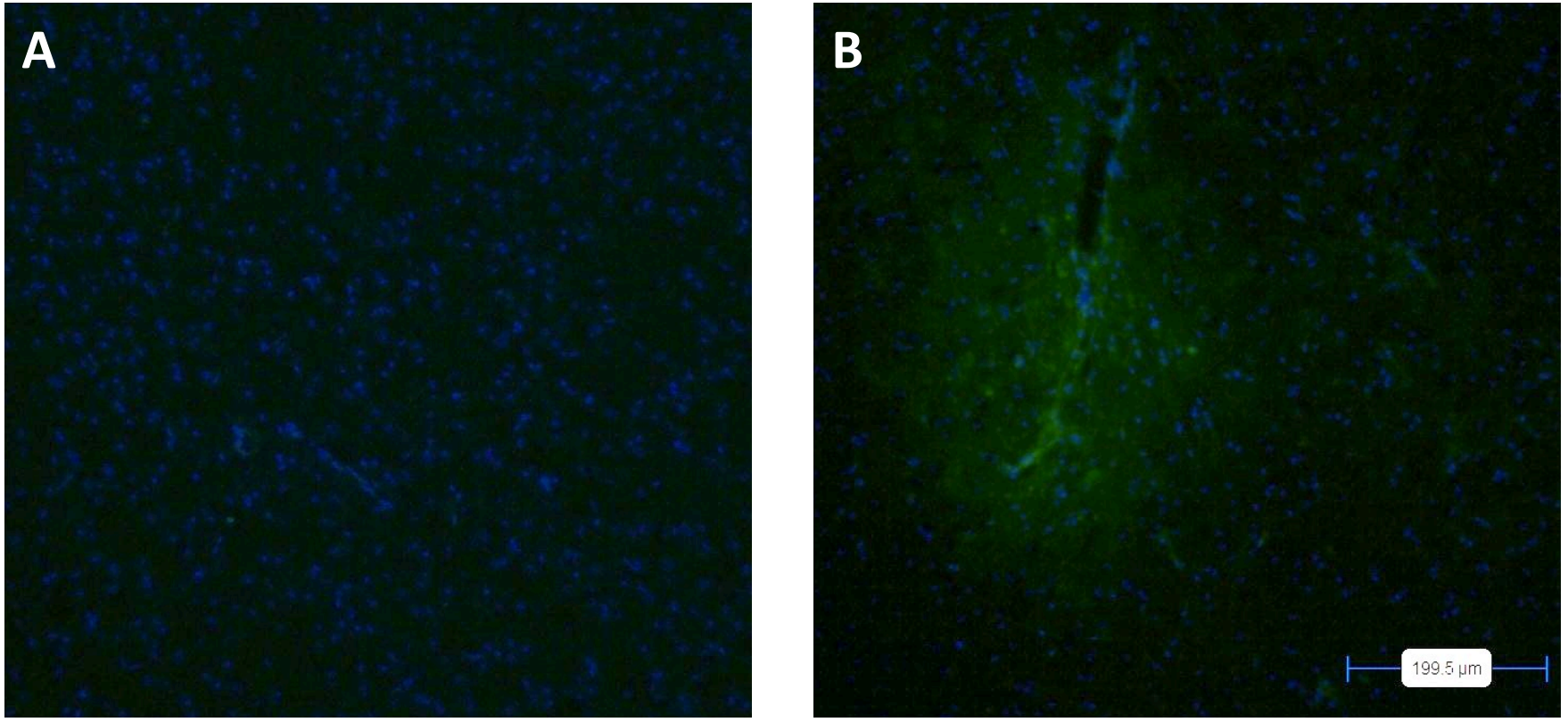
Supplemental data

Supplemental figure 1

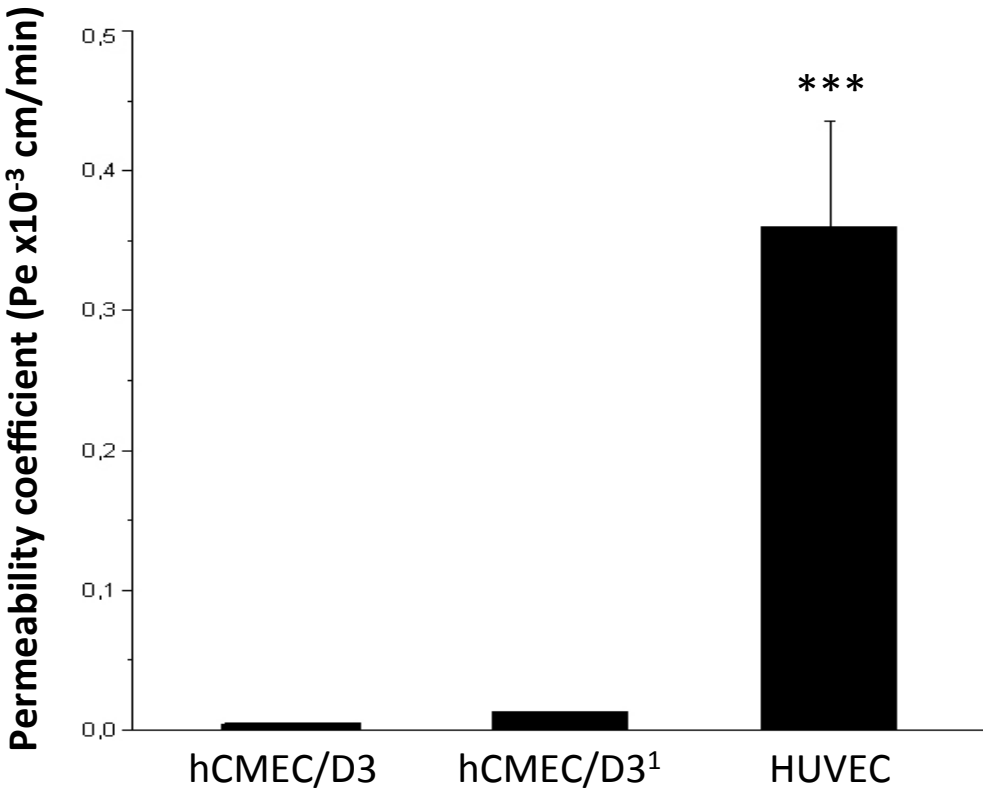


In vivo study for BBB disruption

Representative FITC-dextran perfusion in cortical capillary networks from mice 4 h after MCA occlusion, comparing non-ischemic area (A) to ischemic area (B). Bar = 200µm.

C57BL6/J male mice of 10 weeks (n=4) were anesthetized with isoflurane mixed with air (4% for induction, 1% during surgery), under spontaneous respiration for focal cerebral ischemia. Intraluminal middle cerebral artery (MCA) occlusion was achieved by introducing a monofilament 7.0 (Doccol corporation) through the right internal carotid up to the bifurcation with the MCA. The monofilament was maintained for 4 hours and then was removed to allow reperfusion. Immediately after reperfusion, 200 mL of an intravascular fluorescent marker (FITC-Dextran, Molecular weight: 70000 Da) at a concentration of 12.5 mg/mL was injected through the right internal carotid. One minute after the injection, the mice were sacrificed with intraperitoneal injection of a lethal dose of pentobarbital. The brain was removed and fixed with 3.7% paraformaldehyde overnight and then with 20% sucrose for 24 hours. The brain was frozen in OCT and sectioned (10 mm) at -20°C. Intraparenchymal extravasation of FITC-Dextran was evaluated by fluorescence microscopy. Animal care and experimental protocols were approved by the Animal Ethics Committee of the INSERM-University Paris 7, authorization 2010/13/698-0002

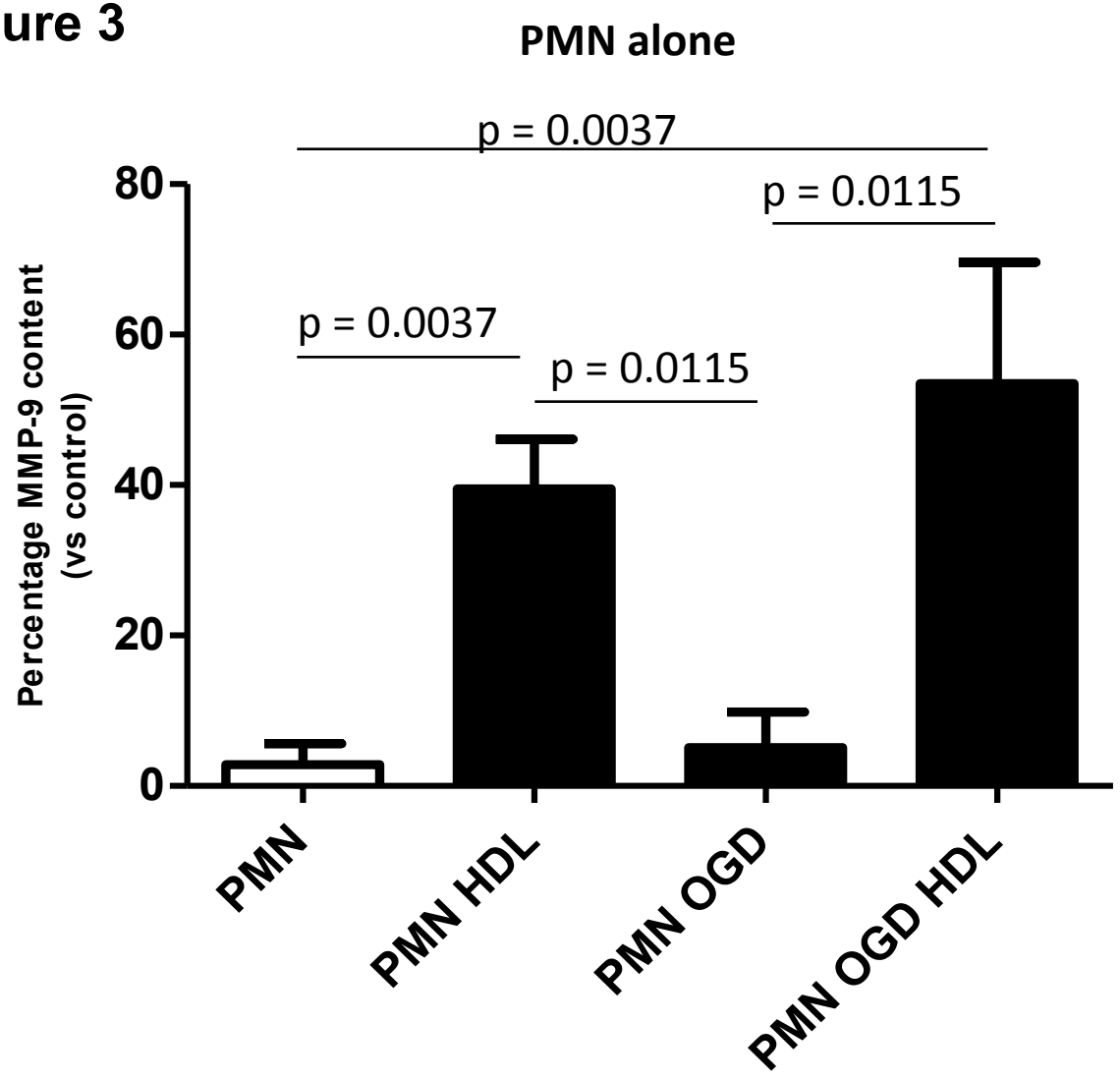
Supplemental figure 2



(n≥9)
(***P<0.0001).

Comparison of permeability of the blood brain barrier (BBB) model used in our study (hCMEC/D3 cells) compared to previously reported results of [1]Weksler et al. (FASEB J. 2005; 19:1872-4) using the same model, and to that of human umbilical vein endothelial cells (HUVEC). Results were obtained using 70kDa-Dextran-FITC in triplicate in 3 independent experiments.

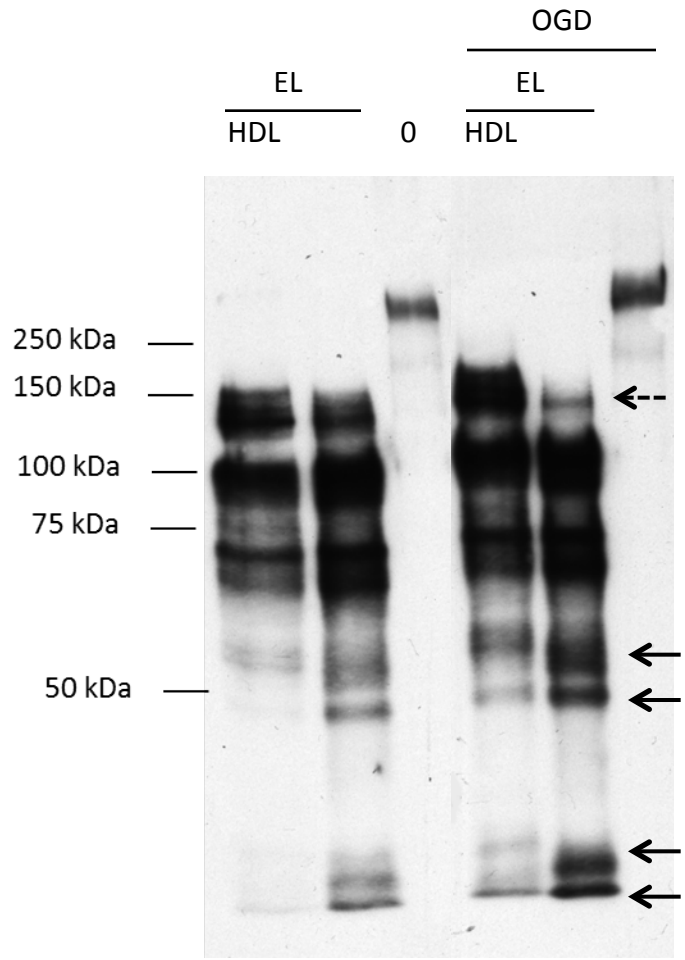
Supplemental figure 3



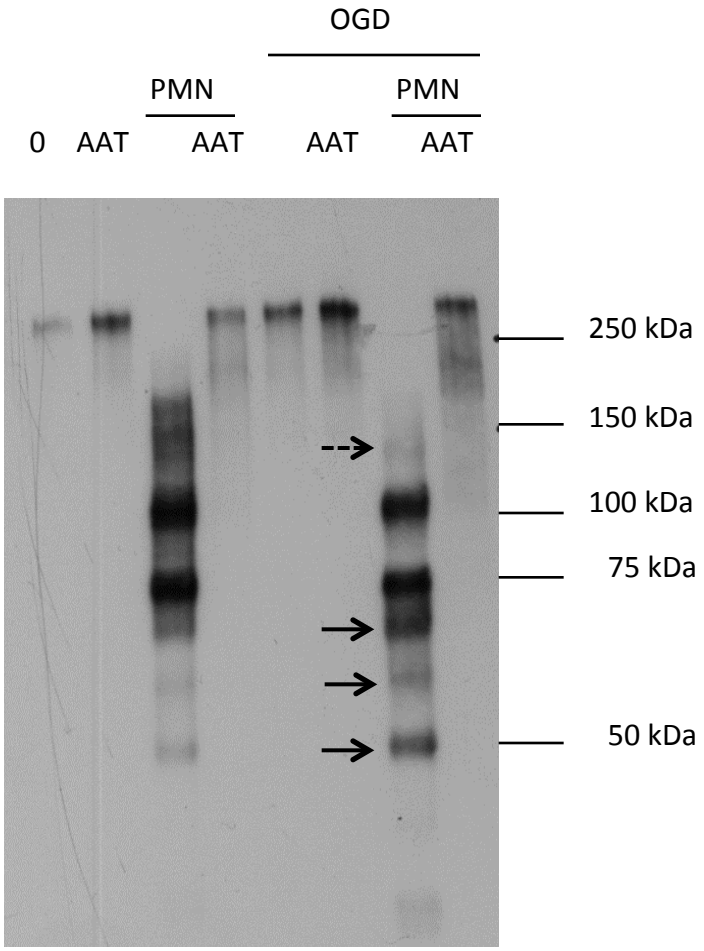
Determination of gelatinolytic activity of MMP-9 in the supernatant of PMNs alone (1.10^6 cells/mL) treated for 4 hours with HDLs (0.4g/L) under control (normoxic and normo-glycemic conditions) or OGD (oxygen-glucose deprivation) conditions (n≥3). After gelatin zymography, the lysis areas corresponding to MMP-9 were quantified by densitometry and expressed as % of MMP-9 detected in the supernatant of PMNs activated by 1µM fMLP (1.10^6 cells/mL). Results were obtained in triplicate in 3 independent experiments.

Supplemental figure 4

A- purified elastase (EL) on cellular fibronectin

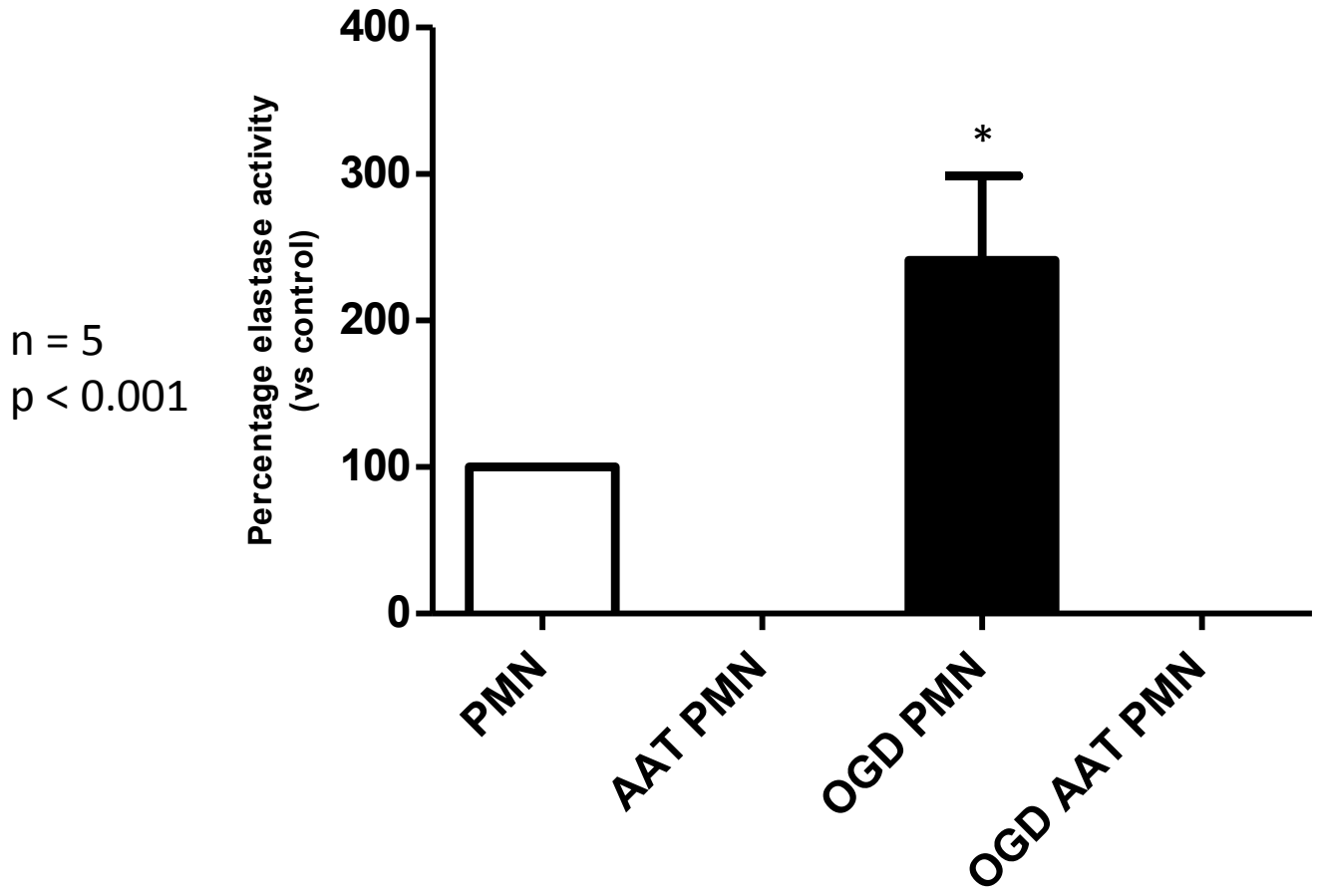


B- AAT PMN-induced fibronectin degradation



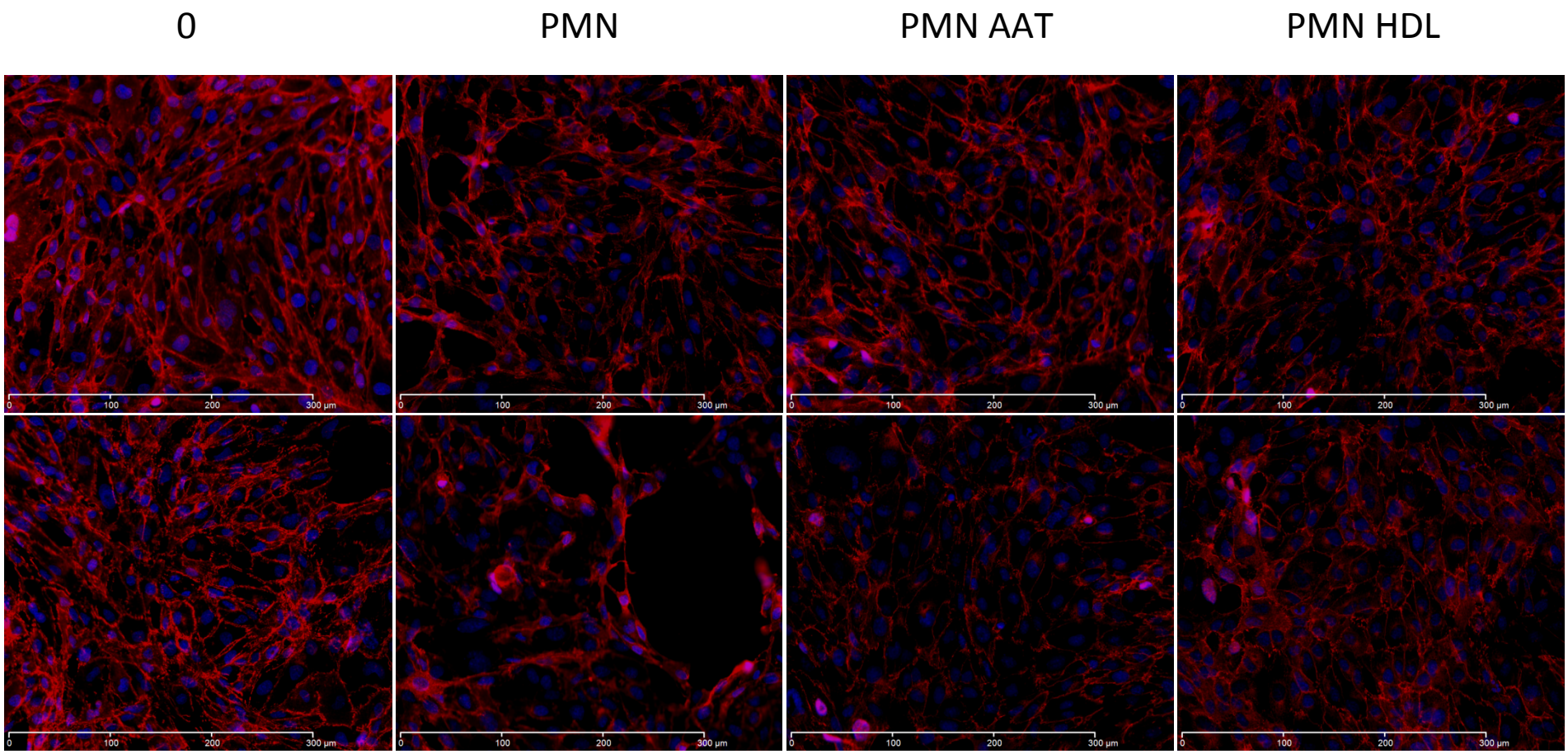
Western Blot for detection of soluble fibronectin/fragments in the supernatant of cells after treatment with A, HDLs (0.4g/L) ± EL (100nM) and B, PMNs (1.10^6 cells/mL) ± alpha-1 anti-trypsin (AAT, 1µM) for 4 hours under OGD (oxygen-glucose deprivation) or non-OGD conditions. Results are representative of three independent experiments. Plain arrows show more intense degradation fragments in OGD versus non-OGD conditions. Dashed arrow shows a decreased amount of high molecular weight fragments of fibronectin.

Supplemental figure 5



Determination of elastase activity in the supernatant of endothelial cells incubated with PMNs (1.106 cells/ mL) ± AAT (1µM) for 4 hours under OGD and non-OGD conditions. No elastase activity could be detected without PMNs. Results are expressed as % of elastase activity detected in the supernatant of endothelial cells + PMNs. *P<0.05 vs all groups.

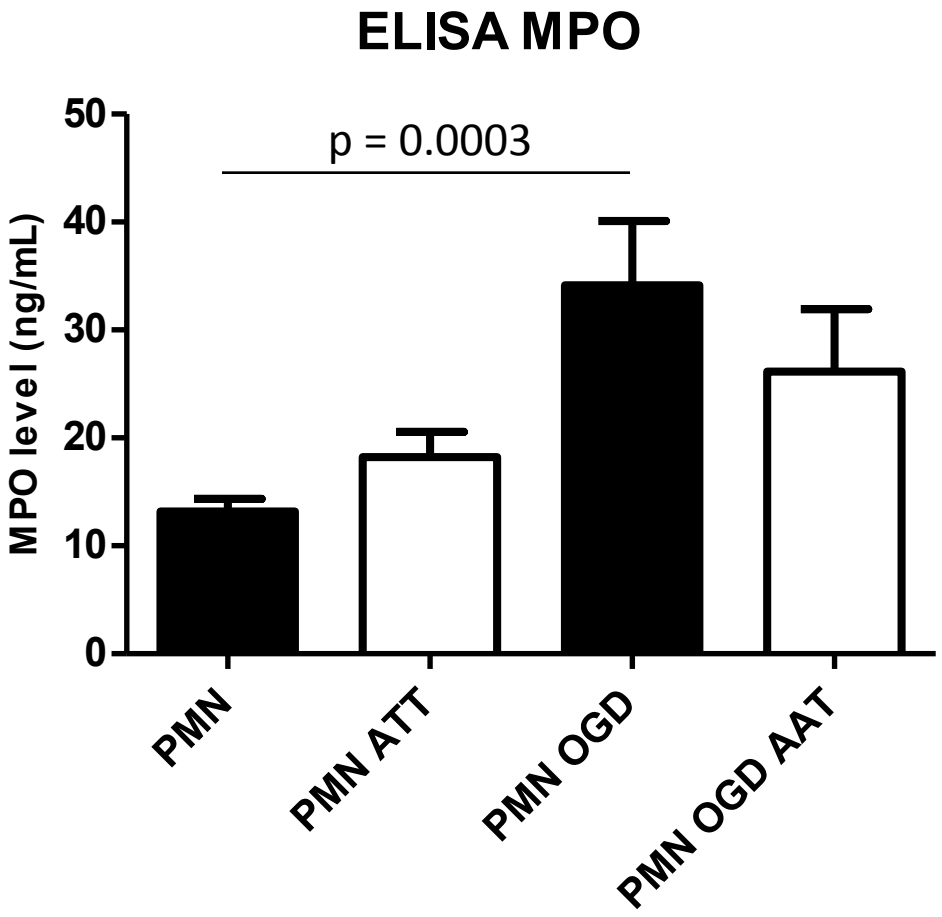
Supplemental figure 6



Immunofluorescent staining of VE-Cadherin (red) after treatment with HDLs (0.4g/L) or AAT (1µM) ± PMN (1.10^6 cells/mL) for 4 hours under OGD and non-OGD conditions. Nuclei are stained with DAPI (blue). Results are representative of three independent experiments.

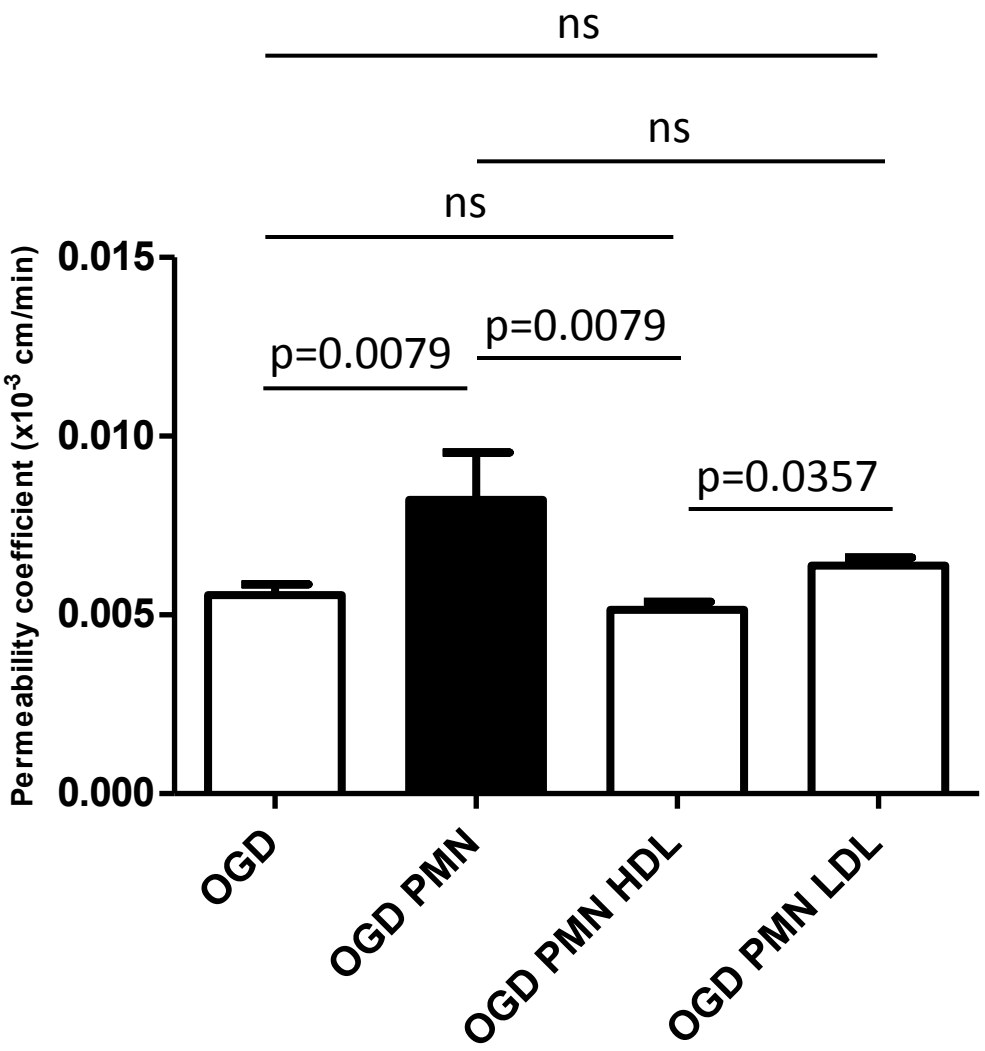
Supplemental figure 7

n ≥ 8



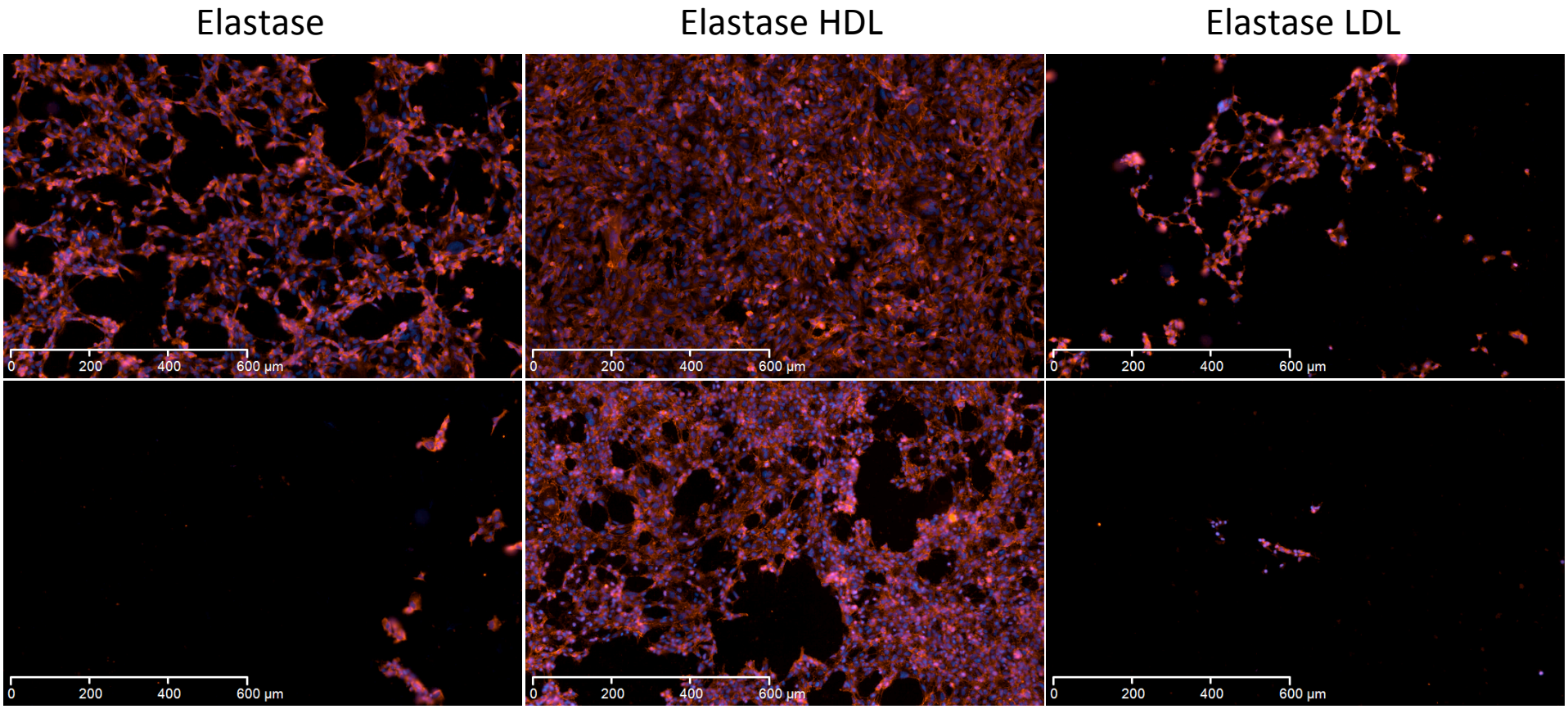
Determination of MPO levels by ELISA in the supernatant of endothelial cells incubated with PMNs (1.10^6 cells/mL) ± AAT (1µM) for 4 hours under OGD and non-OGD conditions.

Supplemental figure 7A



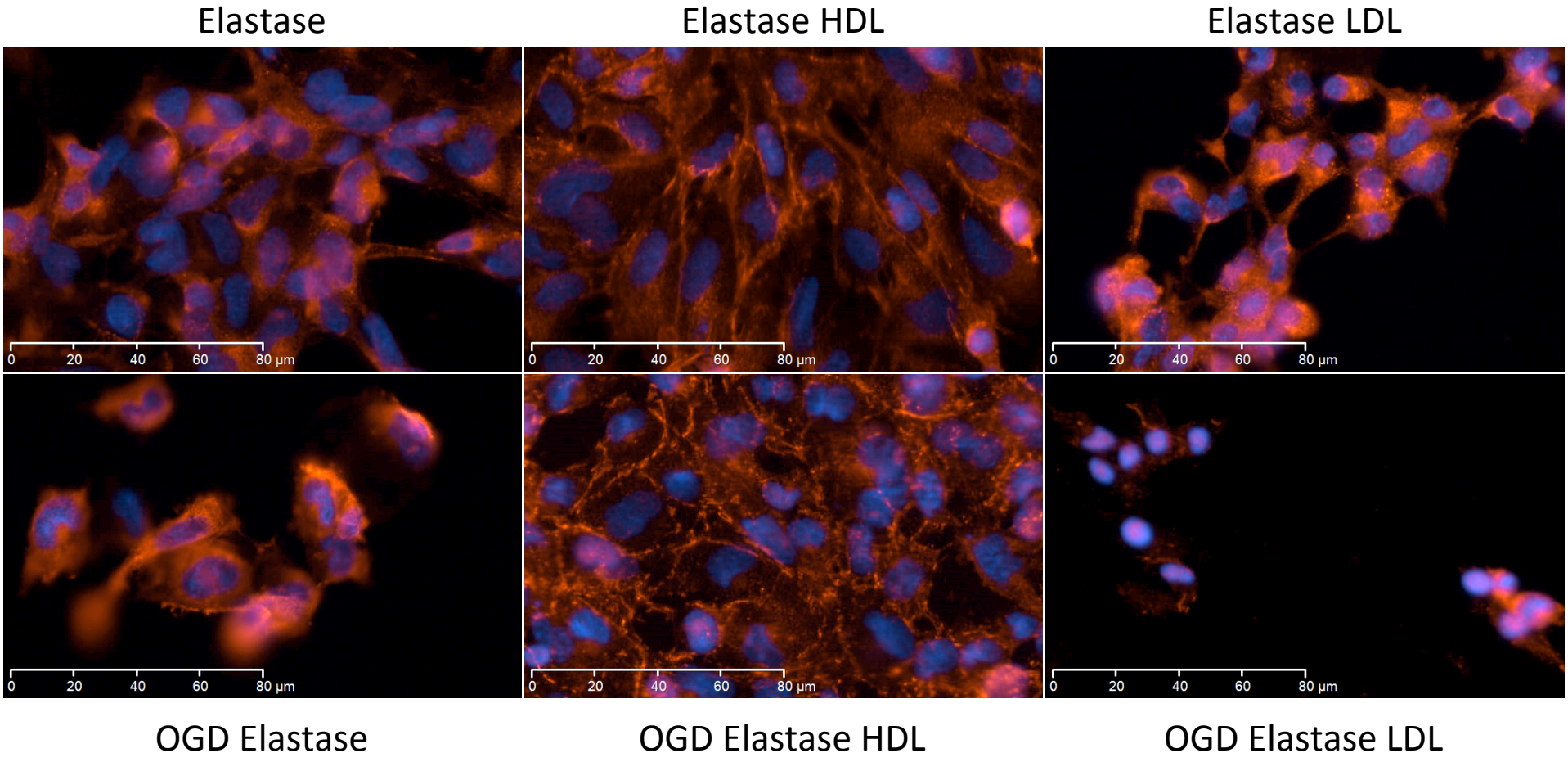
In vitro effects of low density lipoproteins (LDL) on permeability measured in a BBB model (hCMEC/D3 cells) under oxygen-glucose deprivation (OGD) condition. Cells were incubated with HDL (0.4g/L) or LDL (0.4g/L) ± PMNs (1.10^6 cells/mL) for 4 hours (n≥3 for each condition). Results are presented as mean ±SD.

Supplemental figure 8B



Immunofluorescent staining of VE-Cadherin (red) after treatment with elastase ± HDLs (0.4g/L) or LDL (0.4g/L) for 4 hours under OGD and non-OGD conditions. Nuclei are stained with DAPI (blue). Results are representative of three independent experiments.

Supplemental figure 8C



Immunofluorescent staining of VE-Cadherin (red) after treatment with elastase ± HDLs (0.4g/L) or LDL (0.4g/L) for 4 hours under OGD and non-OGD conditions. Nuclei are stained with DAPI (blue). Results are representative of three independent experiments.