Supplementary Material

Molecular Insights Into Neutrophil Biology From the Zebrafish Perspective: Lessons From CD18 Deficiency

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Supplementary Figures

Supplementary Figure 1

CD18

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H. sapiens      MLGLRPPL-LALVGLLSLG--CVLSQECTKFKVSSCRECIESGPGCTWCQKLNFTGPGDPDSIRCDTRPQLLMRGCAADDIMDPTSLAET     87
M. musculus     MLGLRPSLLLALAGLFFLG--SAVSQECTKYKVSSCRDCIQSGPGCSWCQKLNFTGPGEPDSLRCDTRAQLLLKGCPADDIMDPRSIANP     88
D. rerio        ---MHQSVCLKSLLLLLVGTQVYTQEQCLKASVNTCGDCIKSGPGCAWCKELNFTKTGEQEAARCDTVKLLLDRGCSKENIINPQNIFQN     87
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H. sapiens      QED----HNGGQKQLSPQKVTLYLRPGQAAAFNVTFRRAKGYPIDLYYLMDLSYSMLDDLRNVKKLGGDLLRALNEITESGRIGFGSFVD     173
M. musculus     EFD----QRGQRKQLSPQKVTLYLRPGQAAAFNVTFRRAKGYPIDLYYLMDLSYSMLDDLNNVKKLGGDLLQALNEITESGRIGFGSFVD     174
D. rerio        VVNKALVAGKDPVQIQPQEIQLNLRPGMPQTFQLRFKRAEGYPVDLYYLMDLSYSMKDDLANVKNLGNDILEKLNRITGNARIGFGSFVD     177
                  :          *:.**:: * ****   :*:: *:**:***:************ *** ***:**.*:*. **.** ..*********

H. sapiens      KTVLPFVNTHPDKLRNPCPNKEKECQPPFAFRHVLKLTNNSNQFQTEVGKQLISGNLDAPEGGLDAMMQVAACPEEIGWRNVTRLLVFAT     263
M. musculus     KTVLPFVNTHPEKLRNPCPNKEKACQPPFAFRHVLKLTDNSNQFQTEVGKQLISGNLDAPEGGLDAIMQVAACPEEIGWRNVTRLLVFAT     264
D. rerio        KTLLPYTDTNEAKLKHPCSDKNEPCQPAFGFQHVLPLTANGNDFKELVKQQHISGNLDPPEGSLDAIMQAAVCLKEIGWGNSTRLLVLAT     267
                **:**:.:*:  **::** :*:: *** *.*:*** ** *.*:*:  * :* ****** ***.***:**.*.* :**** * *****:**

H. sapiens      DDGFHFAGDGKLGAILTPNDGRCHLEDNLYKRSNEFDYPSVGQLAHKLAENNIQPIFAVTSRMVKTYEKLTEIIPKSAVGELSEDSSNVV     353
M. musculus     DDGFHFAGDGKLGAILTPNDGRCHLEDNMYKRSNEFDYPSVGQLAHKLSESNIQPIFAVTKKMVKTYEKLTEIIPKSAVGELSDDSSNVV     354
D. rerio        DDGFHMAGDGKLAAILEPNKETCQLDKNKYSKSNIWDYPSVGQVARKLEEQNIQPIFAVTKKMETVYTELSKLIPKSAVGVLSEDSNNVV     357
                *****:******.*** **.  *:*:.* *.:** :*******:*:** *.**********.:* ..* :*:::******* **:**.***

H. sapiens      QLIKNAYNKLSSRVFLDHNALPDTLKVTYDSFCSNGVTHRNQPRGDCDGVQINVPITFQVKVTATECIQEQSFVIRALGFTDIVTVQVLP     443
M. musculus     QLIKNAYYKLSSRVFLDHSTLPDTLKVTYDSFCSNGASSIGKSRGDCDGVQINNPVTFQVKVMASECIQEQSFVIRALGFTDTVTVQVRP     444
D. rerio        NLIVEAYNNLTSEVIMAHDALPNFISVKYSSKCTGGKTFS--DNGKCDNVNIGLEVIFDVTVTVDRCINDQSFSIGPLGFNEKLKVNVQT     445
                :** :** :*:*.*:: *.:**: :.*.*.* *:.* :     .*.**.*:*.  : *:*.* . .**::*** *  ***.: :.*:*

H. sapiens      QCECRCRDQSRDRSLCHGKGFLECGICRCDTGYIGKNCECQTQGRSSQELEGSCRKDNNSIICSGLGDCVCGQCLCHTSDVPGKLIYGQY     533
M. musculus     QCECQCRDQSREQSLCGGKGVMECGICRCESGYIGKNCECQTQGRSSQELERNCRKDNSSIVCSGLGDCICGQCVCHTSDVPNKEIFGQY     534
D. rerio        LCECNCGDPPGVHPHCKGQGKVVCGSCKCNDGFIGQLCECTIGKKDEASFKAQCRKDNG-TECEGKGDCVCGRCQCHLTEGN-GHFYGEH     533
                 ***.* *    :  * *:* : ** *:*: *:**: ***    :.. .:: .*****.   *.* ***:**:* ** ::     ::*::

H. sapiens      CECDTINCERYNGQVCGGPGRGLCFCGKCRCHPGFEGSACQCERTTEGCLNPRRVECSGRGRCRCNVCECHSGYQLPLCQECPGCPSPCG     623
M. musculus     CECDNVNCERYNSQVCGGSDRGSCNCGKCSCKPGYEGSACQCQRSTTGCLNARLVECSGRGHCQCNRCICDEGYQPPMCEDCPSCGSHCR     624
D. rerio        CECDDEHCEKFDNKQCAG--HGKCKCGKCECDDGYEGTACHCAKSDNECKIDS-HVCYNRGKCVCNQCECERGYKGPYCKTCPTCQRPCQ     620
                ****  :**:::.: *.*  :* * **** *. *:**:**:* ::   *       * .**:* ** * *. **: * *: ** *   *

H. sapiens      K-YISCAECLKFEKGPFGKNCSAACPGLQLSNN-PVKGRTCKERDSEGCWVAYTLEQQDGMDRYLIYVDESRECVAGPNIAAIVGGTVAG     711
M. musculus     DNHTSCAECLKFDKGPFEKNCSVQCAGMTLQTI-PLKKKPCKERDSEGCWITYTLQQKDGRNIYNIHVEDSLECVKGPNVAAIVGGTVVG     713
D. rerio        Q-SGSCVECFAFQSGPFEKNCSLACKHLTIKIVDKLVKSDCRVKDKEGCWMAFTMTEELGFDLYSVSVLKDRECPEGPNIIAIVGGSLAG     709
                .   **.**: *:.*** ****  *  : :.    :    *: :*.****:::*: :: * : * : * .. **  ***: *****::.*

H. sapiens      IVLIGILLLVIWKALIHLSDLREYRRFEKEKLKSQWNNDNPLFKSATTTVMNPKFAES-     769
M. musculus     VVLIGVLLLVIWKALTHLTDLREYRRFEKEKLKSQWNNDNPLFKSATTTVMNPKFAES-     771
D. rerio        VALIGLLMLLIIKGVLYASDLREWRRFEKDRKHEKTSGTNPLFQNATTTVQNPTFSGDS     768
                :.***:*:*:* *.: : :****:*****:: :.: .. ****:.***** **.*: .
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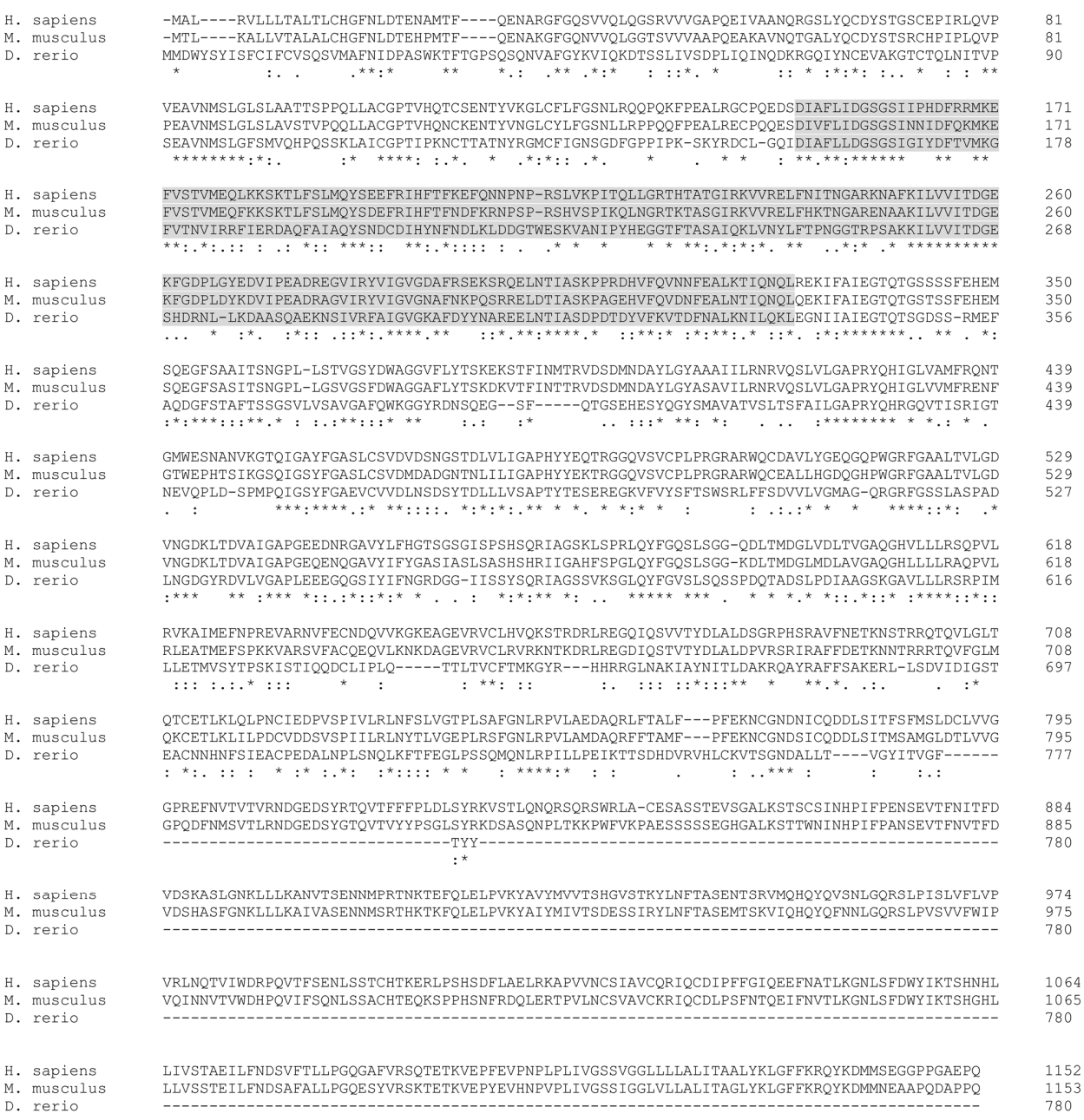
Supplementary Figure 1: Sequence alignment of the human, murine, and zebrafish integrin CD18. The highly conserved I-like domains are shaded in gray. The transmembrane domain is shown in blue. The highly conserved NXXF motifs are highlighted in yellow and green, respectively. Identical (*), strongly similar (:) and weakly similar (.) amino acid residues are indicated below the alignment.

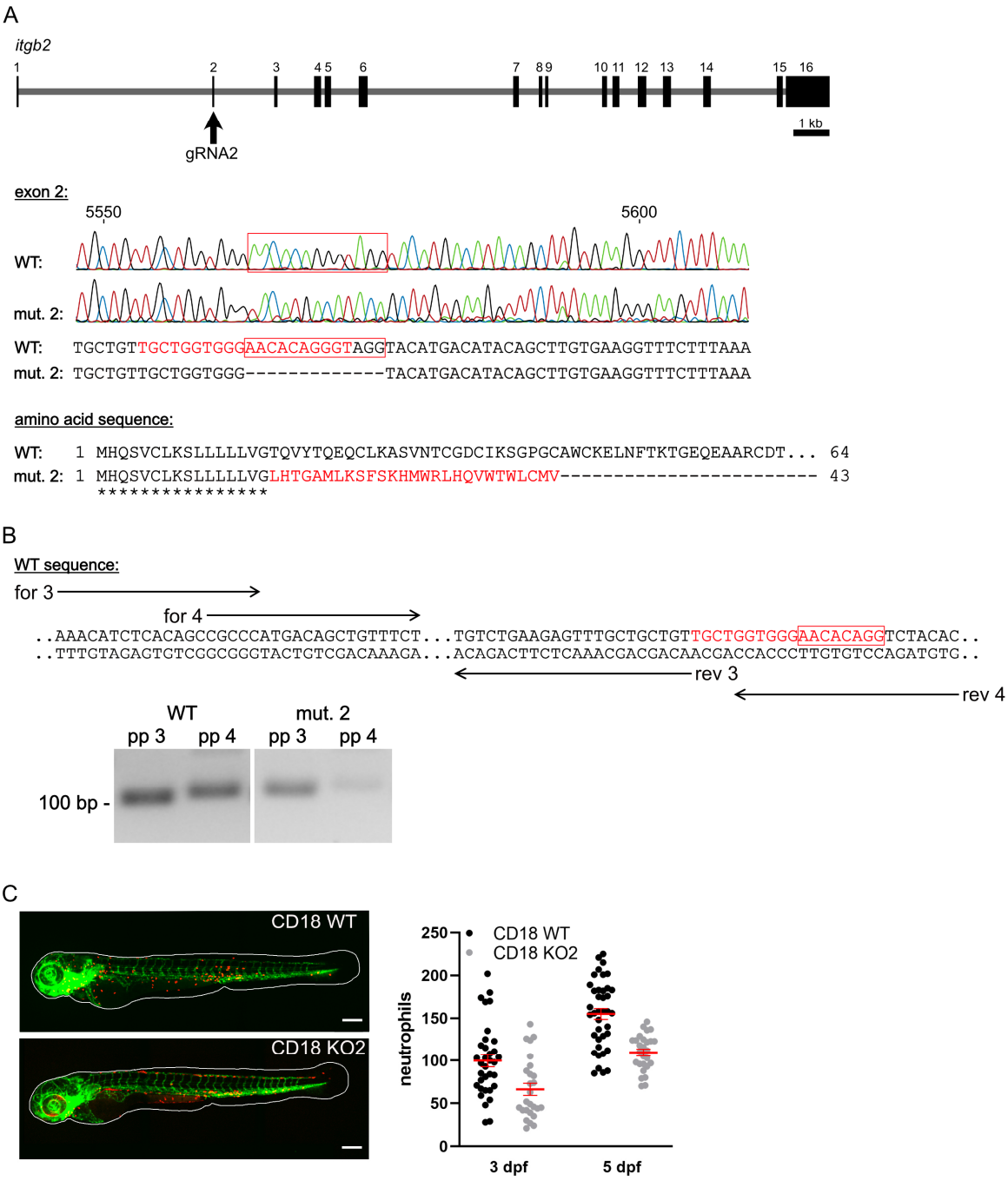
Supplementary Figure 2

CD11b



Supplementary Figure 2: Sequence alignment of the human, murine, and zebrafish integrin CD11b. The I domains are shaded in gray. Identical (*), strongly similar (:) and weakly similar (.) amino acid residues are indicated below the alignment.

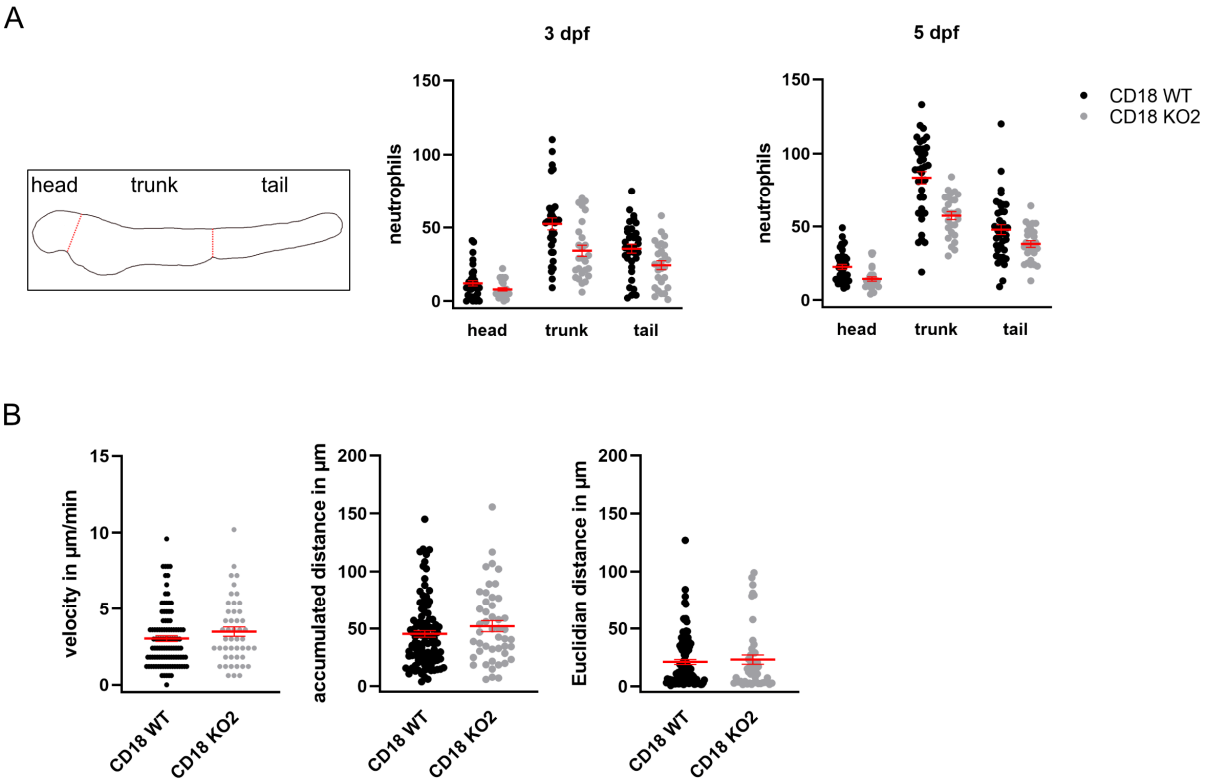
Supplementary Figure 3



Supplementary Figure 3: Generation of CD18 KO2 zebrafish. (**A**) Upper panel: Schematic of *itgb2* gene and target exon 2 of the gRNA2. Middle panel: Sequencing traces and partial genomic sequence of WT and CD18 mutant 2 (mut. 2). Numbers indicate position within the gene. Red boxes show nucleotides deleted in the mutant. Highlighted is the target sequence of the gRNA in red. Lower panel: Predicted amino acid sequence of mutants aligned to WT sequence of the first 64 amino acids. Identical (*) and altered (red) amino acids are indicated. (**B**) Upper panel: Location of primer pairs for PCR

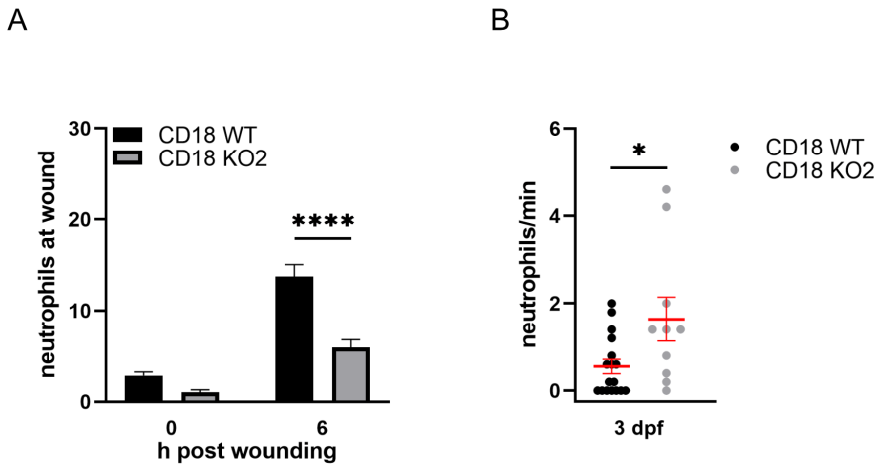
analysis of WT and CD18 mutant mRNA expression analysis. Reverse (rev) primer 3 binds to WT and CD18 mutant sequence, reverse primer 4 only binds the WT sequence. Lower panel: Representative image of agarose gel electrophoresis of PCR products of WT and CD18 mutant cDNA with primer pairs (pp) 3 (forward primer 3, reverse primer 3) and 4 (forward primer 4, reverse primer 4), respectively, from zebrafish larvae at 5 dpf. Expected band sizes are 101 bp for pp 3 and 112 bp for pp 4. (**C**) Left: Exemplary maximum intensity projections of CD18 WT and CD18 KO2 zebrafish larvae at 3 dpf. Endothelial cells are shown in green, neutrophils in red. Scale bars represent 200 µm. Right: Total neutrophil counts in CD18 WT and CD18 KO2 zebrafish larvae at 3 dpf and 5 dpf. Mean ± sem of ≥ 26 individual larvae of ≥ 3 independent experiments.

Supplementary Figure 4



Supplementary Figure 4: Analysis of neutrophil count and random migration at steady state in CD18 KO2. (**A**) Left: Schematic of zebrafish larvae for analyzing neutrophil distribution in head, trunk, and tail. Middle and right: Distribution of neutrophils in CD18 WT and CD18 KO2 zebrafish larvae at 3 dpf and 5 dpf. Mean ± sem of ≥ 26 individual larvae of ≥ 3 independent experiments. (**B**) Mean migration velocity, accumulated and Euclidian distance of individual neutrophils. Mean ± sem of ≥ 47 individual neutrophils of 3 independent experiments. Unpaired t-test.

Supplementary Figure 5



Supplementary Figure 5: Neutrophil trafficking in CD18 KO2 zebrafish. (**A**) Quantification of neutrophil numbers at the transected tail fin in 3 dpf CD18 WT and CD18 KO2 zebrafish larvae. Mean ± sem of ≥ 14 individual larvae of ≥ 2 independent experiments. One-way ANOVA, $p < 0.0001$: ****. Similar results were obtained after 5 dpf. (**B**) Number of neutrophils per min in the circulation 6 h after tail fin transection in CD18 WT and CD18 KO2 zebrafish larvae at 3 dpf. Mean ± sem of ≥ 10 individual larvae of 2 independent experiments. Unpaired t-test, $p < 0.05$: *.

Supplementary Videos

Supplementary Video 1: Time-lapse confocal spinning disc maximum z projections showing random migration of neutrophils (red) in zebrafish CD18 WT and CD18 KO1 larvae at 5 dpf. Endothelial cells are shown in green. Three representative tracks of neutrophils migrating interstitially are shown. Time lapse interval = 60 s. Frame rate = 0.02 frames/s. Scale bar represents 100 μm.

Supplementary Video 2: Time-lapse confocal spinning disc video microscopy showing neutrophils (red) in the posterior caudal vein 6 h post wounding in CD18 WT and CD18 KO1 zebrafish larvae at 5 dpf. Endothelial cells are shown in green. Arrows point out neutrophils detected in the circulation. Time lapse interval = 4.5 s. Frame rate = 0.22 frames/s. Scale bar represents 200 μm.