Supplementary Information

Endomembrane Protein Trafficking Regulated by a *Tv*CyP2 Cyclophilin in the Protozoan Parasite, *Trichomonas vaginalis*

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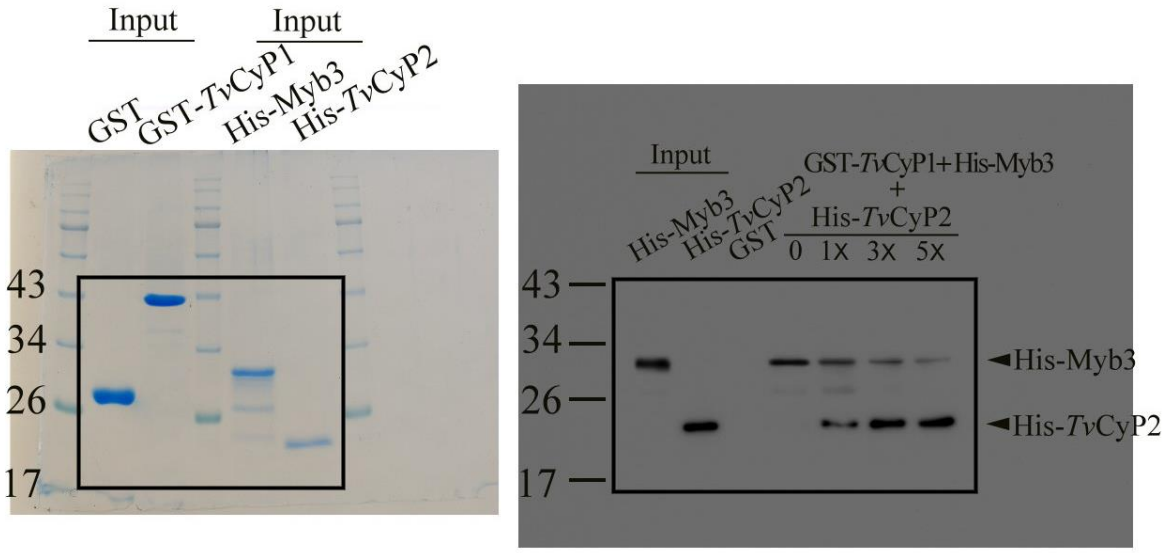
Supplementary Figure 1

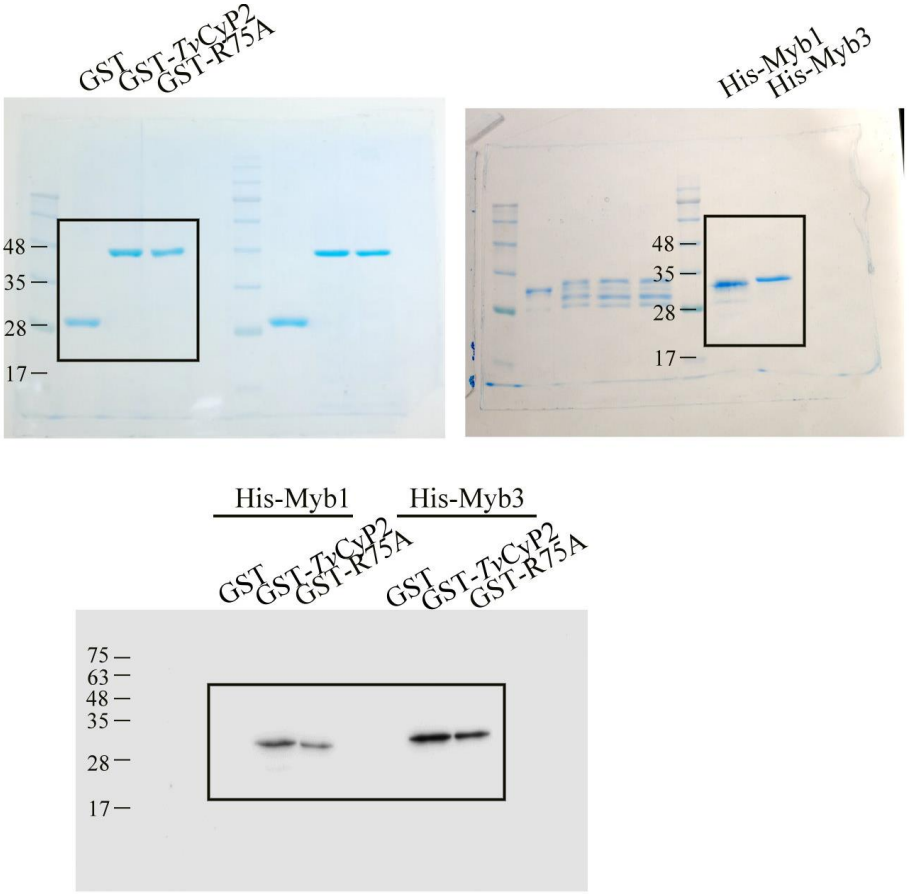
Fig. 3B



Supplementary Figure 1. Raw data from the Coomassie blue gel staining and Western blotting in Figure 3B. The boxed regions are shown in the manuscript. See figure legends in the manuscript for description.

Supplementary Figure 2

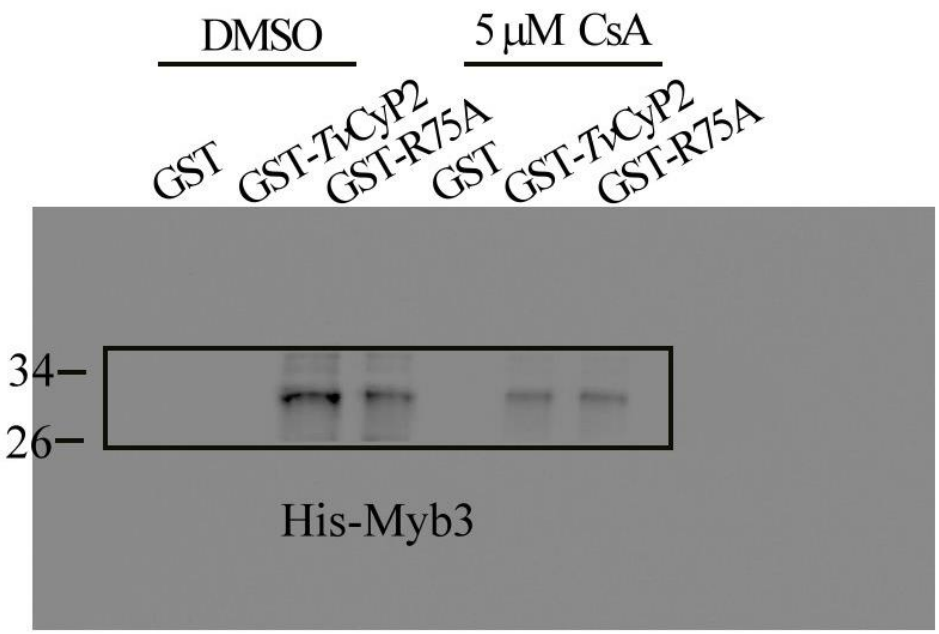
Fig. 3C



Supplementary Figure 2. Raw data from the Coomassie blue gel staining and Western blotting in Figure 3C. The boxed regions are shown in the manuscript. See figure legends in the manuscript for description.

Supplementary Figure 3

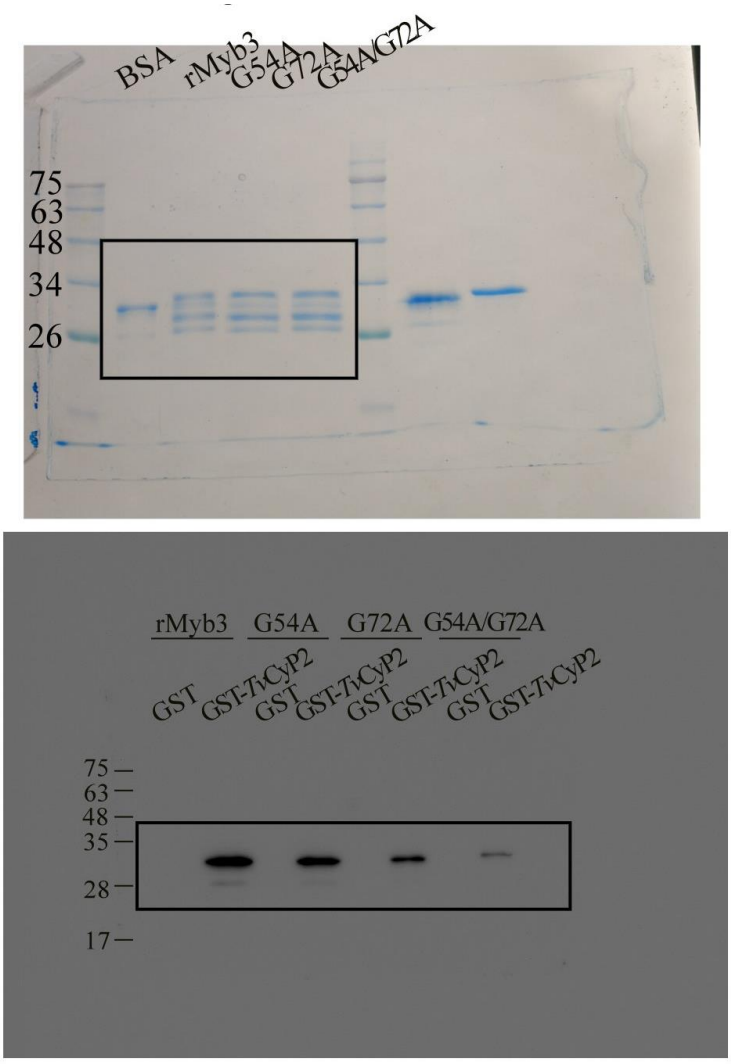
Fig. 3D



Supplementary Figure 3. Raw data from the Western blotting in Figure 3D. The original membrane blot was sliced into pieces as indicated by the molecular weight markers. Boxed regions are shown in the manuscript. See figure legends in the manuscript for description.

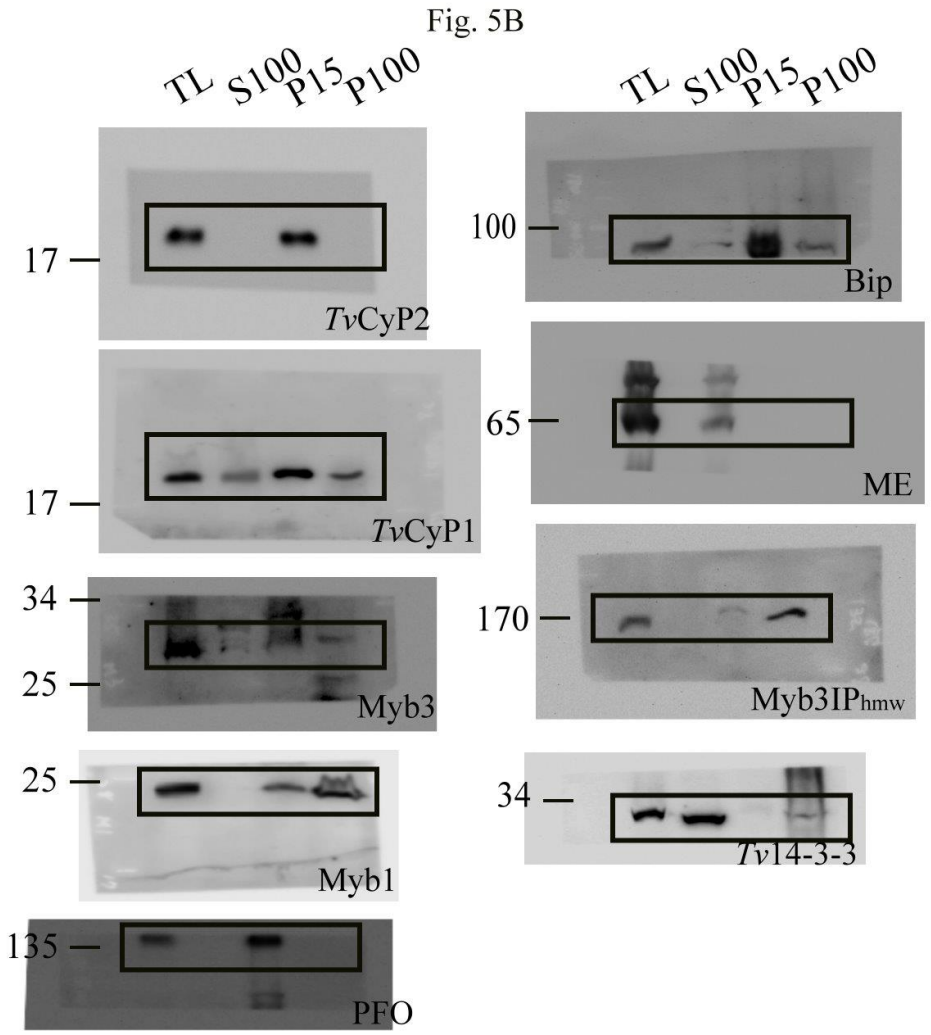
Supplementary Figure 4

Fig. 3E



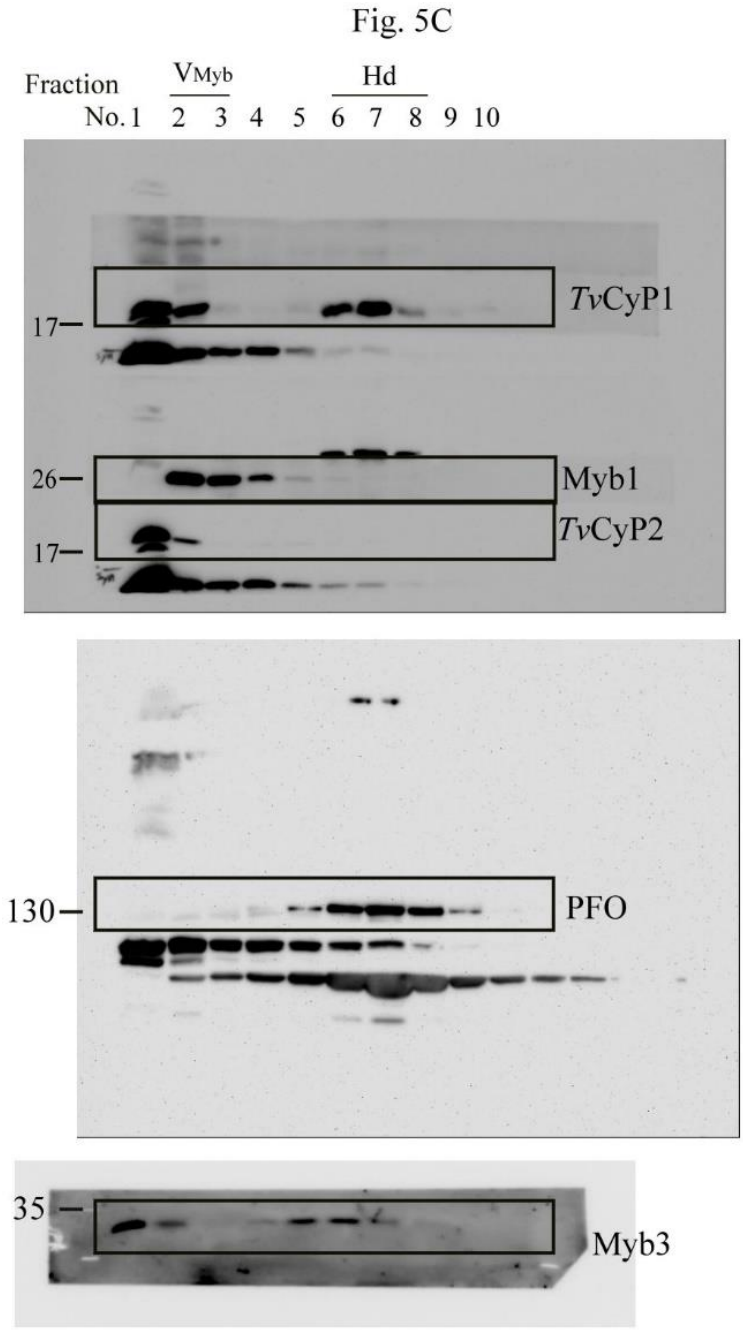
Supplementary Figure 4. Raw data from Coomassie blue gel staining and the Western blotting in Figure 3E. Boxed regions are shown in the manuscript. See figure legends in the manuscript for description.

Supplementary Figure 5



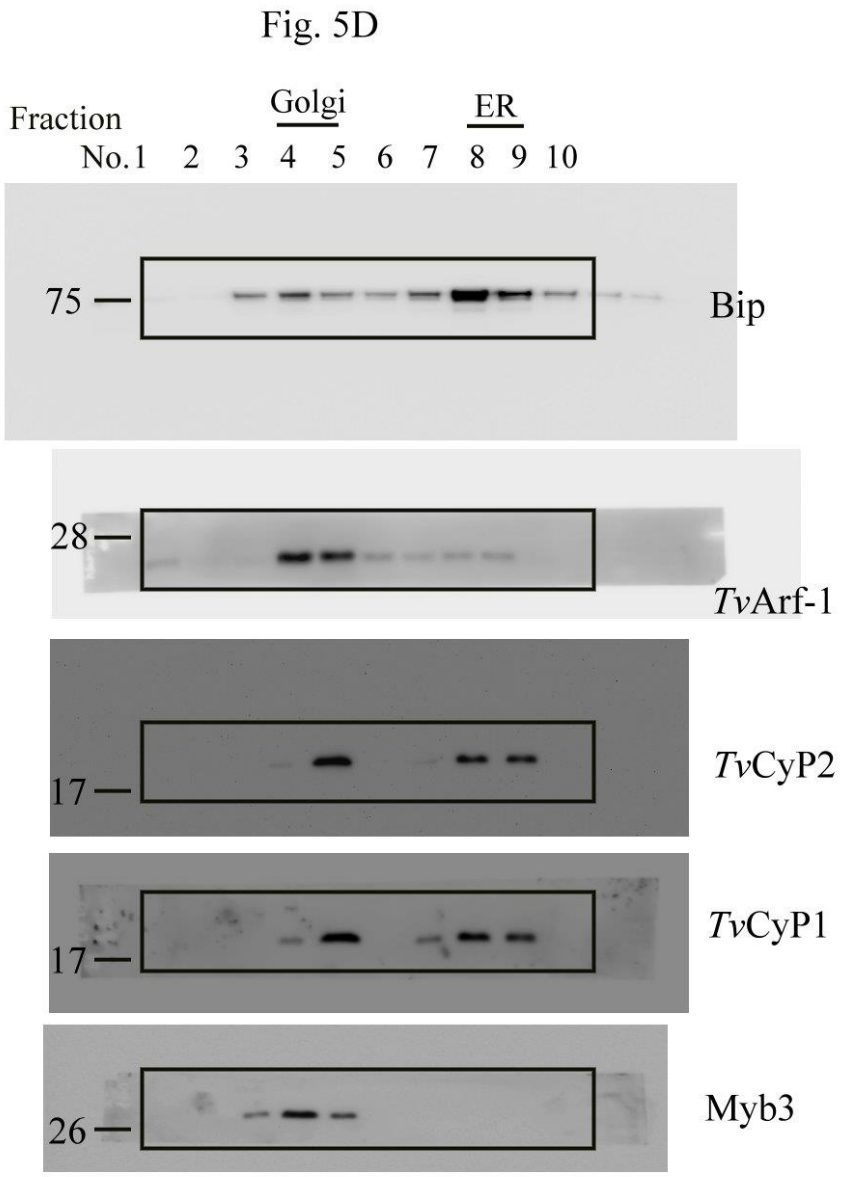
Supplementary Figure 5. Raw data from the Western blotting in Figure 5B. The original membrane blot was sliced into pieces as indicated by the molecular weight markers. Boxed regions are shown in the manuscript. See figure legends in the manuscript for description.

Supplementary Figure 6



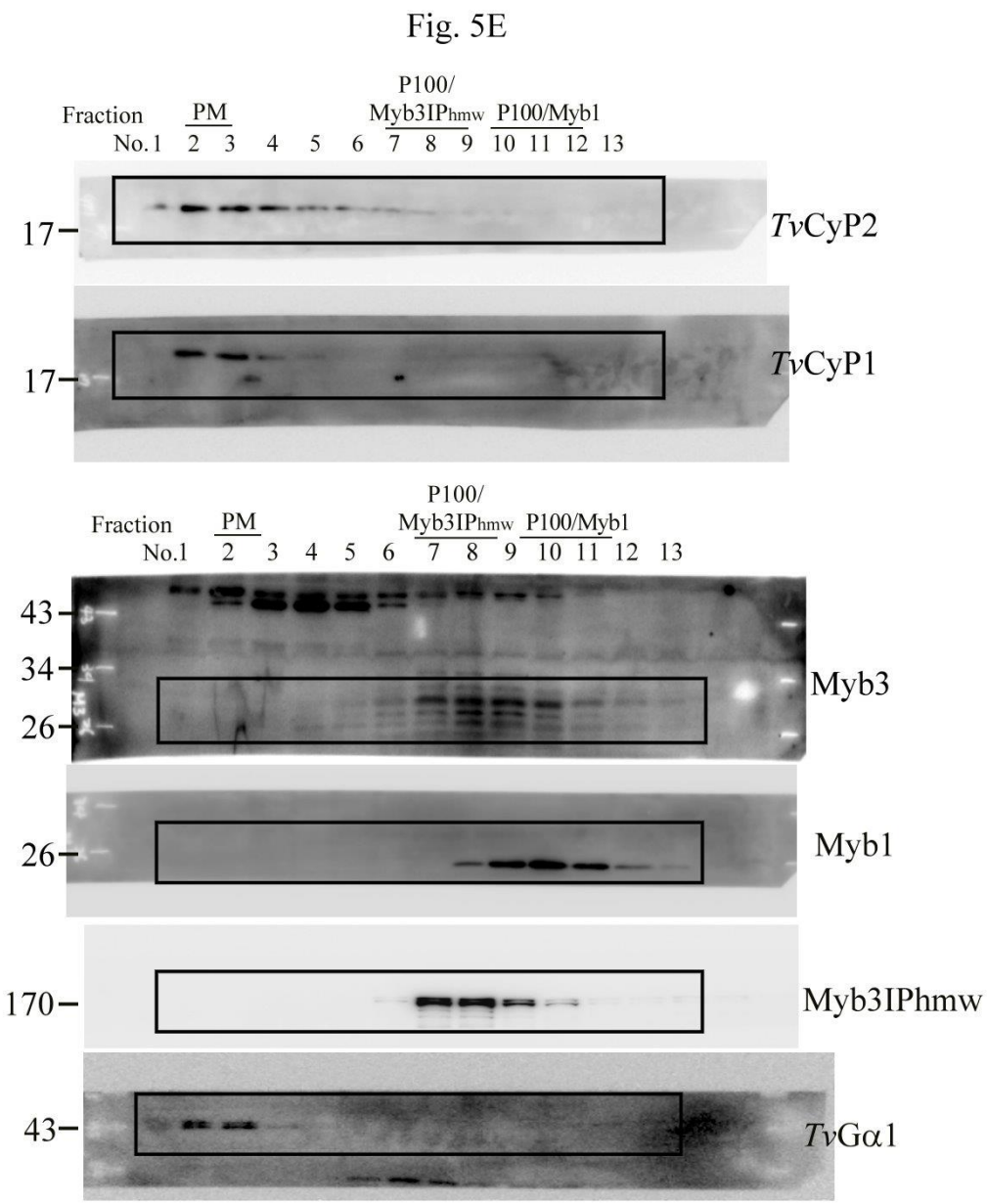
Supplementary Figure 6. Raw data from the Western blotting in Figure 5C. The original membrane blot was sliced into pieces as indicated by the molecular weight markers. Boxed regions are shown in the manuscript. See figure legends in the manuscript for description.

Supplementary Figure 7



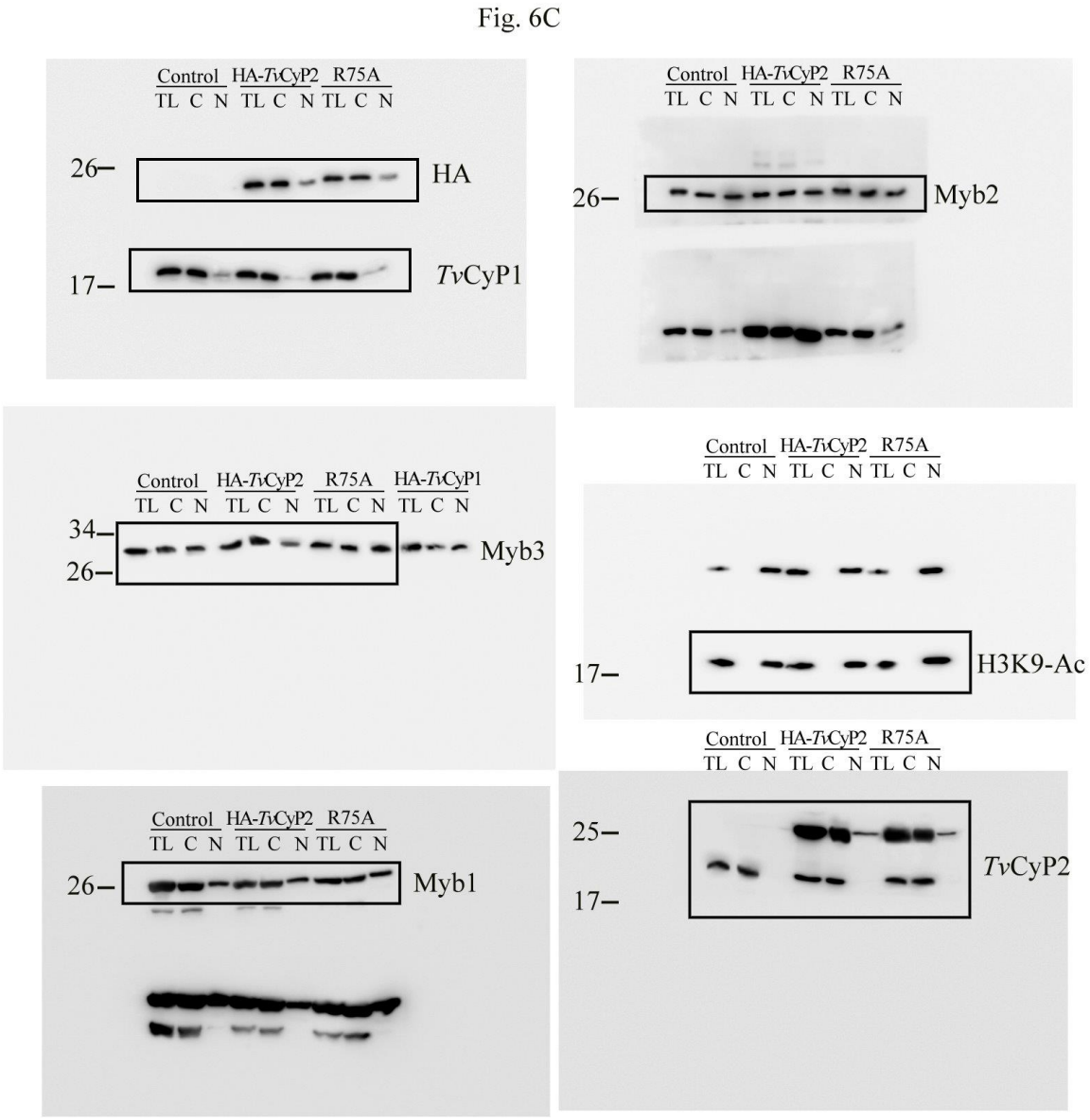
Supplementary Figure 7. Raw data from the Western blotting in Figure 5D. The original membrane blot was sliced into pieces as indicated by the molecular weight markers. Boxed regions are shown in the manuscript. See figure legends in the manuscript for description.

Supplementary Figure 8



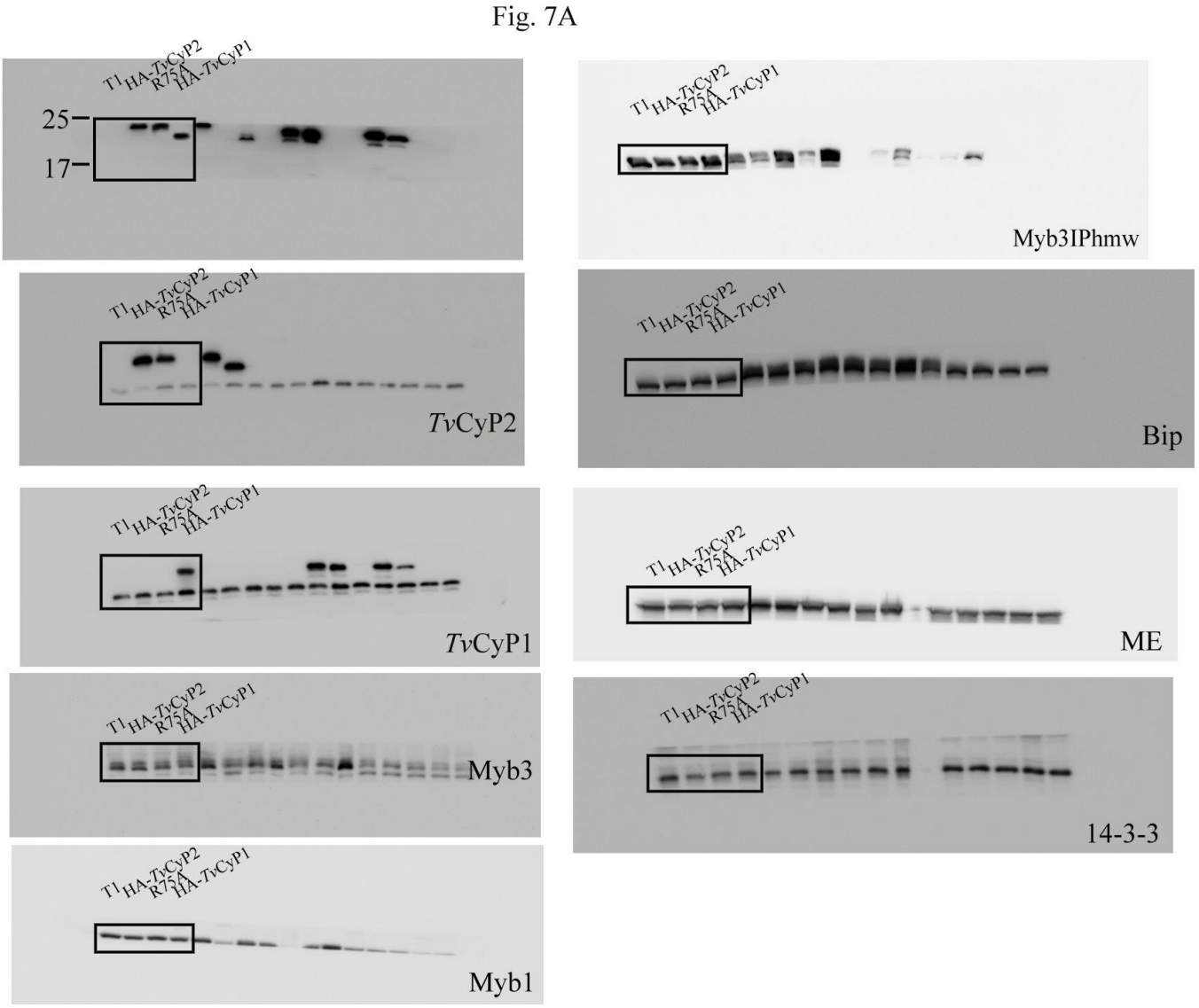
Supplementary Figure 8. Raw data from the Western blotting in Fig. 5E. The original membrane blot was sliced into pieces as indicated by the molecular weight markers. Boxed regions are shown in the manuscript. See figure legends in the manuscript for description.

Supplementary Figure 9



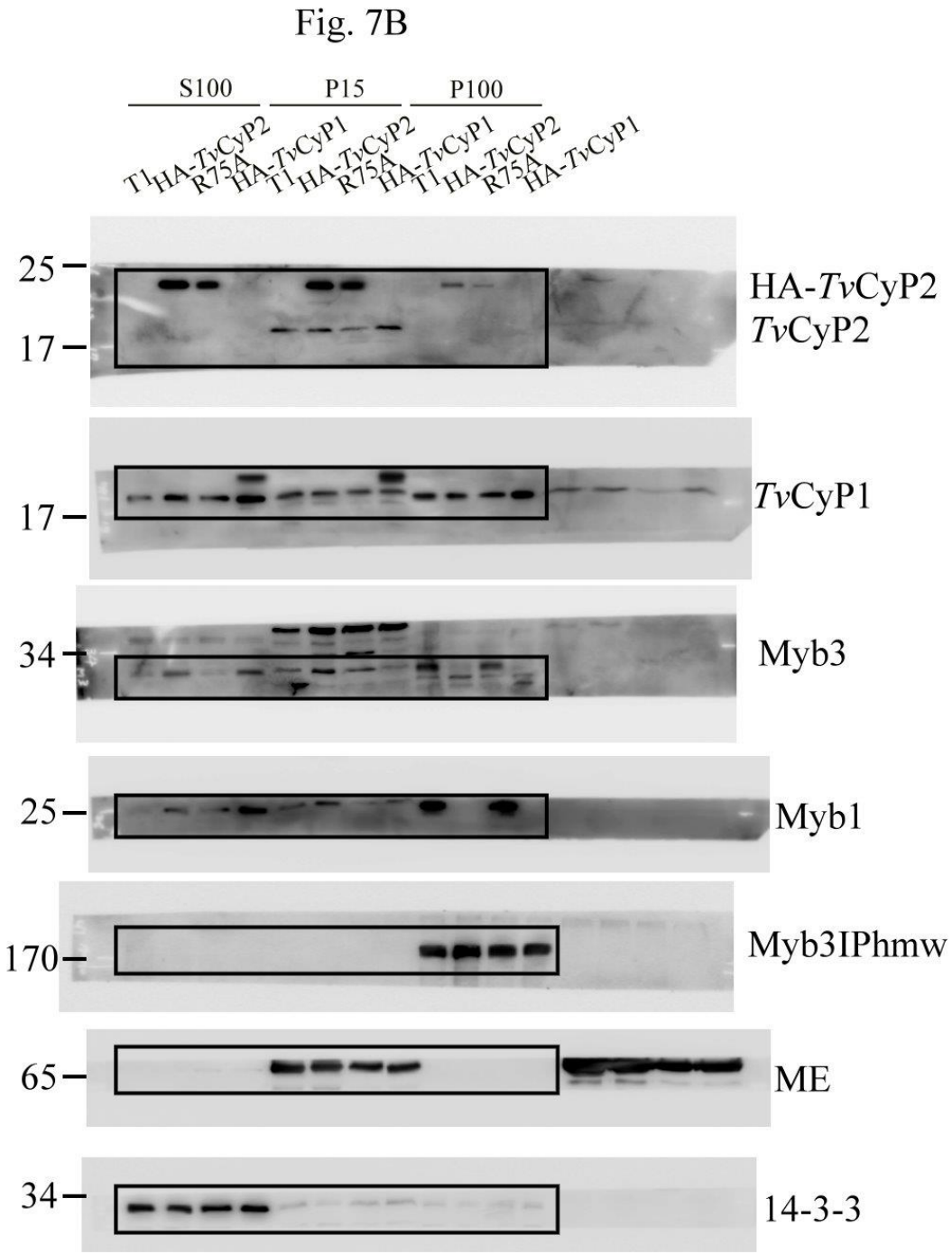
Supplementary Figure 9. Raw data from the Western blotting in Figure 6C. The original membrane blot was sliced into pieces as indicated by the molecular weight markers. Boxed regions are shown in the manuscript. See figure legends in the manuscript for description.

Supplementary Figure 10



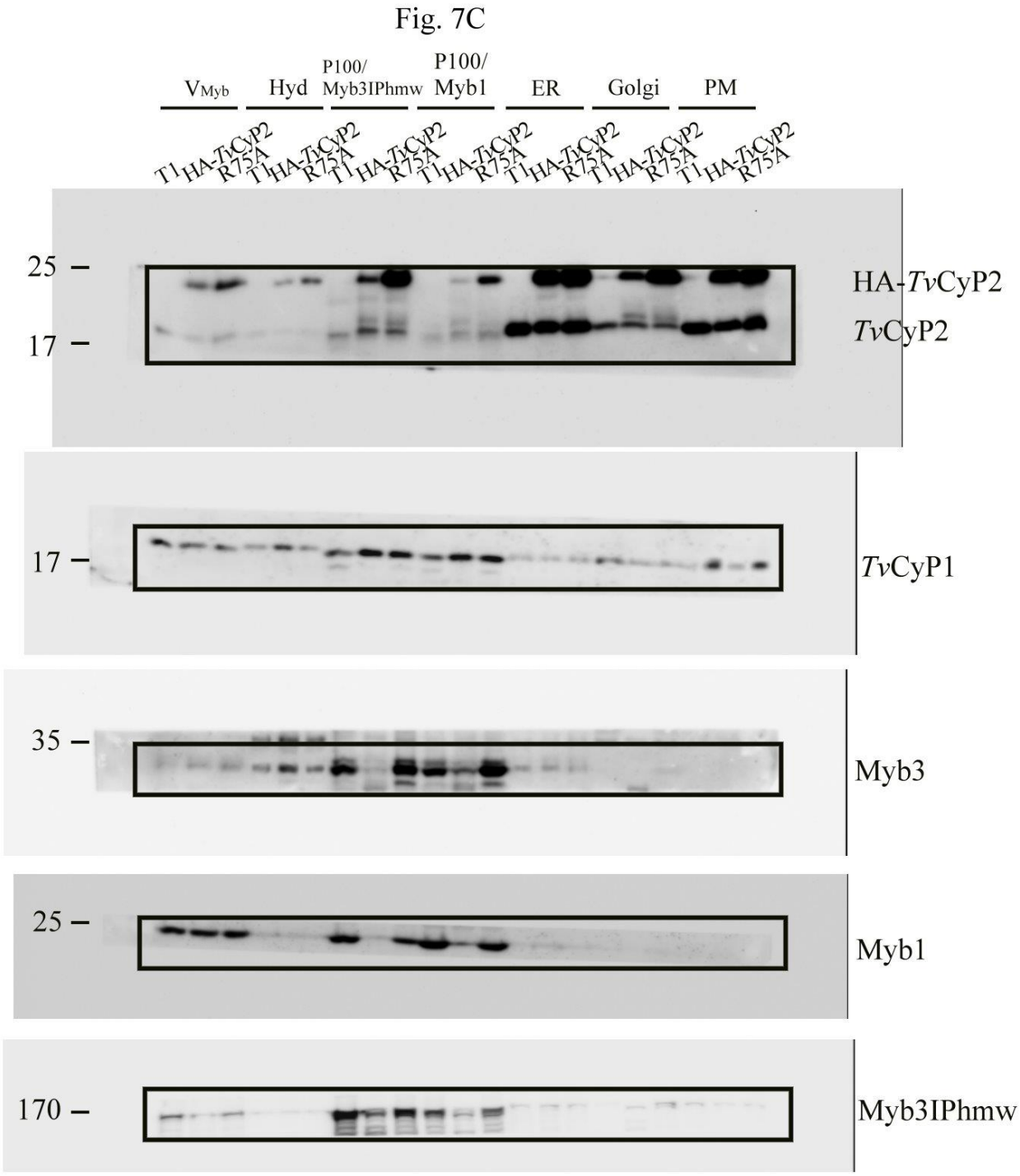
Supplementary Figure 10. Raw data from the Western blotting in Figure 7A. The original membrane blot was sliced into pieces as indicated by the molecular weight markers. Boxed regions are shown in the manuscript. See figure legends in the manuscript for description.

Supplementary Figure 11



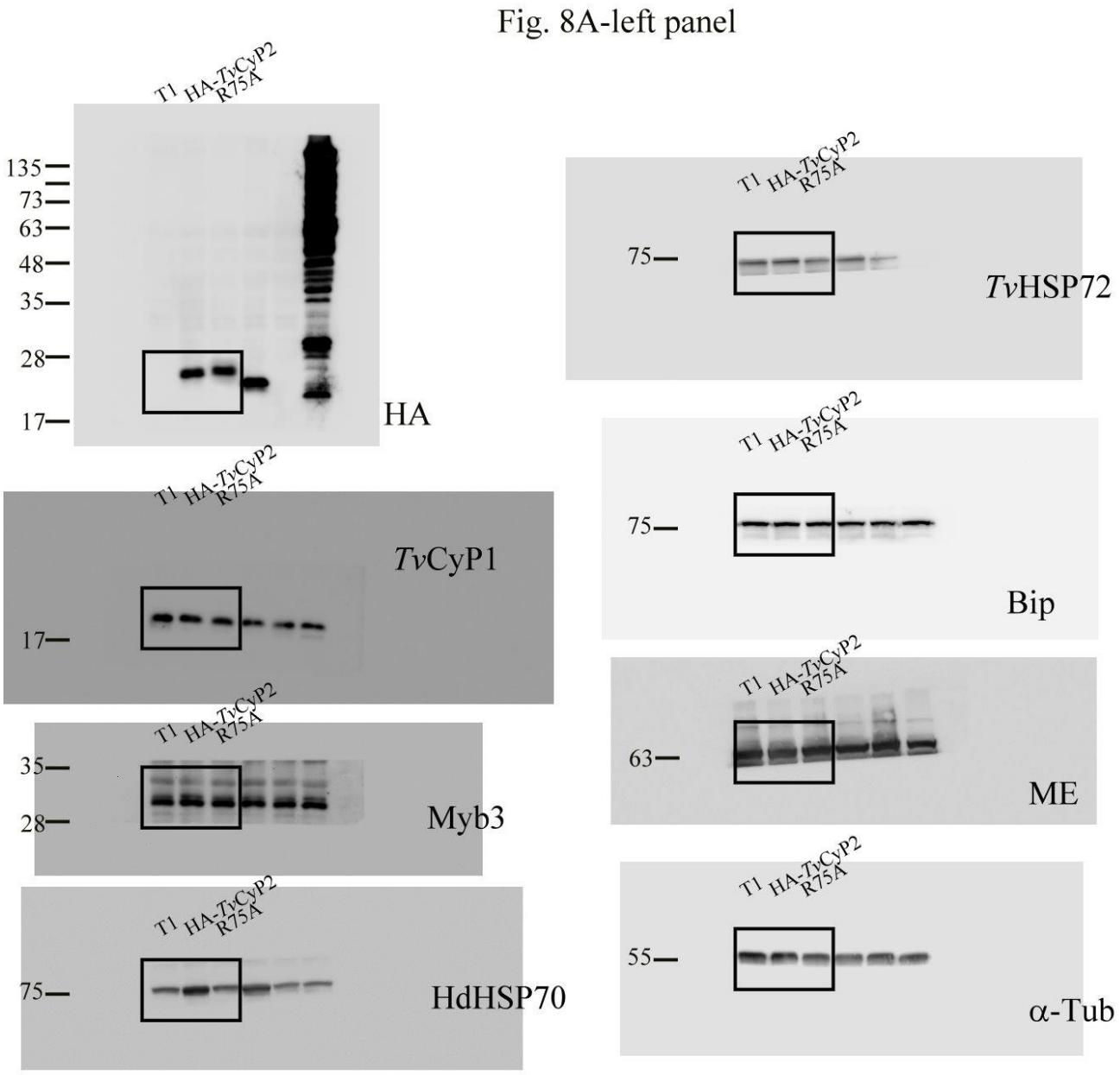
Supplementary Figure 11. Raw data from the Western blot in Figure 7B. The original membrane blot was sliced into pieces as indicated by the molecular weight markers. Boxed regions are shown in the manuscript. See figure legends in the manuscript for description.

Supplementary Figure 12



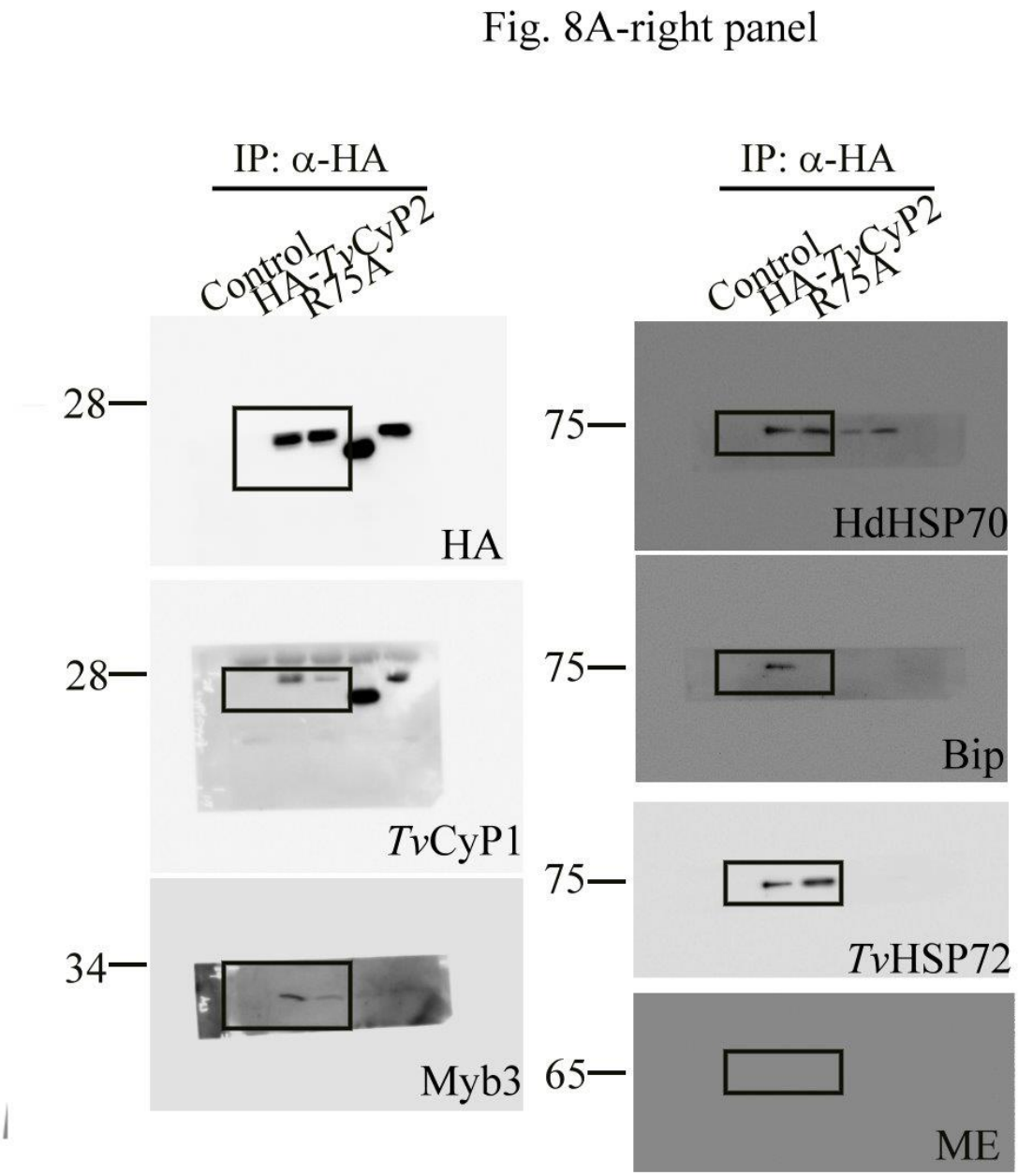
Supplementary Figure 12. Raw data from the Western blotting in Figure 7C. The original membrane blot was sliced into pieces as indicated by the molecular weight markers. Boxed regions are shown in the manuscript. See figure legends in the manuscript for description.

Supplementary Figure 13



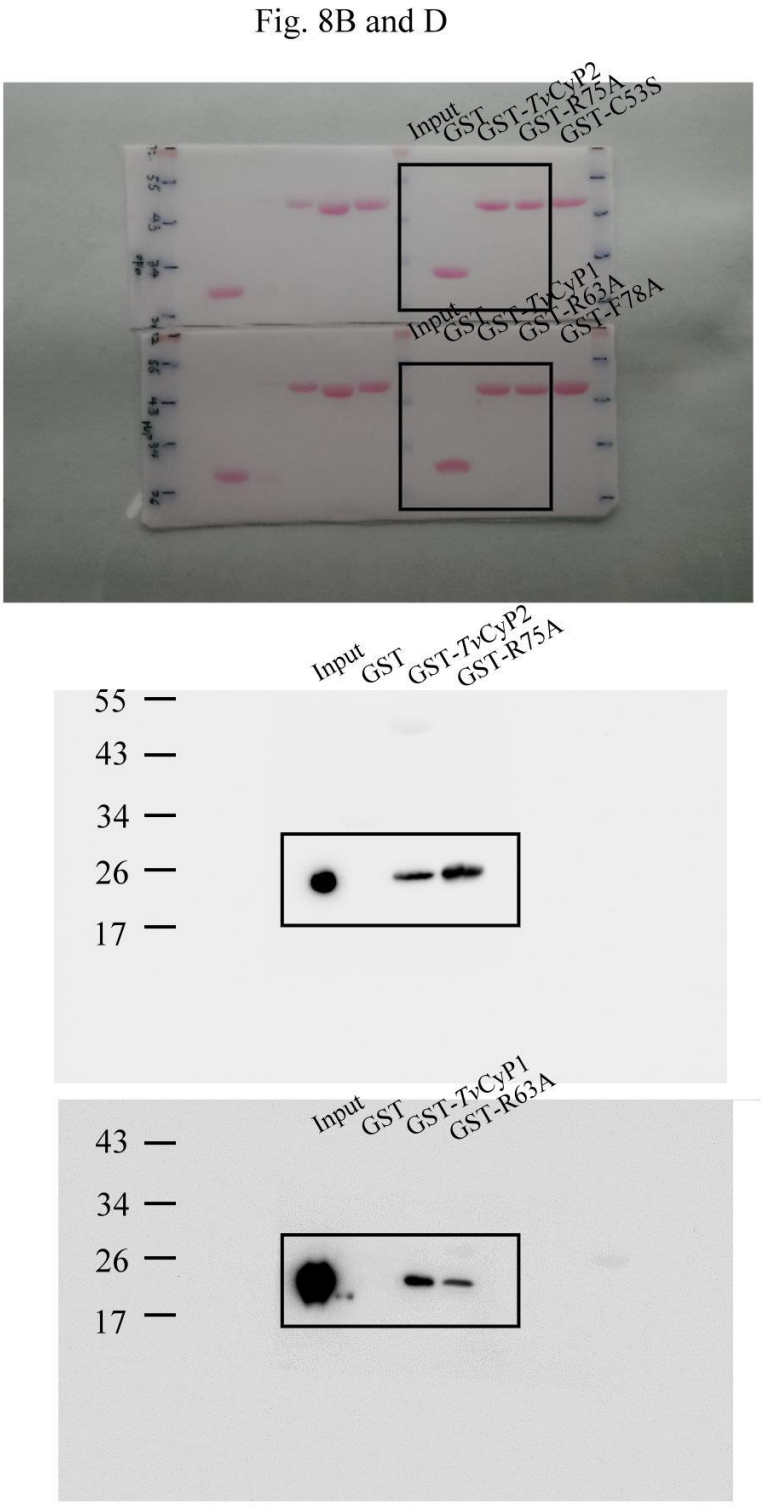
Supplementary Figure 13. Raw data from the Western blotting in the left panel of Figure 8A. The original membrane blot was sliced into pieces as indicated by the molecular weight markers. Boxed regions are shown in the manuscript. See figure legends in the manuscript for description.

Supplementary Figure 14



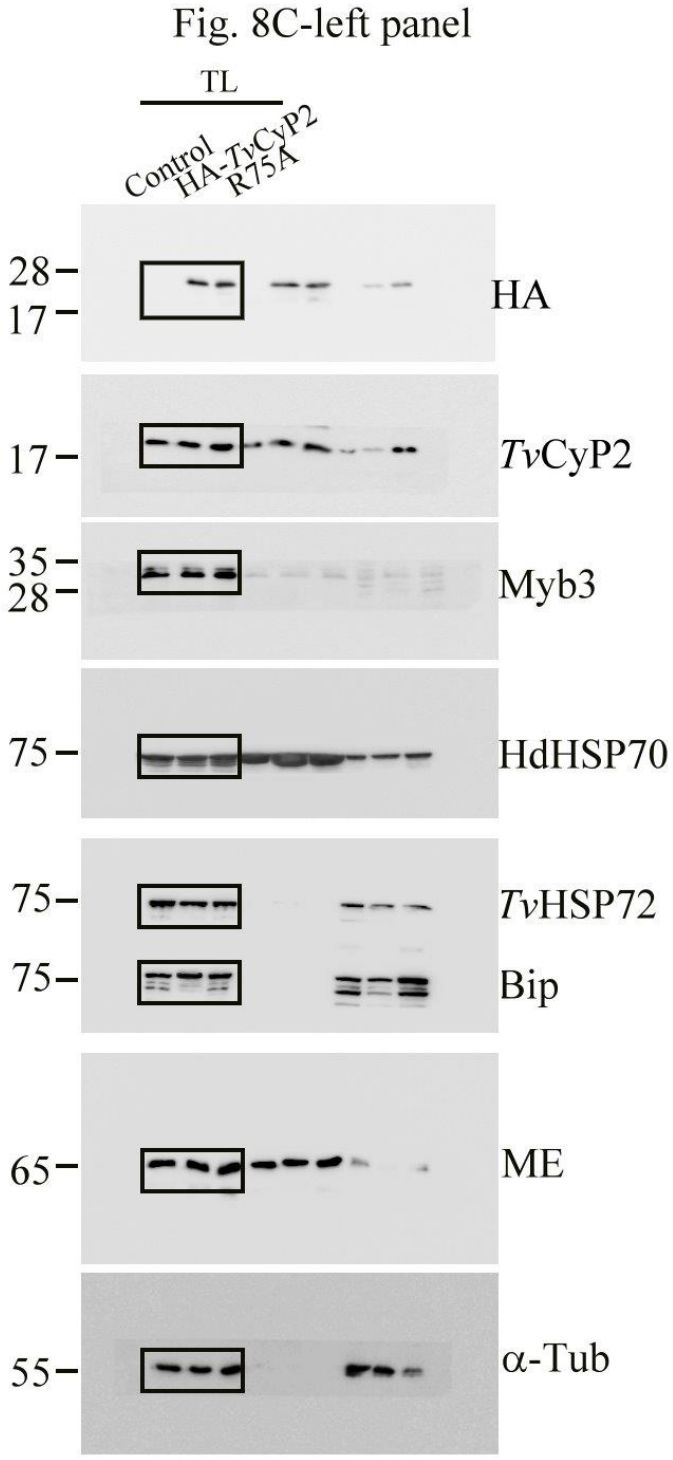
Supplementary Figure 14. Raw data from the Western blotting in the right panel of Figure 8A. The original membrane blot was sliced into pieces as indicated by the molecular weight markers. Boxed regions are shown in the manuscript. See figure legends in the manuscript for description.

Supplementary Figure 15



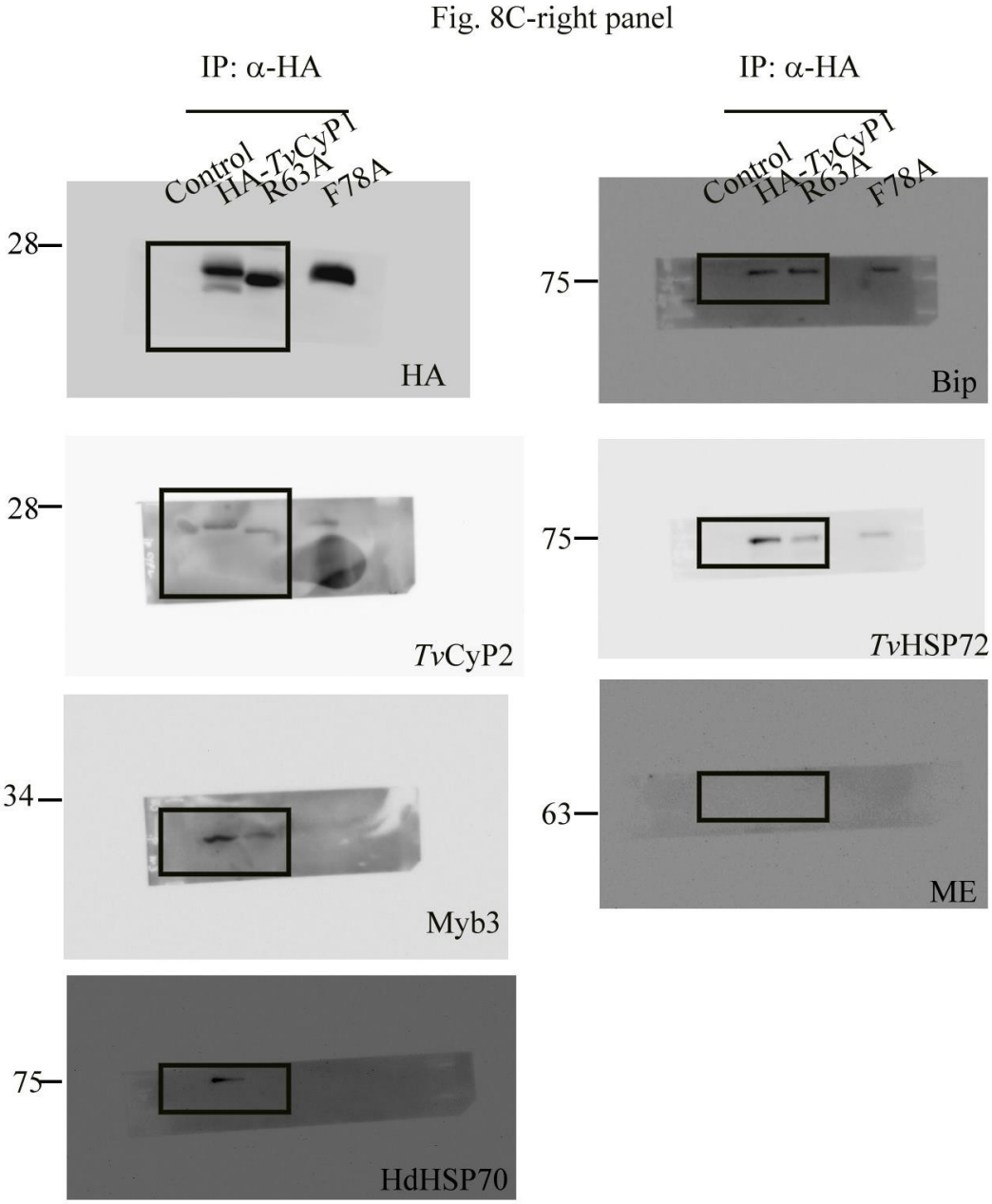
Supplementary Figure 15. Raw data from the Ponceau S staining and Western blotting in Figure 8B and 8D. The original membrane blot was sliced into pieces as indicated by the molecular weight markers. Boxed regions are shown in the manuscript. See figure legends in the manuscript for description.

Supplementary Figure 16



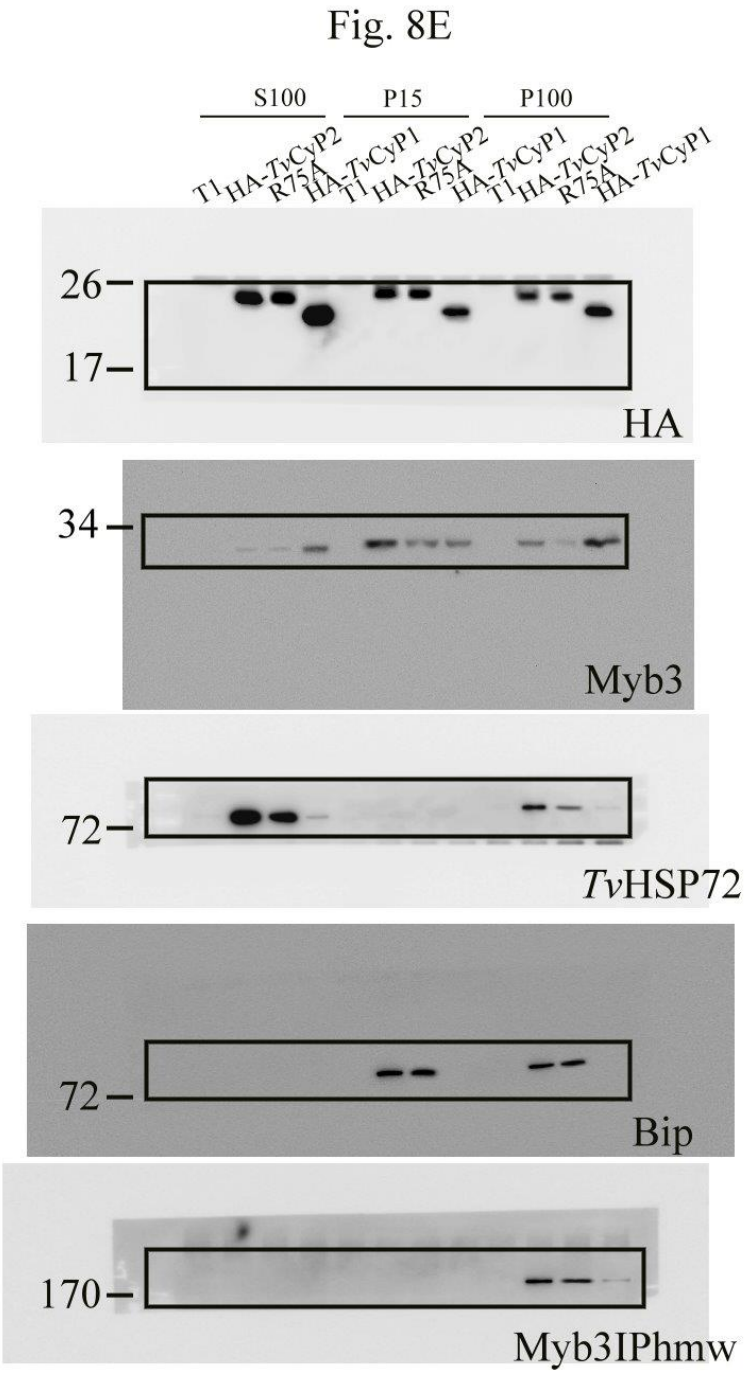
Supplementary Figure 16. Raw data from the Western blotting in the left panel of Figure 8C. The original membrane blot was sliced into pieces as indicated by the molecular weight markers. Boxed regions are shown in the manuscript. See figure legends in the manuscript for description.

Supplementary Figure 17



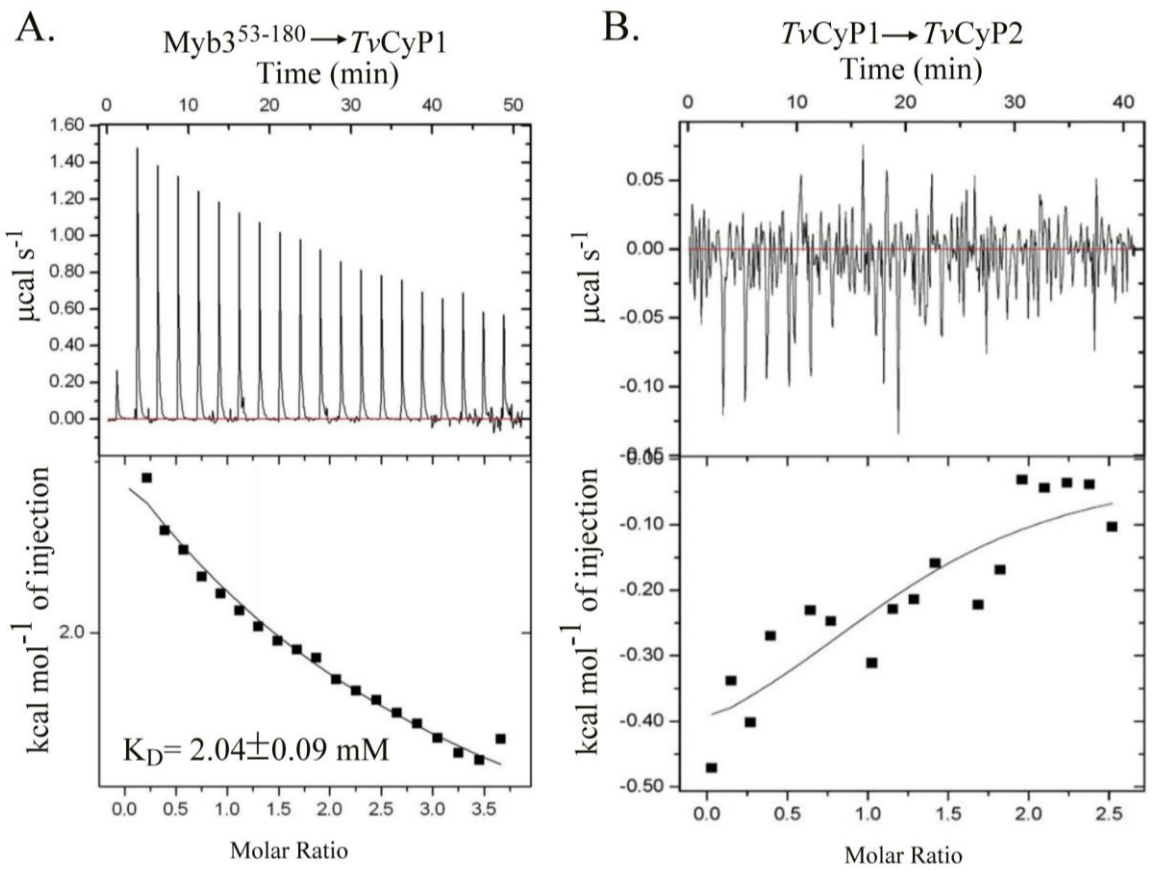
Supplementary Figure 17. Raw data from the Western blotting in the right panel of Figure 8C. The original membrane blot was sliced into pieces as indicated by the molecular weight markers. Boxed regions are shown in the manuscript. See figure legends in the manuscript for description.

Supplementary Figure 18



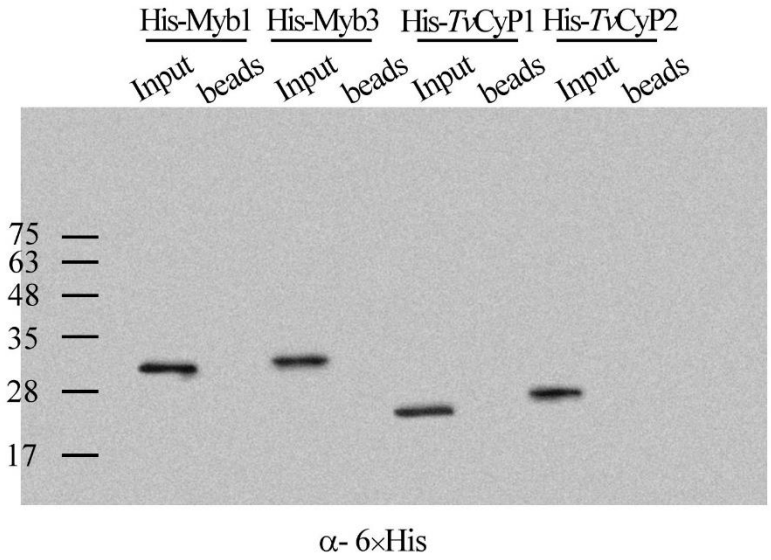
Supplementary Figure 18. Raw data from the Western blotting in Figure 8E. The original membrane blot was sliced into pieces as indicated by the molecular weight markers. Boxed regions are shown in the manuscript. See figure legends in the manuscript for description.

Supplementary Figure 19



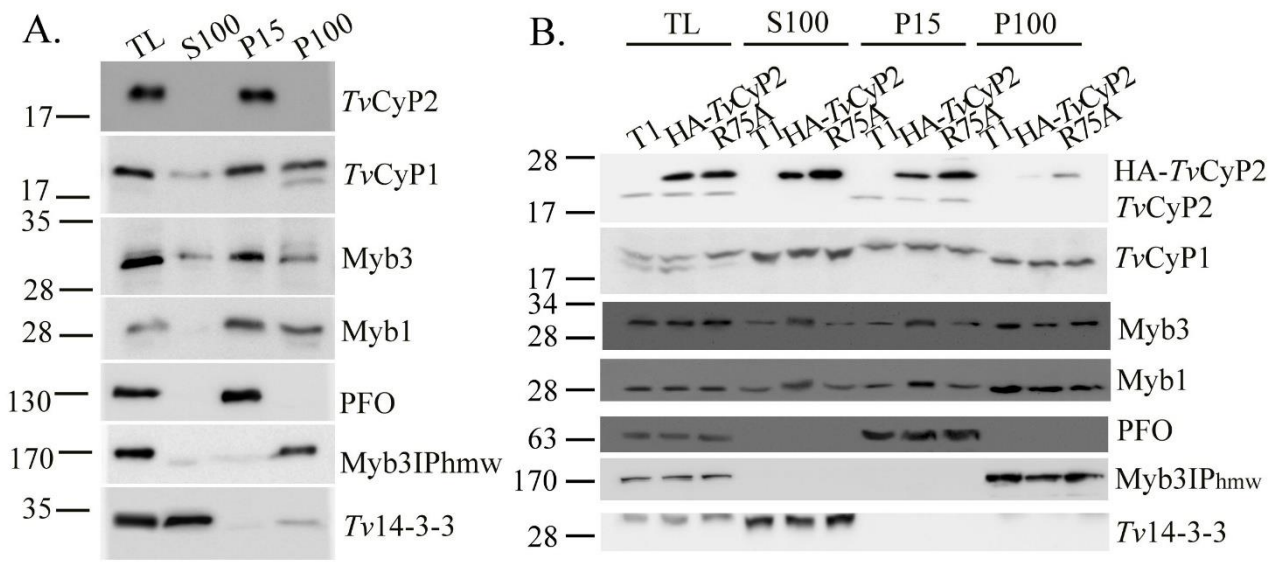
Supplementary Figure 19. The protein interaction measured by ITC. In A, 150 µM of His-*Tv*CyP1 was titrated with increasing amounts of Myb3$^{53-180}$ (1- 20 µM). In B, 180 µM of His-*Tv*CyP1 was titrated with increasing amounts of His-*Tv*CyP2 (18- 360 µM). ITC was performed at 25°C and K_D value with the standard deviation was measured as shown in each panel.

Supplementary Figure 20



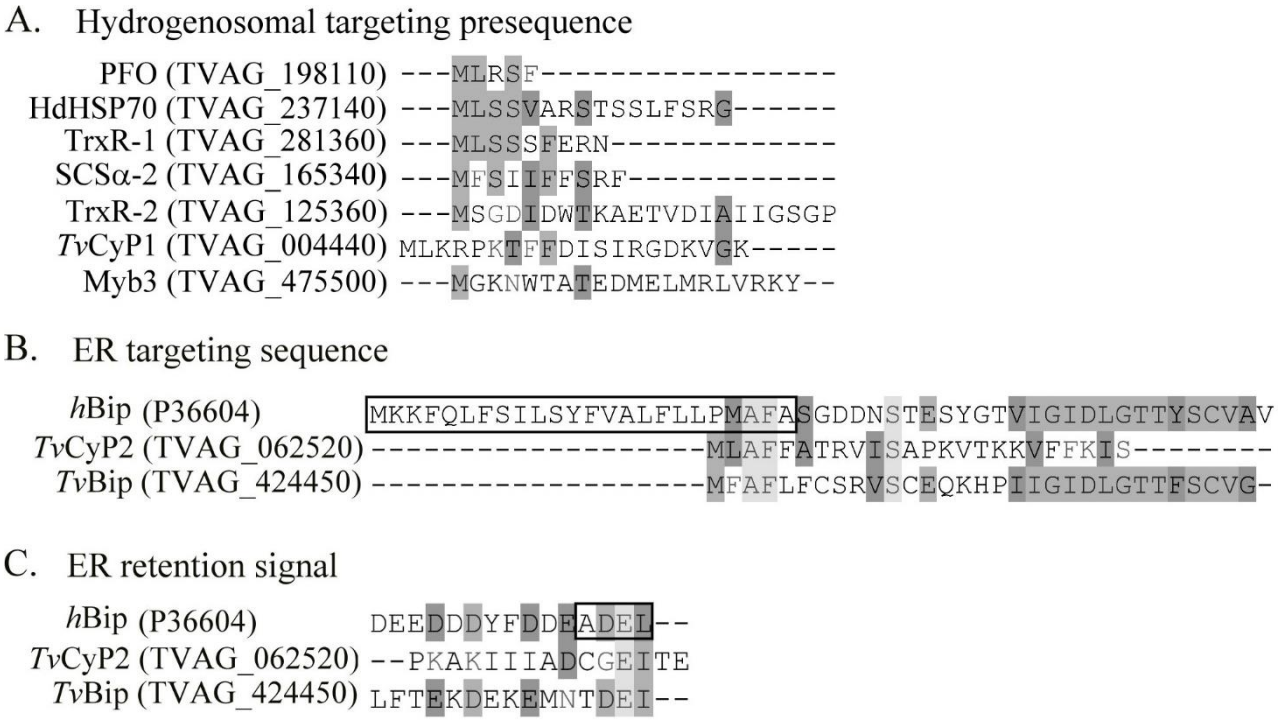
Supplementary Figure 20. Binding specificity of the His-proteins to the glutathione-conjugated beads. Equivalent amount of His-Myb1, His-Myb3, His-*Tv*CyP1, or His-*Tv*CyP2 was incubated with glutathione-conjugated beads for the GST pulldown assay. The pulled down products were analyzed by the Western blotting using the anti-6×His antibody.

Supplementary Figure 21



Supplementary Figure 21. Subcellular localization of *Tv*CyP2. The experiments described in Fig. 5B and Fig. 7B of the original manuscript were repeated, and the results shown here are used in the revised manuscript.

Supplementary Figure 22



Supplementary Figure 22. Signal peptides in the hydrogenosomal or ER proteins. In A, the N-terminal sequences of several hydrogenosomal proteins, including the pyruvate ferrodoxin oxidoreductase (PFO) (TVAG_198110), HdHSP70 (TVAG_237140), thioredoxin reductase 1 (TrxR-1, TVAG_281360), succinyl CoA synthetase alpha subunit 2 (SCSα-2, TVAG_165340), thioredoxin reductase 2 (TrxR-2, TVAG_125360), and *Tv*CyP1 (TVAG_004440) in *T. vaginlais* are aligned. In B and C, the N-terminal (B) or C-terminal (C) sequences of *Tv*CyP2 (TVAG_062520) and *Tv*Bip (TVAG_424450) in *T. vaginalis* and the human *h*Bip (P36604) are aligned. The ER targeting signal at the N-terminus and the retention signal at the C-terminus of *h*Bip are boxed.