Supplementary information for:

Integrated phosphoproteomics for identifying substrates of human Protein Kinase A (*PRKACA*) and its oncogenic mutant *DNAJB1-PRKACA*

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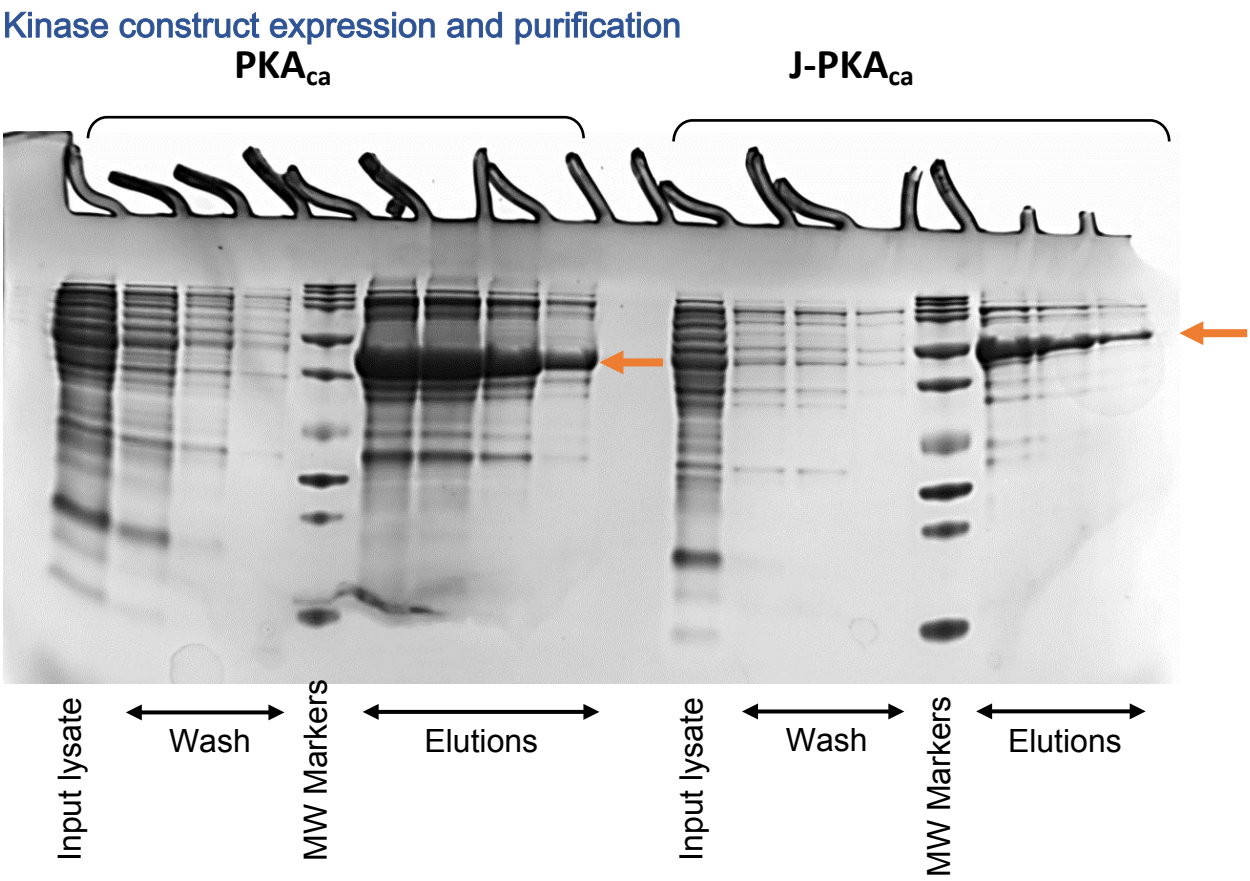
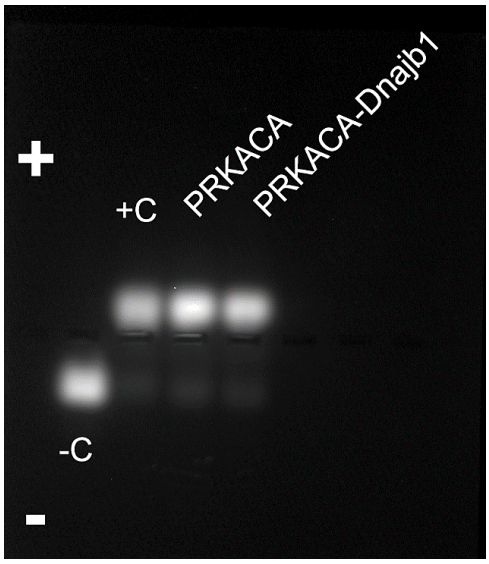


Supplementary Figure S1. PKA_{ca} and J-PKA_{ca} were expressed as described in the main manuscript Methods and purified using Ni-NTA resin column. Shows: SDS-PAGE gel stained with Coomassie stain; arrow shows desired construct.

Kinase activity validation

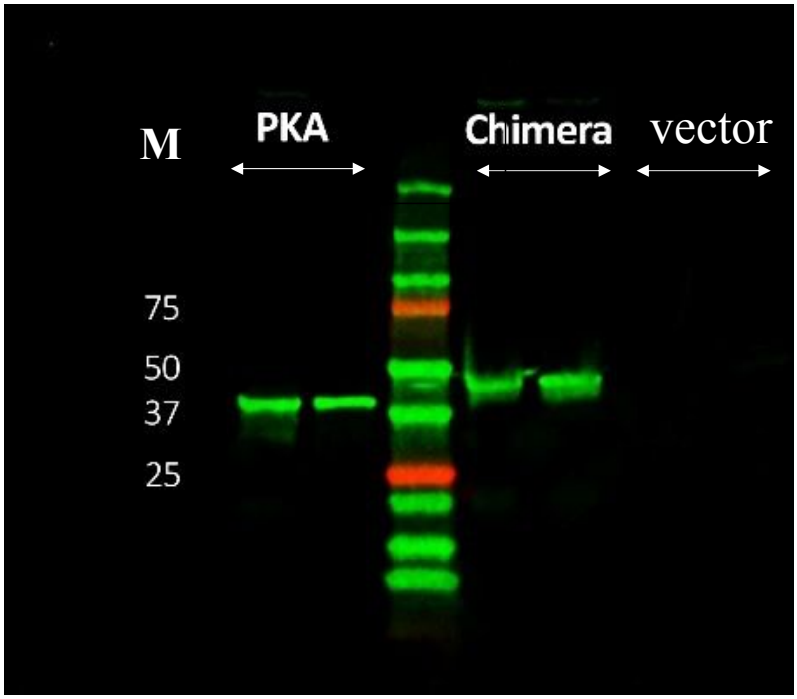
The PepTag® Non-Radioactive Protein Kinase Assay provides a rapid, sensitive and non-radioactive method to detect PKA activity. Phosphorylation by PKA of its specific substrate alters the peptide's net charge from +1 to –1. This change in the net charge of the substrate allows the phosphorylated and non-phosphorylated versions of the substrate to be rapidly separated by electrophoresis on an agarose gel. The phosphorylated species migrates toward the positive electrode, while the nonphosphorylated substrate migrates toward the negative electrode.



Supplementary Figure S2. PepTag® Non-Radioactive Protein Kinase Assays for recombinant kinases used in this work. The samples include -C (negative control, no kinase), +C (positive control, commercial recombinant PKA provided by the kit), PKA_{ca} (PRKACA) and J-PKA_{ca} (PRKACA-Dnajb1).

Western Blotting to validate kinase construct expression in mammalian cells.

After measuring protein concentration of lysates, 50 µg of protein from each sample was reduced with 10 mM DTT and 1× loading buffer (Bio-Rad Cat#161-0710 and Cat.#161-0737) and heated to 95 °C for 5 min. Proteins were separated on 4–15% Mini-PROTEAN® TGX™ Precast Protein Gels and transferred to PVDF membranes. After blocking with TBS-T milk containing 0.1% Tween 20 and fat-free powdered milk for 1 hour the membranes were probed against the proteins of interest using the appropriate primary and secondary antibodies (primary: mouse anti-human PKA-C antibody, Invitrogen #PA5-115762, 1:200 in TBS-T buffer overnight at 4°C; secondary: donkey anti-mouse IR Dye 680, Li-COR #926-68072, 1:5000 in TBS-T buffer, 2h at RT) to verify kinase construct expression (Figure S3).



Supplementary Figure S3. Western Blot of the HEK293 cell lysates analyzed for in-cell phosphoproteomics profiles and for KALIP rephosphorylation experiments. M = MW marker lane. PKA = $PKA_{ca.}$ expressing cells. Chimera = J-PKA_{ca} expressing cells. Vector = vector-only cells.