

Supplementary Figure 1

NF- κ B modulation of EMT in *V12S35Ras* cells. V12S35Ras cells expressing the empty vector control (empty), TD-I κ B α , or CA-IKK-2, were cultivated on porous supports for 7 days in the presence or absence of TGF β (5 ng/ml, day 2-7; Suppl. Figure 1A, B and D), or were cultivated on plastic dishes in the absence of TGF β (Suppl. Figure 1C). (A) Photographs of cultivated cells: regions with strands of spindle-shaped mesenchymal cells are indicated by white dotted lines. Inserts show higher magnifications. (B) Quantitation of areas on porous supports covered by mesenchymal strands as percentage relative to total area (cultures shown in A, day 6). (C) Cells were cultivated on plastic for 3 days (until they reached 90% confluence) in the absence of TGF β . Left panel shows photographs of cultivated cells. Immunostaining for E-cadherin (red, middle panel) or Vimentin (red, right panel) plus DAPI counterstaining (blue) is shown. Note an almost completely transformed mesenchymal phenotype and *de novo* expression of Vimentin in CA-IKK-2-expressing V12S35Ras cells, even in the absence of TGF β . (D) Cells cultivated on porous supports for 7 days +/- TGF β (5 ng/ml, day 2-7) were immunostained for E-Cadherin (red, left panels) or Vimentin (red, right panels) plus DAPI counterstaining (blue). No loss of polarized E-cadherin expression or *de novo* Vimentin expression in TD-I κ B α -expressing V12S35Ras cells, even in the presence of TGF β . Note loss of polarized E-cadherin expression as well as *de novo* Vimentin expression in CA-IKK-2-expressing V12S35Ras cells, even in the absence of TGF β .



Vimentin/DNA

Supplementary Figure 2

TNF α induces EMT in the absence of TGF β (analysis in collagen gels). EpRas cells expressing the empty vector control (empty) or TD-I_KB α were seeded into collagen gels. EpRas-empty cells were either treated with TNF α (40 ng/ml; fresh TNF α was supplied with every medium change) from the beginning of collagen gel culture, or left untreated. Cells were allowed to form structures for 3 days and induced or not induced to undergo EMT by addition of TGF β (5 ng/ml). Left panel: cells as indicated without TGF β for 3 days. Middle panel: cells as indicated without TGF β for 6 days. Right panel: cells as indicated without TGF β for 3 days, plus TGF β treatment for 3 days. Bottom middle and right panels: collagen gel structures were stained for the mesenchymal marker Vimentin (red) plus DAPI counterstaining for DNA (blue). Note Vimentin positive mesenchymal structures in EpRas control cultures treated with TNF α , even in the absence of TGF β (bottom middle panel). The percentage of mesenchymal structures in TNF α -treated EpRas collagen gel cultures in the absence of TGF β , is in the range of 10-20%, which is lower than that in CA-IKK-2-expressing EpRas cells without TGF β treatment (see Figure 5B).