

ADDITIONAL FILE_3

Muñoz-Galván et al., “Downregulation of *MYPT1* increases tumor resistance in ovarian cancers by targeting the Hippo pathway and increasing the stemness”

Figure S1. Copy number alterations and expression of miR-30b. Blox plot showing miR-30b expression levels associated to the copy number alterations observed in patients from the ovarian cancer TCGA database. Statistical differences were analyzed by the Mann-Whitney's *U*-test. ***, $P < 0.001$; ****, $P < 0.0001$.

Figure S2. Downregulation of *MYPT1* decreases Hippo pathway activation. (a) Western blot showing the protein levels of MYPT1, pYAP (S127), YAP and α -tubulin in ES-2, SKOV3 or OVCAR8 ovarian cells expressing *shMYPT1-4* or empty vector (EV). (b) Heatmaps showing the expression fold-change of the Hippo pathway genes in ES-2, SKOV3 or OVCAR8 ovarian cells expressing *shMYPT1-4* or miR-30b compared to cells expressing the EV. Hierarchical clustering of the samples is shown. (c) Heatmaps showing the z-scores of Hippo pathway gene expression in ES-2, SKOV3 or OVCAR8 cells expressing the EV. Hierarchical clustering of the samples is shown. (d) Quantification of MYPT1, NF2 and pNF2 protein levels from western blots in Figure 2h, as well as the phospho-NF2/NF2 ratio. (e) Western blot and quantification of the protein levels of MYPT1, YAP and TAZ either in the cytoplasmic or nuclear fractions in ES-2, SKOV3 or OVCAR8 ovarian cells expressing *shMYPT1-4* or EV. Xiap and hnRNP C1/C2 were used as cytoplasmic and nuclear controls, respectively. (f) Determination of the methylation status of the *NF2* gene promoter in the SKOV3 or OVCAR8 ovarian cancer cell lines. (g) Analysis of the expression by RT-qPCR of several Hippo pathway target genes, including *BIRC5*, *CTGF*, *FGF1* and *GLI2*, in SKOV3 or OVCAR8 ovarian cancer cells expressing *shMYPT1-4* or EV.

Figure S3. Downregulation of *MYPT1* increases tumorigenesis and resistance to platinum in ovarian cancer *in vivo* and *in vitro*. (a) Quantification of the number of clones in ES-2, SKOV3 or OVCAR8 ovarian cell lines carrying EV (dark green) or *shMYPT1-4* (light green). (b) Growth curve of ES-2, SKOV3 or OVCAR8 ovarian cell lines carrying EV (dark green) or *shMYPT1-4* (light green), represented as the accumulation of doubling times. (c) Determination of the IC50 (concentration of drug necessary to induce 50% of cell death) to platinum-derived drugs in cells overexpressing *shMYPT1-4* or EV. (d) Determination of tumor volume and survival after carboplatin treatment of xenografts of SKOV3 cells expressing *shMYPT1-4* or EV. Cohorts of 5 mice each one

were either treated with carboplatin or saline serum once the tumor reached 0.5 cm of diameter, and survival rates were determined. All experiments were repeated at least three times. Data were analyzed using Student's *t*-test. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Figure S4. Representative images of MYPT1, NF2 and YAP immunostaining in xenografts derived from SKOV3 or OVCAR8 ovarian cells expressing *shMYPT1* or EV. Scale bar: 100 μm .

Figure S5. Downregulation of *MYPT1* increases stemness in ovarian cancer. (a) Percentage of paraclones, meroclones and holoclones formed from ES-2, SKOV3 or OVCAR8 ovarian cells expressing *shMYPT1-4* or EV. (b) Quantification of the number and size of tumorspheres formed from ES-2, SKOV3 and OVCAR8 cells expressing *shMYPT1-4* or EV. (c) Same as in b but from single cells. (d) Analysis of the expression of several stemness-associated genes, including *OCT4*, *NANOG* and *SOX2*, as well as *MYPT1* by RT-qPCR in total extracts and tumorspheres from ES-2, SKOV3 or OVCAR8 ovarian cancer cells expressing *shMYPT1-4* or EV. (e) Analysis of the expression of several hippo pathway targets genes, including *BIRC5*, *CTGF*, *FGF1* and *GLI2*, by RT-qPCR in tumorspheres from ES-2, SKOV3 or OVCAR8 ovarian cancer cells expressing *shMYPT1-4* or EV. Average and SD of three independent experiments are shown. Data were analyzed using Student's *t*-test. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Figure S6. CSC surface markers are increased upon *MYPT1* depletion.

Representative plots of CD10+, CD133+ and CD19+ CSC surface markers analyzed by FACS in ES-2, SKOV3 or OVCAR8 ovarian cancer cells expressing *shMYPT1*, miR-30b or EV.

Figure S7. Downregulation of *MYPT1* increases resistance to platinum treatment by inhibiting the Hippo pathway. (a) Determination of the IC50 to platinum drugs in combination with 2 nM of the YAP inhibitor verteporfin (YAPi) in ES-2, SKOV3 or OVCAR8 expressing *shMYPT1-4* or EV. (b) Quantification of the number and size of tumorspheres formed from ES-2, SKOV3 and OVCAR8 ovarian cells expressing *shMYPT1-4* or EV, treated or not with YAPi. (c) Percentage of paraclones, meroclones and holoclones formed from ES-2, SKOV3 or OVCAR8 ovarian cells expressing *shMYPT1*, miR-30b or EV, treated or not with YAPi. (d) Percentage of paraclones,

meroclones and holoclones formed from ES-2, SKOV3 or OVCAR8 ovarian cells expressing *shMYPT1-4* or EV, treated or not with YAPi.

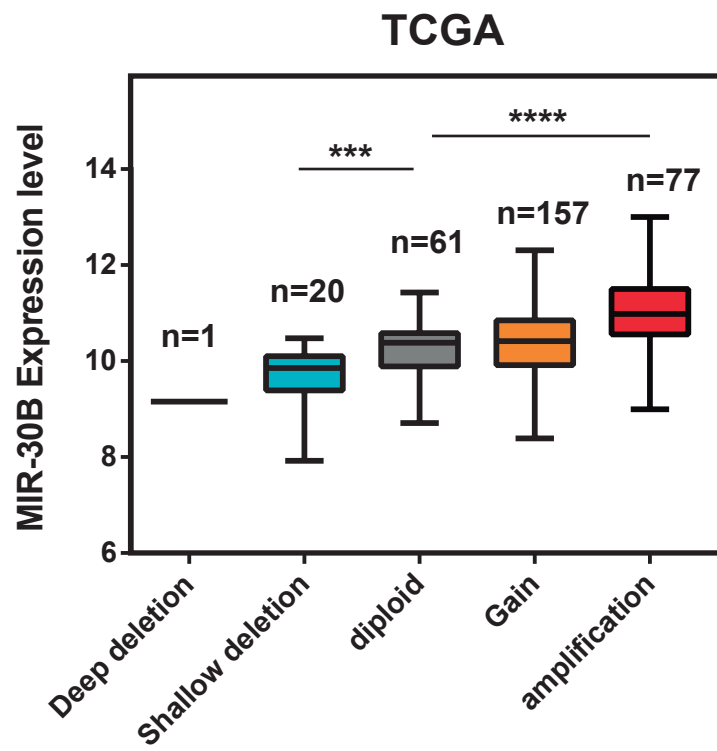


Figure S1

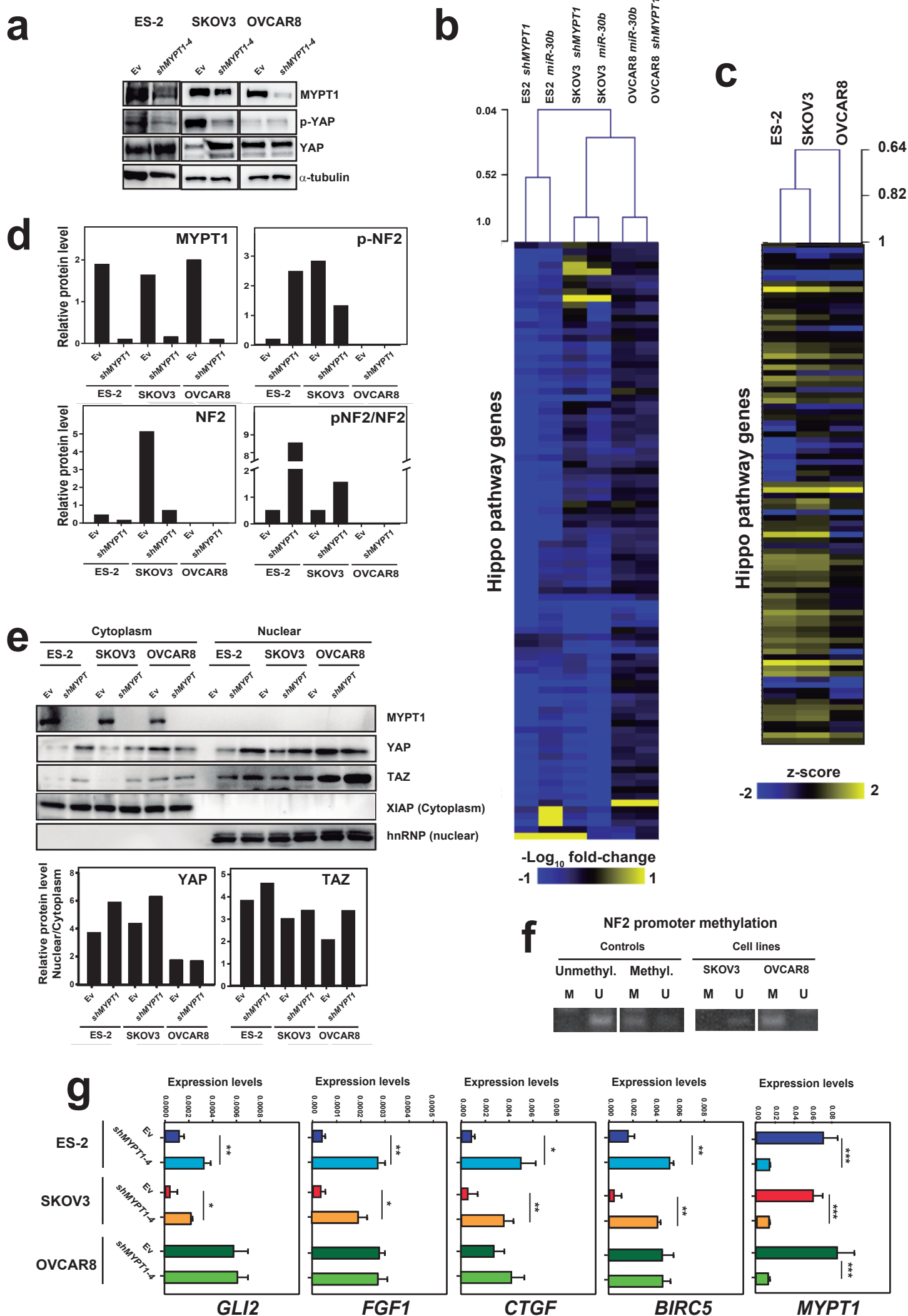


Figure S2

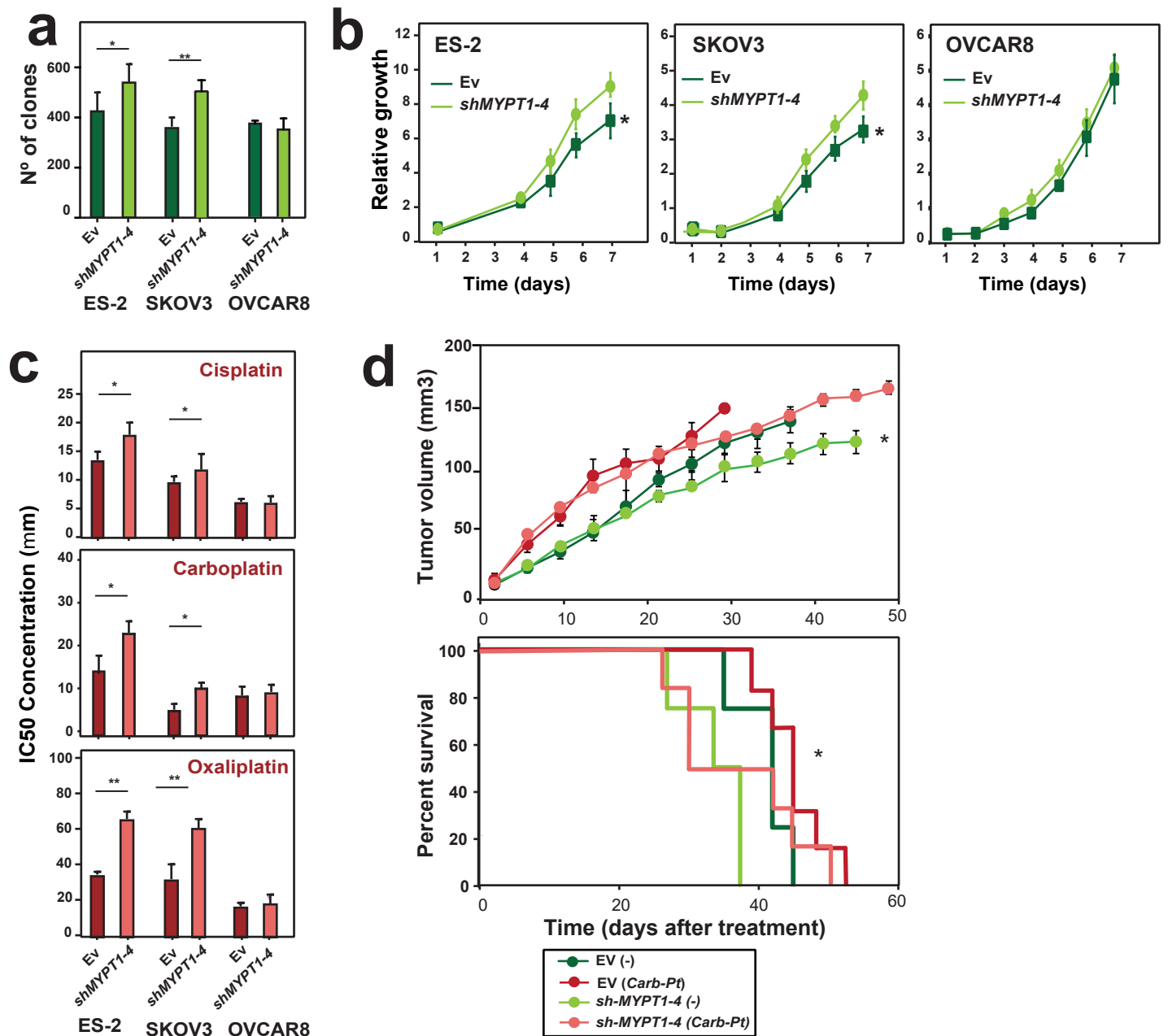


Figure S3

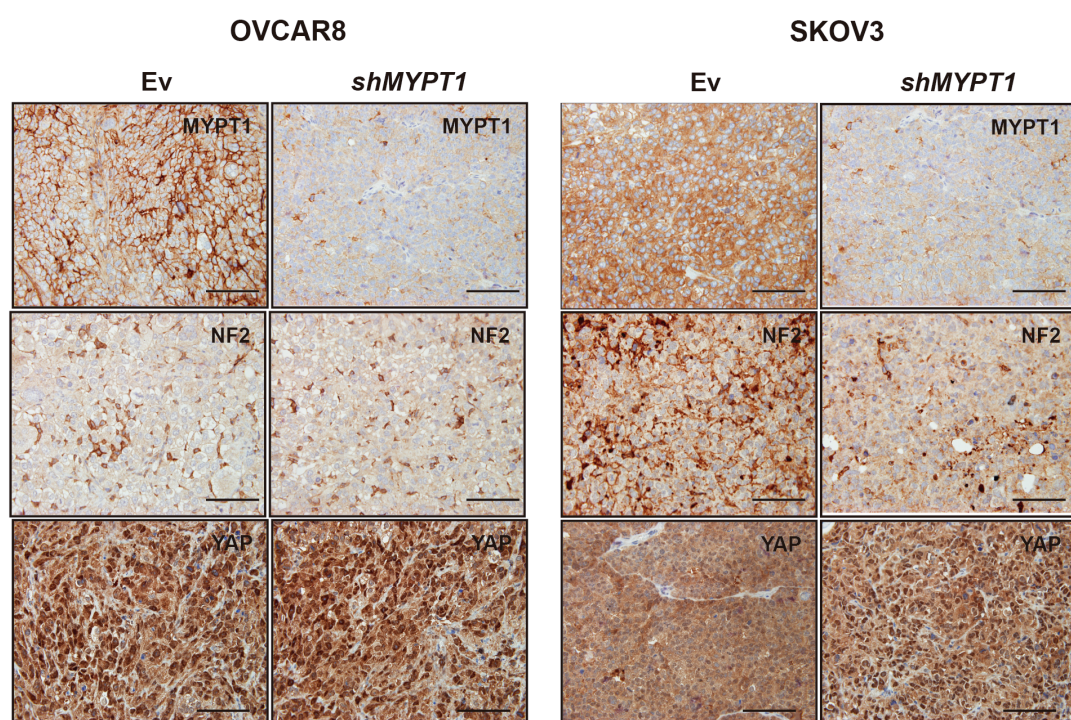


Figure S4

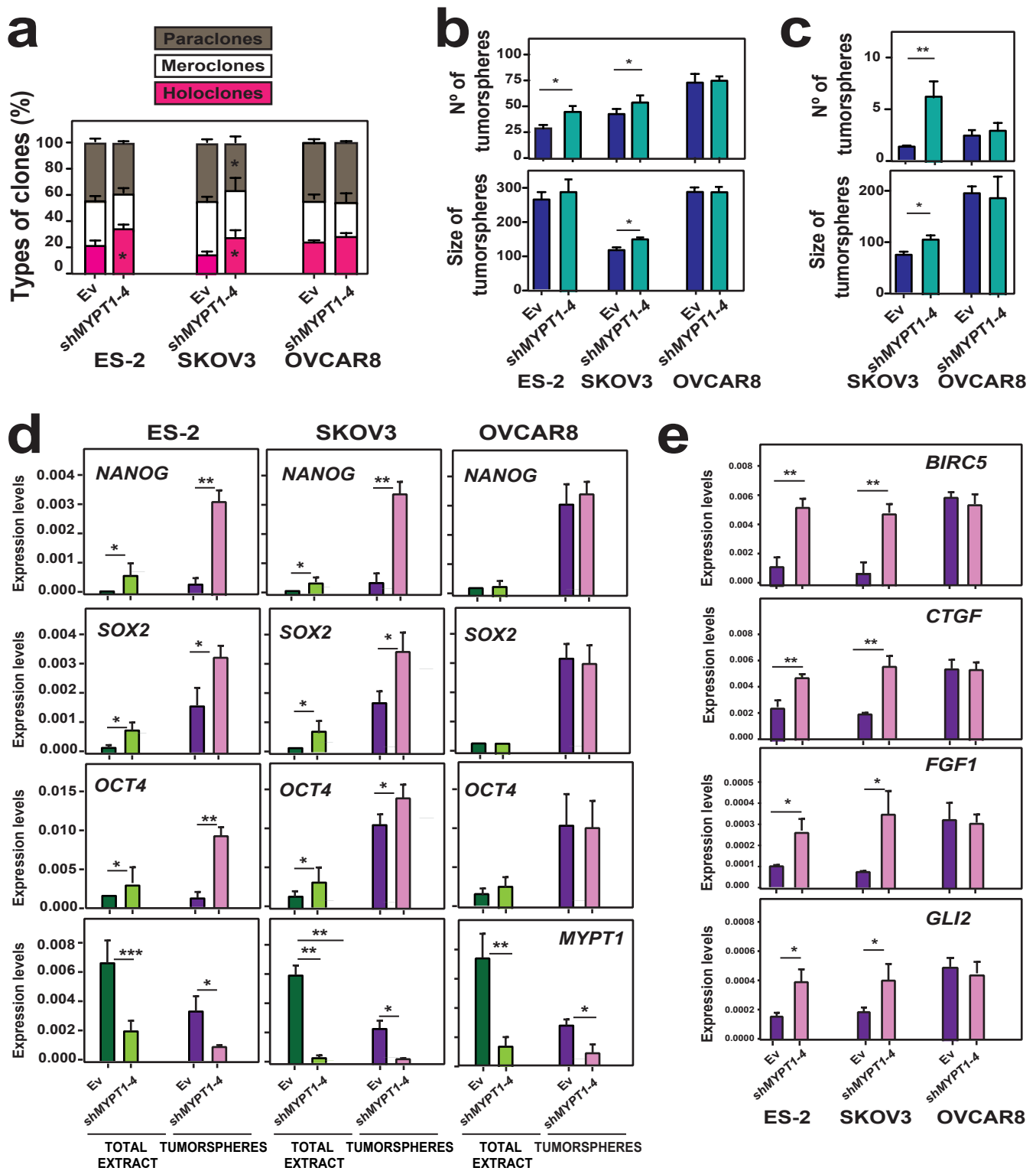
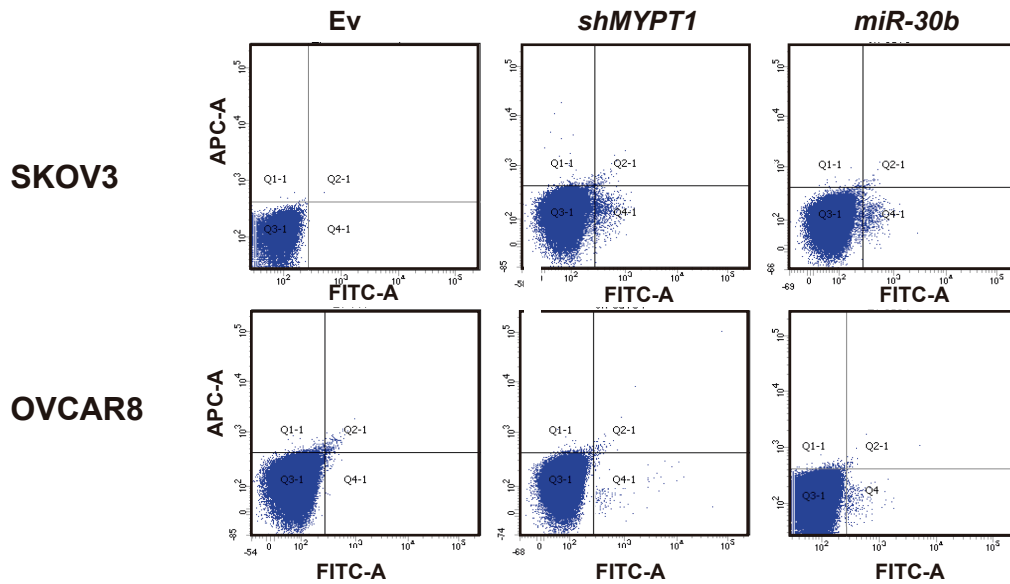
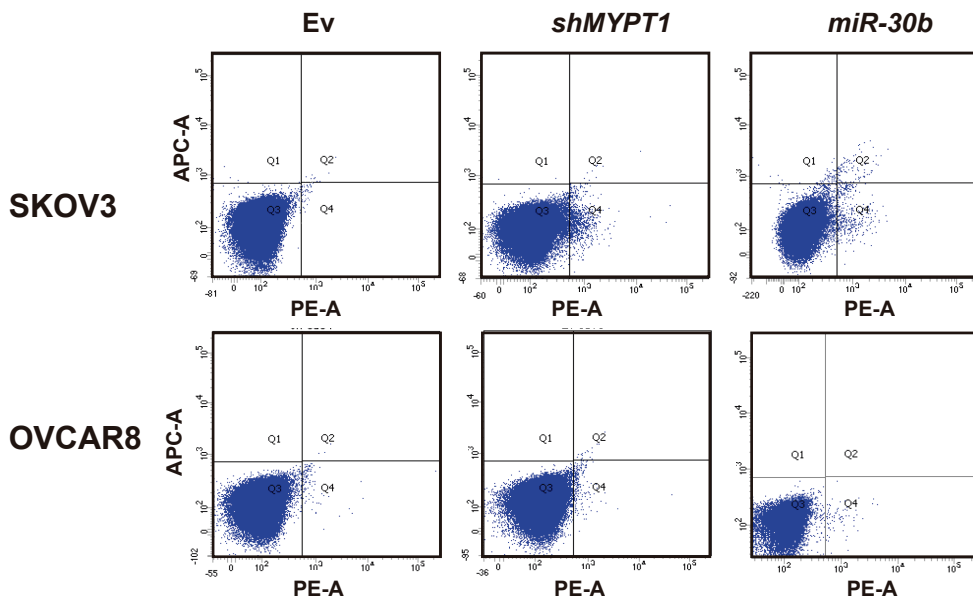


Figure S5

CD10-FITC



CD133-PE



CD19-APC

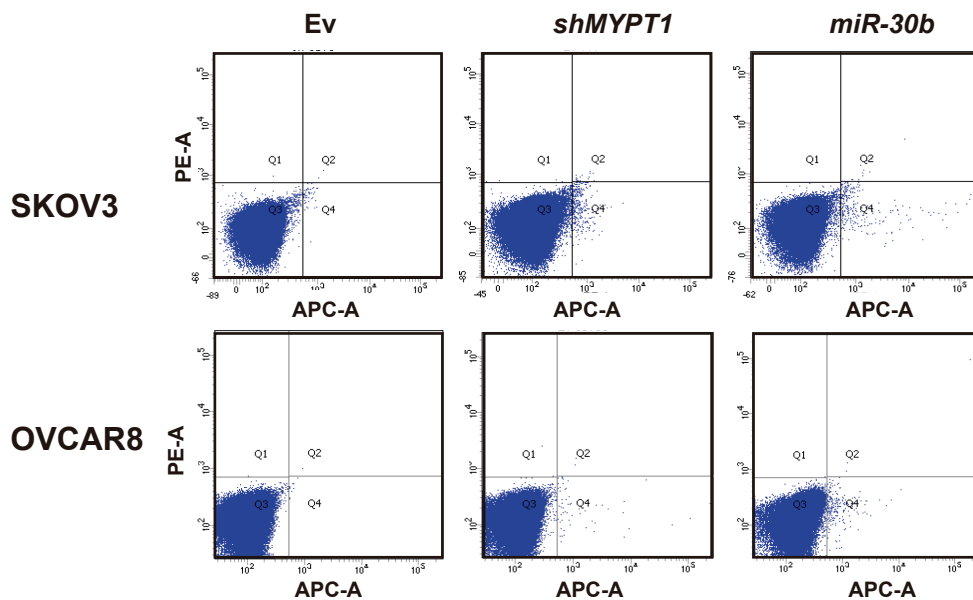


Figure S6

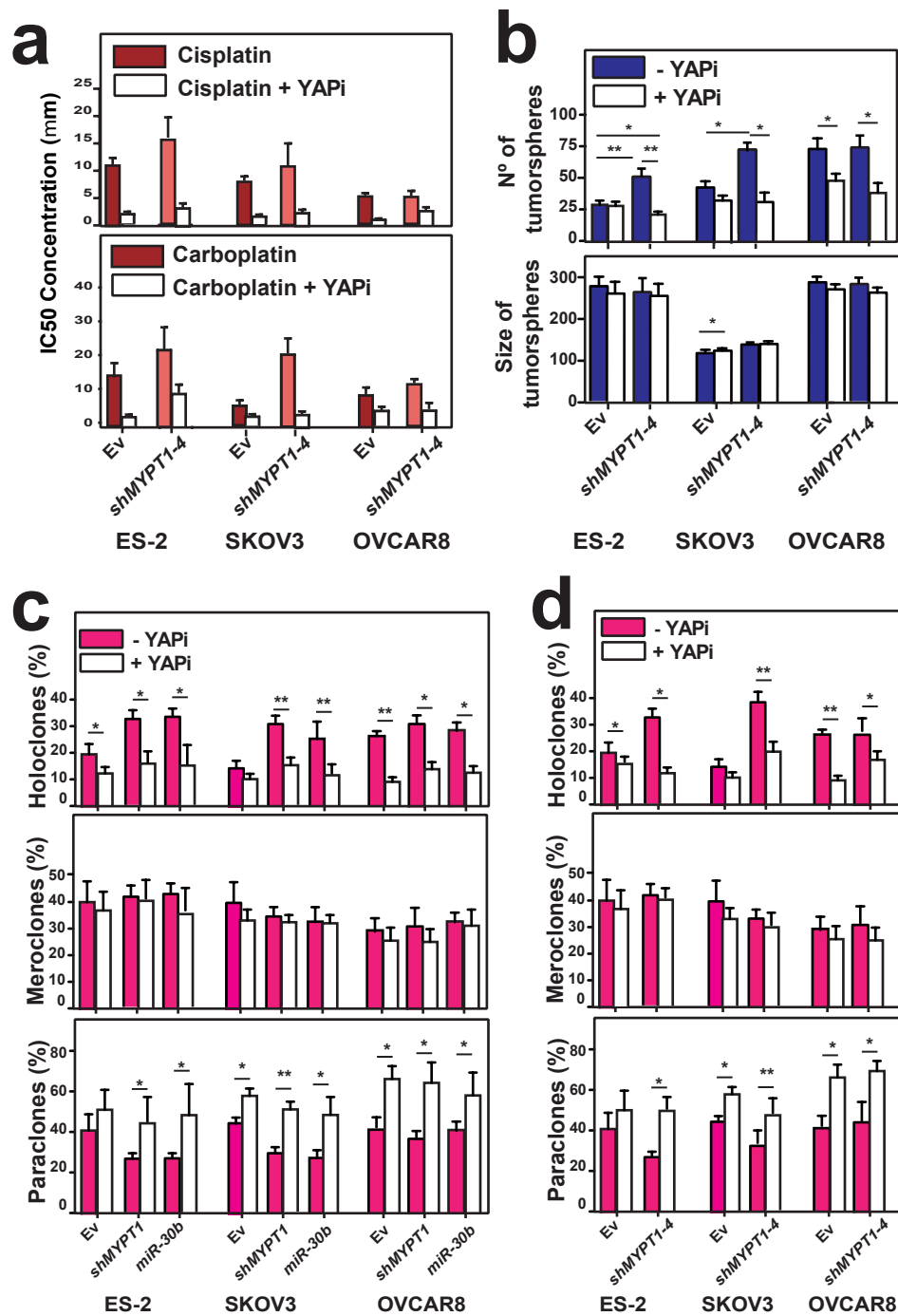


Figure S7