Supplementary Information

The Lipid Receptor G2A (GPR132) Mediates Macrophage Migration in Nerve Injury-Induced Neuropathic Pain

Tabea Osthues [1], Béla Zimmer [2], Vittoria Rimola [2], Kevin Klann [3], Karin Schilling [2], Praveen Mathoor [4], Carlo Angioni [2], Andreas Weigert [4], Gerd Geisslinger [1,2], Christian Münch [5], Klaus Scholich [1,2] and Marco Sisignano [1,2,*]

[1] Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Branch for Translational Medicine and Pharmacology TMP, Theodor-Stern-Kai 7, 60596 Frankfurt am Main, Germany; Tabea.Osthues@ime.fraunhofer.de (T.O.); geisslinger@em.uni-frankfurt.de (G.G.); scholich@em.uni-frankfurt.de (K.S.)

[2] Institute of Clinical Pharmacology, *pharmazentrum frankfurt/ZAFES*, University Hospital, Goethe-University, D-60590 Frankfurt am Main, Germany; Zimmer@med.uni-frankfurt.de (B.Z.); rimola@med.uni-frankfurt.de (V.R.); Schilling@med.uni-frankfurt.de (K.S.); angioni@em.uni-frankfurt.de (C.A.)

[3] Institute of Biochemistry II, Faculty of Medicine, University Hospital, Goethe-University, D-60590 Frankfurt am Main, Germany; klann@em.uni-frankfurt.de

[4] Institute of Biochemistry I, Faculty of Medicine, Goethe-University, D-60590 Frankfurt am Main, Germany, Frankfurt Cancer Institute, D-60590 Frankfurt am Main, Germany; Cardio-Pulmonary Institute, D-60590 Frankfurt am Main, Germany; mathoor@biochem.uni-frankfurt.de (P.M.); weigert@biochem.uni-frankfurt.de (A.W.)

[5] Institute of Biochemistry II, Faculty of Medicine, University Hospital, Goethe-University, D-60590 Frankfurt am Main, Germany; Frankfurt Cancer Institute, D-60590 Frankfurt am Main, Germany; Cardio-Pulmonary Institute, D-60590 Frankfurt am Main, Germany; ch.muench@em.uni-frankfurt.de

* Correspondence: Marco.Sisignano@med.uni-frankfurt.de; Tel.: +49 69-6301-87819

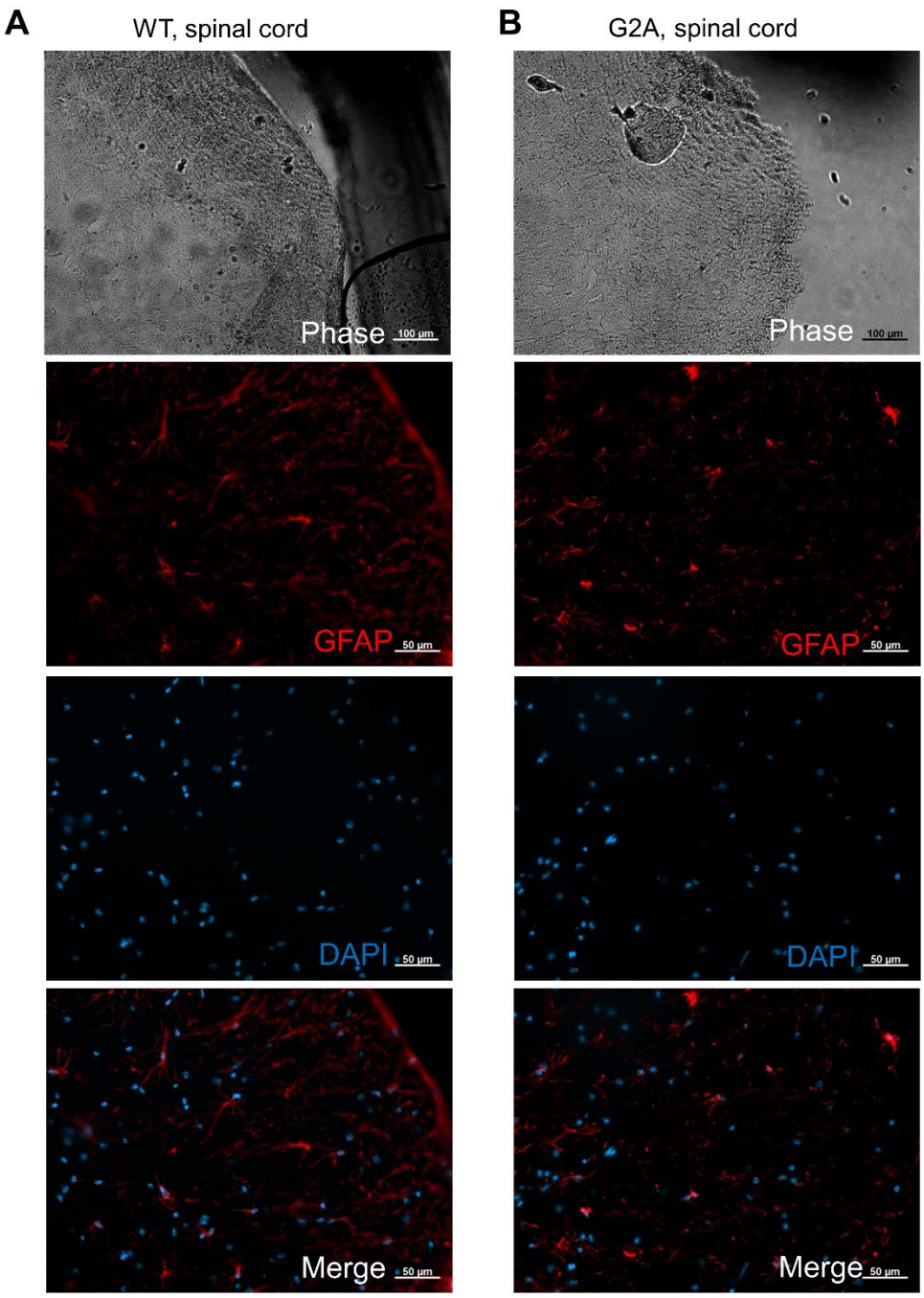

Figure S1. Activated astrocytes in spinal cord 7d after SNI. (**A**) Representative immunohistochemistry images of astrocyte staining (GFAP) of ipsilateral dorsal site of spinal cord in wild-type mice. (**B**) Representative immunohistochemistry images of astrocyte staining (GFAP) of ipsilateral dorsal site of spinal cord in male wild-type mice.

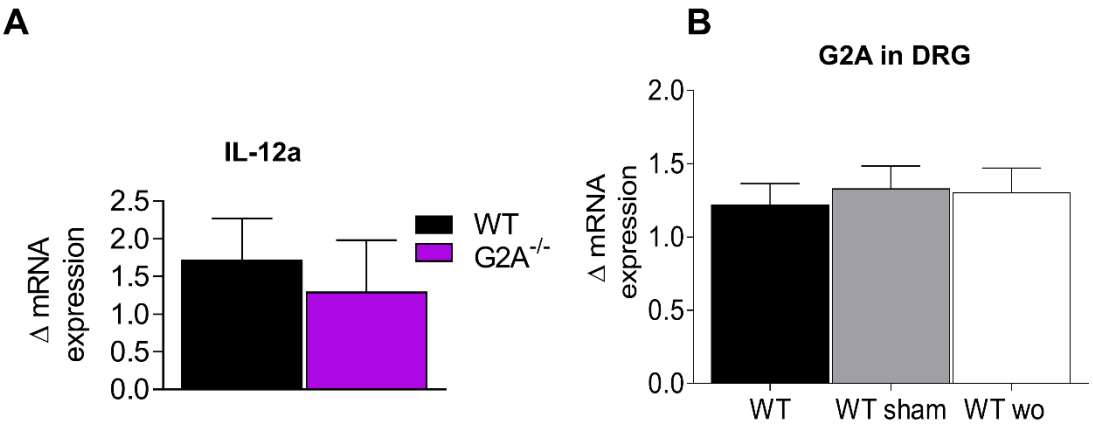

Figure S2. Cytokine and chemokine production in nervous tissue after SNI surgery. (**A**) Relative mRNA expression of IL-12a in DRGs 7d after SNI in WT and G2A$^{-/-}$ mice, n=3 male mice per group. (**B**) Relative mRNA expression of G2A receptor in ipsilateral site of L4 to L6 DRG in wild-type mice (WT) 7 d after SNI, after sham-surgery (WT sham) or without treatment (WT wo). n=8-10 male and female animals per group. WT ipsilateral site is shown in black, WT sham site in grey and WT without any treatment (WT wo) in white. G2A$^{-/-}$ ipsilateral site is depicted in in purple. Data represents mean ± SEM. *$p<0.05$, one-way ANOVA.

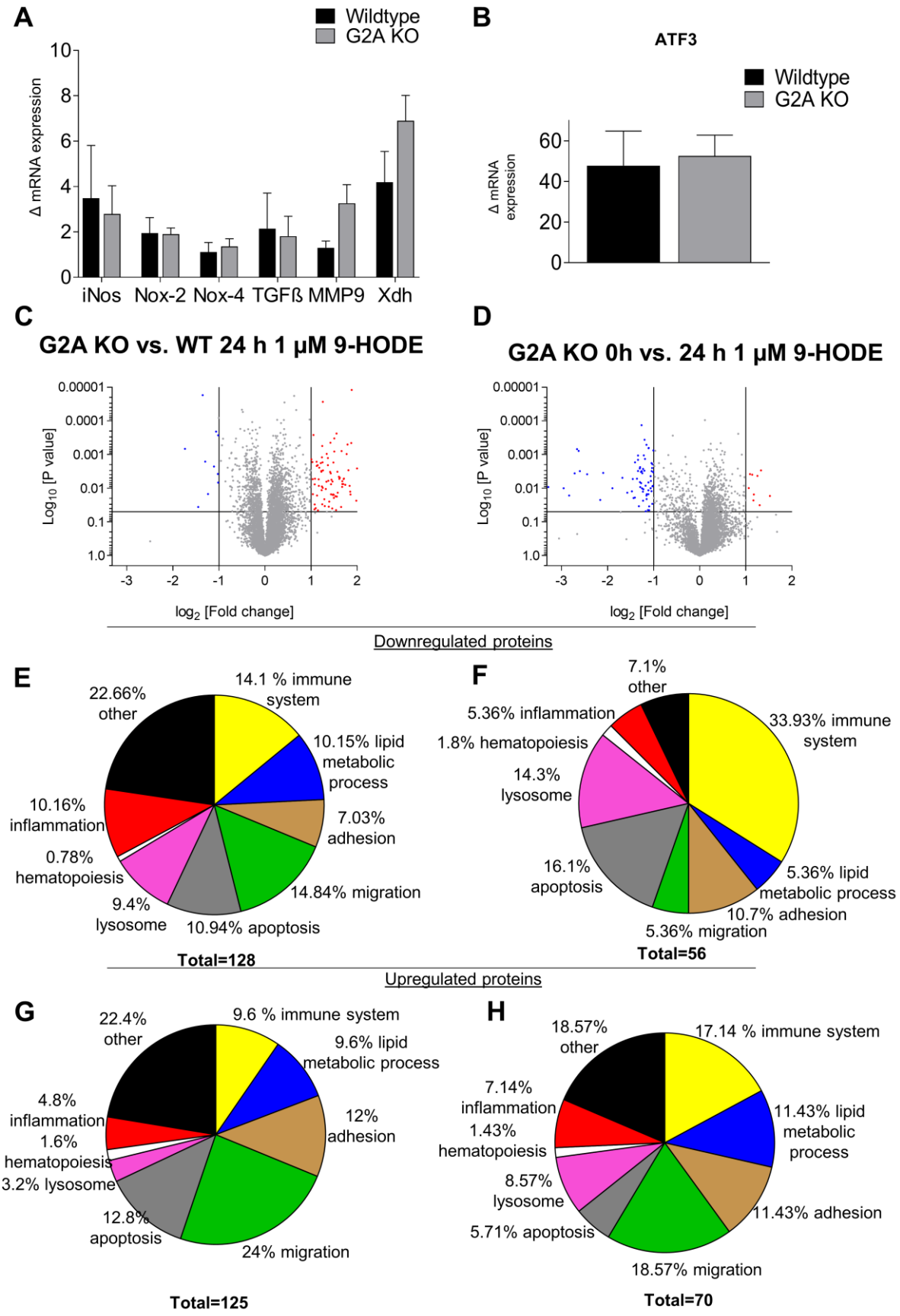

Figure S3. Stress reactions in G2A-deficient mice and in BMDMs after stimulation with 9-HODE. (**A**) Relative mRNA expression of oxidative stress markers in dorsal root ganglia (DRGs) of wild-type (black) and G2A-deficient (G2A-KO, grey) mice 7 d after SNI surgery: inducible nitric oxide synthase (iNOS), NADPH oxidase 2 (NOX-2) and NOX-4, xanthine dehydrogenase (Xdh),

matrixmetalloproteinase 9 (MMP9) and transforming growth factor β (TGFβ). Two-way ANOVA with Bonferroni's post-hoc test. (**B**) Relative mRNA expression of neuronal stress marker activating transcription factor 3 (ATF3) in DRGs of wild-type (black) and G2A-deficient mice (grey) 7 d after SNI surgery. n=4-8 animals per group, male. Data represents mean ± SEM, unpaired t-test. (**C**) Volcano plot of > 6.000 regulated proteins after 24 h stimulation with 1 μM 9-HODE in G2A-deficient BMDMs compared with wild-type mice (WT). (**D**) Volcano plot of > 6.000 regulated proteins in G2A-deficient BMDMs without and after 24 h stimulation with 1 μM 9. Fold change (FC) [log2] is plotted against *P* values [Log10]. Significant downregulated proteins are depicted in blue, red represents upregulated proteins. (**E**) Percentage of downregulated proteins in G2A-deficient BMDMs 24 h after 1 μM 9-HODE stimulation compared to treated WT. (**F**) Percentage of downregulated proteins in G2A-deficient BMDMs 24 h after 1 μM 9-HODE stimulation compared to untreated G2A-deficient BMDMs, clustered in groups. (**G**) Percentage of upregulated proteins in G2A-deficient BMDMs 24 h after 1 μM 9-HODE stimulation compared to treated WT. (**H**) Percentage of upregulated proteins in G2A-deficient BMDMs 24h after 1 μM 9-HODE stimulation compared to untreated G2A-deficient BMDMs, clustered in groups. yellow: immune system, blue: lipid metabolic process, brown: adhesion, green: migration, grey: apoptosis, pink: lysosome, red: inflammation, white: hematopoiesis, black: other.

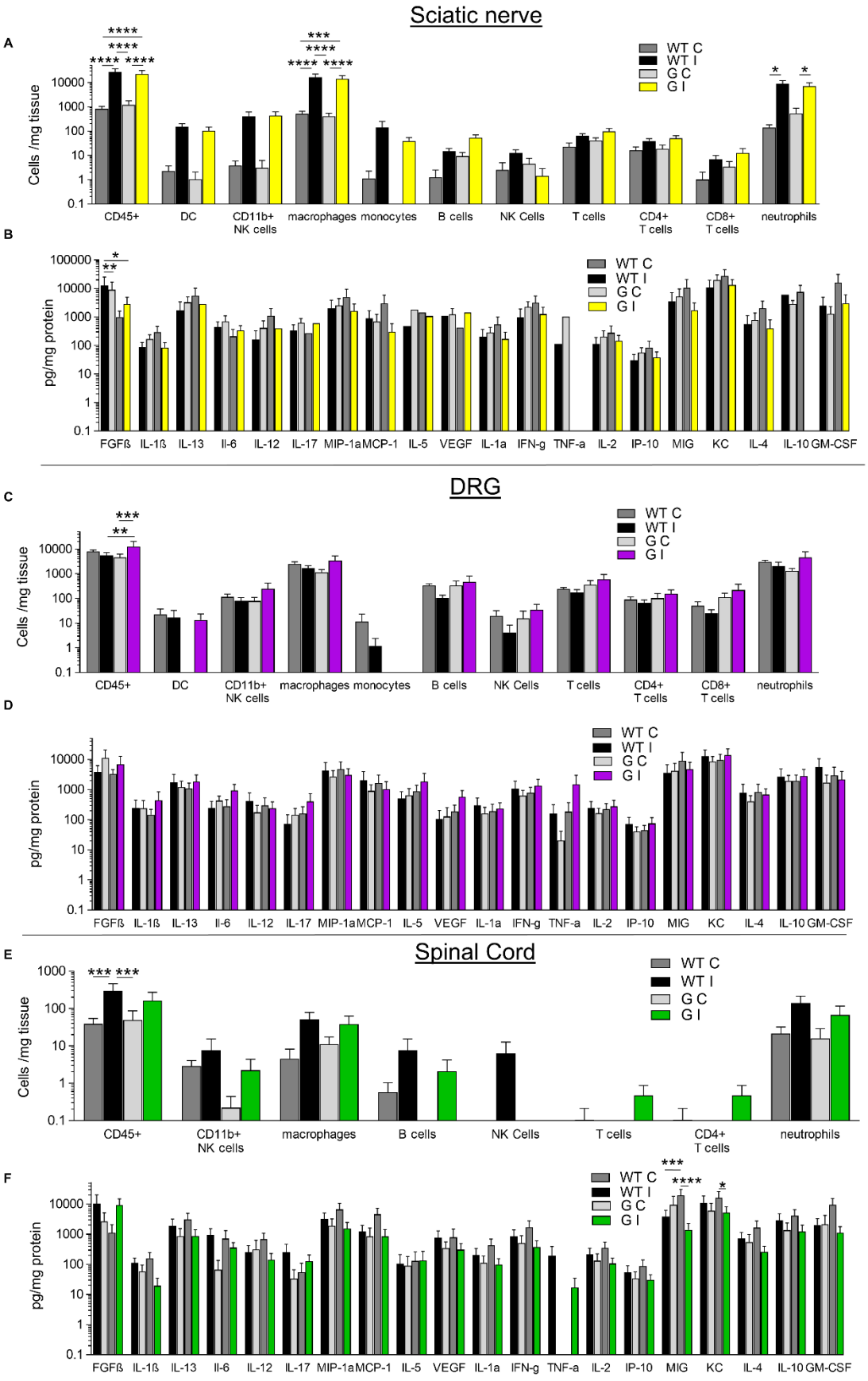

Figure S4. Immune cell number, cytokine and chemokine production in nervous tissue 1d after SNI-treatment. (**A**) Number of immune cells in ipsi- and contralateral site of sciatic nerve (SN) 1 d after SNI surgery in wild-type (WT) and G2A-deficient mice (G2A$^{-/-}$). n=5 animals per group, male and female, Two-way ANOVA with Bonferroni's post-hoc test. (**B**) Concentrations of different

cytokines, chemokines and growth factors in ipsi- and contralateral site of SN 1 d after SNI in wild-type (black) and G2A-deficient mice from n=5 animals per group, male and female, multiple t-test. (**C**) Number of immune cells in ipsi- and contralateral site of L4-L6 dorsal root ganglia (DRG) 1 d after SNI surgery in wild-type and G2A-deficient mice from n=5 animals per group, male and female, Two-way ANOVA with Bonferroni's post-hoc test. (**D**) Concentrations of different cytokines, chemokines and growth factors in ipsi- and contralateral site of L4-L5 DRGs 1 d after SNI surgery in wild-type and G2A-deficient mice from n=5 animals per group, male and female, multiple t-test. (**E**) Number of immune cells in ipsi- and contralateral site of spinal cord 1 d after SNI surgery in wild-type and G2A-deficient mice from n=5 male and female animals per group, Two-way ANOVA with Bonferroni's post-hoc test. (**F**) Concentrations of different cytokines, chemokines and growth factors ipsi- and contralateral site of spinal cord 1 d after SNI surgery in wild-type and G2A-deficient mice. n=5 animals per group, male and female, multiple t-test. Data represents mean ± SEM. Ipsilateral site of WT mice are shown in black. Ipsilateral site in SN is shown in yellow, in DRG in purple and in SC in green. Contralateral site of WT mice are depicted in dark grey, contralateral site of $G2A^{-/-}$ mice is shown in light grey. Abbr.: SEM: standard error of the mean, wo: without treatment, FGF: fibroblast growth factor, IL: interleukin, MIP: macrophage inflammatory protein, MCP: methyl-accepting chemotaxis protein, IFN: interferon, TNF: transforming growth factor, IP: interferon gamma-induced protein, MIG: monokine induced by IFN-gamma, KC: platelet-factor 4-type cytokine, G2A KO: G2A knockout, DRG: dorsal root ganglion, GM-CSF: granulocyte-macrophage colony-stimulating factor, DC: dendritic cells, NK: natural killer.

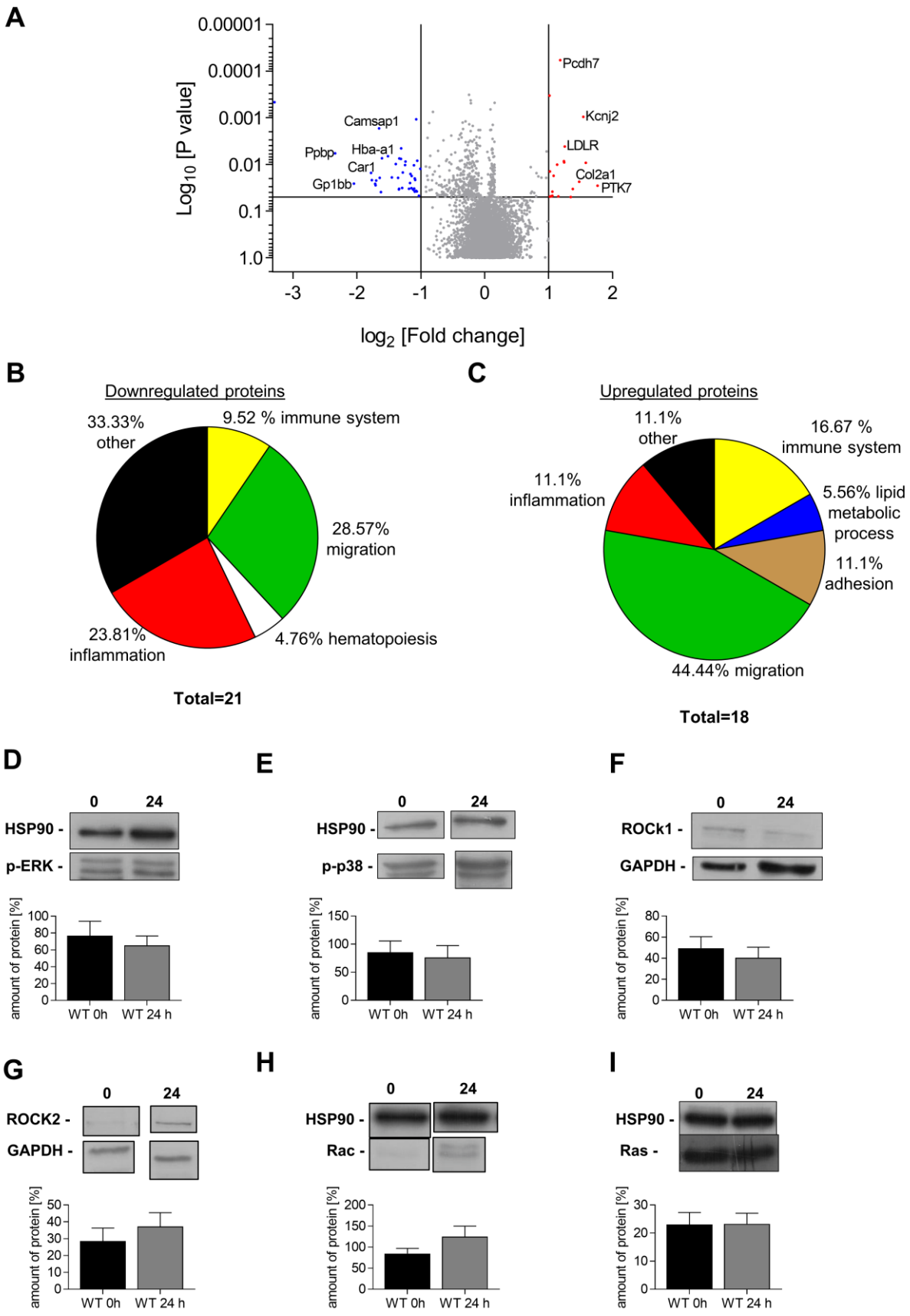

Figure S5. Effect of 9-HODE on migratory signaling pathways. (**A**) Volcano plot of > 6.000 regulated proteins without stimulation in wild-type (WT) BMDMs compared to G2A-deficient (G2A$^{-/-}$) BMDMs. Fold change (FC) [log2] is plotted against *P* values [Log10]. Significant downregulated proteins are depicted in blue, red represents upregulated proteins. Experiments were performed in triplicates,

bone marrow derived macrophages from male mice. (**B**) Percentage of downregulated proteins in untreated WT compared to untreated G2A$^{-/-}$. (**C**) Percentage of upregulated proteins in untreated WT compared to untreated G2A$^{-/-}$, clustered in groups. yellow: immune system, blue: lipid metabolic process, brown: adhesion, green: migration, grey: apoptosis, pink: lysosome, red: inflammation, white: hematopoiesis, black: other. (**D, E**) Representative Western Blots of phosphorylated ERK and p38 in BMDMs stimulated with 1 μM 9-HODE for 0 h (WT 0 h, black) and 24 h (WT 24 h, grey). As endogenous control HSP90 was used. n=5-11. (**F, G**) Representative Western Blots of ROCK1 and ROCK2 in BMDMs stimulated with 1 μM 9-HODE for 0 h (WT 0 h, black) and 24 h (WT 24 h, grey). As endogenous control GAPDH was used. n=5-11 male mice per group. (**H, I**) Representative Western Blots of Rac and Ras in BMDMs stimulated with 1 μM 9-HODE for 0 h (WT 0 h, black) and 24 h (WT 24 h, grey). As endogenous control HSP90 was used. n=8-10 male mice per group. Data represents mean ± SEM, unpaired t-test. Abbr.: SEM: standard error of the mean, HSP: heat shock protein, ROCK: rho-associated protein kinase, Rac: Ras-related C3 botulinum toxin substrate 1, Ras: rat sarcoma, p-38:p-38 mitogen-activated protein kinase , ERK: extracellular signal-regulated kinase.

Supplementary Table 1. Overview of regulated proteins in wild-type BMDMs treated with 1 μM 9-HODE for 24 h. Description according to KEGG pathway database.

Downregulated		Upregulated	
A) Immune system processes			
B2m	Antigen processing and presentation	**Alcam**	Cell adhesion molecule, hematopoiesis, adaptive immune response
Blnk	B cell receptor signaling pathway; Primary immunodeficiency; NF-kappa B signaling pathway; Osteoclast differentiation	**Cd72**	B cell receptor signaling pathway
C3	Complement and coagulation cascades; Viral carcinogenesis; Chagas disease (American trypanosomiasis); Systemic lupus erythematosus; Phagosome	**Lilrb4**	Osteoclast differentiation, B cell receptor signaling pathway
Ccl9	Cytokine-cytokine receptor interaction; Chemokine signaling pathway	**Lyn**	B cell receptor signaling pathway
Ctsl	Apoptosis; Phagosome; Antigen processing and presentation; Lysosome	**Pik3ap1**	B cell receptor signaling pathway, PI3K-Akt signaling pathway
Fcgr	Phagocytosis-promoting receptors, B cell receptor signaling, Natural killer cell mediated cytotoxicity; Staphylococcus aureus infection; Systemic lupus erythematosus; Phagosome; Tuberculosis		
Lgmn	Lysosome; Antigen processing and presentation		
Ly96	NF-kappa B signaling pathway; Toxoplasmosis; Toll-like receptor signaling pathway; Pertussis		
Notch2	Notch signaling pathway; Th1 and Th2 cell differentiation		
Plau	NF-kappa B signaling pathway; Complement and coagulation cascades		
Plin2	PPAR signaling pathway		
Tlr8	Toll-like receptor signaling pathway		
B) Lipid metabolic processes			
Abca1	ABC transporters; Fat digestion and absorption; Lysosomal membrane protein	**Acadvl**	Metabolic pathways; Fatty acid degradation; Fatty acid metabolism
Acaa2	Metabolic pathways; Fatty acid elongation; Fatty acid metabolism; Fatty acid degradation	**Cept1**	Ether lipid metabolism; Glycerophospholipid metabolism
Acadsb	Fatty acid degradation; Valine, leucine and isoleucine degradation; Metabolic pathways; Fatty acid metabolism	**Chkb**	Metabolic pathways; Glycerophospholipid metabolism
Acat1	Metabolic pathways; Fatty acid metabolism; Fatty acid degradation	**Dgkh, Dgkz**	Glycerophospholipid metabolism
Acox1	alpha-Linolenic acid metabolism; Biosynthesis of unsaturated fatty acids; PPAR signaling pathway; Fatty acid degradation; Fatty acid metabolism	**Fads1**	Biosynthesis of unsaturated fatty acids; Fatty acid metabolism
Aldh1b1	Lysine degradation; Pyruvate metabolism; Glycolysis / Gluconeogenesis; Glycerolipid metabolism; Fatty acid degradation;	**Fasn**	Fatty acid biosynthesis; Metabolic pathways; Fatty acid metabolism

	Metabolic pathways; glycerolipild metabolism		
Echs1	Fatty acid metabolism; Fatty acid elongation; Fatty acid degradation; Metabolic pathways; Carbon metabolism; fatty acid elongation	**Gnpat**	Peroxisome; Glycerophospholipid metabolism
Gpcpd1	Glycerophospholipid metabolism	**Lpl**	Glycerolipid metabolism; PPAR signaling pathway
GPx1	Arachidonic acid metabolism; Glutathione metabolism	**Hacd3**	Fatty acid metabolism; Biosynthesis of unsaturated fatty acids; Fatty acid elongation
Hadh	Fatty acid metabolism; Fatty acid elongation; Fatty acid degradation;	**Smpd2**	Sphingolipid metabolism; Sphingolipid signaling pathway
Neu1	Lysosome; Sphingolipid metabolism		
Pcyt1a	Glycerophospholipid metabolism;		
Pld3	Glycerophospholipid metabolism; Metabolic pathways; Ether lipid metabolism		
Sdha	Alzheimer's disease; Huntington's disease; Oxidative phosphorylation; Non-alcoholic fatty liver disease (NAFLD); Metabolic pathways		
Smpd1	Sphingolipid signaling pathway; Sphingolipid metabolism; Lysosome		
Akr1a1	Metabolic pathways; Glycerolipid metabolism; Glycolysis / Gluconeogenesis		
	C) Migration		
CCl6	Chemokine signaling pathway; Cytokine-cytokine receptor interaction	**Actn1**	Tight junction; Regulation of actin cytoskeleton; Focal adhesion; Adherens junction; Leukocyte transendothelial migration; Amoebiasis
Cd5l	inflammatory response, lipid synthesis, migration	**Akt1**	Fc gamma R-mediated phagocytosis; B cell receptor signaling pathway; Chemokine signaling pathway; Toll-like receptor signaling pathway; Focal adhesion; Tight junction; T cell receptor signaling pathway; Ras signaling pathway; mTOR signaling pathway; Jak-STAT signaling pathway
Cxcr4	Axon guidance; Cytokine-cytokine receptor interaction; Intestinal immune network for IgA production; Chemokine signaling pathway; Leukocyte transendothelial migration	**Cadm1**	Neural system cell adhesion
Glg1	Cell adhesion molecules (CAMs)	**Col6a2**	ECM-receptor interaction; Focal adhesion; PI3K-Akt signaling pathway
Grb2	Ras signaling pathway; Focal adhesion; B cell receptor signaling pathway; Gap junction; Chemokine signaling pathway; Natural killer cell mediated cytotoxicity; PI3K-Akt signaling pathway; MAPK signaling pathway	**Ctnna1**	Tight junction; Leukocyte transendothelial migration; Adherens junction

Il16	cytokine signaling pathway, unclassified to CD4	**Ctnnb1**	Leukocyte transendothelial migration; Focal adhesion; Tight junction; Adherens junction
Itga2b	Platelet activation; Hematopoietic cell lineage; Rap1 signaling pathway; Focal adhesion; PI3K-Akt signaling pathway; Regulation of actin cytoskeleton; phagosome	**Elmo1**	Chemokine signaling pathway; Rac protein signal transduction
Itgb3	Focal adhesion; Rap1 signaling pathway; Hematopoietic cell lineage; Platelet activation; ECM-receptor interaction; Regulation of actin cytoskeleton; Phagosome; PI3K-Akt signaling pathway; phagosome	**Epb4.1**	Tight junction
Tmsb4x	Regulation of actin cytoskeleton	**Itga5**	Regulation of actin cytoskeleton; Phagosome; Focal adhesion
Pik3r1	Chemokine signaling pathway; Toll-like receptor signaling pathway; Natural killer cell mediated cytotoxicity; Fc gamma R-mediated phagocytosis; Fc epsilon RI signaling pathway; B cell receptor signaling pathway; PI3K-Akt signaling pathway; Inflammatory mediator regulation of TRP channels; Regulation of actin cytoskeleton; Leukocyte transendothelial migration; TNF signaling pathway; Axon guidance; T cell receptor signaling pathway; Ras signaling pathway	**MyD88**	Toll-like receptor signaling pathway; NF-kappa B signaling pathway; MAPK signaling pathway
Cmklr1	chemotaxis, complement receptor mediated signaling pathway, immune response, inflammatory response	**Mylk**	Calcium signaling pathway; Regulation of actin cytoskeleton; Focal adhesion
		Ncf2	Leukocyte transendothelial migration; Phagosome
		Parvb	Focal adhesion
		Pik3r5	Ras signaling pathway; Regulation of actin cytoskeleton; Chemokine signaling pathway; Natural killer cell mediated cytotoxicity; Leukocyte transendothelial migration; Inflammatory mediator regulation of TRP channels; Focal adhesion
		Plcg2	Natural killer cell mediated cytotoxicity; Ras signaling pathway; NF-kappa B signaling pathway; Inflammatory mediator regulation of TRP channels; Axon guidance; Leukocyte transendothelial migration; B cell receptor signaling pathway
		Ppp1cc	Focal adhesion; Inflammatory mediator regulation of TRP channels; Long-term potentiation; Regulation of actin cytoskeleton
		Prkcb	gap junctions
		Pten	Tight junction; Focal adhesion; regulation of ERK1 and 2 cascade

		Ptk2b	chemokine signaling pathway, positive regulation of ERK1 and 2 cascade
		Rasa1	Axon guidance; MAPK signaling pathway; Ras signaling pathway
		Tgfbr1	Adherens junction; TGF-beta signaling pathway; MAPK signaling pathway; Cytokine-cytokine receptor interaction
		Was	Fc gamma R-mediated phagocytosis; Chemokine signaling pathway; Regulation of actin cytoskeleton; Adherens junction
	D) Cell adhesion		
CD22	Cell adhesion molecules (CAMs); Hematopoietic cell lineage; B cell receptor signaling pathway	**Arpc1b**	actin polymerization, regulation of actin cytoskeleton
H2-Aa	Antigen processing and presentation; Th1 and Th2 cell differentiation; Phagosome; Cell adhesion molecules (CAMs), MHCII; hematopoiesis	**Braf**	Regulation of actin cytoskeleton; Chemokine signaling pathway; Focal adhesion; Long-term depression; Natural killer cell mediated cytotoxicity; Endocrine resistance; Long-term potentiation
H2-Ab1	Phagosome; Th1 and Th2 cell differentiation; Cell adhesion molecules (CAMs); Antigen processing and presentation; MHCII	**Myl6**	regulation of actin cytoskeleton
Icam1	Cell adhesion molecules (CAMs); NF-kappa B signaling pathway; Natural killer cell mediated cytotoxicity;; Leukocyte transendothelial migration; macrophages T-cell adhesion and T cell to target cell		
Parvg	focal adhesion		
Shc1	Natural killer cell mediated cytotoxicity; Chemokine signaling pathway; Focal adhesion; Ras signaling pathway; chemokine signaling pathway		
Stmn1	MAPK signaling pathway		
Thbs1	Focal adhesion; ECM-receptor interaction; Phagosome; PI3K-Akt signaling pathway; Phagosome		
	E) Apoptosis		
Casp8	apoptosis	**Aifm2**	necroptosis
Ct(bs,sa,sb , sc, sd, sf, sg, sl, ss, sz)	Cathepsine, Lysosomal, Apoptosis	**Capn1**	Apoptosis
Fadd	Apoptosis; TNF signaling pathway; Toll-like receptor signaling pathway	**Chuk**	survival factor, (IKK), apoptosis
Gadd45	Apoptosis	**Traf2**	apoptosis, necroptosis
Glul	necroptosis		
Mcl1	Jak-STAT signaling pathway; Apoptosis; PI3K-Akt signaling pathway		

Ppid	necroptosis		
Rbck1	NOD-like receptor signaling pathway, necroptosis		
Spna2	apoptosis		
Vdac1	Calcium signaling pathway;; necroptosis		
	F) Hematopoiesis		
Cd38	Hematopoietic cell lineage	**Cdk6**	PI3K-Akt signaling pathway; Cell cycle; hematopoiesis
Csf1	Hematopoietic cell lineage; TNF signaling pathway; Ras signaling pathway; Cytokine-cytokine receptor interaction; PI3K-Akt signaling pathway		
Gp1bb	hematopoiesis		
	G) Inflammation		
Adcy3	Rap1 signaling pathway; Chemokine signaling pathway; Inflammatory mediator regulation of TRP channels; Longevity regulating pathway;	**Acsl5**	Peroxisome; PPAR signaling pathway
Hgs	Endocytosis; Phagosome	**Bcl2l1**	Jak-STAT signaling pathway; Chronic myeloid leukemia; Ras signaling pathway; PI3K-Akt signaling pathway; NF-kappa B signaling pathway
Mpo	Transcriptional misregulation in cancer; Phagosome	**Bmp2k**	cytokine signaling pathway
		Coro2a	Phagosome
		Pla2g4a	MAPK signaling pathway; Fc gamma R-mediated phagocytosis; Ras signaling pathway; Long-term depression; Inflammatory mediator regulation of TRP channels
		Prkch	Tight junction; Inflammatory mediator regulation of TRP channels;
		Ptpn7	MAPK signaling pathway
		Rab5a	Amoebiasis; Ras signaling pathway; Phagosome;
		Trpv2	Inflammatory mediator regulation of TRP channels; NOD-like receptor signaling pathway
		Vamp3	SNARE interactions in vesicular transport; Phagosome
	H) Lysosome		
Aga	Lysosome; Other glycan degradation	**Ctns**	Lysosome
Dnase2a	Lysosome	**Hgsnat**	Glycosaminoglycan degradation; Lysosome
Gaa	Lysosome	**Scarb1**	Lysosome and phagosome
Galns	Glycosaminoglycan degradation; Lysosome		
Gba	Lysosome; Other glycan degradation		

Gga1	Lysosome		
Gm2a	Lysosome		
Gns	Metabolic pathways; Lysosome		
Idua	Lysosome		
Napsa	Lysosome		
Npc2	Lysosome		
Pla2g15	Lysosome; Glycerophospholipid metabolism		
	I) Other		
Cox7a2	Oxidative phosphorylation	**Dapk3**	Autophagy, ER stress
Map4k1	MAPK signaling pathway	**Pik3c3**	Autophagy; Phagosome; Metabolic pathways
Ndufa2	Oxidative phosphorylation	**Prkcd**	Autophagy
Ndufv	oxidative phosphorylation	**Rps6ka1**	Thermogenesis
Uqcrfs1	Metabolic pathways; Oxidative phosphorylation	**Rptor**	Thermogenesis
		Stx5a	Autophagy

Supplementary Table 2. Overview of regulated proteins in untreated G2A-deficient and wild-type BMDMs. Description according to KEGG pathway description.

Downregulated		Upregulated	
A) Immune system processes			
Sos1	Regulation of actin cytoskeleton; Gap junction; T cell receptor signaling pathway; Chemokine signaling pathway; Fc epsilon RI signaling pathway; Focal adhesion; B cell receptor signaling pathway; Ras signaling pathway; PI3K-Akt signaling pathway; Natural killer cell mediated cytotoxicity	**C1qa**	Complement and coagulation cascades
Tradd	NF-kappa B signaling pathway; TNF signaling pathway	**Plaur**	Complement and coagulation cascades
		Serpinb2	Complement and coagulation cascades
B) Lipid metabolic processes			
		Gpx7	Glutathione metabolism; Arachidonic acid metabolism
C) Migration			
Dock2	Chemokine signaling pathway; Fc gamma R-mediated phagocytosis	**Col6a2**	ECM-receptor interaction; Focal adhesion; PI3K-Akt signaling pathway
Elmo1	Chemokine signaling pathway	**Epb4.1**	tight junctions
Irak4	Toll-like receptor signaling pathway, cytokine signaling pathway	**Flnb**	MAPK signaling pathway; Focal adhesion
Map2k2	MAPK signaling pathway; Toll-like receptor signaling pathway; Natural killer cell mediated cytotoxicity; Long-term potentiation; Regulation of actin cytoskeleton; B cell receptor signaling pathway; T cell receptor signaling pathway; Ras signaling pathway; Gap junction	**Hspb1**	Amoebiasis; MAPK signaling pathway;
Pik3r1	Chemokine signaling pathway; Toll-like receptor signaling pathway; Natural killer cell mediated cytotoxicity; Inflammatory mediator regulation of TRP channels; Focal adhesion; Regulation of actin cytoskeleton; Leukocyte transendothelial migration; TNF signaling pathway; Axon guidance; Ras signaling pathway	**Mylk**	Calcium signaling pathway; Regulation of actin cytoskeleton; Focal adhesion
Was	Fc gamma R-mediated phagocytosis; Chemokine signaling pathway; Regulation of actin cytoskeleton; Adherens junction	**Prkca**	Leukocyte transendothelial migration; Ras signaling pathway; Fc gamma R-mediated phagocytosis; Gap junction; Natural killer cell mediated cytotoxicity; Long-term potentiation; MAPK signaling pathway; Inflammatory mediator regulation of TRP channels; Axon guidance; Calcium signaling pathway; Tight junction

		Serpine1	Complement and coagulation cascades
		Tgfbr1	Adherens junction; TGF-beta signaling pathway; MAPK signaling pathway; Cytokine-cytokine receptor interaction
D) Cell adhesion			
		H2-Aa	Antigen processing and presentation; Th1 and Th2 cell differentiation; Phagosome; Cell adhesion molecules (CAMs), MHCII; hematopoiesis
		Thbs1	Focal adhesion; ECM-receptor interaction; Phagosome; PI3K-Akt signaling pathway; Phagosome
E) Apoptosis			
Bcl2l1	Jak-STAT signaling pathway; Chronic myeloid leukemia; Ras signaling pathway; PI3K-Akt signaling pathway; NF-kappa B signaling pathway		
F) Hematopoiesis			
Cdk6	PI3K-Akt signaling pathway; Cell cycle; hematopoiesis		
G) Inflammation			
Casp1	NOD-like receptor signaling pathway; Cytosolic DNA-sensing pathway; Pertussis	**Ltbp1**	TGF-beta signaling pathway
Nfkb1	Ras signaling pathway; Toll-like receptor signaling pathway; MAPK signaling pathway; B cell receptor signaling pathway; Chemokine signaling pathway; Th1 and Th2 cell differentiation; T cell receptor signaling pathway; NF-kappa B signaling pathway; TNF signaling pathway; PI3K-Akt signaling pathway	**Ube2i**	NF-kappa B signaling pathway
Prkch	Tight junction; Inflammatory mediator regulation of TRP channels		
Rps6ka5	TNF signaling pathway; MAPK signaling pathway		
H) Other			
Atp6v1 (a, b2, e1)	Oxidative phosphorylation; Metabolic pathways; mTOR signaling pathway; Synaptic vesicle cycle; Phagosome	**Ndufab1**	Oxidative phosphorylation
Map4k1	MAPK signaling pathway	**Rps6-ps4; Rps6**	HIF-1 signaling pathway; PI3K-Akt signaling pathway; Insulin signaling pathway
Ndufa11	Oxidative phosphorylation		
Prkacb	MAPK signaling pathway		
Sdhb	Oxidative phosphorylation		