RT-PCR
For RT-PCR, total RNA was isolated by the RNeasy midi kit (QIAGEN). PCR primers used in Fig. 1 A are as follows: Gab1 (forward: 5'-cagttacgacattccgc-caa-3', reverse: 5'-ggaattggctcggtaaagctg-3'); Gab2 (forward: 5'-gaagagtactggcagtgtggat-3', reverse: 5'-caagctttttgacgaagcttt-3'); Gab3 (forward: 5'-acagcttcacccagtgctga-3', reverse: 5'-gtccagggccaaataatccagc-3'); Gab4 (forward: 5'-acagcttcacccagtgctga-3', reverse: 5'-gtccagggccaaataatccagc-3').

The luciferase/GFP reporter assays
The 3'-UTR and its adjacent 200 bp of coding sequence of mouse Gab2 and full-length mouse Gab3 cDNA were subcloned into the pCS2-luciferase vector (Yu et al., 2002) to create Gab2 and Gab3 reporters, respectively. A second Gab2 reporter, pCS2-GFP-Gab2-3'-UTR, was generated by replacement of the luciferase gene in the pCS2-luciferase-Gab2-3'-UTR construct with GFP. shRNA constructs were cotransfected with either reporter into P19 cells. 48 h after transfection, luciferase activity was measured and normalized by ß-gal activity. GFP expression was monitored by fluorescence microscopy.

JNK and p38 in vitro kinase assay
P19 cells were treated with 10 ng/ml bFGF, 1 µM RA alone, or in combination for 0, 0.5, 1, 5, and 10 h. 200 µg of lysates was immunoprecipitated by anti-JNK or anti–phospho-p38 antibody, immune complexes were washed twice in 1×TBS, twice in lysis buffer (0.5 M LiCl and 0.1 M Tris-Cl, pH 8.0), and once with JNK kinase buffer (20 mM Hepes, pH 7.4, 10 mM MgCl₂, 1 mM MnCl₂, 1 mM EGTA, 0.2 mM Na₃VO₄, 1 mM DTT, and 0.03% Brij 35) or p38 kinase buffer (according to the manufacturer’s protocol) and resuspended in 25 µl of kinase buffer containing 5 µCi of γ[³²P]ATP (ICN) and 1 µg of the corresponding substrate, GST-c-Jun, or ATF-2. Incubation was for 30 min at 30°C. Kinase activity was quantified by a PhosphorImager (Bio-Rad Laboratories).

References