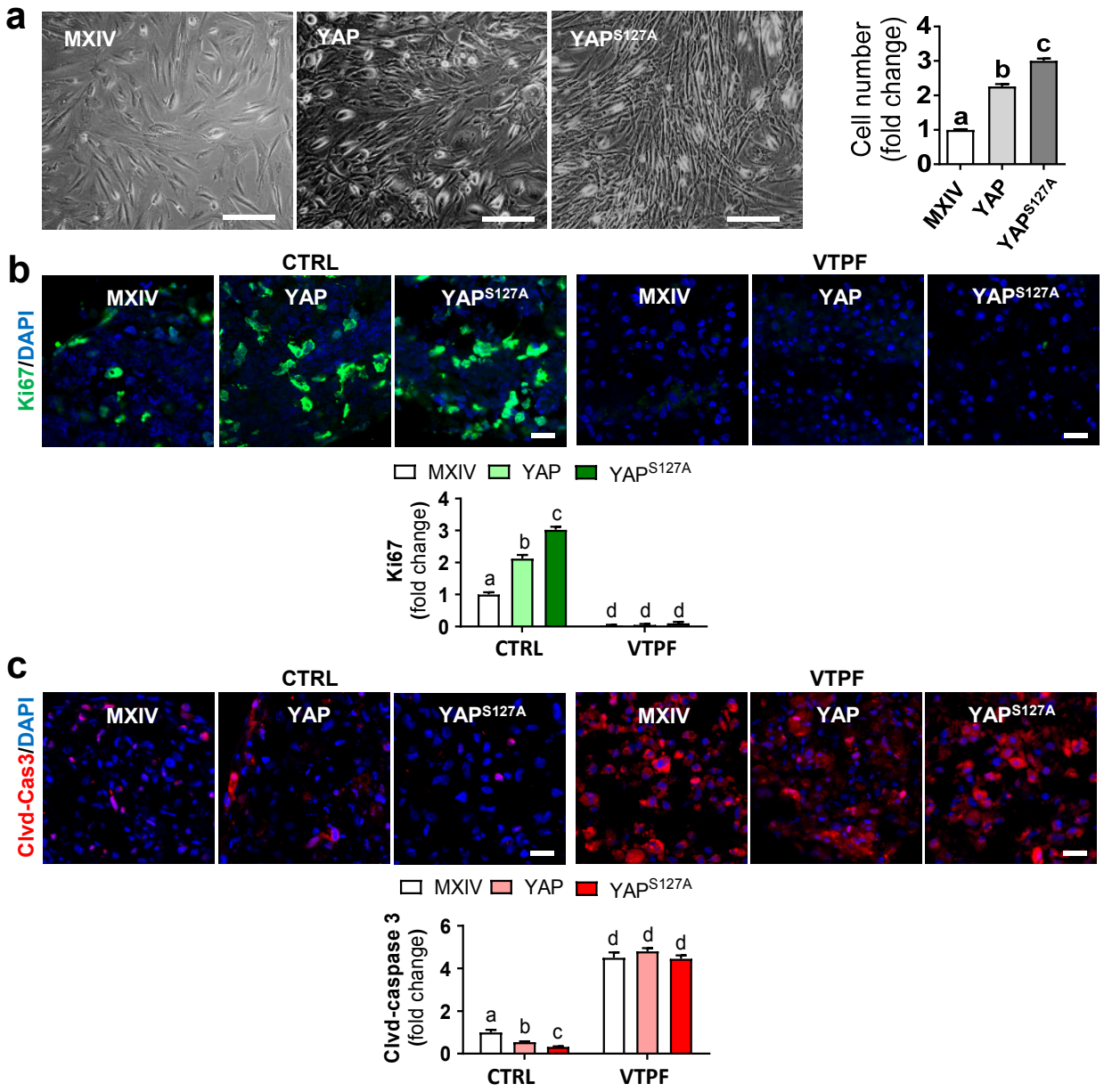
Supplementary Figure



Supplementary figure S1. YAP1 promotes proliferation and supports survival of human granulosa cells. a) Representative images showing morphology of control MXIV, YAP, and YAP^{S127A}-expressing hGCs incubated in the growing medium for 5 days, Scale bar: 200 μm; right: bar graph showing cell number change of control and YAP1-expressing hGCs incubated in the growing medium for 5 days. Each bar represents the mean ± SEM (n = 4). Bars with different letters are significantly different from each other ($P < 0.01$). **b**) Representative images showing Ki67 expression examined by fluorescent immunohistochemistry in spheroids formed by hGC-MXIV, hGC-YAP, and hGC-YAP^{S127A} cells incubated in the presence or absence of 2 μM verteporfin (VP) for 5 days. Scale bar: 20 μm. The bar graph shows the quantitative relative ration of KI67-positive cells. **c)** Representative images showing cleaved caspase 3 (Clvd-Cas 3) examined by fluorescent immunohistochemistry in spheroids formed by hGC-MXIV, hGC-YAP, and hGC-YAP^{S127A} cells incubated in the presence or absence of 2 μM verteporfin for 5 days; The bar graph showing the relative ratio of cells with cleaved caspase 3. Scale bar: 20 μm. Each bar represents the mean ± SEM (n ≥ 4). Bars with different letters are significantly different from each other ($P < 0.05$).

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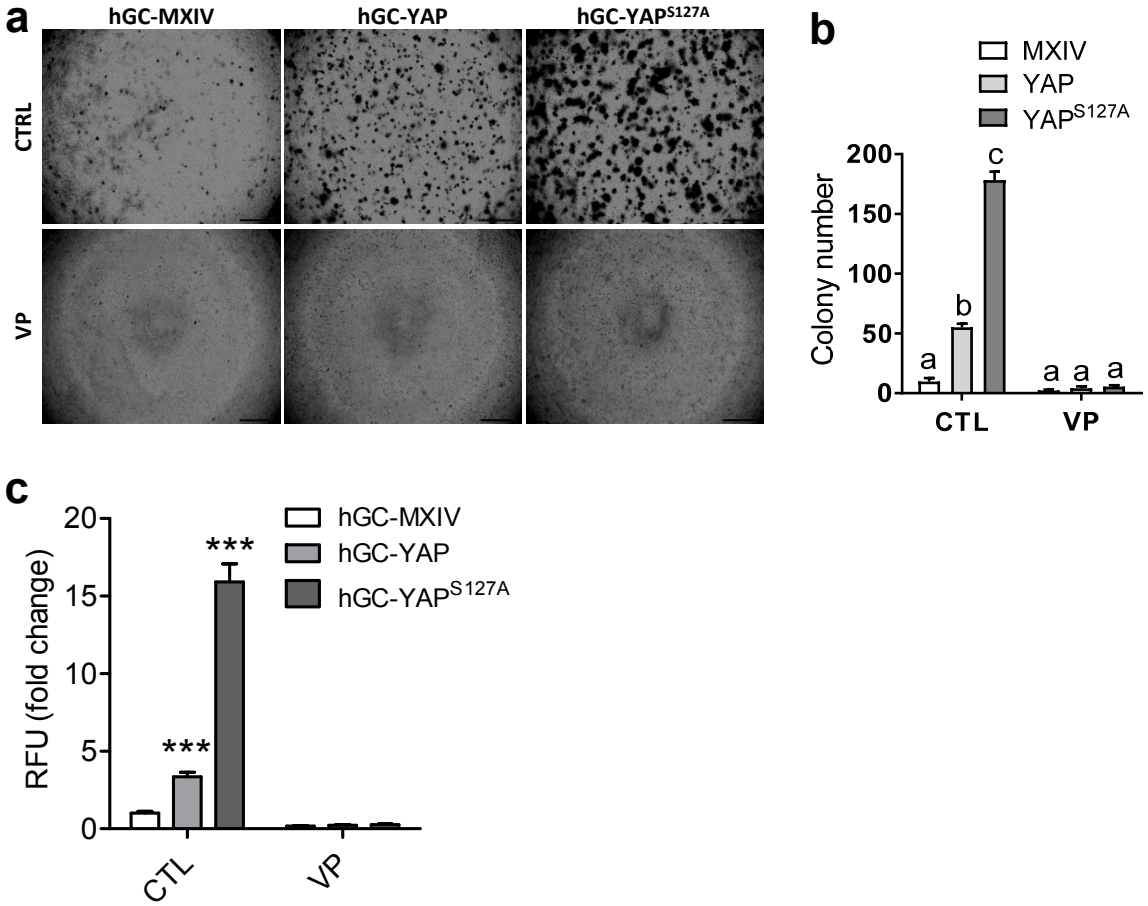
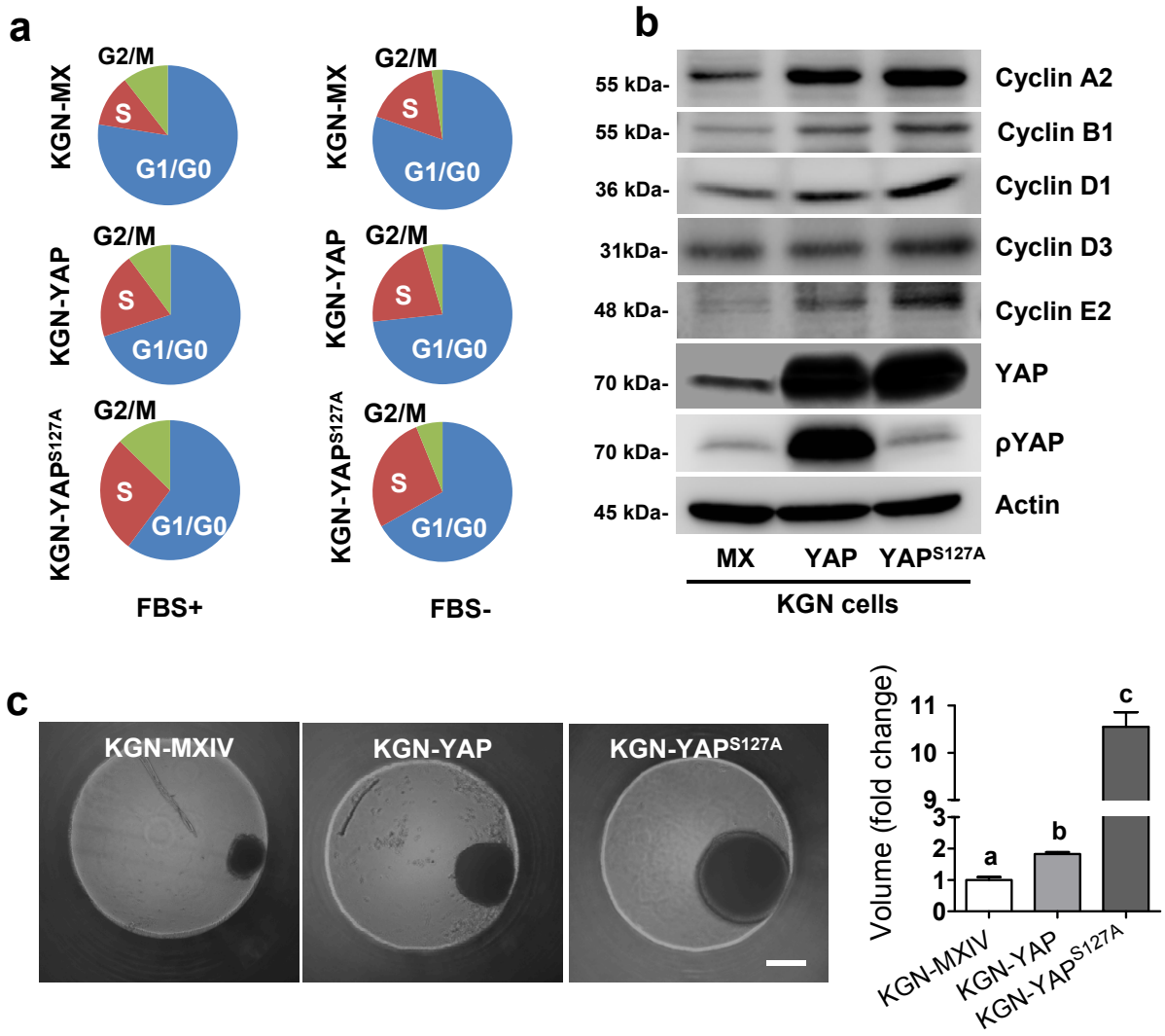
Supplementary Figure



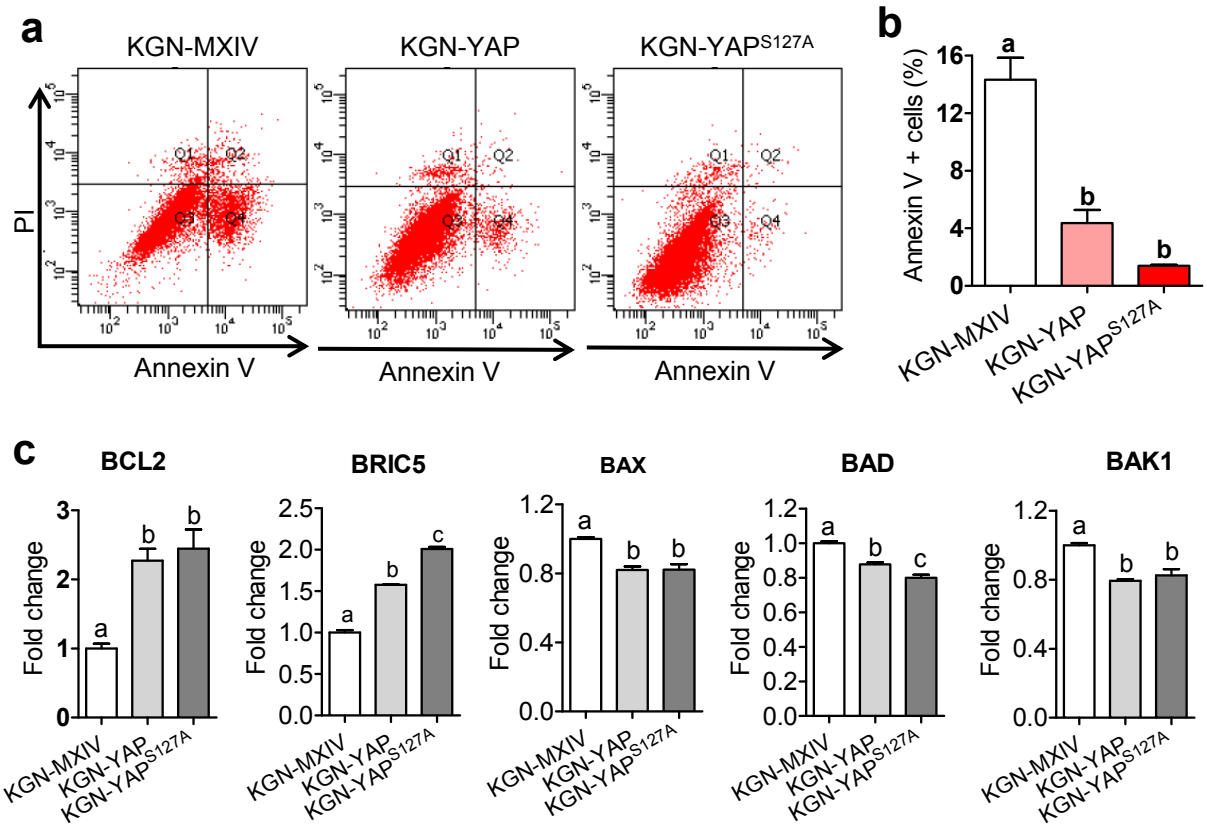
Supplementary figure S2. Hyperactivation of YAP1 is able to transform primary cultured human granulosa cells (hGCs). a) Representative images showing colonies formed by hGC-MXIV, hGC-YAP, and hGC-YAP^{S127A} cells. Cells were incubated in growth medium in a soft agar assay system in the presence or absence of verteporfin (2 μM) for 10 days before colony counting. Scale bar: 500 μm. **b**) Bar graph showing quantitative data of a). Each bar represents the mean ± SEM (n = 4). ***: significantly different from control ($P < 0.001$). **C**) Quantitative analyses of colony formation of hGCs with differential expression of YAP1. hGC-MX, hGC-YAP, or hGC-YAP^{S127A} cells were incubated in growth medium in a fluorescence-based semi-quantitative soft agar assay in the presence or absence of verteporfin (2 μM) for 10 days. Relative colonies formed by these cells were indicated by relative fluorescence unit (RFU). Each bar represents the mean ± SEM (n = 4). Bars with different letters are significantly different from each other ($P < 0.05$).

Supplementary Figure



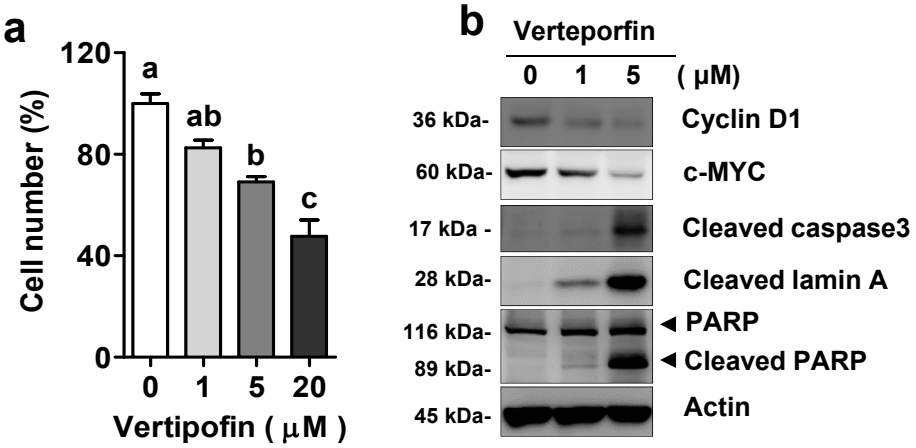
Supplementary figure S3. YAP promotes the proliferation of KGN cells. a). Ectopic expression of YAP or constitutively active YAP (YAP^{S127A}) promotes cell cycle progression in KGN cells. KGN-MX~~IV~~, KGN-YAP and KGN-YAP^{S127A} cells were cultured in growth medium supplemented with (FBS+) or without (FBS-) serum for 5 days. Cell cycle were analyzed by Annexin V-FITC/PI - based flow cytometry. Experiments were repeated for three times and the representative graphs for each group were presented. **b)** Representative blots showing the protein expression levels of Cyclins in KGN cells after ectopic expression of YAP or constitutively active YAP (YAP^{S127A}). Experiments were independently repeated for at least 3 times. **c)** YAP overexpression promotes KGN cell growth in 3D culture: representative pictures showing the spheroids from KGN-MX, KGN-YAP, and KGN-YAP^{S127A} cells; bar graph showing quantitative data for the volume of spheroids. Scale bar: 200 μm. Graph on the right is the quantitative data of the volume of the KGN cell spheroid in each group. Each bar represents the mean ± SEM (n ≥ 5). Bars with different letters are significantly different from each other ($P < 0.05$).

Supplementary Figure



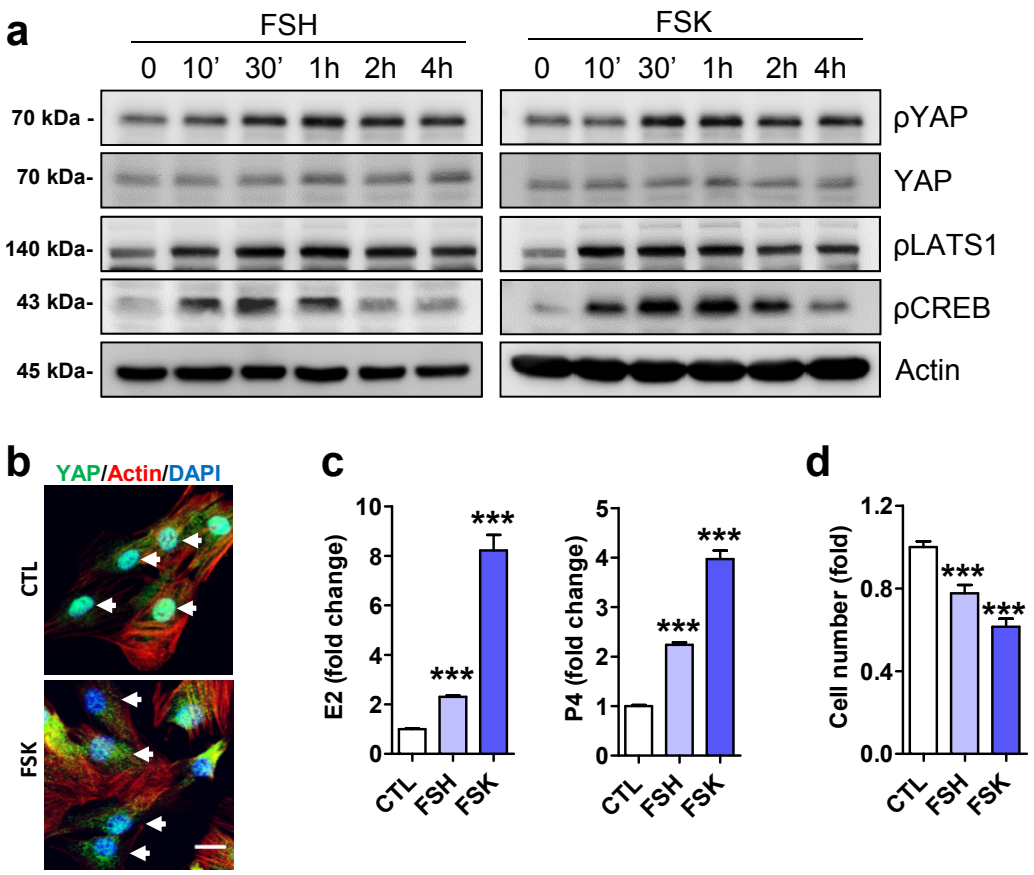
Supplementary figure S4. YAP plays important role in regulating KGN cell survival. a) Representative pictures showing Annexin V-FITC/Propidium Iodide (PI) double staining of KGN-MX, KGN-YAP, and KGN-YAP^{S127A} cells after serum starvation for 4 days. **b**) Quantitative results of **a**). Each bar represents mean + SEM of 3 independent experiments. Bars with different letter are significantly different from each other ($P < 0.05$). **c**) *BCL2*, *BRIC5*, *BAX*, *BAD*, and *BAK1* mRNA expression levels in KGN-MX, KGN-YAP, KGN-YAP^{S127A} cells were detected by RT-PCR. Each bar represents the mean ± SEM (n ≥ 3). Bars with different letters are significantly different from each other ($P < 0.05$).

Supplementary Figure



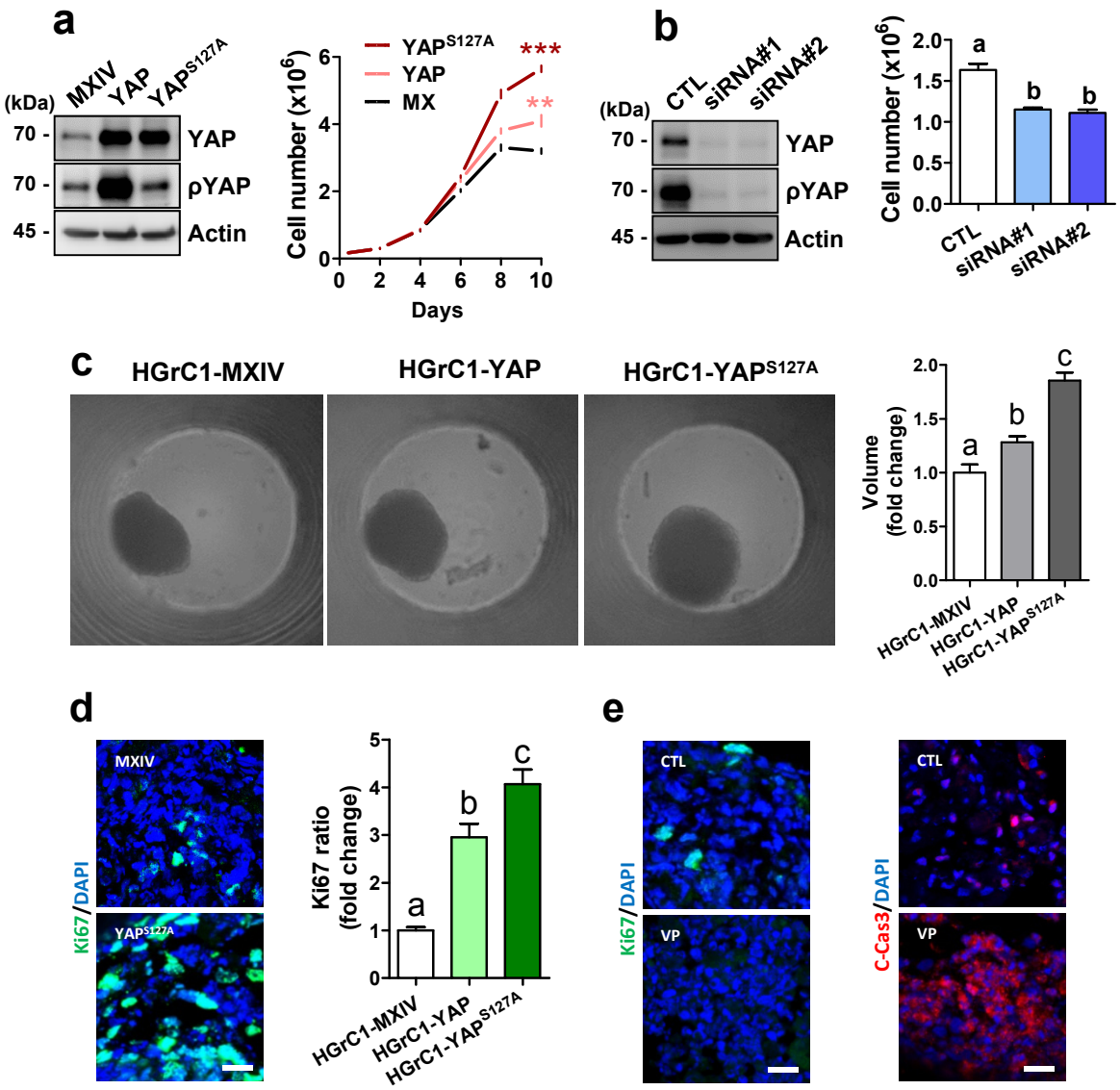
Supplementary figure S5. YAP plays important role in the proliferation and apoptosis of KGN cells. a) YAP inactivation by verteporfin inhibits KGN cell growth: the bar graph showing KGN cell number after incubating in growth medium with increasing concentrations of verteporfin for 5days. Each bar represents the mean ± SEM (n ≥ 3). Bars with different letters are significantly different from each other ($P < 0.05$). **b)** Western blot results showing expression changes of cell proliferation markers and apoptosis markers with different concentration of verteporfin treatment for 5 d.

Supplementary Figure



Supplementary figure S6. FSH and FSK activated the Hippo pathway to suppress YAP activity and induced hormone production, and inhibited cell proliferation. a) FSH (10 ng/ml) and forskolin (FSK, 10 μM) treatment induced phosphorylation of YAP and LATS1 in KGN cells. Phosphorylated CREB was used as a positive control of FSH and FSK action. β-actin was used as a protein loading control. **b)** FSK treatment (10 μM, 60') suppresses YAP activity by retaining YAP in the cytoplasm. YAP protein was stained by fluorescent immunohistochemistry and visualized by a Alexa 488-conjugated second antibody (green). Actin was stained with phalloidin-rodhamine (red). Nuclei was stained by DAPI (blue). Scale bar = 20 μm. **c)** Bar graphs showing production of 17β-estradiol (E2) and progesterone (P4) in KGN cells incubated in growth media for 3 days in the absence (CTL) or presence of FSH (10 ng/ml) or FSK (10 μM). **d)** Bar graphs showing growth of KGN cells incubated for 3 days in the growth media in the absence (CTL) or presence of FSH (10 ng/ml) or FSK (10 μM). Each bar represents the mean ± SEM (n ≥ 3). *** : $P < 0.001$, compared with control (CTL).

Supplementary Figure



Supplementary figure S7. YAP1 promotes proliferation and supports survival of HGrC1 cells in both 2D and 3D culture systems. a) Ectopic expression of YAP1 promotes HGrC1 cell proliferation. Left panel: Western blot showing YAP1 expression in HGrC1-MXIV, HGrC1-YAP, and HGrC1-YAP^{S127A} cells; right panel: growth curves of three cell lines. Each point represents the mean ± SEM (n = 4). **: p <0.01, ***: P < 0.001, compared to the control group in the same day (day 10). **b)** knockdown of YAP1 suppresses growth of HGrC1 cells. Left panel: Western blot showing knockdown of YAP1 protein with YAP1-targeting siRNA#1 and siRNA#2; right panel showing cell number changes in HGrC1 cells with or without knockdown of YA:P1. HGrC1 cells were transfected with non-targeting RNA (CTL) or YAP siRNA#1 and siRNA#2. Cell number was counted 4d after transfection. Each bar represents the mean ± SEM (n = 4). Bars with different letter are significantly different from each other (P < 0.05). **c)** Representative pictures showing the spheroids formed by HGrC1-MXIV, HGrC1-YAP, and HGrC1-YAP^{S127A} cells in the 3D culture system after loading onto a 3D culture system for 5 days; The bar graph showing relative volume of spheroids. Scale bar: 200 μm. **d)** Representative pictures showing Ki67 staining with spheroids formed by HGrC1-MX and HGrC1-YAP^{S127A} cells; Scale bar: 20 μm. bar graph showing ratio of Ki67 positive cells. Each bar represents the mean ± SEM (n ≥ 4). Bars with different letters are significantly different from each other (P < 0.05). **e)** Representative images showing Ki67 (green) and cleaved Caspase 3 (red) expression in spheroids formed by HGrC1 cells cultured in the absence (CTRL) or presence (VP) of verteporfin (2μM) for 5 days. Scale bar: 20 μm.

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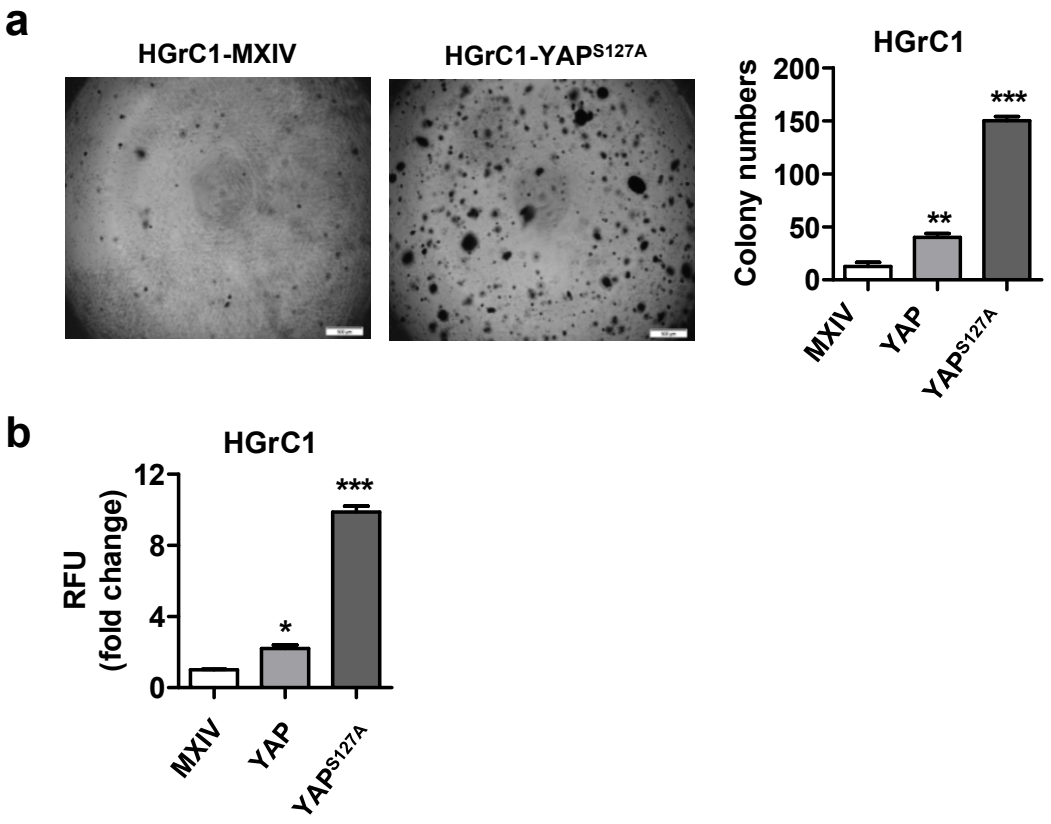
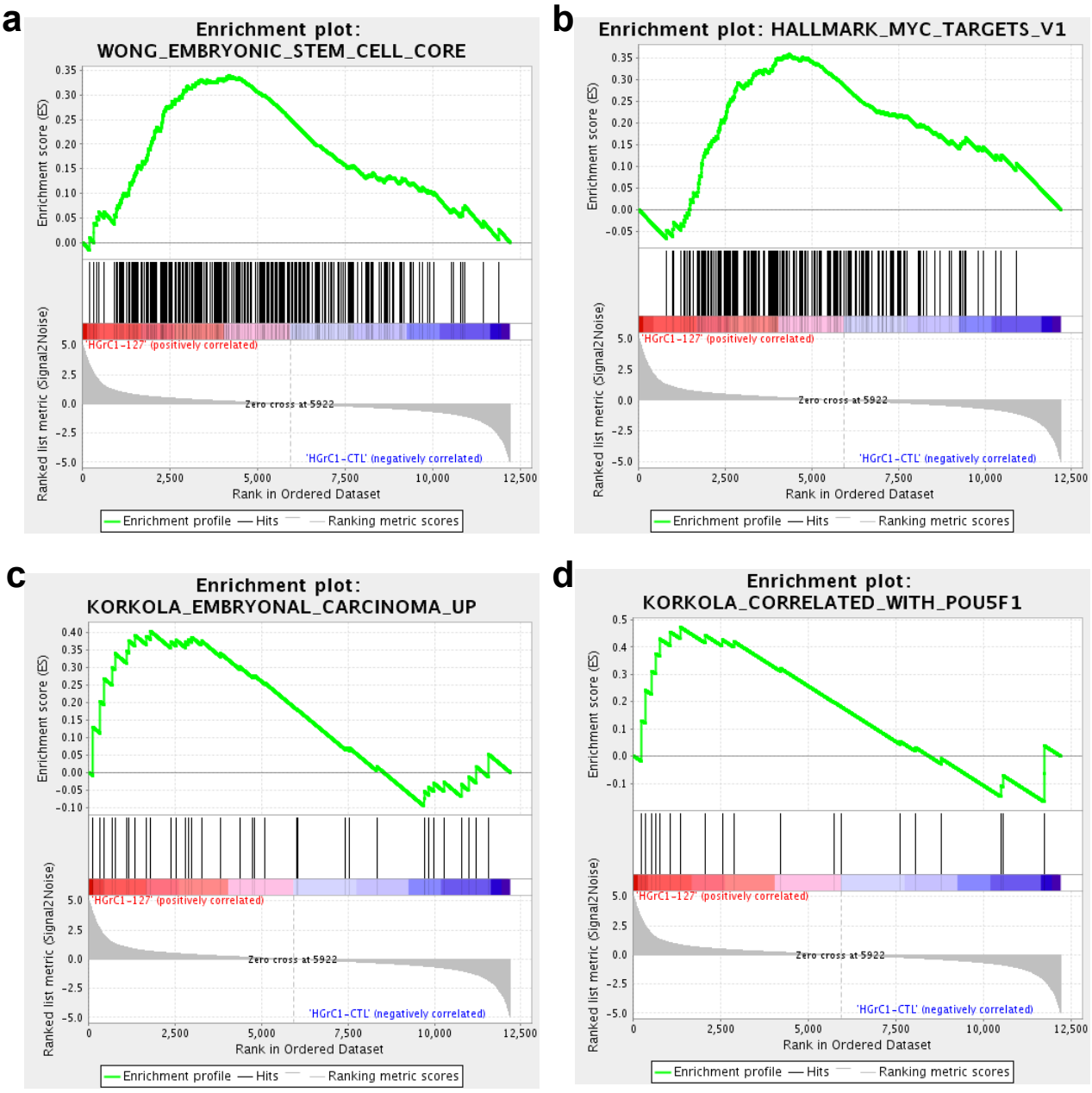
Supplementary Figure



Supplementary figure S8. YAP1 promotes transformation of HGrC1 granulosa cells. a) Representative images showing colonies formed by HGrC1-MX and HGrC1-YAP^{S127A} cells in the soft agar assay. Scale bar: 500 μm. Bar graph on the right showing the quantitative data of colonies formed by HGrC1-MX, HGrC1-YAP, and HGrC1-YAP^{S127A} cells in the soft agar assay**. b**) Relative colony numbers formed by HGrC1-MX, HGrC1-YAP, and HGrC1-YAP^{S127A} cells in a fluorescence-based semi-quantitative colony formation assay. The relative colony number in each cell lines is reflected by relative fluorescent unit (RFU). Each bar represents the mean ± SEM (n ≥ 3), when compared with MXIV control, *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$.



Supplementary figure S9. YAP1 induced enrichment of genes associated with cellular reprogramming and stemness in HGrC1 cells. Gene Set Enrichment Analysis (GSEA) was performed based on the gene expression profiling on HGrC1-MX (HGrC1-CTL, n=3) and HGrC1-YAPS127A cells (n=3) using RNA-seq analysis. **a**) Enrichment of genes-associated with embryonic stem cells from the WONG EMBRYONIC STEM CELL CORE gene sets. **b**) Enrichment of genes-associated with cell reprogramming from the HALLMARK MYC TARGETS-V1 gene sets. **c**) Enrichment of genes-associated with development of undifferentiated cancers from the KORKOLA EMBRYONAL CARCINOMA–UP gene sets. **d**) Enrichment of genes-associated with cell reprogramming (Oct4) from the KORLOLA_CORRELATED_WITH_POU5F1 gene sets.

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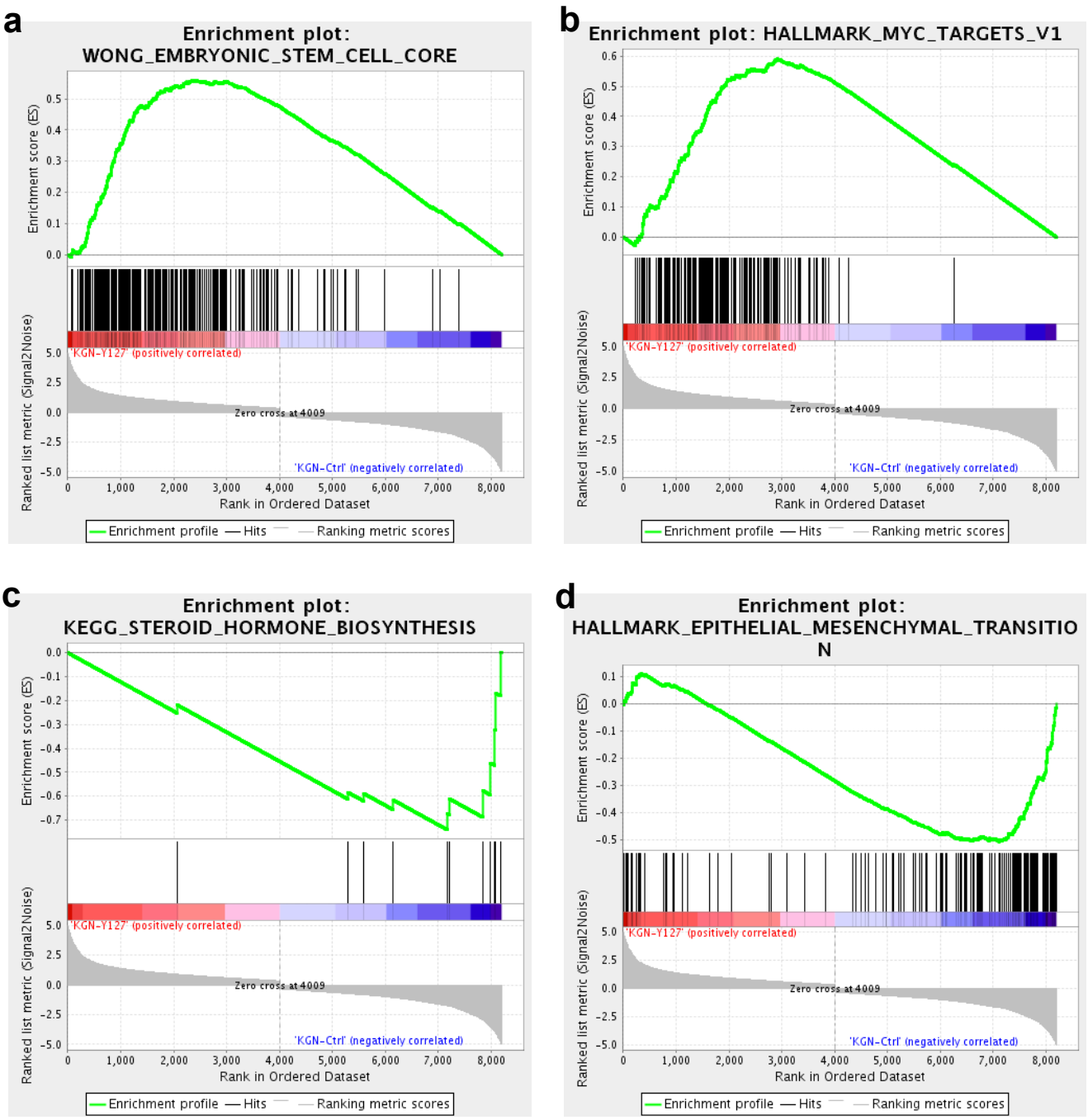

Supplementary figure S10. YAP1 induced enrichment of genes associated with cellular reprogramming and stemness in KGN cells. Gene Set Enrichment Analysis (GSEA) was performed based on the gene expression profiling on KGN-MX (HGrC1-CTL, n=3) and KGN-YAPS127A cells (n=3) using RNA-seq analysis. **a**) Enrichment of genes-associated with embryonic stem cells form the WONG EMBRYONIC STEM CELL CORE gene sets. **b)** Enrichment of genes-associated with cell reprogramming from the HALLMARK MYC TARGETS-V1 gene sets. **c**) Enrichment of genes-associated with granulosa cell differentiation from the KEGG_STEROID-HORMONE_BIOSYNTHESIS gene sets. **d)** Enrichment of genes-associated with cell reprogramming (MET) from the HALLMARK EPITHELIAL MESENCHYMAL TRANSITION gene sets.

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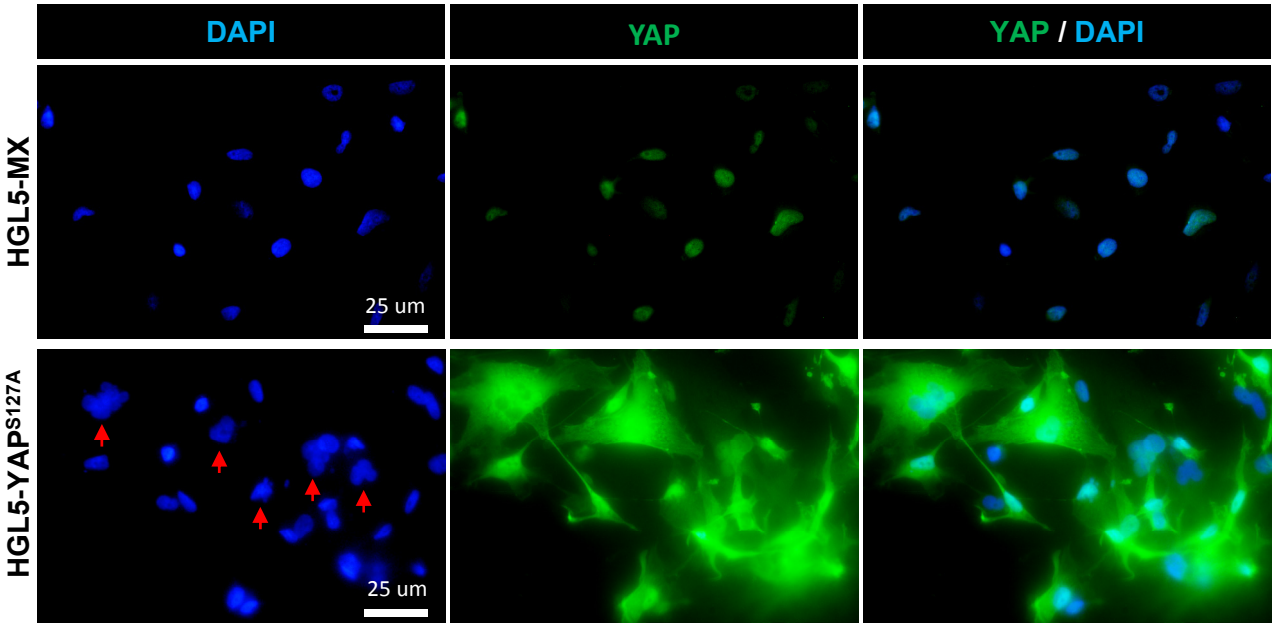
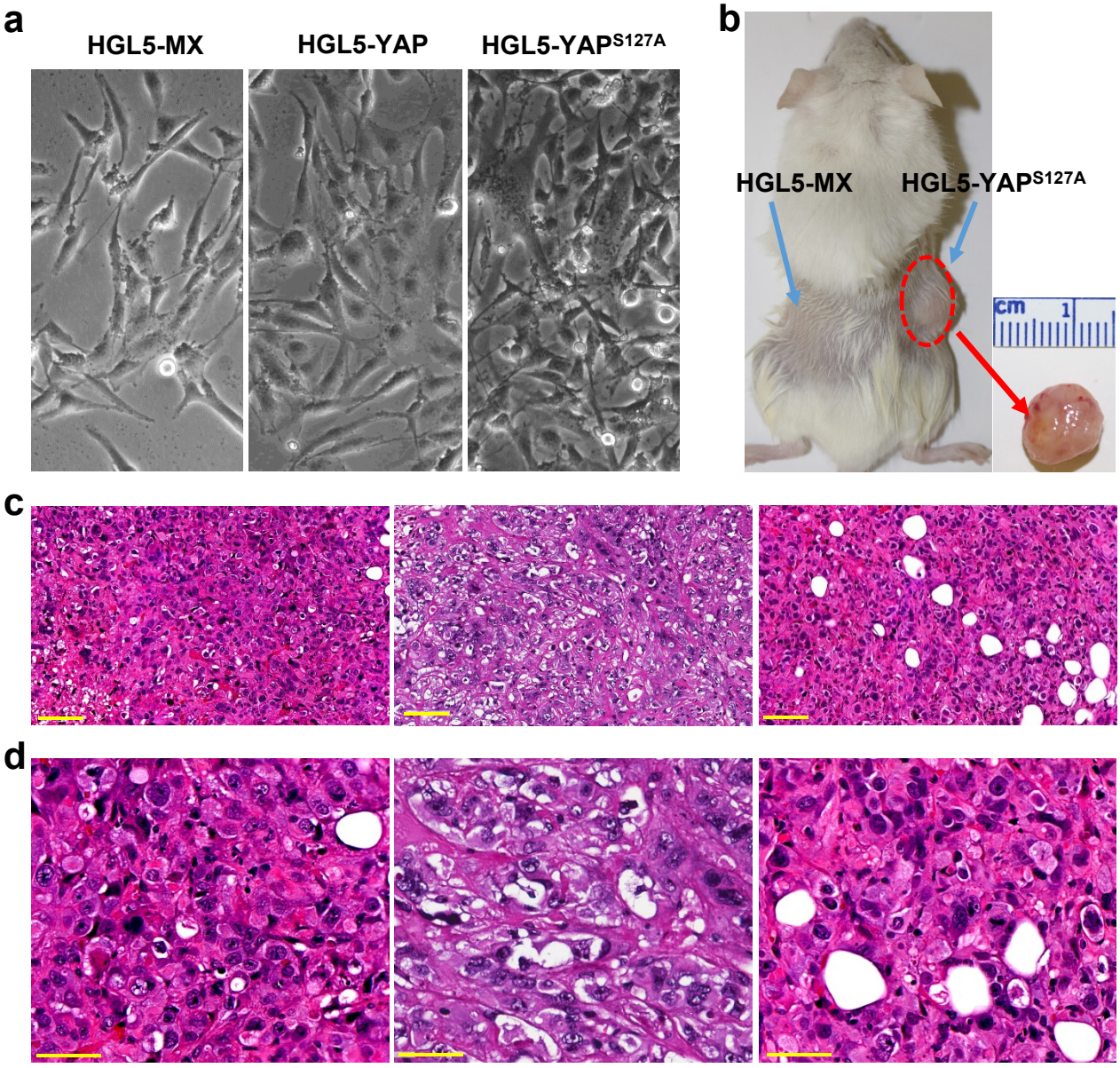
Supplementary Figure



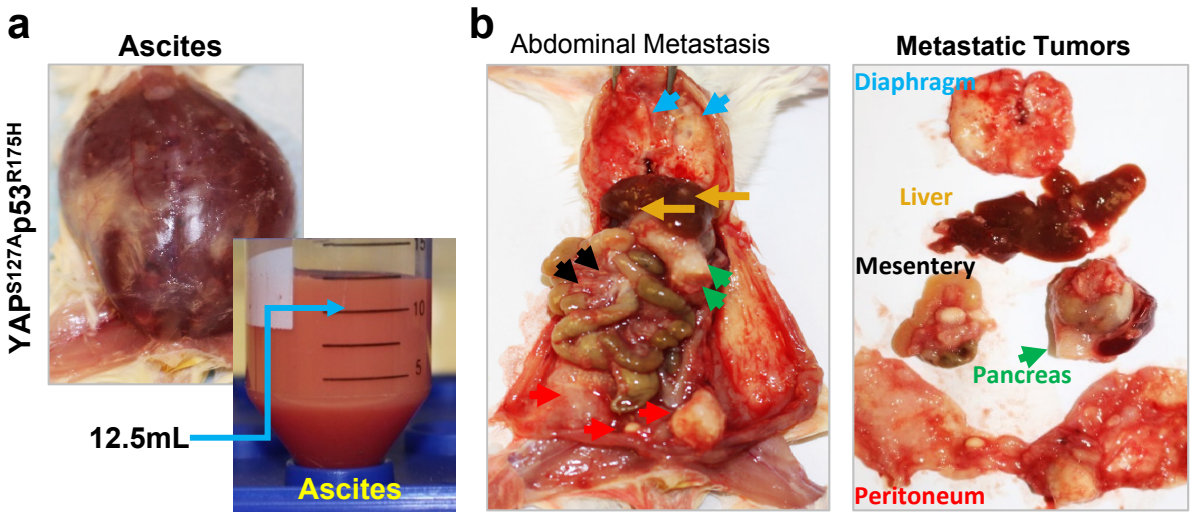
Supplementary figure S11. Hyperactivation of YAP1 in HGL5 cells induced significant nuclear atypia. Green signal indicated YAP1 expression detected by fluorescent immunohistochemistry in HGL5-MX (HGL5 cells transfected with empty MXIV vectors as control) and HGL5-YAP^{S127A} cells (HGL5 cells transfected with vectors expressing YAP^{S127A}, a constitutively active form of YAP1). Nuclei were stained with DAPI (blue). Scale bar: 25μm.

Supplementary Figure



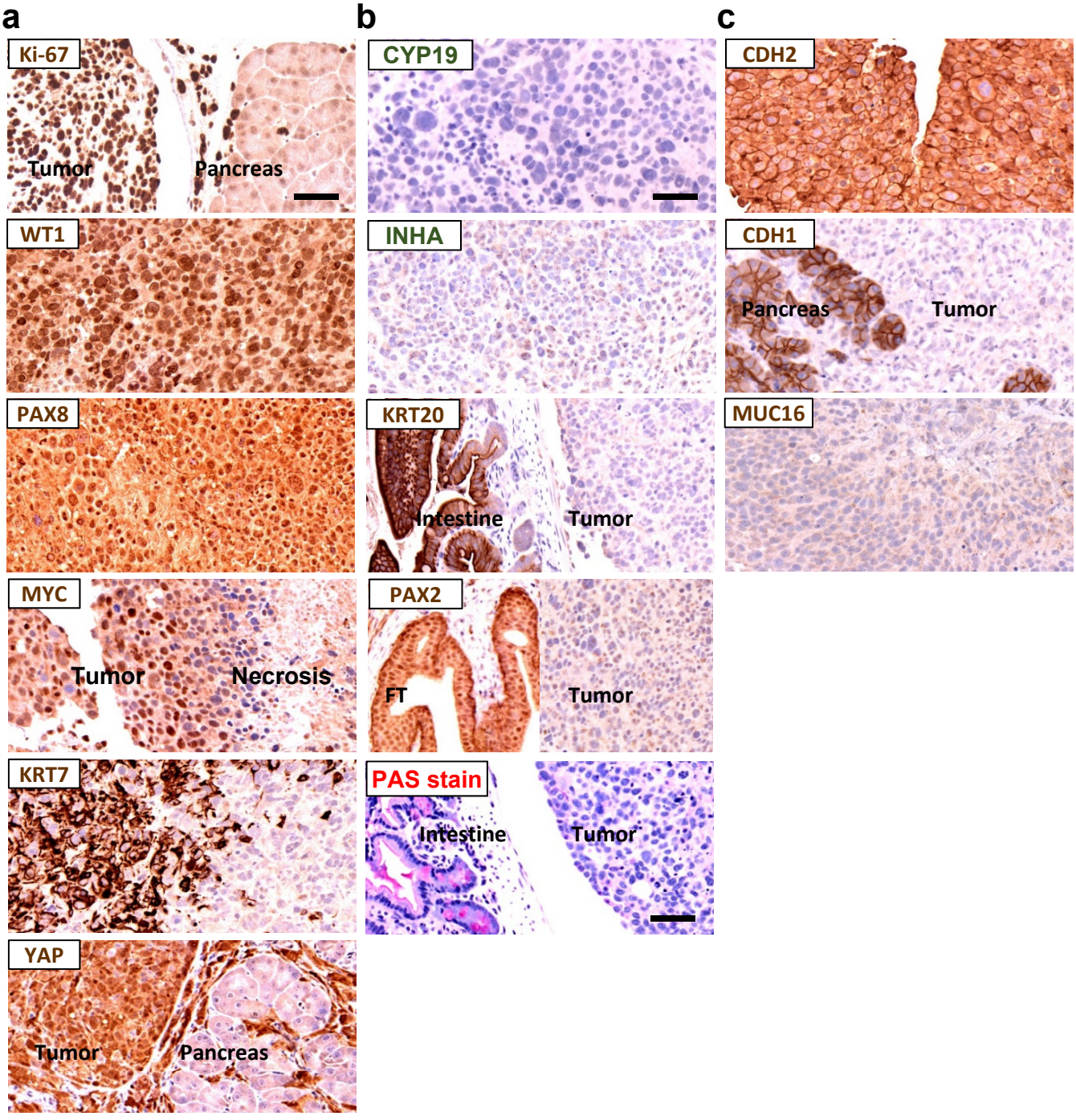
Supplementary figure S12. Hyperactivation of YAP1 induced tumorigenesis in the HGL5 cells. **a**) Morphology of cultured HGL5-MX cells (HGL5 cells transfected with empty MXIV vectors as control), HGL5-YAP cells (HGL5 cells transfected with vectors expressing YAP1), and HGL5-YAP^{S127A} cells (HGL5 cells transfected with vectors expressing YAP^{S127A}, a constitutively active form of YAP1). Please note the rapid multiple layer growth of HGL5-YAP^{S127A} cells. Scale bar: 20μm. **b**) HGL5-YAP^{S127A} cells, not HGL5-MX cells, formed tumor in NGS mice. **c**) representative low magnification images showing the histology of tumors derived from HGL5-YAP^{S127A} cells. Note the nuclear pleomorphism and formation of microcyst. Scale bar: 100μm. **d**)) Representative high-resolution images showing the histology of tumors derived from HGL5-YAP^{S127A} cells. Note the high pleomorphic nuclei, large with coarsely clumped chromatin, and numerous abnormal mitotic figures. Scale bar: 50μm

Supplementary Figure



Supplementary figure S13. Introduction of *TP53*^{R175H} did not affect progression of tumors derived from HGrC1-YAP^{S127A} cells. **a)** Representative images showing accumulation of large amount of ascites in the abdomen of mice carrying tumors derived from HGrC1-YAP^{S127A}/TP53^{R175H} cells. **b)** Representative images showing metastatic spread of tumors derived from HGrC1-YAP^{S127A}/TP53^{R175H} cells in the mouse abdomen organs and tissues, including peritoneum (red arrowhead), mesentery (black arrowhead), diaphragm (blue arrowhead), pancreas (green arrowhead), and liver (orange arrowhead).

Supplementary Figure



Supplementary figure S14. YAP elicits ovarian HGSC-like tumor from HGrC1 cells. a) Molecular features of tumors derived from HGrC1-YAP^{S127A} cells. Representative images showing high expression of Ki-67, WT-1, PAX8, MYC, Keratin 7 (KRT7) and YAP1 proteins in tumor tissues detected by immunohistochemistry. Scale bar: 50 μm. **b)** Representative images showing that tumor tissues are negative for granulosa cell tumor markers (aromatase, inhibin), low-grade serous carcinoma markers (KRT20, PAX2), and PAS staining (Mucinous ovarian carcinoma). **c**) Representative images showing that tumor tissues express high level of N-cadherin (CDH2), but very low (or undetectable) levels of E-cadherin (CDH1) and CA125 (MUC15), which is the molecular feature of the mesenchymal type of High-grade serous ovarian carcinoma. Scale bar: 50 μm.

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