Supplementary Information included with Nature MS 2008-02-01484B by Colantonio et al., entitled "The dynein regulatory complex is required for ciliary motility and otolith biogenesis in the inner ear".

This document contains:

1. Legends for six (6) supplementary figures, labeled "S1" – "S6".
2. Legends for fourteen (14) supplementary videos, labeled "SupMov01"-"SupMov11b".

SUPPLEMENTAL FIGURE AND MOVIE LEGENDS

Supplemental Figure S1. High magnification image of gas11 expression in the otic vesicle. In situ hybridization shows gas11 is expressed throughout the otic vesicle at 8 to 13-somite stages, then becomes concentrated at the poles of the otic vesicle (white arrows) at 15 to 18-somite stages. Beyond the 18-somite stage, the signal in the otic vesicle is not detectable above that in surrounding tissues (not shown). The dorsal/ventral and anterior/posterior axis are shown.

Supplemental Figure S2. *gas11* knockdown results in left-right axis defects. *In situ* hybridizations using *southpaw* riboprobe demonstrate laterality defects in *gas11* morphants at the 16-18 somite stage. The relative numbers of uninjected (n = 119) and *gas11* morphant (n = 138) embryos having each staining pattern are shown.

Supplemental Figure S3. *gas11* is required for tether cilia motility. Brightfield images taken from high-speed videos of tether cilia in control (top) and *gas11* morphant (bottom) embryos. Panels are consecutive frames extracted at 16 ms intervals from supplemental movies 5 (control), and 8 (*gas11* morphant). Lines trace the cilium and are colorized (violet to red) in time

sequence. These are then aligned at the base of the cilium and overlayed in the right-most panel to generate the "combined" image.

Supplemental Figure S4. Morpholino knockdown of *lrrc50* and *lrdr1* disrupts tether cilia motility and causes otolith defects. (a) Table summarizing the effect of *lrrc50* and *lrdr1* knock down. To confirm the specificity of the *lrrc50* morpholino, a control morpholino (5 base pairs mismatch) was injected. Error bars show standard deviation. (b-f) phenotypic comparison between control (b) and morpholino injected embryos (c-f) at 27 hpf. Among multiple otolith defects the most common are: (c) fused otolith, (d) smaller otoliths, (e) mis-positioned otolith, (f) single otolith. Left otic vesicle, anterior to the right. Scale bar: 20 microns.

Supplemental Figure S5. The *lrrc50* splice morpholino interferes with *lrrc50* splicing. (A) RT-PCR with two different primer sets confirms that the *lrrc50* splicing morpholino interferes with *lrrc50* splicing. mRNA from control embryos (Ctrl2) or embryhos injected with 10 ng of splicing MO (Mo-2) were subjected to RT-PCR with the indicated primers for *lrrc50*, or GAPDH as a control. The positions of *lrrc50* primers are shown relative to the intron/exon positions of the *lrrc50* mRNA (not to scale). (B) RT-PCR with *lrrc50* primers (1) x (3) showing a decrease in the abundance of the product from the spliced transcript (147 bp, open arrow) and the appearance of a band corresponding to the size expected for the product from the unspliced transcript in the injected sample (1241 bp, black arrow). GAPDH mRNA levels were unaffected. (C) RT-PCR with *lrrc50* primers (1) x (2) shows the appearance of a band corresponding to the size predicted from the unspliced transcript in the injected sample (1147 bp,

red arrow). The same results were obtained with an independent set of mRNA samples (not shown).

Supplemental Figure S6. Particle displacement in control embryo. White arrows indicate particle direction, illustrating the attractive flow at the base of the cilium. These particle displacements correspond to the particle traces shown in figure 4G.

Supplemental movies

Supplemental movie 1. Three dimensional display of a control inner ear at stage 27 hpf after immunofluorescence labeling of cilia with acetylated tubulin antibody. Notice the two clusters of tether cilia on the lateral side of the inner ear. Side view, anterior on the right. (QuickTime: 1.27 MB)

Supplemental movie 2. Three dimensional display of a *gas11* morphant inner ear at stage 27 hpf after acetylated tubulin immunofluorescence labeling. The two clusters of tether cilia are properly located. Side view, anterior on the right. (QuickTime: 2.30 MB)

Supplemental movie 3. Video shows tether cilia motility in control embryo. This video corresponds to snapshots shown in figure 3A, B. One cilium is attached to the otolith and beating of this cilium causes the otolith to move. A second motile cilium is also evident. Embryo is 20 hpf. Acquisition rate 322 frames/sec played at 20 frame/sec. (QuickTime: 1.30 MB)

Supplemental movie 4. Video shows tether cilia motility in control embryo. Embryo is 24 hpf. Acquisition rate 322 frames/sec played at 20 frame/sec. (QuickTime: 1.50 MB)

Supplemental movie 5. Video shows tether cilia motility in control embryo. Cilium beating causes otolith to move. This video corresponds to snapshots shown in supplemental figure 2 (control). Embryo is 20-22 hpf. Acquisition rate 322 frames/sec played at 20 frame/sec. (QuickTime: 2.95 MB)

Supplemental movie 6. Video shows short cilia in control embryo are not motile. Embryo is 20-22 hpf. Acquisition rate 322 frames/sec played at 20 frame/sec. (QuickTime: 5.9 MB)

Supplemental movie 7. Video shows tether cilia in a *gas11* morphant embryo are not motile. Embryo is 20 hpf. This video corresponds to snapshots shown in figure 3C,D. (QuickTime: 0.83 MB)

Supplemental movie 8. Video shows tether cilia in a *gas11* morphant embryo are not motile. Embryo movement during the video is due to normal embryonic activity. This video corresponds to snapshots shown in supplemental figure 2 (*gas11* MO). Embryo is 20-22 hpf. Acquisition rate 322 frames/sec played at 20 frame/sec. (QuickTime: 8.05 MB)

Supplemental movie 9a. High speed video microscopy of tether cilia in a control embryo at 24 hpf showing vortices in the vicinity of the tether cilia and particle propelling along the growing otolith. Acquisition rate: 322 frames/sec played at 50 frames/sec. (QuickTime: 2.94 MB)

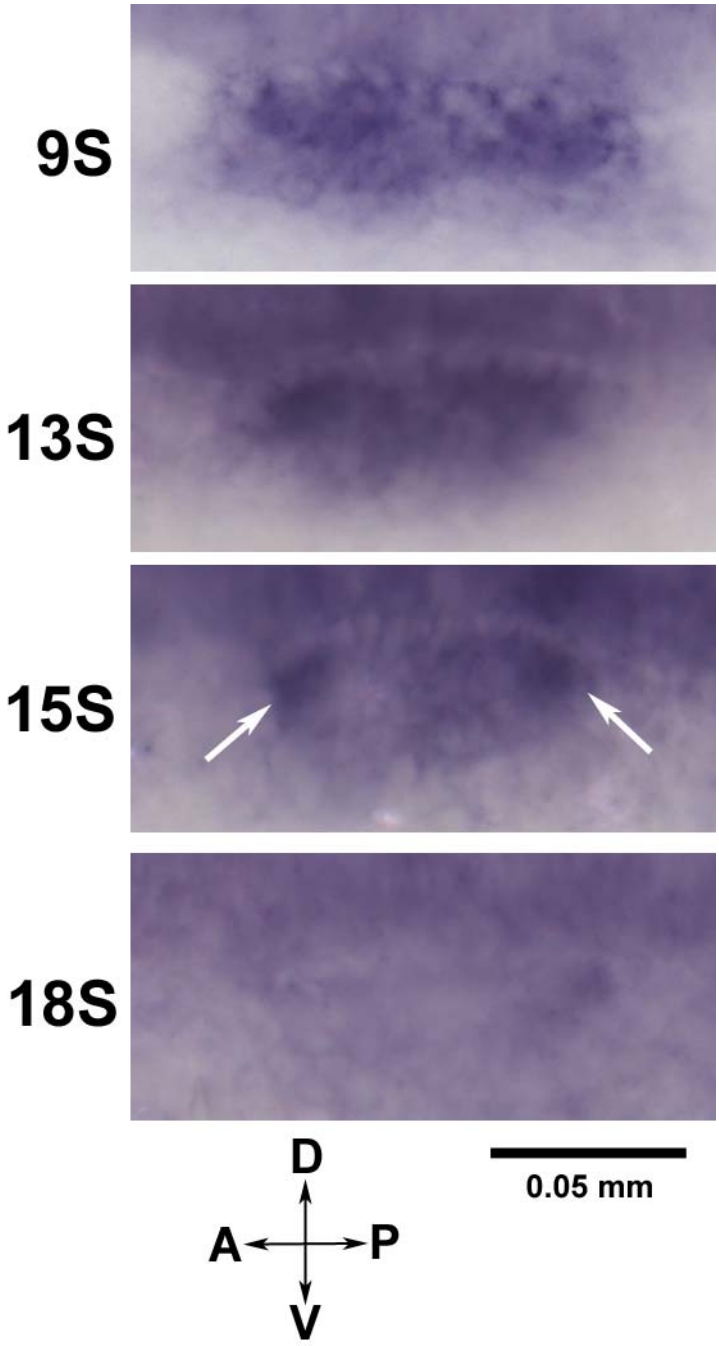
Supplemental movie 9b. Same as 9a with particle tracking. This video corresponds to figure 4A, B. Acquisition rate: 64 frames/sec played at 20 frames/sec. (QuickTime: 10.69 MB)

Supplemental movie 10a. High speed video microscopy of the inner ear in a control embryo at 23 hpf showing high displacement of otolith precursors in the vicinity of tether cilia and low displacement away from tether cilia. Acquisition rate: 322 frames/sec played at 20 frames/sec. (QuickTime: 3.11 MB)

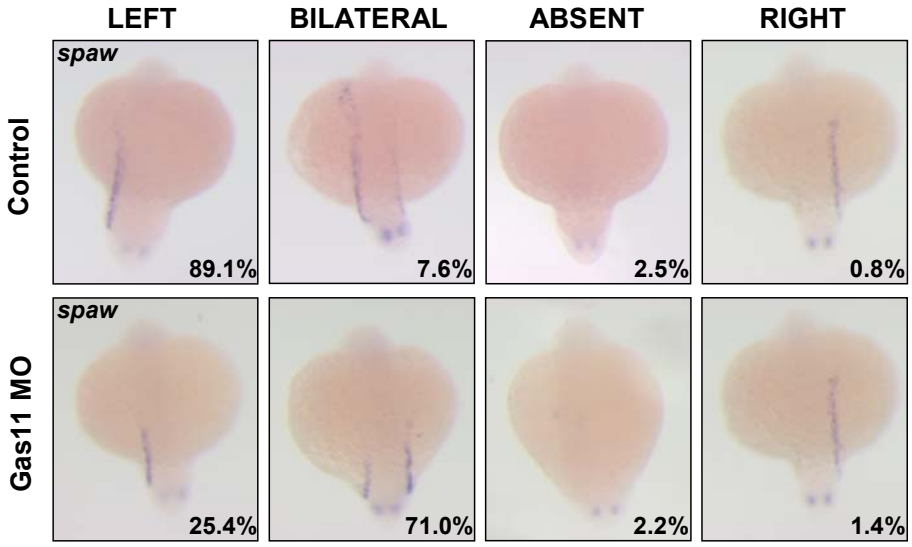
Supplemental movie 10b. Same as supplemental movie 10a with particle tracking. This video corresponds to figure 4G and supplemental Figure S3. Acquisition rate: 64 frames/sec played at 50 frames/sec. (Quicktime: 1.59 MB)

Supplemental movie 11a. High speed video microscopy of tether cilia in a *gas11* morphant at 25 hpf showing very low particle displacement in the vicinity of tether cilia. Acquisition rate: 322 frames/sec played at 20 frames/sec. (QuickTime: 1.36 MB)

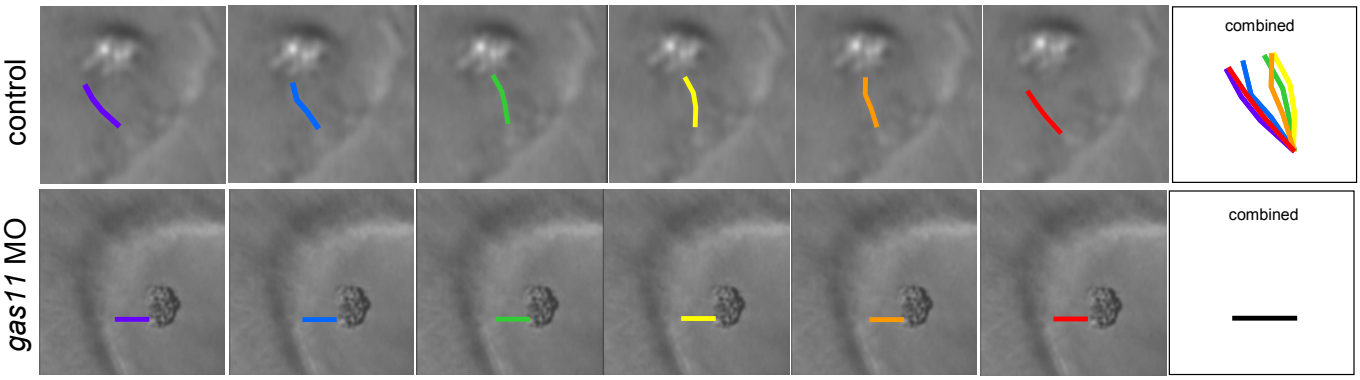
Supplemental movie 11b. Same as 11a with particle tracking. This video corresponds to figure 4D, E. Acquisition rate 64 frames/sec played at 50 frames/sec. (QuickTime: 14.79 MB)



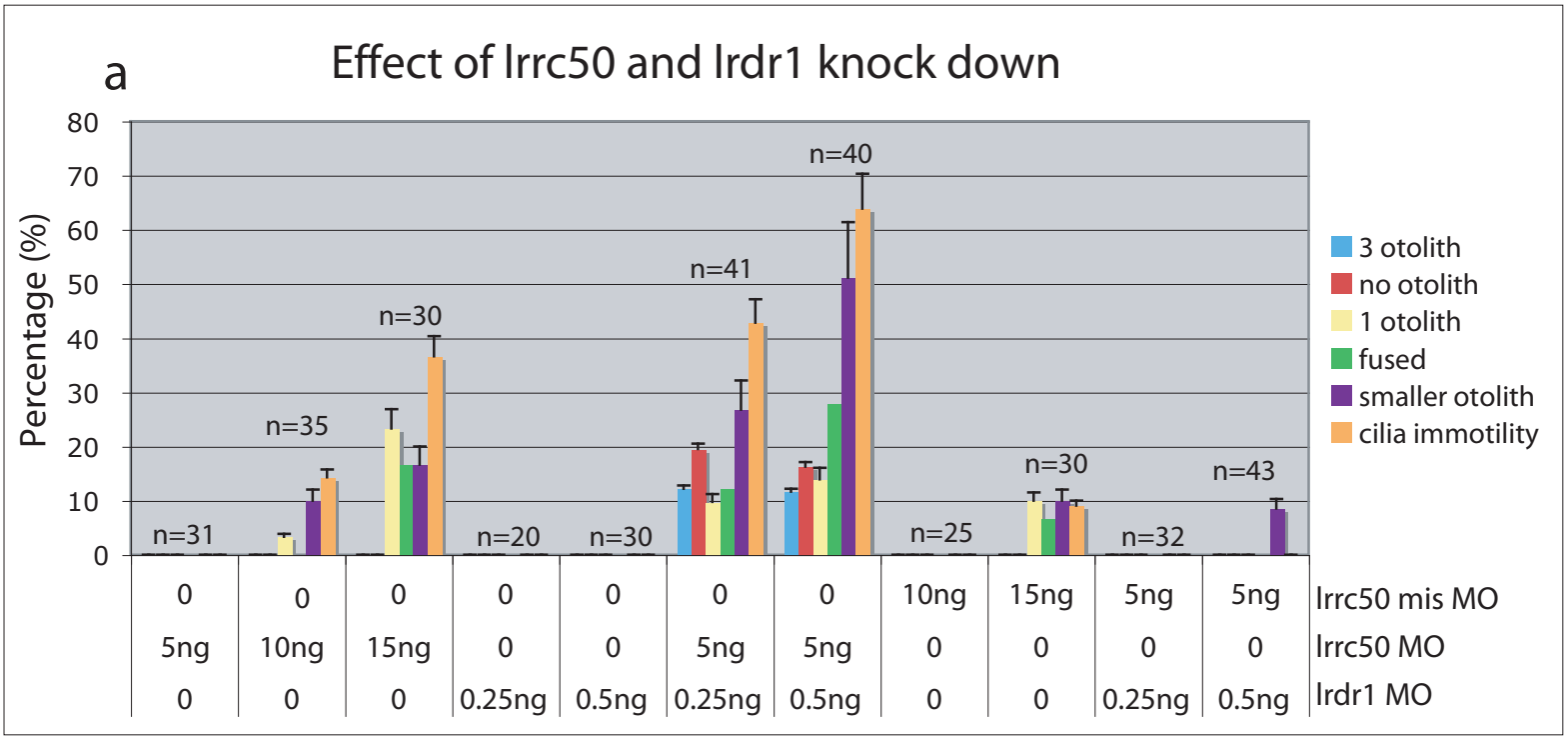
Supplemental Figure S1

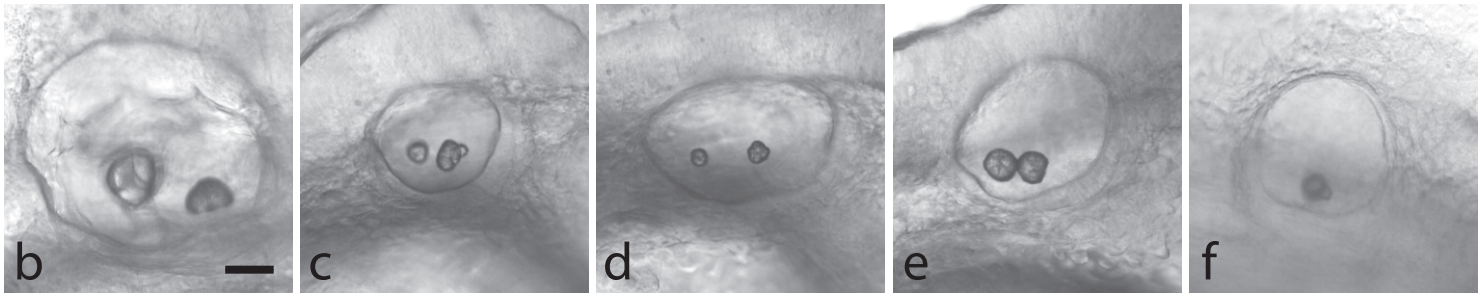


Supplemental Figure S2



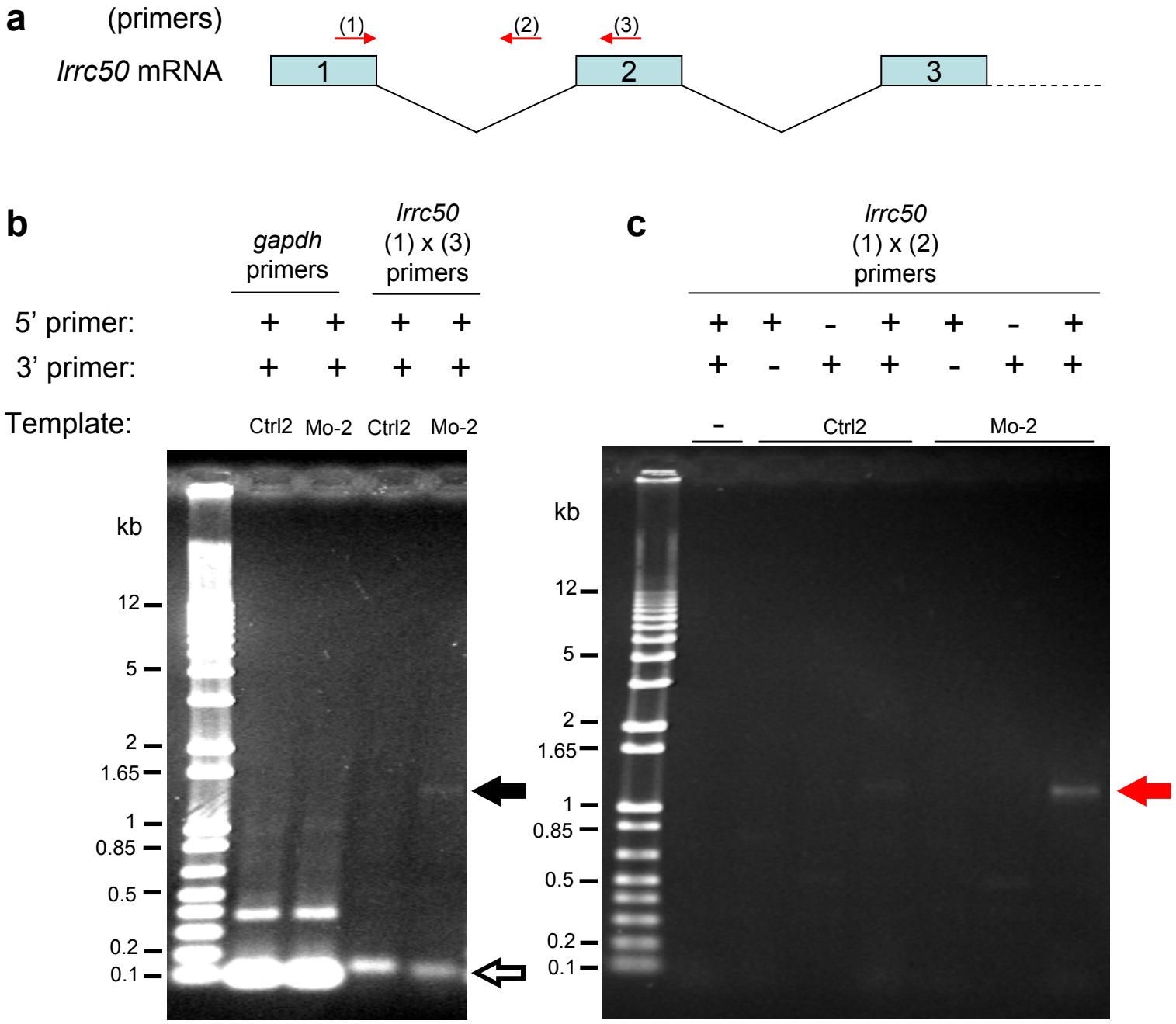
Supplemental Figure S3



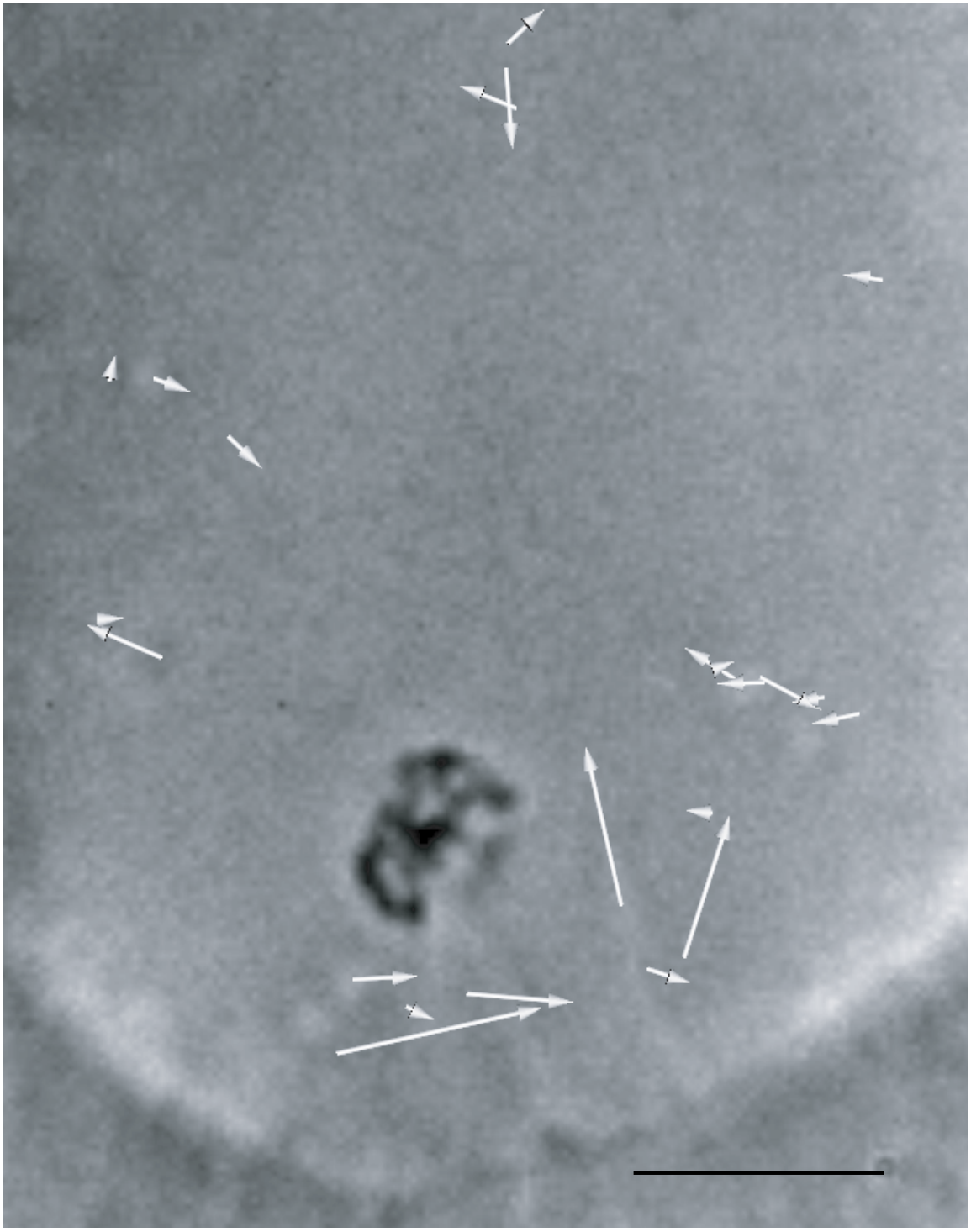


control lrrc50/lrdr1 MO

Supplemental Figure S4



Supplemental Figure S5



Supplemental Figure S6