Figure EV1. Validation of the evi1 morpholino oligonucleotides.

A. Schematic representation of protein domains of the transcription factor Evi1. Blue boxes represent zinc finger motifs in the Evi1 protein. Other functional domains are indicated.

B. The evi1 splice donor MO, depicted by red crosses, target either the 3rd (MO1) or the 6th (MO2) zinc finger in the first zinc finger domain.

C. RT–PCR of evi1 in embryos injected with evi1 MO1 or MO2 indicates splice modification. Corresponding primer pairs are shown. Expected wt bands for MO1 389 bp, in evi1 morphants 2,526 bp. For MO2: expected wt bands 1,100 bp, 5,695 bp for evi1 morphants.

D. No pooling was observed by o-dianisidine staining in evi1 morphants at 38 hpf.

E, E’ Co-injection of capped evi1 mRNA (E) or UAS:mEvi1 (in Tg(fli:1:Gal4FF; UAS:RFP) embryos, E’) together with the evi1 MO rescues the HSC phenotype, shown by restored runx1/c-myb expression in the VDA, marked with black arrowheads. Numbers indicate the amount of embryos with the respective phenotype/total number of embryos analyzed in each experiment. Arrows indicate up- or downregulation of runx1/c-myb in each condition. Lateral views are shown, anterior to the left, dorsal up.
Figure EV2. *evi1* MO2 injection leads to the same phenotype as seen by injection of *evi1* MO1.

A  WISH of c-myb in control- and *evi1* MO2-injected albino (alb) embryos.

B  WISH of l-plastin (upper) and mpo (lower) in control- and *evi1* MO2-injected alb embryos.

C  For each analyzed gene, quantitation of results is shown, displaying the percentages of embryos with normal vs. decreased gene expression for each condition. A Fisher's exact test was applied to calculate statistical significance (**P < 0.001**).

Data information: Gene expression is reduced upon *evi1* MO2 injection as indicated by the black arrows. Lateral views are shown, anterior to the left, dorsal up. Numbers indicate the amount of embryos with the respective phenotype/total number of embryos analyzed in each experiment. Arrows indicate up- or downregulation in each condition.
Figure EV3. Thymus epithelium, notochord, and vasculature are not affected upon evi1 knockdown.

A WISH of foxn1 in the thymus epithelium of both control (left)- and evi1 MO-injected (right) 5 dpf embryos.

B WISH of endothelial-specific flt1 (upper) and flt4 (lower) in both control (left)- and evi1 MO-injected (right) 26 hpf embryos.

C WISH of shh in the notochord of both control- and evi1 MO-injected embryos.

Data information: Lateral views are shown, with anterior to the left, dorsal up. Squares represent enlargements of the region of interest. Numbers indicate the amount of embryos with the respective phenotype/total number of embryos analyzed in each experiment. A minimum of two biological replicates was performed for each marker with at least n = 5 embryos per experiment.
Figure EV4. Unchanged expression of genes involved during the somitic requirement of HSC emergence.

A–E WISH of dlc (A), efnb2a (B), wnt16 (C), foxc1b (D), and twist1b (E) in control-injected (left) or evi1 MO-injected alb embryos (right).

Data information: At least two biological replicates were performed for each experiment. Lateral views are shown, anterior to the left, dorsal up. Numbers indicate the amount of embryos with the respective phenotype/total number of embryos analyzed in each experiment.