SUPPORTING INFORMATION

MATERIALS AND METHODS

Cell-based assays: HEK293T cells were purchased from the American Type Culture Collection and maintained as recommended. Pyrvinium pamoate was purchased from Sigma-Aldrich and dissolved in DMSO (Sigma-Aldrich). VU-WS211 was obtained from Vanderbilt University. Cells were treated with the indicated drugs in medium lacking serum. Plasmids expressing HA-tagged CK1α splice variants were synthesized or mutated through site-directed mutagenesis (Genewiz). Transfections of plasmids were performed using Lipofectamine 2000 transfection reagent (Thermo Fisher Scientific) as per the manufacturer instructions. After transfections or drug treatments, cells were collected and lysed in 2X Laemmli sample buffer (Bio-Rad). The primary antibodies used for immunoblotting include those that recognize phosphorylated β-catenin S45, β-catenin or the HA epitope, from Cell Signaling Technology, or α-tubulin from EMD Millipore. Secondary antibodies recognizing mouse or rabbit IgG were purchased from Jackson ImmunoResearch Laboratories. Immunoblots were quantified using Li-COR image studio software and then analyzed by Prism 6 software.

Protein kinase assays: A non-radioactive kinase assay (ADP-Glo™ assay, Promega) was utilized to evaluate how pyrvinium activates CK1α [1]. Unless otherwise indicated, protein kinase reactions were performed by incubating 40 nM GST-CK1α (Thermo Fisher Scientific), 50 μM dephosphorylated α-casein (Sigma), and 50 μM ATP (Promega), at 30°C. These reactions occurred in kinase buffer (50 mM Tris-HCl pH 7.5, 10 mM $MgCl_2$, 2 mM DTT, 0.1 mM EDTA, 0.01% Brij 35) in the presence of DMSO (vehicle), pyrvinium

(Sigma) or VU-WS211 (Vanderbilt University), and were stopped by incubating on ice, and assayed using the ADP-Glo™ assay. All kinetic responses were plotted and analyzed by Prism 6 software. Calculations: V_{max} (mol/min) = [ADP generated (%) * ATP (mol)] / Time (min). $k_{cat} = V_{max}/[CK1\alpha]$. The %ADP generated was calculated based on a standard ATP-to-ADP conversion curve: y [Kinase activity (RLU)] = 24935*x (%ADP).

Statistical analysis: For the quantification of immunoblots, error bars represent the standard error of the mean (S.E.M.) from three independent experiments. A representative protein kinase assay is shown, from at least three independent repeats. The error bars shown in these experiments represent the range of duplicate reactions. Student's t test and or a two-way Anova analysis were used to determine statistical significance. A p-value ≤ 0.05 was considered statistically significant, and indicated using an asterisk.

REFERENCE:

[1] Li, H., Totoritis, R. D., Lor, L. A., Schwartz, B., Caprioli, P., Jurewicz, A. J., and Zhang, G. (2009) Evaluation of an antibody-free ADP detection assay: ADP-Glo, *Assay Drug Dev Technol 7*, 598-605.