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Supplemental Information

Wilms Tumor 1b Expression Defines a Pro-regenerative Macrophage Subtype and Is Required for Organ Regeneration in the Zebrafish

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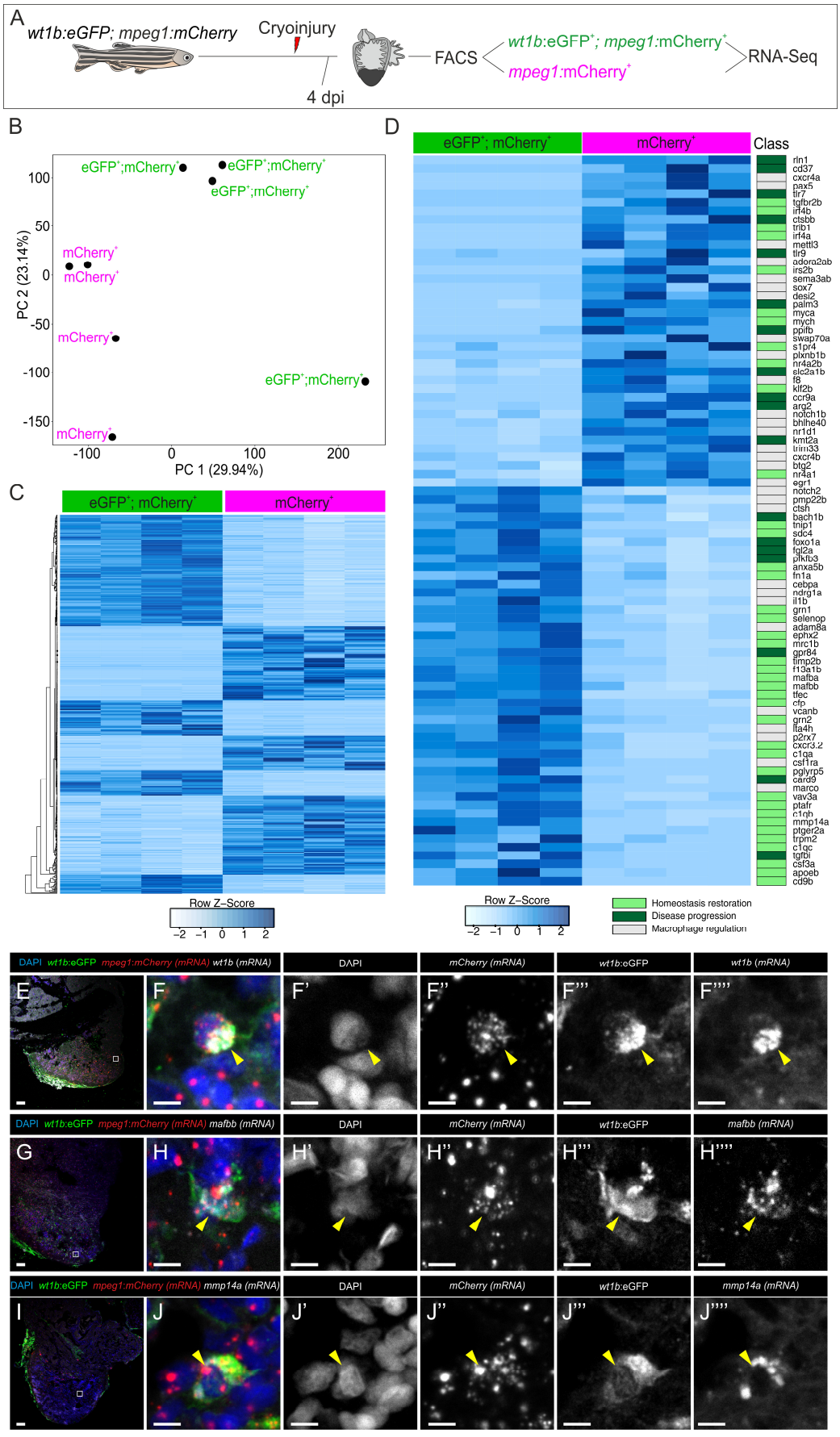

Figure S1. Differential gene expression of *wt1b*-positive macrophages. Related to Figure 2 and Table S1. A, *wt1b*:eGFP^{+};*mpeg1*:mCherry^{+} and *mpeg1*:mCherry^{+} cells were FAC-sorted from cryoinjured adult zebrafish hearts at 4 dpi and their transcriptomes analyzed using RNA-seq. **B**, Principal component analysis of

wt1b:eGFP^{+};*mpeg1*:mCherry^{+} and *mpeg1*:mCherry^{+} cells. **C**, Heatmap indicating all significant differentially expressed genes between both populations. **D**, Heatmap of genes from (C) whose function has been described in macrophages. Classification of gene function in macrophages according to literature: grey, genes involved in macrophage regulation including differentiation, phagocytosis and apoptosis; light green, genes involved in homeostasis restoration; dark green, genes involved in disease progression. **E-J''''**, Validation of RNA-seq target genes by RNAScope *in situ* hybridization followed by anti-GFP immunostaining on cryoinjured *wt1b*:eGFP;*mpeg1*:mCherry heart sections at 4 dpi. Signal from *wt1b*, *mafbb* and *mmp14a* antisense riboprobes co-localizes with *mCherry* mRNA and eGFP signal (arrowheads). Note that large dots for mCherry channels represent background staining, and small dots correspond to signal. dpi, days post injury; FACS, fluorescence-activated cell sorting; PC, principal component; Mφ, macrophage. Scale bars, 50 μm (E, G, I) and 5 μm (F-F'''', H-H'''' and J-J'''').

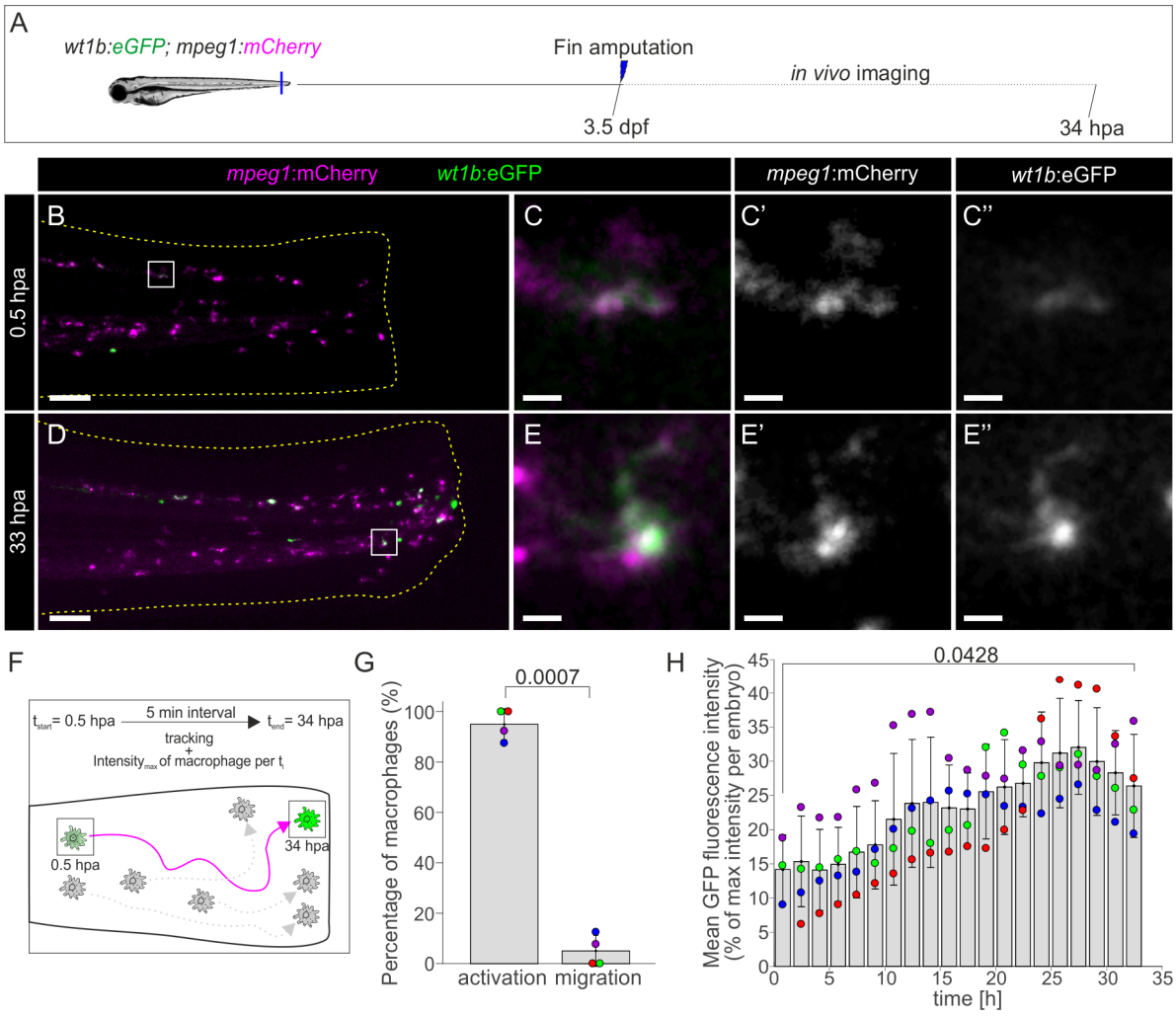

Figure S2. Activation of *wt1b*:eGFP during migration towards the amputation plane. Related to Figure 3. **A**, Caudal fins from *Tg(wt1b:eGFP;mpeg1:mCherry)* zebrafish larvae were amputated at 3.5 dpf and *in vivo* imaging was performed from 0.5 to 34 hpa. **B,** Overview of merged eGFP and mCherry channels at 0.5 hpa. **C–C''**, Merged and single eGFP and mCherry channels of the zoomed views from boxed areas in panel B are shown. **D,** Overview of merged eGFP and mCherry channels at 33 hpa. **E–E''**, Merged and single eGFP and mCherry channels of the zoomed views from boxed areas in panel B are shown. **F**, Scheme of macrophage tracking and fluorescence intensity measurement. *wt1b:*eGFP^{+} macrophages were tracked during the entire movie and the maximal GFP intensity within the macrophage was detected simultaneously. **G**, For each of 41 tracked macrophages in 4 embryos an intensity- and localization-based decision (25 % most anterior location and at least 33 % of the macrophage's maximal intensity) defines whether they upregulate *wt1b:*eGFP during migration or are eGFP^{+} from the initial time of tracking. The graph shows the overall mean percentage of upregulation (activation) and migration as bars plus the percentage for each of 4 embryos. **H**, Overall mean GFP intensity of the 41 tracked macrophages at different time points (grey bars). Colored dots indicate mean GFP intensity ± SD of macrophages in each of the 4 larvae. eGFP levels over time were normalized by maximal measured intensity per larvae. Time-intervals of 1.7 h (20 x 5 min) were binned. Paired t-test was performed. Scale bars: overview images 100 µm (B and D), zoomed views 10 µm (C-C''and E-E'').

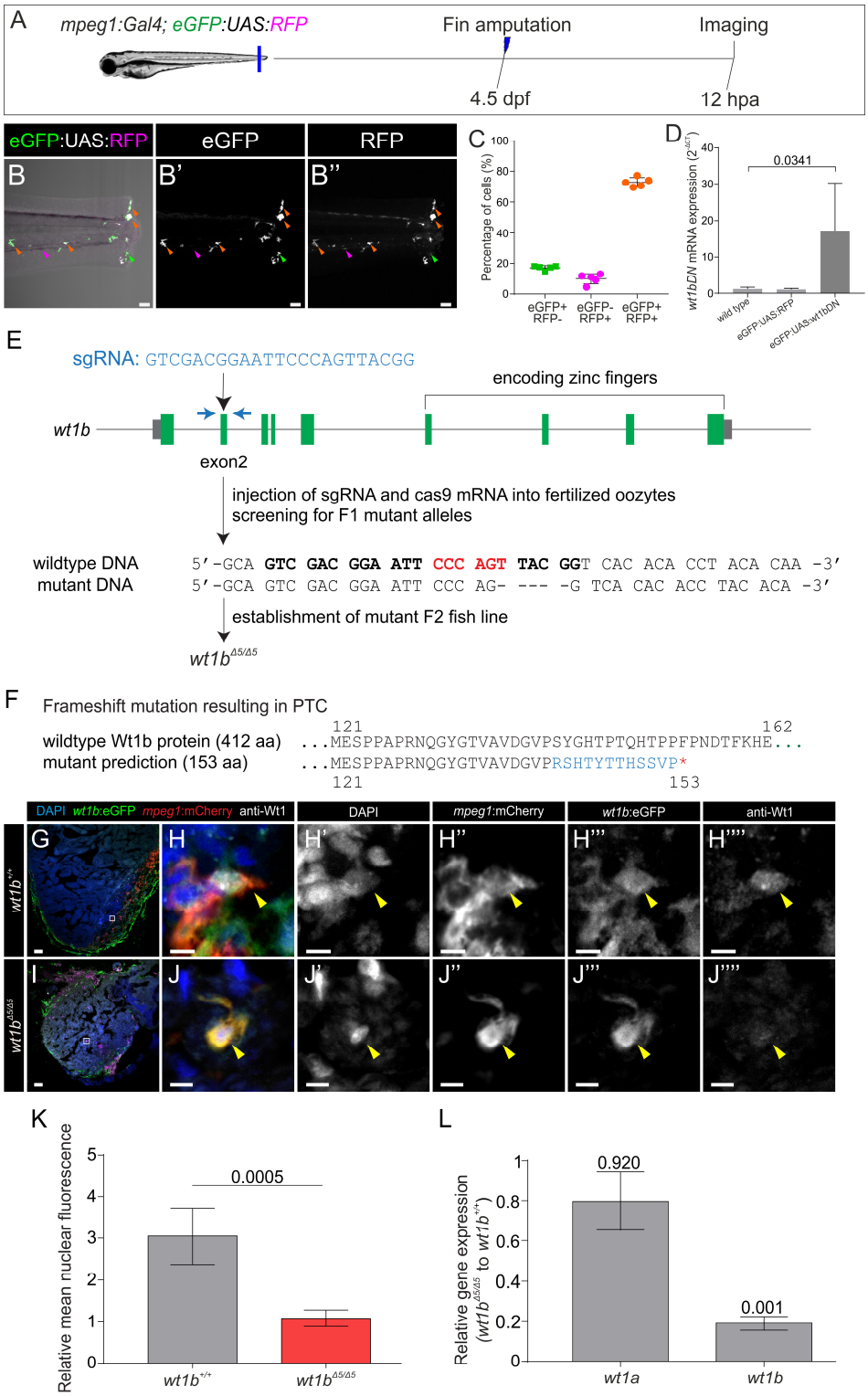

Figure S3. Validation of genetic lines to assess wt1b function. Related to Figures 3, 4 and 5.

A-D, Validation of transgenic lines for macrophage-specific inhibition of *wt1b* function. A, Caudal fins of *Tg(mpeg1:Gal4; GFP:UAS:RFP)* larvae were amputated at 4.5 dpf and eGFP and RFP positive cells imaged at 12 hpa. **B–B'''**, Images of the caudal fin showing RFP and eGFP positive cells. B' and B'' are single channels for eGFP and RFP. Orange arrowheads, double positive cells; magenta arrowheads, RFP^+ cells; and green arrowheads, $eGFP^+$ cells. **C**, Quantification of the percentage of $eGFP^+$, RFP^+ and double-positive cells. Dots indicate individual fish, shown are means ± SD. Note that over 70% of macrophages are double-positive. **D**, Confirmation of *wt1bDN* overexpression using the *Tg(eGFP:UAS:wt1bDN)* line. *Gal4FF* mRNA was injected into 1-cell stage wildtype, embryos from the transgenic control line *Tg(eGFP:UAS:RFP)* or *Tg(eGFP:UAS:wt1bDN)*. qRT-PCR was performed on cDNA obtained from these larvae. Shown are mean values ± SD (n= 4 biological replicates per

condition). Statistical analysis by Mann-Whitney test. **E-L**, Generation of the *wt1b*$^{\Delta 5}$ mutant line. Related to Figures 4 and 5. **E**, Schematic drawing of the *wt1b* gene locus and workflow for *wt1b* mutant generation. A fish line that lacks 5 nucleotides in exon 2 of the *wt1b* gene (*wt1b*$^{\Delta 5}$) was established. Blue arrows indicate the forward and reverse primer positions and the *Bsr1* restriction site, used to detect indels, is highlighted in red. The sgRNA target sequence is shown in bold. **F**, The 5 nucleotides deletion is predicted to result in a frame shift (blue) and a premature termination codon that leads to a truncation of the protein. **G-J''''**, Immunostaining with anti-WT1, anti-GFP and anti-mCherry on cryoinjured heart sections at 4 days postinjury from *wt1b*$^{+/+}$; *wt1b*:eGFP;*mpeg1*:mCherry and *wt1b*$^{\Delta 5/\Delta 5}$; *wt1b*:eGFP;*mpeg1*:mCherry zebrafish. Cell nuclei are counterstained with DAPI. Colocalization (arrowheads) of Wt1 with GFP and mCherry is detected in *wt1b*$^{+/+}$ but absent in *wt1b*$^{\Delta 5/\Delta 5}$. **K**, Quantification of the relative mean Wt1 nuclear fluorescence in images of *wt1b*:eGFP; *mpeg1*:mCherry cells normalized by background signal. Two-tailed unpaired t test. **L**, Quantitative RT-qPCR analysis of *wt1a* and *wt1b* expression in *wt1b*$^{\Delta 5/\Delta 5}$ *vs.* wildtype larvae. Error bars represent standard error. p-values are calculated by pair wise fixed reallocation randomization with REST (n=10). dpf, days post fertilization; hpa, hours post amputation. Scale bars 50 µm (G and I), 40 µm (B-B'') and 5 µm (H-H'''' and J-J'''').

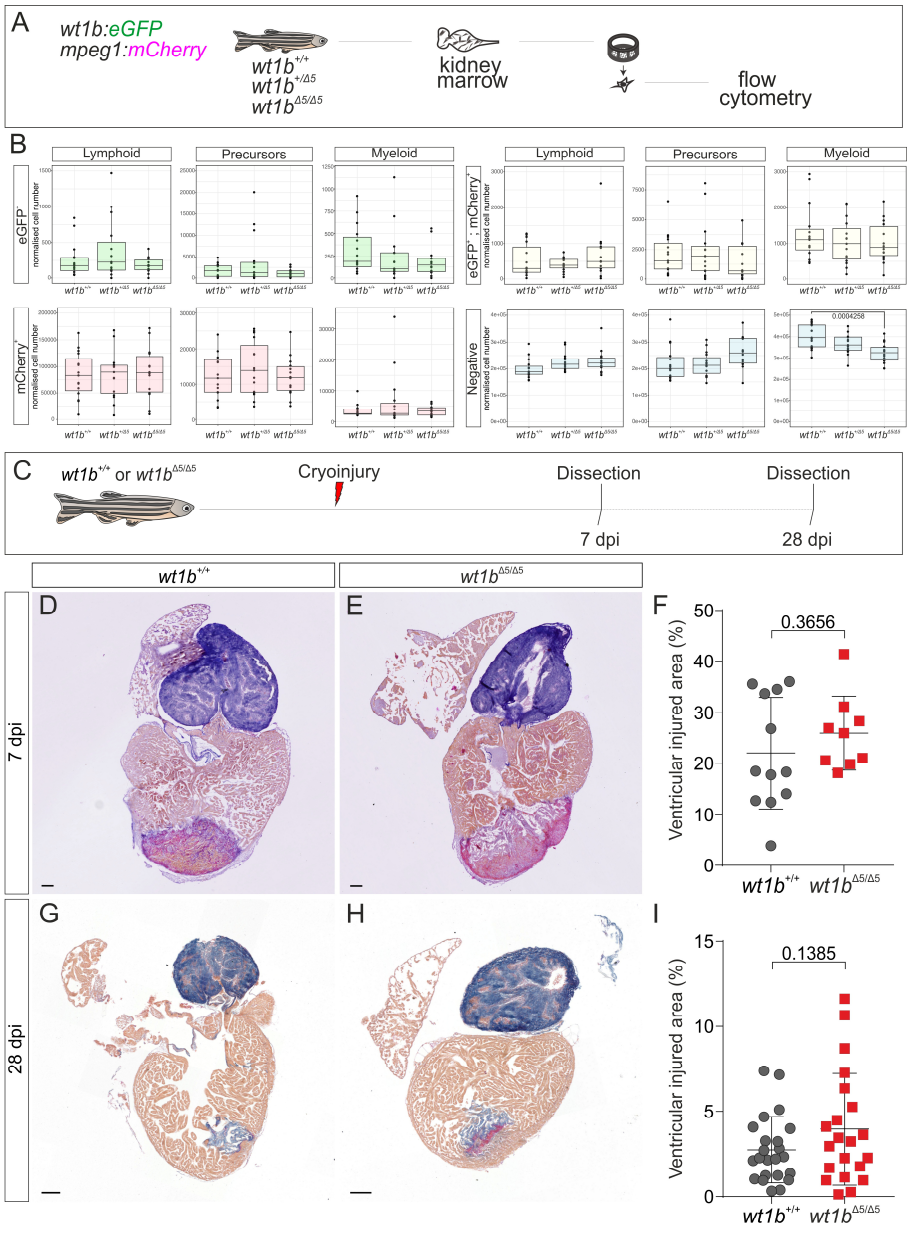

Figure S4. Phenotypic characterization of *wt1b*$^{\Delta5}$ mutants. Related to Figure 4 and Figure 5. Composition of the whole kidney marrow cell populations in *wt1b*$^{\Delta5}$ mutants. **A**, Whole kidney marrow (WKM) cells of *wt1b*$^{+/+}$, heterozygous *wt1b*$^{+/\Delta5}$ or homozygous *wt1b*$^{\Delta5/\Delta5}$ crossed into *a Tg(wt1b:eGFP; mpeg1:mCherry)* background fish were isolated. This is the same experiment as described in Figure 4, but here, eGFP^{+}, mCherry^{+}, double positive populations and the non-fluorescent fraction were analyzed by flow cytometry separately. **B**, Shown are boxplots of normalized cell numbers of cell populations in gate 1 (lymphoid), 2 (precursors) or 3 (myeloid). Normalized cell numbers relate to cell numbers per 10^6 events of living single cells. Negative (non-fluorescent) cell numbers in gate 3 are significantly lower in *wt1b*$^{\Delta5/\Delta5}$ than in *wt1b*$^{+/+}$ by one-way ANOVA followed by a Tukey's post-hoc test. **C-I**, Fibrotic tissue deposition and regeneration in *wt1b*$^{\Delta5}$ mutants. **C,** Ventricular cryoinjury was performed to *wt1b*$^{+/+}$ and *wt1b*$^{\Delta5/\Delta5}$ adult fish and fibrosis assessed at 7 and 28 dpi on sectioned hearts using AFOG histological staining to detect collagen. **D**, **E**, Representative sagittal section of a *wt1b*$^{\Delta5/\Delta5}$ and *wt1b*$^{+/+}$ heart stained with AFOG at 7dpi. **F,** Quantification of injured area versus total ventricular area. Data from two independent experiments. Two-tailed unpaired t test. **G-I**, Representative images and quantification of injured cardiac ventricular area at 28 dpi, as shown in D-F. Two-tailed unpaired t test. dpi, days postinjury. Scale bars, 100 µm.

Primers to genotype wt1bΔ5/Δ5 mutants:	For: 5'-GTGAACTCTTGAAATGTCACTACAAGC-3'
	Rev: 5-ACCGCTGATGAATAAAGGGACTAAC-3'
qPCR primers, wt1b (heart RT-qPCR):	Fw: 5'-GGCCTGGAATCCTGTTAGC-3'
	Rv: 5'-CAGAGGAGGTGCTCCTGAAG-3'
qPCR primers, wt1b (larvae RT-qPCR):	Fw: 5'-TGCTGATCCTCCTTCTAGCC-3'
	Rv: 5'-GAACGGAGGAGTGTGTTGTG-3'
qPCR primers, wt1a (larvae RT-qPCR):	Fw: 5'-AGCCAACCAAGGATGTTCAG-3'
	Rv: 5'-CCTCGTGTTTGAAGGAGTGG-3'
qPCR primer, ef1α (larvae RT-qPCR):	Fw: 5'-AAGAGAACCATCGAGAAGTTCGA-3'
	Rv: 5'-ACCCAGGCGTACTTGAAGGA-3'
qPCR primers, wt1bDN (larvae RT-qPCR):	Fw: 5'-TATTTGCCAGGCTGCATGGA-3'
	Rv: 5'-TCGGGTCCTCGTGTTTGAAG-3'
qPCR primers, ef1α (larvae RT-qPCR):	Fw: 5'-CAGCTGATCGTTGGAGTCAA-3'
	Rv: 5'-TGTATGCGCTGACTTCCTTG-3'

Table S2. List of primers used to genotype wt1bΔ5/Δ5 mutants and to perform RT-qPCRs. Related to STAR Methods and the Key Resources Table.