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Supplemental Information

High-Throughput Assessment of Kinome-wide Activation States

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High-throughput assessment of kinome-wide activation states

Thierry Schmidlin^{1,2}, Donna O. Debets^{1,2}, Charlotte A.G.H. van Gelder^{1,2}, Kelly E. Stecker^{1,2}, Stamatia Rontogianni^{1,2}, Bart L. van den Eshof³, Kristel Kemper⁴, Esther H. Lips⁵, Maartje van den Biggelaar³, Daniel S. Peeper⁴, Albert J.R. Heck^{1,2} and Maarten Altelaar^{1,2,6,#,*}

1 Biomolecular Mass Spectrometry and Proteomics, Bijvoet Center for Biomolecular Research and Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands

2 Netherlands Proteomics Center, Padualaan 8, 3584 CH Utrecht, The Netherlands

3 Sanquin Research, Department of Molecular and Cellular Hemostasis, Amsterdam, the Netherlands.

4 Division of Molecular Oncology, Onco Institute, The Netherlands Cancer Institute, 1066 CX Amsterdam, the Netherlands

5 Department of Molecular Pathology, The Netherlands Cancer Institute, 1066 CX Amsterdam, the Netherlands.

6 Mass Spectrometry and Proteomics Facility, The Netherlands Cancer Institute, 1066 CX Amsterdam, the Netherlands

To whom correspondence should be addressed: m.altelaar@uu.nl

* Lead contact

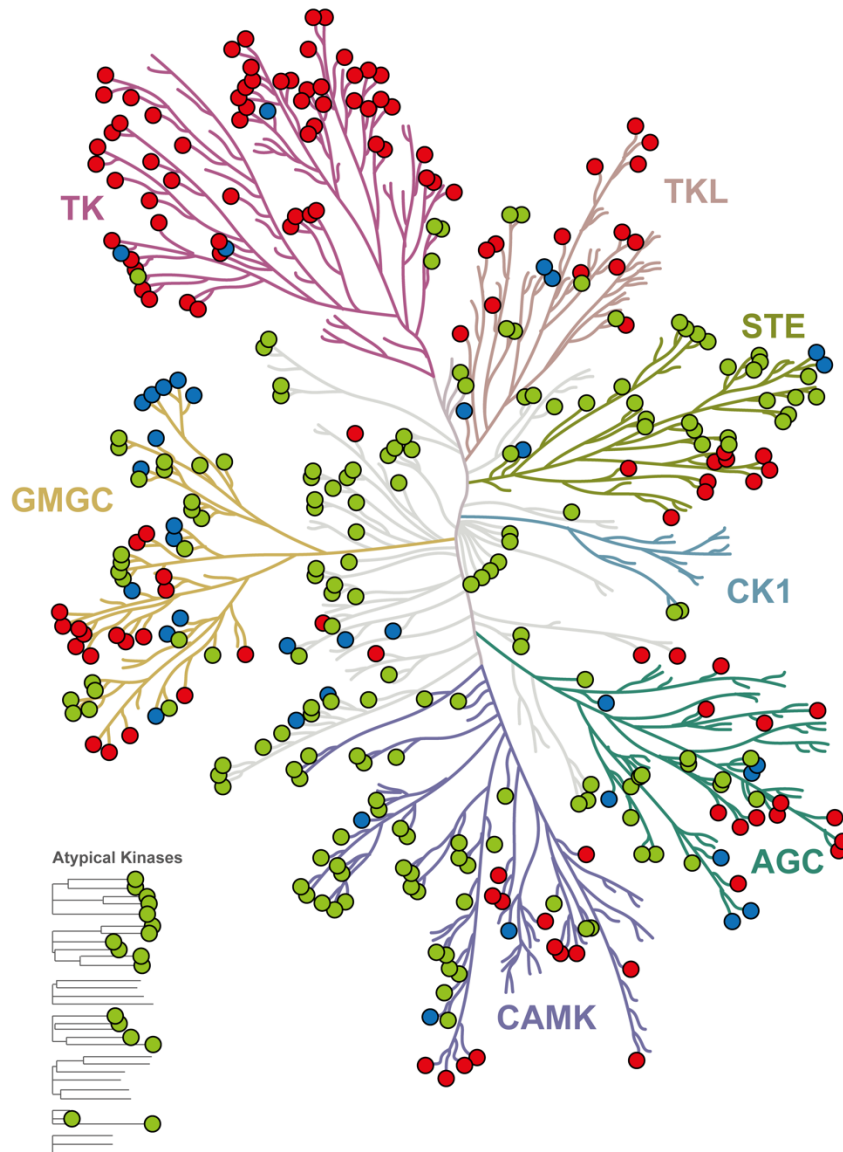


Figure S1, Related to Figure 1: Depiction of the human kinome indicating all kinases currently included in the t-loop screening method (blue circle), kinases being classified as understudied by Collins et al. (green circle) and the overlap of the two (red circles).

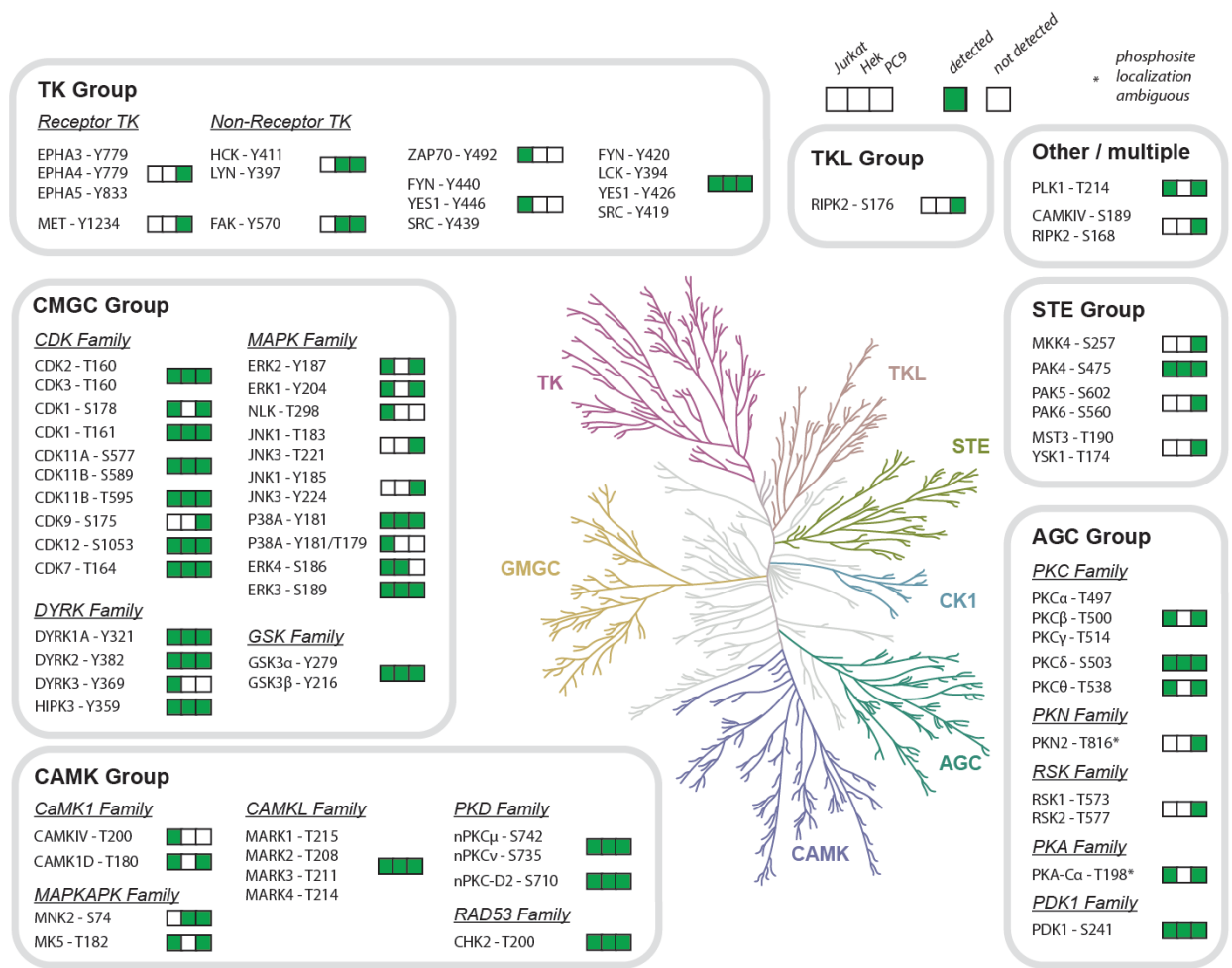


Figure S2, Related to Figure 1: Compendium of t-loop phosphorylations detected in Jurkat, HEK293T and PC9 cell lines cultured under standard conditions without any form of stimulation.

Literature Based Kinase-Substrate Library with Phosphosites

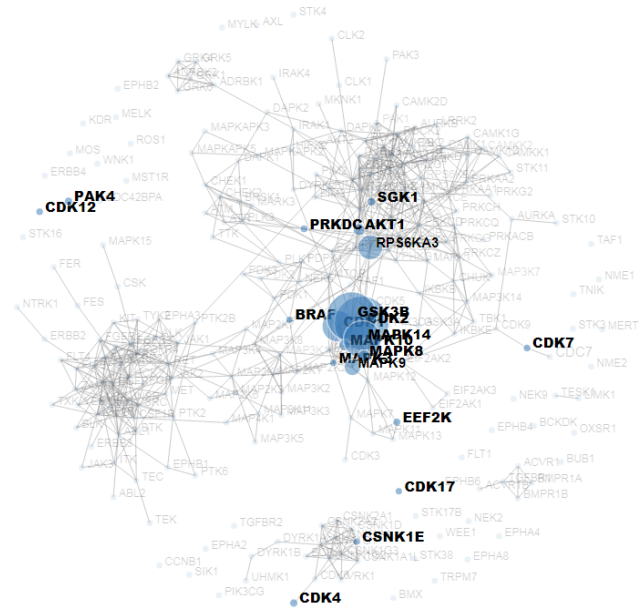
Run Enrichment

LYST_Y3208
 LYST_Y3210
 LYST_Y3215
 ESYT1_Y822
 CPVL_Y266
 SCAF1_Y494
 COG4_Y441
 CBX8_Y106
 BCLAF1_Y284
 DNAH2_Y2902
 DNAH2_Y2989
 ZC3H4_Y158
 SRRM2_Y996
 COL4A3BP_Y291

[Submit](#) [Example](#) [Clear](#)

Library Info:

This library can be used to predict kinase activity from your input set of phosphosites. This library was made by manual curation of kinase-substrate interactions in the literature. The library terms are kinases and the elements are substrates with phosphosites.



Enrichment Results:

Term	P-Value
CDK2	4.86e-32
GSK3B	4.14e-29
MAPK14	3.12e-21
CDK1	2.20e-17
RPS6KA3	3.29e-13
MAPK9	6.41e-8
MAPK8	3.83e-5
AKT1	4.94e-5
MAPK10	9.03e-5
SGK1	6.55e-3
CDK4	1.19e-2
PAK4	1.27e-2
EEF2K	2.08e-2
PRKDC	3.53e-2
CDK7	3.85e-2
CDK12	5.79e-2
CSNK1E	8.34e-2

Found 802 phosphosites in the library

[Download Enrichment Results](#)

[Download Network SVG](#)

MAPK14

TP53_S392, ESR1_T311, BAD_S750, MAX_S490, MAX_S400, STAT4_S721, EEF2K_S396, NCF1_S348, NCF1_S345, MEF2C_S387, MEF2C_T293, MEF2C_T300, ATF2_T69, ATF2_T71, ATF2_S90, DDIT3_S82, DDIT3_S79, ELK1_S383, ELK1_S389, SLC9A1_S723, SLC9A1_S726, SLC9A1_S729,

Figure S3, Related to Figure 2: Screenshot of the KEA2 output reporting kinase activity determined from Jurkat cell lysates subjected to high-pH reversed phase fractionation and subsequent phosphopeptide enrichment analyzed by shotgun LC-MS.

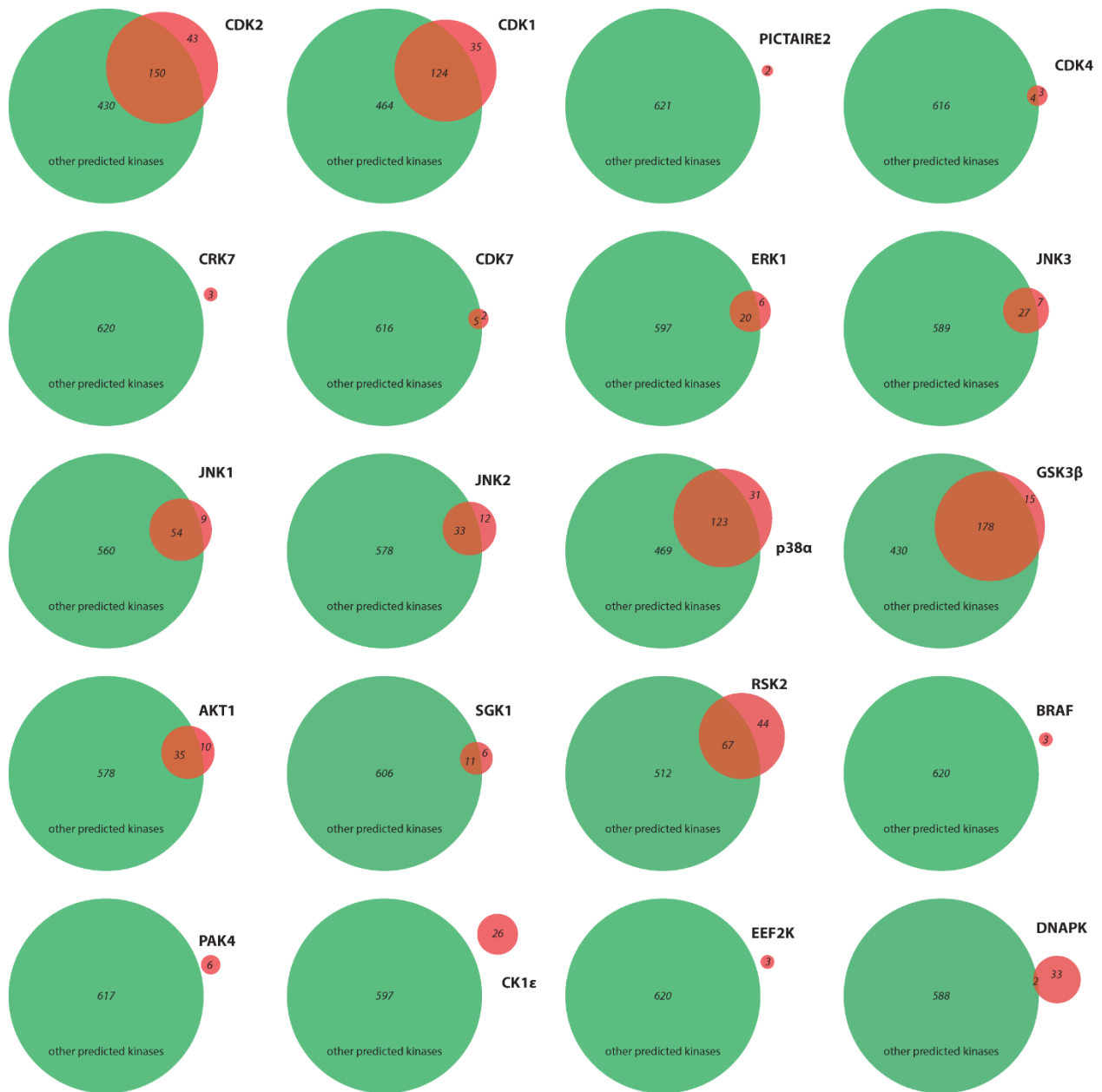


Figure S4, Related to Figure 2: Venn diagrams illustrating the amount of redundancy in kinase-substrate relationships impeding the kinase prediction by the online tool KEA2. Each Venn diagram compares the reported substrates to the reported substrates of the other predicted kinases.

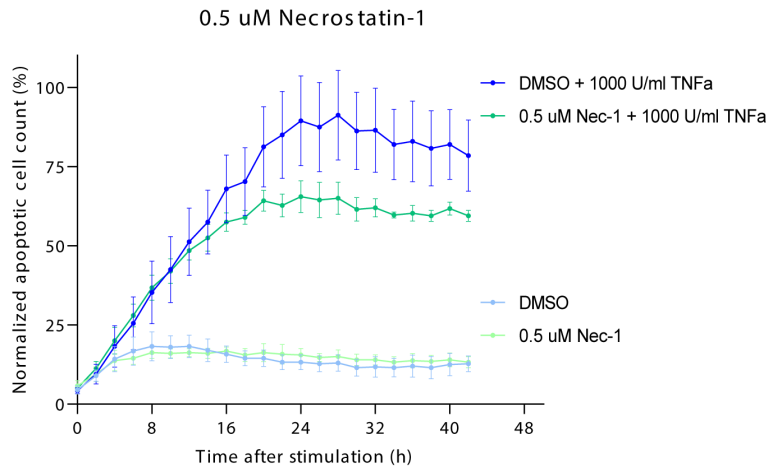
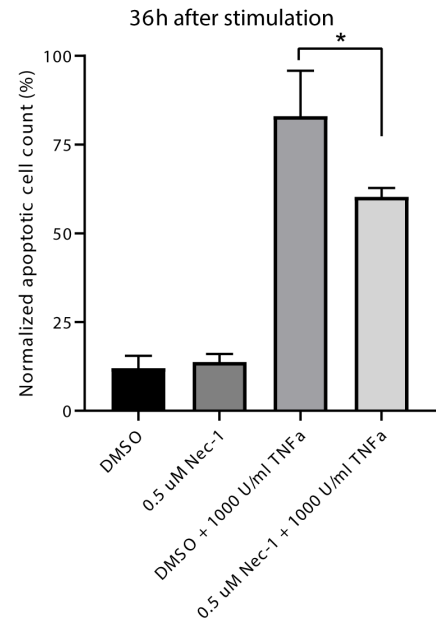
A**B**

Figure S5, Related to Figure 2: Jurkat cell apoptosis upon TNF α treatment: Jurkat cells were grown with and without TNF α stimulation, in presence or absence of necrostatin-1 (Nec-1), and rate of cell death was determined by Caspase-3/7 green apoptosis reagent (n = 4 for all groups, error bars depicting standard deviation). (B) Apoptotic cell count after 36 hours in the different conditions showing a significant reduction in apoptotic cells after TNF α stimulation in the presence of Nec-1.