Swenson et al. Supplemental Data

Supplemental Figure Legends

Supplemental Figure 1. The HECT domain of UBR5 is required for survival at birth. (A) Distribution of pups born from crossing *Ubr5*$^{\Delta HECT/\Delta HECT}$ with E2A^{CRE}. (B) Pups alive at birth per genotype. (C) Genotyping PCR of targeted alleles and deletion in six-week old mice bred to B lymphocyte specific *Mb1*^{Cre} mouse model. PCR was performed on BM.

Supplemental Figure 2. The loss of the HECT domain of *Ubr5* does not impact the transitional B cell population but decreased B cells within the peritoneal cavity. (A) Bar graph of spleen weight. (N=19) (B) An example hematoxylin and eosin stain of spleens. (N=4) (C) Representative flow cytometry plot gated on B220^{+} for transitional cells: T1 cells (B220^{+}CD93^{+}IgM^{+}CD23^{-}), T2 cells (B220^{+}CD93^{+}IgM^{+}CD23^{+}), and T3 cells (B220^{+}CD93^{+}IgM^{-}CD23^{+}). (N=5) (D-E) Bar graphs of percentages and absolute number of transitional cells within the spleen. (F) Representative flowcytometry plot for B2 (B220^{+}CD19^{+}) and B1 (B220^{lo}CD19^{+}) B cells within the peritoneal cavity. (G-H) Bar graphs representing the percentages of B2 and B1 B cells within the peritoneal cavity. (N=5, * P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001)

Supplemental Figure 3. Loss of HECT domain leads to increased splenic cells in G0. (A) Cell cycle breakdown of mature (B220^{+}IgM^{+}IgD^{+}) splenocytes. (N≥7) (B) Ki67 IHC staining of spleen. (N=3, * P<0.05, ** P<0.01, *** P<0.001)

Supplemental Figure 4. CCND1 overexpression coupled with loss of the HECT domain does not promote lymphomagenesis. (A) Bar graph representing the percentages of ProPre, Immature, and Recirculating B cells. (N=6) (B) Bar graph representing the percentages of $B220^+$ cells within the peripheral blood. (N≥9) (C) Bar graph representing the percentages of $CD138^+$ cells within the $B220^+$ cell population. (N≥9) (D) Bar graph of spleen weight. (N≥9) (E) Bar graph of total splenocytes. (N≥6) (F) Bar graph representing the percentage of $B220^+$ cells within the spleen. (N≥9) (G) Bar graph of the percentages of marginal zone and follicular B cells within the spleen. (N≥9) (H) Bar graph representing the percentages of B1, B1a, and B1b percentages within the spleen. (N=6) (I) Bar graphs of the MFI of selected markers of follicular B cells. (N≥9, * P<0.05, ** P<0.01, **** P<0.0001)

Supplemental Figure 5. UBR5 expression in MCL, and activity of UBR5 in mouse model. (A) Western blot of UBR5 and spliceosome components in normal human B cells from tonsil ($B220^+$) as compared to human MCL cell lines that are WT *Ubr5* (JEKO1 and MINO). (B) Western blot of UBR5 and Ubiquitin input and IP for normal IgG or UBR5 in $CD23^+$ B cells from the spleen. (C and D) Graphical representation of the C-terminal portion of WT and ΔHECT UBR5 highlighting important phosphorylation and ubiquitination residues and the catalytic cysteine residue lost upon truncation. (E) Western blot of UBR5 and spliceosome components of $Ubr5^{WT}$ and $Ubr5^{\Delta HECT}$ follicular B cells ($B220^+CD21^+CD23^+$) treated with cycloheximide and either DMSO or MG132 with a table of average half-lives of UBR5 and spliceosome proteins. (F) Western blot of

input and TR-TUBE agarose bead IP for Ubiquitin, U5 spliceosome proteins, and known UBR5 interacting protein RNF168.

Supplemental Material and Methods

Genotyping Primer	Sequence
Ubr5 3' F	5' – GCT CCT CCA GTT CAA ACG GT – 3'
Ubr5 3' R	5' – GAC CCA AAC AAT GAC TCA GAA C – 3'
Ubr5 5' F	5' – GGA TTA AAG GTG TGC GCT GCC – 3'
Mb1 F	5' – CCC GCC TCA CCT GTG AAA A – 3'
Mb1 R	5' – CTG GCA CCA CTG CAC AGA A – 3'
Pan Cre F	5' – CCC AAG AAG AAG AGG AAA GTC – 3'
Pan Cre R	5' – AGA CCA GGC CAG GTA TCT C – 3'

qRT-PCR Primer	Sequence
Gapdh F	5' – CAT GGC CTT CCG TGT TCC TA – 3'
Gapdh R	5' – CTG GTC CTC AGT GTA GCC CAA – 3'
Ubr5 F	5' – TGA GGT TTC TAC GAT CTG TGG C – 3'
Ubr5 R	5' – AAA CAC ACG TTT GCA TTT TCC A – 3'
Ubr5 Hect F	5' – CTC CAG TTC AAA CGG TGG TT – 3'
Ubr5 Hect R	5' – CTG GCA ATG ATG GGC TAG AT – 3'

IHC Antibodies:

Antibody	Company	Ref#
UBR5	Abcam	ab70311
PNA	Vector Laboratories	B-1075

IP and Western Blot Antibodies:

Antibody	Company	Ref#
UBR5	Cell Signaling	65344S
UBR5	Abcam	ab70311
RNF168	Santa Cruz	sc-101125
RNF168	Millipore Sigma	ABE367
EFTUD2	Bethyl	A300-957A
SNRNP200	Abcam	ab176715
ACTIN-HRP	Santa Cruz	sc-47778
DHX15	Protein Tech	12265-1-AP
PRPF8	Abcam	ab79237
LAMIN-A/C	Bethyl	A303-431A

UBIQUITIN	Invitrogen	14-6078-80
Rabbit IgG	Cell Signaling	2729S
TR-TUBE Agarose	LifeSensors	UM401
Rabbit-HRP	Jackson	111-035-144
Mouse-HRP	Jackson	315-035-048

Flow Cytometry Antibodies:

Antibody	Fluorochrome	Clone	Company
B220	BV421, BV510, APC Cy7	RA3-6B2	BioLegend
CD19	APC Cy7, PE Cy7	6D5	BioLegend
IgM	PE, PE Cy7, FITC	RMM-1	BioLegend
IgD	APC, BV711	11-26c.2a	BioLegend
cKit	APC	2B8	BioLegend
CD25	FITC	3C7	BioLegend
CD21/CD35	PE	7E9	BioLegend
CD23	APC, Alexa 700	B3B4, B3B4	BioLegend
CD5	FITC, Per cp Cy 5.5	53-7.3	BioLegend
CD1d	Pac Blue	1B1	BioLegend
CD138	BV421	281-2	BioLegend
CD22	APC	OX-97	BioLegend
CD93	FITC	AA4.1	BioLegend
DAPI			ThermoFisher
Ki67	FITC	16A8	BioLegend
Streptavidin	FITC		Biolegend
CD95	APC	SA367H8	Biolegend

Mass Spectrometry Method:

Samples were loaded onto trap column Acclaim PepMap 100 75µm x 2 cm C18 LC Columns (Thermo Scientific™) at flow rate of 5µl/min then separated with a Thermo RSLC Ultimate 3000 (Thermo Scientific™) from 5-20% solvent B (0.1% FA in 80% ACN) from 10-98 minutes at 300nL/min and 50ºC with a 120 minutes total run time for fractions one and two. For fractions three to six, solvent B was used at 5-45% for the same duration. Eluted peptides were analyzed by a Thermo Orbitrap Fusion Lumos Tribrid (Thermo Scientific™) mass spectrometer in a data dependent acquisition mode using synchronous precursor selection method. A survey full scan MS (from m/z 375-

1500) was acquired in the Orbitrap with a resolution of 120,000. The AGC target for MS2 in iontrap was set as $1x10^4$ and ion filling time set as 150ms and fragmented using CID fragmentation with 35% normalized collision energy. The AGC target for MS3 in orbitrap was set as $1x10^5$ and ion filling time set as 200ms with a scan range of 100-500 and fragmented using HCD with 65% normalized collision energy. Protein identification was performed using proteome discoverer software version 2.2 (Thermo Fisher Scientific) by searching MS/MS data against the UniProt mouse protein database. The search was set up for full tryptic peptides with a maximum of 2 missed cleavage sites. Oxidation, TMT6plex of the amino terminus, GG and GGQ ubiquitination, phosphorylation, and acetylation were included as variable modifications and carbamidomethylation and TMT6plex of the amino terminus were set as fixed modifications. The precursor mass tolerance threshold was set at 10ppm for a maximum fragment mass error of 0.6 Da with a minimum peptide length of 6 and a maximum peptide length of 144. The significance threshold of the ion score was calculated based on a false discovery rate calculated using the percolator node. Protein accessions were put into Ingenuity Pathway Analysis (QIAGEN Inc.) to identify gene symbols and localizations. Gene ontology pathway analysis was performed using DAVID Bioinformatics Database 6.8 using the functional annotation tool.

TR-TUBE IP Method:

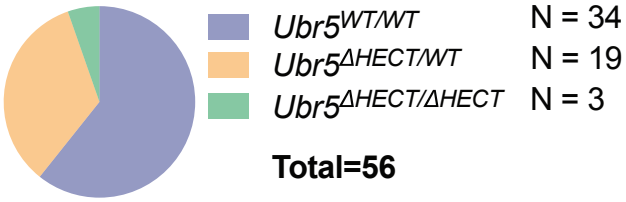
$CD23^+$ splenocytes were lysed in IP lysis buffer containing protease and deubiquitinase inhibitors and sonicated for 10 seconds. A portion of the cleared lysate was taken for input and the remaining lysate was added to TR-TUBE agarose beads (LifeSensors)

(washed 2x in IP lysis buffer) for two hours at room temperature. Beads were washed 5x in IP lysis buffer and then eluted with SDS loading dye at 95°C for 10 minutes.

Swenson et al Supplemental Figure 1

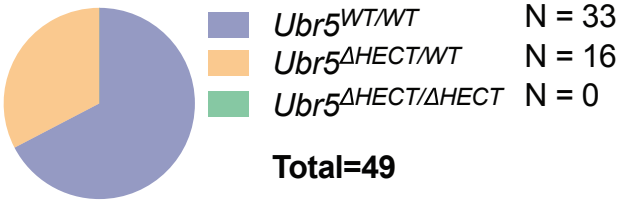
A.

At Birth

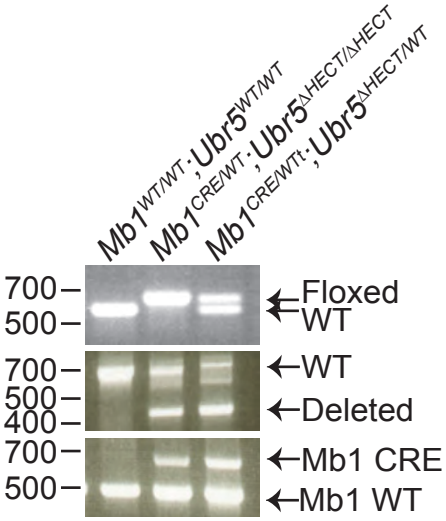


B.

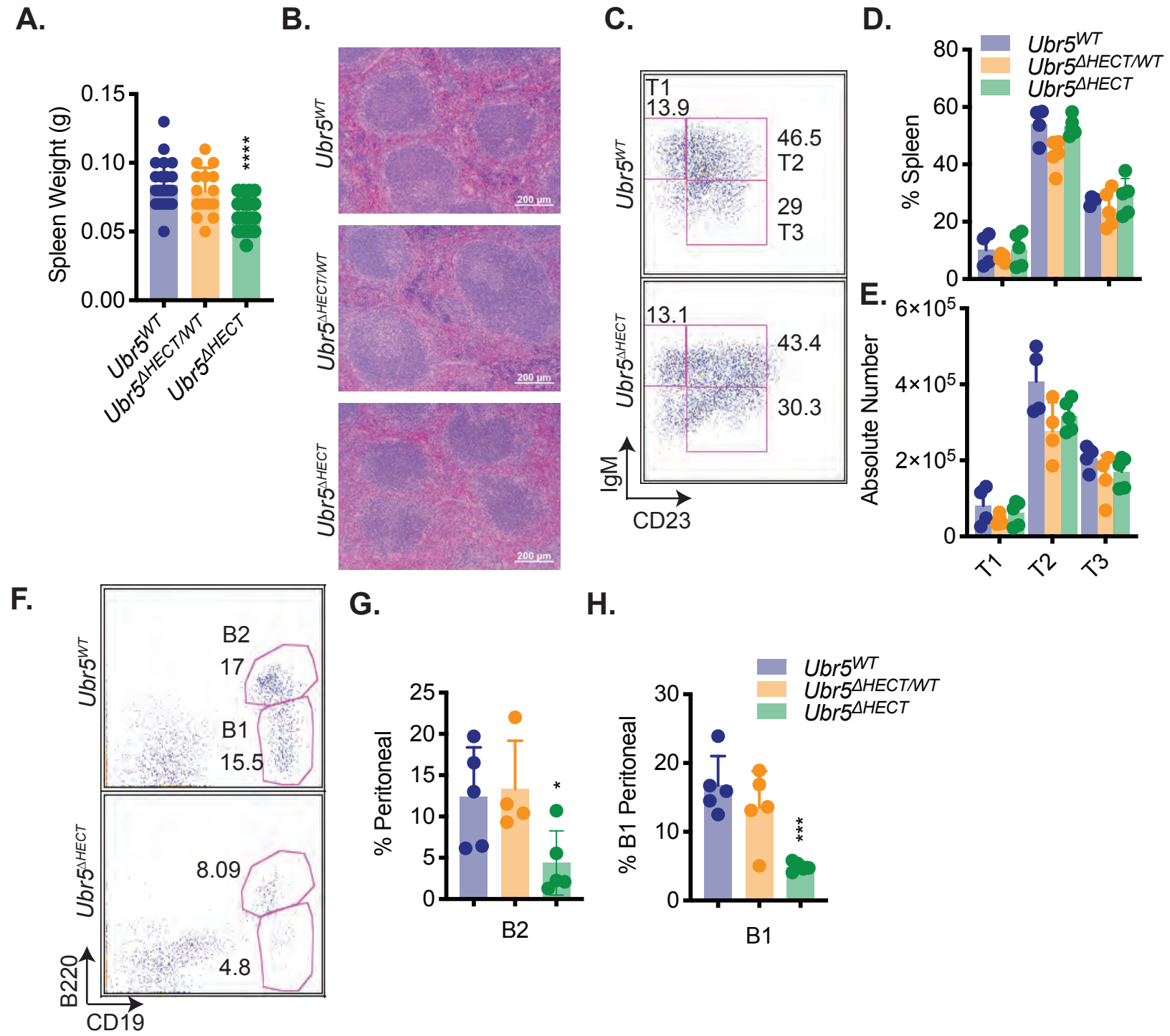
Live at Birth



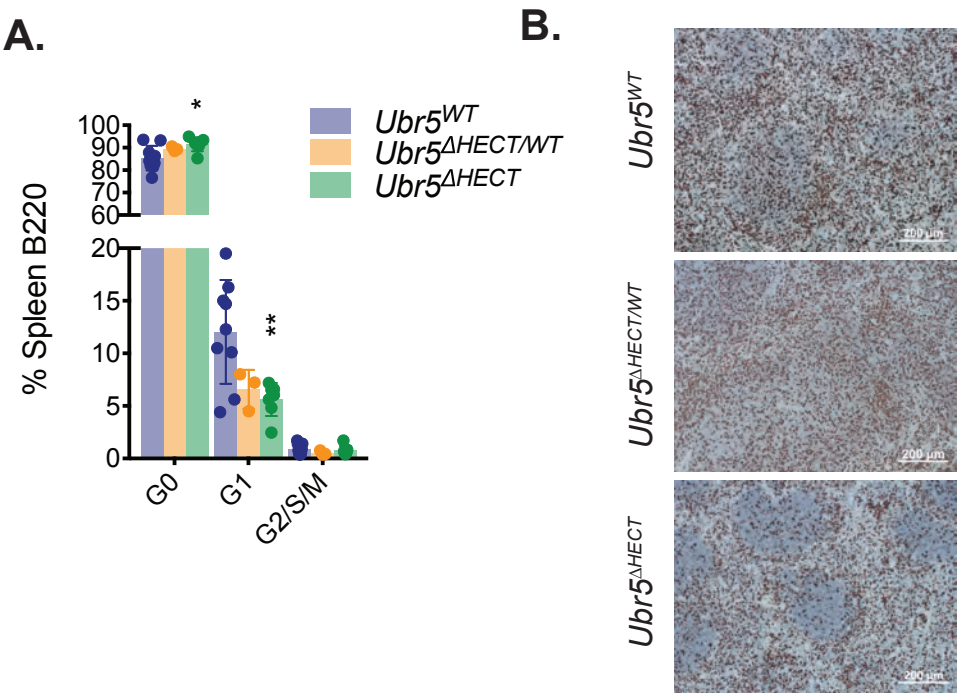
C.



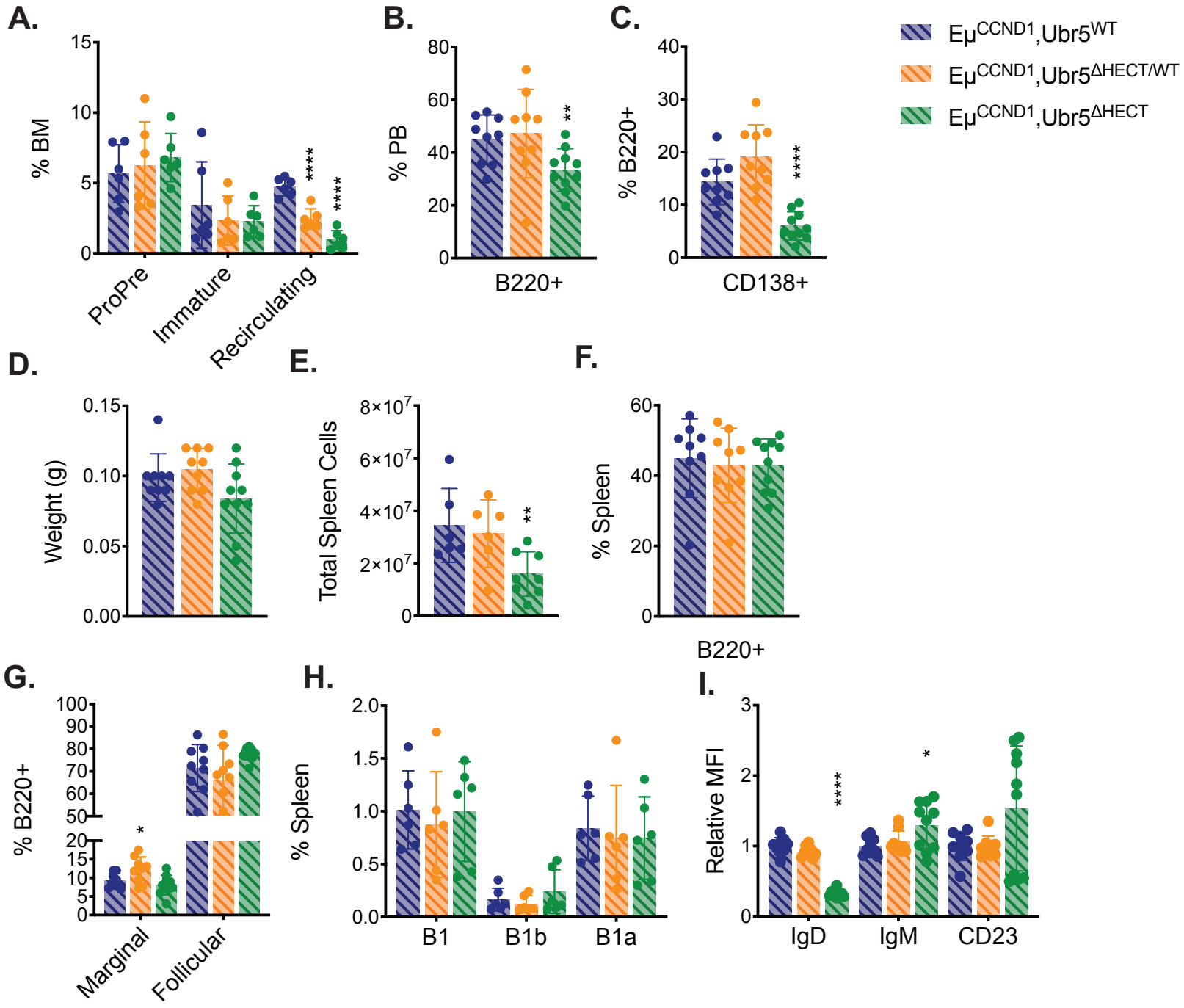
Swenson et al Supplemental Figure 2



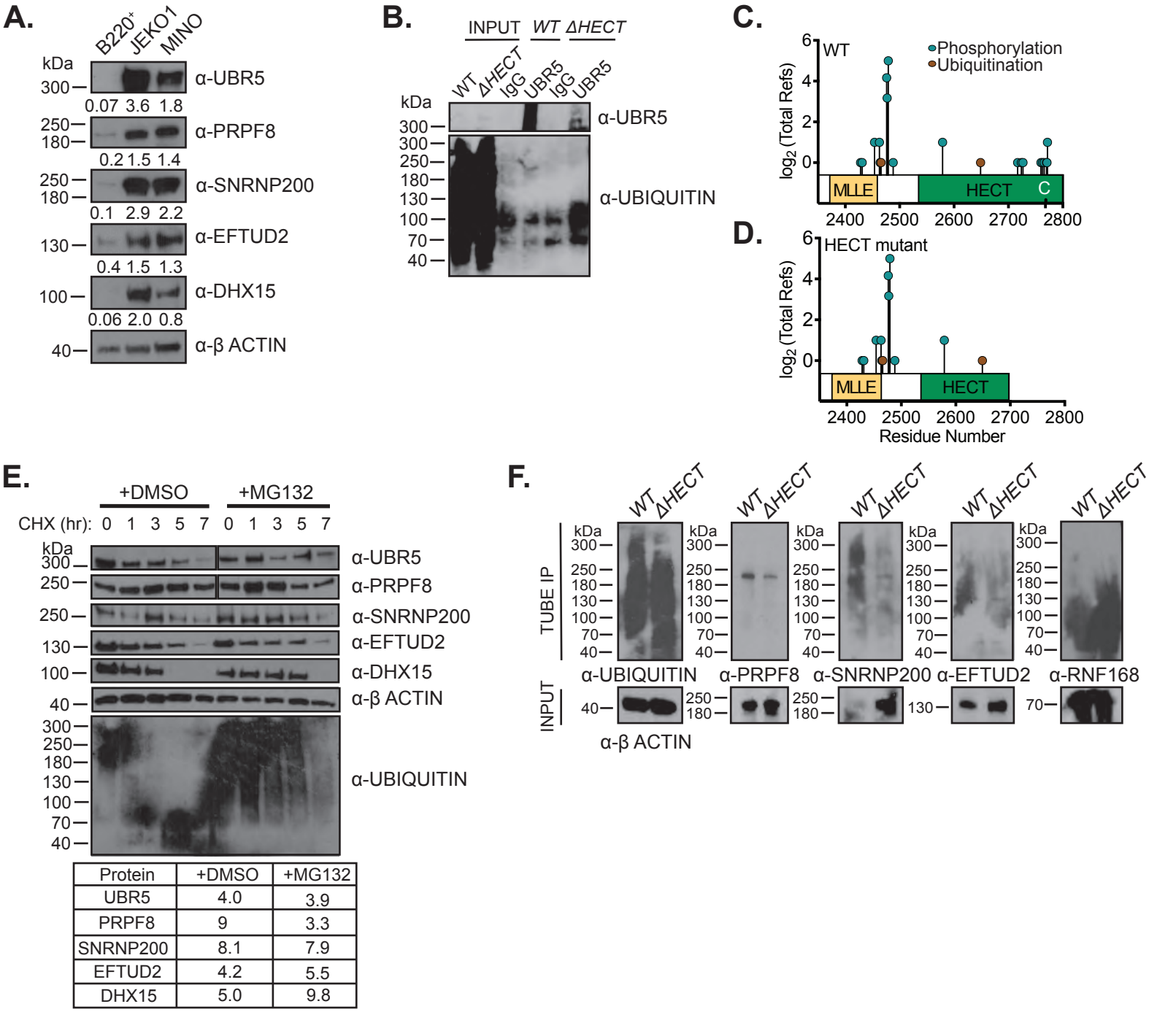
Swenson et al Supplemental Figure 3



Swenson et al Supplemental Figure 4



Swenson et al Supplemental Figure 5



Protein	+DMSO	+MG132
UBR5	4.0	3.9
PRPF8	9	3.3
SNRNP200	8.1	7.9
EFTUD2	4.2	5.5
DHX15	5.0	9.8