Supplemental Materials

Supplemental Inventory

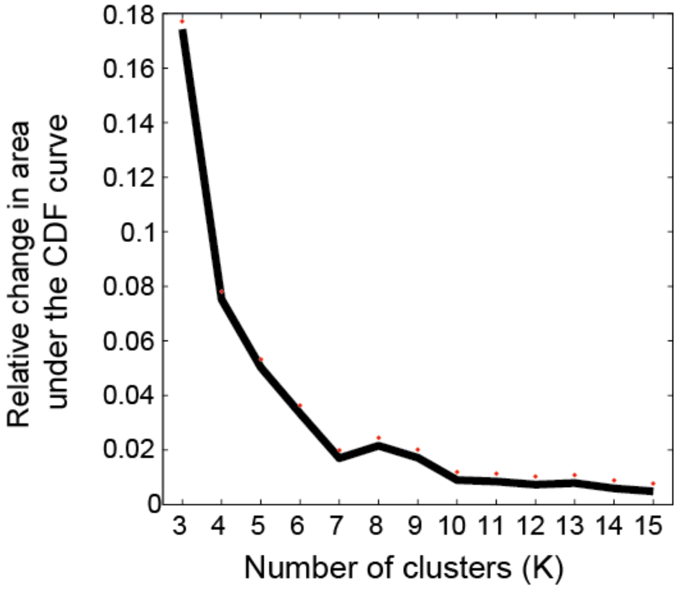

Figure S1. Delta area plot of consensus clustering. Related to Figure 2.

Change in area under the Consensus Cumulative Distribution function (CDF) comparing k and k-1 clusters. CDF shows the cumulative distribution functions of the consensus matrix for each k, estimated by a histogram of 100 bins.

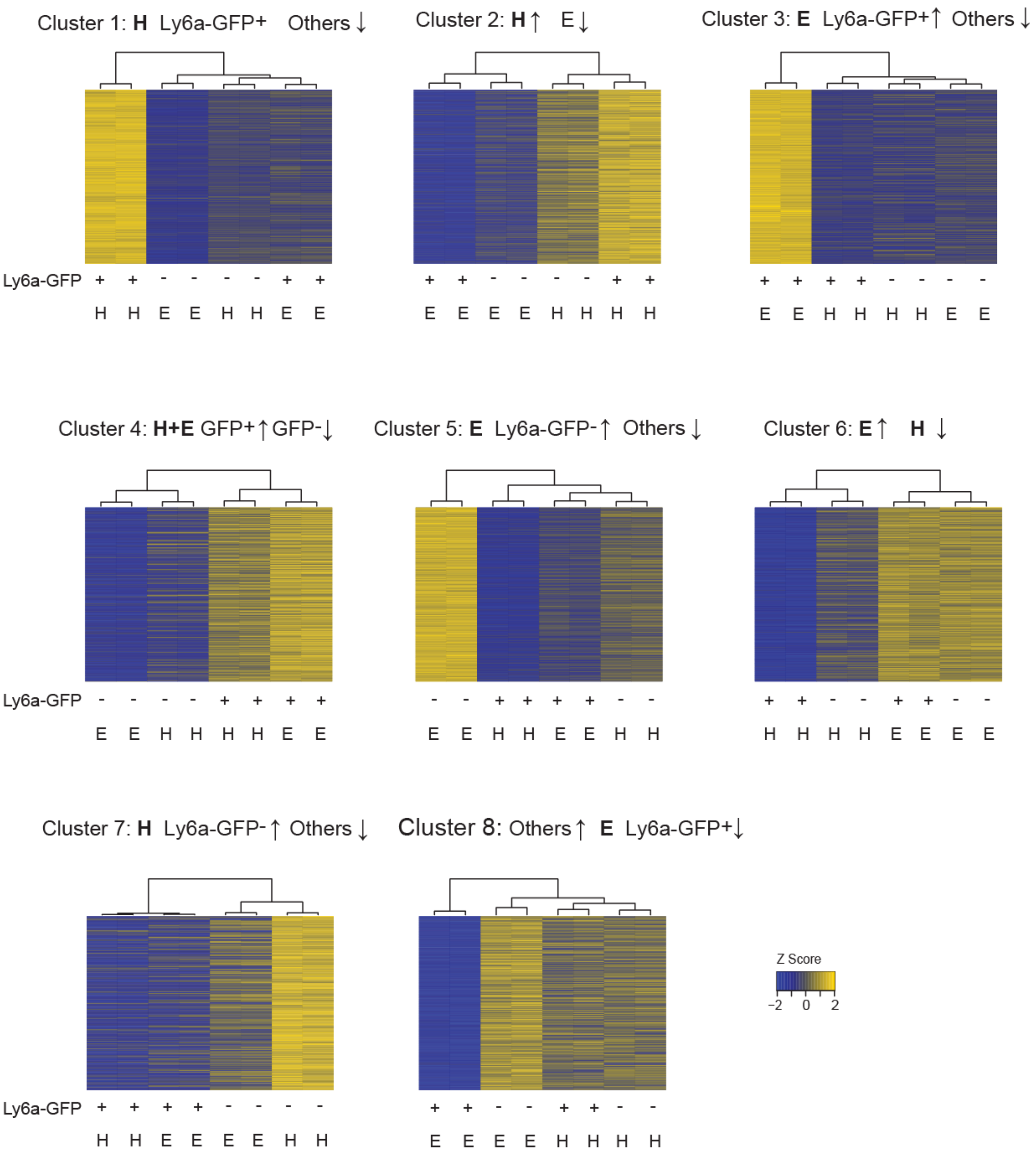

Figure S2. Heatmap of the eight gene clusters resulting from consensus clustering. Shown are Z-score normalized gene expression values. H, hematopoietic cluster; E, endothelium. Related to Figure 2.

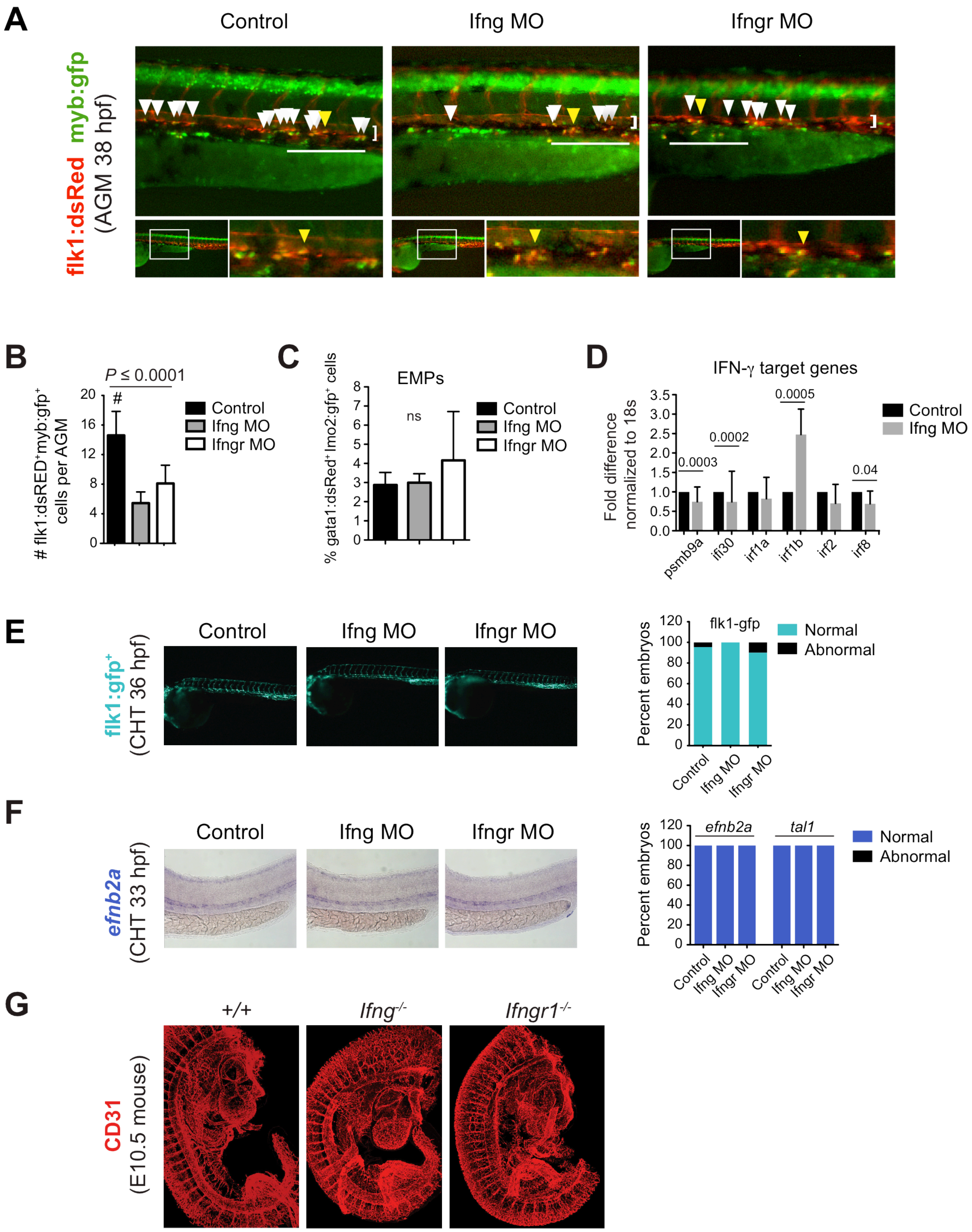

Figure S3. Controls for zebrafish IFN morphants and IFN deficient mouse embryos. Related to Figure 5.

A. Representative images showing the effect of MOs targeting Ifng and Ifngr on numbers of flk1:dsRed$^+$myb:gfp$^+$ cells (white arrowheads) in the AGM at 38 hpf (n≥9/ condition). Picture on top is magnified from the boxed area in the in lower left picture. Area marked by the line in the top picture is amplified in the lower right picture. The yellow arrows point to the same cell in the upper and lower right panels. A bracket marks the aorta.

B. Absolute counts of flk1:dsRed$^+$myb:gfp$^+$ cells from embryos in panel A. *P* value determined by ANOVA and Dunnett's test as described in methods with Control as the comparator (Error bars = SD).

C. Percentage of gata1:dsRed$^+$lmo2:gfp$^+$ EMPs determined by FACS in 30hpf zebrafish embryos. Error bars represent mean ± SD, n=5-8 embryos/ condition, ns = not significant.

D. qPCR for IFN target genes from Ifng morphant zebrafish embryos versus controls normalized to *18s* rRNA (n=2 replicates X 4 experiments, mean ± SD).

E. Representative effects of Ifng and Ifngr knockdown on flk1:gfp$^+$ cells at 36hpf (left) and phenotypic distribution of the effects in flk1:gfp morphant embryos (right) (n ≥10 embryos/ condition x 2 experiments).

F. Representative effects of Ifng and Ifngr knockdown on *efnb2a* at 33hpf (left) and phenotypic distribution of the effects on *efnb2a* and *tal1* (representative data not shown) in morphant embryos (right). n ≥10 embryos/ condition x 2 experiments.

G. Whole mount immunohistochemistry/confocal microscopy of E10.5 mouse embryos stained with an antibody to CD31 to examine the vasculature. The head and body walls are removed to facilitate imaging.

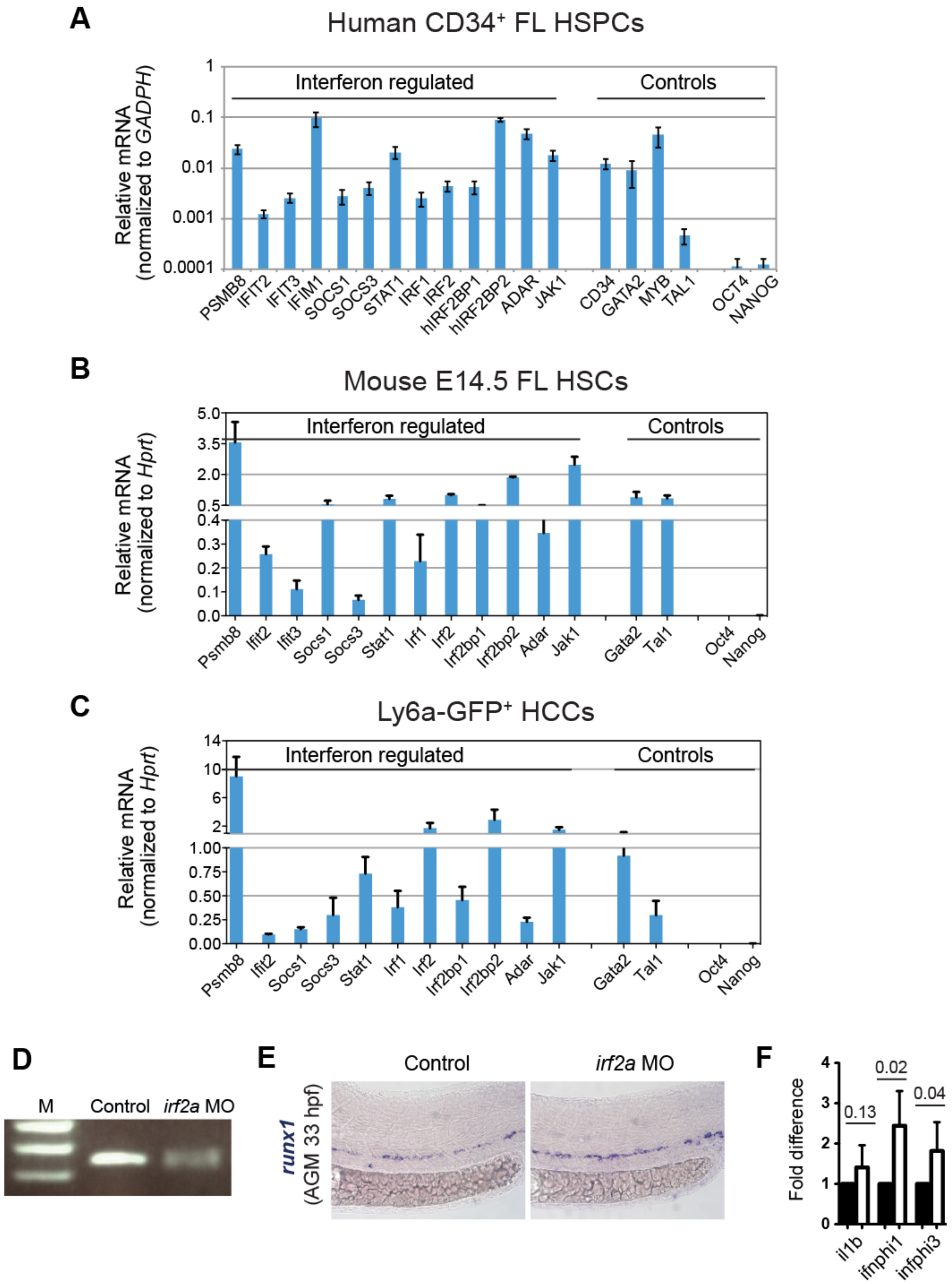
A
Human CD34⁺ FL HSPCs
Relative mRNA (normalized to GADPH)
1
0.1
0.01
0.001
0.0001
Interferon regulated
Controls
PSMB8
IFIT2
IFIT3
IFIM1
SOCS1
SOCS3
STAT1
IRF1
IRF2
hIRF2BP1
hIRF2BP2
ADAR
JAK1
CD34
GATA2
MYB
TAL1
OCT4
NANOG
B
Mouse E14.5 FL HSCs
Relative mRNA (normalized to Hprt)
5.0
3.5
2.0
0.5
0.4
0.3
0.2
0.1
0.0
Interferon regulated
Controls
Psmb8
Ifit2
Ifit3
Socs1
Socs3
Stat1
Irf1
Irf2
Irf2bp1
Irf2bp2
Adar
Jak1
Gata2
Tal1
Oct4
Nanog
C
Ly6a-GFP⁺ HCCs
Relative mRNA (normalized to Hprt)
14
10
6
2
1.00
0.75
0.50
0.25
0.00
Interferon regulated
Controls
Psmb8
Ifit2
Socs1
Socs3
Stat1
Irf1
Irf2
Irf2bp1
Irf2bp2
Adar
Jak1
Gata2
Tal1
Oct4
Nanog
D
M
Control
irf2a MO
E
Control
irf2a MO
runx1 (AGM 33 hpf)
F
Fold difference
4
3
2
1
0
0.13
0.02
0.04
il1b
ifnphi1
infphi3

Figure S4. Expression of IRF2 target genes by qPCR. Related to Figure 6.

A. Expression in hCD34$^+$ FL HSPCs, normalized to *GADPH*. Controls include genes expressed in HSPCs (*CD34, GATA2, MYB, TAL1*), as well as genes specifically expressed in embryonic stem cells (*OCT4, NANOG*) (mean ± SD, n = 3).

B. Expression of IRF2 target genes in phenotypic long term repopulating HSCs (LT-HSCs, CD150$^+$ CD48$^-$ LSK) from E14.5 mouse FL (mean ± SD, n = 3).

C. Expression of IRF2 target genes in Ly6a-GFP$^+$ hematopoietic cluster cells from the E10.5 mouse A+U+V (mean ± SD, n = 3).

D. Representative image of *irf2a* MO validation by RT-PCR indicates strong reduction in expression compared to sibling controls (20 pooled embryos/ condition).

E. WISH for *runx1* in the AGM region of representative embryos injected with MO-1 targeting *irf2a*.

F. qPCR for inflammatory cytokines in *irf2a* morphant embryos normalized to 18s (italics).

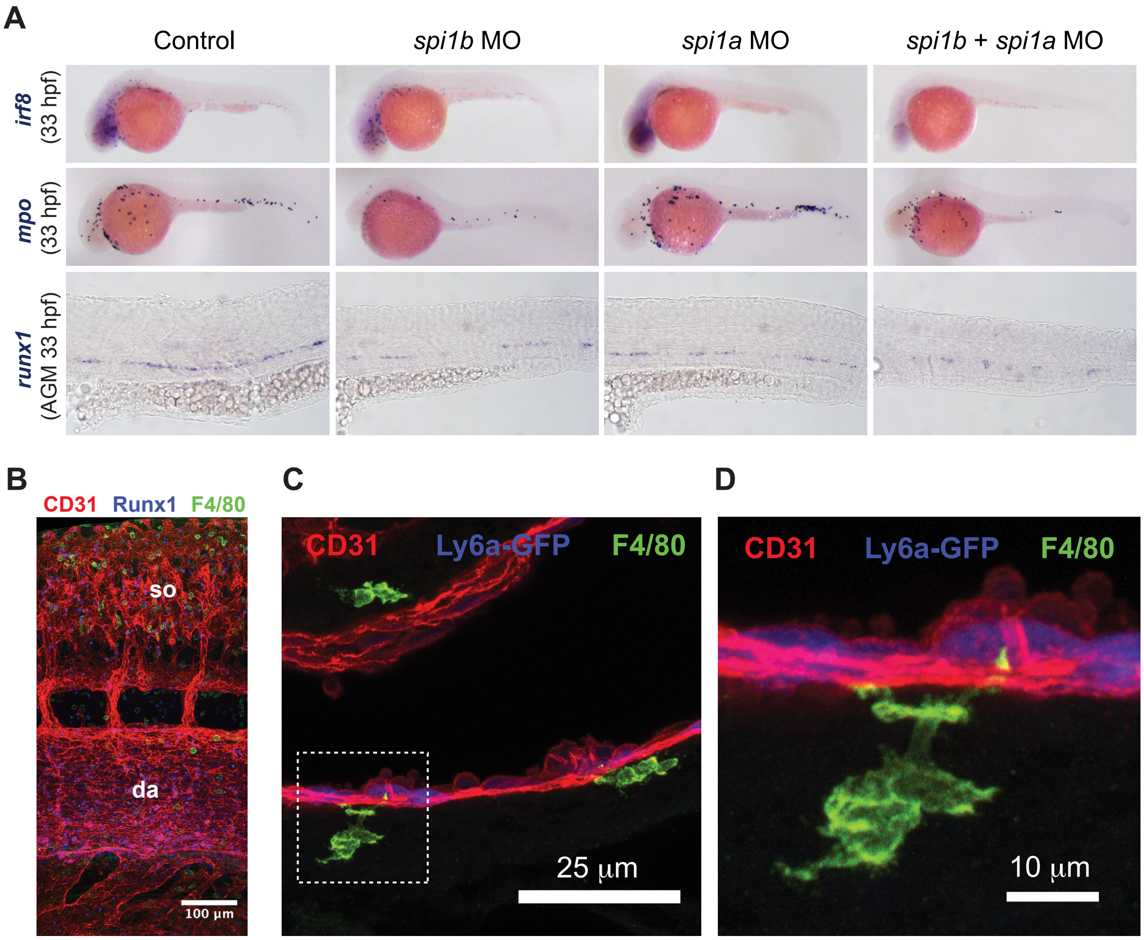

Figure S5. Primitive myeloid cells regulate HSPCs in the AGM region. Related to Figure 7.

A. Representative WISH for *irf8* (marks macrophages), *mpo* (marks granulocytes) in whole zebrafish embryos, and *runx1* in the AGM region following MO knockdown of *spi1a* and *spi1b* alone and in combination.

B. Whole mount immunohistochemistry/confocal microscopy of the mouse AGM region. da, dorsal aorta; so, somite. Image represents a 245 µm section. CD31 labels endothelial and hematopoietic cells, Runx1 labels hematopoietic cells and hemogenic endothelium, F4/80 labels macrophages.

C. Umbilical artery with two F4/80$^+$ macrophages that have inserted processes between Ly6a-GFP$^+$ endothelial cells. Boxed area on left is magnified in panel D. Image is a Z-projection of 15 z-slices with a depth of 22.5 μm.

D. High magnification view of macrophage boxed in panel C.

Table S1. Frequency of progenitors with lymphoid potential in sorted populations from the A+U+V. Related to Figure 1.

Embryonic Age	**HCC Population**[1]	**No. cells/embryo**[2]	**Lymphoid progenitor frequency**[3]	
			B cell	**T cell**
E10.5	Ly6a-GFP$^+$	184	1 in 7	1 in 4
E10.5	Ly6a-GFP$^-$	744	1 in 42	1 in 40
E10.5	Ly6a-GFP$^+$	269	1 in 4	1 in 6
E10.5	Ly6a-GFP$^-$	947	1 in 63	1 in 48
E10.5	Ly6a-GFP$^+$	177	1 in 3	1 in 6
E10.5	Ly6a-GFP$^-$	1096	1 in 41	1 in 36
Average E10.5	Ly6a-GFP$^+$	**210**	**1 in 4.6**	**1 in 5.3**
Average E10.5	Ly6a-GFP$^-$	**929**	**1 in 48.7**	**1 in 41.3**
E11.5	Ly6a-GFP$^+$	340	1 in 10	1 in 19
E11.5	Ly6a-GFP$^-$	1060	0	1 in 716
E11.5	Ly6a-GFP$^+$	310	1 in 13	1 in 16
E11.5	Ly6a-GFP$^-$	1122	0	0
E11.5	Ly6a-GFP$^+$	450	1 in 15	1 in 19
E11.5	Ly6a-GFP$^-$	1354	0	0
E11.5	Ly6a-GFP$^+$	522	1 in 7	1 in 30
E11.5	Ly6a-GFP$^-$	1520	1 in 2260	1 in 2260
Average E11.5	Ly6a-GFP$^+$	**406**	**1 in 10.4**	**1 in 19.9**
Average E11.5	Ly6a-GFP$^-$	**1264**	**1 in 9040**	**1 in 2175**

[1] Data are from three independent experiments for E10.5 embryos and 4 experiments for E11.5 embryos. HCC, hematopoietic cluster cell (CD31$^+$ VEC$^+$ ESAM$^+$ Kit$^+$). No progenitors were found in the CD31$^+$ VEC$^+$ ESAM$^+$ Kit$^-$ endothelial populations (not shown).

[2] Total number of cells with the indicated markers per embryo determined by cell count from sorter.

[3] The frequency of progenitors within each of the sorted populations. B cells were scored as CD19$^+$ B220$^+$, T cells as Th1.1$^+$ CD25$^+$.

Table S2. Highly enriched Gene Ontology terms for each cluster. Related to Figure 2.

Table S3. The genes and their expression in each cluster after consensus clustering. Related to Figure 2.

Table S4. Gene Ontology terms for IRF2 bound genes determined by GREAT. Related to Figure 6.

Supplemental material and methods

Table S5- Morpholino sequences

Morpholino name	Sequence	Citation
IRF8-MO1	AATGTTTCGCTTACTTTGAAAATGG	(Li et al. 2011))
pu.1-MO1 (currently named *spi1b*)	GATATACTGATACTCCATTGGTGGT	(Bukrinsky et al. 2009)
spi1l (currently named *spi1a*)	AGCGACTCACGCTGTGGAGGAACT	(Bukrinsky et al. 2009)
MO1-ifng1-1	TTTCTGTGCTGTGAACCAAGTGATG	(Sieger et al. 2009)
MO1-ifng1-2	TGAAGGCGTTCGCTAAAGTTAGAGT	(Sieger et al. 2009)
IRF2a_e1i1	GTACAGATCTCTCTCCTCACCTCAT	
IRF2a_ATG	CATTCGCATTCTGTCCACTGGCATC	
MO1-il1b	CCCACAAACTGCAAAATATCAGCTT	(Lopez-Munoz et al. 2011)
MO3-tnfa	GGCAGGATTTTCACCTTATGGAGCG	(Qi et al. 2010)
crfb6_ATG	GTCATTTCGACAAATATAAATCCAC	(Aggad et al. 2010)
crfb13_ATG	CAAACCAGGATCTCCAGACATTTTG	(Aggad et al. 2010)
crfb17_ATG	GATGAGACAGATCAATATCCGCATT	(Aggad et al. 2010)
crfb5_i1e2	GGATGCTGGAACACACACACACACA	(Levraud et al. 2007)

Table S6 - Human qPCR primers

Primer target	Primer Sequence	Citation
PSMB8-F	GCAGGCTGTACTATCTGCGAA	
PSMB8-R	AGAGCCGAGTCCCATGTTCAT	
IFIT2-F	GACACGGTTAAAGTGTGGAGG	
IFIT2-R	TCCAGACGGTAGCTTGCTATT	
IFIT3-F	AGAAAAGGTGACCTAGACAAAGC	
IFIT3-R	CCTTGTAGCAGCACCCAATCT	
SOCS1-F	TTTTCGCCCTTAGCGTGAAGA	
SOCS1-R	GAGGCAGTCGAAGCTCTCG	
SOCS3-F	CCTGCGCCTCAAGACCTTC	
SOCS3-R	GTCACTGCGCTCCAGTAGAA	
STAT1-F	CGGCTGAATTTCGGCACCT	
STAT1-R	CAGTAACGATGAGAGGACCCT	
IRF1-F	CTGTGCGAGTGTACCGGATG	
IRF1-R	GGCTGGACTTCGACTTTCTTTC	
IRF2-F	AAAACATGGAAGGCGAATTTCAG	
IRF2-R	CCAGAGATGACTCAACTGGTTC	
IRF2BP1-F	GCGGACTGTCTGGCAGAAC	
IRF2BP1-R	GAAGCGCACATTGAACGGG	
IRF2BP2-F	ACACCCATTTTGTGCAGTGC	
IRF2BP2-R	ACTGGGACAATAGACCTCTCC	
ADAR-F	ATCAGCGGGCTGTTAGAATATG	
ADAR-R	AAACTCTCGGCCATTGATGAC	

JAK1-F	CTTTGCCCTGTATGACGAGAAC	
JAK1-R	ACCTCATCCGGTAGTGGAGC	
CD34-F	CTACAACACCTAGTACCCTTGGA	
CD34-R	GGTGAACACTGTGCTGATTACA	
GATA2-F	GCAACCCCTACTATGCCAACC	
GATA2-R	CAGTGGCGTCTTGGAGAAG	
MYB-F	AACCACACATGCAGCTACCC	
MYB-R	GGTGTTCTCCCAAACAGGAA	
TAL1-F	GTGCAGCTGAGTCCTCCCGC	
TAL1-R	CCGGCTCCCCAAAGAACCCG	
OCT4-F	CTTGAATCCCGAATGGAAAGGG	
OCT4-R	GTGTATATCCCAGGGTGATCCTC	
NANOG-F	TTTGTGGGCCTGAAGAAAACT	
NANOG-R	AGGGCTGTCCTGAATAAGCAG	
GAPDH-F	ACCCAGAAGACTGTGGATGG	
GAPDH-R	TTCAGCTCAGGGATGACCTT	

Table S7- Mouse qPCR primers

Primer target	**Primer Sequence**	**Citation**
Tlr2-F	CATCACCGGTCAGAAAACAA	
Tlr2-R	GCCACCAAGATCCAGAAGAG	
Psd4-F	CTTGGCCTTAGGATCCACTG	
Psd4_R	AAAGGGTGCTTTAGGTGAGC	
Fcrls-F	GATGAGGATGCTGGAGCTGT	
Fcrls-R	GAGGCCCAGTGCAGAAAGTA	
Tabpb-F	CTCAAAGGAGAAGGCCACAG	
Tabpb-R	ATGAGGGTGGCTTCCACA	
Socs3-F	TTTCGCTTCGGGACTAGC	
Socs3-R	GAGCCAGCGTGGATCTG	
Isg20-F	CCACGCCATTTGGTGAA	
Isg20-R	TCCTCCTTCAGGGCATTG	
Ifit2-F	CTGCAGCCCTGCTTTGAG	
Ifit2-R	GCTGTCGCAGATTGCTCTC	
Ifit3-F	AGCCCACACCCAGCTTTT	
Ifit3-R	TGGCACTTCAGCTGTGGA	
Psmb8-F	TTGCTGCAATGCTGTCTACC	
Psmb8-R	CAGCTCCCTTTCCTTCTTCA	
Socs1-F	GCCCCTCGAGTAGGATGG	
Socs1-R	ACGAGGAGGGCTCTGACC	
Adar-F	GGGAAGCCAAAGCCAAAG	
Adar-R	CCTGAGCCTCTGCTGGTT	
Jak1-F	GCCTGCCAGACTCCTGAC	
Jak1-R	CCTTCCAAAGCCCATTCA	
Stat1-F	CACGCTGCCTATGATGTCTC	(Munakata et al. 2012)
Stat1-R	ACGCTTGCTTTTCCGTATGT	(Munakata et al. 2012)
Gata2-F	CCTCCAGCTTCACCCCTAA	
Gata2-R	CAGAGAGGGGTGGCTGTG	

Tal1-F	AGCGGAGGAGTGGAGGTC	
Tal1-R	CCCTCTAGTTGCGGGTCA	
Nanog-F	ACCTCAGCCTCCAGCAGA	
Nanog-R	CCGCTTGCACTTCATCCT	
Oct4-F	GGAGGAAGCCGACAACAA	
Oct4-R	TCCACCTCACACGGTTCTC	
Irf2bp1-F	ACCTCCAAGAGCCCCATC	
Irf2bp1-R	CTGCTCCTCCGTGGTCAT	
Irf2bp2-F	GCAGGTTGTTGGGTTTCG	
Irf2bp2-R	CTTCCGGTTCTGGGGAAG	
Il1b-F	CGGACCCATATGAGCTGAA	
Il1b-R	TGTCGTTGCTTGGTTCTCC	
Ifng-F	CAGCAAGGCGAAAAAGGA	
Ifng-R	GGTGGACCACTCGGATGA	
Tnf-F	GCCACCACGCTCTTCTGT	
Tnf-R	TGAGGGTCTGGGCCATAG	
Ifna-F	CCTGCTGGCTGTGAGGA	
Ifna-R	GGAAGACAGGGCTCTCCAG	
Ifnar1-F	CACGGTCGCTGTAGAAGTAAAG	
Ifnar1-R	TCTCCTCCTCTTCGTTGGAATA	
Irf1-F	CCAAATCCCAGGGCTGAT	
Irf1-R	CGGAACAGACAGGCATCC	
Irf2-F	CTCCGCTCTTCAGAAACTGG	
Irf2-R	GGCACATCGAAAATTTGCTT	
Hprt-F	CTCCTCAGACCGCTTTTTGC	(Cai et al. 2011)
Hprt-R	TAACCTGGTTCATCATCGCTAATC	(Cai et al. 2011)

Table S8- Zebrafish qPCR primers

Primer target	**Primer sequence**	**Citation**
cd44a-F	TGAAGAGAATGAAGAGATGGTGG	
cd44a-R	GCACTCTTACTCGGTCCGTAC	
cd44b-F	AGCGCATCAGGACACCAC	
cd44b-R	TGATCCGTTCTCTGCTGCTG	
crfb1-F	GGGAATATGAAGGCAGTGTCCT	
crfb1-R	GGCTGGTTCCTTTGTGTAGC	
crfb13-F	GTCCACGTGAACCAGTCTGT	
crfb13-R	ACCACAGAACGACGAAAACA	
crfb17-F	TGTGACGCTGCTGGTCATTA	
crfb17-R	GTTTGGGGTCAGCTGGTTCT	
crfb2-F	TGTGCACTCCGAGACTTGTT	
crfb2-R	AGTTCCTCCTCTGCCTCTCC	
crfb5-F	CTGTACTTCCTGTTGGCGCT	
crfb5-R	TCCATATGCTCGCACACACA	
crfb6-F	TGCTGCTTAAATCCGCCTCA	
crfb6-R	TTTCCCTTCTCTGCACGGAC	
ifi30-F	GTTGTGCTCGTCGCCGTGTT	(Sieger et al. 2009)

ifi30-R	GGCGGATTCAGTGCATCGGT	(Sieger et al. 2009)
ifit2-F	TGGACGCTGGTGAAGTTCAA	
ifit2-R	CTGCTGCCGTTTTCACCTTC	
ifn-phi1-F	GTGGCAAGATACGCAAAGCC	
ifn-phi1-R	ATTGACCCTTGCGTTGCTTG	
ifn-phi2-F	GACAGCTCTACAAAATGG AATTTTGG	(Aggad et al. 2010)
ifn-phi2-R	CTGCTTTTGCTACACCGGTT	(Aggad et al. 2010)
ifn-phi3-F	GATTCCGTATAGGCATCTGTCA	(Aggad et al. 2010)
ifn-phi3-R	GCGTCAATGTTCTGGAGTGT	(Aggad et al. 2010)
ifn-phi4-F	GTCAAACACAGACTTCAGCATCA	
ifn-phi4-R	CGAACTGGATCTGCTTCTCA	
ifng1.1-F	GCGCATACAGATTTCGACGG	(Sieger et al. 2009)
ifng1.1-R	TTTTTCTGTGGAGGCCCGAT	(Sieger et al. 2009)
ifng1.2-F	TACAAAGGAGAGGCTGGCAC	
ifng1.2-R	TGCCGTCTCTTGCGTTCTTT	
il1b-F	CCGCTCCACATCTCGTACTC	(Sieger et al. 2009)
il1b-R	CCATCTCCACCATCTGCGAA	(Sieger et al. 2009)
irf1a-F	TCCTAAAACGTGGAAGGCGA	
irf1a-R	TGTTTGTGCTGCAGGTGAAG	
irf1b-F	ACACCATCGACAGCACTCAG	(Lopez-Munoz et al. 2011)
irf1b-R	CTAAGATGGCTTCGCTGTCC	(Lopez-Munoz et al. 2011)
irf2-F	CTGAGAGCGAAACGGACAGC	
irf2-R	GAAGAGGCTCCAGGATGTCC	
irf2a-F	GCCCAGGTATCGACAAACCA	
irf2a-R	CTGCTCCGGGTCCATCTTTT	
irf3-F	AGCCAAACACCCAGAGAACT	
irf3-R	AGCCAAACACCCAGAGAACT	
irf7-F	TCCCCTCTCAGCTCAGATGG	
irf7-R	TCCCCTCTCAGCTCAGATGG	
irf8-F	GAGCCCACTGTCACACTCTG	
irf8-R	ACAAGCCATCTGATCACTGACT	
irf9-F	CGCCAACCCAGCTGTTTAAC	
irf9-R	TGACCTCTTCTATCTGCGCC	
isg15-F	CGGTGATGCTACCGTTGGAA	
isg15-R	TGGCCCTTCTCGTTCTTGAC	
isg20-f	CTCTCTGAAGAAGCTGGCACA	
isg20-R	TTACAGGCTGCAGTCCATCA	
psmb9a-F	GACTCAGAGCAGGCAGGTTT	
psmb9a-R	ACACGATTCACCACTGACTCC	
socs3a-F	CCCCAGCAGCTCAAAGACTT	
socs3a-R	GCAAGAATGGCGCTTCAACA	
socs3b-F	TAGACGTGTCCACCAAACGG	
socs3b-R	TTGAGTGGGAGAACGCTGTC	
stat1a-F	AGAAGGCAGACGAGGGATAC	
stat1a-R	AAAACCCGTTCGAGTCACTC	
stat1b-F	GCTGCTCCAATGAAGGTTCC	

stat1b-R	GCAACGGGTCTTGCAACAG	
stat2-F	ATGTCAAAGAGGGGCCAGTG	
stat2-R	GCTTTCTGGTGGTGGTGAGA	
tnfa-F	AAGGAGAGTTGCCTTTACCGC	(Sieger et al. 2009)
tnfa-R	CGTGCAGATTGAGCGGATTG	(Sieger et al. 2009)

Supplemental References

Aggad D, Stein C, Sieger D, Mazel M, Boudinot P, Herbomel P, Levraud JP, Lutfalla G, Leptin M. 2010. In vivo analysis of Ifn-gamma1 and Ifn-gamma2 signaling in zebrafish. *J Immunol* **185**: 6774-6782.

Bukrinsky A, Griffin KJ, Zhao Y, Lin S, Banerjee U. 2009. Essential role of spi-1-like (spi-1l) in zebrafish myeloid cell differentiation. *Blood* **113**: 2038-2046.

Cai X, Gaudet JJ, Mangan JK, Chen MJ, De Obaldia ME, Oo Z, Ernst P, Speck NA. 2011. Runx1 loss minimally impacts long-term hematopoietic stem cells. *PLoS One* **in press**.

Levraud JP, Boudinot P, Colin I, Benmansour A, Peyrieras N, Herbomel P, Lutfalla G. 2007. Identification of the zebrafish IFN receptor: implications for the origin of the vertebrate IFN system. *J Immunol* **178**: 4385-4394.

Li L, Jin H, Xu J, Shi Y, Wen Z. 2011. Irf8 regulates macrophage versus neutrophil fate during zebrafish primitive myelopoiesis. *Blood* **117**: 1359-1369.

Lopez-Munoz A, Sepulcre MP, Roca FJ, Figueras A, Meseguer J, Mulero V. 2011. Evolutionary conserved pro-inflammatory and antigen presentation functions of zebrafish IFNgamma revealed by transcriptomic and functional analysis. *Molecular immunology* **48**: 1073-1083.

Munakata K, Takashima K, Nishiyama M, Asano N, Mase A, Hioki K, Ohnishi Y, Yamamoto M, Watanabe K. 2012. Microarray analysis on germfree mice elucidates the primary target of a traditional Japanese medicine juzentaihoto: acceleration of IFN-alpha response via affecting the ISGF3-IRF7 signaling cascade. *BMC genomics* **13**: 30.

Qi F, Song J, Yang H, Gao W, Liu NA, Zhang B, Lin S. 2010. Mmp23b promotes liver development and hepatocyte proliferation through the tumor necrosis factor pathway in zebrafish. *Hepatology* **52**: 2158-2166.

Sieger D, Stein C, Neifer D, van der Sar AM, Leptin M. 2009. The role of gamma interferon in innate immunity in the zebrafish embryo. *Dis Model Mech* **2**: 571-581.