Supplemental Experimental Procedures

Production of recombinant proteins. A 25 ml preculture was grown overnight at 37˚C in Luria-Bertani media supplemented with 30 μg/ml kanamycin. This culture was used to inoculate 2 L of LB-Kan media and grown to an OD_{600} of 0.6 before inducement with isopropyl-1-thio-β-D-galactopyranoside to a final concentration of 0.4 mM. Cells were grown for 4 hours at 30 ˚C. After centrifugation for 10 min at 10000xg, the bacteria were resuspended with 5 ml BugBuster reagent (Novagen) per gram of wet cell paste containing 25 units of Benzonase Nuclease (Novagen) per ml of BugBuster reagent and Protease Inhibitor Cocktail Set I (0.5mM AEBSF-HCl, 0.15μM Aprotinin, 1.0μM E-64, 0.5mM EDTA, 1.0μM Leupeptin Hemisulfate, Calbiochem). The cell suspension was incubated on a shaking platform for 20 minutes at room temperature and the insoluble cell debris was removed by centrifugation at 16,000xg for 20 min. A 1.5 vol bed of His-Bind® Resin (Novagen) was applied to an empty disposable column and washed with 3 vol of deioninized water, 5 vol of charge buffer (50mM NiSO4), and 3 vol of Binding buffer (5mM imidazole, 0.5 M NaCl, 20 mM Tris-HCl pH 7.9). The soluble extract was loaded onto the prepared column and washed sequentially with 6 vol wash buffer (0.5 M NaCl, 20mM Tris-HCl pH 7.9) and 10 vol of Binding buffer. The bound protein was eluted with 6 vol of elution buffer (1 M imidazole, 0.5 M NaCl, 20 mM Tris-HCl pH 7.9) and then dialyzed in phosphate-buffered saline. The successive steps of purification were assessed by electrophoresis on a 14% Tris-Glycine polyacrylamide gel (Invitrogen). Protein concentrations were measured by Bradford assay (Bio-Rad).

Branching morphogenesis assays. Cell clusters were prepared as follows: agarose was heated in DME/F12 (final 2%) and 1 ml of the solution was added to each well of 24-well plates. After the agarose gelled, 1.5ml of growth medium was added to each well and incubated for 1 hr at 37º C in a 5% CO_2 incubator. This medium was then discarded and SCp2 cells suspended in 500ul of growth medium containing 2.5U of desoxyribonuclease I (DNase I) were added on top of the agarose gel and incubated at 37º C with gentle rotation (100rpm) for 16 hr, which yielded rounded and well packed cell clusters. Unclustered single cells were removed by centrifugation and the clusters were then washed three times with DME/F12. Cell clusters were embedded in type I collagen gels. Acid-soluble collagen I (Cellagen; Koken, Osaka, Japan) was mixed gently on ice (8 vol) with 1 vol of 10X DME/F12 and 1 vol of 0.1N NaOH. Collagen solution (100 μl) was added into each well of a 48 well plate, which was then incubated at 37º C to allow for polymerization of the basal collagen layer. The cell clusters were suspended in growth medium and 10 μl of the suspension (24-40 clusters) was mixed with 10 μl of protein solution at 0.25 mg/ml and 80 μl of the collagen solution, and poured onto the basal collagen layer and placed at 37º C for gelation. After gelation occurred, 200 μl of growth medium containing 10% protein solution and 50 ng/ml epidermal growth factor (EGF) was added to the media, when indicated. Cultures were maintained at 37º C in 5% CO_2 and media was changed every other day. Branching morphogenesis was assessed using a Nikon Diaphot 300 microscope and clusters were scored as positive when possessing two or more branches of a length at least half the diameter of the central cell cluster.