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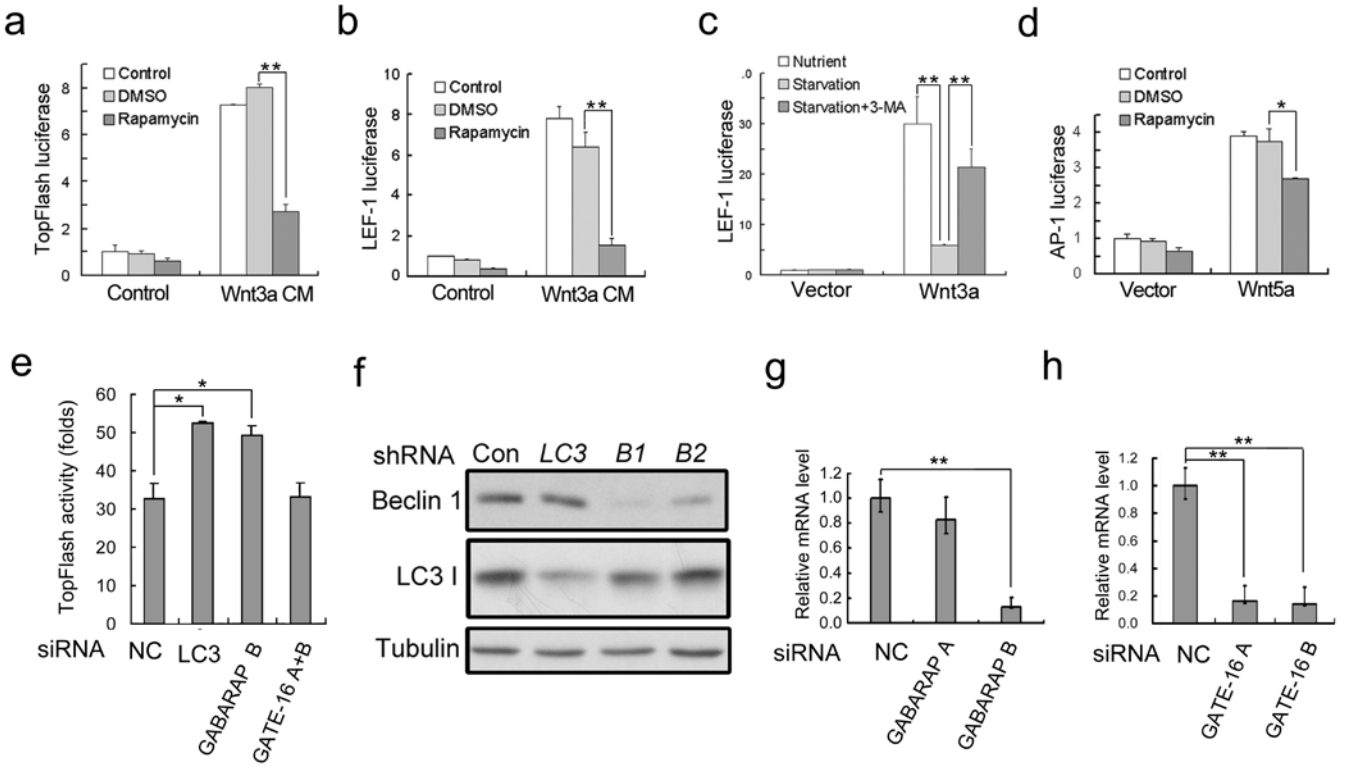

Figure S1 Autophagy attenuates the expression of TopFlash or AP-1 reporter induced by Wnt3a or Wnt5a. (**a, b**) HEK293T cells transfected with TopFlash or LEF-1 luciferase reporter were maintained in control medium or Wnt3a condition medium (CM) overnight, and then treated with DMSO or rapamycin (2μM). After 2h treatment, luciferase activity was measured. The pRL-tk Renilla reporter (5ng) was co-transfected to normalize transfection efficiency. (**c**) HEK293T cells transfected with LEF-1 luciferase reporter with or without Wnt3a were in HBSS solution (starvation) in the absence or presence of 10 mM 3-MA for 2 h, and then luciferase activity was measured. (**d**) HeLa cells transfected with AP-1 luciferase reporter or co-transfected with Wnt5a were treated with DMSO or rapamycin for 2 h, and then luciferase activity was measured. (**e**) Knockdown of LC3 or GABARAP, but not GATE-16 potentiated Wnt signaling. HEK293T cells were transfected with LC3 siRNA, GABARAP siRNA (B), or two GATE-16 siRNA (A, B). At 24 h post-transfection, the cells were transfected with TopFlash reporter and then were maintained in control medium or Wnt3a condition medium (CM) overnight. Then cells were harvested and subjected to luciferase activity assay. (**f**) Efficiency of shRNA-mediated downregulation of LC3 and Beclin 1. HEK293T cells were transfected with LC3 shRNA or two Beclin 1 shRNAs (B1, B2). The cell lysates were subjected to anti-Beclin or anti-LC3 immunoblotting. Tubulin served as a loading control. (**g, h**) Efficiency of siRNA-mediated downregulation of LC3, GABARAP and GATE-16. Reporter assays were performed in triplicate and the data represent the mean ± S.D. after normalized to Renilla activity. All the other data are also presented as the mean ± S.D. (n=3). The asterisks indicate a statistically significant difference (* $p<0.05$; ** $p<0.01$).

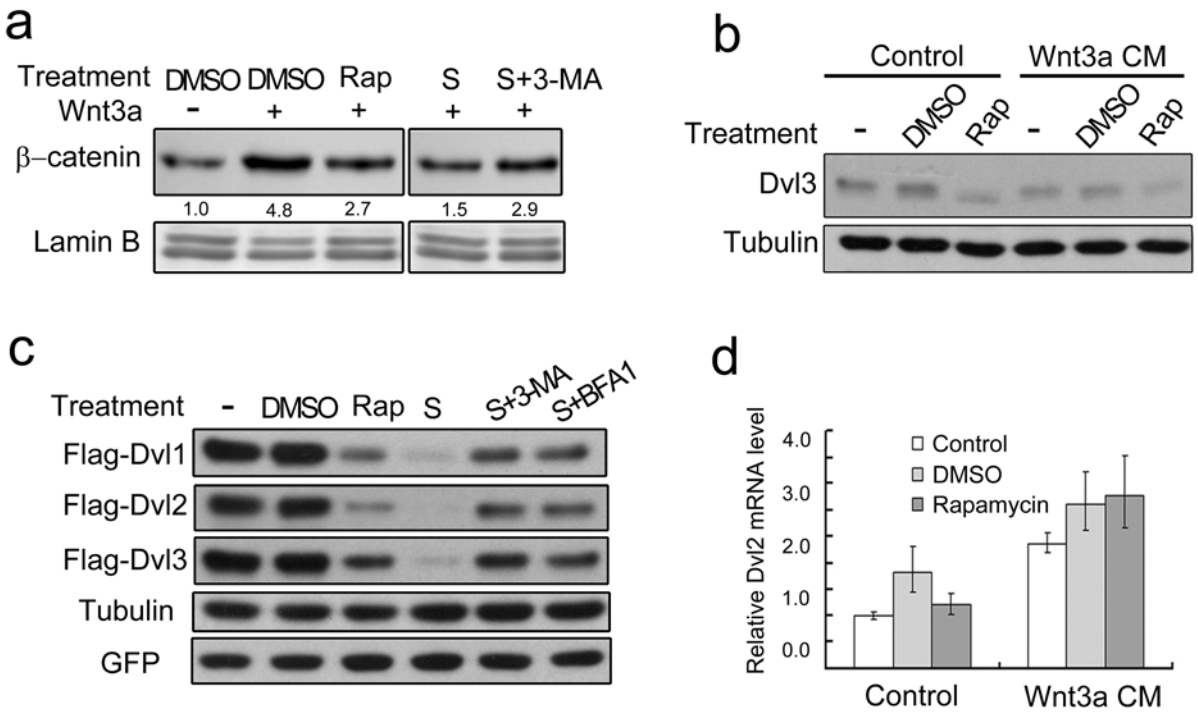

Figure S2 Dvl undergoes autophagy-mediated degradation. (**a**) Effect of autophagy on Wnt3a-induced nuclear accumulation of β-catenin. HEK293T cells transfected with Wnt3a (0.5 μg) were treated with 2 μM rapamycin or starved for 4h with or without 10 mM 3-MA. Then the cell nuclei were harvested for immunoblotting analysis of the endogenous β-catenin protein. Lamin B served as a loading control. (**b**) Rapamycin treatment promoted turnover of endogenous Dvl3 protein. HEK293T cells maintained in complete medium were treated with DMSO or 2 μM rapamycin. Then, cells were harvested for immunoblotting to detect endogenous Dvl3. Tubulin served as a loading control. (**c**) Autophagy induced degradation of ectopically expressed Dvl1, Dvl2, and Dvl3. HEK293T cells transfected with human Flag-Dvl1, -Dvl2, or -Dvl3 were treated with DMSO or 2 μM rapamycin, or placed in DMEM or HBSS solution (starvation, S) for 4h in the absence or presence of 3-MA or BFA1. Then, cells were harvested for anti-Flag immunoblotting. Tubulin served as a loading control. (**d**) Rapamycin had no effect on Dvl2 mRNA levels shown by quantitative RT-PCR. Total RNA was extracted from HEK293T cells treated with DMSO or 2 μM rapamycin for 4 h and subjected to q-RT-PCR. The results are shown as means ± S.D. (n=3).

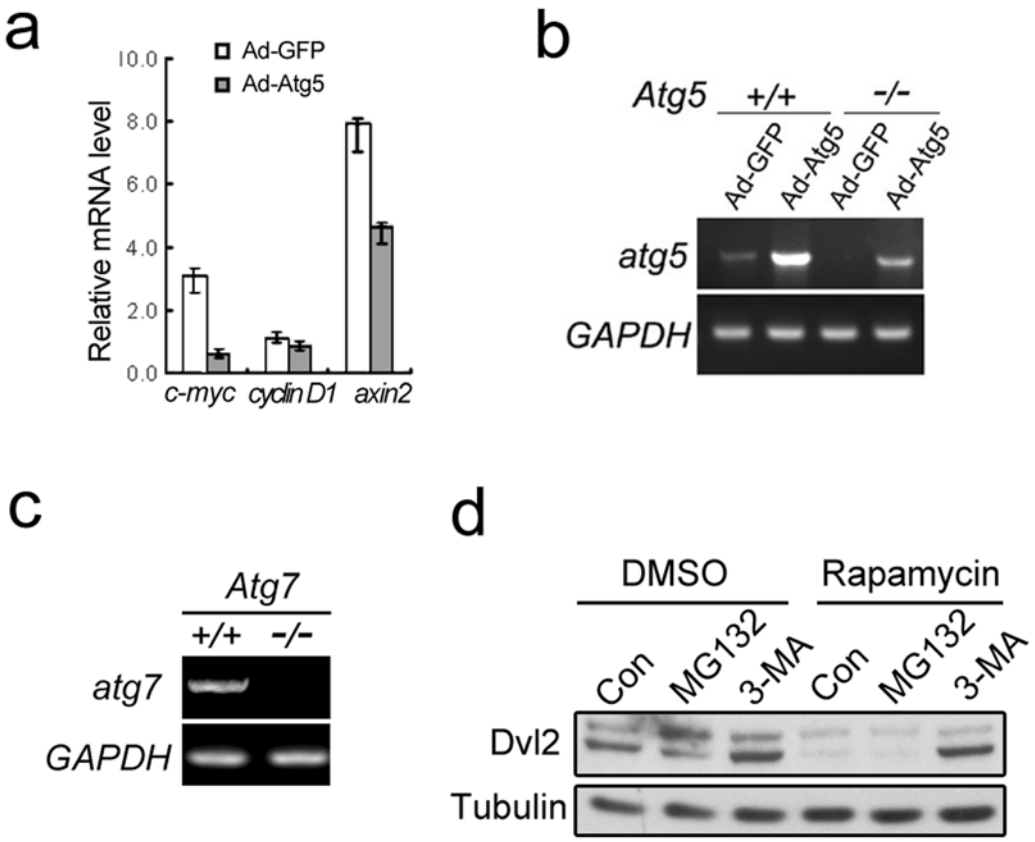

Figure S3 (**a**) Re-introduction of Atg5 in *atg5*$^{-/-}$ cells repressed the induction of Wnt target genes. GFP or Atg5 was re-introduction in *atg5*$^{-/-}$ MEFs, and the cells were then treated with control or Wnt3a condition medium. Total RNA was harvested for q-PCR. The bars indicated the induction folds of target genes under Wnt stimulation. Each experiment was performed in triplicate and the data represent the mean ± S.D. (n=3). (**b**) Atg5 expression was confirmed by PCR. (**c**) Atg7 expression was examined by PCR. (**d**) HEK293T cells were treated with MG132 (1μM) or 3-MA (10 mM) in the absence or presence of rapamycin (2μM) for 4 h, followed by anti-Dvl2 immunoblotting to detect endogenous Dvl2 protein level.

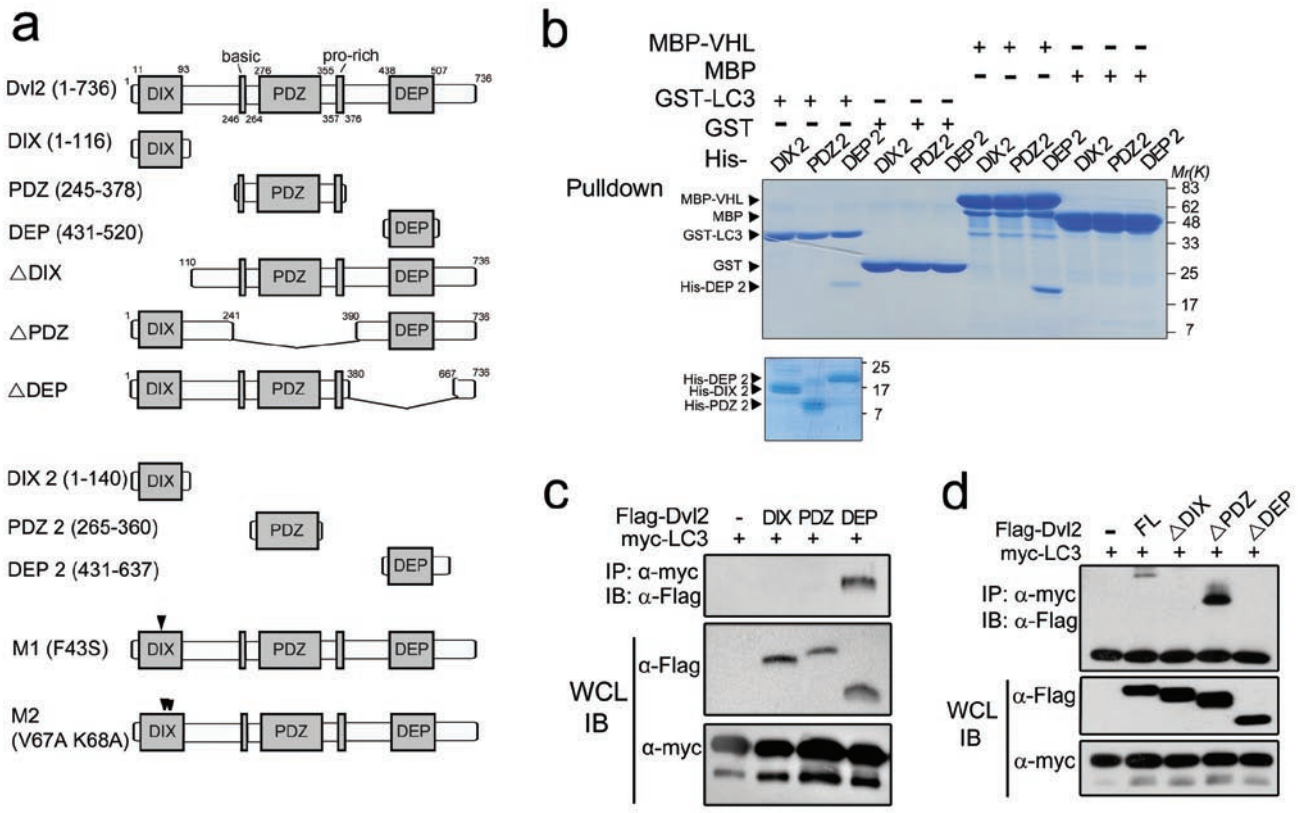

Figure S4 The DEP domain of Dvl2 directly interacts with LC3. (**a**) A schematic representation of wild-type mouse Dvl2 and different domains, deletions or point mutations used in this study. (**b**) Pull-down assay using various recombinant Dvl2 domains and immobilized GST, GST-LC3, MBP, or MBP-VHL. Proteins were detected by Coomassie staining. (**c, d**) Interaction between LC3 and wild-type or various mutants Dvl2. After co-transfected with myc-LC3 and Flag-Dvl2 variants as indicated, HEK293T cells were harvested for anti-myc immunoprecipitation followed by anti-Flag immunoblotting. Total protein expression was confirmed by immunoblotting with whole cell lysates (WCL).

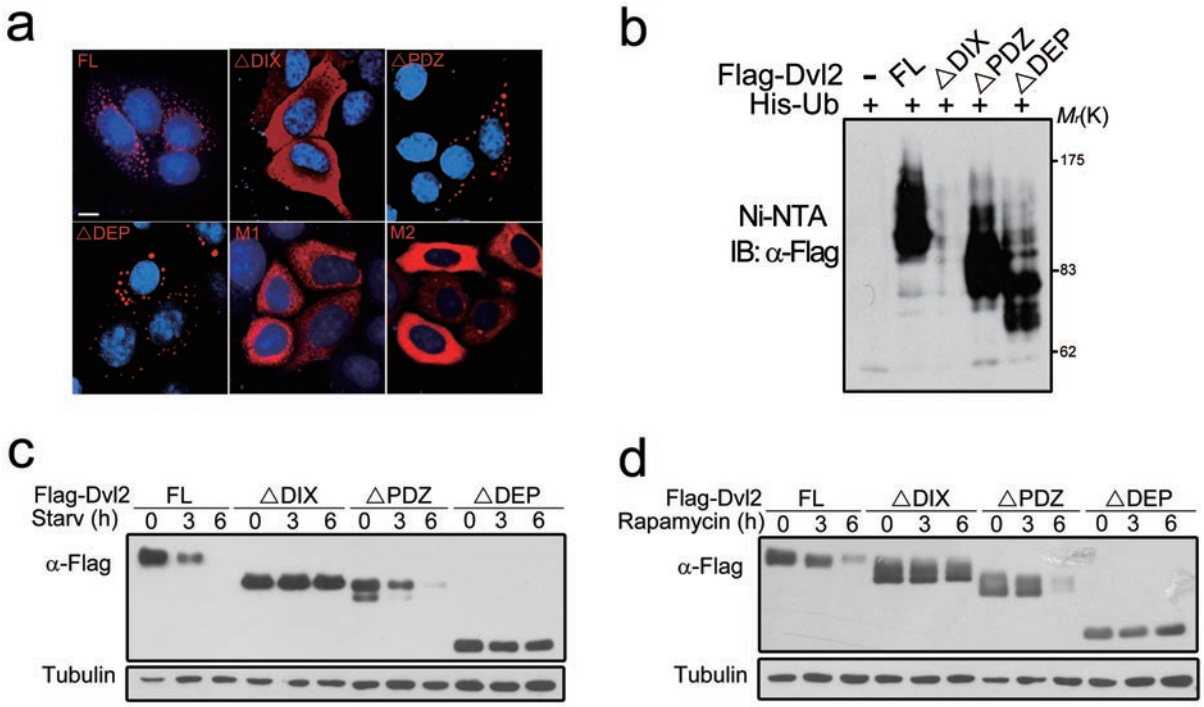

Figure S5 The DIX and DEP domains of Dvl2 are important for autophagy-induced Dvl2 degradation. (**a**) HeLa cells transfected with Flag-tagged full length (FL) and various mutant Dvl2 were processed for immunofluorescence. The nuclei were counter-stained with DAPI (blue). Scale bar: 10μm. (**b**) Ubiquitination of full-length Dvl2 and its variants. HEK293 cells transfected with His-tagged ubiquitin and Flag-tagged Dvl2 variants were harvested for Ni-NTA beads precipitation followed by anti-Flag immunoblotting. (**c, d**) Degradation kinetics of Dvl2 full-length and variants under starvation (**c**) or rapamycin treatment (**d**). HEK293T cells were transfected with the plasmids as indicated. At 36 h post-transfection, cells were washed three times with PBS, and subjected to starvation with HBSS or 2μM rapamycin treatment in the presence of 20mM cycloheximide. At a various time points, cells were harvested for anti-Flag immunoblotting. Total protein expression was detected by immunoblotting. Tubulin served as a loading control.

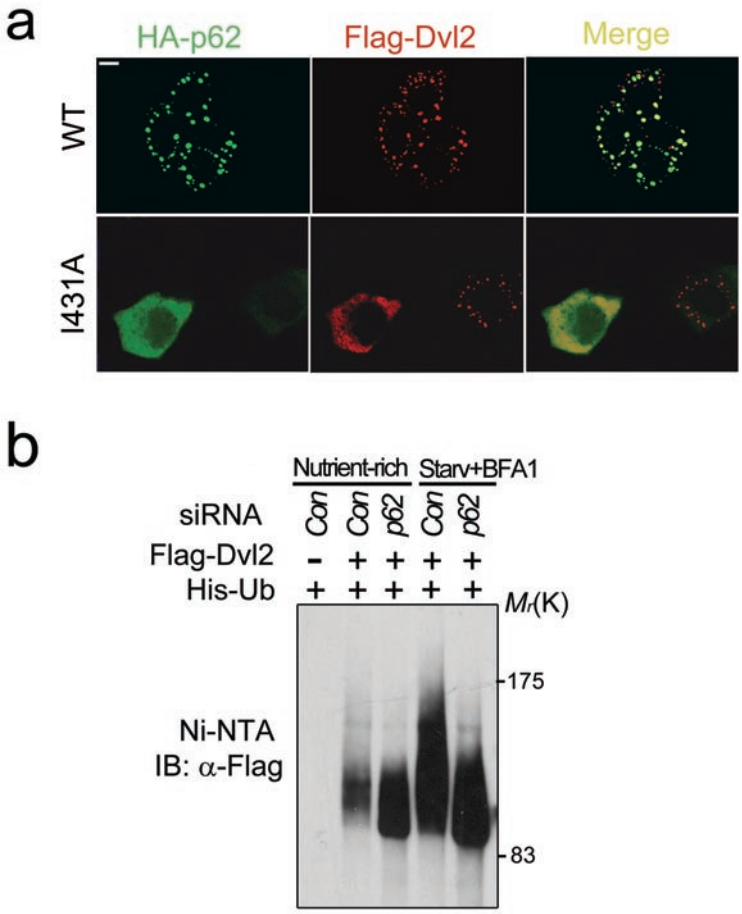

Figure S6 p62 promotes Dvl2 aggregation. (**a**) Immunofluorescence of HeLa cells transfected with Flag-Dvl2 and wild-type or I431A mutant HA-p62. At 36 h post-transfection, the cells were starved for 4 h in the presence of BFA1. Proteins visualized by immunofluorescence with anti-HA (green) and anti-Flag (red) antibodies. Scale bar: 10μm. (**b**) Ubiquitination of Dvl2 in p62-knockdown cells under normal or starvation condition. HEK293T cells transfected with His-tagged ubiquitin and Flag-tagged Dvl2 with control or p62 siRNA. After 36 h post-transfection, the cells were cultured under normal or starvation condition with BFA1 for 4 h, and then harvested in GTN buffer for Ni-NTA beads precipitation, followed by anti-Flag immunoblotting.

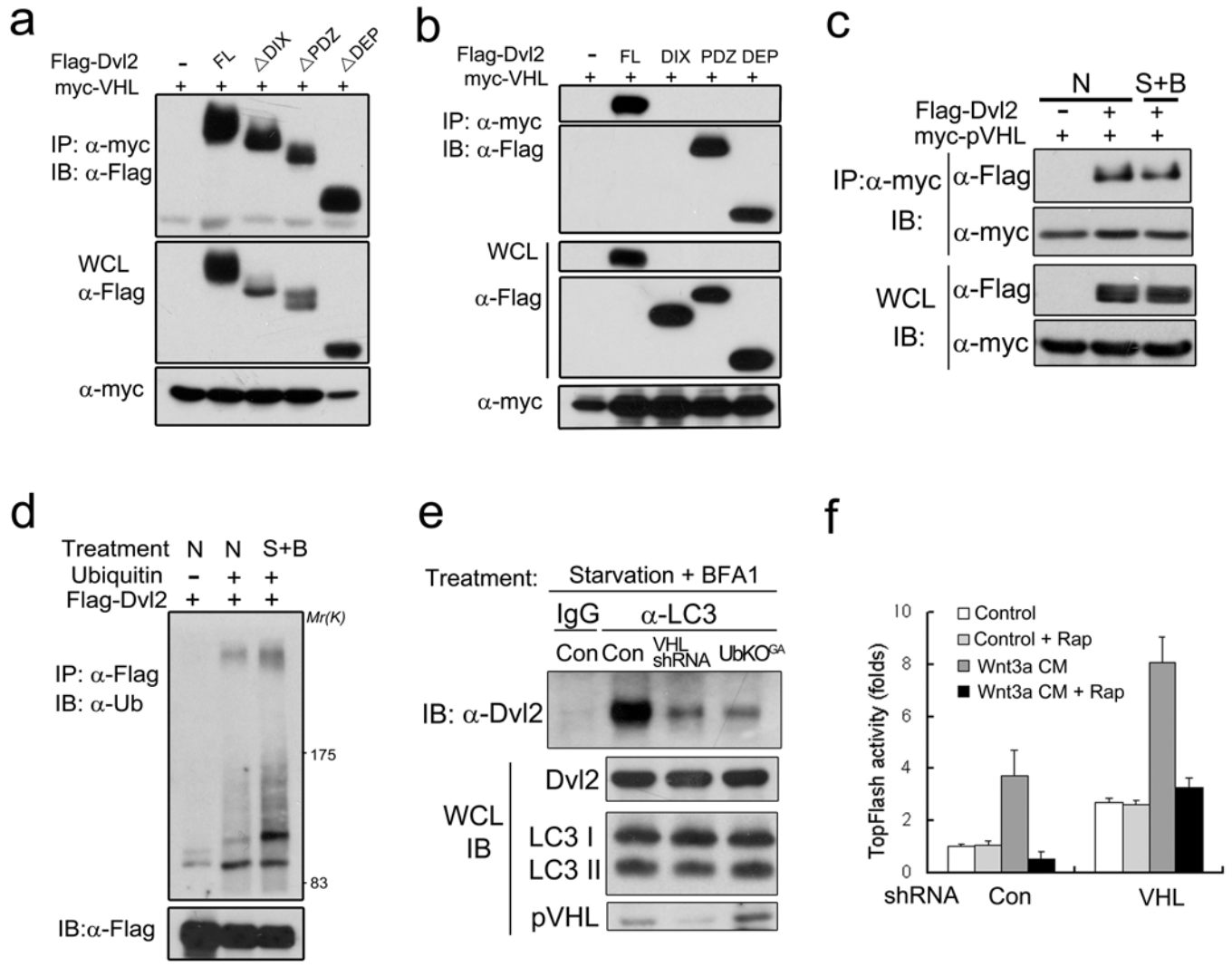

Figure S7 (**a, b**) Interaction between pVHL and Dvl2 mutants. After transfected with myc-VHL together with various mutants of Flag-Dvl2 for 36 h, HEK293T cells were harvested for anti-myc immunoprecipitation followed by anti-Flag immunoblotting. Total protein expression was confirmed by immunoblotting with whole cell lysates (WCL) (lower panels). (**c**) Interaction between Dvl2 and pVHL. After transfected with myc-pVHL and Flag-Dvl2 for 36 h, HEK293T cells were cultured in nutrient-rich medium or starved with BFA1 for 4 h. Cells were harvested for anti-myc immunoprecipitation followed by anti-Flag immunoblotting. Protein expression was confirmed by immunoblotting with whole cell lysates (WCL). (**d**) Ubiquitination of Dvl2. HEK293T cells were transfected with Flag-Dvl2 or together with ubiquitin. After transfection for 36 h, the cells were treated with BFA1 for 6 h under normal or starvation condition. Cell extracts were subjected to anti-Flag immunoprecipitation. The ubiquitinated Dvl2 was revealed using anti-Ub antibody. (**e**) Knockdown of pVHL expression attenuates the LC3-Dvl2 interaction. HeLa cells transfected with either control or VHL shRNA were placed in starvation medium with BFA1 for 4 h. The cell lysates were subjected to anti-LC3 immunoprecipitation followed by anti-Dvl2 immunoblotting. The total protein expression was confirmed by immunoblotting with whole cell lysates (WCL). (**f**) pVHL knockdown promoted TopFlash reporter activity. Each experiment was performed in triplicate and the data represent the mean ± S.D. (n=3).

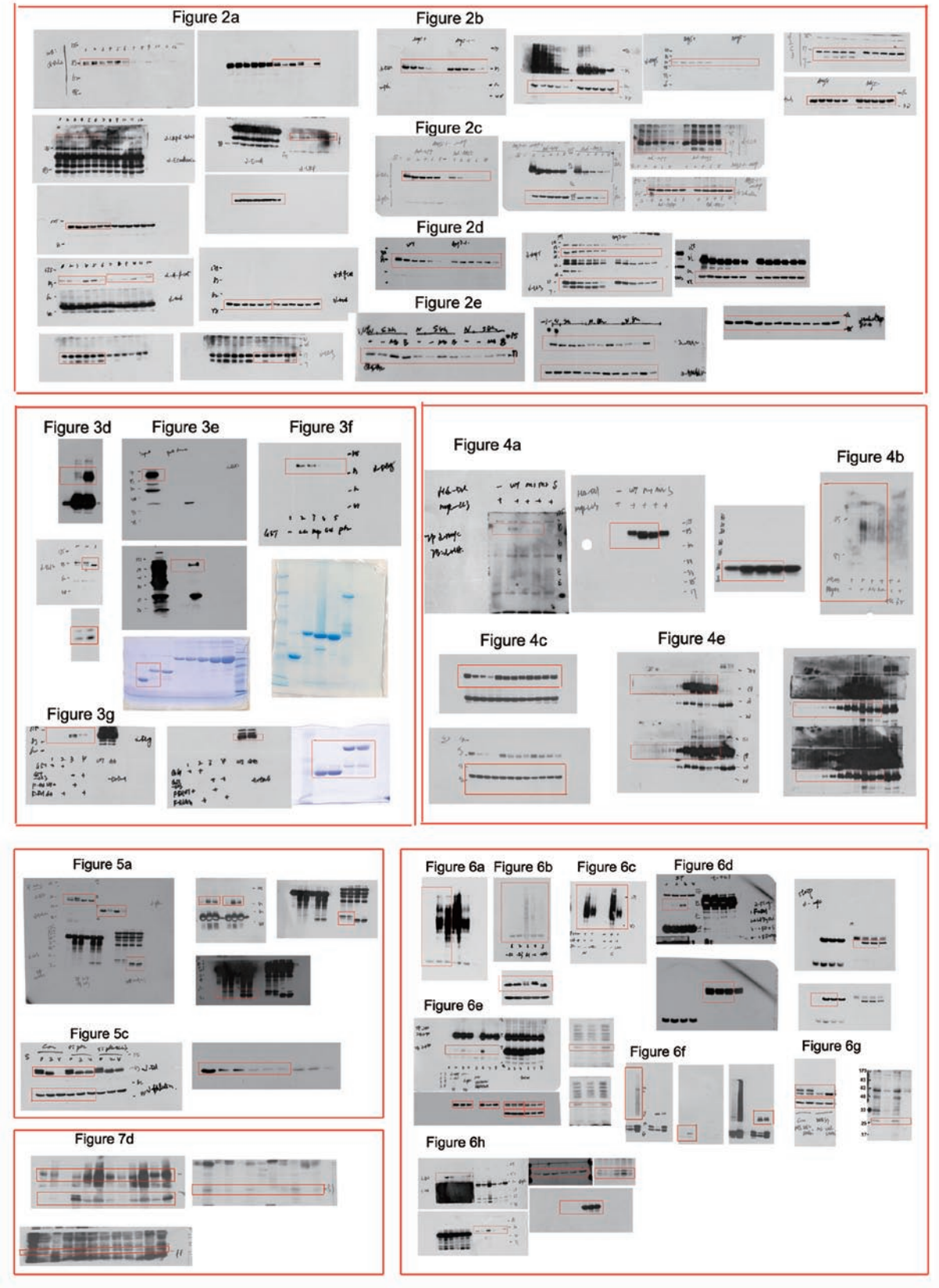

Figure S8 Full scans of immunoblots shown in Figs 2-7.