

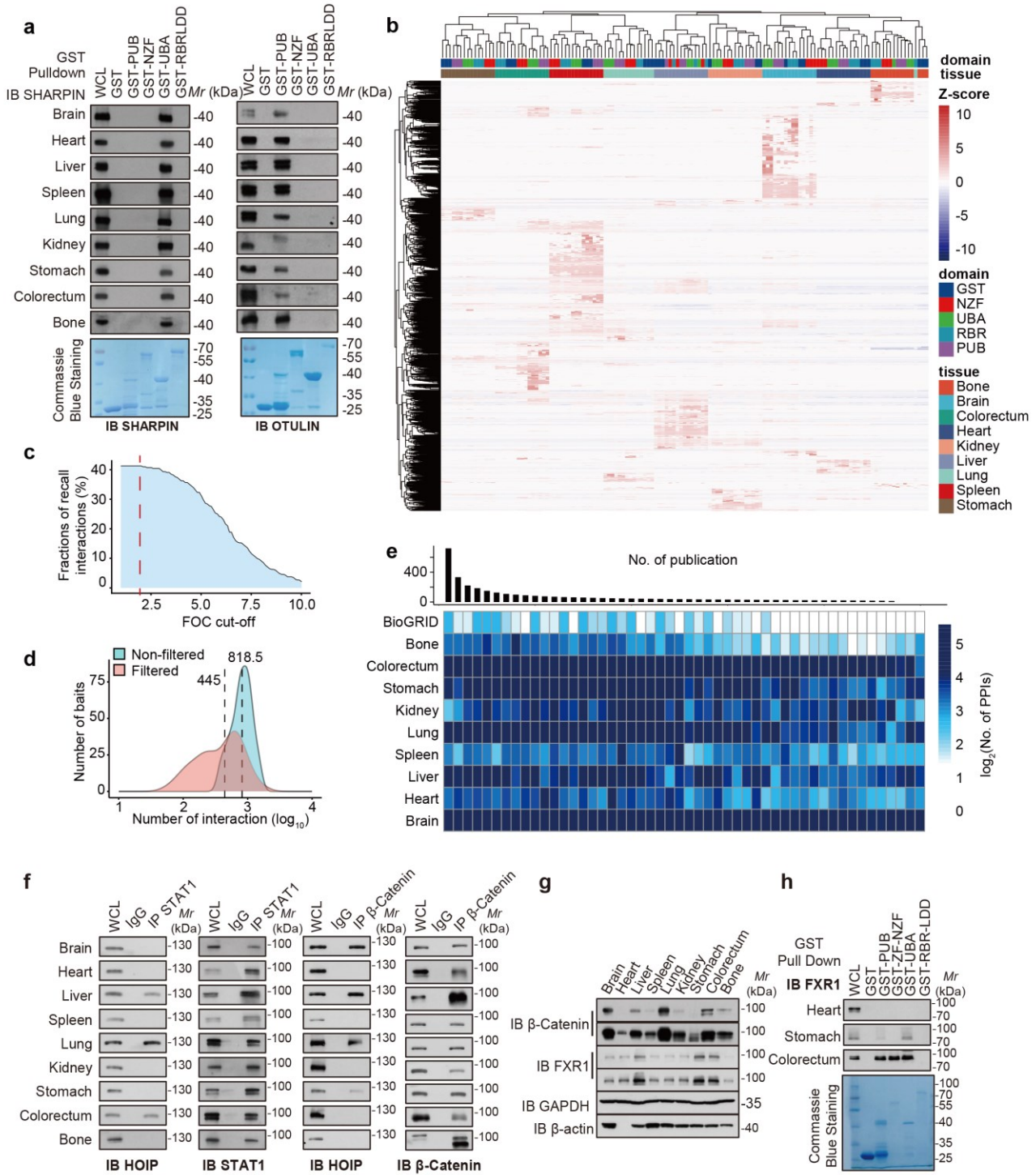
## **Supplementary Information File**

### **Systematic HOIP Interactome Profiling Reveals Critical Roles of Linear Ubiquitination in Tissue Homeostasis**

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**The PDF file includes:**  
**Supplementary Figures 1–9**  
**Unprocessed blots for Supplementary Figures**



**Supplemental Fig. 1 Data filtering and detection of known interactions, related to Fig. 1.**

**a** Immunoprecipitation analysis of canonical interactions, such as OTULIN and SHARPIN between four HOIP domains in various tissues.

**b** Global visualization of HOIP interacting proteins across domains and tissues. Protein abundance is calculated by iBAQ and homogenized by z-score.

**c** Positive discovery rate of the known HOIP interacting proteins with different fold of change (FOC)

cut-offs.

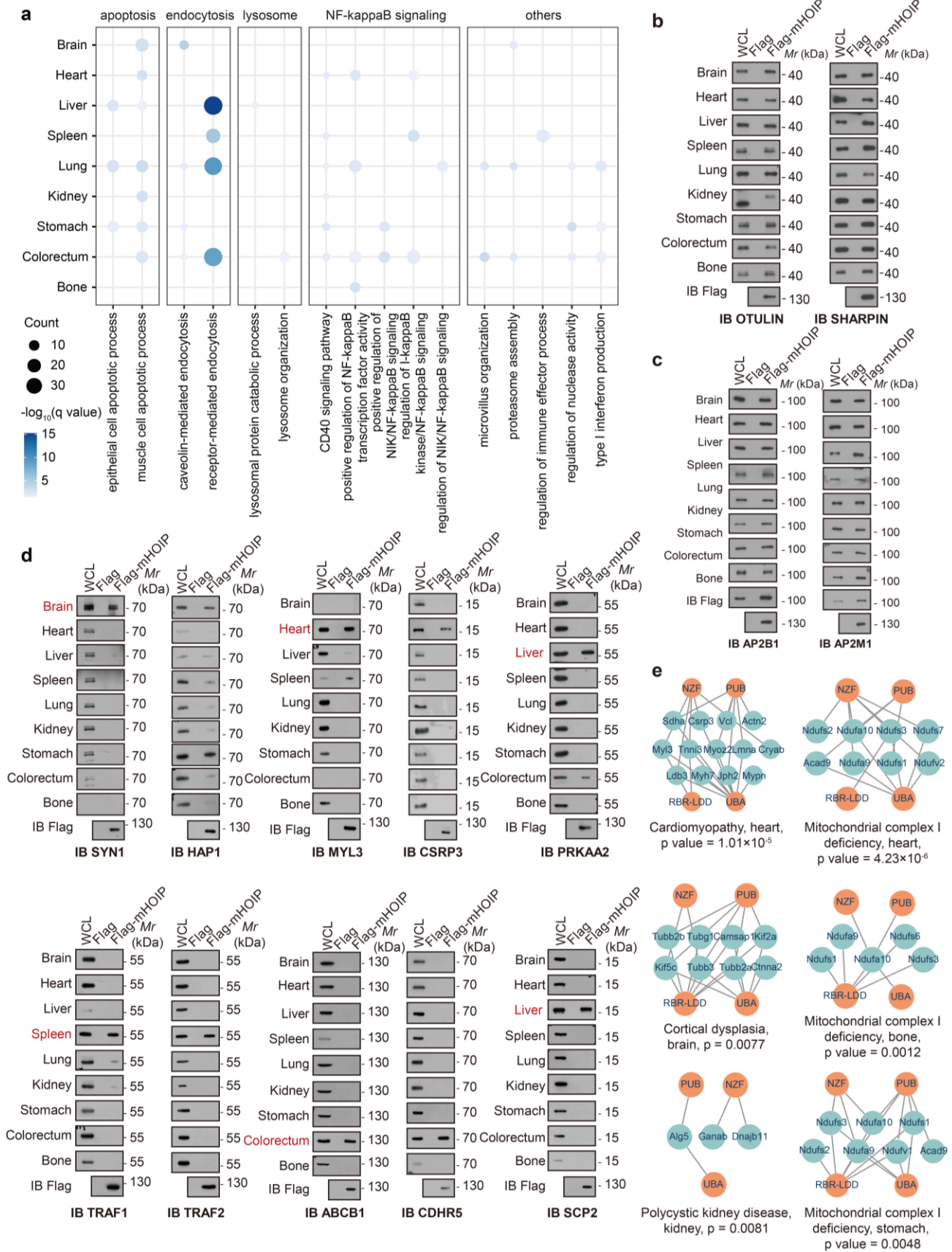
**d** Histograms depicting the number of HOIP interactions before and after filtering.

**e** Number of interactions between proteins binned by number of publications. Histogram shows the median number of publications.

**f** Endogenous immunoprecipitation analysis of STAT1 (or  $\beta$ -Catenin) and HOIP across tissues.

**g** Immunoblot analysis of HOIP,  $\beta$ -Catenin and FXR1 across tissues.

**h** Immunoprecipitation analysis of the known  $\beta$ -Catenin-interacting protein FXR1 between four HOIP domains in heart, stomach and colorectum.



**Supplemental Fig. 2 Identification of tissue-specific HOIP PPIs, related to Fig. 2.**

**a** Functional enrichment analysis of HOIP PPIs identifies a number of Gene Ontology (GO) terms

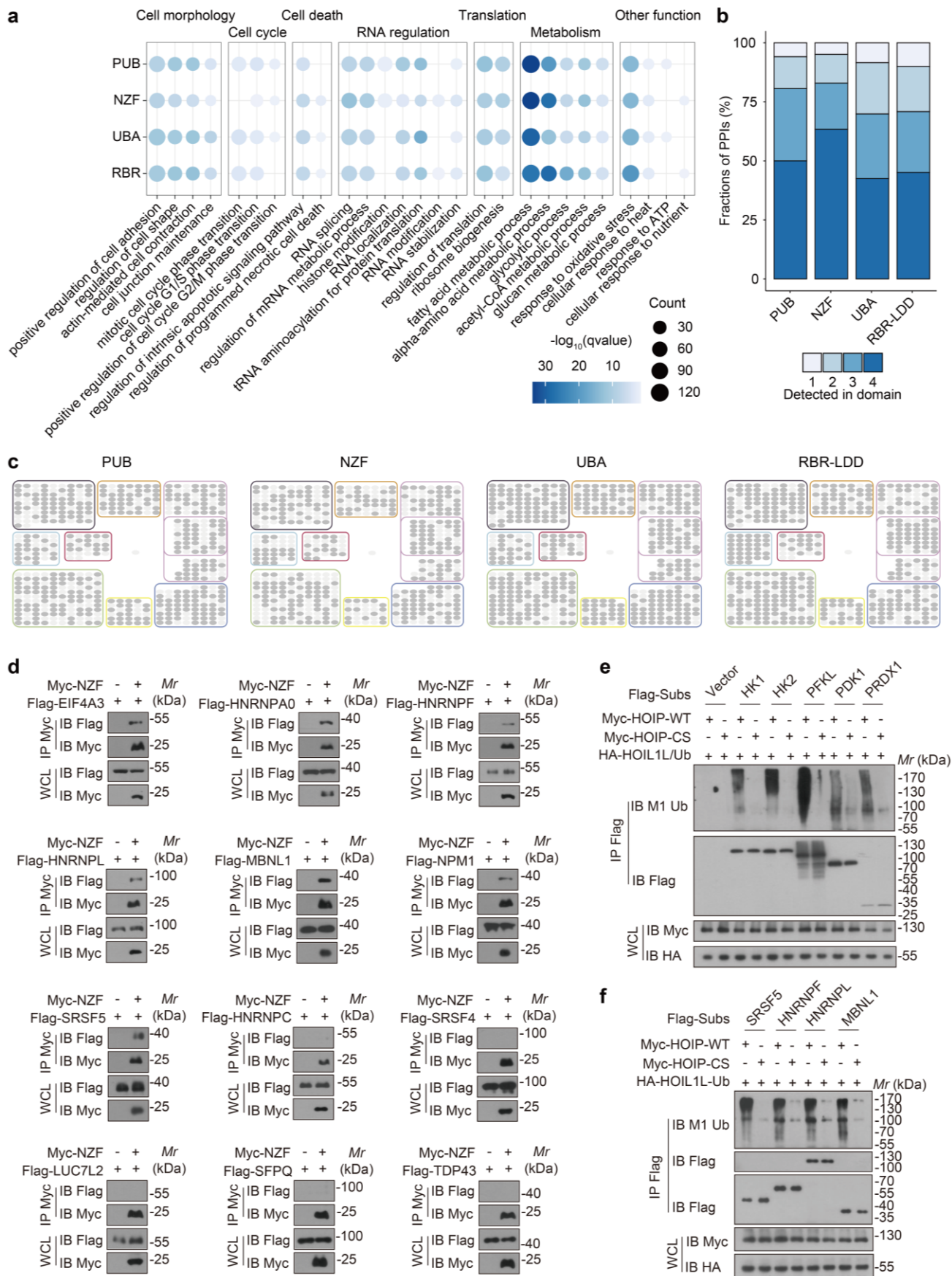
associated with the known functions of HOIP. q value is calculated by Benjamini–Hochberg method.

**b** Immunoprecipitation of ectopic expressed HOIP from HEK293T cells in different tissues and immunoblot with the antibodies of OTULIN and SHARPIN. The ectopically expressed Flag-tagged only peptide was a negative control.

**c** Immunoprecipitation of ectopic expressed HOIP from HEK293T cells in different tissues and immunoblot with the antibodies of AP2B1 and AP2M1. The ectopically expressed Flag-tagged only peptide was a negative control.

**d** Immunoprecipitation of ectopic expressed HOIP from HEK293T cells in different tissues and immunoblot with the antibodies of tissue-specific proteins. The ectopically expressed Flag-tagged only peptide was a negative control.

**e** HOIP interaction modules, in which interactors with the same disease term are present at a statistically significant frequency, are shown. p values are from the Fisher exact test.



Supplemental Fig. 3 Functional landscape of proteins included in the HOIP interaction network,

**related to Fig. 3.**

**a** Functional enrichment analysis of HOIP PPIs identifies a number of Gene Ontology (GO) terms associated with the fundamental cellular functions. q value is calculated by Benjamini–Hochberg method.

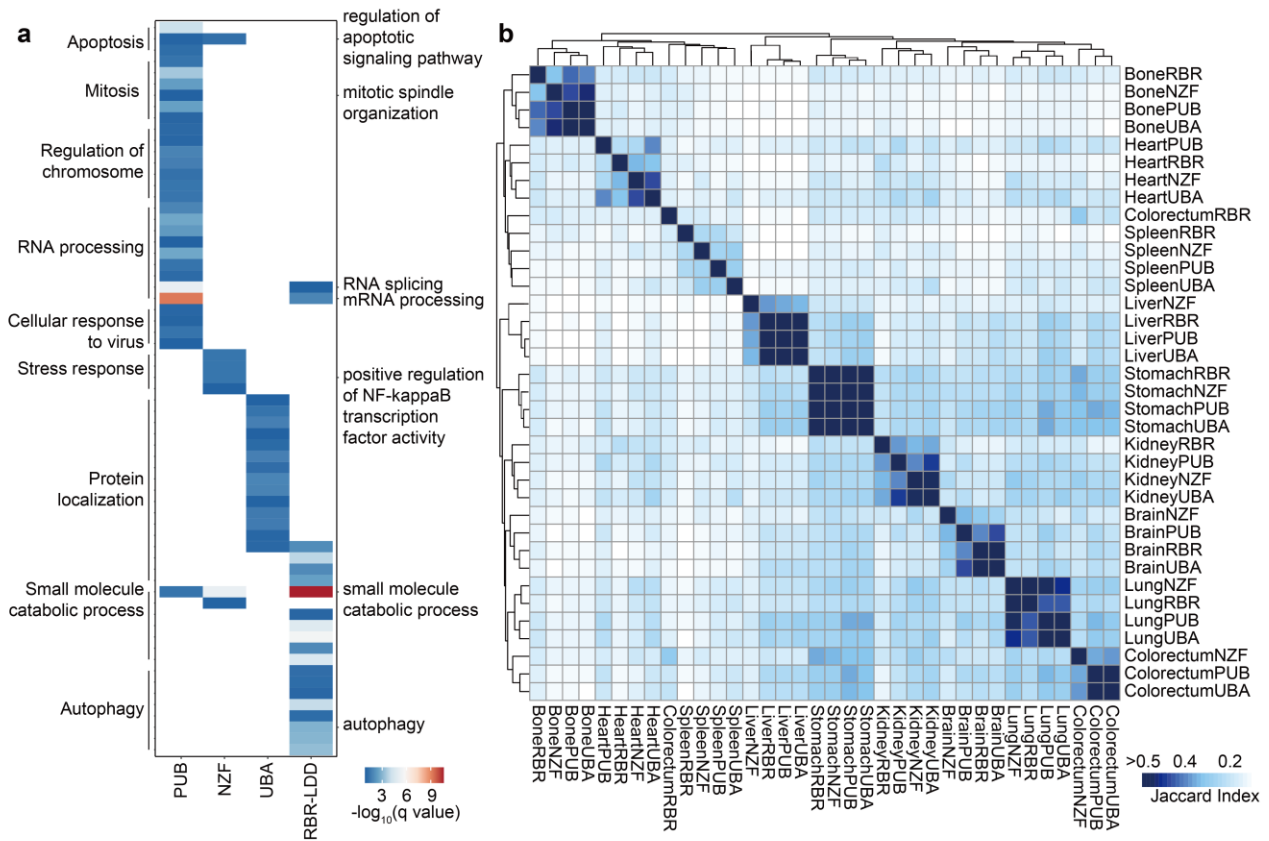
**b** Distribution of the fraction of HOIP PPIs quantified in each domain.

**c** Individual diagrams of domain-dependent interactors. Domain-dependent nodes are shown in gray, whereas domain-independent interactors are depicted in white.

**d** Immunoprecipitation of ectopic expressed Myc-tagged HOIP NZF in lysates from HEK293T cells transfected with indicate Flag-tagged proteins, and immunoblot with the corresponding antibodies. The Myc-tagged vector was a negative control.

**e** Immunoprecipitates of HK1, HK2, PFKL, PDK1 and PRDX1 to detect the linear ubiquitination in HEK293T cells transfected with Myc-tagged HOIP WT or CS (a HOIP inactivate mutant).

**f** Immunoprecipitates of SRSF5, HNRNPF, HNRNPL and MBNL1 to detect the linear ubiquitination in HEK293T cells transfected with Myc-tagged HOIP WT or CS (a HOIP inactivate mutant).

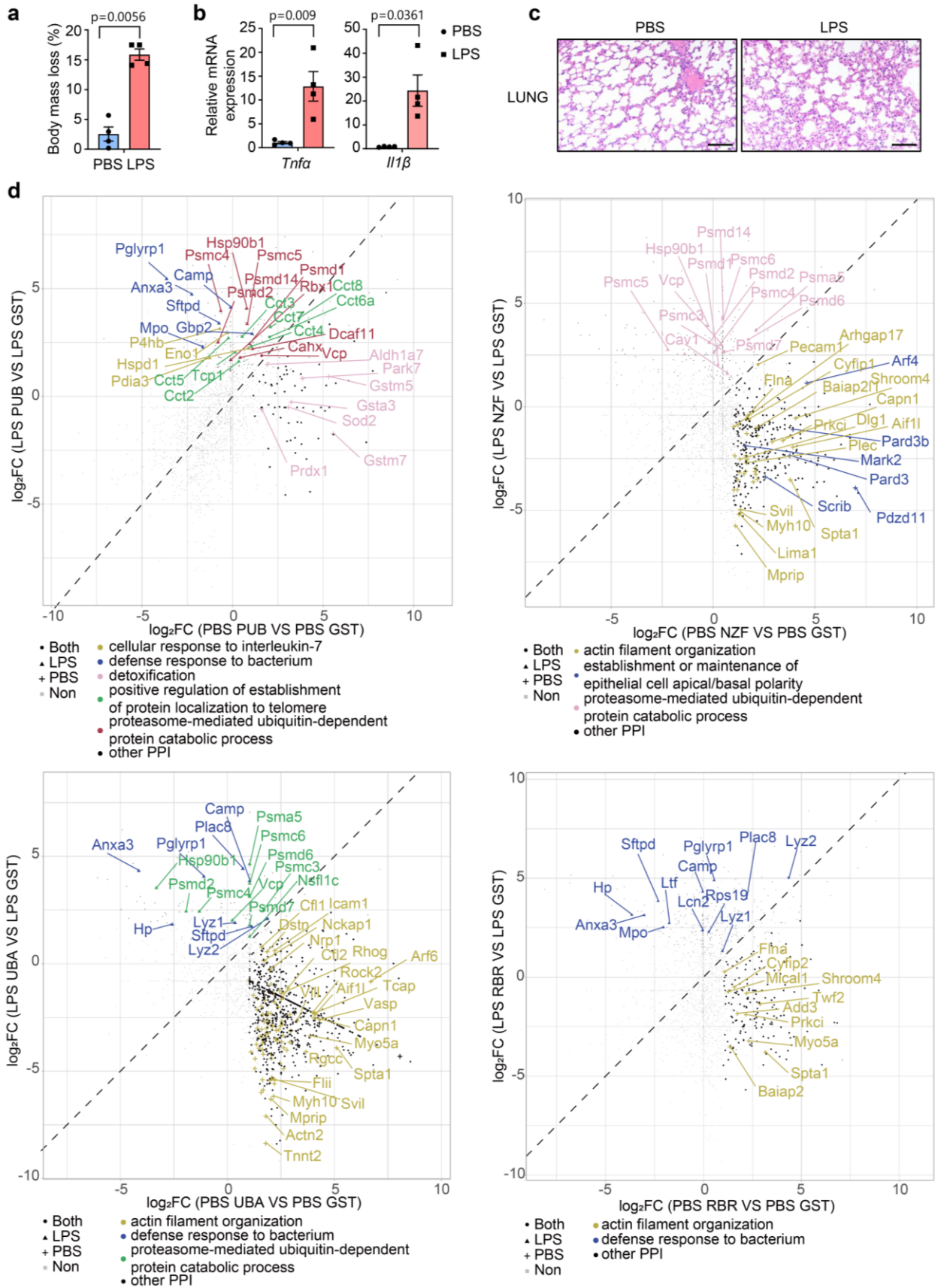


**Supplemental Fig. 4 Functional enrichment of domain-specific HOIP PPIs across tissues, related to Fig. 3.**

**a** Functional enrichment analysis of HOIP PPIs identifies a number of Gene Ontology (GO) terms associated with the domain-specific PPIs. q value is calculated by Benjamini–Hochberg method.

**b** Jaccard index of HOIP PPIs detected by various tissues and domains to show protein coverage between different tissues and domains.





**Supplemental Fig. 5 Functional landscape of HOIP interaction network in lungs from LPS-induced sepsis models, related to Fig. 3.**

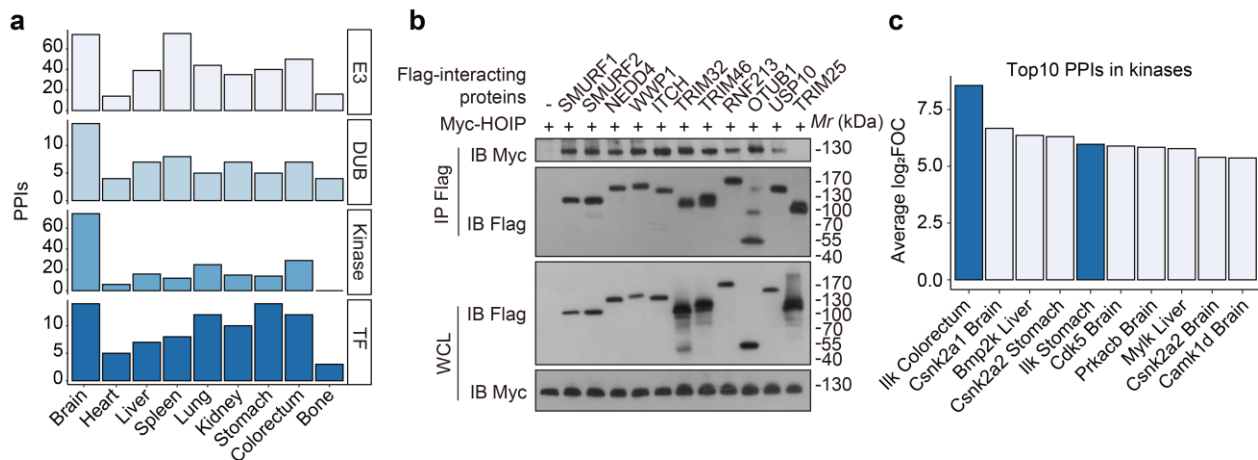
**a** Weight monitoring of mice i.p. injection with or without LPS (12 mg/kg body weight in saline). n = 4 per group.

**b** Quantitative RT-PCR analysis of *Tnfa* and *IL1 $\beta$*  mRNA levels in lungs from LPS-induced sepsis models. n = 4 per group.

**c** H&E staining of lungs from LPS-induced sepsis models. Scale bars, 100  $\mu$ m.

**d** Correlation plot of log<sub>2</sub>FC (HOIP domains/GST) for lungs with or without LPS injection.

Data are shown as the mean  $\pm$  SEM; p values are from the unpaired two-sided t-test.

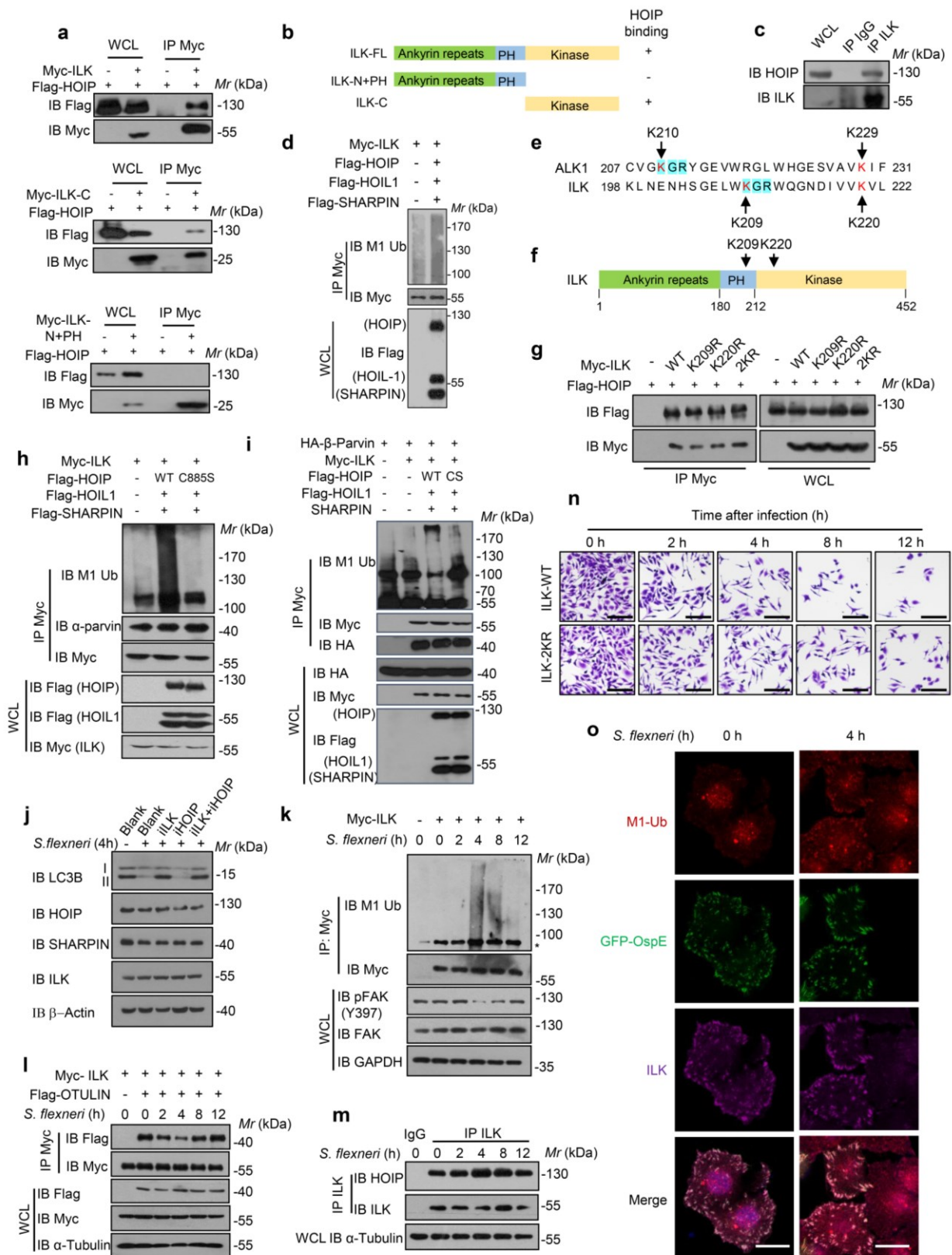


**Supplemental Fig. 6 Functional analysis of HOIP PPIs associated with kinases, E3 ligases, deubiquitinases and transcriptional factors, related to Fig. 4.**

**a** Barchart with the numbers of HOIP PPIs associated with kinases, E3 ligases, deubiquitinases and transcriptional factors.

**b** Immunoprecipitation of ectopic expressed NEDD4-1, WWP1, ITCH, SMURF1, SMURF2, TRIM32, TRIM46, RNF213, OTUB1 and USP10 in HEK293T cells transfected with Myc-tagged HOIP and immunoblot with indicated antibodies.

**c** Representable top 10 HOIP PPIs in the kinases.



**Supplemental Fig. 7** Linearly ubiquitinated ILK is dynamically regulated during *Shigella flexneri* infection, related to Fig. 4.

a HEK293T cells were transfected with Flag-tagged HOIP and Myc-tagged ILK (or ILK truncations), and then immunoprecipitated with the Myc antibody, followed by immunoblotting with the indicated

antibodies.

**b** Schematic representative of the ILK truncations.

**c** Immunoprecipitation of ILK in HEK293T cells and immunoblot with indicated antibodies.

**d** Immunoprecipitation of ILK linear ubiquitination in HEK293T cells transfected with Flag-tagged HOIP, Flag-tagged HOIL1, Flag-tagged SHARPIN and immunoblot with indicated antibodies.

**e** Distribution diagram of potential linear ubiquitination sites of ILK.

**f** Sequence alignment of ILK and ALK1. The horizontal line represents a conservative sequence, arrows indicate linear ubiquitination sites of ALK1 and predicted potential linear ubiquitination sites of ILK.

**g** Immunoprecipitation of ILK and its mutants in HEK293T cells transfected with Myc-tagged ILK wild type (WT) or its mutants K209R, K220R or 2KR (K209R/K220R) and Flag-tagged HOIP, followed by immunoblotting with indicated antibodies.

**h** Cell lysates from HEK293T cells transfected with Myc-tagged ILK, Flag-tagged HOIL-1, Flag-tagged SHARPIN and Flag-tagged HOIP wild type or its catalytically inactivated mutant were immunoprecipitated with the Myc antibody, followed by immunoblotting with indicated antibodies.

**i** Cell lysates from HEK293T cells transfected with HA-tagged  $\beta$ -Parvin, Myc-tagged ILK, Flag-tagged HOIL-1, Flag-tagged SHARPIN and Flag-tagged HOIP wild type or its catalytically inactivated mutant were immunoprecipitated with the Myc antibody, followed by immunoblotting with indicated antibodies.

**j** Immunoblot analysis of the LC3B in *Shigella flexner* infected HeLa cells with 2  $\mu$ M ILK inhibitor ILK-IN-3 and 20  $\mu$ M HOIP inhibitor HOIPin-8.

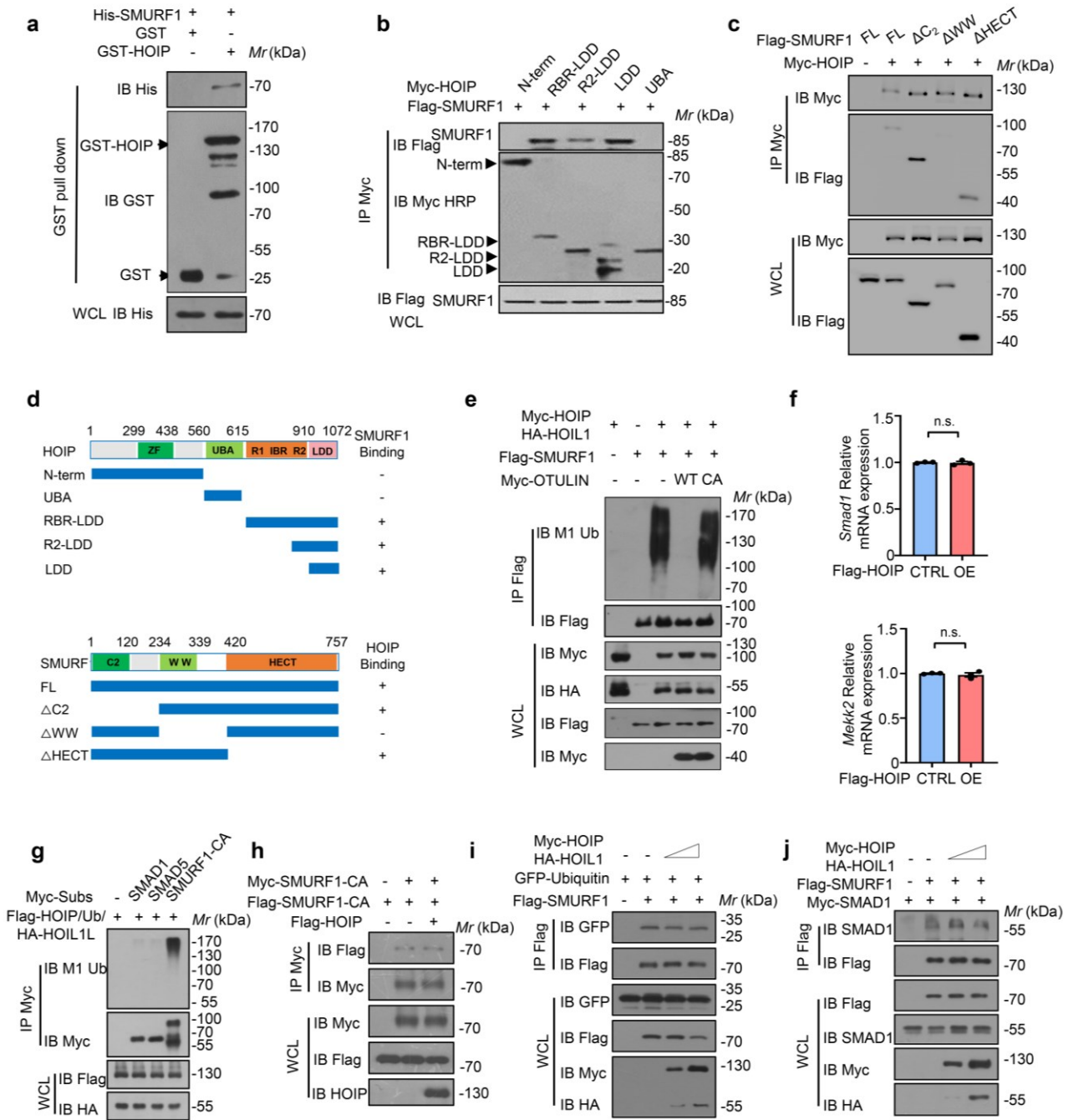
**k** Immunoprecipitation of ILK linear ubiquitination in Myc-tagged ILK overexpressed HeLa cells, infected with *Shigella flexner* for 0 h, 2 h, 4 h, 8 h and 12 h respectively. And immunoblot with indicated antibodies.

**l** Immunoprecipitation of ILK in HeLa cells transfected with Flag-tagged OTULIN and Myc-tagged ILK after *Shigella flexner* infection and immunoblot with indicated antibodies.

**m** Immunoprecipitation analysis of interactions between ILK and HOIP after *Shigella flexner* infection in HeLa cells.

**n** Representative images showing the attached cells of control and ILK-2KR groups infected with *Shigella flexner* for indicated time points, Scale bars, 10  $\mu$ m.

**o** HeLa cells were infected with or without *Shigella flexner* for 4h, then the cells were collected for immunofluorescence with indicated antibodies. Scale bars, 20  $\mu$ m.



**Supplemental Fig. 8 SMURF1 is a HOIP-interacting protein, related to Fig. 5.**

**a** *In vitro* binding assay of purified GST-tagged HOIP and His-tagged SMURF1.

**b** Immunoprecipitates of HOIP and its truncations in HEK293T cells transfected with Flag-tagged SMURF1 and immunoblot with the indicated antibodies.

**c** Immunoprecipitates of SMURF1 and its truncations in HEK293T cells transfected with Myc-tagged HOIP and immunoblot with the indicated antibodies.

**d** Schematic representative of the HOIP-interacting region in SMURF1 and the SMURF1-interacting region in HOIP.

**e** Immunoprecipitates of SMURF1 linear ubiquitination in HEK293T cells transfected with LUBAC and OTULIN mutants.

**f** Analysis of *Smad1* and *Mekk2* genes expression by qPCR in HOIP stably over-expressed (OE) BMSCs.

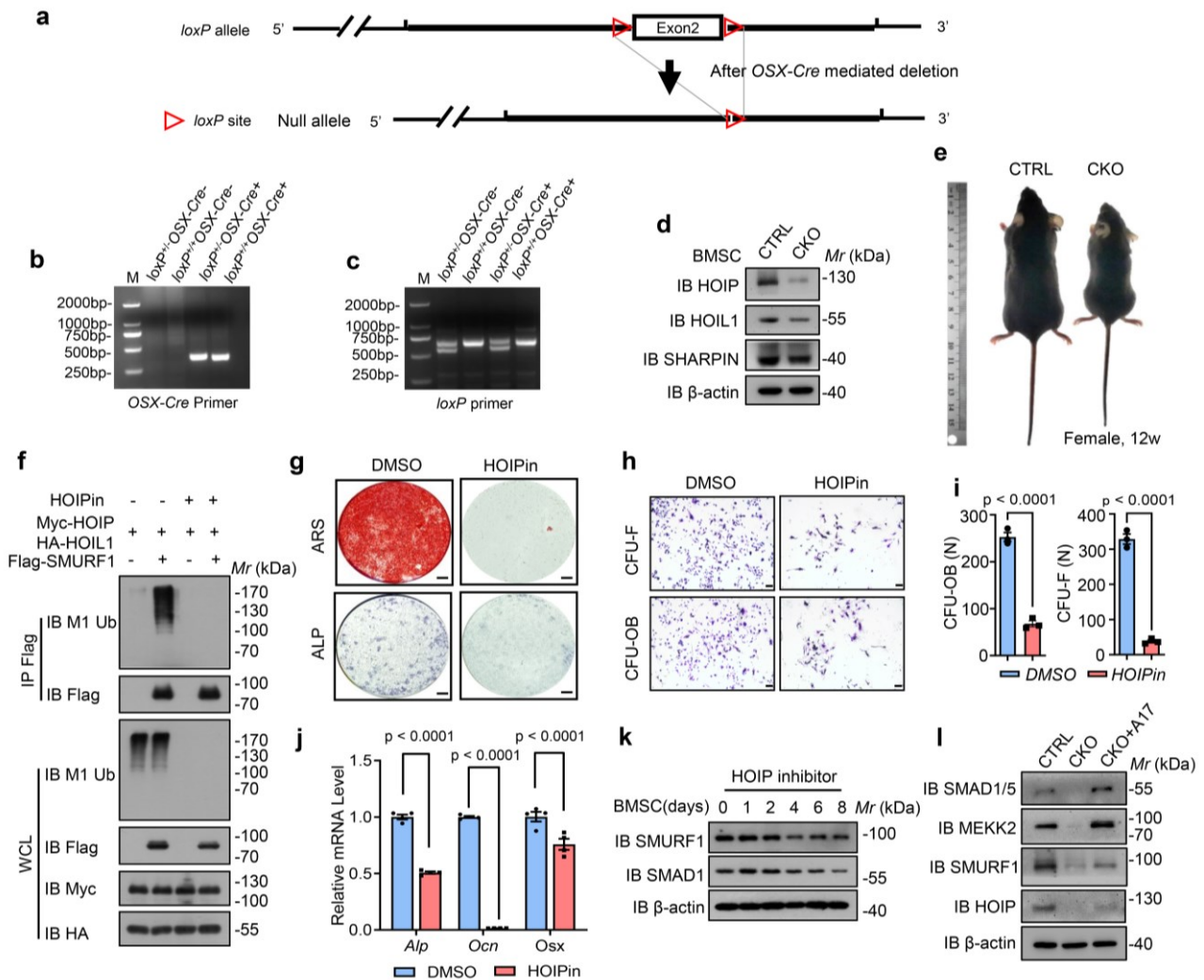
**g** Immunoprecipitates of SMAD1, SMAD5 and SMURF1-C699A's linear ubiquitination in HEK293T cells transfected with LUBAC

**h** Immunoblot analysis of the interaction between Myc-tagged SMURF1-C699A and Flag-tagged SMURF1-C699A in HEK293T cells transfected with Myc-tagged HOIP.

**i** Immunoblot analysis of the interaction between SMURF1 and ubiquitin in HEK293T cells transfected with LUBAC.

**j** Immunoblot analysis of the interaction between SMURF1 and its substrate SMAD1 in HEK293T cells transfected with LUBAC.

Data are shown as the mean  $\pm$  SEM; n = 3 per group, p values are from the unpaired two-sided t-test. n.s., no significant.



**Supplemental Fig. 9 Targeted disruption of the murine *Hoip* gene, related to Fig. 6.**

**a** Diagram of *Hoip* osteoblast specific knockout strategy.

**b, c** PCR analysis of HOIP CTRL and CKO mice tails.

**d** Immunoblot analysis of HOIP, HOIL1 and SHARPIN protein levels in BMSCs from HOIP CTRL and CKO mice.

**e** Representative image of 8-week-old female HOIP CTRL and CKO mice.

**f** Immunoprecipitates of SMURF1 linear ubiquitination in HEK293T cells transfected with LUBAC with or without HOIP inhibitor HOIPin-8.

**g** Representative images of ALP and ARS staining of BMSCs with or without HOIP inhibitor treatment after cultured in osteogenic medium for 14 and 28 days. Scale bars, 2 mm.

**h, i** Representative images and quantification of CFU-OB and CFU-F of BMSCs with or without HOIP inhibitor treatment. Scale bars, 50  $\mu$ m. n = 3 per group.

**j** Immunoblot of SMURF1 in BMSCs with HOIP inhibitor treatment.

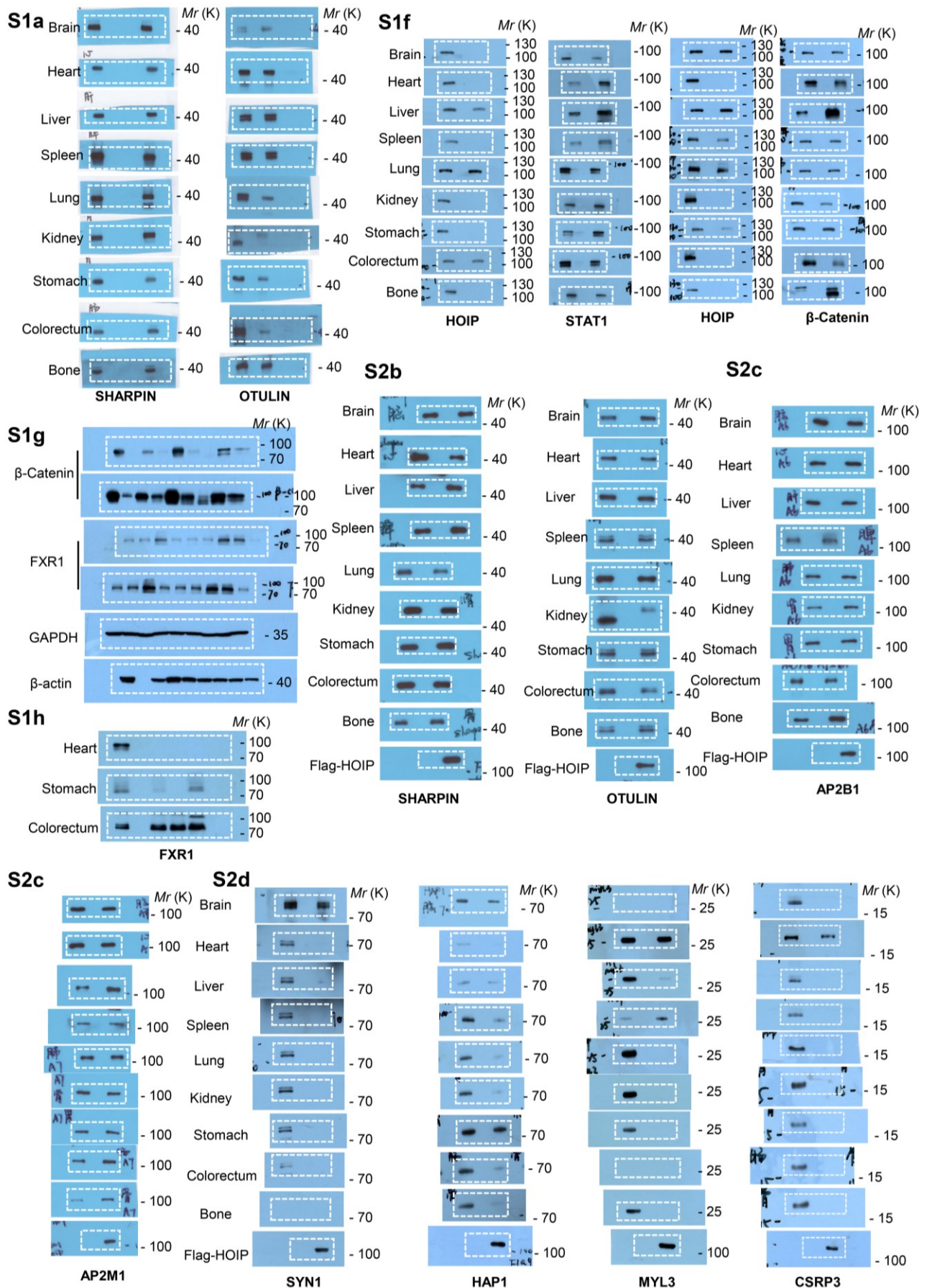
**k** Quantitative RT-PCR analysis of osteogenesis genes mRNA levels in BMSCs with or without HOIP inhibitor treatment. n = 3 per group.

**l** Immunoblot of SMURF1 and its substrate SMAD1/5, MEKK2 in BMSCs from CTRL, CKO and CKO treated with SMURF1 inhibitor A17 groups for 28 days.

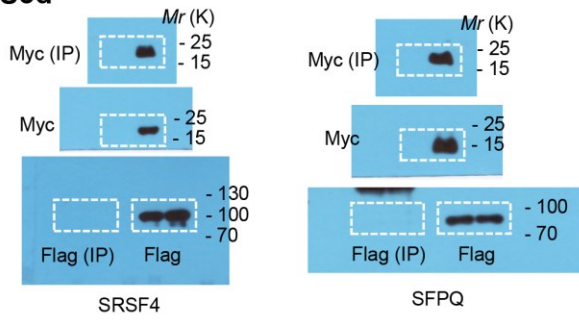
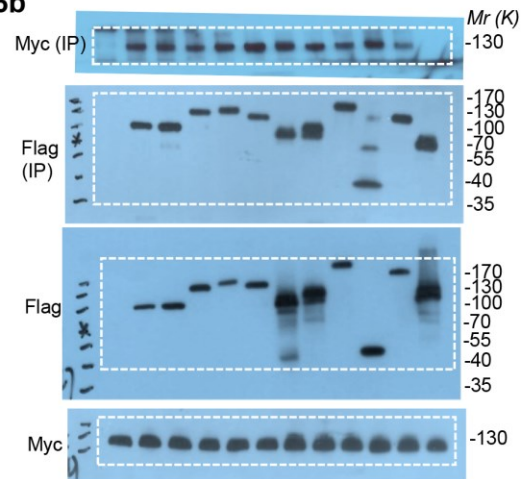
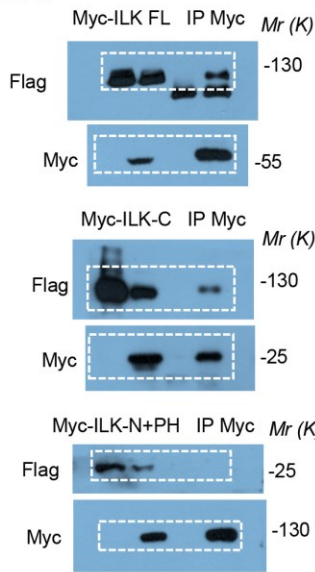
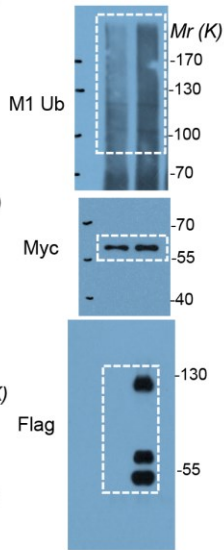
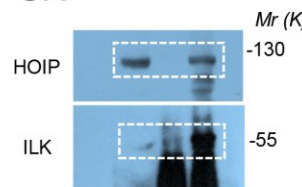
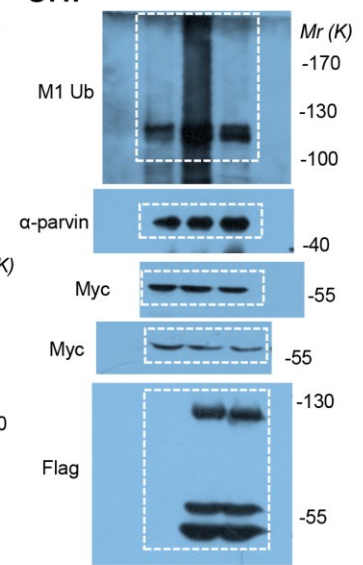
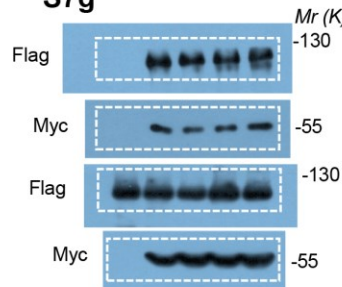
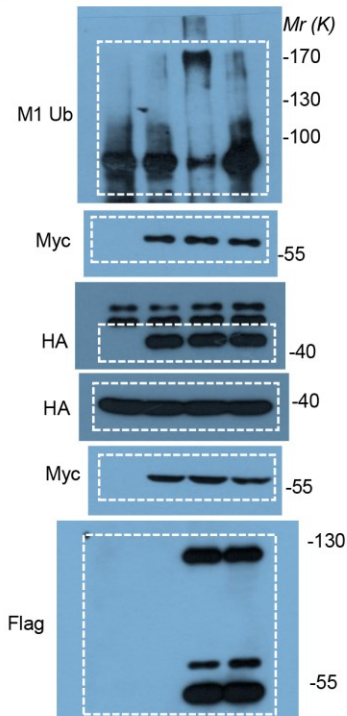
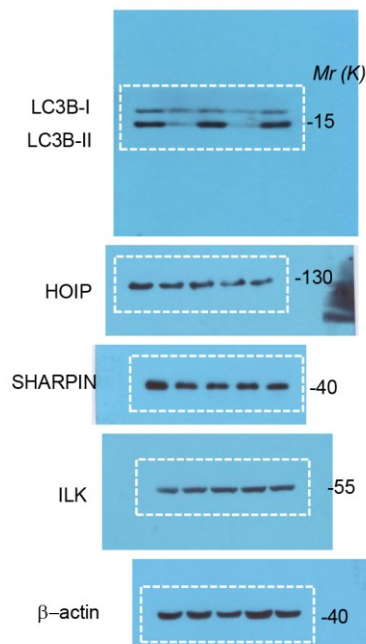
Data are shown as the mean  $\pm$  SEM; p values are from the unpaired two-sided t-test and the two-way ANOVA (Sidak's multiple comparisons test).



## Uncropped blots for Supplementary Figures





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