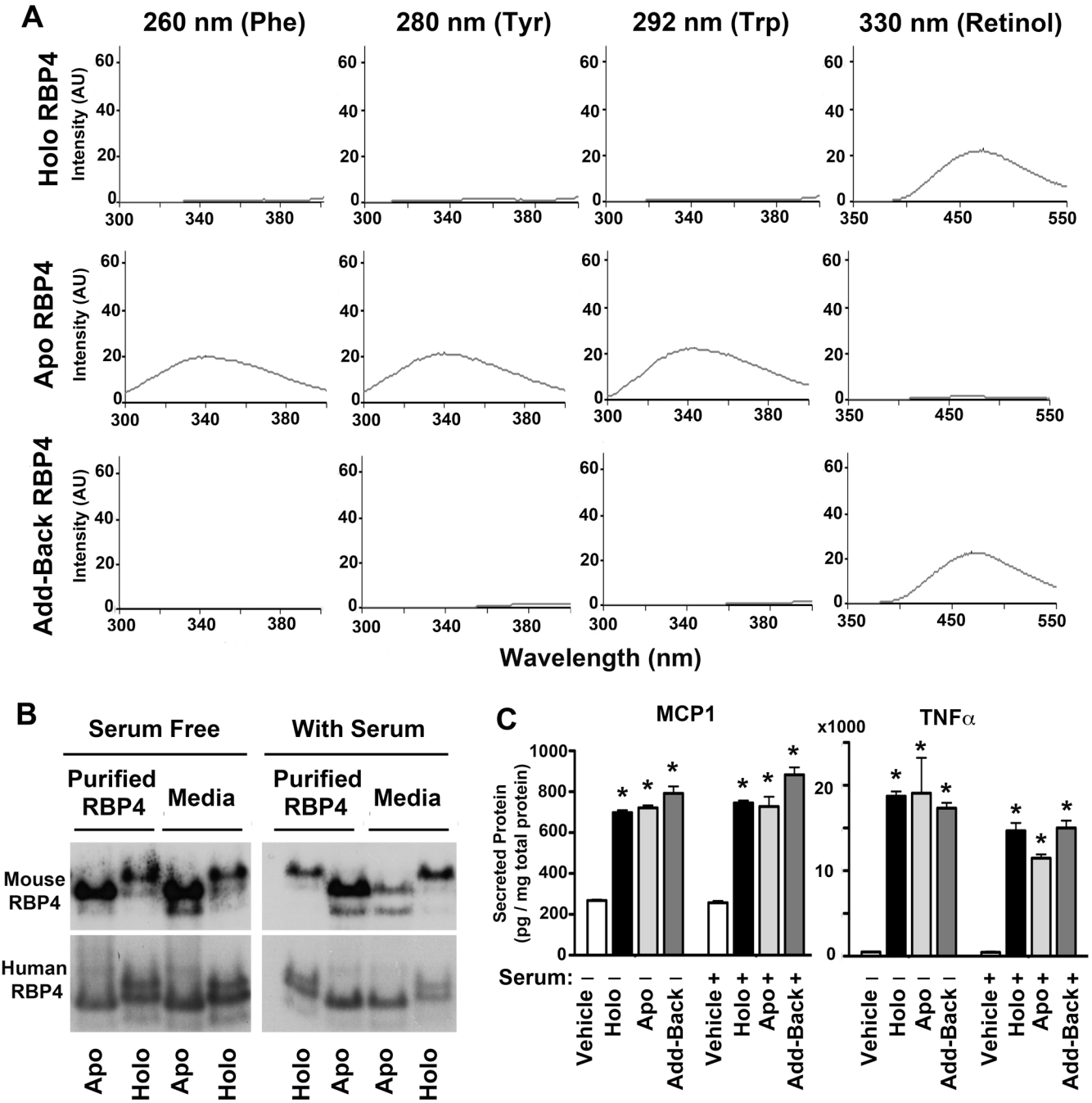

Supplemental Figure 1 The presence of serum in cell culture does not alter retinol binding of RBP4 or cytokine induction by RBP4.

(A)Representative fluorimetry of purified holo-, apo-, and add-back-RBP4. The lack of signal at 260, 280, and 292 nm in holo- (top row) and add-back- (bottom row) RBP4 indicates that the amino acids lining the retinol binding pocket are not able to resonate because the pocket is occupied. The signal at 330 nm indicates that retinol is present. The resonance at 260, 280, and 292 but not at 330 (middle row) indicates that the binding pocket of RBP4 is retinol-free in the apo-RBP4.

(B)Representative western blots of mouse and human apo- and holo-RBP4 after being dissolved in cell culture media and incubated with RAW264.7 macrophages for 20 hours in the absence or presence of 10% fetal bovine serum (FBS). Purified apo- and holo-RBP4 protein that was not exposed to cells or culture media was run for comparison.(C) MCP-1 and TNF-α secretion from RAW264.7 cells stimulated with human holo-, apo-, or add-back RBP4 (50 μg/ml for 20 hours) in the presence or absence of 10% FBS. Similar results were obtained in the presence or absence of serum in primary mouse macrophages. N=3 *p<0.05 versus vehicle-treated by ANOVA.