

Supplementary Material

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

Almke Bader^{1,2†}, Jincheng Gao^{1,2†}, Thibaud Rivière^{1,2}, Bettina Schmid³, Barbara Walzog^{1,2*}, Daniela Maier-Begandt^{1,2*}

¹Institute of Cardiovascular Physiology and Pathophysiology, Biomedical Center, Ludwig-Maximilians-Universität München, Planegg-Martinsried, Germany

²Walter Brendel Center of Experimental Medicine, University Hospital, Ludwig-Maximilians-Universität München, Munich, Germany

³Fish Core Unit, German Center for Neurodegenerative Diseases (DZNE), Munich, Germany

*Correspondence:	Daniela Maier-Begandt, Daniela.maier@lrz.uni-muenchen.de
	Barbara Walzog, Walzog@lrz.uni-muenchen.de

[†]These authors have contributed equally to this work

Supplementary Figure 1 CD18

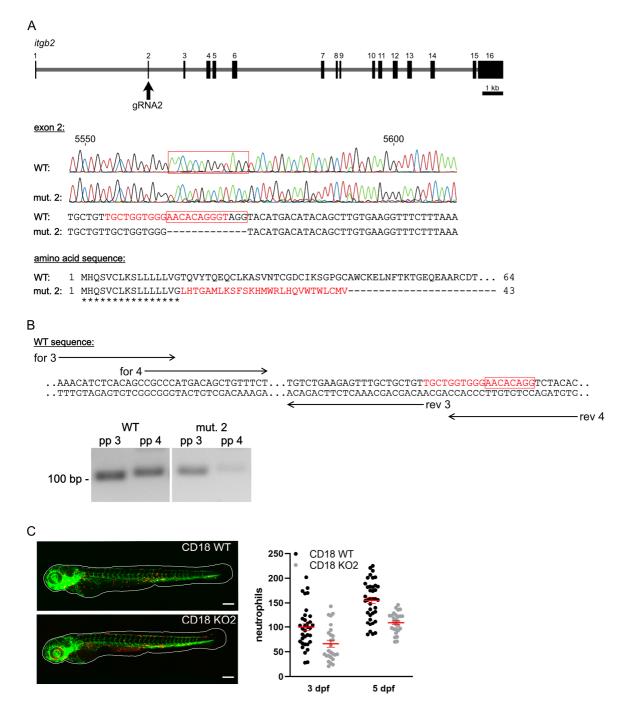
H. sapiens M. musculus D. rerio	MLGLRPPL-LALVGLLSLGCVLSQECTKFKVSSCRECIESGPGCTWCQKLNFTGPGDPDSIRCDTRPQLLMRGCAADDIMDPTSLAET MLGLRPSLLLALAGLFFLGSAVSQECTKYKVSSCRDCIQSGPGCSWCQKLNFTGPGEPDSLRCDTRAQLLLKGCPADDIMDPRSIANP MHQSVCLKSLLLLVGTQVYTQEQCLKASVNTCGDCIKSGPGCAWCKELNFTKTGEQEAARCDTVKLLLDRGCSKENIINPQNIFQN :: : * *: :: :: *: :: :: *: :: :: :: ::	87 88 87
H. sapiens M. musculus D. rerio	QEDHNGGQKQLSPQKVTLYLRPGQAAAFNVTFRRAKGYPIDLYYLMDLSYSMLDDLRNVKKLGGDLLRALNEITESGRIGFGSFVD EFDQRGQRRQLSPQKVTLYLRPGQAAAFNVTFRRAKGYPIDLYYLMDLSYSMLDDLNNVKKLGGDLLQALNEITESGRIGFGSFVD VVNKALVAGKDPVQIQPQEIQLNLRPGMPQTFQLRFKRAEGYPVDLYYLMDLSYSMKDDLANVKNLGNDILEKLNRITGNARIGFGSFVD : *:.**:: * **** :: *:: *:: *:: *:**	173 174 177
H. sapiens M. musculus D. rerio	KTVLPFVNTHPDKLRNPCPNKEKECOPPFAFRHVLKLTNNSNOFQTEVGKQLISGNLDAPEGGLDAMMQVAACPEEIGWRNVTRLLVFAT KTVLPFVNTHPEKLRNPCPNKEKACOPPFAFRHVLKLTDNSNOFQTEVGKQLISGNLDAPEGGLDAIMQVAACPEEIGWRNVTRLLVFAT KTLLPYTDTNEAKLKHPCSDKNEPCOPAFGFQHVLPLTANGNDFKELVKQQHISGNLDPPEGSLDAIMQAAVCLKEIGWGNSTRLLVLAT **:**:.:*: **::** :*:: *** *.*:*** *.*:*** ** *.*:*: * :* ****** ***.***:**	263 264 267
H. sapiens M. musculus D. rerio	DDGFHFAGDGKLGAILTPNDGRCHLEDNLYKRSNEFDYPSVGQLAHKLAENNIQPIFAVTSRMVKTYEKLTEIIPKSAVGELSEDSSNVV DDGFHFAGDGKLGAILTPNDGRCHLEDNMYKRSNEFDYPSVGQLAHKLSESNIQPIFAVTKKMVKTYEKLTEIIPKSAVGELSDDSSNVV DDGFHMAGDGKLAAILEPNKETCQLDKNKYSKSNIWDYPSVGQVARKLEEQNIQPIFAVTKKMETVYTELSKLIPKSAVGVLSEDSNNVV *****:*****************************	353 354 357
H. sapiens M. musculus D. rerio	QLIKNAYNKLSSRVFLDHNALPDTLKVTYDSFCSNGVTHRNQPRGDCDGVQINVPITFQVKVTATECIQEQSFVIRALGFTDIVTVQVLP QLIKNAYYKLSSRVFLDHSTLPDTLKVTYDSFCSNGASSIGKSRGDCDGVQINNPVTFQVKVMASECIQEQSFVIRALGFTDTVTVQVRP NLIVEAYNNLTSEVIMAHDALPNFISVKYSSKCTGGKTFSDNGKCDNVNIGLEVIFDVTVTVDRCINDQSFSIGPLGFNEKLKVNVQT :** :** :*:*.*: *.:**: *.:**:**:*: *:*** * ***:*:	443 444 445
H. sapiens M. musculus D. rerio	QCECRCRDQSRDRSLCHGKGFLECGICRCDTGYIGKNCECQTQGRSSQELEGSCRKDNNSIICSGLGDCVCGQCLCHTSDVPGKLIYGQY QCECQCRDQSREQSLCGGKGVMECGICRCESGYIGKNCECQTQGRSSQELERNCRKDNSSIVCSGLGDCICGQCVCHTSDVPNKEIFQQY LCECNCGDPPGVHPHCKGQGKVVCGSCKCNDGFIGQLCECTIGKKDEASFKAQCRKDNG-TECEGKGDCVCGRCQCHLTEGN-GHFYGEH ***.* * : * *:* : ** *:* : *:* :: :: .:: .	533 534 533
H. sapiens M. musculus D. rerio	CECDTINCERYNGQVCGGPGRGLCFCGKCRCHPGFEGSACQCERTTEGCLNPRRVECSGRGRCRCNVCECHSGYQLPLCQECPGCPSPCG CECDNVNCERYNSQVCGGSDRGSCNCGKCSCKPGYEGSACQCQRSTTGCLNARLVECSGRGHCQCNRCICDEGYQPPMCEDCPSCGSHCR CECDDEHCEKFDNKQCAGHGKCKCGKCECDDGYEGTACHCAKSDNECKIDS-HVCYNRGKCVCNQCECERGYKGPYCKTCPTCQRPCQ **** :**:::: *.* :* * **** *. *:**:**: :	623 624 620
H. sapiens M. musculus D. rerio	K-YISCAECLKFEKGPFGKNCSAACPGLQLSNN-PVKGRTCKERDSEGCWVAYTLEQQDGMDRYLIYVDESRECVAGPNIAAIVGGTVAG DNHTSCAECLKFDKGPFEKNCSVQCAGMTLQTI-PLKKKPCKERDSEGCWIYYTLQQKDGRNIYNIHVEDSLECVKGPNVAAIVGGTVVG Q-SGSCVECFAFQSGPFEKNCSLACKHLTIKIVDKLVKSDCRVKDKEGCWMAFTMTEELGFDLYSVSVLKDRECPEGPNIIAIVGGSLAG . **.**: *:.*** **** * ::. : *::*****::** ::* ::	711 713 709
H. sapiens M. musculus D. rerio	IVLIGILLLVIWKALIHLSDLREYRRFEKEKLKSQWNNDNPLF 769 VVLIGVLLVIWKALTHLTDLREYRRFEKEKLKSQWNNDNPLF 771 VALIGLLMLLIKGVLYASDLREWRRFEKDRKHEKTSGTNPLF 771 VALIGLLMLLIKGVLYASDLREWRRFEKDRKHEKTSGTNPLF 768 :.***::*:*:* *::::::::::::::::::::::::::::::::::::	

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.

CD11b

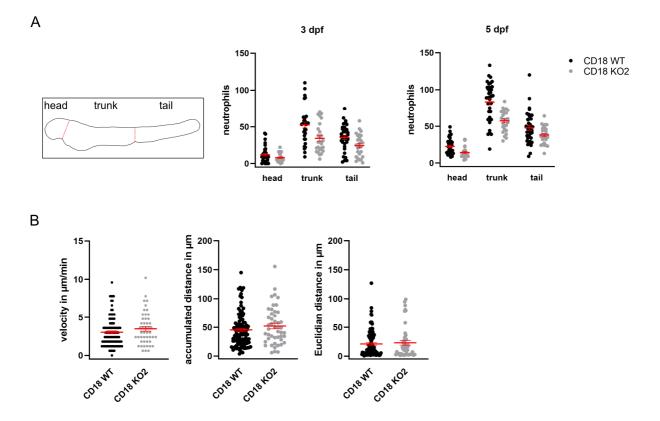
H. sapiens M. musculus D. rerio	-MALRVLLLTALTLCHGFNLDTENAMTFQENARGFGQSVVQLQGSRVVVGAPQEIVAANQRGSLYQCDYSTGSCEPIRLQVP -MTLKALLVTALALCHGFNLDTEHPMTFQENAKGFGQNVVQLGGTSVVVAAPQEAKAVNQTGALYQCDYSTSRCHPIPLQVP MMDWYSYISFCIFCVSQSVMAFNIDPASWKTFTGPSQSQNVAFGYKVIQKDTSSLIVSDPLIQINQDKRGQIYNCEVAKGTCTQLNITVP * : **:* ** *: ** *: ::* ::*: ::* *: ::*	81 81 90
H. sapiens M. musculus D. rerio	VEAVNMSLGLSLAATTSPPQLLACGPTVHQTCSENTYVKGLCFLFGSNLRQQPQKFPEALRGCPQEDSDIAFLIDGSGSIIPHDFRRMKE PEAVNMSLGLSLAVSTVPQQLLACGPTVHQNCKENTYVNGLCYLFGSNLLRPPQQFPEALRECPQQESDIVFLIDGSGSINNIDFQKMKE SEAVNMSLGFSMVQHPQSSKLAICGPTIPKNCTTATNYRGMCFIGNSGDFGPPIPK-SKYRDCL-GQIDIAFLLDGSGSIGIYDFTVMKG *********::::::::::::::::::::::::::::	171 171 178
H. sapiens M. musculus D. rerio	FVSTVMEQLKKSKTLFSLMQYSEEFRIHFTFKEFQNNPNP-RSLVKPITQLLGRTHTATGIRKVVRELFNITNGARKNAFKILVVITDGE FVSTVMEQFKKSKTLFSLMQYSDEFRIHFTFNDFKRNPSP-RSHVSPIKQLNGRTKTASGIRKVVRELFHKTNGARENAAKILVVITDGE FVTNVIRRFIERDAQFAIAQYSNDCDIHYNFNDLKLDDGTWESKVANIPYHEGGTFTASAIQKLVNYLFTPNGGTRPSAKKILVVITDGE **:.*:::::::::::::::::::::::::::::::::	260 260 268
H. sapiens M. musculus D. rerio	KFGDPLGYEDVIPEADREGVIRYVIGVGDAFRSEKSRQELNTIASKPPRDHVFQVNNFEALKTIQNQLREKIFAIEGTQTGSSSSFEHEM KFGDPLDYKDVIPEADRAGVIRYVIGVGNAFNKPQSRRELDTIASKPAGEHVFQVDNFEALNTIQNQLQEKIFAIEGTQTGSTSSFEHEM SHDRNL-LKDAASQAEKNSIVRFAIGVGKAFDYYNAREELNTIASDPDTDYVFKVTDFNALKNILQKLEGNIIAIEGTQTSGDSS-RMEF * :*. :*:: .::*:.******* :::******* :::*******	350 350 356
H. sapiens M. musculus D. rerio	SQEGFSAAITSNGPL-LSTVGSYDWAGGVFLYTSKEKSTFINMTRVDSDMNDAYLGYAAAIILRNRVQSLVLGAPRYQHIGLVAMFRQNT SQEGFSASITSNGPL-LGSVGSFDWAGGAFLYTSKDKVTFINTTRVDSDMNDAYLGYASAVILRNRVQSLVLGAPRYQHIGLVVMFRENF AQDGFSTAFTSSGSVLVSAVGAFQWKGGYRDNSQEGSFQTGSEHESYQGYSMAVATVSLTSFAILGAPRYQHRGQVTISRIGT :*:***:::*** : ::***:* * :::****	439 439 439
H. sapiens M. musculus D. rerio	GMWESNANVKGTQIGAYFGASLCSVDVDSNGSTDLVLIGAPHYYEQTRGGQVSVCPLPRGRARWQCDAVLYGEQGQPWGRFGAALTVLGD GTWEPHTSIKGSQIGSYFGASLCSVDMDADGNTNLILIGAPHYYEKTRGGQVSVCPLPRGRARWQCEALLHGDGHPWGRFGAALTVLGD NEVQPLD-SPMPQIGSYFGAEVCVVDLNSDSYTDLLLVSAPTYTESEREGKVFVYSFTSWSRLFFSDVVLVGMAG-QRGRFGSSLASPAD . : ***:****.:* **:::: *:*:** * * * * *	529 529 527
H. sapiens M. musculus D. rerio	VNGDKLTDVAIGAPGEEDNRGAVYLFHGTSGSGISPSHSQRIAGSKLSPRLQYFGQSLSGG-QDLTMDGLVDLTVGAQGHVLLLRSQPVL VNGDKLTDVAIGAPGEQENQGAVYIFYGASIASLSASHSHRIIGAHFSPGLQYFGQSLSGG-KDLTMDGLMDLAVGAQGHLLLLRAQPVL LNGDGYRDVLVGAPLEEEGQGSIYIFNGRDGG-IISSYSQRIAGSSVKSGLQYFGVSLSQSSPDQTADSLPDIAAGSKGAVLLLRSRPIM :*** ** :*** *:*:*:** *: *:*:** *: ***** ***	618 618 616
H. sapiens M. musculus D. rerio	RVKAIMEFNPREVARNVFECNDQVVKGKEAGEVRVCLHVQKSTRDRLREGQIQSVVTYDLALDSGRPHSRAVFNETKNSTRRQTQVLGLT RLEATMEFSPKKVARSVFACQEQVLNNKDAGEVRVCLRVRKNTKDRLREGDIQSTVTYDLALDPVRSRIRAFFDETKNNTRRRTQVFGLM LLETMVSYTPSKISTIQQDCLIPLQTTLTVCFTMKGYRHHRRGLNAKIAYNITLDAKRQAYRAFFSAKERL-LSDVIDIGST ::: :.:.* ::: * : : : ::::::* * ::: :::::::	708 708 697
H. sapiens M. musculus D. rerio	QTCETLKLQLPNCIEDPVSPIVLRLNFSLVGTPLSAFGNLRPVLAEDAQRLFTALFPFEKNCGNDNICQDDLSITFSFMSLDCLVVG QKCETLKLILPDCVDDSVSPIILRLNYTLVGEPLRSFGNLRPVLAMDAQRFFTAMFPFEKNCGNDSICQDDLSITMSAMGLDTLVVG EACNNHNFSIEACPEDALNPLSNQLKFTFEGLPSSQMQNLRPILLPEIKTTSDHDVRVHLCKVTSGNDALLTVGYITVGF : *:. :: : * :* :: : : :: : : : : : : :	795 795 777
H. sapiens M. musculus D. rerio	GPREFNVTVTVRNDGEDSYRTQVTFFFPLDLSYRKVSTLQNQRSQRSWRLA-CESASSTEVSGALKSTSCSINHPIFPENSEVTFNITFD GPQDFNMSVTLRNDGEDSYGTQVTVYYPSGLSYRKDSASQNPLTKKPWFVKPAESSSSSEGHGALKSTTWNINHPIFPANSEVTFNVTFD 	884 885 780
H. sapiens M. musculus D. rerio	VDSKASLGNKLLLKANVTSENNMPRTNKTEFQLELPVKYAVYMVVTSHGVSTKYLNFTASENTSRVMQHQYQVSNLGQRSLPISLVFLVP VDSHASFGNKLLLKAIVASENNMSRTHKTKFQLELPVKYAIYMIVTSDESSIRYLNFTASEMTSKVIQHQYQFNNLGQRSLPVSVVFWIP	974 975 780
H. sapiens M. musculus D. rerio	VRLNQTVIWDRPQVTFSENLSSTCHTKERLPSHSDFLAELRKAPVVNCSIAVCQRIQCDIPFFGIQEEFNATLKGNLSFDWYIKTSHNHL VQINNVTVWDHPQVIFSQNLSSACHTEQKSPPHSNFRDQLERTPVLNCSVAVCKRIQCDLPSFNTQEIFNVTLKGNLSFDWYIKTSHGHL	1064 1065 780
H. sapiens M. musculus D. rerio	LIVSTAEILFNDSVFTLLPGQGAFVRSQTETKVEPFEVPNPLPLIVGSSVGGLLLLALITAALYKLGFFKRQYKDMMSEGGPPGAEPQ LLVSSTEILFNDSAFALLPGQESYVRSKTETKVEPYEVHNPVPLIVGSSIGGLVLLALITAGLYKLGFFKRQYKDMMNEAAPQDAPPQ	1152 1153 780

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.

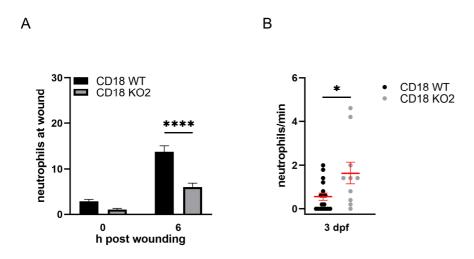


Supplementary Figure 3: Generation of CD18 KO2 zebrafish. (A) Upper panel: Schematic of *itgb2* gene and target exon 2 of the gRNA2. Middle panel: Sequencing traces and partial genomic sequence of WT and CD18 mutant 2 (mut. 2). Numbers indicate position within the gene. Red boxes show nucleotides deleted 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 μ m. Right: Total neutrophil counts in CD18 WT and CD18 KO2 zebrafish larvae at 3 dpf and 5 dpf. Mean \pm sem of \geq 26 individual larvae of \geq 3 independent experiments.



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 \pm sem of ≥ 26 individual larvae of ≥ 3 independent experiments. (B) Mean migration velocity, accumulated and Euclidian distance of individual neutrophils. Mean \pm sem of ≥ 47 individual neutrophils of 3 independent experiments. Unpaired t-test.



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 \pm sem of \geq 14 individual larvae of \geq 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 \pm sem of \geq 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.