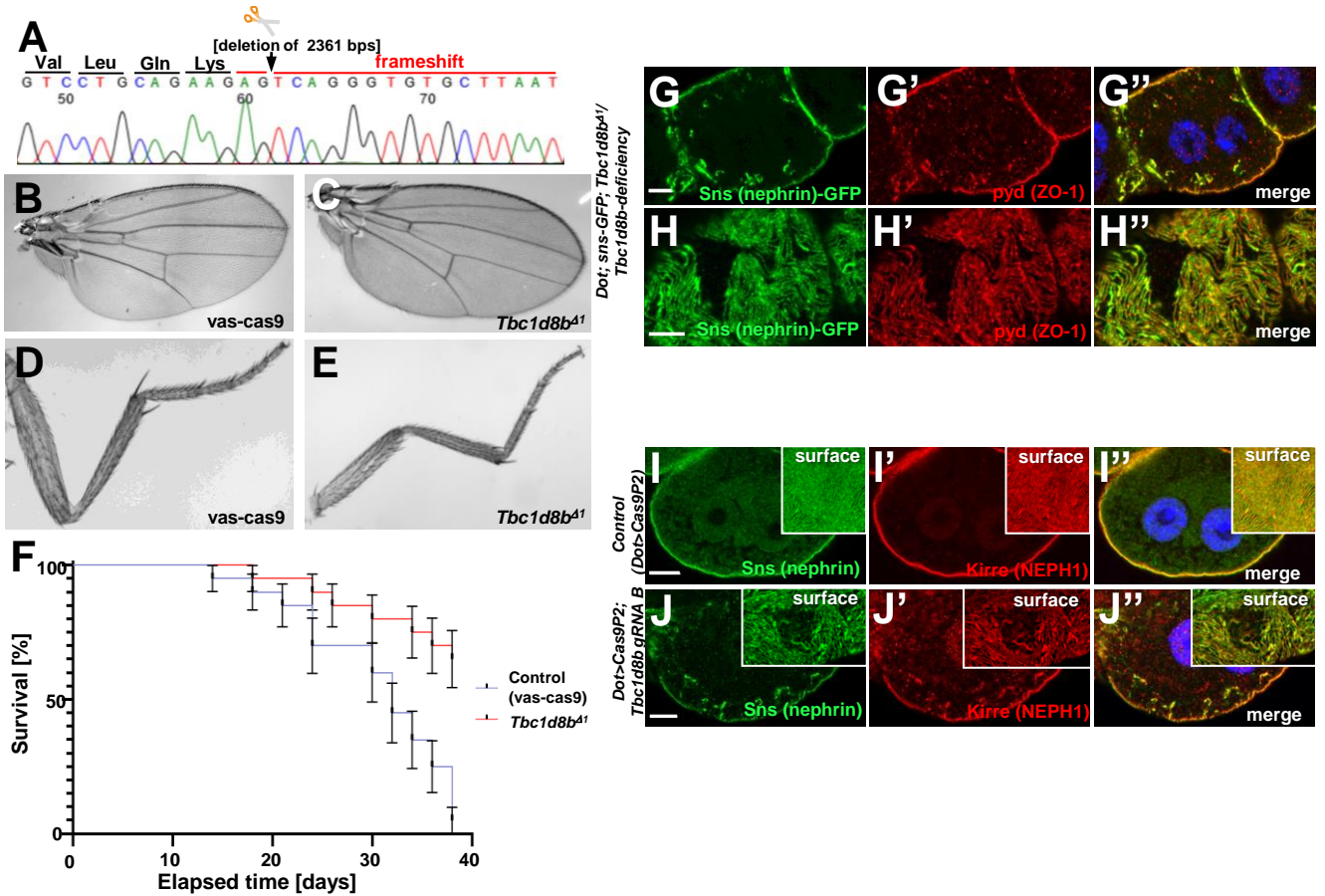


Supplemental Information

- **Supplemental Figure 1. Validation of *Tbc1d8b*^{Δ1}.**
- **Supplemental Figure 2: Loss of *Tbc1d8b* affects nephrin localization and barrier permeability.**
- **Supplemental Figure 3: Microhomology-mediated end joining and localization of Tbc1d8b.**
- **Supplemental Figure 4: Effects of Rab5-RNAi for sequential tracer uptake.**
- **Supplemental Figure 5: Rab5- and Rab7-staining in Tbc1d8b-LOF and validation of Hrs knockdown and antibody..**
- **Supplemental Figure 6: Alignment of human, murine and *Drosophila* *Tbc1d8b* and coexpression with human *Rab11*.**
- **Supplemental Figure 7: Overexpression studies of murine Tbc1d8b.**
- **Supplemental Figure 8: Patient-derived mutations in *TBC1D8B*.**
- **Supplemental Table 1: Primer sequences.**



Supplemental Figure 1: Validation of *Tbc1d8b^{A1}*.

(A) Shown is a Sanger chromatograms of the region of *Tbc1d8b* that is deleted in *Tbc1d8b^{A1}*. The sequence following the indicated cutting site of the respective gRNA aligns 2,361 bp downstream beyond the cutting site of the second gRNA indicating the interjacent sequence was removed.

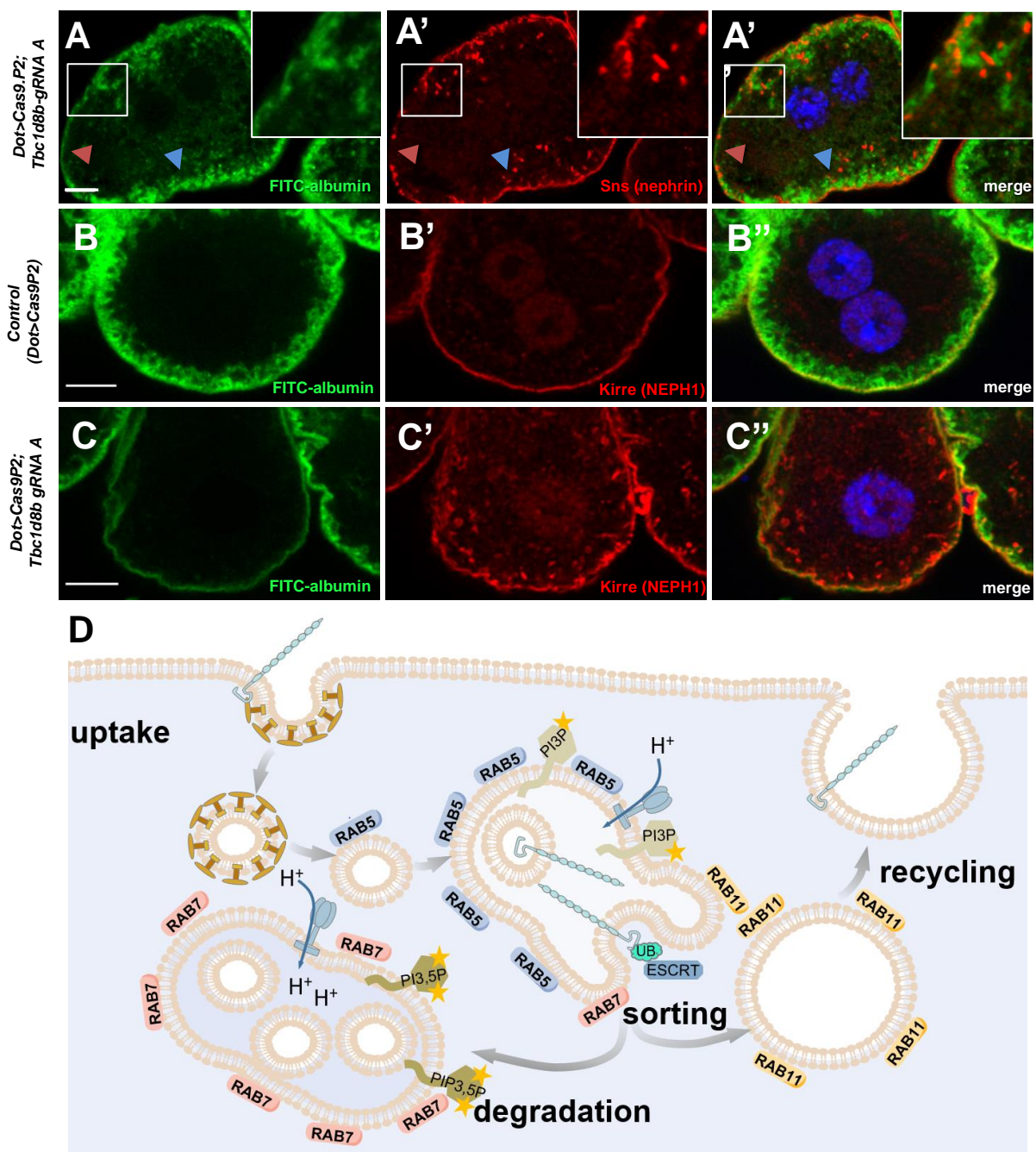
(B-C) Shown are *Drosophila* wings from control (vas-cas9) (B) vs. homozygous *Tbc1d8b^{A1}* flies (C). Wings are formed without overt phenotype in the mutant animals.

(D-E) Mounted legs from *Drosophila* are shown. Legs from homozygous *Tbc1d8b^{A1}* flies (E) show no structural anomaly compared to vas-Cas9 control (D).

(F) Survival of *Tbc1d8b^{A1}* animals is shown in comparison to vas-Cas9 as control.

(G-H'') Shown are cross sections (G-G'') and a tangential sections (H-H'') of nephrocytes that carry the *Tbc1d8b^{A1}* allele heterozygously over an independent deletion spanning several genes. The phenotype is comparable to the phenotype carrying *Tbc1d8b^{A1}* homozygously. This confirms that no second site mutation but the deletion of the *Tbc1d8b* locus is cause of the phenotype. Nephrin is visualized by knock-in of GFP into its genomic locus. Nuclei are marked by Hoechst 33342.

(I-J'') Nephrocytes after a conditional CRISPR/Cas9-mediated loss of *Tbc1d8b* using the same gRNAs employed to generate the stable deletion reveal a similar but more pronounced phenotype (J-J'') compared to control cells (Cas9 without gRNA, I-I'').

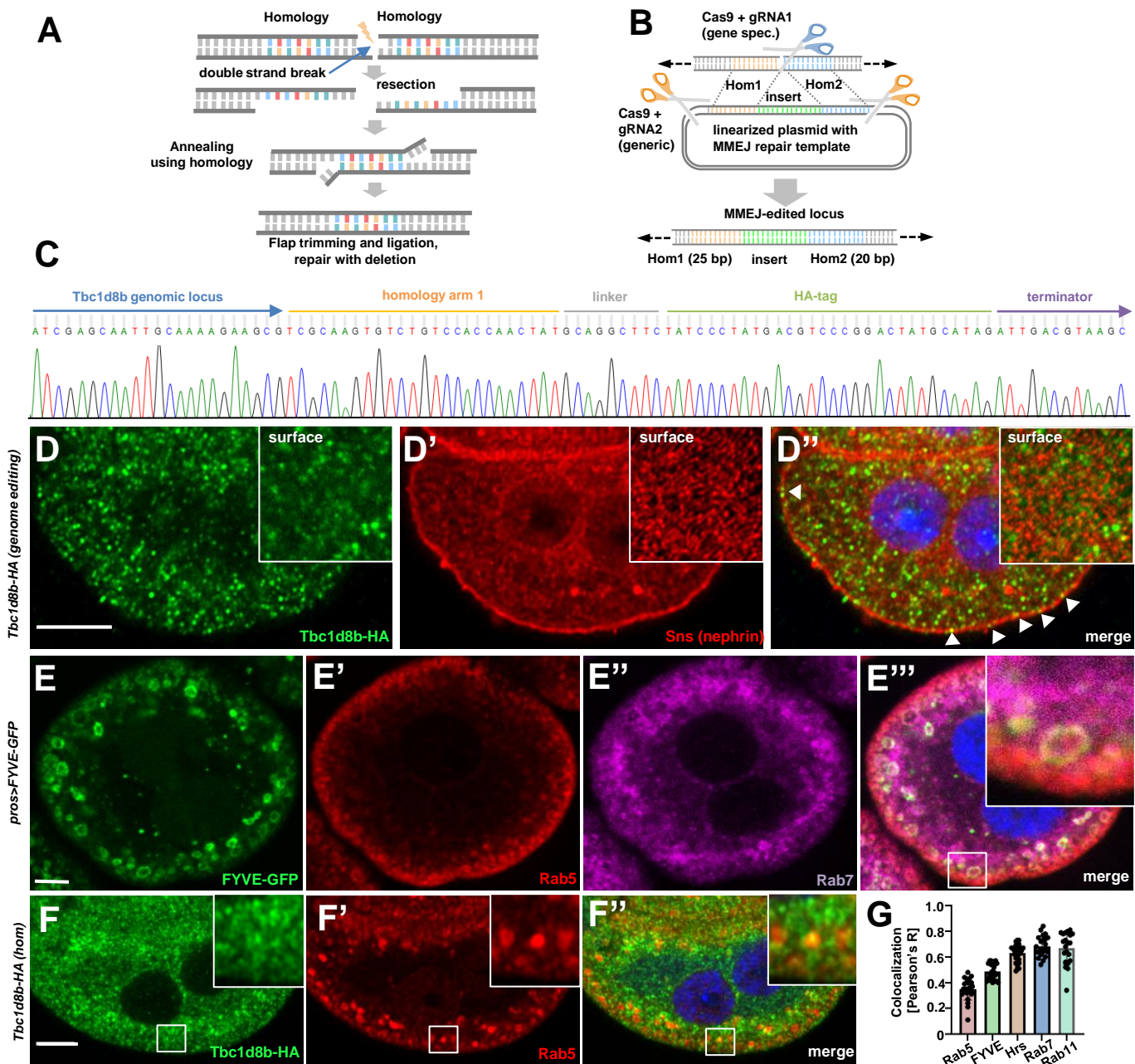


Supplemental Figure 2: Loss of *Tbc1d8b* affects nephrin localization and barrier permeability.

(A-A'') Nephrocytes with CRISPR/Cas9-mediated loss of *Tbc1d8b* were exposed extendedly to endocytic tracer FITC-albumin for 15 min ex vivo before fixation and nephrin (*Sns*) staining. The ectopic nephrin below the cell surface does not colocalize with the endocytic tracer (inset) and tracer endocytosis is diminished in surface sections lacking the slit diaphragm protein (red arrow head) compared to more intact sections (blue arrow head). Nuclei are marked by Hoechst 33342 in blue throughout the figure.

(B-C'') Confocal microscopy images of nephrocytes stained for Neph1 (green) together with labeling of the channels by FITC-albumin *via* passive diffusion after brief fixation. Channels extend below the surface, but are not filled upon loss of *Tbc1d8b*.

(D) Schematic illustrates endocytosis with the major Rab proteins. Rab5 promotes uptake and fusion of vesicles to form early endosomes as sorting stations. Early endosomes mature forming intraluminal vesicles and acidify. The ESCRT complex promotes formation of intraluminal vesicles. Rab11, also present in subdomains of early endosomes, promotes recycling back to the plasma membrane. Mature early endosomes increasingly form PI3P on their surface. The cascade initiated by Rab5 eventually triggers recruitment of Rab7 that promotes inactivation of Rab5, further acidification and formation of intraluminal vesicles and switch from PI3P to PI3,5P.



Supplemental Figure 3: Microhomology-mediated end joining and localization of *Tbc1d8b*.

(A) Schematic illustrates function of microhomology-mediated end joining (MMEJ). A double strand break is repaired by annealing of two homologous regions while the interjacent sequence is deleted.

(B) Schematic indicates principle of MMEJ-mediated genome editing. Two gRNAs are injected together: a generic gRNA (yellow) releases a linearized template with short homologies left and right of the double strand break caused by the gene-specific gRNA (blue). Repair results in insertion of the sequence between the homologies (green).

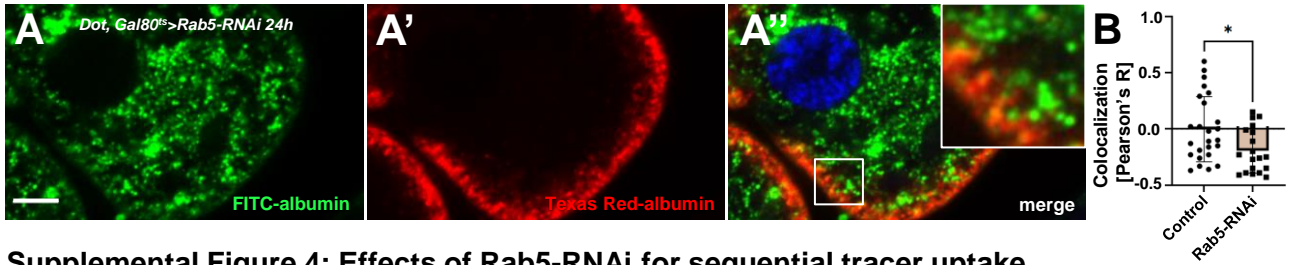
(C) Sanger trace confirms successful MMEJ-mediated knock-in of an HA-tag in frame into the C-terminus of the *Tbc1d8b* locus.

(D-D'') Nephrocytes from animals carrying *Tbc1d8b*-HA homozygously show a regular staining of slit diaphragm protein nephryn (Sns, inset shows tangential section). This suggests the tagged protein is functional. Marginal colocalization of nephryn (Sns) and *Tbc1d8b*-HA is observed on the surface (arrowheads).

(E-E''') A nephrocyte expressing FYVE-GFP is co-stained for early endosomal marker Rab5 and late endosomal marker Rab7. The FYVE-GFP reporters apparently localizes to an intermediate compartment with partial overlap. Nuclei are marked by Hoechst 33342 in blue here and throughout the figure.

(F-F'') Nephrocytes expressing *Tbc1d8b*-HA reveal partial colocalization of the transgene with Rab5 (magnified inset).

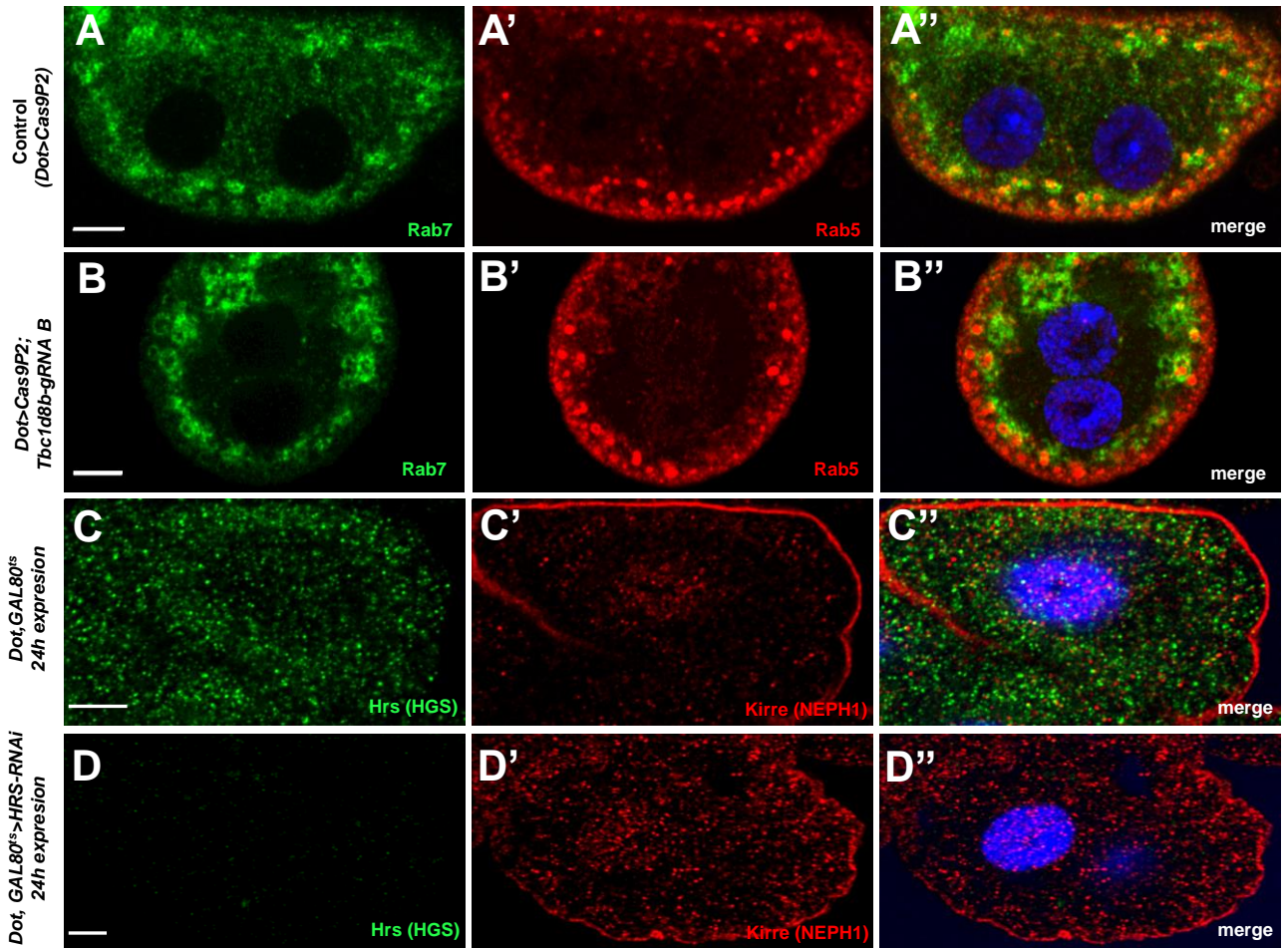
(G) Quantitation of colocalization in nephrocytes between *Tbc1d8b*-HA generated by genome editing and various endosomal compartment markers from immunofluorescence is shown (analogous to panel F and Fig. 3F-I, $n = 7-10$ animals per staining). Pearson's R coefficient ranges from -1 (mutual exclusion) to +1 (complete colocalization).



Supplemental Figure 4: Effects of Rab5-RNAi for sequential tracer uptake.

(A-A'') Sequential tracer endocytosis in nephrocytes expressing Rab5-RNAi shows diffuse localization of the first tracer and decreased colocalization.

(B) Quantitation of data expressed as Pearson's correlation coefficient (mean \pm standard deviation, n=10-12 animals per genotype, $P<0.05$) supports decreased colocalization.



Supplemental Figure 5: Rab5- and Rab7-staining in *Tbc1d8b*-LOF and validation of *Hrs* knockdown and antibody.

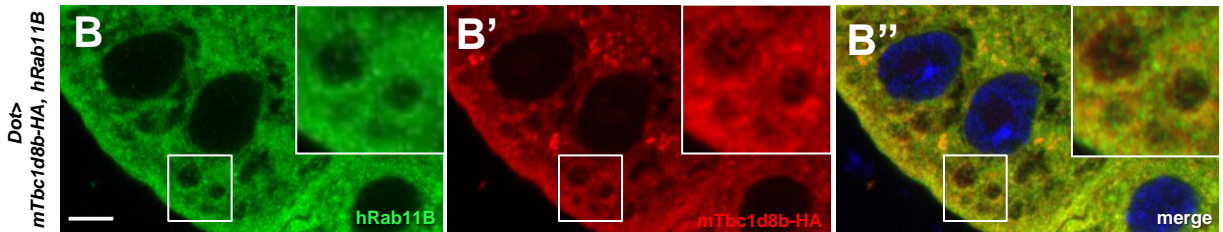
(A-B'') Confocal microscopy images of nephrocytes stained for endosomal markers Rab5 and Rab7. The endosomal markers localize in concentric circular layers with Rab7 showing greater distance to the cell surface. We did not observe an overt difference of the staining pattern comparing control (A-A'') to conditional CRISPR/Cas-mediated loss-of-function of *Tbc1d8b* (B-B''), suggesting that overall endosomal architecture is not disrupted.

(C-C'') Signal from Hrs-staining is diffuse and fine vesicular in the cell.

(D-D'') The signal from anti-Hrs is lost in nephrocytes expressing *Hrs*-RNAi (24h on 31°C) confirming the specificity of the signal and successful knockdown of *Hrs*. Kirre shows mislocalization upon silencing.

A

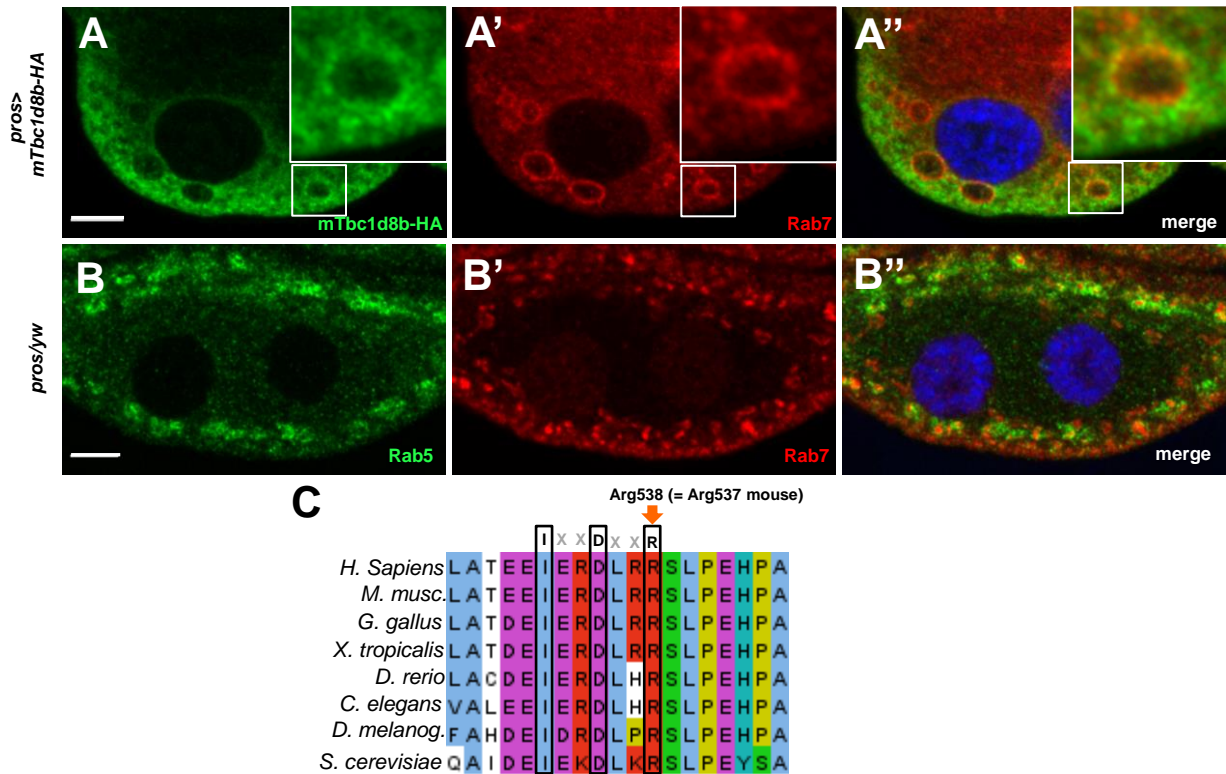
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M.musculus	MNLKPEEVLLKNAKLMLMERSNEYFVLQRIRRGYGEEGGGTLGLVGLTDSVLDAIKV	60
D.melanogaster	MNLQPKELLIPS--AFWIAEHSYFVLQRIRRGYGESIG--FGSLRLVGTDSVMT--KP	54
H.sapiens	APFRILHQTDPDSQVYLSIACGANREETIKHMDLEQNIKMLTSVDSNEDITNFVQGIKIR	120
M.musculus	APFRILHQTDPDSQVYLSIACGANREETIKHMDLEQNIKMLTSVDSNEDITNFVQGIKIR	120
D.melanogaster	APFRILHQTDPSSVSYEATIGTQDETVDWENKANKLRLVDEHENEDEVMTITCKIQ	114
H.sapiens	GLIAEEGKCFKAEEDPEKFRALLKFKCFGLPEKEKLVITYSCSYWIGRVPQGGMLYL	180
M.musculus	GLIAEEGKCFKAEEDPEKFRALLKFKCFGLPEKEKLVITYSCSYWIGRVPQGGMLYL	180
D.melanogaster	SLYTQWQD--TGESADPKVMSKFIQGFQPEERELVSYSATYVANKIPQGGVLY	171
H.sapiens	STNFLSFYSFLLGSEIKLLISWDEVSLEKTSWILTESTHVCSGENHYFSMFLHINQT	240
M.musculus	STNFLSFYSFLLGSEIKLLISWDEVSLEKTSWILTESTHVCSGENHYFSMFLHINQT	240
D.melanogaster	SLHNYCYSYMLGQIEKRTIFAELEDISMANITLYKTIN--NITYNITLFRASDA	227
H.sapiens	YLLMEQLANYAIRRLDKET--FNDPVLV-----NPLQITKRGLENRAHSEQFNAF	290
M.musculus	YLLMEQLANYAIRRLDKET--FNDPVLV-----DPLQITKRGLEYAHSEQFNAF	290
D.melanogaster	HLITEQLNVAIQQLTHDPSPVVDHDSFSLGKSTSKVYLRQLTARQSEEFY	287
H.sapiens	FRLPKGESLKEVHECFLLWPFSSHFNTHGKISENYICFASQDGGQCSVIPLREVLAD	350
M.musculus	FRLPKEETLKEVHECFLLWPFSSHFNTHGKISENYICFASQDGGQCSVIPLREVLAD	350
D.melanogaster	FRLPQSEITDGLKANITHTYSPKIFSGFYLSPKFCFISQDGLSVATPMTTSVVE	347
H.sapiens	KTNDSKSV----IITSGKTAIRFHEVKDFEQLVAKLRRCGAASTQYHDISTELATSS	406
M.musculus	KTNDSKSV----IITSGKTAIRFSELDFEQLVAKLRRCGAASTQYHDISTELATSS	406
D.melanogaster	KDDSGQIRFENQIVITTSSEVPFHAHVDKAVLSSKITDLARV-----HVLPSI	398
H.sapiens	ESTEPSDNFVQSLTSQRECKTVNTEALMTVFHPONLETNSKMLKEKMKQSKMLFA	466
M.musculus	DSTGPSNFEEQPLTCKPKCKTVNTEALMTVFHPONLETNSKMLKEKMKQSKMLFS	465
D.melanogaster	ER----AKYDIS-----HSQTALMNTFKTQFSA--ETIQQEEKVMVWEAHR	441
H.sapiens	ECGRGVSMFRKTKTDLVVRGIPETLRGLWMLFSGAVNDMATNPDYTVVEVQSLGT-C	525
M.musculus	ECGRGVSMFRKTKTDLVVRGIPETLRGLWMLFSGAVNDMATNPDYTVVEVQSLGT-S	524
D.melanogaster	DFRGIGIRFTTDLINLVEGIDKLRQEWILFSGAIDHKNPGLVDELVEKAACIKT	501
H.sapiens	NLATEEIERDLRHSLEPHAFQSDTGISALRRVLTAAYAINPKIGYQGMNLTSLVLLY	585
M.musculus	NLATEEIERDLRHSLEPHAFQSDTGISALRRVLTAAYAINPKIGYQGMNLTSLVLLY	584
D.melanogaster	CFAHDEIDRLRHSLEPHAFQSDTGISALRRVLQAYALRNQVGYQGMNIVSVFLFL	561
H.sapiens	AKEEFAFWLLVAVCEMLPDYFNRRITGALVDQVFEELRDHLPOLTEHTDMTFSSV	645
M.musculus	AKEEFAFWLLVAVCEMLPDYFNRRITGALVDQVFEELRDHLPOLTEHTDMTFSSV	644
D.melanogaster	CDENAFNMLASLCEMLPDYDKVVGQITDQGVNELVETLPHLGHLEQLGVKIRK	621
H.sapiens	SLSNFLTFLTSVLPITESAVNVDCFFYDGTAKILQLGLATLDYMLDKLLTCKDDAEAVTA	705
M.musculus	SLSNFLTFLTSVLPITESAVNVDCFFYDGTAKILQLGLATLDYMLDKLLTCKDDAEAVTA	704
D.melanogaster	SLSNFLTFLTSVLSYESSLHLCDFYEGAKIFMISLQITENHDKLLCQDDGEMLV	681
H.sapiens	LNRFDDIVNTKPSLPINQQGSNVSDKTSHTRVDTDLRESNEKYGN--RYEDIHSM	764
M.musculus	LNRFDDIVNTKPSLPINQQGSNVSDKTSHTRVDTDLRESNEKYGN--RYEDIHSM	763
D.melanogaster	LQNYLEGVMEYQVPPITDKRMKRVQ----TQTVTQLIEAYTKGEDITQQLTEEL	737
H.sapiens	RCINRLVYQTLTEETQVQLRVSSQDVMSLQELDELVIYFKELF----LSCYMLG	819
M.musculus	RCINRLVYQTLTEETQVQLRVSSQDVMSLQELDELVIYFKELF----LSCYMLG	818
D.melanogaster	RMHRLHILTNQDIDNEITVQAYQVPMFMSLHMLLTITREEHALSKLQQQQVQL	797
H.sapiens	CPVLK-----HHDPSPLYEQVQIDCQQRALYHLLSPWAHSANLDSLALW	865
M.musculus	CPGLK-----HHDPSPLYEQVQIDCQQRALYHLLSPWAHSANLDSLALW	864
D.melanogaster	CPLSSETPQLQPSRPIQDAGASGGRYEAYSVEVHTLTETPLRMKVSVD--IGEK	856
H.sapiens	TFRLLDENSDCLINFEKSSAIDIMYNGSFTKLLKLLKHLHPAYTEVSKDASKGDEL	925
M.musculus	TFRLLDENSDCLINFEKSSAIDIMYNGSFTKLLKLLKHLHPAYTEVSKTSSKGDEL	924
D.melanogaster	FLRLTDCKGTGLDFGQLINALGLVCSKNKMKLLVYHLPLLSKAEIERSRPPRPR	916
H.sapiens	SKEEL--LYFSQLHVSYPANEKAEASVHSPKGGKIDQAYLSQWQDELFXVEENTKD	983
M.musculus	STEEEL--LYFSQVYSYPADEKETEESGSPKGGKIDQAYLSQWQDELFXVEENTKD	982
D.melanogaster	TKDDAEAEAFEDFD----NDASESHALPSPSDHNFADDDFA----LISATQHLN	966
H.sapiens	LPRMNSQFIQFSKTYLNLFHEDPEEESLYQAIATVTSLLRMEEVGRKLHSPSSAKGF	1043
M.musculus	LPRMNSQFIQFSKTYLNLFHEDPEEESLYQAIATVTSLLRMEEVGRKLHSPASSATF-	1041
D.melanogaster	LAGISGHTFNDLSRTPHLSVSN--TSSLAH--SSTFYDLPFGQDGAARITPPEAGVG-	1023
H.sapiens	SGTVC--GS-----	1050
M.musculus	---A-RD-----	1044
D.melanogaster	-ADVAGAASSAAAHPLLNVETISNFSQISDLVAATRLERVDSNATDLHSLGSLGYLD	1082
H.sapiens	---GGPSEE-----	1056
M.musculus	---SGPSEG-----	1050
D.melanogaster	QPDGASNSQHSIPHMKENFQLLWRSITEIMGLVQDEEMQRAYENLLELGNISIKPEPS	1142
H.sapiens	-----KTGSHLEKQPC	1067
M.musculus	-----NAESSVKDLP	1061
D.melanogaster	LESFTQLNMGDEQPSNGNPPTPAPEPIGTTLRVALEELRQASGSHITTTSSGDS	1202
H.sapiens	SFREEPQHSFAFEQILASLLNEPALVIRFFKPIDVAKLENARISQLRSRTKM-	1120
M.musculus	SPREEHQHSFAFEQILASLLNEPALVIRFFERPLDLKAKLENASSQLRSRTKM-	1114
D.melanogaster	SNTEISESHISTQFIATVLTNIVIRGFTPIQISEQELQCKRRKCLSTNY	1256



Supplemental Figure 6: Alignment of human, murine and *Drosophila* *Tbc1d8b* and coexpression with human *Rab11B*.

(A) Protein sequences are aligned using Clustal Omega (<https://www.ebi.ac.uk/Tools/msa/clustalo/>).

(B) Co-expression of human *Rab11B* and murine *Tbc1d8b*-HA in *Drosophila* in nephrocytes results in partial colocalization of both expressed transgenes.

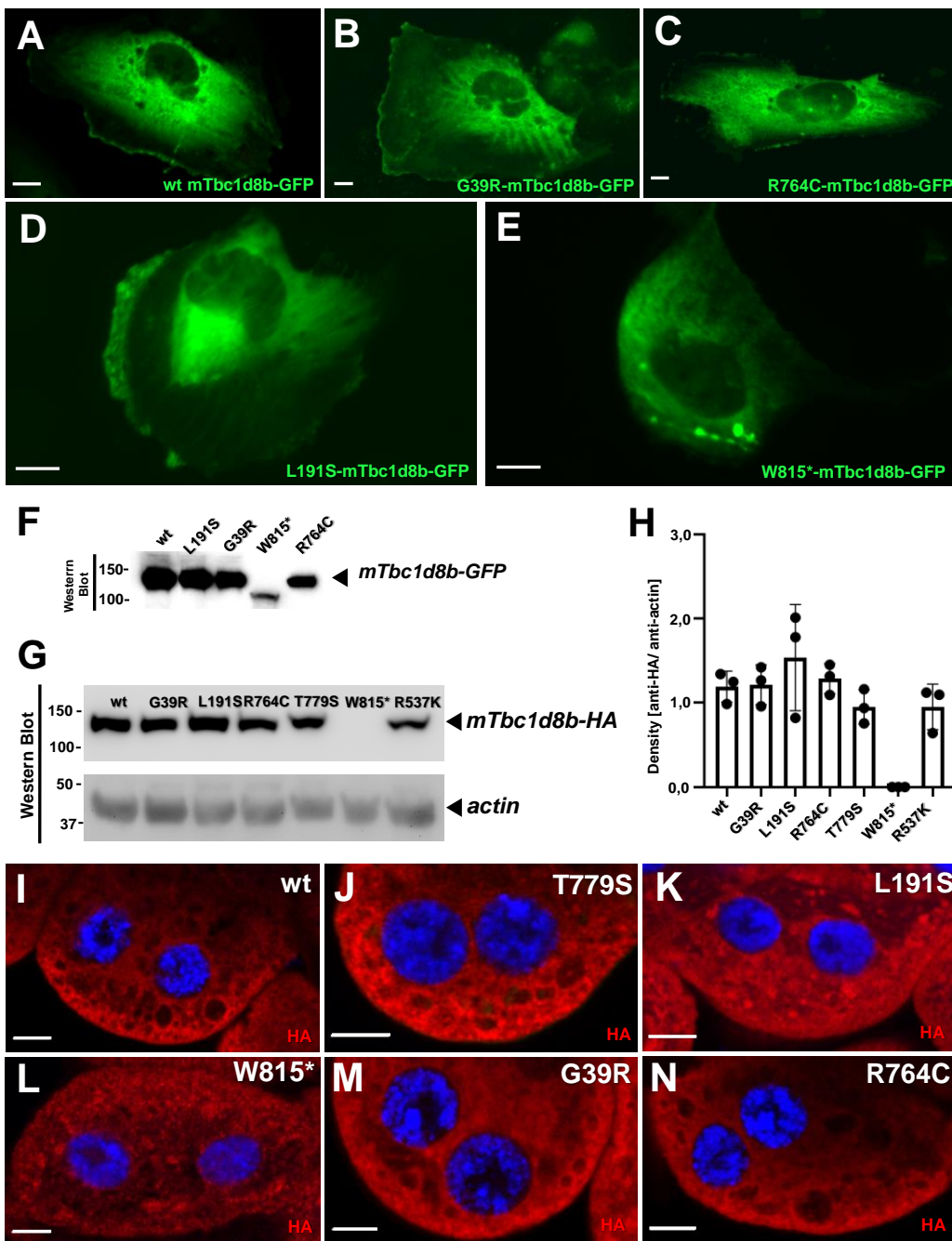


Supplemental Figure 7: Overexpression studies of murine Tbc1d8b.

(A-A'') Staining of nephrocytes overexpressing murine *Tbc1d8b*-HA reveals localization of the mammalian protein includes the outer membrane of enlarged endosomes.

(B-B'') Control nephrocytes occasionally show Rab5 within the larger Rab7 positive vesicles but no extensive colocalization on the outer membrane of late endosomes.

(C) Alignment of TBC1D8B amino acid sequences around residue Arg538 is shown. The sequence reveals a conserved IXXDXXR motif. This suggests this residue is the catalytically essential arginine finger.



Supplemental Figure 8: Patient-derived mutations in *TBC1D8B*.

(A-E) Cultured podocytes were transiently transfected with GFP-tagged variants of *mTbc1d8b*-HA and reporter fluorescence revealed a cytosolic localization (A-D) with the exception of the truncating mutant W815* (E). Scale bars represent 10 μ m. Brightness was adjusted for each image to ensure proper image quality.

(F) Cultured HEK293T-cells were transiently transfected with GFP-tagged variants of *mTbc1d8b*. Immunoblotting confirms decreased expression of the truncating variant in a mammalian cell system.

(G) Immunoblotting from third instar larve expressing the indicated variants of *mTbc1d8b*-HA under control of *prospero* GAL4 (31°C) indicates equal expression except for the truncating mutant W815*.

(H) Quantitation of the density of (G) expressed as ratio (anti-HA/anti-actin) reveals no statistical significant difference except for W815*.

(I-N) Staining the corresponding genotypes from for HA-tag in nephrocytes reveals the subcellular localization of the respective transgenes. Wild type *mTbc1d8b* localizes partially in the cytosol but also in vesicles at the cell periphery (I). Mutant transgenes locate in smaller vesicles across the cell in a mildly divergent pattern for L191S (K) and W815* (L). T779S (J), G39R (M) and R764C (N) did not show a significantly different staining pattern. Laser power was elevated for W815* due to protein lower abundance.

Construct	Direction	Primer sequence
G39F-mTbc1d8b (no tag)	Forward	TACGGGGAAGAAGGAGGGGGAGCTTACAG
G39R-mTbc1d8b (no tag)	Reverse	CTGTAAAGTCGCCGCTCTCTCTGTCGCCGTA
L191S-mTbc1d8b (no tag)	Forward	CAGCTTTCGAGCTCTCTATTCCTCTCGCTAGGATCAGAAATTA
L191S-mTbc1d8b (no tag)	Reverse	TAATTTCTGATCCTAGCGAAGGAATAGAAGCTCAGAAAGTTG
R764C-mTbc1d8b (no tag)	Forward	CTATAGAATATACATAGCATGTGCTGTGCAAAATAGGTTGTATGT
R764C-mTbc1d8b (no tag)	Reverse	ACATAGAACCTTATTGACAGACATGCTATGATATCTTCTATG
W815'-mTbc1d8b (no tag)	Forward	GAGGCTGTTATATCTTGTATTGATTTTGAAGTTGTCGAGGATTGAA
W815'-mTbc1d8b (no tag)	Reverse	TTCAATCTCGGCAAACTCAAATATCAATACAAAGATATAACAGCTC
mTbc1d8b-variants in HA-backbone (except for W815')	Reverse	GAGCTCATAGGATAGAAGCCTGGCATCTTGGTTCTAGACCTTAGC
W815'-mTbc1d8b-HA (no tag W815' into HA-backbone)	Reverse	CGGCGAGAGATTTAGGCAAAATCAGGCTCAATTTCTTC
R537K-mTbc1d8b-HA	Forward	TTTGCCTAAATCTCTCGCGGAACATCCAGCA
generic primer for mTbc1d8b variants	Forward	GTCAAAAAAGCAGGCTTCAACCATGTGCGTGAAGCCCGAGGA
human Rab11B	Reverse	CTTTGTACAAGAAAGCTTGCGTCTATACATCTTGGTTCTAGACCTTAG
Tbc1d8b gRNA B pCFD5_w	Forward	GTCAAAAAAGCAGGCTTCAACCATGTGCGGACCCGGGACGAC
Tbc1d8b-HA MMEJ repair template backbone	Reverse	CTTTGTACAAGAAAGCTTGCGTCTATACAGGTTCTGGCAGAC
Tbc1d8b-HA MMEJ repair template insert PCR 1	Forward	SGCGCCGGGTTGATTCGCGCGCGATGCAAGTGGCGCGCAATCGATCGATCAGTTTAGAGCTAGAAAATAGCAAG
Tbc1d8b-HA MMEJ repair template insert PCR 2	Forward	ATTTTAACTTGCTATTCTAGCTCTAAAACCGTCTCTCTGCAAGACAAATGCACCAGCCGGGAATCGAAACCC
Tbc1d8b-HA MMEJ repair template insert PCR 1	Reverse	AGGACCGAGCTTTCTGTACAAAG
Tbc1d8b-HA MMEJ repair template insert PCR 2	Forward	CCTGTACTGATCTGCGSTTAATGCAATGSGTGAAGCCTGCTTTTGTAC
Tbc1d8b-HA MMEJ pCFD4 for guide RNAs	Reverse	CATTAACCGAATACGATCAGGTGCGAAGTGTCTGTCCACCACTATCGAGGCTCTATCCCTATGACGTCGCCGACTATGCATAGATTGACGTAAAGCTAGACG
Tbc1d8b-HA MMEJ sequencing primer	Forward	AGATCGTCGGCAAGACATCCACCTT
Tbc1d8b-HA MMEJ sequencing primer	Reverse	AAGTGGATGTCTCTTGCCGACGATCTTTACTAGTGCTCTCTATAACT
Tbc1d8b-HA MMEJ pCFD4 for guide RNAs	Forward	CTTTGTACAAGAAAGCTGGGTCCTCAATAGCGAAGATCAGTACAGGACTATGATTATTCAGTAATCCATGACGTTGCGTAATCAGCA
Tbc1d8b-HA MMEJ pCFD4 for guide RNAs	Reverse	TATATAGAAAGATATCCGGGTGAACCTTCGTATGATTAATTCAGTAATTTGGGTTTTAGAGCTAGAAAATAGCAAG
Tbc1d8b-HA MMEJ sequencing primer	Forward	ATTTTAACTTGCTATTCTAGCTCTAAAACGCTACTGATCTTGGGTTAATGCGAGCTTAAATGAAAATAGGTC
Tbc1d8b-HA MMEJ sequencing primer	Reverse	AGAGGAGCTTGGTCAGCGCA
Tbc1d8b (D m.) null allele sequencing primer	Forward	TTACTAGAGATCGTACGGATCTG
Tbc1d8b (D m.) null allele sequencing primer	Reverse	ATAAGCCGACTCTAAAGGTGAAC
Tbc1d8b gRNA A on X_pCFD5_w	Forward	CTCCAGATGGCCGTCGCAATC
Tbc1d8b gRNA A on X_pCFD5_w	Reverse	CGCGCCGGGTTTCGATTCCCGCGCGGATGCACCTACTACAAGGATAAAGTGGGTTTTAGAGCTAGAAAATAGCAAG
Tbc1d8b gRNA A on X_pCFD5_w	Reverse	ATTTTAACTTGCTATTCTAGCTCTAAAACCGTATTCCCGTGGCCACGTCGACCAGCGGGGAATCGAAACCC

Supplemental Table 1: Primer sequences