Supplementary Information

TRBP modulates RLR signaling by inhibiting PKR-mediated antiviral stress granule formation

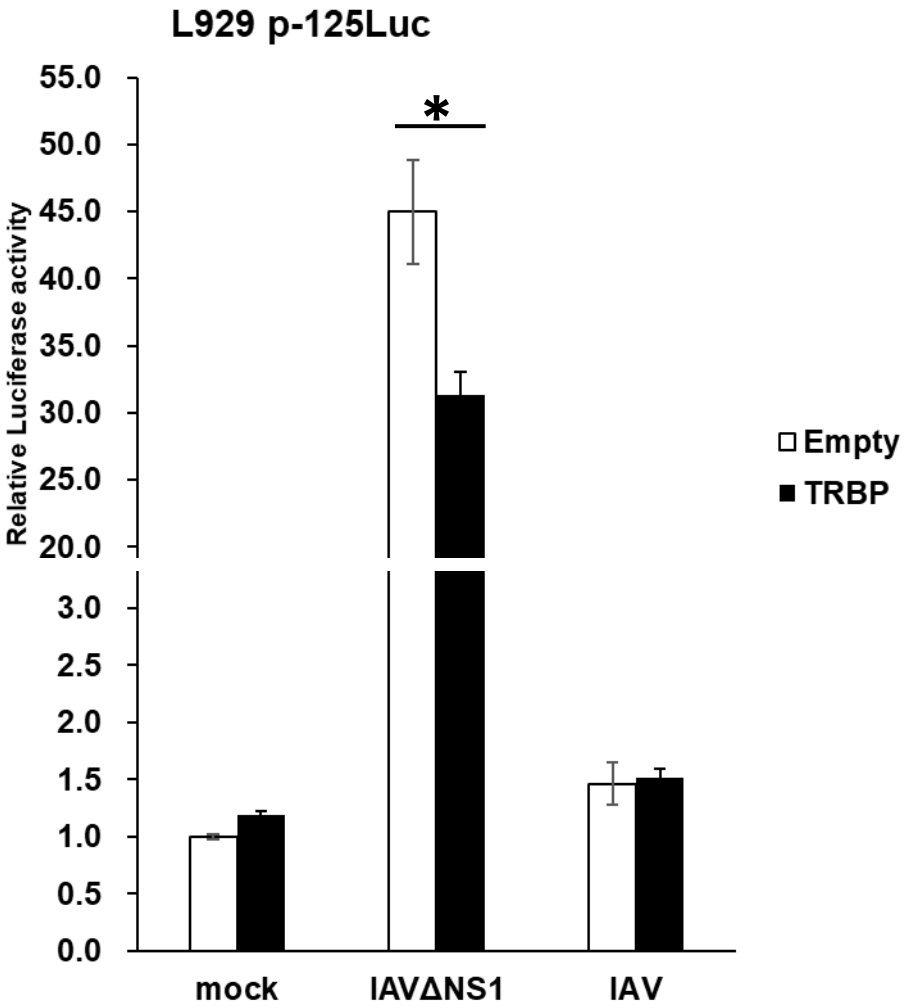
Koji Onomoto[1†], Monami Sakai[1†], Miyu Watanabe[1†], Akira Fukao[2], Yurika Sakamura[2], Mai Miyao[2], Takumi Tomohiro[2], Akio Yamashita[3], Toshinobu Fujiwara[2], Tomoko Takahashi[4], Kumiko Ui-Tei[5,6], Mitsutoshi Yoneyama[1,7]*

[1]Division of Molecular Immunology, Medical Mycology Research Center, Chiba University, Chiba 260-8673, Japan
[2]Laboratory of Biochemistry, Department of Pharmacy, Kindai University, Higashi-Osaka 577-8502, Japan.
[3]Laboratory of Molecular Cell Biology, Department of Pharmaceutical Sciences, Kindai University, Higashi-Osaka, 577-8502, Japan
[4]Department of Biochemistry and Molecular Biology, Graduate School of Science and Engineering, Saitama University, Saitama 338-8570, Japan
[5] NucleoTIDE and PepTIDE Drug Discovery (TIDE) Center, Institute of Science Tokyo, Tokyo 113-8519, Japan
[6]Graduate School of Science, The University of Tokyo, Tokyo 113-0033, Japan
[7]Research Institute of Disaster Medicine, Chiba University, Chiba 260-8670, Japan

Corresponding author
*To whom correspondence should be addressed: Mitsutoshi Yoneyama
Tel: +81-43-226-2797; Fax: +81-43-226-2791
Email: myoneyam@faculty.chiba-u.jp

[†]These authors contributed equally to the paper as first authors.

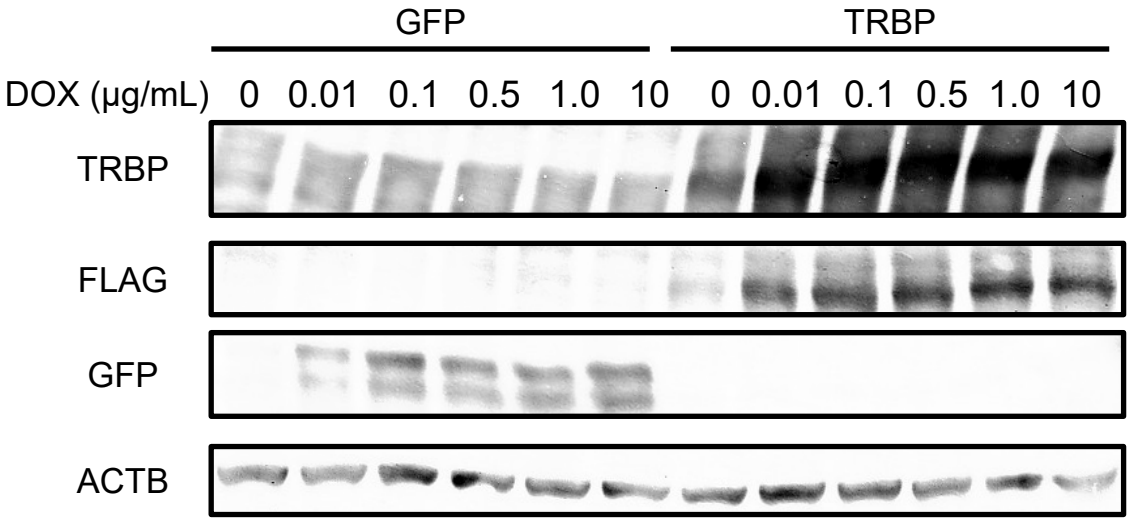
Supplementary Figure 1



Supplementary Figure 1. TRBP did not inhibit IFN signal in IAV WT-infected cells.

L929 cells were transfected with the IFN reporter gene (p-125Luc) and internal control (pRL-tk) vectors, together with the expression vector for empty vector or TRBP. The cells were mock-treated (mock), infected with IAVΔNS1 or IAV WT for 12 h, and subjected to a dual-luciferase assay. At least two independent experiments represent similar results, and the bars indicate the one representative; each bar shows the means and ± the standard deviation (S.D.) of the technical triplicate. P-values were determined using Student's t-test (*$P < 0.05$).

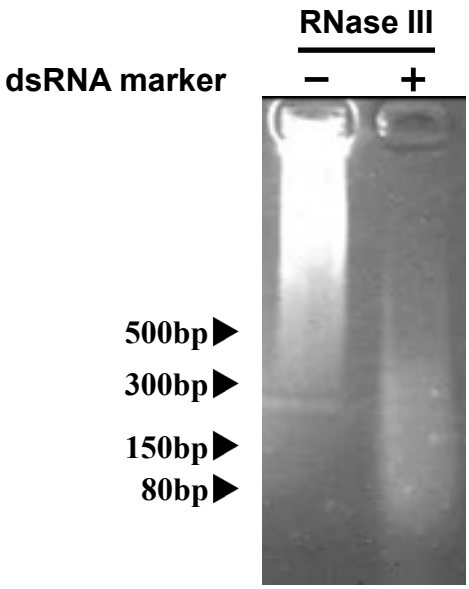
Supplementary Figure 2



Supplementary Figure 2. DOX-induced expression of GFP and TRBP in Flp-In 293 cells

Flp-In 293 CBP-SBP-GFP and FLAG-TRBP cells were treated with DOX 0, 0.01, 0.1, 0.5, 1.0 or 10 μg/mL for 24 h. The cell extracts were prepared and subjected to SDS-PAGE and immunoblotted with antibodies against GFP, FLAG-tag, TRBP, and ACTB.

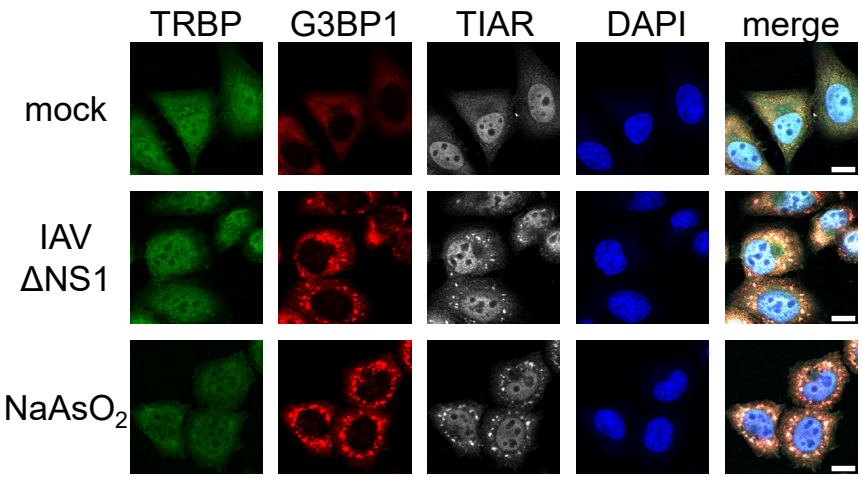
Supplementary Figure 3



Supplementary Figure 3. Preparation of short polyIC with RNase III treatment

PolyIC was treated with RNase III and annealed to produce short polyIC. The polyIC were subjected to agarose gel electrophoresis and stained with ethidium bromide.

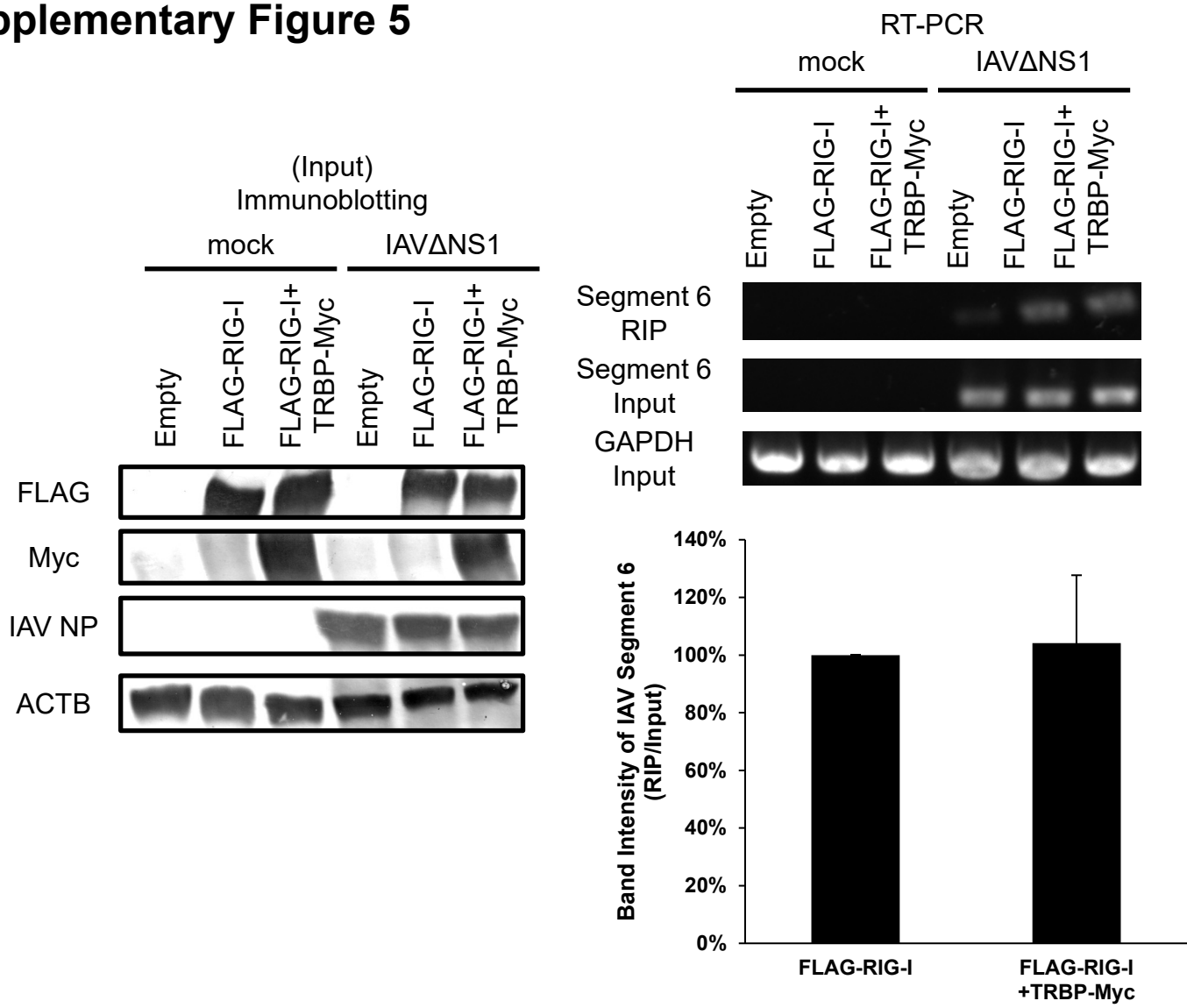
Supplementary Figure 4



Supplementary Figure 4. Localization of endogenous TRBP in SG and avSG

HeLa cells were infected mock-treated, infected with IAVΔNS1 for 12 h, or treated with $NaAsO_2$ (0.5 mM) for 30 min and stained with anti-TRBP (Green), anti-G3BP1 (Red), anti-TIAR (Gray), and DAPI (Blue). The white scale bar corresponds to 10 μm.

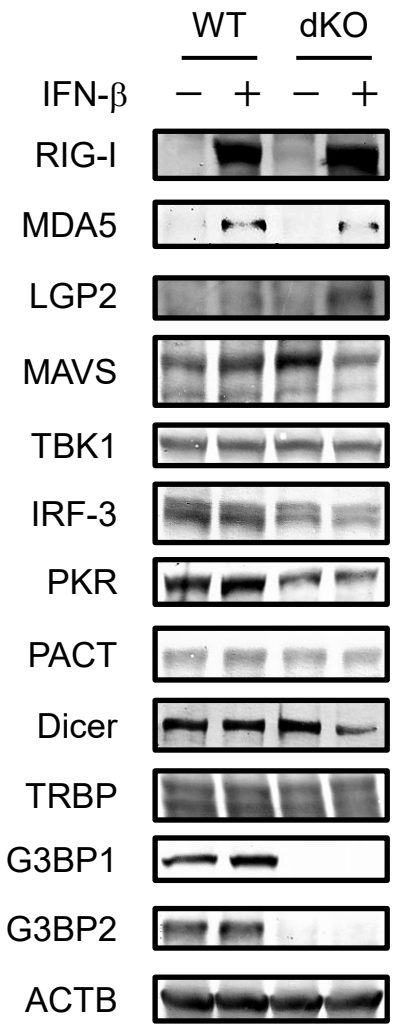
Supplementary Figure 5



Supplementary Figure 5. TRBP did not inhibit RIG-I-mediated detection of IAV RNA.

HEK293T cells were transfected with empty vector, FLAG-tagged RIG-I, or FLAG-tagged RIG-I together with Myc-tagged TRBP WT for 48 h, and the cells were mock-treated or infected with IAVΔNS1 for 15 h. To confirm protein expression, cell extracts were subjected to SDS-PAGE and immunoblotted using antibodies against FLAG-tag, Myc-tag, IAV NP, and ACTB (left panel). Cell extracts were immunoprecipitated with anti-FLAG antibody, and the coprecipitated RNAs were eluted from the beads using the ReliaPrep RNA Mini prep system according to the manufacturer's instructions. RT-PCR was performed using the specific primer sets targeting the IAV gene (Segment 6) and GAPDH to evaluate the RNA level bound to RIG-I (right panel). The intensities of the immunoprecipitated IAV Segment 6 bands were measured using iBright Analysis Software and normalized against the input sample. Data are the mean results of three independent experiments ± standard deviation (S.D.).

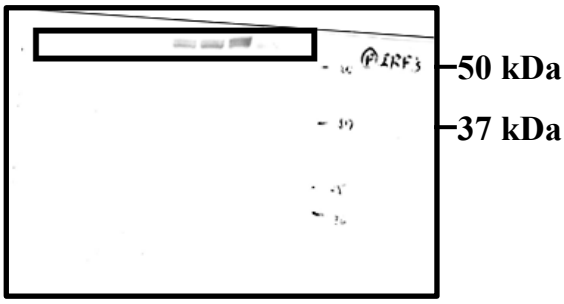
Supplementary Figure 6



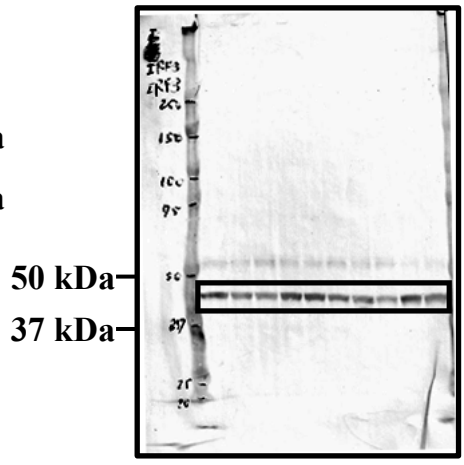
Supplementary Figure 6. Expression patterns of RLR-mediated signal and RNA silencing factors

HEK293T WT and dKO cells were mock-treated or treated with IFN-β (1000 U/mL) for 24 h. Cell extracts were prepared, subjected to SDS-PAGE, and immunoblotted with antibodies against RIG-I, MDA5, LGP2, MAVS, TBK1, IRF-3, PKR, PACT, Dicer, TRBP, G3BP1, G3BP2, and ACTB.

Supplementary Figure 7



pIRF3



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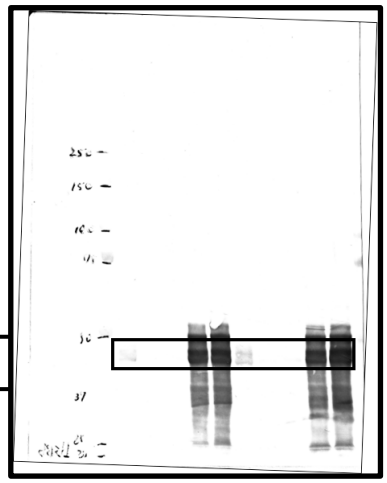
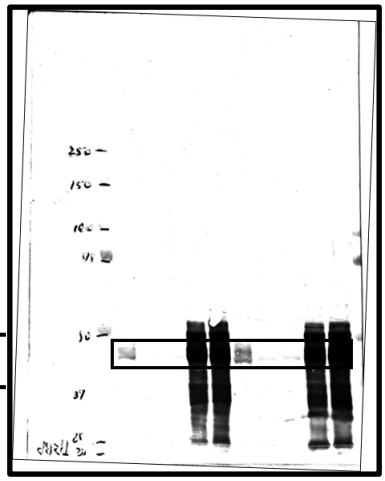
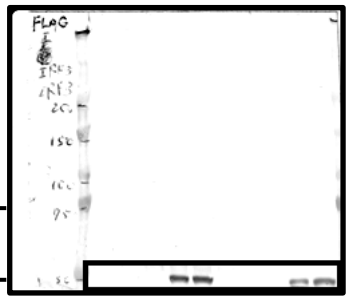


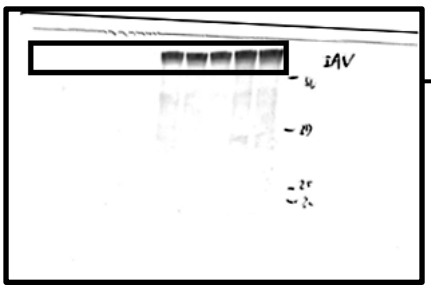
TRBP
(low exposure)



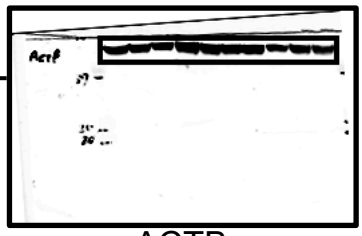
TRBP
(high exposure)



FLAG



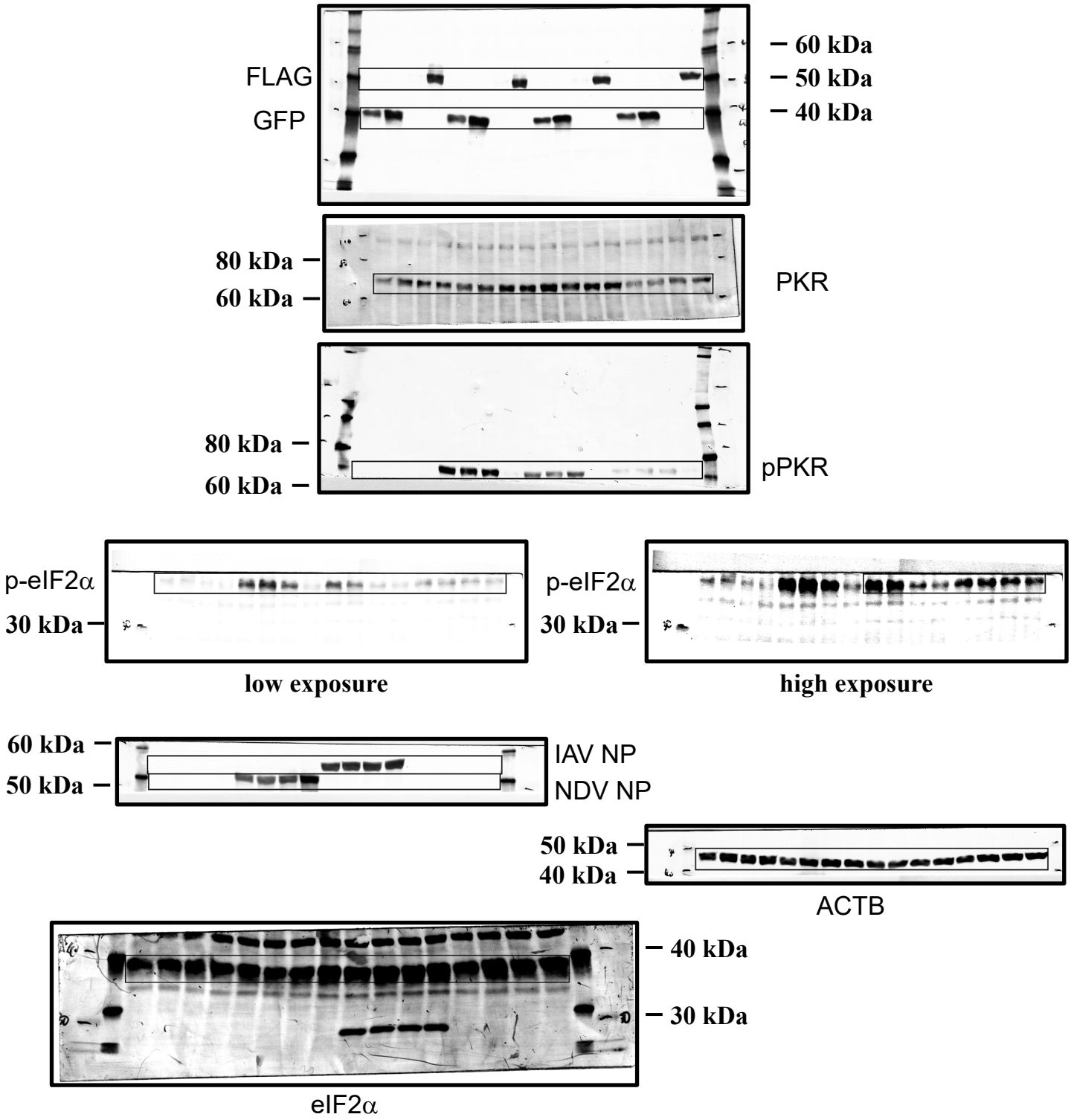
IAV NP



ACTB

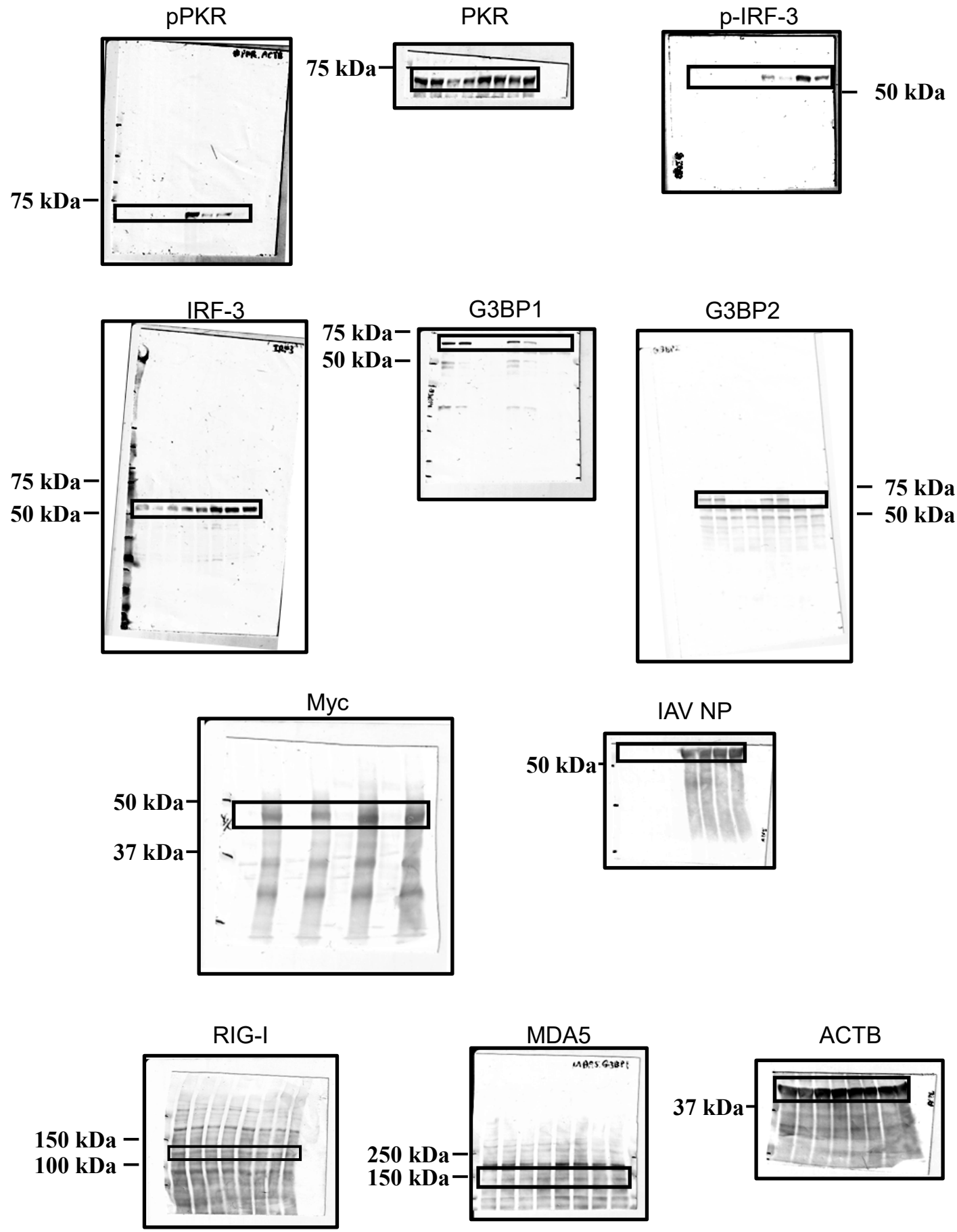
Supplementary Figure 7. Full-size of uncropped SDS-PAGE images in Figure 1e

Supplementary Figure 8



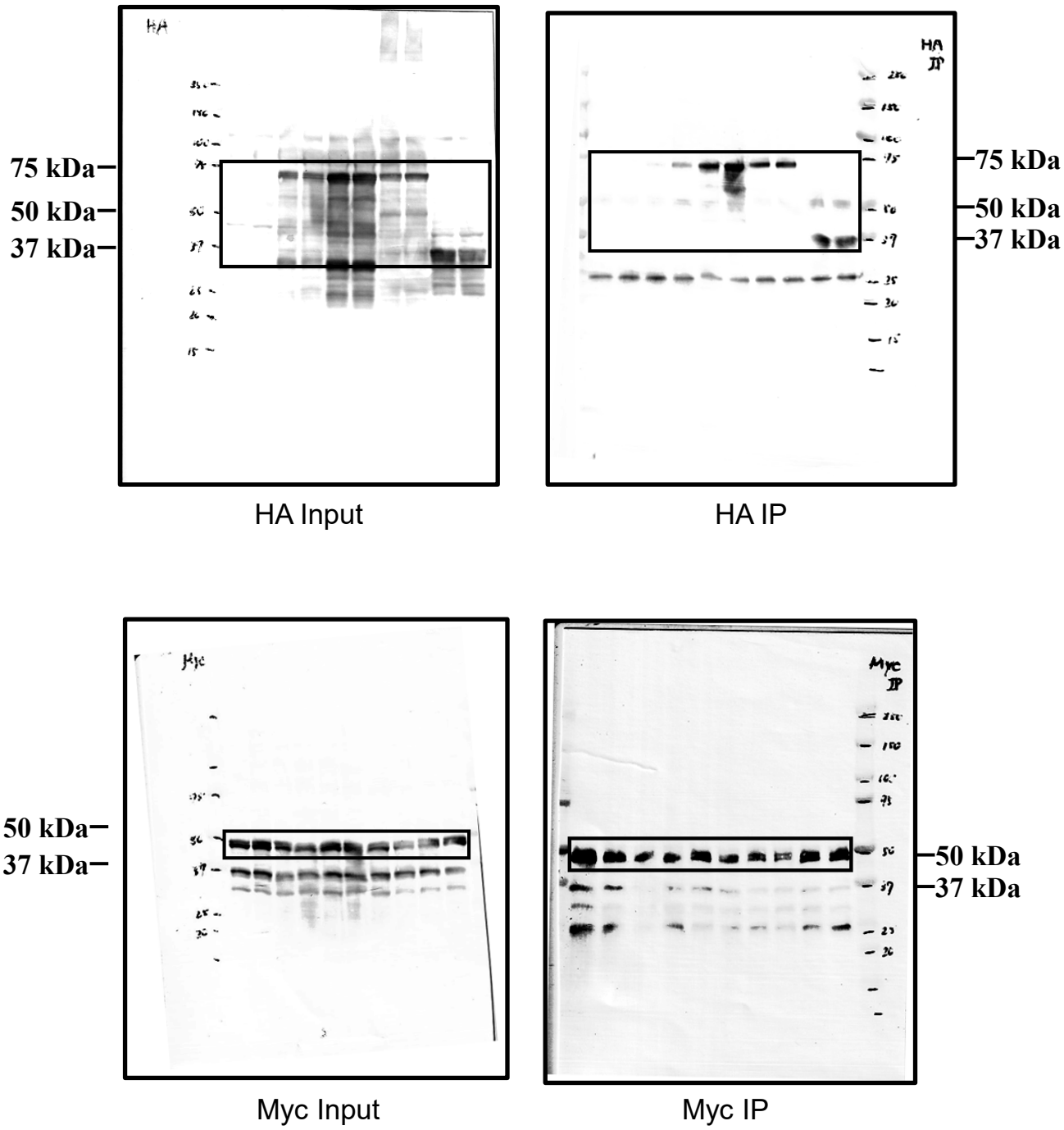
Supplementary Figure 8. Full-size of uncropped SDS-PAGE images in Figure 2a

Supplementary Figure 9



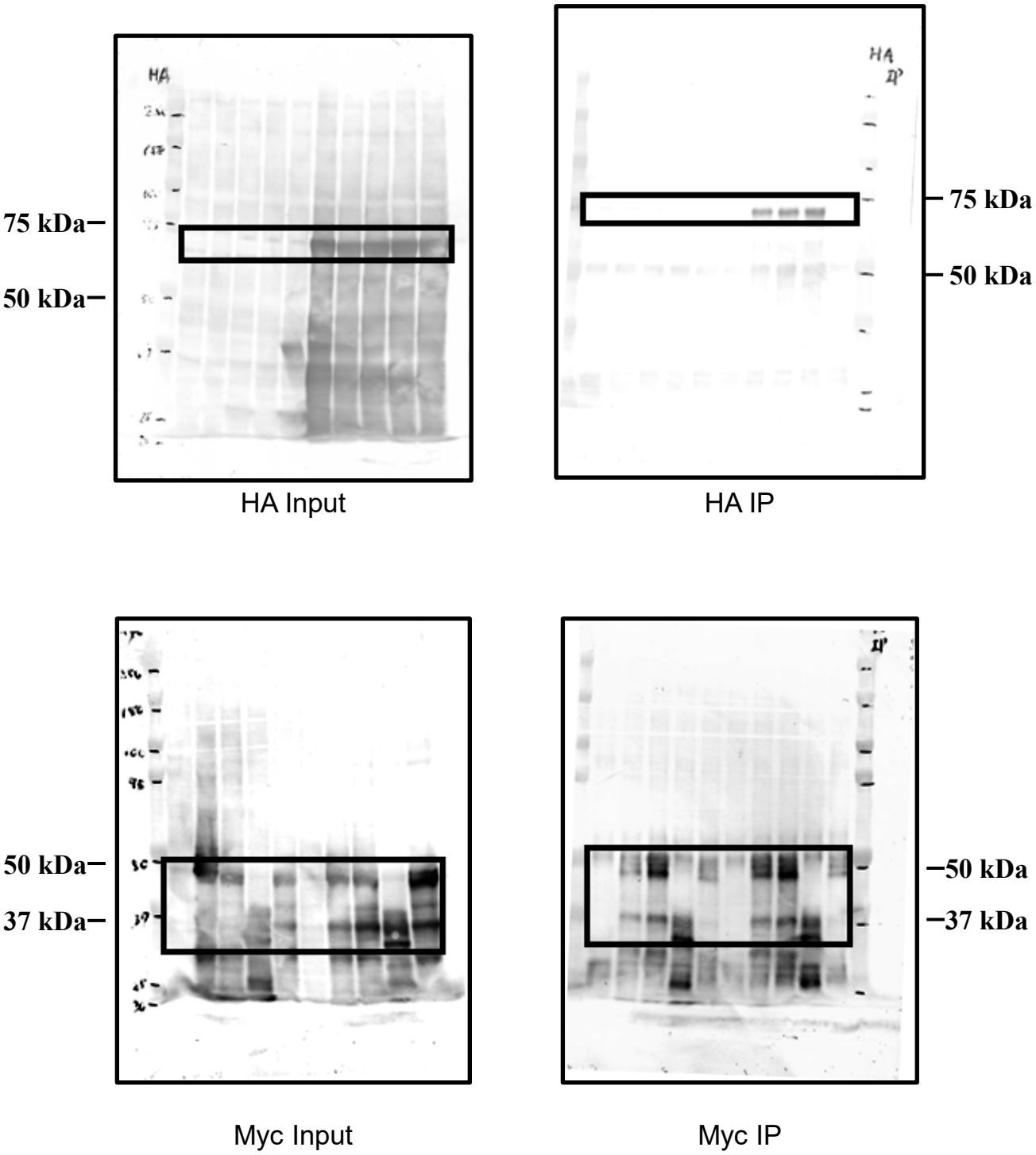
Supplementary Figure 9. Full-size of uncropped SDS-PAGE images in Figure 3b

Supplementary Figure 10



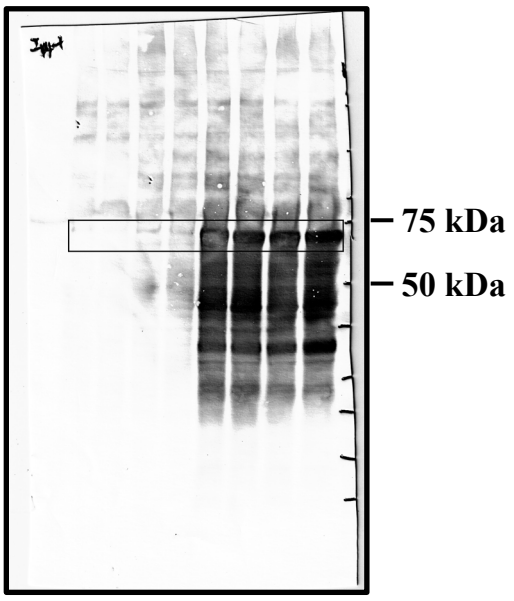
Supplementary Figure 10. Full-size of uncropped SDS-PAGE images in Figure 4a

Supplementary Figure 11



Supplementary Figure 11. Full-size of uncropped SDS-PAGE images in Figure 4c

Supplementary Figure 12



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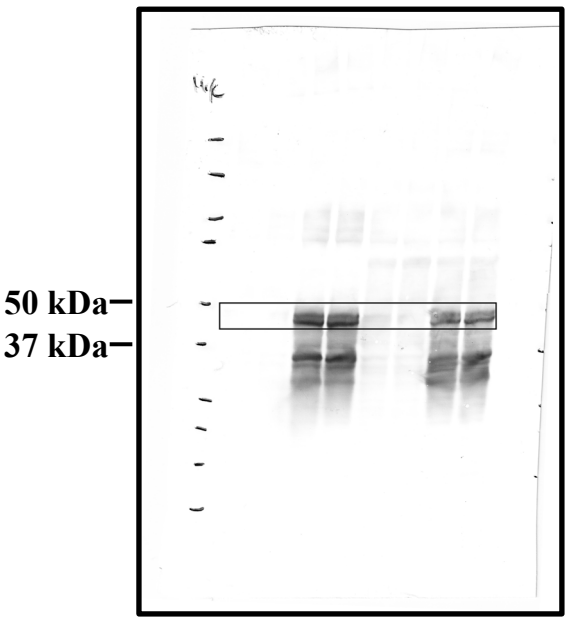
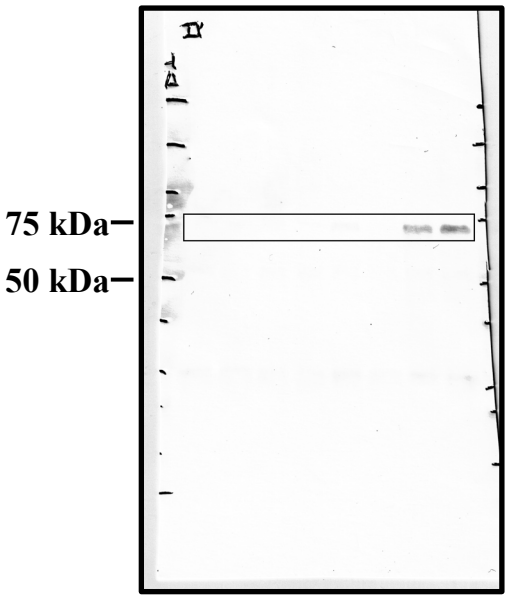


Input Myc



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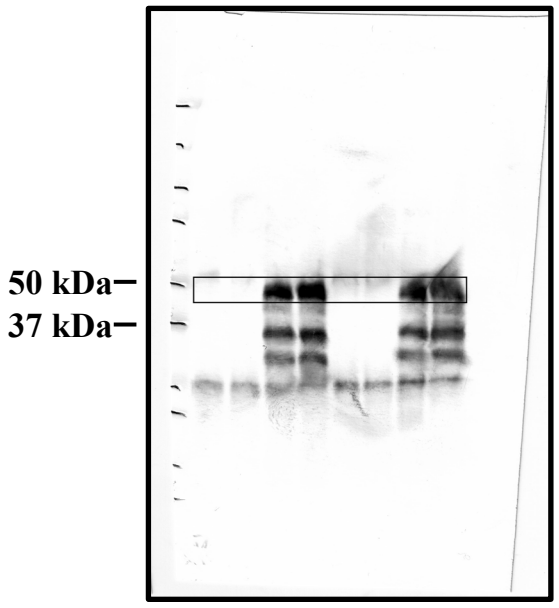

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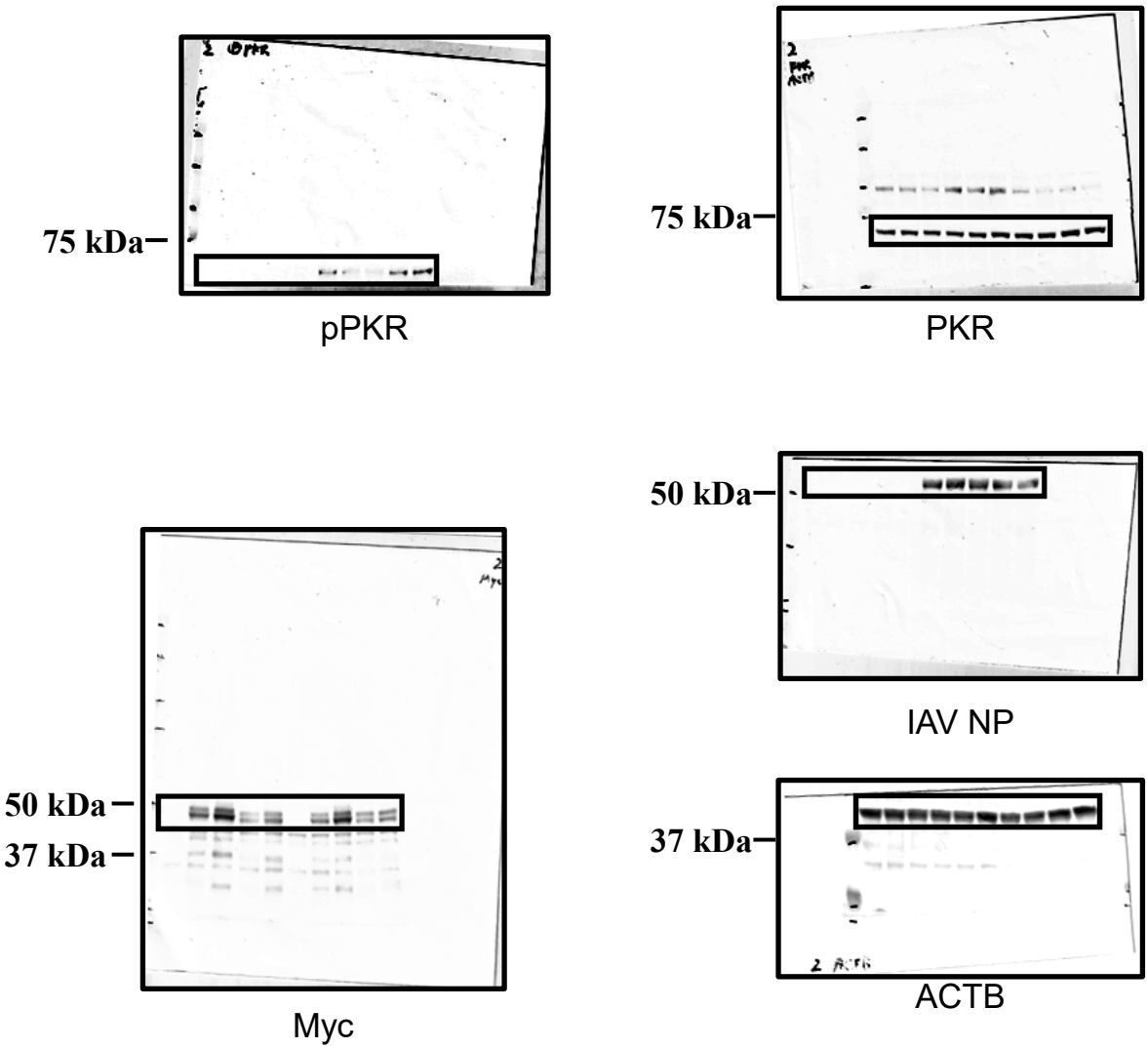
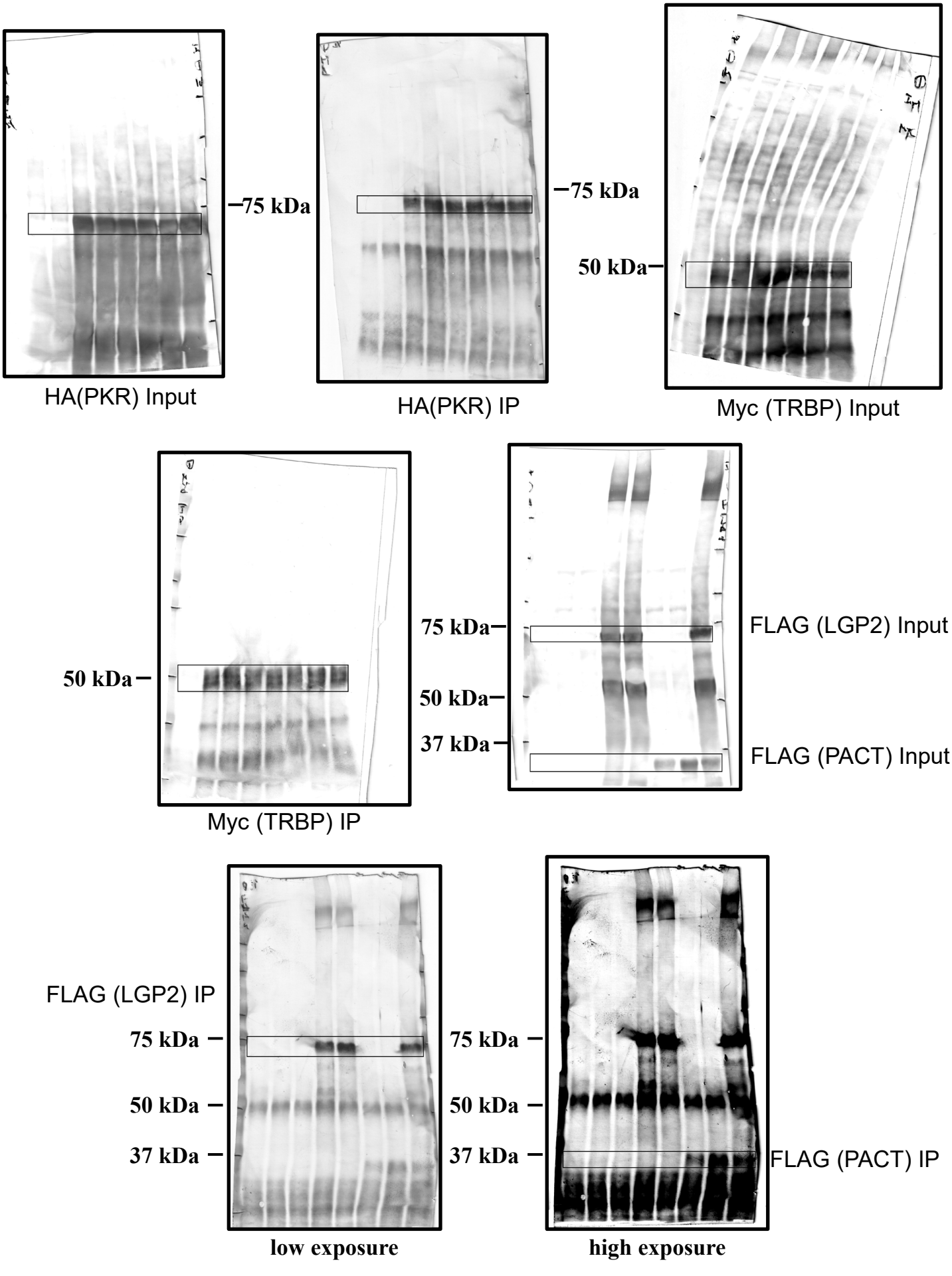
Supplementary Figure 12. Full-size of uncropped SDS-PAGE images in Figure 4d

Supplementary Figure 13



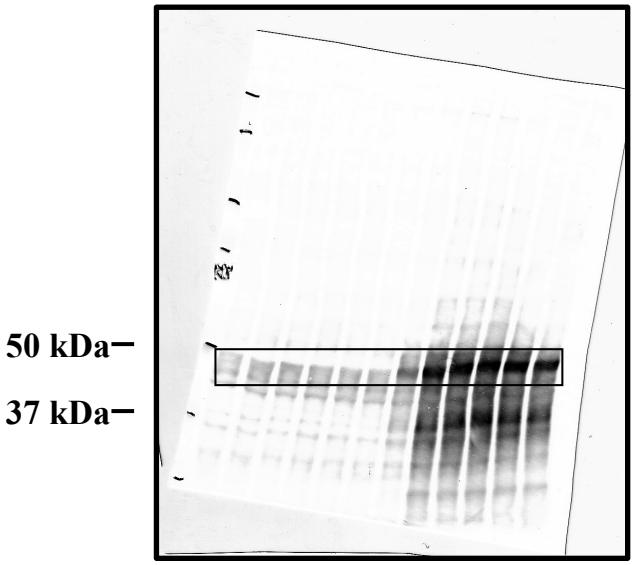
Supplementary Figure 13. Full-size of uncropped SDS-PAGE images in Figure 5b

Supplementary Figure 14

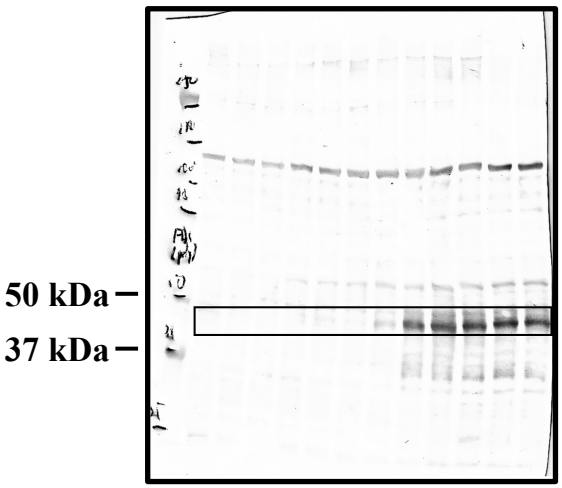


Supplementary Figure 14. Full-size of uncropped SDS-PAGE images in Figure 6a

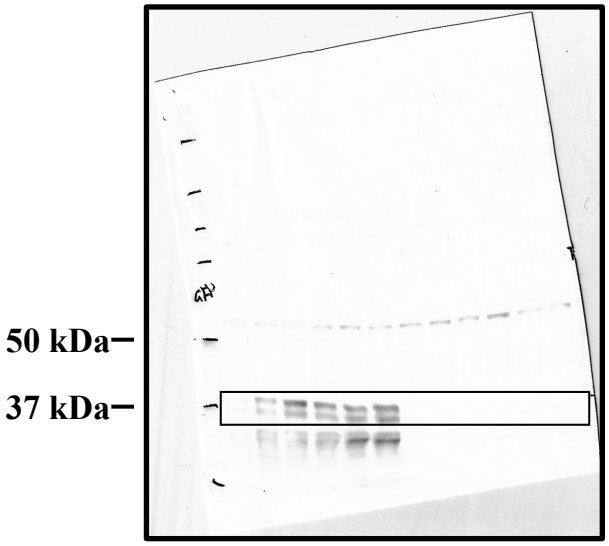
Supplementary Figure 15



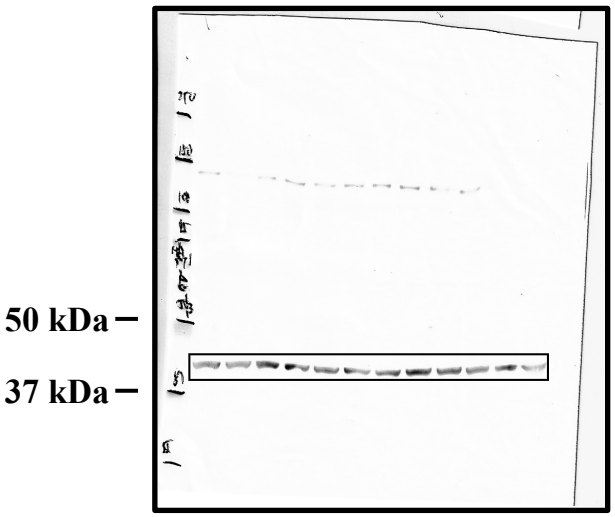
TRBP



FLAG



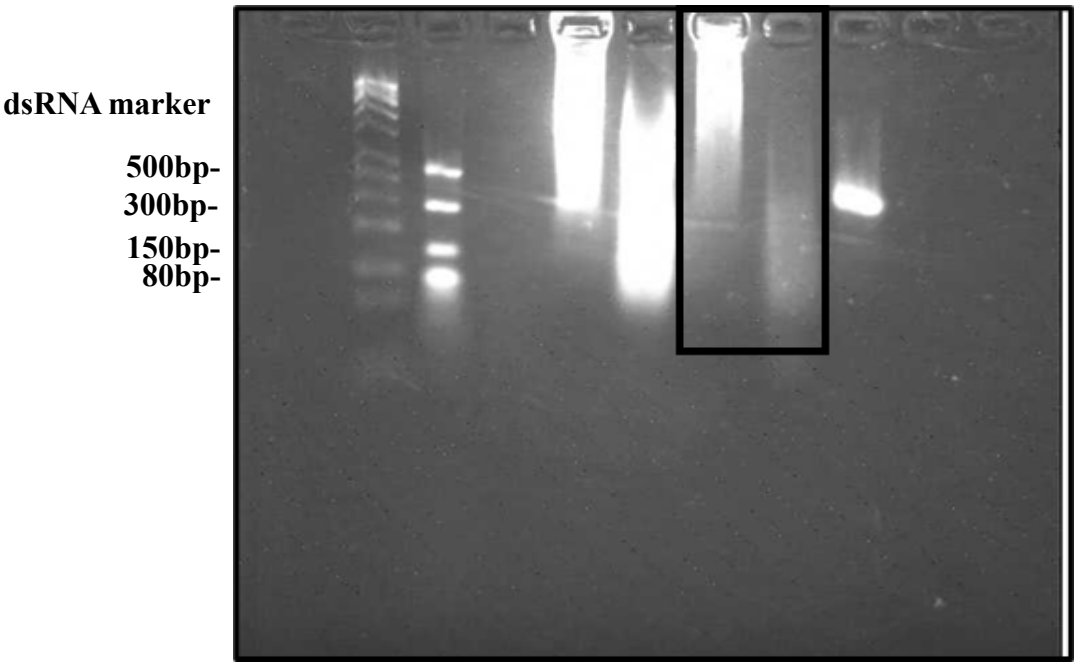
GFP



ACTB

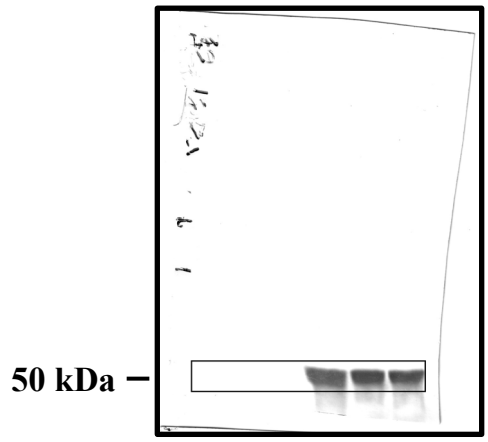
Supplementary Figure 15. Full-size of uncropped SDS-PAGE images in Supplementary Figure 2

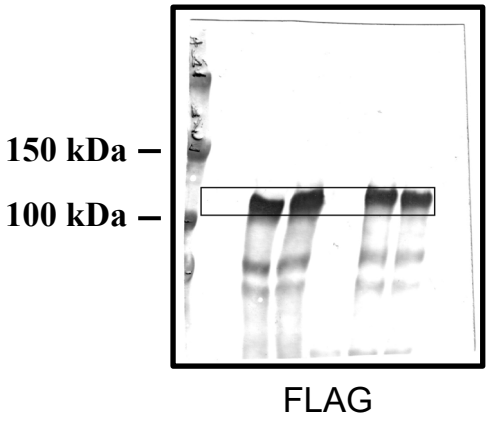
Supplementary Figure 16

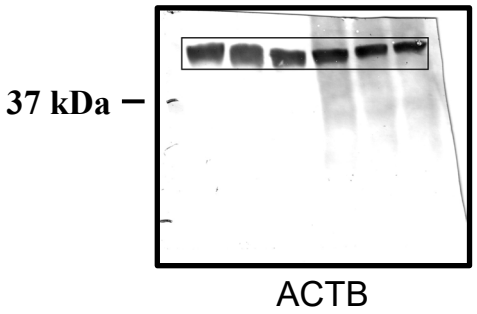


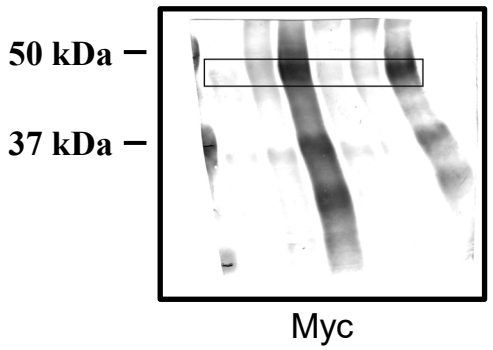
Supplementary Figure 16. Full-size of uncropped Agarose gel image in Supplementary Figure 3

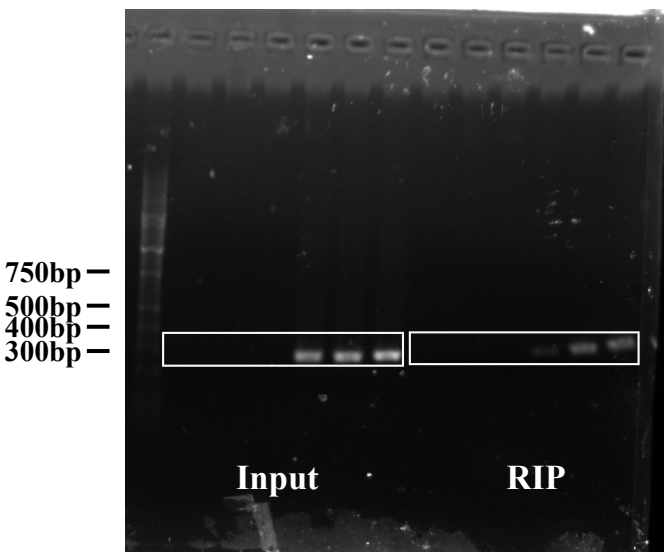
Supplementary Figure 17

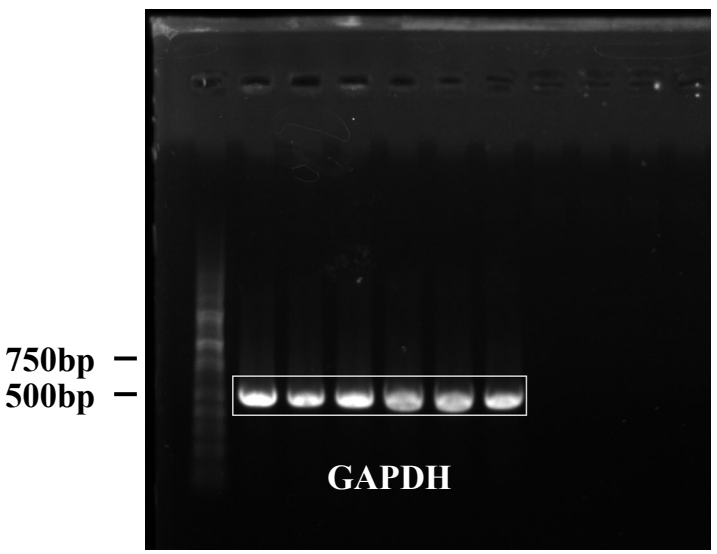






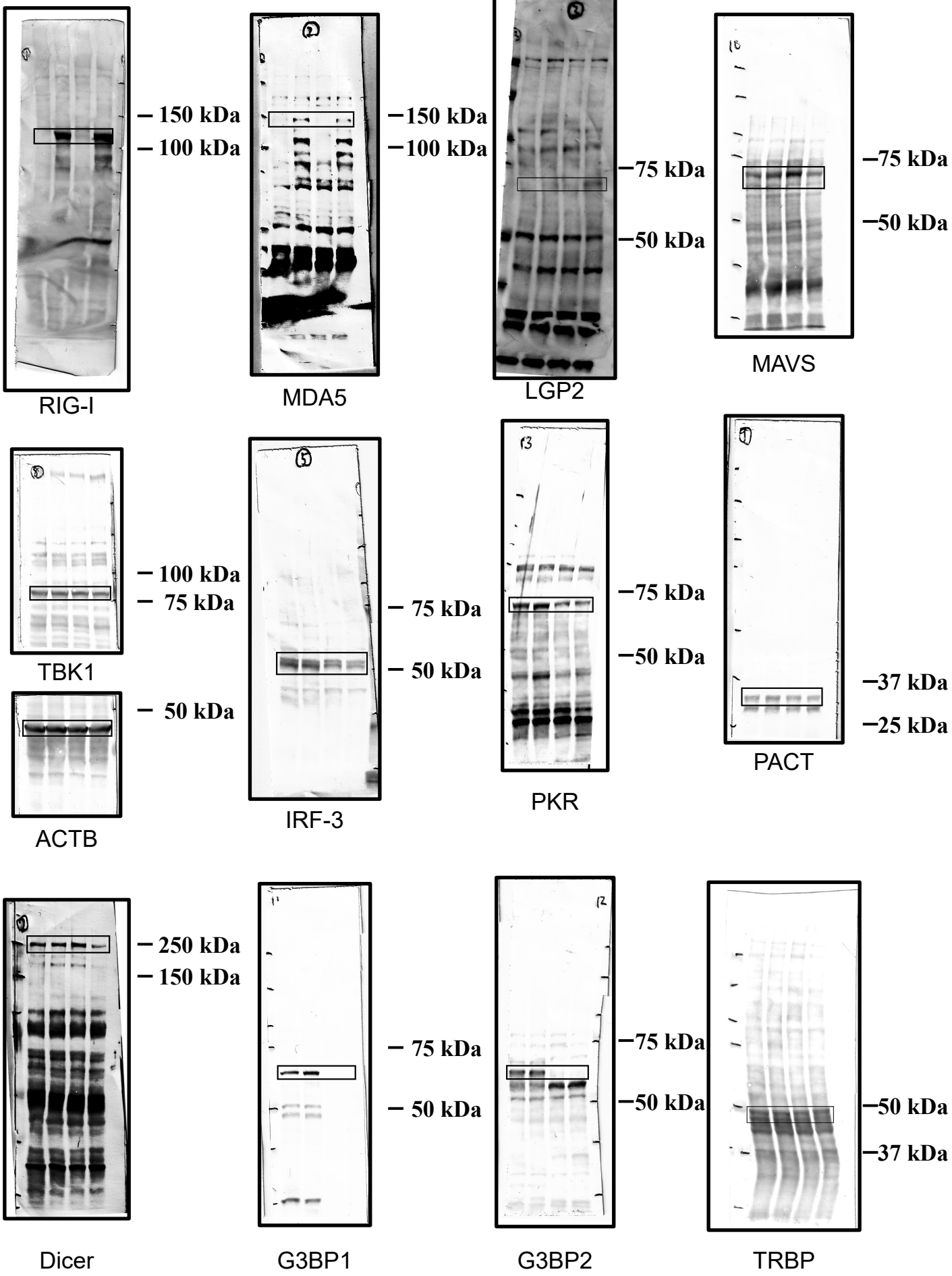






Supplementary Figure 17. Full-size of uncropped SDS-PAGE and agarose Gel images in Supplementary Figure 5

Supplementary Figure 18



Supplementary Figure 18. Full-size of uncropped SDS-PAGE images in Supplementary Figure 6