



Supplemental figure 11. Activation of ERK by MIF. (A) MCF-7 cells were treated with MIF (50 ng/ml) for the times indicated. Activation of ERK is determined by immunoblot analysis using phosphorylation-specific antibodies. The total levels of ERK are also shown. (B) MCF-7 cells were treated with MIF (50 ng/ml) obtained from two different sources (R&D Systems and eBioscience) to compare their abilities to activate ERK. (C) Pre-treatment of MCF-7 cells with a small molecule MIF inhibitor, ISO-1, abolished the activation of ERK by MIF. (D) The secreted MIF from Control, MIF knockdown (shMIF) and MIF over-expressing (f-MIF) cells were immunoprecipitated from the culture media and detected by immunoblot. The cell lysates from these cells were also used to determine the intracellular levels of MIF and activated state of ERK. Total ERK and actin were used as loading controls.