SUPPLEMENTAL INFORMATION

Functional equivalence of the SOX2 and SOX3 transcription factors in the developing mouse brain and testes

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Supplemental Experimental Procedures

gDNA preparation and PCR genotyping

gDNA was collected from tail tissues and extracted using High Pure PCR Template Preparation Kit (Roche). To screen for *Sox3*^{Sox2KI} founders, PCR was performed using F: 5'-CCTGCTGAAACATTCCCTGT-3' and R: 5'-TTCAGCTCCGTCTCCATCAT-3'. PCR products from desired founders were Sanger sequenced to identify specific mutations. Large deletions resulting from two gRNA cuts were detected using primers F: 5'-CCTGCTGAAACATTCCCTGT-3' and R: 5'-ACAAAACCCCGACAGTTACG-3'. For routine genotyping of *Sox3*^{Sox2KI} mice, multiplex PCR was performed using primers F1: 5'-CACAACTCCGAGATCAGCAA-3', F2: 5'-GAACGCATCAGGTGAGAGAAG-3', R1: 5'-CGGCGTTCATGTAGCTCTG-3' and R2: 5'-TTCAGCTCCGTCTCCATCAT-3'. For routine genotyping of *Sox3*^{KO} mice, primers F1: 5'-CAGCATGTACCTGCCACCT-3', F2: 5'-CCCGGATCTGAGCAGGTAT-3' and R: 5'-ACAAAACCCCGACAGTTACG-3' were used.

RNA preparation and qPCR

RNA was extracted using Trizol (Invitrogen), purified using Rneasy mini kit (Qiagen) after DNAse treatment on column using RNase-Free DNase Set (Qiagen). cDNA was generated using a High Capacity RNA-to-cDNA kit (Applied Biosystems) for qPCR analyses using Fast SYBR Green Master Mix (Applied Biosystems) on an Applied Biosystems 7500 StepOnePlus machine. Primers used for qPCR are as follows:

*Act*β F: 5'-CTGCCTGACGGCCAGG-3', R: 5'-GATTCCATACCCAAGAAGGAAGG-3'

Sox2 ORF F: 5’-ACCAGCTCGCAGACCTACAT-3’ R: 5’-TCGGACTTGACCACAGAGC-3’

Sox3 3’UTR F: 5’-AACCTAGGAATCCGGGAAGA-3’ R: 5’-CGTAACTGTCGGGGTTTTGT-3’

Sox2 3’UTR F: 5’-TTCGAGGAAAGGGTTCTTGCTG-3’ R: 5’-CCTTCCTTGTTTGTAACGGTCCT-3’ *Sox1* F: 5’- CCCTCGGATCTCTGGTCA -3’ R: 5’-GCAGGTACATGCTGATCATCTC-3’

Ngn3: F: 5’-CCCCAGAGACACAACAACCT-3’ R: 5’-AGTCACCCACTTCTGCTTCG-3’

Gfra1 F: 5’-ATCGGGCAGTACACATCTCTG-3’ R: 5’-TGTGGTTATGTGGCTGGAGG-3’

Tgfbi F: 5’-TGAAGCGTTCCAAGCCATGC-3’ R: 5’-GATGCCTCCGCTAACCAGGATT-3’

Egr3 F: 5’-TCAACCTCTTCTCCGGCAGC-3’ R: 5’-GATTGGGCTTCTCGTTGGTCA-3’

Scube3 F: 5’-CTGGCACATGACGGACACAAC-3’ R: 5’-CGTAGCTGCCCATCATGTTGAC-3’

Tissue collection and Immunofluorescence

Tissues were fixed overnight in 4% PFA and equilibrated in 30% sucrose. Tissues were then embedded in OCT compound (Sakura Finetek) for cryosectioning using Leica CM1900 at 10-14 μm. For immunohistochemistry, tissue slides were blocked using PBS/0.3% Triton X-100/10% horse serum before primary antibodies were added for overnight incubation at 4 ^{0}C. Slides were then washed in PBS and incubated with secondary antibodies for 2 hours at room temperature. Slides were mounted using Prolong Gold Antifade plus DAPI (Molecular Probes, Invitrogen) and imaged using a Nikon Eclipse Ti microscope. Primary antibodies were SOX3 (R&D

AF2569, 1:200), SOX2 (Santa Cruz SOX2 Y-17 sc-17320, 1:100, used for testes), SOX2 (Millipore Ab5603, 1:1000, used for embryonic brain). Secondary antibodies were donkey anti-goat 488 (Life Technologies) and donkey anti-rabbit TxRed (Life Technologies).

Quantification of SOX2 protein using immunohistochemistry

Immunostaining was described as above and images acquired using an Leica SP5 spectral scanning confocal microscope. The mean SOX3 and SOX2 staining intensity was calculated in at least 100 cells/embryo using NIS Elements Advance Research Software (Nikon). Raw data was transferred to GraphPad Prism 7 where it was transformed such that cells with mean SOX3 intensity 5x above background were deemed SOX3 positive cells. The remaining cells were deemed SOX3 negative. Students unpaired T-test was performed tp test significance.

Calculation of relative *Sox2*/*Sox3* levels

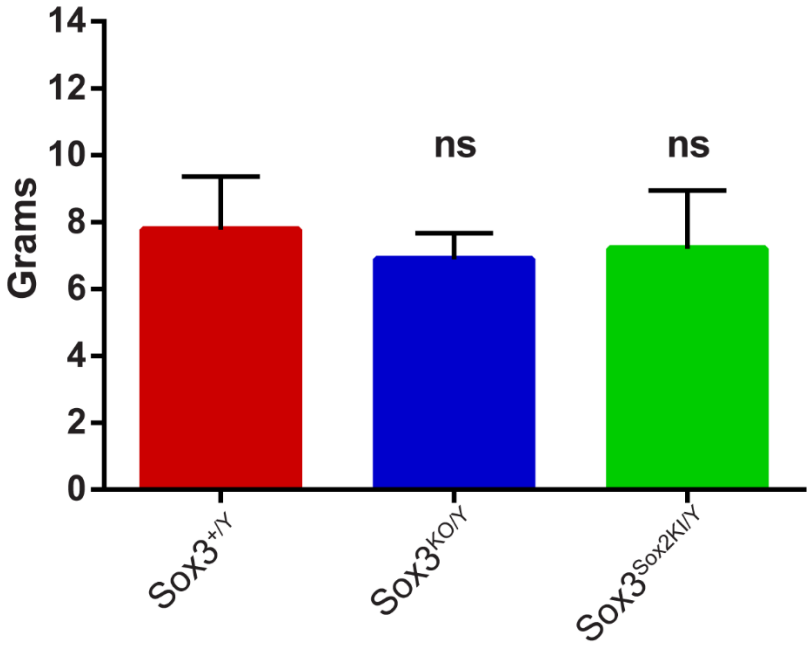
Quantitation of transcripts from the *Sox2* and *Sox3* locus in WT and $Sox3^{Sox2KI}$ reveals that the $Sox3^{Sox2KI}$ locus is producing *Sox2* ORF transcripts at 60% of the normal level of *Sox3* transcript based on a 5'UTR PCR. This additional *Sox2* ORF increases the total *Sox2* ORF transcript pool by approximately 40% in $Sox3^{Sox2KI}$ embryonic heads. Since the rise in *Sox2* transcripts is less than expected based on the contribution from the $Sox3^{Sox2KI}$ locus this implies that the *Sox2* locus contributes approximately 60% of the total *Sox2*/*3* transcript pool in the developing brain. Combined Sox2/3 levels in

Sox3$^{Sox2KI/Y}$ mice can be calculated as follows: *Sox2* locus (60%) + *Sox3* locus (60% x 40%) = 60% + 24% = 84%. This amounts to a 16% reduction compared with WT levels.

Purification of spermatogonia

Testes from post-natal day 6 mice were isolated, de-capsulated, and incubated for 15 min each in 0.5 mg/ml collagenase/DMEM with agitation and then in 0.25% trypsin/EDTA in DMEM. Tubules were dissociated manually by pipetting and washed in 0.5% BSA in DMEM by centrifugation. Cell pellets were resuspended in DMEM and filtered twice through a 70 µm membrane, then separated over a 2-4% BSA gradient. Purified spermatogonia were identified by GCNA1 staining.

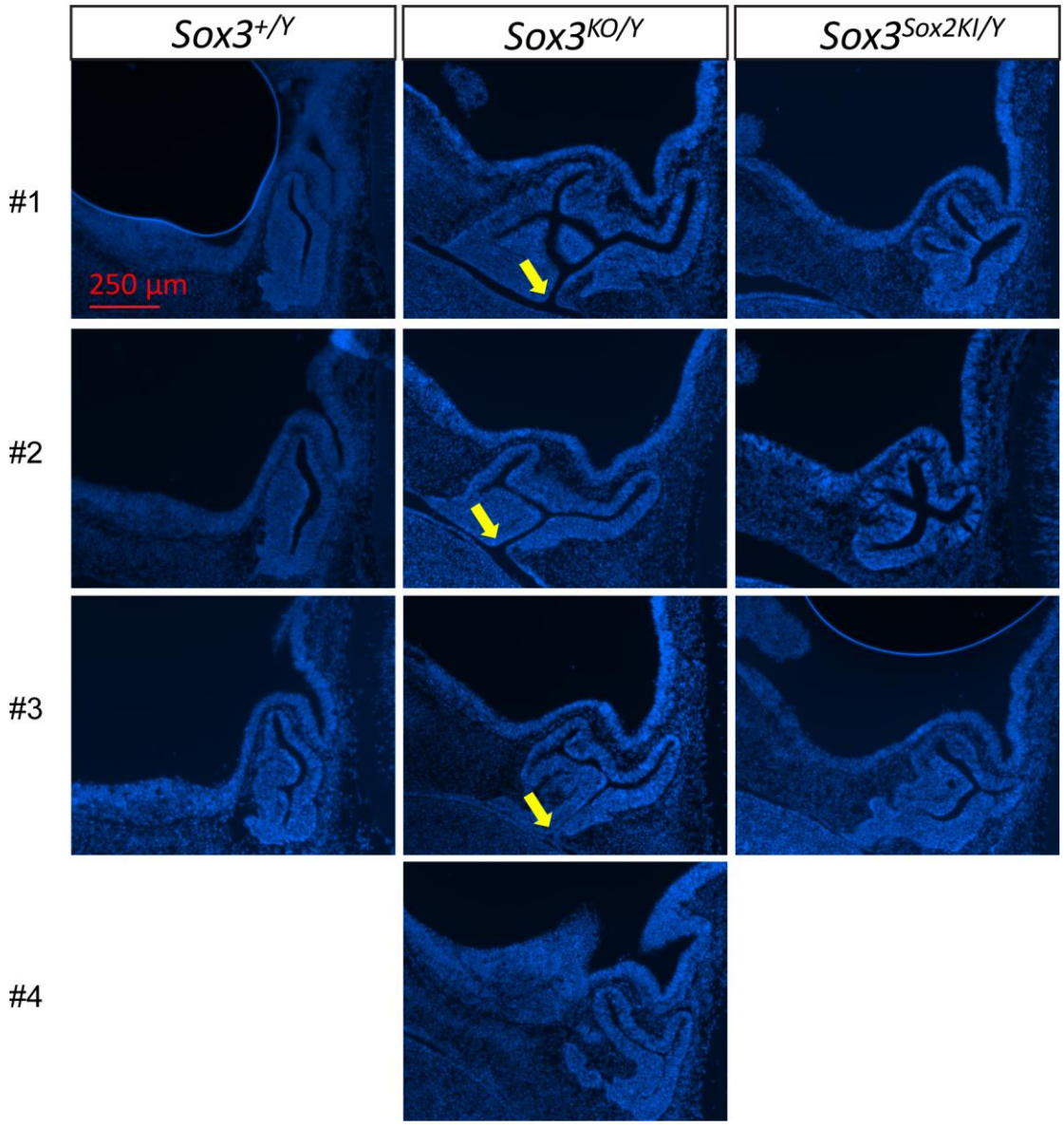
Supplementary Figures



Supplementary Figure 1

Male wean weights were unchanged in mutant mice.

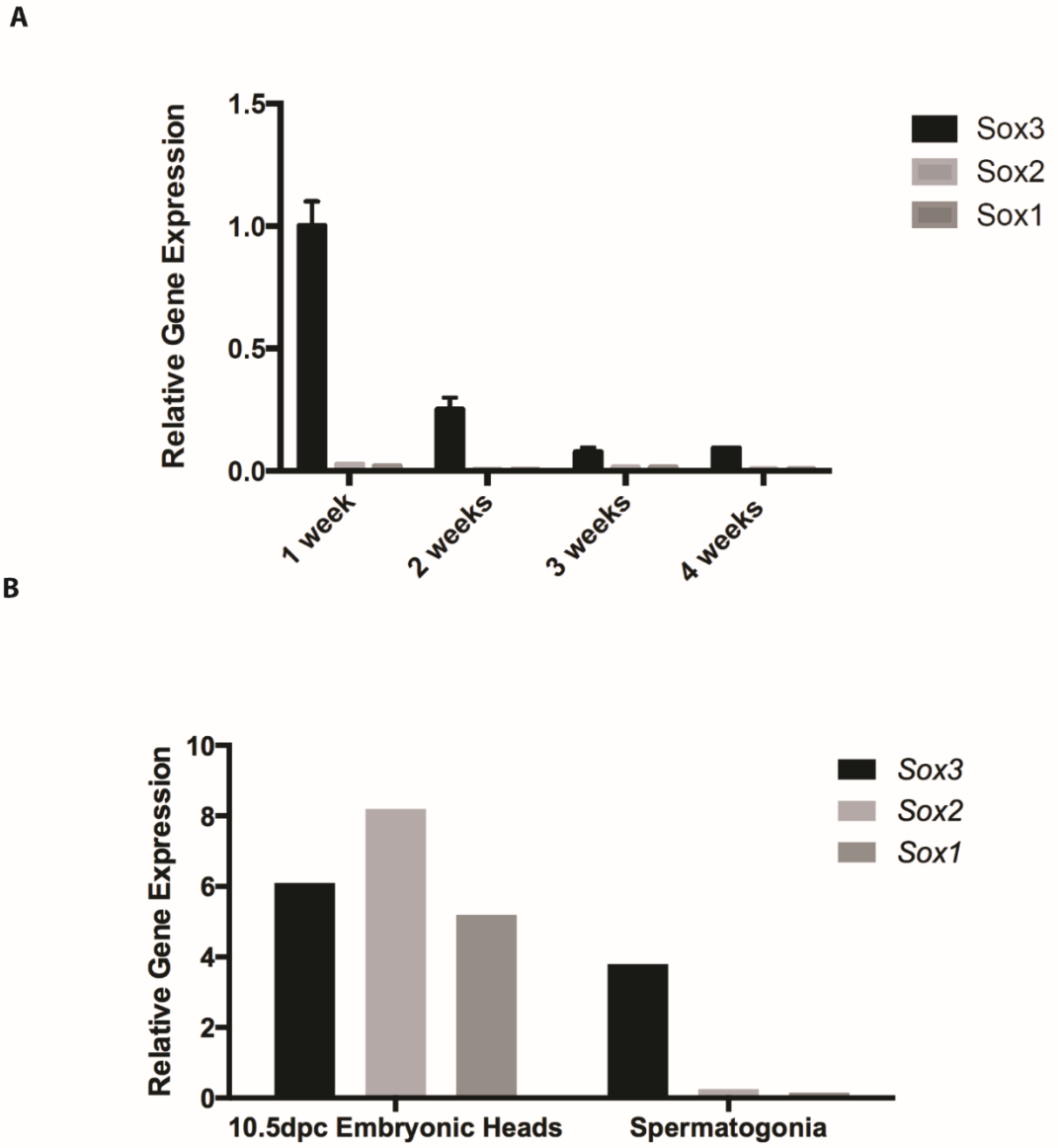
Male mice were weighed at weaning (3 weeks). At least 14 mice of each genotype were weighed and unpaired t-tests were performed to assess pairwise differences with respect to *Sox3*$^{+/}$



Supplementary Figure 2

Sox2KI largely rescues embryonic *Sox3*-null Rathke's pouch

DAPI staining of midline sagittal sections of $Sox3^{+/Y}$, $Sox3^{KO/Y}$ and $Sox3^{Sox2KI/Y}$ 12.5 dpc embryos (*n*=3 per genotype) showing rescue of the $Sox3^{KO/Y}$ pituitary induction phenotype. Arrows indicate failure of the Rathke's pouch to separate from the oral cavity. See Fig. 2C for further details.



Supplementary Figure 3

Sox1 and Sox2 are not detectable in the testes.

(A) Expression of *Sox1*, *Sox2* and *Sox3* was measured by qPCR in the testes at various ages (*n*=3). Expression levels were normalised to *Sox3* levels in 1 week old testes. (B) Expression levels were also compared between 10.5 dpc embryonic heads and purified postnatal day 6 spermatogonia.