Supporting Information

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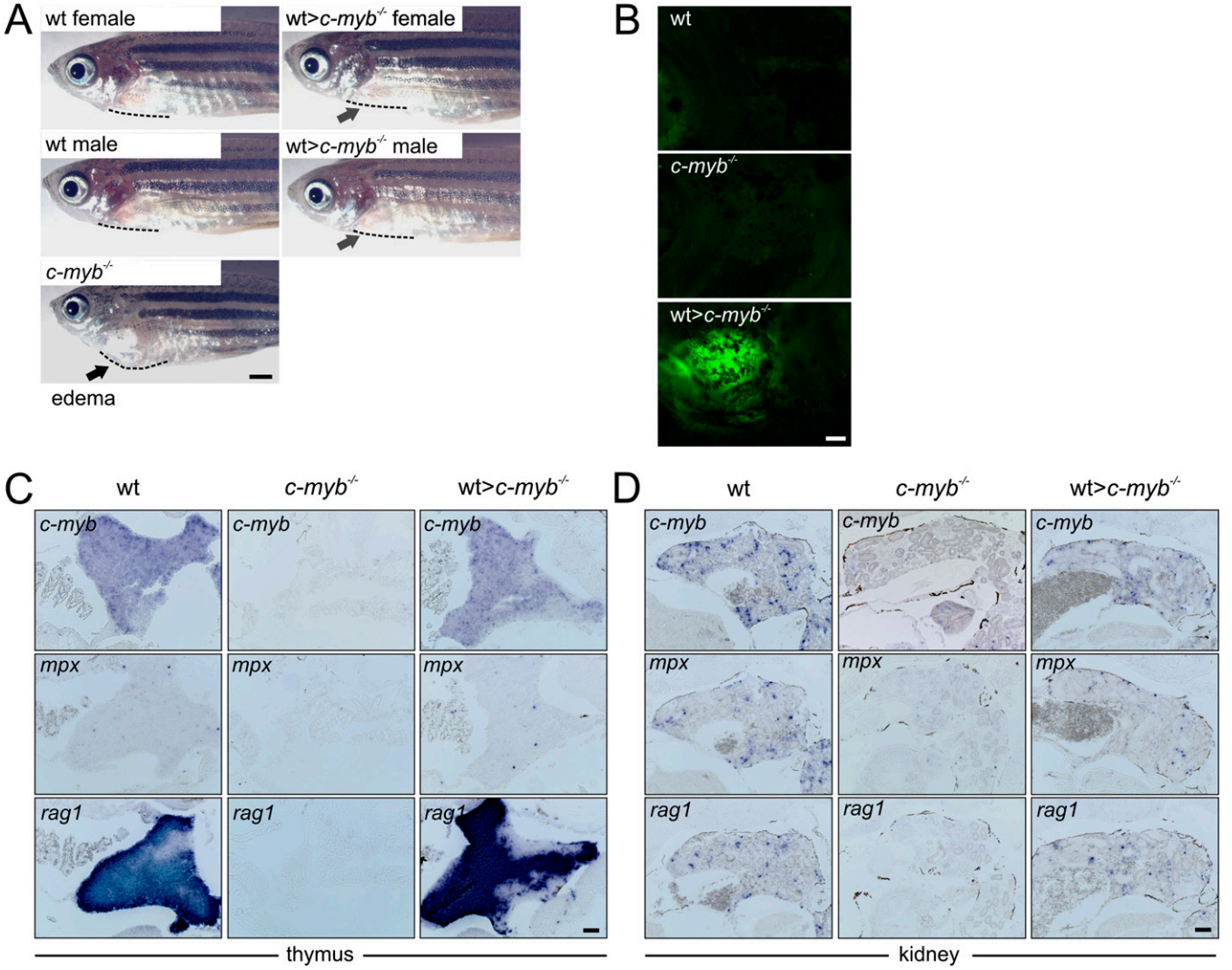

Fig. S1. Characterization of reconstituted *c-myb*$^{-/-}$ mutants. (*A*) Side views of wild-type, *c-myb*$^{-/-}$ transplant recipients and a *c-myb*$^{-/-}$ mutant fish. Note the absence of cardiac edema (resulting in a bulged body curvature) in the transplanted fish; the body curvature is highlighted by dotted lines. (Scale bar, 1 mm.) (*B*) Reconstitution of T-cell development in the thymus of *c-myb*$^{-/-}$ mutant recipients transplanted with *ikaros:eGFP*-transgenic wild-type whole kidney marrow (WKM) cells; note the presence of green fluorescent cells in the transplanted fish. Photographs (side views) were taken 20 d after transplantation. (Scale bar, 50 μm.) (*C* and *D*) Whole-mount RNA in situ hybridization with *c-myb*-, *mpx*-, and *rag*-specific probes on thymus (*C*) and kidney (*D*) sections of wild-type, *c-myb*$^{-/-}$ mutant and *c-myb*$^{-/-}$ transplant recipients (3 wk after transplantation). In mutant tissues, no positive cells were observed for *c-myb*, *mpx*, and *rag1*, while expression patterns in transplanted *c-myb*$^{-/-}$ recipients are indistinguishable from those of their wild-type siblings. *c-myb* is a general marker for hematopoietic cells; *mpx* is a marker for myeloid cells; *rag1* is a marker for developing lymphocytes. (Scale bar, 50 μm.)

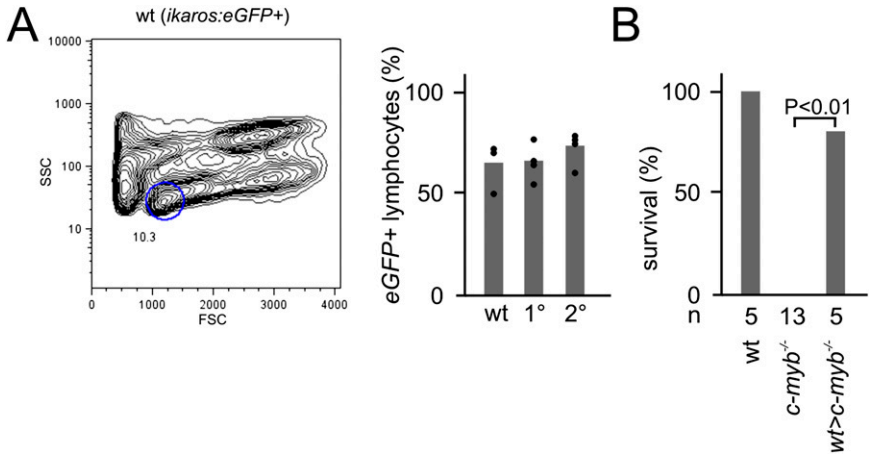

Fig. S2. Lack of host contributions to hematopoietic reconstitution after transplantation. (*A*, *Left*) Flow cytometric profile of WKM of wild-type *ikaros:eGFP* transgenic fish, with the position of the lymphocyte gate indicated (blue circle). FSC, forward light scatter; SSC, side light scatter. (*A*, *Right*) Percentage of green-fluorescent cells in the lymphocyte gate; each dot represents one fish, and the bar denotes average values. wt, wild-type; 1°, recipients of primary transplants, 2°, recipients of secondary transplants. (*B*) Survival rate of fish of the indicated genotype that were fin-clipped at the age of 8 wk.

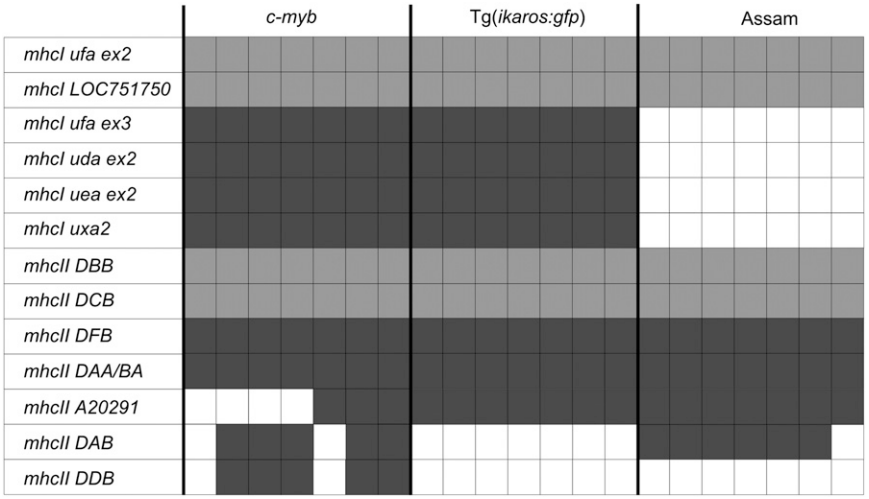

Fig. S3. Allelic diversity of *mhc* genes in zebrafish. The diagram shows the outcome of PCR experiments for several *mhcI* and *mhcII* genes using the primer pairs listed in Table S1; seven embryos each from an in-cross of *c-myb*$^{+/-}$ heterozygous fish, *ikaros:eGFP*-transgenics, and Assam fish were tested. Empty boxes indicate failure of PCR, suggesting sequence diversity occurring at the primer binding site. For the PCR reactions indicated by grayish boxes, amplicons were cloned and sequenced (Fig. 4*A* and Fig. S3).

mhcI_uda
NM_131704.1:
GFYQDSYDGEDFVYLDLKEMRYISPVPQALLTLQKWNDDKAFLAQQINYLSIECIEWLQKYMQYGKSSL

c-myb recipient strain:
GFYQDSYDGEDFVYLDLKEMRYISPVPQALLTLQKWNDDKAFLAQQINYLSIECIEWLQKYMQYGKSSL
GFYQDSYDGEDFVYLDLKEMRYISPVPQALLTLQKWNDDKAFLTQQINYLSIECIEWLQKYMQYGKSSL
GFYQDSYDGEDFVYLDLKEMRYISPVPQALLTLQKWNDDKAFLAQQINYLSIECIEWLRKYMQYGKSSL
GFYQDSYDGEDFVYLDLKEMRYISPVPQALLTLRKWNDDKAFLAQQINYLSIECIEWLQKYMQYGKSSL
GFYQDSYDGEDFVYLDLKEMRYISPVPQALLTLQKWNDDKAFLAQRINYLSIECIEWLQKYMQYGKSSL
ikaros:eGfp wild-type donor strain:
GFYQDSYDGEDFVYLDLKEMRYISPVPQALLTLQKWNDDKAFLAQQINYLSIECIEWLQKYMQYGKSSL
GFYQDSYDGEDFVYLDLKEMRYISPVPQALLTLQKWNDDKAFLAQQINYLSIERIEWLQKYMQYGKSSL
Assam wild type donor strain:
GFRQEGFDGEDFLFLDLDELRYISPVQQGISTVQKWNNNRAYLEARKHYYGTLCIEWLKKYVQYGKSSL
GLNQFGYDGDDLVVLDLKEWSWISPKQQGIVTQNKWNNDRAFTESRRNYLSTICIEWLQKYVQYGKSSL
GFRLEGYDGEDLLALDFEEMRYISPAQKGFKTQQSWNNYKAYLEDDKMYFSNECIEWLKKYLEYGKSSL
GLNQFGYDGDDLVVLDLKEWSWISPKQQGIVTQNKWNNDRAFTESRRNYLSTICIEWLQKCVQYGKSSL

mhcI_ufa
NM_194403.1:
SEIKEVIPRQEWVRGAVDEQFWQRNTQIRSNMHQLFKNNINIAMERFN

c-myb recipient strain:
SEIKEVIPRQEWVRGAVDEQFWQRNTQIRSNMHQLFKNNINIAMERFN
SEIKEVIPRQEWVRGAVDEQFWQRNTQIRSNMHQFFKNNINIAMERFN
ikaros:eGfp wild-type donor strain:
SEIKEVIPRQEWVRGAVDEQFWQRNTQIRSNMHQLFKNNINIAMERFN
SEIKEVIPRQEWVRGAVDEQLWQRNTQIRSNMHQLFKNNINIAMERFN
SEIKEVIPRQEWVRGAVDEQFWQRNTQIRSNIHQLFKNNINIAMERFN
Assam wild type donor strain:
SEIKEVIPRQAWVRGAVDEQFWQRNTQIRSNTHQLFKNNINIAMERFN

mhcII_DBB
U08869.1:
AYNFYPKHIKLTWMRDDKVVTADVMSTKVMADGDWYYQIHSHLEYFPQPGEKISCV

c-myb recipient strain:
AYNFYPKHIKLTWMRDDKVVTADVMSTKVMADGDWYYQIHSHLEYFPQPGEKISCV
AYNFYPKHIKLTWMRDDKVVTADVMSIKVMADGDWYYQIHSHLEYFPQPGEKISCV
AYNFYPKHIKLTWMRDDKVVTADVMSTKVMADGDWYYQIHSHLEYFPQPGGKISCV
ikaros:eGfp wild-type donor strain:
AYNFYPKHIKLTWMRDDKVVTADVMSTKVMADGDWYYQIHSHLEYFPQPGEKISCV
AYNFYPKHIKLTWMRDDKVVTADVMSTKVMADGDWYYQIHSHLKYFPQPGEKISCV
Assam wild type donor strain:
AYNFYPKHIKLTWMRDDKVVTADVMSTKVMADGDWYYQIHSHLEYFPQPGEKISCV
AYNFYPKHIKLTWMRDDKVVTADVMSTKVMADGDWYYQIYSHLEYFPQPGEKISCV
AYNFYPKHIKLTWMRDDKEVTADVMSTKVMADGDWYYQIHSHLEYFPQPGEKISCV
AYNFYPKHIKLTWMRDDKVVTANVMSTKVMADGDWYYQIHSHLEYFPQPGEKISCV

mhcII_DCB
NM_131706.1:
VCSEYDFYPKAIKLTWMRNDKRVTADVTSIEEMADGDWYYQIHSHLEYFPQPGEKISCVVDHASFHKPMI

c-myb recipient strain:
VCSAYDFYPKAIKLTWMRNDKKVTADVTSIEEMADGDWYYQIHSHLEYFPQPGEKISCVVDHASFHKPMI
VCSAYDFYPKGIKLTWMRDDKKVTAELTSSEVMADGDWYYQIHSHLEYFPQPGEKISCVVDHASFHKPMI
VCSAYDFYPKGIKLTWMRDDKKVTAELTSSEVMADGHWHYQIHSYLEYFPQTGEKISCVVDHASSLKPMI
VCSAYDFYPKGIKLTWMRDDKKVTAELTSSEVMADGHWHYQIHSYLEYFPQTGEKISCVVDHASFHKPMI
VCSAYDFYPKGIKLTWMRDDKKVTAELTSSEVMADGHWHYQIHSYLEYFPQTGEKISCVVDRASSLKPMI
ikaros:eGfp wild-type donor strain:
VCSAYDFYPKAIKLTWMRNDKKVTADVTSIEEMADGDWYYQIHSHLEYFPQPGEKISCVVDHASFHKPMI
VCSAYDFYPKAIKLTWMRNDKKVTADVTSIEEMADGDWYYQIHSHLEYFPQPGGKISCVVDHASFHKPMI
VCSAYDFYPKAIKLTWMRNDKKVTADVTSIEEMADGDWYYQIHSHLEHFPQPGEKISCVVDHASFHKPMI
VCSAYDFYPKAIKLTWMRNDKKVTADVTSIEEMADGDWYYQTHSHLEYFPQPGKKISCVVDHASFHKPMI
Assam wild type donor strain:
VCSAYDFYPKAIKLTWMRNDKKVTADVTSIEEMADGDWYYQIHSHLEYFPQPGEKISCVVDHASFHKPMI
VCSAYDFYPKGIKLTWMRDDKKVTAELTSSEVMADGHWHYQIHSYLEYFPQTGEKISCVVDHASSLKPMI
VCSAYDFYPKGIKLTWMRDDKKVTAELTSSEVMADGHWHYQIHSYLEYFPQTGEKISCVVDHASFHKPMI

Fig. S4. Diversity of *mhc* genes in zebrafish. Reference sequences (conceptual translations of nucleotide sequences) for each of the analyzed genes are shown; the identifier refers to the relevant GenBank accession number. Amino acid differences from the reference sequences are highlighted in red. The alleles found in seven individuals each of the *c-myb*-mutant background (*c-myb*) and the two wild-type donor backgrounds (*ikaros:eGFP*-transgenic, Assam) are indicated.

Table S1. Primer sequences

Gene	Forward (5′ > 3′)	Reverse (5′ > 3′)
Globin genes		
α-globin	CAAGGCTGTTGTTAGGC	GCACAGTGTTGTTGTCAG
β-globin	GGCCTGTGGGGAAAGCTC	GTTGTCGGGATCCACATGCAG
MHC genes		
mhc1ufa ex2	GAAAGCATTCTCACACTGTTATCT	GTGTTTGGTTAAAACGTTCCATTG
LOC751750	GAAATGTACGGCTGTGAGTG	CAGGCTGCTCTTTCCATAC
mhc1ufa ex3	CAGTTCATGGTCGGCTGTGA	AAGAATCTTTGCCGTAACCC
mhc1uda ex2	AGGCATACACTCTCTGAAATACTTCTTC	CCCTGTGACTGGTTAAACCTCTCC
mhc1uea ex2	TACACACTCCCTGAGGTACTTC	TGTGCGCCAGTAAAGATCTGAG
mhc1uxa2 ex2	AGGCATACACTCTCTGAAATACTTCTTC	CCCTGTGACTGGTTAAACCTCTCC
mhcII DBB	TAAAACCAGAAGTCTTTGTGCGG	CCCAGTAATARAATCATGGGTTT
mhcII DCB	TGAAACCAGAAGTCATTATTCGG	CCCAGTAATARAATCATGGGTTT
mhcII DFB	CAGATGGATATTATTCATCCACG	CTGCTTTATCAAAGACCGCTGA
mhcII DAA/BA	TAGTTGCTCTTCTGGAGAGTT	GCTCAAGCTGAGCACAGGGAT
A20291/ A20290	GCTCAAGCTGAACACAAGGAT	TTTCTCTAAAGGAGAGTTGTAGGCCTT
mhcII DAB	TACTACCAGAGGTAACAATCAAG	TCCAATCTTTAGTAAGAGGTTG
mhcII DDB	TGAAGCCAACAGTTGTGCTCAGT	CCCAGTAATARAATCATGGGTTT
TCR genes*		
Vd35-Cd (first)	GCTTTTACTGTAAAGGAAGAGGA	TTTATTGATTACAAAGATCAGAGCCTC
Vd35-Cd (second)	GAGACTGTGACCTTCAGCTG	TTTATAGGGGACAAGACAGAC
Vb1.5/17.5-Cb1 (first)	AATGGACAGCTTGATAGAACTGAAC	AAGATGACAAGGCCATACAGTC
Vb1.5/17.5-Cb1 (second)	TGCTTATTCAACCGAACAGAAACATTC	GTCCGCTCTTAGCAATGGTC
Ig genes*		
igVH1-Cm (first)	GATGGACGTGTTACAATTTGG	ACATGAAGGTTGCTGATCCAC
igVH1-Cm (second)	CCTCCTCAGACTCTGTGGTGA	TTGCTGATCCACCTTCTAATTC
Miscellaneous		
ef1α	TATCTCCAAGAACGGACAGAC	GCAAACTTGCAGGCGATGTG
cd79b	CAGGAACTGCACTTCAAGTATCC	CTCATATATGTGGTCATCCTCTGG
lck	TGAATACACCGCCAGAGAAGGTGC	GAGGTTTGCGATCACCTCTGG

*Amplification of rearranged antigen receptor genes was carried out in two successive rounds.