Supplemental Information

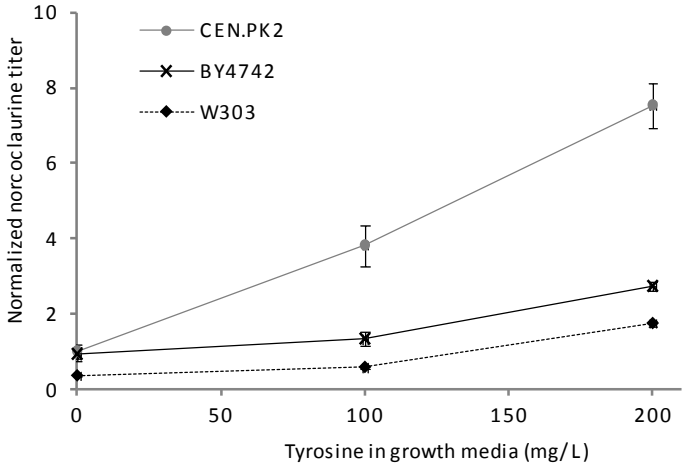

Figure S1 Production of norcoclaurine from fed dopamine in wild-type yeast background strains. Strains were cultured in YNB media containing 100 mM dopamine and indicated concentrations of tyrosine. Norcoclaurine titer was measured from culture supernatant by LC-MS/MS. The results were normalized to the ion count area in CEN.PK2 yeast culture with 0 mg/L tyrosine (9.8 μg/L). Data is reported as the mean ± s.d. of at least 3 independent experiments.

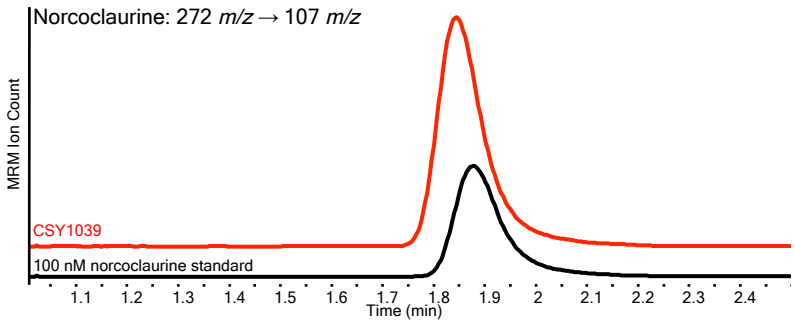

Figure S2 Production of norcoclaurine from fed L-DOPA in CSY1039. CSY1039 produced 49 μg/L norcoclaurine when cultured with 2 mM L-DOPA for 72 hours.

Norcoclaurine was measured in the media with LC-MS/MS in MRM mode and compared to a standard.

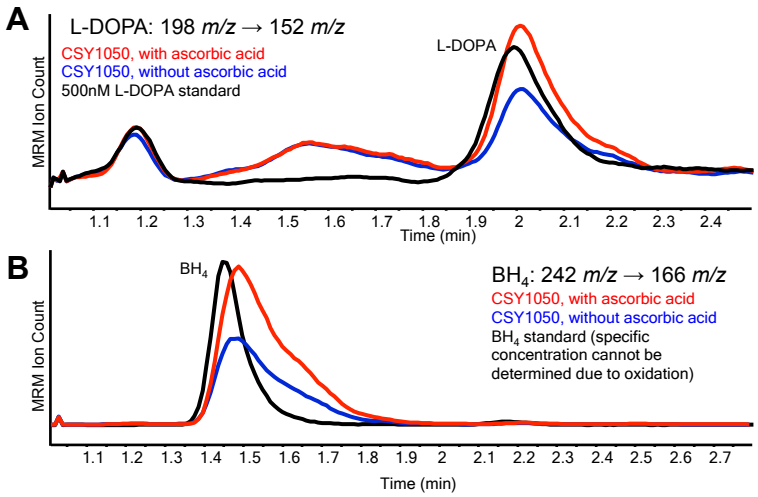

Figure S3 Impact of ascorbic acid on L-DOPA and BH_4 production. (A) Production of L-DOPA in CSY1051 with pCS3231 cultured in media with and without 2 mM ascorbic acid. (B) Production of BH_4 in CSY1051 with wild-type *RnTyrH* (pCS3231) cultured in media with and without 2 mM ascorbic acid. Metabolites were measured in the media with LC-MS/MS in MRM mode and compared to a standard.

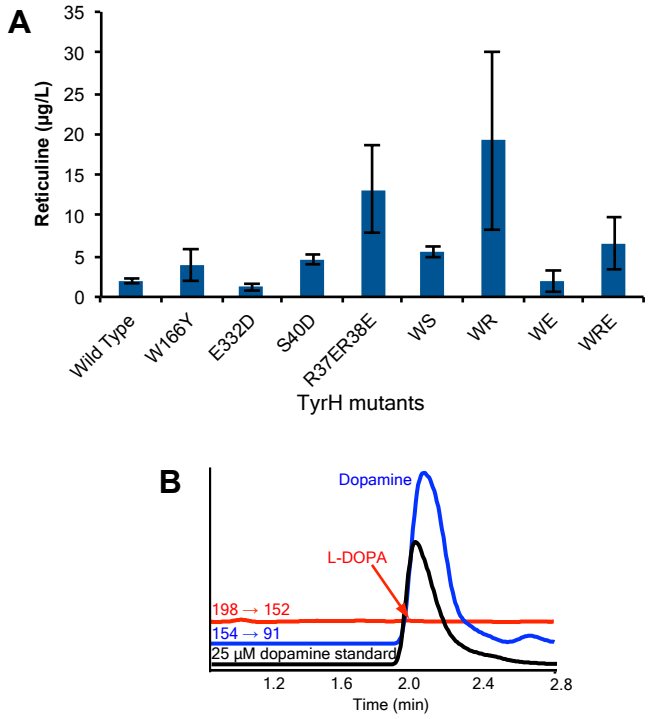

Figure S4 *De novo* production of reticuline in engineered yeast. (A) Reticuline production as a function of *RnTyrH* mutant. CSY1052 strains harboring plasmids with *RnTyrH* mutants (pCS3231-3239) and *CjNCS* (pCS3241) were grown in selective media lacking tyrosine and supplemented with 2 mM ascorbic acid for 96 hours before analysis with LC-MS/MS in MRM mode. Data is reported as the mean ± s.d. of at least 3 independent experiments. (B) Analysis of pathway intermediates from L-DOPA and dopamine in CSY1052 expressing *RnTyrH*^{WR} (pCS3228) and *CjNCS* (pCS3241). Cultures were grown in selective media lacking tyrosine and supplemented with 2 mM ascorbic acid for 96 hours before analysis with LC-MS/MS in MRM.