# ### Material and Methods

# #### Mouse Husbandry

(goal, wild derived inbred strains used for natural genetic variation)

1. (specific strains were obtained from X vendors, 2. housed in X conditions (extra things done for problem breeders, 3. euthanized under X protocol,

wild derived inbred strains used in order to utilize natural genetic variation of house mice (and related species) in this study. The global distribution of inbred mouse strains is reflected in Supplemental Figure 1 (map of mouse sampling)

(X to X mouse strains obtained from Jax labs)

The KAZ/TUA, TOM/TUA, AST/TUA, and HMI/TUA were ordered and cryoderived from BRC at RIKEN (website). (These mice were housed at UW-Madison) <*Mice were euthanized by CO2 asphyxiation upon arrival at the University of Wisconsin - Madison. All work followed protocols approved by the Institutional Animal Care and Use Committee at the University of Wisconsin-Madison.*>

(Gough mice) A colony of mice from Gough island was main

<mice raised in 2-3 UW facilities -- on standard breeder chow>

Some extra breeding steps for problem breeders. Including; nestlets, sunflower seeds, larger rat cages.

# #### Tissue Collection and Immunohistochemistry

(goal, 1. describe how gonad tissue was collected) 2. describe spread process 3. (describe staining process (with DMC1)

(ovaries were collected from neonates less than 24 hours old or embryos day e16 to 21). Ovaries were collected and oocyte slide spreads were

made following (X reference) with some modifications. (

Testes were collected from males age 6 to 12 weeks. The right testes was collected and spermatocyte spreads were perpared following X reference.

Staining of meiocytes was performed following X protocol. (1:50) SCP3 primary antibody (Source), MLH1 pirmary antibody (Source), and centromere primary

incubated on slides for 24 hours. Secondary antibodies ... Washes. Slides were fixed with antifade (source).

*< Spermatocyte spreads were prepared following Peters et al. (1997) with minor adjustments. The tunica albuginea was removed and whole testis was incubated in 3ml of hypotonic solution for 45 minutes. The incubated testis was transferred to 40ul of 100mM sucrose on a microscope slide and torn with fine forceps. Approximately 15ul of cell slurry added to 80ul of a 2% PFA solution was spread onto a glass slide and dried overnight in a humid chamber. Immunohistochemistry followed Anderson et al. (1999) and Koehler et al. (2002). Antibody work and slide blocking were conducted in 1X antibody dilution buffer (ADB), normal donkey serum (Jackson ImmnuoResearch, West Grove PA, USA), 1X PBS, and bovine serum albumin (Sigma-Aldrich, St. Louis, MO, USA), Triton X-100 (Sigma-Aldrich, St. Louis, MO, USA). Each slide was blocked for 30 minutes in ADB before 60ul of a primary antibody mix containing anti-rabbit anti-MLH1 polyclonal antibody to* ***MLH1*** *(Calbiochem, San Diego, CA, USA; diluted 1:50), anti-goat polyclonal antibody to human SYCP3 (R&D Systems, Minneapolis, MN, USA; diluted 1:50), and anti-human polyclonal antibody to CREST (Antibodies Inc, Davis, CA, USA; diluted 1:200) in ADB was incubated for 48 hours at 37 degrees.*

**For slides/spreads for DSB charecterization, DMC1 (X:100) replaced MLH1**

*Slides were washed twice in 50ml ADB between primary and secondary antibody incubations. Slides were incubated overnight at 37 degrees in Alexa Fluor 488 donkey anti-rabbit IgG (Invitrgoen, Carlsbad, CA, USA; diluted to 1:100) and Coumarin AMCA donkey anti-human IgG (Jackson ImmunoResearch, West Grove PA, USA; diluted to 1:200). Alexa Fluor 568 donkey anti-goat (Invitrogen, Carlsbad, CA, USA; diluted 1:100) was incubated at 1:100 for 2 hours at 37 degrees. Slides were washed in 1x PBS, dried, and fixed with Prolong Gold Antifade (Invitrogen, Carlsbad, CA, USA) for at least 24 hours. Mice with at least 10 cells with good staining were included in our analysis. Due to variable quality of spermatocyte spreads four mice were used for quantification of either MLH1 counts or chromosome 1 SC traits instead of both (Supplemental Tables 1 and 2).*>

# #### Image Processing

1. Basic quality criteria, 2. Manual quant pipeline for MLH1,, 3. Quanti pipeline for DMC1 (not completely finished)

Cells were imaged on X microscope. saved as .tif files. Image Processing was done in Photoshop (source).

<*Spermatocytes were imaged using a Zeiss Axioplan 2 microscope with AxioLab camera and Axio Vision software (Zeiss, Cambridge, UK). Preprocessing including cropping, noise reduction, and histogram adjustments, was performed using Photoshop (v13.0). Only cells with a full karyotype (23 autosomes, 1XY), intact bivalents, and clear, distinct MLH1 foci were included for quantification. Image file names were anonymized before manual scoring. We recorded the numbers of MLH1 foci, bivalents with 0 MLH1 foci, and bivalents with signs of asynapsis. We also recorded a quality score (ranging from 1 to 5, with 1 representing high quality), whether or not the X and Y were paired, and whether or not a MLH1 focus was present in the pseudo-autosomal region on the X and Y as quality control measures.*>

# #### Statistical Analysis

1. Software used for analysis 2. (what statistical tools are used)? (difference in analysis of MLH1 and DMC1