

## **SUPPLEMENTAL FIGURE LEGENDS**

**Supplemental Figure 1. Absorption spectrum of purified SLAC.** (A) The absorption spectrum of purified and concentrated SLAC (30 mg mL<sup>-1</sup>; 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.

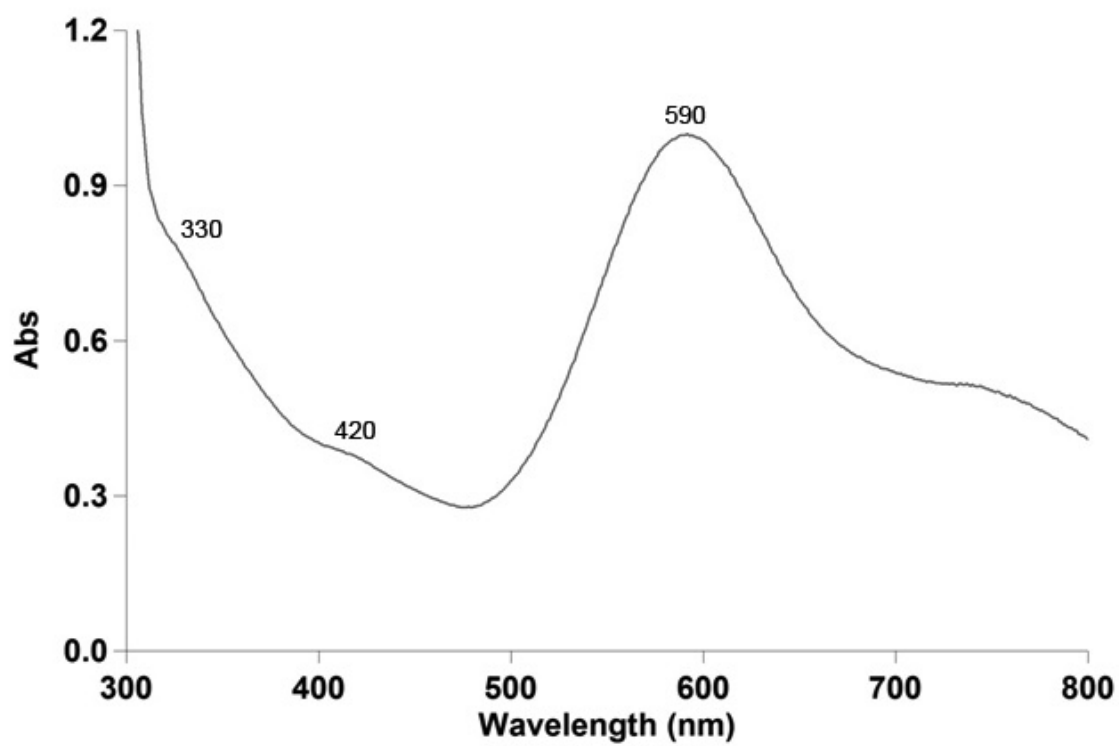
**Supplemental Figure 4. Chemical structures of natural bioactive phenolic substrates.**

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 residues 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  $\mu\text{g mL}^{-1}$  SLAC mutant S292A. Michaelis-Menten curves were plotted using GraphPad Prism5 Software.

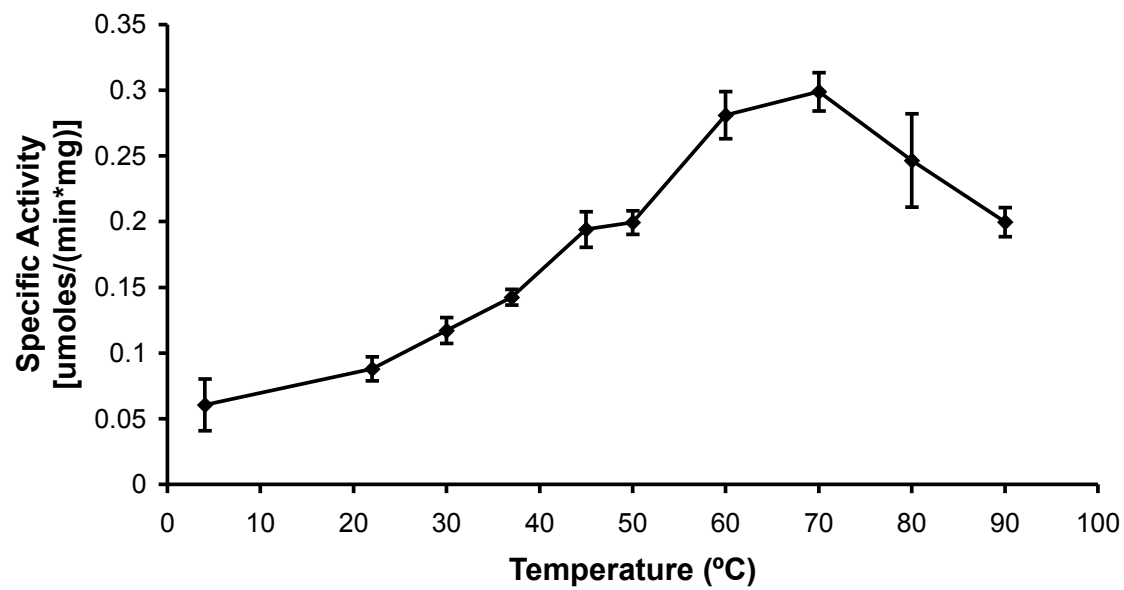


(A)

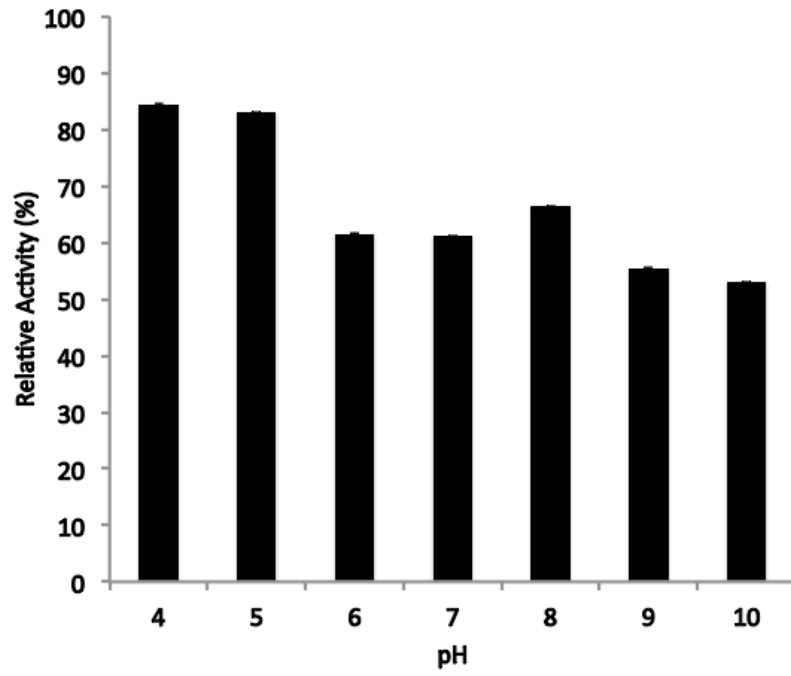


(B)

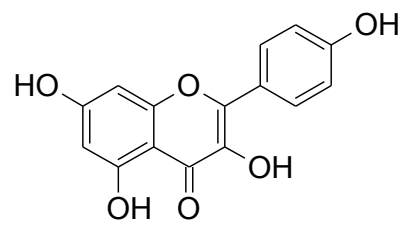
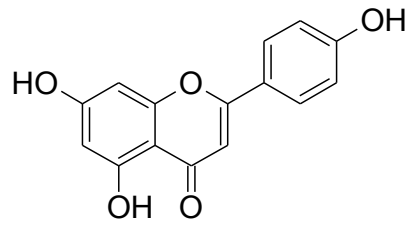
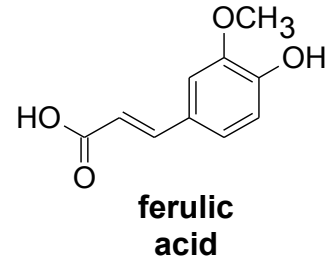
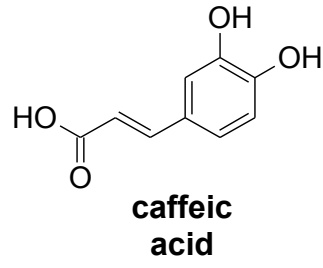
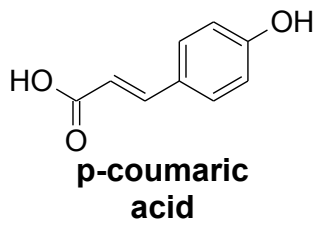
Supplemental Figure 1



Supplemental Figure 2

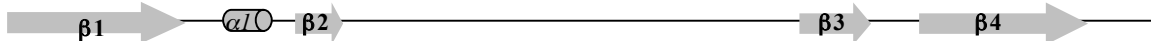


Supplemental Figure 3

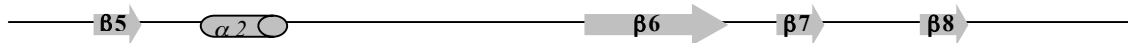


**Supplemental Figure 4**

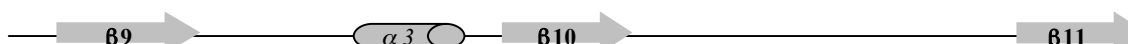
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Svir_oxidase_C7MW31	- MI RKYARTGAFGK KVSRRS MLVGAAGMAAPVVASTVGGTTATAAPN - - RAGDSQGEIR	57
NITEU_oxidase_Q82UE7	MHTRRRNNNPF LYSCLTLFLI LIFLSLSSSLAQAGKVREYWI AAETK DWNYAPTGANQIDL A	60
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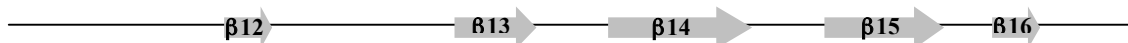
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Svir_oxidase_C7MW31	- RITMYAEELPGGMI GYG - LEP GKATVP - - - - - GPVLEMWEGDT LEI ELVNTDHD	105
NITEU_oxidase_Q82UE7	SDLGVWGT LTYTKRYI GYTDGSYSHPL PQPEWMLG LPTI RAAVGD TI KI HFLNKTD M	120
Cbei_oxidase_A6LXP0	- - - - - VFIKGGWGN - - - - - GSI P - - - - - GPTIQVYP GDFVNI RRVFNKLPE	70



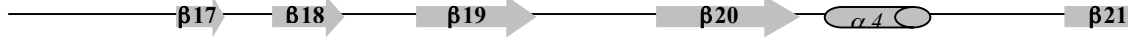
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Svir_oxidase_C7MW31	RLSLIHPHGV EYDTE SDGSPLNDS - - FNNPGERRTYI WRSRAPYQAKDGTWRP - - - - -	155
NITEU_oxidase_Q82UE7	PLSMHPHGV MYDKDNEGADGGKGG - SIPPGERYTYT WI VDQDAGP GP GDP - - - - -	169
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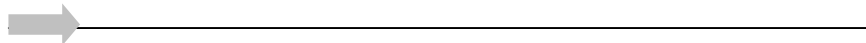
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Svir_oxidase_C7MW31	GSAGYWHYHDHAMGTDHGTGGVLRGLYGLV VRRPGLIP DRQ - - - - - YTIVF	203
NITEU_oxidase_Q82UE7	- SSI VWLYHS HVMAEEVN - - - LGLI GLII TAAGKAYS DKNPAPRDVDQEFIALYMI F	224
Cbei_oxidase_A6LXP0	GLLGGFVILNPNKTKV NKDYLLMQEWHLVGL KKGEEV RPDII YKLDP - - - - - FTNDF	182



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EpoA_oxidase_Q93HV5	N - - - - - DMMI NNRKTAHNSVNF EATVGGDRLEFVMI THGEFYHTFHI HGH RWADNRTG	246
Svir_oxidase_C7MW31	N - - - - - GMTI NNRVAPDAPI FEARLGERVEFI AIGHGDHFHTFHL HAHRWADNRTG	254
NITEU_oxidase_Q82UE7	NEENDEESGLKHAINGRI FGNLSGFETR RQRVRWHLVALGNEVDNHTVHWH - - - GQTV	280
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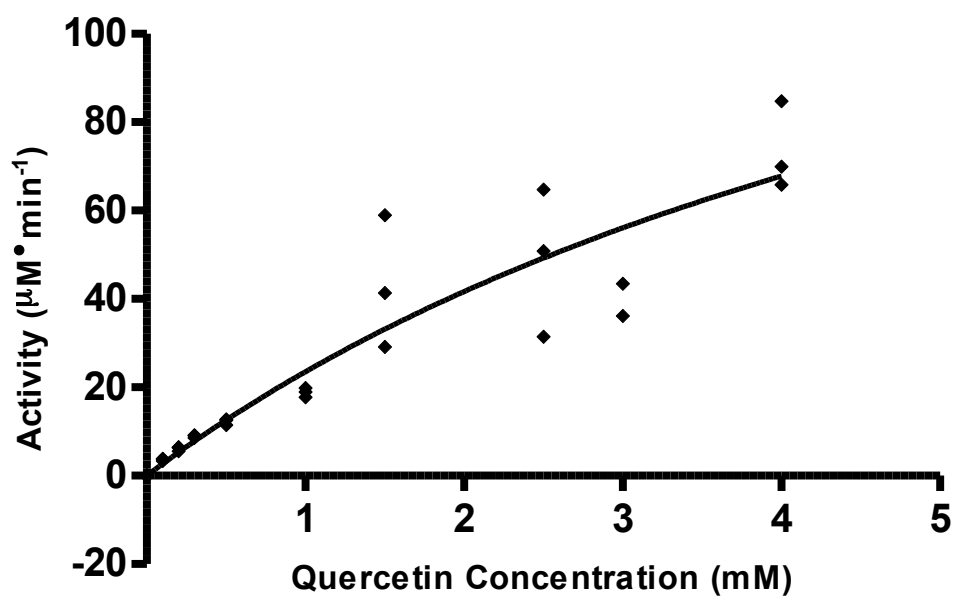


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Svir_oxidase_C7MW31	L LSGPDDPTPI I DNKDLNPGS SFGFQVI AAGEGVGP GAWMYHCHVQFHS DD - - - GMAGIF	310
NITEU_oxidase_Q82UE7	LDHG - - - - RRTDVVEI MPASMTS VDMVPRS - - - PGNWL FHC HVNDHMI A - - - GMATRW	328
Cbei_oxidase_A6LXP0	GNPLDKDNMI LKNTI LVASGETWDVIFKANN - - - PGVWPFHCHI SHHMSNNLT KGTGGMF	290

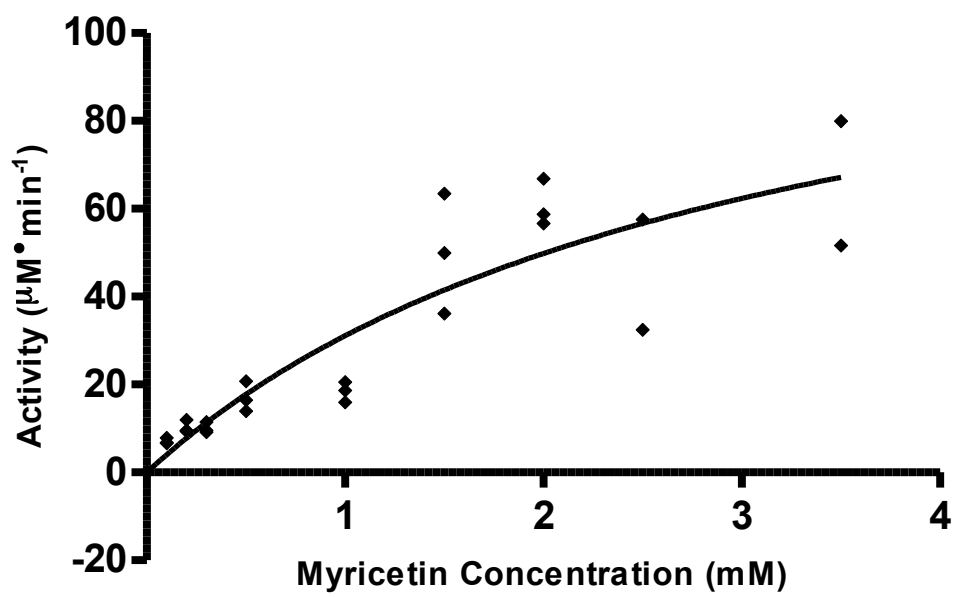


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Svir_oxidase_C7MW31	L VRNEDGSLP - - - - - PGAEALERYRNHG -	334
NITEU_oxidase_Q82UE7	L VK - - - - -	331
Cbei_oxidase_A6LXP0	T TLVYTSNKSS - - - - -	301

Supplemental Figure 5



(A)



(B)

Supplemental Figure 6