Figure S1

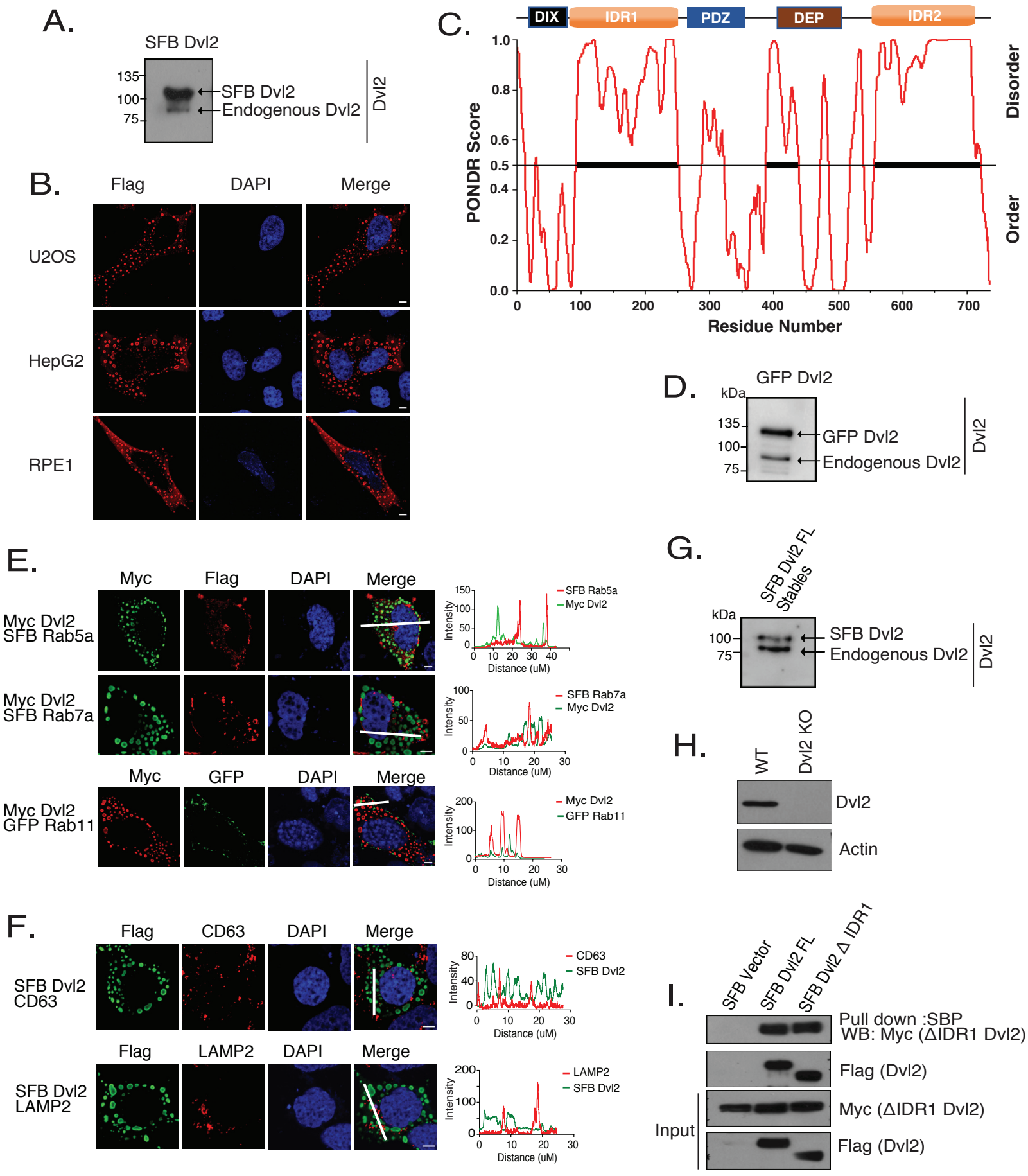
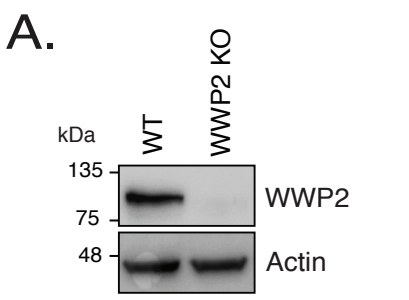
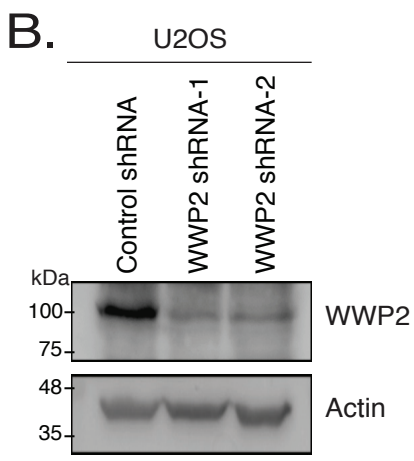
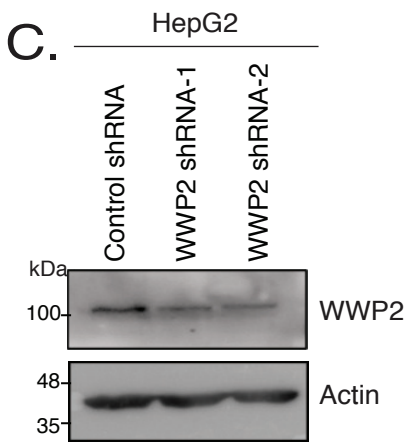


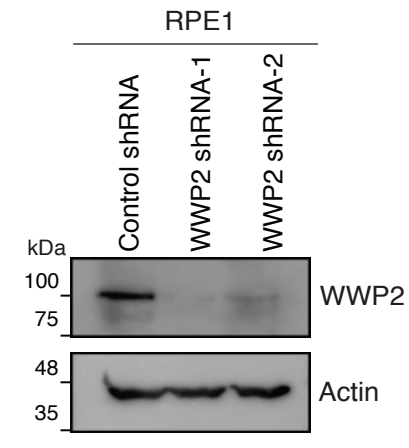
Fig. S1. (a) HEK 293T cells transfected with SFB Dvl2 were lysed and immunoblotted with Dvl2 specific antibody. **(b)** Indicated cell lines transfected with SFB Dvl2 were then fixed and stained with anti-flag antibody. Fixed cells were imaged using a confocal microscope. Scale bar, 5 μM **(c)** The disorder prediction (PONDR) of human Dvl2 showing highly disordered stretches in the N-terminus (IDR1) and C-terminus of the protein (IDR2). **(d)** HEK 293T cells transfected with GFP Dvl2 were lysed and immunoblotted with Dvl2 specific antibody. **(e)** Myc Dvl2 was co transfected with either SFB Rab5a or SFB Rab7a or GFP rab11 in HEK 293T cells. After 24 hours of transfection cells were fixed and stained with indicated antibodies and imaged with a confocal microscope. Fluorescence intensities along the line drawn in a representative cell were obtained using Zen software and the co-localization plots were made using GraphPad. Scale bar,5μM. **(f)** Cells transfected with SFB Dvl2 were fixed and stained by flag antibody along with CD63 or Lamp2 antibodies. Fixed cells were imaged using a confocal microscope. Fluorescence intensities along the line drawn in a representative cell were obtained using Zen software and the co-localization plots were made using GraphPad. Scale bar,5μm. **(g)** HEK 293T cells stably expressing SFB-Dvl2 at near endogenous levels were lysed and immunoblotted with Dvl2 antibody. **(h)** HEK 293T cells transfected with control guide RNA or Dvl2 guide RNA were cultured in puromycin selection media. Dvl2 deletion was confirmed by immunoblotting with Dvl2 antibody. **(i)** Cells were transfected with SFB DVL2 FL or ΔIDR mutant along with Myc ΔIDR Dvl2. Oligomerization of ΔIDR Dvl2 with full length as well as ΔIDR Dvl2 was detected by immunoblotting with anti-myc antibody after incubating the cell lysates with streptavidin sepharose beads.

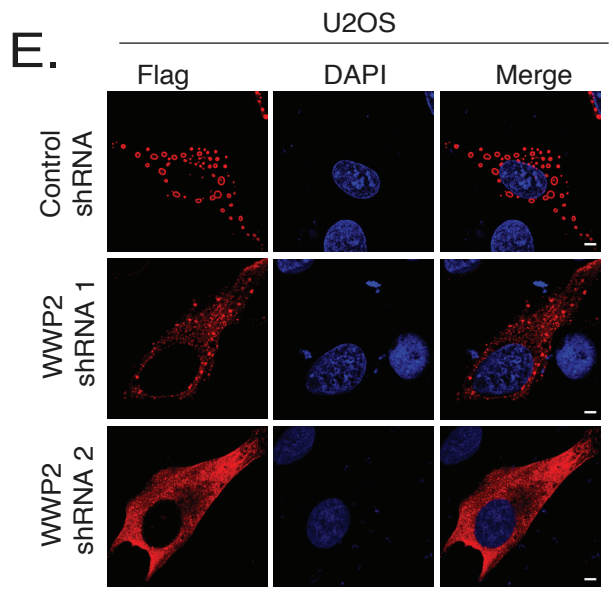
Figure S2

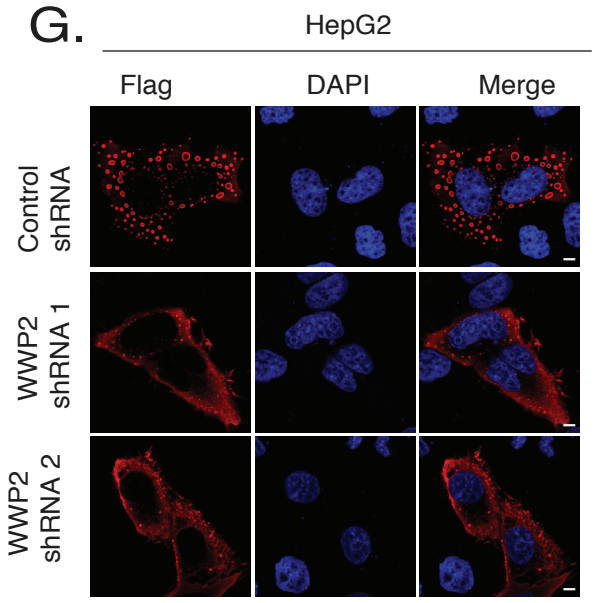


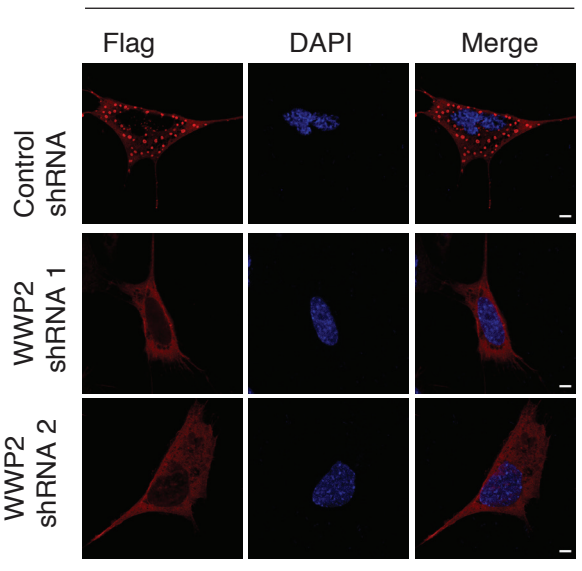


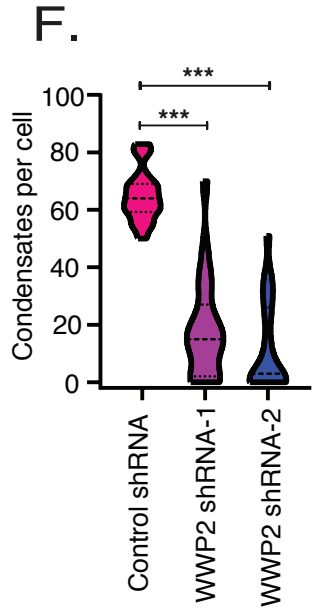


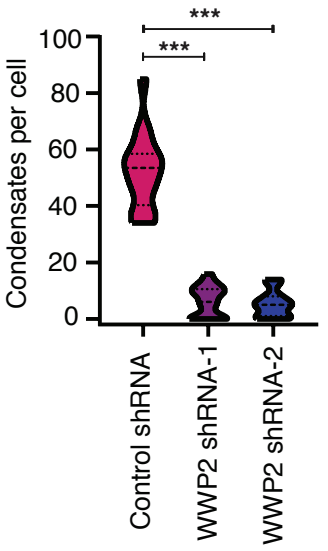












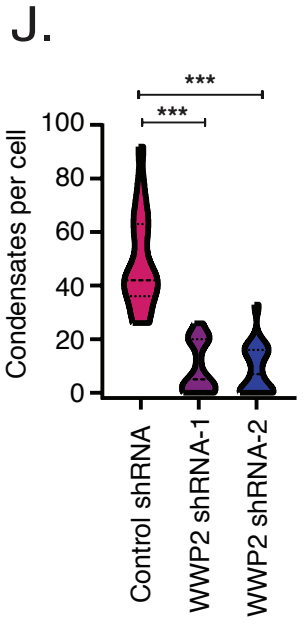
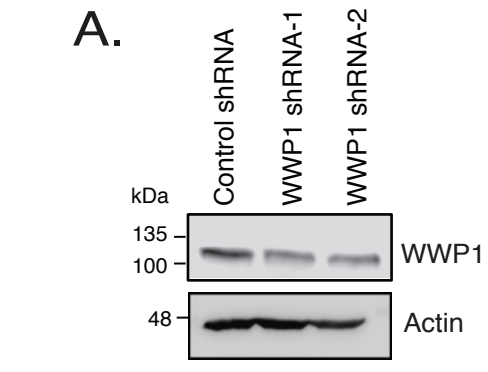
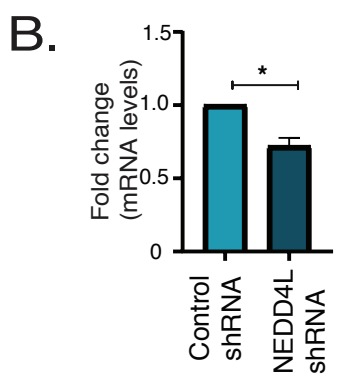
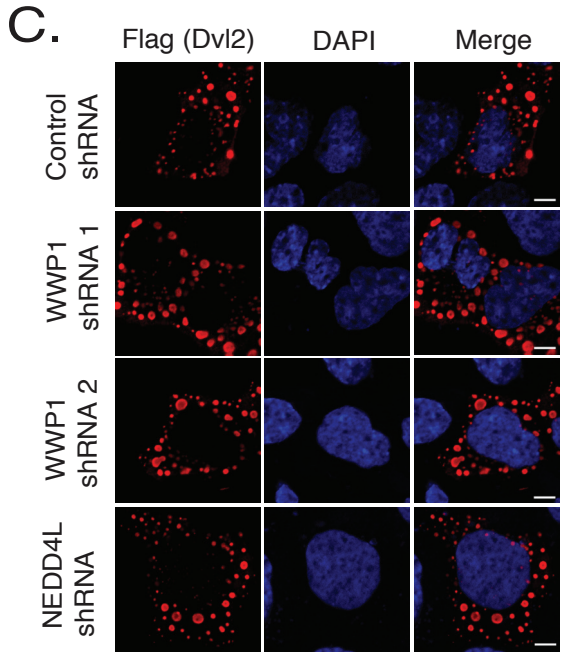


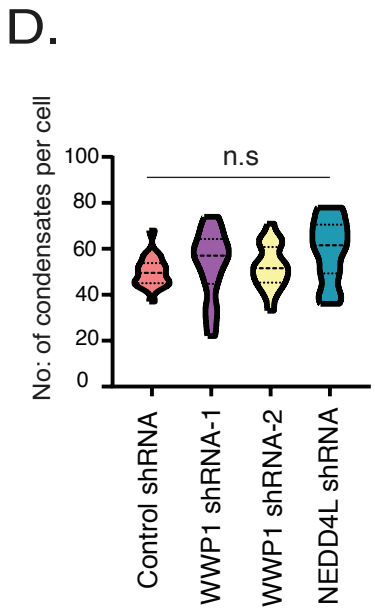
Fig. S2. (a) HEK 293T cells transfected with control guide RNA or WWP2 guide RNA were cultured in puromycin selection media. WWP2 deletion was confirmed by immunoblotting with specific antibody. (b) U2OS cells (c) HepG2 cells and (d) RPE1 cells transduced with either control or two independent WWP2 shRNAs were cultured in puromycin selection media. WWP2 depletion was confirmed by immunoblotting with specific antibody. (e) Control and WWP2 depleted U2OS cells were transfected with SFB Dvl2 for 24 h. Cells were fixed and stained with anti-flag antibody. Images were taken using a confocal microscopy. Scale bar 5µm. (f) Quantification of number of condensates per cell for the indicated conditions was plotted. Solid line represents the median, n=20 *** P<0.001(One-way Anova, Tukey's multiple comparisons test. (g) Control and WWP2 depleted HepG2 cells were transfected with SFB Dvl2 for 24 h. Cells were fixed and stained with anti-flag antibody. Images were taken using a confocal microscopy. Scale bar 5µm. (h) Quantification of number of condensates per cell for the indicated conditions was plotted. Solid line represents the median, n=20 *** P<0.001(One-way Anova, Tukey's multiple comparisons test. (i) Control and WWP2 Knock down RPE1 cells were transfected with SFB Dvl2 for 24 h. Fixed cells were stained with anti-flag antibody and imaged using a confocal microscope. Scale bar 5µm. (j) Quantification of number of condensates per cell for the indicated conditions was plotted. Solid line represents the median, n=20 *** P<0.001(One-way Anova, Tukey's multiple comparisons test.

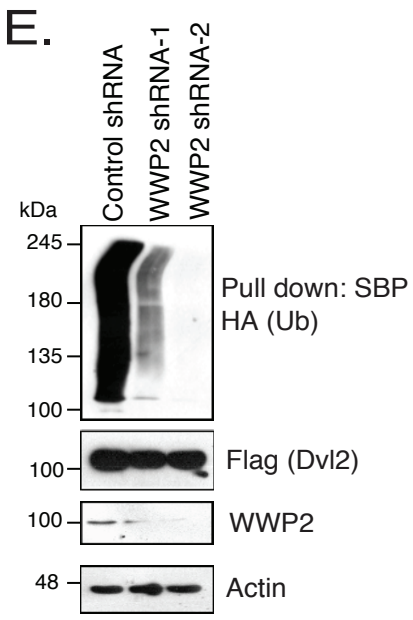
Figure S3

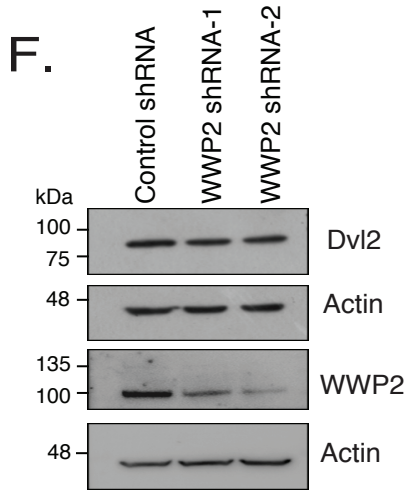


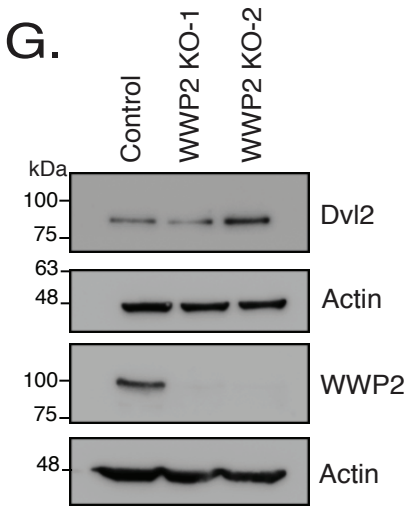












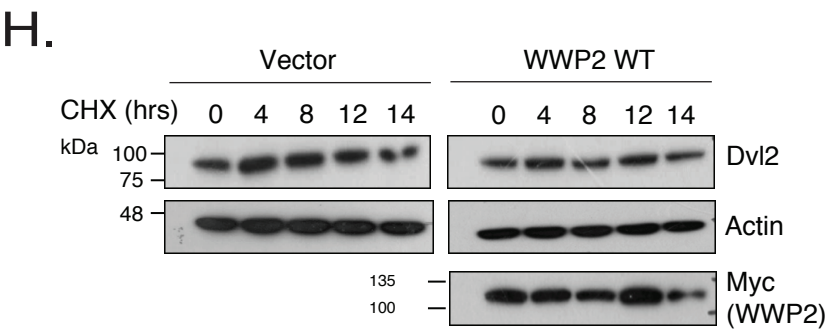


Fig. S3. (a) Cells transduced with control or two independent WWP1 shRNAs were collected after 48h of transduction. Depletion of WWP1 was confirmed by immunoblotting with specific antibody. (b) 293T cells were transduced with either control or Nedd4L shRNA and the Nedd4L depletion was detected using qRT- PCR. (c) Cells transduced with control or indicated shRNAs were transfected with SFB Dvl2. 24h of post transfection, cells were fixed and the condensates were imaged by probing with flag antibody. Images were taken with a confocal microscope, Scale bar 5µm. (d) Quantification of percentage of cells forming condensates, n=100 cells, *p< 0.03 (One-way Anova, Tukey's multiple comparisons test). (e) Cells expressing control or two independent WWP2 shRNAs were co-transfected with SFB Dvl2 and HA Ub for 24 hours. Cells were then lysed in denaturing conditions and the lysate was incubated with streptavidin sepharose beads. Dvl2 Ubiquitination was detected by immunoblotting with HA antibody. (f) Cells transduced with control or WWP2 shRNAs were collected after 48h of transduction. Lysates were tested for the total levels of Dvl2 by immunoblotting with Dvl2 antibody. (g) Wild type or WWP2 KO cells were lysed and tested for the total levels of Dvl2 by immunoblotting with Dvl2 antibody. (h) Empty vector or Myc Wwp2 was transfected in HEK 293T cells. 24hours of post transfection cells were treated with cycloheximide (50µg/mL). Cells were harvested at different time points and Dvl2 levels were detected by immunoblotting.

Figure S4

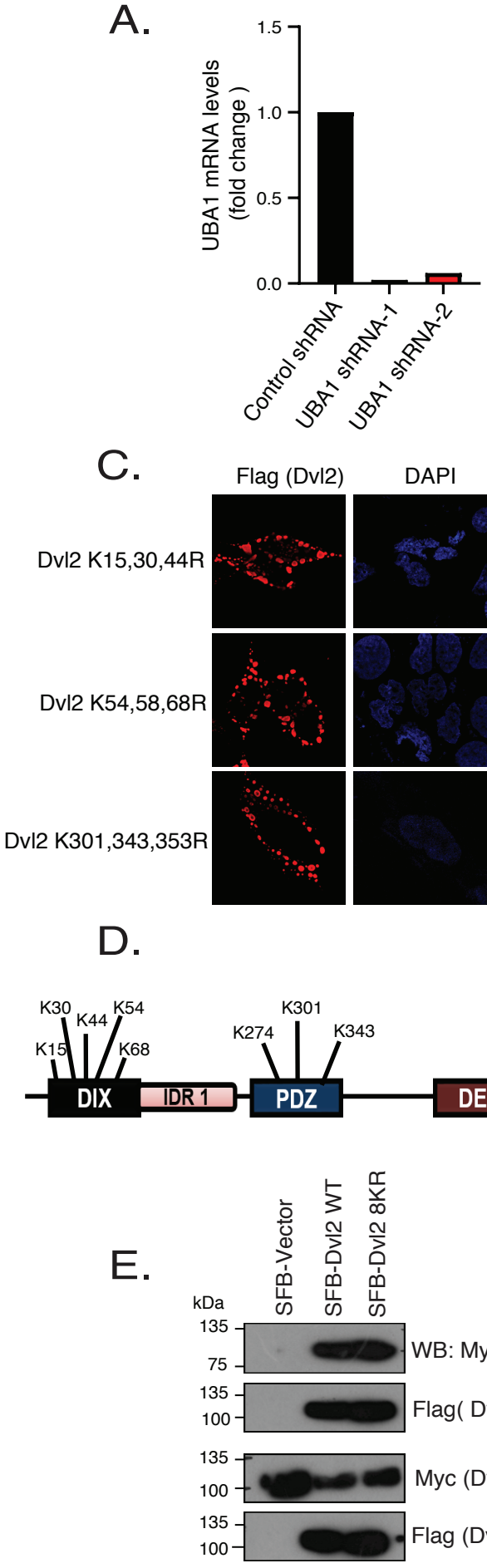
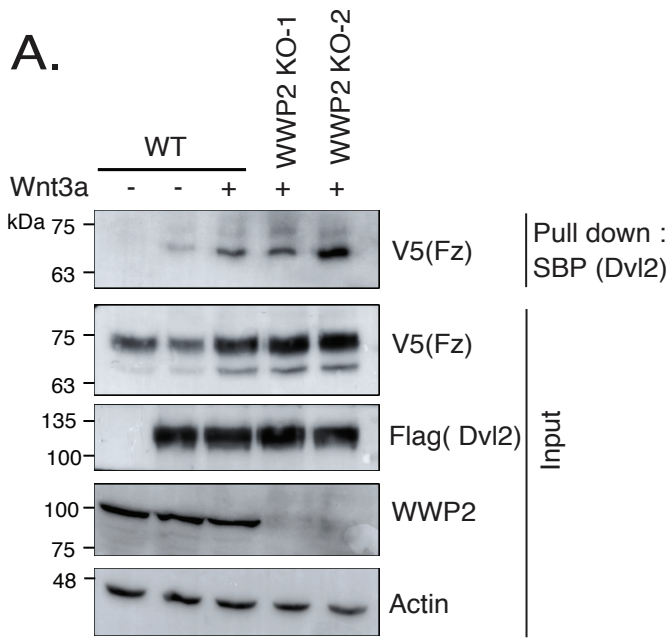
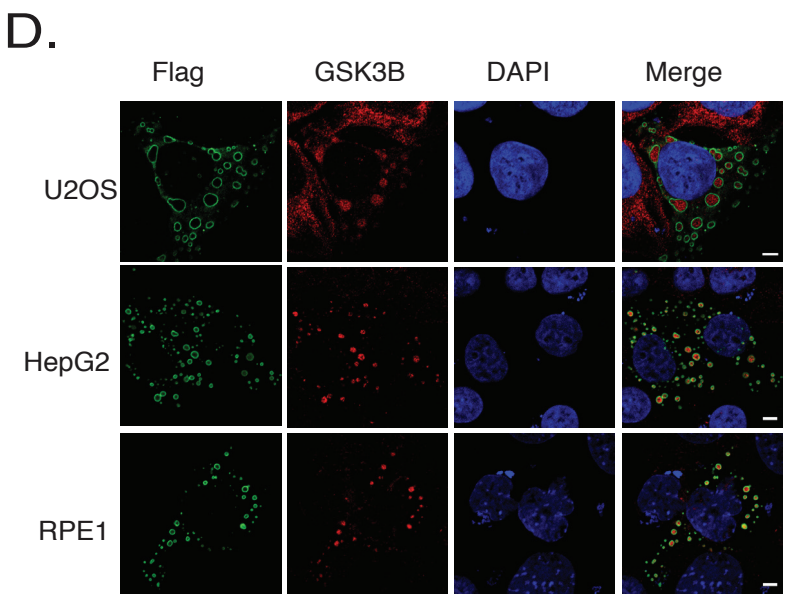
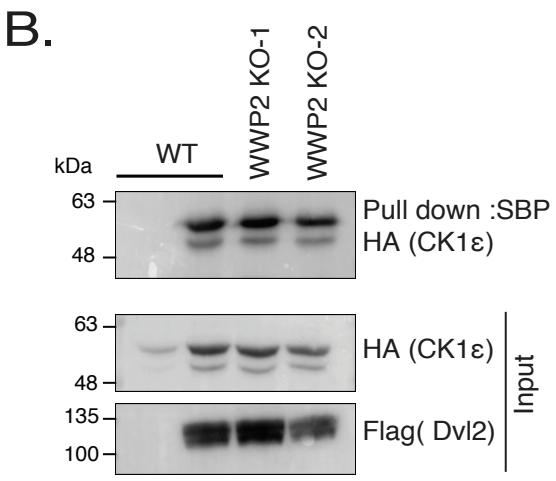


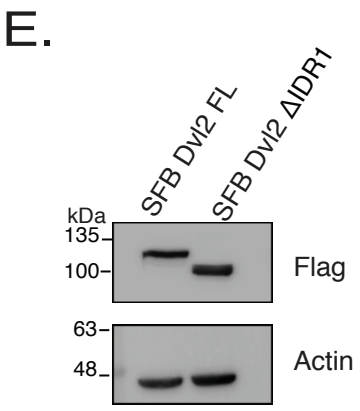
Fig. S4. (a) 293T cells were transduced with either control or two independent UBA1 shRNAs and the level of UBA1 depletion was detected by using qRT- PCR. (b) SFB tagged DVL2 with indicated single lysine mutants were transfected in HEK 293T cells. Dvl2 condensates were captured by imaging with confocal microscope after immunofluorescence staining with Flag antibody. (c) Cells were transfected with SFB tagged Dvl2 with various triple lysine mutants. Dvl2 condensates were captured by imaging with confocal microscope after immunofluorescence staining with Flag antibody. (d) Schematic representation of Dvl2 full length protein with various lysines that are potential sites of WWP2 mediated ubiquitination. (e) Cells were transfected with SFB DVL2 WT or 8KR mutant along with Myc Dvl2. Oligomerization of SFB Dvl2 with Myc Dvl2 was detected by immunoblotting with anti-myc antibody after incubating the cell lysates with streptavidin sepharose beads.

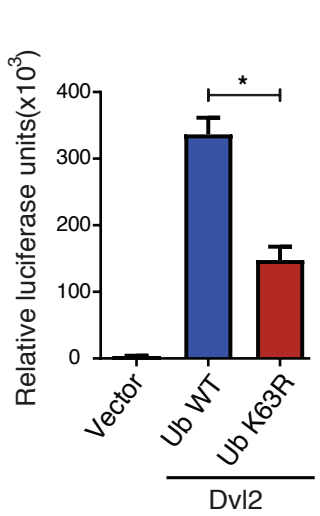
Figure S5

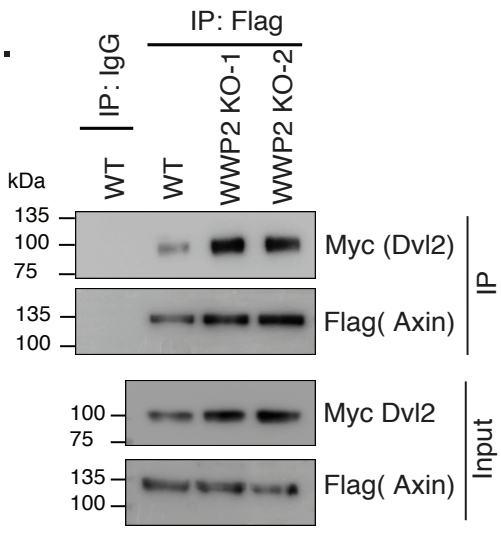


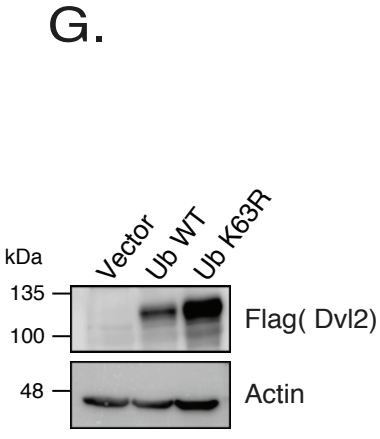












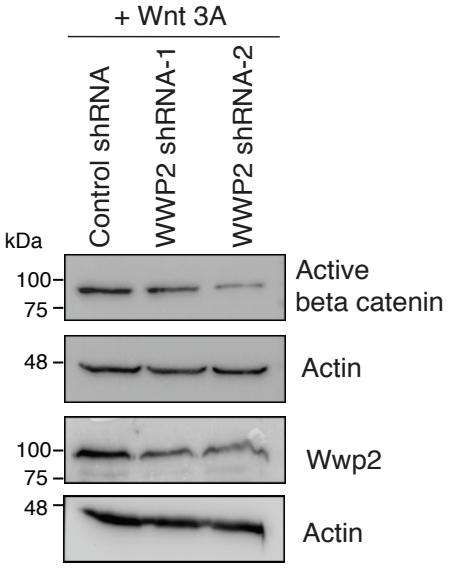


Fig. S5. (a) Wild type (WT) or WWP2 KO cells were co-transfected with SFB-Dvl2 and V5-Fz (Frizzled 5) in the presence and absence of Wnt3a. The association between SFB Dvl2 and V5-Fz were detected by immunoblotting with anti-V5 antibody after incubating the cell lysates with streptavidin sepharose beads. (b) SFB Dvl2 and HA CK1ε were co-transfected in WT and WWP2 KO cells. Cells were then lysed and subjected to streptavidin sepharose pull down and immunoblotted with anti-HA antibody to detect Dvl2-CK1 interaction. (c) WT and WWP2 KO cells were transfected with Myc Dvl2 and Flag Axin. Cells were then lysed and incubated with either IgG or flag antibody bound protein G agarose beads. The interaction of Flag Axin and Myc Dvl2 was detected by immunoblotting with anti-myc antibody. (d) Indicated cells transfected with SFB DVL2 were then fixed and stained with anti-flag and anti -GSK3β antibodies. Fixed cells were imaged under a confocal microscope. Scale bar, 5μM. (e) HEK 293T cells expressing SFB Dvl2 FL and ΔIDR were lysed and immunoblotted with anti-flag antibody (f) HEK 293T cells were co-transfected with SFB Dvl2 and HA Ub-WT or K63R mutant along with TOP FLASH reporter plasmid and Renilla luciferase reporter plasmid. 24h of post transfection, cells were lysed and luciferase assay was performed with Dual Luciferase Kit (Promega). The values were normalized to Renilla luciferase. Error bar indicates standard deviation, *P< 0.05 (One-way Anova, Tukey's multiple comparisons test). (g) Expression levels of SFB Dvl2 in the lysate used for luciferase assay after expressing Ub WT or K63R mutant was detected by immuno blotting with anti-Flag antibody. (h) Cells transduced with control or WWP2 shRNAs for 48h were then treated with Wnt3a ligand for 2h. Cells were lysed in NETN lysis buffer and immunoblotted with non-phospho beta catenin (active beta catenin) antibody and other indicated antibodies.

Table S1. List of Dvl2 associated proteins identified by mass spectrometric analysis after biochemical purification was shown. Proteins with cut off of 3 unique peptides were included in the list.

Protein	Unique peptides
DVL2	89
AP1B1	64
AP2A2	52
AP2B1	46
AP2A1	42
USP7	36
MCM5	35
AP2M1	33
MCM3	33
ATAD3A	32
FOXK1	31
NEDD4L	29
NXN	28
ITCH	27
FOXK2	25
PLEKHA5	22
MCM7	22
PKP3	22
DVL3	20
DVL1P1	19
CSNK1D	19
TAF6L	19
CSNK2A1	18
TRIM28	18
PLK1	18
SLC25A13	18
CSNK1A1	17
VANGL1	17
BAG3	17
WWP2	17
CSNK2A2	16
SNW1	16
NDUFA9	15
MCCC2	14
AAK1	14
SMC2	14
RPL23	13
PRDX1	13
POLD1	13
PLD1	13
CSNK2B	12
GID8	12
CHEK2	12

ATAD3B	12
NUMB	11
TUFM	11
POMGNT2	11
HADHB	11
AIFM1	11
NUMBL	11
CSNK1A1L	10
FKBP8	10
VANGL2	10
SNX8	10
IRS2	10
ARRB2	10
ACAD11	10
NECAP1	9
GTF3C5	9
RFC2	9
PDK3	9
CTNND1	9
AP2S1	9
MARK2	9
PPP2R1A	9
SNIP1	8
ARMC8	8
WWP1	8
SNX9	8
MCCC1	8
KCTD12	8
ABCD3	8
MARK3	8
AP1G1	8
CDK1	8
PFKP	8
AXIN1	8
EEF2	8
PARD3	8
MAEA	8
PRDX2	8
LEMD3	7
SENP2	7
IQGAP1	7
STRN	7
MKLN1	7
RANBP10	7
EPS15L1	7
MARK1	7

PHB2	7
EPS15	7
ARAF	7
TCP1	7
VIM	7
UBA52	6
CSNK1E	6
PCBP1	6
PRDX4	6
YWHAQ	6
ATP5A1	6
TYK2	6
KIF2C	6
CCNB1	6
PGAM5	6
GSK3B	6
RAB11FIP5	6
SMC4	6
PPP1CA	6
IQGAP3	6
CCT2	6
PKM	6
SH3BP4	6
DVL1	5
CNP	5
NFKBIL1	5
TCAF1	5
RANBP9	5
YWHAE	5
TRIM33	5
STX5	5
PIP4K2C	5
SLMAP	5
AURKA	5
BUB3	5
KIF2A	5
MTHFD1	5
EGLN1	4
NECAP2	4
SH2D4A	4
DHRS2	4
CCAR2	4
ANXA2	4
WDR48	4
GULP1	4
RFC4	4

SGPL1	4
DLG3	4
PIK3R4	4
PRPF19	4
STON2	4
ZNF598	4
TK1	4
PPP2R2A	4
NAGK	4
MAPK1	4
FAM83B	4
MRE11	4
CDC7	4
MTERF1	3
PDZD11	3
TANC1	3
KANSL2	3
METTL15	3
YWHAB	3
PELI2	3
YWHAG	3
POLDIP3	3
ARF3	3
CEP78	3
TTK	3