

Figure S1 Verification of *Vdr* knockout by genotyping in mice and KGN cells

(A) mRNA levels by real-time RT-PCR relative to *β-actin* mRNA in ovaries from young and aged mice (n=3). (B) Western blots of GCs proteins showing p53, p21, and *Vdr* expression in 2-month-old and 2-month-old with SPD or SAM treatment. Densitometric analysis was used to assess protein levels relative to *β-actin* (n=3). (C) Representative agarose gel analyses for cVKO mice genotype. (D) Schematic diagram of VKO KGN cells construction. (E) Representative agarose gel analyses for VKO KGN cells *Vdr* knockout identification (deletion length =111bp). (F) Western blots of VKO cell proteins showing *Vdr* expression. (G) Elisa for 25(OH)D₃ and 1,25(OH)₂D levels. (H) Western blots showing *Vdr* expression in KGN cells with or without 1,25(OH)₂D treatment. (I) Western blots of GCs proteins showing *Amh*, p21, and Ki67 expression in Control and cVKO mice. Statistical analysis was performed with an unpaired Student's t-test. Values are means ± SEM. *P < 0.05, **P<0.01, ***P < 0.001.

Figure S2 Aging downregulated the quantity and quality of mitochondria

(A) Heatmap showing changes of genes related to ETC and TCA in aged ovaries. (B) GSEA analysis showed abnormality of mitochondria in VKO cells. (C) Western blots of GCs proteins showing *Atp5a*, *Ndufb8*, *Drp1*, *mt-co1*, *Mfn2* and *Sdhb* expression in Control and cVKO mice. Statistical analysis was performed with an unpaired Student's t-test. Values are means ± SEM. *P < 0.05, **P<0.01, ***P < 0.001.

Figure S3 Loss of *Vdr* disrupts polyamine metabolism

(A) Heatmap showing differential metabolites in VKO cells. (B) Principal-component (PC) analysis of transcriptome data from VKO and WT cells. Blue circles represent samples from the VKO group, and red circles represent samples from the WT group. (C) Volcano plot displays differential metabolites (upregulated, red; downregulated, blue) in VKO cells compared to those from WT. (D) Differential abundance score analysis of differential metabolites (upregulated, blue; downregulated, red) from VKO cells compared to those from WT ones. (E) Predicted Vdr binding site in the *Odc1* promoter region. (F) Western blots of GCs proteins showing *Odc1*, *Ass1*, *Smox* and *Slc7a2* expression in Control and cVKO mice. Statistical analysis was performed with an unpaired Student's t-test. Values are means \pm SEM. *P < 0.05, **P<0.01, ***P < 0.001.

Figure S4 SPD treatment rescued aging induced by *Vdr* loss in the ovaries

(A) SPD levels in the indicated ovaries. (B) Immunofluorescence showing ovary staining for DDX4 and Ki67. (C) SA- β -gal staining for primary ovarian granulosa cells. (D) Immunofluorescence showing ovary staining for *Amh*. (E) Western blots of GCs proteins showing *Amh*, p21, and Ki67 expression in Control and cVKO mice. (F) Gross photograph of ovarian tissue. Statistical analysis was performed with one-way ANOVA for three group. Values are means \pm SEM. *P < 0.05, **P<0.01, ***P < 0.001.

Figure S5 SPD regulated GCs aging by modulating the DNA methylation through DNMTs

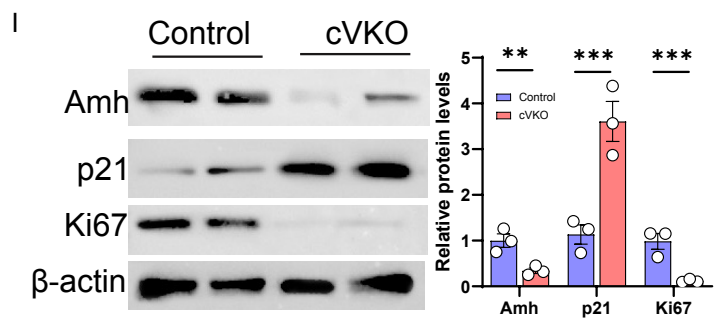
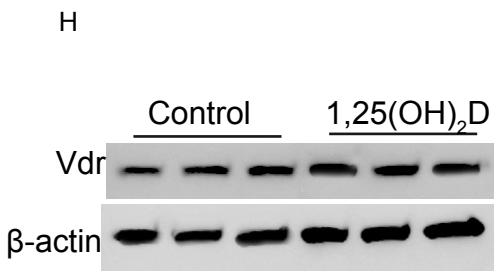
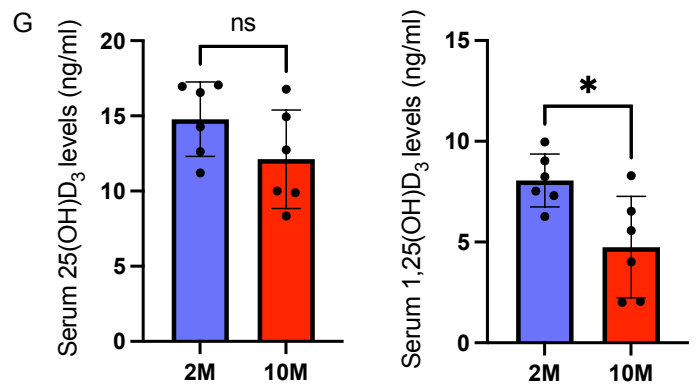
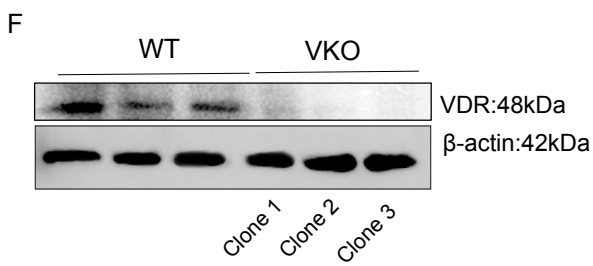
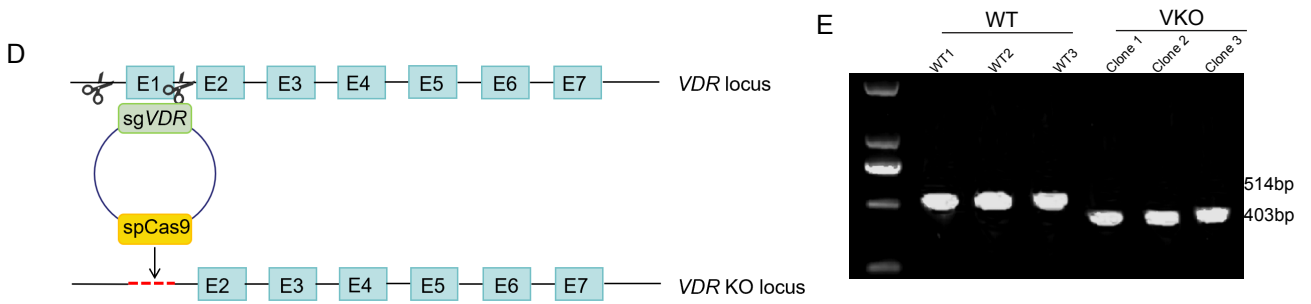
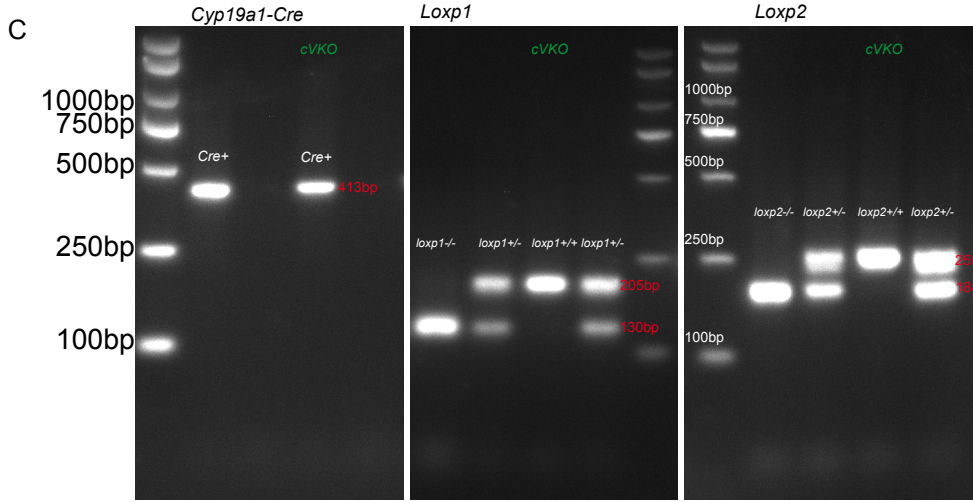
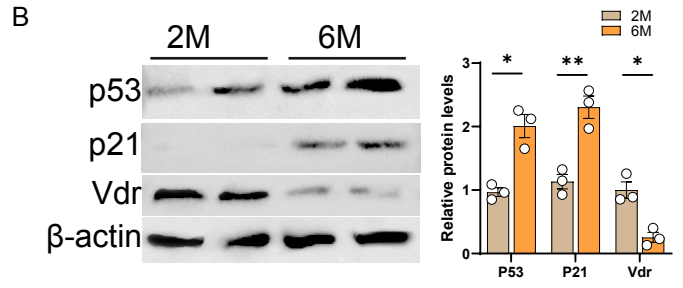
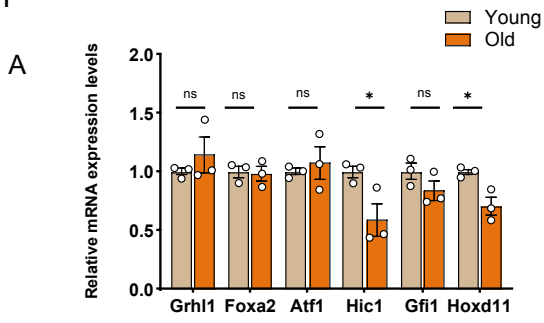
(A) Scatter plot showing correlation between *DNMTs* and *Gdf9* or *Ddx4* expression across transcriptomic datasets. (B) Western blots of KGN cell proteins showing p53, p21, and DNMTs expression. (C) Dot plots showing DNA methylation in KGN cells (n=5). Statistical analysis was performed with one-way ANOVA. Values are means \pm SEM. ***P < 0.001. (D) Western blots of KGN cell proteins showing p21 and p53 expression. Densitometric analysis was used to assess protein levels relative to β -actin (n=3). (E) SA- β -gal staining for KGN cells, and analysis for SA- β -gal positive cells (n=3). Statistical analysis was performed with an unpaired Student's t-test. Values are means \pm SEM. **P < 0.01. (F) Analysis of DNMTs activity in KGN cells (n=3). (G) Representative agarose gel analyses of MSP products from KGN cells and statistical analysis of MSP products. Values are means \pm SEM. **P < 0.01. (H) *p53* and *p21* mRNA levels by real-time RT-PCR relative to β -actin mRNA in KGN cells (n=3). (I) Western blots of KGN cell proteins showing p21, p53, and DNMT3A expression. Densitometric analysis was used to assess protein levels relative to β -actin (n=3). (J) *Amd1* mRNA levels by real-time RT-PCR relative to β -actin mRNA in KGN cells (n=3). Statistical analysis was performed with an unpaired Student's t-test. Values are means \pm SEM. *P < 0.05, **P < 0.01, ***P < 0.001.

Figure S6 SPD or SAM supplementation rescue ovarian aging

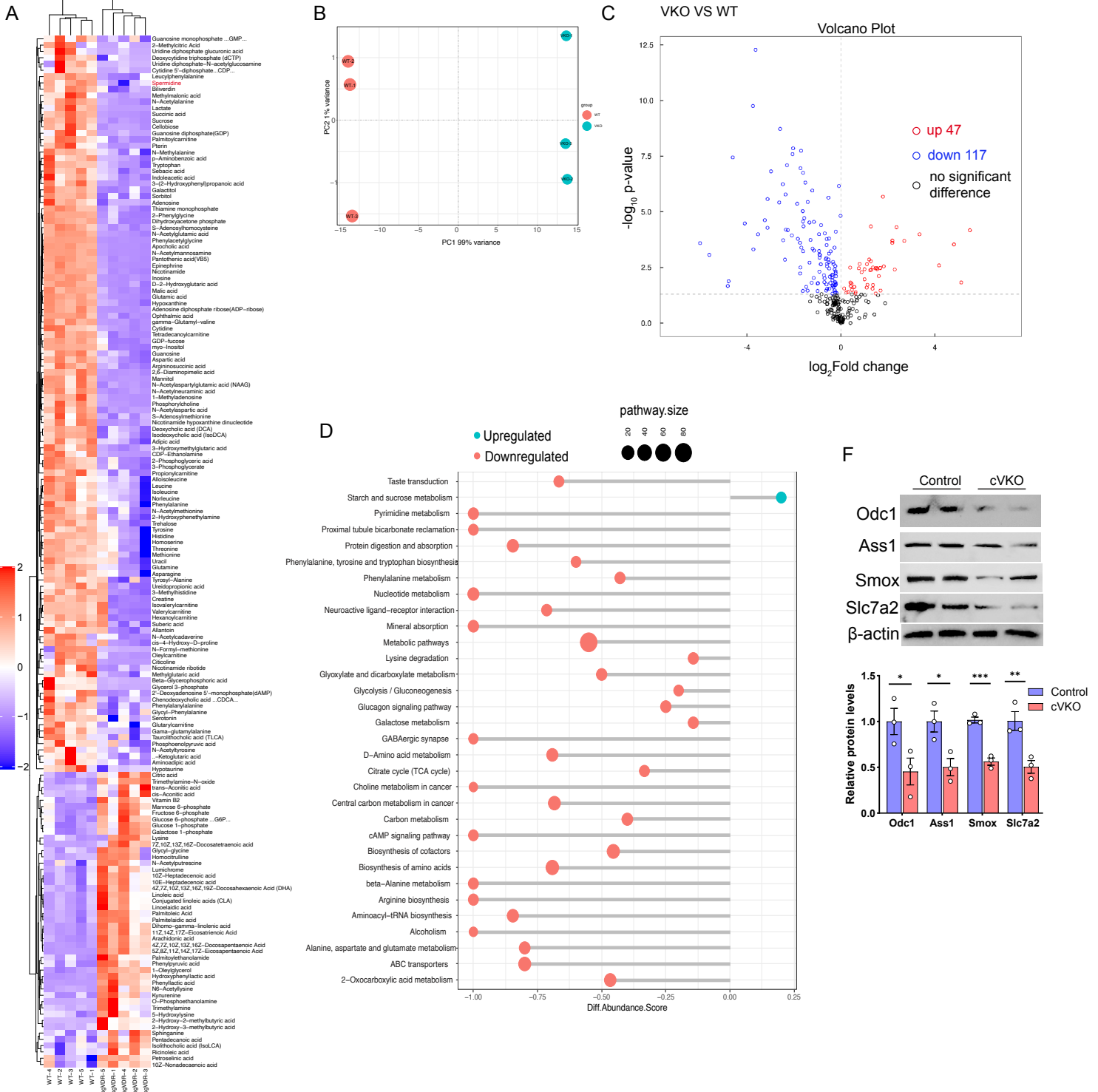
(A) Immunofluorescence showing ovary staining for Amh. (B) Western blots of GCs proteins showing p21, and Amh expression. Densitometric analysis was used to assess protein levels relative to β -actin (n=3). (C) Western blot of ovarian proteins showing

Vdr expression. (D) H&E staining showing ovaries culture in vitro, and analysis of max cross-sectional area. (E) Western blots of GCs proteins showing p53 and p21 expression. Densitometric analysis was used to assess protein levels relative to β -actin (n=3). (F) Gross photograph of ovarian tissue. Statistical analysis was performed with one-way ANOVA for three group. Values are means \pm SEM. **P<0.01, ***P < 0.001.

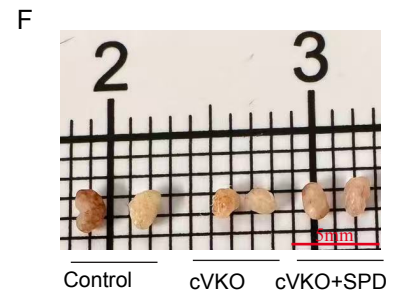
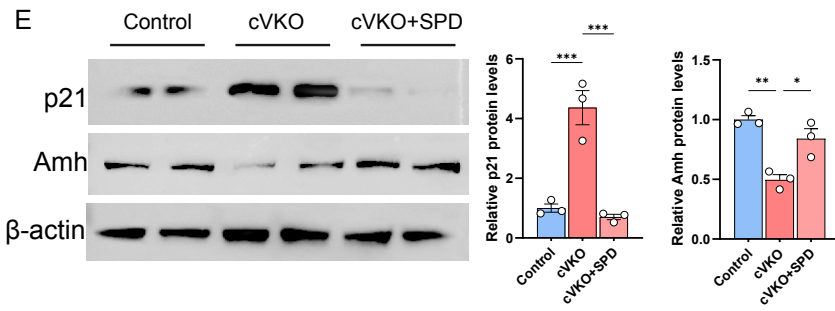
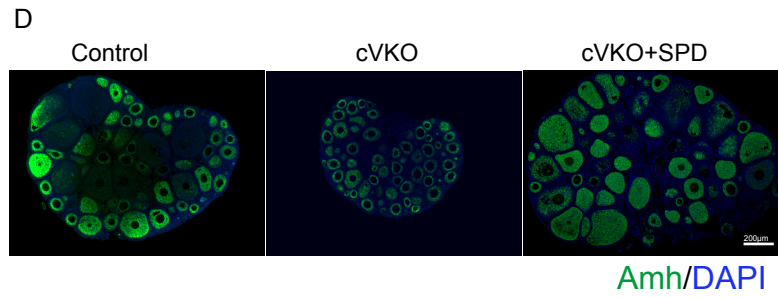
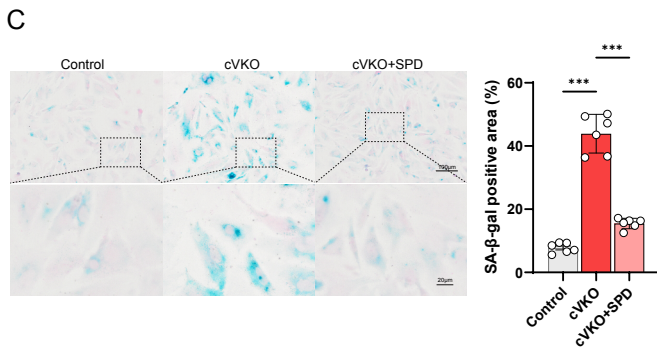
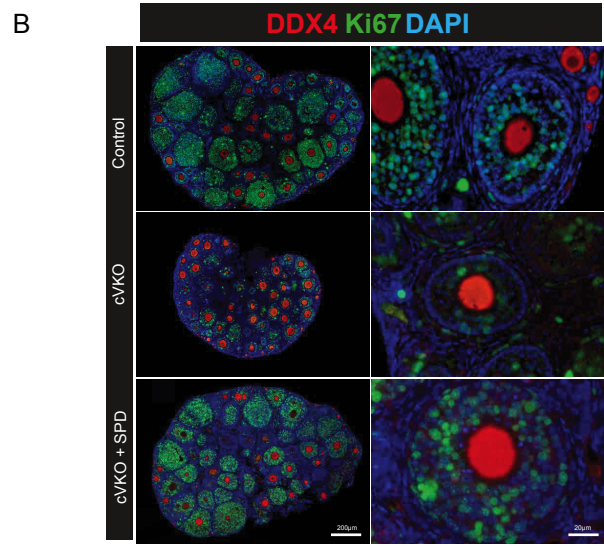
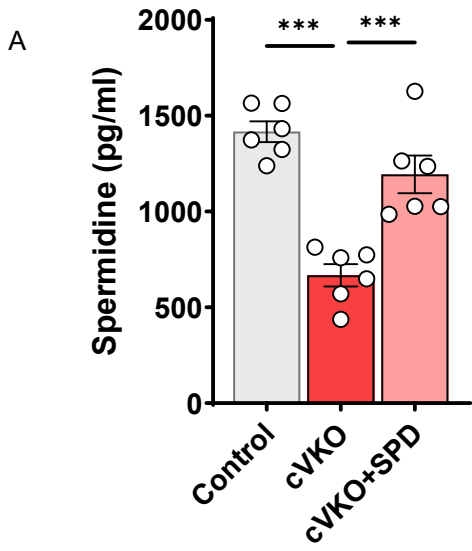
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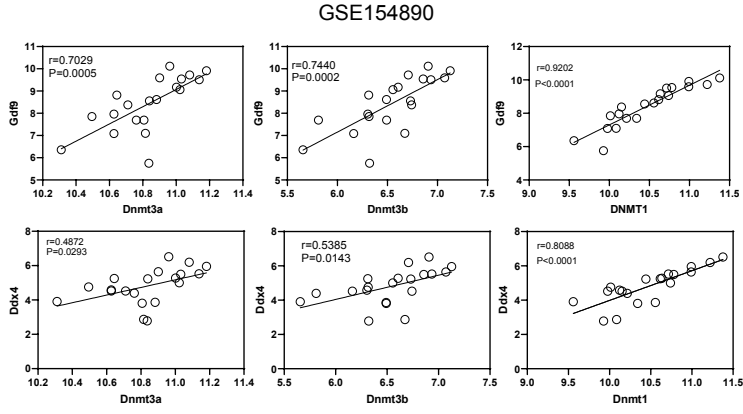
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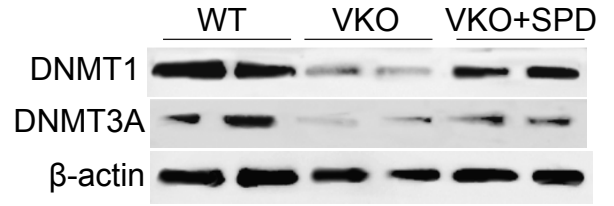
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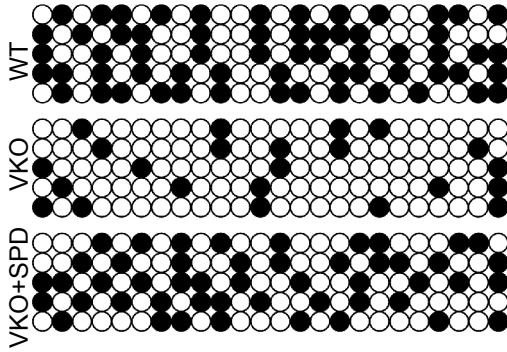
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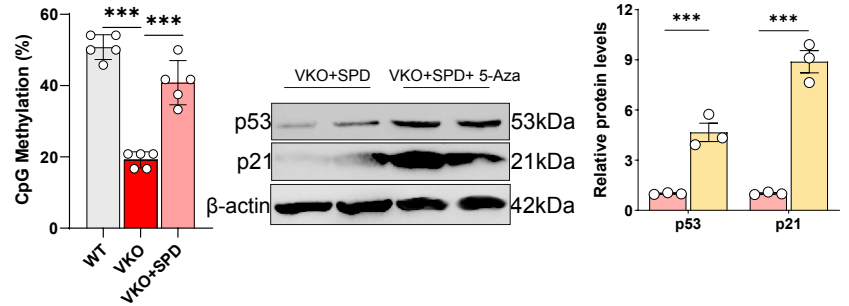
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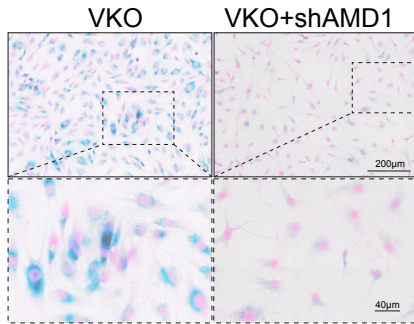
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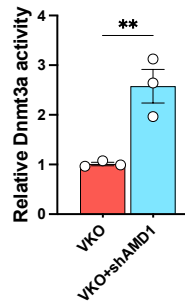
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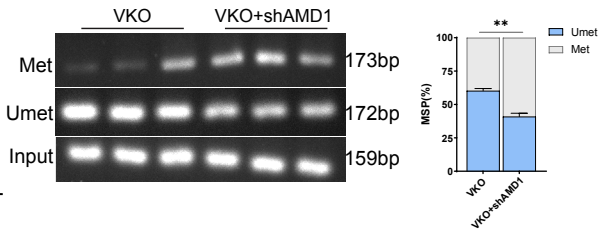
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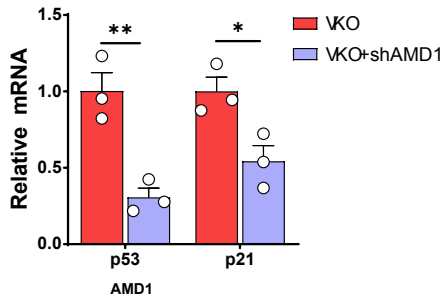
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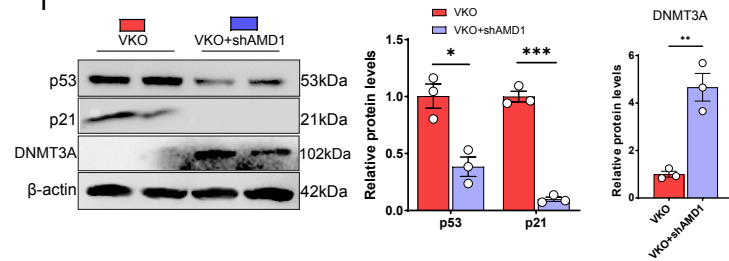
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