SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. Absorption spectrum of purified SLAC. (A) The absorption spectrum of purified and concentrated SLAC (30 mg mL^{-1}; in 50 mM HEPES-K, pH 7.5, 0.5 M NaCl, 0.25 M imidazole, 5% glycerol) was recorded on a Varian Cary 50 spectrophotometer at room temperature. The wavelengths for the major peak and two shoulders are shown on the graph. (B) Corresponding image of the purified and concentrated SLAC preparation.

Supplemental Figure 2. Effect of temperature on SLAC activity. Reactions (200 μL) contained 3.7 μg of SLAC, 1 mM ABTS, and 50 mM sodium acetate (pH 4.0). n = 3; errors indicate standard deviation.

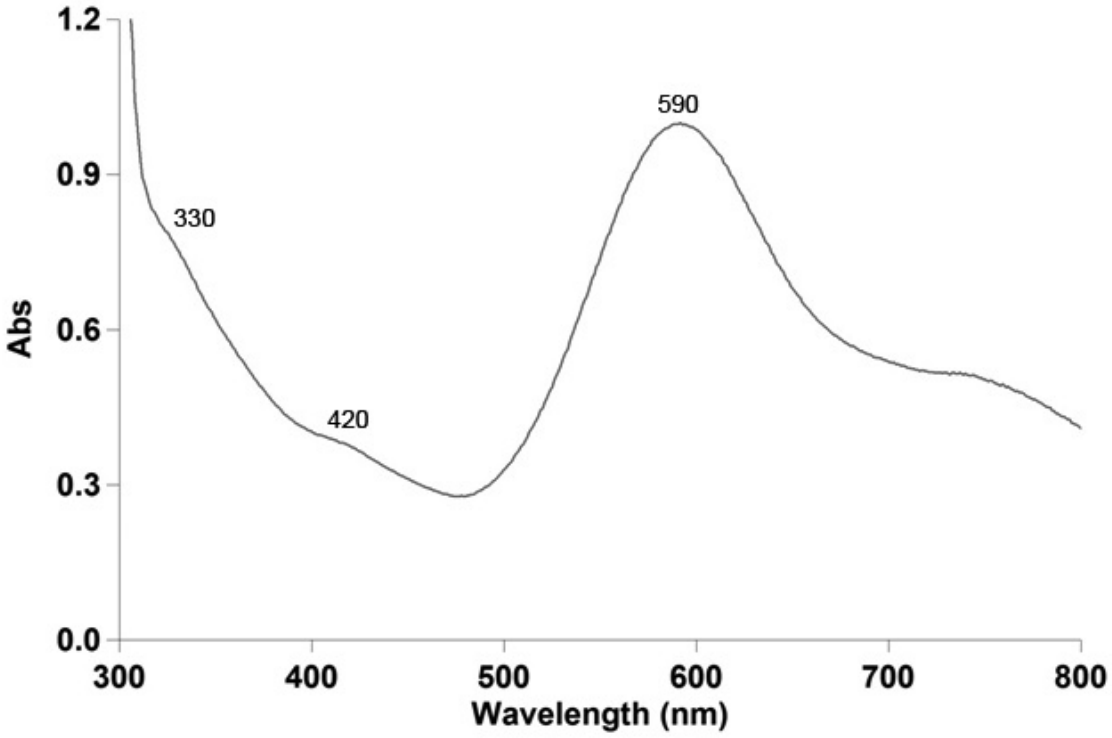
Supplemental Figure 3. pH Stability of SLAC. SLAC was pre-incubated for up to 5 h at room temperature in 50 mM universal buffer solutions (50 mM acetic acid, 50 mM boric acid, 50 mM phosphoric acid) ranging from pH 4.0 to 10.0. Reaction mixtures contained 1.2 μg SLAC, 1 mM ABTS, and were performed at 60 °C for 20 min. n = 3; errors indicate standard deviation.

Supplemental Figure 4. Chemical structures of natural bioactive phenolic substrates. Structures are shown for p-coumaric acid, caffeic acid, ferulic acid, apigenin and kaempferol.

Supplemental Figure 5. Multiple sequence alignment of SLAC and other small multicopper oxidases. The secondary structure of SLAC was assigned by DSSP and is shown above the

alignment. Similar resides are shadowed, and identical residues are boxed. SLAC residues that were mutated to Ala are indicated by filled arrows and numbered above the alignment. The proteins and the accession numbers are SLAC *Streptomyces coelicolor* copper oxidase (Q9XAL8), *Streptomyces griseus* EpoA (Q93HV5), *Saccharomonospora viridis* multicopper oxidase (C7MW31), *Nitrosomonas europaea* multicopper oxidase (Q82UE7), and *Clostridium beijerinckii* multicopper oxidase (A6LXP0).

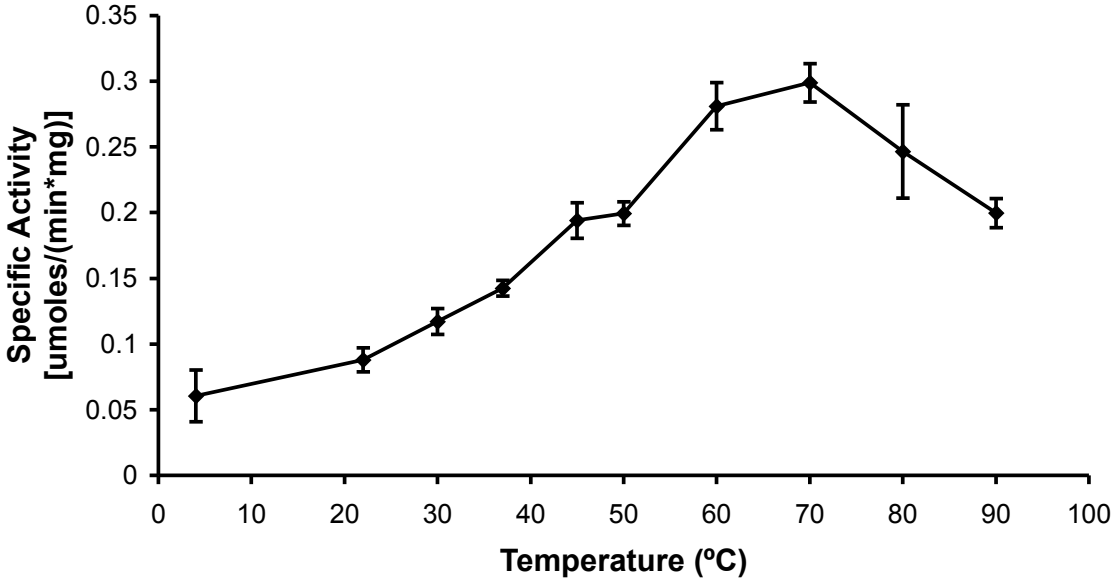
Supplemental Figure 6. Michaelis Menten plots for SLAC mutant S292A on quercetin and myricetin. Enzyme activity corresponding to initial reaction rates was calculated for (A) quercetin and (B) myricetin at substrate concentrations up to the limit of substrate solubility. Reactions contained 6 μg mL^{-1} SLAC mutant S292A. Michaelis-Menten curves were plotted using GraphPad Prism5 Software.



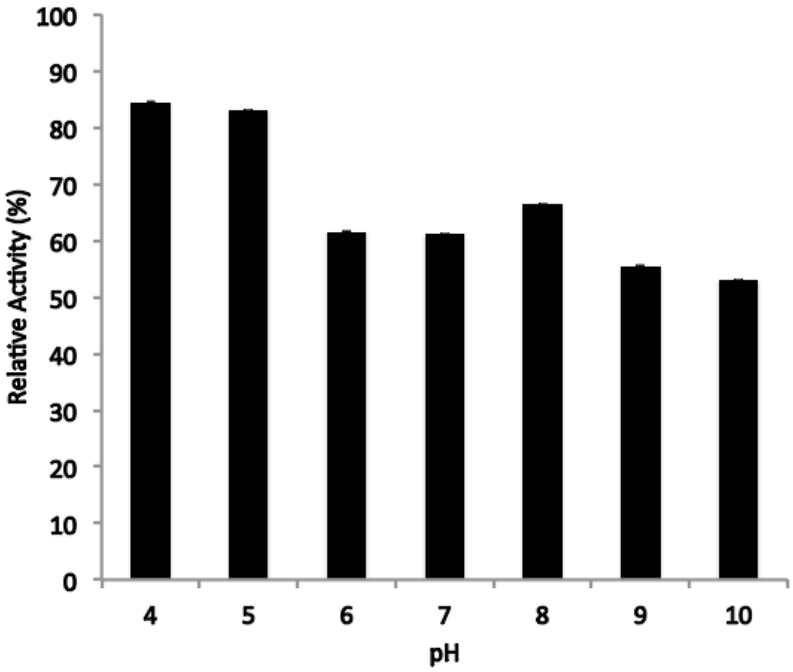
(A)

(B)

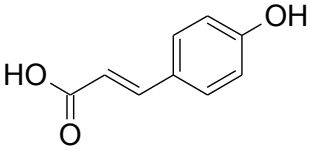
Supplemental Figure 1



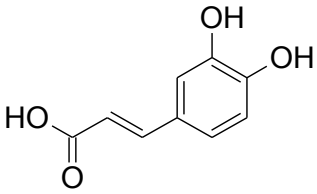
Supplemental Figure 2



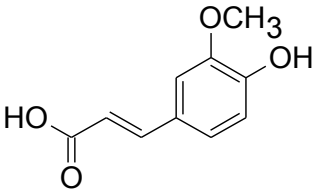
Supplemental Figure 3



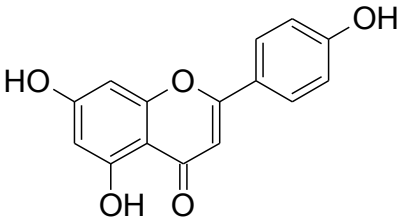
p-coumaric acid



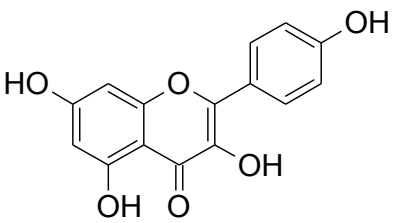
caffeic acid



ferulic acid

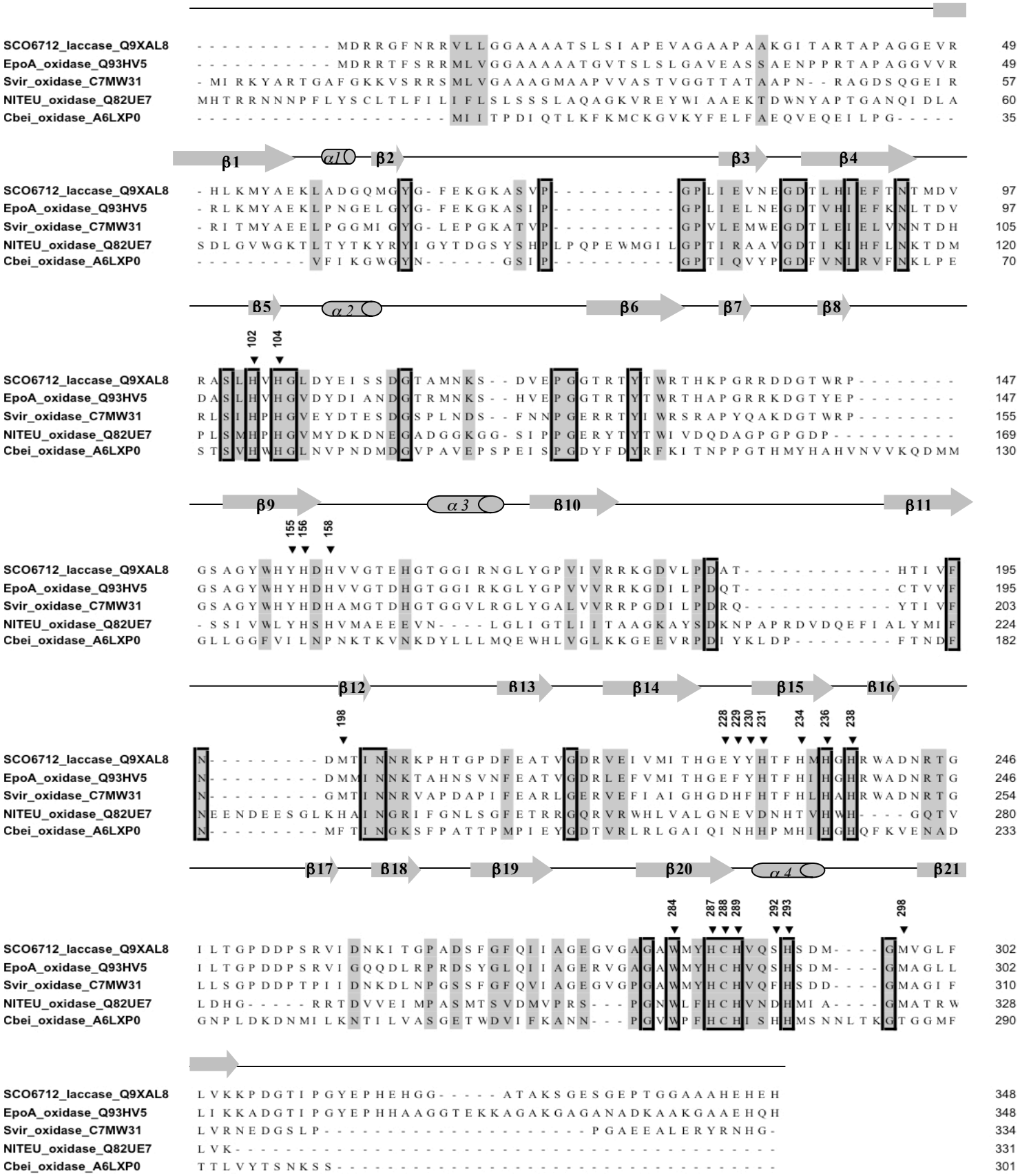


apigenin

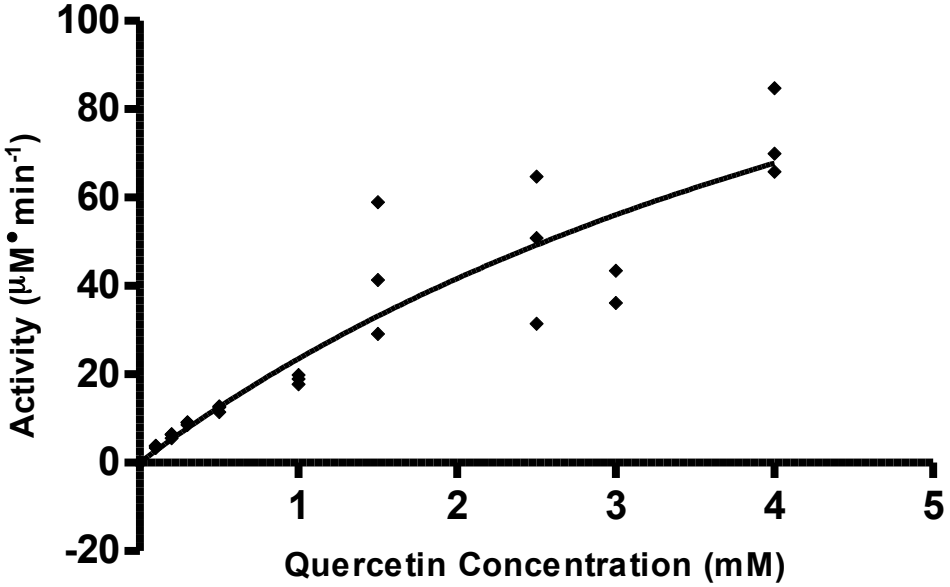


kaempferol

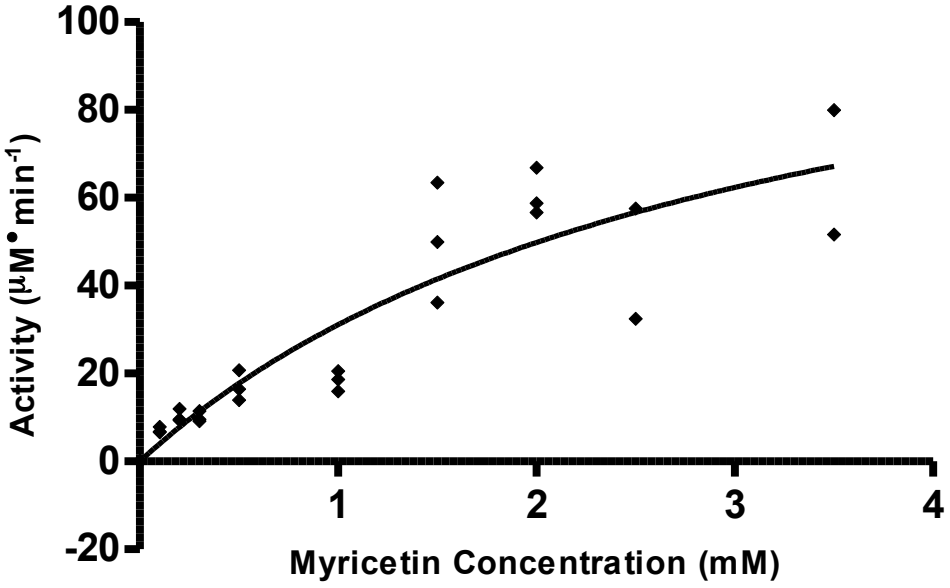
Supplemental Figure 4



Supplemental Figure 5



(A)



(B)

Supplemental Figure 6