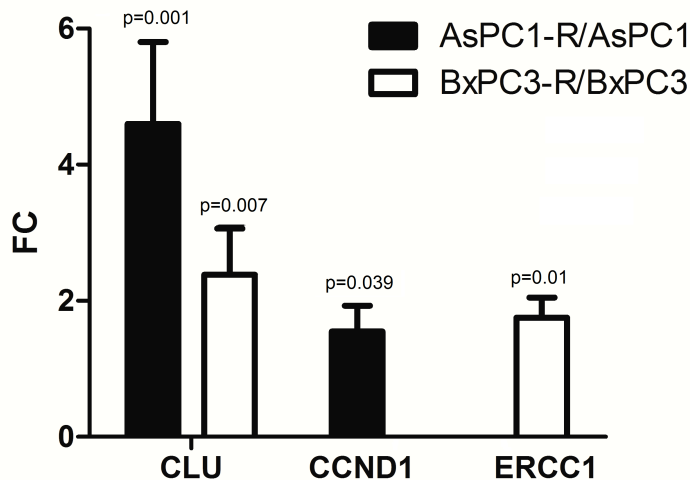


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Supplementary Method

Total cellular RNA was isolated from AsPC1, AsPC1-R, BxPC3 and BxPC3-R cells cultured in drug-free RPMI 1640 medium with 10% FBS, 2 mM L-glutamine, penicillin (100 IU/mL), streptomycin (100 µg/mL) and amphotericin B (0.25 µg/mL) using the RNeasy Mini Kit (Qiagen, Valencia, CA). Purity and concentration of total cell RNA were determined with NanoDrop 1000 Spectrophotometer (Thermo Scientific, Wilmington, DE). RNA specimens were processed with a DNA-free kit (Ambion/ThermoFisher Scientific, Waltham, MA) to remove contaminating DNA and reverse-transcribed using the High Capacity cDNA Reverse Transcription Kit with RNase Inhibitor (Applied Biosystems/ThermoFisher Scientific). qPCR was performed using the CFX96 Real Time PCR Detection System (BioRad, Hercules, CA) using TaqMan assays Hs00156548_m1 for CLU, Hs01012158_m1 for ERCC1, Hs00765553_m1 for CCND1, Hs02758991_g1 for GAPDH and Hs99999903_m1 for ACTB and TaqMan Universal Master Mix II with UNG (all from Applied Biosystems/ThermoFisher Scientific) following the manufacturer's recommendations. All qPCR reactions were performed in triplicate. Threshold cycles (Ct) were determined using single threshold/baseline subtracted curve fit (CFX Manager Software) and averaged across replicates. Fold changes (FC) of expression between resistant and parental cells were determined using the $\Delta\Delta C_t$ method. GAPDH and ACTB were selected as internal controls for AsPC1/AsPC1-R and BxPC3/BxPC3-R cells, respectively, based on microarray data. Standard deviations of FC were calculated using error propagation. Statistical significance of differences among mean ΔC_t values was tested using a two-tailed t-test with Welch's correction. The differences between means were considered significant for $p < 0.05$.

Supplementary Results



FC: Fold change between resistant and parental cells

Supplementary Figures

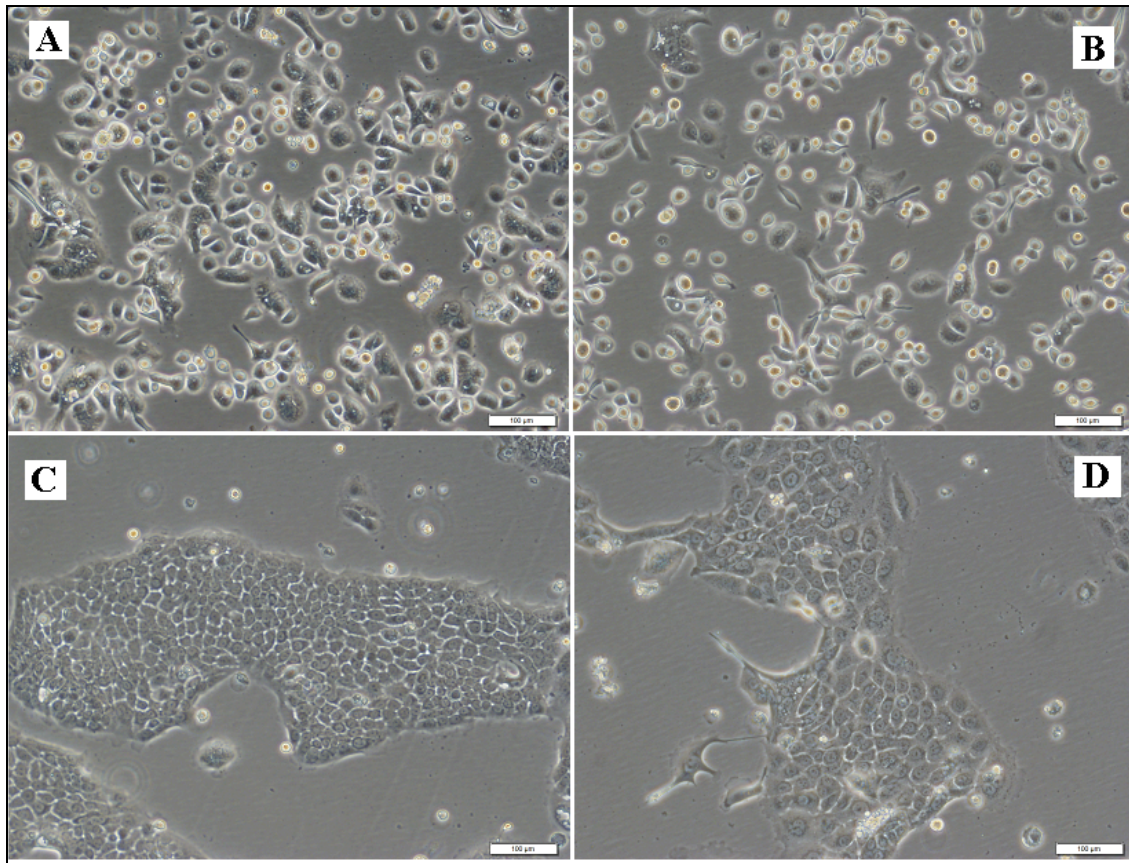


Fig. S1. Morphology of cells in culture examined by phase-contrast microscopy. (A) AsPC1; (B) AsPC1-R; (C) BxPC3; (D) BxPC3-R; magnification = 100 ×; scale bar = 100 µm

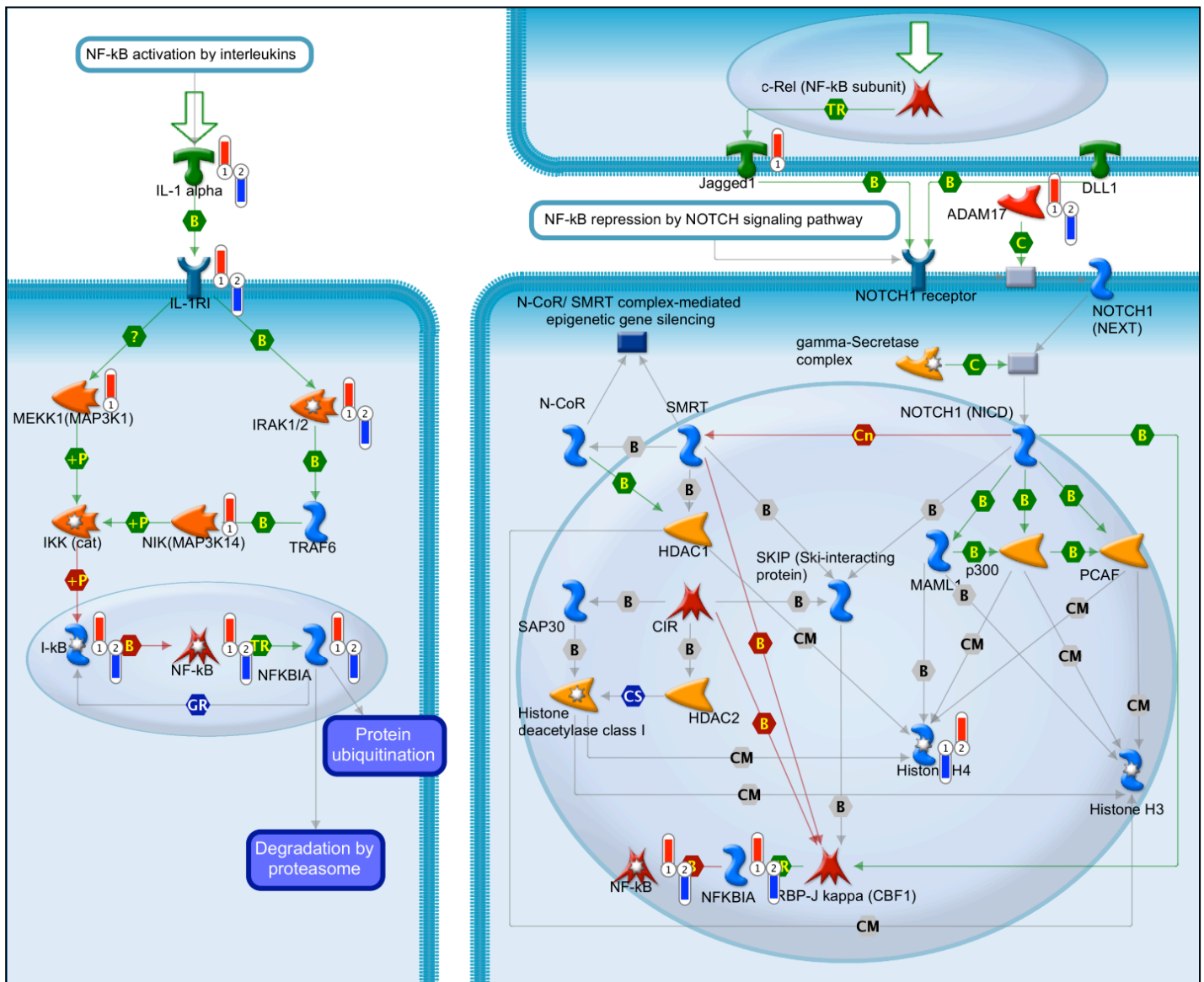


Fig. S2. MetaCore Development_NOTCH1-mediated pathway for NF-κB activity modulation. 1 = AsPC1-R vs AsPC1 cells; 2 = BxPC3-R vs BxPC3 cells; Red thermometer: gene is overexpressed; Blue thermometer: gene under-expressed in resistant cells. For legend see <https://portal.genego.com/legends/MetaCoreQuickReferenceGuide.pdf>

enriched in parental phenotype; Edges: connect significantly overlapping gene sets (width reflects degree of the overlap).

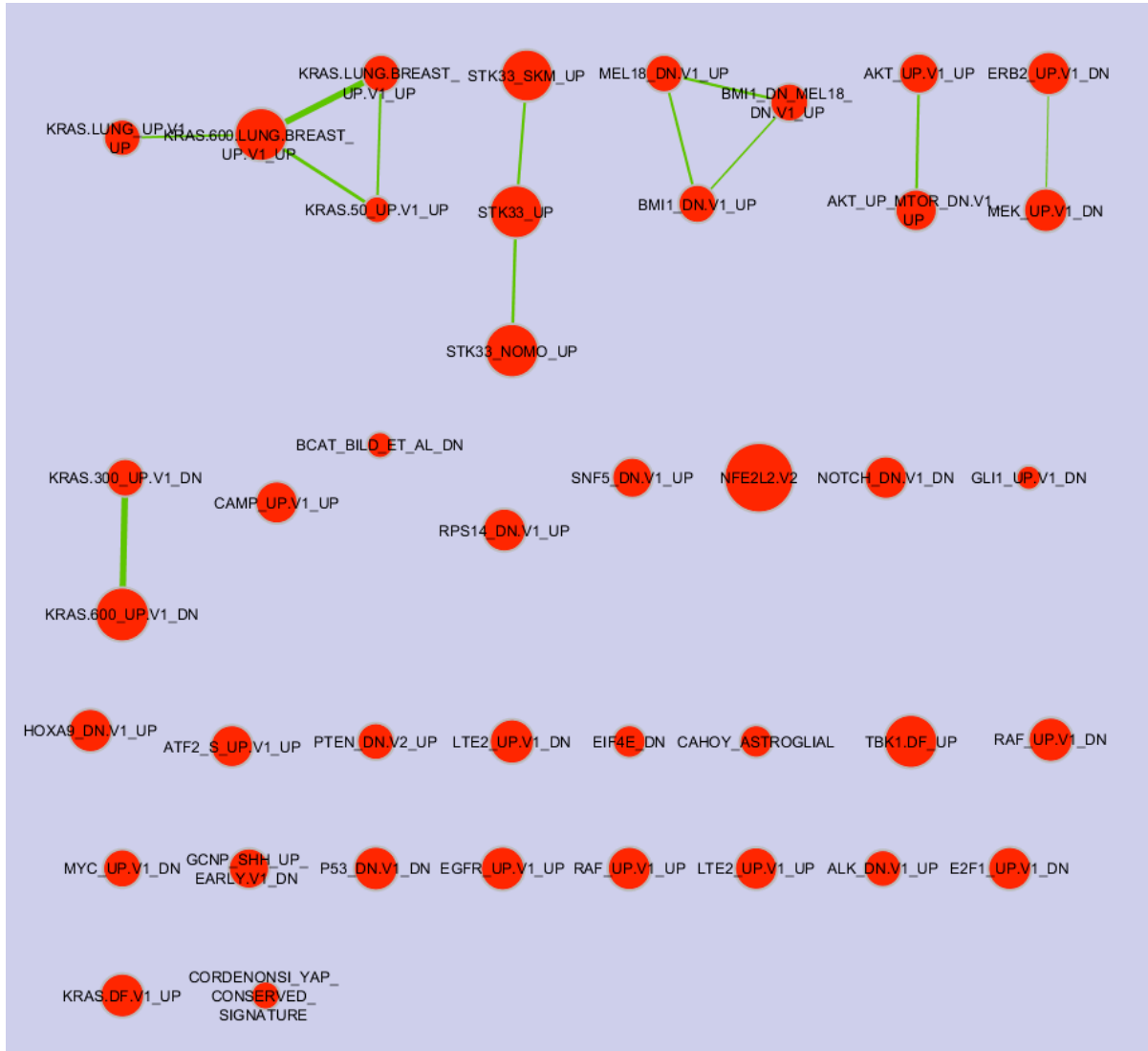


Fig. S5. Enrichment Map visualizing results of GSEA analysis for AsPC1-R/AsPC1 cells; Oncogenic Signatures gene set. Gene set: Oncogenic Signatures (189 gene sets) (<http://www.broadinstitute.org/gsea/msigdb/collections.jsp>); Nodes: gene sets; Node size: number of genes in the gene set; Node color: red = enriched in resistant phenotype; blue = enriched in parental phenotype; Edges: connect significantly overlapping gene sets (width reflects degree of the overlap).

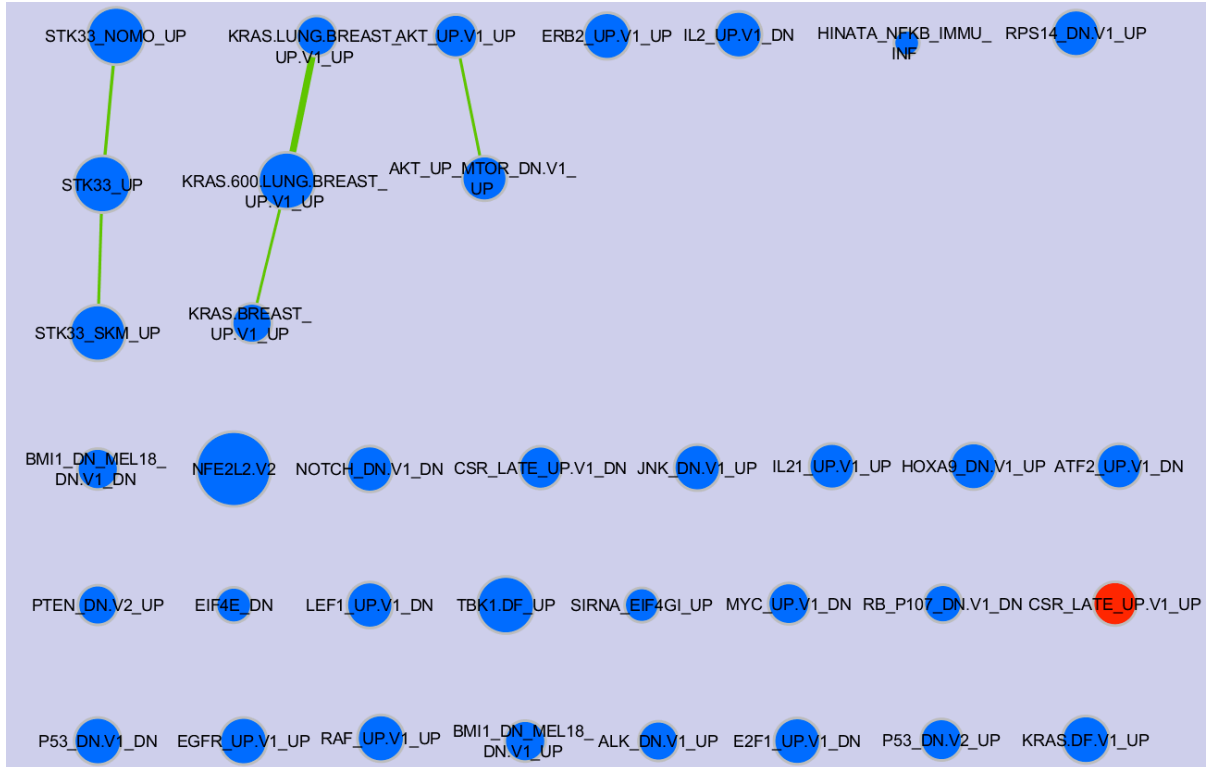


Fig. S6. Enrichment Map visualizing results of GSEA analysis for BxPC3-R/BxPC3 cells; Oncogenic Signatures gene set. Gene set: Oncogenic Signatures (189 gene sets) (<http://www.broadinstitute.org/gsea/msigdb/collections.jsp>); Nodes: gene sets; Node size: number of genes in the gene set; Node color: red = enriched in resistant phenotype; blue = enriched in parental phenotype; Edges: connect significantly overlapping gene sets (width reflects degree of the overlap).

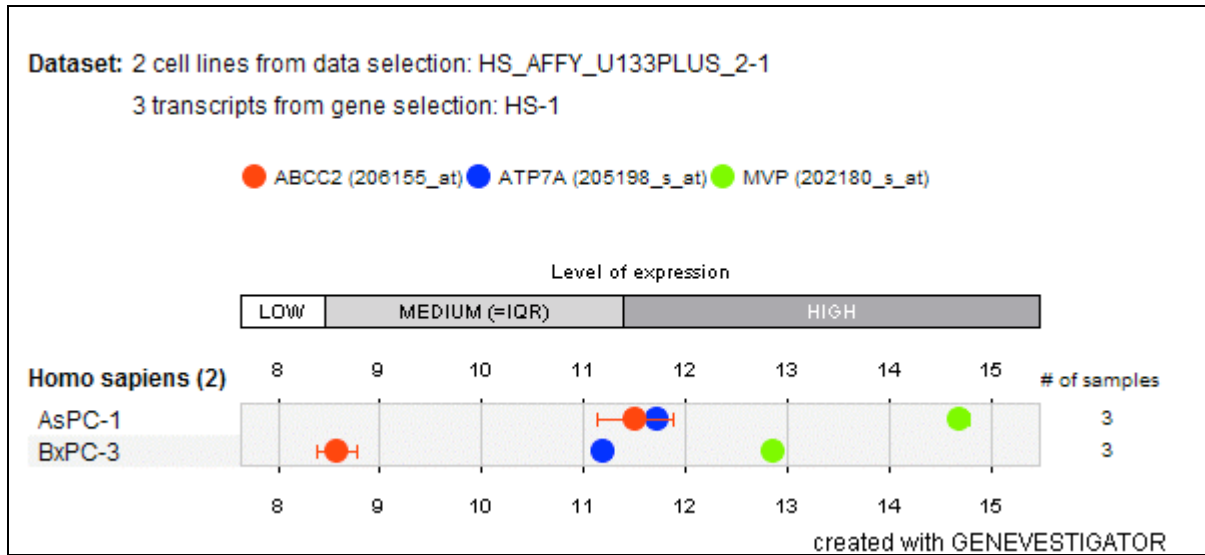


Fig. S7. Normalized expression signals for ABCC2, ATP7A and MVP genes in AsPC1 and BxPC3 cell lines processed from the GSE22973 dataset (Gene Expression Omnibus; www.ncbi.nlm.nih.gov/geo). The data were processed and image generated using the Genevestigator 5-11-02, Release Date 07/26/2016 [1].

[1] Hruz, T.; Laule, O.; Szabo, G.; Wessendorp, F.; Bleuler, S.; Oertle, L.; Widmayer, P.; Gruissem, W.; Zimmermann, P. Genevestigator v3: a reference expression database for the meta-analysis of transcriptomes. *Advances in bioinformatics*, **2008**, *2008*, 420747.

Supplementary Tables

Table S1. List of 72 cisplatin resistance-related genes [1-6]

| CISPLATIN-RESISTANCE RELATED GENES | | | | | | | |
|------------------------------------|--------|----------|----------|-------|--------|---------|-------|
| ABCC1 | CCND1 | ERCC2 | HMGB1 | MARK2 | NFE2L2 | RPL27 | XPA |
| ABCC2 | CD44 | ERCC4 | HSP90AA1 | MLH1 | NQO1 | RPL36 | XRCC1 |
| ADM | CFLAR | GCLC | HSPA1A | MPO | PGK1 | RPL36A | |
| ATP7A | CHEK1 | GCLM | HSPA1B | MSH2 | POLB | SIRT1 | |
| ATP7B | CHEK2 | GGT1 | HSPB1 | MSH6 | POLH | SLC31A1 | |
| BCL2 | CITED2 | GLUT1 | HSPD1 | MT1A | PTGS2 | SLC31A2 | |
| BCL2A1 | CLU | GSTM1 | HSPE1 | MT2A | PTPN21 | SOD1 | |
| BCL2L1 | DHDH | GSTP1 | KAT2B | MVD | RAB8 | TMEM205 | |
| BIRC3 | EHD1 | GSTT2 | KAT5 | MVP | RAD51 | TOP2A | |
| CABIN1 | ERCC1 | HIST1H1A | LRRFIP1 | NAPA | REV1 | XIAP | |

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- [5] Stewart, D.J. Mechanisms of resistance to cisplatin and carboplatin. *Crit Rev Oncol Hematol*, **2007**, *63* (1), 12-31.
- [6] Fujimori, S.; Abe, Y.; Nishi, M.; Hamamoto, A.; Inoue, Y.; Ohnishi, Y.; Nishime, C.; Matsumoto, H.; Yamazaki, H.; Kijima, H.; Ueyama, Y.; Inoue, H.; Nakamura, M. The subunits of glutamate cysteine ligase enhance cisplatin resistance in human non-small cell lung cancer xenografts in vivo. *Int J Oncol*, **2004**, *25* (2), 413-418.

Table S2. Properties of pancreatic ductal adenocarcinoma cell lines selected for the development of cisplatin-resistant cells (reviewed in [1])

| | AsPC1 | BxPC3 |
|---|--|---|
| Origin Specimen for cell isolation Patient Diagnosis Grading | Ascites Female, 62 yr Metastatic adeno- carcinoma of the head of pancreas Poorly differentiated | Primary tumor tissue Female, 61 yr Adenocarcinoma of the body of pancreas, metastasis not found Moderately to poorly differentiated |
| Genotype K-Ras TP53 CDKN2A | Mut Mut WT | WT Mut WT |
| Phenotype Cell line doubling time COX2 expression VEGF expression | 38-40 hrs Low Low | 48-60 hrs High High |

References

- [1] Deer, E.L.; Gonzalez-Hernandez, J.; Coursen, J.D.; Shea, J.E.; Ngatia, J.; Scaife, C.L.; Firpo, M.A.; Mulvihill, S.J. Phenotype and genotype of pancreatic cancer cell lines. *Pancreas*, **2010**, *39* (4), 425-435.

Table S3. MetaCore Pathway Maps enriched by genes up regulated in AsPC1-R vs AsPC1 cells

| # | Maps | Total | In Data | p-value | FDR |
|----|---|-------|---------|-----------|-----------|
| 1 | Immune response_Alternative complement pathway | 34 | 16 | 5.754E-10 | 4.269E-07 |
| 2 | Immune response_Signaling pathway mediated by IL-6 and IL-1 | 29 | 11 | 4.288E-06 | 6.471E-04 |
| 3 | Cell adhesion_Chemokines and adhesion | 86 | 20 | 4.351E-06 | 6.471E-04 |
| 4 | Complement pathway disruption in thrombotic microangiopathy | 24 | 10 | 4.361E-06 | 6.471E-04 |
| 5 | Development_NOTCH1-mediated pathway for NF-KB activity modulation | 30 | 11 | 6.315E-06 | 7.810E-04 |
| 6 | Immune response_Classical complement pathway | 43 | 13 | 1.051E-05 | 9.748E-04 |
| 7 | Immune response_Lectin induced complement pathway | 42 | 12 | 4.348E-05 | 3.226E-03 |
| 8 | Immune response_IL-1 signaling pathway | 39 | 11 | 1.036E-04 | 6.405E-03 |
| 9 | Cell adhesion_ECM remodeling | 48 | 12 | 1.802E-04 | 9.552E-03 |
| 10 | Signal transduction_NF-kB activation pathways | 42 | 11 | 2.148E-04 | 1.063E-02 |
| 11 | IGF family signaling in colorectal cancer | 51 | 12 | 3.332E-04 | 1.448E-02 |
| 12 | Mucin expression in CF airways | 44 | 11 | 3.351E-04 | 1.448E-02 |
| 13 | Immune response_IL-33 signaling pathway | 45 | 11 | 4.138E-04 | 1.616E-02 |
| 14 | Immune response_TLR5, TLR7, TLR8 and TLR9 signaling pathways | 39 | 10 | 5.015E-04 | 1.637E-02 |
| 15 | Immune response_HSP60 and HSP70/ TLR signaling pathway | 46 | 11 | 5.075E-04 | 1.637E-02 |
| 16 | Immune response_MIF-induced cell adhesion, migration and angiogenesis | 40 | 10 | 6.242E-04 | 1.838E-02 |
| 17 | Main growth factor signaling cascades in multiple myeloma cells | 35 | 9 | 9.361E-04 | 2.481E-02 |
| 18 | Immune response_Bacterial infections in normal airways | 36 | 9 | 1.166E-03 | 2.704E-02 |
| 19 | Immune response_Histamine H1 receptor signaling in immune response | 37 | 9 | 1.439E-03 | 3.236E-02 |
| 20 | Immune response_IL-18 signaling | 53 | 11 | 1.788E-03 | 3.492E-02 |

Total: total number of MetaCore network objects in a given MetaCore Map;

In Data: number of MetaCore network objects corresponding to differentially expressed genes; p-values are calculated based on hypergeometric distribution; FDR: false discovery rate.

Table S4. MetaCore Pathway Maps enriched by genes up regulated in BxPC3-R vs BxPC3 cells

| # | Maps | Total | In Data | p-value | FDR |
|---|--|-------|---------|-----------|-----------|
| 1 | Transport_cAMP/ Ca(2+)-dependent Insulin secretion | 18 | 5 | 3.195E-04 | 9.387E-02 |
| 2 | Signal transduction_cAMP signaling | 29 | 6 | 4.514E-04 | 9.387E-02 |
| 3 | Cytoskeleton remodeling_Keratin filaments | 30 | 6 | 5.478E-04 | 9.387E-02 |
| 4 | Development_Thromboxane A2 signaling pathway | 32 | 6 | 7.881E-04 | 9.387E-02 |
| 5 | Regulation of lipid metabolism_Regulation of lipid metabolism by niacin and isoprenaline | 24 | 5 | 1.333E-03 | 9.387E-02 |
| 6 | NETosis in SLE | 25 | 5 | 1.618E-03 | 9.387E-02 |

Total: total number of MetaCore network objects in a given MetaCore Map;

In Data: number of MetaCore network objects corresponding to differentially expressed genes; p-values are calculated based on hypergeometric distribution; FDR: false discovery rate.

Table S5. MetaCore Pathway Maps enriched by genes down regulated in AsPC1-R vs. AsPC1 cells

| # | Maps | Total | In Data | p-value | FDR |
|----|---|-------|---------|-----------|-----------|
| 1 | Regulation of lipid metabolism_Regulation of lipid metabolism via LXR, NF-Y and SREBP | 29 | 9 | 5.339E-07 | 3.283E-04 |
| 2 | Protein folding and maturation_Angiotensin system maturation \ Human version | 26 | 8 | 2.540E-06 | 5.208E-04 |
| 3 | Transcription_Sirtuin6 regulation and functions | 55 | 11 | 3.555E-06 | 5.466E-04 |
| 4 | Development_Role of IL-8 in angiogenesis | 49 | 9 | 5.807E-05 | 7.142E-03 |
| 5 | Cell cycle_Initiation of mitosis | 24 | 6 | 1.772E-04 | 1.816E-02 |
| 6 | NETosis in SLE | 25 | 6 | 2.261E-04 | 1.986E-02 |
| 7 | Reproduction_Progesterone-mediated oocyte maturation | 28 | 6 | 4.387E-04 | 3.372E-02 |
| 8 | Reproduction_GnRH signaling | 53 | 8 | 6.167E-04 | 4.214E-02 |
| 9 | Adiponectin in pathogenesis of type 2 diabetes | 21 | 5 | 8.060E-04 | 4.957E-02 |
| 10 | Development_Delta- and kappa-type opioid receptors signaling via beta-arrestin | 22 | 5 | 1.012E-03 | 5.660E-02 |

Total: total number of MetaCore network objects in a given MetaCore Map;

In Data: number of MetaCore network objects corresponding to differentially expressed genes; p-values are calculated based on hypergeometric distribution; FDR: false discovery rate .

Table S6. MetaCore Pathway Maps enriched by genes down regulated in BxPC3-R vs. BxPC3 cells

| # | Maps | Total | In Data | p-value | FDR |
|----|--|-------|---------|-----------|-----------|
| 1 | Immune response_HMGB1/RAGE signaling pathway | 44 | 15 | 5.386E-07 | 2.332E-04 |
| 2 | Immune response_Alternative complement pathway | 34 | 13 | 6.884E-07 | 2.332E-04 |
| 3 | Immune response_HSP60 and HSP70/ TLR signaling pathway | 46 | 15 | 1.036E-06 | 2.332E-04 |
| 4 | Impaired inhibitory action of lipoxins and Resolvin E1 on neutrophil functions in CF | 21 | 10 | 1.244E-06 | 2.332E-04 |
| 5 | Immune response_IL-17 signaling pathways | 49 | 15 | 2.565E-06 | 3.847E-04 |
| 6 | Immune response_MIF-mediated glucocorticoid regulation | 19 | 9 | 4.602E-06 | 5.752E-04 |
| 7 | Complement pathway disruption in thrombotic microangiopathy | 24 | 10 | 5.581E-06 | 5.980E-04 |
| 8 | Immune response_IL-18 signaling | 53 | 15 | 7.606E-06 | 7.131E-04 |
| 9 | Immune response_Role of PKR in stress-induced antiviral cell response | 49 | 14 | 1.364E-05 | 1.061E-03 |
| 10 | Th17 cells in CF | 43 | 13 | 1.415E-05 | 1.061E-03 |
| 11 | Immune response_C3a signaling | 32 | 11 | 1.683E-05 | 1.148E-03 |
| 12 | Immune response_TLR5, TLR7, TLR8 and TLR9 signaling pathways | 39 | 12 | 2.498E-05 | 1.561E-03 |
| 13 | Immune response_Lipoxins and Resolvin E1 inhibitory action on neutrophil functions | 18 | 8 | 2.864E-05 | 1.652E-03 |
| 14 | Signal transduction_Soluble CXCL16 signaling | 34 | 11 | 3.234E-05 | 1.732E-03 |
| 15 | Immune response_CD40 signaling | 47 | 13 | 4.075E-05 | 2.038E-03 |
| 16 | Immune response_Bacterial infections in normal airways | 36 | 11 | 5.885E-05 | 2.759E-03 |
| 17 | Immune response_TSLP signalling | 32 | 10 | 1.038E-04 | 4.327E-03 |
| 18 | Immune response_Substance P-stimulated expression of proinflammatory cytokines via MAPKs | 32 | 10 | 1.038E-04 | 4.327E-03 |
| 19 | Bacterial infections in CF airways | 33 | 10 | 1.388E-04 | 5.478E-03 |
| 20 | Immune response_TLR2 and TLR4 signaling pathways | 47 | 12 | 1.878E-04 | 7.044E-03 |
| 21 | Development_PEDF signaling | 35 | 10 | 2.388E-04 | 8.528E-03 |
| 22 | Signal transduction_NF-kB activation pathways | 42 | 11 | 2.731E-04 | 9.311E-03 |
| 23 | Immune response_C5a signaling | 38 | 10 | 4.971E-04 | 1.564E-02 |
| 24 | Immune response_Antigen presentation by MHC class II | 10 | 5 | 5.215E-04 | 1.564E-02 |
| 25 | Immune response_IL-33 signaling pathway | 45 | 11 | 5.230E-04 | 1.564E-02 |
| 26 | Immune response_Role of HMGB1 in dendritic cell maturation and migration | 20 | 7 | 5.421E-04 | 1.564E-02 |
| 27 | Immune response_IL-1 signaling pathway | 39 | 10 | 6.229E-04 | 1.710E-02 |
| 28 | Inhibition of neutrophil migration by proresolving lipid mediators in COPD | 46 | 11 | 6.401E-04 | 1.710E-02 |
| 29 | IL-17-induced mucin expression in CF airways | 33 | 9 | 7.154E-04 | 1.710E-02 |
| 30 | Immune response_MIF-induced cell adhesion, migration and angiogenesis | 40 | 10 | 7.738E-04 | 1.710E-02 |
| 31 | Development_TGF-beta-dependent induction of | 40 | 10 | 7.738E-04 | 1.710E-02 |

| | | | | | |
|----|--|----|----|-----------|-----------|
| | EMT via MAPK | | | | |
| 32 | Immune response_T regulatory cell-mediated modulation of antigen-presenting cell functions | 40 | 10 | 7.738E-04 | 1.710E-02 |
| 33 | Fructose metabolism/ Rodent version | 27 | 8 | 7.753E-04 | 1.710E-02 |
| 34 | Fructose metabolism | 27 | 8 | 7.753E-04 | 1.710E-02 |
| 35 | PDE4 regulation of cyto/chemokine expression in arthritis | 34 | 9 | 9.070E-04 | 1.944E-02 |
| 36 | TLR2-induced platelet activation | 22 | 7 | 1.040E-03 | 2.166E-02 |
| 37 | Development_Role of IL-8 in angiogenesis | 49 | 11 | 1.129E-03 | 2.289E-02 |
| 38 | Glycolysis and gluconeogenesis p. 1 | 17 | 6 | 1.308E-03 | 2.581E-02 |

Total: total number of MetaCore network objects in a given MetaCore Map;

In Data: number of MetaCore network objects corresponding to differentially expressed genes;

p-values are calculated based on hypergeometric distribution; FDR: false discovery rate