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online data

SUPPLEMENTARY ONLINE DATA

Thr^{435} phosphorylation regulates RelA (p65) NF-κB subunit transactivation

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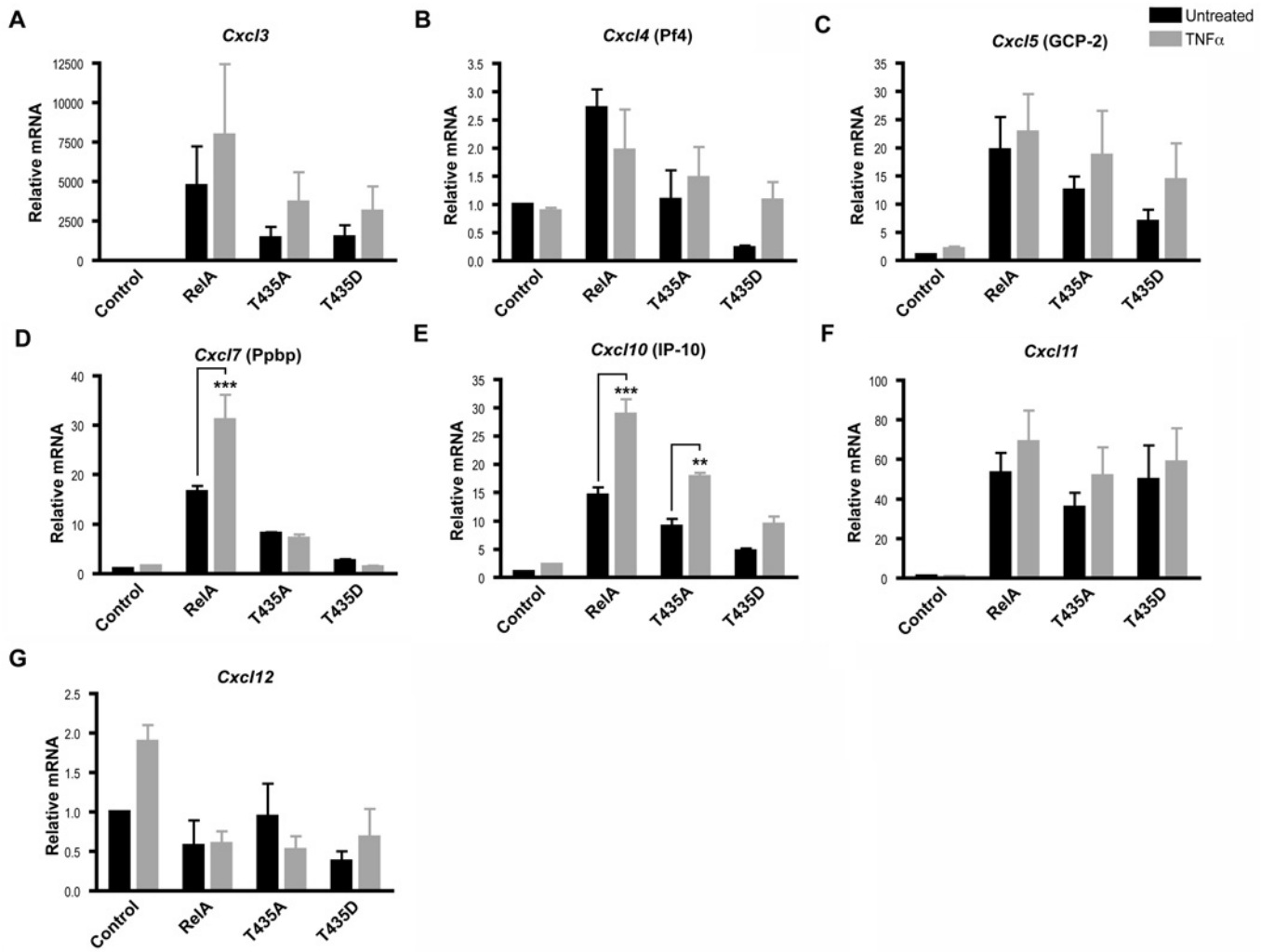

Figure S1 Expression of CXC-chemokine superfamily members

(**A**–**F**) Introduction of RelA into *Rela*$^{-/-}$ MEFs increases expression levels of *Cxcl3*, *Cxcl4* (Pf4), *Cxcl5* (GCP-2), *Cxcl7* (Ppbp), *Cxcl10* (IP-10) and *Cxcl11*. Mutating the Thr^{435} phospho-site reduces the enhanced expression of *Cxcl3, Cxcl4* (Pf4), *Cxcl5* (GCP-2), *Cxcl7* (Ppbp) and *Cxcl10* (IP-10). Furthermore, induction of *Cxcl7* (Ppbp) and *Cxcl10* (IP-10) following TNFα treatment is severely diminished in the T435D cell line. (**G**) Introduction of RelA into the *Rela*$^{-/-}$ MEFs decreases *Cxcl12* expression levels and does not exhibit increased expression after TNFα treatment. RNA was extracted from reconstituted MEF cells, either unstimulated or stimulated with TNFα (40 ng/ml) for 30 min. Total cDNA was prepared and qPCR analysis was performed using primers to mouse (**A**) *Cxcl3*, (**B**) *Cxcl4* (Pf4), (**C**) *Cxcl5* (GCP-2), (**D**) *Cxcl7* (Ppbp), (**E**) *Cxcl10* (IP-10), (**F**) *Cxcl11*, (**G**) *Cxcl12* and *Gapdh* control. For CXC gene family members not shown, we were unable to detect significant levels of expression in these cells. All results were normalized to *Gapdh* levels and are expressed as fold-induction relative to the level of gene expression in the control cell line. Results are the means$\pm$S.E.M., $n = 4$. Two-way ANOVA was performed followed by a Bonferroni post-hoc test to compare replicate untreated and TNFα means, using Prism 4 software (GraphPad). $^{**}P < 0.01$ and $^{***}P < 0.001$.

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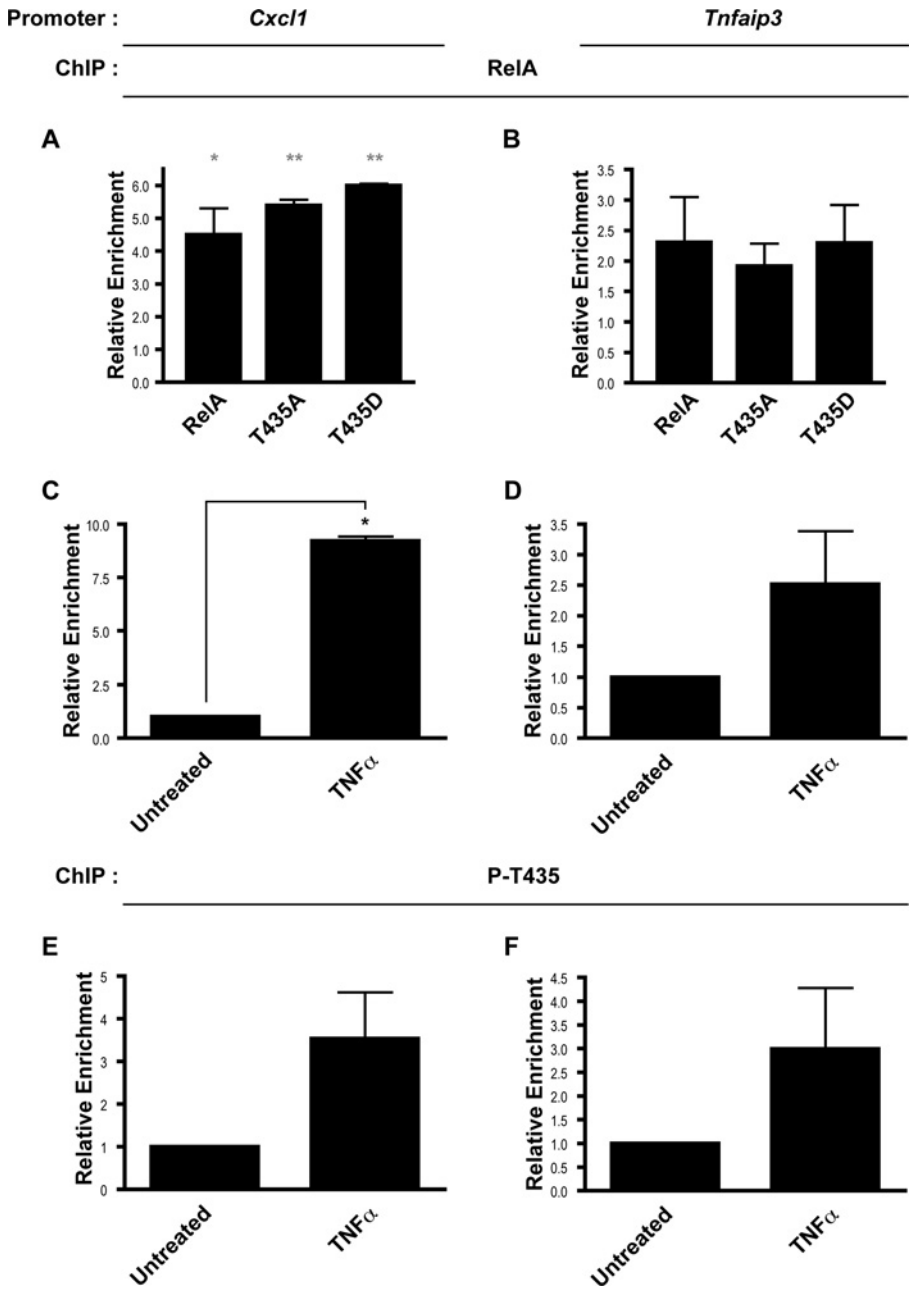

Figure S2 ChIP analysis of the murine *Cxcl1* and *Tnfaip3* promoters in reconstituted and wild-type MEFs

(**A**, **B**) Enhanced binding of RelA to the *Cxcl1* and *Tnfaip3* promoters following introduction of all forms of RelA into *RelA*$^{-/-}$ MEFs. (**C**–**F**) Elevated levels of RelA and RelA phosphorylated at Thr^{435} (P-T435) at the *Cxcl1* and *Tnfaip3* promoters following TNFα treatment in wild-type MEFs. All results were normalized to input levels and control antibodies. Results are the mean enrichment levels relative to untreated cells$\pm$S.E.M., $n = 3$. (**A**, **B**) ANOVA was performed followed by a Tukey–Kramer multiple comparisons test using Prism 4 software (GraphPad). (**C**–**F**) Student's *t* test was performed using Prism 4 software (GraphPad). *$P < 0.05$ and **$P < 0.01$. Grey asterisks indicate *P* values relative to the control cell line.

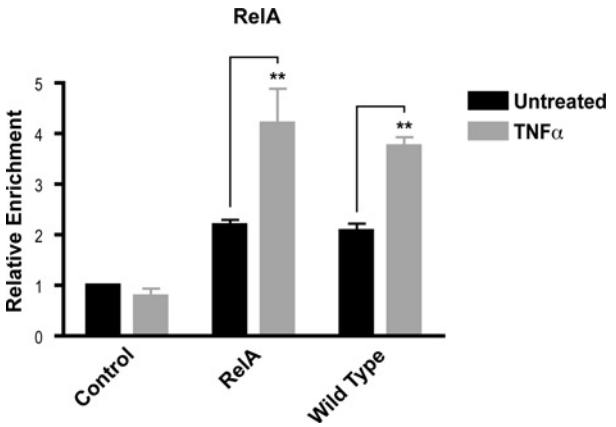

Figure S3 Similar levels of RelA are found at the *Cxcl2* promoter in reconstituted wild-type RelA MEFs and wild-type MEFs

Binding of RelA to the *Cxcl2* promoter increases to similar levels in both reconstituted wild-type RelA and wild-type MEFs following TNFα stimulation. All results were normalized to input levels and control antibodies. Results are the mean enrichment levels relative to untreated control cells$\pm$S.E.M., $n = 3$. Two-way ANOVA was performed followed by a Bonferroni post-hoc test to compare replicate untreated and TNFα means, using Prism 4 software (GraphPad). $^{**}P < 0.01$.

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