Supplementary Information

Proteomic cellular signatures of kinase inhibitor-induced cardiotoxicity

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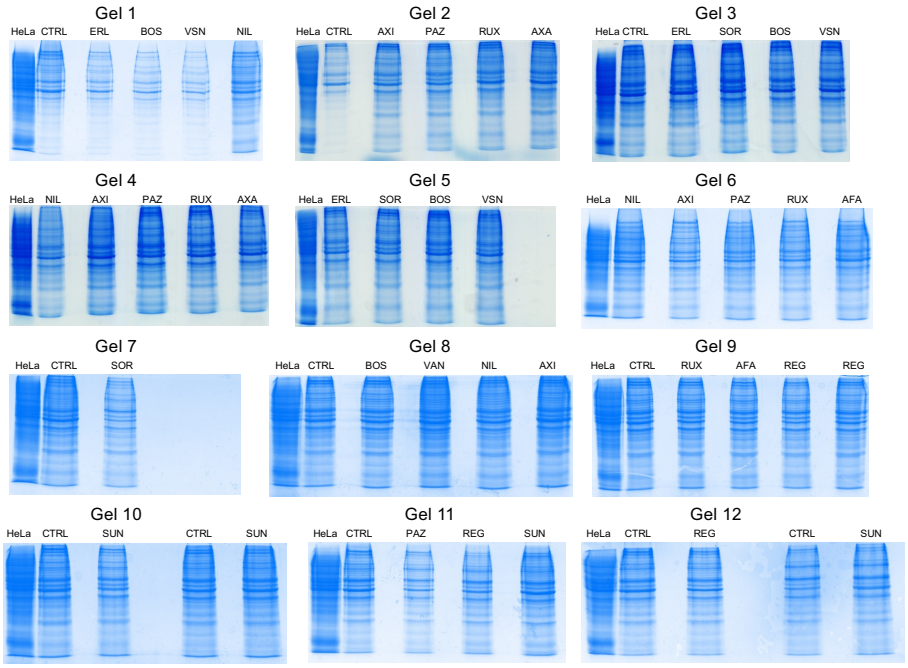
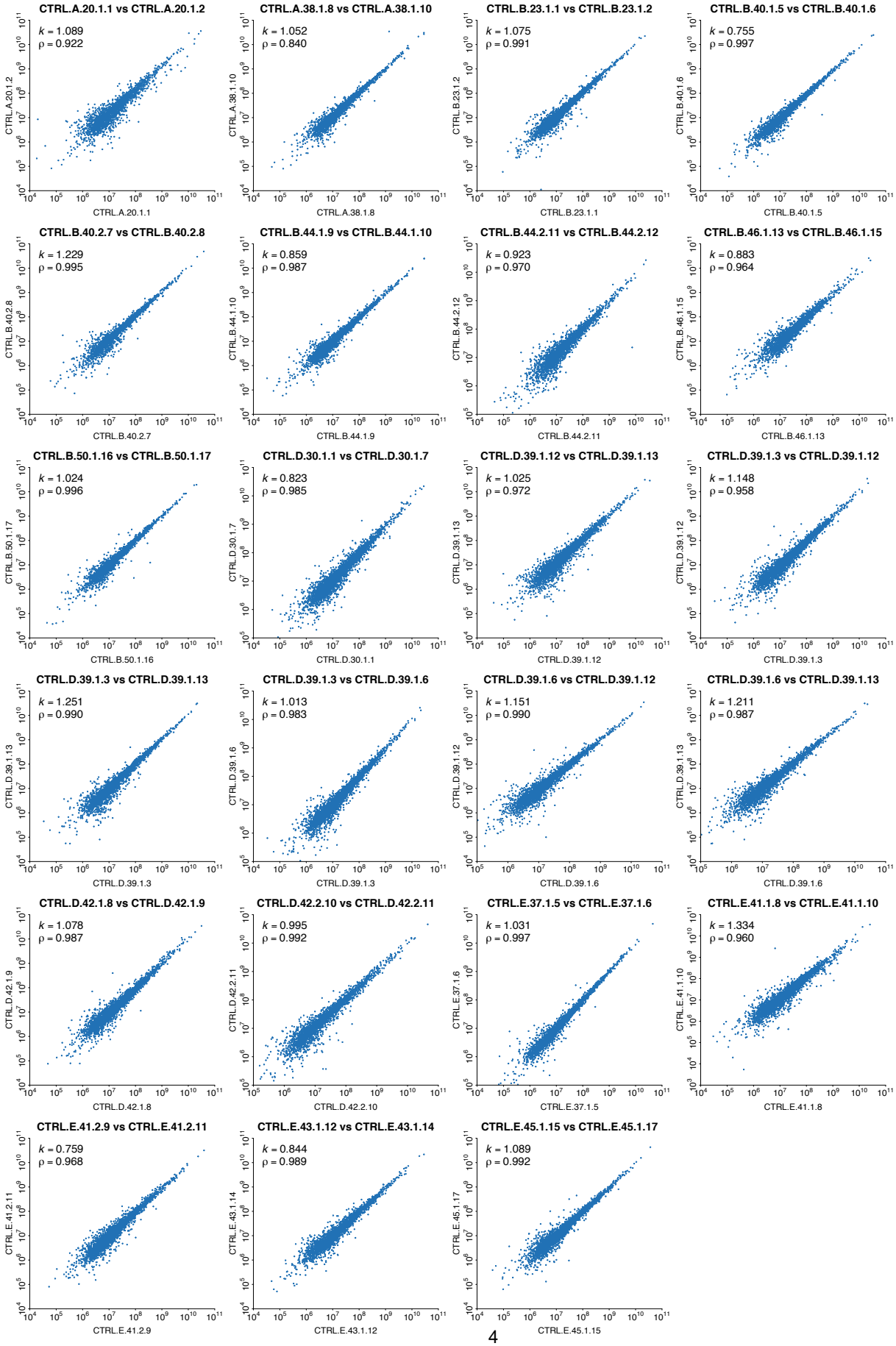
Supplementary File Table of Contents



Supplementary Figure 1. Examples of protein quantification via serial SDS-PAGE. Twenty five micrograms of internally-prepared HeLa cell protein was loaded along with the proteins recovered from the KI treated PMC-D line and separated on 10% SDS-PAGE gels. The gel was stained with Coomassie brilliant blue (CBB) and the staining intensities were measured. The protein CBB densities in drug-treated samples were compared to those of the HeLa cells for the same gel, and the protein amounts in each sample were then estimated. Example protein density measurements for the gels shown here are reported in **Online-only Table 1**.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CTRL.B.40.1.5
CTRL.B.40.2.7 vs CTRL.B.40.2.8
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CTRL.B.44.2.11 vs CTRL.B.44.2.12
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CTRL.B.44.2.11
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CTRL.B.46.1.13
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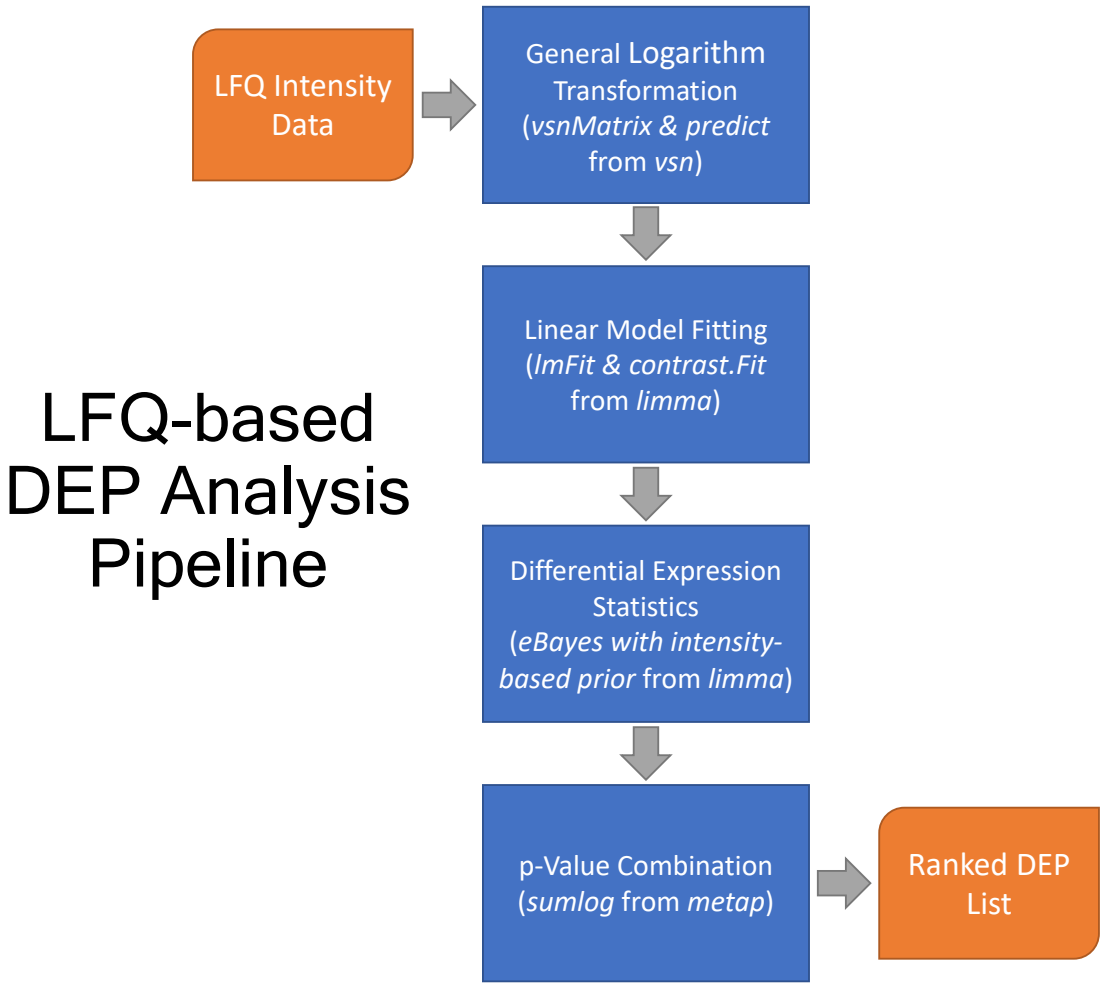
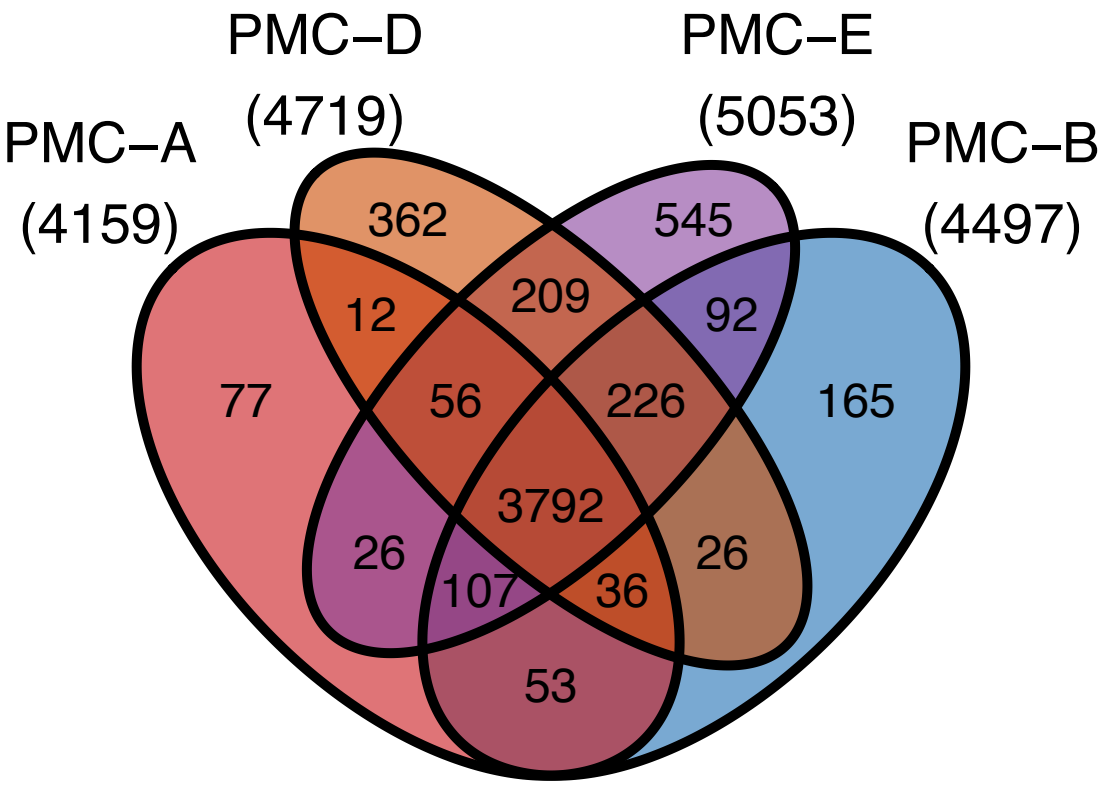
CTRL.E.45.1.15

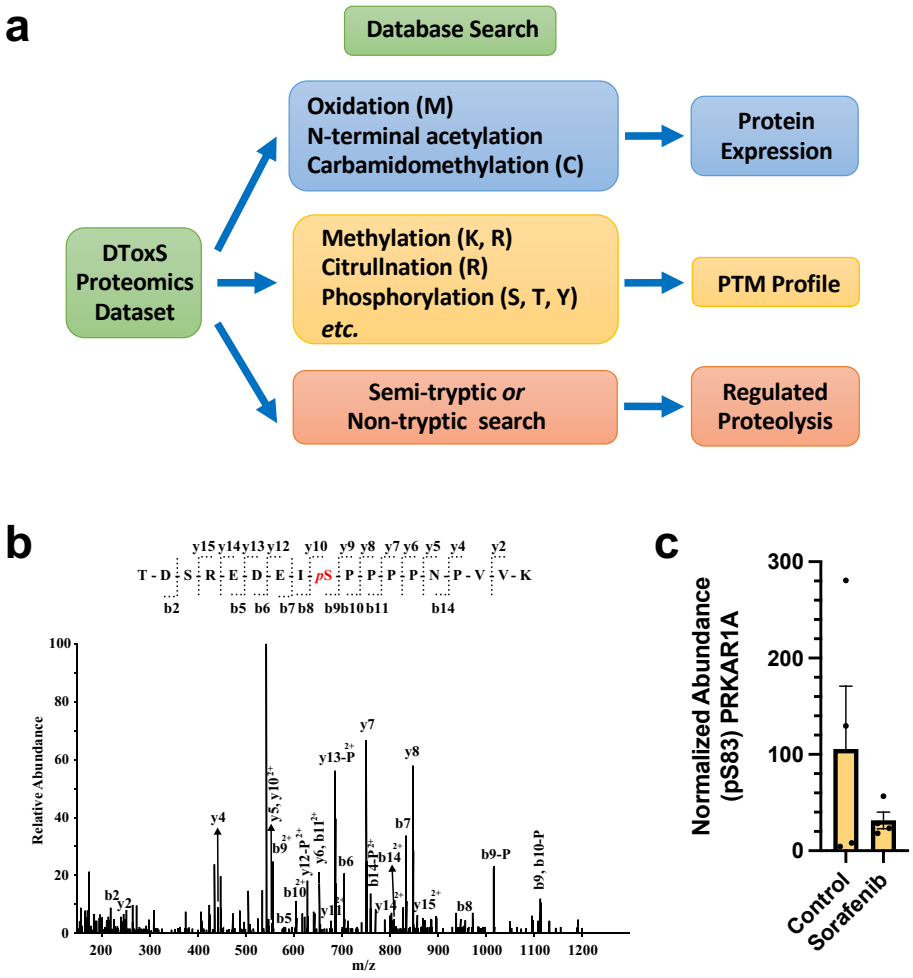
Supplementary Figure 2. Reproducibility of proteomic quantification for technical replicates. Comparison of normalized MS1 LFQ intensities for two technical replicates of the same biological sample that was run on a different SDS-PAGE gel and processed for MS/MS analysis on a different day. Pearson correlations show strong agreement between multiple runs as both coefficients, ρ, and slopes, k, are at or near unity.



Supplementary Figure 3. Workflow for identifying differentially expressed proteins from LFQ intensities.



Supplementary Figure 4. Coverage of identified proteins that map onto genes. Venn diagram of the overlapping and unique proteins across all samples for the four human cardiomyocyte-like cell lines (PMC-A, B, D, E) that map onto genes.



Supplementary Figure 5. Using the complementary proteomics search methods to identify drug-regulated changes of PTMs. (a) The DToxS proteomics dataset can be analyzed with different protein database search methods to obtain different pertinent information, including the changes of protein expression, PTMs and regulated proteolysis of specific peptides. **(b)** Example of a phosphorylation site observed in cardiomyocyte-like human cell line PMC-B treated with multi-kinase inhibitor sorafenib. **(c)** Normalized abundance of the phosphorylated S83 residue for the cAMP dependent kinase A regulatory subunit (PRKAR1A), well known for its functional role in numerous cardiac processes, is significantly reduced in sorafenib treated cells.

Supplementary Table 1. Sample sizes for the KI treated groups per cell line

Drug Treatment	PMC-A	PMC-B	PMC-D	PMC-E
AFA	4	4	4	5
AXI	4	4	4	5
BOS	4	4	4	5
CAB	0	4	0	0
DAB	0	4	0	0
DAS	0	4	0	0
ERL	2	4	4	2
GEF	0	4	4	5
IMA	0	0	0	3
LAP	0	1	0	3
NIL	4	4	4	5
PAZ	4	4	4	5
PON	0	4	0	0
REG	4	4	4	5
RUX	4	4	4	5
SOR	3	3	0	4
SUN	3	4	4	6
TOF	0	4	4	4
TRA	0	4	0	0
TRS	0	4	0	4
VAN	4	3	4	5
VEM	0	3	0	0

* Four human cardiomyocyte-like cell lines; 35 separate experiments; 24 drugs
** Control (CTRL) samples: 13 biological replicates for PMC-A, 23 biological replicates for PMC-B, 13 biological replicates for PMC-D, and 22 biological replicates for PMC-E.