Table S1. Reagents/Resources used in this study.

REAGENT OR RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Guinea pig anti-dFz2C	This study	N/A
Mouse monoclonal anti-β-Tubulin	Developmental Studies Hybridoma Bank	DSHB Cat# E7, RRID:AB_528499
Mouse monoclonal anti-Lamin B	Developmental Studies Hybridoma Bank	DSHB Cat# adl67.10, RRID:AB_528336
Mouse monoclonal anti-Actin	MP Biomedicals	MP Bio Cat# 08691001, RRID:AB_2335127
Mouse monoclonal anti-Rho1	Developmental Studies Hybridoma Bank	DSHB Cat# p1D9 (anti-rho1), RRID:AB_528263
Rabbit polyclonal anti-Arp3	(Stevenson et al., 2002)	N/A
Rabbit polyclonal anti-Arpc1	(Stevenson et al., 2002)	N/A
Rat anti-Cpa	(Amandio et al., 2014)	N/A
Mouse monoclonal anti- mono and polyubiquitinylated conjugates	Enzo Life Sciences	Enzo Life Sciences Cat# BML-PW8810, RRID:AB_10541840
Mouse monoclonal anti-c-myc	Developmental Studies Hybridoma Bank	DSHB Cat# 9E 10, RRID:AB_2266850
Mouse monoclonal anti-wash	Developmental Studies Hybridoma Bank	DSHB Cat# Wash (P3H3), RRID:AB_2239540
Mouse polyclonal anti-CCDC53	(Verboon et al., 2015)	N/A
Mouse polyclonal anti-Strumpellin	(Verboon et al., 2015)	N/A
Mouse polyclonal anti-FAM21	(Verboon et al., 2015)	N/A
Mouse monoclonal anti-Lamin C	Developmental Studies Hybridoma Bank	DSHB Cat# lc28.26, RRID:AB_528339
Rabbit polyclonal anti-Lamin B	(Stuurman et al., 1996)	N/A
Mouse polyclonal anti-SWIP	(Verboon et al., 2015)	N/A
Mouse monoclonal anti-DLG	Developmental Studies Hybridoma Bank	DSHB Cat# 4F3 anti-discs large, RRID:AB_528203
Rabbit polyclonal anti-HRP	Jackson ImmunoResearch Labs	Jackson ImmunoResearch Labs Cat# 323-005-021, RRID:AB_2314648
Mouse monoclonal anti-ATP5a	Abcam	Abcam Cat# ab14748, RRID:AB_301447
Goat anti-Mouse Alexa Fluor 488	Invitrogen	Cat# A-11029, RRID:AB_138404
Goat anti-Rabbit Alexa Fluor 568	Invitrogen	Cat# A-11036, RRID:AB_ 10563566
Goat anti-Guinea pig Alexa Fluor 568	Invitrogen	Cat# A-11075, RRID:AB_141954
Peroxidase-AffiniPure Donkey anti-Mouse IgG (H+L)	Jackson ImmunoResearch Labs	Cat# 715-035-151, RRID:AB_2340771
Peroxidase-AffiniPure Donkey anti-Rabbit IgG (H+L)	Jackson ImmunoResearch Labs	Cat# 711-035-152, RRID:AB_10015282
Peroxidase-AffiniPure Donkey anti- Guinea pig IgG (H+L)	Jackson ImmunoResearch Labs	Jackson ImmunoResearch Labs Cat# 706-035-148, RRID:AB_2340447

Bacterial Strains		
Escherichia coli – Rosetta-gami B(DE3)	Novagen	Cat#71137
Chemicals and Recombinant Proteins		
AlexaFluor-568-conjugated phalloidin	Thermo Scientific	Cat# A12380
SlowFade Gold	Thermo Scientific	Cat# S36937
SuperSignal™ West Pico PLUS Chemiluminescent Substrate	Thermo Scientific	Cat# 34580
NativePaGE™ Sample Buffer (4x)	Invitrogen	Cat# BN2003
NativePaGE™ Cathode Buffer (20x)	Invitrogen	Cat# BN2002
NativePaGE™ Running Buffer (20x)	Invitrogen	Cat# BN2001
NativePAGE™ 3-12% Bis-Tris Protein Gels	Invitrogen	Cat# BN2011BX10
Amersham™ Protran® Western blotting membranes, nitrocellulose	GE Healthcare Life Science	Cat# 10600001
Immobilon-P PVDF Membrane	Millipore	Cat# IPVH00010
Protein G Sepharose®, Fast Flow	Sigma-Aldrich	Cat# P3296-5ML
Series 700 halocarbon oil	Halocarbon Products Corp	N/A
Trypan Blue Solution, 0.4%	Thermo Scientific	Cat# 15250061
Complete EDTA-free Protease Inhibitor cocktail	Sigma-Aldrich	Cat# 11873580001
Commercial Assays		
Pierce™ BCA Protein Assay Kit	Thermo Scientific	Cat# 23227
TnT® Quick Coupled Transcription/Translation System	Promega	Cat# L1170
Cell Lines		
Kc167 cell	Drosophila Genomics Resource Center	DGRC Cat# 1, RRID:CVCL_Z834
Organisms/Strains		
OregonR	Bloomington Drosophila Stock Center	BDSC_25211
P{Sgs3-GAL4.PD}	Bloomington Drosophila Stock Center	BDSC_6870
P{w[+mC]=Mhc-GAL4.K}2	Bloomington Drosophila Stock Center	BDSC_55133
P{w[+mW.hs]=GawB}BG487	Bloomington Drosophila Stock Center	BDSC_51634
wash$^{\Delta 185hz(outX)}$	(Verboon et al., 2018)	N/A
P{TRiP.HMC05339}attP40 (Wash RNAi)	Bloomington Drosophila Stock Center	BDSC_62866
7429R-1 (CCDC53$^{RNAi(1)}$)	NIG-FLY	N/A
7429R-6 (CCDC53$^{RNAi(2)}$)	NIG-FLY	N/A
P{TRiP-HMJ22674}attP40 (Strumpellin$^{RNAi(1)}$)	Bloomington Drosophila Stock Center	BDSC_51906
P{KK101498}VIE-260B (Strumpellin$^{RNAi(2)}$)	Vienna Drosophila Resource Center	FlyBase_FBst0472431
P{GD7643}v31840 (SWIP$^{RNAi(1)}$)	Vienna Drosophila Resource Center	FlyBase_FBst0459246

P{KK101329}VIE-260B (SWIP$^{RNAi(2)}$)	Vienna Drosophila Resource Center	FlyBase_FBst0476085
16742R-3 (FAM21$^{RNAi(1)}$)	NIG-FLY	N/A
16742R-2 (FAM21$^{RNAi(2)}$)	NIG-FLY	N/A
P{TRiP-HMC04816}attP40 (LaminB RNAi)	Bloomington Drosophila Stock Center	BDSC_57501
P{TRiP-HMJ21357}attP40 (Arp3 RNAi)	Bloomington Drosophila Stock Center	BDSC_53972
P{KK108277}VIE-260B (Arpc1 RNAi)	Vienna Drosophila Resource Center	FlyBase_FBst0480998
P{TRiP-HMJ22674}attP40 (CpA^{RNAi})	Bloomington Drosophila Stock Center	BDSC_41952
P{TRiP-GLC01716}attP40 (CpB^{RNAi})	Bloomington Drosophila Stock Center	BDSC_50594
P{w^{+}; GFP-Wash^{WT}}	This study	N/A
P{w^{+}; GFP-Wash$^{\Delta SHRC}$}	This study	N/A
P{w^{+}; GFP-Wash$^{\Delta\Delta LamB}$}	This study	N/A
P{w^{+}; GFP-Wash$^{\Delta Arp2/3}$}	This study	N/A
Oligonucleotides		
Wash-F: CTCTGGTACCGTGTCGCTTCTTGCAGTG	This study	N/A
Wash-R: CTCTGGATCCCAAACGTGCCTCCTTCTC	This study	N/A
ΔΔLamin-F1: CTCGTTGCCTCCGCGGCGGCCATCGCCATCGC TGCACCCGCCCGC	This study	N/A
ΔΔLamin-R1: GCGGGCGGGTGCAGCGATGGCGATGGCCGCC GCGGAGGCAACGAG	This study	N/A
ΔΔLamin-F2: GGAATTTCTGGGAGCGCGGCTCCCGTCGCCGC CGCTGCTGGCAATCCC	This study	N/A
ΔΔLamin-R2: GGGATTGCCAGCAGCGGCGGCGACGGGAGCC GCGCTCCCAGAAATTCC	This study	N/A
ΔArp2/3-F: GACGAGGATGGGTCGAACTAGTGAATA	This study	N/A
ΔArp2/3-R: TATTCACTAGTTCGACCCATCCTCGTC	This study	N/A
ΔSHRC-F: CAAAGGTTGAGGACGCAGCCGCTGCTGTGAAG CGCGCT	This study	N/A
ΔSHRC-F: AGCGCGCTTCACAGCAGCGGCTGCGTCCTCAA CCTTTG	This study	N/A
ΔKRAQ-F: TAAACAATCGTGTGGCGGCCGCTGCGGCAAAA ATCGAT	This study	N/A
ΔKRAQ-R: ATCGATTTTTGCCGCAGCGGCCGCCACACGATT GTTTA	This study	N/A
dFz2c-F: TGTCGGATCCTGATGTGGCGACGCTTCTGGCG GAG	This study	N/A

dFz2c-R: GACAGCGGCCGCTCATACGTGGCTGGCCGCCGG	This study	N/A
Recombinant DNA		
pGEX-dt	(Liu et al., 2009)	
pGEX-dt-dFz2C	This study	
Software and Algorithms		
Fiji	(Schindelin et al., 2012)	http://fiji.sc/
R	R Core Team, 2017	https://www.r-project.org/
Other		
LSM 780 confocal microscope with Airyscan	Zeiss	N/A
JEM-1230 transmission electron microscope	JEOL	N/A
Revolution WD spinning disk confocal microscope	Andor	N/A

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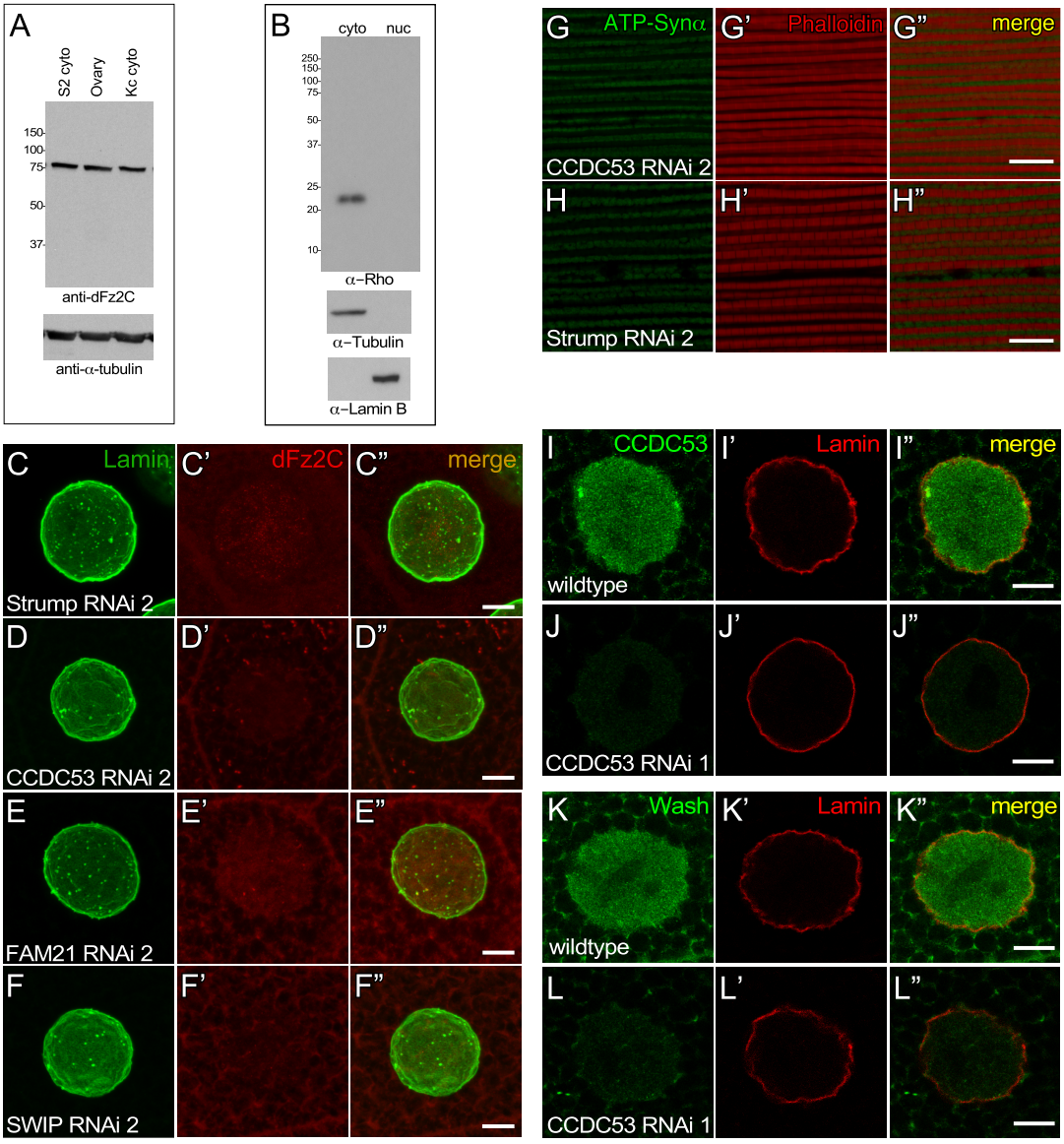

Fig. S1. dFz2C antibody specificity and SHRC mutant characterization. (A) Western blot of S2 cell cytoplasmic, ovary whole cell, and Kc cell cytoplasmic extracts probed with antibodies to dFz2C to show antibody specificity. Loading control is α-Tubulin. (B) Western blot of Kc cell cytoplasmic and nuclear extracts probed with antibodies to Rho1. Blots probed with antibodies to Tubulin and Lamin show cytoplasmic and nuclear purity of extracts. (C-F") Confocal micrograph projections of CCDC53 RNAi 2 (C-C"), Strumpellin RNAi 2 (D-D"), SWIP RNAi 2 (E-E"), and FAM21 RNAi 2 (F-F") larval salivary gland nuclei stained with Lamin B and dFz2C. (G-H") Confocal micrograph projections of adult IFM from CCDC53 RNAi 2 (G-G") and Strumpellin RNAi 2 (H-H") flies aged 21 days then stained with the activity dependent mitochondrial marker ATP-Syn-α and Phalloidin. (I-L") Single slice confocal micrograph from wildtype (I-I", K-K") and CCDC53 RNAi 1 (J-J",L-L") larval salivary gland nuclei co-stained with antibodies to CCDC53 and Lamin B (I-J") or Wash and Lamin B (K-L"). Note that CCDC53 RNAi leads to loss of Wash expression (L-L"). Scale bars: 5 μm in C-F",I-L"; 10 μm in G-H".

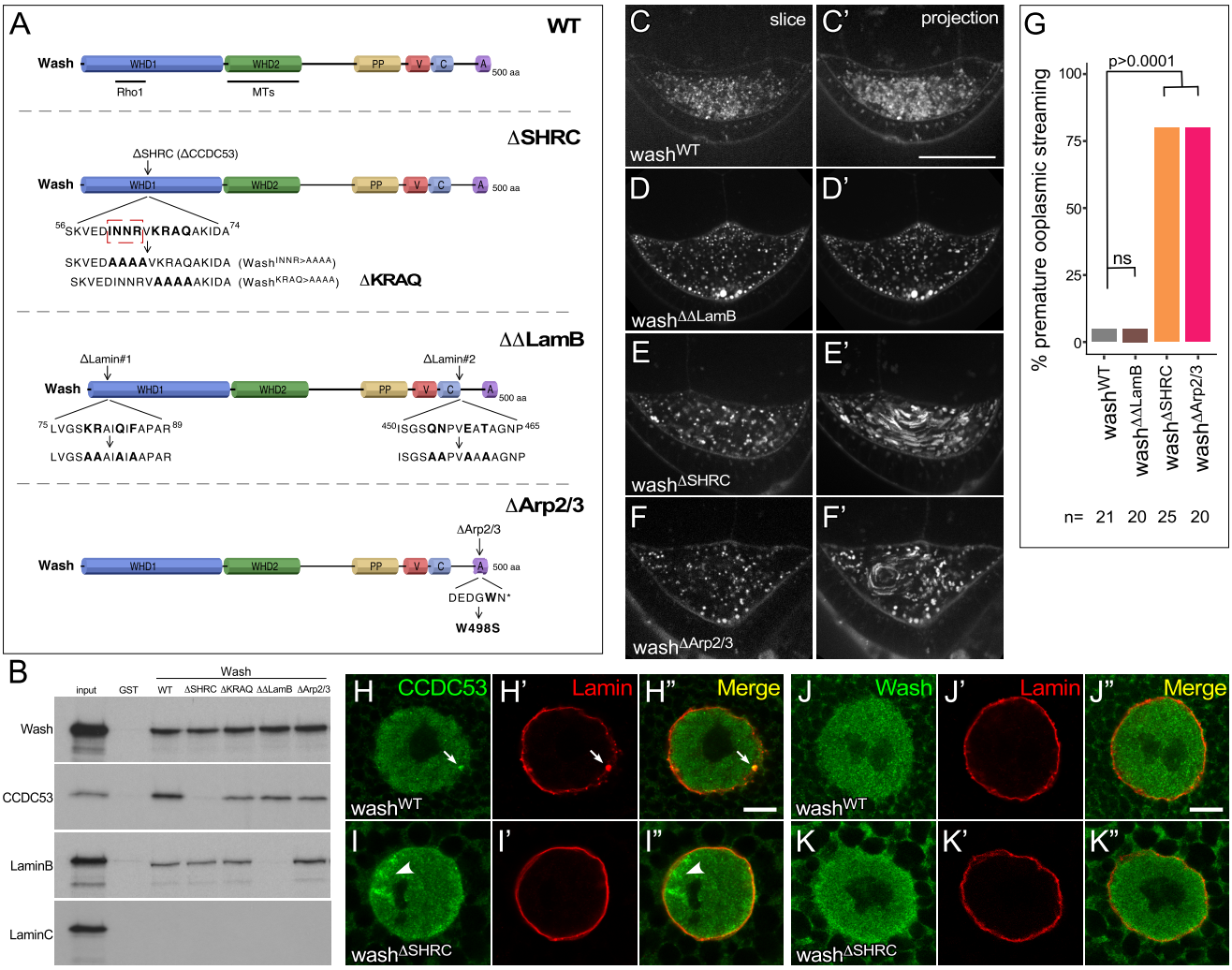

Fig. S2. Wash point mutant characterization. (A) Schematic diagram of the Wash wildtype (WT) rescue and point/substitution mutation constructs used to generate the transgenic lines indicating the position and specific substitution mutations for each construct (not drawn to scale). (B) GST pulldown experiments demonstrating the regions of Wash required for binding to Wash, CCDC53 and Lamin B, as well as the lack of direct binding of Wash by Lamin C. ^{35}S-labeled *in vitro* translated Wash, CCDC53, Lamin B and Lamin C were tested with bacterially purified GST Wash (wildtype or point mutation containing) proteins as indicated. 10% input is shown. (C-F') Single time-point (C-F) and 30 time-point projections (C'-F') of live time-lapse movies of stage 7 oocytes in *wash*^{WT} (C-C'), *wash*$^{\Delta\Delta LamB}$, (D-D'), *wash*$^{\Delta SHRC}$ (E-E'), and *wash*$^{\Delta Arp2/3}$ (F-F'). (G) Quantification of the percentage of stage 7 oocytes exhibiting premature ooplasmic streaming (N= for each genotype indicated on graph). Two-tailed Fishers exact test; p-values indicated. (H-K") Single slice confocal micrograph from *wash*^{WT} (H-H',J-J") and *wash*$^{\Delta SHRC}$ (I-I", K-K") larval salivary gland nucleus co-stained with antibodies to CCDC53 and Lamin B (H-I") or Wash and Lamin B (J-K"). Note CCDC53 enrichment at the nuclear periphery coincident with NE-bud sites (arrow; H-H") that is not observed in *wash*$^{\Delta SHRC}$ mutants (I-I"). Scale bars: 50μm in C-F'; 5μm in H-K".