Figure S1

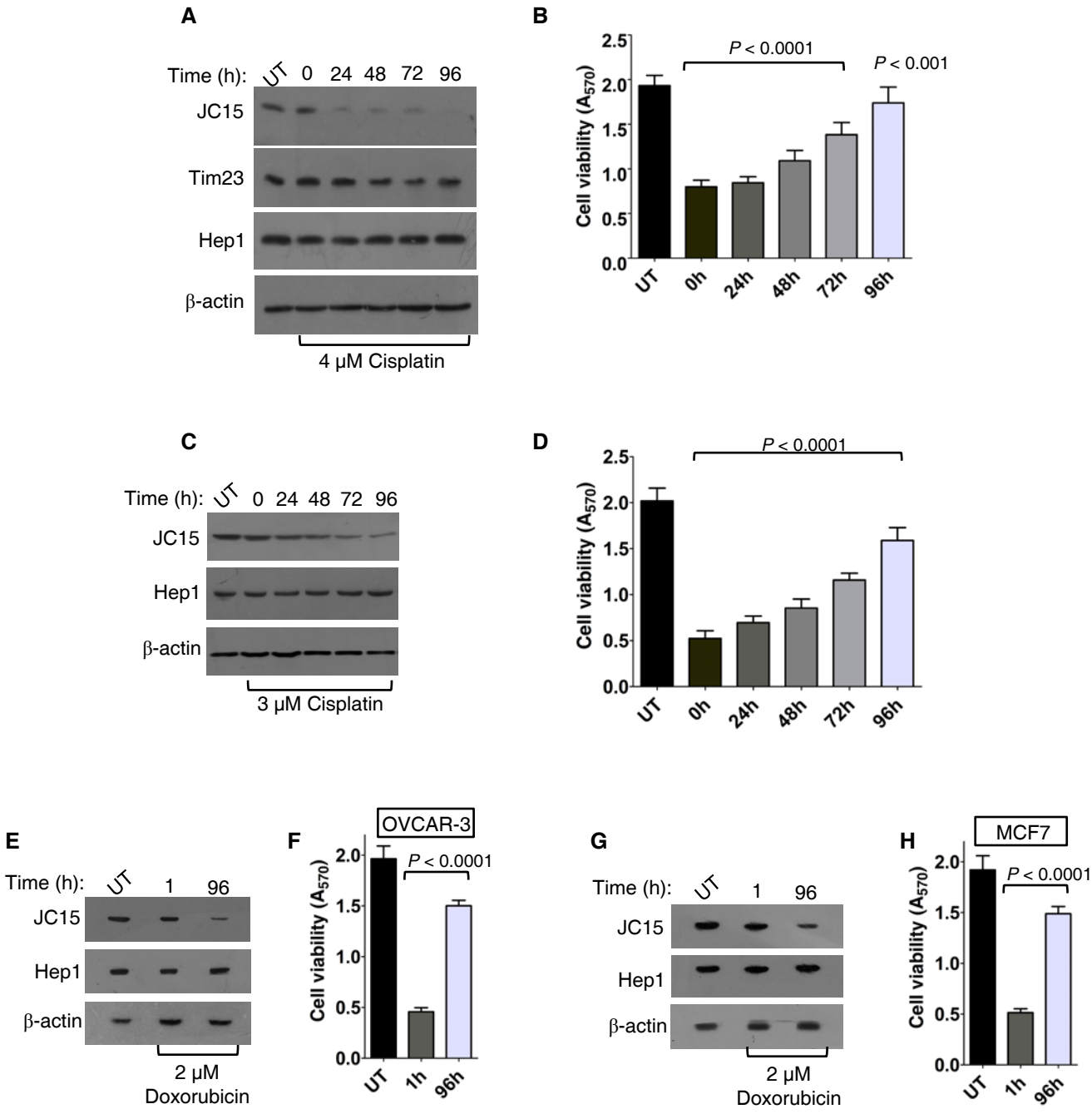

Figure S1 Modulation of cellular sensitivity to chemotherapeutic agents by JC15. **(A)** OVCAR-3 cells were exposed to 4 μM cisplatin for indicated time intervals, followed by immunoblot analysis using anti-JC15 specific antibodies. For loading control, the blot was immunodecorated with antibodies specific to Tim23 and Hep1 for mitochondria and β-actin for equivalency in total cell concentration. As negative control the cells were left untreated (UT) **(B)** OVCAR-3 cells treated with 4 μM cisplatin for 24 h, 48 h, 72 h and 96 h respectively were assayed for cell viabilty using MTT assay by measuring absorbance 570 nm. Untreated cells (UT) was used as negative control. Data shown as mean ± s.e.m. n=8, *P* (two tailed) as highlighted. **(C, D)** Expression pattern of JC15 (C) and relative cell viability (D) was assessed in MCF7 cells subjected to prolonged cisplatin exposure as described above. **(E-H)** Effect of second chemotherapeutic drug doxorubicin on JC15 expression and cell viability. Assessment of cell survivability and protein expression was carried out in OVCAR3 (E-F) and MCF7 (G-H) cells subjected to sustained doxorubicin treatment. Data shown as mean ± s.e.m. n=8, *P* (two tailed) as highlighted.

Figure S2

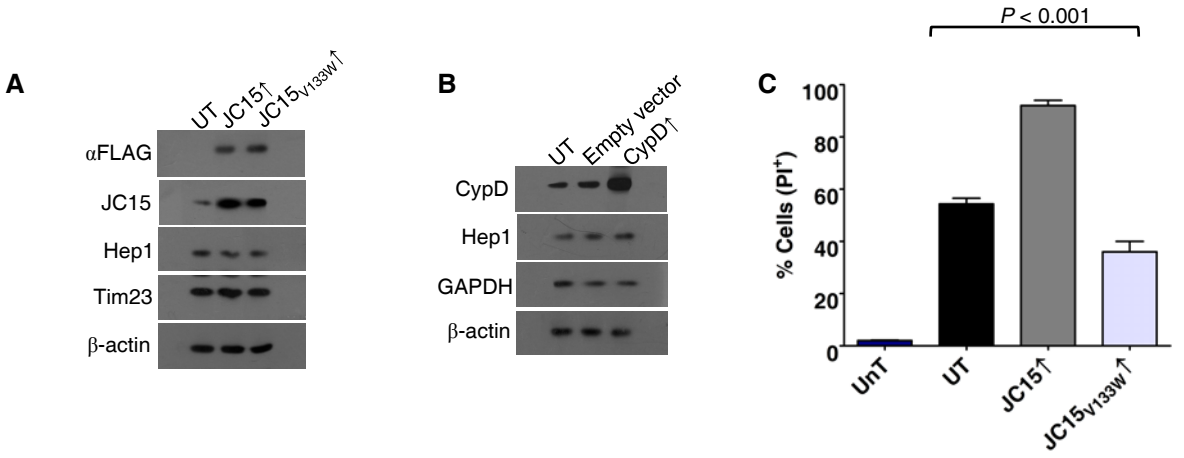

Figure S2 Alteration of JC15 and CypD expression in the cells. **(A, B)** HEK293T cells were transfected with constructs overexpressing wild type and mutant JC15 **(A)** or cyclophilin D **(B)** using Lipofectamine 2000. After 48 h the relative expression of the proteins were assayed by immunoblotting with specific antibodies against cyclophilin D (CypD) and JC15 respectively. Hep1,Tim23 and β-actin served as loading control. **(C)** HEK293T cells overexpressing wild type or mutant JC15 were treated with 10 μM cisplatin for 3 h, followed by propidium iodide (PI) staining. The cells were assayed through flow cytometry to determine the percent cells stained for PI over total normalized cell population. Untransfected cells were used as negative control (UT). Basal cell mortality was determined by PI staining the untransfected untreated cells (UnT). Data represented as mean ± s.e.m. n=3, *P* (two tailed) as highlighted.

Figure S3

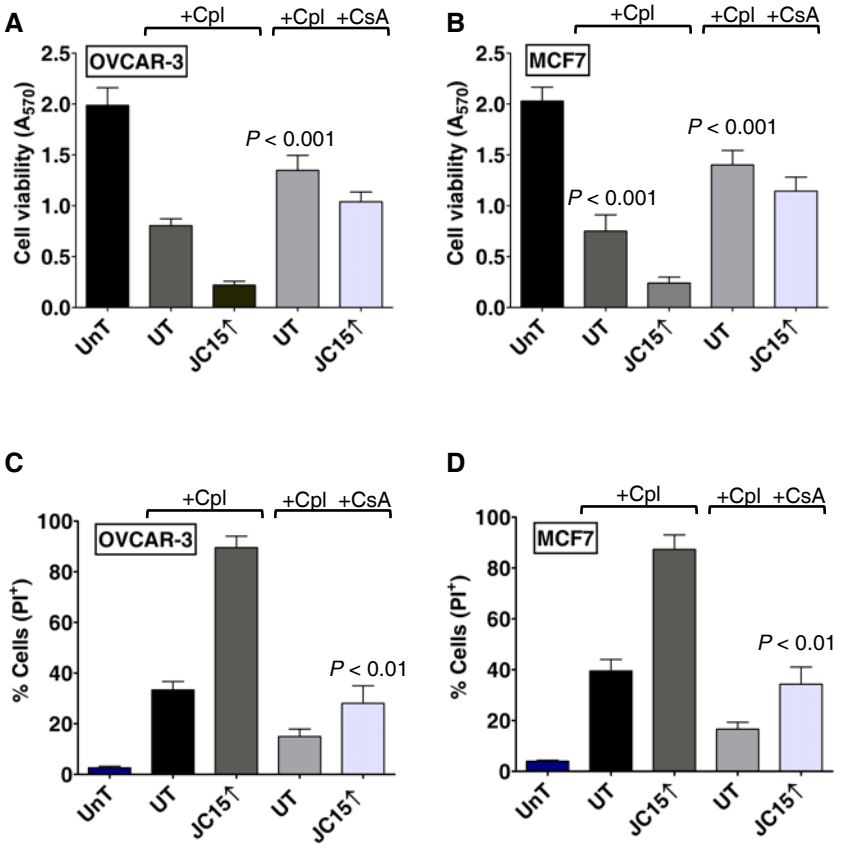

Figure S3 Effect of cyclosporine (CsA) on JC15 overexpressing cells subjected to cisplatin treatment. **(A-D)** OVCAR-3 and MCF7 cells were either left untreated or treated with cisplatin (Cpl) alone or with cyclosporine A prior to cisplatin exposure (Cpl+CsA). Cell viability was measured using MTT assay (A-B) or propidium iodide staining (C-D). Bars represent mean ± s.e.m. n=8, *P* (two tailed) < 0.0001 for (A-B) and mean ± s.e.m. n=3, *P* (two tailed) < 0.001 (C-D) unless otherwise mentioned. UnT- untreated cell control, UT – untransfected cell control, ↑ - exogenous overexpression of the protein.

Figure S4

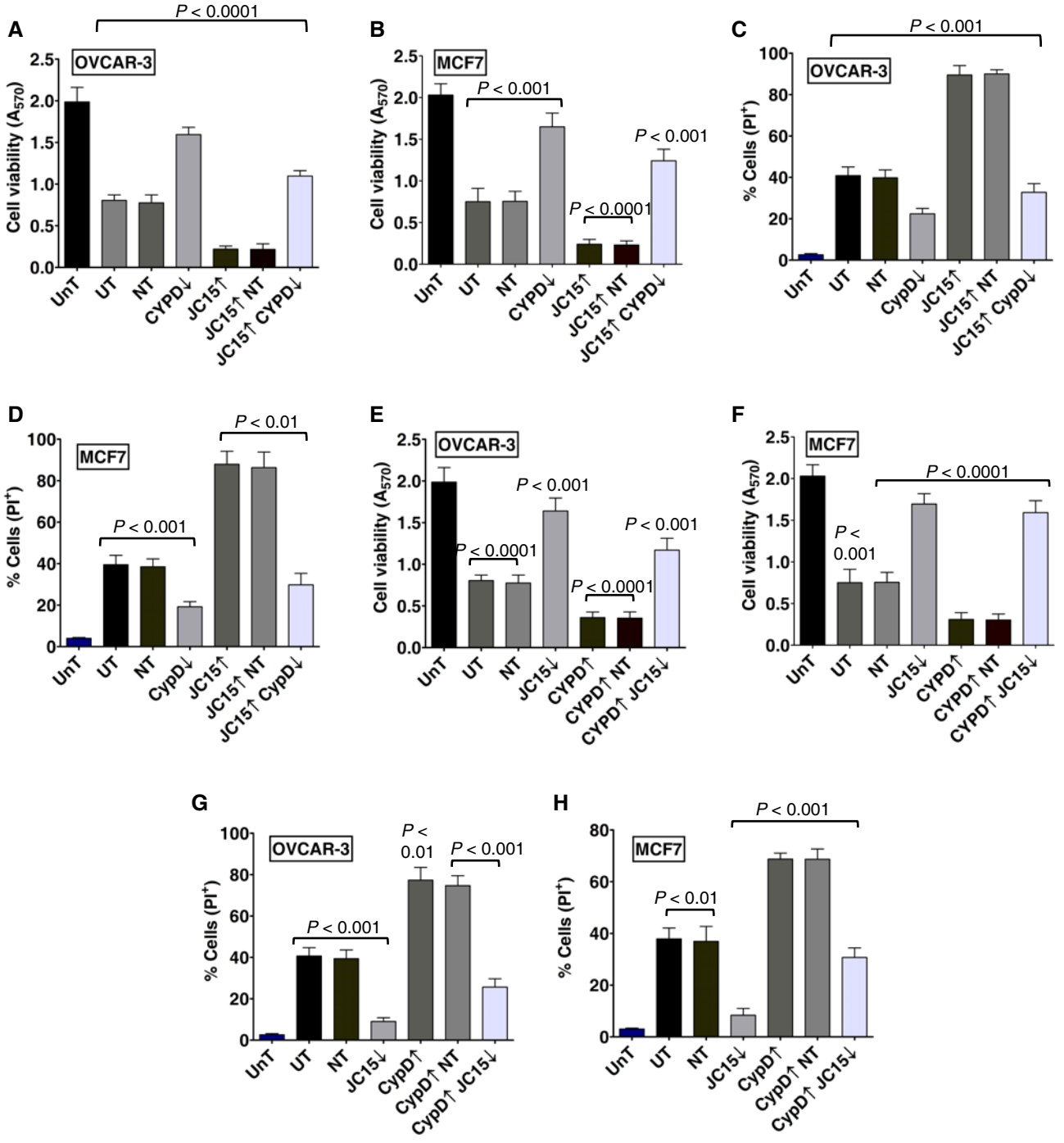

Figure S4 Modulation of cellular sensitivity to chemotherapeutic agents by JC15 in cancer derived cell lines. **(A-B, E-F)** Cell viability of cisplatin treated cells expressing altered amounts of JC15 and CypD was determined using MTT assay and represented as mean ± s.e.m. n=8, *P* (two tailed) as indicated. **(C-D, G-H)** Cells exposed to similar treatment as mentioned in above were screened for relative propidium iodide (PI) uptake through flow cytometry as represented as mean ± s.e.m. n=3, *P* (two tailed) as indicated. UnT- untreated cell control, UT – untransfected cell control, NT - transfected with non-targeting dsiRNA control, ↓ - mRNA downregulated by dsiRNA, ↑ - exogenous overexpression of the protein.

Figure S5

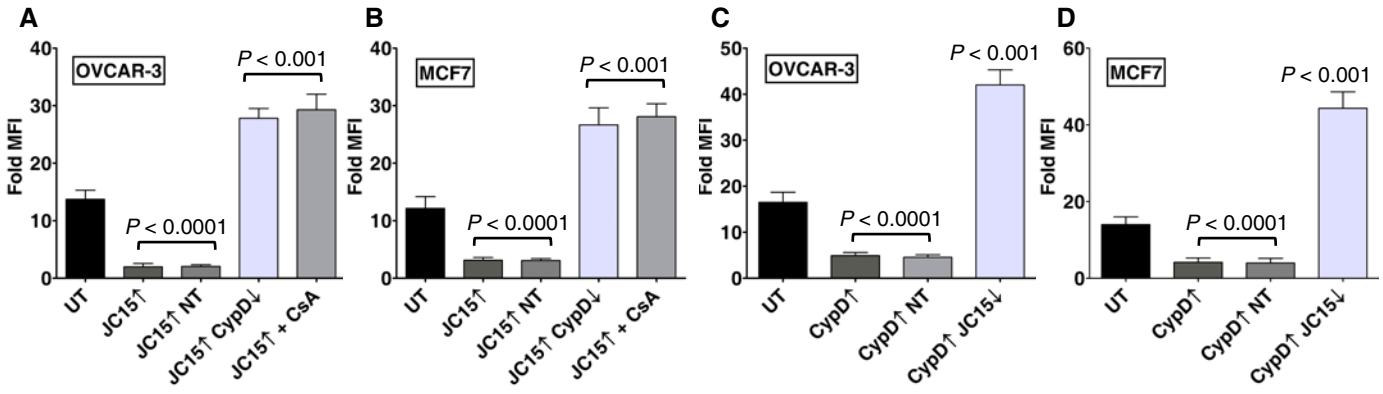

Figure S5 Modulation of mitochondrial membrane potential by JC15 and CypD. **(A-D)** Loss of mitochondrial membrane potential determined by MitoTracker Red staining of cells presenting altered levels of JC15 and CypD. Data is represented as fold mean florescence intensity (MFI) over unstained cells. Bars denote mean ± s.e.m. n=3, *P* (two tailed) as indicated. UnT- untreated cell control, UT – untransfected cell control, NT - transfected with non-targeting dsiRNA control, ↓ - mRNA downregulated by dsiRNA, ↑ - exogenous overexpression of the protein.

Figure S6

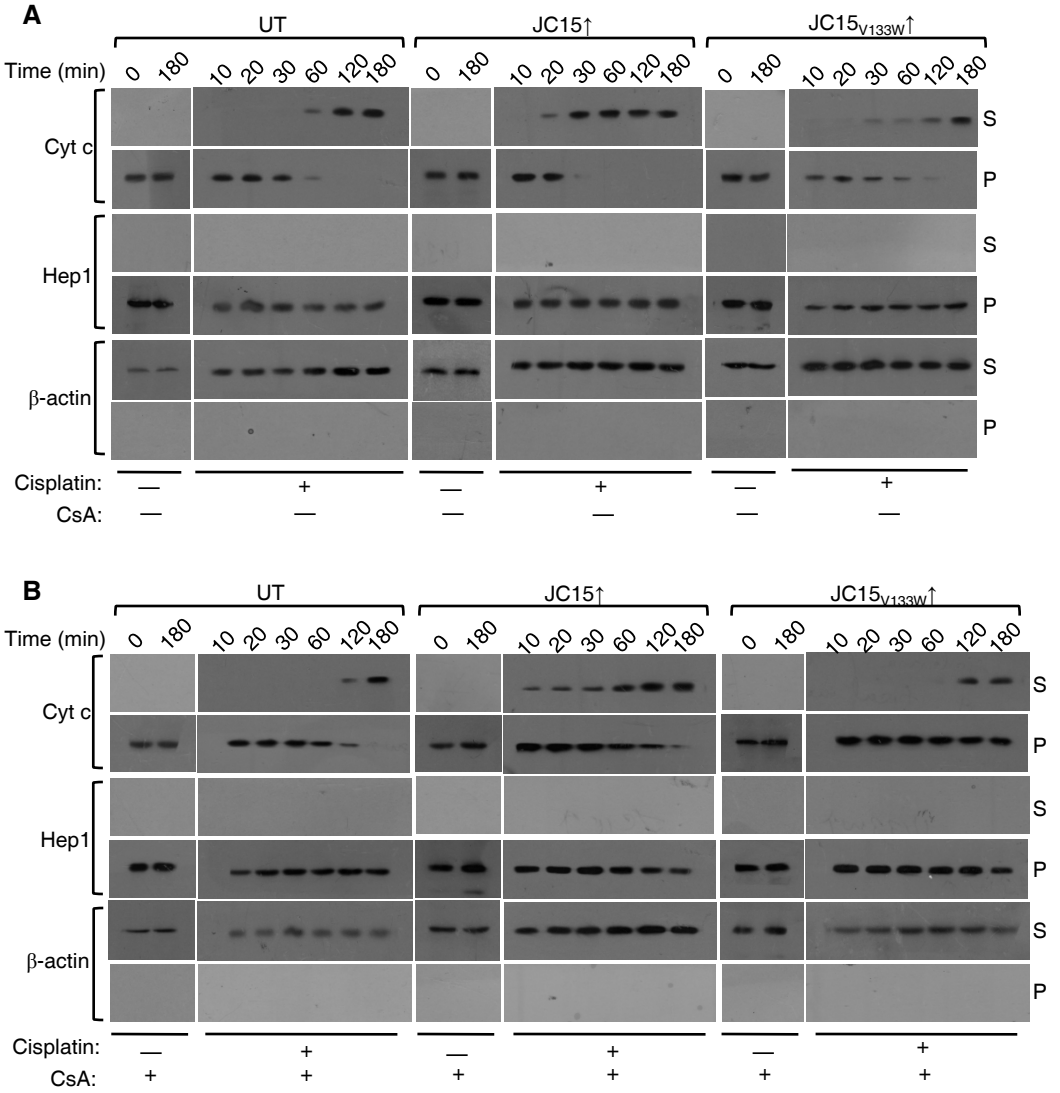

Figure S6 Effect of cyclosporine A on cytochrome c release in cisplatin treated JC15 overexpressing cells. **(A, B)** Untransfected HEK293T cells or cells overexpressing wild type JC15 or $JC15_{V133W}$ were either left untreated **(A)** or pretreated with 1 nM cyclosporin A **(B)** prior to exposure to cisplatin for indicated time periods. Post treatment equivalent amounts isolated mitochondria (P) and cytosolic supernatant (S) were subjected to immunoblot analysis using anti-cytochrome c (Cytc) antibodies. Purity of mitochondrial and cytosolic fractions were adjudged by immunodecorating with mitochondria specific anti-Hep1 and cytosol specific anti- β-actin antibodies. Untreated transfected or untransfected (UT) cells were used as negative control. Hep1 and β-actin served as loading controls for respective fractions.

Figure S7

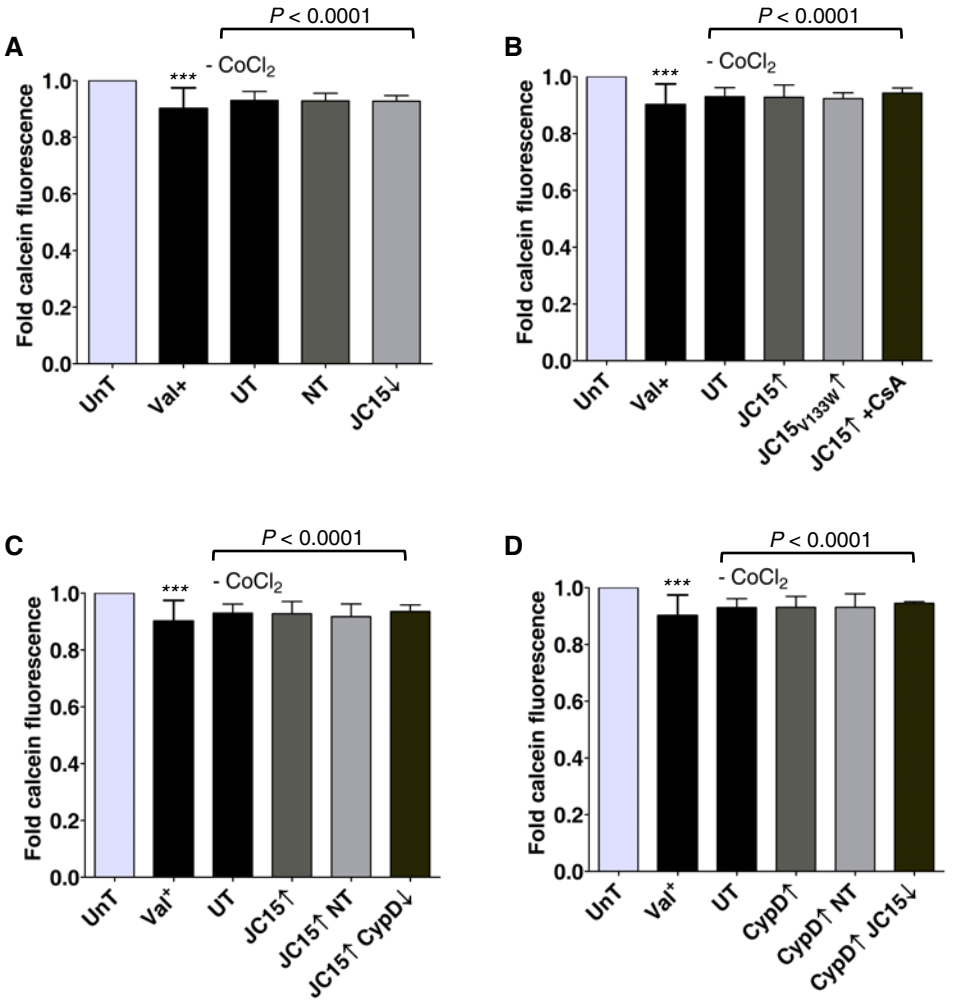

Figure S7 (A-D) To test the equivalency of dye loading, MCF7 cells variably expressing JC15 and CypD were stained with calcein and the relative fluorescence of the dye was quantified in absence Co^{2+}. The data was represented as fold fluorescence over untreated cells and depicted as mean ± s. e. m, $P < 0.0001$. For Val^{+} cells $^{***}P < 0.01$.