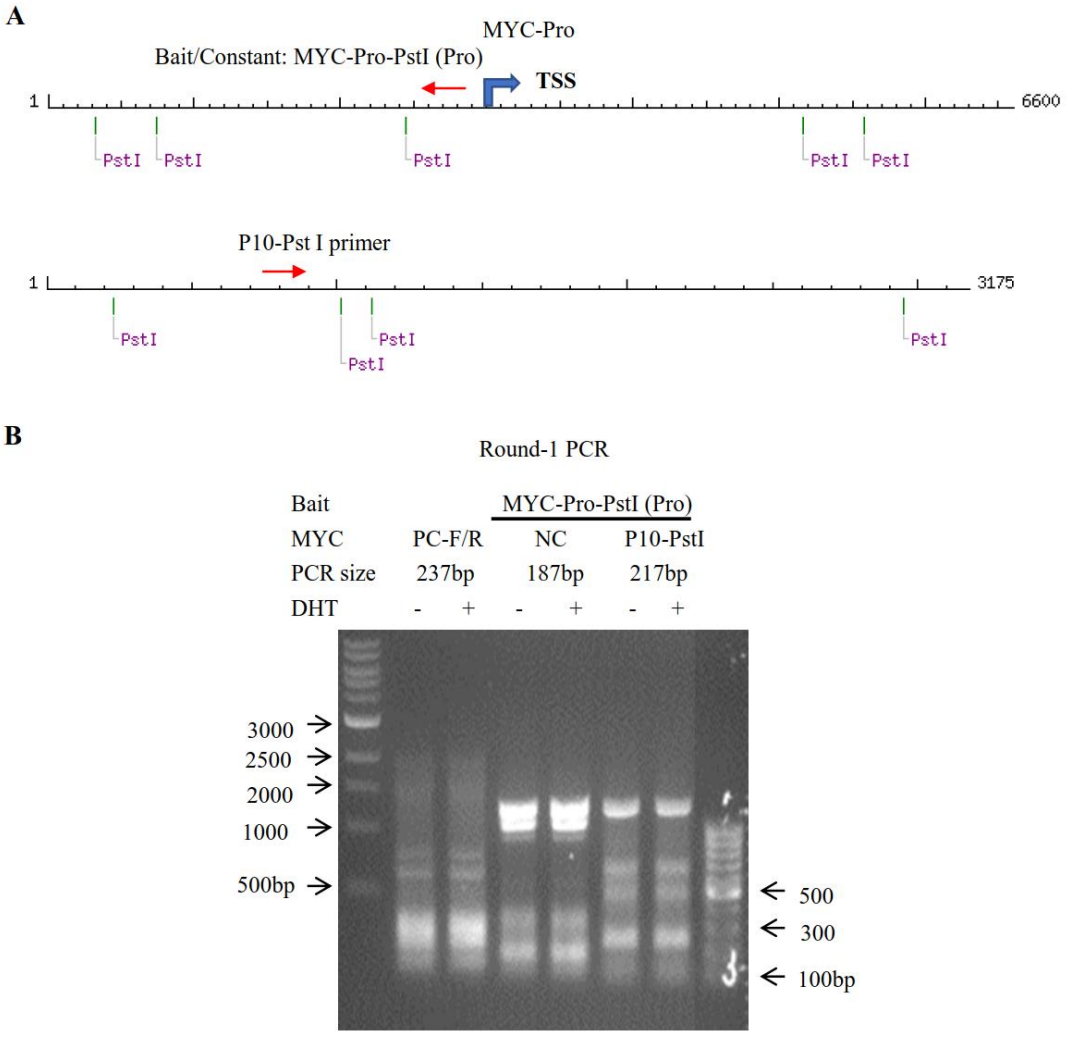
Supplementary information to

Dissecting transcription of the 8q24-MYC locus in prostate cancer recognizes the equilibration between androgen receptor direct and indirect dual-functions

Ju Guo[1*], Zhao Wei[2*], Tianwei Jia[3,4,5], Liyang Wang[6,7], Nuosu Nama[6,8], Jiaqian Liang[9], Xinghua Liao[10], Xiaming Liu[11], Yanfei Gao[12], Xiaoqiang Liu[1], Keshan Wang[6,13,14#], Bin Fu[1#], Shaoyong Shawn Chen[1,6#]



Supplementary Fig.S1. 3C analyses of the chromosomal interactions between MYC promoter and P10 in VCaP cells. A, Schematic showing of the 3C assay strategy based on PstI digestion, aiming to address the interaction between MYC promoter (MYC-Pro) and the ABS P10. Highlighted are the proximal PstI sites and the bait and target primers in 3C PCR analyses. TSS: transcription starting site. **B,** nest-PCR analysis of 3C signals, and this is the round-1 PCR results. PC-F/R: positive loading control primers that target MYC-Pro locus without PstI site in the amplicon. NC: negative control primer that target a distal Chr8 site with proximal PstI site but no AR binding site.

A, Annotation of MYC-Pro (Pro) locus with TSS, a PstI site, and PCR and DNA-Seq primers:

GCCCATTAATACCCTTCTTTCCTCCACTCTCCCTGGGACTCTTGATCAAAGCGCGGCCCTTTCCCCAGCCTTA
GCGAGGCGCCCTGCAGCCTGGTACGCGCGTGGCGTGGCGGTGGGCGCGCAGTGCGTTCTCGGTGTGGAGG
GCAGCTGTTCCGCCTGCGATGATTTATACTCACAGGACAAGGATGCGGTTTGTCAAACAGTACTGCTACGG
AGGAGCAGCAGAGAAAGGGAGAGGGTTTGAGAGGGAGCAAAAGAAAATGGTAGGCGCGCGTAGTTAATT
CATGCGGCTCTCTTACTCTGTTTACATCCTAGAGCTAGAGTGCTCGGCTGCCCGGCTGAGTCTCCTCCCCAC
CTTCCCCACCCTCCCCACCCTCCCCATAAGCGCCCCTCCCGGGTTCCCAAAGCAGAGGGCGTGGGGGAAAA
GAAAAAAGATCCTCTCTCGCTAATCTCCGCCCACCGGCCCTTTATAATGCGAGGGTCTGGACGGCTGAGGA
CCCCCGAGCTGTGCTGCTCGCGGCCGCCACCGCCGGGCCCCGGCCGTCCCTGGCTCCCCTCCTGCCTCGAG
AAGGGCAGGGCTTCTCAGAGGCTTGGCGGGAAAAAG

B, Annotation of P10 site with AR binding region, two Pst I sites in proximity, and PCR and DNA-Seq primers:

TCTGATTAGGAAAGTACAGCATGCTTTCTCCCTCATTTTCTTCTGTTTCCACAGTCTCCTAAGGCTCTAATATT
TCCATAAAATATGGGTTTGTGGAAGGCAATTTGGTTGGAAGGGTTGATTTAAAGTTGTACGGGAGGCCAGGC
ATGGTCACTCACTCCTGTAATCCCAGCACTTTGGGAGGCCAAGGCAGGTGGATCACGAGGTCAAGAGATCGA
GACCATCTTGGCCAACATGGTGAAACCTCGTCTCTACTAAAAATACAAAAATTAGCTGGGCTTGGTGGTGCA
CGCCTGTAGTCCCAGCTACTTGGGAGGCTGAGGCAGGAGAATCACTTGAACCCAGAAGGTGGAAGTTGCAG
TGAGGCAAGGTCGAAGCACTGCACTCCAGCCTGGCGACAGAGCGAGACTCTGTCTCAAAAAATATATATAA
AATAAAGTTGTATGGGAAAAGCATTATACACAAGCTGGAAAAATGACAATAAGACACATAAAGGAAAAGG
GACTGTGGAACTTTCCCTGGGGAAGAGTATATGTCCAGTCCCACCCCAGATATCAGCCTCCAGCCCCCTGGTT
GTCAAACTCTGGGACTGGTGCAGTGACCTCACCAGAAGGCATGAGATCCAGCGAATTATTCAGAATATTGGT
TCTGCTTCGGATGAGATGCCAGTGAGAATATCACCAGCTAACTGCAGGCAGGGAGGAAGTCAATGGTAATT
AAACTTTGTTTCCTTGTACACAGCAGCCAACCGCAAGGTACACAGGAAATACACAGGCAGAAGTTGCCAAG
AGCAACTGCAGATGAAAACTAGTAATAAAATCAGGTACCACTCCAAAAAGAGACACTGGAGAAGAACGAA
ACTAGAGCTATACGGAATTGTGCCTGAGATGGCACACACGCTTATGATGTATCAGGTCACTACCATCCTAAC
ATATCAAACTGAAAATGTCACCCCCATCTGGAGAGTGGGACACGTTTTATTGAAACAATGTTTTCTCTTGTTT
GTTTATATGCTCTGCACTAGTGGCTGGTTCAGTAATAAATCTGTAAGGGCCTTTTTTTAAATAAATAAATAAT
AAATTAGGAAAAAAAAAAGACTGTGGCTCCTATCTTGGGCACTTCTTGCTTTCTGCCTCCCATCAGTCATCCC
AGGGGACGCCAGCTGCCACTTTGATGGCTGCACTTTGGAGAGGCCCACAGAGGAGGAACCAATGTCTCCAG
CCAACAGTCAGCAAAGACCTGAGAAGACCTGAGGCCTTTCCAAAGCCGTGTGAGTGACGCTGGGAGGGAAT
GCTCCCAGAGTCCAGCCTTGAGAAGACCGCAGCTTTGACAGAGAGCTTGAGTGCAGGCTCCAGAGAGACCA
GGAGCAGAACCACCAACTAGGCTGAGCCAGGATTCCTGTCTCTTAGAAGGTGTGAGATAATTAGTGTTTATT
GTTTTCGCTTGCTAACTTTTGGGGTAATTTGTCACACAGCAATAGATAATTAGTATATCATTTTAAAAAATTA
CTCTGGGGCTGGGCACAGTAGCTTATACCTGTAATCCCAATACTTTAGGAGGTCAAGGTGAGAGGATTGCTT
GCGTTCAAGAGTTAGAGACCA

Supplementary Fig.S2. Annotation of MYC-Pro and P10 sites with 3C assay features. A, Alignment of MYC-Pro locus with MYC TSS and transcripts (highlighted in red), a proximal upstream PstI site (highlighted in yellow), and PCR, nest-PCR, and ddPCR primers (underlined) that was examined in the 3C studies. **B,** Alignment of P10 with AR binding peak region (highlighted in red) and two 3C PstI sites in proximity (highlighted in yellow). Also aligned are PCR and DNA-Seq primers (underlined). Based on these two PstI sites, two theoretical major 3C PCR products of MYC-Pro-P10 hybrids were confirmed by DNA electrophoresis (Figure 3D) and the DNA-Seq annotation results (See also Figure 3 and Supplementary Fig.S1 and S3).

A. DNA-Seq based on 3C nest-PCR bands (based on Figure 2B) :

B1:ACCNTGTTCTCCANTACCCGCTGGCTGTCCTGACCCGTGAGGCTGGCTATANNTTGCCCATACAGCTTCAAA
AGCGGATATGATGATCNNAAANTGATGTTGCNNTCNNCACCCNACTCTNNGTCATTCCCCCATTGCCGGGCTA
CCTTCCTTCTTCTGAAGGTCCTGTAATNNCAGGGCCATCCAATAAAACTCTTTATGCATGACATACCCTGCTTTT
GGCATTCGTTGTTATGNTTAAATNNNACTCTTCCCGGCCAGTGACGCCTGGANGTGACACACGTTTGAGACTC
ACCNATTGGGAACTNCTTTCNNTANCCCTCCCATCCNNGCNGCCTTT

B2:AGGGAAGCACGGCTTNNNATGAAAGTGGTTACCNCTTACAGGCGGACTNNTCCGTGAGCAGCTCTGGAGA
AACGTACNGCGCNGTTCCCACGAATGAGTTGGCCCTGGCTGCAGAGGAGAGAGAAGGTCACACTCGGACTCA
CGATCTGTGACAATCAAGGGCTGCGCGGTG

B3:CTATGGGCCACCTGCAATGTGCCAGGCATCGGGGACGGTGCTTCCACAGAGTTGCCTATGCAGGAAACAG
GCATGACTTACGTGCCCAGGCTCAGGGNGGTGCTCAAATCCACCTGCCTTAAAACTCTGNTTTAACACTGTTA
GGAGGTTTTCAATTAACACCGACAACTTTTGGCTCAAAACCAAAAGACAAACACATGCTCCGAGCATGTCGAT
GCCTTGGAGACTAAAGAAAACCCAACAGTCTTCACCTTTGCTTTAGGAAAGGCTGTGCTGTGTGCACTGCAGA
TCTGACTTACTGCAGAGTTCTCCACGAACAGGACAATAAGGACCCATCTGACTTACTGCACAGAGTTCTCCAC
GAACAGGACAATAAGGACCCATCTGACTTACTGCACAGAGTTCTCCACGAACAGGACAATAAGGACCCATCT
GACTTACTGCACAGAGTTCTCCACGAACAGGACAATAAGGACCCATCTGACTTACTGCACAGAGTTCTCCACG
AACAGGACAATAAGGACCCATCTGATTTACTGCAGAGTTTGACAACCANGGG

B4:TCATGATGCACTGCAGTATTTTGTATACCTCTGACCCAACTGNGCTGNCCCAGGGGGGCAGGTATTTTNNT
CTGCTTTGTTCACTGATACTCAATGTCTATNNCATTCNNATNTNGGTGCTGCCCCTCCTCATGCNAATCAGTTTT
TGTCACCTGGAAAAGCTGCAACCATGGGTATCGCCTGCNCCTGGTTGGTGGGGGGGGGCNNCTTAAACAAAAT
ACCACAGTGAGTCATAGTGGGCGTTGAGAGGATCAGGAGTGCTAAGGCGCAAAGGAAAGCAAGCGTGGGCG
GTGG

B5:TGACCAGCACAAGGAGGAGTCCTTCTCATCCCCATTTTATAAGTGACAAGGTGGAGGCTGATATCTGCGGT
GGGCGCGCANTGCGAAGGTGGGTACCCCGGTCGCTGGGCACAGCACANTGNAACTCAACGCTGGCCCCAATG
CTCTTGGTCTCTAACTGAGGCGTGACCTGCGCAGTGGGCGCGCAGTGCG

B6:GCATGANATCCNGCGAATTATTCANATATTGGTTCTGCTTCGGATGAGATGCCAGTGAGAATATCACCAGC
TAACTGCAGGCTGGTACGAACTCAATGNGGCGGTGGTTTGTTTCCTTGAACACAGCAGCCAACCGCAAGGTAC
ACAGGAAATACACAGGCAGAAGTTGCCAAGAGCAACTGCAGCCTGGTACGCGCGTGGCGTGGCGGTGGCGCG
CAGTGCGA

B7:CATGAGNNCCNGCGAATTATTCAGAATATTGGTTCTNCTTCGGATGAGATGCCAGTGAGAATATCACCAGC
TAACTGCAGCCTGGTACGCGCGTGGCGTGGCGGTGGGCGCGCAGTGCGA

B. BLAST annotation results (against human genome and transcripts)

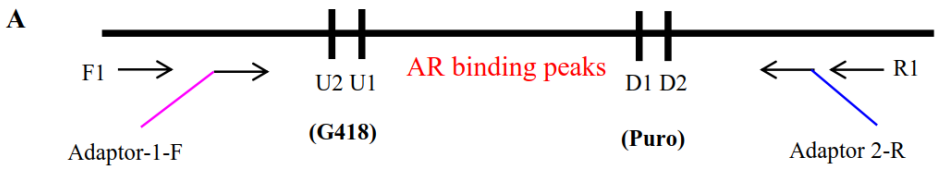
B1, B2, B4, and B5 : Irrelevant to 8q24-MYC locus.

B3: P10 region in fusion to unknown sites via PstI site.

B6: MYC-Pro region in fusion with P10 via a PstI site, which is the second nearest Pst I site in the P10 PCR primer direction. The closest PstI site remained intact.

B7: MYC-Pro region in fusion with P10 via the PstI site immediate to the P10 PCR primer.

Supplementary Fig.S3. DNA-Seq validation of DHT-induced MYC-Pro-P10 looping. A, DNA-Seq read-outs with the PstI site (CTGCAG) highlighted in red and yellow. **B,** DNA-Seq annotation based on BLAST against human genome and transcripts. The overall conclusion: B6 and B7 are both confirmed as MYC-Pro-P10 hybrids fused at two distinct P10-proximal PstI sites, respectively. These findings, together with the ddPCR and SYBR RT-qPCR results, firmly established androgen-motivated spatial interaction between MYC-Pro and P10. (See also Figure 3 and Supplementary Fig.S1-S2).

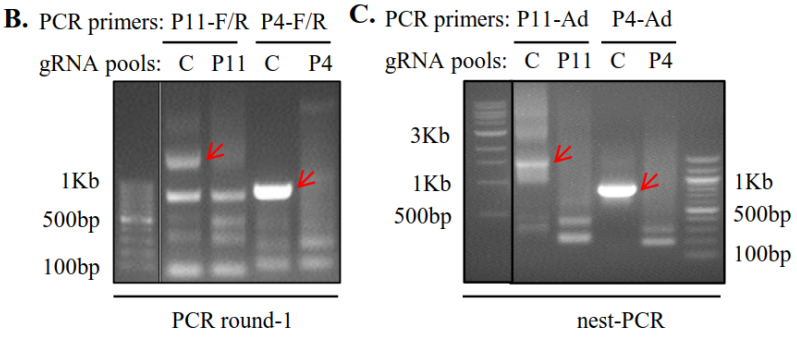


Predicted PCR product sizes (PCR round-1)

P10 locus:	**P11 locus:**	**P4 locus:**
Control gRNA (wild-type)	**Control** gRNA (wild-type)	**Control** gRNA (wild-type)
995bp	1479bp	915 bp
P10 gRNA	**P11** gRNA	**P4** gRNA
371bp	504bp	498bp
313bp	334bp	404bp
317bp	481bp	395bp
259bp	311bp	301bp

Predicted PCR product sizes (PCR round-2, Nest-PCR)

P10 locus:	**P11 locus:**	**P4 locus:**
Control gRNA (wild-type)	**Control** gRNA (wild-type)	**Control** gRNA (wild-type)
941bp	1381bp	813bp
P10 gRNA	**P11** gRNA	**P4** gRNA
316bp	405bp	395bp
258bp	235bp	301bp
262bp	382bp	292bp
204bp	212bp	198bp



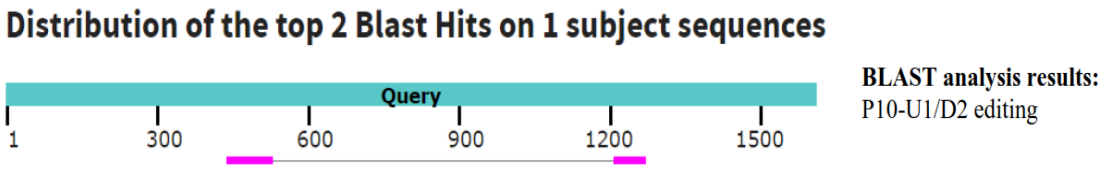
Supplementary Fig.S4. **PCR and DNA-Seq validation of CRISPR/Cas9-mediated genomic KO of P10, P11 and P4 sites. A,** Schematic drawing of KO strategy, nest-PCR primers, and theoretical PCR product sizes based on Cas9 cleavage sites in stable pools. The gene editing and PCR outcomes depend on the cleavage efficacy at U1/U2 site and D1/D2 sites, respectively. **B-C,** two rounds PCR gel imaging results based on control (C) versus P11 and P4 stable KO pools. Arrow heads indicate the wild-type PCR bands. Ad: adaptor. For additional information see Fig.4-5 and Supplementary Figure S5–S7.

A, Annotation of the P10 locus with AR binding peak region, gRNA targets, and PCR and DNA-Seq primers:

TCTGATTAGGAAAGTACAGCATGCTTTCTCCCTCATTTTCTTCTGTTTCCACAGTCTCCTAAGGCTCTAATATTTCC
ATAAAATATGGGTTTGTGGAAGGCAATTTGGTTGGAAGGGTTGATTTAAAGTTGTACGGGAGGCCAGGCATGGT
CACTCACTCCTGTAATCCCAGCACTTTGGGAGGCCAAGGCAGGTGGATCACGAGGTCAAGAGATCGAGACCATC
TTGGCCAACATGGTGAAACCTCGTCTCTACTAAAAATACAAAAATTAGCTGGGCTTGGTGGTGCACGCCTGTAGT
CCCAGCTACTTGGGAGGCTGAGGCAGGAGAATCACTTGAACCCAGAAGGTGGAAGTTGCAGTGAGGCAAGGTCG
AAGCACTGCACTCCAGCCTGGCGACAGAGCGAGACTCTGTCTCAAAAAATATATATAAAATAAAGTTGTATGGG
AAAAGCATTATACACAAGCTGGAAAAATGACAATAAGACACATAAAGGAAAAGGGACTGTGGAACTTTCCCTG
GGGAAGAGTATATGTCCAGTCCCACCCCAGATATCAGCCTCCAGCCCCCTGGTTGTCAAACTCTGGGACTGGTGC
AGTGACCTCACCAGAAGGCATGAGATCCAGCGAATTATTCAGAATATTGGTTCTGCTTCGGATGAGATGCCAGTG
AGAATATCACCAGCTAACTGCAGGCAGGGAGGAAGTCAATGGTAATTAAACTTTGTTTCCTTGTACACAGCAGCC
AACCGCAAGGTACACAGGAAATACACAGGCAGAAGTTGCCAAGAGCAACTGCAGATGAAAACTAGTAATAAAA
TCAGGTACCACTCCAAAAAGAGACACTGGAGAAGAACGAAACTAGAGCTATACGGAATTGTGCCTGAGATGGCA
CACACGCTTATGATGTATCAGGTCACTACCATCCTAACATATCAAACTGAAAATGTCACCCCCATCTGGAGAGTG
GGACACGTTTTATTGAAACAATGTTTTCTCTTGTTTGTTTATATGCTCTGCACTAGTGGCTGGTTCAGTAATAAAT
CTGTAAGGGCCTTTTTTTAAATAAATAAATAATAAATTAGGAAAAAAAAAAAGACTGTGGCTCCTATCTTGGGCAC
TTCTTGCTTTCTGCCTCCCATCAGTCATCCCAGGGGACGCCAGCTGCCACTTTGATGGCTGCACTTTGGAGAGGCC
CACAGAGGAGGAACCAATGTCTCCAGCCAACAGTCAGCAAAGACCTGAGAAGACCTGAGGCCTTTCCAAAGCCG
TGTGAGTGACGCTGGGAGGGAATGCTCCCAGAGTCCAGCCTTGAGAAGACCGCAGCTTTGACAGAGAGCTTGAG
TGCAGGCTCCAGAGAGACCAGGAGCAGAACCACCAACTAGGCTGAGCCAGGATTCCTGTCTCTTAGAAGGTGTG
AGATAATTAGTGTTTATTGTTTTCGCTTGCTAACTTTTGGGGTAATTTGTCACACAGCAATAGATAATTAGTATAT
CATTTTAAAAAATTACTCTGGGGCTGGGCACAGTAGCTTATACCTGTAATCCCAATACTTTAGGAGGTCAAGGTG
AGAGGATTGCTTGCGTTCAAGAGTTAGAGACCA

B, DNA-Seq read-out and alignment:

CATGGATACGGAAGAGCGTCGTGTAGGGAAAGAGTAGTAGGGAAAAGCATTATACACAAGCGGGGAAAATGA
CAATAAAACAAATAAAGGAAAAGGGACTGTGAAAATTTCCGGGGATGAACCAAAGTCTCCAACCAAACGTCA
GCAAACACCTCAAAAGACCTGAGGCCTTTCCAAAGATCGGAAGAGCACACGTCTGAACTCCAGTCAA



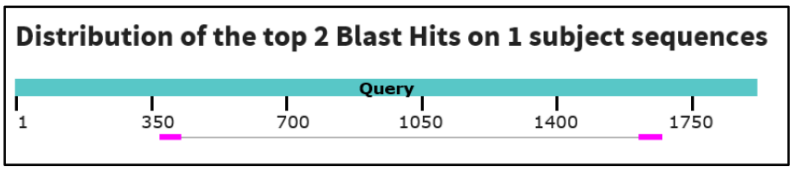
Supplementary Fig.S5. A, Alignment of the P10 site with features assessed in CRIPSR/Cas9 editing study. The AR binding peak region is highlighted in red. Also aligned are gRNA targets (in green) and PCR and DNA-Seq primers (underlined). Highlighted are PCR (F1/R1, in black and adaptor-F/R, in blue) and DNA-Seq primers (adaptor-F/R, in blue). Also colored are gRNA oligos (U2/U1, D1/D2, in green). The theoretical positions of gRNA cleavage sites are: 464 (U2 gRNA), 518 (U1 gRNA), 1141 (D1 gRNA), and 1199 (D2 gRNA), respectively. **B, DNA-Seq validation of CRISPR/Cas9-mediated gene editing in the P10 stable pool.** Blast annotation of one verification DNA-Seq read-out indicated the editing mainly occurred at theoretical positions of U1/D2 gRNA cleavage sites. (See also Figure 4 and supplementary Fig.S4 for additional information).

A, Annotation of the P11 locus with AR binding peak region, AR binding motifs, gRNA targets, and PCR and DNA-Seq primers:

GCCTGGGCAACAAGACCGAAACTCCGTCTCAGATAAAAAACAAACAAACAAACAAAAAAGGCCAACAAATT
TGACCAATACTCCTCAAAACTGGAAAAGCTGGTGACCACCACCCCTAGTGCTCCAATAAAGGGCTGTGGGAA
CAGTTGTGCTCAGGGTGGTACTGGTACAGCCTGGCAGAGGACATGATGCTTGCATAGTGTGGAAAGGCCCTC
TGAGATATTGCTGGGCCTTAGGGGAGGAGGTCCAGGCCTAGAGAGGACTGAAGCCCTCCCCAGGGCATGGT
GTGGACCACGCTGAAGGGAGGTGGGGTTGGAGACACAGCTGGGAGTCAGATCCCAGGGGCTCTCCTGTGTG
ATGCTGAATTGCTGGGAAGGGGATGCCAGCTAGTCTCTTTCTCTGTGCCAACCCTGATTGGTTGGGTGGCTGC
TGAGAGCCTTATGGTGCCCTGGCTCAGTAGGAAGAGTGTGTGTTCATTTAGTGAGTTTGCCAAAGACAAGGG
AAGGGGGTTAGAGAGTAGGTGGCAGATAATGCCGGCCTGGGGATCCTGGAGGTTTTAGGCTGGCAGACCCT
GGCTGAGGCTGTTTTCTGTTCTTTTCCTTAGGCCTTGGGAGACCTTTACCTTTTCAGTGCCTCTAAGACAGGGT
CAGAGCAGTTTGGTGACAAAGTGGAAGTAAACCATGGGTGAAAAACAGCTCTGGGGACCCTCATAGGCTTCC
ACTTATTGAGGATTAGGCAGAACTTGCAGCTTGGCCCAGCTTTCGAAACAAGGTGTTCCCAGGAGGTGGAAA
GACTGGTTTTCAATCAGATAAAGGTAGGATCCTATGAAAAACTCCTAGGGCCTTGCCGTTCCTATGAAGTGGT
AGCTATCATTCACACTCCTAGTTATGCCATCGAGGTAAAAAGAGGTGGAATAAGAATCAGGGACACTTGAAA
ATAAGAAAAGAGAAAGACTGCGTGCATGTGCATGGGTGCACAGCCTGTACATATGTGTGTGTGTGCCCCTGC
GTGCACGTGCATGGGTGCACAGCCTGTACATATGTGTGTGTGTGCCCCTGCGTGCACGTGCGTGGGTGCACA
GCCTGTACATATGTGTGTGTGTGCCCCTGCGTGCACGTGCGTGGGTGCACAGCCTGTACATATGTGTGTGTGT
GCCCCTGCGTGCACGTGCGTGGGTGCACAGCCTGTACATATGTGTGTGTGTGCTCCTGCGTGCACGTGCGTGG
GTGCACAGCCTGTACATATGTGTGTGTGTGCTCCTGCGTGCACGTGCGTGGGTGCACAGCCTGTACATATGTG
TGTGTGTGCTCCTGCGTGCACGTGCGTGGGTGCACAGCCTGTACATATGTGTATTTGTGCTCCTGTGTGCACG
TGCATGGGTGCACAGCCTGTACATATGTGTATGTGTGCTCCTGCTTGCATATGCATGGGTACACAGCCTGTAA
CTACATGTGTGTGAGCCTGTGGGTATGTGAGGGGAGTGAGGGACCCTGAGGGACTAGAAACTTCCTTAAAGG
CCATTTGAGAGAGTGCTGCTTTCTATACCACCCACCCTAGGGCAGTTAGGCAAAGGTGTGCCTCCCTTTAAGA
GGACCTAGCGGTCGGTCTGGTTTACAACATAGGTTATCAGGGAGTAGTACTTTGGCCTGGAAAAGGAACCTT
TATTTTGGAAAAGATTTGTAATCTCTGTGGCTTGTTTGATACACATTGGAAACCTGACTGGAAACTCAGGCCA
CCAGCATCTGTTGGGGGCACATGTTTGCACTTTTGGTTATGATGCCACGGCCCTGGTTCAGGTATCCTAAAGA
AGATAATTAATGATGAAAATGATTGGTGTAGATTTCATACCACAGAGCACTGTGGTTTGTATAGTAAGACAC
AGTGATGGTGTGGACCCATGCAC

B, DNA-Seq read-out and alignment:

ATTTGCGGAAGGGGATGCCAGCTAGTCTCTTTCTCTGTGCCAACCCTAAATTGGTTAGGAGGGGCCGCTCGGT
CCGTCTGGTTTACACCCTAAGTAATCAGGGAGTAGTACTTTGGCCTGGAAGGGGAACCGGATTGGAGCAGCA
CACGTCGGAAGTGGAACAAAGCCCCGGTCTGAGGTCCGAACA



BLAST analysis results:
P11-U2/D2 editing.

Supplementary Fig.S6: A, Annotation of P11 (the central peak out of total 5 AR peaks**) with KO test features.** The AR binding peak region is highlighted in red. Underlined are PCR (F1/R1, in black and adaptor-F/R, in blue) and DNA-Seq primers (adaptor-F/R, in blue). Also colored are gRNA targets (U2/U1, D1/D2, in green). The theoretical positions of gRNA cleavage sites are: 420 (U2 gRNA), 442 (U1 gRNA), 1417 (D1 gRNA), and 1587 (D2 gRNA), respectively. Significantly, the P11 central AR peak contains at least 10 potential AR binding motifs that are highly repetitive, as highlighted in yellow. **B, DNA-Seq validation of CRISPR/Cas9-mediated gene editing at P11.** Blast annotation of DNA-Seq indicated the editing mainly occurred at theoretical positions of U2/D2 gRNA cleavage sites. (See also Figure 5 and supplementary Fig.S4 for additional information).

A, Annotation of the P4 locus with AR binding peak region, gRNA targets, and PCR and DNA-Seq primers:

GTGGCCTCCAGTCTTGAAGTCCACATTCTGAATGTGCCCTCATGTTGACTCCCGGCTTCCTCCAAGCTCAGGAT
TTCCATGTCCCCTGAGGGCCAGTGGCAATAATGCACTGTGGCTCTCTCTCTAGTTGGTAGTGACATGTGAGAG
ATAGCCTGAGCCATGTCTTGGCTTCCCTATGACTTGCTCACTACAATGGATTGTAATTCCATGATGACTATGGA
ACTTCTTTGAGGGGCTTGCAGACTCCCTAGGCTTTTTCTGAGGATTCAAGGATTCCAGTTCTGTTTTACTTACTT
TCTCCTAAATGAGATTAGGTTTCTGTACCCTCCAGCTTGACATTTTACACCAAGGGGTGAGCTGGAACTTGGAT
GGTCATGTTGTACATCTTGAATAGTCATTCTATTCTTTTATCTTTAACACTCAGACTCCTCCCAAGCACTCTGGT
GACTCTCTGAGCCATCGTTTTCCTTCTGTGAGAACTTCCCTGCATCAGTCAGTTCTCACAATTTCTATAATCTGC
TCTGTAAGTGCCTTCAAGTTCTGCCTCAATGGAAGTCACAAGACATTTTTTGTTTGTTTTGTTTTTTTCTCTGTC
AACAAAACCAGCCATCTGTACATCAAAAGCATTTGCCTTCATACCATTGTCTTACTGTATTCTTAGAACAGTAT
TCCAGAACTCAAAGCAACAGAGTCTCTGTGTTCAGACTGGTTCTTCTCGTAAACAAAGGAAAAAATGGTCATT
AATTTTATAGGGGCAGAAAGAAAAGGGACAAGGACTTCGGGAACTAAAGGTTCTGTCTACATGGGGATTTAT
GATACTATTCTCTCAATGATAGATCTGGAAGTTTGATTCACCATGAAAAAAAAACCACACTTTTAAATGCTTA
ATTCCTGTTAAACTTACAAAGAAATTTGACATGCCAAATAATAAACAAAGAATTCAGGAACCAGAGTGGTGA
GAATGATGTGATTTGAGTGAAGATTTTCTAAAGGCTTAATAGGATTTCTTCCCTTTCTCTTATTTTCTGTACCAC
CTAAATTCTAATAACAGAGGACAGTTAACTTTCTGTTATTAGAGCTCTAATCCAGTCACAAAAAGAGAAAAAA
TTAAAGAGAGAAAAGAAGAGAAAAAGGGAAGGGAAGAAAAAAGAAAGAGAAAGGAGGGGAAGGAAGGGA
AGGGAGGGGAGAGGAGAGGAGGGAAGCGAGGGAGAGGAGAAGGAAGGGGAGGGGTGAGATGGTGAGGTGT
GGAGGAAAAGTTAAATATTAAATTTGAACTCAATTGAACATCGACACAAACAATGGTCACCAAGTCCCAGAA
CAGGTTGTGTGTGCCCCTTGAGGCGTTCATCCAGCACTGTTTCAGAGAAATCCCTATTTCAATCTATTCCTATA
CGTTAGTTATTGAAAAGCAATAGACAATCACAAAAAACAAGTTGACCTTTTTGTGTTCCTTGAGCCAAAGGGC
CCTCATGACTGGGCCTCACACCGAATAACTCGTTA

B, DNA-Seq read-out and alignment:

ACCTAATAACAGAAAGTTAACTGTCCTCTGTGGGGGGGGCTCGGGGGGTTAAAAAAAAAAAAAAAAGGGA
CTTTTCAAAATGTCCCCCTGGCCCCCCCATTTTCCCCCCCAAACGGAAAAAGGCCCGGGGGGGGAAAAAGTAA
AAATCTTGTTATGAAAAAAGTTTTTTTTACTCCCCCTGTTTCCCCCCCCCTTTCCCCCCCTAAAAGCAAAAACCTT
TTGTTTGGGCCCCTATAAAAATAATGTCCTTTTTTTCCTTTGTTTACGAAAAAAACCCGTCCGAACCCCGAACC
CCCGTTGCTTTTAGTTCTGGAATACCGTTCCAAAAAAACAGTAAGACAATGGTATGAAGGCAAATGCTTTTGA
TGTACAAATGGGTGGTTTTGTTGACCTAAAAAAAAACAAAACAAACAAAAAATGTCTTGTGACTTCCCTTGGG
GCAAAACTTGGAGGGGTTTTAGTGTTCAAATTTTAAAATTTGTAAGACCTTTCTAAATATGCAAATTTCTCATC
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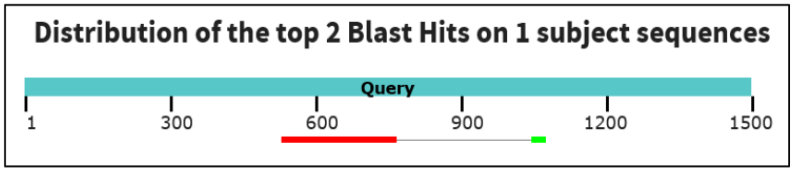
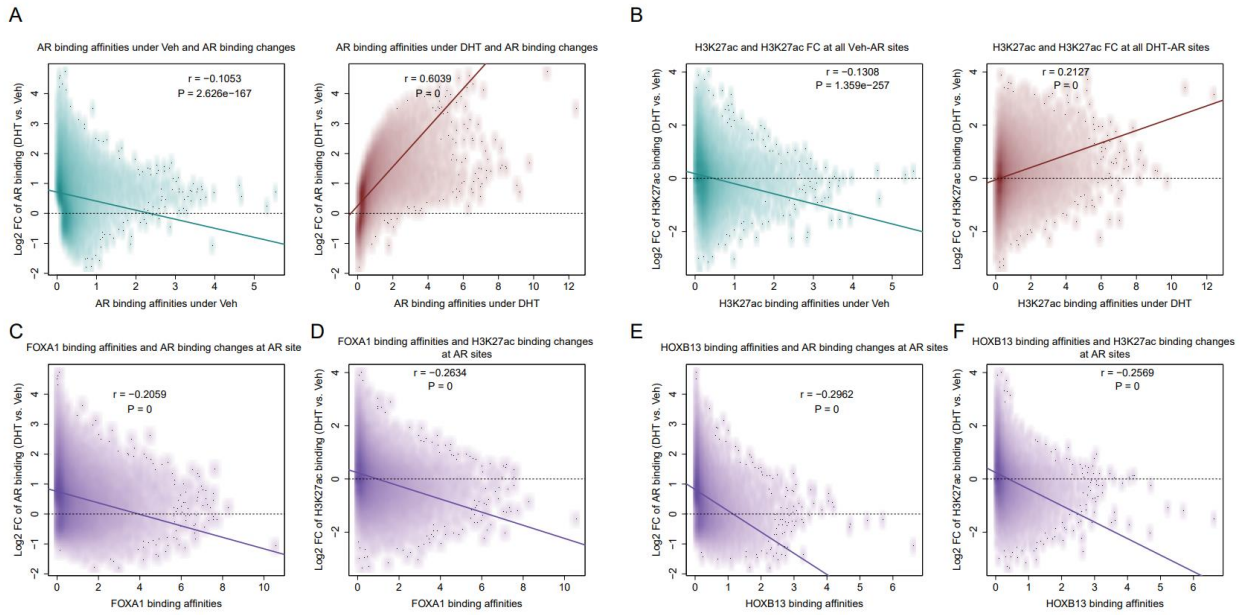


BLAST analysis results:
P4-U1 and D2 editing

Supplementary Fig.S7. A, Annotation of P4 locus with features assessed in CRIPSR/Cas9-KO study. The AR binding peak region is highlighted in red. Also aligned are gRNA and PCR and DNA-Seq primers. Underlined are PCR (F1/R1, in black and adaptor-F/R, in blue) and DNA-Seq primers (adaptor-F/R, in blue). Also colored are gRNA targets (U2/U1, D1/D2, in green). The theoretical positions of gRNA cleavage sites are: 434 (U2 gRNA), 537 (U1 gRNA), 954 (D1 gRNA), and 1048 (D2 gRNA), respectively. **B, DNA-Seq validation of CRISPR/Cas9-mediated gene editing at P4.** Blast annotation of DNA-Seq indicated the editing mainly occurred at theoretical positions of U1/D2 gRNA cleavage sites. See also Figure 5 and supplementary Fig.S4 for additional information.



Supplementary Fig.S8. Bioinformatics analyses of global androgen effects on distribution of AR, H3K27ac, FoxA1 and HoxB13 in VCaP cells. A, The correlation between AR binding affinities and AR binding fold changes (FC: DHT vs. Veh) at all Veh-AR sites and all DHT-AR sites, respectively. **B**, The correlation between H3K27ac signal and H3K27ac signal fold changes (DHT vs. Veh) at all Veh-AR sites and all DHT-AR sites, respectively. **C**, The correlation between FOXA1 binding affinities and AR binding fold changes (DHT vs. Veh) at all AR sites (combination of both Veh and DHT AR sites). **D**, The correlation between FOXA1 binding affinities and H3K27ac signal fold changes (DHT vs. Veh) at all AR sites. **E**, The correlation between HOXB13 binding affinities and AR binding fold changes (DHT vs. Veh) at all AR sites. **F**, The correlation between HOXB13 binding affinities and H3K27ac signal fold changes (DHT vs. Veh) at all AR sites.

Supplementary information

PCR and RT-PCR kits, DNA purification kits and DNA rulers: 1, PCR and nest-PCR kit: Phusion Hot Start Flex DNA Pol (NEB, 0535); 2, RT-qPCR (DNA template) kit: Power SYBR Green PCR Master Mix (Thermo Fisher, Cat. 4367659); 3, DNA purification kit: QIAquick PCR Purification Kit (Cat. No. 28106, Qiagen; Germantown, MD, USA) and Gel purification kit (Qiagen, Cat. No. 28506). 4, 1Kb DNA ladder (New England Biolabs, N3232S); 100 bp DNA Ladder (Thermo Scientific, SM0243).

PCR and RT-PCR parameters and DNA-Seq: The PCR analysis was performed with the Step-One-Plus Real-time PCR system (Applied Biosystems). PCR amplification using Phusion kit (NEB, Catalog 0535) and the thermocycling conditions for PCR round-1 is (1x, 98C, 2min; 40x (98C, 15sec; 63C, 30sec; 72C, 30sec); 1x, 72C, 7min; and hold at 4C) and for nest-PCR is (1x, 98C, 30sec; 40x (98C, 15sec; 63C, 30sec; 72C, 20sec); 1x, 72C, 7min; and hold at 4C). Both runs start with a reaction volume of 50ul and 1.5ul of round-1 PCR products was used as the template for nest-PCR. Nest-PCR products (with regular primers) were purified for regular Sanger sequencing; nest-PCR products (with primers containing adaptors) were purified and submitted for sequencing with the Genewiz Amplicon EZ program.

PCR and RT-PCR primers and probes, and gRNA construct oligos: The uniqueness of all primers and oligos in this study has been validated by BLAST against human genome and transcripts.

1, ChIP-qPCR assay primers:

Myc_pro_F, AGGGCTTCTCAGAGGCTTG

Myc_pro_R, CGGCTCTTCCACCCTAGC

P10-F, CCGCAAGGTACACAGGAAAT

P10-R, CCAGTGTCTCTTTTTGGAGTGG

PCAT1-Pro-F, CTCCGAACCACACATGGATA

PCAT1-Pro-R, CCTTGCCACTTTCCTTTCCT

PCAT1-ABS1-F (P1), GGTCAGGGATGTTGATTTAGC

PCAT1-ABS1-R (P1), AAAGTTTGGGTTTATTGCAAATG

CCAT1-ABS1-F (P4), TCCAGAACTCAAAGCAACAGAG

CCAT1-ABS1-R (P4), CCCTTTTCTTTCTGCCCCTA

CASC8-ABS1-F (P7), AATCCAGCACTGCTTGTGG

CASC8-ABS1-R (P7), CAAATGAGCATGACTGTGTCC

PVT1-Pro-F, GCATGGAGCTTCGTTCAAGT

PVT1-Pro-R, TGTCATTCCAGTGCATGGTT

PVT1-ABS1-F (P11), CCAGCTTTCGAAACAAGGTG

PVT1-ABS1-R (P11), GCAAGGCCCTAGGAGTTTTT

PVT1-ABS2-F (P13), AACAGCCCACAGCATTTTTC

PVT1-ABS2-R (P13), TCACAGTGTTCTGGGTCAGC

PVT1-ABS3-F (P14), TCTCAGTGCTGTGTGCTGTTT

PVT1-ABS3-R (P14), CCTGTTTCTCAGCAGCAACT

PSA-Enh-F, GCCTGGATCTGAGAGAGATATCATC

PSA-Enh-R, ACACCTTTTTTTTTCTGGATTGTTG

NC-F, TCCTGCATTCCATAGCTTTT (Non-specific genomic control site on Chr1)

NC-R, TCTGATCACAATGGAATGAAACT (Non-specific genomic control site on Chr1)

2, PCR primers for 3C assay (round-1 PCR)**:**

3C-MYC-Pro-PstI: AACAGCTGCCCTCCACACCGAGAA

3C-MYC-P10-PstI: TCCCACCCCAGATATCAGCCTCCA

3C-MYC-Pro-PC-F, TTCTCGGTGTGGAGGGCAGCTGTT

3C-MYC-Pro-PC-R, GGTGGGGAAGGTGGGGAGGAGACT

3C-MYC-Pro-NC: AGAGCTCAGCCAAGCTGGCACGAA

3, PCR primers for 3C assay (round-2 PCR, Nest-PCR)**:**

Nest-MYC-Pro-PstI, CGCACTGCGCGCCCACCGC

Nest-MYC-P10-PstI, GCCCCCTGGTTGTCAAACTCTG

Nest-MYC-Pro-PC-F, CCGCCTGCGATGATTTATACTCAC

Nest-MYC-Pro-PC-R, CAGCCGGGCAGCCGAGCACT

Nest-MYC-Pro-NC, GTGGTATTCCCATCATACTCTCTC

4, ddPCR and RT-qPCR primers/probes for 3C assay:

Droplet digital PCR (ddPCR): The ddPCR was performed on the Bio-Rad QX200 AutoDG Droplet Digital PCR System (Bio-Rad, Hercules, CA, USA) that uses a C1000 Touch Thermal Cycler with 96–Deep Well Reaction Module. The ddPCR Probe no UNG cycling conditions: 95 °C/10 min; 40 cycles of 94 °C/30 s and 60 °C/1 min; 98 °C/10 min. The MYC-promoter was used as bait (constant) that was paired with primer specifically located in target regions. Specific probe signal was normalized to that of the copy number reference RPPH1 that does not contain a PstI site in the amplicon.

q3C-MYC-pro-PstI-C, GTATAAATCATCGCAGGCGGAAC

FAM-MYC-Pst-QSY, FAM-TGCAGCCTGGTACGCGCGTGGCGT-QSY

q3C-P10-PstI, CAACCGCAAGGTACACAGGAAAT

Copy number reference is RPPH1 (RNase P).

q3C-hGAPDH-F, CCCTTTCACCATTAGGGACCTTC

q3C-hGAPDH-R, GCTAAGTTTAGCCTGCCTGGTGA

5, gRNA expression constructs and oligos:

Lenti-gRNA-Neo vectors (LGN, Addgene, Catalog. 104992) were constructed to express a pair of upstream sgRNAs (U1/U2); Lenti-gRNA-Puro vectors (LGP, Addgene, Catalog. 104990) were generated to express a pair of downstream sgRNAs (D1/D2), respectively. The cloning is based on vector BsmBI digestion (NEB, R0580L), oligo annealing and ligation with T4 DNA ligase (NEB, M0202).

Control gRNA site (non-specific) oligos:

gRNA-C1-F: CACCGGTAGCGAACGTGTCCGGCGT

gRNA-C1-R: AAACACGCCGGACACGTTCGCTACC

gRNA-C2-F: CACCGGACCGGAACGATCTCGCGTA

gRNA-C2-R: AAACTACGCGAGATCGTTCCGGTCC

P10 site oligos:

P10-gRNA-U1-F: CACCGGGACTGTGGAACTTTCCCTG

P10-gRNA-U1-R: AAACCAGGGAAAGTTCCACAGTCCC

P10-gRNA-U2-F: CACCGGAAAAGCATTATACACAAGC

P10-gRNA-U2-R: AAACGCTTGTGTATAATGCTTTTCC

P10-gRNA-D1-F: CACCGCGTCCCCTGGGATGACTGA

P10-gRNA-D1-R: AAACATCAGTCATCCCAGGGGACGC

P10-gRNA-D2-F: CACCGGACATTGGTTCCTCCTCTGT

P10-gRNA-D2-R: AAACACAGAGGAGGAACCAATGTCC

P11 site oligos:

P11-gRNA-U1-F: CACCGTACTGAGCCAGGGCACCATA

P11-gRNA-U1-R: AAACTATGGTGCCCTGGCTCAGTAC

P11-gRNA-U2-F: CACCGGCCAACCCTGATTGGTTGGG

P11-gRNA-U2-R: AAACCCCAACCAATCAGGGTTGGCC

P11-gRNA-D1-F: CACCGGTACCCATGCATATGCAAGC

P11-gRNA-D1-R: AAACGCTTGCATATGCATGGGTACC

P11-gRNA-D2-F: CACCGCGACCGCTAGGTCCTCTTAA

P11-gRNA-D2-R: AAACTTAAGAGGACCTAGCGGTCGC

P4 site oligos:

P4-gRNA-U1-F: CACCGTCCATTGAGGCAGAACTTGA

P4-gRNA-U1-R: AAACTCAAGTTCTGCCTCAATGGAC

P4-gRNA-U2-F: CACCGGAGTCACCAGAGTGCTTGGG

P4-gRNA-U2-R: AAACCCCAAGCACTCTGGTGACTCC

P4-gRNA-D1-F: CACCGTCACATCATTCTCACCACTC

P4-gRNA-D1-R: AAACGAGTGGTGAGAATGATGTGAC

P4-gRNA-D2-F: CACCGCACCTAAATTCTAATAACAG

P4-gRNA-D2-R: AAACCTGTTATTAGAATTTAGGTGC

P11-M site oligos (targeting multiple AR peaks):

P11-M-gRNA-U1-F, CACCGTTCTCAAGTCCCTATAGAAG

P11-M-gRNA-U1-R, AAACCTTCTATAGGGACTTGAGAAC

P11-M-gRNA-U2-F, CACCGAGGAGTCATGCCCAAGATAC

P11-M-gRNA-U2-R, AAACGTATCTTGGGCATGACTCCTC

P11-M-gRNA-D1-F, CACCGCTCACAAAATACGAAGACGC

P11-M-gRNA-D1-R, AAACGCGTCTTCGTATTTTGTGAGC

P11-M-gRNA-D2-F, CACCGCTGGGTCGCGTGTCCTGGCA

P11-M-gRNA-D2-R, AAACTGCCAGGACACGCGACCCAGC

P1 site oligos:

P1-gRNA-U1-F, CACCGCTAGAATGCGGTCCAAGCAT

P1-gRNA-U1-R, AAACATGCTTGGACCGCATTCTAGC

P1-gRNA-U2-F, CACCGTCGTTAGAGAAGCCAATGCT

P1-gRNA-U2-R, AAACAGCATTGGCTTCTCTAACGAC

P1-gRNA-D1-F, CACCGTTTCCAGCTTCTGGTCTGAT

P1-gRNA-D1-R, AAACATCAGACCAGAAGCTGGAAAC

P1-gRNA-D2-F, CACCGCAGGGGATAAATATCCTAGC

P1-gRNA-D2-R, AAACGCTAGGATATTTATCCCCTGC

CRE2 site oligos:

CRE2-gRNA-U1-F, CACCGCATGGTGAAGGTGGCTAAAG

CRE2-gRNA-U1-R, AAACCTTTAGCCACCTTCACCATGC

CRE2-gRNA-U2-F, CACCGGTAATAGATGGCCTAATATT

CRE2-gRNA-U2-R, AAACAATATTAGGCCATCTATTACC

CRE2-gRNA-D1-F, CACCGATAAGGCAGAGGAAACCTTT

CRE2-gRNA-D1-R, AAACAAAGGTTTCCTCTGCCTTATC

CRE2-gRNA-D2-F, CACCGGAGTTGCTAAATTTGCTAAG

CRE2-gRNA-D2-R, AAACCTTAGCAAATTTAGCAACTCC

P2 site oligos:

P2-gRNA-U1-F, CACCGCTCATGCTGTATTTCTATTA

P2-gRNA-U1-R, AAACTAATAGAAATACAGCATGAGC

P2-gRNA-U2-F, CACCGTGCTAGTCACTACCTTAAAA

P2-gRNA-U2-R, AAACTTTTAAGGTAGTGACTAGCAC

P2-gRNA-D1-F, CACCGTATACATATGATCAATGGAG

P2-gRNA-D1-R, AAACCTCCATTGATCATATGTATAC

P2-gRNA-D2-F, CACCGATATGTCATAGTTGATTCTC

P2-gRNA-D2-R, AAACGAGAATCAACTATGACATATC

6, Primers for PCR and DNA-Seq validation of CRISPR/Cas9-based genomic KO:
P10 site:
Primers for P10 site (round-1 PCR):
P10-F1: TTGAACCCAGAAGGTGGAAG
P10-R1: GTCAAAGCTGCGGTCTTCTC
Adaptor-primers (for P10 site Nest-PCR and DNA-Seq) (Adaptor part is underlined):
Adaptor-P10-F (Ad-P10-F):
ACACTCTTTCCCTACACGACGCTCTTCCGATCTAGCCTGGCGACAGAGCGAGACT
Adaptor-P10-R (Ad-P10-R):
GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTTGGAAAGGCCTCAGGTCTTCTCA
P11 site:
Primers for P11 site (round-1 PCR):
P11-F1: GGTCCAGGCCTAGAGAGGAC
P11-R1: CCAGTCAGGTTTCCAATGTG
Adaptor primers (for P11 site Nest-PCR and DNA-Seq) (Adaptor part is underlined):
Adaptor-P11-F (Ad-P11-F):
ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCTCTCCTGTGTGATGCTGAATTG
Adaptor-P11-R (Ad-P11-R):
GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGTTCCTTTTCCAGGCCAAAGTAC
P4 site:
Primers for P4 site (round-1 PCR):
P4-F1: TCTGAGGATTCAAGGATTCCA
P4-R1: CTTCCTTCCCCTCCTTTCTC
Adaptor primers (for P4 site Nest-PCR and DNA-Seq) (Adaptor part is underlined):
Adaptor-P4-F (Ad-P4-F):
ACACTCTTTCCCTACACGACGCTCTTCCGATCTGAGCTGGAACTTGGATGGTCATGT
Adaptor-P4-R (Ad-P4-R):
GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTCTTTTTGTGACTGGATTAGAGCT
Reference control:
MYC-Ctr-F: TTCTCGGTGTGGAGGGCAGCTGTT

MYC-Ctr-R: GGTGGGGAAGGTGGGGAGGAGACT

PSA-Ctr-F: TGTCCTGGAGAGGGTGGAGGTGGA

PSA-Ctr-R: AGGTGGGCACTGAGACTGCACTGG