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Supplementary Data

Treatment with a γ-Ketoaldehyde Scavenger Prevents Working Memory Deficits in hApoE4 Mice

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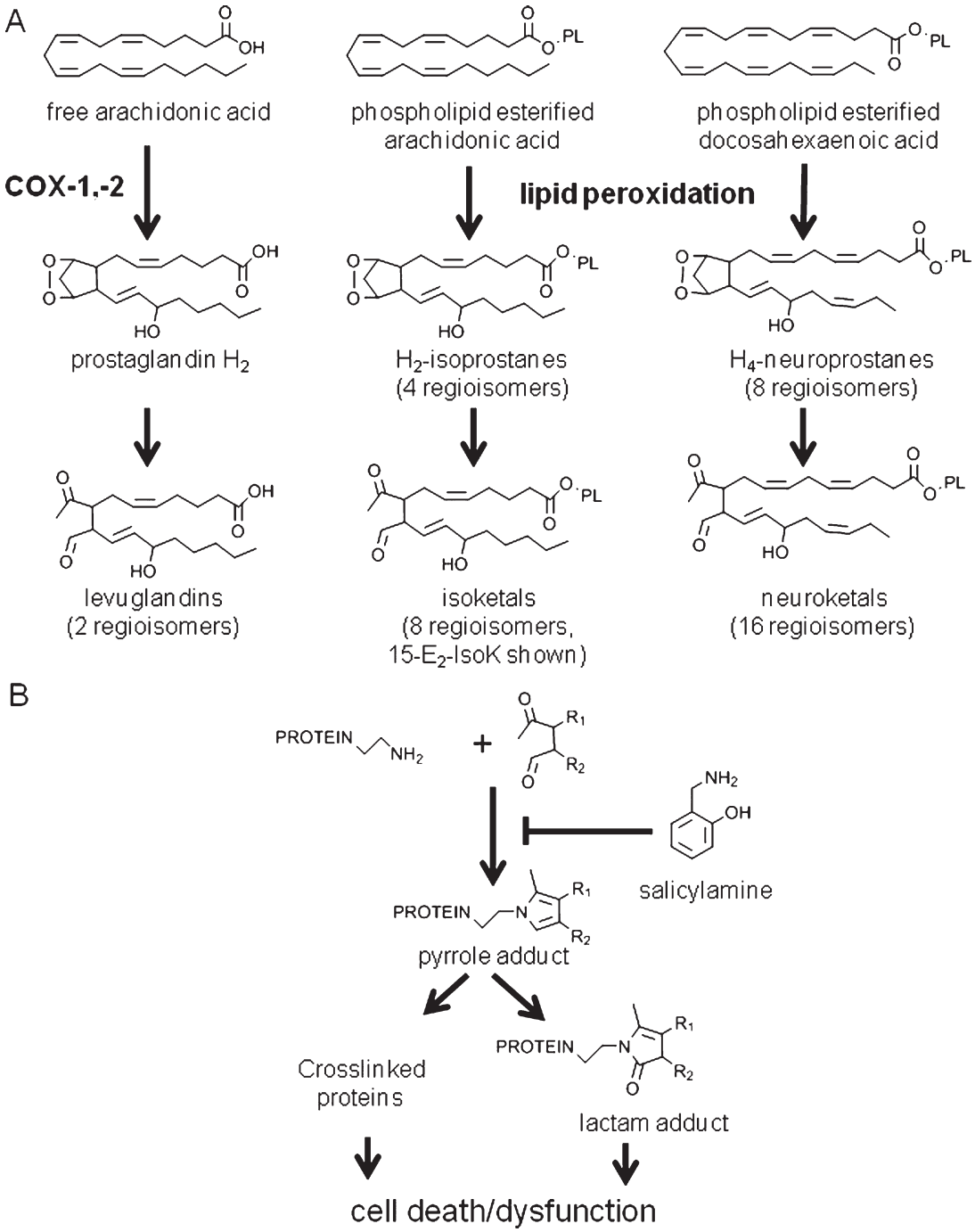
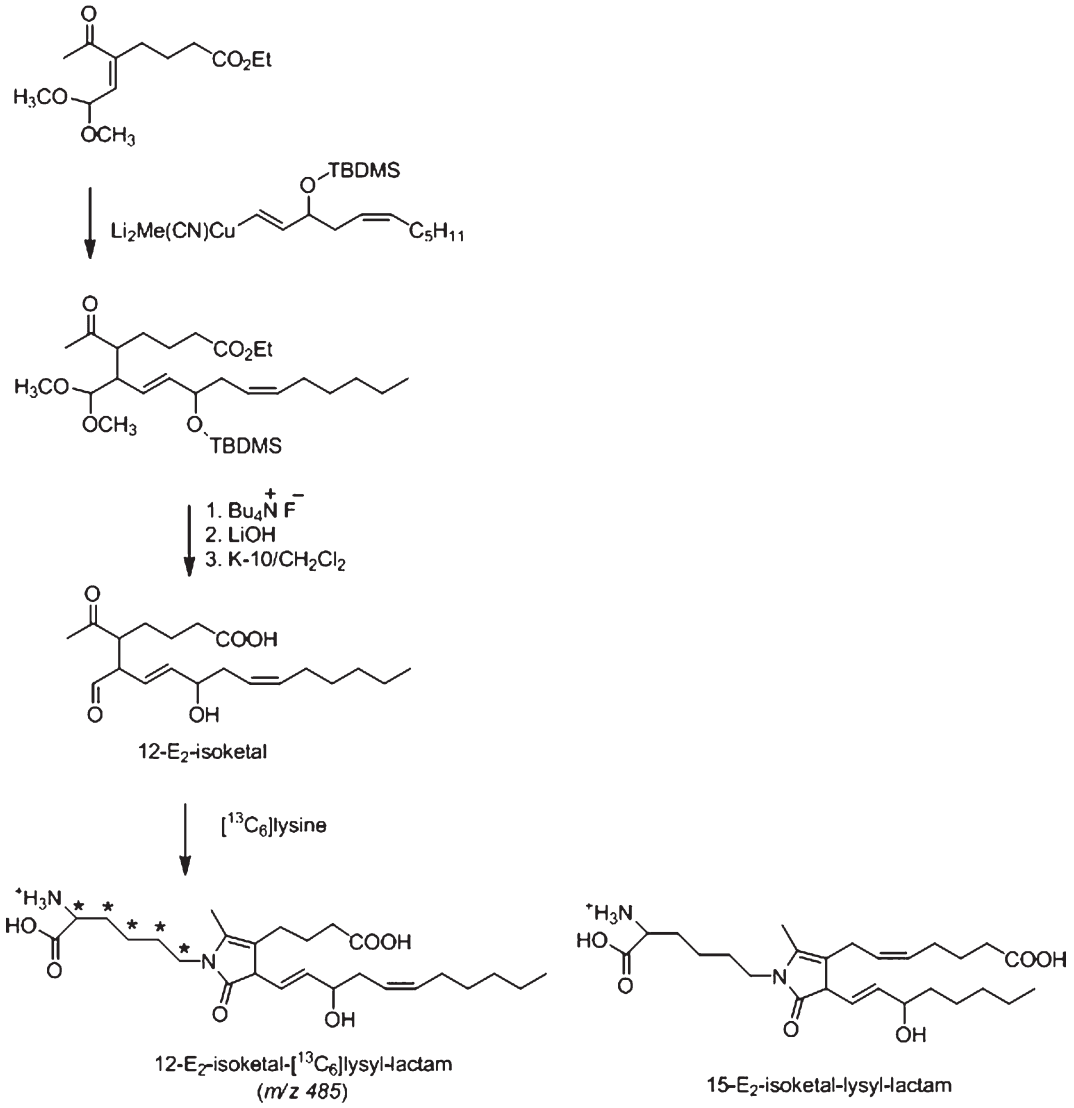
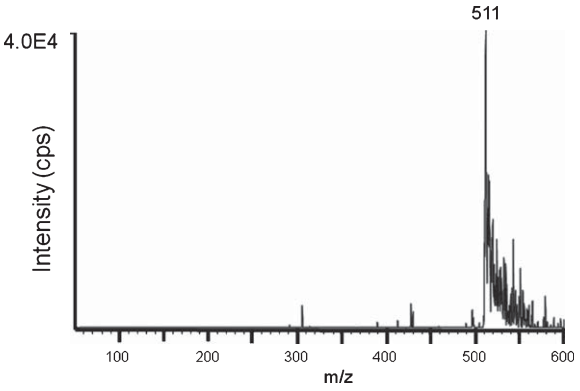


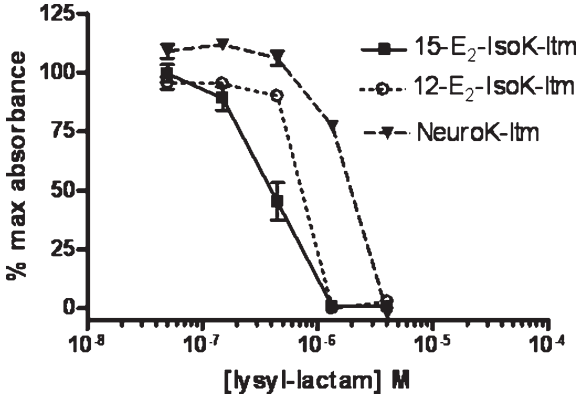
Supplementary Figure 1. Formation of γ-ketoaldehydes and their adducts. A) Arachidonic acid released by $cPLA_2$ is enzymatically converted to the bicyclic endoperoxide prostaglandin H_2 by the action of cyclooxygenase-1 and -2. Arachidonic and docosahexaenoic acid esterified in phospholipids are converted to analogous bicyclic endoperoxides (H_2-isoprostanes and H_4-neuroprostanes, respectively,) by free radical mediated lipid peroxidation. Because free radical can initiate peroxidation at any of the bis-allelic hydrogens, this mechanism forms multiple regioisomers of bicyclic endoperoxides. Each bicyclic endoperoxide regioisomer can non-enzymatically rearrange to form two possible γ-ketoaldehyde regioisomers. When formed from prostaglandin H_2, these γ-ketoaldehydes are called levuglandins, while when formed from H_2-isoprostanes or H_4-neuroprostanes they are given the name isoketals (or isolevuglandins) and neuroketals. B) These γ-ketoaldehydes rapidly react with cellular amines, including protein, to form pyrrole adducts, which in the presence of molecular oxygen mature to form lactam (and hydroxylactam) adducts. Formation of γ-ketoaldehyde adducts leads to cell death and dysfunction which can be inhibited by the selective γ-ketoaldehyde scavenger salicylamine.



Supplementary Figure 2. Synthesis of 12-E_2-isoketal and 12-E_2-isoketal-lysyl-lactam for testing D11 ScFv immunoreactivity. Ethyl 5-acetyl-7,7-dimethoxy-5-hetenoate (obtained from ethyl 5-(diethylphospono)-6-oxoheptanoate and 2,2-dimethoxyacetaldehyde) was treated with the cyanocuprate of 3-(*tert*-butyldimethylsilyloxy)-1(*E*)-5(*Z*)-undecadiene prepared in situ at – 78 °C to generate the fully protected 12-E_2-isoketal. Desilylation, ester hydrolysis, and treatment with montmorillonite K-10 yielded 12-E_2-isoketal, which was then reacted with [$^{13}C_6$] lysine containing tracer amounts of [^{3}H] lysine to yield 12-E_2-isoketal-lysyl-lactam, which was then isolated by HPLC and confirmed by mass spectrometry. The asterisks indicate the positions of the [^{13}C] label in the final molecule. The structure of 15-E_2-isoketal-lysyl-lactam, is shown to illustrate the overall similarity of the two structures, particularly of the lactam ring and the hydroxyl group on the lower side chain.



Supplementary Figure 3. Mass spectral analysis of purified neuroketal-lysyl-lactam used for testing D11 ScFv immunoreactivity. Docosahexaenoic acid was oxidized in the presence of [$^{13}C_6{}^{15}N_2$]lysine with tracer amounts of [^{3}H]lysine, and the resulting lysyl adducts separated by HPLC, and 1 min fractions collected. Because compounds containing [$^{13}C_6{}^{15}N_2$]lysine generate a characteristic *m/z 90* product ion upon collision induced disassociation, we used precursor scanning to analyze HPLC fractions to find fractions containing neuroketal-lysyl-lactam with expected *m/z 511*. The precursor scan of fraction 40 that was used for the D11 ScFv immunoreactivity study is shown. The structure of 17-E_2-NeuroK-lysyl-lactam is shown as an inset, (*) designates the position of ^{13}C and ^{15}N label. Many other neuroketal-[$^{13}C_6{}^{15}N_2$]lysyl-lactam regioisomers with an expected *m/z 511* are generated by oxidation of docosahexaenoic acid, and the specific regioisomer(s) present in fraction 40 was not determined. Total lysyl-lactam adduct in the fraction was calculated based on the amount of [^{3}H] radiolabel.



Supplementary Figure 4. 15-E_2-isoketal-,12-E_2-isoketal-, and neuroketal-lysyl-lactam adducts all show similar affinity for D11 ScFv in competitive ELISA. The pentameric peptide arg-lys-asp-val-tyr (RKDVY) was reacted with 15-E_2-isoketal and then immobilized on 384-well plates as previously described [34]. Varying concentrations of 15-E_2-isoketal-lysyl-lactam (15-E_2-IsoK-ltm, ■), 12-E_2-isoketal-lysyl-lactam (12-E_2-IsoK-ltm, ○), or neuroketal-lysyl-lactam (NeuroK-ltm, ▼) were pre-incubate with D11 ScFv, and then these solutions were added to wells with the immobilized peptide and allowed to equilibrate, the plates washed thoroughly, anti-ScFv secondary antibody conjugated to horseradish peroxidase added, the plates washed thoroughly again, peroxidase substrate (ABTS) added, and absorbance at 405 nm measured by spectrophotometery. 100% absorbance was calculated using the absorbance of solution where no lysyl-lactam was added to D11 ScFv to compete for binding to the immobilized antigen.