Supporting Data

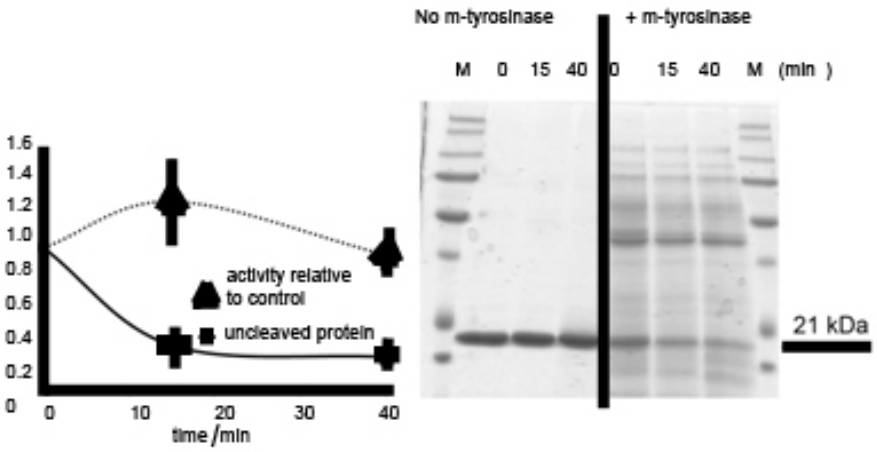

Figure S1. *eDHFR (5 mg/ml) was treated with m-tyrosinase (0.15 mg/ml). At selected time points aliquots were extracted and activity (measured by decrease in A_{340} relative to a no m-tyrosinase treated control) and conversion of reaction were calculated (measured by densitometry of SDS-PAGE gel)*. Control used a similar sample without m-tyrosinase addition. m-Tyrosinase was shown to have no DHFR activity even at concentration 50 times higher than that of the assay mixture. Errors are standard deviations N=3.

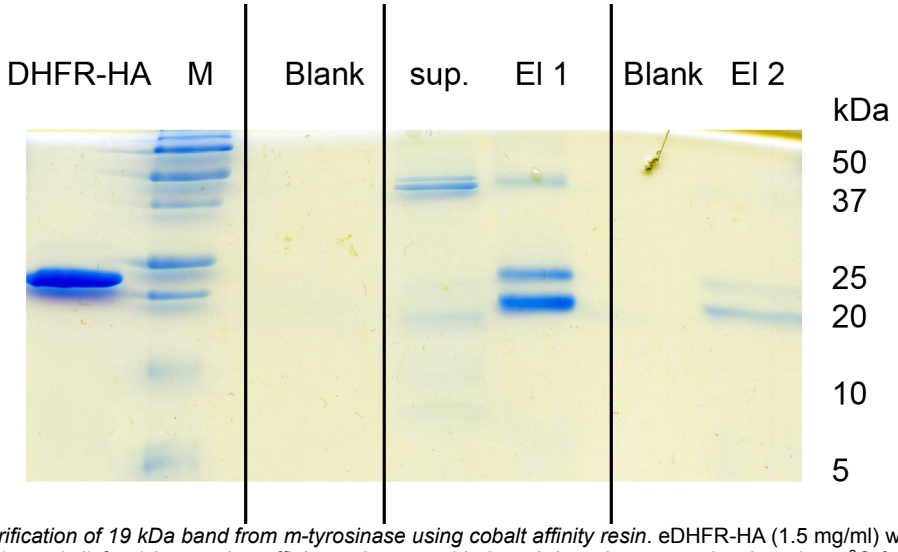

Figure S2. *Purification of 19 kDa band from m-tyrosinase using cobalt affinity resin*. eDHFR-HA (1.5 mg/ml) was treated with m-tyrosinase (0.15 mg/ml) for 1 hour, talon affinity resin was added, and the mixture was incubated at 4^{o}C for 30 minutes. His-tagged proteins were eluted using imidazole. Sup.= supernatant from incubation with beads; El1 = elution with 30 mM imidazole; El2 = elution with 400 mM imidazole.

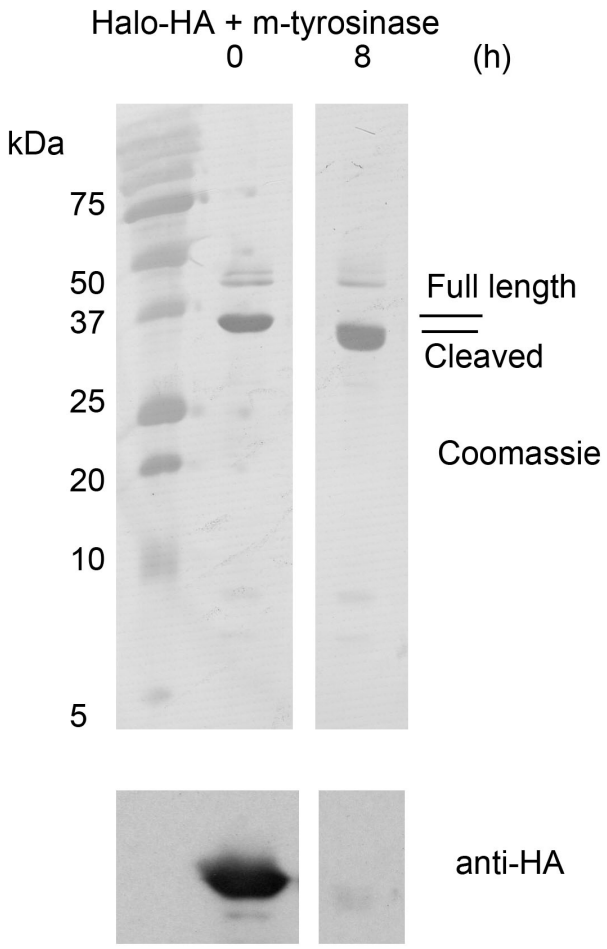

Figure S3. *Cleavage of Halo-HA by m-tyrosinase*. His_6-Halo-HA (3 mg/ml) was treated with m-tyrosinase (0.5 mg/ml). The reaction was analyzed after 8 hours by western blot for HA (lower) and then membrane was stained with Coomassie (upper).

Protein sequence of eDHFR-HA (His6 tag shown in green; HA shown in red)
MGSSHHHHHHSSGLVPRGSHMISLIAALAVDRVIGMENAMPWNLPADLAWFKRNTLNKPVIMGRHTWESIGRPLPGRKNIILSSQ
PGTDDRVTWVKSVDEAIAACGDVPEIMVIGGGRVYEQFLPKAQKLYLTHIDAEVEGDTHFPDYEPDDWESVFSEFHDADAQNSH
SYCFEILERRYPYDVPDYA

Protein sequence of NT-HA (His6 tag shown in green; HA shown in red)
MGSSHHHHHHSSGLVPRGSHYPYDVPDYAMISLIAALAVDRVIGMENAMPWNLPADLAWFKRNTLNKPVIMGRHTWESIGRPLP
GRKNIILSSQPGTDDRVTWVKSVDEAIAACGDVPEIMVIGGGRVYEQFLPKAQKLYLTHIDAEVEGDTHFPDYEPDDWESVFSEF
HDADAQNSHSYCFEILERR

Protein sequence of Halo-HA (His6 tag shown in green; HA shown in red)
MGSSHHHHHHSSGLVPRGSHMAEIGTGFPFDPHYVEVLGERMHYVDVGPRDGTPVLFLHGNPTSSYVWRNIIPHVAPTHRCIA
PDLIGMGKSDKPDLGYFFDDHVRFMDAFIEALGLEEVVLVIHDWGSALGFHWAKRNPERVKGIAFMEFIRPIPTWDEWPEFARET
FQAFRTTDVGRKLIIDQNVFIEGTLPMGVVRPLTEVEMDHYREPFLNPVDREPLWRFPNELPIAGEPANIVALVEEYMDWLHQSPV
PKLLFWGTPGVLIPPAEAARLAKSLPNCKAVDIGPGLNLLQEDNPDLIGSEIARWLSTLEISGYPYDVPDYA

Protein sequence of His6-GFP-TEV-HA-GST-α1 (His6 tag shown in green; TEV shown in black; HA shown in red; GFP shown in brown; GST-α1 shown in blue)
MGSSHHHHHHSSGLVPRGSHMVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVTT
LTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSH
NVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTAAGITLGM
DELYKENLYFQGYPYDVPDYAMAEKPKLHYFNARGRMESTRWLLAAAGVEFEEKFIKSAEDLDKLRNDGYLMFQQVPMVEIDG
MKLVQTRAILNYIASKYNLYGKDIKERALIDMYIEGIADLGEMILLLPVCPPEEKDAKLALIKEKIKNRYFPAFEKVLKSHGQDYLVGN
KLSRADIHLVELLYYVEELDSSLISSFPLLKALKTRISNLPTVKKFLQPGSPRKPPMDEKSLEEARKIFRF

Figure S4. Sequences of proteins used.

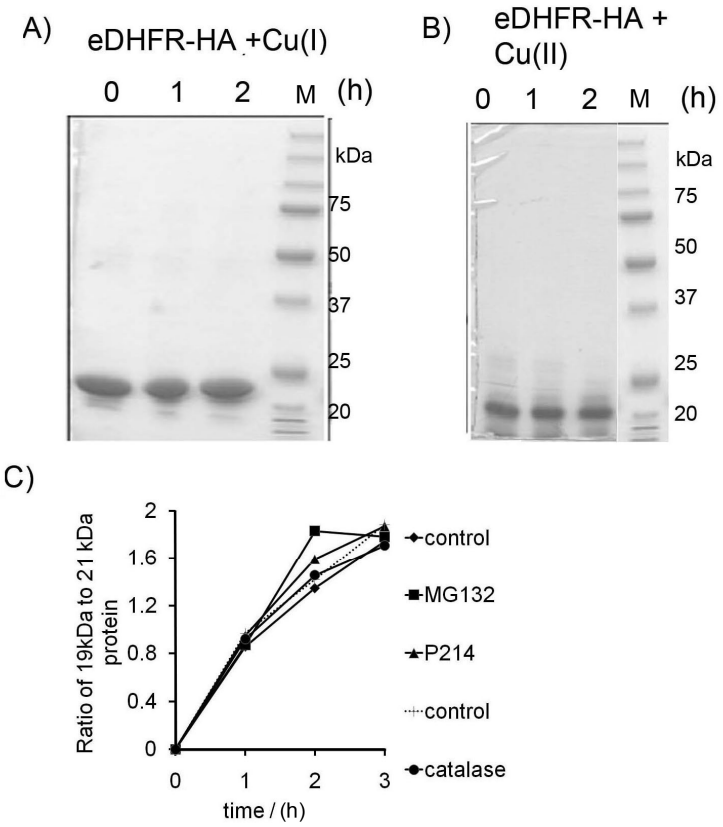

Figure S5. *Protease inhibitors do not affect the cleavage reaction and copper ions do not cause cleavage*. A) Effect of Cu(I) (50 μM) on His_6-eDHFR-HA (1.5 mg/ml). B) Effect of Cu(II) (50 μM) on His_6-eDHFR-HA (1.5 mg/ml). C) Quantitation of Coomassie stained SDS-PAGE gels showing cleavage of eDHFR-HA (1.5 mg/ml) by m-tyrosinaseosinase (0.15 mg/ml) in the presence of different additives (100 μM MG132, 2x Sigma protease inhibitor cocktail P214 [containing: 4-(2-aminoethyl)benzenesulfonyl fluoride (AEBSF), pepstatin A, E-64, bestatin hydrochloride, leupeptin hemisulfate, and aprotinin], 0.5 mg/ml catalase.

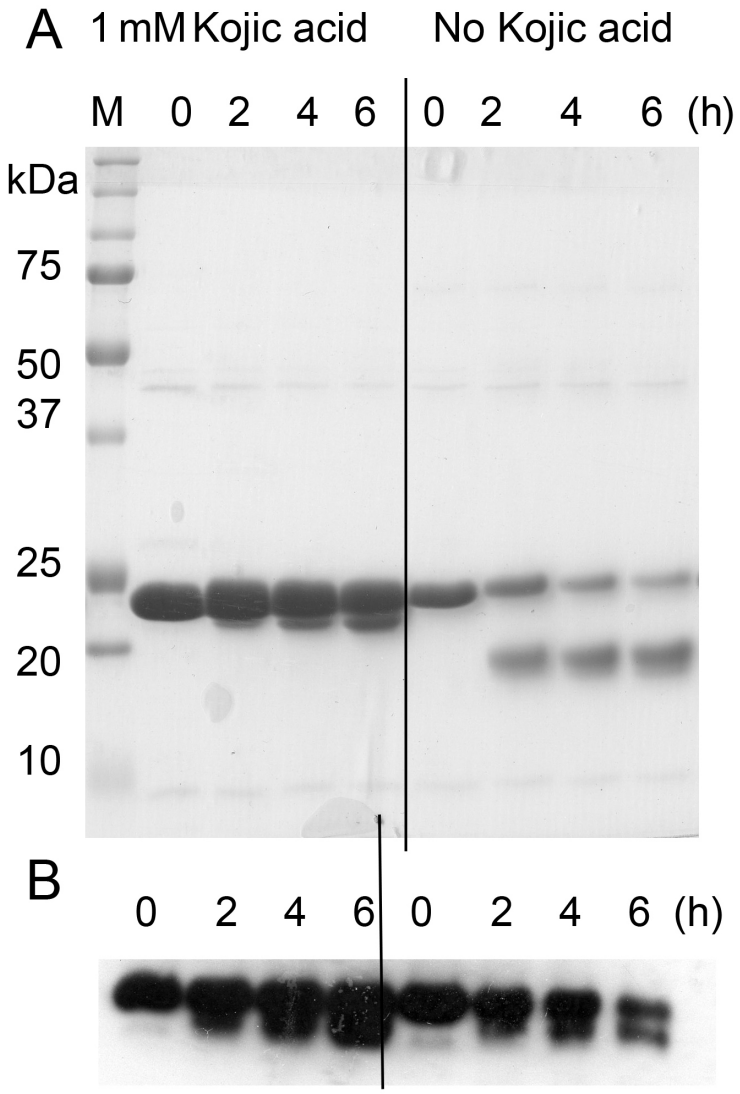

Figure S6. A) 18 % SDS PAGE gel analysis of cleavage of eDHFR-HA (1.5 mg/ml) with m-tyrosinase (0.15 mg/ml) in the presence (left) and absence (right) of Kojic acid (1 mM). B) western blot analysis (anti-HA) of a separate reaction of eDHFR-HA (1.5 mg/ml) with m-tyrosinase (0.15 mg/ml) in the presence (left) and absence (right) of Kojic acid (1 mM).

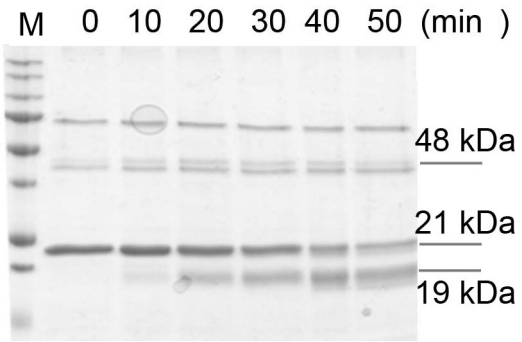

Figure S7. *Time course for cleavage of eDHFR-HA (quantified in main test figure 2C).*eDHFR-HA (1.5 mg/ml) was treated with m-tyrosinase (0.15 mg/ml) and aliquots were removed at the stated time points.

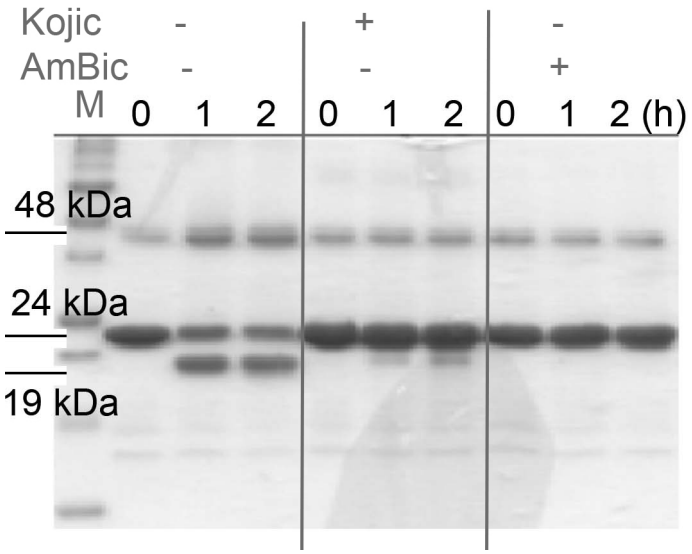

Figure S8. eDHFR-HA (1.5 mg/ml) was treated with m-tyrosinase (0.15 mg/ml). Kojic contained 1 mM kojic acid. AmBic used 50 mM ammonium bicarbonate buffer pH 6.6 instead of phosphate. *Inability to cleave in the presence of AmBic is postulated to be due to interception of the quinone by ammonia.*

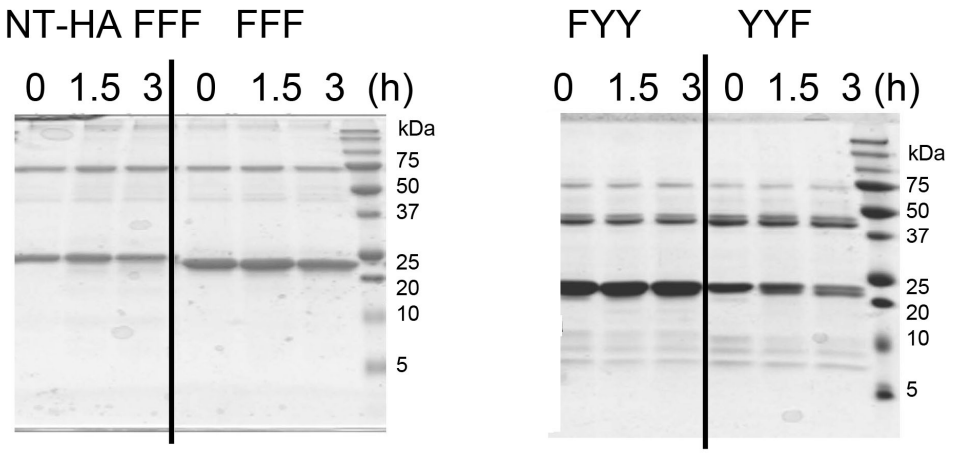

Figure S9. *Mutants treated with m-tyrosinase and analyzed by reducing SDS-PAGE gel*. No 19 kDa cleavage band was observed. NOTE: N-terminal cleavage is observable in the YYF mutant, but presumably occurs in all species although resolution prevents detection.

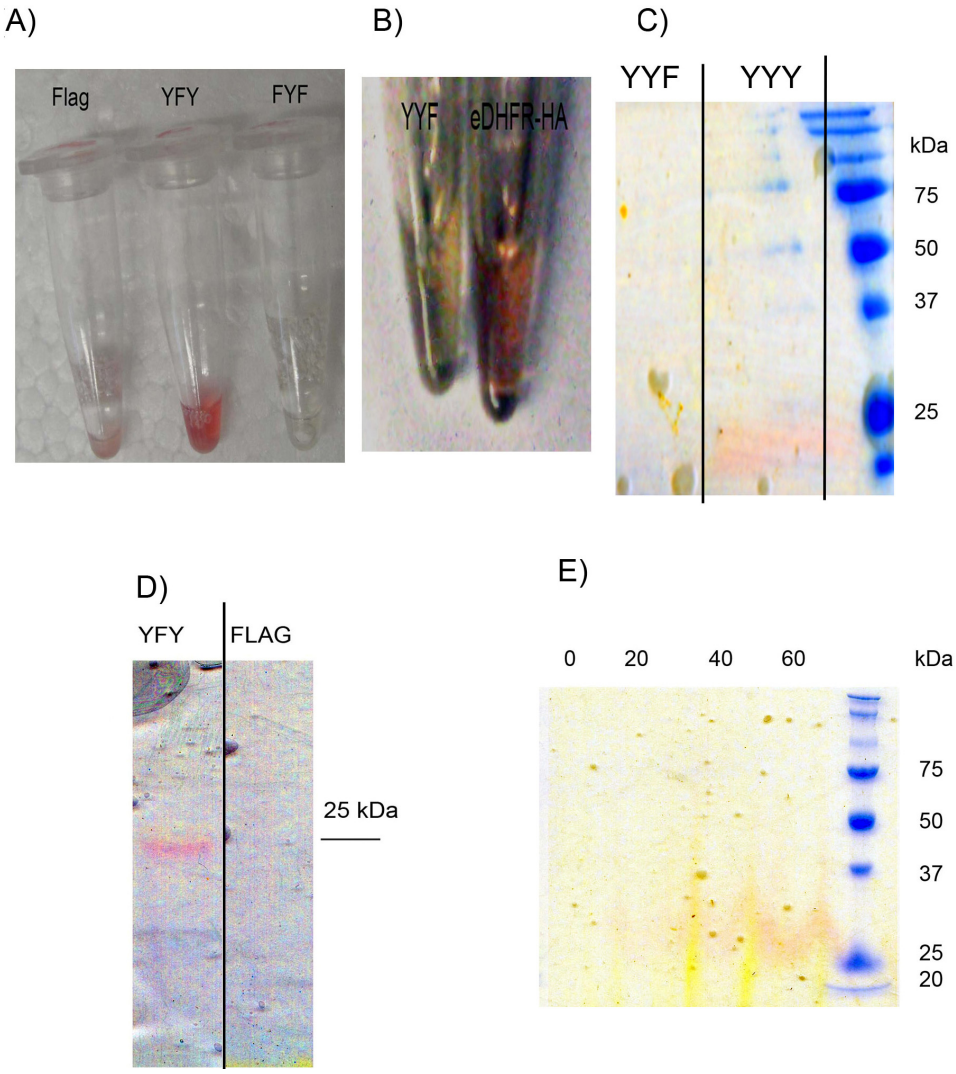

Figure S10. *No quinone is formed from His_6-eDHFR-FLAG (1.5 mg/ml) (referred to as FLAG), FYF or YYF mutants (1.5 mg/ml), but it is from YFY (1.5 mg/ml) mutant in presence of 0.15 mg/ml) m-tyrosinase and Besthorn's reagent (30 μM).* A) and B) Images of a reactions with m-tyrosinase (0.15 mg/ml) and Besthorn's reagent (30 μM) after 3 hours. C) and D) SDS-PAGE analysis of YYF, eDHFR-HA, YFY and FLAG samples (unstained). E) FYY (1.5 mg/ml) was treated with m-tyrosinase (0.15 mg/ml) and Besthorn's reagent (30 μM) and aliquots were extracted at the indicated time points (minutes) and analyzed by SDS-PAGE.

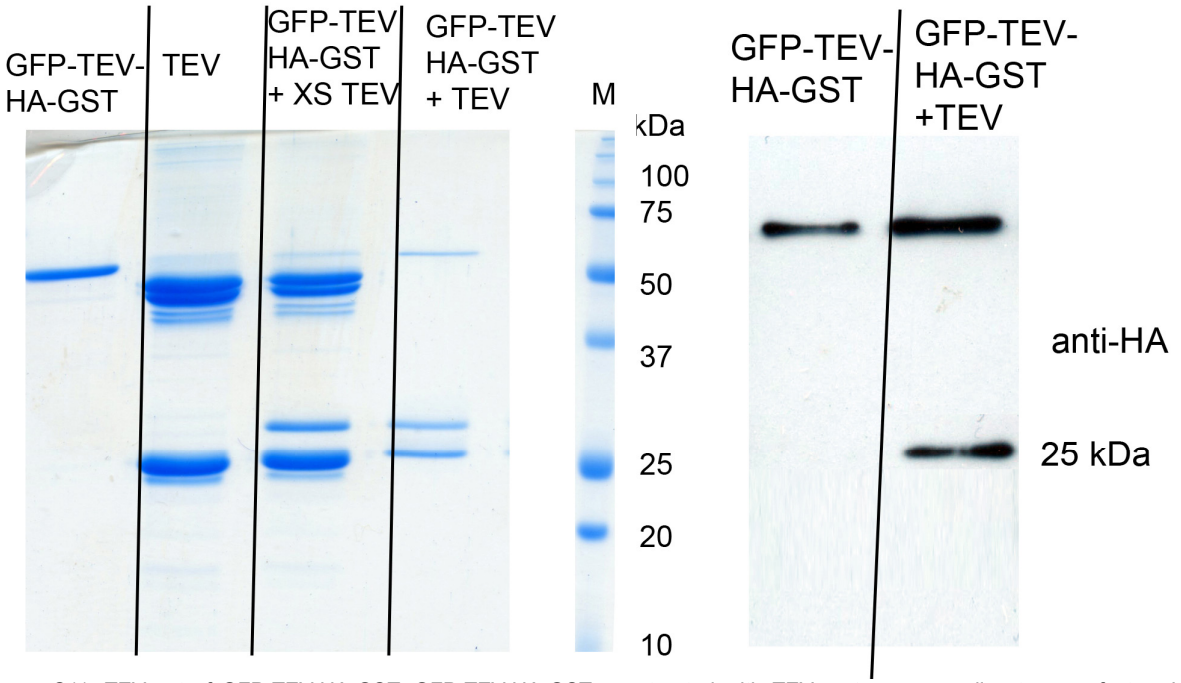

Figure S11. TEV cut of GFP-TEV-HA-GST. GFP-TEV-HA-GST was treated with TEV protease according to manufacturer's instructions. Reaction was analyzed by SDS PAGE (left) and anti-HA blot.

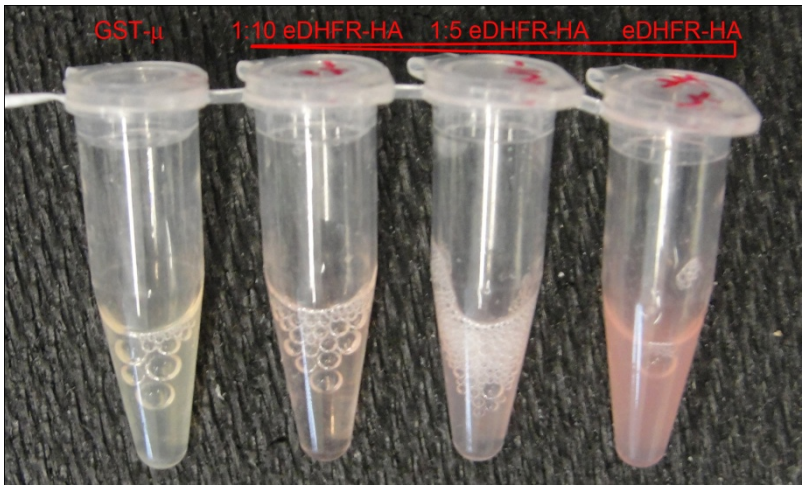

Figure S12. *No quinone is formed in E. coli lysates over expressing human GST-μ whereas even in a ten-fold less concentrated lysate expressing DHFR-HA pink is observed*. m-tyrosinase (0.15 mg/ml) and Besthorn's reagent (30μM) was added to a BL21 DE3 *E. coli* lysate over expressing human GST-μ (30 mg/ml) and the reaction was left for 3 hours (left). Second left to right increasing concentrations of DHFR-HA over expressing BL21 DE3 *E. coli* lysates [1/10 of left; 1/5 left; same concentration (by Bradford analysis) as GST-μ sample].

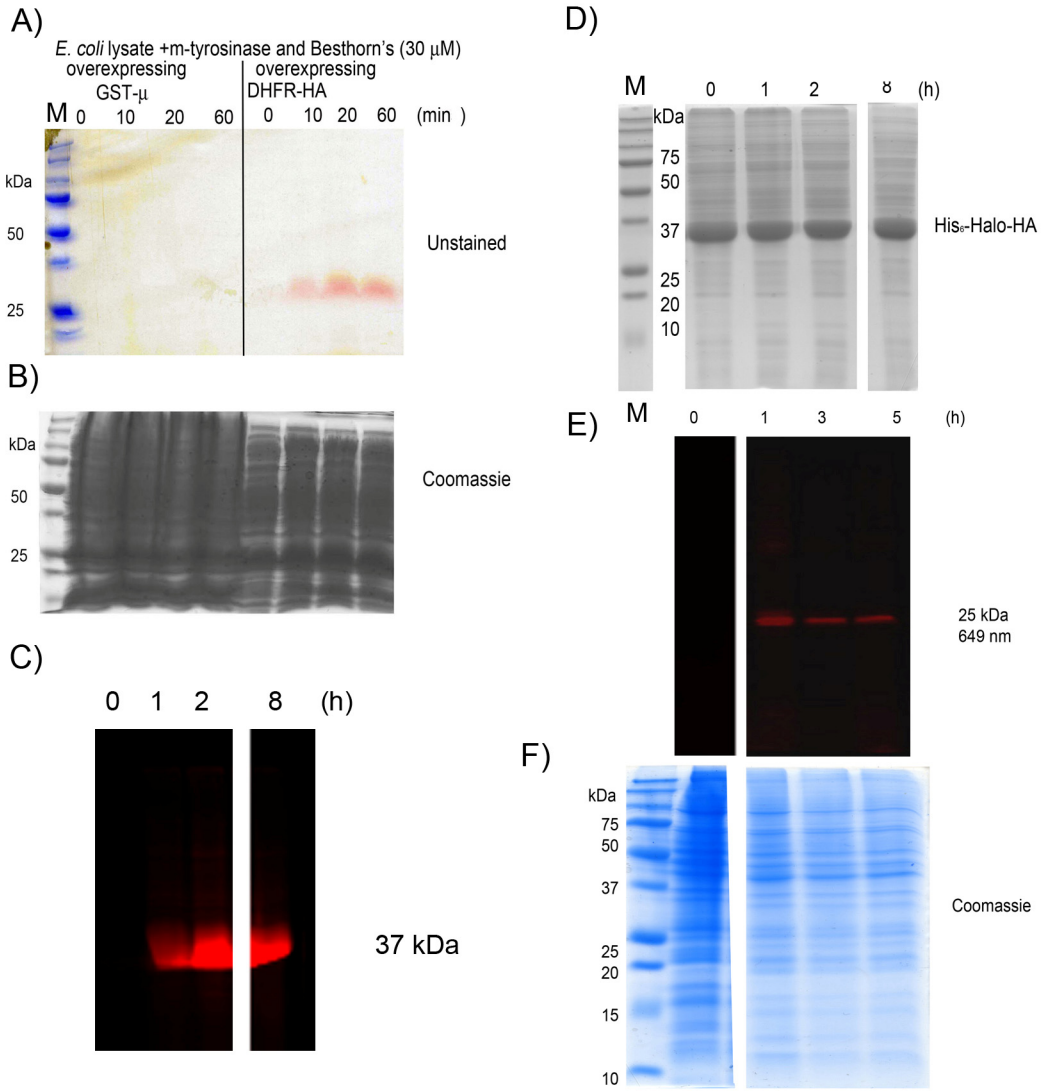

Figure S13. A) Main text fig 7 A) in color. [m-tyrosinase] = 0.15 mg/ml. B) Coomassie staining of A). C) In gel fluorescence (Ex 650nm; Em 670 nm) imaging of an E. coli lysate overexpressing Halo-HA treated with Cy5-hydrazide and m-tyrosinase (0.15 mg/ml). D) Coomassie staining of gel in C). E) In gel fluorescence imaging of a lysate (optimized to 1 mg/mL) of human embryonic kidney 293T cells transiently (Mirus® 2020 as per manufacturer's instructions) transfected with eDHFR-HA (under a CMV promoter) for 24 hours was treated with mTYR (0.15 mg/ml) and Cy5-hydrazide (5 μM) (Upper: Ex 650 nm; Em 670 nm. F) Coomassie staining).

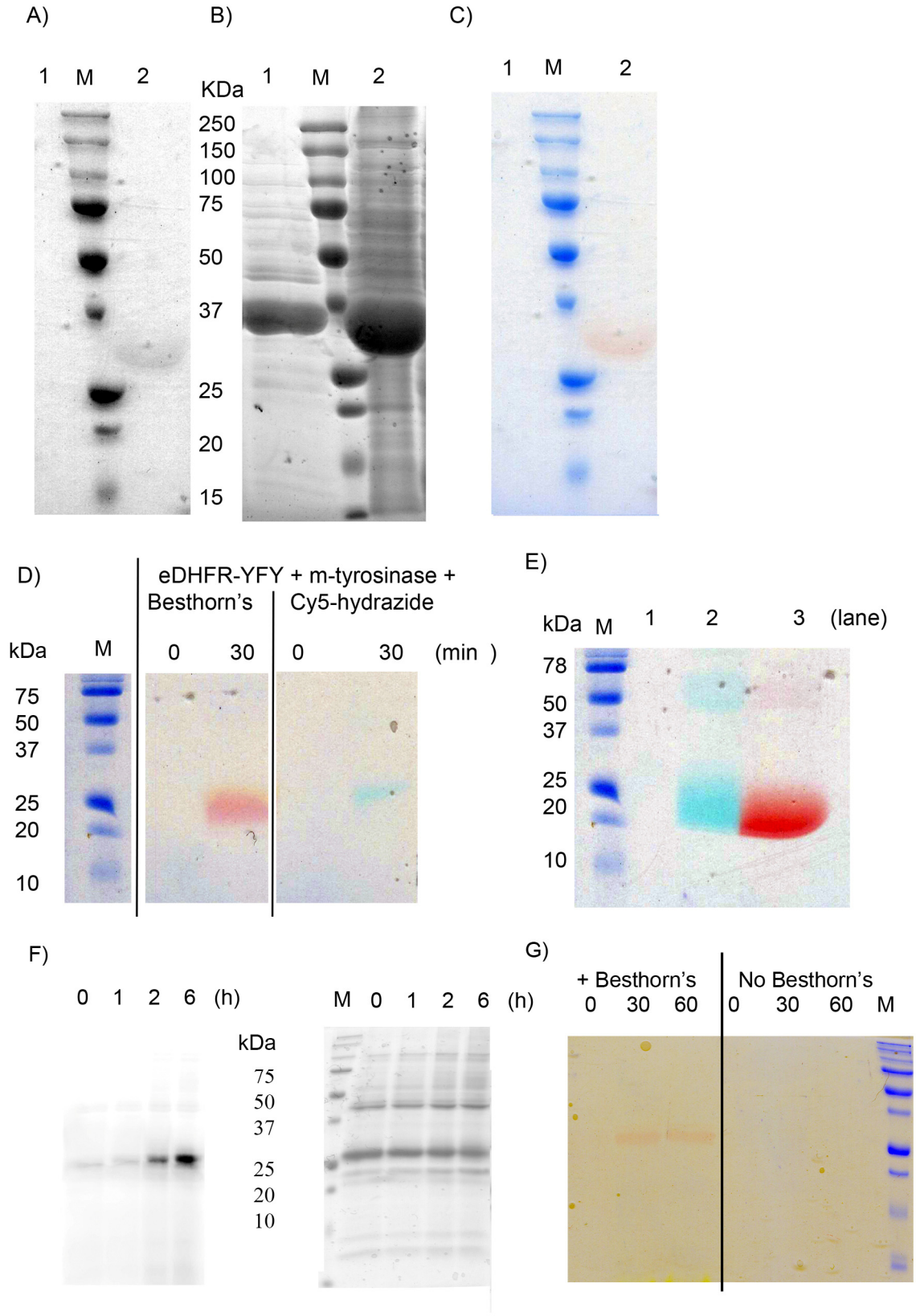

Figure S14. *An E. coli lysate over expressing Halo-HA was treated with Besthorn's reagent and after 12 hours, the reaction was analyzed by SDS-page*. A) unstained gel: **1** purified Halo-HA; **2** Halo-HA overexpressing *E. coli* lysate (30 mg/ml) treated with Besthorn's reagent (30 μM) and mTYR (0.5 mg/ml) for 12 hours. B) as in A) but stained with Coomassie. C) A) in color. D) Main text figure 5 A) in color. E) GST-α1-HA (5 mg/ml) was treated with (lane 1) nothing; (lane 2) m-tyrosinase (0.15 mg/ml) and Cy5-hydrazide (30 μM); (lane 3) Besthorn's reagent (30 μM). The samples were analyzed by SDS-Page and unstained (note in this

instance some aggregation occurred, which was also labeled). F) (left) GST-α1-HA (0.7 mg/ml) was treated with m-tyrosinase (0.15 mg/ml) and Cy5-hydrazide (100 μM) and aliquots were removed, run on SDS-PAGE and visualized using a fluorescence scanner (less aggregation occurred); (right) Coomassie stained gel. G) Figure 3A) in color.

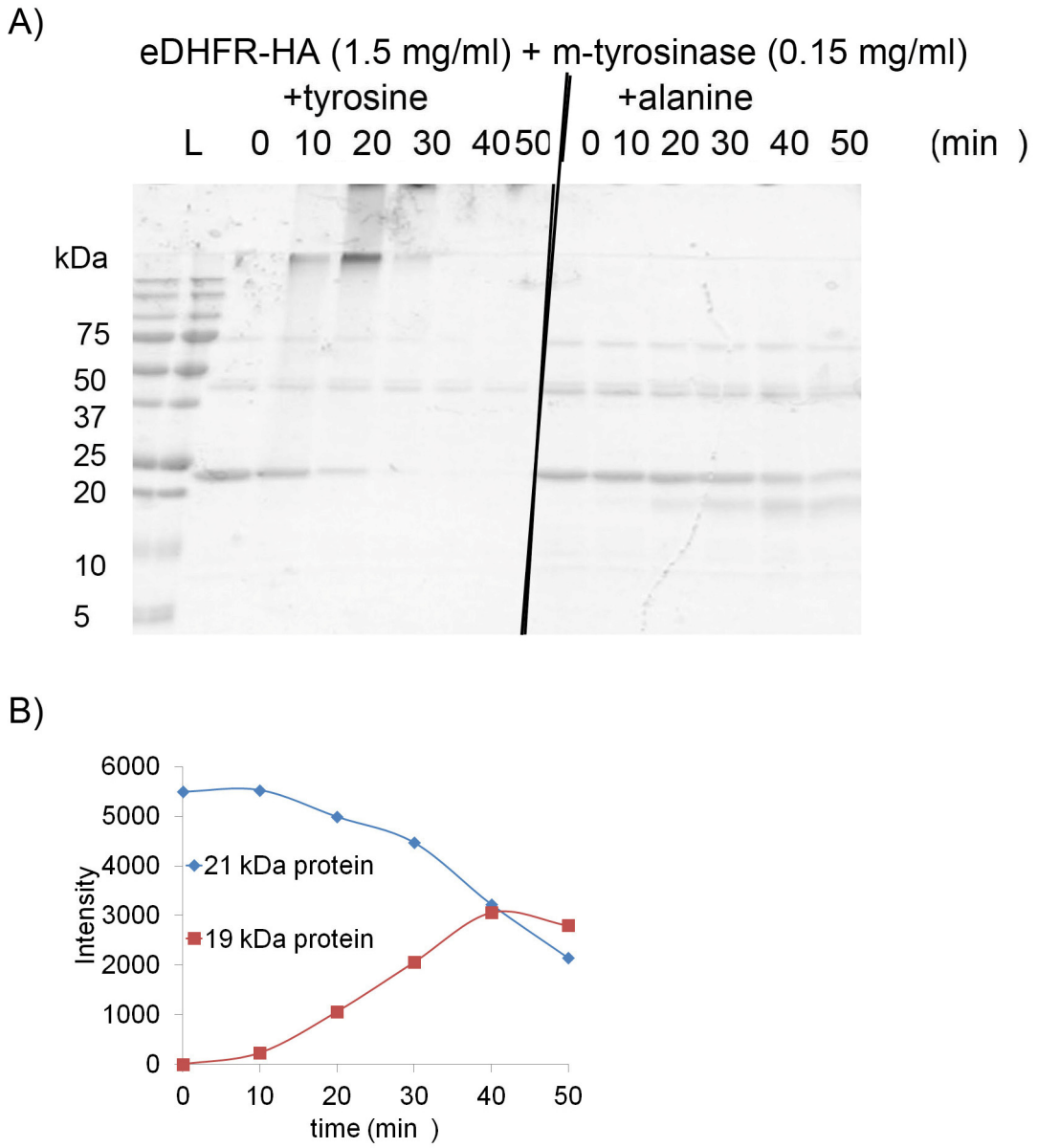

Figure S15. *eDHFR-HA can be incorporated into melanin polymers in the presence of m-tyrosinase*. A) eDHFR-HA (1.5 mg/ml) in the presence of 1 mM either tyrosine or alanine was added to m-tyrosinase (0.15 mg/ml) and aliquots were removed as a function of time and analyzed by SDS-page. B) Quantitation of the cleavage of eDHFR-HA in the presence of alanine.

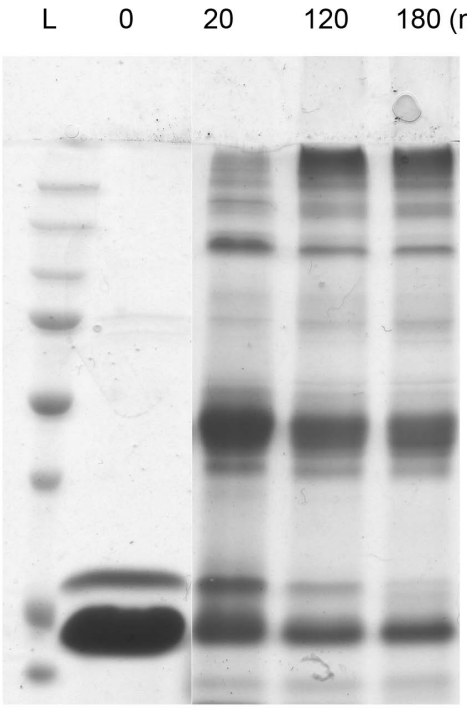

Figure S16. *Representative SDS-PAGE gel of eDHFR-$(GY)_5$ (5 mg/ml) treated with m-tyrosinase (1.5 mg/ml) from fig 8.*

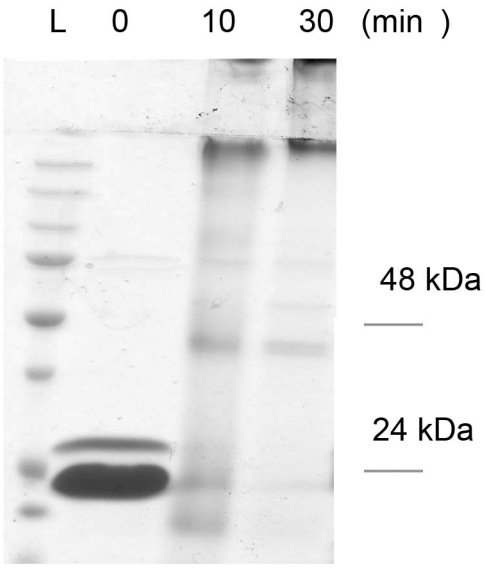

Figure S17. *eDHFR-$(GY)_5$ (1.5 mg/ml) treated with m-tyrosinase (0.15 mg/ml) with 10mM DOPA shows complete aggregation in less than 10 minutes.*

Supplemental method. *LCMS analysis of the small molecule content of the cleavage reaction by LCMS*. eDHFR-HA (1.5 mg/ml) (prepared by desalting as usual described in the main text) was treated with m-tyrosinase (0.15 mg/ml) on a 600 μL scale. After 40 minut6es, the reaction was loaded into a YM-10 microcon (Millipore) and the mixture was spin concentrated (residual volume was approx 50 μL) as per the manufacturer's instructions at 4^{o}C. The flow through was immediately frozen and lyophilized overnight. This was redissolved in water and analyzed by LCMS. An identical experiment was carried out using **YFY** with identical results.