

Title: The Andrographolide analogue 3A.1 synergizes with Taxane derivatives in aggressive metastatic prostate cancers by upregulation of Heat Shock proteins and downregulation of MAT2A-mediated cell migration and invasion

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Supplementary Figure 1.***In vitro* Docetaxel (DTX), Cabazitaxel (CBZ) and andrographolide analogue (3A.1) response in human aggressive prostate cell lines**

A) Bar graphs represent the dose reduction (Reduce Dose-RD, calculated by Dose Reduction Index) for all cell lines treated with the 3A.1+DTX and 3A.1+ CBZ in combination treatment at IC_{75} .

B) Bar graphs represent the dose reduction (Reduce Dose-RD, calculated by Dose Reduction Index) for all cell lines treated with the 3A.1+DTX and 3A.1+ CBZ in combination treatment at IC_{90} .

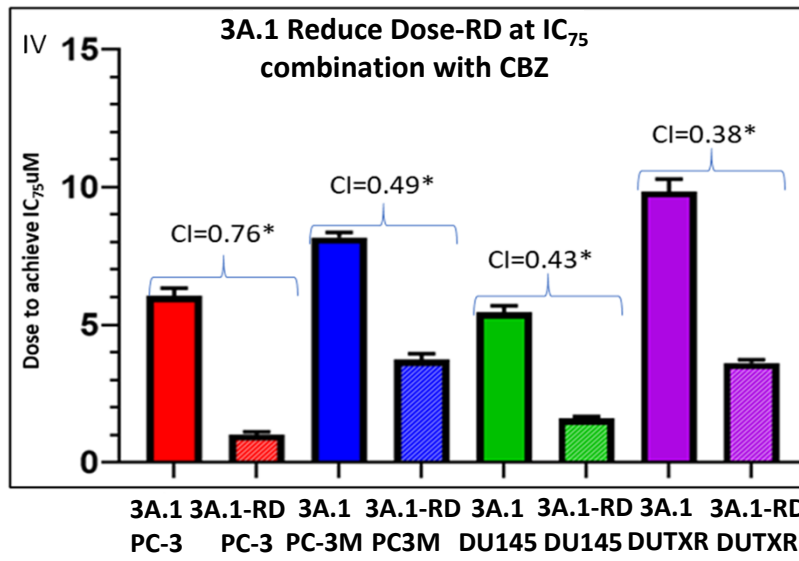
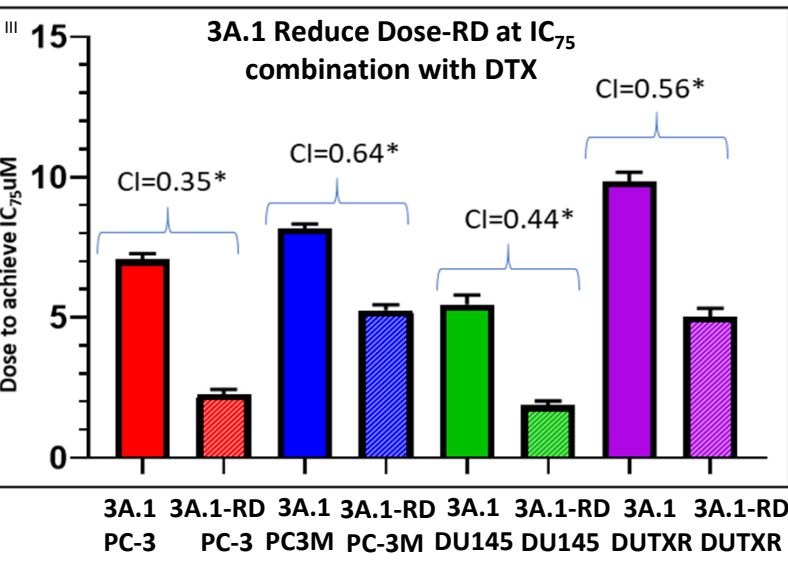
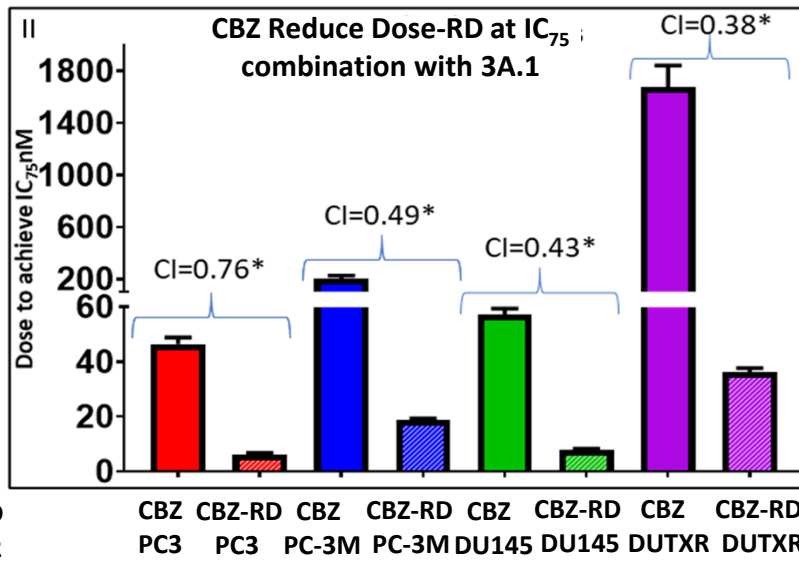
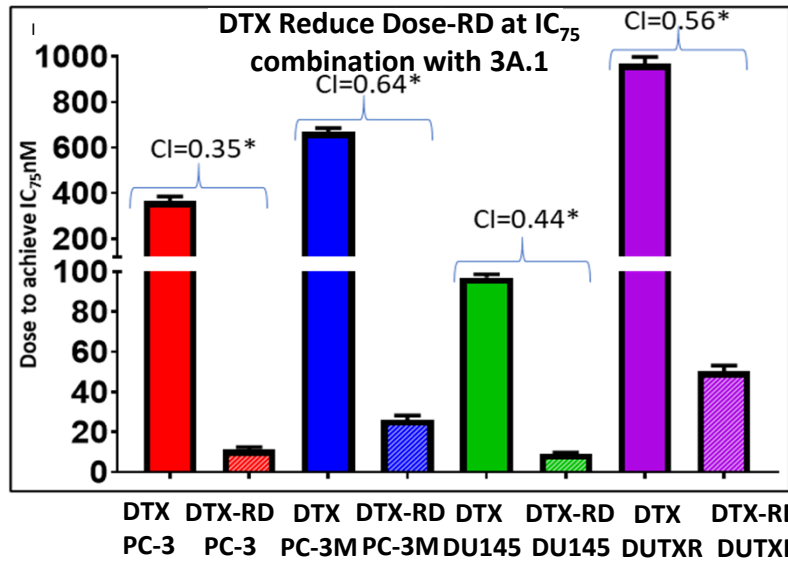
C) Combination Index (CI) DTX, CBZ and 3A.1 observed at IC_{50} , IC_{75} and IC_{90} . The CI value <1 , $=1$, and >1 refer to synergistic, additive and antagonistic effect of the drugs treated in combination, respectively.

To evaluate the pharmacological interactions, percent (%) of cells were affected (fraction affected - Fa) by DTX or CBZ and 3A.1 treatment were analyzed by CompuSyn® version 1.0 software.

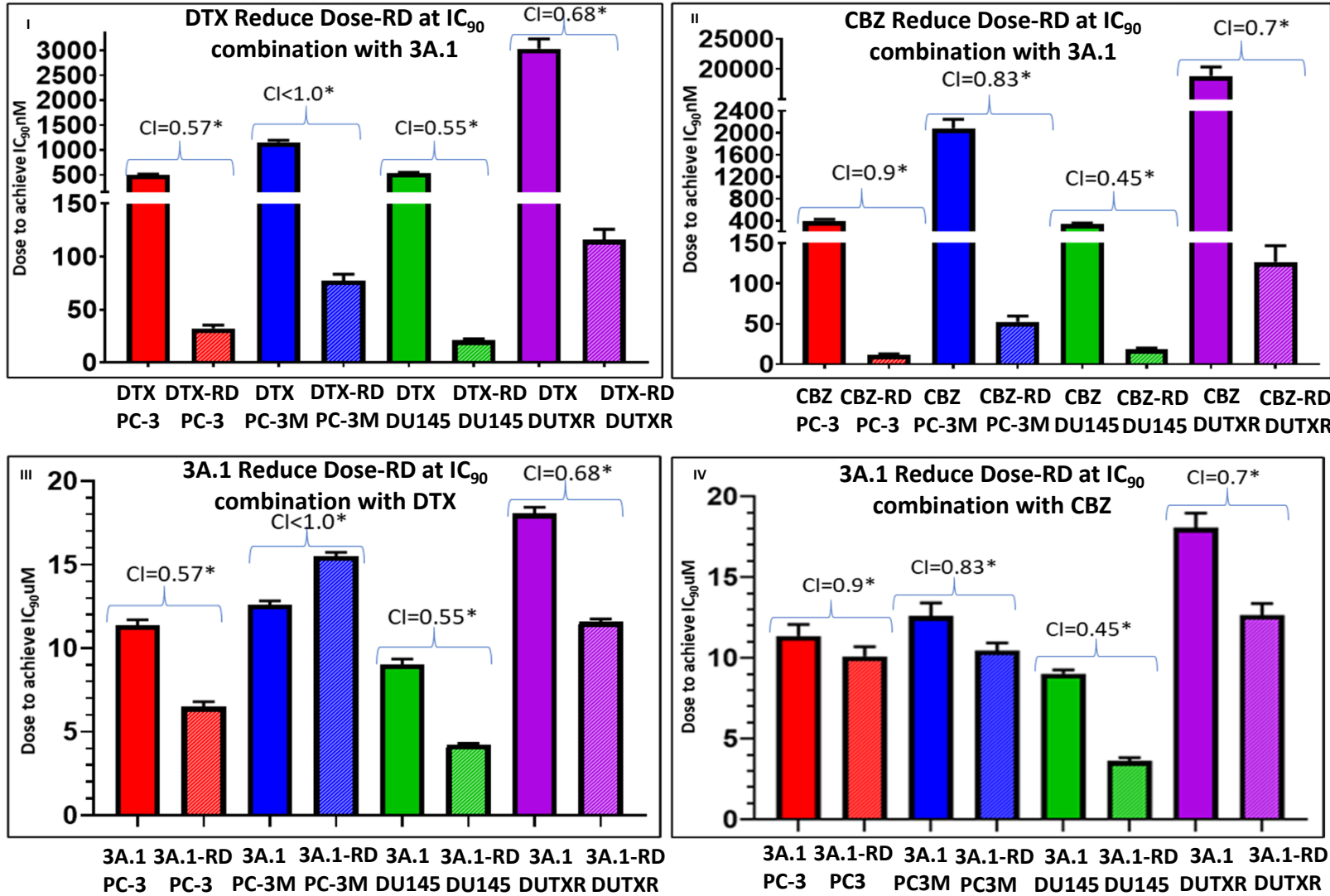
Combination index (CI) value was calculated according to Chou's method

([https://doi.org/10.1016/0065-2571\(84\)90007-4](https://doi.org/10.1016/0065-2571(84)90007-4)). (* = $p \leq 0.05$).

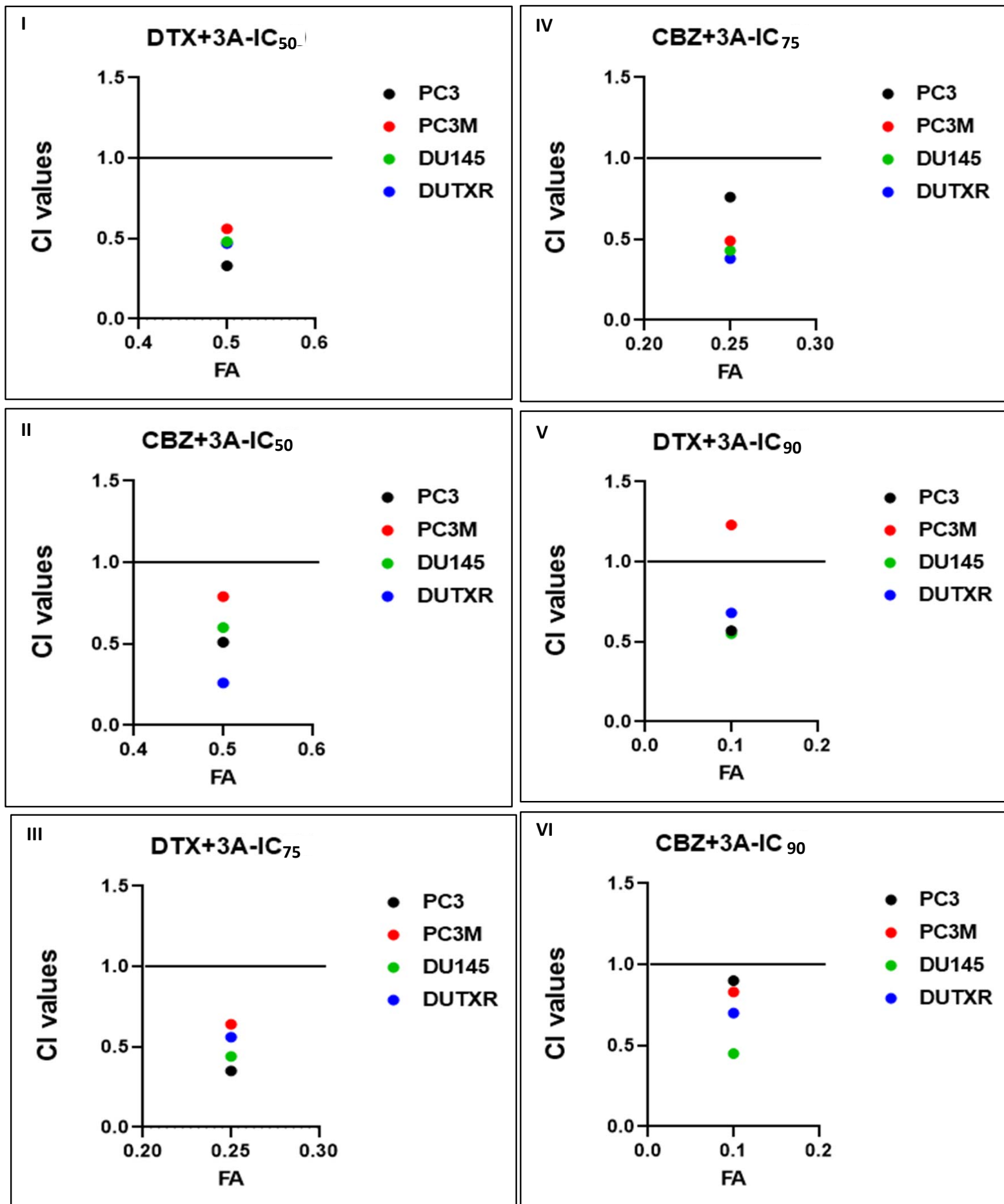
A.



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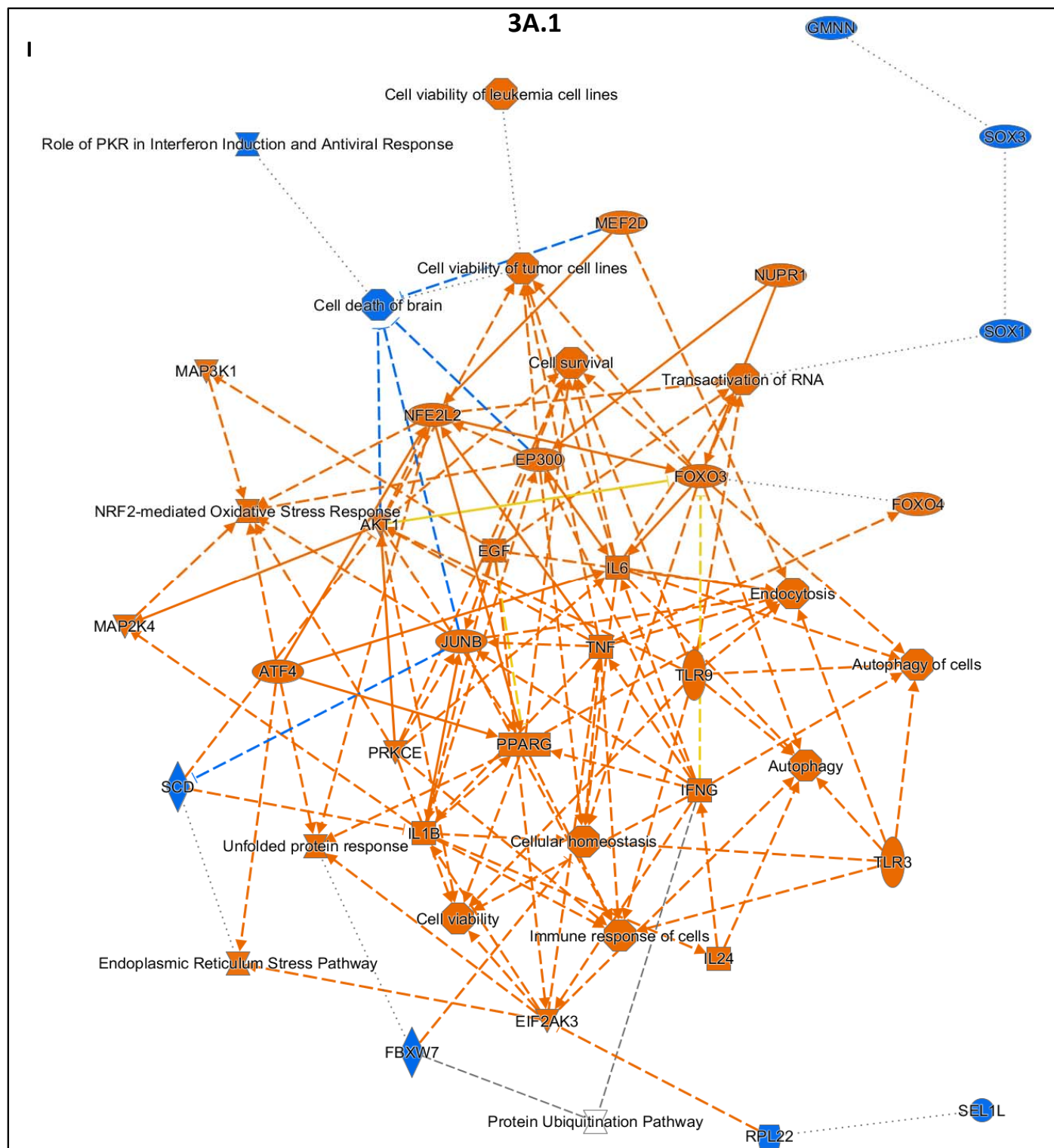


Supplementary Figure 2. Differential gene expression profile

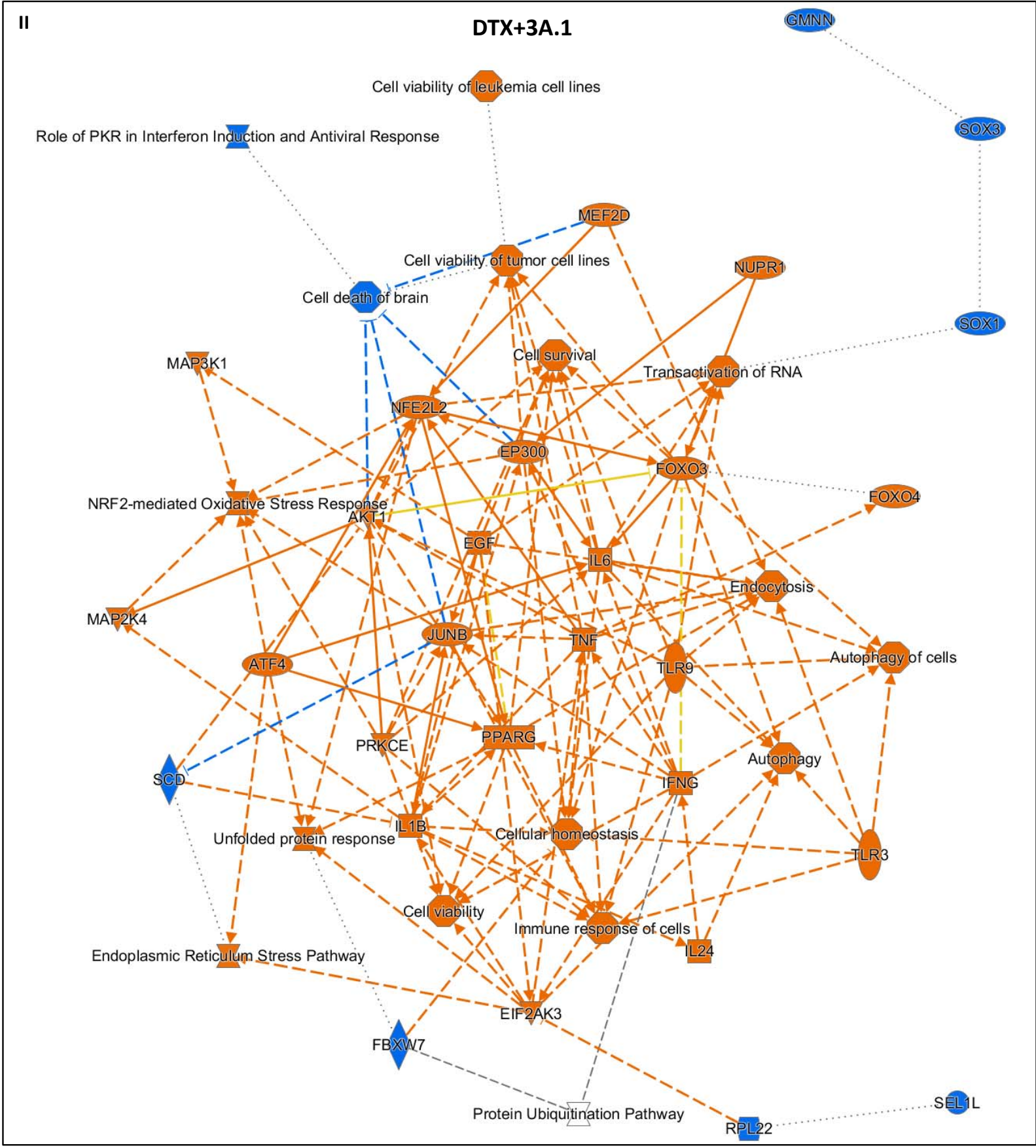
- A. Graphical summary of IPA analysis predicted various canonical pathways, causal network, upstream and downstream regulators (genes) for various treatment regimens 3A.1, DTX, DTX+3A.1, CBZ, CBZ+3A.1 in all cell lines ($p < 0.05$)
- B. Venn diagrams illustrating unique and common DEGs (based on RNAseq gene expression analysis) among 3A.1, DTX, DTX+3A.1, CBZ, CBZ+3A.1 treatments in DU145 vs DUTXR cell lines ($p < 0.05$).

Supplementary Figure 2.

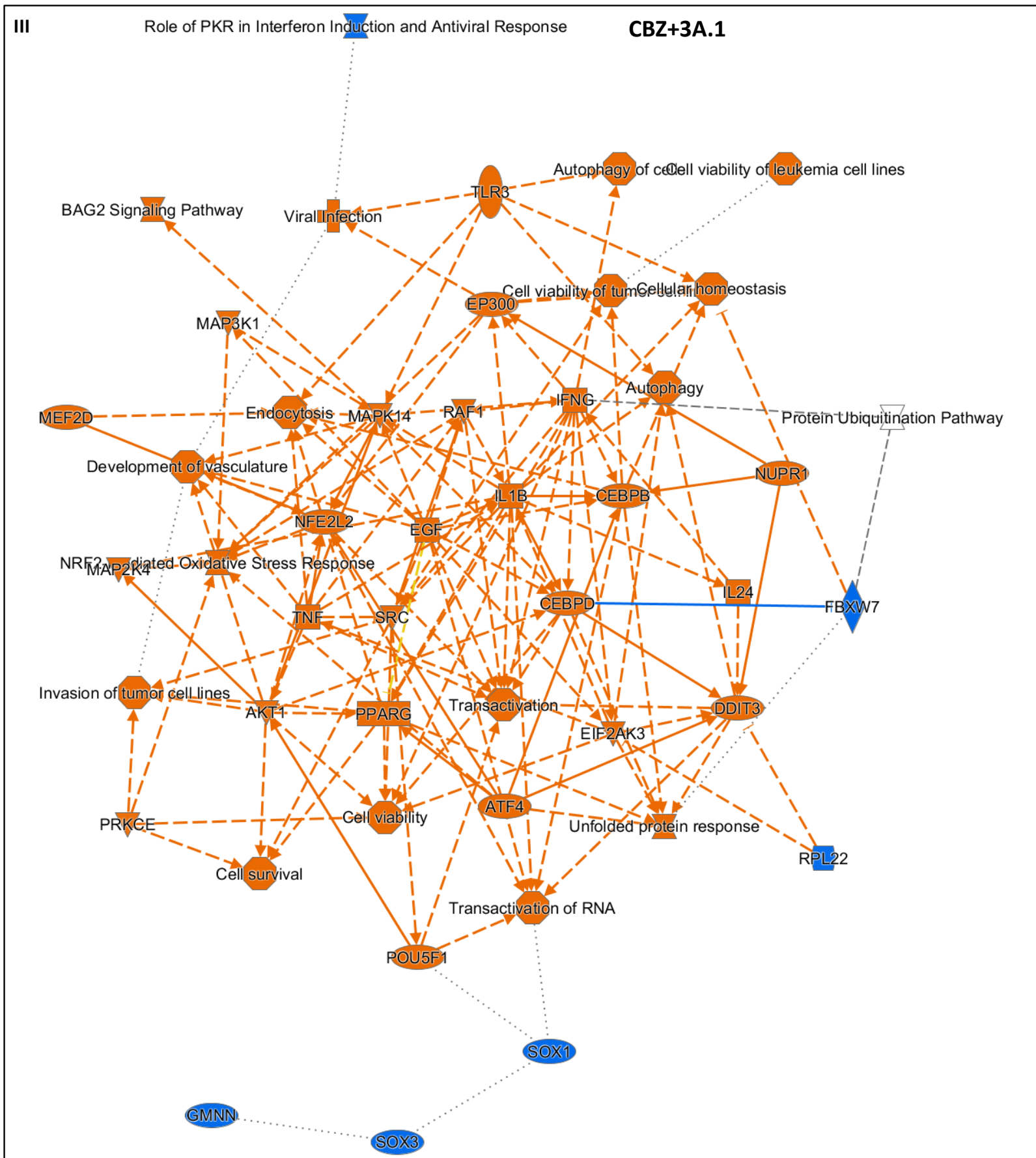
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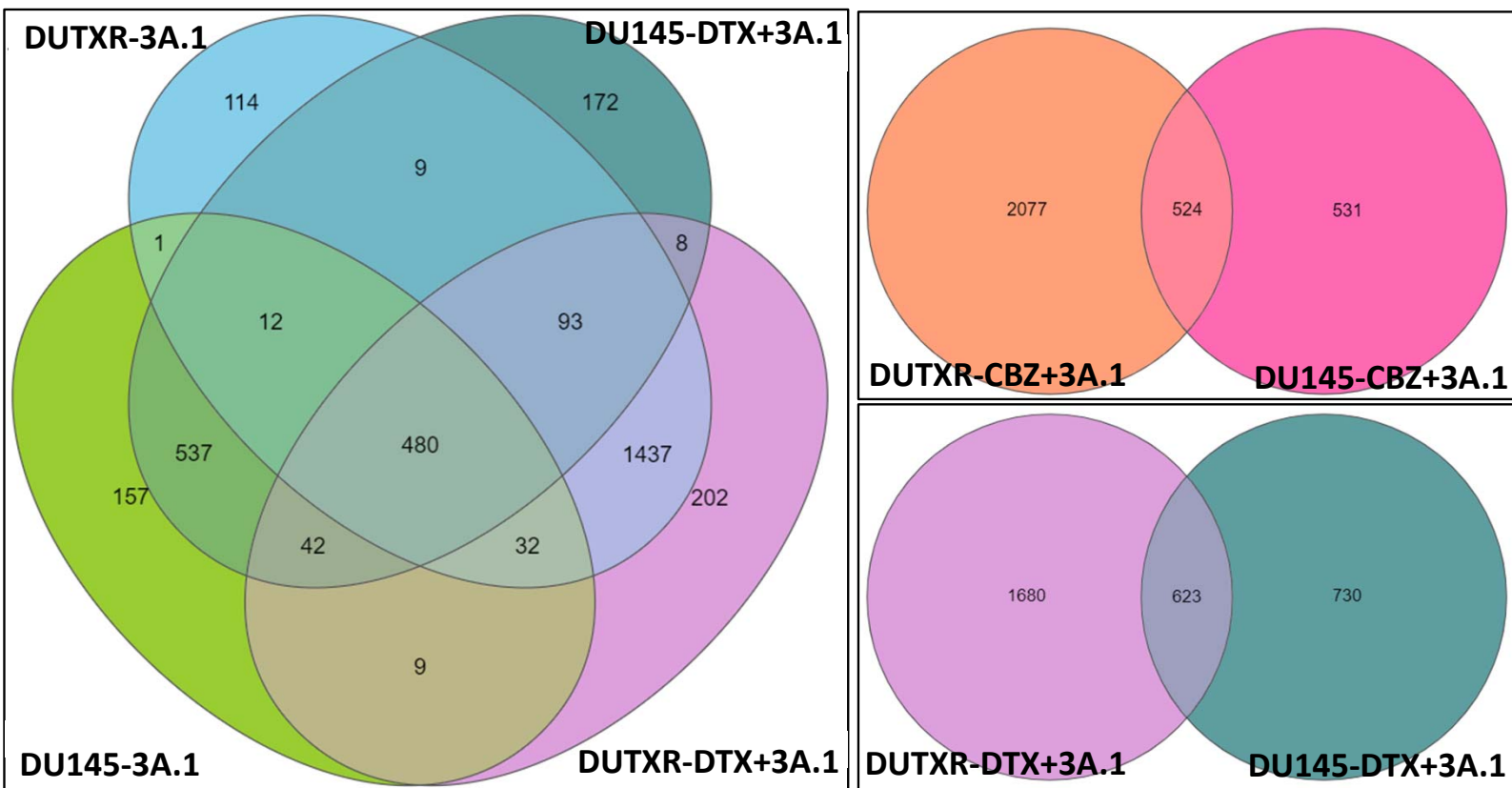
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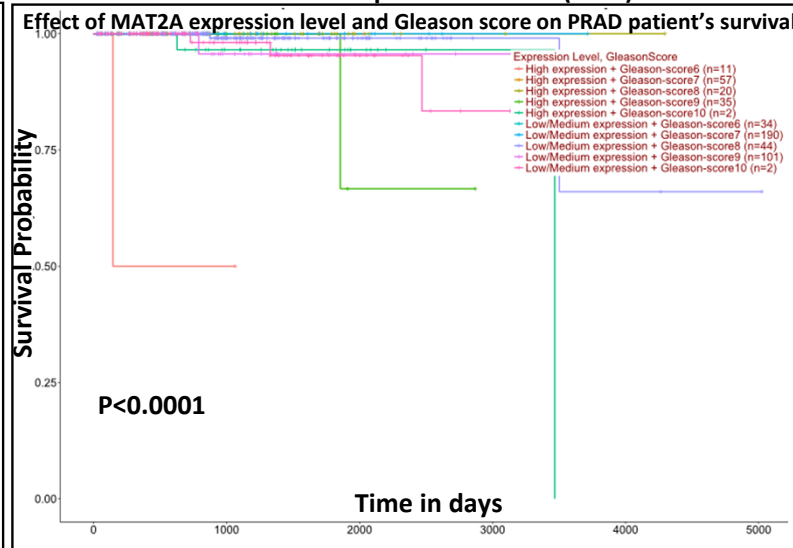
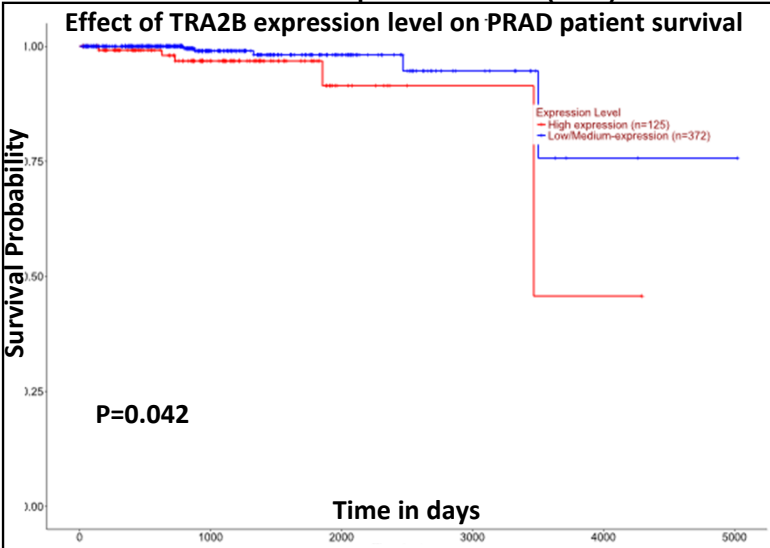
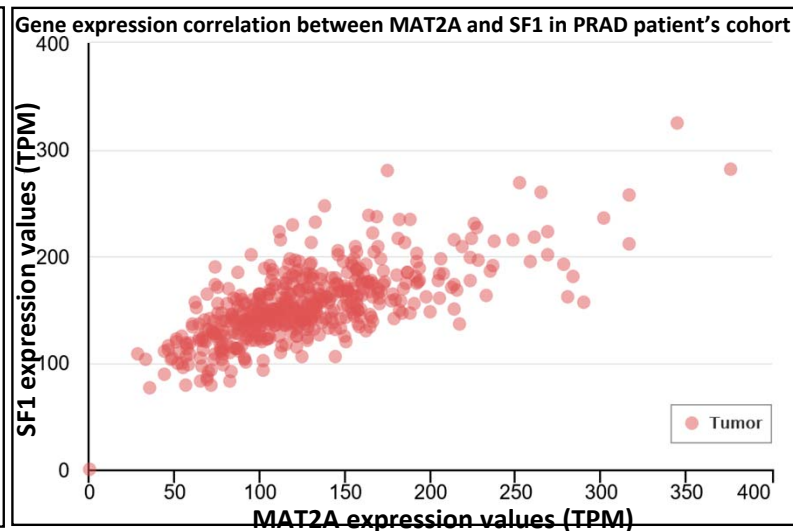
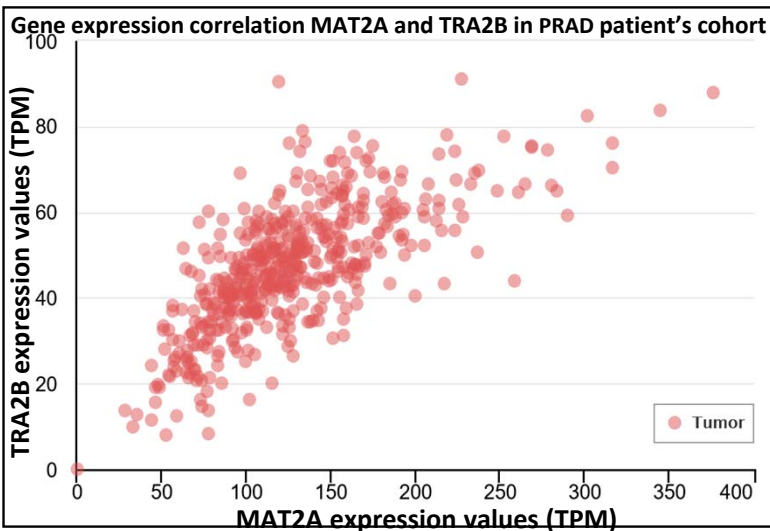
B.



Supplementary Figure 3. Functional analysis of the top DEG (MAT2A) using *in silico* approach (TCGA data)

Genes highly co-expressed ($r > 0.7$) with MAT2A (TRA2B and SF1) are associated with patient survival PRAD patients.

Supplementary Figure 3.



Supplementary Figure 4.

