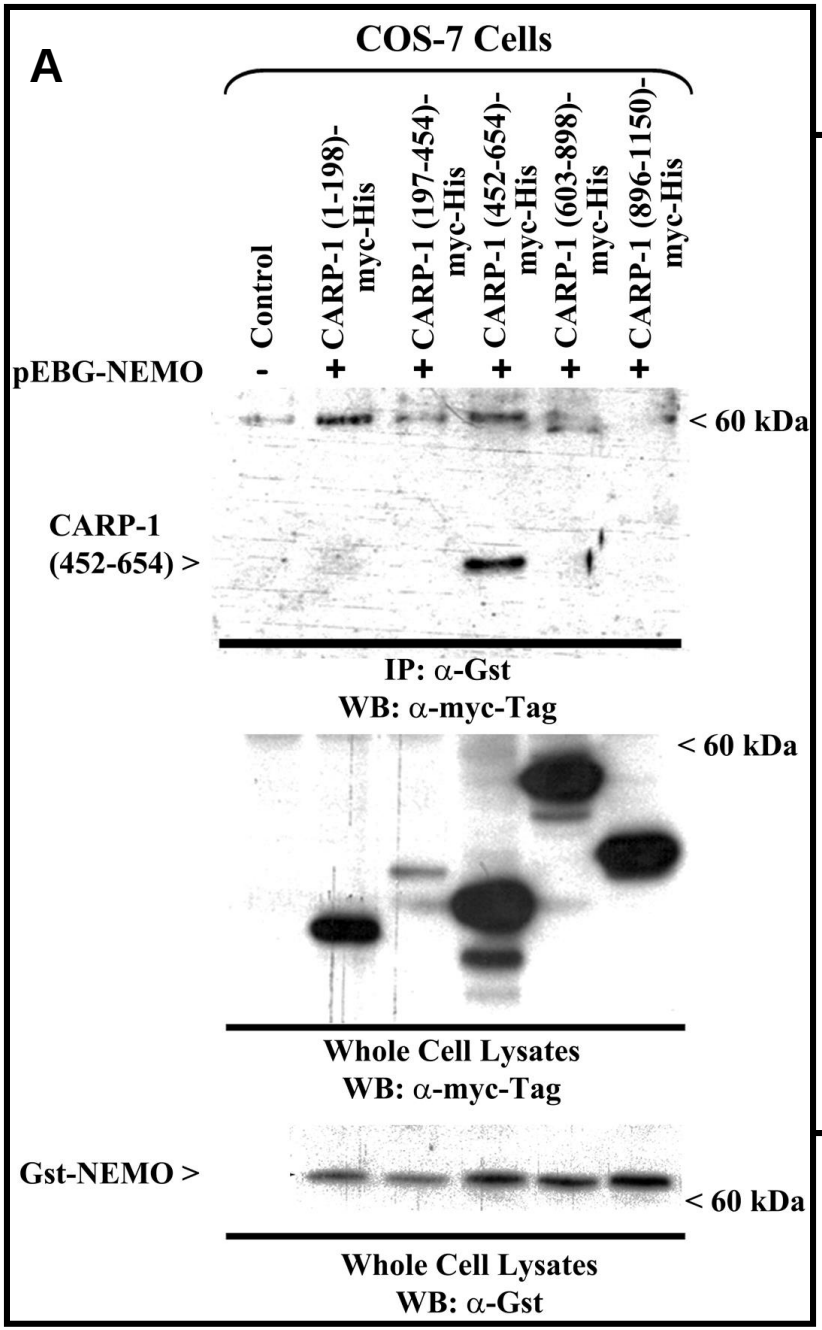
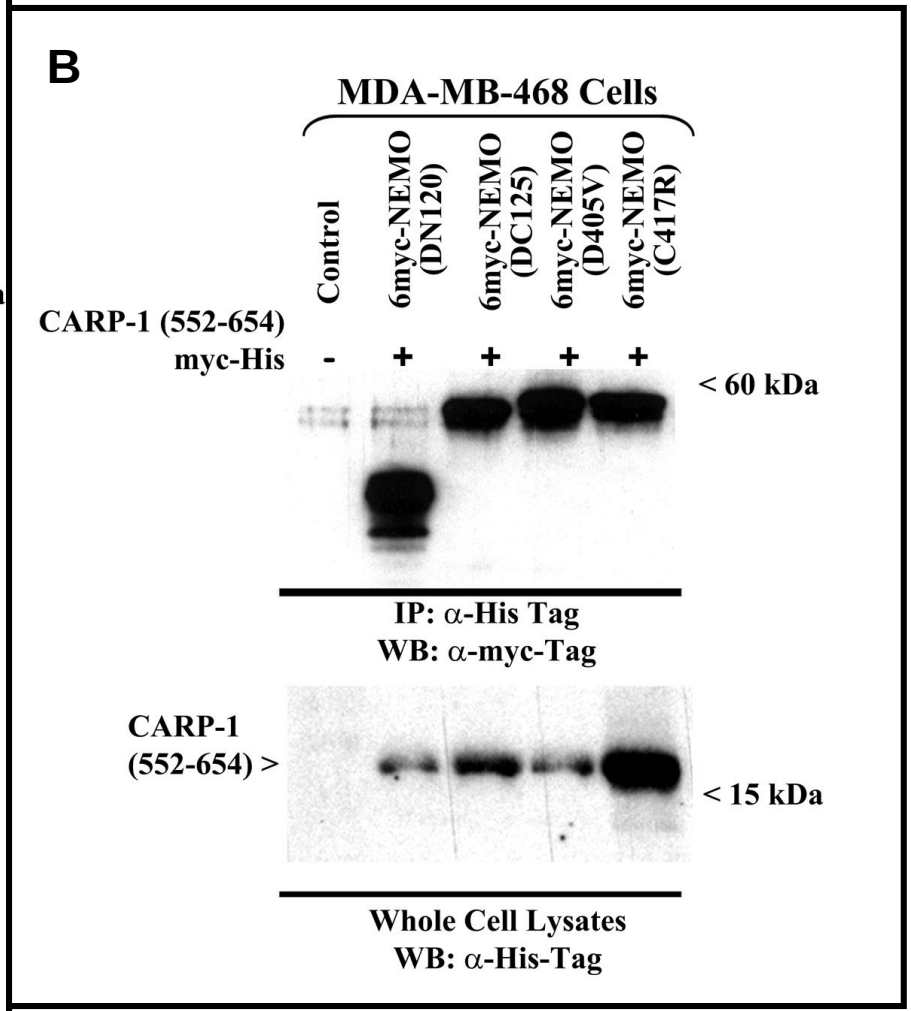
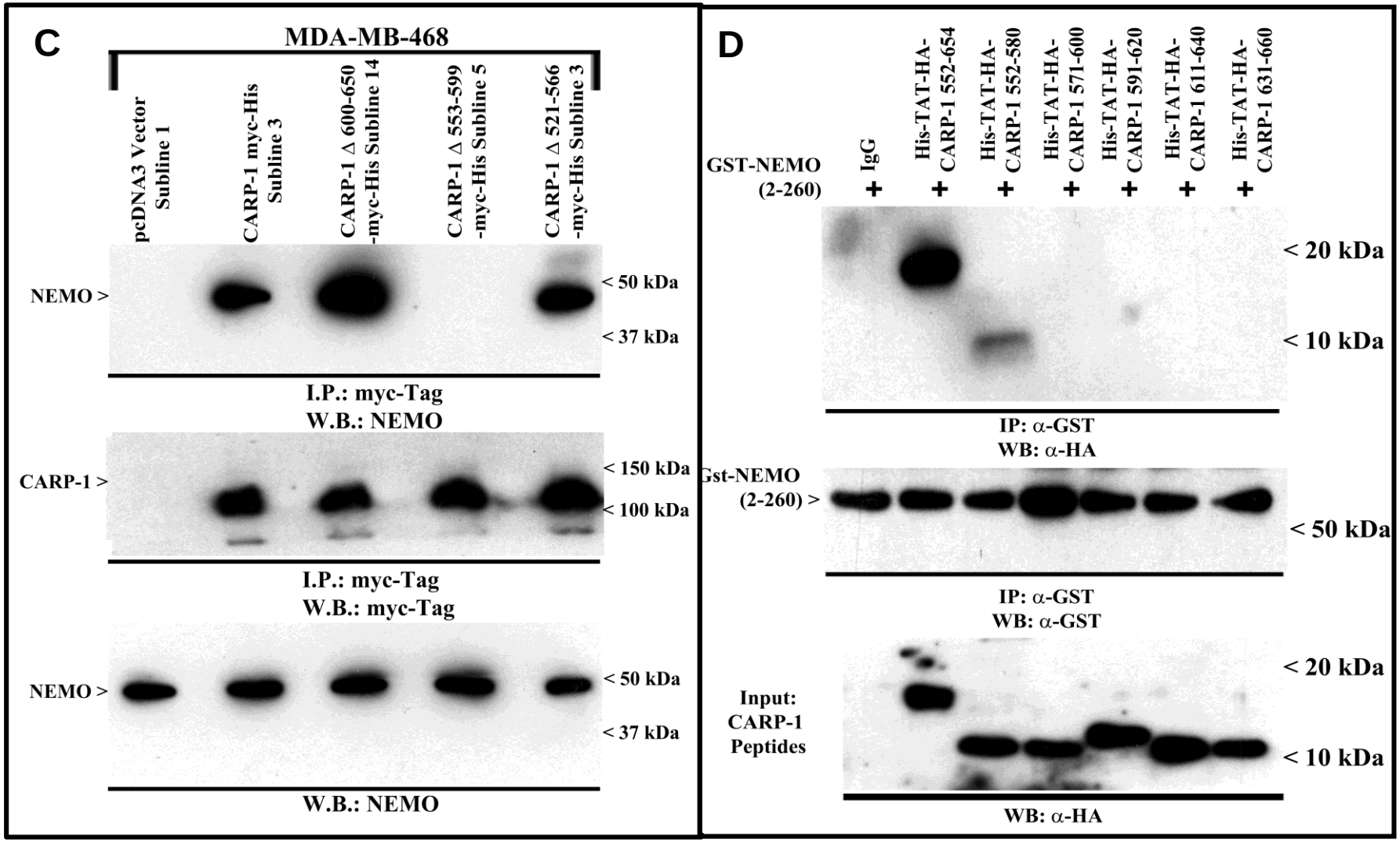


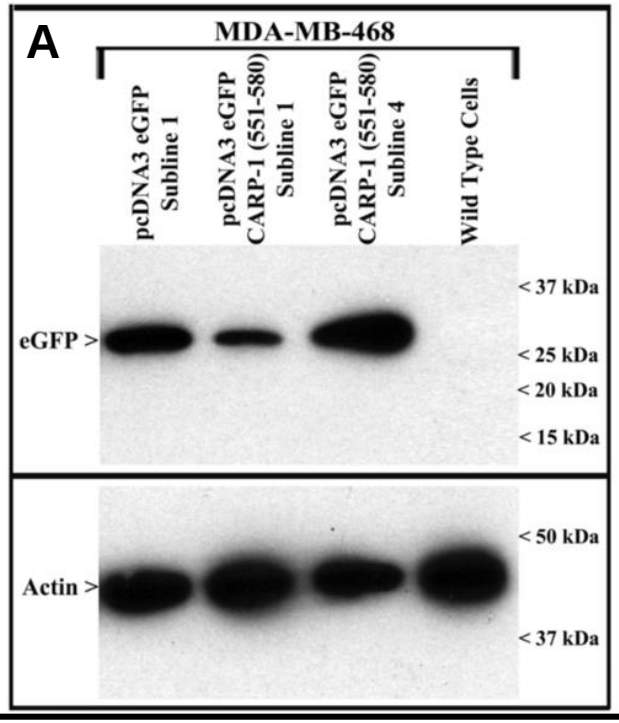


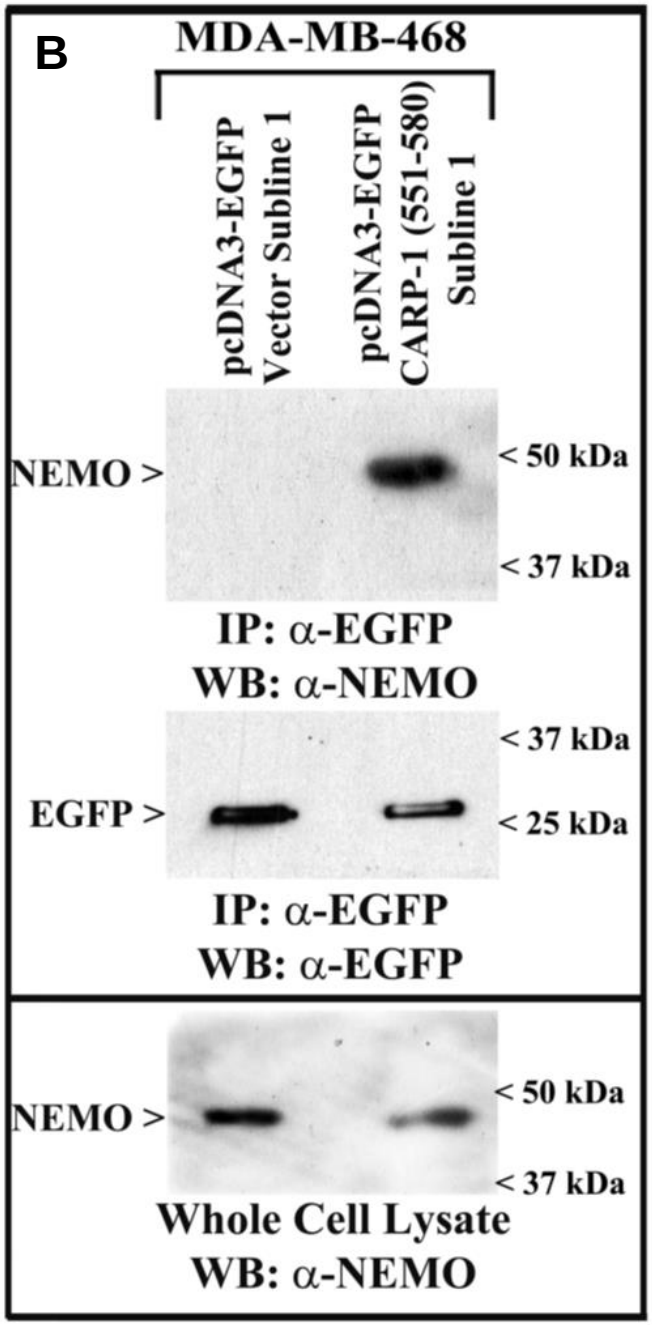
Supplementary Figure 1

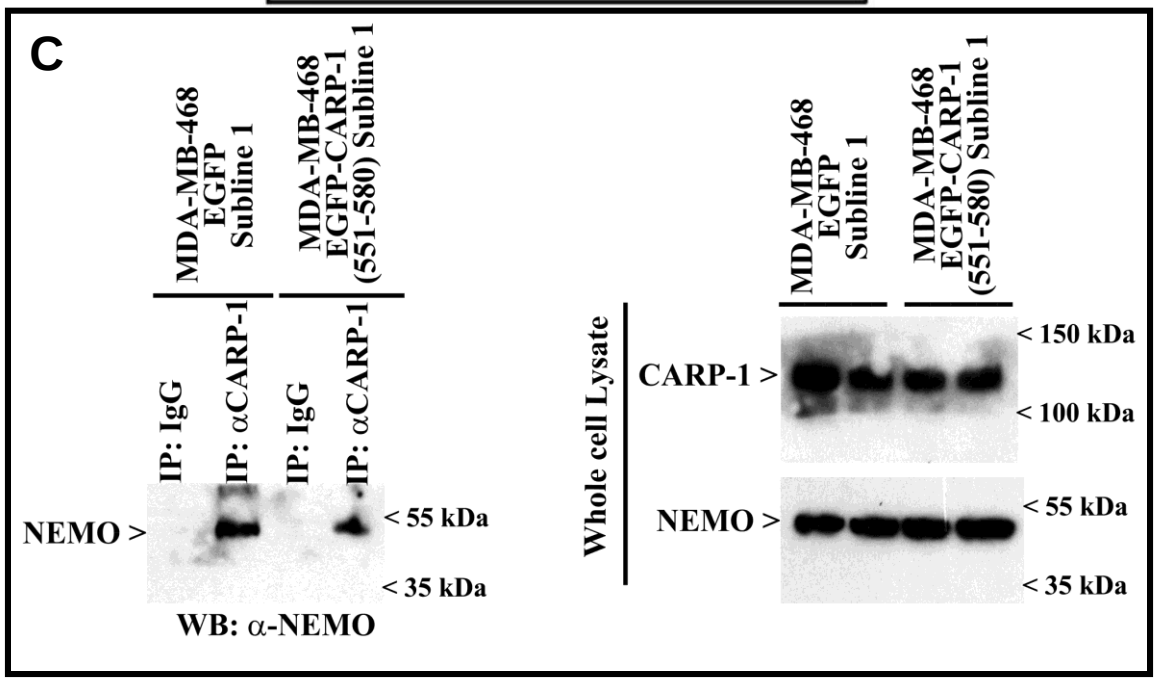


Supplementary Figure 1

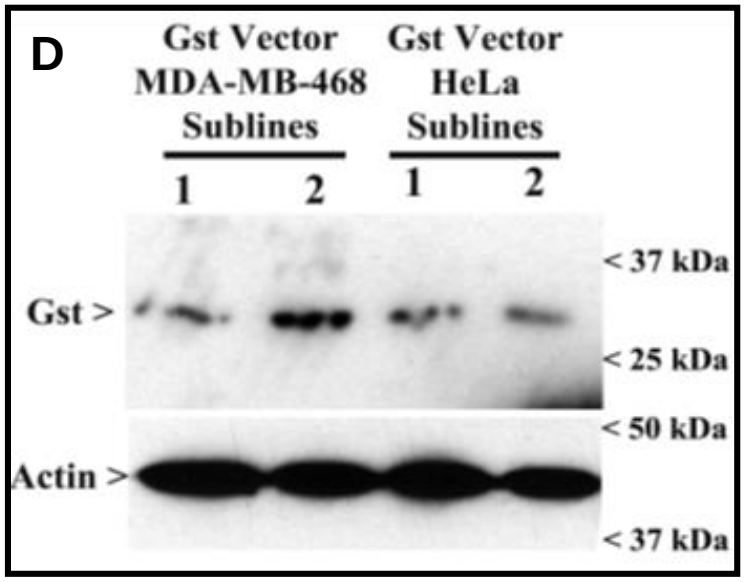
Supplementary Fig. 1: Mapping of minimal epitopes of CARP-1 binding with NEMO. *A.* Noted cells were either untransfected (noted as -) or transfected separately with plasmids expressing indicated, myc-His-tagged CARP-1 mutant proteins in combination with plasmid expressing Gst-tagged NEMO (noted as +). Upper Blot, W.B. analysis of I.P. protein complexes using indicated antibodies. The membrane containing proteins from whole cell lysates was probed with anti-myc-Tag (middle) or anti-Gst (Lower) antibodies for presence of respective fusion proteins. *B.* Noted cells were either untransfected (noted as -) or transfected separately with plasmids expressing indicated, 6 x myc-tagged NEMO mutant proteins in combination with plasmid expressing myc-His-tagged CARP-1 (552-654; noted as +). Upper Blot, Cell lysates were subjected to I.P. using anti-His tag antibodies to precipitate proteins complexed with His-tagged CARP-1 (552-654). The immunocomplexes were then analyzed by W.B. with anti-myc tag antibodies to detect 6xmyc tagged NEMO proteins. The membrane containing proteins from whole cell lysates was probed with anti-His-Tag (Lower) antibodies for presence of CARP-1 (552-654) protein. *C.* Noted cells were first transfected with vector plasmid or plasmids encoding myc-His-tagged WT CARP-1 or its mutants as indicated. The neomycin-resistant, stable sublines were generated and characterized as described before (3, 21). Upper Blot, W.B. analysis of I.P. protein complexes using indicated antibodies. The membrane from upper blot was next probed with myc-Tag (middle) or the membrane containing proteins from whole cell lysates was probed with anti-NEMO (Lower) antibodies. *D,* The Gst-tagged NEMO (2-260) protein, and various His-TAT-HA-tagged CARP-1 peptides were purified following expression in *E.coli* BL-21 cells as described (21). The Gst-NEMO (2-260) protein was immobilized on glutathione sepharose followed by incubation with IgG or indicated CARP-1 peptides. Following stringent washing, the bound proteins were analyzed by WB using anti-HA (Upper) or anti-Gst (middle) antibodies. The lower blot shows respective HA-tagged CARP-1 peptides used as input. Arrowheads on the left or right side of each blot in panels A-D indicate presence of proteins or molecular weight markers, respectively.

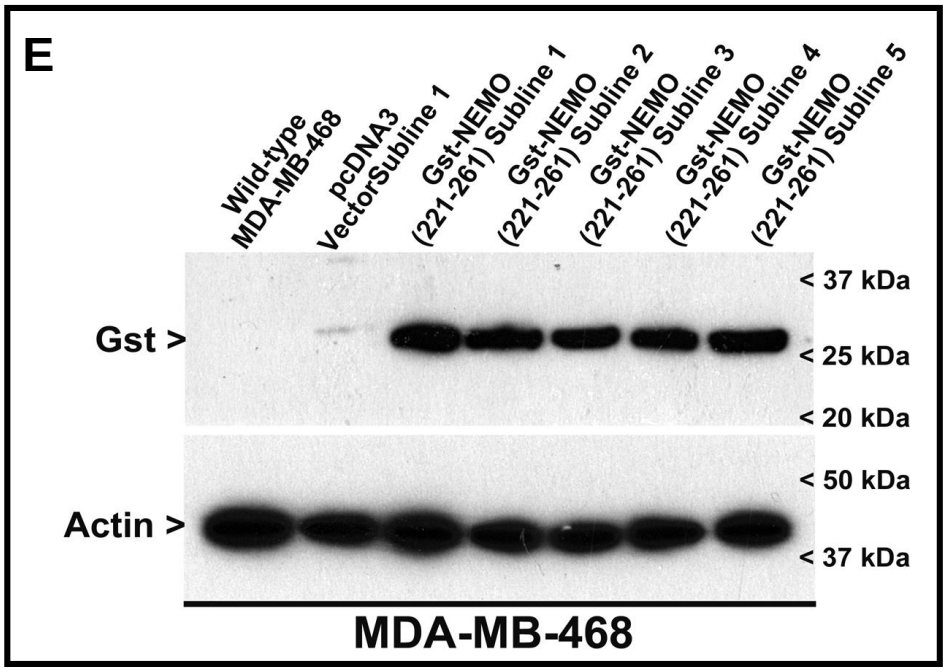


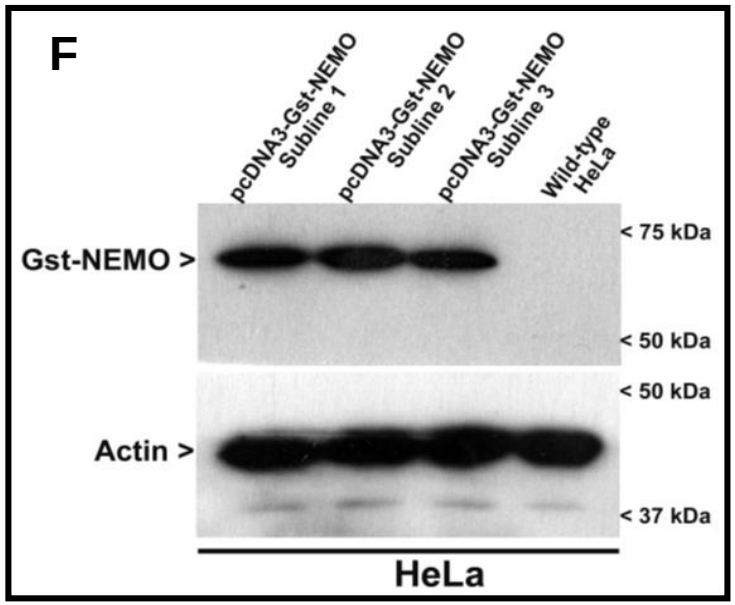


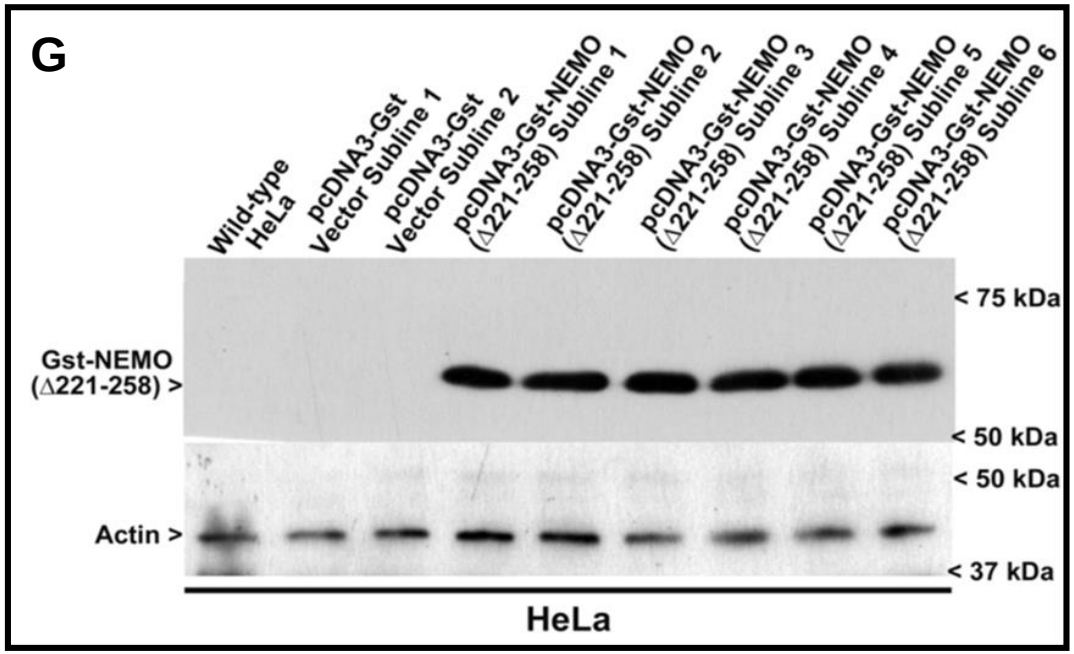


Supplementary Figure 2

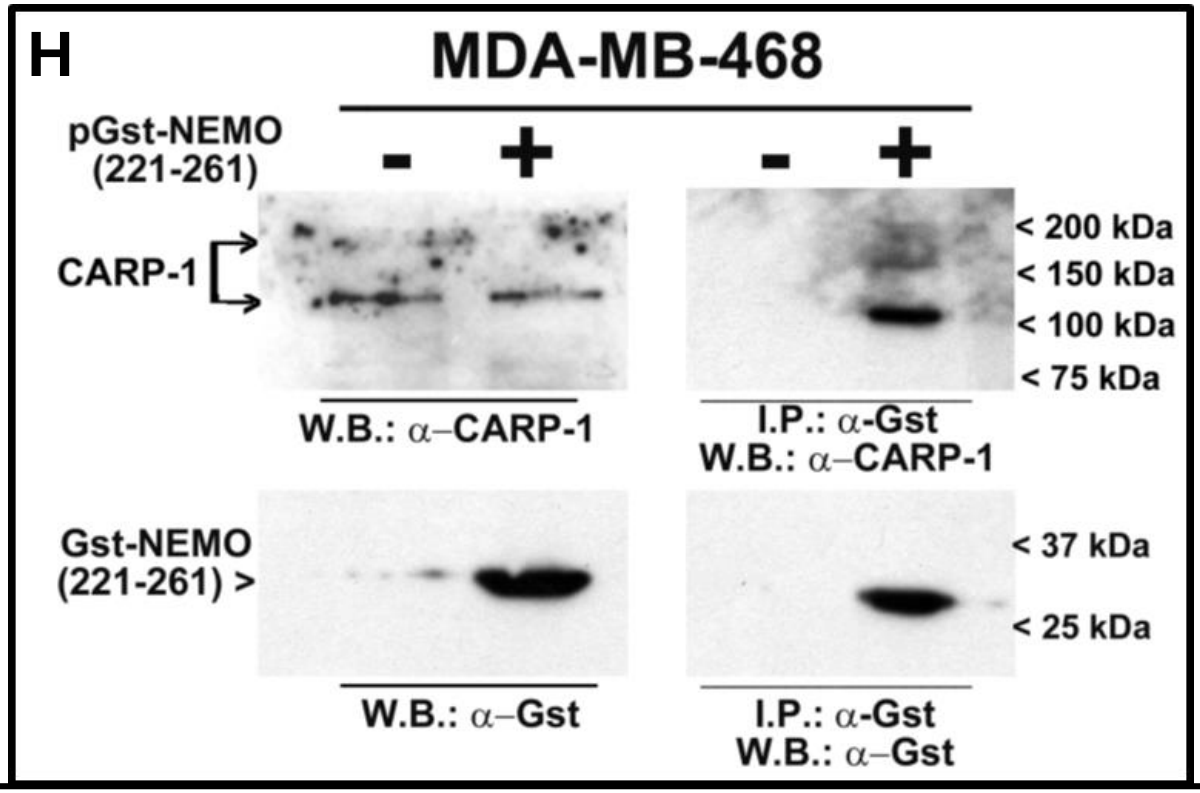


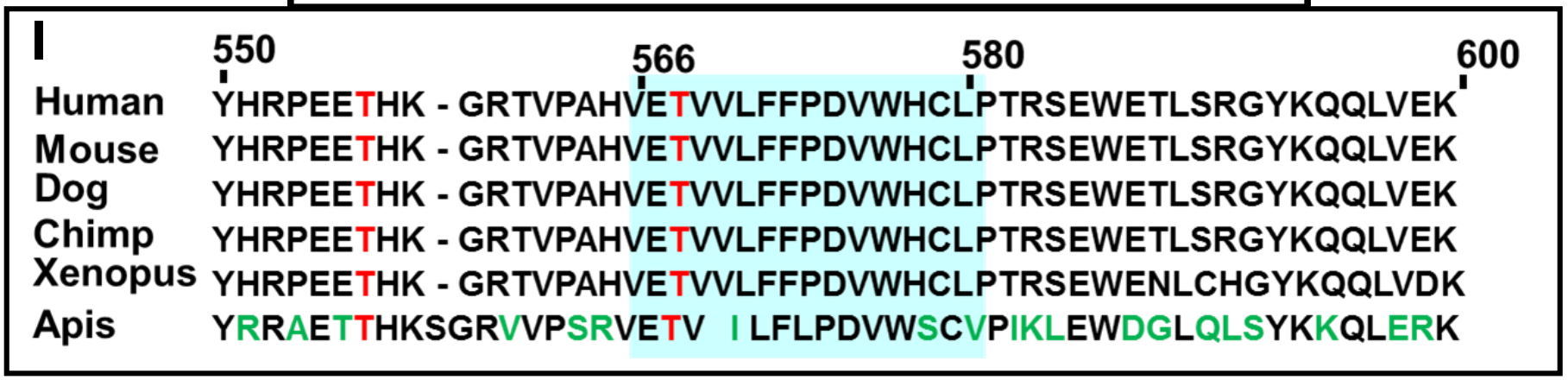






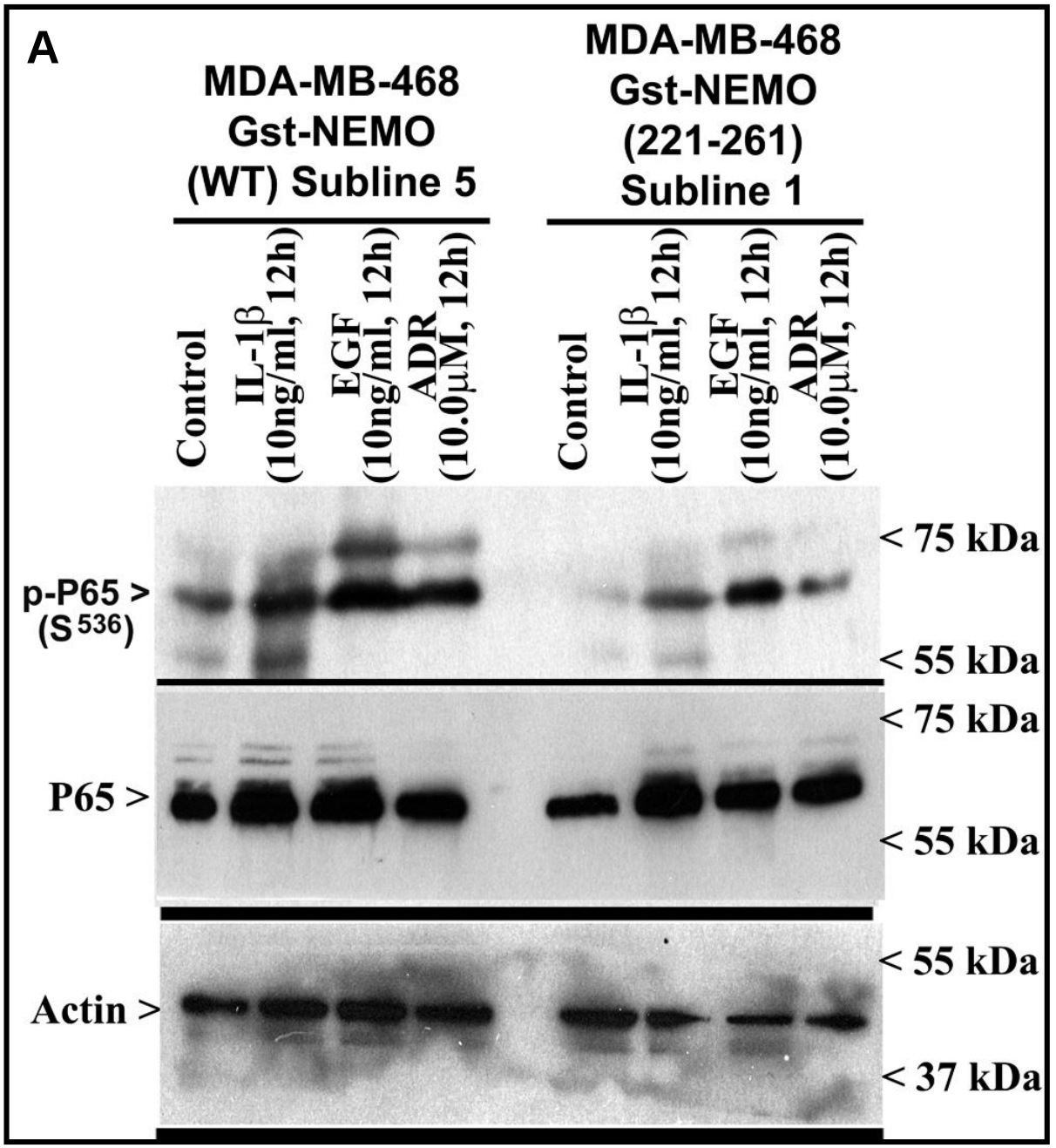
Supplementary Figure 2



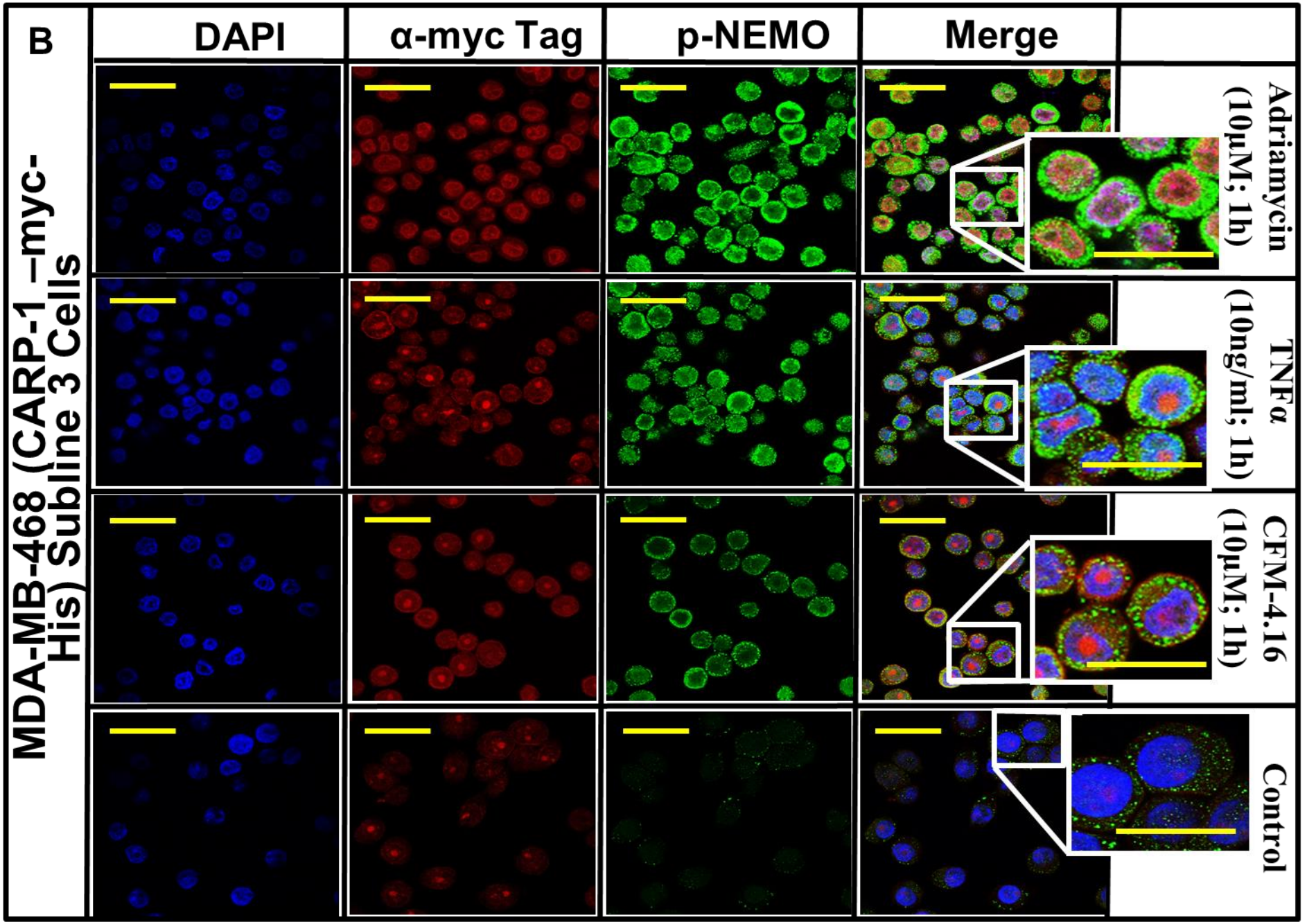


Supplementary Figure 2

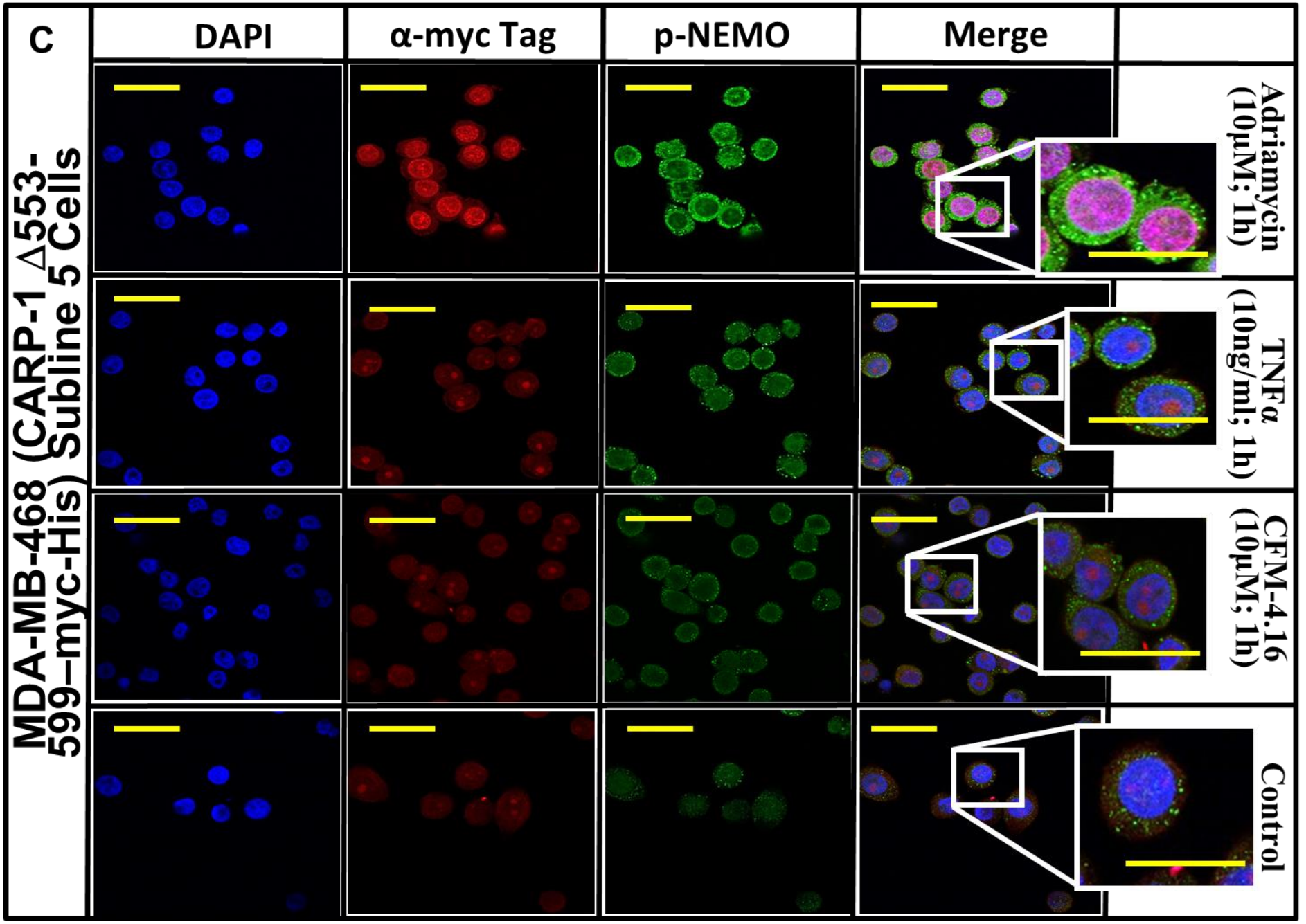
Supplementary Fig. 2: NEMO (221-261) interacts with CARP-1 (551-580). *A, D-G*. Noted cells were either untransfected (noted Wild-type) or transfected separately with indicated plasmids expressing eGFP, eGFP-tagged CARP-1 (551-580) mutant (panel A), Gst, Gst-NEMO (wild-type or mutant) proteins (panels (D-G), and neomycin-resistant, respective stable sublines were generated and characterized as described before (3, 21). Expression of respective, transfected proteins was analyzed by W.B. (upper blots), and each membrane was probed with anti-actin antibodies to assess protein loading (Lower blots). *B, C,* W.B. analysis of I.P. protein complexes from indicated stable sublines using noted antibodies (upper and middle blots in B, Left blot in C). The membrane containing proteins from whole cell lysates was probed with anti-NEMO antibodies (Lower blot in B) or anti-NEMO and anti-CARP-1 antibodies (Right side blots in C). *H,* Indicated cells were either untransfected (noted as -) or transfected with Gst-NEMO (221-261) plasmid (noted as +), and W.B. analysis of I.P. protein complexes was conducted using noted antibodies (upper and lower right blots). The membrane containing proteins from whole cell lysates was probed with anti-CARP-1 (Upper left) or anti-Gst (Lower left) antibodies. Arrowheads on the left or right side of each blot in panels A-G indicate presence of proteins or molecular weight markers, respectively. *I,* Alignment of human CARP-1 (550-600) protein with corresponding regions of various vertebrate and invertebrate CARP-1 proteins deduced from GenBank sequences.



Supplementary Figure 3

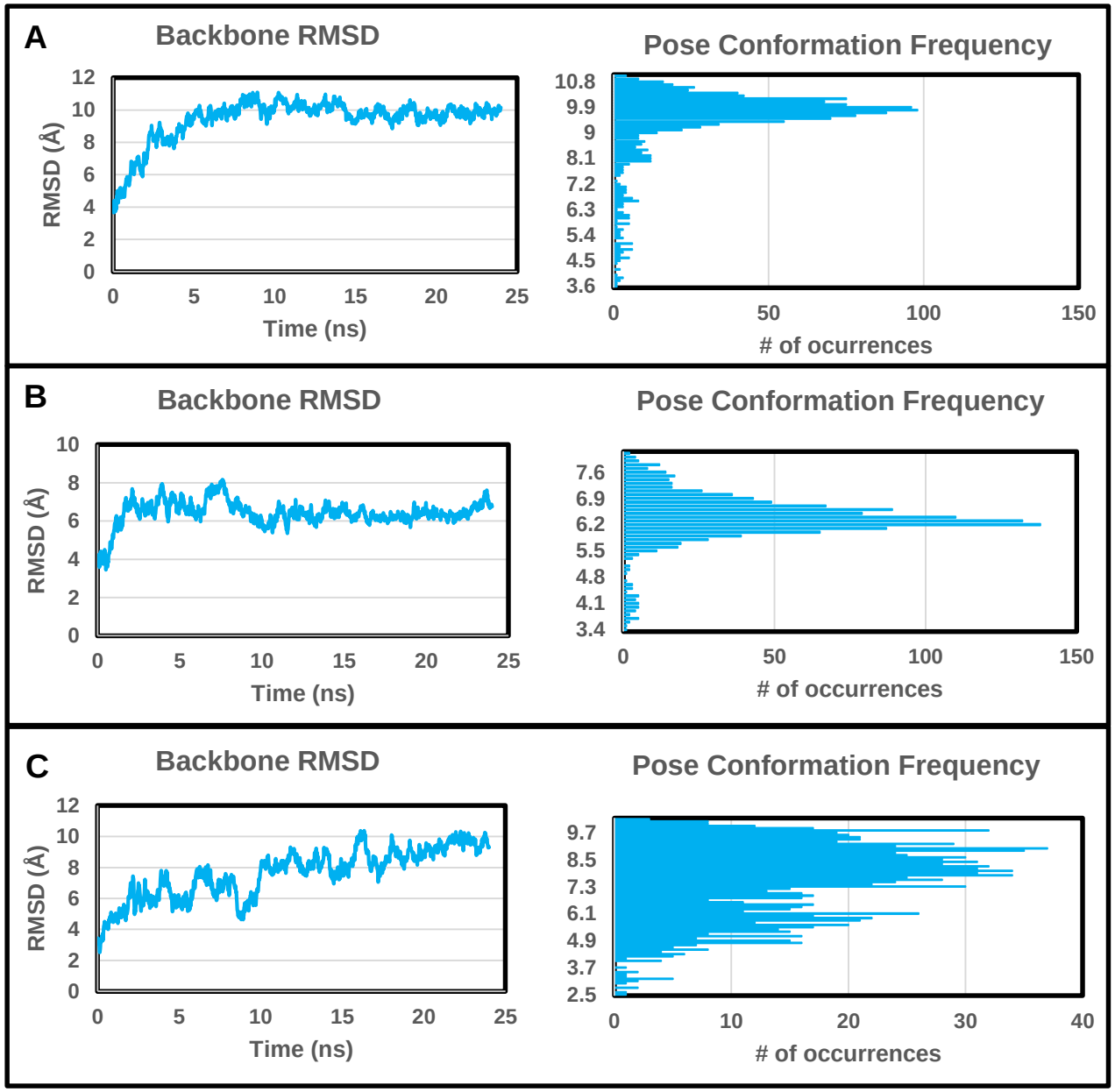


Supplementary Figure 3



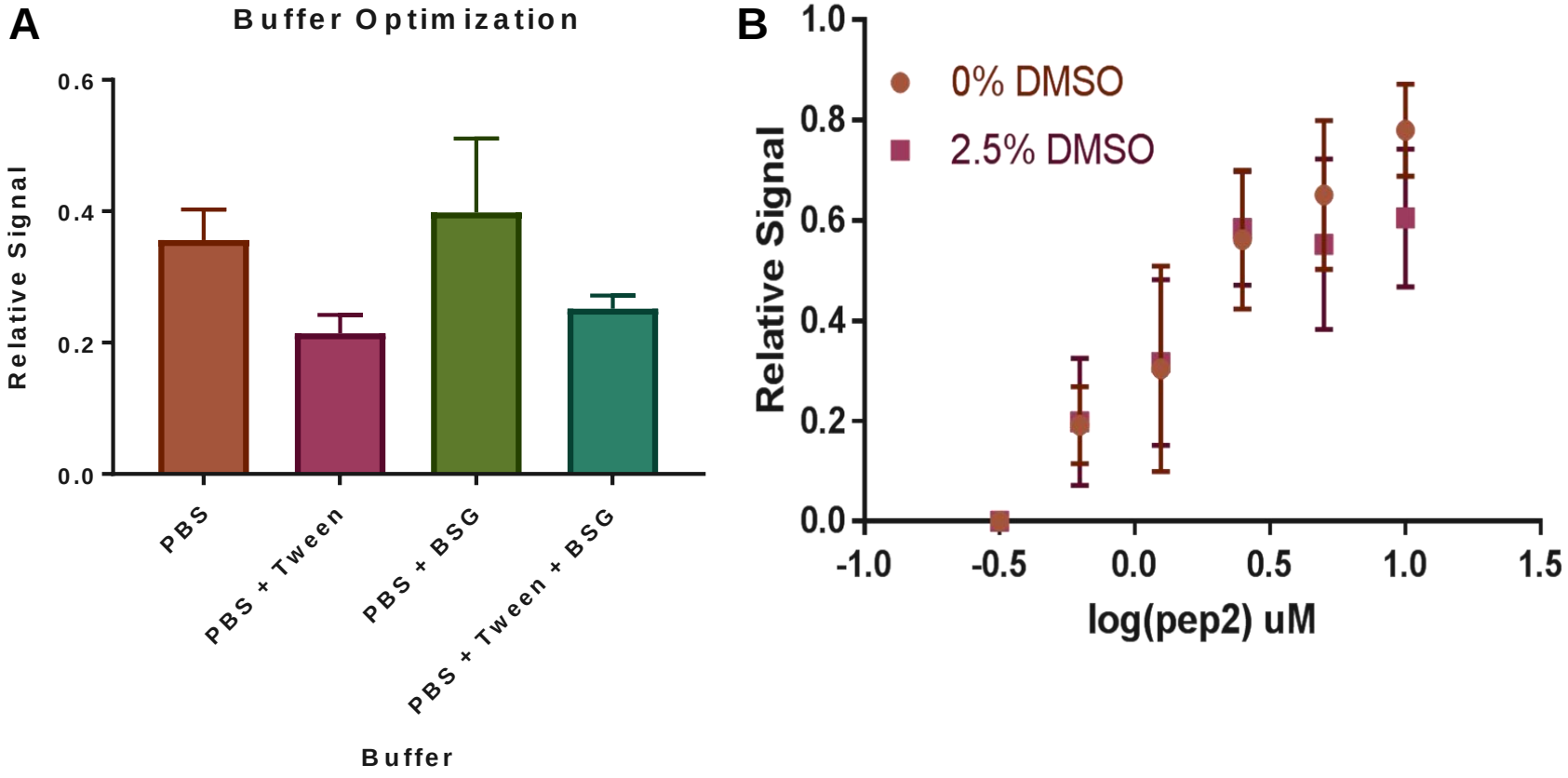
Supplementary Figure 3

Supplementary Fig. 3: Interference of CARP-1 binding with NEMO results in diminished RelA and NEMO phosphorylation. *A*, HBC cells stably expressing Gst-NEMO or Gst-NEMO (221-261) were either untreated (Control) or treated with indicated dose and time of each agent, and W.B. analysis of the cell lysates was carried out using anti-phospho-RelA, anti-RelA, and anti-actin antibodies as in Fig. 3. *B, C,* Indicated cells stably expressing wild-type or mutant CARP-1 protein were either untreated (Control) or treated with noted dose and time of each agent. Cells were then processed for immunofluorescence staining for CARP-1 (red), DAPI (blue), and phosphorylated NEMO (green) as detailed in "Experimental Procedures." Images were taken using Zeiss LSM 510 Meta NLO. Bar, 2 micrometer.



Supplementary Figure 4

Supplementary Fig. 4: Computational analyses of CARP-1 (551-600) binding with NEMO (221-261). Backbone RMSD and conformation histogram analyses for the three top scoring CARP1/NEMO complexes obtained from docking. A, the top scoring pose, B, the second, and C. the third pose. All calculations were done over the 24 ns production run.

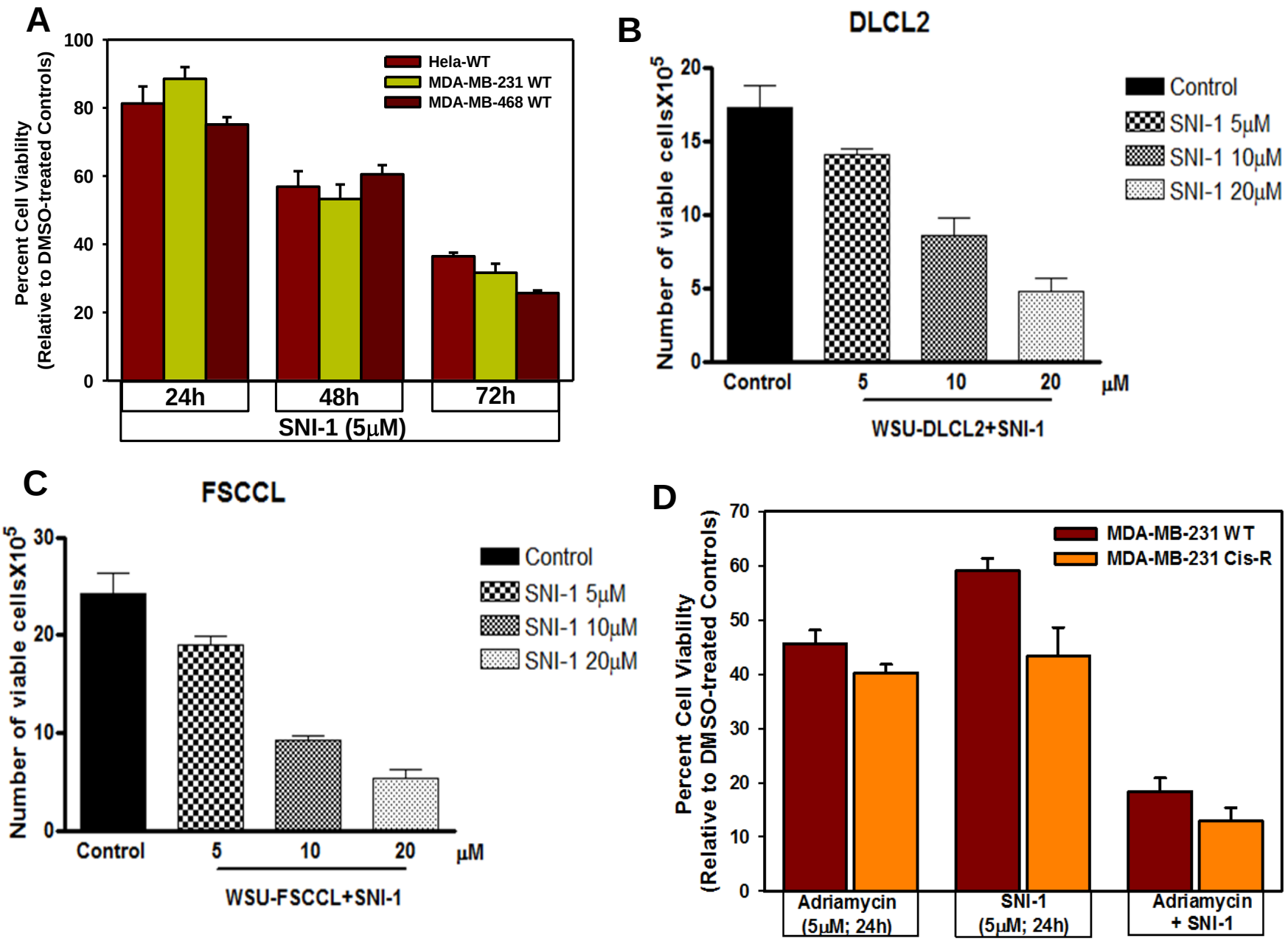


Peptide 1: CARP-1(551-580): HRPEETHKGRTVPAHVETVVLFFPDVWHCL

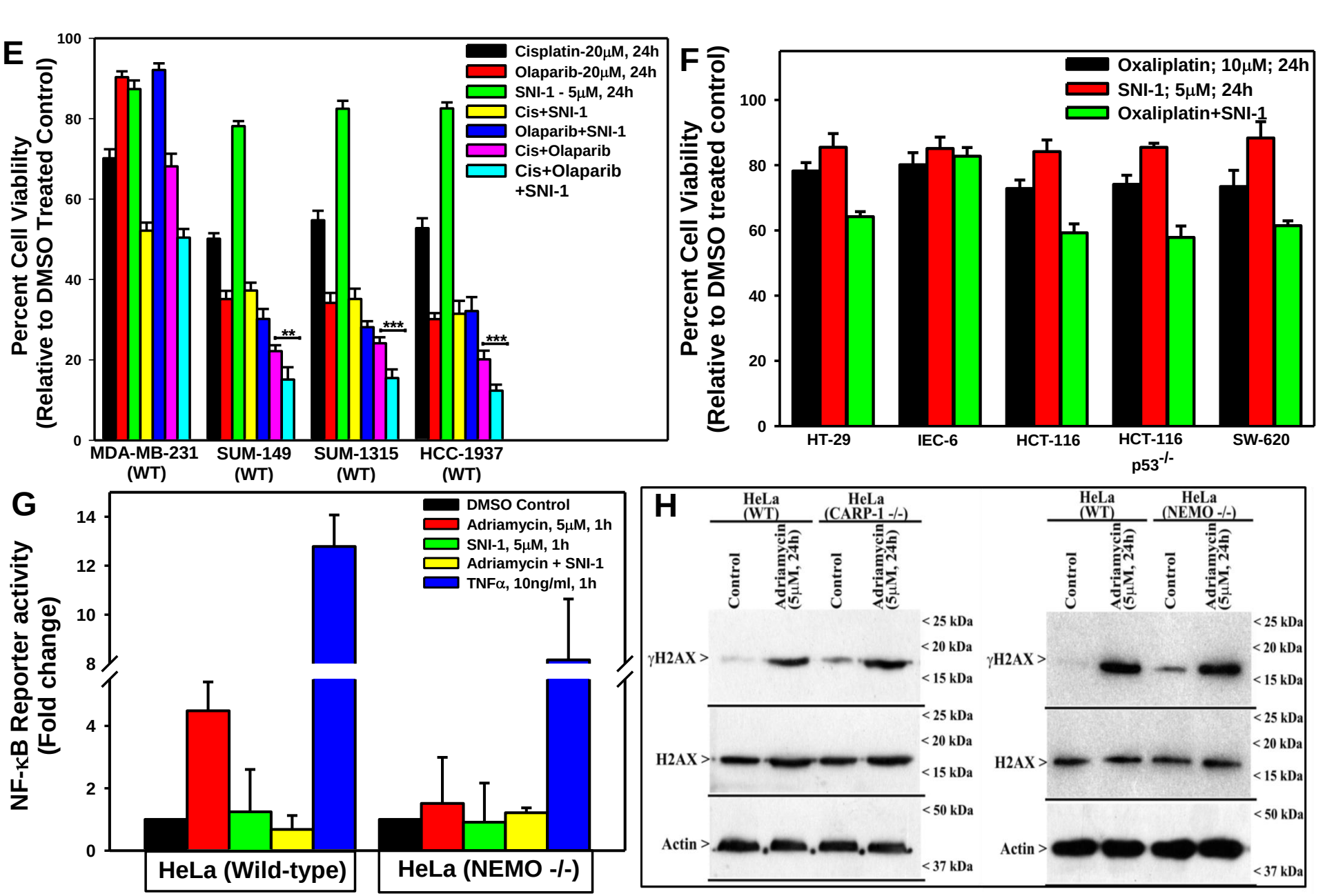
Peptide 2: Biotin-NEMO (221-261): EEKRKLAQLQVAYHQLFQEYDNHIKSSVVGSERKRGMQLE

Supplementary Figure 5

Supplementary Fig. 5: Buffer optimization (*A*) or DMSO tolerance (*B*) of the High-throughput screening assay. The "Experimental Procedures" detail measurements of binding of CARP-1 (551-580) and Biotin-tagged NEMO (221-261), peptides in PBS buffer with or without Tween, BSG, or noted concentrations of DMSO.

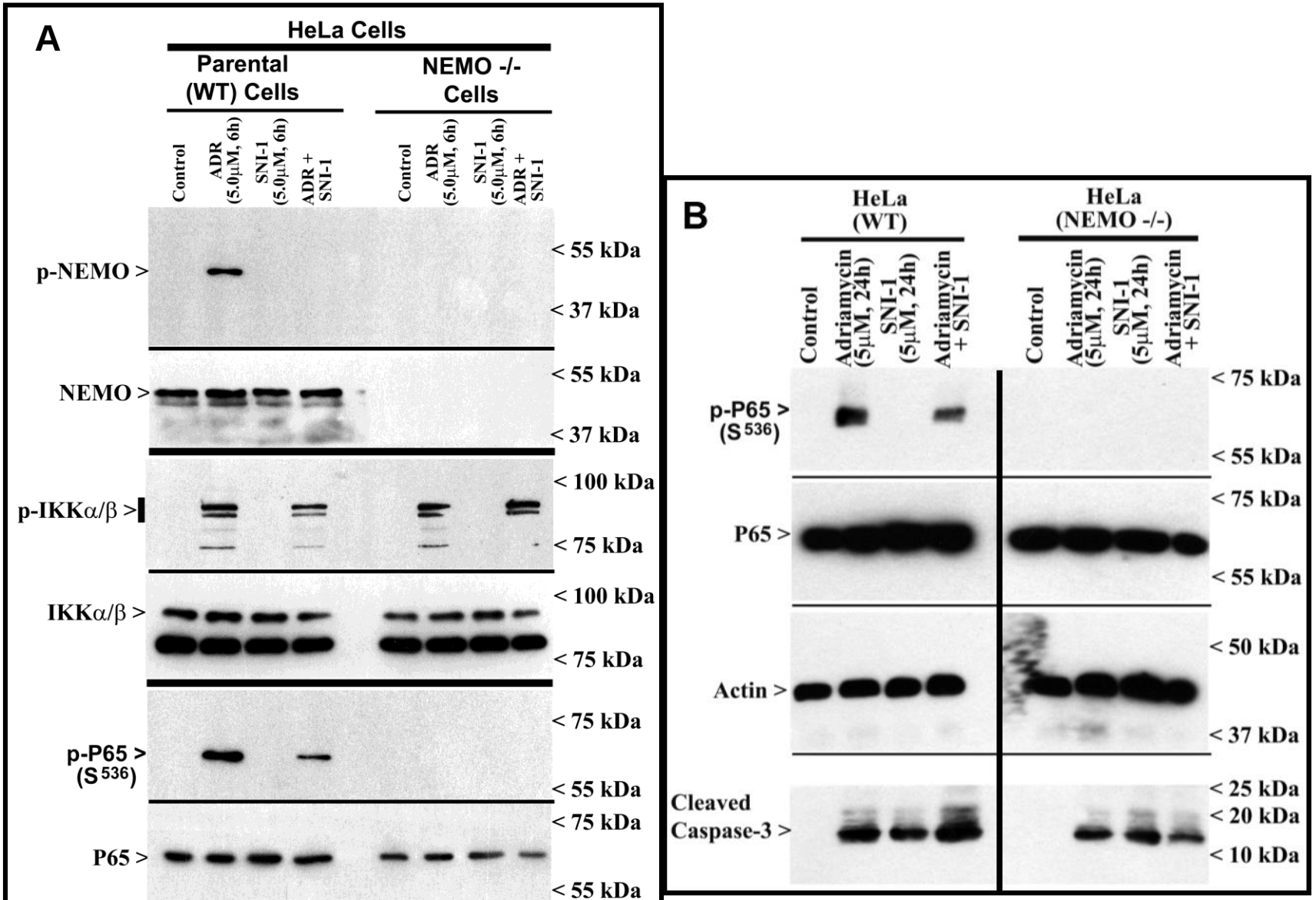


Supplementary Figure 6



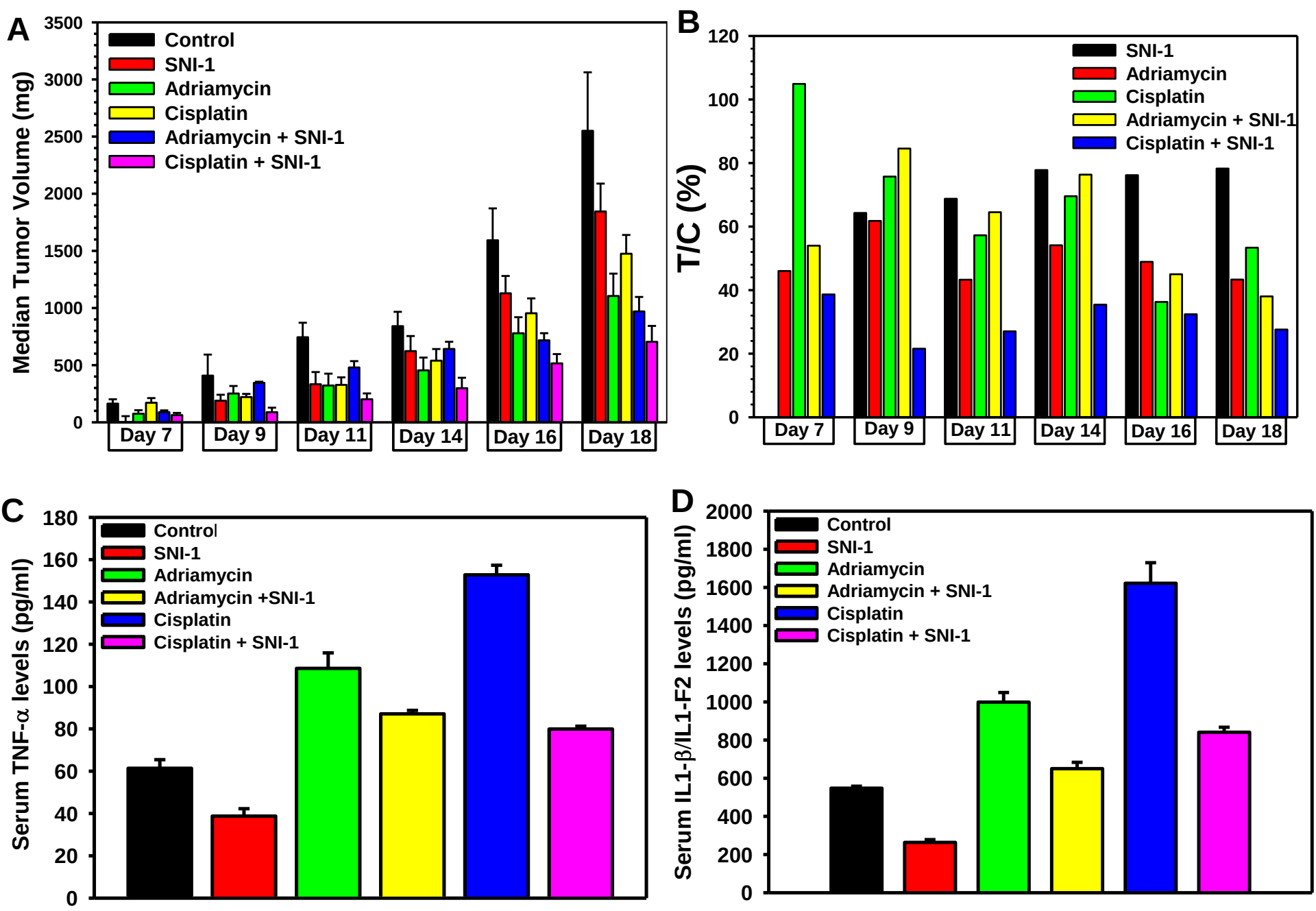
Supplementary Figure 6

Supplementary Fig. 6: SNI-1 inhibits cell growth in time (*A*) and dose-dependent manner (*B, C*), enhances efficacy of genotoxic chemotherapy *in vitro in drug-resistant and BRCA-mutant TNBC*, (*D-E*), colon cancer cells (*F*), while NEMO is required for Adriamycin-dependent transcriptional activation of NF-κB (*G*) but not for γH2AX (*H*). *A, D-F*, Cell viability was determined by MTT assay following treatments of cells with vehicle/DMSO (Control) or indicated time and doses of various agents. The *columns* in each *histogram* indicate percent of live/viable cells relative to their DMSO-treated controls and represent means of two-three independent experiments. *B, C*, The *columns* in each *histogram* indicate number of live/viable cells; *bars*, S.E. ** and ***; p = 0.005 and 0.001, respectively. G, Indicated cell were transfected with NF-κB-TATA-Luc plasmid followed by treatments with time and dose of noted agents as detailed in "Experimental Procedures". The columns in histogram indicate activities of the NF-κB reporter relative to the DMSO-treated controls and represent two separate experiments; *bars*, S.E. *H*, HeLa cells (wild-type, CARP-1 ko, and NEMO-ko) were either untreated (Control) or treated with Adriamycin for indicated dose and time. W.B. analysis of the cell lysates was carried out using anti-γH2AX, anti-H2AX, and anti-actin antibodies. Arrowheads on the left or right side of each blot in panel *H* indicate presence of proteins or molecular weight markers, respectively.



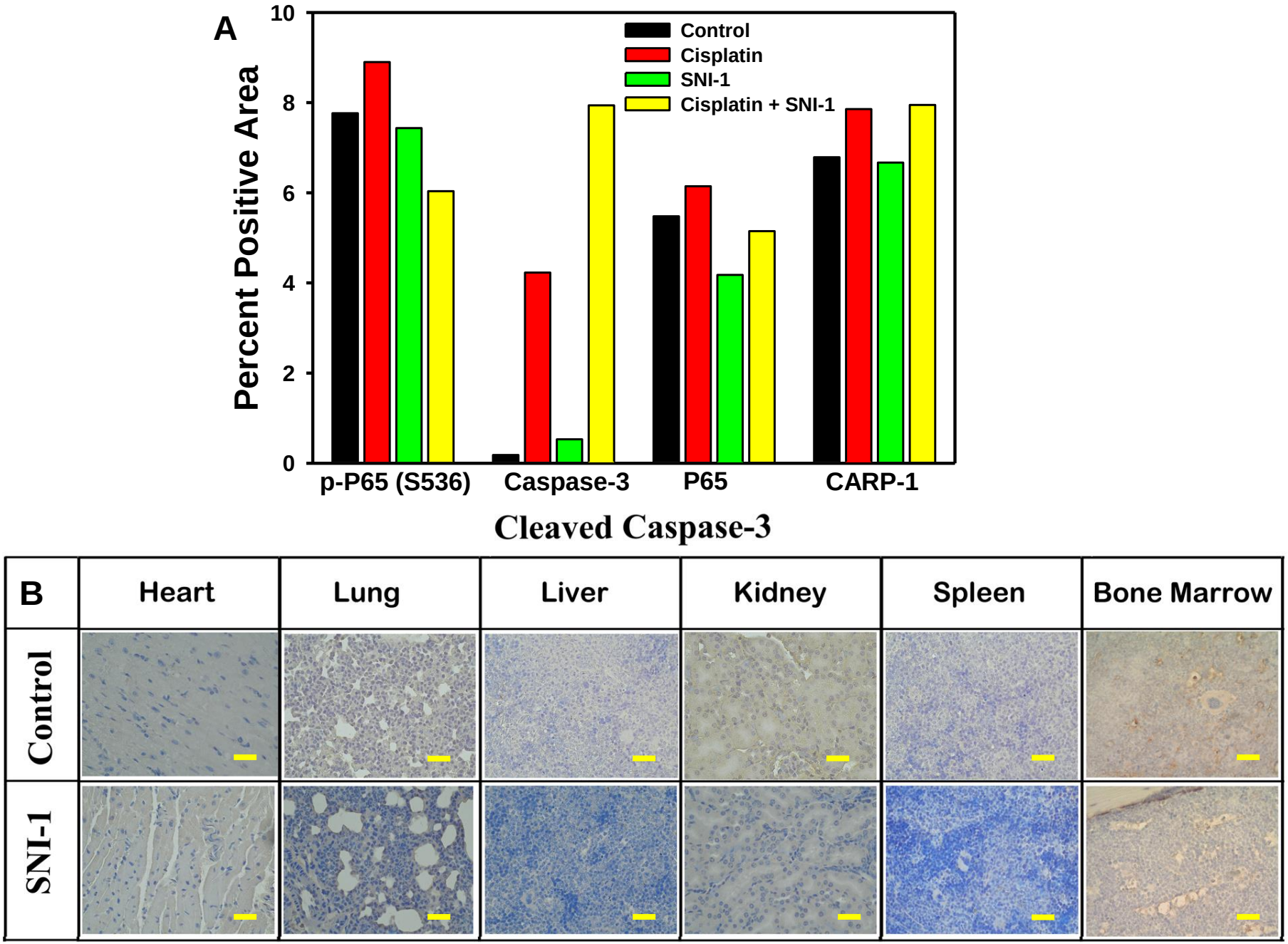
Supplementary Figure 7

Supplementary Fig. 7: Adriamycin activates IKKα/β and caspase-3 independent of NEMO. *A, B,* HeLa cells (wild-type and NEMO-ko) were either untreated (Control) or treated with indicated dose and time of noted agents. W.B. analysis of the cell lysates was carried out using anti-phospho RelA, anti-RelA, anti-cleaved caspase-3, anti-phospho-NEMO, anti-NEMO, anti-phospho-IKKα/β, anti-IKKα/β, and anti-actin antibodies. Arrowheads on the left or right side of each blot in panels *A* and *B* indicate presence of proteins or molecular weight markers, respectively. Vertical bar in *B* denotes autoradiogram splicing.



Supplementary Figure 8

Supplementary Fig. 8: SNI-1 enhances tumor suppression by chemotherapy in part by attenuating systemic levels of pro-inflammatory cytokines and promoting tumor apoptosis. *Histogram* columns showing median tumor volume (A) or percent T/C (B) of the TNBC (4T1) xenograft-bearing mice treated with indicated agents. The xenograft establishment, treatment and analysis procedures were carried out essentially as detailed in "Experimental procedures." *C, D,* serum levels of noted pro-inflammatory cytokines. The *columns* in *histograms* indicate noted systemic cytokine levels in two representative animals from each of the control and treatment groups; *bars,* S.E.



Supplementary Figure 9

Supplementary Fig. 9: *A*, Histogram showing quantitation of staining for indicated proteins in the tumor tissues in Fig. 9D. *B*, SNI-1 administration does not induce apoptosis in various critical organs of 4T1 tumor-bearing mice. Immuno-histochemical staining for presence of cleaved caspase-3 was carried out in indicated murine tissues from a representative animal from control or SNI-1 group as detailed in "Experimental Procedures." Bar, 200 micrometer.