Supplemental Material

Methods

Cell line.
SRF shRNA 2-2 cell line was generated by stable transfection of NIH3T3 with pLKO.1 containing short hairpin RNA (shRNA) directed to SRF; shSRF-2, 5' – GAUGGAGUUCAUCGACAACAA -3'; NIH3T3 cells were used as the control cell line. The lentiviruses were made in Phoenix-ECO cells (ATCC CRL3214). Cells were grown in 5% CO_2 Dulbecco's modified Eagle's medium (DMEM) containing 10% New Born Calf serum (NCS) and 10 μg/ml puromycin (Invivogen). NIH3T3 cells were grown in 5% CO_2 in DMEM supplemented with 10% NCS.

Figure Legend

Figure S1. Effect of SRF depletion on IEG expression.
Control and SRF knockdown cells were starved in 0.2% NCS overnight and then serum induced with 20% NCS for 0, 30, 60 or 120 minutes. Total RNA was isolated from the cells. The levels of the indicated genes were measured using quantitative real time-PCR (qPCR). 18s rRNA levels were used to normalize for overall RNA levels.

Figure S2. Effect of C3 transferase of Id1 reporter gene expression.
NIH3T3 cells were transfected with the indicated reporter genes along with pRLSV40P as an internal control. The next day transfected cells were starved in 0.2% serum overnight and then induced with 20% serum for four hours. As a control, the pGL3-promoter reporter containing the SV40 virus promoter was used. The Fos-pm18 reporter contains the c-fos SRE region with a

mutation in the TCF site, such that it is activated predominantly by the RhoA-MKL pathway [22, 80]. The Id1-1150 reporter is described in figure 3. Firefly luciferase levels were normalized to the Renilla pRLSV40P levels and are the means of three experiments +/- the standard deviation. *** indicates a p-value ≤ 0.05.

Figure S3. Protein synthesis-independent induction of Id1 expression.

NIH3T3 cells were starved in 0.2% serum overnight, treated with or without cycloheximide (50 μg/ml) for 30 minutes and then treated with or without BMP2 (50 ng/ml) for 1 hour. The levels of Id1 mRNA were measured by qPCR with 18s rRNA levels used to normalize the samples. The values are the means of two experiments in duplicate +/- standard deviations.