

**P059** Phosphorylation of Ro52 by Bruton's Tyrosine Kinase (Btk).  
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Ro52 is a 52 kDa protein that contains a RING-finger in its N-terminal domain and is suspected to function as an E3 ligase. Studies have shown that Ro52 is capable of ubiquitinating itself which does not target it for proteosomal degradation. Regulation of Ro52 activity has not been reported to date. Immunoprecipitation of tyrosine phosphorylated proteins from LPS activated THP1 monocytic cells allowed us to identify Ro52 by mass spectrometry. Furthermore, immunoprecipitation of Ro52 from THP1 cells shows phosphorylation in response to stimulation by LPS. Btk is a member of the Tec family tyrosine kinases, which are multi-domain proteins involved in haematopoietic signalling. An association between Ro52 and Btk was confirmed by precipitation of Btk using His-tag Ro52 immobilized on Ni<sup>2+</sup> agarose beads. Bone marrow derived-macrophages from knock-out Btk and wild-type mice show differential phosphorylation of Ro52 upon stimulation by LPS. Three possible phosphor-accepting tyrosines were identified at positions 343, 388 and 393. Mutants of these tyrosines can be used to assess the function of Ro52. Moreover, the Btk inhibitor LFM-A13 can be used to investigate the endogenous tyrosine phosphorylation of Ro52 in cells treated with LPS. Our study may open a new avenue for determining the regulation of Ro52.