Supplementary Figure Legends:

Supplementary Figure 1

Measurement of CSA13 in mouse feces. LC/MS/MS-MRM traces from a sample of mouse fecal extract of a drug-treated animal after treatment with acetic anhydride to convert CSA13 and the internal standard CSA44 to the corresponding triacetylated derivatives.

Supplementary Figure 2

Oral CSA13-Eudragit treatment did not affect fecal microbiome of uninfected mice.

(A) Alpha diversity (richness as measured by Chao1) is shown for fecal samples from uninfected mice (n=4 mice per group) from day 0 to day 10 treated with oral CSA13-Eudragit (10mg/kg) for 10 days. (B) Principal coordinates plot of weighted UniFrac for all mice. The difference of microbial composition was statistically insignificant. (C) Genus-level taxonomic summary of mean abundances for each group. Family is shown for microbes that could only be assigned at the family level.

Supplementary Figure 3

CSA13 mildly ameliorated toxin A-mediated enteritis in mice.

(A) Upper Panel: H&E staining of ileal tissues. Lower Panel: Histology score of ileal tissues. (B) Ileal tissue TNFα mRNA expression (fold). Each group consisted of 8 mice. (C) Macrophages were pretreated with CSA13 (3μM) for 4 hours, followed by exposure to toxin A (0.1μg/ml) for 4 hours. mRNA expression of 84 genes were determined by PCR arrays. The genes significantly changed by toxin A and CSA13 are shown. Results were pooled from three independent experiments. (D) Upper panel: A normal intestinal crypt organoid appears round with hollow center. Toxin A (0.1μg/ml) induced mouse intestinal crypt organoids rupture. CSA13 (3μM) inhibited organoid rupture. Middle panel: Normal fibroblasts have spindle shape. Toxin A-induced cell rounding into small circles. CSA13 prevented cell rounding. Lower panel: Actin cytoskeleton staining. Normal cells have widespread actin network. Toxin A (0.1μg/ml) induced actin disruption (green) to condense spots in

human primary colonic fibroblasts. Cell nuclei are stained in blue (DAPI). CSA13 (3μM) partially inhibited toxin A-mediated actin disruption. Results represent three independent experiments.

Supplementary Figure 4

CSA13 inhibits TNFα secretion, inflammasome activity, and NF-κB phosphorylation in macrophages.

(A) Macrophages were pretreated with CSA13 for 1 hour, followed by exposure to toxin A (0.1μg/ml) for 4 hours. TNFα levels in the conditioned media were measured by ELISA. (B) Macrophages were pretreated with CSA13 for 4 hours, followed by exposure to *C. difficile* conditioned media for 2 hours. Inflammasome activity was detected by a luminescence reader. Results were pooled from three independent experiments. (C) Left Panel: Macrophages were pretreated with CSA13 for 1 hour, followed by exposure to toxin A (0.1μg/ml) for 1 hour. Western blot analysis indicated that CSA13 inhibited toxin A-induced NF-κB phosphorylation that was reversed by NF-κB activator PMA. NF-κB inhibitor CAPE inhibited toxin A-induced NF-κB phosphorylation in macrophages. Right Panel: Quantification of the Western blot analysis. (D) Immunohistochemistry of phosphorylated NF-κB in the mouse colon. Brown spots indicate NF-κB phosphorylation. Black bars indicate 100 microns. (E) Serum starved human colonic epithelial cells were incubated with CSA13. Cell viability was measured by MTS assays. Results were pooled from three independent experiments. (F) Colonic TRPV1 mRNA expression on day 3 post-infection. Each group consisted of 8 mice.

Supplementary Figure 5

Intracolonic administration of CSA13 and CSA44 had similar protective effects in survival and body weight maintenance against *C. difficile* infection.

(A) Experimental plan of *C. difficile* infection. (B) Survival rate on day 3. (C) Body weight change on day 3. Each group consisted of 8 mice. (D) *C. difficile* spores (10^5 cfu) were incubated with CSA44 in BHIS-taurocholate media (1mL) for 24 hours. The viability of *C. difficile* was determined by absorbance reading at 600nm. (E) The same culture (100μL) was inoculated to BHIS-taurocholate-agar and incubated for 24 hours.

The presence of colonies is shown. Negative control showed no colony. Results were pooled from three independent experiments.

Supplementary Figure 6

CSA44 did not protect toxin A-mediated cell rounding and TNFα expression.

(A) Normal mouse 3T3 fibroblasts have spindle shape. Toxin A-induced cell rounding into small circles. CSA44 did not prevent cell rounding. Orange bars indicate 50 microns (B) Macrophages were pretreated with CSA13 and CSA44 for 1 hour, followed by exposure to toxin A (0.1μg/ml) for 4 hours. TNFα levels in the conditioned media were measured by ELISA. (C) Serum starved human colonic epithelial cells were incubated with CSA44. Cell viability was measured by MTS assays. Results were pooled from three independent experiments.

Supplementary Figure 7

Citrulline, retinol, and UDCA have anti-inflammatory effects against toxin A.

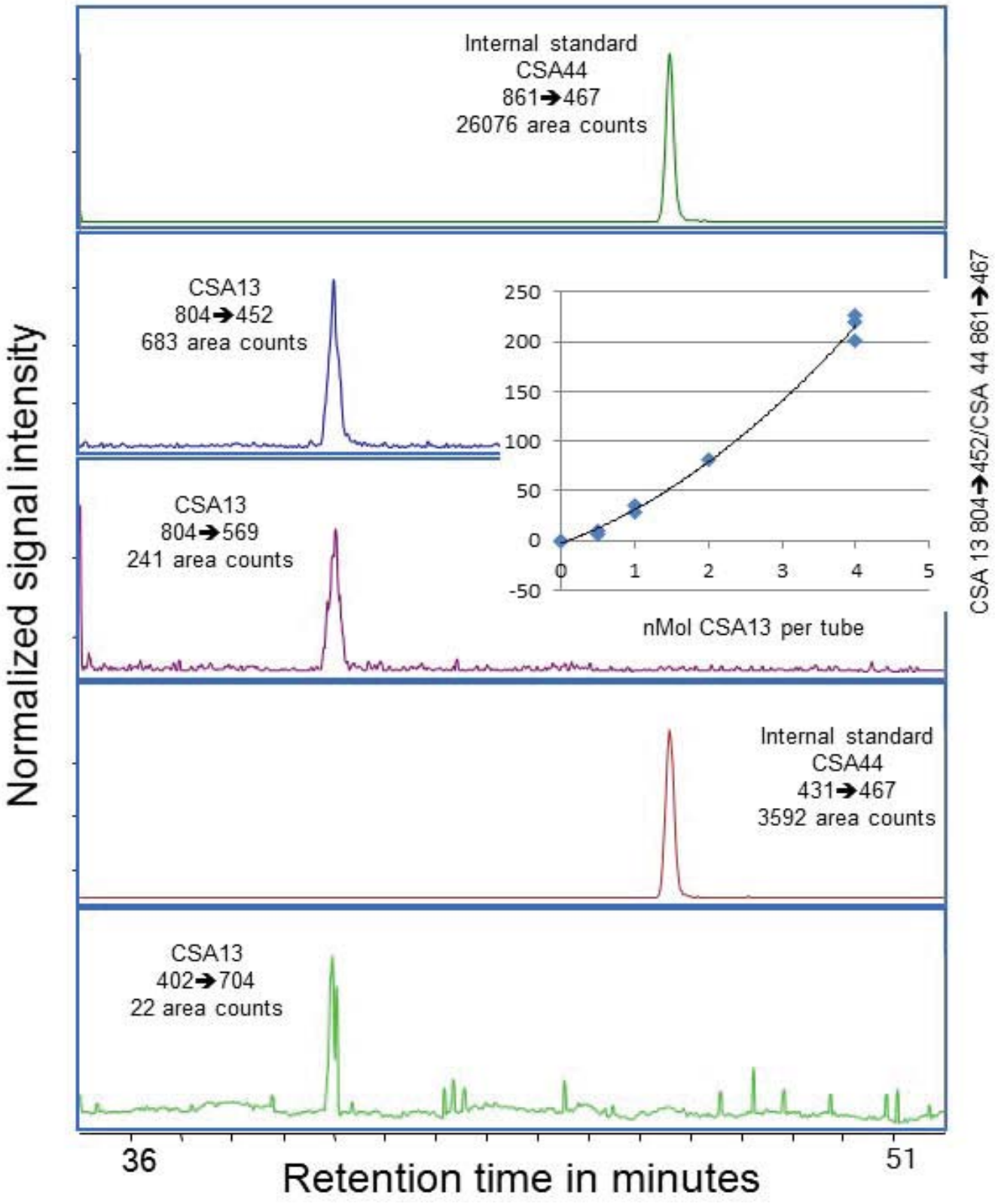
Experimental plan of oral metabolite administration in (A) primary CDI model and (B) vancomycin-dependent relapse model. (C) Mouse 3T3 fibroblasts were pretreated with metabolites for 30 minutes, followed by exposure to toxin A (0.1μg/ml) for 2 hours. Cell rounding was observed and scored. Results are expressed as average values of four independent experiments. (D) Cytotoxicity of toxin in the sterile fecal filtrates. Each group consisted of 8 mice. (E) Mouse Raw264.7 macrophages were pretreated with metabolites for 30 minutes, followed by exposure to toxin A (0.1μg/ml) for 4 hours. TNFα protein levels in the conditioned media were measured by ELISA. Results are expressed as average values of four independent experiments.

Supplementary Figure 8

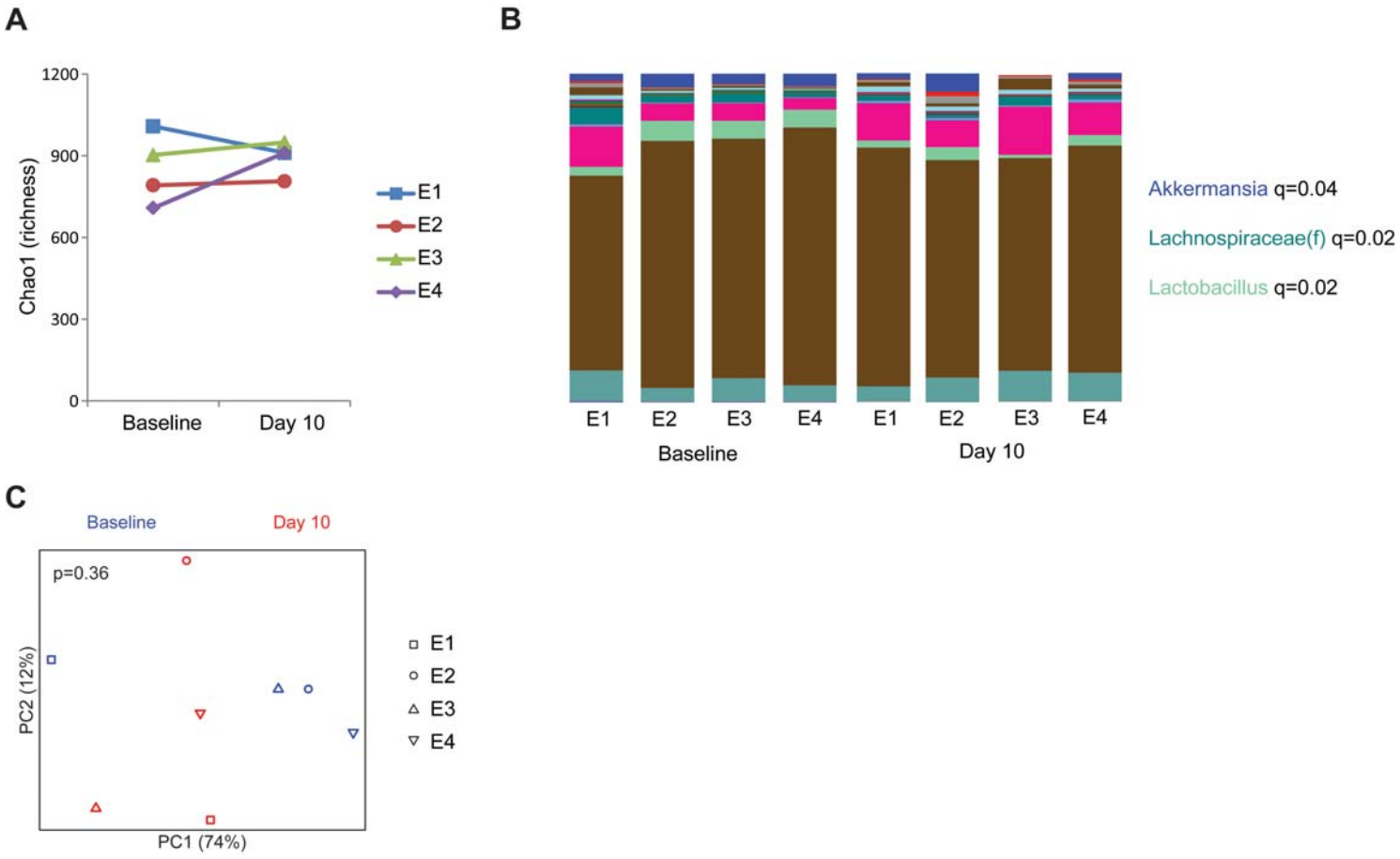
3-Aminoisobutyric acid promoted wound healing of colonic epithelial cells.

Human colonic epithelial NCM460 cells were grown to >95% confluence. The serum-starved cells were scratched by a P200 pipette tip to make a gap. 3-aminoisobutyric acid and toxin A were added to the culture and further incubated for 24 hours. The gap images were taken at 100X magnification. Grey bars of left and right sides were identical in length and served as references for respective treatment groups. 3-aminoisobutyric acid moderately promoted gap closure. The results were pooled from three independent experiments.

Supplementary Figure 1

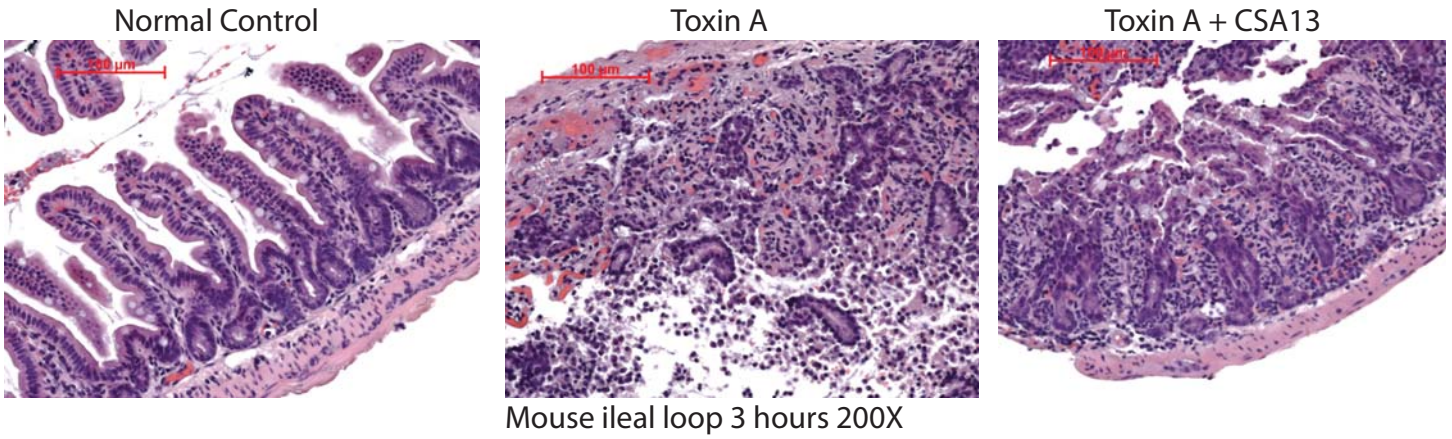


Supplementary Figure 2

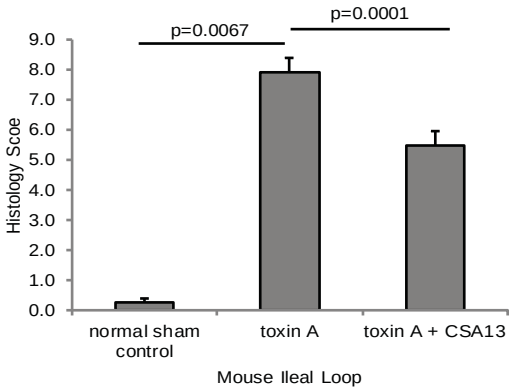


Supplementary Figure 3

A



Mouse ileal loop 3 hours 200X



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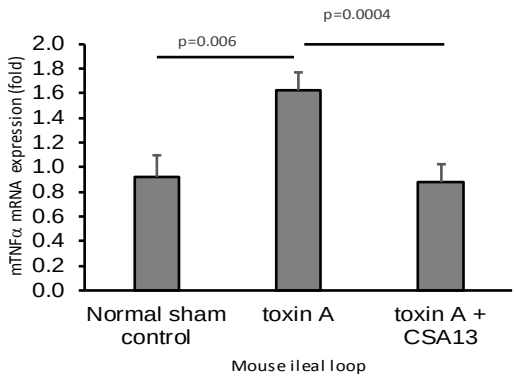
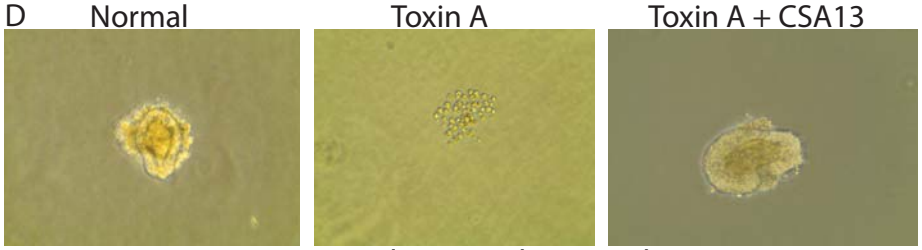


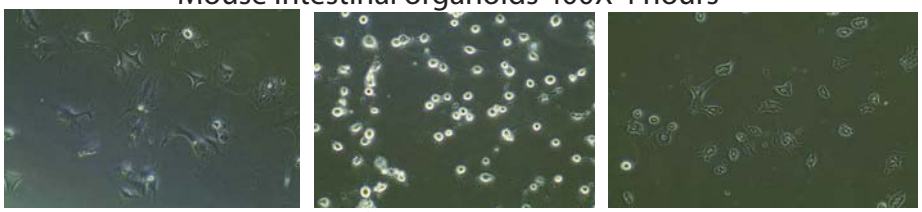
C

PCR array of mouse macrophages 4 hours						
* only statistically significant toxin A-modulated genes are shown						
	mean relative difference to		sem		Compared to control	Compared to toxin A
	toxin A	toxin A + CSA13	toxin A	toxin A + CSA13		
Ikbkb	12.04	0.99	0.14	0.06	p=0.0001	p=0.0001
Map2k3	8.34	4.23	0.60	0.44	p=0.0033	p=0.0052
Mpo	6.07	1.11	0.83	0.03	p=0.0036	p=0.0039
Pycard	2.58	0.94	0.17	0.09	p=0.0007	p=0.001
Rela	3.72	1.01	0.23	0.04	p=0.0003	p=0.0003
Ripk1	7.27	0.97	0.03	0.05	p=0.0001	p=0.0001
Ticam2	3.60	0.54	0.05	0.13	p=0.0001	p=0.0001
Tlr1	0.49	0.43	0.07	9.09	p=0.0019	n.s.
Tnf	1.53	1.65	0.40	0.03	ns	n.s.

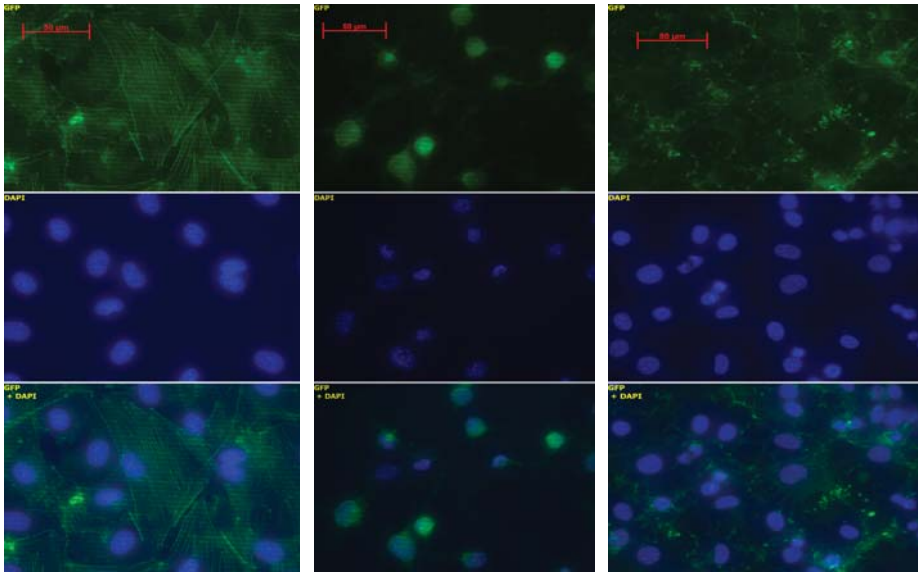
D



Mouse intestinal organoids 400X 4 hours

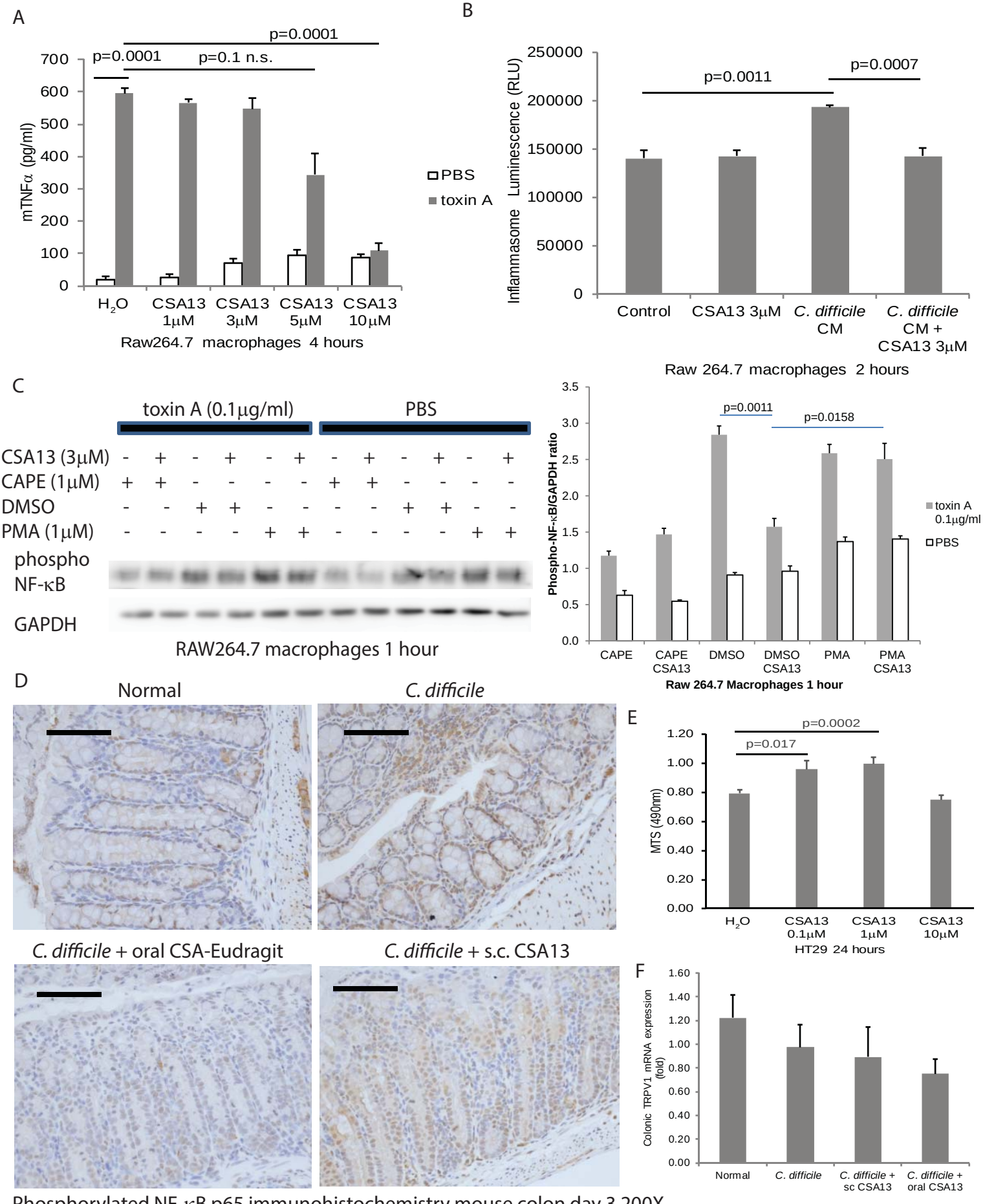


Mouse 3T3 fibroblasts 400X 2 hours



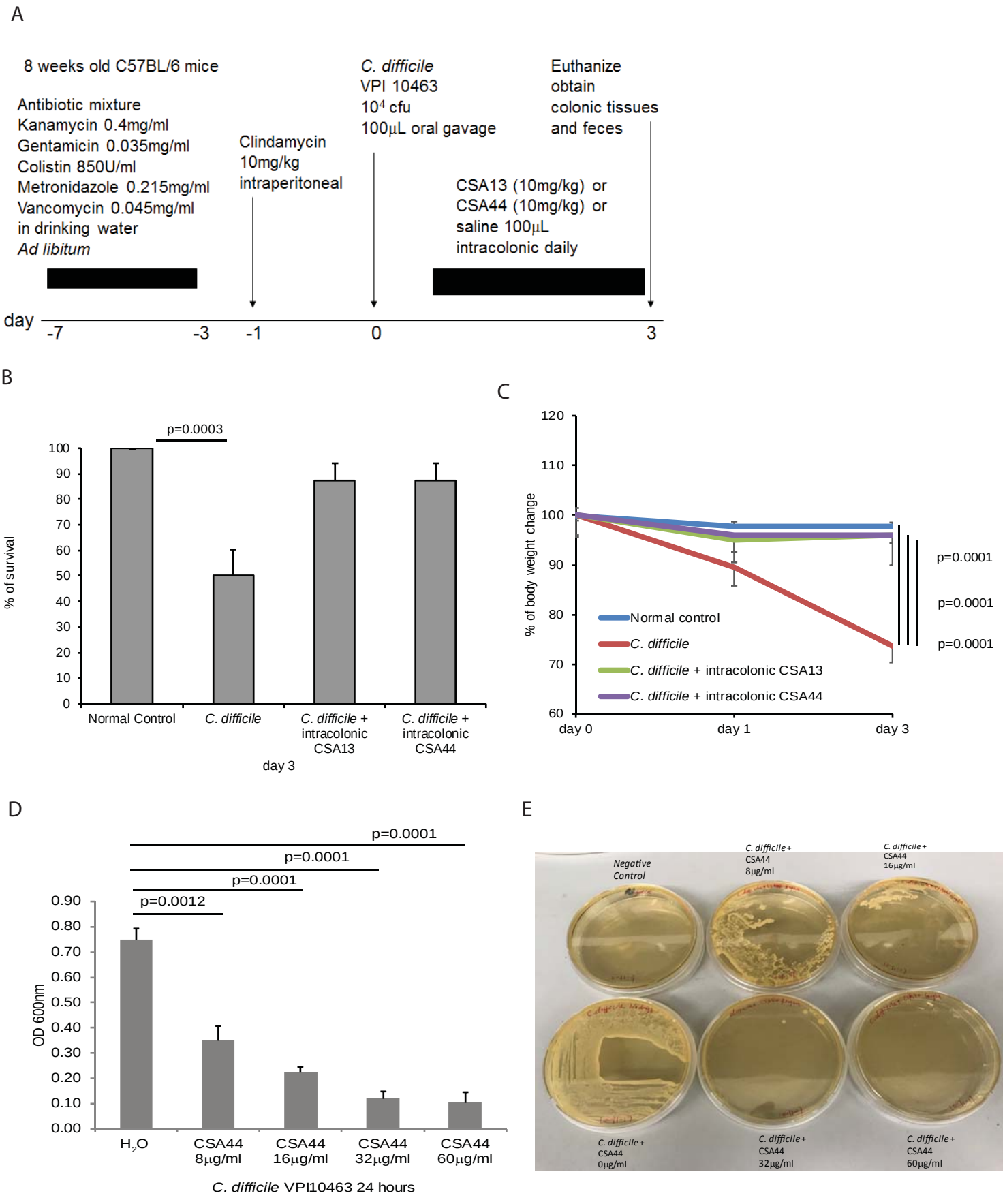
Actin Green staining human CCD18Co fibroblasts 400X 2 hours

Supplementary Figure 4

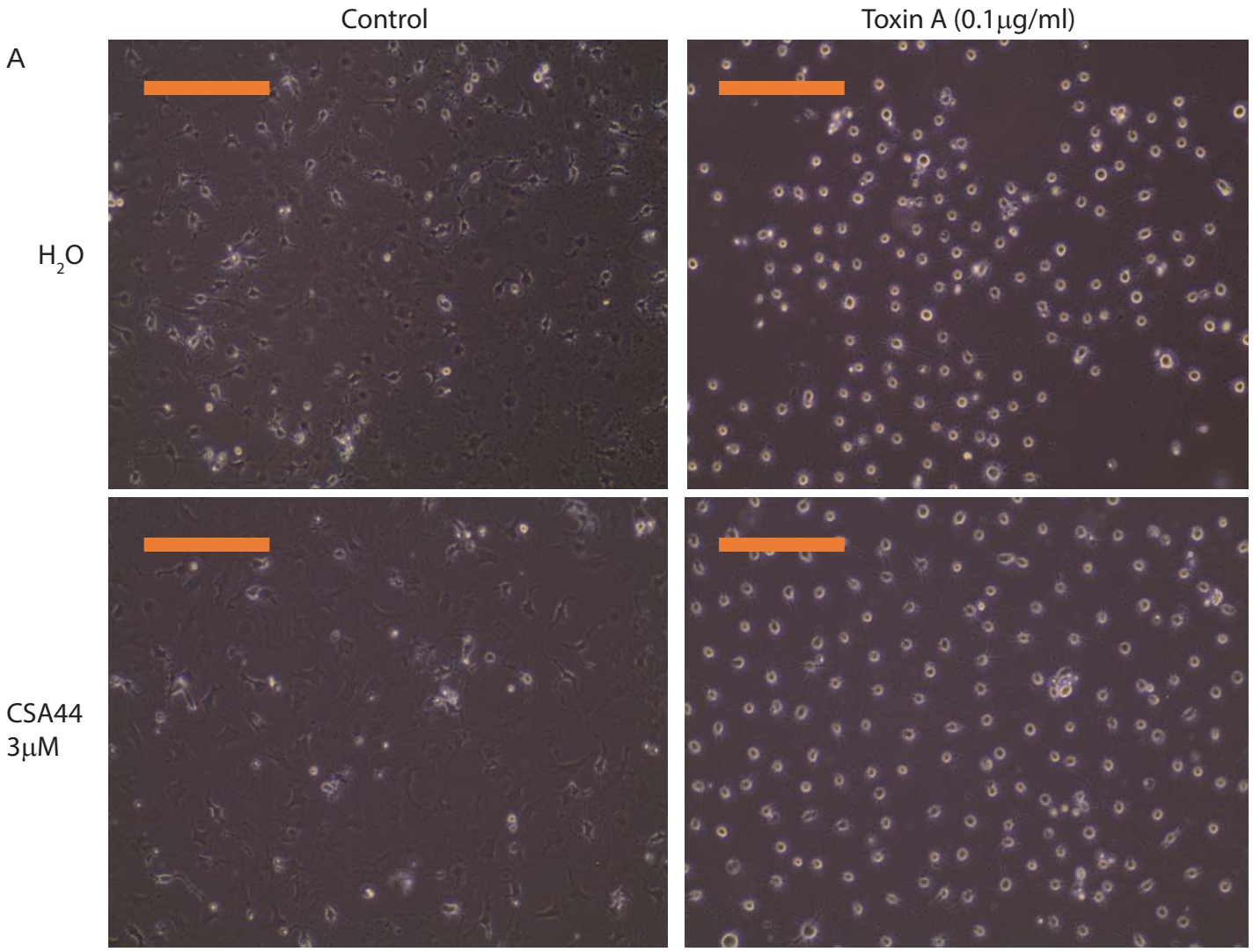


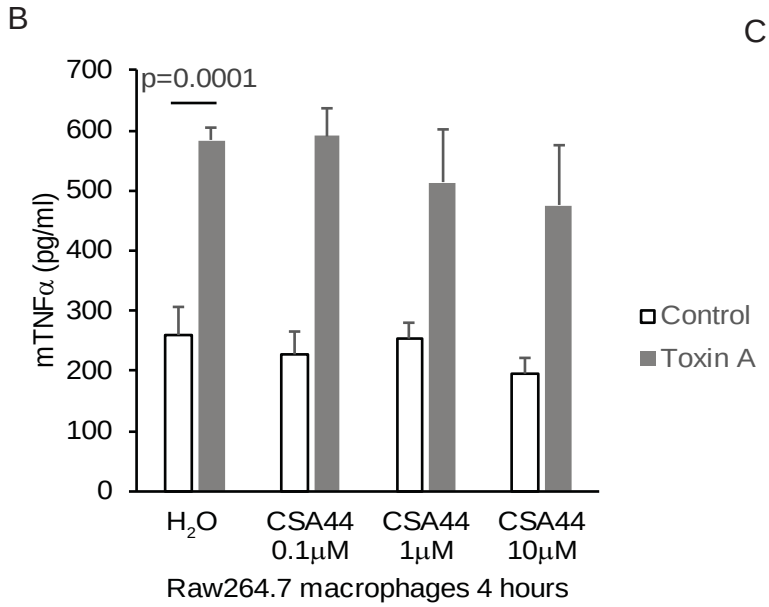
Phosphorylated NF-κB p65 immunohistochemistry mouse colon day 3 200X

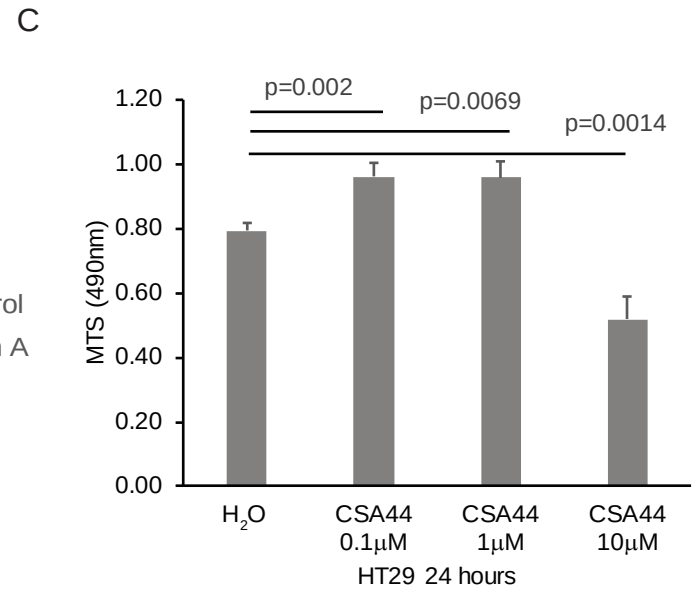
Supplementary Figure 5



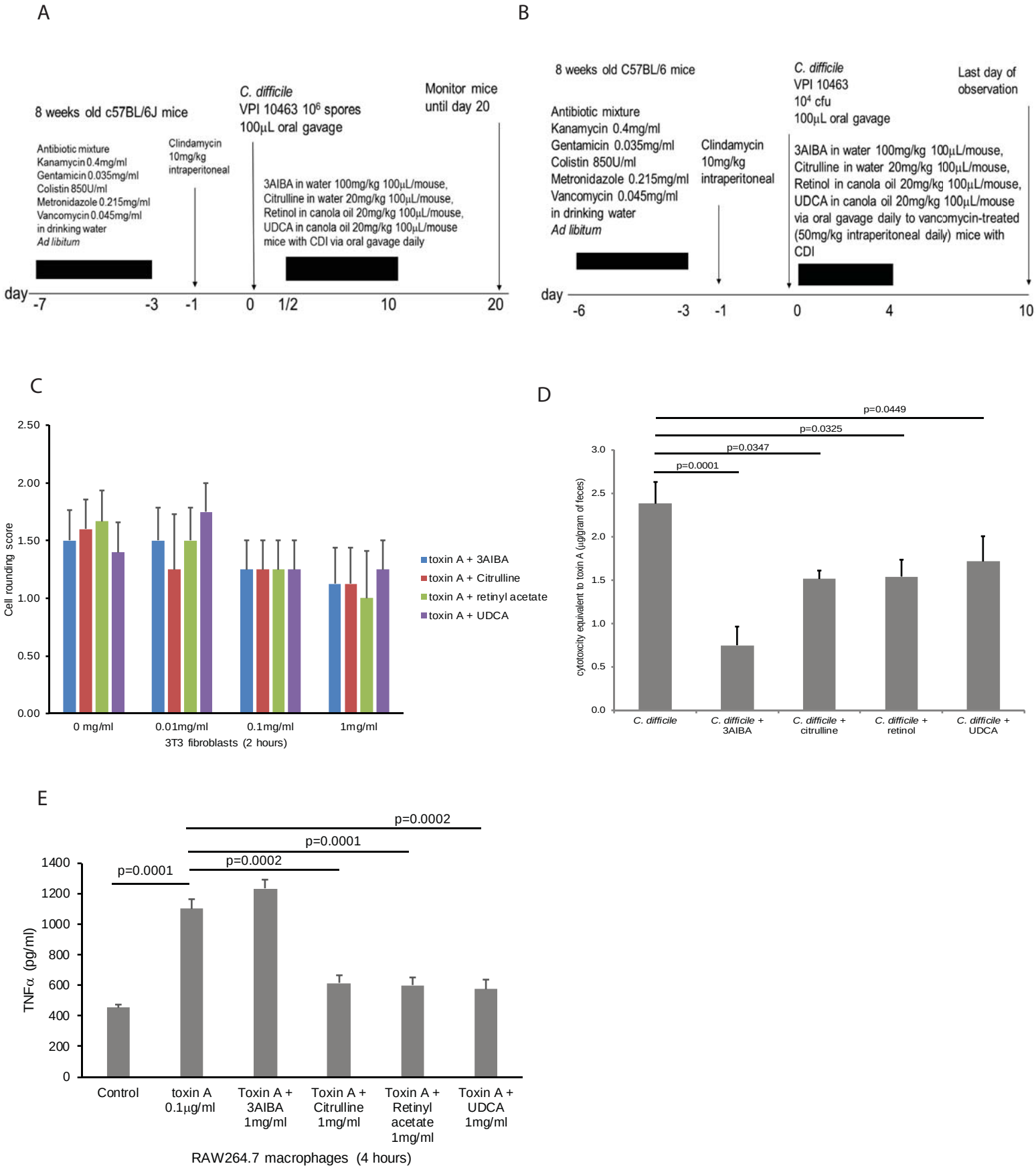
Supplementary Figure 6



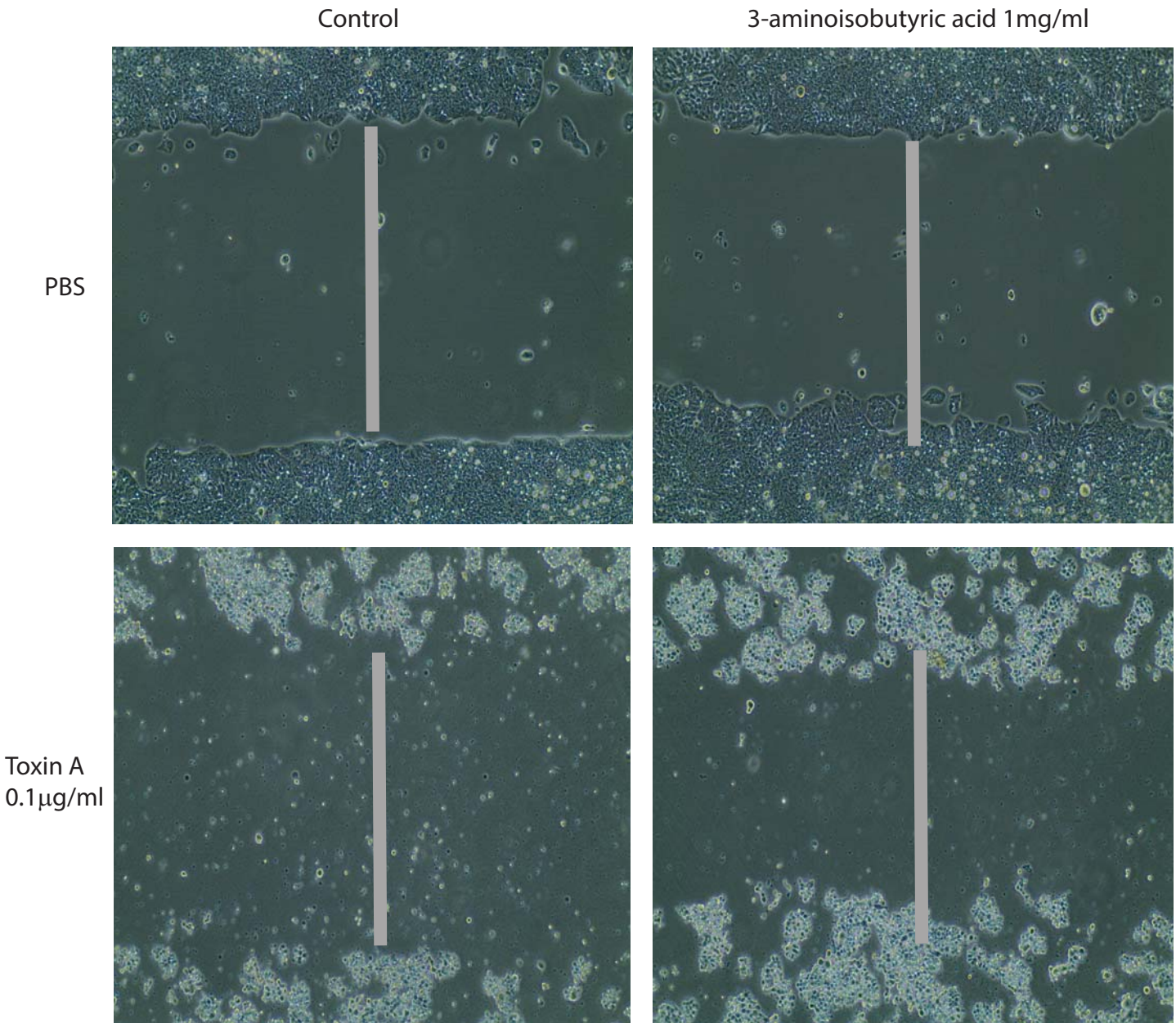




Supplementary Figure 7



Supplementary Figure 8



Wound healing assay NCM460 24 hours