Figure S1. Suppression of Gli2 expression by DOX in LNCaP Gli2shR cells in a dose-dependent manner. LNCaP cells transfected with the Gli2shR plasmid were treated with increasing concentrations of DOX (0.05, 0.1, 0.5 and 1 μM) for 48 h, following which Gli2 expression was analyzed by western blotting. Gli2, Glioma-associated oncogene family zinc finger 2; Gli2shR, vector encoding Gli2 short hairpin RNA. DOX, doxycycline; Ctl, control.

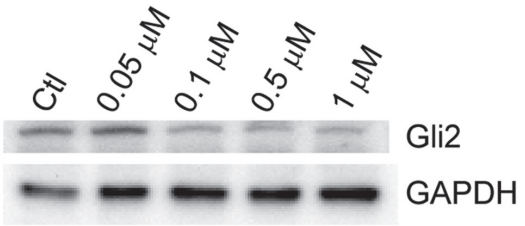

Figure S2. Knockdown of Gli2 expression reduces prostate cancer cell viability in vitro. Gli2 shR1 and matched control shRNA were transfected into LNCaP, 22RV1 and VCaP cells via lentiviral infection. (A) Confirmation of stable Gli2 knockdown in prostate cancer cell lines by reverse transcription-quantitative PCR, using GAPDH as the reference gene. (B) Gli2 knockdown was confirmed on protein level by western blotting. (C) Gli2 knockdown reduced prostate cancer cell viability as measured using MTT assay. Data presented as the mean ± SEM from three experimental replicates. **P<0.01; ***P<0.001 and ****P<0.0001, last three time points. Gli2, Glioma-associated oncogene family zinc finger 2; Ctl, control vector not encoding the shRNA; Gli2shR, vector encoding Gli2 short hairpin RNA; OD, optical density.

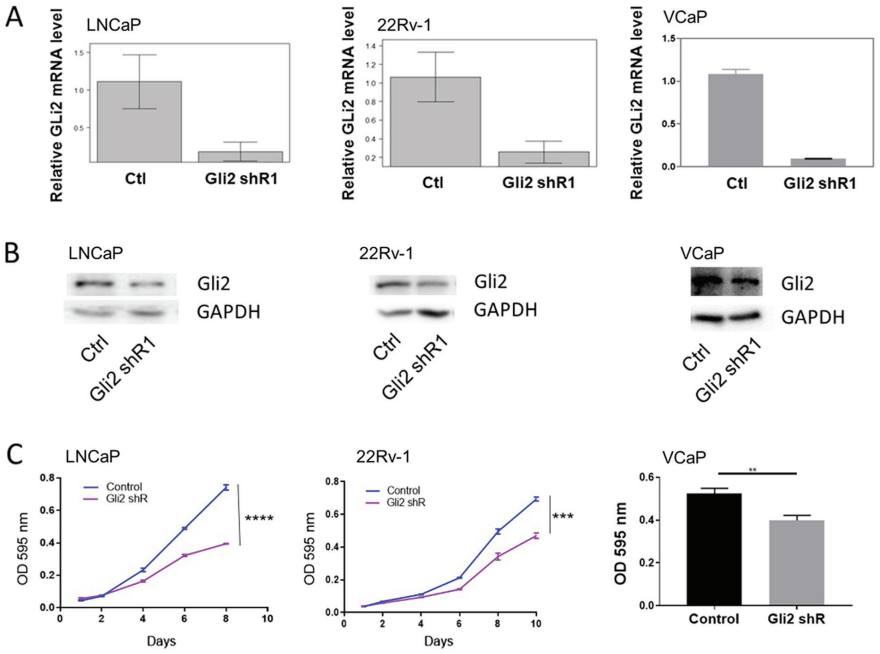

Figure S3. Flow cytometry histograms of the cell cycle data shown in Fig. 1D. The three histograms in each treatment group were from three experimental repeats. Green, G_0/G_1 phase; yellow, S phase; cyan, G_2/M phase. Gli2, Glioma-associated oncogene family zinc finger 2; Ctl, control vector not encoding the shRNA; Gli2shR, vector encoding Gli2 short hairpin RNA; DOX, doxycycline.

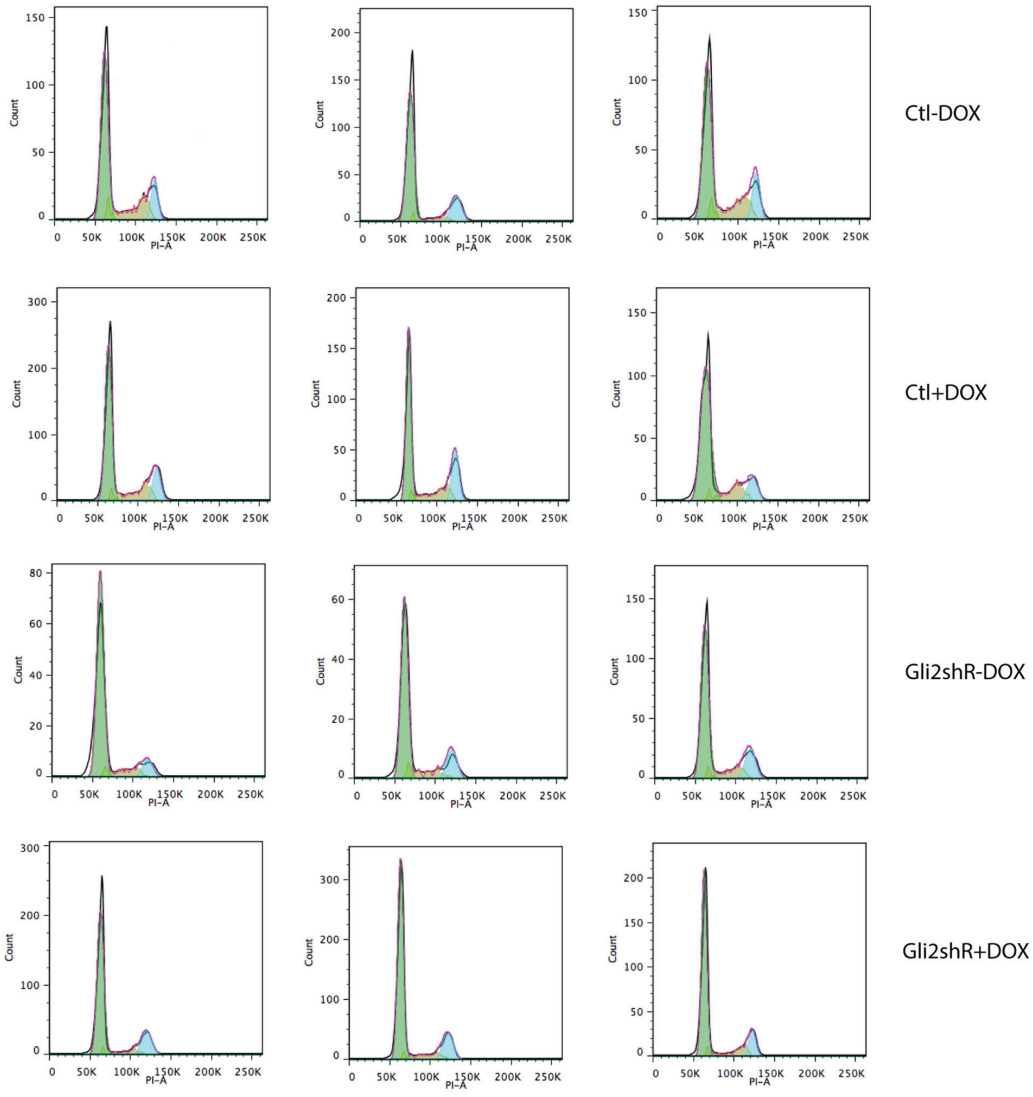

Figure S4. Reversal of LNCaP growth inhibition following Gli2 knockdown by ectopic Gli2ΔN expression. (A) pSDM101-Gli2ΔN or control vector pSDM101 was transfected into LNCaP cells following which Gli2 protein expression was measured by western blotting, whereas (B) Gli mRNA expression was measured using reverse transcription-quantitative PCR. (C) LNCaP Gli2shR/Gli2ΔN and LNCaP Gli2shR/CV cells were treated with 1 μM DOX or DMSO. Relative cell viability was quantified using MTT assay on days 2, 4, 6 and 8. *P<0.05 for the indicated comparisons on all four time points. D, day; Gli2, Glioma-associated oncogene family zinc finger 2; ΔNGli2, Gli2 lacking the lacking the N-terminal repressor domain; CV, control vector pSDM101; Gli2shR, vector encoding Gli2 short hairpin RNA; DOX, doxycycline; OD, optical density.

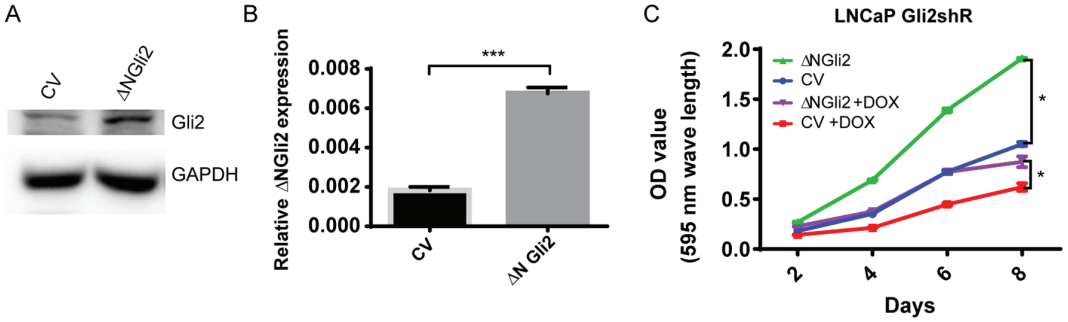

Figure S5. Androgen deprivation results in upregulation of Gli2 expression in LNCaP cells *in vitro* and *in vivo*. (A) Gli2 expression in LNCaP cells were assessed by reverse transcription-quantitative PCR following culture in an androgen-depleted medium supplemented with or without 10 pM synthetic androgen R1881. ***P<0.001 and ****P<0.0001. (B) Eleven SCID mice bearing LNCaP Ctrl xenograft tumors without DOX treatment were castrated when the tumors reached an average size of 200-300 mm^3. Three mice were euthanized on days 1 and 15, respectively, after castration, whilst the remaining mice were euthanized on day 60 in the Ctrl-DOX group in Fig. 5B. Gli2 expression in tumor tissues was observed using immunohistochemical staining. Scale bar, 100 μm. ns, not significant; SCID, severe combined immunodeficient; DOX, doxycycline; Gli2, Glioma-associated oncogene family zinc finger 2; D, days.