# A Vascular Permeability Assay Using An *In Vitro* Human Microvessel Model Mimicking The Inflammatory Condition

Joris Pauty <sup>a,b</sup>, Ryo Usuba <sup>a</sup>, Haruko Takahashi <sup>a</sup>, Junichi Suehiro <sup>c</sup>, Kanoko Fujisawa <sup>a</sup>, Kiichiro Yano <sup>d</sup>, Tomohiro Nishizawa <sup>d</sup>, Yukiko T. Matsunaga <sup>a,b,\*</sup>

<sup>a</sup> Center for International Research on Integrative Biomedical Systems (CIBiS), Institute of Industrial Science, The University of Tokyo, 4-6-1 Komaba, Meguro-ku, Tokyo 153-8505, Japan

<sup>b</sup> LIMMS/CNRS-IIS (UMI 2820), Institute of Industrial Science, The University of Tokyo, 4-

6-1 Komaba, Meguro-ku, Tokyo 153-8505, Japan

<sup>c</sup> Department of Pharmacology and Toxicology, Kyorin University School of Medicine, 6-20-

2, Shinkawa, Mitaka-shi, Tokyo 181-8611, JAPAN

<sup>d</sup> End-Organ Disease Laboratories, R&D Division, Daiichi Sankyo Co., Ltd., 1-2-58, Hiromachi, Shinagawa-ku, Tokyo, 140-8710, Japan

\* Address correspondence to mat@iis.u-tokyo.ac.jp

## Supplementary materials

#### Content:

1. Supplementary method: Immunofluorescence of ZO-1 on a 2D model

2. Figure S1: Immunofluorescence on 2D model showing the effect of the treatments on the

tight junctions and actin cytoskeleton

## 1. Supplementary method

## Immunofluorescence of ZO-1 on a 2D model

The primary antibody targeting ZO-1 (Rabbit pAb, 40-2200) was purchased from Life Technologies - Thermo Fisher Scientific, Co. (Waltham, MA, USA). Immunofluorescence was performed as described in the Materials and Methods section of the manuscript with a dilution 1:100 of the primary antibody.

#### 2. Supplementary Figure



Figure S1. Immunofluorescence on 2D model showing the effect of the treatments on the tight junctions and actin cytoskeleton

(A, B) Immunofluorescence of ZO-1(green), actin cytoskeleton (red) and nucleus (blue) on the 2D-model treated the same way as the microvessel model; scale bars: 20  $\mu$ m. (A) ZO-1 and actin cytoskeleton in absence of treatment. (B) *Left*: effect of 30-min incubation with 10 mM EDTA followed by 60-min incubation with EGM-2; *middle and right*: effect of 30-min treatment with 100 U/mL thrombin after pre-treament with EDTA and incubation with EGM-2 or 200  $\mu$ M 007.