Supplementary Data

Title: MEX3A mediates p53 degradation to suppress ferroptosis and facilitate ovarian cancer tumorigenesis

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Table S1. Short tandem repeat of ovarian teratocarcinoma and clear cell carcinoma cell line.							
STR\Cell Line	**PA-1**	**TOV21G**	**JHOC9**	**RMG-1**	**RMG-2**	**OVISE**	**OVCA-429**
TH01	7,9	8,11	6,9	6,7	6,7	9,9.3	9,9
D5S818	11,11	12,13	10,10	12,12	12,12	10,10	11,12
D13S317	9,10	11,12	10,10	8,12	8,12	11,12	7,12
D7S820	9,9	12,12	8,12	11,11	11,11	11,12	11,12
D16S539	9,12	10,12	12,12	9,10	9,10	9,9	12,12
CSF1PO	9,12	13,15	10,13	10,10	10,10	9,11	12,13
Amelogenin	X,X	X,X	X,X	X,X	X,X	X,X	X,X
VWA	15,17	17,17	17,17	17,18	17,18	18,18	16,18
TPOX	11,11	8,11	8,11	11,11	11,11	8,8	9,11
Match to Test Sample	100%	100%	94%	94%	100%	95.65%	100%
Database	DSMZ	DSMZ	DSMZ	DSMZ	DSMZ	ExPASy	ExPASy

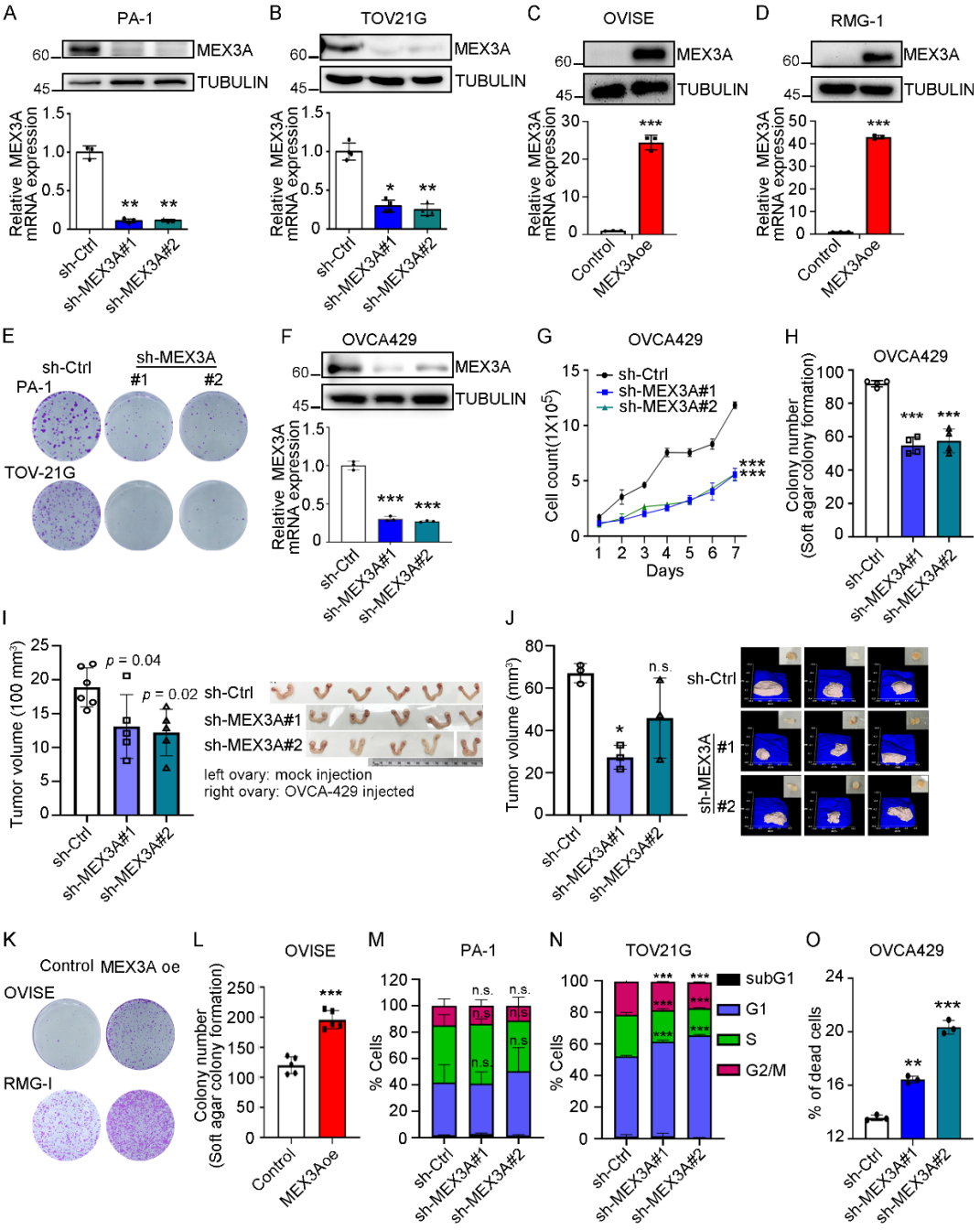

Figure S1. MEX3A expression promoted tumorigenic ability and cell survival in WT p53 OC cells.

(**A, B**) MEX3A protein and mRNA level in PA-1 (A) and TOV21G (B) cells without (sh-Ctrl) or with MEX3A depletion (sh-MEX3A#1 or sh-MEX3A#2) detected by IB and qRT-PCR analyses, respectively. TUBULIN was used as an IB loading control. Blots shown are from one representative experiment of three replicates. RNA18S5 was used as a qRT-PCR internal control. Three independent experiments were performed and data are means ± SD from one representative experiment (n =3). Significant differences are based on unpaired T-test.

(**C, D**) MEX3A protein and mRNA level in OVISE (C) and RMG-1 (D) cells without

(Control) or with MEX3A overexpression detected by IB and qRT-PCR analyses. Data formatting is as described for A.
(**E**) Representative images of clonogenic assay using sh-Ctrl and MEX3A-depleted PA-1 and TOV21G cells.
(**F**) MEX3A protein and mRNA level in OVCA-429 cells without (sh-Ctrl) or with MEX3A depletion (sh-MEX3A#1 or sh-MEX3A#2) detected by IB and qRT-PCR analyses. Data formatting is as described for A.
(**G**) Cell growth assays using MEX3A-depleted (#1 or #2) OVCA-429 cells. Data are shown as mean ± SD with *p* value based on two-way ANOVA-test (n=3, ***$p < 0.001$). The experiments were repeated three times.
(**H**) Soft-agar colony forming assays using MEX3A-depleted (#1 or #2) OVCA-429 cells. Data are shown as mean ± SD with *p* value based on unpaired T-test (n=4, ***$p < 0.001$). The experiments were repeated two times.
(**I**) Orthotopic xenograft models in NOD/SCID mice using control (sh-Ctrl, n=6) or MEX3A-depleted (sh-MEX3A, n=5) OVCA-429 cells. Data are means ± S.D., significant difference is based on unpaired T-test of the tumor size six weeks after the *intra bursa* injection.
(**J**) Subcutaneous xenograft models in NOD/SCID mice using sh-Ctrl or sh-MEX3A OVCA-429 cells (n=3). Photographs and 3D reconstruction from stereographic tumor images are shown. Data are means ± S.D., significant difference is based on unpaired T-test of the tumor size three weeks after the injection.
(**K**) Representative images of clonogenic assay using MEX3A-overexpressing OVISE and RMG-1 cells.
(**L**) Soft-agar colony forming assays using MEX3A overexpressing OVISE cells. Data are shown as mean ± SD with *p* value based on unpaired T-test (n=5, ***$p < 0.001$). The experiments were repeated two times.
(**M**) Cell cycle EdU staining analysis using sh-Ctrl and MEX3A-depleted PA-1 cells. Data are shown as mean ± SD with *p* value based on unpaired T-test (n=3, *$p < 0.05$, **$p < 0.01$). The experiments were repeated three times.
(**N**) Cell cycle EdU staining analysis using sh-Ctrl and MEX3A-depleted TOV21G cells. Data formatting is as described for K.
(**O**) Annexin V/ PI staining assays using MEX3A-depleted (#1 or #2) OVCA-429 cells. Data are shown as mean ± SD with *p* value based on unpaired T-test (n=3, *$p < 0.05$, **$p < 0.01$). The experiments were repeated three times.

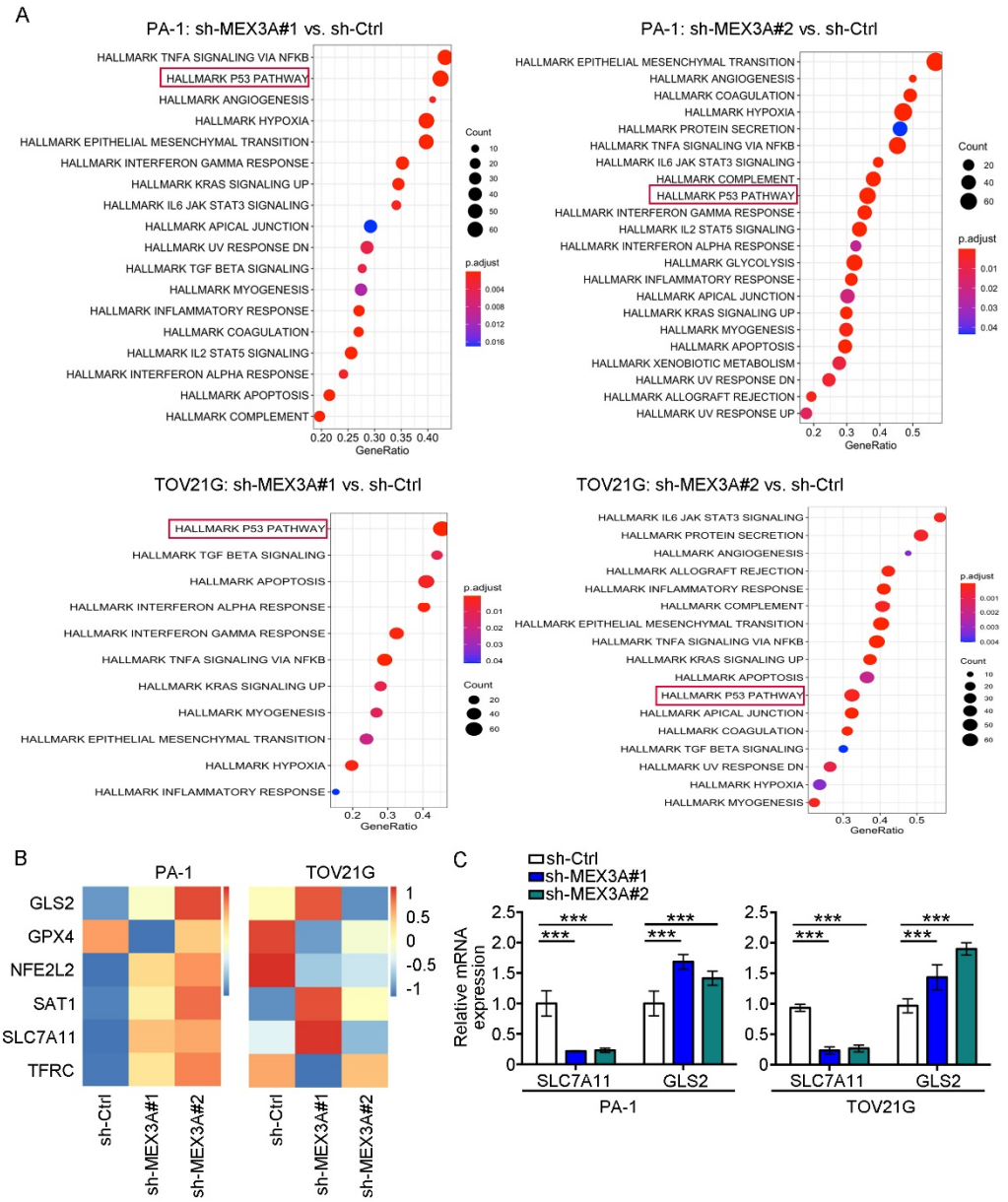

Figure S2. The p53 pathway was enriched in upregulated genes in MEX3A-depleted OC cells expressing WT p53.

(**A**) Gene Set Enrichment Analysis (GSEA) of upregulated genes in MEX3A-depleted PA-1 and TOV21G cells. The p53 pathway was found in the top 15 enriched pathways in all MEX3A-depleted group.

(**B**) Heatmap of ferroptosis related genes in PA-1 and TOV21G cells without or with MEX3A depletion.

(**C**) qRT-PCR analysis of SLC7A11 and GLS2 expression in control (sh-Ctrl) or MEX3A-depleted (sh-MEX3A) PA-1 and TOV21G cells. RNA18S5 was used as an internal control. Three independent experiments were performed and data are means ± SD from one representative experiment (n =3). Significant differences are based on unpaired T-test (***$p < 0.001$).

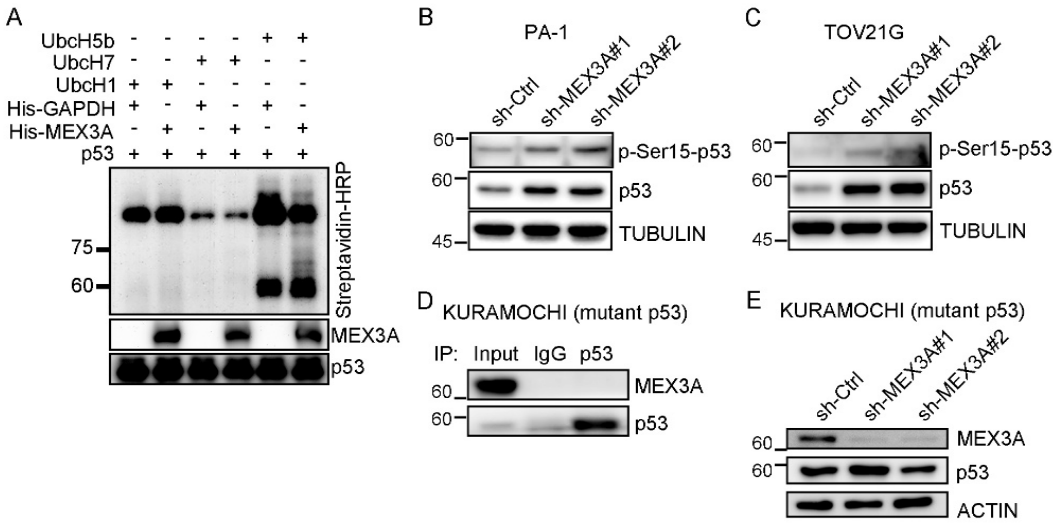

Figure S3. Stabilized WT p53 protein in MEX3A-depleted cells was activated. MEX3A did not interact with or affect the level of mutant p53 protein.
(**A**) *In vitro* ubiquitination of p53 by MEX3A. Purified recombinant p53 protein was incubated with purified E1, E2 UbcH5b, UbcH7 or UbcH1 as well as His-tagged GAPDH or His-tagged MEX3A protein as indicated. The reaction was subjected to anti-Streptavidin, anti-MEX3A and anti-p53 immunoblotting.
(**B, C**) Phosphorylated and total p53 protein in PA-1 (B) and TOV21G (C) cells without (sh-Ctrl) or with MEX3A depletion (sh-MEX3A#1 or sh-MEX3A#2) detected by IB. TUBULIN was used as an IB loading control. Blots shown are from one representative experiment of three replicates.
(**D**) Co-IP assays of MEX3A and mutant p53 using KURAMOCHI cells. Cell lysates were IP with indicated antibodies and analyzed by IB assay. Normal IgG was used as an IP control.
(**E**) MEX3A and p53 protein expression detected by IB using control (sh-Ctrl) or MEX3A-depleted (sh-MEX3A#1 or sh-MEX3A#2) KURAMOCHI cells. ACTIN was used as a loading control. Blots shown are from one representative experiment of three replicates.

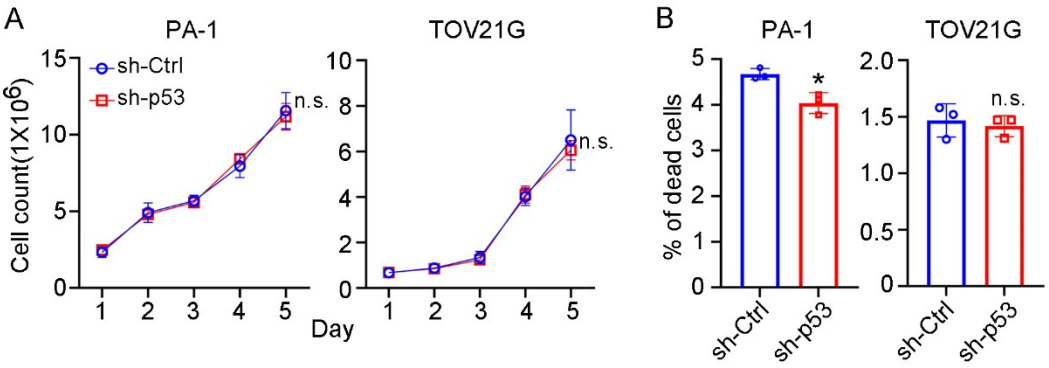

Figure S4. p53 depletion alone did not significantly affect cell proliferation or cell death in MEX3A expressing WT p53 OC cells.
(**A**) Cell growth assays using p53-depleted PA-1 and TOV21G cells. Data are shown as mean ± SD with *p* value based on two-way ANOVA-test (n=3, n.s., not significant). The experiments were repeated three times.
(**B**) Annexin V/ PI staining assays using p53-depleted PA-1 and TOV21G cells. Data are shown as mean ± SD with *p* value based on unpaired T-test (n=3, $*p < 0.05$; n.s., not significant). The experiments were repeated three times.

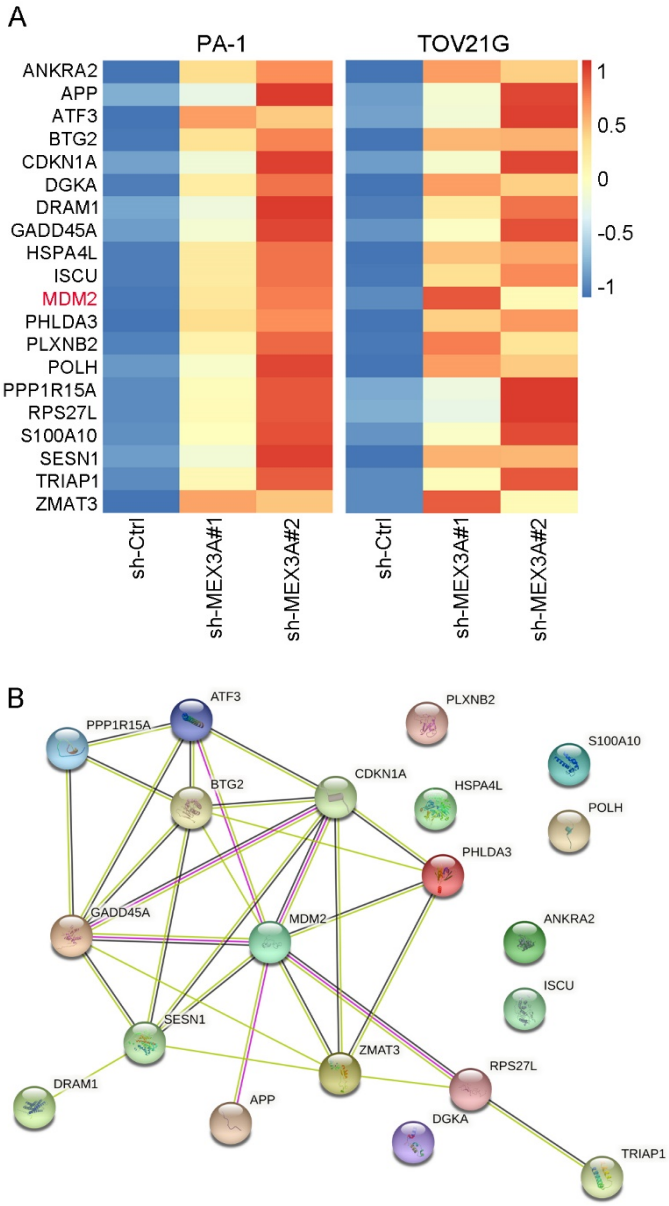

Figure S5. MEX3A and MDM2 in OC.
(**A**) Heatmap of up-regulated genes related to the p53 pathway identified in MEX3A-depleted PA-1 and TOV21G cells.
(**B**) STRING network plot of MDM2 centered protein-protein interactome enriched in MEX3A-depleted cells.