SUPPLEMENTARY MATERIAL

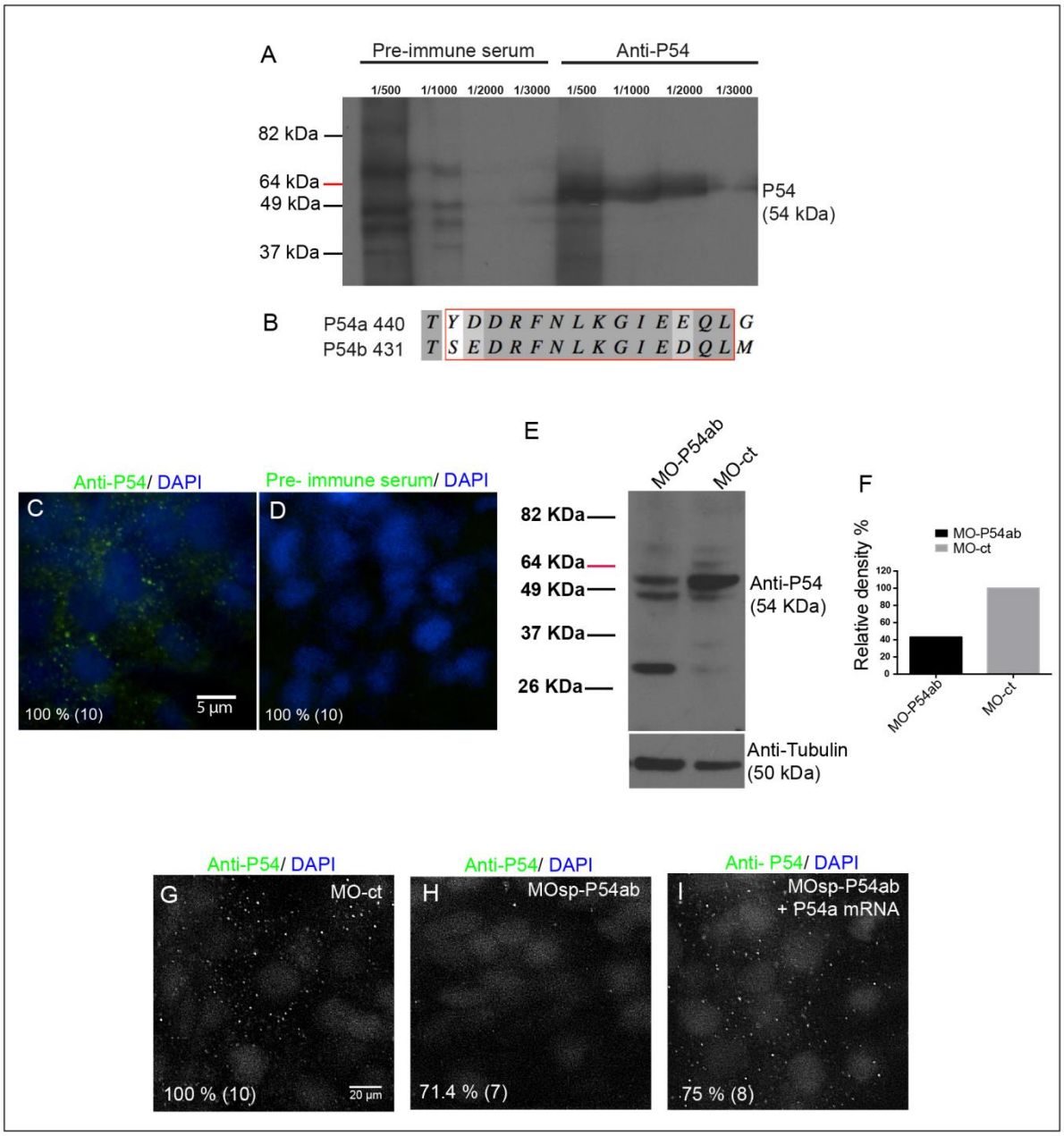

Figure S1. Testing of Anti-P54 serum and P54a and P54b morpholinos. Testing of Anti-P54 serum and P54a and P54b morpholinos. (A) Western blot analysis using protein extracts from 24 hpf WT zebrafish embryos (12 embryos per lane): the rabbit pre-immune serum (dilutions 1/500 – 1/3000) produces no specific signal, whereas the anti-P54 serum

(dilutions 1/500 –1/3000) showed a band at approximately 54 kDa, which is the predicted molecular weight of both P54 RNA helicases from zebrafish. (B) Fragment of a protein alignment between P54a and P54b with the sequence used to synthesize the peptide against anti-P54 serum was generated. (C and D) Wholemount immunostaining of WT embryos at 24 hpf using 1/2000 dilution of anti-P54 serum or rabbit pre-immune serum. (E) Protein extracts from 24 hpf embryos micro-injected with MO-P54ab or MO-ct were tested by Western blotting with the anti-P54 serum, and the MO-P54ab embryos show a reduction in the signal. As a loading control, an anti-tubulin antibody was used. (F) Relative density measured in anti-P54 protein bands in both MO-P54ab and MO-Ct total protein samples, normalized using the anti-Tubulin loading controls. (G – I) Immunostaining of MO-ct or MO-P54ab 24 hpf morphants showed the loss of the P54 signal in the MO-P54ab-treated embryos, but this signal could be recovered by rescuing P54a expression by co-injecting a mix of MO-P54ab and mRNA-P54a. Nuclei were counterstained with DAPI. The percentage of embryos showing the same pattern shown in the figure is indicated in each panel, with the total number of embryos tested indicated in parentheses. Control morpholino (MO-ct), mix of p54a and p54b morpholinos (MO-P54ab), "*in vitro*" synthesized mRNA from p54a (mRNA-P54a).

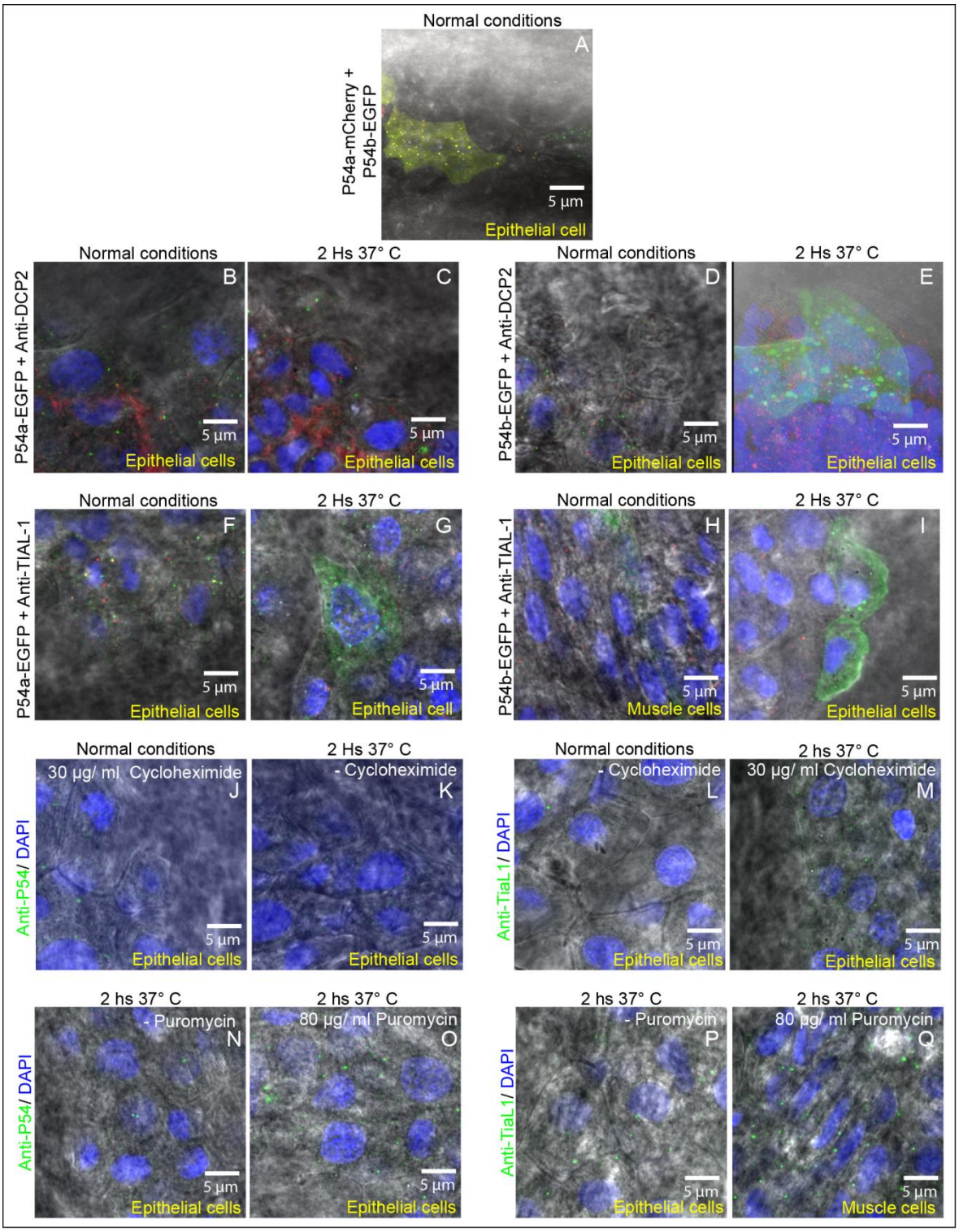

Figure S2. Differential interference contrast (DIC) illumination and cytoplasmic granules in epithelial and muscle cells. In all images we combined DIC illumination and fluorescence microscopy. Nuclei were labeled with DAPI (blue labeling) and cytoplasmic granules with different labels.(A) Cytoplasmic granules of P54a-mCherry and P54b-EGFP fusion reporters in an epithelial cell under normal conditions (same as Fig. 2K). (B and C) Cytoplasmic P54a-EGFP- and anti-Dcp2 labeled granules in an epithelial cell under normal conditions (B same as Fig. 2N) and in heat shock conditions (C same as Fig. 2Q). (D and E) P54b-EGFP- and anti-Dcp2 in cytoplasmic granules in an epithelial cell under normal conditions (D same as Fig. 2T) and in heat shock conditions (E same as Fig. 2W). (F and G) Epithelial cells with cytoplasmic granules containing P54a-EGFP- and labeled with anti-TIAL-1, in normal (F same as Fig. 3G) or heat shock conditions (G same as Fig. 3J). (H) Muscle cell and (I) Epithelial cells, both with cytoplasmic granules containing P54b-EGFP- and anti-TIAL-1, in normal (same as Fig. 3M) or heat shock conditions (same as Fig. 3P), respectively. (J and K) Anti-P54 labeling cytoplasmic granules, in epithelial cells, treated with or without cycloheximide and in normal temperature conditions (same as Fig. 4B) or in heat shock (same as Fig. 4C), respectively. (L and M) Cycloheximide treated and untreated epithelial cells, with anti-P54 labeling cytoplasmic granules, in normal conditions (same as Fig. 4E) or in heat shock (same as Fig. 4H), respectively. (N and O) Cytoplasmic granules labeled with anti-P54 antibody, in epithelial cells, treated with or without puromycin, both in heat shock conditions (same as Fig. 4K and L respectively). (P) Epithelial cell where cytoplasmic granules were labeled with anti-TIAL-1 antibody (same as Fig. 4O) and exposed to a heat shock. (Q) Muscular cell treated with puromycin and in heat shock conditions where cytoplasmic granules were labeled with anti-TIAL-1 antibody. All these images are the same as some Figures 2, 3 and 4 and are presented here with overlapping DIC illumination to observe the type of cell studied.

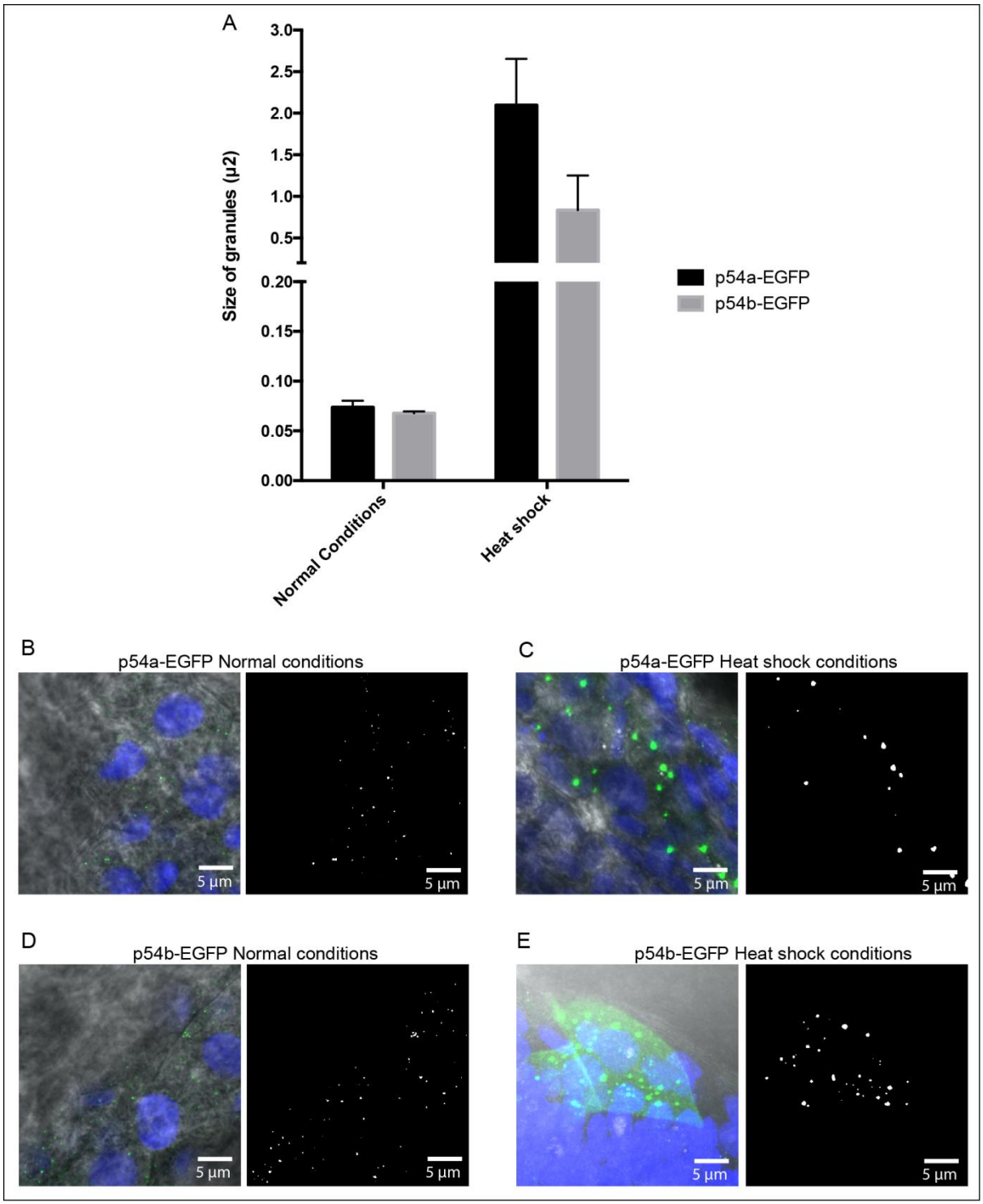

Figure S3. Estimated size for cytoplasmic granules. (A) Graph comparing average area (μm^2) for P54a-EGFP and P54b-EGFP cytoplasmic granules. Area values were estimated

with ImageJ and later converted to diameter values considering that granules have a circular shape. Under normal temperature P54a-EGFP granules have an average area of 0.07 μm^2 and a diameter of 0.30 μm, while P54b-EGFP granules have an average area 0.06 μm^2 and a diameter of 0.29 μm. In heat shock conditions, the average area and diameter for P54a-EGFP cytoplasmic granules was of 2.0 μm^2 and 1.63 μm, respectively. For P54b-EGFP granules, under heat shock the average area and diameter for cytoplasmic granules was of 0.8 μm^2 and 1.03 μm, respectively. (B) Example of P54a-EGFP granules in normal conditions used in our measurements. (C)

Example of P54a-EGFP granules in heat shock. (D) P54b-EGFP granules in normal conditions. (E) P54b-EGFP granules in heat shock.

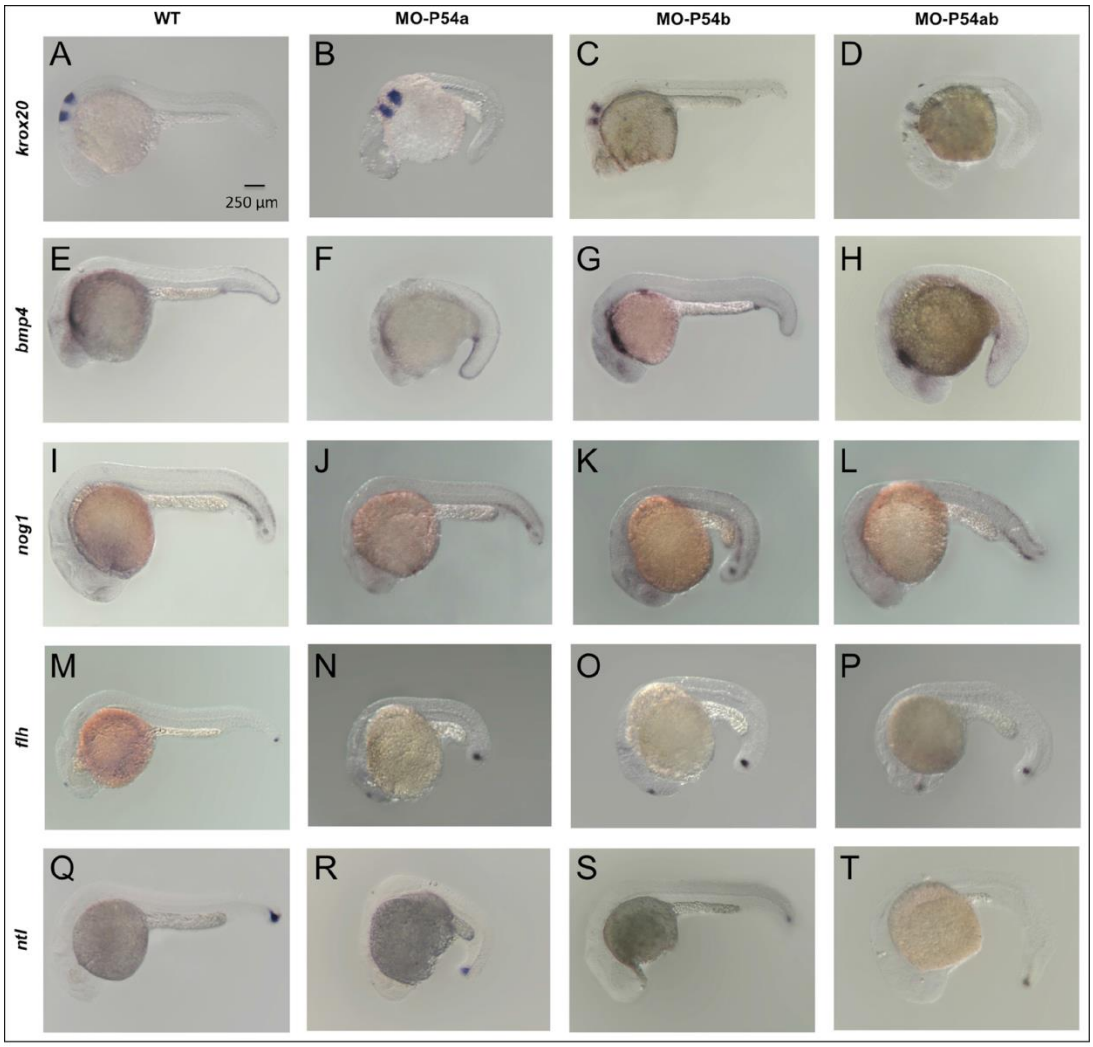

Figure S4. Effects of morpholino knockdown of P54a and P54b RNA helicases in the expression of some markers for zebrafish development.

Whole-mount *in situ* hybridization of WT or morpholino micro-injected 24 hpf zebrafish embryos. (A, E, I, M and Q) show WT embryos. (B, F, J, N and R) Embryos micro-injected with 15 ng of MO-P54a. (C, G, K, O and S) Embryos micro-injected with 9 ng of MO-P54b. (D, H, L, P and T) Embryos micro-injected with a mix of 15 ng of MO-P54a and 9 ng of MO-P54b. Abbreviations: *p54a* morpholino (MO-P54a), *p54b* morpholino (MO-P54b), mix of *p54a* and *p54b* morpholinos (MO-P54ab), "*in situ*" hybridization (ISH), *early growth response 2a* (*krox20*), *bone morphogenetic protein 4* (*bmp4*), *noggin 1* (*nog1*), *floating head or notochord homeobox* (*flh*) and *no tail or brachyury homolog* (*ntl*).

Table S1. Sequences of morpholino oligonucleotides and primers used in this work.

Name	Description	Primer sequence	Temp
MO-P54a	Morpholino	5'-CGGCATTTGCAAGGACTTACTTGAT-3'	-
MO-P54b	Morpholino	5'-TTACATGAAGATTACCTGATACCGC-3'	-
Control MO	Human β-globin morpholino	5'-CCTCTTACCTCAGTTACAATTTATA-3'	-
***p54a* forward**	RT-PCR	5'-AACACAGCCTGCCCAGGTCAAA-3'	64 ºC
***p54a* reverse**	RT-PCR	5'-TGGTTTCTCCCAGCCCATTTCA-3'	64 ºC
***p54b* forward**	RT-PCR	5'-CAAGCCCATGAGCCTTCAGACG-3'	64 ºC
***p54b* reverse**	RT-PCR	5'-TGCCCATCAGCAGTTCCCTCTT-3'	64 ºC
***Actin* forward**	RT-PCR	5'-CATCAGCATGGCTTCTGCTCTGT ATGG-3'	60 ºC
***Actin* reverse**	RT-PCR	5'-GACTTGTCAGTGTACAGAGACAC CCTG-3'	60 ºC
***p54a*Fw1**	Used to clone p54a into pCS2 vector	5'-CGggatccCGATGAGTACAGCCAGA ATGG-3'	44 ºC/ 62 ºC
***p54a*Rv1**	Used to clone p54a into pCS2 vector	5'-CCatcgatGGGGCAGTTTAACCTCCT CTCC-3'	44 ºC/ 62 ºC
***p54a*Fw2**	Used to clone p54a with the Gateway system	5'-GGGGACAAGTTTGTACAAAAAAGCAGG CTTCGAAGGAGATAGAACCATGAGTACA GCCAGAATGGAG-3'	65 ºC
***p54a*Rv2**	Used to clone p54a with the Gateway system	5'-GGGGACCACTTTGTACAAGAAAGCTGG TCCAGTTTAACCTCCTCTCC-3'	65 ºC
***p54b*Fw**	Used to clone p54b with the Gateway system	5'-GGGGACAAGTTTGTACAAAAAAGCAGG CTTCGAAGGAGATAGAACCATGGCTACA GCGAGAACTGAG-3'	65 ºC
***p54b*Rv**	Used to clone p54b with the Gateway system	5'-GGGGACCACTTTGTACAAGAAAGCTGG GTCATTAAGCTCGCCTGTTTTGTG-3'	65 ºC
***nog1* Fw**	Used for ISH	5'-GCTCCTTCAATCGTGATGCTTTCT-3'	58 ºC
***nog1* Rv**	Used for ISH	5'-GCTTCACATCAGAGTCCAGAAACG-3'	58 ºC
***flh* Fw**	Used for ISH	5'-CTATCCGCCCTACAACTTCCAAAC-3'	58 ºC
***flh* Rv**	Used for ISH	5'-AGAGCAATGGCGTGTGTAAGTGAC-3'	58 ºC

Lowercase letters within sequences indicate restriction sites.