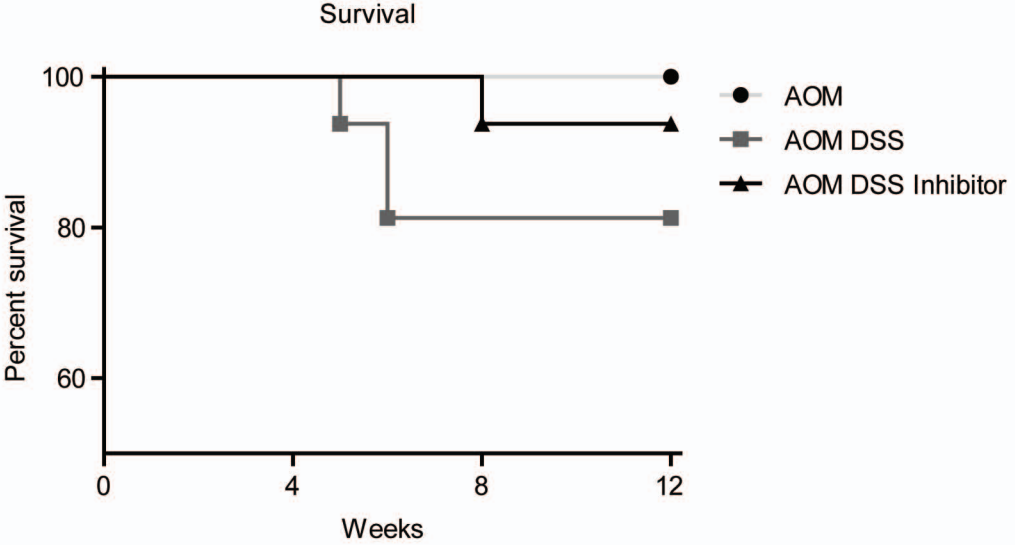
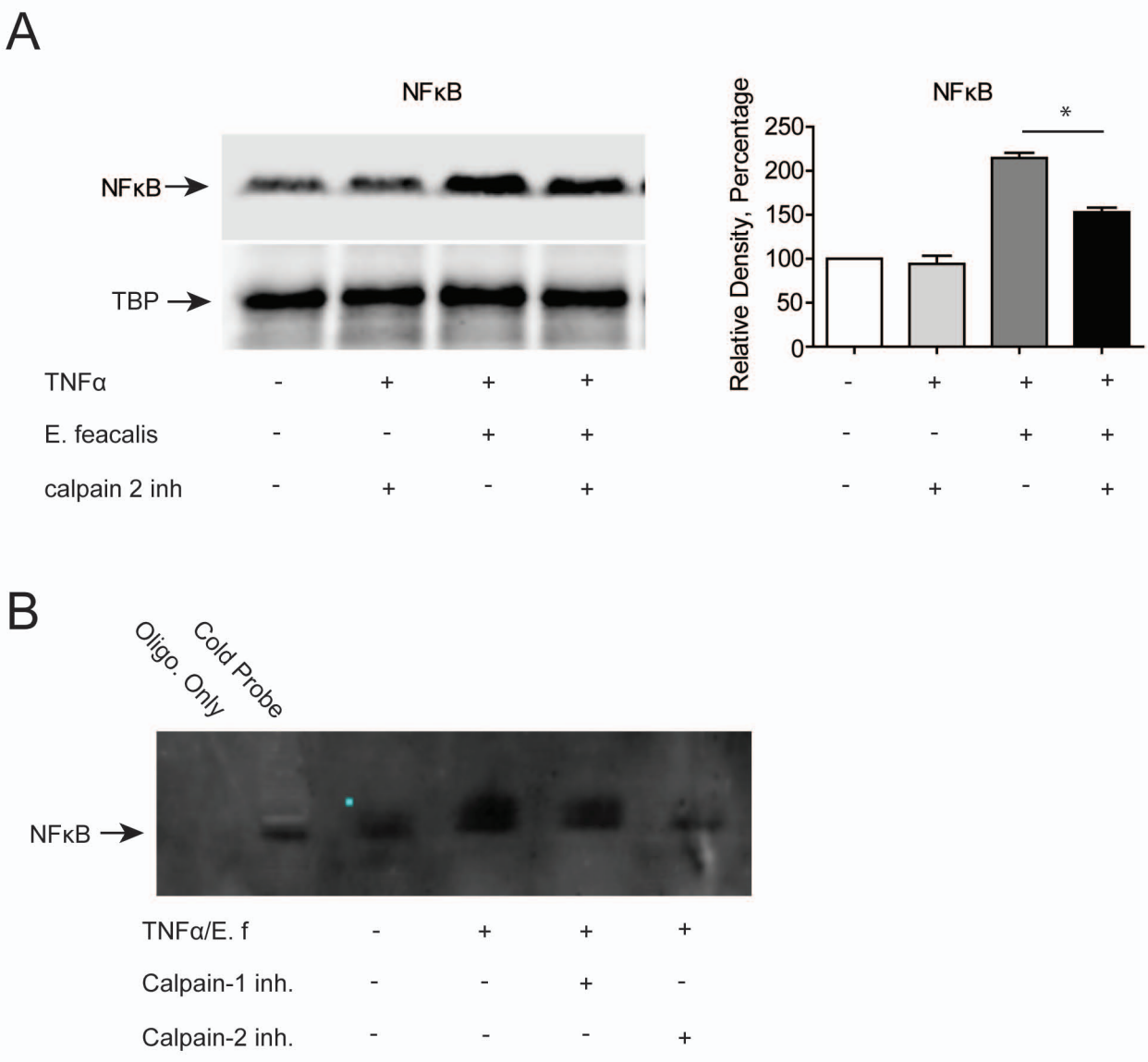
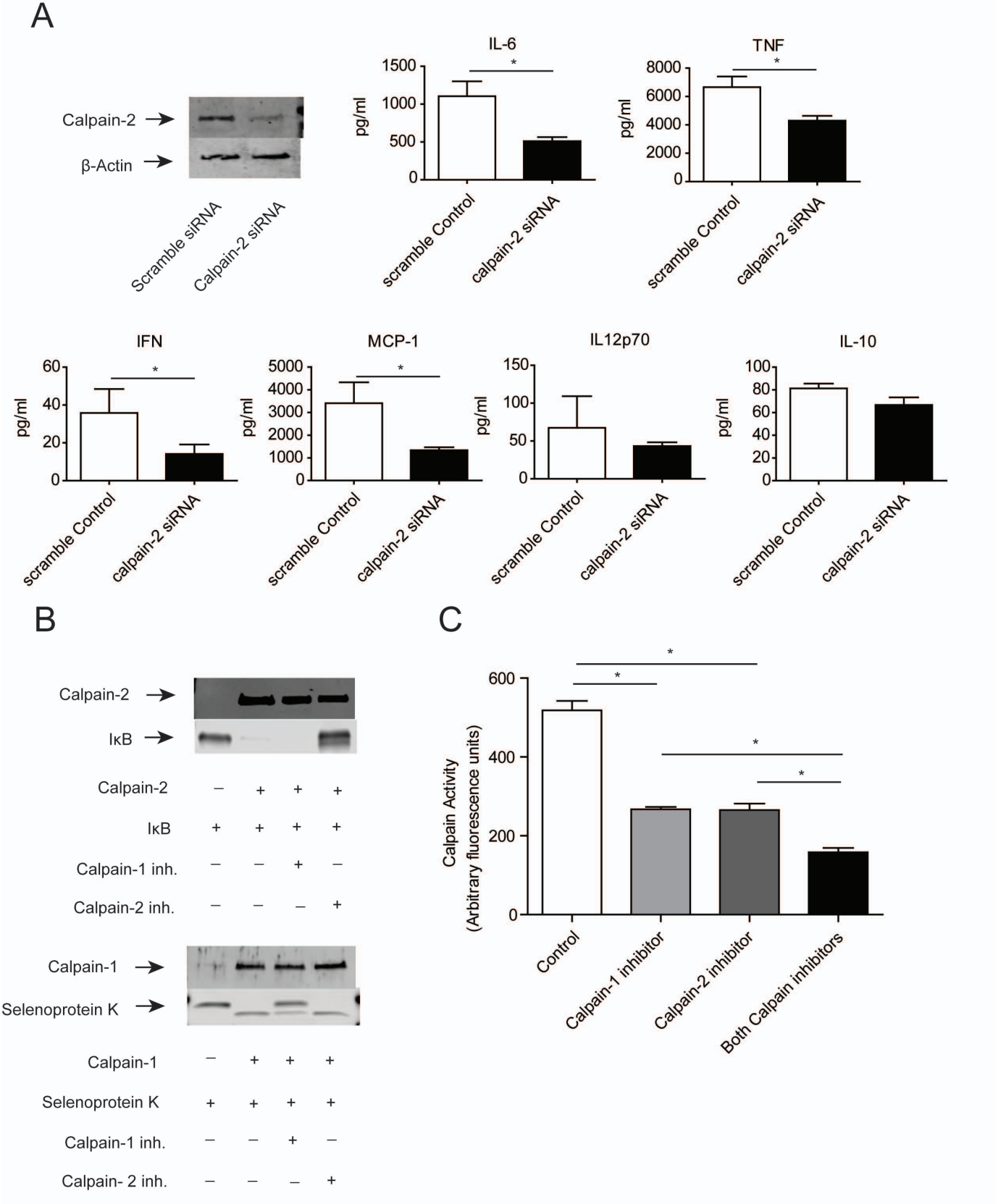


Supplemental Figure 1. A Kaplain-Meier survival curve was generated from an AOM and 2.5% DSS colitis model (n=20). There were no statistically significant differences in survival of the groups, Log-Rank test p=0.2831.



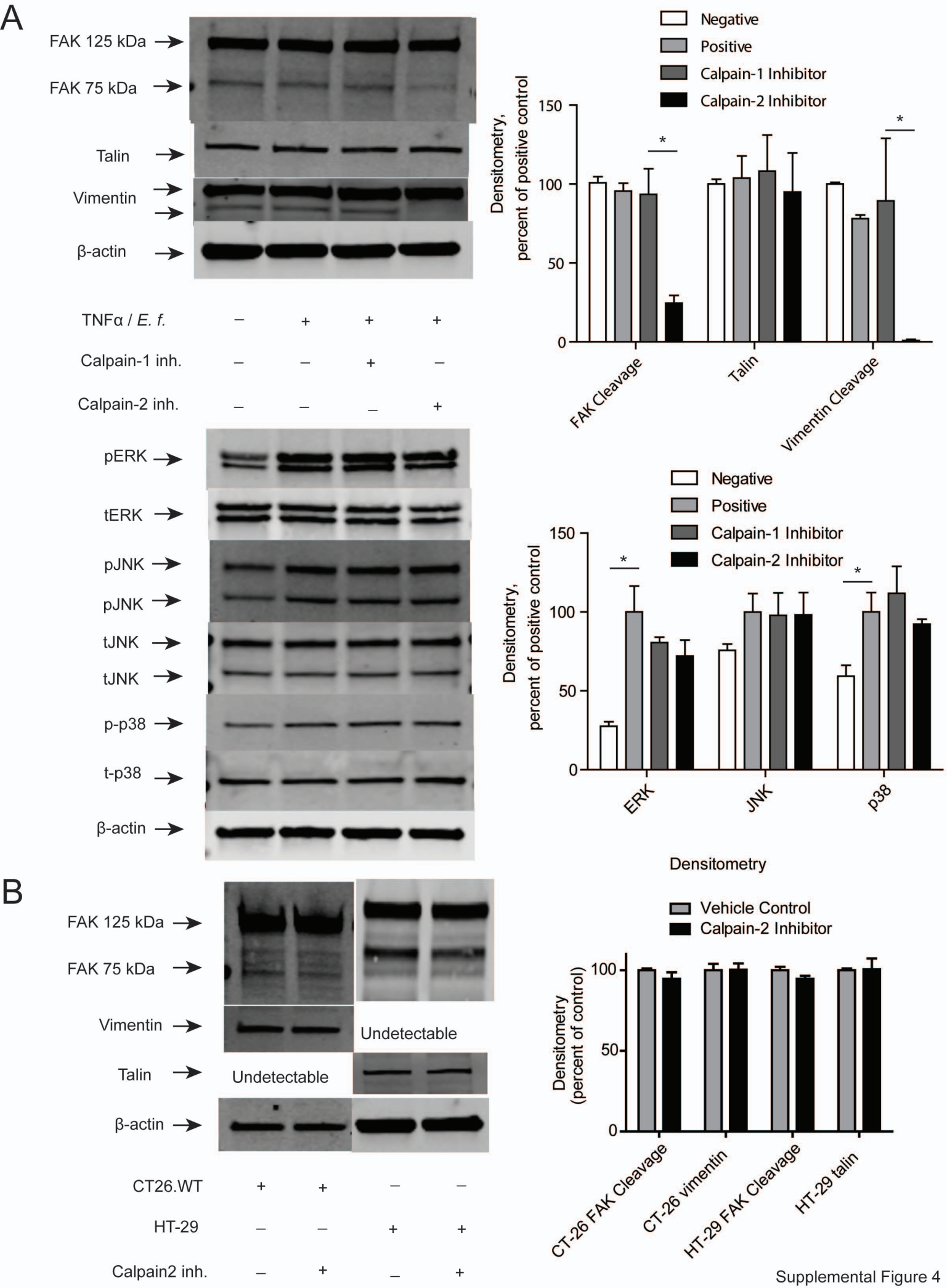
Supplemental Figure 2

Supplemental Figure 2. (A) Levels of NFκB in nuclear fractions were analyzed by Western blot, with β-actin and TATA binding protein serving as loading controls for lysates and nuclear fractions. Representative immunoblots are shown on the left with densitometric analyses shown on the right. (B) NFκB protein levels were detected by anti-NFκB membrane probe after in-gel EMSA assay to verify that the shifted bands of the EMSA could be positively identified as NFκB. Cold probe sample was positive control (TNFα nad treated with 50x unlabeled DNA probe. Data are mean ± SEM from three independent experiments, analyzed by one-way ANOVA followed by Tukey post-test, *p < 0.05.



Supplemental Figure 3

Supplemental Figure 3. Calpain-2 siRNA knock down has similar effects in production of inflammatory cytokines to the Calpain-2 inhibitor. (A) BMDM derived from WT mice treated with calpain-2 siRNA were primed 18 h with TNFα (20 ng/mL) and activated with heat-killed E. faecalis (1 μg/mL) for 1 h. Calpain-2 inhibitor (20 μg/mL) or DMSO as a control were added during both priming and stimulation. Western blots were performed to verify protein KO or siRNA knock down with βctin as loading control. Cytokines in media were measured using a cytometric bead array. (B) Calpain-1 and 2 inhibitors were analysed for specificity with the combination of recombinant calpain-1 (1μg) with Selenoprotein K (50 μg) and calpain-2 (1 μg) with IκB (50 μg) in buffer containing 50 mM Tris-HCl pH 7.5, 10 mM CaCl2, 30 mM NaCl, 5 mM DTT. (C) Calpain-1 and 2 inhibitors were applied (20ug/ml) to lysates from mouse colons after 3 days of DSS treatment. The resulting total calpain activity was measured.



Supplemental Figure 4

Supplemental Figure 4. Calpain-2 inhibition in BMDM and colon cancer cells show different effects on proteolytic activity. (A) Western blots were performed on BMDM treated with DMSO, calpain-1 inhibitor, or calpain-2 inhibitor. These blots were stained with antibodies for the known targets of calpain-2 specific cleavage; FAK, Vimentin, and Talin, with β-actin serving as loading control for lysates. The results show the calpain-2 inhibitor alone decreases degradation products of FAK and Vimentin. (B) CT26.WT and HT-29 colon cancer cells were serum starved in RPMI with 0.2% serum in the presence of calpain-2 inhibitor or DMSO for 1 h and western blots were performed. Representative immunoblots are shown to the left with densitometric analyses shown on the right. Data are mean ± SEM from three independent experiments, analyzed by one-way ANOVA followed by Tukey post-test or students t-