Supplementary Information

Cyp2aa9 regulates haematopoietic stem cell development in zebrafish

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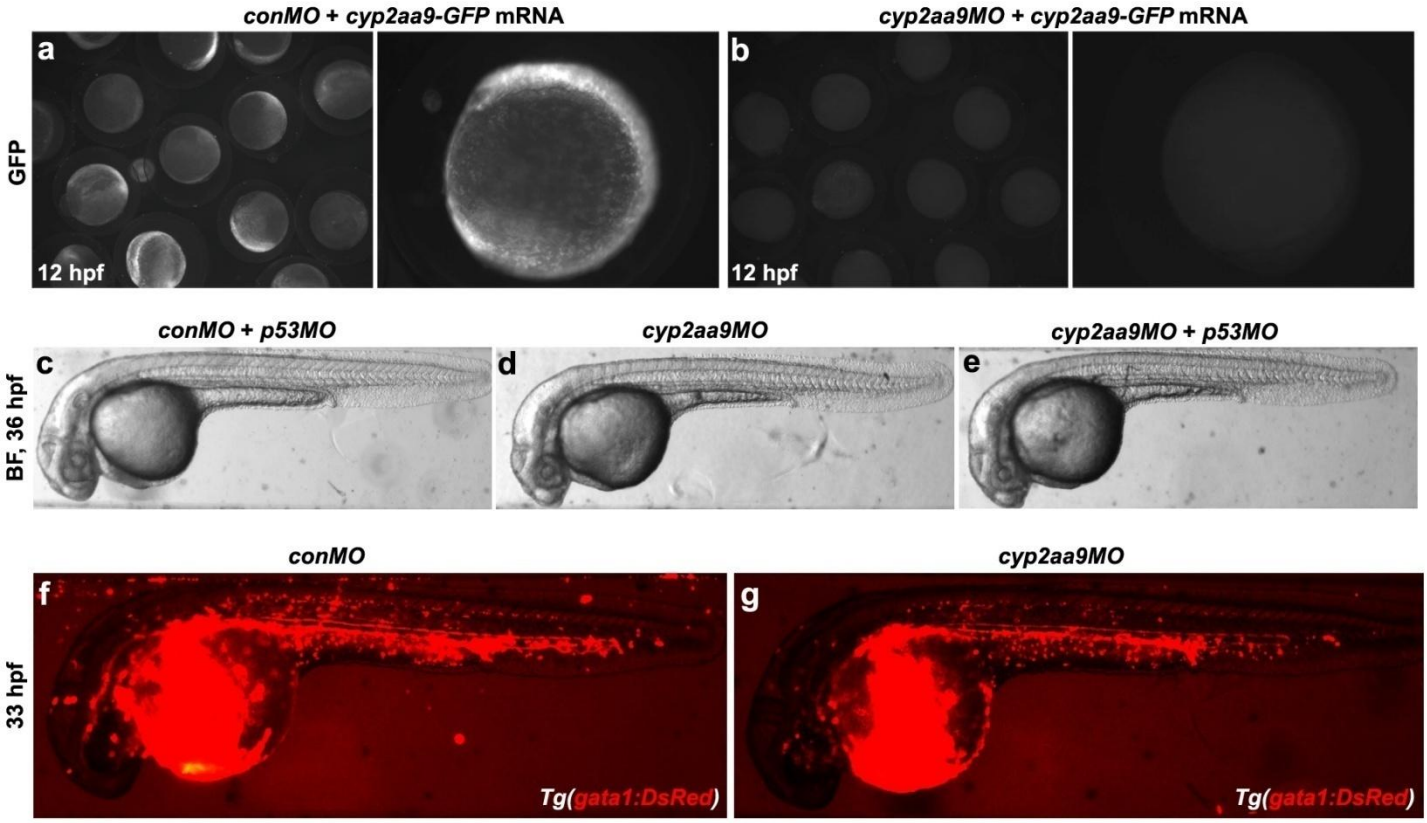
Supplementary Data:

Supplementary Figure S1 to S3

Supplementary Video Legends

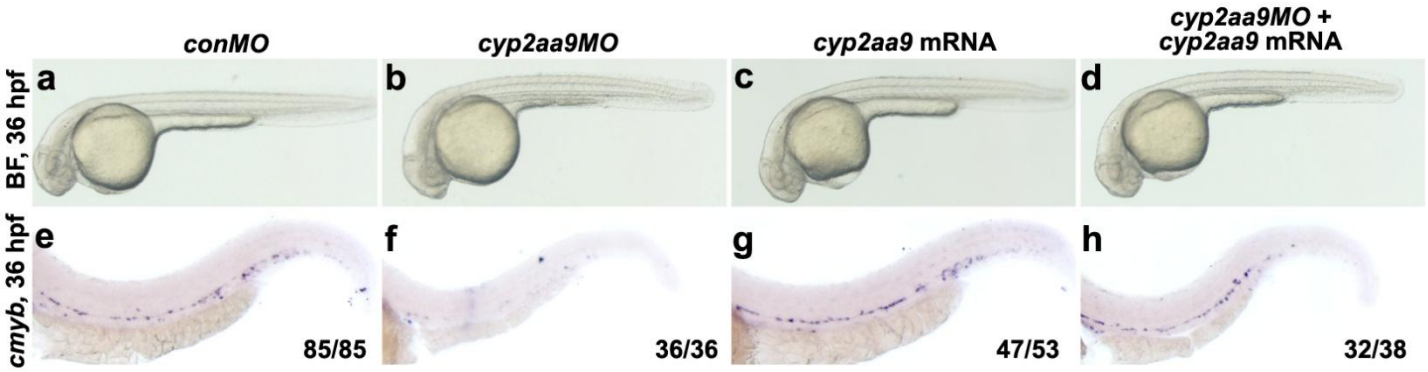
Supplementary Materials and Methods

Supplementary Videos 1 to 2

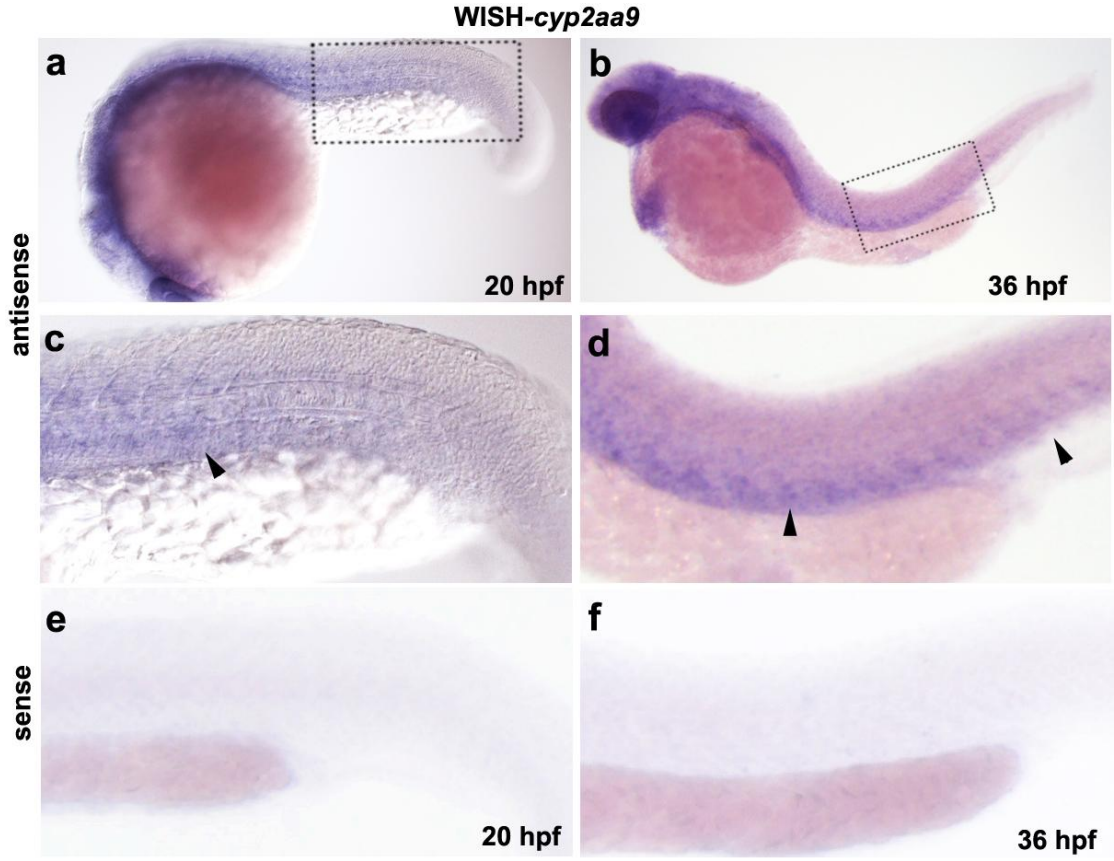


Supplementary Figure S1 (related to Figure 1). Effects of *cyp2aa9MO* is specific.

(**a**-**b**) Translation of *cyp2aa9-GFP* mRNA was inhibited by *cyp2aa9MO*, but not *conMO*. (**c**-**e**) *cyp2aa9MO* did not lead to obvious cell apoptosis as indicated by the co-injection of *p53MO*, excluding off-target effects of *cyp2aa9MO* (**c**, n=30/31; **d**, n=24/25; **e**, n=26/27). (**f**-**g**) The *cyp2aa9* morphants exhibited normal blood circulation under the *Tg(gata1:DsRed)* background (**f**, n=24/24; **g**, n=23/25). **c**-**f**, lateral views, anterior left, dorsal top.



Supplementary Figure S2 (related to Figure 1). Defective *cmyb* expression in the *cyp2aa9* morphants was rescued by the *cyp2aa9* mRNA. (**a**-**h**) As indicated by the *cmyb* expression, defective HSC development in the *cyp2aa9* morphants was rescued by the *cyp2aa9* mRNA. lateral views, anterior left, dorsal top.



Supplementary Figure S3 (related to Figure 1). Expression pattern of *cyp2aa9* in zebrafish embryos. (**a**-**f**) Whole-mount *in situ* hybridization indicated diffused expression of *cyp2aa9* in the mesoderm and tail region (arrowheads) at 20 hpf and 36 hpf (**a**, n=14/17; **b**, n=15/17). Dashed boxs were enlarged in **c**-**d**, which is controlled by the sense probe as shown in **e**-**f**.

Supplementary Video Legends

Supplementary Video 1. Real-time imaging of budding HSCs (arrowheads) in the *Tg(cmyb:GFP, kdrl:mCherryRas)* transgenic embryos injected with *conMO* from 26 hpf to 30 hpf. The durations of the imaging were 4 hours. Scale bar, 50 μm. Related to Fig. 1i, k.

Supplementary Video 2. Real-time imaging of budding HSCs (arrowheads) in the *Tg(cmyb:GFP, kdrl:mCherryRas)* transgenic embryos injected with *cyp2aa9MO* from 26 hpf to 30 hpf. The durations of the imaging were 4 hours. Scale bar, 50 μm. Related to Fig. 1j, l.

Supplementary Materials and Methods

MO and mRNA injections. The *p53MO*[23] were synthesized by the Gene-Tools, LLC. 5 ng *p53MO* plus 5 ng *conMO* or *cyp2aa9MO* were injected. *cyp2aa9-GFP* mRNAs were synthesized from the Not I-linearized *pCS2(+)* plasmids using the mMessage mMachine kit (Ambion) as previously described[42]. Injections were performed as previously described[43]. 70 pg of *cyp2aa9-GFP* mRNA were injected.