

Figure S1 MUC1 activates p65 and forms a complex with IKK β and IKK γ . **a.** Lysates from HeLa and HCT116 cells stably expressing an empty vector or exogenous MUC1 and from ZR-75-1 and MCF-7 cells silenced for endogenous MUC1 were immunoblotted with anti-MUC1-C and anti- β -actin. **b.** Cytosolic fractions from the indicated cells were immunoblotted with anti-p65 and anti- β -actin. **c.** Lysates from HeLa/MUC1 cells were

immunoprecipitated with increasing amounts (10, 20 and 40 μ g) of anti-MUC1-C (left) or a control IgG (right). The precipitates were immunoblotted with anti-MUC1-C (left, upper panel). The MUC1-C or control IgG immunodepleted lysates were immunoblotted with the indicated antibodies. **d.** Potential binding of MUC1-CD, IKK β and IKK γ in a trimolecular complex.

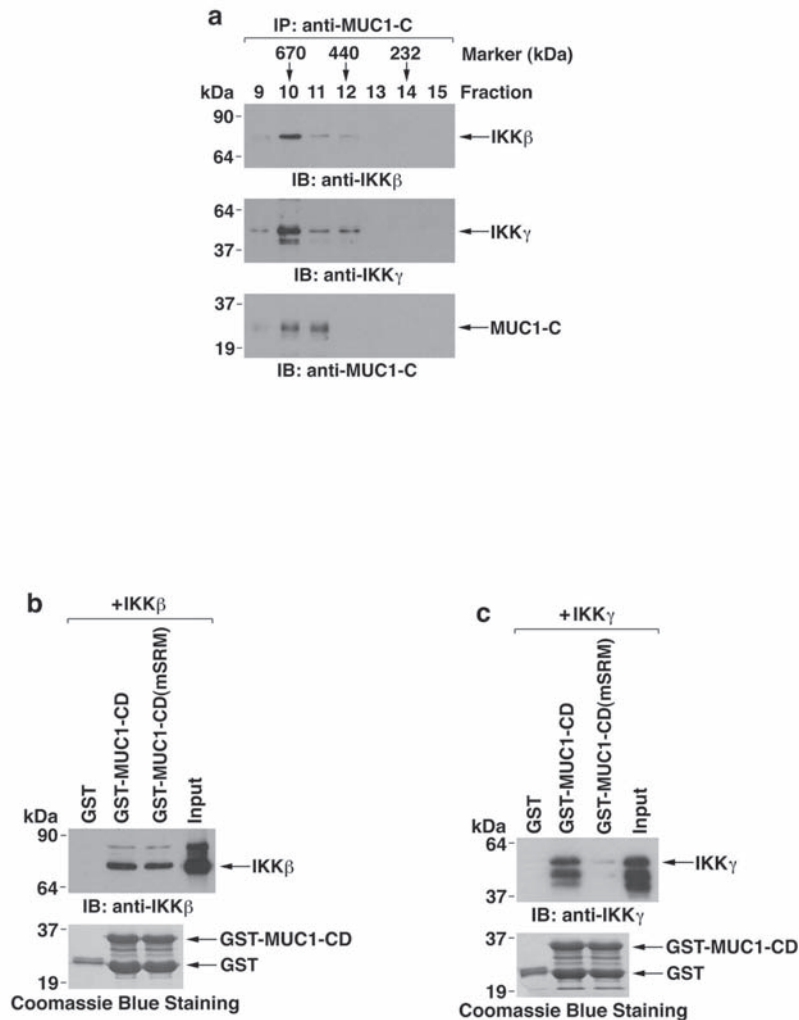


Figure S2 Binding of MUC1-CD to IKK γ , but not IKK β , is abrogated by mutating the SRM. **a.** Lysates from HeLa/MUC1 cells were precipitated with anti-MUC1-C and released by adding MUC1-C peptide. The proteins were separated in a Sephacryl S-200 HR column and the indicated fractions were immunoblotted with the indicated antibodies. **b and c.** GST, GST-MUC1-CD

or GST-MUC1-CD(mSRM) bound to glutathione beads was incubated with purified IKK β (**b**) or IKK γ (**c**). The precipitates were immunoblotted with the indicated antibodies. Input of GST, GST-MUC1-CD and GST-MUC1-CD(mSRM) was assessed by Coomassie blue staining.

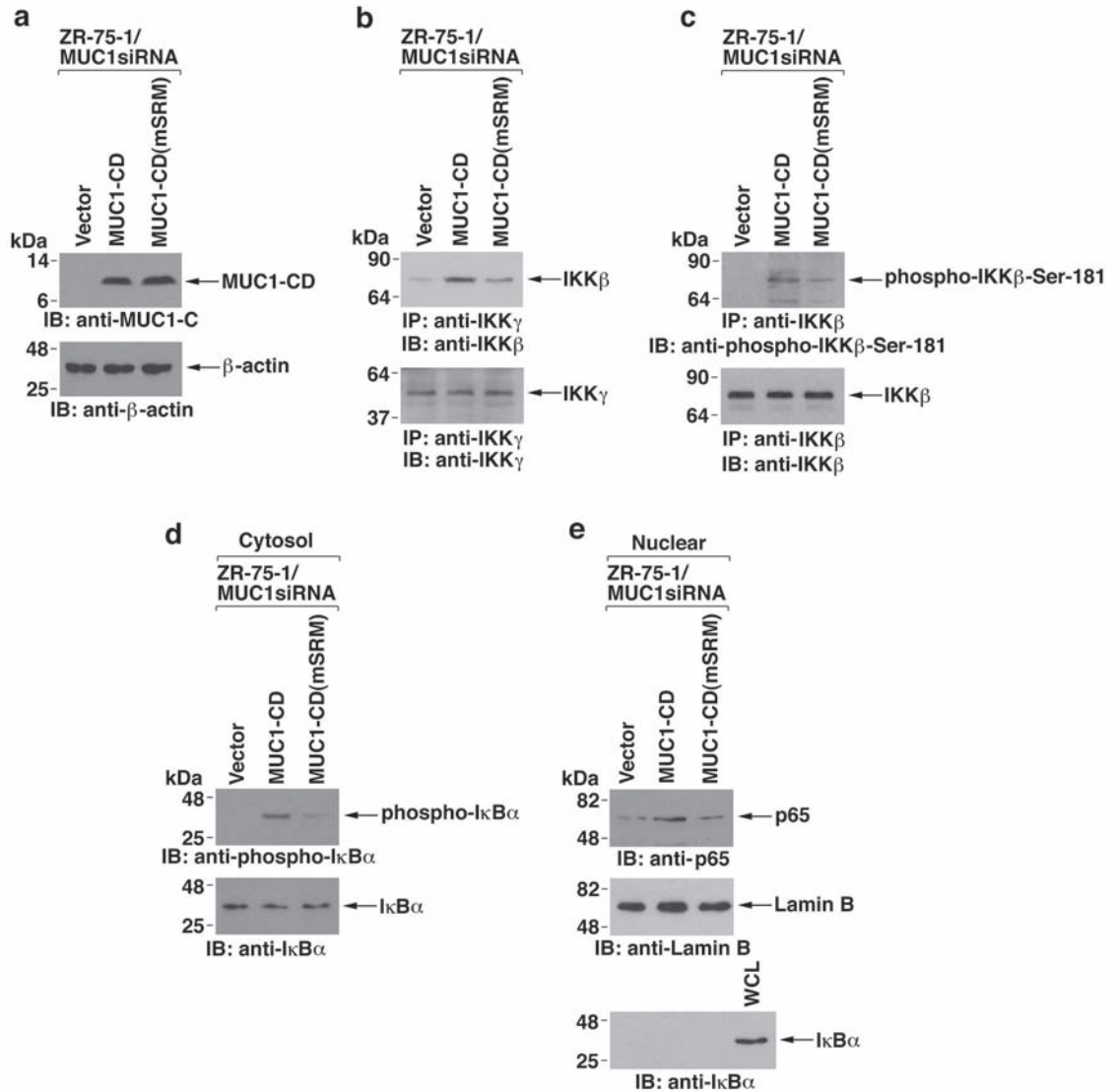
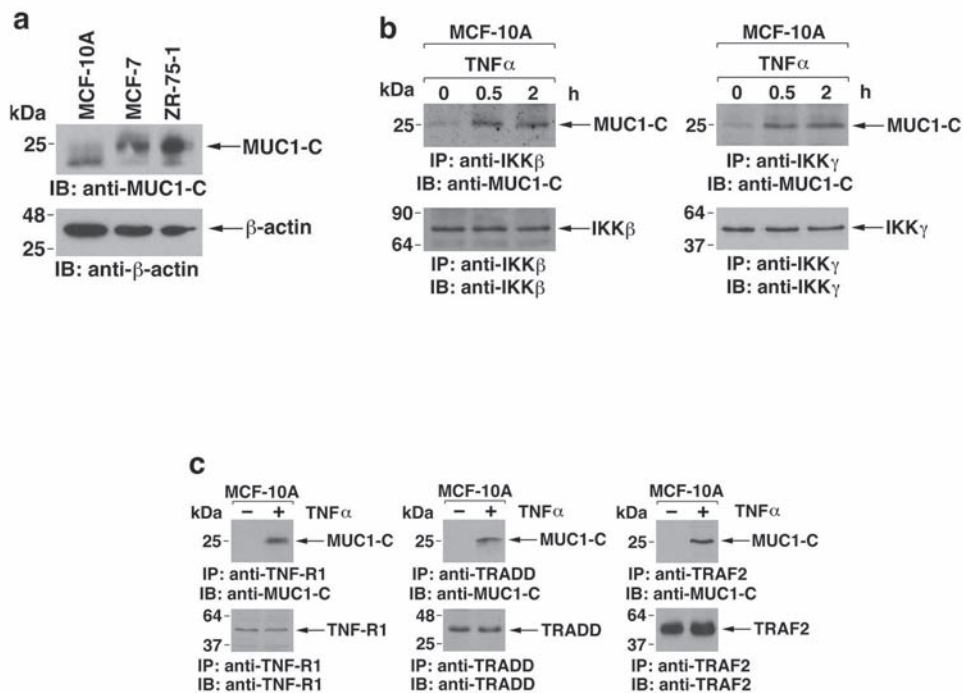


Figure S3 MUC1-CD is sufficient for activation of the IKKβ->NF-κB p65 pathway. ZR-75-1/MUC1siRNA cells were transfected with the empty pIRES-puro2 vector, Flag-MUC1-CD or Flag-MUC1-CD(mSRM) in the presence of Lipofectamine for 48 h. Of note, the MUC1siRNA used to silence endogenous MUC1 in the ZR-75-1 cells targets the extracellular region of MUC1-C and not the cytoplasmic domain. **a.** Lysates from the

indicated cells were immunoblotted with the indicated antibodies. **b.** Anti-IKKγ precipitates from the indicated cells were immunoblotted with the indicated antibodies. **c.** Anti-IKKβ precipitates were immunoblotted with the indicated antibodies. **d.** Cytosolic fractions were immunoblotted with anti-phospho-IκBα and anti-IκBα. **e.** Nuclear fractions were immunoblotted with the indicated antibodies.



C to IKK β and IKK γ and recruitment of MUC1-C to the TNF-R1 complex. a. Lysates from the indicated cells were immunoblotted with anti-MUC1-C and anti- β -actin. **b.** Lysates from MCF-10A cells left untreated or stimulated with 20 ng/ml TNF α for the indicated times were immunoprecipitated with anti-

the indicated antibodies. **c.** MCF-10A cells were left untreated or stimulated with TNF α for 30 minutes. Lysates were immunoprecipitated with anti-TNF-R1 (left), anti-TRADD (middle) or anti-TRAF2 (right). The precipitates were immunoblotted with the indicated antibodies.

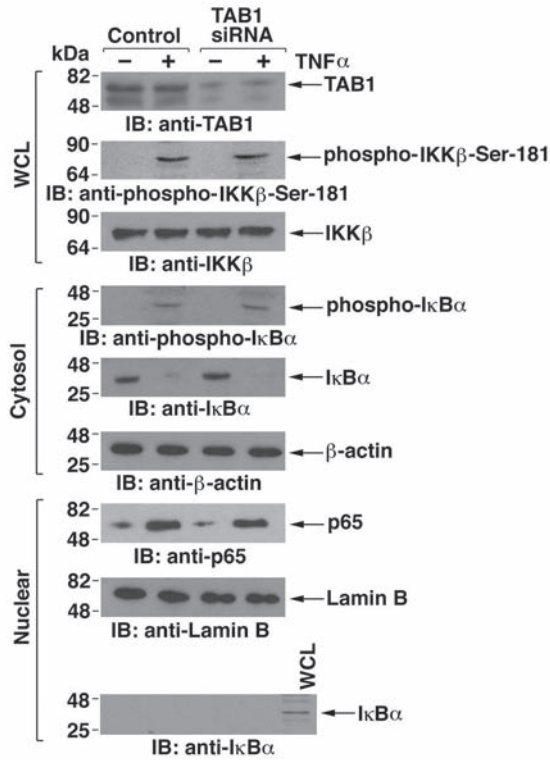


Figure S5 TAB1 is dispensable for TNF α -induced activation of IKK β ->NF- κ B signaling in MCF-10A cells. MCF-10A cells were transfected with control siRNA or TAB1 siRNA pools for 72 h and then stimulated with

TNF α . Whole cell lysates, cytosolic fractions and nuclear fractions were immunoblotted with the indicated antibodies.

Figure S6-1 & 2

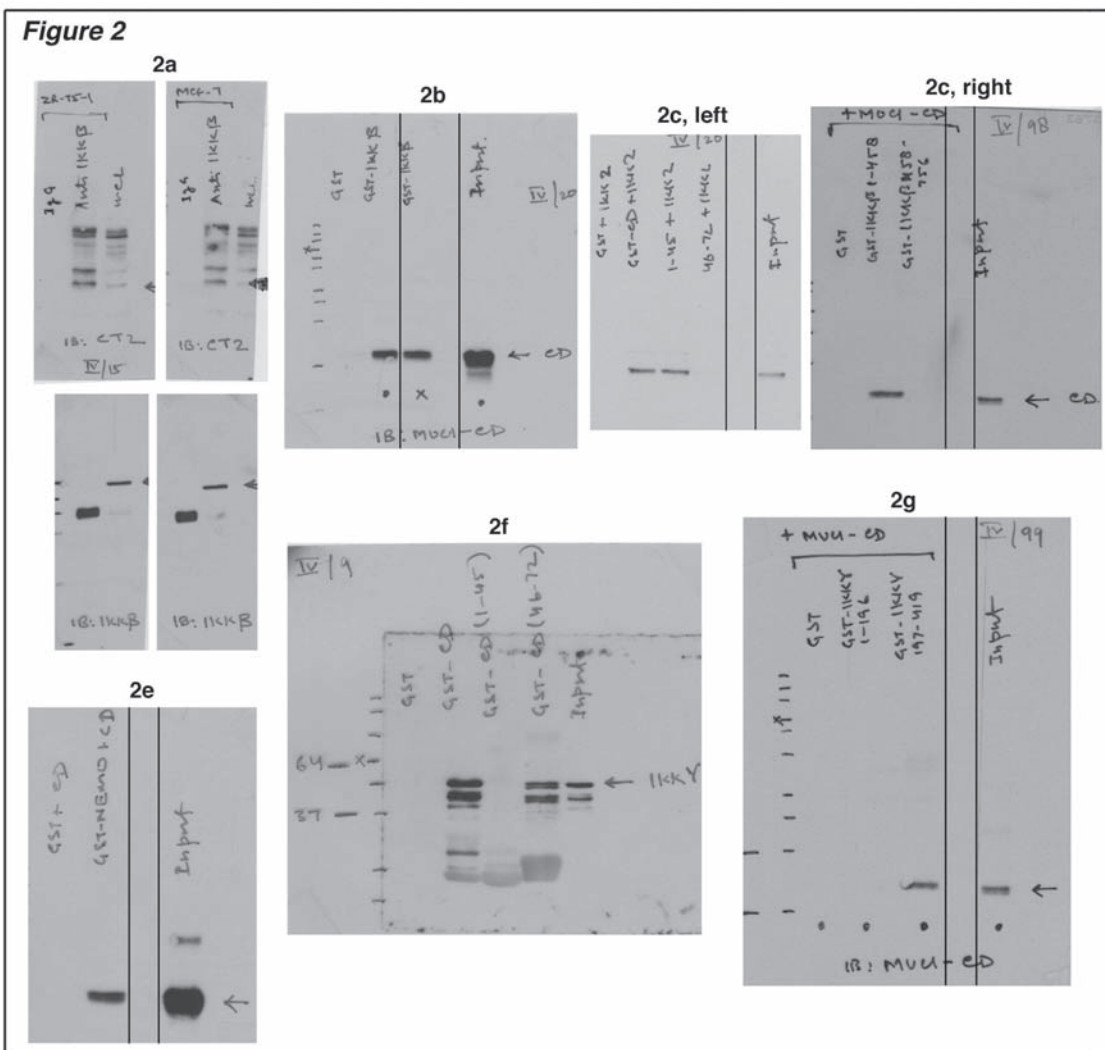
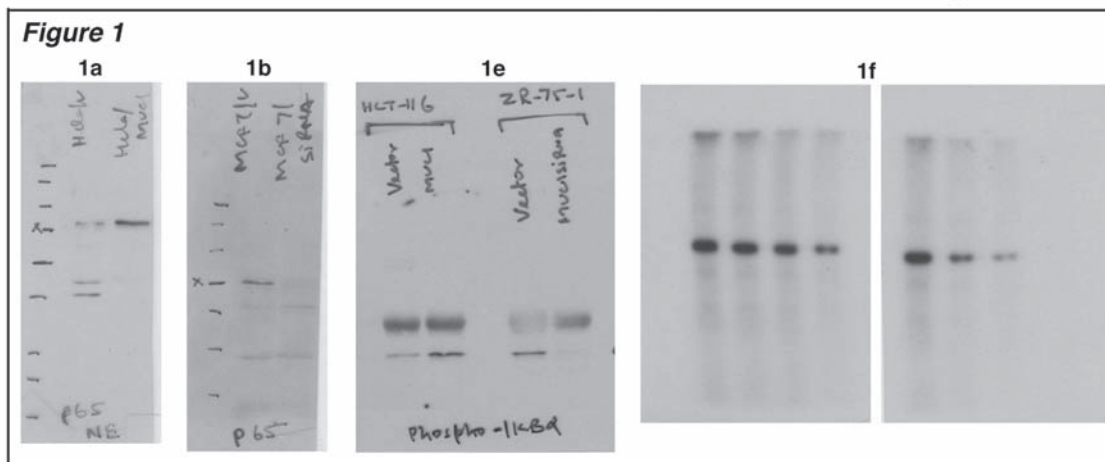


Figure S6-3

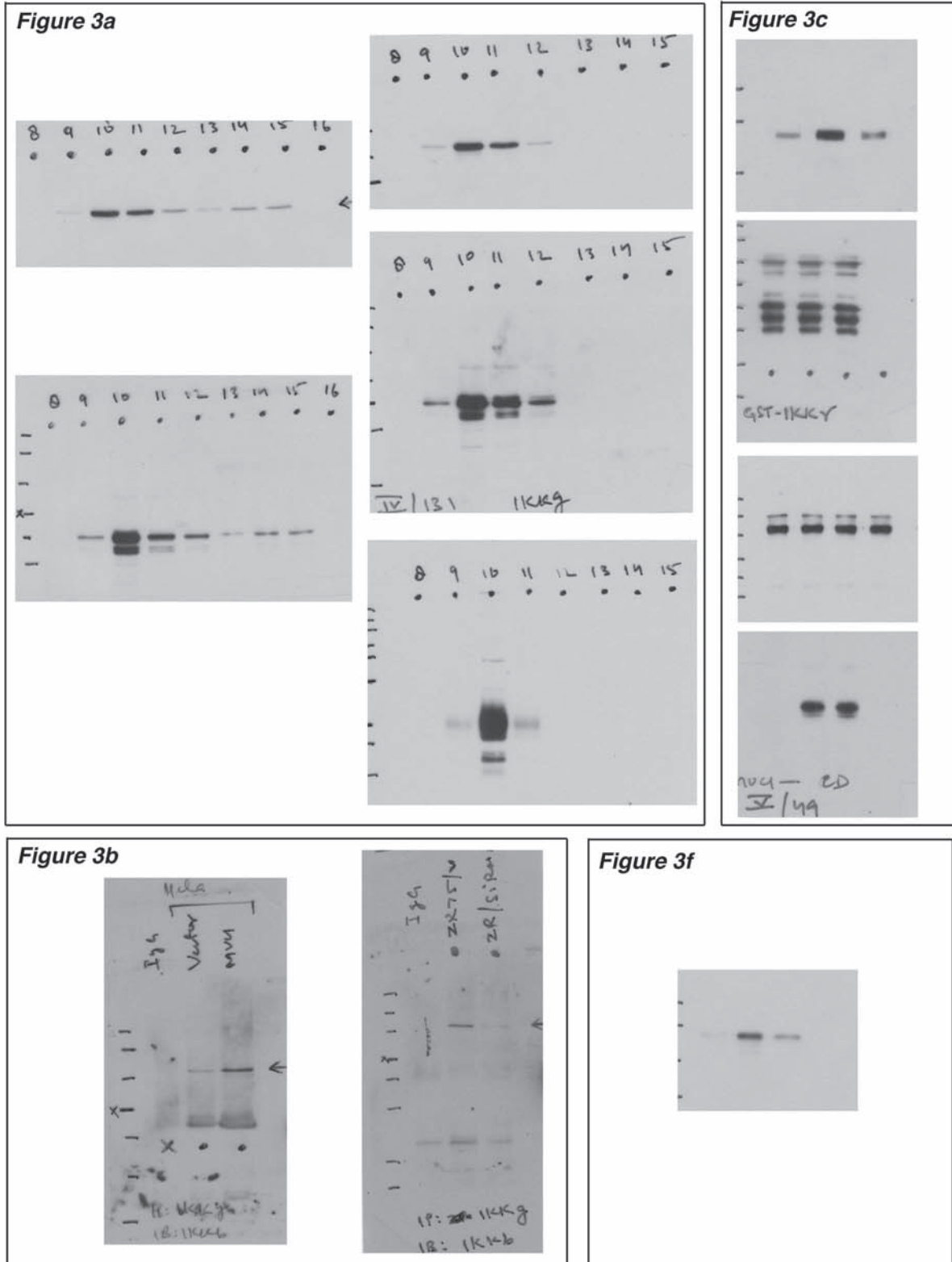
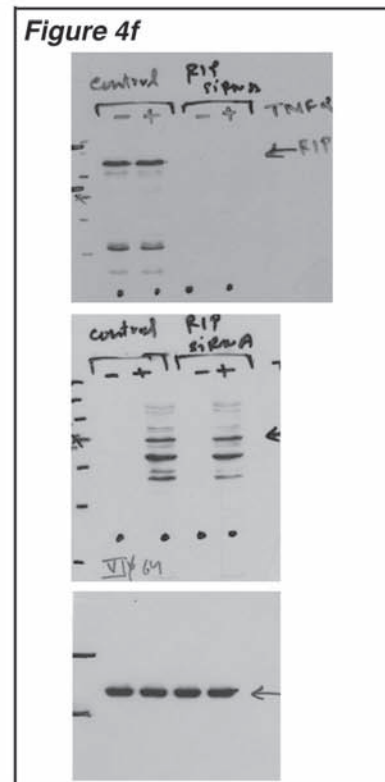
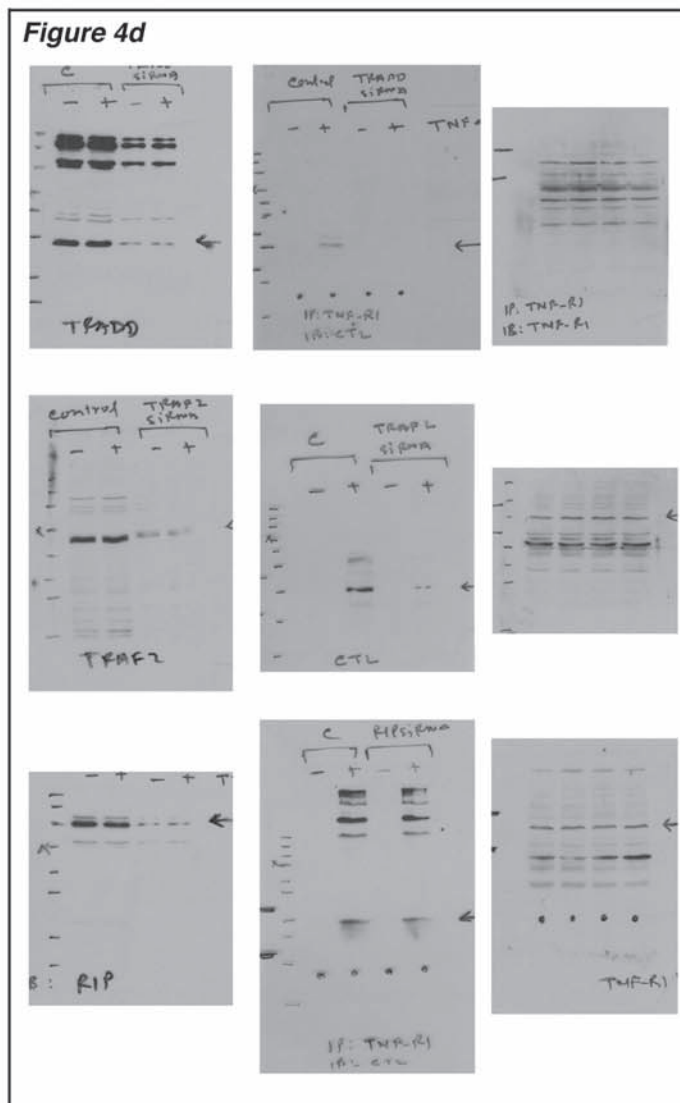
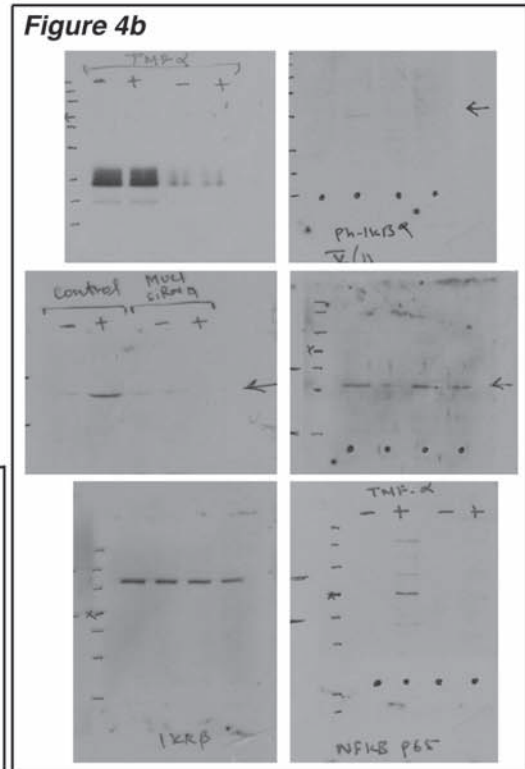
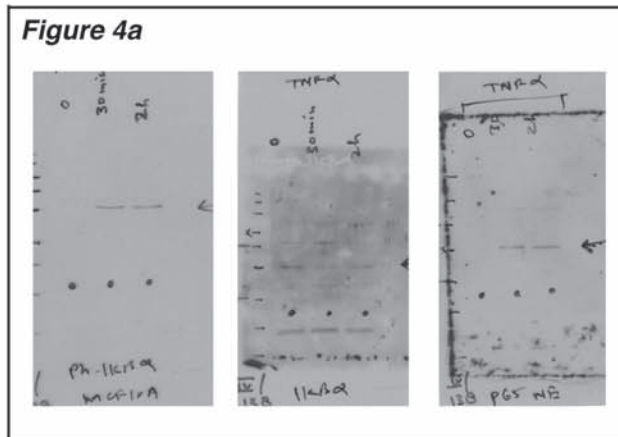


Figure S6-4



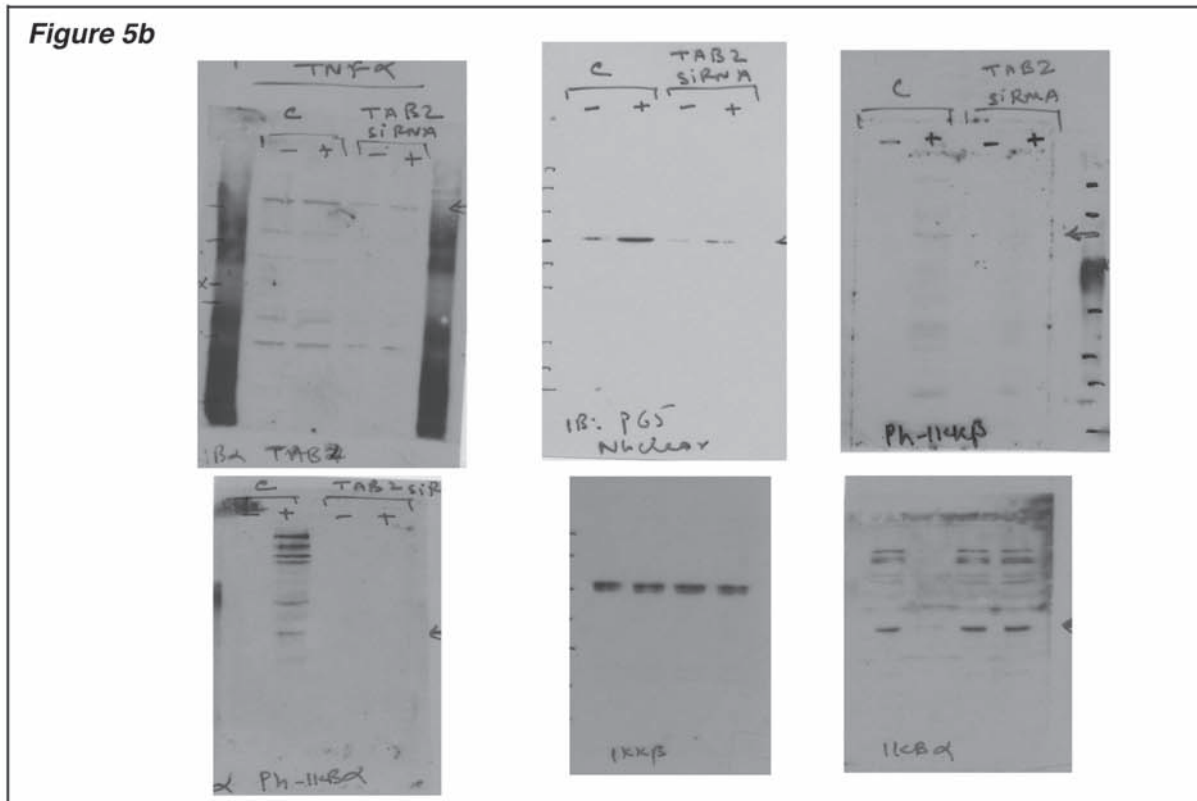
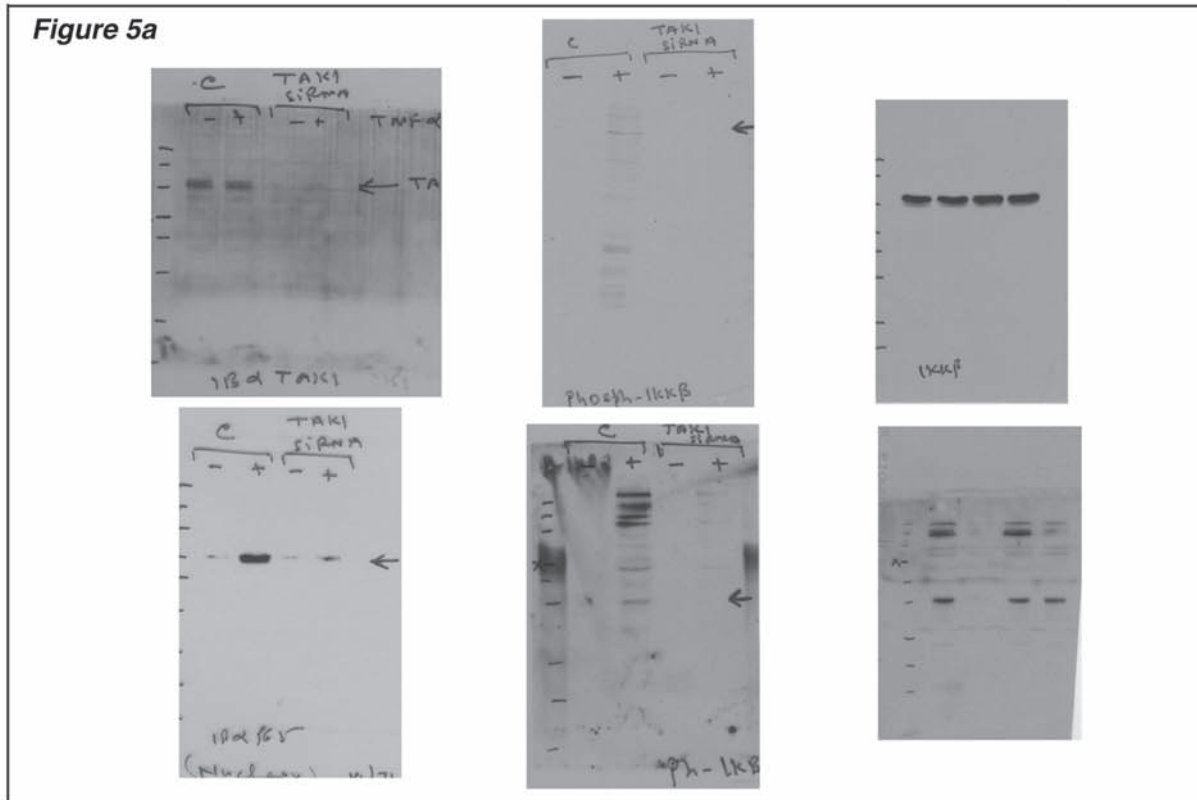


Figure S6 Scans of blots.

Mouse Muc1 siRNA pool (Dharmacon)

1. CAACAGCUCUCCAGUAGUC
2. CUACCAGUCUAGUCUAUAA
3. CCUGAAGACUCUACCAGUA
4. AGUCACAGCUUAUACAGCA

Human MUC1 siRNA pool (Dharmacon)

1. GAUCUGUGGUGGUACAAUU
2. ACCAAGAGCUGCAGAGAGA
3. GAUCGUAGCCCCUAUGAGA
4. GAUUAACCUGACGAUCUC

TRADD siRNA pool (Dharmacon)

1. GGAGGAUGCGCUGCGAAAU
2. GCGAGGGACUGUACGAGCA
3. GGGUCAGCCUGUAGUGAAU
4. GGACGAGGAGCGCUGUUUG

TRAF2 siRNA pool (Dharmacon)

1. GGAGCAUUGGCCUCAAGGA
2. GCAGGUACGGCUACAAGAU
3. CGGUAGAGGGUGAGAAACA
4. GAAGAAGGCAUUUCUAUUU

TAK1 siRNA pool (Dharmacon)

1. GAGGAAAGCGUUUUAUUGUA
2. CCCAAUGGCUUAUCUUACA
3. GGACAGCCAAGACGUAGAU
4. UACACUGGAUCACCAACUA

TAB1 siRNA pool (Dharmacon)

1. GAUGAGCUCUCCGUCUUU
2. GAACAACUGCUUCCUGUAU
3. GGAGAUUGCUGCGAUGAUU
4. GAACUGUCUUUACCACUGA

TAB2 siRNA pool (Dharmacon)

1. GAAGAGAUGAGUACUAAUUU
2. UAAGGGAAGUGCUGAAAUA
3. GGAACAAGGUUUAACUAAU
4. CCUCAAGGCUUUAUGUUU

Non-Targeting Control siRNA pool (Dharmacon)

1. UGGUUUACAUGUCGACUAA
2. UGGUUUACAUGUUGUGUGA
3. UGGUUUACAUGUUUUCUGA
4. UGGUUUACAUGUUUCCUA

(All of the siRNAs from Dharmacon have 3' UU overhangs on both strands and a 5'phosphate on the antisense strand)

RIP1 siRNA pool (Santa Cruz Biotechnology)

1. CGUGAAGAGUUUAAAGAAAtt
2. GACAGAAUGUGGCUUACAAtt
3. GACGAGUUCAUCACUACUAtt