Appendix

A missense mutation in human *INSC* causes peripheral neuropathy

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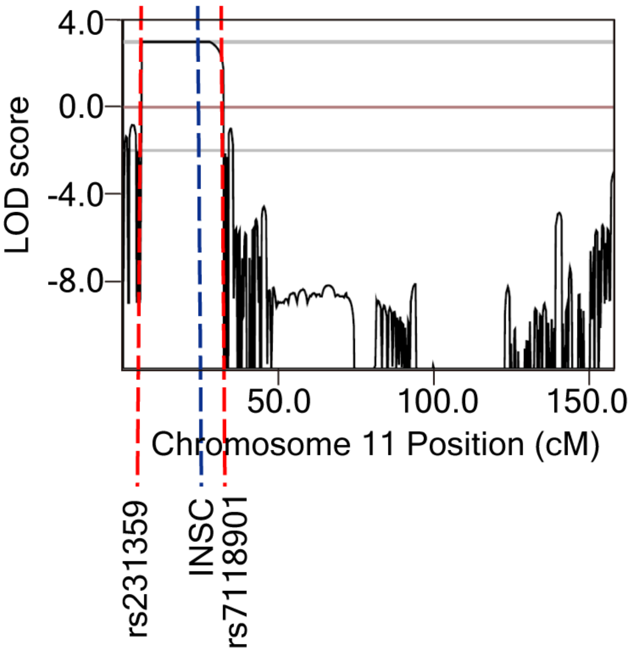
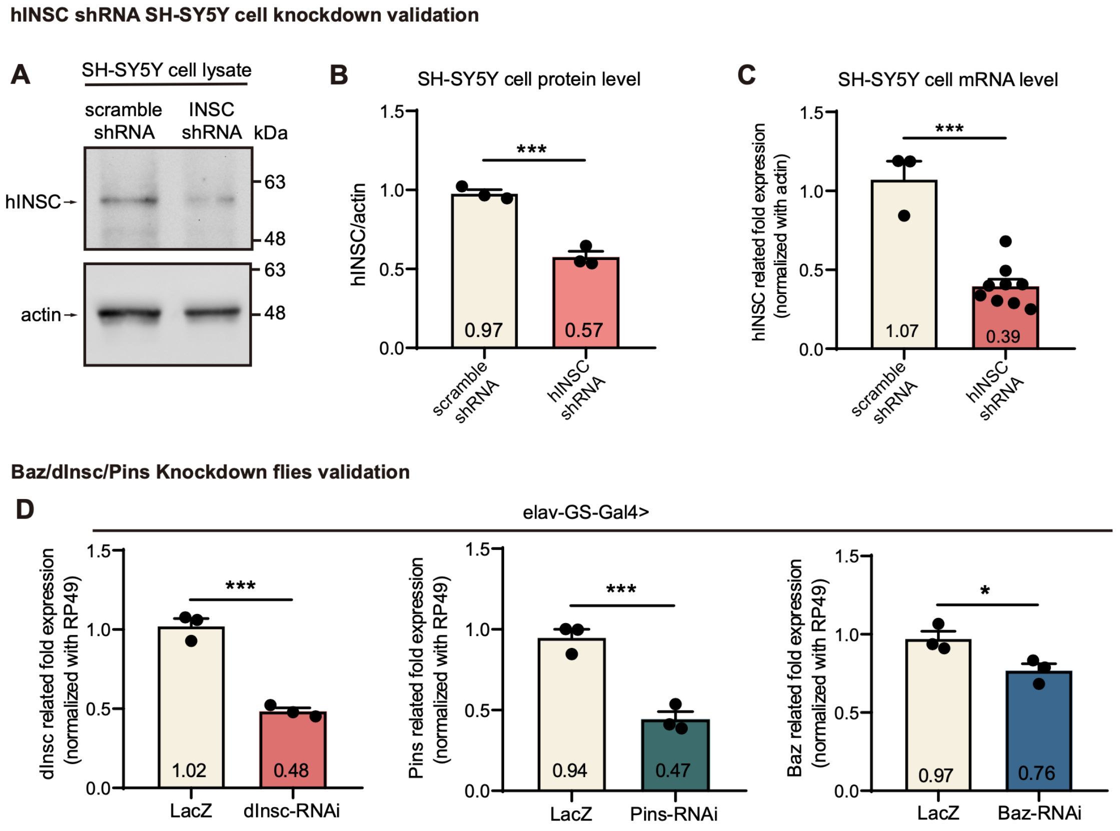
Appendix Figure S1. Clinical pictures of the patients with *INSC* p.Met70Arg.
A. Affected female II-2 aged 59 years, with muscle wasting below the knees.
B. Affected male II-3 aged 57, with pes cavus.
C. Affected male III-3 aged 30, with pes planus.
D. Affected male II-9 aged 49, with pes cavus.



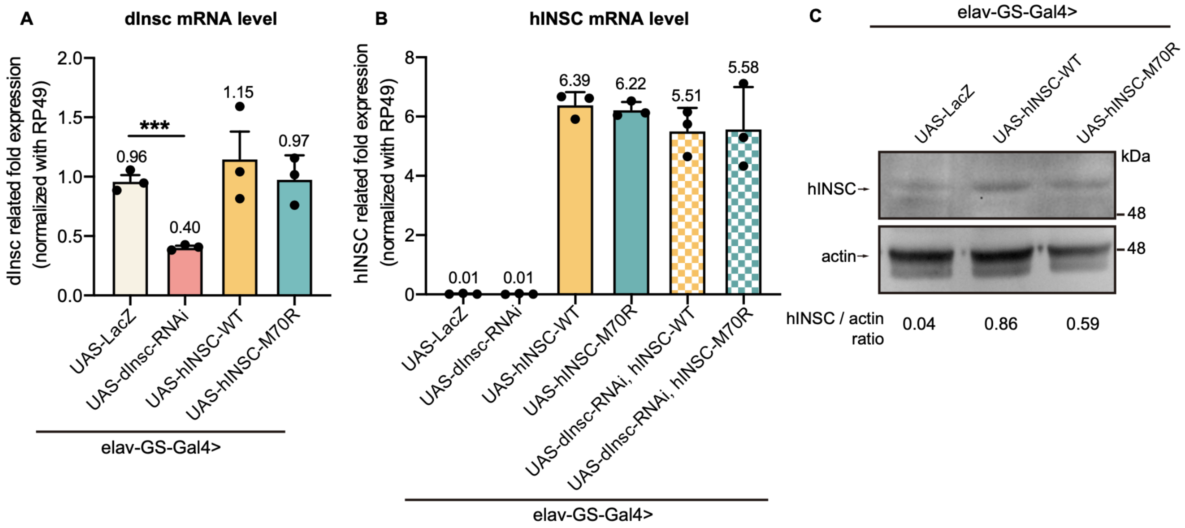
Appendix Figure S2. **Multipoint parametric linkage analysis of CMT2 in the family.** Multipoint parametric linkage analysis revealed three loci with a LOD score of approximately 3, including chr11:2694606-19743250 (GrCh37; Zmax 3.01), chr4:442021-6213622 (Zmax 3), and chr16:72022941-77978326 (Zmax 2.97). The red dotted lines define the flanking markers of the linkage peaks. The blue dotted line shows the location of the *INSC* gene within the chromosome 11 linkage region.



Appendix Fig S3. *hINSC* shRNA knockdown SH-SY5Y cell and *Baz/dInsc/ Pins* knockdown fly validation

A. Representative blot from 3 independent western blottings of SH-SY5Y cell extracts of *hINSC*-shRNA and scramble shRNA control.
B. Quantification of (A).
C. Relative *hINSC* mRNA abundance in SH-SY5Y cell extracts of *hINSC*-shRNA and scramble shRNA control.
D. Relative mRNA abundance in whole fly extracts of *dInsc, Pins* and *Baz* RNAi flies driven by *elav-GS*-Gal4; n = 24 flies/genotype from 3 independent fly crosses.

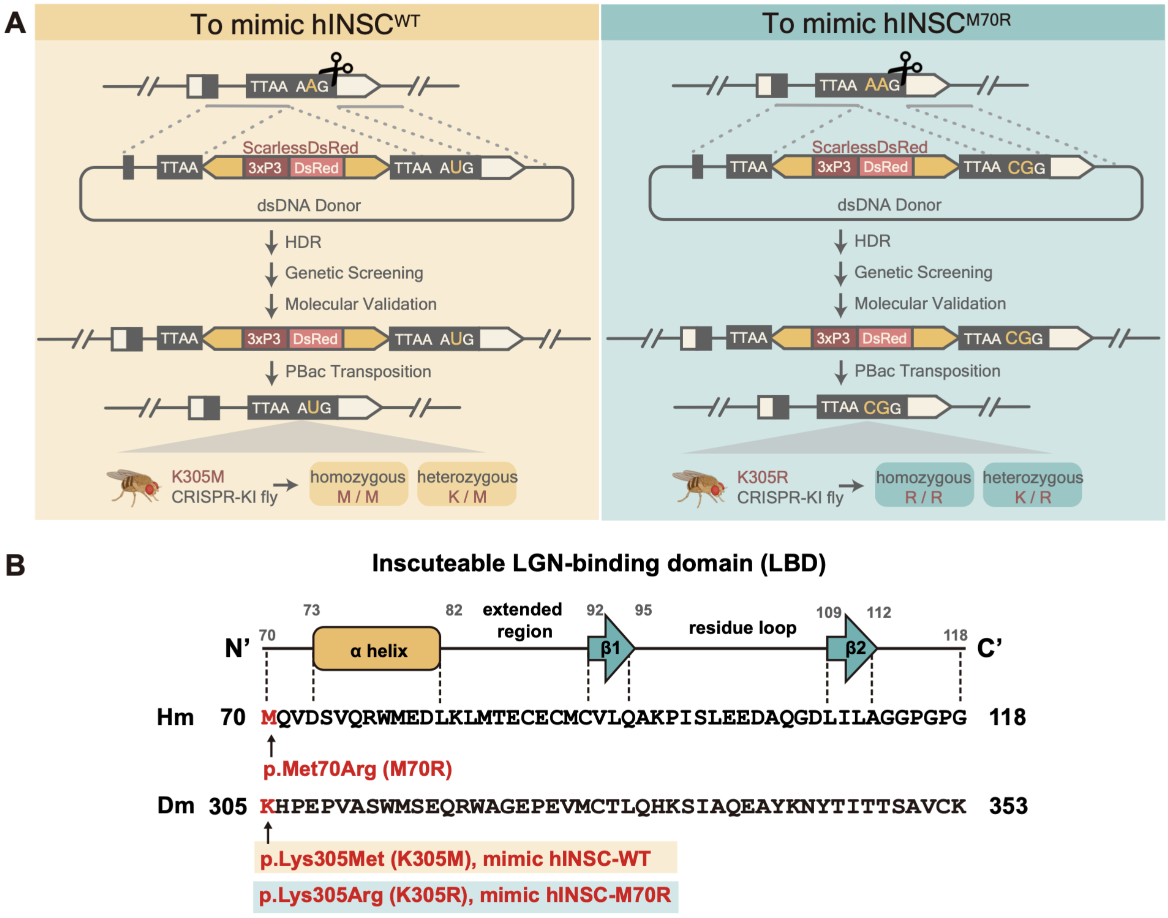
Data information: Error bars indicate mean ± SEM. Statistical analysis was performed using two-tailed Student's t-test. *P < 0.05, **P < 0.01, ***P < 0.001.



Appendix Fig S4. *hINSC* transgenic fly mRNA and protein level validations

A. Relative *dInsc* mRNA abundance in whole fly extracts of the indicated UAS transgenes driven by *elav-GS*-Gal4; n = 12 flies/genotype from 3 independent fly crosses.
B. Relative *hINSC* mRNA abundance in whole fly extracts of the above indicated groups driven by *elav-GS*-Gal4; n = 12 flies/genotype from 3 independent fly crosses.
C. Representative western blotting of whole fly extracts from UAS-$hINSC^{WT}$ and UAS-$hINSC^{M70R}$, and UAS-*LacZ* control, driven by *elav-GS*-Gal4; n = 5 flies/genotypes.

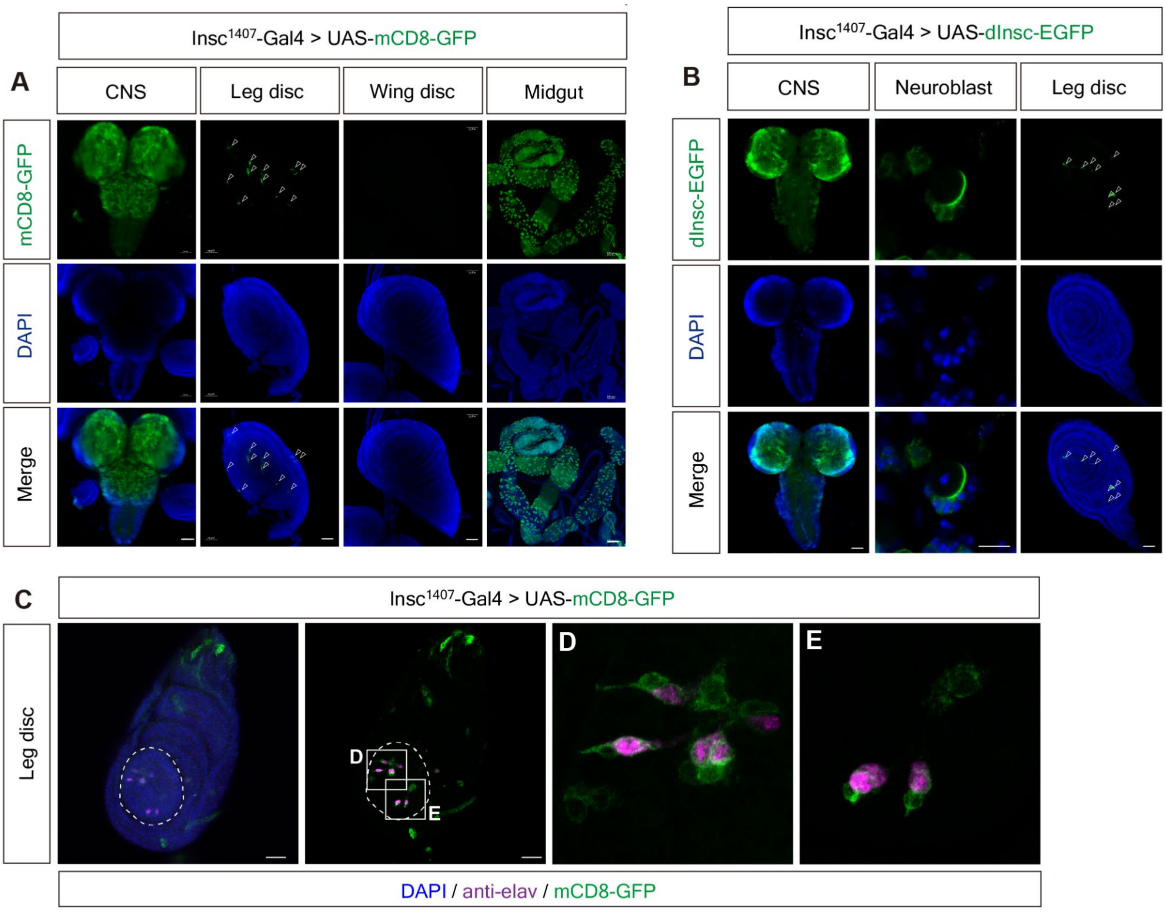
Data information: Error bars indicate mean ± SEM. Statistical analysis was performed using two-tailed Student's t-test. *P < 0.05, **P < 0.01, ***P < 0.001.



Appendix Fig S5. Schematic of a seamless editing knock-in strategy and sequence alignment of LGN binding domain.

A. Schematic presentation of a seamless editing knock-in strategy to generate two alleles, *dInsc*^{K305M} and *dInsc*^{K305R} with CRISPR-Cas9 technique to mimic *hINSC*^{WT} and *hINSC*^{M70R}, respectively.

B. Sequence alignment of human and flies LGN-binding domain of INSC.

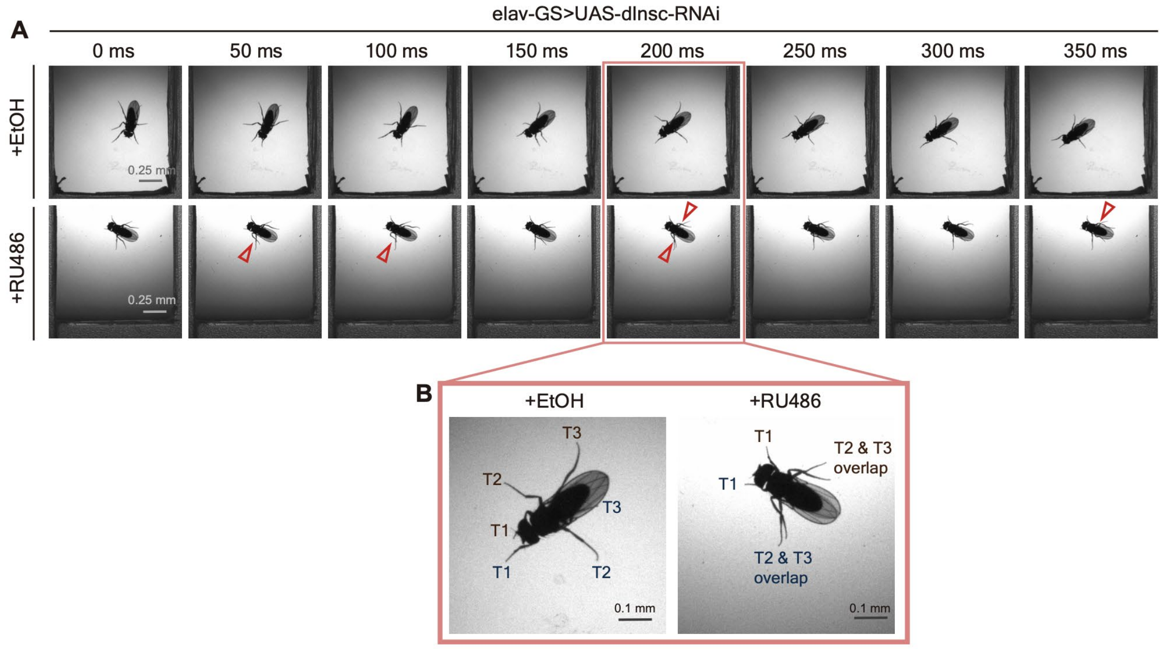


Appendix Fig S6. The expression pattern and protein localization of *Drosophila Inscuteable* in larval tissues.

A. Representative images of CNS, leg disc, wing disc and midgut labeled with *mCD8-GFP* (green) under the control of *dInsc^{1407}*-Gal4 in third instar larvae. (Blue) DAPI staining. Scale bars: 50, 50, 50 and 100 µm from left to right.

B. Representative confocal images of *dInsc-EGFP* (*dInsc^{1407}*-Gal4>UAS-*dInsc-EGFP*) in CNS, neuroblast, and leg disc of third instar larvae. Green indicates *dInsc*-EGFP. (Blue) DAPI staining. Scale bars: 50, 100 and 50 µm from left to right.

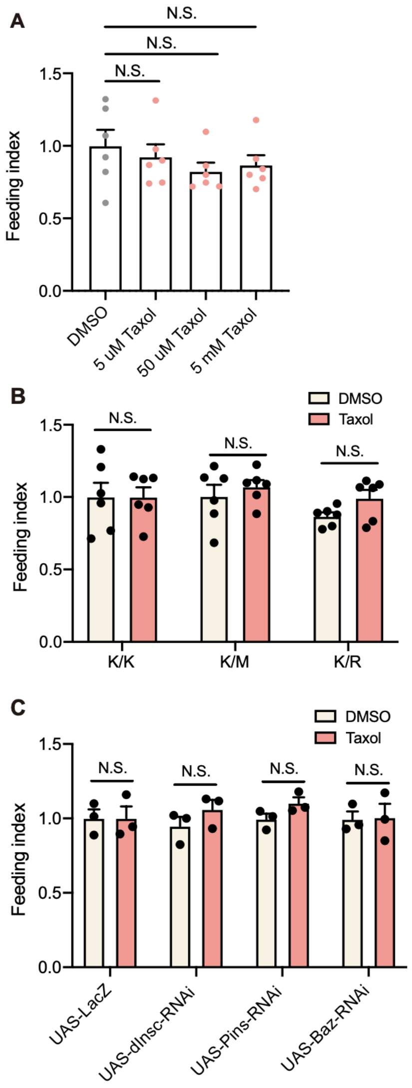
C-E. Representative confocal images of leg disc labeled with *mCD8*-GFP (green) under the control of *dInsc^{1407}*-Gal4 in third instar larvae. (Blue) DAPI staining; (magenta) anti-elav immunofluorescence. Scale bars: 50 µm.



Appendix Fig S7. The time-lapse imaging of the *Drosophila* walking behaviors

A. Snapshots from *Drosophila in vivo* imaging dataset for *elav-GS*>UAS-*dInsc*-RNAi flies fed with RU486 (bottom row), compared with the solvent (EtOH)-fed controls (top row). The interval of time-lapse is 50 ms. Scale bars: 0.25 mm.

B. Magnified images of (A). The RU486-fed flies, but not in solvent-feeding control, showed overlapped T2 and T3 legs (red arrowheads in (A)). Scale bars: 0.1 mm.



Appendix Fig S8. Feeding assay of Taxol feeding in different doses and genetic background

A. Feeding index of *W1118* (*K/K*) flies fed with different doses (5 µM, 50 µM and 5 mM) of Taxol; 24 flies/genotypes from 6 independent technical replicates.
B. Feeding index of *K/K*, *K/M*, *K/R* flies fed with 50 µM Taxol; 24 flies/genotypes from 6 independent technical replicates.
C. Feeding index of UAS-*LacZ*, UAS-*dInsc*-RNAi, UAS-*Pins*-RNAi and UAS-*Baz*-RNAi flies driven by *elav-GS*-Gal4. The flies were fed with 50 µM Taxol; 12 flies/genotypes from 3 independent technical replicates.

Data information: Error bars indicate mean ± SEM. Statistical analysis was performed using two-tailed Student's t-test. *P < 0.05, **P < 0.01, ***P < 0.001.

Appendix Table S1 Nerve conduction studies of the patients carrying *INSC* p.Met70Arg

Patient	Age at exam (y)	Motor nerve conduction studies						Sensory nerve conduction studies		
		Median nerve		Peroneal nerve		Tibial nerve		Median nerve		Sural nerve
		CV, m/s	CMAP, mV	CV, m/s	CMAP, mV	CV, m/s	CMAP, mV	CV, m/s	SNAP, uV	CV, m/s
II-2	59	53.9	6	37.6	0.3	NR	NR	NR	NR	NR
II-3	57	44.4	4.4	ND	ND	ND	ND	NR	NR	ND
II-8	52	56.6	4.7	45	2.6	42.2	2.0	NR	NR	NR
II-9	49	51.5	7	38	3.9	36.8	4.4	45.2	6	NR
III-3	30	55.5	12.2	39.6	2.1	40.1	1.4	NR	NR	NR
III-7	25	51.3	2.7	43.3	3.0	31.7	1.6	NR	NR	NR
III-8	22	54.5	5	41.7	0.7	36.8	0.7	NR	NR	NR
III-10	21	49	15.6	41.1	1.8	39.3	1	NR	NR	NR
Controls (mean ± SD)		58.6 ± 4	12.0 ± 3.9	48.8 ± 3.9	6.5 ± 2.9	49.6 ± 4.5	14.1 ± 4.5	56 ± 9	37.6 ± 16.4	48.3 ± 10.1

Control values are based on a previously published report.[ref]

CV = conduction velocity; CMAP = compound motor action potential; SNAP = sensory action potential; NR = no response; ND = not done; SD = standard deviation.
Ref: Lin KP, Chan MH, Wu ZA. Nerve conduction studies in healthy Chinese: correlation with age, sex, height and skin temperature. Zhonghua Yi Xue Za Zhi (Taipei) 1993;52:293-297.

Appendix Table S2 Bioinformatics analyses and filtering of the whole genome sequencing data

Filtering criteria	Affected individuals with CMT	
	III-3 (S009)	III-8 (Q217)
Total bases sequenced by whole genome sequencing (Mbases)	118,721	120,758
Average depth in targeted region (x)	37.2	38
Percentage of >10x coverage in targeted region (%)	99.5	99.6
Heterozygous variants	30,671	34,709
Shared heterozygous variants in affected individuals	14824	
Variants not present in 1517 Taiwan biobank control genomes	82	
Variants altering coding sequences	1	
Variants completely segregated with CMT phenotype	1 (*INSC* c.209T>G p.Met70Arg)	

CMT: Charcot-Marie-Tooth disease

Appendix Table S3 *Drosophila* stocks

Name	Genotype	Source
Insc^{1407}-Gal4	*w[*]; P{w[+mW.hs]=GawB}insc[Mz1407]*	BDSC_8751
UAS-dInsc-EGFP	*w[*]; P{UAS-dInsc.EGFP}2*	This study
UAS-mCD8-GFP	*y[1] w[*]; Pin[Yt]/CyO; P{w[+mC]=UAS-mCD8::GFP.L}LL6*	BDSC_5130
elav-GS-Gal4	*y[1] w[*]; P{w[+mC]=elav-Switch.O}GSG301*	BDSC_43642
UAS-dInsc-RNAi	*y[1] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.HMJ22537}attP40/CyO*	BDSC_60358
UAS-w-RNAi	*y[1] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.HMS00017}attP2*	BDSC_33623
UAS-LacZ	*y[1] w[1118]; P{w[+mC]=UAS-lacZ.Exel}3*	BDSC_8530
UAS-Pins-RNAi	*y[1] sc[*] v[1] sev[21]; P{y[+t7.7] v[+t1.8]=TKO.GS00670}attP40*	BDSC_77190
UAS-Bazooka-RNAi	*y[1] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.JF01079}attP2*	BDSC_31523
W1118	*W[1118]*	BDSC_3605
Insc$^{\text{InSITE}}$-Gal4	*w[1118]; PBac{w[+mC]=IT.GAL4}insc[1235-G4] sktl[1235-G4]/CyO*	BDSC_65626
UAS-dInsc-WT	*w[*]; P{w[+mC]=UAS-insc.K}3*	BDSC_39676
Iav-Gal4	*w[*]; P{w[+mC]=iav-GAL4.K}3*	BDSC_52273
OK371-Gal4	*w[1118]; P{w[+mW.hs]=GawB}VGlut[OK371]*	BDSC_26160
Insc^{p49}	*insc[P49]/CyO*	DGRC_108467
UAS-hINSC-WT-FLAG	*w[*]; P{UAS-hINSC-WT.FLAG}3*	This study
UAS-hINSC-M70R-FLAG	*w[*]; P{UAS-hINSC-M70R.FLAG}3*	This study
nan-Gal4	*w[*]; P{w[+mC]=nan-GAL4.K}2*	BDSC_24903
UAS-dPins-mCherry	*w[*]; P{UAS-dPins-mCherry}3*	This study
UAS-hNSC-WT-EGFP	*w[*]; P{UAS-hINSC-WT.EGFP}3*	This study
UAS-hNSC-M70R-EGFP	*w[*]; P{UAS-hINSC-M70R.EGFP}3*	This study
UAS-Bazooka-mCherry	*w[*]; P{y[+t7.7] w[+mC]=UAS-baz::mCherry}attP40/CyO*	BDSC_65844
Insc^{1407}-Gal4, UAS-tub-mCherry	*w[*]; P{w[+mW.hs]=GawB}insc[Mz1407]/CyO; P{w[+mC]=UAS-ChRFP-Tub}3*	BDSC_25773
Elav-Gal4	*P{w[+mC]=GAL4-elav.L}2/CyO*	BDSC_8765
PiggyBac	*w[*]; PBac{GFP[ECFP.3xP3]=5pBlueEye.hsp70-*	BDSC_32174

	PBac\T}Dmel2	
CRISPR dInsc K305M KI	*dInsc-K305M-KI*	This study
CRISPR dInsc K305R KI	*dInsc-K305R-KI*	This study
UAS-MTDS (microtubule-destabilizing system)	*w[*];;UAS-MTDS*	This study

Appendix Table S4 *Drosophila* genotype and associated figures

Figures	**Abbreviated genotype**	**Full genotype**
Fig 2A	*elav-GS*-Gal4>UAS-*mCD8-GFP*	*w[*];+/+;P{w[+mC]=elav-Switch.O}GSG301/P{w[+mC]=UAS-mCD8::GFP.L}LL6*
Fig 2A	*elav-GS*-Gal4>UAS-*dInsc*-RNAi	*w[*];P{y[+t7.7] v[+t1.8]=TRiP.HMJ22537}2/+; P{w[+mC]=elav-Switch.O}GSG301*
Fig 2A	*elav-GS*-Gal4>UAS-*w*-RNAi	*w[*];+/+;P{w[+mC]=elav-Switch.O}GSG301/P{y[+t7.7] v[+t1.8]=TRiP.HMS00017}3*
Fig 2A	*elav-GS*-Gal4>UAS-*Pins*-RNAi	*w[*];P{y[+t7.7] v[+t1.8]=TKO.GS00670}2/+; P{w[+mC]=elav-Switch.O}GSG301/+*
Fig 2A	*elav-GS*-Gal4>UAS-*Baz*-RNAi	*w[*];+/+;;P{w[+mC]=elav-Switch.O}GSG301/P{y[+t7.7] v[+t1.8]=TRiP.JF01079}3*
Fig 2B	*dInsc-InSITE*-Gal4> UAS-*dInsc-WT*	*w[*]; PBac{w[+mC]=IT.GAL4}insc[1235-G4] sktl[1235-G4]/+;P{w[+mC]=UAS-insc.K}3/+*
Fig 2B	*dInsc-InSITE*-Gal4 / +	*w[*]; PBac{w[+mC]=IT.GAL4}insc[1235-G4] sktl[1235-G4]/+*
Fig 2B	*dInsc-InSITE*-Gal4> UAS-*hINSC-WT*	*w[*]; PBac{w[+mC]=IT.GAL4}insc[1235-G4] sktl[1235-G4]/+;P{UAS-hINSC-WT.FLAG}3/+*
Fig 2B	*dInsc-InSITE*-Gal4> UAS-*hINSC-M70R*	*w[*]; PBac{w[+mC]=IT.GAL4}insc[1235-G4] sktl[1235-G4]/+;P{UAS-hINSC-M70R.FLAG}3/+*
Fig 2B	*dInsc-InSITE*-Gal4> UAS-*dInsc*-RNAi	*w[*]; PBac{w[+mC]=IT.GAL4}insc[1235-G4] sktl[1235-G4]/P{y[+t7.7] v[+t1.8]=TRiP.HMJ22537}attP40*
Fig 2B	*dInsc-InSITE*-Gal4> UAS-*dInsc*-RNAi, UAS-*hINSC-WT*	*w[*]; PBac{w[+mC]=IT.GAL4}insc[1235-G4] sktl[1235-G4]/P{y[+t7.7] v[+t1.8]=TRiP.HMJ22537}attP40;P{UAS-hINSC-WT.FLAG}3/+*
Fig 2B	*dInsc-InSITE*-Gal4> UAS-*dInsc*-RNAi, UAS-*hINSC-M70R*	*w[illegible]; PBac{w[+mC]=IT.GAL4}insc[1235-G4] sktl[1235-G4]/P{y[+t7.7] v[+t1.8]=TRiP.HMJ22537}attP40;P{UAS-hINSC-M70R.FLAG}3/+*
Fig 2D	*dInsc K305M/+*	w[*];dInsc K305M-KI/+

Fig 2D	*dInsc K305M/dInsc K305M*	w[*];dInsc K305M-KI/dInsc K305M-KI
Fig 2D	*dInsc K305R/+*	w[*];dInsc K305R-KI/+
Fig 2D	*dInsc K305R/dInsc K305R*	w[*];dInsc K305R-KI/dInsc K305R-KI
Fig 2E, Fig EV1A	*insc1407*-Gal4>UAS-*mCD8-GFP*	w[*]; P{w[+mW.hs]=GawB}insc[Mz1407]/+ ; P{w[+mC]=UAS-mCD8::GFP.L}LL6/+
Fig 2I	*Iav*-Gal4 > UAS-*mCD8-GFP*	w[*];+/+; P{w[+mC]=iav-GAL4.K}3/P{w[+mC]=UAS-mCD8::GFP.L}LL6
Fig 2I	*Iav*-Gal4 > UAS-*dInsc*-RNAi	w[*];P{y[+t7.7] v[+t1.8]=TRiP.HMJ22537}2/+; P{w[+mC]=iav-GAL4.K}3/+
Fig 2I	*Iav*-Gal4 > UAS-*Baz*-RNAi	w[*];+/+; P{w[+mC]=iav-GAL4.K}3/P{y[+t7.7] v[+t1.8]=TRiP.JF01079}3
Fig 2I	*Iav*-Gal4 > UAS-*Pins*-RNAi	w[*];+/P{y[+t7.7] v[+t1.8]=TKO.GS00670}2;P{w[+mC]=iav-GAL4.K}3/+
Fig 2J-N	*elav-GS*-Gal4>UAS-*dInsc*-RNAi	w[*];P{y[+t7.7] v[+t1.8]=TRiP.HMJ22537}2/+; P{w[+mC]=elav-Switch.O}GSG301
Fig 3D&E	*nan*-Gal4>UAS-*w*-RNAi	w[*]; P{w[+mC]=nan-GAL4.K}2/+;P{y[+t7.7] v[+t1.8]=TRiP.HMS00017}3/+
Fig 3D&E	*nan*-Gal4>UAS-*dInsc*-RNAi	w[*]; P{w[+mC]=nan-GAL4.K}2/P{y[+t7.7] v[+t1.8]=TRiP.HMJ22537}2;+/+
Fig 3D&E	*nan*-Gal4>UAS-*Pins*-RNAi	w[*]; P{w[+mC]=nan-GAL4.K}2/P{y[+t7.7] v[+t1.8]=TKO.GS00670}2;+/+
Fig 3D&E	*nan*-Gal4>UAS-*Baz*-RNAi	w[*]; P{w[+mC]=nan-GAL4.K}2/+;P{y[+t7.7] v[+t1.8]=TRiP.JF01079}3/+
Fig 3F, Fig EV3A	*dInsc K305M/+*	w[*];dInsc K305M-KI/+
Fig 3F, Fig EV3A	*dInsc K305M/dInsc K305M*	w[*];dInsc K305M-KI/dInsc K305M-KI
Fig 3F	*dInsc K305R/+*	w[*];dInsc K305R-KI/+
Fig 3F, Fig EV3A	*dInsc K305R/dInsc K305R*	w[*];dInsc K305R-KI/dInsc K305R-KI
Fig 3G	*dInsc K305M/+*	w[*];dInsc K305M-KI/+

Fig 3G, Fig EV3B	*dInsc K305M; elav-*Gal4>UAS-*hINSC-WT*	w[*];dInsc K305M-KI/P{w[+mC]=GAL4-elav.L}2;P{UAS-hINSC-WT.FLAG}3/+
Fig 3G, Fig EV2B	*dInsc K305M; elav-*Gal4>UAS-*hINSC-M70R*	w[*];dInsc K305M-KI/P{w[+mC]=GAL4-elav.L}2;P{UAS-hINSC-M70R.FLAG}3/+
Fig 3G, Fig EV2B	*dInsc K305M; elav-*Gal4>UAS-*dInsc-WT*	w[*];dInsc K305M-KI/P{w[+mC]=GAL4-elav.L}2;P{w[+mC]=UAS-insc.K}3/+
Fig 3G, Fig EV3B	*dInsc K305R/+*	w[*];dInsc K305R-KI/+
Fig 3G, Fig EV2B	*dInsc K305R; elav-*Gal4>UAS-*hINSC-WT*	w[*];dInsc K305R-KI/P{w[+mC]=GAL4-elav.L}2;P{UAS-hINSC-WT.FLAG}3/+
Fig 3G, Fig EV2B	*dInsc K305R; elav-*Gal4>UAS-*hINSC-M70R*	w[*];dInsc K305R-KI/P{w[+mC]=GAL4-elav.L}2;P{UAS-hINSC-M70R.FLAG}3/+
Fig 3G, Fig EV2B	*dInsc K305R; elav-*Gal4>UAS-*dInsc-WT*	w[*];dInsc K305R-KI/P{w[+mC]=GAL4-elav.L}2;P{w[+mC]=UAS-insc.K}3/+
Fig 4C-F	*nan*-Gal4>UAS-*hINSC-WT,* UAS-*Pins-mCherry*	w[*]; P{w[+mC]=nan-GAL4.K}2/+;P{UAS-hINSC-WT.FLAG}3/P{UAS-dPins-mCherry}3
Fig 4C-F	*nan*-Gal4>UAS-*hINSC-M70R,* UAS-*Pins-mCherry*	w[*]; P{w[+mC]=nan-GAL4.K}2/+;P{UAS-hINSC-M70R.FLAG}3/P{UAS-dPins-mCherry}3
Fig 4K-L	*iav*-Gal4>UAS-*hINSC-WT,* UAS-*Baz-mCherry*	w[*]; P{w[+mC]=iav-GAL4.K}2/P{y[+t7.7] w[+mC]=UAS-baz::mCherry}2;P{UAS-hINSC-WT.FLAG}3/+
Fig 4K-L	*iav*-Gal4>UAS-*hINSC-M70R,* UAS-*Baz-mCherry*	w[*]; P{w[+mC]=iav-GAL4.K}2/P{y[+t7.7] w[+mC]=UAS-baz::mCherry}2;P{UAS-hINSC-M70R.FLAG}3/+
Fig 5A-C	*Insc1407*-Gal4> UAS-*tubulin-mCherry,* UAS-*w*-RNAi	w[*]; P{w[+mW.hs]=GawB}insc[Mz1407]/+; P{w[+mC]=UAS-ChRFP-Tub}3/P{y[+t7.7] v[+t1.8]=TRiP.HMS00017}3
Fig 5A-C	*Insc1407*-Gal4> UAS-*tubulin-mCherry,* UAS-*dInsc*-RNAi	w[*]; P{w[+mW.hs]=GawB}insc[Mz1407]/P{y[+t7.7] v[+t1.8]=TRiP.HMJ22537}2; P{w[+mC]=UAS-ChRFP-Tub}3/+
Fig 5A-C	*Insc1407*-Gal4> UAS-*tubulin-mCherry,* UAS-*dInsc*-RNAi, UAS-	w[*]; P{w[+mW.hs]=GawB}insc[Mz1407]/P{y[+t7.7] v[+t1.8]=TRiP.HMJ22537}2; P{w[+mC]=UAS-

	hINSC-WT	ChRFP-Tub}3/P{UAS-hINSC-WT.FLAG}3
Fig 5A-C	*Insc1407*-Gal4> UAS-*tubulin-mCherry,* UAS-*dInsc*-RNAi, UAS-*hINSC-M70R*	w[*]; P{w[+mW.hs]=GawB}insc[Mz1407]/P{y[+t7.7] v[+t1.8]=TRiP.HMJ22537}2; P{w[+mC]=UAS-ChRFP-Tub}3/P{UAS-hINSC-M70R.FLAG}3
Fig 5D&E	*W1118*	w[1118]
Fig 5D&E	*dInsc K305M/+*	w[*];dInsc K305M-KI/+
Fig 5D&E	*dInsc K305R/+*	w[*];dInsc K305R-KI/+
Fig 5F&G	*Insc1407*-Gal4> UAS-*tubulin-mCherry,* UAS-*dInsc*-RNAi	w[*]; P{w[+mW.hs]=GawB}insc[Mz1407]/P{y[+t7.7] v[+t1.8]=TRiP.HMJ22537}2; P{w[+mC]=UAS-ChRFP-Tub}3/+
Fig 5F&G	*Insc1407*-Gal4> UAS-*tubulin-mCherry,* UAS-*Baz*-RNAi	w[*]; P{w[+mW.hs]=GawB}insc[Mz1407]/+;P{y[+t7.7] v[+t1.8]=TRiP.JF01079}3/P{w[+mC]=UAS-ChRFP-Tub}3
Fig 5F&G	*Insc1407*-Gal4> UAS-*tubulin-mCherry,* UAS-*Pins*-RNAi	w[*]; P{w[+mW.hs]=GawB}insc[Mz1407]/P{y[+t7.7] v[+t1.8]=TKO.GS00670}2;P{w[+mC]=UAS-ChRFP-Tub}3/+
Fig 6A&D	*dInsc K305M; Insc1407*-Gal4> UAS-*tubulin-mCherry*	w[*];dInsc K305M-KI/P{w[+mW.hs]=GawB}insc[Mz1407];P{w[+mC]=UAS-ChRFP-Tub}3/+
Fig 6A&D	*dInsc K305R; Insc1407*-Gal4> UAS-*tubulin-mCherry*	w[*];dInsc K305R-KI/P{w[+mW.hs]=GawB}insc[Mz1407];P{w[+mC]=UAS-ChRFP-Tub}3/+
Fig 6H, Fig EV5A	*dInsc K305M/+*	w[*];dInsc K305M-KI/+
Fig 6H, Fig EV5A	*dInsc K305R/+*	w[*];dInsc K305R-KI/+
Fig EV4A	*Insc1407*-Gal4> UAS-*tubulin-mCherry*	w[*]; P{w[+mW.hs]=GawB}insc[Mz1407]/+; P{w[+mC]=UAS-ChRFP-Tub}3/+
Fig EV4B	*Insc1407*-Gal4> UAS-*tubulin-mCherry,* UAS-*dInsc*-RNAi	w[*]; P{w[+mW.hs]=GawB}insc[Mz1407]/P{y[+t7.7] v[+t1.8]=TRiP.HMJ22537}2; P{w[+mC]=UAS-ChRFP-Tub}3/+

Appendix Table S5 List of plasmids used in this study

Name	**Source**
pUASt-attB-hINSC^{WT}-FLAG	This study (FLAG tag fused to C terminus)
pUASt-attB-UAS-hINSC^{M70R}-FLAG	This study (FLAG tag fused to C terminus)
pUASt-attB-UAS-hINSC^{WT}-EGFP	This study (EGFP fused to C terminus)
pUASt-attB-UAS-hINSC^{M70R}-EGFP	This study (EGFP fused to C terminus)
pUASt-attB-UAS-dInsc-EGFP	This study (EGFP fused to C terminus)
pUASt-attB-UAS-Pins-mCherry	This study (mCherry fused to C terminus)
pFLAG-hINSC^{WT}	This study (FLAG tag fused to C terminus)
pFLAG-hINSC^{M70R}	This study (FLAG tag fused to C terminus)
pMyc-LGN	This study (Myc tag fused to C terminus)
pHA-PAR3	This study (HA tag fused to C terminus)
pBFv-U6.2	Addgene #138400
pScarlessHD-DsRed	Addgene #64703

Appendix Table S6 List of primers used in this study

Quantitative real-time PCR primers	
Gene	**Primer sequence**
Human INSC	fwd: ACAGTGCTGTCAGCAGGAACTC
	rev: TGAAGGCACCTCTCTGTGACTG
Human beta-Actin	fwd: GCGTCGGTCAATTCAATCTT
	rev: AAGCTGCAACCTCTTCGTCA
Fly Baz	fwd: GACTCGGAGTTGGACTGATGG
	rev: CAGCGTTTGTTACTGCCCTCT
Fly Insc	fwd: GGCGGTTTCTATTCGAGCTTC
	rev: GATGCTGGCTGTCTTTGTGG
Fly Pins	fwd: GCGGAGAATGTGTCCAGTTTG
	rev: GAGTGCGAAGATCCTCGGTTC
Fly RP49	fwd: ACTTCATCCGCCACCAGTCG
	rev: CGGGTGCGCTT [illegible] TTCGATCC
Site-Directed Mutagenesis primers	
Gene	**Primer sequence**
hINSC-M70R	fwd: GCGGCTACACCTGAGGCAGGTGGACTCAGTC
	rev: GACTGAGTCCACCTGCCTCAGGTGTAGCCGC
Molecular cloning primers	
Plasmid	**Primer sequence**
pUASt-attB-UAS-dInsc-EGFP	fwd (BglII site): ATTCGTTAACAGATCTATGTCCTTTCAGCGTAGC rev (XhoI site): TAGAGGTACCCTCGAGTTACTTGTACAGCTCGTCC
pUASt-attB-UAS-dPins-mCherry	fwd (EcoRI site): AGGGAATTGGGAATTCATGTCCTCGCTCTCTGCG
	rev (KpnI site): AAAGATCCTCTAGAGGTACCTTACTTGTACAGCTCGTCCAT

pUASt-attB-UAS-hINSC-WT-FLAG	fwd (EcoRI site): CCCGAATTCCAAAATGAGACGGCCCCCTGGC
	rev (XhoI site): GGGCTCGAGCTACTTGTCATCGTCGTC
pUASt-attB-UAS-hINSC-M70R-FLAG	fwd (EcoRI site): CCCGAATTCCAAAATGAGACGGCCCCCTGGC
	rev (XhoI site): GGGCTCGAGCTACTTGTCATCGTCGTC
pUASt-attB-UAS-hINSC-WT -EGFP	fwd (KpnI site): GTTTTGTGGGATCCGGTACCATGGTGAGCAAGGGCGAG
	rev (KpnI site): AAAGATCCTCTAGAGGTACCTTACTTGTACAGCTCGTCCA
pUASt-attB-UAS-hINSC-M70R -EGFP	fwd (KpnI site): GTTTTGTGGGATCCGGTACCATGGTGAGCAAGGGCGAG
	rev (KpnI site): AAAGATCCTCTAGAGGTACCTTACTTGTACAGCTCGTCCA
pFLAG-hINSC-WT	fwd (EcoRI site): AAATTGAATTCACCATGAGACGGCCCCC
	rev (BamHI site): CGCGGATCCCACAAAACTCTCCTCC
pFLAG-hINSC-M70R	fwd (EcoRI site): AAATTGAATTCACCATGAGACGGCCCCC
	rev (BamHI site): CGCGGATCCCACAAAACTCTCCTCC

Appendix Table S7 List of antibodies used in this study

Name	**Source**	**Identifier**	**concentration**
Rat anti-FLAG	Novus biologicals	Novus Cat# NBP1-06712; RRID:AB_1625981	1:500 (IF), 1:1000 (WB)
Mouse anti-DLG	DSHB	DSHB Cat# 4F3	1:200 (IF)
Rabbit anti-HA	Abcam	Abcam Cat# ab9110; RRID:AB_307019	1:500 (IF), 1:1000 (WB)
Mouse anti-c-Myc	DSHB	DSHB Cat# 9E10; RRID:AB_2266850	1:500 (IF), 1:1000 (WB)
Rat anti-alpha-tubulin	Abcam	Abcam Cat# ab6160; RRID:AB_305328	1:500 (IF)
Mouse anti-acetylated tubulin	Sigma-Aldrich	Sigma-Aldrich Cat# T6793; RRID:AB_477585	1:500 (IF)
Rabbit anti-*Drosophila* Insc antibody	A gift from Dr. Hongyan Wang, Duke-NUS medical school	-	1:1000 (WB)
Rabbit anti-human INSC antibody	Invitrogen	Thermo Fisher Scientific Cat#PA5-58921	1:1000 (WB)
Mouse anti-actin	Biolegend	Biolegend Cat#643807	1:10000 (WB)
Alexa Fluor@-568 Phalloidin	Invitrogen	Thermo Fisher Scientific Cat#A12380	1:500
Alexa Fluor@-488 anti-Mouse IgG	Jackson ImmunoResearch Laboratories	Jackson ImmunoResearch Labs Cat# 715-545-150; RRID:AB_2340846	1:500