#### Supplementary Figure S2





**Figure S1.** Immunoblot analysis of RAC1B and E-cadherin in H6c7 and Capan1 cells. Protein lysates from the benign human pancreatic ductal epithelial cell line H6c7, the human PDAC-derived line Capan1, and Capan2 as control (see Figure 1A), were fractionated by SDS-PAGE, blotted and incubated with antibodies to RAC1B, E-cadherin, and HSP90 to verify equal loading.

**Figure S2.** Concentration of bioactive TGF- $\beta$ 1 in culture supernatants of Colo357 and Panc1 cells as measured by ELISA. Data represent the mean  $\pm$  SD of three experiments. The asterisks indicates a significant difference.

#### Supplementary Figure S4



**Figure S3.** Effect of RAC1B knockdown on *CLDN7* and *EPCAM* expression in Panc1 cells. Panc1 cells were transiently transfected twice with 50 nM each of control (Ctr) siRNA or RAC1B siRNA (R1B). Fortyeight h after the second transfection cells were processed for RNA isolation and qRT-PCR analysis. Data represent the TBP-normalized mean  $\pm$  SD of three experiments. The asterisks indicate significance.



**Figure S4.** Immunoblot detection of RAC1B and RAC1 in Panc1 cells stably expressing a HA-tagged version of RAC1B. Crude protein lysates of three individual clones of Panc1-HA-RAC1B cells as well as of empty vector (V)transfected control cells were fractionated by SDS-PAGE, blotted and incubated with an anti-Rac1 antibody that recognizes both RAC1B (upper band) and RAC1 (lower band). Incubation of the same blot with an antibody to  $\beta$ actin served to verify equal loading.



**Figure S5.** MEK-ERK signaling is involved in TGF- $\beta$ 1 and RAC1B regulation of *CDH1*. Panc1 cells were transfected twice with 50 nM of an siRNA specific to RAC1B (R1B) or a control (Ctr) siRNA, serum-starved overnight and treated with vehicle (0.1% dimethylsulfoxide), or UO126 (10  $\mu$ M) in the absence or presence of TGF- $\beta$ 1 (5 ng/ml) for a period of 48 h. Cells were then subjected to qPCR analysis of *CDH1*. Data represent the TBP-normalized mean  $\pm$  SD of three experiments. The asterisks indicate significance.



**Figure S6.** Effect of RAC1B knockdown on *VIM* expression in Panc1 cells. Panc1 cells were transiently transfected twice with 50 nM each of control (Ctr) siRNA or RAC1B siRNA (R1B) and treated or not with TGF- $\beta$ 1 for 24 h. Cells were then processed for RNA isolation and qPCR analysis of *VIM*. Data represent the TBP-normalized mean ± SD of three experiments. The asterisks indicate significance.



Full uncropped blots from Figure 1A

Full uncropped blots from Figure 4A

🛏 E-cadherin

-Non-specific

band derived

from RAC1B antibody

HSP90

RAC1B

Vimentin

M = molecular weight marker (SM1841, Fermentas/Thermo Fisher Scientific)