Importance of phosphorylation/dephosphorylation cycles on lipid-dependent modulation of membrane protein topology by posttranslational phosphorylation

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Supporting information

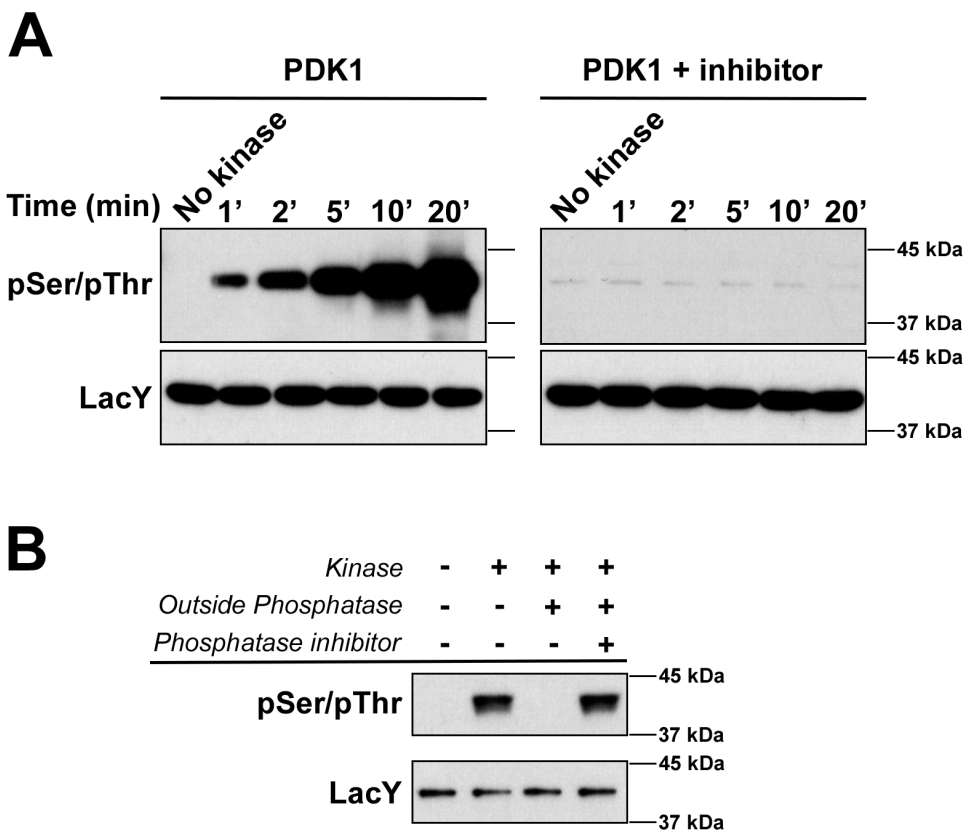

Figure S1. **Inhibition of Kinase (A) and Phosphatase (B) activities using pharmacological modulation**. LacY template -2/+2/+2 containing a single engineered PDK1 kinase sequence in EMD C6 was reconstituted in proteoliposomes made with *E. coli* native lipid composition. (A) For kinase inhibition assay, LacY was incubated at 4°C in the presence or absence (no kinase) of PDK1, with or without its inhibitor GSK 2334470. (B) For phosphatase inhibition assay, LacY was incubated at 4°C for 30 minutes, in the presence or absence of PDK1, phosphatase and phosphatase inhibitor, as indicated. In all cases, kinase and phosphatase were located outside of the proteoliposomes. The phosphorylation state of LacY at the various indicated times was visualized by western blotting using an anti-pSer/pThr antibody after quenching in Laemmli buffer.

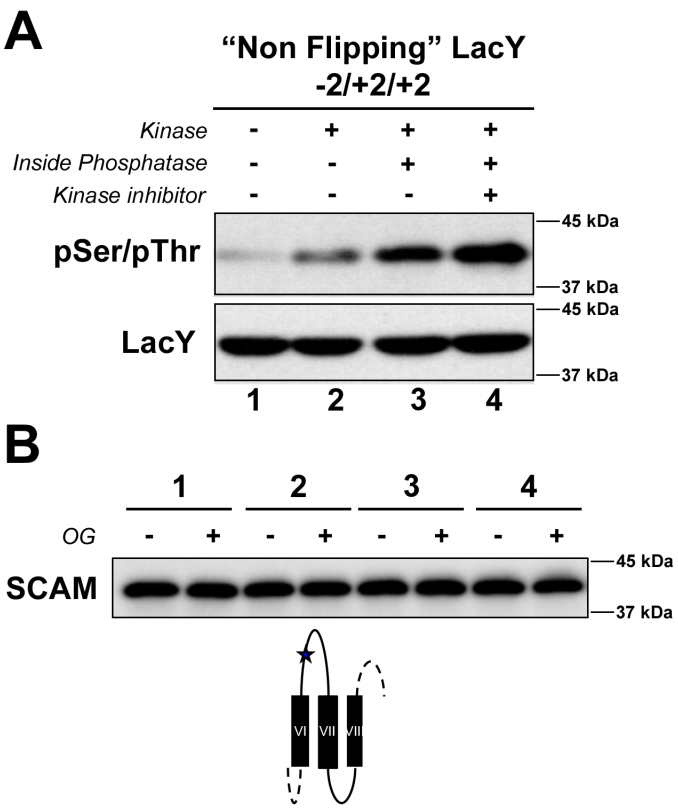

Figure S2. Determination of the phosphorylation state and topology of "non-flippping" LacY upon induction of phosphorylation/dephosphorylation cycles. (A) Determination of the phosphorylation state of LacY template -2/+2/+2 containing a single engineered PDK1 kinase sequence in EMD C6 in the presence or absence of external kinase and its inhibitor, with and without encapsulated phosphatase, as indicated. LacY was reconstituted in proteoliposomes made with total *E. coli* native lipid composition. The phosphorylation state of LacY was visualized by western blotting using an anti-pSer/pThr antibody after quenching in Laemmli buffer. (B) Determination of the orientation of EMD C6 of LacY with altered EMD net charge and containing a single cysteine replacement using $SCAM^{TMD}$ under the various conditions indicated in (A). Proteoliposomes were exposed to MBP before or after addition of OG. The deduced orientation of EMD C6 is also shown.