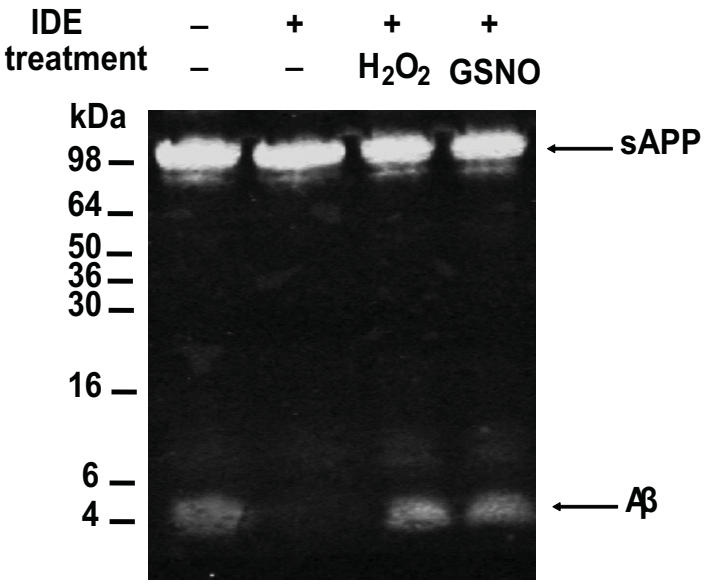

Fig. S1: The ability of native or modified IDE to degrade Aβ produced by HEK293swe.3 cells was performed as previously described (8). Briefly, conditioned medium collected from HEK293APPswe.3 cells (kindly provided by Dr. Sangram S. Sisodia-University of Chicago) was centrifuged at 100,000 x g for 15 min to remove cell debris and membranes, and the supernatant fraction was frozen in aliquots at -20 °C without added protease inhibitors. The conditioned medium (40 µl) was incubated with native or modified IDE enzyme at 37 °C for 30 minutes, and reactions were quenched by addition of a mixture of 4x Laemmli sample buffer. The resulting mixtures were boiled, fractionated on 16% Tris-Tricine gels, and transferred to nitrocellulose membranes. APPs derivatives and Aβ peptides were detected using the Aβ-specific monoclonal antibody 26D6 (24). Bound antibodies were visualized by scanning on a LI-COR Odyssey IR Imager.

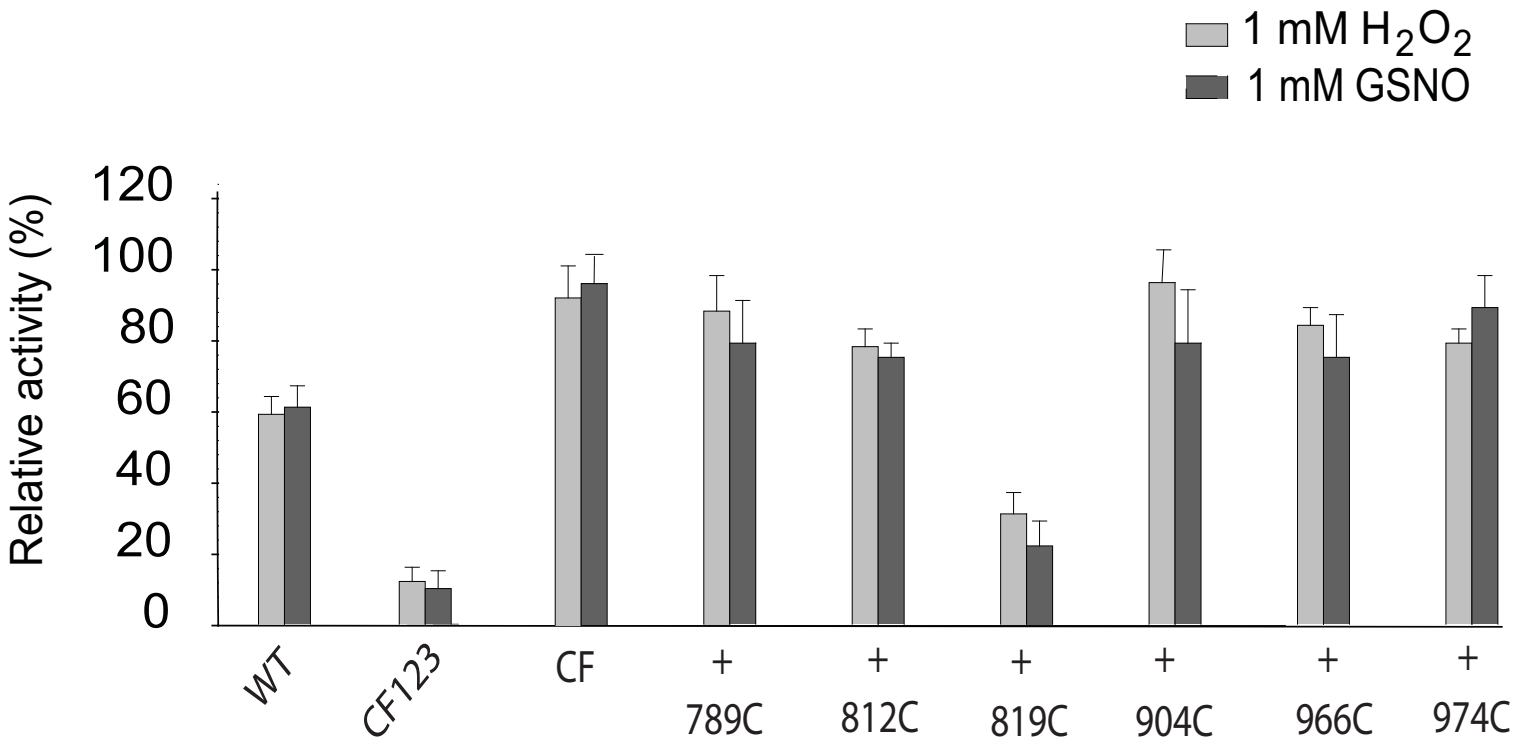

Fig. S2 To unambiguously identify the role of specific cysteine residues of domain 4 in H_2O_2 and GSNO sensitivity, without the complication of the protective cysteine(s) in other domains, we started with the IDE-CF mutant and individually restored the six known cysteines in the domain 4 of IDE (Figure 4A). Using the *fully-purified* IDE mutant proteins, we found that only the IDE mutant that contains the single cysteine in position 819 was highly sensitive to the treatment of 1 mM H_2O_2 or 1 mM GSNO (Figure 4A). Thus, we conclude that in domain 4, the only cysteine that plays a crucial role in H_2O_2 or GSNO mediated inactivation is cysteine 819. However, similar to IDE-CF123, this mutant has higher sensitivity to the treatments with H_2O_2 and GSNO, as compared to IDE-WT. This finding further supported a 'protective' role of cysteine residues in domains 1, 2, and/or 3.

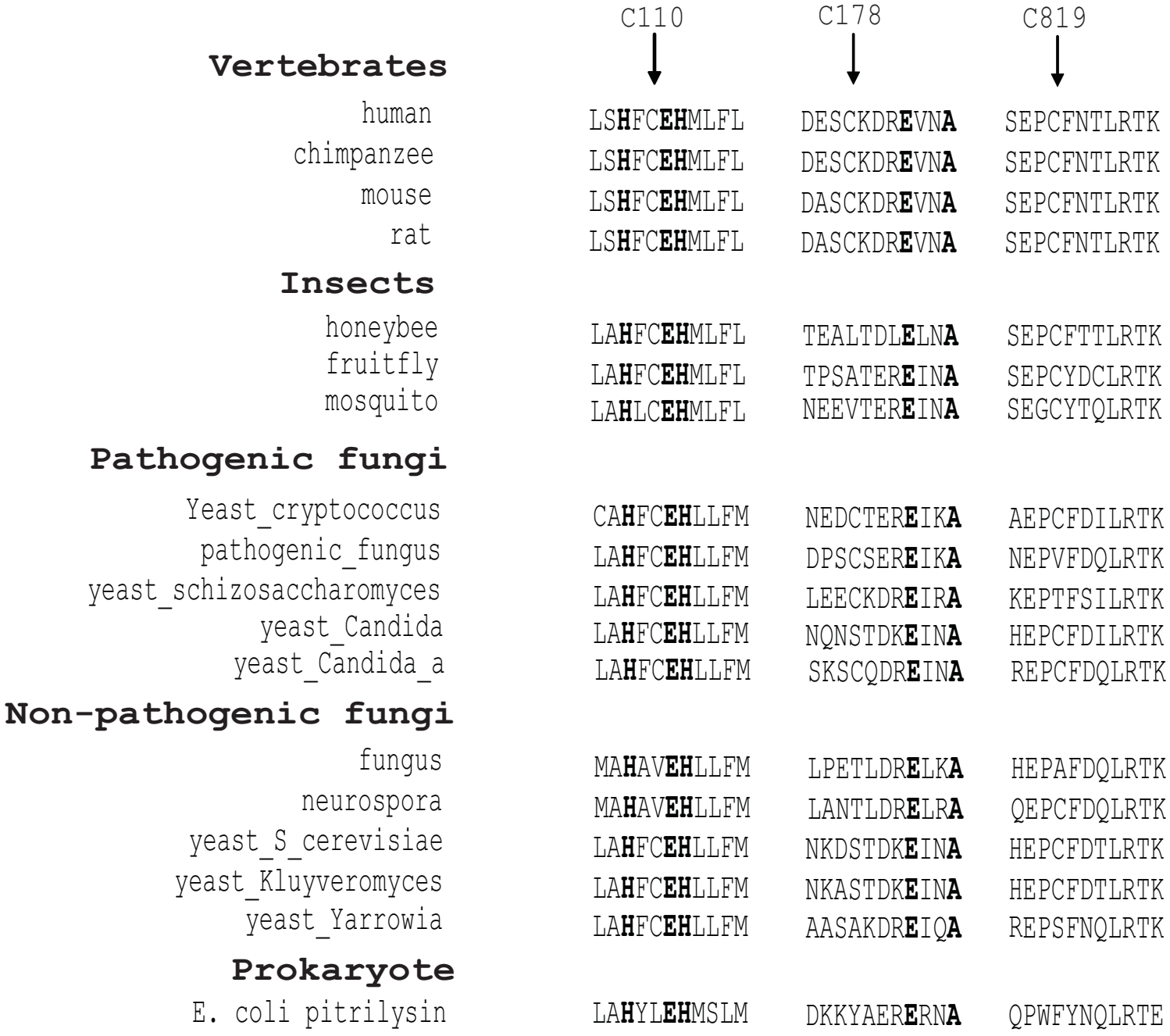

Fig. S3. Sequence alignment of IDE from various species. The alignment was made by AlignX. Completely conserved residues are shown in bold. The positions of Cys-110, Cys-178, and Cys-819 are highlighted. NO synthases produce NO through the conversion of the nitrogen rich amino acid, L-arginine into L-citrulline. Whereas lower eukaryotes typically possess a single NO synthase, higher eukaryotes have evolved three distinct NO synthase genes (eNOS, iNOS, and nNOS) (3). The production of NO is lethal to both bacteria and fungi and is considered part of the immune response to infection. Analysis of the sequence alignment of IDE (Figure 6) indicates that Cys-110 is well conserved among most species, except in *E.coli* and some of the non-pathogenic yeast. Interestingly, except for neurospora, the same species missing Cys-110 are also missing Cys-819, thus under oxidative attack, the activity of IDE in these species is likely preserved. Moreover, some of the pathogenic fungi that do have Cys-110 are missing Cys-819, suggesting that in the presence of ROS/RNS, the solvent exposure and modification of Cys-110 is unlikely to occur. Of the three cysteines, Cys-178 of IDE is the least conserved, present only in higher vertebrates, which have evolved 3 different types of synthases, and in most pathogenic fungi, suggesting that the evolution of this residue might contribute to the generation of a more stable and efficient IDE in an ROS/RNS-rich environment.