The laforin/malin E3-ubiquitin ligase complex ubiquitinates members of the Hsp70 and Hsp90 protein family.

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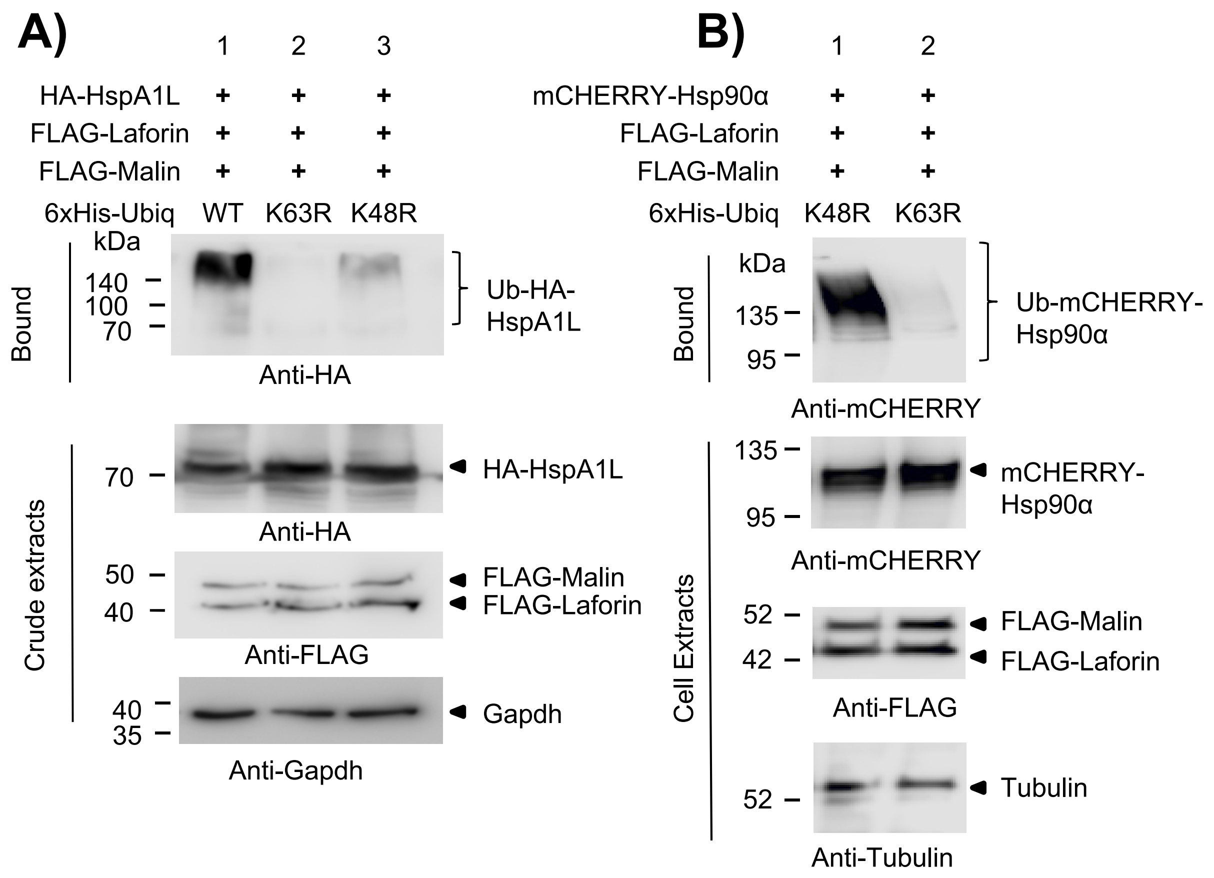
Supplementary Material

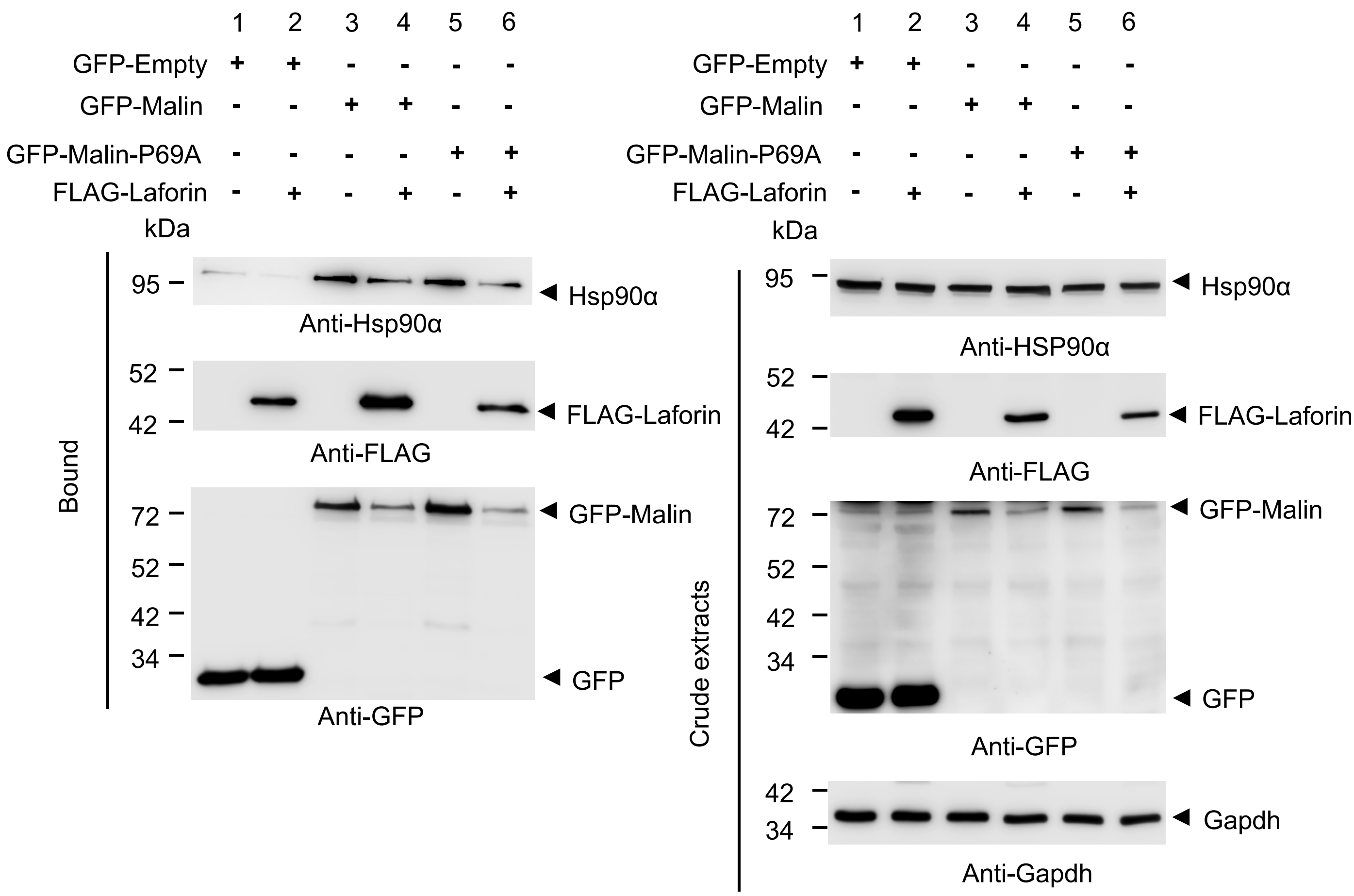
Supplementary Figure S1: Analysis of the topology of the ubiquitination reaction mediated by the laforin/malin complex in HA-HspA1L (A) and mCHERRY-Hsp90α (B). Ubiquitination reactions were performed as in the legend of Figure 2 but using modified forms of ubiquitin that carried K48R or K63R mutations, which prevent the formation of K48- or K63-linked chains, respectively. Representative blots of three independent experiments are shown

Supplementary Figure S2: The laforin/malin complex interacts physically with endogenous Hsp90α. HEK293 cells were co-transfected with the indicated combination of plasmids. Cells were lysed, and 1.5 mg of proteins were subjected to GFP-trap affinity purification. After washing, beads were boiled in loading buffer, and the purified proteins were analyzed by SDS PAGE and Western blot using anti-Hsp90α, anti-GFP, anti-FLAG, and anti-Gapdh antibodies, as indicated adjacent to the corresponding panels. Bound: proteins retained on the resin; Cell Extract: 30 μg of protein were loaded for the total fraction. Representative blots of three independent experiments are shown. See text for details.

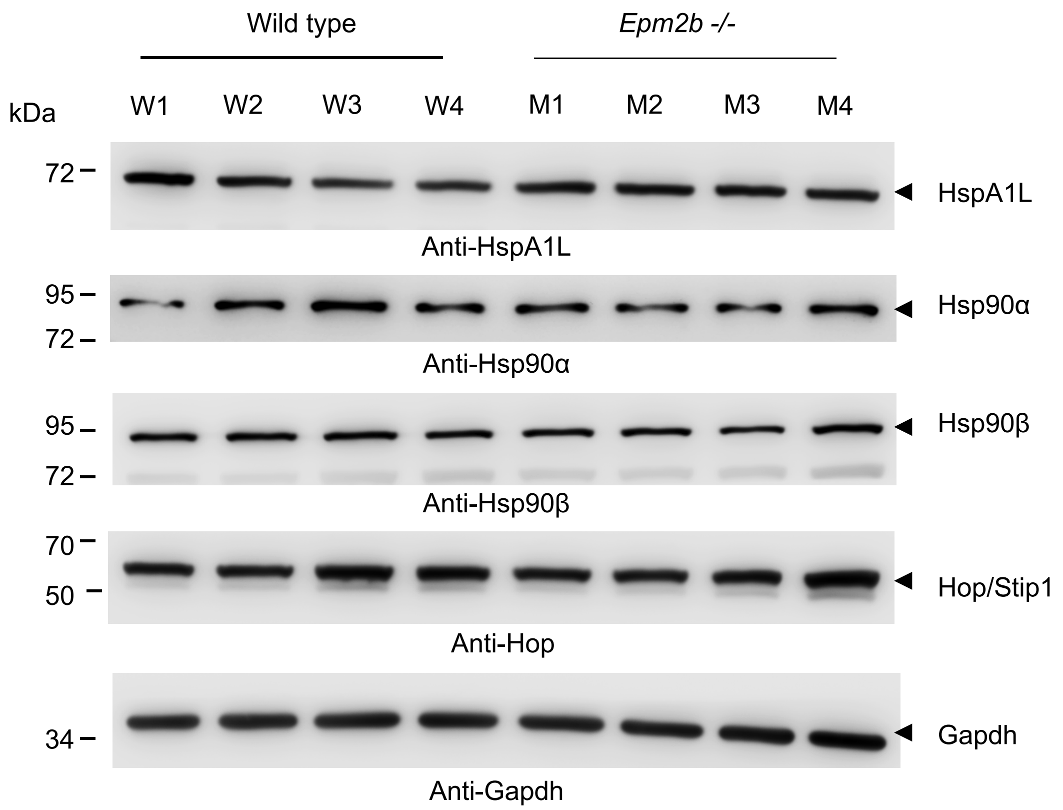
Supplementary Figure S3: Analysis of the levels of endogenous HspA1L, Hsp90α, Hsp90β and Hop/Stip1 in brain crude extracts from wild-type and Epm2b-/- mice of 16 months of age. Crude extracts were prepared as indicated in Materials and Methods and analyzed using the indicated antibodies. Four independent samples from wild-type (W1-W4) and *Epm2b-/-* (Malin-KO; M1-M4) mice were analyzed.



Supplementary Fig. S1



Supplementary Fig. S2



Supplementary Fig. S3

Full-length gels

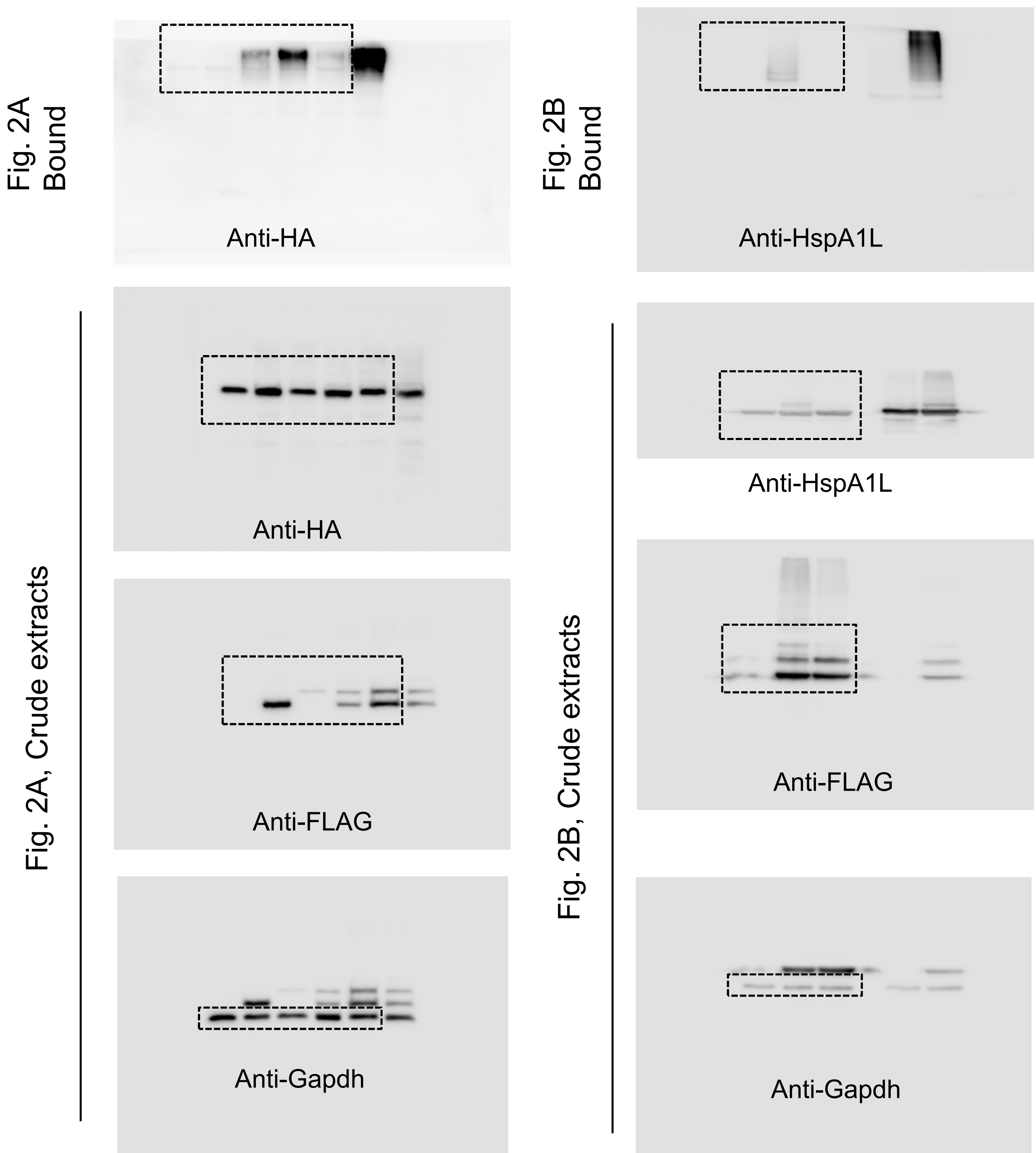


Fig. 2

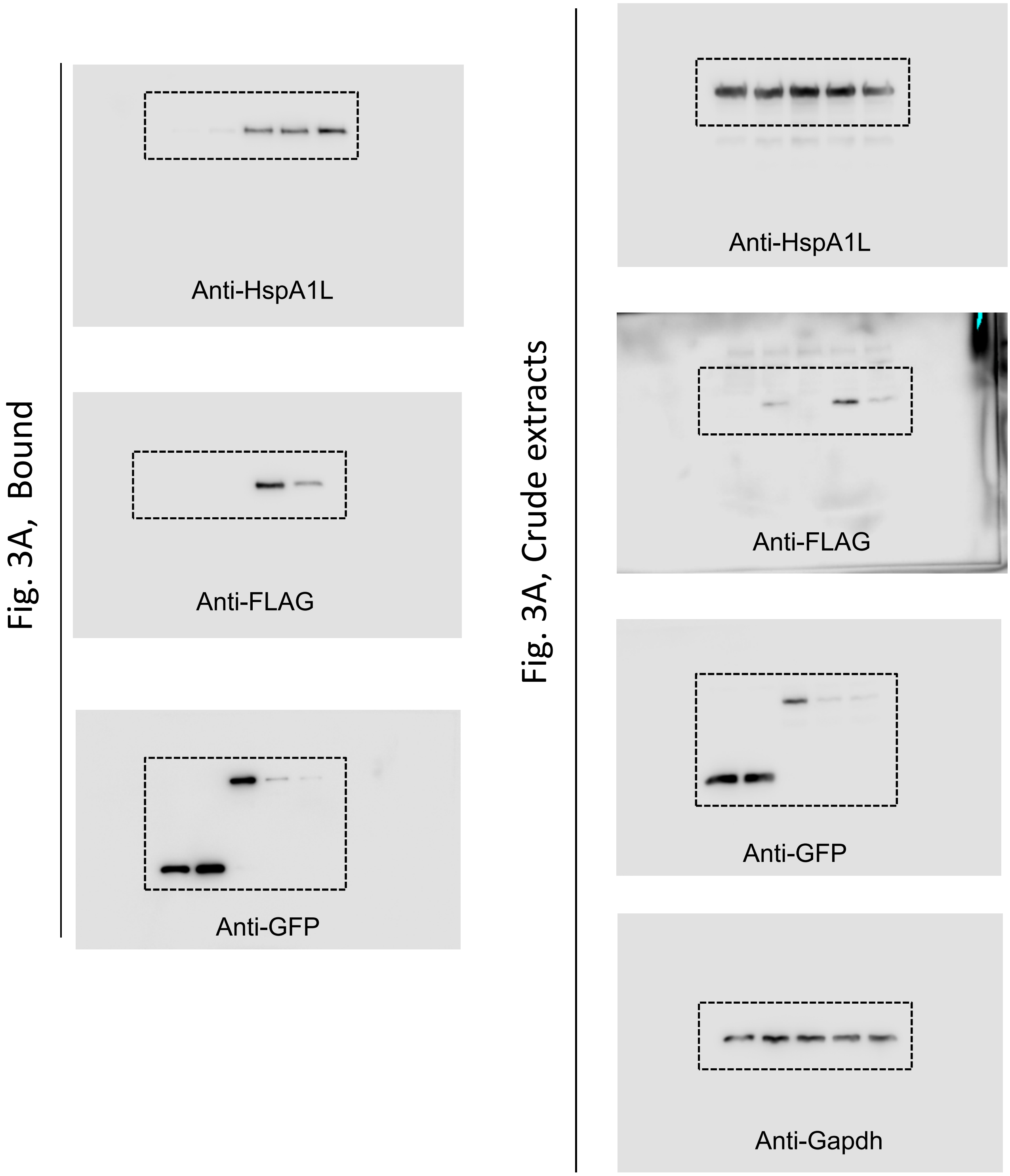


Fig. 3A, Bound
Anti-HspA1L
Anti-FLAG
Anti-GFP
Fig. 3A, Crude extracts
Anti-HspA1L
Anti-FLAG
Anti-GFP
Anti-Gapdh

Fig. 3A

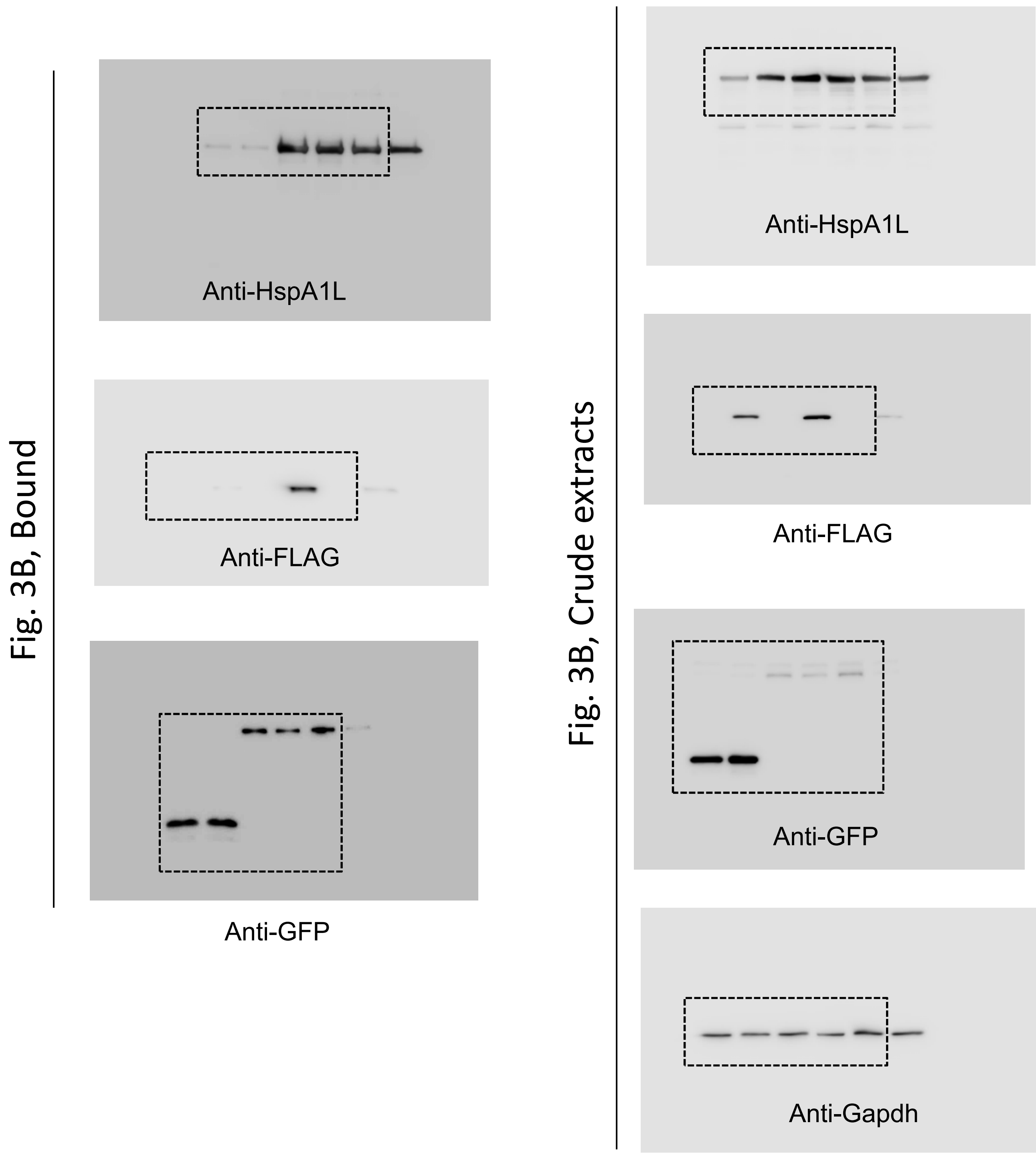


Fig. 3B, Bound
Anti-HspA1L
Anti-FLAG
Anti-GFP
Fig. 3B, Crude extracts
Anti-HspA1L
Anti-FLAG
Anti-GFP
Anti-Gapdh

Fig. 3B

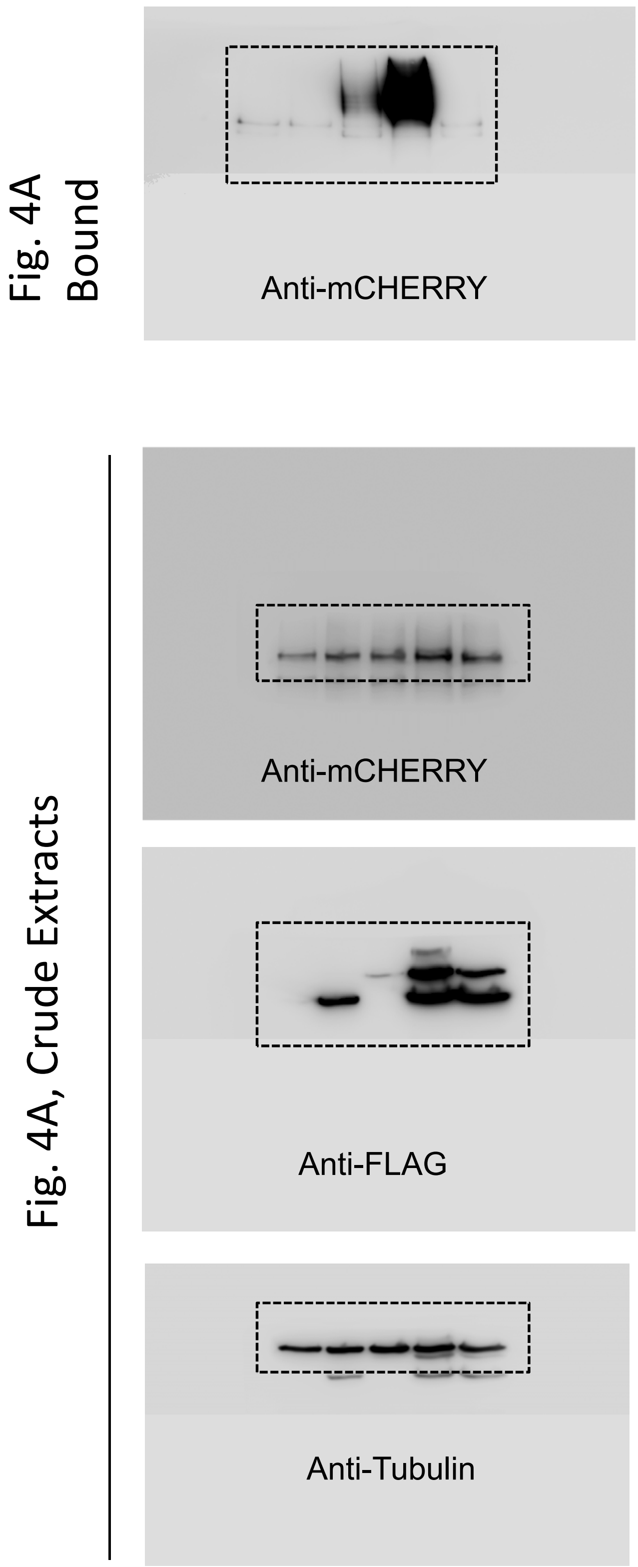


Fig. 4A
Bound
Anti-mCHERRY
Fig. 4A, Crude Extracts
Anti-mCHERRY
Anti-FLAG
Anti-Tubulin

Fig. 4A

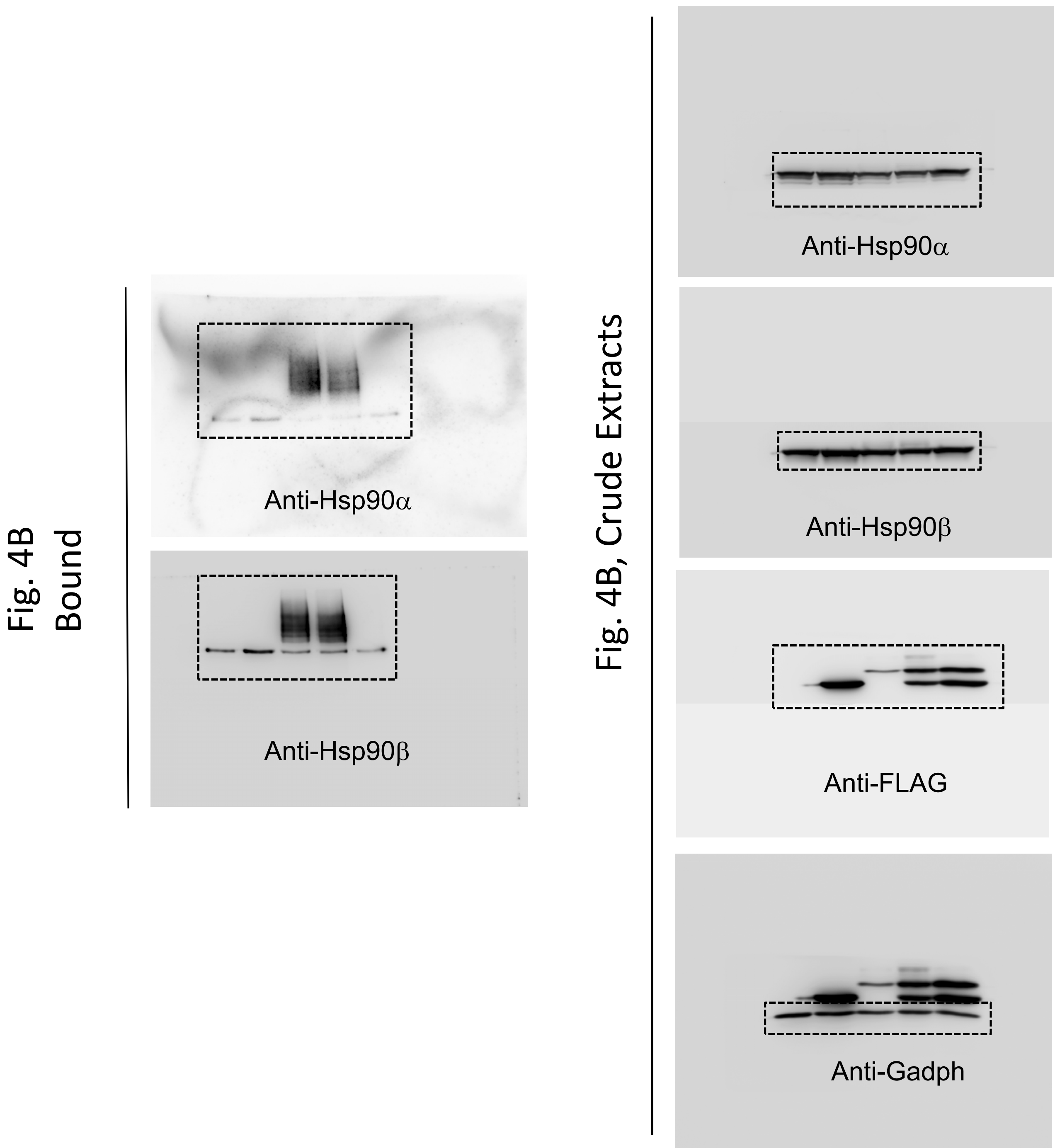


Fig. 4B
Bound
Anti-Hsp90α
Anti-Hsp90β
Fig. 4B, Crude Extracts
Anti-Hsp90α
Anti-Hsp90β
Anti-FLAG
Anti-Gadph

Fig. 4B

Fig. 5A, Bound

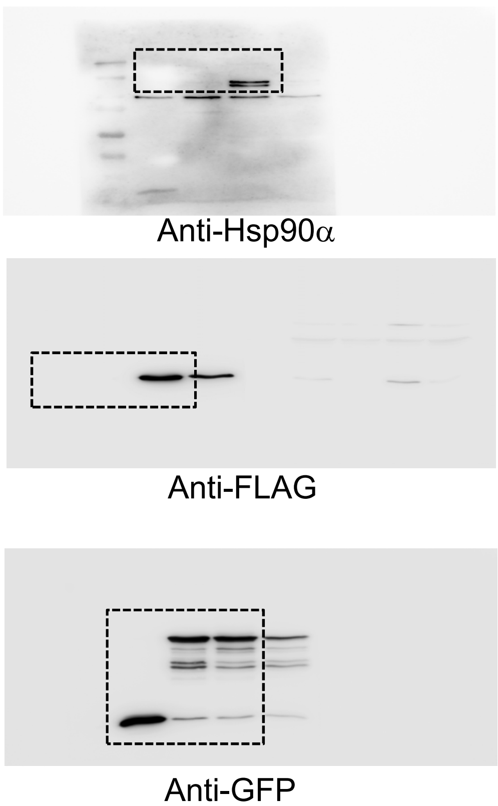


Fig. 5A, Crude Extracts

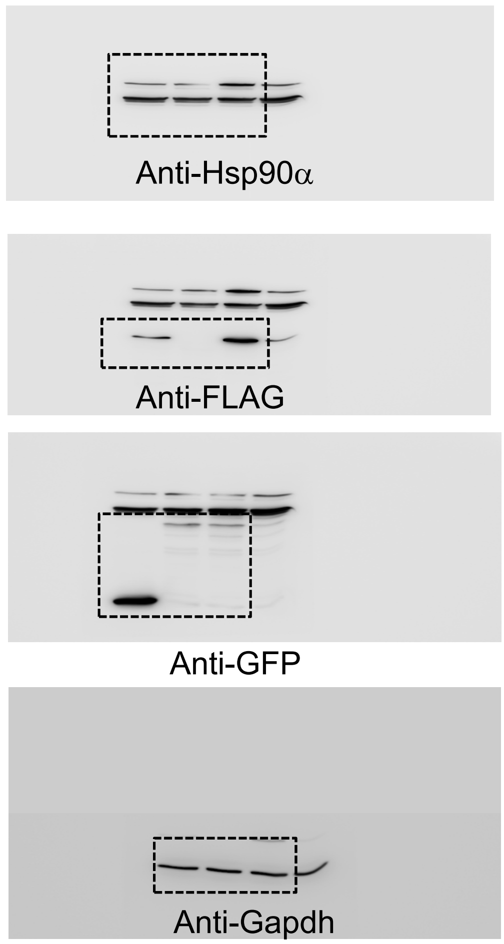


Fig. 5A

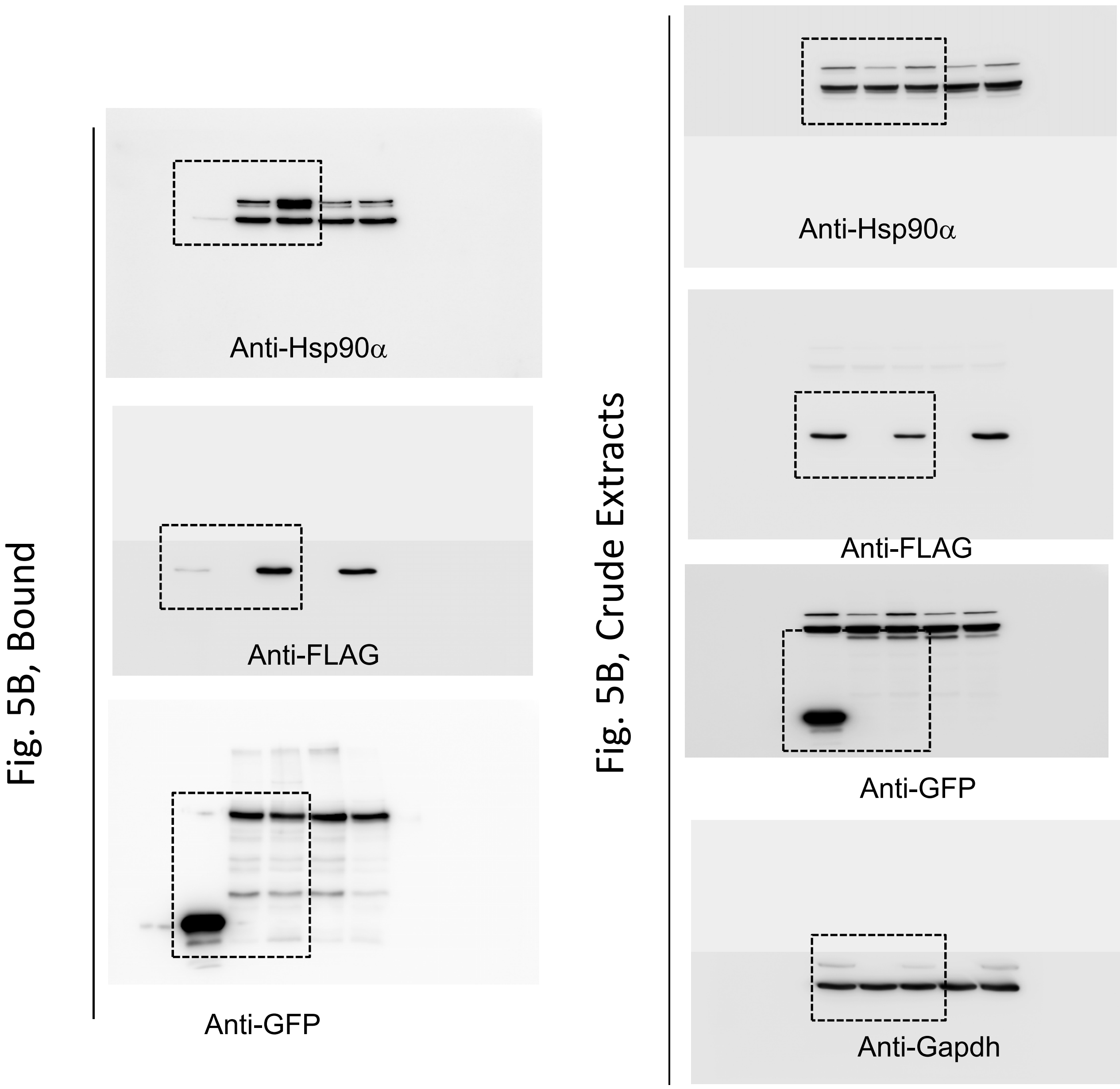


Fig. 5B, Bound
Anti-Hsp90α
Anti-FLAG
Anti-GFP
Fig. 5B, Crude Extracts
Anti-Hsp90α
Anti-FLAG
Anti-GFP
Anti-Gapdh

Fig. 5B

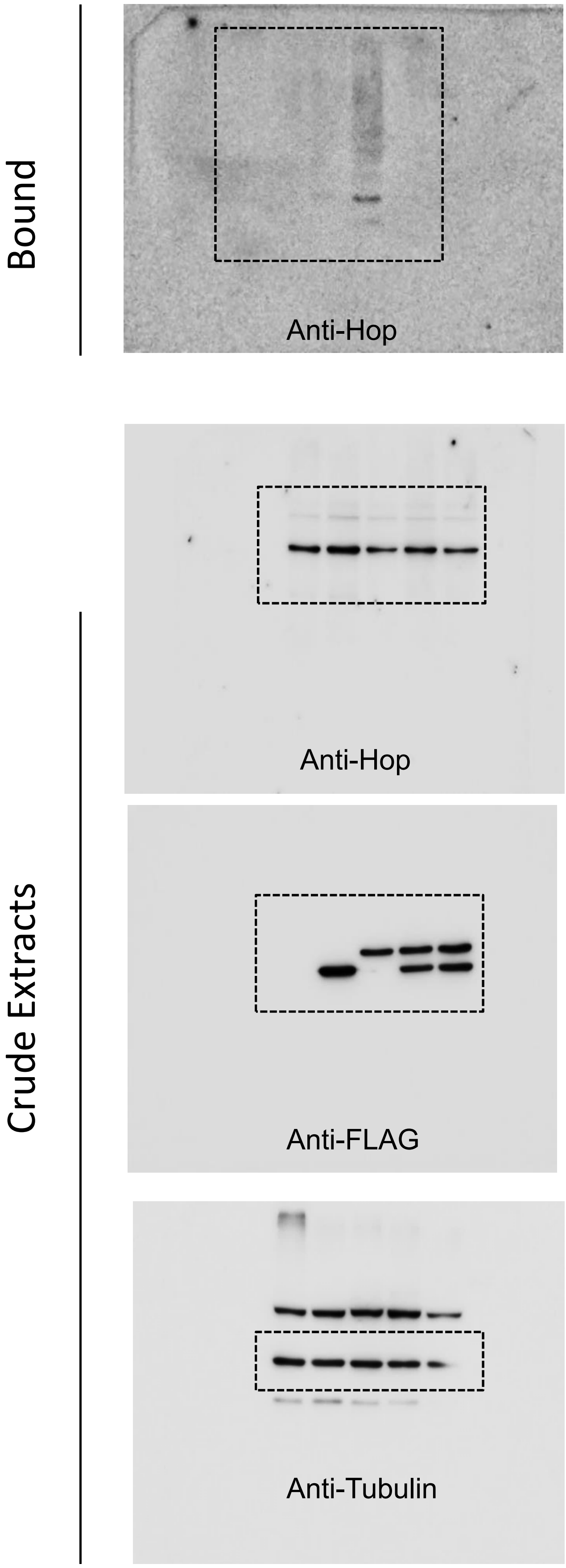

Bound
Crude Extracts
Anti-Hop
Anti-Hop
Anti-FLAG
Anti-Tubulin

Fig. 6

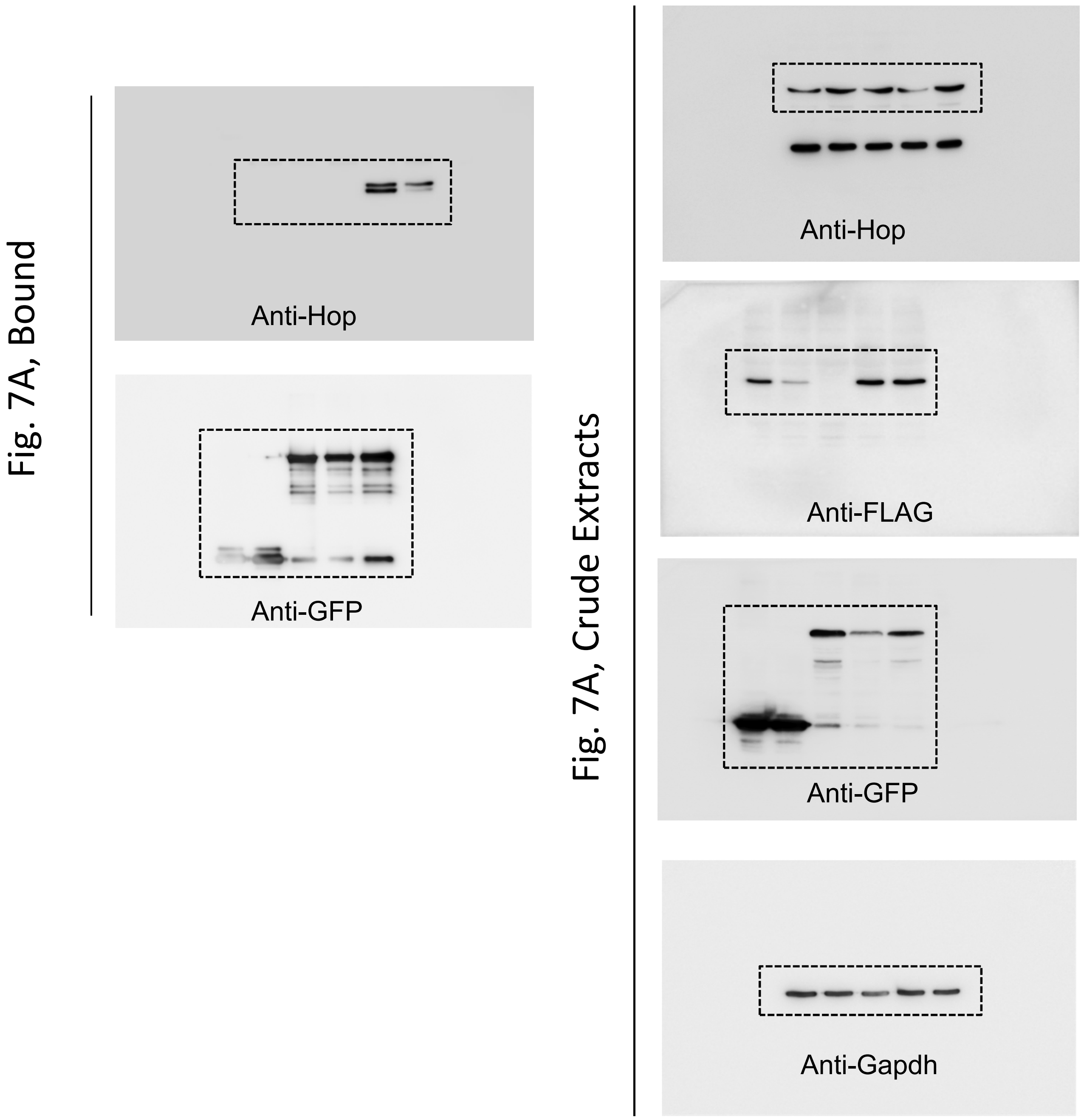


Fig. 7A, Bound
Anti-Hop
Anti-GFP
Fig. 7A, Crude Extracts
Anti-Hop
Anti-FLAG
Anti-GFP
Anti-Gapdh

Fig. 7A

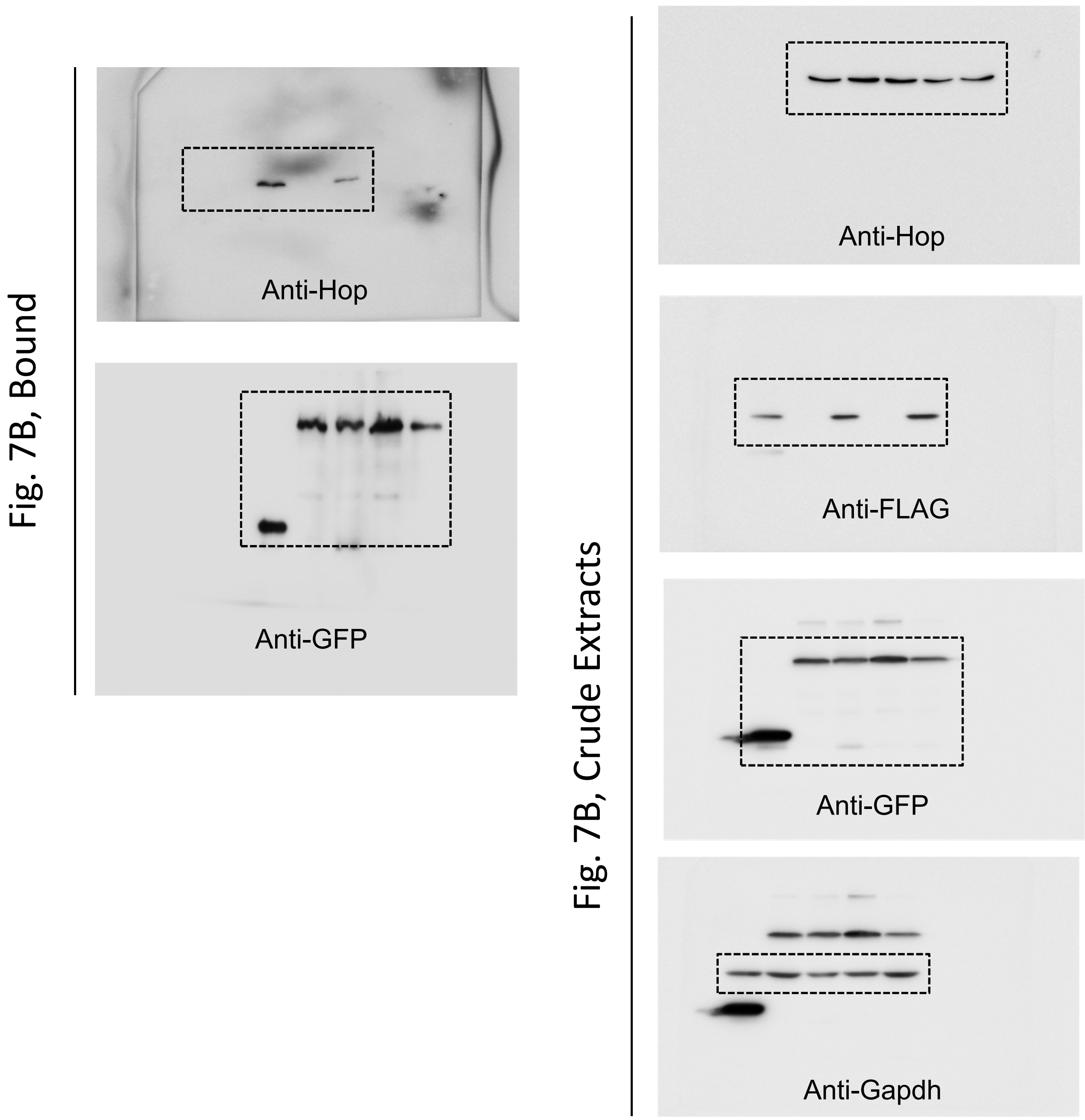
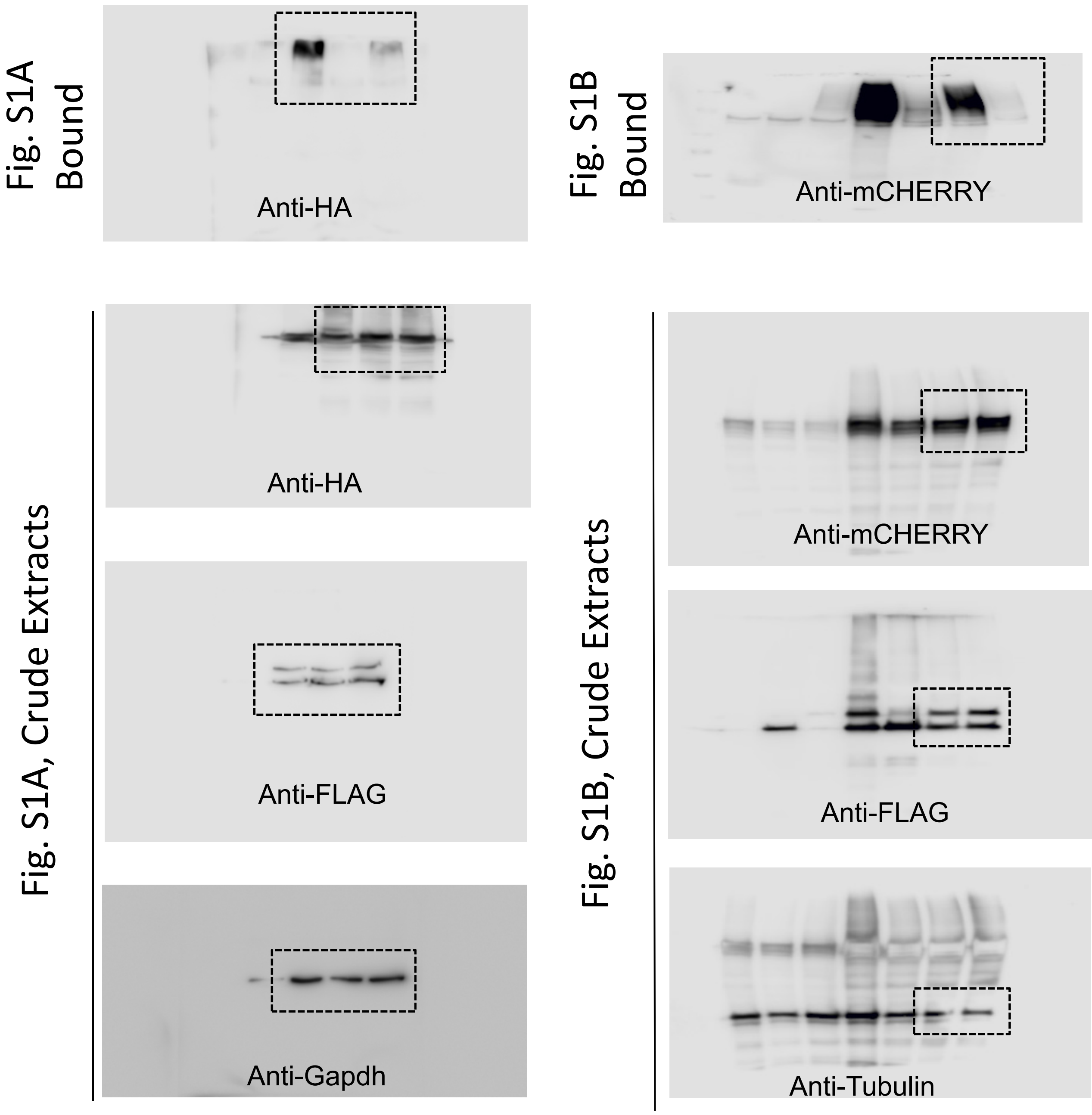
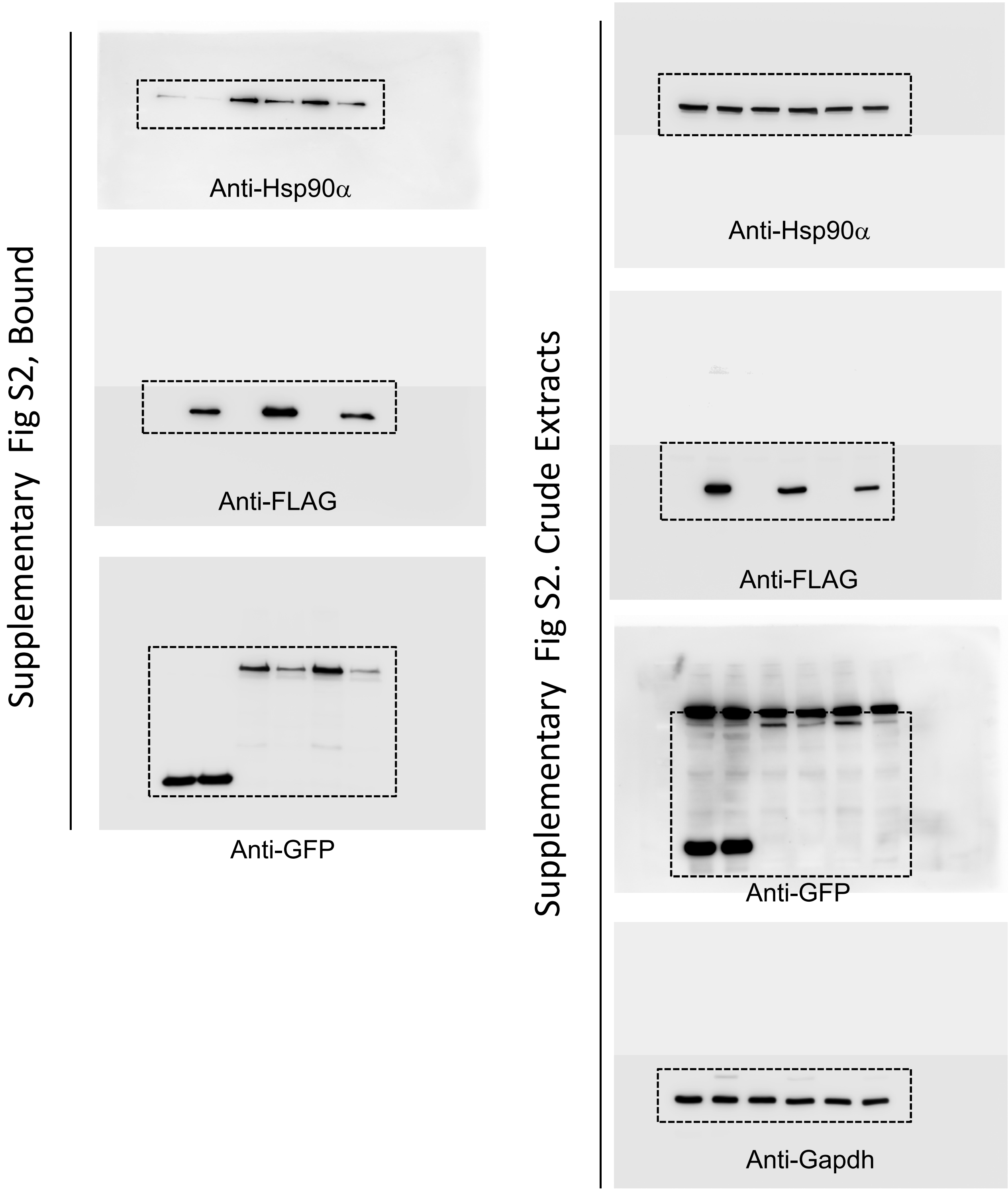


Fig. 7B, Bound
Anti-Hop
Anti-GFP
Fig. 7B, Crude Extracts
Anti-Hop
Anti-FLAG
Anti-GFP
Anti-Gapdh

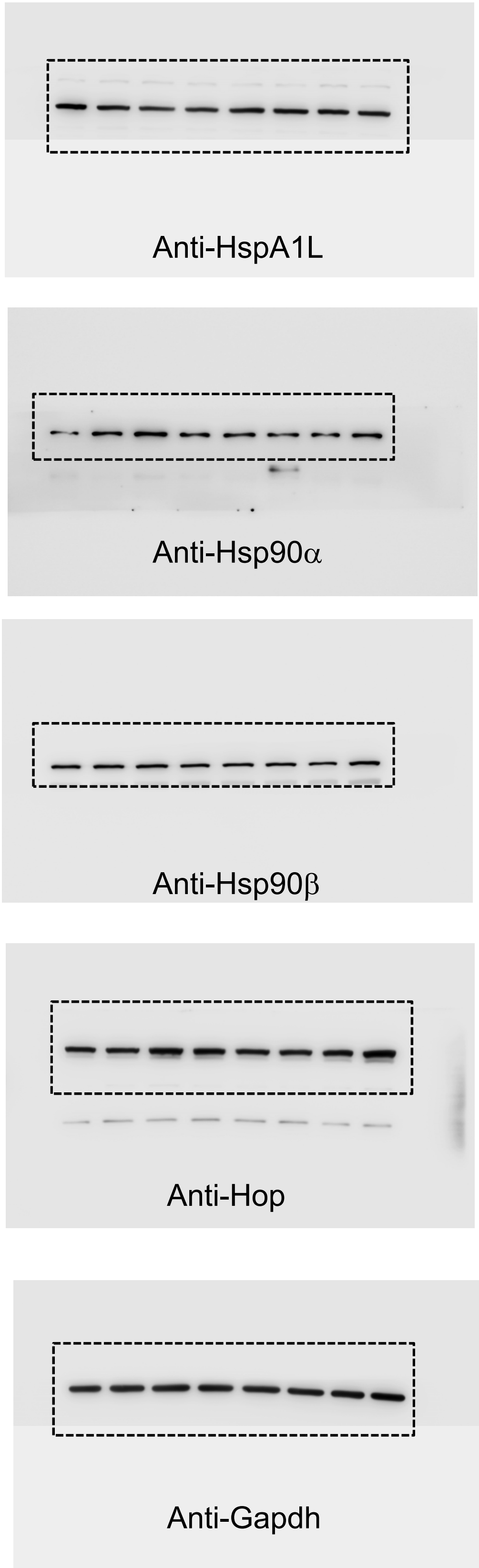
Fig. 7B



Supplementary Fig. S1



Supplementary Fig. S2



Supplementary Fig. S3