

Su et. al. Supplementary Figure 3

A: 0.4 or 2 μg of synthetic AE1 C-terminal peptides with and without phosphorylation of Y⁹⁰⁴ (AE1C-pY⁹⁰⁴ and AE1C-Y⁹⁰⁴) were dotted onto nitrocellulose membrane and detected with anti-AE1 C-terminal antibody Bric155 or anti-phosphotyrosine antibody 4G10 (gift of Dr CE Rudd, Cambridge). This analysis demonstrates that Bric155 (upper panel) recognises non-phospho peptide only, and 4G10 confirms that phospho-peptide is present (lower panel). B: Bric170, an N-terminal AE1 antibody, is able to immunoprecipitate both non-phosphorylated and phosphorylated kAE1 from untreated and pervanadate-treated MDCK cells stably expressing kAE1, respectively, detected by α-rbAE1Ct and αAE1phosY⁹⁰⁴ antibodies (left panel) as previously reported (30). In contrast, Bric155 immunoprecipitates the non-phospho AE1 (right panel). These blots also confirm that kAE1 is not significantly phosphorylated prior to the addition of pervanadate.