

Figure S1. **TRPM8 stimulation by menthol inhibits EC migration and adhesion.** (A) Surface biotinylation experiment performed on HMECs treated or not with 10  $\mu$ M icilin. (B) The effect of menthol on HMEC migration, tubulogenesis, and adhesion. (Bi) Plot of the percentage of migration of HMECs in control condition (growth medium) and treated with 250  $\mu$ M menthol. The asterisk represents significant differences relative control untreated ECs (\*,  $P < 0.05$ , Wilcoxon test). (Bii and Biii) Quantification of tubulogenesis (Bii) or cell adhesion (Biii) of HMECs in control or in the presence of 250  $\mu$ M menthol. \*,  $P < 0.05$  (Wilcoxon test). (C) Efficiency of TRPM8 silencing (72 h after transfection) in HMEC or HUVEC transfected with siRNA specific for TRPM8 (siTRPM8). Bars represent the mean of five experiments  $\pm$  SEM. The efficiency is measured as the percentage of remaining protein compared with cells transfected with a nontargeting siRNAs (siCtrl = 100%) as measured by Western blot and quantified using ImageJ software. On the right is a representative picture of Western blot. Actin was used as a loading control.

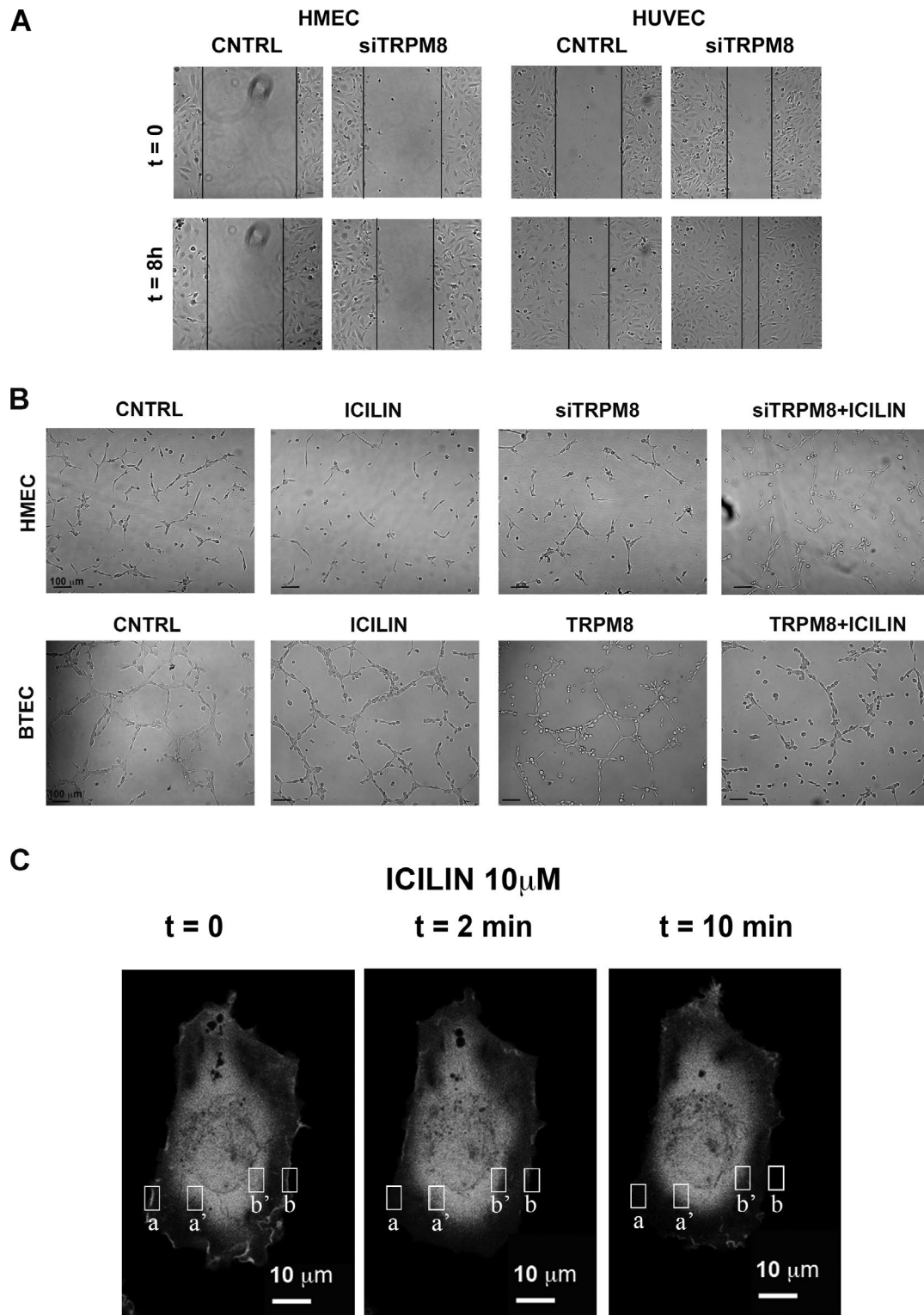


Figure S2. **Endogenous TRPM8 inhibits EC migration and capillary-like structure in Matrigel.** (A) Representative photographs of a wound-healing assay taken at two different time points ( $t = 0$  h, top;  $t = 8$  h, bottom). HMECs or HUVECs transfected with siRNA for TRPM8 200 nM (siTRPM8) or an siRNA for luciferase (CNTRL) 200 nM as a control were treated with control medium or 10  $\mu$ M icilin. Bars, 50  $\mu$ m. (B) Representative photographs of the formation of capillary-like structure in Matrigel by HMECs (top) and BTECs (bottom) at 18 h. HMECs transfected with an siRNA for TRPM8 (siTRPM8) or an siRNA for luciferase (CNTRL) were treated with control medium or 10  $\mu$ M icilin. BTECs transfected with TRPM8 2  $\mu$ g (TRPM8) or 2  $\mu$ g of a GFP control vector (CNTRL) were treated with control medium or 10  $\mu$ M icilin. (C) Quantification of GFP-RBD PM recruitment after icilin 10  $\mu$ M stimulation. Retention of GFP-RBD to the cytosol at different time ( $t$ ) can be given as the relative cytoplasmic retention (R) for a given set of regions of interest as described in Materials and methods. Here, a and b and a' and b' are regions of interest that correspond, respectively to a patch of PM and an adjacent area of cytosol of identical size. For each time imaged (here  $t = 0$ , 2 min, and 10 min), the R factor for the cytosolic retention is calculated as  $R = (I_{cs} - I_m)/I_m$ , where  $I_m$  and  $I_{cs}$  are the fluorescence intensities of the ROIs of membrane and cytosol, respectively, as previously described.

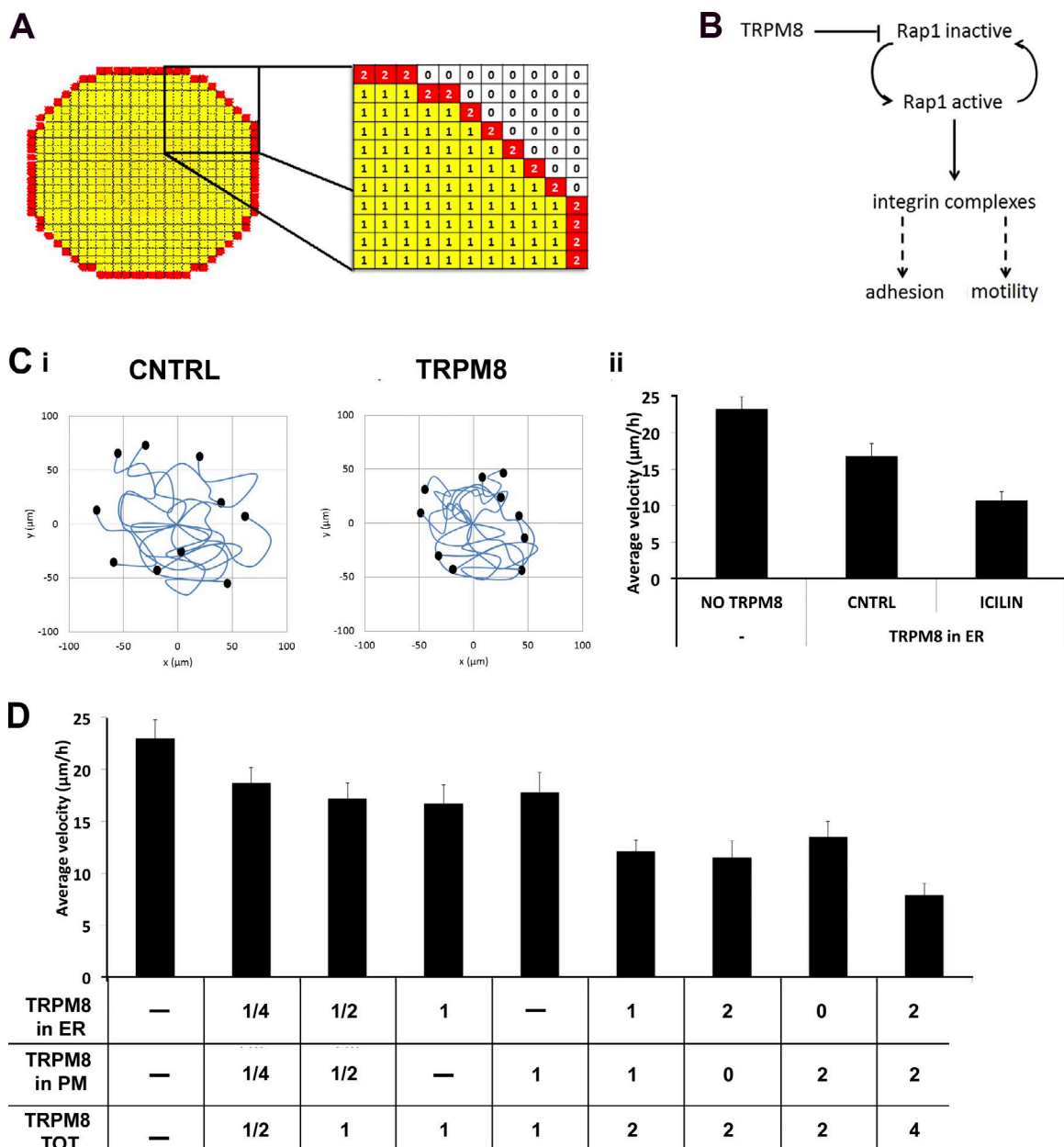


Figure S3. **Multiscale hybrid mathematical model predicts TRPM8 function on EC migration.** (A) Representation of the model cell, compartmentalized in the cytosolic region ( $\Sigma_1$ ) and in the PM ( $\Sigma_2$ ). The extracellular medium is instead identified by spin  $\sigma = 0$ . (B) Diagram of the simplified intracellular cascades included in the model. Rap1 cycle can be inhibited by TRPM8 channels by sequestering the inactive Rap1 form. Active Rap1 mediates the assembly of membrane-bound integrin complexes that enhance cell adhesion and motility. (C) Wind-rose graphs (Ci) and corresponding quantification (Cii) showing 10 migratory tracks (over a time span of 12 h) of simulated cells in the absence or in the presence of basal TRPM8 levels (0.2  $\mu\text{M}$ ). Black circles represent the ending location of cell center of mass. (D) Effect of different cellular locations and quantity of TRPM8 in mean velocity of a simulated cell. In particular, we vary both the total amount of TRPM8 and the ratio between its ER and PM expression. The values are means over 100 simulations. Error bars show standard deviations.

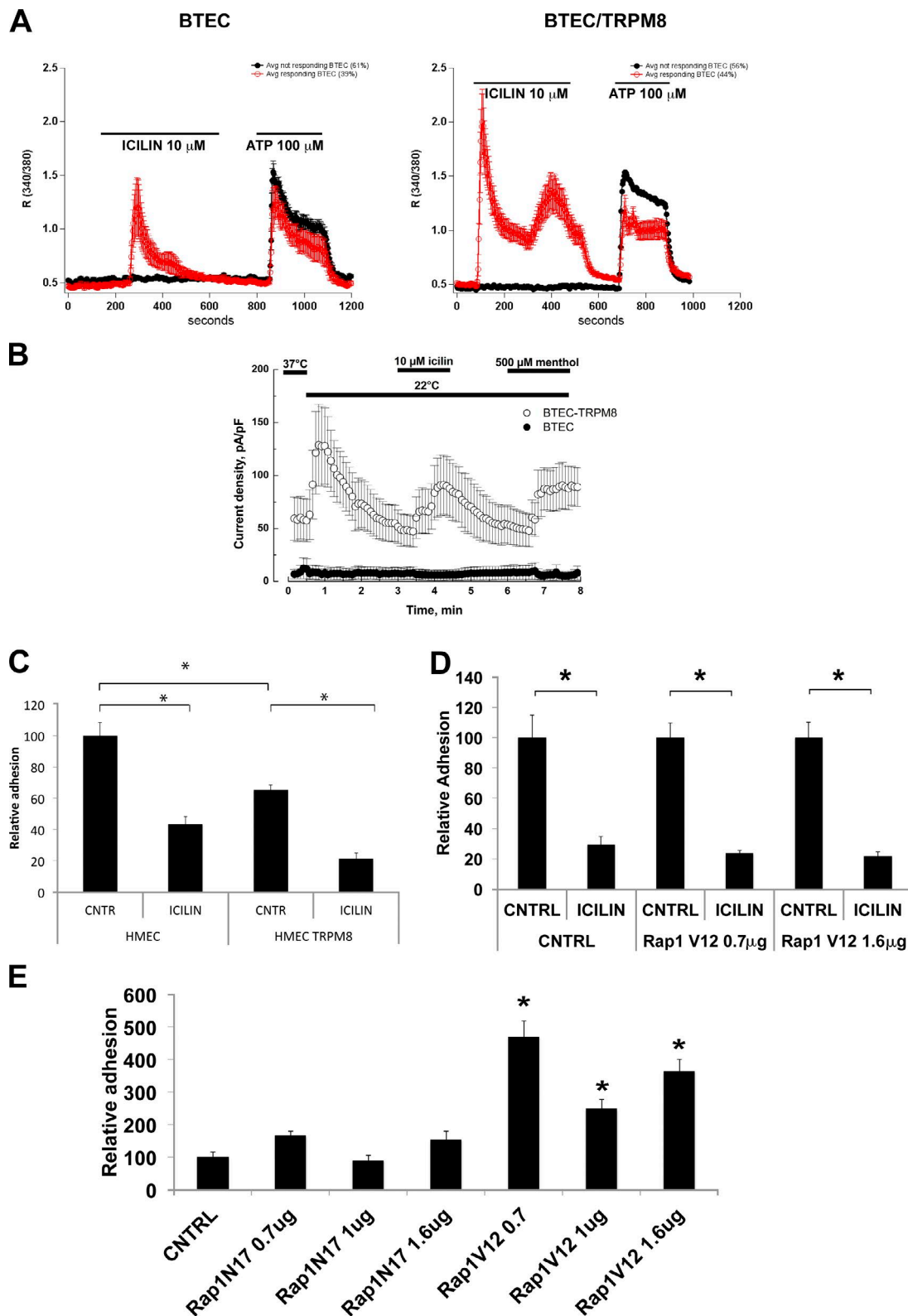


Figure S4. **TRPM8 is functionally expressed in EC.** (A) Time course of mean intracellular  $\text{Ca}^{2+}$  concentration in control (CNTRL) and TRPM8 transfected BTECs. ECs were perfused with 10  $\mu\text{M}$  icilin or 100  $\mu\text{M}$  ATP as a positive control. (B) Representative traces of 10  $\mu\text{M}$  icilin- and 500  $\mu\text{M}$  menthol-evoked currents recorded on BTECs or BTECs overexpressing TRPM8. (C) Quantification of adhesion to FN of HMECs overexpressing TRPM8 (HMEC/TRPM8) or not (HMECs), in the presence or absence of icilin. \*,  $P < 0.05$  (Wilcoxon–Mann–Whitney test). (D) Quantification of adhesion assays on FN coating of HMECs transfected with 0.7, 1.6  $\mu\text{g}$  Rap1V12 and in a control condition treated or not with 10  $\mu\text{M}$  icilin. \*,  $P < 0.05$  (Wilcoxon test). The data are shown as relative adhesion normalizing treatment with icilin to the no-treatment condition. (E) Quantification of adhesion assays on FN coating of HMECs transfected with 0.7, 1, and 1.6  $\mu\text{g}$  Rap1N17 or Rap1V12 plasmids and in control condition. Data are presented as mean  $\pm$  SEM. \*,  $P < 0.05$  (Wilcoxon test). The data are shown as relative adhesion normalizing to control.

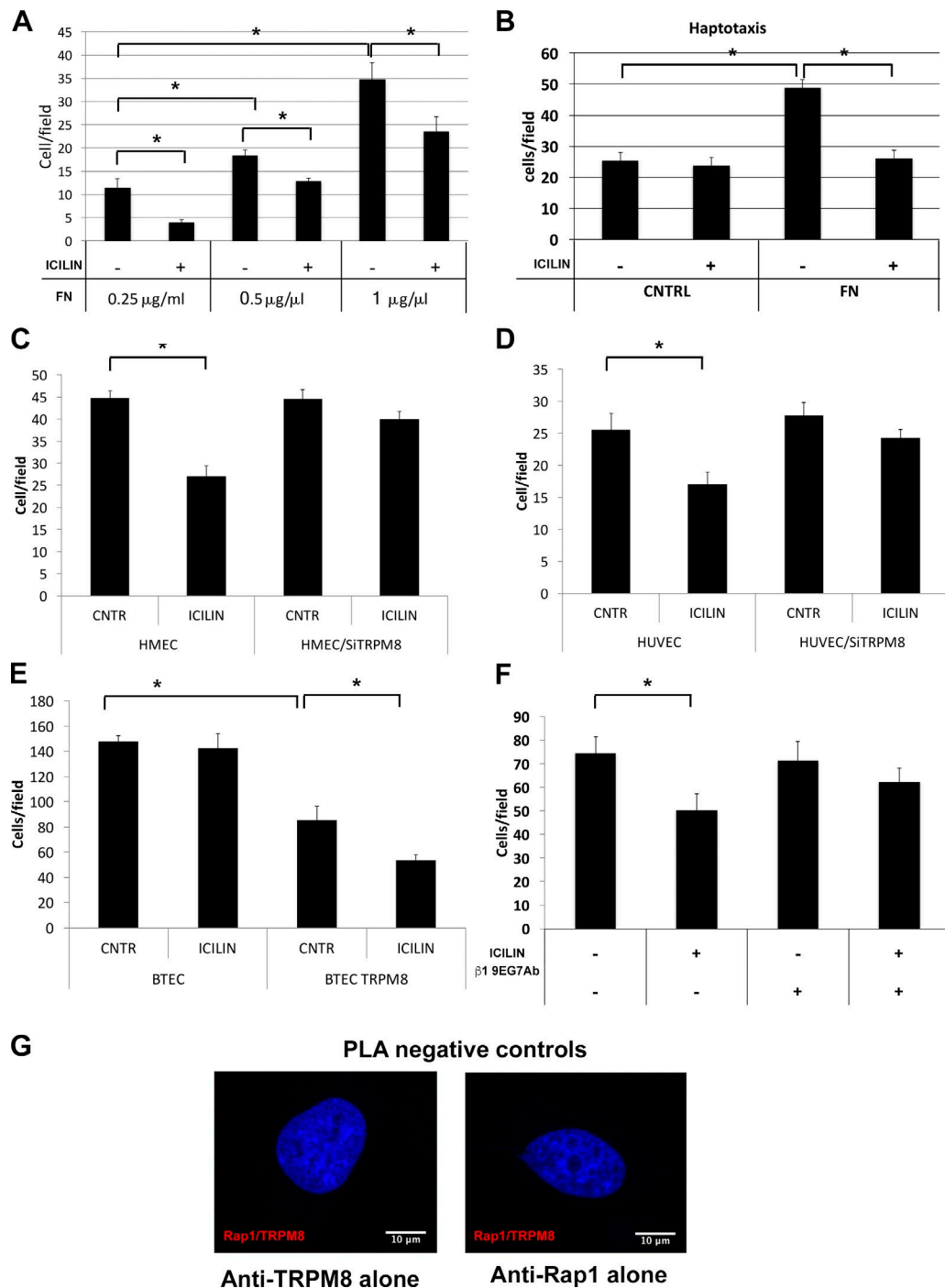


Figure S5. **Endogenous TRPM8 inhibits EC adhesion by inhibiting  $\beta 1$ -integrin.** (A) Quantification of adherent HMECs treated or not with 10  $\mu\text{M}$  icilin on plates coated with 0, 0.25, 0.5, and 1.0  $\mu\text{g}/\text{ml}$  FN. \*,  $P < 0.05$  (Wilcoxon–Mann-Whitney test). (B) Quantification of haptotaxis of HMECs treated or not with 10  $\mu\text{M}$  icilin on 1  $\mu\text{g}/\text{ml}$  FN or control substrate. \*,  $P < 0.05$  (Wilcoxon–Mann-Whitney test). (C) Quantification of adhesion to FN of HMECs silenced for TRPM8 (HMEC/siTRPM8) or not (HMECs), in the presence or absence of icilin. (D) Quantification of adhesion to FN of HUVECs silenced for TRPM8 (HUVEC/siTRPM8) or not (HUVECs), in the presence or absence of icilin. (E) Quantification of adhesion to FN of BTECs overexpressing TRPM8 (BTEC/TRPM8) or not (BTECs), in the presence or absence of icilin. \*,  $P < 0.05$  (Wilcoxon–Mann-Whitney test). (F) TRPM8 acts through the  $\alpha 5\beta 1$  integrin signaling pathway. Quantification of adhesion to FN of HMECs treated (or not) with icilin, the integrin-activating 9EG7 antibody, both in the presence of 0.1 mM  $\text{MnCl}_2$ . Data are presented as mean  $\pm$  SEM. \*,  $P < 0.05$  (Wilcoxon–Mann-Whitney test). Data represent adhesion normalized to the control untreated condition. All data represent not normalized cell adhesion/field. (G) Negative controls for PLA experiments. PLA was performed incubating cells with both primary antibodies separately. No PLA puncta are detectable.

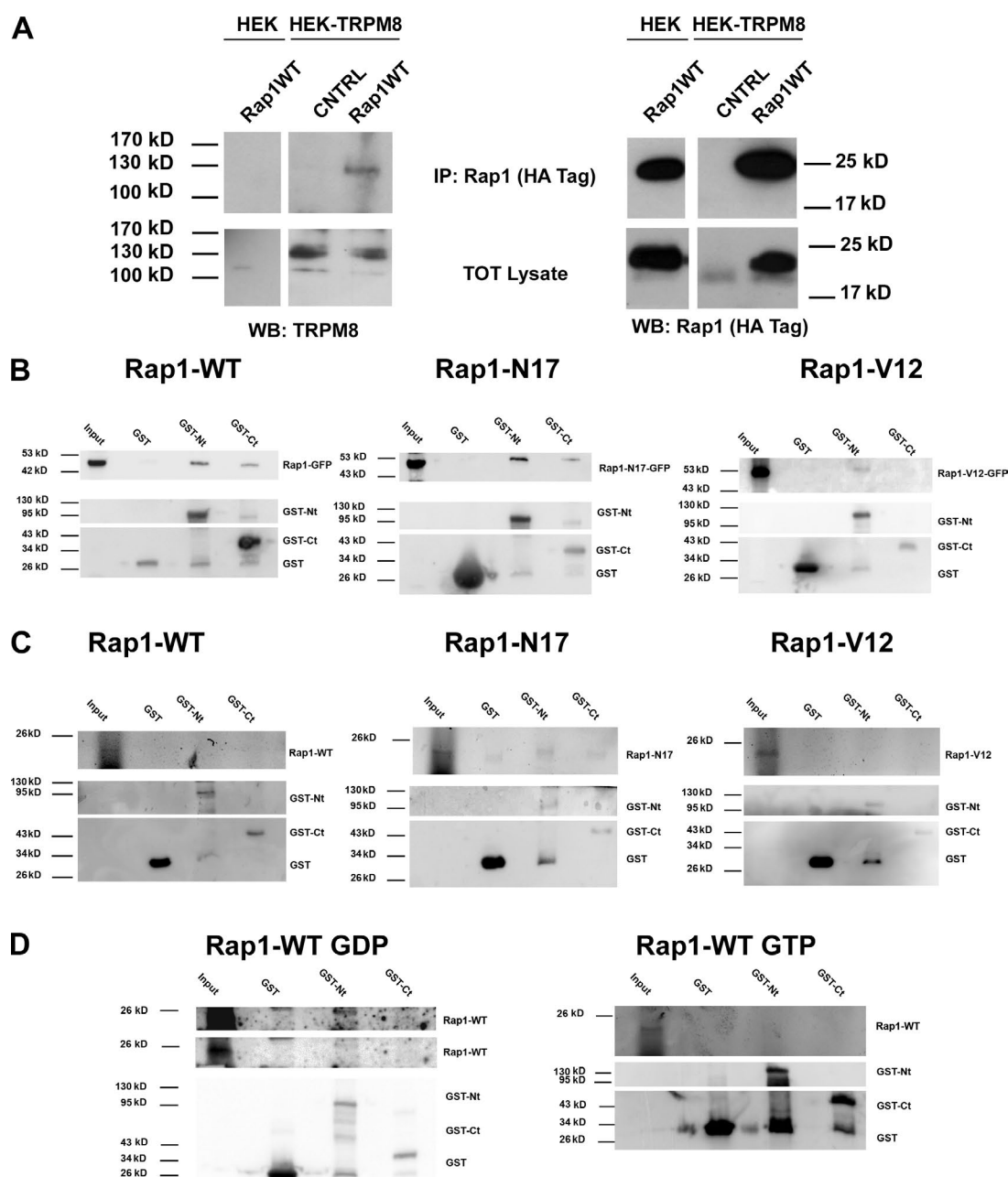
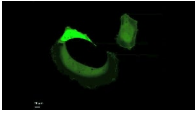
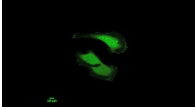


Figure S6. **The GDP-bound form of Rap1 interacts directly with the TRPM8 N terminus.** (A) Representative immunoprecipitation experiments. Expression vectors encoding Rap1-WT-HA were transfected into HEK-overexpressing TRPM8 cells. Cell lysates were immunoprecipitated (IP) with an anti-HA antibody and immunoblotted with antibodies against TRPM8 and HA. (B) Immunoblot against GFP (top) showing the GST pull-down assay between HEK cells lysate overexpressing GFP-Rap1 (left), GFP-Rap1-N17 (middle), or GFP-Rap1-V12 (right) and GST or GST fused to the TRPM8 N-terminal tail (GST-Nt) or C-terminal tail (GST-Ct). 10% of cell lysates were used for the input of the GST pull-down (top panel). Anti-GST or anti-GFP Antibodies were used to visualize the interaction of Rap with GST-fused N- and C-terminal tail. (C) GST pull-down assay of in vitro translated Rap1-WT, Rap1-N17 or Rap1-V12 using FluoroTect and GST or GST fused to the TRPM8 N-terminal tail (GST-Nt) or C-terminal tail (GST-Ct). 10% of the in vitro translated Rap1 was used for the input of the GST pull-down (top). Bodipy-FL-stained gel are shown for the expression of Rap1, whereas GST antibody was used to visualize the GST-fused N- and C-terminal tail. (D) The GST pull-down assay was repeated by loading in vitro translated RAP1-WT with 1 mM GDP (left) or 0.1 mM GTP (right). Anti-GST or anti-Rap1 antibodies were used to visualize the interaction of Rap with GST-fused N- and C-terminal tail. All the gels are representative of at least three independent experiments.



Video 1. **Live-cell imaging by confocal microscopy of ECs expressing GFP-RBD<sub>RalGDS</sub> probe in control (siCNTRL).** The video shows EC expressing GFP-RBD<sub>RalGDS</sub>. 10  $\mu$ M icilin was added after 10 min. The video was acquired by capturing one frame per minute.



Video 2. **Live-cell imaging by confocal microscopy of ECs expressing GFP-RBD<sub>RalGDS</sub> probe in cells silenced for TRPM8 (siTRPM8).** The video shows EC expressing GFP-RBD<sub>RalGDS</sub>. 10  $\mu$ M icilin was added after 10 min. The video was acquired by capturing one frame per minute.