Supplemental Materials

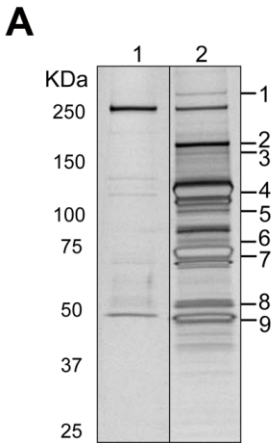
Molecular Biology of the Cell

Yamashita et al.

Yamashita et al.

Supplemental Materials

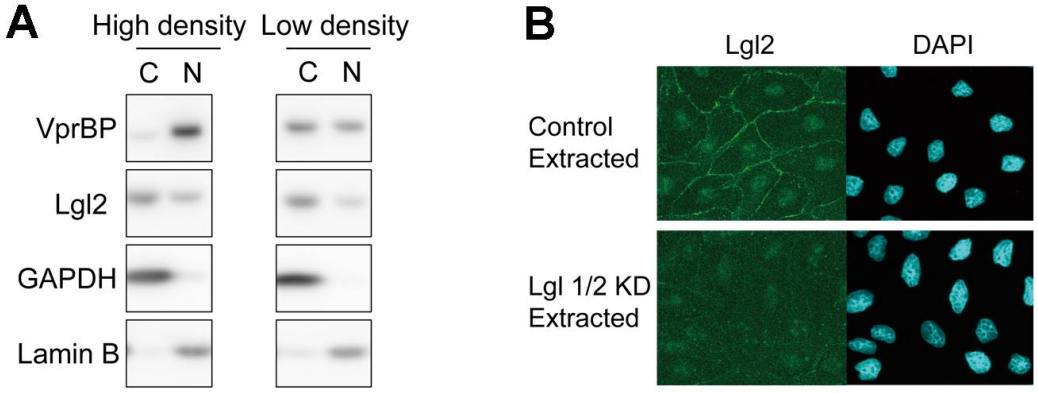
Supplemental Figures S1–S9



B

Band location	*Number of peptides detected*	*Protein name*
#1	2	Lethal giant larvae homolog 2
#2	8	**Vpr-binding protein**
#3	13	Lethal giant larvae homolog 2
#4	102	Lethal giant larvae homolog 2
	5	**damage-specific DNA binding protein 1**
	2	Nuclear autoantigen Sp-100 (Speckled 100 kDa)
	1	Lethal giant larvae homolog 1
#5	16	Lethal giant larvae homolog 2
#6	8	Lethal giant larvae homolog 2
	6	Protein kinase C iota
#7	36	Protein kinase C iota
	33	heat shock 70kDa protein 8
	12	Lethal giant larvae homolog 2
#8	30	beta-tubulin
	7	alpha-tubulin
#9	34	Partitioning defective-6 homolog beta (PAR-6B)
	6	dihydrolipoamide branched chain transacylase E2
	2	Dnj3/Cpr3

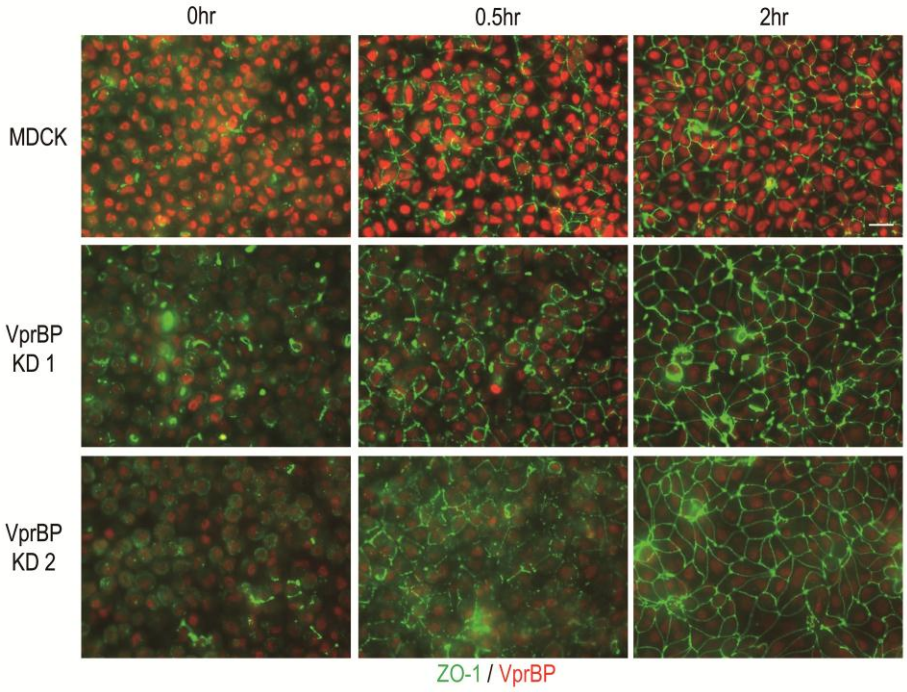
Supplemental Figure S1. Identification of Lgl2-binding proteins by mass spectrometry. (A) Purified SBP-Lgl2 was separated by SDS-PAGE, and co-purified proteins were analyzed by mass spectrometry. Bait and DDB1 were in band #4, and VprBP was in band #2. Note that the bands containing large amounts of protein were not stained completely by this method. (B) List of proteins associated with SBP-Lgl2 identified by mass spectrometry analysis. VprBP and DDB1 are shown in bold.



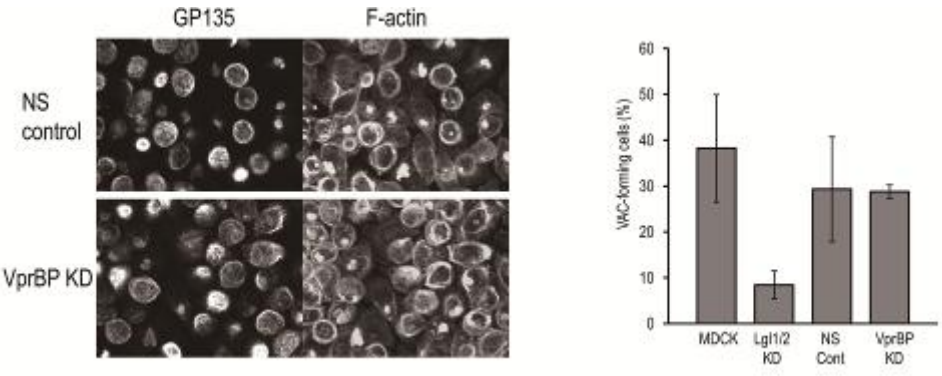
Supplemental Figure S2. Lgl2 and VprBP localize to both the cytosol and the nucleus. (A) Cytoplasmic and nuclear fraction (lane C and N respectively) were prepared from MDCK cells

cultured at high density or low density, respectively, using NE-PER Nuclear and Cytoplasmic Extraction Reagents (Thermo Scientific). (B) Immunofluorescence was performed to control and Lgl1/2 KD MDCK cells which cytoplasmic and membrane component were extracted by using ProteoExtract Subcellular Proteome Extraction kit (Millipore). After taking F1 (cytosolic fraction) and F2 (membrane fraction), cells were fixed and subjected to immunofluorescence.

A

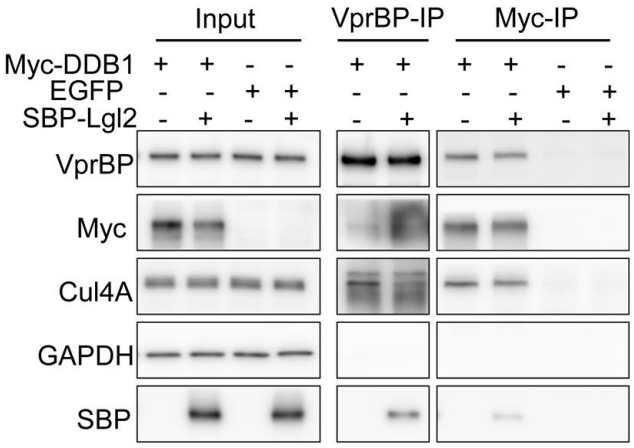


B



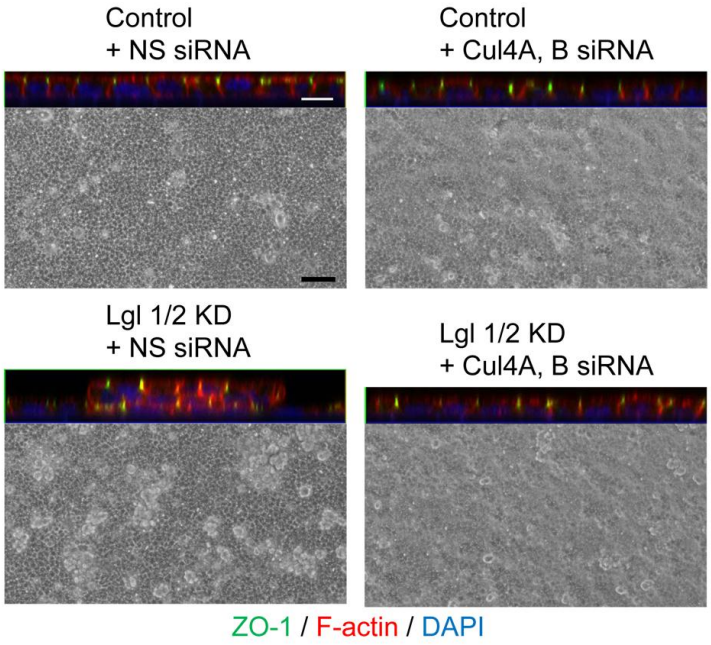
Supplemental Figure S3. VprBP is not critically involved in regulation of cell polarity. (A) Junction formation in normal MDCK or VprBP-depleted MDCK cells was evaluated after the calcium switch; cell junctions are disrupted by calcium depletion, and re-addition of calcium initiates junction formation (Suzuki *et al.*, 2001). Each cell line was incubated for 18 h in low calcium-containing

medium (LC medium: 3 μM Ca^{2+}, 5% FBS) and then cultured in normal medium for the indicated time. Immunofluorescence of ZO-1 was used as a tight junction marker. Note that VprBP-depleted cells did not show a significant delay in junction formation compared with normal cells. Green, ZO-1; Red, VprBP. (B) VprBP-depleted MDCK cells were depolarized by calcium deprivation. VAC (vacuolar apical compartment) is an organelle containing microvilli and apical membrane proteins, and is transiently formed after cell depolarization induced by calcium deprivation. As described previously, Lgl1/2 KD cells maintained an apical membrane domain with a defect in VAC formation (Yamanaka *et al.*, 2006). After incubation for 18 h in LC medium, the cells were fixed and stained using rhodamin-phalloidin and anti-GP135. The cells containing both aggregated F-actin and GP135 staining were counted as VAC-forming cells (n=3). Note that knockdown of VprBP did not significantly affect VAC formation.

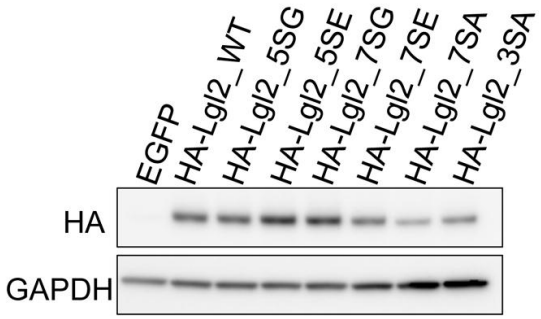


Supplemental Figure S4. The interaction between DDB1 and Cul4A was not largely disrupted by overexpression of Lgl2. The amount of Cul4A co-immunoprecipitated with exogenously expressed Myc tagged DDB1 was assayed because of disability of the DDB1 antibody for immunoprecipitation. Myc-DDB1 or endogenous VprBP was immunoprecipitated from the common lysate of HEK293T cells transfected with Myc-DDB1 and SBP-Lgl2 expression vector (lane2, 6, 8); also immunoprecipitated from the common lysate of cells transfected with Myc-DDB1 and SBP expression vector (lane 1, 5, 7). EGFP was expressed instead of the bait (Myc-DDB1) as a negative control (lane 3, 4, 9, 10).

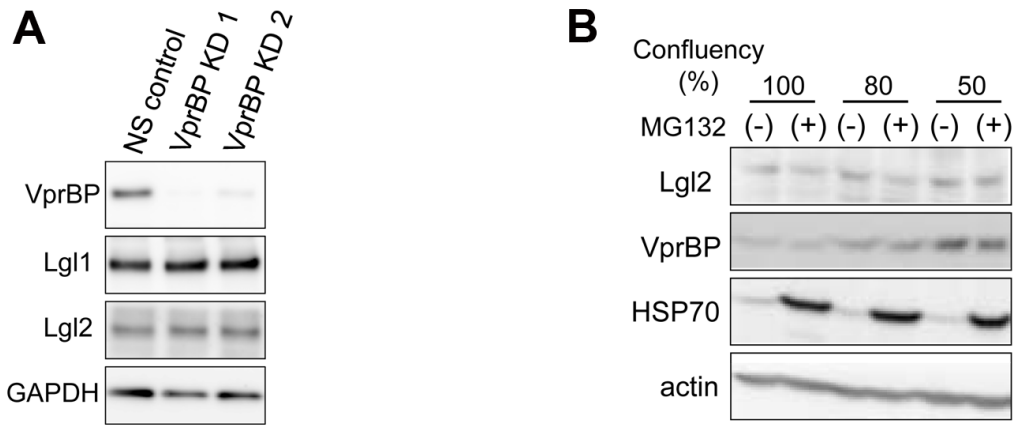
Supplemental Figure S5. Depletion of VprBP upregulates p27 and downregulates Skp2 in HeLa cells. HeLa cells were seeded at low density and transfected with the indicated siRNAs. Cells were harvested after culture for an additional 48 h. The target sequence of canine VprBP siRNA #2 is conserved in humans.



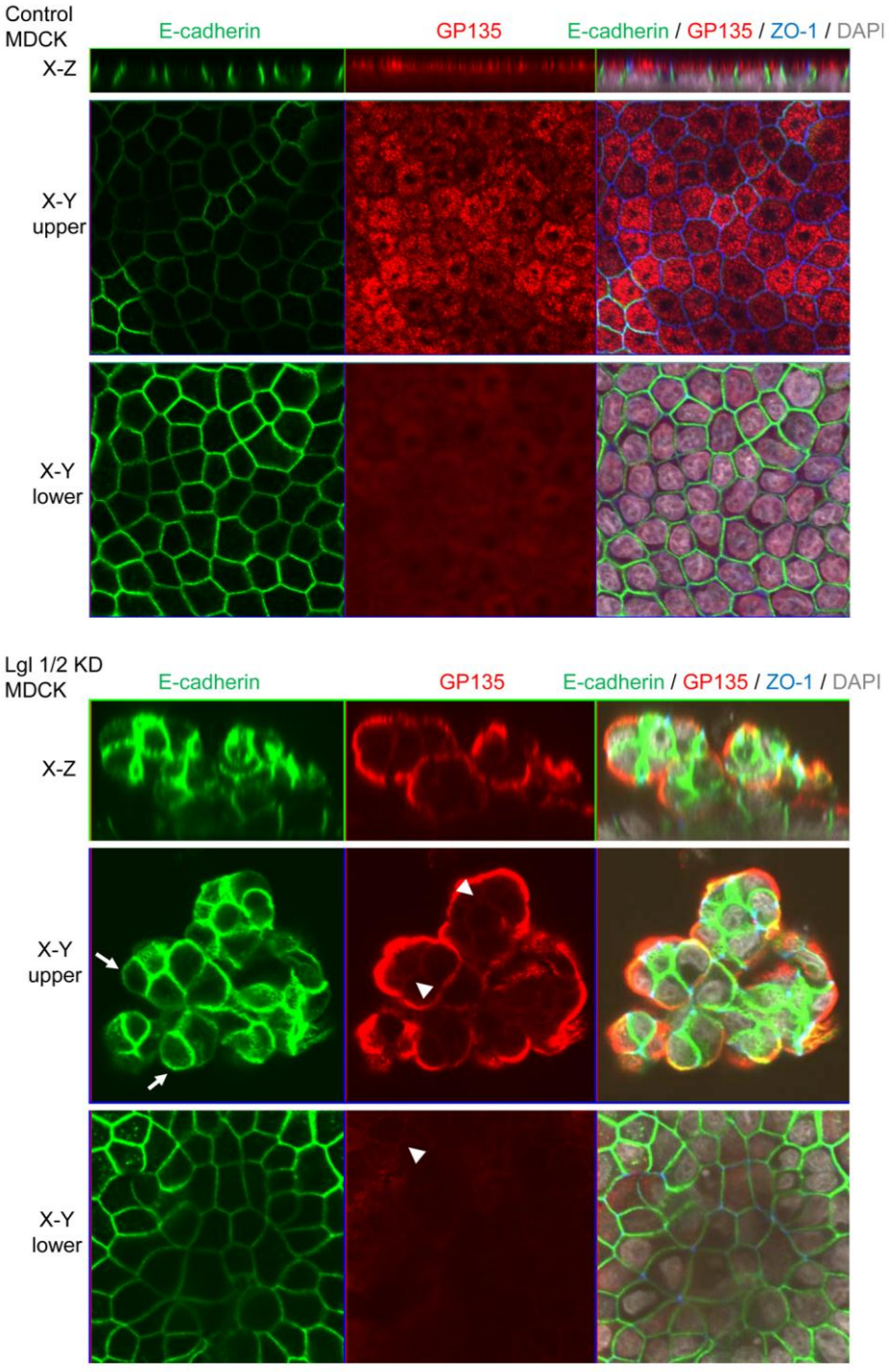
Supplemental Figure S6. Depletion of both Cul4A and Cul4B suppressed formation of multilayered structures in Lgl1/2 KD MDCK cells. Control or Lgl1/2 KD MDCK cells were transfected with non-silencing siRNA (NS) or siRNAs for Cul4A and Cul4B, and cultured until they reached confluency. Phase-contrast images and reconstituted confocal z-axis images of the epithelial sheets are shown. Samples were stained with anti-ZO-1 (green), Rhodamine-phalloidin (red) and DAPI (blue). White and black scale bars represent 10 μm and 30 μm, respectively.



Supplemental Figure S7. Glycine and Glutamate but not Alanine mutants of Lgl2 were well expressed in MDCK cells. MDCK cells were transfected with each expression vectors. After culturing 24 h, cells were lysed in SDS-sample buffer, and samples were analyzed by western blotting. Ser649, 653 and 660 are altered in 3SA mutant.



Supplemental Figure S8 Lgl2 is probably not a substrate of VprBP-DDB1-containing ubiquitin ligases. (A) Protein levels of Lgl2 were analyzed in normal or VprBP-depleted MDCK cells. Note that level of Lgl2 was not significantly affected by depletion of VprBP. (B) MDCK cells were cultured until reaching each indicated confluence and treated with 10 μM of MG132 for 6 h until harvesting. Western blotting analysis showed that protein amount of Lgl2 was not affected by treatment of MG132. Effect of MG132 was confirmed by stabilization of HSP70.



Supplemental Figure S9. Distribution of asymmetrically localizing proteins in Lgl1/2 KD MDCK cell. Cells were cultured to confluence on the Transwell. In control cells, GP135 and E-cadherin exclusively localized to apical and lateral domains respectively. However, in Lgl1/2 KD cells, portion of E-cadherin merged with strong GP135 signal (white arrow), whereas part of GP135 merged with strong E-cadherin signal (white arrow head). This result suggests that cell polarity is compromised in Lgl 1/2 KD MDCK cells