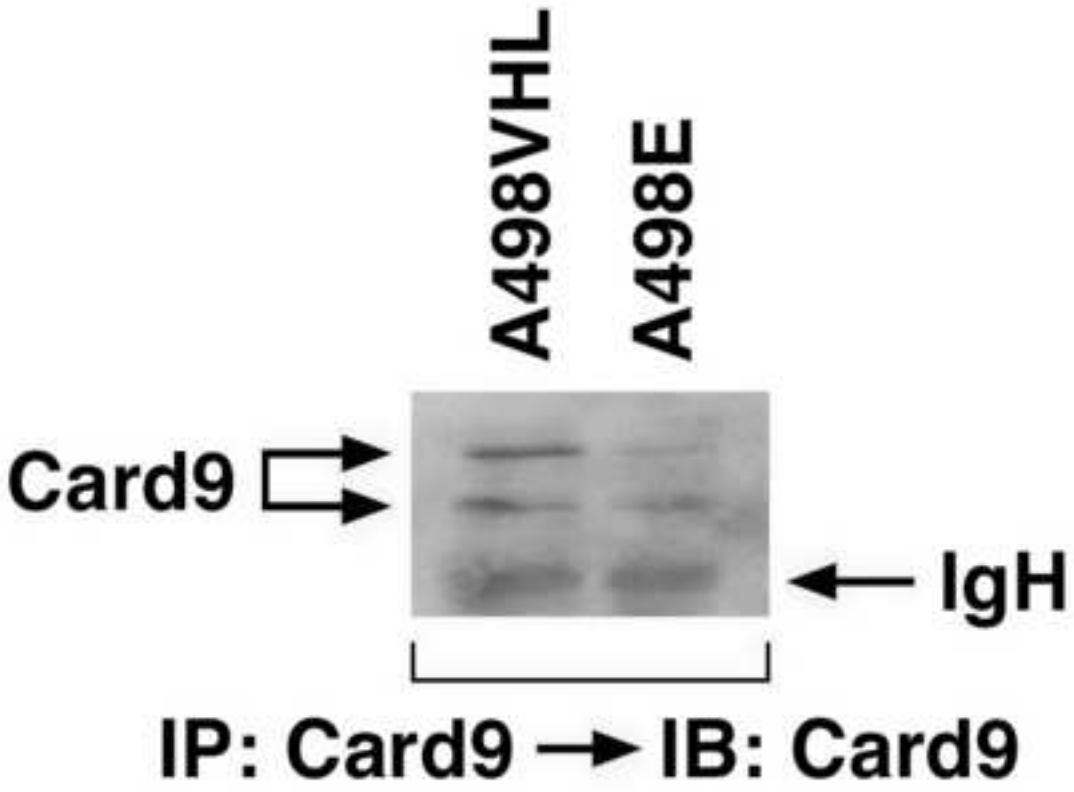
F) Supplemental Fig 1



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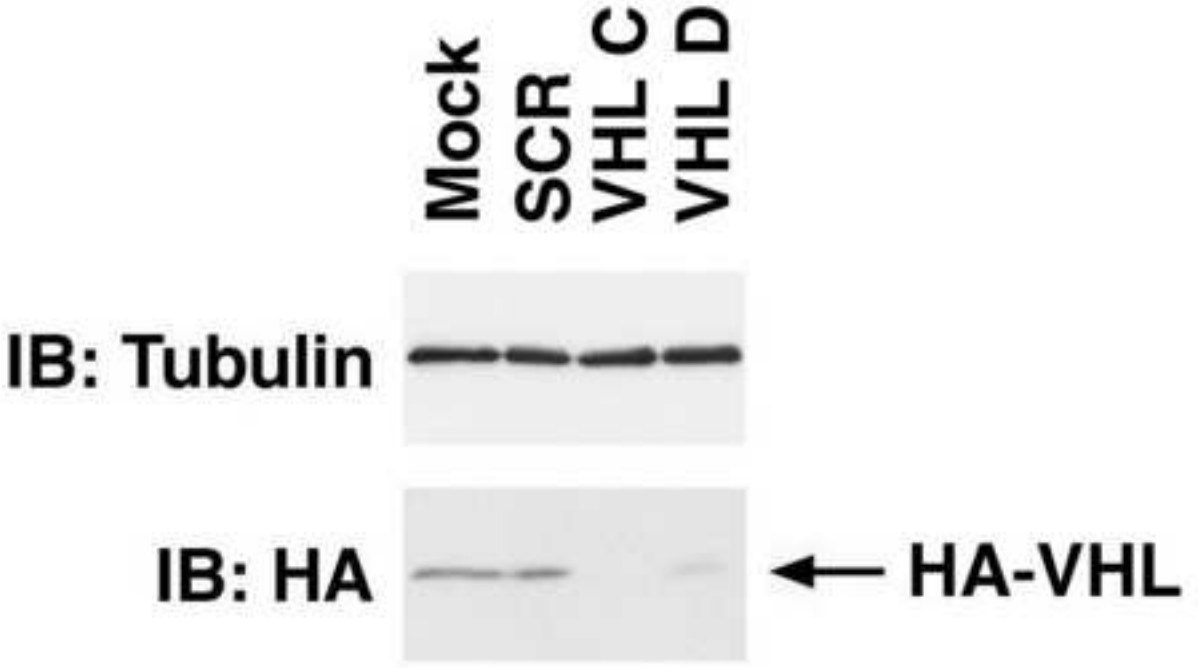

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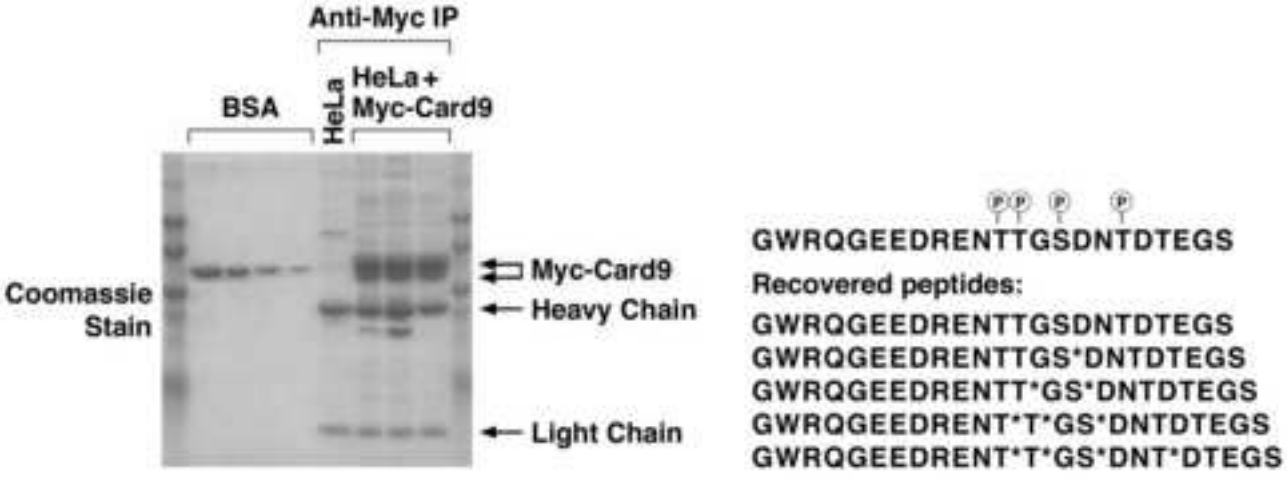

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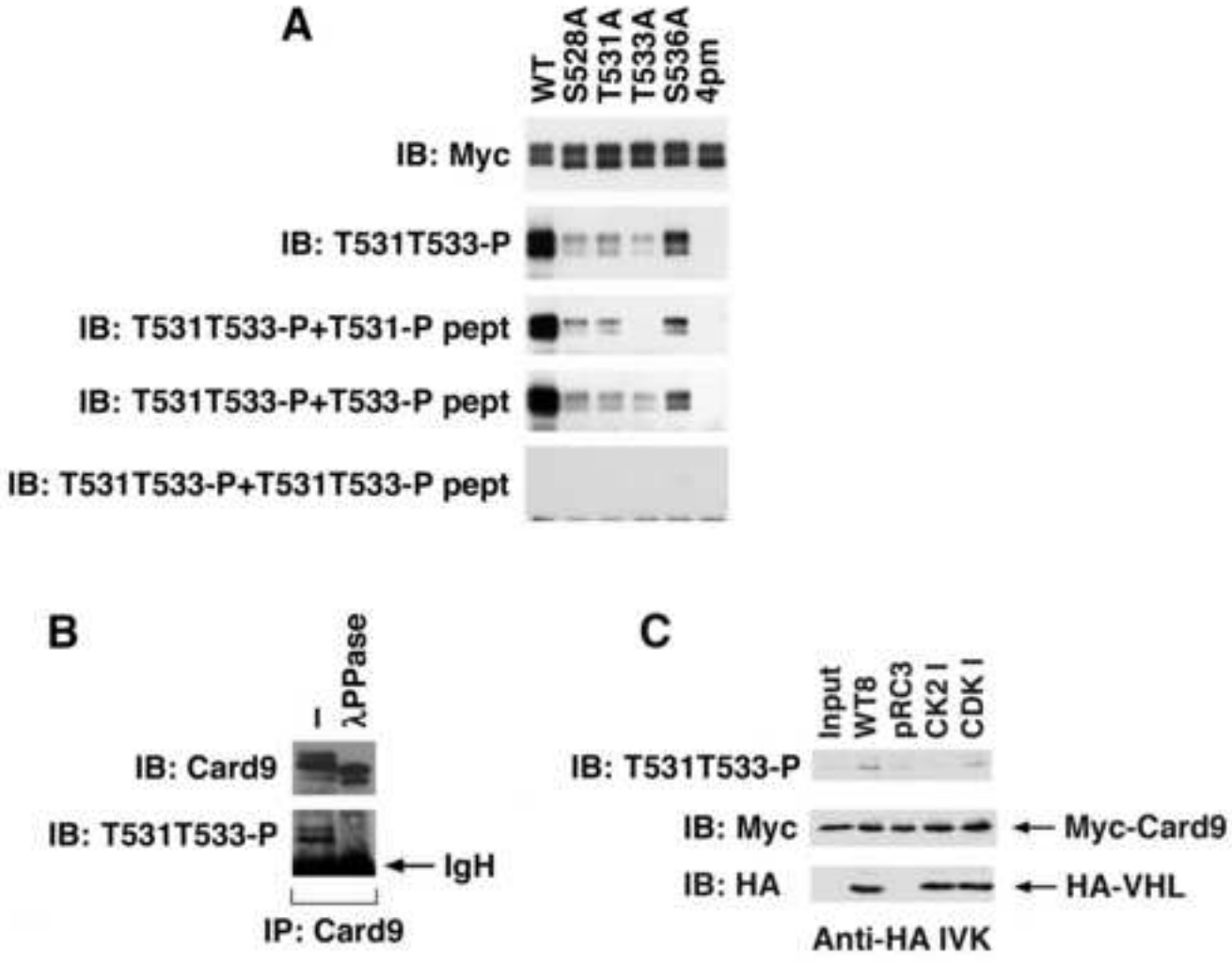

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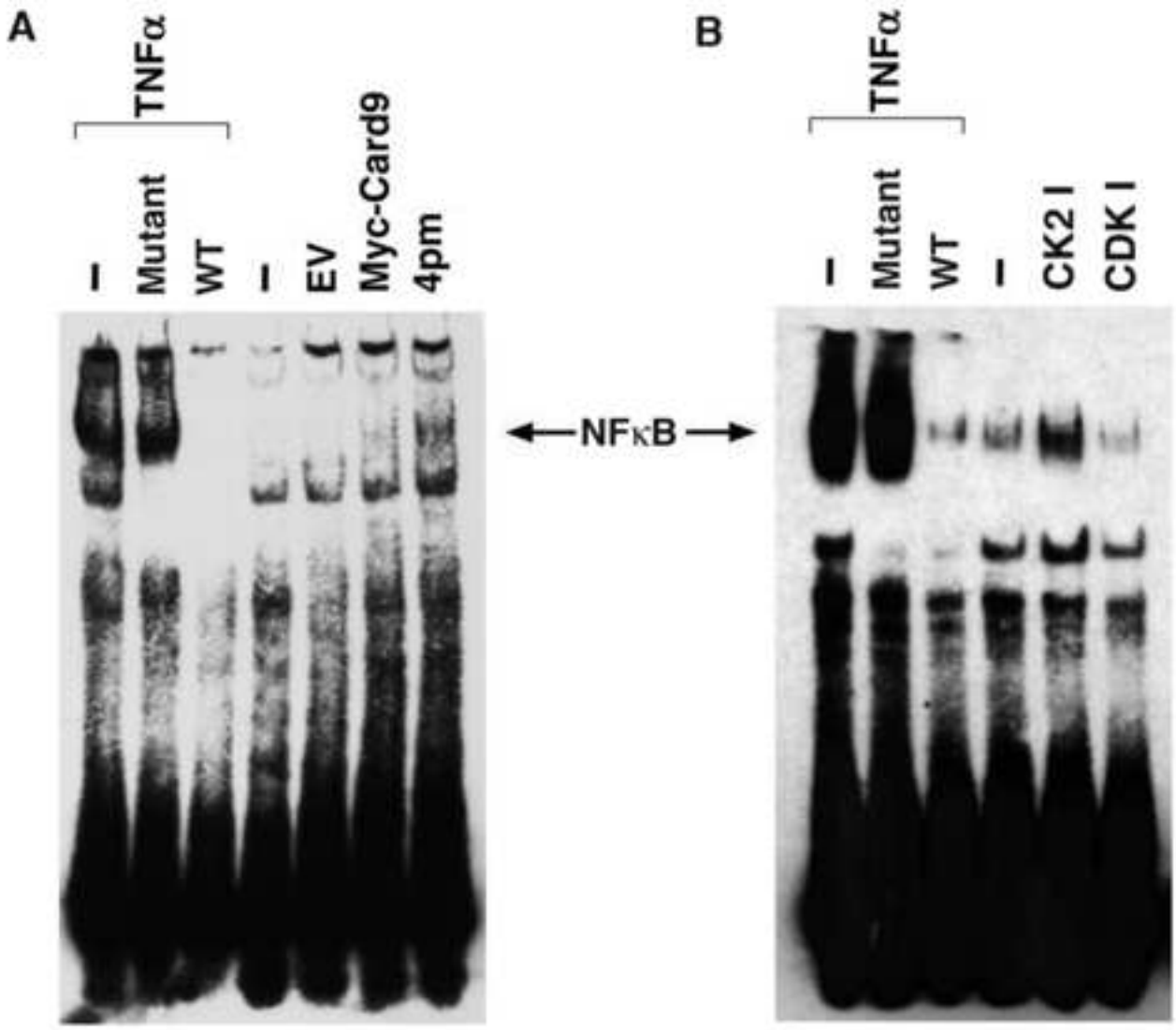

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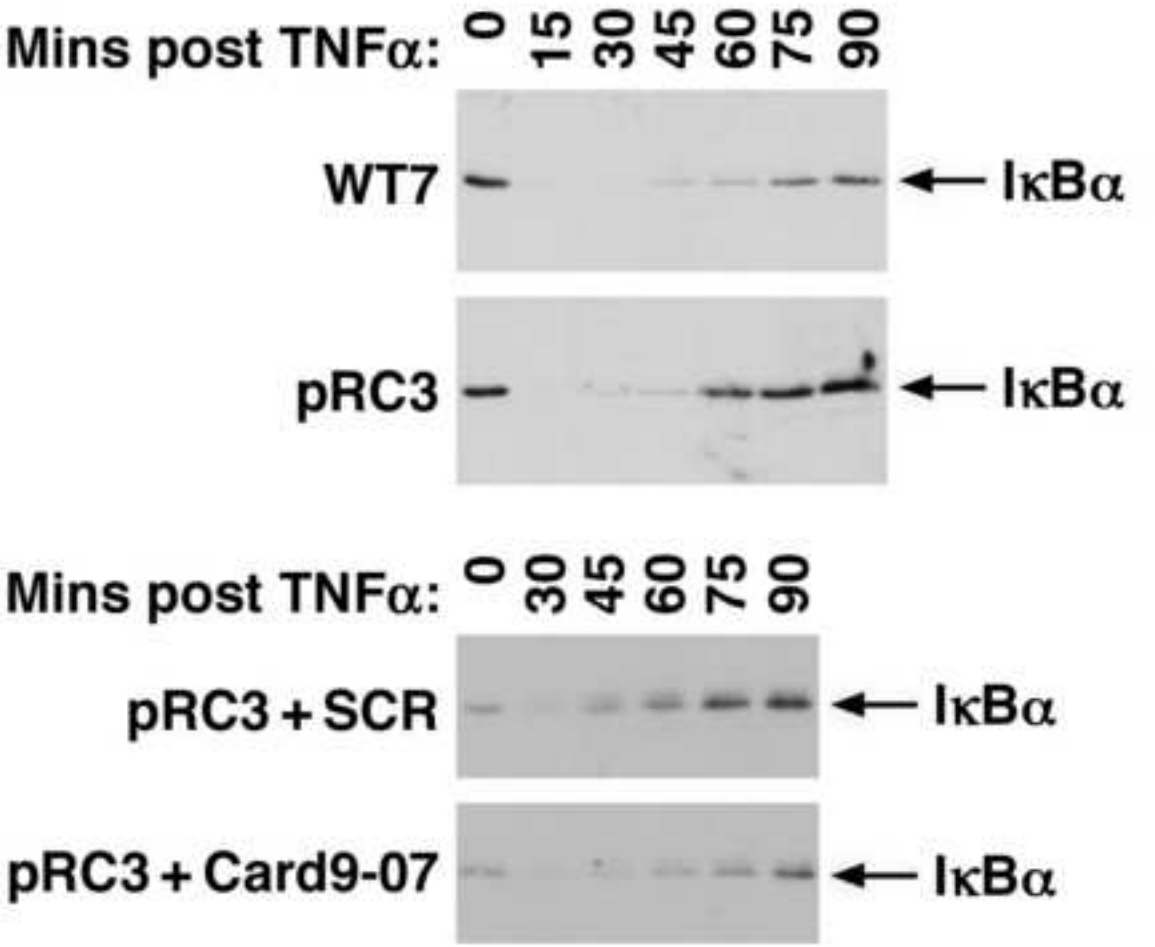

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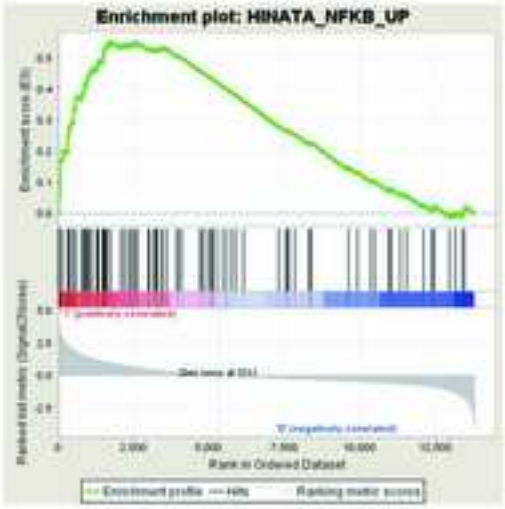
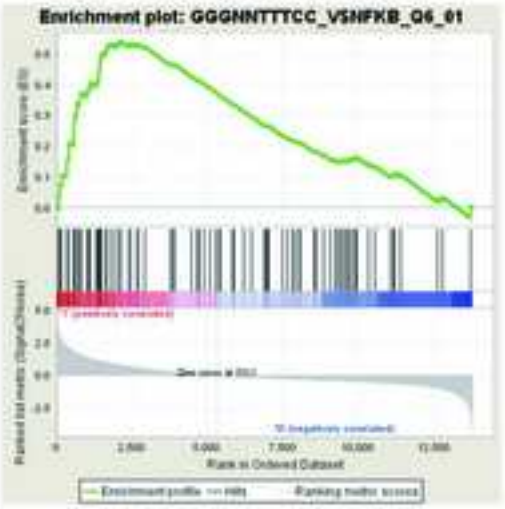
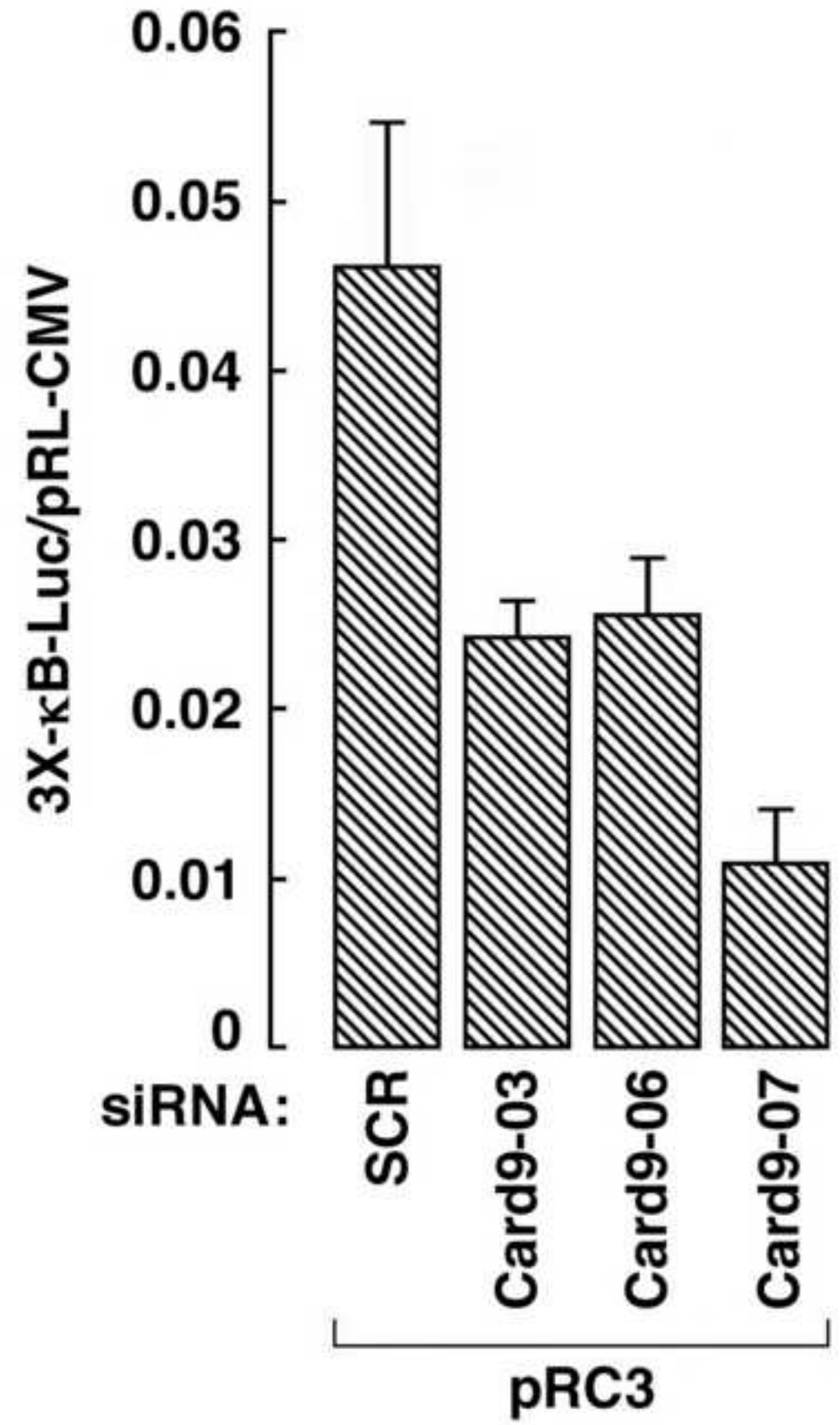


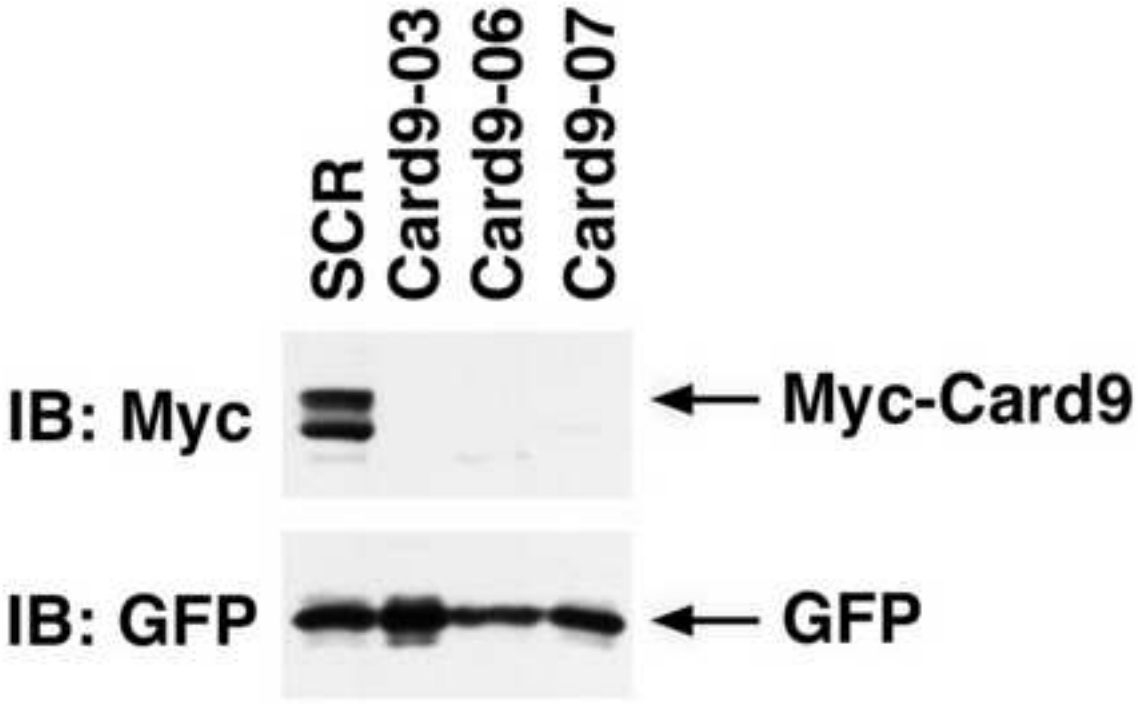


	NF-kB induced genes from Hinata et al.	Genes with conserved NFkB binding motif (Xie et al)
Gene set size	89 genes	91 genes
P value	< .01	< .01
FDR q value	< .01	< .01

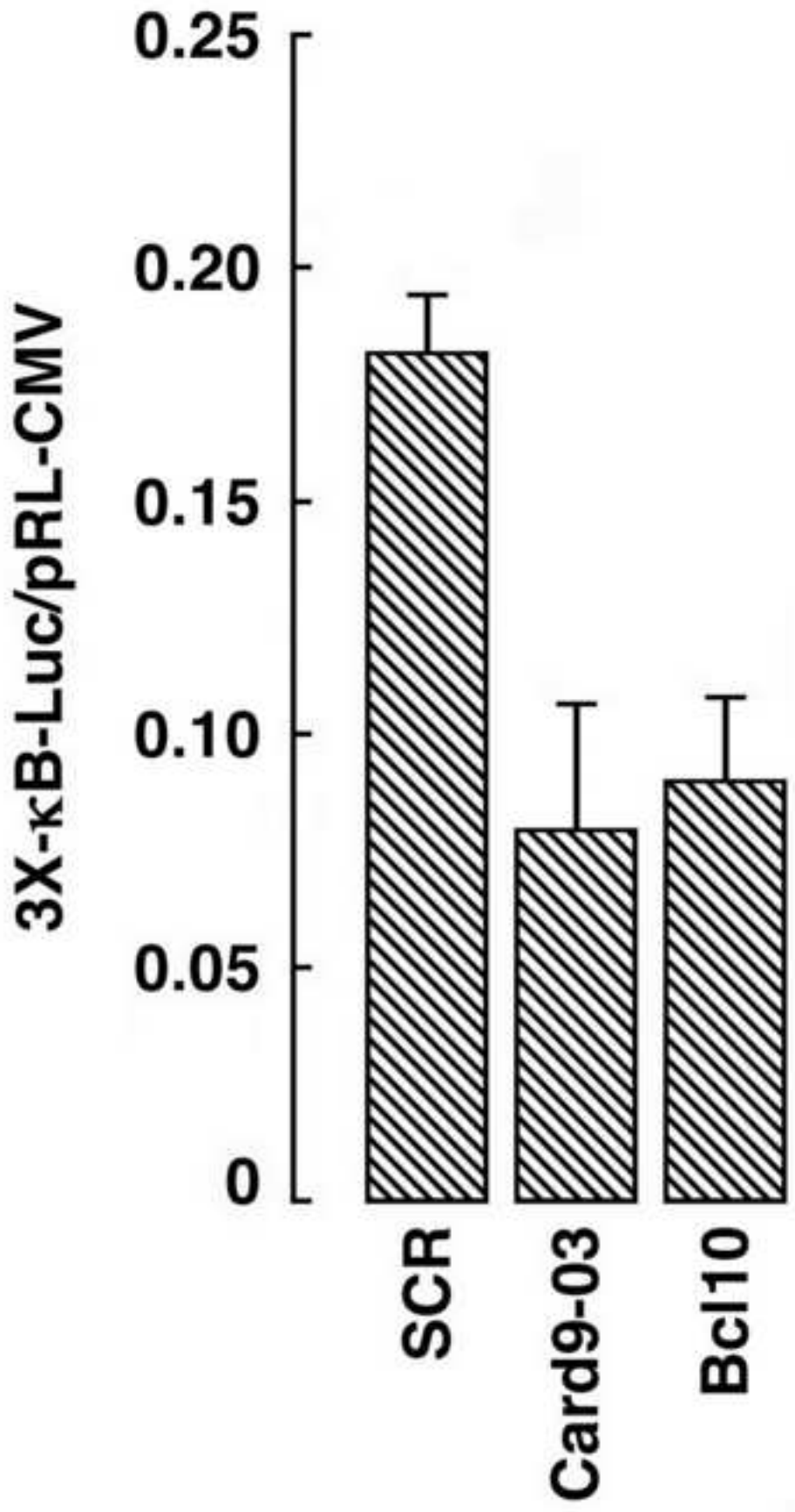
F) Supplemental Fig 8



F) Supplemental Fig 9



F) Supplemental Fig 10



F) Supplemental Fig 11

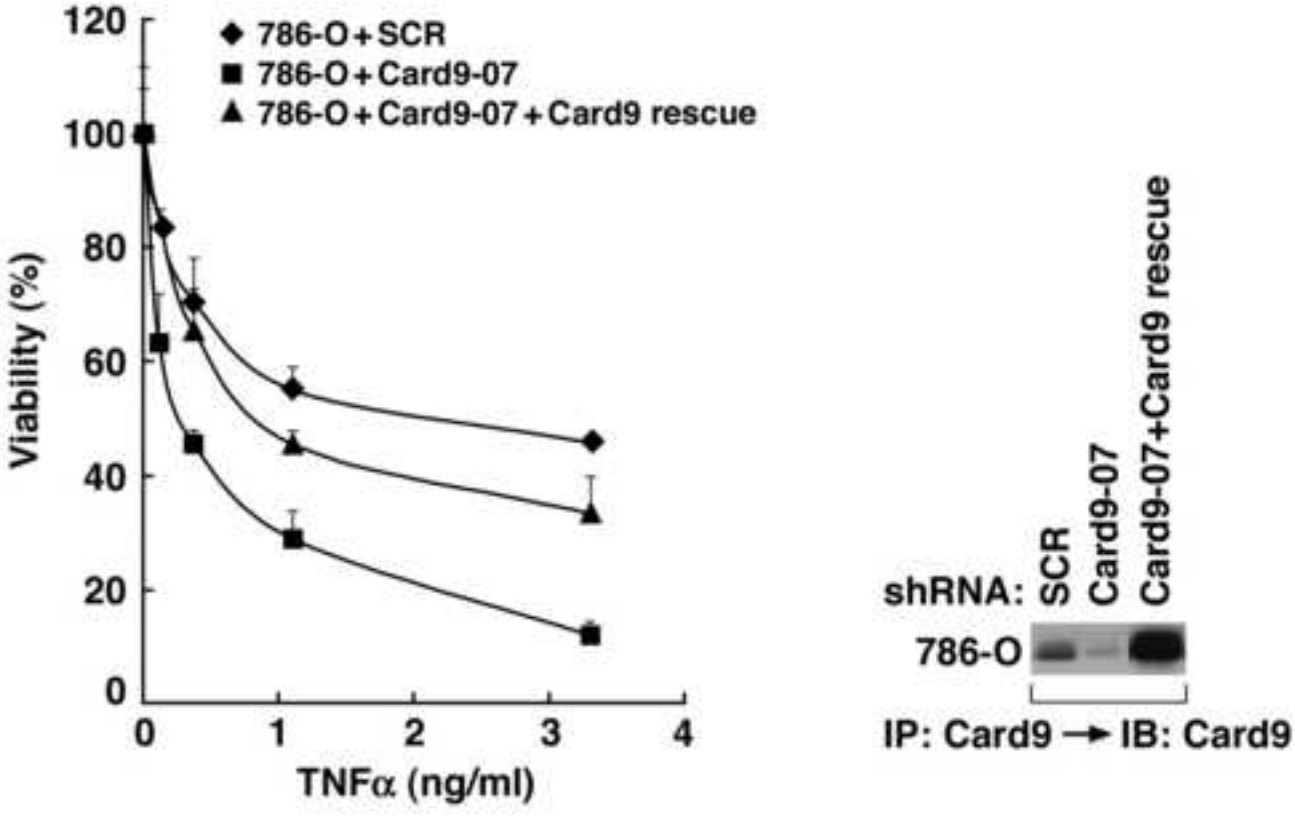


Figure legends for supplementary figures:

Figure S1. Immunoblot analysis of anti-Card9 immunoprecipitates from A498 cells stably infected to produce wild-type pVHL or infected with empty vector.

Figure S2. Validation of VHL siRNA

Immunoblot analysis of HeLa cells stably producing HA-VHL transfected with the indicated siRNAs. SCR = scrambled.

Figure S3. Purification of Myc-Card9 for Mass Spectrometry Analysis

Coomassie blue stained SDS-PAGE gel loaded with 2, 1, 0.5, or 0.2 μg of BSA (lanes 2-5) or with anti-Myc immunoprecipitates derived from HeLa cells transfected with empty vector (lane 6) or pcDNA3-Myc-Card9 (lanes 7-9). Bands labeled Myc-Card9 were excised and analyzed by mass spectrometry. Recovered phosphopeptides (* = phosphoamino acid) corresponding to Card9 residues 514-536 are shown.

Figure S4. Validation of phosphospecific antibody recognizing Card9 peptide phosphorylated at T531 and T533

(A) Immunoblot analysis of anti-Myc immunoprecipitates derived from Hela cells that were transiently transfected to produce the indicated Myc-Card9 variants. Where indicated blocking peptides in which T531, T533, or both were phosphorylated were added as competitors.

(B) Immunoblot analysis of anti-Card9 immunoprecipitates from leukemic THP-1 cells that were not treated or treated with λ protein phosphatase.

(C) Immunoblot analysis of Myc-Card9 after in vitro kinase assays performed with anti-HA immunoprecipitates derived from WT8 cells, which produce HA-pVHL, (lane 2, 4, 5) or pRC3 (empty vector) cells (lane 3). CK2 inhibitor TBB (30 μM) or Cdk inhibitor 3-Amino-1H-pyrazolo[3.4-b]quinoxaline (30 μM) were present during the in vitro kinase assays corresponding to lane 4 and 5, respectively.

Figure S5. Activation of NF-KB DNA-Binding Activity by Card9 Phosphosite Mutant

(A) NF-KB DNA Binding Activity measured by electrophorectic mobility shift assay using nuclear extracts from 293T cells transfected to produce the indicated Card9 variants or, as a positive control, treated with TNFα. EV = empty vector transfectants. Where indicated unlabeled wild-type (WT) or mutant competitor oligonucleotides were added.

(B) NF-KB DNA Binding Activity measured by electrophorectic mobility shift assay using nuclear extracts from Hep3B cells treated with Solvent (DMSO), CK2 inhibitor (TBB, 120uM) and CDK inhibitor (120uM) for 20 hours. TNFα treated cells were used as positive control.

Methods: Cells were suspended and swollen in 200 μl of buffer A (20 mM [HEPES; pH 7.9], 10 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol, pH 7.9) supplemented with protease and phosphatase inhibitors. 0.5% NP-40 was added and the cells were gently vortexed for 10 sec. Following centrifugation, the pelleted nuclei were washed with buffer A, resuspended in 50 μl of buffer C (20 mM HEPES [pH 7.9], 0.4 M NaCl, 1 mM EDTA, 1 mM dithiothreitol) supplemented with protease and phosphatase

inhibitors, and vortexed for 15 min at 4 ^{o}C. The extract was centrifuged and used for EMSA analysis. 5-10 μg nuclear extract was incubated with 1μl 10x binding buffer (100mM Tris-HCl, pH 7.5, 0.5M NaCl, 10mM EDTA, pH 8.0, 50% glycerol), 0.5 μl GTP (60mM), 2 μl BSA (10mg/ml), and 1 μl biotinylated probe (30uM) in a total volume of 10 μl at room temperature for 15 minutes. DNA-protein complexes were resolved on a 6% nondenaturing 0.5X TBE gel, transferred to nitrocellose, and crosslinked by baking and exposure to UV light. Complexes were detected using a Pierce LightShift Chemiluminescent EMSA kit.

The NF-KB specific probe contained sense and antisense oligonucleotides that were 3'-biotinylated. Non-biotinylated duplexes were used as competitors. NF-KB wild-type (sense strand): AGTTGAGGGGACTTTCCCAGGC;

NF-KB mutant (sense strand): AGTTGAGGCGACTTTCCCAGGC.

Figure S6. VHL and Card9 affect IκBα Induction

Anti-IκBα immunoblot analysis of whole cell extracts from pRC3 and WT7 cells before and after treatment with 20 ng/ml TNFα (Top). Bottom panel depicts similar analysis of pRC3 cells infected with lentiviral vector expressing a scrambled shRNA or Card9 shRNA (Card9-07).

Figure S7. Activation of NF-κB by *VHL* inactivation in vivo as Determined by Gene Set Enrichment Analysis

Microarray-based gene expression data from the livers of *VHL* flox/flox; Alb-Cre mice was compared to Alb-Cre control mice using a previously published dataset (Kim et al., 2006). Data were preprocessed and normalized using Robust Multichip Averaging (Bolstad et al., 2003). Gene set enrichment analysis (GSEA) was performed as described (Subramanian et al., 2005). Two sets of NF-kB target genes were used: the first set was determined experimentally by gene expression profiling (Hinata et al., 2003) and the second set was defined computationally as the set of genes with NFkB binding sites in their promoters that are conserved between four mammalian species including human and mouse (Xie et al., 2006). All genes on the array were ranked by signal to noise score according to the comparison of VHL inactivation versus control. The vertical bars correspond to the location of the genes in the gene sets in the ranked list. The enrichment score is shown in green at the top. Statistical significance was determined by random permutation of the gene sets. The expression of both sets of NF-kB target genes were significantly enriched in the livers of *VHL* -/- mice compared with control mice ($p < .01$, FDR < .01 for both gene sets).

Figure S8. Regulation of NF-κB activity by pVHL and Card9

Normalized luciferase values of pRC3 cells transfected with NF-κB-responsive reporter and indicated siRNAs.

Figure S9. Validation of Card9 siRNA

Immunoblot analysis of HeLa cells transfected to produce Myc-Card9 in the presence of the indicated siRNAs. SCR = scrambled.

Figure S10. Increased NF-κB activity in *VHL*-/- Renal Carcinoma Cells Requires Bcl10

Normalized luciferase values of pRC3 cells transfected with NF-κB-responsive reporter in the presence of the indicated siRNAs. SCR = scrambled siRNA.
Card9-03 = Card9 siRNA. Bcl10 = Bcl10 siRNA.

Figure S11. Restoring Card9 expression abrogates the effect of a Card9 shRNA

Viability of the indicated 786-O renal carcinoma derivatives after exposure to TNFα.
SCR = scrambled shRNA. Card9-07 = Card9 shRNA. Card9 rescue = Card9 cDNA with silent mutations in Card9 shRNA binding site.