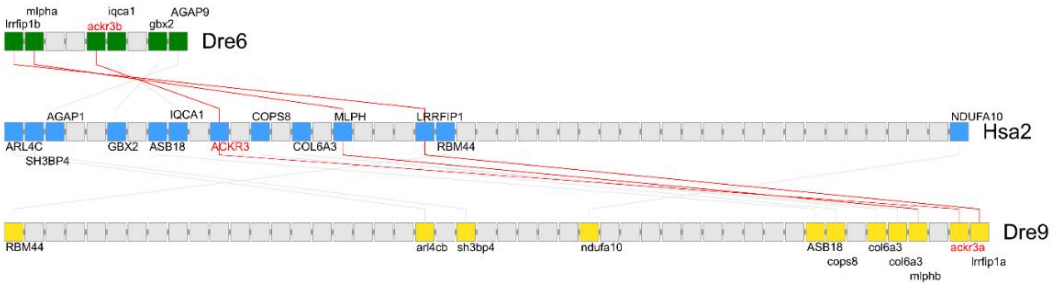
Supplementary Material

A



B

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ACKR3       MDLHLFDYSEP-GNFSDISWPCNSSDCIVVDTVMCPNMPNKSVLLYTLSFIYIFIFVIGM   59
Ackr3a      ------------MWEELNFTDSNNNSSRVEAQMCPASFNRSALLNAMCTLYAFIFVVGL   47
Ackr3b      MSVNVNDFNDILDALGELNFSTLDDNVS--HVEVCHSTFSQRALLYALSVLYIFLFIIGL   58
                          ::.:   ..:    .. :*    .: .** ::. :* *:*::*:

ACKR3       IANSVVVWVNIQAKTTGYDTHCYILNLAIADLWVVLTIPVWVVSLVQHNQWPMGELTCKV   119
Ackr3a      AANALVVWVNMRSQRHYHETHMYILNLAVADLCVVATLPVWVSSLAQGGHWAFGQAACKL   107
Ackr3b      AANALVVWVNVRAERTRYETHLYILNLAIADLCVVATLPVSISSLLQLGHWPFGGAMCKI   118
             **::*****::::   ::** ******:*** ** *:** : ** * .:* :*   **:

ACKR3       THLIFSINLFGSIFFLTCMSVDRYLSITYFTNTPSSRKKMVRRVVCILVWLLAFCVSLPD   179
Ackr3a      THLLFSVNLFASIFFLTCMSVDRYLSVVRFREISHRRGRQVRRLVCAITWLLALFASVPD   167
Ackr3b      THLIFSVNLFSSIFFLTCMSVDRYLSVKLFGDTPSQRKRRTRQIICVGVWLLALIAALPE   178
            ***:**:***.***************:  * :    * : .*:::*  .****: .::*:

ACKR3       TYYLKTVTSASNNETYCRSFYPEHSIKEWLIGMELVSVVLGFAVPFSIIAVFYFLLARAI   239
Ackr3a      TYFLRSVKSQYSHVTLCLPVYPEDNPLQWMVGIQLSFVVLGFAIPFPIIAVSYALLANTL   227
Ackr3b      IYFLQAEKSDHSDAIVCKAVYPVESMKEWTVGIQMSFFMLGFAIPFPVIAVFYVLLANTI   238
             *:*:: .*  ..   *  .** ..  :* :*:::  .:****:** :*** * ***.::

ACKR3       SA-------SSDQEKHSSRKIIFSYVVVFLVCWLPYHVAVLLDIFSILHYIPFTCRLEHA   292
Ackr3a      ASTSTHSPSNGDQDRSVSKKVILMYIVVFIACWAPYHAVLLADALAMLGVLPLGCSSENG   287
Ackr3b      HP-------SVDQERRISRHLIFTYIVVFLVCWLPYHGALLLDTLAFLNVLPFNCTLENA   291
                  . **::  *:::*: *:***:.** *** .:* * :::*  :*: *  *:.

ACKR3       LFTALHVTQCLSLVHCCVNPVLYSFINRNYRYELMKAFIFKYSAKTGLTKLIDASRVSET   352
Ackr3a      LFVALHLTQCLSLLHCCVNPVVYSFAHRHYRYDLMKAFIFKYSTRTGLARLIDGSQGIET   347
Ackr3b      LYAALHLTQCFSLFHCCANPIIYNFINKNYRYDLMKAFIFKYSTKTGLARLIDASHVSET   351
            *:.***:***:**.***.**::*.* :::***:**********::***::***.*:  **

ACKR3       EYSALEQSTK---       362
Ackr3a      EYAMVENNPPTVT       360
Ackr3b      EYSAVENQGPL--       362
            **: :*:.
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C

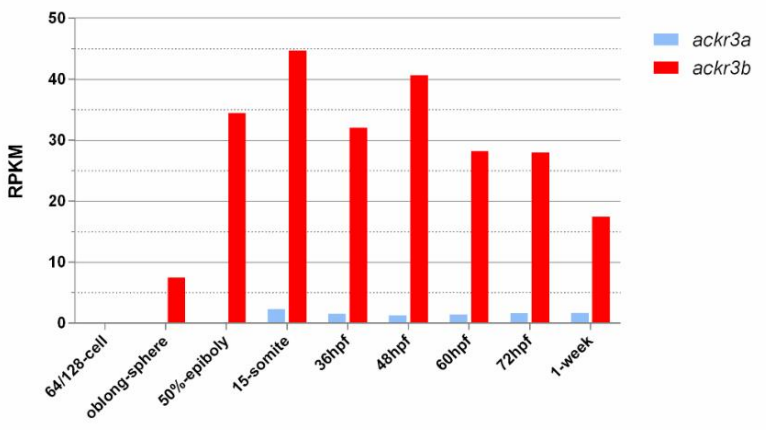

Fig. S1. Analysis of *ackr3* genes in zebrafish. A Composite cluster representation of conserved synteny around the *ACKR3* locus between *Danio rerio* chromosomes 6 (Dre6) and 9 (Dre9) and *Homo sapiens* chromosome 2 (Hsa2) generated using the Synteny Database (50-gene sliding window). Genes are drawn as squares with their order but not their physical location preserved. Colored squares are members of the cluster while grey squares represent genes in the interval that do not have orthologs (or paralogs) in the other segments. Red lines connect orthologous genes within the 3 clusters, gray lines identify orthologous genes between Hsa2 and either Dre6 or Dre9. Six gene pairs can be identified in orthologous pairwise clusters between Hsa2 and Dre6, and 11 gene pairs are present when considering Hsa2 and Dre9. The analysis has been performed based on *Danio rerio* Zv9 and *Homo sapiens* GRCH 37 genome assemblies. B Multiple sequence alignment of human ACKR3, zebrafish Ackr3a and zebrafish Ackr3b polypeptides obtained using the MUSCLE software. In the ClustalW output format, asterisks indicate positions which have a single, fully conserved residue, colons indicate conservation between groups of strongly similar properties while periods indicates conservation between groups of weakly similar properties. Residues are colored according to their physicochemical properties. **C** RNA-Seq data of *ackr3a* (blue bars) and *ackr3b* (red bars) expression at different stages of zebrafish development derived from a systematic study aimed at capturing the transcriptome landscape of zebrafish embryos and larvae [PMID: 23700457]. Gene expression levels are expressed as RPKM (Reads Per Kilobase per Million mapped reads).

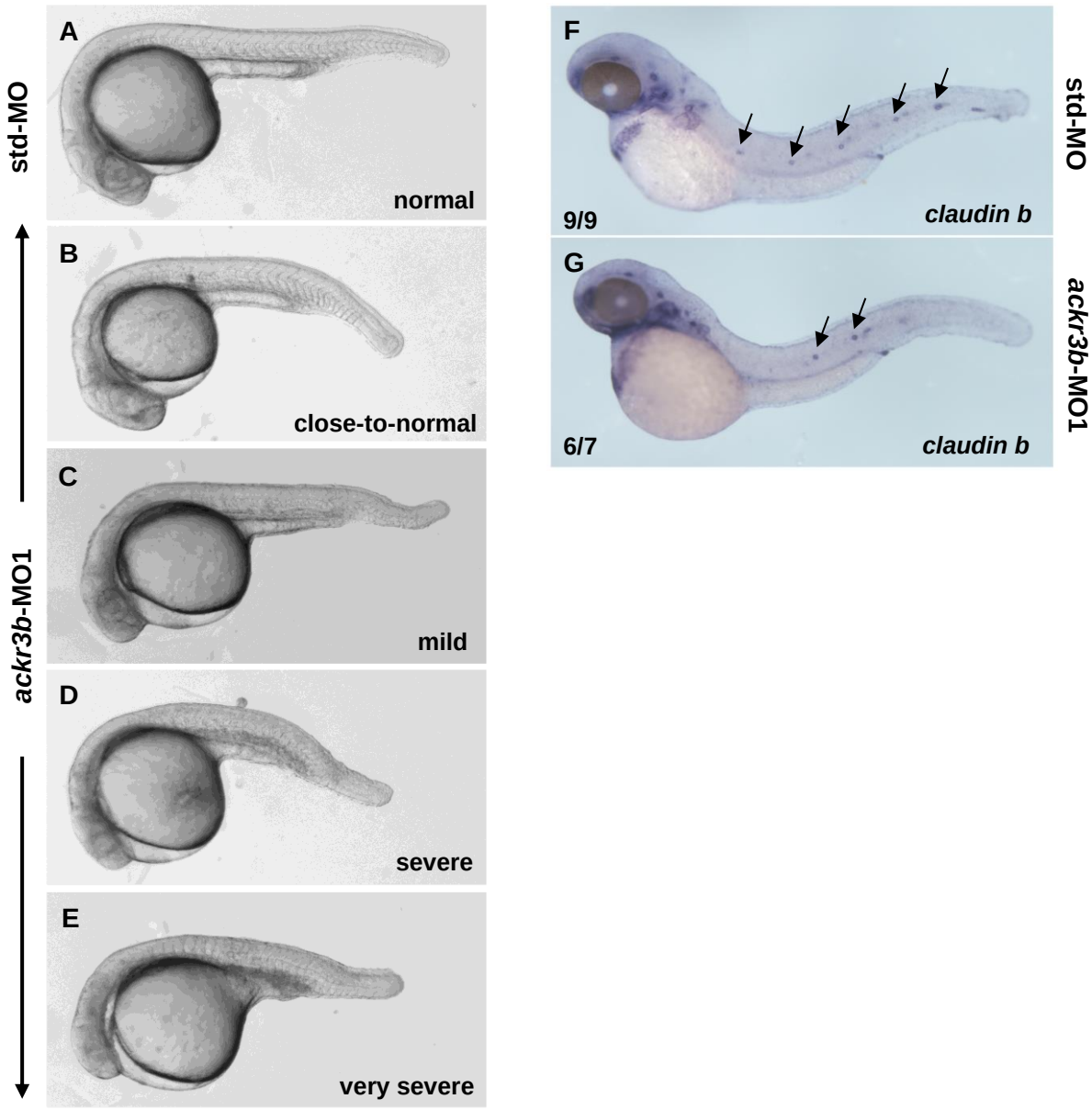

Fig. S2. Effect of *ackr3b* knockdown on zebrafish embryo development. A-E Embryos injected with 0.2 pmoles/embryo of std-MO (**A**) or *ackr3b*-MO1 (**B-E**) were photographed at 28 hpf. Embryos with close-to-normal (**B**) and mild (**C**) phenotype were used for all further analyses whereas severe (**D**) and very severe (**E**) phenotypes were discarded. **F, G** Effect of *ackr3b* knockdown on neuromast migration. WISH analysis of *claudin b* expression was performed at 48 hpf on zebrafish embryos injected with std-MO (**F**) or *ackr3b*-MO1 (**G**) to assess actual *ackr3b* knockdown. Note the impaired neuromast migration in *ackr3b* morphants (arrows in **G**) when compared to the normal positioning observed in control embryos (arrows in **F**). The number of embryos presenting the showed phenotype in respect to the total number of analyzed embryos is shown in both panels.

Cells	- | ECs | -			mock-CHO | ECs | mock-CHO			ACKR3-CHO | ECs | ACKR3-CHO			mock-CHO | ECs | ACKR3-CHO	
CXCL12	-/-	+/-	+/+	-/-	+/-	+/+	-/-	+/-	+/+	-/-	+/+
Migration	**No**	**Yes ←**	**No**	**No**	**Yes ←**	**No**	**No**	**No**	**No**	**No**	**Yes ←**

Table S1. Summary of the results obtained with the µ-slide cell co-culture chemotaxis assay. HUVECs (indicated as ECs) were seeded in the central observation area in the absence or presence of mock-CHO or ACKR3-CHO cells in the lateral reservoirs as indicated. Next, cells were exposed to three different experimental conditions: no CXCL12 (-/-) or addition of 50 ng/ml of CXCL12 to one (+/-) or both (+/+) lateral reservoirs. When present, arrow indicates the direction of HUVEC migration. No: no directional migration.