Materials and Methods

Mice

Calorie restricted mice were started on diet at 8 weeks of age and fed 60% of the amount of food consumed by ad libitum mice for a period of 16 weeks. High fat diet mice were started on a diet at 8 weeks of age that contained 60% Kcal from fat (Research Diets catalog # D12492) for a period of 16 weeks.

MNase Digest

Chromatin from SirT1 f/f (WT) or SirT1 f/f; Stra8-Cre (KO) sonication resistant spermatids (SRS) was isolated by incubating SRS in lysis buffer (50 mM Tris, pH 7.4, 150 mM NaCl, 1% NP-40, 0.5% DOC, and 0.1% SDS). This chromatin was subjected to digestion by incubating with MNase in MNase buffer (10 mM Tris, pH 7.5, 10 mM KCl, and 1 mM CaCl2) at 37 degrees Celsius. Samples were collected as a function of time and the reactions were terminated with 5mM EDTA. DNA was purified and analyzed on an agarose gel subsequently stained with EtBr for visualization.

Meiotic Spreads

Meiotic Spreads were prepared as previously described (Peters et al., 1997). Briefly a cell suspension was prepared in DMEM by tearing apart seminiferous tubules. Large tubule pieces were removed by allowing them to settle in a 15ml falcon tube. Cells from the upper phase were counted and centrifuged for 8 min at 1000 rpm. Cell pellet was resuspended in 1ml of hypotonic buffer (30 mM TrisCl pH 8.2, 50 mM sucrose pH 8.2, 17 mM sodium citrate) and incubated at RT for 7 minutes. Samples were centrifuged again and resuspended in 100 mM sucrose pH 8.2 and 10ul were spread on slides containing a thin film of 1% PFA w/ 0.15% TritionX-100 and allowed to dry in a humidified chamber then stored at -80^{o} C. Slides were washed 3x10 minutes with PBS after removing from the 80^{o} C and then were blocked with 3% BSA + 1% serum + 0.05% TritonX-100 for 30 minutes. Primary antibody was diluted in 1% BSA in PBS and the slides were incubated o/n at 4^{o} C. The next day slides were washed in PBS and

incubated with the appropriate secondary antibody for 1 h at RT and then stained with DAPI. Antibodies used are Scp1 (Abcam 1:250), Scp3 (Santa Cruz 1:100), and phosphor-H2A.X (Millipore 1:5000).

LC-MS/MS

For TP2 acetylation, testis were decapsulated and minced in 1ml of PBS, rotated for 15 minutes at 4^{o} C, transferred to a 15 ml conical tube and allowed to settle on ice for 15 minutes. Supernatant was taken without any pieces of tissue and centrifuged for 10' at 1.5Kxg. Pellet was solubilized in 1ml hypotonic lysis buffer (10mM Tris-Cl pH 8.0, 1mM KCl, 1.5mM $MgCl_2$, 1mM DTT and complete protease inhibitor (Roche)) and rotated for at least 30 minutes at 4^{o} C. Nuclei were pelleted by spinning at 10Kxg for 10 minutes at 4^{0}C and then raised in 400ul 0.2M HCl and rotated overnight at 4^{o}C. Acid soluble samples were centrifuged for 10 minutes at 16Kxg at 4^{0}C, and supernatant was transferred to a fresh tube and 100% TCA was added to a final concentration of 5% and allowed to sit on ice for at least 1 h. Samples were centrifuged for 10 minutes at 16,000g at 4^{o} C and supernatant was mixed with 100% TCA to get a final concentration of 25% TCA and incubated on ice for 1 h to O/N. Samples were pelleted by spinning for 10 minutes at 16,000g at 4^{o} C. Insoluble material was washed 2X with ice cold acetone and allowed to dry. Samples were raised in ddH_2O, reduced, alkylated, and digested with trypsin O/N, then desalted using C18 Ziptips (Millipore) and lyophilized. Samples were labeled with iTRAQ (AB Sciex), loaded onto a C18 pre-column and subsequently attached to an HPLC for LC-MS/MS analysis via ultra-low-flow nano electrospray on an LTQ Orbitrap Elite (Thermo).

HAT Assay

Histone acetyl-transferase activity was determined with a HAT Assay Reagent Kit (Millipore 17-284) using 3H-Acetyl Coenzyme A (American Radiolabeld Chemicals Inc). Activity was determined with 1ug of germ cell nuclear extract.

HDAC Assay

Histone de-acetylase activity was determined with a colormetric HDAC assay kit (Active Motif). Activity was determined with 5ug of germ cell nuclear extract.

Biotin Tracer Studies

Using a biotin tracer (Furuse et al., 2002), we assessed the permeability of the BTB. The biotin tracer studies were performed as described in Meng et al. (Meng et al., 2005). Briefly, 3 or 14 month old SirT1 f/f or SirT f/f; Stra8-Cre mice were anesthetized and injected with 50 µl of 10 mg/ml EZ-Link Sulfo-NHS-LC-Biotin (Pierce Chemical Co.) into the interstitium of exposed testes. The testes were removed after 30 min. Paraffin-embedded 5 µm thick sections were prepared, incubated, and treated with streptavidin conjugated to Alexa Fluor 555 (Invitrogen) for 30 min at room temperature. The sections were rinsed with PBS, stained with DAPI, mounted and observed on an Axio Imager (Zeiss) at 20X magnification.

Statistical analysis

The data presented are means ± SEM. Lines present in the box charts indicate the average of all data points and each point/dot is a single mouse. Data were analyzed by two-way analysis of variance using Origin Pro 8. When the analysis of variance indicated a significant difference, individual differences were explored with two tailed paired *t* test. Statistical significance was determined at the 0.05 level.

References

Furuse, M., Hata, M., Furuse, K., Yoshida, Y., Haratake, A., Sugitani, Y., Noda, T., Kubo, A. and Tsukita, S. (2002). Claudin-based tight junctions are crucial for the mammalian epidermal barrier: a lesson from claudin-1–deficient mice. *The Journal of Cell Biology* **156**, 1099-1111.

Meng, J., Holdcraft, R. W., Shima, J. E., Griswold, M. D. and Braun, R. E. (2005). Androgens regulate the permeability of the blood-testis barrier. *Proc Natl Acad Sci U S A* **102**, 16696-700.

Peters, A. H., Plug, A. W., van Vugt, M. J. and de Boer, P. (1997). A drying-down technique for the spreading of mammalian meiocytes from the male and female germline. *Chromosome Res* **5**, 66-8.

Figure Legends

Supplemental Figure S1

(A) Body Weight, (B) epididymal weight of SirT1 f/f (WT) and SirT1 f/f; Stra8-Cre (KO) mice. Loss of SirT1 in the testis does not change body weight or epididymal weight. Line is the mean and dots are one mouse.

(C) Relative mRNA levels of leydig, sertoli, and germ cell markers from whole testes of SirT1 f/f (WT) and SirT1 f/f; Stra8-Cre (KO) mice. Error bars = SEM.

(D) Western blot analysis of SirT1 exon 4 excision in SirT1 f/f Prm1-Cre mice. SirT1 is not expressed when this Cre is active, therefore we do not observe a shift in SirT1 protein similar to what is observed in the Stra8-Cre; SirT1 f/f sample (last lane).

(E) RFP and GFP fluorescence of testes cross sections from Prm1-Cre mice crossed to the dTomato reporter mouse. The presence of GFP when Cre is present indicates that the Cre is active.

(F) Genotyping for SirT1 exon 4 excision using DNA isolated from sperm. The presence of Cre corresponds with the presence of the pcr product that indicates exon 4 has been deleted (Δ exon 4).

(G) Testis weight and sperm number from SirT1f/f and SirT1f/f;Prm1-Cre mice.

Supplemental Figure S2

Meiotic spreads from SirT1 f/f (WT) and SirT1 f/f; Stra8-Cre (KO) mice stained with Scp1, Scp3, and γH2AX to examine synapsis. There is no apparent defect in leptotene (top left), zygotene (top right), pachytene (bottom left) or diplotene (bottom right) indicating that synapsis and de-synapsis is normal in the KO mice.

Supplemental Figure S3

A) Acetyltransferase activity assay measuring transfer of 3H-AcetylCoA incorporation onto core histones by 1ug of nuclear extract from SirT1 f/f (WT) or SirT1 f/f; Stra8-Cre (KO) germ cells. Error bars =SEM. N=3

B) Immunoblots on NETN soluble lysate from SirT1 f/f (WT) or SirT1 f/f; Stra8-Cre (KO) germ cells isolated from 3 littermate pairs.

C) Immunoblots on NETN soluble lysate from testes isolated from SirT1 f/f mice (WT) and SirT1 f/f; Stra8-Cre (KO) mice 20, 23, 28, 35, or 75 days old.

D) HDAC activity assay using 5ug of nuclear extract from SirT1 f/f (WT) or SirT1 f/f; Stra8-Cre (KO) germ cells. Error bars =SEM and N=3. Each biological replicate was measured in quadruplicate and then normalized to the OD405 obtained from reactions lacking substrate.

Supplemental Figure S4

Transition proteins and protamines are not differentially expressed, nor is transition protein 2 differential acetylated between SirT1 f/f and SirT1 f/f; Stra8-Cre mice.

(A) Transition protein and protamine mRNA is not different in KO mice. Error bars = SEM

(B) The corresponding y and b ions for the peptide (top) that has been manually validated in fragmentation spectra of the tryptic peptide fragment of TFEG(Ac)KVSK from transition protein 2 labeled with iTRAQ. The lysine that is acetylated corresponds to K91 of transition protein 2.

(C) Quantification of AcK91 in SirT1 f/f (WT) vs. SirT1 f/f; Stra8-Cre (KO) using iTRAQ.

Supplemental Figure S5

MNase digestion of chromatin from sonication resistant spermatids islated from SirT1 f/f (WT) and SirT1 f/f; Stra8-Cre (KO) mice. This is a representative gel image of experiments from 3 different littermate pairs aged 3 months.

Supplemental Figure S6

(A) PAS stained cross-sections from 14 month old SirT1 f/f (WT) and SirT1 f/f; Stra8-Cre (KO) mice. Images were acquired with a 20X objective and are representative of N=2.

(B) Relative levels of sertoli cell and leydig cell markers from 20 month old SirT1 f/f (WT) and SirT1 f/f; Stra8-Cre (KO) mice. N=6; error bars = SEM; NS=not significant.

(C) Relative levels of spermatogonia stem cell, undifferentiated spermatogonia, and differentiating spermatogonia cell markers from 20 month old SirT1 f/f (WT) and SirT1 f/f; Stra8-Cre (KO) mice. N=6; error bars = SEM; NS=not significant.

(D) Relative levels of genes involved in formation of sertoli cell tight junctions that make up the blood testes barrier from 20 month old SirT1 f/f (WT) and SirT1 f/f; Stra8-Cre (KO) mice. N=5; error bars = SEM; NS=not significant.

(E) Streptavidin staining of 3 month and 14 month old SirT1 f/f (WT) and SirT1 f/f; Stra8-Cre (KO) testes injected with Sulfo-NHS-LC-LC-Biotin tracer to assess functionality of the blood testes barrier.

Biotin-streptavidin is red and DNA (DAPI) is blue. Images were acquired with a 20X objective and are representative of N=3 for 3 month and N=2 for 14 month old animals.

Supplemental Figure S7

Sirt1 protein levels do not change in response to diet in the testis.

(A) Western blot for SirT1 in testes isolated from mice fed an Ad libitum diet (AL) or a calorie restricted diet (CR).

(B) Western blot for SirT1 in testes isolated from mice fed a normal chow diet (C) or a high fat diet (HFD).

Supplemental Figure S1

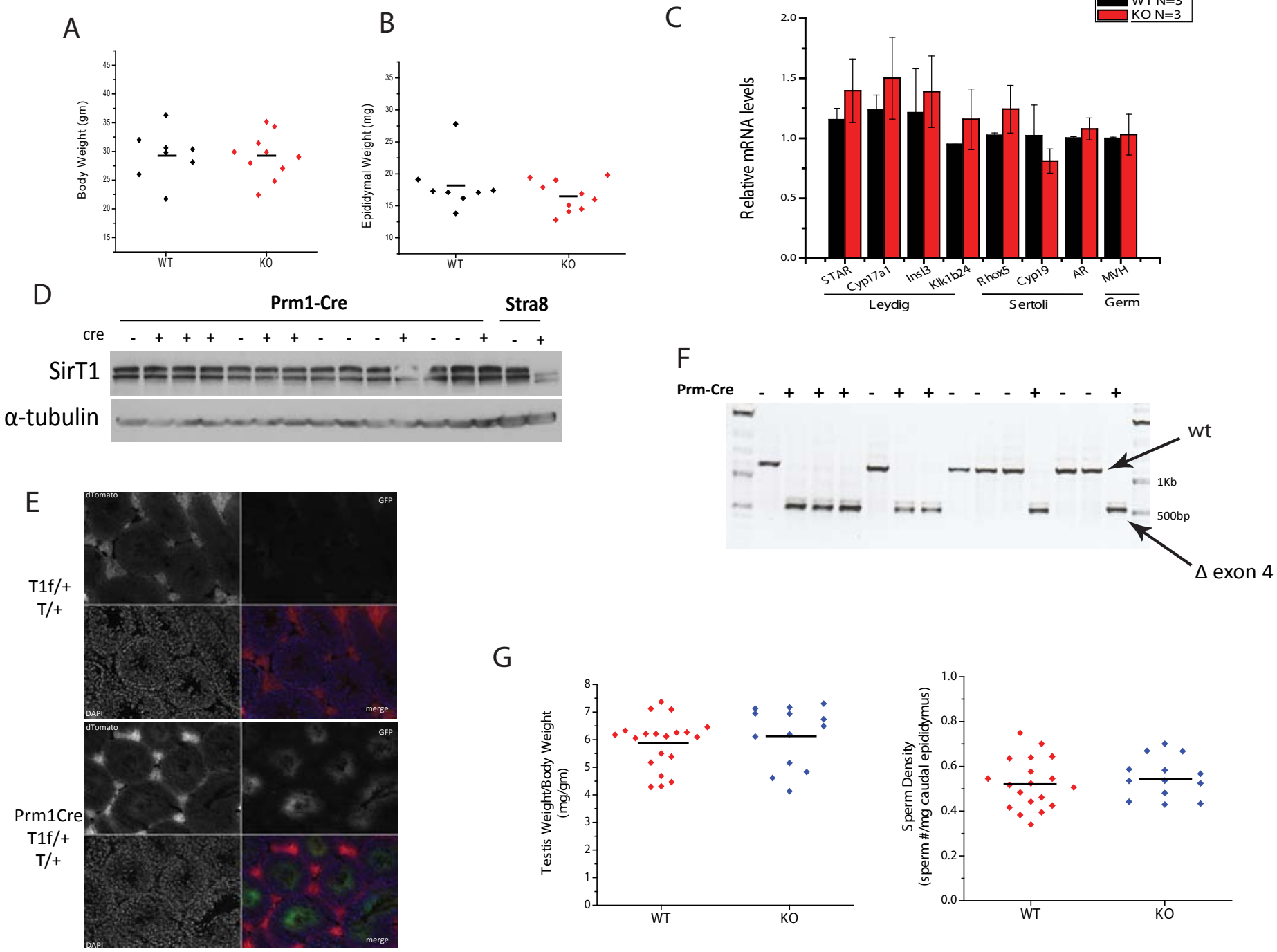


Figure S2

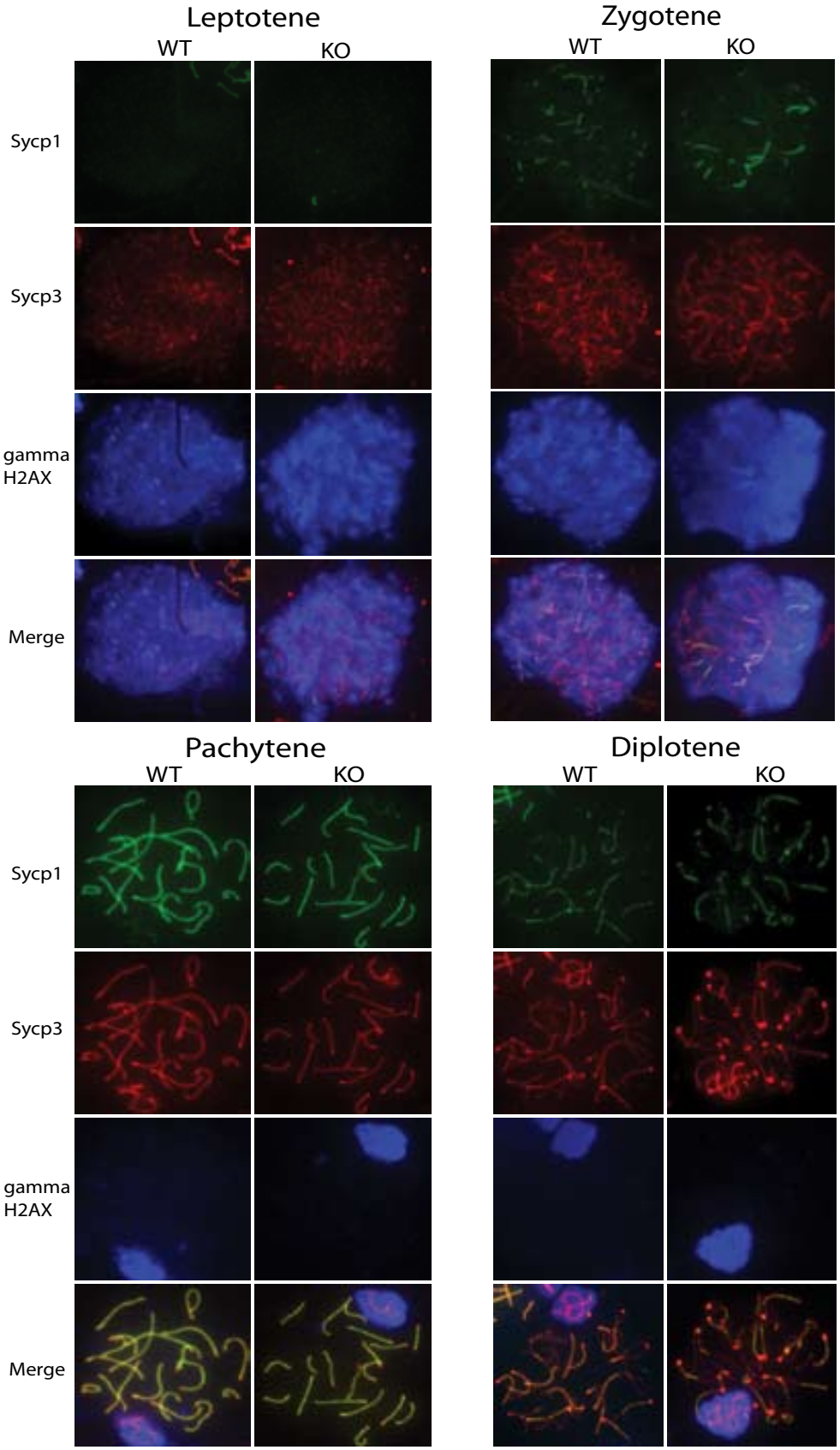

Leptotene
WT
KO
Zygotene
WT
KO
Sycp1
Sycp3
gamma H2AX
Merge
Pachytene
WT
KO
Diplotene
WT
KO
Sycp1
Sycp3
gamma H2AX
Merge

Figure S3

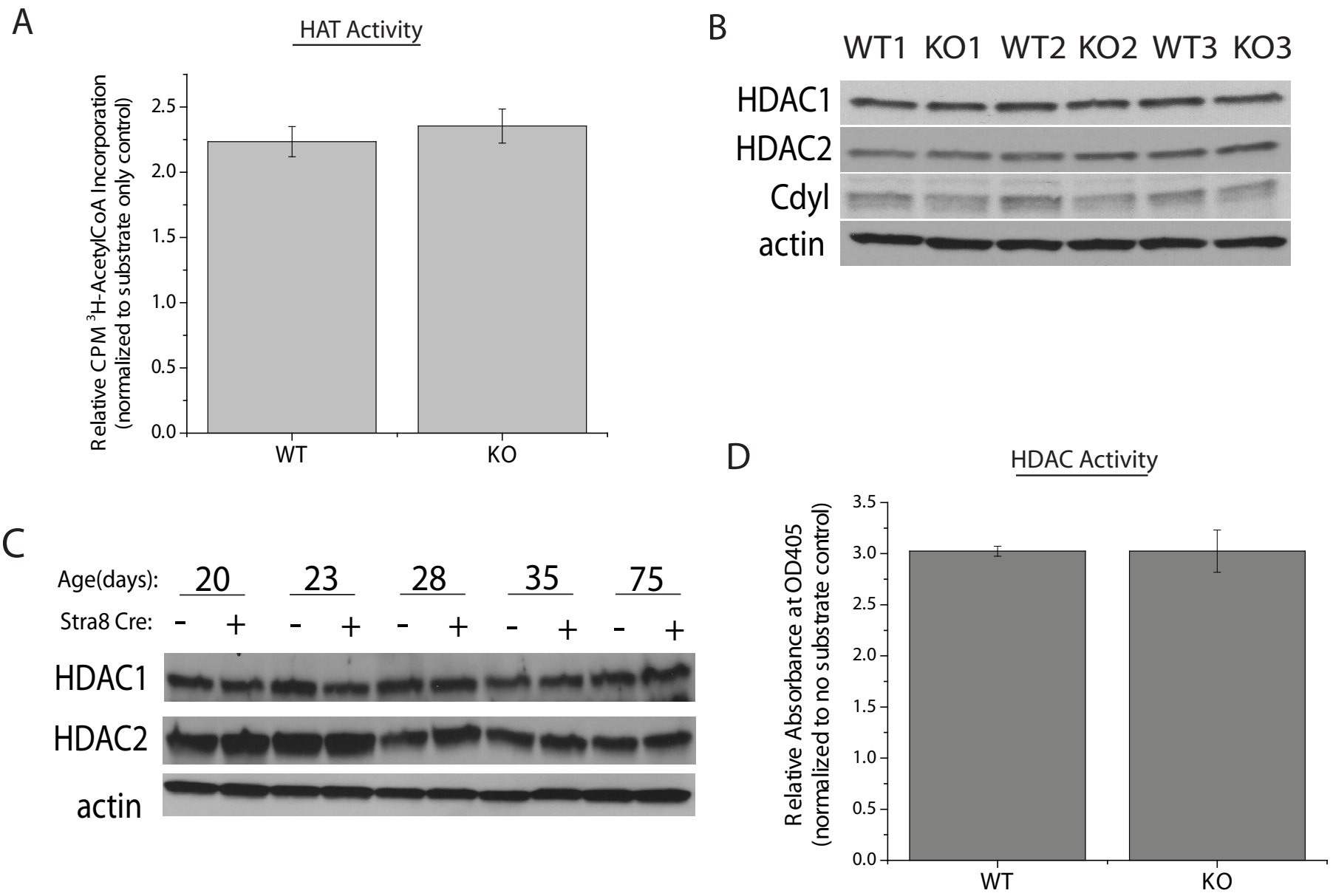
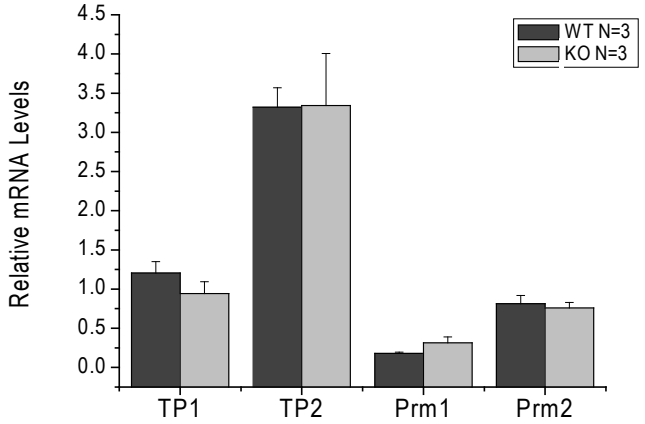
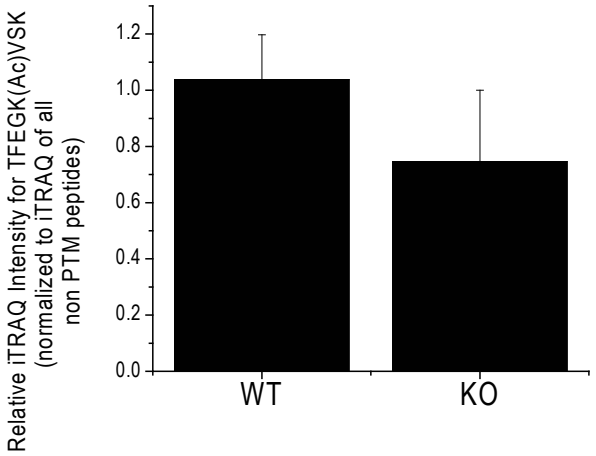

A
HAT Activity
Relative CPM ^{3}H-AcetylCoA Incorporation (normalized to substrate only control)
2.5
2.0
1.5
1.0
0.5
0.0
WT
KO
B
WT1 KO1 WT2 KO2 WT3 KO3
HDAC1
HDAC2
Cdyl
actin
C
Age(days): 20 23 28 35 75
Stra8 Cre: - + - + - + - + - +
HDAC1
HDAC2
actin
D
HDAC Activity
Relative Absorbance at OD405 (normalized to no substrate control)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
WT
KO

Figure S4

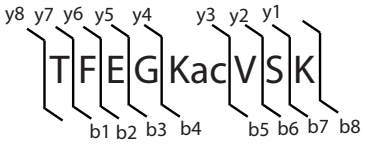
A



C



B



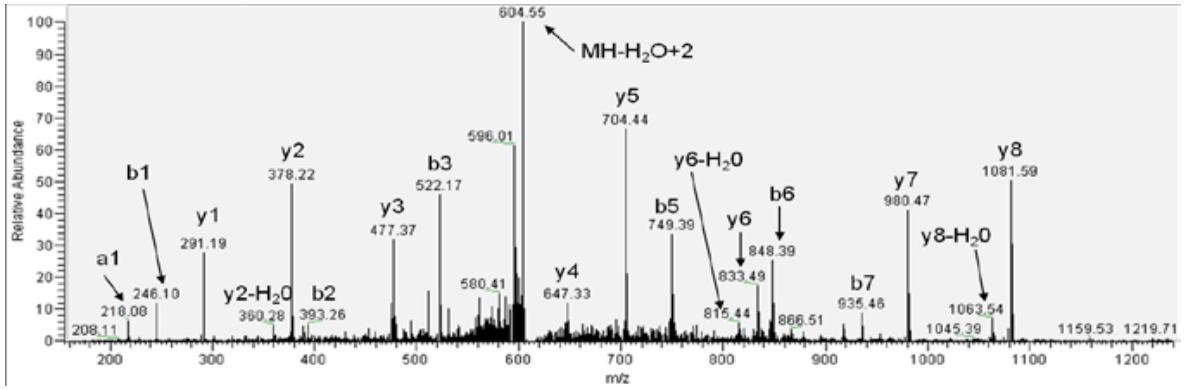


Figure S5

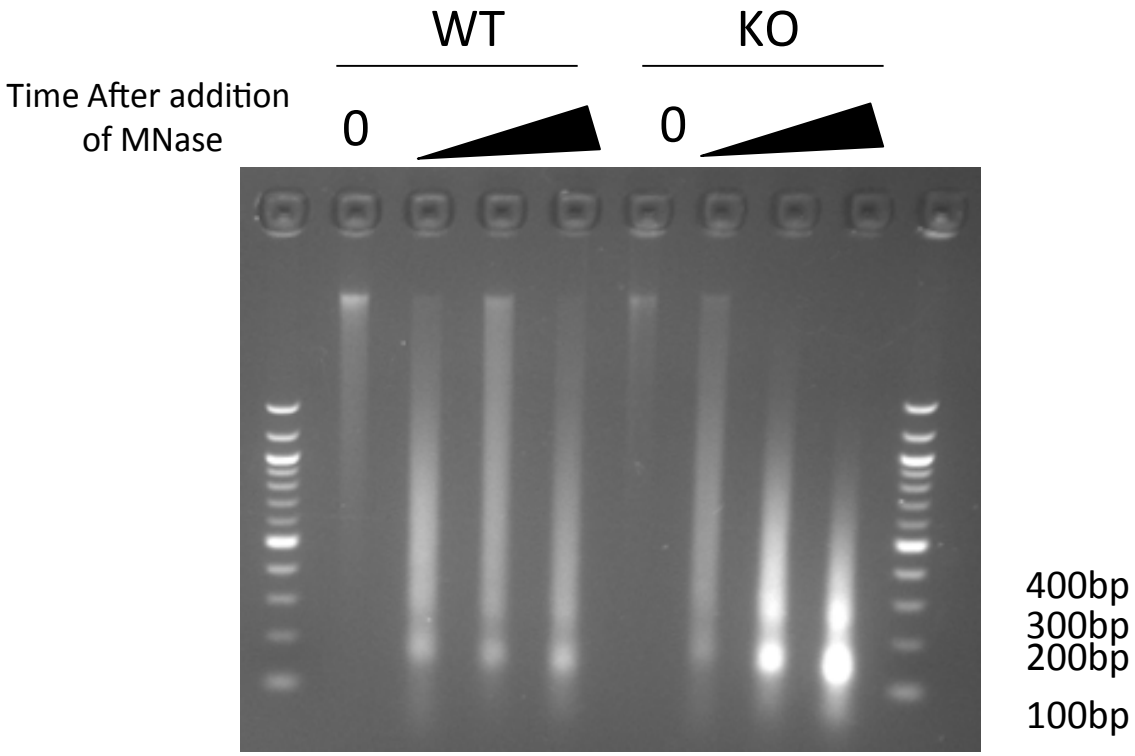

WT
KO
Time After addition of MNase
0
0
400bp
300bp
200bp
100bp

Figure S6

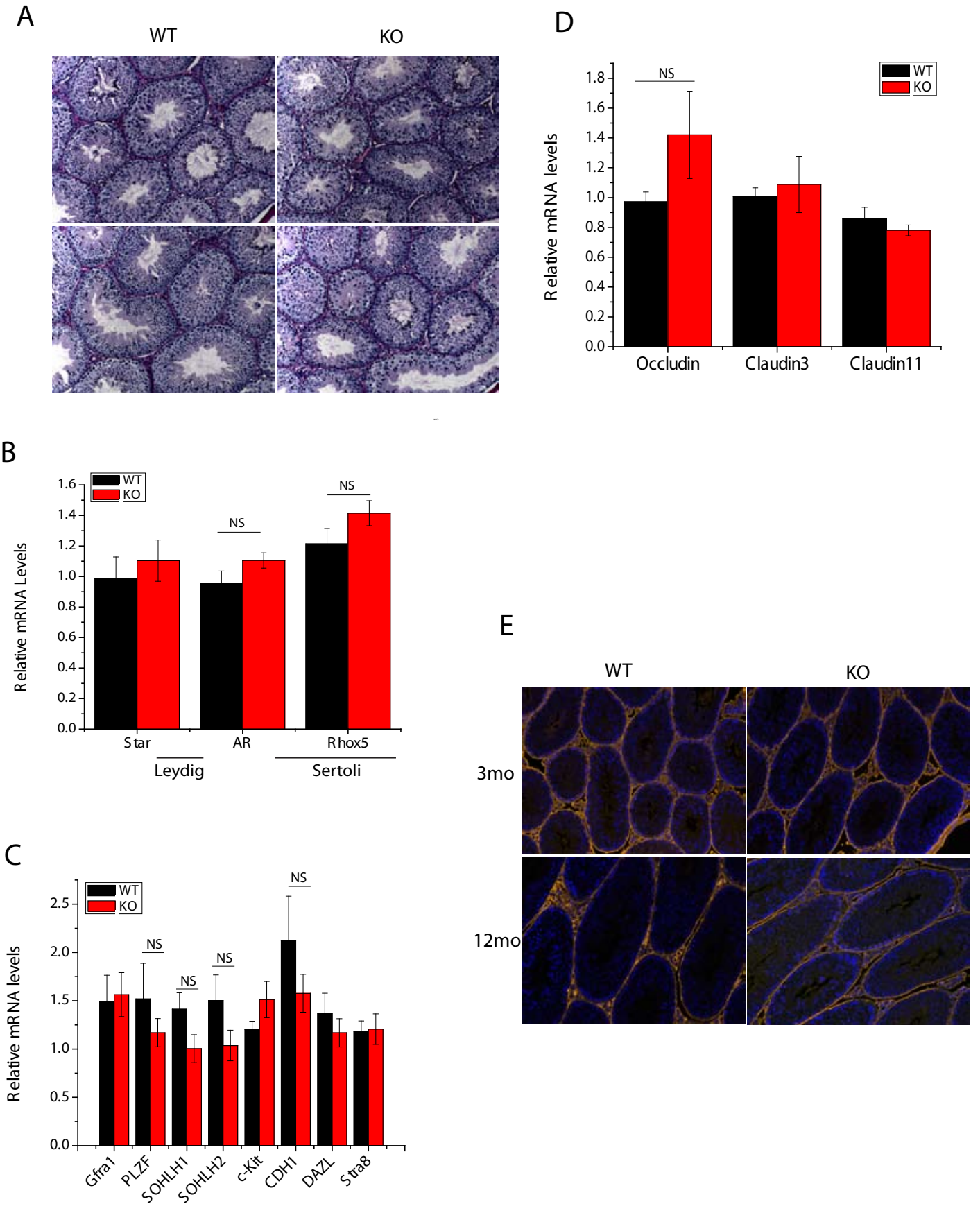

A
WT
KO
D
NS
WT
KO
Relative mRNA levels
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Occludin
Claudin3
Claudin11
B
WT
KO
Relative mRNA Levels
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
NS
NS
Star
AR
Rhox5
Leydig
Sertoli
C
WT
KO
Relative mRNA levels
2.5
2.0
1.5
1.0
0.5
0.0
NS
NS
NS
NS
Gfra1
PLZF
SOHLH1
SOHLH2
c-Kit
CDH1
DAZL
Stra8
E
WT
KO
3mo
12mo

Figure S7