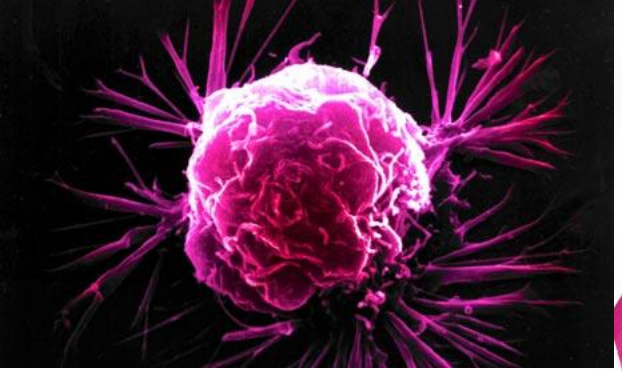

DEAD-box RNA Helicase DP103 as a Biomarker for Therapeutic Response to Docetaxel

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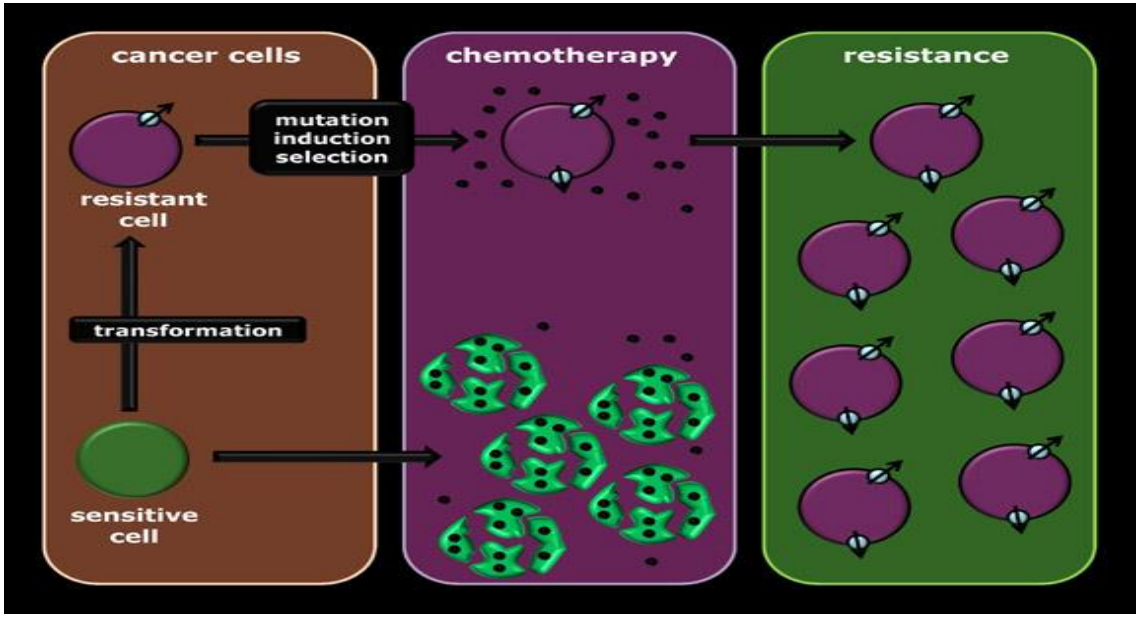
- **School of Biomedical Sciences, Curtin University, WA**

Chemoresistance
'MAN PROPOSES : TUMOR DISPOSES'



- Accounts for over 90% of treatment failures in patients with metastatic breast cancer (Longley et al., 2005)
- Time-to-Progression (**TTP**) of **6-10 months** (Cortes et al., 2007)

Popular Mechanisms Behind Docetaxel Resistance

β-tubulin gene mutations or differential expression

Suppression of pro-apoptotic genes (p53, Bax, Caspases)

Multi-Drug Resistant phenotype (PgP)

Activation of survival genes (PI3K/Akt, Bcl2)

Increased cellular drug detoxification

None has been successfully employed in the clinic to aid as predictive marker of docetaxel-response and -resistance (Camerini et a., 2011)

In Pursuit of New Biomarkers.....

Breast Cancer

Response to Docetaxel

NUS National University of Singapore

CSI SINGAPORE

DEAD Box RNA Helicases

- Belong to **SF2 family** of helicases
- **ATP-dependent helicases,** unwinding dsRNA, RNA-DNA and RNA-protein structures
- **Conserved motifs** in the **central core region,** highly **variable N & C-terminals**
- **D-E-A-D** → characteristic & conserved sequence (**Asp-Glu-Ala-Asp**)

DP103/DDX20/Gemin 3

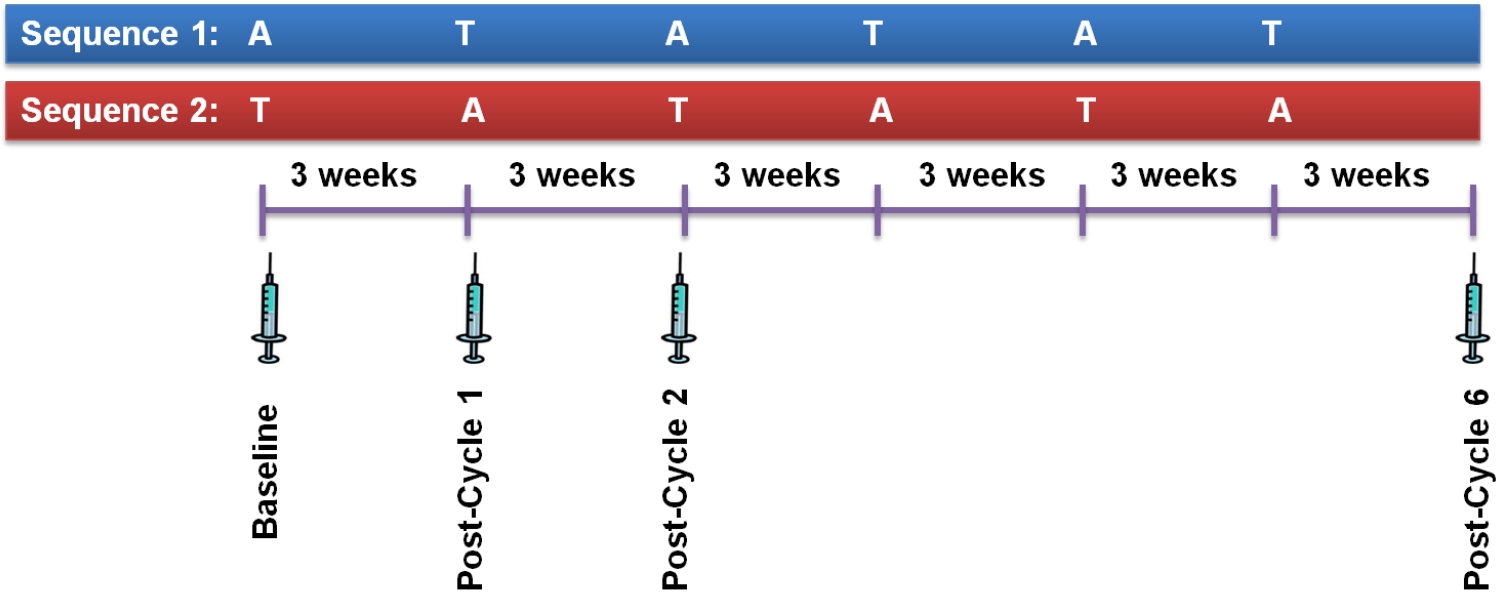
- Interacts with **EBNA2 and EBNA3C** through its C-terminus;
  expression higher in **rapidly proliferating cells** (testis & tonsils) (Grundhoff et al., 1999)
- Component of the **Survival Motor Neuron** complex → snRNP biogenesis & spliceosomal assembly (Charroux et al., 1999, Campbell et al., 2000)
- DP103 **K/O mice → embryonic lethality;**
  homozygous mice failed to survive beyond the 2-cell stage (Mouillet et al., 2008)
- **Transcriptionally represses** Egr2, SF-1 and Ets target genes
  (Gillian and Svaren et al., 2004, Ou et al., 2001, Klappacher et al., 2002)
- **Represses SF-1 activity via sumoylation** (Lee MB et al., 2005)

DEAD Box Protein, DP103: coming ALIVE in Cancer

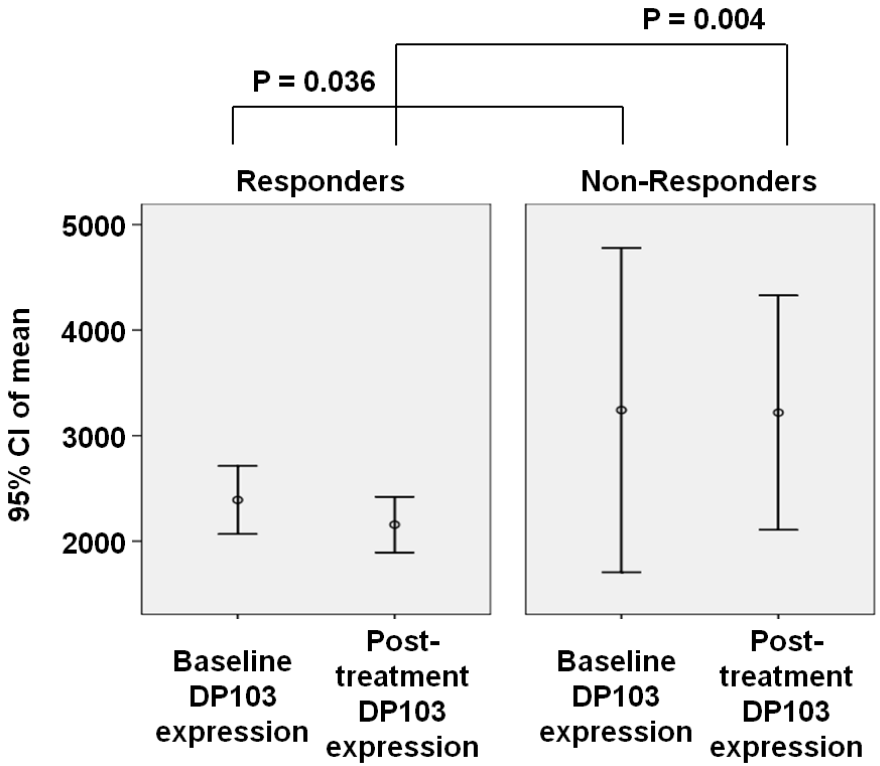
- **Tumor-suppressor** in Hepatocellular Carcinoma (Zender et al., 2008, Takata et al., 2012)
- **Upregulated** in Mantle-Cell Lymphomas (Ghobrial et al., 2005)
- Increases the **metastatic potential of breast cancer** cells through **activation of NFκB,** and being **regulated by NFκB** itself (Shin et al., 2014)

Clinical Setting

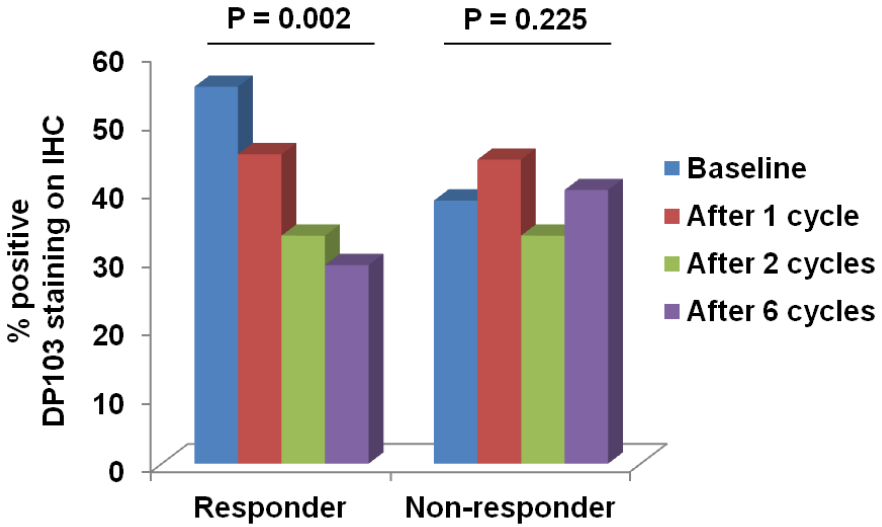
- **Neoadjuvant** phase II study
- 100 patients, with locally advanced or metastatic breast cancer, recruited and randomized to **two alternating sequences of doxorubicin (A) and docetaxel (T),** starting with 75mg/m^2 of either drugs, as an hourly infusion



Clinical Responders exhibit a chemotherapy-induced decrease in DP103 expression

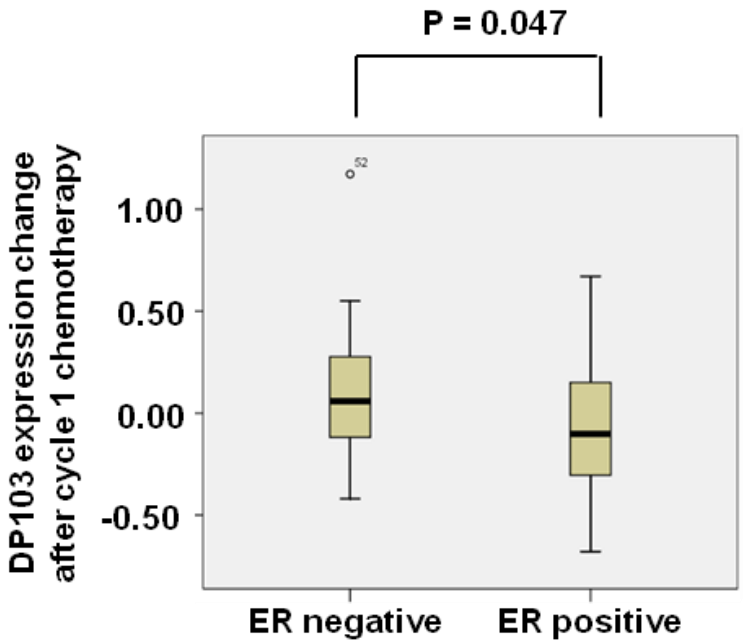


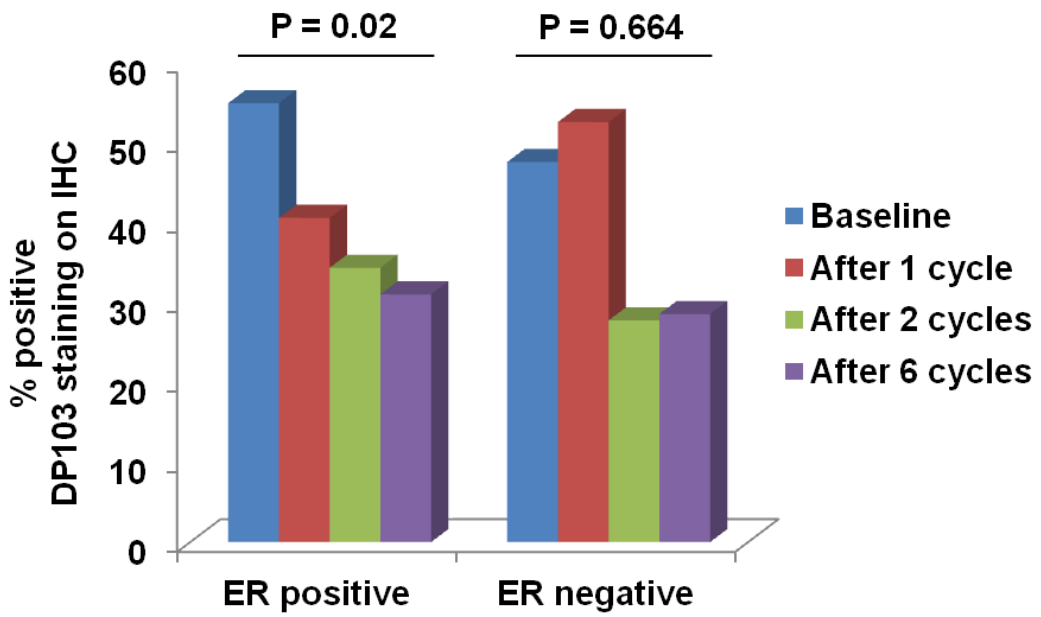
n = 54 48 11 8



n = 49, 42, 39, 31 13, 9, 12, 5

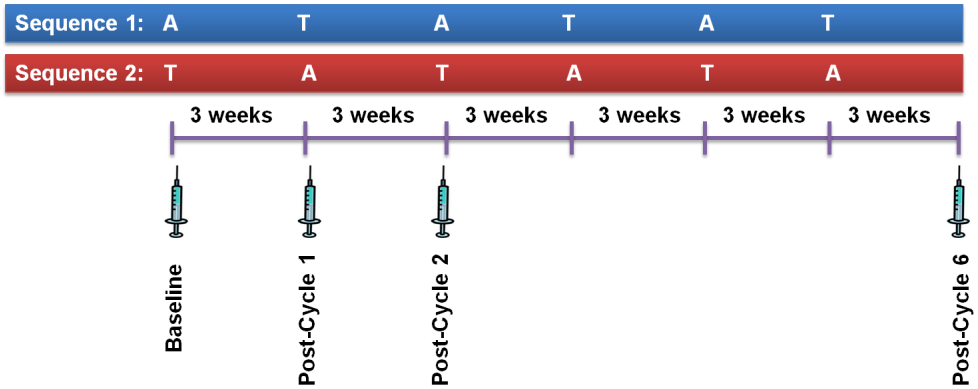
Chemotherapy negatively regulates DP103 expression in ER positive patients

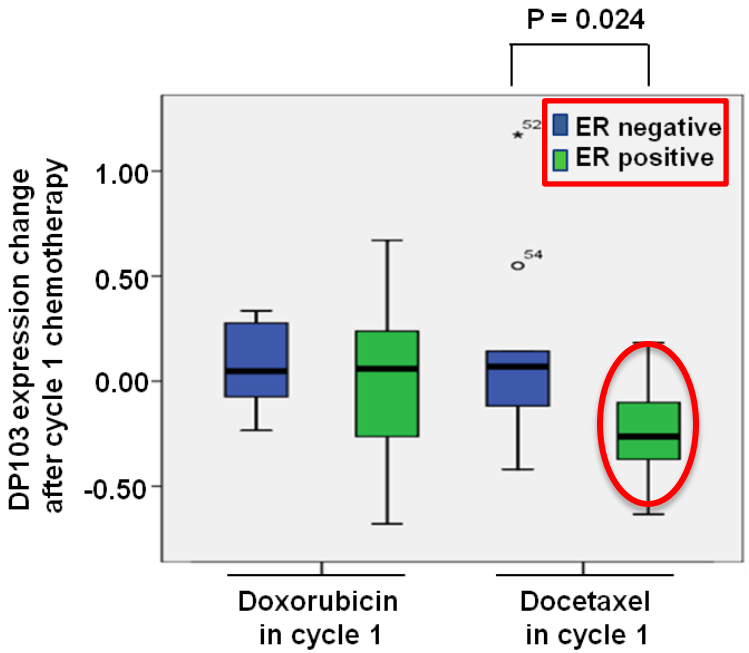




n = 40, 32, 32, 29 21, 19, 18, 7

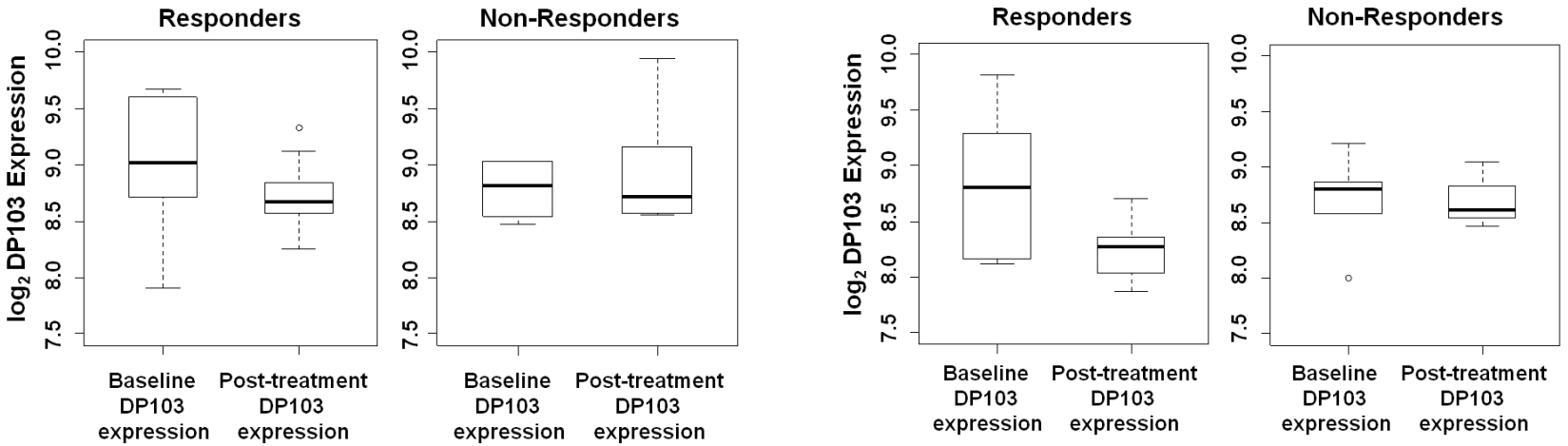
Docetaxel negatively regulates DP103 expression in ER positive patients





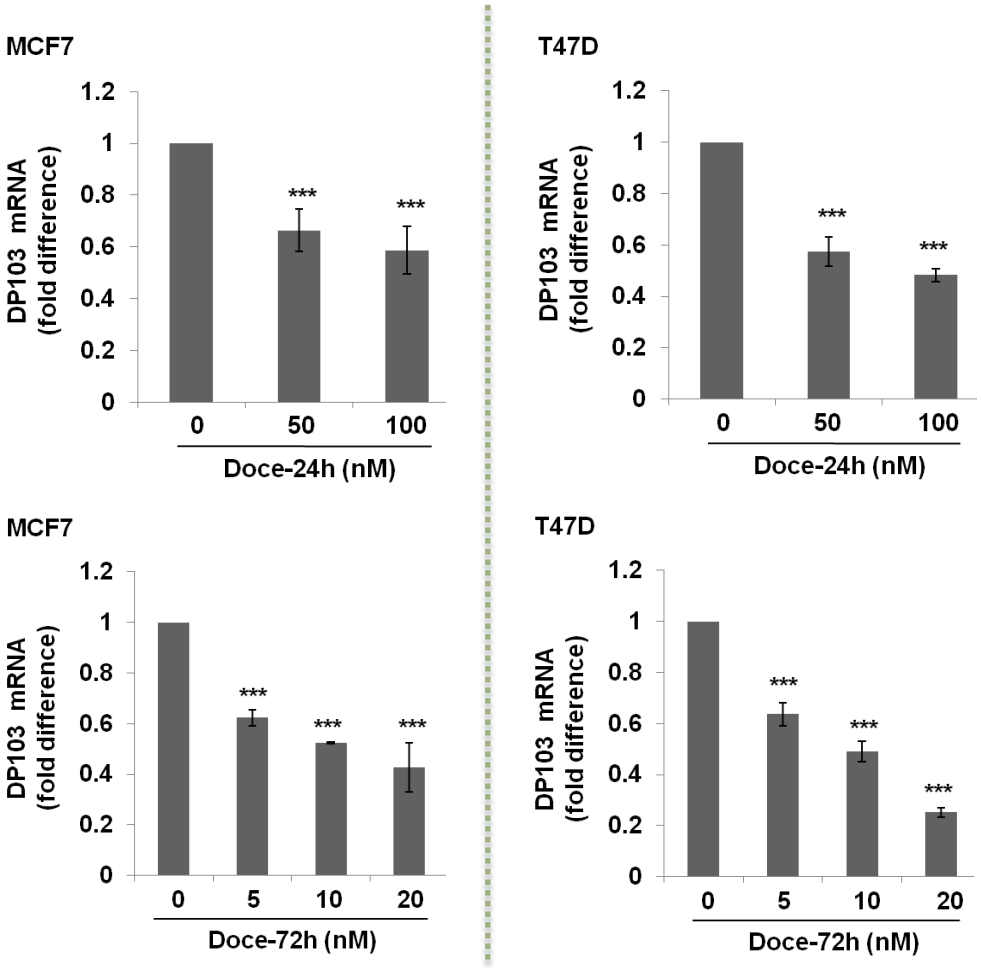
Docetaxel negatively regulates DP103 expression in ER positive patients

GEO gene expression data sets GSE21974 (Left) & GSE18728 (Right)



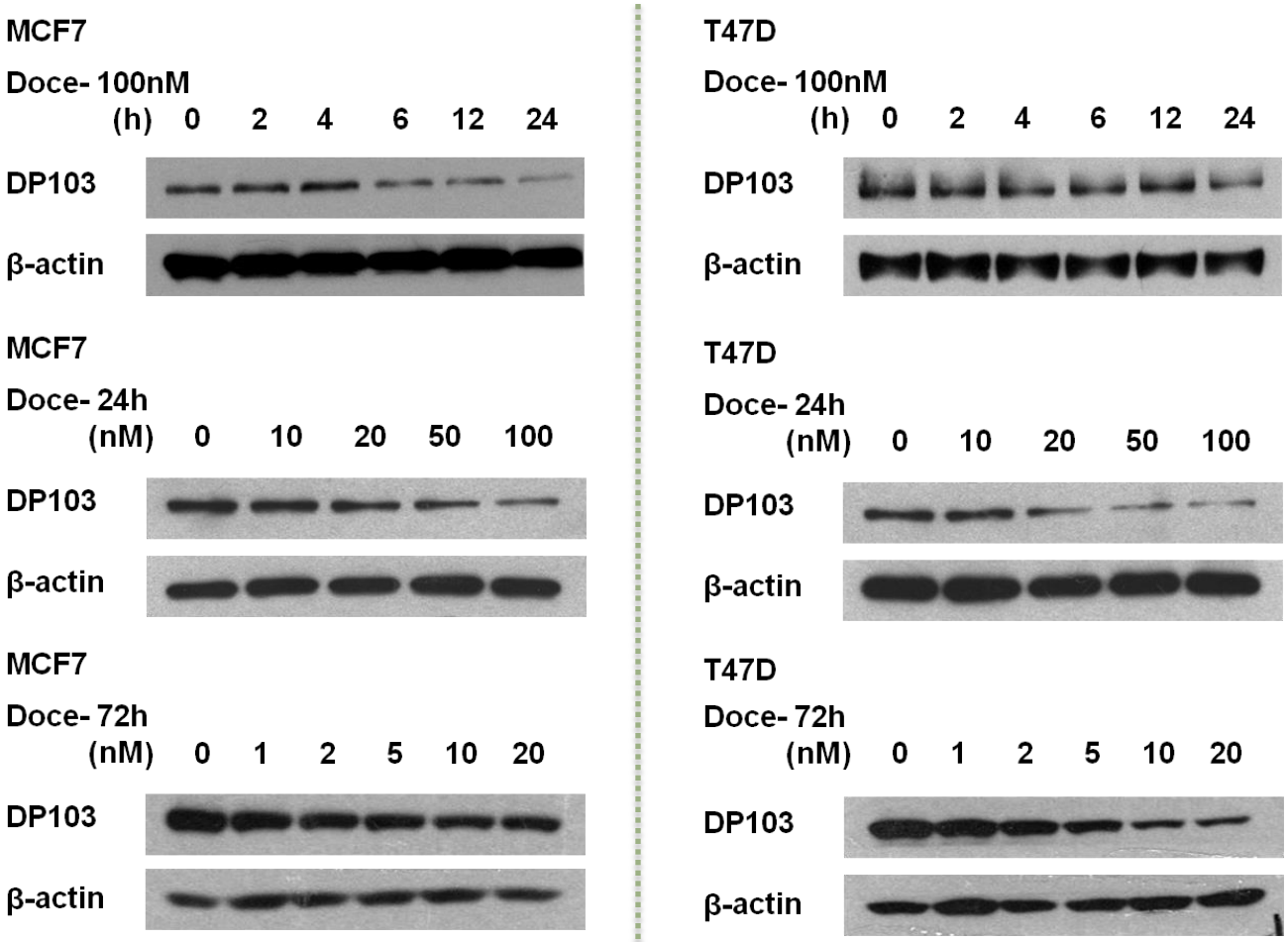
(n=31, p=0.028) **(n=14, p=0.98)** **(n=13, p=0.05)** **(n=8, p=0.96)**

Docetaxel decreases DP103 mRNA expression in ER positive cell lines

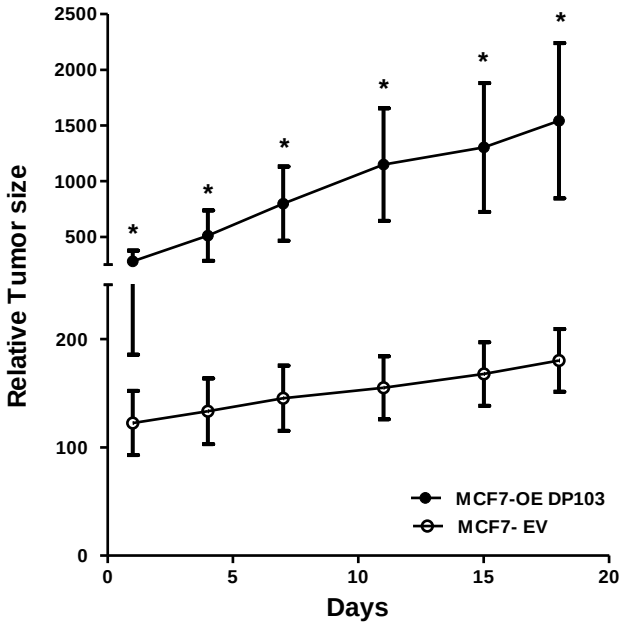


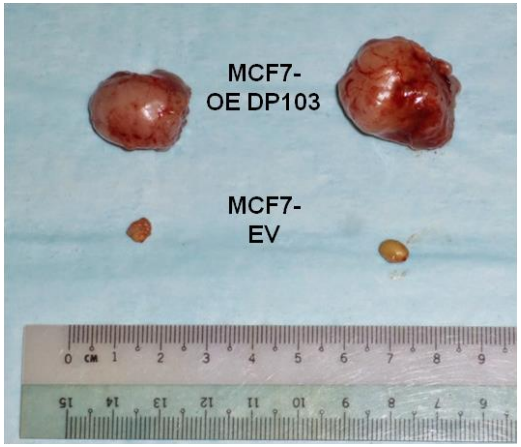
*** denotes P value < 0.001

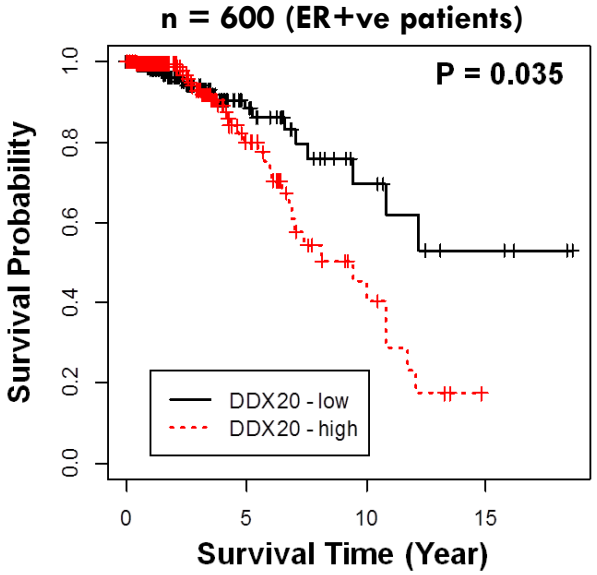
Docetaxel decreases DP103 protein expression in ER positive cell lines



DP103 over-expression increases tumor burden

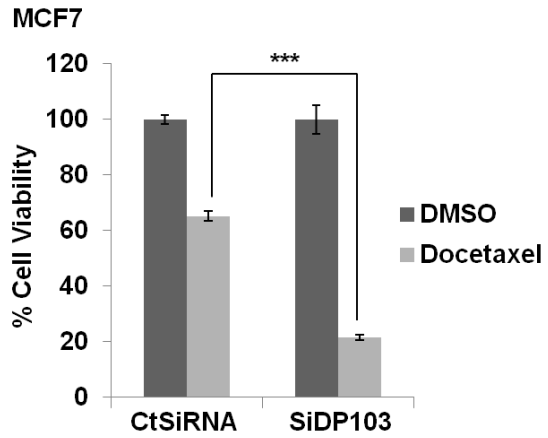


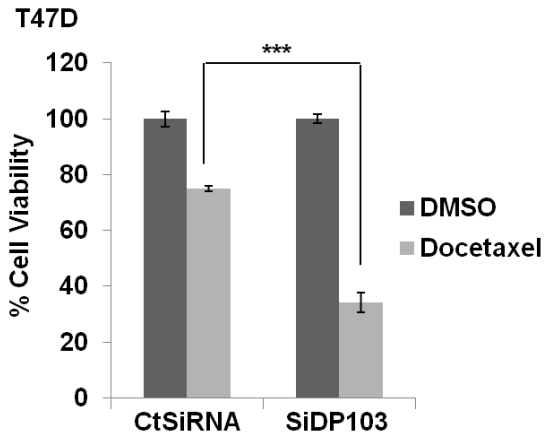


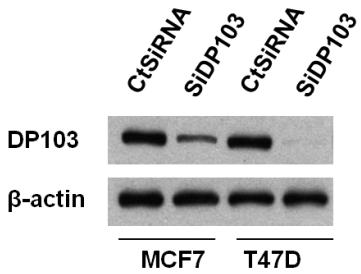


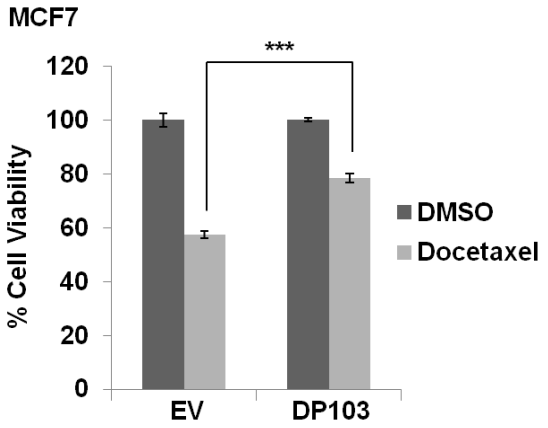
* denotes P value < 0.05

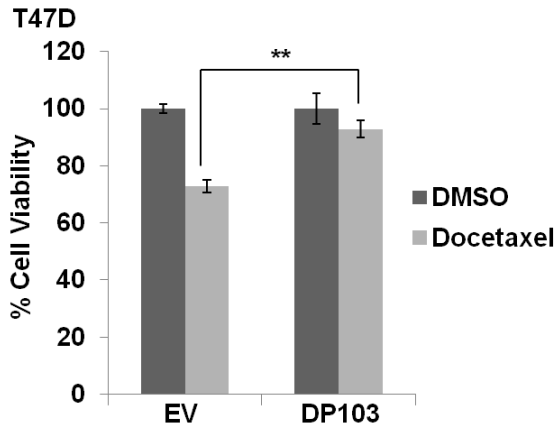
DP103 modulates docetaxel sensitivity

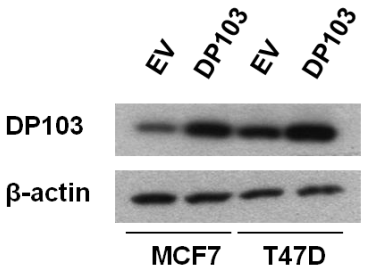








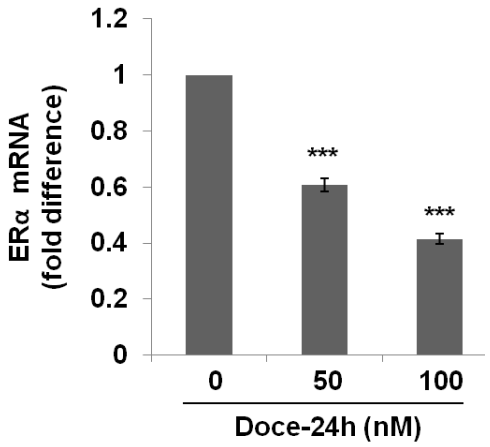




** denotes P value < 0.01, *** denotes P value < 0.001

ERα observes similar docetaxel-induced profile as DP103

MCF7



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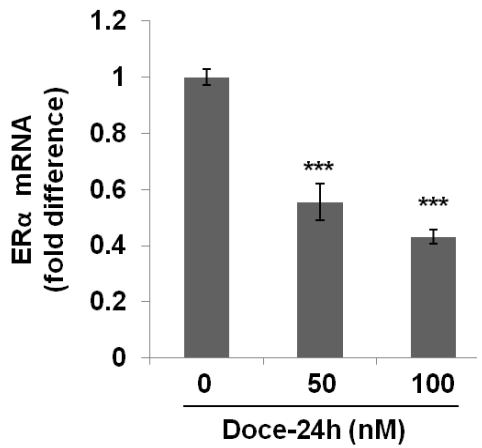

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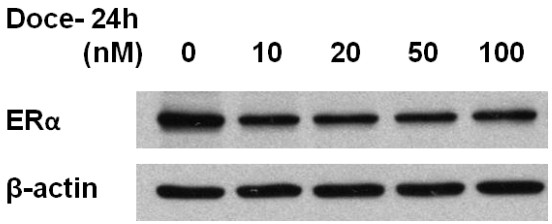

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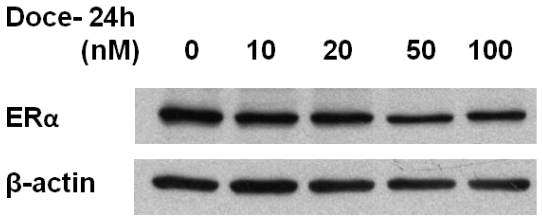

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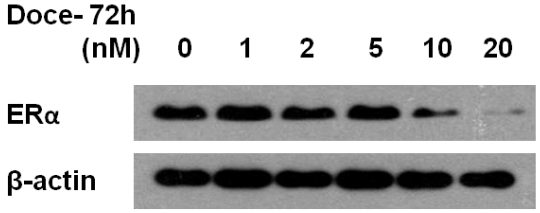

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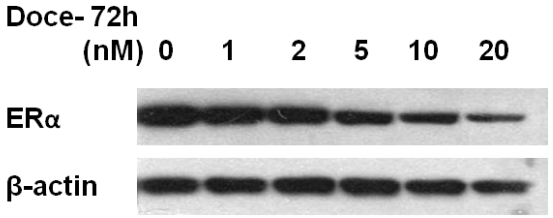
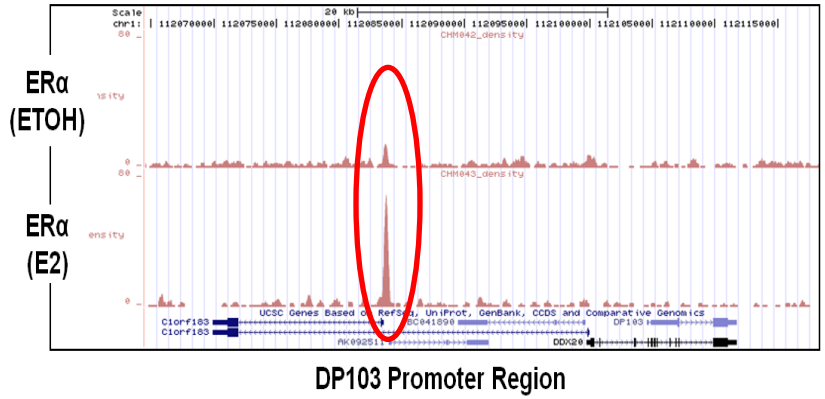
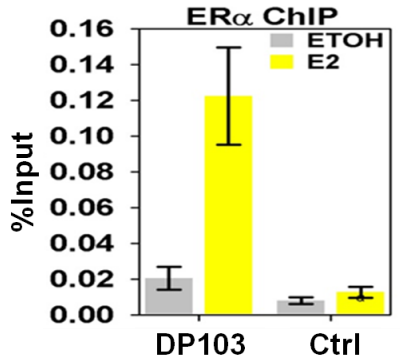
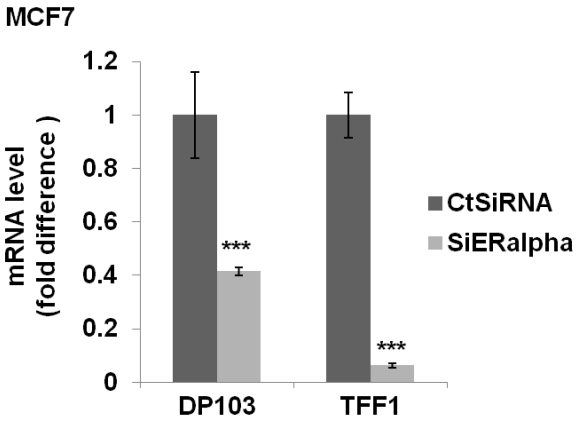


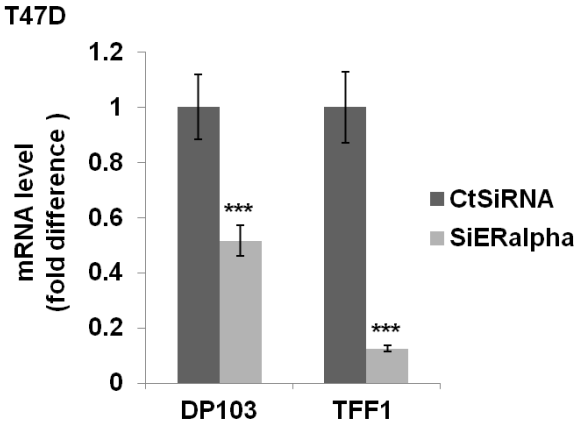
*** denotes P < 0.001

ERα regulates DP103 transcriptionally

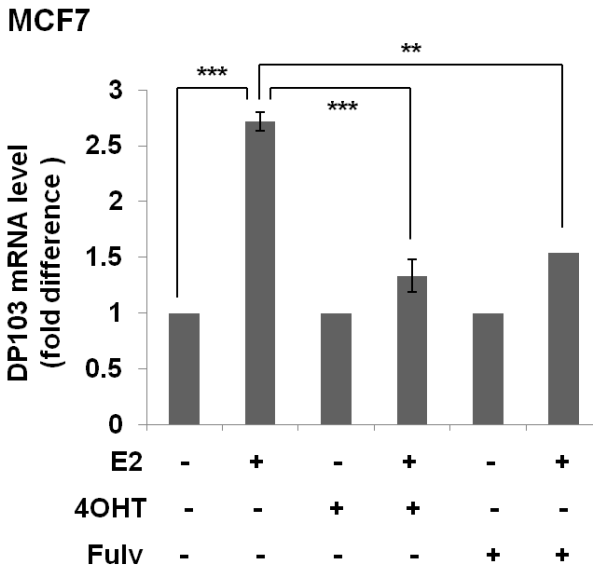


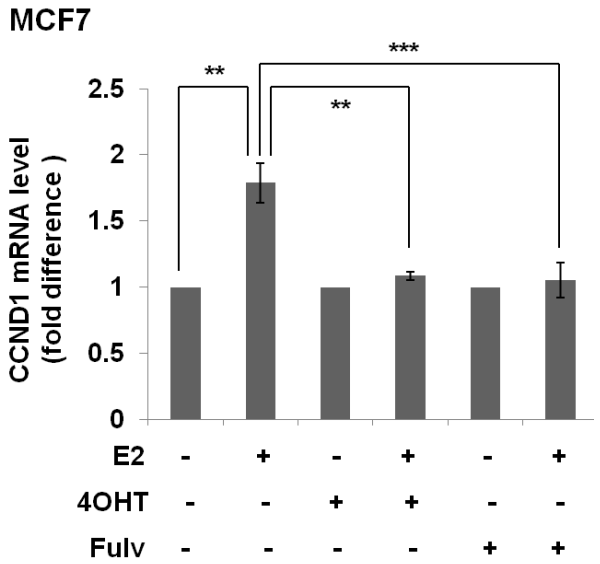






Pharmacological inhibition of ERα abrogates E2-induced DP103 expression

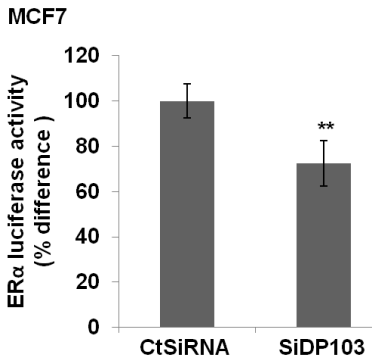


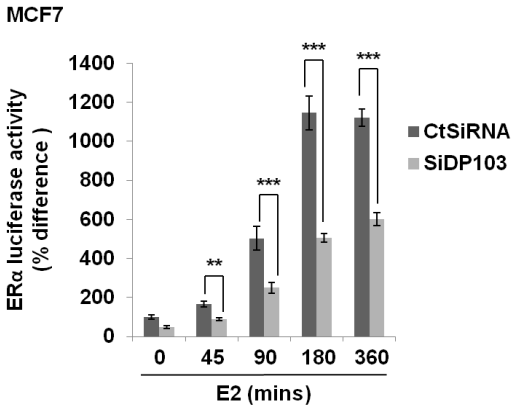


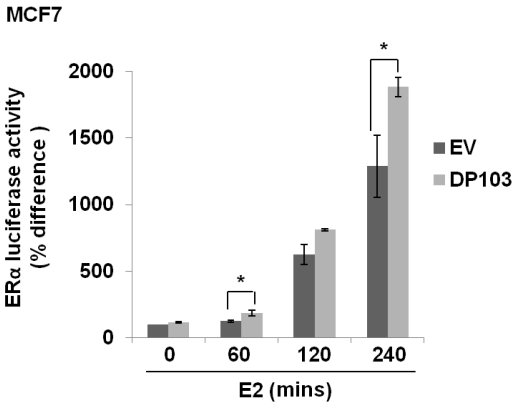
E2 - 17-β-Estradiol
4OHT - 4-hydroxy tamoxifen
Fulv - Fulvestrant

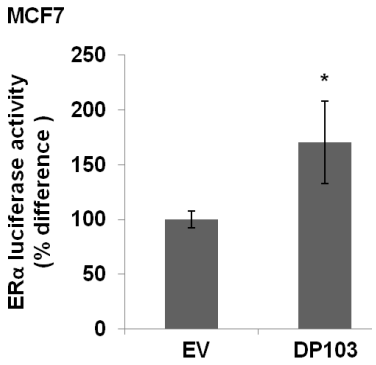
** denotes P < 0.01, *** denotes P < 0.001

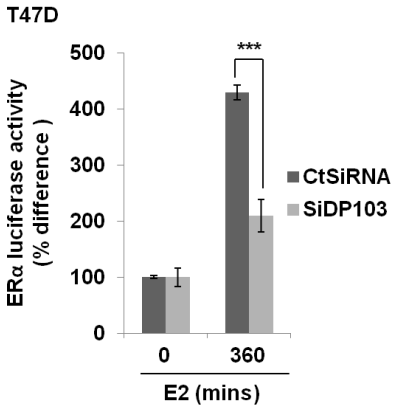
DP103 modulates ER transcriptional activity

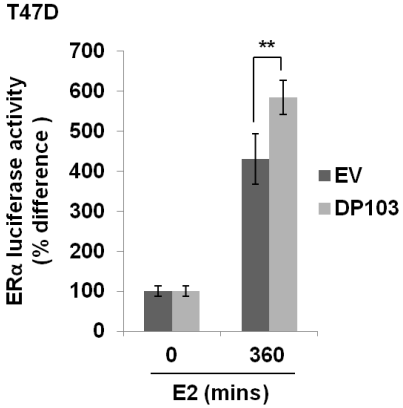


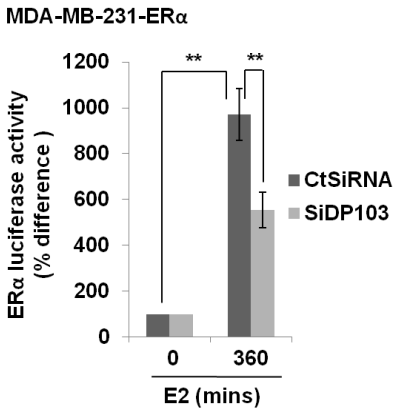






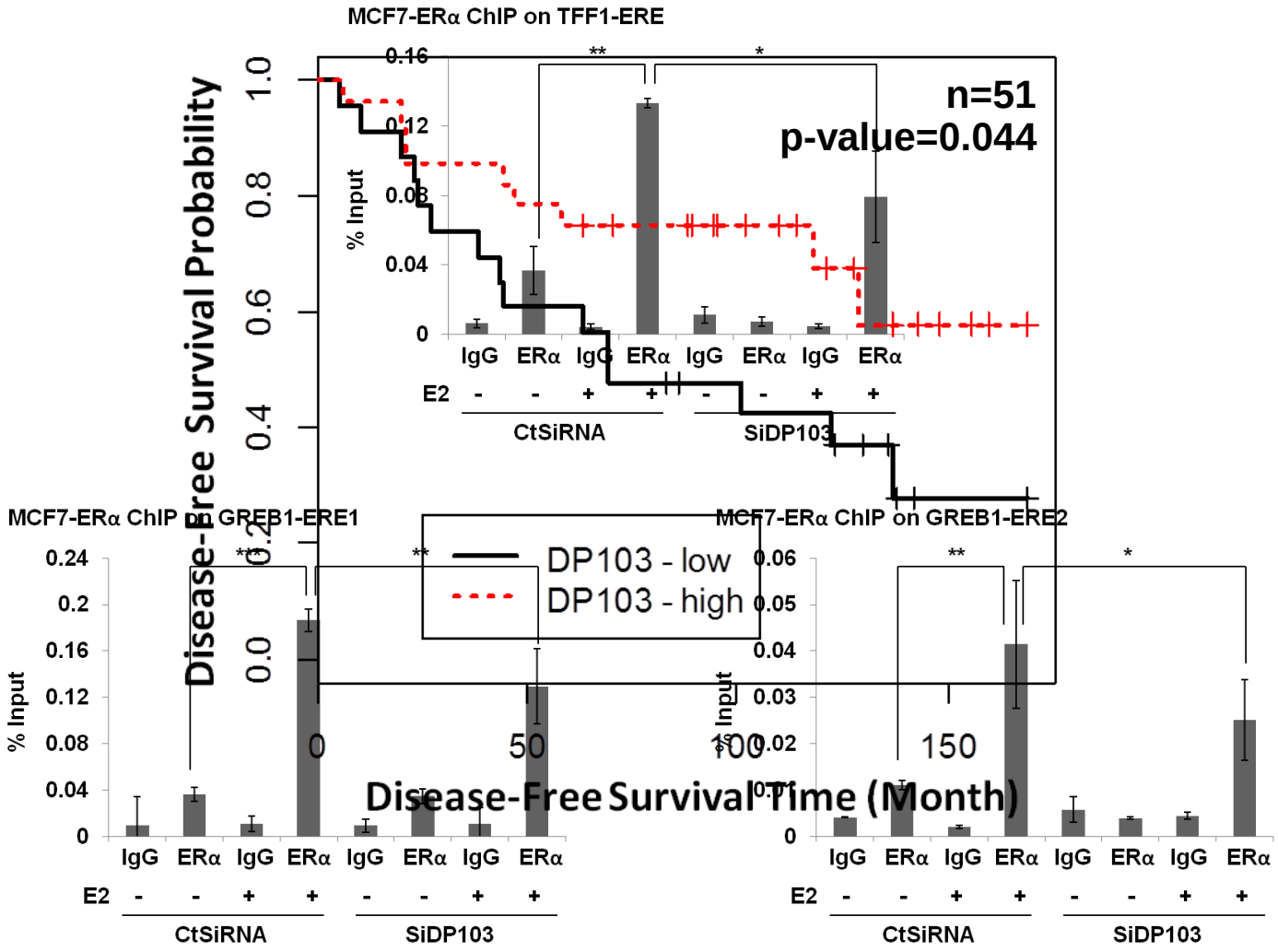






* denotes P value < 0.05, ** denotes P < 0.01, *** denotes P < 0.001

DP103 modulates recruitment of ERα to the promoter

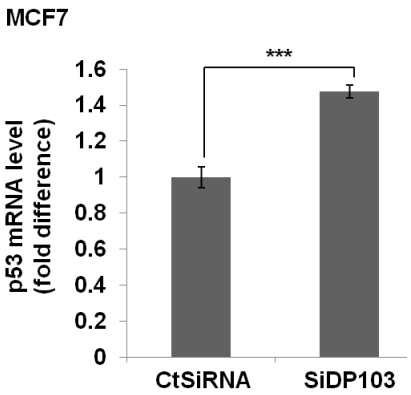


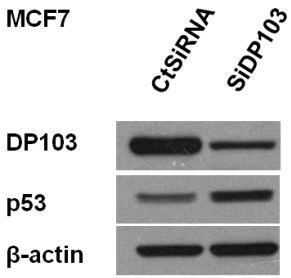
* denotes P value < 0.05, ** denotes P < 0.01, *** denotes P < 0.001

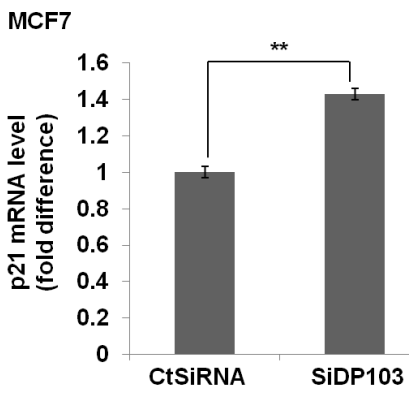
DP103 is a novel repressor of p53

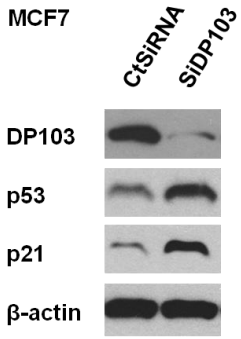
- **DP103** also **represses p53 transcriptional activity** (Cai et al., 2011)

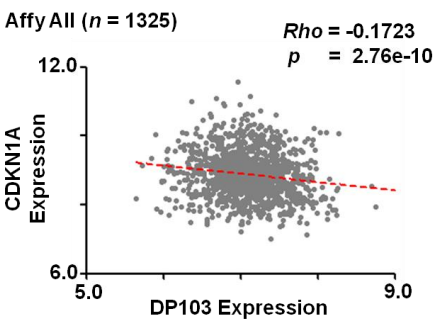
DP103 represses transcriptional regulation of p53

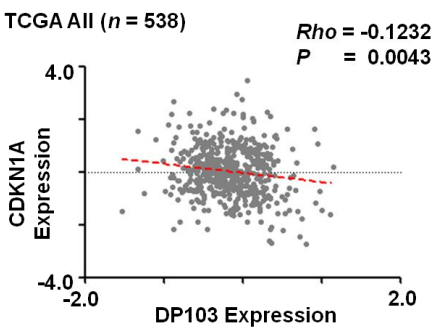


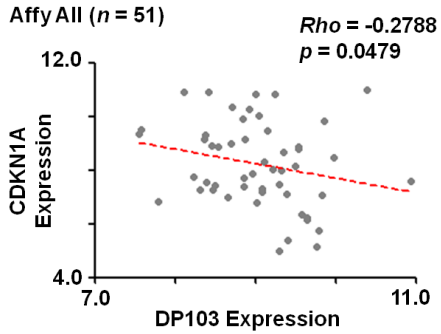


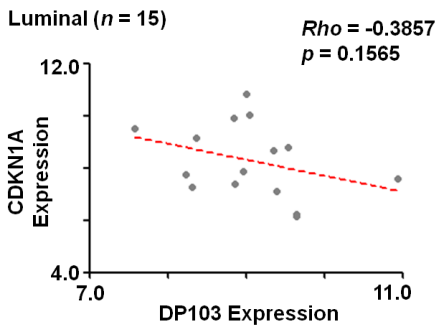






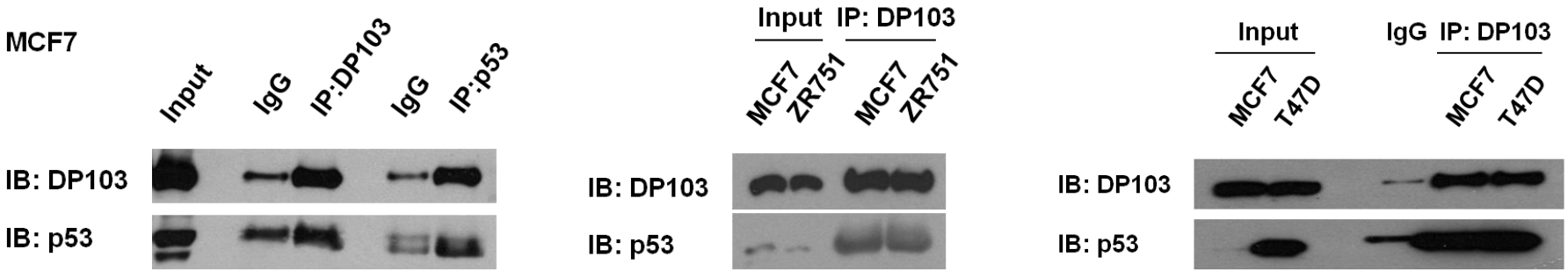






** denotes P < 0.01, *** denotes P < 0.001

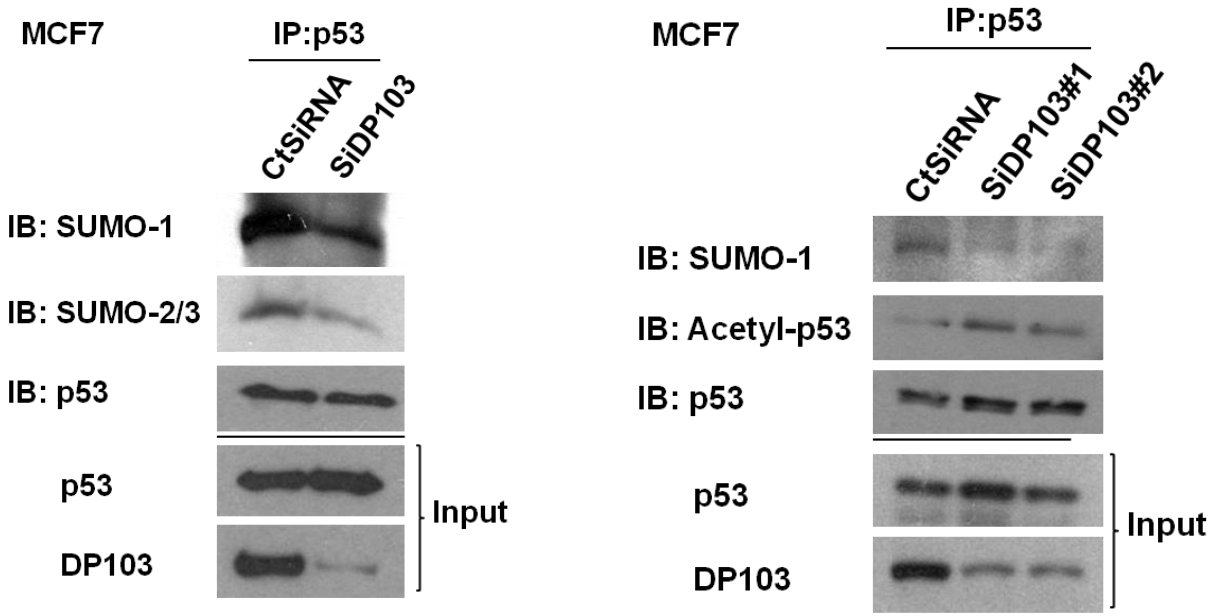
DP103 interacts with p53



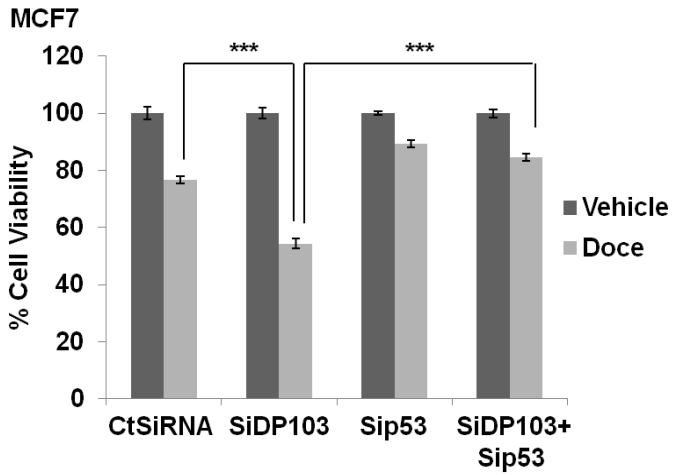
Effect of Sumoylation on p53 transcriptional activity

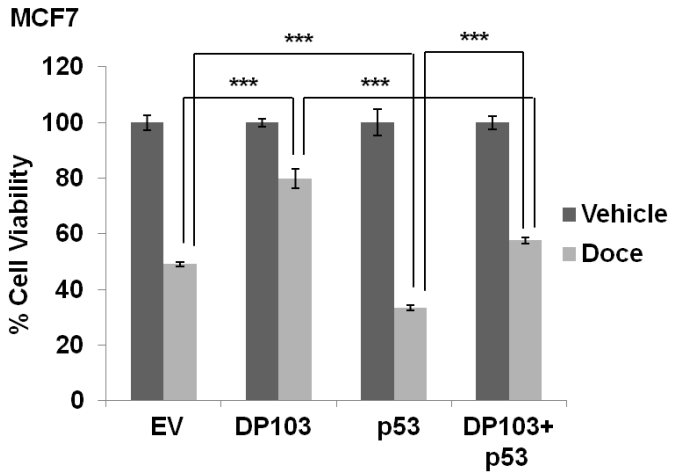
- Conjugation of SUMO ligases to p53 **represses** its activity (Schmidt et al., 2002)
- Ionizing radiation-induced Chk2, **phosphorylates p53**, also **repressing its sumoylation** (Lin J-Y et al., 2004)
- Sumoylation increases **nuclear export of p53**, thus increasing its cytoplasmic retention (Santiago et al., 2013)
- SUMO-1 conjugated p53 fails to activate p53 transcriptional activity, because of its **inability to bind to DNA**
  Sumoylation of p53 recruits transcriptional **co-repressor mSin3A**
  Sumoylation-deficient **K386R exhibits higher transcriptional activity** compared to WT p53
  Sumoylation of p53 at K386 **prevents subsequent acetylation** (and activation) at **K382** (Wu et al., 2009)

DP103 modulates the switch between p53 sumoylation and acetylation



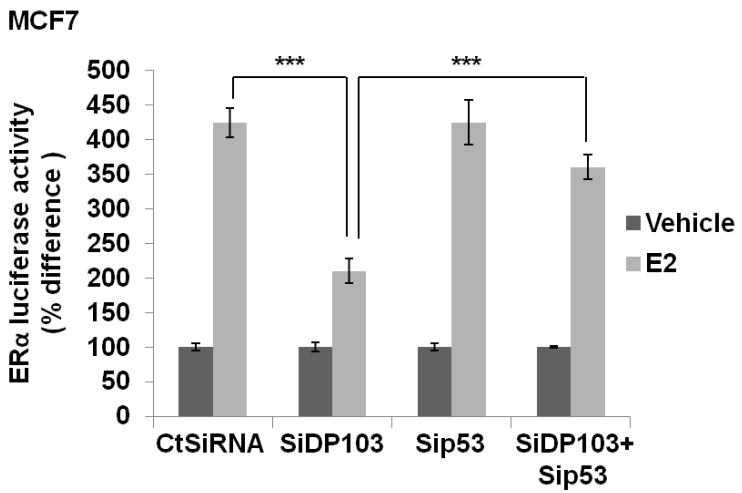
DP103 modulates docetaxel sensitivity through suppression of p53

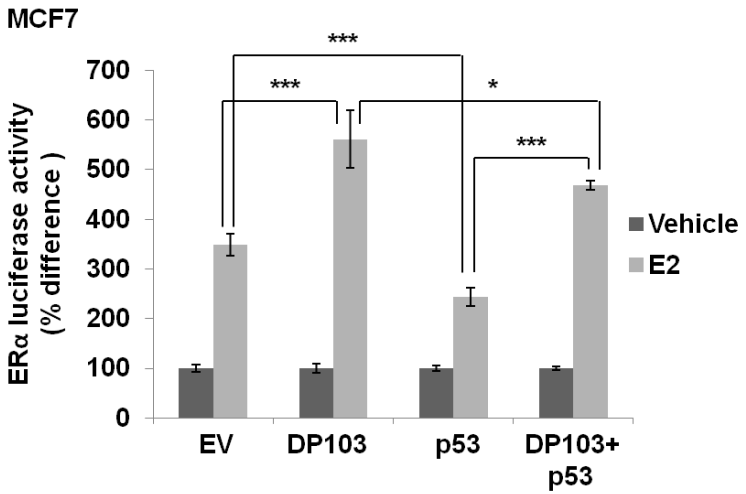




*** denotes P < 0.001

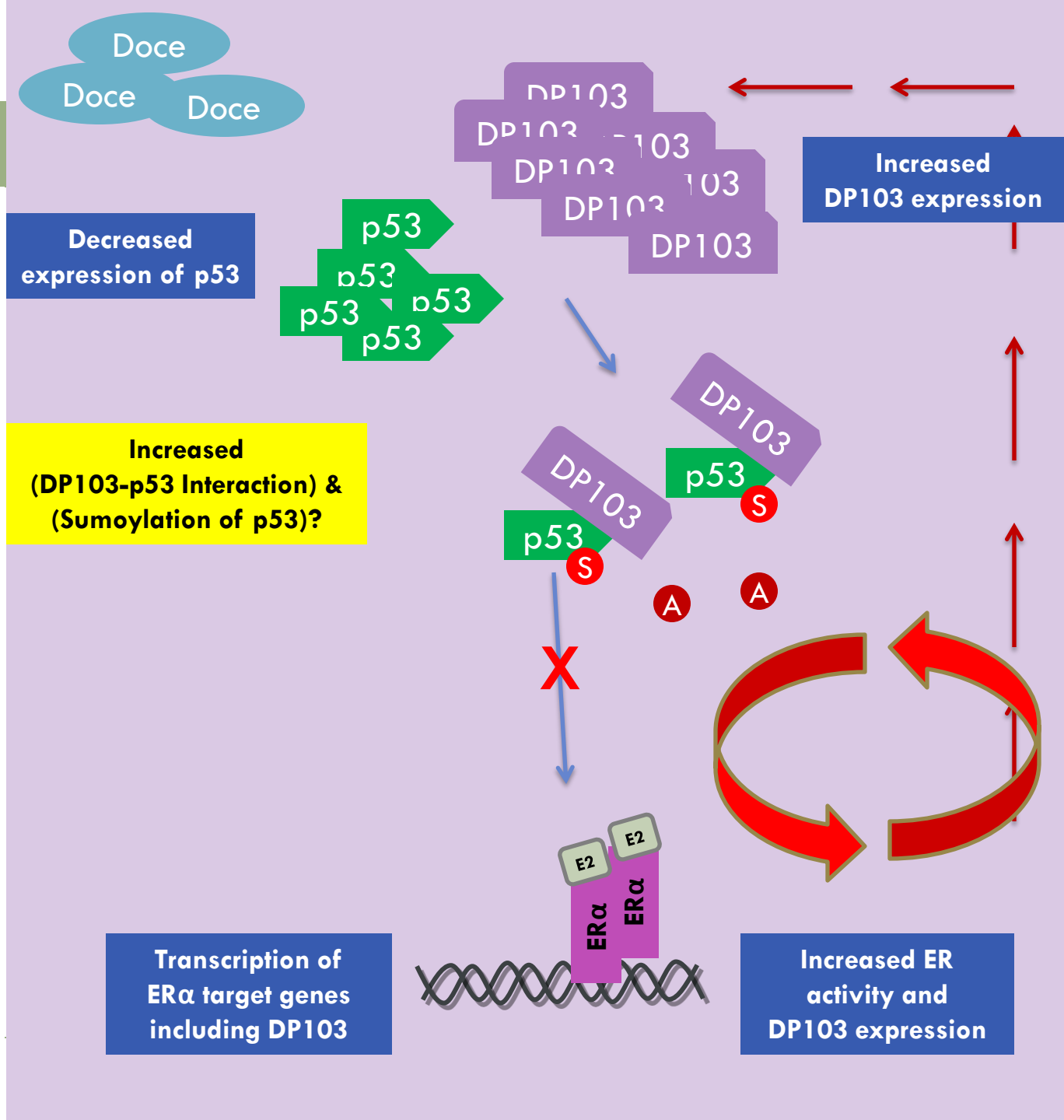
DP103 modulates ER activity through repression of p53





* denotes P < 0.05, ** denotes P < 0.01, *** denotes P < 0.001

Non-Responders



S Sumo group

A Acetyl group

Thank you!