Supplemental information

Supplemental material and methods

Mice Generation and Genotyping.

For generation of Mex-3b null mice, the second exon was flanked by *loxP* sequences, and the Neo selectable marker was removed by transient expression of Cre recombinase in the ES cells. The *Mex-3b* null allele was obtained by breeding conditional floxed *Mex-3b* animals with Nestin-Cre mice expressing Cre in the germ line (Betz et al., 1996). Heterozygous *Mex-3b +/-* animals negative for Cre were bred to each other to obtain the *Mex-3b* knock-out mice. For the generation of Sertoli cells specific *Mex-3b* null mice, conditional *Mex-3b* floxed mice were bred with AMH-Cre mice expressing Cre (Lecureuil et al., 2002).

Embryos and mice were genotyped by multiplex PCR using 3 primers as described in figure 1A:

Mex-3b (1) 5' (GCTCAGTTGGATACCAGCAGC)

Mex-3b (2) 3' (CAAGCATCGTCAGCTGTGTGTAATG)

Mex-3b (3) 5' (GGGCCTTTAACCTCATGGTC)

The wild-type allele produced a band of 433 bp, the floxed allele a band of 559 bp and the knock-out allele a band of 387 bp. Survival of wild type, *Mex-3b +/-* and *Mex-3b -/-* mice was monitored for 60 days after birth.

Transfection and Infection.

For transfection, cells were plated for 24h and plasmids transfected into cells using ExGen 500 (Upstate Biotechnologies) or Jet Pei (Ozyme) according to the manufacturer's instructions. For infections, cells were plated for 24h and lentiviruses were added to the culture media at a MOI of 30 in the presence of polybren (8 µg/ml). Expression of GFP proteins was controlled by microscopy and Western blot.

Histological sections and Staining.

Serial sections (3µm) were stained with Periodic Acid-Schiff and Gill's Hematoxilin solution (Sigma-Aldrich). For immunohistochemistry, sections were deparafinized, deshydrated and incubated with primary antibody (mouse anti-vimentin (1:100,

Sigma-Aldrich)). The ABC MOM and AEC peroxidase substrate kits (Vector laboratories) and Hematoxylin QS were used.

Biotin Tracer Studies.

Mice (5 months old) were anesthetized and their testes exposed. Before injection of 50 µl of 10 mg/ml EZ-Link Sulfo-NHS-LC-Biotin (Pierce Chemical Co.), into the interstitium of one testis and 50 µl of 1 mM $CaCl_2$ in PBS in the other, as a control. After 30 min the animals were euthanized, and their testes were immediately removed and frozen on dry ice. Cryosections of 5-µm thickness were fixed for 10 min with 4% PFA, rinced with PBS and treated with Alexa Fluor 488 streptavidin. The sections were rinsed twice with PBS for 10 min and mounted in DAPI containing mounting medium.

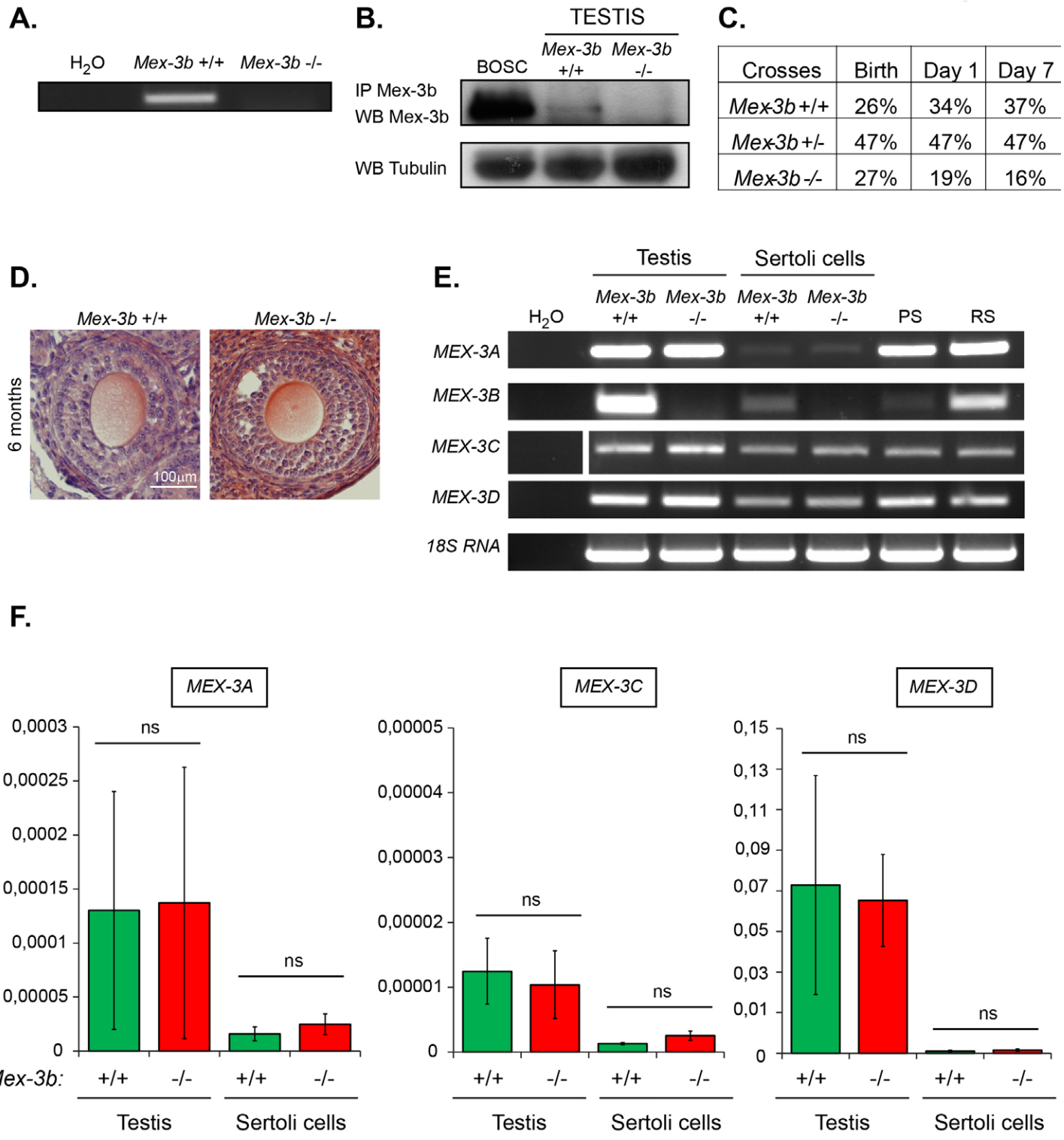

Figure S1. Mex-3b depletion affects viability and fertility in both male and female mice.

(A) RT-qPCR analysis of *Mex-3b* transcripts isolated from MEFs cells. (B) Western blot analysis of endogenous MEX-3B (indicated by black arrow) immunoprecipitated from BOSC cells and from whole mouse testis with the indicated *Mex-3b* genotypes. One representative experiment of three is shown. (C) Table of breeding and survival data of *Mex-3b* deficient mice. The average genotypes of offspring from *Mex-3b +/-* mating at birth to day 7. (D) Hematoxilin and Scarlett Eosin staining of secondary follicle cross section from 6 months-old mice with the indicated *Mex-3b* genotypes.. (E) RT-PCR analysis of the levels of the four distinct *Mex-3* mRNA in the testis of wt and *Mex-3b* deficient mice, purified Sertoli cells and purified pachytene spermatocytes (PS) and round spermatids (RS). (F) RT-qPCR analysis of *Mex-3a, c and d* transcripts isolated from testis and purified Sertoli cells of indicated *Mex-3b* genotypes 3 months-old mice.

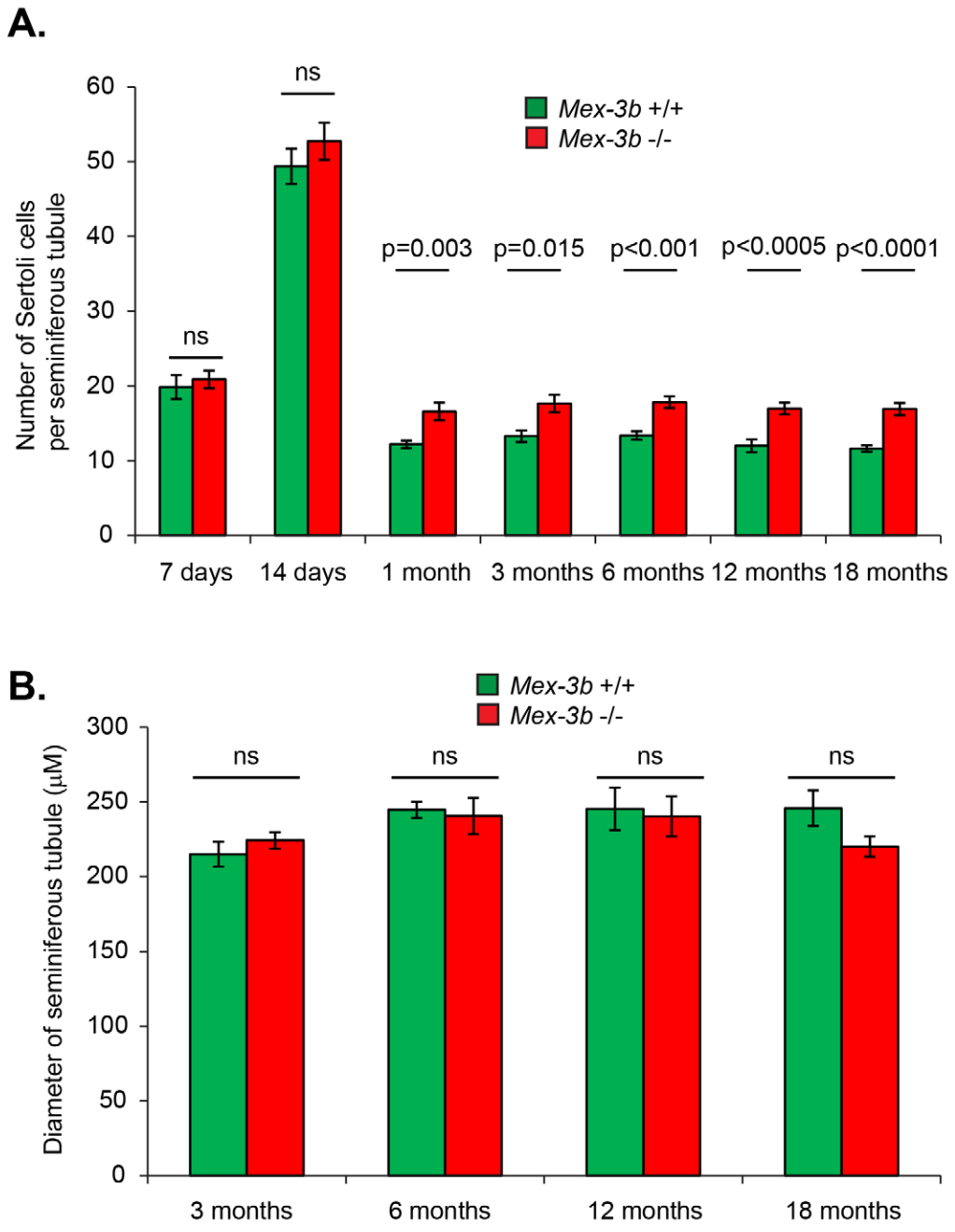

Figure S2. *Mex-3b* deficient seminiferous tubules have more Sertoli cells but an identical diameter compare to control.

(A) Counting of Sertoli cells per seminiferous tubules of wt and *Mex-3b* deficient mice at different ages (d for days and m for months). Sertoli cells were identified as cells positively stained for vimentin (n= 6 mice/group, 30 seminiferous tubules/ mouse). (B) Representation of the mean seminiferous tubules diameter of wt and *Mex-3b* deficient mice at different ages (d for days and m for months) (n= 6 mice/group, 30 seminiferous tubules/mouse).

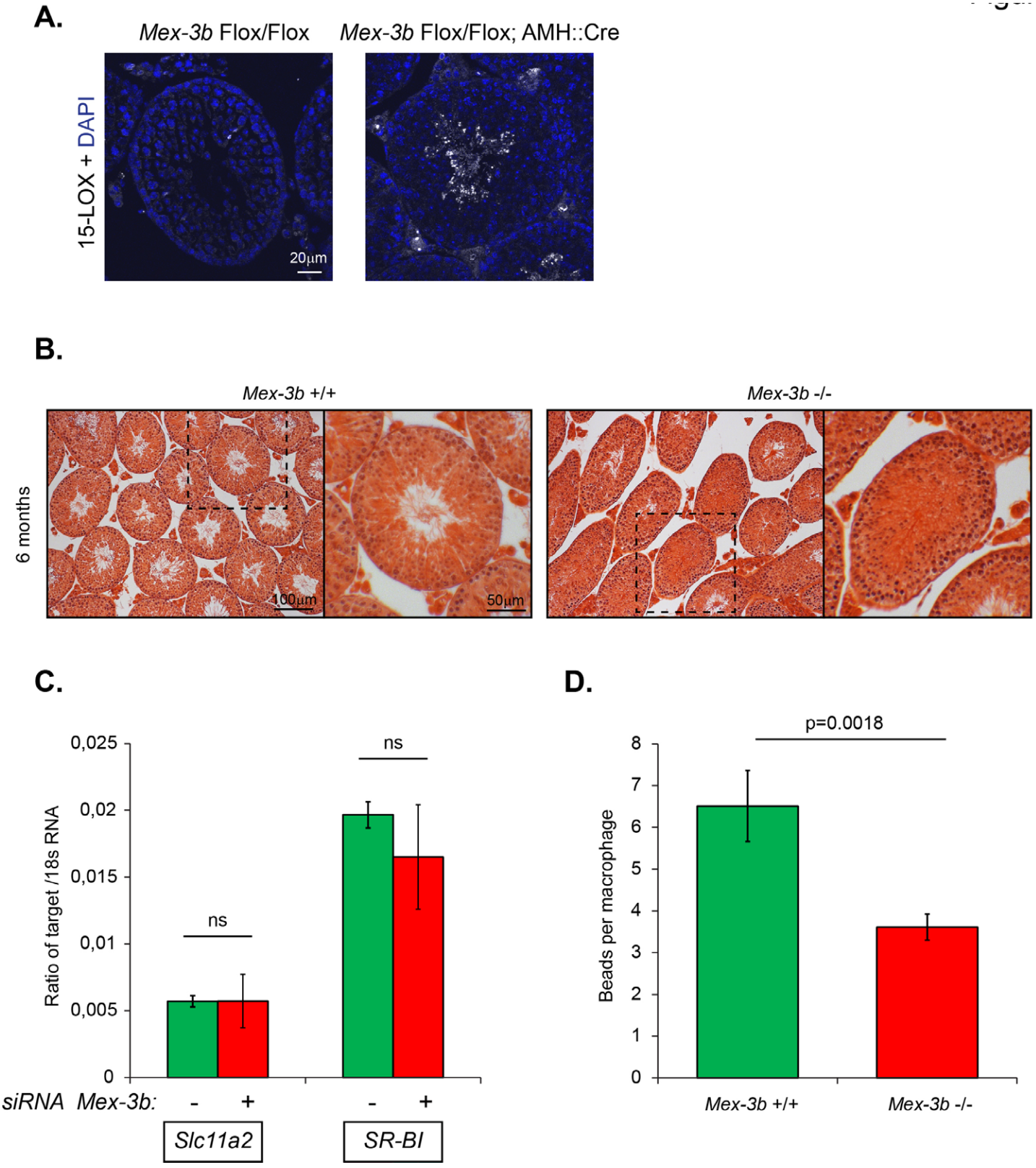

Figure S3. Deletion of *Mex-3b* affects phagocytosis in Sertoli cells.

(A) Immunodetection of 15-Lipoxygenase (15-LOX) on cross-sections of mouse testes with the indicated *Mex-3b* genotypes at 6 months old. (B) Hematoxilin and Scarlett Eosin staining of testis cross section from mice with the indicated *Mex-3b* genotypes. (C) RT-qPCR analysis of *Slc11a* and *SR-BI* transcripts isolated from murine TM4 cells depleted in *Mex-3b* by siRNA. All levels were normalized to the level of *18S* mRNA. One representative experiment of three is shown. Scale bars and P values with s.e.m are indicated. (D). Phagocytosis assay on wild type and *Mex-3b -/-* isolated macrophages Histogram indicates the average numbers of engulfed fluorescent beads per phalloidin-TRITC stained cell (n=100 cells per condition).

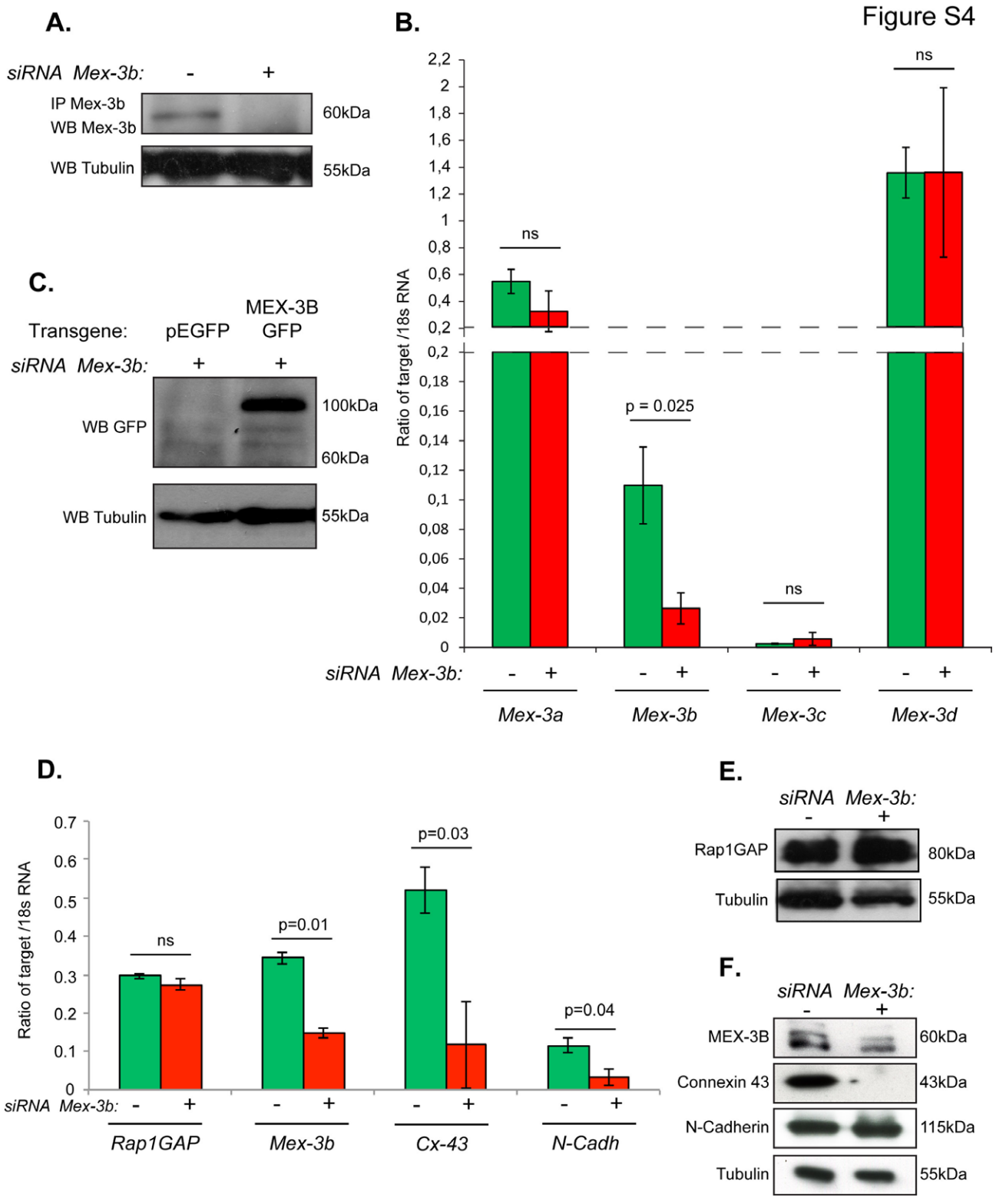

Figure S4. Specific inhibition of mouse *Mex-3b* expression and rescue with human MEX-3B in TM4 cells.

(A) Mex-3b protein level was determined by immunoprecipitation and Western blot analysis in TM4 cells 48h after siRNA treatment. (B) RT-qPCR analysis of *Mex-3a, b, c* and *d* transcripts isolated from murine TM4 cells depleted in *Mex-3b* by siRNA. All levels were normalized to the level of *18S* mRNA. One representative experiment of three is shown. (C) Expression of human MEX-3B-GFP construct in TM4 cells knocked down for mouse *Mex-3b* expression after lentiviruses infection (n=3 experiments). Tubulin was used as a loading control. (D) RT-qPCR analysis of *Rap1GAP, Mex-3b, Connexin-43 and N-Cadherin* transcripts isolated from murine TM4 cells depleted in *Mex-3b* by siRNA (n=3 experiments). All levels were normalized to the level of *18S* mRNA. (E) Western blot analysis of Rap1GAP protein in TM4 cells depleted in *Mex-3b*. Tubulin was used as a loading control. (F) Western blot analysis of MEX-3B, Connexin-43 and N-Cadherin proteins in TM4 cells depleted in *Mex-3b*. Tubulin was used as a loading control.

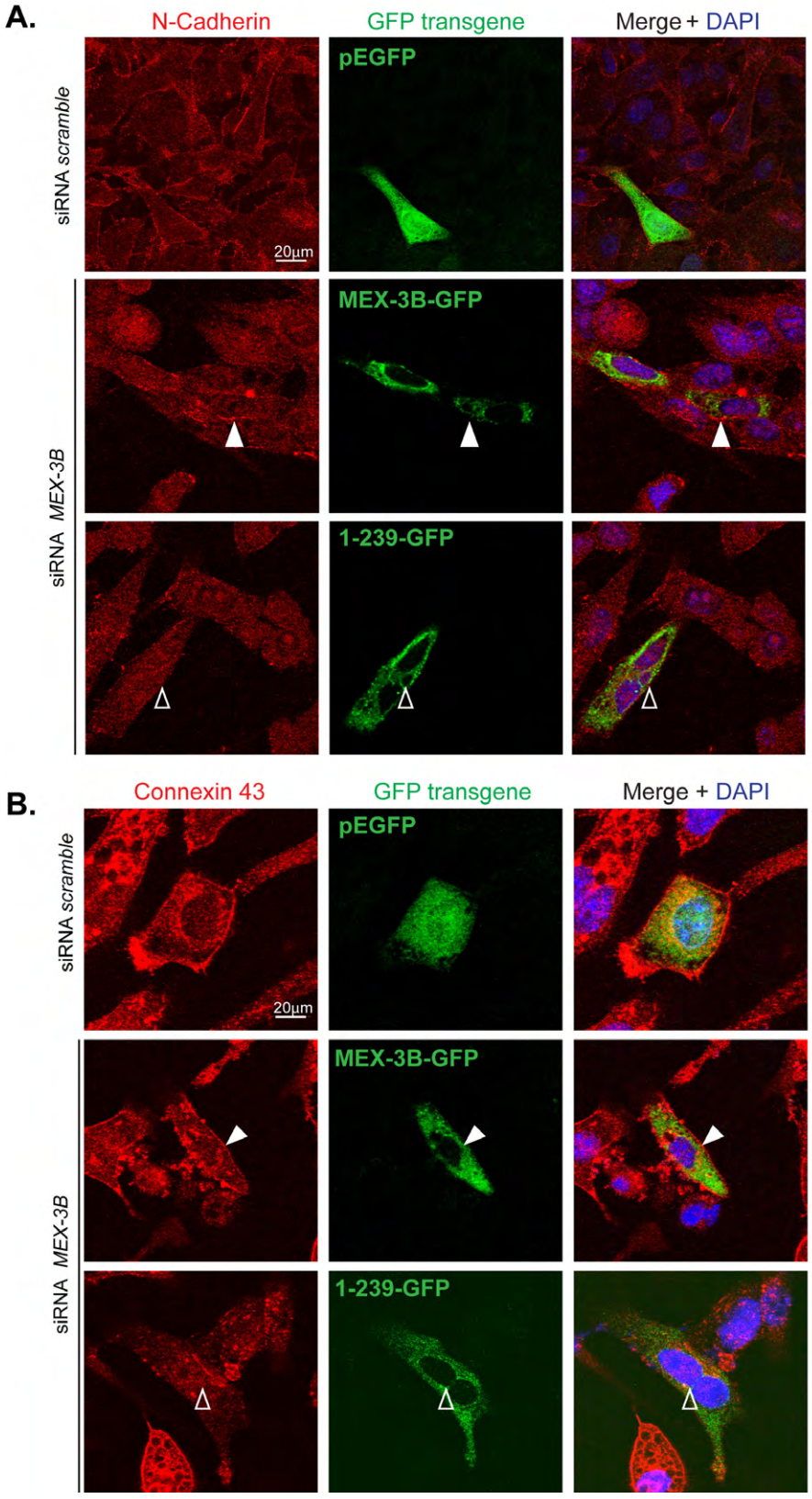

Figure S5. Mex-3b binding to RAP1GAP is required for junctions protein localization.

Confocal microscope images of TM4 cells inhibited for endogenous *Mex-3b* by siRNA and re-expressing the human MEX-3B protein full length or truncated (1-239) fused to GFP. N-cadherin (A) and Connexin 43 (B) were immunostained and counterstained with DAPI. Upon Mex-3b depletion by *si RNA,* the re-expression of the full-length construct allows the relocalization of junction proteins to the membrane (white arrowheads), wheras the re-expression of the 1-239 mutant does not (empty arrowheads).

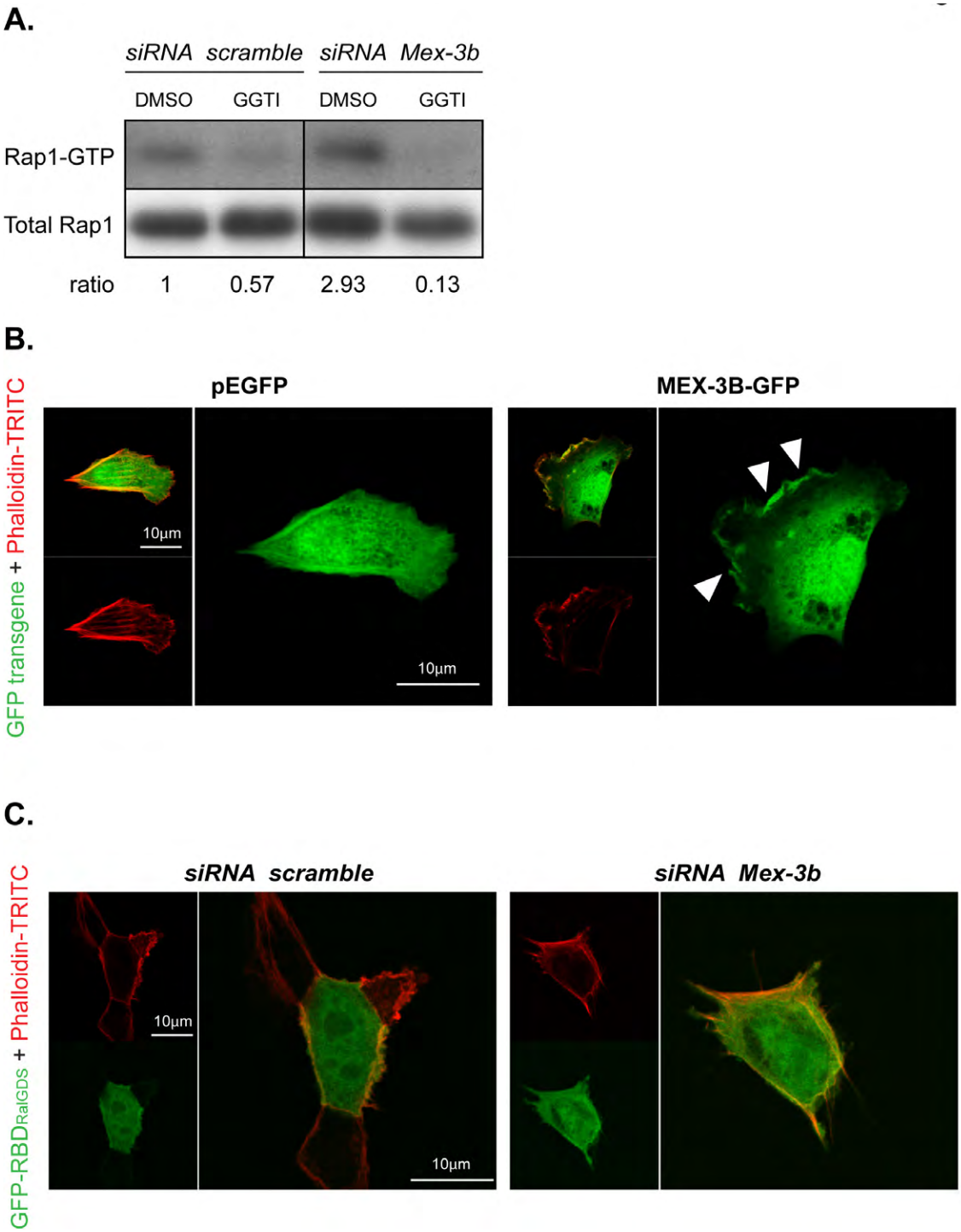

Figure S6. Mex-3b regulates Rap1 activity.

(A) Rap1-GTP pull down from lysates of TM4 cells transfected with either *scramble* or *Mex-3b* siRNA before treatment with the Rap1 inhibitor, GGTI-298. The ratio of Rap1-GTP / Total Rap1 for each condition is indicated. (B) After a short serum stimulation, TM4 cells expressing the indicated GFP constructs were fixed and stained with phalloidin-TRITC prior observation by confocal microscopy. White arrowheads indicate the plasma membrane localization of MEX-3B-GFP.

(C) Localization of GFP-RBD_{ralGDS} in TM4 cells knocked down for *Mex-3b* expression by siRNA after GGTI-298 treatment. Cells were exposed to phalloidin-TRITC prior observation by confocal microscopy.

Betz, U. A., Vosshenrich, C. A., Rajewsky, K. and Müller, W. (1996). Bypass of lethality with mosaic mice generated by Cre-loxP-mediated recombination. *Curr. Biol.* **6**, 1307–16.

Lecureuil, C., Fontaine, I., Crepieux, P. and Guillou, F. (2002). Sertoli and granulosa cell-specific Cre recombinase activity in transgenic mice. *Genesis* **33**, 114–118.

Table S1. RT-PCR primer sequences

	LEFT	RIGHT
Mex-3a	TTGATCAACTCTGCCTCCTG	TCCCAGTCACCATGAACACT
Mex-3b	GAGACTCTGGATGACCAAAGA	CGTTGAGAGCCGTGTTCTTG
Mex-3c	CTGTACGGCGGGGACGATG	ACCAGGCAGATTAGGACTACA
Mex-3d	AGTTGAACGTGATCGGGAGT	CCATCTCCACATCCTCCTTG
Clusterin	AACAGCTTCACCACCACCTC	CGAAGATGCTCAACACCTCA
Transferrin	GGCATCGGACACTAGCATCA	TGCCATCAGGGCAGAGCAAC
Inhibin	TCAGCCCAGCTGTGGTTCCACA	AGCCCAGCTCTTGGAAGGAGAT
LDHA	TTCCACTGCTCCTTGTCTGC	ACAGTCCACACTGCAAGCTG
Vimentin	AAGGAAGAGATGGCTCGTCA	TTGAGTGGGTGTCAACCAGA
18S	CGACGACCCATTCGAACGTCT	GCTATTGGAGCATGGAATTACCG
Rap1GAP	GAAAAGATGCAGGGAAGCAG	GTTGGTGCCTTCAATCCAGT
Slc11a2	TCCCCATTCCTGAGGAGGAG	ATCCGTGGGACCTTGGGATA
SR-BI	TCTTCACTGTCTTCACGGGC	CATGAAGGGTGCCCACATCT
Connexin-43	CTATCTTTGAGGTGGCCTTC	TCGCTCTTTCCCTTAACCCG
N-Cadherin	CGGACTCCGAGGCCCGCTAT	GCCTCCACAGACGCCTGAAGC