

In vivo Monitoring of Transcriptional Dynamics After Lower-Limb Muscle Injury Enables Quantitative Classification of Healing

Carlos A. Aguilar^{1*}, Anna Shcherbina¹, Darrell Ricke¹, Ramona Pop², Christopher T. Carrigan³, Casey A. Gifford², Maria L. Urso^{3,†}, Melissa A. Kottke³, Alexander Meissner²
Distribution A: Public Release

Supplementary Materials

Text S1: Transcriptional Signatures of Inflammation and Immune System Detected in Early Period

Text S2: Transcriptional Signatures Associated With Injured Muscle Tissue Extracellular Matrix

Text S3: Injured Muscle Tissue Microenvironment Regulates Existing and Invading Cellular Migration, Proliferation, Differentiation and Phenotype

Text S4: Activation of Muscle Repair Machinery

Text S5: Late Transcriptional Programs Activated in Response to Traumatic LLMI

Fig. S1. Immuno-histological analysis of muscle after cryo-injury.

Fig. S2. a) Example of reproducibility from RNA-Seq datasets. Biological replicates of *in vivo* isolated RNA samples from 24 hours after injury. Sequencing libraries were prepared from the isolated RNA and a strong correlation ($R^2 = 0.95$) was observed for two representative examples. b) Validation of RNA-Seq data with qPCR comparing the injured versus uninjured samples for the 3 hour time point. c) Validation of RNA-Seq data with qPCR comparing the injured versus uninjured samples for the 10 hour time point. d) Validation of RNA-Seq data with qPCR comparing the injured versus uninjured samples for the 24 hour time point.

Fig. S3. Number of differentially expressed genes.

Fig. S4. Gene Sets Activated Early After Injury (3-24 hrs) a) Enriched KEGG pathways from differentially expressed genes for the early time points (3-24 hrs). The size of the circle corresponds to the number of significant genes with each enriched pathway. b) Heatmap of genes associated metabolism expressed in the early time period. c) Gene expression profile of Arginase 1 (Arg1), which is a molecule emitted by type 2 macrophages to balance inflammatory reactive-oxygen species present in the injured tissue. Red-Injured, Blue-Uninjured

Fig. S5. Examples of alternative splicing in immune network of genes.

Fig. S6. Genes associated with hematopoietic activation, proliferation and anti-inflammatory cytokines activated in early & middle periods.

Fig. S7. Gene sets associated with extra-cellular matrix remodeling & satellite cell activation.

Fig. S8. a) Enriched KEGG pathways from differentially expressed genes for the late time points. The size of the circle corresponds to the number of significant genes with each enriched pathway. b) Heatmaps of gene sets activated in late period categorized by function.

Fig. S9. Area plots of FDR values for Gene Annotation (GO) clusters over time. GO terms were separated into major functional categories, and the IQR was computed for the terms in each group. The grey lines indicate the 25th and 75th percentiles, red lines denote the 25% of gene FDR values, the blue line denotes the median FDR value, and the green line denotes the 75% for FDR.

Fig. S10. Pathway-level scoring results for test datasets from PCA and the timepoint signatures methods. a). Principal component analysis. b) Normalized time point signatures method applied to mean pathway expression values. Timepoint signatures for 6 test samples and a truth training sample. The green curve indicates the control sample that was misclassified by the algorithm at the pathway level. Time point signatures for the 9 time points (blue lines) as well as 6 test samples (red and green lines).

Fig. S11. Differentially expressed gene pathways in injured samples over time.

Table S1. Significant GO clusters (FDR <0.05) identified for differentially expressed genes through the DAVID annotation tool. Each tab represents the annotations derived for each timepoint, clustered by function and sorted by FDR value from most to least significant.

Table S2: KEGG pathways identified for differentially expressed genes (FDR < 0.05). Pathways are listed for each timepoint and sorted by FDR value from most to least significant.

Table S3. Significant GO clusters identified from the 168h time point that were obtained from differentially expressed genes that were determined to contribute the largest variance at that time point.

Text S1: Transcriptional Signatures of Inflammation and Immune System Detected in Early Period

Pro-inflammatory cytokines and chemotactic protein members: IL-1 β , IL-6, IL-18, Tnf α , Osm, Ccl2, Ccr2, Cxcr2, Trem2, S100a8, FDR=2.21e-22 - GO:0034097, response to cytokine

Anti-inflammatory genes: Socs3, IL-1rn, IL-4ra, IL-10ra, IL-13ra1, FDR=4.31e-10 - GO:0050728, negative regulation of inflammatory response

Invading immune cell genes: Cd68, Ly6c, Cd14, Cd163, Mrc1, Ptprc, Cd24a, Cd63, Itgam, Clec7a, Ltf, Irf7, Hp, FDR=7.6e-7 - GO:0050900, leukocyte migration involved in inflammatory response

Cytokines and chemokines: Ym1, S100a9, Spp1, Ccr1, Ccl3, Ccl11, Lcn2, Cxcl5, Ccl12, FDR=3.8e-44 - GO:0001816, cytokine production, FDR=9.05e-14 - GO:0032602, chemokine production

Signaling lymphocytic activation molecule family: Slamf7, Slamf8, Ly9, FDR=3.44e-16 - GO:0046649, lymphocyte activation

Pro-apoptotic loci: Casp1, Casp4, Casp8, Apaf1, Fas, FDR=1.40e-9 - GO:2001235, positive regulation of apoptotic signaling pathway

Anti-apoptotic loci: Xiap, Birc3, Birc5, Api5, Bax, Mcl1, Bcl2l1, FDR=2.17e-23 - GO:0043066, negative regulation of apoptotic process

Text S2: Transcriptional Signatures Associated With Injured Muscle Tissue Extracellular Matrix

Structural components of extra-cellular matrix (ECM) remodeling: Col1a1, Col1a2, Col3a1, Col5a1, Col5a2, Col5a3, Col6a1, Col6a2, Col6a3, Eln, FDR=4.19e-111 - GO:0016043, cellular component organization

Connective tissue: Fbn1, Ctgf, Has1, Hbegf, Fn1, Sparc, Tnc, FDR=6.65e-14 - GO:0061448, connective tissue development

ECM cross-linking enzymes: Lox, Loxl1, Loxl2, Plod3, Leprel2, P4hb

Small leucine-rich proteoglycans: Dcn, Bgn, Kera, Vcan, Lum, Ogn, FDR=3.69e-28 - GO:0036211, protein modification process

Adhesive glycoproteins: Pcolce, Itgbpb1, Lgals1, Dpt, Postn, Emilin2, Efemp2, Igfbp7, FDR=3.36e-16 - GO:0043062, extracellular structure organization

Tgf- β superfamily: Tgf- β i, Tgf- β r1, Tgf- β r2, Tgf- β 1

Formation and stabilization of blood vessels and arterial repair: Ang, Angpt2, Angptl1, Angptl4, Angptl7, Cthrc1, Aif1, FDR=3.12e-10 - GO:0001525, angiogenesis

Contractile fibers: ActB, Actg, Capg, Arpc3, Cofil1, Tln1, Tagln, Vcl, RhoA, FDR=7.8e-5 - GO:0030833, regulate actin filament polymerization, FDR=1.5e-3 - GO:0032273, positive regulation of protein polymerization

Integrin-associated genes: Itga5, Itga7, Itgam, Itgav, Itgb1, Itgb2, FDR=3.34e-5 - GO:0007229, integrin-mediated signaling pathway

Mmps, Adams and Timps: Mmp2, Mmp3, Mmp8, Mmp14, Mmp19, Adam8, Adam15, Adam19, Timp1, Timp2, FDR=6.83e-8 - GO:0048771, tissue remodeling

Text S3: Injured Muscle Tissue Changes from Pro- to Anti-Inflammatory States Several Days After Injury

Innate immunity and microbial recognition: Tlr1, Tlr7, Tlr8, FDR=0.003 - GO:0034121, regulation of toll-like receptor signaling pathway

Antigen presentation and hematopoietic activation and proliferation: Ptprc, IL-21r, IL-17ra, Vav1, Lyz2, Csf1r, Csf2rb, Csf2ra, Il3ra, Il5ra, Ifi204, Aifl, FDR=1.4e-8 - GO:0042110, T cell activation, FDR=8.18e-19 - GO:0030097, Haematopoiesis

Anti-inflammatory cytokines: IL-1rl1, Il-4ra, IL-10ra, Msr1, IL-13ra1, S100a9, FDR=2.9e-10 - KEGG mmu04630, cytokine-cytokine receptor interaction, FDR=9e-3 - GO:0010934, macrophage cytokine production

Phagocytic and complement cascade genes: Cyba, Ncf1, Ncf2, Ncf4, C1qa, C1qb, C1qc, C1ra, C1s, FDR=3.37e-8 - GO:0006956, complement activation, FDR=7e-6 - GO:0006909, phagocytosis

Notch signaling: Dll1, Notch2, Myc, Rbpj, Cdkn1a, FDR = 3.69e-18, GO:0002684, positive regulation of immune system process

Bone morphogenetic proteins: Bmp1, Bmp4, FDR = 0.08, GO:0005125, cytokine activity

Actively proliferating cells: Cdk1, FDR = 9.63e-9, GO:0007049, cell cycle

Text S4: Activation of Muscle Repair Machinery

Satellite cell markers: CD34, Itga7, Itgb1, Sdc3, Sdc4, Sdcbp, Cav1, Cxcr4, Cdh15, Ly6a, FDR=4.933e-5 - GO:0007519, muscle tissue development

Muscle-fusion genes: Tmem8c, Cdh15, Capn2, Capns1, Myof, Cav3, Itgb1, Vcam1, Itga4, FDR=2.5e-6 - GO:0098602, single organism cell adhesion

Basement membrane genes: Col4a1, Col4a2, Dysf, Trim72, Lamc2, Cav3, Ahnak, Anxa1, Anxa2, Anxa5, FDR=4.78e-9 - GO:0061024, membrane organization

Muscle-specific actins, myosins, troponins, and tropomyosins: Actl9, Clrn1, Myh3, Myl4, Myl6b, Cald1, Tpm4, Tnni1, Tnni3, Tnnt1, Tnnt2, Tnncl, Tpm3, Des, Vim

Transcription factors: Itgb2, Rrad, Fos, JunB, FosB, Pbx2, Pbx3, Dusp5, MyC, BMyc, Mt3, Egr1, Egr2, Sgms2, Runx1, Grn, Pdlim3, Csrp3, Usf1, Arid5b, Naca, Ankrd1, Ankrd2, Atf3, Atf4, Atf6, Xbp1, Tead4, Nfe2l2, FDR=2.46e-10 - GO:0006351, transcription, DNA-templated

Text S5: Late Transcriptional Programs Activated in Response to Traumatic LLM

Chemotaxis genes: Ccl6, Ccl8, Ccl9, Cxcl16, Fcer1g, Fcgr3, FDR=1.9e-5

Mast cell activation: Fyb, Fcer1g, Fcgr3, Fcgr2b, Lat2, Lcp2, FDR=3.33e-5 - GO:0045576, mast cell activation

Fc receptors: Fcer1g, Fcgr1, Fcgr3, Fcgr2b, Clec7a, Colec12, Hck, Sirpa, Slc11a1, Vav1, FDR=0.035 for KEGG mmu04664, Fc epsilon RI signaling pathway.

Lectins: Clec12a, Clec4a1, Clec4a2, Clec4a3, Clec4n, Clec7a, Clec10a.

Collagen fibril organization: Adamts14, Col3a1, Col5a1, Col5a2, Dpt, Lox, and Anxa2, FDR = 1e-7)

ECM receptors: VLA proteins (α 2, α 5, α 8, α 9, α 10, α 11), cytoadhesins (β 3, AII- β), and proteoglycans (Cd44, Sdc3, Sv2), KEGG ECM-receptor interaction pathway, FDR = 5.7e-7

Angiogenesis genes: Col4a1, Col4a2, Robo4, Vash1, Sema5a, Sox18, GO:0001525, FDR = 3.24e-14

Muscle contraction and muscle system processes: Cacna1s, Myom1, Myh2, Myh4, Myh7, Nos1, Kcnma1, Ryr1, Trim63, FDR=6.7e-4 and 1.6e-3, respectively.

Ossification regulation: Gpnmb, Bglap, Sparc, Spp1, Runx2, Bmp1, Bmp5, Smad1

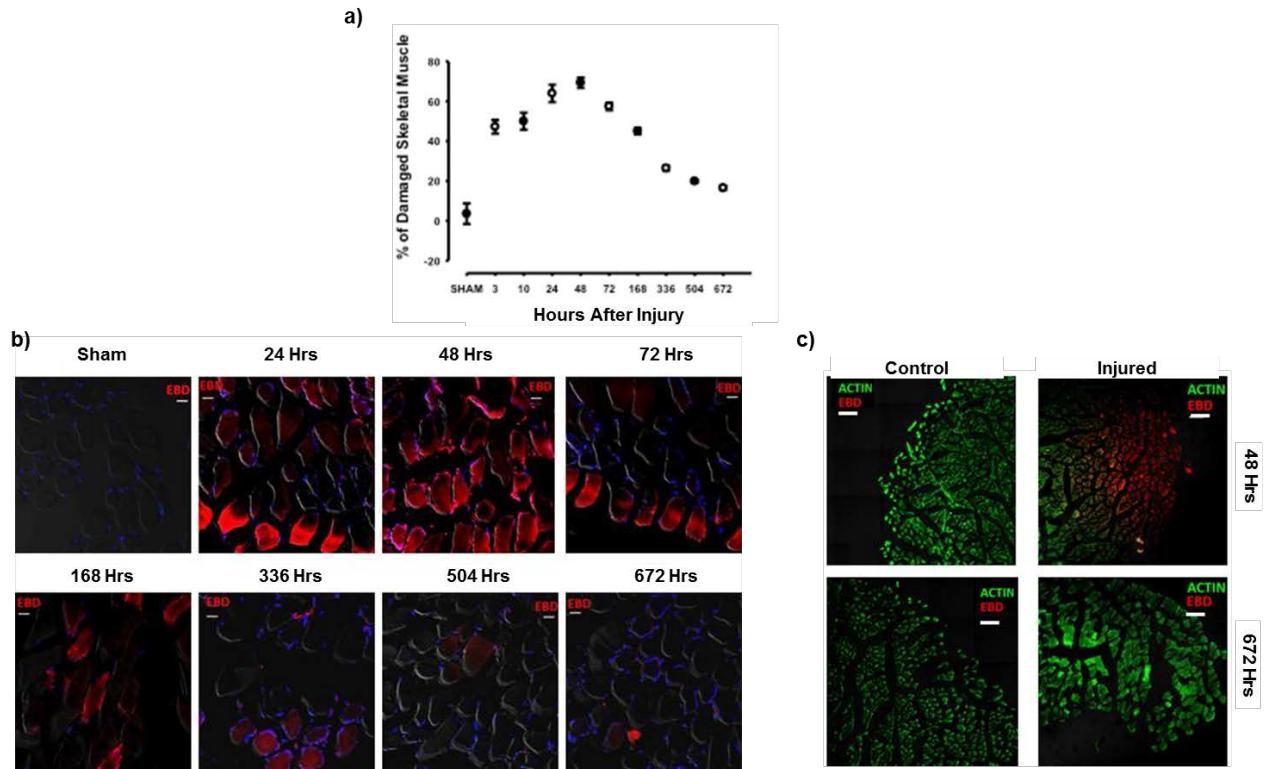


Fig. S1. Immuno-histological analysis of muscle after cryo-injury. a) Quantitative analysis of immuno-histological images revealed a gradual increase in tissue damage until 48 hours, indicating secondary damage to the muscle tissue occurred after the initial cryo-injury. b) Representative histological images of the injured TA stained with Evans Blue Dye (EBD – red) and DNA (blue) after several time points. c) Representative immuno-histological images at 48 hours and 672 hours after injury, whereby the green is stained for actin and red is stained for evans blue dye.

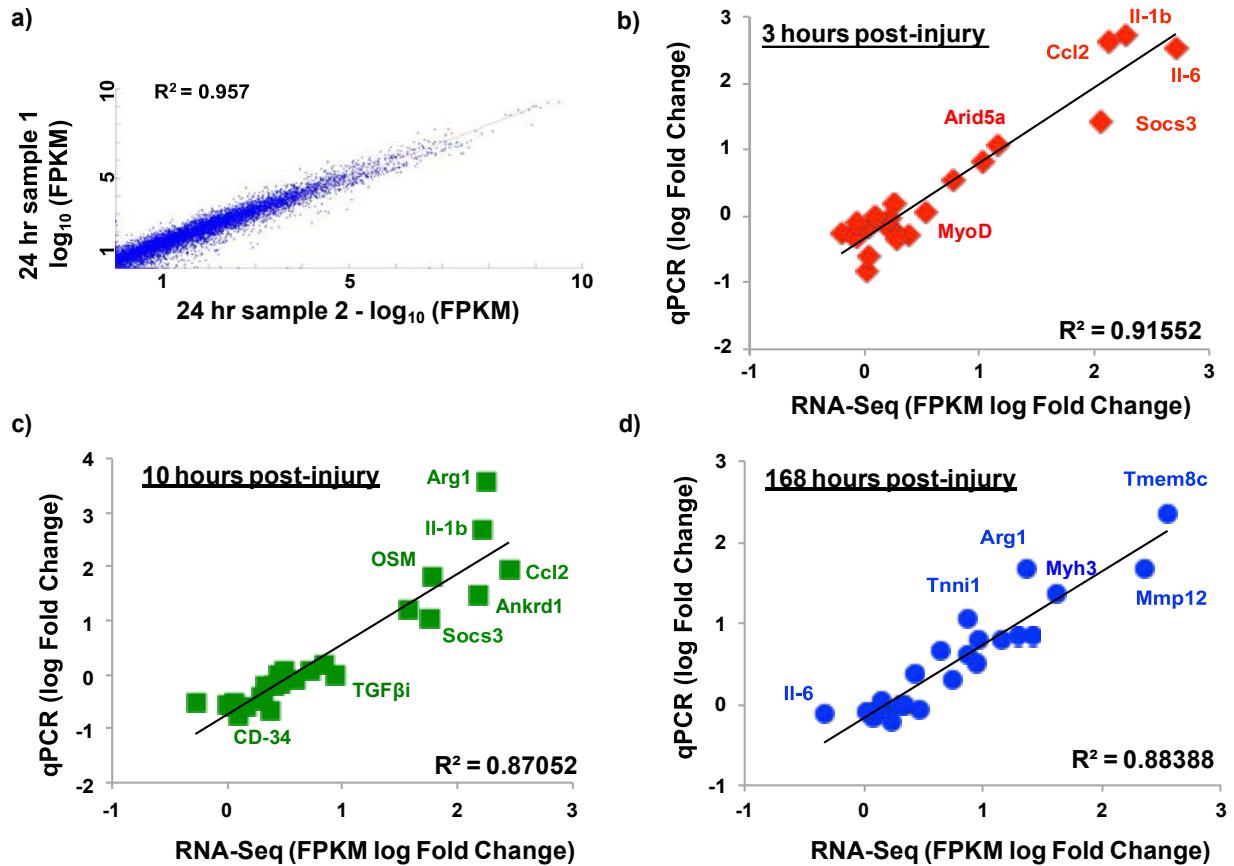


Fig. S2. a) Example of reproducibility from RNA-Seq datasets. Biological replicates of *in vivo* isolated RNA samples from 24 hours after injury. Sequencing libraries were prepared from the isolated RNA and a strong correlation ($R^2 = 0.95$) was observed for two representative examples. b) Validation of RNA-Seq data with qPCR comparing the injured versus uninjured samples for the 3 hour time point. c) Validation of RNA-Seq data with qPCR comparing the injured versus uninjured samples for the 10 hour time point. d) Validation of RNA-Seq data with qPCR comparing the injured versus uninjured samples for the 168 hr time point.

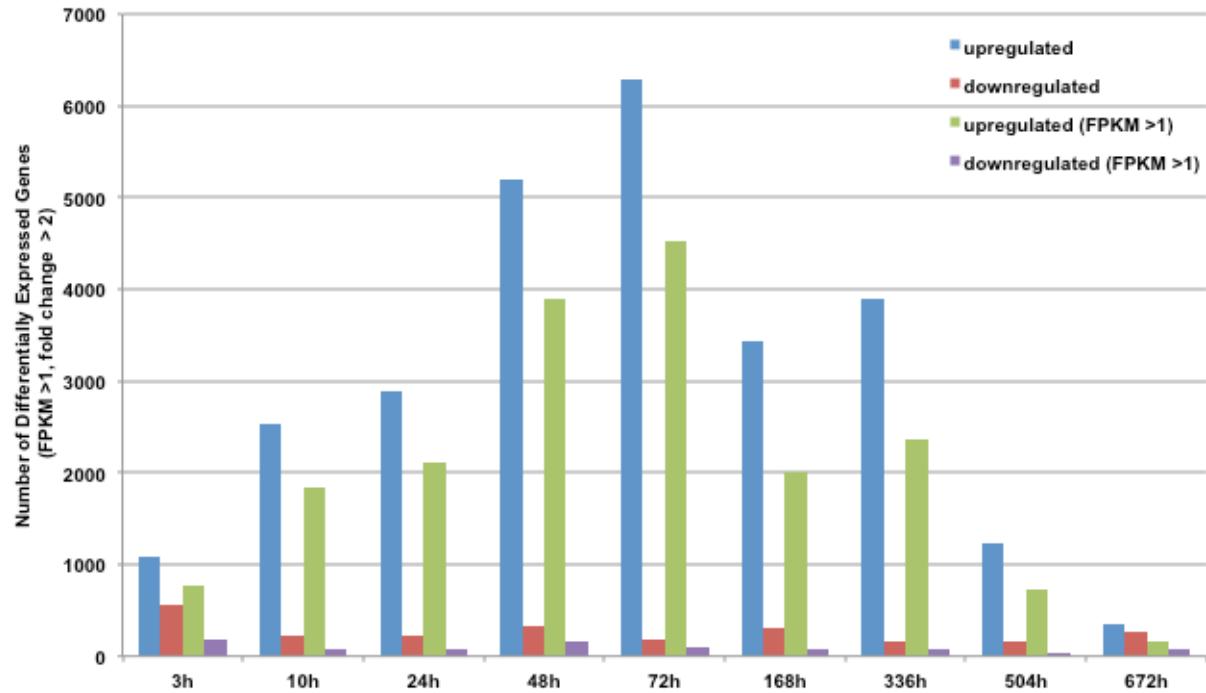


Fig. S3. Number of differentially expressed genes

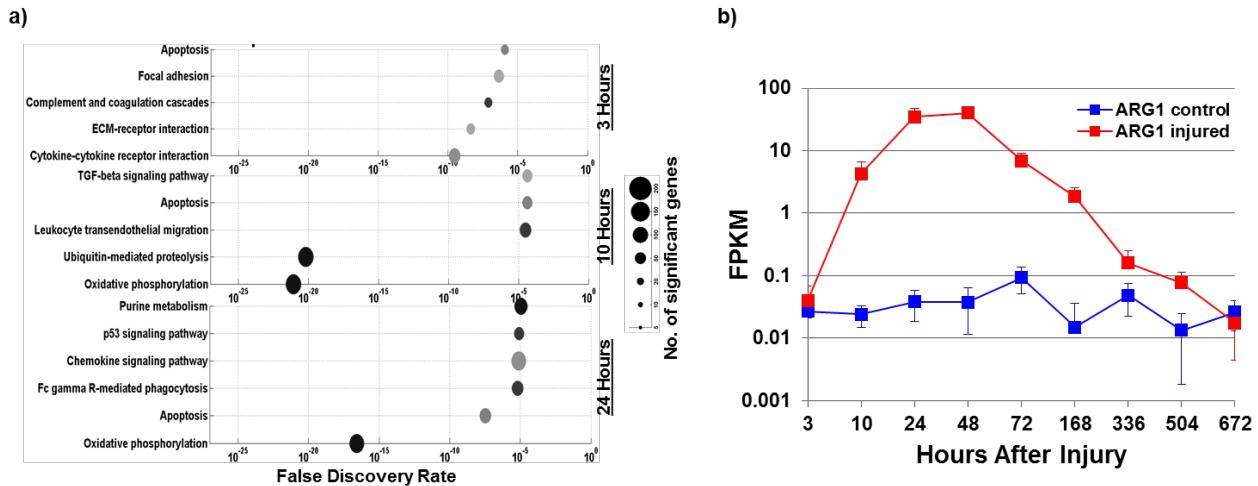


Fig. S4. Gene Sets Activated Early After Injury (3-24 hrs) a) Enriched KEGG pathways from differentially expressed genes for the early time points (3-24 hrs). The size of the circle corresponds to the number of significant genes with each enriched pathway. b) Heatmap of genes associated with metabolism expressed in the early time period. c) Gene expression profile of Arginase 1 (Arg1), which is a molecule emitted by type 2 macrophages to balance inflammatory reactive-oxygen species present in the injured tissue. Red-Injured, Blue-Uninjured.

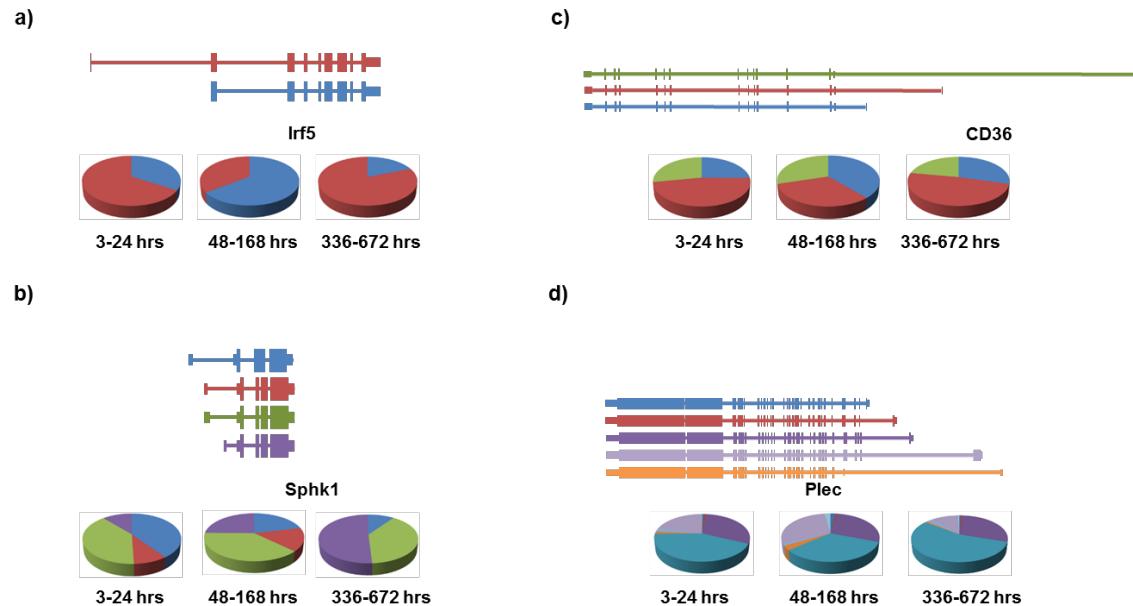


Fig. S5. Examples of alternative splicing in immune network of genes as determined by Cufflinks.

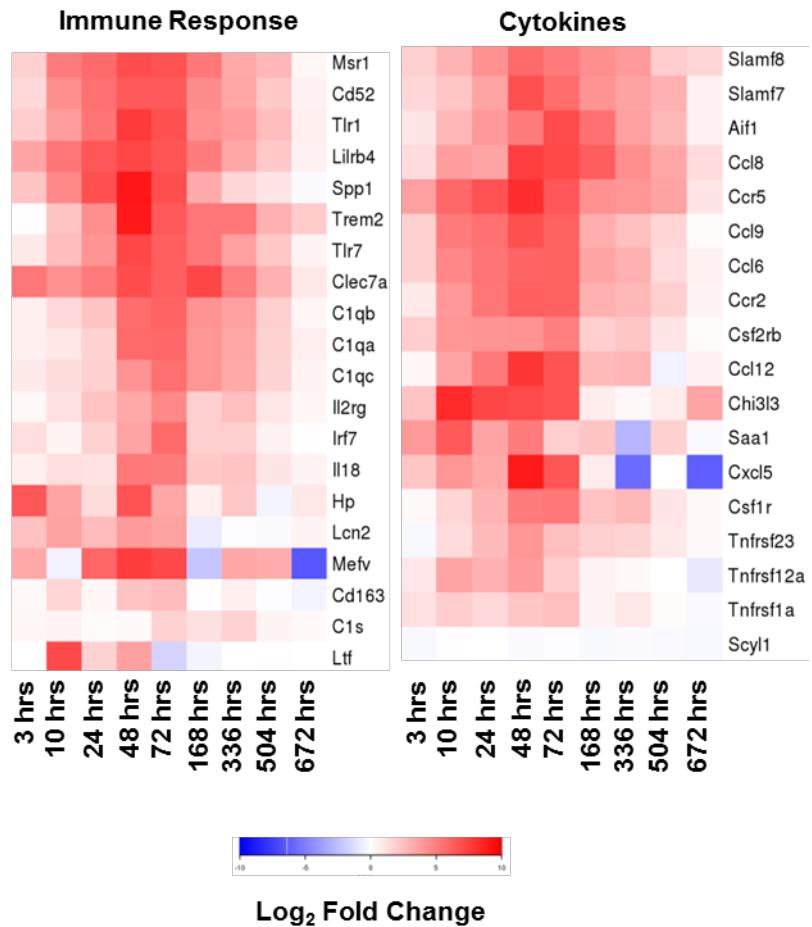


Fig. S6. Genes associated with hematopoietic activation, proliferation and anti-inflammatory cytokines activated in early & middle periods.

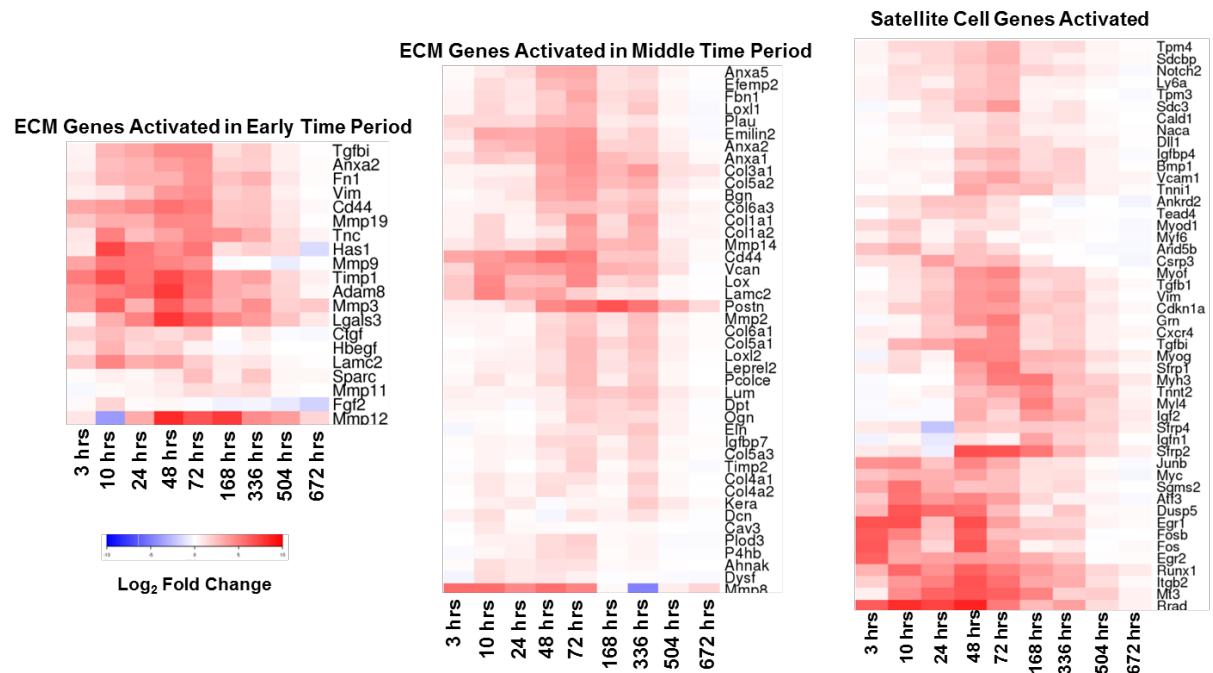


Fig. S7. Gene sets associated with extra-cellular matrix remodeling & satellite cell activation

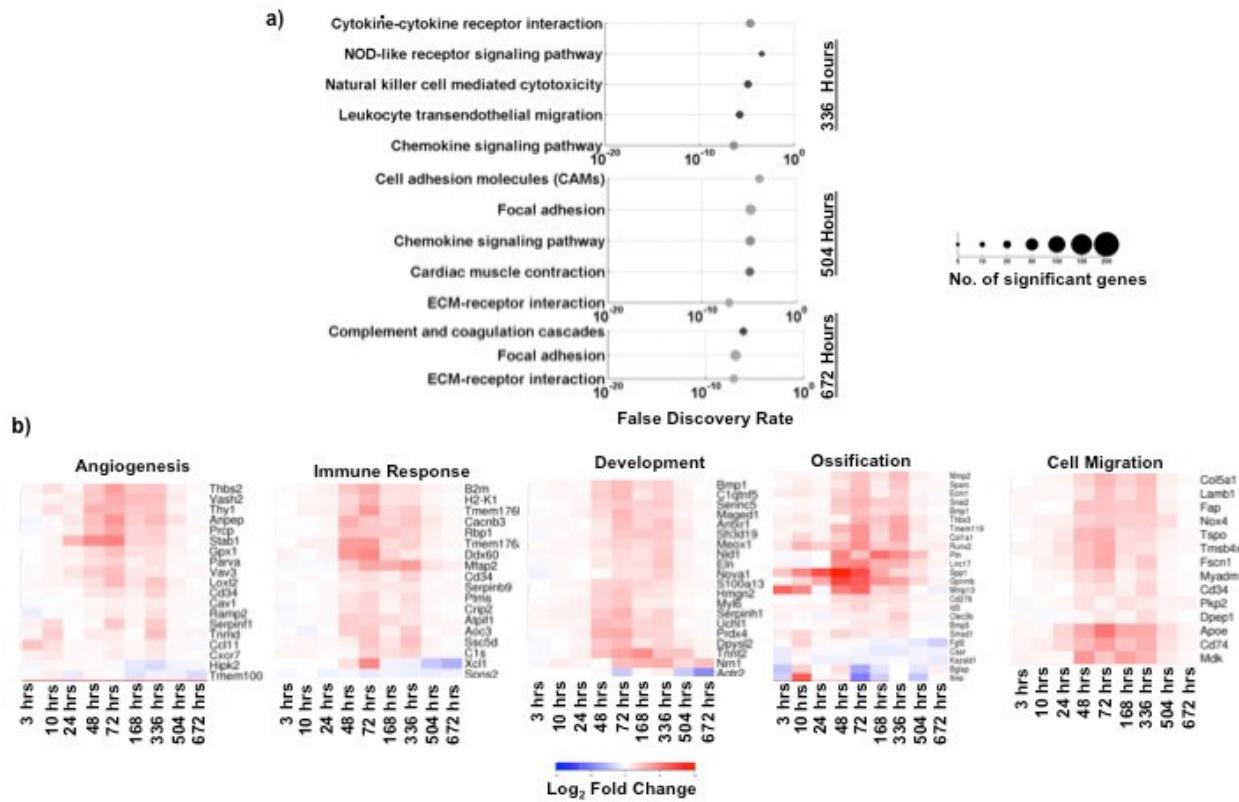


Fig. S8. a) Enriched KEGG pathways from differentially expressed genes for the late time points. The size of the circle corresponds to the number of significant genes with each enriched pathway. b) Heatmaps of gene sets activated in late period categorized by function.

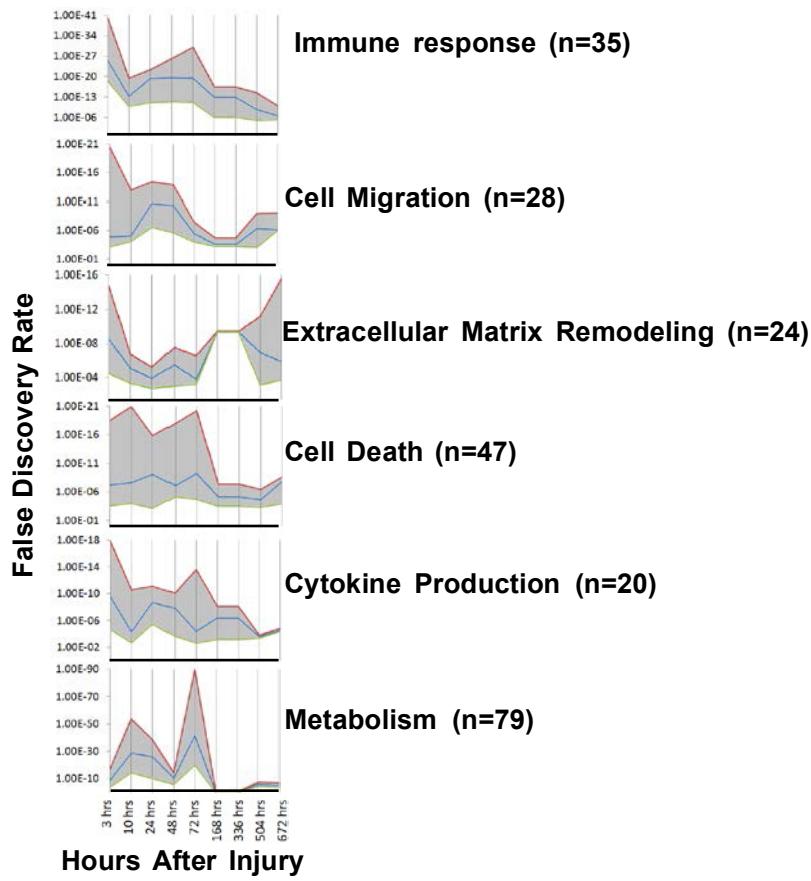


Fig. S9. Area plots of FDR values for Gene Annotation (GO) clusters over time. GO terms were separated into major functional categories, and the IQR was computed for the terms in each group. The grey lines indicate the 25th and 75th percentiles, red lines denote the 25% of gene FDR values, the blue line denotes the median FDR value, and the green line denotes the 75% for FDR.

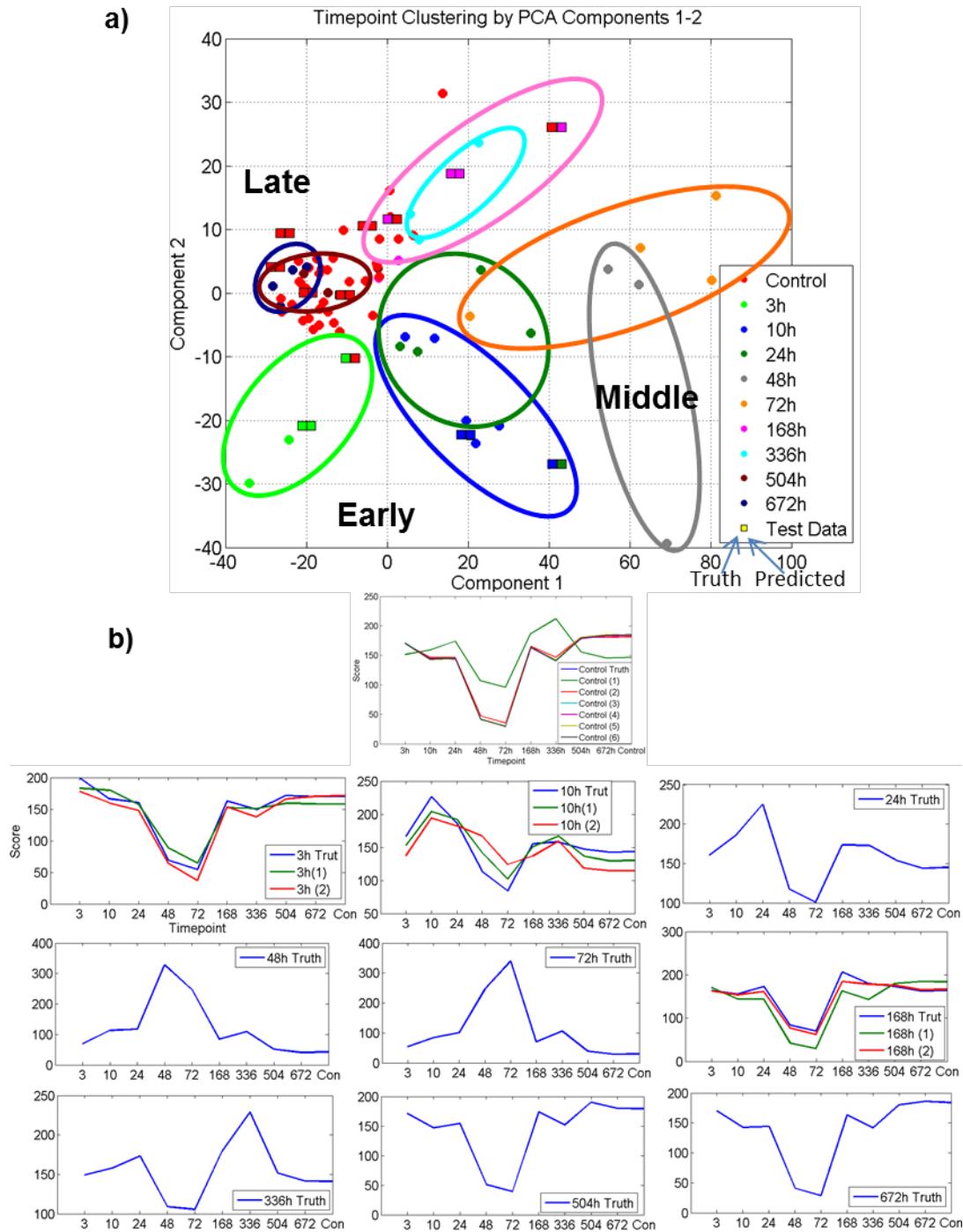


Fig. S10. Pathway-level scoring results for test datasets from PCA and the timepoint signatures methods. a) Principal component analysis. b) Normalized time point signatures method applied to mean pathway expression values. The top panel represents the timepoint signatures obtained for the uninjured control datasets for 6 test samples and a truth training sample. The green curve indicates the control sample that was misclassified by the algorithm at the pathway level. The next panels are derived from time point signatures for the 9 time points (blue lines) as well as 6 test samples (red and green lines).

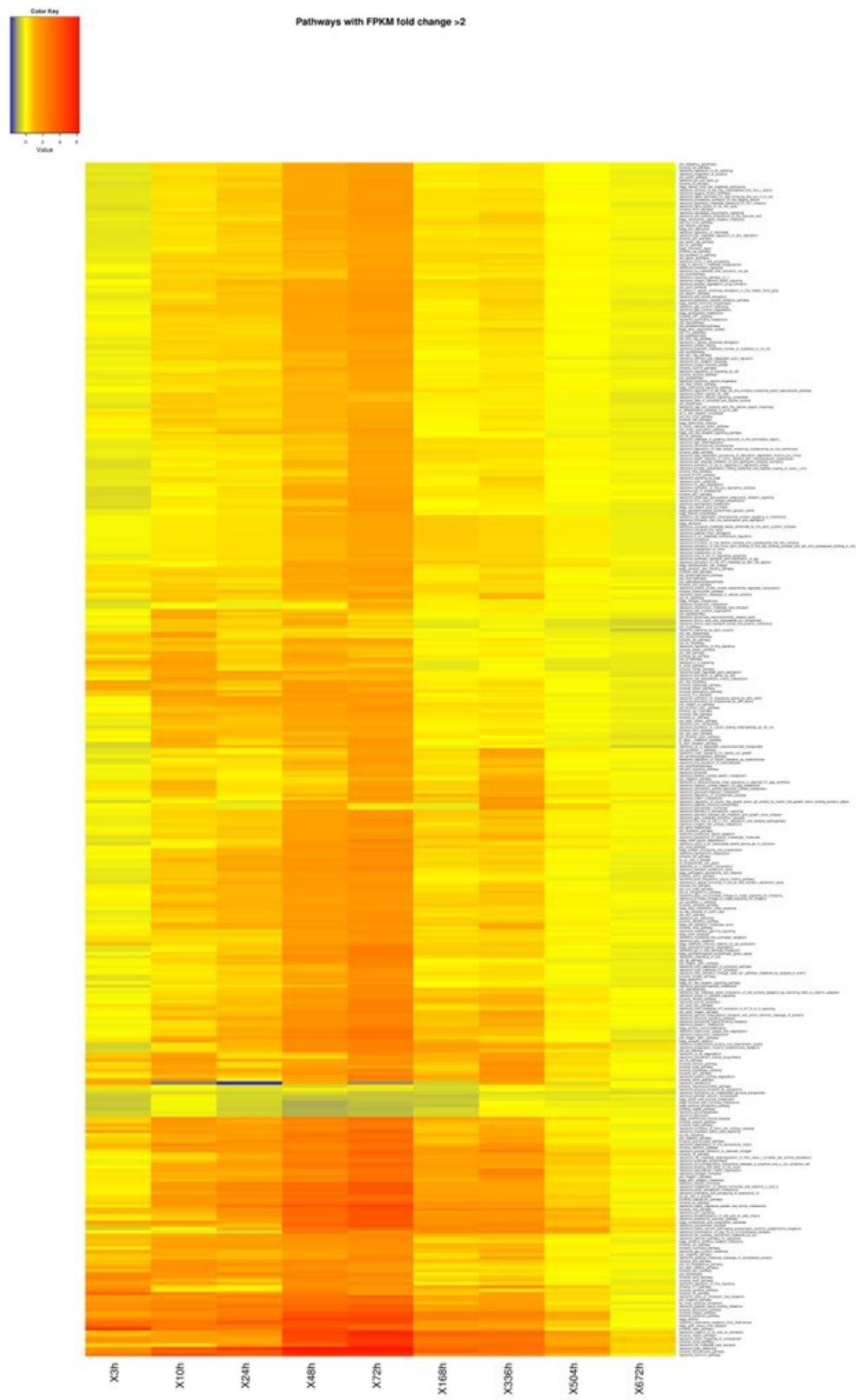


Fig. S11. Differentially expressed gene pathways in injured samples over time.

Table S1.A GO Terms upregulated 3 hours post injury

| | | | | | | | | | | | | |
|-----------------------|---------------------------------------|-------|------|----------|---|------------|----------|-----------|-----------------|-------------|-----------|-------------|
| GOTERM_BP_FAT | GO:0007155*cell adhesion | 76 | 7.01 | 5.20E-11 | PVR, NRPF2, CADM5, ATP1B2, CLN3, POFN1, DGR3, TGFBR2, GEF1F1, CD44, C9orf1, CTGF, TGFBI, CSF3R, 2VK, ADAM1, COL11A2, BOC, CYBB, C8orf1, DMD, FBN1, FCHM, HNRNPA01, BCALN, NUDT11, CD84, HES1, TNFRSF1B, CAV1, LAMP2, DCDC2D, ITGB1, TNFRSF12A, TNC, NEDD1, SORBS2, SORBS3, CDH4, ITGAM, ITGB1L, VCAN, CDR, PURL1, ACT, COMB, MYBPN, C04G2, COLE4A1, THBS1, SEL1L, THBS2, THBS3, FN1, THBS4, DPT, SPP1, COL18A1, PTPRC, SELP, SVEP1, TNXB, PUFK, SEL, PCDH12, NID1, ITGA3, ITGA4, EMILIN2, COL16A1, LAMA4, COL14A1, SNED1, ITGAS, CLIC7A, BMPR1B | 823 | 561 | 13588 | 2.24 | 1.57E-07 | 0.00 | 9.36E-08 |
| Annotation Cluster 8 | Enrichment Score: 5.749336180757243 | | | | | | | | | | | |
| Category | Term | Count | % | PValue | Genes | List Total | Pop Hits | Pop Total | Fold Enrichment | Bonferroni | Benjamini | FDR |
| GOTERM_MF_FAT | GO:0008009*chemokine activity | 14 | 1.29 | 1.36E-07 | CXCL1, CCL3, CXCL2, CXCL12, CXCL9, CC19, PF4, CC17, CCL6, CC124, CCL11, CXCL14, PPBP, CXCL16 | 788 | 38 | 13288 | 6.21 | 1.16E-04 | 0.00 | 2.10E-04 |
| GOTERM_MF_FAT | GO:0042379*chemokine receptor binding | 14 | 1.29 | 1.93E-07 | CXCL1, CCL3, CCL6, CC124, CXCL2, CXCL9, CC19, PF4, CC17, CCL6, CC124, CCL11, CXCL14, PPBP, CXCL16 | 788 | 39 | 13288 | 6.05 | 1.63E-04 | 0.00 | 2.99E-04 |
| Annotation Cluster 9 | Enrichment Score: 5.746125904859628 | | | | | List Total | Pop Hits | Pop Total | Fold Enrichment | Bonferroni | Benjamini | FDR |
| Category | Term | Count | % | PValue | Genes | | | | | | | |
| GOTERM_BP_FAT | GO:0006915*apoptosis | 56 | 5.17 | 1.28E-06 | RASSF5, CSRNP1, CASP12, SUF1, APAF1, DRAM1, PDCD7 | 823 | 465 | 13588 | 1.99 | 0.003849547 | 0.00 | 0.002303428 |
| GOTERM_BP_FAT | GO:0006915*apoptosis | 56 | 5.17 | 1.28E-06 | NUAK2, NFKB1, CXCR2, TGFBR2, SHB, TMEM173, CAP34, BAG3, CAP38, FA5, CAP31, MYC, BCL10, LTRB, NLRP3, BCL111, DDT4, OSM, TMFRSF10B, RIPK3, GAD0456, RIPK3, TNFRSF1A, EIP2AK2, GAD0458, PPP1R15A, PEG3, MCL1, LITAF, TNFRSF12A, PML, STK17B, ARF6, IRAK3, TNFRSF1A, PRUNE2, PEG10, TMFRSF1B, COMP, MAP3K1, SH3GAS, ZCH12A, INPP5D, ERG1, PHLDA1, CFAR, ACTC1, BIRC3, NFKB1L, CIDE1, NUAK2, NFKB1, CXCR2, TGFBR2, SHB, TMEM173, CAP34, BAG3, CAP38, FA5, CAP31, MYC, BCL10, LTRB, NLRP3, BCL111, DDT4, OSM, TMFRSF10B, RIPK3, GAD0456, RIPK3, TNFRSF1A, EIP2AK2, GAD0458, PPP1R15A, PEG3, MCL1, LITAF, TNFRSF12A, PML, STK17B, ARF6, IRAK3, TNFRSF1A, PRUNE2, PEG10, TMFRSF1B, COMP, MAP3K1, SH3GAS, ZCH12A, INPP5D, SRGN, PHLDA1, CFAR, ACTC1, BIRC3, NFKB1L, CIDE1, RASSF5, CSRNP1, CASP12, SUF1, APAF1, DRAM1, PDCD7 | 823 | 519 | 13588 | 1.91 | 0.005805099 | 0.00 | 0.003476951 |
| GOTERM_BP_FAT | GO:0016265*death | 60 | 5.54 | 1.93E-06 | NRA42, BIRC3, NFKB1L, CIDE1, RASSF5, CSRNP1, CASP12, APAF1, DRAM1, PDCD7 | 823 | 507 | 13588 | 1.92 | 0.005808084 | 0.00 | 0.00347744 |
| Annotation Cluster 10 | Enrichment Score: 4.535473351581223 | | | | | List Total | Pop Hits | Pop Total | Fold Enrichment | Bonferroni | Benjamini | FDR |
| Category | Term | Count | % | PValue | Genes | | | | | | | |
| GOTERM_BP_FAT | GO:0008219*cell death | 59 | 5.44 | 1.93E-06 | NUAK2, NFKB1, CXCR2, TGFBR2, SHB, TMEM173, CAP34, BAG3, CAP38, FA5, CAP31, MYC, BCL10, LTRB, NLRP3, BCL111, DDT4, OSM, TMFRSF10B, RIPK3, GAD0456, RIPK3, TNFRSF1A, EIP2AK2, GAD0458, PPP1R15A, PEG3, MCL1, LITAF, TNFRSF12A, PML, STK17B, ARF6, IRAK3, TNFRSF1A, PRUNE2, PEG10, TMFRSF1B, COMP, MAP3K1, SH3GAS, ZCH12A, INPP5D, SRGN, PHLDA1, CFAR, ACTC1, BIRC3, NFKB1L, CIDE1, NUAK2, NFKB1, CXCR2, TGFBR2, SHB, TMEM173, CAP34, BAG3, CAP38, FA5, CAP31, MYC, BCL10, LTRB, NLRP3, BCL111, DDT4, OSM, TMFRSF10B, RIPK3, GAD0456, RIPK3, TNFRSF1A, EIP2AK2, GAD0458, PPP1R15A, PEG3, MCL1, LITAF, TNFRSF12A, PML, STK17B, ARF6, IRAK3, TNFRSF1A, PRUNE2, PEG10, TMFRSF1B, COMP, MAP3K1, SH3GAS, ZCH12A, INPP5D, SRGN, PHLDA1, CFAR, ACTC1, BIRC3, NFKB1L, CIDE1, RASSF5, CSRNP1, CASP12, SUF1, APAF1, DRAM1, PDCD7 | 823 | 507 | 13588 | 1.92 | 0.005808084 | 0.00 | 0.00347744 |
| GOTERM_BP_FAT | GO:0012501*programmed cell death | 56 | 5.17 | 2.18E-06 | NRA42, BIRC3, NFKB1L, CIDE1, RASSF5, CSRNP1, CASP12, APAF1, DRAM1, PDCD7 | 823 | 473 | 13588 | 1.95 | 0.006542704 | 0.00 | 0.003920182 |
| Annotation Cluster 10 | Enrichment Score: 4.535473351581223 | | | | | List Total | Pop Hits | Pop Total | Fold Enrichment | Bonferroni | Benjamini | FDR |
| Category | Term | Count | % | PValue | Genes | | | | | | | |
| GOTERM_BP_FAT | GO:0010941*regulation of cell death | 66 | 6.09 | 3.28E-07 | NUAK2, FOXO1, PNP, TGFB2, BDNF, CASP4, APOE, BAG3, HMOX1, CHST11, CAP38, FA5, CAP31, MYC, BCL10, PML, NLRP3, BCL111, AORB2, HIF1A, TNFRSF10B, BTG2, BTG1, RIPK3, IFH1, GFR3, MCL1, PML, STK17B, HSPA1B, HSPA1B, NR3C1, SOX9, C074, ADA, GCH1, IRAK3, ALDH1A3, AGT, SH3GAS, BCL3, FCE1R16, INPP5D, SKN1, ANGPTL4, SPP1, BMP4, COL18A1, CFAR, PTPRC, PTEN6, KIF10, TGFBR1, SPHK1, NRA42, BIRC3, NFKB1L, CIDE1, NOTCH2, COKNIA, NUPR1, CASP12, APAF1, ID3, PDCD7, IFI204 | 823 | 563 | 13588 | 1.94 | 9.88E-04 | 0.00 | 5.91E-04 |
| GOTERM_BP_FAT | GO:0042981*regulation of apoptosis | 65 | 6.00 | 3.70E-07 | NUAK2, FOXO1, PNP, TGFB2, BDNF, CASP4, APOE, BAG3, HMOX1, CHST11, CAP38, FA5, CAP31, MYC, BCL10, PML, NLRP3, BCL111, AORB2, HIF1A, TNFRSF10B, BTG2, BTG1, RIPK3, IFH1, GFR3, MCL1, PML, STK17B, HSPA1B, HSPA1B, NR3C1, SOX9, C074, ADA, GCH1, IRAK3, ALDH1A3, AGT, SH3GAS, BCL3, FCE1R16, INPP5D, SKN1, ANGPTL4, SPP1, BMP4, COL18A1, CFAR, PTPRC, PTEN6, KIF10, TGFBR1, SPHK1, NRA42, BIRC3, NFKB1L, CIDE1, NOTCH2, COKNIA, NUPR1, CASP12, APAF1, ID3, PDCD7, IFI204 | 823 | 553 | 13588 | 1.94 | 0.001115723 | 0.00 | 6.67E-04 |

Table S1.8 GO Terms upregulated 10 hours post injury

| | | | | | | | | | | | | |
|---------------|---|-----|------|----------|--|------|-----|-------|------|----------|----------|----------|
| GOTERM_BP_FAT | GO:0051173 ⁺ positive regulation of nitrogen compound metabolic process | 121 | 4.22 | 2.33E-06 | PTPRC, IL6, CEBPD, CEBPA, TBX1, AIFI, STAT3, IRF5, IRF1, PBAK1, RIMM15, NFA, ARAP1 | 2080 | 526 | 13588 | 1.50 | 9.57E-03 | 2.67E-04 | 4.34E-03 |
| | | | | | MYOD1, THRB, TGFBS, TRPB1, CITED2, KDM1A, ARFGEF1, PTEN, PGP1B, CEPBD, RAR, RBL10, RSLA, SRSK, ARNTL, SRSK, MED10, PRAPC1C1, PTEN, HIF1A, ZFP462, JUN, VEGFA, SRSK, VGL2L, NFE2L2, FUS, HMG2B, ADOR2B, CTLL, SOX4, ITGB1, SPFH, SOX9, PLAGL1, SC11A1, TEAD4, AGRN, RUNX1, RUNX2, RUNX3, TRPS2, BMP4, KLF6, BMP2, ESRRA, EPAS1, KLF1, MAFB, MAMLL1, SMADS, CREBBP, NR42, EGR2, HSPD1, SMAD1, NR43, KLF15, TEAD3, SREBF2, ATFA, SAL1, EAF1, CSNP1, KLF2, KLF4, PPARA, CHURC1, FOXP1, E14, FOS, SDPR, YAP1, MYC, FGFR2, RHOG, SERTAD1, EGR1, AR, EGR2, HSPD1, SMAD1, NR43, KLF15, TEAD3, SREBF2, ATFA, SAL1, EAF1, CSNP1, FOXO1, INGS, ABILM2, Q522L, CEPBD, CEPBA, TBX1, AIFI, STAT3, IRF5, IRF1, PBAK1, RIMM15, NFA, ARAP1 | 2080 | 526 | 13588 | 1.50 | 9.57E-03 | 2.67E-04 | 4.34E-03 |
| GOTERM_BP_FAT | GO:0045944 ⁺ positive regulation of transcription by RNA polymerase II promoter | 89 | 3.10 | 2.36E-06 | PYD1, PPARA, CHURC1, THRB, TGFBS, TRPB1, CITED2, KDM1A, PGP1B, TRPB3, RARA, RBL10, RELA, SRSK, ARNTL, SRSK, MED10, PRAPC1C1, HIF1A, ZFP462, JUN, VEGFA, SRSK, VGL2L, NFE2L2, FUS, HMG2B, CYTL, SOXA, SPFH, SOX9, PLAGL1, SC11A1, TEAD4, AGRN, RUNX1, RUNX2, TRPS2, BMP4, KLF6, ESRRA, BMP2, EPAS1, KLF1, MAFB, MAMLL1, SMADS, CREBBP, NR42, EGR2, NR41, SMAD1, NR43, KLF15, TEAD3, SREBF2, ATFA, SAL1, EAF1, CSNP1, KLF2, KLF4, PPARA, CHURC1, FOXP1, E14, FOS, SDPR, YAP1, MYC, FGFR2, RHOG, SERTAD1, EGR1, AR, EGR2, TOPORS, HMGAL, SMO, MURC, CAND1, FOXC1, INGS, ABILM2, ABILM3, G152, NUP11, KIT, PRDM16, TRPS1A, REL, NFAT5, BCL3, MYOG, CEPBA, BCKDHA, MYF6, L6, CEPBP, CEPBD, TBX1, AIFI, STAT3, IRF5, IRF1, PBAK1, RIMM15, NFA, ARAP1 | 2080 | 358 | 13588 | 1.62 | 9.72E-03 | 2.64E-04 | 4.41E-03 |
| GOTERM_BP_FAT | GO:0045941 ⁺ positive regulation of transcription | 111 | 3.87 | 2.36E-06 | PYD1, THRB, TGFBS, TRPB1, CITED2, KDM1A, MY088, TRPB3, RARA, RBL10, RELA, SRSK, ARNTL, SRSK, MED10, PRAPC1C1, HIF1A, ZFP462, JUN, VEGFA, SRSK, VGL2L, NFE2L2, FUS, HMG2B, CYTL, SOXA, SPFH, SOX9, PLAGL1, SC11A1, TEAD4, AGRN, RUNX1, RUNX2, TRPS2, BMP4, KLF6, ESRRA, BMP2, EPAS1, KLF1, MAFB, MAMLL1, SMADS, CREBBP, NR42, EGR2, NR41, SMAD1, NR43, KLF15, TEAD3, SREBF2, ATFA, SAL1, EAF1, CSNP1, KLF2, KLF4, PPARA, CHURC1, FOXP1, E14, FOS, SDPR, YAP1, MYC, FGFR2, RHOG, SERTAD1, EGR1, AR, EGR2, TOPORS, HMGAL, SMO, MURC, CAND1, FOXC1, INGS, ABILM2, URCR3, ABILM3, G152, NUP11, KIT, PRDM16, TRPS1A, REL, NFAT5, BCL3, MYOG, CEPBA, BCKDHA, MYF6, L6, CEPBP, CEPBD, TBX1, AIFI, STAT3, IRF5, IRF1, PBAK1, RIMM15, NFA, ARAP1 | 2080 | 475 | 13588 | 1.53 | 1.21E-02 | 2.98E-04 | 5.52E-03 |
| GOTERM_BP_FAT | GO:0010628 ⁺ positive regulation of gene expression | 113 | 3.94 | 3.77E-06 | PTPRC, IL6, CEPBD, CEPBA, TBX1, AIFI, STAT3, IRF5, IRF1, PBAK1, RIMM15, NFA, ARAP1 | 2080 | 488 | 13588 | 1.51 | 1.54E-02 | 3.38E-04 | 7.02E-03 |
| | | | | | MYOD1, THRB, TGFBS, ANKRD1, CITED2, KDM1A, MY088, TRPB3, RARA, RBL10, RELA, SRSK, ARNTL, SRSK, MED10, PRAPC1C1, HIF1A, ZFP462, JUN, VEGFA, SRSK, VGL2L, NFE2L2, FUS, HMG2B, CYTL, SOXA, SPFH, SOX9, PLAGL1, SC11A1, TEAD4, AGRN, RUNX1, RUNX2, TRPS2, BMP4, KLF6, ESRRA, BMP2, EPAS1, KLF1, MAFB, MAMLL1, SMADS, CREBBP, NR42, EGR2, HSPD1, SMAD1, NR43, KLF15, TEAD3, SREBF2, ATFA, SAL1, EAF1, CSNP1, KLF2, KLF4, PPARA, CHURC1, FOXP1, E14, FOS, SDPR, YAP1, MYC, FGFR2, RHOG, SERTAD1, EGR1, AR, EGR2, TOPORS, HMGAL, SMO, MURC, CAND1, FOXC1, INGS, ABILM2, URCR3, ABILM3, G152, NUP11, KIT, PRDM16, TRPS1A, REL, NFAT5, BCL3, MYOG, CEPBA, BCKDHA, MYF6, L6, CEPBP, CEPBD, TBX1, AIFI, STAT3, IRF5, IRF1, PBAK1, RIMM15, NFA, ARAP1 | 2080 | 516 | 13588 | 1.44 | 2.15E-02 | 4.10E-04 | 9.82E-03 |
| GOTERM_BP_FAT | GO:0006357 ⁺ regulation of transcription from RNA polymerase II promoter | 136 | 4.74 | 5.37E-06 | NFA, IRD24 | 2080 | 510 | 13588 | 1.49 | 2.74E-02 | 5.06E-04 | 1.26E-02 |
| | | | | | MYOD1, THRB, TGFBS, TRPB1, CITED2, KDM1A, MY088, TRPB3, RARA, RBL10, RELA, SRSK, ARNTL, SRSK, MED10, PRAPC1C1, PTEN, HIF1A, ZFP462, JUN, VEGFA, SRSK, VGL2L, NFE2L2, FUS, HMG2B, CYTL, SOXA, SPFH, SOX9, PLAGL1, SC11A1, TEAD4, AGRN, RUNX1, RUNX2, TRPS2, BMP4, KLF6, ESRRA, BMP2, EPAS1, KLF1, MAFB, MAMLL1, SMADS, CREBBP, NR42, ESRRA, NR41, SMAD1, NR43, KLF15, TEAD3, SREBF2, ATFA, SAL1, EAF1, CSNP1, KLF2, KLF4, PPARA, CHURC1, FOXP1, E14, FOS, SDPR, YAP1, MYC, FGFR2, RHOG, SERTAD1, EGR1, AR, EGR2, TOPORS, HMGAL, SMO, MURC, CAND1, FOXC1, INGS, ABILM2, URCR3, ABILM3, G152, NUP11, PRDM16, TRPS1A, REL, NFAT5, BCL3, MYOG, CEPBA, BCKDHA, MYF6, L6, CEPBP, CEPBD, TBX1, AIFI, STAT3, IRF5, IRF1, PBAK1, RIMM15, NFA, ARAP1 | 2080 | 510 | 13588 | 1.49 | 2.74E-02 | 5.06E-04 | 1.26E-02 |
| GOTERM_BP_FAT | GO:0045955 ⁺ positive regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic proc | 116 | 4.04 | 6.74E-06 | TBX1, AIFI, STAT3, IRF5, IRF1, PBAK1, RIMM15, NFA, ARAP1 | 2080 | 510 | 13588 | 1.49 | 2.74E-02 | 5.06E-04 | 1.26E-02 |
| | | | | | MYOD1, THRB, TMB1, TGFBS, TBLA, TGFBI, CITED2, KDM1A, MY088, TRPB3, RARA, RBL10, ICDS1, RSLA, SRSK, ARNTL, SRSK, MED10, PRAPC1C1, PTEN, HIF1A, ZFP462, JUN, VEGFA, SRSK, VGL2L, NFE2L2, FUS, HMG2B, CYTL, SOXA, SPFH, SOX9, PLAGL1, SC11A1, TEAD4, AGRN, RUNX1, RUNX2, TRPS2, BMP4, KLF6, ESRRA, BMP2, EPAS1, KLF1, MAFB, MAMLL1, SMADS, CREBBP, NR42, ESRRA, NR41, SMAD1, NR43, KLF15, TEAD3, SREBF2, ATFA, SAL1, EAF1, CSNP1, KLF2, KLF4, PPARA, CHURC1, FOXP1, E14, FOS, SDPR, YAP1, MYC, FGFR2, RHOG, SERTAD1, EGR1, AR, EGR2, TOPORS, HMGAL, SMO, MURC, CAND1, FOXC1, INGS, ABILM2, URCR3, ABILM3, G152, NUP11, PRDM16, TRPS1A, REL, NFAT5, BCL3, MYOG, CEPBA, BCKDHA, MYF6, L6, CEPBP, CEPBD, TBX1, AIFI, STAT3, CCN, IRG, IRF1, PBAK1, RIMM15, NFA, ARAP1 | 2080 | 530 | 13588 | 1.47 | 3.91E-02 | 6.87E-04 | 1.80E-02 |
| GOTERM_BP_FAT | GO:0010557 ⁺ positive regulation of macromolecule biosynthetic process | 119 | 4.15 | 9.65E-06 | TBX1, AIFI, STAT3, CCN, IRG, IRF1, PBAK1, RIMM15, NFA, ARAP1 | 2080 | 530 | 13588 | 1.47 | 3.91E-02 | 6.87E-04 | 1.80E-02 |

Table S1.C GO Terms upregulated 24 hours post injury

| | | | | | | | | | | | | |
|---------------|--------------------------------------|----|------|----------|---|------|-----|-------|------|----------|----------|----------|
| GOTERM_MF_FAT | GO:0005096-GTPase activator activity | 56 | 1.96 | 3.05E-07 | SH3BP1, ARAP1, IQGAP2, ARHGAP15, TBC1D19, IQGAP1, TBC1D16, MMHA1, ARHGAP22, ARHGAP20, RIN1, TIAM2, ARHGAP1, RANBP1, ARHGAP11A, ARHGAP9, BCR, ARHGAP28, SIPA1L2, THY1, ARHGAP25, ARHGAP30, ACAP1, RIN1, RIN3, ARFGAP2, ARHGDIG, ARFGAP3, RAP1GAP, TBC1D9, GPNM3, SIPA1, MYO9B, FANCI, ARAP1, DOCK2, RGS12, GMIP, GPML1, RASA3, ARHGDA, ARHGDI8, TBC1D2, TBC1D8, TBC1D10C, ABR, TBC1D10A, RGS18, RGS16, RACGAP1, RGS14, USPENL, ADAP2, RGS2, CHNL, GRTP1, | 1935 | 192 | 13288 | 2.00 | 4.32E-04 | 2.70E-05 | 5.03E-04 |
|---------------|--------------------------------------|----|------|----------|---|------|-----|-------|------|----------|----------|----------|

Table S1.D GO Terms upregulated 48 hours post injury

Table S1.E GO Terms upregulated 72 hours post injury

| | | | | | | | | | | | | |
|---------------|---|-----|-------|----------|--|------|------|-------|------|----------|----------|----------|
| GOTERM_MF_FAT | GO:0032553"ribonucleotide binding | 349 | 12.28 | 2.89E-10 | KIF18B, CENPE, SMC2, CSG2, SMCA, GCK, GSPZ, TEP1, APAF1, IAK3, H2' OMA, CKD2 | 1916 | 1796 | 13288 | 1.35 | 3.83E-07 | 1.28E-07 | 4.73E-07 |
| GOTERM_MF_FAT | GO:0017076"purine nucleotide binding | 358 | 12.60 | 1.12E-09 | TET2, CENPE, SMC2, CSG2, SMCA, GCK, GSPZ, TEP1, APAF1, IAK3, H2' OMA, CKD2 | 1916 | 1871 | 13288 | 1.33 | 1.49E-06 | 2.98E-07 | 1.84E-06 |
| GOTERM_MF_FAT | GO:0000166"nucleotide binding | 397 | 13.97 | 7.17E-08 | TET2, CENPE, SMC2, CSG2, SMCA, GCK, GSPZ, TEP1, APAF1, IAK3, H2' OMA, CKD2 | 1916 | 2183 | 13288 | 1.26 | 9.50E-05 | 1.06E-05 | 1.17E-04 |
| GOTERM_MF_FAT | GO:0005524"ATP binding | 270 | 9.50 | 1.51E-06 | APAF1, JAK3, H2' OMA, CKD2 | 1916 | 1443 | 13288 | 1.30 | 2.00E-03 | 1.11E-04 | 2.47E-03 |
| GOTERM_MF_FAT | GO:0032559"adeny ribonucleotide binding | 271 | 9.54 | 2.85E-06 | GCK, TEP1, APAF1, IAK3, H2' OMA, CKD2 | 1916 | 1460 | 13288 | 1.29 | 3.77E-03 | 1.99E-04 | 4.67E-03 |

| | | | | | | | | | | | | | | | |
|-----------------------|---|----------|------|---|---|-------|--|------------|----------|-----------|-----------------|------------|-----------|----------|----------|
| | | | | RNASEL, PARK2, PTEN, PRKAT1, TTK, PTPN22, RDRC, AURKB, PRKG2, TGFBI1, BTK, PRKAR2B, APP, UQCRC10, UQCRC11, MAST2, MAST3, MAST4, MAST5, MAST6, MAST7, MAST8, MAST9, MAST10, MAST11, MAST12, MAST13, MAST14, MAST15, MAST16, MAST17, MAST18, MAST19, MAST20, MAST21, MAST22, RPLP0, LRRK1, FGRF8, FOR, GRM2, NRK2, STX10, SH3D1, PTK7, ACVR1, MARK1, CHX11, EPHA2, IRAK4, IRAK5, VIK3, PTPR8, CSF1R, TFC, PTPN7, GP92, MDF1, FCER1A, PTPN6, GP91, PTPN18, TAOK1, HOF, EPHA2, EPHA5, INBEK, EPHA7, DUSP28, P2RX7, PLX4, PLX3, PLK2, PLX1, GRK4, MERTK, ATPE, IMPA2, EPHA2, EPHA5, SYNJ1, FES, CAMKK1, CAMK2, CNE1, SH2D1B1, ATP5I, ATP5O, MAST1, CSK, SH1, ATP6VOVD2, COK1S, ATPK5, ATP5H, CDK14, ATPK2, ATP5I, CDC7, COKL, LYN, PHKG1, LIMK1, NDUFV2, COK6, PKDC, PRK, PRKCA, PRKCB, MAST3, OSM, ATM, PIA1, CONNEXIN44, DUSP1, EP300, NOUFA1, PRM1A, ATP9G1, ISCP10, GALR2, GALR1, STAT4, PRK2, PRK2B, BUB1, DCLX1, TES, TCRG1, PTPRC, ALPK1, PTPRE, RLT, POK3, HCK, PTPRA, NOUFA1, AXL, ATPS1, COK1, GSG2, CDC25B, DUSP5, CCNB1, RPSKA6, DUSP4, RPSKA4A, GCK, ATPA3, RPSKA1, PYN, GYK, JAK3, DUSP9, CIT, | | | | | | | | | | | |
| GOTERM_BP_FAT | GO:0006793-phosphorus metabolic process | 175 | 6.16 | 5.82E-06 | CDK20, DUSP6 | | | | 2002 | 866 | 13588 | 1.37 | 2.33E-02 | 2.36E-04 | 1.08E-02 |
| Annotation Cluster 35 | Enrichment Score: 2.5545766691173992 | Category | Term | Count % | PValue | Genes | | List Total | Pop Hits | Pop Total | Fold Enrichment | Bonferroni | Benjamini | FDR | |
| GOTERM_BP_FAT | GO:0033559-unsaturated fatty acid metabolic process | 19 | 0.67 | 6.23E-07 | FCER1A, TBKAS1, PTG52, NCF1, POPN, FASD1, EPHK2, LTC4S, CD74, GGTS, TNFRSF1A, PTG15, PTGES, ALOX5AP, ALOX5, NCF2, NCF1, POPN, LTC4S, CD74, GGTS, TNFRSF1A, PTG15, PTGES, ALOX5AP, ALOX5, | | | 2002 | 36 | 13588 | 3.58 | 2.53E-03 | 3.05E-05 | 1.16E-03 | |
| GOTERM_BP_FAT | GO:0006690-lipid metabolism process | 17 | 0.60 | 7.22E-06 | HPGD, RNPEP, HPGD, MGST2 | | | 2002 | 34 | 13588 | 3.39 | 2.89E-02 | 2.76E-04 | 1.34E-02 | |
| Annotation Cluster 40 | Enrichment Score: 2.3291168681773002 | Category | Term | Count % | PValue | Genes | | List Total | Pop Hits | Pop Total | Fold Enrichment | Bonferroni | Benjamini | FDR | |
| GOTERM_BP_FAT | GO:0005077-negative regulation of immune response | 14 | 0.49 | 2.33E-05 | SPN | | | 2002 | 26 | 13588 | 3.65 | 9.02E-02 | 8.00E-04 | 4.33E-02 | |
| Annotation Cluster 43 | Enrichment Score: 2.1580191024363786 | Category | Term | Count % | PValue | Genes | | List Total | Pop Hits | Pop Total | Fold Enrichment | Bonferroni | Benjamini | FDR | |
| GOTERM_BP_FAT | GO:0031341-regulation of cell killing | 16 | 0.56 | 5.44E-06 | KLRB18, IL4, C3, TLR4, CLX1L, ADA, GPX1, TNFRSF1A, CD47, NOD2, TNFRSF1B, AOAH, APOH, FCER1G, LBP, THBS1, SPN, CD28, ZFP96, FCER1A, SELP, CLN3, PLEK, CD276, AGER, FCGR1, | | | 2002 | 30 | 13588 | 3.62 | 2.19E-02 | 2.23E-04 | 1.01E-02 | |
| GOTERM_BP_FAT | GO:0001910-regulation of leukocyte mediated cytotoxicity | 16 | 0.56 | 5.44E-06 | KLRB18, LAG3 | | | 2002 | 30 | 13588 | 3.62 | 2.19E-02 | 2.23E-04 | 1.01E-02 | |
| Annotation Cluster 44 | Enrichment Score: 2.143499635516042 | Category | Term | Count % | PValue | Genes | | List Total | Pop Hits | Pop Total | Fold Enrichment | Bonferroni | Benjamini | FDR | |
| GOTERM_BP_FAT | GO:0032101-regulation of response to external stimulus | 33 | 1.16 | 1.87E-05 | FCGR3, ANXA2, CD99B, FGR2B, NPY, FABP7 | | | 2002 | 103 | 13588 | 2.17 | 7.33E-02 | 6.62E-04 | 3.49E-02 | |
| Annotation Cluster 50 | Enrichment Score: 2.0226420719661573 | Category | Term | Count % | PValue | Genes | | List Total | Pop Hits | Pop Total | Fold Enrichment | Bonferroni | Benjamini | FDR | |
| GOTERM_MF_FAT | GO:0005077-monovalent inorganic cation transmembrane transporter activity | 33 | 1.16 | 1.79E-07 | SLC9A8, ATP5E, SLC36A1, COX7B, COX7C, ATP5G1, UQCRC1, COX5A, COX5B, UQCRC0, UQCRC10, UQCRC11, COX6B1, ATP5L, ATP5O, COX6B2, ATP6VOVD2, ATP5H, ATP5L, TCRG1, COX7A2, COX8B, SLC9A8, ATP5E, SLC36A1, COX7B, COX7C, ATP5G1, UQCRC1, COX5A, COX5B, UQCRC0, UQCRC10, UQCRC11, COX6B1, ATP5L, ATP5O, COX6B2, ATP6VOVD2, ATP5H, ATP5L, TCRG1, COX7A2, COX8B, | | | 1916 | 87 | 13288 | 2.63 | 2.37E-04 | 1.97E-05 | 2.93E-04 | |
| GOTERM_MF_FAT | GO:0015078-hydrogen ion transmembrane transporter activity | 31 | 1.09 | 4.92E-07 | COTXA1, ATP5F1, CQXIC, ATP5EVA, UQCRC1, COX6A2, ATP5O, UQCRCB | | | 1916 | 82 | 13288 | 2.62 | 6.51E-04 | 4.07E-05 | 8.05E-04 | |

Table S1.F GO Terms upregulated 168 hours post injury

| Annotation Cluster 1 | | Enrichment Score: 14.55639806337477 | | | | | | | | | | | |
|-----------------------|--|--------------------------------------|-------|----------|---|--|------------|----------|-----------|-----------------|------------|-----------|----------|
| Category | Term | Count | % | PValue | Genes | | List Total | Pop Hits | Pop Total | Fold Enrichment | Bonferroni | Benjamini | FDR |
| GOTERM_BP_FAT | GO:0006955="immune response" | 49 | 14.16 | 6.60E-20 | LYMV, TLR2, CD161, CLOC, TLRL, IL10, CPFL, QDQ12, AF231705, NCFL, C4B, H2, DDM81, C1QA, C1QB, IIL8BP9, CCSP, JAH3, CXCR1, ULR84, CCSP, QAS1A, H12R, G4S10, CD300BL, CD300BL, C12L, LTA1, C19L, C1QB, OAS2, C027, SLC11A1, FGR8A, FCFR1G, CQD, CD7, TLRL3, MYO13, CFC130W, FCGR1, C180, C1C2, FGR3, CCL12, CD300A, PLMG2, IRPF, CLECTA, XCL1, C014 | | 249 | 471 | 13588 | 5.68 | 1.03E-19 | 1.03E-19 | 1.03E-19 |
| GOTERM_BP_FAT | GO:0006952="defense response" | 37 | 10.69 | 5.48E-14 | C1C2, FGR3, CCL12, CD300A, PLMG2, IRPF, CLECTA, XCL1, C014 | | 249 | 448 | 13588 | 4.51 | 8.55E-11 | 4.28E-11 | 9.15E-11 |
| GOTERM_BP_FAT | GO:0006954="inflammatory response" | 26 | 7.51 | 4.19E-13 | C1C2, FGR3, CCL12, CCBS, INR, CCR2, CLECTA, CD14, OM2 | | 249 | 225 | 13588 | 6.31 | 6.53E-10 | 2.18E-10 | 6.99E-10 |
| GOTERM_BP_FAT | GO:00069611="response to wounding" | 29 | 8.38 | 3.92E-11 | CC12, LY86, TLR2, CBLB, ITGB2, C1QC, TLRL, CC17, CFP, SLC11A1, PYCARD, TPFD, PLEK, GATM, NCFS, C4B, TLRL3, SAA3, FGR1, CD180, FGR3, C1QA, C1QB, CCL2, CCBS, CCR2, CLECTA, CD14, OM2, CD14 | | 249 | 347 | 13588 | 4.56 | 6.12E-08 | 1.53E-08 | 6.55E-08 |
| Annotation Cluster 2 | | Enrichment Score: 4.591123660755224 | | | | | | | | | | | |
| Category | Term | Count | % | PValue | Genes | | List Total | Pop Hits | Pop Total | Fold Enrichment | Bonferroni | Benjamini | FDR |
| GOTERM_BP_FAT | GO:0047424="defense response to bacterium" | 13 | 3.76 | 6.20E-07 | LY22, FGR, NCFL1, CAMP, HCK, TLRL, MYO1F, FGR1, IL10, SLC11A1, CCRS, IRPF, FCFR1G | | 249 | 108 | 13588 | 6.57 | 9.66E-04 | 6.90E-05 | 1.03E-03 |
| GOTERM_BP_FAT | GO:0009617="response to bacterium" | 15 | 4.34 | 1.08E-06 | LY22, FGR, NCFL1, CAMP, HCK, TLRL, MYO1F, FGR1, IL10, SLC11A1, CCRS, IRPF, PLG2, FCFR1G | | 249 | 157 | 13588 | 5.21 | 1.69E-03 | 1.13E-04 | 1.81E-03 |
| Annotation Cluster 3 | | Enrichment Score: 4.990722469347588 | | | | | | | | | | | |
| Category | Term | Count | % | PValue | Genes | | List Total | Pop Hits | Pop Total | Fold Enrichment | Bonferroni | Benjamini | FDR |
| GOTERM_BP_FAT | GO:0008189="positive regulation of cytokine production" | 11 | 3.18 | 1.60E-07 | C083, SLC11A1, KRE1, PYCARD, TLRL2, FCFR1G, CLECTA, CASP1, TLRL7, SASH3, CD14 | | 249 | 62 | 13588 | 9.68 | 2.49E-04 | 2.27E-05 | 2.67E-04 |
| GOTERM_BP_FAT | GO:0008187="regulation of cytokine production" | 15 | 4.34 | 2.44E-07 | SASH3, CD14 | | 249 | 139 | 13588 | 5.89 | 3.80E-04 | 3.16E-05 | 4.07E-04 |
| Annotation Cluster 4 | | Enrichment Score: 4.97611947155325 | | | | | | | | | | | |
| Category | Term | Count | % | PValue | Genes | | List Total | Pop Hits | Pop Total | Fold Enrichment | Bonferroni | Benjamini | FDR |
| GOTERM_BP_FAT | GO:0042330="taxis" | 15 | 4.34 | 1.00E-08 | CM381, CCL2, CCL9, C19L, ITGB2, C17L, C1C17, FGR3, CCL12, CORO1A, RAC2, CX3CR1, FCFR1G, XCR1, C1X1 | | 249 | 109 | 13588 | 7.51 | 1.69E-05 | 1.88E-06 | 1.81E-05 |
| GOTERM_BP_FAT | GO:0006935="chemotaxis" | 15 | 4.34 | 1.00E-08 | CM381, CCL2, CCL9, C19L, ITGB2, C17L, C1C17, FGR3, CCL12, CORO1A, RAC2, CX3CR1, FCFR1G, XCR1, C1X1 | | 249 | 109 | 13588 | 7.51 | 1.69E-05 | 1.88E-06 | 1.81E-05 |
| GOTERM_MF_FAT | GO:0008009="chemokine activity" | 7 | 2.02 | 2.84E-05 | C1C2, CCL2, C19L, C1C17, FGR3, CCL12, CORO1A, RAC2, CX3CR1, FCFR1G, XCR1, C1X1 | | 214 | 38 | 13288 | 11.44 | 9.14E-03 | 4.58E-03 | 3.84E-02 |
| GOTERM_MF_FAT | GO:0042379="chemokine receptor binding" | 7 | 2.02 | 3.32E-05 | C1C2, CCL2, C19L, C1C17, FGR3, CCL12, CORO1A, RAC2, CX3CR1, FCFR1G, XCR1, C1X1, C1C17, C1C17 | | 214 | 39 | 13288 | 11.14 | 1.07E-02 | 3.56E-03 | 4.48E-02 |
| Annotation Cluster 5 | | Enrichment Score: 4.734364155733175 | | | | | | | | | | | |
| Category | Term | Count | % | PValue | Genes | | List Total | Pop Hits | Pop Total | Fold Enrichment | Bonferroni | Benjamini | FDR |
| GOTERM_BP_FAT | GO:0001775="cell activation" | 24 | 6.94 | 1.31E-10 | FYBL, PLEX, MYO1F, RORC, SPFH1, CD10, WAS, FGR3, CC4B, SLC11A1, BC12A1D, CD86, ULR83, PLG2, CX3CR1, KRE1, FCFR1G, C1D4, IIL2B, HELLS, RHOM, LCPL2 | | 249 | 246 | 13588 | 5.32 | 2.04E-07 | 4.08E-08 | 2.19E-07 |
| GOTERM_BP_FAT | GO:0045321="leukocyte activation" | 22 | 6.36 | 5.46E-10 | FYBL, MYO1F, RORC, ITGB2, SPFH1, CD10, WAS, FGR3, CC4B, SLC11A1, BC12A1D, CD86, ULR83, PLG2, CX3CR1, KRE1, FCFR1G, C1D4, IIL2B, HELLS, RHOM, LCPL2 | | 249 | 219 | 13588 | 5.48 | 8.52E-07 | 1.42E-07 | 9.12E-07 |
| GOTERM_BP_FAT | GO:0002274="methylene leukocyte activation" | 10 | 2.89 | 9.07E-09 | FYBL, SLC11A1, ULR83, CX3CR1, MYO1F, FCFR1G, SPFH1, LCPL2, RHOM, FGR3, RORC, ITGB2, SPFH1, CD10, WAS, CC4B, SLC11A1, BC12A1D, CD86, BC12A1D, PLG2, FCFR1G, C1D4, IIL2B, HELLS, RHOM | | 249 | 35 | 13588 | 15.59 | 1.41E-05 | 1.77E-06 | 1.51E-05 |
| GOTERM_BP_FAT | GO:0046649="lymphocyte activation" | 15 | 4.34 | 1.09E-09 | RHOM | | 249 | 191 | 13588 | 4.29 | 1.68E-02 | 8.46E-04 | 1.81E-02 |
| Annotation Cluster 6 | | Enrichment Score: 4.102642809152643 | | | | | | | | | | | |
| Category | Term | Count | % | PValue | Genes | | List Total | Pop Hits | Pop Total | Fold Enrichment | Bonferroni | Benjamini | FDR |
| GOTERM_BP_FAT | GO:0002684="positive regulation of immune system process" | 21 | 6.07 | 1.15E-09 | C4B, IIL2B, CD1D1, CLOC, FCFR1G, FGR3, C1P, C1QA, SLC11A1, CD83, BC12A1D, CORO1A, PLG2, CX3CR1, KRE1, C1D4, IIL2B, C1C17, SASH3 | | 249 | 201 | 13588 | 5.56 | 1.79E-06 | 2.56E-07 | 1.92E-06 |
| GOTERM_BP_FAT | GO:0050778="positive regulation of immune response" | 16 | 4.62 | 2.64E-08 | CFER1G, CLECTA, SASH3 | | 249 | 136 | 13588 | 6.42 | 4.11E-05 | 4.40E-05 | |
| GOTERM_BP_FAT | GO:0045087="innate immune response" | 13 | 3.76 | 5.60E-07 | C4B, TLRL3, TU2R, CD1D1, CLOC, FCFR1G, FGR3, C1QD, C1QA, SLC11A1, BC12A1D, CD86, ULR83, PLG2, CX3CR1, KRE1, FCFR1G, C1D4, IIL2B, HELLS, RHOM, LCPL2 | | 249 | 107 | 13588 | 6.63 | 8.73E-04 | 6.72E-05 | 9.35E-04 |
| GOTERM_BP_FAT | GO:0045854="positive regulation of response to stimulus" | 16 | 4.62 | 1.06E-06 | CFER1G, CLECTA, SASH3 | | 249 | 186 | 13588 | 4.69 | 2.50E-05 | 1.56E-05 | 2.68E-05 |
| GOTERM_BP_FAT | GO:0002252="immune effector process" | 13 | 3.76 | 3.24E-06 | CFER1G, CLECTA, SASH3 | | 249 | 126 | 13588 | 5.63 | 5.04E-04 | 2.97E-04 | 5.41E-04 |
| GOTERM_BP_FAT | GO:0045853="immune response" | 11 | 3.18 | 4.91E-06 | CFER1G, CLECTA, SASH3 | | 249 | 89 | 13588 | 6.74 | 7.63E-04 | 4.03E-04 | 8.20E-04 |
| GOTERM_BP_FAT | GO:0004147="receptor-mediated immunity" | 11 | 3.18 | 4.91E-06 | C1QA, SLC11A1, C1C17, NCFL1, C1QD, ULR83, MYO1F, FCFR1G, FGR3, C1QD, C1QA, SLC11A1, BC12A1D, CD86, BC12A1D, PLG2, FCFR1G, C1D4, IIL2B, HELLS, RHOM, LCPL2 | | 249 | 89 | 13588 | 6.74 | 7.63E-04 | 4.03E-04 | 8.20E-04 |
| GOTERM_BP_FAT | GO:0002529="adaptive immune response" | 10 | 2.89 | 2.17E-05 | C1QA, SLC11A1, C1C17, ULR83, PLG2, FCFR1G, C1D4, IIL2B, HELLS, RHOM, LCPL2 | | 249 | 84 | 13588 | 6.50 | 3.33E-02 | 1.61E-03 | 3.63E-02 |
| GOTERM_BP_FAT | GO:0002469="adaptive immune response based on somatic recombination of immune receptors built fr | 10 | 2.89 | 2.17E-05 | C1QA, C1QB, SLC11A1, ULR83, PLG2, FCFR1G, C1D4, IIL2B, HELLS, RHOM, LCPL2 | | 249 | 84 | 13588 | 6.50 | 3.33E-02 | 1.61E-03 | 3.63E-02 |
| Annotation Cluster 7 | | Enrichment Score: 3.0962877308218895 | | | | | | | | | | | |
| Category | Term | Count | % | PValue | Genes | | List Total | Pop Hits | Pop Total | Fold Enrichment | Bonferroni | Benjamini | FDR |
| GOTERM_BP_FAT | GO:0050778="positive regulation of immune response" | 16 | 4.62 | 2.64E-08 | CFER1G, CLECTA, SASH3 | | 249 | 136 | 13588 | 6.42 | 4.11E-05 | 4.11E-06 | 4.40E-05 |
| GOTERM_BP_FAT | GO:0048584="positive regulation of response to stimulus" | 16 | 4.62 | 1.60E-06 | CFER1G, CLECTA, SASH3 | | 249 | 186 | 13588 | 4.69 | 2.50E-03 | 1.56E-04 | 2.68E-03 |
| Annotation Cluster 12 | | Enrichment Score: 2.13327429095495 | | | | | | | | | | | |
| Category | Term | Count | % | PValue | Genes | | List Total | Pop Hits | Pop Total | Fold Enrichment | Bonferroni | Benjamini | FDR |
| GOTERM_BP_FAT | GO:0006909="phagocytosis" | 8 | 2.31 | 2.77E-05 | SLC11A1, HCK, IRPF, FCFR1G, CLECTA, MEGF10, FCFR1G, FGR3 | | 249 | 49 | 13588 | 8.91 | 4.23E-02 | 1.96E-03 | 4.62E-02 |
| Annotation Cluster 13 | | Enrichment Score: 2.048358806290177 | | | | | | | | | | | |
| Category | Term | Count | % | PValue | Genes | | List Total | Pop Hits | Pop Total | Fold Enrichment | Bonferroni | Benjamini | FDR |
| GOTERM_MF_FAT | GO:0019955="cytokine binding" | 11 | 3.18 | 1.38E-06 | IIL8BP, BCR5, CSFR2B2, IL10RA, CCR2, CX3CR1, CSFR2B, IL2RG, CX1R, CSFR2A | | 214 | 88 | 13288 | 7.76 | 4.45E-04 | 4.45E-04 | 1.86E-03 |

Table S1.G GO Terms upregulated 336 hours post injury

Table S1.H GO Terms upregulated 504 hours post injury

Table S1.I GO Terms upregulated 672 hours post inj

Table S2.A KEGG pathways significant at 3 hours

| Category | Term | | Count | % | PValue | Genes | List | Total | Pop | Hits | Pop Total | Fold Enrichment | Bonferroni | Benjamini | FDR |
|--------------|---|--|-------|------|----------|---|--|-------|------|------|-----------|-----------------|------------|-----------|-----|
| KEGG_PATHWAY | mmu04060:Cytokine-cytokine receptor interaction | | 49 | 4.52 | 2.9E-10 | OSMR, CXCR2, IL17RA, TGFBR2, CXCR4, IL1RAP, CSF2R, CSF2RB, IL1B, FAS, IL13RA1, CSF2RA, EGFR, LTRB, INHBB, OSM, TNFRSF10B, PPBP, CCR2, PDGFR, CXCL1, CSF1, IL1R2, CCL3, IL1R1, CCL2, CSF2RB2, TNFRSF12A, CSF1, CR1, IL4RA, CXCL2, CXCL9, CCL9, P44, CNTR, CCL7, CCL6, CCL24, TNFRSF1A, TNFRSF1B, IL10RA, BMP2, TGFBRL1, TGFBR2, CCL11, CXCL14, | 434 | 244 | 5738 | 2.66 | 4.88E-08 | 4.88E-08 | 3.59E-07 | | |
| KEGG_PATHWAY | mmu04512:ECM-receptor interaction | | 25 | 2.31 | 4.36E-09 | TNC, COL3A1, SDC4, SDC2, CD44, COMP, COL6A2, COL6A1, COL11A2, THBS1, THBS2, THBS3, THBS4, SPP1, FN1, TNXB, COL4A1, ITGA3, ITGA4, COL5A2, LAMA4, TGAS1, COL1A2, | 434 | 83 | 5738 | 3.98 | 7.20E-07 | 3.60E-07 | 5.30E-06 | | |
| KEGG_PATHWAY | mmu04610:Complement and coagulation cascades | | 22 | 2.03 | 7.69E-08 | PLAT, C7, CSAR1, MASP1, C3, C4B, CFB, F13A1, SERPING1, C1S, C1QC, PLAU, C1QA, C1QB, C1RA, THBD, F3, SERPINE1, CFH, | 434 | 75 | 5738 | 3.88 | 1.27E-05 | 4.23E-06 | 9.33E-05 | | |
| KEGG_PATHWAY | mmu04510:Focal adhesion | | 37 | 3.41 | 4.52E-07 | CFD, PROS1, PLAU, MYL2, PGF, TNC, COL3A1, RAC2, COMP, COL6A2, COL6A1, PIK3R5, ZYX, PAK1, COL11A2, THBS1, THBS2, THBS3, FN1, SPP1, THBS4, ACTB, EGFR, COL4A1, TNXB, ACTN1, ITGA3, ITGA4, FLNB, BIRC3, FLNB, COL5A2, LAMA4, ITGA5, FYN, PDGFR, COL1A2, RAP1B, LAMC2, COL1A1 | 434 | 198 | 5738 | 2.47 | 7.45E-05 | 1.86E-05 | 5.48E-04 | | |
| KEGG_PATHWAY | mmu04210:Apoptosis | | 22 | 2.03 | 1.18E-06 | MAP3K14 | CXCL1, ADCY4, CCL3, CCL2, GNAI1, CCR1, CXCL2, CCL9, CXCL9, NFKBIA, NFKB1, CXCR2, PF4, GNG11, CCL7, CCL6, CCL24, RAC2, TIAM1, PTX2B, CXCR4, PIK3R5, PAK1, LYN, RELA, STAT3, | 434 | 87 | 5738 | 3.34 | 1.95E-04 | 3.89E-05 | 1.43E-03 | |
| KEGG_PATHWAY | mmu04062:Chemokine signaling pathway | | 33 | 3.04 | 4.28E-06 | STAT2, CCL11, PPBP, CXCL14, CXCL16, CCR1, RAP1B | IL1R2, IL1R1, FGFR3, MRAS, DUSP10, HSPA1A, NFKB1, HSPA1B, TGFB2, FOS, TNFRSF1A, BDNF, RAC2, HSPA2, MAP3K2, MAP3K1, JUND, MAP3K8, IL1B, FAS, PAK1, RAPGEF2, MYC, EGFR, RELA, TGFBRL1, TGFB2, FLNC, FLNB, DUSP5, MAP4KA, DUSP2, GADD45G, NTRK2, PDGFR, HSPB1, RAP1B, | 434 | 182 | 5738 | 2.40 | 7.05E-04 | 1.18E-04 | 5.19E-03 | |
| KEGG_PATHWAY | mmu04010:MAPK signaling pathway | | 41 | 3.78 | 1.37E-05 | GADD45B, MAP3K14, GADD45A, CD14, PLA2G4E | GADD45B, MAP3K14, GADD45A, CD14, PLA2G4E | 434 | 265 | 5738 | 2.05 | 2.26E-03 | 3.23E-04 | 1.66E-02 | |

Table S2.B KEGG pathways significant at 10 hours

Table S2.C KEGG pathways significant at 24 hours

Table S2.D KEGG pathways significant at 48 hours

Table S2.E KEGG pathways significant at 72 hours

| | | | | | | | | | | | | |
|--------------|-----------------------------------|----|------|----------|---|------|----|------|------|-------------|----------|-------------|
| KEGG_PATHWAY | mim05212:Pancreaticcancer | 47 | 0.65 | 9.20E-06 | E2F1, E2F2, PGF, NFKB1, BCL2L1, TGF β 1, TGF β 2, AKT1, ACRV1B, RAC2, CASP9, RAC3, RALB, RALA, PIK3CA, PIK3RS, EGF, FIGF, AKT3, AKT2, PIK3CG, TRP53, EGFR, PLD1, RELA, ARHGEEF6, TGF β R1, PIK3CD, TGF β R2, SMAD4, RAF1, BRC2, SMAD3, CDK6, STAT1, CDK4, STAT3, RALGDS, RAD51, VEGFB, CCND1, ARAF, VEGFA, MAPK3, MAPK9, MAPK8, IKBKB, TGF β 1, TGF β 2, AKT1, FOS, ACRV1B, CASP3, CASP9, PIK3CA, MYC, AKT3, AKT2, EGFR, PIK3CG, PIK3CD, CYCS, CCND1, JUN, MAPK3, PDGFRA, MAPK9, MAPK8, GRB2, TCF7L2, RAC2, RAC3, SOS1, BCL2, SOS2, PIK3RS, DVL1, FZD9, TRP53, TC7, MSH3, MSH2, TGF β R2, SMAD4, FZD1, RAF1, SMAD3, BIRC5, FZD2, FZD5, APP11, FZD4, FZD7, RALGDS, FZD6, DVL1, BAX, GSK3B, ARAF, E2F1, E2F2, GRB2, STAT5B, NFKBIA, NFKB1, BCL2L1, TGF β 1, TGF β 2, AKT1, ACRV1B, GAB2, SOS1, SOS2, PIK3CA, SHC1, PIK3RS, RUNX1, MYC, AKT3, AKT2, PIK3CG, TRP53, CTPB1, ICR, MAP2K2, RELA, TGF β R1, CBL, PIK3CD, TGF β R2, SMAD4, RAF1, SMAD3, CDK6, MECOM, CDK4, PTPN11, HRAS1, NRAS1, | 2203 | 72 | 5738 | 1.70 | 0.001774079 | 9.86E-05 | 0.011463648 |
| KEGG_PATHWAY | mim05210:Colorectalcancer | 53 | 0.73 | 2.43E-05 | BIRC5, FZD2, FZD5, APP11, FZD4, FZD7, RALGDS, FZD6, DVL1, BAX, GSK3B, ARAF, E2F1, E2F2, GRB2, STAT5B, NFKBIA, NFKB1, BCL2L1, TGF β 1, TGF β 2, AKT1, ACRV1B, GAB2, SOS1, SOS2, PIK3CA, SHC1, PIK3RS, RUNX1, MYC, AKT3, AKT2, PIK3CG, TRP53, CTPB1, ICR, MAP2K2, RELA, TGF β R1, CBL, PIK3CD, TGF β R2, SMAD4, RAF1, SMAD3, CDK6, MECOM, CDK4, PTPN11, HRAS1, NRAS1, | 2203 | 86 | 5738 | 1.61 | 0.004670485 | 2.46E-04 | 0.030220506 |
| KEGG_PATHWAY | mim05220:Chronic myeloid leukemia | 48 | 0.67 | 2.60E-05 | CCND1, CDKN1A, HDAC2, HDAC1, ARAF, MAPK3, MDM2, IKBKB | 2203 | 76 | 5738 | 1.65 | 0.005013764 | 2.51E-04 | 0.032446929 |

Table S2.F KEGG pathways significant at 168 hours

| Category | Term | Count | % PValue | Genes | List Total | Pop Hits | Pop Total | Fold Enrichment | Bonferroni | Benjamini | | FDR |
|--------------|--|-------|------------------|--|------------|----------|-----------|-----------------|-------------|-----------|-------------|-----|
| | | | | | | | | | | | | |
| KEGG_PATHWAY | mmu04062:Chemokine signaling pathway | 18 | 0.65 4.47E-07 | CCL2, FGR, NCF1, HCK, CCL9, WAS, CCL7, CCL17, PRKCB, CCL12, CCR5, RAC2, CCR2, CX3CR1, PIK3R5, XCL1, XCR1 | 129 | 182 | 5738 | 4.40 | 4.78E-05 | 4.78E-05 | 5.02E-04 | |
| KEGG_PATHWAY | mmu04670:Leukocyte transendothelial migration | 14 | 0.50 1.93E-06 | NCF2, NCF1, NCF4, ITGB2, PRKCB, VCAM1, CYBA, CYBB, EZR, RAC2, PLCG2, CLDN2, PIK3R5, RHOB, BID, FCGR4, ITGB2, NCR1, PRKCB, FCGR3, CD48, RAC2, PLCG2, FCER1G, PIK3R5, LCP2, TYROBP | 129 | 119 | 5738 | 5.23 | 2.06E-04 | 1.03E-04 | 0.002168346 | |
| KEGG_PATHWAY | mmu04650:Natural killer cell mediated cytotoxicity | 13 | 0.47 1.46E-05 | C1QA, C1QB, C7, CD66, C4B, FCGR4, H2-DMB1, HIST1H4I, C1QC, FCGR1, IL10, FCGR3 | 129 | 122 | 5738 | 4.74 | 0.001563801 | 5.22E-04 | 0.016439609 | |
| KEGG_PATHWAY | mmu05322:Systemic lupus erythematosus | 12 | 0.43 1.55E-05 | CL2, CSF2RB2, CCL9, CCL8, IL10, CCL7, CCL17, CCL12, CCR5, IL10RA, CCR2, CX3CR1, CSF2RB, IL2RG, C1QA, C1QB, C7, CD66, C4B, FCGR4, H2-DMB1, HIST1H4I, C1QC, FCGR1, IL10, FCGR3 | 129 | 103 | 5738 | 5.18 | 0.001652768 | 4.13E-04 | 0.017375573 | |
| KEGG_PATHWAY | mmu04060:Cytokine-cytokine receptor interaction | 18 | 0.65 2.48E-05 | IL12B, XCL1, XCR1, CSF2RA | 129 | 244 | 5738 | 3.28 | 0.00265068 | 5.31E-04 | 0.027879105 | |

Table S2.G KEGG pathways significant at 336 hours

| Category | Term | Count | % | PValue | Genes | List Total | Pop Hits | Pop Total | Fold Enrichment | Bonferroni | Benjamini | FDR |
|--------------|---------------------------------------|-------|------|----------|--|------------|----------|-----------|-----------------|-------------|-----------|-------------|
| KEGG_PATHWAY | mmu04512:ECM-receptor interaction | 18 | 2.36 | 7.85E-08 | COL1A1, THBS2, THBS3, SPP1, FN1 IBSP, TNC, COL3A1, ITGB5, ITGA4, COL5A3, COL5A2, SDC2, COL5A1, CD44, COL1A2, COL6A2, COL6A1, | 254 | 83 | 5738 | 4.90 | 1.08E-05 | 1.08E-05 | 9.24E-05 |
| KEGG_PATHWAY | mmu05416:Viral myocarditis | 16 | 2.10 | 1.24E-05 | MYH13, MYH14, ABL2 H2-K1, TGAL, MYH3, H2-D1, H2-DMB1, ITGB2, MYH7, H2-AB1, MYH8, CD86, H2-EB1, SGCD, H2-AA, | 254 | 94 | 5738 | 3.85 | 0.001713391 | 8.57E-04 | 0.014623985 |
| KEGG_PATHWAY | mmu04062:Chemokine signalling pathway | 23 | 3.01 | 1.34E-05 | PARD3, CCL2, ADCY7, NCF1, STAT5B, CXCL9, CCL9, CCL8, GNG11, PF4, CCL6, CCL12, CCR5, ADCY9, TIAM1, CXCR4, GNG10, CXCL16, GSK3B, CCR2, SOS2, PAK1, SHC2 | 254 | 182 | 5738 | 2.85 | 0.001841954 | 6.14E-04 | 0.015722211 |
| KEGG_PATHWAY | mmu04510:Focal adhesion | 24 | 3.15 | 1.64E-05 | IBSP, FLT1, TNC, COL3A1, ITGB5, ITGA4, COL5A3, COL5A2, COL5A1, GSK3B, BCL2, SOS2, COL6A2, GRLF1, COL1A2, COL6A1, COL1A1, PAK1, EGF, SHC2, THBS2, THBS3, SPP1, FN1 | 254 | 198 | 5738 | 2.74 | 0.002258263 | 5.65E-04 | 0.019279342 |

Table S2.H KEGG pathways significant at 504 hours

| Category | Term | Count | % | PValue | Genes |
|--------------|--------------------------------------|-------|------|----------|---|
| KEGG_PATHWAY | mmu04512:ECM-receptor interaction | 18 | 2.36 | 7.85E-08 | COL1A1, THBS2, THBS3, SPP1, FN1 IBSP, TNC, COL3A1, ITGB5, ITGA4, COL5A3, COL5A2, SDC2, COL5A1, CD44, COL1A2, COL6A2, COL6A1, H2-K1, TGAL, MYH3, H2-D1, H2-DMB1, ITGB2, MYH7, H2-AB1, MYH8, CD86, H2-EB1, SGCD, H2-AA, |
| KEGG_PATHWAY | mmu05416:Viral myocarditis | 16 | 2.10 | 1.24E-05 | MYH13, MYH14, ABL2 PARD3, CCL2, ADCY7, NCF1, STAT5B, CXCL9, CCL9, CCL8, GNG11, PF4, CCL6, CCL12, CCR5, ADCY9, |
| KEGG_PATHWAY | mmu04062:Chemokine signaling pathway | 23 | 3.01 | 1.34E-05 | TIAM1, CXCR4, GNG10, CXCL16, GSK3B, CCR2, SOS2, PAK1, SHC2 IBSP, FLT1, TNC, COL3A1, ITGB5, ITGA4, COL5A3, COL5A2, COL5A1, GSK3B, BCL2, SOS2, COL6A2, |
| KEGG_PATHWAY | mmu04510:Focal adhesion | 24 | 3.15 | 1.64E-05 | GRLF1, COL1A2, COL6A1, COL1A1, PAK1, EGF, SHC2, THBS2, THBS3, SPP1, FN1 |

Table S2.H KEGG pathways significant at 504 hours

| List Total | Pop Hits | Pop Total | Fold Enrichment | Bonferroni | Benjamini | FDR |
|------------|----------|-----------|-----------------|-------------|-----------|-------------|
| 254 | 83 | 5738 | 4.90 | 1.08E-05 | 1.08E-05 | 9.24E-05 |
| 254 | 94 | 5738 | 3.85 | 0.001713391 | 8.57E-04 | 0.014623985 |
| 254 | 182 | 5738 | 2.85 | 0.001841954 | 6.14E-04 | 0.015722211 |
| 254 | 198 | 5738 | 2.74 | 0.002258263 | 5.65E-04 | 0.019279342 |

Table S2.1 KEGG pathways significant at 672 hours

| Category | Term | Count | % | PValue | Genes | List Total | Pop Hits | Pop Total | Fold Enrichment | Bonferroni | Benjamini | FDR |
|--------------|--|-------|------|----------|--|------------|----------|-----------|-----------------|------------|-----------|----------|
| KEGG_PATHWAY | mmu04512:ECM-receptor interaction | 17 | 2.51 | 7.58E-08 | IBSP, TNC, COL3A1, ITGA4, COL5A2, SDC2, COL5A1, CD44, COMP, COL1A2, COL6A2, COL6A1, COL1A1, THBS2, COL1A1, THBS1, FN1 | 223 | 83 | 5738 | 5.27 | 1.08E-05 | 1.08E-05 | 8.96E-05 |
| KEGG_PATHWAY | mmu04510:Focal adhesion | 26 | 3.85 | 1.21E-07 | IBSP, CAV2, CAV1, MYL2, TNC, COL3A1, BCL2, COMP, COL6A2, COL6A1, PAK1, EGF, COL11A1, THBS2, FN1, VAV3, IGF1, ITGA4, COL5A1, PDGFRA, COL1A2, GRIP1, COL1A1, PARVA | 223 | 198 | 5738 | 3.38 | 1.72E-05 | 8.62E-06 | 1.44E-04 |
| KEGG_PATHWAY | mmu04610:Complement and coagulation cascades | 15 | 2.22 | 7.70E-07 | C3AR1, CFB, F13A1, SERPING1, C1S, C1QC, C1Q8, C1RA, CD55, F3, CFH, C2, CFD, PROS1 | 223 | 75 | 5738 | 5.15 | 1.09E-04 | 3.64E-05 | 9.10E-04 |

Table S3. Significant GO clusters identified from the 168h time point that were obtained from differentially expressed genes that were determined to contribute the largest variance at that time point.

| Cluster 1: Enrichment Score: 11.54 | | | | | |
|------------------------------------|---------------------------------|------------|----------|----------|--|
| Annotation | GO Term | Gene Count | P-value | FDR | |
| GOTERM_BP_FAT | vasculature development | 85 | 5.20E-14 | 9.70E-11 | |
| GOTERM_BP_FAT | blood vessel development | 83 | 1.00E-13 | 1.90E-10 | |
| GOTERM_BP_FAT | blood vessel morphogenesis | 69 | 4.30E-12 | 8.00E-09 | |
| GOTERM_BP_FAT | angiogenesis | 48 | 3.00E-09 | 5.60E-06 | |
| Cluster 2: Enrichment Score: 11.31 | | | | | |
| Annotation | GO Term | Gene Count | P-value | FDR | |
| GOTERM_CC_FAT | extracellular matrix | 100 | 2.50E-14 | 3.60E-11 | |
| SP_PIR_KEYWORD | extracellular matrix | 76 | 7.40E-14 | 1.10E-10 | |
| GOTERM_CC_FAT | proteinaceous extracellular m | 96 | 9.40E-14 | 1.40E-10 | |
| GOTERM_CC_FAT | extracellular region part | 179 | 1.10E-09 | 1.60E-06 | |
| GOTERM_CC_FAT | extracellular matrix part | 37 | 1.50E-08 | 2.20E-05 | |
| Cluster 3: Enrichment Score: 9.38 | | | | | |
| Annotation | GO Term | Gene Count | P-value | FDR | |
| KEGG_PATHWAY | lysosome | 53 | 2.40E-12 | 3.00E-09 | |
| SP_PIR_KEYWORD | lysosome | 52 | 5.60E-10 | 8.30E-07 | |
| GOTERM_CC_FAT | lysosome | 60 | 1.00E-09 | 1.50E-06 | |
| GOTERM_CC_FAT | lytic vacuole | 60 | 1.30E-09 | 1.90E-06 | |
| GOTERM_CC_FAT | vacuole | 64 | 6.80E-09 | 9.90E-06 | |
| Cluster 4: Enrichment Score: 7.85 | | | | | |
| Annotation | GO Term | Gene Count | P-value | FDR | |
| GOTERM_BP_FAT | vesicle-mediated transport | 121 | 2.70E-10 | 5.00E-07 | |
| GOTERM_BP_FAT | membrane invagination | 61 | 2.10E-09 | 3.90E-06 | |
| GOTERM_BP_FAT | endocytosis | 61 | 2.10E-09 | 3.90E-06 | |
| GOTERM_BP_FAT | membrane organization | 76 | 3.10E-08 | 5.70E-05 | |
| SP_PIR_KEYWORD | endocytosis | 31 | 1.60E-05 | 2.40E-02 | |
| Cluster 5: Enrichment Score: 7.18 | | | | | |
| Annotation | GO Term | Gene Count | P-value | FDR | |
| GOTERM_BP_FAT | actin filament-based process | 61 | 1.10E-10 | 2.00E-07 | |
| GOTERM_BP_FAT | actin cytoskeleton organization | 57 | 5.60E-10 | 1.00E-06 | |
| GOTERM_BP_FAT | actin filament organization | 23 | 6.70E-06 | 1.20E-02 | |
| GOTERM_BP_FAT | cytoskeleton organization | 76 | 4.80E-05 | 8.80E-02 | |