

## Supplementary Material.

Figure 1S: Changes in combined backbone  $^1\text{H}$  and  $^{15}\text{N}$  chemical shifts of wild-type S100B caused by the H15A, D23A, H25, C84A, H85A, or  $\Delta 89-91$  mutations. Comparisons were made for apo-S100B for all of the mutations except for D23A, in which case comparisons were made for  $\text{Ca}^{2+}$ -S100B. For the most part, the mutations were found to affect only residues spatially nearby the mutation. The one exception being the C84A mutation, which had wide spread perturbations throughout the protein indicative of a larger structural change. The asterisk (\*) indicates residues for which the chemical shift values could not be determined due to exchange broadening effects due to the mutation.

