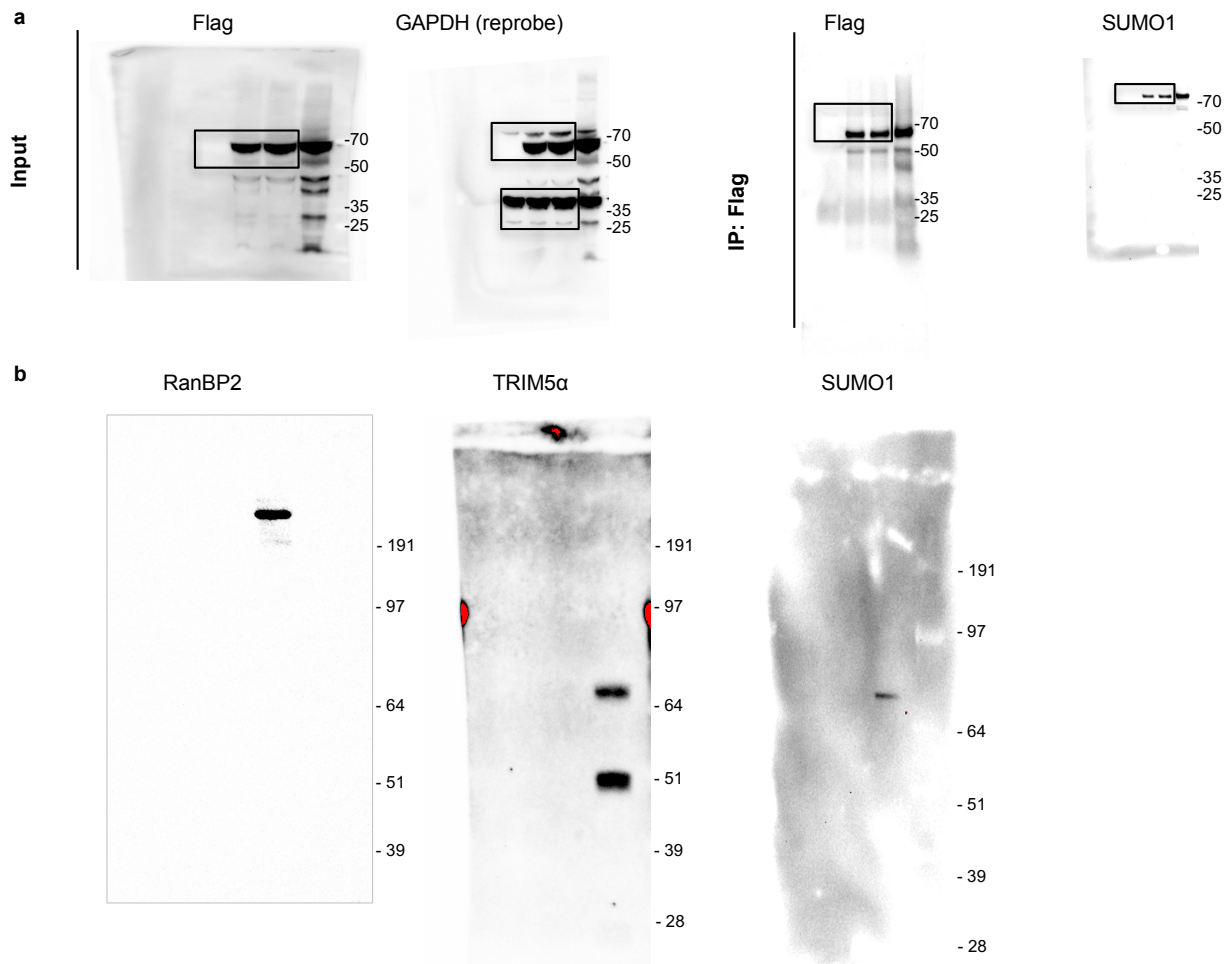


Supplementary Figure 1.

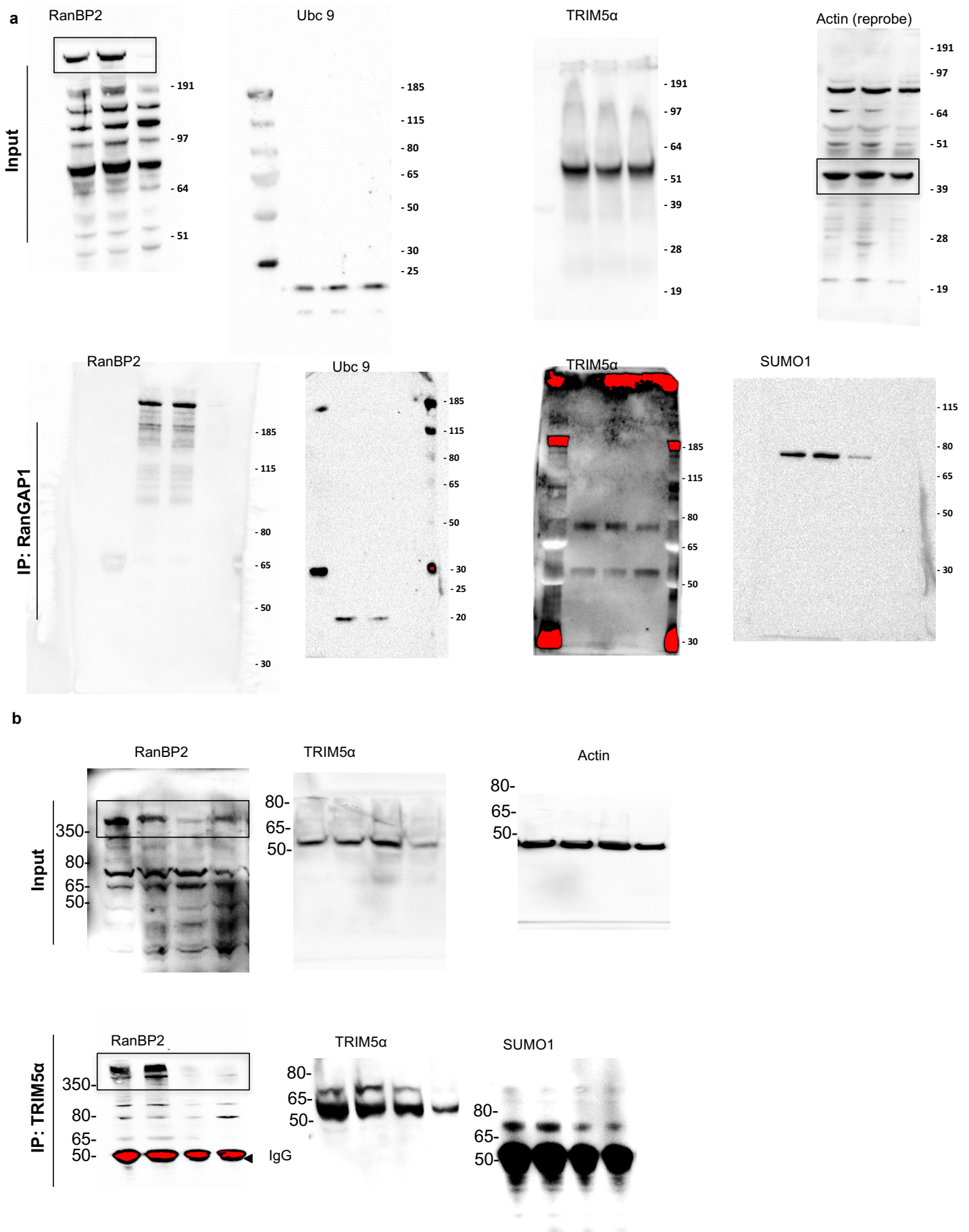
a, RanBP2 SUMOylates TRIM5 α *in vitro*. *In vitro* SUMO assay was performed by combining His-tagged HR-TRIM5 α (40 kDa), and the RanBP2 domain previously identified to contain E3 SUMO ligase activity of RanBP2 (RanBP2 Δ FG, Pichler et al., 2002). Following *in vitro* SUMO assay, multiple SUMO modified TRIM5 α bands, that appeared at ca. 15 kDa increments were visible in both SUMO1 and His blots, demonstrating that RanBP2 Δ FG is sufficient to mediate TRIM5 α SUMOylation *in vitro*.

b, Full length RanBP2 stimulates SUMOylation of recombinant TRIM5 α *in vitro*. Human recombinant TRIM5 α (HR-TRIM5 α , 40 kDa) produced in *E. coli* was incubated with full-length human RanBP2 isolated from Hela cytosol. *In vitro* SUMOylation, performed in a minimal reaction assay containing RanBP2, TRIM5 α , E1, E2, SUMO1 and ATP for 30 min at 30°C, generated two high molecular weight bands above the ca. 40 kDa unmodified band, at apparent mobilities of ca. 55 kDa and 70 kDa, indicating SUMO modification. Original blots are provided in Supplementary Figure 6).



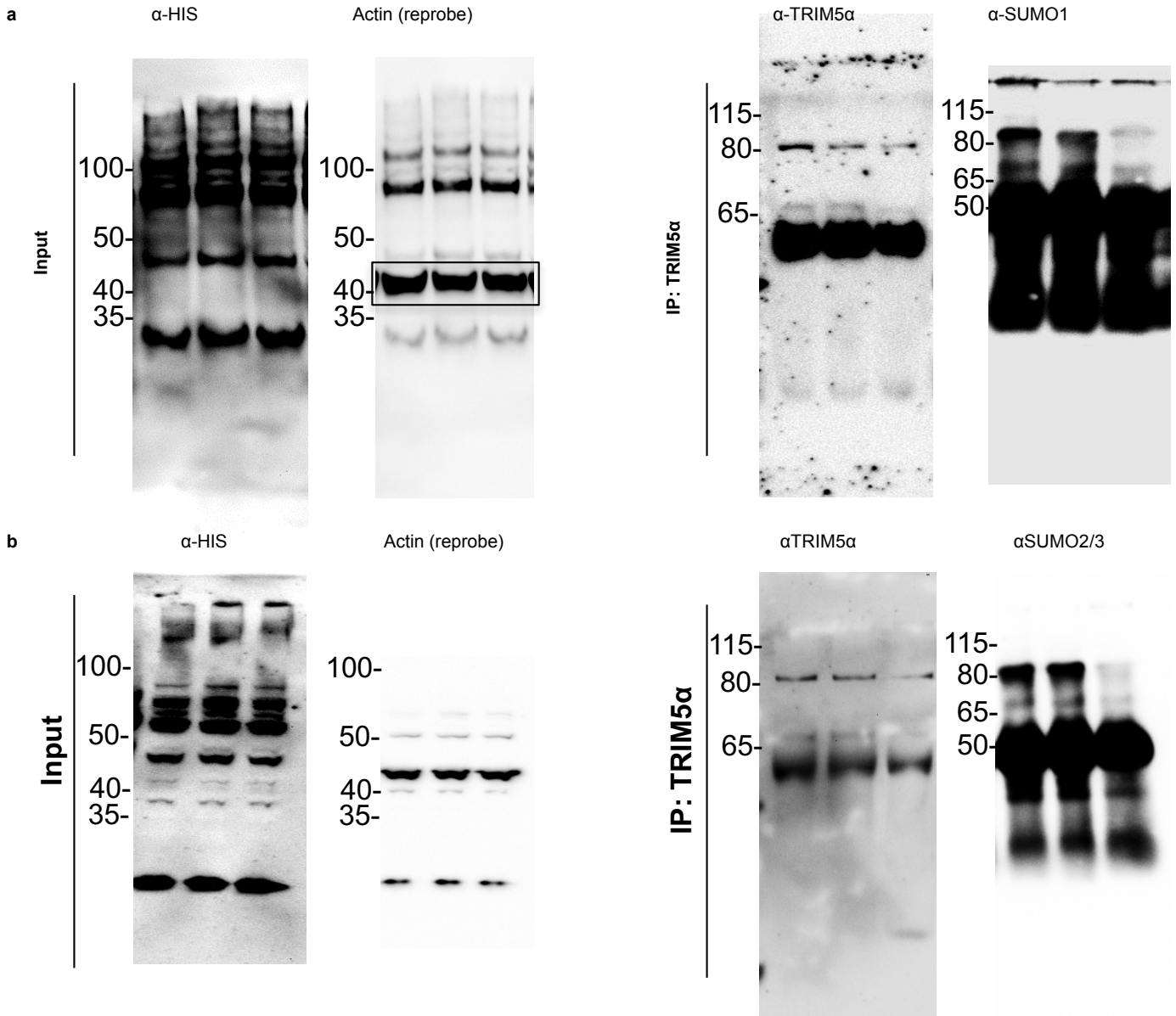
Supplementary Figure 2.

a, Original Western blots for Fig. 1a. **b**, Original Western blots for Fig. 1d.



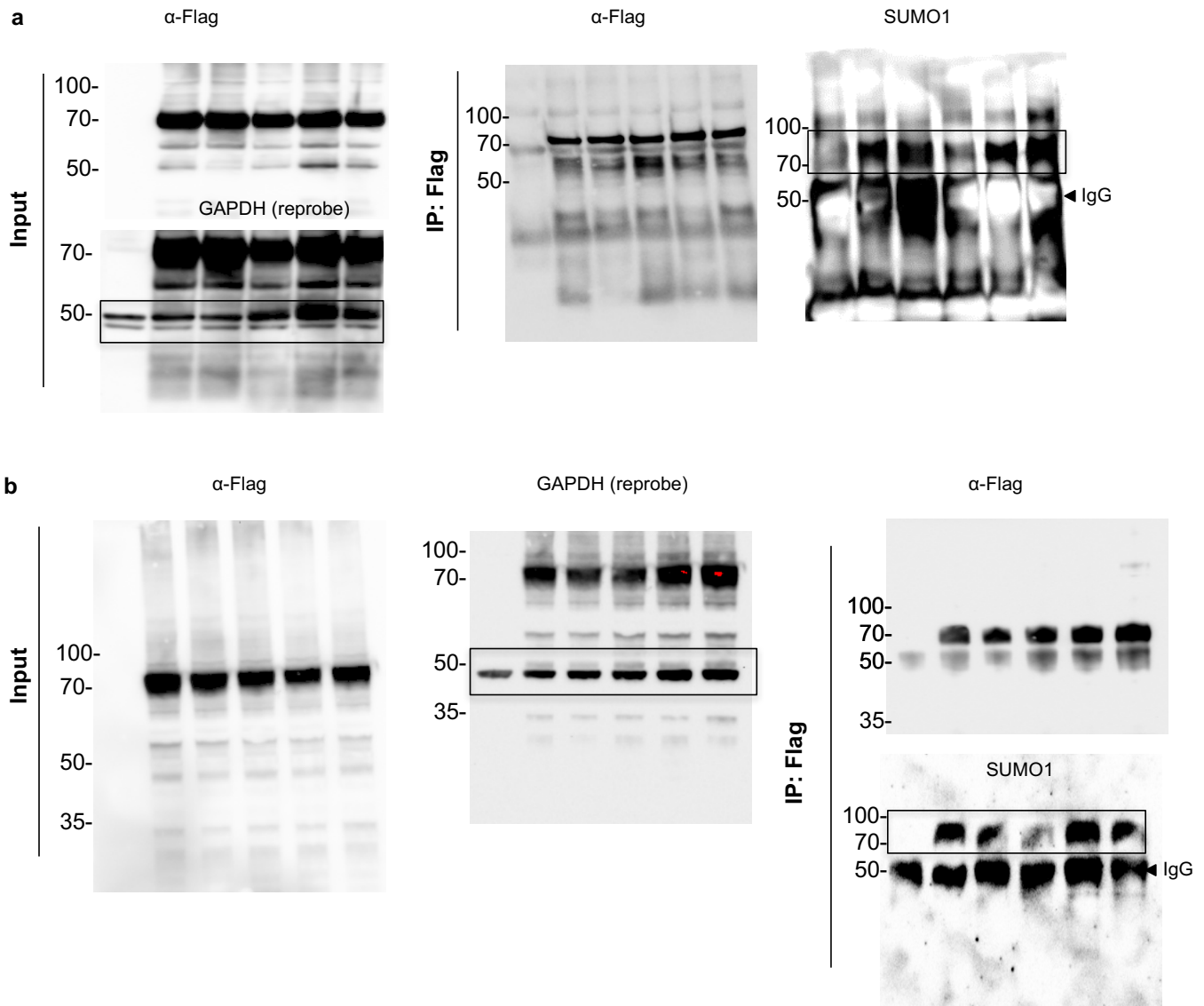
Supplementary Figure 3.

a, Original Western blots for Fig. 2a. b, Original Western blots for Fig. 2b.



Supplementary Figure 4.

a, Original Western blots for Fig. 3d. **b**, Original Western blots for Fig. 3e.

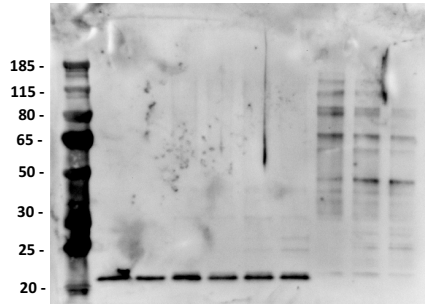


Supplementary Figure 5.

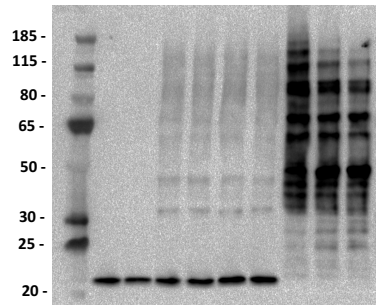
a, Original Western blots for Fig. 6b. **b**, Original Western blots for Fig. 6c.

a

α-SUMO1

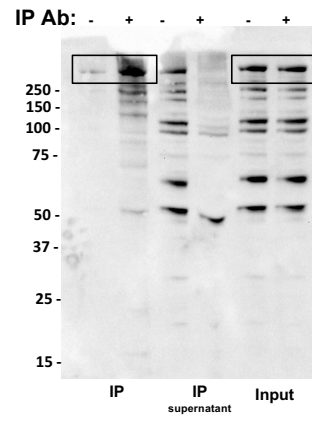


α-HIS

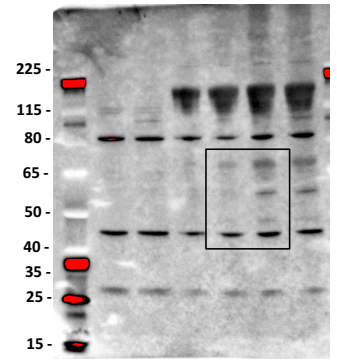


b

α-RanBP2



IVSA: α-TRIM5α



Supplementary Figure 6.

a, Original Western blots for Supplementary Figure 1a. **b**, Original Western blots for Supplementary Figure 1b.

Supplementary References:

Pichler, A., Gast, A., Seeler, J. S., Dejean, A. & Melchior, F. The nucleoporin RanBP2 has SUMO1 E3 ligase activity. *Cell* **108**, 109-120 (2002).