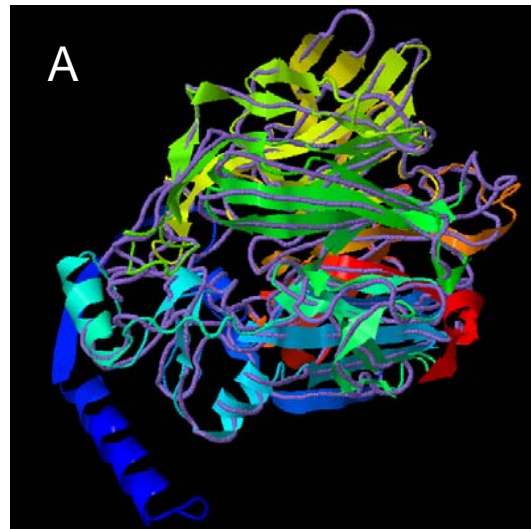


**Supplemental table S1.** Matrix of similarity between the different *Citrus clementina* CCD4-like family members based on deduced amino acid and sequences alignment (%).

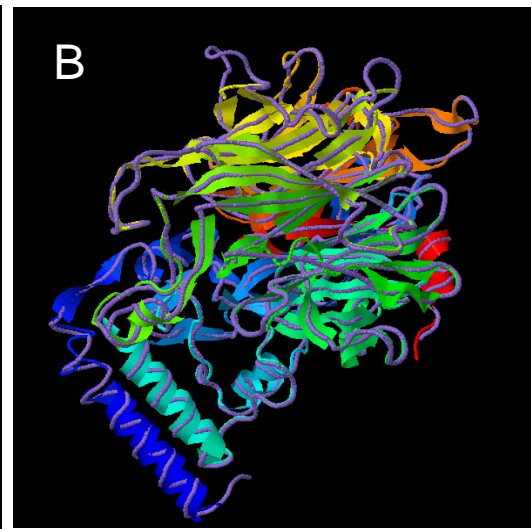
	CCD4a	CCD4b1	CCD4b2	CCD4c
CCD4a	100			
CCD4b1	48	100		
CCD4b2	48	88	100	
CCD4c	60	45	45	100
CCD4d	39	32	30	49

**Supplemental Figure S1.** Best 3D models of *Citrus* CCD4b1 by using I-TASSER online platform by using as templates the 3D structure of CCO (PBD: 2biwA) from *Synechocystis* (A) and VP14 (PBD: 3npeA) from maize (B) which are showed overlapped in purple ribbons. The C-score obtained for each model is indicated below the images. C-score is a confidence score for estimating the quality of predicted models by I-TASSER. It is calculated based on the significance of threading template alignments and the convergence parameters of the structure assembly simulations. C-score is typically in the range of [-5,2], where a C-score of higher value signifies a model with a high confidence and vice-versa.

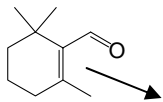
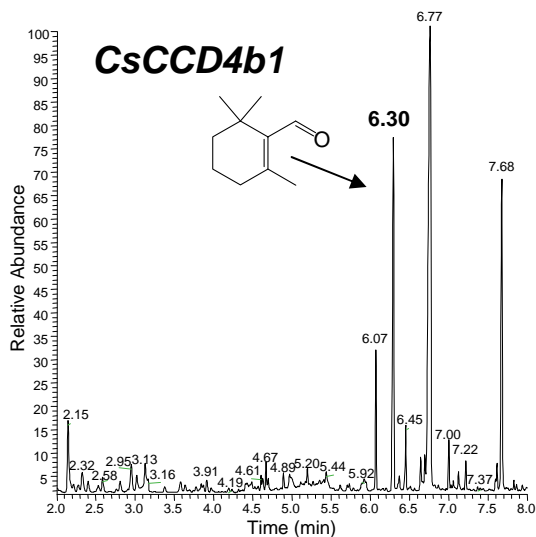
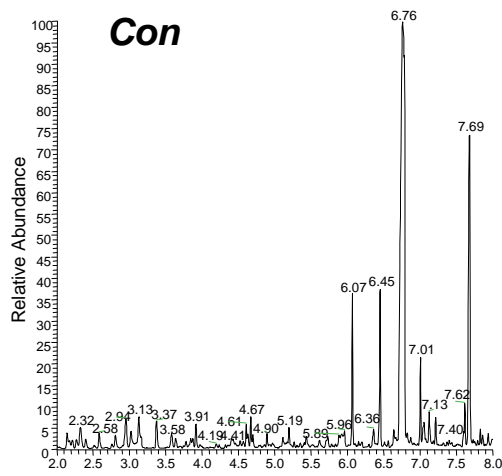
Zhang Y. (2008). I-TASSER server for protein 3D structure prediction. BMC Bioinformatics. 9: 40;. Roy A., Kucukural A. and Zhang Y.(2010). I-TASSER: a unified platform for automated protein structure and function prediction. Nature Protocols 5: 725-738.



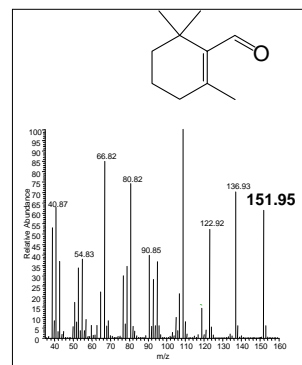
C-score 0.77



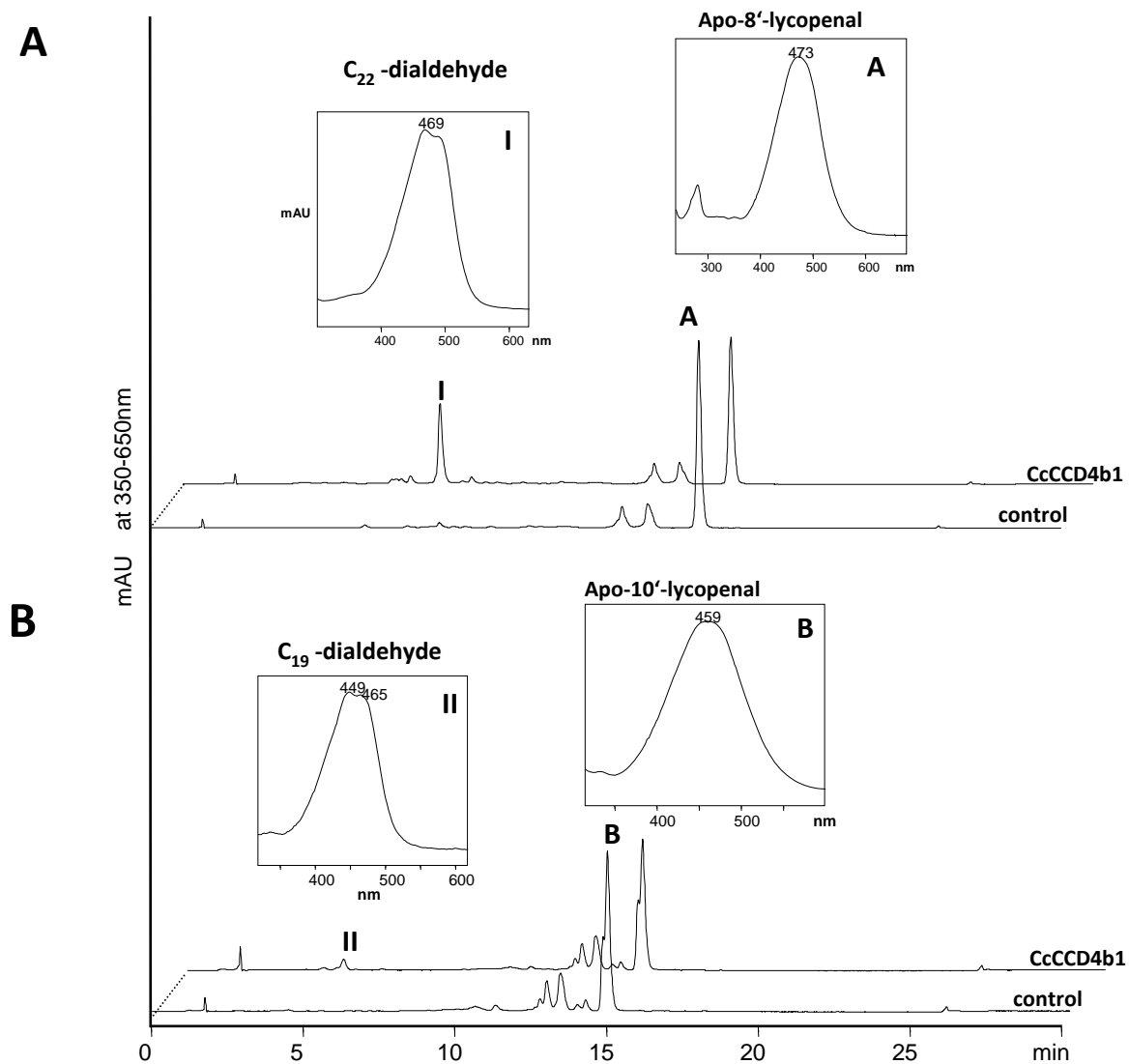
C-score 1.96



**$\beta$ -cyclocitral**



**Supplemental Figure S2.** GC-MS analysis of *in vitro* enzymatic activity of citrus CCD4b1. Gas phase of *in vitro* assays performed with  $\beta$ -carotene or  $\beta$ -cryptoxanthin and crude lysate of thioredoxin-CCD4b1 expressing *E. coli* cells (CCD4b1) and control lysate obtained from thioredoxin expressing cells (Con) was extracted using an open loop and applied to GC-MS analysis. The total ion current revealed a new compound formed by thioredoxin-CCD4b1, which eluted at min 6.30. Mass spectrum of this product identified it as  $\beta$ -cyclocitral.



**Supplemental Figure S3.** *In vitro* CCD4b1 enzymatic assays using apo-lycopenals as substrates (A, Apo-8'-lycopenal (C<sub>30</sub>); B apo-10'-lycopenal (C<sub>27</sub>)).

**Figure S4.** Relative transcript levels of *CCD4a* in the peel of Navel sweet orange, Clementine mandarin and the hybrid Fortune mandarin during fruit development and ripening.

