

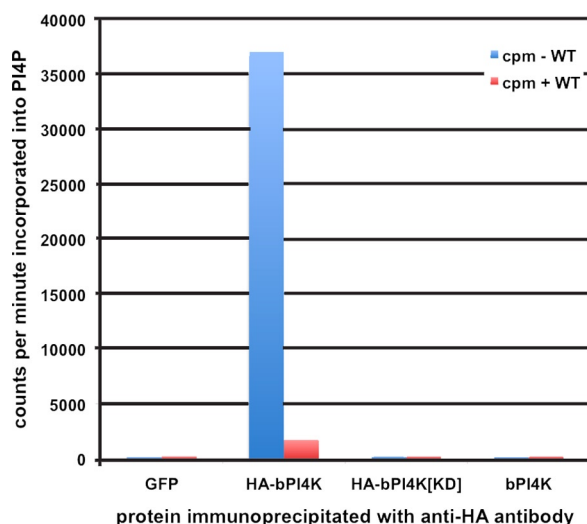
Polevoy et al., <http://www.jcb.org/cgi/content/full/jcb.200908107/DC1>

Figure S1. **Kinase-dead PI4K $\beta$  lacks detectable catalytic activity in vitro.** GFP, HA-tagged bovine PI4K $\beta$  (HA-bPI4K), HA-tagged kinase-dead bovine PI4K $\beta$  (HA-bPI4K[KD]), or untagged bovine PI4K $\beta$  (bPI4K) were expressed in COS-7 cells, immunoprecipitated with anti-HA antibody and subjected to in vitro kinase assays in the presence or absence of the PI4K $\beta$  inhibitor wortmannin (WT; see Materials and methods). Incorporation of  $^{32}\text{P}$  into PI4P is indicated as counts per minute. HA-bPI4K showed PI4K activity that was inhibited by WT, whereas HA-bPI4K[KD] had no activity in this assay. Equivalent amounts of HA-bPI4K and HA-bPI4K[KD] were immunoprecipitated, as determined by immunoblotting with anti-HA antibody (not depicted). The data shown represent an average of the counts obtained from two identical samples in a single experiment. Similar results were obtained in a second independent experiment. Note that because the mammalian proteins are 98% identical over their entire length (801 amino acids) (Balla et al., 1997; Meyers and Cantley, 1997), wild-type and kinase-dead (D656A) human and bovine PI4K $\beta$ s would be expected to behave in an identical manner.