A

```
HumanFIP5          1 MALVRGAEPAAGPSRWLPTHVQVTVLRARGLRGKSSGAGSTSDAYTVIQVGREKYSTSVV
DogFIP5            1 MALVRGAEPAPGPSRWLPTHVQVTVLRARGLRGKSSGAGSTSDAYTVIQVGREKYSTSVV
ZebrafishFip5a     1 MSLAKS-D---EDQRWVPTHVQVTVLRARGLRAKG--KHGTSDVYTIIQLGKEKYSTCVM
ZebrafishFip5b     1 MPLISLDD---EEQRWVPTHVNVTVLRARALRTKG--KQGSRYVYTIIQVGKEKYTTGLV

HumanFIP5         61 EKTHGCPEWREECSFELPPGALDGLLRAQEADAGPAPWAASSAAACELVLTTMHRSLIGV
DogFIP5           61 EKTPGCPEWREECSFELPPGALDGLLRAQEADAGSAPWAAGSAAACELVLTTMHRSLIGV
ZebrafishFip5a    55 EKTT-DPEWGEECSFELQPGILEEEGR--------DAY---PPGSGDLTLTVMHRALIGL
ZebrafishFip5b    56 EKAE-EPQWGEECAFELLPGLLEAGGT--------SAY---PPGSSNLVFTVMHRVLIGL

HumanFIP5        121 DKFLGQATVALDEVFGAGRAQHTQWYKLHSKPGKKEKERGEIEVTIQFTRNNLSASMFDL
DogFIP5          121 DKFLGQATVALDEVFGAGRAQHTQWYKLHSKAGKKEKERGEIQVTIQFTRNNLSASMFDL
ZebrafishFip5a   103 DVFLGQAVLPLHKAFQDRKSKKNEWHRLHSKTGKKEKERGELQLSVQFTRHNLTASMYDL
ZebrafishFip5b   104 DVFLGQTIVPLDKVFQEGTCPRNEWLKLHSKAGRKEKERGELQVTIQFTRNNMTASMYDL

HumanFIP5        181 SMKDKPRSPFSKIRDKMKGKKKY-DLESASAILPSSA-------------------I-ED
DogFIP5          181 SMKDKPRSPFSKIKDKMKGKKKF-DLESASAILPSSA-------------------L-ED
ZebrafishFip5a   163 SMKDKPRSAFDKLRERMRAKKRPAEEDSSSAIVPGGYGALARMRGRLP-SDGGGEEDYED
ZebrafishFip5b   164 TVKDKPRSAFGKLKDRVTGKKRD--VESSSAVLPGRYAALSGSVGPPFAGDGGSYEA-SE

HumanFIP5        220 PDLGSLGKMGKAKGFFLRNKLRKSSLTQSNTSLGSDSTLSSASGSLAYQGPGA----ELL
DogFIP5          220 PELGSLGKMGKAKGFFLRNKLRKSSLTQSNTSLGSDSTLSSASGSLAYQGPGT----ELL
ZebrafishFip5a   222 -DEGGEARRSKMRSFFLRGRLRKSSDTRSSTSLGSESSESSSRGGSLSPTAGISVVVSDL
ZebrafishFip5b   221 -EDGVEEHRSKVKDFFLKGKLRKNSDTRSCSSLASDSSMASSAGDPFIPV--------EI

HumanFIP5        276 TRSPSRSSWLSTEGGRDSAQSPK--LFTHKRTYSDEANQMRVAPPRALLDLQGHLDA---
DogFIP5          276 THSPSRSSWLSTEGGRDSTQSPK--LLTHKRTYSDEASQMRVAPPRSLLDLQGHLDA---
ZebrafishFip5a   281 SNSPSNSSNLTADNSPEHTVAPSPQVSPVRHVMYD----ISLPVPHS------MMSDNDT
ZebrafishFip5b   272 PRTPIYSSRVMEPFRMDTEEAIK--VMTHKRAHSDEASKITCVPRPS-----PAVEN---

HumanFIP5        331 -ASRSSLCVNGSHIYNEEPQGPVR---------HRSSISGSL-------------------
DogFIP5          331 -ASRSSLCVNGSHIYNEEPQAPLR---------HRSSISGPF-------------------
ZebrafishFip5a   331 PILLPSVCVNGNPVETS----PLTHHPPTLVLQH-PQQESTKPITQSGQPQATKLPAKPE
ZebrafishFip5b   322 -LSQSTLCINGSHIYSSEPVSPKSP---SAIPAKRSLLEKCA-------------------

HumanFIP5        363 ----------------------PSSGSLQAVSSRFSEEGPR---STDDTWPRGSRSNSSS
DogFIP5          363 ----------------------PPSSSLHSVSFRPAEEGSR---PTDDSGGRGSRSTSSS
ZebrafishFip5a   386 KSQESKPRPEPRLPALGVLQKGSLSLSLQNLSRQ-GKEKQNGGPVD---GRRWSFDKPGE
ZebrafishFip5b   360 ----------------------PLSRSLQNLTRR-GEDSQK---SD---GRRWSIDKSKK

HumanFIP5        398 EAVLGQEELSAQAKVLAPGASHPGEEEGARLPEGKPVQV--ATPIVASSEAVAEKEGARK
DogFIP5          398 EMLPGQEELSSQAKVLATGTSRSGEEEGARLPEGKPVQV--ATPLVASSESVAEKEGARK
ZebrafishFip5a   442 E-------------EKAAIVA-ALEHAGR--VTDEPVNETVIRAG------------ETE
ZebrafishFip5b   391 E-------------DLETNAA-QSQTQGSTIVDGKPVQA--AGAVD-----------VLD

HumanFIP5        456 EERKPRMGLFHHHHQGLSRSELGRRSSLGEKGGPIL-GASPHHSSSGEEKAKSSWFGLRE
DogFIP5          456 EERKPRMGLFHHHHQGLSRSELGRRGSLGEKGGPTQ-GASPHHSSGGEEKAKSSWFGLRE
ZebrafishFip5a   474 TQGKKRRGLFSH----------GKGDSAGKG--PITSKEETEHAQPLVEVKHKGWFSS--
ZebrafishFip5b   424 KGKKLRKTLFSS----------GRSDSLPAK--P-----EQGQVSAPVEGRRRGWFGS--

HumanFIP5        515 AKDPTQKPSPHPVKPLSAAPVEGSPDRKQSRSSLSIALSSGLEKLKTV-TSGSIQPVTQA
DogFIP5          515 AKEPTQKPSPHPVKPLSAASLEGSPDKKQSRSSLSTALSSGLEKLKTV-TSGSVQPVAPA
ZebrafishFip5a   520 -KDSHSKPSPHPVKPLTPPDE----KRSEGR--------SVLEKLKSTIHSGRSDA----
ZebrafishFip5b   465 -GDSQNKPSPHPVKPLTNNTLQGE-KKAESR--------SVLEKLKSTINPGRSALATTA

HumanFIP5        574 PQAGQMVDTKRLKDSAVLDQSAKYYHLTHDELISLLLQRERELSQRDE------------
DogFIP5          574 PHVGQTVDTKRLKDSGVLDQSAKYYHLTHDELISLLLQRERELSQRDE------------
ZebrafishFip5a   563 ------------DKKPLVEGGGSYYHLNHSELVNLLIQRDMELRQEREEYEKRGMLLEKR
ZebrafishFip5b   515 EEE--------KQQLSLMEARAHYQNMTNMELIALLLQQELEIKKQRAETEVQVVMLEKR

HumanFIP5        622 ---------HVQELESYIDRLLVRIMETSPTLLQIPPGPPK
DogFIP5          622 ---------HVQELESYIDRLLVRIMETSPTLLQIPPDPPK
ZebrafishFip5a   611 ETDLKKMKLLIKDLEDYIDTLLVRIMEQTPTLLQVRPK-MK
ZebrafishFip5b   567 DAELKKMKVQVRDLEDYIDKLLVRIMEQTPTLLQVRGR-LK
```

B

Fip5b Exon 2

WT Fip5b: WLKLHSKAGRKEKERGELQVTIQFTRNNMTASMYDLTVKDKPRSAFGKLKDRVTGKKRDVESSSAVLPGRYA
ALSGSVGPPFAGDGGSYEASEEDGVEEHRSKVKDFFLKGKLRKNSDTRSCSSLASD

Fip5b^{CO40}: ALSGSVGPPFAGDGGSYEASEEDGVEEHGVEEVRSKTSFStop

Fip5b^{CO43}: ALSGSVGPPFAGDGGSYEASEEDGVEEHRSKTSFStop

C

Fip5a Exon 1

WT Fip5a: MSLAKSDEDQRWVPTHVQVTVLRARGLRAKGKHGTSDVYTIIQLGKEKYSTCVMEKTTDPEWGEECSFEL

Fip5a^{CO35}: MSLAKSDEDQRWVPTHVQVTVLRARGLREGQTRHQRRVHHHPAGQGEILHVRDGEDYRSGMGRGMLVStop

Fip5a^{CO38}: MSLAKSDEDQRWVPTHVQVTVLRARGLRVTStop

Figure S1. (A) Protein alignments for human FIP5, dog FIP5, and zebrafish paralogs Fip5a and Fip5b. The yellow highlighted region denotes the C2 domain and the blue highlighted region denotes the Rab-binding domain. (B) Fip5b exon 2 sequence in wild-type, *fip5b*^{CO40} mutant, and *fip5b*^{CO43} mutant alleles. Red amino acids show where mutants differ from wild-type allele. (C) Fip5a exon 1 sequence in wild-type, *fip5a*^{CO35} mutant, and *fip5a*^{CO38} mutant alleles. Red amino acids show where mutants differ from wild-type allele.

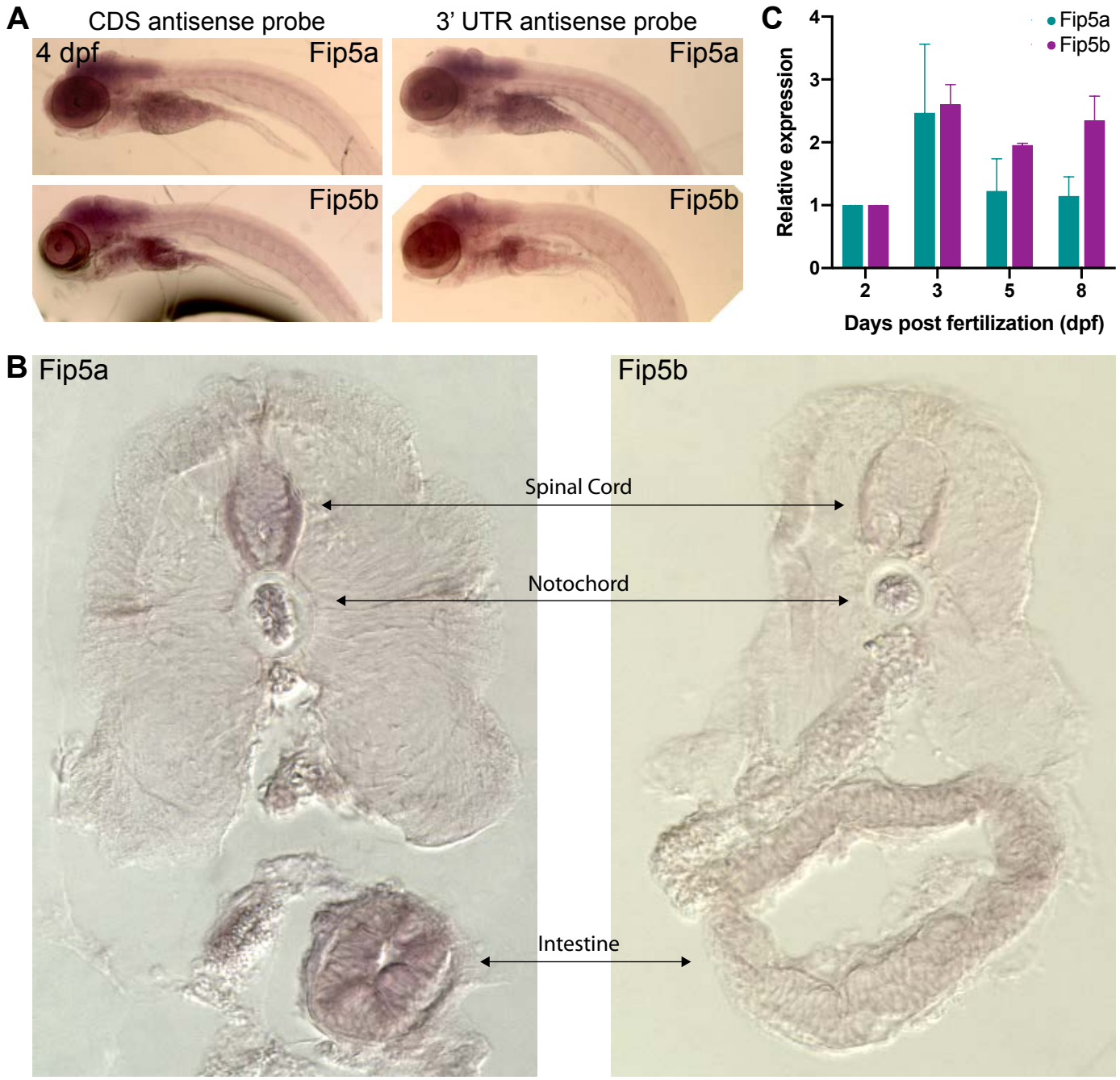

Figure S2. *In situ* hybridization on 4 dpf larvae with antisense probes for the coding sequences of *fip5a* and *fip5b* (left panel) and the 3' UTR sequences of *fip5a* and *fip5b* (right panel). (B) Representative cross sections of *fip5a* and *fip5b* antisense coding sequence probes. (C) qRT-PCR measuring *fip5a* and *fip5b* transcript levels at 2, 3, 5, and 8 dpf normalized to levels at 2 dpf. Three independent experiments were performed. All plots show mean ± s.e.m.

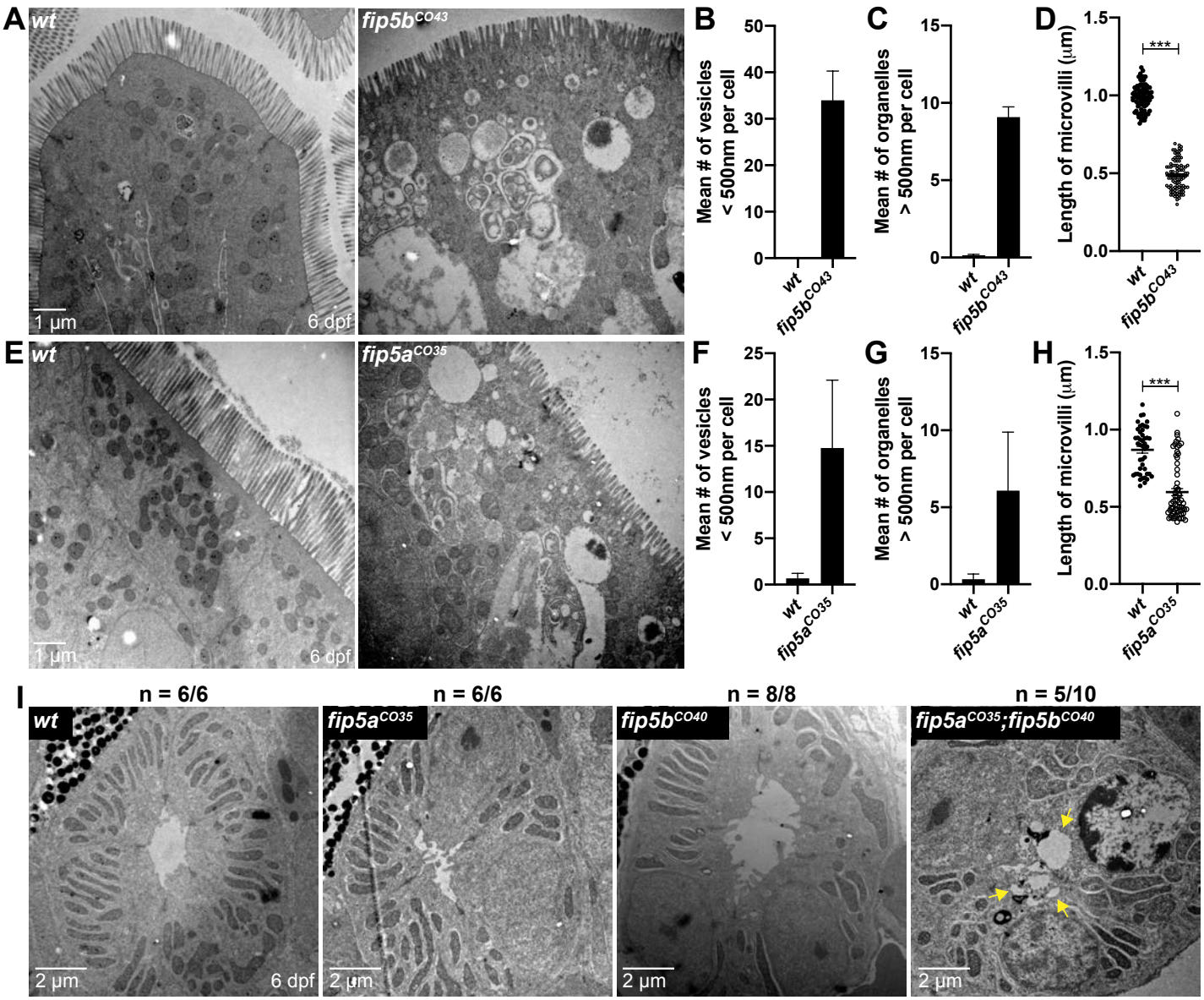

Figure S3. All following images are representative cross sections through midgut region on 6 dpf larvae. Wild-type siblings are used as controls. For A-G, three separate animals for each condition were analyzed. (A) Electron micrographs showing wild-type and *fip5b*^{CO43} mutant larvae. (B) Quantitation of less than 500nm apical vesicles. (C) Quantitation of greater than 500nm organelles. (D) Quantitation of midgut microvilli length. Each dot represents a single microvillus length combined across three animals. (E) Electron micrographs showing wild-type and *fip5a*^{CO35} mutant larvae. (F) Quantitation of less than 500nm apical vesicles. (G) Quantitation of greater than 500nm organelles. (H) Quantitation of midgut microvilli length. Each dot represents a single microvillus length combined across three animals. (I) Electron micrographs of kidneys in wild-type, *fip5b*^{CO40} mutant, *fip5a*^{CO35} mutant, and *fip5a*^{CO35}*; fip5b*^{CO40} double mutant larvae. N indicates number of representative kidneys out of total number of kidneys analyzed. Arrows point to multiple lumens in *fip5a*^{CO35}*; fip5b*^{CO40} double mutant larvae. All plots show mean ± s.e.m. A t-test was used for Gaussian data and a Mann-Whitney test for all other statistics. ***P < 0.0005.

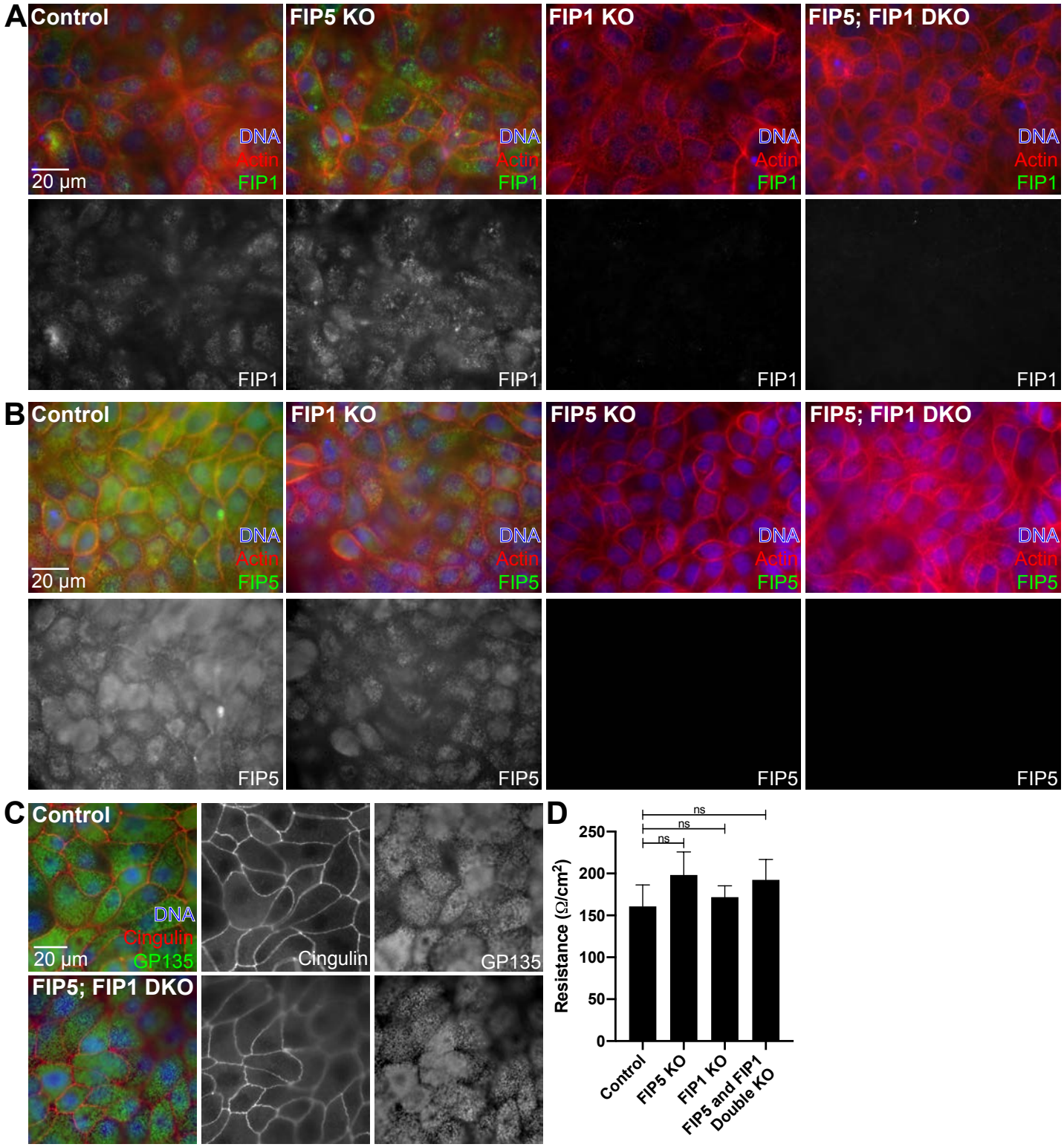

Figure S4. (A) Wild-type, FIP5 KO, FIP1 KO, and FIP5 and FIP1 double KO MDCK cells grown in polarized monolayers and stained for Hoechst (blue), Phalloidin (red) and Fip1 (green). (B) Wild-type, FIP1 KO, FIP5 KO, and FIP5 and FIP1 double KO MDCK cells grown in polarized monolayers and stained for Hoechst (blue), Phalloidin (red) and Fip5 (green). (C) Wild-type and FIP5 and FIP1 double KO MDCK cells grown in polarized monolayers and stained for Hoechst (blue), the tight junction marker Cingulin (red) and the apical membrane marker GP135 (green). (D) Trans-epithelial resistance measurements on wild-type, FIP5 KO, FIP1 KO, and FIP5 and FIP1 double KO MDCK cells grown in polarized monolayers from three biological replicates.

Table S1. Primer sequences. Primers were designed using the NCBI/Primer-BLAST tool.

Fip5a ISH For Antisense	TACAACAAACGCCTCCGCTA
Fip5a ISH Rev Antisense	TAATACGACTCACTATAGGGCGCGTTGTGCAACAAAAACC
Fip5b ISH For Antisense	GAAGCGCTCCGTCCCAAATA
Fip5b ISH Rev Antisense	TAATACGACTCACTATAGGGTGATTCACTACAATCTCAGACCTCA
Fip5a genotyping For	CCACTGTCTTATGTGCCCGT
Fip5a genotyping Rev	TGCTCTTCCGATCCTGAAAGG
Fip5b genotyping For	GAGAGCTACAGGTCACCATCC
Fip5b genotyping Rev	GCTGTAAATCGGTGTTCTGGG
Fip5aExon1gRNAolig1	TAGGCCCGAGGGTTGCGCGCGA
Fip5aExon1gRNAolig2	AAACTCGCGCGCAACCCTCGGG
Fip5bExon2gRNAolig1	TAGGTGGAAGAACACCGGAGTA
Fip5bExon2gRNAolig2	AAACTACTCCGGTGTTCTTCCA
FIP5Bset1For qPCR	GGCAAACTATTGTTCCGCTCG
FIP5Bset1Rev qPCR	TTGTTGCGGGTGAACTGGAT
FIP5Bset2For qPCR	AAATCCAGGACGATCTGCTCT
FIP5Bset2Rev qPCR	CGCTGCTTCTTGATCTCCAAT
Rpl13aFor qPCR	TCTGGAGGACTGTAAGAGGTATGC
Rpl13aRev qPCR	AGACGCACAATCTTGAGAGCAG
GAPDHFor qPCR	GTGGAGTCTACTGGTGTCTTC
GAPDHRev qPCR	GTGCAGGAGGCATTGCTTACA
FIP1KO gRNA	GTGATAACCCAAGGGCACTG
FIP5KO gRNA	GGGTTCATTTGGGGTCACAT