

Figure S1. c-Myc activity determined using the the Jung c-Myc-target signature positively correlates with AR level in human prostate cancer samples. Pearson's correlation coefficient analysis showing a positive correlation between c-Myc activity score and AR level in 159 mCRPC samples from 3 cohorts (A), a meta dataset of 1642 primary prostate cancer samples (B), and the TCGA dataset of 500 primary prostate cancer samples (C). The c-Myc activity score was computed as the sum of z-scores for the Jung c-Myc-target signature.



Figure S2. GSEA showing enrichment of the hallmark Myc_targets_v2 and androgen_response pathways in mCRPC samples (n = 159) that express a high level of AR.

Α

- Enrichment profile — Hits

Ranking metric scores



Figure S3. GSEA showing enrichment of the hallmark Myc_targets_v1, Myc_targets_v2, and androgen_response pathways in primary prostate cancer samples that express a high level of AR. A, a meta dataset of 1642 primary prostate cancer samples; B, the TCGA dataset of 500 primary prostate cancer samples.

Ranking metric scores

Enrichment profile — Hits

Ranking metric scores

- Enrichment profile — Hits



Figure S4. c-Myc activity, calculated using the Jung c-Myc-target signature, positively correlates with AR-FL, -V7, -V9, and -V3 levels in 159 mCRPC samples in Pearson's correlation coefficient analysis. The AR-FL, -V7, -V9, and -V3 levels were quantified as normalized number of RNA-seq reads spanning AR exons 7-8, 3-CE3, 3-CE5, and 2-CE4 splice junctions, respectively. The c-Myc activity score was computed as the sum of z-scores for the Jung c-Myc-target signature.



Figure S5. c-Myc level and activity positively correlate with AR activity in human prostate cancer samples. Pearson's correlation coefficient analysis showing a positive correlation between c-Myc level and AR activity score (top panels) and between c-Myc and AR activity scores (bottom panels) in the TCGA dataset of 500 primary prostate cancer samples (A & B), a meta dataset of 1642 primary prostate cancer samples (C), and 159 mCRPC samples from 3 cohorts (D). The c-Myc and AR activity scores were computed as the sum of z-scores for the Schuhmacher c-Myc-target signature and the Nelson AR-target signature, respectively, in A and as the sum of z-scores for the Jung c-Myc-target signature and the Bluemn AR-target signature, respectively, in **B to D**.



Figure S6. GSEA showing enrichment of the hallmark Myc_targets_v1 and Myc_targets_v2 pathways in mCRPC samples (n = 159) that express a high level of c-Myc.



ank -0.50

0

10.000

20.000

- Enrichment profile — Hits

30.000

Rank in Ordered Dataset

Primary PC - 7 studies (n = 1642) Enrichment plot: HALLMARK_ANDROGEN_RESPONSE Enrichment plot: HALLMARK_MYC_TARGETS_V1 Enrichment plot: HALLMARK_MYC_TARGETS_V2 NES=1.689 NES=1.792 0.40 NES=1.321 0.6 0.6 0.35 0.30 0.25 p-value=0.011 (<u>)</u> 0.5 p-value=0.006 p-value=0.17 <u>()</u> 0.5 e.0.4 9.0 O.4 FDR q=0.017 FDR q=0.009 FDR q=0.16 ichment 0.3 0.2 0.20 0.15 0.10 t 0.3 Ē 0.2 ш_{0.1} <u>لة</u> 0.1 0.05 0.0 0.0 0.00 (Pearson) (Pearson (Pearson) 1.00 1.00 1.00 Myc_high Myc high Myc low Myc low Myc high Mvc low 0.75 0.75 0.75 0.50 0.50 0.50 netric metric metric Zero cross at 7693 Zero cross at 7693 Zero cross at 7693 0.25 0.25 0.25 0.00 0.00 0.00 list list list -0.25 -0.25 -0.25 Ranked - 0.22 -0.50 -0.50 'ENSG00000136997 neg' (negatively co 'ENSG00000136997_neg' (negatively co 'ENSG00000136997_neg' (negatively cor 17,50 2,500 5,000 7.500 10.000 12,500 15,000 17,50 ß 2,500 5,000 7,500 10.000 12,500 15,000 17,50 Ra 2,500 5,000 7,500 10,000 12,500 15,000 Rank in Ordered Dataset Rank in Ordered Dataset Rank in Ordered Dataset Enrichment profile — Hits Ranking metric scores Enrichment profile — Hits Ranking metric scores Enrichment profile — Hits Ranking metric scores В TCGA primary PC (n = 500) Enrichment plot: HALLMARK_MYC_TARGETS_V1 Enrichment plot: HALLMARK_ANDROGEN_RESPONSE Enrichment plot: HALLMARK_MYC_TARGETS_V2 0.7 0.8 NES=1.843 NES=2.004 0.8 NES=2.017 0.7 0.6 3 0.7 0.6 e (ES) p-value=0.01 p-value=0.0 p-value=0.0 0.5 SCOLE 0.0 0.5 FDR q=0.014 FDR q=0.0 0.4 FDR q=6.3E-4 t 0.4 번 0.4 0.3 ₿ 0.3 Ĕ 0.3 LU 0.2 0.2 0.2 Ē 0.1 0.1 0.10.0 0.0 0.0 1.00 1.00 1.00 Myc high Pear Myc high Myc high Myc low Pear Myc low Myc low 0.75 0.75 0.75 0.50 0.50 0.50 Zero cross at 32234 Zero cross at 32234 Zero cross at 32234 0.25 0.25 0.25 0.00 0.00 0.00 ŝ ist ist -0.25 · -0.25 ਸ਼ -0.25 p

Figure S7. GSEA showing enrichment of the hallmark androgen response, Myc targets v1, and Myc targets v2 pathways in primary prostate cancer samples that express a high level of c-Myc. A, a meta dataset of 1642 primary prostate cancer samples; **B**, the TCGA dataset of 500 primary prostate cancer samples.

30.000

Rank in Ordered Dataset

'MYC_neg' (negatively correlated)

Ranking metric scores

50.000

40.000

-0.50

10.000

20.000

Enrichment profile — Hits

30.000

Rank in Ordered Dataset

Rank

60.000

'MYC_neg' (negatively correlated)

Ranking metric scores

50.000

60.000

40.000

'MYC_neg' (negatively correlated)

Ranking metric scores

40.000

50,000

-0.50

0

10.000

20.000

- Enrichment profile — Hits

Rank

60.000



Figure S8. Knockdown of c-Myc does not affect CMV-promoter

activity. Luciferase assay showing no change in CMV-promoter activity after c-Myc knockdown. At 24 h after shCtrl- or shMyclentivirus transduction, 22Rv1 cells were transiently transfected with the pGL4-CMV-Luc construct in bulk and reseeded in triplicate 24 h post transfection for luciferase assay.



Figure S9. c-Myc inhibition leads to reduced expression of AR-FL and AR-V7 as well as their target genes. A and B, qRT-PCR (A) and Western blot analysis (B) showing a downregulated expression of AR-FL and AR-V7 mRNA and protein in 22Rv1 cells after treatment with the 10058-F4 c-Myc inhibitor. C, qRT-PCR analysis showing a reduced expression of AR-FL and AR-V7 targets, PSA, UBE2C, CROT, and SOX9, but not the non-AR target PCP4 in 22Rv1 cells after treatment with 10058-F4. *, P < 0.05 from the control cells.