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# Supplementary information

## Highly effective identification of drug targets at proteome level by pH-dependent protein precipitation

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Table S1 pH values corresponding to the molar concentration of different acidic agents

| | 1mM | 3mM | 6mM | 9mM | 12mM | 15mM | 17mM | 20mM |
|---|---|---|---|---|---|---|---|---|
| **Vc** | 7.01 | 6.66 | 6.02 | 5.03 | 4.23 | 3.90 | 3.77 | 3.62 |
| **HCl** | 7.01 | 6.61 | 5.91 | 4.52 | 3.37 | - | - | - |
| **FA** | 7.01 | 6.60 | 5.80 | 4.07 | 3.50 | - | - | - |

| | 1mM | 2mM | 3mM | 4mM | 5mM | 6mM |
|---|---|---|---|---|---|---|
| **CA** | 6.70 | 6.20 | 5.68 | 4.93 | 4.40 | 3.78 |

The different concentrations of Vc, CA, HCl and FA were added in 10 mM PBS buffer to simulate the environment of the protein solution in Fig. S1.

Table S2 Subunits of 19 S regulatory particle were stabilized by AMP-PNP

| IDs | Gene | Protein name | Ratio H/L-1 | Log2-1 | Ratio H/L-2 | Log2-2 |
|---|---|---|---|---|---|---|
| P62333 | PRS10 | 26S proteasome regulatory subunit 10B | 0.19 | 2.43 | 0.11 | 3.15 |
| P62191 | PRS4 | 26S proteasome regulatory subunit 4 | 0.14 | 2.88 | 0.14 | 2.79 |
| P17980 | PRS6A | 26S proteasome regulatory subunit 6A | 0.17 | 2.57 | 0.21 | 2.23 |
| P43686 | PRS6B | 26S proteasome regulatory subunit 6B | 0.21 | 2.25 | 0.14 | 2.83 |
| P35998 | PRS7 | 26S proteasome regulatory subunit 7 | 0.10 | 3.27 | 0.15 | 2.72 |
| P62195 | PRS8 | 26S proteasome regulatory subunit 8 | 0.13 | 2.93 | 0.15 | 2.70 |
| O00231 | PSD11 | 26S proteasome non-ATPase regulatory subunit 11 | 0.17 | 2.52 | 0.17 | 2.58 |
| O00232 | PSD12 | 26S proteasome non-ATPase regulatory subunit 12 | 0.24 | 2.05 | 0.21 | 2.27 |
| Q9UNM6 | PSD13 | 26S proteasome non-ATPase regulatory subunit 13 | 0.16 | 2.68 | 0.17 | 2.55 |
| O00487 | PSDE | 26S proteasome non-ATPase regulatory subunit 14 | 0.17 | 2.57 | 0.17 | 2.52 |
| Q99460 | PSMD1 | 26S proteasome non-ATPase regulatory subunit 1 | 0.14 | 2.87 | 0.18 | 2.46 |
| Q13200 | PSMD2 | 26S proteasome non-ATPase regulatory subunit 2 | 0.11 | 3.15 | 0.13 | 2.90 |
| O43242 | PSMD3 | 26S proteasome non-ATPase regulatory subunit 3 | 0.21 | 2.22 | 0.22 | 2.17 |
| P55036 | PSMD4 | 26S proteasome non-ATPase regulatory subunit 4 | 0.26 | 1.94 | 0.21 | 2.27 |
| Q15008 | PSMD6 | 26S proteasome non-ATPase regulatory subunit 6 | 0.30 | 1.74 | 0.28 | 1.82 |
| P51665 | PSMD7 | 26S proteasome non-ATPase regulatory subunit 7 | 0.29 | 1.77 | 0.28 | 1.82 |
| P48556 | PSMD8 | 26S proteasome non-ATPase regulatory subunit 8 | 0.20 | 2.35 | 0.18 | 2.49 |

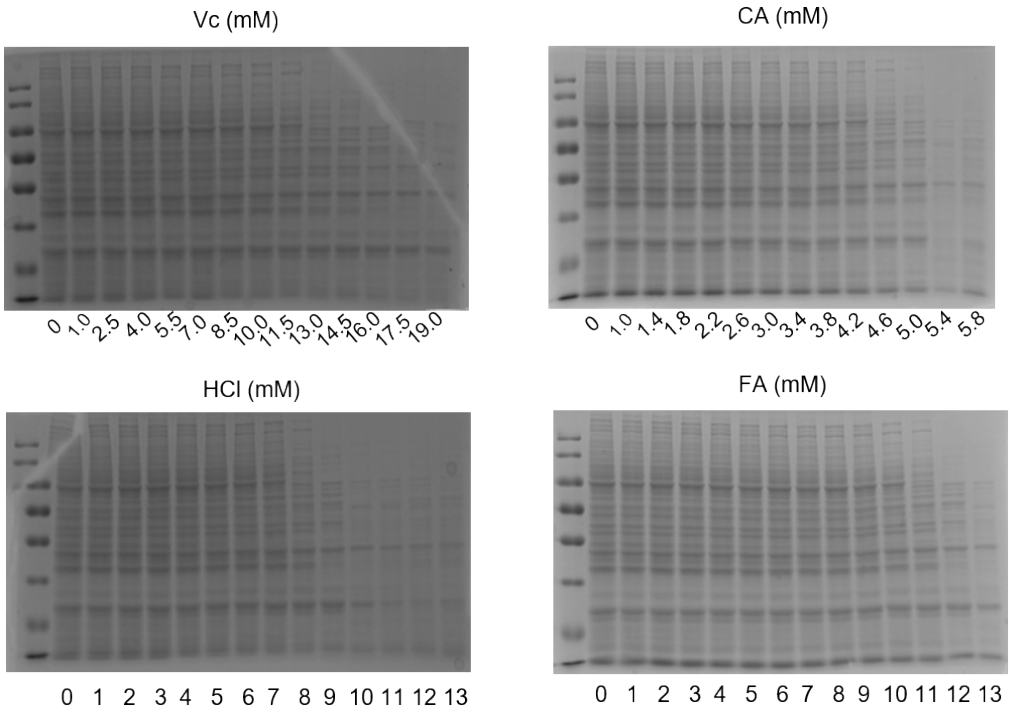


Fig. S1 Acidic agents-induced denaturation of the K562 proteome. K562 lysates were treated with 14 increasing concentrations of different acidic agents. The proteins in soluble fractions were separated by SDS-PAGE and visualized with Coomassie staining.

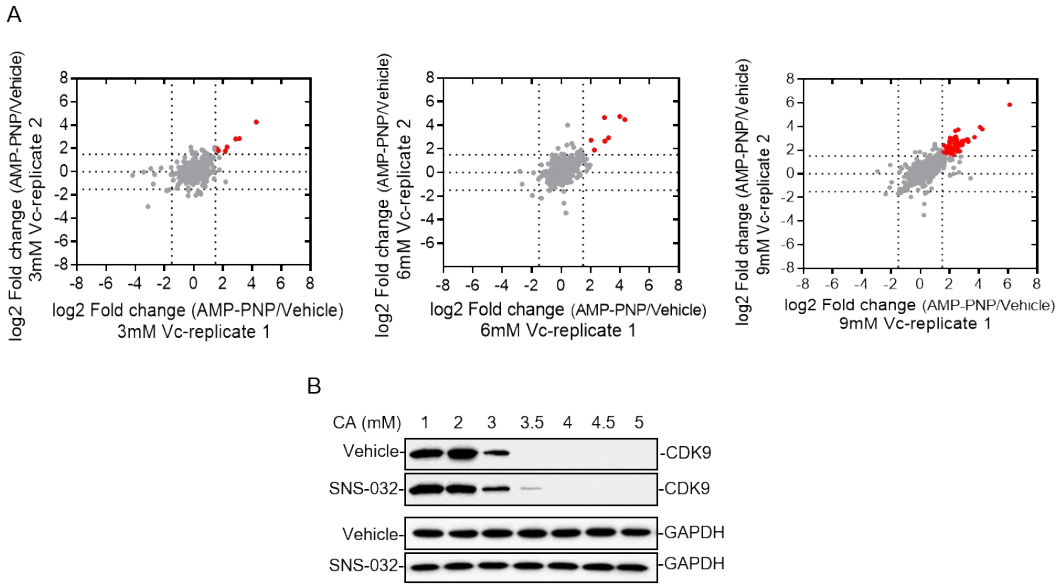


Fig. S2 Identification of target protein of small molecules AMP-PNP and SNS-032. (A) Identification of stabilized proteins in 3mM, 6mM and 9mM Vc-treated samples by AMP-PNP based on quantitative proteomics. (B) Western blotting confirmed the known target CDK9 was stabilized by SNS-032 in 293T cell lysate.

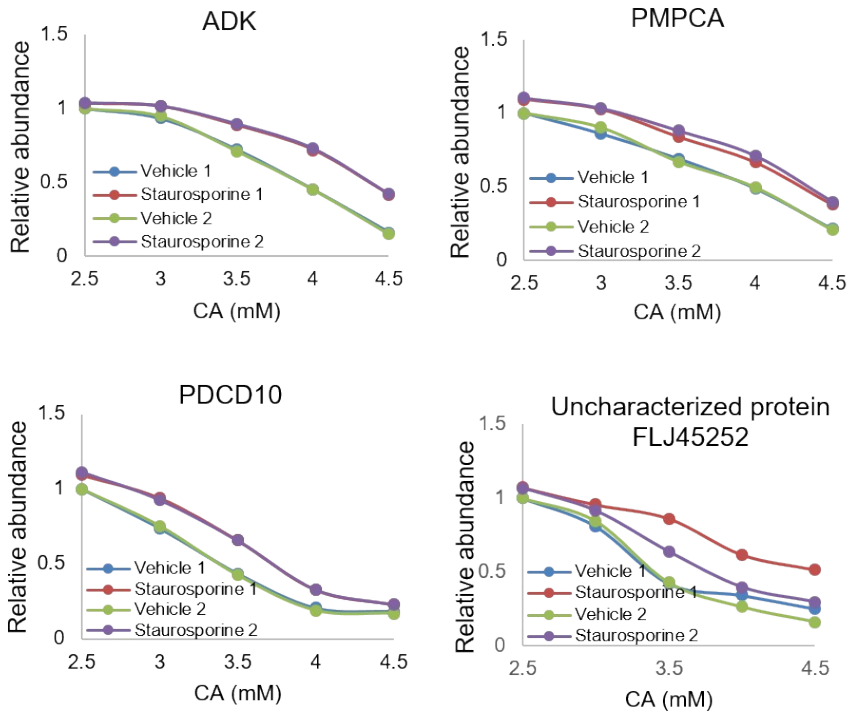


Fig. S3 Examples of stability shift curves for non-protein kinase such as ADK, PMPCA, PDCD10 and uncharacterized protein FLJ45252 in the presence or absence of staurosporine.

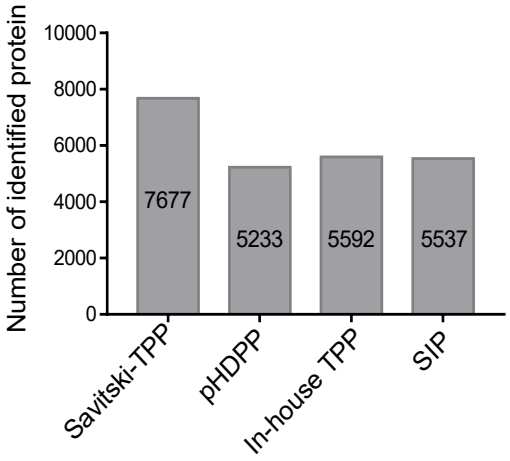


Fig. S4 Number of total protein quantified by TPP-Savitski [1], pHDPP, in-house TPP and SIP approaches.

[1] Savitski, M.M., et al., *Tracking cancer drugs in living cells by thermal profiling of the proteome.* Science, 2014. **346**(6205): 1255784.

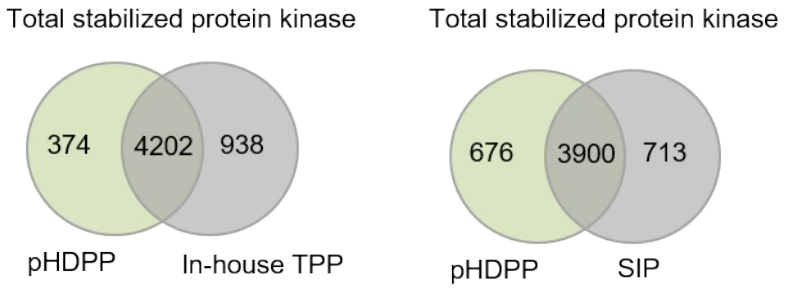


Fig. S5 Overlapped protein in the filtering datasets of pHDPP with in-house TPP and SIP.

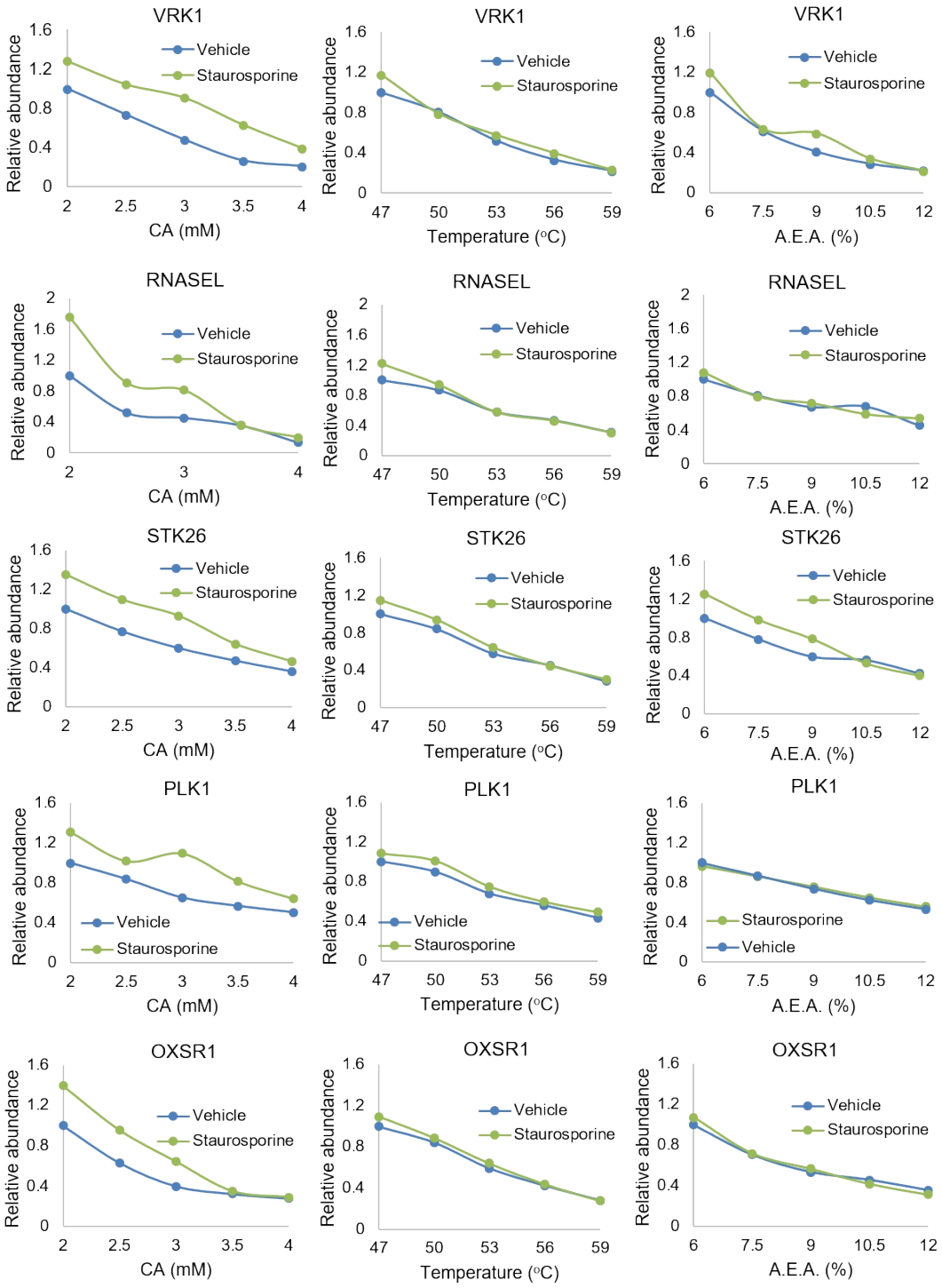


Fig. S6 Examples of stability shift curves where protein kinases were stabilized by staurosporine in pHDPP but not stabilized in in-house TPP and SIP approaches.

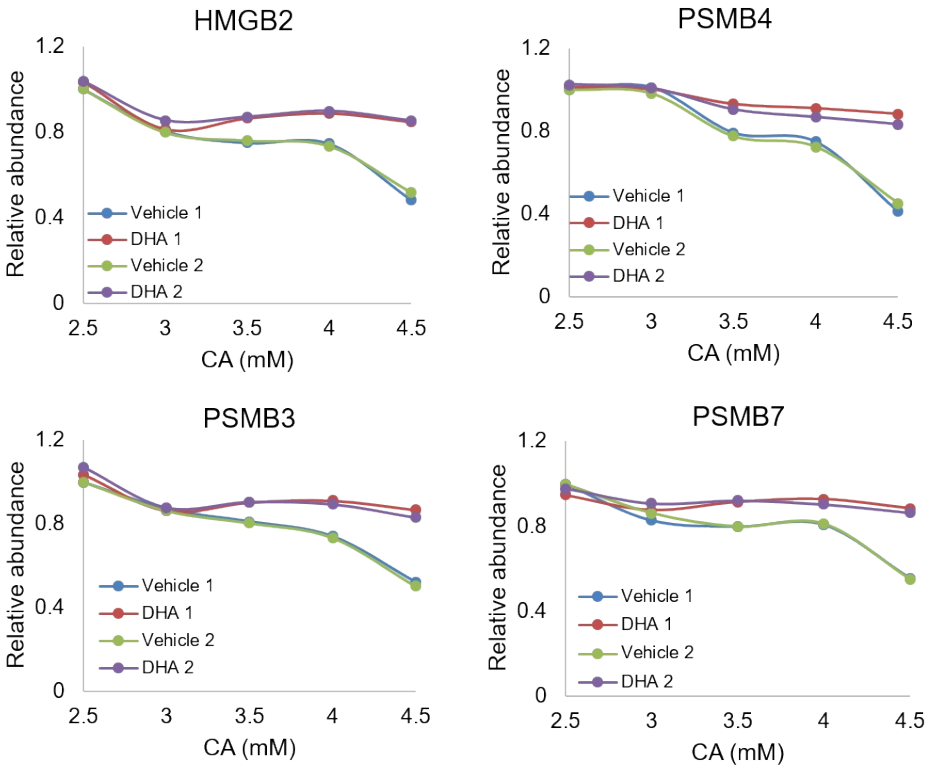


Fig. S7 Examples of stability shift curves for HMGB2, PSMB3, PSMB4 and PSMB7 related to apoptosis pathway in DHA experiment.