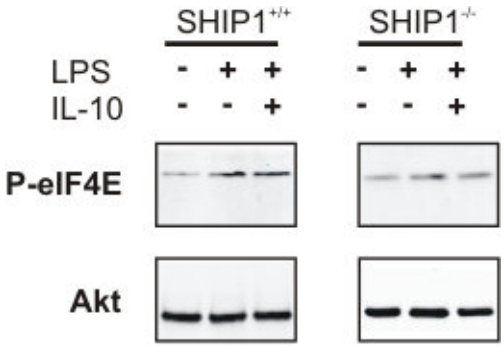
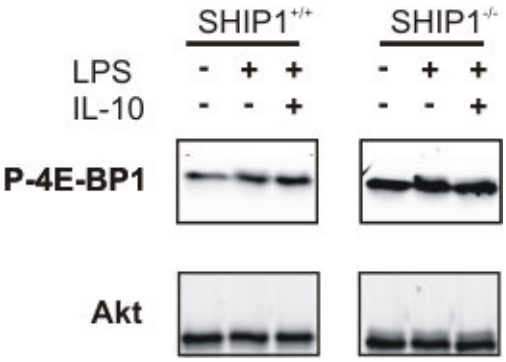
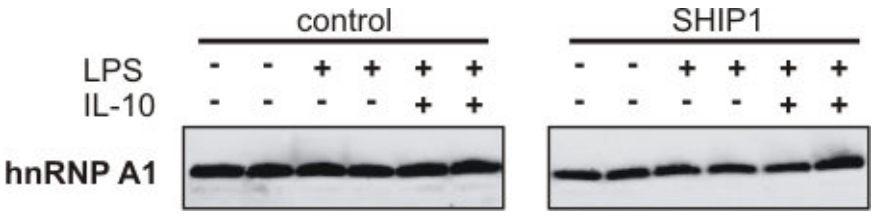
Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3



SUPPLEMENTAL FIGURE LEGENDS

SUPPLEMENTAL FIG 1. IL-10 does not inhibit eIF4E phosphorylation. Immunoblot analysis of cell lysates of peritoneal macrophages isolated from wildtype ($SHIP1^{+/+}$) or SHIP1 knockout ($SHIP1^{-/-}$) mice stimulated with 1 ng/ml LPS in the absence or presence of 100 ng/ml IL-10 for 1 hour. Representative results from three experiments.

SUPPLEMENTAL FIG 2. IL-10 does not inhibit 4E-BP1 phosphorylation. Immunoblot analysis of cell lysates of peritoneal macrophages isolated from wildtype ($SHIP1^{+/+}$) or SHIP1 knockout ($SHIP1^{-/-}$) mice stimulated with 1 ng/ml LPS in the absence or presence of 100 ng/ml IL-10 for 1 hour. Representative results from two experiments.

SUPPLEMENTAL FIG 3. IL-10 does not affect the interaction between hnRNP A1 and TNFα ARE. Immunoblot analysis of affinity pull down assays using a TNFα ARE oligonucleotide to isolate hnRNP A1 from SHIP1 siRNA cells induced for knockdown (SHIP1) or cells without knockdown induction (control). Cells were stimulated with 1 ng/ml LPS in the absence or presence of 100 ng/ml IL-10 for 30 min (details described in Supplemental Experimental Procedures). Representative results from three experiments.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

TNFα ARE pull downs - siRNA lentivirus-transduced cells were treated with 2 μg/ml of doxycycline 48 hours prior to stimulation, where the cells were re-plated at a density of 2×10^6 cells in a 6-well tissue culture plates at ~32 hours. Cells were stimulated with 1 ng/ml LPS +/- 100 ng/ml IL-10 for 30 mins and then rinsed with 2-3 ml cold PBS. Cells were lifted in 300 μl of buffer containing 50 mM Tris-HCl (pH 7.4), 150 mM potassium chloride, 1.5 mM magnesium chloride, 0.5 mM dithiothreitol, 0.5% Nonidet P-40, 10% v/v glycerol, 1X EDTA-free Protease Inhibitor Cocktail (Roche Diagnostics, Mississauga ON, Canada), 1X PhosSTOP (Roche Diagnostics, Mississauga ON, Canada) and 50 U/ml RNase Protector (Roche Diagnostics, Mississauga ON, Canada) with a cell scraper and lysed for 30 min at 4 ^{o}C. The soluble lysates were collected by centrifugation at 12,000 *g* for 15 min. Yeast tRNA (Sigma, Burlington, ON) was added to the clarified lysate to a final of 0.1 mg/ml and 100 μl of the mixture was added to 30 pmol of 5' biotinylated RNA oligos corresponding to the TNFα ARE sequence (UUAUUAUUUAUUAUUUAUUUAUUAUUUAUUUAUUU) or a non-specific sequence (AAGCUUGGGCUGCAGGUCGACUCUAGAGGAACCUA) and incubated end-over-end at 4 ^{o}C for 2 hours. The mixture was then added to 50 μl slurry of nuclease-free BioMag streptavidin magnetic beads and incubated end-over-end at 4 ^{o}C for 1.5 hours. The beads were then washed three times with 150 μl buffer containing 50 mM Tris-HCl (pH 7.4), 100 mM potassium chloride, 1.5 mM magnesium chloride, 0.5 mM dithiothreitol, 0.2 mM EDTA, and 10% v/v glycerol. Proteins were eluted by the addition of 2X Laemmli solubilization buffer and analyzed by immunoblotting using anti-hnRNP A1 (Sigma, Burlington, ON).