## Appendix 1

84.12% of the proteins were between 0-100 kDa (*Figure S1A*). After proteolysis by trypsin, 95.1% of the protein could be detected by mass spectrometry, indicating that the enzymatic efficiency was extraordinarily high (*Figure S1A*). In general, the peptides of the protein profile are dominated by 2+ and 3+, and in our study the peptides of 2+ and 3+ accounted for 81.2% (*Figure S1A*). For the spectra and peptides identified to the protein, it is generally believed that the greater the number, the higher the confidence of the protein. In this result, 68.83% of the proteins had more than 2 spectra to support the identification, and 60.68% of the proteins had 2 unique peptides to support the identification (*Figure S1B*). We used the target-decoy method for false positive evaluation. The protein identification threshold was false discovery rate (FDR)<1%, and the FDR of the peptide-spectrum match (PSM) and protein were evaluated separately (*Figure S1B*).

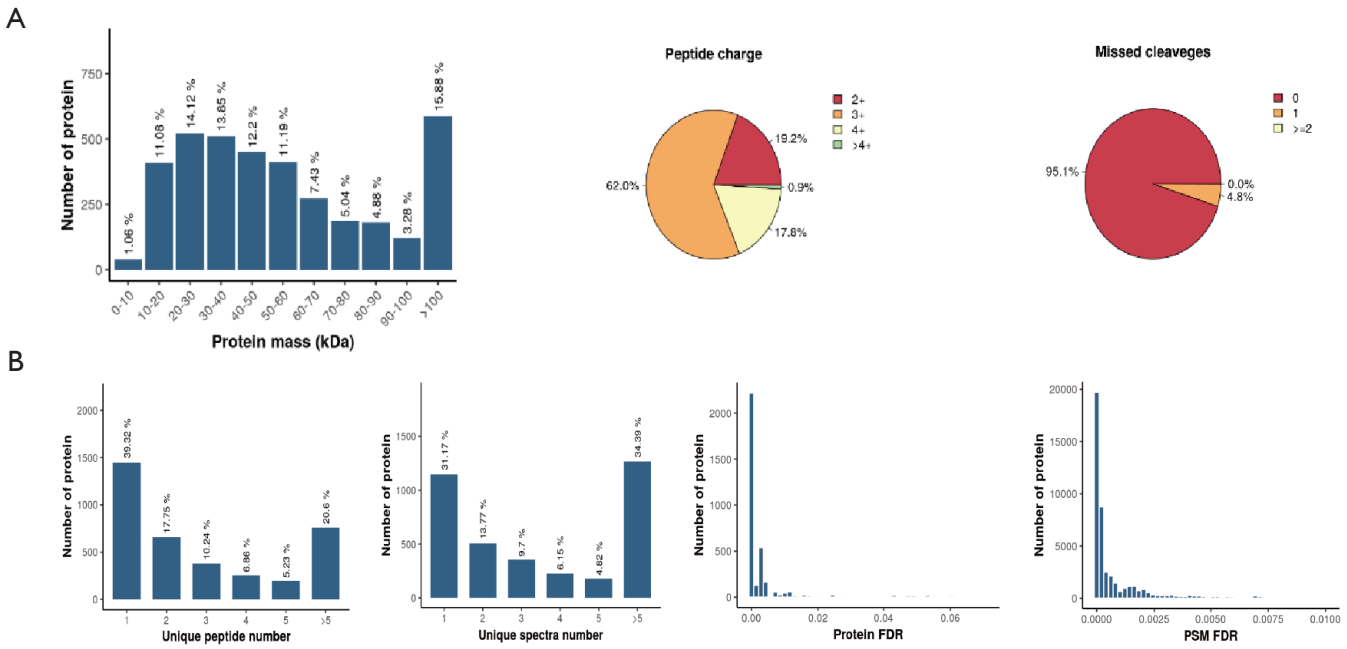


**Figure S1** Quality control of the data identified by proteomics in rat bladder tissue. (a) Protein molecular weight distribution diagram, peptide charge distribution diagram and missed cleaveges distribution diagram. (b) Protein identification unique peptide number distribution diagram, protein identification unique spectra number distribution diagram, protein false discovery rate and peptide-spectrum match false discovery rate.


https://dx.doi.org/10.21037/tau-21-351

## Appendix 2

We display the top 20 proteins with increased or decreased expression and their fold change in the following table.

**Table S1** The top 20 increased differentially expressed protein (DEPs) in ketamine treated rat urine samples compared to control group

| Proteins(up) | Fold change |
|---|---|
| Lipoxygenase homology domains 1 (Loxhd1) | 3.739952982 |
| Serine/threonine-protein phosphatase 1 regulatory subunit 10(Ppp1r10) | 3.541575787 |
| Keratin, type I cytoskeletal 10(Krt10) | 2.984604082 |
| DAP3-binding cell death enhancer 1 (Dele1) | 2.899067973 |
| GTPase activating protein testicular GAP1 (tGap1) | 2.708722676 |
| Fc fragment of IgG-binding protein (Fcgbp) | 2.591026386 |
| LBH domain-containing 2 (Lbhd2) | 2.543003004 |
| Keratin, type II cytoskeletal 1 (Krt1) | 2.54024553 |
| Serum albumin (Alb) | 2.27004498 |
| Echinoderm microtubule-associated protein-like 5 (Eml5) | 2.243260225 |
| Deoxyribonuclease-1 (Dnase1) | 2.163559813 |
| AT-rich interaction domain 3C (Arid3c) | 2.135045788 |
| Capping protein regulator and myosin 1 linker 2 (Carmil2) | 2.11468956 |
| Meprin A subunit alpha (Mep1a) | 1.994876567 |
| Keratin, type II cytoskeletal 6A (Krt6a) | 1.961711449 |
| Cubilin OS=Rattus norvegicus (Cubn) | 1.94926884 |
| Urinary protein 1 | 1.938451945 |
| Neuronal apoptosis inhibitory protein (Naip) | 1.92289812 |
| Lactase-phlorizin hydrolase (Lct) | 1.921361909 |
| Collagen alpha-1(I) chain OS=Rattus norvegicus (Col1a1) | 1.889831845 |
| Coatomer protein complex, subunit zeta (Copz2) | 1.871099763 |


https://dx.doi.org/10.21037/tau-21-351

**Table S2** The top 20 decreased differentially expressed protein (DEPs) in ketamine treated rat urine samples compared to control group

| Proteins (down) | Fold change |
|---|---|
| Mast cell protease 1 (Mcpt1) | 0.35559608 |
| Mast cell protease 2 (Mcpt2) | 0.391628906 |
| Acyloxyacyl hydrolase (Aoah) | 0.475499379 |
| Zero beta-globin (Fragment) | 0.494807389 |
| Phosphatase and actin regulator (Phactr3) | 0.495260588 |
| Parvalbumin alpha (Pvalb) | 0.496477493 |
| GIPC PDZ domain-containing family, member 3 (Gipc3) | 0.502031679 |
| Aly/REF export factor (Alyref) | 0.529845212 |
| Alpha globin (Hba-a3) | 0.535662499 |
| Malonyl-CoA decarboxylase, mitochondrial (Mlycd) | 0.536544844 |
| Desmuslin (DMN) | 0.558401104 |
| Nesprin-1 | 0.559887553 |
| Keratin, type I cytoskeletal 14 (Krt14) | 0.571095288 |
| Four and a half LIM domains 3 (Fhl3) | 0.594009377 |
| Voltage-dependent calcium channel subunit alpha-2/delta-3 (Cacna2d3) | 0.594103368 |
| HECT domain E3 ubiquitin protein ligase 4 (Hectd4) | 0.599442607 |
| Olfactory receptor OS=Rattus norvegicus (Olr174) | 0.602178973 |
| Actin, beta-like 2 OS=Rattus norvegicus (Actbl2) | 0.60514843 |
| Alpha-synuclein OS=Rattus norvegicus (Snca) | 0.611749025 |
| Phosphorylase b kinase regulatory subunit (Phkb) | 0.611764341 |
| Galectin-5 (Lgals5) | 0.621122069 |


https://dx.doi.org/10.21037/tau-21-351

**Appendix 3**

We compared the DEPs and the COG database to obtain annotations for each protein, and macroscopically recognize which COG the identified protein belongs to. We also predicted the possible functions of these proteins and classified them through their functions. The functional classification with the largest number of proteins were general function prediction only, with 39 proteins involved; the second functional classification was posttranslational modification, protein turnover, chaperones (34 proteins), translation, ribosomal structure and biogenesis (29 proteins), signal transduction. mechanisms (19 proteins) (*Figure S2A*).

After getting the EggNOG (evolutionary genealogy of genes: Non-supervised Orthologous Groups) annotation for each protein, we done EggNOG statistics on the protein to identify which EggNOG the protein belongs to. And the database mainly divided into five categories: cellular processes and signaling, energy production and conversion, information storage and processing, metabolism, poorly characterized. The results showed that DEPs involed most in cellular processes and signaling (618 proteins), accounting for 48% of all identified proteins, in cellular processes and in signaling, proteins were mostly involved in signal transduction mechanisms (167 proteins), followed by posttranslational modification, protein turnover, chaperones (137 proteins), intracellular trafficking, secretion, and vesicular transport (121 proteins), cytoskeleton (95 proteins) (*Figure S2B*).

Proteins can only perform their specific functions in specific subcellular locations. We obtained subcellular localization annotations for each protein and performed statistical analysis. The results showed that the number of proteins was highest in cytoplasm (254 proteins), followed by nucleus (86 proteins), extracellular (130 proteins) (*Figure S2C*). After annotating parallel statistical analysis of the transcription factors of each identified protein, the number of proteins involved in transcription cofactors was the highest (20 proteins), followed by zf-C2H2 (4 proteins), chromatin remodeling factors (3 proteins) (*Figure S2D*).


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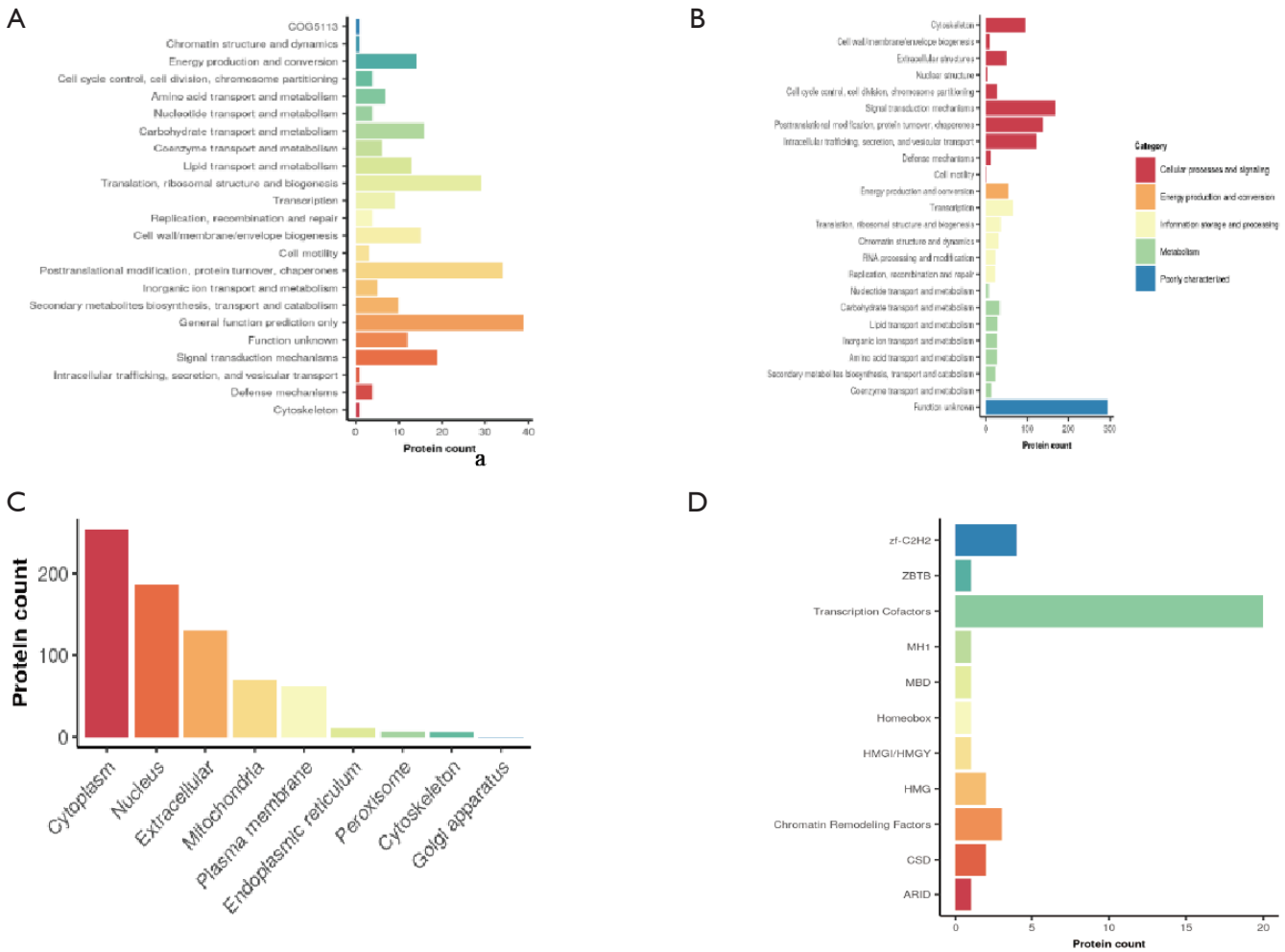


**Figure S2** Functional annotation of DEPs. (a) Statistics of COG entries of DEPs. (b) Statistics of EggNOG entries of DEPs. (c) Statistics of subcellular localization entries of DEPs. (d) Statistics of transcription factors entries of DEPs.


https://dx.doi.org/10.21037/tau-21-351

## Appendix 4

In the platelet activation pathway, 64 proteins are annotated, 19 of which are differentially expressed, P=0.03212282. For information about each protein and its expression, see *Table S4* and *Figure S3*.

**Table S3** KEGG annotation for platelet activation

| Pathway | Diff Proteins with pathway annotation (662) | All Proteins with pathway annotation (3394) | Pvalue | Qvalue | Pathway ID | Level 1 | Level 2 |
|---|---|---|---|---|---|---|---|
| Platelet activation | 19 (2.87%) | 64 (1.89%) | 0.03212282 | 0.7091730262 | ko04611 | Organismal Systems | Immune system |

**Table S4** All proteins differentially expressed in the platelet activation pathway

| Pathway | Proteins |
|---|---|
| Platelet activation | tr\|D4ACS0\|D4ACS0_RAT, tr\|A0A0G2JU01\|A0A0G2JU01_RAT,sp\|Q63538\|MK12_RAT, sp\|P62836\|RAP1A_RAT, sp\|P02454\|CO1A1_RAT, tr\|Q9R1X8\|Q9R1X8_RAT, sp\|P10824\|GNAI1_RAT, tr\|Q45QM8\|Q45QM8_RAT, sp\|P14480\|FIBB_RAT, tr\|G3V852\|G3V852_RAT, sp\|P61589\|RHOA_RAT, tr\|A1L114\|A1L114_RAT, tr\|Q45QN0\|Q45QN0_RAT, tr\|M0R4J7\|M0R4J7_RAT, tr\|D3ZRN3\|D3ZRN3_RAT, tr\|F1LS40\|F1LS40_RAT, sp\|Q76K24\|ANR46_RAT, tr\|D4ACC2\|D4ACC2_RAT, tr\|F1LQ3\|F1LQT3_RAT |


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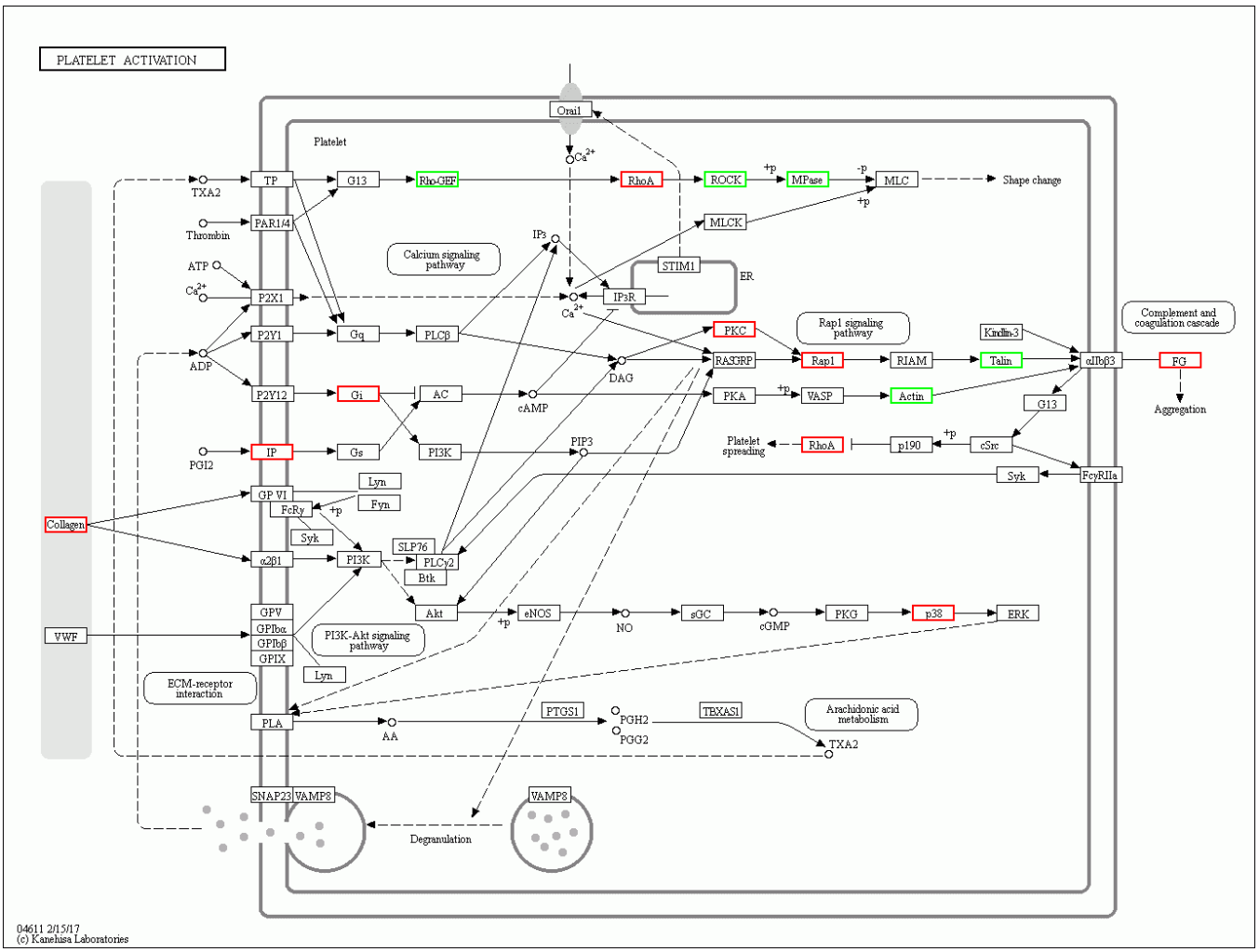


**Figure S3** The KEGG pathway diagram of the platelet activation pathway.


https://dx.doi.org/10.21037/tau-21-351