Appendix for

Map7/7D1 and Dvl form a feedback loop that facilitates microtubule remodeling and Wnt5a signaling.

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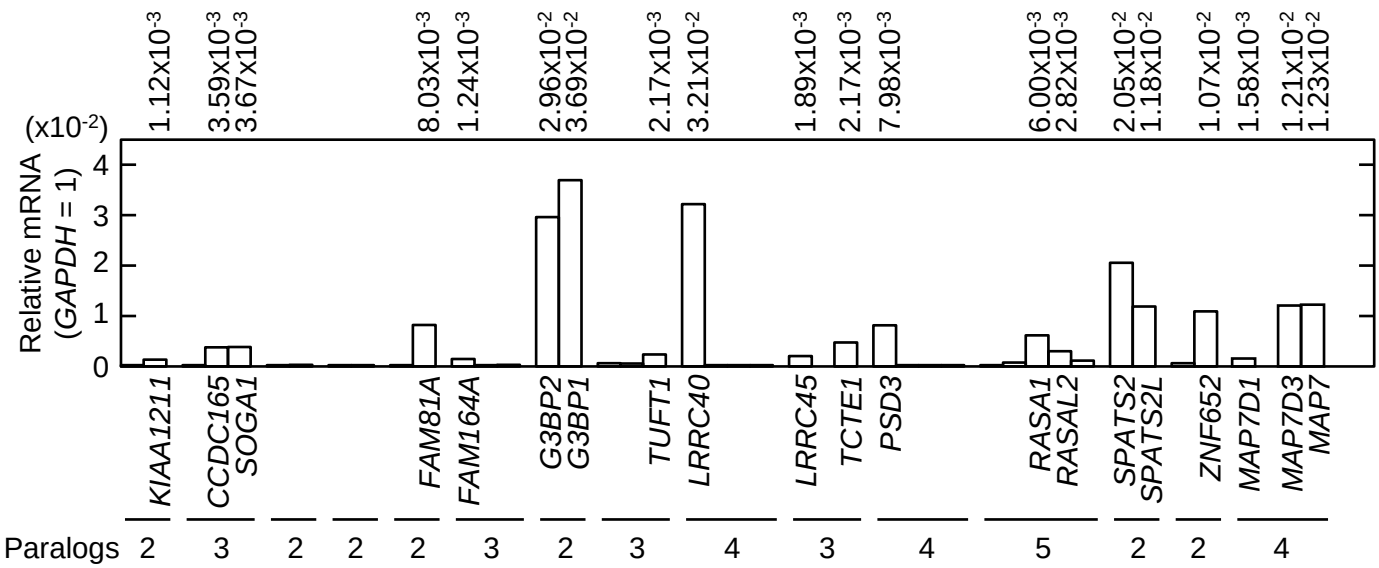
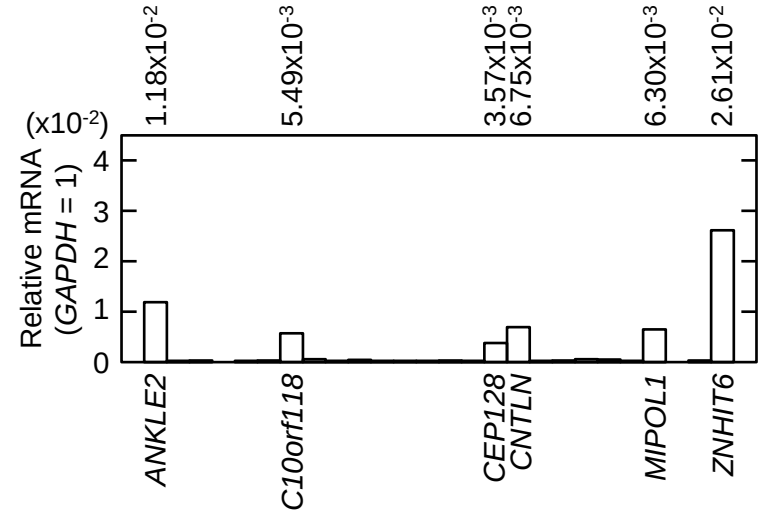
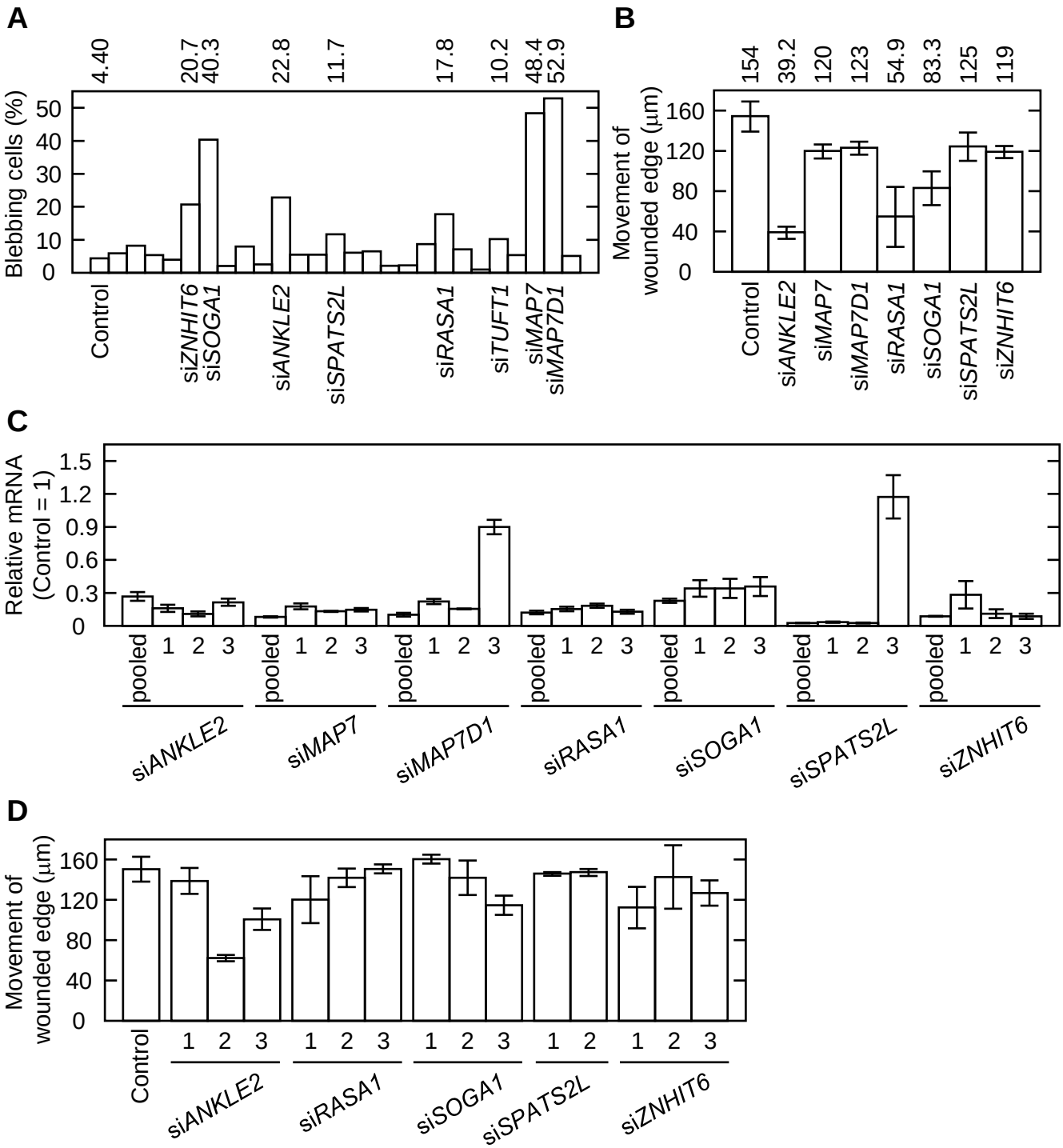
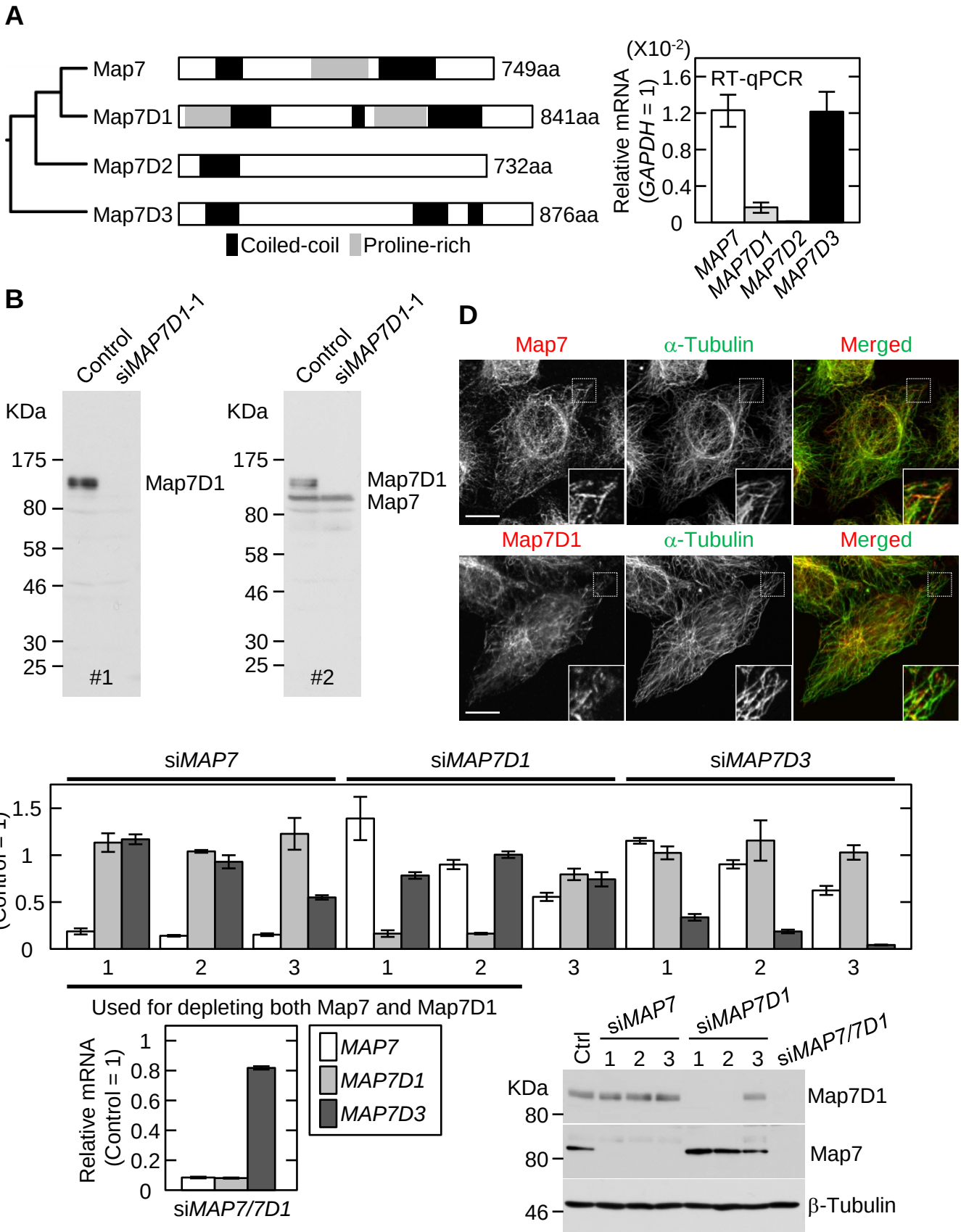
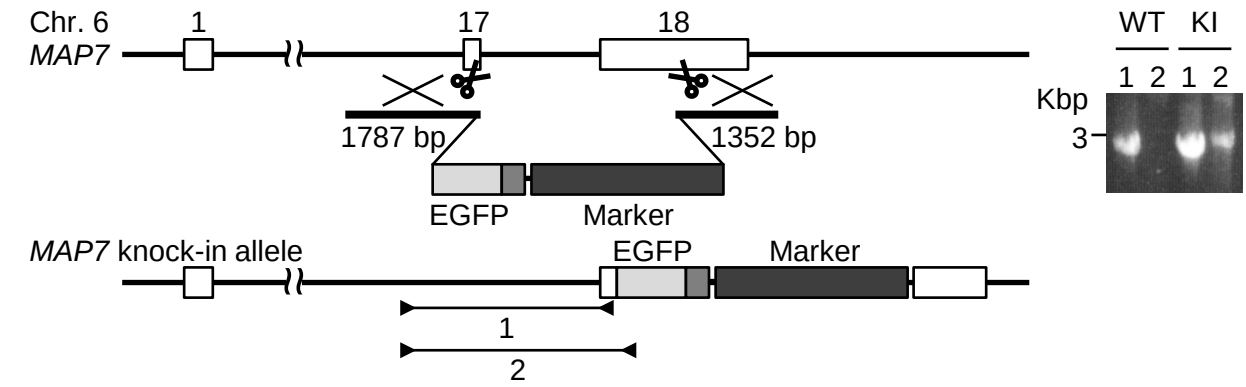

(x10-2)
Relative mRNA (GAPDH = 1)
0
1
2
3
4
1.12x10-3
3.59x10-3
3.67x10-3
8.03x10-3
1.24x10-3
2.96x10-2
3.69x10-2
2.17x10-3
3.21x10-2
1.89x10-3
2.17x10-3
7.98x10-3
6.00x10-3
2.82x10-3
2.05x10-2
1.18x10-2
1.07x10-2
1.58x10-3
1.21x10-2
1.23x10-2
KIAA1211
CCDC165
SOGA1
FAM81A
FAM164A
G3BP2
G3BP1
TUFT1
LRRC40
LRRC45
TCTE1
PSD3
RASA1
RASAL2
SPATS2
SPATS2L
ZNF652
MAP7D1
MAP7D3
MAP7
Paralogs
2
3
2
2
2
3
2
3
4
3
4
5
2
2
4


(x10-2)
Relative mRNA (GAPDH = 1)
0
1
2
3
4
1.18x10-2
5.49x10-3
3.57x10-3
6.75x10-3
6.30x10-3
2.61x10-2
ANKLE2
C10orf118
CEP128
CNTLN
MIPOL1
ZNHIT6

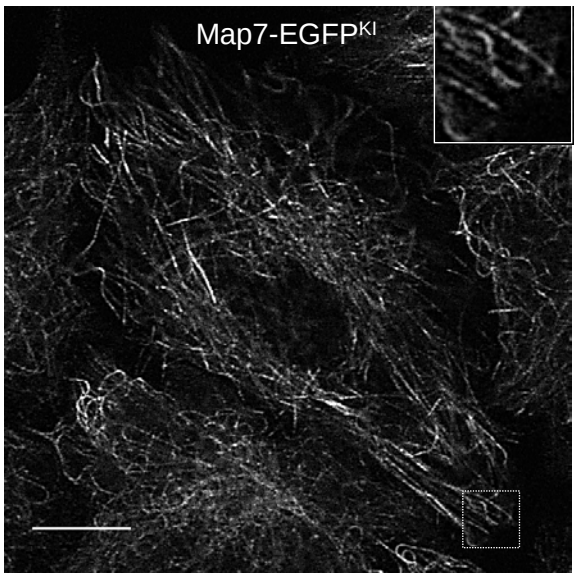

A
4.40
20.7
40.3
22.8
11.7
17.8
10.2
48.4
52.9
Blebbing cells (%)
0
10
20
30
40
50
Control
siZNHIT6
siSOGA1
siANKLE2
siSPATS2L
siRASA1
siTUFT1
siMAP7
siMAP7D1
B
154
39.2
120
123
54.9
83.3
125
119
Movement of wounded edge (μm)
0
40
80
120
160
Control
siANKLE2
siMAP7
siMAP7D1
siRASA1
siSOGA1
siSPATS2L
siZNHIT6
C
Relative mRNA (Control = 1)
0
0.3
0.6
0.9
1.2
1.5
pooled
1
2
3
siANKLE2
siMAP7
siMAP7D1
siRASA1
siSOGA1
siSPATS2L
siZNHIT6
D
Movement of wounded edge (μm)
0
40
80
120
160
Control
1
2
3
siANKLE2
siRASA1
siSOGA1
siSPATS2L
siZNHIT6

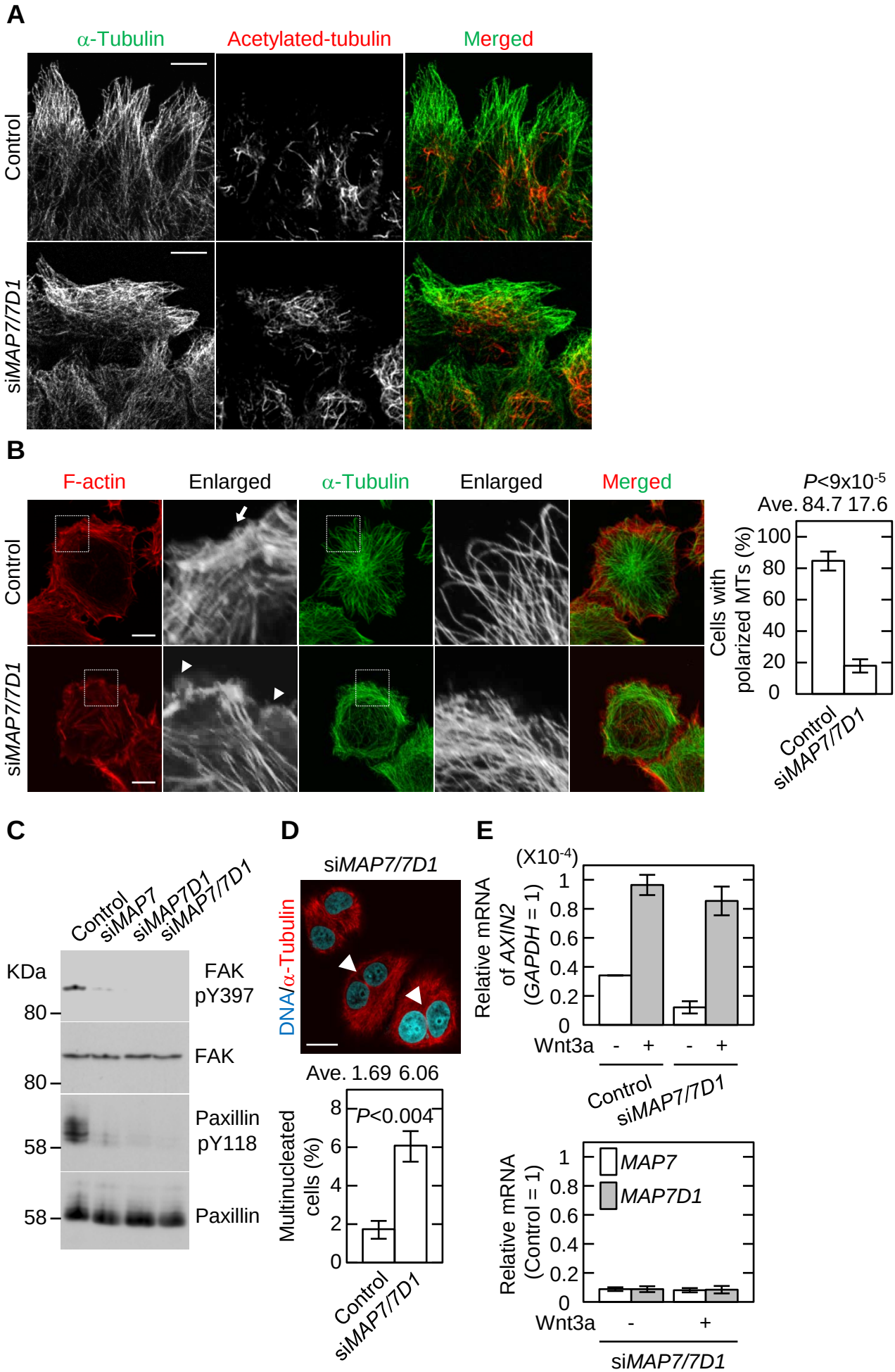

A
Map7
Map7D1
Map7D2
Map7D3
749aa
841aa
732aa
876aa
Coiled-coil
Proline-rich
(X10-2)
RT-qPCR
Relative mRNA (GAPDH = 1)
MAP7
MAP7D1
MAP7D2
MAP7D3
B
Control
siMAP7D1-1
KDa
Map7D1
Map7D1
Map7
#1
#2
D
Map7
α-Tubulin
Merged
Map7D1
α-Tubulin
Merged
C
siMAP7
siMAP7D1
siMAP7D3
Relative mRNA (Control = 1)
Used for depleting both Map7 and Map7D1
siMAP7/7D1
MAP7
MAP7D1
MAP7D3
Ctrl
siMAP7
siMAP7D1
siMAP7/7D1
KDa
Map7D1
Map7
β-Tubulin

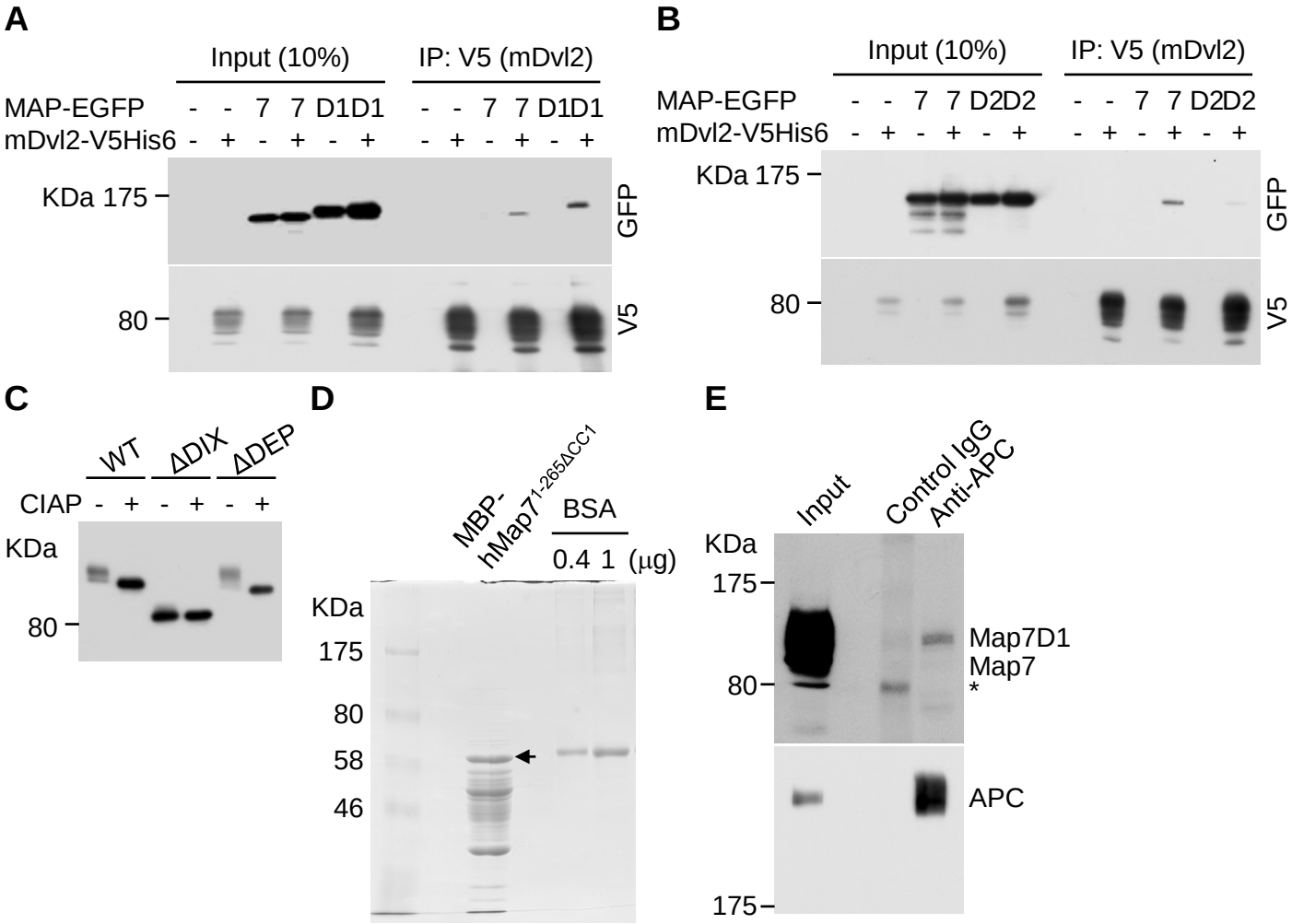
A

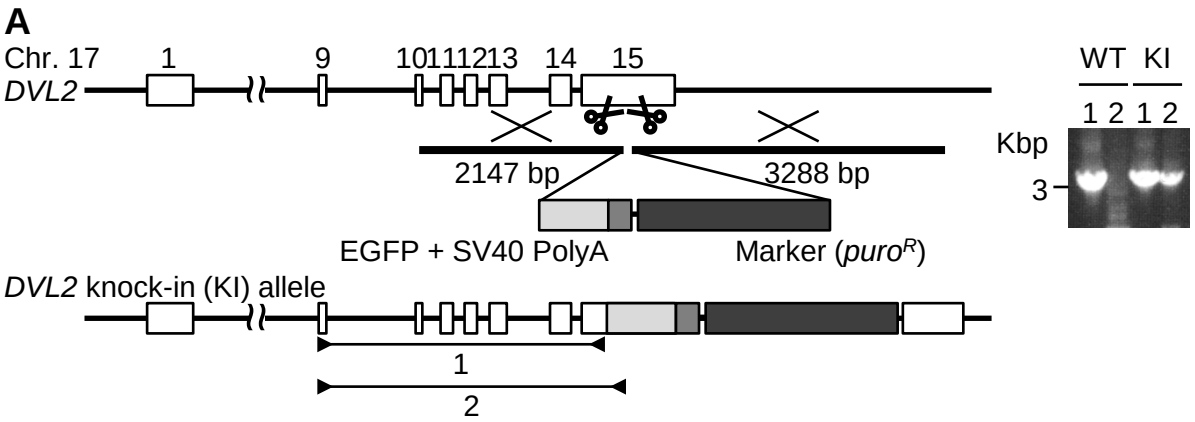

Chr. 6
MAP7
1
17
18
1787 bp
1352 bp
EGFP
Marker
MAP7 knock-in allele
EGFP
Marker
1
2
WT
KI
1 2 1 2
Kbp
3

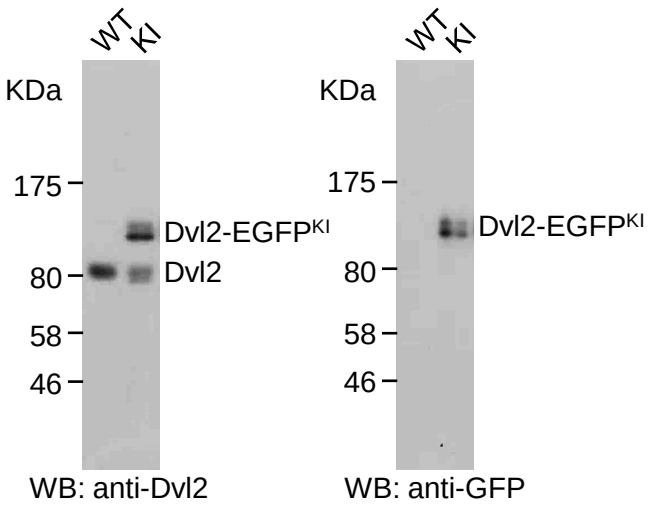
B

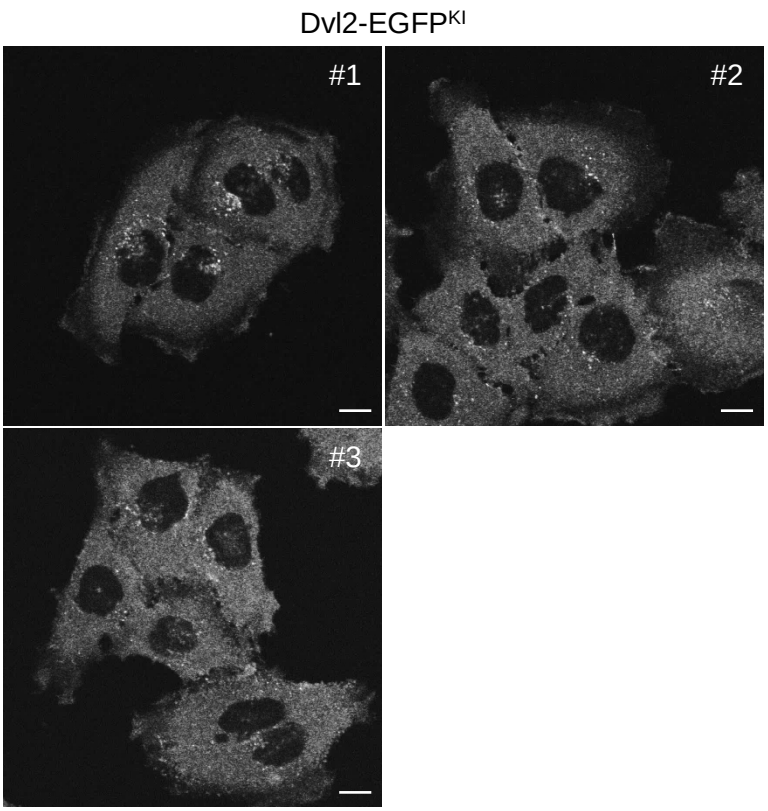

Map7-EGFPKI

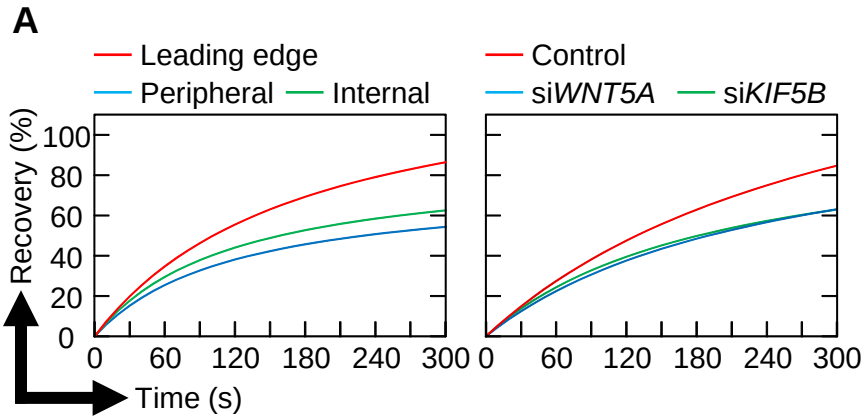

A
α-Tubulin
Acetylated-tubulin
Merged
Control
siMAP7/7D1
B
F-actin
Enlarged
α-Tubulin
Enlarged
Merged
Control
siMAP7/7D1
P<9x10-5
Ave. 84.7 17.6
Cells with polarized MTs (%)
100
80
60
40
20
0
Control
siMAP7/7D1
C
Control
siMAP7
siMAP7D1
siMAP7/7D1
KDa
80
80
58
58
FAK pY397
FAK
Paxillin pY118
Paxillin
D
siMAP7/7D1
DNA/α-Tubulin
Ave. 1.69 6.06
P<0.004
Multinucleated cells (%)
8
6
4
2
0
Control
siMAP7/7D1
E
(X10-4)
Relative mRNA of AXIN2 (GAPDH = 1)
1
0.8
0.6
0.4
0.2
0
Wnt3a - + - +
Control
siMAP7/7D1
Relative mRNA (Control = 1)
MAP7
MAP7D1
1
0.8
0.6
0.4
0.2
0
Wnt3a - +
siMAP7/7D1

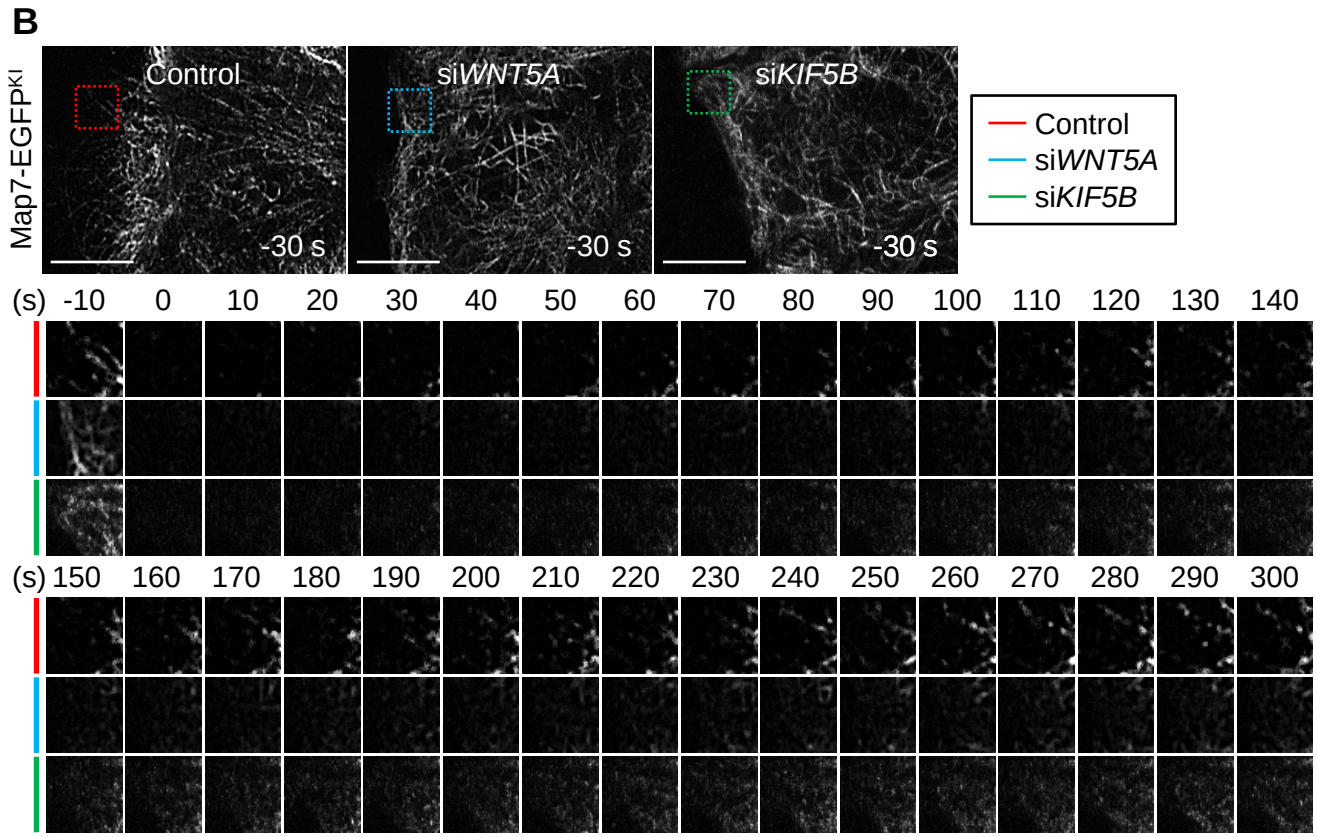

A
Input (10%)
IP: V5 (mDvl2)
MAP-EGFP - - 7 7 D1D1 - - 7 7 D1D1
mDvl2-V5His6 - + - + - + - + - + - +
KDa 175
80
GFP
V5
B
Input (10%)
IP: V5 (mDvl2)
MAP-EGFP - - 7 7 D2D2 - - 7 7 D2D2
mDvl2-V5His6 - + - + - + - + - + - +
KDa 175
80
GFP
V5
C
WT
ΔDIX
ΔDEP
CIAP - + - + - +
KDa
80
D
MBP-hMap7 1-265ΔCC1
BSA
0.4 1 (μg)
KDa
175
80
58
46
E
Input
Control IgG
Anti-APC
KDa
175
80
Map7D1
Map7
*
APC
175

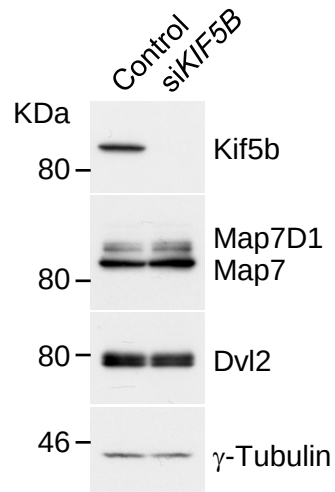

A
Chr. 17
DVL2
1
9
10
11
12
13
14
15
2147 bp
3288 bp
EGFP + SV40 PolyA
Marker (puroR)
DVL2 knock-in (KI) allele
1
2
WT
KI
1 2 1 2
Kbp
3

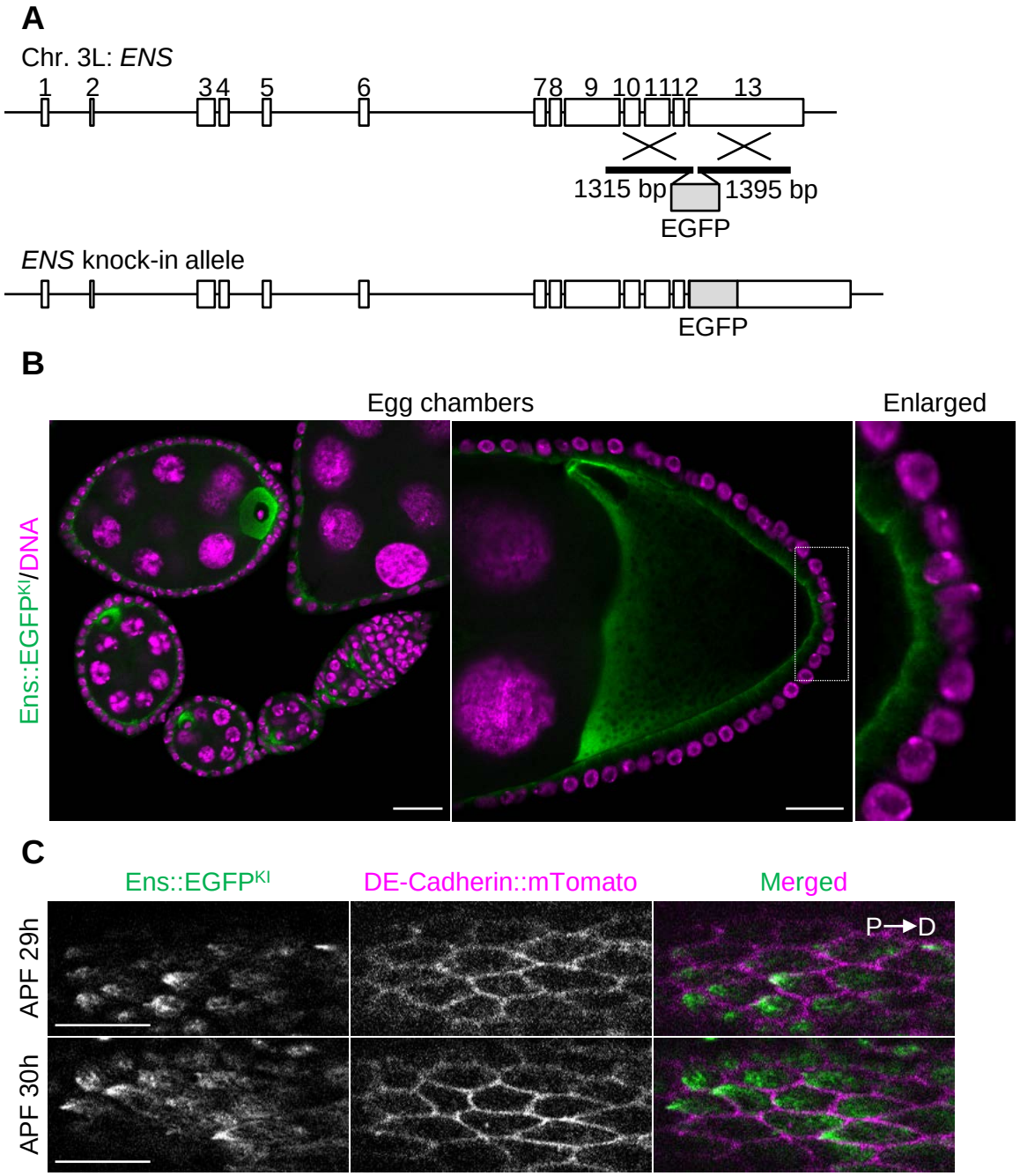

B
WT
KI
KDa
175
80
58
46
Dvl2-EGFPKI
Dvl2
WB: anti-Dvl2
WB: anti-GFP


C
Dvl2-EGFPKI
#1
#2
#3


A
Leading edge
Peripheral
Internal
Control
siWNT5A
siKIF5B
Recovery (%)
100
80
60
40
20
0
0
60
120
180
240
300
Time (s)


B
Map7-EGFPKI
Control
siWNT5A
siKIF5B
-30 s
Control
siWNT5A
siKIF5B
(s) -10 0 10 20 30 40 50 60 70 80 90 100 110 120 130 140
(s) 150 160 170 180 190 200 210 220 230 240 250 260 270 280 290 300


C
Control
siKIF5B
KDa
80
Kif5b
80
Map7D1
Map7
80
Dvl2
46
γ-Tubulin


A
Chr. 3L: ENS
1 2 3 4 5 6 7 8 9 10 11 12 13
1315 bp
1395 bp
EGFP
ENS knock-in allele
EGFP
B
Egg chambers
Enlarged
Ens::EGFPKI/DNA
C
Ens::EGFPKI
DE-Cadherin::mTomato
Merged
APF 29h
APF 30h
P→D

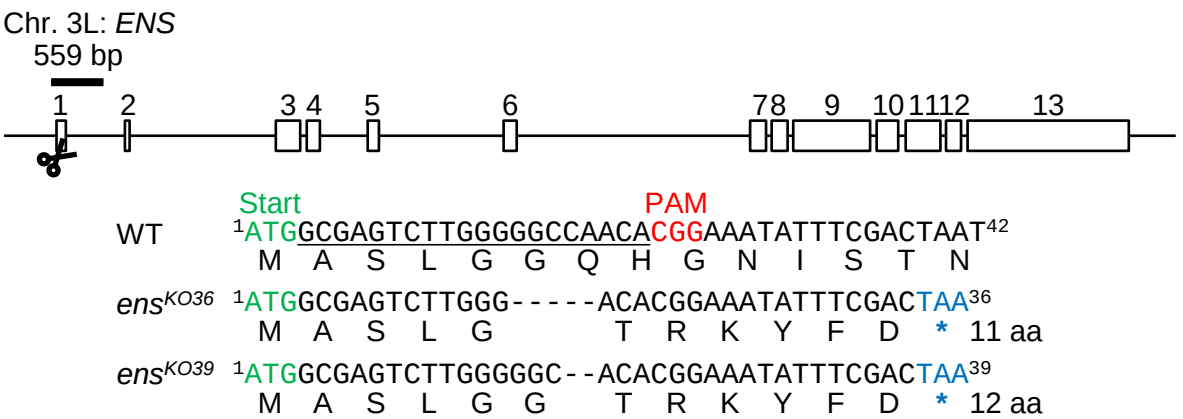
A

Female: *Df(3L)ens*$^{\Delta 3277}$		Hemizygous	Heterozygous	Mendelian inheritance	Lethality (%)
Male:	*ens*^{KO36}	82	703	351.5	76.7
	ens^{KO39}	43	682	341	87.4
	ens$^{\Delta C}$	46	412	206	77.7

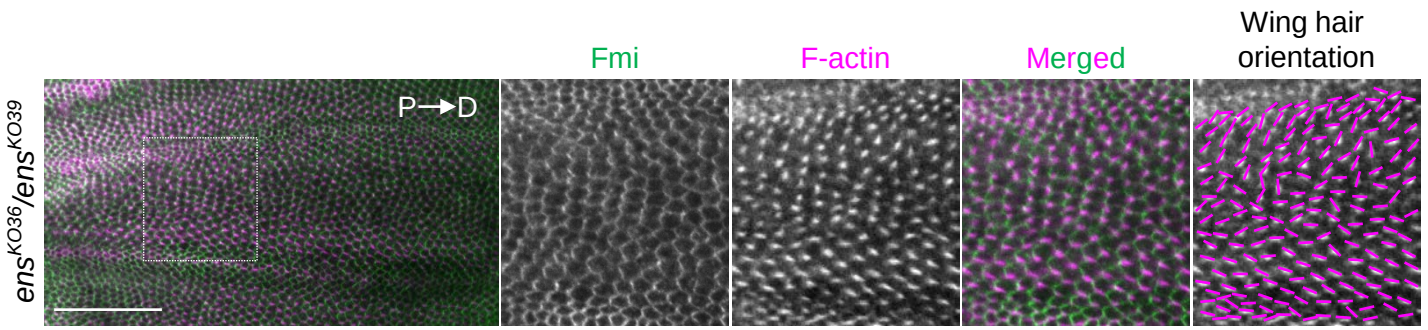
	Homozygous	Heterozygous	Mendelian inheritance	Lethality (%)
ens^{KO36}	2	1091	545.5	99.6
ens^{KO39}	0	559	144.5	100
ens$^{\Delta C}$	98	271	135.5	27.7

Female	Male	Trans-heterozygous	Heterozygous	Mendelian inheritance	Lethality (%)
ens^{KO36}	*ens*^{KO39}	0	501	250.5	100
ens^{KO39}	*ens*^{KO36}	0	562	281	100

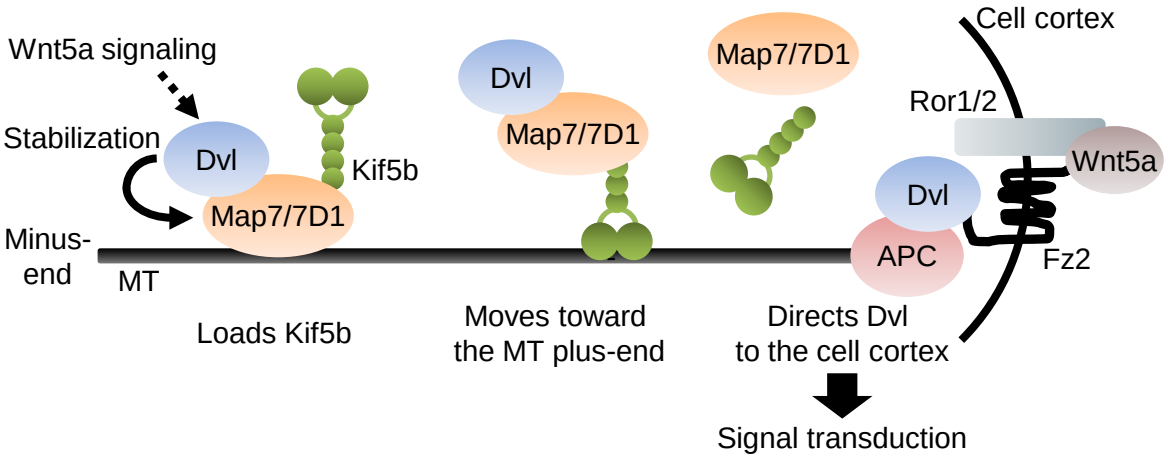
B



C



D



Appendix Figure legends

Appendix Figure S1. Expression levels of candidate genes in HeLa cells.

The expression levels of 69 genes for uncharacterized MT co-sedimented proteins and their paralogs were analyzed in HeLa cells by RT-qPCR. The relative mRNA level of each gene was normalized to the *GAPDH* expression. The primer list is shown in Appendix Table S4. Data are from two independent experiments and represent the average.

Appendix Figure S2. siRNA-based screen in HeLa cells to discover MT-binding protein(s) potentially involved in the Wnt/PCP signaling pathway.

A. Primary screening by cell spreading assays using siRNA pools for the indicated genes. Data represent the percentage of blebbing cells 60 min after being replated on a fibronectin-coated glass-bottom dish (total number of blebbing cells divided by total cell number, from four independent experiments). The siRNA list is shown in Appendix Table S5.

B. Wound healing assay using siRNA pools for the indicated genes. Data are from four independent experiments and represent the average distance moved by the wounded edge 6 h after wounding, ± S.D.

C. RT-qPCR analysis for the depletion efficiency of the indicated siRNAs. At 72 h post-transfection with control or the indicated siRNAs, the mRNA level of each gene was quantified and normalized to the *GAPDH* expression. The mRNA level relative to that of control cells is shown. Data are from three independent experiments and represent the average ± S.D.

D. Wound healing assay of cells transfected with each validated siRNA individually. Data are from three independent experiments and represent the average distance moved by the wounded edge 6 h after wounding, ± S.D.

Appendix Figure S3. Map7 and its paralog, Map7D1 belong to the MAP7 family.

A. Schematic structures of MAP7 family proteins (left). Expression levels of MAP*7* family genes in HeLa cells. The mRNA level of each gene normalized to the *GAPDH* expression is shown.

B. Specificity of anti-Map7D1 antibodies was evaluated by immunoblotting. HeLa cells were transfected with the indicated siRNAs. To detect Map7D1 alone, we used antibody #1, which hardly recognized Map7. Because antibody #2 recognized both Map7D1 and Map7, we used #2 to detect both of these proteins.

C. Specificity of siRNAs against Map7, Map7D1, or Map7D3. At 72 h post-transfection with control or the indicated siRNAs, cells were lysed, and the expression levels of *MAP7*, *MAP7D1*, and *MAP7D3* were quantified by normalization to the *GAPDH* expression. Relative mRNA levels to control cells are shown. In addition, specificity of siRNAs against Map7 or Map7D1 was confirmed by immunoblotting (the right of the bottom panel). The double depletion of Map7 and Map7D1 was achieved by transfecting cells with a mixture of three validated siRNAs against Map7 and the two against Map7D1.

D. Subcellular localization of endogenous Map7 or Map7D1 in non-migrating HeLa cells. To see the co-localization of MTs, fixed cells were co-immunostained for α-tubulin. Scale bars, 10 μm.

Data information: Data are from three independent experiments and represent the average ± S.D in A and C.

Appendix Figure S4. Generation of Map7-EGFP knock-in HeLa cells.

A. Schematic representation of the strategy to generate Map7-EGFP knock-in (Map7-EGFP^{KI}) HeLa cells by the CRISPR-Cas9 technique (left). The knock-in (KI) was confirmed by PCR using genomic DNAs derived from parental HeLa cells (WT) or Map7-EGFP^{KI} HeLa cells (KI) as a template. The positions of primer sets detecting the control (1) or KI (2) allele are shown at the right panel.

B. An image of the Map7-EGFP^{KI} localization in living cells (see Movie EV1). Scale bars, 10 μm.

Appendix Figure S5. Phenotypes of Map7/7D1-depleted HeLa cells.

A. Control and Map7/7D1-depletd migrating cells stained for acetylated-tubulin. Cells were fixed 1 h after wounding, and stained with anti-α-tubulin (green) and anti-acetylated-tubulin (red) antibodies.

B. Peripheral MTs in the indicated cells during cell adhesion. Cells were fixed 1 h after being replated on fibronectin-coated cover glass, and stained with an anti-α-tubulin antibody and Phalloidin. Map7D/7D1 depletion caused a decrease in the proportion of cells with polarized MT arrays that were induced by cell adhesion. Cells with Map7/7D1-depletion failed to form lamellipodia (arrow), and instead exhibited membrane blebbing (arrowheads). Graph shows the percentage of cells with polarized MT arrays.

C. Kinetics of FA turnover during cell adhesion was evaluated in the indicated cells by measuring the cell adhesion-dependent activation of FAK. Cells were lysed 1 h after being replated on a fibronectin-coated dish. Map7/7D1-depleted cells exhibited decreased auto-phosphorylation of FAK on Tyr397 (pY397) and FAK-dependent phosphorylation of Paxillin on Tyr118 (pY118) during cell adhesion.

D. Image of multinucleated cells induced by depleting Map7/7D1. Cells were stained with DAPI (DNA) and an anti-α-tubulin antibody. Graph shows the percentage of multinucleated cells in the indicated cell types.

E. The Wnt3a-induced expression of *AXIN2* in the indicated cells. Cells were lysed 8 h after addition of buffer or purified Wnt3a (20 ng/ml). Expression level of *AXIN2* transcripts was quantified by normalization to the *GAPDH* expression at the top panel. Depletion efficiency of Map7/7D1 was shown at the bottom panel.

Data information: Scale bars, 10 μm in A, B, and D. Data are from three independent experiments and represent the average ± S.D in B, D, and E. Statistical significance was tested with the Student's *t*-test in B and D.

Appendix Figure S6. Specific complex formation between Map7/7D1 and Dvl.

A. Lysates from HeLa cells co-expressing hMap7-EGFP or mMap7D1-EGFP with mDvl2-V5His$_6$ were immunoprecipitated with an anti-V5 antibody, and the immunoprecipitates were probed with anti-GFP and anti-V5 antibodies.

B. Lysates from HeLa cells co-expressing hMap7-EGFP or rat (r) Map7D2-EGFP with mDvl2-V5His$_6$ were immunoprecipitated with an anti-V5 antibody, and the immunoprecipitates were probed with anti-GFP and anti-V5 antibodies.

C. The phosphorylation state of the indicated Dvl2 mutants. Lysates from HeLa cells expressing various mutants of mDvl2-EGFP were immunoprecipitated with an anti-GFP antibody, and the immunoprecipitates were treated with (+) or without (-) alkaline phosphatase (CIAP).

D. The amount of MBP-hMap7$^{1\text{-}265\Delta CC1}$ was determined on SDS polyacrylamide gels with Coomassie brilliant blue staining. Arrow shows the intact MBP-hMap7$^{1\text{-}265\Delta CC1}$.

E. Lysates from HeLa cells were subjected to immunoprecipitation with control IgG or an anti-APC antibody, and analyzed by immunoblotting with an anti-Map7/7D1 or anti-APC antibody.

Appendix Figure S7. Generation of Dvl2-EGFP knock-in HeLa cells.

A. Schematic representation of Dvl2-EGFP knock-in (Dvl2-EGFP^{KI}) HeLa cell generation by the CRISPR-Cas9 technique. The KI was confirmed by PCR using genomic DNA derived from parental HeLa cells (WT) or Dvl2-EGFP^{KI} HeLa cells (KI) as a template (right panel). The positions of the primer sets for detecting the control (1) or KI (2) allele are shown at the bottom of the left panel.

B. Dvl2-EGFP^{KI} expression was confirmed by immunoblotting. Lysates derived from the indicated cells were probed with anti-Dvl2 (left panel) and anti-GFP (right panel) antibodies. The number of KI events on the pseudo-triploid HeLa genome was estimated by comparing the band intensity of Dvl2 with that of Dvl2-EGFP^{KI}. In this experiment, a cell strain in which EGFP was inserted into two of the three *Dvl2* loci was used.

C. Localization patterns of Dvl2-EGFP^{KI} in three independent clones. The same localization pattern was seen in all of the clones. Scale bars, 10 μm.

Appendix Figure S8. Wnt5a signaling and the Kinesin-1 member Kif5b promote directional Map7 movement toward the MT plus-end.

A. Graph shows fitted curves from the indicated data sets, related to Fig. 7.

B. FRAP analysis of Map7-EGFP^{KI} at the leading edge of the indicated cells after wounding. Cropped images of Map7-EGFP^{KI} at the leading edge of the indicated cells after wounding, related to Fig. 7B.

C. Confirmation of the depletion efficiency of si*KIF5B*. Lysates derived from the indicated cells were separated by SDS-PAGE, and immunoblotted for Kif5b, Map7/7D1, and Dvl2. Kif5b depletion did not affect the expression of Map7/7D1 or Dvl. The blot was reprobed for γ-tubulin as a loading control.

Appendix Figure S9. Generation of Ens::EGFP knock-in fly strains.

A. Schematic representation of the generation of an Ens::EGFP knock-in (Ens::EGFP^{KI}) fly by the CRISPR-Cas9 technique.

B. Images of Ens::EGFP^{KI} egg chambers. Egg chambers were counterstained with DAPI (DNA). Enlarged image shows the Ens::EGFP^{KI} localization to the apical region in the follicle cell epithelium.

C. Single channel images for live pupal wings in Ens::EGFP^{KI} pupae expressing DE-Cadherin::mTomato at 29 and 30 h APF. P, proximal side; D, distal side.

Data information: Scale bars, 10 μm in B and D

Appendix Figure S10. Generation of *ens* null mutants.

A. Lethality of different *ens* mutants. A few *ens*^{KO} *neoFRT80B*/*Df(3L)ens*$^{\Delta 3277}$ hemizygous escapers were eclosed, all of which died immediately after eclosion. In addition, *ens*^{KO} *neoFRT80B*/*ens*^{KO}

neoFRT80B homozygous or transheterozygous mutants died before eclosion. In contrast, $ens^{\Delta C}$ *neoFRT80B*/*Df(3L)*$ens^{\Delta 3277}$ hemizygous mutants survived up to two weeks after eclosion, and increased numbers of $ens^{\Delta C}$ *neoFRT80B*/$ens^{\Delta C}$ *neoFRT80B* homozygous mutants were eclosed, suggesting that the remaining N-terminal portion encoded by the $ens^{\Delta C}$ allele allowed the organisms to grow to the adult stage.

B. Schematic representation of the generation of *ens* null mutants by the CRISPR-Cas9 technique. The deletions in the two *ens* null alleles are shown.

C. Pupal wings of ens^{KO36}/ens^{KO39} mutants at 32-34 h APF were stained with an anti-Fmi antibody and Phalloidin. P, proximal side; D, distal side. High magnification views of boxed area (intervein region between L3 and L4 veins) are shown at right. Magenta lines in right side panels indicate wing hair orientation. Because ens^{KO36}/ens^{KO39} mutants died before eclosion, we obtained their pupae by selecting third instar larvae, which were identified by the loss of GFP fluorescence from the balancer chromosome. Note that defects in wing hair orientation in ens^{KO36}/ens^{KO39} pupae were virtually identical to those in wing hair orientation in ens^{KO36}/*Df(3L)BSC735* pupae. Therefore, off-target mutations in ens^{KO36} or ens^{KO39} chromosomes should not affect planar cell polarity on wing epithelium. Scale bar, 10 μm.

D. Proposed model for the actions of Map7/7D1 in Wnt5a signaling. See Discussion in detail.

Appendix Table S1. Primary antibodies used in this study.

Company	Name, catalog number	Used for (dilutions)
Absea	Rat anti-APC (KT45), 030903E07	IF (1:200)
Bioresource	Rabbit anti-FAK pY397, 44-624G	IB (1:3000)
BD Biosciences	Mouse anti-Clathrin heavy chain, 610500	IB (1:5000)
	Mouse anti-FAK, 610088	IB (1:3000), IF (1:250)
	Mouse anti-EB1, 610535	IF (1/500)
	Mouse anti-Paxillin, 610052	IB (1:3000), IF (1:250)
Chromotek	Rat anti-RFP, 6g6-100	IF (1:200)
Cell Signaling Technology	Rabbit anti-Dvl2, 3216	IB (1:3000), IP
	Rabbit anti-Paxillin pY118, 2541	IB (1:3000)
	Rabbit anti-Wnt5a/b, 2530	IB (1:1500)
DSHB*	Mouse anti-Fmi, #74	IF (1:20)
GeneTex	Rabbit anti-Kinesin (Kif5b), GTX104874	IB (1:3000)
MBL	Mouse anti-GFP, M048-3	IB (1:2000)
	Mouse anti-MBP, M091-3	IB (1:3000)
	Rabbit anti-GFP, 598	IB (1:5000), IF (HeLa, 1:500; Fly, 1:200), IP
	Rabbit anti-GST, PM013	IB (1:3000)
Millipore	Rabbit anti-Detyrosinated tubulin, AB3201	IB (1:3000)
Nacalai	Mouse anti-V5, 04434-36	IB (1:3000), IP
	Rat anti-GFP, 04404-84	IF (HeLa, 1:500)
R&D systems	Goat anti-Fz6, AF1526	IF (1:100)
Santa Cruz Biotechnology	Rabbit anti-APC (C-20), sc-896	IB (1:1500), IP
Sigma-Aldrich	Mouse anti-Acetylated tubulin, T7451	IB (1:5000), IF (1:500)
	Mouse anti-α-tubulin (DM1A), T6199	IF (1:1000)
	Mouse anti-β-tubulin, T0198	IB (1:5000)
	Mouse anti-γ-tubulin (GTU-88), T6557	IB (1:5000)
	Rabbit anti-Flag, F7425	IF (1:500)
	Rabbit anti-Map7, SAB1408648	IB (1:3000), IF (1:250)
T. Fujimori's lab	Guinea pig anti-Celsr1 [1]	IF (1/100)
	Rat anti-E-cadherin [2]	IF (1/2)
Made in-house	Rabbit anti-Map7D1#1	IB (1:5000), IF (1:500)
	Rabbit anti-Map7D1#2	IB (1:5000)

IB, Immunoblotting; IF, Immunofluorescence; IP, Immunoprecipitation

*, The Developmental Studies Hybridoma Bank

Appendix Table S2. siRNAs used in this study except for the siRNA-based screen.

Name	Sequence (sense)
Randomized control [3]	5′-CAGUCGCGUUUGCGACUGGTT-3′
MAP7 3’ UTR-1	5′-AUUAAUUGUAGAAAUUCUCAU-3′
MAP7 3’ UTR-2	5′-UAUUUGAUUAGUUUAUCUGGA-3′
DVL1 [3]	5′-GGAGGAGAUCUUUGAUGACTT-3′
DVL2 [3]	5′-GGAAGAAAUUUCAGAUGACTT-3′
DVL3 [3]	5′-GGAGGAGAUCUCGGAUGACTT-3′
WNT5A [3]	5′-GUUCAGAUGUCAGAAGUAUTT-3′
APC [3]	5′-GAGCGGCAGAAUGAAGGUCAA-3′
KIF5B-1 [4]	5′-GCAAGAAGUAGAUCGCAUATT-3′
KIF5B-2 [4]	5′-GCUGUCAAUUAUGAUCAGATT-3′
KIF5B-3 [4]	5′-GACAUGUAGCAGUUACAAATT-3′

Appendix Table S3. sgRNA sequences used in this study.

Name	Sequence (sense)
*MAP7*_KI-1	5′-GTTCAGACACAGCAGACTGC-3′
*MAP7*_KI-2	5′-GTTGACTGTGTTTGGCACAC-3′
*DVL2*_KI-1	5′-CAATCCCAGCGAGTTCTTTG-3′
*DVL2*_KI-2	5′-GTGCTTGCTCTTACAGTGCC-3′
*ens*_KO-1	5′-GCGAGTCTTGGGGGCCAACA-3′
*ens*_KO-2	5′-GGACCTCCGCTGACCCCAAA-3′
*ens*_KI-1	5′-ACTATCAGCAGTGACTAGCG-3′
*ens*_KI-2	5′-ACGATCCCCGATCTCTGAGG-3′

Appendix Table S4. Primer sequences for RT-qPCR used in this study.

Primer name	Sequences
C2orf55+	TCCCGGATGAGGAGAAGG
C2orf55-	GTGTTTCAGGCAGCTCTTG
KIAA1211+	CAGTCCAGCACGCCCTA
KIAA1211-	CGTGCTTCTTCTCCTCCAA
C6orf174+	ACTGAGGCAACAGCTCATA
C6orf174-	TCATTGTCCTCCTCTGTGG
CCDC165+	TGAAACACTGGCGGCAA
CCDC165-	CCCACTGGAAAGCCACA
SOGA1+	CTAAGGAGATTCTTCTGGCAAA
SOGA1-	CATTGTCTTCAGCTCGGTA
CCDC9+	CCCTGGAGGAGTCTGAG
CCDC9-	TGCTTCCAGCGCAGGTA
C15orf52+	AGGAGCCACCAGAAACT
C15orf52-	TCCTTCATATCCCACCTTCG
CCDC38+	TCCTCAGGGAGTATATGAAATATGG
CCDC38-	GATGCCTTCCTTGTTACTTGAAT
CCDC37+	TTGAAGACACTCTGAAGCATTAC
CCDC37-	CTGGTGTGGAGTTAACACTG
FAM81B+	CGGCCACAGGAACTAAC
FAM81B-	TCTCAATTTGCCGGTGC
FAM81A+	TTGAACAAAGAACAGCAGGC
FAM81A-	TGGTGGTTACTGTCTCTGTGA
FAM164A+	GGATCTTCACGATTACCGC
FAM164A-	GTGGTGTGGAATTTCGGG
FAM164B+	CGATCAGCAAAGCAGTGT
FAM164B-	TGCAGAAATTAGTATGTCGCTC
FAM164C+	CAGAAGGATCCAGACGC
FAM164C-	ATCCTCTCTCCTGTCTCTACTAA
G3BP2+	ACCAGAAGTTCAATCTCAGC
G3BP2-	TGATGACTATCTGGATAGCGAATTA
G3BP1+	GTGGAGCTGTTCCAGTTAC
G3BP1-	GGGAGGAATATTTATTCGTTGTTCT
GCOM1+	AGCCTAGAGGAGAAAGACC
GCOM1-	CTGATACCTTTCCTTGTCAGAAT
CCDC68+	TCTTAGAAATGAACAAAGAGAATGAAGTAT
CCDC68-	ACCTGGAGTAATTGTTTACTGTC
TUFT1+	GATGGAGACGGAGCATCA
TUFT1-	GTGGATCTTCTCCCGCA
LRRC40+	TACGGTCCTTGGAAAGGC
LRRC40-	AGGACTTCTTGTGTTCCTTTAC
LRRC30+	CGAGGTCCAGAAACTCAATC
LRRC30-	CAGGCAGTTCATGTTGACAAA
LRRD1+	GTGCAATGTTGGAATGCC
LRRD1-	ATATGTTATTAAGATGTGAGATACAGTCAG

LRRIQ4+	TGACCTGGACGAGAACAAA
LRRIQ4-	GGAGGAGGTTGTGGGATAA
LRRC45+	GCAGAGCGGGAGTCTAA
LRRC45-	GCTGACATGCTGGTCATC
C14orf166B+	CTTCAAGGAAGACTCCGCA
C14orf166B-	GCTCAGATCCAGTGACG
TCTE1+	CAGCGAGGGAGAGATGG
TCTE1-	AAGGAGAGGCAGTCACG
PSD3+	TATACGGAGGACTCCACCGA
PSD3-	AATGCGCTGTTGTATCATTG
PSD+	TCCCTGCCACTCAGAGGA
PSD-	TGCCCATTGGACAAGGT
PSD2+	GGGTCCTCACACACTTCTC
PSD2-	ATTGCTGACAGGACATCTTT
PSD4+	AGCTTCTTCTCCAGAGCCTA
PSD4-	AGTGAAGACTGTGAAGCATCTA
RASAL3+	GCTTCTACTGAGGACTGTGA
RASAL3-	AGCTTGAGAACACGATGC
DAB2IP+	GCTGGAGCAGAGCATAG
DAB2IP-	ATCGTTCTCAATCACCATCTT
RASA1+	GTTCTGTCTATGTCGTTCATGAT
RASA1-	AAATGCCTGCAGACCTT
RASAL2+	GTTTCCCAACTTGATAAGGGT
RASAL2-	GGACTGGAGTTATGTTCAGTG
SYNGAP1+	CATCGCAGACAGGCTTATC
SYNGAP1-	AAGTCCTCCTTTGAGGTAAAC
SPATS2+	GCAGAATGGTGTCTCTGAT
SPATS2-	TTTACTGGTGGCGAGGG
SPATS2L+	AATTAAGCACTTTGTCAGCGA
SPATS2L-	CAGGGAGTTCTTGAGGAATAG
ZBTB47+	AGAAGAGGAAGAGGAAGGT
ZBTB47-	TGATCCTGCTCACTAGGC
ZNF652+	CTAAGCGTAAGAAGCGGG
ZNF652-	CAAATCTGCATGCGCCTA
MAP7D1+	GGACAAGGAGCGGGAAA
MAP7D1-	TGTGGAGGGAGAGGATGG
MAP7+	AACTCTTTGTAACACCACCT
MAP7-	GGTTGTCTTGCTTTGGGA
MAP7D2+	GGCATTCCTAAGAGACCATC
MAP7D2-	CTTTCCTCTTGGGCATGT
MAP7D3+	CAAGTGTGGATGCACCC
MAP7D3-	TCCATGCTCATCTCAGAATCA
ANKLE2+	GTAAGTTTGGAAATGCAGATGTAG
ANKLE2-	CCTTTAAATACTCTCTGATCCGC
C1orf49+	TAAACAAGAAGGGCGGTTTAC
C1orf49-	TTCCTTCATAATTTCCACAAATTCG
C2orf16+	ACTATGGAAAGGAAGCTTTGT

C2orf16-	GGCTGAGAAATAGTAACTGGAC
C5orf49+	TCAGAAGTTGCACCGAGAT
C5orf49-	GCCAAAGTCCCGGTTTAG
C6orf97+	AAATCGCAGCCCTCCTTA
C6orf97-	TCCTTTCCCAACTGTTCAACAA
C10orf68+	GCAGTTAAAGAGAAAGAGTTACCC
C10orf68-	GGTTGACTCCTTCATTAGACC
C10orf118+	AAGGAAGGCGAAACGACTA
C10orf118-	TGTTGTTTCTTTGAGTTTATCTTTGG
C16orf45+	CAGAACTTGGTCGCCAT
C16orf45-	TTGTCTTCTTCTTGCTCCCT
CCDC13+	TTGTCTGTCTATCCAGACCC
CCDC13-	GTTCCGAGACCTCATGC
CCDC40+	CCATCATGAAGGAGGAAGAAA
CCDC40-	TGCAGGGTGAGCCTGTA
CCDC67+	ACTTGAATCATCTTATTTGCCTTCTATTA
CCDC67-	GATGAAGCTCTTCTGTTAGGTC
CCDC83+	CCAGGTATCCAGTGCTACA
CCDC83-	GCAATTCCAAAGACATTTCTTCAG
CCDC146+	GCTAAGAGGAATTTGGCCC
CCDC146-	CTTTCATTTCTTTAACAATGTTGGTGTA
CCDC147+	TCGAACAGCACAAAGAAACC
CCDC147-	CCGCCTTAAGCATGTTCTTAT
CEP112+	AACATGAAACTGTTACAAACCAAATA
CEP112-	GCTTTCTTTGAAGTTCTGATTCC
CEP128+	CTGTATGCAGCATTACAACAAATAG
CEP128-	AATTCTTAACTTCCAATTCAAGGTC
CNTLN+	TGTGGAATGAACTGGCATATTT
CNTLN-	GCTGTTCTTCTCTTCTCTCTG
CXorf58+	AGTCCAAAGTAACTGATATAATGGAT
CXorf58-	ACATTAGCATTGTCCTGGG
FHAD1+	ACAGAGTGAAGGAAGCATTAG
FHAD1-	GGGTTAAATGGTTCTCCAGAT
FSIP1+	ATGAACTTGCAGTCACCC
FSIP1-	CCATATTTCTTTCACCATCACG
LOC100271840+	CTAATGAAGATGGAGTCCACG
LOC100271840-	GATCAGCGATGCCCTTG
LRRCC1+	CCTTGTTGAACAGCTAGACC
LRRCC1-	ATTTCCTTTAGCCTATCTGTGG
MIPOL1+	AGCAGAAATTGGCTAAAGAAGATAA
MIPOL1-	TAACAGCTTCATCACGTTCCT
RGSL1+	ACATGAAGGAAATGGACTATAGG
RGSL1-	GCCATCTTTGGGTTCTTGT
ZNF626+	CAAAGCCTTTAACCACTCTTG
ZNF626-	TAGGGTTTCTCTCCAGTATGATT
ZNHIT6+	GGAATTGATGCATGGAGAGT
ZNHIT6-	GCTTCTTCTGTACCACAAGT

GAPDH+	CCTGTTCGACAGTCAGCCG
GAPDH-	CGACCAAATCCGTTGACTCC

Appendix Table S5. siRNA sequences for the siRNA-based screen used in this study.

NCBI gene symbol	siRNA Target Sequence
G3BP1	AACCCTGGTTCCAACAGAATG
G3BP1	CAGGAGGAGTCTGAAGAAGAA
G3BP1	CAGAAAGAAATCCACAGGAAA
G3BP2	AAGAGCTGCAAGAGAGCGAGA
G3BP2	CAGGGATATTAGGCGCAATGA
G3BP2	CCACCTCGTGTGCGTGAACAA
C10orf118	CAGCCTGTCATTGAATCTAAA
C10orf118	AAGGAAATTATTAATCGCCAA
C10orf118	ACACTACTTAACACTCCTAAA
CEP128	CAGGACCGTGTAATTGCATTA
CEP128	AACGAGCGTTGGAGAAACAAT
CEP128	CACAGGGTATTAAACGAATGA
ZNHIT6	CACCGTCTAGCCACAGGAGAA
ZNHIT6	CACGTTGTATGCGATATTCCT
ZNHIT6	CTCCTAAGTGATTATCGATTT
SOGA1	TTCCTATGCCTCTGAGATCAA
SOGA1	CACGCCCAATGAGTACATCAA
SOGA1	ACGCTGTTTGTGACTGTAGTA
ZC2HC1A	CCCGAAATTCCACACCACCTA
ZC2HC1A	AACAGGCAGCACGTATTAGTA
ZC2HC1A	TGCCATGAGTGTGGGACTAAA
CNTLN	CACAGCTGAAAGTATATCATA
CNTLN	AAGGAGTGTGTACAGAACAAA
CNTLN	CTGCACTGACCTGCTAAATGA
FAM81A	ATCCGTCAGAAGTATGATGTA
FAM81A	ACAGCCGTCTATGAAAGCATA
FAM81A	AAGGCTCAATATCTGCGTTGA
ANKLE2	ATCGTCAAAGCCGGATTGAAA
ANKLE2	CCGTTACGTGGTGGACCTGTA
ANKLE2	AAGGAGCGGATCAGAGAGTAT
SOGA2	ACCCACGAGCTCAGCAAGTTT
SOGA2	CCGACTCCTCCTGGTACCTAA
SOGA2	CTGCAGTACCGTCTTCGGAAA
KIAA1211	CAGGATCCACAACATGAGCAA
KIAA1211	ACCATCGAAAGTGTCAACTTA
KIAA1211	CAGCCGCTGTGGATAACGTTA
SPATS2L	CACCGTTTCTCTAACTAGATA
SPATS2L	TAGATATCGCGTCATGATTAA
SPATS2L	AAGATCTATGCAGTTAGATCA
LRRC40	AAGATGATGGACCTAGCCAAA
LRRC40	AAGGAATTGCACGTAGGTGAA
LRRC40	CAACATCGTCACTTCTATTAA
LRRC45	CTTGATGGAGACTATTGATAA

LRRC45	CTCCGAAAGCCTGCGCATCAA
LRRC45	CCGCACTCACGTCCTCAGCAA
TCTE1	CAAGACCCTCCTGGAATTTGA
TCTE1	CCCACCGTTGACCACTACCAA
TCTE1	CACAGCTCTCAAAGCCTTCAA
MIPOL1	ATGGGTAGCTATAAGGTTACA
MIPOL1	AACGGGATGCTGCCTTGTCTA
MIPOL1	CAGGGCAGAGATCAACGAATT
PSD3	AAGCGTGAAGATCGTAGGATT
PSD3	AAGGACGTCGATGAGTACAAA
PSD3	CAGGGACTTCTGGATAGGCTA
RASA1	CAGCTCCCATATACCATTAAA
RASA1	ACGGACCTGTCCCGTGATTTA
RASA1	CAGACCTAATAGGTTATTACA
RASAL2	CGGCGACTGGAGGAATATGAA
RASAL2	CTCGTGGGCTGCCTAAACTAA
RASAL2	AACCTTCGCAGGACAGTTCAA
SPATS2	TACGTGCAATAGTTCCTAATA
SPATS2	CACAGTGTCTCTTGCACGGTA
SPATS2	CGGTATCGAGTTGTAGTTAAA
TUFT1	AAGTAGATAGGCATCAGAGTA
TUFT1	CTGAGGTGGACACCTGTATAA
TUFT1	CAGACGGAGCACGAGCACCTA
ZNF652	ACCAGCATTCTTAGGCGATAA
ZNF652	GCAGATCATAGTGGAGGTAAA
ZNF652	AGAGTGAGCCTTTAAATCATA
MAP7	CCTCTTCATCTGCAACTTT
MAP7	GGAGAGAAAGAAGCGACTT
MAP7	ATGAGAATTTCTACAATTA
MAP7D1	AGCGTCTGGAGGAGATCAT
MAP7D1	ATGAAGAGGACTCGGAAGT
MAP7D1	CCAAGGGGCGGGTTCGGAG
MAP7D3	AGCACTAACAGGCAAATCC
MAP7D3	GCTTTGAGCCAAAGGCATA
MAP7D3	GCCAATAAACGATCTGCAT

Appendix Table S6. Fly strains used in this study.

Used in	Genotype
Fig. 8C and Appendix Fig. S9C	*w; shg::mTomato; ens::EGFP^{KI}*
Fig. 9A	*Oregon-R*
Fig. 9A	*w; Df(3L)BSC735/TM6B P{Dfd-EYFP}3 Sb Tb ca*
Fig. 9A and Appendix Fig. S10C	*w; ens^{KO36} P{neoFRT}80B/TM6B P{Dfd-EYFP}3 Sb Tb ca*
Fig. 9C and Fig. EV2	*w; dsh::EGFP*
Fig. 9C and Fig. EV2	*w; dsh::EGFP; ens^{KO36} P{neoFRT}80B/ TM3, P{sChFP}3 Sb*
Fig. 9C and Fig. EV2	*w; dsh::EGFP; ens^{KO39} P{neoFRT}80B/ TM3, P{sChFP}3 Sb*
Appendix Fig. S9B	*w; ens::EGFP^{KI}*
Appendix Fig. S10A	*w; ens^{KO36} P{neoFRT}80B/TM3 Sb Ser*
Appendix Fig. S10A	*w; ens^{KO39} P{neoFRT}80B/TM3 Sb Ser*
Appendix Fig. S10A	*w; ens$^{\Delta C}$ P{neoFRT}80B/TM3 Sb Ser*
Appendix Fig. S10A	*w; Df(3L)ens$^{\Delta 3277}$, Chd64$^{\Delta 3277}$ ens$^{\Delta 3277}$ P{neoFRT}80B/TM3 Sb^{1}*
Appendix Fig. S10C	*w; ens^{KO39} P{neoFRT}80B/TM6B P{Dfd-EYFP}3 Sb Tb ca*

Appendix References

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