



Supplemental Figure 1. NF- κ B subunit binding at the EAAT2 promoter. A) EAAT2 promoter -583 site. NF- κ B DNA binding was assayed using the NoShift Transcription Factor Assay kit (Novagen) according to manufacturer's protocol. Briefly, a double-stranded, biotin end-labeled oligo was incubated with H4 nuclear extracts from cells treated with TNF or EGF. Samples were incubated with antibodies to p65 (Rockland), p50 (Santa Cruz, SC-7178), p52 (SC-298), cRel (SC-71X) or RelB (SC-226). Competition assays to determine binding specificity were performed with p65 and p50 antibodies with 100X excess of a mutant oligo (comp). Colorimetric assays were performed with HRP-conjugated secondary antibody and tetramethylbenzidine (TMB) substrate. Absorbance was read at 450 nm. See materials and methods for oligonucleotide sequences. B) Binding to the EAAT2 promoter -334, -272, -251, +133 and +265 sites. Experiments were performed as described for (A). Similar results were seen for EGF (data not shown).