

# Appendix

A palmitoyl transferase chemical genetic system to map ZDHHC-specific S-acylation

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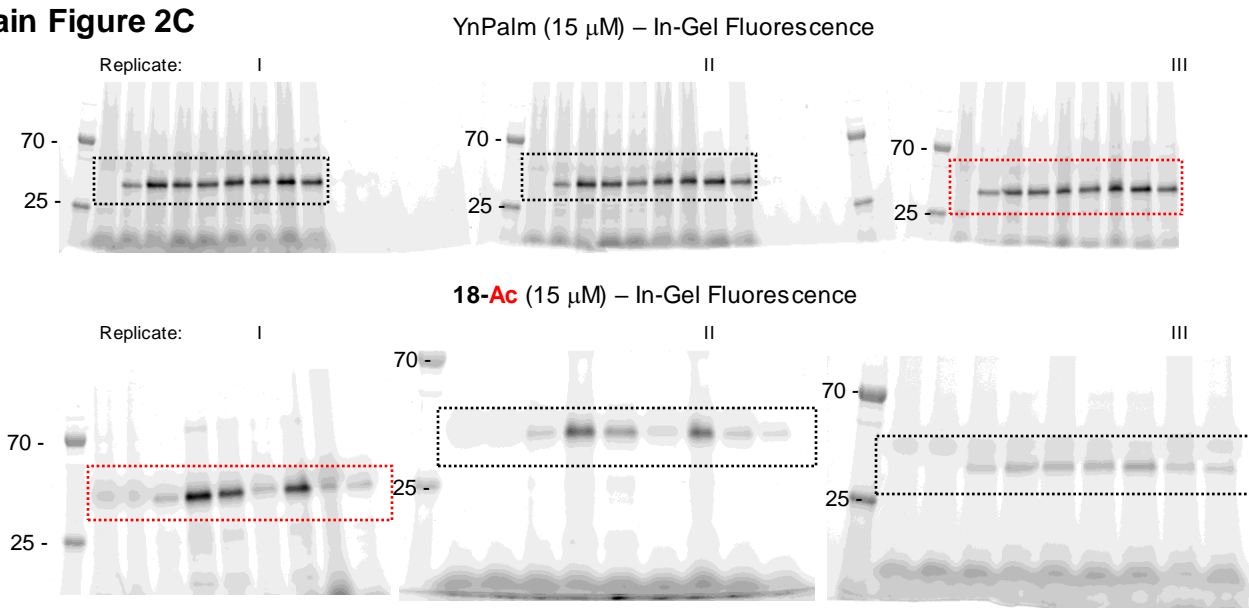
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2. Uncropped gels, blots and replicates from Extended Figures. 20 – 31

# Appendix – Main Figures

## Uncropped Gels and Blots

### Main Figure 2C



### Main Figure 2E

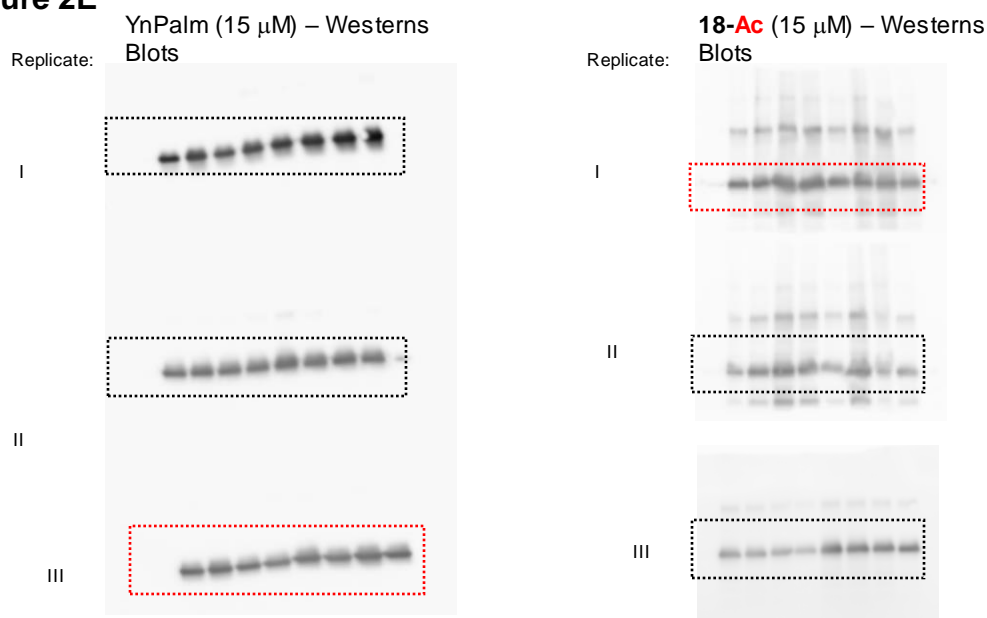


Figure A1. Uncropped gels and blots for Main Figure 2C,E. Areas within red borders are in the main figure

### Main Figure 2G

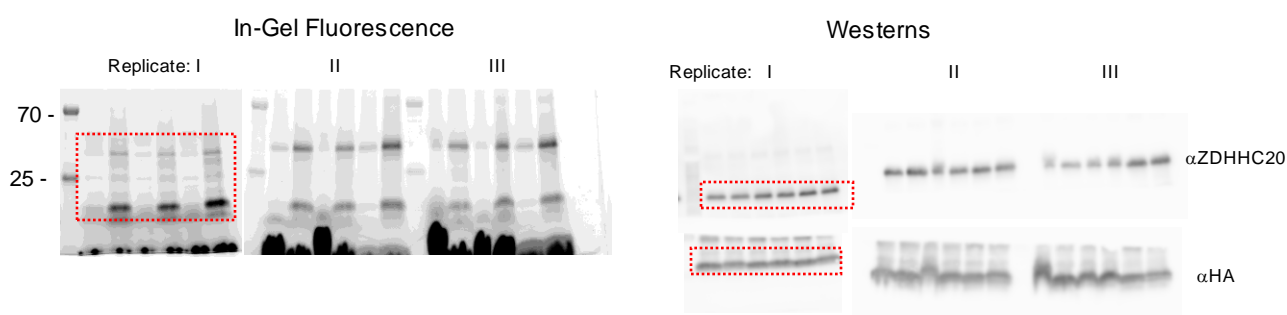
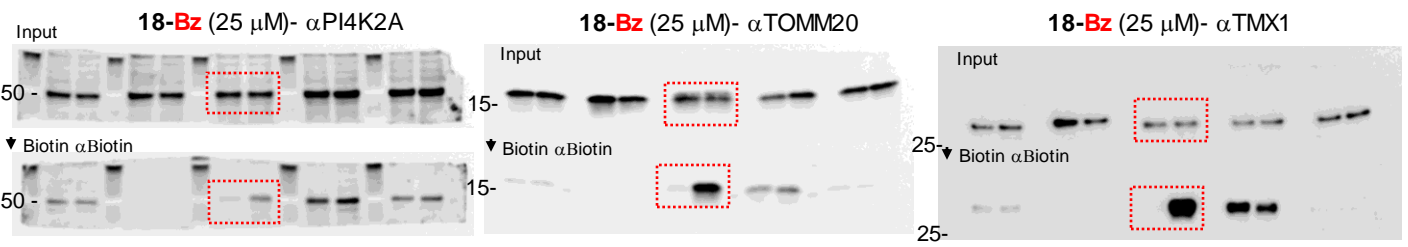


Figure A2. Uncropped gels and blots for Main Figure 2G. Areas within red borders are in the main figure

## Main Figure 3F

C18Bz – Westerns Blots for ZDHHC(wt) and ZDHHC (M)



## Main Figure 3G

YnPal – Westerns Blots for ZDHHC(wt) and ZDHHC (M)

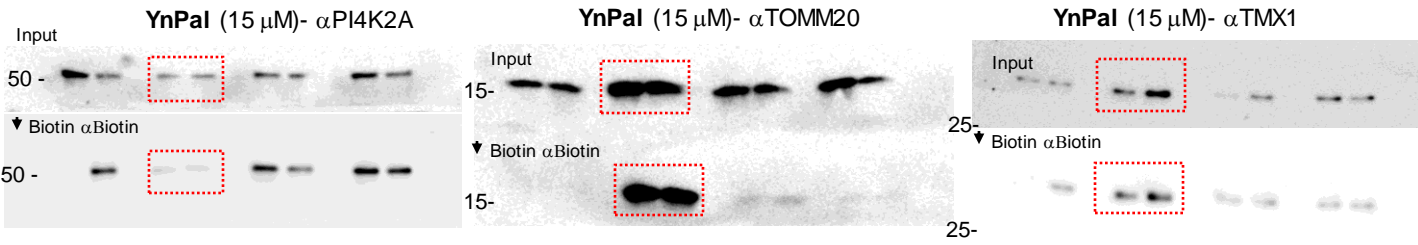
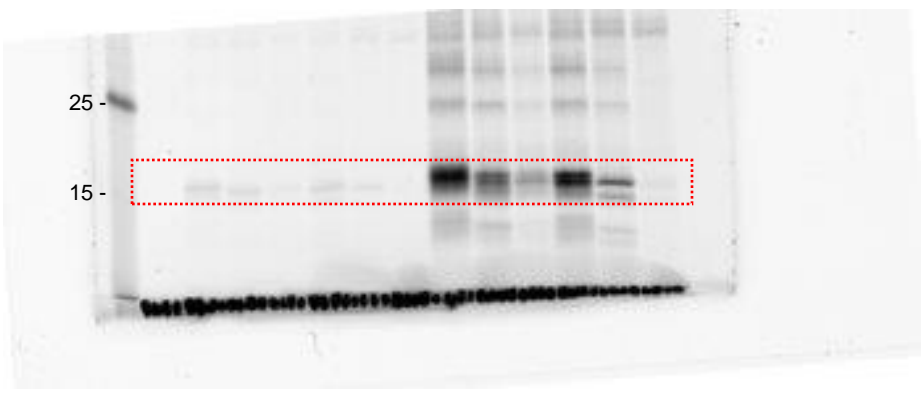


Figure A3. Uncropped gels and blots for Main Figure 3F and 3G. Areas within red borders are in the main figure

**Main Figure 4A**

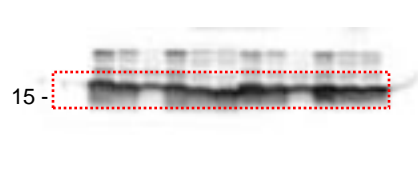
IFITM3 Rep 1

IFITM3 Rep 1 TAMRA



IFITM3 Rep 1 HA IP

IFITM3 Rep 1 HA input



IFITM3 Rep 1 tubulin

IFITM3 Rep 1 FLAG input

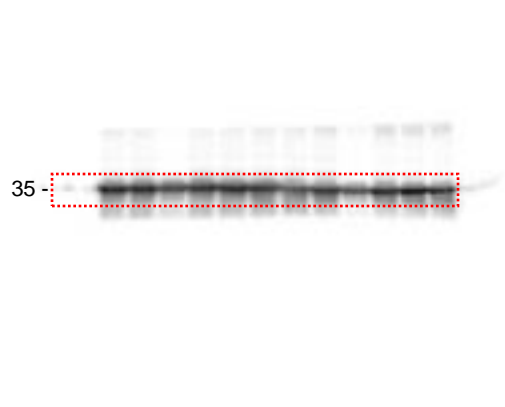


Figure A4. Uncropped gels and westerns for Main Figure 4A, replicate set 1. Areas within red borders are in the main figure.

# Main Figure 4A

IFITM3 Rep 2

N-HA-IFITM3 Cys to Ala mutants	E	W	71	72	105	71/72	71/72/105	W	71	72	105	71/72	71/72/105
C-FLAG-zDHHC20	-	W	W	W	W	W	W	<b>M</b>	<b>M</b>	<b>M</b>	<b>M</b>	<b>M</b>	<b>M</b>
C18-Bz 15 $\mu$ M	+	+	+	+	+	+	+	+	+	+	+	+	+

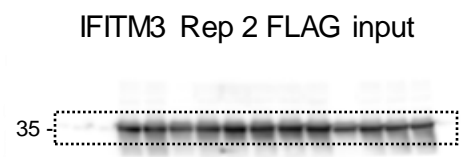
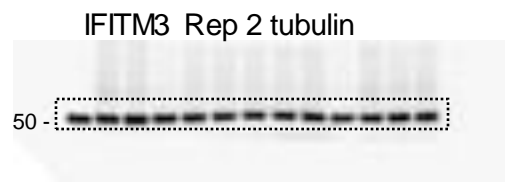
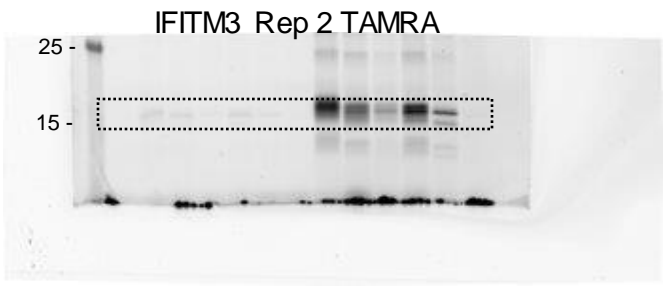
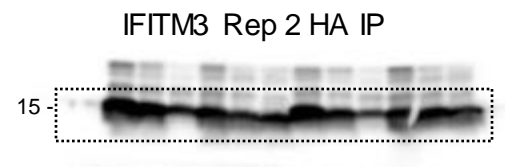
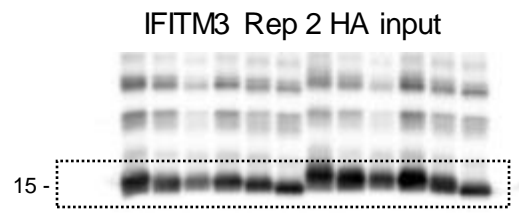
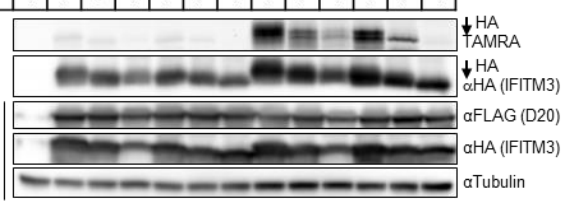
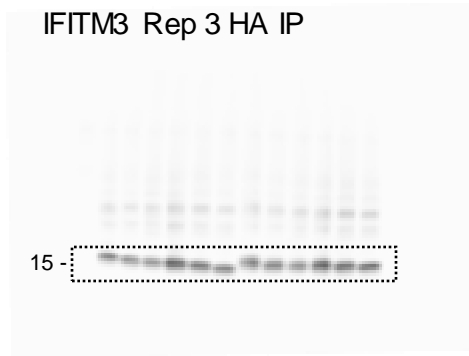
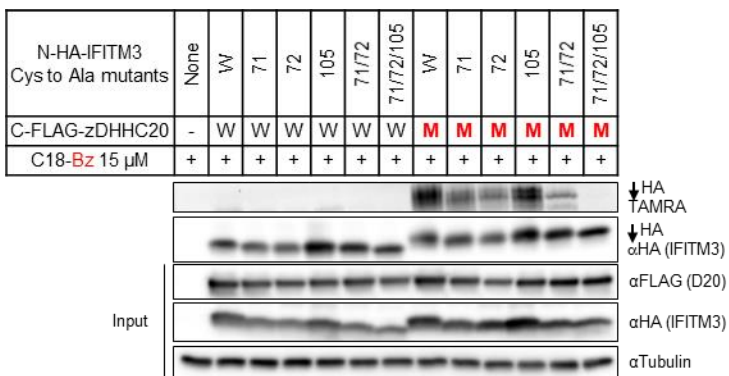


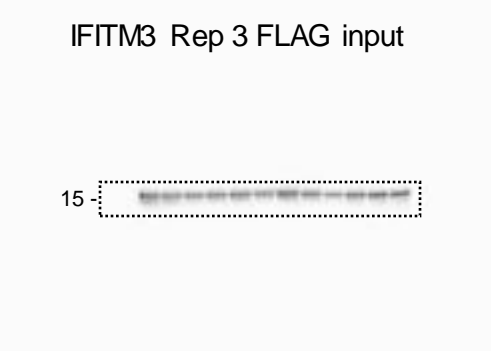
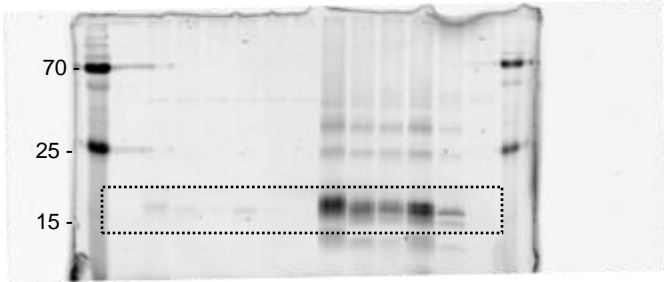
Figure A5. Uncropped gels and westerns for Main Figure 4A, replicate sets 2–3. Areas within red borders are in the main figure.

**Main Figure 4A**

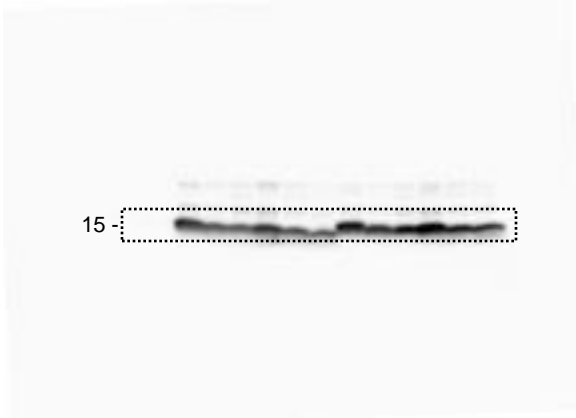
IFITM3 Rep 3



IFITM3 Rep 3 TAMRA



IFITM3 Rep 3 HA input



IFITM3 Rep 1 tubulin

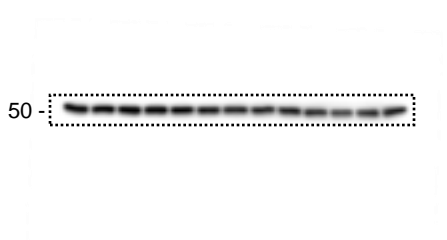
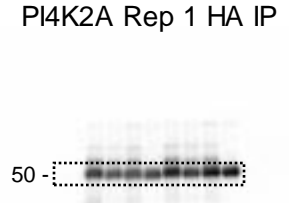
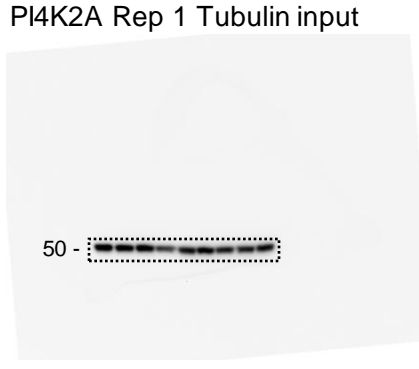
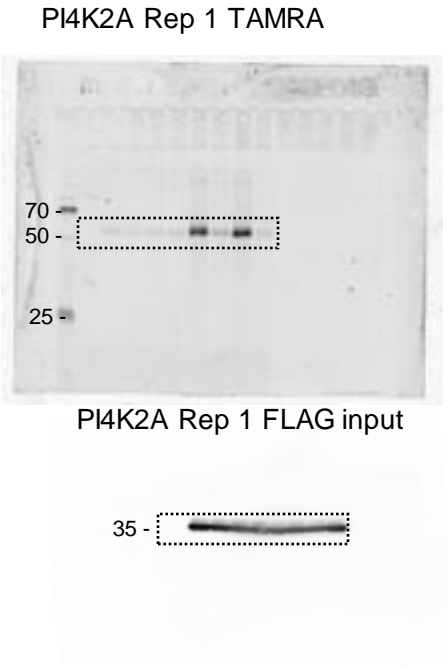


Figure A6 cont. Uncropped gels and westerns for Main Figure 4A, replicate sets 2 – 3. Areas within red borders are in the main figure.

**Main Figure 4B**

**PI4K2A Rep 1**



**PI4K2A Rep 2**

N-HA-PI4K2A Cys to Ala mutants	None	W	174 175	177 178	174 175 178	WT	174 175	177 178	174 175
C-FLAG-zDHHC20	-	W	W	W	W	M	M	M	M
C18-Bz 15 μM	+	+	+	+	+	+	+	+	+

Input

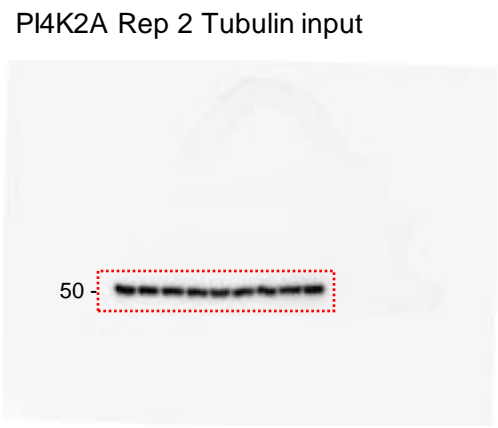
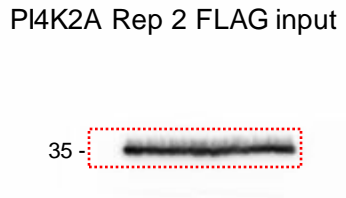
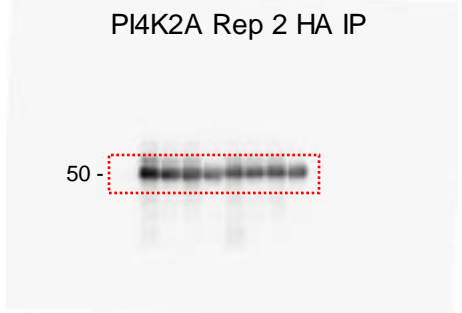
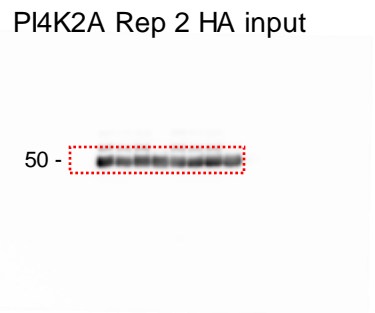
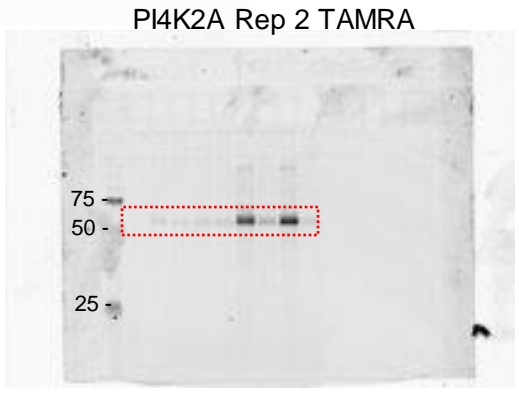
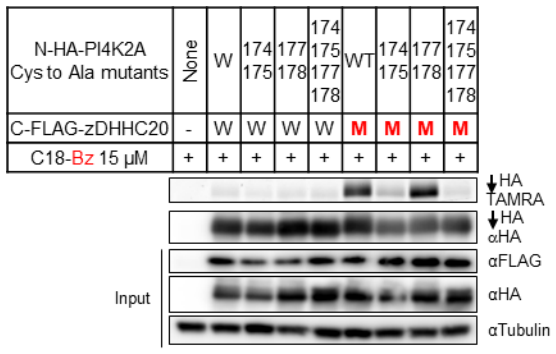


Figure A7. Uncropped gels and westerns for Main Figure 4B, replicate sets 1 – 2. Areas within red borders are in the main figure.

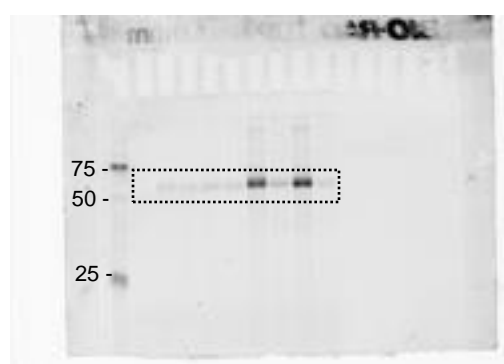


**Main Figure 4B**

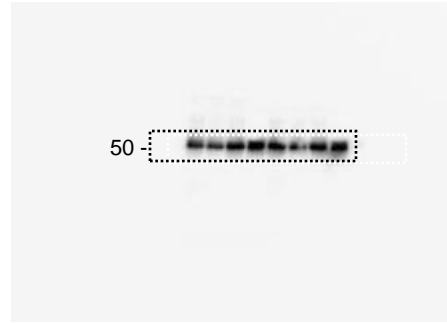
PI4K2A Rep 3



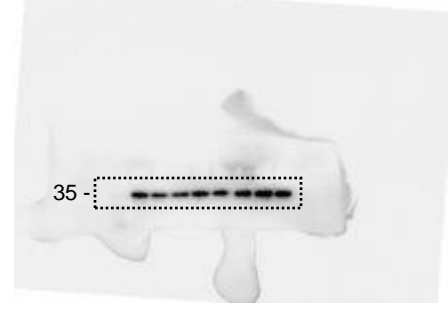
PI4K2A Rep 3 TAMRA



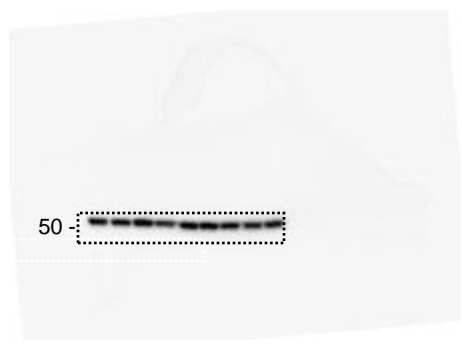
PI4K2A Rep 3 HA input



PI4K2A Rep 3 FLAG input



PI4K2A Rep 3 tubulin input



PI4K2A Rep 3 HA IP

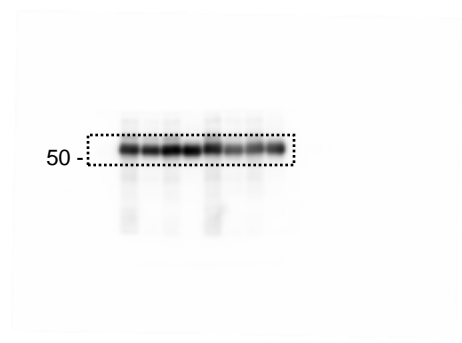


Figure A8. Uncropped gels and westerns for Main Figure 4B, replicate set 3. Areas within red borders are in the main figure.

**Main Figure 4C**

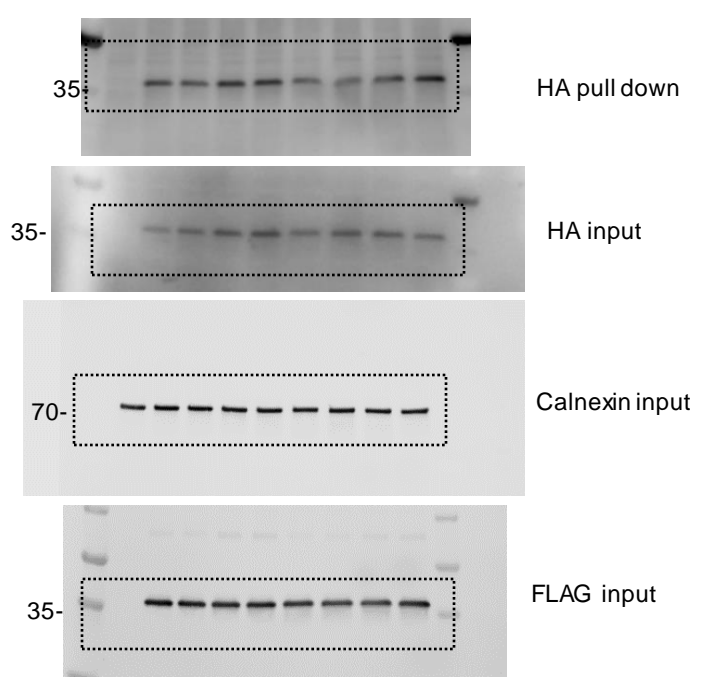
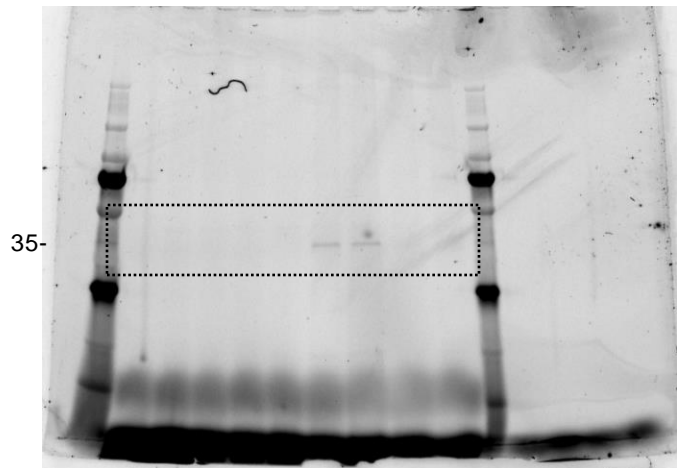
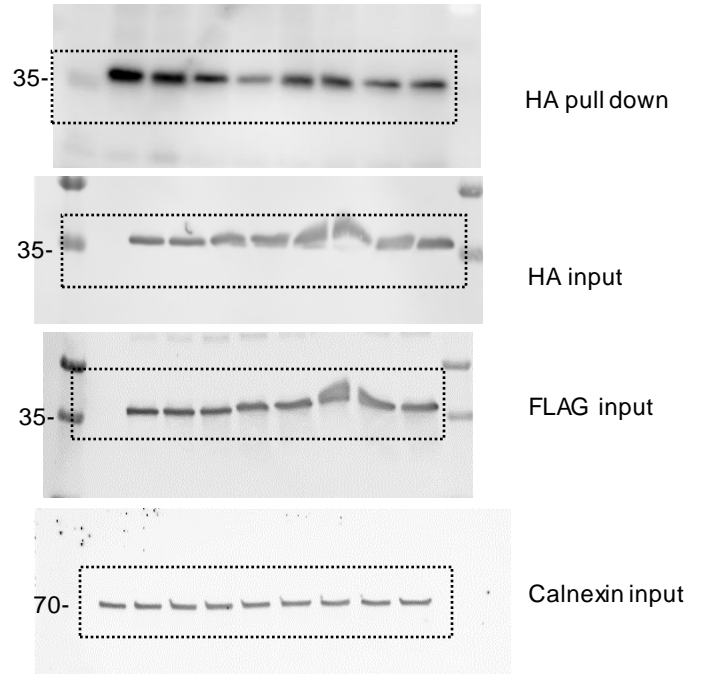
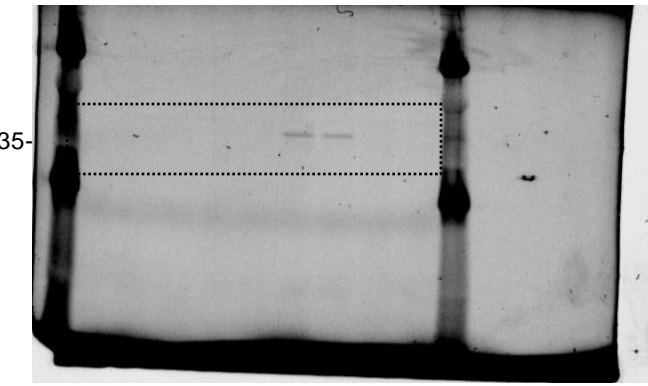
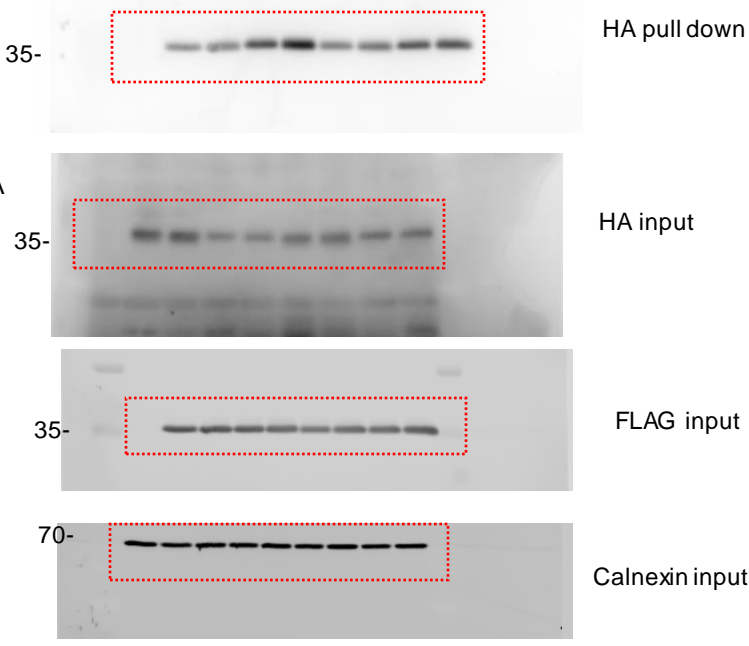
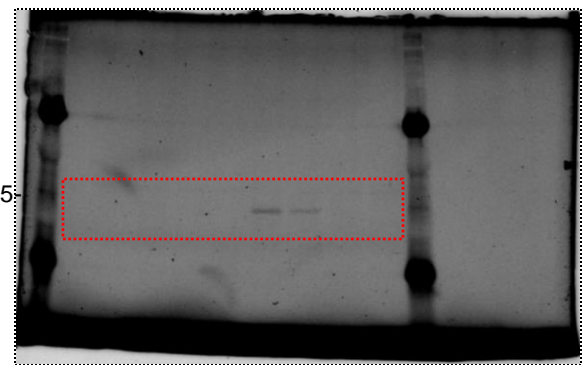


Figure A9. Uncropped gels and westerns for Main Figure 4c. Areas within red borders are in the main figure.

**Main Figure 4D**

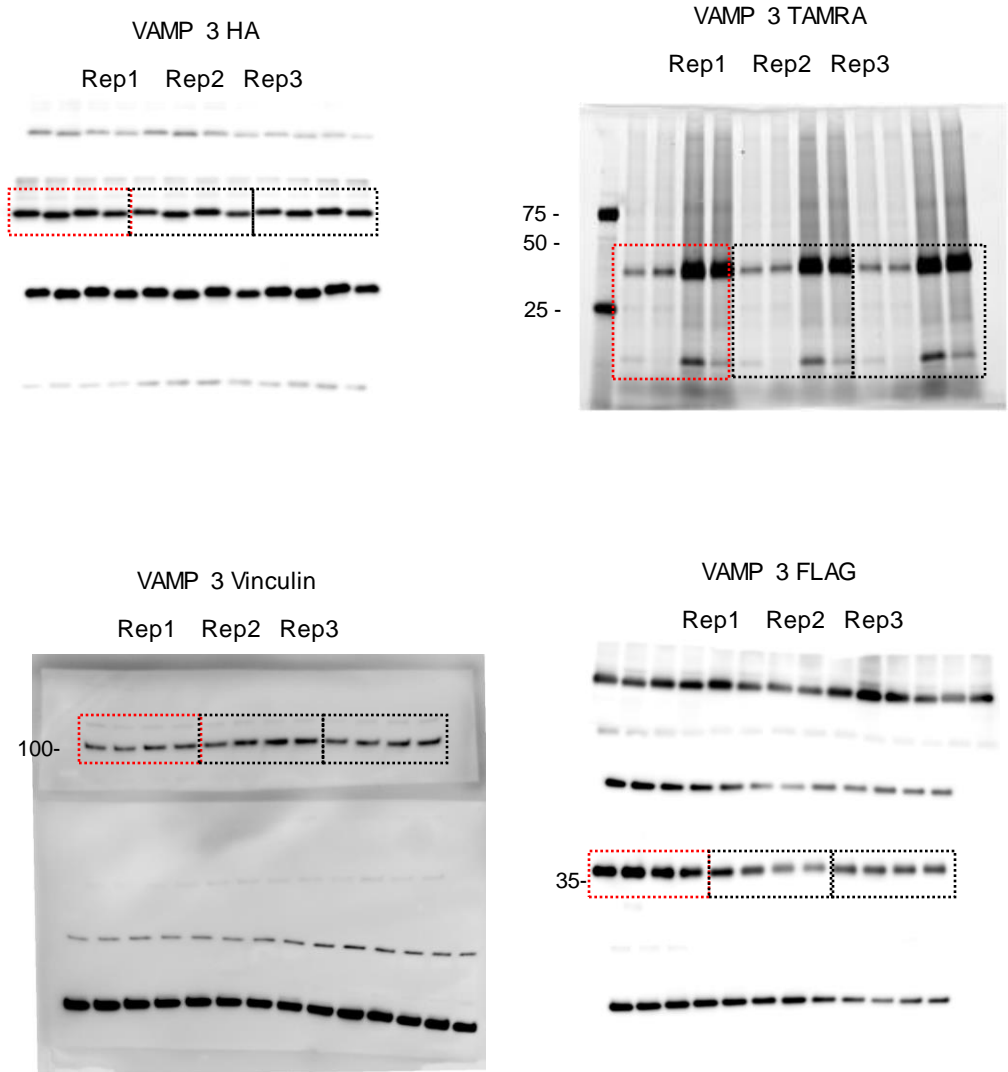


Figure A10. Uncropped gels and westerns for Main Figure 4D. Areas within red borders are in the main figure.

**Main Figure 4E**

BCAP31 – Replicates 1 - 3

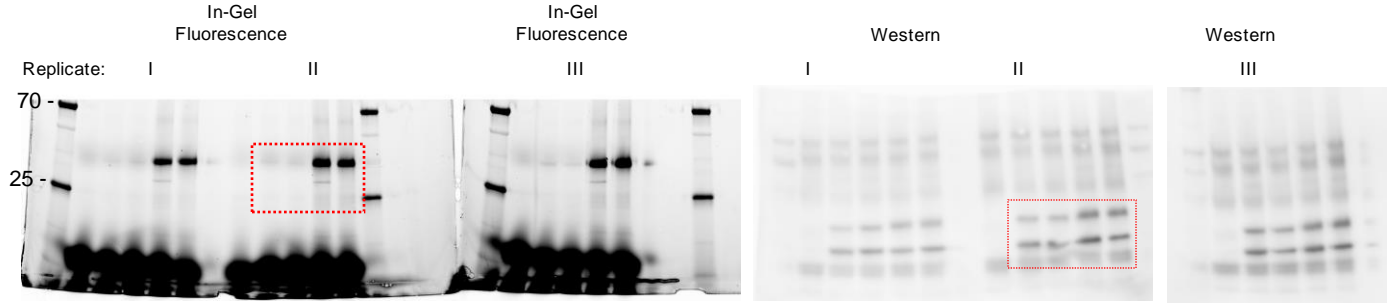


Figure A11. Uncropped gels and westerns for Main Figure 4E. Areas within red borders are in the main figure.

**Main Figure 4F**

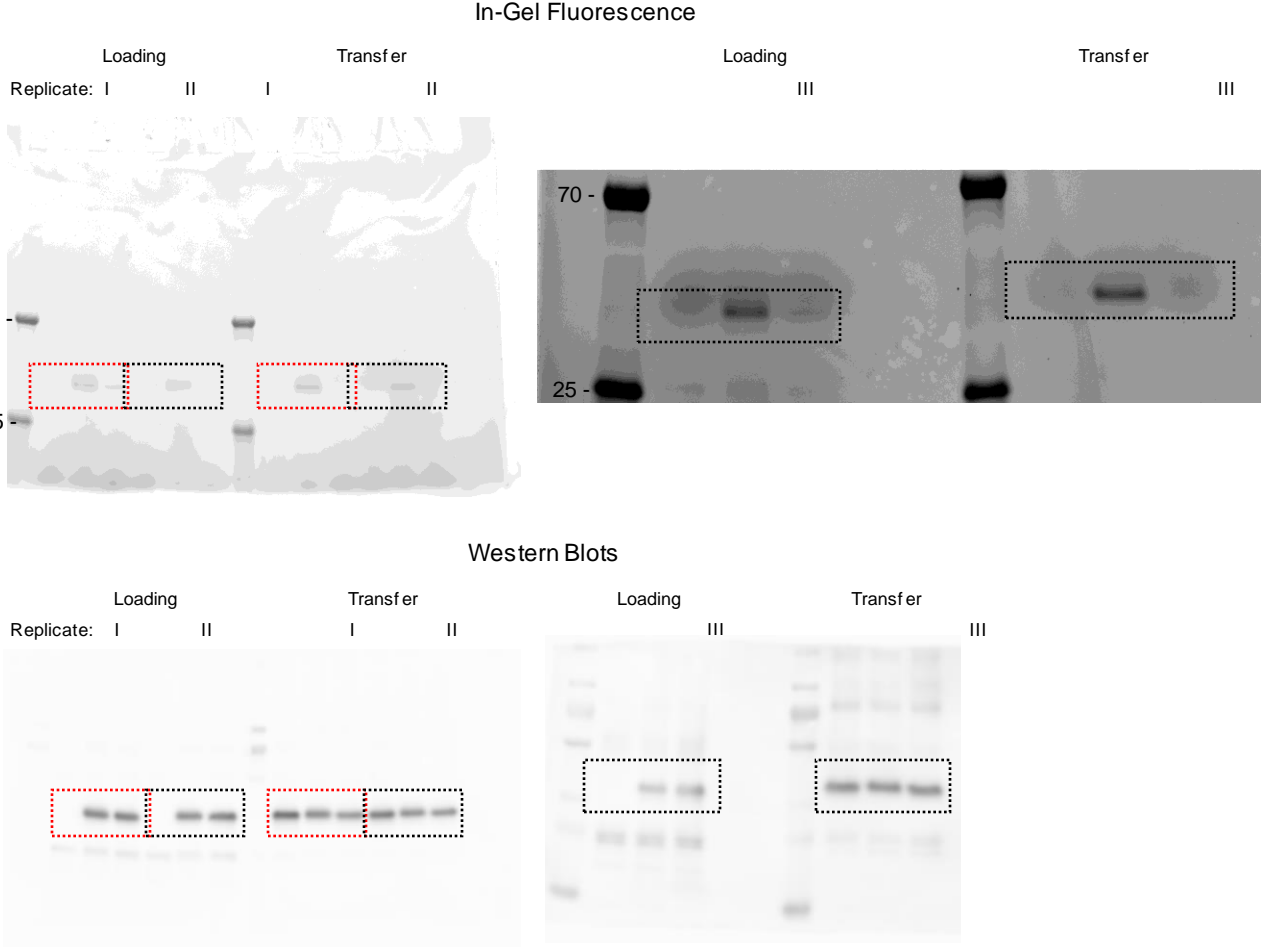


Figure A12. Uncropped gels and westerns for Main Figure 4F, replicate sets 1 - 3. Areas within red borders are in the main figure.

# Main Figure 5A

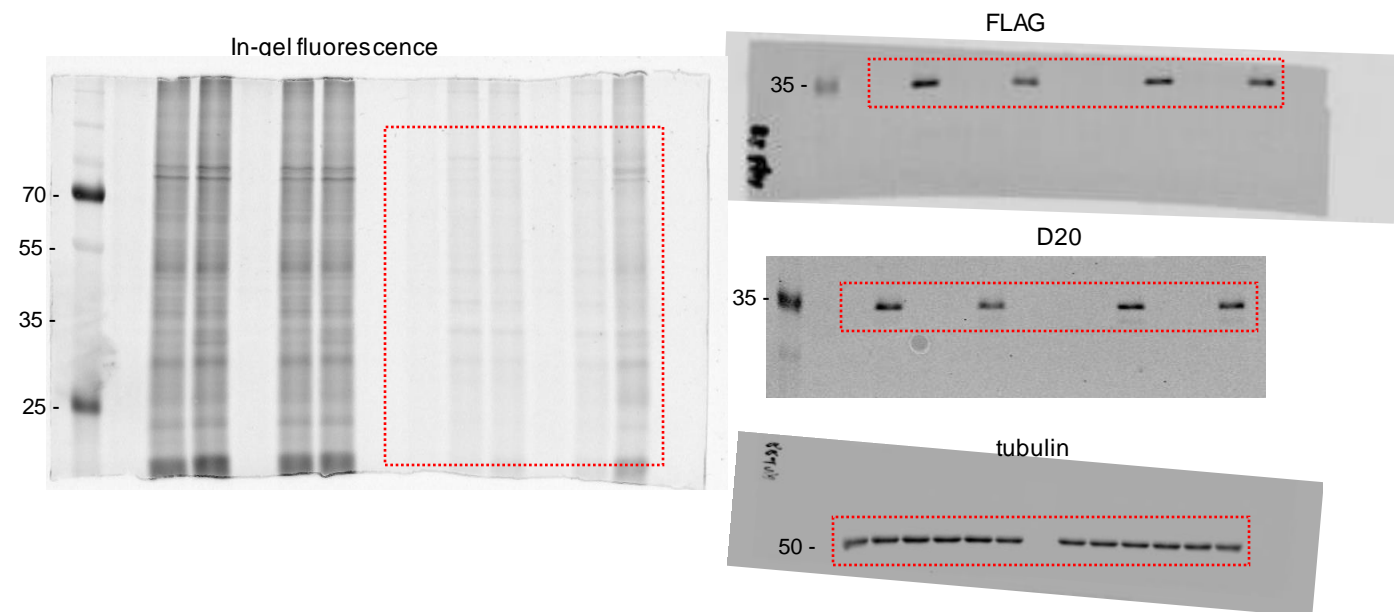
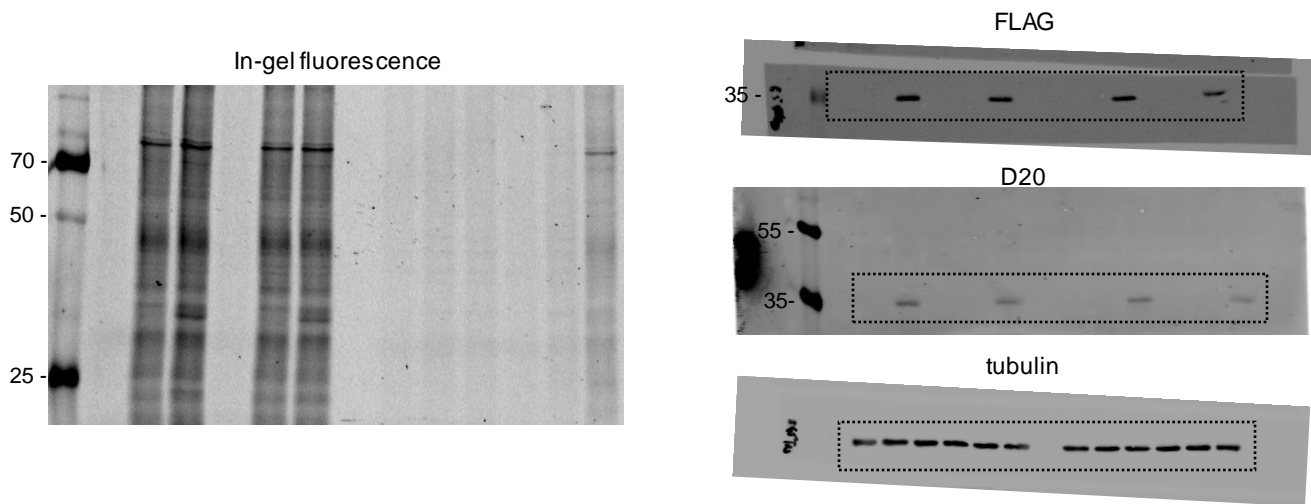
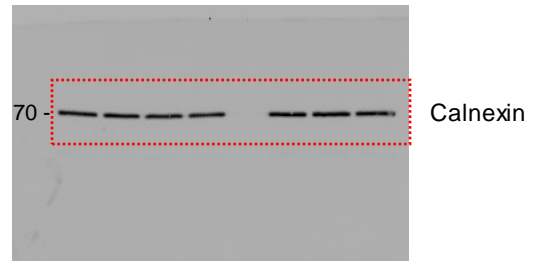
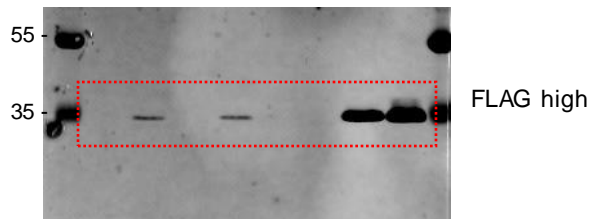
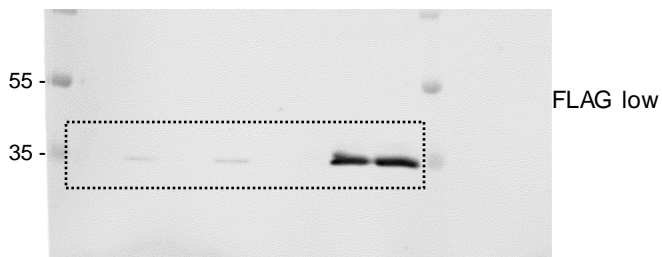
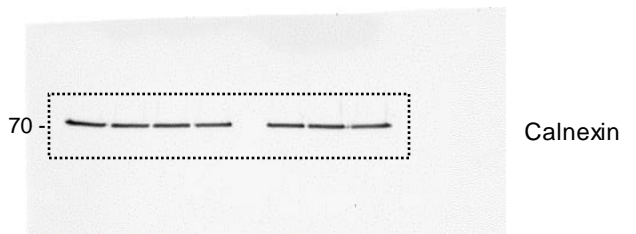
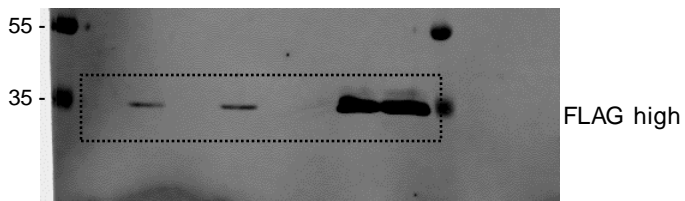


Figure A13. Uncropped gels and westerns for Main Figure 5F. Areas within red borders are in the main figure.

### Main Figure 5B rep1



### Main Figure 5B rep2



### Main Figure 5B rep3

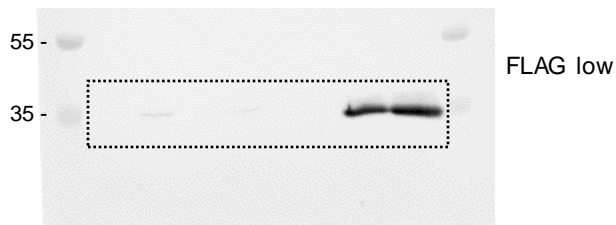
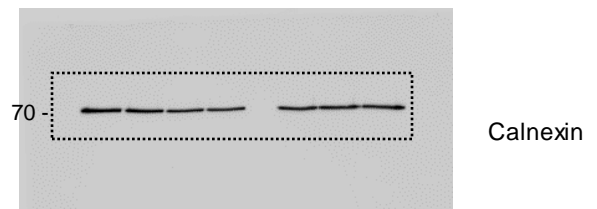
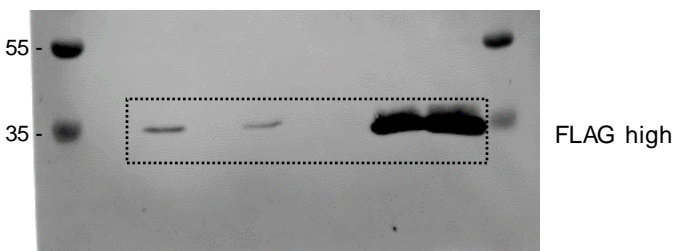
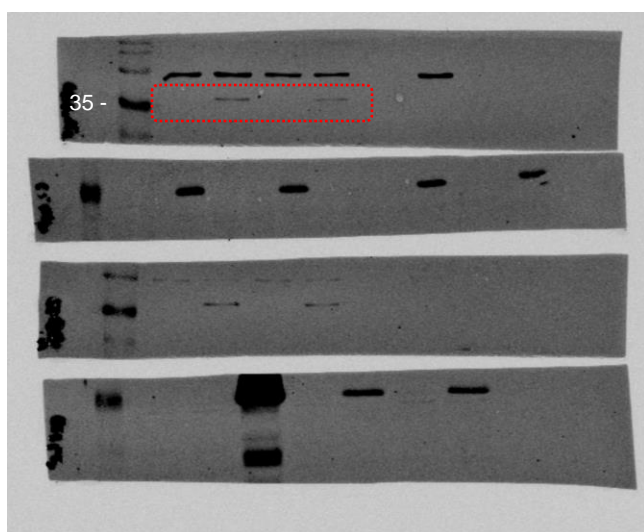
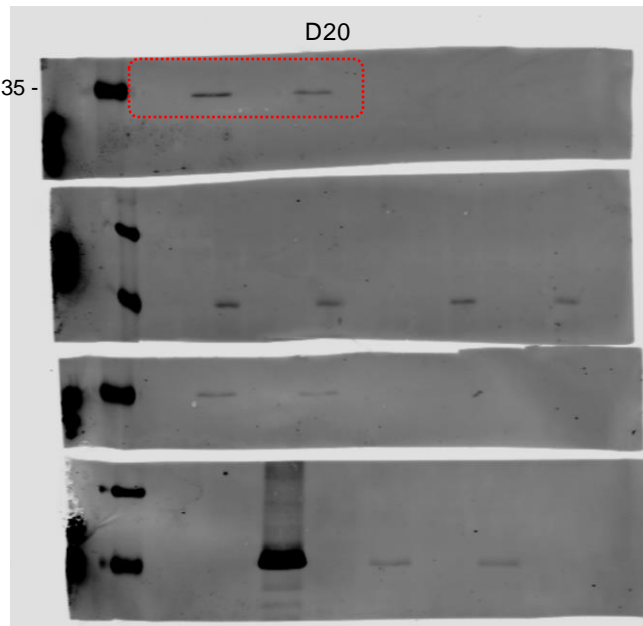
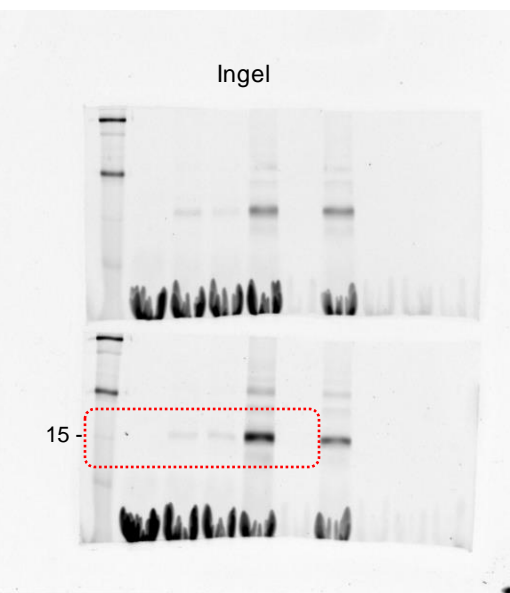


Figure A14. Uncropped gels and westerns for Main Figure 5B. Areas within red borders are in the main figure.

# Main Figure 5C

Replicate 1



FLAG

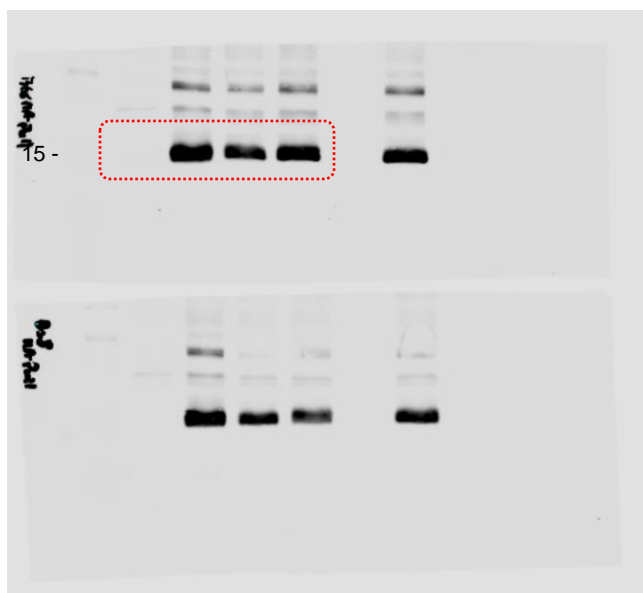
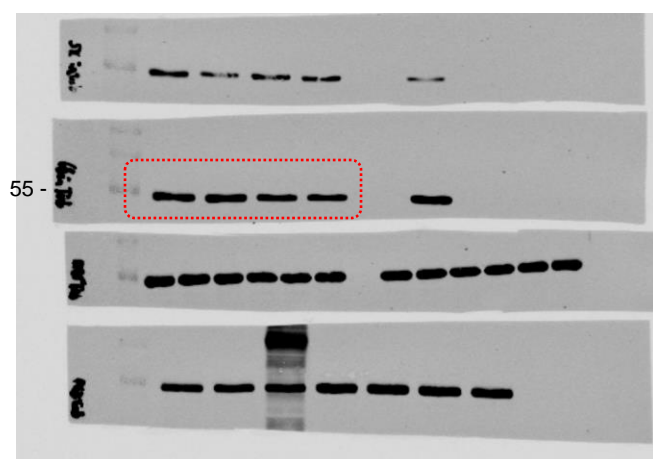


Figure A15. Uncropped gels and westerns for Main Figure 5C. Areas within red borders are in the main figure.

# Main Figure 5C

Replicate 2

Ingel

15-

15-

HA pull

D20

35-

Tub

FLAG

5-

Figure A16. Uncropped gels and westerns for Main Figure 5C. Areas within red borders are in the main figure.



# Main Figure 5C

Replicate 3

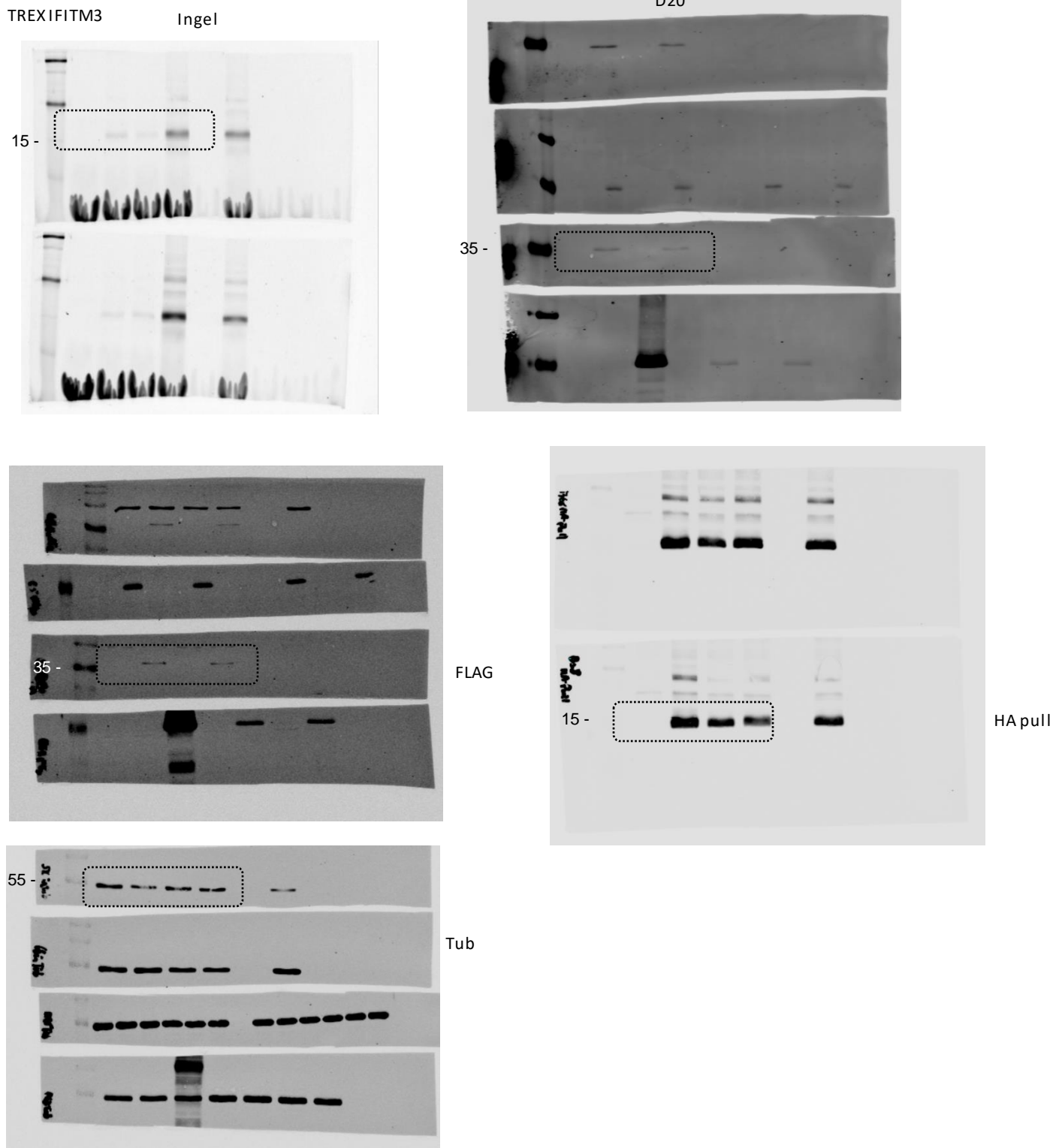
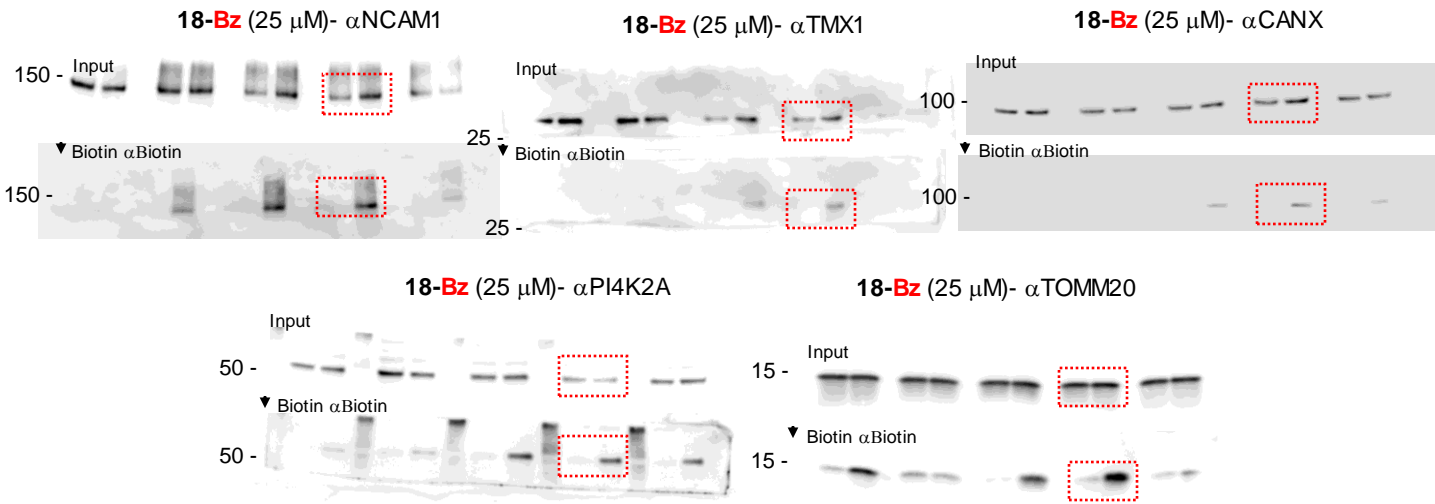


Figure A17. Uncropped gels and westerns for Main Figure 5C. Areas within red borders are in the main figure.

## Main Figure 5E

C18Bz – Westerns Blots for Trex ZDHHC(wt) and ZDHHC (M)



## Main Figure 5F

YnPal – Westerns Blots for Trex ZDHHC(wt) and ZDHHC (M)

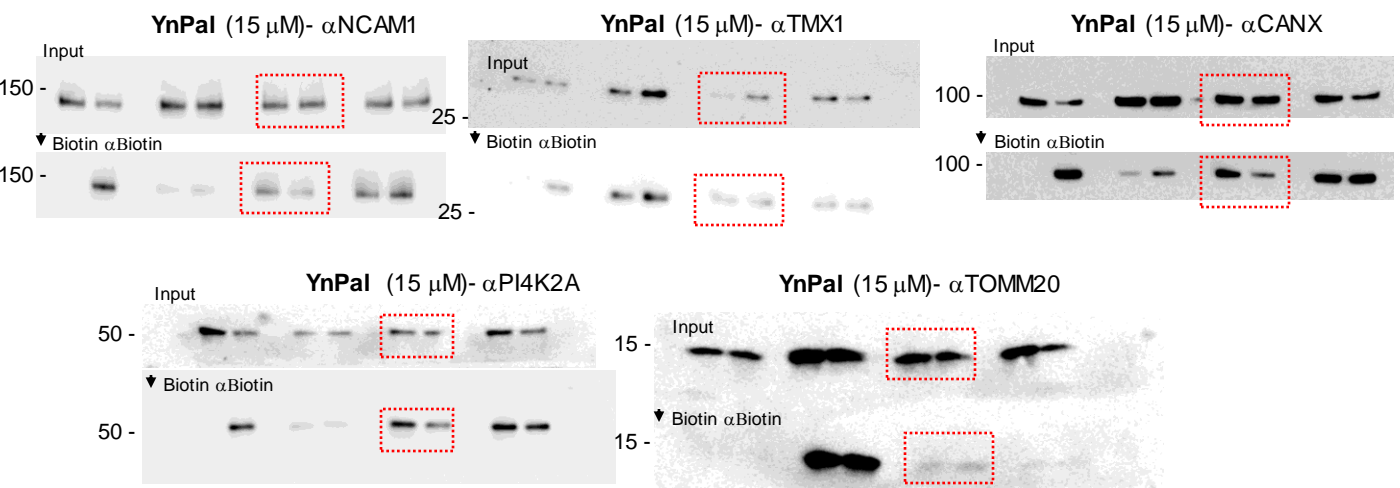


Figure A18. Uncropped gels and westerns for Main Figure 5E and 5F. Areas within red borders are in the main figure.

# Main Figure 6B

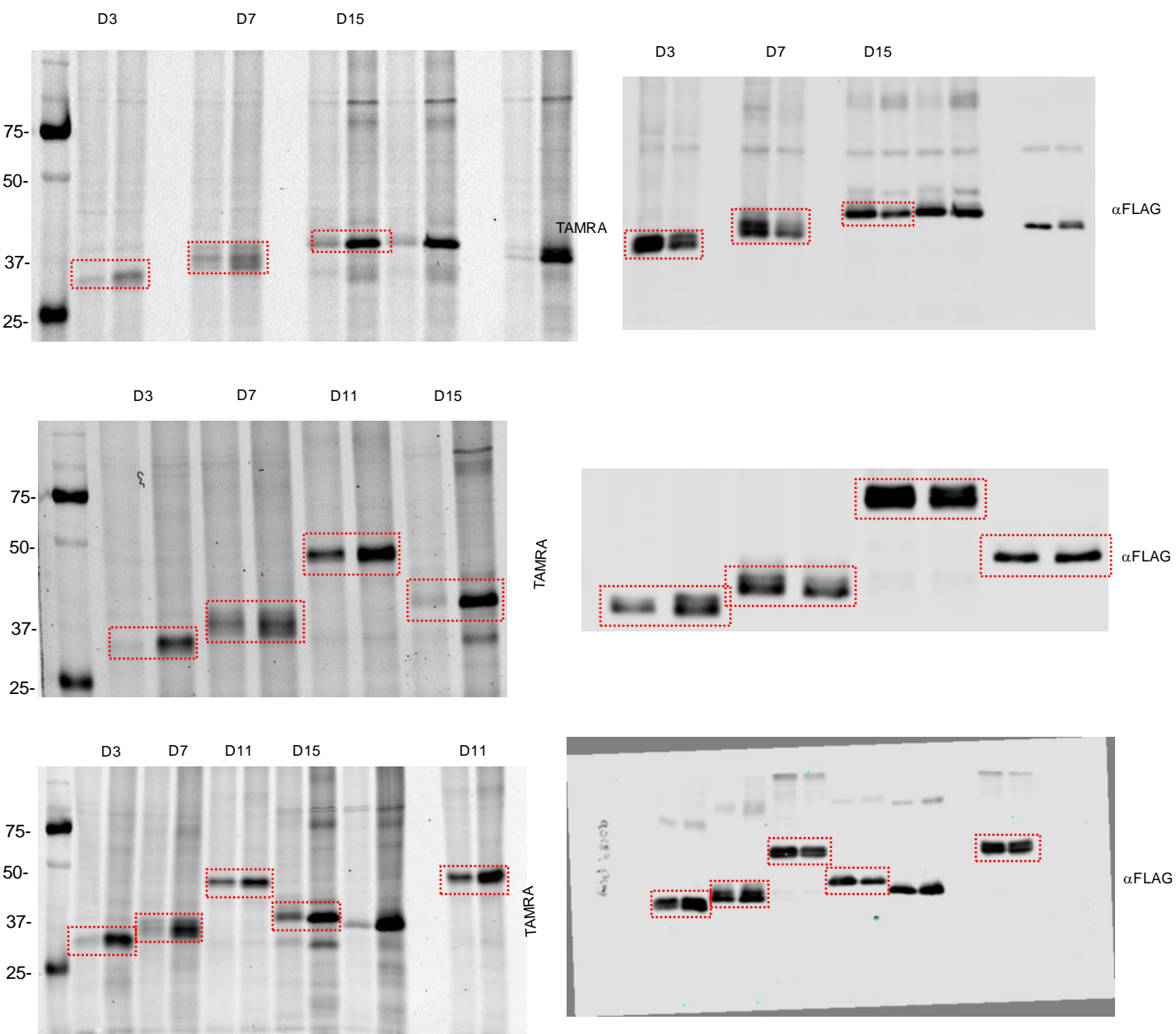
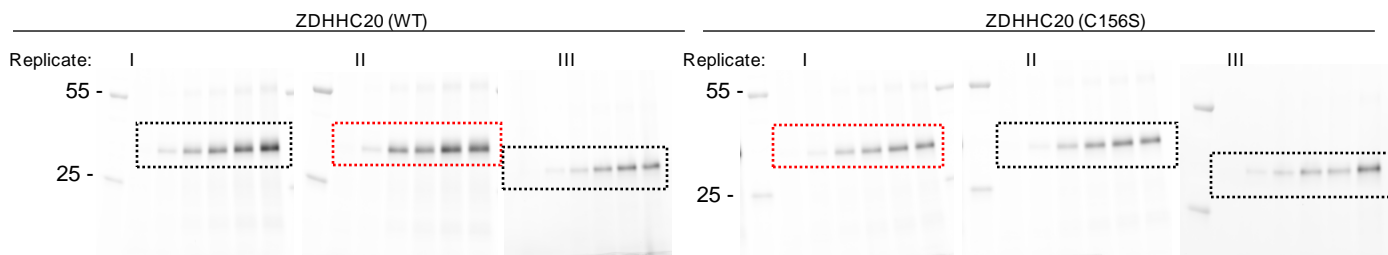


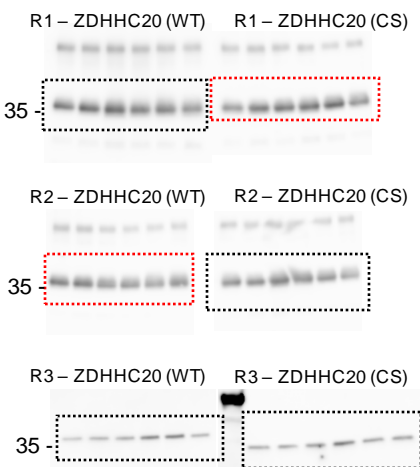
Figure A19. Uncropped gels and westerns for Main Figure 6B. Areas within red borders are in the supplementary figure.

# Extended Data Figure 1A and E

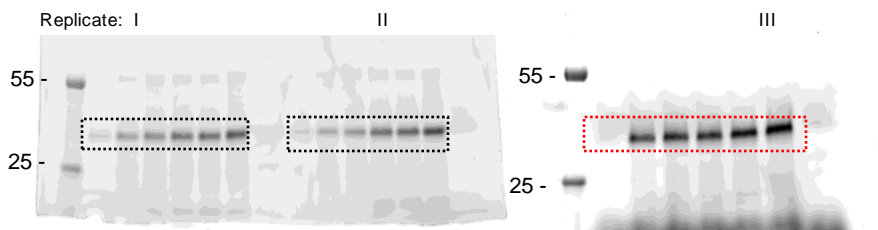
## YnPalm Dose Response – In-Gel Fluorescence



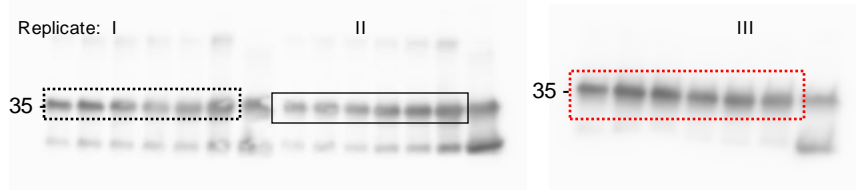
### 1A YnPalm Dose Response – Westerns



### 1E YnPalm Time Course – In-Gel Fluorescence

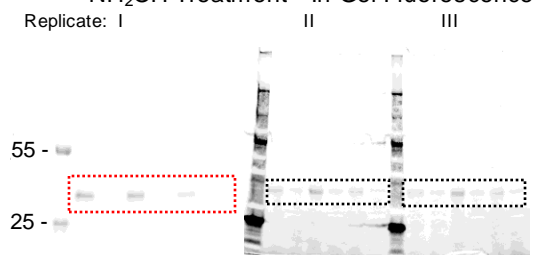


### YnPalm Time Course – Westerns

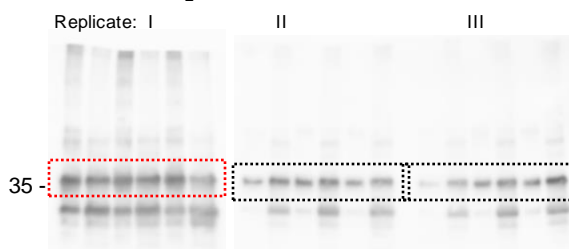


## Extended Data Figure 1C

### NH<sub>2</sub>OH Treatment – In-Gel Fluorescence



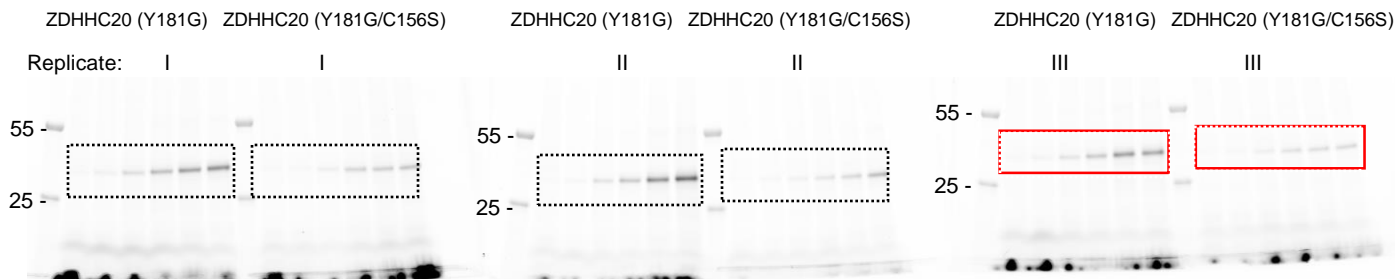
### NH<sub>2</sub>OH treatment – Westerns



## Extended Data Figure 1G

### YnPalm Dose Response

#### In-gel Fluorescence



#### Western blots

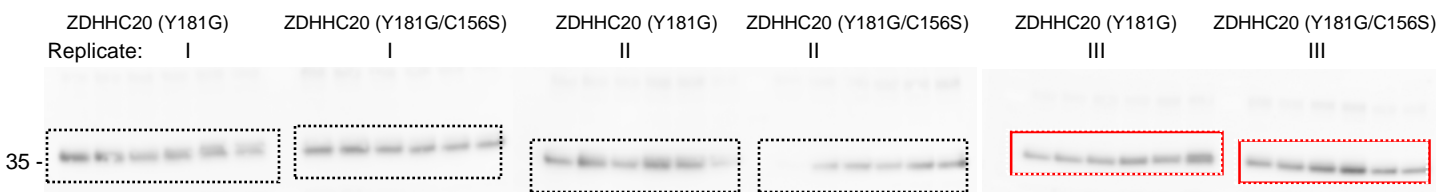


Figure A20. Uncropped gels and blots for Extended Data Figure 1A,C,E,G. Areas within red borders are in the supplementary figure.

# Extended Data Figure 1I and K

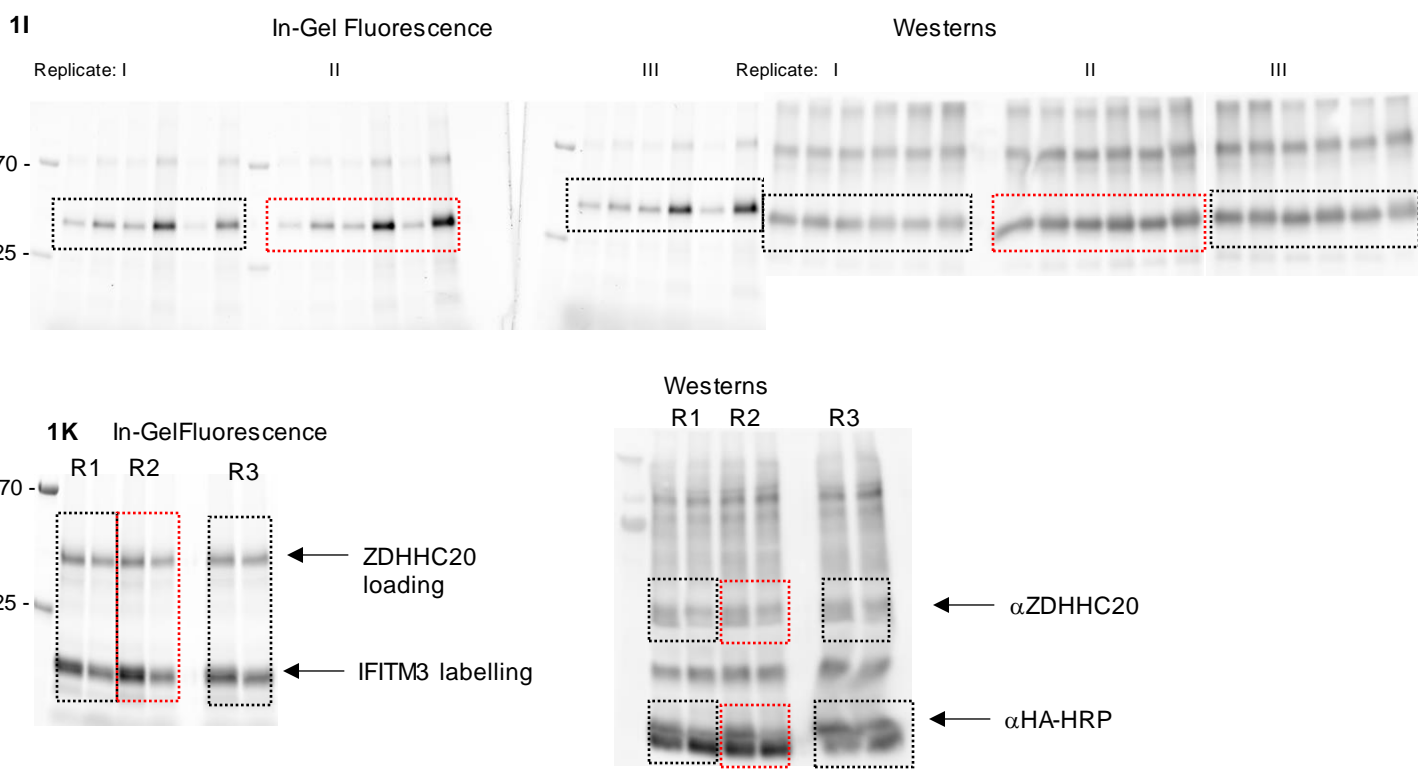
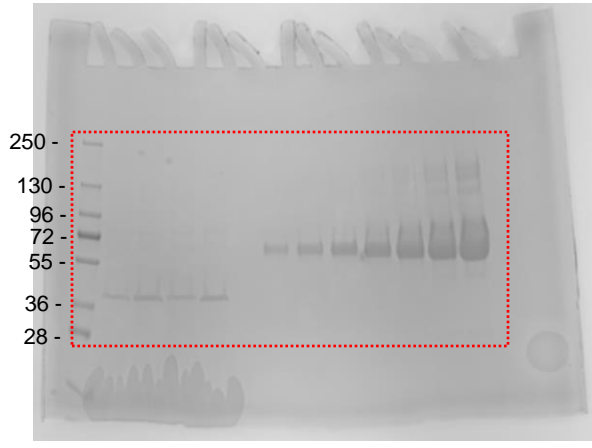


Figure A21. Uncropped gels and blots for Extended Data Figure 1I,K. Areas within red borders are in the supplementary figure.

# Extended Data Figure 2A

Replicate 1



Replicate 3

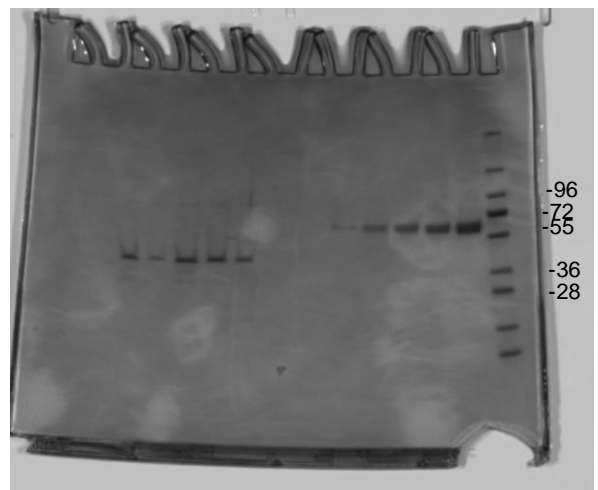
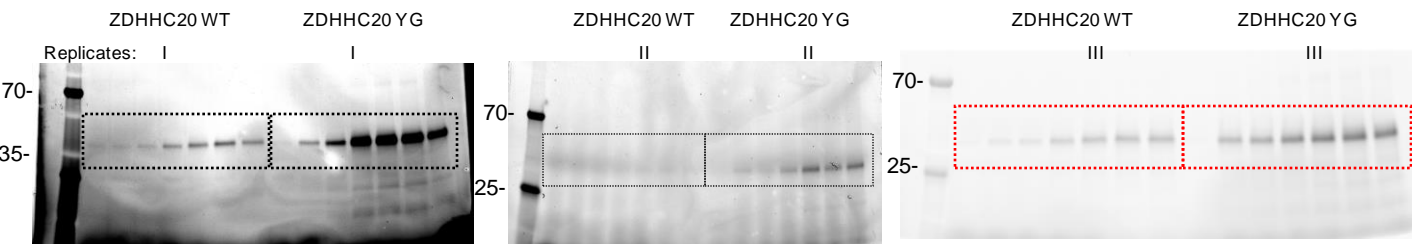


Figure A22. Uncropped gels and blots for Extended Data Figure 2A. Areas within red borders are in the supplementary figure.

## Extended Data Figure 3A

### 18-Bz Dose Response In-Gel Fluorescence



### Westerns

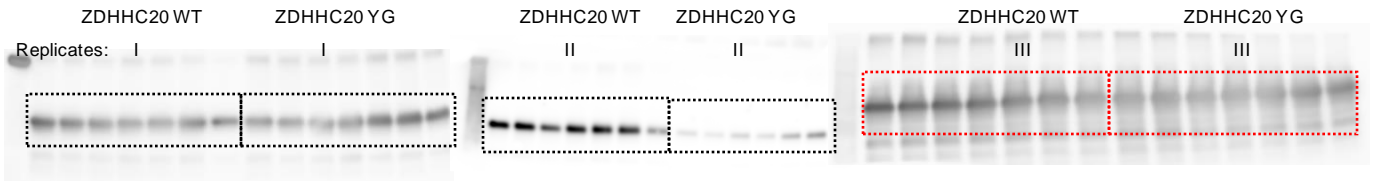
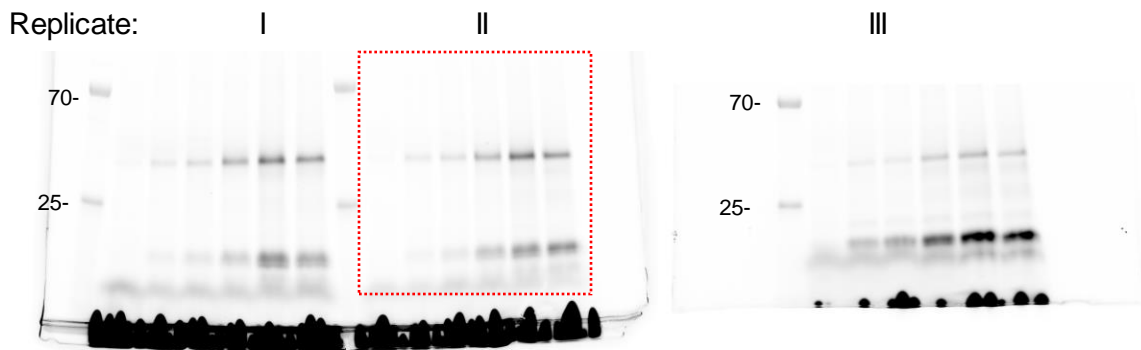


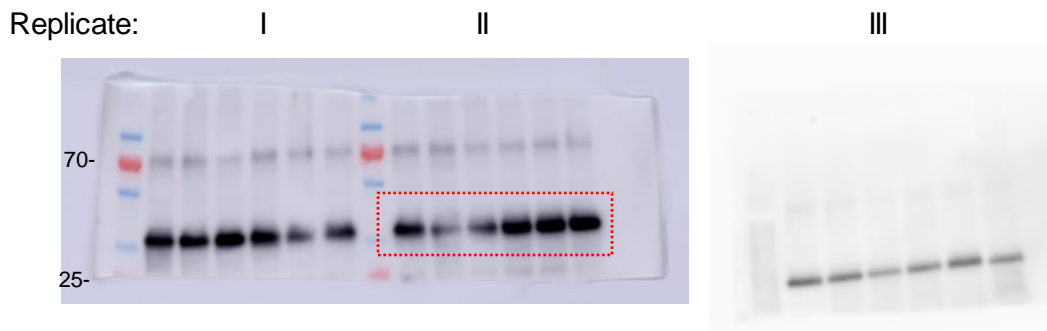
Figure A23. Uncropped gels and westerns for Supplementary Figure 3A. Areas within red borders are in the supplementary figure.

## Extended Data Figure 3C

### 18-Bz Time Course (Y181G) In-gel Fluorescence



### Western-blot (Flag-IP, anti-zDHHC20)



### Western-blot (HA-IP, anti-HA)

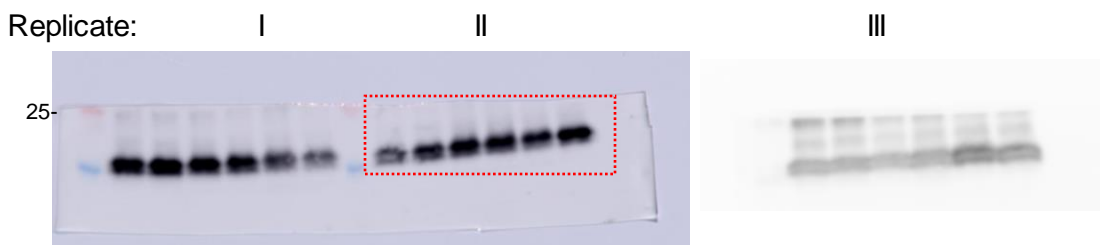


Figure A24. Uncropped gels and westerns for Supplementary Figure 3C. Areas within red borders are in the supplementary figure. Replicates I and II for HA-IP and Flag-IP are overlays of the chemiluminescence scan and the white light image.

# Extended Data Figure 6A

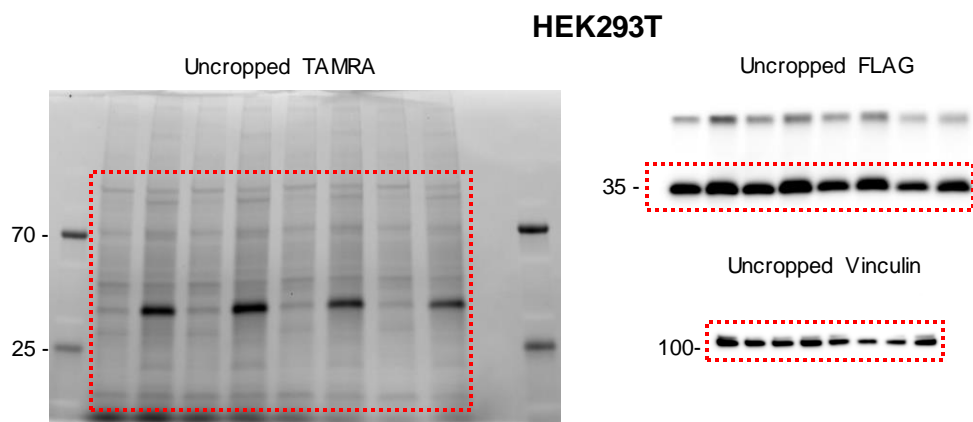


Figure A25. Uncropped gels and westerns for Extended Data Figure 6A. Areas within red borders are within the supplementary figure.

# Extended Data Figure 6D

## PANC1

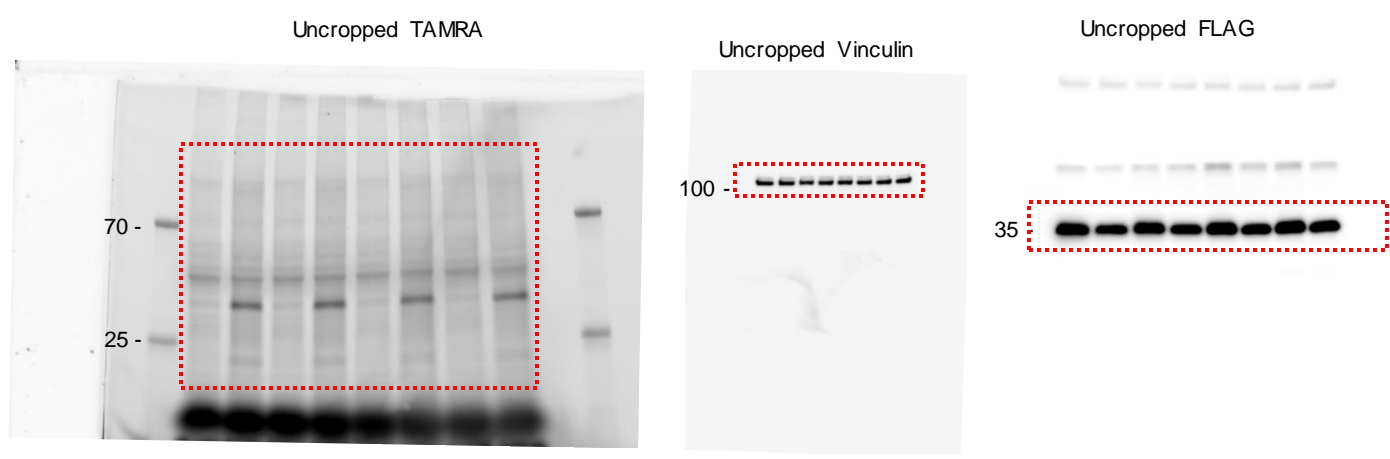


Figure A26. Uncropped gels and westerns for Extended Data Figure 6D. Areas within red borders are in the supplementary figure.

# Extended Data Figure 6E

## MDA-MB-231

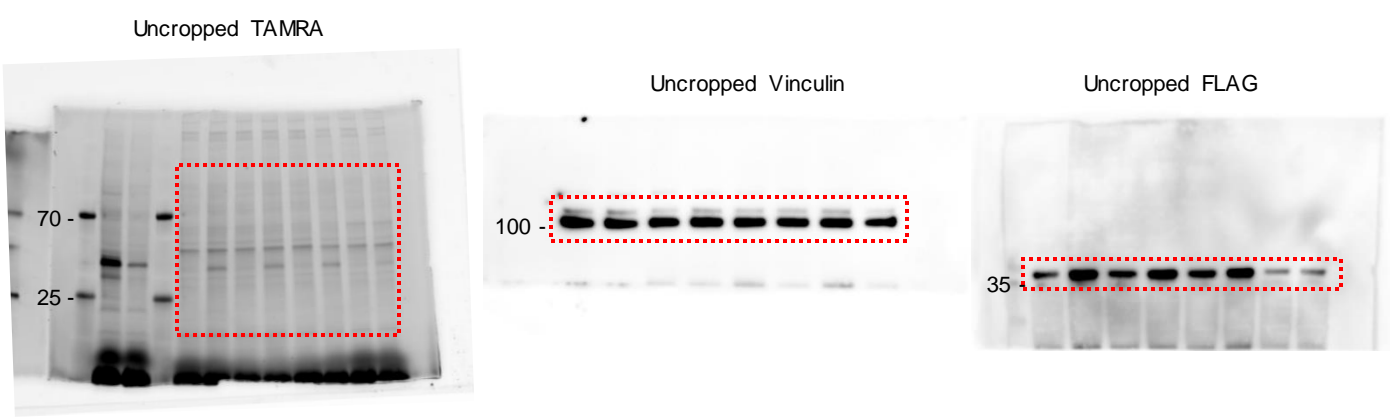


Figure A27. Uncropped gels and westerns for Extended Data Figure 6E. Areas within red borders are in the supplementary figure.



**Extended Data Figure 3E**

18-Bz: Time Course  
In-Gel Fluorescence

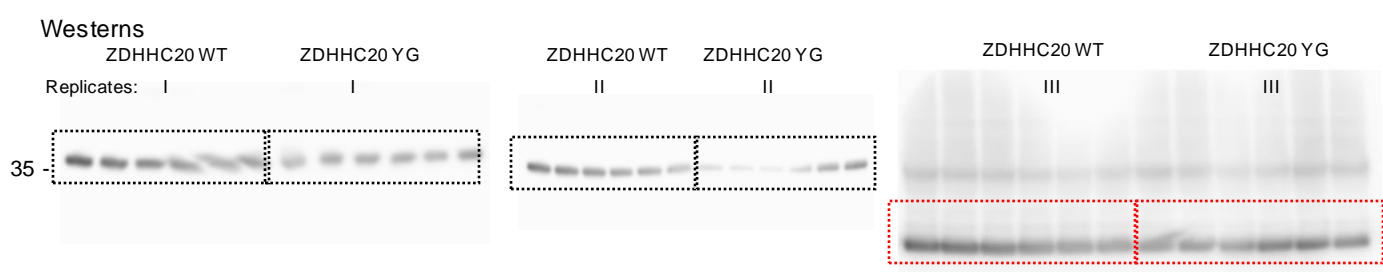
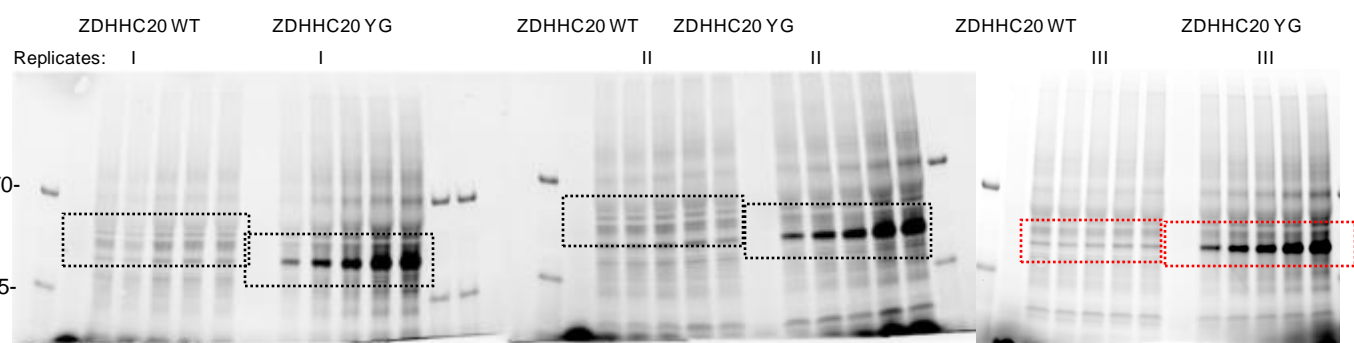


Figure A28. Uncropped gels and westerns for Extended Data Figure 3E. Areas within red borders are in the supplementary figure.

**Extended Data Figure 3G**

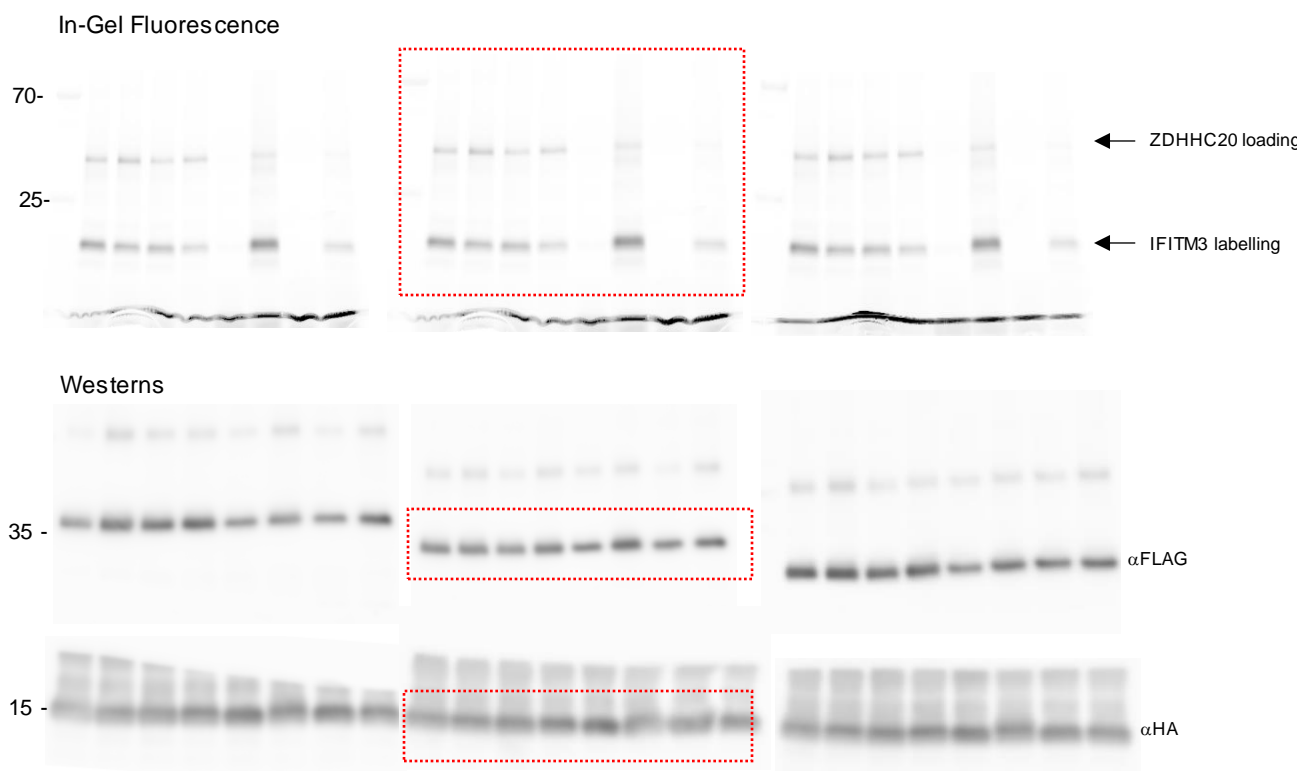


Figure A29. Uncropped gels and westerns for Extended Data Figure 3G. Areas within red borders are in the supplementary figure.

# Extended Data Figure 6G

Validation of HA-PTRH2 S-acylation by ZDHHC20

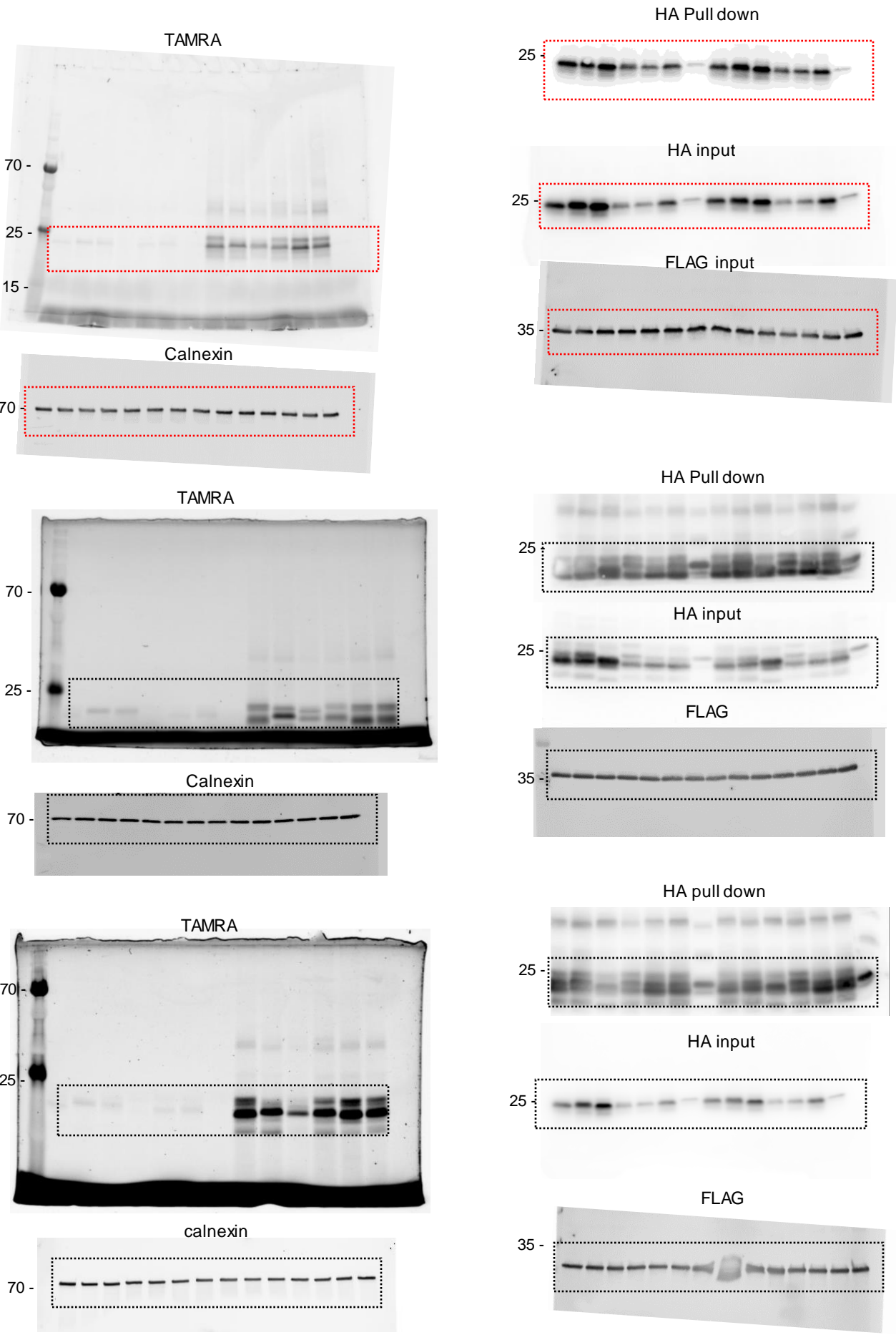


Figure A30. Uncropped gels and westerns for Extended Data Figure 6G. Areas within red borders are in the supplementary figure.

### Extended Data Figure 7A



Figure A31. Uncropped westerns for Extended Data Figure 7A. Areas within red borders are in the supplementary figure.

### Extended Data Figure 7B

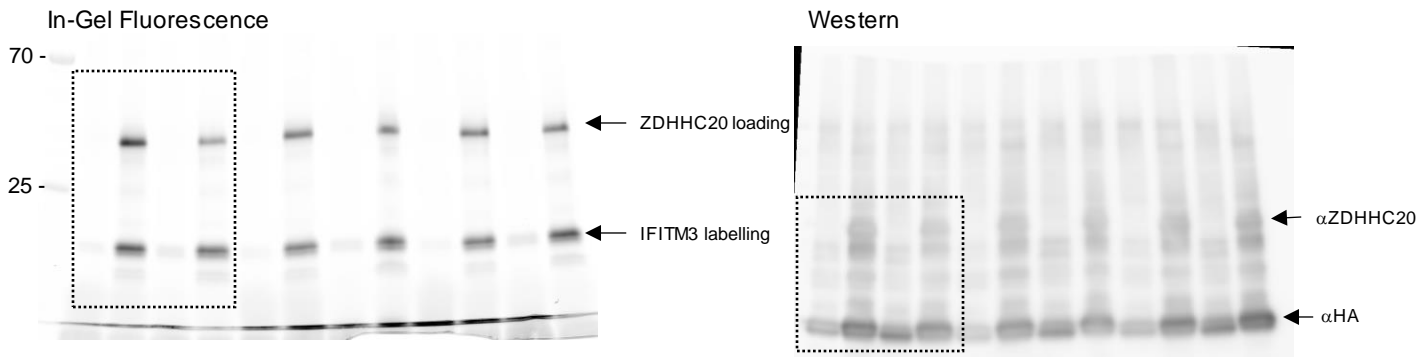


Figure A32. Uncropped gels and westerns for Extended Data Figure 7B. Areas within red borders are in the supplementary figure.

### Extended Data Figure 7D

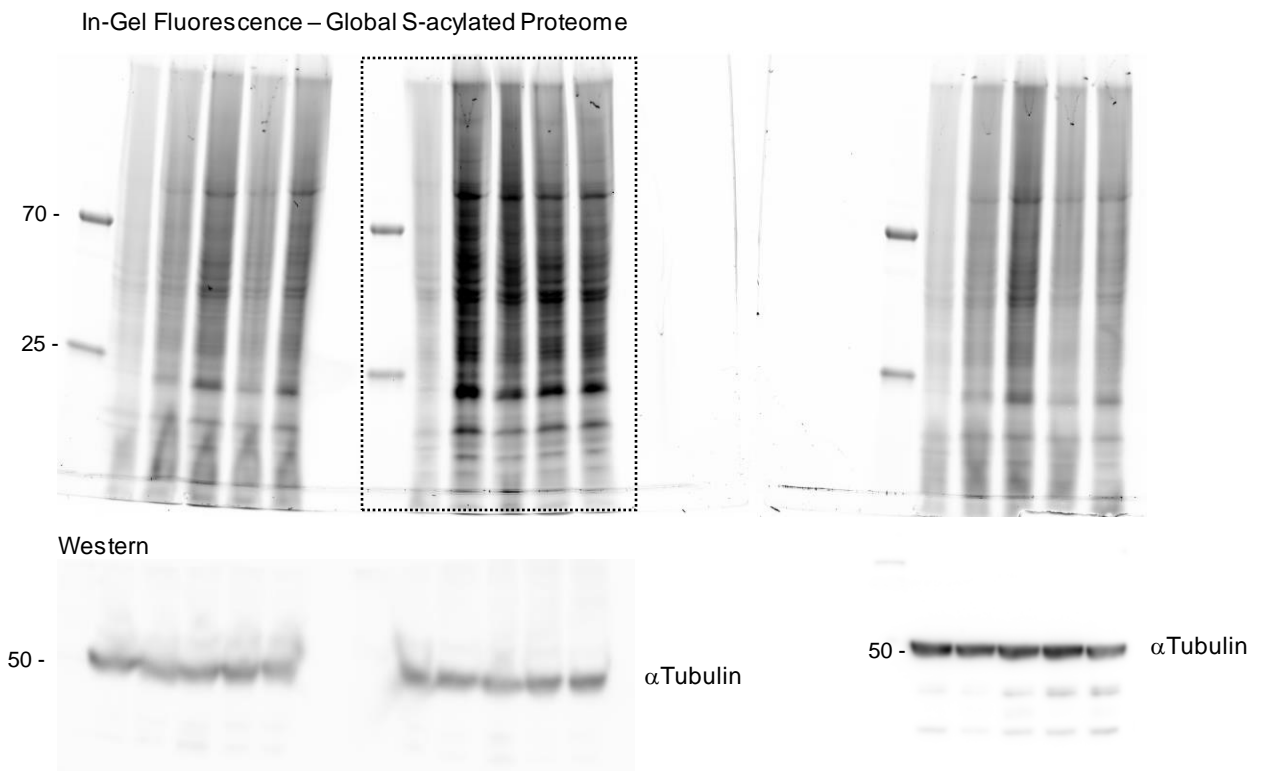
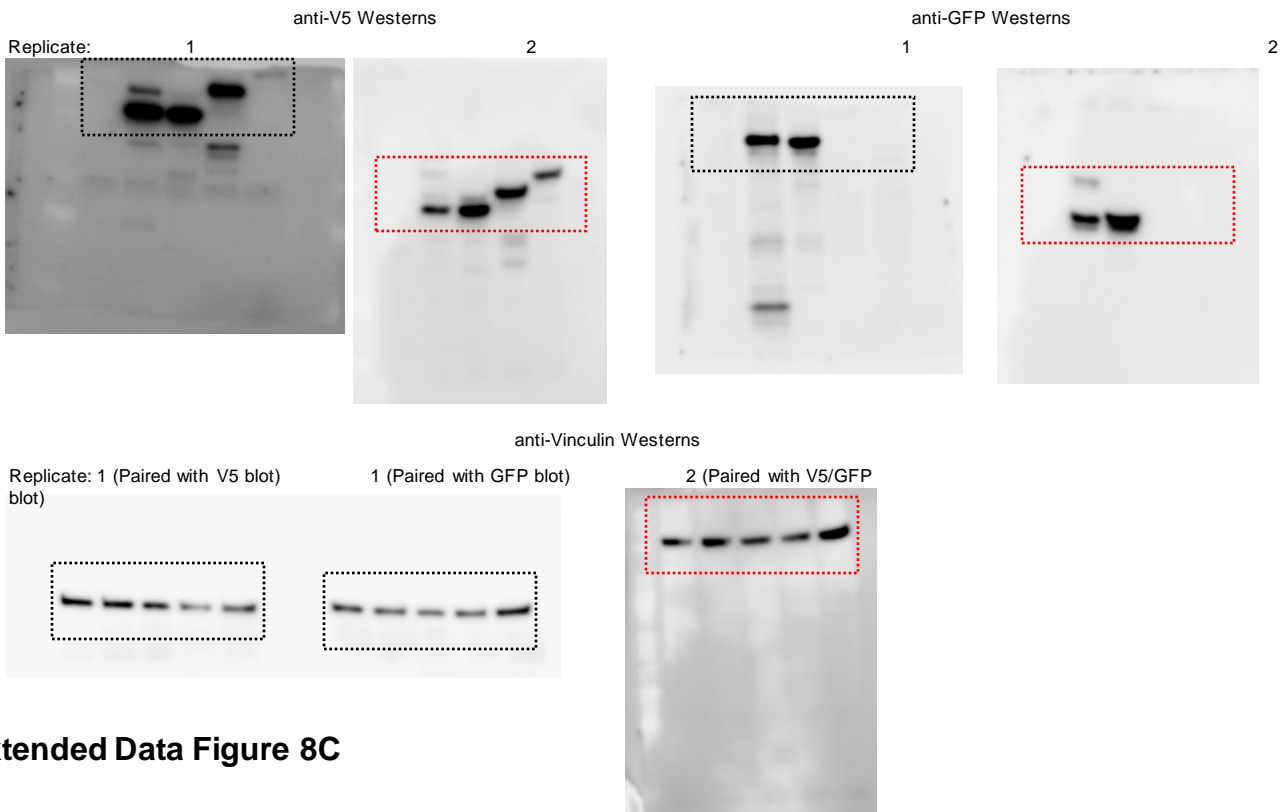


Figure A33. Uncropped gels Extended Data Figure 7D. Areas within red borders are in the supplementary figure.

# Extended Data Figure 8B



# Extended Data Figure 8C

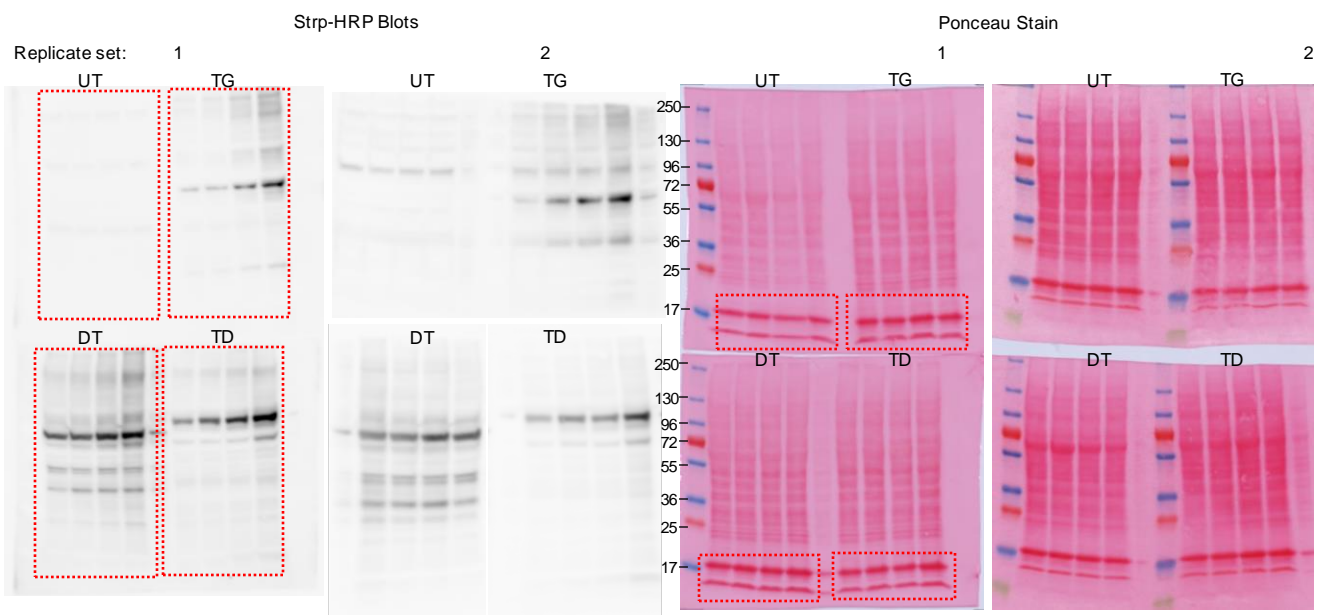
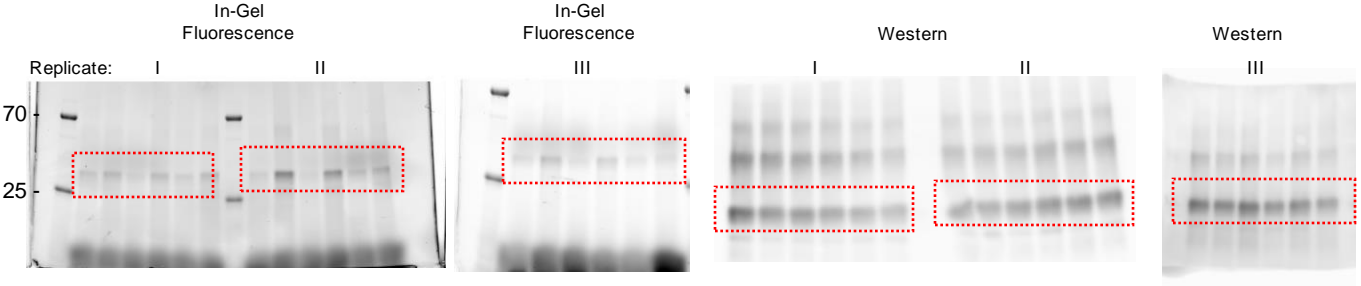


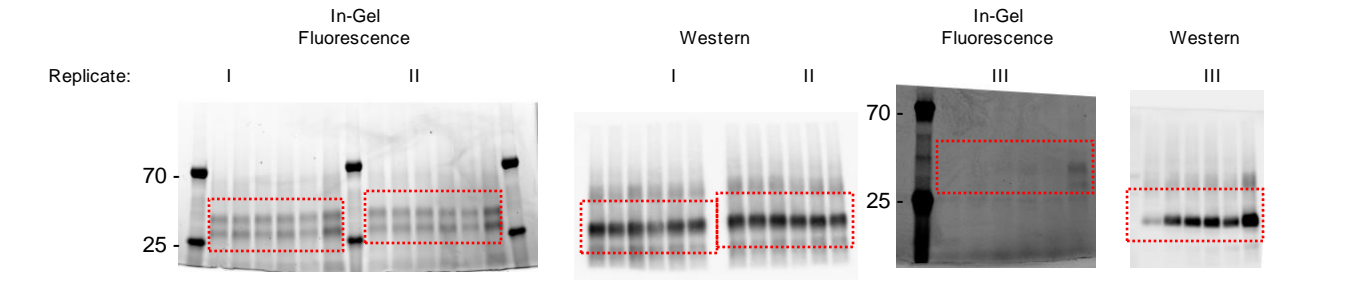
Figure A34. Uncropped westerns and blots for Extended Data Figure 8B-C. Areas within red borders are in the supplementary figure.

# Extended Data Figure 9A-G

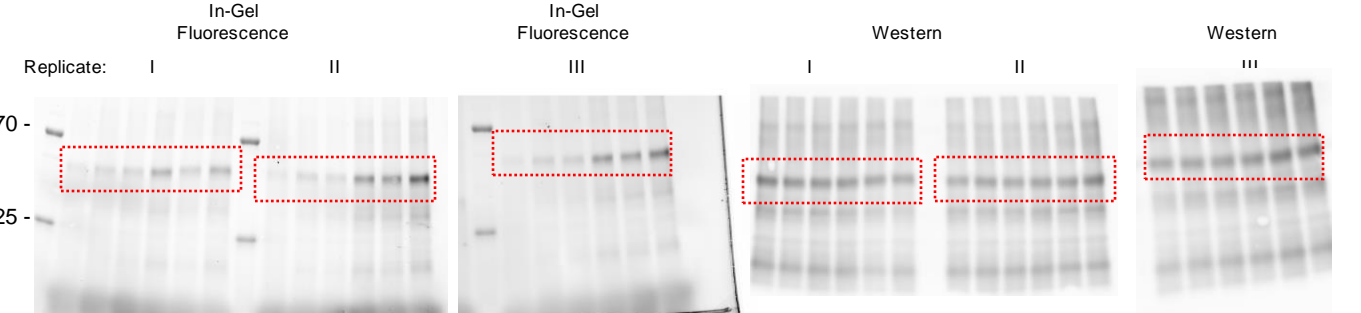
FLAG-ZDHHC3 (41 kDa): WT vs M (I182G)



FLAG-ZDHHC7 (38 kDa): WT vs M (L57G)



FLAG-ZDHHC11 (49 kDa): WT vs M (M181A)



FLAG-ZDHHC15 (39 kDa): WT vs M (Y184G)

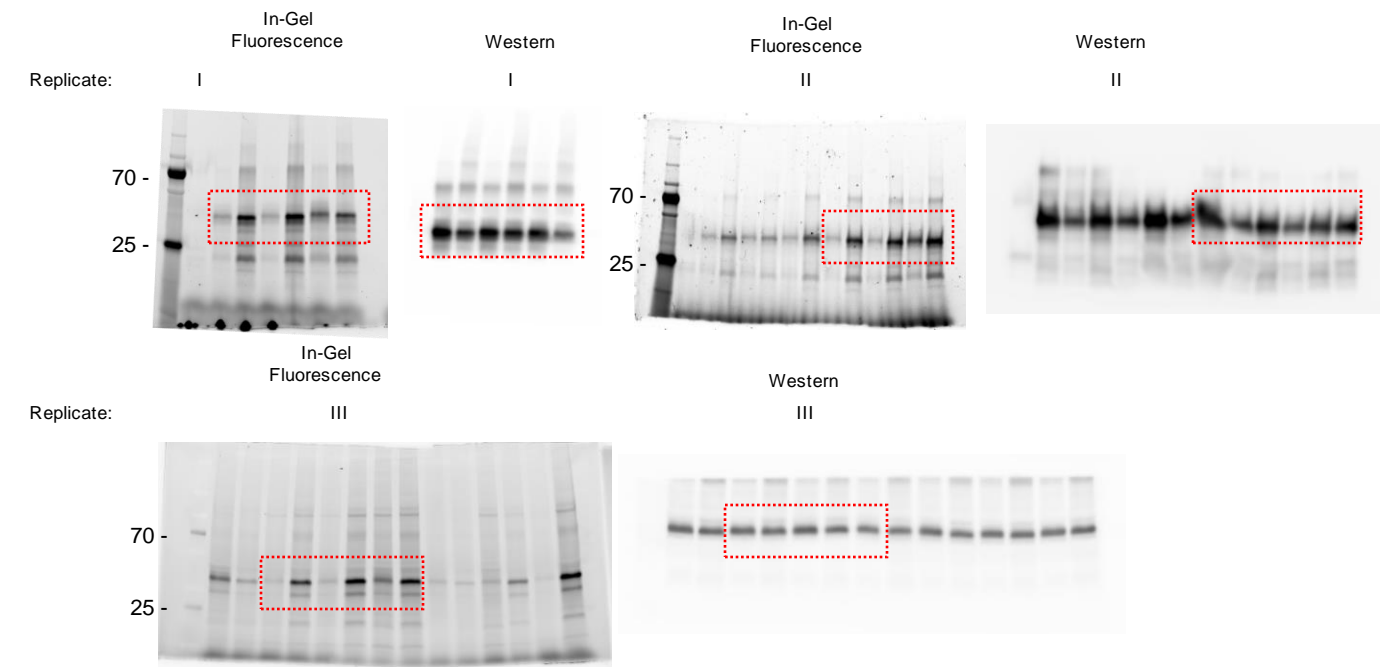


Figure A35. Uncropped gels and westerns for Extended Data Figure 9A-G. Areas within red borders are in the supplementary figure.

# Extended Data Figure 9I

ZDHHC7, ZDHHC15, and ZDHHC 3 and ZDHHC11 NH<sub>2</sub>OH treatment

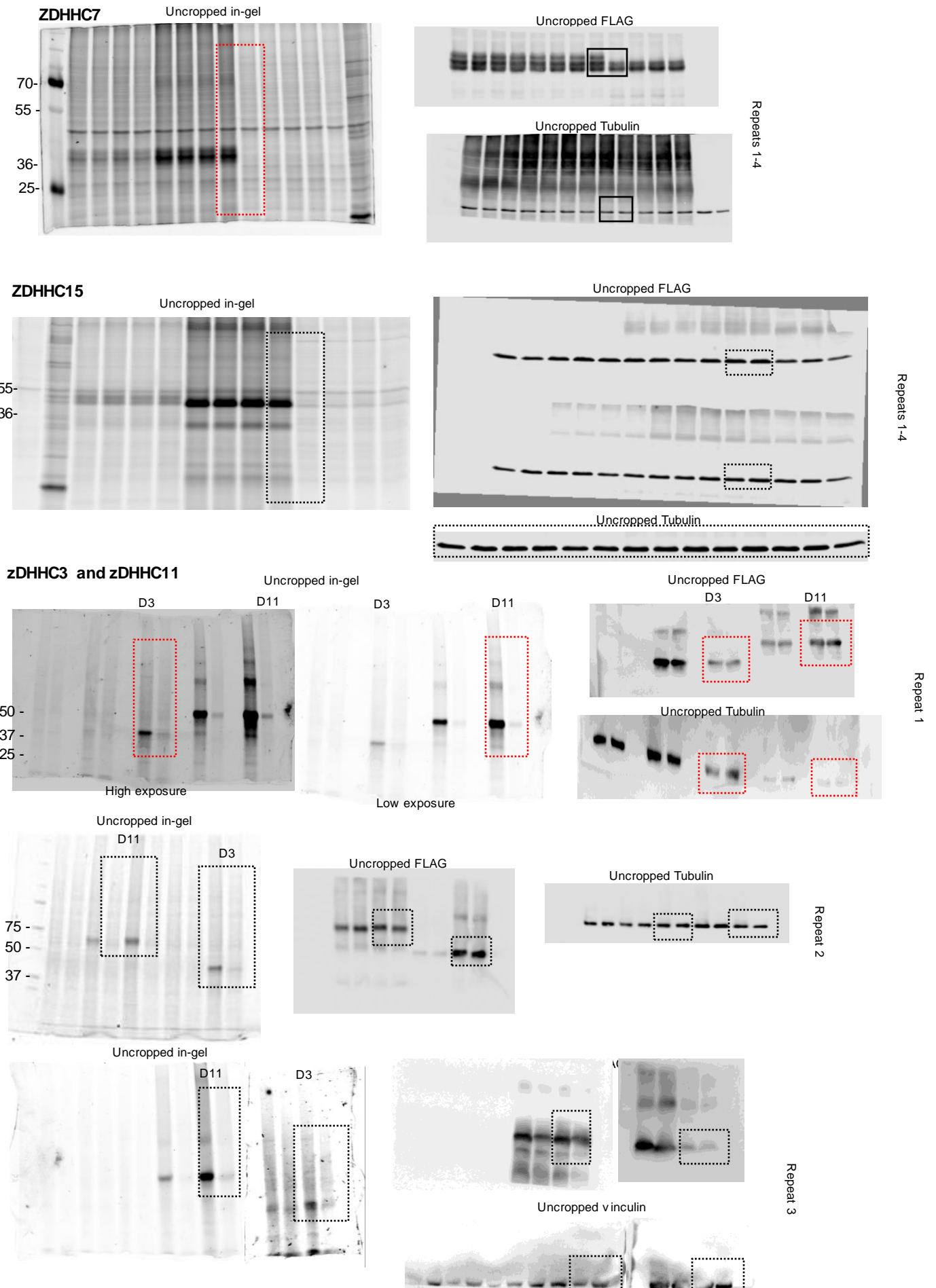


Figure A36. Uncropped gels and westerns for Extended Data Figure 8I. Areas within red borders are in the supplementary figure.

**Extended Data Figure 10 A-E**

**10A**

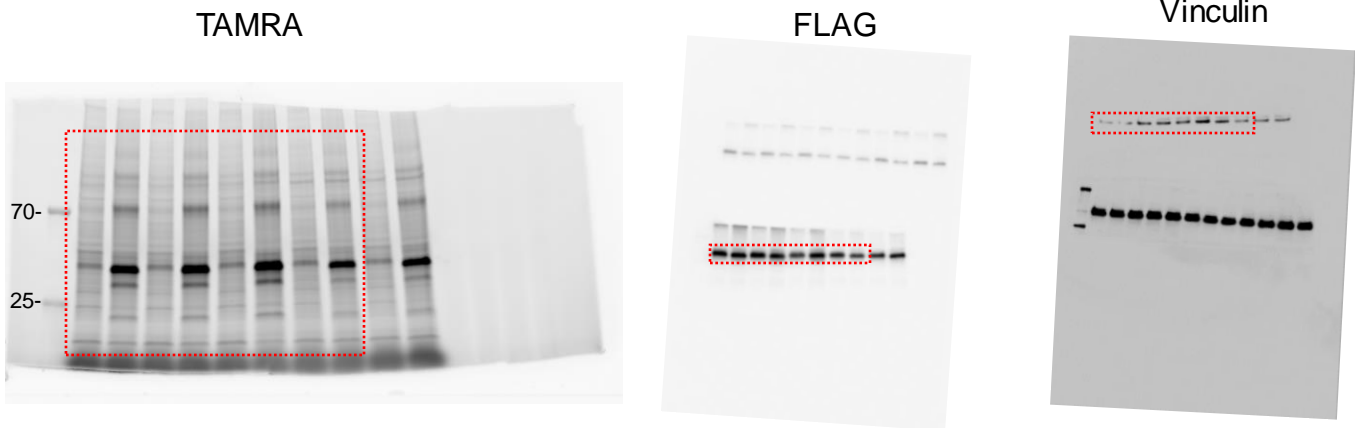


Figure A37. Uncropped gels and westerns for Extended Data Figure 9A. Areas within red borders are in the supplementary figure.

**10C**

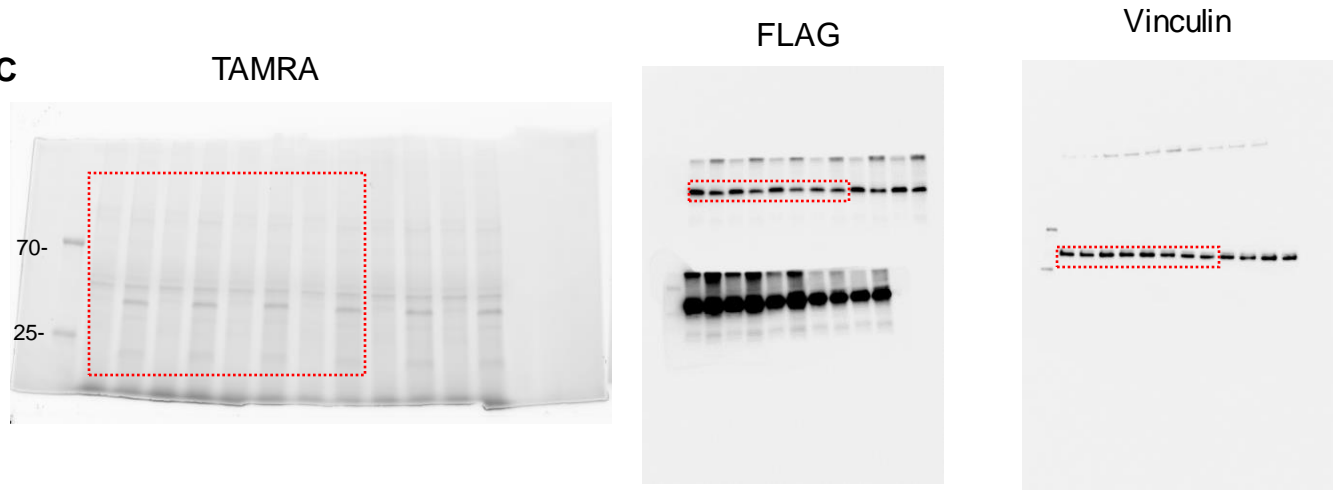


Figure A38. Uncropped gels and westerns for Extended Data Figure 9C. Areas within red borders are in the supplementary figure.

**10E**

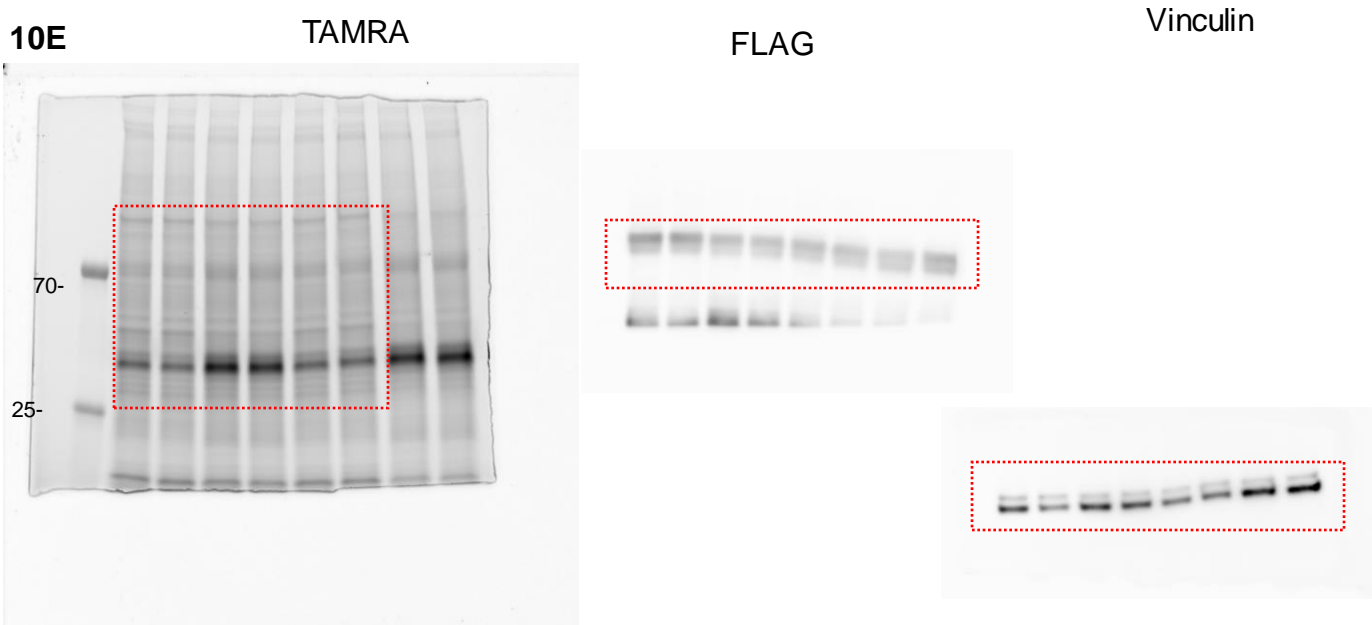


Figure A39. Uncropped gels and westerns for Extended Data Figure 9E. Areas within red borders are in the supplementary figure.