Supplemental File Legends

Fig. S1. Regeneration was assayed over the course of seven days. To quantitatively assess regeneration, two measurements were taken: (A) the first measurement was taken from the cloacal opening to the amputation plane immediately following amputation and (B) the second measurement was taken from the cloacal opening to the tip of the tail following 7 days of regeneration. Measurement A was subtracted from measurement B as a measure of regenerative growth. For qualitative analysis there were four possible outcomes for the assay. (B) Embryos exhibited a morphologically normal, regenerated tail, indicating the compound had no effect on regeneration. (C) Some regeneration occurred but the resulting tail was morphologically abnormal or not completely regenerated. (D) No regenerative outgrowth occurred beyond the amputation plane. (E) Embryos showed systemic toxicity, identified by general atrophy, lethargy and/or tissue degeneration (amputation plane indicated by black dashed line).

Fig. S2. Control means and standard deviations are depicted by the solid black and dashed grey lines (respectively) on each plot. (A) All broad scale (Tier 1) blockers of chloride channels resulted in delayed and/or reduced regeneration. (B) CaCCs are indicated by both the tier 2 blocker, CaCCinh-A01, and the tier 3 drugs Benzbromarone (Tmem16A/B) and T16Ainh-A01 (Tmem16A). (C) Ligand gated chloride channel blockers resulted in complete inhibition of regeneration at higher concentrations. (D) Blockade of volume regulated anion channels was lethal or caused systemically toxic at all concentrations tested. (E & F) Neither ClC of CFTR blockade had any effect on regeneration (* indicates $p < 0.05$ compared to control measurements; error bars are standard deviations).

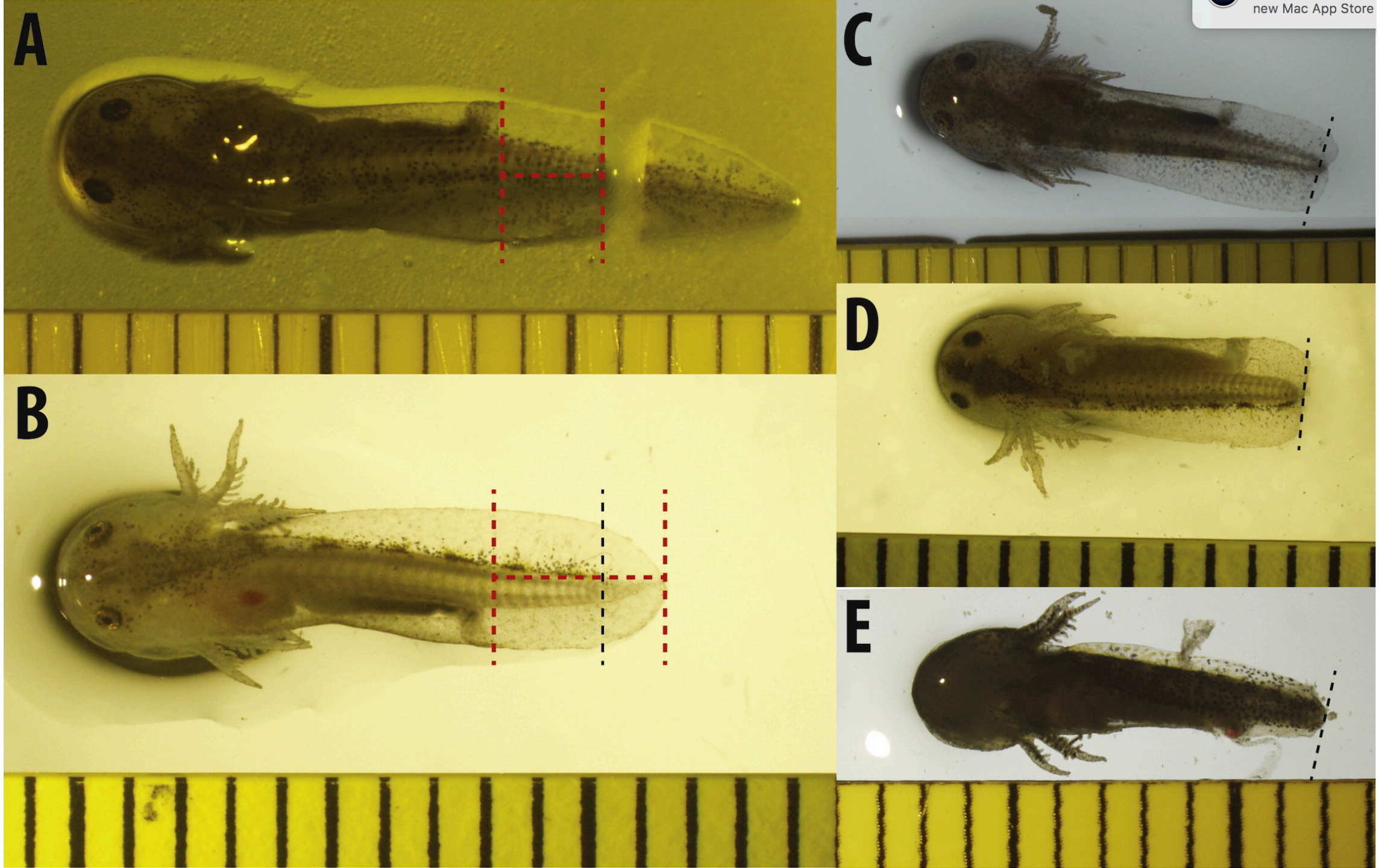
Fig. S3. Control means and standard deviations are depicted by the solid black and dashed grey lines (respectively) on each plot. (A) Broad scale potassium channel blockade using Tetraethylammonium (TEA) reduced regenerative outgrowth. (B) Tail regeneration was completely inhibited in a dose dependent manner by the KV Channel blocker 4-Aminopyridine (4-AP). (C & D) The more specific inhibitors of KV Channels were less effective at non-lethal concentrations with Cytochalasin B affecting regeneration the most (* indicates $p < 0.05$ compared to control measurements).

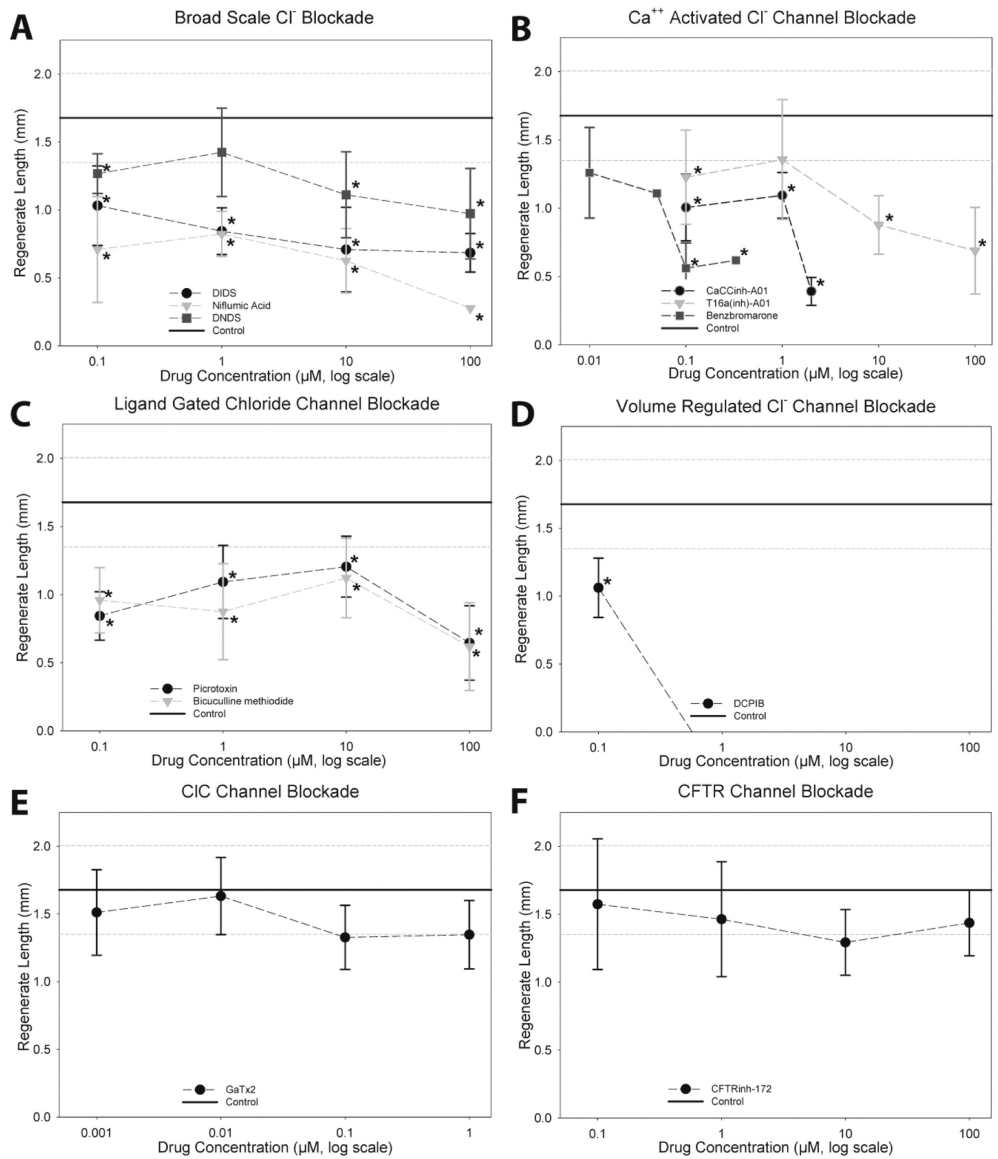
Fig. S4. Control means and standard deviations are depicted by the solid black and dashed grey lines on each plot. Broad scale sodium channel blockade was accomplished with either Amiloride, Lidocaine or Tetrodotoxin. None of these drugs affected regenerative growth, although tetrodotoxin was toxic at higher concentrations (* indicates $p < 0.05$ compared to control measurements).

Fig. S5. Control means and standard deviations are depicted by the solid black and dashed grey lines on each plot. Broad scale calcium channel blockade was accomplished with Bepridil HCl which was lethal or toxic at all concentrations tested. The more specific inhibitors of CaV Channels were more informative exhibiting reductions from either Amlodipine or Diltiazem (A). Complete inhibition was observed in animals treat with ω-conotoxin MVIIC but interestingly ω-agatoxin IVA had no effect. (* indicates $p < 0.05$ compared to control measurements; error bars represent standard deviation)

Fig. S6. Control means and standard deviations are depicted by the solid black and dashed grey lines on each plot. Ion pumps and transporters were examined only in tier 3 of the screen. H/K pump inhibitors resulted in reduced regeneration and Na/K pump inhibitors resulted in complete inhibition of regeneration. SERCA inhibitors inhibited regeneration but were systemically toxic to embryos (* indicates $p < 0.05$ compared to control measurements).

Fig. S7. To confirm NR staining of phagocytes, embryos were injected IP with either Encapsome® (B) for vehicle control or Clodrosome®(C) for macrophage depletion. Macrophage depletion (A&C, n = 17 each group) significantly reduced the number of NR + cells at the wound site following amputation compared to control embryos (A&B, n = 15) (* indicates $p < 0.05$ compared to control measurements; error bars are standard deviations; amputation plane indicated by black dashed line).


A
B
C
D
E


A
Broad Scale Cl- Blockade
Regenerate Length (mm)
Drug Concentration (μM, log scale)
DIDS
Niflumic Acid
DNDS
Control
B
Ca++ Activated Cl- Channel Blockade
CaCCinh-A01
T16a(inh)-A01
Benzbromarone
Control
C
Ligand Gated Chloride Channel Blockade
Picrotoxin
Bicuculline methiodide
Control
D
Volume Regulated Cl- Channel Blockade
DCPIB
Control
E
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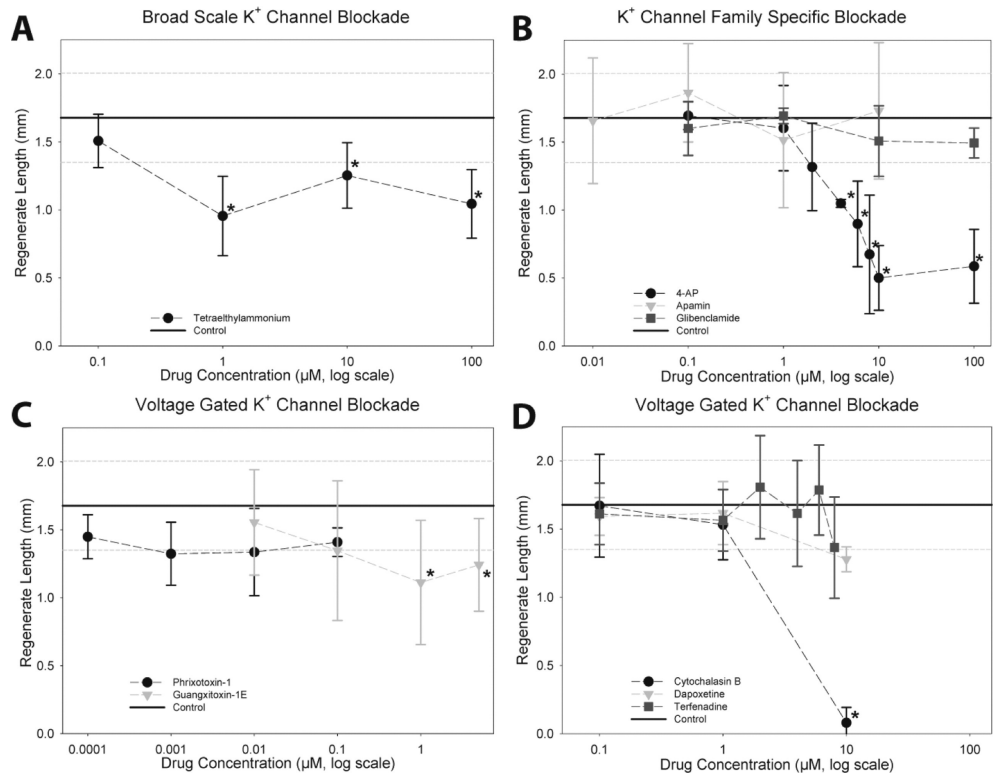
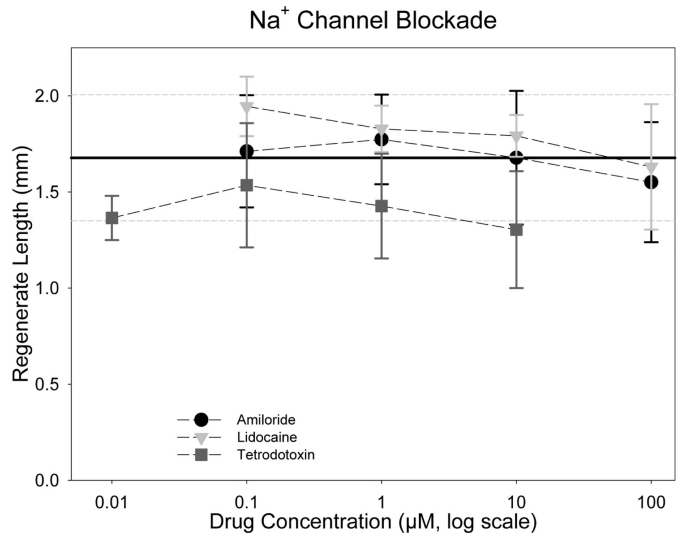
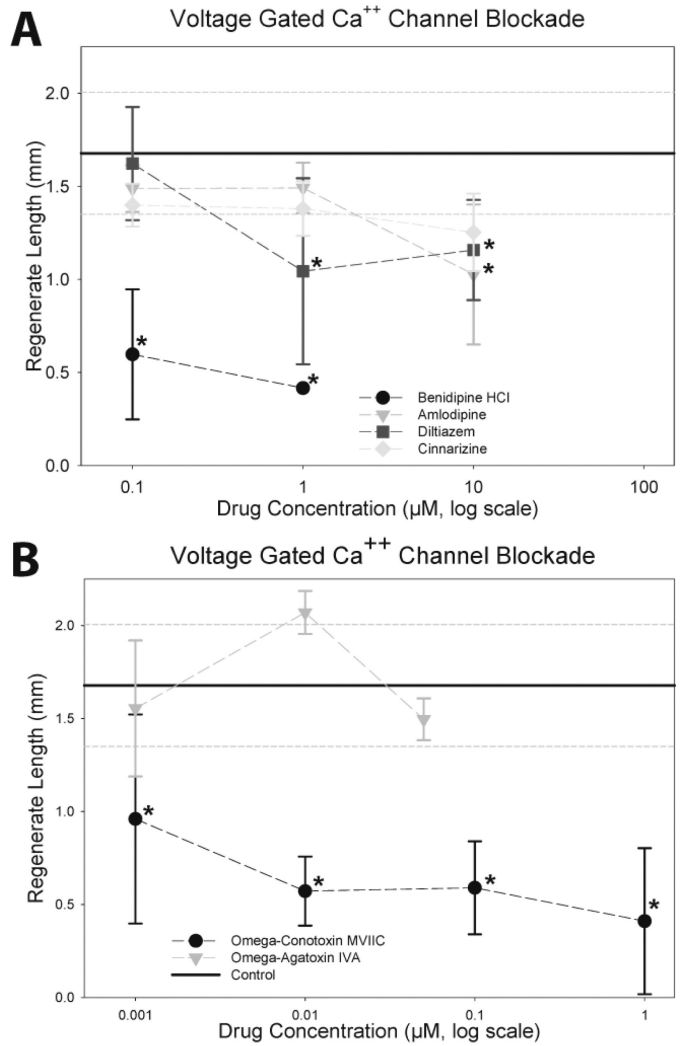
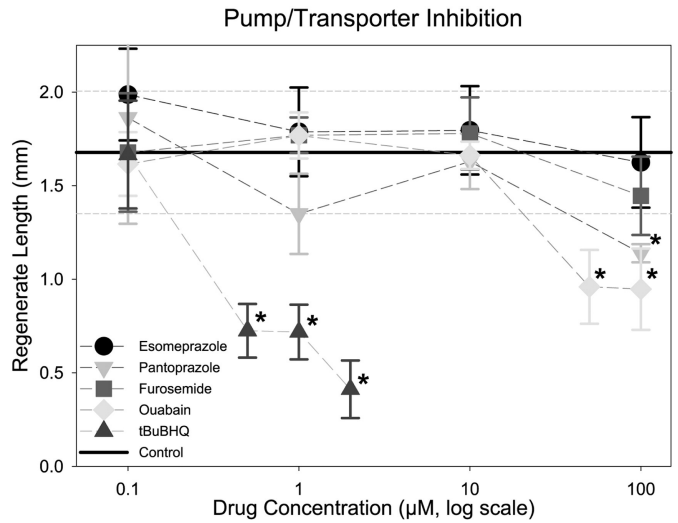
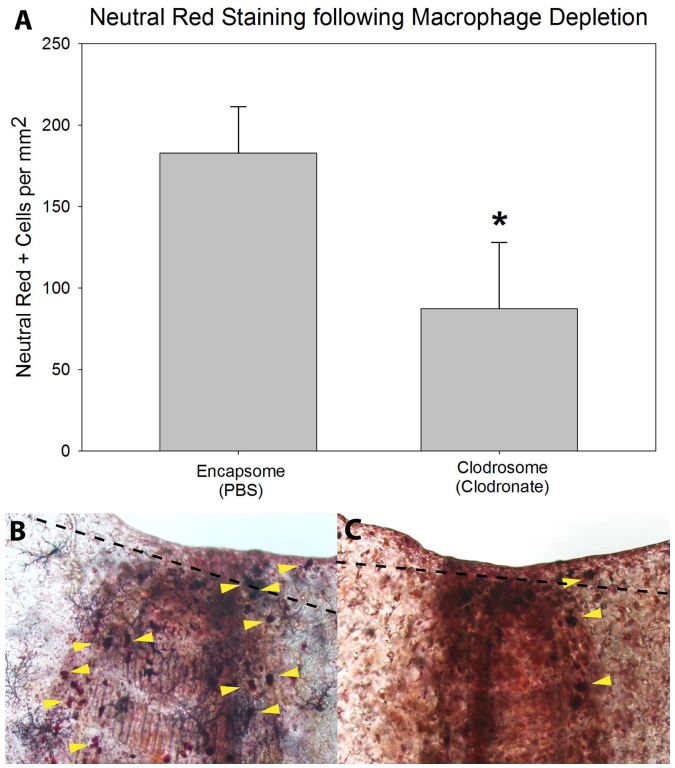
Control
F
CFTR Channel Blockade
CFTRinh-172
Control


A
Broad Scale K+ Channel Blockade
Regenerate Length (mm)
Drug Concentration (μM, log scale)
Tetraethylammonium
Control
B
K+ Channel Family Specific Blockade
Regenerate Length (mm)
Drug Concentration (μM, log scale)
4-AP
Apamin
Glibenclamide
Control
C
Voltage Gated K+ Channel Blockade
Regenerate Length (mm)
Drug Concentration (μM, log scale)
Phrixotoxin-1
Guangxitoxin-1E
Control
D
Voltage Gated K+ Channel Blockade
Regenerate Length (mm)
Drug Concentration (μM, log scale)
Cytochalasin B
Dapoxetine
Terfenadine
Control


Na+ Channel Blockade
Regenerate Length (mm)
2.0
1.5
1.0
0.5
0.0
0.01
0.1
1
10
100
Drug Concentration (µM, log scale)
Amiloride
Lidocaine
Tetrodotoxin


A
Voltage Gated Ca++ Channel Blockade
Regenerate Length (mm)
2.0
1.5
1.0
0.5
0.0
0.1
1
10
100
Drug Concentration (μM, log scale)
Benidipine HCl
Amlodipine
Diltiazem
Cinnarizine
B
Voltage Gated Ca++ Channel Blockade
Regenerate Length (mm)
2.0
1.5
1.0
0.5
0.0
0.001
0.01
0.1
1
Drug Concentration (μM, log scale)
Omega-Conotoxin MVIIC
Omega-Agatoxin IVA
Control


Pump/Transporter Inhibition
Regenerate Length (mm)
2.0
1.5
1.0
0.5
0.0
0.1
1
10
100
Drug Concentration (μM, log scale)
Esomeprazole
Pantoprazole
Furosemide
Ouabain
tBuBHQ
Control
*
*
*
*
*
*


A
Neutral Red Staining following Macrophage Depletion
Neutral Red + Cells per mm2
250
200
150
100
50
0
*
Encapsome
(PBS)
Clodrosome
(Clodronate)
B
C