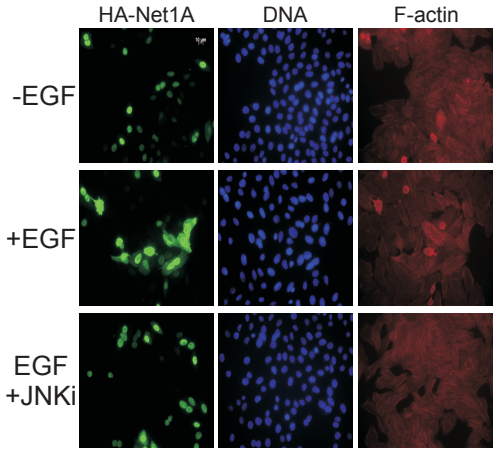
SUPPLEMENTARY FIGURES



Supplementary Fig. 1. Immunofluorescence imaging of MCF7 cells at 40X magnification. MCF7 cells were transfected with HA-Net1A and serum starved overnight. Some cells were stimulated with EGF (100 ng/ml, 15 min) in the presence or absence of JNK inhibitor (SP600125, 10 μM). HA-Net1A is shown in green, DNA in blue, and F-actin in red. Representative images are shown.

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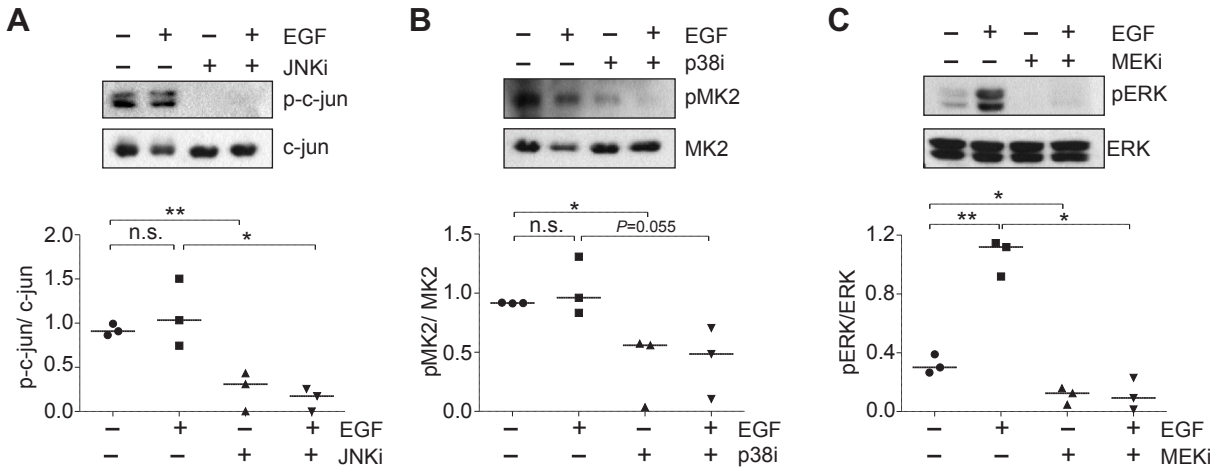

Supplementary Fig. 2. Effects of JNK, p38, and MEK inhibitors on corresponding downstream signaling. MCF7 cells were transfected with HA-Net1A and serum starved overnight. Some cells were stimulated with EGF (100 ng/ml) for 15 min. Prior to EGF stimulation some cells were pretreated with (A) JNK inhibitor (SP600125, 10 μM), (B) p38 inhibitor (SB202190, 10 μM), or (C) MEK inhibitor (UO126, 10 μM) for 30 min. Cells were then lysed for Western blot analysis to detect p-c-jun, c-jun (A), p-MK2, MK2 (B), p-ERK1/2, and ERK1/2 (C). Signal intensities were quantified using ImageJ. Quantification is from three independent experiments. Bars are median values. * = $P<0.05$; ** = $P<0.01$. n.s. = not significant.

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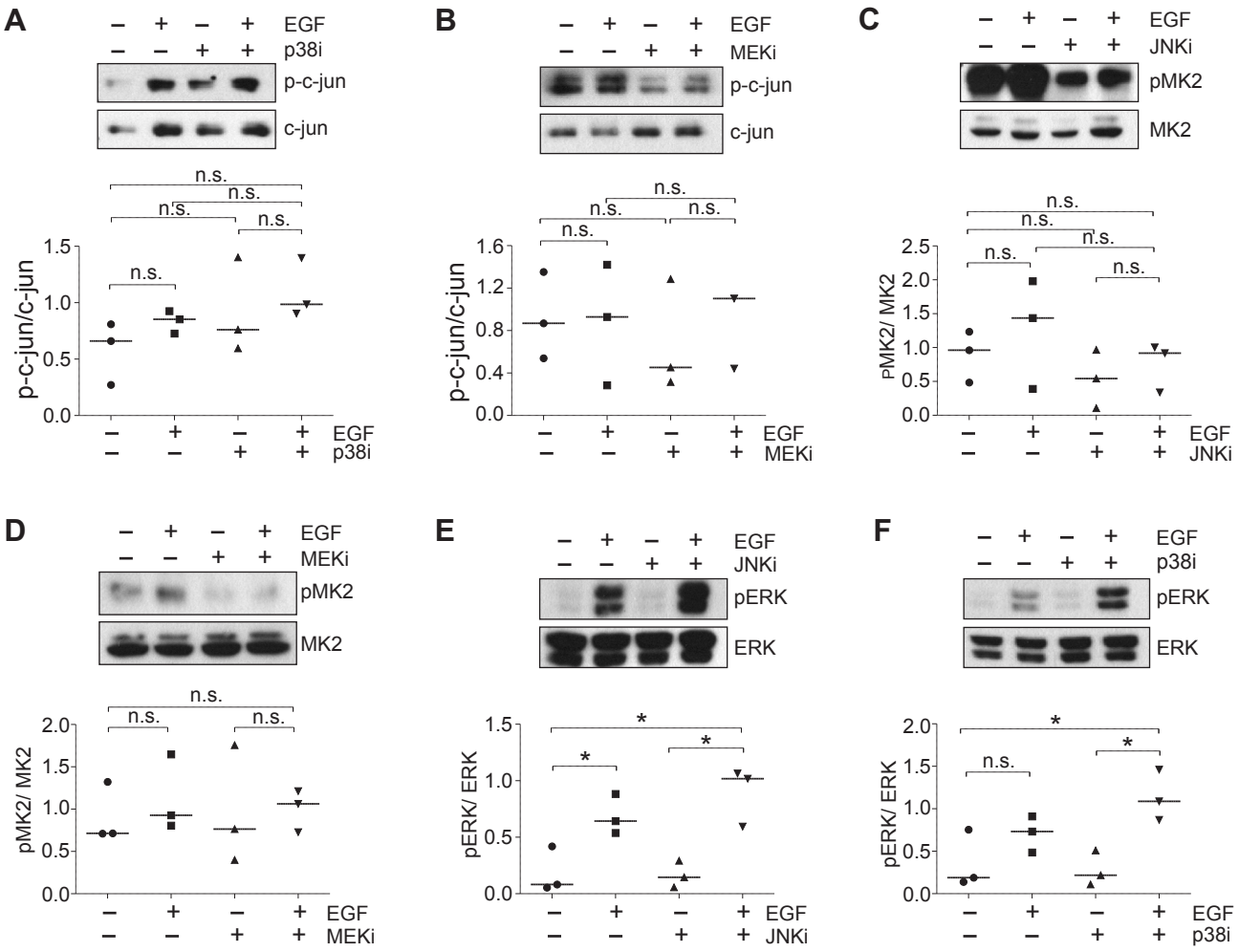
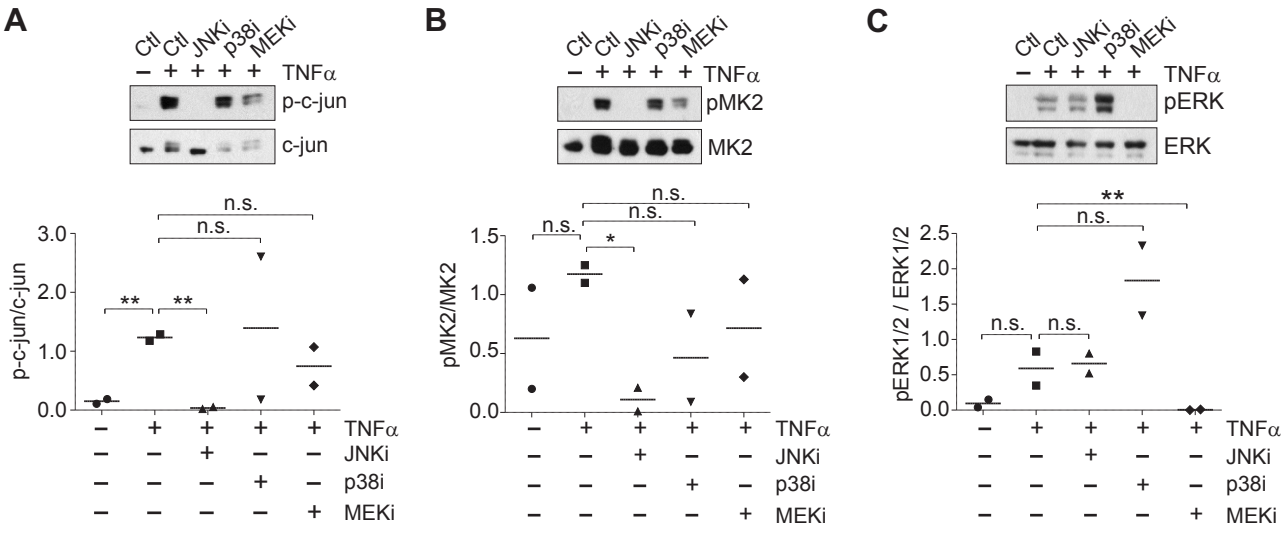


Supplementary Fig. 3. Effects of JNK, p38, and MEK inhibitors on signaling within different MAPK pathways. MCF7 cells were transfected with HA-Net1A and serum starved overnight. Some cells were then stimulated with EGF (100 ng/ml) for 15 min. Prior to EGF stimulation some cells were pretreated with (A, F) p38 inhibitor (SB202190, 10 μM), (B, D) MEK inhibitor (UO126, 10 μM), or (C, E) JNK inhibitor (SP600125, 10 μM) for 30 min. Cells were then harvested for Western blot analysis to detect p-c-jun, c-jun, p-MK2, MK2, p-ERK1/2, and ERK1/2. Signal intensities were quantified using ImageJ. Quantification is from three independent experiments. Bars are median values. * = P<0.05. n.s. = not significant.



Supplementary Fig. 4. Effects of TNF-α and MAPK inhibitors on JNK, p38, and ERK activity. MCF7 cells were transfected with HA-Net1A and serum starved overnight. Some cells were then stimulated with TNF-α (10 ng/ml) for 30 min. Prior to TNF-α stimulation some cells were pretreated with JNK inhibitor (SP600125, 10 μM), p38 inhibitor (SB202190, 10 μM), or MEK inhibitor (UO126, 10 μM) for 30 min. Representative Western blots (top) and quantification of phospho-c-jun, phospho-MK2, and pERK1/2 (bottom). Quantification is from two independent experiments. Bars are median values. * = P<0.05; ** = P<0.01. n.s. = not significant.

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