In-depth proteomics identifies a role for autophagy in controlling ROS-mediated endothelial permeability

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Supporting Information

Table S1. Proteins identified and quantified in HUVECs (total lysate) forming a tightly confluent monolayer.

Table S2. Gene Ontology (GO) category enrichment analysis of proteins regulated when HUVECs form a tightly confluent monolayer

Table S3. Proteins identified and quantified in HUVECs (total lysate) cultured confluent for 4 days upon 4h bafilomycin treatment.

Table S4. Proteins identified and quantified in HUVECs (serum-free conditioned medium) cultured confluent for 4 days upon 4h bafilomycin treatment.

Table S5. Proteins identified and quantified in HUVECs (total lysate) silenced for ATG5.

Table S6. Two dimension (2D) Gene Ontology (GO) category enrichment analysis upon autophagy inhibition with si-ATG5 or bafilomycin.

Figure S1. Proteomic analyses. (A) Time resolved SILAC-based proteomic analysis used to identify cellular processes regulated in HUVEC while assembling into a physiologically permeable monolayer. (B) SILAC-based proteomic analysis used to identify proteomic alterations which occur in HUVEC upon acute/short term or chronic/long term inhibition of autophagy. Short term inhibition was triggered by 4h bafilomycin treatment while long term inhibition (4 days) by silencing ATG5 (si-ATG5). Control = DMSO-treated cells; siNT = non targeting siRNA.

Figure S2. Autophagy inhibition increases the permeability of EC monolayer. (A-B) Endothelial cell barrier functionality measured by FITC-albumin permeability upon ATG5 siRNA (A) or bafilomycin (bafi) treatment (B). (C) Increased FITC-albumin permeability due to autophagy blockade by bafilomycin is rescued by scavenging the ROS with NAC and vitamin C (NAC + Vit C).