Supplementary Material

The Role and Mechanism of CRT0066101 as An Effective Drug for Treatment of Triple-Negative Breast Cancer

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Figure S1

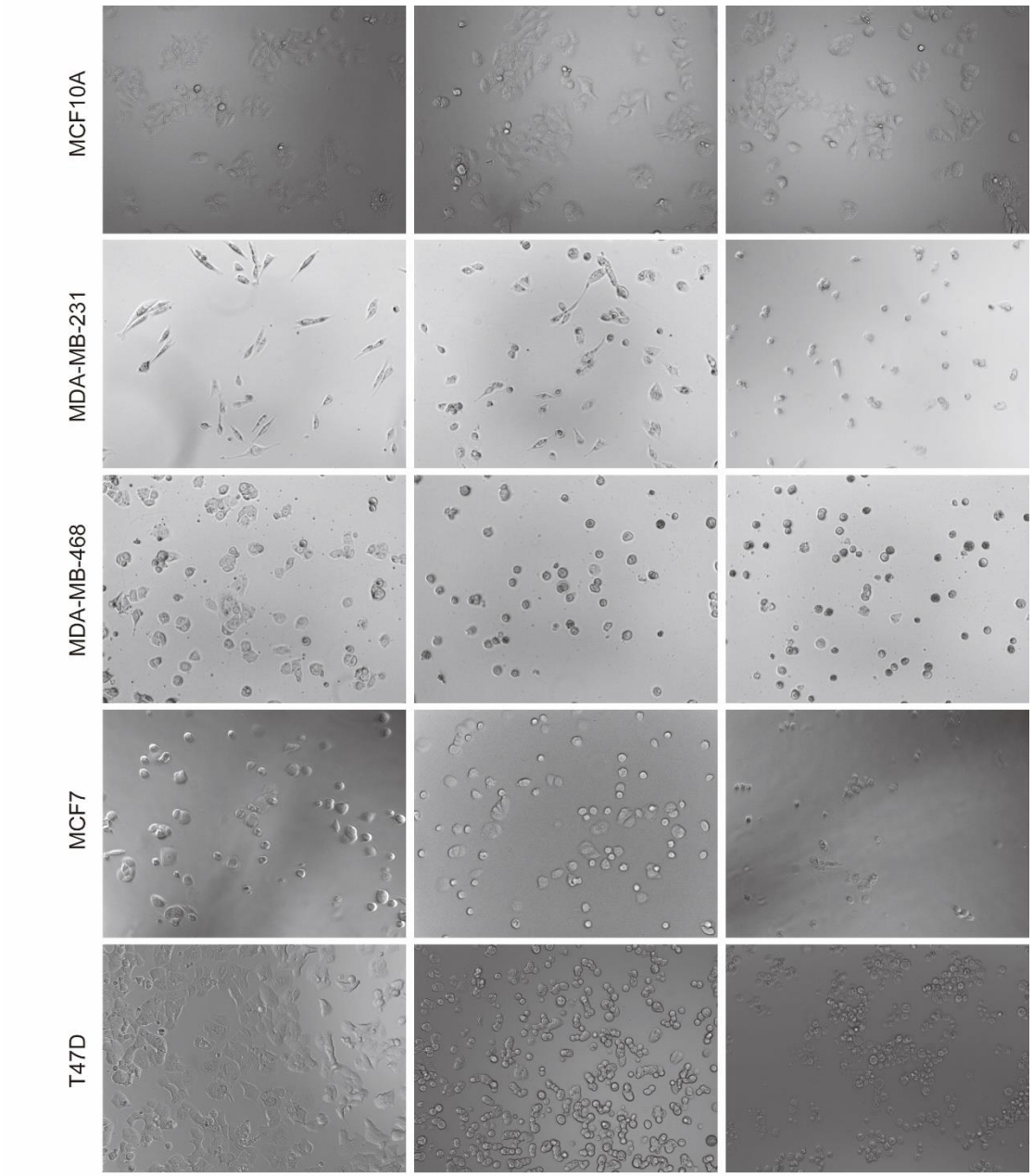
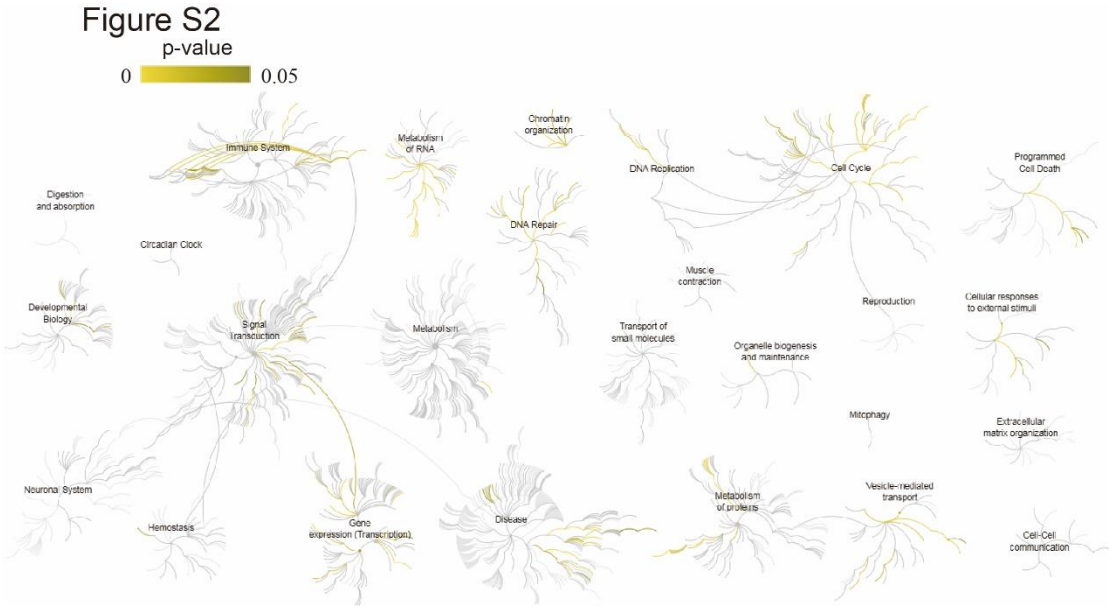


Figure S2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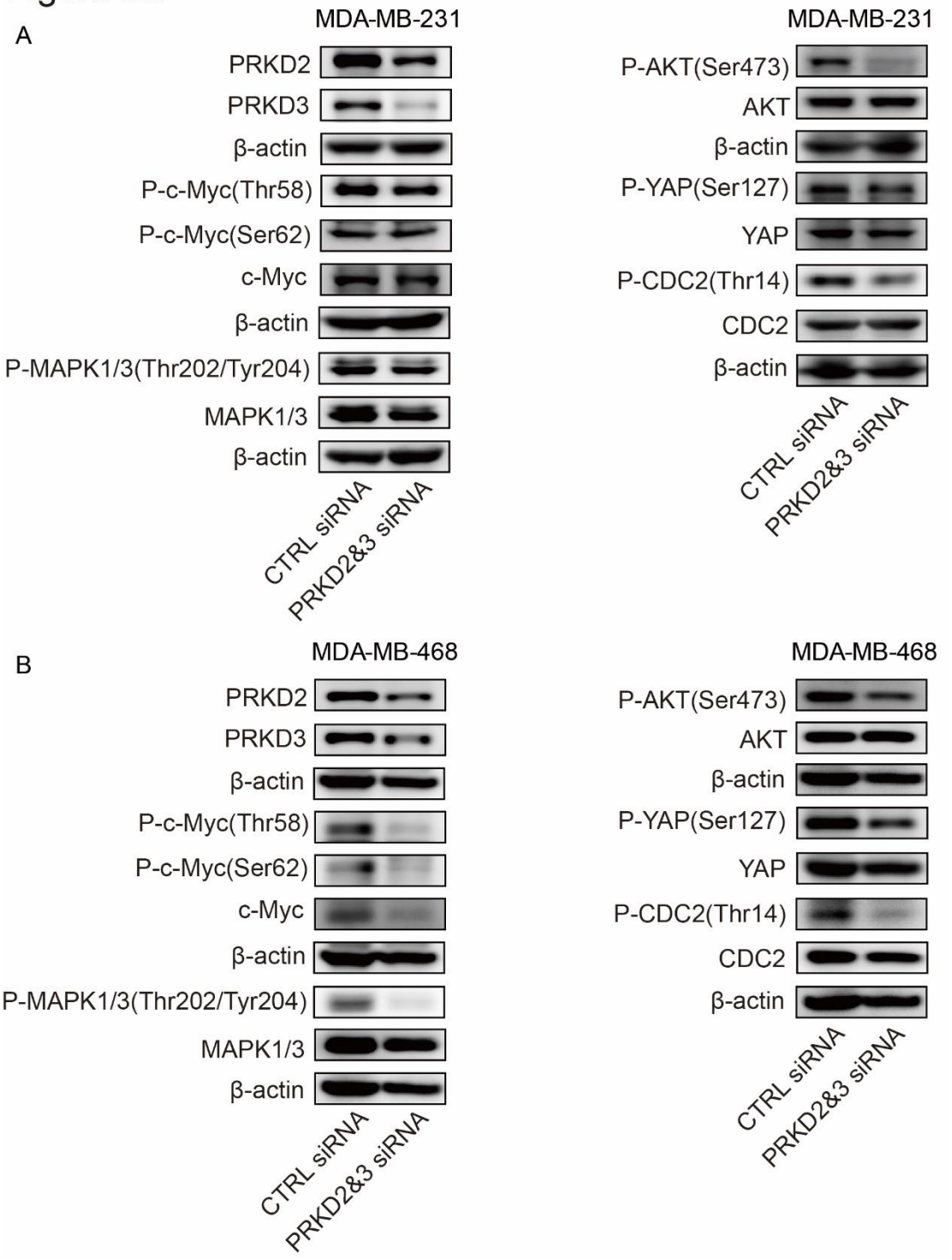
Immune System
Metabolism of RNA
Chromatin organization
DNA Replication
Cell Cycle
Programmed Cell Death
Digestion and absorption
DNA Repair
Circadian Clock
Muscle contraction
Developmental Biology
Signal Transduction
Metabolism
Transport of small molecules
Reproduction
Cellular responses to external stimuli
Organelle biogenesis and maintenance
Mitophagy
Extracellular matrix organization
Neuronal System
Hemostasis
Gene expression (Transcription)
Disease
Metabolism of proteins
Vesicle-mediated transport
Cell-Cell communication

Figure S3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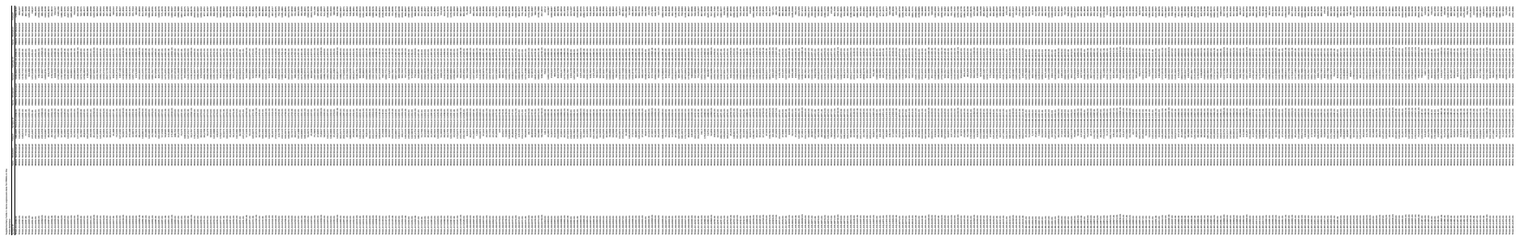
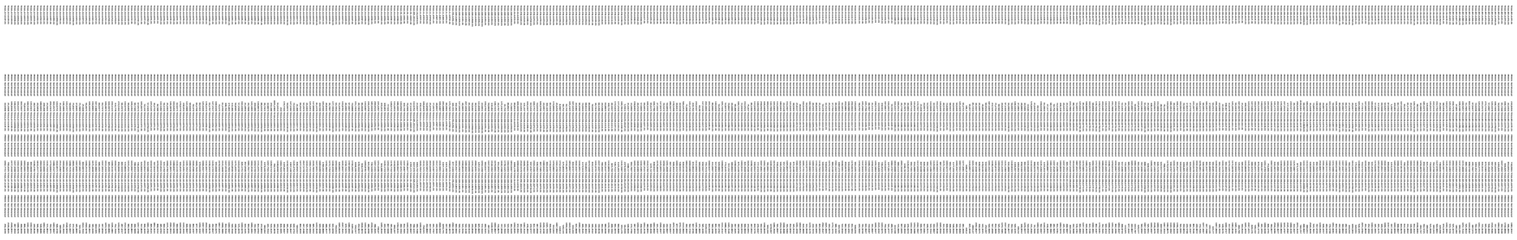
MDA-MB-231
PRKD2
PRKD3
β-actin
P-c-Myc(Thr58)
P-c-Myc(Ser62)
c-Myc
β-actin
P-MAPK1/3(Thr202/Tyr204)
MAPK1/3
β-actin
CTRL siRNA
PRKD2&3 siRNA
MDA-MB-231
P-AKT(Ser473)
AKT
β-actin
P-YAP(Ser127)
YAP
P-CDC2(Thr14)
CDC2
β-actin
CTRL siRNA
PRKD2&3 siRNA
B
MDA-MB-468
PRKD2
PRKD3
β-actin
P-c-Myc(Thr58)
P-c-Myc(Ser62)
c-Myc
β-actin
P-MAPK1/3(Thr202/Tyr204)
MAPK1/3
β-actin
CTRL siRNA
PRKD2&3 siRNA
MDA-MB-468
P-AKT(Ser473)
AKT
β-actin
P-YAP(Ser127)
YAP
P-CDC2(Thr14)
CDC2
β-actin
CTRL siRNA
PRKD2&3 siRNA

Figure S1. Cells were incubated with increasing concentrations (0, 1, and 3 μM) of CRT0066101 for 24 h.

Figure S2. Reactome analysis of phosphoproteins regulated by PRKD.

Figure S3. Protein and phosphorylation levels of key regulators in TNBC. (A) MDA-MB-231 and (B) MDA-MB-468 cell lines were treated with siPRKD2 and siPRKD3 or control siRNA (CTRL siRNA) for 3 days. The protein and phosphorylation levels of PRKD, MYC, MAPK1/3, AKT, YAP, and CDC2 were analyzed by western blot.





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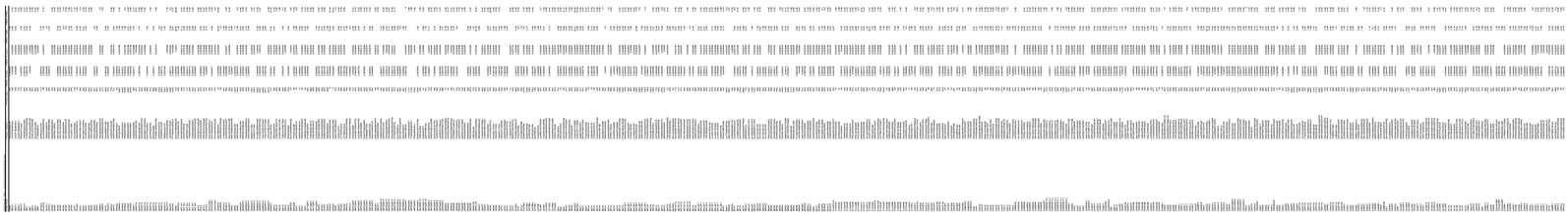
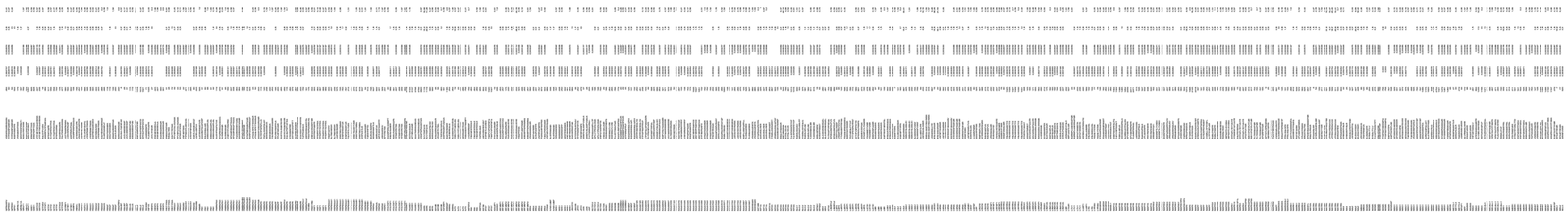
Supplementary Table 2.Gene expression data for PRKDs in TNBC

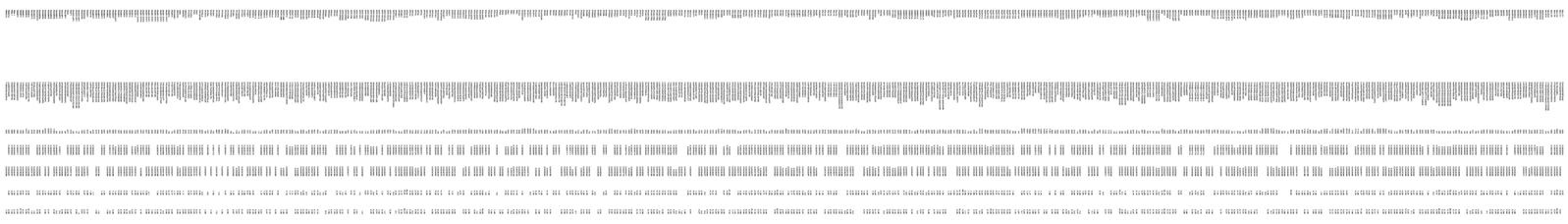
Gene Name	SUM185PE	MDAMB453	HCC2185	BT20	BT549	HBL100	HCC38	HCC70	HCC1143	HCC1187	HCC1500	HCC1937	HCC2157	HCC3153	HS578T	MCF10A	MCF12A	MDAMB157	MDAMB231	MDAMB435	MDAMB436	MDAMB468	SUM149PT	SUM159PT	SUM1315
PRKD1	3,4299	3,4389	3,2412	3,0791	3,1025	3,4776	3,2362	3,0749	3,2184	3,4282	3,2807	3,1087	3,2149	3,0838	3,5724	3,3277	2,9888	3,2209	3,1243	3,6134	3,5864	2,9966	3,2557	3,6494	3,123
PRKD2	7,3791	7,0602	7,7302	7,9408	6,226	6,9478	7,9131	8,1211	7,3995	8,3859	8,2069	7,4	5,9297	7,0843	6,8559	6,8104	7,6628	6,4787	6,7203	5,8634	6,074	7,6287	6,9767	6,6584	5,883
PRKD3	5,5263	4,2822	6,1613	5,1677	5,8715	4,7359	5,6974	6,9345	5,7985	6,4442	7,5142	5,8694	4,9798	5,6684	5,5401	6,2281	5,9311	6,0256	6,3109	6,8037	6,0347	6,6554	6,2977	5,8284	5,5213

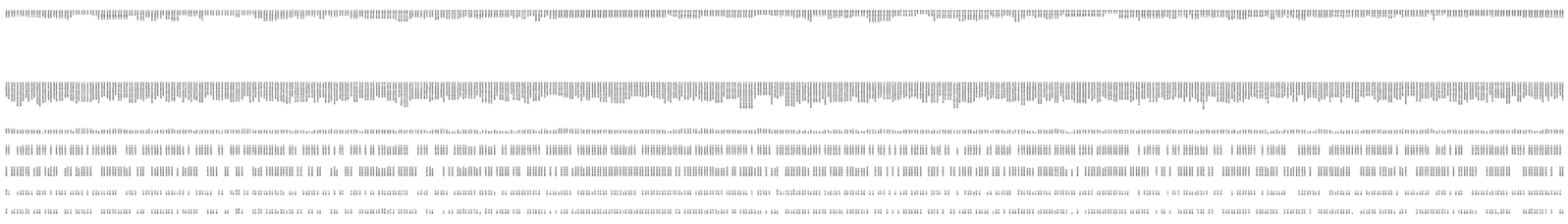
Supplementary Table 3.Information for breast cancer specimens collected from Jinling Hospital

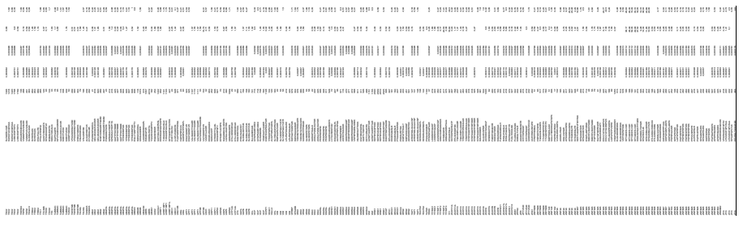
Sample Number.	Invasive breast cancer calssification	ER	PR	CerbB2	Ki-67
T1	Ductal carcinoma II	+	+	-	15%
T2	Ductal carcinoma III	-	-	-	30%
T3	Ductal carcinoma III	+	+	+	40%
T4	Ductal carcinoma III	+	-	+	60%
T5	Ductal carcinoma III	-	-	-	40%
T6	Ductal carcinoma I	+	-	+	50%
T7	Ductal carcinoma III	+	+	+	60%
T8	Ductal carcinoma II	+	+	+	40%
T9	Ductal carcinoma II	-	+	+	45%
T10	Ductal carcinoma II	+	+	+	30%
T11	Ductal carcinoma I	+	+	+	20%
T12	Ductal carcinoma II	+	+	+	40%
T13	Ductal carcinoma III	+	+	+	20%
T14	Ductal carcinoma II	+	-	+	20%
T15	Ductal carcinoma II	+	+	+	80%
T16	Ductal carcinoma III	-	-	-	25%
T17	Ductal carcinoma III	-	-	+	60%
T18	Ductal carcinoma II	+	-	-	30%
T19	Ductal carcinoma I	-	-	-	60%
T20	Ductal carcinoma II	+	+	+	40%
T21	Ductal carcinoma II	+	+	-	20%
T22	Ductal carcinoma II	-	-	-	50%
T23	Ductal carcinoma II	+	-	+	50%
T24	Ductal carcinoma II	+	+	+	20%
T25	Ductal carcinoma III	-	-	+	60%
T26	Ductal carcinoma II	+	-	+	20%
T27	Ductal carcinoma II	+	+	+	25%
T28	Ductal carcinoma II	+	-	+	10%
T29	Ductal carcinoma III	+	-	+	20%
T30	Ductal carcinoma I	+	+	-	10%
T31	Ductal carcinoma II	+	-	+	40%
T32	Ductal carcinoma III	+	+	+	70%
T33	Ductal carcinoma I	+	+	-	50%
T34	Ductal carcinoma II	+	+	+	80%

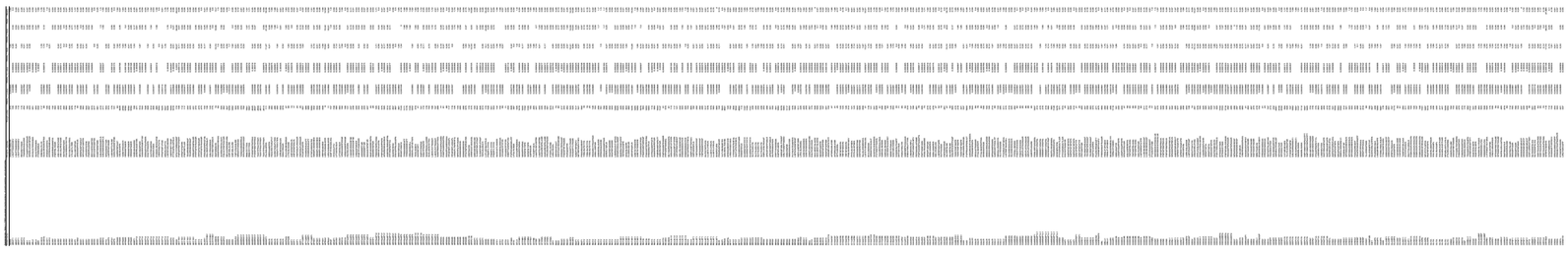


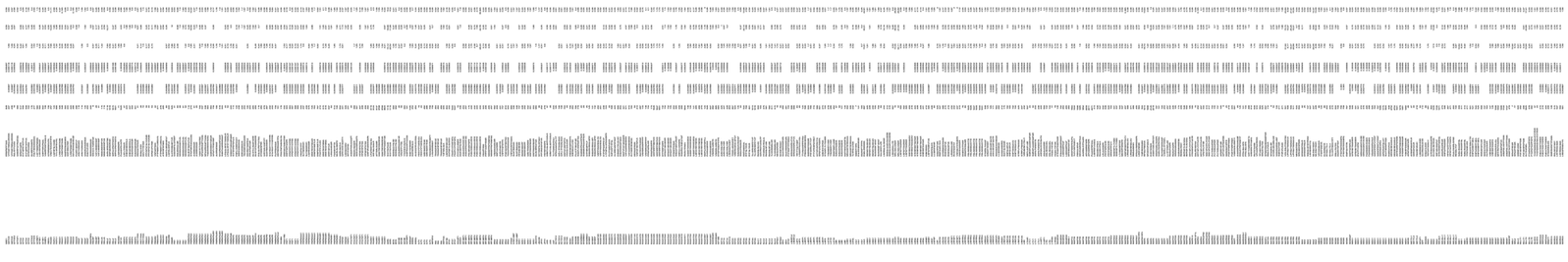


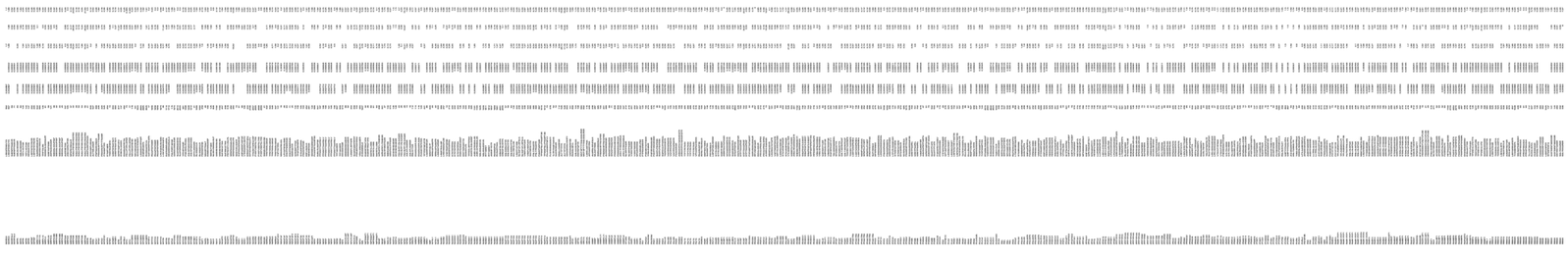


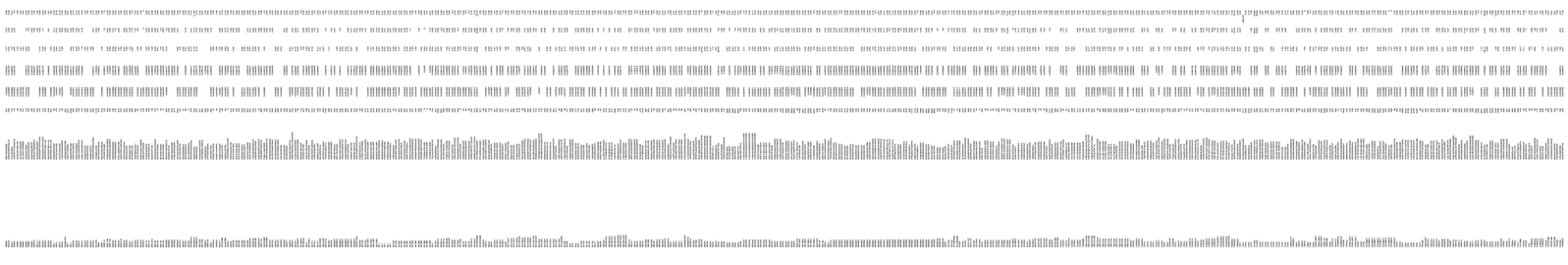


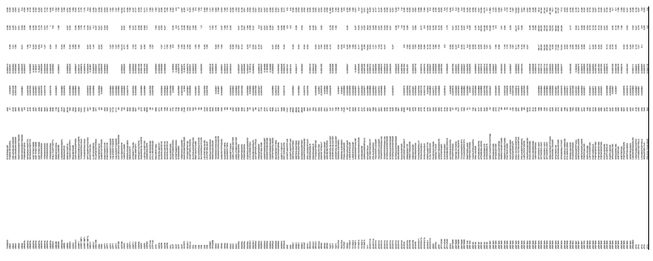












Supplementary Table 6. Biological events of enriched pathways in PRKD-regulated reactomes

Biological events	Pathway name	Entities pValue	Entities FDR
Metabolism of RNA	Processing of Capped Intron-Containing Pre-mRNA	1,11E-16	1,58E-13
Metabolism of RNA	mRNA Splicing - Major Pathway	4,66E-15	3,32E-12
Metabolism of RNA	mRNA Splicing	5,76E-14	2,73E-11
Metabolism of RNA	Metabolism of RNA	6,14E-13	2,18E-10
Metabolism of RNA	Transport of Mature mRNAs Derived from Intronless Transcripts	7,60E-07	8,96E-05
Metabolism of RNA	Transport of Mature Transcript to Cytoplasm	9,91E-07	1,08E-04
Metabolism of RNA	Transport of Mature mRNA Derived from an Intronless Transcript	2,26E-06	2,13E-04
Metabolism of RNA	Transport of the SLBP Dependant Mature mRNA	2,30E-05	0,001357027
Metabolism of RNA	Transport of Mature mRNA derived from an Intron-Containing Transcript	3,57E-05	0,001856284
Metabolism of RNA	Transport of the SLBP independent Mature mRNA	6,62E-05	0,003092238
Metabolism of RNA	tRNA processing in the nucleus	7,03E-05	0,003092238
Metabolism of RNA	mRNA 3'-end processing	8,22E-05	0,003371015
Cell Cycle	Cell Cycle, Mitotic	8,68E-12	2,47E-09
Cell Cycle	Cell Cycle	1,20E-11	2,85E-09
Cell Cycle	Nuclear Envelope Breakdown	1,81E-07	3,68E-05
Cell Cycle	M Phase	4,17E-07	6,59E-05
Cell Cycle	Nuclear Pore Complex (NPC) Disassembly	1,06E-05	8,40E-04
Cell Cycle	Mitotic Prophase	1,30E-05	8,70E-04
Cell Cycle	Mitotic Metaphase and Anaphase	1,55E-05	9,92E-04
Cell Cycle	Mitotic Anaphase	2,88E-05	0,001614838
Cell Cycle	Cell Cycle Checkpoints	2,27E-04	0,006961128
Cell Cycle	Mitotic Prometaphase	3,62E-04	0,009645473
Cell Cycle	Clearance of Nuclear Envelope Membranes from Chromatin	3,71E-04	0,009645473
Metabolism of Proteins	SUMO E3 ligases SUMOylate target proteins	3,38E-07	5,98E-05
Metabolism of Proteins	SUMOylation	6,33E-07	8,96E-05
Metabolism of Proteins	SUMOylation of DNA damage response and repair proteins	2,75E-06	2,42E-04
Metabolism of Proteins	SUMOylation of RNA binding proteins	9,59E-06	7,96E-04
Metabolism of Proteins	SUMOylation of chromatin organization proteins	1,27E-05	8,70E-04
Metabolism of Proteins	Activation of the mRNA upon binding of the cap-binding complex and eIFs, and subsequent binding to 43S	5,77E-05	0,00288417
Metabolism of Proteins	Translation initiation complex formation	7,65E-05	0,003291014
Metabolism of Proteins	Ribosomal scanning and start codon recognition	1,38E-04	0,004925998
Disease	Interactions of Rev with host cellular proteins	3,30E-05	0,001781518
Disease	Rev-mediated nuclear export of HIV RNA	6,99E-05	0,003092238
Disease	Nuclear import of Rev protein	1,14E-04	0,004273925
Disease	NS1 Mediated Effects on Host Pathways	1,16E-04	0,004273925
Disease	HIV Life Cycle	2,26E-04	0,006961128
Disease	Vpr-mediated nuclear import of PICs	2,41E-04	0,00721589
Disease	Host Interactions with Influenza Factors	2,89E-04	0,008373555
Gene expression	Regulation of TP53 Activity through Acetylation	1,14E-04	0,004273925
Gene expression	TP53 Regulates Transcription of DNA Repair Genes	1,41E-04	0,004925998
Gene expression	Formation of RNA Pol II elongation complex	1,52E-04	0,005160326
Gene expression	RNA Polymerase II Transcription Termination	1,66E-04	0,005485817
Gene expression	Cleavage of Growing Transcript in the Termination Region	1,66E-04	0,005485817
Gene expression	RNA Polymerase II Transcription Elongation	3,22E-04	0,009325931
Programmed Cell Death	Apoptotic execution phase	7,50E-07	8,96E-05
Programmed Cell Death	Apoptotic cleavage of cellular proteins	2,30E-05	0,001357027
Immune System	ISG15 antiviral mechanism	3,52E-04	0,009506104
Immune System	Antiviral mechanism by IFN-stimulated genes	3,52E-04	0,009506104
Signaling Transduction	Regulation of PTEN gene transcription	1,13E-06	1,14E-04
Cellular responses to external stimuli	Cellular response to heat stress	1,30E-05	8,70E-04
Vesicle-mediated transport	Membrane Trafficking	1,14E-04	0,004273925
DNA repair	Resolution of Abasic Sites (AP sites)	2,32E-04	0,006961128