**Supplementary Information**

**Supplementary Figure and Table Legends**

**Supplementary Figure 1. Pkm isoform expression in normal murine prostate and prostate tumors.**

**A.** Representative H&E and immunohistochemistry (IHC) assessment of Pkm1 and Pkm2 (brown) expression in prostate and adjacent tissue from 1-month-old wild-type mice. 1, seminal vesicle; 2, anterior prostate; 3, urethra; 4, ventral prostate; 5, dorsal prostate; 6, bladder. Scale bar for low power images = 2 mm, all others = 200m.

**B.** Representative H&E and IHC assessment of Pkm1 and Pkm2 (brown) expression in control tissues known to express Pkm2 (Colon), Pkm1 (Muscle), or neither Pkm1 nor Pkm2 (Liver)(32). IHC assessment of pancytokeratin (PanCK) (brown) and vimentin (pink) expression in colon, and synaptophysin (brown) expression in pancreas, is also shown as controls for those antibodies. Scale bar = 200m.

**C.** Representative H&E and IHC assessment of Pkm1, Pkm2, pancytokeratin (PanCK), vimentin, and synaptophysin expression in prostate tissue harvested from *Ptenpc-/-* mice of the indicated age. Scale bar = 300m.

**D.** Representative IHC staining of Pkm1or Pkm2 (brown), and AR (pink), in prostate tumors harvested from 6-month-old *Ptenpc-/-* mice. Scale bar = 200m.

**Supplementary Figure 2. Pkm1 conditional allele characterization.**

**A.** Southern blot screening of embryonic stem cell clones for homologous recombination of the *Pkm1* targeting construct using the 5’ probe indicated in Fig. 2A. Insertion of a novel KpnI site results in a new ~5.0 kb fragment from the targeted locus following digestion of genomic DNA. Two clones with successful integration are marked with an arrowhead.

**B.** PCR genotyping of genomic DNA from *Pkm1*+/+ (+/+), *Pkm1*fl/+ (f/+), and *Pkm1fl/fl* (f/f) mice as indicated. Genotyping primers anneal at sites indicated in Fig. 2A to produce amplicons of 509 bp from the Pkm wild-type allele (*Pkm+*) and 577 bp from the conditional Pkm allele with flanking LoxP sites (*Pkm1fl*).

**Supplementary Figure 3. Histopathology and IHC assessment of *Pkm1;Ptenpc-/-*prostate tissue.**

**A.** Swimmers plot summarizing results from histopathological analysis of prostate tissue harvested from *Ptenpc-/-, Pkm1*;*Ptenpc-/-,* and *Pkm2*;*Ptenpc-/-* mice at the indicated ages. Open bars represent mice with no evidence of neoplasia, hatched bars represent mice with prostate intraepithelial neoplasia (PIN), and filled bars represent mice with invasive cancer.

**B.** Representative H&E and IHC assessment of Pkm1, Pkm2, pancytokeratin (PanCK), vimentin, and synaptophysin expression in prostate tissue harvested from *Pkm1;Ptenpc-/-*mice of the indicated age. Scale bar = 300m.

**C.** Representative IHC staining of Pkm1or Pkm2 (brown), and AR (pink), in prostate tumors harvested from 6-month-old *Pkm1;Ptenpc-/-*mice. Scale bar = 200m.

**Supplementary Figure 4. Histopathology and IHC assessment of *Pkm2;Ptenpc-/-*prostate tissue.**

**A.** Representative H&E and IHC assessment of Pkm1, Pkm2, pancytokeratin (PanCK), vimentin, and synaptophysin expression in prostate tissue harvested from *Pkm2;Ptenpc-/-*mice of the indicated age. Scale bar = 300m.

**B.** Representative IHC staining of Pkm1or Pkm2 (brown), and AR (pink), in prostate tumors harvested from 6-month-old *Pkm2;Ptenpc-/-* mice. Scale bar = 500m.

**Supplementary Figure 5. Assessment of DNA replication stress in normal prostate and prostate tumors.**

**A.** Representative IHC staining for phospho-Chk1 (brown) in anterior prostate tissue harvested from6-month-old wild-type (WT) and *Ptenpc-/-* mice. No primary antibody control is shown in inset. Scale bar = 200m.

**B.** Representative IHC staining for phospho-Chk1 in prostate tissue/tumors harvested from *Ptenpc-/-, Pkm1*;*Ptenpc-/-*, and *Pkm2*;*Ptenpc-/-* mice of the indicated age. Scale bar = 300m

**Supplementary Figure 6. Pkm2** **activator treatment reduces *Ptenpc-/-* mouse prostate tumor growth.**

**A.** Waterfall plot showing the maximal change in prostate tumor volume as assessed by MRI for

*Ptenpc-/-* mice treated twice a day for one month with vehicle or 50 mg/kg TEPP-46 as indicated. These data are from the same study presented in Fig. 6A, but display the max change in tumor volume present in each prostate lobe.

**B.** Representative Ki-67 (brown) IHC staining of anterior prostate tumors from *Ptenpc-/-* mice treated with vehicle or TEPP-46 twice a day for one month. Scale bar = 100μm.

**Supplementary Table 1. Impact of pyruvate kinase isoform expression on metabolite levels in prostate tissue from *Ptenpc-/-* mice.**

Relative levels (mean peak area) for all metabolites measured by LC/MS that were significantly different (p<0.05 by Student’s t-test) in a comparison of prostate tissue harvested from 6-month-old

*Pkm2*;*Ptenpc-/-* and *Ptenpc-/-* mice, or prostate tissue harvested from 6-month-old *Pkm1*;*Ptenpc-/-* and *Ptenpc-/-* mice as indicated. (*Ptenpc-/-*, n=10; *Pkm2*;*Ptenpc-/-*,n=6; and *Pkm1*;*Ptenpc-/-*, n=6). The p value is shown for each significant difference in metabolite levels.