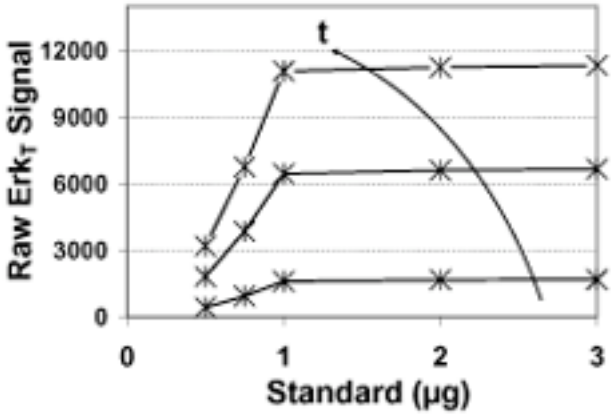
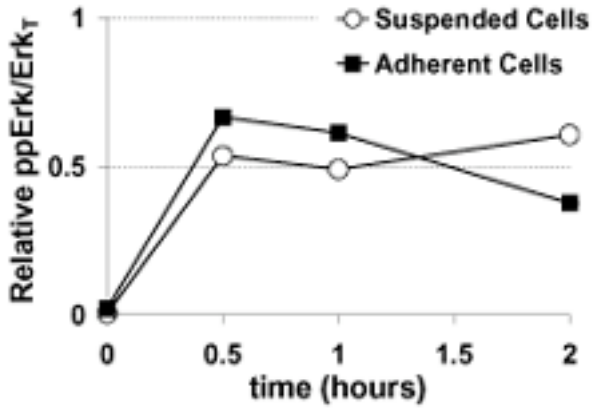
SUPPLEMENTAL MATERIAL

Supplemental Figure 1



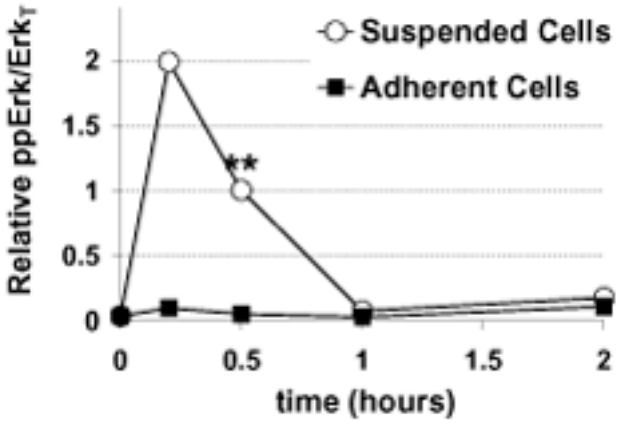
Supplemental Figure 1: Blot saturation occurs even when operating within the detection limit of the imaging system. Integration time (or, exposure time) is the amount of time that the camera acquires the chemiluminescent signal from the Western blot. The line marked "t" denotes increasing integration time. Although signal strength increases with increasing image integration time, blot saturation is observed for all three integration times. Thus, reducing the exposure time does not eliminate blot saturation.

Supplemental Figure 2



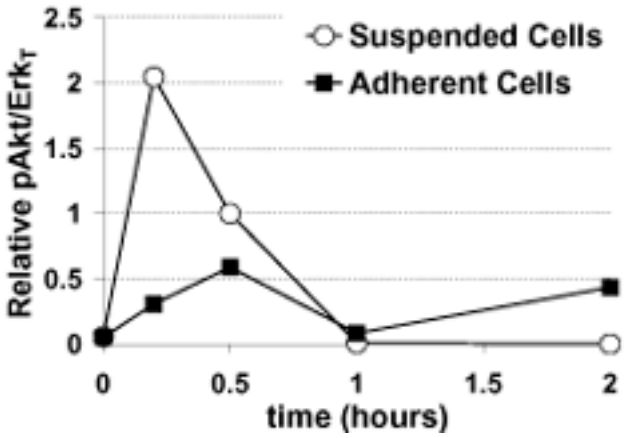
Supplemental Figure 2: bFGF does not induce Erk signaling in an adhesion-dependent manner for any portion of the time course. Cells that were either maintained in suspension on PH (empty circles) or allowed to adhere on FN (filled squares) for 2.5 h were stimulated with 1000 pM bFGF for the indicated times prior to lysing. The relative amount of active Erk (ppErk) normalized to the equal-loading control, total Erk (Erk_T), is reported for the different treatment conditions. The graph represents a single experiment.

Supplemental Figure 3



Supplemental Figure 3: At the critical bFGF concentration of 1 pM, suspended cells strongly induce Erk activation, while Erk activation remains near basal in adherent cells. NIH-3T3 cells were prepared as described in the legend to Figure 2. After being maintained in suspension by PH (empty circles) or allowed to adhere on FN (filled squares) for 2.5 h, cells were stimulated with the critical concentration of 1 pM bFGF. The relative amount of active Erk (ppErk) normalized to the equal-loading control, total Erk (Erk_T), is reported for the different treatment conditions. The graph represents one to three independent experiments, with $n > 1$ for the 0 and 30' time point. The double asterisk denotes that 1 pm bFGF-mediated ERK activation in suspended and adherent cells is statistically different ($P < 0.01$).

Supplemental Figure 4



Supplemental Figure 4: At the critical bFGF concentration of 1 pM bFGF, suspended cells also strongly induce Akt activation, while Akt activation remains near basal in adherent cells. NIH-3T3 cells were prepared and analyzed identically to those in Figure 2. Total cell lysates were assayed by immunoblot analysis using antibodies specific to total Erk (Erk_T) and phosphorylated Akt (pAkt). The relative amount of active Akt (pAkt) normalized to the equal-loading control, total cellular Erk (Erk_T), is reported for the different treatment conditions. The graph represents a single experiment.