Supplementary Figure Legends, IMMUNITY-D-14-00831R1
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Figure S1 (related to Figure 1). Knockdown of MCPIP1 enhances IL-17-dependent gene expression. a. ST2 cells were transfected in triplicate with siRNAs against the indicated genes as described (Garg et al., 2013). Cells were treated ± IL-17 for 3 h. *Zc3h12a* and *Csf3* mRNA were assessed by qPCR. **p*<0.05 compared to IL-17-treated siRNA control. ‡ p<0.05 compared to untreated siRNA control. Data are expressed as fold-change relative to the untreated scrambled control. **b**. Lysates from ST2 cells transfected with the indicated siRNAs were analyzed by immunoblotting for MCPIP1 (top) and β-tubulin (bottom). Densitometry indicated a 50% reduction in protein expression between control and *Zc3h12a*$^{+/-}$ cells. **c.** Primary fibroblasts from WT or *Zc3h12a*$^{+/-}$ mice were stimulated with IL-17 for 4 or 8 hours and IL-6 in supernatants were assessed by ELISA. *p<0.05 relative to IL-17-treated WT controls. ‡ p<0.05 compared to untreated WT controls. Experiments were performed a minimum of twice. **d.** Primary fibroblasts from *Zc3h12a*$^{-/-}$ or WT littermates were treated with IL-17 for 24 h and IL-6 assessed by ELISA.*p<0.05 compared to IL-17-treated controls. ‡ *p*<0.05 compared to untreated WT controls. Data presented as mean ± SEM. All experiments were performed a minimum of twice.

Figure S2 (related to Figures 2-3). MCPIP1 knockout but not haploinsufficient mice show signs of inflammation in kidney and lung. Tissue from lung (**a**) or kidney (**b**) from WT (littermates), *Zc3h12a*$^{+/-}$ or *Zc3h12a*$^{-/-}$ mice (n=2-5) (Liang et al., 2010) were analyzed for the indicated genes by qPCR. Each symbol represents 1 mouse. **c.** The indicated mice were infected i.v. with *Candida albicans* and survival was assessed over 14 d.

Figure S3 (related to Figure 5). MCPIP1 regulation of transcription factors downstream of IL-17 signaling. ST2 cells were transfected with siRNAs against MCPIP1, stimulated with IL-17 for 3 h, and analyzed for expression of the indicated genes by qPCR. **p*<0.05 compared to unstimulated control siRNA. ‡ p<0.05 compared

to IL-17-treated control siRNA. Data are presented as mean ± SEM. Experiments were performed a minimum of twice.

Figure S4 (related to Figures 5-6). Iκbζ-dependent IL-17 target genes. a-b. ST2 cells were transfected with the indicated siRNA (targeting IκBζ, MCPIP1, Roquin-1, Roquin-2 or a scrambled control), treated with IL-17 for 3 h and the indicated genes were evaluated by qPCR normalized to GAPDH. Data expressed as fold-change relative to the untreated control siRNA. * $p<0.05$ compared to control siRNA treated with IL-17. ‡ $p<0.05$ compared to MCPIP1 siRNA treated with IL-17. & $p<0.05$ compared to MCPIP1 knockdown alone. # $p<0.05$ compared to control siRNA samples. Experiments were performed 1-2 times.

Figure S5 (related to Figures 6-7). MCPIP1 does not degrade Act1 and does not co-IP with IL-17RA. a. Mouse Flag-tagged MCPIP1 was co-expressed in HEK293T cells with WT Act1 or the indicated mutants lacking the SEFIR domain (ΔSEF) or the U-box motif of the ubiqutin ligase domain (Liu et al., 2009), all Myc-tagged. Expression of Act1 and MCPIP1 were assessed by immunoblotting. **b.** HEK293T cells were transfected with MCPIP1± TLR4 (left) or TNFRp60 (right) tagged with HA. Lysates were assessed by immunoblotting. **c** MCPIP1 was co-expressed in HEK293T cells with TRAF6. Expression was assessed by immunoblotting. **d.** HEK293T cells were transfected with Myc-tagged IL-17RA (Ho et al., 2010) together with RNase deficient mutants of MCPIP1. Lysates were immunoprecipitated with Myc to pull down IL-17RA and blotted for MCPIP1 (top) or IL-17RA (middle). Expression of MCPIP1 in whole cell lysates (WCL) was confirmed (bottom). Experiments were performed a minimum of twice. **e.** ST2 cells were transfected with siRNAs against the indicated genes. Cells were treated ± IL-17 for 3 h. *Tnfaip3* (encoding A20) and *Usp25* mRNA were assessed by qPCR.

Figure S6 (related to Figures 6-7). MCPIP1 degrades IL-17RA through an N-terminal domain sequence. a. Schematic diagram of murine IL-17RA. Residues are indicated in black. Nucleotide (nt.) designation is indicated in red. **b-c.** The indicated HA-tagged IL-17RA mutants (Maitra et al., 2007) were co-expressed with MCPIP1.

Whole cell lysates were immunoblotted for HA or Myc (to detect IL-17RA). **d**. Pooled fractions of HEK293T cell lysates transfected with His-tagged MCPIP1 were analyzed for expression of recombinant protein prior to dialysis. Experiments were performed a minimum of twice. *e. In vitro* transcribed mRNAs encoding *Il6* 3' UTR, *Il17ra* (nucleotides 1-775) or *Traf3ip2* (Act1) were incubated with water, buffer or recombinant MCPIP1 for 1 h at 30°C. Transcripts were analyzed on a denaturing agarose gel. Size markers are indicated. **f.** Spinal cords from *Zc3h12a*$^{+/+}$ or *Zc3h12a*$^{+/-}$ mice (n=5-6) subjected to EAE were analyzed for *Il17ra* expression by qPCR. Data expressed relative to *Gapdh*. **p*<0.05 by Students t-test. Experiments were performed a minimum of twice. **g**. *Zc3h12a*$^{+/+}$ and *Zc3h12a*$^{+/-}$ mice (n=1-4) were subjected to EAE and spinal cords evaluated by flow cytometry at days 8, 14 and 20. Representative IL-17RA staining on microglia (CD11b^{+}CD45^{lo} population) is indicated (taken from day 20). Bottom: Mean fluorescence intensity (MFI) of IL-17RA staining in microglia harvested at days 8, 14 or 20 from unchallenged mice or mice subjected to EAE is indicated.

References

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Liang, J., Saad, Y., Lei, T., Wang, J., Qi, D., Yang, Q., Kolattukudy, P.E., and Fu, M. (2010). MCP-induced protein 1 deubiquitinates TRAF proteins and negatively regulates JNK and NF-kappaB signaling. J Exp Med *207*, 2959-2973.

Liu, C., Qian, W., Qian, Y., Giltiay, N.V., Lu, Y., Swaidani, S., Misra, S., Deng, L., Chen, Z.J., and Li, X. (2009). Act1, a U-box E3 ubiquitin ligase for IL-17 signaling. Sci Signal *2*, ra63.

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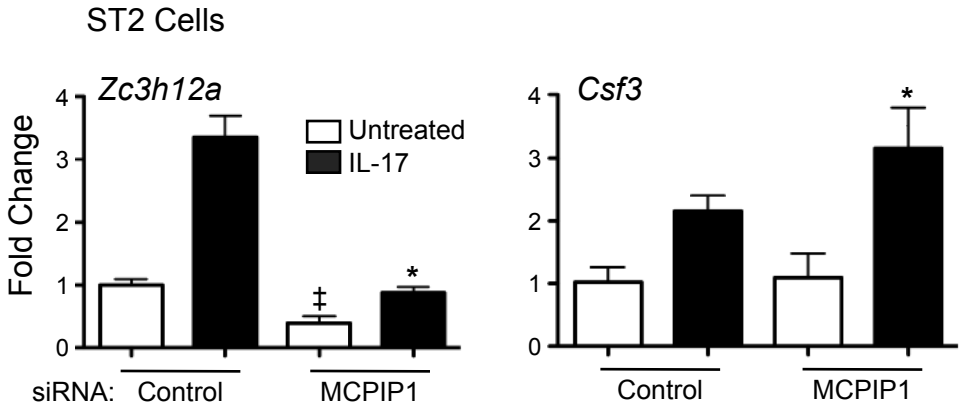
Supplementary Figure 1 (Garg et al.)

a.



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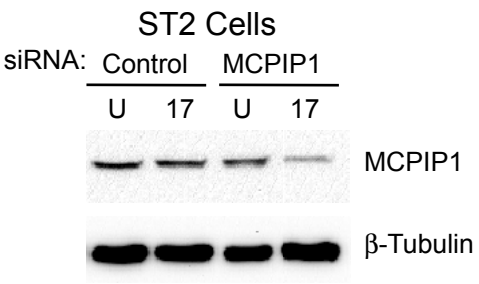

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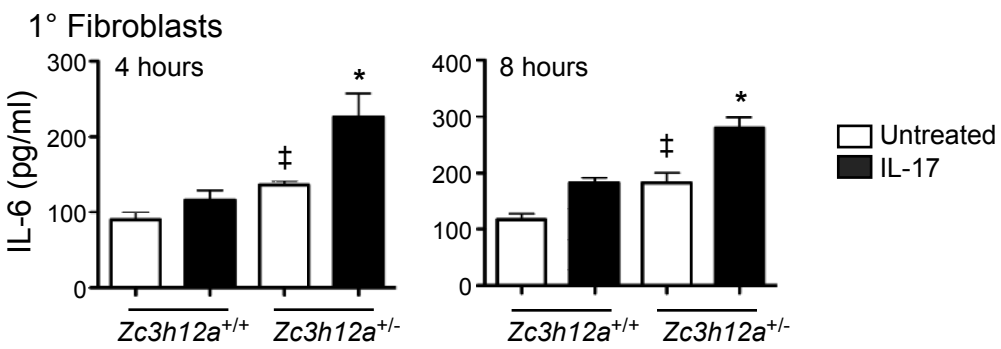

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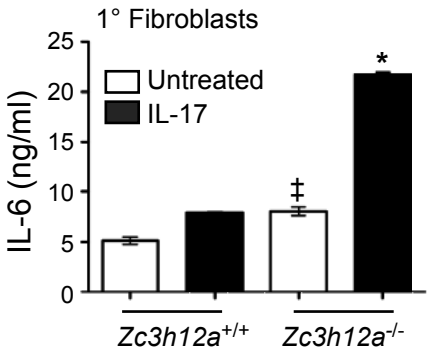
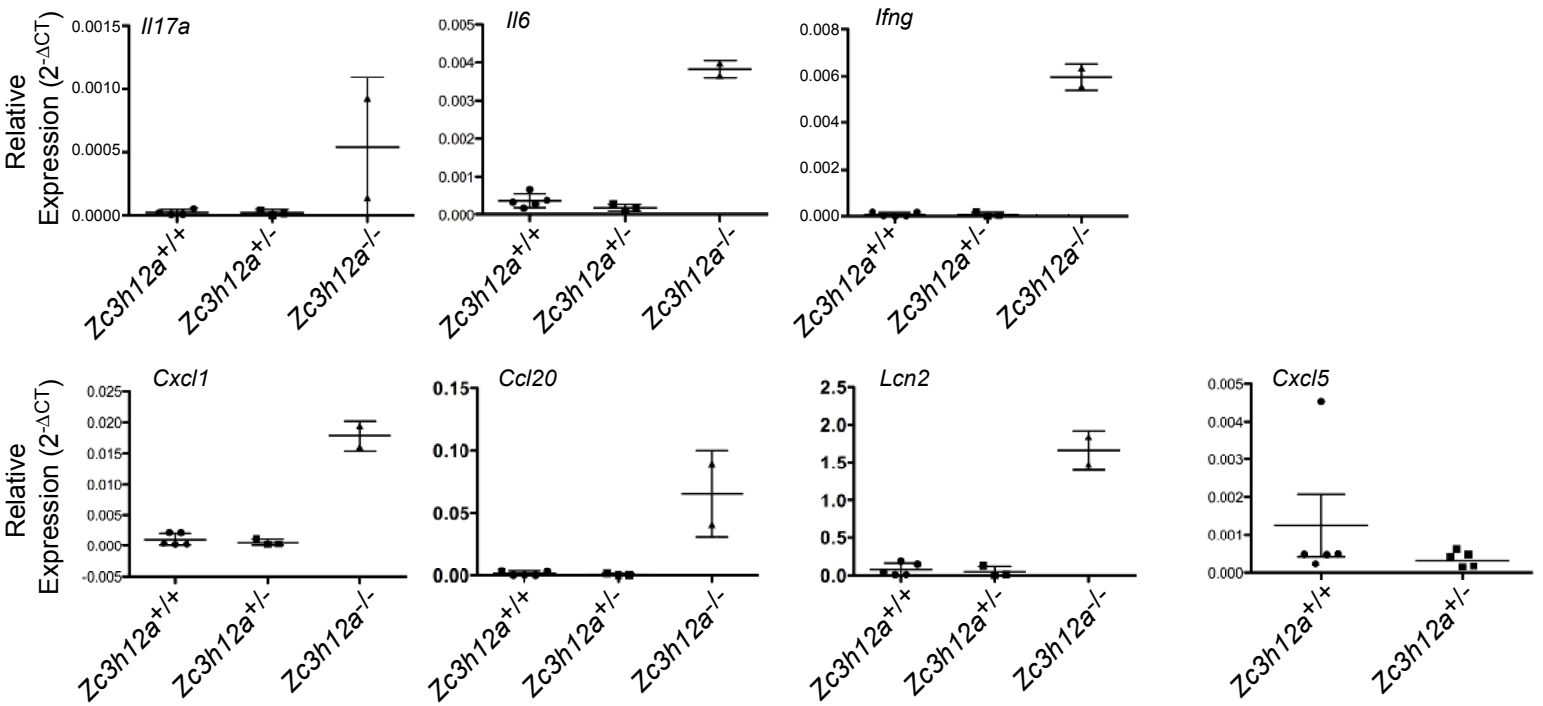


Supplementary Figure 2 (Garg et al.)

a. Lung



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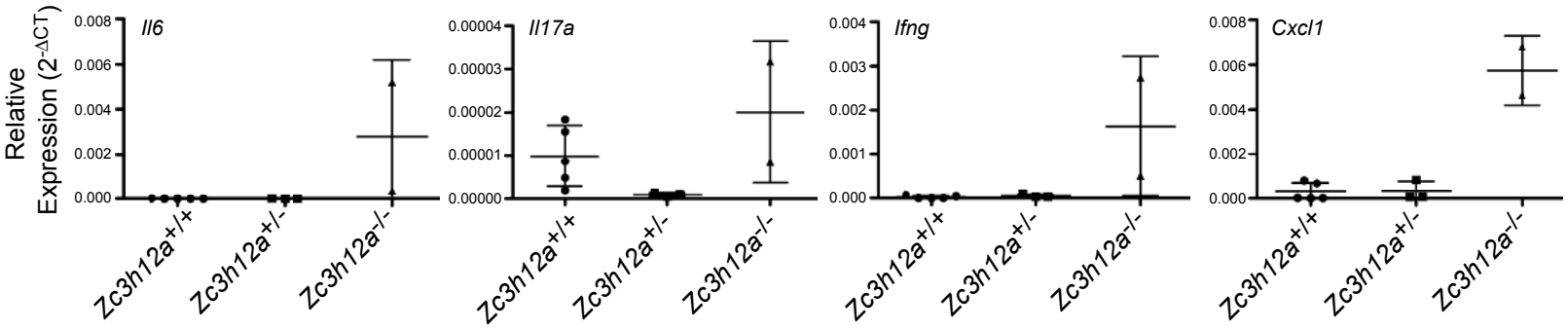

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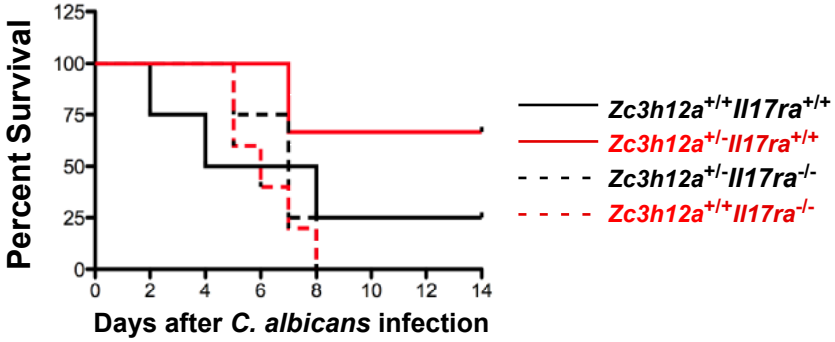
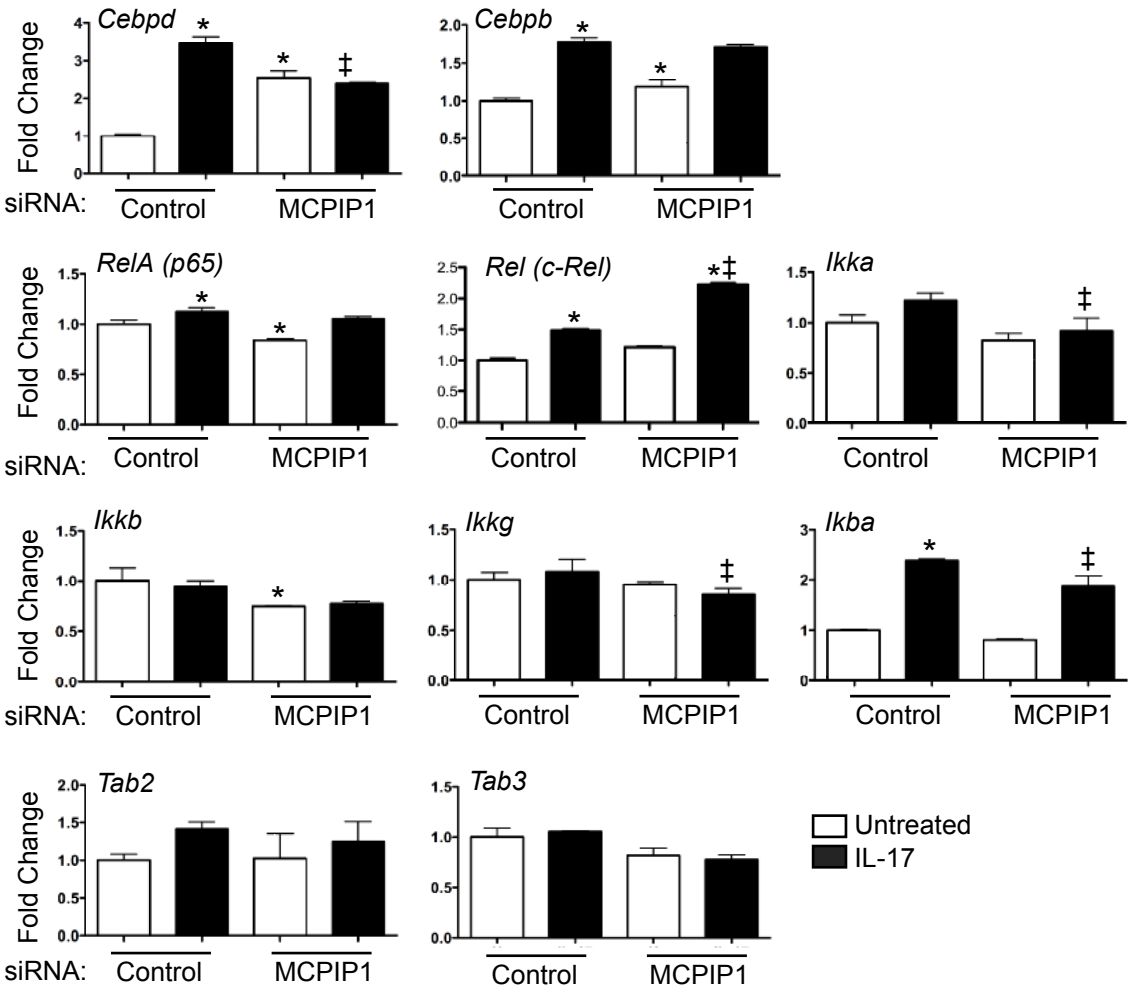
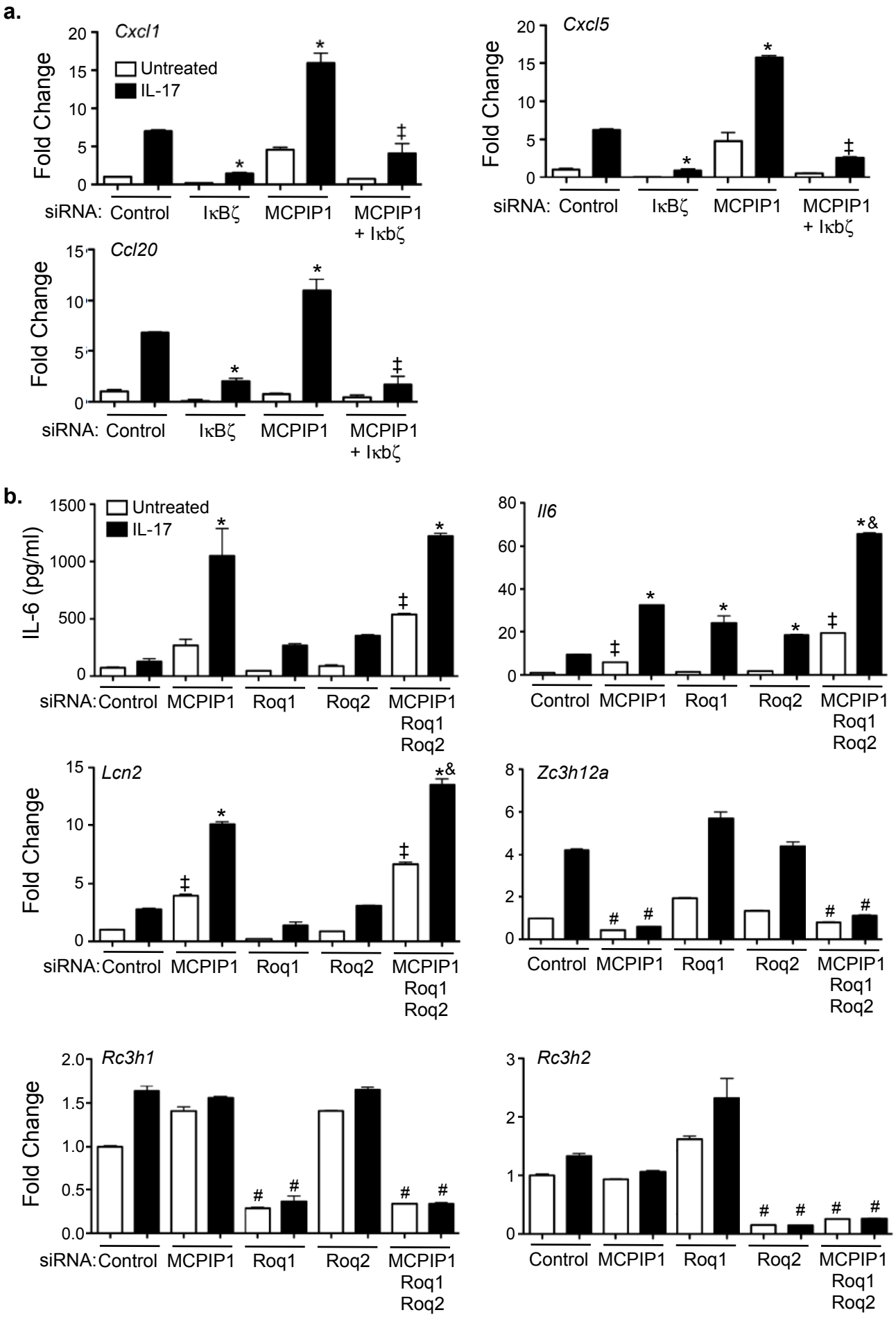


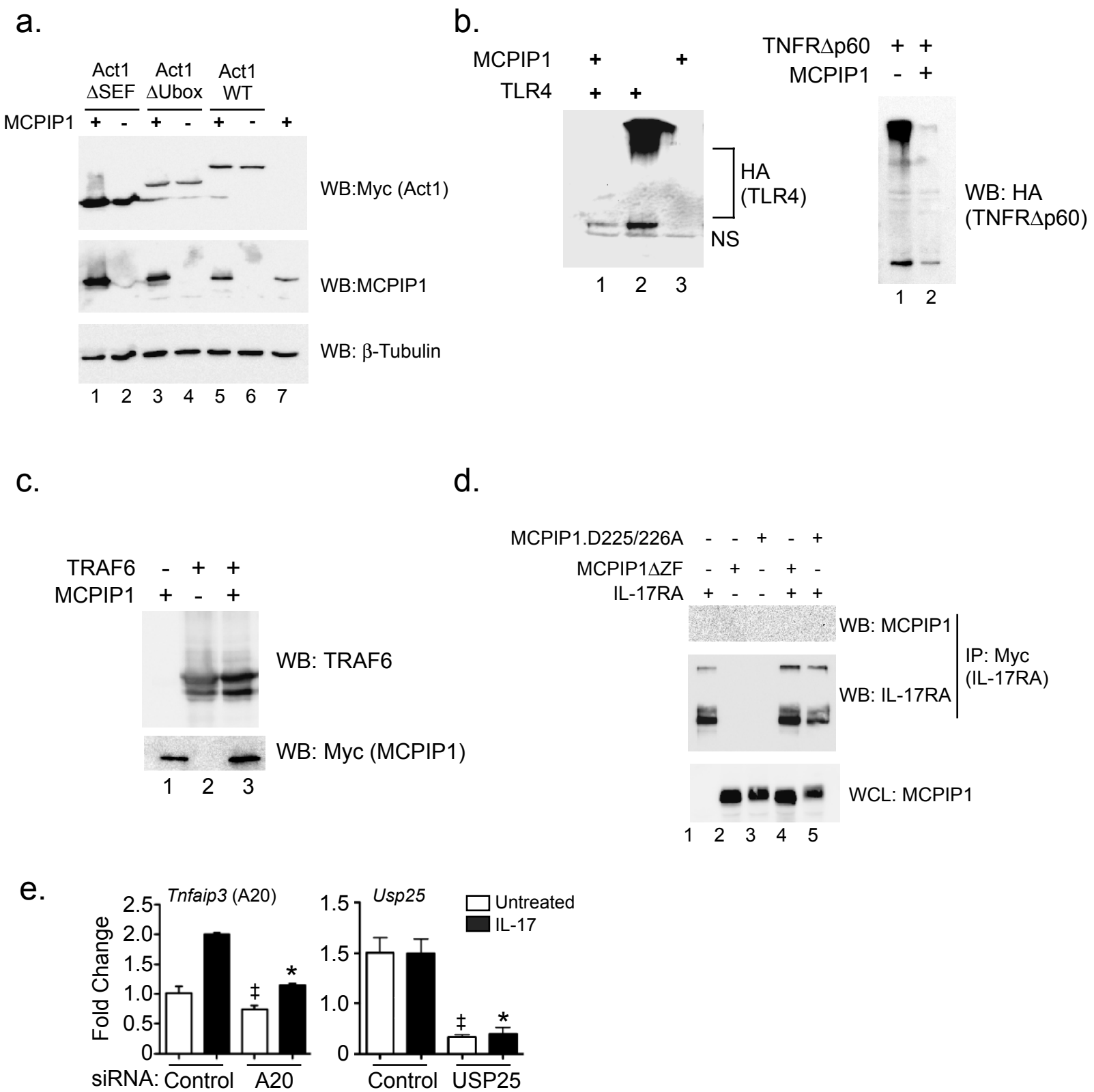
Supplementary Figure 3 (Garg et al.)



Supplementary Figure 4 (Garg et al.)



Supplementary Figure 5 (Garg et al.)



Supplementary Figure 6 (Garg et al.)