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Response by Mittal et al to Letter Regarding Article, "Neutrophil Activation of Endothelial Cell-Expressed TRPM2 Mediates Transendothelial Neutrophil Migration and Vascular Injury"

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If the intent of the letter by Gagat and Grzamka is to point out that actin cytoskeleton remodeling induces vascular barrier disruption is an important phenomenon, we fully agree[1]. Its quite clear tight junctions (TJs) have a role in regulating endothelial barrier in the brain endothelial cells although their role is less important in the continuous endothelium such as in the heart and lungs[1]. Recent knock out mouse model studies[2,3] (including ours[4]) have provided compelling evidence that adherens junctions (AJs) or VE-cadherin junctions are important in regulating leukocyte transmigration and vascular inflammation. Our study[4] focused exclusively on the role of endothelial TRPM2 in signaling PMN trans-endothelial migration. We addressed the mechanism by which PMN-derived ROS activate TRPM2 mediated Ca^{2+} influx in endothelial cells and thereby disrupt the VE-cadherin junctions to promote PMN migration[4].

The authors in referring to their unpublished work appear to be making the point that “reorganization of F-actin polymerization into linear stress fibers inhibits formation of intercellular junctions and/or generates forces breaking down cell-cell connections". We did not disagree. But we did not address this issue, and thus cannot comment on it. Also they mention that "cellular response on hydrogen peroxidase is dismissed after knockdown of *Trpm2*." It’s not a matter of dismissing this work rather we didn't study the response to peroxiodase! They further mention “AJ proteins, and probably TJ proteins, interact(s) with prominent thick bundles of cytoplasmic actin filaments that represent stress fibers, what may rather suggest strong anchoring of cells as a result of loss AJs continuity". We studied AJs, the primary barrier in endothelial cells regulating permeability and trafficking of leukocytes[5,6]. While the transcellular route of leukocyte transmigration is important, its contribution relative to the paracellular pathway is substantially less[7]. We appreciate learning about their results in human carotid artery endothelial cells (pHCAECs) in which they studied the response to TNF-α, and apparently obtained data similar to ours. Our results obtained using genetic mouse models and human microvascular endothelial cells

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provide fundamental insights how PMNs trigger their own migration and vascular inflammation through activating the endothelial cell-expressed TRPM2 channel.

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