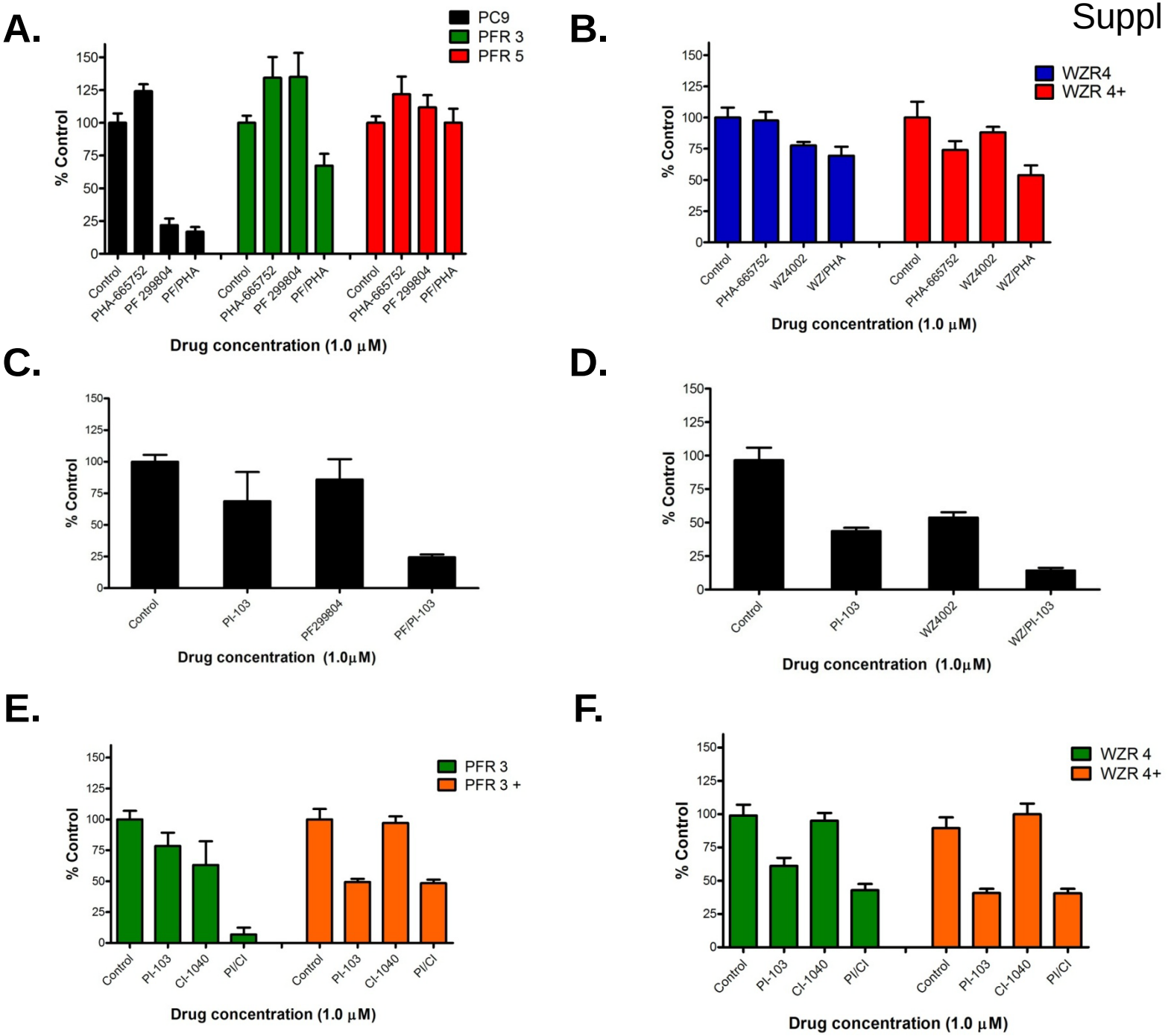
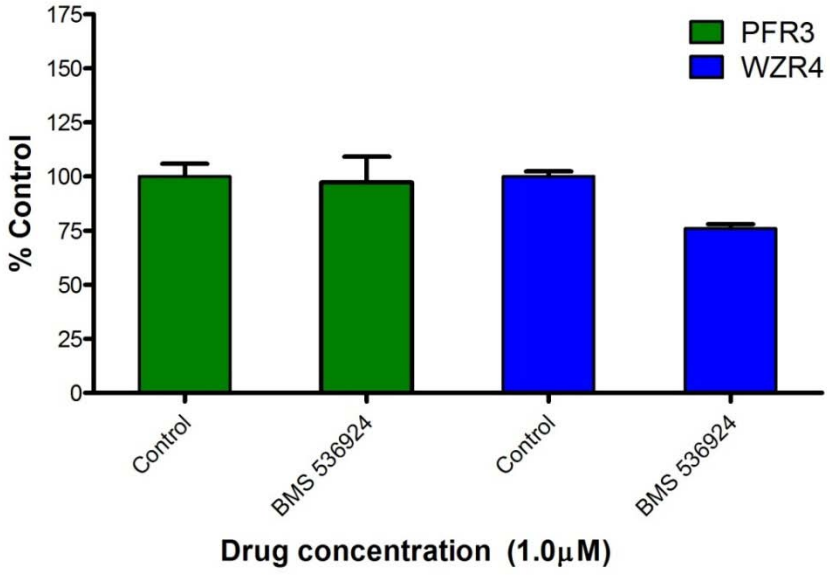
Suppl Figure 1

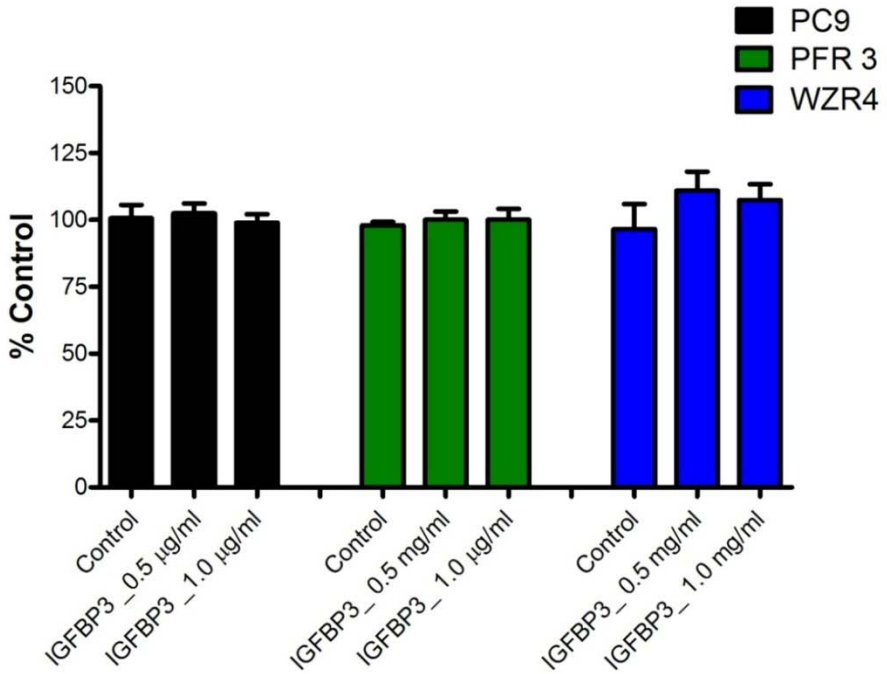


Suppl Figure 2

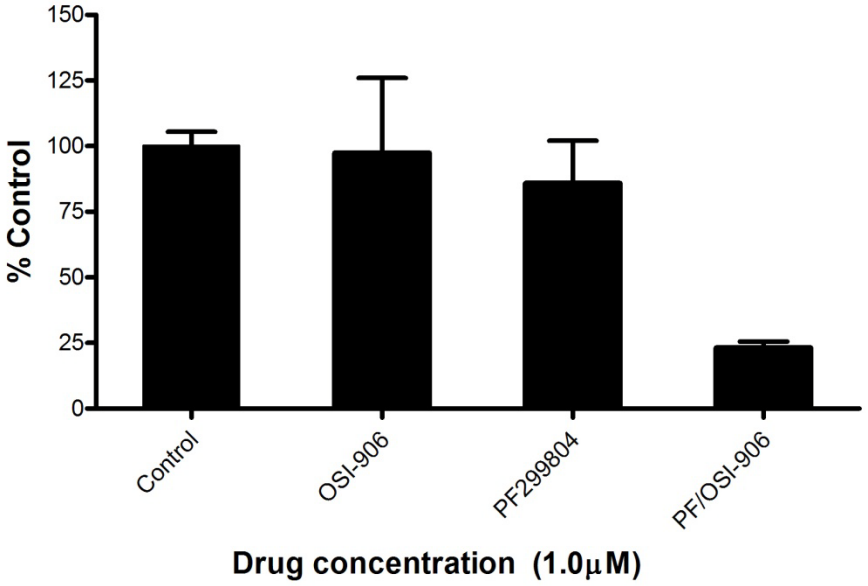
A.



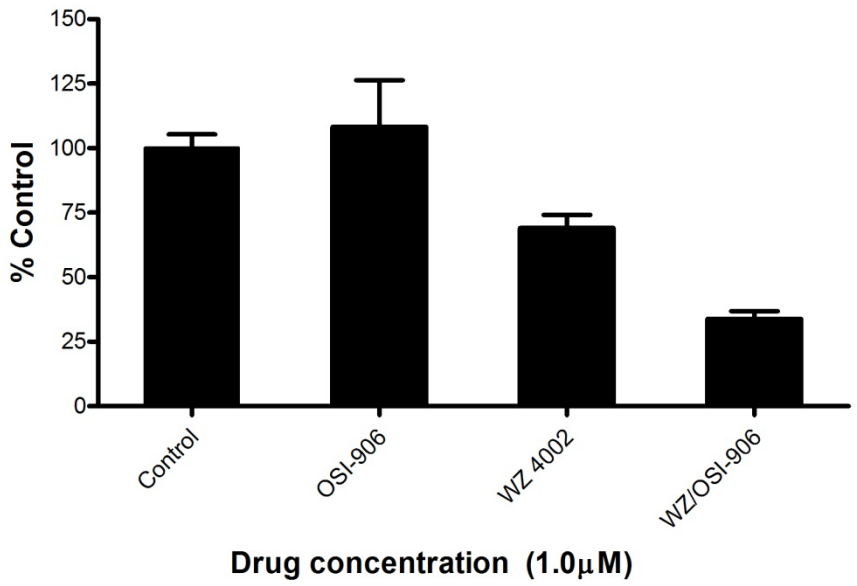
B.



A.

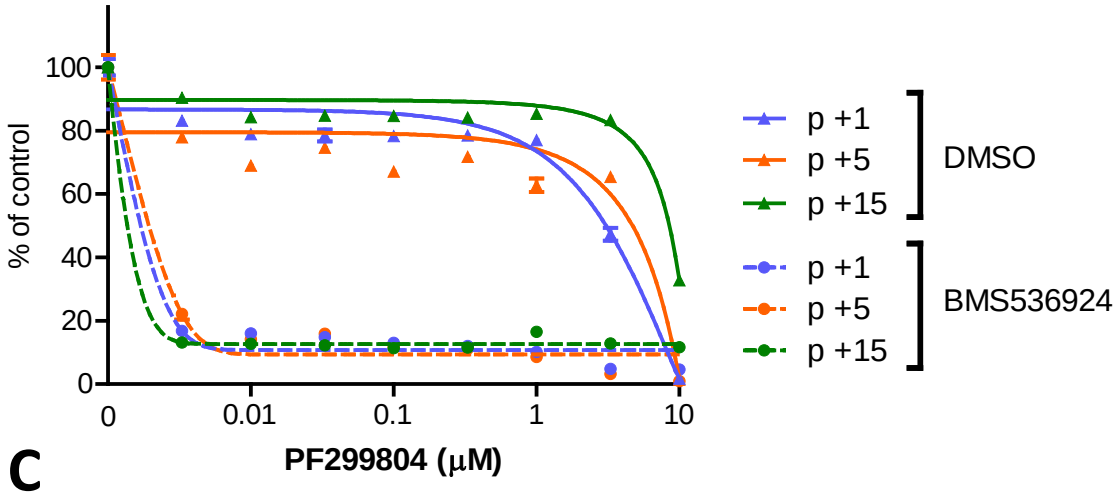

150
125
100
75
50
25
0
% Control
Control
OSI-906
PF299804
PF/OSI-906
Drug concentration (1.0μM)

B.

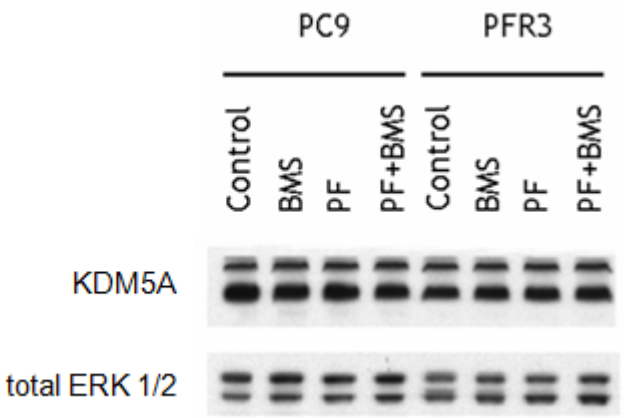

150
125
100
75
50
25
0
% Control
Control
OSI-906
WZ 4002
WZ/OSI-906
Drug concentration (1.0μM)

Suppl Figure 4

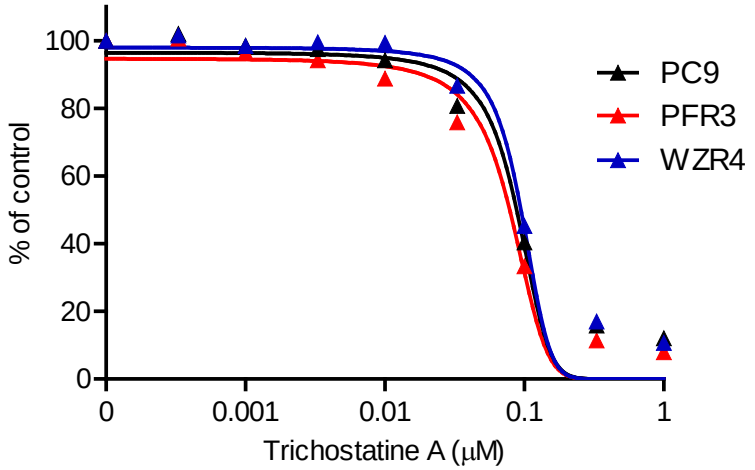
A

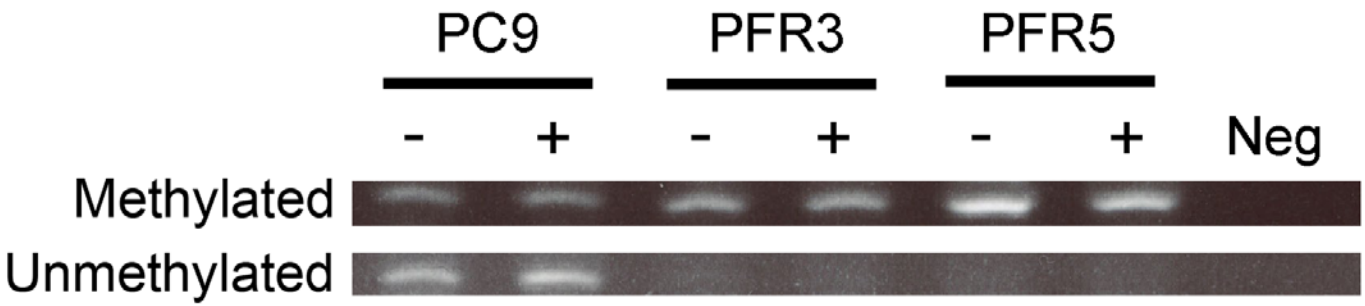


B

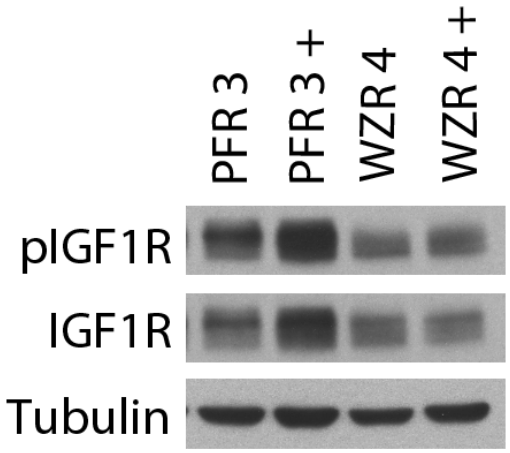


C



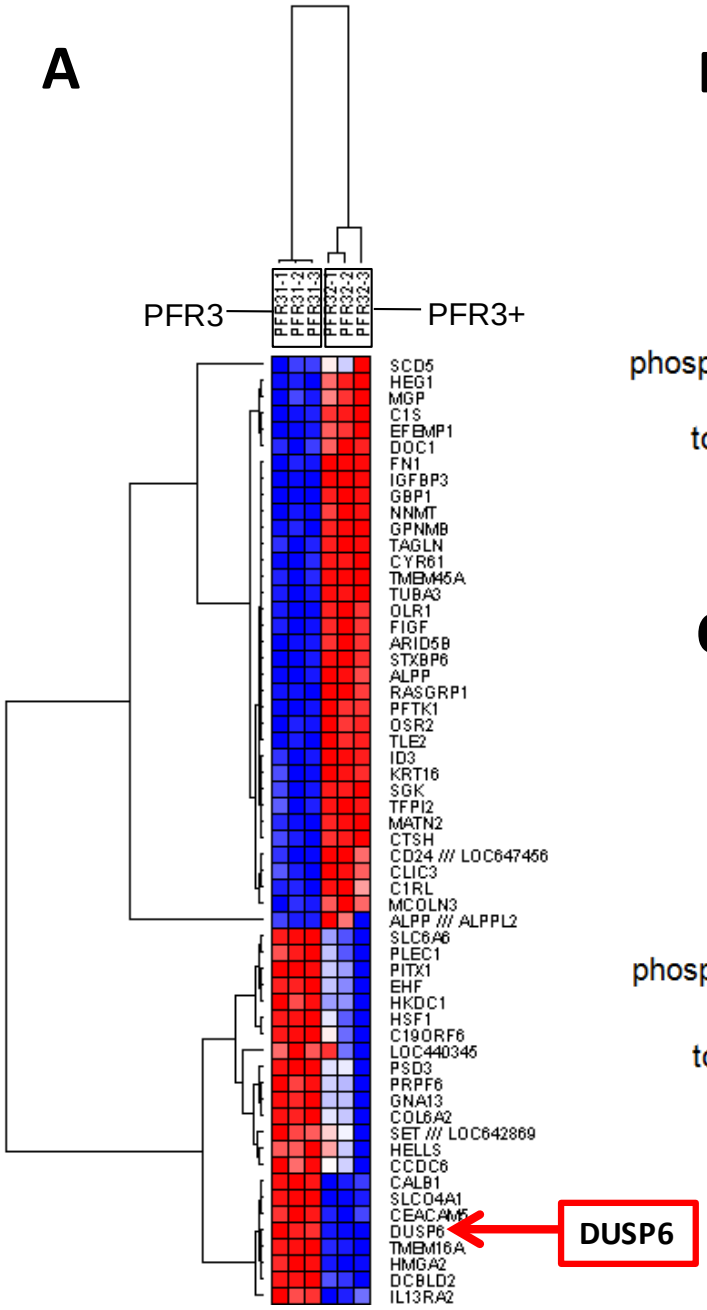

PC9
PFR3
PFR5
- + - + - + Neg
Methylated
Unmethylated

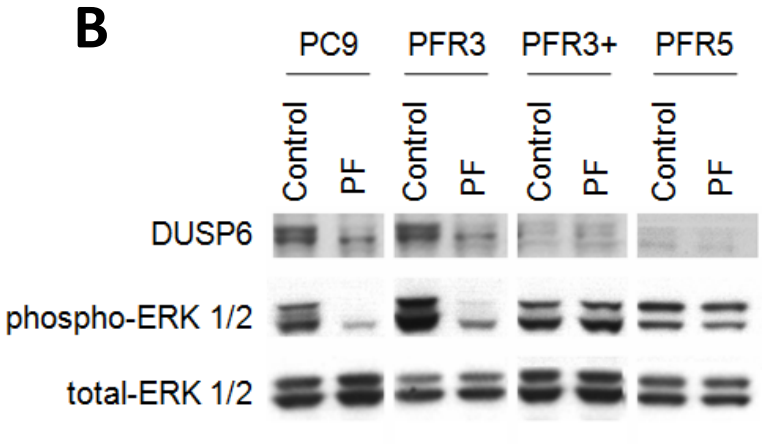
Suppl Figure 6

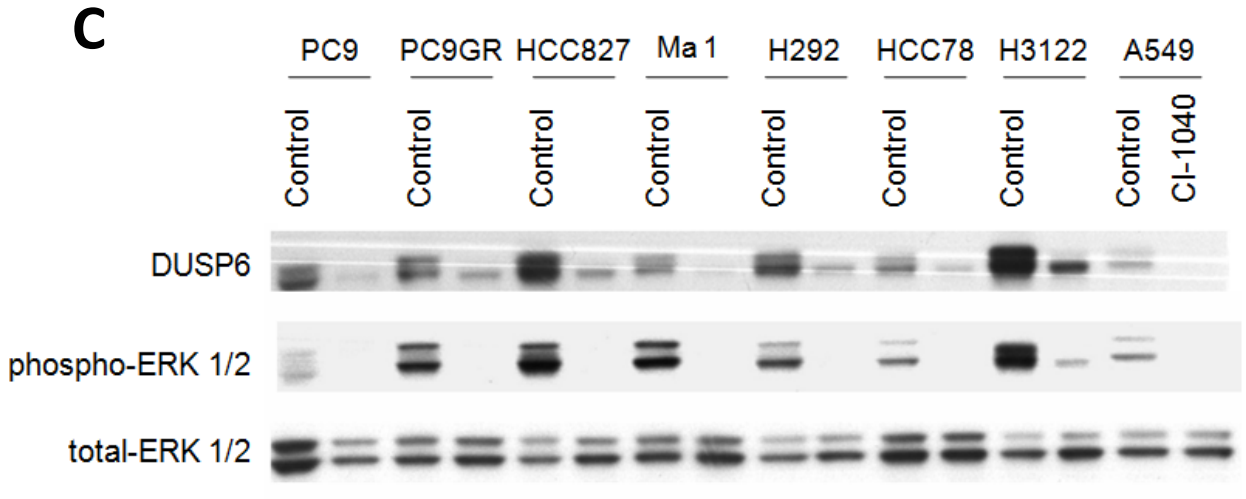


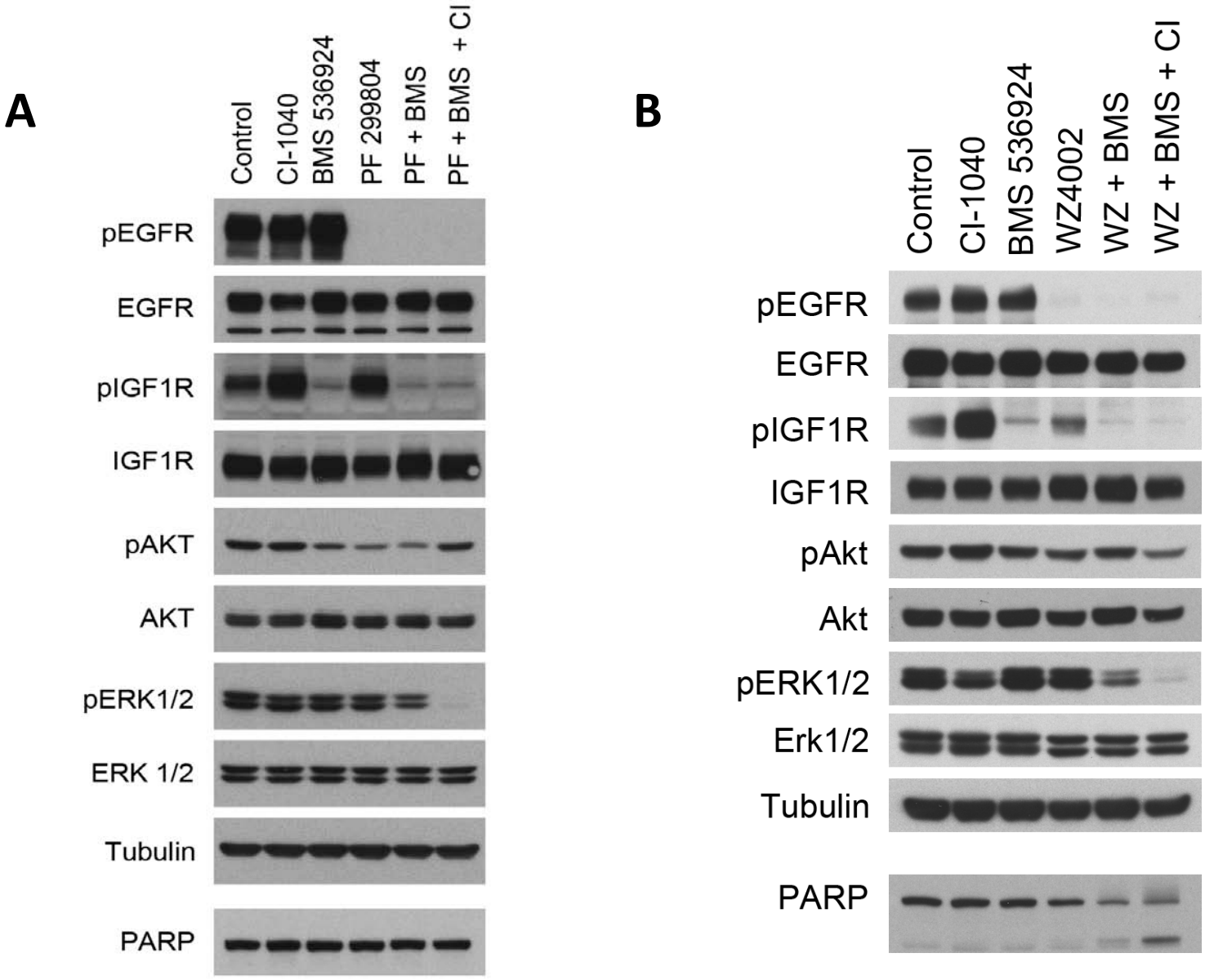
Suppl Figure 7

% of control


A
PFR3
PFR3+
PFR31-1
PFR31-2
PFR31-3
PFR32-1
PFR32-2
PFR32-3
SCD5
HEG1
MGP
C1S
EFEMP1
DOC1
FN1
IGFBP3
GBP1
NNMT
GPNMB
TAGLN
CYR61
TMEM45A
TUBA3
OLR1
FIGF
ARID5B
STXBP6
ALPP
RASGRP1
PFTK1
OSR2
TLE2
ID3
KRT16
SGK
TFPI2
MATN2
CTSH
CD24 /// LOC647456
CLIC3
C1RL
MCOLN3
ALPP /// ALPPL2
SLC6A6
PLEC1
PITX1
EHF
HKDC1
HSF1
C19ORF6
LOC440345
PSD3
PRPF6
GNA13
COL6A2
SET /// LOC642869
HELLS
CCDC6
CALB1
SLCO4A1
CEACAM5
DUSP6
TMEM16A
HMGA2
DCBLD2
IL13RA2
DUSP6


B
PC9
PFR3
PFR3+
PFR5
Control
PF
DUSP6
phospho-ERK 1/2
total-ERK 1/2


C
PC9
PC9GR
HCC827
Ma 1
H292
HCC78
H3122
A549
Control
CI-1040
DUSP6
phospho-ERK 1/2
total-ERK 1/2


A
Control
CI-1040
BMS 536924
PF 299804
PF + BMS
PF + BMS + CI
pEGFR
EGFR
pIGF1R
IGF1R
pAKT
AKT
pERK1/2
ERK 1/2
Tubulin
PARP
B
Control
CI-1040
BMS 536924
WZ4002
WZ + BMS
WZ + BMS + CI
pEGFR
EGFR
pIGF1R
IGF1R
pAkt
Akt
pERK1/2
Erk1/2
Tubulin
PARP

Supplementary Figure Legends

Figure S1. PI3K inhibition restores sensitivity to PF299804 and WZ4002. A. MET inhibition does not restore sensitivity to PF299804. Cells were treated with PHA-665,752, PF299804 or with the combination of both drugs at the indicated concentrations, and viable cells were measured after 72 hours of treatment and plotted relative to untreated controls. **B.** WZR4 and WZR4 + cells were treated as in A. **C.** PFR3 cells were treated with PI-103, PF299804 or with the combination of both drugs at the indicated concentrations, and viable cells were measured after 72 hours of treatment and plotted relative to untreated controls. **D.** WZR4 cells were treated as in C. **E.** PFR3 and PFR3+ cells were treated with PI-103, CI-1040 or with the combination of both drugs at the indicated concentrations, and viable cells were measured after 72 hours of treatment and plotted relative to untreated controls. **F.** WZR4 and WZR 4+ cells were treated as in E.

Figure S2. IGF1R inhibition alone does not effect growth of PFR or WZR cells. **A.** PFR3 and WZR4 cells were treated with BMS 536924 at the indicated concentration, and viable cells were measured after 72 hours of treatment and plotted relative to untreated controls. **B**. PC9, PFR3 and WZR4 cells were treated with IGFBP3 at the indicated concentrations, and viable cells were measured after 72 hours of treatment and plotted relative to untreated controls.

Figure S3. OSI-906 restores sensitivity to PF299804 and WZ4002 in the PFR and WZR cells. **A.** PFR3 cells were treated with OSI-906 alone, PF299804 alone or with the combination of both drugs at the indicated concentration, and viable cells were measured after 72 hours of treatment

and plotted relative to untreated controls. **B.** WZR cells were treated with OSI-906 alone, WZ4002 alone or with the combination of both drugs as in A.

Figure S4. PFR3 cells do not share the characteristics of "drug-tolerant cells" A. Sensitivity of PFR3 cells to the combination of BMS536924 (1 μM) and PF299804 (indicated concentrations) is maintained after 15 passages. Viable cells were measured after 72 hours of treatment. **B.** PFR3 cells do not have elevated levels of the chromatin-modifying enzyme KDM5A/RBP2/Jarid1A compared to parental PC9 cells. Cells were treated for 6 hours with either PF299804 (PF, 1 μM) alone, BMS536924 (BMS, 1 μM) alone or with both agents. Cells were lysed, and the indicated proteins were detected by immunoblotting. **C.** PFR and WZR cells are not more sensitive to the HDAC inhibitor trichostatin A than parental PC9 cells. Viable cells were measured after 72 hours of treatment.

Figure S5. IGFBP3 promoter methylation is present in the PFR3 and PFR5 cells. Methylation specific PCR was used to evaluate methylation status at IFGBP3 locus in PC9, PFR3 and PFR5 cells. Both methylated and unmethylated promoter sequences are detected in the parental PC9 cells. In contrast, only methylated IGFBP3 sequences are identified in the PFR3 and PFR5 cells.

Figure S6. IGF1R and pIGFR1R levels are similar in the PFR3 and PFR3+, and WZR4 and WZR4+ cells. Cell extracts were immunoblotted to detect the indicated proteins.

Figure S7. PFR5 and PFR5+ cells grow in a similar fashion. Equal numbers of PFR5 and PFR5+ cells were grown in the absence or presence of PF299804. Colonies were counted after 15 days of treatment with or without PF299804 (1 μM).

Figure S8. Drug resistant PFR3+ and PFR5 cells exhibit loss of DUSP 6. A. Comparison of expression profiles of PFR3 and PFR3+ cells. DUSP6 is one of the most downregulated genes in the PFR3+ cells. **B.** The indicated cell lines were treated with PF299804 (1 μM) for 6 hours. Cells were lysed, and the indicated proteins were detected by immunoblotting. **C.** The indicated cell lines were treated with CI-1040 (1 μM) for 6 hours. Cells were lysed, and the indicated proteins were detected by immunoblotting.

Figure S9. Effect of EGFR inhibitors (PF299804 or WZ4002) alone, BMS 53924 alone, CI-1040 alone or the combination of all 3 agents on cell signaling in the PFR3 + (**A.**) and WZR4 + (**B.**) cells. Cells were lysed, and the indicated proteins were detected by immunoblotting.

Type of file: figure
Label: Figure 7
Filename: CortotetalSupplfigure7.pdf

Suppl Figure 7