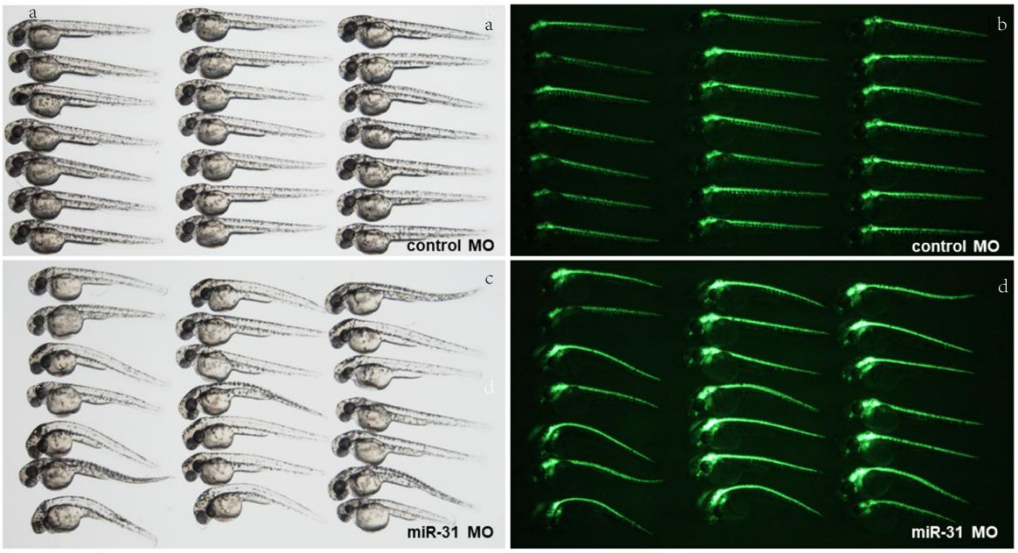
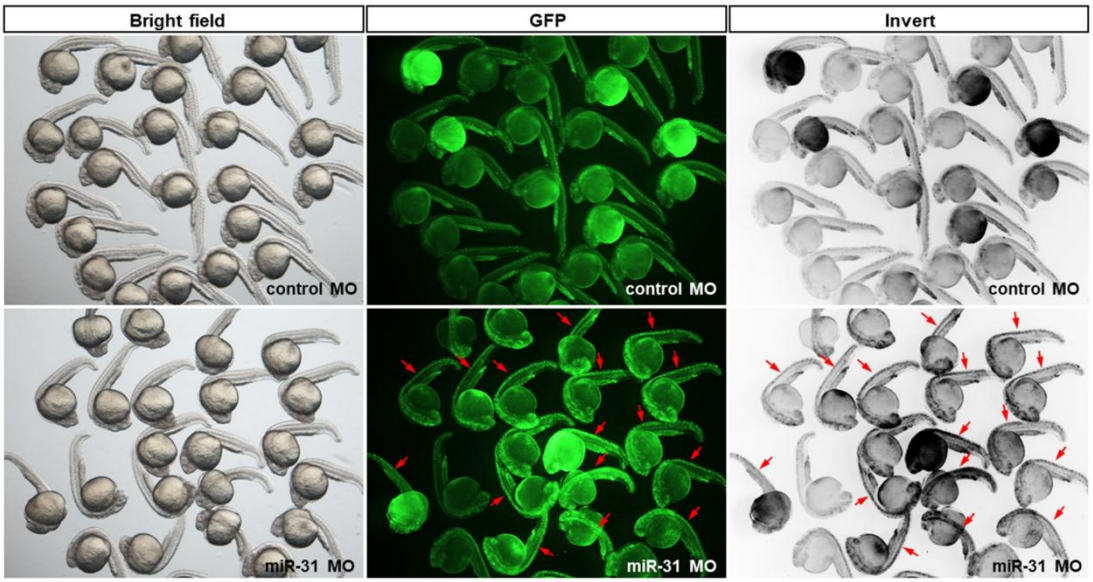
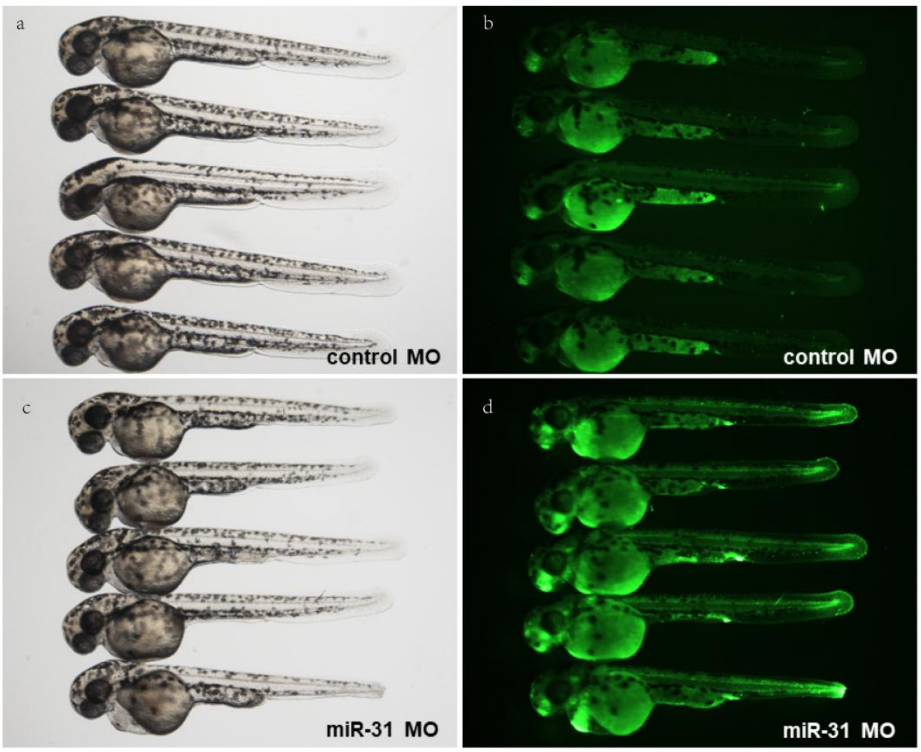
**Supplementary Fig S1 miR-31 knockdown induces abnormal motor neuron outgrowth.** Tg (hb9:EGFP) zebrafish embryos were injected with 8 ng of control or miR-31 MO. **(a-d)** Bright-field and fluorescent images of Tg(hb9:EGFP) the embryos used in this experiment at 48 hpf.



**Supplementary Fig S2. miR-31 knockdown induces CNS-specific apoptosis.** Embryos injected with control or miR-31 MO were stained with acridine orange (AO) at 26 hpf. Apoptotic cells are visible as bright green spots or black spots. Less bright homogenous green or black is unspecific background staining. **(a-c)** Control zebrafish exhibited few or no apoptotic cells in the central nervous system (CNS). In contrast, significantly increased staining was observed throughout the CNS in zebrafish injected with miR-31 MO (**D-F**, red arrows).



**Supplementary Fig S3. Morpholino knockdown of miR-31 induces apoptosis in the CNS and tail.** Embryos injected with control **(a-b)** or miR-31 MO **(c-d)** were stained with acridine orange (AO) at 48 hpf. Apoptotic cells are visible as bright green spots or red spots, and less bright homogenous green or black is unspecific background staining. **(a-b)** Control MO zebrafish exhibited few or no apoptotic cells in the whole body. In contrast, significantly increased staining was observed throughout the CNS and tail in zebrafish injected with 8 ng of miR-31 MO (**c-d**).



**Supplementary Fig S4. Morpholino knockdown of miR-31 induces abnormal heart development.** Zebrafish embryos were injected with control **(a,c)** or miR-31 MO **(b,d)**. Bright-field images were acquired at 8 hpf. miR-31-knockdown caused pericardial edema (blue arrow, B) compared with normal heart (Red arrow, A), and the blood could not be pumped out.