Supplemental Information

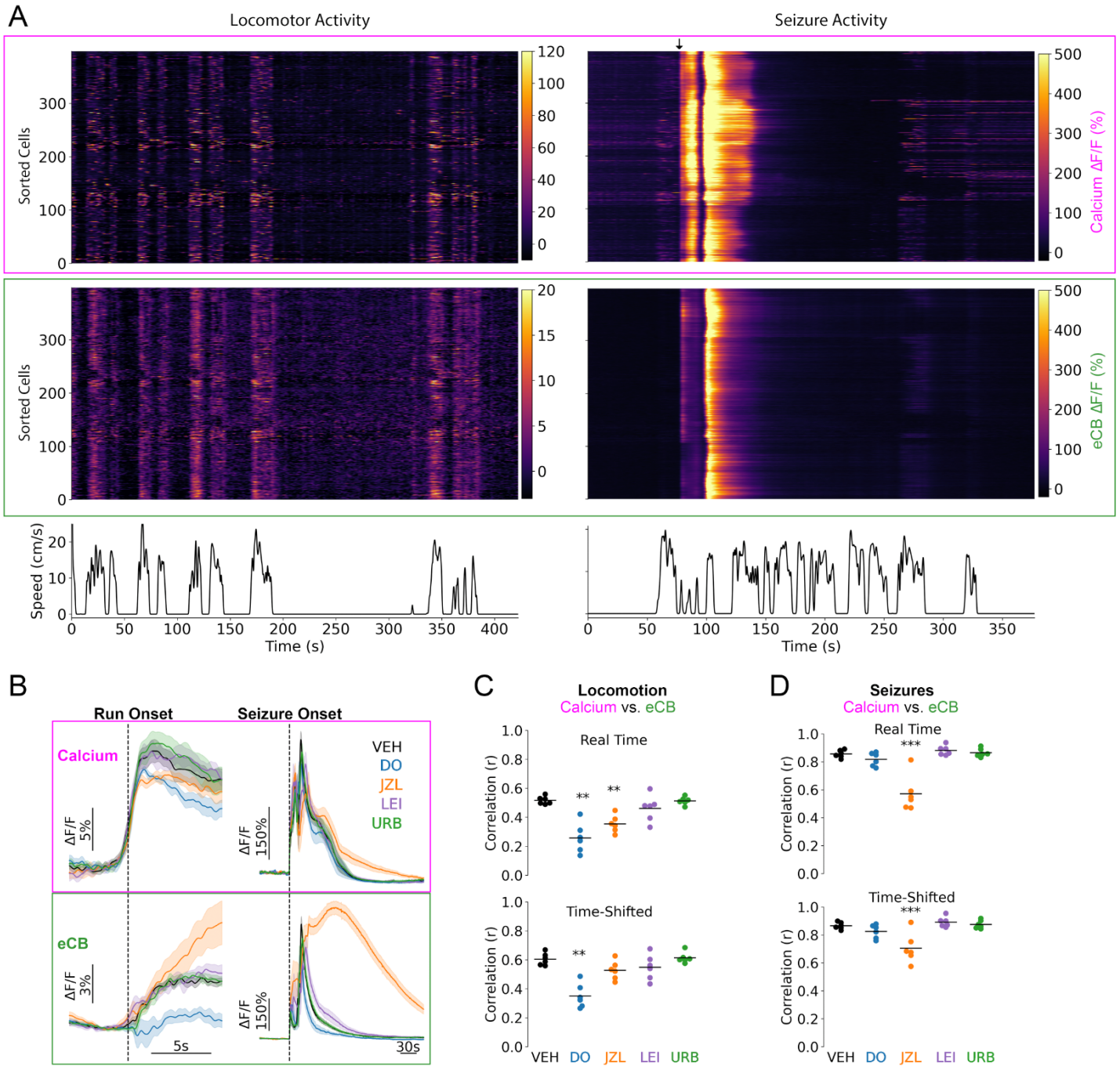

Figure S1. Pharmacological sensitivity of single cell calcium and eCB dynamics during locomotor and seizure activity. Related to Figure 1 and Figure 2.

(A) Heatmaps showing single cell calcium (top, magenta outline) and eCB (bottom, green outline) traces for locomotor and seizure activity from a representative mouse (vehicle treatment). Cells are sorted based on calcium activity during the seizure using the rastermap algorithm (github.com/MouseLand/rastermap). For both locomotor activity and seizure activity, similar activity is reported by both sensors demonstrating correlation at the single cell level. Note that the color scale is increased for seizure traces showing both excessive and synchronous activity during seizures with both sensors.

(B) Mean run onset- and seizure onset-triggered averages (n=6 mice per drug) across drug treatments. Calcium is plotted on top with eCB on the bottom for locomotion (left) vs. seizures (right). Note the reduced vs. increased endocannabinoid signal with 2-AG synthesis and hydrolysis inhibition,

respectively, by DO and JZL. Data are mean +/- sem and smoothed with a narrow gaussian filter for visualization purposes (sigma = 2 frames).
(C) Real time (top) vs. time-shifted (bottom) correlation values for calcium vs. eCB activity during locomotion, averaged across cells in each session (n=6 mice per drug). In real time, DO and JZL reduced the correlation between cellular calcium and eCB (within-subject ANOVA $F_{4,5}$=16.46, Dunnett's post-test **p<0.01). When the traces were adjusted to optimize for the time-shift, only DO reduced this correlation (within-subject ANOVA $F_{4,5}$=14.81, Dunnett's post-test **p<0.01).
(D) Analysis from B performed on seizure traces in real time and shifted time. JZL reduced the correlation between cellular calcium and eCB (within-subject ANOVA $F_{4,5}$=25.63 and $F_{4,5}$=9.58, respectively; Dunnett's post-test ***p<0.01).

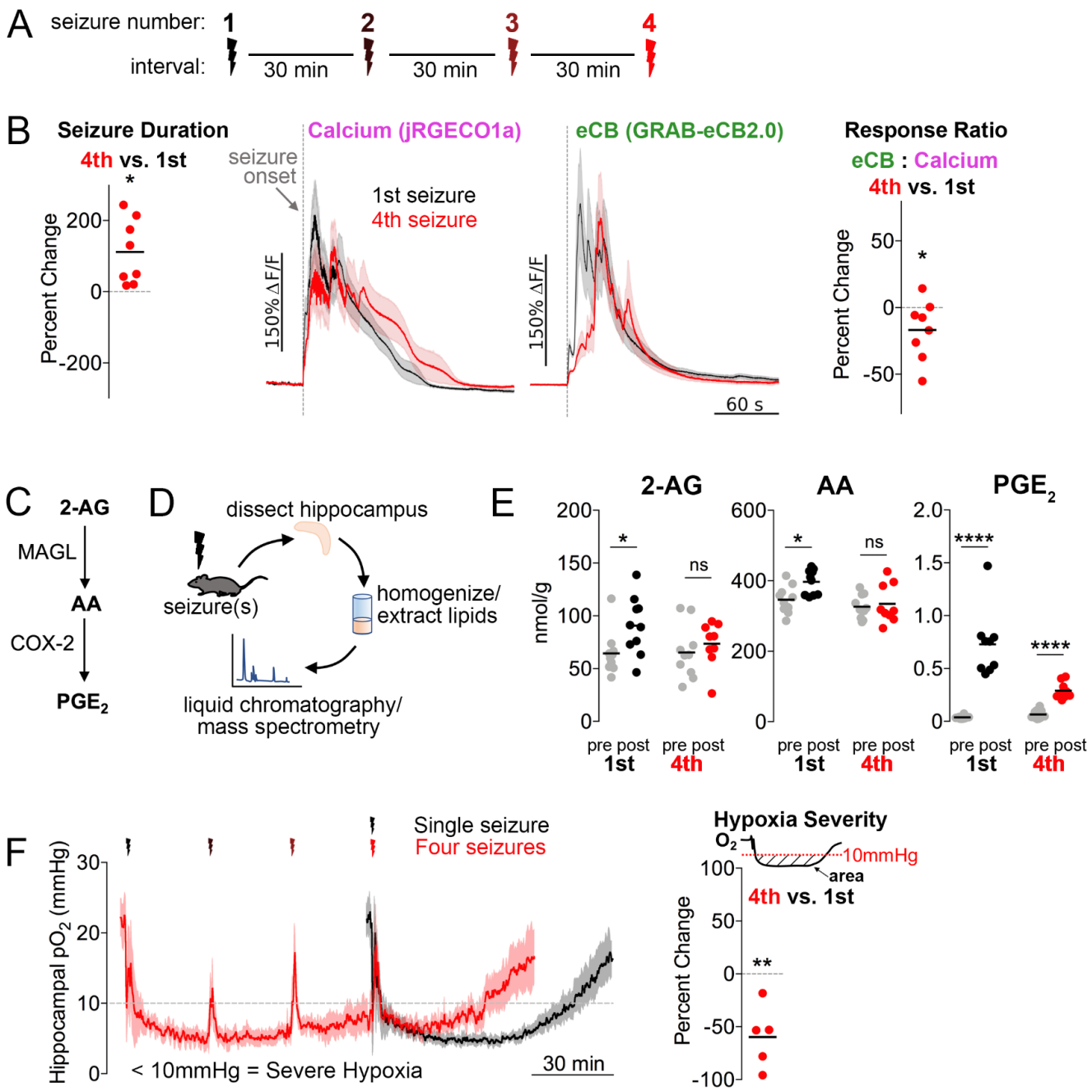

Figure S2. Repeated seizures diminish the endocannabinoid-prostaglandin pathway, worsen seizures, and attenuate postictal hypoxia. Related to Figure 3.

(A) Four seizure protocol.

(B) the fourth seizure was longer than the first seizure (left, t_7=3.50). Calcium (middle-left) and eCB (middle-right) responses to the first and fourth seizures. The eCB to calcium ratio (area under curve) decreased upon the fourth seizure (right, t_7=1.23).

(C) the proposed molecular pathway leading to seizure-induced prostaglandin production.

(D) LC/MS on tissue collected immediately after single or four seizures was used to quantify lipid levels.

(E) 2-AG (left) increased after a first (t_{18}=2.44), but not a fourth seizure (t_{17}=0.78, p=0.45). AA (center) increased after a first (t_{18}=3.07), but not a fourth seizure (t_{17}=0.39, p=0.35). PGE_2 (right) increased after a single seizure (t_{18}=6.77) and a fourth seizure (t_{17}=7.98), but to lesser extent.

(F) hippocampal oxygen was recorded following single or four consecutive seizures. The fourth seizure was associated with less severe hypoxia (area below 10mmHg, t_4=4.98).

*p<0.05, ** p<0.01, ***p<0.001, ****p<0.0001.

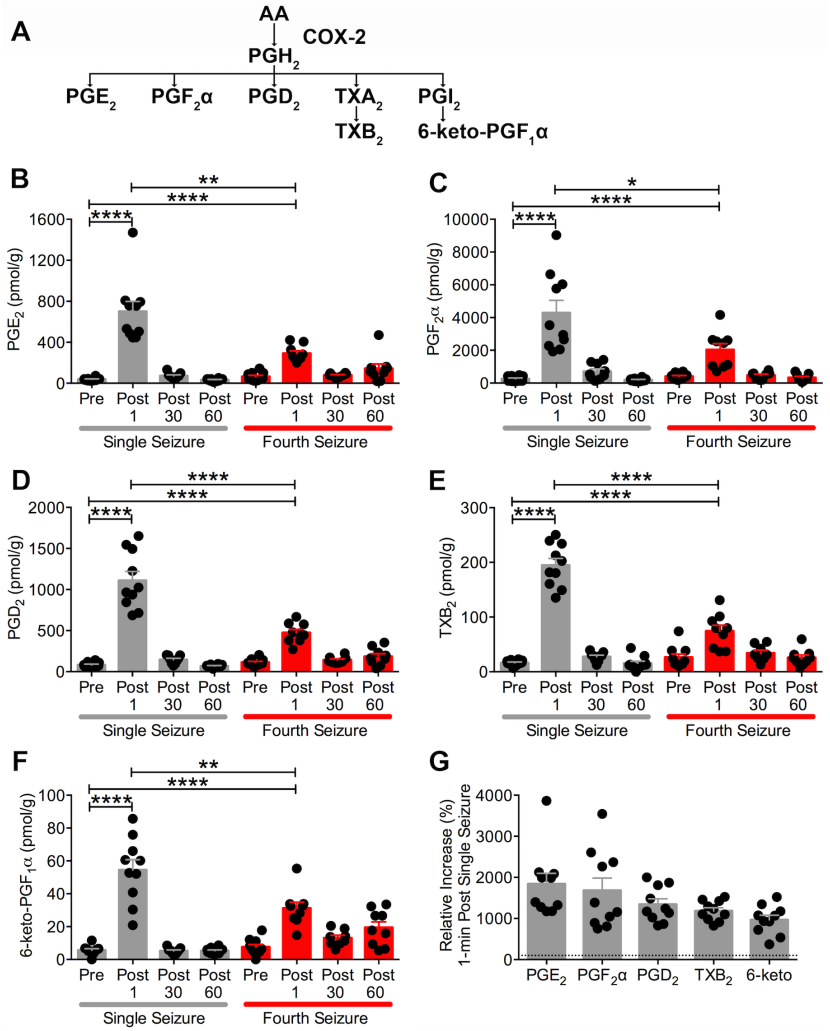

Figure S3. Prostanoid changes relative to single or four consecutive seizures. Related to Figure 3.

(A) Schematic of COX-2 metabolites from arachidonic acid. PGE_2 $PGF_2\alpha$, PGD_2 and the stable metabolites of TXA_2 and PGI_2, TXB_2 and 6-keto-$PGF_1\alpha$, respectively, were measured with mass spectrometry.

(B) PGE_2 levels with respect to single (grey) or fourth (red) seizures. PGE_2 was significantly increased following single (t_{18}=6.770) and repeated seizures (t_{17}=10.00). The immediate increase following a fourth seizure was significantly less than immediately following a single seizure (t_{17}=3.857).

(C) $PGF_2\alpha$ levels were significantly increased following single (t_{18}=5.246) and repeated seizures (t_{17}=4.908). The immediate increase following a fourth seizure was significantly less than immediately following a single seizure (t_{17}=2.546).

(D) PGD_2 levels were significantly increased following single (t_{18}=9.222) and repeated seizures (t_{17}=9.754). The immediate increase following a fourth seizure was significantly less than immediately following a single seizure (t_{17}=5.151).

(E) TXB_2 (TXA_2 metabolite) levels were significantly increased following single (t_{18}=14.09) and repeated seizures (t_{17}=5.693). The immediate increase following a fourth seizure was significantly less than immediately following a single seizure (t_{17}=7.231).

(F) 6-keto-$PGF_1\alpha$ (PGI_2 metabolite) levels were significantly increased following single (t_{18}=7.710) and repeated seizures (t_{17}=7.109). The immediate increase following a fourth seizure was significantly less than immediately following a single seizure (t_{17}=3.121).

(G) Normalized percent change immediately following a single seizure relative to the pre-seizure baseline condition (100%, dotted line). Relative increase reveals that PGE_2 > $PGF_2\alpha$ > PGD_2 > TXB_2 > 6-keto-$PGF_1\alpha$.