Supplementary material

Tab S1. The 44 common differentially expressed proteins caused by storage temperature by General iTRAQ.

NO	Uniprot ID	Genename	Founction	Location	Log_2FoldChange	
					10°C 5d vs 22°C 5d	-80°C+D 5d vs 22°C 5d
1	O95433	AHSA1	protein binding	intracellular organelle	-1.61	-0.94
2	O00483	NDUFA4	oxidoreductase activity	organelle membrane	-1.11	-2.62
3	P18136	P18136	immunoglobulin receptor binding	cell periphery	-0.69	0.73
4	Q9UNF0	PACSIN2	cytoskeletal protein binding	plasma membrane region	-0.67	-1.11
5	O00534	VWA5A	-	intracellular organelle part	-0.60	-0.64
6	P25705	ATP5A1	protein binding	intracellular organelle	0.59	0.89
7	P09486	SPARC	collagen binding	plasma membrane	0.63	1.26
8	B2RUZ4	SMIM1	-*	extracellular organelle	0.64	0.65
9	Q9H3K6	BOLA2	-	extracellular organelle	0.68	0.59
10	P10124	SRGN	protein binding	intracellular organelle	0.70	1.08
11	P01009	SERPINA1	glycoprotein binding	endoplasmic reticulum	0.72	1.15
12	P02787	TF	transferrin receptor binding	plasma membrane region	0.74	1.25
13	P01834	IGKC	antigen binding	extracellular organelle	0.75	1.22
14	P01024	C3	protein binding	extracellular organelle	0.75	0.88
15	P01137	TGFB1	-	-	0.76	1.06
16	P00751	CFB	catalytic activity	extracellular organelle	0.77	2.34
17	O75947	ATP5H	hydrolase activity	intracellular organelle	0.80	0.60
18	Q8NEU8	APPL2	protein binding	extracellular region part	0.80	0.98
19	P01859	IGHG2	protein binding	extracellular organelle	0.80	0.75
20	P01857	IGHG1	protein binding	extracellular organelle	0.83	1.19
21	P07225	PROS1	protein binding	endoplasmic reticulum	0.84	0.95
22	Q15388	TOMM20	protein transporter activity	endoplasmic reticulum	0.85	-1.04
23	P05067	APP	peptidase inhibitor activity	endoplasmic reticulum	0.86	1.08
24	P10909	CLU	hydrolase activity	endoplasmic reticulum	0.94	1.14
25	P0C0L4	C4A	peptidase regulator activity	extracellular organelle	0.97	0.78
26	Q14766	LTBP1	signaling receptor activity	extracellular region part	0.98	1.28
27	P99999	CYCS	hydrolase activity	organelle envelope lumen	1.10	1.33
28	P30084	ECHS1	catalytic activity	intracellular organelle	1.16	2.23
29	P11277	SPTB	cytoskeletal protein binding	intracellular organelle	1.17	1.08
30	P02760	AMBP	enzyme inhibitor activity	intracellular organelle	1.18	1.29
31	P69905	HBA1	oxygen transporter activity	intracellular organelle	1.24	-0.62
32	P02790	HPX	cofactor transporter activity	intracellular organelle	1.36	0.69
33	P02671	FGA	protein binding	endoplasmic reticulum	1.43	1.76
34	P02652	APOA2	cholesterol transporter activity	endoplasmic reticulum	1.44	1.35

35	P12259	F5	cation binding	plasma membrane	1.53	1.71
36	P02679	FGG	cation binding	intracellular organelle	1.65	1.94
37	P07996	THBS1	cytokine binding	endoplasmic reticulum	1.65	2.61
38	P02675	FGB	protein binding	intracellular organelle	1.71	2.29
39	P04275	VWF	glycoprotein binding	endoplasmic reticulum	1.75	2.15
40	P02749	APOH	enzyme regulator activity	plasma lipoprotein particle	1.82	2.97
41	P02776	PF4	chemokine receptor binding	intracellular organelle	1.90	3.12
42	Q13201	MMRN1	-	intracellular organelle	2.03	2.39
43	P04003	C4BPA	poly(A) RNA binding	membrane	2.24	1.43
44	P02647	APOA1	enzyme activator activity	endoplasmic reticulum	5.36	1.31

*The corresponding function or loaction was not significantly mapped

Tab S2. 13 common differentially expressed proteins caused by storage temperature by Phosphorylation iTRAQ.

NO	Uniprot ID	Genename	Founction	Location	Log_2FoldChange	
					22°C 5d vs 22°C 0d	10°C 5d vs 22°C 0d
1	Q6VY07	PACS1	protein binding	membrane-bounded organelle	0.63	0.69
2	Q15555	MAPRE2	cytoskeletal protein binding	cytoskeleton	0.69	1.26
3	Q00013	MPP1	nucleotide kinase activity	intracellular	0.70	0.85
4	Q12846	STX4	enzyme activator	synaptic vesicle	0.76	0.72
5	P13861	PRKAR2A	kinase regulator activity	plasma membrane region	0.81	0.88
6	O75396	SEC22B	syntaxin binding	endoplasmic reticulum	0.82	0.70
7	Q16512	PKN1	protein binding	intracellular organelle	0.86	1.22
8	P53621	COPA	protein binding	organelle membrane	0.92	0.75
9	P24844	MYL9	cytoskeletal protein	actin cytoskeleton	0.93	0.78
10	Q13442	PDAP1	nucleic acid binding	-	1.05	0.86
11	P08567	PLEK	protein kinase binding	plasma membrane region	1.22	1.62
12	Q6ZTN6	ANKRD13D	-*	membrane	1.48	1.00
13	P37802	TAGLN2	cytoskeletal protein binding	extracellular organelle	1.52	0.68

*The corresponding function or loaction was not significantly mapped

Figure S1

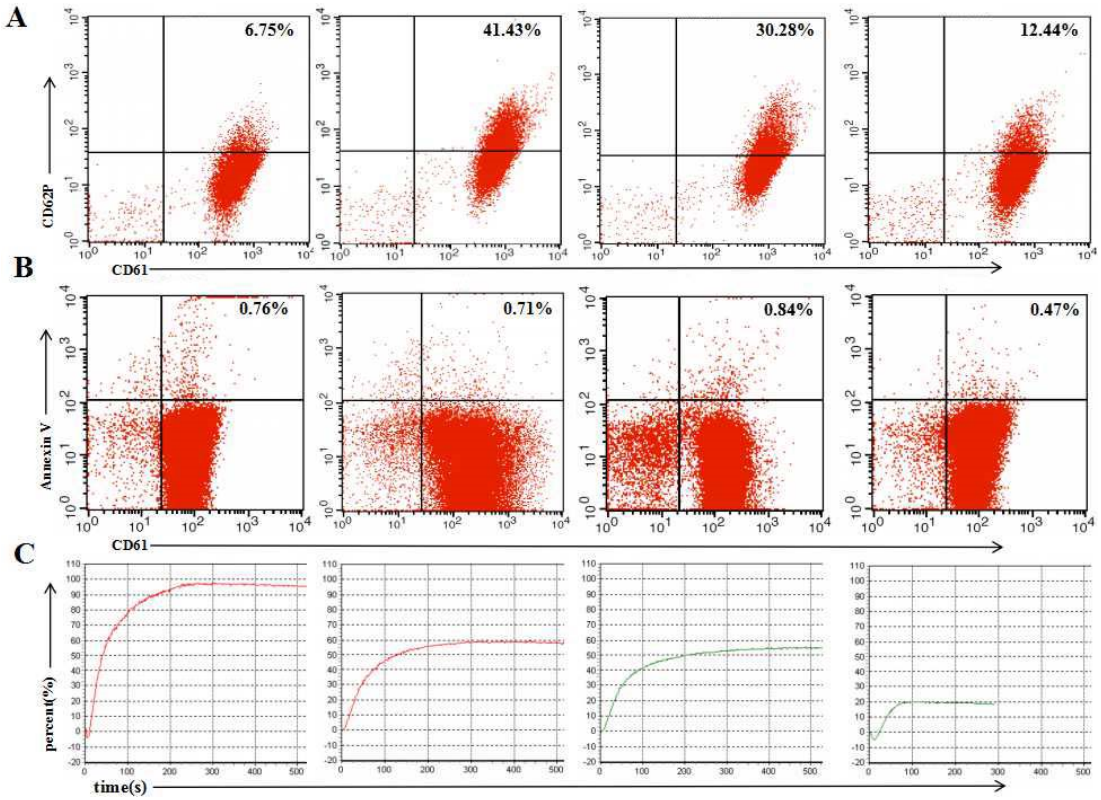


Fig S1. The Storage condition could affect the activation and aggregation of platelets. (A). CD62P surface expression as measured with flow cytometry. (B). PS exposure measured as Annexin V binding with flow cytometry. (C). Themaximal aggregationof each group.

Figure S2

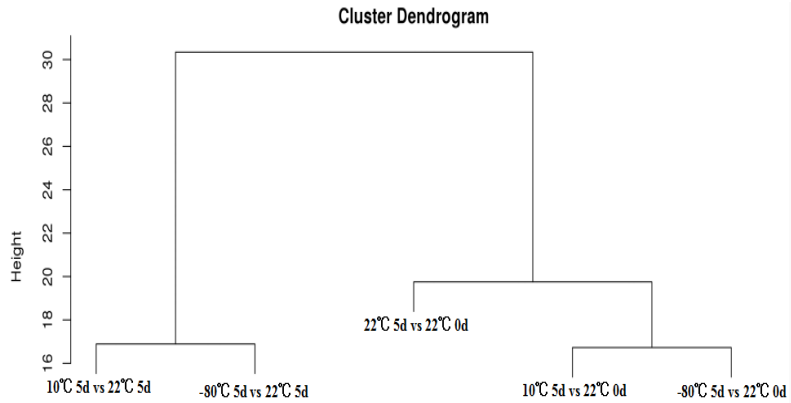


Fig S2. Hierarchical clustering analysis of differentially expressed proteins between groups

Figure S3

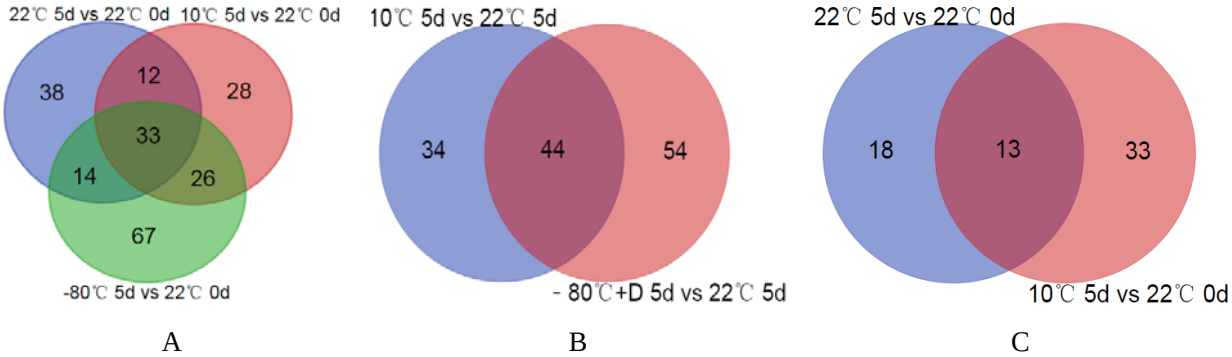
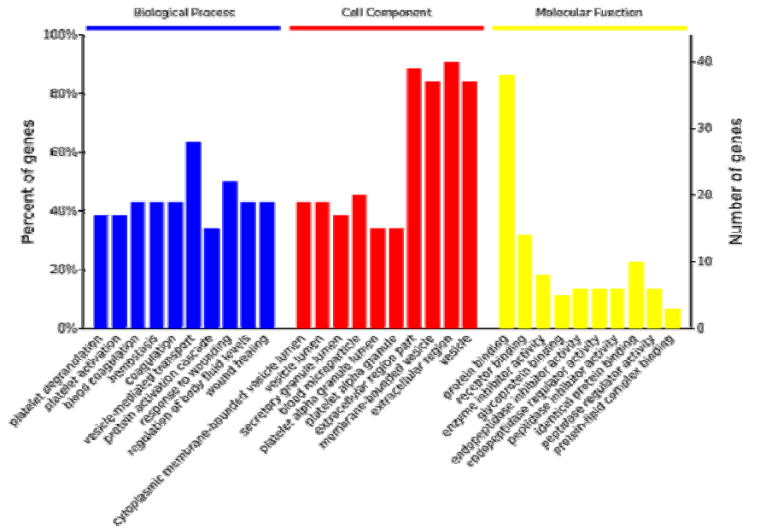
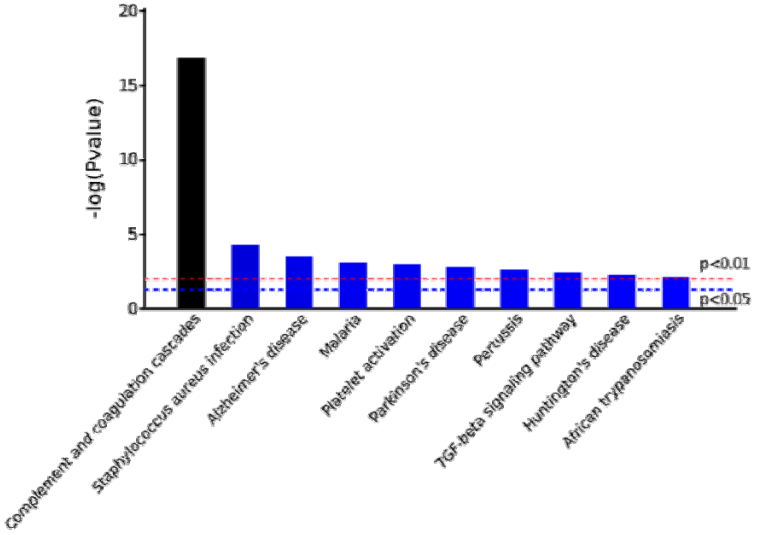


Fig S3. Venn diagram representing the overlap of the differentially expressed proteins by General iTRAQ (A,B) and byPhosphorylation iTRAQ (C). (A). Storage time caused 33 common differential proteins between22°C 5d vs 22°C 0d, 10°C 5d vs 22°C 0d and -80°C+D 5d vs 22°C 0d. (B). Storage temperature caused 44 common differential proteins between10°C 5d vs 22°C 5d, -80°C+D 5d vs 22°C 5d. (C). Storage time caused 13 common differential expression proteins by Phosphorylation of iTRAQ between 22°C 5d vs 22°C 0d, 10°C5d vs 22°C 0d.

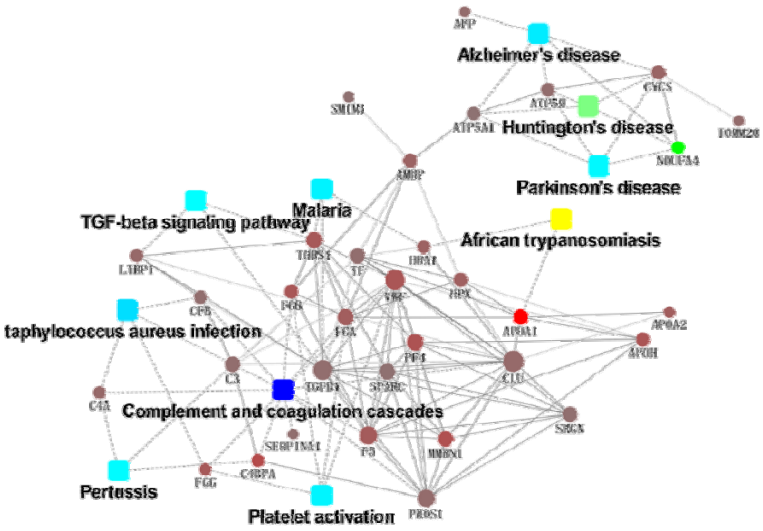
1 *Figure S4*



A



B



C

Fig S4. Bioinformatics analysis of the 44 common differentially expressed proteins caused by storage temperatureby General iTRAQ.

(A). Gene Ontology analysis. The colored bar graph showing the Biological Process(blue bar), Cellular Component (red bar) and Molecular Functions (yellow bar), over-represented in the identified platelet proteome, as determined by the IPA software. The y-axis shows the -log (p-value) associated with the predicted functional enrichment.

(B). KEGG Pathway analysis. The x-axis shows the pathways. The y-axis shows the -log (p-value) associated with the predicted functional enrichment.

(C). Platelet Protein-Protein Interaction (PPI) network. Each platelet protein is shown as a colored dot and interaction between any two proteins was shown as a gray colored edge.

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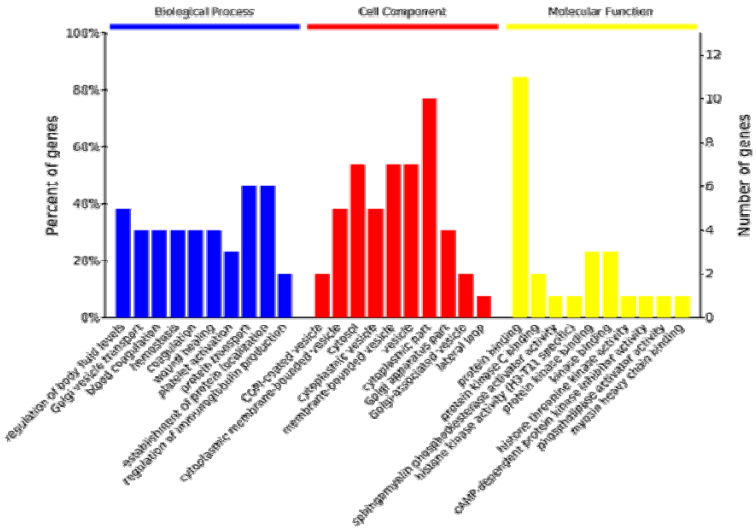
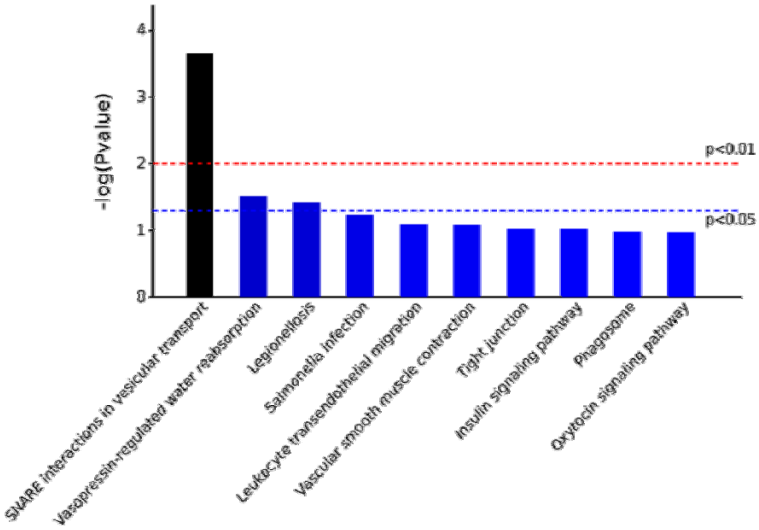
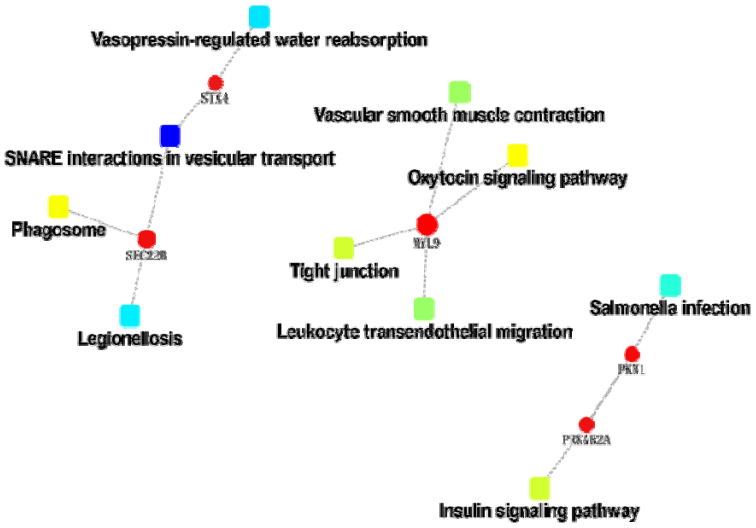
1 *Figure S5*



A



B



C

Fig S5. Bioinformatics analysis of the 13 common differentially expressed proteins caused bystorage time by Phosphorylation of iTRAQ.

(A).Gene Ontology analysis. The colored bar graph showing the Biological Process(blue bar), Cellular Component (red bar) and Molecular Functions (yellow bar), over-represented in the identified platelet proteome, as determined by the IPA software. The y-axis shows the -log (p-value) associated with the predicted functional enrichment.

(B). KEGG Pathway analysis. The x-axis shows the pathways. The y-axis shows the -log (p-value) associated with the predicted functional enrichment.

(C). Platelet Protein-Protein Interaction (PPI) network. Each platelet protein is shown as a colored dot and interaction between any two proteins was shown as a gray colored edge.

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Supplemental Methods

Protein extraction, quantization, digestion and iTRAQ labeling

Platelet proteins was collected, and protein concentrations were measured using a MicroBCA protein assay kit (Pierce, Rockford, IL). For each sample, 100 µg of protein was dissolved in a dissolution buffer (AB Sciex, Foster City, CA, USA). After being volume metered (30 µL), alkylated and trypsin-digested (modified trypsin (Sigma, St. Louis, MO), 37°C), the samples were labeled following the manufacturer's instructions for the double iTRAQ Reagents 4-plex kit ((AB Sciex), group A-113, group B-114, C-115, D-116) and the phosphorylation iTRAQ Reagents 8-plex kit ((AB Sciex), group A-113, 114; group B-115, 116; group C-117, 118). Samples were each labeled with iTRAQ reagents with molecular masses of 113, 114, 115, 116, 117, and 118 Da.

Phosphopeptide enrichments and LC-MS analysis

After labeling, all samples were mixed with TiO_2 phosphobind Buffer, and the phosphopeptides were enriched following the manufacturer's instructions of Phosphopeptides Enrichment Kit No. 539772. The enriched phosphopeptides were analyzed using a Triple TOF 5600 mass spectrometer (AB SCIEX, USA). Mass spectrometer data acquisition was performed with a Triple TOF 5600 System fitted with a Nanospray III source (AB SCIEX, USA) and a pulled quartz tip as the emitter (New Objectives, USA). Data were acquired using an ion spray voltage of 2.5 kV, curtain gas of 30 PSI, nebulizer gas of 5 PSI, and an interface heater temperature of 150°C. For information dependent acquisition (IDA), survey scans were acquired in

250 ms and as many as 35 product ion scans were collected if they exceeded a threshold of 150 counts per second (counts/s) with a 2+ to 5+ charge-state. The total cycle time was fixed to 2.5 s. A rolling collision energy setting was applied to all precursor ions for collision-induced dissociation (CID). Dynamic exclusion was set for Kof peak width (18 s), and the precursor was then refreshed off the exclusion list.

Protein Identification and Quantification

The iTRAQ data were processed with Protein Pilot Software v.5.0 (AB SCIEX, USA) against the *Homo sapiens* database using the Paragon algorithm. Protein identification was performed with the search option of emphasis on biological modifications. The database search parameters were the following: the instrument was a TripleTOF 5600, iTRAQ quantification, cysteine modified with iodoacetamide, phosphorylation emphasis, biological modifications were selected as ID focus, and trypsin digestion. For false discovery rate (FDR) calculation, an automatic decoy database search strategy was employed to estimate the FDR using the PSPEP (Proteomics System Performance Evaluation Pipeline Software, integrated in the ProteinPilot Software). The FDR was calculated as the number of false positive matches divided by the number of total matches. Then, the iTRAQ was chosen for protein quantification with unique peptides during the search, and peptides with global FDR values less than 1% were considered for further analysis. Within each iTRAQ run, differentially expressed proteins were determined based on the ratios of differently labeled proteins. Finally, for differential expression analysis, fold change was calculated, and proteins with a fold change of 1.5 or 0.67 were considered to be significantly differentially expressed.

P value<0.05.