SUPPLEMENTAL DATA

Supplemental Figure S1:

(A) BEAS-2B cells were transfected or not (NT) with siRNA control (siNeg) or siRNA against RhoB (siB1 or siB2). Cells were lysed and immunoblotted with anti-RhoB, anti-RhoA or anti-actin. **(B)** Expression of RhoC mRNA was studied using real time PCR. **(C)** To analyze proliferation, $9x10^3$ cells were seeded per well in 12-well plates on day 0 in medium containing 10 % FCS and counted every day. Each point is the average of 3 individual measurements. Data are representative of 2 independent experiments.

Supplemental Figure S2: Inhibition of RhoB by siRNA increases migration of HBE-135 cells.

(A) Cells were transfected with siRNA, then transduced or not with an adenoviral vector encoding RhoB at a MOI of 5:1 (Rescue). 48 h later, cells were lysed and immunoblotted with anti-RhoB or anti-actin. **(B)** $140x10^3$ cells were plated on a 35 mm dish, first coated with collagen type I. 8 h after scratching with a p200 tip, the ability of cells to migrate into the cleared section was monitored. **(C)** Percentage of migration was defined by 3 measures of lengthwise migration.

Supplemental Figure S3: Inhibition of RhoB by siRNA increases motility and invasion of HBE-135 cells.

(A) $25x10^3$ cells/well were added in 500 µL serum-free DMEM in the upper compartment of the transwell. The bottom chamber was filled with 750 µL of DMEM supplemented with 10 % FCS. After 24 h incubation at 37°C, cells on the upper surface of the filter were wipped off and the filter fixed and stained. **(B)** Percentage migration is represented. **(C)** $32x10^3$ cells/well in 500 µL serum-free DMEM were added in the upper compartment of the chamber after coating with Matrigel. The bottom chamber was filled with 750 µL of DMEM

supplemented with 10 % FCS. 48 h later, cells were fixed and stained. **(D)** Percentage invasion is represented.

Supplemental Figure S4: Inhibition of RhoB by shRNA increases motility and invasion of BEAS-2B.

(A) Cells were transfected with siRNA, then transduced or not with an adenoviral vector encoding RhoB at a MOI of 5:1 (Rescue). 48 h later, cells were lysed and immunoblotted with anti-RhoB or anti-actin. **(B)** $140x10^3$ cells were plated on a 35 mm dish, first coated with collagen type I. 16 h after scratching with a p200 tip, the ability of cells to migrate into the cleared section was monitored. Percentage of migration was defined by 3 measures of lengthwise migration. **(C)** $25x10^3$ cells/well were added in 500 µL serum-free DMEM in the upper compartment of the transwell. The bottom chamber was filled with 750 µL of DMEM supplemented with 10 % FCS. After 24 h incubation at 37°C, cells on the upper surface of the filter were wipped off and the filter fixed and stained. Percentage migration is represented. **(D)** $32x10^3$ cells/well in 500 µL serum-free DMEM were added in the upper compartment of the chamber after coating with Matrigel. The bottom chamber was filled with 750 µL of DMEM supplemented with 10 % FCS. 48 h later, cells were fixed and stained. Percentage invasion is represented.

Supplemental Figure S5: Inhibition of AKT and ERK by pharmacological inhibitors and siRNA in BEAS-2B cells

(A) After treatment with DMSO (control), PD 98059, LY294002 or Triciribin cells were lysed and immunoblotted with antibodies against phosphorylated and total ERK and Akt. **(B)** After transfection with siRNA specific for each Akt isoform, cells were lysed and immunoblotted with antibodies against each Akt isoform.

Supplemental Figure S6: Role of AKT and ERK pathways in RhoB regulation of invasion in BEAS-2B cells.

$32x10^3$ cells/well in 500 µL DMEM serum-free were added in the upper compartment of the chamber coated with Matrigel. The bottom chamber was filled with 750 µL of DMEM supplemented with 10% FCS. 48 h later, cells were fixed and stained1 h after treatment with DMSO (control), PD 98059, LY294002 or Triciribin **(A)** or after transfection with siRNA against Akt isoforms **(B)**.

Supplemental Figure S7: Role of AKT and ERK pathways in RhoB regulation of migration and invasion in HBE-135 cells.

(A) 48 h after transfection with siRNA**,** cells plated on collagen I were lysed and immunoprecipitated with antibody against Akt1, Akt2 or Akt3, followed by immunoblotting of the immunoprecipitates. **(B)** $140x10^3$ cells were plated on a 35 mm dish, first coated with collagen I and co-transfected with siNeg (black box) or siB2 (grey box) and siRNA against Akt isoforms. 8 h after scratching with a p200 tip, the ability of cells to migrate into the cleared section was monitored and the percentage migration defined by 3 measures of lengthwise migration. **(C)** $32x10^3$ cells/well in 500 µL DMEM serum-free were added in the upper compartment of the chamber after coating with Matrigel. The bottom chamber was filled with 750 µL of DMEM supplemented with 10% FCS. 48 h later, cells were fixed and stained. Percentage invasion is represented.

Supplemental Figure S8: Role of Rac1 in RhoB regulation of migration in HBE-135 cells.

(A) 48 h after transfection with siRNA, cells were lysed and a GST-pull down were performed to precipitate Rac-GTP. **(B)** $140x10^3$ cells were plated on a 35 mm dish, first coated with collagen I and co-transfected with siNeg (black box) or siB2 (grey box) and siRNA against Rac1. 16 h after scratching with a p200 tip, the ability of cells to migrate into the cleared section was monitored and the percentage migration defined by 3 measures of lengthwise migration. **(C)** 48 h after transfection with siNeg (black box) or siB2 (grey box) and siRNA against Rac1, $32x10^3$ cells/well in 500 µL DMEM serum-free were added in the upper

compartment of the chamber after coating with Matrigel. The bottom chamber was filled with 750 µL of DMEM supplemented with 10% FCS. 48 h later, cells were fixed and stained.

Supplemental Figure S9: Inhibition of RhoB did not amplify K-RasV12 transformation of BEAS-2B cells

(A) BEAS-2B cells transformed (BKR C1) or not (WT) with K-Ras and transduced with Adenovirus control (Adeno cont) or coding for RhoB (Adéno RhoB) or with lentivirus control –Lenti cont) or coding for shRhoB (Lenti shB2) or not (NT) were lysed and immunobloted with anti-RhoB, K-Ras or Actin. **(B)** $8x10^3$ BEAS-2B cells transformed by K-RasV12 cells and transduced with control lentivirus (BKR C1 Lenti cont) or coding for shB2 (BKR C1 Lenti shB2) were plated onto 0.6% agar layer in 0.3% agar-containing medium. The growth was evaluated by staining with MTT. Data are representative of 2 independent experiments. **(C)** $10x10^6$ BEAS-2B cells WT or expressing K-RasV12 (BKR C1) and transduced with the lentivirus were implanted into the left flank of nude mice. The results are expressed as mean size of tumors (mm3) from a group of 5 mice. **(D)** 48 h after Transduction, $32x10^3$ cells/well in 500 µL DMEM serum-free were added in the upper compartment of the chamber after coating with Matrigel. The bottom chamber was filled with 750 µL of DMEM supplemented with 10% FCS. 48 h later, cells were fixed and stained.

Supplemental Figure S10: Role of Akt1 isoform in BKR migration

(A) 48 h after transduction with adenovirus control (Adeno cont) or coding for RhoB (Adeno RhoB)**,** cells plated on collagen I were lysed and immunoprecipitated with antibody against Akt1 followed by immunoblotting of the immunoprecipitates. **(B)** $140x10^3$ cells were plated on a 35 mm dish, first coated with collagen I and transduced with adenovirus and transfected with siRNA against Akt1 isoform. 8 h after scratching with a p200 tip, the ability of cells to migrate into the cleared section was monitored and the percentage migration defined by 3 measures of lengthwise migration. **(C)** $32x10^3$ cells/well in 500 µL DMEM serum-free were added in the upper compartment of the chamber after coating with Matrigel. The bottom

chamber was filled with 750 µL of DMEM supplemented with 10% FCS. 48 h later, cells were fixed and stained. Percentage invasion is represented.

Supplemental Figure S11: Inhibition of RhoB expression does not enhance transformation of BEAS-2B cells transformed by K-Ras clone 2 but enhances their migration and invasion properties

(A) BEAS-2B cells transformed (BKR C2) or not (WT) by K-RasV12 were transduced with control adenovirus (Adeno cont) or adenovirus encoding RhoB (Adeno RhoB), before being lysed and immunoblotted with anti-RhoB or anti-actin. **(B)** To study the doubling time, $9x10^3$ cells were seeded per well in 12-well plates on day 0 in medium containing 10 % FCS, and then counted every day in triplicate. **(C)** $140x10^3$ BEAS-2B cells stably transfected with K-RasV12 (BKR C2) or not (WT) and then transduced with adenovirus control (Adeno cont) or coding for RhoB (Adeno RhoB) were plated on a 35 mm dish, and coated first with collagen type I. 48 h later, cells were wounded with a p200 tip. 8 h after scratching, the ability of cells to migrate into the cleared section was monitored. Percentage migration was defined by 3 measures of lengthwise migration. **(D)** $32x10^3$ cells/well in 500 µL DMEM serum-free were added in the upper compartment of the chamber after coating with Matrigel. The bottom chamber was filled with 750 µL of DMEM supplemented with 10% FCS. 48 h later, cells were fixed and stained. Percentage invasion is represented.