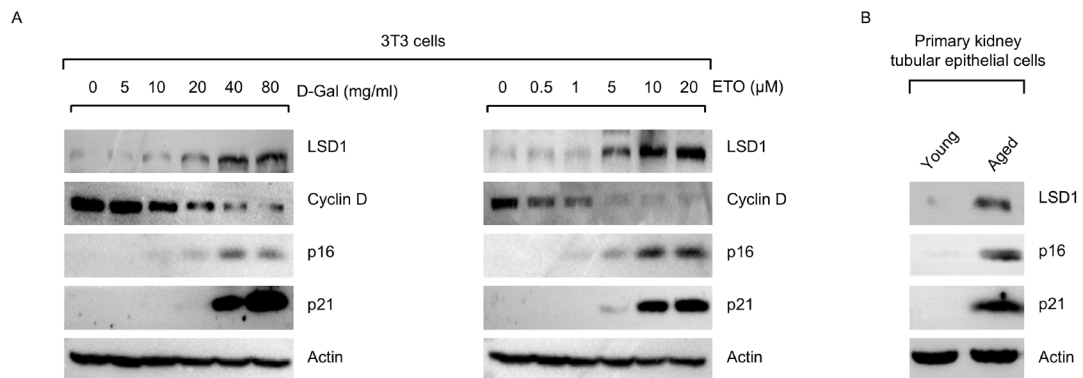


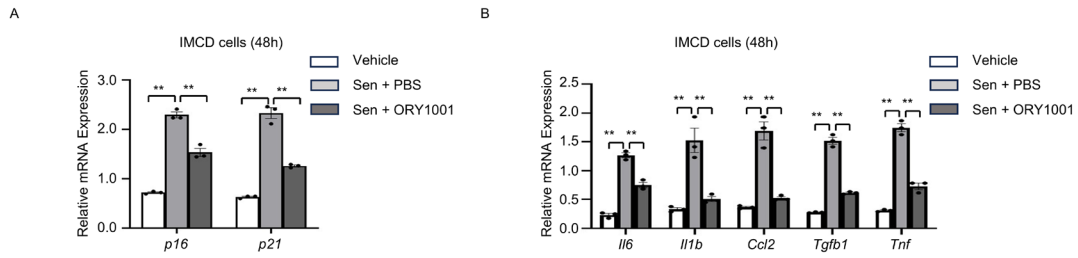
Supplementary Figures and Legends

Supplementary Figure 1



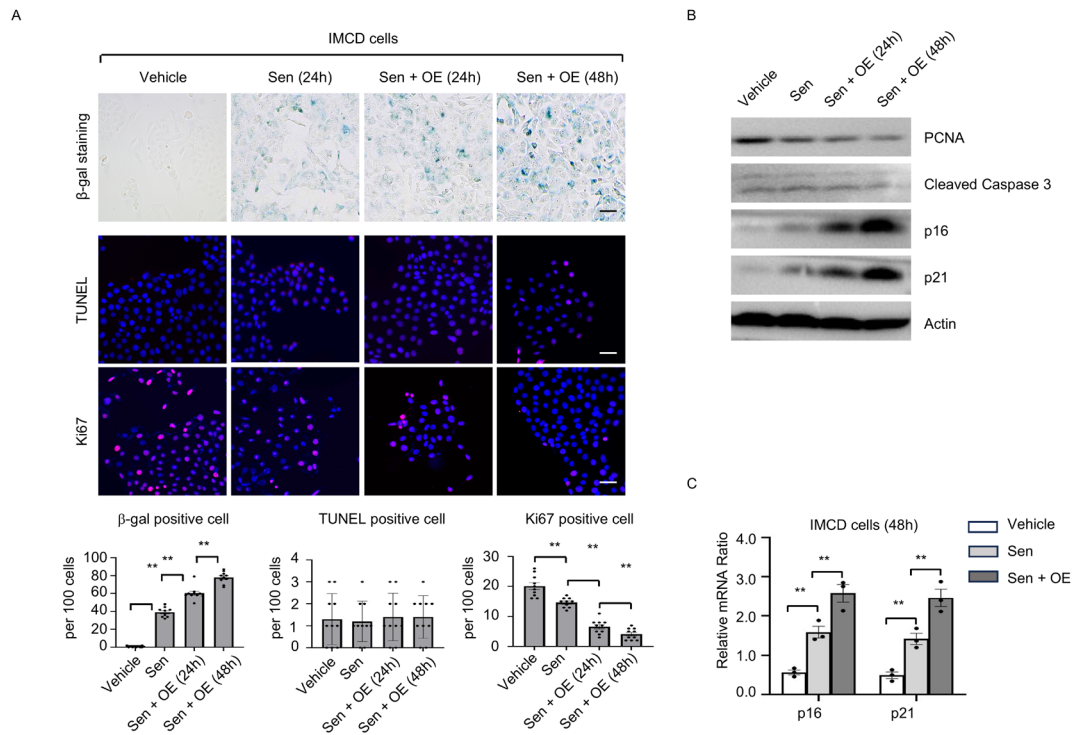
Supplementary Figure 1. The expression of LSD1 is increased in senescent 3T3 and primary kidney tubular epithelial cells. (A) Western blot analysis of the levels of LSD1, Cyclin D, p16 and p21 in mouse 3T3 cells treated with different dose of D-Gal and etoposide (ETO). n=3. (B) Primary tubular epithelial cells (TECs) were isolated from 2-month-old mice (young) and 22-month-old mice (aged). Western blot analysis of the levels of p16 and p21 in primary tubular epithelial cells (TECs) isolated from kidneys of 2-month-old mice (young) and 22-month-old mice (aged). n=3.

Supplementary Figure 2



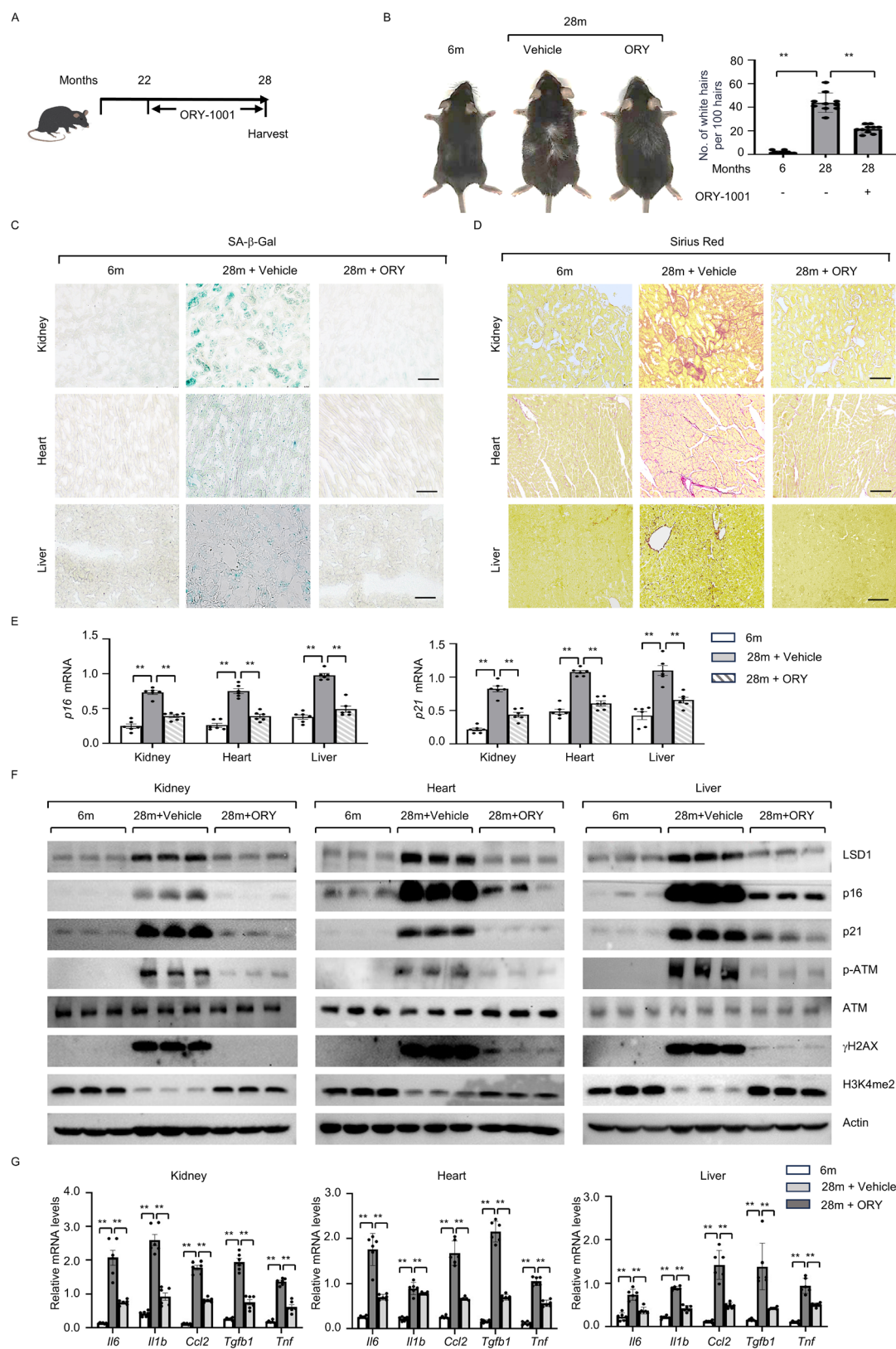
Supplementary Figure 2. Inhibition of LSD1 reduces cellular senescence and the expression of senescence-associated secretory phenotype (SASP) factors in mouse IMCD cells. (A, B) qRT-PCR analysis of *p16* and *p21* mRNAs (A) and Key SASP cytokines, including *Il6*, *Il1b*, *Ccl2*, *Tgfb1* and *Tnfa*. (B) in mouse IMCD cells treated with or without ORY1001 following ETO treatment. (Statistical analysis of the three independent expression; ** $p < 0.01$; one-way ANOVA)

Supplementary Figure 3



Supplementary Figure 3. LSD1 overexpression enhances cellular senescence in a time dependent manner. (A) Representative images and quantification of SA- β -gal staining (*top*), TUNEL assay (*middle*), and Ki67 staining (*bottom*) in mouse IMCD cells overexpressing LSD1 at 24 hours and 48 hours after ETO (5 μ M) treatment. bar=50 μ m. (The percentage of positive cells was quantified using the same software in 10 randomly selected fields; ** $p < 0.01$; one-way ANOVA) (B) Western blot analysis of LSD1, PCNA, cleaved caspase-3, p21, and p16 in GFP-LSD1-overexpressing senescent mouse IMCD cells at 24 and 48 hours post-ETO treatment. Represent data from 3 independent experiments are shown. (C) qRT-PCR analysis of *p16* and *p21* mRNAs in mouse IMCD cells with or without LSD1 overexpression at 48 hours after ETO (5 μ M) treatment. (All experiments were repeated at least 3 times; * $p < 0.05$; one-way ANOVA)

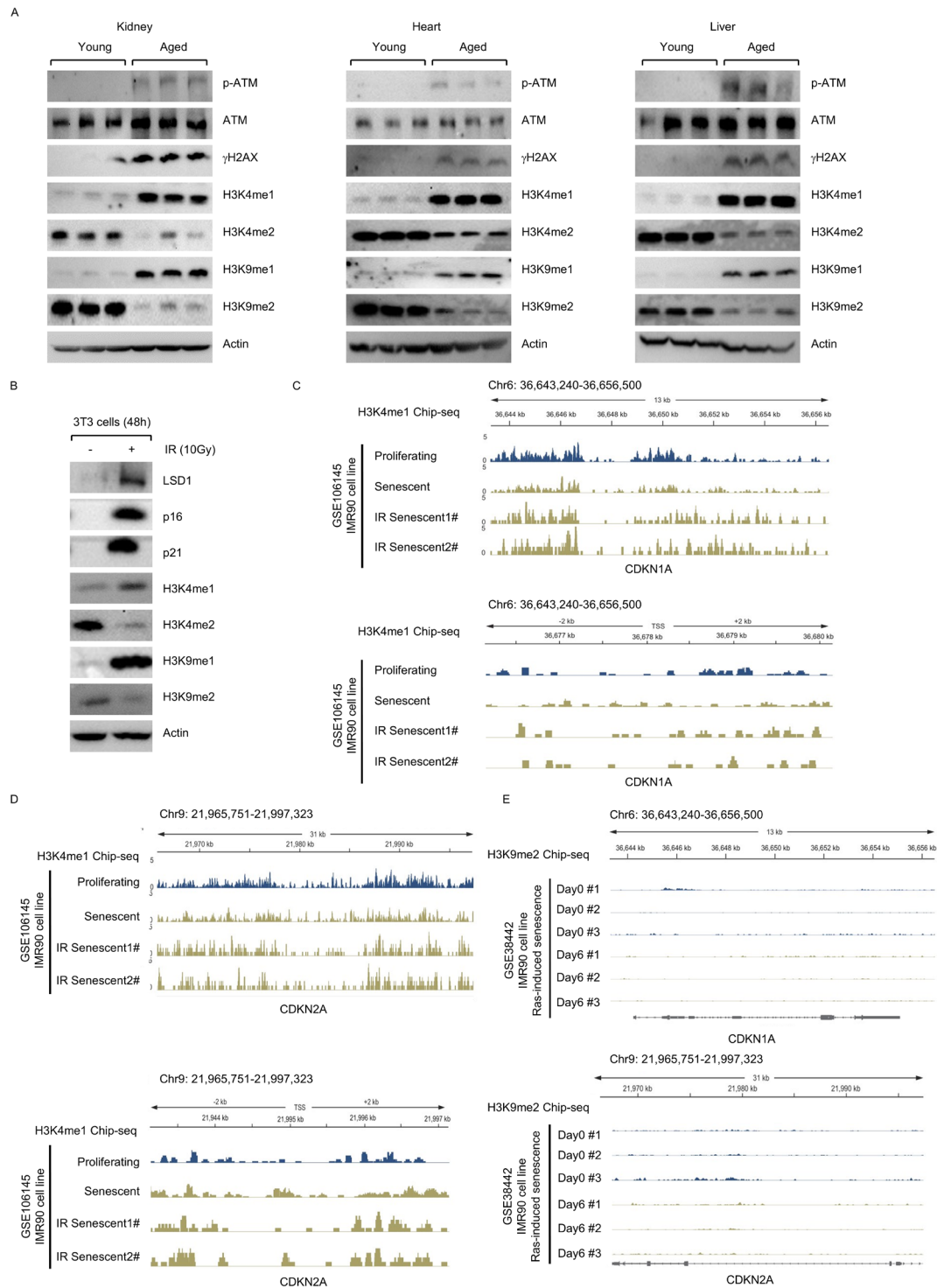
Supplementary Figure 4



Supplementary Figure 4. Treatment with ORY-1001 decreases natural aging-induced DNA damage in mouse organs and protects hair graying. (A) Schematic of the ageing

and ORY-1001 treatment regimen in C57BL/6 mice (treatment from 22 to 28 months of age). **(B)** Representative images of mice from the young (6-month-old), aged (28-month-old), and aged plus ORY-1001 treatment groups. Quantification of gray hair is shown as the number of gray hairs per 100 strands (mean $\pm$ SEM). (n=10; ** $p < 0.01$; one-way ANOVA) **(C-D)** SA- β -gal staining **(C)** and Sirius Red staining **(D)**, analysis in mouse organs, including kidneys (bar=100 μ m); heart (bar=100 μ m); and livers (bar=50 μ m) from aged and aged plus ORY-1001 treated groups. n=6. **(E)** qRT-PCR analysis of *p16*, *p21* mRNAs in kidney, heart and liver tissues. n=6. **(F)** Western blot analysis of the levels of LSD1, p16, p21, phospho-ATM (p-ATM), total ATM, and in mouse organs, including kidney (*left*), heart (*middle*) and livers (*right*) from aged and aged plus ORY-1001 treated groups. n=6. **(G)** qRT-PCR analysis of *Il6*, *Il1b*, *Ccl2*, *Tgfb1* and *Tnfa* mRNAs in kidney, heart and liver tissues. (n=6/group; ** $p < 0.01$; one-way ANOVA)

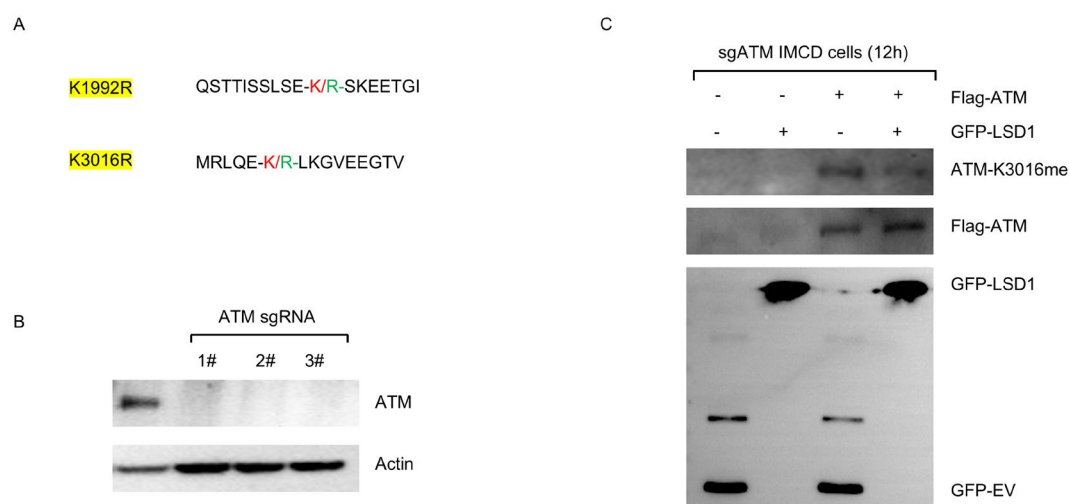
Supplementary Figure 5



Supplementary Figure 5. DNA damage signaling and histone methylation are altered in aged organs and senescent cells. (A) Western blot analysis of the levels of phospho-ATM (p-ATM) and total ATM, H3K4me1/2, H3K9me1/2 in mouse organs, including kidneys (*left*), heart (*middle*) and livers (*right*) from young (2-month-old) and aged (22-24-month-old) mice.

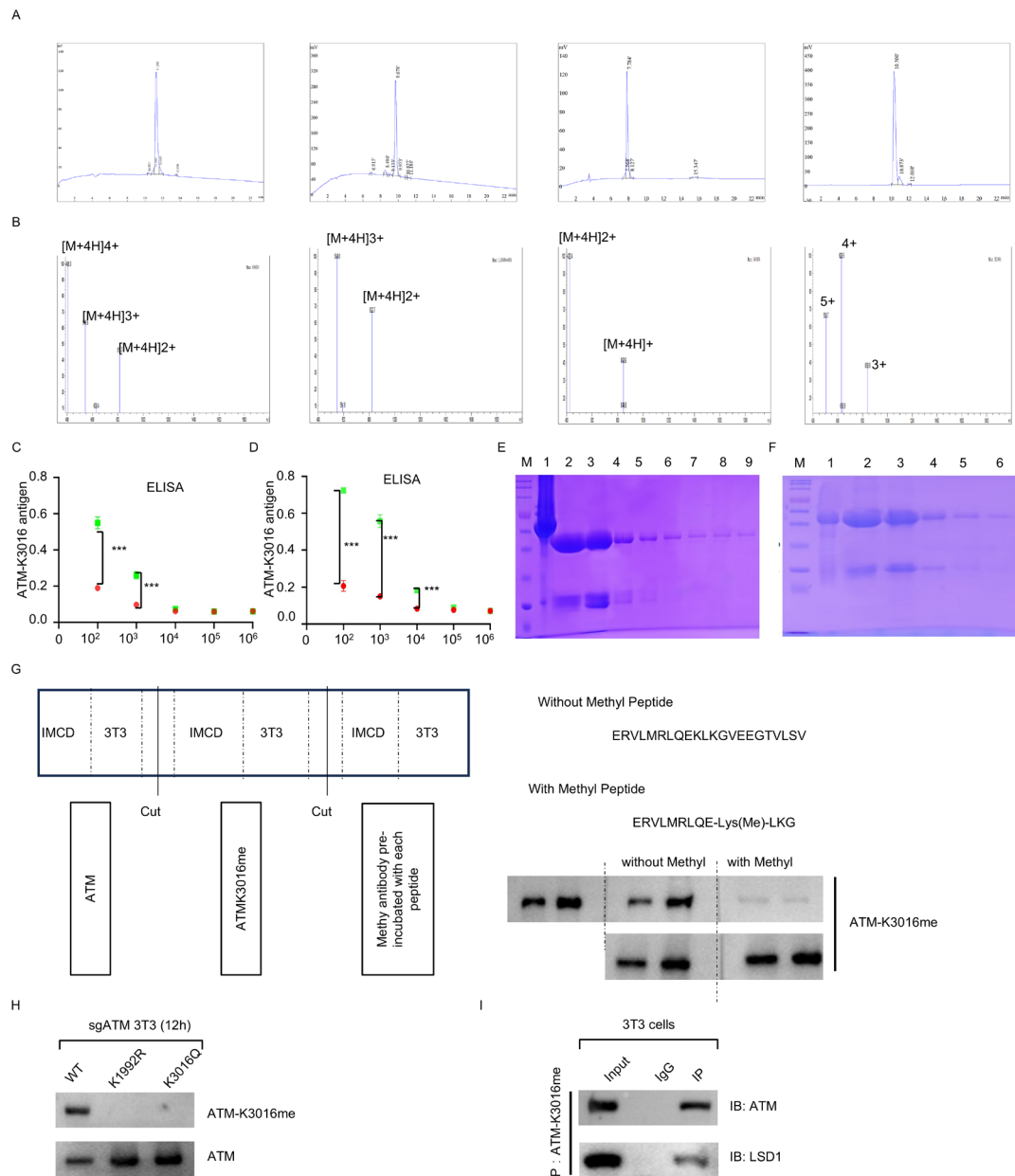
(B) Western blot analysis of LSD1, p16, p21, H3K4me1, H3K4me2, H3K9me1, H3K9me2 in mouse 3T3 cells with or without IR treatment for 48 hours. (C-D) Integrative Genomic Viewer (IGV) tracks showing H3K4me1 ChIP-seq signals at the *CDKN1A* (C) and *CDKN2A* (D) locus in human fibroblast cells (IMR90) under different statuses (GSE106415). (E) Integrative Genomic Viewer (IGV) showing the H3K9me2 ChIP-seq signals at the *CDKN1A* locus in human fibroblast cells (IMR90) in different statuses (GSE38442).

Supplementary Figure 6



Supplementary Figure 6. Generation of ATM knockout cell lines. (A) Alignment of human ATM amino acid sequences surrounding lysine 1981 and lysine 3016. Substitution of Lysine (R) with Arginine (K) was indicated. (B) Western blot analysis of ATM expression in mouse 3T3 cells transfected with three sgRNAs targeting ATM (sgATM). (C) Western blot analysis of the levels of ATM-K3016 methylation, Flag-tagged ATM and GFP-tagged LSD1 in ATM knockout mouse IMCD cells transfected with the indicated plasmids at 12 hours post-IR.

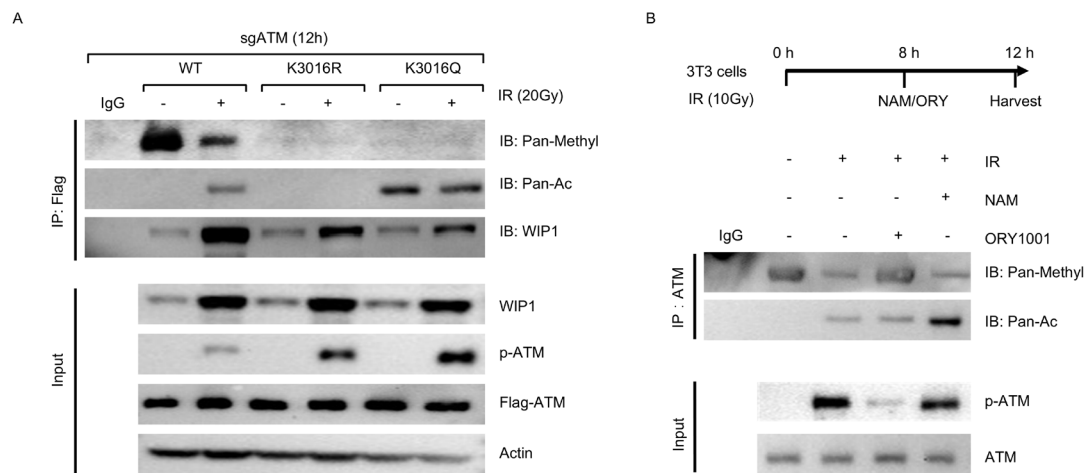
Supplementary Figure 7



Supplementary Figure 7. Generation and validation of the ATM-K3016me antibody. (A) High-performance liquid chromatography (HPLC) analysis confirmed the successful synthesis of three methylated peptides and one unmethylated peptide. (B) Mass Spectrometry (MS) analysis further validated the successful synthesis of three methylated peptides and one unmethylated peptide surrounding lysine 3016. (C) ELISA analysis detects the immunogenicity titer of serum ATM-K3016me following four polypeptide immunizations in rabbit, comparing peptide-immunized group (*green*) and control (*red*) groups. HRP-conjugated goat anti-rabbit IgG (100 μ l) (diluted 1:15000) was added to each well, followed

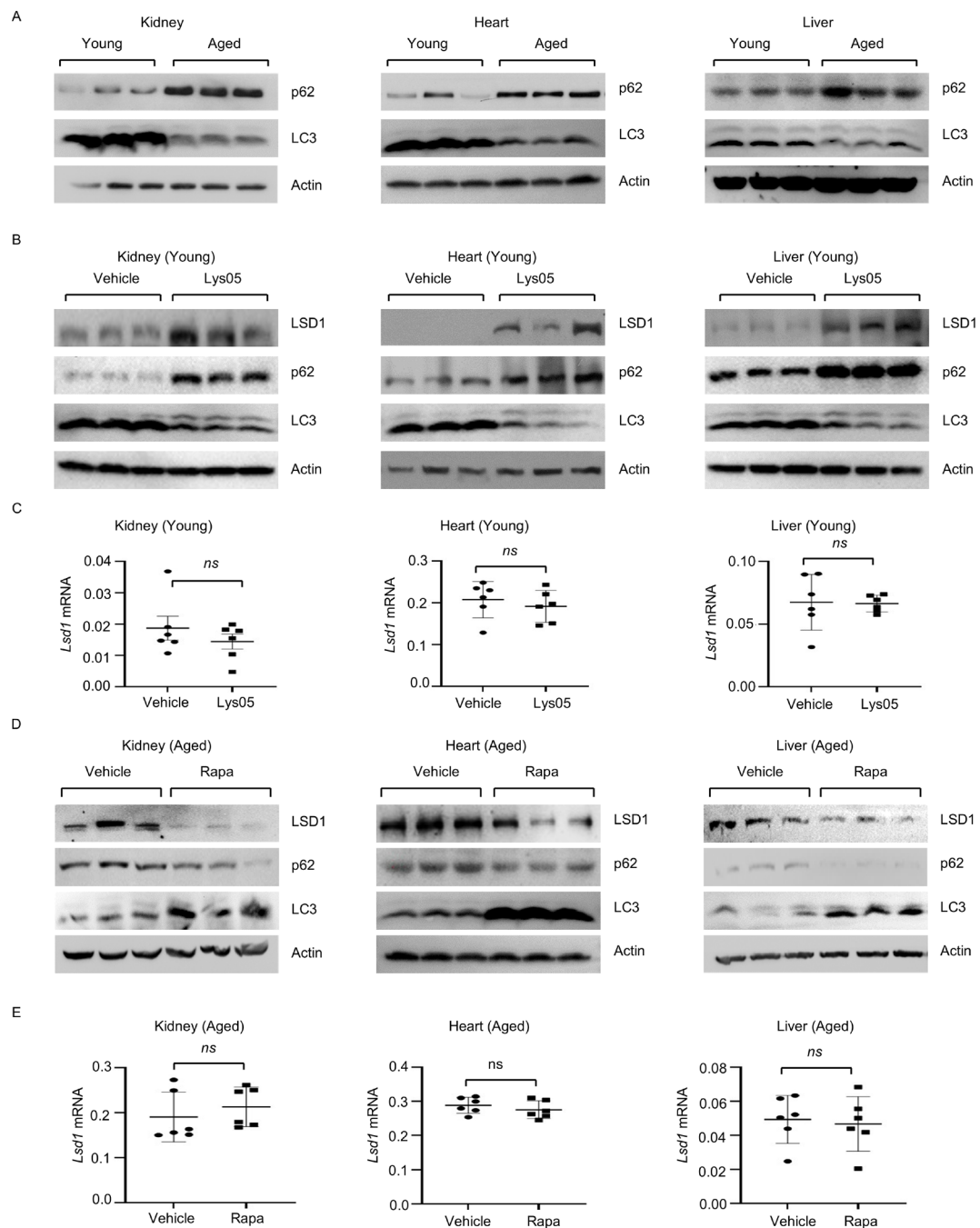
by the addition of 100 μ l of tetramethylbenzidine (TMB) substrate solution. Absorbance readings were taken at a wavelength of 450 nm. **(D)** ELISA analysis detects the immunogenicity titer of serum following five peptide immunizations in rabbit. **(E)** The antibodies were purified by Protein A/G beads and the purity of the antibodies was assessed by SDS-PAGE. **(F)** SDS-PAGE analysis of the purity of methylated peptide antigens following affinity purification. **(G)** Peptide competition assay for ATM-K3016me antibody. Cell lysates (50 μ g) from IMCD cells and 3T3 cells were run on 6% SDS-PAGE gels, transferred to PVDF membranes, and then immunoblotted with ATM-K3016 antibody pre-incubated with unmethylated and methylated peptides for 60 minutes at room temperature. Membranes were processed and exposed under identical conditions. **(H)** Western blot analysis of the levels of ATM-K3016 methylation in ATM knockout mouse 3T3 cells transfected with the indicated plasmids. **(I)** Immunoprecipitation assay assessing the interaction between ATM-K3016 methylated and LSD1 using ATM-K3016me antibody. *** $p < 0.001$.

Supplementary Figure 8



Supplementary Figure 8. The acetylation and methylation of ATM at K3016 is detected upon DNA damage. (A) Immunoprecipitation assay detecting the methylation and the acetylation of ATM, as well as the interaction between Flag-tagged ATM and WIP1, in ATM knockout 3T3 cells transfected with the indicated plasmids with or without IR (20 Gy) treatment. **(B)** Immunoprecipitation assay detecting the methylation and acetylation of ATM following NAM or ORY treatment at 8 hours post IR (10 Gy) treatment for 4 hours in 3T3 cells.

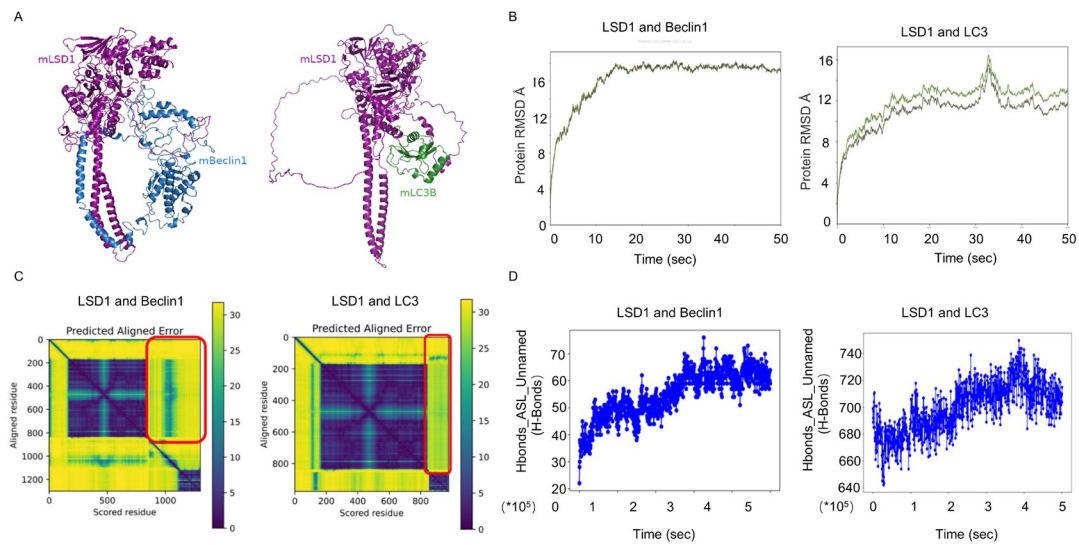
Supplementary Figure 9



Supplementary Figure 9. Autophagy modulates the stability of LSD1 protein in young and aged mouse organs. (A) Western blot analysis of the expression of p62 and LC3 in mouse organs, including kidneys (left), hearts (middle), and livers (right), collected from 20-22-months-old mice (*aged*) compared to that in organs collected from 2-month-old mice (*young*). (B) Western blot analysis of the expression of LSD1, p62 and LC3 in organs, including kidneys (*left panel*), heart (*middle panel*) and livers (*right panel*) collected from 2-

month-old mice treat with Lys05 or vehicle. (C) qRT-PCR analysis of *Lsd1* mRNA in mouse organs, including kidneys, hearts and livers, treated with Lys05 and vehicle. (n=6/group; ** $p < 0.01$; unpaired, 2-tailed Student's *t* test) (D) Western blot analysis of the expression of LSD1, p62 and LC3 in organs, including kidneys (*left panel*), heart (*middle panel*) and livers (*right panel*), collected from aged mice treated with rapamycin and vehicle. (E) qRT-PCR analysis of *Lsd1* mRNA in aged organs, including kidneys, hearts and livers, treated with rapamycin and vehicle. (n=6/group; ns, not significant; unpaired, 2-tailed Student's *t* test)

Supplementary Figure 10



Supplementary Figure 10. LSD1 interacts with LC3 and Beclin1 by molecular dynamics simulation experiments. (A) Uniport acquired the complete structural models of mLSD1 (purple), mBeclin1 (blue) and mLC3B (green). (B) The Root Mean Square Deviation (RMSD) value of the protein backbone of LSD1 and LC3 as well as LSD1 and Beclin1, which was calculated based on backbone atoms. (C) The calculation of the predicted aligned error (pAE) value between LSD1 and Beclin1 as well as LSD1 and LC3 by using AlphaFold multimer. (D) The detection of the dynamic changes of hydrogen bonds between LSD1 and Beclin1 as well as LSD1 and LC3B by using the molecular dynamics simulation-driven method.