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Supplementary Materials for

WDFY4 is required for cross-presentation in response to viral and tumor antigens

Derek J. Theisen[1†], Jesse T. Davidson IV[1,2†], Carlos G. Briseño[1], Marco Gargaro[3], Elvin J. Lauron[4], Qiuling Wang[5], Pritesh Desai[1,5],Vivek Durai[1], Prachi Bagadia[1], Joshua R. Brickner[1], Wandy L. Beatty[5], Herbert W. Virgin[1], William E. Gillanders[2,6], Nima Mosammaparast[1], Michael S. Diamond[1,5,7.8], L. David Sibley[5], Wayne Yokoyama[4], Robert D. Schreiber[1,8], Theresa L. Murphy[1], Kenneth M. Murphy[1,9*]

Correspondence to: kmurphy@wustl.edu

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Materials and Methods

Mice

All mice were bred and maintained in specific pathogen-free facilities according to institutional guideline protocols approved by the Animal Studies Committee of Washington University in St. Louis. WT C57BL/6 (WT), C57BL/6-Tg(TcraTcrb)1100Mb/J (OT-I), C57BL/6-Tg(TcraTcrb)425Cbn/J (OT-II) were purchased from The Jackson Laboratory. C57BL/6NF-*Wdfy4*$^{em1(IMPC)J}$/J (Stock no. 029334) were generated through the KOMP^2 project at The Jackson Laboratory. MHCI TKO mice ($K^{b-/-}D^{b-/-}\beta2m^{-/-}$) were originally provided by H.W. Virgin and T. Hansen (Washington University, St. Louis, MO (*74*)). 4-14 week old mice that were age and sex matched were used for all experiments. For bone marrow chimeras B6.SJL (B6.SJL-PtprcaPepcb/BoyJ) were purchased from Charles River Laboratories. Chimeras were generated by lethally irradiating mice and injecting them with 4-6 million bone marrow cells i.v. Genotyping primers for *Wdfy4*$^{em1(IMPC)J}$/J are a: TTCTGCAGCAGGACAGACAC, b: TTTAGGAGCAGGTTGCCATC, c: ATGCCCTCTTCCCAACTTCT.

For bone marrow chimeras B6-Ly5.1/Cr (Charles River) mice were γ-irradiated with 1050rad and allowed to recover one day before i.v. injection of 50/50 mixes of *Wdfy4*$^{+/+}$/*Batf3*$^{-/-}$ or *Wdfy4*$^{-/-}$/*Batf3*$^{-/-}$ bone marrow. Mice rested for eight weeks post bone marrow injection before use in tumor experiments.

Antibodies and Flow Cytometry

Flow cytometry and cell sorting were completed on a FACS CantoII or FACS Aria Fusion instrument (BD) and analyzed using FlowJo analysis software (Tree Star). Staining was performed at 4ºC in the presence of Fc block (2.4G2; BD) in magnetic-activated cell-sorting

(MACS) buffer (PBS + .5% BSA + 2mM EDTA). The Following antibodies were used. From BD Biosciences: CD117 (2B8), CD135 (A2F10.1), Ly6C (AL-21), MHCI (AF6-88.5), CD4 (RM4-5), CD8α (53-6.7), CD11b (M1/70), B220 (RA3-6B2), CD64 (X54-5/7.1), CD19 (1D3), CD95 (Jo2), CD3 (145-2C11), CD45 (30-F11); from Tonbo Biosciences: MHCII (M5/114.15.2), CD44 (IM7), CD45.1 (A20), CD45.2 (104), CD11c (N418); from Biolegend: CD103 (2E7), XCR1 (ZET), CD115 (AFS98), Ter119 (Ter-119), Ly6G (1A8), TCRβ (H57-597), GL-7 (GL7), CXCR5 (L138D7), PD-1 (29F.1A12), CD3 (145-2C11), CD8 (53-6.7), CD4 (RMA4-5), CD44 (IM7), CD16/32 (93) ; from eBiosciences: TCRVα2 (B20.1), CD90.1 (HIS51), CD90.2 (53-2.1), IL-12p40 (C17.8), F4/80 (BM8), SIINFEKL/H-2K^b (25-D1.16), CD317 (eBio927); from Invitrogen: CD172α (P84), TNFα (MP6-XT22), CD45 (30F11), IgD (11-26c).
For immunofluorescence, the following antibody was purchased from Invitrogen: Alexa Fluor 488 goat anti-mouse IgG (H+L). Anti-Rab43 (2E6) was produced in house as described previously (*32*). From abcam: anti-SQSTM1 (ab56416), anti-Rab7 (ab50533), anti-Lamp1(ab24170), anti-β2m (ab75853), anti-calnexin (ab22595), anti-EEA1 (ab2900). From Santa Cruz: anti-Tap1 (B-8). From IBA Lifesciences: StrepMAB-Classic.

H-2K^b-TSYKFESV tetramers were produced in the Immunomonitoring Laboratory within the Center for Human Immunology and Immunotherapy Programs (Washington University). West Nile tetramers: D^b-WNV NS4B, I-A^b-WNV E 641-655, I-A^b-WNV NS3 372-386, I-A^b-WNV NS3 111-125 were generated by the NIH Tetramer Core Facility.

Cell sorting

Splenic and Flt3L DCs were sorted as $B220^{-}MHCII^{+}CD11c^{+}CD24^{+}CD172\alpha^{-}$ (cDC1) and $B220^{-}MHCII^{+}CD11c^{+}CD24^{-}CD172\alpha^{+}$ (cDC2). Monocytes were sorted from the bone marrow as $Ly6G^{-}CD19^{-}Ter119^{-}CD11c^{-}CD115^{+}CD117^{-}Ly6C^{+}CD11b^{+}$. OT-1 cells were sorted from the spleen as $B220^{-}CD11c^{-}CD4^{-}CD8^{+}CD45.1^{+}V\alpha2^{+}$. OT-II cells were sorted from the spleen as $B220^{-}CD11c^{-}CD8^{-}CD4^{+}CD45.1^{+}V\alpha2^{+}$. Infected DCs for CRISPR screen were sorted as $CD90.1^{+}$. Splenic B cells were sorted as $B220^{+}Thy1.2^{-}CD11c^{-}$.

DC Preparation

To harvest DCs from lymphoid tissues, organs were digested in 250µg/ml collagenase B (Roche) and 30 U/ml DNase I (Sigma-Aldrich) for 30-60min at 37ºC with stirring in complete IMDM + 10% FCS (I10F). Erythrocytes (RBCs) were lysed using ACK lysis buffer (150mM ammonium chloride, 10mM potassium bicarbonate, and .1mM EDTA). Before sorting DCs were enriched using CD11c microbeads (Miltenyi Biotech). For peripheral tissue DCs, organs were digested with collagenase D (Roche) and DNaseI (Sigma-Aldrich) for 1hr at 37ºC with stirring in 5ml cIMDM.

BM culture with Flt3L

Tibias, femurs, and hips from mice were crushed into MACS buffer and RBCs were removed with ACK lysis buffer. BM cells were cultured at 2x10^6 cells/ml in cIMDM containing 5% FLT3L for 8-10 days. Loosely adherent cells were harvested for analysis.

moDC preparation

Tibias, femurs , and hips were crushed into macs buffer and depleted of $B220^+$ and $Ly6G^+$ cells using MagniSort streptavidin beads (Thermo Fisher). Sorted monocytes were cultured in 2ml I10F with 20ng/ml GM-CSF and 20ng/ml IL-4 (Peprotech) for 4 days. Loosely adherent cells were harvested for analysis

sgRNA vector design

The ires-GFP fragment from the MSCV-ires-GFP retrovirus vector was replaced with Thy1.1 cDNA from MSCV-ires-Thy1.1 retrovirus vector (*75*) using Xho1 and EcoR1 digests to produce Thy1.1- RV. Two PCR DNA fragments containing the hU6 promoter and gRNA scaffold from pSpCas9(BB)-2A-GFP (PX458) (a gift from Feng Zhang (Addgene plasmid #48138) (*76*)) and a Bbs1 stuffer fragment from pLentiCrisprV1, provided by Feng Zhang (Addgene plasmid #49535) were sequentially cloned into the T easy vector (Promega). An EcoR1 fragment from the resulting plasmid was cloned into the EcoR1 site of Thy1.1-RV to produce Thy1.1-hU6-gRNA-Bbs1 stuffer-RV. To produce retroviral guide plasmids, annealed oligonucleotides with Bbs1 compatible overhangs and containing guide target sequences as described (*77*) were ligated into Bbs1 digested Thy1.1-hU6-gRNA-Bbs1 stuffer-RV.

Antigen presentation assays

In vivo cross-presentation assays were performed as described previously (*8*). MHCI TKO splenocytes were osmotically loaded with 10mg/ml soluble ovalbumin (Worthington Biochemical Corporation), irradiated at 1350rad, and 500,000 cells were injected i.v. into mice that had been injected with 500,000 CFSE-labeled OT-I T cells one day prior. After 3 days, spleens were harvested, mashed, and analyzed for CFSE dilution of OT-I cells. *In vitro* cross-

presentation assays were performed using 10,000 sorted cDCs and 25,000 CFSE-labeled OT-I cells co-cultured for 3 days before analysis of CFSE dilution.

HKLM-OVA (a gift from H. Shen, University of Pennsylvania, Philadelphia, PA) and soluble OVA were prepared as described previously (*32*), OVA-loaded splenocytes were prepared as described above. Bm1 T OVA cells (*72*) (a gift from C. Reis e Sousa, Francis Crick Institute, London, UK) were UVC irradiated with 240mJ/cm^2 one day prior to plating with sorted cDCs and OT-1.

For direct-presentation assays, cDC1 and cDC2 were sorted from day 10 Flt3L cultures as described above. Cells were then osmotically loaded with 10mg/ml soluble ovalbumin (Worthington Biochemical Corporation) and co-cultured for 3 days with CFSE-labeled OT-I cells. For alternative direct presentation bone marrow was infected with viruses containing an empty vector or an OVA expression plasmid before culturing in Flt3L to generate DCs. 7 days after infection cDC1 and cDC2 were either stained for K^b-SIINFEKL complexes (25-D1.16) or cultured with CFSE-labeled OT-1 T cells for 3 days and assayed for T cell proliferation.

For B cell antigen presentation assays, B cells were sorted from spleens as described above. 50K B cells were then co-cultured for 3 days with CFSE-labeled OT-II cells and 100µg/ml soluble ovalbumin (Worthington Biochemical Corporation) with or without 1µg/ml LPS from *Escherichia coli* (O55:B5; Sigma-Aldrich).

For FACS of K^b-SIINFEKL, Day 8 Flt3L cultured DCs were cultured for 48 additional hours with either 0.1mg/ml soluble OVA or 1x10^6 OVA-loaded irradiated splenocytes prepared as described above.

<u>Cross-presentation screen</u>

Retroviral vectors were transfected into Platinum-E cells as described (*78*) and supernatant containing virus was collected after 2 days. Sorted $Lin^{-}cKIT^{hi}$ cells from the bone marrow of Cas9-expressing mice (a gift from Feng Zhang (*45*)) were infected with viral supernatants with 2μg/ml polybrene at 2,250r.p.m. for at least 1hr. Cells were then grown in Flt3L culture for 7 days before sorting for $Thy1.1^{+}$ cells. Sorted cells were then diluted to a concentration of ~10K cells per well and cocultured with 10^{7} HKLM-OVA and 25K CFSE-labeled OT-I T cells for 3 days before analysis for CFSE dilution.

DC turnover assay

Mice were injected daily with 1mg BrdU and spleens were harvested on days 2, 4, and 6 for flow cytometry. Spleens were digested as described above and then fixed and stained according to the BrdU Flow Kit procedure (BD). pDCs were identified as $CD11c^{low}B220^{+}Bst2^{+}$ and B cells were identified as $CD11c^{-}B220^{+}$.

In vitro TLR Stimulation

200K Flt3L cultured DCs were plated and stimulated with either: 1μg/ml LPS from *Escherichia coli* (O55:B5; Sigma-Aldrich), 50μg/ml PolyI:C (Sigma-Aldrich), 63nM ODN1826, class B CpG oligonucleotide (CpG; InvivoGen), 2.5μg/ml STAg (prepared as previously described (*79*)) or 10^{7} HKLM-OVA (prepared as previously described (*32*)). Cells were stimulated for 1hr, treated with 1μg/ml brefeldin A (Sigma-Aldrich), and then incubated 4hrs. Cells were washed, prepared for surface staining, fixed in 4% paraformaldehyde (Electron Microscopy Sciences), and then permeabilized with .5% saponin for intracellular staining.

For analysis of CD80/86 expression cells were prepared as described above and incubated with 1µg/ml LPS from *Escherichia coli* (O55:B5; Sigma-Aldrich) before surface staining.

DQ-Ovalbumin degradation assay

200K cells from day 9 Flt3L cultures were incubated with either 10µg/ml soluble OVA (Worthington Biochemical Corporation), 10µg/ml DQ-Ovalbumin (Thermo Fisher), or 10µg/ml DQ-OVA + 5µg/ml MG132 (Sigma-Aldrich) for either 5, 15, 30, or 60min. Cells were washed at the indicated timepoints with PBS and surface stained for DC markers before analysis for $FITC^+$ cells.

HKLM phagocytosis assay

1 million cells from day 10 Flt3L cultures were harvested and plated with either $2x10^8$ AlexaFluor647 (generated as described previously (*32*)) labeled HKLM-OVA or no antigen at either 4ºC or 37ºC for 4hrs. After incubation DCs were enriched to remove HKLM using CD45.2-biotin [104] (eBiosciences) and MojoSort separation (BioLegend) and stained for DC markers for flow cytometry.

WDFY4 overexpression constructs

The cDNA fragments for 4 individual domains of murine WDFY4 were synthesized as gene blocks (Thermo Fisher). A Twin-Strep® tag was placed at the 5' end of Fragment 1. Silent mutations were introduced in all fragments to remove endogenous BamHI sites. Gene blocks were synthesized with the following 5'/3' restriction enzyme sites: BamHI/AatII (Fragment 1), AatII/BstEII (Fragment 2), BstEII/AgeI (Fragment 3), AgeI/SpeI (Fragment 4). Each gene block

was cloned individually then in sequence into pBluescript (Addgene) and then cloned into MSCV-IRES-Thy1.1 (*75*) using 5' BglII site and 3' blunt end to produce MSCV-TwinStrep-WDFY4-IRES-Thy1.1 vector.

For proteomics and co-immunoprecipitation experiments, 2XFLAG tagged fragments of domains 1-4 were created using PCR and the above constructs as template. Each fragment was cloned into MSCV-IRES-Thy1.1 using a 5' BglII site and either a 3' SalI site or 3' blunt end.

Immunoprecipitation and mass spectrometry

JAWSII cells (ATCC) stably expressing constructs for empty vector or Flag-tagged GFP, FL1, FL2, FL3, or FL4 were grown up to 50-100 million cells and harvested. Cells were lysed using lysis buffer (1% NP-40, 20mM Tris, 127mM NaCl, 10% Glycerol, 2mM EDTA, 1mM EGTA, 50mM NaF) with protease inhibitors for 30min and spun to clear. Cleared lysates were incubated for 3.5hrs with magnetic beads conjugated to an anti-Flag antibody [M2] (Sigma) in Tap wash buffer (50mM Tris, 100mM KCl, 5mM $MgCl_2$, .2mM EDTA, 10% Glycerol, .1% NP-40, .2mM PMSF, 1mM DTT) before washing. Proteins were eluted from beads using a 3xFlag peptide (Sigma) for 1hr and precipitated using tricholoroacetic acid before submitting for mass spectrometry. LC-MS/MS was performed at the Taplin Mass Spectometry Facility (Harvard Medical School) using an LTQ Orbitrap Velos Pro ion-trap mass spectrometer (Thermo Fisher) and Sequest software. GO terms were generated using the ClueGO plugin for Cytoscape.

For immunoprecipitation in HEK293 cells, cells were transiently transfected with *Trans*IT-LT1 (Mirus) and lysates were prepared 24-48hrs after transfection as described above. Cleared lysates were incubated for 2hrs with magnetic beads conjugated to an anti-Flag antibody [M2] (Sigma)

in Tap wash buffer before washing with Tap wash buffer and elution by boiling in sample buffer containing beta-mercaptoethanol.

Western Blot

For Western analysis, the following antibodies were purchased from Abcam: anti-hsp90 beta (ab32568); from Millipore Sigma: anti-FLAG (M2); from Santa Cruz Biotechnology, Inc: anti–β-actin (C4).

Whole-cell extracts or immunoprecipitates were denatured in Laemmli sample buffer at 95°C for 5 min. Samples were run on a 4-20% Mini-Protean TGX precast gels (Bio-Rad) and transferred onto a nitrocellulose membrane (Bio-rad). Immunoblots were blocked in TBS containing 3% BSA or 5% nonfat milk and 0.1% Tween 20 at room temperature for 1 h and then incubated with primary antibody overnight at 4°C. After washing, membranes were incubated with goat anti–rabbit IgG (H + L) or goat anti–mouse IgG (H + L) conjugated to horseradish peroxidase (Jackson ImmunoResearch Laboratories, Inc.) for 1 h at room temperature in 5% nonfat milk and 0.1% Tween 20 TBS. Then, membranes were washed and developed with SuperSignal West Pico Plus Chemiluminescent Substrate (Thermo Fisher Scientific).

Tumor transplantation

The MCA-induced fibrosarcoma 1969 used in this study was generated in a female C57BL/6 $Rag2^{-/-}$ mouse, was tested for mycoplasma, and was banked at low-passage as previously described (*15,80*). Tumor cells derived from frozen stocks and propagated in vitro in RPMI media supplemented with 10% FCS (Hyclone) were washed three times with PBS, resuspended at a density of 6.67 x 10^6 cells/ml in endotoxin-free PBS and then 150 µl was injected

subcutaneously into the flanks of recipient mice. Tumor growth was measured with a caliper and expressed as the area based on two perpendicular diameters.

For FACS of tumor infiltrating cells tumors were harvested either at day 6 or after 20-25 days of growth, chopped using a razor blade, and digested in Collagenase B (Roche), Collagenase D (Roche), and DNaseI (Sigma-Aldrich) for 45min at 37ºC with constant agitation.

Microarrays

Tumors were injected into mice as described above and allowed to grow for 6 days. On day 6 mice were harvested and inguinal lymph nodes from either the flank with the tumor (draining) or the opposite flank (non-draining) were harvested, digested, and sorted for cDC1 ($MHCII^+CD11c^+XCR1^+$). RNA was extracted and purified using the Nucleospin xs RNA isolation kit (Macherey-Nagel), amplified with the Ovation Pico WTA System (NuGEN), and hybridized to Mouse Gene 1.0 ST arrays (Affymetrix). Expression values were analyzed after robust multiarray average (RMA) and quantile normalization using ArrayStar 4 software (DNASTAR).

Infection and Immunization

For *Toxoplasma gondii,*10-14 week old mice were injected with the type II Prugniaud strain of *T. gondii* expressing a firefly luciferase and GFP transgene (provided by J. Boothroyd, Stanford University, Palo Alto, CA) at a dose of 200 tachyzoites intraperitoneally per mouse as described previously (*25*). Mice were monitored over 30 days for survival and weight loss.

For cowpox, age- and sex- matched mice were used at 8-10 weeks of age. Mice were anesthetized with ketamine/xylazine and were inoculated intranasally (i.n.) with 5000 pfu of

BAC-derived cowpox Brighton Red (*81*) in 30 μL of minimum essential medium (MEM) (Mediatech). At 7 days post-infection, lungs were harvested, minced, and digested with 22.4 μg/mL DNase I (type II; Sigma) and 0.7 mg/mL collagenase (Sigma) at 37°C for 30 minutes. Digested lungs were then passed through 70-μm cell strainers and red blood cells were lysed (0.15 M NH_4Cl, 10 mM $KHCO_3$, and 0.1 mM EDTA). Spleens were passed through 70-μm strainers without digestion and red blood cells were lysed. Cells were stained with Fixable Viability Dye eFlour 506 (eBioscience) prior to cell surface staining.

For SRBC immunization $2x10^8$ sheep red blood cells (Colorado Serum Company) were injected i.p. into 8-10 week old mice. Spleens were harvested seven days later and analyzed for germinal center B cell and T_{fh} development.

For West Nile Virus, age- and sex-matched mice were used at 10-12 weeks of age. Mice were infected with 10^2 PFU of West Nile Virus via foot pad injection. After eight days spleens were harvested and analyzed for CD8 and CD4 T cell responses. Cells were stained with Fixable Viability Dye eFluor 506 (eBioscience) prior to cell surface staining.

Confocal microscopy

JAWSII cells or DCs from day 10 Flt3L cultures were allowed to adhere to Alcian blue (Electron Microscopy Sciences) coated coverslips at 500K cells/ coverslip. Cells were fixed with 4% paraformaldehyde, quenched with .4M glycine, and blocked using BlockAid (Invitrogen) with .2% saponin. Cells were stained overnight in PBS + .2% saponin + 2% FBS at 4ºC, washed with PBS + .2% saponin three times and stained with secondary antibodies for at least 2hrs at 4ºC in PBS + .2% saponin + 2% FBS. Coverslips were mounted in ProLong Gold Antifade with DAPI (Invitrogen). Labeled HKLM-OVA was prepared as described (*32*).

Images were acquired on a Nikon A1Rsi confocal microscope using a 60x oil immersion objective and analyzed using ImageJ.

Statistics

Statistical analysis was performed using GraphPad Prism (GraphPad Software). Two-way ANOVA with Tukey's multiple comparison test or Mann-Whitney U test was performed to determine statistical significance.

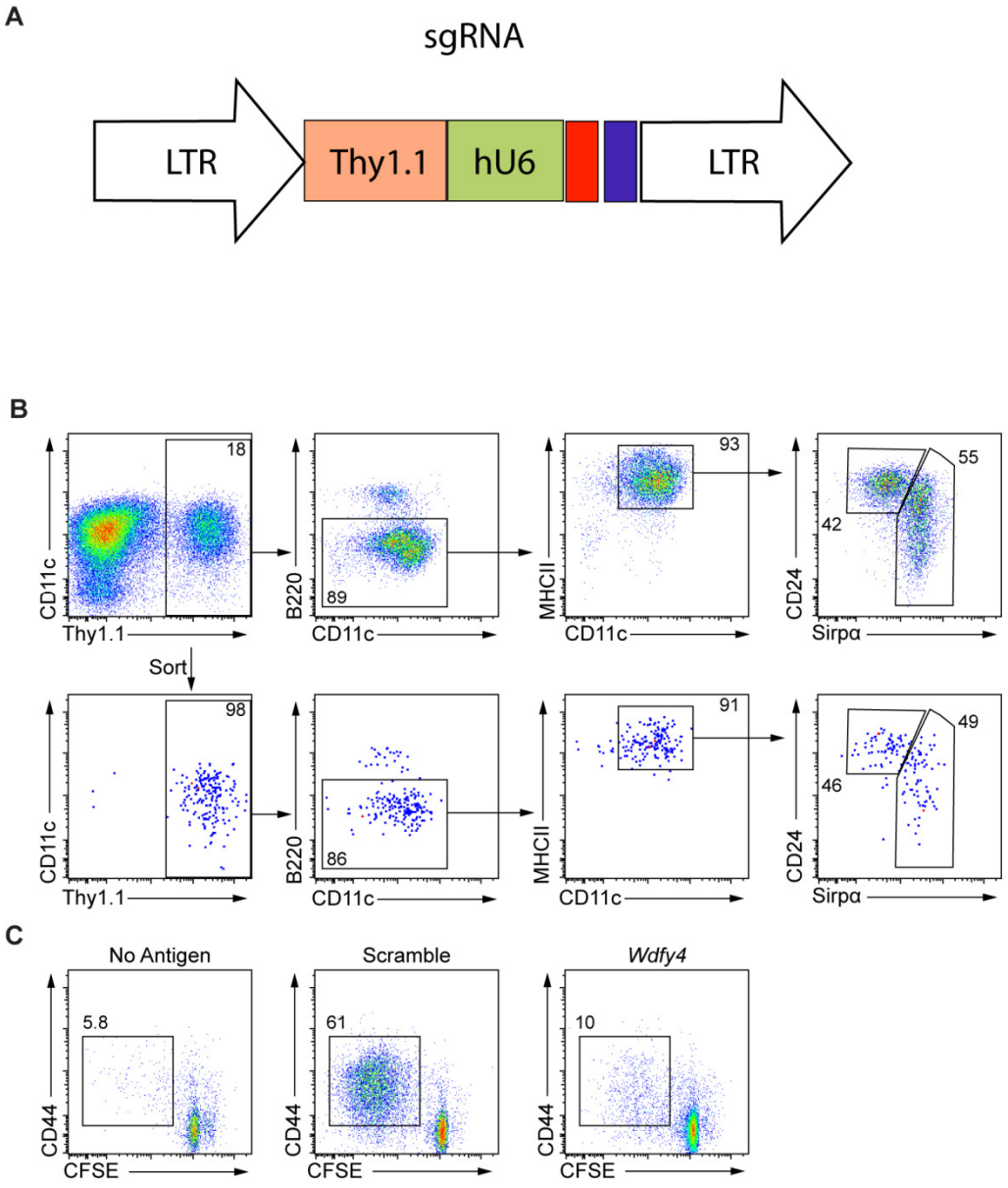

Figure S1. Functional cross-presentation screen using CRISPR/Cas9.

(**A**) A vector was constructed to express a single sgRNA (red/blue) under control of an internal human U6 promoter (green). This cassette is encoded on the sense strand downstream of the Thy1.1 marker (*75*). (**B**) Sorting strategy and gating of infected cDCs after Flt3L culture. DCs were sorted as Thy1.1^{+} and analyzed by flow cytometry for expression of B220, CD11c, MHC-II, CD24, and Sirpα to verify normal development after target knockout. Sorted Thy1.1^{+} cells

were then tested for cross-presentation of cell-associated antigen. **(C)** Example flow cytometry of OT-I T cells 3 days after co-culture with sgRNA infected DCs and HKLM-OVA. OT-I cells were pre-gated as $CD45.1^{+}CD8^{+}V\alpha2^{+}$ and then analyzed by CD44 and CFSE.

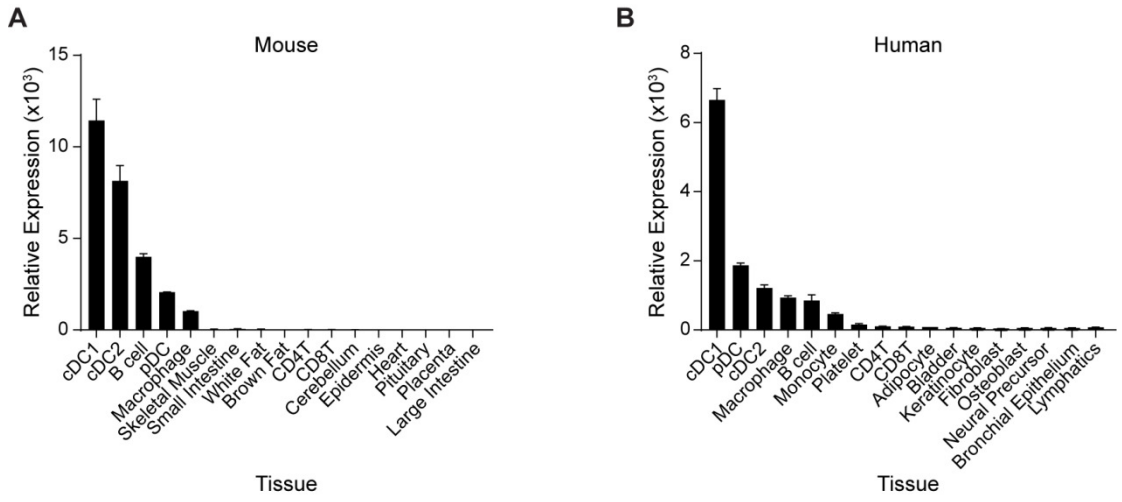

Figure S2. Expression profile of *Wdfy4*. (**A**) Mouse gene expression profile of *Wdfy4* in various tissues. (**B**) Human gene expression profile of *Wdfy4* in various tissues.

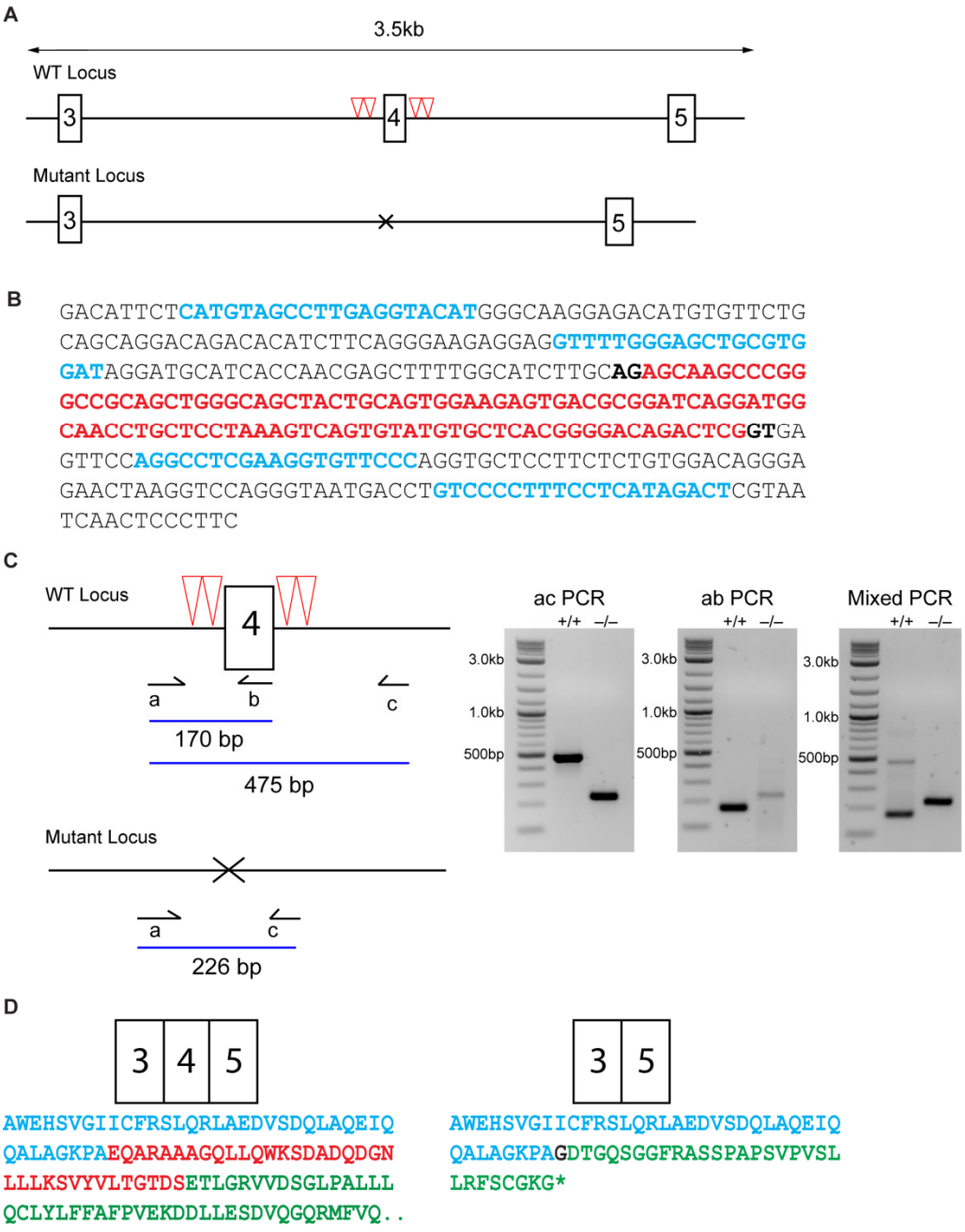

Figure S3. Characterization of *Wdfy4*$^{-/-}$ mice. (**A**) *Wdfy4*$^{-/-}$ mice were generated by the Knockout Mouse Phenotyping Program (KOMP) at The Jackson Laboratory. The schematic shows the WT and targeted *Wdfy4* locus for exons 3 to 5, showing the locations of the four sgRNAs (red triangles) used for CRISPR/Cas9 to delete exon 4. (**B**) Shown is the genomic

sequence surrounding exon 4 (black), exon 4 coding region (red), the flanking splice AG acceptor and GT splice donor nucleotides (bold), and the sequences of the four sgRNAs used for CRISPR/Cas9 targeting (blue). (**C**) Genotyping strategy for identifying *Wdfy4*$^{+}$ and *Wdfy4*$^{-}$ alleles uses the oligonucleotide primers (a-c), whose locations relative to exon 4 are indicated. The WT allele is identified as a 170 bp PCR product generated with primers a and b. The targeted allele is identified from the 226 bp PCR product with a and c. (**D**) Shown are the protein sequence of Wdfy4 encoded by exons 3 through 5 of the WT locus (left) and the mutant Wdfy4 protein resulting from the splicing of exon 3 to exon 5 (right) in the targeted locus. Because the coding region of exon 4 contain 107 nucleotides, splicing from exon 3 to 5 results in a shift in the reading from in exon 5 leading to the early translational terminal in exon 5 (*).

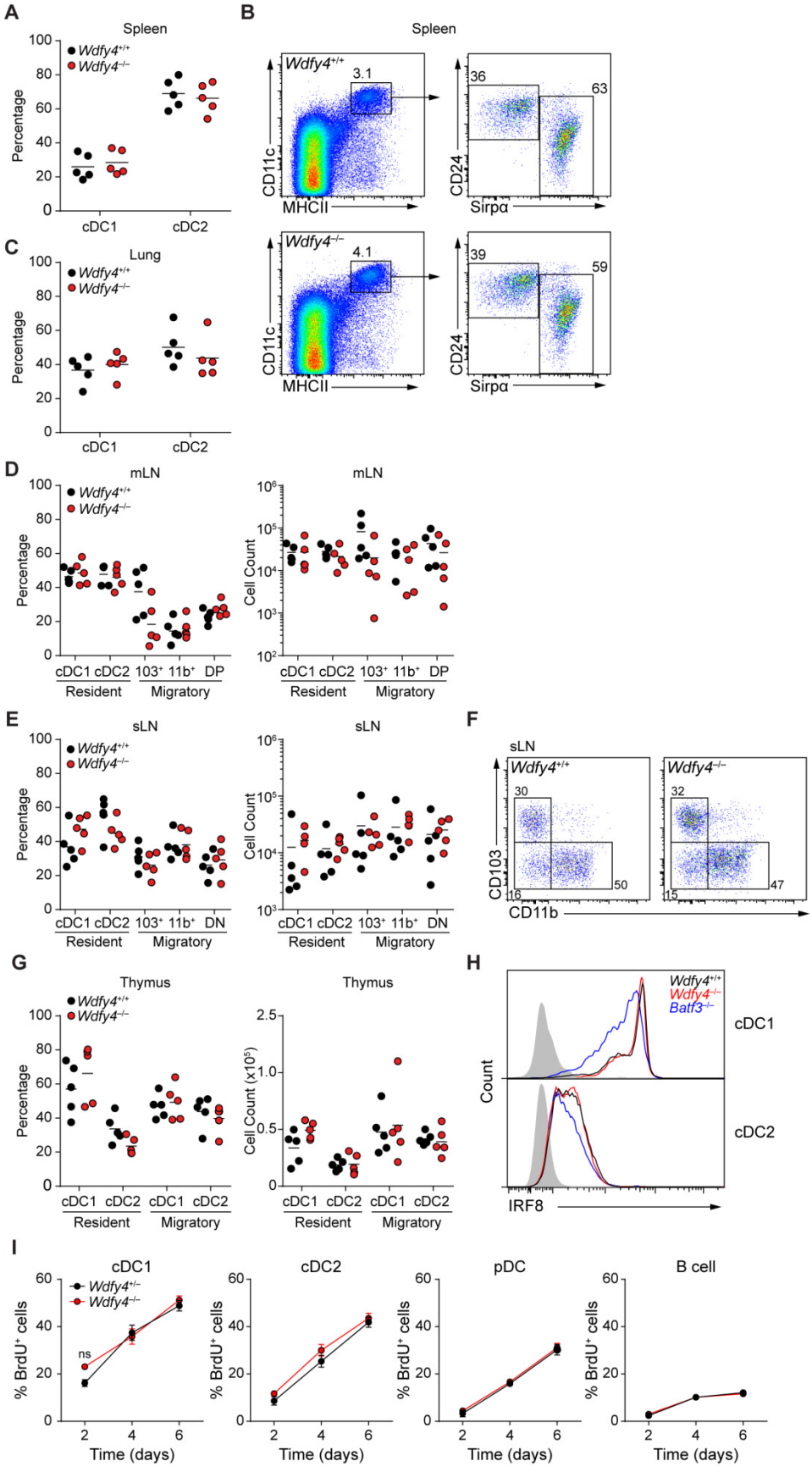
A
Spleen
Wdfy4+/+
Wdfy4−/−
Percentage
cDC1
cDC2
B
Spleen
Wdfy4+/+
3.1
CD11c
MHCII
36
63
CD24
Sirpα
Wdfy4−/−
4.1
39
59
C
Lung
D
mLN
Cell Count
cDC1 cDC2 103+ 11b+ DP
Resident
Migratory
E
sLN
cDC1 cDC2 103+ 11b+ DN
F
CD103
CD11b
30
50
16
32
47
15
G
Thymus
Cell Count (x10⁵)
H
Batf3−/−
Count
IRF8
I
cDC1
cDC2
pDC
B cell
% BrdU+ cells
ns
Time (days)

Figure S4: Normal DC development in *Wdfy4*$^{-/-}$ mice. (**A**) Percentages of cDC1 (B220^{-} CD11c^{+}MHCII^{+}CD24^{+} Sirpα^{-}) and cDC2 (B220^{-}CD11c^{+}MHCII^{+}CD24^{lo} Sirpα^{+}) from spleen of *Wdfy4*$^{+/+}$ and *Wdfy4*$^{-/-}$ mice. Dot indicates one mouse, bar indicates mean. (**B**) Representative flow cytometry plots from spleen of *Wdfy4*$^{+/+}$ and *Wdfy4*$^{-/-}$ mice. Pregated as B220^{-}. (**C**) Percentages of cDC1 (CD45^{+}CD64^{-}B220^{-}CD11c^{+}MHCII^{+}CD24^{+} Sirpα^{-}) and cDC2 (CD45^{+}CD64^{-}B220^{-}CD11c^{+}MHCII^{+}CD24^{lo} Sirpα^{+}) from lung of *Wdfy4*$^{+/+}$ and *Wdfy4*$^{-/-}$ mice. Dot indicates one mouse, bar indicates mean. (**D-E**) Migratory (CD11c^{lo} MHC-II^{hi}) and resident (CD11c^{hi} MHC-II^{lo}) cDC1 (resident: CD24^{+}Sirpα^{-}, migratory: CD103^{+}CD11b^{-}) and cDC2 (resident: CD24^{-}Sirpα^{+}, migratory: CD11b^{+}CD103^{-}) from mLN (D) or sLN (E) were distinguished and shown as a percentage of total cDCs (left) or total cell numbers (right). Dot indicates one mouse, bar indicates mean (**F**) Representative flow cytometry plots of migratory sLN DCs from *Wdfy4*$^{+/+}$ and *Wdfy4*$^{-/-}$ mice. Pregated B220^{-}CD11c^{lo}MHCII^{hi}. (**G**) Thymic migratory (CD11c^{lo} MHC-II^{hi}) and resident (CD11c^{hi} MHC-II^{lo}) cDCs were distinguished within cDC1 (resident: CD24^{+}Sirpα^{-}, migratory: CD103^{+}Sirpα^{-}) and cDC2 (resident: CD24^{-}Sirpα^{+}, migratory: CD103^{-}Sirpα^{+}) and shown as a percentage of total cDCs (left) or total cell numbers (right). Dot indicates one mouse, bar indicates mean. No statistically significant differences in cDC subsets were identified between WT and *Wdfy4*$^{-/-}$ mice for any tissues tested. **(H)** Intracellular staining for IRF8 in splenic cDCs from *Wdfy4*$^{+/+}$, *Wdfy4*$^{-/-}$, and *Batf3*$^{-/-}$ mice compared to *Irf8*$^{-/-}$ control (gray histogram). (**I**) BrdU incorporation in indicated cell types over time after daily injections of *Wdfy4*$^{+/-}$ and *Wdfy4*$^{-/-}$ mice. Data shown as mean ± SEM of three independent experiments. ns. Not significant by 2-way ANOVA.

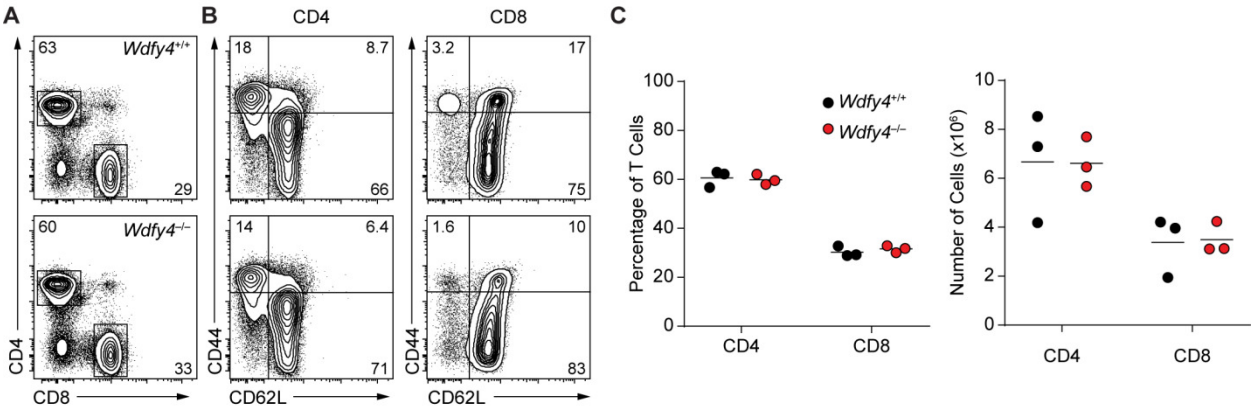

Figure S5: Normal T cell development in *Wdfy4*$^{-/-}$ mice. (**A-C**) TCRβ^{+} T cells from spleens of *Wdfy4*$^{+/+}$ and *Wdfy4*$^{-/-}$ mice are shown for CD4 and CD8 expression (A), and for activation markers CD44 and CD62L (B) for CD4 (left) and CD8 (right) T cells respectively. No significant differences in percentages or total numbers of CD4 or CD8 T cells were found between *Wdfy4*$^{+/+}$ and *Wdfy4*$^{-/-}$ mice (C). Each dot indicates one mouse, horizontal bars indicate mean.

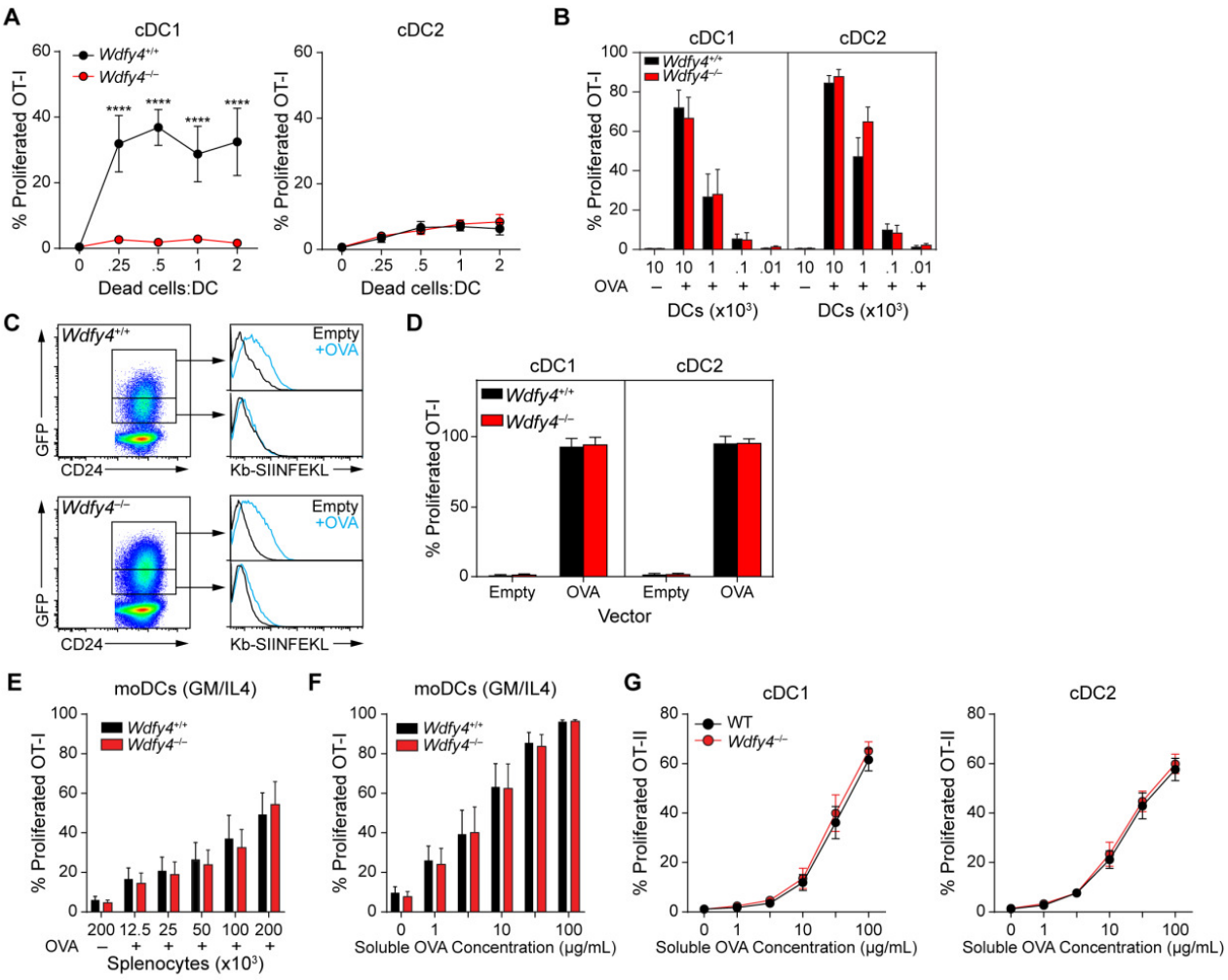

Figure S6: WDFY4 acts specifically in cDC1-mediated cross-presentation.

(A) Sort-purified cDC1 and cDC2 from spleens of WT and *Wdfy4*$^{-/-}$ mice were assayed for presentation of UVC-irradiated OVA-expressing cells (bm1) to OT-1 T cells. Activated T cells gated as CFSE^{-}CD44^{+}. **(B)** Sorted cDC1 and cDC2 from WT or *Wdfy4*$^{-/-}$ Flt3L cultures were osmotically loaded with 10mg/ml soluble OVA and then diluted to indicated cell concentrations and cultured with 25K CFSE-labeled OT-I T cells for three days and analyzed for CFSE dilution (CFSE^{-}CD44^{+}). Control DCs were osmotically shocked but given no OVA (**C**) Representative flow cytometry plots showing infection of OVA-GFP virus (left) and expression of K^{b}-SIINFEKL complexes (right) in WT or *Wdfy4*$^{-/-}$ cDC1 (B220^{-}CD11c^{+}MHCII^{+}CD24^{+}Sirpα^{-})

compared to empty vector control. (**D**) OT-I proliferation ($CFSE^{-}CD44^{+}$) of WT or *Wdfy4*$^{-/-}$ cDCs after infection with empty vector or OVA-GFP vector. (**E-F**) MoDCs generated from sorted BM monocytes from WT or *Wdfy4*$^{-/-}$ mice were put in culture for four days with 20ng/ml GM-CSF and IL-4. 25K moDCs were cultured for three days with 25K CFSE-labeled OT-I cells and the indicated concentrations of OVA- or PBS- loaded splenocytes (E) or soluble OVA (F). OT-I activation measured as $CFSE^{-}CD44^{+}$. **(G)** Sort purified cDC1 and cDC2 from spleens of WT or *Wdfy4*$^{-/-}$ mice were cultured for three days with CFSE-labeled OT-II T cells and the indicated concentrations of soluble OVA. Data is shown as percentage of $CFSE^{-}CD44^{+}$ OT-II cells. For all figures data shown as mean ±SEM from three independent experiments, ****$P<0.0001$ using 2-way ANOVA with Tukey's multiple comparisons test.

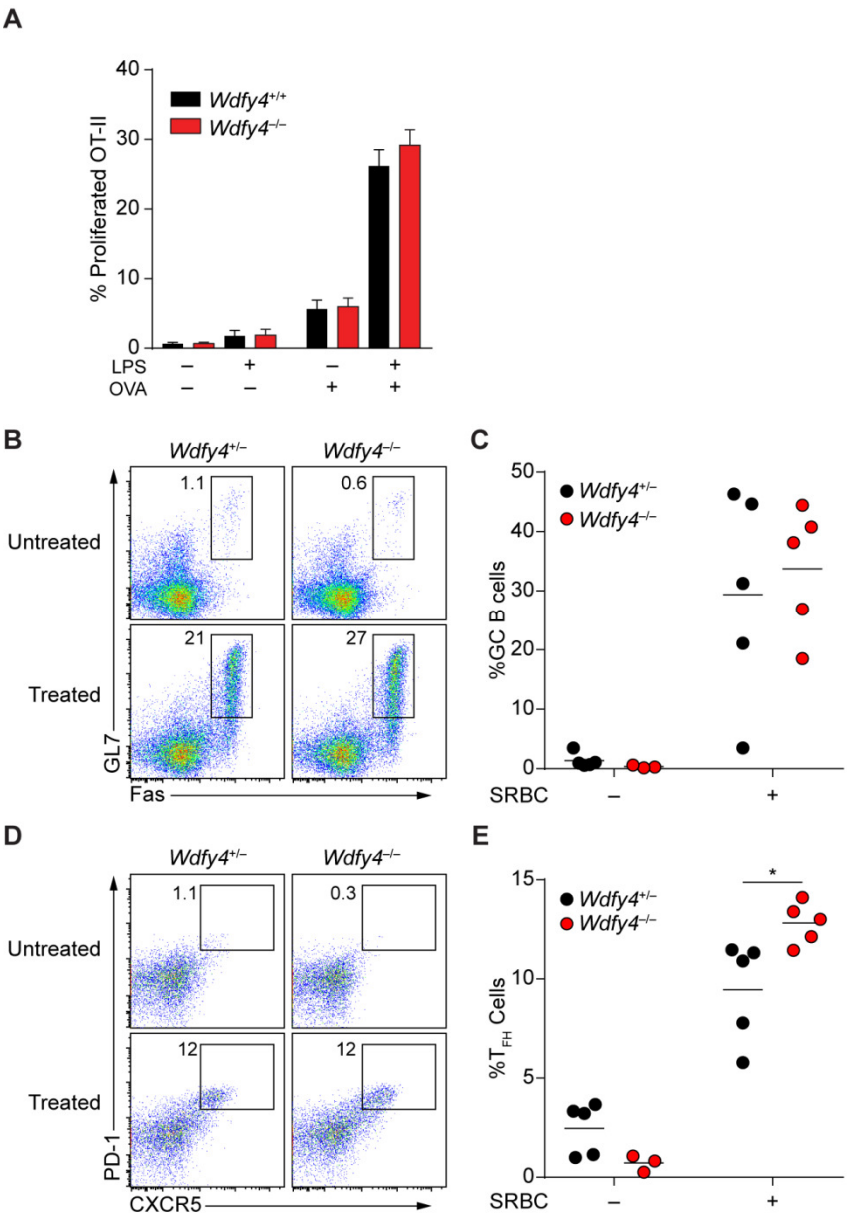

Figure S7: *Wdfy4*$^{-/-}$ mice are capable of inducing normal B cell responses.

(A) 50K sorted B220^{+} B cells were co-cultured for three days with 25K CFSE-labeled OT-II T cells and 100µg/ml soluble OVA with or without 1µg/ml LPS and assayed for CFSE dilution (CFSE^{-}CD44^{+}). Data shown as mean ± SEM of three independent experiments. **(B-C)** *Wdfy4*$^{+/-}$ and *Wdfy4*$^{-/-}$ mice were injected i.p. with sheep red blood cells and spleens were analyzed seven days later for induction of germinal center B cells, summarized in (C). Cells were pre-gated on

$B220^{+}IgD^{-}CD19^{+}$. Each dot indicates one mouse, bar indicates mean. **(D-E)** *Wdfy4*$^{+/-}$ and *Wdfy4*$^{-/-}$ mice were injected i.p. with sheep red blood cells and spleens were analyzed seven days later for induction of T_{FH} cells, summarized in (E). Cells were pre-gated on $B220^{-}TCR\beta^{+}CD4^{+}CD62L^{-}CD44^{+}$. Each dot indicates one mouse, bar indicates mean. * $P<.05$ by 2-way ANOVA.

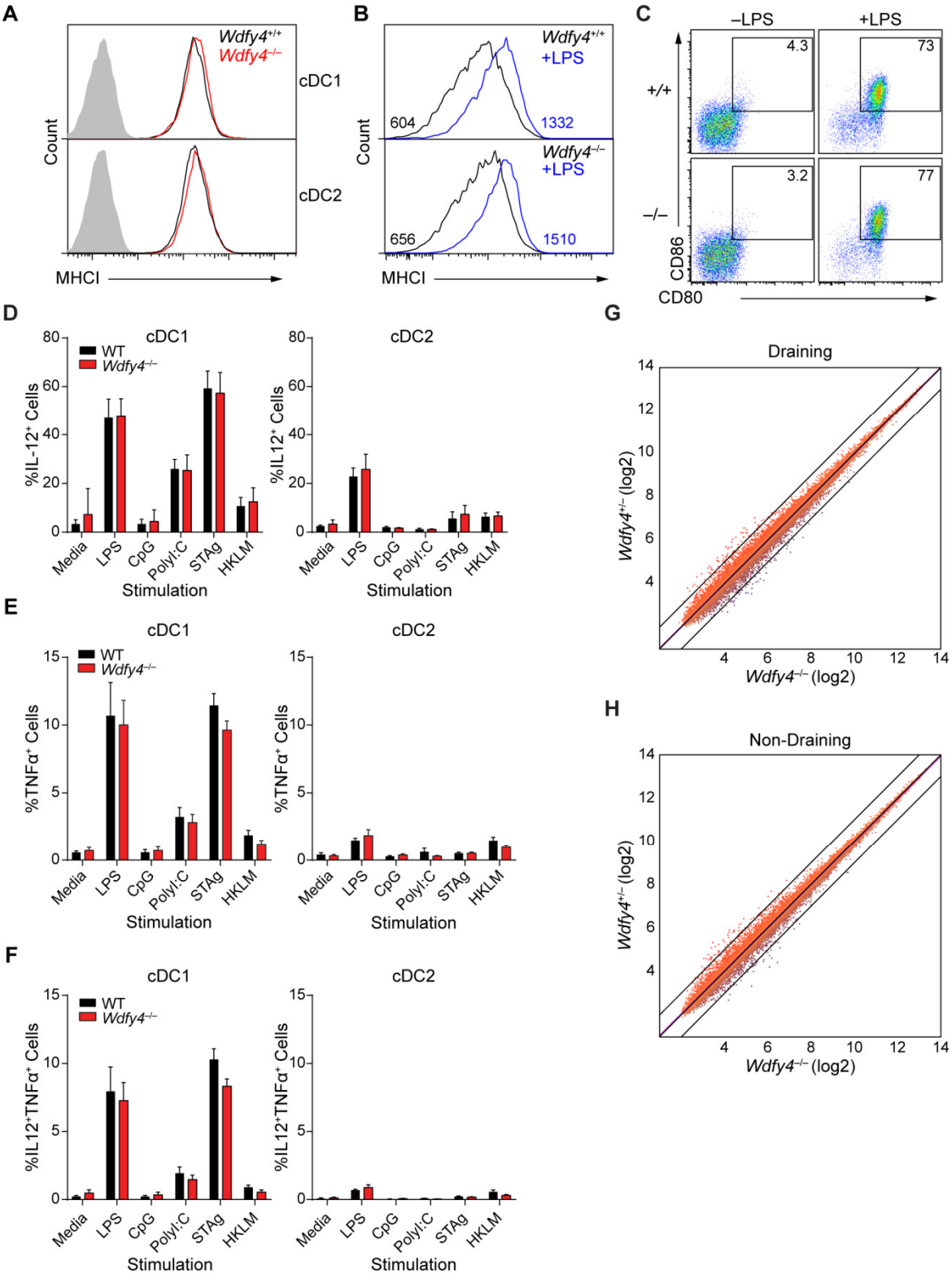

Figure S8: *Wdfy4*-deficient cDCs are capable of producing signals for T cell priming

(**A**) Flow cytometry of splenic cDC1 and cDC2 from WT or *Wdfy4*$^{-/-}$ mice for MHC-I compared to MHC-I TKO control mice (gray histogram). Pre-gated on cDC1(B220^{-} CD11c^{+}MHCII^{+}CD24^{+}Sirpα^{-}) or cDC2 (B220^{-}CD11c^{+}MHCII^{+}CD24^{-}Sirpα^{+}) (**B**) Flow cytometry of day eight Flt3L cultured cDC1 from WT or *Wdfy4*$^{-/-}$ mice for MHC-I with either no treatment (black line) or four hours of culture with 1μg/ml LPS (blue bar). Numbers indicate MFI. Pre-gated on cDC1 (B220^{-}CD11c^{+}MHCII^{+}CD24^{+}Sirpα^{-}). **(C)** Representative flow cytometry of expression of CD80 and CD86 in cDC1 cells generated from Flt3L cultured bone marrow of indicated genotypes after eight days. Cells were stimulated for four hours with 1μg/mL LPS to induce activation. Pre-gated on cDC1 (B220^{-}CD11c^{+}MHCII^{+}CD24^{+}Sirpα^{-}) . (**D-F**) Production of IL-12p40 (D), TNFα(E), or IL-12p40 and TNFα (F) by cDC1 (left, B220^{-} CD11c^{+}MHCII^{+}CD24^{+}Sirpα^{-}) or cDC2 (right, B220^{-}CD11c^{+}MHCII^{+}CD24^{-}Sirpα^{+}) generated in Flt3L cultures for nine days to indicated stimuli. Data shown as mean ± SEM for three independent experiments. (**G-H**) Gene expression data from *Wdfy4*$^{+/-}$ or *Wdfy4*$^{-/-}$ cDC1 from the draining (G) or non-draining (H) inguinal sLN from tumor bearing mice 6 days after injection of 1x10^{6} regressor fibrosarcoma cells s.c. n=4 mice per condition. Lines indicate 2-fold changes.

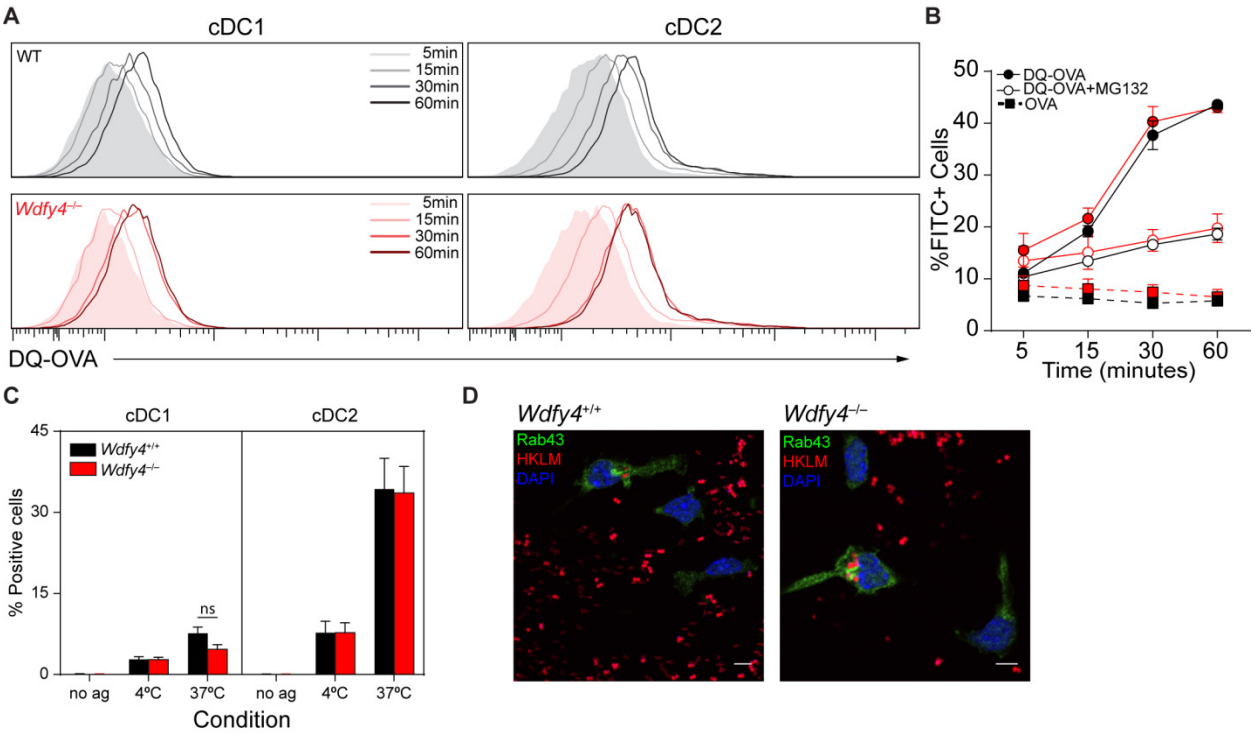

Figure S9: *Wdfy4*-deficient cDCs can take up and degrade antigen.

(A) Representative flow cytometry plots of DQ-OVA (10µg/ml) degradation by cDC1 (left, $B220^{-}CD11c^{+}MHCII^{+}CD24^{+}Sirp\alpha^{-}$) or cDC2 (right, $B220^{-}CD11c^{+}MHCII^{+}CD24^{-}Sirp\alpha^{+}$) generated in Flt3L cultures for nine days, read out by $FITC^{+}$ fluorescence. **(B)** Percentage of $Wdfy4^{+/+}$ (black) or $Wdfy4^{-/-}$ (red) $FITC^{+}$ cDCs after culture with unlabeled OVA (10µg/ml), DQ-OVA (10µg/ml), or DQ-OVA (10µg/ml) + MG132 (5µg/ml) for indicated times. data shown as mean ±SEM from three independent experiments. (**C**) Representative microscopy images of Flt3L generated DCs from indicated genotypes allowed to uptake HKLM-647 for 4 hours and stained for Rab43 (green) to distinguish cDC1. (**D**) Quantification of percentage of $HKLM\text{-}647^{+}$ cDC1 (left, $B220^{-}CD11c^{+}MHCII^{+}CD24^{+}Sirp\alpha^{-}$) or cDC2 (right, $B220^{-}CD11c^{+}MHCII^{+}CD24^{-}Sirp\alpha^{+}$) after four hours of culture of 1 million Flt3L generated DCs from BM of indicated genotypes with $2x10^{8}$ bacteria at indicated temperatures. Data shown as mean ± SEM from three independent experiments.

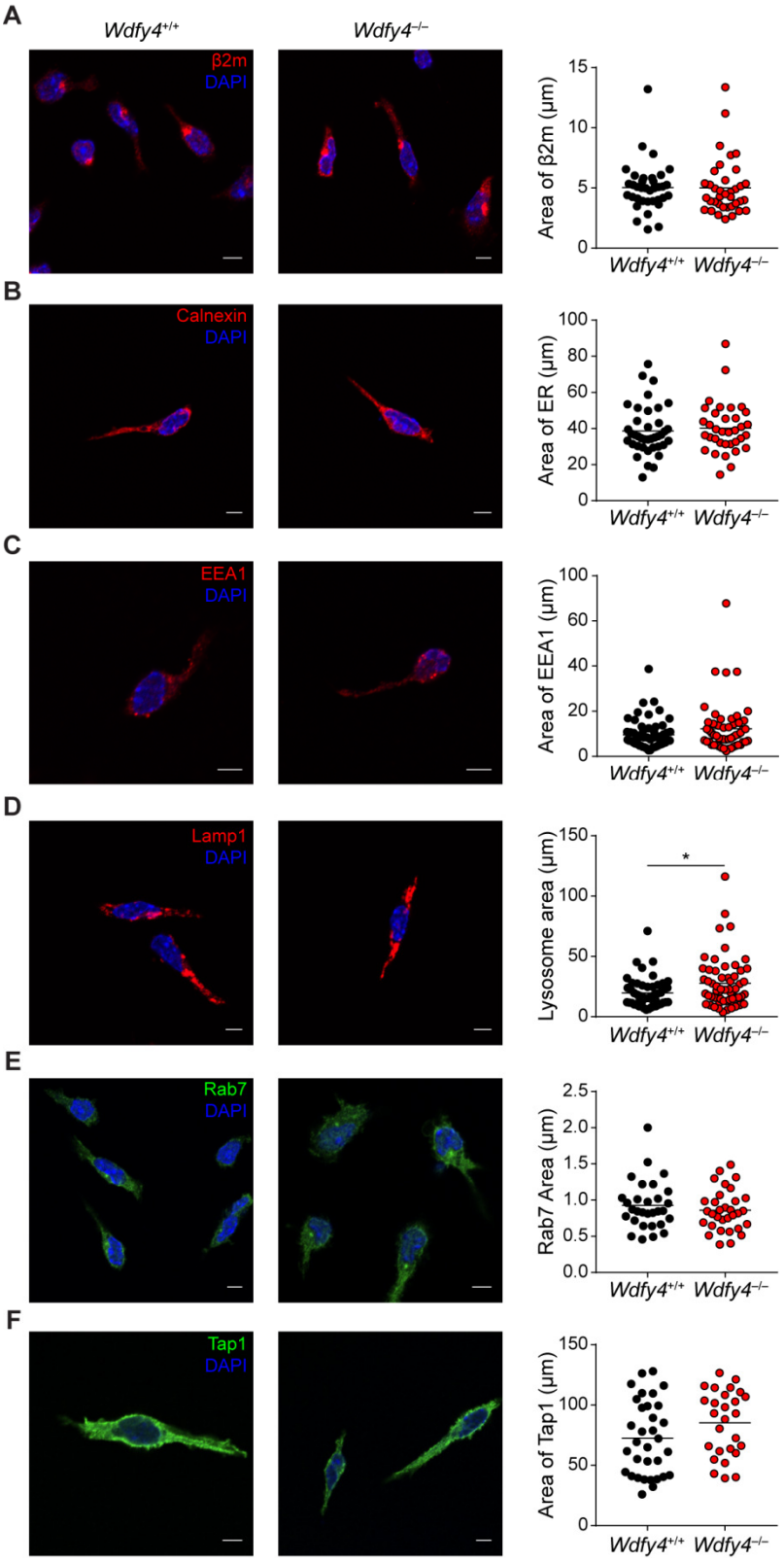
A
Wdfy4+/+
Wdfy4−/−
β2m
DAPI
Area of β2m (μm)
B
Calnexin
DAPI
Area of ER (μm)
C
EEA1
DAPI
Area of EEA1 (μm)
D
Lamp1
DAPI
Lysosome area (μm)
*
E
Rab7
DAPI
Rab7 Area (μm)
F
Tap1
DAPI
Area of Tap1 (μm)

Figure S10: *Wdfy4*-deficient cDCs have normal cellular structures

(**A-F**) Confocal microscopy of day 10 Flt3L-cultured DCs attached to alcian blue coated coverslips for four hours. Cells were fixed, permeabilized, and stained with antibodies against β2m (A), Calnexin (B), EEA1 (C), Lamp1 (D), Rab7 (E), or Tap1 (F) which were secondary stained with antibodies conjugated to AF488 (green) or AF647 (red) before mounting on slides using Prolong Gold antifade + DAPI (blue). Scale bars indicate 5μm. Staining quantified for each stain on left, each dot indicates one cell, bar indicates mean. n.s. not significant ($P>.05$); * $P<.05$ by Mann-Whitney U Test.

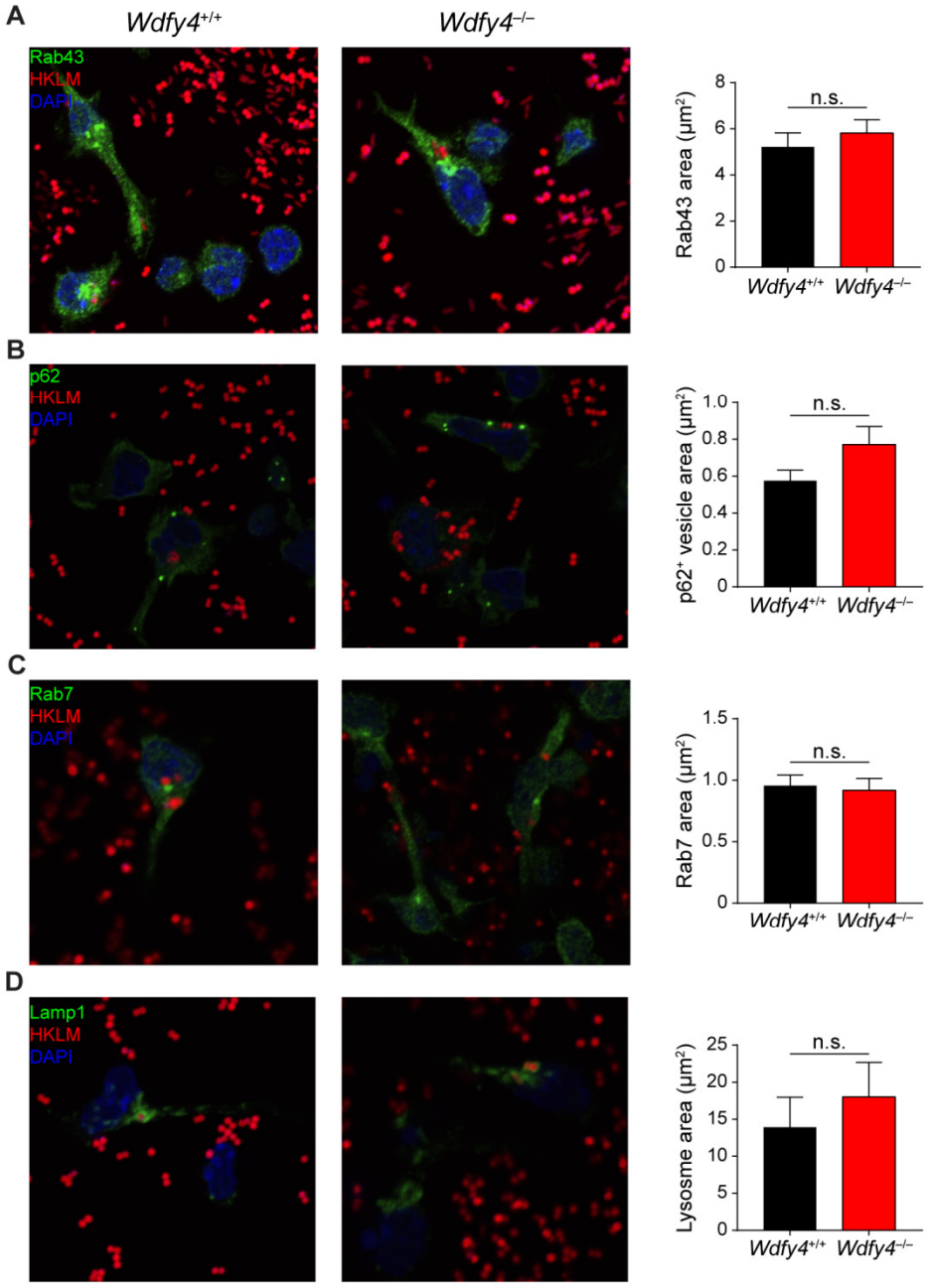

Figure S11: *Wdfy4*-deficient cDCs have normal cellular structures after antigen uptake.

(A-D) Confocal microscopy of day 10 Flt3L-cultured DCs attached to alcian blue coated coverslips for four hours in the presence of AF647-labeled HKLM-OVA (red). Cells were fixed, permeabilized, and stained with antibodies against RAB43 (A), p62 (B), Rab7 (C), or Lamp1 (D) which were secondary stained with antibodies conjugated to AF488 (green) before mounting on slides using Prolong Gold antifade + DAPI (blue). Area of staining quantified for each stain on left, shown as mean ± SEM. n.s. not significant $P>.05$ by Mann Whitney U Test.

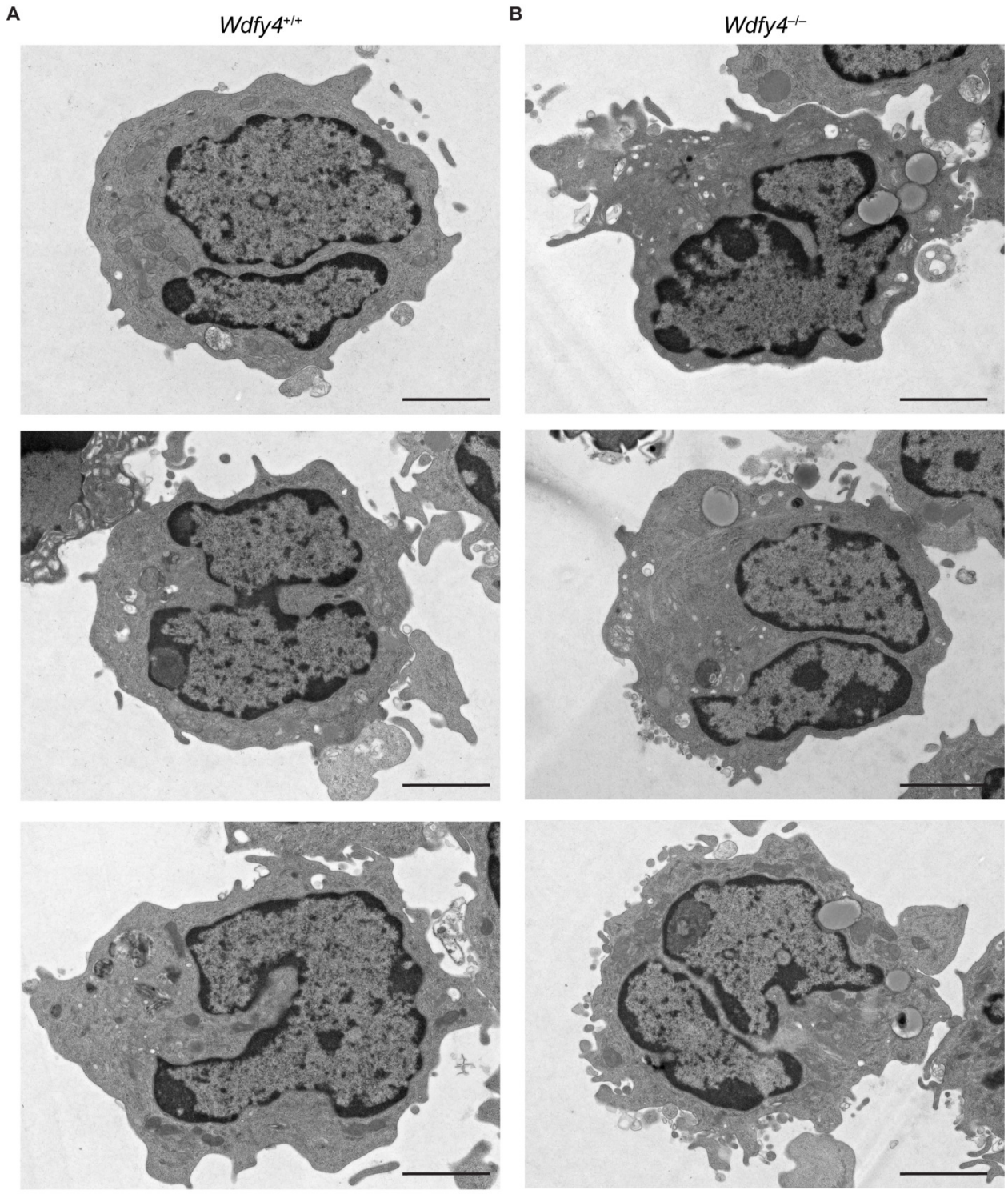

Figure S12: *Wdfy4*-deficient cDC1 have increased presence of intracellular lipid bodies.

(A-B) Representative EM images of *Wdfy4*$^{+/+}$ (A) or *Wdfy4*$^{-/-}$ (B) cells analyzed by electron microscopy. Scale bars indicate 2µm.

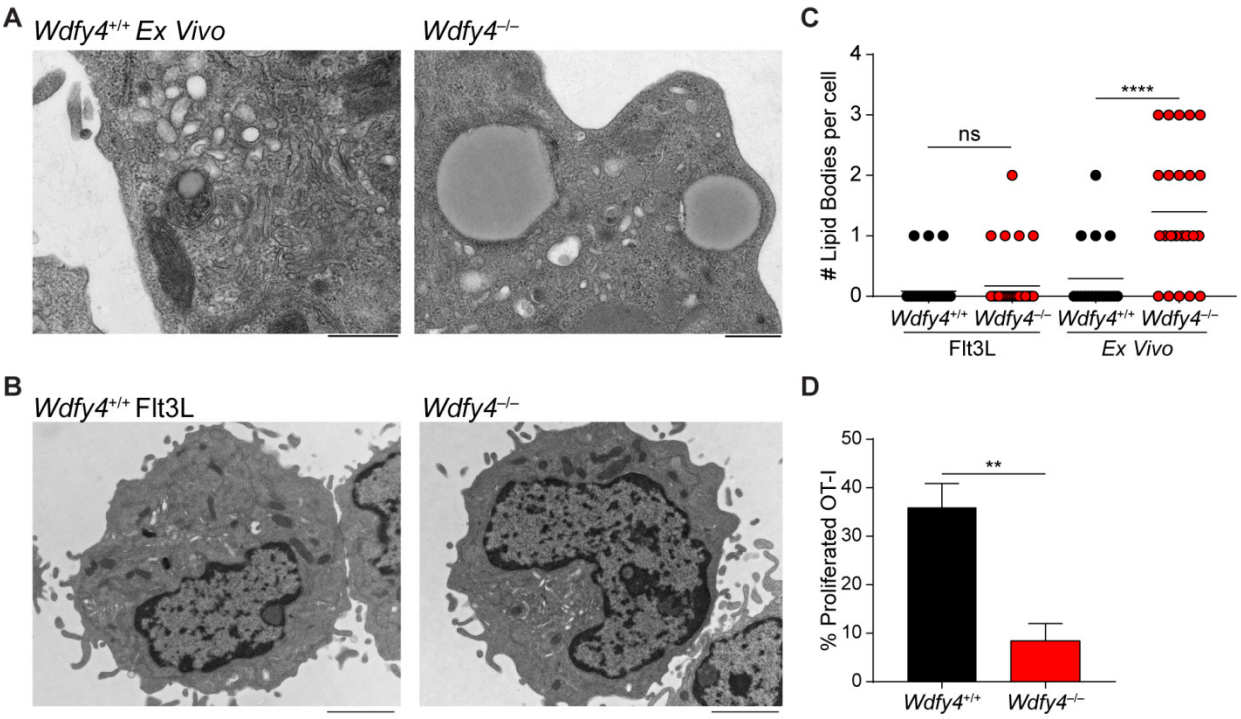

Figure S13: Presence of lipid bodies in *Wdfy4*-deficient cDC1 does not explain cross-presentation defect

(**A**) Representative EM images of *Wdfy4*$^{+/+}$ or *Wdfy4*$^{-/-}$ cDC1 taken *ex vivo* from spleen, scale bars indicate 500nm (**B**) Representative EM images of *Wdfy4*$^{+/+}$ or *Wdfy4*$^{-/-}$ cDC1 from Flt3L cultures, scale bars indicate 2µm (**C**) Quantification of the number of lipid bodies per cell for indicated samples. Each dot indicates one cell, bar indicates mean. (**D**) Cross-presentation of 10^7 HLKM-OVA to OT-1 T cells by cDC1 from *Wdfy4*$^{+/+}$ or *Wdfy4*$^{-/-}$ Flt3L cultures. T cell activation assayed as CFSE$^-$CD44$^+$. Bars indicate mean ±SEM, ns, not significant (P>0.05); ** P<.01, **** P<0.0001 using One-way ANOVA (C) or Mann-Whitney U Test (D).

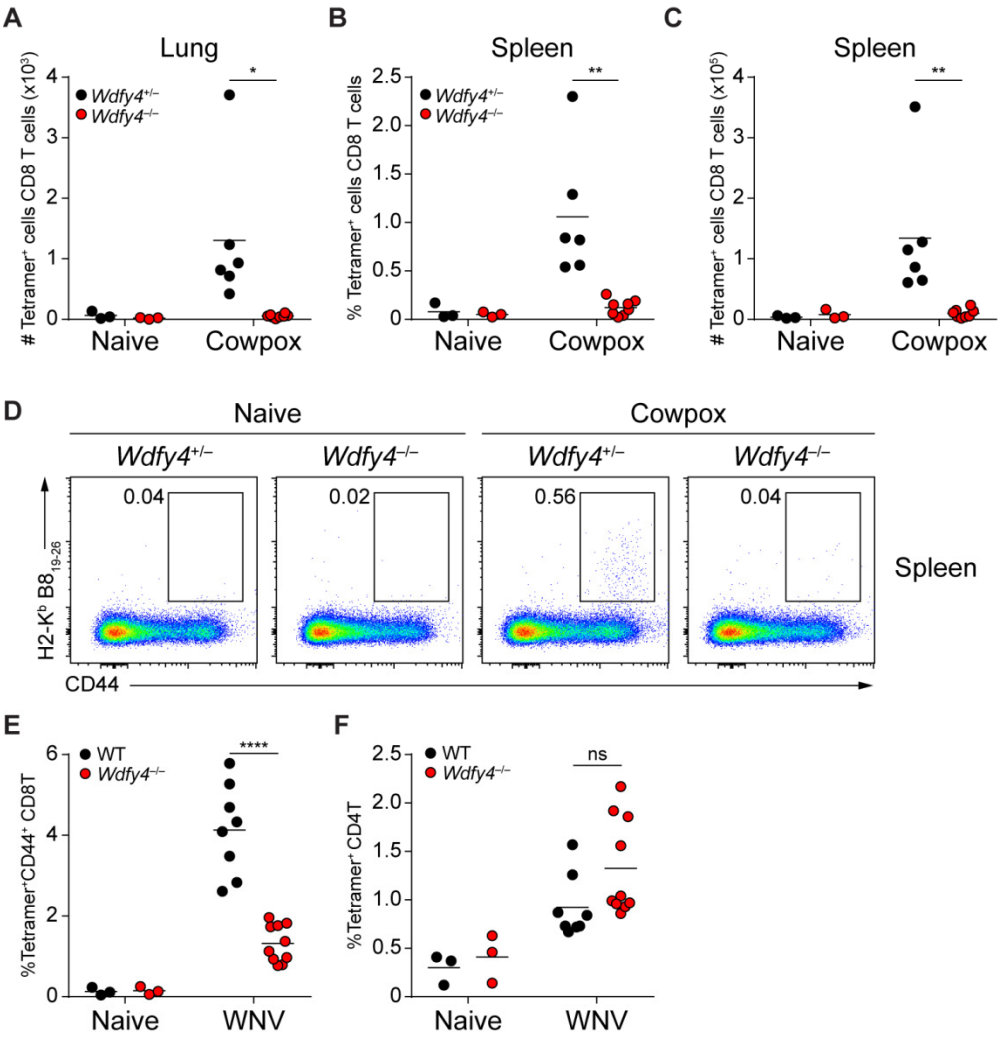

Figure S14: *Wdfy4*-deficient mice cannot prime CD8 T cells to cowpox virus infection.

(A-C) Quantification of number (A,C) or percentage (B) of tetramer^{+}CD8 T cells from lung (A) or spleen (B,C) of infected or uninfected (naïve) *Wdfy4*$^{+/-}$ or *Wdfy4*$^{-/-}$ mice. Each dot represents one mouse, bars indicate mean. **(D)** Representative flow cytometry of CD8 T cells (pregate CD4^{-} CD3^{+}CD8^{+}) from the spleen of naïve or infected *Wdfy4*$^{+/-}$ or *Wdfy4*$^{-/-}$ mice. (**E**) Percentage of Tetramer^{+}CD44^{+} cells within CD8 T cell population in spleen of mice eight days after West Nile Virus infection. (**F**) Percentage of Tetramer^{+} cells within CD4 T cell population in spleen of mice eight days after West Nile Virus infection. Each dot indicates one mouse, bar indicates

mean, ns, not significant, *P<0.05; **P<0.01, ****P<.0001 using 2-way ANOVA with Tukey's multiple comparisons test.

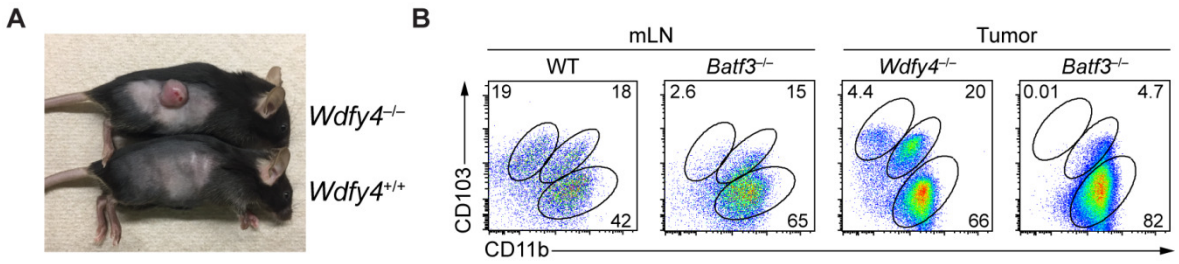

Figure S15: Analysis of tumors from *WDFY4*$^{-/-}$ or *Batf3*$^{-/-}$ mice.

(**A**) Representative images of tumors from WT and *Wdfy4*$^{-/-}$ mice. (**B**) Representative flow cytometry plots indicating *Batf3*-dependent populations in mLN (pre-gate B220^{-} CD11c^{+}MHCII^{+}) compared to WT (left) and representative flow cytometry plots showing the presence of *Batf3*-dependent DC subsets in tumors (pre-gate B220^{-}CD3^{-}NK1.1^{-}CD11c^{+}MHCII^{+}) of *Wdfy4*$^{-/-}$ mice compared to *Batf3*$^{-/-}$ mice after at least 20 days of growth (right).

Gene	Name	cDC1 Expression	cDC2 Expression	Steady State cDC1	Activated cDC1	DC Specificity	Other immune cells	cDC1/cDC2	Steady State/Activated
Leprel1	leprecan-like1 (prolyl 3-hydroxylase 2)	644.254	35.759			cDC1	none	18.01655527	
Ifi205	interferon induced gene 205	2242.841	131.696	8456.33	3061.67	cDC1	none	17.03044132	2.76199917
Hepacam2	HEPACAM family member 2	621.812	47.902	1672	85.33	cDC1	peritoneal B1	12.98091938	19.59451541
P2ry14	Purinergic receptor P2Y, G-protein coupled	831.49	69.269	658.67	18.33	cDC1, pDC	Mast cells, lung populations	12.00378236	35.933988
Pdia5	protein disulfide isomerase associated 5	468.177	39.275	486.67	106.33	cDC1	none	11.92048377	4.576977335
Naaa	N-acylethanolamine acid amidase	2377.942	217.601	9833	238.33	cDC1	monocytes	10.92799206	41.25791969
Cadm1	cell adhesion molecule 1	870.215	84.588	1114.33	133.67	cDC1	red pulp, liver macrophages	10.28768856	8.336425526
Mpeg1	Macrophage expressed gene 1	1196.745	133.271	12902.33	10090.67	cDC1, pDC	monocytes, macrophages	8.979785 5	1.278639575
Fam40b	family with sequence similarity 40, member B	332.686	39.912	622.67	2346.33	CD103+ cDC1	none	8.335488074	0.265380403
Ppef2	Protein phosphatase, EF-hand calcium binding domain 2	126.067	16.441	25	12.67	cDC1	monocytes	7.667842589	1.973164957
Fnip2	folliculin interacting protein 2	453.869	59.559	677	246	cDC1	Thio-stimulated macrophages	7.620493964	2.75203252
Naga	N-acetyl galactosaminidase, alpha	1068.988	142.464	2549	735	cDC1	none	7.503565813	3.468027211
Laptm4b	lysosomal-associated protein transmembrane 4B	540.675	73.444	907.67	4162.33	Activated cDCs	peritoneal macrophages	7.361731387	0.218067765
Ppt1	palmitoyl-protein thioesterase 1	3361.065	531.101	10595	454.67	cDC1	none	6.328485542	23.30261508
Unc119b	unc-119 homolog	295.312	53.769	694	43.33	cDC1	several	5.492235303	16.01661666
Fam149a	Family with sequence similarity 149, member A	268.271	49.625	276.67	39.67	cDC1	none	5.405964736	6.974287875
Fmnl2	formin-like 2	780.582	145.949			All but cDC2	red pulp MFs, PMNs	5.348320304	
Ppap2a	phosphatidic acid phosphatase type 2A	286.48	55.916	1163	172	cDC1	red pulp macrophages	5.123399385	6.761627907
Gclc	glutamate-cysteine ligase, catalytic subunit	481.738	95.955	653.33	2642.33	cDC1	pre-B cells	5.020457506	0.247255263
Clec1a	C-type lectin domain family 1, member a	206.398	42.421			cDC1	none	4.865467575	
Gatm	Glycine Amino-transferase	195.992	42.056	3569	313	cDC1	Some macrophages	4.660262507	11.40255591
Fndc7	fibronectin type III domain containing 7	191.991	41.929	104.67	22.33	cDC1	none	4.5789549	4.687416032
Trpm2	transient receptor potential cation channel subfamily M, member 2	328.227	74.946	233.67	103	cDC1	red pulp macrophages, PMNs	4.37951325	2.268640777
Arhgap42	Rho GTPase activating protein 42	184.369	43.355	22.33	22.67	cDC1	none	4.252542959	0.985002206
Sdad1	SDA1 domain containing 1	620.645	147.671	826.33	59.33	cDC1	several	4.202890209	13.92769257
Aif1	allograft inflammatory factor 1	1121.587	267.869			cDC1	monocytes, macrophages	4.187072786	
ApoL7c	apolipoprotein L 7C	2251.617	538.871	1949	6805.33	cDC1	none	4.178397056	0.286393165
Ece1	endothelin converting enzyme 1	865.587	212.008	907	517	cDC1	RP MFs, monocytes	4.082803479	1.754352031
MNDA	Myloid cell nuclear differentiation antigen	1188.085	316.086			cDC1	macrophages	3.75873971	

Plekha5	pleckstrin homology domain containing, family A member 5	251.762	69.818	319	97.67	cDC1	NK cells	3.605975536	3.266100133
Slamf8	SLAM family member 8	501.5	145.173	1646.33	42	cDC1	monocytes	3.454499115	39.19833333
Itpripl1	inositol 1,4,5-triphosphate receptor interacting protein-like 1	338.432	98.738	22.67	17.67	cDC1	none	3.427576009	1.282965478
Fuca1	fucosidase, alpha-L- 1, tissue	1113.487	324.88			cDC1	B cells, monocytes	3.42737934	
Cxx1c	CAAX box 1 homolog C	268.097	83.251	99	36.67	cDC1	none	3.220345702	2.699754568
Zfp367	zinc finger protein 367	254.079	81.728			cDC1	germinal center B cells, activated NK cells	3.108836629	
Ocstamp	Osteoclast stimulatory transmembrane protein	274.506	88.39			cDC1	Osteoclasts	3.105622808	
Txndc15	thioredoxin domain containing 15	407.018	139.142			cDC1	none	2.925198718	
Tmtc3	transmembrane and tetratricopeptide repeat	139.514	48.782			cDC1	red pulp macrophages, monocytes	2.859948342	
Zdhhc23	zinc finger, DHHC domain containing 23	236.277	82.976			cDC1	monocytes, red pulp macrophages	2.847534227	
Rasgrp3	RAS, guanyl releasing protein 3	914.018	324.428	853.33	133.33	cDC1	B cells	2.817321563	6.400135003
Dbn1	drebrin 1	204.241	74.652	78.33	30	cDC1	none	2.735907946	2.611
Tmem39a	transmembrane protein 39a	543.552	200.512	636.33	2751.67	cDC1	none	2.7108203	0.231252294
Mctp1	multiple C2 domains, transmembrane 1	779.042	303.359	484.67	36	cDC1	B cells, monocytes, PMNs	2.568053033	13.46305556
Ttc39a	tetratricopeptide repeat domain 39A	131.534	51.643			cDC1	none	2.546986039	
Tifab	TRAF-interacting protein with forkhead box	551.118	230.354	213.67	18.67	All	Monocytes	2.392482874	11.44456347
Tspan33	tetraspanin 33	196.332	84.5			cDC1	none	2.323455621	
Fzd1	frizzled homolog 1	147.277	64.989	635	73	cDC1	peritoneal macrophages	2.266183508	8.698630137
Wdfy4	WD repeat and FYVE domain containing 4	1829.575	826.605	4710	598	cDC1	B cells	2.213360674	7.876254181
Arhgap18	Rho GTPase activating protein 18	459.167	209.475	434.33	36	cDC1	B cells, NK cells, macrophages	2.191989498	12.06472222
Map2k6	mitogen-activated protein kinase kinase 6 (MEK6)	255.027	120.483	121	41.33	cDC1	none	2.116705261	2.927655456
Arhgap22	Rho GTPase activating protein 22	170.321	82.048	138.33	599	cDC1	none	2.075870222	0.230934891
Mtmr4	myotubularin related protein 4	249.61	122.059			cDC1	none	2.044994634	
Epsti1	Epithelial stromal interaction 1	1082.682	532.351	22.67	531.67	cDC1	NK cells, T cells	2.033774709	0.042639231
Hps5	Hermansky-Pudlak syndrome 5 homolog	233.46	115.889			cDC1	monocytes	2.014513888	
Pfkfb3	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3	361.188	181.874	462.33	3914	CD103+ cDC1	monocytes	1.985924321	0.118122126
Tbc1d8	TBC1 domain family, member 8	1089.281	558.685	403.67	1471.67	All	monocytes	1.949723001	0.274293829
Ap1s3	adaptor-related protein complex AP-1, sigma 3	586.278	311.206			cDC1	B cells	1.883890413	
Alms1	Alstrom syndrome 1	131.586	70.269	44	30.33	cDC1	B cell progenitors	1.872603851	1.450708869
Fam190a	family with sequence similarity 190, member A	106.323	57.573			cDC1	none	1.846751081	

Srsf11	serine/arginine-rich splicing factor 11	669.647	365.266			cDC1	several	1.833313257	
Lrrc40	leucine rich repeat containing 40	207.079	114.077			cDC1	B cells	1.815256362	
Plbd1	phospholipase B domain containing 1	4539.941	2563.829	10627.33	421.67	cDC1	monocytes, PMNs	1.770765913	25.20295492
Mpzl2	myelin protein zero-like 2 (EVA1)	43.303	24.835	383.33	165.33	cDC1	none	1.743627944	2.318574971
Ptcd2	pentatricopeptide repeat domain 2	430.296	254.133			cDC1	none	1.693192147	
Dock5	dedicator of cytokinesis 5	817.625	492.62	321.67	82.33	cDC1	monocytes, PMNs, NK cells, activated T cells	1.659747879	3.907081258
Pik3cb	phosphatidylinositol 3-kinase, catalytic, beta polypeptide	891.243	565.375	671.67	319.67	cDC1	PMNs, monocytes	1.576374972	2.101135546
Cdk14	cyclin-dependent kinase 14	433.634	277.568	711.33	251.33	cDC1	none	1.562262222	2.830263001
Lrrc18	leucine rich repeat containing 18	624.324	411.68	27.67	27.67	cDC1	none	1.5165274	1
Havcr2	Hepatitis A virus cellular receptor 2	886.503	590.72	791.33	35.67	cDC1	Macrophages	1.500716075	22.18474909
Erlin1	ER lipid raft associated 1	461.626	313.719	2605	2147.33	cDC1	monocytes	1.471463316	1.213134451
Rala	v-ral simian leukemia viral oncogene homolog A (ras related)	656.41	467.7	658	274.67	cDC1	none	1.403485144	2.395601995
Sh3bp1	SH3-domain binding protein 1	398.673	286.586			cDC1	monocytes	1.391111222	
Wdr86	WD repeat domain 86	189.862	139.383	87.33	51.67	cDC1	none	1.362160378	1.690149023
Cacnb3	calcium channel, voltage depedent, beta 3 subunit	78.588	62.484	66.33	2514	cDC1	none	1.257729979	0.026384248
Lrrk2	leucine-rich repeat kinase 2	644.256	545.687	578.67	146.33	cDC1	B cells, PMNs	1.180632854	3.954554773
Insm1	Insulinoma associated 1	67.877	58.615	16	1762.33	Activated DCs	none	1.158014166	0.00907889
Dock7	dedicator of cytokinesis 7	199.721	175.659			cDC1	B cells, macrophages, monocytes	1.136981311	
Tbc1d9	TBC1 domain family, member 9	741.905	668.595			cDC1	monocytes	1.109647844	
Slamf7	SLAM family member 7	2651.531	2419.749			cDC1	B cells, NK cells, activated T cells	1.095787621	
Fscn1	fascin homolog 1, actin bundling protein	84.504	77.395	31	567.33	CD103+ cDC1	none	1.091853479	0.054641919
Bloc1s2	biogenesis of lysosome-related organelles complex-1, subunit 2	519.446	476.41			All	none	1.090333956	
Cdc14a	CDC14 cell division cycle 14 homolog A	543.912	513.91	803.33	323	cDC1	PMNs, gamma-delta T cells	1.058379872	2.487089783
Clu	Clusterin	38.817	37.812	18	1978.67	Activated cDC1	None	1.026578864	0.00909702
Rogdi	rogdi homolog	718.925	735.088	537.67	1075	All	PMNs, monocytes	0.978012156	0.50015814
Gcfc1	GC-rich sequence DNA binding factor 1	554.587	590.824			All	several	0.938667014	
ETV3	ets variant gene 3	58.411	62.481	274.33	1571.33	All cDC	several	0.934860198	0.174584588
Serpinb1a	Serine peptidase inhibitor beta 1 subunit a	35.016	54.243	29	835.67	Activated DCs	B cells, macrophages, neutrophils	0.645539517	0.034702694
FoxP4	forkhead box P4	120.622	196.225	24	69.33	Activated DCs	none	0.614712702	0.346170489
Tbc1d4	TBC1 domain family, member 4	134.851	298.759	947	1899.33	All cDCs	none	0.451370503	0.498596874

Gpr126	G-protein coupled receptor 126	25.589	137.69	90	1165	Activated cDCs	macrophages	0.185845014	0.077253219

Table S1: Expression profiles of candidate genes for CRISPR screen

Expression values taken from previously published resources (*82-84*).

	Gene	Primer 1	Primer 2
A	WDFY4	CACCGATCTGCTTCGCCAACGATGG	AAACCCATCGTTGGCGAAGCAGATC
B	WDFY4	CACCGAAAGACTGAGGACAGACCCG	AAACCGGGTCTGTCCTCAGTCTTTC
C	Fam149a	CACCGGAAGGTCGCTGTGTTGGACC	AAACGGTCCAACACAGCGACCTTCC
D	Lrrk2	CACCGCATGGCCAGTGGCGCCTGTC	AAACGACAGGCGCCACTGGCCATGC
E	Fam190a	CACCGTCTCCCGGTTGCCAATATTC	AAACGAATATTGGCAACCGGGAGAC
F	Pdia5	CACCGTGGGGGCTGCTACTGGCGAT	AAACATCGCCAGTAGCAGCCCCCAC
G	Txndc15	CACCGGGGCCTTCCGGCCCACGGCC	AAACGGCCGTGGGCCGGAAGGCCCC
H	Ttc39a	CACCGGGGCTGTCATGCACTGGTCC	AAACGGACCAGTGCATGACAGCCCC
I	Mtmr4	CACCGGGGCATCGGTTGCCCGGCCC	AAACGGGCCGGGCAACCGATGCCCC
J	Ece1	CACCGGCGGACCGTGTGGTCGCCGC	AAACGCGGCGACCACACGGTCCGCC
K	Ece1	CACCGGGTGCAGCATGCGGACCGTG	AAACCACGGTCCGCATGCTGCACCC
L	Tex2	CACCGCCGGCAATGACAAGTCTGAA	AAACTTCAGACTTGTCATTGCCGGC
M	Tex2	CACCGTGATGGTTTTGGCATGTCAA	AAACTTGACATGCCAAAACCATCAC
N	Cacnb3	CACCGTGTAGGAGTCGGCTGAACCC	AAACGGGTTCAGCCGACTCCTACAC
O	Rogdi	CACCGCGCGGCGCTCGCAGCCATCG	AAACCGATGGCTGCGAGCGCCGCGC
P	Srsf11	CACCGGCAGTGGTCCCCAGCGCGCC	AAACGGCGCGCTGGGGACCACTGCC
Q	Srsf11	CACCGAGCTGCTACATGATTCAATC	AAACGATTGAATCATGTAGCAGCTC
R	Strip2	CACCGTGCCGCGGGGTCGTCCATGC	AAACGCATGGACGACCCCGCGGCAC
S	Strip2	CACCGAAACGGCTCTTCATTATGTG	AAACCACATAATGAAGAGCCGTTTC
T	Etv3	CACCGGAAAGCAGGCTGTAGCATCG	AAACCGATGCTACAGCCTGCTTTCC
U	Etv3	CACCGCTACAAAGCCGAGTCGTCGC	AAACGCGACGACTCGGCTTTGTAGC
V	Foxp4	CACCGCCGTCTGGTCAGAACGGCGT	AAACACGCCGTTCTGACCAGACGGC
W	Foxp4	CACCGACAATCAGGTCGGCTCCGTC	AAACGACGGAGCCGACCTGATTGTC
X	Insm1	CACCGCCCGTGTCCTACCGGGTCCG	AAACCGGACCCGGTAGGACACGGGC
Y	Insm1	CACCGGGTCACTGTCCTCGCCGCCG	AAACCGGCGGCGAGGACAGTGACCC
Z	Naaa	CACCGCCACCAGGGCCGCGTGCCAT	AAACATGGCACGCGGCCCTGGTGGC
AA	Naaa	CACCGTACTGTCGCTGTCCGGTCCC	AAACGGGACCGGACAGCGACAGTAC
AB	Sdad1	CACCGGCGGGACCCGCCGGCCTACG	AAACCGTAGGCCGGCGGGTCCCGCC
AC	Sdad1	CACCGCCGGCGGGTCCCGCTTGATC	AAACGATCAAGCGGGACCCGCCGGC

AD	Gpr126	CACCGTGTTTGACACTCTCGGGAAG	AAACCTTCCCGAGAGTGTCAAACAC
AE	Gpr126	CACCGGGAGACGTAAAGGTACCGGA	AAACTCCGGTACCTTTACGTCTCCC
AF	Rala	CACCGTCTGCAGTTCATGTACGACG	AAACCGTCGTACATGAACTGCAGAC
AG	Rala	CACCGTCGCAGCTACCGCGGACTTC	AAACGAAGTCCGCGGTAGCTGCGAC
AH	Rnf115	CACCGCGGGGCTCTTACCGGTAGTT	AAACAACTACCGGTAAGAGCCCCGC
AI	Rnf115	CACCGCATGGCGGAGGCCTCGGCGG	AAACCCGCCGAGGCCTCCGCCATGC
AJ	Slamf8	CACCGCAACGGCCATGTGGTCCCTC	AAACGAGGGACCACATGGCCGTTGC
AK	Slamf8	CACCGTCACGCACTTGGAAGCCCGG	AAACCCGGGCTTCCAAGTGCGTGAC
AL	Tmem39a	CACCGGCCGCCGACCAACGTCTGTA	AAACTACAGACGTTGGTCGGCGGCC
AM	Tmem39a	CACCGATACAGACGTTGGTCGGCGG	AAACCCGCCGACCAACGTCTGTATC
AN	Laptm4b	CACCGCCGCACCGGCACCATCCTGC	AAACGCAGGATGGTGCCGGTGCGGC
AO	Laptm4b	CACCGGGCTACTTACGGTGCATACA	AAACTGTATGCACCGTAAGTAGCCC
AP	Laptm4b	CACCGCCAGGTACCAGACGCCCAGC	AAACGCTGGGCGTCTGGTACCTGGC
AQ	Ppt1	CACCGTGGCGTCGTCCTGTTCGCGG	AAACCCGCGAACAGGACGACGCCAC
AR	Ppt1	CACCGAGATGGCGTCGTCCTGTTCG	AAACCGAACAGGACGACGCCATCTC
AS	Unc119b	CACCGAGCCGTCGTCGGGCGTGTGT	AAACACACACGCCCGACGACGGCTC
AT	Unc119b	CACCGCCGGCTCAAGGCGCGACGCC	AAACGGCGTCGCGCCTTGAGCCGGC
AU	Fndc7	CACCGTAGAATGGACTACCGTGCCA	AAACTGGCACGGTAGTCCATTCTAC
AV	Fndc7	CACCGGCAATAGTATCACCGTAGAA	AAACTTCTACGGTGATACTATTGCC
AW	Leprel1	CACCGGCGACTATGAGCGAGCGGTG	AAACCACCGCTCGCTCATAGTCGCC
AX	Leprel1	CACCGGAGCAGTCACCGGCGCCTAC	AAACGTAGGCGCCGGTGACTGCTCC
AY	Ifi205	CACCGGAAACGTGGTATATTTCTCT	AAACAGAGAAATATACCACGTTTCC
AZ	Ifi205	CACCGTGAAGCCGAAGATGAGACCT	AAACAGGTCTCATCTTCGGCTTCAC
BA	Hepacam2	CACCGCCGCTGCAGTTCACCGATGA	AAACTCATCGGTGAACTGCAGCGGC
BB	Hepacam2	CACCGAACGGAGCCTAGCAAGTATT	AAACAATACTTGCTAGGCTCCGTTC
BC	Cadm1	CACCGGGATCAGTGCCGCGGCCGAA	AAACTTCGGCCGCGGCACTGATCCC
BD	Cadm1	CACCGGCCGCACACTGGGATCCGCT	AAACAGCGGATCCCAGTGTGCGGCC
BE	Mpeg1	CACCGCTTGGAGAAACGGGTACCAC	AAACGTGGTACCCGTTTCTCCAAGC
BF	Mpeg1	CACCGGAAGCTGTTCATGGCGCAGA	AAACTCTGCGCCATGAACAGCTTCC
BG	Naga	CACCGGTTACAGCGGAAGCGTTCCC	AAACGGGAACGCTTCCGCTGTAACC

BH	Naga	CACCGCCGCTGTAACATCGACTGTG	AAACCACAGTCGATGTTACAGCGGC
BI	Gclc	CACCGCGCCTCCATTCAGTAACAAC	AAACGTTGTTACTGAATGGAGGCGC
BJ	Gclc	CACCGCGATGTTCTTGAGACTCTGC	AAACGCAGAGTCTCAAGAACATCGC
BK	Aif1	CACCGGAGCCAAAGCAGGGATTTGC	AAACGCAAATCCCTGCTTTGGCTCC
BL	Aif1	CACCGAAAAGCTTTTGGACTGCTGA	AAACTCAGCAGTCCAAAAGCTTTTC
BM	Apol7c	CACCGAGTGGCCCCAGACTTATTTC	AAACGAAATAAGTCTGGGGCCACTC
BN	Apol7c	CACCGGTTGGTTGGCACCAGGTTCC	AAACGGAACCTGGTGCCAACCAACC
BO	Arsb	CACCGCTTCGTGCTGGCGGACGACC	AAACGGTCGTCCGCCAGCACGAAGC
BP	Arsb	CACCGGGTCATCCGCACGCCGCACC	AAACGGTGCGGCGTGCGGATGACCC
BQ	Ocstamp	CACCGTCCTTGGCCAGTCTTATCGC	AAACGCGATAAGACTGGCCAAGGAC
BR	Ocstamp	CACCGGGTCAGTAGTTCGTTACAGC	AAACGCTGTAACGAACTACTGACCC
BS	Plbd1	CACCGGCGATGTGCCACCGCAGCCC	AAACGGGCTGCGGTGGCACATCGCC
BT	Plbd1	CACCGGCCAGCCAGAGCGACCCAAC	AAACGTTGGGTCGCTCTGGCTGGCC
BU	Anpep	CACCGTCTGTCGGTGGTCTACGCTC	AAACGAGCGTAGACCACCGACAGAC
BV	Anpep	CACCGGAGGTGCTGCCCGGGAGCGT	AAACACGCTCCCGGGCAGCACCTCC
BW	Tbc1d8	CACCGTTCGTCTTGCAGCGGCGCCG	AAACCGGCGCCGCTGCAAGACGAAC
BX	Tbc1d8	CACCGGGCGCCGCGGGCACGGCGAG	AAACCTCGCCGTGCCCGCGGCGCCC
BY	Epsti1	CACCGTCCATCTCTCGGGACCACGC	AAACGCGTGGTCCCGAGAGATGGAC
BZ	Epsti1	CACCGGACCATGTACACCCGCAGTA	AAACTACTGCGGGTGTACATGGTCC
CA	Clu	CACCGGGAGATTCAGAACGCCGTCC	AAACGGACGGCGTTCTGAATCTCCC
CB	Clu	CACCGCAAGGACTTGCGCTCTGCGT	AAACACGCAGAGCGCAAGTCCTTGC
CC	WDFY1	CACCGCGGCGTGATCACCGCTAGCG	AAACCGCTAGCGGTGATCACGCCGC
CD	WDFY1	CACCGTGATCACCGCTAGCGAGGAC	AAACGTCCTCGCTAGCGGTGATCAC
CE	WDFY2	CACCGTGGATCTCCGCCGCCATCGG	AAACCCGATGGCGGCGGAGATCCAC
CF	WDFY2	CACCGGGGTGTCATCAGCGTCTCGG	AAACCCGAGACGCTGATGACACCCC
CG	WDFY3	CACCGTAGGCCTGATGCACCTCCGC	AAACGCGGAGGTGCATCAGGCCTAC
CH	WDFY3	CACCGAGCCCGCAAGACAACGCCTT	AAACAAGGCGTTGTCTTGCGGGCTC
CI	P2ry14	CACCGCATTCCCGTGTTGTACGGTA	AAACTACCGTACAACACGGGAATGC
CJ	P2ry14	CACCGACGGTATGGTCTTCATCACG	AAACCGTGATGAAGACCATACCGTC
CK	Fnip2	CACCGCTCCTTGGGCGGCCGGGCCT	AAACAGGCCCGGCCGCCCAAGGAGC

CL	Fnip2	CACCGCCGGCTCCTCCTTGGGCGGC	AAACGCCGCCCAAGGAGGAGCCGGC
CM	Fmnl2	CACCGGGAGCGCTTTGCCATCGTGC	AAACGCACGATGGCAAAGCGCTCCC
CN	Fmnl2	CACCGCACGTTGTGCGCCTTGAAAT	AAACATTTCAAGGCGCACAACGTGC
CO	Ppap2a	CACCGCAAGACGCGGCTGCCGTACG	AAACCGTACGGCAGCCGCGTCTTGC
CP	Ppap2a	CACCGCAGCCGCGTCTTGTCGAACA	AAACTGTTCGACAAGACGCGGCTGC

Table S2: sgRNA sequences used in this study

sgRNA were designed using MIT CRISPR Design Tool (*85*). Blue nucleotides indicated cloning sites

Sample	Proteins Enriched (2/3)																										
FL1	Actbl2	Actl6a	Aifm2	Arpc1b	Arpc5	Atp5j	Bag6	Card9	Cpsf6	Dctn4	Dgat1	Dhrs7b	Eif3g	Ganab	Hdac6	Mapk6	Mroh1	Nomo1	Nup160	Pfkfb3	Psmd4	Ran	Rps25	Sort1	Tmed9	Vps50	Wdfy4
FL2	Actbl2	Actl6a	Actr3	Aifm2	Arpc2	Atg13	Atp5j	Bag6	Cd44	Cers2	Clta	Dctn4	Ddx23	Fip1l1	Ganab	Gvin1	Hdac6	Hspa1b	Hspa2	Hspa8	IgG1_bovi	Kif5c	Lrch4	Mroh1	Mybbp1a	Myo1d	Nt5c2
	Pabpc1	Pfkfb3	Pls3	Psmd11	Psmd8	Rps19	S100a4	Sf3b3	Smarcd1	Sort1	Spin1	Tfrc	Thrap3	Trappc8	Ulk1	Vps41	Vwa8	Wdfy4									
	Myo1c	Myo5a	Myo6	Pfkfb3	Plec	Ppp1ca	Ppp1r18	Sept11	Siglec1	Snrpd2	Sort1	Sptbn1	Tcirg1	Tpm1	Tpm2	Tpm3	Wdfy4										
FL3	Acta2	Actb	Actbl2	Actn1	Actn4	Actr3	Ahnak2	Ap2b1	Arhgap9	Arpc2	Arpc4	Atp6v0a1	Atp6v0d1	Atp6v0d2	Atp6v1f	Cald1	Clta	Cltc	Cpsf6	Iqgap1	Lima1	Lsp1	Myh11	Myh9	Myl12a	Myl12b	Myl6
FL4	Abcd2	Acap2	Acta2	Actb	Actbl2	Actl6a	Actn4	Actr3	Adm	Ahnak	Ahnak2	Ap2a1	Ap2a2	Ap2b1	Apobr	Araf	Arfgef2	Arhgap9	Arpc2	Arpc4	Atp6v0d1	Atp6v0d2	Atp6v1a	Atp6v1f	Bag6	Cald1	Calm2
	Ccdc88a	Cct3	Cct6a	Cd44	Cd68	Cfl2	Chil4	Clns1a	Clta	Cltc	Copg2	D1Pas1	Dhrs7b	Dnaja1	Efhd2	Ganab	Hcls1	Hsp90ab1	Hspa1b	Hspa2	Hspa5	Hspa8	Hyou1	Iqgap1	Kif5c	Lima1	Lsp1
	Mpc2	Mpeg1	Mroh1	Msn	Myh10	Myh11	Myh9	Myl12a	Myl12b	Myl6	Myl6b	Myo1c	Myo1e	Myo1f	Plec	Plod3	Ppp1ca	Psma1	Psma4	Rpl24	Rplp1	Rps27a	Sdhb	Sec61a1	Sfxn3	Siglec1	Sort1
	Stip1	Tcirg1	Tcp1	Thy1	Tmed9	Tpm3	Txn	Uqcrc1	Vps13c	Vps53	Wdfy4																

Table S3: Proteins enriched in mass spectrometry

List of proteins found enriched at least 2-fold of control samples in at least two of three mass spectrometry runs

SUID	% Associated Genes	Associated Genes Found	GOID	GOTerm	Group PValue	Nr. Genes	Term PValue
2427	1.388888836	[Actr3, Arpc1b, Arpc2, Arpc5, Hdac6, Hspa1b, Hspa8, Pls3]	GO:0097435	supramolecular fiber organization	{Group2=7.689871029925464E-5}	8	5.34E-04
2428	1.134644508	[Actr3, Arpc1b, Arpc2, Arpc5, Clta, Cpsf6, Hdac6, Hspa1b, Hspa8, Psmd11, Psmd4, Psmd8, Rps19, Ulk1, Vps41]	GO:0071822	protein complex subunit organization	{Group2=7.689871029925464E-5}	15	1.45E-05
2429	0.994694948	[Actr3, Arpc1b, Arpc2, Arpc5, Clta, Cpsf6, Dgat1, Hdac6, Hspa1b, Psmd11, Psmd4, Psmd8, Rps19, Ulk1, Vps41]	GO:0065003	macromolecular complex assembly	{Group2=7.689871029925464E-5}	15	6.78E-05
2430	0.749375522	[Actl6a, Actr3, Atg13, Bag6, Clta, Dctn4, Hdac6, Hspa8, Kif5c, Mybbp1a, Myo1d, Nup160, Psmd8, Ran, Sort1, Tmed9, Vps41, Vps50]	GO:0051641	cellular localization	{Group1=6.057261404642146E-4}	18	6.06E-04
2431	0.980392158	[Actl6a, Atg13, Clta, Dctn4, Hdac6, Hspa8, Kif5c, Mybbp1a, Myo1d, Nup160, Psmd8, Ran, Sort1, Vps41, Vps50]	GO:0046907	intracellular transport	{Group1=6.057261404642146E-4}	15	8.00E-05
2432	0.755857885	[Actr3, Arpc1b, Arpc2, Arpc5, Atg13, Bag6, Clta, Cpsf6, Dgat1, Hdac6, Hspa1b, Hspa2, Pls3, Psmd11, Psmd4, Psmd8, Ran, Rps19, Ulk1, Vps41]	GO:0044085	cellular component biogenesis	{Group2=7.689871029925464E-5}	20	2.22E-04
2433	0.99765259	[Actr3, Arpc1b, Arpc2, Arpc5, Clta, Cpsf6, Dgat1, Hdac6, Hspa1b, Hspa8, Psmd11, Psmd4, Psmd8, Rps19, Smarcd1, Ulk1, Vps41]	GO:0043933	macromolecular complex subunit organization	{Group2=7.689871029925464E-5}	17	1.80E-05
2434	1.357466102	[Actr3, Arpc1b, Arpc2, Arpc5, Clta, Hdac6, Hspa1b, Psmd11, Psmd4, Psmd8, Rps19, Vps41]	GO:0034622	cellular macromolecular complex assembly	{Group2=7.689871029925464E-5}	12	2.22E-05
2435	0.579547584	[Actl6a, Actr3, Aifm2, Arpc1b, Arpc2, Arpc5, Atg13, Bag6, Cd44, Cers2, Clta, Cpsf6, Dgat1, Hdac6, Hspa1b, Hspa2, Hspa8, Kif5c, Mapk6, Pls3, Psmd11, Psmd4, Psmd8, Ran, Rps19, Smarcd1, Sort1, Spin1, Tmed9, Ulk1, Vps41]	GO:0016043	cellular component organization	{Group0=1.8401552771690126E-4}	31	1.84E-04

Table S4: GO Terms for FL1/FL2 fragments

Enriched GO terms generated by ClueGO for proteins enriched in FL1 and FL2 samples after mass spectrometry

SUID	% Associated Genes	Associated Genes Found	GOID	GOTerm	Group PValue	Nr. Genes	Term PValue
1253	2.917771816	[Actl6a, Ccdc88a, Cct3, Cct6a, Hcls1, Hsp90ab1, Iqgap1, Msn, Myo1c, Sptbn1, Tcp1]	GO:1903829	positive regulation of cellular protein localization	{Group3=1.7911444457370406E-9, Group5=5.400723098059407E-11, Group6=9.289432501882645E-13}	11	9.53E-06
1254	3.378378391	[Actr3, Arpc2, Arpc4, Cfl2, Hcls1, Hspa1b, Hspa8, Lima1, Myo1c, Sptbn1]	GO:1902903	regulation of supramolecular fiber organization	{Group6=9.289432501882645E-13}	10	6.99E-06
1255	3.472222328	[Actn1, Actn4, Actr3, Arpc2, Arpc4, Ccdc88a, Cfl2, Hcls1, Hsp90ab1, Hspa1b, Hspa8, Lima1, Myh10, Myh11, Myo1c, Plod3, Sptbn1, Tpm1, Tpm2, Tpm3]	GO:0097435	supramolecular fiber organization	{Group6=9.289432501882645E-13}	20	6.00E-11
1256	3.063457251	[Acta2, Actr3, Adm, Arpc2, Arpc4, Cfl2, Clns1a, Hcls1, Hsp90ab1, Lima1, Msn, Myo1c, Plod3, Sptbn1]	GO:0090066	regulation of anatomical structure size	{Group6=9.289432501882645E-13}	14	2.88E-07
1257	1.739788175	[Actr3, Ap2b1, Arpc2, Arpc4, Atp6v0a1, Cct3, Cfl2, Clta, Cltc, Cpsf6, Hcls1, Hsp90ab1, Hspa1b, Hspa8, Lima1, Msn, Myh11, Myo1c, Plod3, Psma4, Sept11, Sptbn1, Tcp1]	GO:0071822	protein complex subunit organization	{Group2=4.007090667982035E-10, Group6=9.289432501882645E-13}	23	9.31E-07
1258	1.167434692	[Acta2, Actb, Actr3, Adm, Arpc2, Arpc4, Atp6v0a1, Atp6v0d1, Atp6v0d2, Atp6v1a, Bag6, Calm2, Cct3, Cct6a, Cd44, Cfl2, Clns1a, Hcls1, Hsp90ab1, Hspa5, Hspa8, Lima1, Mpc2, Msn, Myh10, Myh9, Myl12b, Myo1c, Myo5a, Myo6, Plod3, Rps27a, Sfxn3, Sptbn1, Tcp1, Thy1, Tpm1, Txn1]	GO:0065008	regulation of biological quality	{Group0=3.0590363954994164E-6}	38	3.06E-06
1259	2.017937183	[Actl6a, Calm2, Ccdc88a, Cct3, Cct6a, Cltc, Dnaja1, Hcls1, Hsp90ab1, Iqgap1, Mpc2, Msn, Myo1c, Myo5a, Sptbn1, Tcp1, Thy1, Txn1]	GO:0060341	regulation of cellular localization	{Group3=1.7911444457370406E-9, Group5=5.400723098059407E-11}	18	2.33E-06
1260	1.794453502	[Actl6a, Actn4, Actr3, Ap2a1, Ap2a2, Ap2b1, Atp6v0d1, Atp6v0d2, Calm2, Ccdc88a, Clta, Cltc, Copg2, Hcls1, Hsp90ab1, Hspa8, Hyou1, Kif5c, Mpc2, Msn, Myh10, Myh9, Myo1c, Myo5a, Myo6, Rps27a, Sec61a1, Sort1, Sptbn1, Thy1, Tmed9, Txn1, Vps53]	GO:0051649	establishment of localization in cell	{Group5=5.400723098059407E-11}	33	6.48E-10
1261	1.706910849	[Acap2, Actl6a, Actn4, Actr3, Ap2a1, Ap2a2, Ap2b1, Atp6v0d1, Atp6v0d2, Bag6, Calm2, Ccdc88a, Cct3, Cct6a, Clta, Cltc, Copg2, Dnaja1, Hcls1, Hsp90ab1, Hspa5, Hspa8, Hyou1, Iqgap1, Kif5c, Mpc2, Msn, Myh10, Myh9, Myo1c, Myo5a, Myo6, Rps27a, Sec61a1, Sort1, Sptbn1, Tcp1, Thy1, Tmed9, Txn1, Vps53]	GO:0051641	cellular localization	{Group5=5.400723098059407E-11}	41	7.85E-12
1262	2.678571463	[Actr3, Arpc2, Arpc4, Ccdc88a, Cfl2, Cltc, Hcls1, Hspa1b, Lima1, Myo1c, Myo1f, Sptbn1]	GO:0051493	regulation of cytoskeleton organization	{Group6=9.289432501882645E-13}	12	8.66E-06
1263	1.281746984	[Abcd2, Actl6a, Actn4, Actr3, Adm, Ap2a1, Ap2a2, Ap2b1, Apobr, Araf, Arfgef2, Atp6v0a1, Atp6v0d1, Atp6v0d2, Atp6v1a, Atp6v1f, Bag6, Calm2, Ccdc88a, Cct3, Cct6a, Clns1a, Clta, Cltc, Copg2, Dnaja1, Hcls1, Hsp90ab1, Hspa2, Hspa5, Hspa8, Hyou1, Kif5c, Mpc2, Msn, Myh10, Myh9, Myo1c, Myo1e, Myo1f, Myo5a, Myo6, Psma1, Rps27a, Sec61a1, Sfxn3, Siglec1, Sort1, Sptbn1, Tcp1, Thy1, Tmed9, Txn1, Vps53]	GO:0051234	establishment of localization	{Group5=5.400723098059407E-11}	54	6.22E-11
1264	1.642178059	[Actl6a, Actn4, Actr3, Arpc2, Arpc4, Ccdc88a, Cct3, Cct6a, Cfl2, Hspa1b, Hspa5, Iqgap1, Msn, Myo1c, Psma1, Tcp1, Thy1, Tmed9, Vps13c]	GO:0051130	positive regulation of cellular component organization	{Group4=4.007090667982035E-10, Group6=9.289432501882645E-13}	19	2.27E-05
1265	1.375332713	[Actl6a, Actn4, Actr3, Arpc2, Arpc4, Ccdc88a, Cct3, Cct6a, Cd44, Cfl2, Cltc, Hcls1, Hspa1b, Hspa2, Hspa5, Hspa8, Iqgap1, Lima1, Msn, Myh10, Myh9, Myl12b, Myo1c, Myo1f, Psma1, Sptbn1, Tcp1, Thy1, Tmed9, Tpm1, Vps13c]	GO:0051128	regulation of cellular component organization	{Group4=4.007090667982035E-10, Group6=9.289432501882645E-13}	31	1.24E-06
1266	1.83006537	[Actl6a, Actn4, Ap2a1, Ap2a2, Ap2b1, Atp6v0d1, Atp6v0d2, Ccdc88a, Clta, Cltc, Copg2, Hcls1, Hsp90ab1, Hspa8, Hyou1, Kif5c, Mpc2, Msn, Myh10, Myo1c, Myo5a, Myo6, Rps27a, Sec61a1, Sort1, Sptbn1, Txn1, Vps53]	GO:0046907	intracellular transport	{Group5=5.400723098059407E-11}	28	1.35E-08
1267	1.446855903	[Actl6a, Actn4, Ap2a1, Ap2a2, Ap2b1, Arfgef2, Bag6, Cct6a, Clta, Cltc, Copg2, Dnaja1, Hcls1, Hsp90ab1, Hspa8, Mpc2, Myh9, Myo1c, Myo5a, Myo6, Sec61a1, Sptbn1, Tcp1, Tmed9, Txn1, Vps53]	GO:0045184	establishment of protein localization	{Group5=5.400723098059407E-11}	26	4.94E-06
1268	1.525821567	[Actr3, Ap2b1, Arpc2, Arpc4, Atp6v0a1, Cct3, Cfl2, Clns1a, Clta, Cltc, Cpsf6, Hcls1, Hsp90ab1, Hspa1b, Hspa8, Lima1, Msn, Myh11, Myo1c, Plod3, Psma4, Rpl24, Sept11, Snrpd2, Sptbn1, Tcp1]	GO:0043933	macromolecular complex subunit organization	{Group2=4.007090667982035E-10, Group6=9.289432501882645E-13}	26	1.84E-06
1269	2.402957439	[Actr3, Ap2b1, Arpc2, Arpc4, Atp6v0a1, Clta, Cltc, Hcls1, Hsp90ab1, Hspa1b, Myh11, Myo1c, Sptbn1]	GO:0043623	cellular protein complex assembly	{Group6=9.289432501882645E-13}	13	1.14E-05
1270	1.738570452	[Acap2, Actl6a, Ap2a1, Ap2a2, Ap2b1, Bag6, Ccdc88a, Cct3, Cct6a, Clta, Cltc, Copg2, Dnaja1, Hcls1, Hsp90ab1, Hspa5, Hspa8, Iqgap1, Msn, Myo1c, Myo5a, Myo6, Sec61a1, Sptbn1, Tcp1, Thy1, Txn1]	GO:0034613	cellular protein localization	{Group3=1.7911444457370406E-9, Group5=5.400723098059407E-11}	27	7.82E-08
1271	2	[Acap2, Actl6a, Bag6, Ccdc88a, Cct3, Cct6a, Dnaja1, Hcls1, Hsp90ab1, Hspa5, Hspa8, Msn, Sec61a1, Sptbn1, Tcp1, Txn1]	GO:0033365	protein localization to organelle	{Group3=1.7911444457370406E-9, Group5=5.400723098059407E-11}	16	1.03E-05
1272	1.653611779	[Actl6a, Actr3, Arpc2, Arpc4, Ccdc88a, Cct3, Cct6a, Cfl2, Cltc, Hcls1, Hspa1b, Lima1, Msn, Myo1c, Myo1f, Sptbn1, Tcp1, Tmed9, Vps13c]	GO:0033043	regulation of organelle organization	{Group3=1.7911444457370406E-9, Group4=4.007090667982035E-10, Group6=9.289432501882645E-13}	19	2.06E-05
1273	1.348819733	[Acap2, Actl6a, Actn4, Ap2a1, Ap2a2, Ap2b1, Apobr, Arfgef2, Bag6, Ccdc88a, Cct3, Cct6a, Clta, Cltc, Copg2, Dnaja1, Hcls1, Hsp90ab1, Hspa5, Hspa8, Iqgap1, Kif5c, Mpc2, Msn, Myh9, Myo1c, Myo5a, Myo6, Plod3, Sec61a1, Sptbn1, Tcp1, Thy1, Tmed9, Txn1, Vps53]	GO:0033036	macromolecule localization	{Group5=5.400723098059407E-11}	36	2.30E-07
1274	3.416149139	[Actr3, Arpc2, Arpc4, Ccdc88a, Cfl2, Hcls1, Lima1, Myh9, Myo1c, Myo1f, Sptbn1]	GO:0032970	regulation of actin filament-based process	{Group6=9.289432501882645E-13}	11	2.12E-06
1275	3.426791191	[Actr3, Arpc2, Arpc4, Cfl2, Clns1a, Hcls1, Hsp90ab1, Lima1, Msn, Myo1c, Sptbn1]	GO:0032535	regulation of cellular component size	{Group6=9.289432501882645E-13}	11	2.05E-06
1276	3.25900507	[Actb, Actn1, Actn4, Actr3, Arpc2, Arpc4, Ccdc88a, Cfl2, Hcls1, Lima1, Myh10, Myh11, Myh9, Myo1c, Myo1f, Sptbn1, Tpm1, Tpm2, Tpm3]	GO:0030036	actin cytoskeleton organization	{Group4=4.007090667982035E-10, Group6=9.289432501882645E-13}	19	5.66E-10
1277	3.495440722	[Acap2, Actb, Actn1, Actn4, Actr3, Arpc2, Arpc4, Ccdc88a, Cfl2, Hcls1, Lima1, Myh10, Myh11, Myh9, Myl6, Myl6b, Myo1c, Myo1f, Myo5a, Sptbn1, Tpm1, Tpm2, Tpm3]	GO:0030029	actin filament-based process	{Group6=9.289432501882645E-13}	23	1.44E-12
1278	1.515151501	[Actn1, Actn4, Actr3, Ap2b1, Arpc2, Arpc4, Atp6v0a1, Atp6v0d1, Bag6, Ccdc88a, Cct3, Cfl2, Clns1a, Clta, Cltc, Cpsf6, Hcls1, Hsp90ab1, Hspa1b, Hspa2, Iqgap1, Lima1, Msn, Myh10, Myh11, Myo1c, Myo6, Plec, Plod3, Psma4, Rpl24, Sept11, Snrpd2, Sptbn1, Tcp1, Thy1, Tpm1]	GO:0022607	cellular component assembly	{Group2=4.007090667982035E-10, Group6=9.289432501882645E-13}	37	4.41E-09
1279	2.093802452	[Actn4, Adm, Ap2a1, Ap2a2, Ap2b1, Apobr, Arfgef2, Clta, Cltc, Copg2, Hspa8, Hyou1, Msn, Myh10, Myh9, Myo1e, Myo1f, Myo5a, Myo6, Psma1, Siglec1, Sort1, Sptbn1, Tmed9, Vps53]	GO:0016192	vesicle-mediated transport	{Group1=7.193960081190489E-9}	25	7.19E-09
1280	1.121704936	[Actb, Actl6a, Actn1, Actn4, Actr3, Adm, Ap2b1, Arfgef2, Arpc2, Arpc4, Atp6v0a1, Atp6v0d1, Bag6, Ccdc88a, Cct3, Cct6a, Cd44, Cfl2, Clns1a, Clta, Cltc, Cpsf6, Hcls1, Hsp90ab1, Hspa1b, Hspa2, Hspa5, Hspa8, Iqgap1, Kif5c, Lima1, Lsp1, Msn, Myh10, Myh11, Myh9, Myl12b, Myo1c, Myo1e, Myo1f, Myo5a, Myo6, Plec, Plod3, Psma1, Psma4, Rpl24, Rps27a, Sec61a1, Sept11, Snrpd2, Sort1, Sptbn1, Tcp1, Thy1, Tmed9, Tpm1, Tpm2, Tpm3, Vps13c]	GO:0016043	cellular component organization	{Group2=4.007090667982035E-10, Group4=4.007090667982035E-10}	60	4.01E-10
1281	1.401050806	[Actl6a, Actn4, Ap2a1, Ap2a2, Ap2b1, Arfgef2, Clta, Cltc, Copg2, Dnaja1, Hcls1, Hsp90ab1, Hspa8, Mpc2, Myh9, Myo1c, Myo5a, Myo6, Sec61a1, Sptbn1, Tcp1, Tmed9, Txn1, Vps53]	GO:0015031	protein transport	{Group5=5.400723098059407E-11}	24	2.20E-05
1282	2.321724653	[Actl6a, Actr3, Arpc2, Arpc4, Ccdc88a, Cct3, Cct6a, Cfl2, Hspa1b, Msn, Myo1c, Tcp1, Tmed9, Vps13c]	GO:0010638	positive regulation of organelle organization	{Group3=1.7911444457370406E-9, Group4=4.007090667982035E-10, Group6=9.289432501882645E-13}	14	7.49E-06
1283	5.29801321	[Actr3, Arpc2, Arpc4, Cfl2, Hcls1, Lima1, Myo1c, Sptbn1]	GO:0008064	regulation of actin polymerization or depolymerization	{Group6=9.289432501882645E-13}	8	2.31E-06
1284	3.89221549	[Actn1, Actn4, Actr3, Arpc2, Arpc4, Cfl2, Hcls1, Lima1, Myo1c, Sptbn1, Tpm1, Tpm2, Tpm3]	GO:0007015	actin filament organization	{Group6=9.289432501882645E-13}	13	5.11E-08
1285	1.979345918	[Actb, Actn1, Actn4, Actr3, Arpc2, Arpc4, Ccdc88a, Cfl2, Cltc, Hcls1, Hspa1b, Lima1, Lsp1, Myh10, Myh11, Myh9, Myo1c, Myo1f, Sptbn1, Thy1, Tpm1, Tpm2, Tpm3]	GO:0007010	cytoskeleton organization	{Group4=4.007090667982035E-10, Group6=9.289432501882645E-13}	23	9.39E-08
1286	1.169415236	[Actb, Actl6a, Actn1, Actn4, Actr3, Arfgef2, Arpc2, Arpc4, Atp6v0d1, Bag6, Ccdc88a, Cct3, Cct6a, Cfl2, Cltc, Hcls1, Hsp90ab1, Hspa1b, Hspa2, Lima1, Lsp1, Msn, Myh10, Myh11, Myh9, Myo1c, Myo1f, Rpl24, Rps27a, Sec61a1, Sort1, Sptbn1, Tcp1, Thy1, Tmed9, Tpm1, Tpm2, Tpm3, Vps13c]	GO:0006996	organelle organization	{Group2=4.007090667982035E-10, Group4=4.007090667982035E-10, Group6=9.289432501882645E-13}	39	1.95E-06
1287	2.4866786	[Actn4, Adm, Ap2a1, Ap2a2, Ap2b1, Apobr, Clta, Cltc, Myh9, Myo1e, Myo6, Psma1, Siglec1, Sort1]	GO:0006897	endocytosis	{Group1=7.193960081190489E-9}	14	3.41E-06

Table S5: Go Terms for FL3/FL4 fragments

Enriched GO terms generated by ClueGO for proteins enriched in FL3 and FL4 samples after mass spectrometry

Gene Symbol	Sum Intensity MT	Sum Intensity FL-4		Gene Symbol	Sum Intensity MT	Sum Intensity FL-4		Gene Symbol	Sum Intensity MT	Sum Intensity FL-4		Gene Symbol	Sum Intensity MT	Sum Intensity FL-4		Gene Symbol	Sum Intensity MT	Sum Intensity FL-4
Actb	6.20E+09	7.60E+10		Psmc2	1.10E+07	8.70E+06		Prpf8	1.00E+06	1.70E+06		Ccdc22	NF	2.20E+05		Sel1l	7.60E+04	NF
Acta2	1.20E+09	7.00E+09		Eif3e	5.10E+06	8.70E+06		Jak1	3.50E+06	1.70E+06		Srp72	NF	2.20E+05		Hist1h1a	NF	NF
Wdfy4	1.30E+05	1.60E+09		Dnajb11	3.70E+06	8.70E+06		Eif3g	2.20E+06	1.70E+06		Scarb2	NF	2.20E+05		Snrpg	NF	NF
Stk38	1.80E+09	1.60E+09		Psma4	5.30E+05	8.60E+06		Ap2a1	NF	1.70E+06		Ndufs7	NF	2.20E+05		Babam2	1.20E+05	NF
Myh9	1.30E+08	1.20E+09		Hadhb	7.50E+06	8.50E+06		Psmd4	2.10E+06	1.70E+06		Tmed2	NF	2.10E+05		Mta1	3.60E+05	NF
Mthfd1l	1.50E+09	1.10E+09		Naglu	1.50E+07	8.40E+06		Atp6v1d	5.10E+05	1.70E+06		Clcc1	NF	2.10E+05		Sptan1	NF	NF
Prmt5	1.20E+09	1.10E+09		Actr1b	9.60E+06	8.40E+06		Tuba4a	7.40E+05	1.70E+06		Rplp1	NF	2.10E+05		Acat1	NF	NF
Eif4b	1.10E+09	7.80E+08		Capza1	1.40E+07	8.20E+06		Atp6v0a1	NF	1.70E+06		Rps6	2.70E+05	1.90E+05		Zyx	NF	NF
Ighg1	6.40E+08	6.80E+08		Sfn	2.80E+06	8.20E+06		Hnrnph1	3.00E+06	1.60E+06		Ywhab	5.60E+04	1.90E+05		Stag2	NF	NF
KV2A7	4.40E+08	6.50E+08		Eif3m	7.60E+06	8.10E+06		Ywhaz	1.60E+06	1.60E+06		Tpm4	NF	1.80E+05		Tubal3	3.80E+05	NF
Hspa8	2.30E+07	6.10E+08		Eif3l	6.60E+06	8.00E+06		Atp6v1f	NF	1.60E+06		Rpl14	NF	1.80E+05		Gsn	NF	NF
Flna	3.00E+08	5.70E+08		Psmd12	1.00E+07	7.90E+06		Alyref2	9.50E+05	1.60E+06		Thy1	NF	1.80E+05		Ddx5	2.60E+05	NF
Vcp	3.90E+08	5.40E+08		Ahnak2	3.70E+06	7.80E+06		Snrpe	6.10E+05	1.60E+06		Hspa4	1.00E+05	1.70E+05		Atp5l	NF	NF
Tuba1a	2.70E+08	4.30E+08		Psmc4	7.20E+06	7.70E+06		Vps16	1.90E+06	1.50E+06		Bag6	NF	1.70E+05		Epg5	NF	NF
Atp5b	2.60E+08	4.20E+08		Atp5f1	9.20E+06	7.60E+06		Psmb4	5.20E+06	1.50E+06		Kdm1a	5.10E+04	1.60E+05		Igfbp2	NF	NF
Wdr77	4.80E+08	4.20E+08		Psmb3	4.20E+06	7.60E+06		Itgb2	2.00E+06	1.50E+06		Arfgef2	NF	1.60E+05		Hist2h2aa1	NF	NF
Atp5a1	1.60E+08	3.40E+08		Sdha	2.80E+06	7.50E+06		Sept9	1.30E+07	1.50E+06		Samhd1	2.30E+05	1.50E+05		Rpp30	2.70E+05	NF
Tubb3	1.80E+08	3.30E+08		Tyms	6.00E+06	7.40E+06		Rbbp4	2.00E+06	1.50E+06		Aldh3a2	2.60E+05	1.40E+05		Bckdk	3.60E+05	NF
Hspa5	1.10E+08	2.30E+08		Rrbp1	1.90E+06	7.30E+06		Ndufa13	NF	1.50E+06		Mic13	NF	1.40E+05		Mapk6	2.40E+05	NF

Myl6	2.60E+07	2.20E+08		Hcls1	2.60E+06	7.30E+06		Hsp90b1	2.40E+06	1.40E+06		Smc1a	3.20E+05	1.30E+05		Ikbkb	1.70E+05	NF
Ankfy1	2.10E+08	2.10E+08		Gpnmb	6.90E+06	7.30E+06		Kpnb1	1.20E+06	1.40E+06		Rps5	8.30E+05	1.30E+05		Hnrnpab	1.60E+05	NF
Gsn	7.40E+07	2.10E+08		Canx	6.90E+06	7.20E+06		Sec61a1	5.00E+05	1.40E+06		Slc8b1	NF	1.30E+05		Gatad2b	NF	NF
Tubb5	1.30E+08	2.00E+08		Sdf2l1	4.40E+06	7.20E+06		Psmb6	9.40E+05	1.40E+06		Atxn10	2.50E+05	1.20E+05		Ndufa8	NF	NF
Eef1a1	1.00E+08	1.80E+08		Mccc2	7.40E+06	7.10E+06		Ran	NF	1.40E+06		Sec31a	NF	1.20E+05		Ybx1	1.40E+05	NF
Tubb2b	7.80E+07	1.70E+08		Rbm3	3.60E+06	6.90E+06		Clta	NF	1.40E+06		Gcn1	NF	1.10E+05		Marco	2.10E+05	NF
Myl12b	1.60E+07	1.50E+08		Calu	6.70E+06	6.80E+06		Rps28	NF	1.40E+06		Tom1	NF	1.00E+05		Ap2m1	NF	NF
Myh10	1.70E+07	1.50E+08		Slc25a1	6.10E+06	6.70E+06		Fasn	3.00E+06	1.30E+06		Eif2s3y	9.30E+05	9.30E+04		Tmem165	NF	NF
IGKC	2.10E+08	1.50E+08		Ssr4	7.10E+06	6.70E+06		Myo18a	2.20E+06	1.30E+06		Smarcd1	NF	9.20E+04		Erh	9.10E+04	NF
Tubb4a	2.60E+07	1.30E+08		Rps10	7.30E+06	6.70E+06		Lima1	NF	1.30E+06		Cdc42bpb	NF	9.20E+04		Gid8	NF	NF
Tmod3	2.10E+08	1.20E+08		Psmd3	1.10E+07	6.60E+06		Arl1	1.20E+06	1.30E+06		Wdr7	NF	8.90E+04		Isoc2a	1.10E+05	NF
Vim	6.20E+07	1.20E+08		Uqcrc2	1.30E+06	6.60E+06		Actn1	NF	1.30E+06			6.70E+04	8.50E+04		Npm1	NF	NF
Ppib	5.20E+07	1.10E+08		Slain2	6.70E+06	6.60E+06		Cd68	NF	1.30E+06		Ldha	NF	8.10E+04		Ndufb10	3.60E+05	NF
Ighg1	1.30E+08	1.10E+08		Rps18	4.80E+06	6.50E+06		Rpl31	3.30E+05	1.30E+06		Washc2	NF	7.70E+04		Dhcr7	1.60E+05	NF
Slc25a5	3.40E+07	1.00E+08		Ap2b1	2.00E+05	6.30E+06		Cfl2	NF	1.30E+06		Pcyt1a	NF	7.70E+04		Washc4	1.20E+05	NF
Flii	6.40E+07	9.90E+07		Alb	4.10E+06	6.30E+06		Eif2b5	1.30E+06	1.20E+06		Cpsf2	NF	6.40E+04		Rps15a	NF	NF
Cltc	NF	9.70E+07		Lrp1	1.30E+07	6.20E+06		Eif2b4	1.20E+06	1.20E+06		Mtnd5	NF	6.40E+04		Eif2s2	3.50E+05	NF
Cct3	3.20E+07	9.30E+07		Myof	4.70E+06	6.20E+06		Hnrnpa2b1	3.20E+05	1.20E+06		Stt3a	NF	5.40E+04		Ndufb5	NF	NF
Hspa2	7.80E+06	9.10E+07		Rock2	2.80E+06	6.00E+06		Pycr2	NF	1.20E+06		Lrrk2	NF	3.70E+04		Des	NF	NF
Hsp90ab1	9.20E+06	8.70E+07		Eif2s1	5.70E+06	6.00E+06		Eif2ak3	2.90E+06	1.10E+06		Araf	NF	2.50E+04		Syngr1	NF	NF
Slc25a4	7.50E+07	8.30E+07		Arcn1	6.40E+06	5.90E+06		Dctn2	3.70E+06	1.10E+06		Stom	NF	1.90E+04		Tpm3	NF	NF
Prpf31	1.20E+08	8.10E+07		Eif3h	7.60E+06	5.80E+06		Qpctl	3.20E+06	1.10E+06		GFP	NF	NF		Tpm2	NF	NF
Tuba1c	4.00E+07	8.10E+07		Myo1c	1.10E+06	5.70E+06		Glud1	1.30E+06	1.10E+06		Snrnp200	2.00E+06	NF		Pls1	NF	NF
Cct8	3.10E+07	7.40E+07		Anxa1	4.50E+06	5.70E+06		Ruvbl2	7.10E+05	1.10E+06		Colgalt1	2.40E+06	NF		Atg13	NF	NF

Cct6a	2.80E+07	7.30E+07		Hspd1	3.10E+06	5.60E+06		Atp5j	NF	1.10E+06		Phb2	7.00E+05	NF		Ppp1r18	NF	NF
Cct2	4.30E+07	7.20E+07		Msn	2.70E+06	5.60E+06		Atp6v1h	6.70E+05	1.10E+06		Ruvbl1	2.70E+06	NF		Rpl22	3.70E+05	NF
Ahnak	1.00E+07	6.90E+07		Arpc4	2.40E+05	5.60E+06		Camk2d	NF	1.10E+06		Eftud2	1.00E+06	NF		Rab5b	NF	NF
Flnc	5.30E+07	6.80E+07		Isg15	1.80E+06	5.50E+06		Eif2b1	1.80E+06	1.00E+06		Rb1cc1	9.80E+05	NF		Trappc4	2.10E+05	NF
Iqgap1	6.80E+06	6.60E+07		Lpcat3	4.20E+06	5.50E+06		Ndufa9	1.10E+06	1.00E+06		Eno1	NF	NF		Pcca	2.20E+05	NF
Actn4	3.50E+05	6.40E+07		Psma7	8.80E+06	5.40E+06		Lasp1	1.80E+05	9.90E+05		Ccar1	1.00E+06	NF		Gatad2a	3.00E+05	NF
Thrap3	6.40E+07	6.20E+07		Psma5	3.40E+06	5.40E+06		Mtatp8	1.30E+06	9.80E+05		Mob1b	5.40E+06	NF		Ifit1	NF	NF
Tcp1	2.70E+07	5.90E+07		Mpeg1	NF	5.40E+06		Ctsd	1.50E+06	9.40E+05		Aldh2	1.60E+06	NF		Lnp	3.60E+04	NF
Ivns1abp	3.10E+07	5.40E+07		Hist1h2ad	2.90E+06	5.40E+06		PRAG1	3.80E+06	9.10E+05		Ttc7b	8.40E+05	NF		Atad3	NF	NF
Hspa1b	6.40E+06	5.40E+07		Myo1e	2.10E+06	5.20E+06		Uqcrfs1	1.10E+06	9.10E+05		Pfkfb3	1.40E+05	NF		Fbl	NF	NF
Hnrnpk	4.40E+07	5.30E+07		Psmd13	1.20E+07	5.00E+06		Git2	6.30E+05	9.10E+05		Sort1	6.50E+05	NF		Ikbkap	NF	NF
Txn	2.10E+07	4.70E+07		Arpc2	NF	5.00E+06		Spata31	NF	8.90E+05		Nomo1	1.40E+06	NF		Rpl19	6.80E+04	NF
Eif3b	1.50E+07	4.60E+07		Stip1	NF	5.00E+06		Psma1	5.10E+06	8.50E+05		Dock4	6.60E+05	NF		Spag9	8.80E+04	NF
Hspa9	2.50E+07	4.30E+07		Ssr1	2.20E+06	4.90E+06		Ccdc88a	3.20E+05	8.50E+05		Gvin1	2.70E+05	NF		Rhog	NF	NF
Myh11	7.00E+06	3.90E+07		Rac2	2.00E+06	4.90E+06		Vps53	NF	8.40E+05		Dock10	4.70E+05	NF		Ddx23	NF	NF
Stk38l	7.00E+07	3.80E+07		Ina	NF	4.90E+06		Ccr1	1.90E+06	8.30E+05		Ndufv1	6.70E+05	NF		Fam47e	1.20E+05	NF
Cct5	2.00E+07	3.60E+07		Ezr	3.60E+06	4.80E+06		S100a10	1.00E+06	8.20E+05		Map3k7	1.40E+05	NF		Ndufs4	2.70E+05	NF
Lrrfip1	1.80E+07	3.40E+07		Capzb	1.90E+07	4.70E+06		Tmc5	NF	8.10E+05		Tubb6	3.30E+06	NF		Lman1	1.40E+05	NF
Cct4	1.40E+07	3.10E+07		Rps27	2.20E+06	4.70E+06		Hyou1	7.60E+05	8.00E+05		Psmb7	5.00E+05	NF		Eif5a2	NF	NF
Acot9	4.70E+07	3.10E+07		Eif3c	7.50E+06	4.60E+06		Fth1	NF	8.00E+05		Tln1	NF	NF		Vps52	2.10E+05	NF
Erh	2.90E+07	3.10E+07		Glyr1	2.90E+06	4.60E+06		Pls3	NF	7.90E+05		Lgals3bp	1.40E+06	NF		Commd9	7.10E+04	NF
Plod3	1.00E+07	3.00E+07		Ppm1a	9.10E+05	4.60E+06		Rcn2	8.20E+05	7.70E+05		Prdx4	NF	NF		Vps8	NF	NF
Atp5o	1.40E+07	2.90E+07		Atp6v1e1	2.60E+06	4.60E+06		Sf3b1	1.00E+05	7.60E+05		Sept8	1.50E+05	NF		Tlr3	1.30E+05	NF
Ppm1b	1.70E+07	2.80E+07		Actr3	NF	4.50E+06		Cald1	NF	7.60E+05		Nup160	1.50E+05	NF		Exoc5	NF	NF

Arf5	1.70E+07	2.80E+07		Hnrnpf	2.30E+06	4.50E+06		Fip1l1	NF	7.40E+05		Eif2b2	1.60E+06	NF		Klc4	NF	NF
Myo1g	8.30E+05	2.70E+07		Arhgdia	NF	4.50E+06		Adrm1	6.80E+05	7.40E+05		Capzb	2.00E+06	NF		Rras	NF	NF
Cct7	1.60E+07	2.70E+07		Pon2	2.30E+06	4.40E+06		Eppk1	NF	7.40E+05		Vdac2	NF	NF		Psmb9	1.80E+05	NF
Tab1	1.80E+07	2.60E+07		Psma3	1.60E+06	4.40E+06		Actl6a	NF	7.30E+05		Ganab	NF	NF		Atp6v0c	NF	NF
Myl6b	3.40E+06	2.60E+07		Rps27a	NF	4.40E+06		Huwe1	1.20E+06	7.20E+05		Actr10	5.40E+05	NF		Smarcc2	NF	NF
Capza2	3.20E+07	2.50E+07		Ap2a2	NF	4.30E+06		Hnrnpl	4.70E+05	7.20E+05		Plek	9.10E+05	NF		Cul1	NF	NF
Pi4ka	1.70E+07	2.40E+07		Rpl24	NF	4.30E+06		Camk2a	3.10E+05	7.20E+05		Pccb	6.20E+05	NF		Ankrd50	NF	NF
Clns1a	1.80E+07	2.40E+07		Psmb8	1.40E+06	4.20E+06		Cdc42	9.20E+05	7.10E+05		Cpsf1	3.10E+05	NF		Twf1	NF	NF
Gapdh	1.30E+07	2.40E+07		Psmd6	4.70E+06	4.10E+06		Kdelr2	2.60E+05	6.90E+05		Eif3k	1.80E+05	NF		Wdr26	NF	NF
Hadha	2.40E+07	2.30E+07		Myo5a	NF	4.10E+06		Nsf	9.30E+05	6.80E+05		Hnrnpu	4.30E+05	NF		Sptbn1	NF	NF
Mycbp	1.60E+07	2.30E+07		Psmc6	7.80E+06	4.00E+06		Timm50	4.80E+05	6.80E+05		Smarca2	NF	NF		Mrps15	2.20E+05	NF
Eef1d	1.40E+07	2.20E+07		Sept2	3.90E+06	3.90E+06		Gatd1	2.60E+05	6.80E+05		Mob2	2.70E+06	NF		Vps41	NF	NF
Psmd11	1.80E+07	2.10E+07		Lgals1	1.70E+06	3.90E+06		Spin1	1.10E+06	6.70E+05		Tagln2	3.80E+05	NF		Ndufa2	1.10E+05	NF
Tufm	1.20E+07	2.10E+07		Gigyf1	NF	3.90E+06		Eif2b3	1.20E+06	6.70E+05		Gpx4	4.60E+05	NF		Mrps26	1.10E+05	NF
Chil3	9.10E+06	2.10E+07		Fam126a	3.50E+06	3.80E+06		Vps18	6.50E+05	6.70E+05		Acp5	3.30E+06	NF		Aldoa	NF	NF
Tcirg1	NF	1.90E+07		Atp6v0d1	NF	3.80E+06		Ywhag	4.00E+05	6.40E+05		Rps24	6.40E+05	NF		Dctn4	NF	NF
Eif3f	9.30E+06	1.90E+07		Ndufa4	9.00E+06	3.80E+06		Vps51	NF	6.40E+05		Ulk1	3.40E+05	NF		Hdac6	NF	NF
Rpl38	1.90E+07	1.90E+07		P4hb	4.30E+06	3.70E+06		Trim21	3.10E+06	6.30E+05		Hist1h1d	2.20E+06	NF		Slc37a2	NF	NF
Rps3	1.40E+07	1.80E+07		Tpm3	NF	3.70E+06		Mroh1	NF	6.30E+05		Tmed10	6.60E+05	NF		Rpsa	9.90E+04	NF
Slc25a11	7.50E+06	1.70E+07		Actr3b	3.90E+05	3.70E+06		Adgre1	7.70E+05	6.20E+05		Tpm1	NF	NF		Elmo1	1.20E+05	NF
Acot10	2.10E+07	1.70E+07		Ahnak2	6.00E+05	3.60E+06		Arhgef6	NF	6.20E+05		Myo6	NF	NF		4930430A15Rik	NF	NF
Anxa2	7.80E+06	1.70E+07		D1Pas1	4.90E+05	3.60E+06		Efr3a	1.90E+05	6.10E+05		Slc25a10	4.90E+05	NF		Fis1	7.60E+04	NF
Eef1b	1.80E+07	1.70E+07		Atp6v0d2	NF	3.50E+06		Rnf213	4.70E+05	6.00E+05		Ndufs3	6.30E+05	NF		Rhot1	6.80E+04	NF
Eif3a	1.90E+07	1.60E+07		Fmnl1	1.70E+06	3.40E+06		Aifm2	NF	5.90E+05		Rps4x	3.30E+05	NF		Mrps23	9.70E+04	NF

Atp6v1a	5.30E+06	1.60E+07		Psmd8	4.30E+06	3.40E+06		Sbf1	3.10E+05	5.80E+05		Hba	NF	NF		Rab38	NF	NF
Cct6b	5.60E+06	1.60E+07		Dnaja2	2.10E+06	3.40E+06		Ctsb	NF	5.80E+05		Afg3l2	3.30E+05	NF		Pkm	NF	NF
Tpi1	8.00E+06	1.60E+07		Hist1h1e	1.20E+06	3.40E+06		Taf4	9.40E+05	5.70E+05		Copz1	7.50E+04	NF		Pfkfb4	NF	NF
Actbl2	NF	1.60E+07		Hdac1	3.20E+06	3.30E+06		Pcbp1	NF	5.70E+05		Ndufv2	4.80E+05	NF		Tmed5	1.30E+05	NF
Copa	1.70E+07	1.50E+07		Psma6	3.80E+06	3.30E+06		Ap5b1	NF	5.70E+05		Sf3b4	NF	NF		Dpf2	NF	NF
Rock1	1.90E+07	1.50E+07		Actr1a	4.30E+06	3.30E+06		Dock2	1.40E+06	5.60E+05		Trappc3	5.00E+05	NF		Cd2bp2	NF	NF
Eef1g	1.20E+07	1.50E+07		Psmd14	4.90E+06	3.20E+06		Vps13c	2.30E+05	5.50E+05		Ncl	3.10E+05	NF		Aldh3b1	NF	NF
Sqor	6.00E+06	1.50E+07		Slc25a3	1.50E+06	3.20E+06		Ndufs8	1.40E+07	5.40E+05		Mcfd2	NF	NF		Tapbp	NF	NF
Lsp1	NF	1.50E+07		Sept7	2.40E+06	3.20E+06		Cd44	NF	5.20E+05		Mybbp1a	1.50E+05	NF		Vwa8	NF	NF
Prdx1	3.70E+06	1.50E+07		Psmb1	4.30E+06	3.10E+06		Ppp1ca	1.60E+05	5.10E+05		Ndufs2	2.90E+05	NF		Pde4dip	1.60E+05	NF
Rps20	1.00E+07	1.50E+07		Rbm10	6.70E+05	3.10E+06		Dab2	NF	5.00E+05		Rab1b	3.30E+05	NF		Glg1	9.00E+04	NF
Snrpd1	1.60E+07	1.50E+07		Arpc3	NF	3.10E+06		Gsr	8.70E+05	4.90E+05		Myo1d	NF	NF		Mrps11	7.50E+04	NF
Psmd1	2.60E+07	1.40E+07		Thy1	NF	3.10E+06		Kif5c	NF	4.90E+05		Dgat1	3.10E+05	NF		Fcgr3	NF	NF
Rpn2	5.80E+06	1.40E+07		Rbbp7	4.10E+06	3.00E+06		Ugp2	NF	4.90E+05		Tspo	NF	NF		Atad5	3.70E+05	NF
Nt5c2	2.70E+07	1.40E+07		Arf2	1.70E+06	3.00E+06		Psmb10	NF	4.80E+05		Aldh16a1	1.50E+05	NF		Mdm4	NF	NF
Atp6v1b2	5.80E+06	1.40E+07		Fau	9.40E+05	3.00E+06		Dpy30	1.30E+06	4.70E+05		Ftl2	NF	NF		Hydin	NF	NF
Psmd7	1.60E+07	1.40E+07		Lrrfip2	1.50E+06	2.90E+06		Rpl9	3.90E+05	4.70E+05		G3bp1	3.90E+05	NF		Vwa5a	NF	NF
Myo1f	6.20E+06	1.40E+07		Vars	4.10E+06	2.80E+06		Sart3	2.90E+05	4.60E+05		Arpc1b	NF	NF		Sars2	NF	NF
Rpl23	4.80E+06	1.40E+07		Asph	1.90E+06	2.80E+06		Tm9sf3	7.80E+05	4.60E+05		Eef2	1.20E+05	NF		Spns1	1.20E+05	NF
Dhx57	3.00E+07	1.30E+07		Efhd2	NF	2.80E+06		Rps7	8.10E+05	4.50E+05		Smarcd2	NF	NF		Card9	NF	NF
Mccc1	1.20E+07	1.30E+07		Dhx38	4.60E+06	2.70E+06		Plbd2	NF	4.50E+05		Mbd2	3.60E+05	NF		Slc25a18	9.40E+04	NF
Atp5h	1.00E+07	1.30E+07		Bclaf1	7.00E+06	2.70E+06		Mroh1	NF	4.40E+05		Smarcc1	NF	NF		Tmem184b	NF	NF
Coro1c	7.20E+06	1.30E+07		Dock8	2.60E+06	2.70E+06		Ddx3x	NF	4.30E+05		Trappc5	3.70E+05	NF		Tbc1d20	NF	NF
Atp5c1	4.90E+06	1.30E+07		Bdh1	5.40E+05	2.70E+06		Tmed9	NF	4.30E+05		Rps2	NF	NF		Znf639	NF	NF

Prph	1.20E+07	1.30E+07		Cyc1	1.80E+06	2.70E+06		Elmo2	7.20E+05	4.20E+05		Exoc1	1.50E+05	NF		Commd5	NF	NF
Copb1	1.10E+07	1.20E+07		Lyz2	1.10E+06	2.70E+06		Tubb4b	7.20E+05	4.20E+05		Vps50	7.20E+04	NF		Hprt1	NF	NF
Rpn1	6.70E+06	1.20E+07		Ptprc	1.80E+06	2.60E+06		Mrpl12	1.50E+05	4.20E+05		Setx	NF	NF		Micall1	NF	NF
Siglec1	NF	1.20E+07		Sdhb	8.40E+05	2.60E+06		Vps33a	NF	4.10E+05		C3	5.60E+05	NF		Rtn3	NF	NF
Pabpc1	7.00E+06	1.20E+07		Fus	1.90E+06	2.50E+06		Dhx15	4.70E+05	4.00E+05		Rbm6	2.60E+05	NF		Tfrc	NF	NF
Myl12a	1.50E+06	1.20E+07		Aifm1	NF	2.50E+06		Cers2	5.10E+05	4.00E+05		Clns1a	1.90E+06	NF		Arl11	NF	NF
Usp15	1.90E+07	1.10E+07		Tpm3	NF	2.50E+06		Mbd3	1.60E+05	3.90E+05		Eif1ax	1.90E+06	NF		Akap13	NF	NF
Psmc3	9.70E+06	1.10E+07		Uqcrc1	NF	2.50E+06		Slc39a7	NF	3.90E+05		Sh3bgrl2	1.20E+06	NF		S100a4	NF	NF
Lcp1	1.10E+07	1.10E+07		Scyl2	2.10E+06	2.40E+06		Mpc2	NF	3.90E+05		Snrpd2	2.10E+06	NF		Lrch4	NF	NF
Ddost	7.50E+06	1.10E+07		Snrpb	2.70E+06	2.40E+06		Adgrl1	NF	3.90E+05		Vapa	8.80E+05	NF		Ttll3	NF	NF
Psma2	1.00E+07	1.10E+07		Copg2	9.60E+05	2.40E+06		Acap2	NF	3.80E+05		Dhx36	8.50E+05	NF		Gba2	NF	NF
Cap1	3.50E+06	1.10E+07		Atp2a2	1.40E+06	2.30E+06		Akap8	3.80E+05	3.80E+05		Zfp58	3.30E+06	NF		Rps25	NF	NF
Arl8b	1.20E+07	1.10E+07		Snrpd3	4.80E+06	2.30E+06		Lnpep	8.20E+05	3.60E+05		Ahnak	5.50E+05	NF		Top2a	NF	NF
Actr2	NF	1.10E+07		Apobr	7.20E+05	2.30E+06		Sept11	NF	3.60E+05		Serbp1	3.70E+05	NF		Lgals2	NF	NF
Hist1h4a	2.30E+06	1.10E+07		Ddb1	4.00E+06	2.20E+06		Acaca	3.80E+05	3.50E+05		Usp11	1.30E+06	NF		Ap3b1	NF	NF
Prmt5	3.30E+07	1.10E+07		Eif3i	4.40E+06	2.20E+06		Rps19	2.60E+05	3.50E+05		Spin2c	6.30E+05	NF				
S100a11	5.50E+06	1.10E+07		Trpv2	1.60E+06	2.20E+06		Arpc5l	NF	3.50E+05		Rpl10	2.70E+05	NF				
Psmd2	1.20E+07	1.00E+07		Hist1h2bk	NF	2.20E+06		Slc25a12	9.30E+04	3.30E+05		Psmb9	2.10E+05	NF				
Copb2	9.30E+06	1.00E+07		Dnaja1	7.70E+05	2.20E+06		Otud4	1.10E+06	3.10E+05		Akap5	8.60E+06	NF				
Psmc1	1.40E+07	1.00E+07		Lonp1	4.00E+06	2.10E+06		Nsfl1c	6.80E+05	3.10E+05		Mre11	2.50E+05	NF				
Morc3	1.40E+07	1.00E+07		Sfxn3	NF	2.10E+06		Ugt1a2	NF	3.10E+05		Ywhae	6.40E+05	NF				
Chil4	3.20E+06	1.00E+07		Atp5d	1.60E+06	2.10E+06		C1qbp	3.90E+05	3.00E+05		Ndufa5	4.90E+05	NF				
Anapc1	NF	1.00E+07		Ttc7a	3.60E+06	2.00E+06		Cyfip2	NF	3.00E+05		Atp1b3	3.10E+05	NF				
Psmc5	1.60E+07	9.90E+06		Eif3d	3.30E+06	2.00E+06		Naca	NF	2.90E+05		Synj1	NF	NF				

Cfl1	8.00E+06	9.80E+06		Unc93b1	2.30E+06	2.00E+06		Mta2	8.50E+05	2.70E+05		Eif2ak3	5.70E+05	NF				
Copg1	1.10E+07	9.70E+06		Atp6v1c1	NF	2.00E+06		Sf3b3	NF	2.60E+05		Arf4	1.80E+06	NF				
Synj1	5.10E+06	9.60E+06		Dhrs7b	4.10E+05	2.00E+06		Trappc8	NF	2.60E+05		Arpc5	NF	NF				
Ipo8	1.20E+07	9.40E+06		Diaph1	1.50E+06	1.90E+06		Nbeal2	NF	2.60E+05		Atxn7l3	NF	NF				
Ckap5	6.70E+06	9.30E+06		Cope	1.50E+06	1.90E+06		Fam45a	NF	2.50E+05		Hbb-b1	NF	NF				
Plec	5.00E+05	9.00E+06		Atp1a1	3.60E+05	1.90E+06		Arhgap9	NF	2.40E+05		P2rx4	2.00E+05	NF				
Psmb2	5.20E+06	9.00E+06		Ndufs1	1.90E+06	1.80E+06		Cops4	NF	2.40E+05		Pigk	2.30E+05	NF				
Myo1f	4.30E+06	8.90E+06		Acly	4.00E+05	1.80E+06		Cdc37	1.20E+05	2.30E+05		Tmem33	3.70E+05	NF				
Calm2	3.70E+06	8.90E+06		Dctn1	1.10E+06	1.80E+06		Ctsa	5.10E+05	2.20E+05		Ccdc130	NF	NF				
Rps16	8.70E+06	8.80E+06		Psmd10	1.10E+06	1.80E+06		Ipo7	8.40E+04	2.20E+05		Capza1	6.00E+05	NF				

Table S6: Sum intensities of mass-spectrometry targets for empty and FL4

Representative analysis of proteins pulled down after flag purification of JAWSII cells expressing either empty vector or Flag-tagged FL4 fragment. NF= Not Found.