

## **PIR-B/Lilrb3 Negative regulation of innate myeloid cell activation**

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**Introduction:** The inflammatory bowel diseases (IBD) Crohn's disease (CD) and ulcerative (UC) colitis are chronic relapsing gastrointestinal (GI) inflammatory diseases that are driven by an aberrant intestinal inflammatory response associated with exaggerated T-cell and macrophage (MØ's) activation directed against luminal antigens. Recently a novel family of molecules known as inhibitory receptors that negatively-regulate the activating signal associated with inflammatory responses has been identified. Paired immunoglobulin-like receptor B (Pir-B/Lilrb3), an inhibitory receptor, is thought to play a vital role in the negative regulation of myeloid cell-derived pro-inflammatory cytokine production. Although the exact mechanism remains unclear, our previous studies indicate that the inhibition of pro-inflammatory cytokine production by Lilrb3 involves a conserved TRAF6-binding domain within the cytoplasmic tail of Lilrb3 and resultant gene expression.

**Methods:** To determine the involvement of Lilrb3 TRAF-6 binding domain in the regulation of innate immune activation, we transfected NIH3T3 Fibroblasts cells with WT Lilrb3-GFP tagged (WT-Lilrb3) or a mutant Lilrb3 with point mutations in the consensus-TRAF6 binding site (Lilrb3<sup>ΔTRAF6</sup>), and assessed NfκB activation (IκB-α degradation and reappearance) and cytokine production following LPS stimulation.

**Result:** Western blot analysis revealed that LPS-stimulation induced IκB-α degradation at 30 minutes and reappearance at three hours in both WT-Lilrb3 and Lilrb3<sup>ΔTRAF6</sup>-NIH 3T3 cells. However, the level of IκB-α degradation was significantly stronger in the Lilrb3<sup>ΔTRAF6</sup>-NIH 3T3 cells compared with the WT-Lilrb3 NIH 3T3 cells.

**Conclusion:** We conclude that the TRAF-6 domain located within the cytoplasmic tail of Lilrb3 contributes to the Lilrb3 inhibitory activity and increased proinflammatory cytokine production.

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