

Supplemental Information

Figure S1

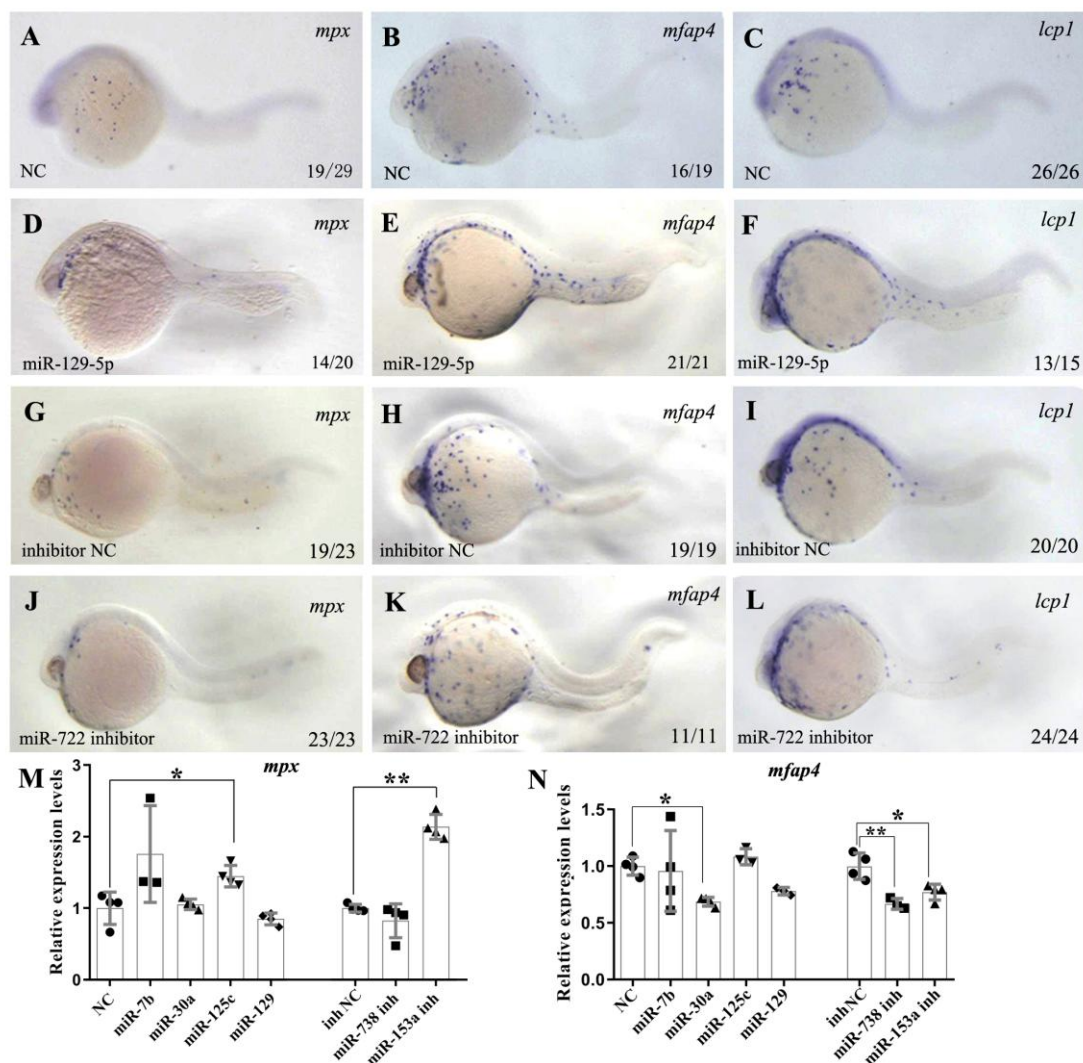


Figure S1. Overexpression of the other 7 miRNAs doesn't reduce the formation of both granulocytes and macrophages in zebrafish primitive myelopoiesis together. (A-L) Whole mount *in situ* hybridized embryos showing the expressions of *mpx* (A, D, G, J), *mfap4* (B, E, H, K), and *lcp1* (C, F, I, L) in the 26 hpf embryos microinjected with NC (A-C) or inhibitor NC (G-I) were similar to the ones microinjected with miR-129-5p mimic (D-F) or miR-722 inhibitor (J-L). Embryos were viewed laterally and positioned head left. The images in panels (A, B, C) were also used in Figure 1 (B, D, F) because these results were from one ISH experiment and shared the same negative controls. (M-N) qRT-PCR results showing the expressions of *mpx* (M) in the embryos microinjected with mimics of miR-7b, miR-30a, miR-125c, and miR-129-5p, or inhibitors of miR-738 were similar to their control embryos, and the expressions of *mfap4* (N) in the embryos microinjected with mimics of miR-7b, miR-125c, and miR-129-5p, or miR-135a inhibitor were similar to the embryos microinjected with NC or inhibitor NC, respectively. However, the expressions of *mpx* (M) in the embryos microinjected with miR-125c mimic or miR-135a inhibitor were increased while the

expressions of *mfap4* (N) in the embryos microinjected with miR-30a mimic, miR-738 inhibitor or miR-153a inhibitor were all reduced compared to their controls. The gene expression levels in NC group was normalized as 1.0 and the fold changes of gene expression levels in the embryos microinjected mimics or inhibitors were shown relative to NC group (shown in Y-axis). NC: mimic negative control; inhNC: inhibitor negative control; *: $P < 0.05$, **: $P < 0.01$.

Figure S2

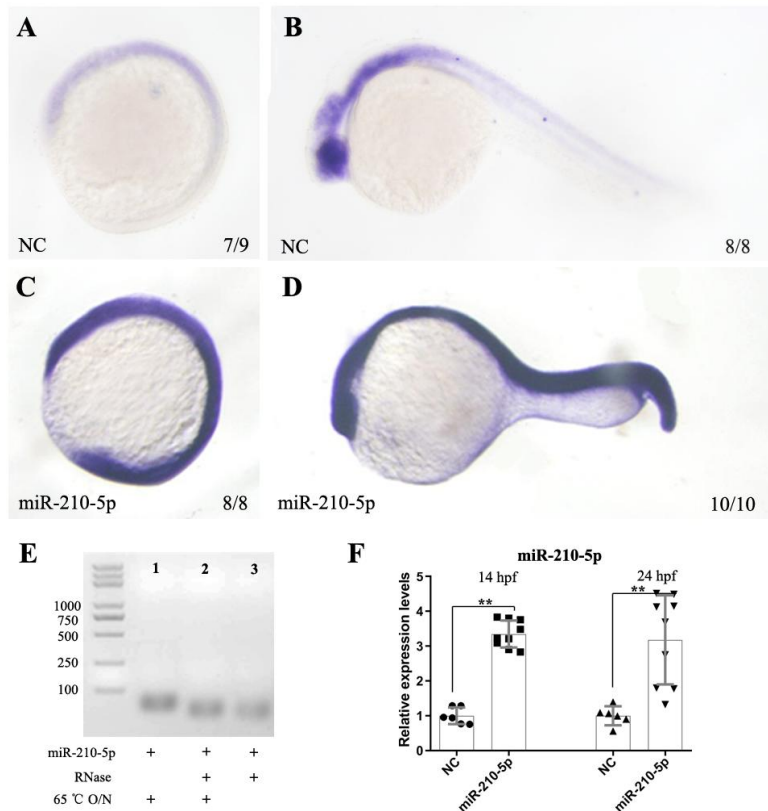


Figure S2. The amount of mature miR-210-5p was significantly increased in the embryos microinjected with miR-210-5p mimic. (A-D) Whole mount *in situ* hybridization results showing the expression of miR-210-5p was significantly increased in the embryos microinjected with miR-210-5p mimic (C, D) compared to the ones microinjected with NC (A, B) at 14 hpf (A, C) and 26 hpf (B, D), respectively. (E) *In vitro* assay showing the miR-210-5p mimic was stable after overnight incubation with RNase A at 65°C. The *in vitro* reaction was performed by incubating the dsRNA (miR-210-5p mimic) with RNase A under the same conditions (without adding yeast tRNA) as the experiment of whole mount *in situ* hybridization. The 1st lane (in the leftmost) shows the DNA maker. The 2nd lane (labeling with 1) shows the miR-210-5p mimic was stable in the solution without RNase A after incubation overnight at 65°C. The 3rd lane (labeling with 2) shows the miR-210-5p mimic was stable in the solution with RNase A (final concentration: 50 µg/ml) after incubation overnight at 65°C. The 4th lane (labeling with 3) shows the miR-210-5p mimic was stable in the solution with RNase A (final concentration: 50 µg/ml) after incubation overnight at room temperature. The smaller size of miR-210-5p occurred in the 3rd and 4th lanes (labeling with 3 and 4) compared with the 2nd lane (labeling with 2) was due to the removal of the 2 nt 3'-overhang in each strand of the dsRNA (see Table S1) by RNase A (4 nt shorter than the "wild type" miR-210-5p mimic). (F) qRT-PCR results showing the amount of mature miR-210-5p was significantly increased in the embryos microinjected with miR-210-5p mimic compared to the ones microinjected with NC at 14 hpf and 26 hpf, respectively. The expression level of mature miR-210-5p in NC group was normalized as 1.0 and the fold change of miR-210-5p expression level in miR-210-5p mimic microinjection group was shown relative to NC group (shown in Y-axis), respectively. miR-210-5p: miR-210-5p mimic; RNase: RNase A; O/N: overnight; NC: mimic negative control; **: $P < 0.01$.

Figure S3

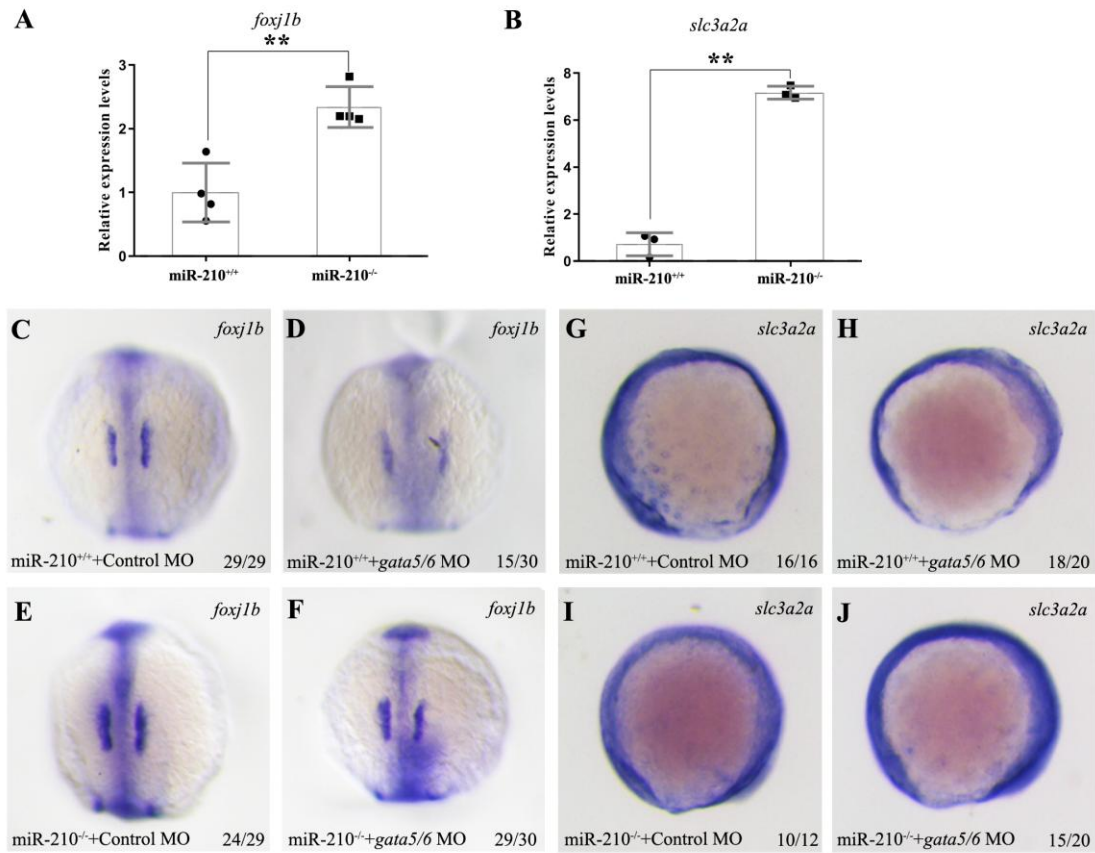


Figure S3. The expressions of *foxj1b* and *slc3a2a* were significantly increased in *miR-210* knockout embryos and the increased expressions were inhibited in the *gata5/6* morphants. (A-B) qRT-PCR results showing the expressions of *foxj1b* and *slc3a2a* were significantly increased in the *miR-210* knockout embryos. The expression level of genes in *miR-210*^{+/+} embryos was normalized as 1.0 and the fold change of the gene expression levels in *miR-210*^{-/-} embryos was shown relative to *miR-210*^{+/+} embryos (shown in Y-axis), respectively. **: $P < 0.01$. (C-J) The expressions of *foxj1b* and *slc3a2a* were reduced in the *gata5/6* morphants (D, H) compared with the embryos microinjected with control MO (C, G). However, knocking out *miR-210* dramatically increased the expressions of *foxj1b* and *slc3a2a* (E, I) compared to the wild type embryos (C, G). The reduced expressions of *foxj1b* and *slc3a2a* in the *gata5/6* morphants (D, H) were effectively rescued in the *miR-210* knockout embryos (F, J). Embryos were *in situ* hybridized at 14 hpf and viewed dorsally (C-F) or laterally (G-J). Embryos were positioned head top (C, F) or left (G-J).

Table S1. Sequences of miRNA mimics, miRNA inhibitors and primers used for sgRNA synthesis, RT-PCR and qRT-PCR

Name	Sequence
miR-129-5p mimic	Guide strand: CUUUUUGCGGUCUGGGCUUGCU Passenger strand:CAAGCCCAGACCGCAAAAAGUU
miR-210-5p mimic	Guide strand: AGCCACUGACUAACGCACAUUG Passenger strand:AUGUGCGUUAGUCAGUGGCUUU
miR-7b mimic	Guide strand: UGGAAGACUUGUGAUUUUGUU Passenger strand:CAAAAUCACAAGUCUUCCAU
miR-30a mimic	Guide strand: UGUAAACAUUCCCGACUGGAAG Passenger strand: UCCAGUCGGGAAUGUUUACAU
miR-125c mimic	Guide strand: UCCCUGAGACCCUAACUCGUGA Passenger strand:ACGAGUUAGGGUCUCAGGGAAU
miR-722 inhibitor	AAUCUGAAACGUUUCUGCAAAAAA
miR-153a inhibitor	GAUCACUUUUGUGACUAUGCAA
mimic negative control	Guide strand: UUCUCCGAACGUGUCACGUTT Passenger strand: ACGUGACACGUUCGGAGAATT
inhibitor negative control	CAGUACUUUUGUGUAGUACAA
sgRNA1F	TAATACGACTCACTATAAGTTCCGATGGCACAGGAAAGTTTTAGAGCTA GAAATAGC
sgRNA2F	TAATACGACTCACTATAGACAGCGGCTAACCAGCTTTGTTTTAGAGCTA GAAATAGC
sgRNAR	AAAAAAGCACCGACTCGGTGCC AC
<i>gata4</i> -FL-F	GCTCGTGGAGAATAATCGC
<i>gata4</i> -FL-R	TGCGTTTATGCCAGAATCAG
<i>gata6</i> -FL-F	CCTCATTGTGGACCCTACC
<i>gata6</i> -FL-R	AATTCAGCCTCAAGATCACC
<i>foxj1b</i> -FL-F	CCGCTCAAACATACGAGTTA
<i>foxj1b</i> -FL-R	CGTGTATATGTCATGCGTTC
<i>slc3a2a</i> -FL-F	GTCCGCCGGCTTGAAACAGA
<i>slc3a2a</i> -FL-R	TGTGTCTAGCCCGGAATGG
<i>stard3</i> -FL-F	GCTGATTCTGGGATGTGCTC
<i>stard3</i> -FL-R	CGATAGGTCACCGGTACGAG
<i>mpx</i> -qRT-F	GGGGCAGAAGAAGAAAGTCC
<i>mpx</i> -qRT-R	CCCTTGCTAAACTCTCATCTCG
<i>lcp1</i> -qRT-F	GAAGCTCTGATCGCTCTGCT
<i>lcp1</i> -qRT-R	CCCTTGCTAAAC TCTCATCTCG
<i>mfap4</i> -qRT-F	ATGAAGAGAACGGAGGATGG
<i>mfap4</i> -qRT-R	CACATTCCCGAATCCTCTCT
<i>actb1</i> -qRT-F	TTCCTTCCCTGGGTATGGAATC
<i>actb1</i> -qRT-R	GCACTGTGTTGGCATAACAGG
<i>tall1</i> -qRT-F	GGAGATGCGGAACAGTATGG

<i>tal1</i> -qRT-R	GAAGGCACCGTTCACATTCT
<i>lmo2</i> -qRT-F	GGACGCAGGCTTTACTACAAAC
<i>lmo2</i> -qRT-R	CCGGATCCTCTTTTCACAGGAA
<i>spi1b</i> -qRT-F	GGGCAGTTTTTAACCAAAGATCA
<i>spi1b</i> -qRT-R	CCCAAGAGTGATCGTTCTGAC
<i>foxj1b</i> -qRT-F	TTATAGCAACGAGGACGAGCAG
<i>foxj1b</i> -qRT-R	ATCCCTCACAAAACGCATAGCC
<i>rnasel3</i> -qRT-F	TGTCAAGAAGAGCAAGTCATTTTC
<i>rnasel3</i> -qRT-R	TTCTGCTGGTTGACCGTAAG
<i>slc3a2a</i> -qRT-F	TAAAGGTCAGTGCCGGAGA
<i>slc3a2a</i> -qRT-R	AACCAGTAAAGCCCAACGAG
<i>stard3</i> -qRT-F	GCTGATTCTGGGATGTGCT
<i>stard3</i> -qRT-R	GGCGTTTTAAAGAAGCAATGG
miR-210-5P-qRT	AGCCACTGACTAACGCACATTG
<i>foxj1b</i> -3utr-F	ACGCATGACATATACACGCA
<i>foxj1b</i> -3utr-R	CTGGAATTGATTTTGCGGAG
<i>slc3a2a</i> -3utr-F	GTCATTATTTACTCGGCTCC
<i>slc3a2a</i> -3utr-R	TCTGTACAGAGATCAGCTTC
<i>slc3a2a</i> -3UTR-F	agatcgccgtgtaattctagaGTCATTATTTACTCGGCTCC
<i>slc3a2a</i> -3UTR-R	gccggccgccccgactctagaTCTGTACAGAGATCAGCTTC
<i>foxj1b</i> -3UTR-F	agatcgccgtgtaattctagaACGCATGACATATACACGCA
<i>foxj1b</i> -3UTR-R	gccggccgccccgactctagaCTGGAATTGATTTTGCGGAG
F- <i>foxj1b</i> -3UTR-MT	GCGGAAAGATCGCCGTGTAATTCTAGAAcgcacatgacatatacacgca
R- <i>foxj1b</i> -3UTR-MT	AAGCGGCCGCGCCCGCCCGACTCTAGAAgtacagattgatctttttat
F- <i>slc3a2a</i> -3UTR-MT	GCGGAAAGATCGCCGTGTAATTCTAGAAcacacacagacactgttta
R- <i>slc3a2a</i> -3UTR-MT	AAGCGGCCGCGCCCGCCCGACTCTAGAAatcctcatttgccattttat