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Supplementary: The Influence of Capsaicin on the Integrity of Microvascular Endothelial Cell Monolayers

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Digital holographic microscopy (DHM)

For imaging studies with DHM, cells were seeded in Petri dishes with glass lid (ibidi µ-Dish with glass lid, ibidi GmbH, Munich, Germany) in supplemented ECM at a density of 2.1 x 105 cells/dish and were allowed to attach overnight. The following day the medium was replaced by ECM lacking fetal calf serum but containing 50 µM capsaicin in 20 mM HEPES buffer. DHM imaging was carried out using an inverted microscope (iMIC, Till Photonics, Gräfelfing, Germany) with an attached DHM module [1] with an incubator set at 37°C. The coherent light source was a Nd:YAG laser (Compass 315 M-100, Coherent, Lübeck, Germany, λ =532 nm). The digital holograms of single confluent cell layers were recorded continuously every 9 min using a 20x microscope lens (Zeiss LD Acroplan 20x/0.4 Korr). Quantitative phase images were reconstructed from the digitally-captured holograms as previously described [2, 3]. Three independent measurements were taken in each experiment.

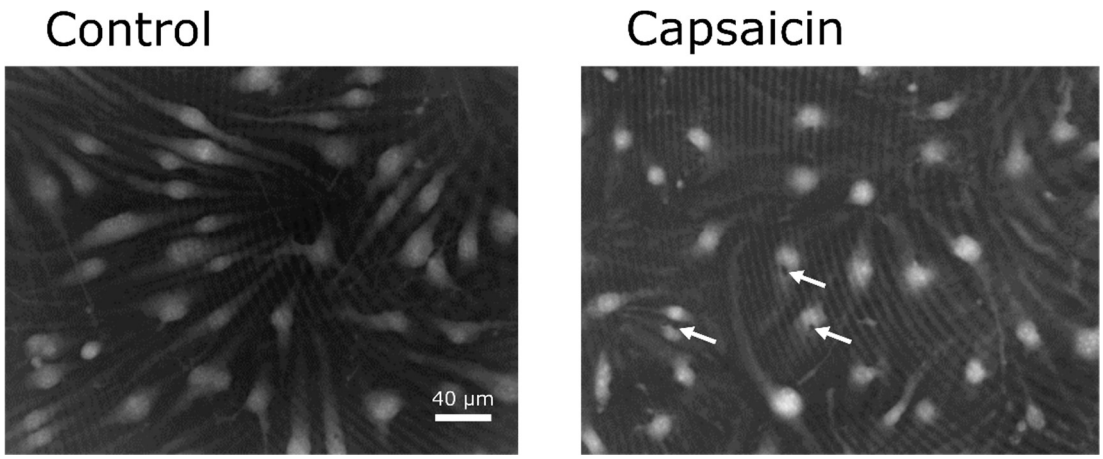

Figure S1. Representative DHM quantitative phase images of cEND cell monolayers. Cells remained untreated or were treated with 50 µM capsaicin for 16 h. Arrows indicate morphological changes.

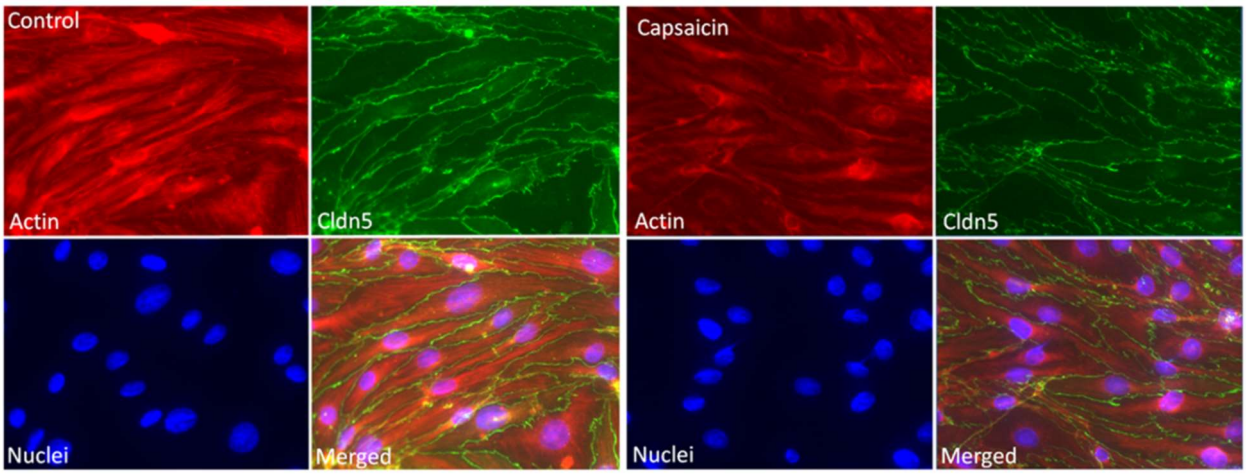

Figure S2. SIFM images of primary mouse brain microvascular endothelial cells treated with capsaicin. Cells remained untreated or were treated with 100 µM capsaicin for 12 h. Nuclei were stained with DAPI (blue), claudin 5 was stained using specific antibodies (green) and actin was stained with TRITC-phalloidin (red), magnification 400x.

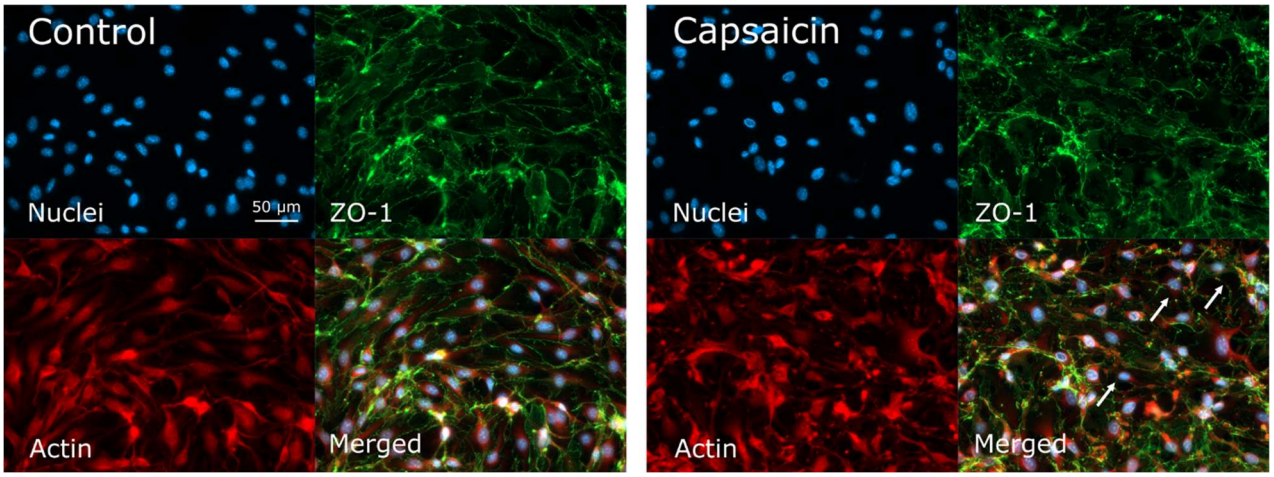

Figure S3. SIFM images of cEND cells treated with capsaicin. Cells remained untreated or were treated with 100 µM capsaicin for 12 h. Nuclei were stained with DAPI (blue), zonula occludens 1 (ZO-1) was stained using specific antibodies (green) and actin was stained with TRITC-phalloidin (red). Arrows indicate changes in protein localization.

Literature Cited

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