Chapter 5:
Identification of novel feedback regulators of TNF-α-induced NF-κB pathway: Screening of TRIMs

Chronic inflammation is due to the failure in resolving the pathways activating inflammatory mediators and effectors. Therefore, better understanding of regulatory mechanisms controlling pro-inflammatory pathways can provide better insight into chronic inflammatory conditions. TNF-α-induced gene expression has been categorized in early, mid and late response but it is now known whether late response genes also regulate the pathway in a feedback manner to achieve amplification or resolution (Tian et al., 2005). PTM by ubiquitination may have critical role during late response regulation of NF-κB pathway. This study systematically identifies late response TNF-α activated TRIM E3 ligases and their role in regulation of TNF-α-induced NF-κB activation.

5.1 TRIMs are late response TNF-α-induced genes and inhibits TNF-α-induced NF-κB activation

Several ubiquitin E3 ligases are involved in activation of TNF-α mediated NF-κB pathway, but to our knowledge role of E3 ligase in negative feedback regulation of TNF-α induced NF-κB had not been investigated. TNF-α-induced temporal expressions of NF-κB target genes are crucial for optimal inflammatory response and resolution of inflammation. To identify feedback regulators of TNF-α-induced NF-κB pathway a two step screening was performed. Firstly, to identify late expressing TRIMs the expression of TRIMs in HEK293 was monitored at 10 hours. The mRNA expression of several TRIM genes (TRIM1, 2, 3, 8, 9, 15, 16,21, 31, 37, 38, 39, 41, 44, 46, 47 and 55) increased greater >2 folds in TNF-α treated cells (Figure 5.1A). The expression of TRIM1, 2, 15 and 16 using different set of primers was reconfirmed in HEK293 cells (Figure 5.1B).

TNFAIP3/A20 and IκBα are only NF-κB activated genes which regulate the pathway in a negative feedback manner and these belong to the early response genes (Afonina et al., 2017; Liu et al., 2017). Therefore, it is highly

likely that genes transcribed during the 'Late' response may have negative feedback regulatory functions to inhibit inflammation.

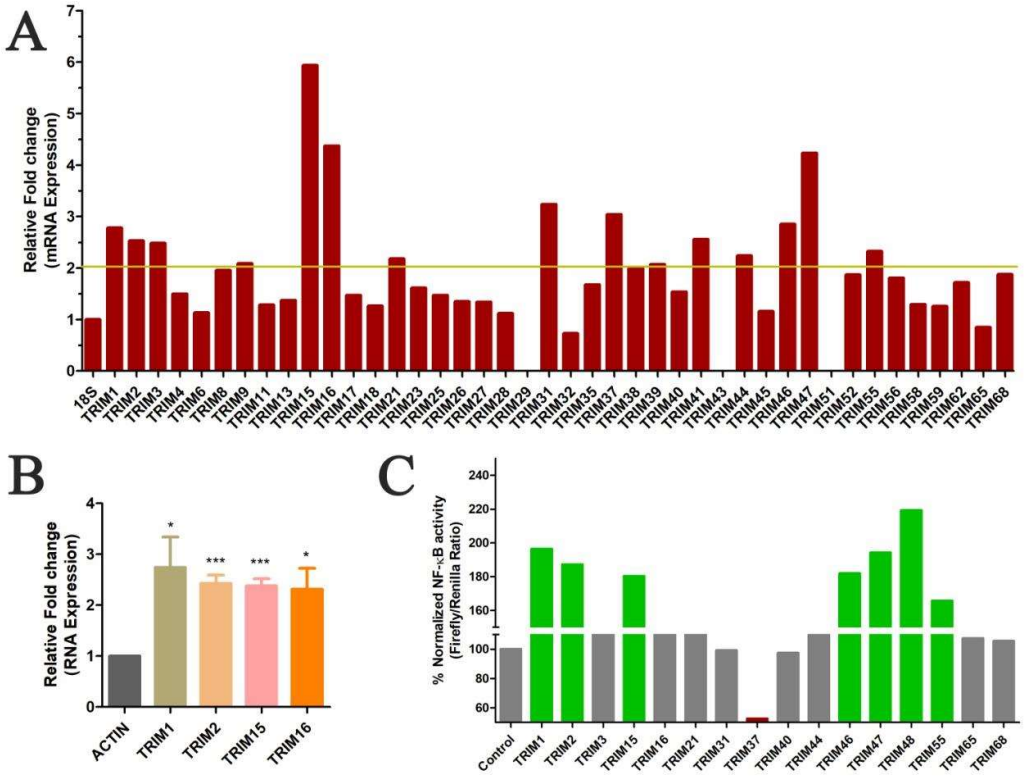

Figure 5.1: TRIMs are feedback regulators of TNF-α-induced NF-κB pathway. *(A) TNF-α induces expression of TRIM genes. HEK293 cells were treated with TNF-α (10 ng/ml) and cDNA was synthesized from isolated RNA. mRNA expression of indicated TRIM genes was checked by real time PCR using specific TaqMan probes. (B) mRNA expression of indicated TRIM genes was checked by indicated specific TRIM primers using SYBR green chemistry and real time PCR. (C) TRIMs regulate TNF-α-induced NF-κB activation. siRNA targeting indicated TRIM genes were co-transfected with NF-κB Firefly and Renilla reporter constructs in HEK293 cells. NF-κB Firefly and Renilla reporter activity was recorded after 10 hours of TNF-α treatment. Asterisk (*) and (***) indicates fold change statistically significant from vector; p value <0.05 and <0.001(respectively), SEM of minimum three independent experiments.*

Thus, the role of TNF-α-induced TRIMs in negative regulation of NF-κB pathway was analyzed. Indicated TRIMs were knocked down using siRNA and NF-κB reporter activity in presence/absence of TNF-α was monitored. The knock down of TRIM1, 2, 15, 46, 47, 48 and 55 enhanced TNF-α-induced NF-κB activation, whereas TRIM37 knock down reduced the activity (Figure 5.1C).

5.2 Discussion:

Cells keep inflammatory pathway under stringent control and it can be inferred that late response target genes activated by the pathway itself may have important regulatory functions. Therefore this study systematically identified late response TNF-α-induced TRIMs that can regulate the proinflammatory NF-κB pathway. The study identified several TRIMs (TRIM1, 2, 3, 8, 9, 15, 16, 21, 31, 37, 38, 39, 41, 44, 46, 47 and 55) which are late response TNF-α activated genes. Interestingly most of the late response TRIMs: TRIM1, 2, 15, 46, 47, 48 and 55 inhibit the TNF-α-induced NF-κB pathway, suggesting that these members of TRIM family proteins may act as feedback regulator of TNF-α-induced NF-κB pathway. These results further warrant more focused investigation of late response genes and their role in feedback regulation of TNF-α-induced NF-κB pathway in different pathophysiological conditions.