Expanded View Figures

Figure EV1. Related to Fig 1, SAGA and USP22 inducibly bind to the promoter region of ER stress response genes.

A, B ChIP-qPCR for USP22 at the CHOP and ERP70 loci before and after Thaps treatment, with and without shRNA-mediated depletion of USP22. Three independent experiments are represented as mean ± SEM, with significance measured by Student's *t*-test. ***$P < 0.005$. X-axis labels on *CHOP* indicate coordinates relative to the TSS.

C ChIP-qPCR for USP22 and ATXN7L3 at bound (*GRP78*, *SEL1L*) and unbound (*FNDC3B*) genes. Three independent experiments are represented as mean ± SEM. *Desert* serves as a negative control.

D Venn diagram overlap of SAGA peaks prior to filtering for ER stress responsiveness as measured by increases in SAGA subunit ChIP-seq signal following Thaps treatment.

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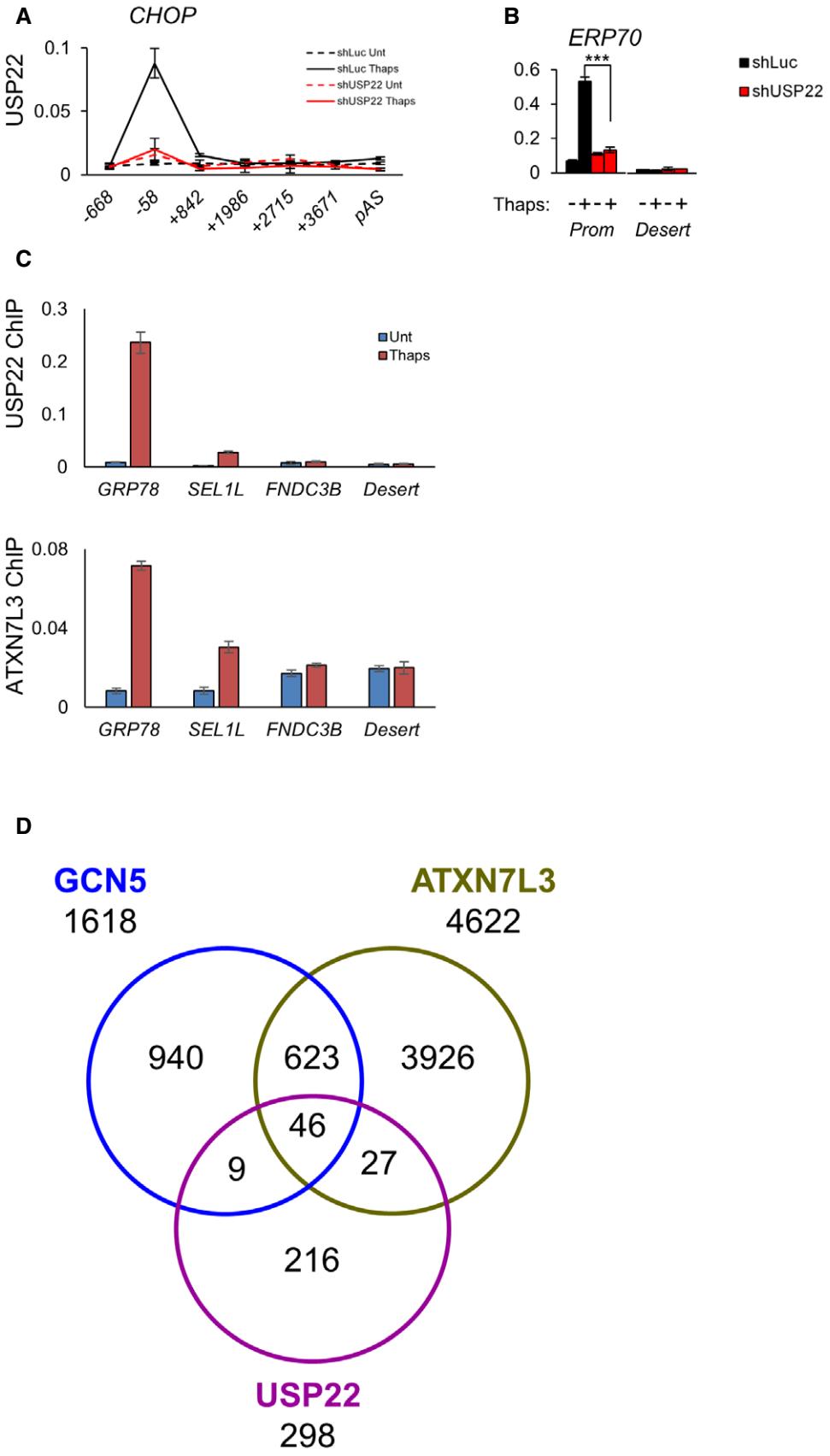
A
CHOP
USP22
0.1
0.05
0
shLuc Unt
shLuc Thaps
shUSP22 Unt
shUSP22 Thaps
-668
-58
+842
+1986
+2715
+3671
pAS
B
ERP70
***
0.6
0.4
0.2
0
shLuc
shUSP22
Thaps: -+-+ -+-+
Prom Desert
C
USP22 ChIP
0.3
0.2
0.1
0
Unt
Thaps
GRP78 SEL1L FNDC3B Desert
ATXN7L3 ChIP
0.08
0.04
0
GRP78 SEL1L FNDC3B Desert
D
GCN5
1618
ATXN7L3
4622
940
623
3926
46
9
27
216
USP22
298

Figure EV1.

Figure EV2. Related to Fig 3, USP22 is required for transcription of ER stress response genes.

A Cells were treated for 2 h with 100 nM Thapsigargin in the presence or absence of shRNAs targeting USP22. Whole-cell lysate was subjected to immunoblotting with the indicated antibodies.

B HCT116 cells were treated with 100 nM Thapsigargin for the indicated times in the presence or absence of shRNA targeting USP22. RNA was isolated and subjected to qRT–PCR against the indicated transcripts. Three independent experiments are represented as mean ± SEM.

C, D Cells were treated as in (A) and subjected to ChIP-qPCR for ATF4 and XBP1s at the indicated gene enhancers and promoters. Three independent experiments are represented as mean ± SEM.

Source data are available online for this figure.

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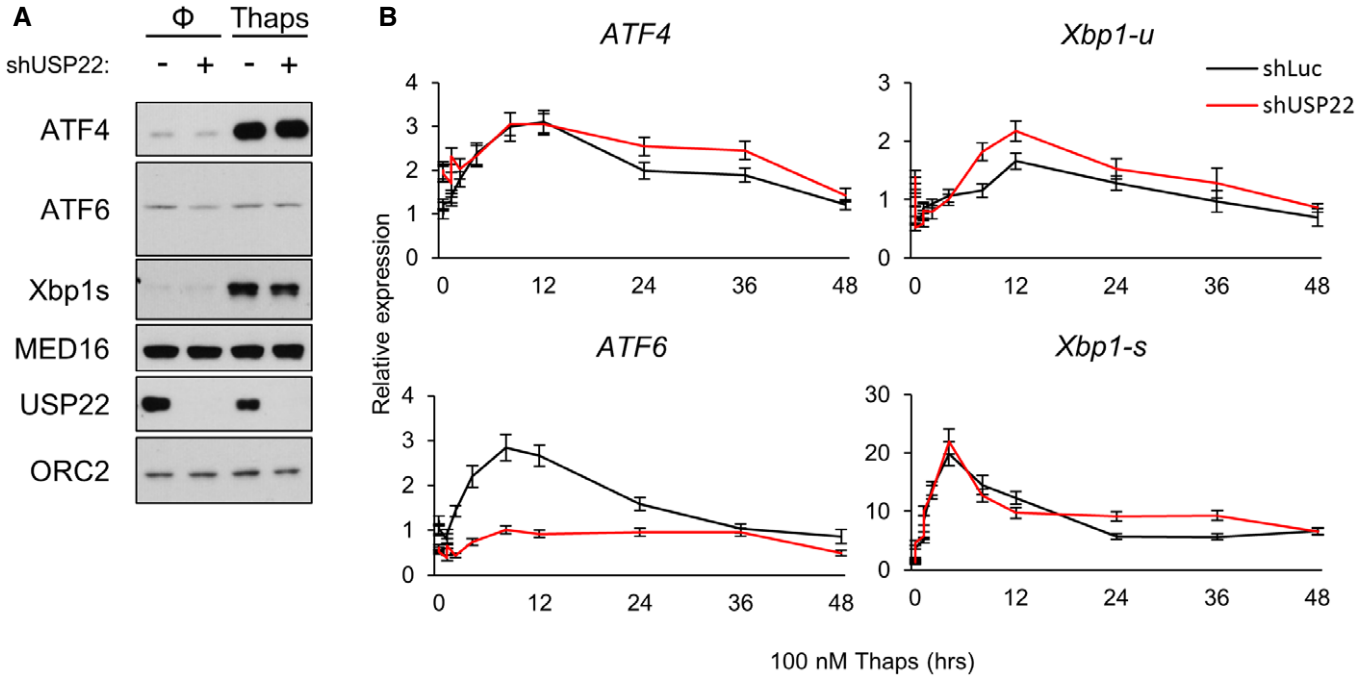

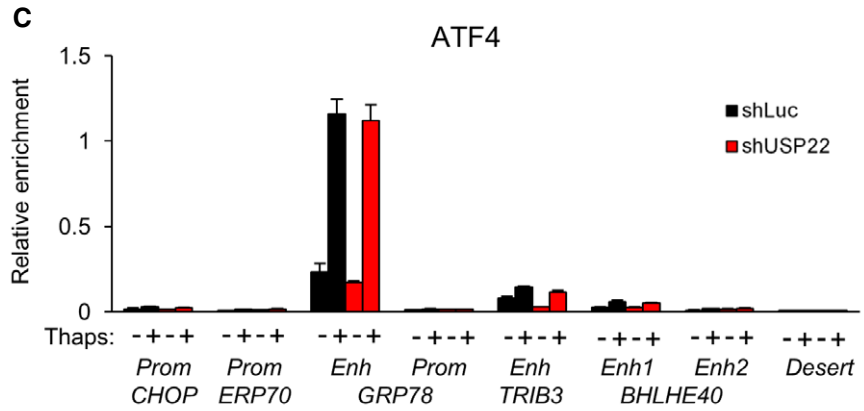

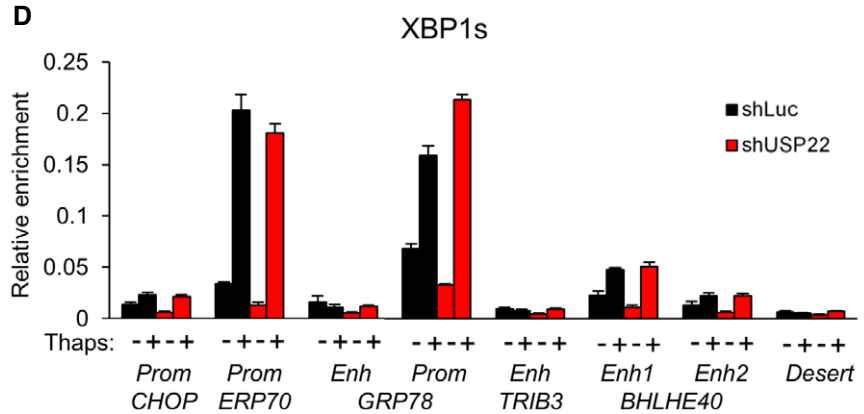

Figure EV2.

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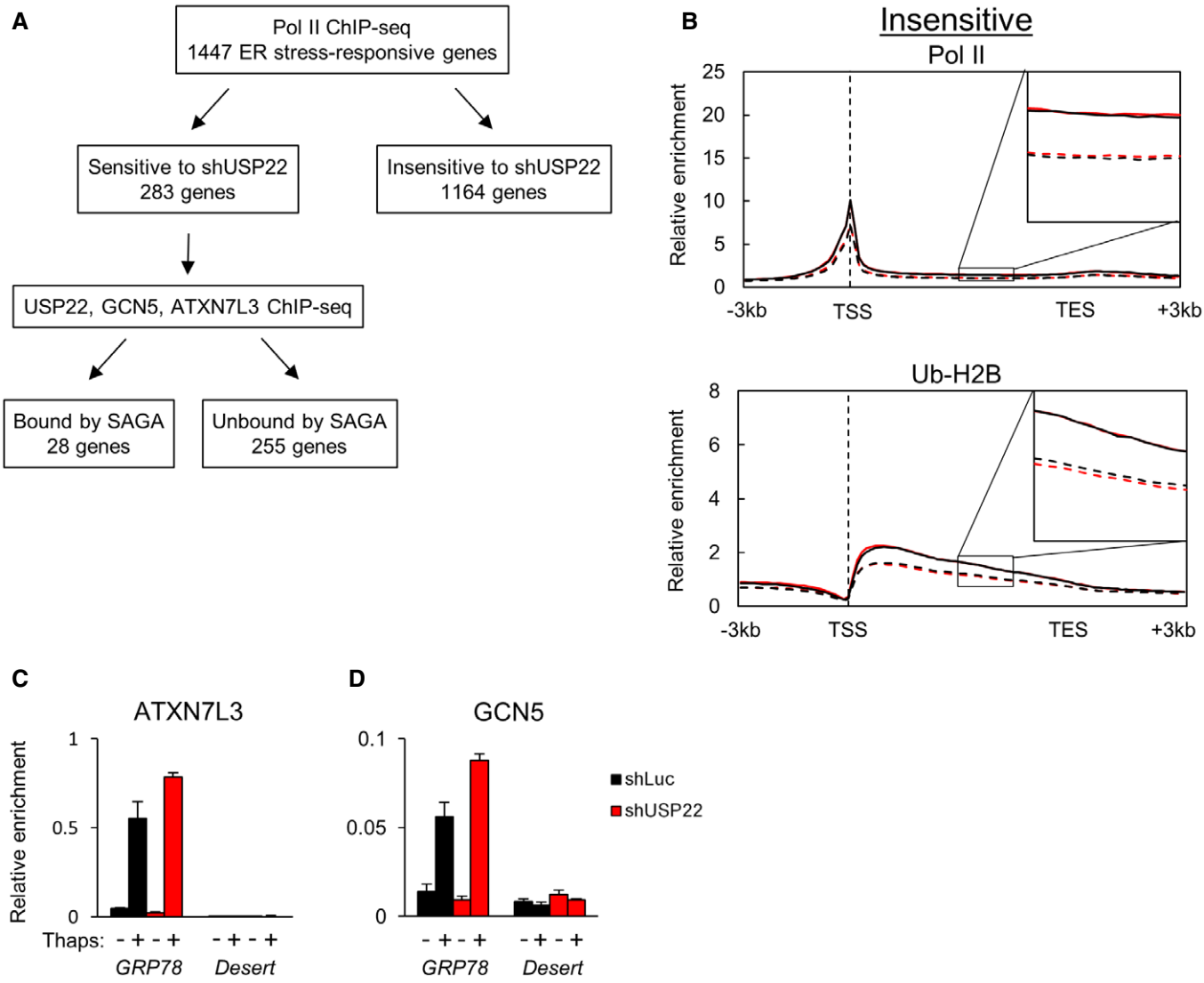

Figure EV3. Related to Fig 4, USP22 is required for efficient recruitment of Pol II to activator-induced target genes independent of Ub-H2B.

A Flowchart depicting ChIP-seq subsetting.

B Metagene profiles for Pol II (top) and Ub-H2B (bottom) ChIP-seq for all genes not directly bound by USP22 and insensitive to USP22 knockdown, termed "Insensitive" genes.

C, D ChIP-qPCR for ATXN7L3 and GCN5 at the indicated loci, before and after ER stress, in the presence and absence of shRNA targeting USP22, represented from three independent experiments as mean ± SEM.

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Figure EV4. Related to Fig 6, deubiquitylation of Pol II is controlled by USP22.

A High-throughput proteomic analysis of Pol II following depletion of USP22, with significance measured by Student's *t*-test. Specific ubiquitylated lysines detected are highlighted in red text.

B HCT116 cells were treated for 2 h with 100 nM Thapsigargin in the presence or absence of shRNA targeting USP22. Cells were harvested and fractionated, and fractions were subjected to immunoblotting with the indicated antibodies. Loading controls ORC2 and β-tubulin are duplicate images from Fig 6.

C *In vitro* deubiquitylation UbiTest of endogenously ubiquitylated Pol II. HCT116 cells were treated with MG132 in the presence or absence of shRNA targeting USP22. Lysates were generated using buffer containing protease cocktail inhibitor, pan-DUB inhibitor PR619, and the JAMM protease inhibitor *o*-phenanthroline. Ubiquitylated proteins were purified on ubiquitin-binding resin, and eluates were either undigested (lanes 1 and 2) or digested with USP2 to strip polyubiquitin (lanes 3 and 4) and reduce target proteins to unit length. Digestion reactions were subjected to immunoblotting with the indicated antibodies. shRNA control USP22 and loading controls actin and Ub are duplicate images from Fig 6.

D *In vitro* deubiquitylation of endogenously ubiquitylated proteins. HCT116 cells were treated as in (C). Ubiquitylated proteins were purified on ubiquitin-binding resin, and eluates were either undigested (lane 3) or digested with non-specific DUB ("USP2", lane 4) or human DUB module ("USP22", lane 5) to reduce target protein ubiquitylation. Digestion reactions were subjected to immunoblotting with the indicated antibodies.

Source data are available online for this figure.

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A

Protein	Fold Change shUSP22 : shLuc	Site	Peptide	p-value
POLR2A	2.3	707	TYQDIQNTIKK	0.046
	2.9	767	SLSEYNNFKSMVVSGAK	0.014
	4.0	775	SMVVSGAKGSK	0.038
	2.1	796	INISQVIAVVGQQNVEGKR	0.043
	2.4	976	VIFPTGDSKVVLPCNLLR	0.041
	2.2	1019	KLVIVNGDDPLSR	0.006
	2.8	1014	LPSDLHPIKVVEGVK	0.040
	2.6	1155	AKDILCR	0.003

K = ubiquitylated lysine

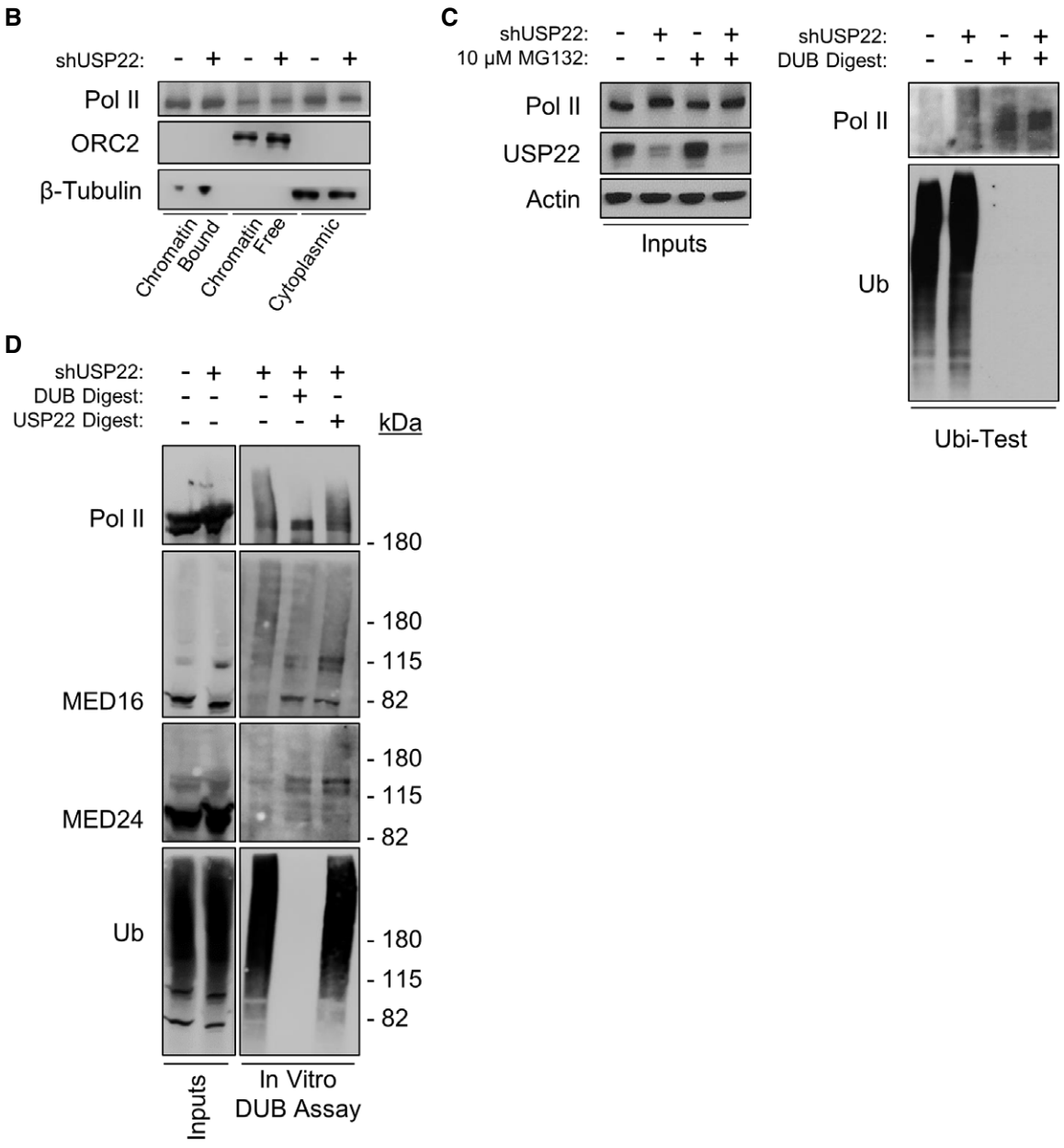

Figure EV4.

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