miR-137 mediates the functional link between c-Myc and EZH2 that regulates cisplatin resistance in ovarian cancer

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Running Title: c-Myc-miR-137-EZH2 axis regulates cisplatin resistance

Abstract

Platinum drugs are used in first-line to treat ovarian cancer, but the most of patients eventually generate resistance. Although both c-Myc and EZH2 have been implicated in regulating cisplatin resistance in ovarian cancer, the interplay between these two regulators is poorly understood. Using RNA sequence analysis (RNA-seq), for the first time we find that miR-137 level is extremely low in cisplatin resistant ovarian cancer cells, correlating with higher levels of c-Myc and EZH2 expression. Further analyses indicate that in resistant cells c-Myc enhances the expression of EZH2 by directly suppressing miR-137 that targets EZH2 mRNA, and increased expression of EZH2 activates cellular survival pathways, resulting in the resistance to cisplatin. Inhibition of c-Myc-miR-137-EZH2 pathway re-sensitizes resistant cells to cisplatin. Both *in vivo* and *in vitro* analyses indicate that cisplatin treatment activates c-Myc-miR-137-EZH2 pathway. Importantly, elevated c-Myc-miR-137-EZH2 pathway in resistant cells is sustained by dual oxidase maturation factor 1 (DUOXA1)-mediated production of reactive oxygen species (ROS). Significantly, clinical studies further confirm the activated c-Myc-miR-137-EZH2 pathway in platinum drug resistant or recurrent ovarian cancer patients. Thus, our studies elucidate a novel role of miR-137 in regulating c-Myc-EZH2 axis that is crucial to the regulation of cisplatin resistance in ovarian cancer.

Keywords: miR-137/c-Myc/EZH2/cisplatin resistance/ovarian cancer/DUOXA1

Introduction

Ovarian cancer is one of the most lethal malignant gynecological cancers. Platinum agents for example carboplatin and cisplatin are the standard chemotherapy for ovarian cancer patients[1]. However, nearly 80% of patients with high response to initial treatment develop recurrent tumors within 2 years and eventually generate acquired resistance against platinum drugs[2, 3]. Platinum drugs are believed to cause purine bases crosslink on the nuclear DNA, leading to DNA damage, and subsequently inducing cell death[4] Additionally, cisplatin treatment also generates cytotoxicity by inducing production of ROS[5, 6]. The reason why cells generate resistance to cisplatin is complicated. The previous studies have elucidated multiple mechanisms including p53 inactivation, decrease in intracellular accumulation of cisplatin, increase in metabolic inactivation of cisplatin and enhanced repair activity of cisplatin-induced DNA damage, etc. [7, 8].

MicroRNAs (miRNAs) are 19 to 25 noncoding nucleotides, which can pair with sequence-specific base on 3′ UTR of the target mRNA, resulting in the translational repression[9]. In addition, miRNAs also can cause direct mRNA cleavage[9]. miRNAs participate in many bio-metabolism activities and biological processes, including response to chemotherapy[10-14]. Multiple studies have indicated the potential regulatory effects of miRNAs in different types of drug-resistant cancers[15-18]. miR-137 also has been found involved in regulation of multiple pathways by targeting EZH2, MITF, TGFA, EGFR and CXCL12[19-22]. However, whether and how miR-137 participates in platinum resistance in ovarian remains unknown.

Enhancer of zeste homologue 2 (EZH2), with the histone methyl transferase (HMTase) activity, belongs to the member of polycomb repressive complex 2 (PRC2). EZH2 can regulate histone methylation that is responsible for gene expression and repression. The disruption of balance in histone methylation causes oncogenesis and metastasis in many types of cancers[23-25]. EZH2 has been found to participate in cisplatin resistance in ovarian cancer[26]. c-Myc is found to act as a transcription factor which is link with oncogenesis by targeting genes that in charge of cell proliferation and apoptosis[27]. It is found that 30–60% of human ovarian tumors have amplification of c-Myc gene[28, 29]. The expression level of c-Myc shows a strong association with chemoresistance in many cancers such as osteosarcoma, friend erythroleukemia, urinary bladder tumor, melanoma, lung carcinoma, hepatocellular carcinoma, and ovarian cancer[30-38]. Although both c-Myc and EZH2 have been implicated in chemoresistance, whether or not there is a functional link between these two proteins is unknown[26, 39].

In this study, for the first time we found a significant decrease of miR-137 in cisplatin resistant ovarian cancer. miR-137 regulates cisplatin resistance by linking c-Myc to EZH2, which in turn activates cellular survival pathways. Specifically, in resistant cells c-Myc promotes expression of EZH2 by recruiting EZH2 to miR-137 promoter region to suppress miR-137 expression which target EZH2. Inhibition of c-Myc-miR-137 axis re-sensitizes resistant cells to cisplatin. *In vivo* and *in vitro* studies indicate that c-Myc-miR-137-EZH2 axis is activated in response to cisplatin treatment. Moreover, increased production of ROS promotes cisplatin resistance by activating c-Myc-miR-137-EZH2 pathway. The clinical evidences also demonstrate the activation of c-Myc- miR-137-EZH2 axis in the most tested

platinum recurrent or resistant ovarian tumors, and the elevated EZH2 or c-Myc shows a strong correlation with the poor prognosis in ovarian cancer patients treated with platinum drugs.

Results

Identification of miR-137-EZH2 axis in cisplatin resistant ovarian cancer cells by genomic sequencing

To elucidate the new mechanisms governing cisplatin resistance in ovarian cancer cells, we generated ovarian cancer cells resistant to cisplatin by using procedures as previously described[40]. Cell viability analyses revealed that the IC50 against cisplatin in IGROV1 CR cells was increased by 8-fold when compared to its sensitive parent IGROV1 cells (Fig. 1A, left). To ensure the potential pathways that could be confirmed in cell models more close to human patients, we obtained a paired ovarian cancer cells, PEO1 and PEO4, which are directly derived from the same patient before and after the patient developed resistance to platinum drugs [41]. Indeed, cell survival analysis indicated that the IC50 against cisplatin in resistant PEO4 cells was increased by 4-fold as compared with cisplatin sensitive PEO1 cells (Fig. 1A, right).

In order to identify the genes that regulate cisplatin resistance in ovarian cancer cells, we examined gene expression by conducting RNA sequencing (RNA-seq) using IGROV1 and IGROV1 CR cells. From this analysis, we found a total of 993 upregulated genes and 835 downregulated genes in IGROV1 CR cells when compared with IGROV1 cells (Fig. 1B). We are interested in the identification of miRNAs that regulate resistance against cisplatin in

ovarian cancer cells. To do this, the expression levels of total of seven miRNAs were found changed in resistant cells as compared with cisplatin sensitive cells (Fig. 1B and C). We were particularly interested in miR-137 because its level was undetectable in IGROV1 CR cells but highly expressed in IGROV1 (Fig. 1C). We therefore assumed that the targeted pathways by miR-137 should be upregulated in resistant ovarian cancer cells, which may play an important role in regulating cisplatin resistance. To confirm this hypothesis, we firstly detected the miR-137 expression in two paired sensitive and resistant cells by quantitative PCR (qPCR). Indeed, miR-137 expression was dramatically downregulated in both resistant cells, IGROV1 CR and PEO4, compared to their sensitive counterparts (Fig. 1D). Since miR-137 has been reported to target multiple genes including EZH2, MITF, TGFA, EGFR and CXCL12, we next examined the expression of these genes by qPCR[19-22, 42]. Although expressions of MITF, TGFA, EGFR and CXCL12 were not increased in resistant IGROV1 CR cells (Supplementary Fig. S1), EZH2 expression showed a significantly higher level in resistant cells lines IGROV1 CR and PEO4 compared to their sensitive counterparts (Fig. 1E). Consistently, EZH2 protein levels were also dramatically elevated in both resistant cells (Fig. 1F). Similar results were observed in another pair of cisplatin sensitive and resistant ovarian cell lines OV90 and OV90 CR (Supplementary Fig. S7). We next examined and compared all other miRNAs that potentially target EZH2 from RNA-seq data and fount that miR-137 appeared to be the only one that was dramatically downregulated in resistant ovarian cancer cells (Supplementary Fig. S9). Taken together, using RNA-Seq analysis, we have identified the miR-137-EZH2 axis that are significantly altered in cisplatin resistant cells and might regulate resistance against cisplatin in ovarian cancer cells.

miR-137 regulates resistance against cisplatin by targeting EZH2 expression in ovarian cancer cells

Given that expression level of miR-137 is low whereas EZH2 levels is high in resistant cells, we hypothesized that forced expression of miR-137 could reduce the expression of EZH2, resulting in re-sensitizating the resistant cells to cisplatin. To confirm this hypothesis, we transfected miR-137 mimic into two resistant cells IGROV1 CR and PEO4. Our results showed that forced expression of miR-137 dramatically downregulated EZH2 protein levels in both cells (Fig. 2A). Strikingly, forced expression of miR-137 also downregulated cell survival pathways as indicated by reduced phosphorylation of AKT at Thr 473, ERK1/2 at Thr 202/204, as well as expression for Bcl-xL, an anti-apoptosis factor (Fig. 2A)[43, 44]. To further confirm these results, we transfected miR-137 inhibitor into two sensitive IGROV1 and PEO1 cells, and found that inhibiting expression of miR-137 dramatically upregulated EZH2 expression in both cells (Supplementary Fig. S5A and B). Consistently, inhibition of miR-137 also increased the phosphorylation levels of AKT and ERK1/2, and elevated the Bcl-xL expression levels, as well as cell survival (Supplementary Fig. S5A and B). Taken together, these results indicate that miR-137 is critical to regulate cell survival by regulating EZH2 as well as cell survival pathways in resistant cells.

Using cell proliferation assay we found that forced expression of miR-137 was able to re-sensitize both resistant cells IGROV1 and PEO4 to cisplatin (Fig. 2B and C). Consistently, the clonogenic survival assay proved us the evidence that forced expression of miR-137 together with cisplatin dramatically reduced the survival of both resistant cells (Fig. 2D and

E, Supplementary Fig. S2C and D). Moreover, miR-137 was found to promote the apoptotic cell death of IGROV1 CR cells in the presence of cisplatin as indicated via an increase of cleaved PARP, an apoptotic marker (Fig. 2F). Together, these results indicate that miR-137 is critical in regulating cisplatin resistance in ovarian cancer cells.

miR-137 has been found to target multiple pathways[19-22]. Thus, it is possible that miR-137 may regulate cisplatin resistance by targeting proteins other than EZH2. To confirm that EZH2 is a major target of miR-137 in resistant ovarian cancer cells, we examined whether ectopic expression of EZH2 could restore downregulated cell survival pathway in the cells with miR-137 forced expression. To this end, we transfected control vector or vector expressing EZH2 into resistant cells with forced expression of miR-137 mimic. The forced expression of miR-137 reduced the phosphorylation of AKT, ERK and Bcl-xL expression (Fig. 2G, compare lane 1 and 2), and ectopic expression of EZH2 could increase the phosphorylation level of AKT and ERK as well as the expression level of Bcl-xL (Fig. 2G, compare lane 3 and 4). Significantly, downregulated phosphorylation of AKT and ERK as well as Bcl-xL expression by overexpression of miR-137 was partially restored by ectopic expression of EZH2 (Fig. 2G, compare lane 2 and 5), indicating that miR-137 regulates cell survival by targeting EZH2 in cisplatin resistant cells.

EZH2 inhibition synergizes with cisplatin in resistant ovarian cancer cells

Considering that EZH2 is dramatically upregulated in resistant ovarian cancer cells, we hypothesized that EZH2 inhibitor and cisplatin should have synergy in resistant cell lines. To prove our hypothesis, we treated resistant cells IGROV1 CR and POE4 by using an EZH2

specific inhibitor called GSK343, together with cisplatin. Cell proliferation assay showed an excellent synergy of GSK343 with cisplatin in both resistant cells as indicated by the combination index (CI < 1) (Fig. 3A and B). CI index is used for quantitative definition of synergism (CI<1), additive effect (CI=1), and antagonism (CI>1) between two drugs.[45]. In order to eliminate the off-target effect of the EZH2 inhibitor GSK343, we depleted EZH2 by siRNA and found that depletion of EZH2 re-sensitized both resistant cells IGROV1 CR and POE4 to cisplatin (Fig. 3C and D). Consistently, clonogenic survival analyses indicated that depletion of EZH2 by siRNA could suppress cell survival of both resistant cells (Fig. 3E and F, Supplementary Fig. S3A and B). To further elucidate the role of EZH2 in regulating of resistance against cisplatin, we examined whether EZH2 could affect cell survival pathway in resistant cells. Results showed that phosphorylation levels of AKT and ERK as well as the expression levels of EZH2 and Bcl-xL were increased in IGROV1 CR and PEO4 cells compared to their counterparts (Fig. 3G). Significantly, depletion of EZH2 by siRNA significantly reduced the phosphorylation of AKT and ERK as well as expression of Bcl-xL, indicating that EZH2 is a critical factor to sustain the activated cell survival pathway in resistant cells (Fig. 3H). Furthermore, EZH2 depleted cells treated with cisplatin showed an increase in cell apoptosis as indicated by the elevated level of cleaved PARP (Fig. 3I).

c-Myc suppresses miR-137 expression in cisplatin resistant ovarian cancer cells

Having found a novel miR-137-EZH2 axis in regulating resistance against cisplatin in ovarian cancer cells, we next probed the molecular mechanism how miR-137 is regulated in resistant cell lines. To accomplish this goal, we first examined the JASPAR database to identify

transcription factors that can target miR-137 promoter region. Strikingly, c-Myc was found as a strong candidate that binds to miR-137 promoter region. To explore whether c-Myc indeed binds to miR-137 promoter region in ovarian cancer cells, we performed ChIP assay with control IgG, c-Myc or EZH2 antibody. The distal promoter of miR-137 locates at 5.5kb upstream of miR-137 host gene[46]. It was recently identified an internal promoter adjacent to the miR-137 sequence and confirmed as the promoter of the miR-137 gene. (Fig. 4A)[47]. ChIP analyses showed that c-Myc was enriched at both distal and internal promoter regions of miR-137 but not at a negative control region on miR-571 promoter, indicating c-Myc indeed binds to miR-137 promoters (Fig. 4B). Surprisingly, ChIP assay also indicated a strong association of EZH2 with both promoter regions (Fig. 4B), suggesting a possible role of EZH2 in regulating miR-137 expression (See results in Fig. 5A).

To test how c-Myc regulates expression of miR-137, we first examined the c-Myc levels in resistant cells. Strikingly, c-Myc was overexpressed in both resistant cells IGROV1 CR and PEO4 cells compared to their sensitive counterparts (Fig. 4C). Consistently, mRNA level of c-Myc was found upregulated in both resistant cells (Fig. 4D). Significantly, depleting of c-Myc by siRNA dramatically increased miR-137 levels in both resistant cells (Fig. 4E), indicating that the expression of miR-137 is suppressed by c-Myc in resistant cells.

Since c-Myc regulates miR-137 expression in resistant cells, we expected that depleting of c-Myc should downregulate EZH2 and cell survival pathway. Indeed, depletion of c-Myc by siRNA decreased expression of EZH2 and Bcl-xL, as well as activation of AKT and ERK as indicated by reduced phosphorylation of AKT at 473 and ERK at 202/204 (Fig.

4F). Consistent with these results, depletion of c-Myc re-sensitized both resistant cells to cisplatin (Fig. 4G and H).

To confirm that c-Myc regulates EZH2 via directly targeting miR-137, we ectopically expressed c-Myc in both resistant cells followed by transfecting cells with miR-137. Strikingly, ectopic expression of c-Myc increased EZH2 levels, which was suppressed by miR-137 forced expression in both resistant cells (Fig. 4I, compare lane 4 and 5). Taken together, our results indicate that c-Myc controls EZH2 expression by directly regulating miR-137 expression in cisplatin resistant ovarian cells.

c-Myc recruits EZH2 to miR-137 promoter to suppress miR-137 transcription

Encouraged by the finding that EZH2 also binds to miR-137 promoter region (Fig. 4B), we next tested whether EZH2 regulates miR-137 expression. Interestingly, depletion of EZH2 by siRNA significantly increased miR-137 expression in IGROV1 cells (Fig. 5A), suggesting that EZH2 also suppresses miR-137 expression.

Since both c-Myc and EZH2 bind to miR-137 promoters and suppresses miR-137 expression, we next explored whether the association of EZH2 with miR-137 promoter is c-Myc dependent. To this goal, we conducted ChIP assay to indentify the binding of EZH2 to miR-137 promoters in c-Myc knockdown cells by siRNA. Significantly, the PCR results suggested that the EZH2 enrichment at miR-137 promoter was abolished by c-Myc depletion in IGROV1 cells (Fig. 5B). On the other hand, depletion of EZH2 didn't affect the c-Myc association with miR-137 promoters (Fig. 5C). All these data indicate that there is a

c-Myc>miR-137>EZH2>miR-137 loop that is critical to regulate cell survival pathways in cisplatin resistant cells.

Cisplatin activates c-Myc-miR-137-EZH2 pathway *in vivo* and *in vitro*

Given the activation of c-Myc-miR-137-EZH2 pathway in resistant cells and participated in regulating cisplatin resistance in ovarian cancer cells, we hypothesized that c-Myc-miR-137-EZH2 pathway may be activated in response to cisplatin treatment in sensitive cells, which could explain why patients eventually develop cisplatin resistance through activating this pathway. To confirm the above hypothesis, we treated IGROV1 cells with cisplatin at different concentrations and examined miR-137 expression level by using qPCR and Western blotting. The qPCR analysis revealed that miR-137 expression was downregulated in a cisplatin dose-dependent manner (Fig. 6A left panel). Consistently, EZH2 as well as c-Myc expression levels were increased after cisplatin treatment (Fig. 6A right panel and 6B), indicating that cisplatin indeed activates c-Myc-miR-137-EZH2 pathway in sensitive cells. Significantly, we also observed the activation of ERK, pAKT as well as overexpression of Bcl-xL in IGROV1 cells after cisplatin treatment (Fig. 6B).

To further assess whether c-Myc-miR-137-EZH2 pathway is activated *in vivo* after a cisplatin treatment, the IGROV1 xenograft mice were administrated with different doses of cisplatin (0.5, 1 and 2 mg/kg) for 2 weeks. IHC analysis showed that the expression levels of c-Myc and EZH2 were upregulated in tumor samples treated with cisplatin (Fig. 6C and D). Similar to ovarian cancer cells treated with cisplatin, IGROV1 xenograft tumors also

exhibited a dramatically reduction of miR-137 level after cisplatin treatment (Fig. 6E and Supplementary Fig. S10).

Taken together, both *in vivo* and *in vitro* results indicate that cisplatin treatment could activate c-Myc-miR-137-EZH2 pathway. These results provide us with the molecular insights of how ovarian cancer patients generate resistance to platinum drugs over a period time of therapy.

DUOXA1-mediated production of ROS activates c-Myc-miR-137-EZH2 pathway

The next question is why and how activated c-Myc-miR-137-EZH2 pathway is sustained in cisplatin resistant cells. Since cisplatin can induce the production of ROS which has been linked to cell survival, we evaluated the ROS level in paired cisplatin sensitive and resistant ovarian cancer cells using ROS cell staining assay[5, 6, 48-50]. Interestingly, the ROS level was significant increased in IGROV1 CR and PEO4 cells compared to IGROV1 and PEO1 cells respectively (Fig. 7A, Supplementary Fig. S4A). Next, we treated sensitive cells IGROV1 and PEO1 with either cisplatin (0~1μM) or carboplatin (0~10μM). Cisplatin or carboplatin treatment remarkably increased ROS production (Supplementary Fig. S8A-D). To explore whether ROS regulates c-Myc-miR-137-EZH2 pathway in cisplatin resistant cell line, cells were treated with ROS inhibitor YCG063 and the results showed that inhibition of ROS indeed reduced c-Myc as well as EZH2 expression level (Fig. 7B and D). Significantly, inhibition of ROS by YCG063 dramatically increased miR-137 level in both resistant cells (Fig. 7C). Consistently, YCG063 also led to a declined phosphorylation level of ERK and

ART as well as the expression level of Bcl-xL (Fig. 7D). Thus, increased level of ROS is critical to sustain the activation of c-Myc-miR-137-EZH2 pathway in cisplatin resistant cells.

To explore how ROS level is upregulated in cisplatin resistant cells, we analyzed the RNA-seq data to figure out the changes in ROS related gene. We identified a total of 7 ROS related genes upregulated in IGROV1 CR cells when compared to IGROV1 cells (Fig. 7E). Among these genes, the expression level change of DUOXA1 is the most significant upregulated. DUOXA1 is critical in regulating ROS production and upregulation of DUOXA1 level can promote ROS production[51]. We therefore examined the expression of DUOXA1 and found that DUOXA1 showed a higher level in resistant cells (Fig. 7F). To explore the role of DUOXA1 in regulating c-Myc-EZH2 pathway in cisplatin resistant cells, we knocked down DUOXA1 by two independent siRNAs and found that DUOXA1 deficiency reduced ROS levels (Fig. 7H). More importantly, DUOXA1 deficiency downregulated the expression levels of EZH2 and c-Myc (Fig. 7G). Taken together, our finding suggests that elevated ROS production mediated by the overexpression of DUOXA1 sustains activated c-Myc-miR-137-EZH2 pathway in cisplatin resistant ovarian cells.

Clinical evidence of activated c-Myc-miR-137-EZH2 in platinum resistant ovarian cancer patients

Inspired by the finding of the correlation between c-Myc-miR-137-EZH2 activation and platinum resistance in ovarian cancer patients, we further evaluated the correlation between c-Myc and EZH2 expression and the survival rate in patients with ovarian cancer receiving platinum-based therapy. Given that c-Myc and EZH2 were upregulated in cisplatin resistant

cells, we hypothesize that the levels of c-Myc and EZH2 are inversely correlated with survival rate. To confirm this hypothesis, we analyzed the data from 15 datasets of ovarian cancer patients and compared the expressions of c-Myc and EZH2 genes with survival rate in these patients by using Kaplan-Meier plotter. Indeed, patients with platinum drug treatment history exhibited a worse 5-year FPS rate (EZH2: 19.8 months versus 14.93 month, n=459, p=0.0035, c-Myc : 23.4 months versus 18.6 months, n=1435, p=0.00017) when expression levels of c-Myc or EZH2 were high (Fig. 8A and B). Consistently, low level of miR-137 correlates with lower PFS (23.5 months versus 11.1 months, n=33, $p<0.05$) in patient samples from our collection (Fig. 8C). In addition, analyses from 15 datasets indicated that higher level of Bcl-xL correlated with worse 5-year FPS rate (Supplementary Fig. S6).

To directly correlate c-Myc-miR-137-EZH2 pathway with tumor resistance or recurrence in ovarian patients, we collected samples from the same patient before and after the patient developed resistance or recurrence. We obtained and analyzed 7 pairs of samples from patients who were given platinum-based chemotherapy with recurrent tumor within 11-14 month after treatment. We found that 71.43% (5 out of 7) and 83.33% (6 out of 7) patients have elevated c-Myc and EZH2 expression levels after tumor recurrence (Fig. 8D and E). To evaluate miR-137 level in platinum-sensitive and resistant patients, we performed qPCR assay and compared miR-137 level in 65 drug sensitive and 34 drug resistant patients. We observed that the drug resistant group had significantly lower miR-137 level than that in drug sensitive group (Fig. 8F). These clinical data strongly indicate that c-Myc-miR-137-EZH2 pathway is activated in resistant ovarian cancer tumors, which is highly correlated with survival of ovarian cancer patients.

Discussion

In this study, using RNA-seq analysis and a series of functional and biochemical assays, we have identified a novel c-Myc-miR-137-EZH2 axis playing an important role in regulating cisplatin resistance in ovarian cancer. Specifically, we found that in cisplatin resistant ovarian cancer cells, the elevated ROS production is responsible for increased expression of c-Myc, which activates expression of EZH2 by suppressing miR-137. High expression level of EZH2 promotes cisplatin resistance by activating cell survival pathways[26, 52-55]. In fact, our finding is consistent with previous findings of that EZH2 acts a crucial epigenetic regulator governing diverse gene expression in many aspects of human cancers such as tumor initiation, metastasis, chemoresistance, invasiveness and angiogenesis[56]. Interestingly, c-Myc helps to recruit EZH2 to the promoter region of miR-137 to suppress its expression in resistant cells, providing a feedback loop for activation of EZH2 (Fig. 8G). This discovery not only explains why miR-137 level is extremely low in cisplatin resistant ovarian cancer cells, but also elucidates a novel c-Myc-miR-137-EZH2 axis in the regulation of cisplatin resistance in ovarian cancer. Importantly, this novel molecular mechanism has been confirmed in ovarian cancer patients with acquired cisplatin resistance. Thus, tumors with low level of miR-137 in ovarian cancer patients are more likely to exhibit cisplatin resistance, suggesting that miR-137 might be a useful biomarker of resistance to cisplatin in ovarian cancer. Further studies using larger pool of clinical ovarian cancer samples are required to test this possibility.

Our study indicates that cisplatin treatment suppresses miR-137 expression *in vitro* and *in vivo*. Clearly ovarian cancer cells reply cisplatin treatment by downregulating miR-137

expression, resulting activation of EZH2, which in turn, promotes cellular survival pathways. Thus, miR-137 acts as a key factor to mediate the activation signaling from c-Myc to EZH2 in cisplatin resistant ovarian cancer cells. Given that both c-Myc and EZH2 participate in cisplatin resistance in ovarian cancer[26, 39], targeting miR-137 provides us with an alternative approach to overcome cisplatin resistance in ovarian cancer cells by blocking the linkage between c-Myc and EZH2.

High levels of c-Myc or EZH2 correlate with poor prognosis in ovarian cancer patients receiving platinum-based therapy. It is unknown whether there is a link between c-Myc and EZH2, which may relate to cisplatin resistance in ovarian cancer. It has been proved that c-Myc could regulate EZH2 expression by directly binding to its promoter in fibroblasts[57]. However, it is not the same case in cisplatin resistant ovarian cancer, in which miR-137 mediates the interaction between c-Myc and EZH2. It remains unknown why c-Myc regulates EZH2 expression by targeting miR-137 in cisplatin resistant ovarian cancer cells. One possibility is that c-Myc might be regulated by other pathways in cisplatin resistant ovarian cancer cells and such regulatory pathway may promote c-Myc to bind to the promoter region of miR-137, rather than EZH2 promoter region. Further analyses on the c-Myc activity in cisplatin resistant ovarian cancer cells are expected to test this possibility. On the other hand, we have found that the high production of ROS is commonly seen in cisplatin resistant ovarian cancer cells. Importantly, we demonstrated that the high level of ROS is sustained by the upregulation of DUOXA1 which is a key NADPH oxidases involving in hydrogen peroxide production[58]. Co-treatment of ROS inhibitor YCG063 could completely impair the functions of c-Myc-miR-137-EZH2 in regulating cisplatin resistance in ovarian cancer cells.

This suggests application of either pharmaceuticals such as ROS inhibitor or genetic manipulation of miR-137 levels could effectively inhibit cisplatin resistance in ovarian cancers.

In summary, our current study underscores a novel signaling cascade of c-Myc-miR-137-EZH2 in regulating cisplatin resistance in ovarian cancer cells. Importantly, we proposed a unique molecular mechanism by which the upregulation of DUOXA1 maintains high level of ROS which, in turns, activates c-Myc-miR-137-EZH2 signaling pathway in cisplatin resistant ovarian cancer. These findings provide a scientific base for exploring either miR-137 as a biomarker or targeting ROS and c-myc-miR-137-EZH2 cascade in impeding cisplatin resistance in ovarian cancers.

Materials and methods

Antibodies and reagents

Antibodies specifically recognizing EZH2 (5246S), c-Myc (9402S), PARP (9542S), p-ERK (4370S), ERK (4695S), p-AKT Ser 473 (4060s), AKT (4691S), Bcl-xL (2764S) were from Cell Signaling Technology; HA (H9658), β-actin (5441) were from Sigma-Aldrich; c-Myc for IHC (ab32072) was from Abcam; DUOXA1 (26632-1-AP) was from Proteintech. Cis-Diamineplatinum(II) dichloride (Cisplatin, Sigma-Aldrich) was dissolved in sterile 0.9% saline for the following *in vitro* and in *vivo* assay. YCG 063 (Calbiochem) was dissolved in DMSO for the *in vitro* assay.

Cell culture

The human ovarian cancer PEO1 or PEO4 cells (Sigma) was maintained in RPMI-1640 containing 10% Fetal Bovine Serum (FBS). IGROV1 cells (a generous gift form Wei Zheng) was cultured in DMEM containing 10% FBS. OV90 (ATCC) was cultured in the medium

containing MCDB 105 and M199 (Volume ratio 1:1) and 10% FBS supplemented. All the cells were cultured at 37 °C in 5% CO_2.

Resistance cell line establishment

Both IGROV1 CR and OV90 CR cisplatin resistant cells were established following the previous report[40, 59]. After five-six months of cisplatin treatment for 6 cycles, resistant cell lines were established. We only use the early-passage (<10 passages) of them in the following study.

Cell viability assay

Cell viability was detected by using Sulforhodamine B (SRB) assay as previously published[45, 60, 61].

Clonogenic Assay

The detailed procedure of clonogenic assay is provided in Supplementary methods.

Transfection

The detailed procedure of transfection assay is provided in Supplementary methods.

Western blotting

Western blot was performed as previous published[62, 63 64].

Genome-wide RNA-sequencing (RNA-seq)

The detailed approach was as we described previously[59, 60]. Genes with false discovery rate (FDR) < 0.05, fold of change > 2 or < 0.5 were considered as differentially expressed genes when comparing that in IGROV1 or IGROV1 CR cells.

RT-qPCR

The detailed procedure of RT-qPCR assay is provided in Supplementary methods.

Chromatin Immunoprecipitation (ChIP)

The detailed procedure of ChIP assay is provided in Supplementary methods.

Animal experiments

The detailed procedure of Animal experiments is provided in Supplementary methods.

Immunohistochemistry

The detailed procedure of Immunohistochemistry is provided in Supplementary methods.

Reactive Oxygen Species (ROS) detection

ROS level was measured by using CellROX Orange reagent (Thermo Fisher Scientific) according to the manufacturer's protocol. ROS intensity was calculated by using Image-ProPlus Software.

Ovarian cancer patients

The details of ovarian cancer patient information are provided in Supplementary methods.

Statistical analysis

GraphPad Prism 7.0 (https://www.graphpad.com/scientific-software/prism/) was used to incorporate One-way ANOVA and Student's t test to analyze statistical parameters.

Column graph represents mean of samples and the error bar represents standard deviation. Significant difference was defined as $P < 0.05$. Kaplan Meier curves for PFS of ovarian cancer patient were obtained from database online (http://kmplot.com), and comparisons were evaluated by using log-rank test.

Conflicts of interest

The authors declare no conflict of interest.

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Figure legends

Fig. 1 Identification of miR-137-EZH2 axis by genomic sequencing.

(A) Cell viability in IGROV1 and IGROV1 CR cells (left), PEO1 and PEO4 (right) treated with different gradient doses of cisplatin for three days. Then SRB assay was performed and IC_{50} values were calculated. Data were represent as mean ± SD (n=3).

(B) Volcano plot showing up-regulated and down-regulated genes identified from RNA-Seq experiments using IGROV1 and IGROV-1 CR cells. A change is considered significantly if the change is > 2-fold with a p-value < 0.05. Genes with very significant p-values ($p<1\times10^{-5}$) were indicated.

(C) Hierarchical clustering of miRNAs with a >2-fold change in IGROV1 CR compared to IGROV1 cells. Data were normalized by z-score in different samples. Different colors represent the expression levels of genes.

(D) miR-137 expression levels in IGROV1 and IGROV1 CR cells (left), PEO1 and PEO4 cells (right) were measured by qPCR. Data were represent as mean ± SD (n=3). *p < 0.05 and **p < 0.01.

(E) EZH2 mRNA levels of in IGROV1 and IGROV1 CP cells (left), PEO1 and PEO4 cells (right) were measured by qPCR. Data were represent as mean ± SD (n=3). ***p < 0.001.

(F) EZH2 protein levels in IGROV1 and IGROV1 CP cells (left), PEO1 and PEO4 cells (right) were measured by western blot analysis.

Fig. 2 miR-137 regulates cisplatin resistance via targeting EZH2 in resistant cells.

(A) Control miRNA (Ctr.) or miR-137 mimic was transfected in IGROV1 CR (left) or PEO4 (right) cells for 48h. Then cells were harvested to detect indicated proteins by using western blot analysis.

(B-C) Control miRNA (Ctr.) or miR-137 mimic was transfected in IGROV1 CR (left) or PEO4 (right) cells for 48h. Then cells were treated with different gradient doses of cisplatin for three days. Then SRB assay was performed. Data were represent as mean ± SD (n=3). *p < 0.05, **p < 0.01 and ***p < 0.001.

(D-E) Control miRNA (Ctr.) or miR-137 mimic was transfected in IGROV1 CR (left) or PEO4 (right) cells for 48h. Then cells were treated with cisplatin and clonogenicity was detected 10-14 days after cisplatin treatment. Colonies were fixed and stained with Crystal violet. Data were represent as mean ± SD (n=3). **p < 0.01 and ***p < 0.001.

(F) Control miRNA (Ctr.) or miR-137 mimic was transfected in IGROV1 CR cells for 24h. Then cells were treated with 4μM cisplatin for 48h and harvested to detect indicated proteins by using western blot analysis.

(G) Control vector or vector expressing EZH2 was transfected for 48h in the IGROV1 CR cells with forced expression of miR-137 mimic. Then cells were harvested to detect indicated proteins by using western blot analysis.

Fig. 3 EZH2 inhibition has the synergy with cisplatin in resistant cells.

(A-B) The synergistic effects of EZH2 inhibitor (GSK343) and cisplatin on resistant cell lines. Concentrations of EZH2 inhibitor (GSK343) and cisplatin as well as the CI index were indicated. Data were represent as mean ± SD (n=3).

(C-D) Control siRNA (siGl2) or EZH2 siRNA was transfected for 48h in IGROV1 CR and PEO4 cells. Then cells were treated with different gradient doses of cisplatin for three days. Then SRB assay was performed. Data were represent as mean ± SD (n=3). *p < 0.05, **p < 0.01 and ***p < 0.001.

(E-F) Control siRNA (siGl2) or EZH2 siRNA was transfected for 48h in IGROV1 CR and PEO4 cells. Then cells were treated with cisplatin and clonogenicity was detected 10-14 days after cisplatin treatment. Colonies were fixed and stained with Crystal violet. Data were represent as mean ± SD (n=3). **p < 0.01 and ***p < 0.001.

(G) IGROV1, IGROV1 CR (left) or PEO1 and PEO4 (right) cells were harvested to detect indicated proteins by using western blot analysis.

(H) Control siRNA (siGl2) or EZH2 siRNA was transfected for 48h in IGROV1 CR (left) and PEO4 (right) cells. Then cells were harvested to detect indicated proteins by using western blot analysis.

(I) Control siRNA (siGl2) or EZH2 siRNA was transfected for 48h in IGROV1 CR. Then cells were treated with 5μM cisplatin for 24h and were harvested to detect indicated proteins by using western blot analysis.

Fig. 4 c-Myc negatively regulates miR-137 expression in resistant cells.

(A) Schematic of promoter region of miR-137 gene.

(B) ChIP assay to detect the association of c-Myc or EZH2 with promoter regions of the miR-137. IGROV1 CR cells were harvested and cell lyses were immunoprecipitated with IgG, anti-c-Myc or EZH2 antibodies. c-Myc or EZH2 associated DNA were examined by PCR.

(C) c-Myc protein levels in IGROV1, IGROV1 CR (left), or PEO1 and PEO4 (right) cells.

(D) The mRNA levels of c-Myc in IGROV1, IGROV1 CR (left), or PEO1 and PEO4 (right) cells. Data were represent as mean ± SD (n=3). *p < 0.05 and ***p < 0.001.

(E) siGl2 or sic-Myc was transfected for 48h in IGROV1 CR (left) and PEO4 (right) cells. Then cell were harvested to detect miR-137 level by qPCR. Data were represent as mean ± SD (n=3). ***p < 0.001.

(F) siGl2 or sic-Myc was transfected for 48h in IGROV1 CR (left) and PEO4 (right) cells. Then cell were harvested to detect indicated proteins by using western blot analysis.

(G-H) siGl2 or sic-Myc was transfected for 48h in IGROV1 CR and PEO4 cells. Then cells were treated with different gradient doses of cisplatin for three days. Then SRB assay was performed. Data were represent as mean ± SD (n=3). **p < 0.01 and ***p < 0.001.

(I) Control vector or vector expressing c-Myc was transfected for 48h in the IGROV1 CR cells with forced expression of miR-137 mimic. Then cells were harvested to detect indicated proteins by using western blot analysis.

Fig. 5 c-Myc recruits EZH2 to miR-137 promoter to suppress miR-137 transcription.

(A) miR-137 expression level in EZH2 depleted IGROV1 CR cells. siGl2 or siEZH2 was transfected for 48h in IGROV1 CR cells Then cells were harvested to detect miR-137 levels (left) and EZH2 level (right).

(B) ChIP assay to detect the association of EZH2 with promoter region of the miR-137 in c-Myc knockdown cells. siGl2 or sic-Myc was transfected for 48h in IGROV1 CR cells. Then cells were harvested and cell lyses were immunoprecipitated with IgG, anti-c-Myc or EZH2 antibodies. c-Myc or EZH2 associated DNA were examined by qPCR using primers for distal promoter (left) or internal promote (middle). c-Myc level was detected by using western blot analysis (right).

(C) ChIP assay to detect the association of c-Myc with promoter region of the miR-137 in EZH2 knockdown cells. siGl2 or siEZH2 was transfected for 48h in IGROV1 CR cells. Then cells were harvested and cell lyses were immunoprecipitated with IgG, anti-c-Myc or EZH2 antibodies. c-Myc or EZH2 associated DNA were examined by qPCR using primers for distal

promoter (left) or internal promote (middle). EZH2 level was detected by using western blot analysis (right).

In all panels of this figure, data were represent as mean ± SD (n=3). p* < 0.05, ***p < 0.001 and n.s., not significant.

Fig. 6 Cisplatin activates c-Myc-miR-137-EZH2 pathway *in vivo* and *in vitro*.

(A-B) IGROV1 cells were treated with different gradient doses of cisplatin for four days. Then cells were harvested to detect miR-137 level and indicated protein levels.

(C-D) Representative HE staining and IHC images of IGROV1 xenograft tumors treated with with different gradient doses of cisplatin. Quantification of IHC staining for c-Myc and EZH2 in tumor samples.

(E) miR-137 expression level in IGROV1 xenograft tumor samples.

In all panels of this figure, data were represent as mean ± SD (n=3). p* < 0.05, p** < 0.01 and ***p < 0.001.

Fig. 7 ROS regulates c-Myc-miR-137-EZH2 pathway.

(A) Quantification of ROS production in IGROV1, IGROV1 CR (left) or PEO1 and PEO4 (right) cells. Data were represent as mean ± SD (n=3). ***p < 0.001.

(B) ROS production was stained and quantified in ROS inhibitor YCG063 (20 μM) treated IGROV1 CR (left) and PEO4 (right) cells. Data were represent as mean ± SD (n=3). ***p < 0.001.

(C) miR-137 level in YCG063 treated IGROV1 CR (left) or PEO4 (right). Data were represent as mean ± SD (n=3). *p < 0.05 and **p < 0.01.

(D) The indicated proteins were detected in YCG063 treated IGROV1 CR (left) or PEO4 (right).

(E) Heatmap of ROS related genes. Data were normalized by z-score in different samples. Different colors represent the expression levels of genes. Fold change bar on the right showed that DUOXA1 was the most significant upregulated gene.

(F) DUOXA1 protein levels were detected in IGROV1, IGROV1 CR cells (left), or PEO1 and PEO4 cells (right).

(G) siGl2 or siDUOXA1 was transfected for 48h in IGROV1 CR (left) or PEO4 (right) cells. Then cells were harvested to detect indicated proteins by using western blot analysis.

(H) siGl2 or siDUOXA1 was transfected for 48h in IGROV1 CR (upper) and PEO4 (below) cells. ROS production was stained and quantified by using Image-ProPlus Software. Data were represent as mean ± SD (n=3). **$p < 0.01$.

Fig. 8 Clinical evidence of activated c-Myc-miR-137-EZH2 in platinum treated ovarian cancer patients.

(A-B) Kaplan-Meier survival curves that present correlation between EZH2 (A) or c-Myc (B) expression level and 5-year progression-free survival rates (PFS) in ovarian cancer patients. 459 and 1435 patients were analyzed for EZH2 expression-PFS and c-Myc expression-PFS respectively. Comparisons were evaluated by using log-rank test. P values, 95 % confidence interval in parentheses and hazard ratios (HR) are indicated.

(C) Kaplan–Meier survival curves indicating correlation between miR-137 expression level and 5-year PFS rate of 33 ovarian cancer patients.

(D) IHC quantification of EZH2 and c-Myc in the primary and recurrent tumor samples from seven patients (P1–P7) received platinum-based therapy.

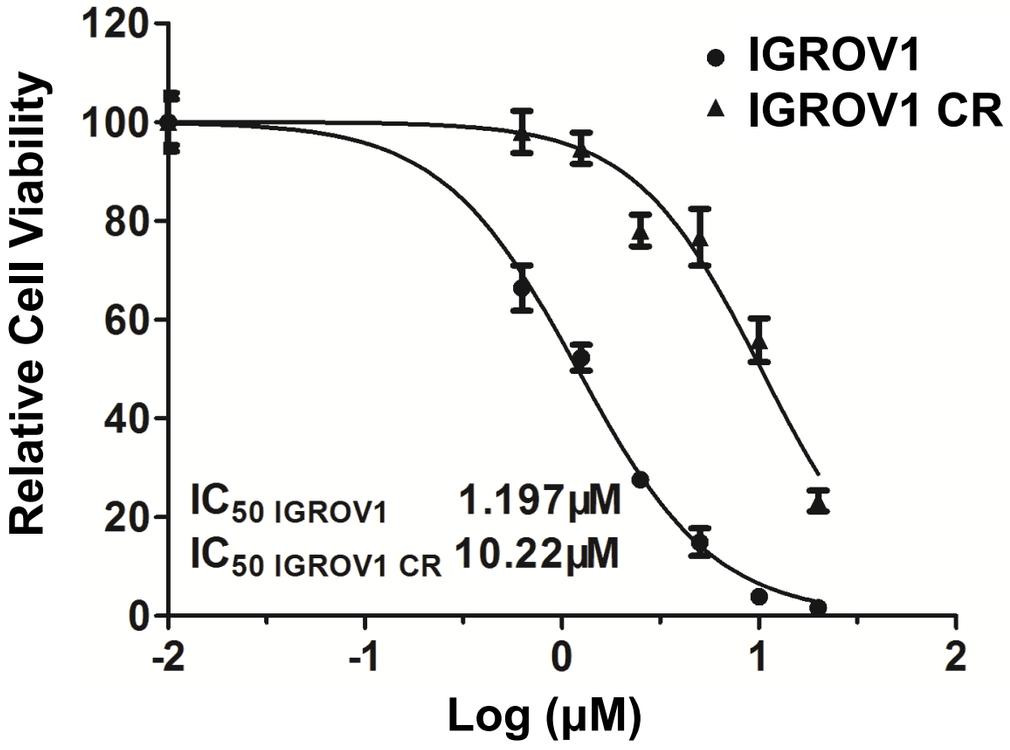
(E) Representative IHC images of EZH2 and c-Myc in the primary and recurrent tumor samples from the same patient 1, patient 3 and patient 5.

(F) Comparison of the miR-137 levels measured by qPCR in platinum-sensitive and-resistant ovarian cancer patients.

(G) Working model of activated c-Myc-miR-137-EZH2 pathway in regulating cisplatin resistance in ovarian cancer.

Fig. 1

A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IGROV1 CR
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Log (μM)
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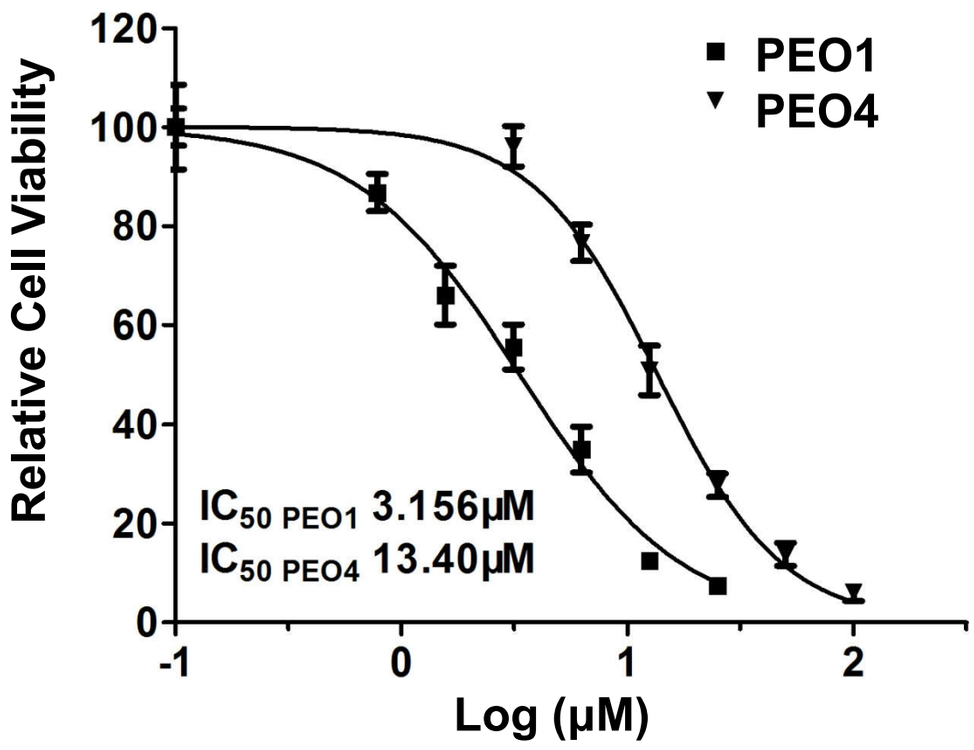
IC50 IGROV1 CR 10.22μM


PEO1
PEO4
Relative Cell Viability
Log (μM)
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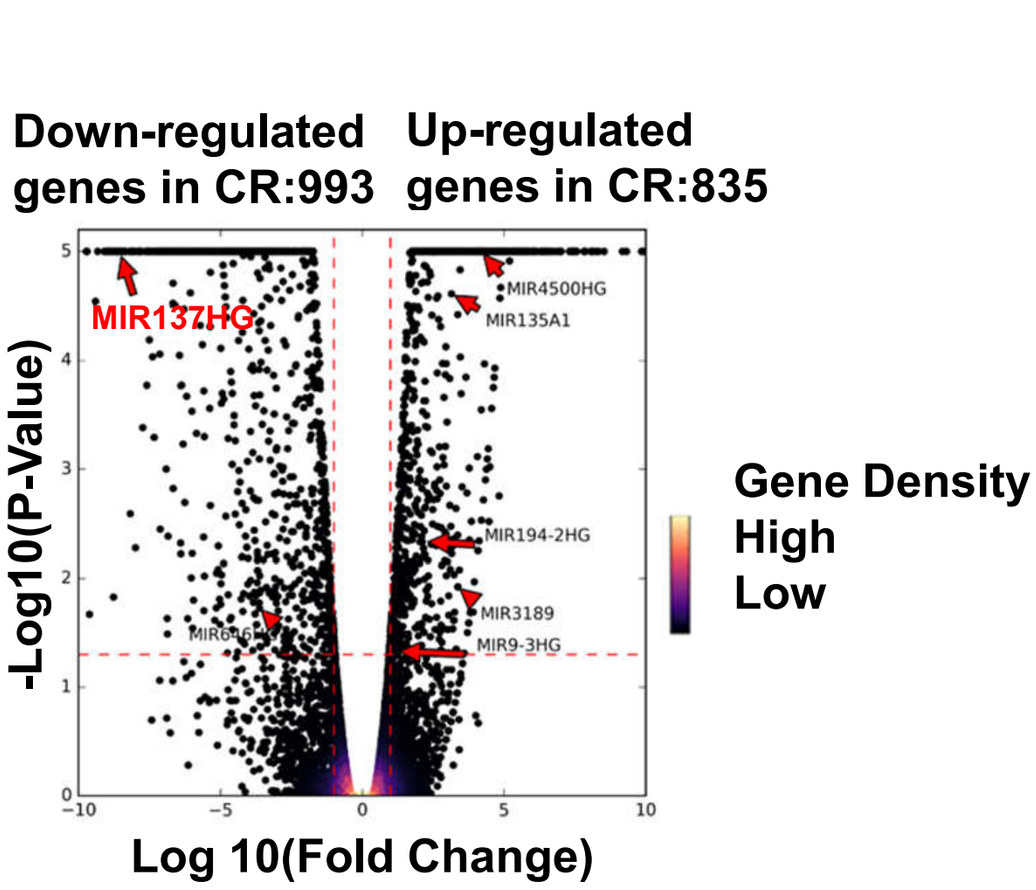
IC50 PEO4 13.40μM

B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MIR4500HG
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MIR3189
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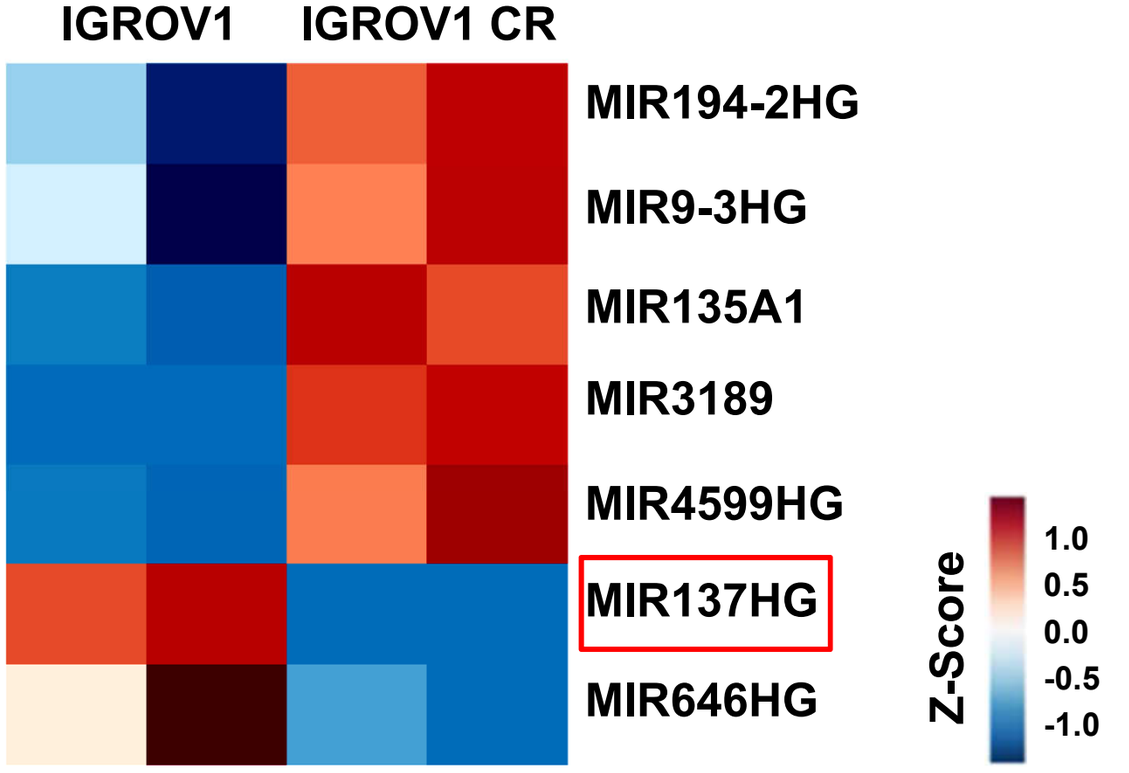
Log 10(Fold Change)
Gene Density
High
Low

C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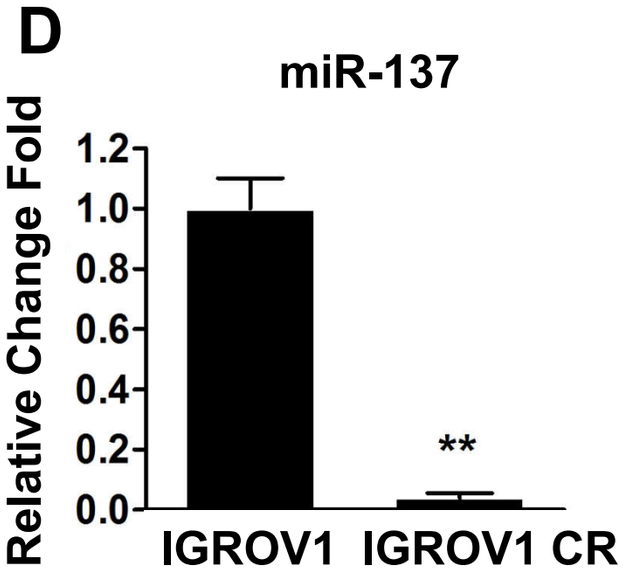
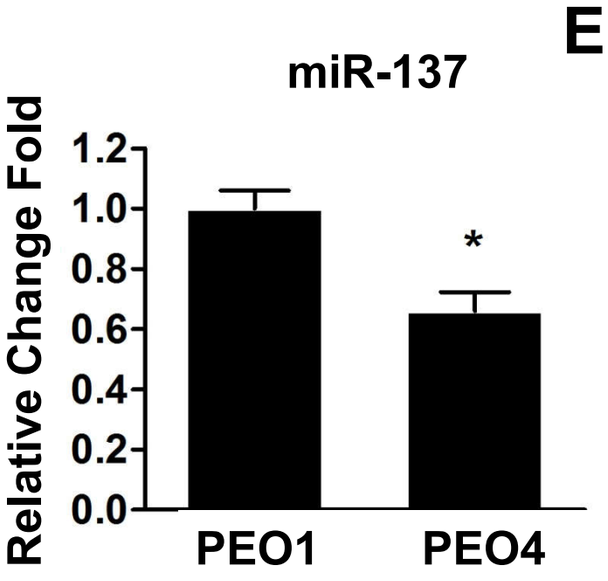
0.5
0.0
-0.5
-1.0

D


miR-137
Relative Change Fold
**
IGROV1
IGROV1 CR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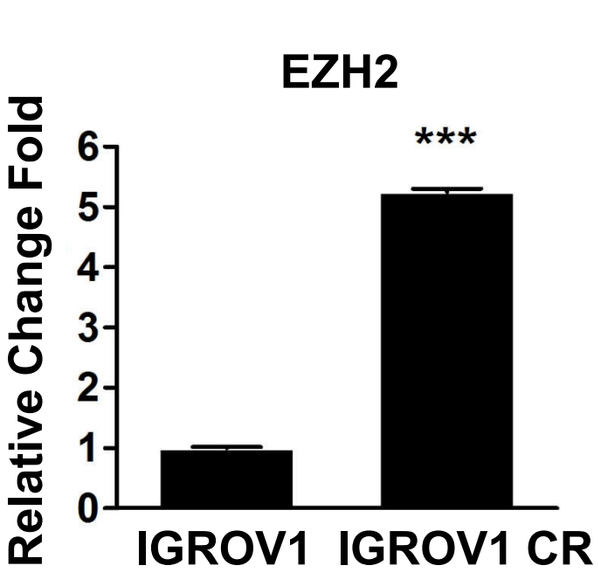
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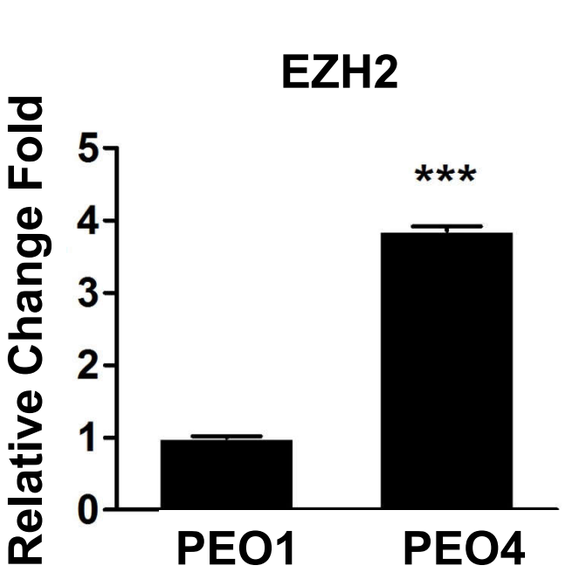

EZH2
Relative Change Fold
***
PEO1
PEO4

F

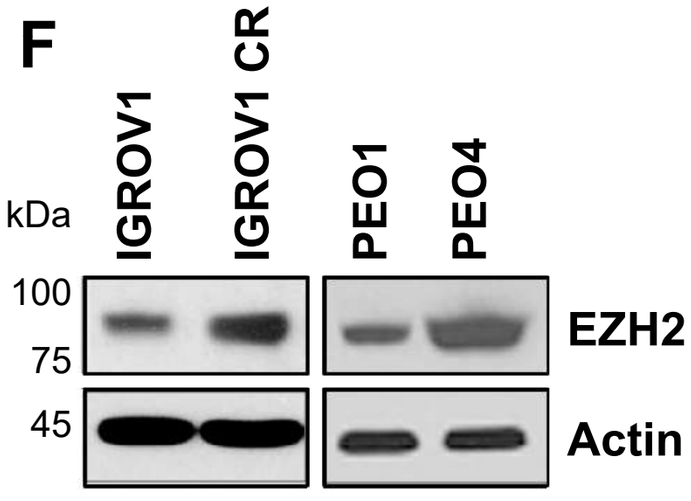
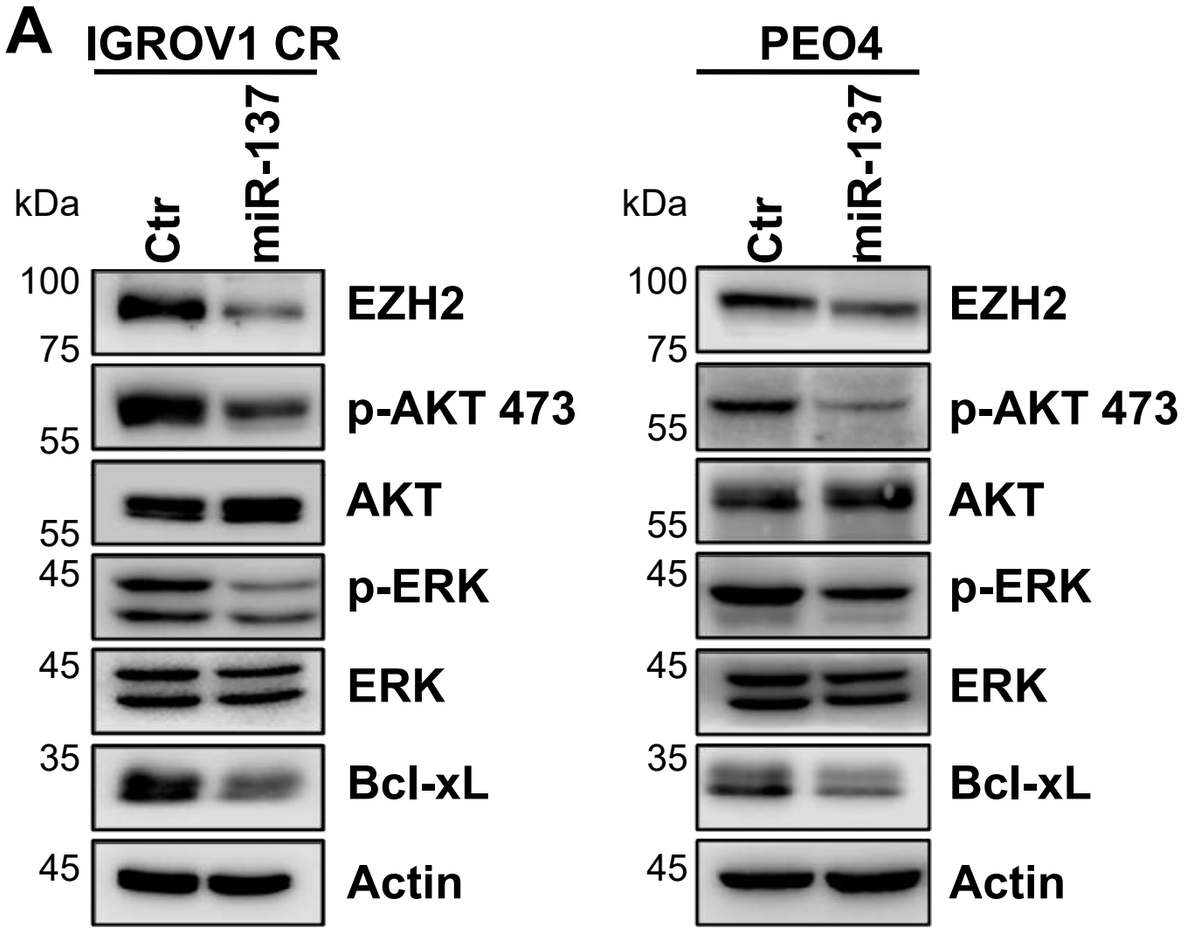
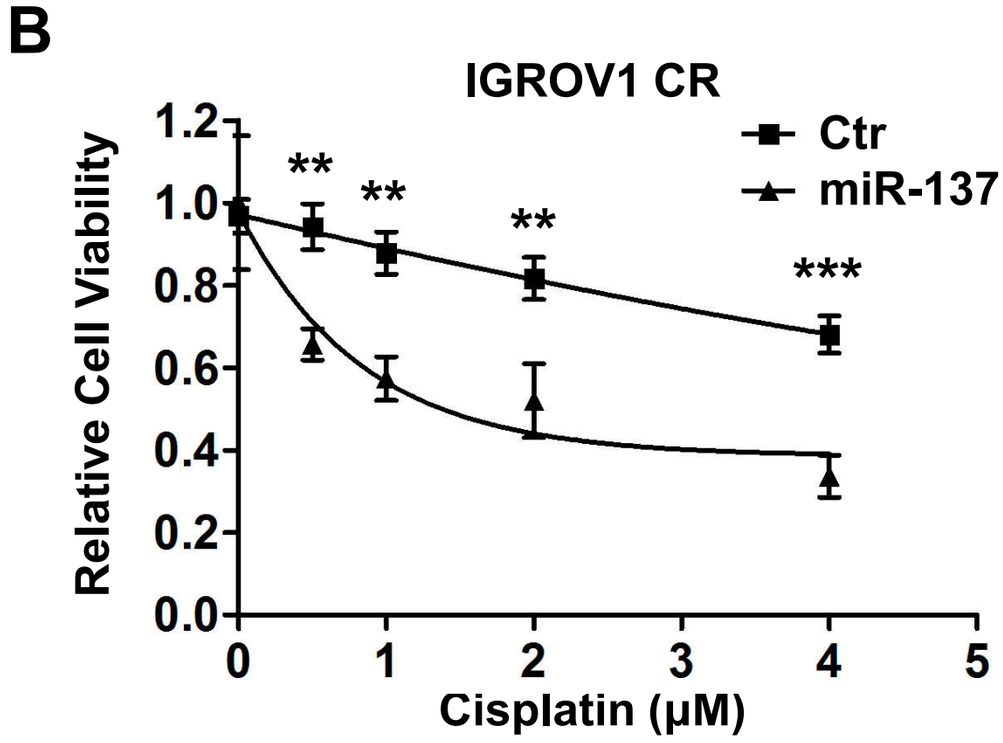
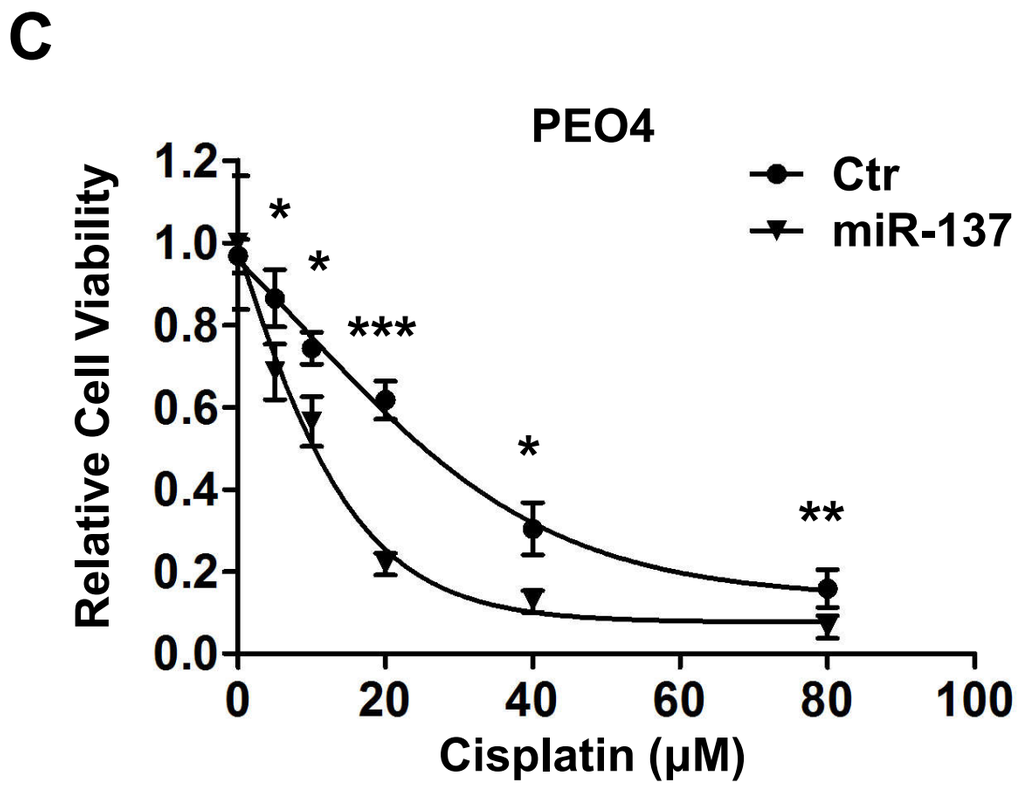
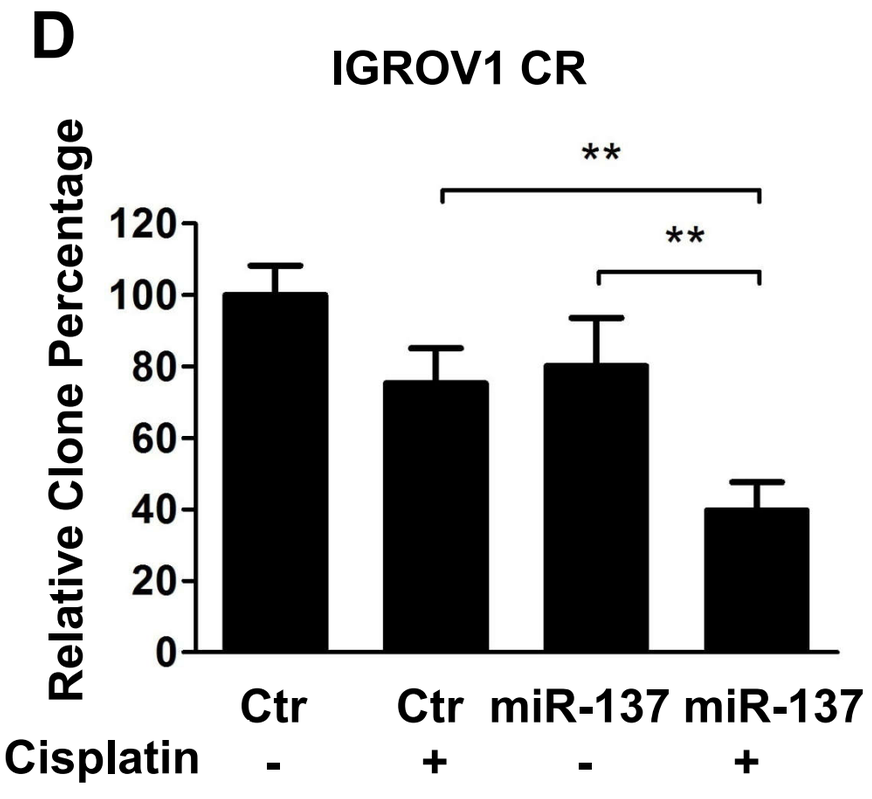

IGROV1
IGROV1 CR
PEO1
PEO4
kDa
100
75
45
EZH2
Actin

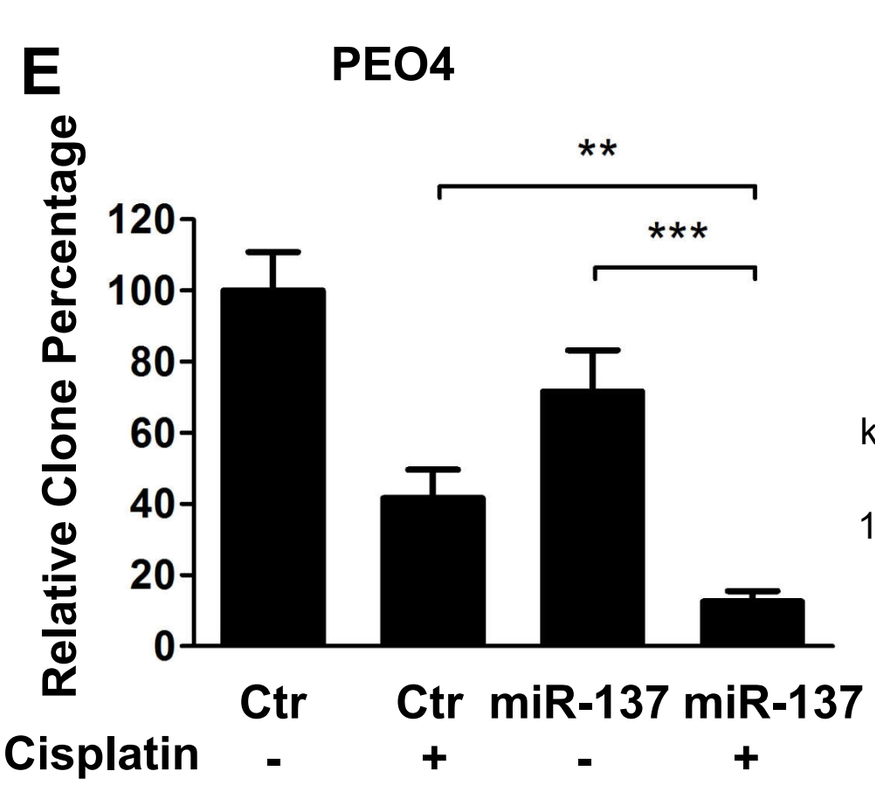
Fig. 2

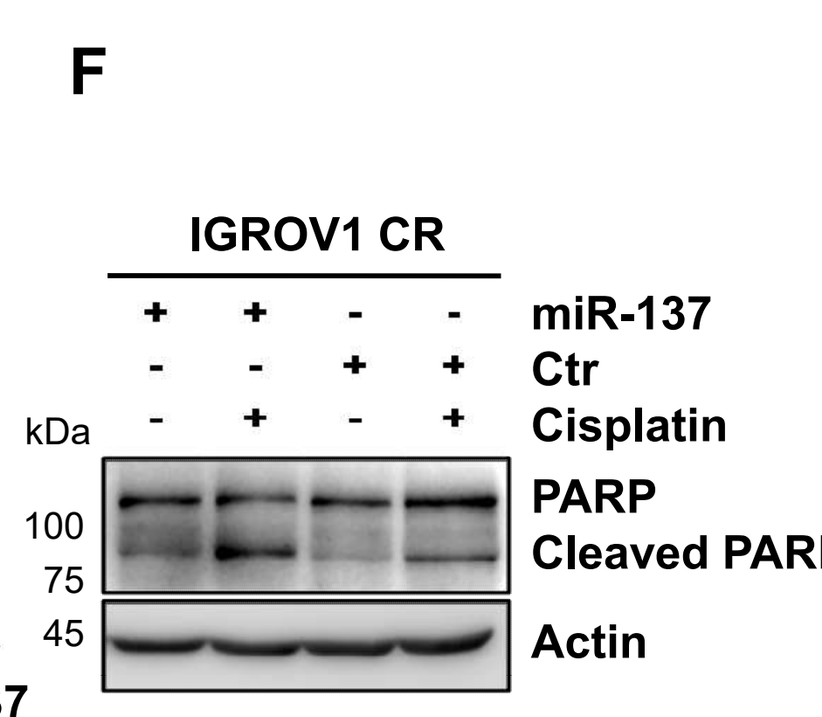

A
IGROV1 CR
PEO4
kDa
Ctr
miR-137
100
75
55
45
35
EZH2
p-AKT 473
AKT
p-ERK
ERK
Bcl-xL
Actin


B
IGROV1 CR
Ctr
miR-137
Relative Cell Viability
1.2
1.0
0.8
0.6
0.4
0.2
0.0
**
**
**
***
0
1
2
3
4
5
Cisplatin (μM)


C
PEO4
Ctr
miR-137
Relative Cell Viability
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
*
***
*
**
0
20
40
60
80
100
Cisplatin (μM)


D
IGROV1 CR
Relative Clone Percentage
120
100
80
60
40
20
0
**
**
Ctr
Ctr
miR-137
miR-137
Cisplatin
-
+
-
+


E
PEO4
Relative Clone Percentage
120
100
80
60
40
20
0
**
***
Ctr
Ctr
miR-137
miR-137
Cisplatin
-
+
-
+


F
IGROV1 CR
+
+
-
-
miR-137
-
-
+
+
Ctr
kDa
-
+
-
+
Cisplatin
100
75
45
PARP
Cleaved PARP
Actin

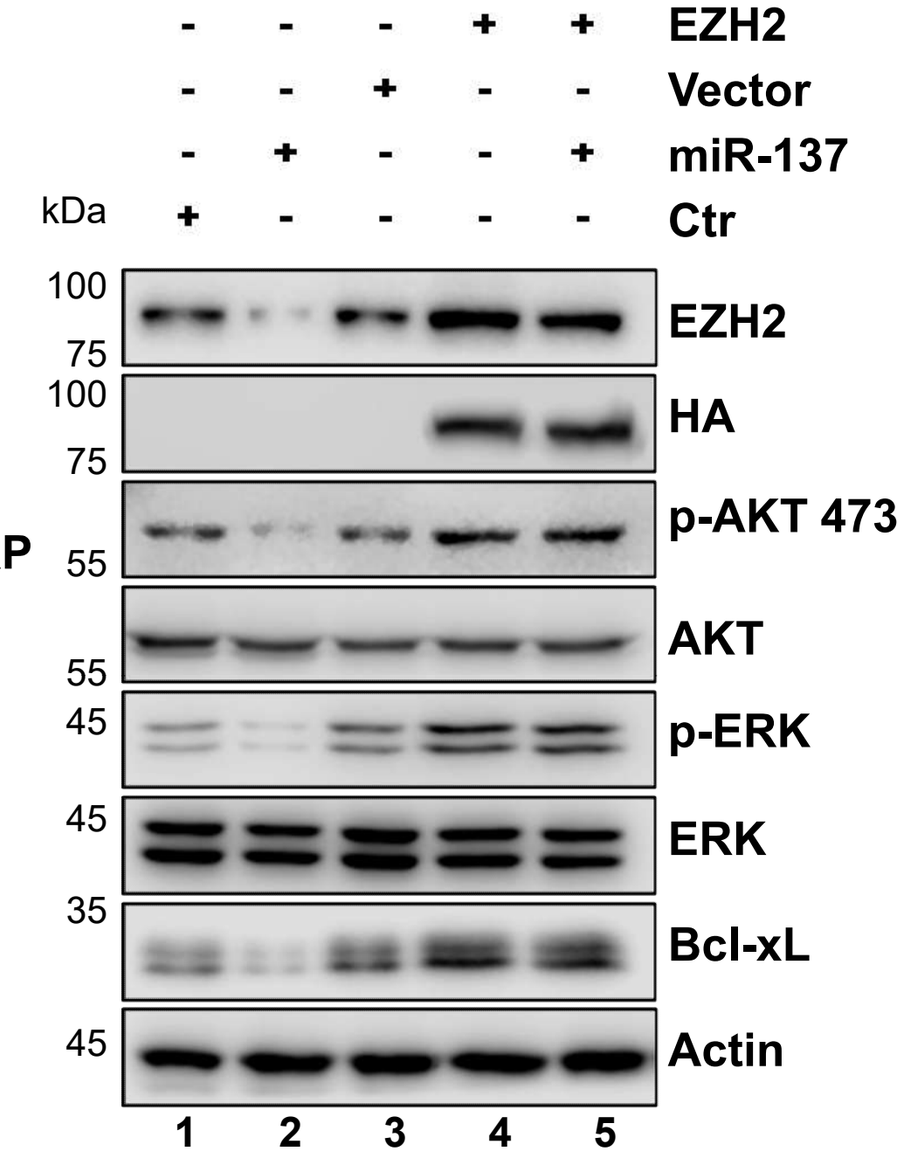
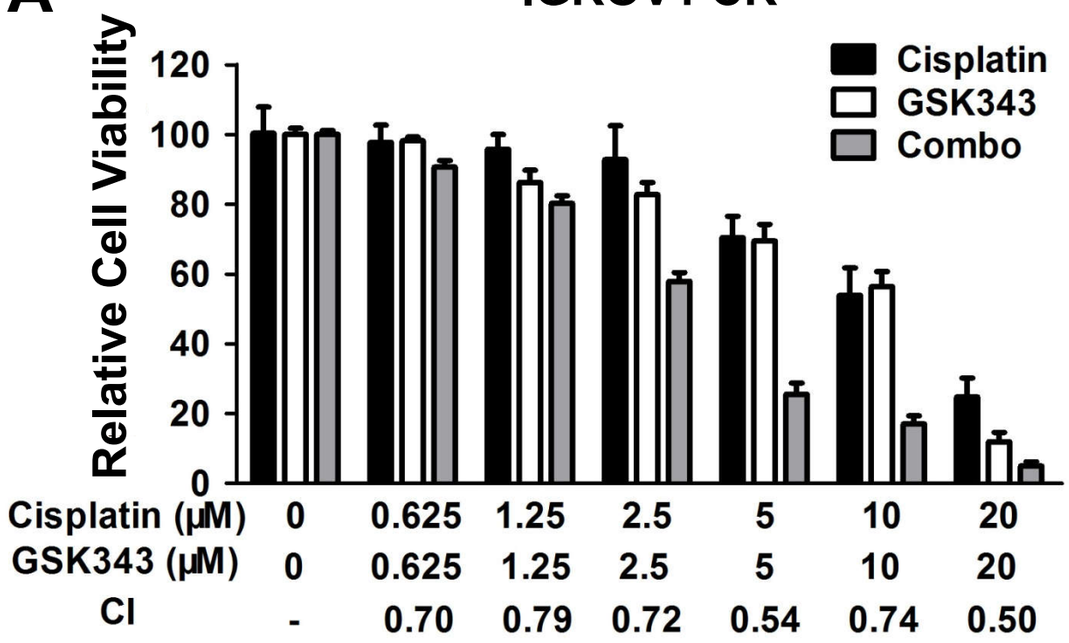
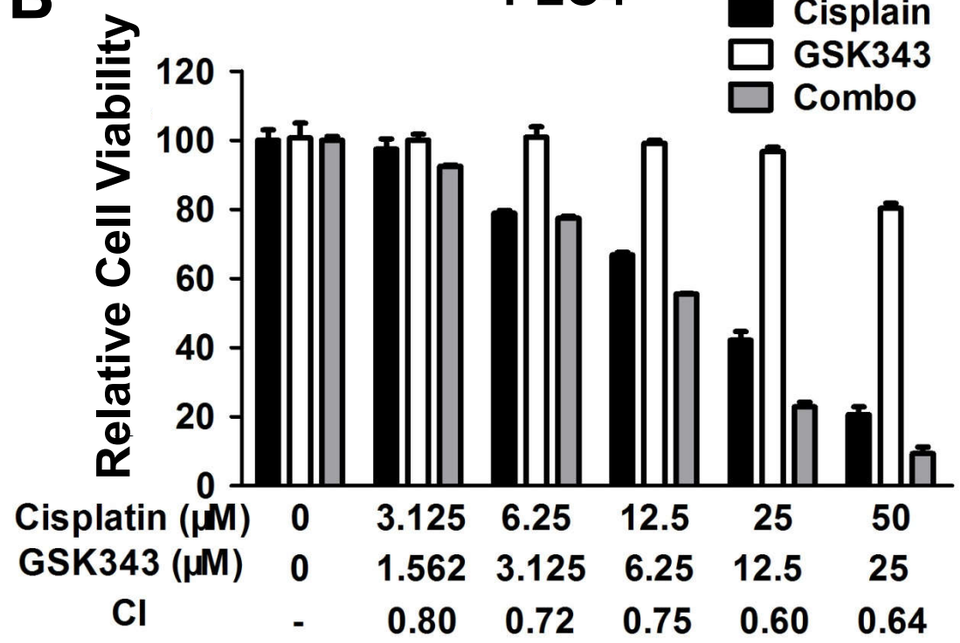

G
IGROV1 CR
-
-
-
+
+
EZH2
-
-
+
-
-
Vector
-
+
-
-
+
miR-137
kDa
+
-
-
-
-
Ctr
100
75
EZH2
100
75
HA
p-AKT 473
55
AKT
55
45
p-ERK
45
ERK
35
Bcl-xL
45
Actin
1
2
3
4
5

Fig. 3

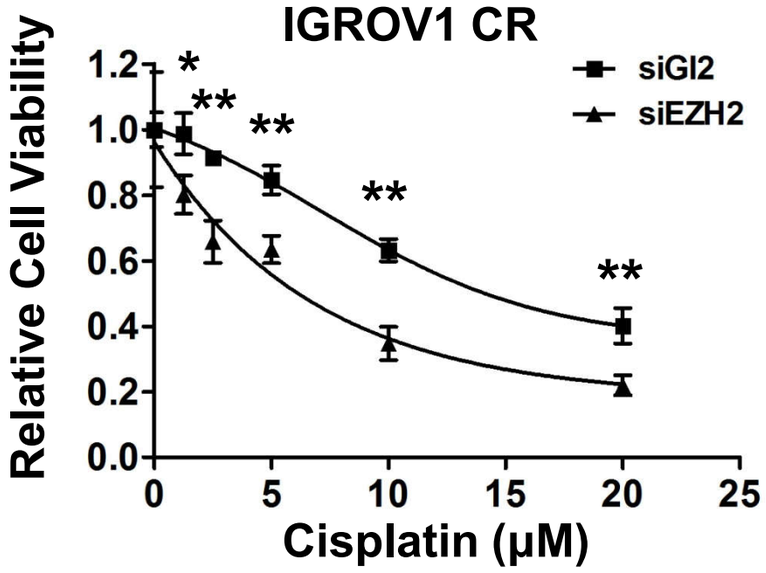
A



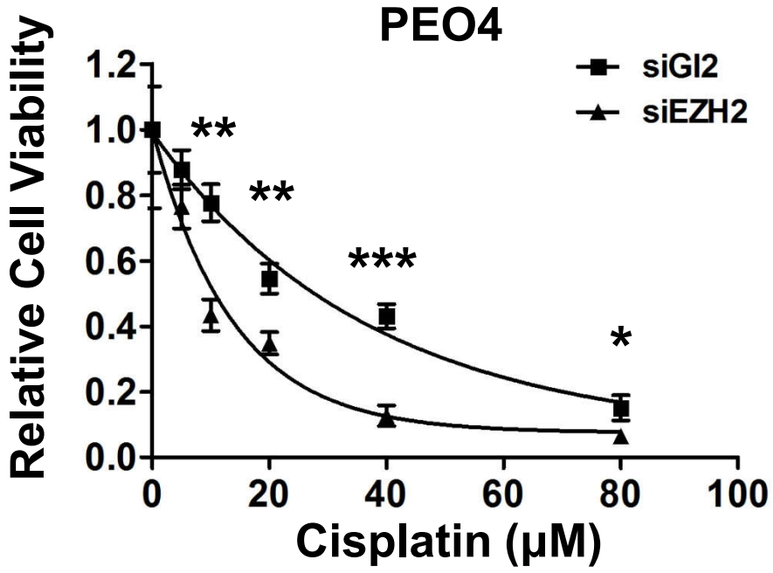
B



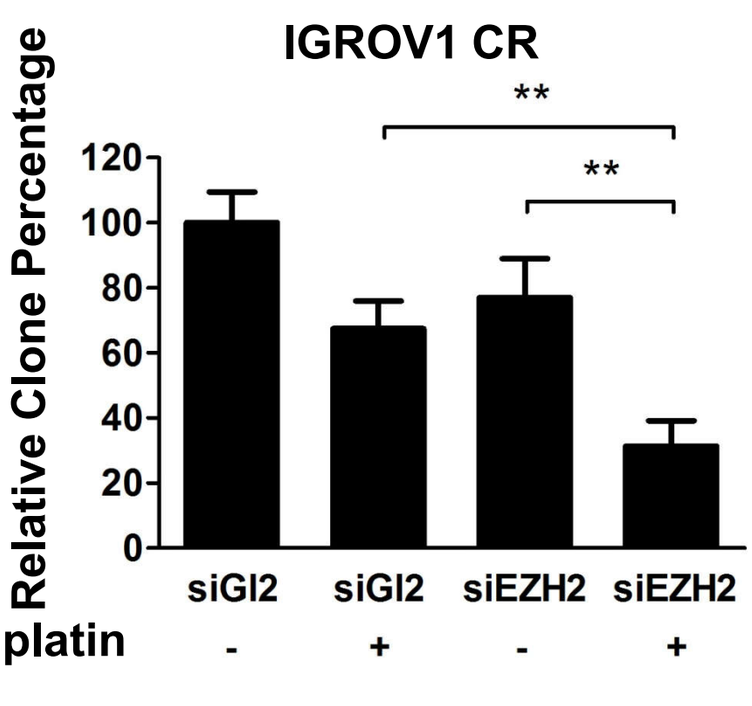
C



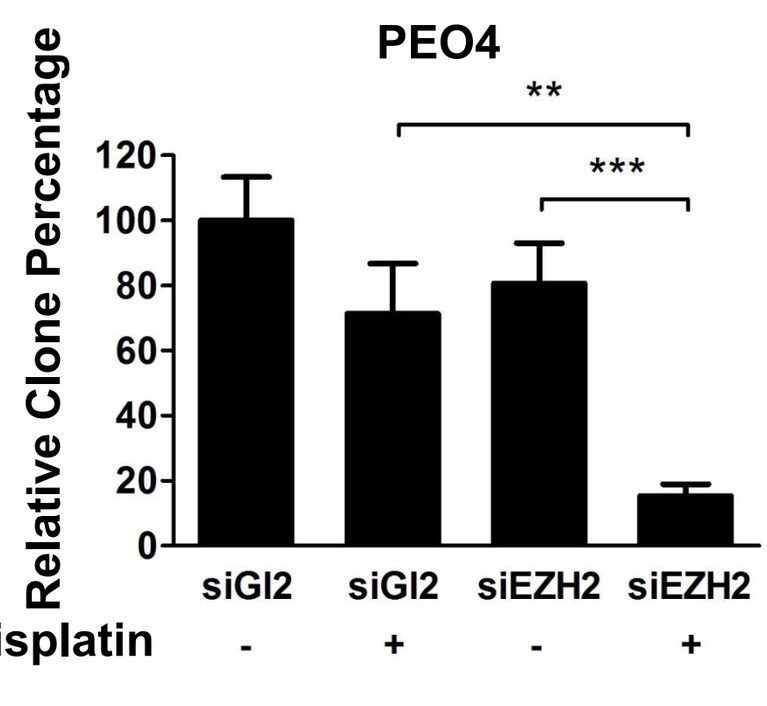
D



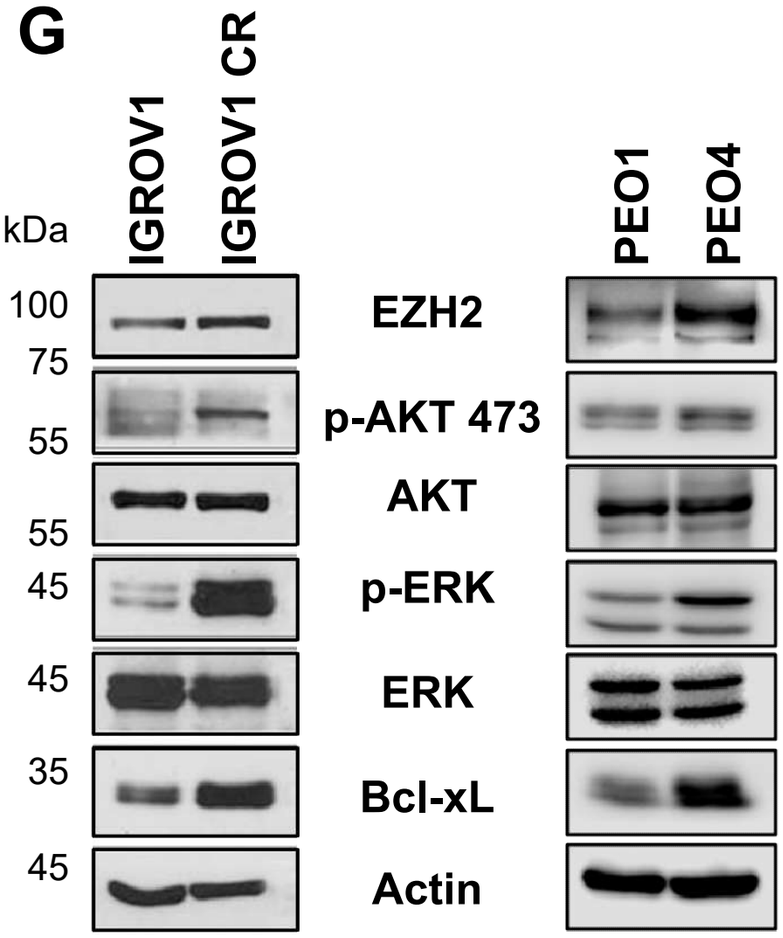
E



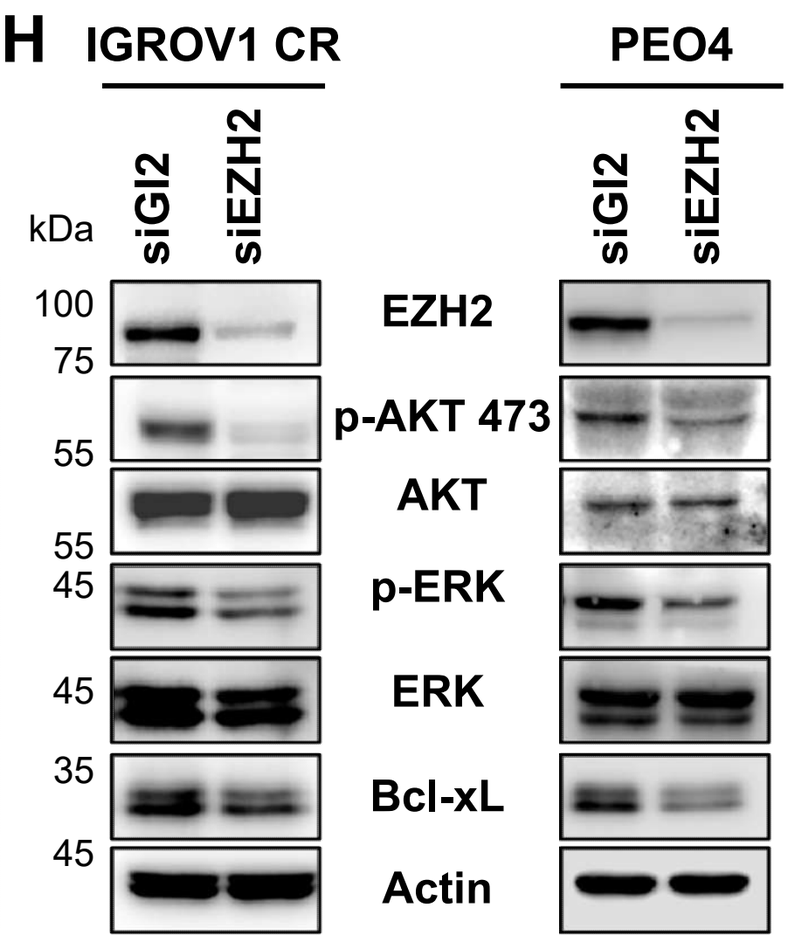
F



G



H



I

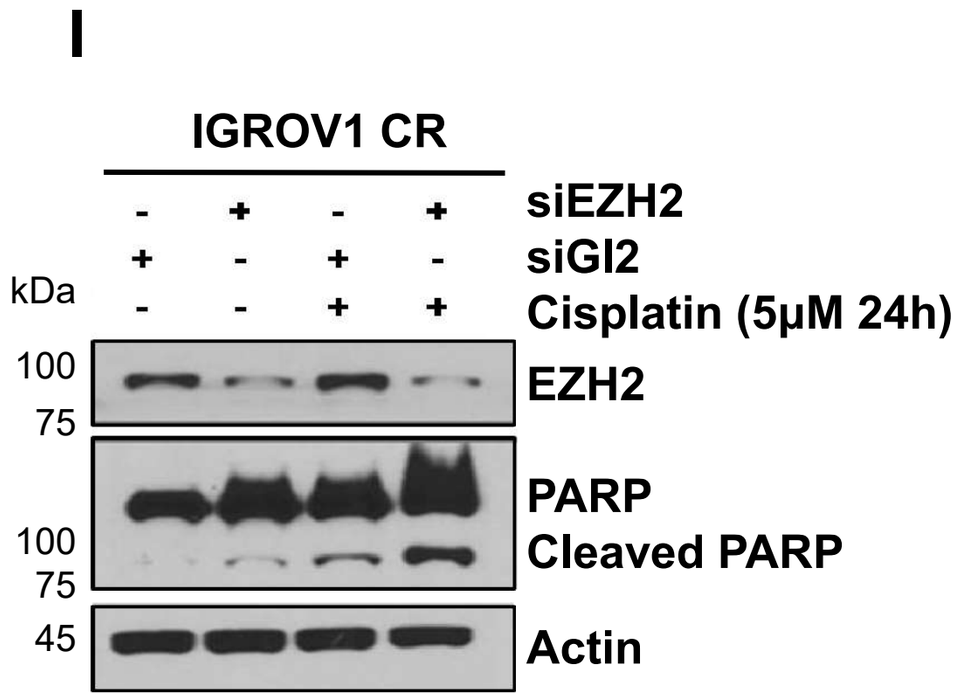
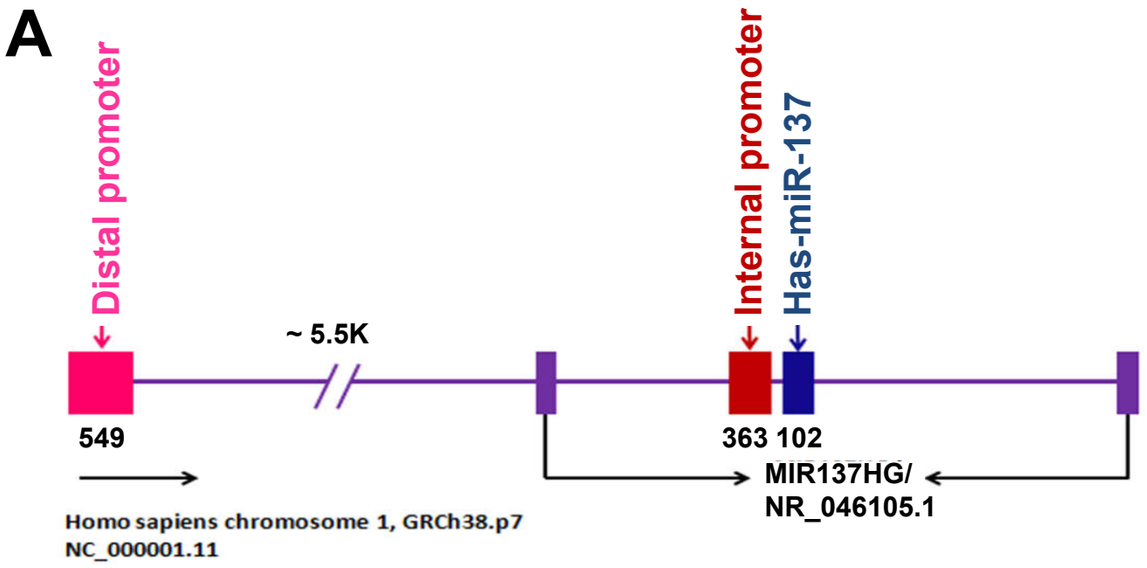
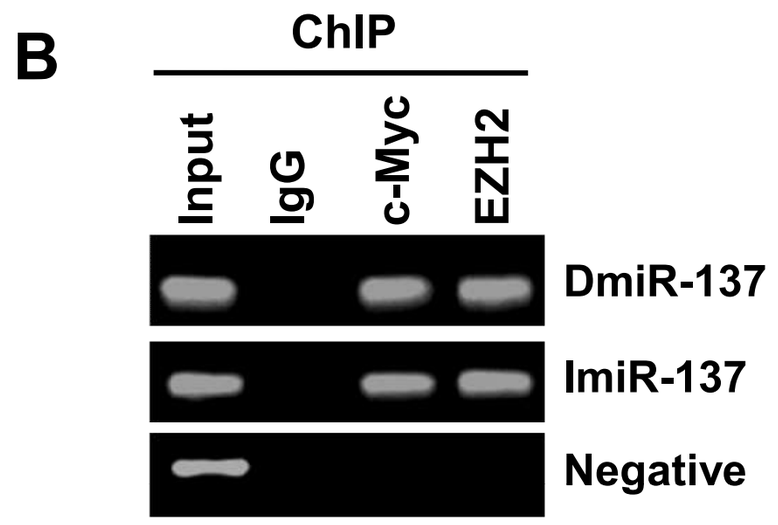
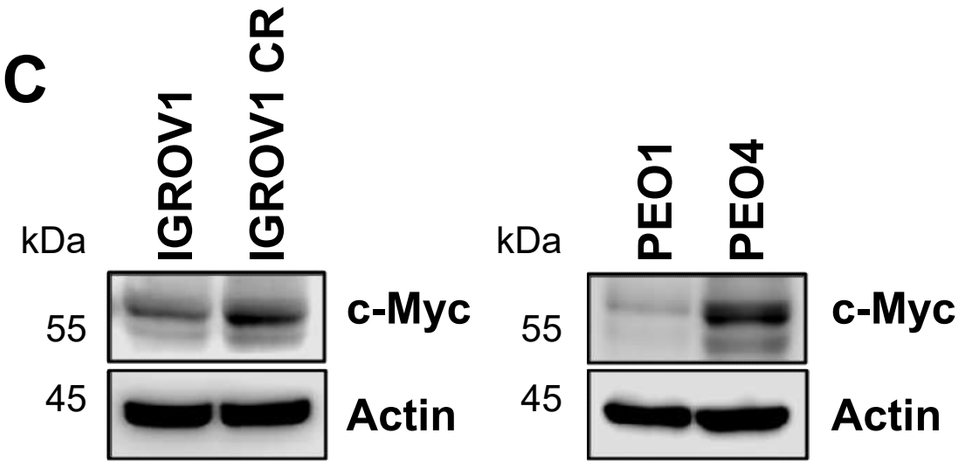
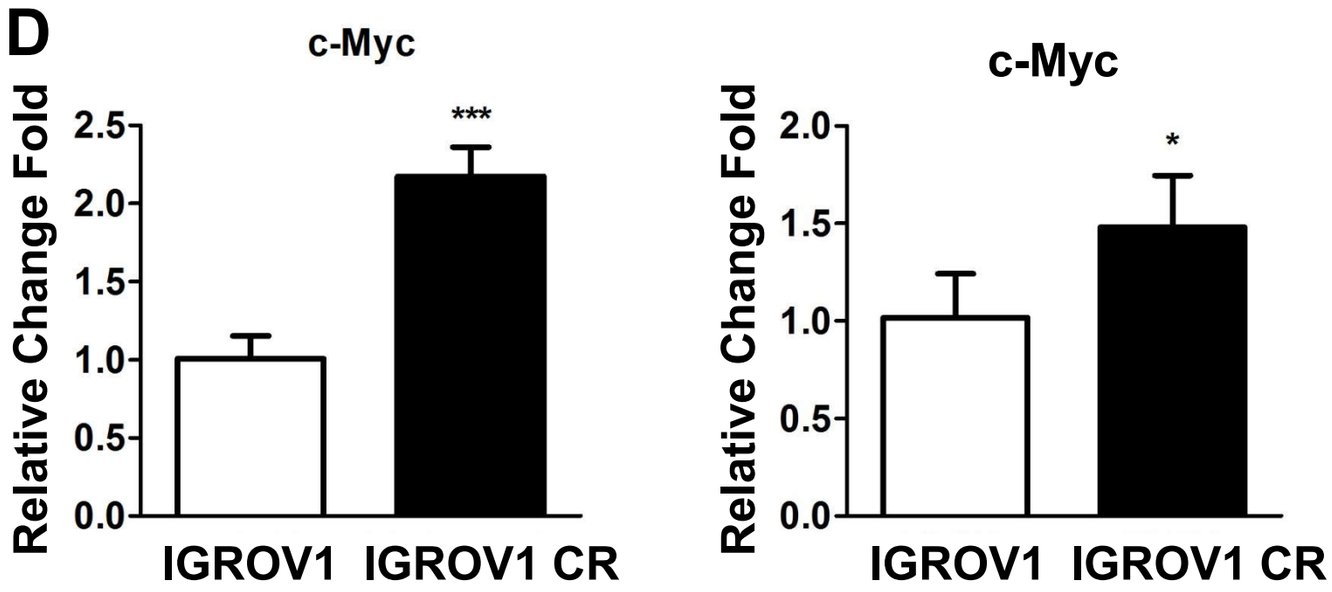


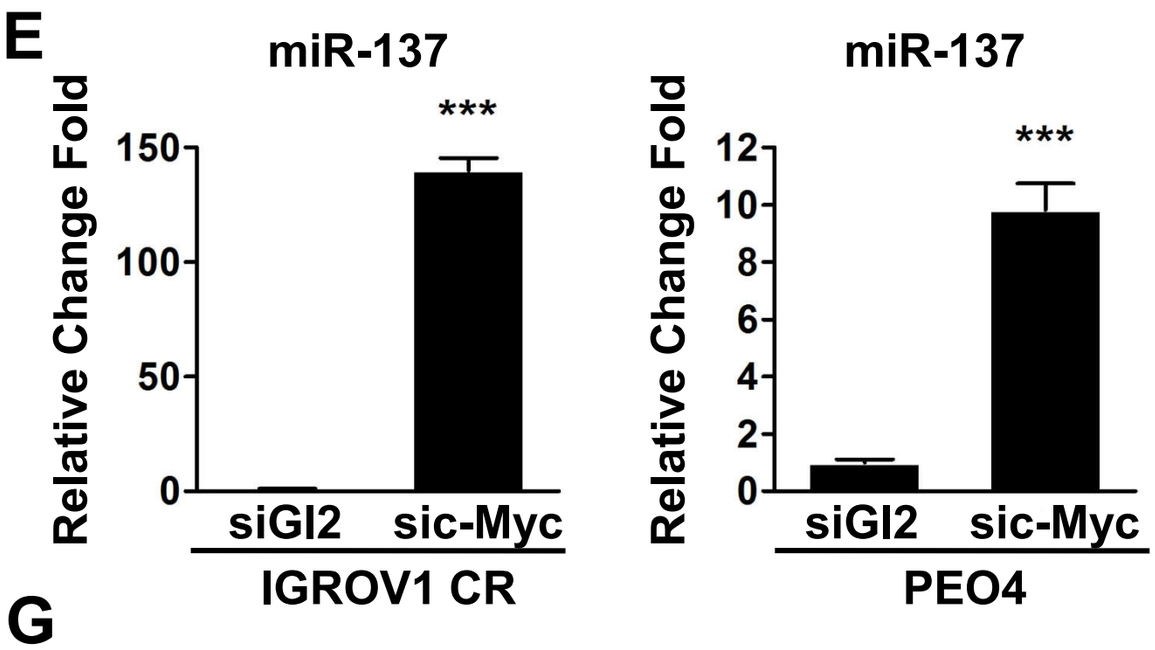
Fig. 4

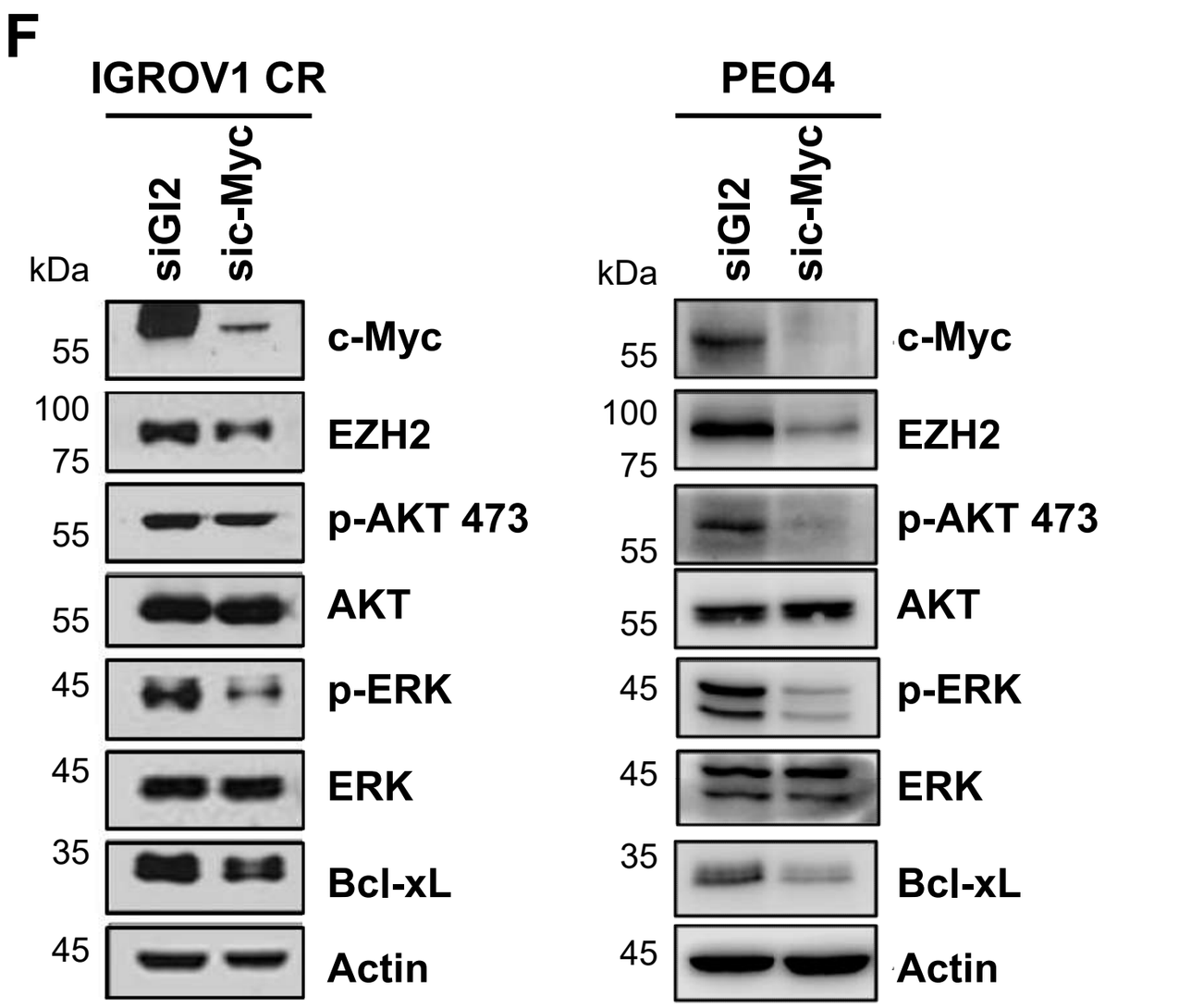

A
Distal promoter
~ 5.5K
Internal promoter
Has-miR-137
549
363 102
MIR137HG/
NR_046105.1
Homo sapiens chromosome 1, GRCh38.p7
NC_000001.11

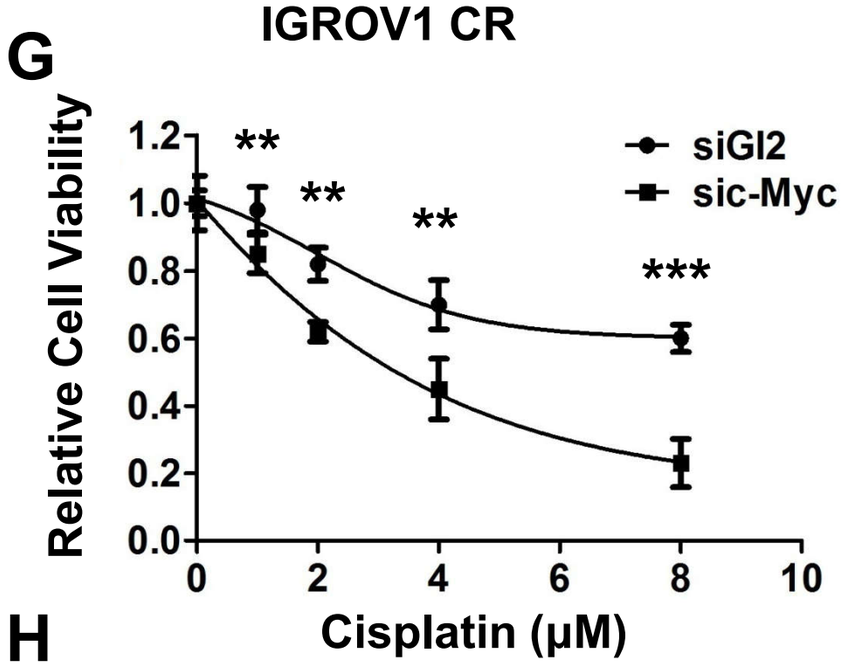

B
ChIP
Input
IgG
c-Myc
EZH2
DmiR-137
ImiR-137
Negative

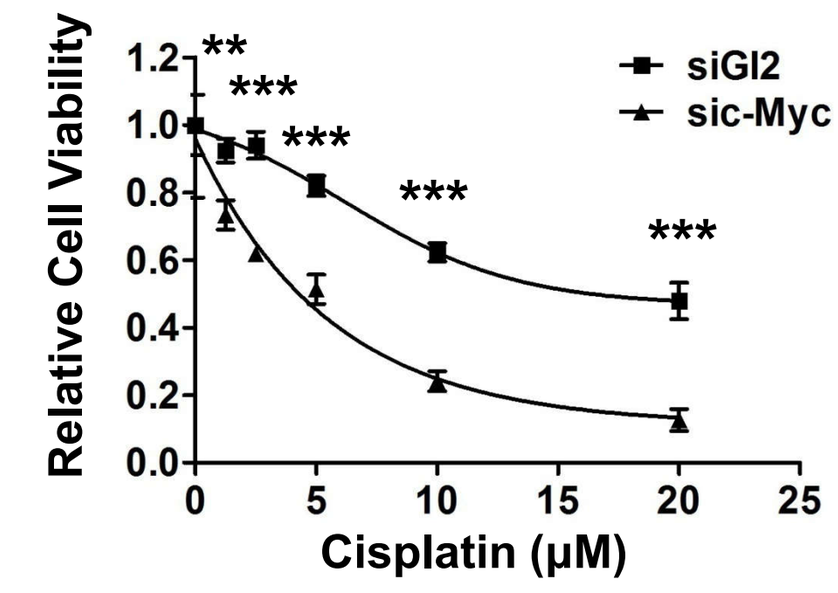

C
IGROV1
IGROV1 CR
kDa
55
45
c-Myc
Actin
PEO1
PEO4


D
c-Myc
Relative Change Fold
***
*
IGROV1
IGROV1 CR


E
miR-137
Relative Change Fold
***
siGl2
sic-Myc
IGROV1 CR
PEO4


F
IGROV1 CR
PEO4
siGl2
sic-Myc
kDa
c-Myc
EZH2
p-AKT 473
AKT
p-ERK
ERK
Bcl-xL
Actin


G
Relative Cell Viability
siGl2
sic-Myc
Cisplatin (μM)


H
Relative Cell Viability
siGl2
sic-Myc
Cisplatin (μM)

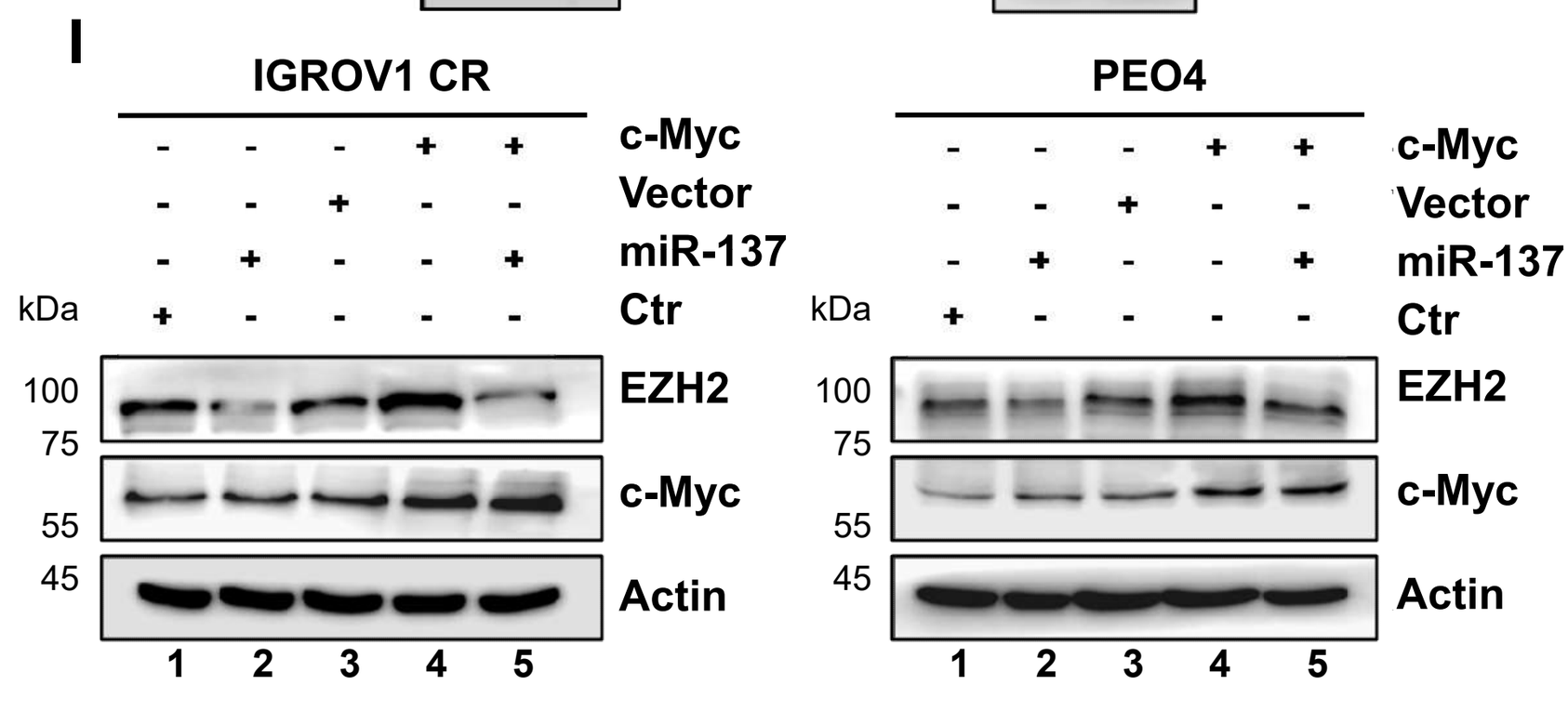
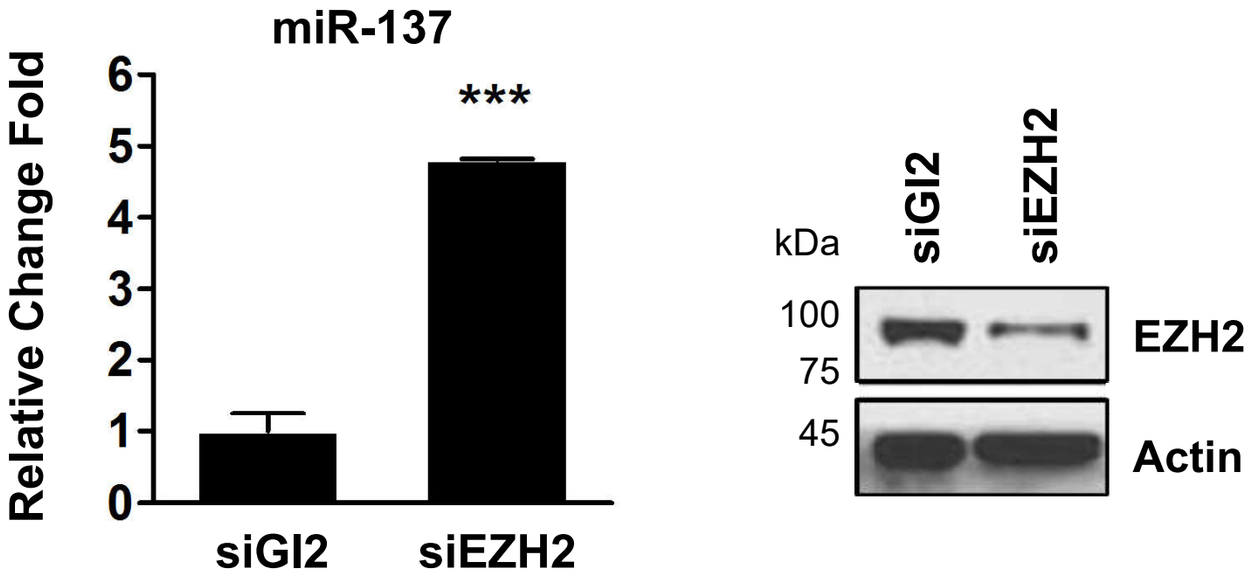
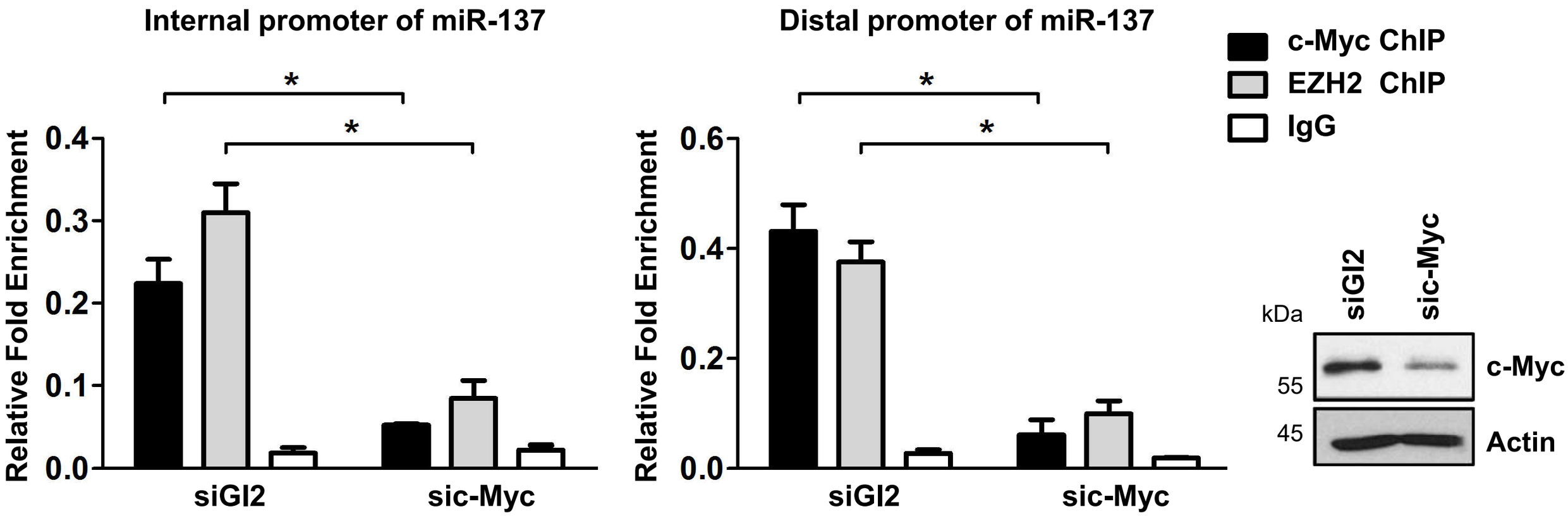

I
IGROV1 CR
PEO4
c-Myc
Vector
miR-137
Ctr
kDa
EZH2
c-Myc
Actin
1 2 3 4 5

Fig. 5

A


miR-137
Relative Change Fold
6
5
4
3
2
1
0
***
siGl2
siEZH2
kDa
siGl2
siEZH2
100
75
EZH2
45
Actin

B


Internal promoter of miR-137
Distal promoter of miR-137
c-Myc ChIP
EZH2 ChIP
IgG
Relative Fold Enrichment
0.4
0.3
0.2
0.1
0.0
0.6
*
siGl2
sic-Myc
kDa
siGl2
sic-Myc
55
c-Myc
45
Actin

C

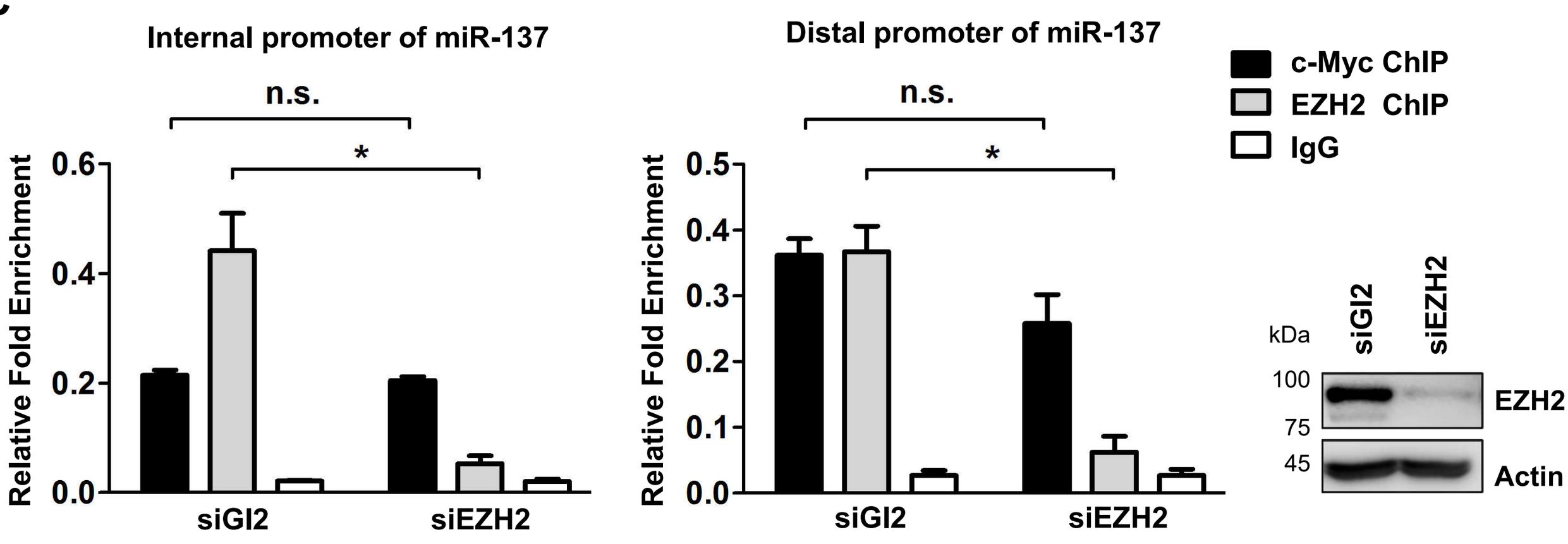
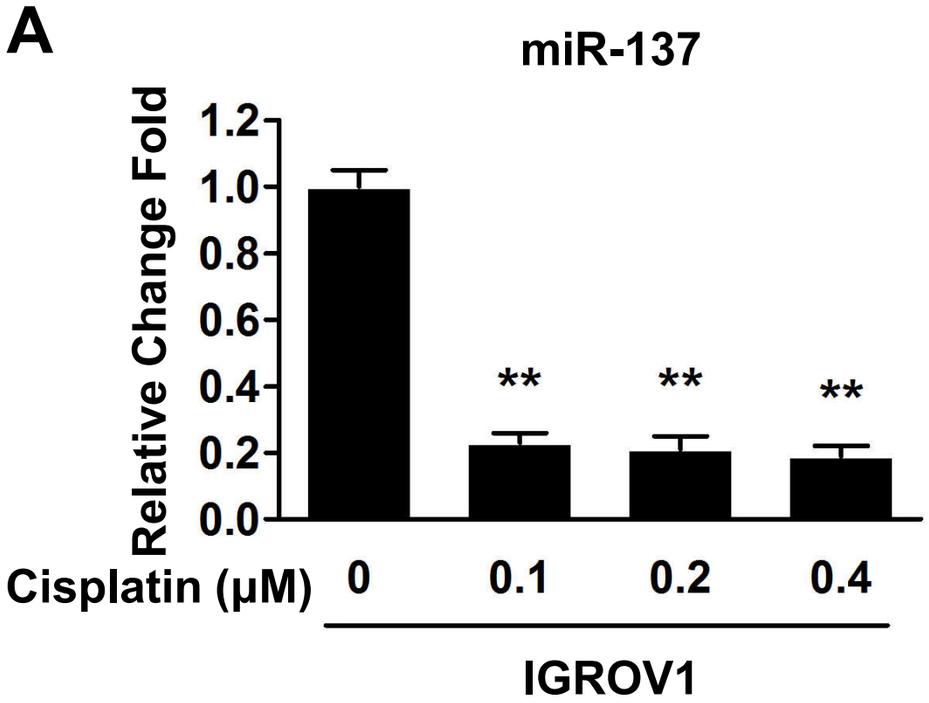
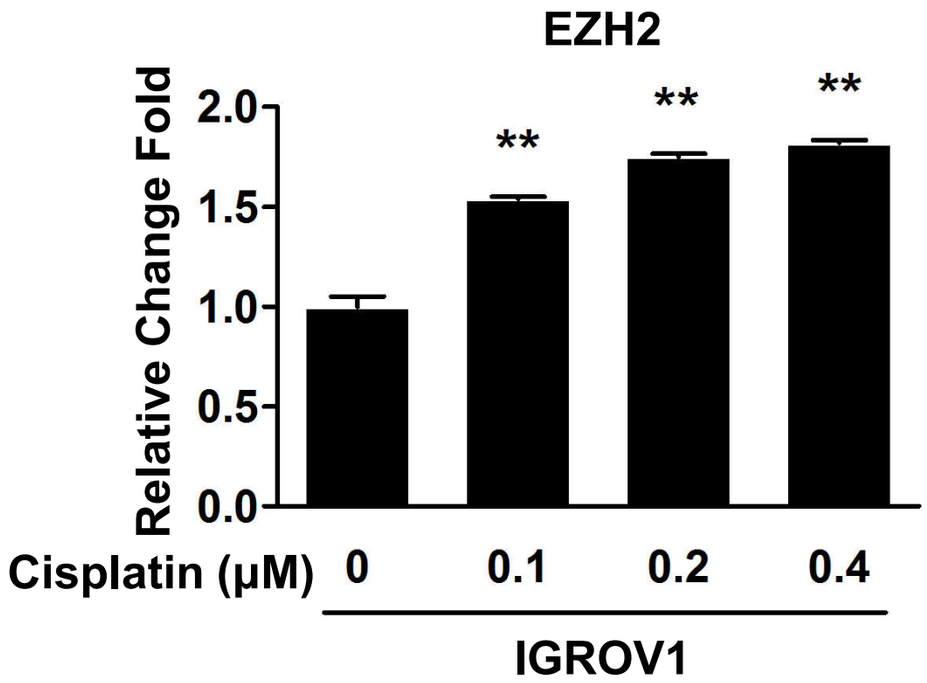

Internal promoter of miR-137
Distal promoter of miR-137
n.s.
*
c-Myc ChIP
EZH2 ChIP
IgG
Relative Fold Enrichment
0.6
0.4
0.2
0.0
0.5
0.3
0.1
siGl2
siEZH2
kDa
siGl2
siEZH2
100
75
EZH2
45
Actin

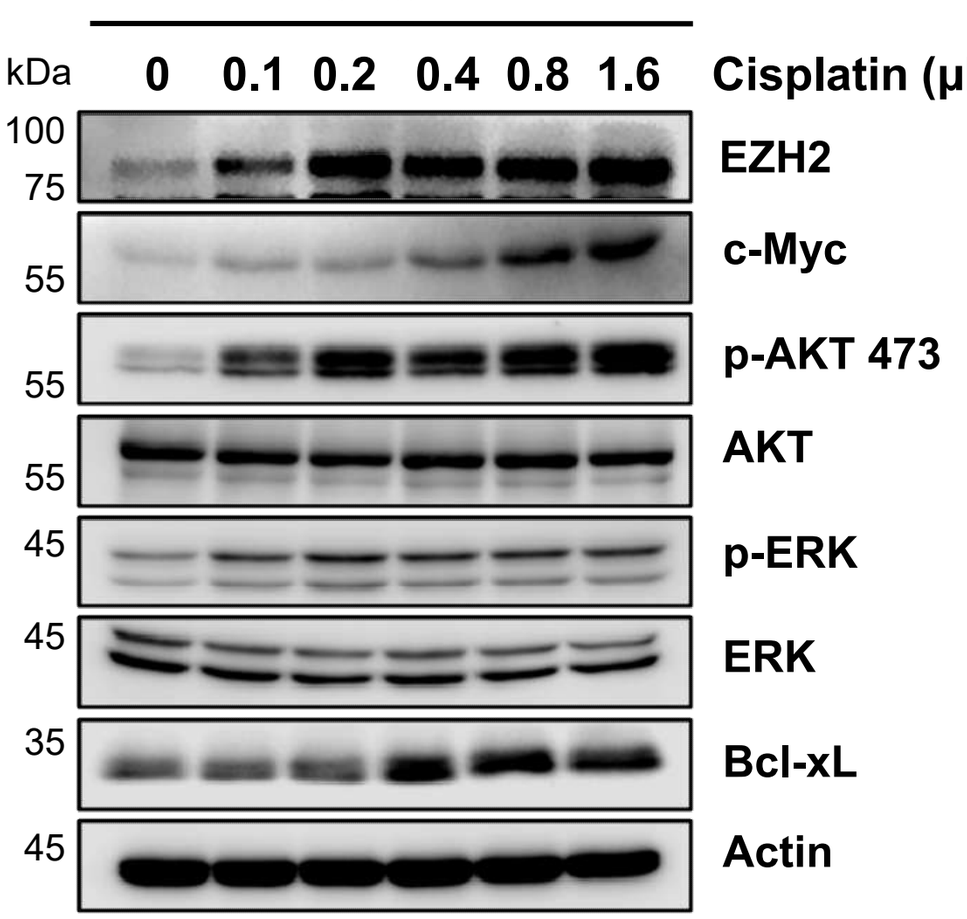
Fig. 6

A

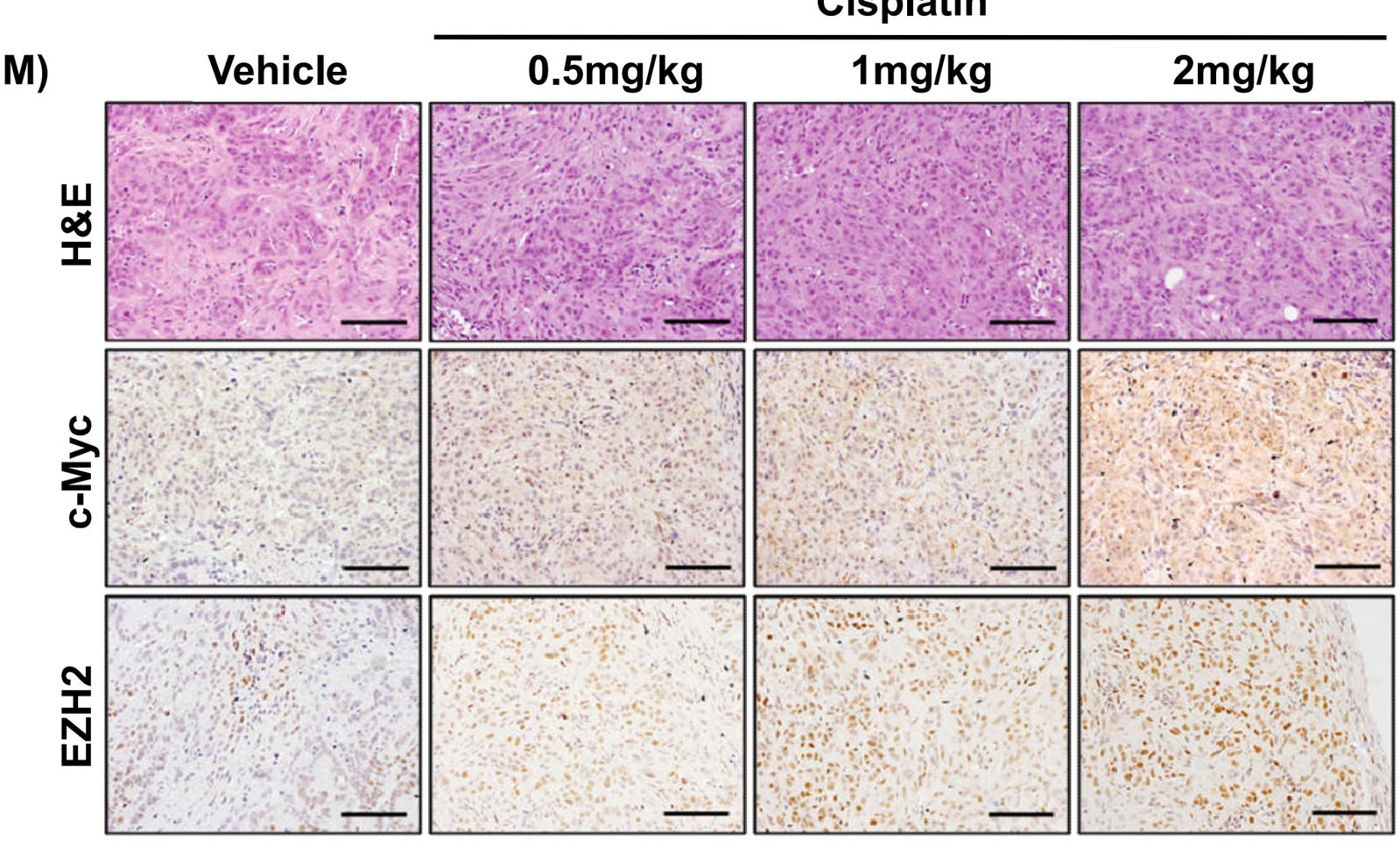

miR-137
Relative Change Fold
1.2
1.0
0.8
0.6
0.4
0.2
0.0
**
**
**
Cisplatin (μM)
0
0.1
0.2
0.4
IGROV1


EZH2
Relative Change Fold
2.0
1.5
1.0
0.5
0.0
**
**
**
Cisplatin (μM)
0
0.1
0.2
0.4
IGROV1

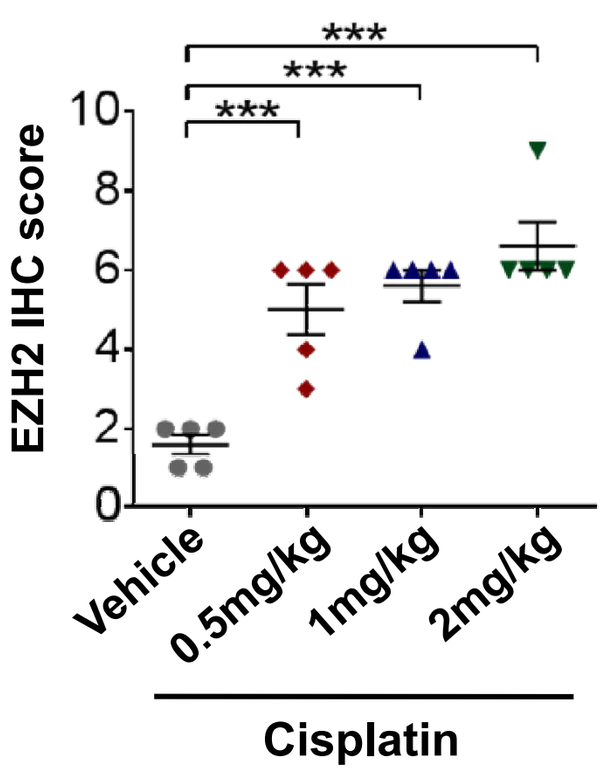
B

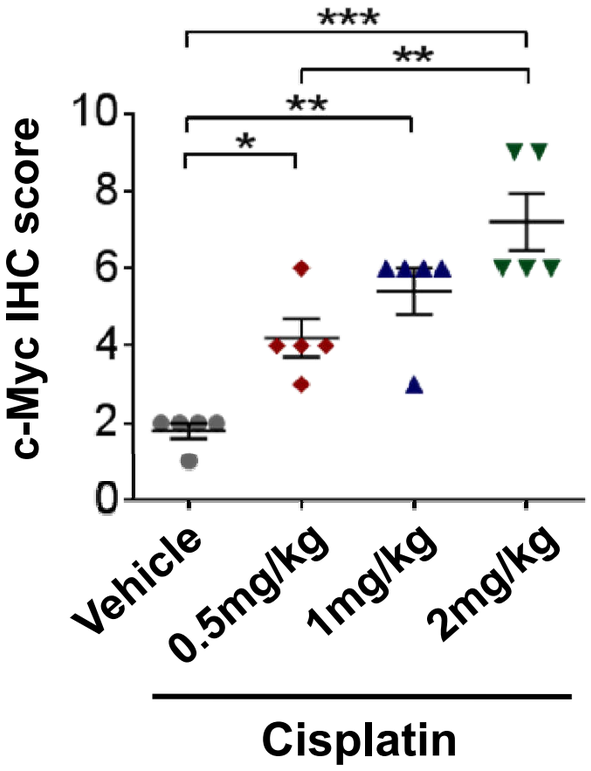

IGROV1
kDa
0
0.1
0.2
0.4
0.8
1.6
Cisplatin (μM)
100
75
EZH2
55
c-Myc
55
p-AKT 473
55
AKT
45
p-ERK
45
ERK
35
Bcl-xL
45
Actin

C


IGROV1 Xenograft
Cisplatin
Vehicle
0.5mg/kg
1mg/kg
2mg/kg
H&E
c-Myc
EZH2

D


EZH2 IHC score
10
8
6
4
2
0
***
***
***
Vehicle
0.5mg/kg
1mg/kg
2mg/kg
Cisplatin


c-Myc IHC score
10
8
6
4
2
0
***
**
**
*
Vehicle
0.5mg/kg
1mg/kg
2mg/kg
Cisplatin

E

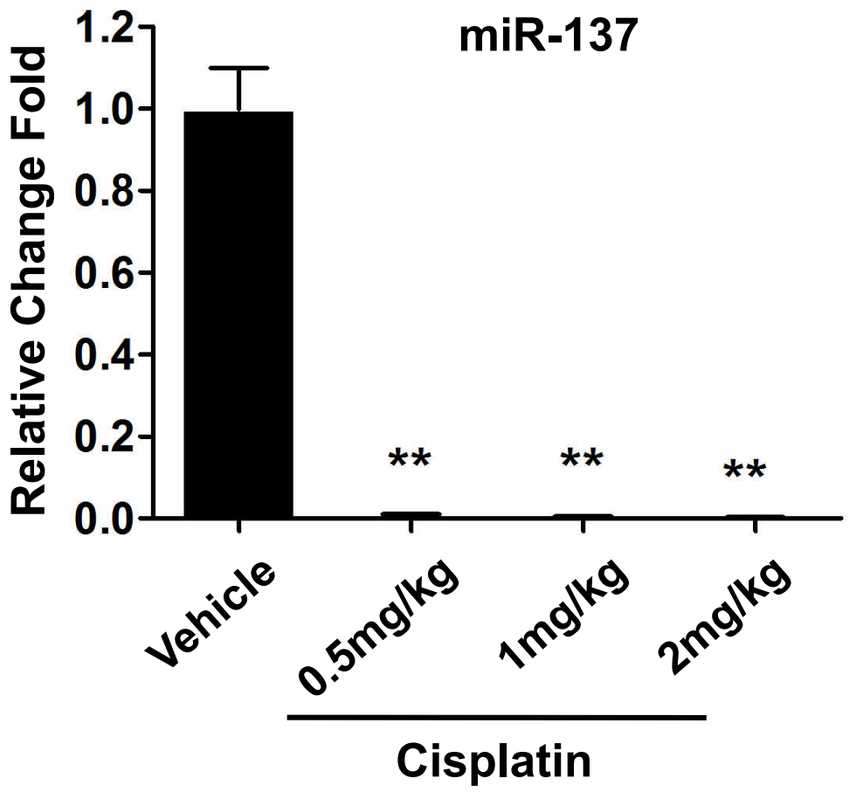
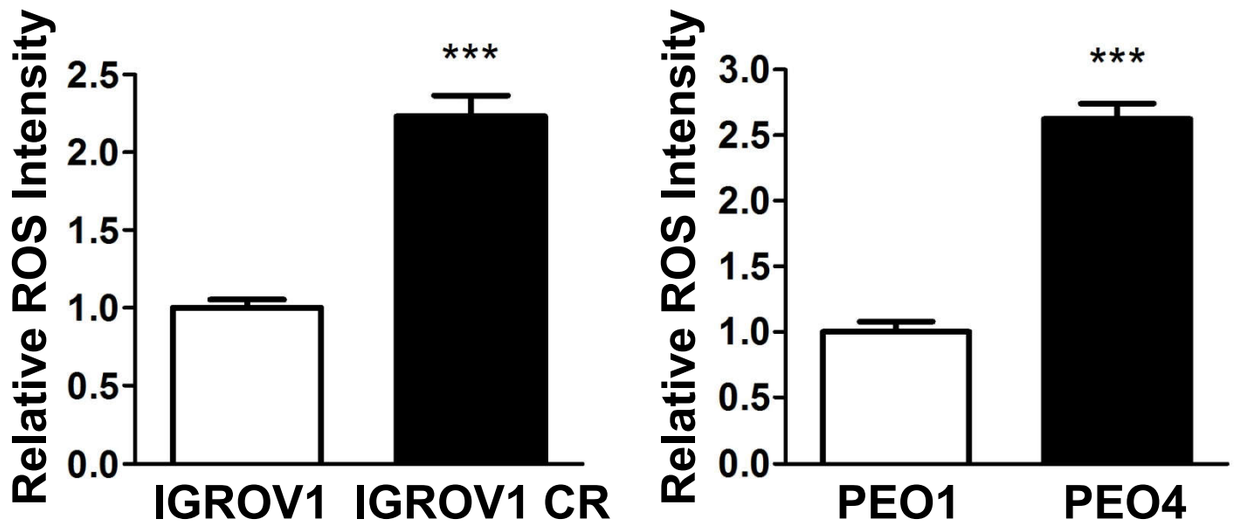
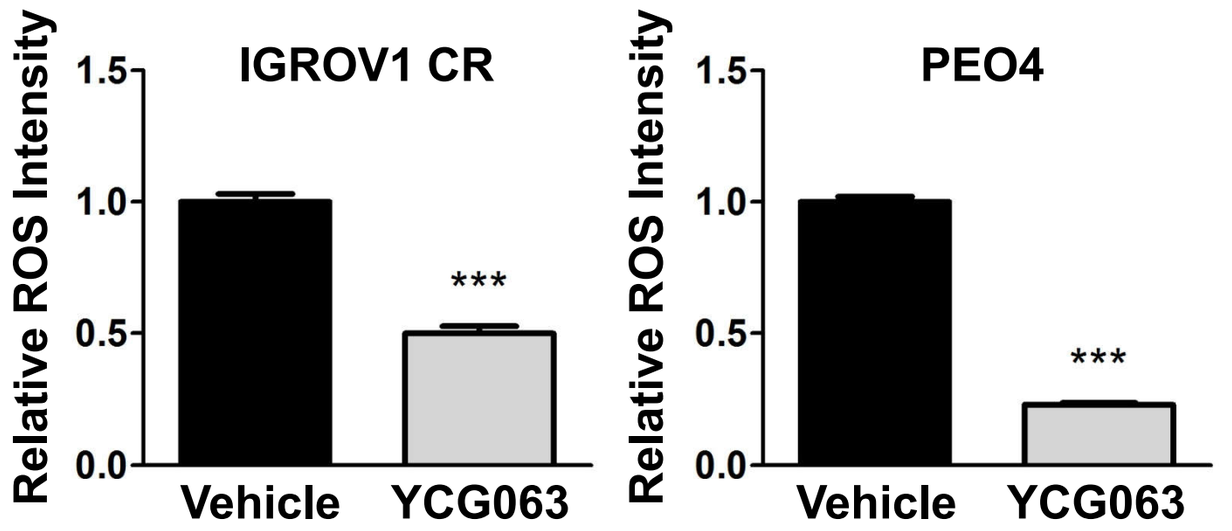

miR-137
Relative Change Fold
1.2
1.0
0.8
0.6
0.4
0.2
0.0
**
**
**
Vehicle
0.5mg/kg
1mg/kg
2mg/kg
Cisplatin

Fig. 7

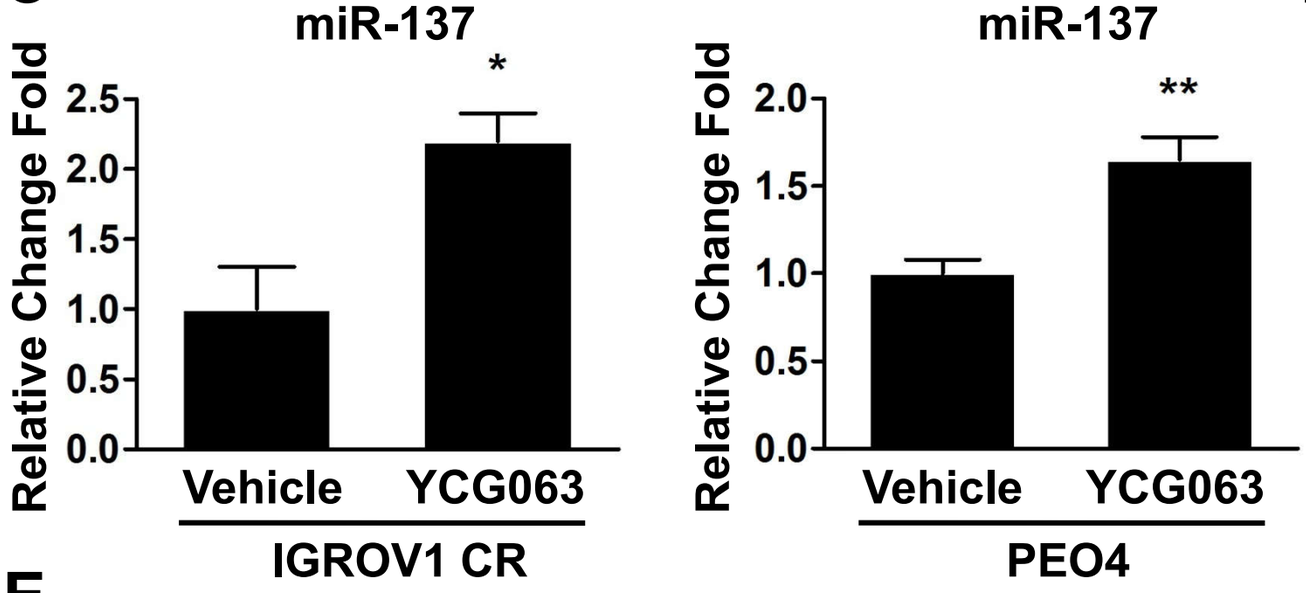
A



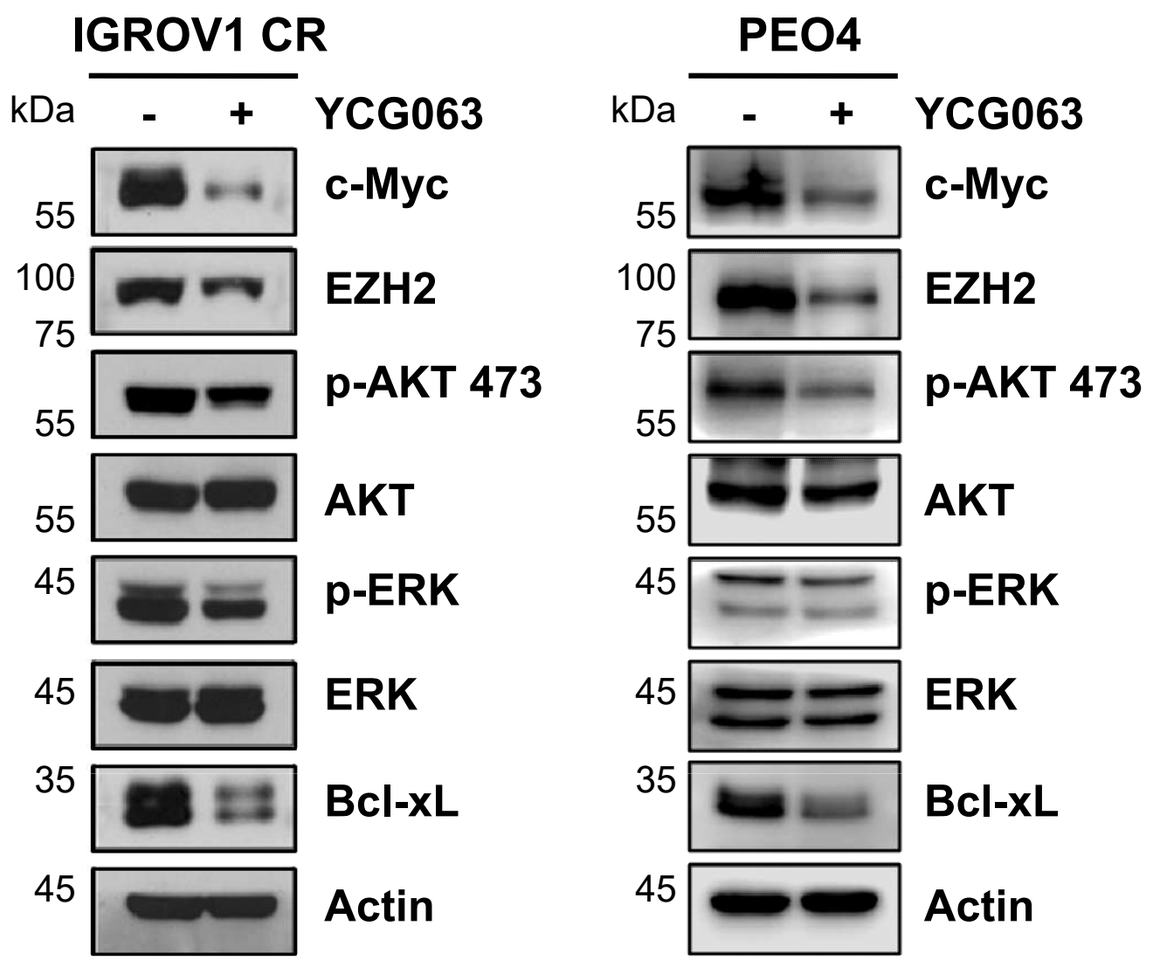
B



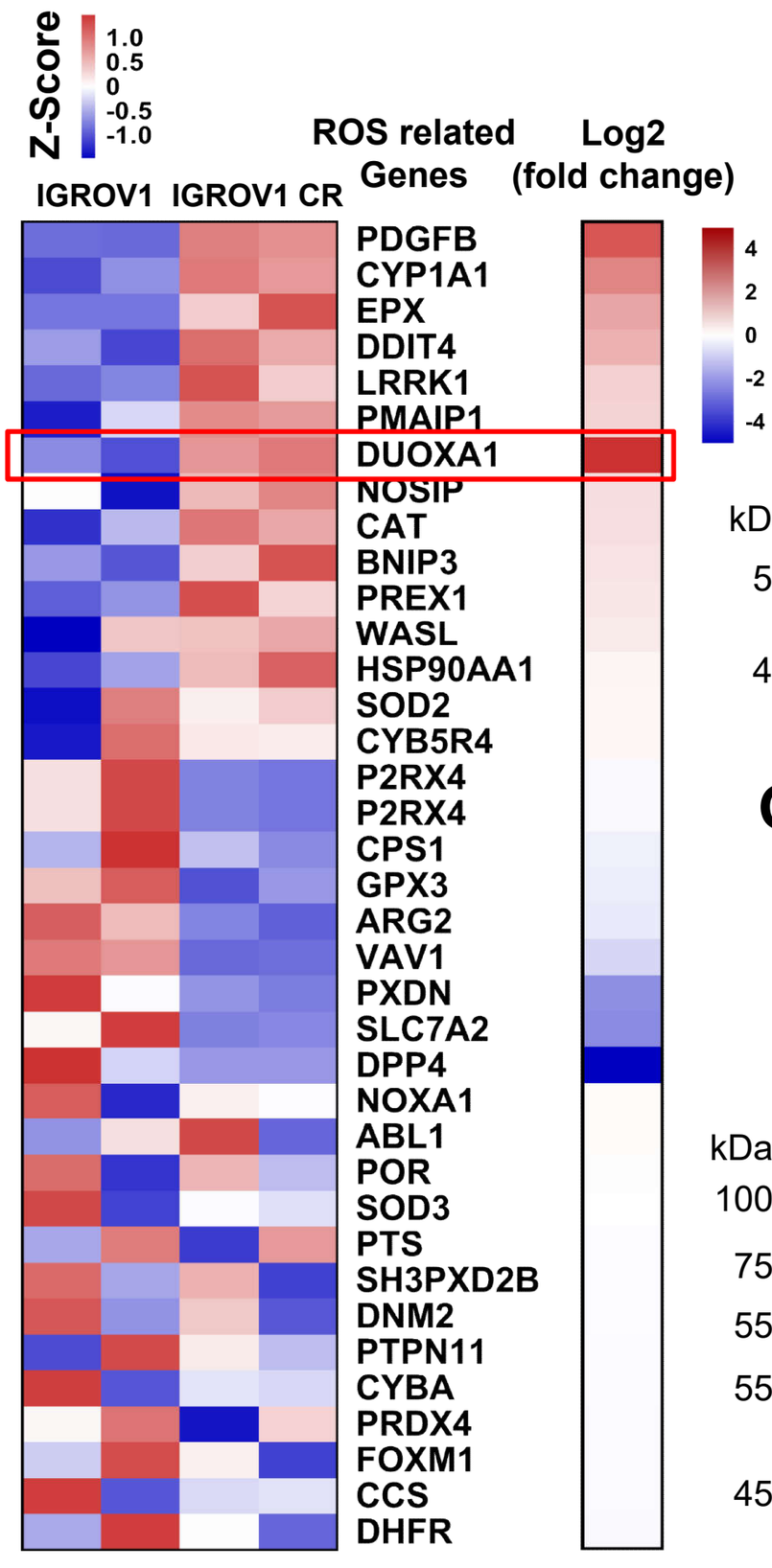
C



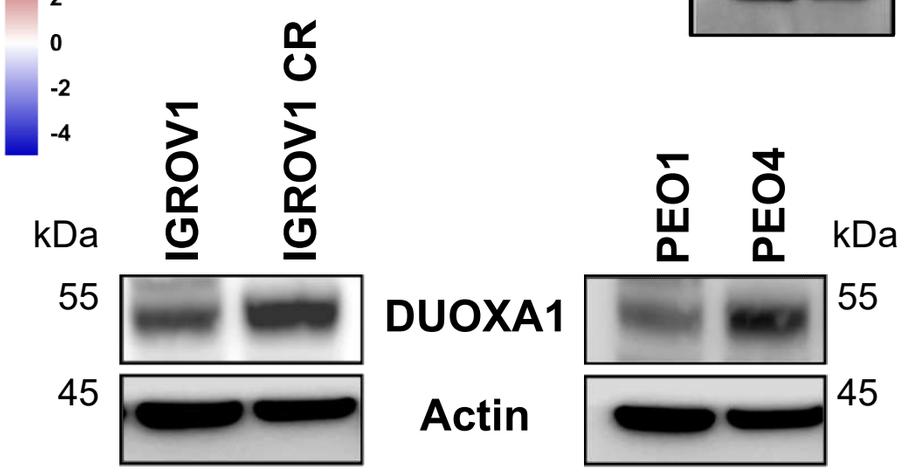
D



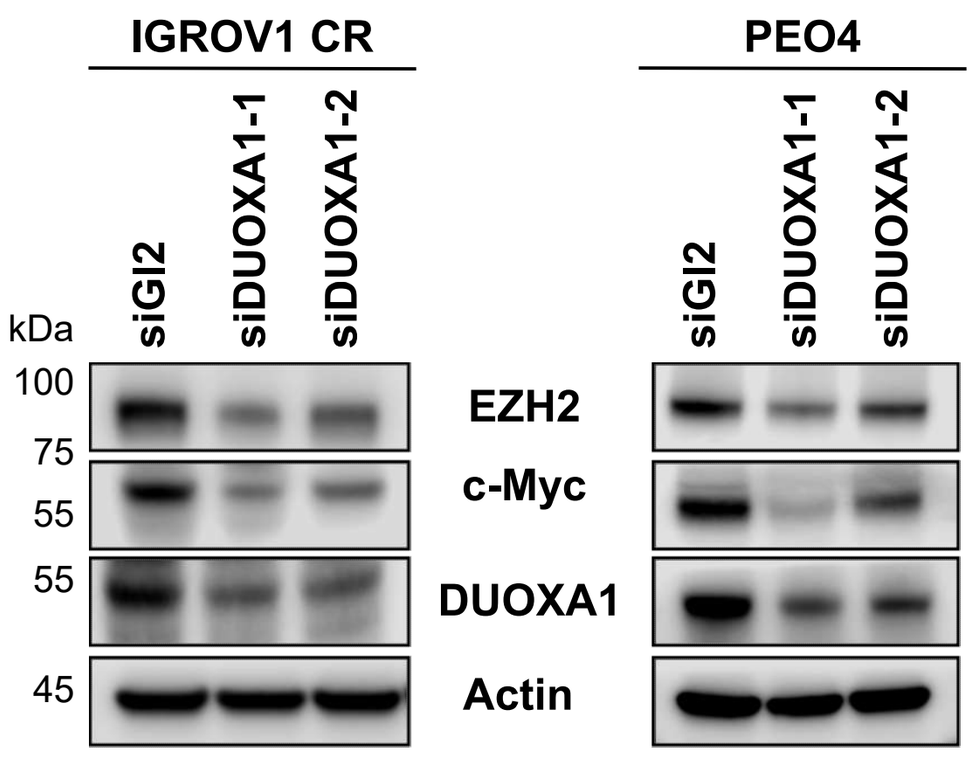
E



F



G



H

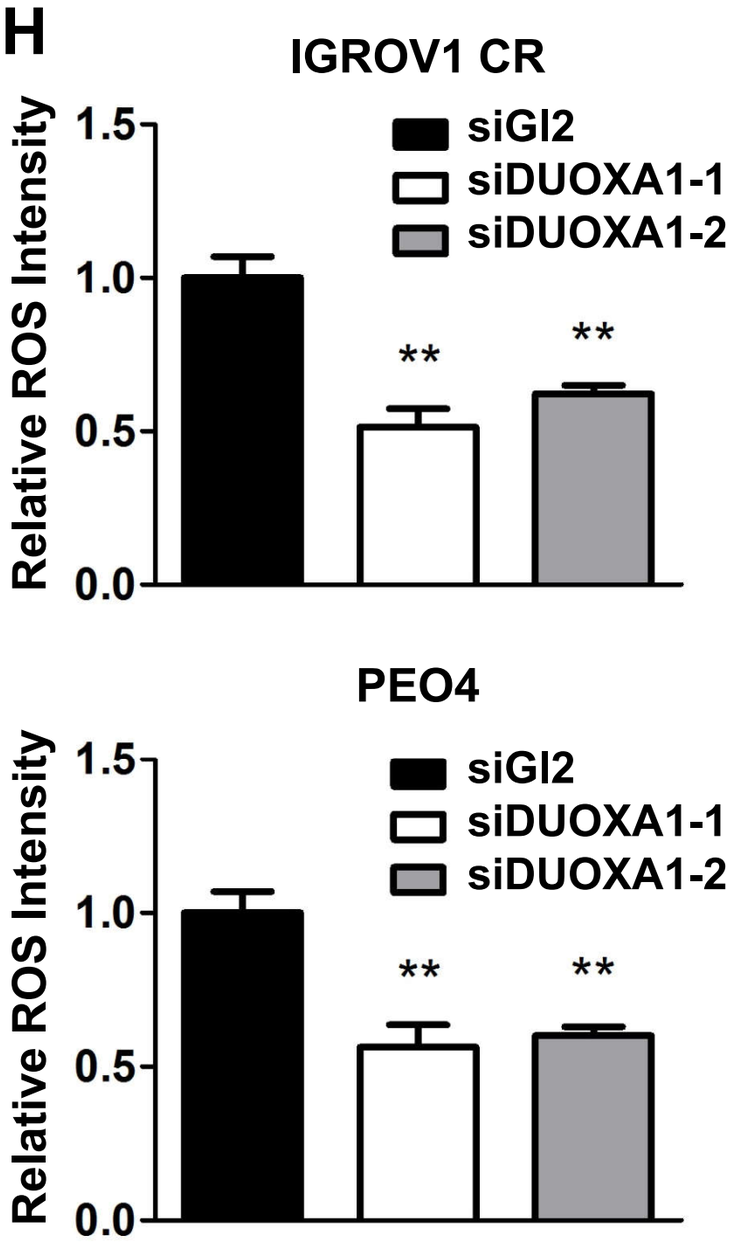
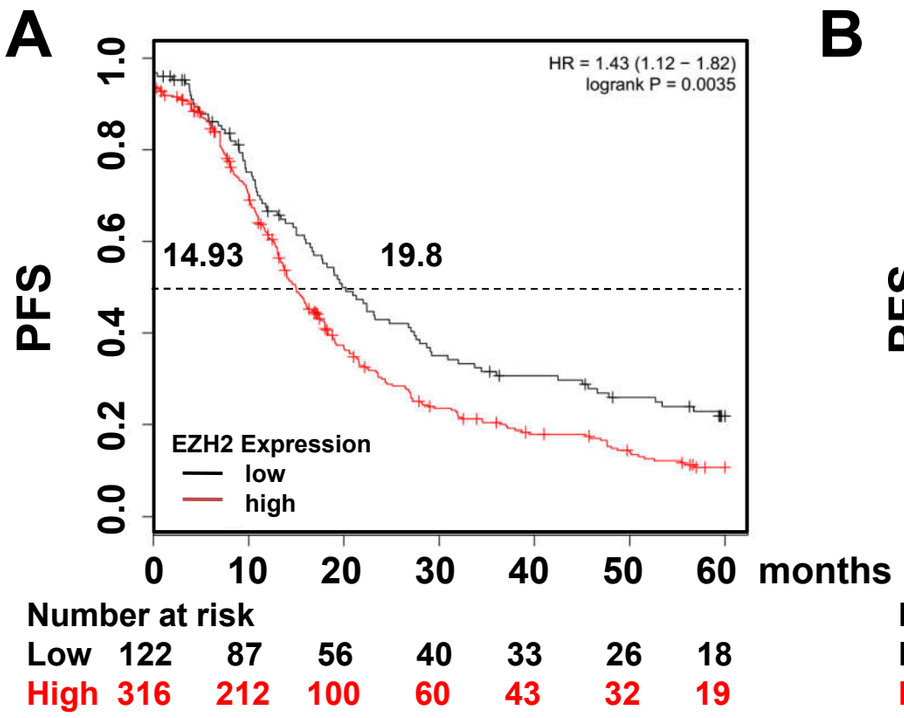
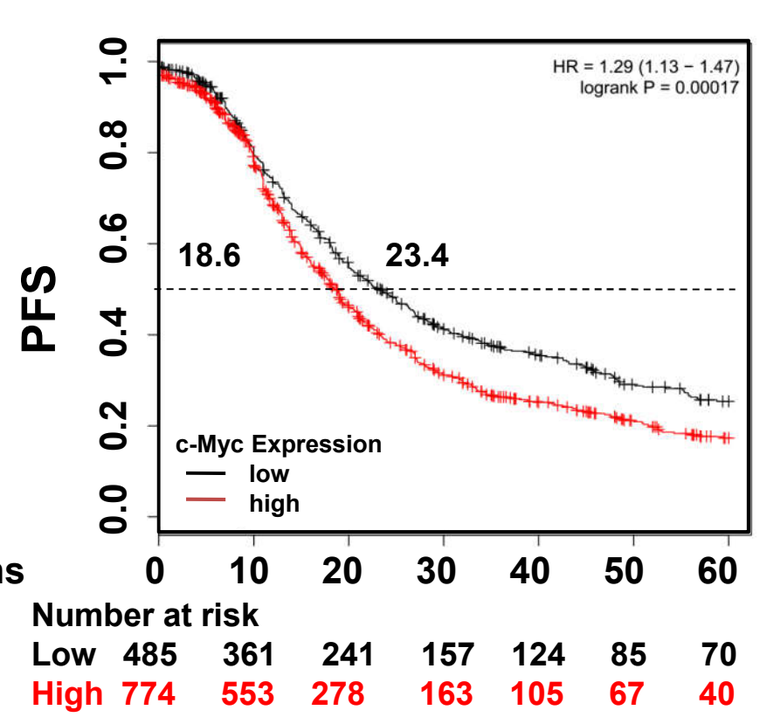
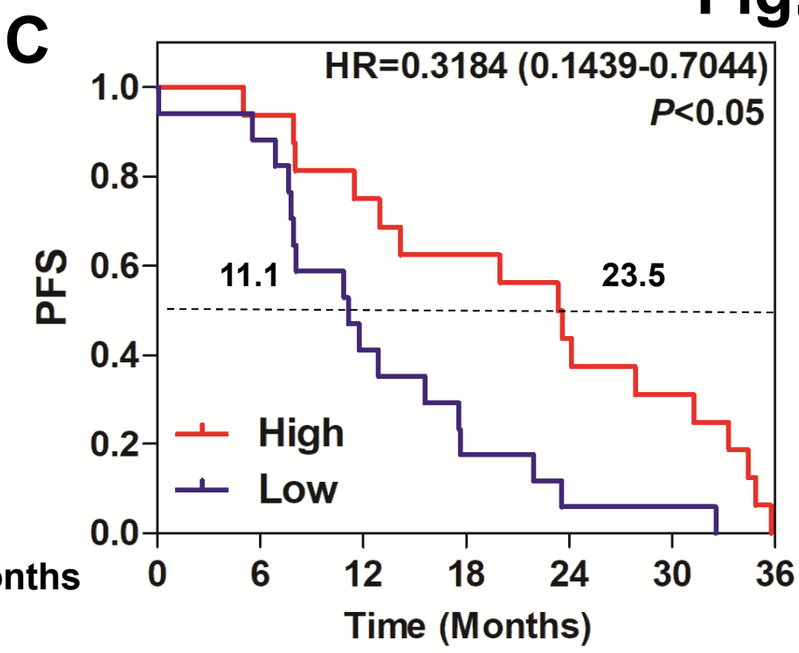
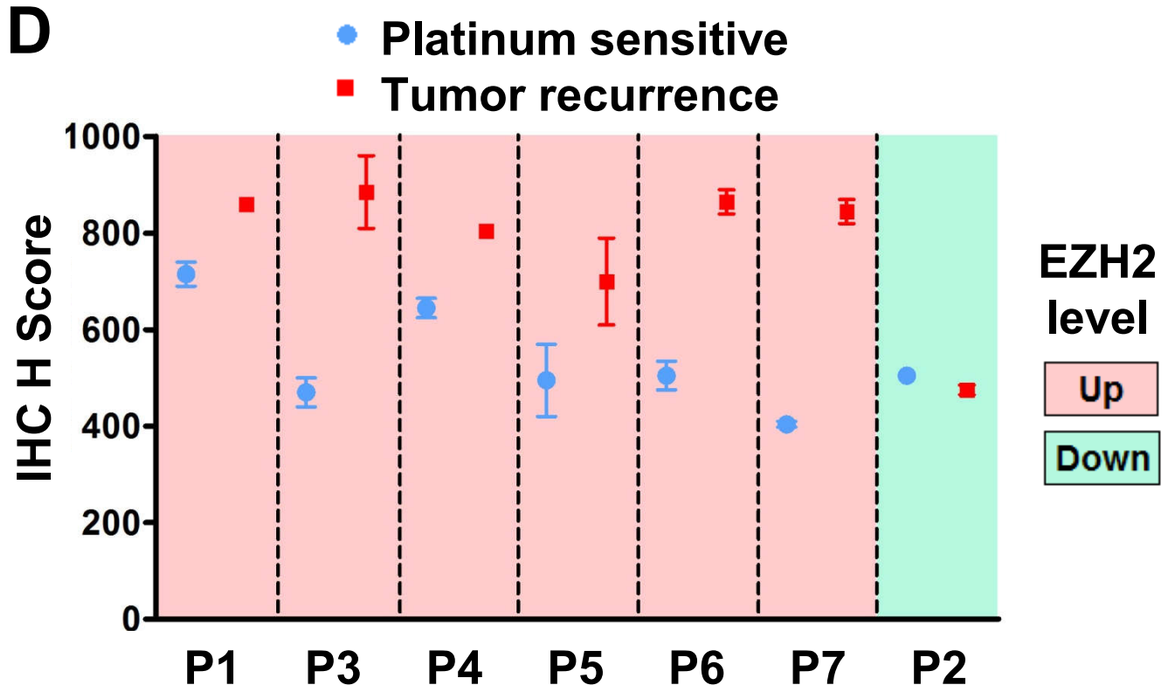


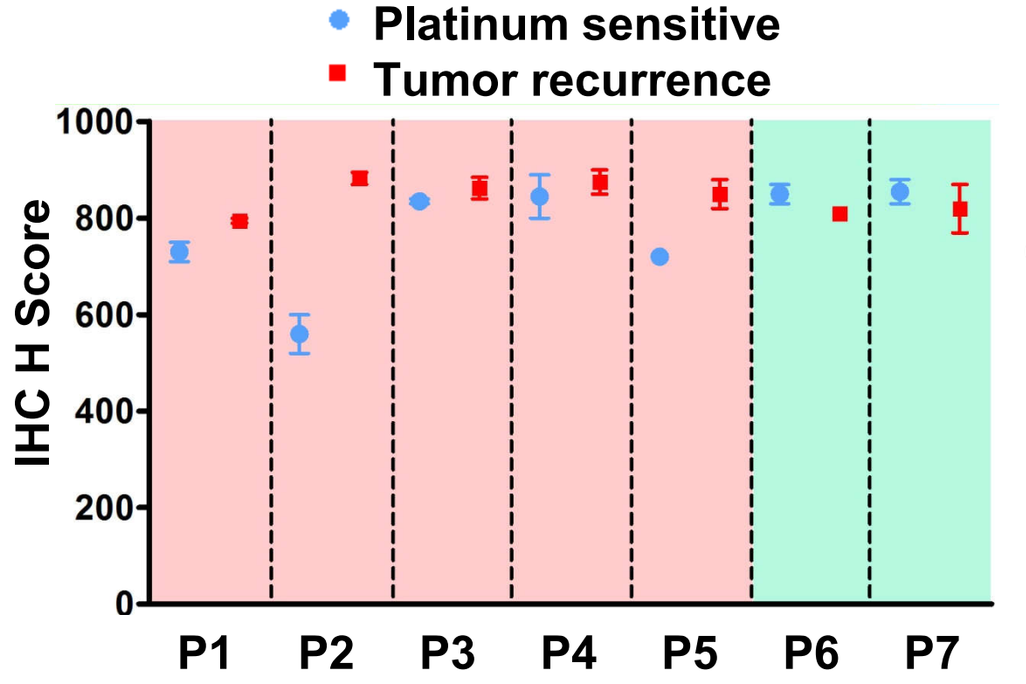
Fig. 8

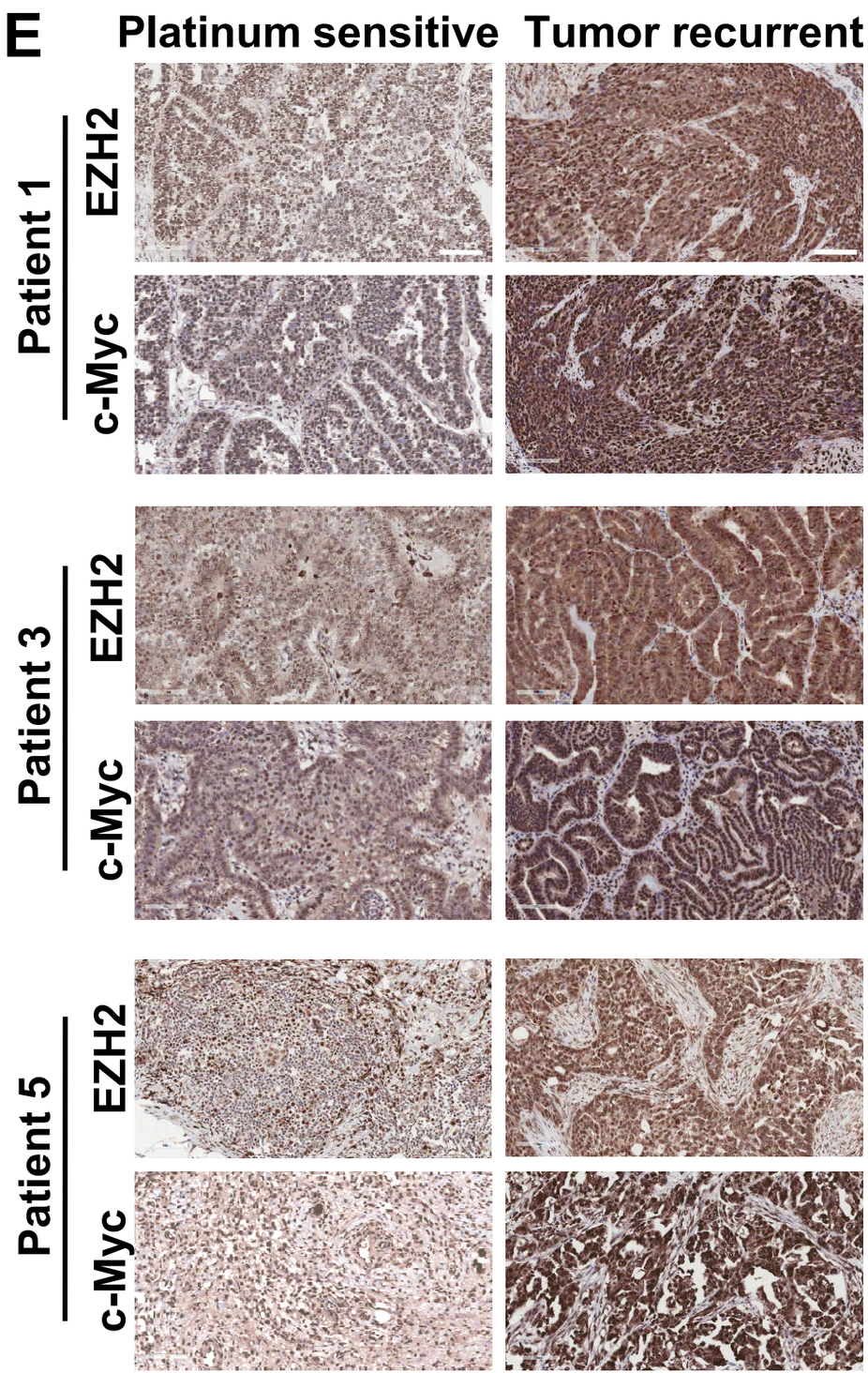

A
HR = 1.43 (1.12 − 1.82)
logrank P = 0.0035
PFS
14.93
19.8
EZH2 Expression
low
high
0 10 20 30 40 50 60 months
Number at risk
Low 122 87 56 40 33 26 18
High 316 212 100 60 43 32 19

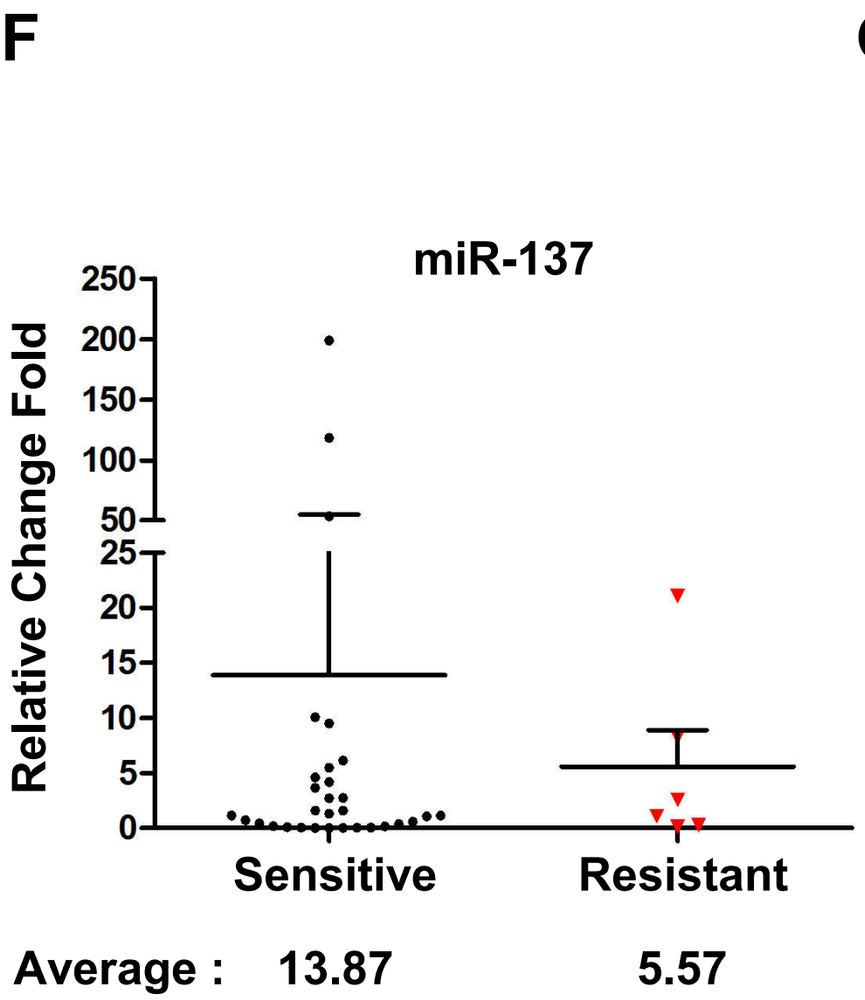

B
HR = 1.29 (1.13 − 1.47)
logrank P = 0.00017
PFS
18.6
23.4
c-Myc Expression
low
high
0 10 20 30 40 50 60 months
Number at risk
Low 485 361 241 157 124 85 70
High 774 553 278 163 105 67 40

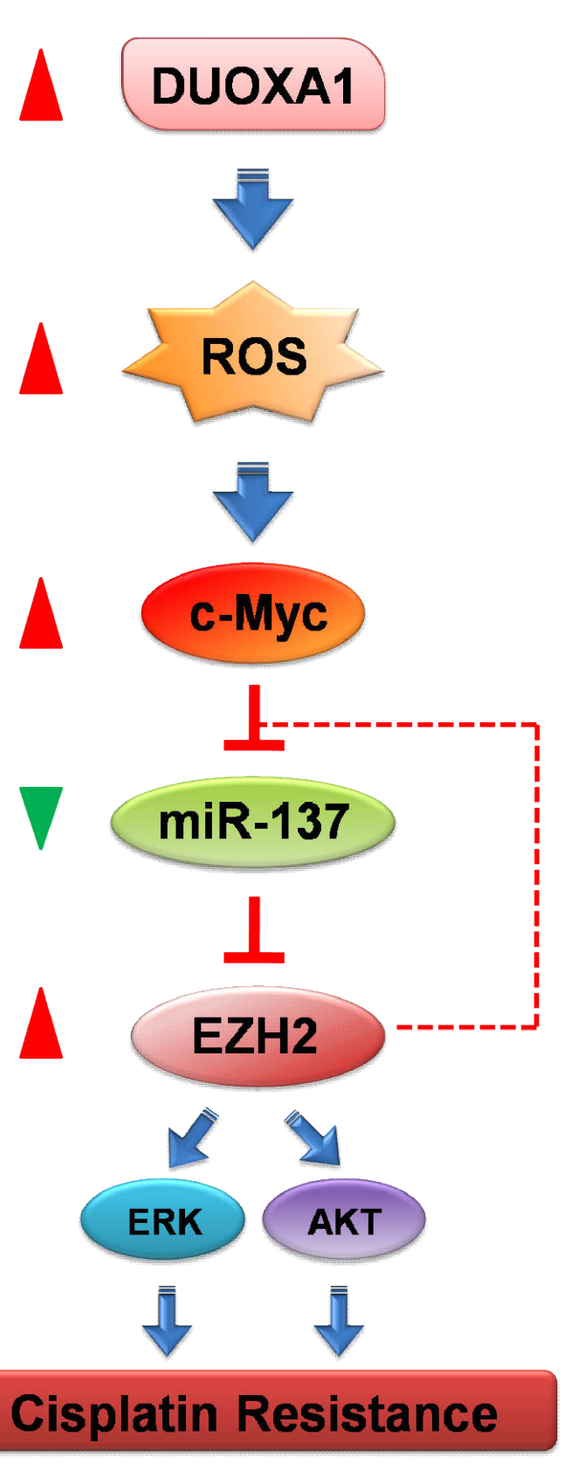

C
HR=0.3184 (0.1439-0.7044)
P<0.05
PFS
11.1
23.5
High
Low
Time (Months)


D
Platinum sensitive
Tumor recurrence
IHC H Score
EZH2 level
Up
Down
P1 P3 P4 P5 P6 P7 P2


Platinum sensitive
Tumor recurrence
IHC H Score
c-Myc level
Up
Down
P1 P2 P3 P4 P5 P6 P7


E
Platinum sensitive
Tumor recurrent
Patient 1
EZH2
c-Myc
Patient 3
EZH2
c-Myc
Patient 5
EZH2
c-Myc


F
miR-137
Relative Change Fold
Sensitive
Resistant
Average : 13.87 5.57


G
DUOXA1
ROS
c-Myc
miR-137
EZH2
ERK
AKT
Cisplatin Resistance