

Toll-like receptor adaptor protein TIRAP has specialized roles in signaling, metabolic control and leukocyte migration upon wounding in zebrafish larvae

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Supplementary material

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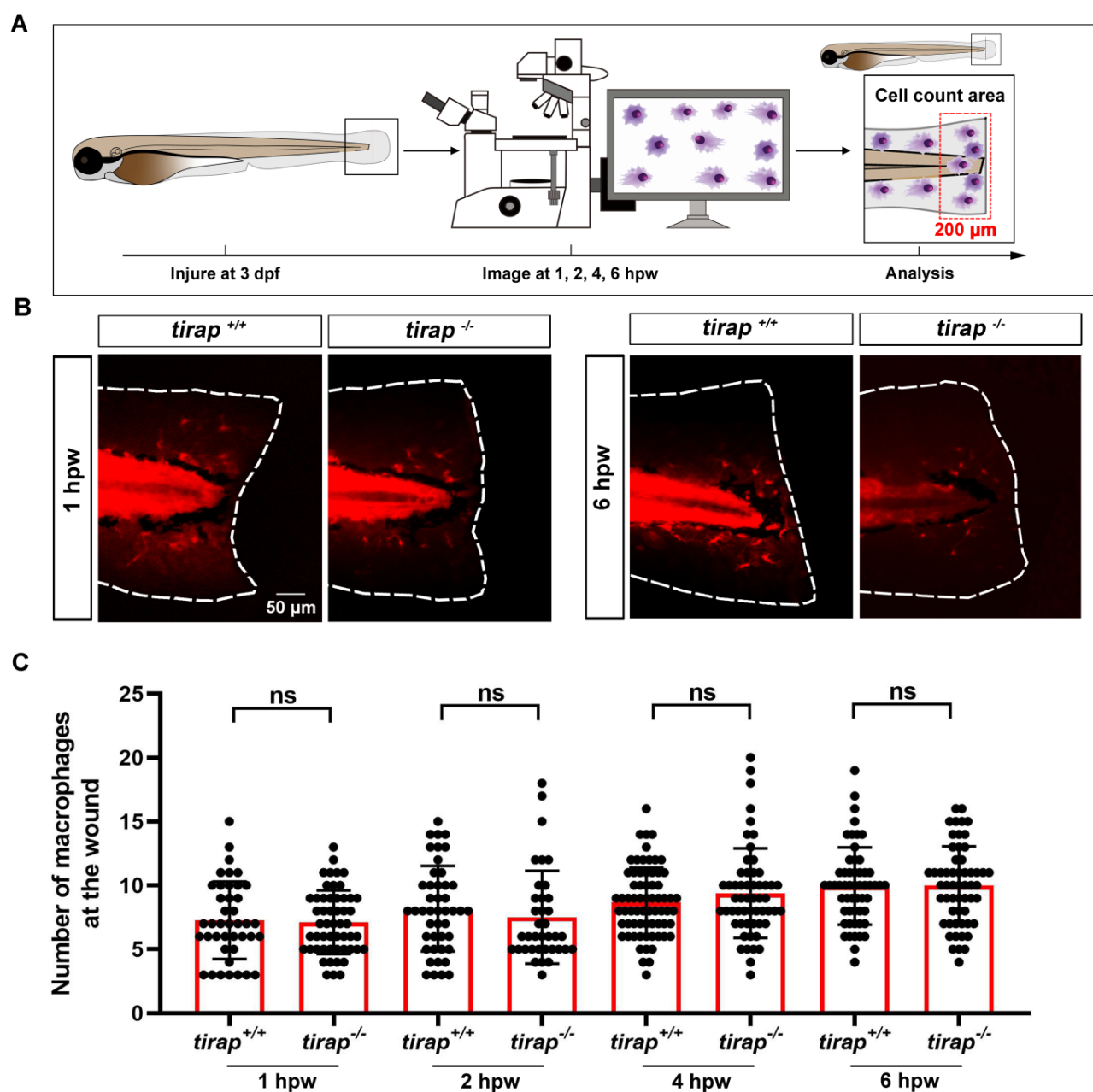


Figure S1. The number of recruited macrophages upon tail wounding in zebrafish larvae. (A) Schematic representation of experiment. The zebrafish larvae were imaged at designated times 1 hour post wounding (hpw), 2hpw, 4 hpw and 6 hpw. (B) Representative images of *tirap*^{+/+} and *tirap*^{-/-} at 1 hpw and 6 hpw. Scale bar: 50 μ m. (C) Number of macrophages recruited to tail region upon tail wounding at 1, 2, 4, 6 hpw. The number of macrophages at 1 hpw from two independent experiments for *tirap*^{+/+} (n=41) and *tirap*^{-/-} (n=53). At 2 hpw for *tirap*^{+/+} (n=45) and *tirap*^{-/-} (n=37), at 4 hpw for *tirap*^{+/+} (n=68) and *tirap*^{-/-} (n=56) and at 6 hpw for *tirap*^{+/+} (n=55) and *tirap*^{-/-} (n=56) were based on three independent experiments. Statistical significant difference was determined by nonparametric tests: Mann-Whitney and Kolmogorov-Smirnov, ns, non-significant, *, $P < 0.05$, **, $P < 0.01$, ****, $P < 0.0001$.

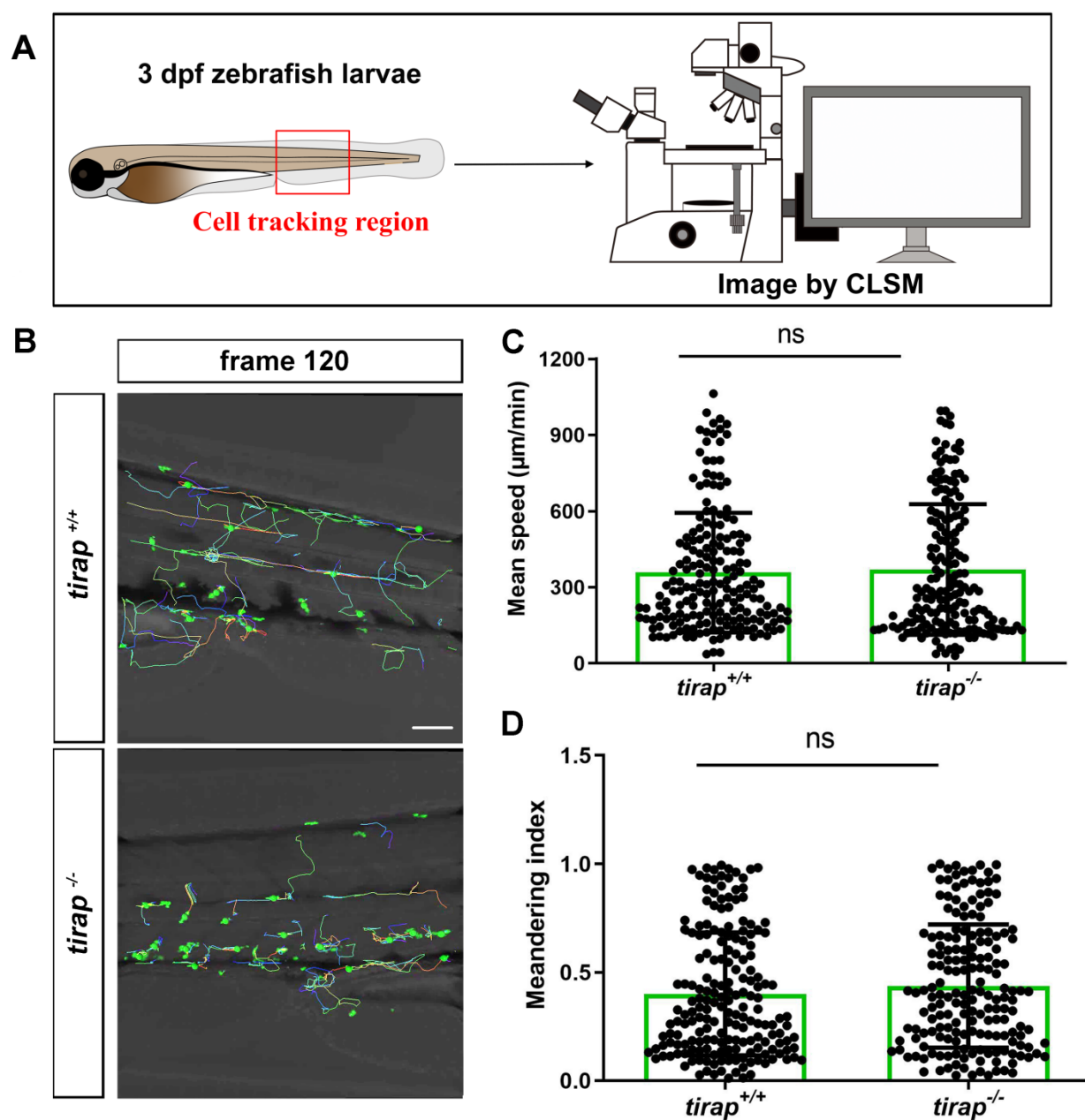


Figure S2. Quantification of neutrophil behaviour under unchallenged conditions. (A) Schematic representation of experiment. (B) 23
 Representative image of neutrophil behavior at frame 120. (C-D) Quantification of basal neutrophil migratory capability of 3 dpf 24
tirap larvae at caudal hematopoietic tissue (CHT). Mean speed (C) and meandering index (D) were quantified using IMARIS 25
 Software (Imaris (RRID:SCR_007370)). The mean speed of neutrophils is measured as total displacement of neutrophils divided 26
 by the number of frames. Data is combined from 2 independent experiments for both *tirap*^{+/+} (N=4) and *tirap*^{-/-} (N=4). Statistical 27
 significant difference was determined by unpaired t test with Welch's correction, ns, non-significant. 28

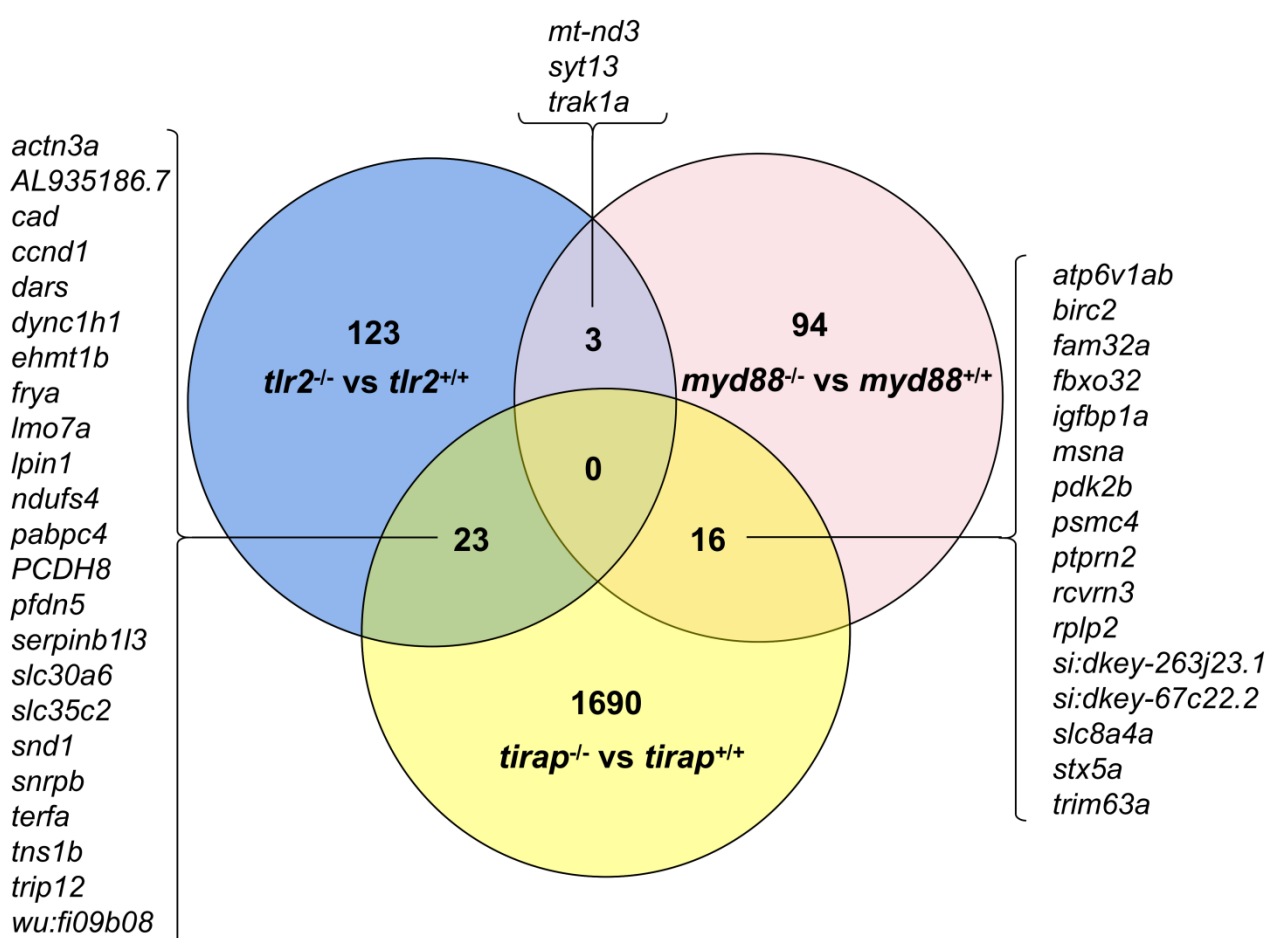


Figure S3. Venn diagram of DEGs from *tirap^{-/-}* vs *tirap^{+/+}* group, *tlr2^{-/-}* vs *tlr2^{+/+}* group and *myd88^{-/-}* vs *myd88^{+/+}* group. DEGs from *tirap^{-/-}* vs *tirap^{+/+}* group and *myd88^{-/-}* vs *myd88^{+/+}* group were assessed by padj value less than 0.05. DEGs from *tlr2^{-/-}* vs *tlr2^{+/+}* group were assessed by svalue less than 0.05 [23].

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Table S1. The qRT-PCR primers used in this study

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Primers	Sequences	Application
<i>il1b</i> -F	5'-GAACAGAATGAAGCACATCAAACC-3'	qRT-PCR
<i>il1b</i> -R	5'-ACGGCACTGAATCCACCAC-3'	qRT-PCR
<i>il6</i> -F	5'...TTCATGAGTCTCGCTGACCC...3'	qRT-PCR
<i>il6</i> -R	5'...GAGGAGTGCTGATCCTGACC...3'	qRT-PCR
<i>il8</i> -F	5'...TGTGTTATTGTTTTCTGGCATTTC...3'	qRT-PCR
<i>il8</i> -R	5'...GCGACAGCGTGGATCTACAG...3'	qRT-PCR
<i>il10</i> -F	5'...GGAGACCATTCTGCCAACAGC...3'	qRT-PCR
<i>il10</i> -R	5'...TCTTGCAATTCACCATATCCCG...3'	qRT-PCR
<i>tnfa</i> -F	5'...AGACCTTAGACTGGAGAGATGAC...3'	qRT-PCR
<i>tnfa</i> -R	5'...CAAAGACACCTGGCTGTAGAC...3'	qRT-PCR
<i>cebpb</i> -F	5'...GCAGGCAACCTATCACCTACATAC...3'	qRT-PCR
<i>cebpb</i> -R	5'...CGCAAGTTTCACCGACTACAAGT...3'	qRT-PCR
<i>fosl1a</i> -F	5'...CTCAGCCCTCCCAATCACATCT...3'	qRT-PCR
<i>fosl1a</i> -R	5'...TACTTTCGCCGCAGCCATT...3'	qRT-PCR
<i>ppial</i> -F	5'...TGAGCCGCAACAGTAATC...3'	Reference
<i>ppial</i> -R	5'...AAGGGAAAAGGAAGTGAAAG...3'	Reference

Table S2. Differentially expressed genes (DEGs) in the representative pathway in *tirap*^{-/-} versus *tirap*^{+/+} zebrafish larvae groups.

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Pathway	Ensembl ID	Gene name	log2 Fold Change	padj-Value	Description
TLR signaling	ENSDARG00000099184	<i>map2k6</i>	-1.510915968	0.000740717	mitogen-activated protein kinase kinase 6
	ENSDARG00000070573	<i>mapk3</i>	-1.668589839	4.00E-05	mitogen-activated protein kinase 3
	ENSDARG00000023110	<i>mapk7</i>	-1.376786107	0.015633315	mitogen-activated protein kinase 7
	ENSDARG00000042725	<i>cebpb</i>	-1.887025193	6.25E-05	CCAAT enhancer binding protein beta
	ENSDARG00000074378	<i>junba</i>	-1.628961987	0.003284556	JunB proto-oncogene, AP-1 transcription factor subunit a
	ENSDARG00000020133	<i>jdp2b</i>	-2.287062064	0.000234706	Jun dimerization protein 2b
	ENSDARG00000067850	<i>jund</i>	-1.188760964	0.012711251	JunD proto-oncogene, AP-1 transcription factor subunit
	ENSDARG00000037116	<i>cxcl12a</i>	-1.235618795	0.025460758	chemokine (C-X-C motif) ligand 12a (stromal cell-derived factor 1)
	ENSDARG00000004196	<i>tnfsf10l</i>	-1.24722621	0.02313739	TNF superfamily member 10, like
	ENSDARG00000098377	<i>tnfrsf11b</i>	7.489464879	0.023864106	tumor necrosis factor receptor superfamily, member 11b
	ENSDARG00000037553	<i>il1rapl2</i>	8.105000555	0.00416451	interleukin 1 receptor accessory protein-like 2
	ENSDARG00000074667	<i>akt1s1</i>	1.279698923	0.005125106	AKT1 substrate 1 (proline-rich)
	ENSDARG00000011219	<i>akt2</i>	1.2700915	0.00966533	v-akt murine thymoma viral oncogene homolog 2
	ENSDARG00000087205	<i>akt3b</i>	8.297200628	1.50E-05	v-akt murine thymoma viral oncogene homolog 3b
Glycolysis and TCA cycle	ENSDARG00000060504	<i>pfkla</i>	6.037617359	0.02513267	phosphofructokinase, liver a
	ENSDARG00000054191	<i>pgk1</i>	1.065360736	0.023951103	phosphoglycerate kinase 1
	ENSDARG00000039093	<i>got1</i>	1.463607063	0.02926267	glutamic-oxaloacetic transaminase 1, soluble
	ENSDARG00000042010	<i>pklr</i>	-1.376344872	0.00580824	pyruvate kinase L/R
	ENSDARG00000059054	<i>pdk2b</i>	1.197079899	0.046865975	pyruvate dehydrogenase kinase, isozyme 2b
	ENSDARG00000076308	<i>pdp1</i>	7.881587716	0.014198245	pyruvate dehydrogenase phosphatase catalytic subunit 1
	ENSDARG00000043371	<i>mdh2</i>	0.960102902	0.036123462	malate dehydrogenase 2, NAD (mitochondrial)
	ENSDARG00000005359	<i>sucla2</i>	1.140335947	0.048819898	succinate-CoA ligase, ADP-forming, beta subunit
	ENSDARG00000103364	<i>cs</i>	1.082934649	0.004366919	citrate synthase
	ENSDARG00000055652	<i>aclyb</i>	-2.434685739	0.025909141	ATP citrate lyase b
Autophagy	ENSDARG00000040657	<i>wipi1</i>	-1.401387052	0.033030684	WD repeat domain, phosphoinositide interacting 1
	ENSDARG00000075139	<i>hdac5</i>	-2.190324537	0.031243992	histone deacetylase 5
	ENSDARG00000086458	<i>hdac10</i>	-1.105818447	0.035662922	histone deacetylase 10

	ENSDARG00000062518	<i>ULK1A</i>	9.354760047	5.73E-07	unc-51 like autophagy activating kinase 1a
	ENSDARG00000040277	<i>FBXO32</i>	1.768701643	0.003885178	F-box protein 32
	ENSDARG00000055292	<i>ATG9B</i>	1.368894618	0.007208796	autophagy related 9B
	ENSDARG00000052818	<i>SMCR8A</i>	7.776146188	0.038195224	Smith-Magenis syndrome chromosome region, candidate 8a
	ENSDARG00000097245	<i>SOGA3A</i>	9.984814829	2.00E-08	SOGA family member 3a
	ENSDARG00000037871	<i>WIP12</i>	2.352236748	1.07E-05	WD repeat domain, phosphoinositide interacting 2
	ENSDARG00000037560	<i>MTM1</i>	6.044536193	0.001407213	myotubularin 1
	ENSDARG00000056842	<i>USP30</i>	7.613615523	0.016816102	ubiquitin specific peptidase 30
	ENSDARG00000036338	<i>VPS11</i>	1.562483319	0.013571365	vacuolar protein sorting 11
Ribosome	ENSDARG00000053457	<i>RPL23</i>	-1.31832882	0.011619987	ribosomal protein L23
	ENSDARG00000099104	<i>RPL24</i>	-1.221610002	0.031143469	ribosomal protein L24
	ENSDARG00000005791	<i>RPL28</i>	-1.221797114	0.027097835	ribosomal protein L28
	ENSDARG00000077717	<i>RPL29</i>	-1.367320533	0.002203954	ribosomal protein L29
	ENSDARG00000035871	<i>RPL30</i>	-1.211077303	0.023579704	ribosomal protein L30
	ENSDARG00000036316	<i>RPL39</i>	-1.09012718	0.048576788	ribosomal protein L39
	ENSDARG00000011201	<i>RPLP21</i>	-1.218283147	0.023013393	ribosomal protein, large P2, like
	ENSDARG00000036298	<i>RPS13</i>	-1.340170625	0.014103277	ribosomal protein S13
	ENSDARG00000030602	<i>RPS19</i>	-1.174866323	0.041930897	ribosomal protein S19
	ENSDARG00000025850	<i>RPS21</i>	-1.109696313	0.021019913	ribosomal protein S21
	ENSDARG00000041811	<i>RPS25</i>	-1.219167475	0.027363843	ribosomal protein S25
	ENSDARG00000037071	<i>RPS26</i>	-1.167312016	0.026665233	ribosomal protein S26
	ENSDARG00000041232	<i>RPS29</i>	-1.222203934	0.014053931	ribosomal protein S29
	Oxidative phosphorylation	ENSDARG00000052840	<i>NDUFS4</i>	-1.151745645	0.038414796
ENSDARG00000042469		<i>NDUFA12</i>	-1.559292267	0.010102699	NADH:ubiquinone oxidoreductase subunit A12
ENSDARG00000045230		<i>COX6B1</i>	-1.088023429	0.014277173	cytochrome c oxidase subunit 6B1
ENSDARG00000027355		<i>SLC25A4</i>	1.220084402	0.048494089	solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 4
Calcium regulation	ENSDARG00000042552	<i>CACNA1SB</i>	1.391654176	0.02922106	calcium channel, voltage-dependent, L type, alpha 1S subunit, b
	ENSDARG00000078169	<i>CACNA2D2B</i>	8.211738729	0.00663626	calcium channel, voltage-dependent, alpha 2/delta subunit 2b
	ENSDARG00000063006	<i>CACNG7A</i>	7.349292843	0.034747364	calcium channel, voltage-dependent, gamma subunit 7a
	ENSDARG00000099241	<i>PPP2CB</i>	-1.464703532	0.004401995	protein phosphatase 2, catalytic subunit, beta isozyme

ENSDARG00000102009	<i>ppp2r2d</i>	-2.224775103	9.14E-07	protein phosphatase 2, regulatory subunit B, delta
ENSDARG00000059083	<i>ppp2r5ca</i>	2.239352665	0.006191032	protein phosphatase 2, regulatory subunit B', gamma a
ENSDARG00000031200	<i>ppp2r5cb</i>	1.32778729	0.01328537	protein phosphatase 2, regulatory subunit B', gamma b
ENSDARG00000043406	<i>slc8a1b</i>	9.089887066	1.58E-05	solute carrier family 8 (sodium/calcium exchanger), member 1b
ENSDARG00000055154	<i>slc8a4a</i>	7.727366289	0.015953285	solute carrier family 8 (sodium/calcium exchanger), member 4a
ENSDARG00000097339	<i>slc9a1b</i>	7.833197174	0.008092416	solute carrier family 9 member A1b
ENSDARG00000012684	<i>atp2b1a</i>	-1.571028762	0.00190273	ATPase plasma membrane Ca ²⁺ transporting 1a
ENSDARG00000023445	<i>atp2b3b</i>	-2.237791493	5.07E-08	ATPase plasma membrane Ca ²⁺ transporting 3b
ENSDARG00000079564	<i>myh7</i>	1.452430669	0.029242822	myosin heavy chain 7
ENSDARG00000099959	<i>smyhc1</i>	0.974799495	0.041781027	slow myosin heavy chain 1
ENSDARG00000103969	<i>smyhc2</i>	2.241942444	4.94E-07	slow myosin heavy chain 2
ENSDARG00000100825	<i>calm3a</i>	-1.809712548	2.21E-06	calmodulin 3a (phosphorylase kinase, delta)
ENSDARG00000115657	<i>prkcaa</i>	-1.077143247	0.010520081	protein kinase C, alpha, a
ENSDARG00000033184	<i>prkar2aa</i>	-1.041567526	0.034767684	protein kinase, cAMP-dependent, regulatory, type II, alpha A
ENSDARG00000053586	<i>creb1a</i>	-1.357761461	0.021504733	cAMP responsive element binding protein 1a

Table S3. Differentially expressed genes (DEGs) in the representative pathway in *myd88*^{-/-} versus *myd88*^{+/+} zebrafish larvae groups.

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Pathway	Ensembl ID	Gene name	log2 Fold Change	padj-Value	Description
TLR signaling	ENSDARG00000006409	<i>mapk12b</i>	4.820780436	0.022554316	mitogen-activated protein kinase 12b
Glycolysis and TCA cycle	ENSDARG00000020364	<i>fbp1b</i>	1.753309151	0.036384064	fructose-1,6-bisphosphatase 1b
	ENSDARG00000059054	<i>pdk2b</i>	1.938947554	5.01E-06	pyruvate dehydrogenase kinase, isozyme 2b
Ribosome	ENSDARG00000112656	<i>rpl36a</i>	-1.469863763	0.013985859	ribosomal protein L36A
	ENSDARG00000006413	<i>rpl38</i>	-1.472779904	0.019617391	ribosomal protein L38
	ENSDARG00000101406	<i>rplp2</i>	-1.394220944	0.040024964	ribosomal protein, large P2
Oxidative phosphorylation	ENSDARG00000076318	<i>atp6v1ab</i>	1.732618721	0.000799351	ATPase H ⁺ transporting V1 subunit Ab
	ENSDARG00000063914	<i>mt-nd3</i>	-1.350342543	0.025536145	NADH dehydrogenase 3, mitochondrial
	ENSDARG00000110786	<i>ndufb4</i>	-9.567162039	5.37E-08	NADH:ubiquinone oxidoreductase subunit B4
	ENSDARG00000103101	<i>ndufc1</i>	-1.588330341	0.020847424	si:ch211-235e9.6
	ENSDARG00000069464	<i>cox7a1</i>	-2.271411629	0.002852318	cytochrome c oxidase subunit 7A1
Calcium regulation	ENSDARG00000055154	<i>slc8a4a</i>	6.94081136	0.010673858	solute carrier family 8 (sodium/calcium exchanger), member 4a
	ENSDARG00000053617	<i>camk2a</i>	4.238435243	0.044672365	calcium/calmodulin-dependent protein kinase II alpha
	ENSDARG00000046079	<i>cacng6b</i>	1.865932062	0.017048177	calcium channel, voltage-dependent, gamma subunit 6b
	ENSDARG00000033683	<i>tpma</i>	-1.398430746	0.012743848	alpha-tropomyosin

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Table S4. 6 significantly altered metabolites in *tirap*^{-/-} versus *tirap*^{+/+} zebrafish larvae groups.

HMDB ID	Metabolite name	log2 Fold Change	P value
HMDB0000162	Proline	-0.972289172	0.0017707
HMDB0059655	2-Hydroxyglutarate	-0.933348095	0.00534
HMDB0000191	Asparagine	-3.248860793	0.0056096
HMDB0000122	Glucose	-0.998747512	0.0071217
HMDB0000187	Serine	-4.390942773	0.016276
HMDB0000904	Citrulline	-2.326894348	0.022583

Table S5. 5 significantly altered metabolites in *myd88*^{-/-} versus *myd88*^{+/+} zebrafish larvae groups.

HMDB ID	Metabolite name	log2 Fold Change	P value
HMDB0000187	Serine	1.374635145	0.039828
HMDB0000517	Arginine	0.61653258	0.014019
HMDB0059655	2-Hydroxyglutarate	0.552629638	0.0090725
HMDB0000191	Aspartate	-0.562953575	0.023516
HMDB0000687	Leucine	-2.106980756	0.03105