SUPPLEMENTARY INFORMATION

Impact of Genetic Variation on Human CaMKK2 Regulation by Ca^{2+}-Calmodulin and Multisite Phosphorylation

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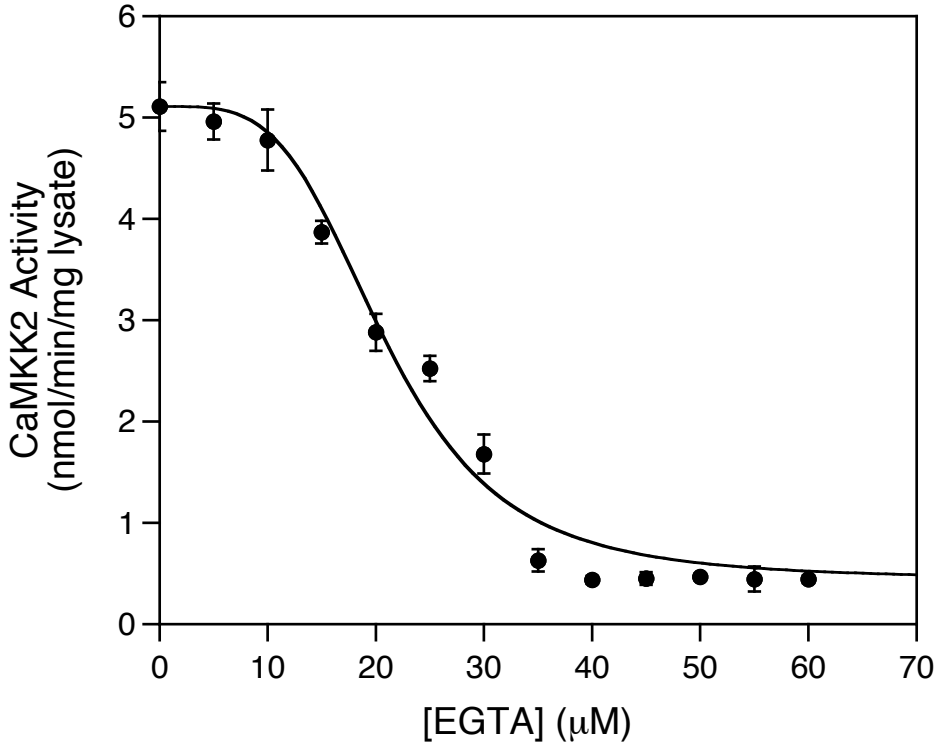
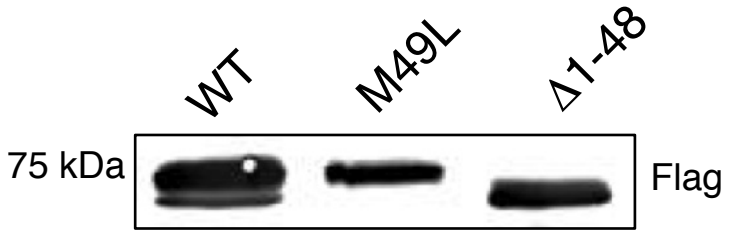
SUPPLEMENTARY FIGURES



Supplementary Figure 1: CaMKK2 activity measured in the presence of CaM and increasing concentrations of EGTA.

CaMKK2 activity was measured in the presence of 200 μM ATP, 1 μM CaM and increasing concentrations of EGTA, in order to determine the EGTA concentration required to chelate contaminating Ca^{2+} in the CaM preparation for measuring Ca^{2+}-independent autonomous activity. CaMKK2 activity was measured using the CaMKKtide peptide substrate assay and data were fitted to the equation: Activity = Minimum Activity + ((Maximal Activity - Minimum Activity)/1 + $([EGTA]/IC_{50})^{h}$). Data are presented as mean ± SEM; n=3.



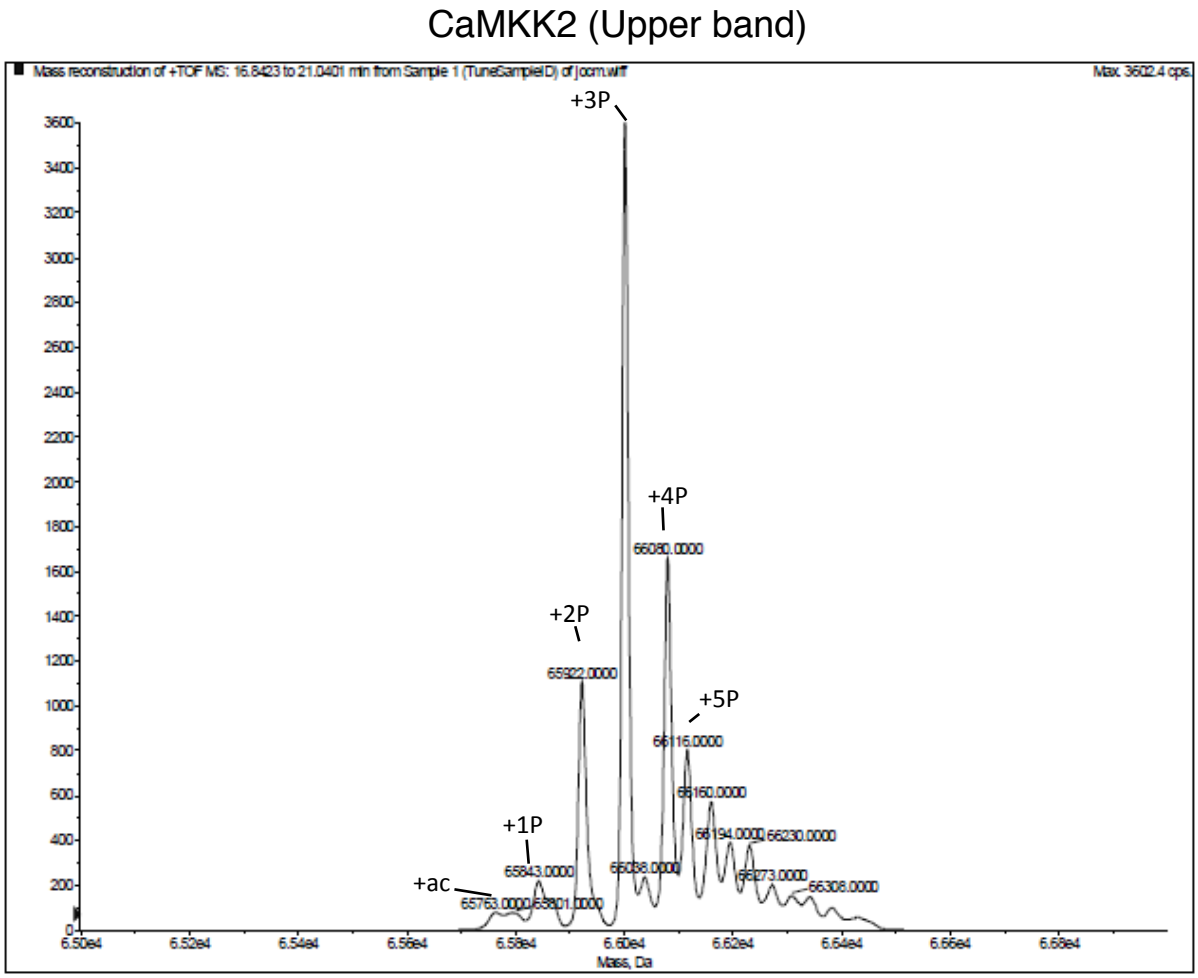
Supplementary Figure 2: CaMKK2 undergoes alternative translation initiation from Met49.

Immunoblot analysis of WT CaMKK2, as well as a M49L point mutant and Δ1-48 deletion mutant. The proteins were visualised using a rabbit anti-Flag antibody, and goat anti-rabbit IgG IRDye680 fluorescently labeled secondary antibody on a Infrared Imager.

Supplementary Figure 3: Phosphorylation profile of full length CaMKK2 and the shorter alternative Met49 initiated translation species.

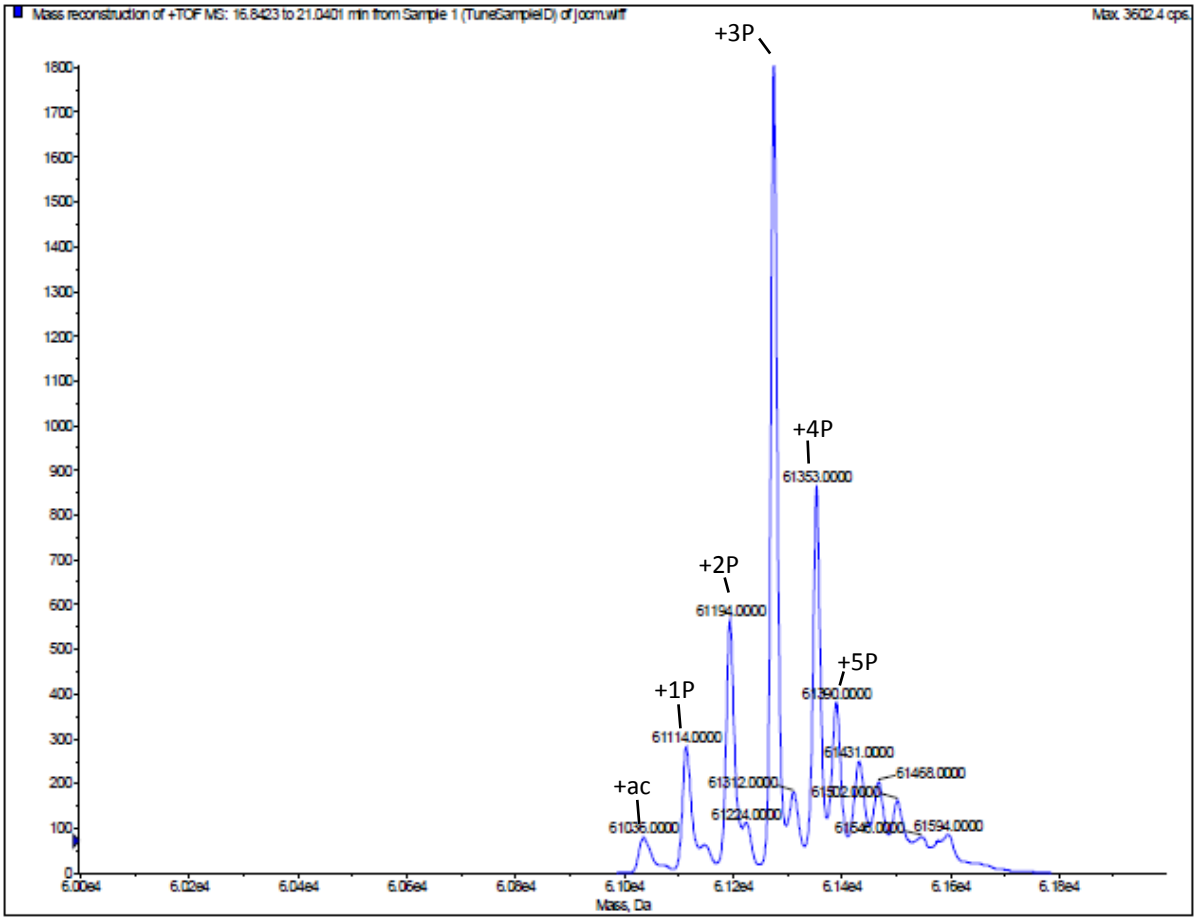
De-convoluted whole-protein TOF spectra of recombinant Flag-tagged human CaMKK2 expressed in COS7 cells under standard culture conditions (DMEM +10% FBS). (**a**) TOF spectra of full length CaMKK2 (upper band). (**b**) TOF spectra of the alternative Met49 initiated translation species (lower band). P denotes the number of phosphate modifications.

a



b

CaMKK2 (Lower band)



Supplementary Figure 4: The G87R mutation blocks Thr85 autophosphorylation.

ESI-tandem mass spectrometry analysis of Thr85 autophosphorylation in WT and G87R mutant CaMKK2. The representative extracted ion chromatograms in each panel show the proportion of Thr85-phosphorylated peptide (pThr85) to non-phosphorylated peptide (Thr85) from a tryptic digest of wild-type (upper panels) and G87R mutant CaMKK2 (lower panels), determined at 0 min (Control) and 40 min after incubation with 50 μM Ca^{2+}, 1 μM CaM and 200 μM MgATP (Autophosphorylation).