sensor candidates with cpEGFP inserted in the indicated PGE2 receptor backbones. The dotted lines in the merged images were used to measure the correlation between the green and red fluorescence signals. Scale bar, 20 μm. **(B)** Line-scan traces of the green (cpEGFP) and red (RFP-CAAX) channels in the images shown in (A). **(C)** Summary of the correlation between the green and red fluorescence signals in order to confirm membrane trafficking of the various $GRAB_{PGE2}$ sensor candidates. n = 33–36 cells/4 wells for EP1–EP4 respectively. **(D)** Peak $\Delta F/F_0$ plotted against maximum brightness for the indicated $GRAB_{PGE2}$ candidates. The candidate with the highest performance (PGE2-0.1) is indicated which was the selected candidate for further optimization. All summary data are presented as the mean ± the standard error of the mean (SEM). Data were analyzed using one-way ANOVA followed by Tukey's test for multiple comparisons. Where indicated, n.s., not significant ($p > 0.05$), ***$p < 0.001$.

A



B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1   M E T D T L L L W V L L L W V P G S T G D T S L Y K K V G T T G G N A S N D S Q S E D C E T R Q W L  50
51  P P G E S P A I S S V M F S A G V L G N L I A L A L L A R R W R G D V G C S A G R R S S L S L F H V 100
                PGE2mut: 113^2.54W
101 L V T E L V F T D L L G T C L I S P V V L A S Y A R N Q T L V A L A P E S R A C T Y F A F A M T F F 150
151 S L A T M L M L F A M A L E R Y L S I G H P Y F Y Q R R V S R S G G L A V L P V I Y A V S L L F C S 200
201 L P L L D Y G Q Y V Q Y C P G T W C F I R H G R T A Y L Q L Y A T L L L L L I V S V L A C N F S V I 250
                Mutation sites
251 L N L I R M H R R Y R R Q L N G W P G E P A P A G P R D T D A L D L E E G G N V Y I K A D K Q K N G 300
301 I K A N F Q I R H N I E D G G V Q L A Y H Y Q Q N T P I G D G P V L L P D N H Y L S V Q S K L S K D 350
351 P N E K R D H M V L L E F V T A A G I T A G M D E L Y K G G T G G S M V R K G E E L F T G V V P I L 400
401 V E L D G D V N G H K F S V S G E G E G D A T E G K L T L K F I C T T G K L P V P W P T L V T T L T 450
451 Y G V Q C F S R Y P D H M K Q H D F F K S A M P E G Y I Q E R T I F F K D D G N Y K T R A E V K F E 500
501 G D T L V N R I E L K G I D F K E D G N I L G H K L E Y N T G A A A R M A E H I D H L I L L A I M T 550
551 I T F A V C S L P F T I F A Y M N E T S S R K E K W D L Q A L R F L S I N S I I D P W V F A I L R P 600
601 P V L R L M R S V L C C R I S L R T Q D A T Q T S C S T Q S D A S K Q A D L *
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Figure S2. The structure and mutation sites in the PGE2-1.0 sensor, related to Figure 1.

(A) Structure of the PGE2-1.0 sensor, with the EP2 GPCR and cpEGFP components shown in purple and green, respectively. The linker and cpEGFP regions in which mutations were introduced to generate PGE2-1.0 are indicated. **(B)** The amino acid sequence of the PGE2-1.0 sensor, with the sensor's domains shown above. The mutation sites for generating the sensor are indicated by red boxes, and the $T113^{2.54}W$ mutation introduced to create the ligand-insensitive PGE2mut version from PGE2-1.0 is indicated by a black box. Note that the amino acid numbering system used here corresponds to the start of the IgK leader sequence.

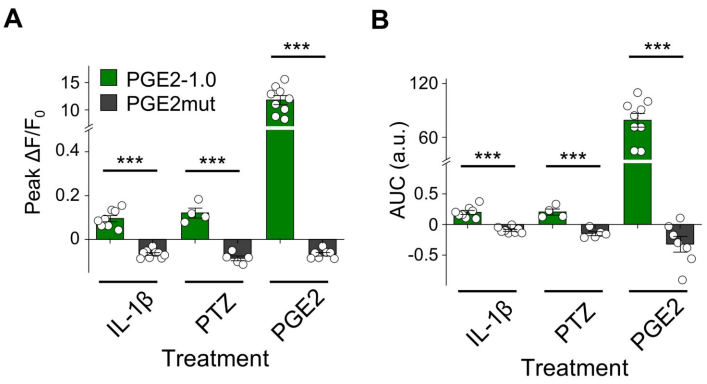
A
B
PGE2-1.0
PGE2mut
Peak ΔF/F0
AUC (a.u.)
***
IL-1β
PTZ
PGE2
Treatment

Figure S3. Summary of the change in PGE2-1.0 and PGE2mut fluorescence measured in brain slices in response to IL-1β, PTZ, and PGE2 perfusion, related to Figure 2.

(A) Summary of the peak change in PGE2-1.0 and PGE2mut fluorescence in brain slices in response to IL-1β (n = 8/6 (8 slices from 6 mice) for PGE2-1.0 sensor, n = 8/3 for PGE2mut), PTZ (n = 4/3 for PGE2-1.0, n = 5/3 for PGE2mut), and PGE2 (n = 9/7 for PGE2-1.0 and n = 7/4 for PGE2mut) perfusion. **(B)** Summary of the area under the curve (AUC) of the change in PGE2-1.0 and PGE2mut fluorescence measured 4 min after perfusing IL-1β (n = 8/6 for PGE2-1.0, n = 8/4 for PGE2mut) or PTZ (n = 4/3 for PGE2-1.0, n = 5/4 for PGE2mut), and 10 min after perfusing PGE2 (n = 9/7 for PGE2-1.0, n = 7/4 for PGE2mut). All summary data are presented as the mean ± SEM. Data were analyzed using Student's t-test. Where indicated, ***$p < 0.001$.

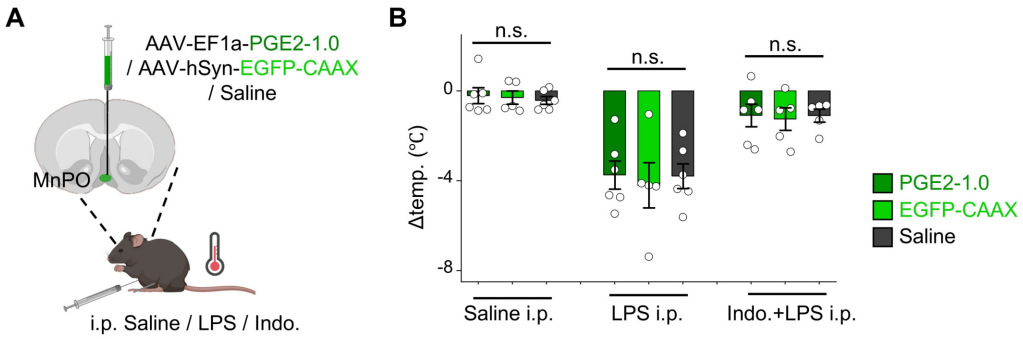
A
AAV-EF1a-PGE2-1.0
/ AAV-hSyn-EGFP-CAAX
/ Saline
MnPO
i.p. Saline / LPS / Indo.
B
n.s.
n.s.
n.s.
0
-4
-8
Δtemp. (℃)
Saline i.p.
LPS i.p.
Indo.+LPS i.p.
PGE2-1.0
EGFP-CAAX
Saline

Figure S4. The expression of PGE2-1.0 does not disrupt the normal physiological temperature response of mice, related to Figure 3.

(A) Schematic diagram depicting the strategy for injecting virus; the animal's skin temperature was also recorded during the experiment. **(B)** Summary of the mouse skin temperature change following i.p. injection of saline, 1 mg/kg body weight LPS, or 1 mg/kg body weight LPS following a pre-injection of 15 mg/kg body weight indomethacin. n = 6 mice for the PGE2-1.0 and saline group respectively; n = 5 for the EGFP-CAAX group. All summary data are presented as the mean ± SEM. Data were analyzed using one-way ANOVA followed by Tukey's test for multiple comparisons. Where indicated, n.s., not significant ($p > 0.05$).

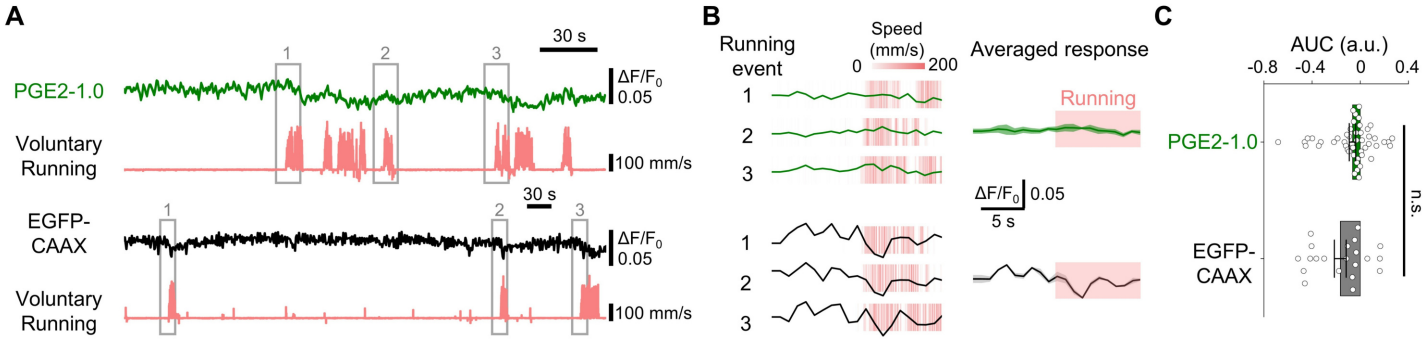
A
30 s
1
2
3
PGE2-1.0
ΔF/F0
0.05
Voluntary
Running
100 mm/s
30 s
EGFP-
CAAX
ΔF/F0
0.05
Voluntary
Running
100 mm/s
B
Running
event
Speed
(mm/s)
0
200
Averaged response
Running
ΔF/F0
0.05
5 s
C
AUC (a.u.)
-0.8
-0.4
0
0.4
PGE2-1.0
EGFP-
CAAX
n.s.

Figure S5. Using the PGE2-1.0 sensor to detect cortex PGE2 dynamics during voluntary running, related to Figure 3.

(A) Example trace of the fluorescence changes of PGE2-1.0 (upper panel) or EGFP-CAAX (lower panel) and simultaneously recorded voluntary running speed. Grey boxes indicate representative running events (1–3) that are expanded in (B). **(B)** (Left) Traces of individual running events. (Right) Averaged fluorescent response aligned to the onset and duration of the running period (indicated by the red shaded box). The upper panel shows PGE2-1.0 responses, and the lower panel shows EGFP-CAAX responses. **(C)** Summary of the area under the curve (AUC) of the change in PGE2-1.0 fluorescence (n = 49/6 (49 running events from 6 mice)) and EGFP-CAAX (n = 21/4) measured for 10 s after running onset. The summary data are presented as the mean ± SEM. Data were analyzed using Student's t-test. Where indicated, n.s., not significant ($p > 0.05$).

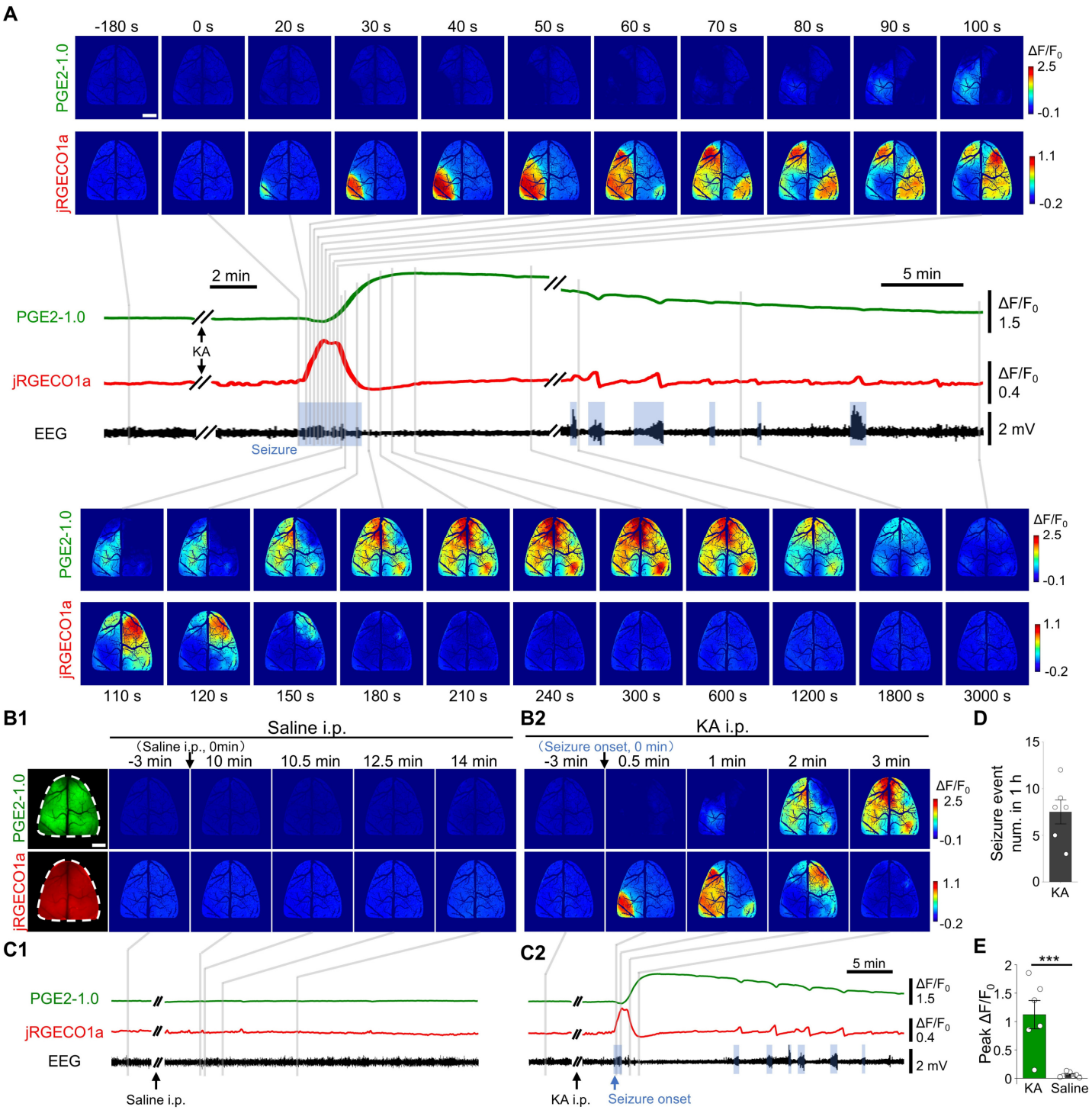
A
-180 s
0 s
20 s
30 s
40 s
50 s
60 s
70 s
80 s
90 s
100 s
PGE2-1.0
jRGECO1a
ΔF/F0
2.5
-0.1
1.1
-0.2
2 min
5 min
KA
EEG
Seizure
1.5
0.4
2 mV
110 s
120 s
150 s
180 s
210 s
240 s
300 s
600 s
1200 s
1800 s
3000 s
B1
Saline i.p.
(Saline i.p., 0min)
-3 min
10 min
10.5 min
12.5 min
14 min
B2
KA i.p.
(Seizure onset, 0 min)
-3 min
0.5 min
1 min
2 min
3 min
C1
C2
Saline i.p.
KA i.p.
Seizure onset
D
Seizure event num. in 1 h
KA
E
Peak ΔF/F0
***
KA
Saline

Figure S6. The PGE2-1.0 sensor reliably detects seizure-induced changes in PGE2 *in vivo*, related to Figure 3.

(A) A high-temporal resolution series of representative images and traces of the change in PGE2-1.0 and jRGECO1a fluorescence in response to an i.p. injection of KA (10 mg/kg body weight). Note the different time scales before and after the break. Scale bars, 1 mm. **(B)** Representative images of the change in PGE2-1.0 and jRGECO1a fluorescence in response to an i.p. injection of Saline (B1) and KA (10 mg/kg body weight) (B2). **(C)** The representative traces of the change in PGE2-1.0 and jRGECO1a fluorescence, together with the corresponding EEG signal recorded in Saline (C1) and KA (C2) injected mouse. The blue shaded boxes in the EEG trace indicate epileptic discharges. **(D)** The summary of epileptiform seizure events within 1 h after the first seizure event in KA-induced seizure model. n = 6 mice. **(E)** Summary of the peak change in PGE2-1.0 fluorescence measured after injecting KA (n = 6 mice, replotted with data from Figure 4C) or Saline (n = 7 mice). The summary data are presented as the mean ± SEM. Data were analyzed using Student's t-test. Where indicated, ***$p < 0.001$.

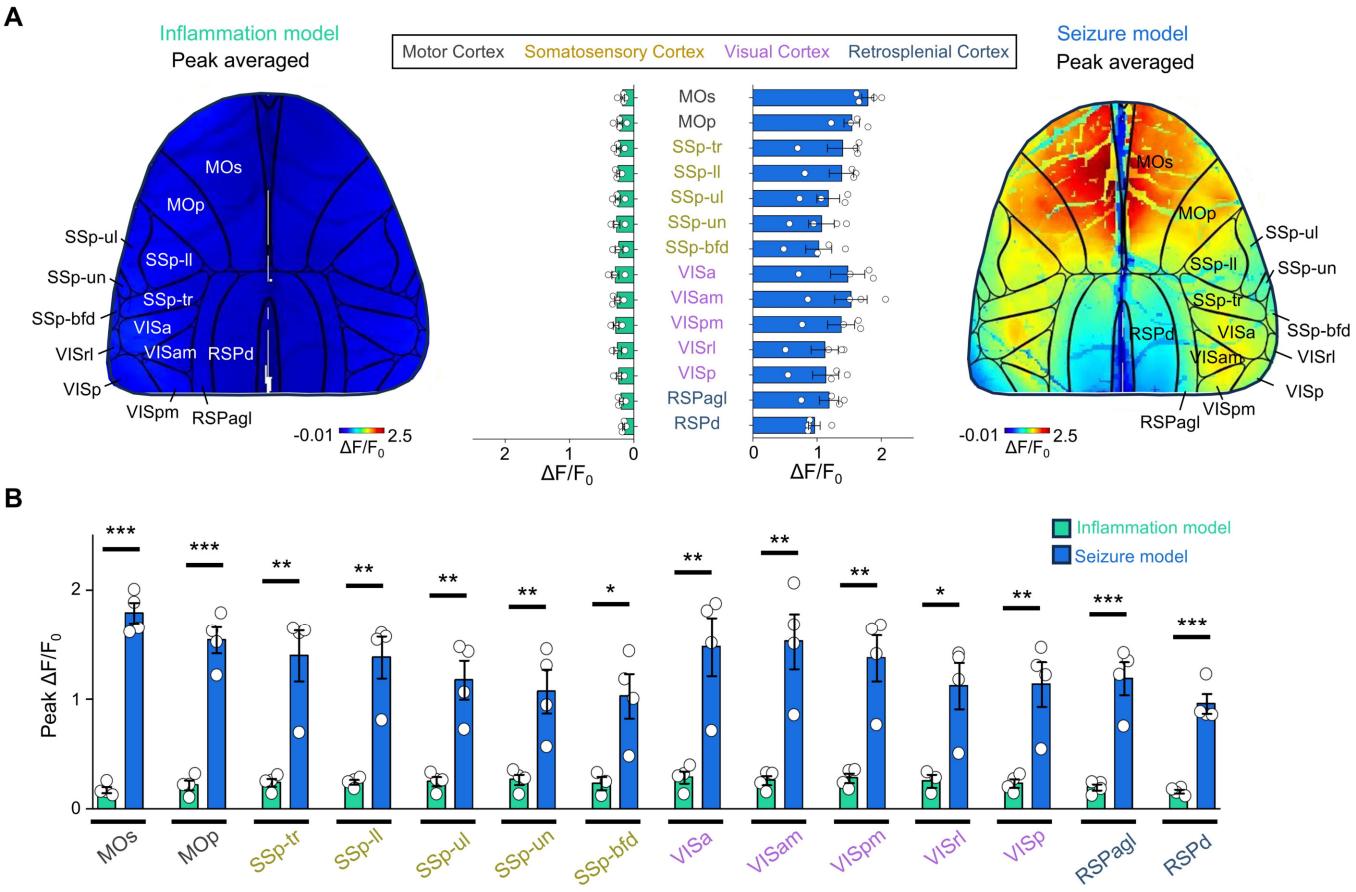
A
Inflammation model
Peak averaged
Motor Cortex
Somatosensory Cortex
Visual Cortex
Retrosplenial Cortex
Seizure model
Peak averaged
MOs
MOp
SSp-ul
SSp-ll
SSp-un
SSp-tr
SSp-bfd
VISa
VISrl
VISam
RSPd
VISp
VISpm
RSPagl
-0.01
2.5
ΔF/F0
2
1
0
B
Inflammation model
Seizure model
Peak ΔF/F0
***
**
*

Figure S7. Replotted data from Figure 4D with adjusted scale and statistical analysis, related to Figure 4.

(A) Pseudo-color images depicting the average peak increase in PGE2-1.0 fluorescence measured during inflammation (left) and seizure (right) in same scale (replotted data from Figure 4D). **(B)** Summary of the peak responses measured in the indicated cortical structures with statistical analysis (replotted data from Figure 4D). All summary data are presented as the mean ± SEM. Data were analyzed using Student's t-test. Where indicated, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.