Introduction:
Endothelial dysfunction plays a major role in the sepsis related organ dysfunction, and is featured by vascular leakage. AMP-activated protein kinase (AMPK) is known to regulate actin cytoskeleton organization and interendothelial junctions (IEJs), contributing to endothelial barrier integrity. We have already demonstrated its role in defence against sepsis induced hyperpermeability [1], but the underlying mechanisms remain unknown. This project aims to identify molecular targets involved in the beneficial action of AMPK against endothelial barrier dysfunction.

Methods:
Experiments have been performed in human microvascular dermal endothelial cells. α1AMPK activity has been modulated via the use of a specific siRNA or treatment by two pharmacological AMPK activators (AICAr, 991). We have investigated the effect of this modulation on the expression/phosphorylation of Connexin 43 (Cx43) and Heat shock protein 27 (HSP27), two proteins playing a key role in maintenance of IEJs and actin dynamics respectively.

Results:
We show that α1AMPK is required to sustain the level of Cx43 expression as it was drastically reduced in cells transfected with a siRNA targeting specifically α1AMPK. Regarding HSP27, its expression level was not affected by α1AMPK deletion. However, both AMPK activators increased its phosphorylation on Ser82, in an α1AMPK-dependent manner, while they had no effect on Cx43. Our results also reveal that HSP27 phosphorylation concurred with the appearance of actin stress fibers at the periphery of cells, suggesting a beneficial role for p-HSP27 as well as F-actin stress fibers in vascular barrier function through reinforcing the endothelial tethering.

Conclusion:
Our work identifies the regulation of Cx43 expression and HSP27 phosphorylation as potential protective responses underlying the beneficial action of AMPK against endothelial barrier dysfunction. AMPK could consequently represent a new therapeutic target during sepsis.

References: