Methods

Animals. The *Nrb02*$^{L-L/-}$ mice used were previously described (8). Mice used in this study were maintained on a mixed background (C57BL/6J/129sv) and housed in temperature-controlled rooms with 12h light/dark cycles. Mice had *ad libitum* access to food and water. Mice were injected subcutaneously, since the first day of life during five days, with diethylstilbestrol (DES, Sigma-Aldrich, L'Isle D'Abeau, France), estradiol benzoate (EB, Sigma-Aldrich) or vehicle (oil tocopherol-stripped). For both EB and DES the same vehicle was used. For both EB and DES, mice were exposed to 0µg, 0.35µg; 0.5µg; 0.75µg; 1.5µg; 2.5µg or 5µg. This study was conducted in accordance with current regulations and standards approved by Institut National de la Santé et de la Recherche Médicale Animal Care Committee.

Histology. Testes from 10-week-old mice were collected, formalin-fixed and embedded in paraffin, and 5 µm-thick sections were prepared and stained with hematoxylin/eosin (n=3-5 animals per group).

Immunohistochemistry

Paraffin sections of Bouin-fixed testis were sectioned at 5 µm. The sections were mounted on positively charged glass slides (Superfrost plus), deparaffinized, rehydrated, treated 20 min at 93–98°C in citric buffer (0.01 M, pH 6), rinsed in osmosed water (2 x 5 min), and washed (2 x 5 min) in Tris-buffered saline. Immunohistochemistry was conducted according to the manufacturer's recommendations as described earlier (27). Slides were then counterstained with hematoxylin. The antibodies used in this study were Tubulin3 (Covance, MMS435P), Cyclin-a1 (Santa Cruz Biotechnology, sc-15383) and Smad6 (Santa Cruz Biotechnology, sc-6034).

Endocrine Investigations. Testosterone concentration was measured on heparin-treated plasma.

Intratesticular testosterone was extracted as previously described (27). A commercial kit (Assay Designs) was used for the assays. To have a better view of the DES effect on testosterone, and avoid confusion due to the difference in basal testosterone level in wild-

type and *Nr0b2* knock-out mice (8), we have decided to express testosterone as a percentage of the vehicle treated mice for each genotypes. Vehicle treated mice were arbitrarily set at 100%.

Plasma LH and FSH measurements

Plasma concentrations of LH and FSH were measured by radioimmunoassay using reagents supplied by the NIADDK with all samples for each hormone assayed in duplicate in one assay (67). The reference preparations used were rat LH RP-3 and rat FSH RP-3, and the minimum detectable concentrations were 0.2 and 3 ng/ml for LH and FSH respectively. The intraassay coefficients of variation were less than 6% (65).

Real-Time RT-PCR. RNA from testis samples were isolated using trizol® (Invitrogen Corporation, Carlsbad, CA). cDNA was synthesized from total RNA with the SuperScript II First-Strand Synthesis System (Life Technologies) and random hexamer primers. The real-time PCR measurement of individual cDNAs was performed using SYBR green dye to measure duplex DNA formation with the Roche Lightcycler system. The sequences of the primers are reported in supplemental data as tables 1 and 2. Standard curves were generated with pools of testis cDNA from animals with different genotypes and/or treatments. Results were analysed using the ct method.

Q-PCR experiments were performed on samples from animals treated with either 0.5µg (data not shown) or 0.75µg because there was no significant difference in the testicular weight with these doses. This suggests that there was no alteration of the cell content of the testis allowing the comparison with vehicle treated mice.

Western blot. Protein were extracted from tissues using the SDS lysis buffer described in the ChIP protocol from Upstate (Upstate Biotechnology Inc). The antibodies used in this study were monomethyl H3K9 (Abcam, ab9045), dimethyl H3K9 (Upstate, 07-441), H3 (Upstate, 05-499), Activated caspase-3 (abcam ab52294), Total caspase-3 (Cell Signalling, #9665), Pcna (SantaCruz Biotechnology, C-20, sc-9857) and Actin (Jackson's Laboratory). They were used in TBS, 0.1%tween, 3% BSA.

Transient transfection. Rar and Nr0b2 plasmids have been described previously (8). F9

cells, provided by Chambon P. (IGBMC, Strasbourg, FRANCE), were transfected with lipofectamin (Invitrogen Corporation) in 6-well plates. RAR (25ng), provided by Rochette-Egly C. (IGBMC, Strasbourg, FRANCE), was transfected with an increasing amount of pCMV-Nr0b2 plasmid (0 to 800ng). The quantity of DNA was maintained constant by the addition of empty pCMV vector to a total amount of 1000 ng of DNA per well. 24 hours after transfection, cells were treated with either 10^{-6}M RA or vehicle (1/1000). Then, cells were harvested 24h after, mRNA or protein extractions were performed.

TUNEL analysis and KI67 staining. TUNEL experiments were performed as previously described (27) on 5 µm of testis fixed in PFA 4%. For the Ki-67 staining, slides were then incubated with anti-Ki67 1/500 (Interchim) overnight at 4°C, then were incubated 1h at room temperature with a goat anti-rabbit secondary antibody (1/250). In each testis, at least 100 random seminiferous tubules were counted. Results are expressed as the number of TUNEL-positive or Ki-67 positive cells per 100 seminiferous tubules.

Chromatin Immuno-Precipitation (ChIP). ChIP was carried out following the protocol provided by the manufacturer (Upstate Biotechnology Inc). In vitro experiments were performed from 10^6, and in vivo ChIP were performed from 10-day-old testis of 3 mice. Samples were cross-linked in 1% formaldehyde for 10 minutes at 37°C. Cells were washed twice with cold PBS1X containing PMSF 1mM and 1X anti-protease mix. Chromatin was sheared to ± 200–1000 bp by sonication. After preclearing, 100µL were taken as input material. Then, 2 µg of the antibody against anti-Flag (M2, Sigma-Aldrich) or RARγ (Proteintech Group, Inc, 11424-1-AP) were added for an overnight incubation. Protein A agarose/Salmon DNA (Upstate Biotechnology Inc) (60µl per IP) was used to recover the immune complexes. Washes and elutions were performed in accordance with the protocol provided by the manufacturer (Upstate Biotechnology Inc). DNA was reverse-crosslinked by adding 100µl of Chelex 10% (Biorad) and boiling 10min. Samples were cooled down to room temperature. 1µl of proteinase K (10mg/ml) was added to the samples and incubated 1h at 55°C. Samples were then boiled 10min. Samples were centrifuged twice for 3min at 13200

rpm and supernatants were pooled (final volume of 150μl). Quantitative-PCR reactions were performed using 3μl of the pooled supernatants.

Statistical Analysis. For statistical analysis, two-way analysis of variance was performed. When significant effects of treatment or genotype or their interactions were obtained, multiple comparisons were made with Tukey's test. All numerical data are represented as mean ± SEM. Significant difference was set at $p < 0.05$.

Supplemental data

Suppl-1.

(**A**) Testicular mRNA expression of *Nr0b2*, *Nr3a1*, *Nr3a2*, *Nr3b1*, *Nr3b2* and *Nr3b3* normalized to *β-actin* levels in whole testis of C57Bl/6J mice of 1, 3, 5 and 90 days (n=3 per group). (**B**) Testicular mRNA expression of *Nr0b2*, normalized to *β-actin* levels in whole testis of 10-day-old $Nr0b2^{+/+}$ (open bars) and $Nr0b2^{L-/L-}$ (black bars) males exposed to 0.75µg of estradiol benzoate (n= 6). (**C**) Weights of testis, epididymis, seminal vehicles and liver normalized to body weight in 10-week-*old* $Nr0b2^{+/+}$ and $Nr0b2^{L-/L-}$ exposed neonatally to 0 or 0.75µg of estradiol benzoate (EB) (n=6-14 per group). All data are expressed as mean ± SEM. * denotes significant difference with the vehicle treated group ($p<0.05$). # denotes significant difference with wild-type mice treated with same dose of EB. (**D**) Spermatozoa count in the tail of epididymis of 10-week-old $Nr0b2^{+/+}$ and $Nr0b2^{L-/L-}$ males exposed to 0 or 0.75µg of EB (n=6-14 per group). All data are expressed as mean ± SEM. * denotes significant difference with the vehicle treated group ($p<0.05$). # denotes significant difference with wild-type mice treated with same dose of EB. **(E)** Spermatozoa count in the tail of epididymis of 10-week-old $Nr0b2^{+/+}$ and $Nr0b2^{L-/L-}$ males exposed neonatally to 0 or 5µg of DES, 50X of ICI or 5µg of DES+50XICI (n=6-14 per group). All data are expressed as mean ± SEM. * denotes significant difference with the vehicle treated group ($p<0.05$). **(F)** Weights of testis, epididymis and seminal vehicles normalized to body weight in 10-week-old $Nr0b2^{+/+}$ and $Nr0b2^{L-/L-}$ exposed to 0 or 5µg of DES, 50X of ICI or 5µg of DES+50XICI (n=6-14 per group). All data are expressed as mean ± SEM. * denotes significant difference with the vehicle treated group ($p<0.05$).). # denotes significant difference with wild-type mice treated with same dose.

Suppl-2.

(**A**) Immunohistology of Smad6 was used as a marker of post-meiotic germ cells on testes from 10-week-old $Nr0b2^{+/+}$ and $Nr0b2^{L-/L-}$ mice exposed to 0 or 5 µg of DES (n=6 per group). (**B**) Immunohistochesmistry for proliferation marker Ki-67 was performed on testes from 10-week-old of $Nr0b2^{+/+}$ and $Nr0b2^{L-/L-}$ mice exposed to 0, 0.35, 0.5, 0.75, 1.5 and 5 µg of DES

(n=6 per group). Cell proliferation analysis in tubules of *Nr0b2*$^{+/+}$ and *Nr0b2*$^{L-/L-}$ mice exposed to 0, 0.35, 0.5, 0.75, 1.5 and 5 µg of DES (n=6 per group) as evaluated by Ki-67 staining. The number of positive cells per 100 seminiferous tubules are indicated (n= 6). (**C**) Pcna immunoblotting performed on whole testis of 10-week-old *Nr0b2*$^{+/+}$ and *Nr0b2*$^{L-/L-}$ mice exposed to 0 or 5 µg of DES (n=6 per group). Quantification of Pcna accumulation compared to Actin. Vehicle treated mice were arbitrarily fixed at 100%. (**D**) Apoptosis in 10-week-old *Nr0b2*$^{+/+}$ and *Nr0b2*$^{L-/L-}$ mice exposed neonatally to 0 or 2.5µg of EB (n=6 per group) as analysed by TUNEL staining. Representative micrographs of a testis of *Nr0b2*$^{+/+}$ and *Nr0b2*$^{L-/L-}$ exposed with 0 and 2.5µg of DES. (**E**) Quantification of the TUNEL analyses. Numbers are indicated as number of positive cells per 100 seminiferous tubules (n=4-6). Vehicle treated mice were arbitrarily set at 100%. *; p<0.05. (**F**) Intratesticular testosterone levels in 10-week-old *Nr0b2*$^{+/+}$ and *Nr0b2*$^{L-/L-}$ mice exposed to 0 or 2.5µg of EB (n= 6 to10 per group). Vehicle treated mice were arbitrarily fixed at 100%. (**G**) Intratesticular testosterone levels in 10-week-old *Nr0b2*$^{+/+}$ and *Nr0b2*$^{L-/L-}$ mice exposed to 0 or 5µg of DES, 50X of ICI or 5µg of DES+50XICI (n= 6 to10 per group). Vehicle treated mice were arbitrarily fixed at 100%.

Suppl-3.

(**A**) Testicular mRNA expression of *Stra8*, *Dmc1* and *Cyclin-a1* normalized to *β-actin* levels in 6-day-old *Nr0b2*$^{+/+}$ and *Nr0b2*$^{L-/L-}$ mice exposed to 0 or 0.75µg of EB (n=10 to 15 per group). Vehicle treated mice were fixed at 100%. *; p<0.05. (**B**) Testicular mRNA expression of *Nanos3*, *Cyclin-d2*; *Oct3/4* normalized to *β-actin* levels in 10-day-old *Nr0b2*$^{+/+}$ and *Nr0b2*$^{L-/L-}$ mice exposed to 0 or 0.75µg of EB (n=10 to 15 per group). Vehicle treated mice were fixed at 100%. *; p<0.05. (**C**) Immunoblot of activated Pcna performed on 10-day-old *Nr0b2*$^{+/+}$ and *Nr0b2*$^{L-/L-}$ mice exposed to 0 or 0.75 µg of DES (n=6 per group). Quantification of Pcna protein accumulation compared to that of Actin. The value of vehicle treated mice was set at 100%. *; p<0.05. (**D**) Testicular mRNA expression of *Star*, *Cyp17*, *Pem* and *Osp* normalized to *β-actin* levels in *Nr0b2*$^{+/+}$ and *Nr0b2*$^{L-/L-}$ 10-day-old mice exposed to 0 or 0.75µg of EB (n=5 to 7 per group). Vehicle treated mice were fixed at 100%. *; p<0.05. (**E**) Testicular mRNA

expression of *Nr3a1*; *Insl3*, *Ren1* normalized to *β-actin* levels in 10-day-old *Nr0b2*$^{+/+}$ and *Nr0b2*$^{L-/L-}$ mice exposed to 0 or 0.75µg of EB (n=10 to 15 per group). Vehicle treated mice were fixed at 100%. *; p<0.05.

Suppl-4.

(**A**) Testicular mRNA expression of *G9a* normalized to *β-actin* levels in whole testis of 6 and 10-day-old *Nr0b2*$^{+/+}$ and *Nr0b2*$^{L-/L-}$ mice exposed to 0 or 0.75µg of EB (n=10 to 15 per group). *; p<0.05. Vehicle treated mice were arbitrarily fixed at 100%. (**B**) Immunoblot of G9a performed on 10-day-old *Nr0b2*$^{+/+}$ and *Nr0b2*$^{L-/L-}$ mice exposed to 0 or 0.75 µg of EB (n=9 per group). Quantification of G9a protein accumulation compared to Actin. Vehicle treated mice were arbitrarily fixed at 100%. *; p<0.05. (**C**) Immunoblots of mono- and dimethyl-H3K9 (H3K9me1 or H3K9me2) performed on 10-day-old *Nr0b2*$^{+/+}$ and *Nr0b2*$^{L-/L-}$ mice exposed to 0 or 0.75 µg of EB (n=9 per group). Quantification of mono- and di methyl-H3K9 accumulation was performed versus total H3. Vehicle treated mice were fixed at 100%. *; p<0.05. These lanes were run on the same gel but were non-contiguous. (**D**) Testicular mRNA expression of G9a target genes *Chst11*, *290*, *110*, *Iap*, *291* and *Wfdc15a* normalized to *β-actin* levels in whole testis of 10-day-old *Nr0b2*$^{+/+}$ and *Nr0b2*$^{L-/L-}$ mice exposed to 0 or 0.75µg of EB (n=10 to15 per group). Vehicle treated mice were fixed at 100%. *; p<0.05.

Suppl-5.

(**A**) Plasma estrogen levels in 10-week-old *Nr0b2*$^{+/+}$ and *Nr0b2*$^{L-/L-}$ mice (n=6). (**B**) Testicular mRNA expression of *Nr3a1*, *Nr3a2*, *Insl3*, and *Ren1* normalized to *β-actin* levels in whole testis of 10-week-old *Nr0b2*$^{+/+}$ and *Nr0b2*$^{L-/L-}$ mice (n=6 to 8 per group). (**C**) Testicular mRNA expression of *Nr3a1*, *Nr3a2*, *Insl3*, *Ren1* and *Greb-1* normalized to *β-actin* levels in whole testis of 10 days old *Nr0b2*$^{+/+}$ and *Nr0b2*$^{L-/L-}$ mice (n=6 to 8 per group). (**D**) Testicular mRNA expression of *Stra8* normalized to *β-actin* levels in whole testis of 10-week-old *Nr0b2*$^{+/+}$ and *Nr0b2*$^{L-/L-}$ mice exposed to 0 or 0.75µg of DES (n=10 per group). *; p<0.05. Vehicle treated mice were arbitrarily fixed at 100%. (**E**) Testicular mRNA expression of *G9a* normalized to *β-actin* levels in whole testis of 10-week-old *Nr0b2*$^{+/+}$ and *Nr0b2*$^{L-/L-}$ mice exposed to 0; 0.35;

0.5; 0.75µg of DES (n=10 per group). *; p<0.05. Vehicle treated mice were arbitrarily fixed at 100%. (**F**) Immunoblot of G9a performed on 10-week-old *Nr0b2*$^{+/+}$ and *Nr0b2*$^{L-/L-}$ mice exposed to 0 or 0.75 µg of DES (n=6 per group). Quantification of G9a protein accumulation compared to Actin. Vehicle treated mice were arbitrarily fixed at 100%. *; p<0.05. These lanes were run on the same gel but were non-contiguous.

Table1.

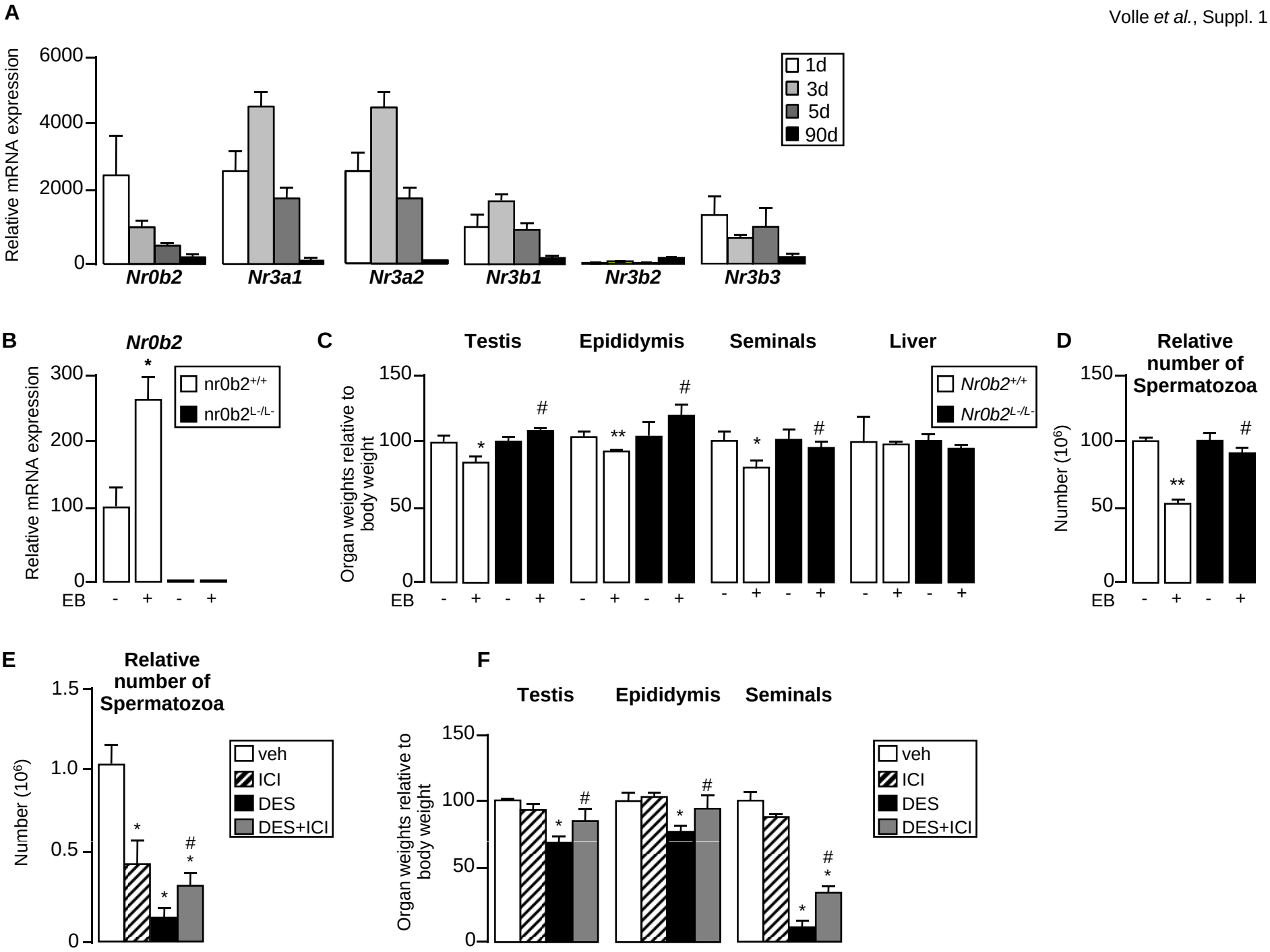
List of primer sequences used for the mRNA analyses.

	Forward	**Reverse**
Star	TGTCAAGGAGATCAAGGTCCTG	CGATAGGACCTGGTTGATGAT
Cyp11a1	CTGCCTCCAGACTTCTTTCG	TTCTTGAAGGGCAGCTTGTT
3bhsd	ATGGTCTGCCTGGGAATGAC	ACTGCAGGAGGTCAGAGCT
Cyp17a1	CCAGGACCCAAGTGTGTTCT	CCTGATACGAAGCACTTCTCG
Nr3c4	AATGAGTACCGCATGCACAA	GGAGCTTGGTGAGCTGGTAG
Pem	GCCTGGGAGTCAAGGAA	GCCACTATCCTTGTCCCCATCA
Osp	GATTGGCATCATCGTCACAACG	AGCCAGCAGAATAAGGAGCAAC
Nr3a1	CATATGATCAACTGGGCAAAGA	ACTCCGGAATTAAGCAAAATGA
Nr3a2	TCTTTGCTCCAGACCTCGTT	GTGTCAGCTTCCGGCTACTC
Insl3	ACTGATGCTCCTGGCTCTGG	GGAGATGTCTCTGCTCTAGC
Nr5a1	TGCAGAATGGCCGACCAG	TGGCGGTAGATGTGGTC
Nr5a2	CTCTTGATTCTCGATCACATTTACC	CCAGGAACTTGAGACATACAAACTC
Nr0b1	TGCACTTCGAGATGATGGAG	ATCTGCTGGGTTCTCCACTG
Nr0b2	CGATCCTCTTCAACCCAGATG	AGGGCTCCAAGACTTCACACA
Oct3/4	AAGTTGGAGAAGGTGGAACC	TGATCCTCTTCTGCTTCAGC
Nanos3	TGCAGGCAAAAAGCTGACC	CTTCCTGCCACTTTTGGAAC
Cyclind2	CGATGATTGCAACTGGAAGC	TTCAGCAGCAGAGCTTCGAT
Stra8	GTTTCCTGCGTGTTCCACAAG	CACCCGAGGCTCAAGCTTC
Dmc1	CCATATCACTACTGGGAGC	GTACTGCTTCATGGTCTAC
Cyclina1	GATGTGTATGAAGTCGACACC	GTGGGGTCAACCAGCATTGG
Ren1	GATGGACAGAAGGAGGATGC	ACTCATAGAGGCTGTGAATCC
Greb1	CTTCCTTACGCTCCAACACCC	TCTTCCTCATTGTGTGCTCG
G9a	ATGTCCAAACCTAGCAACG	TGCATGTCATCACTCATGCG
Akr1c13	ATCTCTGTTGGACACTGTGG	ACTTGACCAATCCTGCATCC
Akr1c12	ATACCTTCCAGCAGAGTTCC	CAGCATCTGAGTCACATCAC
Chst11	GTCCCATCCTGGCCCACCTCTTTG	CCTGGAGGAACATTTCACGCATCTG
Wfdc15a	TAGAGCAACTAGGAAAGGAG	GCATCTGACAGTAGTAGAAGC
Mei1	CTTCATTCACGCTGACATCC	TATCAGGCAGTTGTAGCTGG
Sefb42	CATCCTGGTGACATCATTGC	TGCACAGCTTCCACATCTTC
290	GCCTAACTACAAGCAGAGAAGAG	GGAGTACTACTTTGAGCAGCAC
291	AGCAGGAAAGACACAACACCA	GAGACTGTGAGCCCAAGTAGG
110	GCAAGCGAAGAAAGATGTACGAAA	AAGGATTGCTTAGGTTGGCTACAT
Iap	TCAGGTGATGTGGAGCTCAG	GCATACATCCCTGCACACAC
Nr3b1	ACTGCCACTGCAGGATGAG	CACAGCCTCAGCATCTTCAA
Nr3b2	GGGAGCTTGTGTTCCTCATC	ATCTCCATCCAGGCACTCTG
Nr3b3	GATGAGCCTCCTCCAGAGTG	TGCACAGCTTCCACATCTTC

Table 2.

List of primer sequences used for the ChIP experiment studies.

	Forward	**Reverse**
290	GCCTAACTACAAGCAGAGAAGAG	GGAGTACTACTTTGAGCAGCAC
291	AGCAGGAAAGACACAACACCA	GAGACTGTGAGCCCAAGTAGG
110	GCAAGCGAAGAAAGATGTACGAAA	AAGGATTGCTTAGGTTGGCTACAT
wfdc15a	GGATAACCAGCAGGGAAGCTGAGG	TCTCTAGGTGCCTGAACCTACAGC
282	TGGTCTCCATCGTCCCCAAG	TTTGCCAGGTTATGAGGATTTGTG
g9a-neg	AACGTTGCGGAACATGAACC	TCAGGTAGTTCACGATGTCC
g9a-RARE	TGGTGCTGGCATAAACCATGC	GGGAATTGTAGTCTTCTAGG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4000
2000
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1d
3d
5d
90d
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Nr3b1
Nr3b2
Nr3b3
B
Nr0b2
Relative mRNA expression
300
200
100
0
nr0b2+/+
nr0b2L-/L-
EB - + - +
C
Testis
Epididymis
Seminals
Liver
Organ weights relative to body weight
150
100
50
0
Nr0b2+/+
Nr0b2L-/L-
EB - + - + - + - + - + - + - + - +
D
Relative number of Spermatozoa
Number (10⁶)
150
100
50
0
EB - + - +
E
Relative number of Spermatozoa
Number (10⁶)
1.5
1.0
0.5
0
veh
ICI
DES
DES+ICI
F
Testis
Epididymis
Seminals
Organ weights relative to body weight
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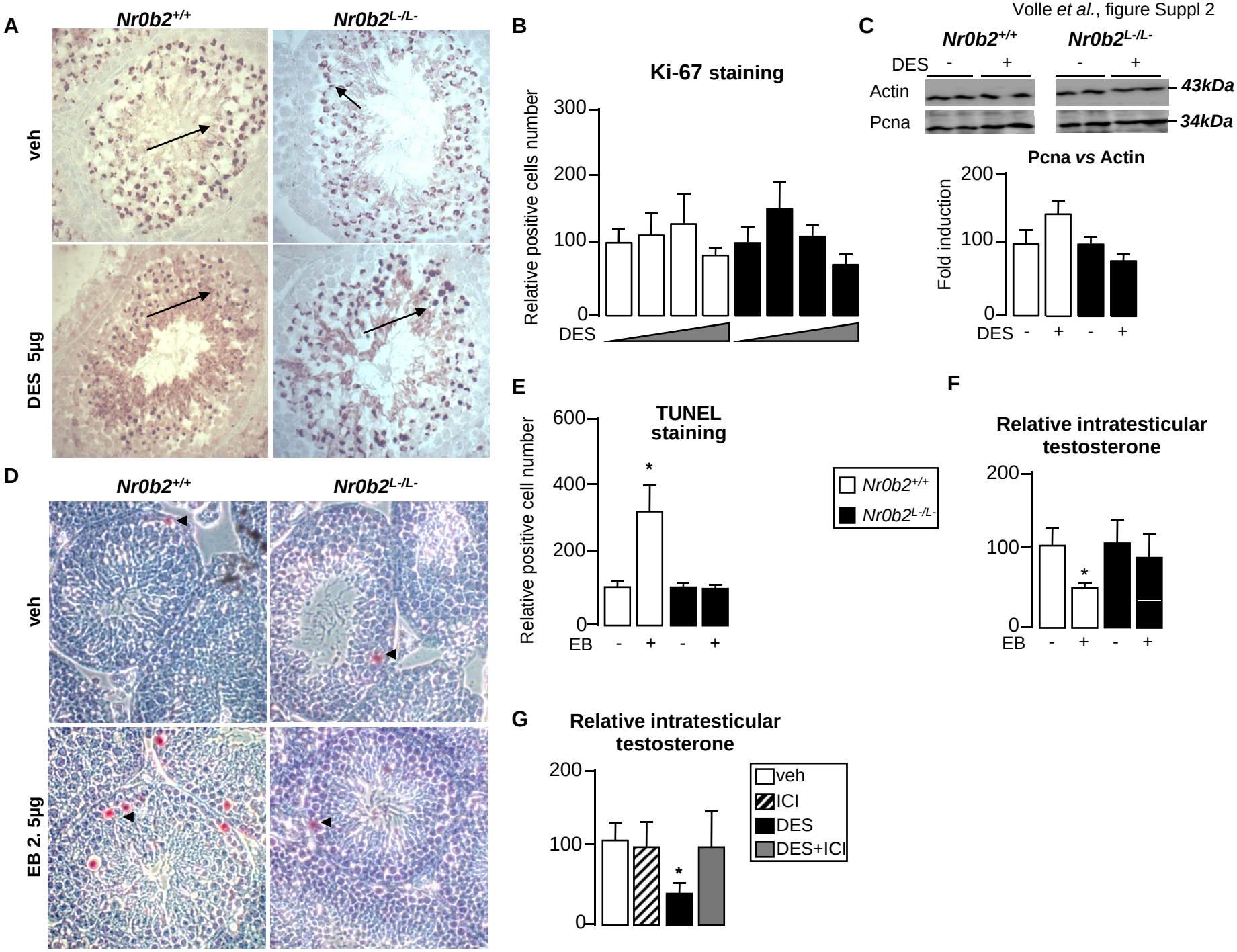
100
50
0
veh
ICI
DES
DES+ICI


A
Nr0b2+/+
Nr0b2L-/L-
veh
DES 5µg
B
Ki-67 staining
Relative positive cells number
300
200
100
0
DES
C
Nr0b2+/+
Nr0b2L-/L-
DES - + - +
Actin
Pcna
43kDa
34kDa
Pcna vs Actin
Fold induction
200
100
0
DES - + - +
D
Nr0b2+/+
Nr0b2L-/L-
veh
EB 2. 5µg
E
TUNEL staining
Relative positive cell number
600
400
200
0
*
EB - + - +
Nr0b2+/+
Nr0b2L-/L-
F
Relative intratesticular testosterone
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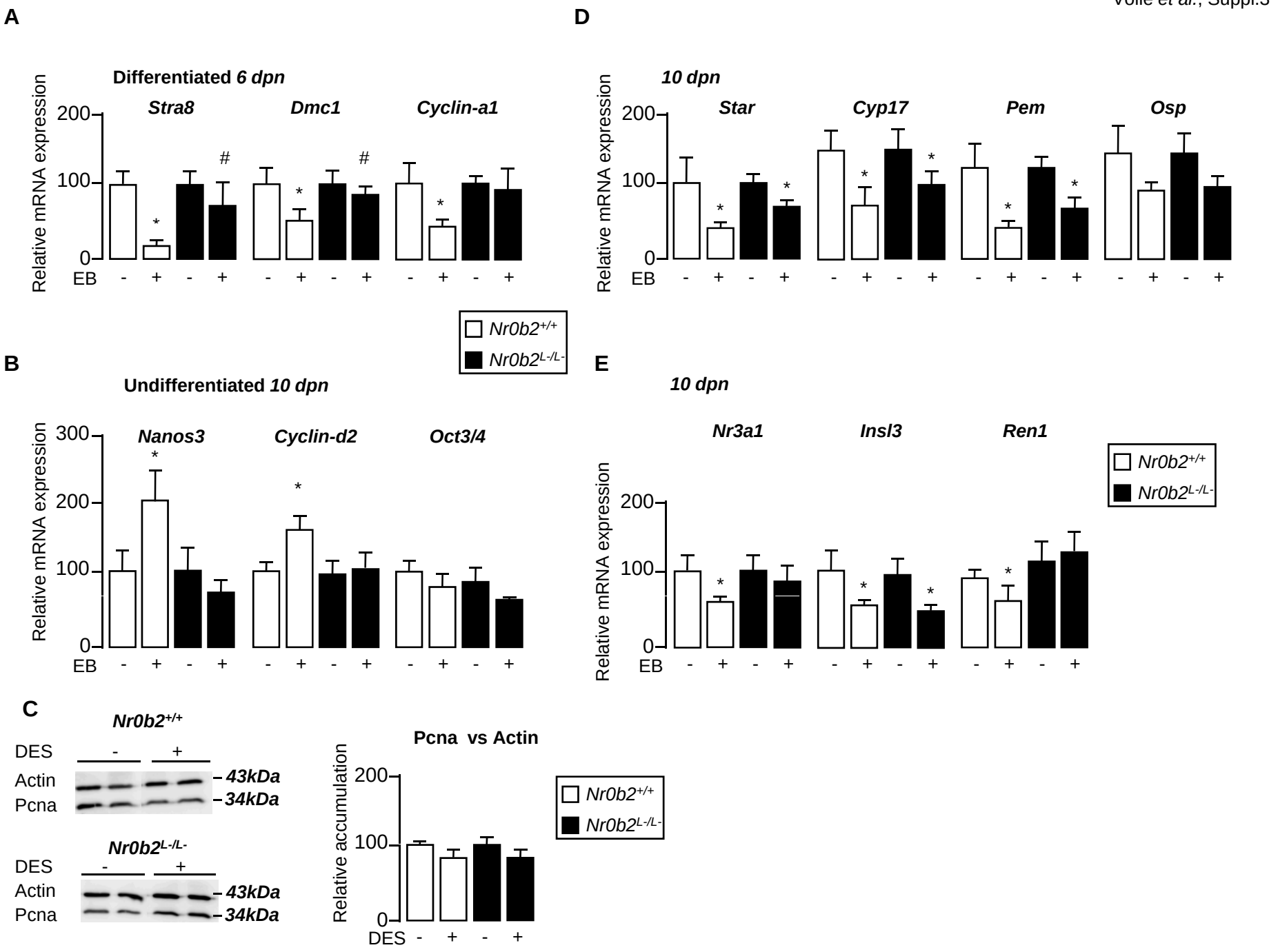
DES
DES+ICI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Dmc1
Cyclin-a1
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10 dpn
Star
Cyp17
Pem
Osp
Relative mRNA expression
200
100
0
EB - + - + - + - + - + - + - + - +
Nr0b2+/+
Nr0b2L-/L-
B
Undifferentiated 10 dpn
Nanos3
Cyclin-d2
Oct3/4
Relative mRNA expression
300
200
100
0
EB - + - + - + - + - + - +
E
10 dpn
Nr3a1
Insl3
Ren1
Relative mRNA expression
200
100
0
EB - + - + - + - + - + - + - + - +
Nr0b2+/+
Nr0b2L-/L-
C
Nr0b2+/+
DES - +
Actin
Pcna
43kDa
34kDa
Nr0b2L-/L-
DES - +
Actin
Pcna
43kDa
34kDa
Pcna vs Actin
Relative accumulation
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100
0
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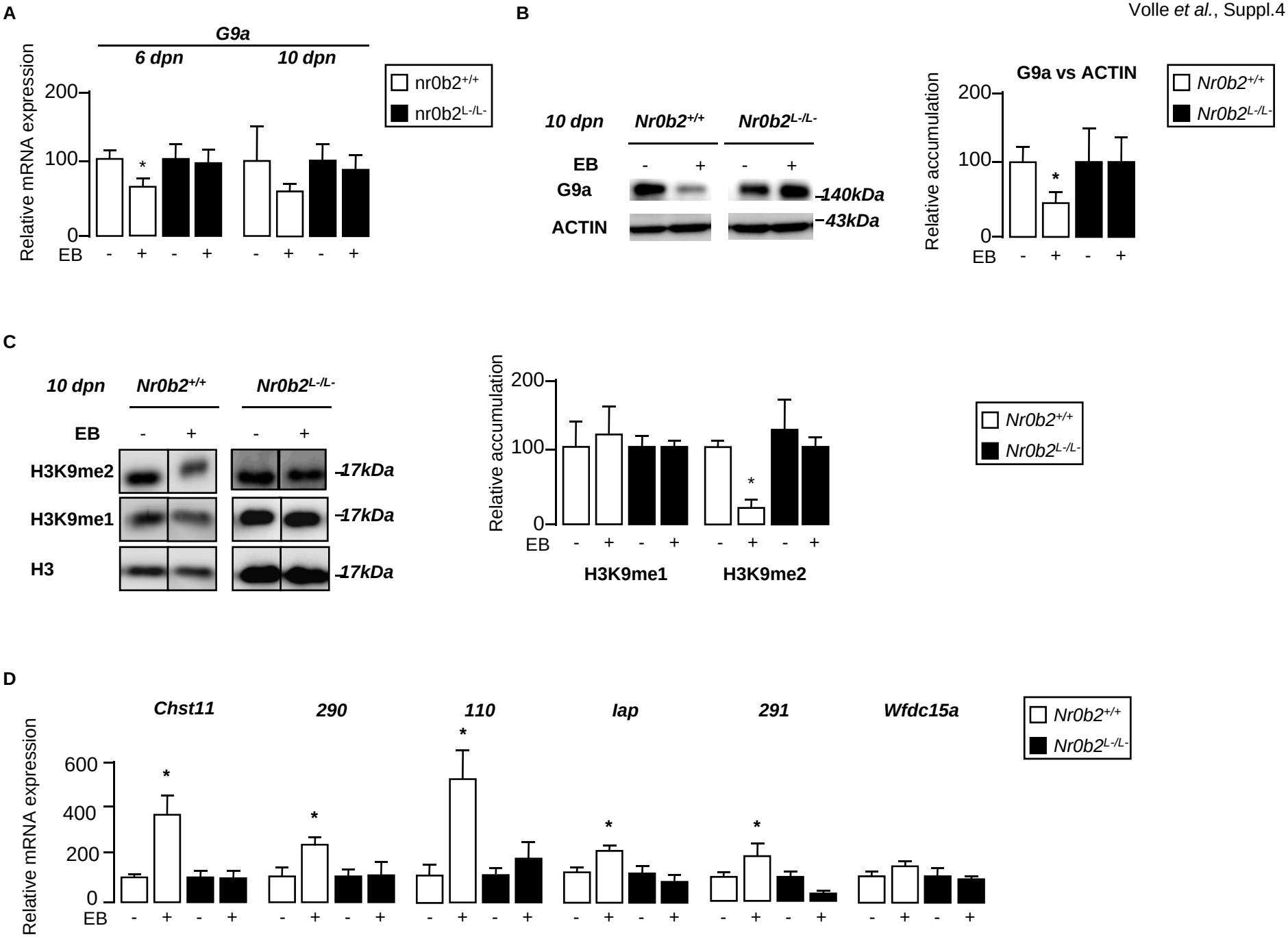
Nr0b2+/+
Nr0b2L-/L-


A
G9a
6 dpn
10 dpn
Relative mRNA expression
200
100
0
EB - + - + - + - +
nr0b2+/+
nr0b2L-/L-
B
10 dpn
Nr0b2+/+
Nr0b2L-/L-
EB - + - +
G9a
ACTIN
140kDa
43kDa
G9a vs ACTIN
Relative accumulation
200
100
0
EB - + - +
Nr0b2+/+
Nr0b2L-/L-
C
10 dpn
Nr0b2+/+
Nr0b2L-/L-
EB - + - +
H3K9me2
H3K9me1
H3
17kDa
17kDa
17kDa
Relative accumulation
200
100
0
EB - + - + - + - +
H3K9me1
H3K9me2
Nr0b2+/+
Nr0b2L-/L-
D
Chst11
290
110
Iap
291
Wfdc15a
Relative mRNA expression
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200
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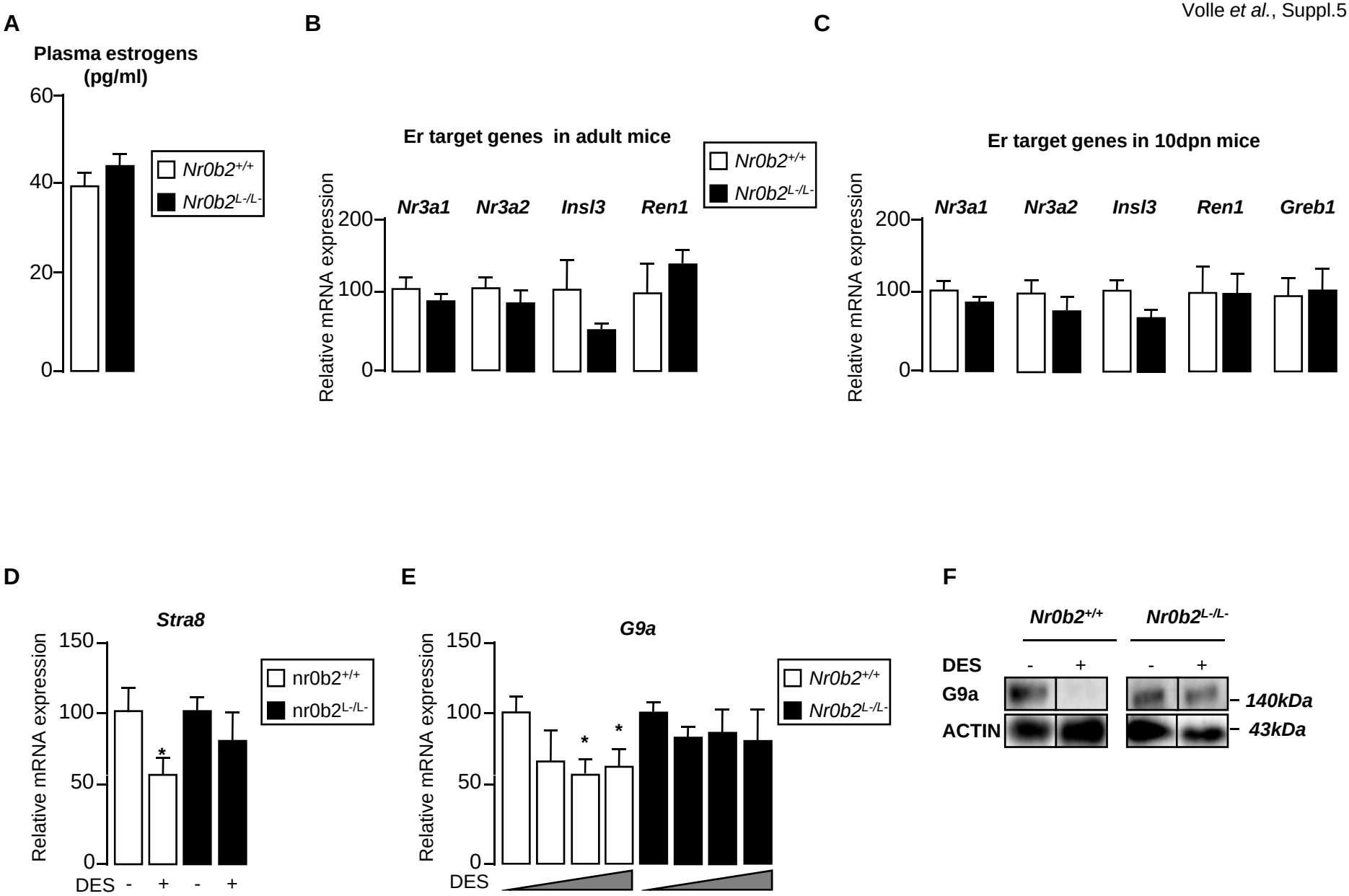
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20
0
Nr0b2+/+
Nr0b2L-/L-
B
Er target genes in adult mice
Relative mRNA expression
200
100
0
Nr3a1
Nr3a2
Insl3
Ren1
Nr0b2+/+
Nr0b2L-/L-
C
Er target genes in 10dpn mice
Relative mRNA expression
200
100
0
Nr3a1
Nr3a2
Insl3
Ren1
Greb1
D
Stra8
Relative mRNA expression
150
100
50
0
*
nr0b2+/+
nr0b2L-/L-
DES - + - +
E
G9a
Relative mRNA expression
150
100
50
0
*
*
Nr0b2+/+
Nr0b2L-/L-
DES
F
Nr0b2+/+
Nr0b2L-/L-
DES - + - +
G9a
ACTIN
140kDa
43kDa