SUPPLEMENTARY INFORMATION

Anti-Tumor Activity of the IGF-1/IGF-2-Neutralizing Antibody Xentuzumab (BI 836845) in Combination with Enzalutamide in Prostate Cancer Models

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# ABBREVIATIONS

AKT, AKT serine/threonine kinase; ANOVA, analysis of variance; AR, androgen receptor; AR-FL, full-length AR; AR-V7, AR variant 7; ATCC, American Type Culture Collection; Bad, Bcl2-associated agonist of cell death; Bak, Bcl-2 antagonist/killer; Bax, Bcl-2-like protein 4 (BCL2 associated X, apoptosis regulator); Bcl-XL, B-cell lymphoma-extra large; Bim, Bcl-2-like protein 11 (BCL2L11; BCL2 like 11); CDC20, cell division cycle protein 20; CDK1, cyclin-dependent kinase 1; cDNA, complementary DNA; EGF(R), epidermal growth factor (receptor); ENZA, enzalutamide; ERG, ETS related gene (ETS transcription factor ERG); FBS, fetal bovine serum; FDR, false discovery rate; FKBP5, FK506 binding protein 5 (FKBP prolyl isomerase 5); FoxO1, Forkhead box protein O1; FoxO3a, Forkhead box protein O3a; GAPDH, glyceraldehyde-3-phosphate-dehydrogenase; GSEA, gene set enrichment analysis; HPRT1, hypoxanthine phosphoribosyltransferase 1; IGF, insulin-like growth factor; IGF-1R, insulin-like growth factor type 1 receptor (IGF1R; insulin like growth factor 1 receptor); IGV, Integrative Genomics Viewer; INSR, insulin receptor; NT, non-targeting; phospho-, phosphorylated; PARP, poly(adenosine diphosphate-ribose) polymerase; PBS, phosphate-buffered saline; PSA, prostate-specific antigen (KLK3; kallikrein-related peptidase 3); PTEN, phosphatase and tensin homolog; PVDF, polyvinylidene difluoride; qPCR, quantitative polymerase chain reaction; qRT-PCR, quantitative reverse transcription polymerase chain reaction; RRID, Research Resource Identifier; RT-PCR, reverse transcription polymerase chain reaction; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; siRNA, small interfering RNA; STR, short tandem repeat; TGI, tumor growth inhibition; T2E, TMPRSS2-ERG; TMPRSS2, transmembrane serine protease 2; UBE2C, ubiquitin-conjugating enzyme E2 C; XENT, xentuzumab.

# SUPPLEMENTARY METHODS

## Xentuzumab: amino acid sequence and structure

Xentuzumab (BI 836845; immunoglobulin G1-lambda1, anti-[*Homo sapiens* IGF-1 [insulin-like growth factor 1, somatomedin C] and IGF-2 [insulin-like growth factor 2, somatomedin A] humanized monoclonal antibody):   
gamma1 heavy chain (1-447) [humanized VH (*Homo sapiens* IGHV3-23\*03 (88.80%) -(IGHD) -IGHJ5\*01) [8.8.10] (1-117) -IGHG1\*01, Gm17,1 (CH1 (118-215), hinge (216-230), CH2 (231-340), CH3 (341-445), CHS (446-447)) (118-447)], (220-215')-disulfide with lambda1 light chain (1'-216') [humanized V-LAMBDA (Homo sapiens IGLV1-40\*01 (88.20%) -IGLJ2\*01) [8.3.11] (1'-110') -IGLC2\*01 A43>G (154) (111'-216')]; dimer (226-226'':229-229'')-bisdisulfide.

Heavy chain:

QVELVESGGG LVQPGGSLRL SCAASGFTFT SYWMSWVRQA PGKGLELVSS 50

ITSYGSFTYY ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARNM 100

YTHFDSWGQG TLVTVSSAST KGPSVFPLAP SSKSTSGGTA ALGCLVKDYF 150

PEPVTVSWNS GALTSGVHTF PAVLQSSGLY SLSSVVTVPS SSLGTQTYIC 200

NVNHKPSNTK VDKKVEPKSC DKTHTCPPCP APELLGGPSV FLFPPKPKDT 250

LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY 300

RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT 350

LPPSRDELTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS 400

DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE ALHNHYTQKS LSLSPGK 447

Light chain:

DIVLTQPPSV SGAPGQRVTI SCSGSSSNIG SNSVSWYQQL PGTAPKLLIY 50

DNSKRPSGVP DRFSGSKSGT SASLAITGLQ SEDEADYYCQ SRDTYGYYWV 100

FGGGTKLTVL GQPKAAPSVT LFPPSSEELQ ANKATLVCLI SDFYPGAVTV 150

AWKGDSSPVK AGVETTTPSK QSNNKYAASS YLSLTPEQWK SHRSYSCQVT 200

HEGSTVEKTV APTECS 216

Disulfide bridges location:

Intra-H (C23-C104) 22-96 144-200 261-321 367-425   
22''-96'' 144''-200'' 261''-321'' 367''-425''

Intra-L (C23-C104) 22'-89' 138'-197'

22'''-89''' 138'''-197'''

Inter-H-L (h 5-CL 126) 220-215' 220''-215'''

Inter-H-H (h 11, h 14) 226-226' 229-229''

N-glycosylation sites:

H CH2 N84.4:

297, 297''

Fucosylated complex bi-antennary CHO-type glycans

## Cell culture conditions

DuCaP and LNCaP cells were cultivated and assayed in RPMI1640 medium supplemented with 10% fetal bovine serum (FBS) and 2 mM L-glutamine. VCaP cells were maintained in Dulbecco’s Modified Eagle Medium supplemented with 10% FBS, 2 mM L-glutamine and 0.1 nM R1881 androgen. Assays (except for the analysis of androgen receptor [AR] signaling markers) were performed using growth medium without R1881. MDA PCa 2b cells were grown in F-12K medium with 20% FBS, 10 ng/mL epidermal growth factor (EGF), 5 μg/mL insulin, 25 ng/mL cholera toxin, 5 μM ethanolamine, 100 pg/mL hydrocortisone, and 45 nM selenious acid. Assays were performed using growth medium without EGF and insulin. PC-3 cells were cultivated and assayed in F-12K medium with 10% FBS.

## Research Resource Identifiers (RRIDs) for cell lines

LNCaP (clone FGC): ATCC Cat# CRL-1740, RRID:CVCL\_1379

MDA PCa 2b: ATCC Cat# CRL-2422, RRID:CVCL\_4748

PC-3: ATCC Cat# CRL-1435, RRID:CVCL\_0035

VCaP: ATCC Cat# CRL-2876, RRID:CVCL\_2235

DuCaP: Provided by Prof. Dr Helmut Klocker, University Hospital Innsbruck, Austria, RRID:CVCL\_2025

## Western blot analysis

Whole-cell lysates were prepared using complete lysis buffer from Cell Signaling or Mesoscale Discovery. Equal amounts of total protein were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and then transferred onto polyvinylidene difluoride (PVDF) membrane and hybridized to a specific primary antibody (Supplementary Table S2A) and horseradish peroxidase-conjugated secondary antibody (DakoCytomation) for subsequent detection by enhanced chemiluminescence (Amersham GE Healthcare).

Quantitative analysis of protein bands was performed using the image analysis software ImageQuant TL 8.1 (GE Healthcare Life Sciences), normalized to the beta-actin (ß-actin [ACTB; actin beta]) loading control and presented as relative intensity to vehicle.

## Simple WesternTM Assay

Whole-cell lysates (see Western blot analysis) were mixed with sample diluent and Fluorescent 5X Master Mix to obtain a final protein concentration of 0.4 µg/µl, analyzed and quantified with a Simple WesternTM assay using the WesTM instrument by ProteinSimple as previously described (1). All steps of the assay were performed according to the manufacturer’s protocol. The chemiluminescent signals for phospho-Bcl2-associated agonist of cell death (phospho-Bad; S112) and phospho-Bad (S136) were detected and quantified using Compass software (Version 3.1.7; ProteinSimple). Values for specific protein expression were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) loading control.

## RNA isolation and quantitative polymerase chain reaction (qPCR) analysis of in vitro samples

Total RNA was extracted using the QIAshredder kit (Qiagen) for cell homogenization, and the RNeasy kit including DNase digestion (Qiagen) for RNA isolation. Complementary DNA (cDNA) synthesis was performed using the SuperScript® VILO™ cDNA Synthesis Kit (Life Technologies) according to the manufacturer’s protocol, using 100 ng RNA for each reaction. For each sample, qPCR using QuantiTect Multiplex PCR Kit (Qiagen) was run in duplicate, and each reaction contained 2 µL of cDNA in a total volume of 25 µL using a StepOnePlus™ Real-Time PCR instrument (Applied Biosystems). The androgen receptor-splice variant 7 (AR-V7) TaqMan primers and probe were as follows: Forward: 5′-TGT CGT CTT CGG AAA TGT TAT GA-3′; reverse: 5′-TCA TTT TGA GAT GCT TGC AAT TG-3′; probe: FAM-TCT GGG AGA AAA ATT-MGB. Other specific TaqMan primer probe sets are listed in Supplementary Table S2B. All were purchased from Applied Biosystems.

Conditions for qPCR were 8 min at 95°C, followed by 45 cycles of 45 seconds at 94°C and 45 seconds at 60°C. Target mRNA levels were normalized against hypoxanthine phosphoribosyltransferase 1 (HPRT1) levels. The ∆∆Ct method was used to compare the relative expression, and fold change in gene expression relative to untreated control was calculated. qPCR data are represented as mean ± standard deviation for repeats.

## RNA interference

VCaP cells were seeded in 96 well plates. After 24-hour incubation at 37°C, cells were transfected with 20 nM phosphatase and tensin homolog (PTEN) ON-TARGETplus SMARTpool small interfering RNA (siRNA) (Dharmacon). The transfection reagent Lipofectamine RNAiMAX (Invitrogen) was used according to the manufacturers’ instructions. At the time of transfection, cells were treated with 0.1 µM xentuzumab (XENT), 1 µM enzalutamide (ENZA) or XENT+ENZA. After 72-hour incubation at 37°C, cell viability was measured using the CellTiter-Glo® Luminescent Assay (Promega).

To investigate the efficiency of silencing, knockdown of PTEN mRNA was quantified using qPCR, whilst knockdown of the protein was visualized by Western blot analysis. 24 hours after seeding, cells were transfected with 20 nM PTEN ON-TARGETplus SMARTpool siRNA, with transfection reagent alone (Mock) or with ON-TARGETplus Non-targeting (NT) siRNA (Thermo Scientific). VCaP cells were cultured for a further 72 hours before being lysed for Western blot analysis or processed for qPCR by FastLane Cell Multiplex Kit (Qiagen) according to the manufacturer’s instructions. Western blot analyses using PTEN antibody were performed as described above. The FastLane cell lysates were used directly in real-time one-step reverse transcription-PCR (RT-PCR) using PTEN TaqMan primers and probe (Applied Biosystems). Conditions for qPCR were 20 min at 50°C and 15 min at 95°C, followed by 45 cycles of 20 seconds at 95°C and 45 seconds at 60°C. PTEN mRNA levels were normalized against HPRT1 levels and presented as fold change to Mock control.

## Statistical analysis of in vitro activity

Statistical differences between means for the different groups were evaluated with GraphPad Prism software (GraphPad Software Inc.) using one-way analysis of variance (ANOVA) followed by pairwise t tests. The *P* values generated by the ttest were adjusted for multiple comparisons. Adjusted *P* values <0.05 were considered to be statistically significant.

## Analysis of PTEN status of LuCaP 96CR

Total RNA was isolated using the RNeasy Plus Universal Mini kit (Qiagen, #73404). RNA-Seq sequencing libraries were prepared using the TruSeq RNA Library Preparation Kit v2 (Illumina) and subsequently sequenced on the Illumina HiSeq 2500 system using a paired-end 100bp protocol. Sequencing reads from grafted samples were filtered into human and mouse reads using Disambiguate based on mappings to hg38 and mm10. Filtered reads from the RNA-Seq experiment were processed with a pipeline building upon the implementation of the ENCODE “Long RNA-seq” pipeline: Reads were mapped against the Homo sapiens (human) genome hg38/GRCh38 (primary assembly, excluding alternate contigs) using the STAR (v2.5.2b) aligner. For quantification, transcript annotations from Ensembl version 86 were used, which corresponds to GENCODE 25. Samples were quantified with the above annotations, using RSEM (v1.3.0) and featureCount (v1.5.1). Quality controls were implemented using FastQC (v0.11.5), picardmetrics (v0.2.4) (available online at: https://github.com/slowkow/picardmetrics), and dupRadar (v1.0.0) at the respective steps.

## LuCaP 96CR tumor explantation

Three LuCaP tumors (450, 500, and 900 mm³) were explanted from sacrificed host animals, soaked in gentamicin for 5 min, rinsed in 1× phosphate-buffered saline (PBS), and cut into ~20 mg sized pieces for subcutaneous transplantation.

The LuCaP 96CR patient-derived xenograft model (2) is derived from LuCaP 96 and is an AR wild-type, TMPRSS2-ERG (T2E) fusion-negative, prostate-specific antigen (PSA [KLK3; kallikrein-related peptidase 3])-moderate model of human prostate cancer that exhibits insulin-like growth factor type 1 receptor (IGF-1R) expression, and is non-responsive to docetaxel.

## Analysis of in vivo activity

Data were collected and calculated by Studylog Study Director Software and uploaded into Microsoft Excel for further analysis. Tumor volume was calculated based on the formula: tumor volume = length × width × height × 0.5236. Tumor growth inhibition (TGI) was calculated based on the formula: TGI = 100 × {1–[(treatedfinal day–treatedday1) / (controlfinal day–controlday1)]}. The tumor sizes of mice sacrificed were carried forward for calculation until 75% of all mice were sacrificed. Tumor volume of the individual groups was only shown as long as 75% of all mice were still alive.

One-sided decreasing Mann–Whitney tests were used to compare tumor volumes (efficacy) or PSA levels. *P* values were adjusted for multiple comparisons according to Bonferroni–Holm. Kaplan–Meier survival curves were calculated using Prism version 3.0 (GraphPad, La Jolla, CA); the same program was used to calculate corresponding *P* values using a log-rank (Mantel–Cox) test.

## RNA isolation and qPCR analysis of tumor samples

For qPCR, total RNA was obtained from tumors using TRIzol (Life Technologies). RNA was converted to cDNA using SuperScript First-Strand Synthesis System according to the manufacturer’s protocol, with random primers (Life Technologies). Relative quantitative RT-PCR (qRT-PCR) was then performed using a ViiA 7 Real-Time PCR system (Life Technologies) and iTaq Universal SYBR Green Supermix (Bio-Rad Laboratories). qPCR data were analyzed using ViiA 7 Software (Life Technologies). Target mRNA levels were normalized against RPL13A levels. The ∆∆Ct method was used to compare the relative expression, and a relative quantification value was achieved.

## RNA-Seq and gene set enrichment analysis (GSEA)

RNA concentration, purity and integrity were assessed by NanoDrop (Thermo Fisher Scientific Inc.) and Agilent Bioanalyzer. RNA-Seq libraries were constructed from 1 µg total RNA using the Illumina TruSeq Stranded mRNA LT Sample Prep Kit according to the manufacturer’s protocol. Barcoded libraries were pooled and sequenced on the Illumina HiSeq 2500, generating 50 bp paired end reads. Sequencing reads were mapped to the hg19 human and mm10 mouse genomes using TopHat version 2.0.12 (3). Sequences aligning to the mouse genome deriving from potential contamination with mouse tissue were removed from the analysis as previously described (4). Gene-level abundance was quantified from the filtered human alignments in R using the Genomic Alignments Bioconductor package (5). Differential expression was assessed using transcript abundances as inputs to the edgeR Bioconductor package in R (6). For edgeR analysis, genes filtered for an expression level of ≥1 count per million reads in at least two samples were used to calculate expression differences using an exact test with a negative binomial distribution, applying a significance level of 0.05 with Benjamin–Hochberg false discovery rate (FDR) adjustment.

Gene expression results were ranked by their edgeR statistics and used to conduct GSEA, to determine patterns of pathway activity in different treatment groups (7). We utilized the curated pathways from within the MSigDB version 6.0. The Reactome pathway database was used for GSEA of expression patterns in established signaling pathways.

## Immunohistochemistry (IHC) analysis

LuCaP 96CR tumors were formalin fixed and embedded in paraffin. Sections of 10 µm thickness were prepared for hematoxylin and eosin staining in addition to IHC. For better antigen retrieval, slides were placed in a 0.01 M citrate buffer for 25 min and then blocked with 5% serum in PBS. Primary and secondary antibodies were diluted in 5% serum/PBS. The human-specific anti-AR N-terminus antibody AR441 (Santa Cruz Biotechnology), the anti-human AR C-terminus antibody clone SP242 (Spring Bioscience), and the AR variant 7 (AR-V7)-specific anti-AR antibody EPR15656 (Abcam) were used for detecting AR-FL and AR-V7. Statistical analysis was conducted using a one-way ANOVA followed by a Tukey test.

# SUPPLEMENTARY TABLES AND FIGURES

**Supplementary Table S1.**

Dates of mycoplasma testing and STR analysis

|  |  |  |  |
| --- | --- | --- | --- |
| **Cell line** | **Experiment dates** | **Mycoplasma tests** | **STR analysis** |
| VCaP | 28 June 2012  2 April 2013  7 May 2013  24 June 2013  18 July 2013  30 March 2015  23 November 2016  28 November 2016  19 August 2019  2 September 2019 | 23 May 2012  27 June 2012  30 January 2015  4 August 2015  1 October 2015  11 September 2019  11 October 2019 | 9 July 2012  5 March 2014  16 October 2019 |
| LNCaP (FGC) | 5 August 2013  3 March 2015  10 July 2017 | 27 June 2012  3 January 2015  25 March 2015 | 10 June 2013 |
| MDA PCa 2b | 6 December 2012  30 March 2015  9 August 2017  25 September 2017 | 27 June 2012  28 August 2012  30 January 2015  June 2015  3 August 2017 | 3 April 2012  31 August 2015  21 September 2015  20 November 2017 |
| DuCaP | 15 November 2012  31 July 2017  2 August 2017 | 10 October 2012  3 August 2017 | 5 November 2012  9 April 2018 |
| PC-3 | 5 June 2012  31 July 2017  2 August 2017 | 23 May 2012  27 June 2012 | 27 March 2012  2 December 2013 |

**Supplementary Table S2.**

(A) Primary antibodies used for Western blot or Simple WesternTM analysis and their respective RRIDs; (B) TaqMan primer and probe sets used for real-time qPCR

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **A) Antibodies** | **Cat No.** | **Supplier** | **RRID** | |
| β-Actin loading control | 4967 | Cell Signaling Technology | Cell Signaling Technology Cat# 4967, RRID:AB\_330288 | |
| β-Actin loading control | 3700 | Cell Signaling Technology | Cell Signaling Technology Cat# 3700, RRID:AB\_2242334 | |
| GAPDH loading control | 2118 | Cell Signaling Technology | Cell Signaling Technology Cat# 2118, RRID:AB\_561053 | |
| AKT | 9272 | Cell Signaling Technology | Cell Signaling Technology Cat# 9272, RRID:AB\_329827 | |
| AR | 5153 | Cell Signaling Technology | Cell Signaling Technology Cat# 5153, RRID:AB\_10691711 | |
| Bak | 12105 | Cell Signaling Technology | Cell Signaling Technology Cat# 12105, RRID:AB\_2716685 | |
| Bax | 5023 | Cell Signaling Technology | Cell Signaling Technology Cat# 5023, RRID:AB\_10557411 | |
| Bcl-XL | 2762 | Cell Signaling Technology | Cell Signaling Technology Cat# 2762, RRID:AB\_10694844 | |
| Bim | 2933 | Cell Signaling Technology | Cell Signaling Technology Cat# 2933, RRID:AB\_1030947 | |
| CDK1 | 9116 | Cell Signaling Technology | Cell Signaling Technology Cat# 9116, RRID:AB\_2074795 | |
| Cleaved Caspase-3 | 9661 | Cell Signaling Technology | Cell Signaling Technology Cat# 9661, RRID:AB\_2341188 | |
| ERG | 97249 | Cell Signaling Technology | Cell Signaling Technology Cat# 97249, RRID:AB\_2721841 | |
| FKBP5 | 12210 | Cell Signaling Technology | Cell Signaling Technology Cat# 12210, RRID:AB\_2797846 | |
| FoxO1 | 14952 | Cell Signaling Technology | Cell Signaling Technology Cat# 14952, RRID:AB\_2722487 | |
| FoxO3a | 99199 | Cell Signaling Technology | Cell Signaling Technology Cat# 99199, RRID:AB\_2800315 | |
| IGF-1R | 3027 | Cell Signaling Technology | Cell Signaling Technology Cat# 3027, RRID:AB\_2122378 | |
| PARP | 9542 | Cell Signaling Technology | Cell Signaling Technology Cat# 9542, RRID:AB\_2160739 | |
| Phospho-AKT (S473) | 4060 | Cell Signaling Technology | Cell Signaling Technology Cat# 4060, RRID:AB\_2315049 | |
| Phospho-Bad (S112) | 5284 | Cell Signaling Technology | Cell Signaling Technology Cat# 5284, RRID:AB\_560884 | |
| Phospho-Bad (S136) | 4366 | Cell Signaling Technology | Cell Signaling Technology Cat# 4366, RRID:AB\_10547878 | |
| Phospho-FoxO1 (T24)/FoxO3a (T32) | 9464 | Cell Signaling Technology | Cell Signaling Technology Cat# 9464, RRID:AB\_329842 | |
| Phospho-FoxO3a (S253) | 9466 | Cell Signaling Technology | Cell Signaling Technology Cat# 9466, RRID:AB\_2106674 | |
| Phospho-IGF-1R/phospho-INSR | 3024 | Cell Signaling Technology | Cell Signaling Technology Cat# 3024, RRID:AB\_331253 | |
| PSA | 2475 | Cell Signaling Technology | Cell Signaling Technology Cat# 2475, RRID:AB\_2797601 | |
| PTEN | 9559 | Cell Signaling Technology | Cell Signaling Technology Cat# 9559, RRID:AB\_390810 | |
| TMPRSS2 | ab92323 | Abcam | Abcam Cat# ab92323, RRID:AB\_10585592 | |
| UBE2C | A-650 | Novus Biologicals/Boston Biochem | Boston Biochem Cat# A-650, RRID:AB\_10693825 | |
| **B) Genes** | | | | **Assay ID** |
| CDK1 (cdc2) | | | | Hs00938777\_m1 |
| CDC20 | | | | Hs00426680\_mH |
| ERG | | | | Hs01554629\_m1 |
| FKBP5 | | | | Hs01561006\_m1 |
| PSA (KLK3) | | | | Hs02576345\_m1 |
| PTEN | | | | Hs02621230\_s1 |
| TMPRSS2 | | | | Hs01120965\_m1 |
| UBE2C | | | | Hs00964100\_g1 |

**Supplementary Table S3**

**.** Gene set enrichment analysis (GSEA) Molecular Signatures Database (MSigDB) C2: Canonical pathways with FDR <0.05

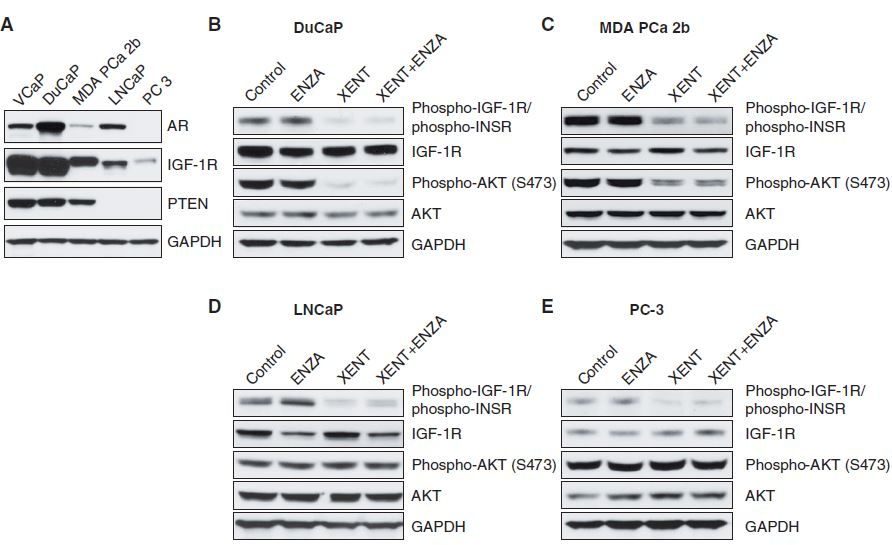
|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Name** | **Size** | **ES** | **NES** | **NOM**  ***P* value** | **FDR**  **q-value** | **FWER**  ***P* value** | **Rank at max** |
| **A) Negatively enriched** | | | | | | | |
| PID\_HIF1\_TFPATHWAY | 54 | -0.60008 | -2.30368 | 0 | 0 | 0 | 3495 |
| KEGG\_ENDOCYTOSIS | 160 | -0.47299 | -2.23447 | 0 | 0.001264 | 0.002 | 3268 |
| KEGG\_DORSO\_VENTRAL\_AXIS\_FORMATION | 19 | -0.69927 | -2.10496 | 0 | 0.012143 | 0.029 | 1965 |
| PID\_ECADHERIN\_STABILIZATION\_PATHWAY | 38 | -0.58437 | -2.10215 | 0 | 0.009107 | 0.029 | 2947 |
| NABA\_MATRISOME | 437 | -0.39896 | -2.07504 | 0 | 0.011522 | 0.044 | 3168 |
| REACTOME\_CELL\_JUNCTION\_ORGANIZATION | 53 | -0.53604 | -2.05879 | 0 | 0.011483 | 0.052 | 3676 |
| KEGG\_ARRHYTHMOGENIC\_RIGHT\_VENTRICULAR\_CARDIOMYOPATHY\_ARVC | 47 | -0.55332 | -2.05333 | 0 | 0.010586 | 0.056 | 3408 |
| PID\_GMCSF\_PATHWAY | 30 | -0.5995 | -2.0525 | 0 | 0.009263 | 0.056 | 3155 |
| PID\_INTEGRIN3\_PATHWAY | 24 | -0.63947 | -2.05142 | 0 | 0.008368 | 0.057 | 3779 |
| REACTOME\_REGULATION\_OF\_INSULIN\_SECRETION | 62 | -0.51124 | -2.04562 | 0 | 0.008411 | 0.063 | 3397 |
| KEGG\_CIRCADIAN\_RHYTHM\_MAMMAL | 13 | -0.75736 | -2.03196 | 0 | 0.010241 | 0.085 | 1706 |
| REACTOME\_POTASSIUM\_CHANNELS | 44 | -0.54361 | -2.01533 | 0 | 0.013023 | 0.116 | 4068 |
| NABA\_ECM\_AFFILIATED | 86 | -0.47085 | -2.01288 | 0 | 0.012511 | 0.12 | 2492 |
| NABA\_MATRISOME\_ASSOCIATED | 308 | -0.39656 | -1.9957 | 0 | 0.014673 | 0.148 | 3168 |
| REACTOME\_SIGNALING\_BY\_EGFR\_IN\_CANCER | 96 | -0.45679 | -1.99493 | 0 | 0.013863 | 0.15 | 2170 |
| REACTOME\_INHIBITION\_OF\_INSULIN\_SECRETION\_BY\_ADRENALINE\_NORADRENALINE | 19 | -0.65603 | -1.99115 | 0.003521 | 0.013862 | 0.159 | 3375 |
| PID\_IL8\_CXCR2\_PATHWAY | 21 | -0.64145 | -1.97893 | 0 | 0.015935 | 0.191 | 4810 |
| KEGG\_DILATED\_CARDIOMYOPATHY | 51 | -0.51727 | -1.97734 | 0 | 0.015541 | 0.196 | 3683 |
| PID\_TXA2PATHWAY | 41 | -0.54023 | -1.97642 | 0 | 0.014986 | 0.2 | 2946 |
| BIOCARTA\_IL2\_PATHWAY | 19 | -0.64398 | -1.97405 | 0 | 0.014675 | 0.207 | 2946 |
| REACTOME\_EGFR\_DOWNREGULATION | 24 | -0.62561 | -1.97295 | 0.001873 | 0.014216 | 0.21 | 1726 |
| PID\_IL8\_CXCR1\_PATHWAY | 17 | -0.66804 | -1.96941 | 0 | 0.014648 | 0.227 | 3944 |
| PID\_AJDISS\_2PATHWAY | 39 | -0.53417 | -1.96242 | 0.001934 | 0.015488 | 0.248 | 2313 |
| REACTOME\_G\_PROTEIN\_BETA\_GAMMA\_SIGNALLING | 20 | -0.63931 | -1.95304 | 0 | 0.016873 | 0.276 | 3375 |
| REACTOME\_INTEGRATION\_OF\_ENERGY\_METABOLISM | 84 | -0.45707 | -1.9528 | 0 | 0.016198 | 0.276 | 3739 |
| KEGG\_GAP\_JUNCTION | 67 | -0.4777 | -1.9431 | 0 | 0.017068 | 0.299 | 2025 |
| REACTOME\_CELL\_CELL\_JUNCTION\_ORGANIZATION | 33 | -0.55145 | -1.93635 | 0 | 0.018014 | 0.323 | 3676 |
| REACTOME\_INHIBITION\_OF\_VOLTAGE\_GATED\_CA2\_CHANNELS\_VIA\_GBETA\_GAMMA\_SUBUNITS | 14 | -0.68587 | -1.93294 | 0.001792 | 0.018217 | 0.337 | 3707 |
| REACTOME\_G\_PROTEIN\_ACTIVATION | 16 | -0.65871 | -1.92956 | 0 | 0.018287 | 0.348 | 3375 |
| PID\_HDAC\_CLASSIII\_PATHWAY | 23 | -0.59285 | -1.9179 | 0 | 0.020891 | 0.406 | 2313 |
| SIG\_BCR\_SIGNALING\_PATHWAY | 37 | -0.52971 | -1.91623 | 0 | 0.020539 | 0.412 | 2946 |
| PID\_CD8\_TCR\_DOWNSTREAM\_PATHWAY | 33 | -0.54728 | -1.91318 | 0 | 0.020715 | 0.423 | 1965 |
| REACTOME\_INTEGRIN\_CELL\_SURFACE\_INTERACTIONS | 56 | -0.49303 | -1.91015 | 0 | 0.020959 | 0.437 | 4591 |
| REACTOME\_G\_BETA\_GAMMA\_SIGNALLING\_THROUGH\_PLC\_BETA | 14 | -0.68515 | -1.90643 | 0.001852 | 0.021593 | 0.452 | 3375 |
| PID\_SYNDECAN\_4\_PATHWAY | 24 | -0.59735 | -1.90641 | 0.001773 | 0.020976 | 0.452 | 3031 |
| ST\_INTEGRIN\_SIGNALING\_PATHWAY | 68 | -0.46994 | -1.8983 | 0 | 0.022292 | 0.475 | 2451 |
| REACTOME\_THROMBOXANE\_SIGNALLING\_THROUGH\_TP\_RECEPTOR | 15 | -0.67084 | -1.8962 | 0.001938 | 0.022302 | 0.486 | 3375 |
| BIOCARTA\_ERK\_PATHWAY | 25 | -0.58842 | -1.89155 | 0 | 0.023058 | 0.502 | 2342 |
| KEGG\_FOCAL\_ADHESION | 147 | -0.4095 | -1.88944 | 0 | 0.022884 | 0.508 | 3180 |
| PID\_A6B1\_A6B4\_INTEGRIN\_PATHWAY | 41 | -0.52448 | -1.88857 | 0 | 0.022434 | 0.509 | 4265 |
| PID\_MET\_PATHWAY | 76 | -0.45184 | -1.88746 | 0 | 0.022284 | 0.513 | 2183 |
| KEGG\_PRION\_DISEASES | 23 | -0.58652 | -1.88419 | 0 | 0.022917 | 0.534 | 2215 |
| REACTOME\_DEVELOPMENTAL\_BIOLOGY | 287 | -0.3753 | -1.88375 | 0 | 0.022561 | 0.536 | 4526 |
| REACTOME\_CIRCADIAN\_CLOCK | 46 | -0.50162 | -1.88218 | 0.001808 | 0.022448 | 0.54 | 1706 |
| REACTOME\_PLATELET\_ACTIVATION\_SIGNALING\_AND\_AGGREGATION | 140 | -0.41053 | -1.87976 | 0 | 0.022508 | 0.549 | 3456 |
| BIOCARTA\_INTEGRIN\_PATHWAY | 35 | -0.53135 | -1.87697 | 0 | 0.022347 | 0.553 | 2947 |
| KEGG\_AXON\_GUIDANCE | 102 | -0.43363 | -1.87513 | 0 | 0.022268 | 0.557 | 2720 |
| BIOCARTA\_AT1R\_PATHWAY | 28 | -0.55612 | -1.87422 | 0 | 0.022064 | 0.562 | 1965 |
| NABA\_ECM\_REGULATORS | 110 | -0.41401 | -1.87384 | 0 | 0.021766 | 0.563 | 3168 |
| KEGG\_BLADDER\_CANCER | 37 | -0.51656 | -1.86935 | 0 | 0.022574 | 0.581 | 1965 |
| PID\_IGF1\_PATHWAY | 30 | -0.55006 | -1.86457 | 0.001845 | 0.023603 | 0.604 | 4387 |
| BIOCARTA\_TOB1\_PATHWAY | 8 | -0.78494 | -1.85671 | 0.004024 | 0.025554 | 0.642 | 3037 |
| PID\_SYNDECAN\_1\_PATHWAY | 28 | -0.54799 | -1.85649 | 0.003831 | 0.025095 | 0.642 | 3168 |
| REACTOME\_G\_BETA\_GAMMA\_SIGNALLING\_THROUGH\_PI3KGAMMA | 17 | -0.62693 | -1.85503 | 0 | 0.025143 | 0.647 | 3375 |
| PID\_THROMBIN\_PAR1\_PATHWAY | 35 | -0.52716 | -1.85032 | 0 | 0.025904 | 0.672 | 3199 |
| PID\_ANTHRAX\_PATHWAY | 10 | -0.73237 | -1.84832 | 0.005725 | 0.02624 | 0.689 | 2369 |
| PID\_ENDOTHELIN\_PATHWAY | 46 | -0.49908 | -1.8464 | 0 | 0.026455 | 0.703 | 3462 |
| ST\_MYOCYTE\_AD\_PATHWAY | 21 | -0.59446 | -1.84612 | 0.001825 | 0.026108 | 0.705 | 4064 |
| REACTOME\_AXON\_GUIDANCE | 183 | -0.38468 | -1.83869 | 0 | 0.0277 | 0.731 | 4526 |
| REACTOME\_THROMBIN\_SIGNALLING\_THROUGH\_PROTEINASE\_ACTIVATED\_RECEPTORS\_ PARS | 23 | -0.58699 | -1.83521 | 0.007366 | 0.028423 | 0.745 | 3375 |
| PID\_DELTA\_NP63\_PATHWAY | 34 | -0.52004 | -1.83439 | 0.003676 | 0.028285 | 0.748 | 1251 |
| KEGG\_CYTOKINE\_CYTOKINE\_RECEPTOR\_INTERACTION | 106 | -0.41557 | -1.83259 | 0 | 0.028329 | 0.757 | 3235 |
| KEGG\_MELANOGENESIS | 73 | -0.44791 | -1.83213 | 0.001805 | 0.028057 | 0.758 | 3462 |
| KEGG\_NOTCH\_SIGNALING\_PATHWAY | 41 | -0.49146 | -1.83212 | 0 | 0.027619 | 0.758 | 3272 |
| KEGG\_LYSOSOME | 112 | -0.41097 | -1.83182 | 0 | 0.027272 | 0.761 | 4368 |
| REACTOME\_THE\_ROLE\_OF\_NEF\_IN\_HIV1\_REPLICATION\_AND\_DISEASE\_PATHOGENESIS | 20 | -0.59195 | -1.8291 | 0.001894 | 0.027574 | 0.768 | 1075 |
| BIOCARTA\_SPRY\_PATHWAY | 17 | -0.61914 | -1.82582 | 0.003891 | 0.028095 | 0.78 | 1965 |
| BIOCARTA\_MCALPAIN\_PATHWAY | 21 | -0.57543 | -1.82509 | 0.005952 | 0.02794 | 0.782 | 1965 |
| PID\_INTEGRIN1\_PATHWAY | 40 | -0.5047 | -1.82332 | 0.001866 | 0.02831 | 0.79 | 3156 |
| KEGG\_ARACHIDONIC\_ACID\_METABOLISM | 33 | -0.51966 | -1.82266 | 0 | 0.028155 | 0.791 | 1843 |
| REACTOME\_SHC1\_EVENTS\_IN\_EGFR\_SIGNALING | 15 | -0.65543 | -1.81812 | 0.003683 | 0.029393 | 0.802 | 1965 |
| KEGG\_LONG\_TERM\_DEPRESSION | 48 | -0.48531 | -1.81699 | 0 | 0.029383 | 0.804 | 4958 |
| BIOCARTA\_PTDINS\_PATHWAY | 22 | -0.57343 | -1.81228 | 0.007105 | 0.030455 | 0.823 | 5158 |
| REACTOME\_CELL\_CELL\_COMMUNICATION | 84 | -0.42897 | -1.81206 | 0 | 0.03018 | 0.826 | 4079 |
| REACTOME\_SIGNALING\_BY\_ROBO\_RECEPTOR | 25 | -0.54883 | -1.81139 | 0.00354 | 0.029993 | 0.827 | 5158 |
| BIOCARTA\_RANKL\_PATHWAY | 10 | -0.71384 | -1.80936 | 0.00365 | 0.030289 | 0.834 | 815 |
| NABA\_CORE\_MATRISOME | 129 | -0.40239 | -1.80681 | 0 | 0.030787 | 0.843 | 3540 |
| REACTOME\_GABA\_B\_RECEPTOR\_ACTIVATION | 24 | -0.54979 | -1.80359 | 0.00578 | 0.031434 | 0.851 | 3707 |
| PID\_NFAT\_TFPATHWAY | 22 | -0.58707 | -1.80288 | 0.001789 | 0.031304 | 0.856 | 3588 |
| REACTOME\_BMAL1\_CLOCK\_NPAS2\_ACTIVATES\_CIRCADIAN\_EXPRESSION | 31 | -0.52921 | -1.80246 | 0.003623 | 0.031143 | 0.856 | 2313 |
| PID\_ARF6\_PATHWAY | 26 | -0.54217 | -1.79986 | 0.001894 | 0.031625 | 0.86 | 3474 |
| REACTOME\_OPIOID\_SIGNALLING | 57 | -0.45812 | -1.79979 | 0 | 0.031239 | 0.86 | 3462 |
| REACTOME\_INTEGRIN\_ALPHAIIB\_BETA3\_SIGNALING | 23 | -0.56926 | -1.7983 | 0.001883 | 0.031343 | 0.865 | 4300 |
| REACTOME\_RESPONSE\_TO\_ELEVATED\_PLATELET\_CYTOSOLIC\_CA2\_ | 54 | -0.46057 | -1.7927 | 0.001802 | 0.032947 | 0.878 | 3856 |
| REACTOME\_PLATELET\_HOMEOSTASIS | 48 | -0.46828 | -1.79005 | 0 | 0.033748 | 0.884 | 3375 |
| REACTOME\_SIGNALING\_BY\_RHO\_GTPASES | 91 | -0.41954 | -1.78845 | 0.001669 | 0.034043 | 0.889 | 4421 |
| PID\_RETINOIC\_ACID\_PATHWAY | 28 | -0.52855 | -1.78405 | 0.003656 | 0.034989 | 0.9 | 2313 |
| BIOCARTA\_IL3\_PATHWAY | 13 | -0.6567 | -1.78035 | 0.010695 | 0.036173 | 0.911 | 3006 |
| REACTOME\_HEMOSTASIS | 306 | -0.35295 | -1.77726 | 0 | 0.036893 | 0.919 | 3031 |
| REACTOME\_ADP\_SIGNALLING\_THROUGH\_P2RY12 | 14 | -0.6355 | -1.77314 | 0.011321 | 0.037963 | 0.927 | 3375 |
| REACTOME\_ALPHA\_LINOLENIC\_ACID\_ALA\_METABOLISM | 11 | -0.67783 | -1.76928 | 0.009747 | 0.038941 | 0.934 | 2937 |
| REACTOME\_NEF\_MEDIATES\_DOWN\_MODULATION\_OF\_CELL\_SURFACE\_RECEPTORS\_BY\_RECRUITING\_THEM\_TO\_CLATHRIN\_ADAPTERS | 17 | -0.59222 | -1.76823 | 0.009728 | 0.038885 | 0.936 | 836 |
| REACTOME\_INWARDLY\_RECTIFYING\_K\_CHANNELS | 18 | -0.59943 | -1.76614 | 0.010733 | 0.039058 | 0.938 | 3707 |
| REACTOME\_ADHERENS\_JUNCTIONS\_INTERACTIONS | 17 | -0.6132 | -1.76464 | 0.005825 | 0.039295 | 0.942 | 2122 |
| PID\_TAP63\_PATHWAY | 47 | -0.46586 | -1.76119 | 0.001792 | 0.040117 | 0.948 | 3794 |
| PID\_S1P\_META\_PATHWAY | 18 | -0.59149 | -1.75523 | 0.00738 | 0.041972 | 0.954 | 2719 |
| SA\_TRKA\_RECEPTOR | 14 | -0.63429 | -1.75168 | 0 | 0.04312 | 0.961 | 3351 |
| KEGG\_ERBB\_SIGNALING\_PATHWAY | 72 | -0.42471 | -1.7515 | 0 | 0.042743 | 0.961 | 2397 |
| PID\_TGFBR\_PATHWAY | 51 | -0.46018 | -1.75049 | 0.005386 | 0.042708 | 0.962 | 1653 |
| BIOCARTA\_EGF\_PATHWAY | 29 | -0.51546 | -1.7502 | 0.003636 | 0.042355 | 0.962 | 1965 |
| PID\_TCR\_PATHWAY | 46 | -0.46853 | -1.75004 | 0.003597 | 0.041998 | 0.962 | 3456 |
| KEGG\_CELL\_ADHESION\_MOLECULES\_CAMS | 66 | -0.43508 | -1.74943 | 0 | 0.041892 | 0.963 | 2040 |
| REACTOME\_PROSTACYCLIN\_SIGNALLING\_THROUGH\_PROSTACYCLIN\_RECEPTOR | 12 | -0.64335 | -1.74857 | 0.007421 | 0.041763 | 0.964 | 4592 |
| REACTOME\_DEGRADATION\_OF\_THE\_EXTRACELLULAR\_MATRIX | 10 | -0.69592 | -1.74817 | 0.003745 | 0.041506 | 0.966 | 147 |
| BIOCARTA\_CBL\_PATHWAY | 10 | -0.69549 | -1.74803 | 0.007905 | 0.041228 | 0.966 | 4026 |
| BIOCARTA\_CCR3\_PATHWAY | 17 | -0.59995 | -1.74551 | 0.005671 | 0.041808 | 0.97 | 3344 |
| NABA\_ECM\_GLYCOPROTEINS | 98 | -0.40375 | -1.74548 | 0 | 0.041417 | 0.97 | 3468 |
| KEGG\_ADHERENS\_JUNCTION | 66 | -0.42683 | -1.74404 | 0.001805 | 0.041569 | 0.971 | 3332 |
| BIOCARTA\_IL2RB\_PATHWAY | 34 | -0.4935 | -1.74245 | 0.003604 | 0.041858 | 0.972 | 3413 |
| REACTOME\_SIGNAL\_AMPLIFICATION | 21 | -0.56559 | -1.73668 | 0.009398 | 0.044198 | 0.977 | 3375 |
| KEGG\_MAPK\_SIGNALING\_PATHWAY | 201 | -0.35938 | -1.73611 | 0 | 0.044014 | 0.979 | 3563 |
| WNT\_SIGNALING | 71 | -0.4201 | -1.73448 | 0 | 0.044232 | 0.98 | 3433 |
| KEGG\_LONG\_TERM\_POTENTIATION | 52 | -0.45015 | -1.73433 | 0.003442 | 0.043873 | 0.98 | 3381 |
| REACTOME\_NITRIC\_OXIDE\_STIMULATES\_GUANYLATE\_CYCLASE | 14 | -0.62416 | -1.73079 | 0.008993 | 0.044791 | 0.982 | 2084 |
| REACTOME\_EXTRACELLULAR\_MATRIX\_ORGANIZATION | 42 | -0.47212 | -1.72852 | 0.003466 | 0.045369 | 0.983 | 3030 |
| REACTOME\_REGULATION\_OF\_GENE\_EXPRESSION\_IN\_BETA\_CELLS | 10 | -0.67407 | -1.72569 | 0.013436 | 0.046273 | 0.984 | 4393 |
| REACTOME\_SIGNALING\_BY\_ERBB2 | 84 | -0.40713 | -1.72385 | 0 | 0.046677 | 0.985 | 2170 |
| PID\_TCR\_RAS\_PATHWAY | 12 | -0.65495 | -1.72332 | 0.007767 | 0.046409 | 0.985 | 1965 |
| REACTOME\_NGF\_SIGNALLING\_VIA\_TRKA\_FROM\_THE\_PLASMA\_MEMBRANE | 117 | -0.38443 | -1.72332 | 0.001745 | 0.04603 | 0.985 | 2170 |
| REACTOME\_NEURONAL\_SYSTEM | 159 | -0.36639 | -1.72273 | 0 | 0.045865 | 0.985 | 4103 |
| BIOCARTA\_CDMAC\_PATHWAY | 13 | -0.63281 | -1.72242 | 0.018868 | 0.0456 | 0.985 | 1965 |
| BIOCARTA\_CARDIACEGF\_PATHWAY | 13 | -0.63521 | -1.71967 | 0.012797 | 0.046498 | 0.987 | 2635 |
| SA\_B\_CELL\_RECEPTOR\_COMPLEXES | 24 | -0.53861 | -1.71945 | 0.01083 | 0.046232 | 0.987 | 2946 |
| PID\_IL2\_1PATHWAY | 49 | -0.45323 | -1.71825 | 0 | 0.046424 | 0.988 | 1997 |
| BIOCARTA\_GLEEVEC\_PATHWAY | 22 | -0.5415 | -1.7164 | 0.007394 | 0.046644 | 0.99 | 2752 |
| BIOCARTA\_HER2\_PATHWAY | 18 | -0.57745 | -1.7148 | 0.009074 | 0.047031 | 0.99 | 2632 |
| REACTOME\_NUCLEAR\_RECEPTOR\_TRANSCRIPTION\_PATHWAY | 34 | -0.48379 | -1.71373 | 0.007619 | 0.047178 | 0.99 | 1181 |
| PID\_PDGFRB\_PATHWAY | 118 | -0.38342 | -1.71135 | 0.001634 | 0.047818 | 0.99 | 2927 |
| BIOCARTA\_EGFR\_SMRTE\_PATHWAY | 11 | -0.66927 | -1.71005 | 0.015504 | 0.048108 | 0.991 | 1013 |
| REACTOME\_SIGNALING\_BY\_GPCR | 268 | -0.34067 | -1.7097 | 0 | 0.047872 | 0.991 | 3494 |
| REACTOME\_CLASS\_B\_2\_SECRETIN\_FAMILY\_RECEPTORS | 45 | -0.45598 | -1.70621 | 0.007168 | 0.04914 | 0.992 | 3828 |
| KEGG\_PHENYLALANINE\_METABOLISM | 13 | -0.6322 | -1.70427 | 0.003906 | 0.049654 | 0.992 | 2392 |
| KEGG\_RENAL\_CELL\_CARCINOMA | 62 | -0.42826 | -1.70332 | 0.001779 | 0.049704 | 0.992 | 2313 |

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **B) Positively enriched** | | | | | | | |
| REACTOME\_METABOLISM\_OF\_NON\_CODING\_RNA | 47 | 0.658999 | 2.63575 | 0 | 0 | 0 | 2799 |
| REACTOME\_TRANSPORT\_OF\_MATURE\_MRNA\_DERIVED\_FROM\_AN\_INTRONLESS\_TRANSCRIPT | 32 | 0.697238 | 2.538822 | 0 | 0 | 0 | 2799 |
| KEGG\_AMINOACYL\_TRNA\_BIOSYNTHESIS | 41 | 0.653384 | 2.480004 | 0 | 0 | 0 | 3635 |
| REACTOME\_REGULATION\_OF\_GLUCOKINASE\_BY\_GLUCOKINASE\_REGULATORY\_PROTEIN | 26 | 0.71311 | 2.463918 | 0 | 0 | 0 | 2775 |
| REACTOME\_NEP\_NS2\_INTERACTS\_WITH\_THE\_CELLULAR\_EXPORT\_MACHINERY | 27 | 0.719692 | 2.434926 | 0 | 0 | 0 | 1947 |
| REACTOME\_TRNA\_AMINOACYLATION | 42 | 0.640207 | 2.412254 | 0 | 0 | 0 | 3635 |
| REACTOME\_TRANSPORT\_OF\_MATURE\_TRANSCRIPT\_TO\_CYTOPLASM | 52 | 0.595791 | 2.395252 | 0 | 0 | 0 | 2958 |
| REACTOME\_TRANSPORT\_OF\_RIBONUCLEOPROTEINS\_INTO\_THE\_HOST\_NUCLEUS | 27 | 0.686221 | 2.352406 | 0 | 0 | 0 | 2775 |
| REACTOME\_G2\_M\_CHECKPOINTS | 41 | 0.594985 | 2.27661 | 0 | 1.61E-04 | 0.002 | 5316 |
| REACTOME\_CYTOSOLIC\_TRNA\_AMINOACYLATION | 24 | 0.654521 | 2.204073 | 0 | 5.56E-04 | 0.008 | 1692 |
| REACTOME\_INTERACTIONS\_OF\_VPR\_WITH\_HOST\_CELLULAR\_PROTEINS | 32 | 0.614339 | 2.196081 | 0 | 5.05E-04 | 0.008 | 2775 |
| PID\_FANCONI\_PATHWAY | 47 | 0.55281 | 2.193226 | 0 | 4.63E-04 | 0.008 | 1773 |
| REACTOME\_ACTIVATION\_OF\_ATR\_IN\_RESPONSE\_TO\_REPLICATION\_STRESS | 35 | 0.589441 | 2.170877 | 0 | 5.89E-04 | 0.011 | 5316 |
| REACTOME\_MITOCHONDRIAL\_TRNA\_AMINOACYLATION | 21 | 0.668687 | 2.145402 | 0 | 8.47E-04 | 0.017 | 3635 |
| REACTOME\_ANTIVIRAL\_MECHANISM\_BY\_IFN\_STIMULATED\_GENES | 64 | 0.506923 | 2.137085 | 0 | 8.36E-04 | 0.018 | 2775 |
| REACTOME\_GLUCOSE\_TRANSPORT | 34 | 0.573547 | 2.118632 | 0 | 0.001314 | 0.029 | 2775 |
| REACTOME\_EXTENSION\_OF\_TELOMERES | 26 | 0.623496 | 2.103514 | 0 | 0.001929 | 0.045 | 3119 |
| REACTOME\_E2F\_MEDIATED\_REGULATION\_OF\_DNA\_REPLICATION | 32 | 0.577264 | 2.099189 | 0 | 0.001863 | 0.046 | 2695 |
| PID\_BARD1\_PATHWAY | 29 | 0.60749 | 2.097111 | 0 | 0.001802 | 0.047 | 4258 |
| REACTOME\_LATE\_PHASE\_OF\_HIV\_LIFE\_CYCLE | 97 | 0.446205 | 2.041886 | 0 | 0.004112 | 0.112 | 3905 |
| REACTOME\_MITOTIC\_M\_M\_G1\_PHASES | 162 | 0.409729 | 2.038857 | 0 | 0.004049 | 0.116 | 4841 |
| KEGG\_HOMOLOGOUS\_RECOMBINATION | 27 | 0.594074 | 2.032579 | 0 | 0.004299 | 0.128 | 3387 |
| REACTOME\_DNA\_REPLICATION | 181 | 0.406568 | 2.030515 | 0 | 0.004142 | 0.128 | 4841 |
| REACTOME\_CELL\_CYCLE\_CHECKPOINTS | 110 | 0.440681 | 2.030232 | 0 | 0.00397 | 0.128 | 5316 |
| KEGG\_RNA\_DEGRADATION | 56 | 0.497756 | 2.029865 | 0 | 0.003865 | 0.13 | 4198 |
| REACTOME\_PROCESSING\_OF\_CAPPED\_INTRON\_CONTAINING\_PRE\_MRNA | 134 | 0.425223 | 2.028775 | 0 | 0.003743 | 0.131 | 2799 |
| KEGG\_DNA\_REPLICATION | 35 | 0.558003 | 2.025146 | 0 | 0.003812 | 0.139 | 4207 |
| REACTOME\_MRNA\_PROCESSING | 153 | 0.416813 | 2.022874 | 0 | 0.003803 | 0.142 | 2842 |
| REACTOME\_NFKB\_ACTIVATION\_THROUGH\_FADD\_RIP1\_PATHWAY\_MEDIATED\_BY\_CASPASE\_8\_AND10 | 12 | 0.740819 | 2.019892 | 0 | 0.003794 | 0.145 | 2139 |
| REACTOME\_DNA\_REPAIR | 103 | 0.445924 | 2.011224 | 0 | 0.003878 | 0.151 | 3596 |
| BIOCARTA\_ATRBRCA\_PATHWAY | 20 | 0.647757 | 2.010915 | 0 | 0.003776 | 0.152 | 1773 |
| KEGG\_MISMATCH\_REPAIR | 22 | 0.622907 | 2.00454 | 0 | 0.004071 | 0.168 | 3160 |
| REACTOME\_CELL\_CYCLE | 365 | 0.368604 | 2.003285 | 0 | 0.004034 | 0.172 | 4163 |
| REACTOME\_ACTIVATION\_OF\_THE\_PRE\_REPLICATIVE\_COMPLEX | 29 | 0.56564 | 1.993371 | 0 | 0.004488 | 0.194 | 5506 |
| REACTOME\_DOUBLE\_STRAND\_BREAK\_REPAIR | 21 | 0.611162 | 1.990921 | 0 | 0.00448 | 0.2 | 3985 |
| PID\_ATR\_PATHWAY | 39 | 0.522885 | 1.980379 | 0 | 0.00501 | 0.226 | 3427 |
| PID\_AURORA\_B\_PATHWAY | 37 | 0.544663 | 1.980288 | 0.002179 | 0.004874 | 0.226 | 5531 |
| REACTOME\_HIV\_LIFE\_CYCLE | 107 | 0.425065 | 1.979594 | 0 | 0.00482 | 0.228 | 3905 |
| REACTOME\_DNA\_STRAND\_ELONGATION | 30 | 0.557885 | 1.955257 | 0 | 0.006506 | 0.299 | 3943 |
| REACTOME\_G1\_S\_SPECIFIC\_TRANSCRIPTION | 16 | 0.664341 | 1.9522 | 0.002183 | 0.006637 | 0.306 | 3473 |
| REACTOME\_PHOSPHORYLATION\_OF\_THE\_APC\_C | 17 | 0.628569 | 1.937416 | 0.002088 | 0.007688 | 0.354 | 3244 |
| REACTOME\_TELOMERE\_MAINTENANCE | 56 | 0.473293 | 1.92752 | 0.002331 | 0.008398 | 0.384 | 3747 |
| PID\_ATM\_PATHWAY | 33 | 0.535061 | 1.925451 | 0 | 0.008378 | 0.392 | 4032 |
| REACTOME\_CELL\_CYCLE\_MITOTIC | 298 | 0.3621 | 1.913623 | 0 | 0.009281 | 0.433 | 4841 |
| REACTOME\_RNA\_POL\_I\_TRANSCRIPTION\_INITIATION | 24 | 0.579112 | 1.909893 | 0.002088 | 0.009417 | 0.444 | 3531 |
| REACTOME\_DEADENYLATION\_DEPENDENT\_MRNA\_DECAY | 42 | 0.501251 | 1.904544 | 0 | 0.009869 | 0.47 | 4122 |
| REACTOME\_CHROMOSOME\_MAINTENANCE | 93 | 0.423225 | 1.901474 | 0 | 0.009907 | 0.475 | 3119 |
| REACTOME\_MRNA\_DECAY\_BY\_3\_TO\_5\_EXORIBONUCLEASE | 11 | 0.71466 | 1.893028 | 0.002088 | 0.010698 | 0.507 | 3321 |
| REACTOME\_APC\_CDC20\_MEDIATED\_DEGRADATION\_OF\_NEK2A | 21 | 0.595263 | 1.889829 | 0 | 0.01085 | 0.521 | 2360 |
| REACTOME\_PROCESSING\_OF\_INTRONLESS\_PRE\_MRNAS | 14 | 0.639905 | 1.886508 | 0.002188 | 0.011008 | 0.535 | 2799 |
| REACTOME\_FANCONI\_ANEMIA\_PATHWAY | 20 | 0.601588 | 1.882145 | 0 | 0.011366 | 0.556 | 4258 |
| REACTOME\_RNA\_POL\_I\_TRANSCRIPTION\_TERMINATION | 21 | 0.600807 | 1.873901 | 0.004556 | 0.012475 | 0.599 | 3531 |
| REACTOME\_REGULATION\_OF\_MITOTIC\_CELL\_CYCLE | 75 | 0.424209 | 1.852129 | 0 | 0.015438 | 0.675 | 5124 |
| REACTOME\_ASSOCIATION\_OF\_LICENSING\_FACTORS\_WITH\_THE\_PRE\_REPLICATIVE\_COMPLEX | 13 | 0.643994 | 1.848953 | 0 | 0.015677 | 0.687 | 2695 |
| REACTOME\_APC\_C\_CDC20\_MEDIATED\_DEGRADATION\_OF\_CYCLIN\_B | 19 | 0.582709 | 1.839639 | 0.002381 | 0.017087 | 0.72 | 3244 |
| REACTOME\_INHIBITION\_OF\_THE\_PROTEOLYTIC\_ACTIVITY\_OF\_APC\_C\_REQUIRED\_FOR\_THE\_ONSET\_OF\_ANAPHASE\_BY\_MITOTIC\_SPINDLE\_CHECKPOINT\_COMPONENTS | 18 | 0.594152 | 1.819944 | 0.002075 | 0.020563 | 0.809 | 2360 |
| REACTOME\_CONVERSION\_FROM\_APC\_C\_CDC20\_TO\_APC\_C\_CDH1\_IN\_LATE\_ANAPHASE | 16 | 0.61531 | 1.817739 | 0.004338 | 0.020741 | 0.817 | 2360 |
| REACTOME\_G2\_M\_DNA\_DAMAGE\_CHECKPOINT | 9 | 0.723511 | 1.817508 | 0.005941 | 0.020395 | 0.817 | 3985 |
| KEGG\_SPLICEOSOME | 123 | 0.385005 | 1.809761 | 0 | 0.02179 | 0.842 | 3710 |
| REACTOME\_BILE\_ACID\_AND\_BILE\_SALT\_METABOLISM | 13 | 0.625028 | 1.803955 | 0.002222 | 0.022538 | 0.856 | 1601 |
| REACTOME\_PROCESSIVE\_SYNTHESIS\_ON\_THE\_LAGGING\_STRAND | 15 | 0.602442 | 1.793279 | 0.008602 | 0.02469 | 0.884 | 3119 |
| REACTOME\_POST\_TRANSLATIONAL\_MODIFICATION\_SYNTHESIS\_OF\_GPI\_ANCHORED\_PROTEINS | 24 | 0.528359 | 1.774269 | 0.004211 | 0.029377 | 0.926 | 1461 |
| REACTOME\_MRNA\_3\_END\_PROCESSING | 33 | 0.495641 | 1.769847 | 0 | 0.030117 | 0.936 | 2958 |
| REACTOME\_UNWINDING\_OF\_DNA | 11 | 0.654989 | 1.768442 | 0.010823 | 0.030092 | 0.939 | 3943 |
| REACTOME\_GENERIC\_TRANSCRIPTION\_PATHWAY | 297 | 0.33403 | 1.762697 | 0 | 0.03141 | 0.949 | 3491 |
| REACTOME\_M\_G1\_TRANSITION | 76 | 0.406917 | 1.758302 | 0 | 0.03238 | 0.958 | 5316 |
| REACTOME\_LAGGING\_STRAND\_SYNTHESIS | 19 | 0.567742 | 1.75573 | 0.004082 | 0.032593 | 0.96 | 3119 |
| REACTOME\_METABOLISM\_OF\_RNA | 248 | 0.338846 | 1.750657 | 0 | 0.033726 | 0.967 | 3690 |
| KEGG\_NON\_HOMOLOGOUS\_END\_JOINING | 11 | 0.668454 | 1.750291 | 0.008333 | 0.033308 | 0.967 | 2749 |
| REACTOME\_TRANSCRIPTION | 169 | 0.355684 | 1.743584 | 0 | 0.035096 | 0.976 | 3852 |
| REACTOME\_E2F\_ENABLED\_INHIBITION\_OF\_PRE\_REPLICATION\_COMPLEX\_FORMATION | 10 | 0.683231 | 1.735738 | 0.006211 | 0.037376 | 0.982 | 2612 |
| REACTOME\_HOMOLOGOUS\_RECOMBINATION\_REPAIR\_OF\_REPLICATION\_INDEPENDENT\_DOUBLE\_STRAND\_BREAKS | 16 | 0.575918 | 1.726167 | 0.010965 | 0.04002 | 0.987 | 3985 |
| REACTOME\_MITOTIC\_PROMETAPHASE | 82 | 0.391371 | 1.721559 | 0 | 0.041059 | 0.988 | 4819 |
| REACTOME\_MITOCHONDRIAL\_PROTEIN\_IMPORT | 48 | 0.436157 | 1.719532 | 0 | 0.041252 | 0.989 | 4736 |
| KEGG\_GLYCOSYLPHOSPHATIDYLINOSITOL\_GPI\_ANCHOR\_BIOSYNTHESIS | 24 | 0.524449 | 1.717303 | 0.006186 | 0.041539 | 0.991 | 1451 |
| REACTOME\_RNA\_POL\_II\_TRANSCRIPTION | 97 | 0.378033 | 1.710178 | 0.002375 | 0.043599 | 0.993 | 4718 |
| REACTOME\_POL\_SWITCHING | 13 | 0.60032 | 1.700395 | 0.012346 | 0.046951 | 0.995 | 3119 |
| REACTOME\_SYNTHESIS\_OF\_BILE\_ACIDS\_AND\_BILE\_SALTS\_VIA\_24\_HYDROXYCHOLESTEROL | 7 | 0.749758 | 1.697346 | 0.008475 | 0.047548 | 0.996 | 1183 |
| REACTOME\_CLEAVAGE\_OF\_GROWING\_TRANSCRIPT\_IN\_THE\_TERMINATION\_REGION\_ | 42 | 0.44975 | 1.696662 | 0.002227 | 0.047222 | 0.996 | 2958 |
| KEGG\_BASE\_EXCISION\_REPAIR | 33 | 0.468528 | 1.690663 | 0.002203 | 0.049329 | 0.996 | 3974 |
| REACTOME\_PROCESSING\_OF\_CAPPED\_INTRONLESS\_PRE\_MRNA | 23 | 0.523683 | 1.689565 | 0.013544 | 0.049237 | 0.996 | 2799 |

**Supplementary Fig. S1**

**.**

A) Protein expression of AR, IGF-1R, and PTEN in prostate cancer cell lines. B–E) IGF signaling (24-hour treatment in growth medium containing FBS, without androgen or growth factor supplementation): Effect of ENZA (1 µM) and XENT (0.1 µM), alone or in combination, on IGF-1R and AKT phosphorylation status in prostate cancer cells.



**Supplementary Fig. S2**

**.**

Analysis of PTEN knockdown by qPCR and Western blot. VCaP cells were transfected with either PTEN siRNA, non-targeting (NT) siRNA, or transfection reagent alone (Mock). A) PTEN mRNA levels were determined after 3 days by qPCR and normalized to HPRT1 levels. Fold changes relative to Mock control (n = 3, mean ± standard deviation) are shown. B) Protein lysates prepared 3 days post-transfection were analyzed for PTEN expression by Western blot analysis.



**Supplementary Fig. S3**

**.**

AR signaling: Effect of XENT and ENZA, alone or in combination, on transcript and protein levels in VCaP cells. Cells were seeded in medium with 10% charcoal-stripped serum and treated with inhibitors for 24 hours (qPCR) or 48 hours (Western blots). Synthetic androgen R1881 (0.1 nM) was added 2 hours after treatment start. A) Transcript/protein levels of AR-regulated target genes. B) Transcript/protein levels of cell cycle regulators (AR-V7 target genes).

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**Supplementary Fig. S4.**

Induction of apoptosis in VCaP cells. A) Effect of 1 µM XENT and 10 µM ENZA, alone or in combination, on caspase 3/7 activity. Cells were incubated with inhibitors in FBS-containing medium (without androgen or growth factor supplementation) for 96 hours. Caspase 3/7-mediated apoptosis was detected using IncuCyte™ Caspase-3/7 Reagent. *P* values were calculated using pairwise t-tests (adjusted for multiplicity) following a one-way ANOVA. B) Effect of 0.1 µM XENT and 1 µM ENZA, alone or in combination, on total protein levels of apoptotic regulators.

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## Supplementary Fig. S5.

AR-FL and AR-V7 mRNA and protein expression in LuCaP 96CR PDX. A) qPCR analysis of AR-FL and AR-V7 mRNA. B) IHC staining with an AR-V7-specific antibody and quantitative analysis of AR-V7 IHC. C) IHC staining with AR-FL N-terminus and C-terminus antibodies and quantitative analysis of IHC. Nuclear AR-V7 and AR-FL were quantified using a score obtained from the number of positive nuclei per 200 cells per section × intensity, graded on a scale of 0 to 3. *P* values were calculated using Tukey tests conducted following a one-way ANOVA.



## Supplementary Fig. S6.

Paired end, unstranded RNA-Seq profile matching to the *PTEN* locus in LuCaP 96CR. A) IGV genome browser view of GRCh38 mapped transcriptomics data showing the coverage, the junctions, and the raw reads track (red: forward 5’ to 3’ mapping, blue: reverse 3’ to 5’ mapping). The *PTEN* locus, including exons, is shown in blue, and the phosphatase domain is shown in red. A dotted blue box highlights *PTEN* exon 3. B) Amino acid sequence of the canonical *PTEN* coding sequence. The phosphatase domain is colored in red, the deleted exon 3 sequence is boxed in blue, and the active site is boxed in green.

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