

Melatonin inhibits lipid accumulation to repress prostate cancer progression by mediating the epigenetic modification of CES1

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Running Head: Melatonin repress prostate cancer progression

Figures: 9 main figures; 2 supplementary tables; 6 supplementary figures

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Supplementary Figure Legends

Figure S1. MLT inhibits lipid accumulation in the blood and in PCa cells. (A-C) Flow diagram of the studies assessed by the systematic review and meta-analysis. (D) AR-V7 mRNA expression in prostate cancer cell lines as determined by real-time PCR. (E-F) IHC assays and statistical analysis of the IRS scores were performed to detect the levels of Ki67 in the Rm-1 cell xenograft tumor tissues and TUNEL assays were performed to detect the apoptosis rate of the tumor tissue cells. Representative images are shown. (G) IHC staining of Rabbit IgG (R-IgG) as negative control in the paraffin-embedded prostate tissue arrays. T: prostate tumor tissue; N: normal prostate tissue.

Figure S2. CES1 is significantly regulated in melatonin-treated PCa cells (A) Volcano map of up- and downregulated genes in C4-2 cells treated with MLT (1 mM). And the number of DEGs in the C4-2 cells treated with MLT (1 mM). (B) C4-2 and 22RV1 cells were transfected with siMT1. MT1 protein levels were determined by Western blot analysis. Densitometry and statistical analysis. Representative images are shown. (C) Heat map of differential lipid metabolites in the C4-2 cells treated with MLT based on those in the control cells (DMSO treated) according to the results of the LC-MS/MS lipidomic assay. Only statistically significant changes ($p < 0.05$, $VIP > 1$) are presented. (D) C4-2 and 22RV1 cells were treated with 1 mM MLT for 48 h. CES1, DHRSX, HNF4A, SLC7A10, IGFBP1, ALDH1A2, ACSBG2, ZIC1, and SLC38A4 protein levels were determined by Western blotting. Densitometry and statistical analysis. (E) After transfection with siMT1, C4-2 and 22RV1 cells were treated with MLT (1 mM) for 48 h. Western blot analysis was performed to determine the levels of CES1 protein expression. Densitometry and statistical analysis. Representative images are shown.

Figure S3. CES1 is a key gene in PCa, and associated with lipid accumulation. MLT inhibited cell invasion and reversed enzalutamide resistance by CES1. (A) The receiver operating characteristic (ROC) curves of CES1 in PCa. (B) Multivariate analyses of clinical pathological factors of the disease. (C) Kaplan–Meier analysis of the associations of the level of CES1 mRNAs with OS in the TCGA PCa ($n = 497$) and

Taylor databases (n = 150). (D) GSEA of the correlation of the CES1 mRNA levels and lipid accumulation-related pathways in a TCGA PCa cohort. (E) After transfection with siMT1, C4-2 and 22RV1 cells were treated with MLT (1 mM) for 48 h. Western blot analysis was performed to determine the levels of CES1 protein expression. Densitometry and statistical analysis. Representative images are shown. (F) Cell migration was evaluated in C4-2 and 22RV1 cells with CES1-knockout by Transwell assays. The results are presented as the mean \pm SEM of 3 independent experiments. (G) GSEA of the correlation of the CES1 mRNA levels and lipid metabolism-related pathways in the TCGA PCa cohort. (H) TG and T-CHO contents were measured in C4-2 and C4-2-ENZR cells. The graphs represent the means \pm SEM. (I) The mRNA levels of PPARa-related genes (CPT1C and ME1) are dramatically upregulated in the C4-2 cells treated with MLT. The mRNA levels of steroid hormone metabolism-related genes (CYPA11A1 and STARD4) were downregulated in C4-2 cells treated with MLT. (J) GSEA of the correlations of the CES1 mRNA levels and cholesterol metabolism-related pathways in a TCGA PCa cohort. (K) WB assays were used to measure the CES1 expression in the C4-2-ENZR cells treated with MLT.

Figure S4. MLT regulates the methylation of the CES1 promoter via SIRT1-mediated DNMT1 deacetylation. (A) The sequences of CGIs in the CES1 promoter regions were examined experimentally by BSP. (B) The sequence of DNMT1 binding to DNA is predicted by CisBP. (C) C4-2 and 22RV1 cells were transfected with siSIRT1, and the control siRNA (siNC). SIRT1 protein levels were determined by WB. Densitometry and statistical analysis. Representative images are shown. (D) Densitometry and statistical analysis of the data of Figure 7H.

Figure S5. MLT inhibition of tumor growth and reversal of ENZ resistance of CPRC are mediated by CES1 expression in vivo. (A) IHC and TUNEL assays were performed to detect the levels of CES1, Ki67, PPARa, PERK, and caspase-3 in the C4-2 cell xenograft tumors and the apoptosis rate of the tumor cells. Representative images are shown. IHC staining of Rabbit IgG (R-IgG) as negative control. (B) IHC and TUNEL assays were performed to detect the levels of CES1, Ki67, CYP11A1, and PSA in the C4-2-ENZR cell xenograft tumor tissues and the apoptosis rate of the tumor tissue

cells. IHC staining of Rabbit IgG (R-IgG) as negative control. Representative images are shown. (C) 22RV1 cells were transfected with a siRNA targeting PPAR α , CYP11A1, PERK, Caspase 3 or PSA, with a control siRNA (siNC). Western blot was performed to test the antibody validation.

Figure S6. MLT inhibited tumor growth and reversed of ENZ resistance in animal CRPC models with a circadian rhythm disorder. (A-B) IHC assays and statistical analysis of the IRS scores of IHC staining were performed to detect the levels of Ces1, Ppara, Perk, caspase-3, Cyp11a1, and Psa in the Rm-1 cell xenograft tumor tissues. IHC staining of Rabbit IgG (R-IgG) as negative control. Representative images are shown. Statistical analysis of the IRS scores. (C) IHC staining of Rabbit IgG (R-IgG) as negative control.

Table S1

Review1: Influence of MLT levels on the risk of prostate cancer

n = the sample of high levels MLT in the events

N = the total sample of the events

Case = prostate cancer sample

Control = non-prostate cancer sample

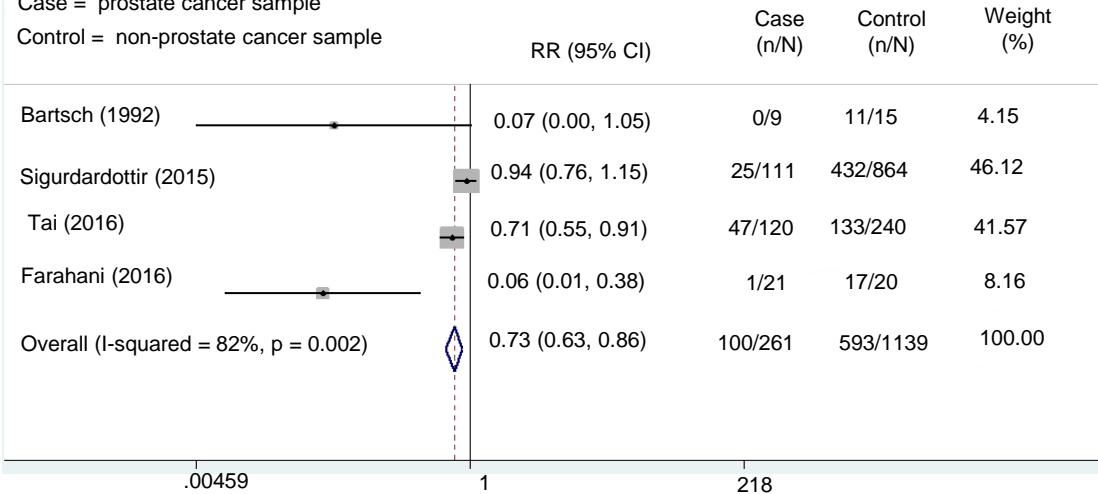
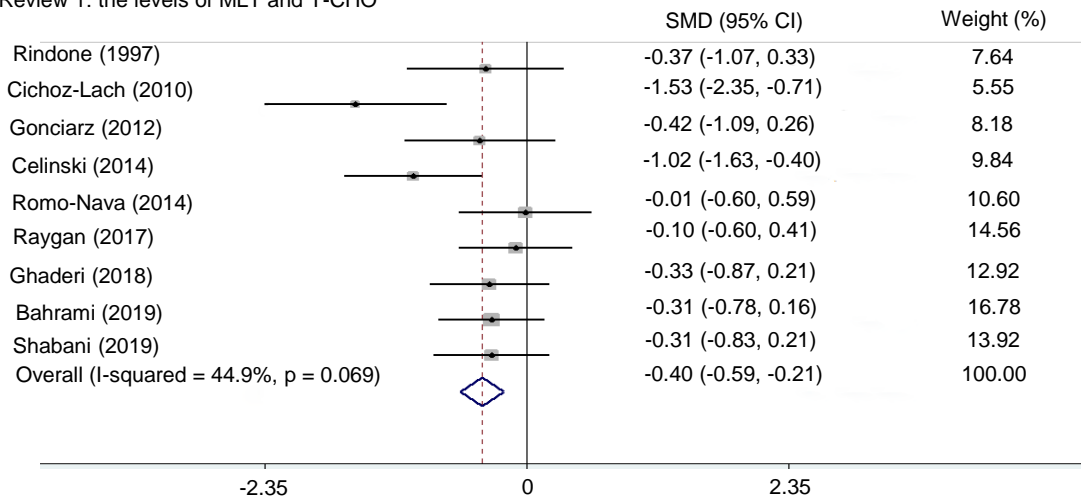
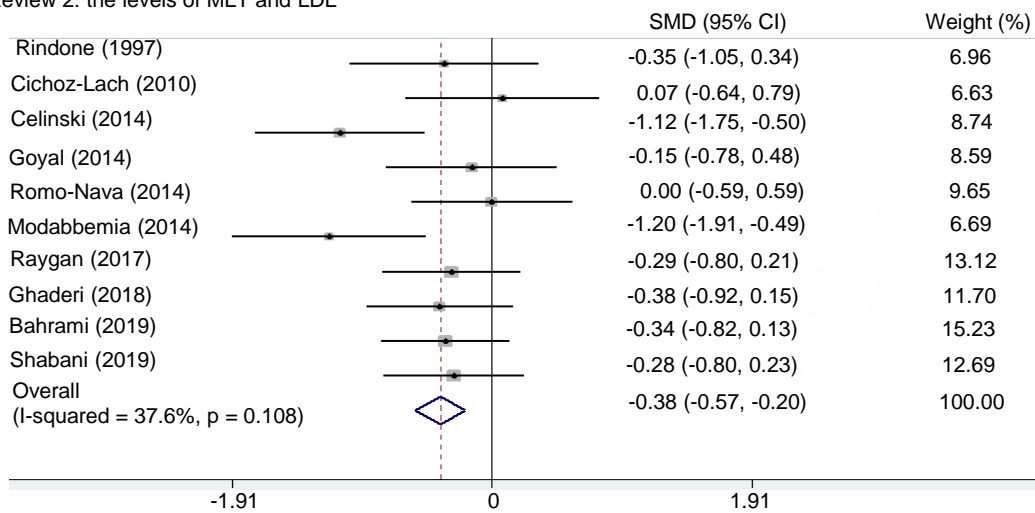


Table S2

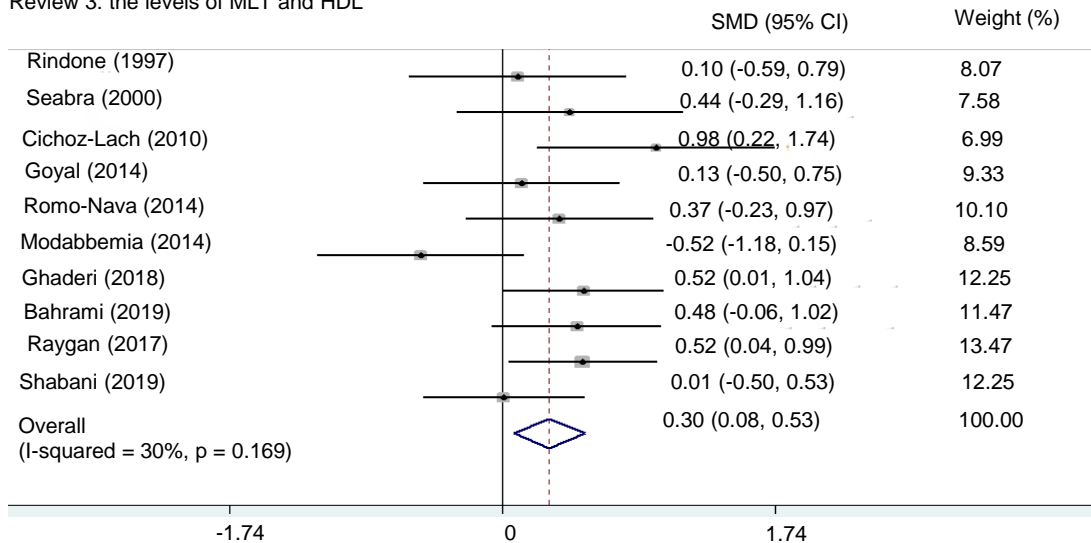
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Review 2: the levels of MLT and LDL

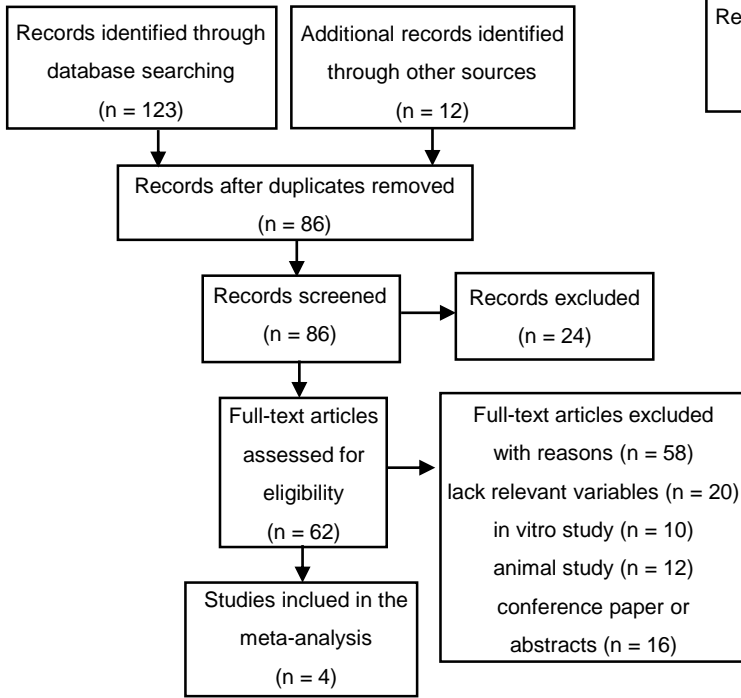


Review 3: the levels of MLT and HDL



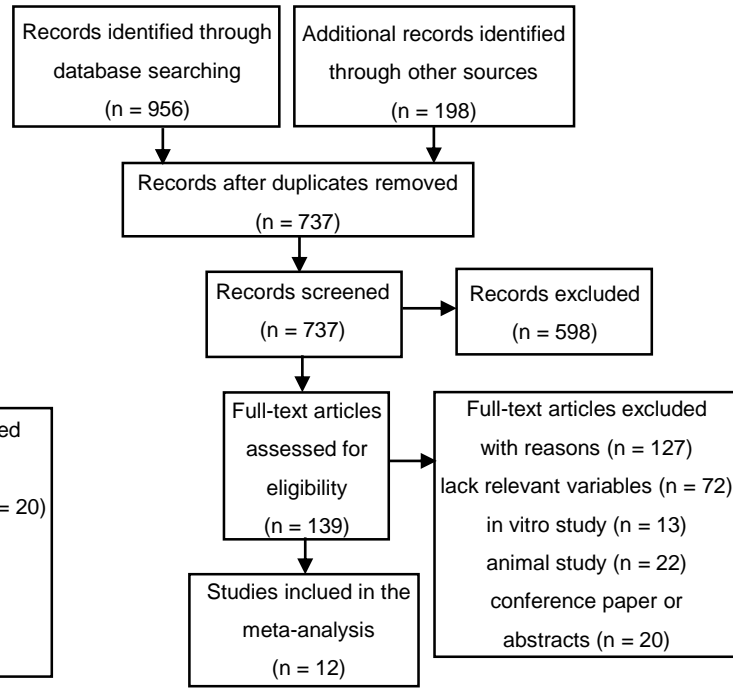
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Influence of MLT levels on the risk of prostate cancer

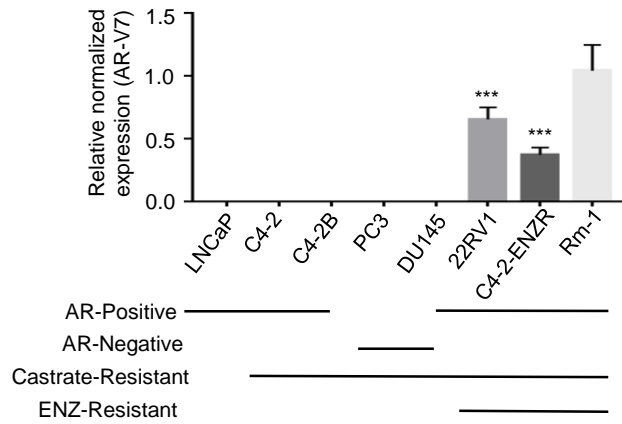


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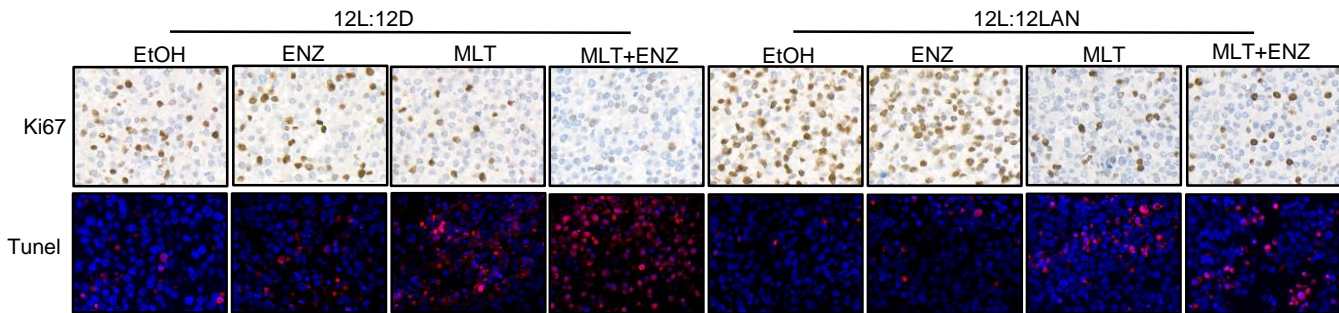
The levels of MLT and lipid metabolism



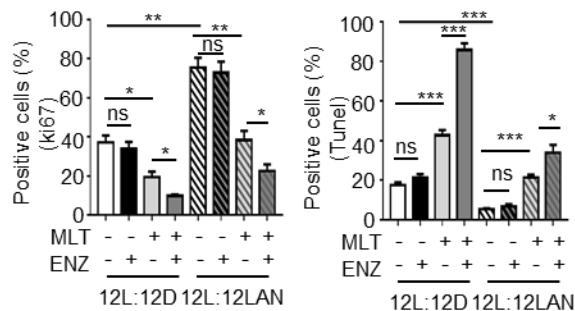
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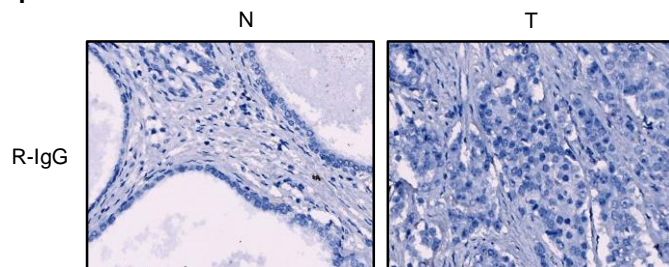
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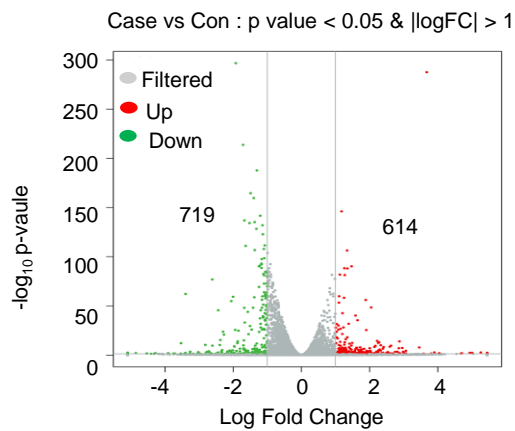
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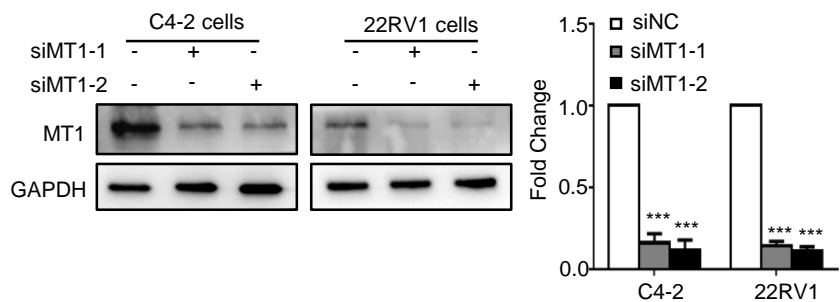
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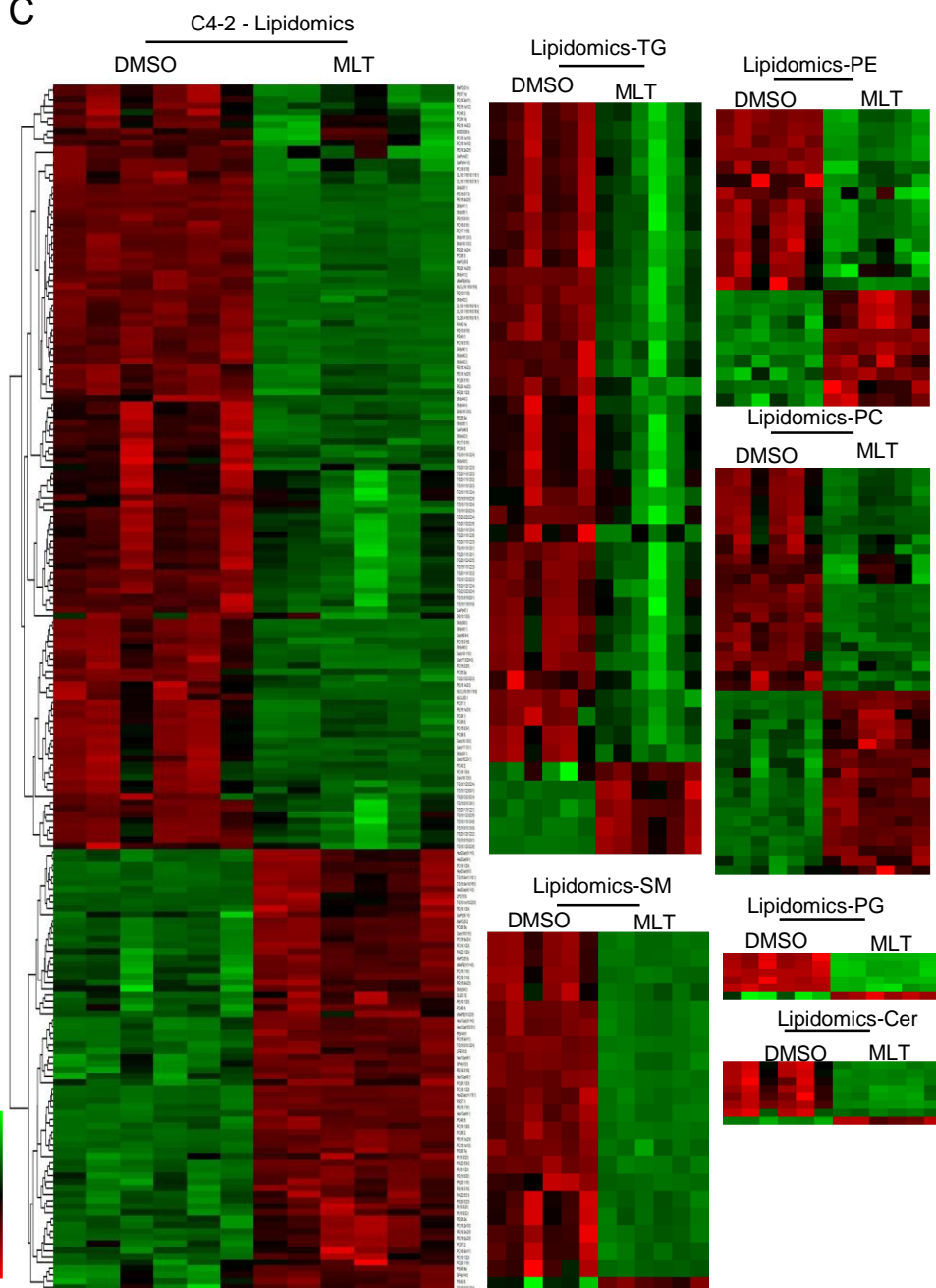
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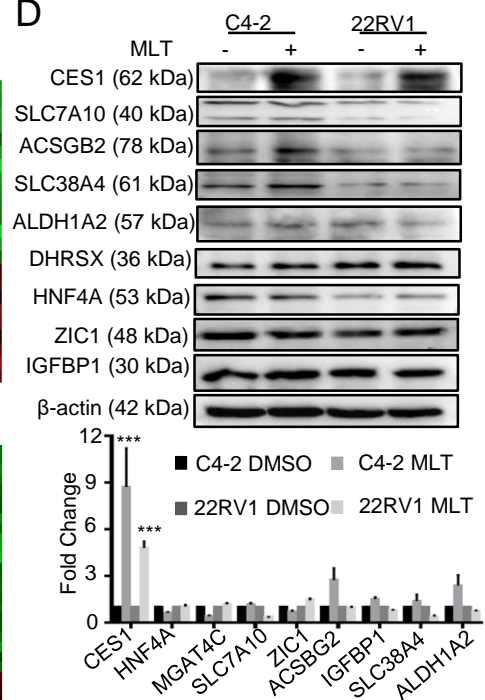
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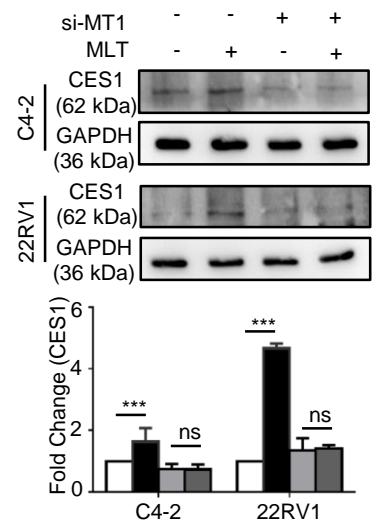
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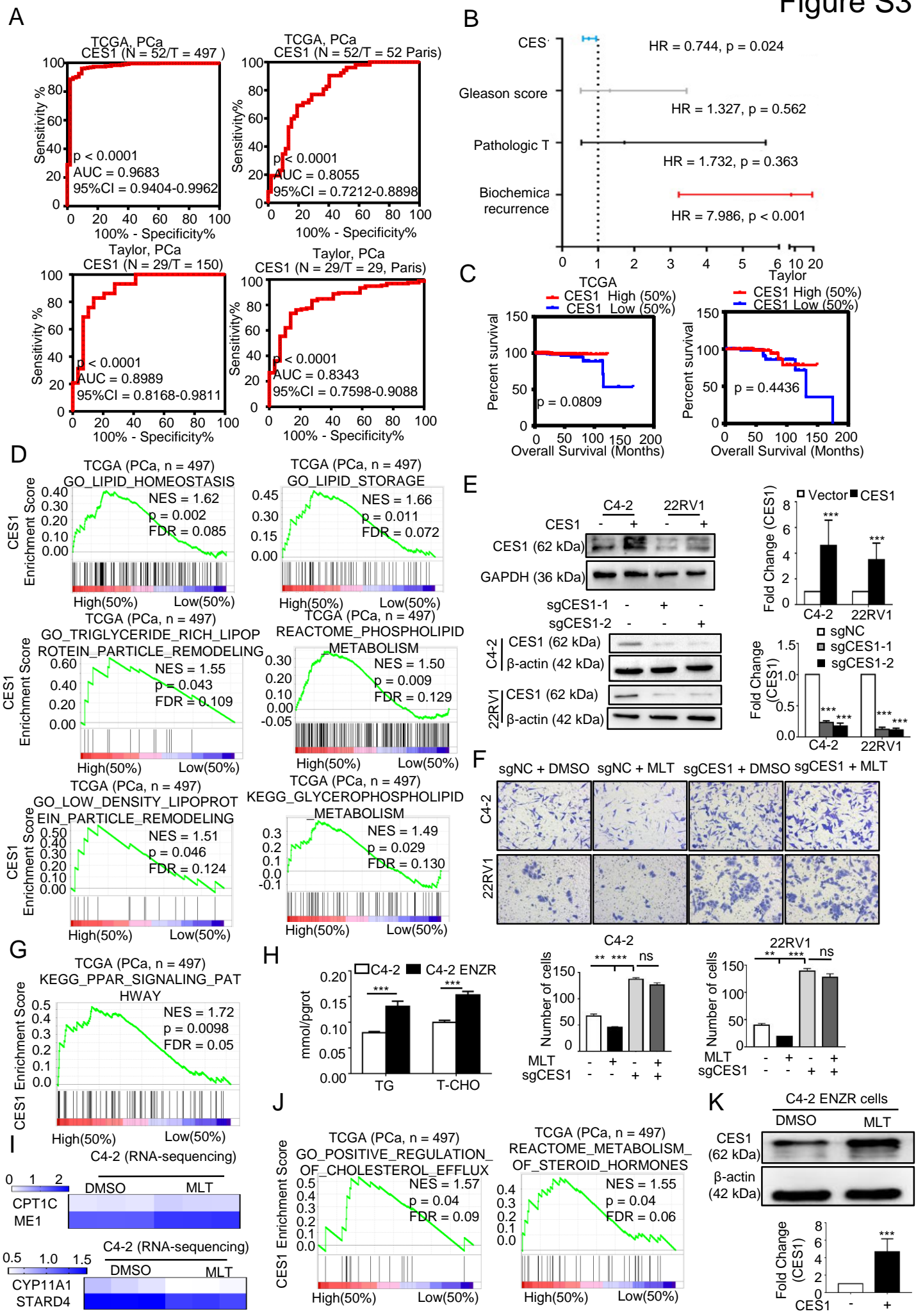
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TG (Triglyceride)
 PE (Phosphatidylethanolamine)
 PC (Phosphatidylcholine)
 SM (Sphingomyelin)
 Cer (Ceramide)



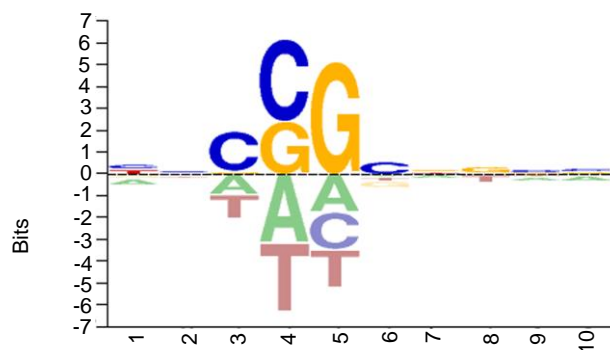
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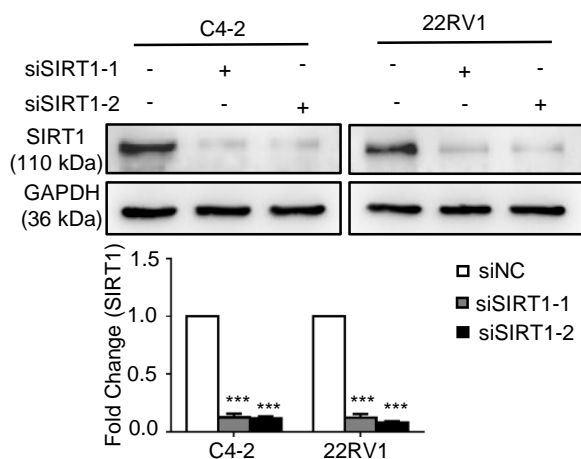
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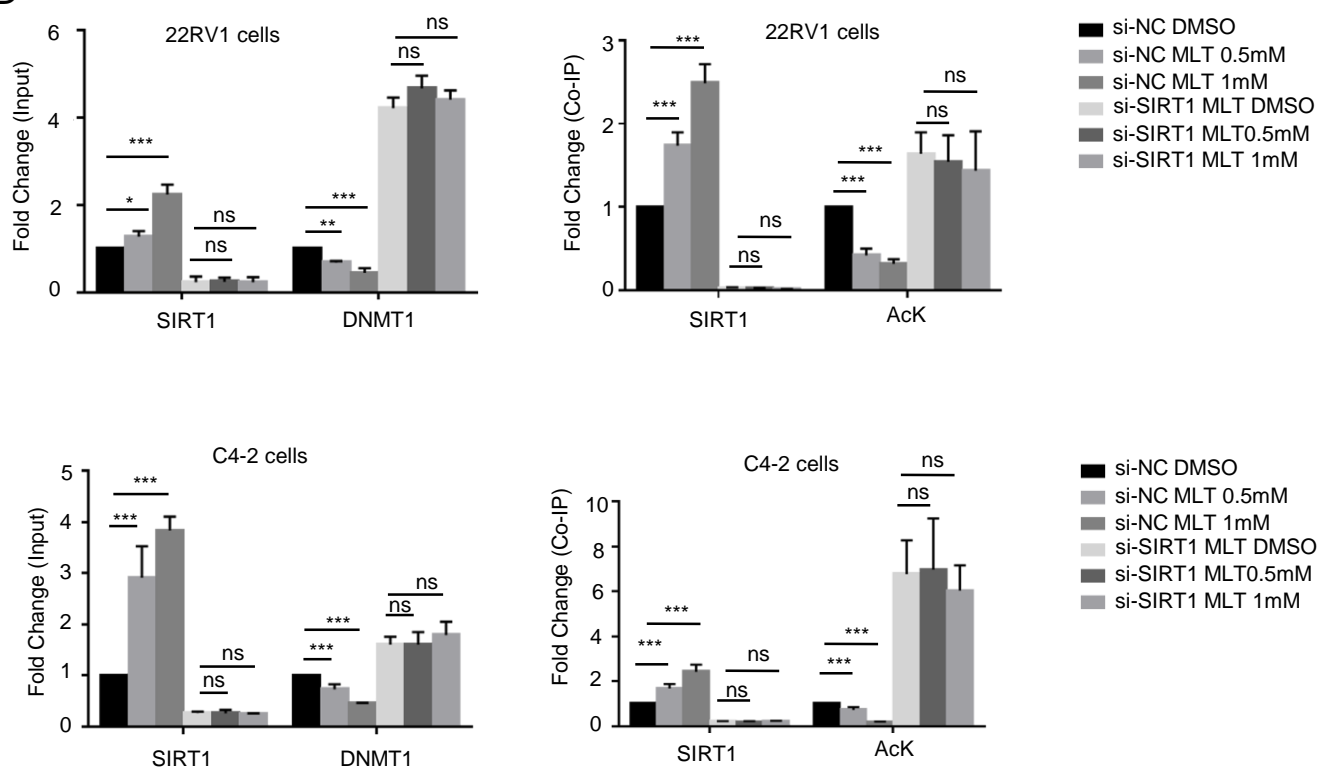
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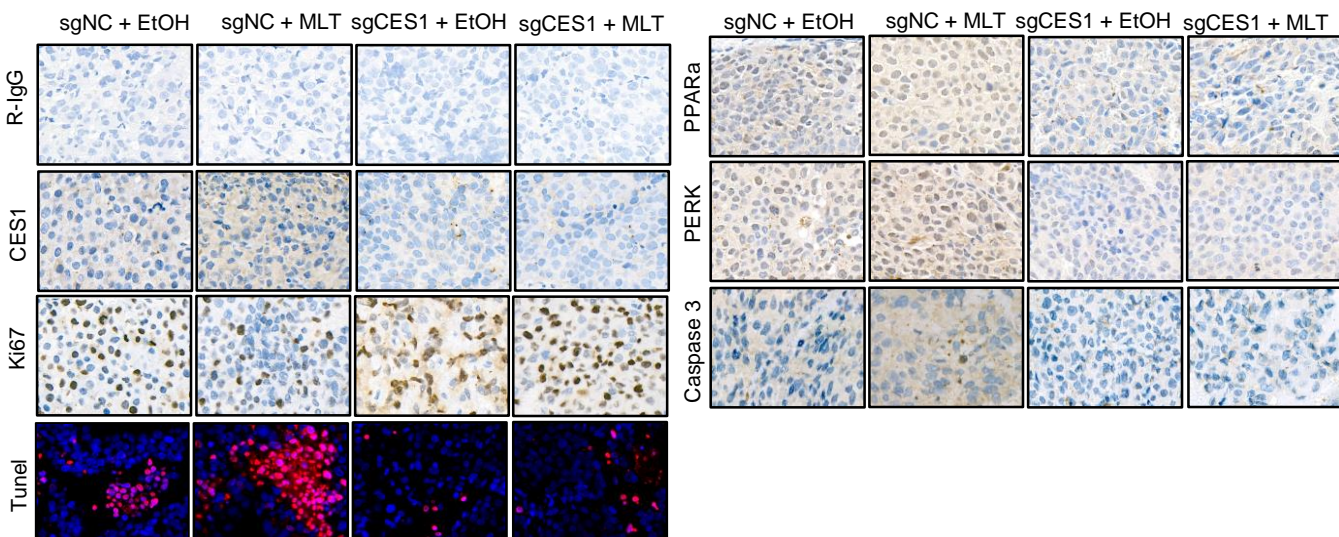
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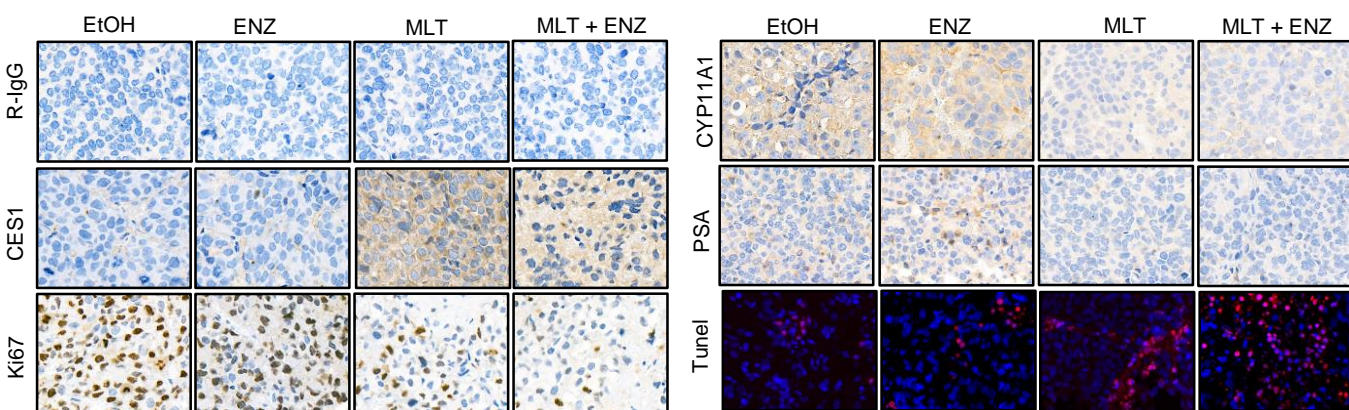
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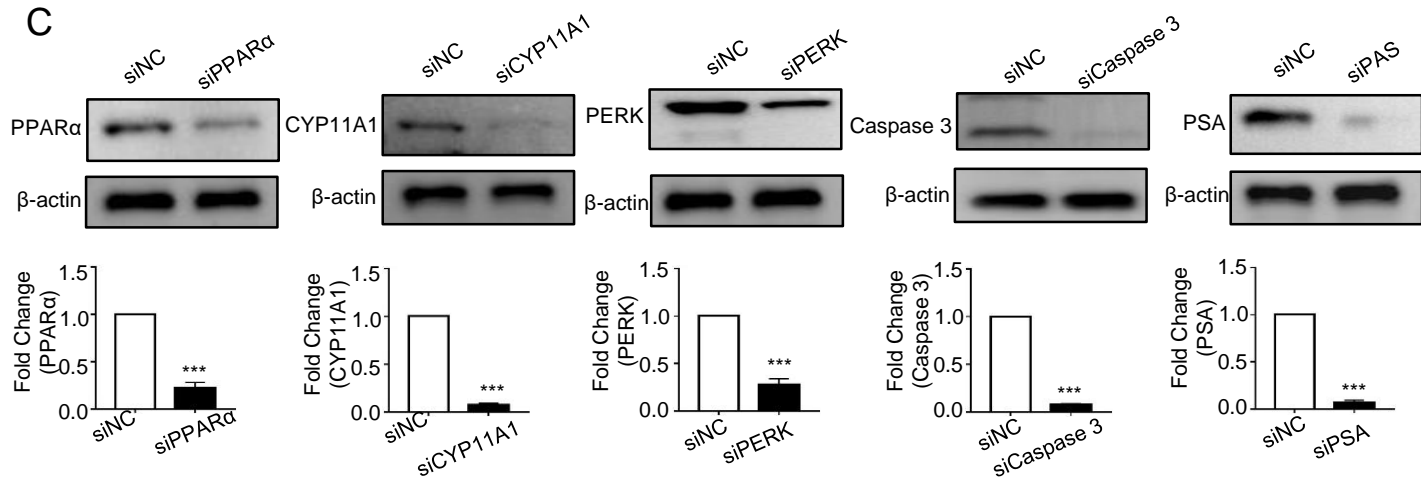
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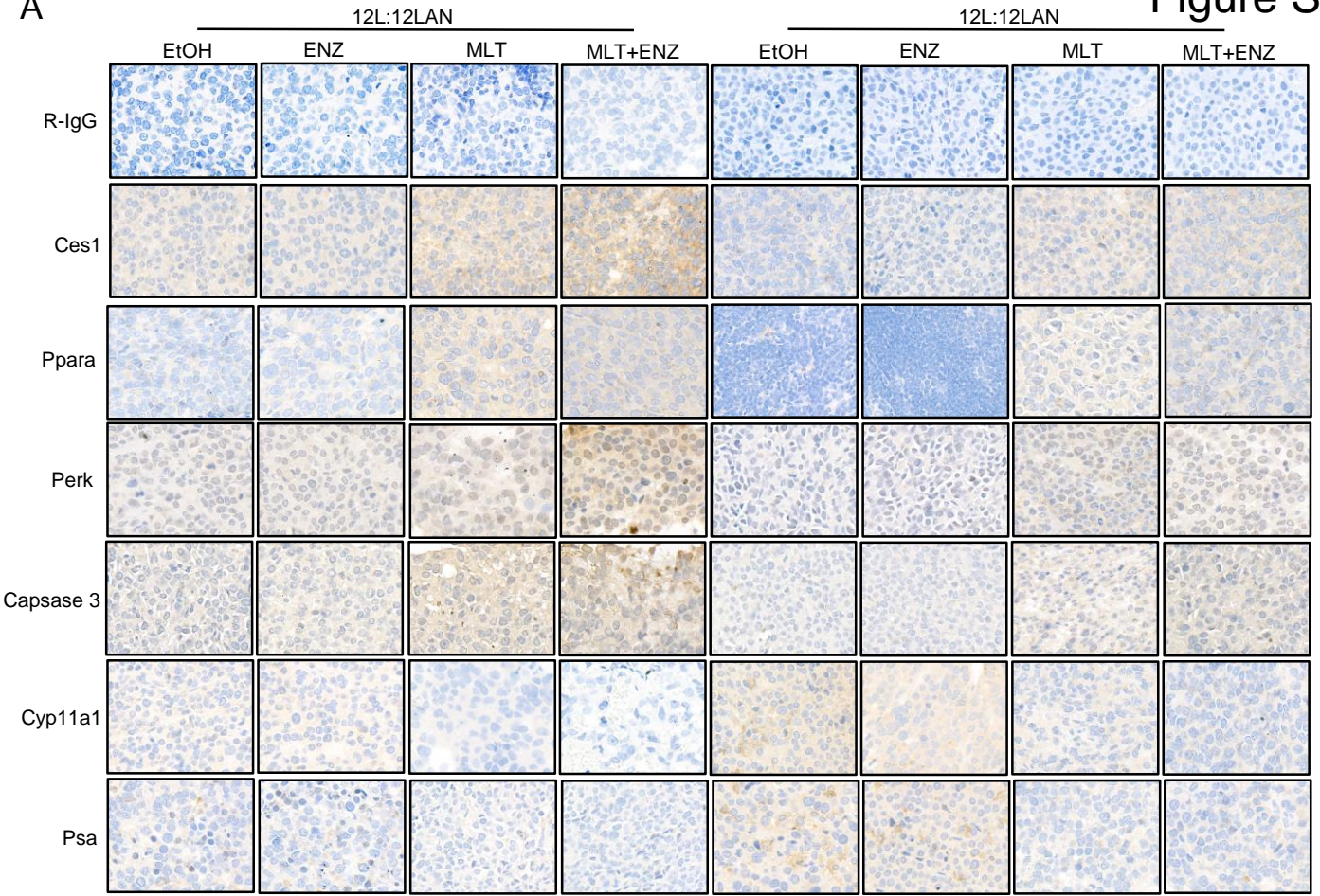
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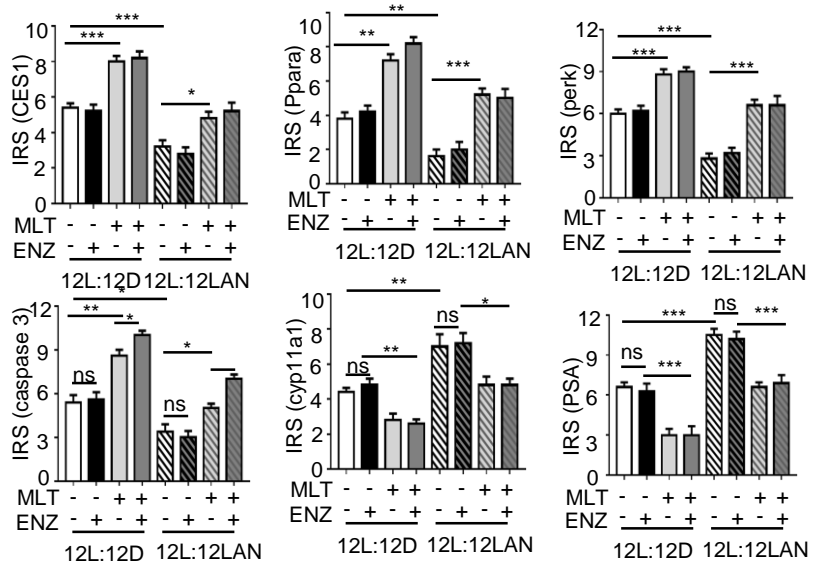
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