Supplementary Figures

S Figure 1 Identification of Ser^{829} and validation of a new $pSer^{829}$ antibody (pPC2)

A. $pSer^{829}$ can be detected using a commercial PKA phospho-substrate antibody which recognises the sequence (RRXS*/T*, where *=phosphorylation). HEK cells were transfected with HA-tagged PC2, PC2 was immunoprecipitated with anti-HA antibody and detected with PKA substrate antibody (top) or HA antibody (bottom). Mutation of Ser^{829} to Ala abolished recognition by the antibody.

B. A custom rabbit polyclonal phosphospecific antibody to Ser^{829} (pPC2) was generated to the phosphopeptide (SGHSSRRRGpSISSG). (Top panel) Recombinant Thio-CT2 protein (amino acids 700-968) was phosphorylated *in vitro* using recombinant PKA. The pPC2 antibody recognised CT2 only after incubation with PKA. Equal loading was confirmed using an antibody to the Thio tag. (Bottom panel) HEK cells transfected with HA-PKD2 were lysed and treated with λ-phosphatase (+) or buffer (-) for 90 min. The pPC2 antibody signal was lost after dephosphorylation demonstrating its specificity for $pSer^{829}$.

C. The pPC2 antibody recognises wild type PC2 but not a S829A mutant by immunofluorescence. HEK cells were transiently transfected with HA-PC2 or HA- PC2 S829A and detected with primary antibodies to either HA (green) or pPC2 (red). The pPC2 antibody detects PC2 but not the S829A mutant. Magnification (x60).

D. Ser^{829} phosphorylation is increased in full-length and truncated PC2 by mutations in a C-terminal coiled coil domain. HEK cells were transfected with wild-type or mutant PC2 constructs, where mutations in the C-terminal coiled coil domain (4M) abolish dimerisation and hence binding to endogenous PC1. A full-length PC2 construct (PC2), a construct lacking the N-terminus (PC2 224-968) or one just comprised of the C-terminal domain (PC2 681-968, CT2) were expressed in HEK cells. The presence of endogenous Ser^{829} phosphorylation in HEK cells is shown by a single band (*) around ~110kDa in all lanes while the heterologous protein varied in molecular weights as predicted. All three constructs showed increased detection by pPC2 in the presence of 4M. Equivalent loading was demonstrated using a C terminal PC2 antibody (1A11).

S Figure 2 The subcellular localisation and expression of pSer^{829}varies between dividing and non-dividing cells

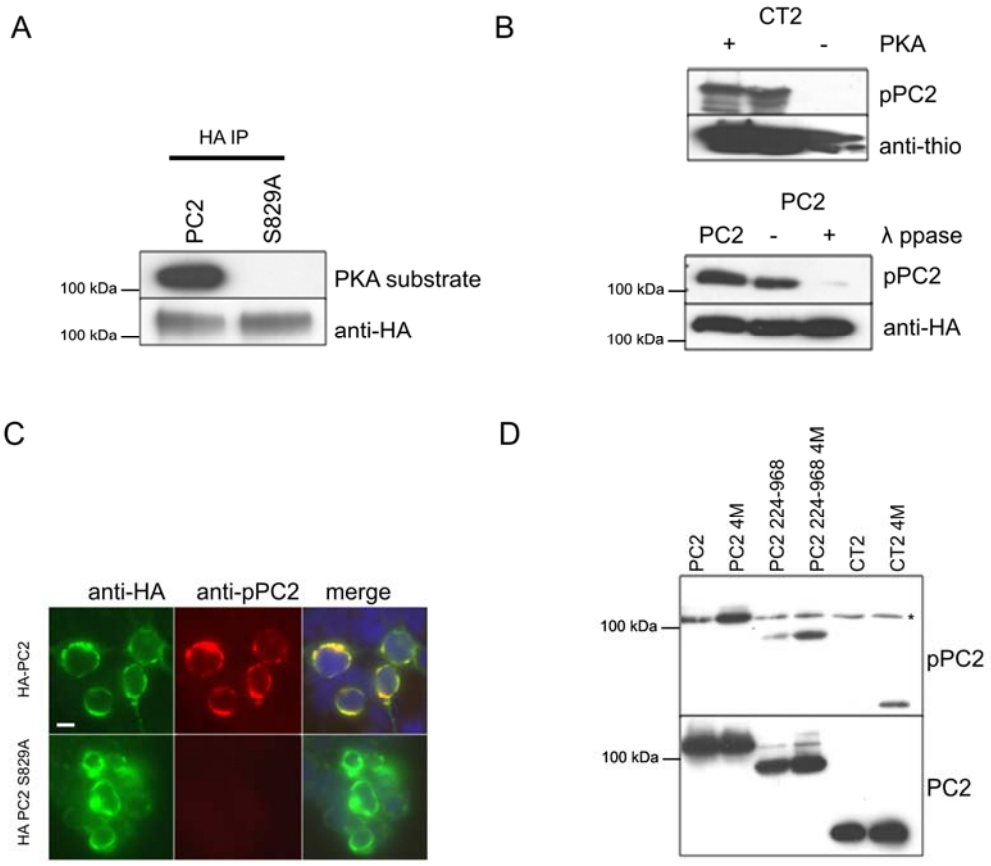
A. Endogenous PC2 localised to centrosomes in both dividing and non-dividing cells as well as to primary cilia. PC2 was detected by IF using a previously characterised antibody, p30 and overlapped with γ-tubulin and acetylated tubulin. Magnification (x60)

B. pSer^{829} (pPC2) was specifically localised to the centrosomes of mitotic MDCK II cells. It was not detected in the centrosomes of interphase cells or in primary cilia. Magnification (x60).

S Figure 3 Ser^{829} does not regulate PC2 homo or heterodimerisation

A. Mutation of Ser^{829} does not affect PC2 homodimerization. Under non-reducing conditions, HA-PC2 forms higher molecular weight oligomers and this is not altered in S829A or S829D.

B. Mutation of Ser^{829} does not affect PC1-PC2 heterodimerisation. HEK cells were co-transfected with PC1-FLAG and Pk-PC2 or Pk-PC2 S829A or Pk-PC2S829D. Lysates (0.5mg protein) were immunoprecipitated using epitope-tagged (FLAG or Pk) antibodies and detected with 7e12 (PC1) or Pk-tag (PC2) antibodies.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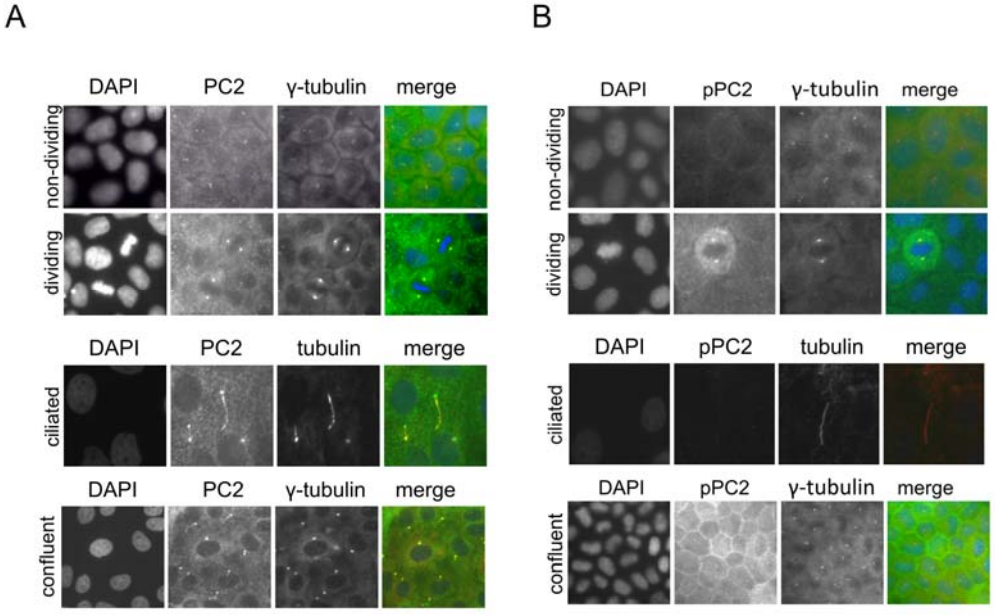
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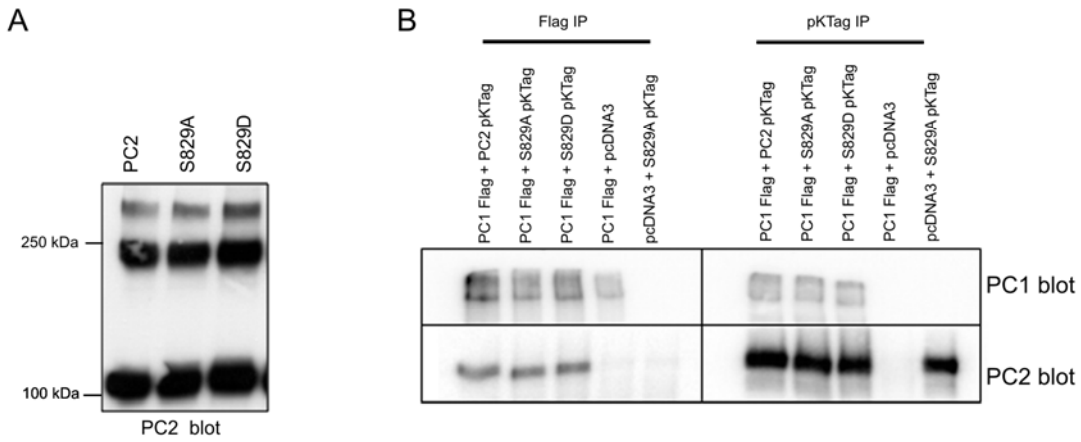
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S829D
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PC1 Flag + S829D pKTag
PC1 Flag + pcDNA3
pcDNA3 + S829A pKTag
PC1 Flag + PC2 pKTag
PC1 Flag + S829A pKTag
PC1 Flag + S829D pKTag
PC1 Flag + pcDNA3
pcDNA3 + S829A pKTag
PC1 blot
PC2 blot