Supplementary Materials: Pregnenolone 16-Alpha Carbonitrile, an Agonist of Rodent Pregnane X Receptor, Regulates Testosterone Biosynthesis in Rodent Leydig Cells

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RT-PCR analysis: Reverse transcription was performed with the iScript cDNA Synthesis Kit (Bio-Rad). Polymerase chain reaction was performed for 40 cycles by using the PerfeCTa SYBR Green FastMix (Quanta BioSciences) and CFX96 Touch Real-Time PCR Detection System (Bio-Rad). Transcripts of the rPXR and mPXR were amplified using the gene-specific primers **(Table S1)**. PCR products were run on 2% agarose gels and detected by ethidium bromide. Purified PCR products were sequenced to verify the identity of the RT-PCR products.

Table S1. Forward (F) and reverse (R) primers used for RT-PCR of rPXR and mPXR.

Gene/Primer sequence	Gene Bank Accession no
rPXR F: 5′-TGATCATGTCTGATGCCGCTG-3′ R: 5′-GAGGTTGGTAGTTCCAGATGCTG-3′	NM_052980
mPXR F: 5′-GGGATAGGGTTACAGCACGA-3′ R: 5′-CCACCGCCATAGTTCTCATC-3′	NM_010936

Cell viability assays: Cell viability assays were performed in MA-10 cells. The cells were treated with DMSO or 10 μM PCN for 48 h before measuring cell viability using CellTiter-Glo Luminescent Cell Viability Assays (Promega) (Abbott et al., 2019; Flannery et al., 2020; Pondugula et al., 2015a; Pondugula et al., 2015b; Pondugula et al., 2015c).

Cell morphology analysis: Cell morphology of MA-10 cells was visualized after treating the cells DMSO or 10 μM PCN for 72 h using a Revolve 4 microscope (ECHO).

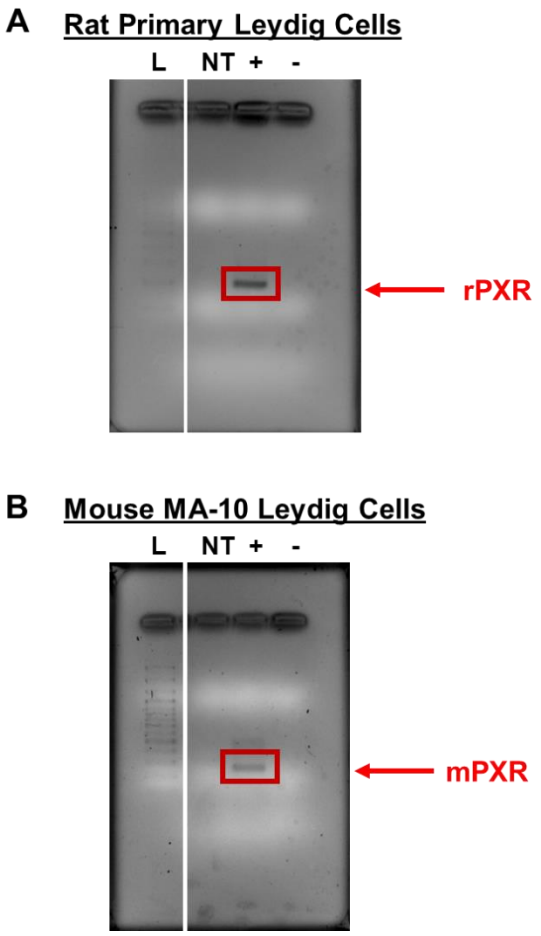


Figure S1. PXR mRNA expression in rodent LCs. Determination of the transcript for rPXR in rat primary LCs (A) and mPXR in mouse MA-10 cells (B). RT-PCR products were run on a 2% gel. Bands were observed at the expected size in reverse transcription (+), but no signal was observed in negative reverse transcription (-) control and no-template (NT) control. Identity of the band was verified by sequence analysis. L, 100 bp ladder.

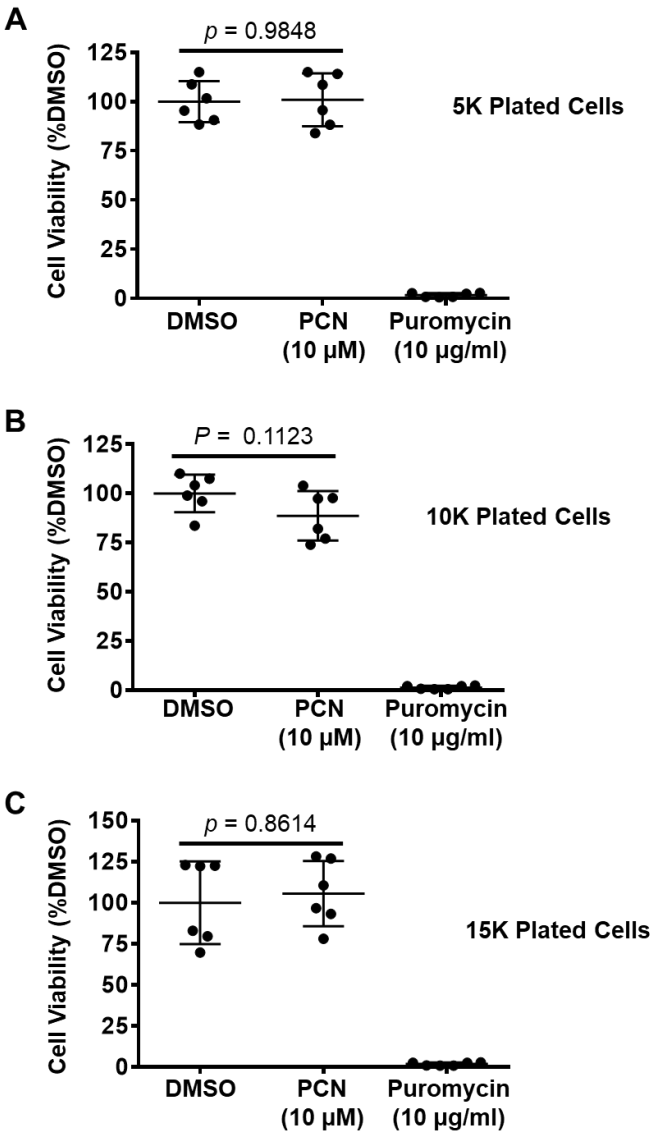


Figure S2. Effect of PCN on viability of MA-10 cells. MA 10 cells were seeded at 5K (A), 10K (B), and 15K (C) densities and incubated overnight. The cells were then treated with DMSO or 10 µM PCN for 48 h before measuring viability by using CellTiter-Glo reagent. Puromycin was used as a positive control for cytotoxicity. Viability of DMSO-treated cells was expressed as 100%. Results are shown as mean ± SD. * $p < 0.05$; compared with DMSO by ANOVA and a Tukey's multiple comparisons test.

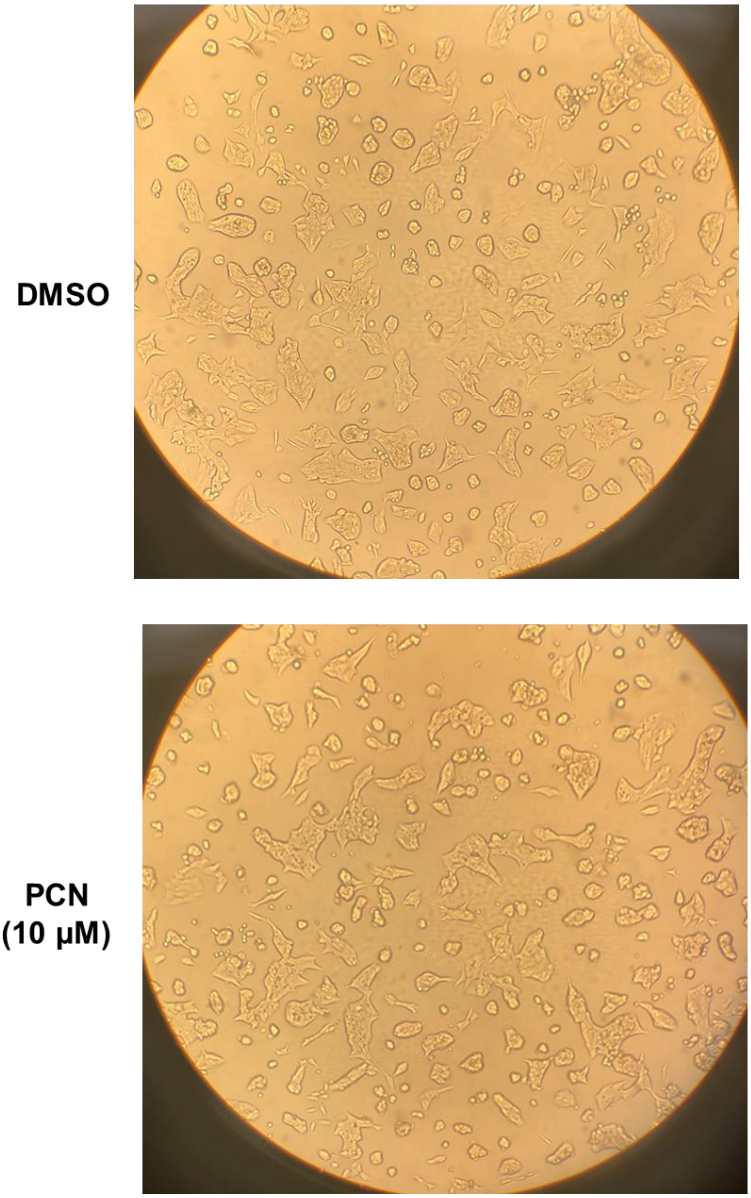


Figure S3. Effect of PCN on morphology of MA-10 cells. MA 10 cells were seeded and incubated overnight. The cells were then treated with DMSO or 10 μM PCN for 72 h before taking images using a Revolve 4 microscope (ECHO). The representative images are shown.

References

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