SUPPLEMENTARY ONLINE MATERIAL

Results

The enzymatically active portion of mouse tyrosinase, involving residues 1-470, is buried within melanosome and separated by a single-pass trans-membrane segment (residues 474-496) from the C-terminus (residues 498-529) which is exposed to cytoplasm (UniProtKB: http://www.uniprot.org/uniprot/P14679). The structural alignment of *Streptomyces Castaneoglobisporus* tyrosinase, plant catechol oxidase, and octopus hemocyanin was obtained using the UCSF Chimera and shown in Fig. 1S. The structural alignment suggests that the consensus sequence matches the mouse *Tyr* sequence as shown in Fig. 2S. A Simple Modular Architecture Research Tool (SMART) (http://smart.embl-heidelberg.de/) analysis recognizes a tyrosinase domain in positions 170-403 with an E-value=$1.3E^{-58}$, suggesting that the plant catechol oxidase sequence matches residues 77-453 with an even better score, E-value=$2.0E^{-61}$. As suggested by previous models, the core of the tyrosinase catalytic domain (residues 170-403) modeled by the structure of the four-helix bundle, maintains the dinuclear copper binding center (*42*, *45*). Two sets of three histidine residues are located in conserved positions to maintain a dinuclear copper center (CuA and CuB) in bacterial tyrosinase, plant catechol oxidase, and hemocyanin, as shown in Fig. 1S and 2S. In the mouse tyrosinase, CuA and CuB copper ions are coordinated by histidine residues thorough Nε atoms in positions H180, H202, and H211, and in positions H363, H367, and H390, respectively, similar to that described elsewhere (*40*).

In our analysis, *Streptomyces Casneoglobisporus* and plant catechol oxidase share the same bicopper active site characterized by six critical histidines in the enzyme's active site (*40*). Although mouse tyrosinase demonstrates an identity (similarity) to plant catechol oxidase of

20% (35%), slightly lower than that of *Streptomyces Casneoglobisporus* tyrosinase at 23% (37%), in regions containing the tyrosinase catalytic domain, its homology covers a broader sequence range at the N-terminus. This is particularly helpful in predicting the structure of the N-terminal EGF/laminin-like motif (residues 60-90 and higher). So, whereas *Streptomyces Casneoglobisporus* tyrosinase is a slightly better model of the area surrounding the bicopper active site, plant catechol oxidase is a better model of the EGF/laminin-like motif.

Methods: Molecular Modeling.

The minimization procedure and molecular dynamics (MD) simulations were performed with the Impact module of the Maestro program package (version 8.0.308, Schrodinger, Inc., New York, NY, USA). Hydrogen atoms were added to the structure of mouse tyrosinase (Fig. 3S) and the structure was regularized by an energy minimization procedure using the OPLS_2005 potentials, the 12 Å non-bonded cut-offs, the distance-dependent dielectric constant and 100 steepest descent steps of minimization followed by 200 steps of conjugated gradient in the presence of 7135 SPC water molecules on the final step. MD trajectories were calculated in a periodic rectangular box of explicit SPC water molecules. The structures of the enzyme and EGF-like domains were equilibrated using the SPC water boxes for the tyrosinase enzyme domain and EGF-like motif. All bonds were constrained by the linear constraint solver algorithm. Temperature was kept constant to 298.15 K. Isotropic pressure coupling of the system and fast particle-mesh Ewald electrostatics were applied. Solvent was equilibrated by 20 ps of solute positions restrained MD (20 000 of 1 fs steps). Finally, the quality of the predicted structure was tested with the program Procheck(*67*).

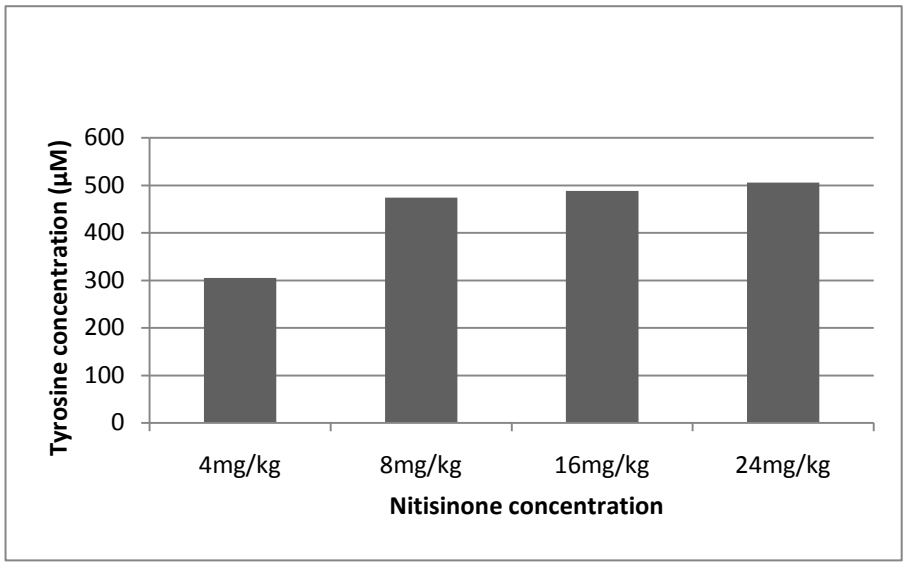

Figure S1: Dose-response of oral nitisone given qod by oral gavage to 3-4 month old C57BL/6-*Tyr*$^{c\text{-}h/c\text{-}h}$ mice. Plasma tyrosine concentrations were measured after one month.

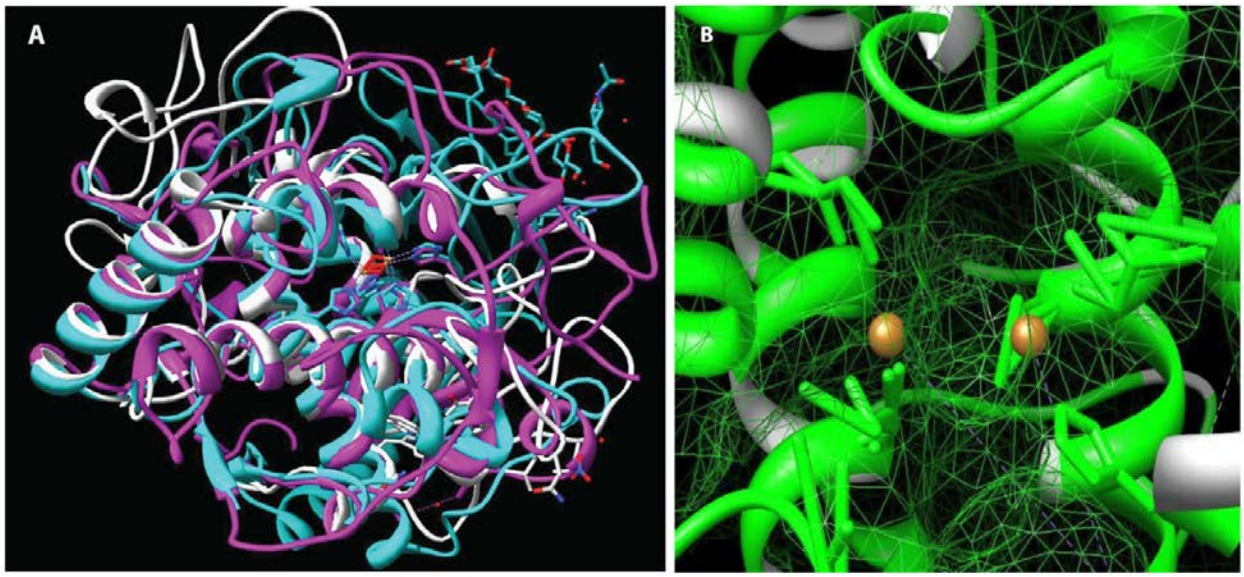

Figure S2**.** (A) Protein structure superposition of plant catechol oxidase (PDB file: 1bt3), *Octopus* hemocyanin (PDB file: 1js8) and *S.Casneoglobisporus* tyrosinase (PDB file: 1wxc). These structures are shown by magenta, cyan and light gray, respectively. Protein structures are superimposed using the MatchMaker procedure incorporated in the UCSF Chimera, build 1.4.1(*60*). The superposition of histidine side chains involved in di-copper site is shown in the center in red. (B) Close-up of the active site of mouse tyrosinase including the dicopper center (orange spheres). The area highlighted in green is the hydrophobic surface felt to form the initial binding site for tyrosine to the enzyme.

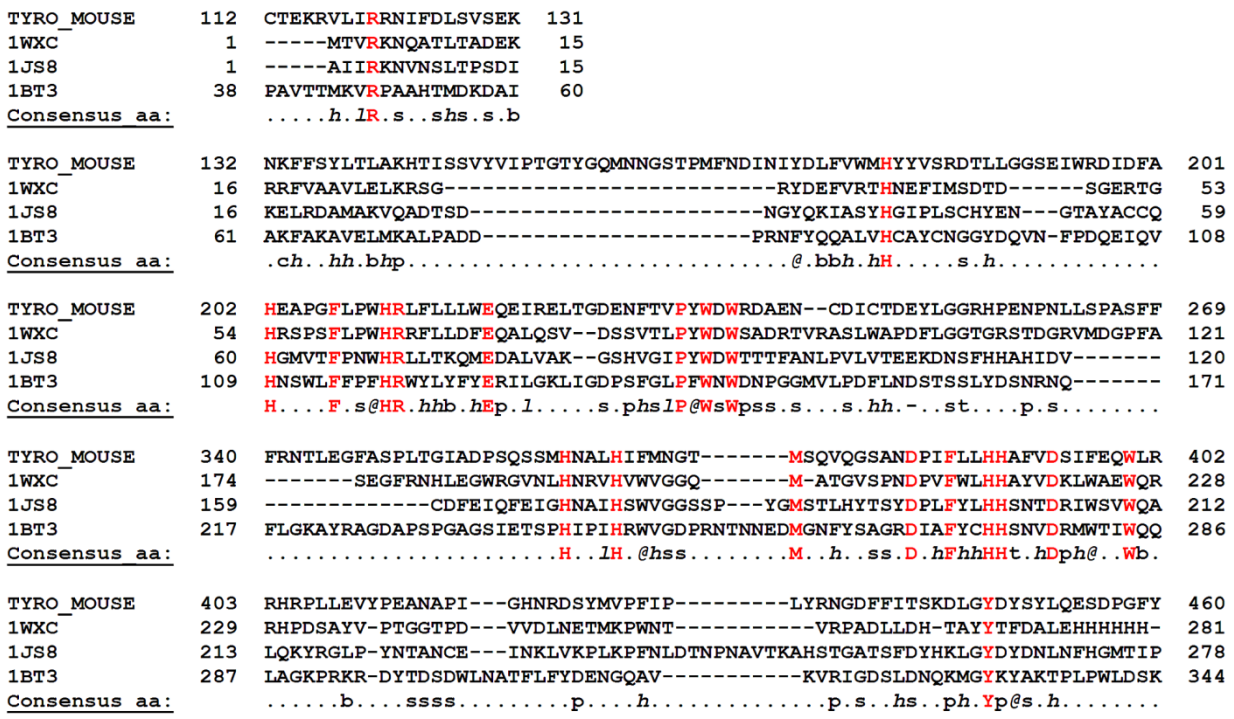

Figure S3. A fragment of multiple sequence alignment of mouse tyrosinase, plant catechol oxidase, *Octopus* hemocyanin, and *Streptomyces Casneoglobisporus* tyrosinase demonstrates a conserved pattern of amino acid residues involved in formation of the dinuclear copper center. Defined by 3D-structure superposition conserved residues are shown in red. The alignment is obtained using the protein structure superposition shown in Figure S2 of Supplementary Material and the PROMALS3D multiple alignment engine (Pei et al. 2008). The UniProtKB accession number for the mouse tyrosinase, TYRO_MOUSE, is P11344. The PDB files 1wx4, 1bt3, and 1js8 are structures of *S. Castaneoglobisporus* tyrosinase, the plant catechol oxidase, and functional unit of the octopus hemocyanin, respectively.