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Research Article

Generation of a novel *Stra8*-driven Cre recombinase strain for use in pre-meiotic germ cells in mice†

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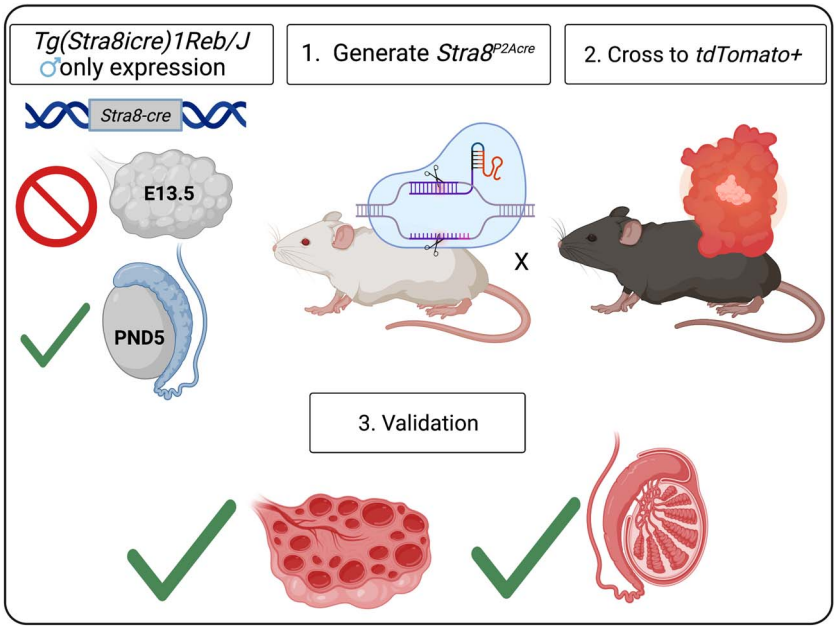
Abstract

The development of oocytes occurs over a broad time frame, starting at the earliest stages of embryogenesis and continuing into adulthood. Conditional knockout technologies such as the Cre/loxP recombination system are useful for analyzing oocyte development at specific stages, but not every time frame has appropriate Cre drivers, for instance, during oocyte meiotic initiation through early prophase I in the embryo. Here, we generated a novel knockin mouse line that produces a bicistronic transcript from the endogenous *Stra8* locus that includes a "self-cleaving" 2A peptide upstream of *cre*. This allows for high efficiency cleavage and production of both proteins individually and results in expression of *cre* in both male and female gonads at the biologically relevant stage. Fluorescent reporter analysis confirms that this line recapitulates endogenous *Stra8* expression in both sexes and does not affect fertility of heterozygous nor homozygous mice. This line, named *Stra8*^{P2Acre}, adds to the repertoire of germ-cell specific *cre* driver lines and, importantly, allows for deletion of target genes during key embryonic oocyte developmental stages, including early events in meiosis.

Summary Sentence

Generation of a novel cre recombinase knockin to the *Stra8* locus allows production of *Stra8* and *cre* without affecting fertility.

Graphical Abstract



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Introduction

Genetic recombineering has wide-spread use for analysis of germ cells, which includes temporally restricted gene deletion, as well as activation of reporter lines and cell lineage analysis. While not expansive, a number of cre recombinase lines are available for use in germ cells that differ in their expression patterns [1–4]. For embryonic primordial germ cells, this includes *TNAP-cre* and *Prdm1 (Blimp1)-cre,* which initiate expression in early embryogenesis, and *Vasa (Ddx4)-cre*, which initiates expression at E15.5 [1, 5–7]. Commonly used transgenic *cre* driver lines that target oocytes include growth and differentiation factor-9 (*Gdf9*)-*icre* and zona pellucida-3 (*Zp3*)-*cre*, and these initiate Cre activity postnatally [4, 8]. *Gdf9-icre* is expressed in oocytes of primordial follicles in postnatal day 3 mouse ovaries, while *Zp3-cre* is expressed from the primary follicle stage and both are exclusive to the female germ line [8]. Few cre lines are available for use at the early stages of germ cell meiosis and progression during prophase I during embryonic ovary development. *Figla-cre* begins expression in oocytes at embryonic (E) day 14.5 and has been used to examine perinatal oocyte dynamics [9], but is downstream of the time period for meiotic initiation at E13.5.

One limitation to using transgenic cre lines developed with partial promoter sequences is that germ cell-specific enhancers are often unmapped and may not be included; thus, *cre* expression may not fully recapitulate endogenous expression. The use of a partial promoter for generating previous *Stra8*-cre lines provides such an example. *Stra8* is expressed in both male and female germ cells and is required for initiation of meiosis, and both male and female homozygous knockout mice ($Stra8^{-/-}$) are sterile [10, 11]. The expression of *Stra8* in female germ cells begins at embryonic day 13.0 (E13.0), reaches maximum expression levels at E14.5, and returns to low expression by E16.5 [12]. In contrast, *Stra8* expression in male germ cells begins at postnatal day 5 (PND5) and continues in premeiotic germ cells throughout spermatogenesis [13, 14]. However, the previously developed *Stra8-cre*, which, like many transgenes, contains a partial promoter sequence, only functions in males [15], thus limiting its use.

The enhancer sequence required for fetal ovary expression of *Stra8* has recently been mapped to an element 2.9 kb upstream of *Stra8* [16] and lies outside the 1.4 kb promoter sequence used to generate *Stra8-cre*. We hypothesized that this is the underlying reason as to why *Stra8-cre* expresses only in the male gonad. Therefore, we generated a new cre driver that utilizes the endogenous *Stra8* locus but takes advantage of CRISPR-Cas9 engineering and the self-cleaving P2A peptide to create a novel cre "knockin" line that does not disrupt *Stra8* expression. This new cre line, $Stra8^{P2Acre}$, has Cre activity that mimics endogenous *Stra8* expression in both males and females and does not affect fertility. Therefore, $Stra8^{P2Acre}$ extends the repertoire of stage-specific cre recombinase driver lines to support studies of meiotic initiation and prophase I progression in both male and female mice.

Materials and methods

Ethics Statement

All studies were performed in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals with prior approval from the Baylor College of Medicine Institutional Animal Careand Use Committee.

Generation of transgenic $Stra8^{P2Acre}$ mice

All experimental procedures were performed in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and with prior protocol approval from the Institutional Animal Care and Use Committee at Baylor College of Medicine. The $Stra8^{P2Acre}$ allele was generated via CRISPR/Cas9-mediated genome editing in mouse pronuclear stage zygotes. Overlapping SpCas9 guide target were identified on the sense and antisense strands at the translation stop site of *Stra8* [5′ TTGACGATCTGTAAAGCAGG (AGG), GRCm38 Chr6: 34939157–34939179; and 5′ CCTGCTTTACAGATCGTCAA (AGG), GRCm38 Chr6: 34939154–34939176] using the Wellcome Trust Sanger Institute Genome Editing database [17]. A plasmid donor with a 760 bp 5′ homology arm and 860 bp 3′ homology arm flanking a P2A-Cre insert was synthesized by Azenta to facilitate homology-directed repair insertion of P2A-Cre immediately 5′ to the translational stop site of *Stra8*. C57BL/6 J embryos ($N = 397$) were microinjected with 4 ng/μl circular plasmid donor and pre-complexed 30 ng/μl SpCas9 (PNABio) and 10 ng/μl total sgRNA (Synthego). A total of 72 liveborn animals were subsequently PCR genotyped for site-specific integration using primers outside of the homology arms and primers specific to Cre recombinase (5′ homology arm: P1 5′- *acctcagagagctttcaggc* and P2 5′-*ggcaaacggacagaagcatt*; 3′ homology arm: P1 5′- *agagatacctggcctggtct* and P2 5′-*tttaaatggcagcacagggg*). Expected bands were 1164 base pairs (bp) for the 5′ arm and 1182 bp for the 3′ arm. A total of four putative founders were identified and bred to generate N1 animals. Homology arm PCR was used to verify allele transmission and Sanger sequencing across the homology arms and P2A-Cre insert was used to verify allele integrity. N1 animals from one founder were used to establish the $Stra8^{P2Acre}$ line. For breeding, $Stra8^{P2Acre/+}$ was maintained on the male line, which avoids recombination in oocytes, as this will lead to the generation of the null allele globally in offspring [6].

Mouse genotyping

Genomic DNA was extracted from ear or tail clippings and genotyped by PCR. Generic Cre primers were used to identify presence or absence of the cre (Supplementary Table S1). To distinguish between $Stra8^{+/+}$, $Stra8^{P2Acre/+}$, and *Stra8*-cre $^{P2Acre/P2Acre}$ genotypes, three allele-specific primers were generated (P1, P2, and P4) (Supplementary Table S1). PCR reactions were run for 35 cycles (95°C, 30s; 60°C, 45 s; 72°C, 1 min) and product electrophoresed on a 2% agarose gel. Expected bands were 241 bp (WT) and 335 bp for the $Stra8^{P2Acre}$ allele.

Tissue collection

Embryos were collected from timed pregnant females with the plug day counted at embryonic day (E) 0.5. At the time of collection, adult mice were anesthetized by isoflurane (Abbott Laboratories, Abbott Park, IL) inhalation and euthanized by cervical dislocation followed by sternal clipping. Embryos were extracted from the uterus and euthanized by decapitation prior to gonad removal. Newborn and postnatal mice prior to PND7 were anesthetized by hypothermia prior to decapitation and gonad harvest.

Whole mount immunofluorescence of gonads

To test for cre activity, *Stra8*$^{P2Acre/+}$ males were crossed to the fluorescent protein reporter strain, *Rosa26Sor*$^{tm9(CAG\text{-}tdTomato)Hze}$ (IMSR_JAX:007909) homozygous females. Ovaries and testes were collected at the indicated time points and fixed in 4% paraformaldehyde (PFA) (Sigma Aldrich, St. Louis, MO) for 1 h at room temperature (RT) on a rotating platform. Tissues were incubated in blocking buffer containing 5% normal donkey serum (Vector Laboratories, Newark, CA) in 1× phosphate-buffered saline (PBS) (Invitrogen, Carlsbad, CA) with 0.25% Triton-X (Sigma Aldrich) at RT for 2 h to prevent non-specific signal. Then, tissues were incubated in primary antibodies: rabbit anti-tdTomato 1:500 (Rockland, Lot#46317/600–401-379), rat anti-TRA98 1:500 (Abcam, Lot #GR17382–8/ab82527) and/or rabbit anti-STRA8 (Abcam, ab49602) in blocking buffer at RT overnight. The following day, tissues were washed in 1× PBS before incubating in secondary antibodies (Alexa Fluor Rabbit 594, Alexa Fluor Rat 488) (Vector Laboratories) 1:1000 in 1× PBS at RT overnight. After washing in 1× PBS, tissues were incubated in 4′,6-diamidino-2-phenylindole (1:1000 in 1× PBS, Sigma Aldrich) at RT for 10 min. Finally, tissues were mounted with VECTASHIELD medium (Vector Laboratories) and cover slipped for storage overnight at 4°C to dry completely before imaging.

Tissue processing and immunofluorescence of cryo-embedded ovary and testis

Ovaries and testes were collected at the indicated time points and fixed in 4% PFA for 1 h at RT on a rotating platform. Whole tissues were subjected to an increasing sucrose gradient over two days to prevent ice crystal formation. Tissues were imbedded in OCT jelly (Sakura Finetek U.S.A., Torrance, CA), flash frozen in liquid nitrogen, and stored at −80°C until sectioning. Cryoblocks were sectioned serially (10 μm thick) in a Cryostat (Leica CM1850) machine and stained using the same protocol as whole mount immunofluorescence.

Quantitative PCR analysis

RNA was extracted from three pools of six embryonic ovaries from six biological replicates and four un-pooled PND5 testes using the Applied Biosciences PicoPure RNA Isolation Kit. cDNA was then prepared using the Applied Biosciences High-Capacity RNA-to-cDNA Kit. Real-time quantitative PCR (RT-qPCR) was performed in a 96-well plate using the StepOnePlus Real-time PCR system (Applied Biosystems). Primer sequences are listed in Supplementary Table S2. Data were analyzed by the ΔΔCT method using *Gapdh* for sample normalization. Fold change values were calculated relative to the WT samples with the mean of the WT sample set to "1".

Fertility analysis

Mating pairs of a *Stra8*$^{P2Acre/+}$, *Stra8*$^{P2Acre/P2Acre}$, and control (*Stra8*$^{+/+}$) littermates of both sexes were housed in continuous breeding cages after reaching sexual maturity (6 weeks for females, 8 weeks for males) with wild type mice of the same genetic background (*C57Bl/6 J;129S7/SvEvBrd* F1). The presence of newborn pups was monitored daily for 3 months, and all resulting litters were weaned and genotyped at 21 days. The number of pups per litter, as well as litters per month, were recorded for $n = 3$–4 per genotype per sex.

Statistical analysis

Statistical analysis was performed with GraphPad Prism v.9. Fertility data were reported as mean±standard error of the mean (s.e.m.) with a minimum of three biological replicates per genotype. P values were considered significant if $P < 0.05$.

Results

For generation of a germ-cell specific cre recombinase line for meiotic initiation and progression in both male and females, we targeted the *Stra8* locus but designed the construct to maintain endogenous *Stra8* expression (Figure 1). A *P2A-cre* cassette was inserted into the last coding exon (10) immediately 5′ to the *Stra8* translation stop codon. This will result in expression of a single transcript for *Stra8-P2A-Cre*, which is then translated into two separate proteins because the self-cleaving P2A sequence induces a ribosomal "skip" site. Thus, the upstream protein (STRA8) is effectively split from the downstream protein (Cre recombinase) and should allow for unaffected STRA8 function, which is necessary for both male and female fertility. Seventy-two potential founders were initially screened by PCR for *cre*, with ten mice genotyping as *cre* positive. Additional screening was performed using 5′ and 3′ homology arm PCRs, which indicated that four mice showed the correct molecular weight bands. Three of these four were then bred to wild type mice (F1 C57Bl6;129SvEv) to verify germ line transmission. Heterozygous offspring underwent genomic sequencing to validate the knockin allele. Once validated, one founder line was chosen for breeding.

To validate functionality of the knockin allele, *Stra8*$^{P2Acre/+}$ males were crossed to *Rosa26*$^{Sortm9(CAG\text{-}tdTomato)Hze}$ (herein called tdTomato+) homozygous reporter females [18], in which transcription of the tdTomato fluorescent reporter is prevented by a *loxP*-flanked stop cassette (Figure 1). Expression of cre removes the stop cassette, resulting in production of tdTomato, which can be measured by direct or indirect immunofluorescence. We analyzed spatiotemporal activity of Cre recombinase by confocal fluorescent imaging in gonads from embryos (E12.5, E13.5, E15.5, E17.5, and E19.5), neonates (PND0), and adults. Prior studies established that endogenous *Stra8* initially expresses in a small population of germ cells in the anterior region of the female gonad at E13.5 and is not expressed in the embryonic testis [10]. In males aged E12.5-E19.5, no tdTomato fluorescence was detected in the testis of cre negative or cre positive mice (*i.e.* *Stra8*$^{+/+}$ *tdTomato* + or *Stra8*$^{P2Acre/+}$ *tdTomato+)* (data not shown). In females, at E12.5, no tdTomato fluorescence was detected in *Stra8*$^{P2Acre/+}$ *tdTomato* + ovaries, as expected (data not shown). However, in cre positive ovaries at E13.5, tdTomato fluorescence was identified in a small number of cells located in the anteromedial region of the ovary, consistent with the current understanding of oogenesis as a spatiotemporally asynchronous phenomenon (Figure 2A) [16]. The tdTomato+ cells co-localized with the germ cell marker, TRA98 (Figure 2B). Quantification of *cre* mRNA expression by RT-qPCR showed significantly increased levels in *Stra8*$^{P2Acre/+}$ E13.5 ovaries compared to wild type (cre neg) littermates, further indicating cre production in the correct time frame (Supplementary Figure S1). tdTomato fluorescence was widespread in *Stra8*$^{P2Acre/+}$ *tdTomato* + oocytes of E15.5

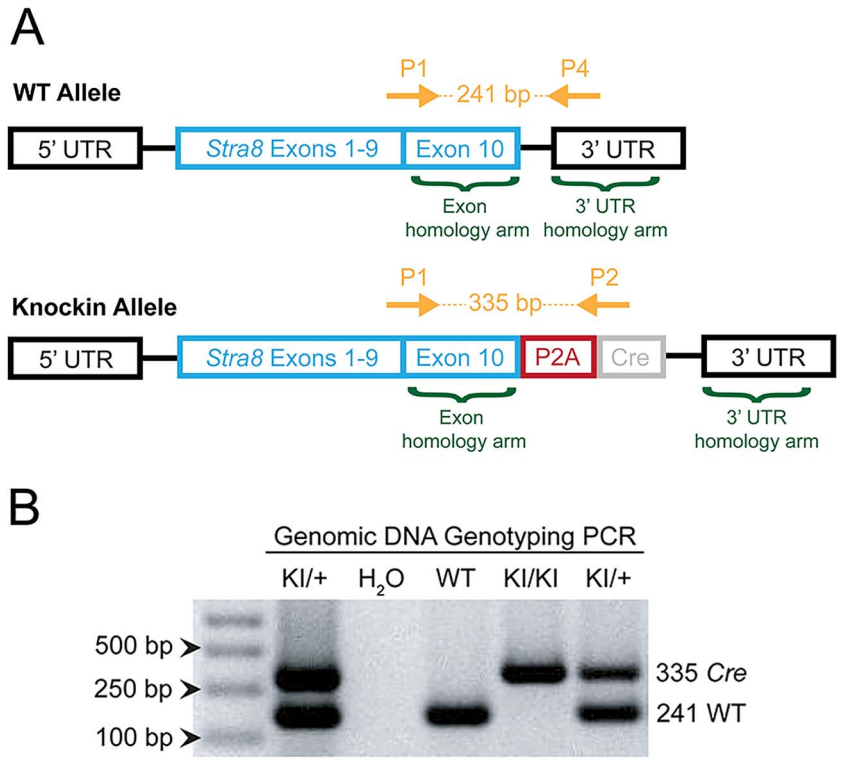

Figure 1. Schematic representation for generation of the *Stra8*^{P2Acre} knockin allele. (A) The endogenous *Stra8* transcript (top) showing location of the homology arms for CRISPR/Cas9 targeting. The *Stra8*^{P2Acre} allele produces a bicistronic transcript that includes a self-cleaving P2A site and *cre recombinase* expression cassette in frame to exon 10 of *Stra8*, keeping the endogenous coding sequence and 5′ and 3′ UTR intact. (B) Genotyping PCR for offspring from a *Stra8*$^{P2Acre/+}$ × *Stra8*$^{P2Acre/+}$ using primers annotated above. Agarose gel of amplification products from genomic DNA PCR using insert-specific primers distinguish between wild-type (WT), heterozygous (KI/+), or homozygous knockin (KI/KI). The H2O lane is the negative control without genomic DNA.

ovaries (Figure 3) and remains detectable in oocytes of non-growing (primordial) and growing follicles in the newborn (PND0), and in oocytes of *Stra8*$^{P2Acre/+}$ *tdTomato* + adult ovaries, but not in cre negative littermates (Figure 4 and Supplementary Figure S2).

In the testis of *Stra8*$^{P2Acre/+}$ *tdTomato* + mice, tdTomato was first detected at PND3 in a small number of cells (Figure 5A). A subset of these cells was positive for the germ cell marker, mouse VASA homolog (MVH; also called DDX4) (Figure 5B). No tdTomato signal was detected in cre negative littermates (Figure 5E). PND5 testes showed more widespread tdTomato in germ cells within the seminiferous tubules, with localization that is consistent with spermatogonia and spermatocyte expression (Figure 6A). No tdTomato is detected cre negative littermates (Figure 6E). *Cre* expression was significantly ($P < 0.03$) upregulated in *Stra8*$^{P2Acre/+}$ testes as compared to cre negative littermates (Supplementary Figure S1). These data are in line with previously reported data regarding *Stra8* gene and protein expression patterns during postnatal testis development [14, 15].

Because *Stra8* is absolutely required for meiotic progression in spermatogenesis and oogenesis [10], we verified that the *P2A-cre* knockin cassette did not affect fertility. As our reporter studies utilized heterozygous mice, fertility trials were conducted using heterozygous *Stra8*$^{P2Acre/+}$ males and females but also with homozygous *Stra8*$^{P2Acre/P2Acre}$ females mated to wild type mice. The average pups per month and pups per litter were compared to *Stra8*$^{+/+}$ littermates mated to wild type mice from the same genetic background. After 3 months, there was no significant difference between the genotypes for pups per litter or litters per month, indicating no effect of the *P2A-cre* knockin cassette on fertility of *Stra8*$^{P2Acre/+}$ mice (Figure 7). Finally, we verified that the *P2A-cre* knockin cassette did not affect the endogenous levels of STRA8 protein or *Stra8* mRNA expression (Supplementary Figure S3).

Discussion

We successfully generated a novel *Stra8*-cre transgenic mouse line that expresses in both male and female germ cells at the appropriate developmental time points. This mouse line will be useful for conditional deletion of genes involved in pre-meiotic female germ cells during embryonic ovary development and also provides an additional cre recombinase drive line for the male germline. Thus, in the female, use of this line provides an additional resource for analyzed effects of loss-of-function genetic studies as oocytes initiate meiosis and without affecting earlier oogonia or primordial germ cell development.

The previous transgenic line, *Tg(Stra8-cre)*^{1Reb}, contains a 1.4 kb promoter of the *Stra8* locus and expresses in males beginning at PND3 in spermatogonia through pre-leptotene stage spermatocytes with no expression in embryonic or adult ovaries [15]. Choice of this promoter was based on transgene expression in spermatogonial stem cells with the same 1.4 kb promoter fused to the enhanced green fluorescent protein [15, 19]. It was hypothesized that this 1.4 kb promoter

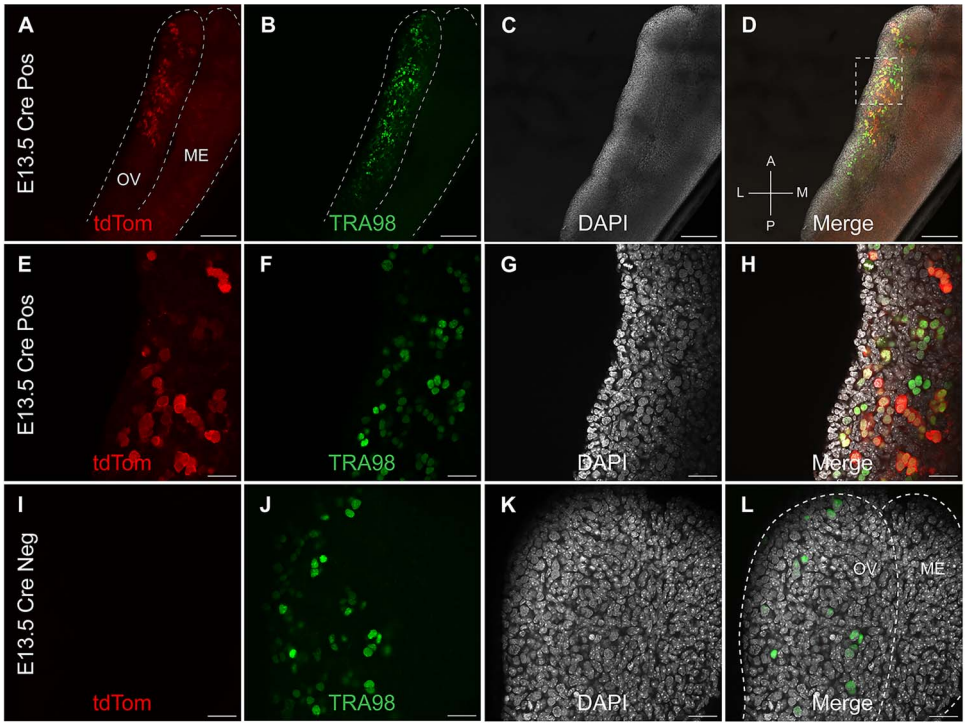

Figure 2. *Stra8*$^{P2Acre/+}$ is active in germ cells in E13.5 ovary. (A) Whole mount ovary immunofluorescence using an antibody against tdTomato (tdTom; red) indicated recombination by Cre in anteromedial ovary (OV), but not the mesonephros (ME), in *Stra8*$^{P2Acre/+}$ *tdTomato*+ (A–H) compared to cre negative littermates (I–L). (B, F, J) Tissue was co-stained with an antibody against the germ cell marker, TRA98 (green) and counterstained with DAPI (C, G, K). (D, H, L) Merged image of tdTomato, TRA98, and DAPI. Panels E–H show zoomed-in images of ovary region boxed in panel D. Images were taken on an LSM 780 confocal microscope. Anatomic position is shown by the cross in panel D, with A, anterior; P, posterior; L, lateral; M, medial. Scale bar = A–D, 100 μm; E-L, 20 μm.

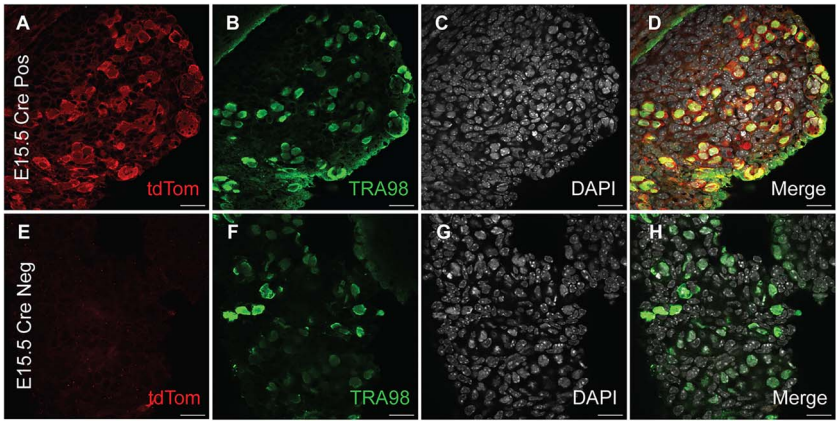

Figure 3. *Stra8*$^{P2Acre/+}$ shows widespread recombination in germ cells in the E15.5 ovary. Widespread activity of *Stra8*-driven cre recombinase shown in tdTomato reporter mouse ovaries (*Stra8*$^{P2Acre/+}$ *tdTomato*+) (A–D) but not control ovaries (*Stra8* $^{+/+}$ *tdTomato*+) (E–H) at E15.5. Red fluorescence (tdTom) indicates activity of *Stra8*$^{P2Acre/+}$. Whole mount ovaries were co-stained with the germ cell marker TRA98 (green) (B, F) and counterstained with DAPI (gray; C, G). Merged images are shown in D, H. Images were taken on an LSM 780 Confocal Microscope. Scale bar = 20 μm.

lacked enhancers necessary for female expression [15]. Functional female-specific enhancers were subsequently mapped to between 1.5 kb and 2.9 kb, although it is yet unclear if this promoter fragment contains all necessary elements to recapitulate the complexity of endogenous *Stra8* expression [16].

During ovary development, E12.5 starts a critical window for meiotic initiation in female germ cells, which occurs in a wave from E12.5 to E16.5 [20]; there are no *cre* lines currently available for that time frame. For instance, *Vasa-cre (Ddx4-cre)* begins expression in ovaries between E15 and E18 [6] and *Figla-cre* begins to be expressed at E14.5 [9]. Developmentally earlier *cre* driver lines, such as *TNAP-cre* and *Prdm1/Blimp1-cre* target primordial germ cells [5, 7], and thus may produce phenotypes prior to meiotic initiation depending on the requirement of the gene in question. While *Stra8* represents an ideal candidate promoter as its expression is limited to germ cells, it is also important to maintain endogenous *Stra8* expression as male and female *Stra8*$^{-/-}$ are sterile [10]. Therefore, to maintain fidelity of the *Stra8* promoter for Cre production and to not affect endogenous *Stra8* expression, we generated a knockin allele using CRISPR/Cas9 system into exon 10, thus preserving both the 5′ and 3′ untranslated regions, which may also contain regulatory elements. Our knockin design generates a bicistronic transcript (*Stra8* and

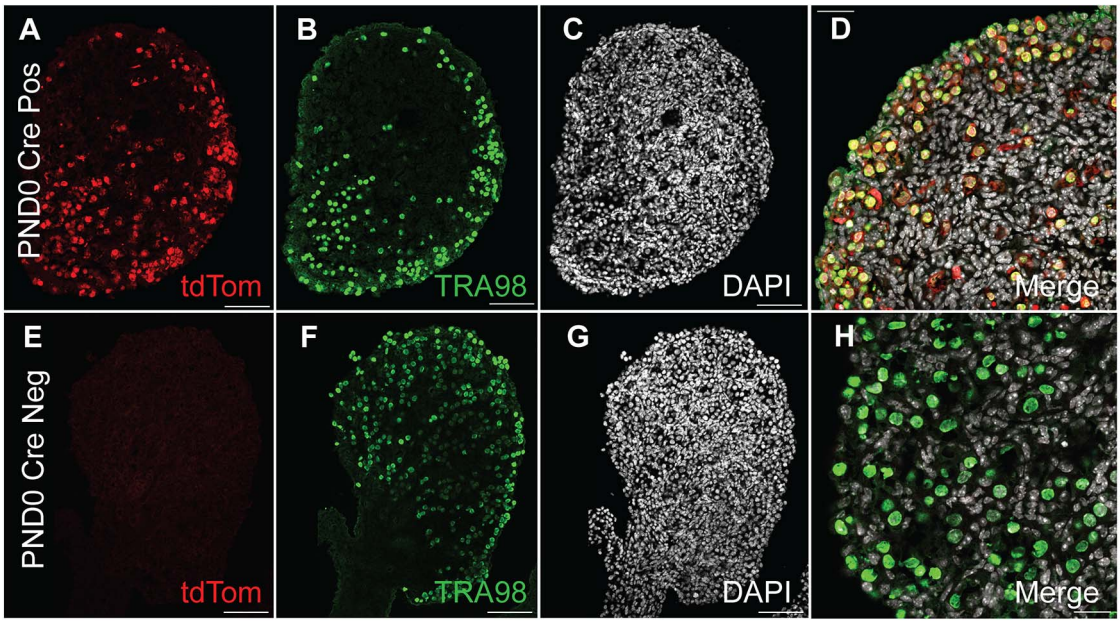

Figure 4. Widespread recombination in oocytes of *Stra8*$^{P2Acre/+}$ tdTomato+ ovary at PND0. (A–D) Example of immunofluorescent imaging of *Stra8*$^{P2Acre/+}$ tdTomato+ (Cre Pos) and control ovaries (tdTomato+ Cre Neg) (E–H) at PND0 ovary for tdTomato (A) the germ cell marker, TRA98 (B), and counterstained with DAPI (C). Red fluorescence (tdTomato) (A, E) indicates recombination. Immunofluorescent co-staining was performed on ovary cryosections, and images taken on an LSM 780 confocal microscope. Exposure times were the same for both cre negative and cre positive sections. Scale bar = A–C, E–G, 50 μm; D, H, 20 μm.

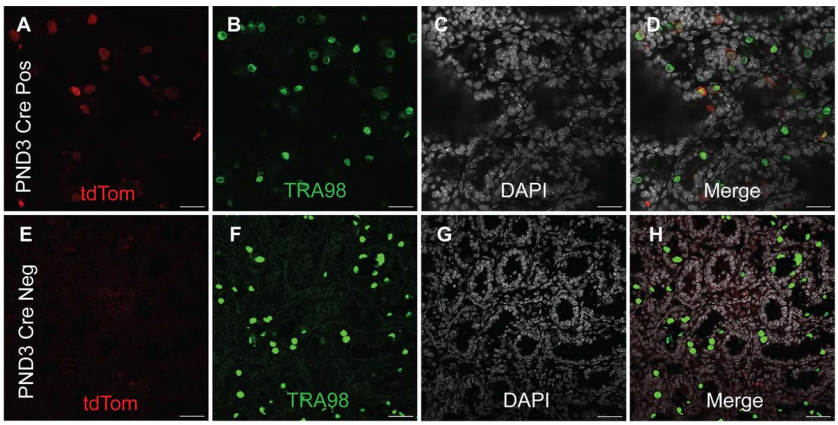

Figure 5. Recombination of *tdTomato*+ is detectable at PND3 in *Stra8*$^{P2Acre/+}$ *tdTomato*+ testes in germ cells. (A) tdTomato immunofluorescence in PND3 *Stra8*$^{P2Acre/+}$ *tdTomato*+ testis cryosections co-stained for TRA98 to mark germ cells (B) and DAPI (C) with merged images shown in panel D. (E) tdTomato immunofluorescence in PND3 wild type (cre neg) littermate cryosections with TRA98 (F), DAPI (G) and merged images (H) shown for comparison. Red fluorescence indicates recombination by the enzymatic actions of *Stra8*-cre, thus showing endogenous *Stra8* expression. Germ cells are marked with the anti-TRA98 antibody. Images were taken on an LSM 780 confocal microscope. Scale bar = 20 μm.

cre) with the self-cleaving P2A peptide, which is shown to be highly efficient in mice [21]. The self-cleaving 2A peptides mediate cleavage of proteins during translation by causing ribosome skipping and have better levels of downstream protein production than sequences that mediate internal ribosome entry [22]. The placement of the P2A sequence in the last *Stra8* coding exon results in an additional 20 amino acids onto STRA8, which has the potential to affect STRA8 function. However, our fertility analysis of heterozygous males and females, as well as homozygous mice, suggests it has no effect on reproductive capacity, unlike global deletion of *Stra8* [10], and it neither affected S*tra8* protein nor mRNA production.

By crossing *Stra8*^{P2Acre} to the fluorescent *tdTomato* loxP reporter line, we were able to show that cre expression from the *Stra8*^{P2Acre} knockin allele successfully recombines a loxP flanked cassette in the endogenous germline pattern for both males and females. This includes embryonic expression at E13.5 in female gonads. Even though *Stra8*^{P2Acre} cre expression should be limited in females to the time frame of E12.5-E16.5, Cre is a long-lived protein in oocytes, which can result in global deletion of *loxP* flanked alleles in offspring if carried on the female, similar to other oocyte-expressed cre lines [6]. Therefore, we suggest a maintenance breeding scheme of a male *Stra8*$^{P2Acre/+}$ or *Stra8*$^{P2Acre/P2Acre}$, unless using the cre line to generate a global null allele.

In conclusion, we have generated a new cre driver line utilizing endogenous expression from the *Stra8* locus in both male and female germ cells. This line will be a useful tool to analyze gene function in embryonic ovaries and postnatal testis as early as meiotic initiation and continuing for later stages of germ cell development.

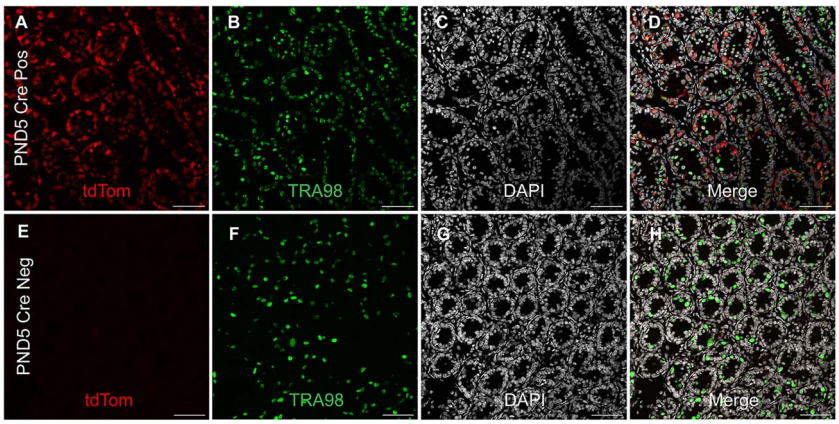

Figure 6. Recombination of *tdTomato*+ is detectable at PND5 in *Stra8*$^{P2Acre/+}$ testes. (A) Endogenous tdTomato fluorescence in PND5 *Stra8*$^{P2Acre/+}$ *tdTomato*+ testis cryosections, co-stained with (B) TRA98 to mark germ cells and (C) DAPI with merged images shown in panel D. Endogenous tdTomato fluorescence in PND5 wild type (cre neg) littermate cryosections shown in panel E with (F) TRA98, (G) DAPI and (H) merged images shown for comparison. Red fluorescence indicates recombination by the enzymatic actions of *Stra8*-cre, thus showing endogenous *Stra8* expression. Images were taken on an LSM 780 confocal microscope. Scale bar = 50 μm.

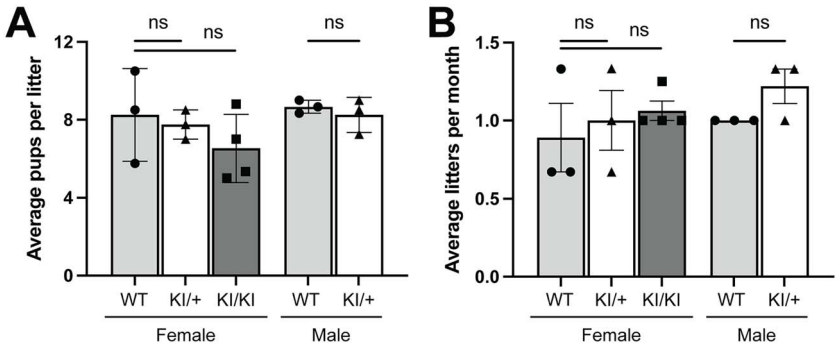

Figure 7. *Stra8*^{P2Acre} mice have normal fertility. (A) Average pups per litter and (B) average litters per month calculated for $n = 3$–4 breeding animals. Shown are data for wild type (WT) cre negative, heterozygous *Stra8*$^{P2Acre/+}$ (KI/+) and homozygous *Stra8*$^{P2Acre/P2Acre}$ (KI/KI) littermates bred to WT. Both graphs shown as mean + s.e.m. Error bars are not visible on control male litters per month bars due to values of 1 ± 0. Analysis by one-way ANOVA with multiple comparisons indicated no significant difference (ns) between genotypes.

Authors' contributions

S.A.P. conceived the idea. D.G.L, J.D.H., and S.A.P designed the study. A.A.A. and E.S. performed experiments. A.A.A., J.D.H., and S.A.P interpreted data. A.A.A, D.G.L, J.D.H., and S.A.P. wrote and edited the manuscript.

Conflict of Interest: The authors have declared that no conflict of interest exists

Data availability

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials and by request to the corresponding authors.

Supplementary data

Supplementary data are available at *BIOLRE* online.

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