1 **Supplementary Note**

2

3 **Details of HMM algorithm used to identify CO and NCO events**

4 Using the information from the filtered strain-informative SNPs, we developed a Hidden Markov
5 Model (HMM) to infer the strain origin of each broad segment of the genome. In our HMM, the
6 three possible emitted genotype states B6/B6, B6/CAST and CAST/CAST are represented by 0,
7 1 and 2, respectively (i.e. the number of CAST allele copies at each strain-informative SNP site).
8 Similarly, the hidden states representing background strain origin are encoded as 0, 1 and 2
9 copies of a CAST haplotype. Emitted states may be different from hidden states due to
10 sequencing errors or real converted events (e.g. observing a homozygous CAST genotype on an
11 otherwise heterozygous CAST/B6 background). A natural initial stationary distribution is (0.25,
12 0.5, 0.25) corresponding to state triple (0, 1, 2). The state transition between two sites is driven
13 by recombination events, with the distance between two different states following an exponential
14 distribution with a rate parameter equal to twice the recombination rate. Here we adopted a
15 genome-wide average constant recombination rate of $r=0.625*10^{-8}$ per base pair per generation[1,2].
16 Thus, the probability of recombination from site i to site j can be written as follows:

17 $$P_{ij}=1-\exp(-2rD_{ij}), \quad (1)$$

18 where P_{ij} and D_{ij} stand for the recombination probability and distance between site i and j,
19 respectively. The transition probability matrix from site i to site j is as follows:

20 $$\mathbf{P}_{ij}=(1-P_{ij})\mathbf{I_3}+P_{ij}\mathbf{Q}, \quad (2)$$

21 where I_3 is the 3×3 identity matrix and Q stands for the conditional transition matrix with the
22 entry q_{mn} (m=0,1,2; n=0,1,2) describing the transition probability from state m to state n:

23 $$\mathbf{Q}=\begin{bmatrix} 0 & 1 & 0 \\ 1/2 & 0 & 1/2 \\ 0 & 1 & 0 \end{bmatrix}. \quad (3)$$

24 There is no transition from state 0 to state 2, or vice versa, because it's unlikely that two

25 independent recombination events would happen at exactly the same position with a small

26 sample size. Conditional on there being a recombination event, state 0 or state 2 transitions to

27 state 1 with probability 1, and state 1 transitions to either state 0 or state 2 with equal probability.

28

29 Here we defined the emission probabilities from each hidden state by using the quality metrics

30 from GATK for states 0, 1 and 2. Given state *g* in each site *t*, GATK provides a quality score S

31 for three states as follows:

32 $$s_g^t = -10 \log_{10} \frac{p(D|G_t=g)}{\max_{k=0,1,2} p(D|G_t=k)}, \quad (4)$$

33 where $p(D|G_t = g)$ is the probability that we observe the data D, conditional on the hidden state

34 G_t being *g*. Since for each site *t*, the maximum score is constant, we can inversely infer the

35 probability of observing different states with a constant scale factor:

36 $$p(D|G_t = g) \propto 10^{-\frac{s_g}{10}}. \quad (5)$$

37 In our analysis, the scaling parameter was arbitrarily set to 1.

38

39 We applied the forward-backward algorithm to infer the posterior distribution of hidden states.

40 Starting with prior state probabilities (0.25, 0.5, 0.25) at the first site, the forward probability of

41 state *j* after seeing the first t sites is

42 $$A_t(j) = \sum_{i=0}^{2} \alpha_{t-1}(i) p_{ij}(t-1) e_j(t), \quad (6)$$

43 where p_{ij}(t-1) is the $(i,j)^{th}$ element of transition matrix **P** at site t-1, and $e_j(t) = p(D|G_t = j)$ is the

44 emission probability conditioned on state *j* at site t given by equation 5. At the same time, we

45 define a backward chain with an initialised probability (1, 1, 1) at the end of the site using the

46 following:

47 $$\beta_t(j) = \sum_{k=0}^{2} \beta_{t+1}(k) p_{jk}(t) e_j(t+1), \quad (7)$$

48 and the probability of hidden state j, given the observed data (j=0,1,2) at site t is

49 $$p_t(j) = \frac{\alpha_t(j)\beta_t(j)}{\sum_{i=0}^{2} \alpha_t(i)\beta_t(i)}. \quad (8)$$

50 Finally, we can calculate the stationary distribution of states 0, 1 and 2 for each strain-
51 informative SNP site given the sequencing data, and for each site we choose the hidden state
52 with maximum probability as the real strain background state at that site. Finally, because we
53 wish to compare genotypes to this background state to identify NCO events (using additional
54 filters), we smoothed the resulting initial background estimation, by reverting inferred changes in
55 background spanning <50 SNPs to the broader inferred background state (such changes were
56 tested as potential NCO events, instead).

57

58 **Details of algorithm used to attribute *Prdm9* control in hybrids**

59 The *Prdm9* alleles in the hybrid mouse in this work are *Prdm9*^{Hum} and *Prdm9*^{Cast}. Using DSB
60 maps from other samples (B6 [3], B6$^{B6/Hum}$, B6$^{Hum/Hum}$, B6$^{-/-}$ [3], B6xCAST, PWD, B6xCAST$^{Hum/Cast}$,
61 (B6xCAST)F2$^{B6/Hum}$, PWDxB6, B6xPWD, PWDxB6$^{Hum/PWD}$, B6xPWD$^{Hum/PWD}$), we were able to
62 classify DSB hotspots as being under the control of either the humanized or CAST *Prdm9* allele
63 in the hybrids. We thank Anjali Hinch for suggesting the following procedure.

64

65 We create a superset of hotspots, which are combined across mice such that hotspots that have
66 their centres within 600 bp of each other are considered to represent the same hotspot. For each
67 hotspot in this superset, we then create a maximal set of *Prdm9* variants that could potentially be
68 responsible for activating it. For example, if a hotspot overlaps hotspots in B6xCAST and
69 B6xCAST$^{Hum/Cast}$, the potential set of alleles that could activate the hotspot is *Prdm9*^{B6},

70 *Prdm9*^{Cast}, and *Prdm9*^{Hum}. From the maximal set, we reduce to a minimal set of alleles that can
71 explain all of the mice in the set. In the example above it is *Prdm9*^{Cast}. Hotspots for which the
72 minimal set consists of a single *Prdm9* variant are inferred to be activated by it. There are two
73 special cases: Hotspots in the B6$^{-/-}$ are said to arise from a dummy allele *Prdm9*^{KO}. Hotspots that
74 overlap with these hotspots are assigned the allele "KO". *Prdm9*^{Cast} and *Prdm9*^{PWD} have
75 similarities in their zinc finger arrays and a large number of overlapping hotspots. If the maximal
76 set contains more than one of these variants, we treat them as equivalent. It is not always
77 possible to reduce the minimal set to a single *Prdm9* variant. For example, if a hotspot is found
78 in B6xCAST, B6xCAST$^{Hum/Cast}$ and B6^{Hum}, then no single *Prdm9* variant can explain all the
79 hotspots. The maximal set cannot be reduced from *Prdm9*^{B6}, *Prdm9*^{Cast} and *Prdm9*^{Hum}. In this
80 case, we take the following approach to assign alleles in the B6xCAST$^{Hum/Cast}$ mouse that is of
81 interest in this work:

82 1. For hotspots where the minimal set contains both *Prdm9*^{Cast} and *Prdm9*^{Hum}, we
83 say the allelic type is "*unknown*" or "*MULT*".

84 2. For hotspots where the minimal set contains *Prdm9*^{Cast}, but not *Prdm9*^{Hum}, the
85 allelic type is "*CAST*".

86 3. For hotspots where the minimal set contains *Prdm9*^{Hum}, but not *Prdm9*^{Cast}, the
87 allelic type is "*HUM*".

88

89 **Further details of testing and characterizing the bias towards GC in NCO events**

90 To test for the presence of GC-bias in NCO events, we first combined inherited and *de novo*
91 NCO events inferred to be under *Prdm9*^{Hum} control, and occurring within (<1 kb from) DSB
92 hotspots identified using our DMC1 data. This identified a total of 1,011 SNPs within such NCO
93 tracts. We focus only on human-controlled events because the resulting hotspots are newly
94 introduced, and so unlike *Prdm9*^{Cast}-controlled DSB hotspots cannot have been influenced by
95 historical recombination, e.g. generating an excess of mutations towards G/C carried on the
96 CAST genome.
97
98 Initially, we simply tested for an overall "GC bias" by testing for an excess of SNPs converted
99 from A/T towards G/C vs. the converse direction, yielding strong evidence (59.9% towards GC,
100 p=3.7x10^{-9} by 2-sided binomial test). This bias occurs despite the great majority of our detected
101 NCO events (encompassing 99% of all converted SNPs) representing simple, contiguous
102 converted tracts. This raises the question of whether multiple co-converted SNPs still show a GC
103 bias.
104
105 We therefore separately tested for directional GC bias of converted SNPs where the adjacent
106 SNPs in our set (among the 14,334,181 genome-wide) were either both non-converted, or where
107 at least one of the adjacent SNPs was converted. These represent conversion of a single isolated
108 SNP, vs. SNPs falling within a multiple-SNP tract, respectively. We observed a strong
109 conversion bias for isolated SNPs (68.1% towards GC, p=1.9x10^{-15}). In contrast there is no
110 evidence of bias whatsoever for SNPs within multiple-SNP tracts (50.4% towards GC, p=0.921).
111 This difference is highly significant (p= 1.1x10^{-7}, by Fisher's Exact Test). Thus, GC-biased gene

112 conversion appears to effectively exclusively influence the shortest conversion tracts, of single
113 SNPs. Below, we analyse occasional complex events that are observed.
114
115 We separated NCO events into bins according to sex, underlying hotspot heat and hotspot
116 symmetry, and NCO position relative to the PRDM9 binding motif (Supplementary Table 3 and
117 Supplementary Fig. 4): no differences in either the lack of bias in multiple-SNP tracts, or the
118 quantitative GC-bias of single-SNP NCO events, were observed. For symmetry, we split single-
119 SNP NCO events into four quartiles based on their proportion of DMC1 signal coming from the
120 B6 chromosome, ignoring SNPs within 20 bp of the centre of an identified (humanized) PRDM9
121 binding motif because such SNPs might be involved in driving (a)symmetry itself. No impact of
122 symmetry on the bias is seen, so GC-bias is not driven by DSB initiation bias.
123
124 To distinguish whether GC-bias is prevented by, or else prevents, multiple-SNP conversion
125 tracts, we tested for a relationship between the strength of bias and the distance of a SNP to other
126 SNPs in hybrid mice. SNPs nearby other heterozygous SNPs tend to be co-converted with those
127 SNPs, meaning they will normally lie within multiple-SNP tracts, if converted. Therefore, if GC-
128 bias is prevented by multiple-SNP conversion SNPs, such SNPs will show little or no bias. We
129 therefore binned SNPs according to their distance to the nearest flanking SNP and plotted the
130 strength of GC-bias for each bin (Fig. 4b). SNPs very near to other SNPs show no GC-bias,
131 whilst those >100 bp from the nearest marker show approximately the 68% bias among all
132 single-SNP conversion tracts. The extremely high observed rate of co-conversion of nearby
133 marker pairs (95.1% of adjacent markers within 10bp of a converted marker are also converted)

134 implies that the number of SNPs in a conversion tract influences biased repair processing of
135 DSBs towards or away from GC, rather than some process operating the other way around.
136
137 Thus, the strength of GC-bias depends on local SNP density, implying that the same SNP where
138 one allele is A/T and the other is G/C will have different conversion rates and biases in different
139 individuals, depending on alleles at surrounding SNPs. In humans, SNP densities are low
140 (roughly 1 SNP per kb in Europeans) and so multiple-SNP conversion tracts (other than very
141 long, typically complex tracts) are unusual; therefore, the similar GC-biases observed for single-
142 SNP conversion tracts, of 68%, imply a common process might act in both species.
143
144 We reasoned that we could leverage the "non-biased" NCO events with longer tracts to
145 understand whether the bias towards GC might depend on the invading (i.e. donor) haplotype, or
146 the recipient haplotype (in which the DSB occurred). There are 12 possible combinations of
147 donor and recipient alleles: we estimated underlying (i.e. "non-biased") proportions of each from
148 the multi-SNP NCO events (we averaged e.g. A<-G and T<-C mutations via obvious strand
149 symmetry to yield 6 pooled types, in generating confidence intervals). In single-SNP observed
150 NCO events, we then plot the fraction of each of the 12 possible types, divided (normalised) by
151 these underlying proportions (Fig. 4c). We calculated 95% confidence intervals (CIs) for the
152 odds of each of the 12 possibilities relative to the multi-SNP tracts (binomial test; 95% CI were
153 calculated from 6 pooled event types, after strand flipping, to aid power, though we show
154 estimates for all 12 single-SNP NCO types for completeness). As an alternative, we used all
155 markers not involved in gene conversion events but immediately adjacent to converted markers
156 to estimate the background probabilities of different SNP types within hotspots in the same way.

157 This yielded essentially identical results (Supplementary Fig. 4b), with no significant difference
158 in SNP composition as measured by underlying proportions between non-converted markers and
159 markers in multi-SNP conversion tracts ($p=0.59$ by Chi-squared test with 5 d.f).

160

161 We observed odds ratios >1 for all event types involving a recipient allele which is an A or T,
162 and odds ratios <1 for all event types involving a recipient allele which is G or C. This suggests a
163 bias driven by the potential recipient allele, i.e. the chromatid which the DSB occurs on. At DSB
164 sites, it is possible in principle for nearby SNPs to be successfully converted from the
165 homologous chromosome, or a potential conversion rejected, e.g. by resolution of heteroduplex
166 DNA in favour of one background or the other. Given we do not observe mutations
167 towards/away from GC bases altering the DSB rate, the observed GC-bias of NCO events is
168 most simply explained by a tendency for the rejection of conversion of single SNPs, if the
169 *recipient* haplotype has a G or a C at the same position, e.g. through the action of MMR, BER or
170 NER proteins on heteroduplex DNA. The relative odds of conversion occurring is approximately
171 half of that if the recipient haplotype (that on which the DSB occurred) carries an A or T allele at
172 the same position, so it is a strong effect. (We cannot determine whether the bias impacts only G
173 or C bases, or both, because we do not observe strand for our NCO events.) Importantly, this
174 rejection does not seem to obviously depend on the type of the *donor* allele (other than it
175 mismatching). For example, G->C conversions appear to occur at the lower rate while A->T
176 conversions occur at the higher rate. As a caveat, there are relatively few such events, because
177 these transversion mutations are relatively rare, so the different rates observed for these events
178 do not reach statistical significance ($p>0.05$).

179

180 In conclusion, our data imply a mechanism of GC-biased gene conversion arising through
181 identification of mismatching bases in the donor haplotype relative to the recipient, sometimes
182 leading to rejection of the donor allele if the *recipient* allele is a G/C base pair (Fig. 6). However,
183 this "checking" process normally only occurs if there is just a *single* mismatching base within the
184 potential conversion tract, so that potential multi-SNP conversion tracts show no bias.
185
186 Finally, we reasoned that rare observed complex recombination events (i.e. non-contiguous NCO
187 and CO tracts) might be explained by every occasional ability of the same process to "escape",
188 and act even in multi-SNP conversion tracts. We observed 12 SNPs not undergoing conversion
189 but flanked by converted SNPs, i.e. within complex NCO events. We reasoned that the non-
190 converted SNPs might be "rejected" by the above biased process, if in rare cases it is able to
191 operate even in the context of a multi-SNP potential conversion tract. If so, we would predict the
192 *non-converted* markers should tend to show bias, with G/C bases on the recipient chromosome.
193 Strikingly, we find all 12 SNPs show G/C bases on the potential recipient chromosome (and
194 varying bases on the potential donor chromosome). The probability of observing this pattern by
195 chance is approximately $1/2^{12}= 0.0002$. It is interesting that several complex events show >1
196 such SNP, so perhaps "escape" acts at the level of the entire event. Similarly, we observe 7
197 complex *de novo* CO events. All involve a single "missing" SNP (not uniquely identifiable in 2
198 cases), which may therefore be explained as a rejected conversion of one SNP. In 6/7 cases this
199 missing SNP has a G/C base on the potential recipient chromosome. Taken together, this yields a
200 p-value among all complex events of p= 0.00004 (Binomial test, 1-sided). A previous human
201 study[4] found a similar bias of up to 100% in apparently converted sites within complex
202 crossovers, which also involved single SNPs, so it appears this phenomenon may extend to

203 humans. Therefore, complex recombination events can be reinterpreted as otherwise normal,
204 continuous-tract events, but where a SNP is "rejected" for conversion, by a near 100% GC-
205 biased process. Moreover, this process involves rejection of bases where the potential recipient
206 chromosome carries a G or a C, exactly as in NCO GC-bias.
207
208 Therefore, the apparently distinct phenomena of GC-biased NCO events, and the occurrence of
209 occasional complex NCO and CO events, might be explained by a common underlying model of
210 biased repair, involving rejection of single "incoming" SNPs where the existing allele is a G or C
211 base. Given it does not correlate with DSB initiation, this phenomenon most plausibly arises via
212 biased heteroduplex repair machinery. Under this model, the bias appears to be close to 100%,
213 but it does not impact all NCO events, yielding a maximal observed NCO bias (identical in
214 humans, and in mice) of around 68%.
215
216 We inferred the rate at which a strongly GC-biased repair process would have to occur to yield
217 the observed GC-biased gene conversion rate at single-SNP sites (68% of converted sites being
218 A/T to G/C). Given the results in complex NCO events, we assume this process has a GC bias
219 close to 100%, which prevents conversion where the recipient chromosome is a G/C. However,
220 that process only acts some of the time, say with probability p, while the normal strand-biased
221 process occurs with probability 1-p. Then, given there's a single-SNP mismatch in a tract, and
222 assuming the mismatch is GC->AT as often as AT->GC, each with probability q=0.5:
223 $\mathrm{P(conversion\ GC\ to\ AT)} = q(1-p)$ and
224 $\mathrm{P(no\ conversion\ GC\ to\ AT)} = qp$, and
225 $\mathrm{P(conversion\ GC\ to\ AT)} = q$,

226 with the probability of no conversion from AT to GC being small, given estimates of the number

227 of DSBs versus the number of CO/NCO events per meiosis.

228 Given our data,

229 $$0.68 = \text{P(conversion AT to GC | conversion observed)} = \frac{q}{q+q(1-p)} = \frac{1}{2-p}.$$

230 Solving this yields p=0.53. Thus, the data can be explained by simple model in which a distinct

231 mismatch repair process acts to prevent gene conversion at 53% of sites where the recipient

232 chromosome contains a G/C at a single mismatch site (or, for example, at 100% of sites where

233 the recipient chromosome contains a G at a single mismatch site; Fig. 6).

234

235 **Details of algorithm used to estimate the number of autosomal DSBs in a single meiosis**

236 **repairing using the homologue**

237 We assume that the average number of DSBs per meiosis resolving as NCO events is K. Because

238 each NCO affects only one of four chromatids, only one quarter of them will be seen in a single

239 offspring.

240

241 We take F2 animals as an example; an identical approach was used for F5 events. Twenty-two

242 meioses occurred, to generate 11 F2 animals. If D is SNP density near DSBs, L is average NCO

243 tract length, and "Power" represents the power we have to detect a particular SNP within a NCO

244 event, then if N is the number of converted sites observed, we have:

$$E(N) = \frac{\text{K}}{4} * 22 * \text{Power} * \text{L} * \text{D}$$

245 Values for N, L, "Power" and D together allow estimation of K. We observe 0.0072 SNPs per bp

246 within hotspots, and N=240 distinct converted sites in total; moreover, we estimate tract length

247 L=30, and a power of 74.3% for these animals. This yields an estimate of $\widehat{K} = 274$ DSBs
248 resolving as NCO events, per meiosis.
249
250 For CO events, we have near 100% power to observe these, and half of all recombination CO
251 events are transmitted to a particular offspring. Therefore, based on 295 observed CO events in
252 these mice, the (sex-averaged) estimated number of CO events is 295x2/22=26.8 per meiosis.
253
254 The sum of these numbers is the total number of autosomal events repairing using the
255 homologous chromosome, per meiosis (we neglect the X-chromosome in this calculation). To
256 obtain CIs for the number of NCOs, COs and the total number of recombination events per
257 meiosis and for the NCO to CO ratio, we performed bootstrapping as to estimate the tract length
258 of NCOs. For each bootstrapped sample (of 10,000), we obtained the number of NCOs and
259 number of COs, and used these to re-estimate the total number of recombination events and the
260 NCO/CO ratio.
261
262 **Rejection sampling algorithm for COs and NCOs, construction of Fig. 5 and**
263 **Supplementary Fig. 6, and testing for impacts of asymmetry on event resolution**
264 In testing for impacts of asymmetry on the number of NCO and CO events observed, relative to
265 expectations from ChIP-seq data, we allowed for the following factors.
266 (i) Asymmetric hotspots have higher SNP density around binding motifs so have higher
267 power to identify NCOs (CO events are not affected). We corrected this in our
268 analyses of how symmetry impacts the number of observed NCO events in different

hotspot types, by incorporating SNP density information to estimate power to detect events in each hotspot.

(ii) We tested for differences in the impacts of symmetry in COs vs, NCOs, males vs. females, *de-novo* versus inherited events, and for *Prdm9*^{Hum}-controlled versus *Prdm9*^{Cast}-controlled events. Because the CAST allele has co-evolved with the *castaneus* genome, some impacts of symmetry on recombination event resolution might be impacted (see below).

(iii) Strand can be identified for NCO events but not for CO events, allowing us to analyse events at asymmetric hotspots initiating on each strand separately. In particular, we can identify whether the homologue is strongly versus weakly marked by H3K4me3, for a given event.

In all analyses, we focused on NCO and CO events occurring in the subset of hotspots containing an identified motif, and with well-defined estimated heat on each strand for both H3K4me3 and DMC1.

To correct for SNP density in our analyses of how symmetry impacts the number of observed NCO events in different hotspot types, we directly leveraged SNP density information to estimate (relative) power to detect events in each hotspot. For the hotspots that contain an identified motif, we give each SNP near the motif (<1 kb) a weight according to its location relative to the motif. The weight is defined using the distribution of NCOs around motifs (Fig. 3d), and so estimates the probability a NCO event initiating within the hotspot will incorporate this SNP (up to a constant of proportionality). Therefore, this quantifies the over-representation

292 of this hotspot compared to the true number of DSBs resolving as NCO events occurring within
293 it. Summing this weight over all SNPs then yields the relative power to detect bases falling
294 within NCO events in each hotspot (so if there are no SNPs in a hotspot, the power to detect
295 NCO events is zero, while hotspots with many SNPs near the motif itself have highest power).
296 This yields a weight w_i for hotspot i. Multiplying the original hotspot heat (from DMC1 or
297 H3K4me3) gives a power-corrected heat for the hotspot, used to define expectations for
298 observable NCO events, and compare to actual observed NCOs.
299
300 *Construction of Fig. 5a-b and Supplementary Fig. 6a-d:*
301 For these figures, we compare CO and NCO events, for events of different types (e.g. male vs.
302 female) and in different categories (e.g. PRDM9^{Cast} versus PRDM9^{Hum} controlled). To compare
303 NCO and CO events, we used the weights w_i and rejection sampling. Within a hotspot and event
304 category, we started with all observed events, and associated weight w_i for event i. Because NCO
305 events are over-represented on average w_i-fold, relative to the ChIP-seq observations and to CO
306 events, we retained NCO events with probability $\min\{\alpha/w_i,1\}$, and CO events/hotspots with
307 probability $\min\{1, w_i/\alpha\}$, where α is any constant. For any hotspot, the probability of retaining a
308 NCO event is then immediately w_i/α times lower than that of a CO event, and so this perfectly
309 reverses the over-representation of this hotspot in observed, versus initiated, NCO events (the
310 constant α only impacts the overall number of NCO vs. CO events retained, not their spread, so
311 does not impact the validity of this point). In practice, we used α=0.7 to retain similar numbers
312 of CO and NCO events.
313

314 We then obtain comparable lists of hotspots with various H3K4me3 heats and symmetries, and
315 DMC1 heats and symmetries, as well as observed NCO and CO events, in any given category.
316 For Fig. 5a and Supplementary Fig. 6a, c, we next ordered hotspots by their H3K4me3
317 symmetry, defining 3 bins with equal expected number of events, according to DMC1-predicted
318 overall heat of each hotspot. We order by H3K4me3 in order that our symmetry estimates are
319 independent of the estimated heats; in practice, ordering by DMC1 symmetry made almost no
320 difference to results (not shown). We compared the binned predictions to the actual number of
321 events of different types observed – both NCO, and COs. We also obtained 95% CIs of the
322 fraction of observed events in each category by bootstrapping events 1000 times. To obtain p-
323 values for asymmetric hotspots, we obtained exact binomial p-values, to test the null hypothesis
324 that the true proportion of events occurring in the asymmetric hotspots bin is 1/3.
325
326 For Fig. 5b and similar for Supplementary Fig. 6, we performed the same analysis, but now
327 ordered hotspots by their DMC1 symmetry, defining 3 bins with equal expected number of
328 events, according to H3K4me3-predicted overall heat of each hotspot. This tests whether
329 H3K4me3-defined heats, which measure the extent of PRDM9 binding in each bin, accurately
330 predict where CO and NCO events occur. As before, though less strongly because DMC1 shows
331 inflation in asymmetric hotspots (Supplementary Fig. 5g), we observe fewer events of all types
332 in asymmetric hotspots, relative to expectations.
333
334 *Construction of Supplementary Fig. 6e-h:*
335 This group of panels is constructed as Supplementary Fig. 6a-d, but studies only CO events, so
336 no rejection sampling was required. Rather than symmetry, we order hotspots based on their

337 H3K4me3 or DMC1-estimated average homologous heat, and predict events within bins using
338 their overall signal of DMC1 or H3K4me3. We separate hotspots depending on whether they are
339 human-controlled or CAST-controlled. Homologous heat provides slightly stronger signals than
340 symmetry itself, implying CO events avoid weak hotspots as well as asymmetric hotspots, i.e. all
341 hotspots where the homologous chromosome is bound weakly.
342
343 *Construction of Supplementary Fig. 6i-l:*
344 This group of panels is constructed as for Supplementary Fig. 6a-d, but studies only NCO events.
345 To account for power, we therefore resampled hotspots with weights proportional to w_i, and
346 compare observed NCO events to expectations under this resampling. For NCO events, we can
347 determine which homologue they occurred on. This allows us to test whether "homologous heat"
348 (see "**Hotspot symmetry estimates**"), i.e. the strength of DMC1/H3K4me3 signal on the
349 homologous chromosome, might more strongly determine whether NCO events occur than our
350 overall single symmetry measure for a hotspot. We therefore now separated the two homologues
351 for each hotspot, resulting in a predicted (DMC1 or H3K4me3) heat for each homologue, as well
352 as two complementary homologous heats. We ordered hotspots by this homologous heat (as in
353 Fig. 5, we defined the fraction of events occurring on each homologue using the independent
354 ChIP-seq data) and then used predicted heat to bin hotspots so that (as in Fig. 5) 1/3 of events are
355 predicted to occur in each bin. We again separate hotspots depending on whether they are
356 PRDM9^{Hum}-controlled or PRDM9^{Cast}-controlled, and otherwise proceed as in Fig. 5.
357
358 This revealed a strengthened signal relative to previous tests – few events are seen in hotspots
359 with low homologous heat, i.e. where PRDM9 does not bind the homologous chromosome,

360 implying NCO events strongly avoid occurring on both the hot allele of highly asymmetric
361 hotspots, and at very weak hotspots. Conversely, NCO events occur preferentially on the cold
362 allele of asymmetric hotspots, or strong hotspots more generally. Again, this occurs for both
363 $Prdm9^{Hum}$ and $Prdm9^{Cast}$.
364
365 Notably, although both differ significantly from expectations from both DMC1 and H3K4me3
366 data, we see somewhat stronger signals for $Prdm9^{Hum}$ than $Prdm9^{Cast}$. Asymmetry in $Prdm9^{Cast}$-
367 controlled hotspots is largely the result of evolutionary erosion, which can only occur when NCO
368 or CO events themselves occur. Therefore, hotspots which evolve by chance to become
369 asymmetric are preferentially sampled from those more active for these marks. This predicts that
370 asymmetric hotspots for this allele may tend to have higher NCO and CO rates relative to
371 PRDM9 binding strength as measured by H3K4me3, compared to these rates at random hotspots.
372 In contrast, asymmetry at $Prdm9^{Hum}$-controlled hotspots is mainly due to SNPs occurring at
373 random within the PRDM9 binding motifs inside these hotspots, so is immune to biases in NCO
374 and CO rate, and this seems likely to explain our observation.
375
376
377
378
379
380
381
382

383 **References**

384 1. Jensen-Seaman, M. & Furey, T. Comparative recombination rates in the rat, mouse, and
385 human genomes. *Genome Res.* 528–538 (2004).

386 2. Brunschwig, H. *et al.* Fine-scale maps of recombination rates and hotspots in the mouse
387 genome. *Genetics* **191,** 757–764 (2012).

388 3. Brick, K., Smagulova, F., Khil, P., Camerini-Otero, R. D. & Petukhova, G. V. Genetic
389 recombination is directed away from functional genomic elements in mice. *Nature* **485,**
390 642–645 (2012).

391 4. Arbeithuber, B., Betancourt, A. J., Ebner, T. & Tiemann-Boege, I. Crossovers are
392 associated with mutation and biased gene conversion at recombination hotspots. *Proc.*
393 *Natl. Acad. Sci.* **112,** 201416622 (2015).

394