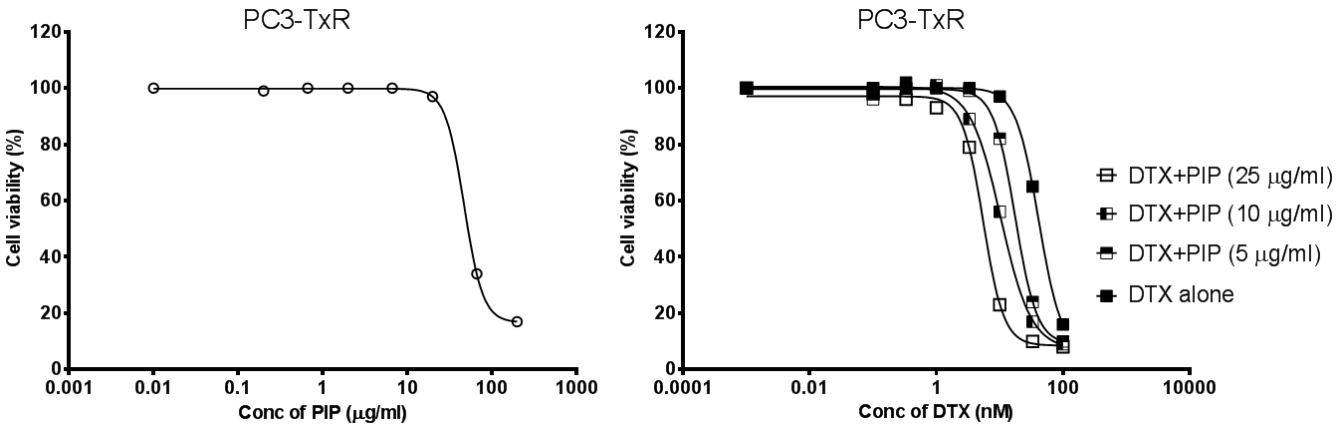
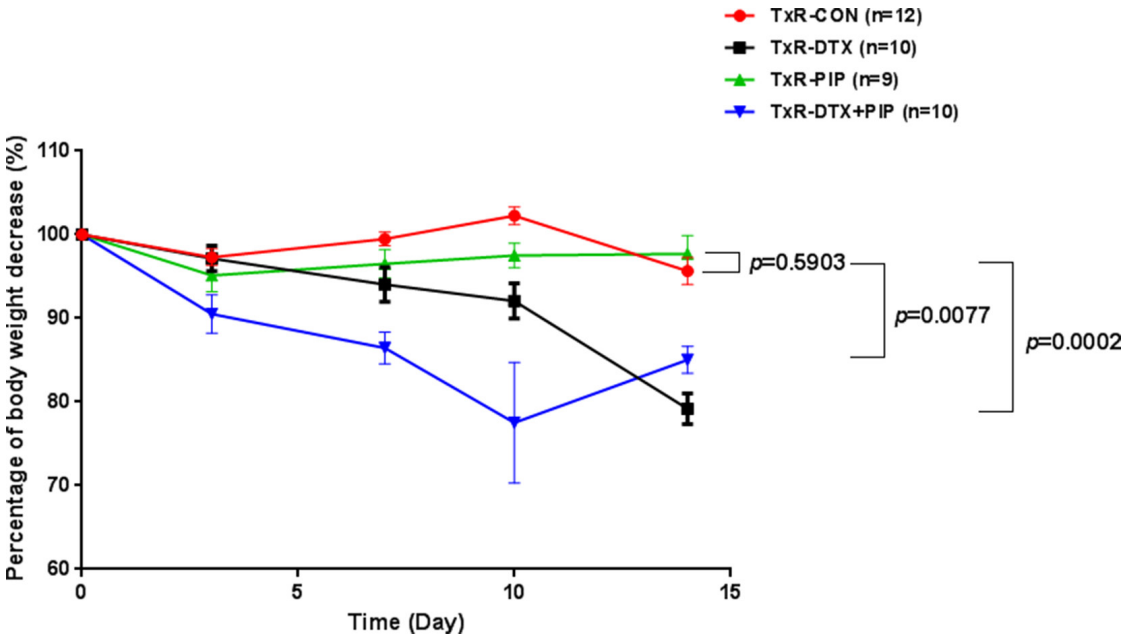
# Enhanced anti-tumor efficacy and mechanisms associated with docetaxel-piperine combination- *in vitro* and *in vivo* investigation using a taxane-resistant prostate cancer model

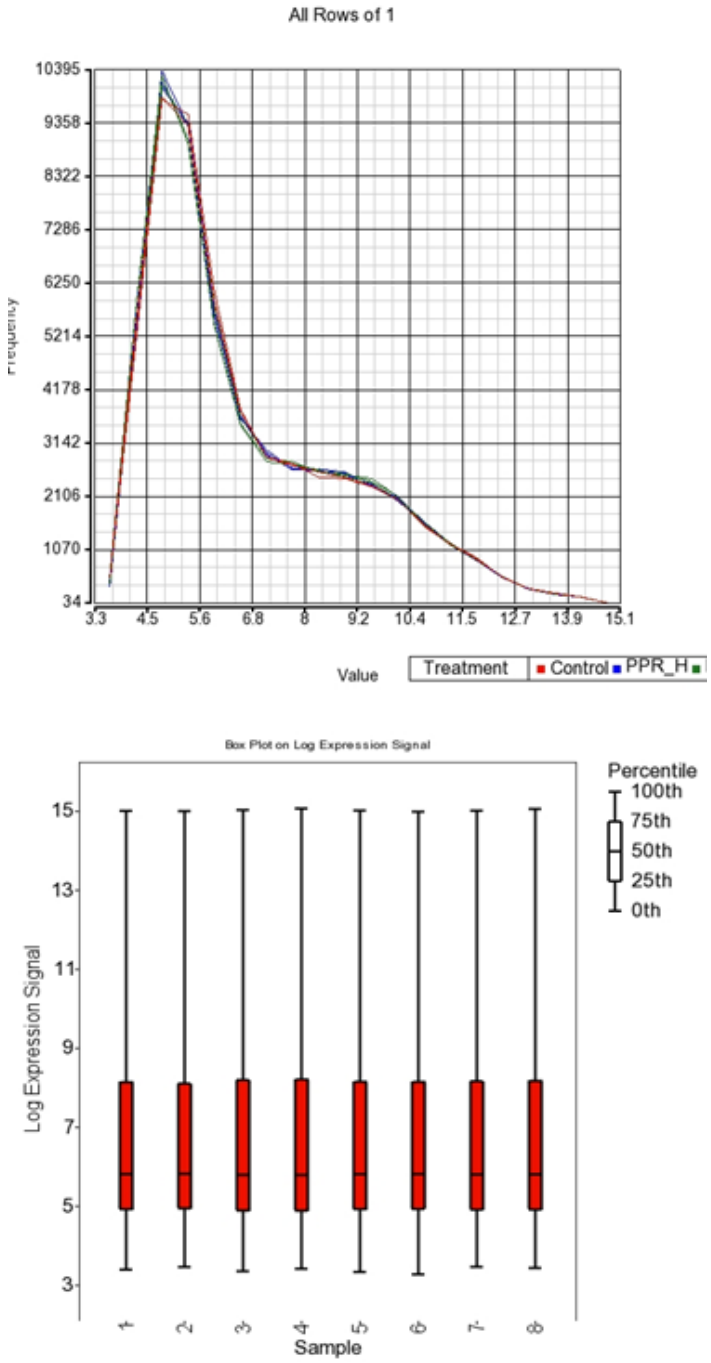
## SUPPLEMENTARY MATERIALS



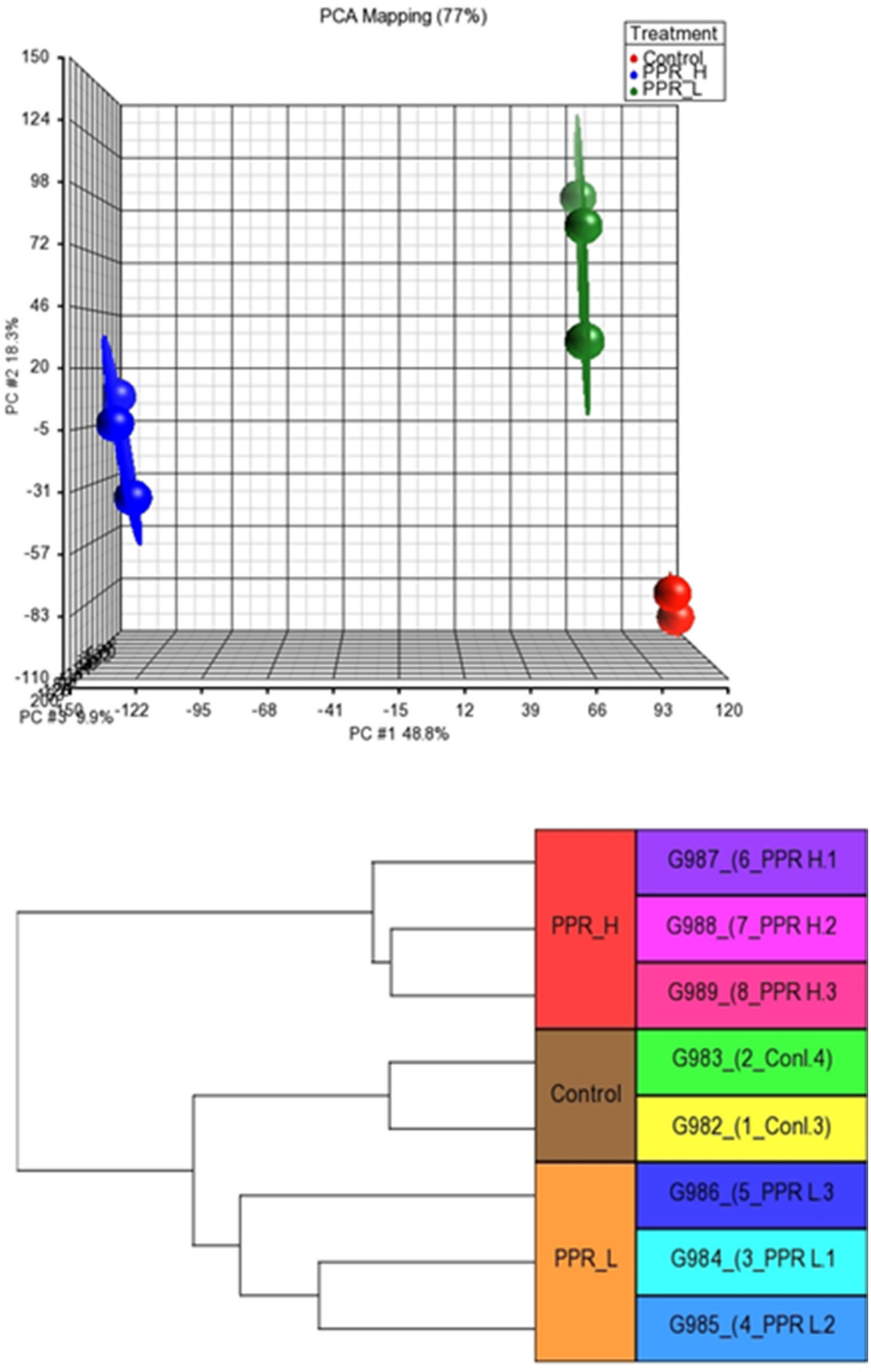

**Supplementary Figure 1: Cell viability (measured from triplicate samples) of PC3-TxR cells.** (**A**) The cytotoxicity of PIP on PC3-TxR (IC50 47.3 µg/ml); (**B**) The cytotoxicity of DTX on PC3-TxR alone or in combination with PIP at concentrations of 5, 10, and 25 µg/ml (IC50 values of DTX are 41.4, 17.6, 10.4, and 5.5 nM for DTX alone, DTX + 5 µg/ml PIP, DTX + 10 µg/ml PIP, and DTX + 25 µg/ml PIP, respectively). Abbreviation: DTX = docetaxel; PIP = piperine.




**Supplementary Figure 2: Plot of percentage of body weight decrease (%) versus time (Day) in mice with TxR tumor xenografts after treatment with saline (*n* = 12), DTX (20 mg/kg, *n* = 10), PIP (50 mg/kg, *n* = 9), or co-administration of DTX and PIP (20 mg/kg DTX and 50 mg/kg PIP, *n* = 10).** Abbreviation: DTX = docetaxel; PIP = piperine.




**Supplementary Figure 3: Sample histograms (upper) and intensity distribution (bottom) of microarray data.**




**Supplementary Figure 4: PCA and hierarchical cluster analysis.**

**Supplementary Table 1: IPA results for piperine high concentration treatment vs. control**

| Top canonical pathways | | | | |
|---|---|---|---|---|
| **Regulation** | **Name** | ***p*-value** | **Overlap** | |
| **Up** | Unfolded protein response | 8.27E-06 | 13.2% | (7/53) |
| | Adipogenesis pathway | 5.24E-05 | 7.4% | (9/122) |
| | Cysteine Biosynthesis/Homocysteine Degradation | 1.93E-04 | 100.0% | (2/2) |
| | Role of Macrophages, Fibroblasts and Endothelial Cells in Rheumatoid Arthritis | 2.05E-03 | 3.9% | (11/282) |
| | VDR/RXR Activation | 4.39E-03 | 6.5% | (5/77) |
| **Down** | 14-3-3-mediated Signaling | 1.12E-04 | 5.2% | (8/154) |
| | Germ Cell-Sertoli Cell Junction Signaling | 2.13E-04 | 4.7% | (8/169) |
| | Sertoli Cell-Setoli Cell Junction Signaling | 3.42E-04 | 6.1% | (6/98) |
| | p53 Signaling | 3.52E-04 | 7.8% | (5/64) |
| | Remodeling of Epithelial Adherens Junctions | | | |

| Top upstream regulators | | | |
|---|---|---|---|
| **Regulation** | **Upstream eegulator** | ***p*-value of overlap** | **Predicted activation** |
| **Up** | tosedostat | 7.18E-19 | Activated |
| | ATF4 | 2.55E-13 | Activated |
| | TRIB3 | 1.67E-11 | Inhibited |
| | miR-30c-5p (and other miRNAs w/seed GUAAACA) | 4.12E-11 | Inhibited |
| | thapsigargin | 5.20E-11 | Activated |
| **Down** | miR-23a-3p (and other miRNAs w/seed UCACAUU) | 1.91E-11 | Activated |
| | miR-124-3p (and other miRNAs w/seed AAGGCAC) | 1.08E-10 | Activated |
| | miR-19b-3p (and other miRNAs w/seed GUGCAAA) | 2.94E-08 | Activated |
| | miR-200b-3p (and other miRNAs w/seed AAUACUG) | 1.33E-07 | Activated |
| | miR-26a-5p (and other miRNAs w/seed UCAAGUA) | 2.01E-07 | Activated |

**Supplementary Table 2: IPA results for piperine low concentration treatment vs. Control**

| Top canonical pathways | | | | |
|---|---|---|---|---|
| **Regulation** | **Name** | ***p*-value** | **Overlap** | |
| **Up** | HIF1 Signaling | 2.45E-03 | 3.00% | (3/100) |
| | Tight Junction Signaling | 9.69E-03 | 1.80% | (3/164) |
| | Hutington's Disease Signaling | 2.25E-02 | 1.30% | (3/225) |
| | Guanosine Nucleotides Degradation III | 3.44E-02 | 7.70% | (1/13) |
| | Urate Biosynthesis/Inosine 5′-phosphate Degradation | 3.70E-02 | 7.10% | (1/14) |
| **Down** | Bupropion Degradation | 7.28E-04 | 8.30% | (2/24) |
| | Acetone Degradation I (to Methylglyoxal) | 7.90E-04 | 8.00% | (2/25) |
| | HMGB1 Signaling | 9.84E-04 | 2.50% | (3/118) |
| | Atherosclerosis Signaling | 9.84E-04 | 2.50% | (3/118) |
| | Estrogen Biosynthesis | 1.55E-03 | 5.70% | (2/35) |
| **Top upstream regulators** | | | | |
| **Regulation** | **Upstream regulator** | ***p*-value of overlap** | **Predicted activation** | |
| **Up** | PPARG | 1.58E-08 | Activated | |
| | troglitazone | 2.02E-06 | Activated | |
| | fluticasone | 4.15E-06 | | |
| | PLAGL1 | 6.72E-06 | | |
| | Insulin | 6.74E-06 | | |
| **Down** | CD40 | 1.84E-10 | | |
| | 5-hydroxytryptamine | 4.10E-10 | Inhibited | |
| | TREM1 | 5.36E-10 | | |
| | TNF | 6.54E-10 | | |
| | U0126 | 9.03E-10 | | |

**Supplementary Table 3: Drug administrations for each treatment group with different cell xenografts**

| Treatment Groups | Cell xenografts | Drug administration |
|---|---|---|
| PC3-CON | PC3 | Vehicle, Daily, p.o. |
| PC3-DTX | PC3 | DTX, 20 mg/kg, once a week, i.v. |
| TxR-CON | PC3-TxR | Vehicle, Daily, p.o. |
| TxR-DTX | PC3-TxR | DTX, 20 mg/kg, once a week, i.v. |
| TxR-PIP | PC3-TxR | PIP, 50 mg/kg, daily, p.o. |
| TxR-DTX+PIP | PC3-TxR | DTX at 20 mg/kg (once a week, i.v.) and PIP at 50 mg/kg (daily, p.o.) |

Abbreviation: DTX = docetaxel; PIP = piperine.