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Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Data were collected from mouse testis (seminiferous tubules explants) of wild-type and selected knockout mouse strains. A Zeiss Axioplan 2ie microscope fitted with a 63X 1.4NA objective, a Roper CoolSnap camera, and ZEN 2.6 (blue edition) was used to acquire 3D time-lapse movies. A Zeiss Axioplan 2ie microscope was used to image mouse spermatocyte chromosome spreads using immunofluorescence. For FISH experiments, imaging was performed on an LSM880 laser scanning confocal microscope equipped with a 63X oil-immersion objective and no pixel binning, yielding a final pixel size of 0.13 µm. We imaged 4 fields per coverslip with a z-stack of varying thicknesses at 0.2-µm intervals. Laser power and gain were optimized per experiment to ensure good signal-to-noise ratios. The number of biological replicates (n), conditions, and treatments are reported in the figure legends and Methods.

Data analysis	3D-time lapse data were converted using Imaris File Converter, and analysis (e.g., quantification) was performed with Imaris 10.0.0 (\Program Files\Bitplane\Imaris 10.0.0\). When relevant, RStudio and custom code were used to calculate specific parameters of chromosome interactions (e.g., stage transitions using the changepoint package in R and an inflection-detection approach based on the Chow test statistic). The distance between the chromosome axes in the wild-type and selected mutant spermatocyte chromosome spreads is described in Methods. For FISH analysis, Individual nuclei were segmented from tubules in 3D using CellPose. For this purpose, we first used CellPose "Human-in-the-loop" training to proofread the segmentation results on 149 images, using these updated ground truths to improve performance. Our custom model weights and all analysis pipelines are available on GitHub. Procedures used after segmentation, including fluorescent FISH spots, 2D spot edges assessment, and distance measurement, are described in Methods. Graphing and statistical tests were performed in GraphPad Prism; statistical tests and multiple-comparisons corrections are reported in the corresponding figure legends and Methods. For data involving FISH experiments, graphs were generated using ggplot2 (https://ggplot2.tidyverse.org). All custom-made code used in this paper is available at https://github.com/OMRF/pezza-lab-stepwise-pairing-and-fast-transition-regulated-by-recomb-drive-meiotic-chrom-interactions1. DOI: https://doi.org/10.5281/zenodo.21681400. The data underlying the figures can be reproduced from the Source Data file provided with this paper, which contains raw data.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

Data availability. The authors declare that all data supporting the findings of this study are available within the paper and its supplementary information files. All data generated in this study are available in the Source Data provided with this paper.

Code availability. All customer code used in this paper has been deposited at: https://github.com/OMRF/pezza-lab-stepwise-pairing-and-fast-transition-regulated-by-recomb-drive-meiotic-chrom-interactions1. DOI: https://doi.org/10.5281/zenodo.21681400.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	No. The study did not involve human participants, human participant data, or primary human biological samples. All experiments were performed in mouse seminiferous tubules.
Reporting on race, ethnicity, or other socially relevant groupings	*Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status).* *Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.)* *Please provide details about how you controlled for confounding variables in your analyses.*
Population characteristics	*Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."*
Recruitment	*Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.*
Ethics oversight	*Identify the organization(s) that approved the study protocol.*

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No statistical methods were used to pre-determine sample size. Sample sizes were chosen based on prior experience with these assays and to ensure reproducible effects across independent experiments. “n” denotes the number of individual samples within a biological replicate. The number of biological replicates, which varies by assay, is also reported.
Data exclusions	Data were excluded only for pre-established technical quality reasons. In 3D time-lapse data, events were excluded by not passing the minimum time resolution, GFP signal/resolution, splitting of lacO-GFP-lacI spots, or absence of active chromosome movements. For FISH data, individual cell events were excluded when the failed pre-specified filters for insufficient 3D resolution and/or the cell stage could not be determined. Pre-established exclusion criteria are described in the main text and under Methods.
Replication	Key findings were confirmed in at least three independent biological replicates. 3D time-lapse and fixed samples (FISH on whole-mounted seminiferous tubules and chromosome spreads) experiments were repeated in independent mice of a relevant background (e.g., seminiferous tubules containing specific types of spermatocytes, or knockout mice of selected recombination proteins). Direction of effects was consistent across repeats; variability is shown in the figures.
Randomization	Randomization was not applicable. Experimental groups were defined by staging or genotype. Samples were processed in parallel using identical protocols and acquisition settings, and analyses used predefined quantification workflows applied uniformly across conditions.
Blinding	Blinding was not performed; samples were defined by treatment/genotype. Analyses used predefined, objective pipelines applied uniformly across conditions.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	Antibody Source Western blot dilution Immuno-labeling dilution α-tubulin Proteintech, 66031-1-Ig 1:5,000 LacI Kerafast, EG1501 1:500 1:100 SYCP3 Pezza Lab. 1:300 SHOC1 Pezza Lab. 1:100 HFM1 Pezza Lab. 1:100
Validation	Antibodies used in this study are listed in Supplementary Table 2 (including source, catalog number/origin, and concentration used according to the use). Antibodies were validated by the manufacturers for the indicated applications and/or used in accordance with our prior or peer-reviewed studies; specificity was additionally supported by appropriate controls (e.g., absence in knockout mouse). Antibodies against SYCP3 raised in chicken, and HFM1 and SHOC1 raised in Rabbit has been reported in PMID: 23555294 and PMID: 29742103, respectively.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	Funder mice carrying lacO insertions in chromosome 8 and chromosome 9, and mice carrying lacI-GFP fusion, were crossed with C57BL/6J mice for several generations. LacO(chr. 8/9)-lacI-GFP used to visualize RPMs or long-term chromosome interactions was generated by crossing mice with either chromosome 8 or chromosome 9 marked with lacO with mice carrying lacI-GFP. Additional mouse strains used in this work: mice carrying the Mlh1, Msh4, Spo11, Dmc1, Hfm1, Shoc1, and Hop2 null alleles. Animals were housed in standard rodent-approved cages, with ad libitum access to food and water and 12:12 light/dark cycle.

3D-time-lapse experiments and experiments with fixed samples used 1.5-2-month-old male mice.
Synchronization of male germ cell development was performed according to a previously described protocol described in Methods. Briefly, 2 days post-partum, male mice were orally administered a solution of the retinoic acid biosynthesis inhibitor WIN 18,446 (MedChemExpress, HY-W011094) once daily for 6 days total. After a one-day rest period, each mouse received a subcutaneous injection of retinoic acid solution. Testes were collected at specific time points following RA injection for further analyses.

Wild animals

Provide details on animals observed in or captured in the field; report species and age where possible. Describe how animals were caught and transported and what happened to captive animals after the study (if killed, explain why and describe method; if released, say where and when) OR state that the study did not involve wild animals.

Reporting on sex

Experiments were focused in mouse spermatocytes. Only males were used in these studies.

Field-collected samples

For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field.

Ethics oversight

All experiments were approved by the Oklahoma Medical Research Foundation IACUC committee (protocol no. 12-55).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks

Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.

Novel plant genotypes

Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.

Authentication

Wild type, transgenic (carrying lacO, lacI), and knockout mice strains were authenticated by established locus specific genotyping, western blot, or by miscroscopy.