SUPPLEMENTARY MATERIALS AND METHODS

Cell Lines and Reagents

Smo-responsive mouse preosteoblast cells, MC3T3-E1 (ATCC) were cultured in Alpha Minimum Essential Medium (Hyclone), supplemented with 10%FBS and streptomycin/penicillin. PCa cells, 22Rv1 (Vancouver Prostate Centre), C42 (Vancouver Prostate Centre), PC3 (Vancouver Prostate Centre) and R1-J1 (from Dr. Scott Dehm, University of Minnesota) were maintained in phenol red-free RPMI 1640, supplemented with 10%FBS and streptomycin/penicillin.; VCaP cells (ATCC) were cultured in Dulbecco's Modified Eagle's Medium (Hyclone), supplemented with 10% FBS and streptomycin/penicillin. Smo agonist, SAG, was purchased from Calbiochem (Etobicoke, ON, Canada).

Plasmid vectors

The vector expressing myc-tagged Gli3-FL and Gli2-C ($Gli2aa_{628-1586}$) vectors were previously described[29]. To generate mutGli2-C, Quick Change II XL Site-Directed Mutagenesis Kit (Stratagene) and the same mutagenesis primers (for $mutGli2aa_{628-897}$) were utilized to introduce substitutions of Arginine (RR) for Aspartate (DD) residues in Gli2-C (mut Gli2-C).

Quantitative real-time PCR (qPCR)

Various prostate cancer cells were characterized for the expression of Gli homologues, Gli1, Gli2 and Gli3. RNA extraction, cDNA synthesis and qPCR were performed following standard protocols as described previously. Additional primers used are as follows, human(h) Gli2, forward, 5'- CCC CTA CCG ATT GAC ATG CG-3', reverse, 5'- ACA GAA

TGA GGC TCG TAA TGG T-3'; hGli3, forward, 5'-CGA ACA GAT GT GAG CGA GAA-3', reverse, 5'-GGC TGC ATA GTG ATT GCG T-3'.

Supplementary Figure legends

Figure S1. Transcriptionally active AR enhances Gli transcriptional activity in *Smo*-responsive mouse preosteoblast MC3T3-E1 cells. MC3T3-E1 cells were co-transfected with Gli-luc and pCMV-eGFP, along with control, empty vector (-), AR-FL (-FL) or AR-V7 (-V7) vectors. Transfected cells were cultured in PBS for 24h followed by CS-FBS in the presence or absence of 1nM R1881, or 200 nM, or both, for additional 48h. Cell extracts were subjected to luciferase reporter assay and values were normalized to GFP intensity. * $P<0.05$, *** $P<0.001$.

Figure S2. Gli3 is the predominant Gli homologue in PCa cells. RNAs were extracted from a variety of PCa cells maintained in their respective culture media. Endogenous expressions of Gli homologues (Gli1, Gli2, Gli3) were examined through qPCR and relative fold changes were shown by normalizing to endogenous Gli1 mRNA levels.

Figure S3. Mutations in phosphorylation sites of Gli2 C-terminus blocked its co-activation on AR transcription. 293FT cells were co-transfected with PGC-luc, pCMV-eGFP and AR-FL, along with control (-), Gli2-C or mutGli2-C. Cells were cultured in CS-FBS in the presence or absence R1881, as indicated. Protein extracts were assayed for luciferase activities and values were normalized to GFP intensity. *** $P<0.001$.

Figure S4. Truncated AR (AR-V7) also stabilizes Gli3-FL in AR+ and AR- PCa cells. (**A**) AR-FL+ LNCaP cells were transiently transfected with control (empty) vector or AR-

V7 vector, and were cultured in CS-FSB for 72h. (Left) Protein extracts were resolved in SDS-PAGE and a western blot was probed for Gli3, AR and GAPDH as indicated. Results show a loss of Gli3-R and an increase in Gli3-FL with expression of AR-V7 (Right) Endogenous Gli target (Gli1 and Ptch1) mRNAs were evaluated by qPCR and normalized to control vector transfected cells. Results show that both were increased with expression of AR-V7. (**B**) AR- PC3 cells were transiently transfected with control vector or with AR-FL (in the presence of 1nM R1881) or AR-V7 vector (in the absence of R1881). (Left) Western blots show an increase in Gli-FL with overexpression of AR-FL (+androgen) or AR-V7 (-androgen) that is confirmed by denisitometric evaluation of the Gli3-FL band (Right).

Figure S5. Androgen-independent LNCaP-AI cells express higher ratio of Gli-FL/Gli3R, compared to androgen-dependent LNCaP cells. Protein lysates were extracted from (**A**) parental LNCaP and LNCaP-AI cells, or (**B**) LNCaP and LNCaP-AI cells transiently transfected with control vector or myc-Gli3 vector, and were resolved in SDS-PAGE and the western blot was probed for Gli3, AR, GAPDH or Vinculin as indicated. ns, non-specific.