Supplemental Figure Legends

Supplemental Figure 1. p38 and MK2 are activated by toxin exposure. A) T84 cells were exposed to TcdA at the indicated concentrations for 2 hrs. Cell lysates were subjected to SDS-PAGE then immunoblotted for phospho-p38, phospho-Hsp27, or non-glucosylated Rac1. B) T84 cells were treated with 500 ng/ml TcdA for the indicated times then cell lysates and immunoblotting were performed as above.

Supplemental Figure 2. Toxin-induced MK2 activation is p38-dependent. T84 cells were pre-incubated either with 0.1% DMSO or with p38 inhibitors SB203580 (2.65 μM), p38 inhibitor III (0.5 μM), JNK kinase inhibitor SP600125 (25 μM) or MK2 inhibitor PHA-781089 (20 μM) for 30 mins then exposed to either no toxin or TcdA at 500 ng/ml for 2 hrs. Cell lysates were subjected to SDS-PAGE followed by immunoblotting for phospho-p38, phospho-Hsp27, or non-glucosylated Rac1.

Supplemental Figure 3. TcdA also induces p38 and MK2-dependent IL-8 release. HT-29 cells were pre-incubated with p38 inhibitor SB203580 (2.65 μM) or MK2 inhibitor PHA-781089 (20 μM) for 30 mins then exposed to TcdA (500 ng/ml) or TcdB (20 ng/ml) for 12 hrs. The supernatants were harvested and IL-8 concentration determined by ELISA.

Supplemental Figure 4. p38 and MK2 inhibition do not interfere with toxin transport or activity in Hela fibroblast cells. Hela cells were pre-incubated with either 0.1% DMSO, p38 inhibitor SB203580 (2.65 μM), MK2 inhibitor PHA-781089 (20 μM), bafilomycin (5 μM) or ammonium chloride (20 mM). TcdB was then added at a concentration of 20 ng/ml and the cells were incubated a further 2 hrs. Cell lysates were subjected to SDS-PAGE and immunoblotting for actin or non-glucosylated Rac1.

Figure 1- Supplemental

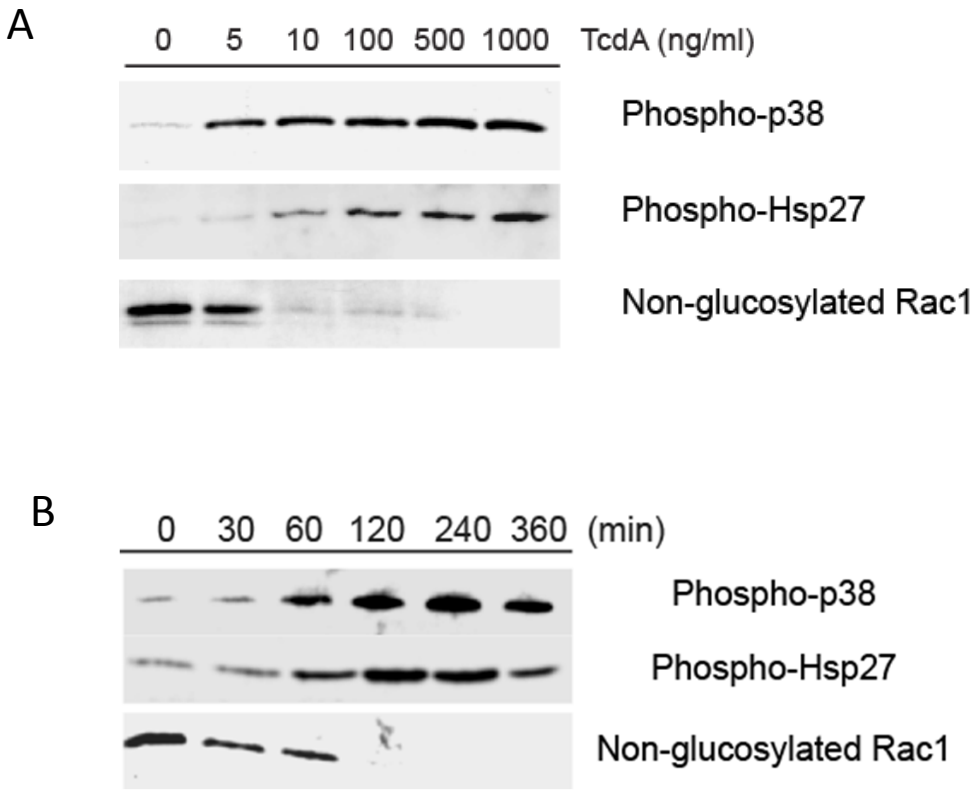

Figure 2 - Supplemental

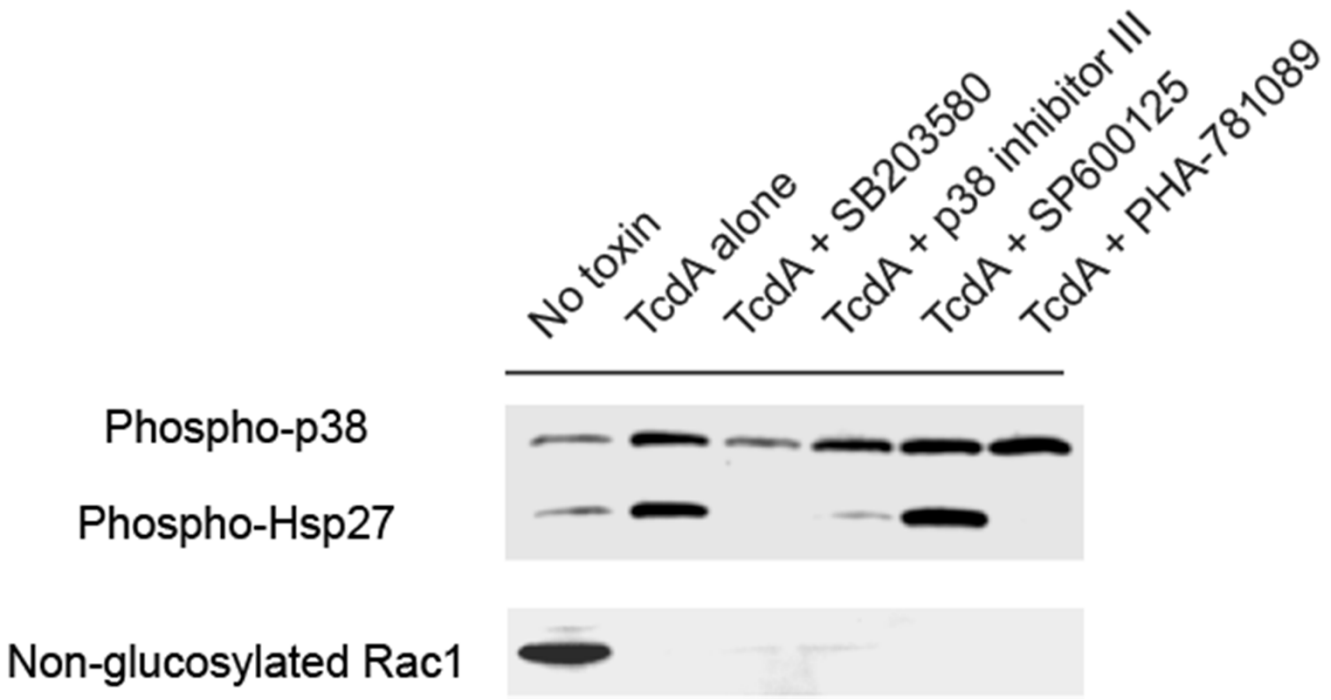

Figure 3 - Supplemental

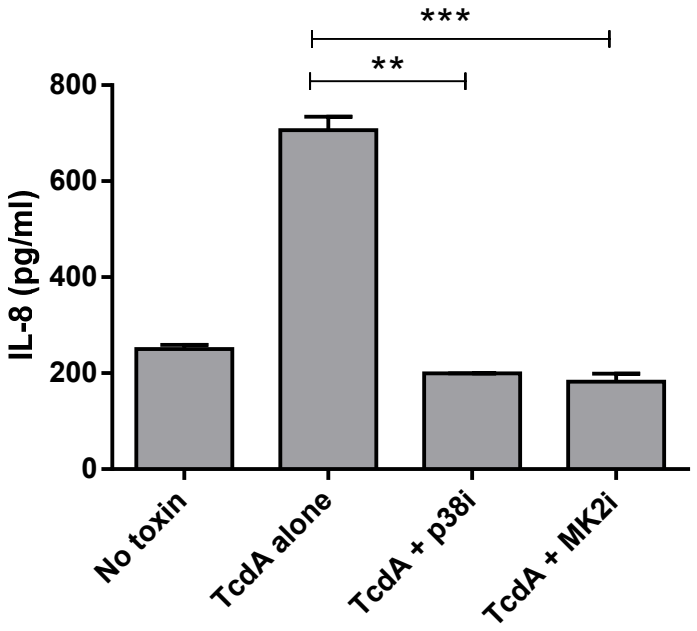

Figure 4 - Supplemental