

Supplemental Figure Legends

FIG. S1. The level of active MLK3 declines in cytoplasmic extracts of SKOV3 cells treated with GA. SKOV3 cells were treated with GA (10 μ M) for the indicated time periods and cytosolic cell extracts were immunoblotted with anti-phospho-MLK3 (p-MLK3), anti-MLK3, and anti- β -Actin antibodies.

FIG. S2. CHIP is required for GA-induced degradation of MLK3 in HEY1B and TOV21G ovarian cancer cells. (A) HEY1B (left panel) and TOV21G (right panel) cells were transfected with non-specific or CHIP siRNA oligos and then treated with GA (10 μ M) for 3 or 6 h. The cell lysates were immunoblotted with anti-MLK3, anti-CHIP, and anti- β -Actin antibodies. The MLK3 band intensity for each time point was normalized to the band intensity for β -Actin, and expressed as a percent of the value for the untreated cells. (B) HEY1B (left panel) and TOV21G (right panel) cells were untreated or treated with GA for 6 h. MLK3 mRNA transcript levels were analyzed by quantitative RT-PCR, normalized to the β -Actin transcript levels, and expressed as a percent of the control, untreated cells. The values represent the means \pm SD, n=3. No statistically significant differences in mRNA levels were observed between the GA-treated and untreated cells. A p-value less than 0.05 was considered statistically significant. (C) HEY1B (left panel) or TOV21G (right panel) cells were untreated or treated with MG132 (10 μ M) and GA (10 μ M) for 6 h. Immunoblotting of whole cell extracts was performed with the indicated antibodies.

FIG. S3. MLK3 is required for sorbitol-dependent activation of JNK. SKOV3 cells were transfected with non-specific or MLK3 siRNA ds oligos, exposed to heat shock (42 °C) (A) or 0.25 M sorbitol (B) for the indicated time periods, and cell extracts were immunoblotted with the antibodies as indicated.

FIG. S4. Effect of TNF α on the endogenous level of MLK3 protein in SKOV3 cells. SKOV3 cells were treated with TNF α (20 ng/ml) for the indicated time periods, and whole cell extracts were immunoblotted with the antibodies as indicated.