Supplementary Materials
*Molecular Biology of the Cell*
Rbaibi *et al*.

**Supplementary Figure Legends**

**Fig. S1. Confirmation of phenotypes in additional CRISPR/Cas9 *Cubn*, *Lrp2*, and *Dab2* KO clones.** (A) Allelic indel sequences for a duplicate set of CRISPR/Cas9 *Cubn* KO, *Lrp2* KO, and *Dab2* KO clones relative to control nucleotide sequences. (B) Equivalent amounts (15 μg) of cell lysates from the duplicate set of control, *Cubn* KO, *Lrp2* KO, and *Dab2* KO clones were western blotted with antibodies against cubilin, megalin, Dab2, EEA1, and Rab11a. (C) Duplicate filters of parental OK cells and control and KO clones were incubated for 30 min with apically added 40 μg/mL AlexaFluor-647 albumin, washed, and cell-associated fluorescence quantified by spectrofluorimetry. The mean +/- SD, normalized to parental OK cells is plotted.

**Fig. S2. Clathrin, Rab5, and Rab7 are similarly distributed in control and CRISPR/Cas9 *Cubn*, *Lrp2*, and *Dab2* KO clones.** Control and CRISPR/Cas9 KO clones were fixed and stained to reveal clathrin heavy chain, Rab5, or Rab7. Images for Rab5 and Rab7 were deconvoluted to match the processing of markers shown in Fig. 6. Maximum projections of representative fields are shown, and xz sections are provided below (with actin staining in red) to confirm the subapical distribution of the compartment markers. Scale bars: 5 μm.

**Fig. S3. Colocalization of albumin and dextran in endocytic apical compartments.** OK cells were incubated with Alexa Fluor-488 albumin and Alexa Fluor-647 Dextran for 3 min at 37ºC, then fixed and imaged by confocal microscopy. Scale bar: 5 μm.

**Fig. S4. Quantitation of EEA1 compartments and area in control and CRISPR/Cas9 *Cubn*, *Lrp2*, and *Dab2* KO clones.** Control and KO cells were fixed and stained to detect EEA1 and imaged by confocal microscopy. The number of EEA1-positive compartments (A) and the total EEA1-positive area (B) in cell lines was quantified from five images each in three independent experiments (each plotted in a different color) as described in Methods.

**Fig. S5. Surface expression of cubilin in parental OK cells and control and CRISPR/Cas9 KO clones.** OK cells and control and CRISPR/Cas9 *Cubn*, *Lrp2*, and *Dab2* KO clones were apically biotinylated, quenched, and solubilized, Ten percent of the lysate was reserved to calculate total cubilin, and biotinylated proteins were recovered using streptavidin-agarose. Total (T) and surface (S) cubilin were quantified after western blotting. Panel A shows a representative blot; MW= molecular mass markers. (B) Data from 3-4 experiments for each clone (two independent blots per experiment) were normalized to the fraction of cubilin at the apical membrane of control cells in each blot. One-way ANOVA p values vs. Control: OK cells 0.9172; *Lrp2* KO, 0.6516; *Cubn* KO, 0.966; *Dab2* KO, >0.9999.

**Fig. S6. Electron microscopy of control and *Lrp2* KO mice.** Cortical kidney sections from 4 month old female C57/Bl6 mice [$Lrp2^{flox/flox}$;$EMX\text{-}Cre^{-/-}$ (A,C) and $Lrp2^{flox/flox}$;$EMX\text{-}Cre^{+/-}$ (B,D)] were processed for EM and imaged. (A,B) Apical endocytic compartments in the majority of cells in PT cross-sections are readily visible at low magnification. Scale bars: 2 μm, (C,D) The similar array of apical endocytic compartments is evident in high magnification images of PT cells in *Lrp2* KO and control mice. Scale bars: 1 μm.

**Table S1. Antibodies used this study.**

| Antigen | dilution | Source |
|---|---|---|
| **Western Blotting** | | |
| Megalin (rabbit polyclonal) | 1:20000 | MC-220 (Zou et al., PMID 15180987) |
| Cubilin (rabbit polyclonal) | 1:5000 | 27445 (Ren et al., PMID 32200668) |
| Dab-2 (rabbit monoclonal) | 1:2000 | Cell Signaling, 12906 |
| Rab11a (rabbit polyclonal) | 1:1000 | Abcam, ab65200 |
| Early endosome antigen 1 (EEA1; mouse monoclonal) | 1:500 | Santa Cruz, sc-365652 |
| | | |
| Clathrin Heavy Chain (CHC; mouse monoclonal) | 1:200 | CHC5.9, Progen 61017 |
| | | |
| **Immunofluorescence Primary Antibodies** | | |
| Megalin | 1:1000 | MC-220 |
| EEA-1 | 1:50 | Santa Cruz, sc-365652 |
| Rab5 (rabbit polyclonal) | 1:500 | Abcam, ab218624 |
| Rab7 (rabbit polyclonal) | 1:100 | Cell Signaling 9367 |
| CHC | 1:25 | Progen, 61017 |
| Cubilin (sheep polyclonal) | 1:200 | R&D Systems, AF3700 |
| Lamp1 (rat monoclonal) | 1:100 | DSHB, 1D4B |
| Rab11a | 1:500 | Abcam, ab65200 |
| HA.11 Tag (mouse monoclonal) | 1:500 | BioLegend, 901501 |
| **Immunofluorescence Secondary Antibodies** | | |
| Alexa Fluor 647 goat anti-Mouse IgG | 1:500 | Invitrogen, A21236 |
| Alexa Fluor 647 Donkey anti-Sheep IgG | 1:500 | Jackson ImmunoResearch, 713-605-147 |
| Alexa Fluor 647 goat anti-Rat IgG | 1:500 | Invitrogen, A21247 |
| Alexa Fluor 647 goat anti-Rabbit IgG | 1:500 | Invitrogen, A21245 |
| Alexa Fluor 488 goat anti-Mouse IgG | 1:500 | Invitrogen, A11029 |
| Alexa Fluor 488 Fab Fragment Goat Anti-Rabbit | 1:500 | Jackson ImmunoResearch, 111-547-003 |

# Fig. S1

**A**

### *Cubn* KO clone 6

| | |
|---|---|
| Wild Type | 5′ – TATT**GAGTTTAGGACTGGGACCCATGG**AAAGATCAAAATGA – 3′ |
| Allele 1 | 5′ – TATTG ––––––––––––––––––CCATGGAAAGATCAAAATGA – 3′ |
| Allele 2 | 5′ – TATTG ––––––––––––––––––CCATGGAAAGATCAAAATGA – 3′ |

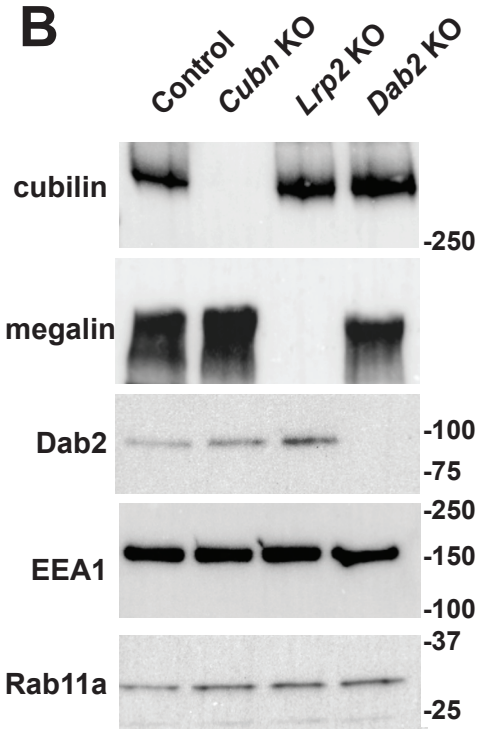
### *Lrp2* KO clone 3

| | |
|---|---|
| Wild Type | 5′ – GCTGTGTGTACCCCCC**CTT–GTCAGCAGTATCAATTT**ACCTGCC - 3′ |
| Allele 1 | 5′ – GCTGTGTGTACCCCCCTTCGTCAGCAGTATCAATTTACCTGCC – 3′ |
| Allele 2 | 5′ – GCTGTGTGTACCCCCCTTGGTCAGCAGTATCAATTTACCTGCC – 3′ |

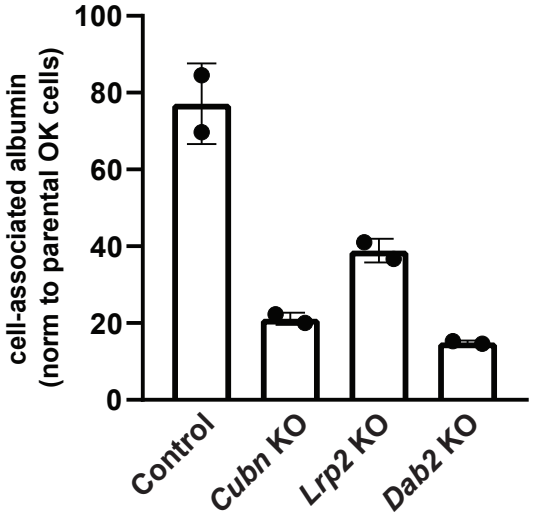
### *Dab2* KO clone 7

| | |
|---|---|
| Wild Type | 5′ – CTAATACC**TGGTCATCACAAGCCTCCAT**GGGGAACCCTTTTCA – 3′ |
| Allele 1 | 5′ – CTAATACCTGGTCATCACAAGCC ––CATGGGGAACCCTTTTCA – 3′ |
| Allele 2 | 5′– CTAATACCTGGTCATCACAAGCCT – CATGGGGAACCCTTTTCA – 3′ |

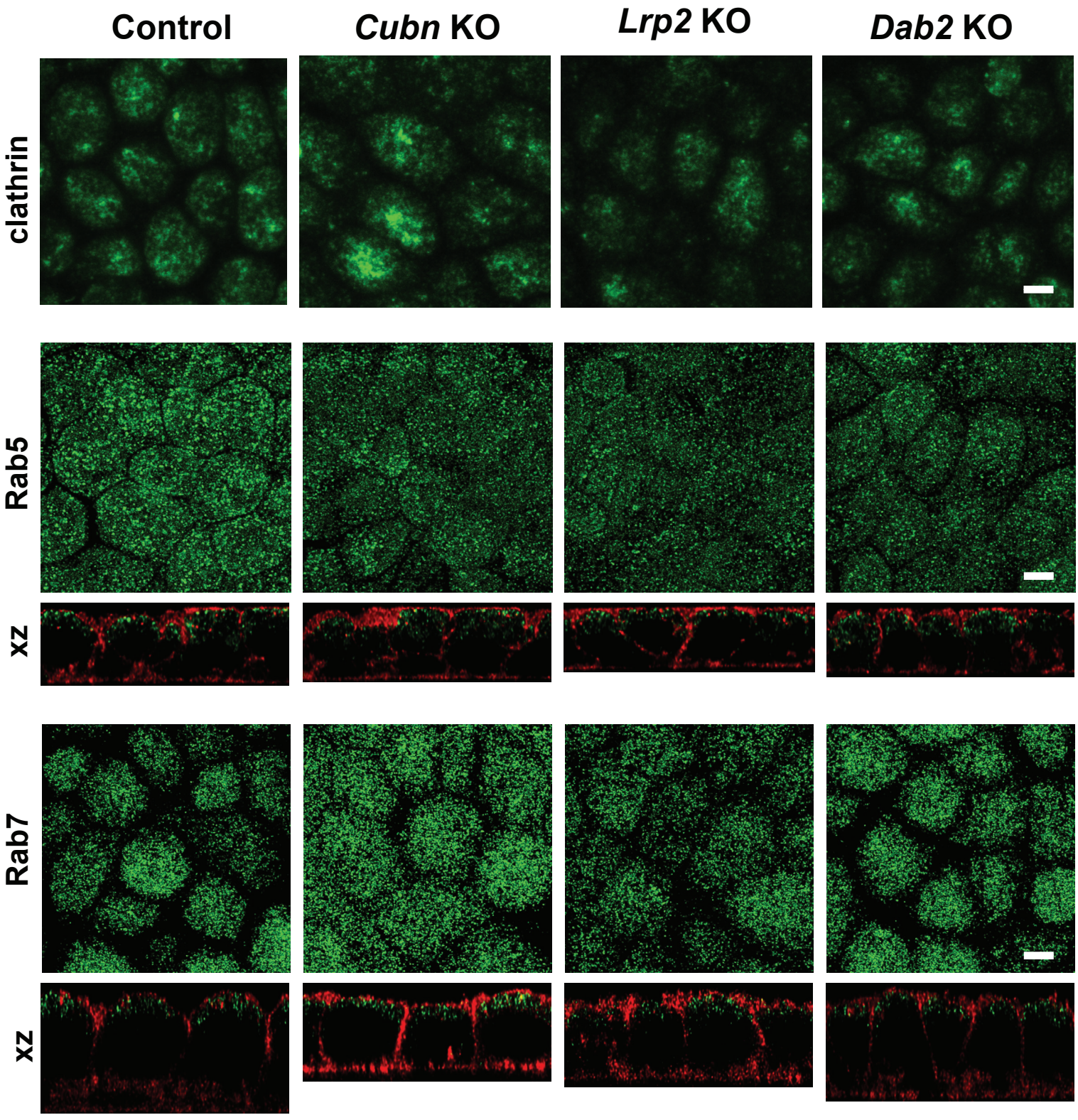
**B**



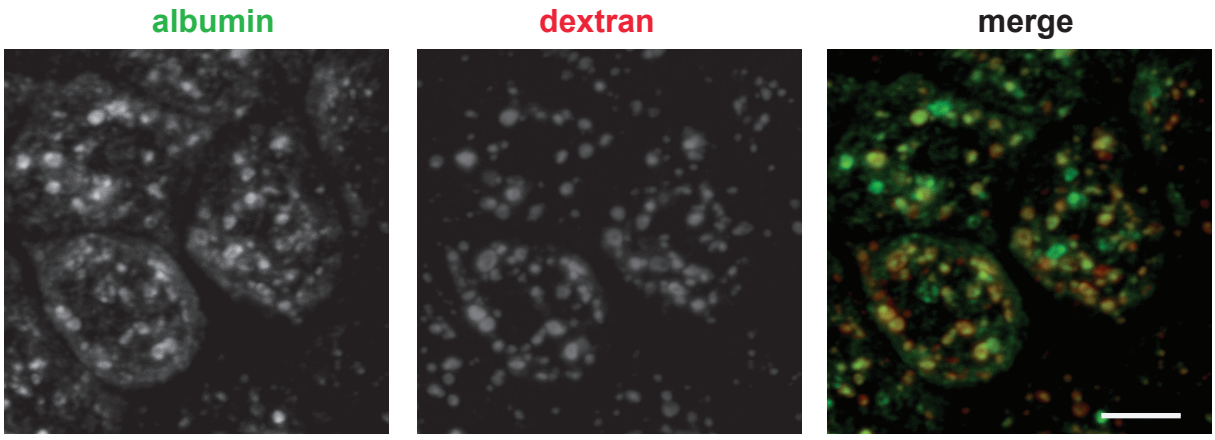

**C**



# Fig. S2



# Fig. S3



# Fig. S4

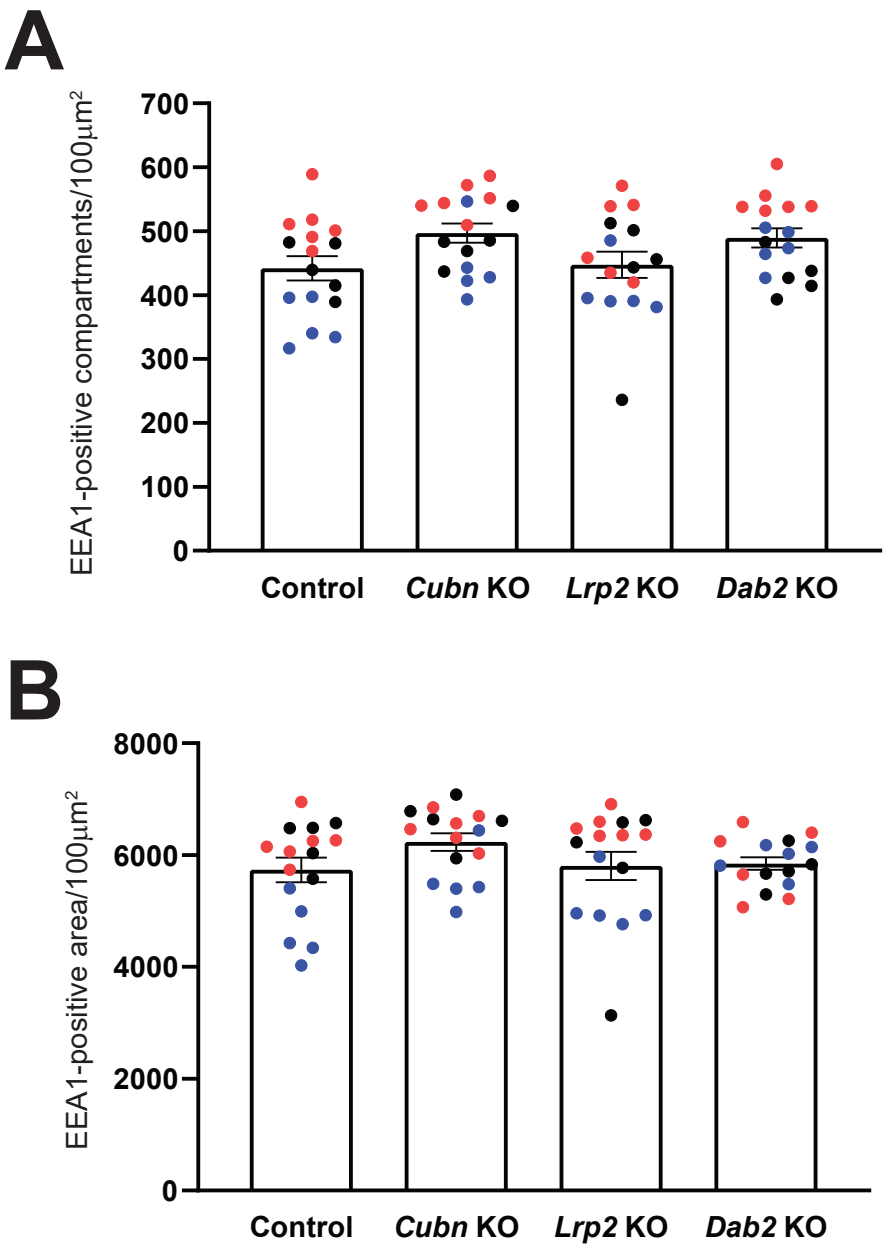
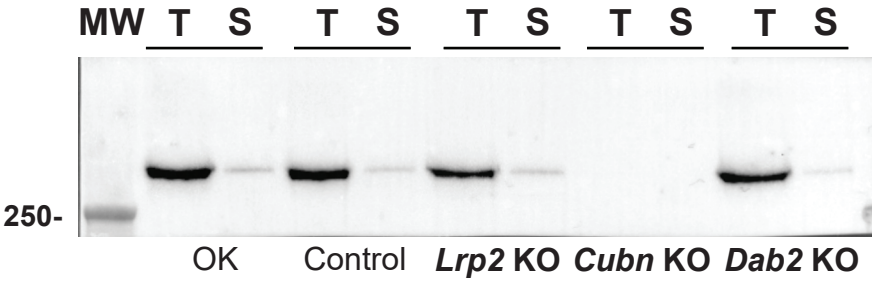
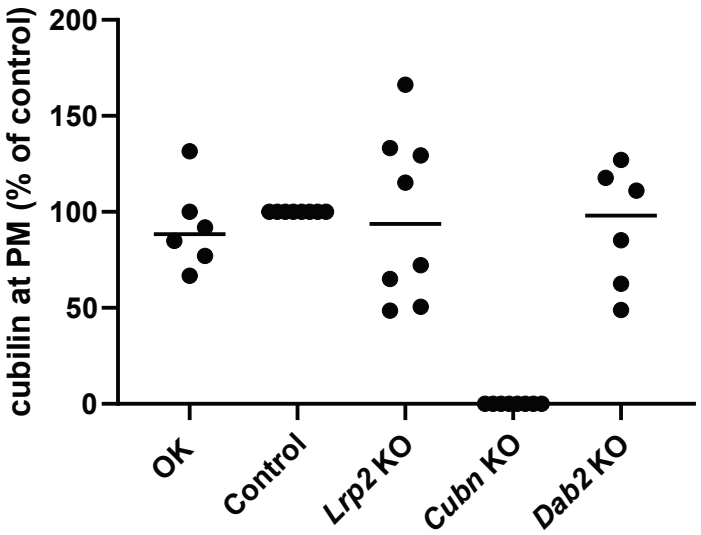

A
EEA1-positive compartments/100µm²
700
600
500
400
300
200
100
0
Control
Cubn KO
Lrp2 KO
Dab2 KO
B
EEA1-positive area/100µm²
8000
6000
4000
2000
0
Control
Cubn KO
Lrp2 KO
Dab2 KO

Fig. S5

A


MW
T S
T S
T S
T S
T S
250-
OK
Control
Lrp2 KO
Cubn KO
Dab2 KO

B


cubilin at PM (% of control)
200
150
100
50
0
OK
Control
Lrp2 KO
Cubn KO
Dab2 KO

# Fig. S6