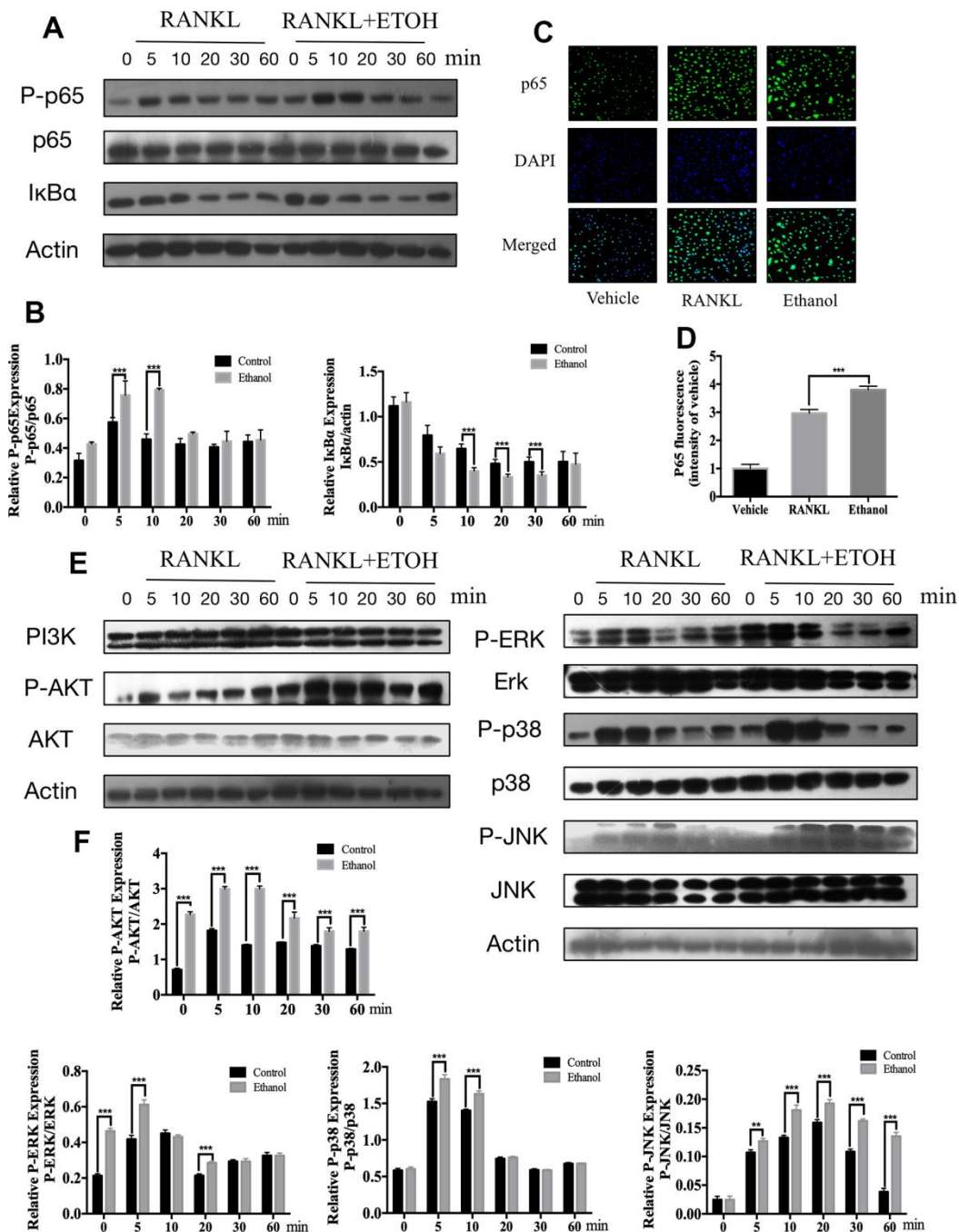


SUPPLEMENTARY FIGURE



Supplementary Figure 1. Ethanol stimulates RANKL-induced activation of the NF-κB, MAPK, and PI3K/AKT pathways, ultimately increasing NFATc1 expression. Total cellular proteins were extracted from BMMs pretreated with 50 mM ethanol for 1 h and then stimulated with 100 ng/mL RANKL for the indicated times. (A) Ethanol promoted RANKL-induced phosphorylation and p65 phosphorylation. (B) Expression levels of p-p65, relative to those of total p65, and IκBα, relative to those of β-actin, were determined using ImageJ software. (C) Representative immunofluorescence images of p65 localization (green) in BMMs treated with ethanol and stimulated with RANKL, showing that ethanol promoted p65 nuclear translocation. Nuclei were counterstained with DAPI (blue). (D) Relative frequency of p65 under each experimental condition was quantified using ImageJ software ($n = 3$). (E) Ethanol increased the phosphorylation of AKT, ERK, p38, and JNK. (F) Relative changes in the phosphorylation status of AKT, ERK, p38, and JNK were determined by densitometric analysis of each phosphorylated band and expressed as a ratio to its total protein counterpart using ImageJ software. Data are the mean \pm SD. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ compared to the respective controls.