SCF^{D3-D14} Ubiquitin Ligase Exploits Structural Plasticity to Coordinate Strigolactone Perception and Signaling

Nitzan Shabek[1,2,‡], Fabrizio Ticchiarelli[3], Haibin Mao[1,2], Thomas R. Hinds[1], Ottoline Leyser[3] & Ning Zheng[1,2,*]

[1]*Department of Pharmacology and* [2]*Howard Hughes Medical Institute, Box 357280, University of Washington, Seattle, WA 98195.*

[3]*The Sainsbury Laboratory, University of Cambridge, Bateman Street, Cambridge, CB2 1LR, United Kingdom*

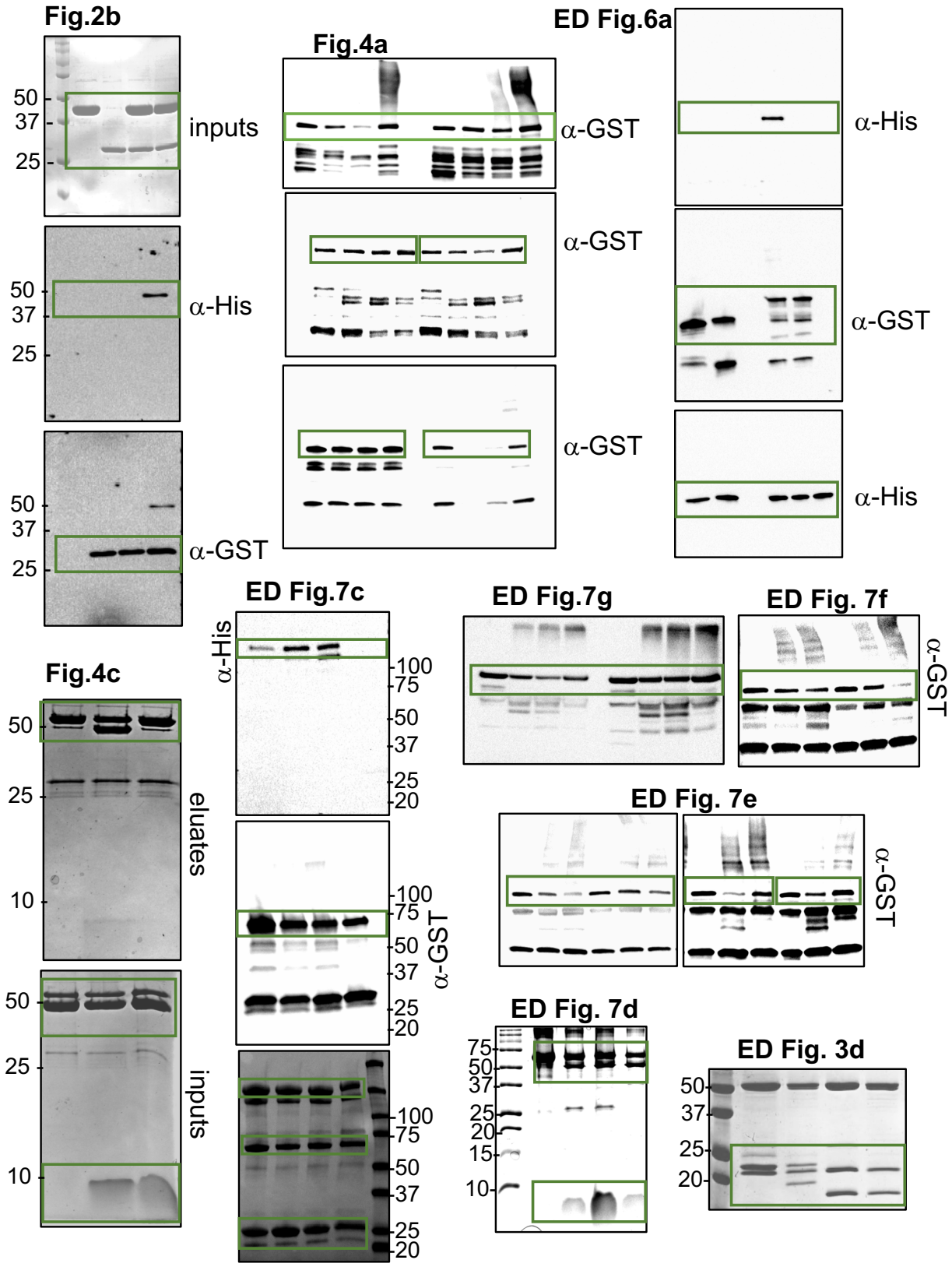
‡ *Department of Plant biology, University of California-Davis, Davis CA 95616*

**Correspondence should be addressed to: nzheng@uw.edu*

Supplementary Information

1. Uncropped SDS-PAGE gel scans: Supplementary Figure 1
2. Supplementary Discussion

Supplemental Figure 1.



Supplementary Figure 1. Gel source data. Uncropped gel scans for Figure 2b, Figure 4a, Figure 4c, Extended Data Figure 3d, Extended Data Figure 6a, Extended Data Figure 7c-g. Where indicated, size marker represents molecular weight in kDa. Green box indicates gel region used for figures.

Supplementary Discussion

1. Our analysis of YLG-induced assembly of the $SCF^{D3\text{-}D14}$-D53-D2 complex and its stalled D14 activity suggest that the inactive CLIM-bound form of D14 stabilized by D3-ASK1 might be able to engage D53 (Extend Data Figure 7i). While we cannot rule out the possibility that CLIM-bound D14 in its closed conformation represents another signaling-competent form of the hydrolase, its binding mode to D53 is most likely to be distinct from that of D3-CTH-bound D14 in its open conformation. This is expected because D53-D14 interaction depends on SL and a mutant D14 defective in adopting the closed conformation has an intact hormone-dependent D53 binding activity[1]. Structural analysis of D53-loaded $SCF^{D3\text{-}D14}$ complexes induced by both hydrolysable and non-hydrolyzable SL mimetics will shed a light on the mechanism of action of natural hormone. Of note, mutual stabilization of CLIM-bound D14 and D3 with its retrieved CTH might also convert the $SCF^{D3\text{-}D14}$ complex into an inactivated state, which is able to present D14 for poly-ubiquitination and degradation[2,3].

2. The C-terminal residue of an LRR-type F-box protein is rarely conserved among different orthologs, unless it is critically involved in the regulation of the SCF substrate receptor function[4]. The unique pocket on D3-ASK1 that anchors the invariant D3 extreme C-terminal Asp residue hints at a regulatory site, where a putative second signaling molecule could bind and modulate the topology of D3-CTH (Extended Data Fig. 1a-c). Such a regulatory mechanism of the $SCF^{D3\text{-}D14}$ E3 would be analogous to the potentiation of SCF^{TIR1} and SCF^{COI1} by inositol polyphosphate cofactors for perceiving the phytohormones auxin and jasmonate[5,6].

Supplemetary References

1 Yao, R. *et al*. DWARF14 is a non-canonical hormone receptor for strigolactone. *Nature* **536**, 469-473, doi:10.1038/nature19073 (2016).

2 Chevalier, F. *et al*. Strigolactone promotes degradation of DWARF14, an alpha/beta hydrolase essential for strigolactone signaling in Arabidopsis. *Plant Cell* **26**, 1134-1150, doi:10.1105/tpc.114.122903 (2014).

3 Hu, Q. *et al*. DWARF14, A Receptor Covalently Linked with the Active Form of Strigolactones, Undergoes Strigolactone-Dependent Degradation in Rice. *Front Plant Sci* **8**, 1935, doi:10.3389/fpls.2017.01935 (2017).

4 Xing, W. *et al*. SCF(FBXL3) ubiquitin ligase targets cryptochromes at their cofactor pocket. *Nature* **496**, 64-68.

5 Tan, X. *et al*. Mechanism of auxin perception by the TIR1 ubiquitin ligase. *Nature* **446**, 640-645, doi:10.1038/nature05731 (2007).

6 Sheard, L. B. *et al*. Jasmonate perception by inositol-phosphate-potentiated COI1-JAZ co-receptor. *Nature* **468**, 400-405, doi:10.1038/nature09430 (2010).