

A non-denatured whey protein supplement (Immunocal®) protects neurons from mitochondrial oxidative stress and delays disease onset in the mutant SOD1 mouse model of ALS

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Introduction:

Amyotrophic lateral sclerosis (ALS) is a devastating neuromuscular disease caused by the death of spinal and cortical motor neurons and retraction of motor axons from the neuromuscular junctions. Mitochondrial oxidative stress (MOS) appears to be a major factor contributing to motor neuron death in ALS and therefore, novel agents targeting MOS could provide effective therapies for this debilitating disease. We have investigated a unique, non-denatured whey protein supplement (Immunocal®) that contains high concentrations of cystine, a precursor for the synthesis of glutathione (GSH). We have tested this whey supplement for neuroprotection *in vitro* from MOS and *in vivo* for its effects on ALS disease onset and progression. In primary cultures of rat cerebellar granule neurons (CGNs), pre-treatment with the whey supplement completely protected against HA14-1, a Bcl-2 inhibitor which induces GSH-sensitive, intrinsic apoptosis. Moreover, the protective effects of the whey supplement were blocked by co-incubation with buthionine sulfoximine, an inhibitor of GSH synthesis. The whey supplement also displayed significant protection against an array of MOS-inducing agents in CGNs, including sodium nitroprusside (SNP), copper chloride (CuCl₂), and aluminum chloride (AlCl₃). In accordance with these findings in CGNs, the whey supplement also significantly protected Chinese hamster ovary (CHO) cells from MOS and intrinsic apoptosis induced by overexpression of amyloid precursor protein. Finally, daily administration of the whey supplement in drinking water (*ad libitum*) beginning at 60-days-old significantly delayed disease onset by approximately 10 days in the G93A mutant SOD1 mouse model of ALS. Taken together, these findings indicate that the whey supplement significantly protects from MOS via an induction of *de novo* GSH synthesis. This strategy of enhancing endogenous GSH levels via intake of a nutritional supplement may be beneficial in mitigating disease symptoms in ALS.

Figure 1: Immunocal® protects CGNs from apoptosis induced by the Bcl-2 inhibitor HA14-1

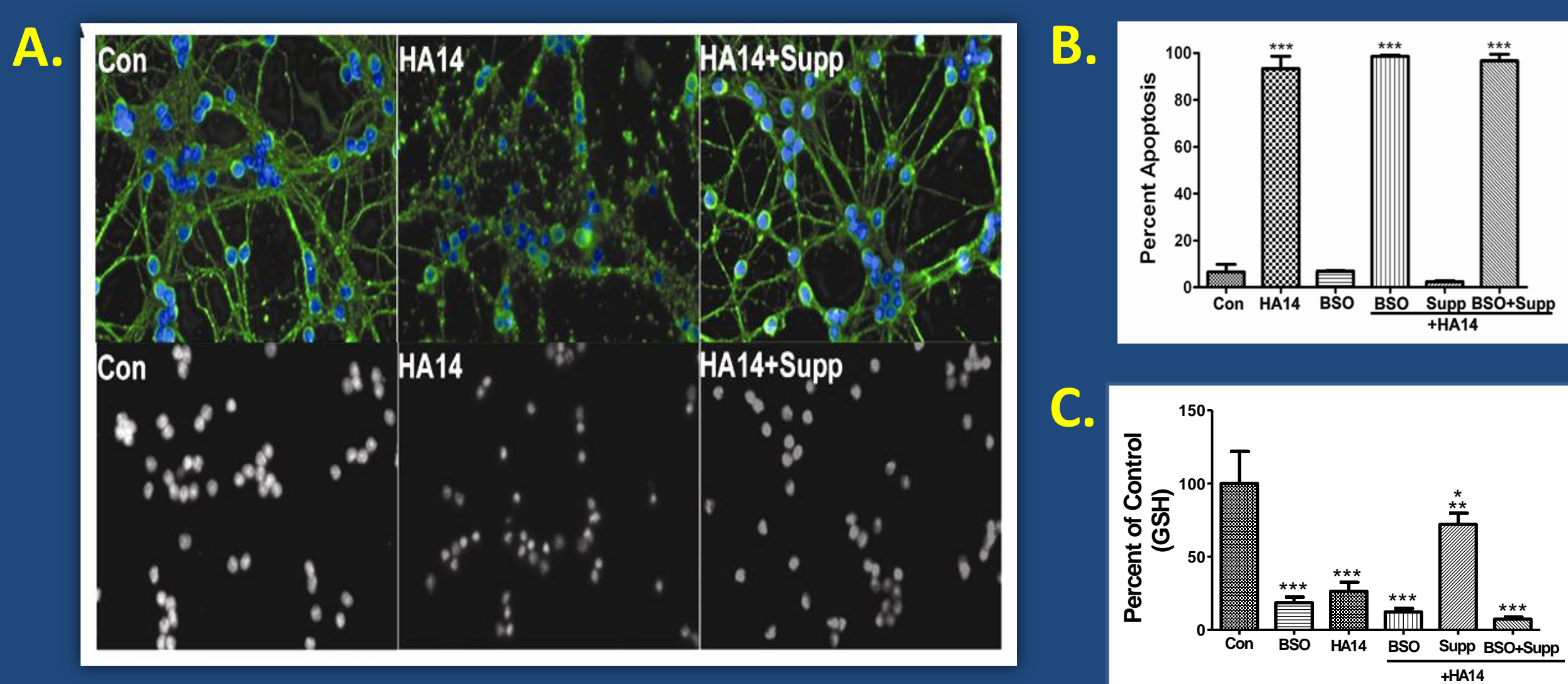
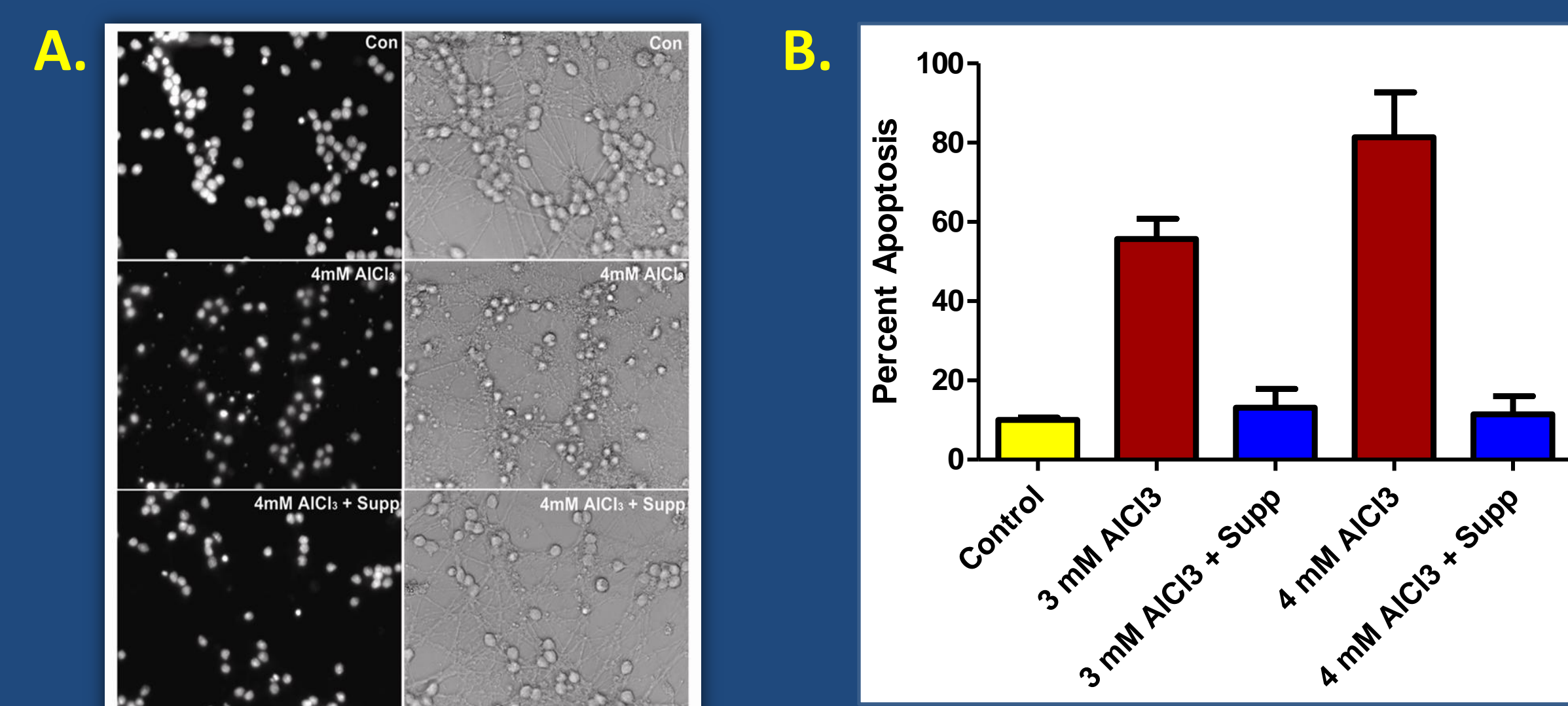


Figure 2: Immunocal® protects CGNs from apoptosis induced by AlCl₃



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Figure 3: Immunocal® protects CGNs from sodium nitroprusside (SNP)

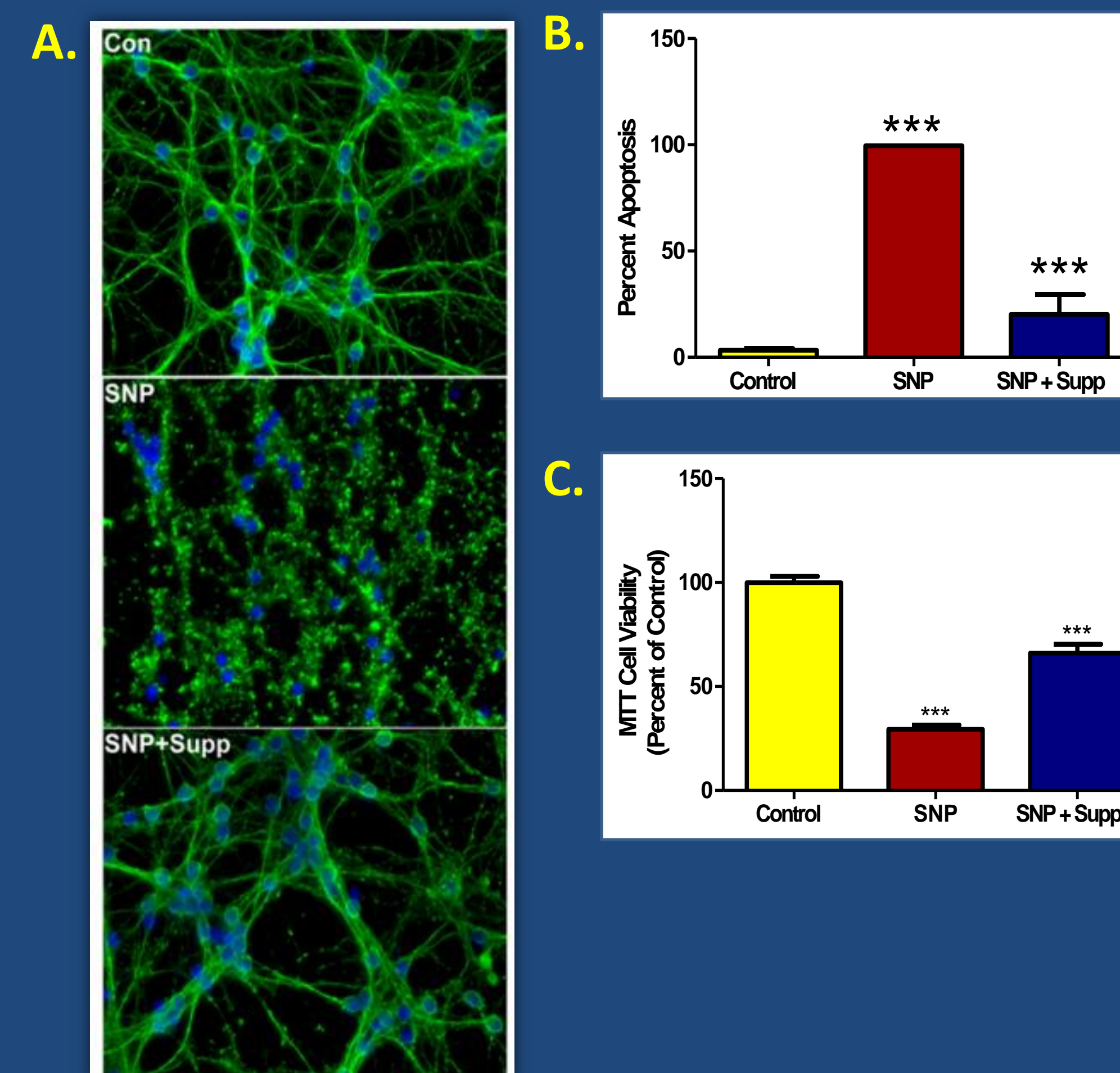


Figure 4: Immunocal® protects from CuCl₂ induced apoptosis and lipid peroxidation

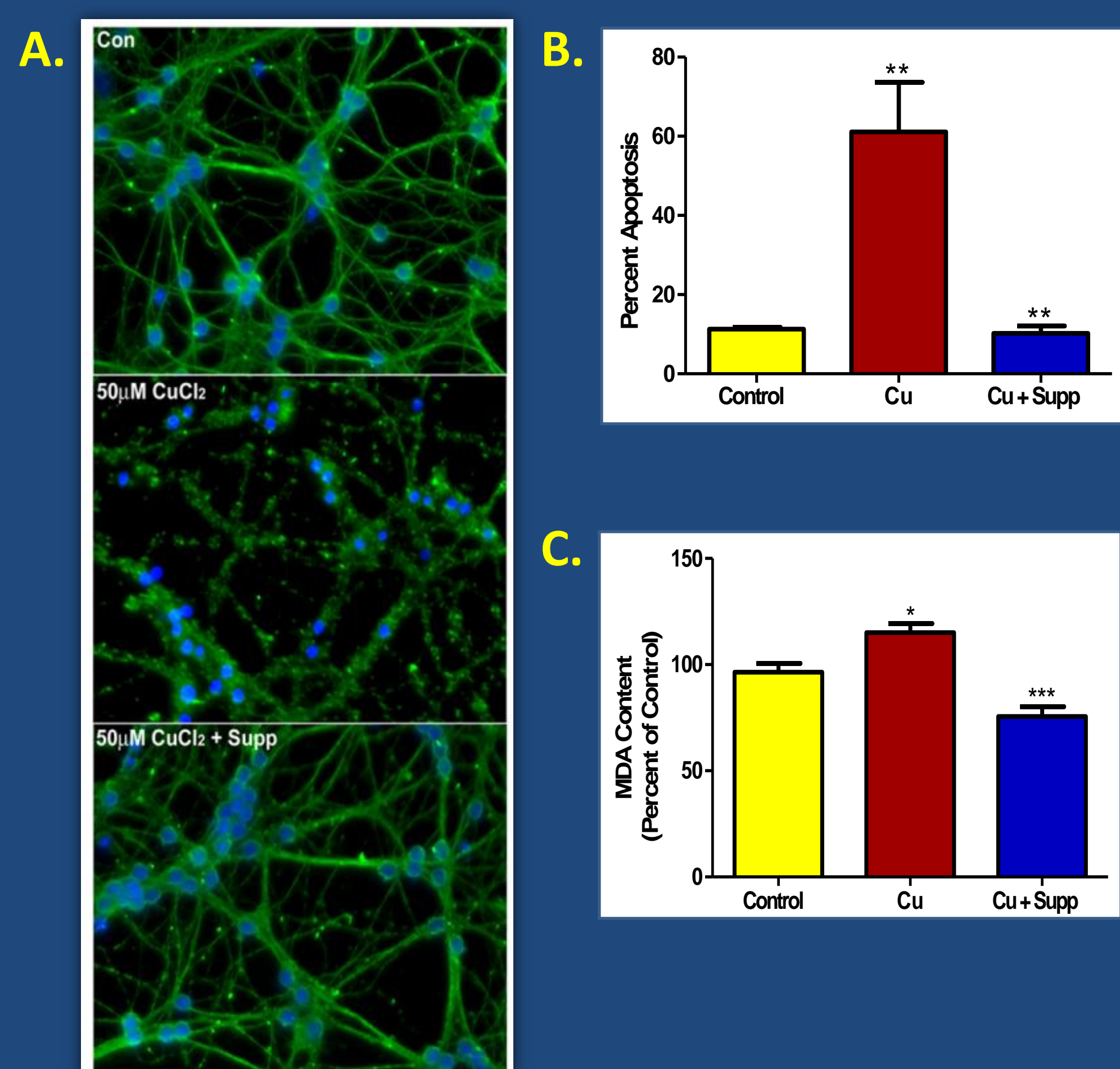


Figure 5: Immunocal® decreases active caspase-3 in CHO cells overexpressing WT APP

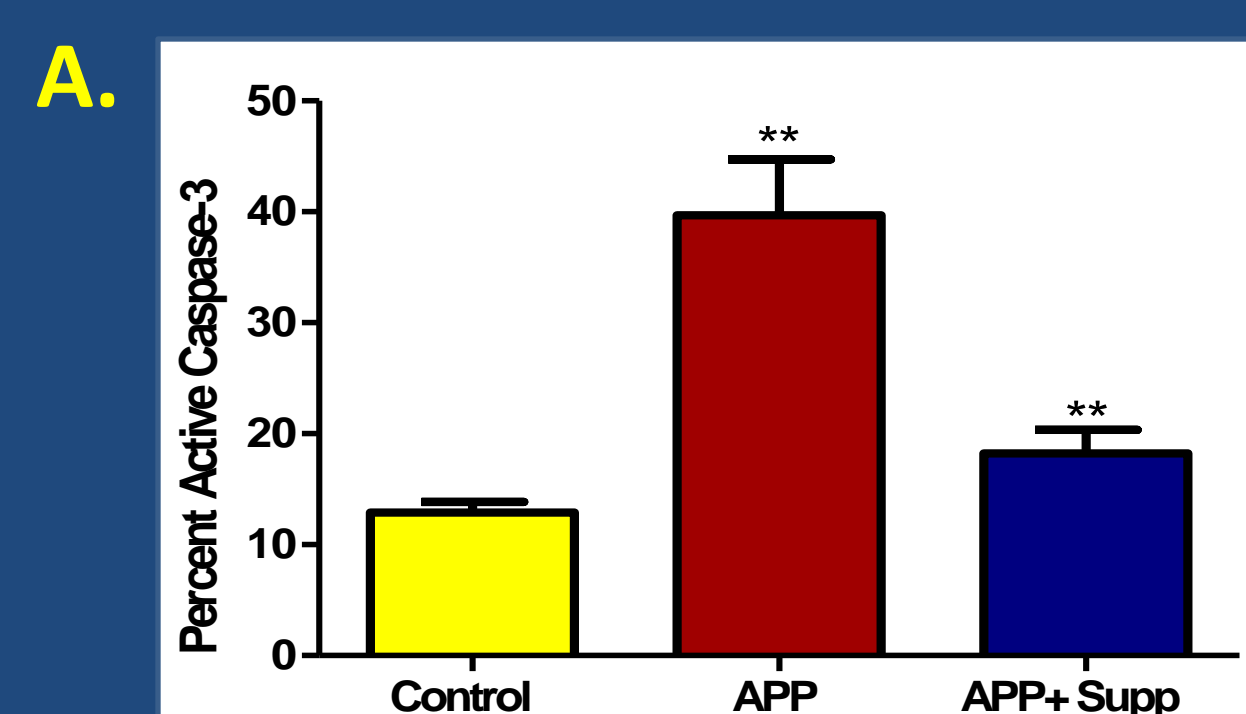


Figure 6: Immunocal® delays clinical disease onset in the G93A mutant SOD1 mouse model of ALS and delays the decline of grip strength

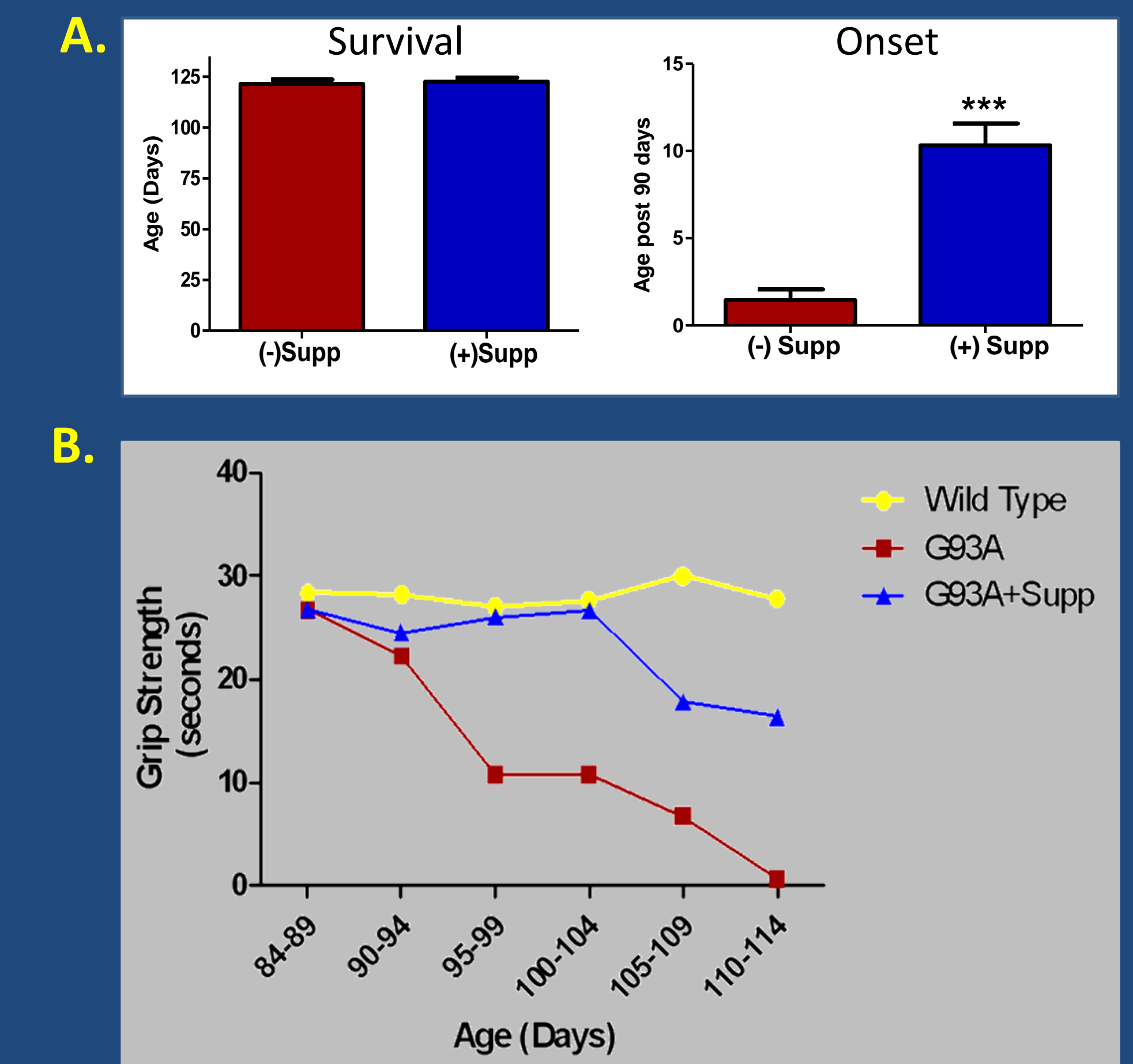
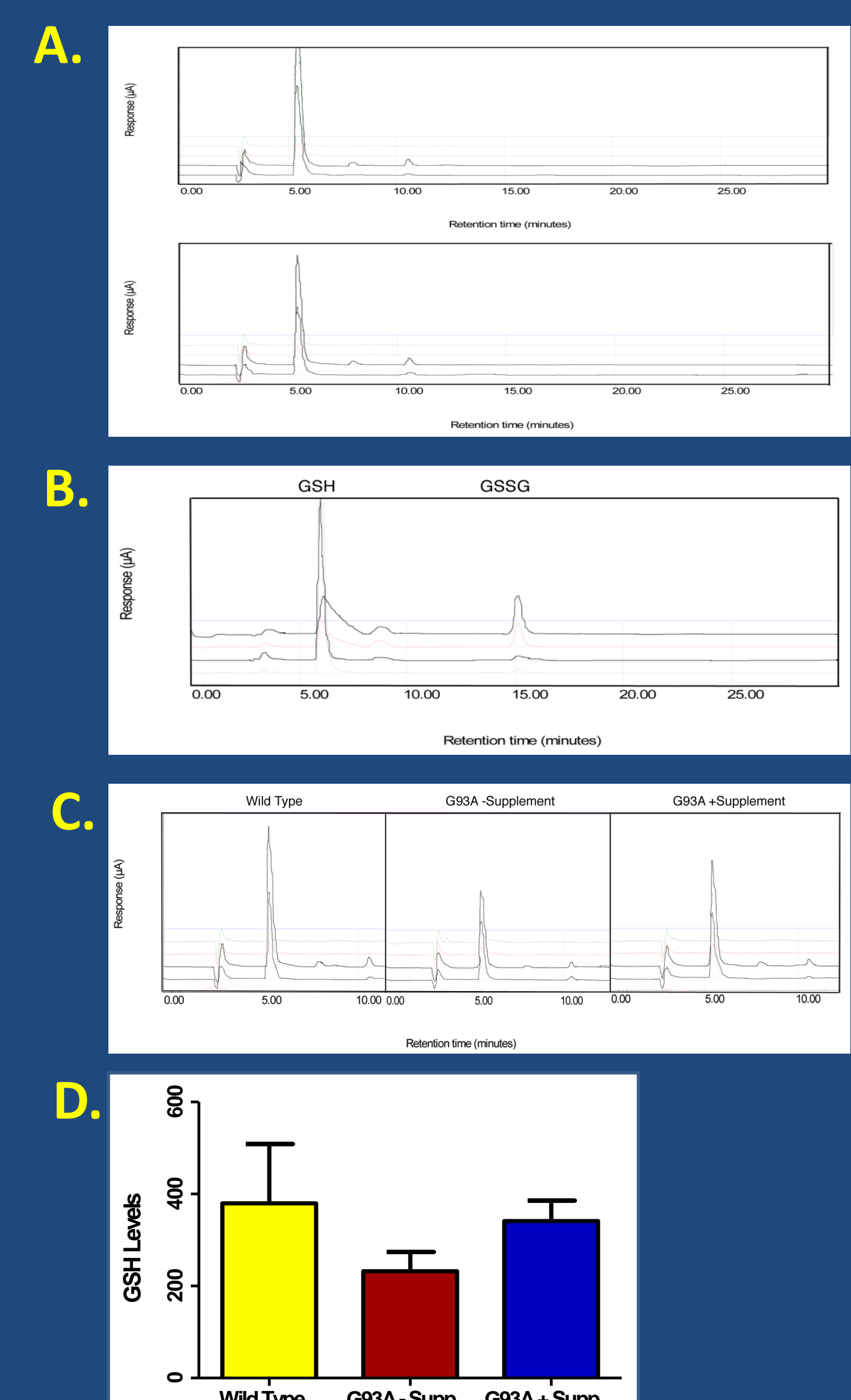


Figure 7: HPLC-ECD shows Immunocal® increases GSH concentrations in whole blood from G93A mutant SOD1 mice



Conclusions and Future Directions:

These data indicate that the non-denatured whey protein supplement, Immunocal®, significantly protects from MOS via an induction of *de novo* GSH synthesis in relevant cell models and delays disease onset in the G93A mutant SOD1 mouse. Future goals include investigating Immunocal® in combination with riluzole to potentially delay onset and increase lifespan in the G93A mutant SOD1 mouse model.