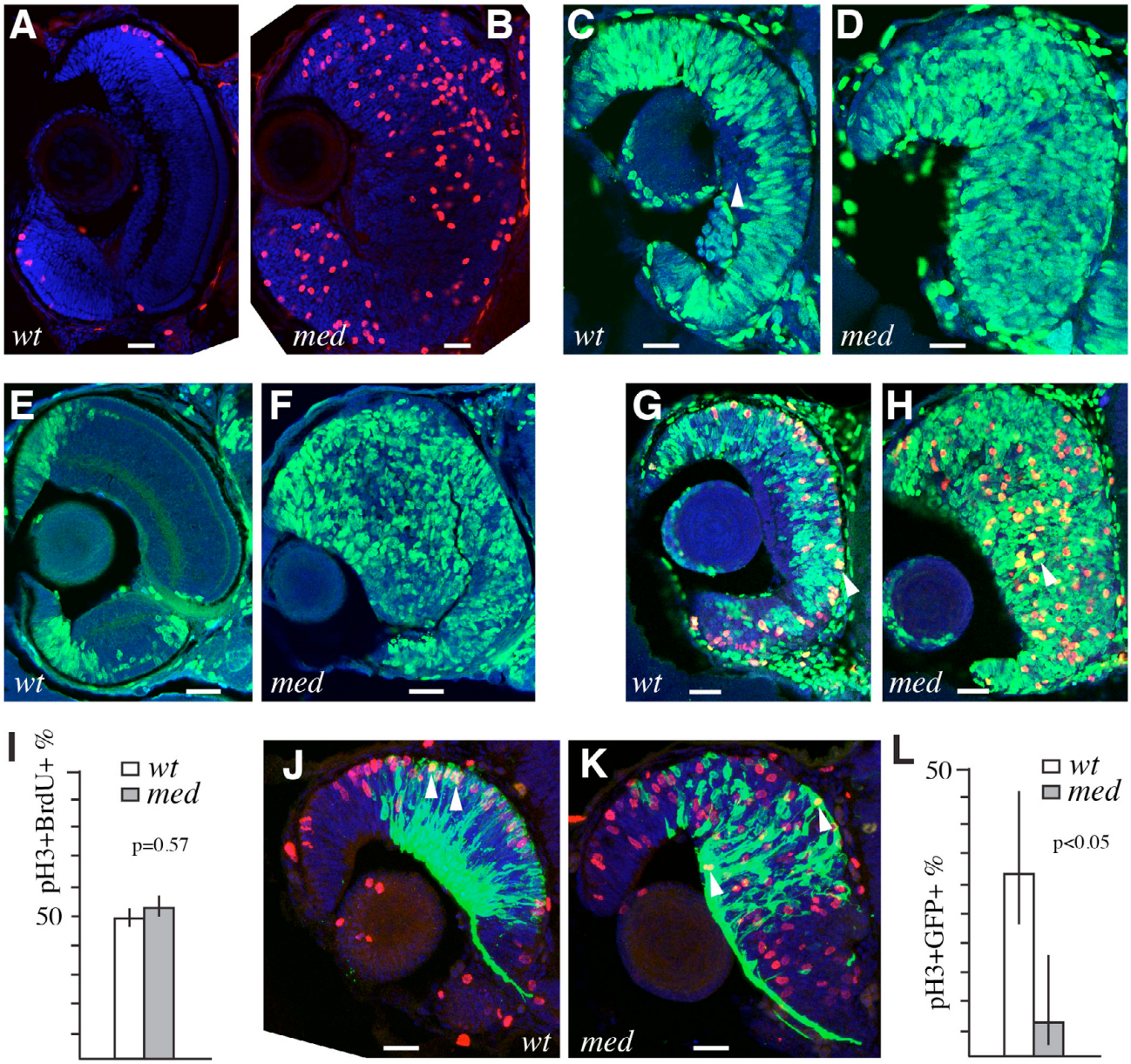

Fig. S1. Proliferation and cell cycle exit are affected by the *med* mutation. (**A**,**B**) M-phase nuclei are visualized by α-pH3 labeling in wild-type (A) and mutant (B) 4 dpf retinae. The central retina of the wild type, where differentiating neurons reside, is free of pH3 labeling. In the mutant, pH3 labeling is detectable in all regions and the ratio of pH3-positive nuclei is increased (see Fig. 1G). (**C**,**D**) BrdU labeling for 2 hours in wild-type (C) and mutant (D) retinae at 2.8 dpf. In the wild type, a small region of the central retina, where presumptive ganglion cells are differentiating, is free of BrdU labeling (arrowhead), whereas the mutant retina is ubiquitously labeled. (**E**,**F**) BrdU incorporation for 12 hours starting at 4 dpf. In the wild type (E), BrdU incorporation is restricted to the peripheral retina where proliferating RPCs reside, whereas in the mutant BrdU incorporation is detectable in all regions of the retina. (**G**,**H**) BrdU (green) and pH3 (red) double labeling at stage 27 to examine S- to M-phase cell cycle progression. BrdU was injected at stage 27 and after 9 hours embryos were fixed and stained for pH3 and BrdU. Examples of double-positive nuclei (yellow) are indicated (arrowheads). (**I**) The ratio of pH3 and BrdU double-positive to total pH3-positive nuclei was analyzed to determine the relative number of cells that progress from S to M phase during the 9 hours of BrdU incorporation. This ratio is similar in both wild type and mutant, indicating that cell cycle progression proceeds normally in mutant RPCs. (**J**,**K**) The expression of ath5 was used to visualize RPCs undergoing the final cell division prior to neuronal differentiation (Poggi et al., 2005; Yamaguchi et al., 2010) by crossing a stable transgenic line expressing GFP under the control of the ath5 regulatory region [ath5::GFP (del Bene et al., 2007)] into the *med* mutant. In this context, cells undergoing a neurogenic division are positive for both pH3 (red, M phase) and GFP (green, ath5). In both mutant and wild type, GFP expression was first detectable in central RPCs at stage 26 and subsequently spread across the retina to more peripheral regions (del Bene et al., 2007) (data not shown). This indicates that neurogenesis is normally initiated in the mutant retina. At stage 27, we quantified the ratio of pH3/GFP double-positive nuclei (arrowhead) to total pH3 nuclei to analyse cell cycle exit. (**L**) Quantification the ratio of pH3/GFP double-positive to total pH3-positive cells to analyze the relative number of cells exiting the cell cycle by neurogenic divisions. In the wild type, 33% of the pH3-positive cells are also GFP positive, in the mutant this ratio is significantly reduced to 6.8%, indicating an almost fivefold reduction of cell cycle exit. (A-H,J,K) Confocal transversal sections, nuclei are stained with DAPI (blue). Scale bars: 25 μm in A-H,J,K.

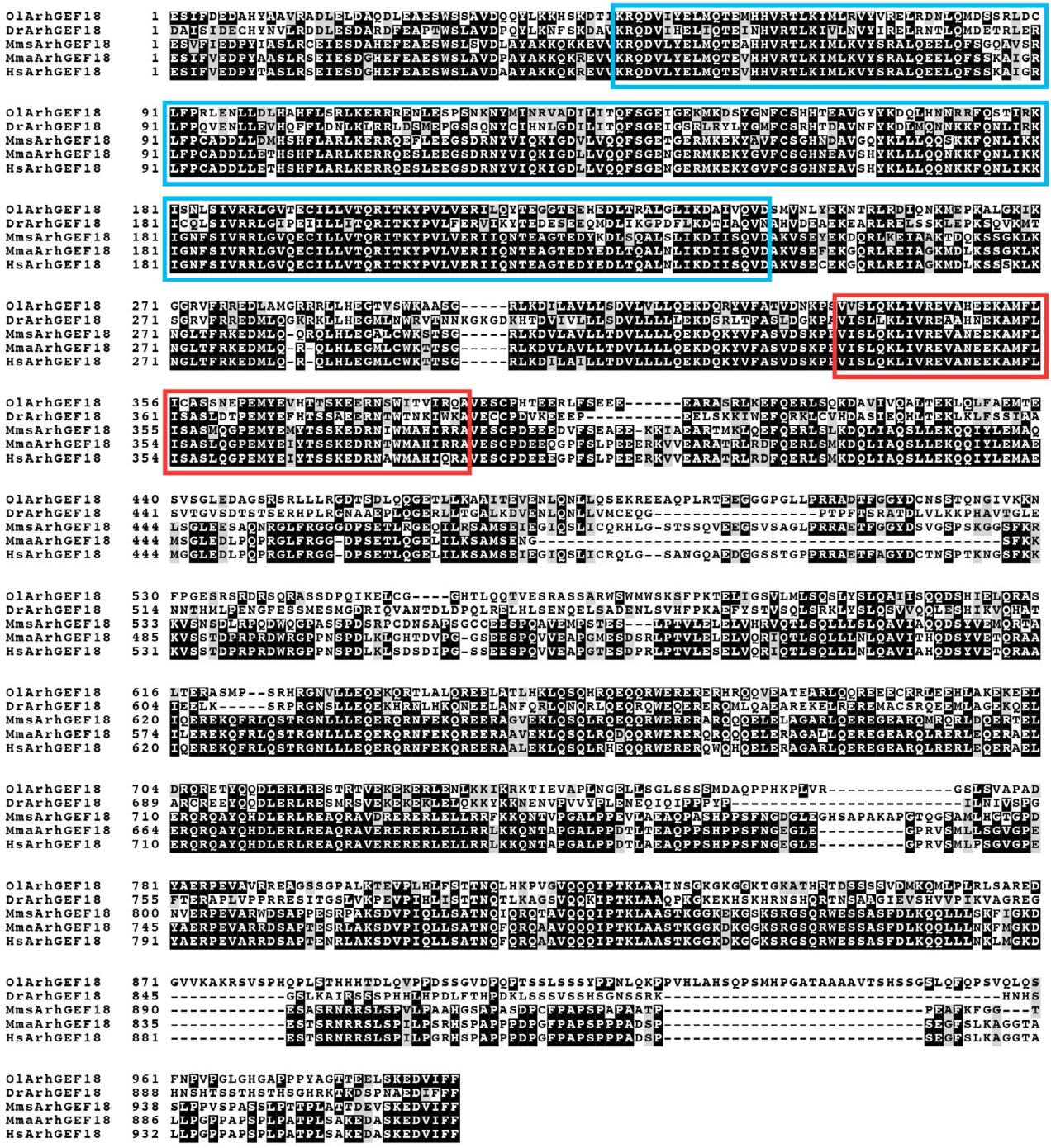

Fig. S2. Vertebrate ArhGEF18 homologues are conserved. Alignments of medaka (*Oryzias latipes*, OlArhGEF18: GenBank JN868075), zebrafish (*Danio rerio*, DrArhGEF18: Uniprot Q6NSP2), mouse (*Mus musculus*, MmsArhGEF18: GenBank EDL21902), macaca (*Macaca mulatta*, MmaArhGEF18: NCBI XP_001095927) and human (*Homo sapiens*, HsArhGEF18: NCBI NP_056133) proteins. The conserved DH (blue) and PH (red) domains are indicated. The DH domain is 60.2% identical to that of the zebrafish homologue and 65.8% to the DH domain of the murine and human homologue. The PH domain is 67.3 identical to that of zebrafish and 71.2% and 69.2% to that of mouse and humans, respectively. The C-terminal part of 604 amino acids is less well conserved, ranging from 39.5% (zebrafish) to 41.2% (human) identity.

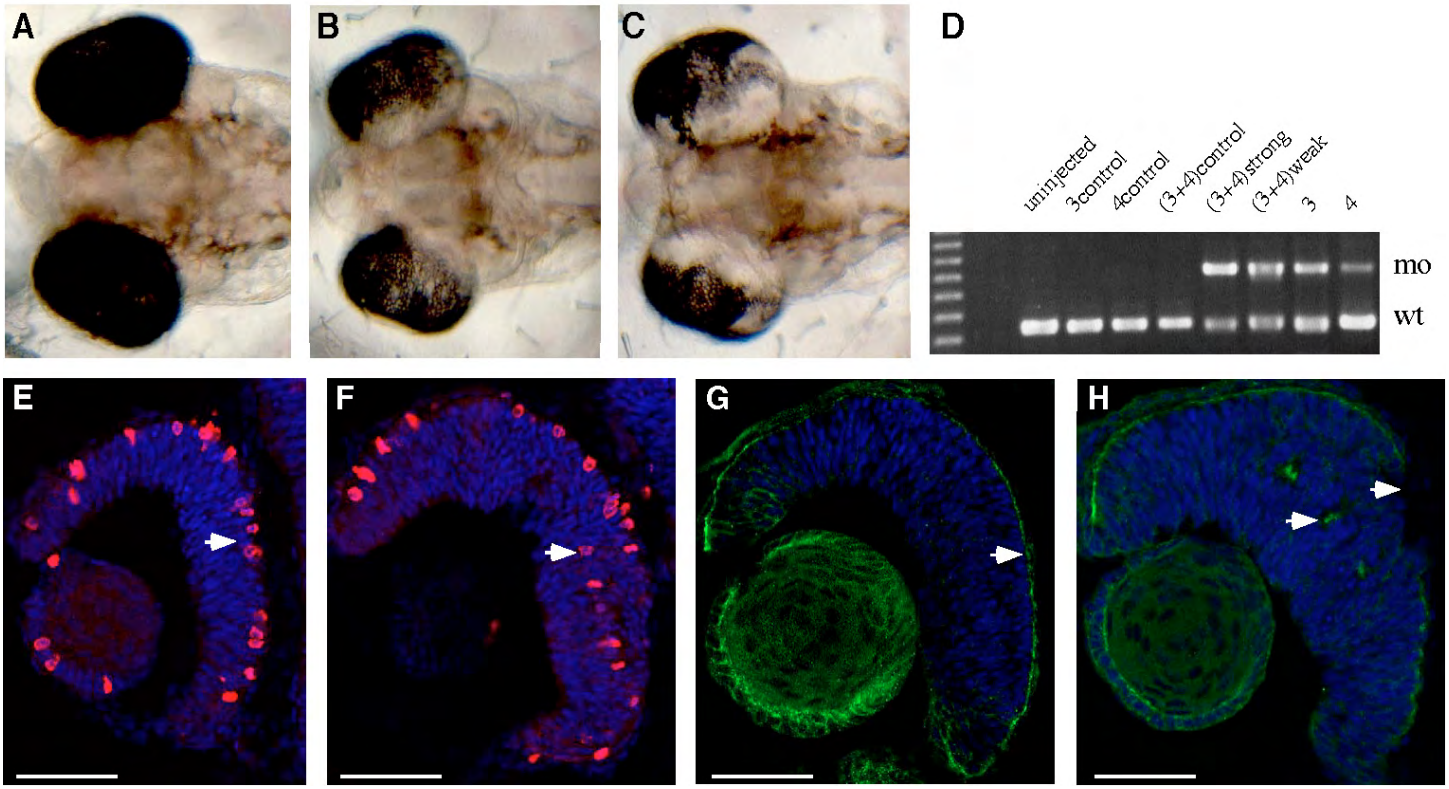

Fig. S3. *OlArhGEF18* morpholino injection phenocopies the *med* mutation. (**A-C**) Dorsal views of control-injected embryos (A), morpholino 3+4-injected embryos with weak phenotype (B) and morpholino 3+4-injected embryos with strong phenotype (C) at 4 dpf. (**D**) RT-PCR analysis of *OlArhGEF18* mRNA of morpholino and uninjected embryos. Uninjected and control morpholino-injected embryos show the wild-type transcript (lower band: wt); injection of morpholino 3, morpholino 4 and morpholino 3+4 results in splicing inhibition. The amount of unspliced transcript (upper band: mo) correlates with the severity of the phenotype. (**E**,**F**) Position of M-phase nuclei as visualized by α-PH3 antibody-labeling in control morpholino- (E) and morpholino 3+4- (F) injected embryos at stage 25 in the retina. Morpholino 3+4 injection results in mislocalized nuclei (F, arrowheads), whereas in the wild type, nuclei are apically localized (E). (**G**,**H**) aPKC localization in control morpholino- (G) and morpholino 3+4- (H) injected embryos at stage 27. Apical aPKC localization in the retina is severely perturbed in morpholino 3+4-injected embryos (arrowheads). (E-H) Nuclei are stained with DAPI (blue). Scale bars: 50 µm in E,F. Morpholino sequences: MO3, TGAATGCAAATGTGGGTGCTTACCA; MO3C, TCAATGGAAATCTGGGTCCTTAGCA; MO4, CAGCTCTGTGAGACGACACCAGTGT; MO4C, CACCTCTCTGACACGAGACCACTGT.

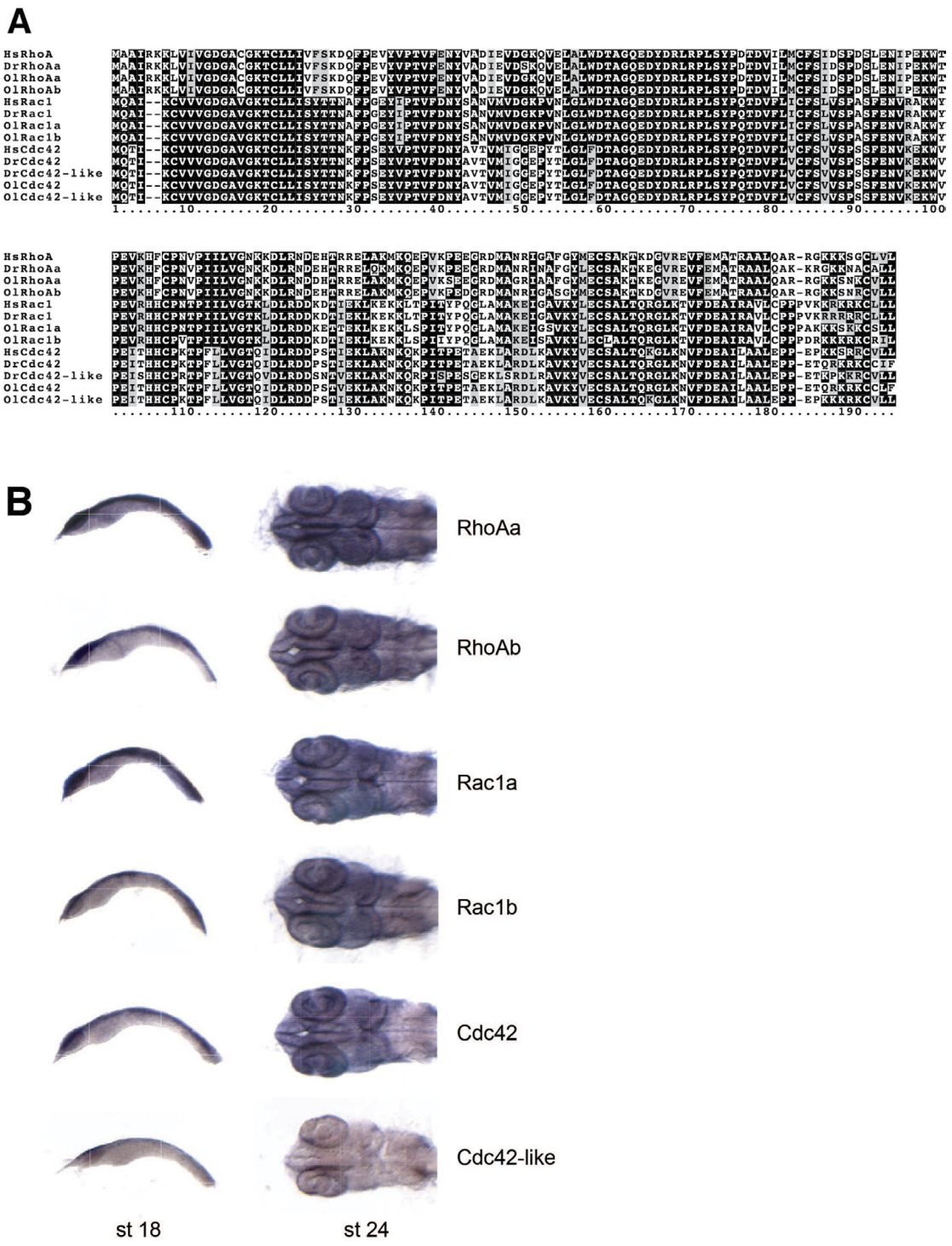

Fig. S4. Vertebrate RhoA, Rac1 and Cdc42 GTPases are highly conserved and ubiquitously expressed in medaka embryos at neurula and organogenesis stages. (**A**) Protein sequence comparison of human RhoA (Hs RhoA), Rac1 (HsRac1), Cdc42 (HsCdc42); zebrafish RhoAa (DrRhoAa), Rac1 (DrRac1), Cdc42 and Cdc42-like (DrCdc42, DrCdc42-like); and medaka RhoAa and RhoAb (OlRhoAa, OlRhoAb), Rac1a and Rac1b (OlRac1a, OlRac1b), and Cdc42 and Cdc42-like (OlCdc42, OlCdc42-like) are shown. Medaka RhoAa and RhoAb are 95% and 96% identical to human RhoA. Medaka Rac1a and Rac1b share 97% and 96% identity with human Rac1. Medaka Cdc42 and Cdc42-like are 96% and 99% identical, respectively, to human Cdc42. Most variable amino acids are located in the C-terminal region of the respective GTPases. All amino acids that have been implicated in GEF binding (Karnoub et al., 2001; Oleksy et al., 2006; Rossman et al., 2002; Worthylake et al., 2000) are completely conserved in these GTPases. OlRhoAa FOE002-P00007-DPE-F_I14 (GenBank: AM298929), OlRhoAb FOE002-P00007-DPE-F_M18 (GenBank: AM299029), OlRac1a FOE002-P00022-DPE-F_N10 (GenBank: AM304480), OlRac1b FOE002-P00013-DPE-F_P20 (GenBank: AM301286), OlCdc42 FOE002-P00024-DPE-F_A03 (GenBank: AM304876) and OlCdc42-like FOE002-P00008-DPE-F_C14 (GenBank: AM299156) were identified by BLAST searches from a medaka unigene cDNA library (Souren et al., 2009) using sequences of zebrafish homologues. (**B**) Whole-mount in situ hybridization expression analysis of *RhoAa*, *RhoAb*, *Rac1a*, *Rac1b*, *Cdc42* and *Cdc42-like* GTPases in wild-type medaka embryos at neurula (stage 18) and organogenesis (stage 24) stages. With the exception *of Cdc42-like*, all GTPases are ubiquitously expressed in the developing medaka embryo.

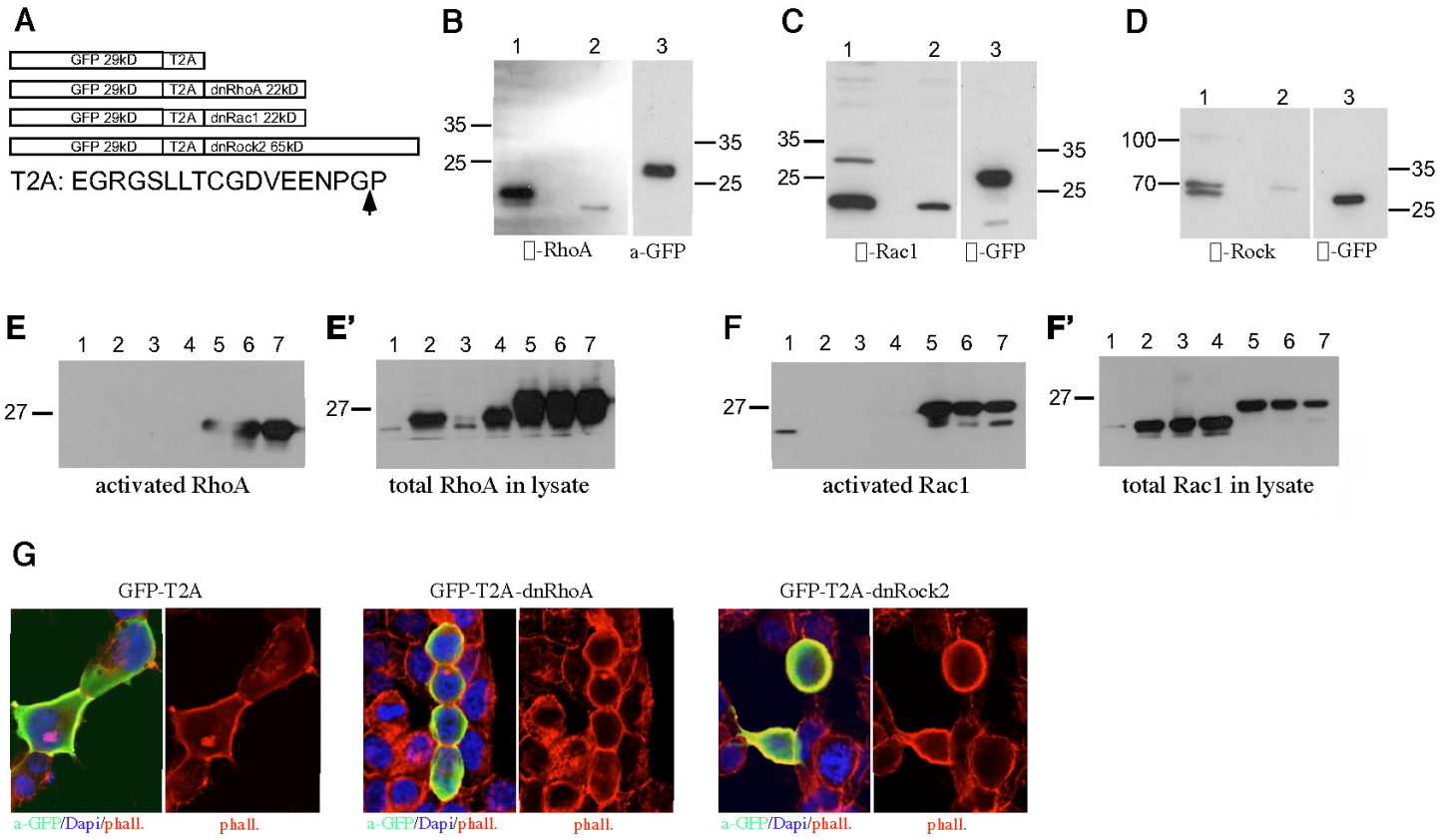

Fig. S5. T2A peptide separates GFP and a C-terminal polypeptide into two functional proteins. (**A**) Scheme of the GFP-T2A expression constructs. N-terminal GFP and the C-terminal dominant-negative protein were linked in-frame by the *Thosea asigna* virus T2A peptide, where a ribosomal skip mechanism results in the 'cleavage' of GFP and dominant-negative protein, respectively. This allows us to co-express these two proteins from a single vector (Szymczak et al., 2004). The expected sizes of the respective 'cleavage'-products in kDa is indicated. The amino acid sequence of the *Thosea asigna* virus T2A peptide and the 'cleavage'-site is shown. (**B-D**) Western blot analysis of 24 hpf medaka embryos injected at the one-cell stage with RNA encoding GFP-T2A-dominant-negative-RhoA (GFP-T2A-dnRhoA; B), GFP-T2A- dominant-negative-Rac1 (GFP-T2A-dnRac1; C) and GFP-T2A- dominant-negative-Rock2 (GFP-T2A-dnRock2; D). Lane 1, lysate of injected embryos probed with the indicated antibodies; lane 2, lysate of uninjected embryos probed with the indicated antibodies; lane 3, lysate of injected embryos probed with α-GFP antibody. The protein sizes are indicated in kDa. Expression of GFP-T2A-dnRhoA (B), GFP-T2A-dnRac1 (C) and GFP-T2A-dnRock2 (D) in medaka embryos results in the cleavage of GFP and the respective C terminal protein. (**E**,**F**) GTPase activation in HEK293 cells in response to GFP-T2A-dnRhoA and constitutive activated RhoA (E,E′) and GFP-T2A-dnRac1 and constitutive activated Rac1 (F,F′). Lanes are as follows: 1, untransfected; 2-4, GFP-T2A-dominant negative GTPase; 5-6, constitutive activated GTPase. (E) GFP-T2A-dnRhoA is inactive as determined by the absence of binding to GST-RBD (lanes 2-4), whereas activated RhoA strongly binds GST-RBD (lanes 5-7). (E′) Total amount of RhoA in the cell lysate. (F) GFP-T2A-dnRac1 is inactive as determined by the absence of binding to GST-Pak1 (lanes 2-4), whereas activated Rac1 shows strong binding. (F′) Total amount of Rac1 in the cell lysate. (**G**) HEK298 cells expressing GFP-T2A (negative control), GFP-T2A-dnRhoA and GFP-T2A-dnRock2 were stained for GFP (α-GFP) and actin (phalloidin), respectively. Cells expressing GFP-T2A-dnRhoA and GFP-T2A-dnRock2 delaminate and round up, thus indicating that dnRhoA and dnRock2 efficiently block RhoA and Rock2 activity, respectively (Barberis et al., 2005).

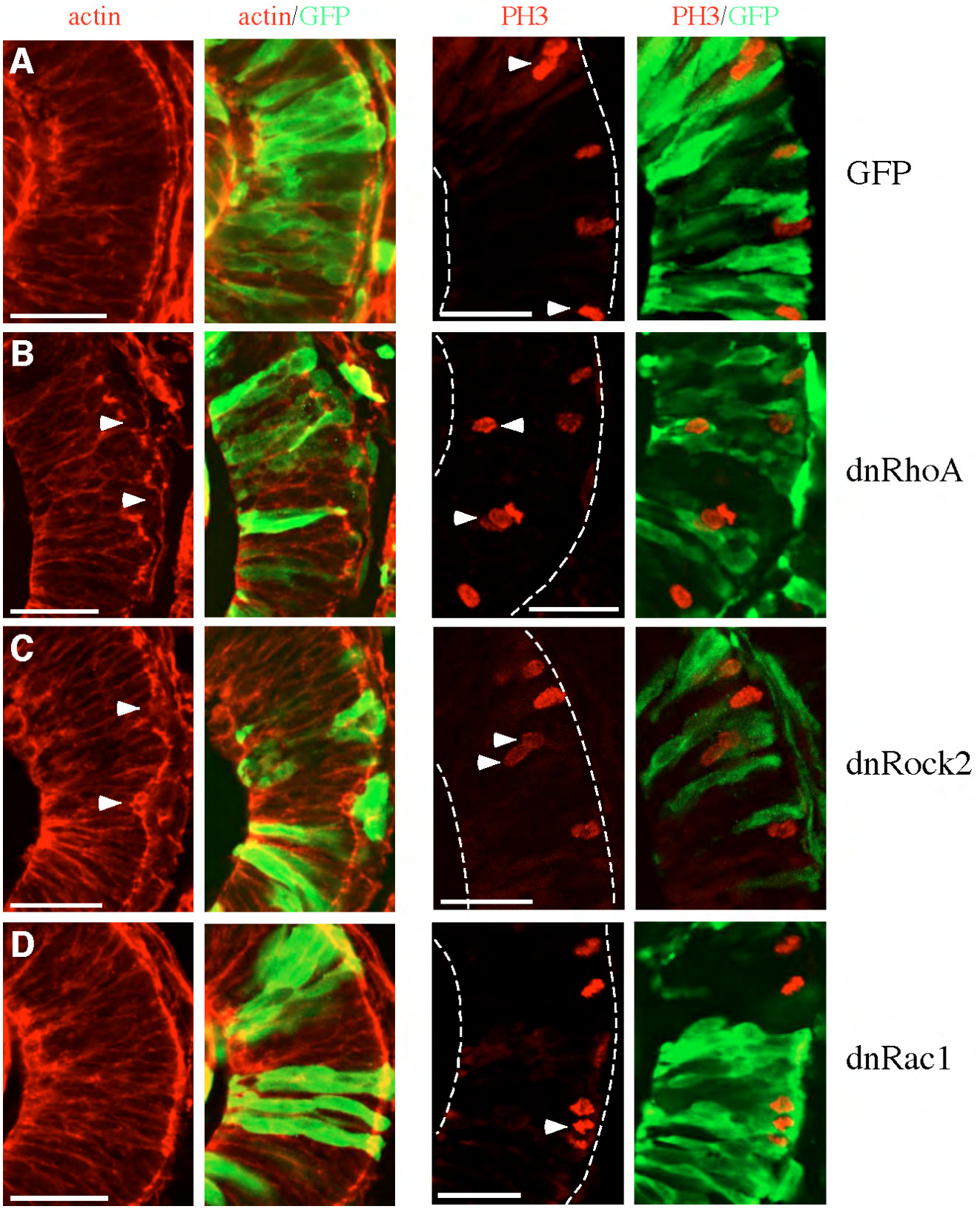

Fig. S6. RhoA and Rock2 but not Rac1 are required for cortical actin localization and apical localization of M-phase nuclei. (**A-D**) Representative examples of confocal transversal sections of embryos at stage 25 expressing GFP (A), dominant-negative RhoA (B), dominant-negative Rock2 (C) or dominant-negative Rac1 (D) under the control of a heat-shock promoter. Expressing cells are labeled with GFP (green). The retinal neuroepithelium is outlined by a broken line in column PH3. In cells expressing dominant-negative RhoA (B) and dominant-negative Rock2 (C) cortical actin localization is disturbed. Apical actin bundles are mislocalized or absent (arrowheads in column actin of B,C). In addition, apical localization of M-phase nuclei is affected by expression of dominant-negative RhoA (B) and dominant negative Rock2 (C). Nuclei of mitotic cells visualized by α-PH3 antibody staining are often displaced basally in expressing cells (arrowheads in column PH3 of B,C). Cortical actin localization and apical localization of M-phase nuclei are not affected by expression of dominant-negative Rac1 (D). Scale bars: 25 μm.