Supplemental Material

Diarrhea caused by ErbB tyrosine kinase inhibitors involves activation of basolateral K^+ channels and apical CFTR Cl^- channels in intestinal epithelium

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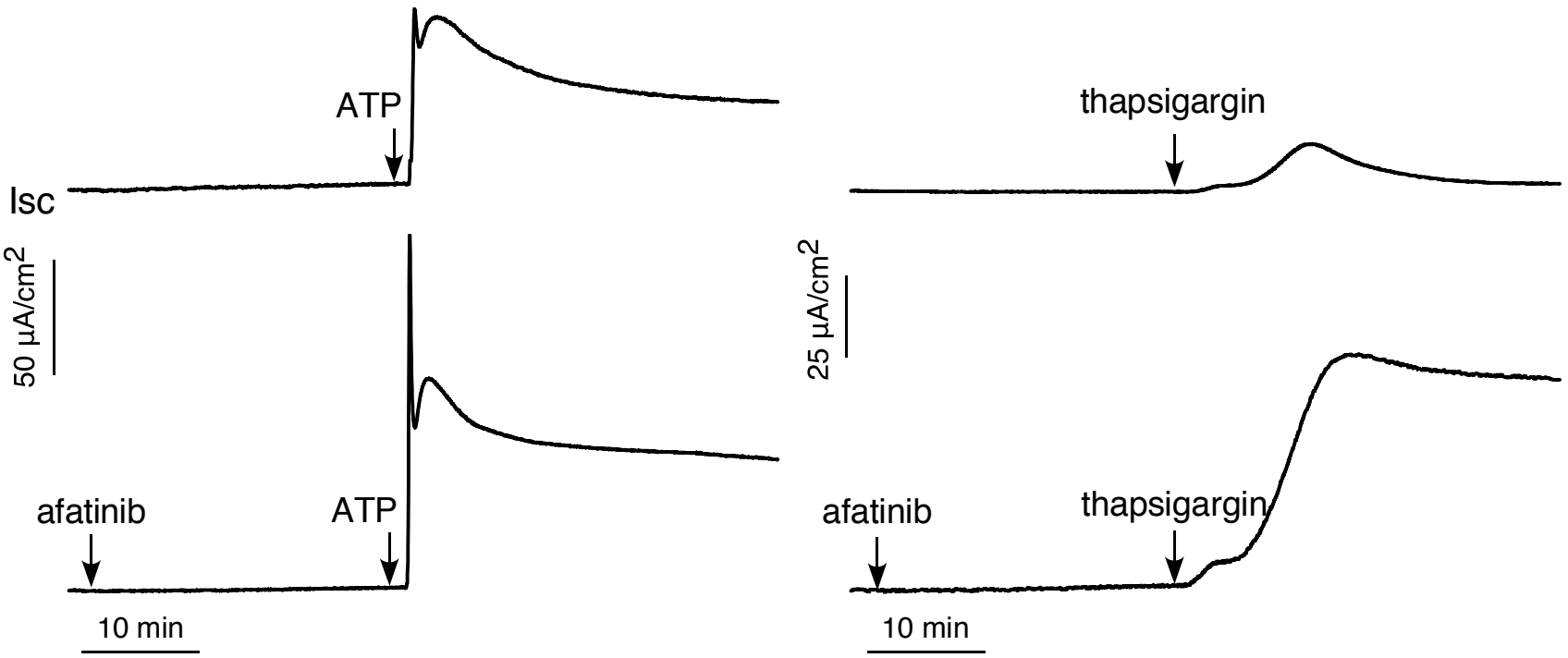
Supplementary Figure 1. Afatinib amplifies ATP- and thapsigargin-induced short-circuit current in T84 cells. Responses shown to 20 μM afatinib added 25 min prior to 100 μM ATP (left) or 2 μM thapsigargin (right). Representative of 3 sets of experiments.

Supplementary Figure 2. Inhibition of Ca^{2+}- and cAMP-activated K^+ channels in T84 cells by clotrimazole and senicapoc. A. (left) Short-circuit current in response to 2 μM thapsigargin (following 25 min pretreatment with 20 μM afatinib), followed by indicated concentrations of clotrimazole or senicapoc. (right) Inhibition concentration-dependence (mean ± S.E.M.). (B) (left) Short-circuit current in response to 10 μM forskolin followed by clotrimazole or senicapoc. (right) Inhibition concentration-dependence (mean ± S.E.M.).

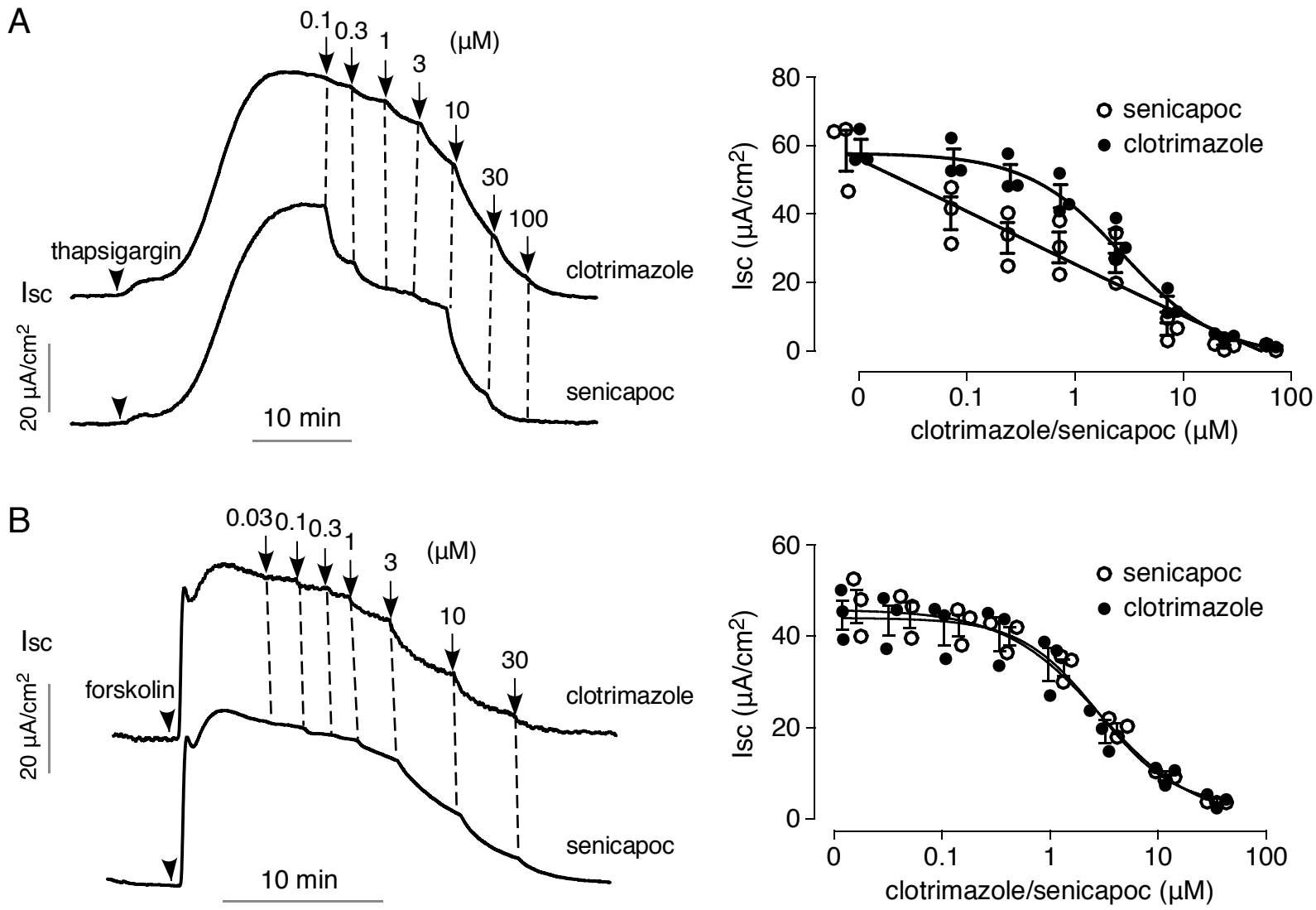
Supplementary Figure 3. Effect of ERK and PKC inhibitors on short-circuit current in T84 cells after treatments with EGF or BAPTA-AM. (A) Responses shown to 100 ng/ml EGF followed by 20 μM afatinib, 10 μM PKC inhibitor (Ro 31-8220), 10 μM ERK inhibitor (GDC-0994), alone and together, added 25 min prior to 100 μM carbachol. (B) Following 30 min incubation with 30 μM BAPTA-AM, short-circuit current showing following 20 μM afatinib, 10 μM PKC inhibitor, 10 μM ERK inhibitor, 10 μM BPO-27, followed by 100 μM carbachol, as indicated. Representative of 2 sets of experiments.

Supplementary Figure 4. Intestinal histology of afatinib-treated rats. Sprague-Dawley rats were treated orally with afatinib (60 mg/kg) for 6 days. H&E staining showing minor injury up to day 4 with villus blunting in ileum. By day 6 scattered epithelial disruption was seen in jejunum (black arrowheads) and villus atrophy in ileum.

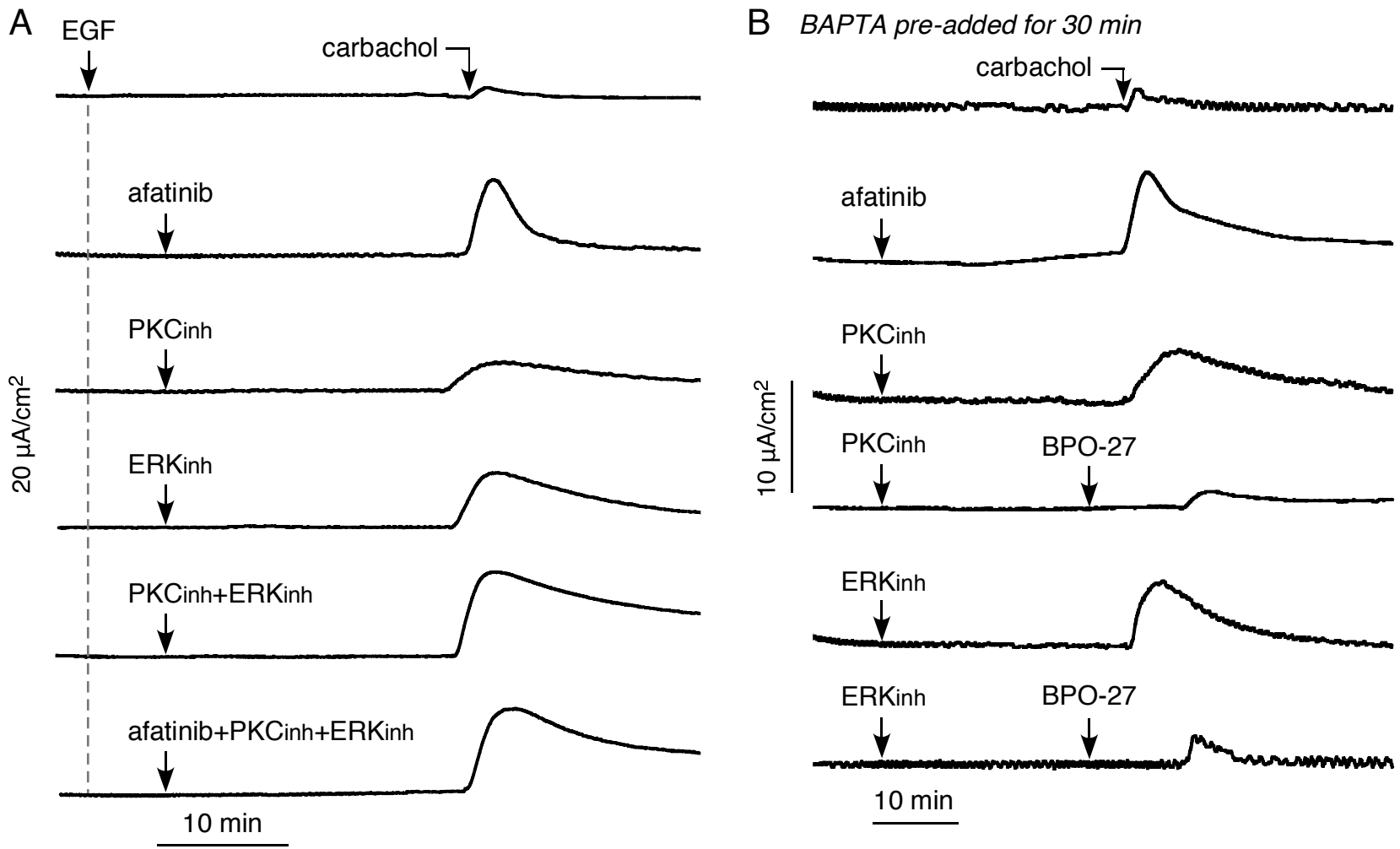
Supplementary Figure 5. Percentage change in rat body weight in afatinib diarrhea corresponding to data in Figure 9B-E (mean ± S.E.M., * $P < 0.05$).



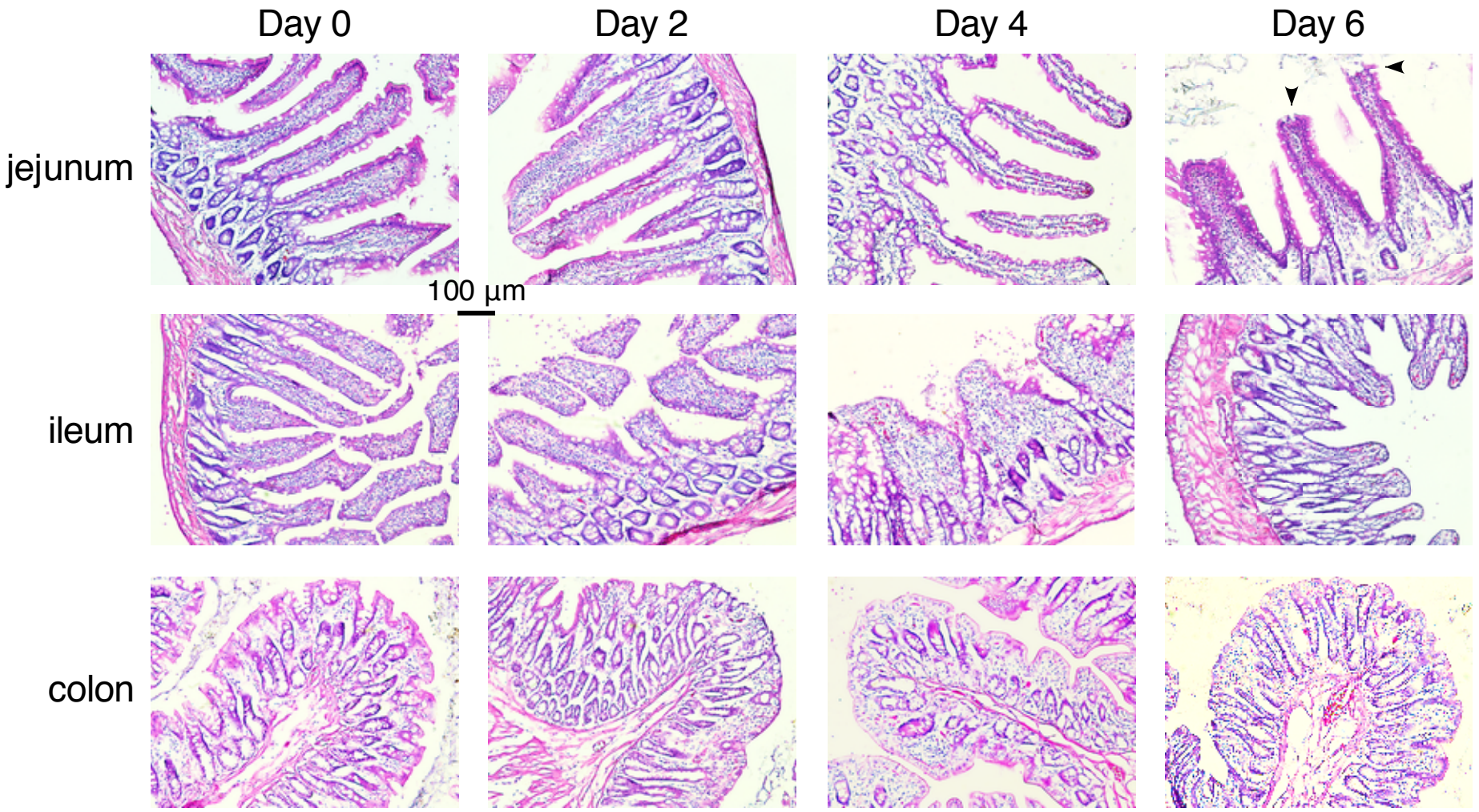
Supplementary Figure 1



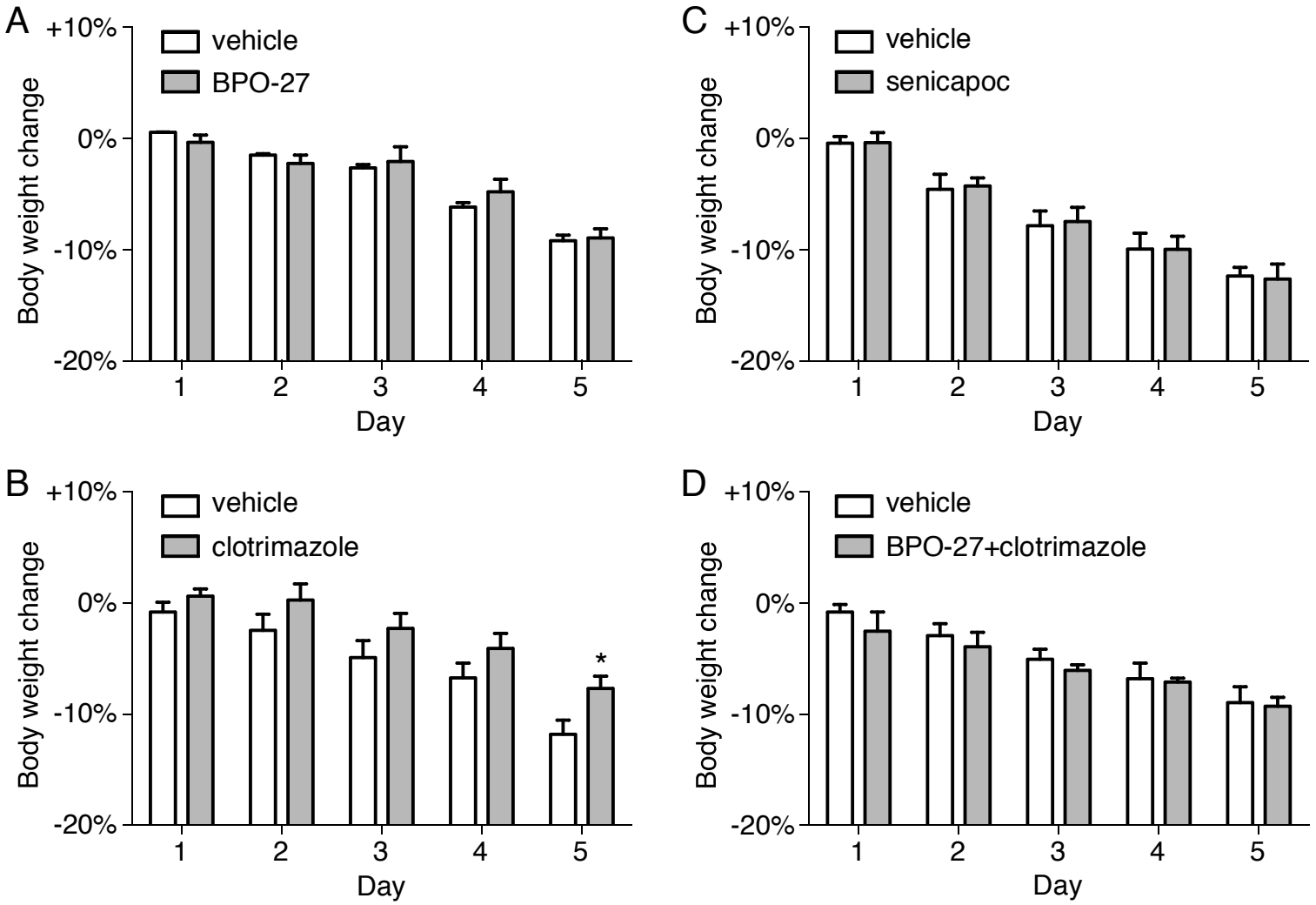
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5