AKT isoforms have discrete expression in Triple Negative breast cancers and roles in cisplatin sensitivity.

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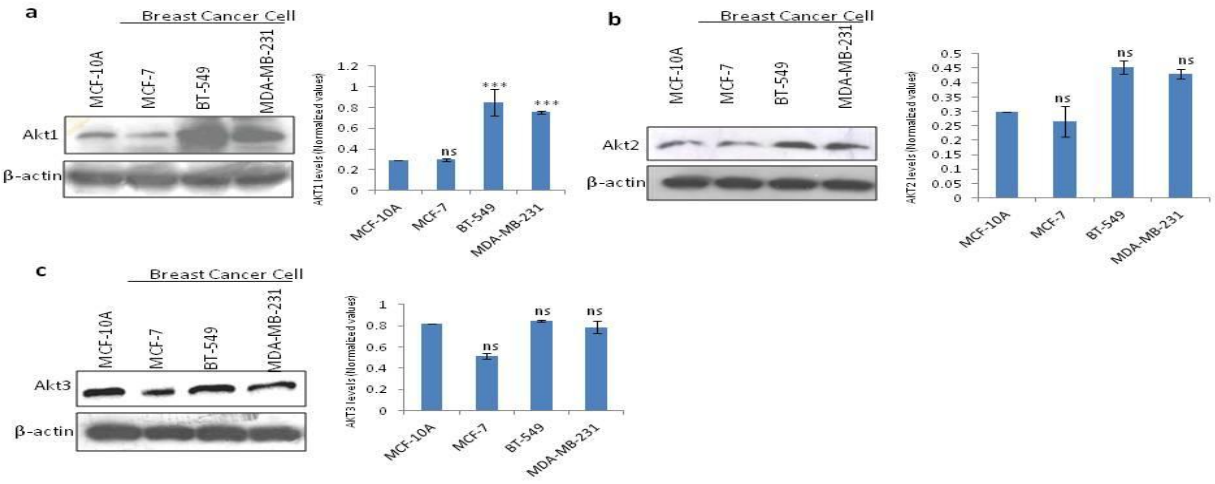
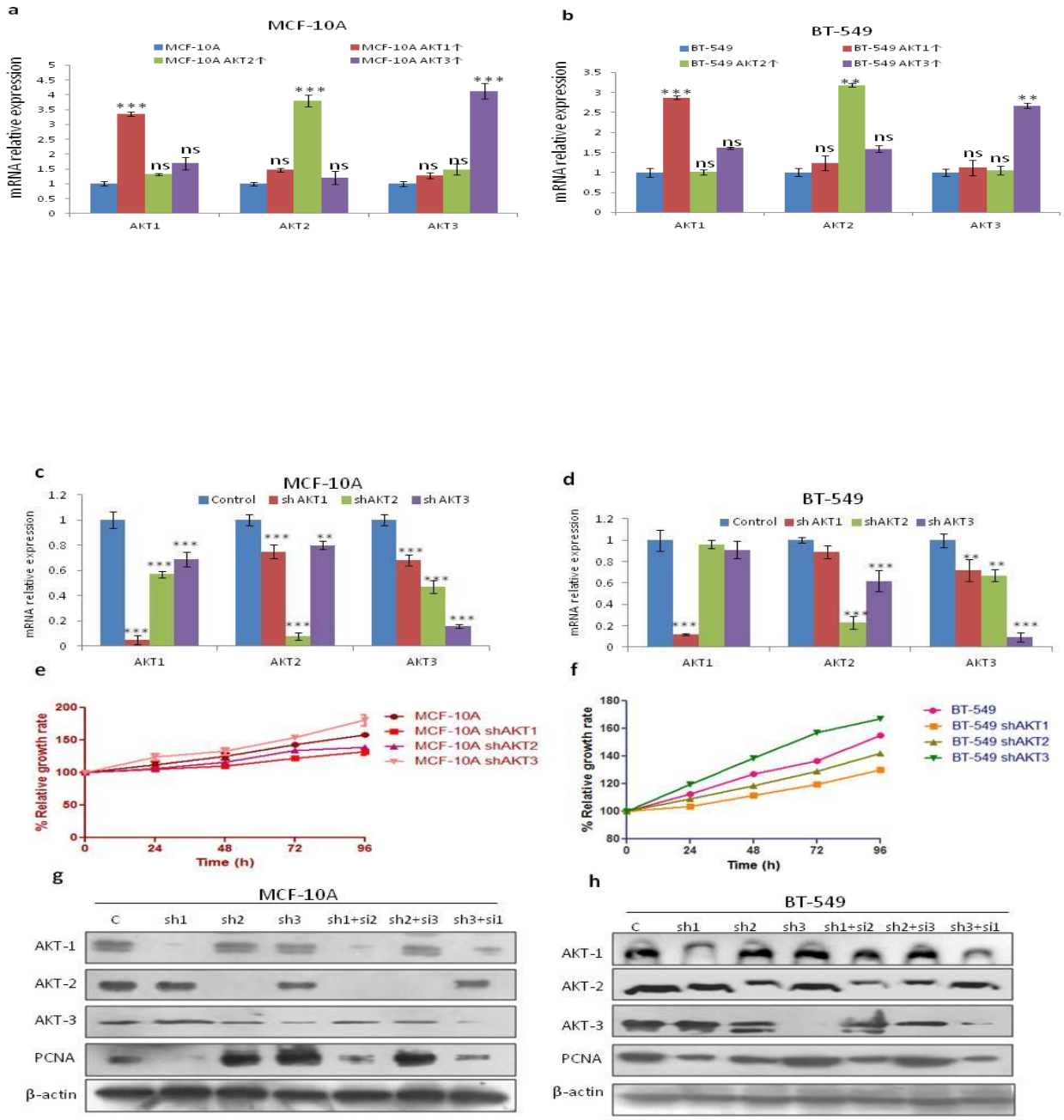
Additional file 1; Supplementary Figure

Fig. S1



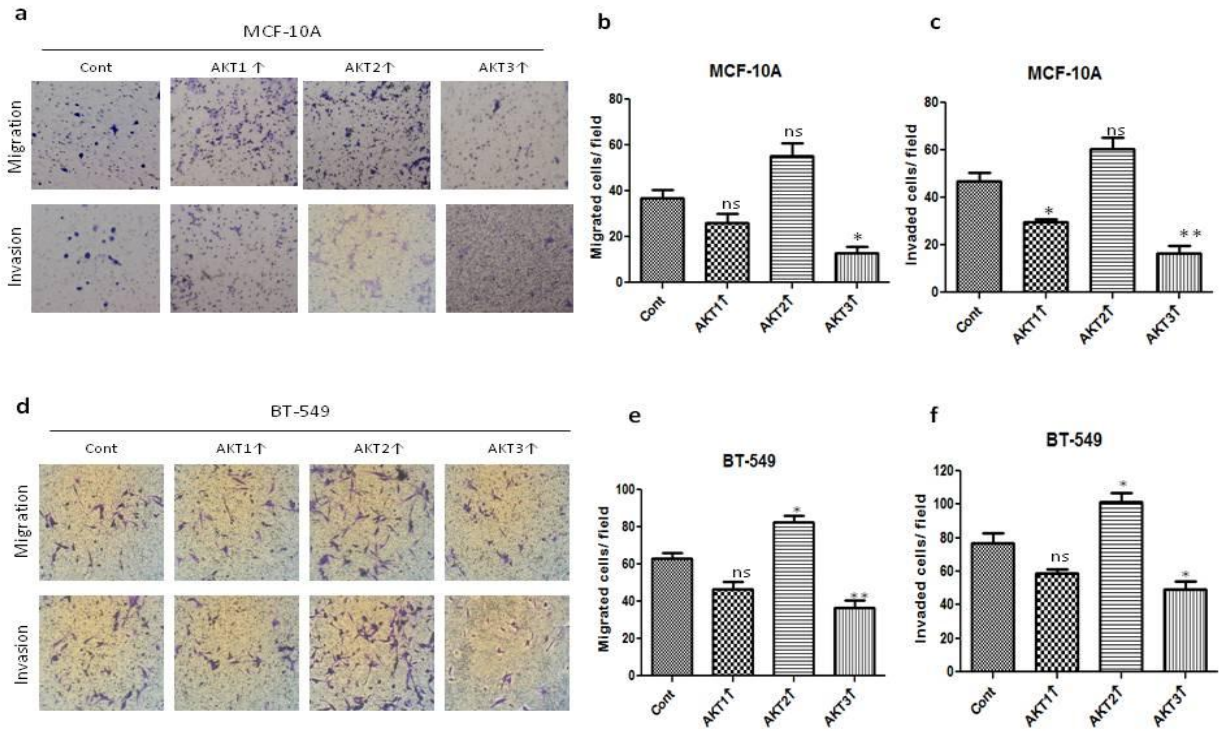
Supplementary Figure S1. Western blot images correlating the expression of AKT1, AKT2 and AKT3 isoforms with malignant breast cancer. (a-c) AKT isoform differential expression between breast normal and cancer cell lines. Data are presented as the mean ± standard deviation of three independent experiments.

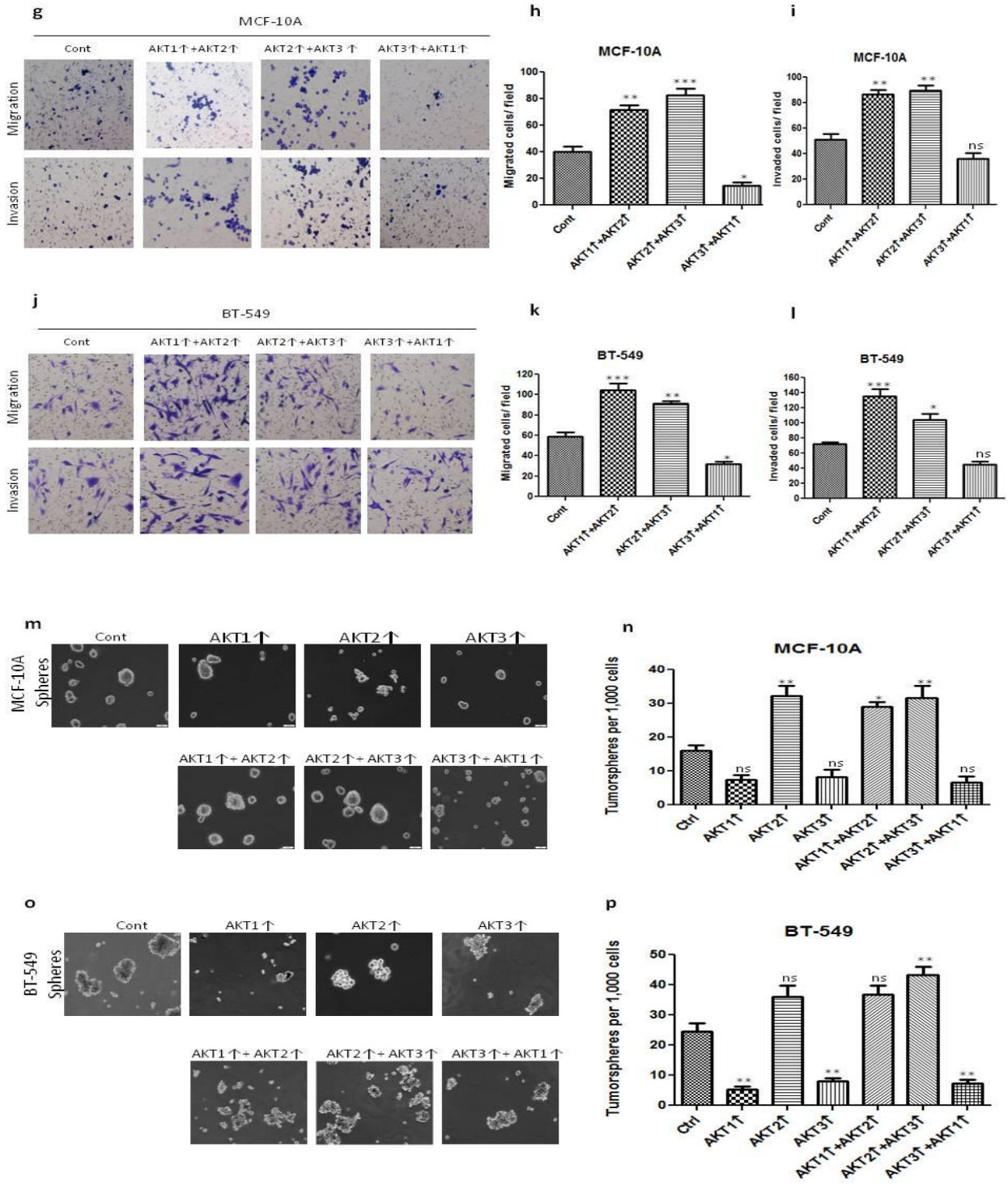
Fig. S2


a
MCF-10A
MCF-10A
MCF-10A AKT1↑
MCF-10A AKT2↑
MCF-10A AKT3↑
mRNA relative expression
AKT1
AKT2
AKT3
b
BT-549
BT-549
BT-549 AKT1↑
BT-549 AKT2↑
BT-549 AKT3↑
mRNA relative expression
AKT1
AKT2
AKT3
c
MCF-10A
Control
sh AKT1
shAKT2
sh AKT3
mRNA relative expression
AKT1
AKT2
AKT3
d
BT-549
Control
sh AKT1
shAKT2
sh AKT3
mRNA relative expression
AKT1
AKT2
AKT3
e
% Relative growth rate
Time (h)
MCF-10A
MCF-10A shAKT1
MCF-10A shAKT2
MCF-10A shAKT3
f
% Relative growth rate
Time (h)
BT-549
BT-549 shAKT1
BT-549 shAKT2
BT-549 shAKT3
g
MCF-10A
C
sh1
sh2
sh3
sh1+si2
sh2+si3
sh3+si1
AKT-1
AKT-2
AKT-3
PCNA
β-actin
h
BT-549
C
sh1
sh2
sh3
sh1+si2
sh2+si3
sh3+si1
AKT-1
AKT-2
AKT-3
PCNA
β-actin

Supplementary Figure S2. AKT1 promotes cell proliferation in MCF-10A and BT-549 cell lines. qPCR analysis confirming the overexpression of AKT1, AKT2 and AKT3 isoforms expression in (a) MCF-10A and (b) BT-549 cells. qPCR analysis confirming the shRNA-mediated downregulation of AKT1, AKT2 and AKT3 expression in (c) MCF-10A and (d) BT-549 cells. (e) Cell viability was analyzed by MTT at different time intervals in MCF-10A and in (f) BT-549. (g) Western blot analysis of PCNA in individual and dual AKT isoform silencing in MCF-10A and (h) BT-549. All the results show the means of the three independent experiments. Error bars indicate SD. Columns, mean; bars, SD with ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$ versus control.

Fig. S3

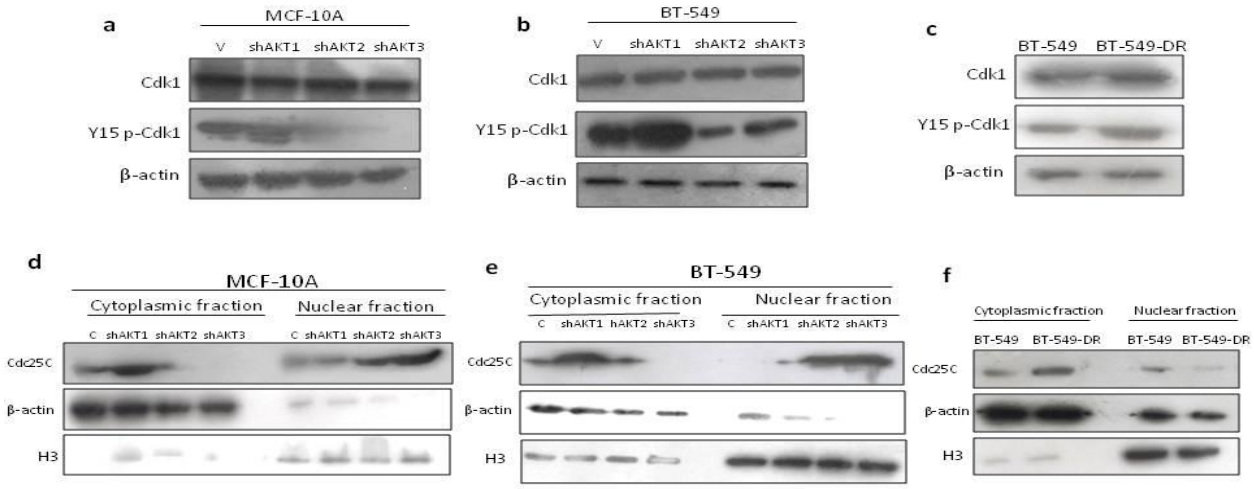




Supplementary Figure S3. AKT2 isoform is correlated with aggressiveness of cells and contributes to metastasis. (a-f) Effect of migration and invasion in MCF-10A and BT-549 cells with the overexpressed AKT isoforms. (g-l) Representative images of migration and invasion analysis in dual AKT isoform overexpression in MCF-10A and BT-549 cells. (m-n)

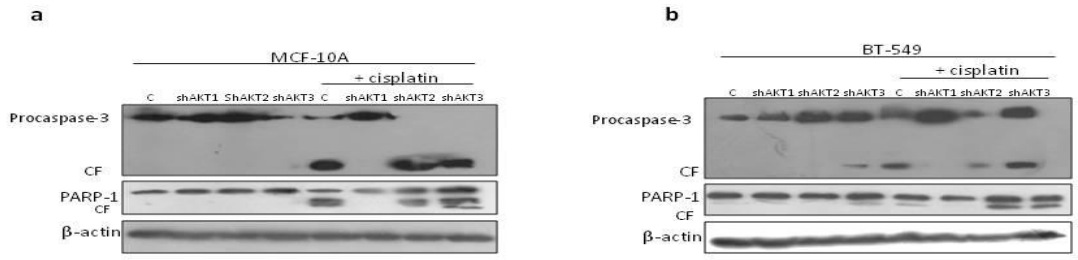
Representative images of the mammospheres formed by overexpressed variants of AKT isoforms in MCF-10A. (o-p) Representative images of the mammospheres formed by overexpressed variants of AKT isoforms in BT-549. The results are shown as mean ± SD of one representative experiment (from three independent experiments) performed in triplicate. Statistically significant differences (***) $p < 0.001$, (**) $p < 0.01$, (*) $p < 0.05$ are indicated.

Fig. S4



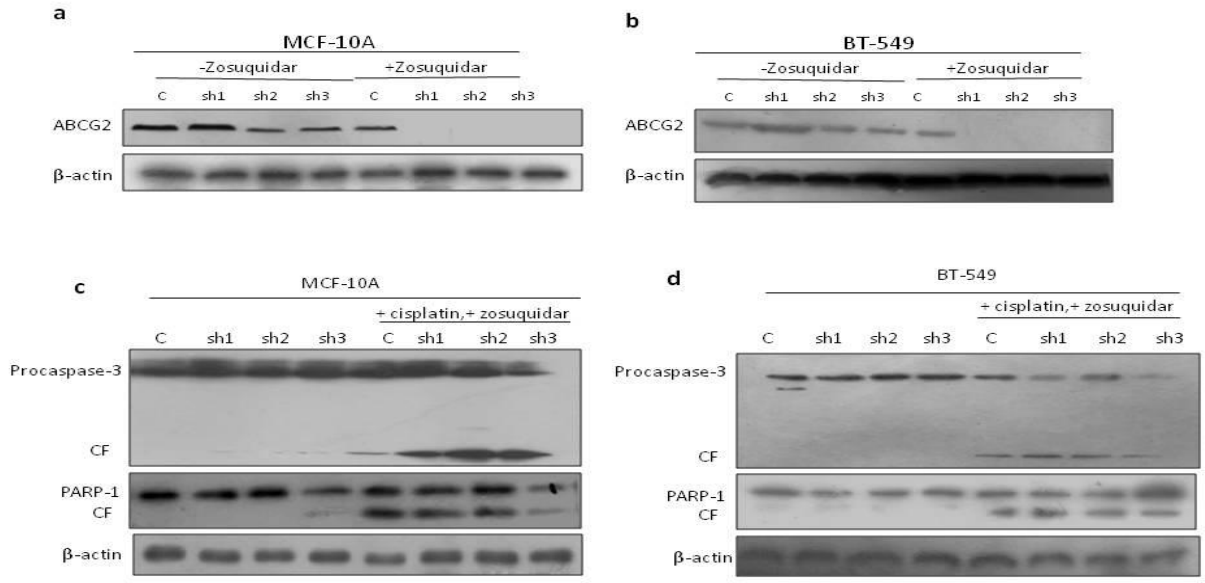
Supplementary Figure S4. AKT1 knockdown leads to prolonged G2 arrest. (a-c) Western blotting images of Cdk1 and Y15p-Cdk1 in (a) MCF-10A AKT isoforms knockdown variants, (b) BT-549 AKT isoforms knockdown variants, (c) BT-549 and BT-549-DR cells. (d-f) Western blot analysis of Cdc25C in subcellular fractions of (d) AKT isoforms knockdown variants in MCF-10A, (e) Akt isoforms knockdown variants in BT-549, (f) BT-549 and BT-549-DR cells.

Fig. S5



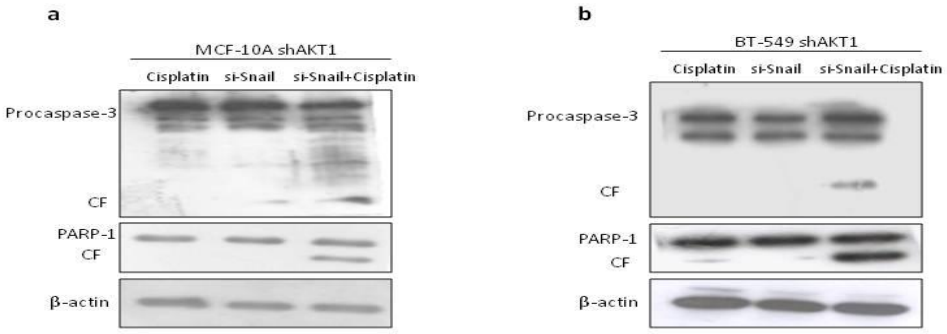
Supplementary Figure S5. AKT1 knockdown abrogated cisplatin sensitivity. (a-b) Western blot analysis of caspase-3 and PARP-1 in presence and absence of cisplatin in (a) MCF-10A and (b) BT-549.

Fig. S6



Supplementary Figure S6. Zosuquidar, a p-gp pump drug efflux inhibitor promoted the apoptotic effect of cisplatin in knockdown of AKT1 (shAKT1). (a-b) Western blot analysis of ABCG2 expression by P-gp inhibitor, zosuquidar. (c-d) Western blot images of apoptotic protein, Procaspase-3 and PARP-1 in AKT isoforms knockdown in (c) MCF-10A and (d) BT-549 respectively. Following 24 h pre-treatment with 2.5 µM zosuquidar, cells were exposed to cisplatin.

Fig. S7



Supplementary Figure S7. Western blot images of the expression of anti-apoptosis. (a) Knockdown of Snail in shAKT1 MCF-10A and (b) in BT-549 altered the expression of apoptotic-related proteins, Procaspase-3 and PARP-1.