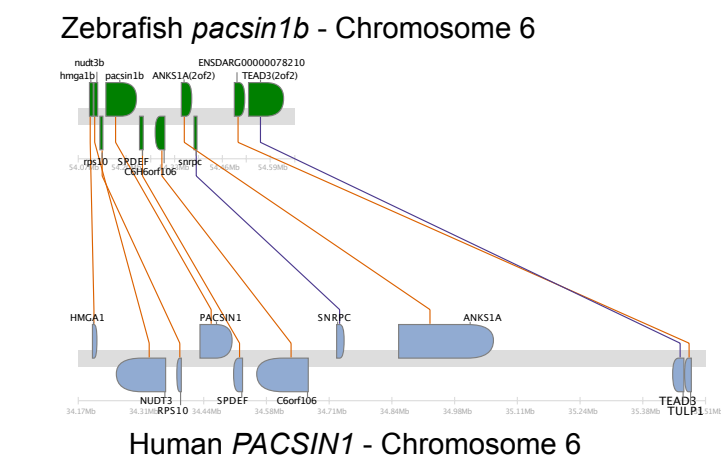


C

F-BAR domain

ch211-51c14-1	11	-EDANNQSFWMPGNVYHOTV	KRTEDSFQACNDIVACFOERAR
zgc:91999	10	-EDINKTSFWMPIYVIRY	ORTSESYQACRDIACMKLRAR
pacsin3	8	QDVGSDSFWEPGNYKRT	KRLDDGKRLCNELVSCFOERAR
pacsin2	9	-IDVSSDSFWEPGNYKRSY	KRLDDGKRLCNELVSCFOERAR
pacsin1a	12	--DEAMDSFWEPGNYKRAY	KRLDDGKRLCNELVSCFOERAR
pacsin1b	12	--DETSDSFWEPGNYKRAY	KRLDDGKRLCNELVSCFOERAR
ch211-51c14-1	51	VERQYARQQLSEWSAKWKP	LVDSAPLYGSLLRWQCFLSSAD
zgc:91999	50	VERQYARQLTQSDKWKSI	TESTRPLYGSLLRWQCFLSSSTE
pacsin3	49	IEKGSQQLSDWARKWRG	IYVEKGSQYGLERAWHAFMNAAD
pacsin2	49	IEKGSQQLSDWARKWRG	IYVEKGSQYGLERAWHAFMNAAD
pacsin1a	51	IEKGSQQLSDWARKWRG	IYVEKGSQYGLERAWHAFMNAAD
pacsin1b	51	IEKGSQQLSDWARKWRG	IYVEKGSQYGLERAWHAFMNAAD
ch211-51c14-1	91	RLSSLSHSICRALVSE	DGRIRLFWOKETFFKKI
zgc:91999	91	RLSALHTESSISOSL	VSEDEGRIRLFWOKETFFKKI
pacsin3	90	RLSEIRMLLENLV	IEDSEKIRNWQDAPHKQMG
pacsin2	90	RLSEIRMLLENLV	IEDSEKIRNWQDAPHKQMG
pacsin1a	92	KVSELHQEVKRNLMN	EDVEKVNQWQDYHKKQ
pacsin1b	92	KVSELHQEVKRNLMN	EDVEKVNQWQDYHKKQ
ch211-51c14-1	133	DFEFTGFSSRAQKP	WAKRLKLEKARSAPFKACR
zgc:91999	132	DLLETAFSSRAQKP	WVKKRLLEKARAVYHKS
pacsin3	131	DADEGFRRAQKP	WVKKLEVESTKKEVHSA
pacsin2	131	EAEDEGFRRAQKP	WAKKLKEMETMKKAVHT
pacsin1a	133	EAEDEGFRRAQKP	WAKKLKEMETMKKAVHT
pacsin1b	133	EAEDEGFRRAQKP	WAKKLKEMETMKKAVHT
ch211-51c14-1	173	THAOGNPDIAIEKQ	RRIQIEEELAHQETEVKRA
zgc:91999	173	NOARKLADLSESKI	SKIQQTREKATOBERRKAR
pacsin3	172	THAKADPTKSOD	EVRFKFDRLKTKTOBAE
pacsin2	172	NSSKLDN-SNPEAC	RKLOEKVEKQOEVQRK
pacsin1a	174	A--DASVTDPDQ	KLLEKTEKQDVKRAK
pacsin1b	174	ANSRGEASVTDPD	QKLQEKVDKKNQVQRAK
ch211-51c14-1	214	VTRYAPRYMEESE	TFDQSEERKRKLSFIRK
zgc:91999	214	VSGFAPRYMESE	VFDSQEEERKRKLSFIRK
pacsin3	213	LNRGNPRYME	MEQVFEIIEQAEKRL
pacsin2	212	LDRVTPDYME	NMEQVFEIIEQAEKRL
pacsin1a	211	LSKCRPPYME	MEQVFDCCQHEVVKR
pacsin1b	215	LNKCTPPDYME	NMEVVFDCQHEVVKR
ch211-51c14-1	255	DVTNNE	SVKAVYNELHDTLMSIS
zgc:91999	255	DIANNESI	KAVYSELHHTLMSIS
pacsin3	254	DLSSNDRF	FRALVRLDQGTISAAND
pacsin2	253	DLSSNHK	FTTVVHTLQDTIQGAD
pacsin1a	252	NLTEENQ	NVAAVYREFERTILAANT
pacsin1b	256	NLTESQSY	ATVYRELERTIVSASA



E

Variable region

Si: ch211-51c14	283	LRWWKNT	HGGPMTDWPQF	EWTPDKKKKKG
zgc:91999	282	LRWWKNT	HGGPMTDWPQF	EWTPDKKKKKG
pacsin3	281	LRWNRN	THGGPMTDWPQF	EWTPDKKKKKG
pacsin2	280	LKWFRN	SHGGPMTDWPQF	EWTPDKKKKKG
pacsin1a	283	LKWFRN	SHGGPMTDWPQF	EWTPDKKKKKG
pacsin1b	279	LKWFRN	SHGGPMTDWPQF	EWTPDKKKKKG
Si: ch211-51c14	314	KKEVEQ	KAAVME--RSVM----	
zgc:91999	313	GKKP	RAHKGTD--KAVM----	
pacsin3	312	RKDRNS	RSDDVVTITNIVSAGDE	
pacsin2	311	RREFTR	RRPAGCVTLTATSHP	
pacsin1a	310	KREK	KKPD--GVAPAT--	
pacsin1b	314	KKEVKR	RNHGDVTLTQVTHG	
Si: ch211-51c14		-----	-----	
zgc:91999	342	-----	TTRAGKDVSSD	
pacsin3	336	PAVSS	SEAACINPFDDDDDD	
pacsin2	336	GVS	SYDKNQY--Y--S	
pacsin1a	345	GVS	SYDKNQY--Y--S	
pacsin1b		-----	-----	
Si: ch211-51c14		-----	-----	
zgc:91999	364	AANGV	DDEEK	
pacsin3	372	THSS	PVKHEEAVQA	
pacsin2	362	-----	-----	
pacsin1a	371	-----	-----	
pacsin1b		-----	-----	
Si: ch211-51c14		-----	-----	
zgc:91999	403	DDDEE	ESCNPFSSSSANGN	
pacsin3	363	N-----	ETNGGANSPFEED	
pacsin2	372	S-----	ASATNGGNPFEE	
pacsin1a		-----	-----	
pacsin1b		-----	-----	

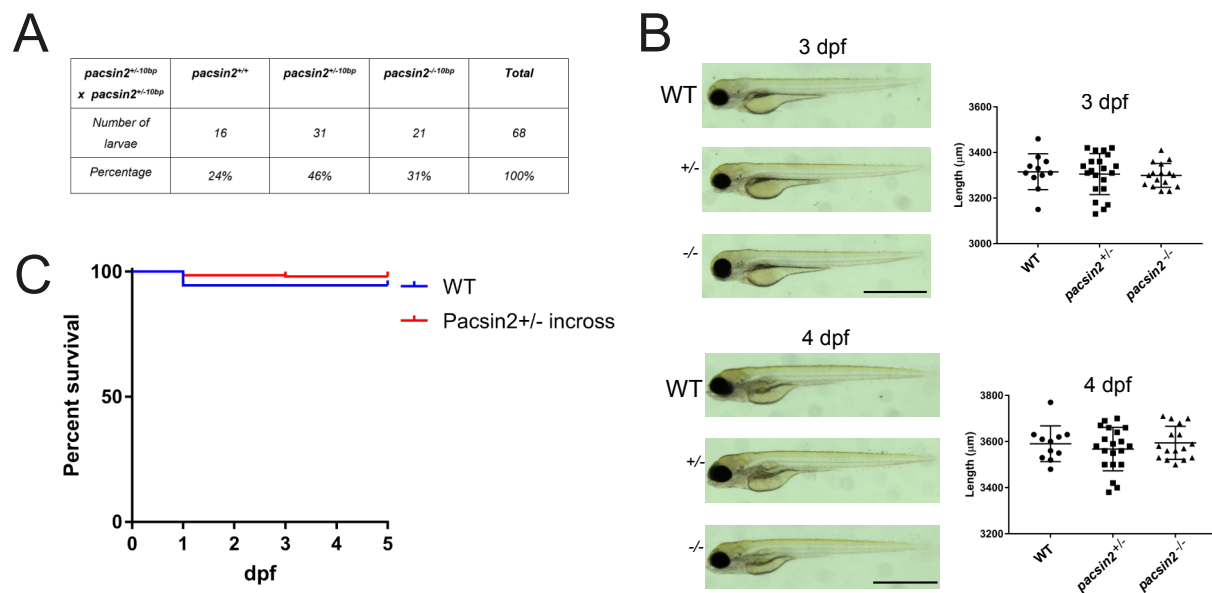
D

SH3 domain

ch211-51c14-1	330	CGVVRV	RAYVYVGGQ	TFDEL
zgc:91999	329	CGVVRV	RAYVYVGGQ	TFDEL
pacsin3	374	VVGVVR	RAYVYVGGQ	TFDEL
pacsin2	430	EMCV	VVRAYVYVGGQ	TFDEL
pacsin1a	397	CGVVRV	RAYVYVGGQ	TFDEL
pacsin1b	386	SKGVVR	RAYVYVGGQ	TFDEL
ch211-51c14-1	360	DED	QGNCRGM	DCCKEGLYPAN
zgc:91999	361	DED	QGNCRGM	DCCKEGLYPAN
pacsin3	406	ED	QGNCKGQL	TSCEKGLYPAN
pacsin2	462	ED	QGNCKGRL	SDCTVGLYPAN
pacsin1a	462	ED	QGNCKGRL	SDCTVGLYPAN
pacsin1b	418	ED	QGNCKGRL	SDCTVGLYPAN

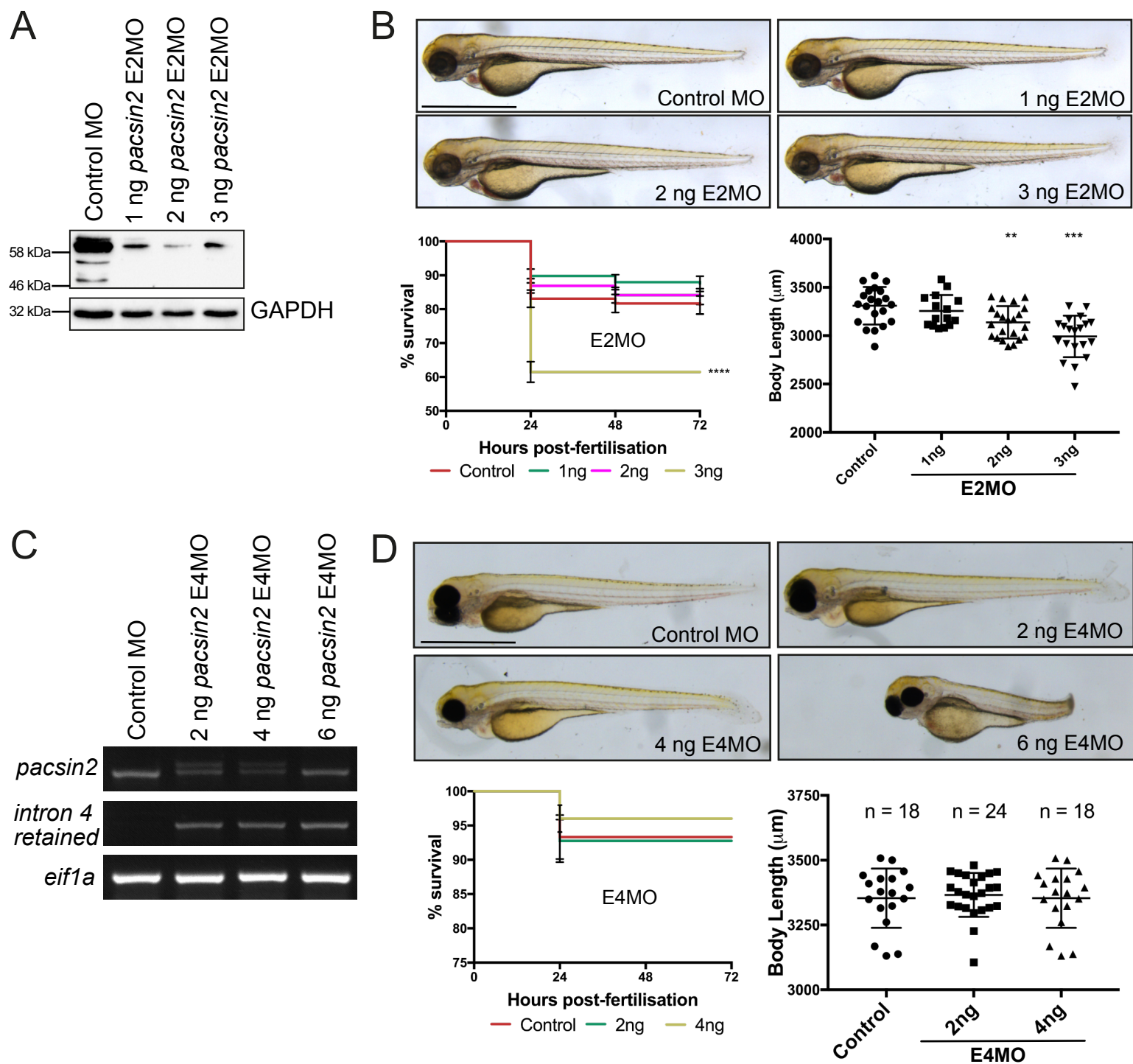
Morgan et al, Fig S1

Fig. S1. Evolutionary conservation of pacsins in zebrafish. (A) Phylogenetic tree from multiple sequence alignments of human and zebrafish *pacsin* family members. (B) Synteny traces using a 50-gene window demonstrates high levels of chromosome arrangement conservation between regions in zebrafish housing *pacsin1a* (top) and *pacsin1b* (bottom) compared to chromosome 6 in human containing *PACSIN 1*. (C-E) Multiple protein sequence alignment of the F-BAR (C), SH3 domain (D) and variable region (E) across all zebrafish *pacsin* family members. Green, blue and red boxes in (C) outline regions of conserved basic residues on the concave face important for lipid-binding, conserved *pacsin*-specific wedge-loop residues, and conserved basic residues on the convex surface important for membrane binding, respectively. Blue and red boxes in (D) highlight conservation of critical residue pairs that form two proline-binding pockets, and the green box indicates conservation of acidic residues in the specificity pocket. The blue box in (E) highlights the minimal NPF motif, and red boxes indicate extended NPF motifs of the form NPF-[D/E]-[D/E]-[D/E].



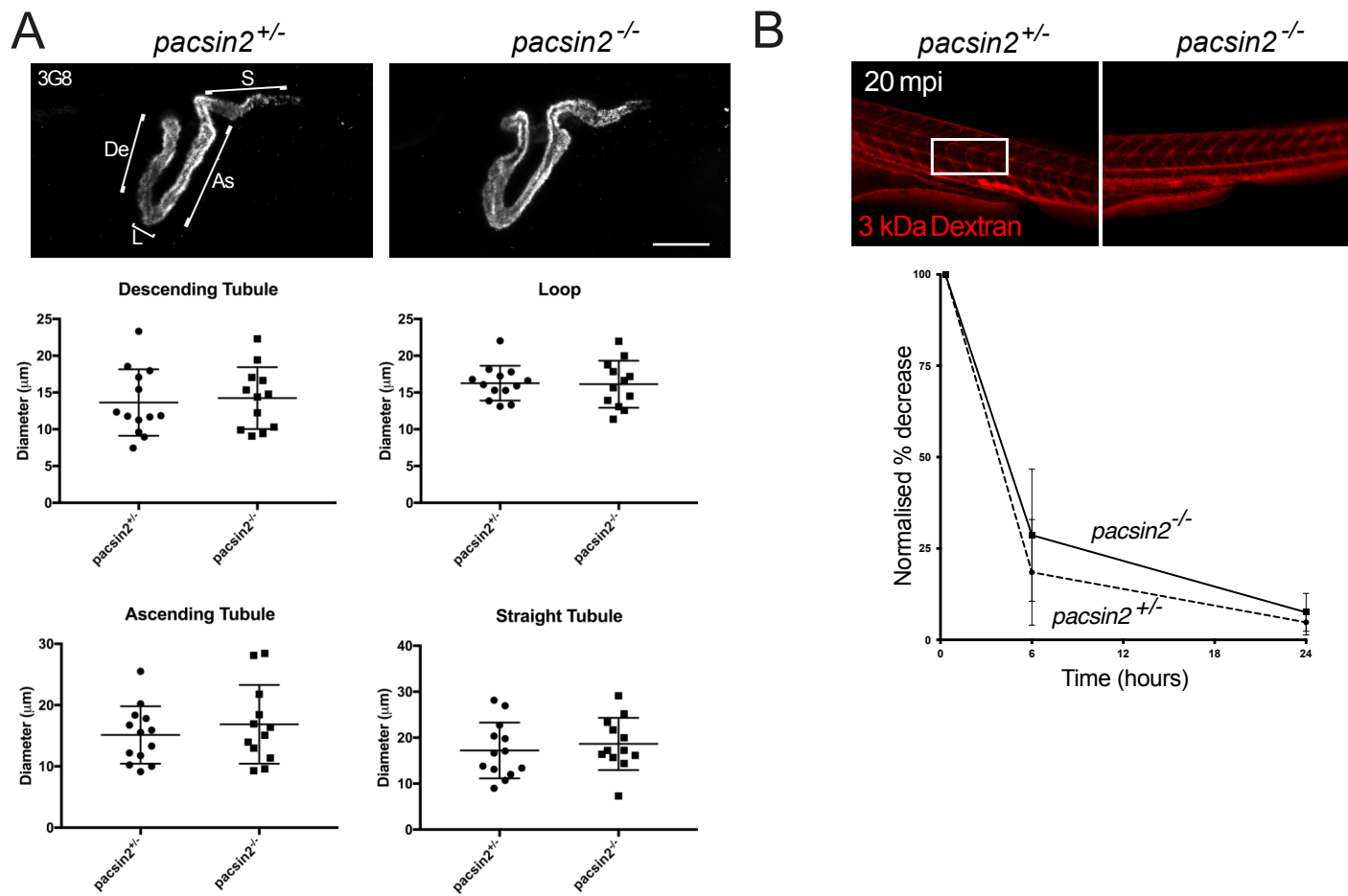
Morgan et al, Fig S2

Fig. S2. Homozygous *pacsin2*^{-10bp/-10bp} mutants are viable and have normal morphology. (A) Larvae produced by in-crossing *pacsin2*^{+/-10bp} zebrafish are at the expected Mendelian ratio. (B) Left, brightfield images of WT, heterozygous *pacsin2*^{+/-10bp} and homozygous *pacsin2*^{-10bp/-10bp} mutants at 3 dpf (top) and 4 dpf (bottom). Right, body lengths of 3 dpf (top) and 4 dpf (bottom) larvae (n =11-16) as indicated. Scale bars, 1 mm. (C) Survival of WT, heterozygous *pacsin2*^{+/-10bp} and homozygous *pacsin2*^{-10bp/-10bp} mutants up to 5 dpf (n=11-16).



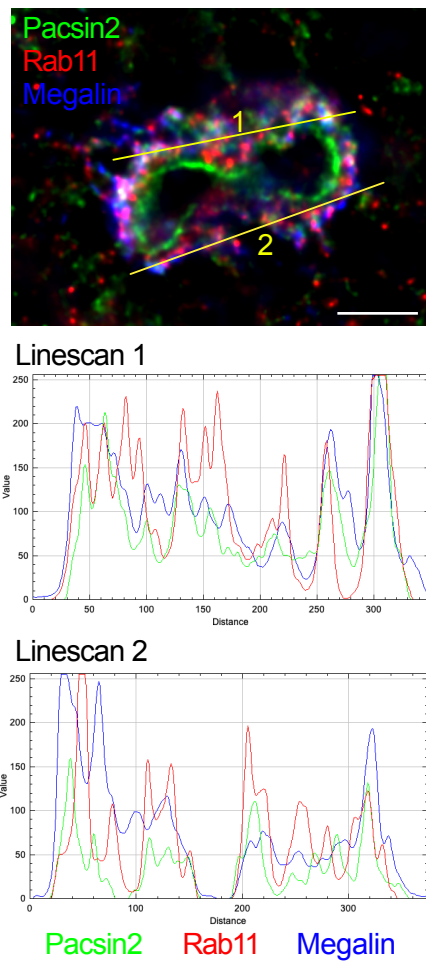
Morgan et al, Fig S3

Fig. S3. *Pacsin2* morphant zebrafish develop and grow normally. (A) 3 dpf larvae injected with 3 ng control morpholino (MO) or 1 – 3 ng *pacsin2* E2MO were analyzed by Western blot with antibodies to *pacsin2* or GAPDH. (B) Top, brightfield images of morphants at 3 dpf. Bottom left, survival of morphants treated with control or increasing doses of *pacsin2* E2MO over 72 hpf. Statistical test used was log-rank (Mantel-Cox) test. **** $p < 0.0001$. $n = 213$ -257 embryos per condition. Bottom right, body length of morphants at 3 dpf larvae. One-way ANOVA followed by Dunnett's multiple comparison was performed. * $p = 0.04$, ** $p = 0.003$. $n = 20$ larvae per condition. Error bars = SD; scale bar, 1 mm. (C) RT-PCR of total mRNA extracted from control or *pacsin2* E4MO morphants injected with 2 ng, 4 ng or 6 ng morpholino. (D) Top, brightfield images of morphants at 3 dpf. Bottom left, survival of morphants treated with control or increasing doses of *pacsin2* E4MO over 72 hpf. Statistical test used was log-rank (Mantel-Cox) test. **** $p < 0.0001$. $n = 60$ -100 embryos per condition. Bottom right, body length of morphants at 3 dpf larvae. One-way ANOVA followed by Dunnett's multiple comparison was performed. $n = 18$ -24 larvae per condition. Scale bar, 1 mm.



Morgan et al, Fig S4

Fig. S4. Loss of *pacsin2* does not affect pronephros formation or dye filtration. (A) Top, whole-mount immunofluorescence performed on 4 dpf *pacsin2*^{+/-} and *pacsin2*^{-/-} larvae using 3G8, an apical brush border proximal tubule-specific marker. De = Descending tubule, L = Loop, As = Ascending tubule, S = straight tubule. Scale bar = 50 µm. Bottom, tubule diameters were measured at the widest part of each region marked in (A) and data analyzed using Unpaired t-test, $p > 0.05$ in all cases. $n = 13$ and 12 larvae for *pacsin2*^{+/-} and *pacsin2*^{-/-}, respectively. Error bars = SD. (B) Top, *pacsin2*^{+/-} and *pacsin2*^{-/-} larvae were injected simultaneously with 3 kDa dextran (red) and imaged 20 minutes post-injection (mpi) to assess successful injections. Bottom, images were taken at 20 mpi, 6 hpi and 24 hpi and fluorescence measured in the circulatory system. Two-way ANOVA followed by Sidak's multiple comparisons test, $p > 0.05$. $n = 5$ and 9 larvae for *pacsin2*^{+/-} and *pacsin2*^{-/-}, respectively.



Morgan et al, Fig S5

Fig. S5. Intensity line-scan analysis of pacsin2 immunolabelling in the proximal tubule. Top, transverse cryosection of proximal tubule immunolabeled for Pacsin2 (green), Rab11 (red) and megalin (blue) in 3 dpf wild-type larvae (image taken from Fig 4A and converted to RGB), with positions of the intensity line-scans across the sub-apical region indicated in yellow. Bottom, the plots of signal intensity (y-axis) against relative distance (x-axis) are shown for each line, as indicated. Scale bar, 5 μ m.