Fig. S1

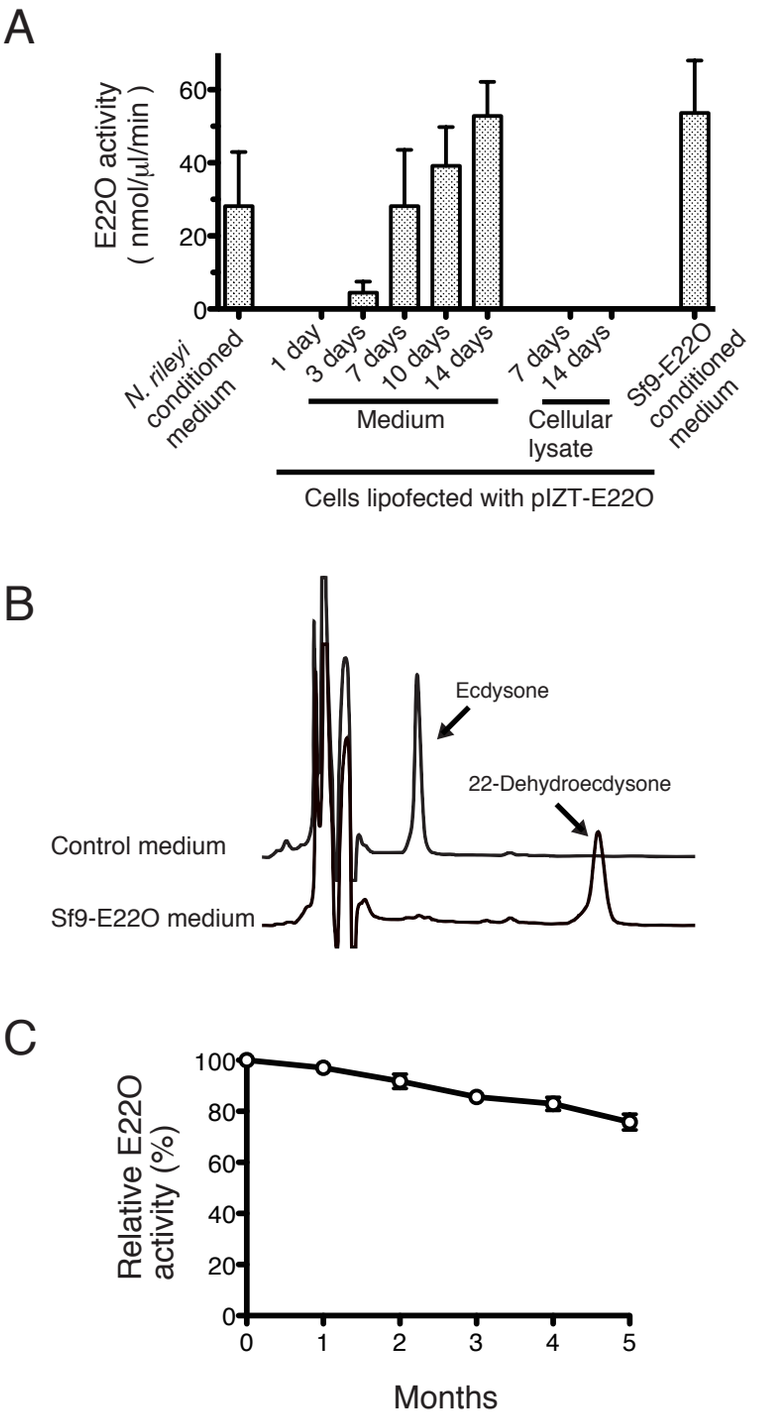

Fig. S1. **E22O activity in various media and cellular lysates.** *A*, Comparison of the ecdysone-oxidizing activity found in the *N. rileyi* conditioned media, in the media and cellular lysates of Sf9 cells transiently expressing the E22O gene, and in the conditioned media of the Sf9-E22O cells. Each sample was appropriately diluted and then incubated with 100 μM of ecdysone. Amount of 22-dehydroecdysone produced was measured after 10 min. Error bars represent SD (N=4-8). *B*, Chromatograms showing the oxidation of ecdysone by the conditioned medium of Sf9 and Sf9-E22O cells. Each medium was mixed with an equal volume of 200 μM ecdysone solution, incubated for 10 min, and then analyzed by HPLC. *C*, Changes in the ecdysone-oxidizing activity of the Sf9-E22O conditioned media stored at 4°C. Error bars represent SEM (N=4).

Fig. S2

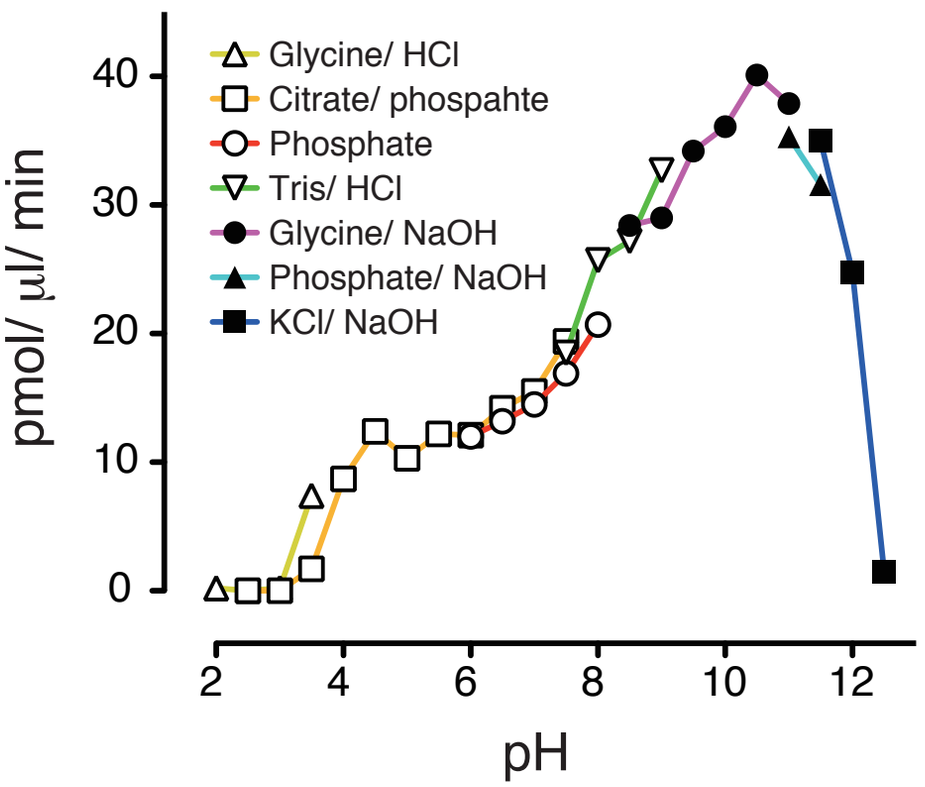

Fig. S2. **pH-dependent activity of E22O.** Appropriately diluted *N. rileyi* conditioned medium was incubated with 50 µM of ecdysone in various buffers and the amount of 22-dehydroecdysone produced under each experimental condition was measured after 10 min. N=2.

Fig. S3

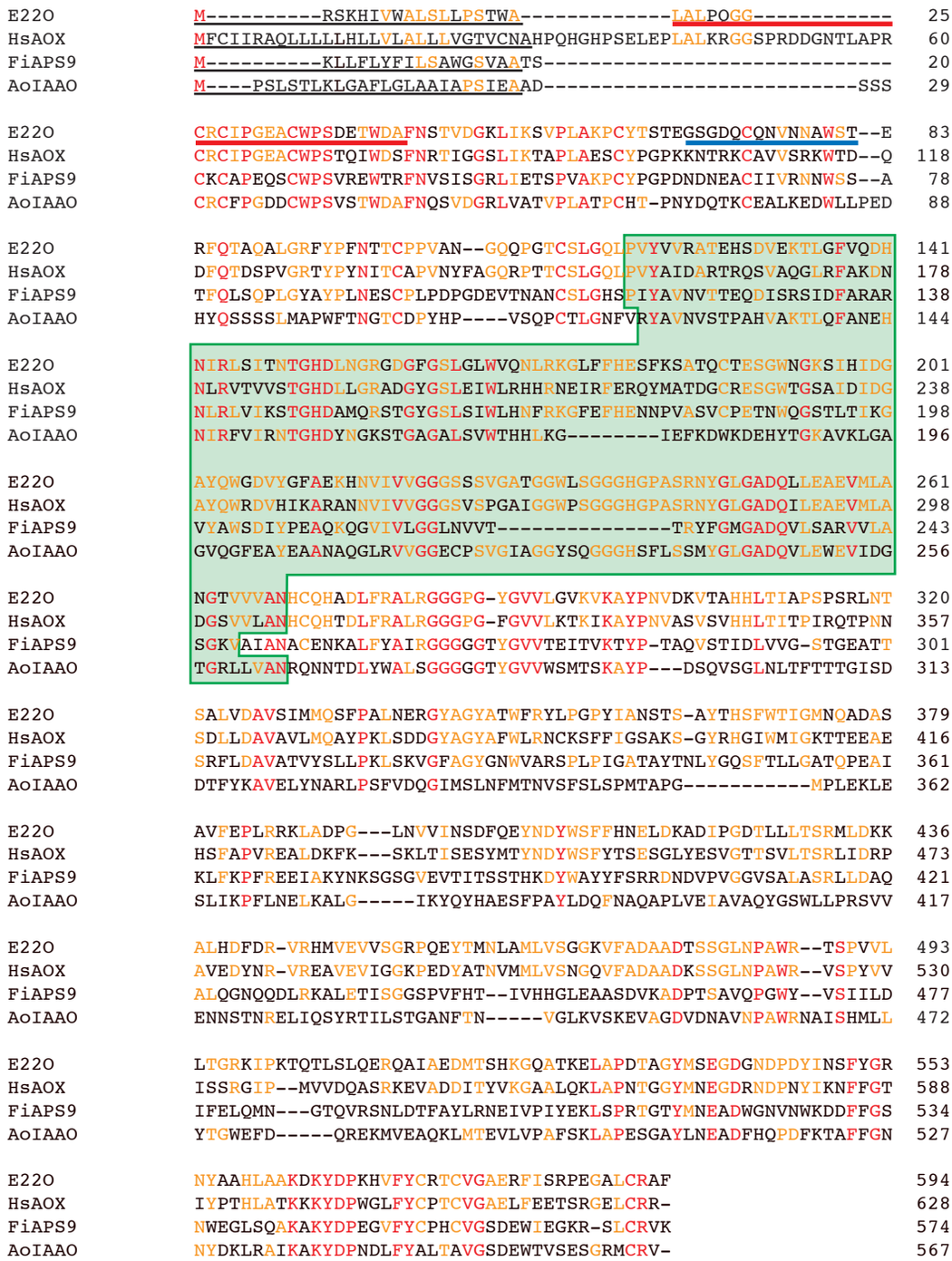

Fig. S3. **Sequence alignment of E22O with three fungal oxidases.** Protein sequences of E22O (this study), *Hypomyces subiculosus* alcohol oxidase (HsAOX, GenBank accession number: ACD39759), *Fusarium incarnatum* APS9 (FiAPS9, GenBank accession number: ACZ66255) and *Aspergillus oryzae* isoamyl alcohol oxidase (AoIAAO, GenBank accession number: BAB13480) were aligned. Amino acid residues conserved in all four proteins are shown in red letters and those conserved between E22O and one or two of the oxidases are shown in orange letters. Putative secretion signals of all four proteins, the N-terminal amino acid sequences of the purified E22O and its limited V8-proteolysis product are underlined using black, red and blue lines, respectively. The putative FAD binding domains in all four enzymes are shown within a green box.

Fig. S4

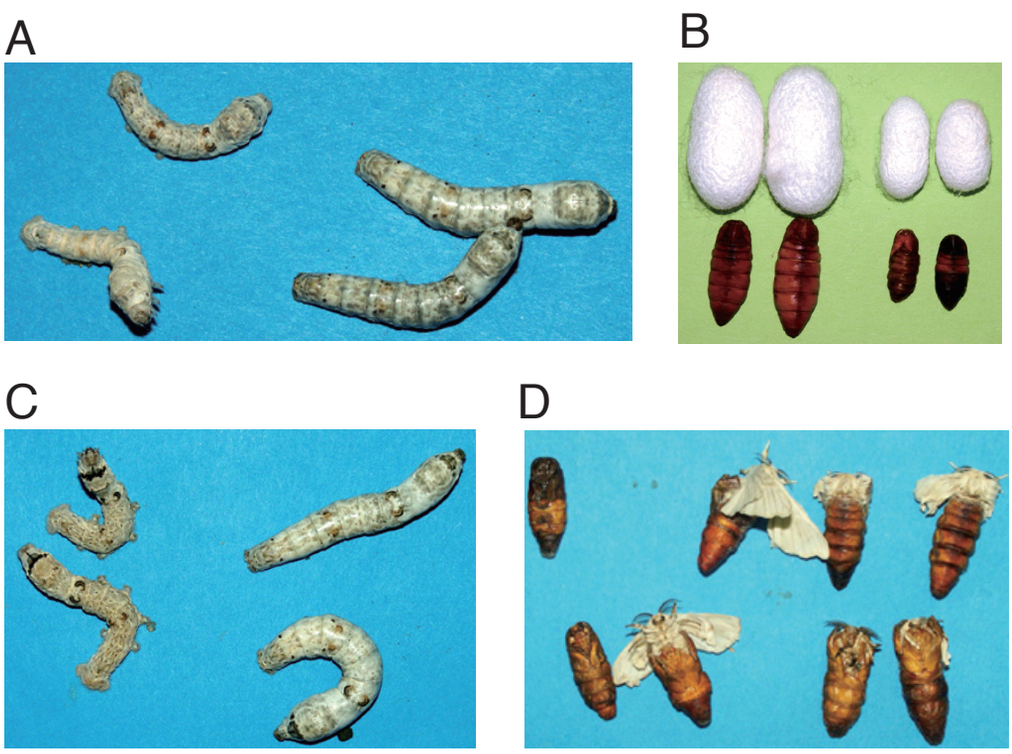

Fig. S4. **Effects of E22O gene expression on the growth of silkworm.** *A*, Larvae lipofected with pIZT-E22O at the beginning of the 3rd instar continued eating and grew much bigger than the pIZT/V5-his-lipofected control. Left: control 4th instar larvae just after the ecdysis, right: *E22O*-expressing 3rd instar larvae. *B*, Some of the 3rd instar larvae that were lipofected with pIZT-E22O pupated precociously after molting to 4th instar larvae. Left: control pupae metamorphosed from the 5th instar larvae, right: precocious pupae. *C*, Larvae lipofected with pIZT-E22O at the beginning of the penultimate (4th) instar also continued eating and grew much bigger than the pIZT/V5-his-lipofected control. Note that some of them precociously started spinning in the instar but none of them completed metamorphosis. Left: control 5th instar larvae just after the ecdysis, right: *E22O*-expressing 4th instar larvae. *D*, Lipofection of pIZT-E22O into the spinning last instar larvae did not prevent their pupation but prevented adult emergence. Upper left: dead pupae; lower left: dead pharate adult; upper and lower right 1-3 rows: unsuccessful emergence.