### **Supplementary Information**

# Intestinal intermediate filament polypeptides in *C. elegans*: Common and isotype-specific contributions to intestinal ultrastructure and function

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**Supplementary Figure S1.** Graphic representation of gene models proposed for the *ifc-2* gene locus and their corresponding protein sequences. (a) The *ifc-2* gene consists to two partially overlapping transcriptional units. Promoter 1 drives transcription starting at exon 1 and generates variants *ifc-2a*, *ifc-2b* and *ifc-2c*, which encode polypeptides with different carboxytermini. Promoter 2 drives transcription starting at exon 11 and generates variants *ifc-2a*, and *ifc-2e*, which encode polypeptides with different carboxytermini in the same way as *ifc-2d* and *ifc-2b*, respectively. Differentially translated regions are highlighted in red. The position and types of tags integrated into the *ifc-2* locus are shown for alleles *qp110* and *kc16*. (b) shows the amino acid sequences for all IFC-2 isoforms. Identical sequences are color-coded for better comparison.



**Supplementary Figure S2.** Immunoblot detects polypeptides IFC-2a::YFP and IFC-2e::YFP produced from *ifc-2* allele *kc16*. Cytoskeletal protein extracts were prepared from wild-type N2, *ifb-2a::cfp* reporter strain BJ49 and strain BJ316 containing a YFP-encoding cassette that is inserted into the 3'-end of *ifc-2a* and *ifc-2e*. Polypeptides were separated by 10% SDS gel electrophoresis, transferred onto PVDF membrane and reacted with anti-GFP antibodies. Position and size in kDa of co-electrophoresed molecular weight markers are shown at left of each blot. One can detect a major species expected for the IFC-2e::YFP fusion (calculated size 83 kDa; black arrowhead in a, b, b') and a minor species expected for the IFC-2a::YFP fusion (calculated size 155 kDa), which is only visible after long exposure (red arrowhead in b'). Since IFB-2a::CFP is produced at much higher levels, 15 times less BJ49 lysate was loaded. Note that the apparent molecular weights are consistently higher than the calculated molecular weights of all IF polypeptides (calculated molecular weight of IFB-2a::CFP 89 kDa). The asterisk marks a non-specific signal that is present both in wild type and in BJ316.

IFC-2a/e::YFP



**Supplementary Figure S3.** Only promoter 2 of the *ifc-2* gene locus is active in the intestine. The maximum intensity fluorescence images show a comparison of the fluorescence in strain BJ316 producing tagged IFC-2a/e::yfp variants from allele *ifc-2(kc16)* (left column) and BK531 producing an aminoterminally 3xFLAG- and GFP-tagged IFC-2a/b/c reporter (corresponding to GFP::EXC-2 in <sup>1</sup>; right column). While IFC-2a/e::YFP is localized in the intestine (black arrow), the excretory canal (orange arrow), corpus and posterior bulb of the pharynx (red and green arrow, respectively), pharyngeal-intestinal valve (blue arrow) and interfacial uterine cells (pink arrow), no intestinal expression can be observed for IFC-2a/b/c, which instead presents a very prominent localization in the intestinal-rectal valve (similar regions marked with black arrowheads). Scale bars: 50 µm.

#### a ifb-2(kc14)

cgacttcatca <mark>tG TCG GCG GTT AGT TAT TCG ATG CAC AGA ACC ACC ACA A[T]C TAC ATC CTC ATC ACA CGG AGG TGT CTC AGC CGG CCA TGC CGC TGA GGAGTTC</mark>
GTTGCCTCTGCCGAGCGCGAGAAGCAAGAGATGCAGCAGTTGAACTCTCGCCTTGAAGTTTACATCAGCCGTGTCCGTCAACTTGAGCAGCGAAGAGCATTGGATTGGATTGGACACCCCTCCGAGGATCACTTG
GAAATGACATCGGACAGATCAAGTTCAAATTCAACGATTCGTTGGTCAAAGTTCGCCGTGAGATCTCAGAAGCTCATTCTGGAACTTATTGGAGTTGAAGGTCGACAGATTGAGAGATGATTGAATGACTACAG
ACACAGATATGAAGAGGCCCGTCGTGAAGTTGAGCGTGAGAAGACCACTTGGGGAGGAGCTATTTCACAAGCACAAGCTGAGCTTGACACAAAGTCCCGCCATTTGGCGCGAGAGAGA
GCTGAACAAGATCAACTCTATCTTCAATTGGCTGCCGCCAAGGATGAGCTTGACGCCGCCAATTGTTGATCGCCGCCGCCTTCAAGCTGAGAAGATGACCTCAAGATTGAGCTGGAATTCTTGGGAAGAATCCACTCTC
AAGAAATCACTGAGCTTCGCACTCTTCTTGCTCAAGCTCCAGCCGACCAAGAGAGTTCTTCAAGAATGAAT
TGAGACAATCTTCCAGAGCAAGATTAGCGCCGTCGAATCATCAATCGTCTCCAAGAACGAGGCTGCCGTTTTCCGTCAAGAGGAGATCAGAAAGATGAACGAGAGCATCACCACATTGCGAGCCAAGCTCAGCGAGCTC
GAAGCCCGTAACTCTGCTTTGGAGAGAGAGAGCAACACCACCCCAGATTCAACTCGGAGAAGATCAAAGAGCTTACGAATCAGAGCTTCACAAGAGAGACAATGCCCTCCGATTTATGCGTGAAGACTGCCAGACTTTGA
TTGCTGAACTTCAAGCTCTTCTTAACACCAAACAAACTTTGGATACTGAGATTGCCATCTACAGAAAGCTTGTCGAGTCCGAGGAAGGA
AAGATTGGTACCAGTCGAGCAAGATCACTGGGACTCTGGAGAGGTTCAAACTCGTTCCTCATTCAAGAGACACGCTAAGGGAAATGTGAGCATCGTTGAGTGCGATCCACAAGGAAAGTACATCATTCTTGAAAACACC
AGTGGATCAGTTGCCGAAGATGTAAGCAACTTTGAAATCCGTCGTGTTATTGATGGAGTTCAAGCCTTCGTTTTCCGTCTCCCATCTCACTTAGTTATTCAACAACACGGACATCTAAGGATCTACGGACGCAATTCAG
GAGGAATTAACTCACCAGACTCAATTGTGATGGAATCTCACCCATCATGGGGACAAGGAGGACAAGTTGAAACATTCTTGTACAATAGTCACGGAATTGAGAAGGCTTCGCACATCCAGACGACGGCGCCGCTTCTTC
$\frac{CCGTTAA}{CG}$ gaaateteeatetaategattettettegatgaatttaatetegaaatgeeeattgaateettteettgetgeetteeateea
ttgttttttgaaataaattgttcaatattg

# b ifc-2(kc15)

gagaaacggagaaacacagcgaccagcgccaaactgattacagttggcattatgttgaaggcataggtggagtcgcaagggttgcctataaaacattgtctaatcagtgccattttctcattttttatcattattaact
tctaaaatcgacaaaaaaaaaccaaaaa <mark>ATG TCG ACT TAC GCG GCC TAT ACT ACT TCT ACT TAT CAA GGT CGT GGA CTT GCC AGT GGA ACA TAT GCT <del>TCTGGCTTTGGCCAGCTTG</del></mark>
T <del>CAGTGGAATGTC</del> ATC TGC TGC TGC CAT CTG CAC AAC CCA AAT CCG  <mark>TGA</mark>  CGCGCGTGAACGTGAGAAGAGAGAGAAATTGGTCTCTTGAACGATCGTCTCGCGGATTACATTGAGAAGAGAGAAG
GGAAGCTCAAAACCGATGCCTCAGCCACGATATTGATATTCTTCGCAACGGATTCAGCGGTGGAGGTCATGTATCTGGACTCTTTGACGCTGAGATCAACCAAAGCATATTCTTGAGCAAAACAACCGCTCACCGC
TCCACATTTGAAAGAGACATCACTGGTCTCTCCGCGGAGATTGAAGTGTTCAGAAAGAA
CTGAAATTTCACTTTTCAAAAGAAAGAACGATCCGTATTGTTGAGGAAGACGTCATCAGAATTCGCAGAGAAAATGACGGAATTTACAATGAAATCGCCAGAATCAAGCAGCTTACCCATAACGAAATTGCTCTGAAGAATGA
GCGCAGCTTGAATGTCTCCGATCTTCTTCAGCGCATCAACCTCCTACAAACGGAGAACAACGTCAGAATCGAGCAAGAACTTGTCTTCATCCGCCGTGACACCACTGCCGACAACAGCGTGATTACTTCAGACATGAGCTT
CAAGCCGCCATTCGTGACATCCGTGCCGATTATGAAGCCATCAGCATCCGCAACCGTCAAGACATTGAAGTGTGGTACCGTGAGCAAATCAGAAAGATTCAGGAACGAATCAGGACGCATCAGCACCCAGACCTCTACAAGG
AAGAACTCATCTCCATCCGCACGACCGTGACAAATGTCCGAAGCAGACTTGCCGAGGTTGAAGGAAG
TGCCGAGAGAGACGCCCAGATTGCGACCCTCCGTGACCAGTGCACAGAGCTCAGCATTCAAATGGAGAAACTCTGTGACAACGAAATTTCCCTCCGCGCCCGAAATCGAGCGCTATCGCATCCTCCTCAACGGAGCAAAC
GTGACAACGTACACCTCCAATACGCACGGAAGCGGCTCTGGAATCGCAGTCGGTGGTGTGTTGTTGGAGGATCAACCCGCGTTATATCTCAAACCACGAGAACCCACAGCAGCAGCAGCAGCAATACGAGCTACTCAAATGTCCCTG
CCTCTAGAGGCGGCTACTCCATCAGTGGGAACGTTGGAGGAATTTCAGTTGGAGGAACCATTGGATCGCACGGAGCTTCGGCCCATGCGACAGGAGGAATCATTGGCTCTGGAGTTCAGGCGCACAGAGGAAGTGGTAC
GTATCAACAGAGCCA <mark>T</mark> CACTCCTACTCCTCCTCCAAATCAC <mark>CGGTCCA</mark> TGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCGGCGACGTAAACGGCCACAAGTTCAGCGTGCCC
GGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCTTCGGCCACGCTGCAGTGCTTCGCCCGCTACCCCGACC
ACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGGCAACTACAAGACCCGCGCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCAT
CGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCAC
AACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAAACACCCCCCATCGGCGACGGCCCCCGTGCTGCTGCTGCCCCACCAACCA
ΑΤΓ ΑΓΑΤGET ΓΓΕΓ ΤΕ GAGT ΤΓ ΕΤΕΑΓ ΓΕΓ ΓΕΓΕ Ε GEAT CAT ΤΓ Ε GEAT GAE CAE CAE CAE CAE CAE CAE CAE CAE CAE C

### C ifp-1(kc18)

cgc <mark>ATG GATTCCGCTAACGCTAGGGATTGTCTA</mark> CT ACA TCT GGC AAG AGC  <u>TAA</u>  GCTCTCTGAGCGACAAGACCTTGTTCAGCTCAACGATTAGGGATATCATCGAACATGTCCACTATATGGAAGC
TGAGCACACTGCTTTGGAACACGACTACAATTTGCTAAAAAGTGGAGTCCAGAGCGACAGTAGTGGGAATTAATGAAATTTACAATGTTGAGATCAGGACTGTCAGAATCGGGAATTGAAGAAATTAACCGAAGCCGGCAC
GAACTACTAAATGAGCAAACCCAGTTGTCCCATCAAGTCAAAGAAGCCGAACAACTTTGGAGACACACTGCAAAATCAGCTTTGGGGGTTCCCAAAGAGGTTGATGATGAGATCCGAAAGAAGCGTTGACAAAGAAGCTGAAGAAGCAAAGAAGCAAAGAAGAAGAAGAAAAAAAA
AAGATTGTATCAACTAAGAAGAATCAAGTATATGGAGGATAAGCTCAGACTGATCAAACAGAACAATGGAAGAATTTTTGAGCATATTAATTTGATGAGAATG <mark>AGAAAAGATCAAGCTGTCAGCTCGCAACAAGAATA</mark>
TCTTCTTCGCAAGAATGAGTTGTTGCACAGTATCCGAAATATGGAGGAAGACAACAAAAAGATTATCATGAATGA
GCAATTGCTGATATTCGTGCTGATTATGAGGCTAACAGAATTGAGAAACGAAGAAGAAATTCGAATTGCCTTGGAAAGGGAAATTCGTATGGACAAGCGAGGAGTAGTTTGCTATGACAAACTGAGAGAGG
AGCTITCGATTGTTAAGAACAATCTITCTGGATTGCAGAAACAAGTITCTGAAGTTGAAATCAGAAACAATTCTCTCACTCAACAAATTGAATTCTATCGTCTGGAAATTTCCGGAAAATAACAAGCTITTCGAGGATAC
TTTGGAATTCAAAATAAAGGAAATTGAGAAAGATGAGAGAGA
GGAGATCCTCGTGCAAATGCGCCAAGTACTCAAGTTATAGCAAGCGCGCCTTAGTCGACAGAACTACAGCAGCATTTCTGACCACCATAGCCGAACTTCTTCTGGAATAGTTCAGCACACTGCTCATCACGCTGTGAGAG
ATTCCTCGTCGTCCAGAACTGGTACCACTATTCATGAGACCATCACCACGCCAATTCCAATTCCTGCTACACTTCTTGTTCAACCAATTCGTGAATATACTTATTCGAGGGACGCGAGCCCGATTCGTCCATCATA
TACTCCGTACCAGCAGGAGTCTCGTGCTGATAGCCGATCTTCTCTCAGGGAGAGAATTCAAATCGAGACTCTCCAAGGCACACTTCCGGTCTGCCATCGGTTGAAACAATTCGTGCTTCAGATAACAACCCCGA
TTAGTGAGAGACCTTAGCGCGGGAGGTCAGATTAACACAACACAGATTAACAATCCATACGCGTCTCGTACTCCAACTTCATCGGTCAATGATAGGATTGCAAAGCGTCGTGATAGTGAAATTCGTCGCAACGATG
CTTTACATCAACCTTATCCAATTGGTGGTGCCGTTCCTCGAGTTGAGCCTACAATTTTTAATGAACGTGCTCCCGGTTACACTCATATTAATAGCAACTTCCCGCTAATTACGCCAGTGACTGAAGGAGCTACTGTCCC
CTCATACTACGATACTTTTAATCGGAACTCGAGGCCAAAGAGGGCCACATCATTCGAGCTATCATGCAGCAACAGGCTCAGTGAGCAATAGCATCTCCACTACTCTAATGAAGGATCAAATTGAACATAGCAGTAGCA
TCCGATAGTGTTGACGAGGACAACTTCCAGAGATTCACTAGATGGTACAAGGGGAGAGTGAAGATTTCGGATGTGCAGATTTTGTCAACTTGTAAATAGAAGCTCGAAAAAATCGGCAGACGTTGGTGGGCTTCA
AACTTATTCACGAGTTTGGAAACAAGAGCGTATATGTTGATCTTCCAGTTGGATTGATCCTTGCACCCAAGGATAGCTTGAAGATCTACGCTCGTGGAGCAACTCATGAAAGGAGGAGCAGTTATTGCTGAAATTGACTT
CTTCGATACCACCATACCAATACATCAATACATCAGAAACACGAATAATGAGGTGAAAATCGTGGTTTGTTT
ctgataacatgataattctaacaaaaatatattttattt
attcaatcccagtaattcgtatttctttcatattctgaatttaaaataaacctattact

#### **d** kcls41[ifp-1p::ifp-1[16-19del]::egfp \*kcls40]

cgcATG GAT TCC GCT AAC GCTAGG ATT GTC TAC TAC ATC TGG CAA GAG CTA AGC TCT CTG AGC AAG ACC TTG TTC AGC TCA ACG ATC AGT TTG TGG ATA TCA
TCG AAC ATG TCC ACT ATA TGG AAG CTG AGC ACA CTG CTT TGG AAC ACG ACT ACA ATT TGC  <u>TAA</u>  AAAGTGGAGTCCAGAGGCGACAGTAGTGGAATTAATGAAATTTACAATGTTGAGAT
CAGGACTGTCAGATCGGGAATTGAAGAAATTAACCGAAGCCGGCACGAACTACTAAATGAGCAAACCCAGTTGTCCCATCAAGACGCCGAACAACTTTGGAGACACACTGCAAAAATCAGCTTTGGGGGTTCCC
AAAGAGGTTGATGATGAGGTTCCATAGAATCTGTCATATCAAGATGGAAGATTGTATCACTAAGAGAAGAATCAAGTATATGGAGGATAAGCTCAGACTGATCAAACAGAACAATGGAAGAATTTTTGAGCATATTAATT
TGATGAGAATGAGAAAAGATCAAGCTGTCAGTCTGCAACAAGAATATCTTCTTCGCAAGAATGAGTTGTTGCACAGTATCCGAAATATGGAGGAAGACAACAAAAAGATTATCATGAAGCACAAATACTTTGTTCG
TGATCGCAATGCGGATCGTCACGTCTTCCGCGATCAACTTCGCAAGGCAATTGCTGATATTCGTGCTGATTATGAGGCTAACAGATTGAGAAACGAAGAAAATTCGAATTCGCTTGGAAAGGGAAATCCATCGAATG
AATACCACTATGCCAGGAGTAGTTTGCTATGACAAACTGAGAGAGGAGCTTTCGATTGTTAAGAACAATCTTTCTGGATTGCAGAAACAAGTTTCTGAAGTTGAAATCAGAAACAATTCTCACTCA
TCTATCGTCTGGAAATTTCGGAAAATAACAAGCTTTTCGAGGATACTTTGGAATTCAAAATAAAGGAAATTGAGAAGATGAGAGAGA
CTTGCAAAAGGAAATCGCAAAATATCGGGAACTTCTCGACAGATCTGGAGATCCTCGTGCAAATGCGCCAAGTACTCAAGTTATAGCAAGCGCGCGTTAGTCGACAGAACTACAGCAGCATTTCTGACCACCACCATAGCCGA
ACTTCTTCTGGAATAGTTCAGCACACTGCTCATCACGCTGTGGGGGAGATTCCTCGTCGTCCGGACTGGTACCACTATTCATGAGACCATCACCGCCAATTCCAATTCCTGCTACACCTGTTCAACCAATTC
GTGAATATACTTATTCGAGGGACGCGAGCCCGATTCGTCCATCATATACTCCGTACCAGCAGGAGTCTCGTGCTGATAGCCGATCTTCTCTCAGGGAGAGATTCAAATCGAGACTCTCCAAGGCACACTTCCGGTTCT
GCCAAGCCATTCGGTTGAAACAATTCGTGCTTCAGATAACACCCCGATTAGTGAGAGACCTTAGCGCGGGAGGTCAGATTAACACAACACAGATTAACAATCCATACGCGTCTCGTACTCCAACTTCATCGGTCAATGAT
AGGATTGCAAGCGAACGTCGTGATAGTGAAATTCGTCGCAACGATGCTTTACATCAACCTTATCCAATTGGTGGTGCCGTTCCTCGAGTTGAGCCTACAATTTTTAATGAACGTGCTCCCGGTTACACTCATATTAATA
GCAACTTCCCGCTAATTACGCCAGTGACTGAAGGAGCTACTGTCCCCTCATACTACGACACTTTTAATCGGAACTCGAGCCAAAGAGGGGCCACATCATTCGAGCTATCATGCAGCAACAGGCTCAGTGAGCAATAGCAT
CTCCACTACTCACTCAATGAAGGATCAATTGAACATAGCAGTAGTTCCGATAGTGTTGACGAGGACAACTTCCAGAGATTCACTAGATGGTACAAGGGGAGAGTGAAGATTTCGGATGTGACTGCAGATTTTGTTCAA
CTTGTAAATAGAAGCTCGAAAAAATCGGCAGACGTTGGTGGCTTCAAACTTATTCACGAGTTTGGAAACAAGAGCGTATATGTTGATCTTCCAGTTGGATCGACCTAGAGGATAGCTTGAAG <mark>ATCTACGCTC</mark>
GT6GAGCAACTCATGAAAGAGGAGCAGTTATTGCTGAAATTGACTTCTCCGATACCAACCA
<mark>AGATGCAGACCATCATCAT</mark> GAAGTGCATACCAATCAGGACCCGCTGGATGAAGTCCACACAAACCAAGATCCACTCGATACTAGT <mark>ATGAGTAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTA</mark>
GATGGTGATGTTAATGGGCACAAATTTTCTGTCAGTGGAGAGGGGGGGG
TAACTAACCCTGATTATTTAAATTTTCAGCCAACACTTGTCACTACTTCTGTTATGGTGTTCAATGCTTCTCGAGATACCCAGATCATATGAAACAGCATGACTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACA
GGAAAGAACTATATTTTTCAAAGATGACGGGAACTACAAGACACGTAAGTTTAAACAGTTCGGTACTAACTA
GAGTTAAAAGGTATTGATTTAAAGAAGATGGAAACATTCTTGGACACAAATTGGAATACAACTATAACTCACACAATGTATACATCATGGCGGACAAACAA
TACTAACTAACTAACTAATCTGATTTAAATTTTCAGAACTTCAAAATTAGACACAACATTGAAGATGGAAGCGTTCAACTAGCAGACCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGTCCTTTTACCAGACAACCA
TTACCTGTCCACAAATCTGCCCTTTCGAAAGATCCCAAACGAAAGAAGAGACCACATGGTCCTTCTTGAGTTTGTAACAGCTGCTGGGATTACAAATGGCATGGATGAACTATACAAATCCGGAAGTTCCTATTCTCTA
GAAAGTATAGGAACTTCCCTCGAGGATTATAAAGACCACGATGGAGACTATAAAGATCATGACATTGACTACAAGGATGACGACGACGACAAGTGAtacattactaatgccaaattt

Supplementary Figure S4. Genetic alterations of alleles *ifb-2(kc14)*, *ifc-2(kc15)*, *ifp-1(kc18)* and kcls41[ifp-1p::ifp-1[16-19del]::egfp \*kcls40]. (a) shows the spliced gene model of ifb-2 isoform a, (b) the gene model of *ifc-2e* with carboxyterminal *yfp* tag highlighted in green (*ifc-*2(kc16); linker in blue, silent mutation of the PAM motif in purple, bold and underlined), (c) the spliced gene model of *ifp-1* isoform a and (d) the gene model of integrated *ifp-1p::ifp-1::egfp* reporter fusion (kc/s40). Note the carboxyterminal egfp tag with artificial introns highlighted in green. Exons shown in alternating colors, non-translated regions in grey letters and translational start codon in green according to <u>www.wormbase.org</u> (Release WS272). (a) Mutant allele *ifb-2(kc14)* contains a thymidine insertion at position 41 (bracketed in red) leading to a premature stop in the second exon (TGA underlined) resulting in a truncated 29 amino acid-long protein encompassing only 13 of the most aminoterminal amino acids of IFB-2a. The mutation also affects all other known IFB-2 isoforms, which share the same start codon and the aminoterminal part encoded by the first five exons. (b) Mutant allele *ifc-2(kc15)* contains a 32 base pair deletion in the first exon (crossed out) causing a premature stop (TGA underlined) resulting in a truncated 34 amino acid-long protein encompassing only 23 of the most aminoterminal amino acids of IFC-2d and e. (c) Deletion allele ifp-1(kc18) carries a 26 base pair deletion in the first exon (crossed out) causing a premature stop (TAA underlined) leading to a truncated 6 amino acid-long protein encompassing no amino acids of all IFP-1 isoforms. (d) kcls41[ifp-1p::ifp-1[16-19del]::egfp \*kcls40] contains a four base pair deletion after position four causing a frameshift in the second exon leading to a truncated 52 amino acid protein encompassing only four of the most aminoterminal amino acids of IFP-1::EGFP fusion protein.



**Supplementary Figure S5.** IFC-1 is not essential for intestinal lumen maintenance and IFB-2 distribution. (a-c") The micrographs show a comparison of the intestinal IFC-1::EGFP fluorescence encoded by extrachromosomal reporter *ifc-1::egfp* in control strain BJ324 (a) and *ifc-1(RNAi)* animals (b; corresponding interference contrast images in a', b'; merged images in a'', b'') and the IFB-2a::CFP fluorescence in *ifc-1(RNAi)* animals of strain BJ49 (c; corresponding interference contrast image in c''; merged image in c''). Note that *ifc-1(RNAi)* efficiently downregulates IFC-1::EGFP but does not affect lumen morphology and IFB-2a::CFP distribution. Scale bars: 25 µm.



**Supplementary Figure S6.** Loss of function mutation of *ifd-1* does not affect intestinal lumen morphogenesis whereas loss of function mutation of *ifd-2* induces luminal widening and cytoplasmic invaginations. The micrographs depict a comparison of anti-IFC-2 (a, b, c) and anti-IFB-2 immunostaining (a', b', c'; merged images in a'', b'', c'') in fixed intestines isolated from adult wild-type N2 (a-a'') and mutant strains BJ374 and BJ375 carrying mutant allele *ifd-1(ok2404)* (b-b'') and *ifd-2(bz187)* (c-c''), respectively. Scale bars: 20 µm.



**Supplementary Figure S7.** IFP-1 is not essential for intestinal lumen maintenance and IFB-2 distribution. (a-d) The micrographs show a comparison of the intestinal IFP-1::EGFP fluorescence (a, c) that is produced from the integrated fosmid-derived reporter *ifp-1::egfp* and anti-IFB-2 immunostaining (a', b') in isolated intestines either in a wild-type control background (strain BJ312) or mutant *ifp-1(kc18)* background (strain BJ320) together with a comparison of the intestinal lumen morphology detected by interference contrast microscopy (b, d). The CRISPR/cas9-mediated *ifp-1* mutations efficiently abrogates all IFP-1::EGFP fluorescence (compare a and c) but does not affect IFB-2 immunofluorescence (compare a', c'). Note also that interference contrast images do not reveal morphological changes in the mutant intestines (b, d). (e-f'') The micrographs depict IFB-2a::CFP and IFP-1::EGFP fluorescence together with corresponding differential interference contrast images (e', f'; merged images in e'', f'') in reporter strains BJ49 (e-e'') and BJ312 (f-f'') after treatment with *ifp-1(RNAi)*. Scale bars: 20  $\mu$ m in a-f''.



**Supplementary Figure S8.** Full-length blot referring to Figure 7g. The broken line indicates the position of the cutting line, which was generated to separate the upper part that was reacted with anti-IFB-2 antibodies and the lower part that was reacted with anti-actin antibodies. The corresponding co-electrophoresed and blotted marker lane is shown at left.



**Supplementary Figure S9.** Full-length blots referring to Supplementary Fig. S2 with corresponding co-electrophoresed and blotted marker lanes.

# Supplementary Table S1. Strains used in this study.

STRAIN	GENOTYPE	Generation	SOURCE/REF	USE
N2	Wild type		CGC	Wild-type control
DP38	unc-119(ed3)III		CGC	Microinjection
BJ49	kcls6[ifb-2p::ifb-2a::cfp]IV	Microinjection of plasmid <i>pifb-2::cfp</i> in N2 followed by UV- induced integration and 5x outcrossing with N2	2	Carboxyterminally tagged IFB- 2::CFP reporter
BJ324	kcEx78[ifc-1p::ifc-1::egfp; unc- 119(ed3)+];unc-119(ed3)III	Microinjection of fosmid clone 18076040888727174 F05 (transgeneome.mpi-cbg.de) in DP38	This study	Carboxyterminally tagged IFC- 1::EGFP reporter
BJ316	ifc-2(kc16[ifc-2a/e::yfp])X	Microinjection of targeting plasmid #4110 and repair plasmid #4102 in N2 followed by screening for knockin animals in F2 and 6x outcrossing with N2	3	Carboxyterminally tagged IFC- 2::YFP reporter
BJ145	kcls6[ifb-2p::ifb-2a::cfp]IV; ifc- 2(kc16[ifc-2a/e::yfp]))X	Cross of BJ49 with BJ316	This study	IFB-2::CFP/IFC- 2::YFP double reporter
BJ251	kcEx70[ifd-2p::ifd-2::egfp;unc- 119(ed3)+];unc-119(ed3)III	Microinjection of fosmid clone 095794875301489 H04 (transgeneome.mpi-cbg.de) in DP38	This study	Carboxyterminally tagged IFD- 2::EGFP reporter
BJ312	kcls40[ifp-1p::ifp-1::egfp]IV	Microinjection of fosmid clone 23762707929976346 G11 (transgeneome.mpi-cbg.de) in DP38 followed by X-ray induced integration and 3x outcrossing with N2	This study	Carboxyterminally tagged IFP- 1::EGFP reporter
RB1742	ifb-1(ok2227)II		CGC	ifb-1 knockout
BJ309	ifb-2(kc14)II, kcls7[ifb-2p::ifb- 2a[40-41insT]::cfp] *kcls6]IV	Microinjection of knockout plasmid #4113 and selection plasmid pCFJ104 in BJ49 followed by screening for knockout animals in F2 and 4x outcrossing with BJ49	3	ifb-2 knockout
BJ318	ifc-2(kc15)X	Microinjection of knockout plasmid #4123 and selection plasmid pCFJ104 in BJ316 followed by screening for knockout animals in F2 and 4x outcrossing with BJ316	This study	ifc-2 knockout
BJ374	ifd-1(ok2404)X	Cross of strain ZB4795 ( <i>ifd-</i> 2( <i>bz</i> 187[ <i>del</i> 816275-815271]); <i>bzls</i> 166[ <i>Pmec-4::mCherry1</i> ]; <i>ifd-1(ok2404)</i> ) (from Monica Driscoll and Meghan Arnold) with N2	This study	ifd-1 knockout
BJ375	ifd-2(bz187)X	Cross of strain ZB4795 ( <i>ifd-</i> 2( <i>bz</i> 187[ <i>del</i> 816275-815271]); <i>bzls</i> 166[ <i>Pmec-4::mCherry</i> 1]; <i>ifd-1(ok2404)</i> ) (from Monica Driscoll and Meghan Arnold) with N2	This study; Arnold et al., manuscript in preparation	ifd-2 knockout

BJ320	ifp-1(kc18)X;kcls41[ifp-1p::ifp- 1[16-19del]::egfp *kcls40]X	Microinjection of knockout plasmid #4135 and selection plasmid pCFJ104 in BJ312 followed by screening for knockout animals in F2	This study	<i>ifp-1</i> knockout
BK531	exc-2(qp111[Pexc- 2::gfp::3Xflag::exc-2])X		1	Aminoterminally tagged EXC- 2::GFP reporter
BK36	qpls11[vha-1p::GFP + unc- 119(+)]l;unc-119(ed3)III		CGC, <sup>4</sup>	VHA-1P::GFP reporter
BJ338	ifc-2(kc15)X;qpls11[vha- 1p::GFP + unc-119(+)] l; unc- 119(ed3)III	Cross of BJ318 with BK36	This study	VHA-1P::GFP reporter in <i>ifc-2</i> knockout
RZB213	plst-1(msn190[plst-1::gfp])IV		5	Carboxyterminally tagged PLST- 1::GFP reporter
BJ344	plst-1(msn190[plst-1::gfp]) IV;ifb-2(kc14)II	Cross of BJ309 with RZB213	This study	PLST-1::GFP reporter in <i>ifb-2</i> knockout
BJ359	plst-1(msn190[plst-1::gfp]) IV;ifc-2(kc15)X	Cross of BJ318 with RZB213	This study	PLST-1::GFP reporter in <i>ifc-2</i> knockout
	plst-1(tm4255)IV		5	plst-1 knockout

# Supplementary Table S2. Phenotype summary.

	ifo-1(kc2)	sma-5(n678)	ifb-2(kc14)	ifc-2(kc15)	ifp-1(kc18)
Endotube	Loss with junctional aggregates <sup>6</sup>	Discontinuous 7	Complete loss <sup>3, this study</sup>	Thinning <sup>this study</sup>	n.d.
Lumen	Widening <sup>6</sup>	Multiple cytoplasmic invaginations and widening <sup>7</sup>	Occasional widening this study	Occasional widening this study	Normal <sup>this</sup> study
Microvilli	Highly disordered <sup>6</sup>	Highly disordered <sup>7</sup>	Slightly disordered this study	Rarefied this study	n.d.
Embryonic lethality	None 6,8	None <sup>7</sup>	None this study	None this study	n.d.
Larval arrest	Yes <sup>3,8</sup>	Yes <sup>3</sup>	None 3, this study	None this study	n.d.
Time of development	Increased <sup>8</sup>	Increased <sup>7</sup>	Slightly increased <sup>3, this</sup> study	Normal this study	n.d.
Brood size	Reduced <sup>6,8</sup>	Reduced <sup>7</sup>	Reduced <sup>3, this study</sup>	Reduced this study	n.d.
Life span	Reduced <sup>8</sup>	Reduced <sup>7</sup>	Increased <sup>3, this study</sup>	Reduced this study	n.d.
Cry5B-sensitivity					
- Larval arrest	100% <sup>3</sup>	82.09% <sup>3</sup>	88.41% <sup>3, this study</sup>	52.17% <sup>this study, *</sup>	n.d.
- Time of development	n.a. <sup>3</sup>	9.92 days <sup>3</sup>	8.77 days <sup>3, this study</sup>	8.10 days this study	n.d.
- Survival	3	_ 3	_ 3, this study	+ <sup>this study</sup>	n.d.
Oxidative stress - Survival	3	_ 3	_ 3, this study	this study	No difference to N2 <sup>this study</sup>
Osmotic stress	+++ 3	+ 3	++ 3, this study	++ this study	No difference to N2 <sup>this study</sup>

n.d., not determined; n.a., not applicable; \*no difference to N2

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