

Supplement Materials

**„Influence of Shear Stress, Inflammation and BRD4 Inhibition on
Human Endothelial Cells: A Holistic Proteomic Approach“**

Jarauschk et al.

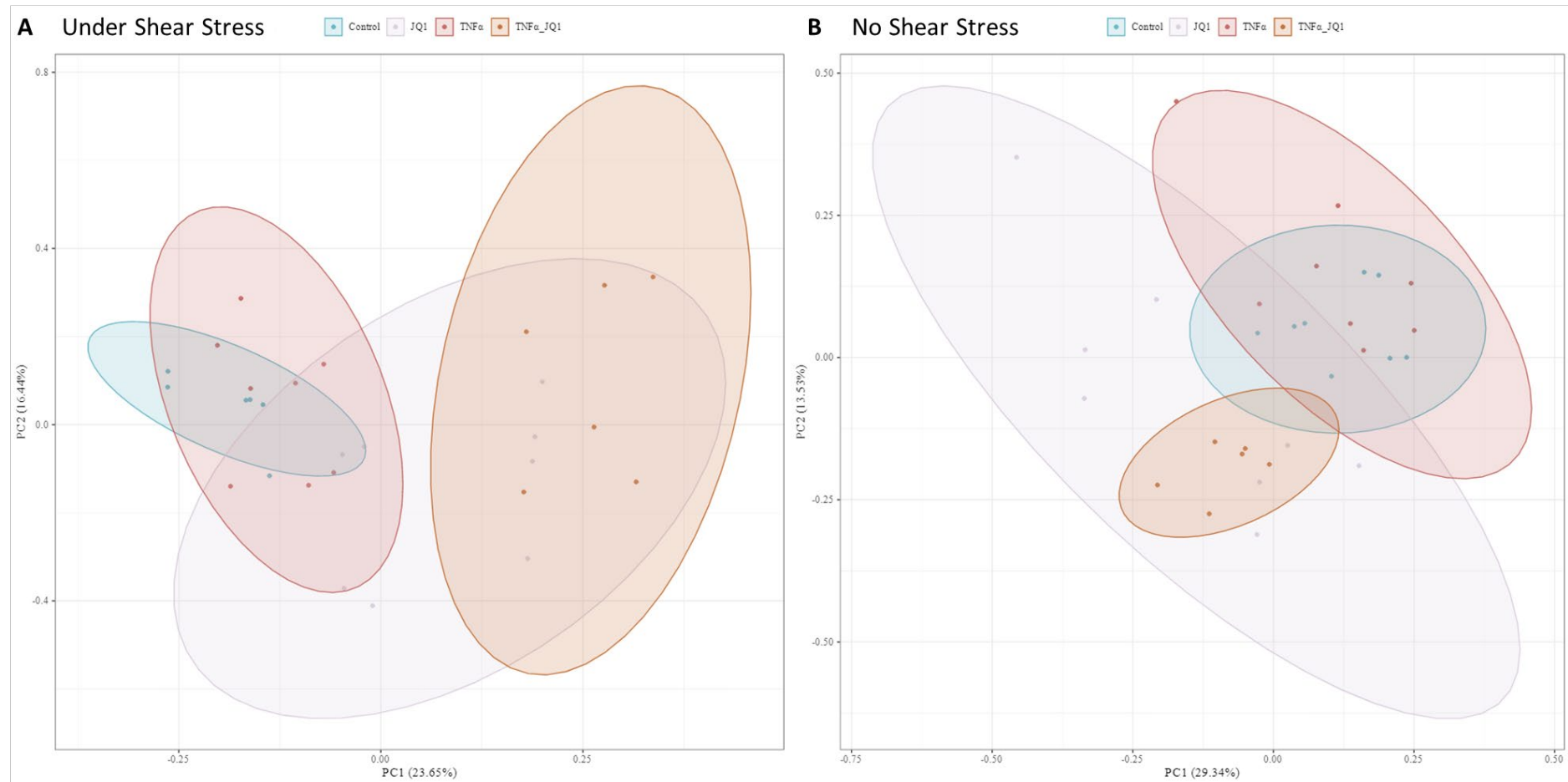


Figure S1. Principal component analysis of all groups under and without SS. (A) A PCA of all groups under and without shear stress (SS) was performed. The first two principal components are shown. The groups treated with TNF α + JQ1 show distinct clustering, whereas the groups treated

only with JQ1 did not separate as well. **(B)** Under no SS all groups treated with JQ1 show a pattern distinctive from groups that did not receive JQ1 treatment.

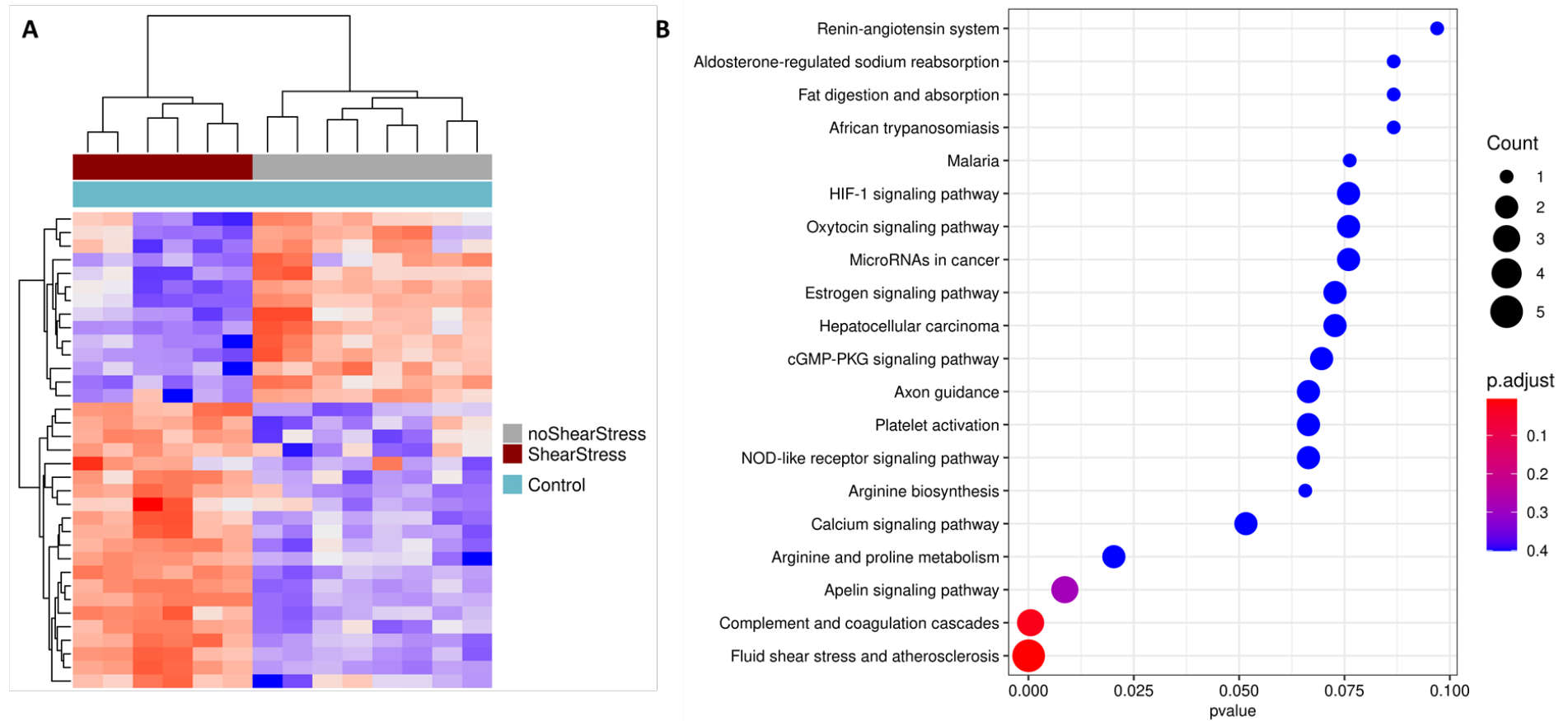
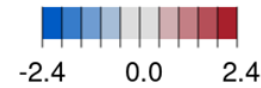
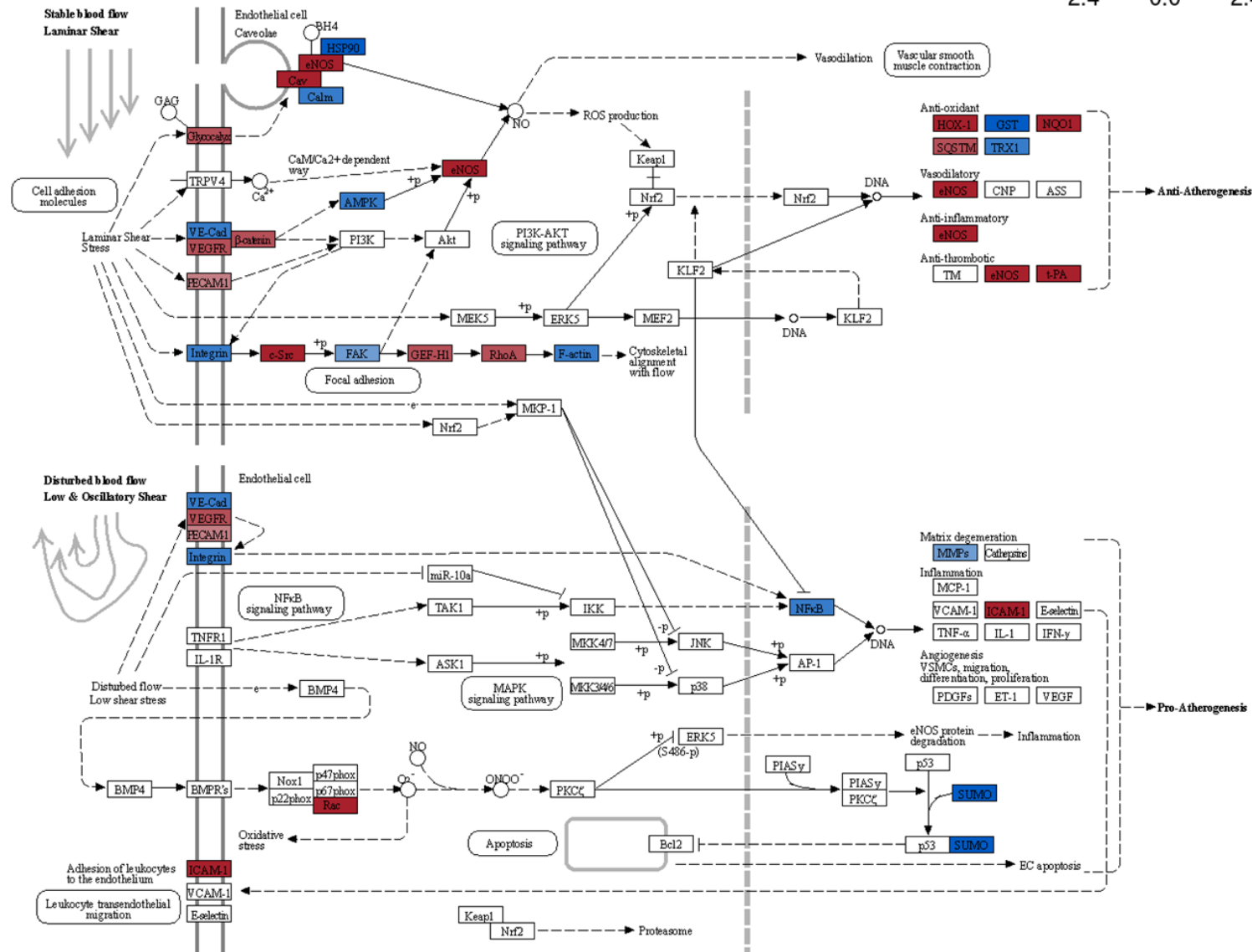


Figure S2. Heat map of all tested proteins between control groups under SS vs. no SS and overrepresented KEGG-pathways. (A) Heat map of all proteins between control group under shear stress (SS) or not were selected. The colours show normalized expression values for the differentially expressed proteins (rows) across samples (columns). Both rows and columns are ordered by hierarchical clustering. The clustering is visualized using dendrograms at the top and at the left. The treatment groups show distinct clustering. (B) Overrepresented Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways are ordered according to significance (most significantly enriched pathway at the bottom) and coloured by p value. The size of the points represents the count of proteins per pathway. The significantly differential pathways “Fluid shear stress and atherosclerosis” and “Complement and coagulation cascades” are represented at the bottom.

Shear Stress vs. Control



FLUID SHEAR STRESS AND ATHEROSCLEROSIS



Data on KEGG graph
Rendered by Pathview

Figure S3. Proteins in the overrepresented KEGG-pathway “Fluid shear stress and atherosclerosis” coloured by log fold change from the comparison between shear stress and control groups. All measured proteins mapped to this pathway are coloured by log fold change (blue for downregulated compared to control, red for upregulated).

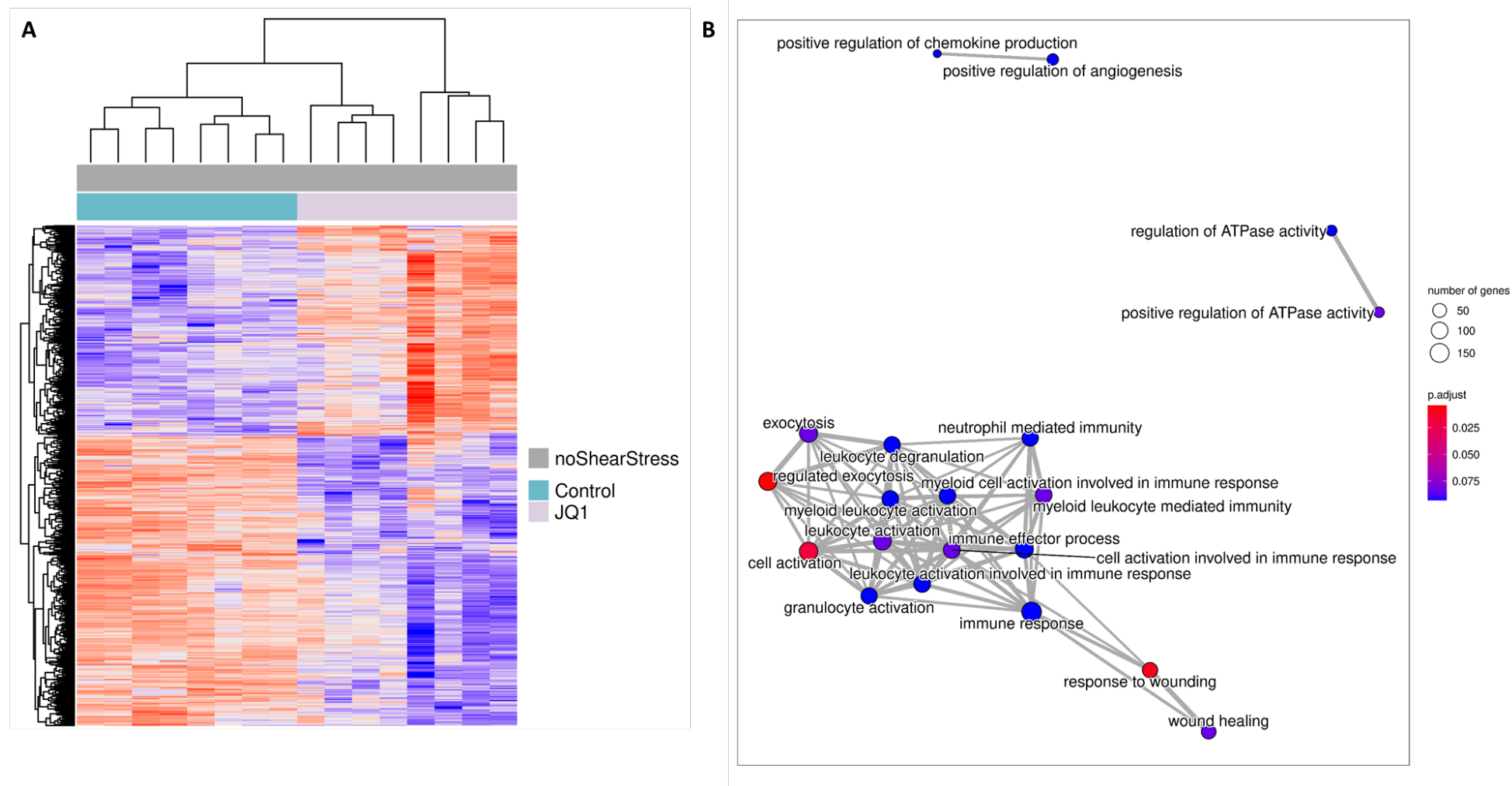


Figure S4. Heat map of all tested proteins under JQ1 without SS in comparison to control and enrichment network-map of the top 20 overrepresented GO terms. (A) All proteins between control and JQ1 treatment without shear stress (SS) were selected. The colours show

normalized expression values for the differentially expressed proteins (rows) across samples (columns). Both rows and columns are ordered by hierarchical clustering. The clustering is visualized using dendrograms at the top and at the left. The treatment groups show distinct clustering. **(B)**

The dots represent the most overrepresented gene ontology (GO) terms with sizes according to the number of proteins associated with the term. The colour of the dots represents the statistical significance according to the adjusted p value. The width of the connecting lines is according to the number of shared proteins. “Cell activation” and “regulated exocytosis” are closely associated.

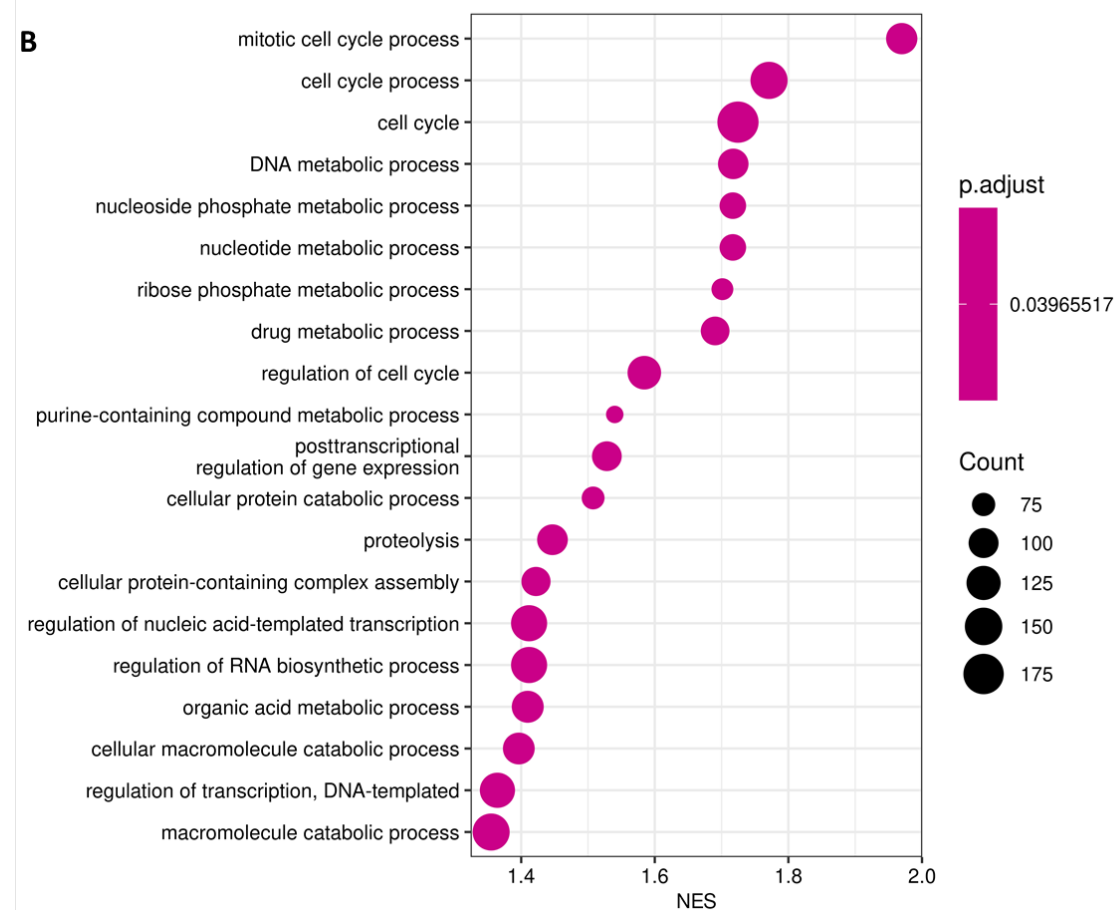
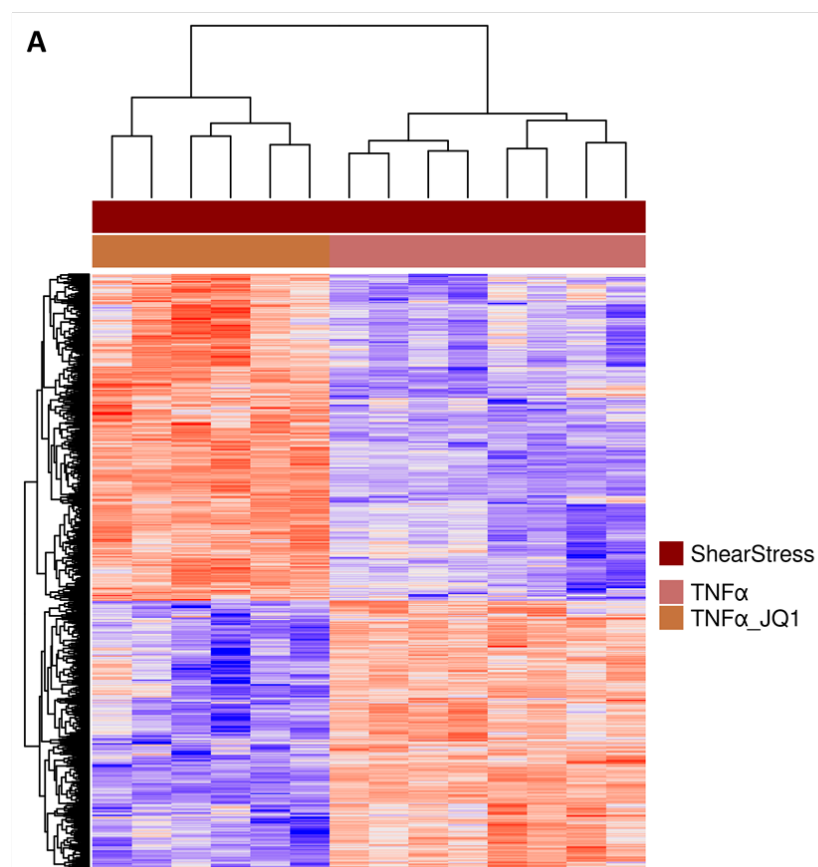


Figure S5. Heat map of all tested proteins in the TNFα + JQ1 under SS vs. TNFα under SS treatment group and the top 20 enriched GO terms.

(A) Significantly differentially expressed proteins between TNFα and TNFα + JQ1 treatment groups under shear stress (SS). The colours show normalized expression values for the differentially expressed proteins (rows) across samples (columns). Both rows and columns are ordered by

hierarchical clustering. The clustering is visualized using dendrograms at the top and at the left. The treatment groups show distinct clustering. **(B)**

The top 20 overrepresented gene ontology (GO) terms comparing TNF α and TNF α + JQ1 treatment groups under SS are ordered according to the normalized enrichment score (most enriched term at the top) and coloured by the adjusted p value. The size of the points represents the size of the term. As can be seen, GO terms related to cell cycle and metabolic processes are most strongly enriched.

Table S1. Top 20 significantly differentially expressed proteins discovered among all treatment groups by ANOVA-like testing ordered according to significance.

Protein	Average Expression	p value	Adjusted p value	Protein Descriptions	Ensgene
SLC9A3R2	7.39847331	1.53234E-27	5.08125E-24	Na(+)/H(+) exchange regulatory cofactor NHE-RF2	ENSG00000065054
FTH1	10.629059	6.26614E-26	1.03893E-22	Ferritin heavy chain	ENSG00000167996
FN1	11.51576524	1.85509E-25	1.56534E-22	Isoform 1 of Fibronectin	ENSG00000115414
FTL	9.622760966	1.88822E-25	1.56534E-22	Ferritin light chain	ENSG00000087086
EIF5	8.513574672	4.54818E-25	3.01635E-22	Eukaryotic translation initiation factor 5	ENSG00000100664
HMOX1	11.46183291	1.96162E-24	1.08412E-21	Heme oxygenase 1	ENSG00000100292
P4HA1	9.454305828	2.36744E-23	1.12149E-20	Prolyl 4-hydroxylase subunit alpha-1	ENSG00000122884
NRP1	7.887329534	4.82122E-23	1.9984E-20	Neuropilin-1	ENSG00000099250
TNFSF4	8.577782052	5.69281E-23	2.09749E-20	Tumor necrosis factor ligand superfamily member 4	ENSG00000117586
HBA1	10.32080764	2.76832E-22	9.17974E-20	Hemoglobin subunit alpha	ENSG00000206172
SERPINH1	12.67097379	3.97193E-22	1.19736E-19	Serpin H1	ENSG00000149257
PTPRB	7.834447345	1.63651E-21	4.52222E-19	Receptor-type tyrosine-protein phosphatase beta	ENSG00000127329
GPX8	9.666582483	1.94209E-20	4.83723E-18	Probable glutathione peroxidase 8	ENSG00000164294
RAB5B	9.464157828	2.04225E-20	4.83723E-18	Ras-related protein Rab-5B	ENSG00000111540
SORBS2	7.765468741	5.81692E-20	1.28593E-17	Sorbin and SH3 domain-containing protein 2	ENSG00000154556

SQSTM1	9.594663069	7.12894E-20	1.47747E-17	Sequestosome-1	ENSG00000161011
CD9	10.00665303	7.94997E-20	1.55071E-17	Tetraspanin	ENSG00000010278
CAV1	11.53978357	2.09811E-19	3.86519E-17	Caveolin-1	ENSG00000105974
ICAM1	9.617511569	2.89168E-19	4.98981E-17	Intercellular adhesion molecule 1	ENSG00000090339
C1QTNF5	9.101523	3.00953E-19	4.98981E-17	Complement C1q tumor necrosis factor-related protein 5	ENSG00000223953

Table S2. Significantly up- or downregulated proteins under shear stress vs. control in relation to all discovered proteins. 35 proteins are significantly differentially expressed. 21 are up- and 14 are downregulated under shear stress.

Control vs. Shear Stress	Non significant	significant	
Down-Regulated	1553	14	
Up-Regulated	1728	21	
Σ	3281	35	Total: 3316

Table S3. Significantly up- or downregulated proteins in the JQ1 without shear stress group vs. control in relation to all discovered proteins.

962 proteins are significantly differentially expressed. 404 are up- and 558 downregulated under JQ1.

Control vs. JQ1	Non significant	significant	
Down-Regulated	1194	558	
Up-Regulated	1160	404	
Σ	2354	962	Total: 3316

Table S4. Significantly up- or downregulated proteins in the JQ1 + TNF α with shear stress group vs. TNF α with SS in relation to all discovered proteins. 955 proteins are significantly differentially expressed. 526 are up- and 429 downregulated under JQ1 + TNF α .

TNF α vs. JQ1 + TNF α under Shear Stress	Non significant	significant	
Down-Regulated	1062	429	
Up-Regulated	1299	526	
Σ	2361	955	Total: 3316

Table S5. Top 20 significantly differentially expressed proteins discovered in the shear stress vs. control group ordered according to significance.

Protein	Average Expression	p value	Adjusted p value	Protein Descriptions	Ensgene
SLC9A3R2	7.39847331	3.47565E-13	1.15253E-09	Na(+)/H(+) exchange regulatory cofactor NHE-RF2	ENSG00000065054
HBA1	10.32080764	9.39887E-13	1.55833E-09	Hemoglobin subunit alpha	ENSG00000206172
PLAT	7.433489845	1.18065E-09	1.30502E-06	Tissue-type plasminogen activator	ENSG00000104368
HMOX1	11.46183291	1.5531E-08	1.28752E-05	Heme oxygenase 1	ENSG00000100292
STING1	8.738845293	2.38028E-08	1.5786E-05	Stimulator of interferon response cGAMP interactor 1	ENSG00000184584
NOS3	7.073176879	7.74897E-08	4.2826E-05	Nitric oxide synthase. endothelial	ENSG00000164867
ITPR3	7.42922369	1.72222E-07	8.15841E-05	Inositol 1,4,5-trisphosphate receptor type 3	ENSG00000096433
DNASE1L1	7.406114017	3.46328E-07	0.000143553	Deoxyribonuclease-1-like 1	ENSG00000013563
HYAL2	8.708874914	2.28588E-06	0.000842221	Hyaluronidase-2	ENSG00000068001
PLXNA2	7.72602269	8.62245E-06	0.002859204	Plexin-A2	ENSG00000076356
NQO1	7.474098828	1.21544E-05	0.003663984	NAD(P)H dehydrogenase [quinone] 1	ENSG00000181019
LXN	8.313569621	2.2551E-05	0.006231583	Latexin	ENSG00000079257
TPI1	11.45701069	3.17862E-05	0.008080394	Triosephosphate isomerase	ENSG00000111669
F5	8.466895328	3.48665E-05	0.008080394	Coagulation factor V	ENSG00000198734
GYG1	7.465747086	3.65518E-05	0.008080394	Glycogenin-1	ENSG00000163754
PLEC.Q15149_3	9.633755621	5.64375E-05	0.011312555	Isoform 3 of Plectin	
DPYSL2	10.64734467	5.79956E-05	0.011312555	Dihydropyrimidinase-related protein 2	ENSG00000092964
PLEC	11.58752569	6.46601E-05	0.011911834	Plectin	ENSG00000178209
PLPP3	8.038891931	8.09454E-05	0.01401305	Phospholipid phosphatase 3	ENSG00000162407
PODXL	8.255463603	8.45178E-05	0.01401305	Podocalyxin	ENSG00000128567

Table S6. Top 20 significantly differentially expressed proteins discovered in the JQ1 without shear stress vs. control group ordered according to significance.

Protein	Average Expression	p value	Adjusted p value	Protein Descriptions	Ensgene
SQSTM1	9.594663069	4.21271E-17	1.39694E-13	Sequestosome-1	ENSG00000161011
NRP1	7.887329534	2.26174E-16	3.74997E-13	Neuropilin;Neuropilin-1	ENSG00000099250
EIF5	8.513574672	3.64909E-16	4.03346E-13	Eukaryotic translation initiation factor 5	ENSG00000100664
FTH1	10.629059	9.64039E-16	7.99188E-13	Ferritin heavy chain	ENSG00000167996
RAB5B	9.464157828	1.15573E-14	7.66481E-12	Ras-related protein Rab-5B	ENSG00000111540
FTL	9.622760966	1.61977E-14	8.95191E-12	Ferritin light chain	ENSG00000087086
OGDH	9.473688552	4.13929E-14	1.96084E-11	2-oxoglutarate dehydrogenase, mitochondrial	ENSG00000105953
NQO1	7.474098828	6.41831E-14	2.66039E-11	NAD(P)H dehydrogenase [quinone] 1	ENSG00000181019
GTF2I	8.301597414	1.50104E-13	5.53051E-11	General transcription factor II-I	ENSG00000263001
P4HA1	9.454305828	2.44369E-13	8.10329E-11	Prolyl 4-hydroxylase subunit alpha-1	ENSG00000122884
GPX8	9.666582483	5.94626E-13	1.79253E-10	Probable glutathione peroxidase 8	ENSG00000164294
PLAT	7.433489845	1.61796E-12	4.47096E-10	Tissue-type plasminogen activator	ENSG00000104368
PI4K2A	7.497889259	2.43798E-12	6.21872E-10	Phosphatidylinositol 4-kinase type 2-alpha	ENSG00000155252
A2M	9.852662879	4.1507E-12	9.83122E-10	Alpha-2-macroglobulin	ENSG00000175899
PTPRB	7.834447345	6.24441E-12	1.38043E-09	Receptor-type tyrosine-protein phosphatase beta	ENSG00000127329
CCAR2	9.185597207	8.39148E-12	1.73914E-09	Cell cycle and apoptosis regulator protein 2	ENSG00000158941
AP2M1	10.23082283	1.85962E-11	3.62736E-09	Isoform 2 of AP-2 complex subunit mu	ENSG00000161203
DPYSL2	10.64734467	5.49059E-11	1.01149E-08	Dihydropyrimidinase-related protein 2	ENSG00000092964
MAGED2	8.564668431	5.93056E-11	1.03504E-08	Melanoma-associated antigen D2	ENSG00000102316
SLC9A3R2	7.39847331	9.63849E-11	1.59806E-08	Na(+)/H(+) exchange regulatory cofactor NHE-RF2	ENSG00000065054

Table S7. Top 20 significantly differentially expressed proteins discovered in the JQ1 + TNF α under shear stress SS vs. TNF α under shear stress group ordered according to significance.

Protein	Average Expression	p value	Adjusted p value	Protein Descriptions	Ensgene
EIF5	8.513574672	4.58533E-19	1.5205E-15	Eukaryotic translation initiation factor 5	ENSG00000100664
FTL	9.622760966	1.24105E-17	2.05765E-14	Ferritin light chain	ENSG00000087086
FTH1	10.629059	3.55049E-17	3.25015E-14	Ferritin heavy chain	ENSG00000167996
SLC9A3R2	7.39847331	3.92057E-17	3.25015E-14	Na(+)/H(+) exchange regulatory cofactor NHE-RF2	ENSG00000065054
SERPINH1	12.67097379	5.09695E-17	3.3803E-14	Serpin H1	ENSG00000149257
TNFSF4	8.577782052	1.66871E-15	9.2224E-13	Tumor necrosis factor ligand superfamily member 4	ENSG00000117586
STING1	8.738845293	6.79797E-15	3.2203E-12	Stimulator of interferon response cGAMP interactor 1	ENSG00000184584
P4HA1	9.454305828	3.76871E-14	1.53815E-11	Prolyl 4-hydroxylase subunit alpha-1	ENSG00000122884
SORBS2	7.765468741	4.1747E-14	1.53815E-11	Sorbin and SH3 domain-containing protein 2	ENSG00000154556
PLSCR4	8.406305397	5.40921E-14	1.72575E-11	Phospholipid scramblase 4	ENSG00000114698
FNDC3B	8.104490586	5.84412E-14	1.72575E-11	Fibronectin type III domain-containing protein 3B	ENSG00000075420
NEK9	6.827641138	6.24516E-14	1.72575E-11	Serine/threonine-protein kinase Nek9	ENSG00000119638
MYADM	10.08777569	2.4017E-12	6.11818E-10	Myeloid-associated differentiation marker	ENSG00000179820
EOGT	9.176582431	2.59305E-12	6.11818E-10	EGF domain-specific O-linked N-acetylglucosamine transferase	ENSG00000163378
IGFBP7	8.813039707	2.76757E-12	6.11818E-10	Insulin-like growth factor-binding protein 7	ENSG00000163453
PCNA	9.368632103	3.45943E-12	7.08945E-10	Proliferating cell nuclear antigen	ENSG00000132646
MOGS	10.73190186	3.63452E-12	7.08945E-10	Mannosyl-oligosaccharide glucosidase	ENSG00000115275
CD9	10.00665303	4.13328E-12	7.61441E-10	Tetraspanin	ENSG00000010278
RALA	10.97131834	4.74958E-12	8.00418E-10	Ras-related protein Ral-A	ENSG00000006451
GCLM	8.503469345	4.82761E-12	8.00418E-10	Glutamate-cysteine ligase regulatory subunit	ENSG00000023909