Figure S1

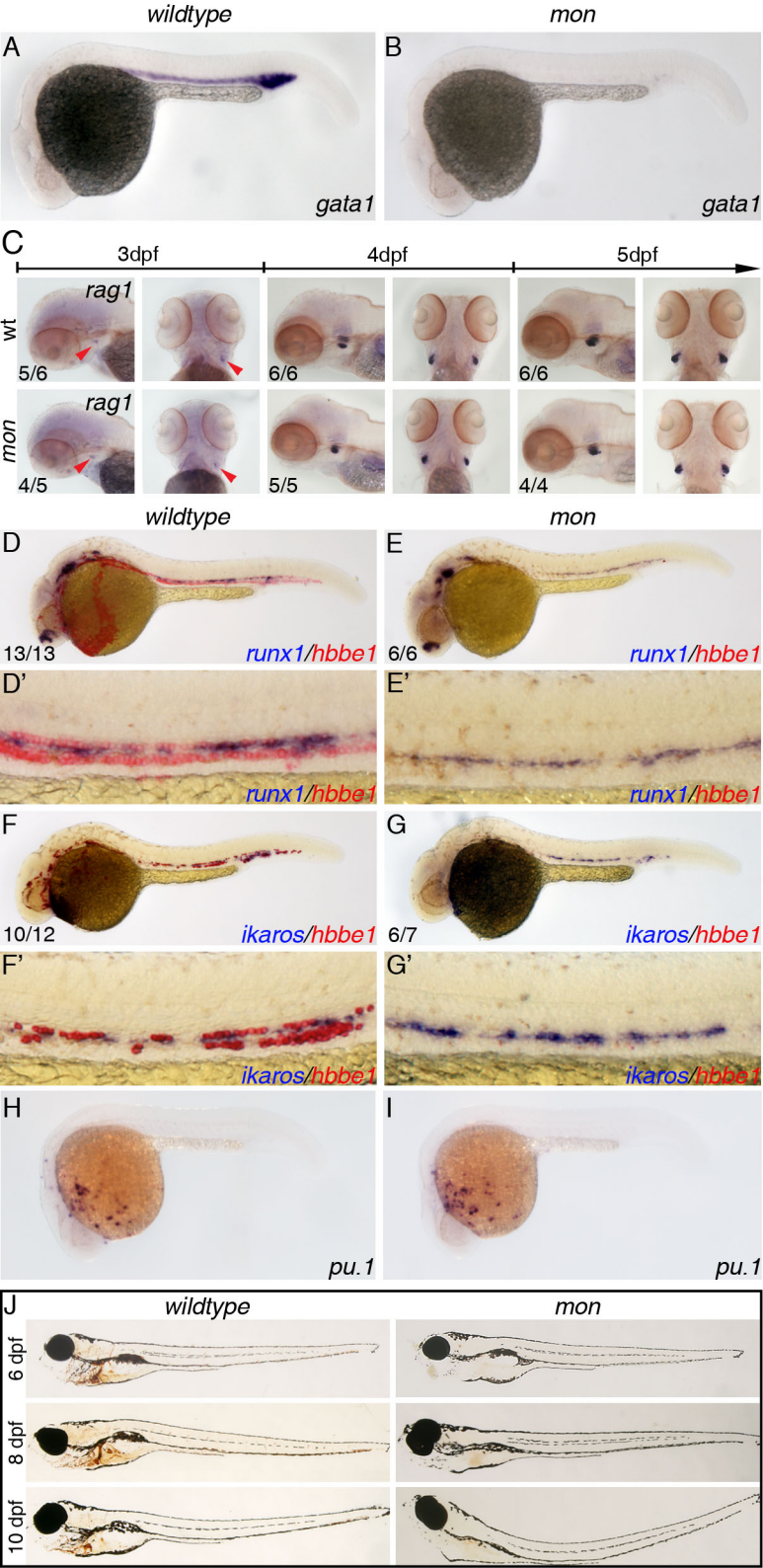

wildtype
mon
A
gata1
B
gata1
C
3dpf
4dpf
5dpf
rag1
wt
5/6
6/6
6/6
rag1
mon
4/5
5/5
4/4
wildtype
mon
D
13/13
runx1/hbbe1
E
6/6
runx1/hbbe1
D'
runx1/hbbe1
E'
runx1/hbbe1
F
10/12
ikaros/hbbe1
G
6/7
ikaros/hbbe1
F'
ikaros/hbbe1
G'
ikaros/hbbe1
H
pu.1
I
pu.1
J
wildtype
mon
6 dpf
8 dpf
10 dpf

Figure S2

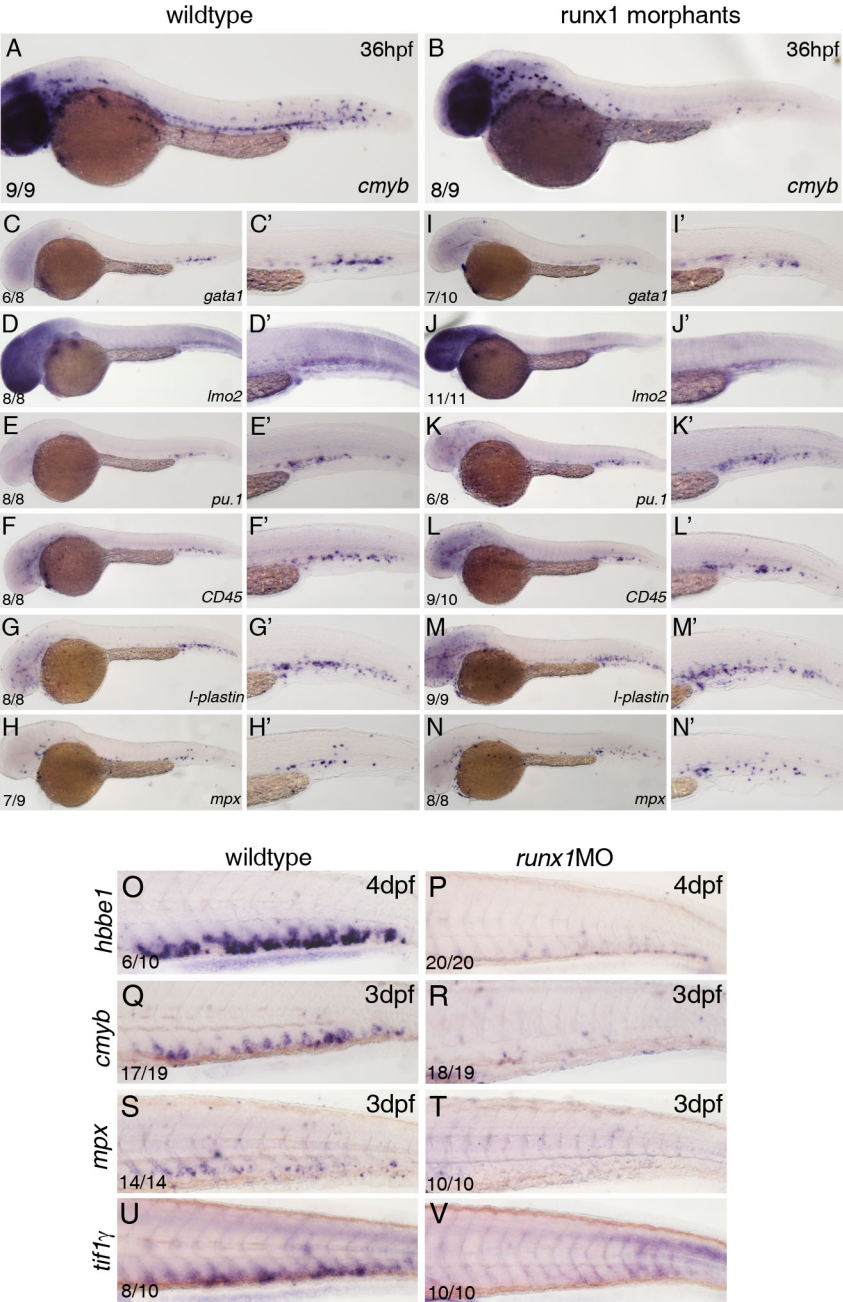

wildtype
runx1 morphants
A
36hpf
9/9
cmyb
B
36hpf
8/9
cmyb
C
6/8
gata1
C'
I
7/10
gata1
I'
D
8/8
lmo2
D'
J
11/11
lmo2
J'
E
8/8
pu.1
E'
K
6/8
pu.1
K'
F
8/8
CD45
F'
L
9/10
CD45
L'
G
8/8
l-plastin
G'
M
9/9
l-plastin
M'
H
7/9
mpx
H'
N
8/8
mpx
N'
wildtype
runx1MO
hbbe1
O
4dpf
6/10
P
4dpf
20/20
cmyb
Q
3dpf
17/19
R
3dpf
18/19
mpx
S
3dpf
14/14
T
3dpf
10/10
tif1γ
U
8/10
V
10/10

Figure S3

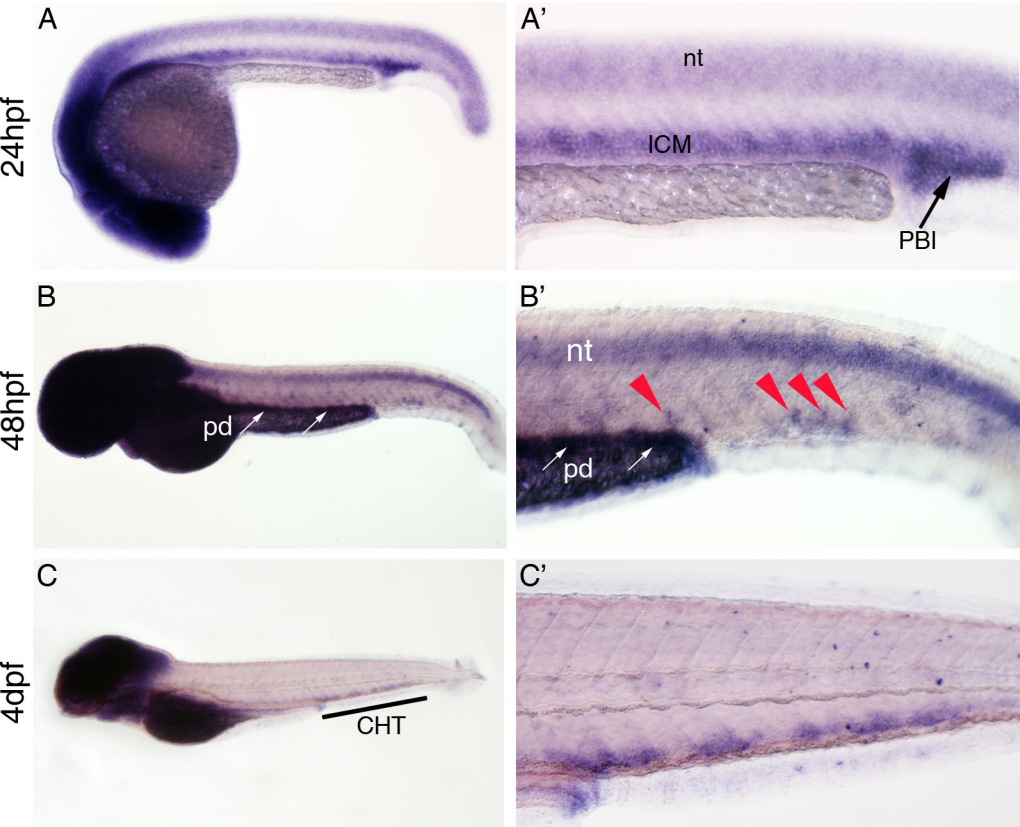


Figure S4

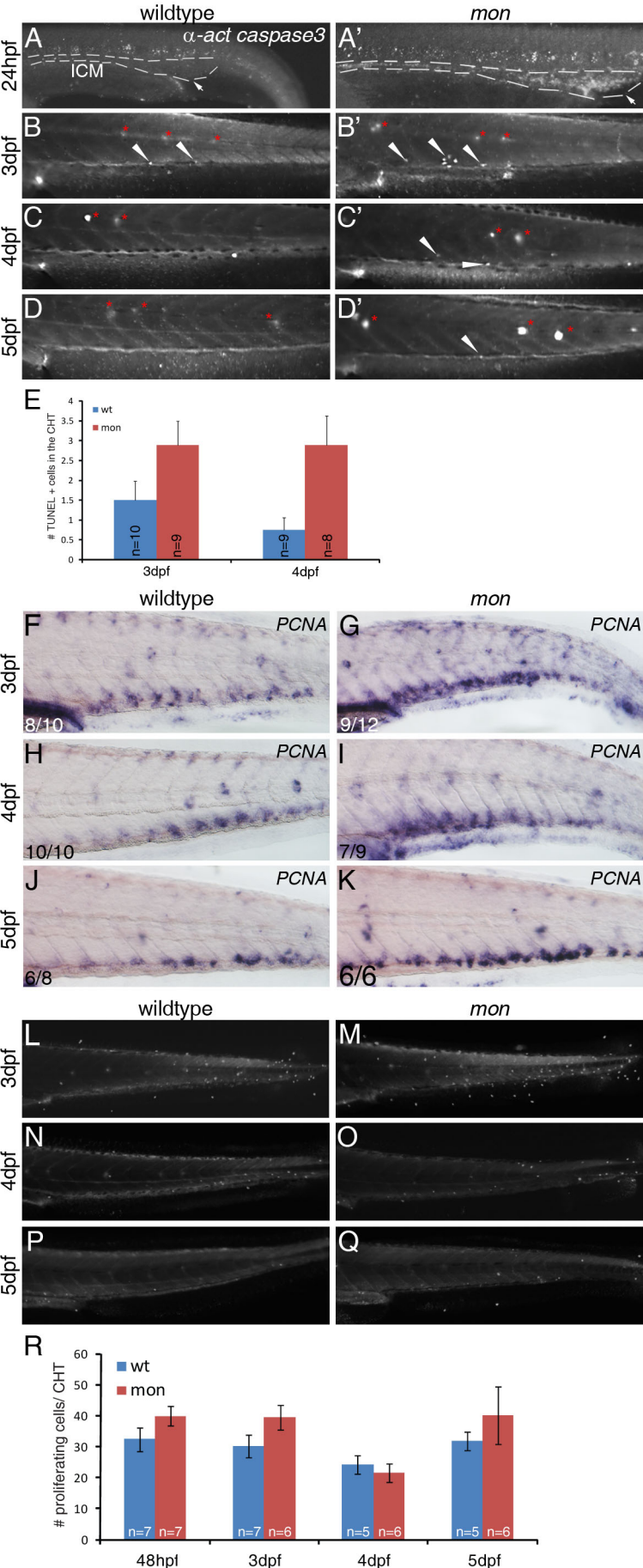

wildtype
mon
A
α-act caspase3
ICM
A'
24hpf
B
B'
3dpf
C
C'
4dpf
D
D'
5dpf
E
wt
mon
# TUNEL + cells in the CHT
n=10
n=9
n=9
n=8
3dpf
4dpf
wildtype
mon
F
PCNA
8/10
G
PCNA
9/12
H
PCNA
10/10
I
PCNA
7/9
J
PCNA
6/8
K
PCNA
6/6
wildtype
mon
L
M
N
O
P
Q
R
wt
mon
# proliferating cells/ CHT
n=7
n=7
n=7
n=6
n=5
n=6
n=5
n=6
48hpf
3dpf
4dpf
5dpf

Figure S5

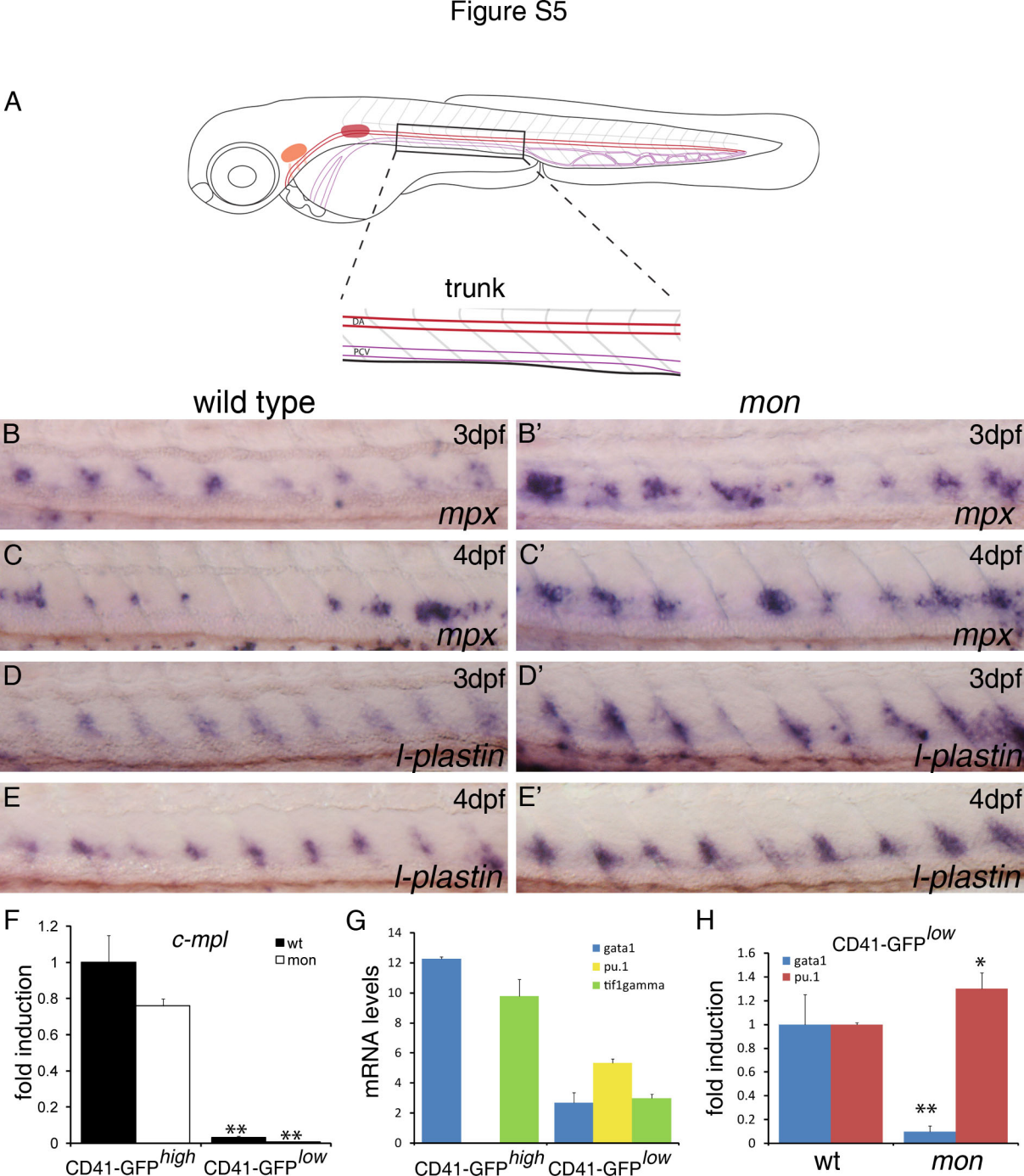
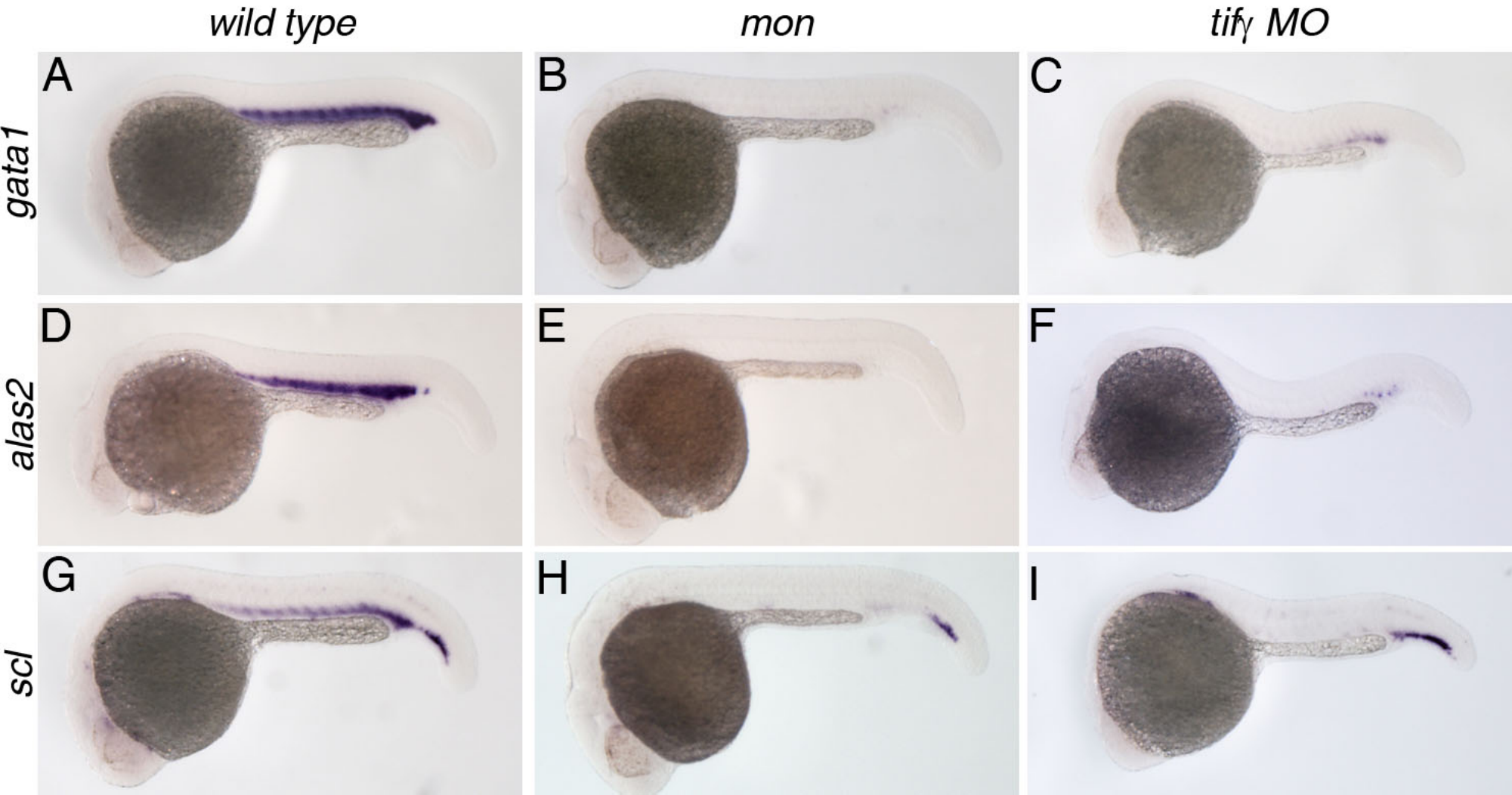

A
trunk
DA
PCV
wild type
mon
B
3dpf
mpx
B'
3dpf
mpx
C
4dpf
mpx
C'
4dpf
mpx
D
3dpf
l-plastin
D'
3dpf
l-plastin
E
4dpf
l-plastin
E'
4dpf
l-plastin
F
c-mpl
wt
mon
fold induction
1.2
1
0.8
0.6
0.4
0.2
0
**
**
CD41-GFP high
CD41-GFP low
G
mRNA levels
14
12
10
8
6
4
2
0
gata1
pu.1
tif1gamma
CD41-GFP high
CD41-GFP low
H
CD41-GFP low
gata1
pu.1
fold induction
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
**
*
wt
mon

Figure S6



Supplementary Figure Legends

Figure S1, related to Fig.1-2 – Expression of definitive HSC, primitive erythroid and myeloid markers in wild type and *mon* mutants. (A) *gata1* is expressed in the ICM and (B) is absent in the ICM in *mon* embryos at 24hpf. (C) Time course of *rag1* expression in the thymus from 3 to 5 days post fertilization (dpf). Wild type (wt) and *mon* larvae are shown. Double *in situ* hybridization for *runx1* (blue) and *hbbe1* at 30pf in wild type (D, D') and *mon* embryos (E, E'). Double *in situ* hybridization for *ikaros* (blue) and *hbbe1* at 30pf in wild type (F, F') and *mon* embryos (G, G'). We found no difference in *runx1* or *ikaros* expression between wild type and *mon*, while *hbbe1*$^{+}$ cells were clearly absent in *mon* embryos. (H) At 24hpf, anterior *pu.1* expression is equivalent between wildtype and (I) *mon* embryos. (J) o-dianisidine staining in wild type and *mon* at 6, 8 and 10dpf. Note the absence of o-dianisidine^{+} erythrocytes in *mon* as compared to wild type.

Figure S2, related to Fig.1-3 – Runx1 depletion by a *runx1*-targeted morpholino does not affect erythro-myeloid progenitor (EMP) marker expression at 36hpf. (A) *in situ* hybridization showed that *cmyb* is strongly expressed in the dorsal aorta (DA) of wild type embryos. (B) *runx1* morphants show no *cmyb* expression in the DA. *In situ* hybridization for (C, C') *gata1*, (D,D') *lmo2*, (E,E') *pu.1*, (F,F') *CD45*, (G,G') *l-plastin*, (H, H') mpx in wild type and (I,I') *gata1*, (J,J') *lmo2*, (K,K') *pu.1*, (L,L') *CD45*, (M,M') *l-plastin* and (N,N') mpx in runx1 morphants. No obvious differences were found for any of the markers analyzed. Panels with " ' " are magnifications of the tail region. Erythroid and myeloid cells present in the CHT are derived from definitive HSCs. (O) Analysis of *hbbe1* expression at 4dpf in wild type and (P) wild type injected with *runx1* MO. (Q) Analysis of *cmyb* expression at 3dpf in wild type and (R) wild type injected with *runx1* MO. (S) Analysis of *mpx* expression at 3dpf in wild type

and (T) wild type injected with *runx1* MO. (U) Analysis of *tif1γ* expression at 4dpf in wild type and (V) wild type injected with *runx1* MO. The number of embryos analysed are shown in each panel.

Figure S3, related to Fig. 2 and Fig. 5 – Analysis of *tif1γ* expression. (A, A') – Expression of *tif1γ* at 24hpf in the intermediate cell mass (ICM) and posterior blood island (PBI). (B, B') - Expression of *tif1γ* at 48hpf in the head region, the neural tube (nt) and CHT (red arrowheads). Expression was also found in the pronephric duct (pd, white arrows). (C, C') *Tif1γ* is clearly expressed in the caudal haematopoietic tissue (CHT) at 4dpf.

Figure S4, related to Fig.2-3 – Analysis of apoptosis and proliferation in the CHT of *mon* mutants. (A-D') Detection of apoptotic cells with an antibody against activated caspase 3 (*α-act caspase3*). (A) At 24hpf, no obviously apoptotic cells are present in the ICM (outlined). (A') By contrast, most of the cells in the ICM are apoptotic in *mon* embryos. White arrow points to the cloaca. (B-D) From 3 to 5 dpf, very few apoptotic cells were found in the CHT of wild type embryos (white arrows). (B'-D') Some apoptotic cells were present in *mon* embryos at 3 and 4dpf (white arrows). Red asterisks mark *act caspase3* staining on the lateral line (in wild type and mutants). (E) Quantification of apoptotic cells by TUNEL assay at 3 and 4dpf in wild type and *mon*. At 3 dpf, *mon* mutants showed an average of 3 apoptotic cells (n=9) as compared to 1.5 cells (n=10) in wild type embryos. At 4 dpf, 2.5 apoptotic cells (n=8) were found in *mon* as compared to 1 cell (n=9) in wild type embryos. (F-K) Proliferation analysis by *in situ* hybridization to proliferating cell nuclear antigen (PCNA). *mon* express somewhat higher levels of PCNA in the CHT from 3 to 5 dpf (G,I,K) when compared to wild type larvae (F,H,J). (L-Q) Analysis of proliferation in the CHT from 3-5dpf by immunochemistry against phosphorylated Histone H3 (pH3). (R) Quantification of the number of pH3+ cells in the CHT

from 48hpf to 5dpf. Average cell numbers ± s.d. are shown. The number of embryos analysed (*n*) is shown in the histogram columns (E, R) or on each panel (F-K).

Figure S5, related to Fig.3 and Fig. 5 – Expression of myeloid markers is increased in the trunk of *mon* as compared to wild type larvae. (A) Schematic representation of a zebrafish larvae at 3dpf, showing a magnification of the trunk region. *mpx* is expressed in the trunk region at 3 (B) and 4dpf (C). Increased expression of *mpx* in the trunk in *mon* at 3 (B') and 4dpf (C'). *L-plastin* is expressed in the trunk region at 3 (D) and 4dpf (E). Increased expression of *l-plastin* in the trunk in *mon* at 3 (D') and 4dpf (E'). (F) Quantitative RT-PCR analysis of *c-mpl* expression in CD41-gfp^{high} and CD41-gfp^{low} cell populations in wildtype or *mon* mutants at 3dpf. *c-mpl* was highly enriched in the CD41-gfp^{high} population. Expression of *c-mpl* in the CD41-gfp^{high} population was set to 1 for comparison. (G) Expression analysis of *gata1*, *pu.1* and *tif1*γ in CD41-gfp^{high} and CD41-gfp^{low} wildtype cells in the CHT at 3dpf. *pu.1* mRNA was absent, whereas *gata1* and *tif1*γ were expressed at similar levels in CD41-gfp^{high}; all three genes were expressed at lower levels in CD41-gfp^{low} (HSCs) cells . Absolute quantification is shown. (H) Expression analysis of *gata1* and *pu.1* in wildtype and *mon* CD41-gfp^{low} cells in the CHT at 3dpf. Both genes are were downregulated in this cell population, albeit the effect on *gata1* expression was more prominent than that of *pu.1*. Expression of each gene in wt CD41-gfp^{low} cells was set to 1 for comparison. All data are shown as average ± standard deviations. **- $P<0.0007$; *- $P<0.02$.

Figure S6, related to Fig. 6– *tif1*γ MO knockdown phenocopies the gene expression defects of *mon* mutants. (A) Expression of *gata1* in wild type, (B) *mon* mutants and (C) *tif1*γ morphants. (D) Expression of *alas2* in wild type, (E) *mon* mutants and (F) *tif1*γ morphants. (G) Expression of *scl* in wild type, (H) *mon* mutants and (I) *tif1*γ morphants.