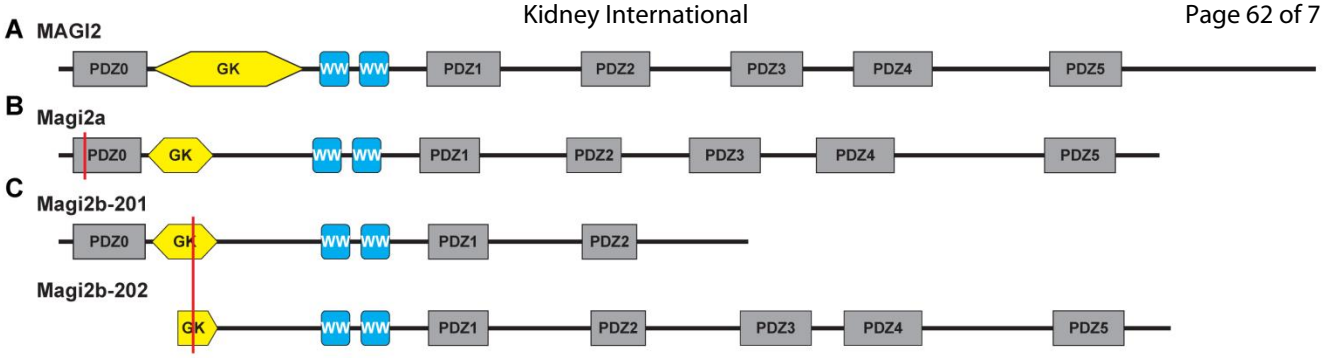
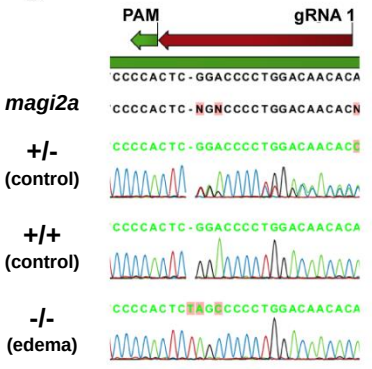
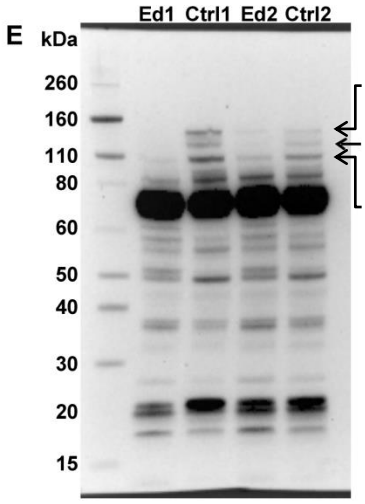
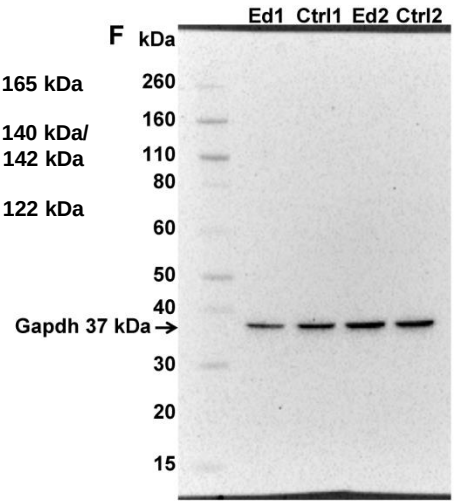
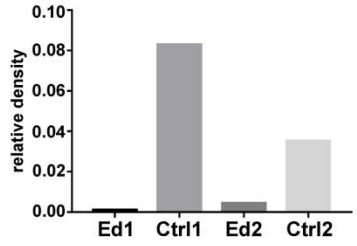

A MAGI2
PDZ0
GK
WW
WW
PDZ1
PDZ2
PDZ3
PDZ4
PDZ5
B Magi2a
C Magi2b-201
Magi2b-202


D
PAM
gRNA 1
magi2a
+/-
(control)
+/+
(control)
-/-
(edema)


E
Ed1 Ctrl1 Ed2 Ctrl2
kDa
260
160
110
80
60
50
40
30
20
15
Magi2a-201 165 kDa
Magi2a-202 140 kDa/
Magi2b-202 142 kDa
Magi3b-202 122 kDa


F
Ed1 Ctrl1 Ed2 Ctrl2
kDa
260
160
110
80
60
50
40
30
20
15
Gapdh 37 kDa


G
relative density
0.10
0.08
0.06
0.04
0.02
0.00
Ed1
Ctrl1
Ed2
Ctrl2


H
Peptide_MAGI2_Ab
Dr_magi2a-202
Dr_magi2b-202
Dr_magi1a-201
Dr_magi1b-203
Dr_magi3a-202
Dr_magi3b-202



**Suppl. Fig. S1**

**Supplemental Figure S1. Zebrafish orthologues of human *MAGI2.***

(**A**) The human MAGI2 protein features six PDZ domains (grey), one Guanylate Kinase-like (GK, yellow) domain and two WW domains (blue).

(**B-C**) Predicted domain structure of zebrafish Magi2a and Magi2b. More evidence exists that *magi2a* is the functional orthologue of human *MAGI2*. However, *magi2b* remains a potential orthologue. The red bar indicates the CRISPR target translated to the protein structure.

(**B**) Zebrafish Magi2a features the same domain structure as human MAGI2.

(**C**) For zebrafish *magi2b*, two incomplete putative transcripts are annotated where *magi2b-201* would represent a C-terminally truncated protein and *magi2b-202* an N-terminally truncated protein. Therefore, *magi2b* was targeted by CRISPR in the earliest overlapping region of both potential transcripts (red intersecting line).

(**D**) Sanger sequencing confirms homozygosity for *magi2a* (*c.69_71delinsGCTA,* p.Pro24Leufs*76) KO larvae with an edema phenotype (see **Fig. 3**). Data shown represent the genotypes observed in both control (*magi2a*$^{-/+}$ and *magi2a*$^{+/+}$) and edema (*magi2a*$^{-/-}$) cohorts.

(**E**) Immunoblot for Magi2a on protein lysates from pooled zebrafish larvae from the truncating allele *magi2a* (*c.69_71delinsGCTA,* p.Pro24Leufs*76) at 6 dpf. Larvae were sorted by phenotype, tail clipped for individual genotyping, but pooled for protein extraction. Edema samples (Ed1, Ed2) consisted of only *magi2a*$^{-/-}$ larvae, control samples (Ctrl1, Ctrl2) of a mix of *magi2a*$^{-/+}$ and *magi2a*$^{+/+}$. For *magi2a* two transcripts exist, resulting in predicted proteins of 140 kDa (UniProt/ID: Q5VSD9) and 165 kDa (UniProt-ID: F1Q897) which could explain the disappearance of the upper double band in the KO larvae. Cross-reactivity of the used antibody with epitopes of the paralogues proteins Magi2b (UniProt-ID: A0A2R8QA68; 142 kDa) or Mag3b, as mapped in **H**, may explain residual or additional bands of >100 kDa.

(**F**) Immunoblot for Gapdh as loading control for **E**. No stripping was performed between **E** and **F**.

(**G**) Densitometry for **E**/**F**. The relative density of Magi2a-202 140 kDa bands normalized by the corresponding Gapdh bands is displayed.

(**H**) Clustal analysis of predicted proteins of zebrafish *magi1a/b*, *magi2a/b* and *magi3a/b* transcript variants, aligned to the human MAGI2 peptide that was used for the generation of antibody ARP61404_P050 (Aviva Systems Biology, San Diego, CA). Red rectangles indicate potential cross-reacting epitopes (i.e., 4 consecutive amino acids) of Magi2b or the paralogues Magi1a, Magi1b, Magi3a and Magi3b.