Supplementary Material

Alternative splicing of pericentrin contributes to cell cycle control in cardiomyocytes

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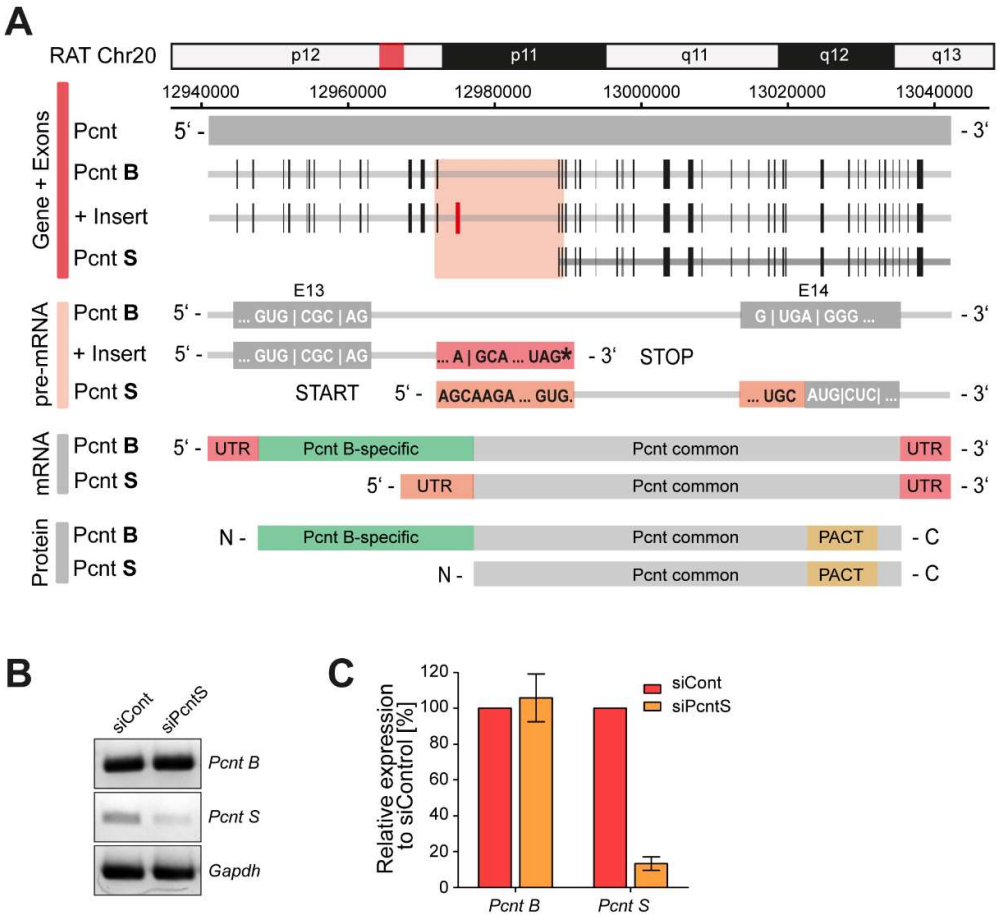

Figure S1. Overview of alternative splicing of PCNT and challenges for specific depletion of Pcnt S. (**A**) Alternative splicing of rat *Pcnt* results in the insertion of an intronic region in the *Pcnt* gene between exon 13 and 14, which becomes the first exon encoding the isoform Pcnt S (Gene + Exon). This exon contains a stop codon and consequently initiation of *Pcnt S* translation occurs in a downstream start codon resulting in the truncation of the first 232 amino acids at the N-terminal region of the longer Pcnt B isoform. The new 5′-UTR is constituted of the sequence between stop codon and new start codon, in part consisting of a coding sequence for *Pcnt B* (pre-mRNA). The lack of specific regions in the expression of *Pcnt S* presents a major challenge for specific Pcnt S depletion and precludes Pcnt S-specific antibody staining. To specifically deplete Pcnt S we used a siRNA against the specific part of the 5′-UTR of the *Pcnt S* mRNA (mRNA). (**B**) RT-PCR of *PcntB*, *PcntS* and *Gapdh* utilizing RNA from P3 cardiomyocytes transfected with the corresponding siRNA as indicated. (**C**) Quantification of B, as *Pcnt* band intensity normalized to *Gapdh* band intensity.

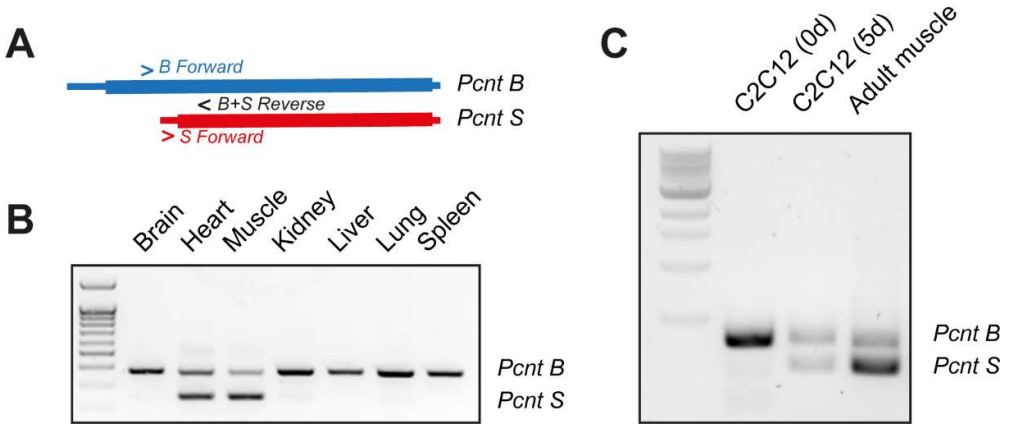

Figure S2. RT-PCR using three primers to detect *Pcnt B* and *Pcnt S*. (**A**) Scheme of the primers used. Forward-primer specific for *Pcnt B* is shown in blue, forward-primer specific for *Pcnt S* in red, and the reverse primer detecting both isoforms is shown in black. (**B**,**C**) Three primer RT-PCR from RNA isolated from the indicated adult mouse tissues (B) or from C2C12 at myoblast stage (0d), or after 5 days of differentiation (5d) in comparison to adult muscle tissue (C).

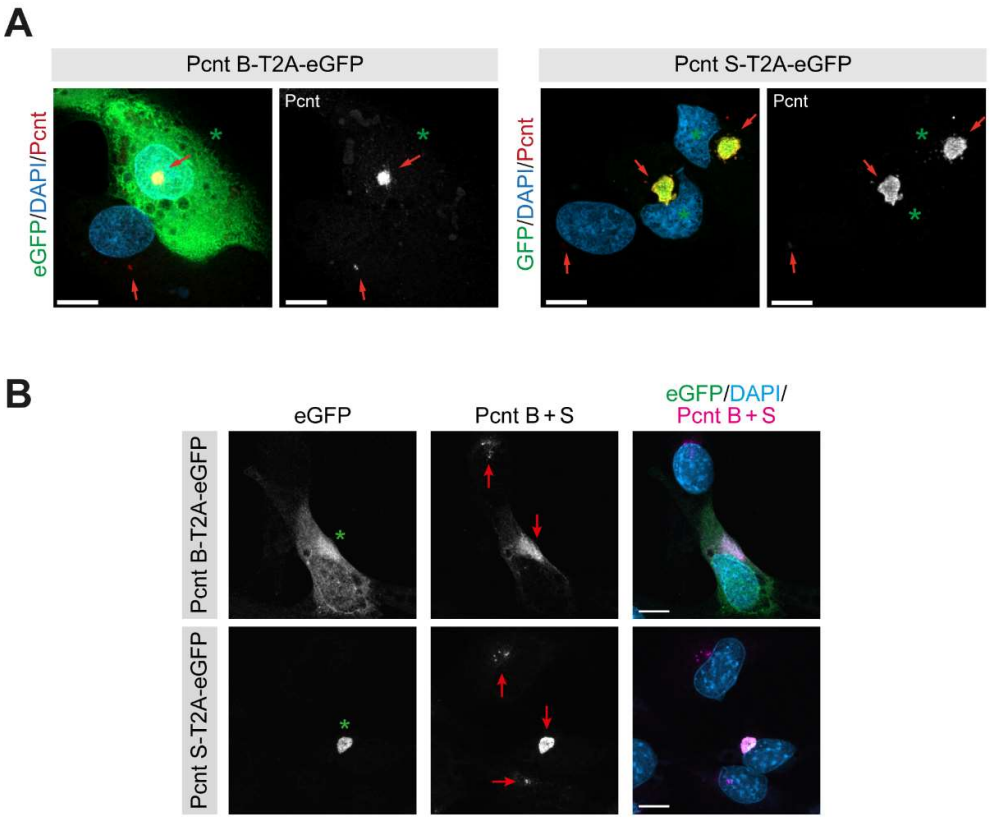

Figure S3. Verification of Pcnt expression after ectopic expression in ARPE-19 adult retinal pigment epithelial cells and C2C12 myoblasts. Representative examples of ARPE-19 cells (A) or C2C12 myoblasts (B) transfected with bi-cistronic plasmids encoding Pcnt B-T2A-eGFP or Pcnt S-T2A-eGFP (GFP, green) and immunostained with anti-Pcnt antibody (red). Nuclei were visualized with DAPI (DNA, blue). Green asterisk: GFP+ cell; Red arrow: Pcnt staining. Scale bars: 10 μm.

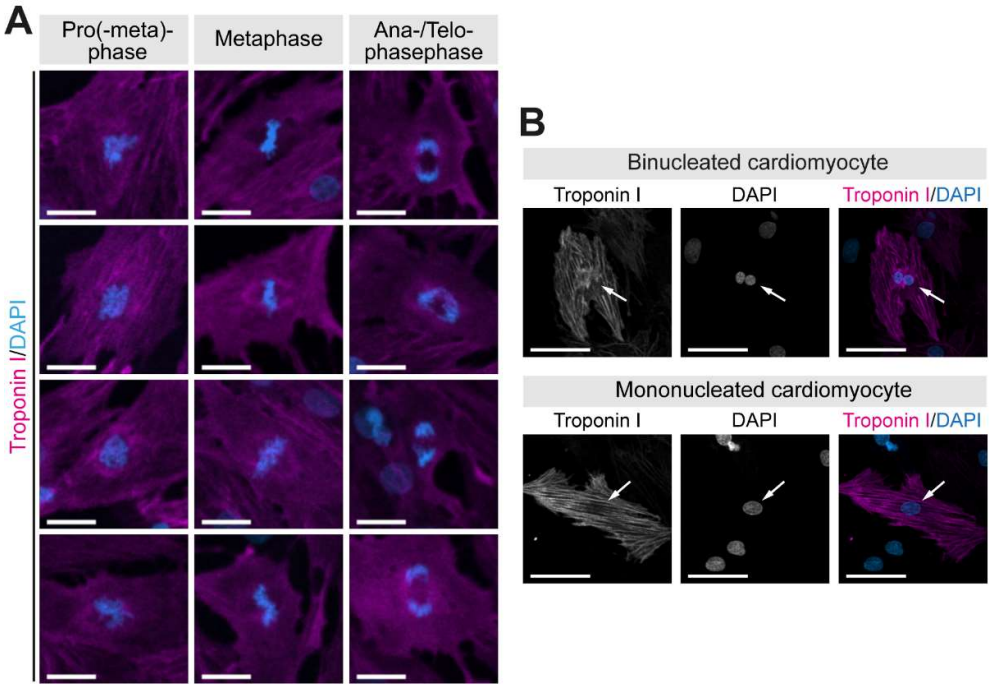

Figure S4. Representative examples of different cell cycle stages of cardiomyocytes. (**A**) Representative examples of cardiomyocytes (troponin I) in pro(-meta)phase, metaphase and ana-/telophase. Chromosomes were visualized with DAPI (DNA). Scale bars: 20 μm. (**B**) Representative examples of mono- and binucleated cardiomyocytes (troponin I). Nuclei were visualized with DAPI (DNA). White arrows: cardiomyocyte nuclei. Scale bars: 50 μm.