SUPPLEMENTARY INFORMATION

Figure legends:

Figure S1. Translocation of endogenous β2-chimaerin. Staining using an anti-β2-chimaerin antibody and FITC-coupled secondary antibody (green) and the membrane marker RFP-CAAX (red) in response to EGF. Endogenous levels in HeLa cells are below levels of detection. (A) HEK 293 cells were transfected with an expression vector for RFP-CAAX. Twenty four h later cells were treated with EGF (100 ng/ml, 5 min), fixed and stained. (B) Similar experiments carried out in Neuro-2a cells, which express high levels of β2-chimaerin are shown.

Figure S2. Association of β2-chimaerin with active Rac.
(A, B) COS-1 cells were transfected with pEBG vectors encoding for various forms of GST-fused Rac, as indicated in the figures, and 18 h later cells were infected with the β2-chimaerin AdV (MOI=10 pfu/cell, 16 h). GST-Rac variants were pulled-down with glutathione Sepharose 4B beads, and the levels of HA-β2-chimaerin in the beads were determined by anti-HA immunoblotting.
(C) Co-immunoprecipitation of active Rac and β2-chimaerin. COS-1 cells were co-transfected with a plasmid encoding for an activated Rac mutant (pCEFL-AU5-Q61L-Rac1) or empty vector, and pEGFP-β2-chimaerin. β2-chimaerin was detected in AU5 immunoprecipitates using an anti-GFP antibody.

Figure S3. Association of ΔEIE-β2-chimaerin with active Rac (G12VRac1). COS-1 cells were transfected with pEBG vectors encoding for either GST-T17N-Rac or GST-G12V-Rac1, and 18 h later cells were infected with the β2-chimaerin AdV (MOI=10 pfu/cell, 16 h). GST-Rac mutants were pulled-down with glutathione Sepharose 4B beads, and the levels of HA-β2-chimaerin in the beads were determined by anti-HA immunoblotting.

Figure S4. Translocation of ΔEIE-β2-chimaerin in HeLa cells expressing T17N-Rac1. HeLa cells were transfected with pEGFP-ΔEIE-β2-chimaerin, and 24 h later infected with an AdV
encoding for T17N-Rac (Myc-tagged) in serum-free medium (MOI =10 pfu/cell, 20 h). Cells were then treated with EGF (100 ng/ml, 5 min) and fixed.
(A) Localization of pEGFP-ΔEIE-β2-chimaerin was determined by confocal microscopy.
(B) Expression of Myc-T17N-Rac1 by Western blot using an anti-Myc antibody (Sigma).

Figure S5. Effect of increasing MOIs of β2-chimaerin AdV in HeLa cells on Rac-GTP, RhoA-GTP and Cdc42-GTP levels.
HeLa cells were infected with different MOIs of a recombinant HA-β2-chimaerin-AdV (0.3-30 pfu/cell). After 24 h, cells were incubated in serum-free medium for 20 h, and then EGF (100 ng/ml) was added for 1 min. Left panel, representative experiment. Right panel, levels of GTP-bound protein normalized to total Rac1, Cdc42 or Rho A. Results are expressed as percentage of the EGF response in control (non-infected) cells. Values are expressed as the mean ± S.E. of 3 independent experiments.

Figure S6. Effect of dual α2-chimaerin and β2-chimaerin RNAi on EGF-induced activation of Rac in HeLa cells.
HeLa cells were transfected with dsRNAs for both α2-chimaerin (50 nM) and β2-chimaerin (50 nM) or with a control duplex (CTL, 100 nM). Forty eight h later, cells were stimulated with EGF (100 ng/ml) for the times indicated in the figure, and Rac-GTP levels were determined using the PBD pull-down assay. Target sequences for β2-chimaerin and control are described in the main text. For α 2-chimaerin, the targeting sequence for RNAi was AAACATATGCCAGTCCTGAAA. Note the significant extension in the duration of the EGF response in cells subject to α2-chimaerin + β2-chimaerin RNAi. A full effect could only be observed when the two chimaerin isoforms were knocked-down in HeLa cells, but not after depletion of individual chimaerins (data not shown).
(A) Representative pull-down assay.
(B) Densitometric analysis of Rac-GTP levels normalized to total Rac in each case (n=5).
Results were expressed as fold-increase relative to non-transfected cells.
(C) Representative Western blot using a chimaerin antibody that detects both α2- and β2-chimaerins, 48 h after transfection of HeLa cells with dsRNAs.
(D) Representative RT-PCR showing the depletion of α2-chimaerin and β2-chimaerin in HeLa cells. Primers used for β2-chimaerin and GAPDH RT-PCR are described in the main text.
Primers for α2-chimaerin RT-PCR are as follows: GCTTTGAGTCCATCCACGAT and CCTACCCACAGTCTCTTTT.