

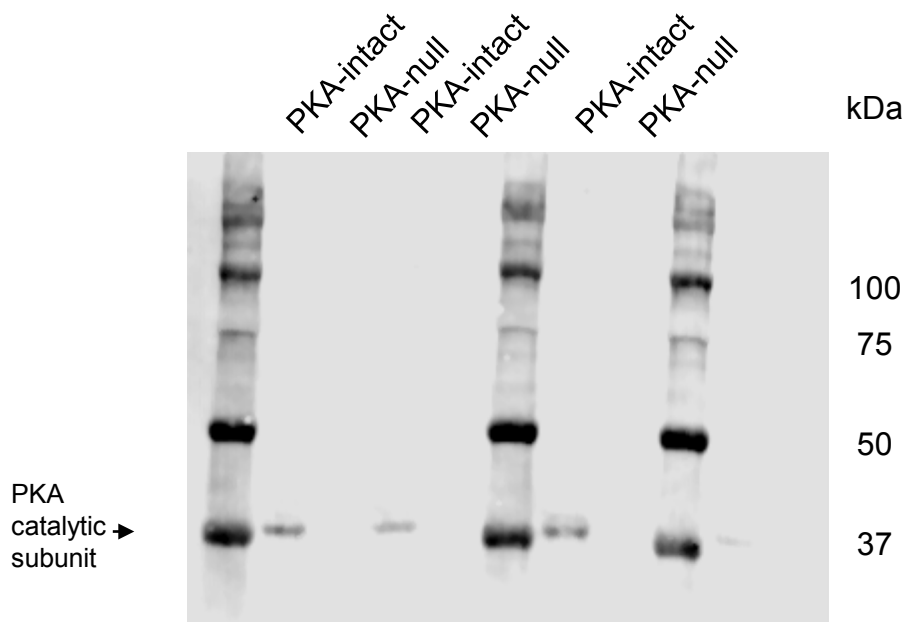
**Supplementary Figures for
Phosphorylation Changes in Response to Kinase Inhibitor H89
in PKA-Null Cells**

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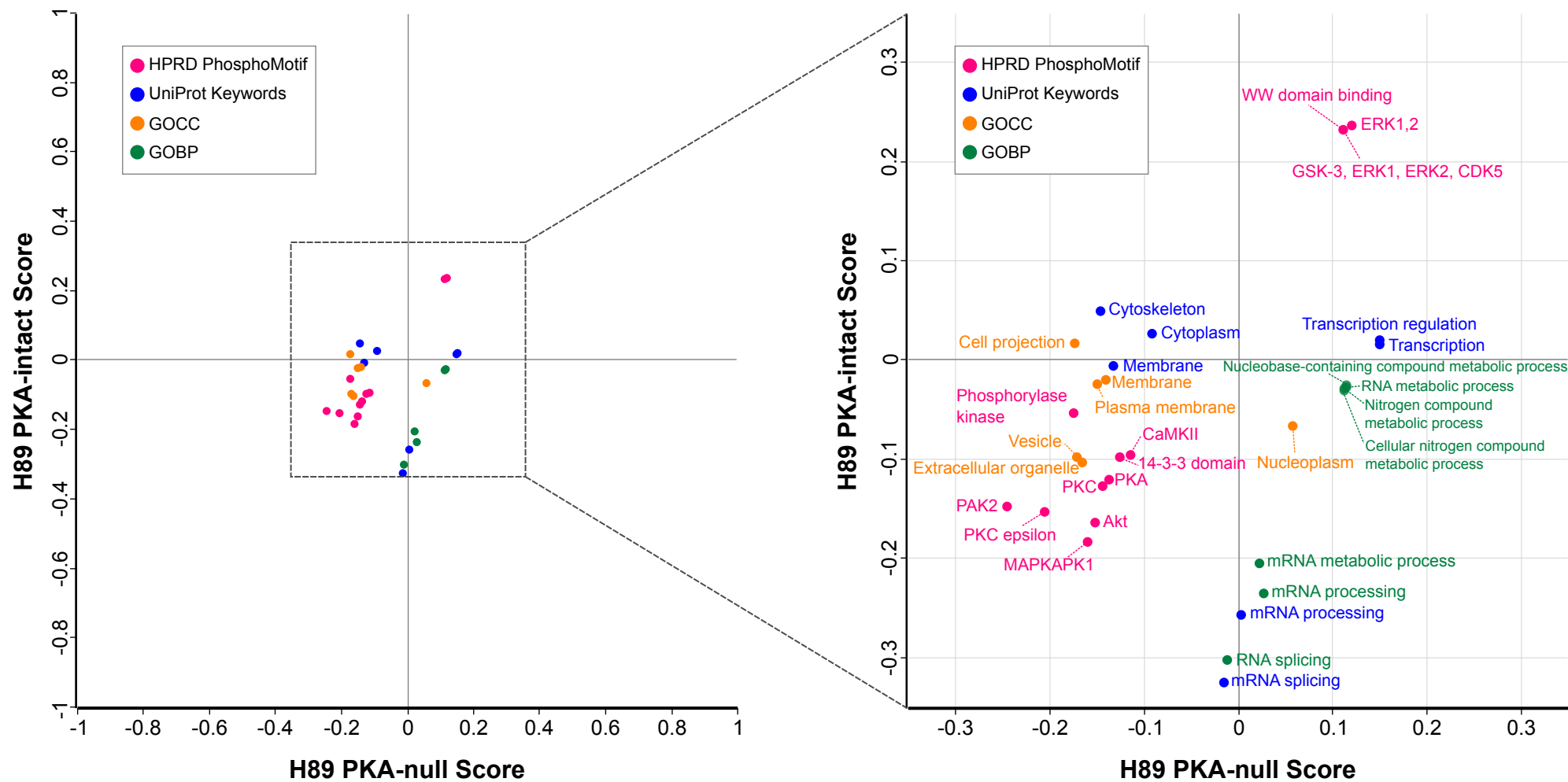
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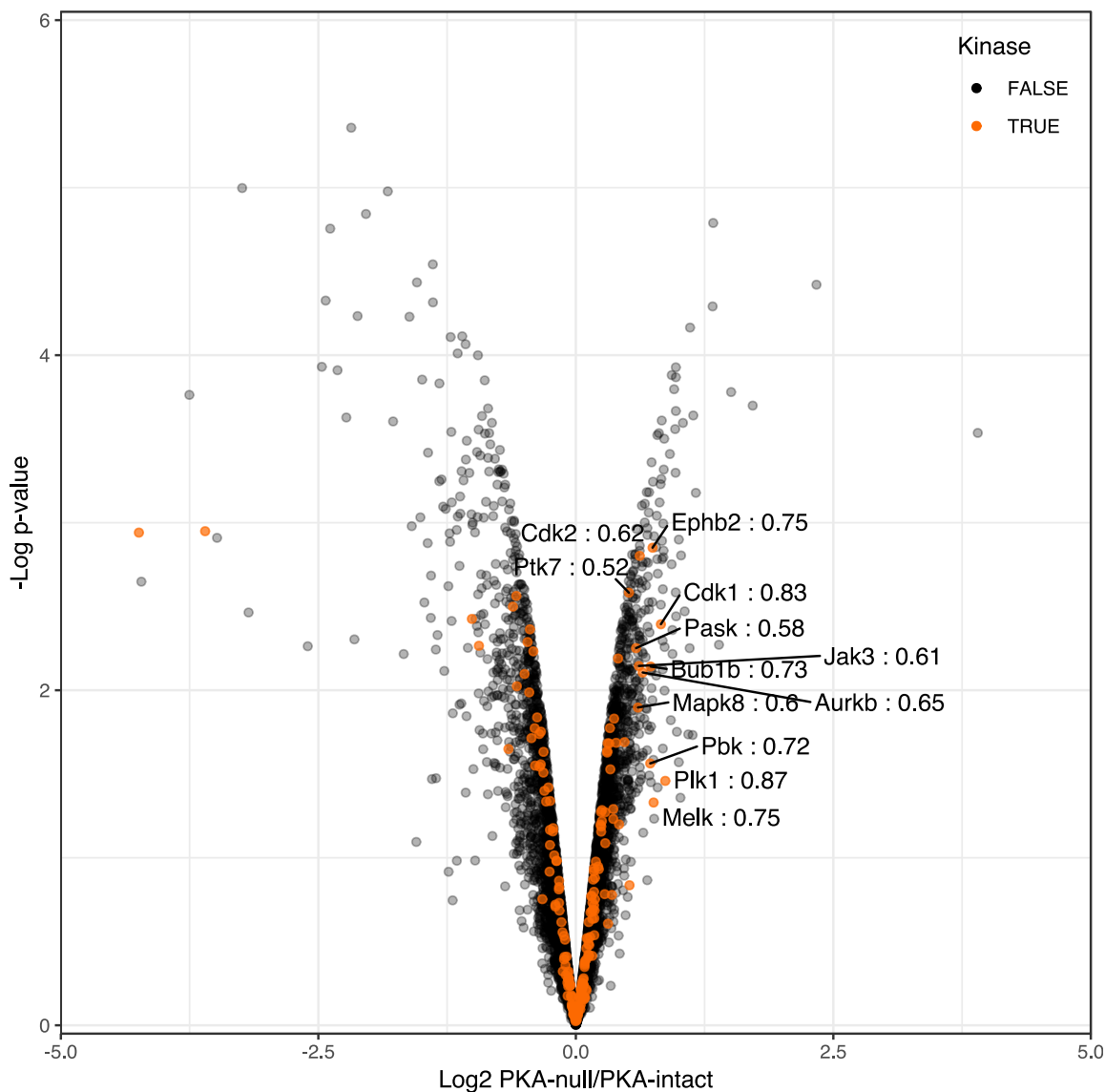


Supplementary Figure S1. Immunoblot using a PKA antibody recognizing both PKA catalytic proteins shows absence of PKA catalytic subunits in PKA-null cells.



Supplementary Figure S2. 2D annotation enrichment analysis.

To compare effect H89 on phosphoproteome of PKA-intact and PKA-null cells, each phosphoprotein was annotated with HPRD PhosphoMotif, gene ontology, KEGG, Pfam, GSEA, Corum, InterPro, PRINTS, PROSITE, Reactome, and SMART terms. 2D annotation enrichment analysis was performed using Perseus software. Terms with adjusted p-value < 0.01 are shown in the scatter plot. X and y-axes correspond to enrichment score which calculated from rank of effect size of H89 on phosphosites (range from -1 to 1). All significant terms are clustered around the origin, suggesting minimal enrichment in any category. Consistent with sequence logos generated from significantly changed phosphosites, several non-PKA basophilic kinase motifs were enriched with negative score (decrease by H89) and proline-directed kinase motifs were enriched with positive score (increase by H89) in both cell types.



Supplementary Figure S3. Up-regulated kinases in PKA-null cells. A volcano plot shows log₂ ratio of protein abundance (normalized log₂ TMT reporter ion intensity) of PKA-null over PKA-intact cells (control group) on x-axis and negative of log₁₀ p-value (moderated t-test, LIMMA) on y-axis. Kinases are colored in orange. Up-regulated kinases with p-value < 0.05 and log₂ ratio > 0.5 are labeled with gene symbol and its log₂ ratio value.

Gene	Protein	Protein name	Position	β_{H89} in PKA-intact	Centralized sequence
Snrnp200	Q6P4T2	U5 small nuclear ribonucleoprotein 200 kDa helicase	2131, 2133, 2135	0.44	VDVKEAE TDS SDS___ VKEAE TDS SDS_____ EAE TDS SDS
Chd2	E9PZM4	Chromodomain-helicase-DNA-binding protein 2	156, 165	0.41	EKWKQDP SE DEQEQQ DEQEQGT SA ESEAEQ
Iws1	Q8C1D8	Protein IWS1 homolog	248, 263	0.39	ELPKPRV SD SESEDP QKGPASD SEA EDASR
Mcm3	P25206	DNA replication licensing factor MCM3	722	0.37	PQVHTPK T DDSQEKT
Sept9	Q80UG5	Septin-9	85	0.31	VDSL SQR SPKPSLRR
Llg1	A0A0R4J0S4	Lethal(2) giant larvae protein homolog 1	988, 989	0.31	ESCEGSP SS AHSKRA SCEGSP SS AHSKRAD
Prr12	E9PYL2	Proline-rich protein 12	1555	0.30	TAAVCGE T DEEAGES
Mphosph10	Q810V0	U3 small nucleolar ribonucleoprotein protein MPP10	244	0.29	DLFEDID S DESEGG
Hnrnpa1	Q5EBP8	Heterogeneous nuclear ribonucleoprotein A1	22	0.29	KLFIGGL S FETTDES
Srsf7	Q8BL97	Serine/arginine-rich splicing factor 7	210	0.26	ASLRRSR S GSIIISR

Supplementary Table S1 : Phosphorylation sites with largest positive β_{H89} in PKA-intact cells.

Gene	Protein	Protein name	Position	β_{H89} in PKA-null	Centralized sequence
Chd2	E9PZM4	Chromodomain-helicase-DNA-binding protein 2	156, 165	0.26	EKWKQDPSEDEQEQQ DEQEQGTSAESEAEQ
Iws1	Q8C1D8	Protein IWS1 homolog	248, 263	0.25	ELPKPRVSDSESEDP QKGPASDSEAEADASR
Ptpn14	Q62130	Tyrosine-protein phosphatase non-receptor type 14	700, 705, 719	0.25	PQYHHKKTFSDATML KKTFSDATMLIHSSE ESEEEEEETLEAAPQV
Prr12	E9PYL2	Proline-rich protein 12	1555	0.25	TAAVCGETDEEAGES
8030462 N17Rik	Q0VAW6	RIKEN cDNA 8030462N17 gene	322, 323	0.23	NEEINIASSDSEVEI EEINIASSDSEVEIV
Utp18	Q5SSI6	U3 small nucleolar RNA-associated protein 18 homolog	115, 118	0.22	RGQLHGSSDESEVEN LHGSSDESEVENEAK
Llg1	A0A0R4J0S4	Lethal(2) giant larvae protein homolog 1	988, 989	0.22	ESCEGSPSSAHSKRA SCEGSPSSAHSKRAD
Mcm3	P25206	DNA replication licensing factor MCM3	722	0.21	PQVHTPKTDDSQEKT
Wrnip1	Q91XU0	ATPase WRNIP1	91, 92	0.20	ATPTAAESSEGEGER TPTAAESSEGEGER
Nucks1	Q80XU3	Nuclear ubiquitous casein and cyclin-dependent kinase substrate 1	144	0.20	LMEDDDDDSDYGSSKK

Supplementary Table S2 : Phosphorylation sites with largest positive β_{H89} in PKA-null cells.