Chapter 6:
MID2/TRIM1 transcriptionally inhibits TRAF2 oncogene and negatively regulates TNF-α-induced NF-κB pathway

The ubiquitination and editing of ubiquitin chains of the unique targets at different steps of NF-κB is major mechanism of modulation in a given pathophysiological conditions (Wertz and Dixit, 2010). E3 ligases are known to recognize the target and define the topology of ubiquitin on recruited substrate. Different types of E3 Ligases are recruited in specific pathophysiological conditions to modulate NF-κB pathway by ubiquitination of key regulators at the different step leading to either stabilization or degradation. Previous work had shown the regulation of TNF-α-induced NF-κB pathway by distinct class of RING E3 Ligase called as TRIM (Tomar and Singh, 2014; Tomar et al., 2012b). Suggesting that TRIMs act at different levels of TNF-α-induced NF-κB signaling cascade regulating NF-κB pathway (Tomar and Singh, 2014; Tomar et al., 2012b).

Ubiquitination and recruitment of E3 ligases at different steps of NF-κB had been studied; however, their role in resolution of TNF-α-induced NF-κB had not been well studied. NF-κB activating oncogene TRAF2 is an E3 ligase essential for assembly of signaling platform for activation of NF-κB pathway. TNF-α receptor-associated death domain (TRADD) recruits TRAF2 to the membrane bound TNF-α-TNFR1 complex which ubiquitinates RIP1, in turn recruits other components of NF-κB signaling pathway (Wang et al., 1998; Wertz and Dixit, 2010). Moreover, TRAF2 induced NF-κB activation has been associated with several epithelial cancers (Shen et al., 2015). The modulation of TNF-α-induced NF-κB pathway downstream of TRAF2 in different pathophysiological conditions is not known.

The screening identified TRIMs as potential regulator of TNF-α induced NF-κB pathway and showed several TRIMs are induced in presence of TNF-α and act as negative feedback regulator of TNF-α-induced NF-κB pathway. Therefore, the role of MID2/TRIM1 in regulation of NF-κB pathway was further characterized.

6.1 MID2/TRIM1 is a 'late' response NF-κB target gene

TNF-α-induced expression of TRIMs and their implication in regulation of NF-κB had not been well elucidated hence TNF-α induced TRIMs and their role in modulation of NF-κB pathway was investigated. Firstly, the expression of MID2/TRIM1 during "Mid" (4 hours) and "Late" (10 hours) response was analyzed using qRT-PCR in presence of TNF-α. It was observed that the transcript levels of MID2/TRIM1 increased 6 folds in both HEK293 (Figure 6.1 A) and MCF-7 cells (Figure 6.1 B) treated with TNF-α at 10hrs, whereas no significant increase in mRNA expression was observed after 4 hours TNF-α treatment (Figure 6.1 A & B).

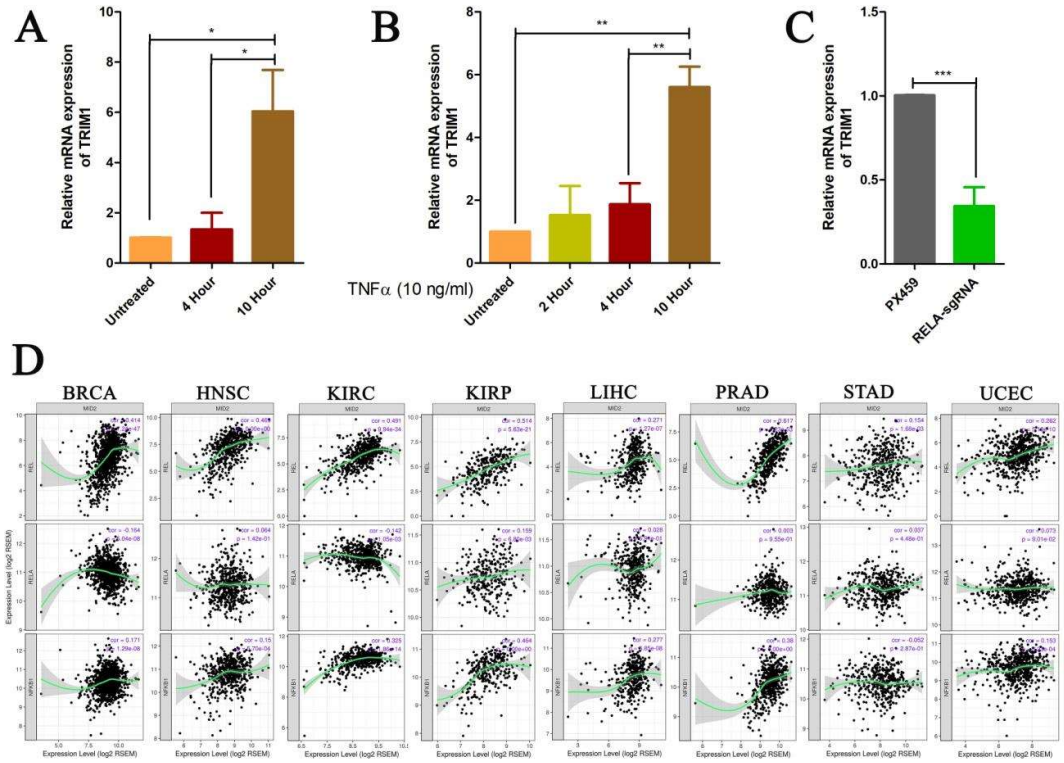

Figure 6.1: MID2/TRIM1 is a 'late' response NF-κB target gene. *TNF-α induces expression of MID2/TRIM1. (A) HEK293 & (B) MCF-7 cells were treated with TNF-α for indicated time and mRNA expression of MID2/TRIM1 was analyzed using qRT-PCR. (C) RelA/p65 knockdown inhibits MID2/TRIM1*

expression. HEK293 cells were transfected with control vector or RelA-sgRNA and after 24 hours of transfection, MID2/TRIM1 mRNA expression was analyzed using qRT-PCR. (D) Expression correlation between canonical NF-κB subunits and MID2/TRIM1 was checked using "Correlation" module of TIMER web server. Asterisk (*), (**) and (***) indicates fold change statistically significant from control; p value <0.05, <0.01 and <0.001(respectively), SEM of minimum three independent experiments.

To further confirm whether MID2/TRIM1 is a NF-κB target gene; RelA/p65 was knocked down using CRISPR/Cas9. Interestingly, knockdown of RelA/p65 significantly reduced the expression of MID2/TRIM1 in HEK293 cells (Figure 6.1 C). Further, the expression correlation between canonical subunits of NF-κB (REL, RELA and NFKB1) and MID2/TRIM1 was analyzed using "Correlation" module of the Tumor IMmune Estimation Resource (TIMER) web server (Li et al., 2017). Interestingly, high degree of positive correlation was observed between REL (c-Rel) and NFKB1 canonical subunits of NF-κB and MID2/TRIM1 expression (Figure 6.1 D) in Breast invasive carcinoma (BRCA), Head and Neck Squamous Cell Carcinoma (HNSC), Kidney Renal Clear Cell Carcinoma (KIRC), Kidney Renal Papillary Cell Carcinoma (KIRP), Liver Hepatocellular Carcinoma (LIHC), Prostate Adenocarcinoma (PRAD), Stomach Adenocarcinoma (STAD) and Uterine Corpus Endometrial Carcinoma (UCEC) tissues. These evidences suggest that MID2/TRIM1 is NF-κB target gene.

6.2 TNF-α inhibits turnover of MID2/TRIM1

TRIMs belong to RING family ubiquitin E3 ligases hence they may auto-ubiquitinate and regulate their own turnover either through Ubiquitin Proteasome system (UPS) or autophagy (Roy et al., 2018; Tomar et al., 2012a).

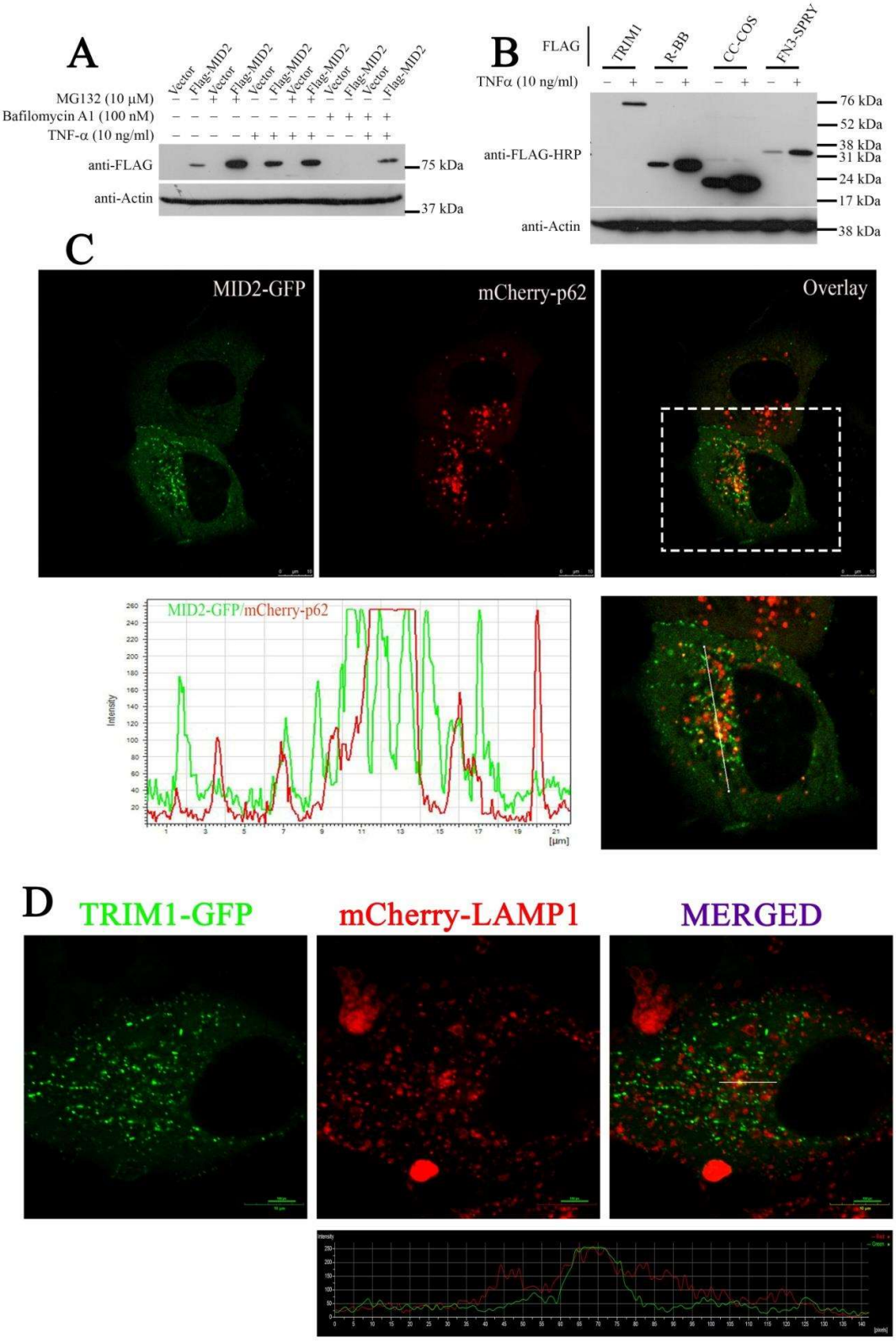

Figure 6.2 (I): TNF-α inhibits turnover of MID2/TRIM1. *MID2/TRIM1 turnover is regulated by UPS and autophagy. (A) Control vector or Flag-TRIM1*

was transfected in HEK293 cells and after 24 hours of transfection treated as indicated. The protein levels of MID2/TRIM1 were monitored by western blotting using anti-Flag antibody. MID2/TRIM1 is stabilized in presence of TNF-α (B) Vector or indicated flag tagged TRIM1 constructs were transfected in HEK293 cells and treated with TNF-α for 10 hours. Western blotting was performed to check the levels of flag tagged proteins. (C) TRIM1-GFP and mCherry-p62/SQSTM1 (D) TRIM1-GFP and mCherry-LAMP1 co-transfected cells were grown on cover slip. Cells were fixed and monitored under confocal microscope.

Moreover, stabilization of TRIMs in stimuli specific conditions may determine their functional outcome (Roy et al., 2018; Tomar et al., 2012a; Versteeg et al., 2013). Therefore, the effect of TNF-α on MID2/TRIM1 expression and turnover was monitored using western blotting. HEK293 cells were transfected with flag-tagged full length MID2/TRIM1 and treated as indicated. It was observed that 80 kDa band corresponding to MID2/TRIM1 increased in TNF-α, MG132 (inhibitor of UPS) and TNF-α-MG132 co-treated cells (Figure 6.2 (I) A). Interestingly, no band of 80 kDa corresponding to MID2/TRIM1 was observed in cells treated with Bafilomycin-A1 (Inhibitor of autophagosome-lysosome fusion), whereas the band reappeared in cells co-treated with TNF-α-Bafilomycin-A1 (Figure 6.2 (I) A) suggesting the turnover through autophagy in normal conditions and is stabilized in presence of TNF-α. The co-localization of MID2/TRIM1 with autophagosome marker p62/SQSTM1 and lysosomal protein LAMP1 was also checked. The GFP puncta of TRIM1-GFP showed co-localization with mCherry tagged p62/SQSTM1 (Figure 6.2 (I) C) and mCherry tagged LAMP1 (Figure 6.2 (I) D). These evidences suggest the turnover of MID2/TRIM1 through both UPS and autophagy pathways.

The stabilization and turnover of MID2/TRIM1 in presence of TNF-α was further checked. Flag tagged full length MID2/TRIM1, RING-B box, CC-COS or

FN3-SPRY domains of MID2/TRIM1 were expressed in HEK293 cells and monitored their expression by western blotting. Interestingly, full length (FL) MID2/TRIM1 only appeared in TNF-α treated cells (Figure 6.2 (I) B), indicating high turnover of full length MID2/TRIM1. The level of bands corresponding to RING-B-box, CC-COS or FN3-SPRY domains of MID2/TRIM1 also increased in MG132 treated cells (Figure 6.4 D).

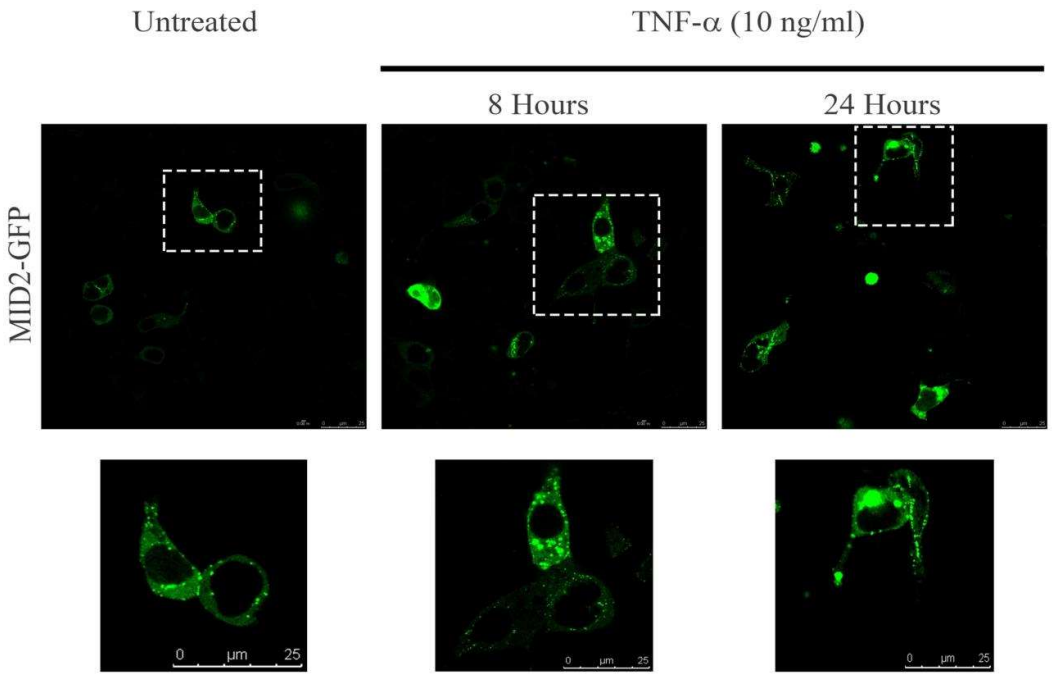

Figure 6.2 (II): TNF-α stabilizes MID2/TRIM1 temporally. *MID2/TRIM1-GFP was transfected in HEK293 cells and after 24 hour of transfection cells were treated with TNF-α as indicated. Cells were fixed with 4% Paraformaldehyde (PFA) and observed under fluorescent microscope.*

Next, the dynamics and stabilization of MID2/TRIM1 was monitored using fluorescent microscopy. HEK293 cells were transfected with MID2/TRIM1-GFP and treated with TNF-α for indicated time. The GFP fluorescent intensity increased after 8 hour and 24 hour of TNF-α treatment as compared to untreated cells (Figure 6.2 (II)). These results indicate that MID2/TRIM1 has high turnover and its expression is stabilized in presence of TNF-α.

6.3 MID2/TRIM1 inhibits TNF-α-induced NF-κB activation

The effect of MID2/TRIM1 on TNF-α-induced NF-κB activation was analyzed using NF-κB luciferase reporter assay. The expression of MID2/TRIM1 inhibited TNF-α-induced NF-κB activity compared to control (Figure 6.3 A) whereas, knockdown of MID2/TRIM1 using sgRNA (Figure 6.3 B & C) or siRNA (Figure 6.3 D & E) increased as compared to control.

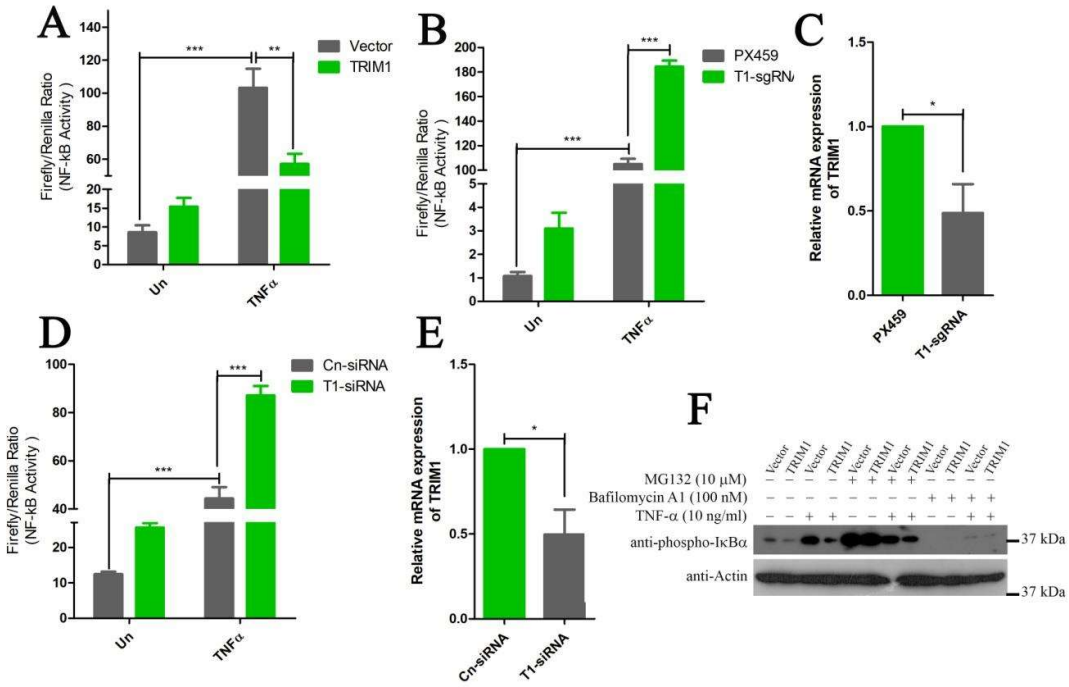

Figure 6.3: MID2/TRIM1 inhibits TNF-α-induced NF-κB activation. *NF-κB reporter constructs were co-transfected with control, MID2/TRIM1 (A), TRIM1-sgRNA (B) or TRIM1-siRNA (D) in HEK293 cells. The cells were treated with TNF-α for 10 hours and NF-κB activity was measured by Dual glow luciferase reporter assay. HEK293 cells were transfected with control vector or TRIM1-sgRNA (C), control siRNA or TRIM1-siRNA (E). Cells were collected and MID2/TRIM1 expression was analyzed using qRT-PCR. (F) Control vector or MID2/TRIM1 was transfected in HEK293 cells and were treated as indicated. Western blotting was performed to check the p-IκBα levels. Asterisk (*), (**) and (***) indicates NF-κB activity statistically significant from control; p value*

<0.05, <0.01 and <0.001 (respectively), SEM of minimum three independent experiments.

To further check the effect of MID2/TRIM1 on inhibition of TNF-α-induced NF-κB activity, HEK293 cells were transfected with either control vector or MID2/TRIM1 and treated with TNF-α, MG132, Bafilomycin-A1; or in combination. The band of 40 kDa corresponding to phospho-IκBα decreased in untreated and TNF-α treated cells transfected with MID2/TRIM1 compared to vector control (Figure 6.3 F). Interestingly the p-IκBα levels decreased in MG132 and TNF-α-MG132 co-treated cells (Figure 6.3 F) whereas, no difference in p-IκBα levels was observed in cells treated with Bafilomycin-A1 and TNF-α-Bafilomycin-A1 co-treated cells (Figure 6.3 F). These results suggest that MID2/TRIM1 inhibits TNF-α-induced NF-κB activity by reducing phosphorylation of IκBα.

6.4 MID2/TRIM1 mediated E3 ligase activity regulates TNF-α-induced NF-κB activity

TRIM proteins regulate NF-κB activity by regulating ubiquitination of the target substrates and activate/inhibit downstream signaling pathway. The role of MID2/TRIM1 as E3 ligase is not known hence we analyzed ligase activity by transfecting HEK293 cells with control or MID2/TRIM1 and treatment with MG132. MID2/TRIM1 expression significantly enhanced the total ubiquitination compared to control (Figure 6.4 A) and ubiquitin conjugates of higher molecular weight increased in presence of MG132 (Figure 6.4 B). Interestingly, the level of total ubiquitinated proteins decreased in MID2/TRIM1 transfected in presence of TNF-α (Figure 6.4 B), whereas increased in presence of MG132 co-treated cells (Figure 6.4 B). This strongly suggests that MID2/TRIM1 enhanced turnover of ubiquitinated proteins in presence of TNF-α cells through UPS.

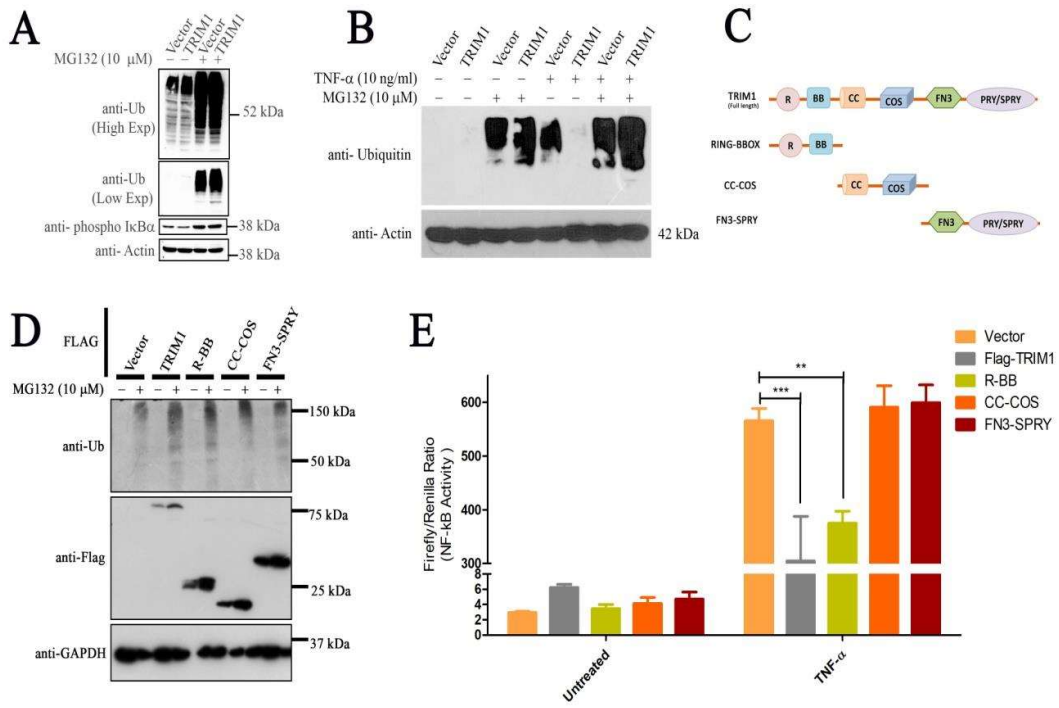

Figure 6.4: MID2/TRIM1 mediated E3 ligase activity regulates TNF-α-induced NF-κB activity. *(A & B) HEK293 cells were transfected with control vector or MID2/TRIM1 and treated as indicated for 10 hours. Western blotting was performed and indicated antibodies were used to check the proteins levels. (C) Schematic representation of MID2/TRIM1 domain deletion constructs used in the study. (D) Indicated TRIM constructs were transfected in HEK293 cells and treated with MG132 for 10 hours. Western blotting was performed and indicated antibodies were used to check the proteins levels. (E) Vector or indicated MID2/TRIM1 constructs were co-transfected with NF-κB reporter constructs. After 24 hours of transfection cells were treated with TNF-α for 10 hours and NF-κB activity was measured by Dual glow luciferase reporter assay. Asterisk (***) indicates NF-κB activity statistically significant from control; p value <0.001, SEM of minimum three independent experiments.*

RING and B-box domains of TRIM proteins are known to possess E3 ligase activity; therefore, it was analyzed whether E3 ligase activity of MID2/TRIM1 is

essential for NF-κB inhibition. The cells were transfected with control full length MID2/TRIM1, RING-B-box domain, CC-COS domain or FN3-SPRY domain of MID2/TRIM1 (Figure 6.4 C), and monitored total cellular ubiquitination (Figure 6.4 D) and NF-κB activity (Figure 6.4 E). The expression of both full length MID2/TRIM1 and only RING-B-box domain of MID2/TRIM1 significantly increased the ubiquitinated protein pool in MG132 treated cells compared to control (Figure 6.4 D), whereas transfection of CC-COS and FN3-SPRY domain showed low ubiquitination as compared to full length MID2/TRIM1 (Figure 6.4 D). Similarly the transfection of full length and RING-B-box domain inhibited TNF-α-induced NF-κB activity as compared to vector transfected cells (Figure 6.4 E). The transfection of MID2/TRIM1 lacking RING-B-box domains failed to inhibit TNF-α-induced NF-κB activity (Figure 6.4 E) suggesting that E3 ligase activity is essential for inhibition of TNF-α-induced NF-κB activity.

6.5 MID2/TRIM1 acts at TRAF2 to regulate TNF-α-induced NF-κB activity and often downregulated in cancers

Membrane bound E3 ligase complex composed of TRAF2, kinase complex TAB-TAK and IKK complex plays critical role in activation of TNF-α-induced NF-κB pathway (Figure 6.5 (I) A). Moreover, the inhibitory effect of MID2/TRIM1 on IκBα phosphorylation suggested that MID2/TRIM1 may act either of the mentioned complexes to regulate TNF-α-induced NF-κB activation. Therefore, to identify the step regulated by MID2/TRIM1, flag-tagged MID2/TRIM1 with control vector, were co-transfected with TRAF2 or TAK1. The expression of TRAF2 significantly induced NF-κB activity in untreated cells compared to control vector (Figure 6.5 (I) B) whereas co-transfection of MID2/TRIM1 failed to reduce the TRAF2-induced NF-κB activity (Figure 6.5 (I) B).

The co-transfection of MID2/TRIM1 significantly reduced NF-κB activity compared to TRAF2 transfected cells treated with TNF-α (Figure 6.5 (I) B), suggesting it inhibits NF-κB activity only in cells stimulated with TNF-α. It was also observed that MID2/TRIM1 expression did not inhibit NF-κB activity in TAK1 transfected cells compared to control (Figure 6.5 (I) B). NF-κB

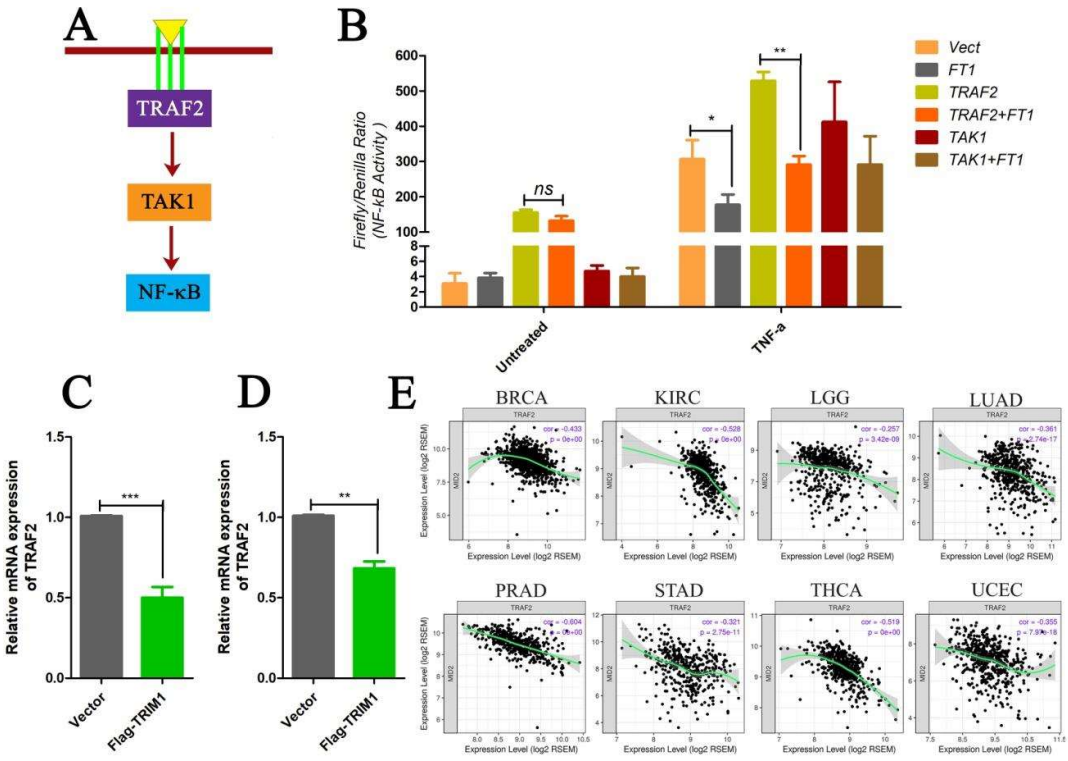

Figure 6.5 (I): MID2/TRIM1 acts at TRAF2 to regulate TNF-α-induced NF-κB activity. *(A) Schematic showing major steps of NF-κB pathway. (B) Vector, Flag-TARF2, Flag-TAK1 and MID2/TRIM1 constructs were co-transfected with NF-κB reporter constructs. The cells were treated with TNF-α for 10 hours and NF-κB activity was measured by Dual glow luciferase reporter assay. HEK293 (C) and MCF-7 (D) cells were transfected with vector control or MID2/TRIM1 and mRNA expression of MID2/TRIM1 was checked using qRT-PCR. (E) Expression correlation between MID2/TRIM1 and TRAF2 was checked using "Correlation" module of TIMER web server. Asterisk (*), (**) and (***) indicates*

fold change statistically significant from control; p value <0.05, <0.01 and <0.001 (respectively), SEM of minimum three independent experiments.

NF-κB target gene TRAF2 is an NF-κB activating oncogene in epithelial cancers and inhibitor of apoptosis and necroptosis (Petersen et al., 2015; Shen et al., 2015; Wang et al., 1998). Therefore, the effect of MID2/TRIM1 on TRAF2 expression was analyzed. Interestingly, the expression of TRAF2 was reduced in TRIM1 transfected cells HEK293 (Figure 6.5 (I) C) and MCF-7 cells (Figure 6.5 (I) D). To further verify the effect of MID2/TRIM1 on TRAF2 expression; the expression correlation of MID2/TRIM1 and TRAF2 in various cancers tissue was analyzed using TIMER web server. Interestingly, a high degree of negative correlation between MID2/TRIM1 and TRAF2 expression (Figure 6.5 (I) E) was observed. This Indicated MID2/TRIM1 may have association with cancers.

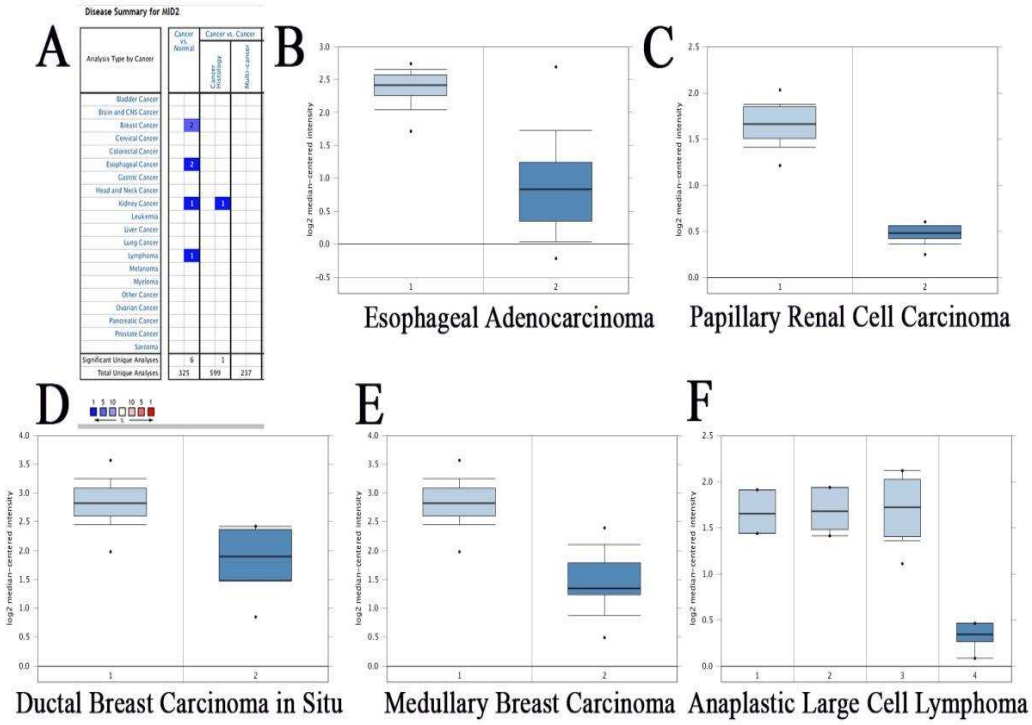

Figure 6.5 (II): MID2/TRIM1 expression is down regulated in several cancers. *(A) Disease summary of MID2/TRIM1. Expression of MID2/TRIM1 Expression of MID2/TRIM1 in Esophageal Adenocarcinoma (B), Papillary Renal*

Cell Carcinoma (C), Ductal Breast Cancer In Situ (D), Medullary Breast Carcinoma (E) and Anaplastic Large Cell Lymphoma (F) compared to control tissue.

Therefore, Oncomine database was explored to check expression of MID2/TRIM1 in different cancers. Interestingly it was observed that MID2/TRIM1 expression was reduced in Esophageal Adenocarcinoma, Papillary Renal Cell Carcinoma, Ductal Breast Cancer In Situ, Medullary Breast Cancer and Analplastic Large Cell Lymphoma (Figure 6.5 (II) B, C, D, E & F) further suggesting a functional correlation may exist between MID2/TRIM1 and TRAF2 expression in various cancers. Together these results suggest that MID2/TRIM1 inhibits NF-κB activity in TNF-α stimulated cells possibly by reducing the expression of TRAF2.

Further, the GEPIA web server was used for analyzing the RNA sequencing expression data of tumors and normal samples from the TCGA and the GTEx projects (Tang et al., 2017). Interestingly, the expression of MID2/TRIM1 was significantly reduced in tumor tissues of Bladder Urothelial Carcinoma (BLCA), Cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), Colon adenocarcinoma (COAD), Rectum adenocarcinoma (READ), Skin Cutaneous Melanoma (SKCM), Uterine Corpus Endometrial Carcinoma (UCEC) and Uterine Carcinosarcoma (UCS) compared to normal tissue (Figure 6.5 (III) A & B). Moreover, only one cancer, Thymoma (THYM) showed more expression of MID2/TRIM1 in tumor tissue compared to control (Figure 6.5 (III) A & B).

As MID2/TRIM1 expression negatively correlates with TRAF2 expression, the differential expression of MID2/TRIM1 and its correlation with TRAF2 in normal and tumor tissues using GEPIA web server was also analyzed. Interestingly MID2/TRIM1 expression was more in normal tissue compared to cancer tissues (Figure 6.5 (III) C), whereas the expression of TRAF2 was more in

cancer tissues compared to control (Figure 6.5 (III) C), suggesting negative correlation of MID2/TRIM1 and TRAF2 has implication in various cancers.

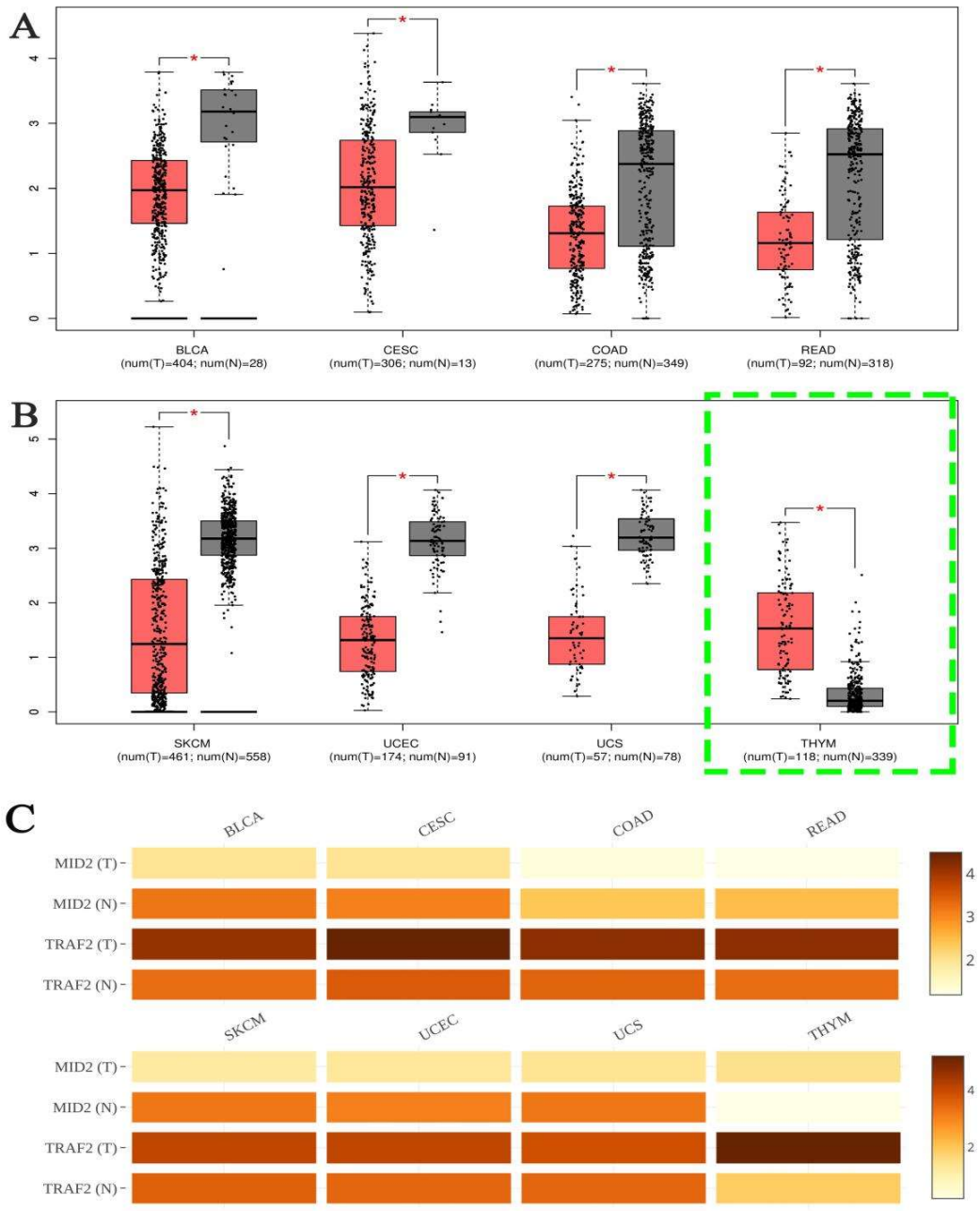

Figure 6.5 (III) : MID2/TRIM1 is down regulated in several cancers and shows inverse correlation with TRAF2.. *(A & B) MID2/TRIM1 expression in normal and tumor tissues of indicated cancers were plotted using GEPIA web*

server. (C) Expression of MID2/TRIM1 and TRAF2 in normal and tumor tissue were analyzed for indicated cancers using GEPIA web server.

These results clearly show that MID2/TRIM1 mediated downregulation of TRAF2 expression and its role in inhibition of TNF-α-induced NF-κB pathway may regulate the oncogenic transformation of cells.

6.6 Discussion

TNF-α-induced NF-κB pathway is a major proinflammatory pathway; often found dsyregulated in cancers, therefore it is important to study its spatio-temporal regulation (Hayden and Ghosh, 2014; Oeckinghaus and Ghosh, 2009; Taniguchi and Karin, 2018). Temporal expression of TNF-α-induced genes, especially the 'early' response feedback regulators like IκBα and TNFAIP3/A20 (Tian et al., 2005; Werner et al., 2008) plays a critical role in biphasic NF-κB response and NF-κB oscillations (Nelson et al., 2004). These early feedback regulators determine the duration of the first and the second phase and contribute to the biphasic response. The current report finds MID2/TRIM1 as a NF-κB target gene specifically expressed during 'Late' response, concomitantly, inhibits NF-κB pathway. Suggesting MID2/TRIM1 is a novel feedback regulator of NF-κB pathway, required for restriction of long-term NF-κB activation.

Stimuli specific expression, stabilization, localization of TRIM proteins have been reported and this suggest that TRIMs have high turnover and stabilizes in a given pathophysiological stimuli (Jiang et al., 2017; Rajsbaum et al., 2008; Roy et al., 2018; Tomar et al., 2012a; Versteeg et al., 2013). It was observed that similar to other TRIMs (Roy et al., 2018; Versteeg et al., 2013), MID2/TRIM1 has high turnover through both UPS and autophagy pathway in normal conditions. Interestingly, the current study also shows that TNF-α not only increases the mRNA expression but also inhibits its turnover. Full length

MID2/TRIM1 showed increased turnover compared to only RING-Bbox, Coiled-coil-COS and FN3-SPRY domain, suggesting that it may auto ubiquitinate and regulate its own turnover through autophagy and UPS.

MID2/TRIM1 is stabilized in different pathophysiological conditions including TNF-α. The subcellular localization of MID2/TRIM1 is known to be localized to microtubule and cytoplasm, however it was observed that in presence of TNF-α it forms distinct puncta which had been previously reported (Short et. al. 2002). TRIMs are known to form distinct protein complexes of higher order structure recruiting other proteins to form dynamic signalosomes in response to a given pathophysiological condition like TNF-α. It is possible that MID2/TRIM1 may be stabilized in different pathophysiological conditions including TNF-α regulating NF-κB. TRIMs modulate several pathways including TNF-α-induced NF-κB pathway by promoting their ubiquitination mediated stabilization or degradation of substrates involved in the signaling cascade (Li et al., 2011; Rajsbaum et al., 2014b; Tomar and Singh, 2014). The current study shows that MID2/TRIM1 possesses E3 ligase activity which is essential for NF-κB inhibition by ubiquitination of critical substrate for regulation of NF-κB pathway.

Previously reports had shown that TRIM13 is recruited at IKK complex (Tomar and Singh, 2014), TRIM8 (Li et al., 2011) and TRIM38 (Hu et al., 2014) at TAB-TAK complex in TNF-α-induced NF-κB pathway. This study identifies that MID2/TRIM1 possibly acts at the membrane bound TRADD-TRAF complex and inhibits NF-κB activation. This strongly suggest that TRIMs are developing as unique modifiers of TNF-α-induced NF-κB pathway however their role had been implicated in innate immune response during viral infections (Uchil et al., 2008; van Gent et al., 2018). The modulation of TNF-α-induced NF-κB pathways suggest its possible role in different chronic disease conditions like rheumatoid arthritis, psoriasis and prostate cancer (Ardizzoia et al., 1992;

Arican et al., 2005; Michalaki et al., 2004; Thilagar et al., 2018) where TNF-α levels are high. Interestingly, during tumorigenesis TNF-α is known to be enhanced in the tumor microenvironment, which acts as an important selection pressure to either activate specific gene program aiding in tumor progression (Ardizzoia et al., 1992; Ham et al., 2016). Moreover, during tumor evolution it had been observed that tumor suppressors are either mutated or lost, and oncogenes are either amplified or acquire dominant mutation leading to over activation (Jia and Zhao, 2019; Lee and Muller, 2010). Moreover, the study shows that MID2/TRIM1 expression is significantly reduced in various cancer tissues like Bladder Urothelial Carcinoma (BLCA), Cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), Colon adenocarcinoma (COAD), Rectum adenocarcinoma (READ), Skin Cutaneous Melanoma (SKCM), Uterine Corpus Endometrial Carcinoma (UCEC) and Uterine Carcinosarcoma (UCS) compared to control. TRAF2 is an NF-κB target gene and an oncogene over-expressed in several cancers (Shen et al., 2015; Wang et al., 1998). TRAF2 expression is enough to induce NF-κB activation hence may play crucial role in different stages of tumor progression. Interestingly, negative correlation between MID2/TRIM1 and TRAF2 expression had been observed across different tumors suggesting unique step during tumorigenesis. This hypothesis is further supported by previous report where MID2/TRIM1 had been shown to regulate cell cycle (Gholkar et al., 2016). Interestingly, MID2/TRIM1 depletion led to Astrin stabilization during cytokinesis, cytokinetic defects, multinucleated cells, and cell death hence during tumor progression MID2/TRIM1 down regulation may lead to genomic instability and tumor progression. This further strengthens the hypothesis that MID2/TRIM1 may act as potential tumor suppressors in specific cancer. This hypothesis needs to be further validated.

In summary, the study identifies MID2/TRIM1 as a NF-κB target gene and a novel regulator of TNF-α-induced NF-κB pathway acting at TRAF2 level. Further, TRAF2 is known to be important for the both canonical and non-canonical pathway NF-κB, hence MID2/TRIM1 mediated modulation of TRAF2 may emerge as unique modulation site in TNF-α-induced NF-κB pathway in different cancer. The study also provides MID2/TRIM1's association with cancers however its implication in different inflammatory conditions and contribution to various tumors of different origin needs to be further explored.