



Online Suppl. Fig. 1. NFATc3 significantly regulates iNOS expression and moderately affects NF-κB activation: HLL BMDM cells were pretreated with ethanol or QNZ; 11R-VEET or 11R-VIVIT and stimulated with 100 ng/ml LPS. The cell lysates were analyzed for (A) expression of iNOS, (B) Cell culture supernatants were analyzed for NO release as described in Figure 1 C, cell lysates from same experiment were analyzed for Lucifer's reporter activity using Promega Luciferase Assay. * $p < 0.01$ LPS compared to control or ** $p < 0.001$ 11R-VIVIT/QNZ + LPS compared to LPS treatment alone.

Supplementary Data

The transcription factor NFATc3 modulates the function of macrophages in sepsis

Ravi Ranjan¹, Jing Deng², Sangwoon Chung², Yong Gyu Lee², Gye Young Park³, Lei Xiao¹, Myungsoo Joo⁴, John W. Christman² and Manjula Karpurapu²

Supplementary Method

The HLL BMDM were treated either with LPS (100 ng/ml) alone or and with ethanol (control), 11R-VIVIT (5 μ M), and NF- κ B inhibitor - QNZ (10 nM) for 12 h. Cells were lysed with 1xRIPA buffer, and protein were immuno-blotted for iNOS and β -actin. Culture supernatants were used for enumerating the nitrite amount. Luciferase activity was measured by adding 20 μ l cell lysate to 100 μ l of freshly prepared luciferase assay buffer (Promega, WI, USA), with a Glomax20/20 luminometer (Promega) and expressed NF- κ B Luciferase units.

Supplementary Result

To check the specificity of the inhibitor peptide 11R-VIVIT, the BMDM from HLL mice were cultured and treated with the NFAT inhibitor peptide 11R-VIVIT, and the NF- κ B inhibitor QNZ [6-Amino-4-(4-phenoxyphenylethylamino)quinazoline]. QNZ inhibits the activation of the transcription factor NF- κ B and has been used to investigate NF- κ B signaling. The HLL BMDM isolated from the transgenic reporter mouse strain HLL, is engineered to carry the proximal 5'-human immunodeficiency virus (HIV-1) long terminal repeat (LTR) driving the expression of *Photinus* luciferase complementary DNA (cDNA) which is sensitive to NF- κ B mediated signaling [1]. The 11R-VIVIT treatment significantly decreased the expression of iNOs, whereas with the QNZ it was moderate (Supplementary Figure 1A), similar results were observed with the nitrite release (Supplementary Figure 1B). However the 11R-VIVIT marginally decreased the luciferase activity, whereas QNZ treatment totally abrogated luciferase expression (Supplementary Figure 1C).

Reference

- 1 Blackwell TS, Yull FE, Chen CL, Venkatakrisnan A, Blackwell TR, Hicks DJ, Lancaster LH, Christman JW, Kerr LD: Multiorgan nuclear factor kappa b activation in a transgenic mouse model of systemic inflammation. *Am J Respir Crit Care Med* 2000;162:1095-1101.