Supplementary Information

Transcriptomic profiling of human cardiac cells predicts protein kinase inhibitor-associated cardiotoxicity

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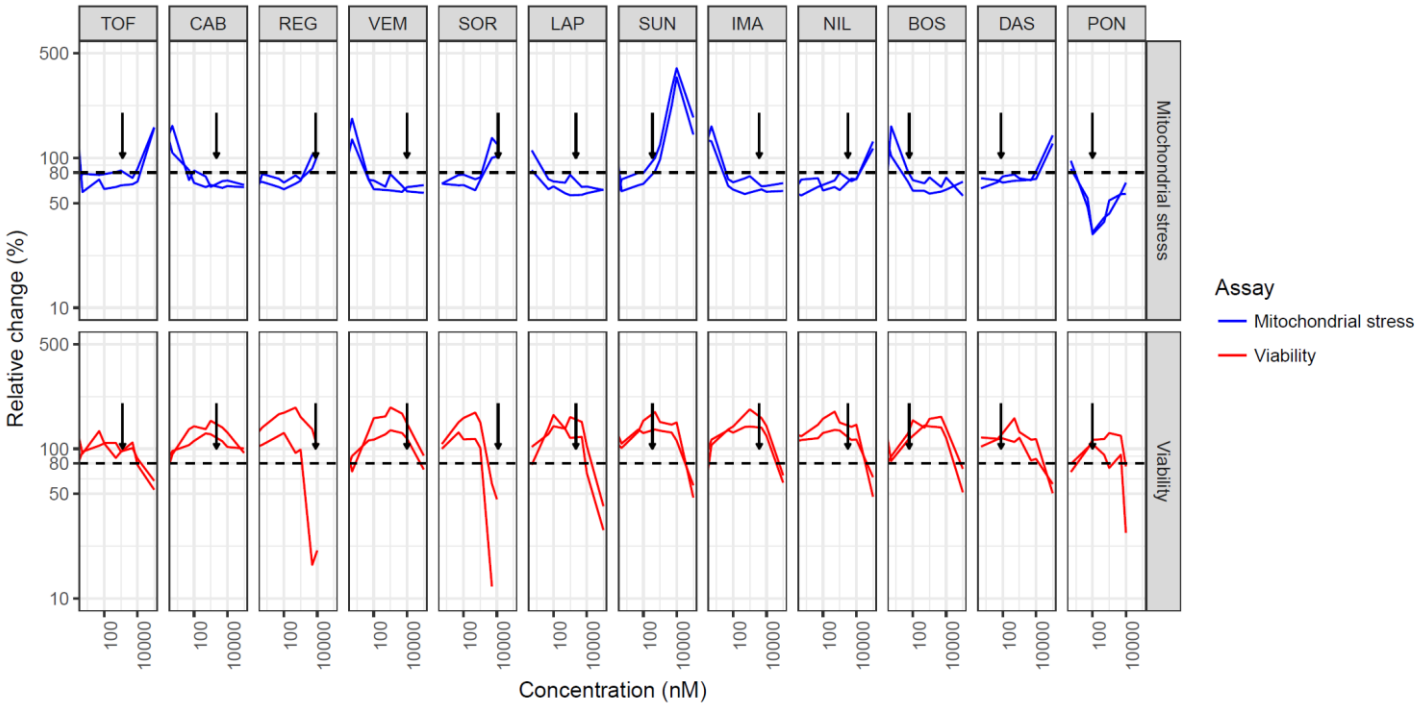
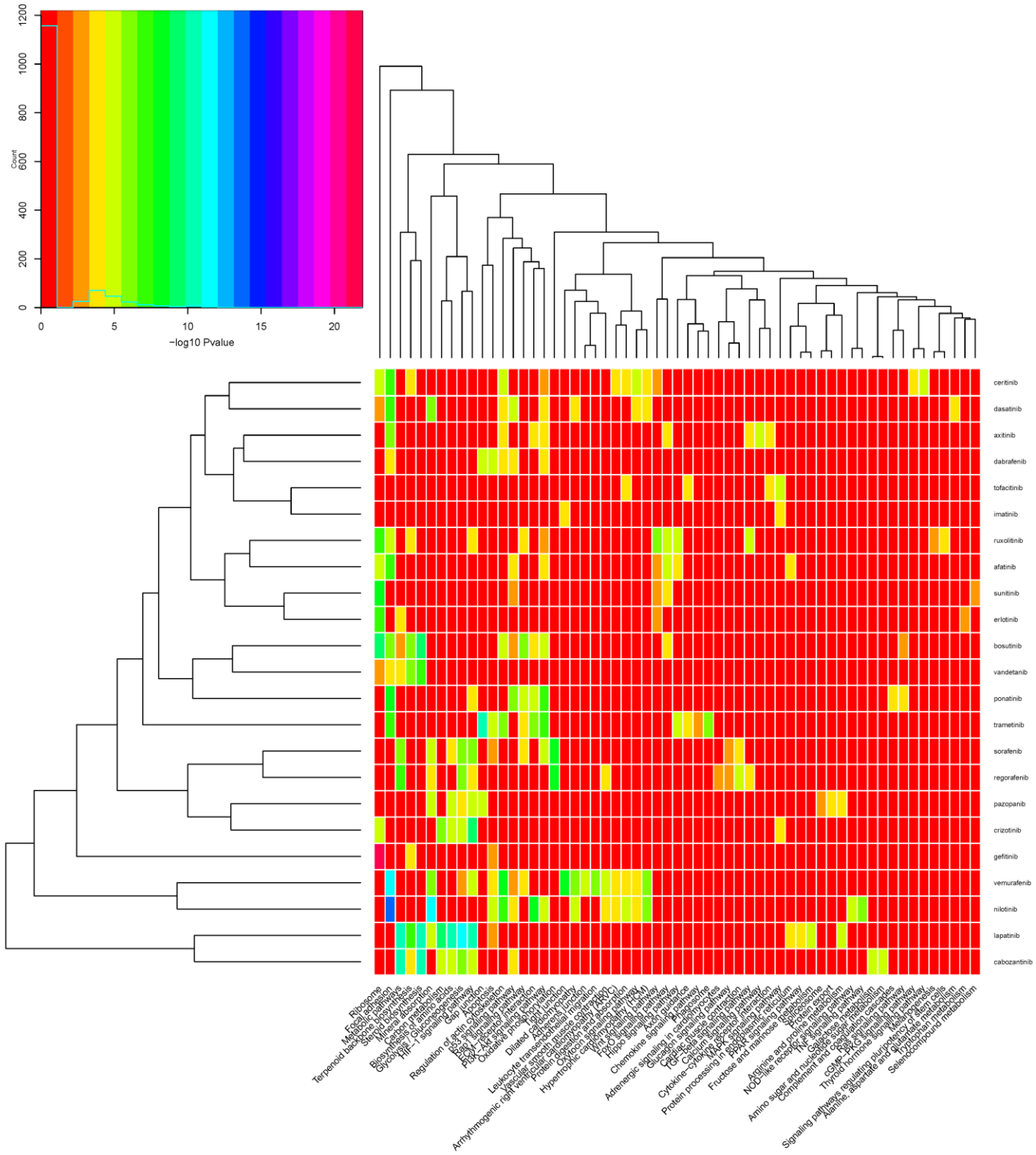
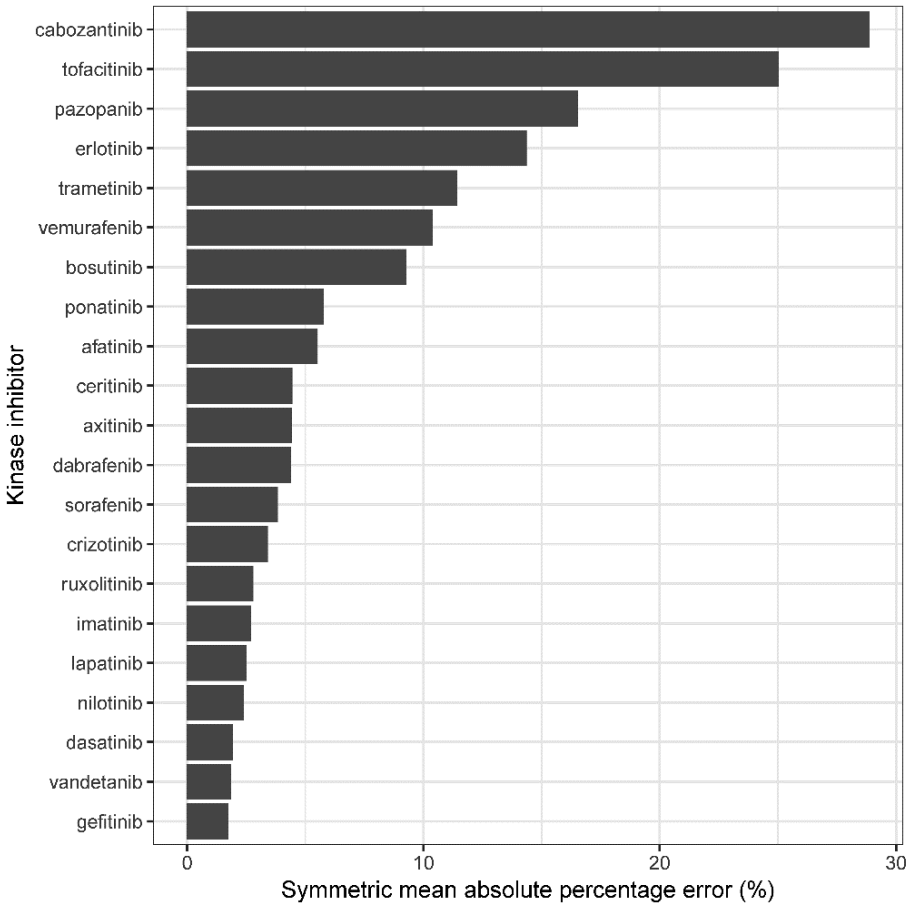
Supplementary Figures



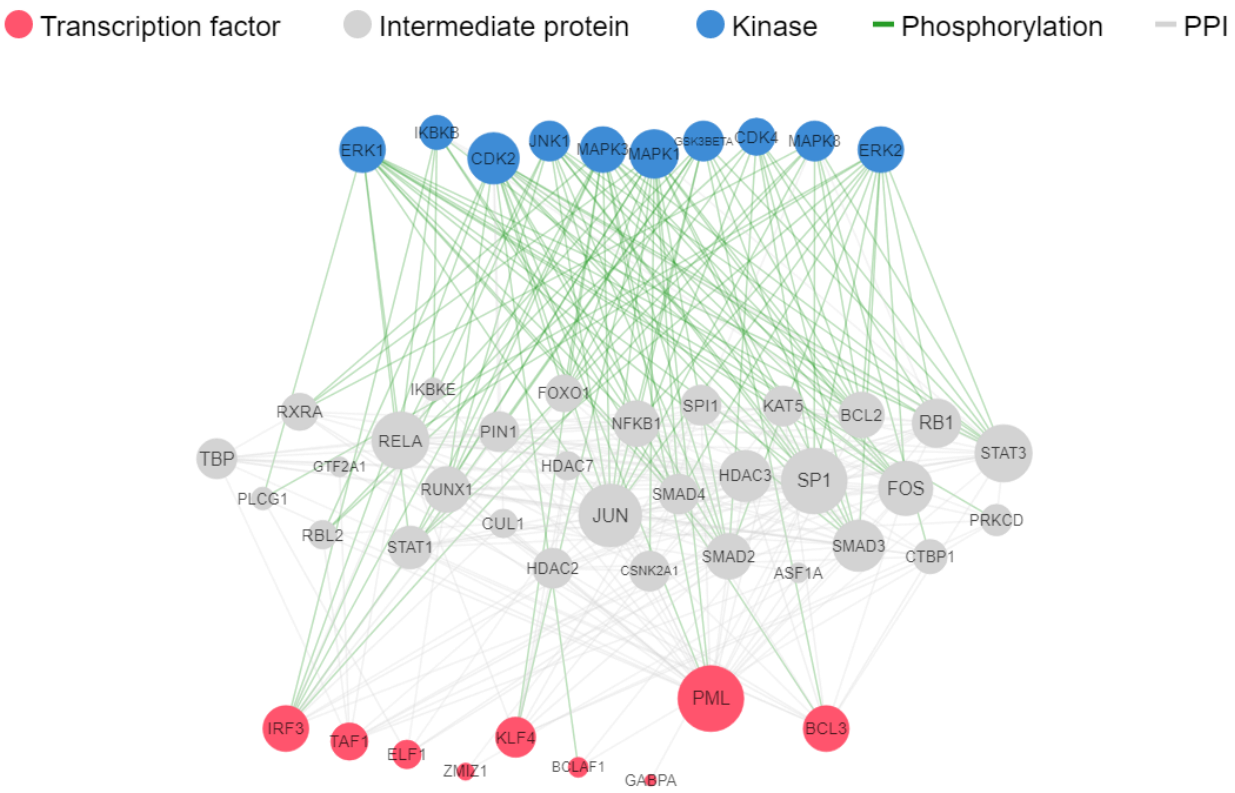
Supplementary Figure 1. Dose-response experiments for multiple selected kinase inhibitors sorted by FAERS-based clinical risk scores (left is low risk; right is high risk), measuring relative change from control in mitochondrial stress and viability. Therapeutic concentrations are indicated with vertical arrows.



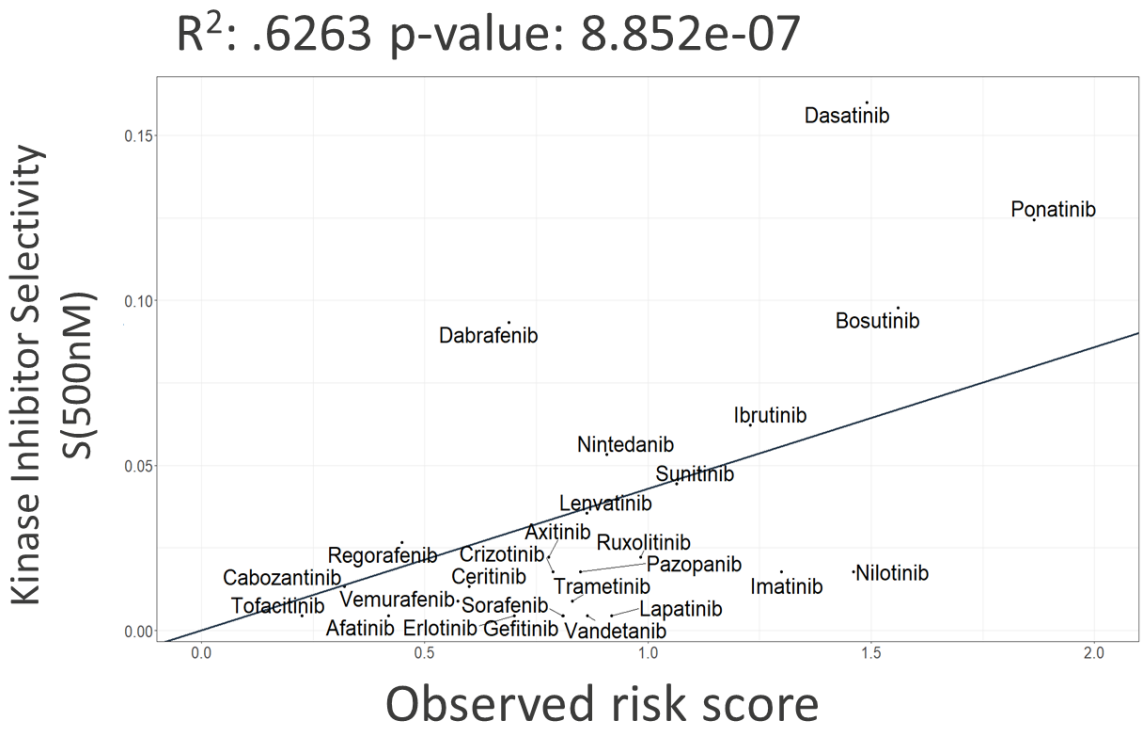
Supplementary Figure 2. Enrichment analysis of pathways in KEGG databased for each of the 23 kinase inhibitors (KIs) across 4 PromoCell cardiomyocyte cell lines based on the top 250 genes for each KI ranked by P-value.



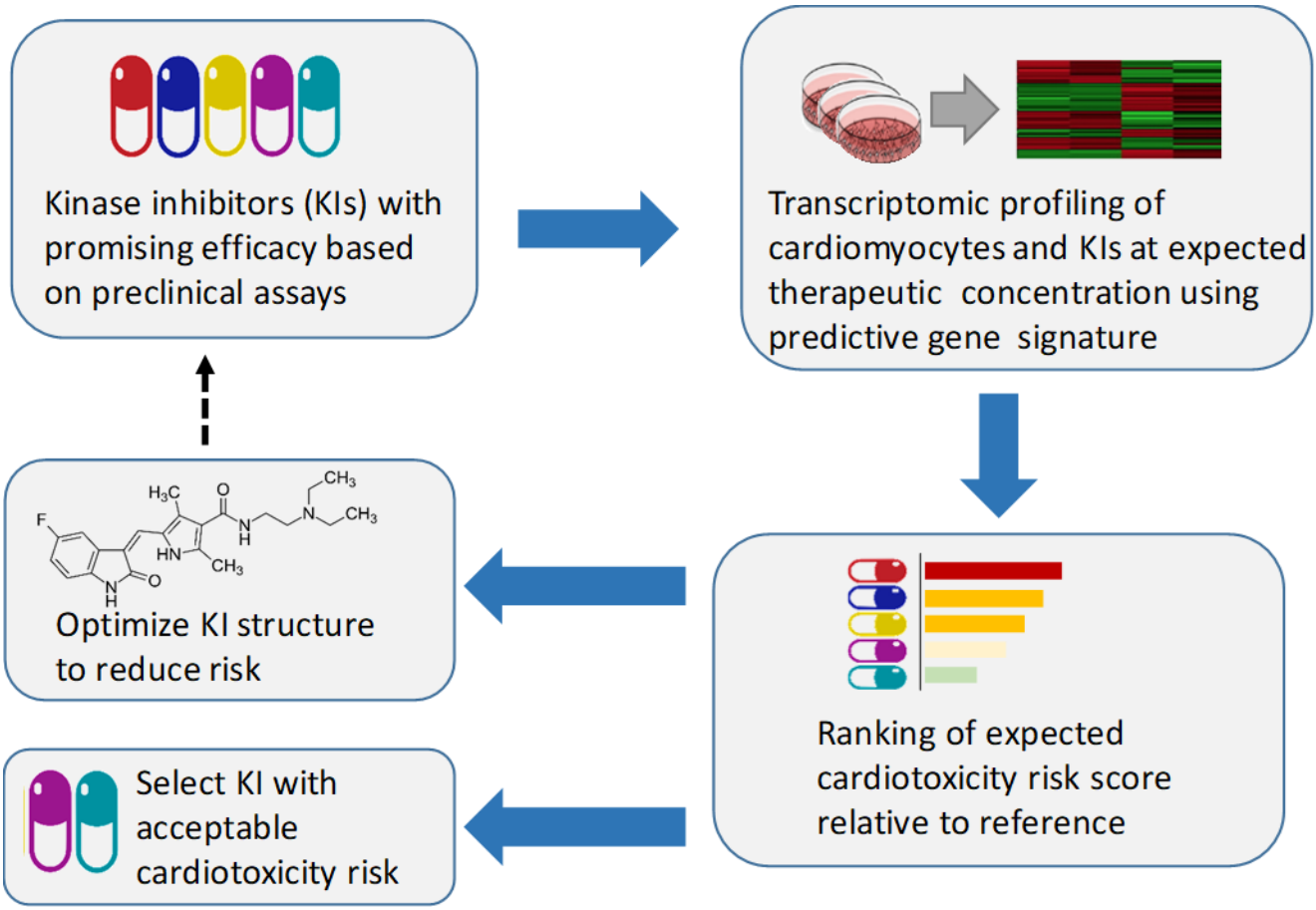
Supplementary Figure 3. Percentage of mean prediction error for left-out drugs during cross-validation. and intermediate proteins that are associated with the cardiotoxicity signature.



Supplementary Figure 4. Expression-to-kinase protein-protein interaction network analysis indicating key kinases, transcription factors



Supplementary Figure 5. Observed risk score vs kinase inhibitor selectivity at 500nM. The Wald statistic was used to test the association between kinase selectivity and observed risk score.



Supplementary Figure 6. Flowchart for implementation of transcriptomic cardiotoxicity signatures in preclinical drug development to reduce risk of cardiotoxicity.

Supplementary Tables

Supplementary Table 1. Typical concentrations used for experiments (exposure for 48h). Concentrations were selected based on the mean maximum drug concentrations reported in clinical studies. For dose escalation studies the highest concentration was used. All drugs were purchased from Aldrich Market Select division of Sigma, and drug purity was confirmed by HPLC-MS analysis, as described in the SOP deposited at the DToxS website.

Drug	Purity	In Vitro (μM)	Reference
Afatinib	98.80	0.05	[3334]
Axitinib	98.00	0.2	[3536]
Bosutinib	98.00	0.1	[37]
Cabozantinib	99.10	2	[38]
Ceritinib	98.85	1	[39]
Crizotinib	98.47	0.25	[40]
Dabrafenib	99.61	2.5	[4142]
Dasatinib	98.36	0.1	[4344]
Erlotinib	99.32	3	[4546]
Gefitinib	99.25	1	[47]
Imatinib	99.48	5	[48]
Lapatinib	99.00	2	[49]
Nilotinib	99.77	3	[5051]
Pazopanib	96.13	10	[52]
Ponatinib	97.29	0.1	[53]
Regorafenib	99.58	1	[54]
Ruxolitinib	99.38	1	[5556]
Sorafenib	99.73	0.5	[57]
Sunitinib	97.13	1	[5859]
Trametinib	99.24	0.1	[60]
Tofacitinib	99.85	1	[61]
Vandetanib	99.73	0.333	[62]
Vemurafenib	98.08	2	[63]

Supplementary Table 2. Overview of heart cell perturbation experiments across drugs, cell lines, plates, and numbers of replicates per experiment for which mRNAseq profiling data was included in this analysis.

Drug	**Nr. of cell lines**[a]	**Nr. of plates**[b]	**Number of Biological replicates/cell line**	
			Minimum	Maximum
Afatinib	4	2	3	4
Axitinib	4	1	4	4
Bosutinib	4	2	4	4
Cabozantinib	3	1	3	4
Ceritinib	4	2	3	4
Crizotinib	4	1	4	4
Dabrafenib	4	1	3	4
Dasatinib	4	2	3	4
Erlotinib	4	2	3	4
Gefitinib	4	1	4	4
Imatinib	4	1	3	4
Lapatinib	2	1	4	4
Nilotinib	4	1	3	4
Pazopanib	4	2	3	4
Ponatinib	4	2	3	4
Regorafenib	3	2	3	4
Ruxolitinib	4	2	3	4
Sorafenib	3	3	3	4
Sunitinib	4	3	3	5
Tofacitinib	4	2	3	4
Trametinib	4	2	3	4
Vandetanib	4	2	4	4
Vemurafenib	4	2	3	4

[a]Derived from different human donors.

[b]In most cases drug perturbation experiments were conducted on multiple plates that were processed in a single RNAseq-run, in order to limit the influence of potential batch-effects.

Supplementary Table 3. List of 26 genes part of the cardiotoxicity gene signature.

Coefficient	Estimate
(Intercept)	0.71042676
PRKRIP1	0.2087493
DDX3Y	-0.14451405
RPL13P5	-0.10065338
DHRS4	0.08840433
ZNF567	-0.19108461
SCO1	0.22498281
ZSCAN25	0.30633667
ZNF776	0.20654469
SNX14	-0.22973802
ZNF611	-0.06748435
MFN2	0.56373084
DSTNP2	-0.16016064
PMS2	0.18801529
NFATC2IP	-0.16092359
UBE2Q2	-0.44148014
ARHGAP12	-0.25426077
TBC1D16	-0.02704898
FAM45BP	0.33178699
GOLPH3L	-0.12777427
BCAT2	0.10332328
URM1	0.31541242
CSE1L	0.17069461
DNAJB14	0.46071526
SNORD47	-0.03746744
CASK	-0.08538788
FASTKD2	0.31397776

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