CRISPR targeting of MEIOTIC-TOPOISOMERASE VIB-dCas9 to a recombination hotspot is insufficient to increase crossover frequency in Arabidopsis

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Abstract

During meiosis, homologous chromosomes pair and recombine, which can result in reciprocal crossovers that increase genetic diversity. Crossovers are unevenly distributed along eukaryote chromosomes and show repression in heterochromatin and the centromeres. Within the chromosome arms crossovers are often concentrated in hotspots, which are typically in the kilobase range. The uneven distribution of crossovers along chromosomes, together with their low number per meiosis, creates a limitation during crop breeding, where recombination can be beneficial. Therefore, targeting crossovers to specific genome locations has the potential to accelerate crop improvement. In plants, meiotic crossovers are initiated by DNA double strand breaks (DSBs) that are catalysed by SPO11 complexes, which consist of two catalytic (SPO11-1 and SPO11-2) and two non-catalytic subunits (MTOPIVIB). We used the model plant *Arabidopsis thaliana* to target a dCas9-MTOPIVIB fusion protein to the 3a crossover hotspot via CRISPR. We observed that this was insufficient to significantly change meiotic crossover frequency or pattern within 3a. We discuss the implications of our findings for targeting meiotic recombination within plant genomes.

1 Introduction

2
3 Meiosis is a specialized eukaryotic cell division where a single round of DNA replication and
4 two rounds of chromosome segregation result in haploid gametes required for sexual
5 reproduction (Mercier et al. 2015; Villeneuve and Hillers 2001). During prophase I of meiosis,
6 homologous chromosomes undergo programmed recombination, which can result in
7 reciprocal crossover (Mercier et al. 2015; Villeneuve and Hillers 2001). Crossovers contribute
8 to genetic variation in progeny and result in new haplotypes, which can allow combination of
9 useful traits in crop species (Taagen et al. 2020). However, recombination frequency and
10 pattern can significantly limit breeding, as crossovers are relatively low per meiosis (typically
11 1-2 per chromosome) and show a highly uneven distribution (Taagen et al. 2020; Mercier et
12 al. 2015). For example, crossovers in wheat, barley and maize occur predominantly in the sub-
13 telomeric regions (Darrier et al. 2017; Rodgers-Melnick et al. 2015; Higgins et al. 2012;
14 Mascher et al. 2017), which can cause linkage drag in low-recombination regions that are
15 under selection. Therefore, technology to increase global crossover numbers, or induce
16 recombination at loci of choice, have the potential to substantially accelerate crop breeding.

17
18 Crossovers are initiated by double-strand breaks (DSBs) catalysed by the conserved
19 transesterase SPO11 (Keeney et al. 1997; Bergerat et al. 1997). SPO11 is a homolog of the
20 archaeal topoisomerase VI catalytic A subunit that acts with non-catalytic B subunits in A₂B₂
21 heterodimers (Robert et al. 2016; Bouuaert and Keeney 2016). In Arabidopsis, two non-
22 redundant homologs of the topoisomerase VI A subunit, SPO11-1 and SPO11-2, are required
23 to generate meiotic DSBs (Stacey et al. 2006; Grelon et al. 2001; Hartung et al. 2007). The
24 meiotic topoisomerase VIB-like subunits, MTOPVIB, interact with both SPO11-1 and SPO11-
25 2 to catalyse meiotic DSBs in Arabidopsis and rice (Bouuaert and Keeney 2016; Vrielynck et
26 al. 2016; Fu et al. 2016). During catalysis, SPO11 becomes covalently bound to DNA and is
27 then removed bound to a short oligonucleotide, via endonuclease activities (Neale et al. 2005;
28 Choi et al. 2018). The resulting DSB 5'-end is then digested by exonucleases to produce 3'
29 overhanging single-strand DNA (ssDNA) at each end of the DSB (Hunter 2015). Meiotic
30 ssDNA associates with the recombinases RAD51 and DMC1 to promote ssDNA strand
31 invasion of a homologous chromosome or a sister chromatid (Hunter 2015). Invasion of
32 homologous DNA generates a displacement loop (D-loop), which allows extension of the 3'
33 ssDNA via DNA synthesis using the homologous DNA sequence as a template (Hunter 2015).

34
35 Following interhomolog or intersister strand invasion, alternative DNA repair pathways are
36 followed during meiosis (Hunter 2015). First, the D-loop may be disassociated from the
37 invaded template and returned to the parental chromosomes, where it is repaired as a non-
38 crossover (Hunter 2015). If DNA synthesis occurred over a polymorphic site following inter-
39 homolog strand invasion this may result in a gene conversion (Hunter 2015). In plants, non-
40 crossover repair is promoted via the activity of several non-redundant proteins that include the
41 FANCM, RECQ4A and RECQ4B helicases, FIGL1 and FLIP1 (Crismani et al. 2012; Girard et
42 al. 2015; Fernandes et al. 2018; Séguéla-Arnaud et al. 2015). Alternatively, capture of the
43 second resected 3' end, followed by DNA synthesis, can form a double Holliday junction joint
44 molecule (dHJ-JM) (Hunter 2015). The Class I pathway acts to stabilize dHJs and promotes
45 their resolution as a crossover (Börner et al. 2004; Chelysheva et al. 2012, 2007; Higgins et
46 al. 2008, 2004; Macaisne et al. 2011, 2008; Wijeratne et al. 2006; Manhart and Alani 2016;
47 Jackson et al. 2006). In Arabidopsis, an estimated ~150-250 DSBs mature into ~10 crossovers
48 per meiosis, with the remaining DSBs repaired as non-crossovers (Rowan et al. 2019; Ferdous
49 et al. 2012; Wijnker et al. 2013). This indicates that the anti-crossover pathways mediate repair
50 of the majority of meiotic DSBs as non-crossovers.

51
52 Chromosome structure, chromatin and epigenetic information also exert a significant influence
53 on meiotic recombination. At the fine-scale, meiotic DSBs and crossovers tend to cluster in
54 narrow (kilobase) regions called hotspots (Choi and Henderson 2015). In plants and budding
55 yeast, meiotic DSB hotspots frequently occur in nucleosome-depleted regions associated with

gene control regions (He et al. 2017; Pan et al. 2011; Choi et al. 2018). Furthermore, RNA-directed DNA methylation and elevated nucleosome occupancy are sufficient to suppress crossovers within an Arabidopsis recombination hotspot (Yelina et al. 2015). Meiotic DSB formation and repair occur in the context of proteinaceous chromosome axis, which underpins meiotic chromosome architecture (Zickler and Kleckner 1999). Sister chromatids are organized as linear arrays of chromatin loops connected to the axis (Zickler and Kleckner 1999). In plants, the chromosome axis includes the HORMA domain protein ASY1 (a homolog of yeast Hop1) and its interacting partners ASY3 and ASY4, which promote DMC1-mediated interhomolog synapsis and recombination (Armstrong et al. 2002; Sanchez-Moran et al. 2007; Chambon et al. 2018; Ferdous et al. 2012). The axis also includes cohesin complexes containing the meiosis-specific REC8 α -kleisin subunit, which coheres sister chromatids and anchors the chromatin loops to the axis (Cai et al. 2003; Chelysheva et al. 2005). As prophase I progresses, the chromosomes synapse and the synaptonemal complex is installed between them, coincident with crossover maturation (Zickler and Kleckner 1999; Hunter 2015).

Work in budding yeast has shown that tethering SPO11, or its interacting partners, using DNA binding domains is sufficient to create recombination hotspots *de novo* (Acquaviva et al. 2013; Pecina et al. 2002). In recent years, several technologies have emerged with the potential to tether factors of interest to specific loci. For example, translational fusions of SPO11 with zinc finger domains, TAL repeats and dCas9 have been used to target meiotic DSBs to loci of choice in budding yeast (Sarno et al. 2017). In this study, we targeted an MTOPVIB-dCas9 fusion protein to the previously characterized 3a crossover hotspot in *Arabidopsis thaliana*. The catalytically dead *Streptococcus pyogenes* Cas9 (dCas9) carries two amino acid substitutions (D10A and H841A) that abolish its endonuclease activity, but do not impair its ability to bind target DNA via guide RNAs (Qi et al. 2013). We used high-resolution crossover mapping to determine 3a recombination frequency and distribution with and without MTOPVIB-dCas9 targeting. We did not observe significant changes to crossover frequency or pattern with the 3a hotspot compared to wild type. This indicates that recruitment of the DSB machinery to Arabidopsis hotspots is insufficient to change crossover recombination.

Results

MTOPVIB-dCas9 functionally complements *mtopvib*

Meiotic DSBs catalysed by Arabidopsis SPO11-1, SPO11-2 and MTOPVIB are essential to initiate crossover formation (Fig. 1A) (Grelon et al. 2001; Vrielynck et al. 2016; Stacey et al. 2006; Hartung et al. 2007). We translationally fused *Streptococcus pyogenes* dCas9 to the C-terminus of Arabidopsis MTOPVIB and asked whether the fusion protein complements the function of wild type MTOPVIB (Fig. 1B-F). We expressed an MTOPVIB-dCas9 translational fusion gene under the control of the endogenous MTOPVIB promoter and terminator, in an *mtopvib-2* (hereafter, *mtopvib*) null mutant background (Vrielynck et al. 2016). Crossovers physically link homologous chromosomes during prophase I of meiosis ensuring balanced chromosome segregation. Therefore, an absence of meiotic DSBs and crossovers in *mtopvib* (or *spo11-1* and *spo11-2*) mutants leads to unbalanced, aneuploid gametes and almost complete sterility (Fig. 1B-1C) (Grelon et al. 2001; Vrielynck et al. 2016; Stacey et al. 2006; Hartung et al. 2007). For example, we observed an average of $\sim 1.9 \pm 1.7$ seeds per fruit (silique) in *mtopvib*, compared to $\sim 64.5 \pm 8.2$ in the wild type (Two-tailed t-test, $P < 0.00001$) (Fig. 1B-1C and Supplemental Table 1). In contrast, MTOPVIB-dCas9 *mtopvib* shows an average seed set of 66.1 ± 3.3 seeds per silique that was not significantly different from wild type (Two-tailed t-test, $P = 0.63$) (Fig. 1B-1C, Supplemental Table 1), indicating that the MTOPVIB-dCas9 fusion protein functionally complements *mtopvib*. To further confirm this, we used fluorescent crossover reporters to measure genetic distances (crossover frequency) in two intervals; CTL2.10, an interstitial region on chromosome 2, and CTL5.1, a sub-telomeric region on chromosome 5 (Wu et al. 2015), in MTOPVIB-dCas9 *mtopvib* and wild type (Figure 1D-1F and Supplemental Tables 2 and 3). We found that mean genetic distances in these

intervals were not significantly different between wild type and *MTOPVIB-dCas9 mtopvib* (Whitney-Mann tests, $P=0.54$ and 0.68 , respectively). This further demonstrates that the MTOPVIB-dCas9 fusion protein is functional and supports a normal level of crossover.

Targeting MTOPVIB-dCas9 to the 3a meiotic crossover hotspot

We chose to target MTOPVIB-dCas9 to the 3a crossover hotspot (Yelina et al. 2012, 2015; Choi et al. 2013), which is located in a sub-telomeric region of chromosome 3 (Fig. 2A, 2B and Supplemental Table 4). 3a is a 5.8 kb region with a genetic distance of ~ 0.2 cM (33.3 cM/Mb) in F_1 hybrids between Col-0 (hereafter, Col) and Ler-0 (hereafter, Ler) *Arabidopsis thaliana* accessions (Yelina et al. 2012, 2015; Choi et al. 2013). Crossover rates within 3a are up to ~ 17 -fold higher than the chromosome 3 average of 4.77 cM/Mb in male meiosis (Giraut et al. 2011). We chose to target the 3a hotspot first because data from budding yeast showed that tethering SPO11 to recombination hotspots leads to additional DSB formation (Sarno et al. 2017), whereas tethering to recombination 'cold' regions exhibited variable and less predictable stimulations (Robine et al. 2007; Ito et al. 2014; Panizza et al. 2011; Sarno et al. 2017; Pan et al. 2011). Second, 3a crossover levels are below their potential maximum in wild type, as we have previously shown a $\sim 40\%$ increase in 3a crossover frequency in *met1* mutants (Yelina et al. 2012). Third, we have an established 'pollen typing' assay that allows us to measure 3a crossover rates and fine-map crossover positions in this region (Yelina et al. 2012, 2015; Choi et al. 2013). We designed guide RNAs (gRNAs) to target three regions within 3a: (i) the At3g02880 promoter and 5' end (hereafter, 3a-P), (ii) the At3g02880 gene body (hereafter, 3a-B), and (iii) the intergenic region between At3g02880 and At3g02885 (hereafter, 3a-I) (Fig. 2A, 2B and Supplemental Tables 4-5). Notably these regions vary in nucleosome occupancy, which is a major determinant of meiotic DSB levels in *Arabidopsis* (Fig. 2A) (Choi et al. 2018).

Testing gRNA gene editing efficiency using catalytically active Cas9

We designed a total of 18 gRNAs within 3a, six targeting each of the three regions within 3a (3a-P, 3a-B and 3a-I), with the rationale that multiple gRNAs may increase the efficiency of targeting, compared to a single gRNA (Fig. 2A-2F and Supplemental Table 5) (Chavez et al. 2016; Sarno et al. 2017). To simultaneously express six gRNAs using one T-DNA construct, we used an approach successfully employed in *Arabidopsis*, rice and wheat, where multiple gRNAs are expressed as part of a tRNA-gRNA synthetic transcript (Xie et al. 2015; Hui et al. 2019; Wang et al. 2018). We designed and assembled six tandemly arranged pre-tRNA-gRNA modules differing only in the sequences of gRNA spacers (Fig. 2C). pre-tRNA-gRNA synthetic transcripts mimic native tRNA-snoRNA43 transcripts in plants, allowing RNase P and Z to cleave the tRNA structure and release mature gRNAs (Fig. 2C) (Phizicky and Hopper 2010; Xie et al. 2015). We tested the efficiencies of *in silico* designed gRNAs by co-expressing 6 $\times$ (pre-tRNA-gRNA) cassettes targeting 3a-P, 3a-B or 3a-I with catalytically active *S. pyogenes* Cas9 in wild type Col (Fig. 2D-2F) (Schiml et al. 2016). We transformed *Cas9-gRNA-P*, *Cas9-gRNA-B* and *Cas9-gRNA-I* constructs into *Arabidopsis* and analysed gene editing events within 3a in T_1 progeny. T_1 individuals are usually chimeric due to somatic gene editing events (Hui et al. 2019). Using PCR amplification across the gRNA target sites, we observed deletions in the respective target regions in 8.9, 12.1 and 25.8% of T_1 progeny of *Cas9-gRNA-P*, *Cas9-gRNA-B* and *Cas9-gRNA-I*-transformed plants (Fig. 2D-2F and Supplemental Table 6). Sanger sequencing of these PCR products confirmed deletions associated with 17 of the 18 tested gRNAs (Fig. 2D-2F and Supplemental Figures S1, S2, and Supplemental Table 6).

In addition, we generated a synthetic 6 $\times$ (pre-tRNA-gRNA) construct to express previously reported gRNAs targeting 6 *Arabidopsis* genes (At1g69320, At1g26600, At2g27250, At3g27920, At4g25530 and At5g35620) outside 3a to use as a negative control (Yamaguchi et al. 2017; Hahn et al. 2017; Pyott et al. 2016; Gallego-Bartolomé et al. 2018). We refer to this construct as *Cas9-non-3a-gRNA*. We transformed *Cas9-non-3a-gRNA* into wild type Col

and observed gene editing events in the target genes in ~4-50% of the T₁ progeny (Supplemental Figure S3 and Supplemental Table 7). In summary, we obtained a set of gRNAs robustly targeting the Arabidopsis genome within and outside the 3a crossover hotspot.

Analysis of 3a crossovers in the presence of MTOPVIB-dCas9 and gRNAs

We next asked whether combining *MTOPVIB-dCas9* and gRNAs that target *3a-P*, *3a-B* or *3a-I* would affect 3a crossover rates or distribution. Crossover detection at 3a hotspot relies on the segregation of DNA sequence polymorphisms through meiosis (Yelina et al. 2012, 2015; Choi et al. 2013). As *MTOPVIB-dCas9 mtopvib* lines were in the Col background, we generated transgenic lines expressing gRNAs in a different Arabidopsis accession, Ws-4 (hereafter, Ws), that was also heterozygous for a *mtopvib* mutation (*mtopvib-1*) (Vrielynck et al. 2016). The resulting lines, each of which carried a 6×(pre-tRNA-gRNA) transgene targeting *3a-P*, *3a-B* or *3a-I*, or six Arabidopsis genes outside 3a, were called *gRNA-P*, *gRNA-B*, *gRNA-I* or *non-3a gRNA*. Ws had a single nucleotide polymorphism (SNP) in position -3 relative to PAM in a target site of one of the *gRNA-B*-specific gRNAs, the remaining 17 3a-specific gRNAs we used targeted regions without any polymorphisms between Col and Ws. We crossed *MTOPVIB-dCas9 mtopvib* in the Col background to *gRNA-P*, *gRNA-B* and *gRNA-I* lines in the Ws background. We then identified F₁ progeny that were *mtopvib* null mutants and that expressed both the *MTOPVIB-dCas9* and gRNA transgenes (Fig. 3A, Supplemental Figure S4). We also crossed Col *MTOPVIB-dCas9 mtopvib* to Ws *MTOPVIB/mtopvib* to generate a 'no gRNA' F₁ population as a negative control.

Given the 3a crossover rate of ~0.2 cM, to characterise ~100 crossover events, it is necessary to assay ~50,000 meioses. To achieve this we employed 'pollen typing', which is a PCR-based assay used to amplify and quantify crossover and parental molecules from pollen DNA (Fig. 3B) (Drouaud and Mézard 2011; Choi et al. 2017). To perform pollen typing, we first extract genomic DNA from F₁ pollen. The pollen DNA contains 3a parental and crossover molecules distinguishable by DNA sequence polymorphisms between the accessions (Col and Ws) (Fig. 3B). We perform two rounds of allele-specific PCR, using primers that anneal to polymorphic sites, to specifically amplify crossover or parental molecules (Fig. 3B). For quantification, we use titration where pollen template DNA is diluted until approximately half of PCR amplification reactions are negative (Drouaud and Mézard 2011; Choi et al. 2017). We also Sanger sequenced the amplified crossover molecules to map internal crossover locations within the 3a hotspot (Drouaud and Mézard 2011; Choi et al. 2017).

We employed pollen typing to measure 3a crossover frequency (genetic distance) and observed ~0.13-0.15 cM in Col/Ws F₁s in the absence of gRNAs (Fig. 3C and Supplemental Table 8). We observed no significant crossover rate changes in F₁ populations expressing *gRNA-B*, *gRNA-I* or *gRNA-P* (0.189 cM, chi-square test, *P*=0.44, 0.175 cM, *P*=0.64 and 0.152 cM, *P*=0.96, respectively), compared to negative controls (0.155 cM and 0.131 cM) (Fig. 3C and Supplemental Table 8). We Sanger sequenced between 77 and 90 crossover molecules for each F₁ population and found that crossover profiles were very similar in the presence or absence of gRNAs targeting 3a (Fig. 3D-3F and Supplemental Table 9). In all cases we observed lower crossover frequencies at the telomere-proximal end and higher crossover frequencies towards the centromere-proximal end of 3a (Fig. 3D-3F and Supplemental Table 9). These data indicate that targeting MTOPVIB-dCas9 to 3a does not have a strong effect on crossover rate or distribution.

In Arabidopsis, a minority of meiotic DSBs (~5-10%) are repaired as crossovers (Choi et al. 2018; Serrentino and Borde 2012; Copenhaver et al. 1998; Chelysheva et al. 2012, 2007; Giraut et al. 2011; Salomé et al. 2012). Non-crossovers are an alternative outcome of meiotic DSB repair and, therefore, we asked whether targeting MTOPVIB-dCas9 to 3a could result in increased non-crossovers, measured via gene conversion. To detect gene conversion we

used four F₂ populations, which were the progeny of Col/Ws F₁ expressing either *gRNA-P*, *gRNA-B* or *gRNA-I*, or 'no gRNA' as a negative control (Figure 4A). We employed a Kompetitive Allele-Specific PCR (KASP) assay to distinguish between SNP alleles (Figure 4B). We designed 12 KASP assays to distinguish between Col and Ws alleles within, as well as up to 5.2 kb up- and 4.5 kb downstream of 3a. Physical distances between the markers used for KASP assays ranged from 0.7 to 2.0 kb, with an average of 1.3 kb (Supplemental Table 10). Initially, we used ~83-96 F₂ individuals for each of the four F₂ populations and detected 2 gene conversion events in the F₂ population expressing *gRNA-P* and none in the other three populations, including the 'no gRNA' negative control. Next we increased *gRNA-P* and 'no gRNA' F₂ population sizes to the total of ~470 individuals each but did not detect any additional gene conversion events (Fig. 4C-4E and Supplemental Table 11). Therefore, we did not observe any gene conversion events in *gRNA-B*, *gRNA-I* or the negative control, but observed two gene conversions out of 469 F₂ individuals in *gRNA-P*. Next we performed a combination of Sanger sequencing and KASP assays at additional SNPs to confirm our initial results and to determine gene conversion tract lengths. We found that one of the gene conversion events, which had a Ws to Col to Ws genotype, occurred in a low polymorphism region and its tract length could vary from a minimum of 1 to a maximum of 1,763 bp. The other gene conversion event, which had a Col to Ws to Col genotype, occurred in a region more densely covered with polymorphisms. Its conversion tract could vary from a minimum of 729 to a maximum of 1,503 bp (Fig. 4E, Supplemental Table 12). Interestingly, both gene conversion events occurred 1.3-3 kb downstream of *gRNA-B* at adjacent, non-overlapping polymorphisms (Fig. 4E and Supplemental Tables 11-12).

Discussion

In this study we targeted Arabidopsis MTOPVIB, which is essential for initiation of meiotic DSBs, to the crossover hotspot 3a via CRISPR. We confirmed that the MTOPVIB-dCas9 translational fusion functionally complements the *mtopvib* mutant. We also confirmed the functionality of the gRNAs we used via catalytically active Cas9 mutagenesis at target loci. The 3a crossover hotspot is a 5.8 kb sub-telomeric interval with recombination up to ~20 times higher than the chromosome average (Yelina et al. 2012, 2015; Choi et al. 2013). We chose 3a hotspot as a target firstly because 3a crossover rates are amenable to manipulation. For example, we have previously shown that recruitment of heterochromatic features, including DNA methylation and H3K9me2, reduces 3a crossover rates by ~2-3 fold (Yelina et al. 2015). 3a crossover rates are also not at their maximum level in wild type, as genome-wide loss of DNA methylation results in a ~40% increase in 3a crossover frequency (Yelina et al. 2012). Secondly, because studies in budding yeast have shown that tethering SPO11 to recombination hot spots leads to a more robust DSB induction compared to targeting SPO11 to DSB cold spots (Sarno et al. 2017; Ito et al. 2014). Mapping of SPO11-1-oligonucleotides in Arabidopsis has revealed that they accumulate at higher levels in nucleosome-free regions (Choi et al. 2018). Therefore, we chose to target MTOPVIB-dCas9 to three locations within 3a that vary in the nucleosome occupancy levels. We observe very modest and statistically insignificant increases to crossover frequencies and a very similar crossover topology within 3a in the presence of *gRNA-P*, *gRNA-B* or *gRNA-I* compared to the negative controls.

To explain these results, it is important to note that although DSBs and crossovers correlate positively at the chromosome-scale, there are also regions where the relationship is less strong (He et al. 2017; Choi et al. 2018). Meiotic DSB repair in Arabidopsis is a multi-step process with only ~5-10% of DSBs typically maturing into crossovers (Choi et al. 2018; Serrentino and Borde 2012; Copenhaver et al. 1998; Chelysheva et al. 2012, 2007; Giraut et al. 2011; Salomé et al. 2012). This is in stark contrast to budding yeast where over a half of meiotic DSBs are repaired as crossovers (Pan et al. 2011; Mancera et al. 2008). This could explain why tethering of SPO11 to DSB hotspots in yeast robustly increases recombination (Sarno et al. 2017). Another explanation for our results is that although MTOPVIB is targeted to the 3a locus, any additional DSBs are repaired via non-crossover pathways. Counter to this,

we also did not measure a significant increase in gene conversions in MTOPVIB-Cas9. Specifically, we observed two gene conversion events at a frequency of ~0.21% per SNP each following targeting of MTOPVIB-dCas9 by *gRNA-B* only. Both gene conversion events occurred 1.3-3 kb downstream of the *gRNA-B* target site and did not overlap with each other or *gRNA-B*. The gene conversion frequency we observed is similar to the previously reported Arabidopsis gene conversion frequencies of 0.017-0.55% per SNP at a meiotic crossover hotspot (Drouaud et al. 2013). However, it is important to note that non-crossovers are only detectable when they lead to gene conversions. In Arabidopsis, detectable gene conversion rates are extremely low, with an average of 1.7 per meiosis and are around 100-150 base pairs in length (Lu et al. 2012; Wijnker et al. 2013). The 3a SNPs measured for gene conversion are spaced 0.7 to 2 kilobase apart. Hence, it is possible that many gene conversions that occur within these intervals would not be detectable. Alternatively, the lack of increased gene conversion frequency upon targeting MTOPVIB-dCas9 may imply that meiotic DSB repair occurs using the sister chromatid as a template (Cifuentes et al. 2013; Yao et al. 2020).

In conclusion, we show that targeting MTOPVIB to an Arabidopsis meiotic recombination hotspot via CRISPR/dCas9 leads to no significant changes in 3a crossover frequency or pattern. This highlights the complexity of plant meiotic recombination control. We propose that combined recruitment of crossover designation factors and modulation of DSB repair pathways to favour crossovers could be alternative strategies to boost plant crossovers in specific genome locations.

Materials and methods

Plant material and genotyping

Arabidopsis lines used in this study were Col-0, *mtopvib-1* (EDA42 line, Ws-4 accession), *mtopvib-2* (GABI_314G09, Col-0 accession) (Vrielynck et al. 2016), *CTL 2.10* and *CTL 5.1* (Wu et al. 2015), which were obtained from the Eurasian Arabidopsis Stock Centre (uNASC) and Arabidopsis Biological Resource Centre (ABRC). Plants were grown under long day conditions (16 h light/8 h dark) at 20 °C, as previously described (Yelina et al. 2015). Plant transformation was performed by floral dipping (Zhang et al. 2006). PCR genotyping of *mtopvib-1* and *mtopvib-2* was performed as described (Vrielynck et al. 2016). PCR genotyping of *mtopvib-2* complemented with *MTOPVIB-dCas9* transgenes was performed with MTOP-genot-compl-F and MTOP-genot-compl-R oligonucleotides. Oligonucleotides are listed in Supplemental Table 13.

In silico gRNA design and *in vitro* testing

gRNAs were *in silico* designed using E-CRISP (Heigwer et al. 2014) (<http://www.e-crisp.org/E-CRISP>), CRISPR-P (Lei et al. 2014) (<http://crispr.hzau.edu.cn/CRISPR2>) and CRISPR-MIT (crispr.mit.edu, now obsolete) online tools. gRNAs spacer sequences and Arabidopsis genome target coordinates are listed in Supplemental Table 5. gRNA efficiencies of *in silico* designed gRNAs were tested in an *in vitro* CRISPR/Cas9 assay. Briefly, DNA fragments corresponding to *3a-P*, *3a-B* and *3a-I* and harbouring gRNA target sites were PCR-amplified using Arabidopsis genomic DNA and oligonucleotides listed in Supplemental Table 13. gRNAs were obtained by *in vitro* transcription using MEGAscript T7 Transcription Kit (ThermoFisher Scientific). DNA templates for *in vitro* transcription were PCR-amplified using pEn-Chimera vector and oligonucleotides listed in Supplemental Table 13. 300 ng of gRNA transcript was bound to a purified Cas9 protein (New England Biolabs) for 10 minutes at 25° C, followed by the addition of 300 ng of target DNA and incubation at 37 °C for 1 hour. gRNA transcripts were then cleaved by 0.3 µg/µl RNase A for 5 minutes at 37 °C. DNA fragments were separated on a 1.5% agarose gel stained with Midori Green Advance DNA Stain (Geneflow) to visualize the presence or absence of CRISPR/Cas9-induced target DNA cleavage. gRNAs that led to target

DNA cleavage in *in vitro* assays were used to generate constructs for Arabidopsis transformation.

Cloning

To generate *MTOPVIB-dCas9*, a full genomic sequence of *MTOPVIB* (At1g60460) including a 2385 bp region upstream of the ATG start codon and a 294 bp region downstream of the TAG stop codon was PCR amplified with oligonucleotides MTOPVI-Prom-Sall-F and MTOPVI-Term-NotI-R and cloned between *Sall* and *NotI* restriction endonuclease sites into pGreen0029 vector (Addgene), to yield the pGreen-gMTOPVIB construct. A *XbaI* restriction endonuclease site in the 7th intron of *MTOPVIB* was mutagenized by digesting pGreen-gMTOPVIB with *XbaI* restriction enzyme, end-filling the resulting 5' overhang using Klenow fragment and religating to yield pGreen-gMTOPVIBΔ*XbaI*. An *Ascl* restriction site was introduced in front of the ATG start codon by amplifying a part of the *MTOPVIB* promoter region with MTOPVI-*NheI*-F and MTOPVI-*Ascl*-R oligonucleotides and cloning the resulting fragment into *NheI*- and *NcoI*-digested pGreen-*Ascl*-gMTOPVIBΔ*XbaI*. A GGSGGS linker, a nuclear localisation signal, two hemagglutinin (2×HA) epitope tags and *XbaI* and *BamHI* restriction sites were introduced at the C-terminus of *MTOPVIB* upstream of the TAG stop codon by cloning a double-strand DNA fragment resulting from annealing MTOPVIB-C-HA-top and MTOPVIB-C-HA-bottom oligonucleotides into a *PstI*-digested pGreen-*Ascl*-gMTOPVIBΔ*XbaI*. The resulting construct was called pGreen-gMTOPVIB-C-NLS-2×HA.

Catalytically inactive Cas9 (dCas9) was generated via PCR-site-directed mutagenesis. Briefly, Cas9 coding sequence was amplified from hSpCas9 plasmid, kindly provided by Prof Jian-Kang Zhu (Feng et al. 2013), in a multiplex PCR reaction using Cas9-1stMut-F, dCas9-1stMut-R, dCas9-2ndMut-F, dCas9-2ndMut-R primers and a Phusion DNA polymerase. Following PCR amplification methylated template plasmid DNA carrying wild type Cas9 was digested with *DpnI* restriction endonuclease, PCR products carrying mutated dCas9 were ligated and transformed into *E.coli* DH5α strain. Mutations leading to D10A and H840A amino acid substitutions in the Cas9 coding sequence were confirmed by Sanger sequencing. Next, dCas9 was PCR amplified with dCas9-*XbaI*-F and dCas9-*BamHI*-R oligonucleotides and cloned into *XbaI*- and *BamHI*- digested pGreen-gMTOPVIB-C-NLS-2HA to yield MTOPVIB-dCas9. Oligonucleotide sequences are provided in Supplemental Table 13.

To generate Cas9-gRNA-P, Cas9-gRNA-B, Cas9-gRNA-I and Cas9-non-3a-gRNA constructs, 6×(pre-tRNA-gRNA) PCR products were amplified using oligonucleotides listed in the Supplemental Table 13 and as described (Xie et al. 2015), digested with *FokI* restriction endonuclease and cloned into *BbsI*-digested pEn-Chimera vector (kindly provided by Prof Holger Puchta) behind the Arabidopsis U6 (*AtU6*) promoter. Fragments containing *AtU6*:6×(pre-tRNA-gRNA) were transferred from pEn-Chimera into binary pDe-CAS9 vector (kindly provided by Prof Holger Puchta) as described (Schiml et al. 2016).

To generate gRNA-P, gRNA-B, gRNA-I and non-3a-gRNA constructs, 6×(pre-tRNA-gRNA) fragments were PCR amplified as described above, digested with *FokI* restriction endonuclease and cloned into *BbsI*-digested pChimera vector, kindly provided by Prof Holger Puchta, behind *AtU6* promoter. Fragments containing *AtU6*:6×(pre-tRNA-gRNA) were excised from the resulting vectors with *AvrII* restriction endonuclease and cloned into a *XbaI*-digested binary vector pGreen0229.

Detection of CRISPR/Cas9-induced mutations

3a-P, *3a-B* and *3a-I* genetic intervals were PCR amplified from Arabidopsis T₁ genomic DNA or wild type Col using oligonucleotides listed in Supplemental Table 13. The resulting PCR products were separated on a 1% agarose gel and stained with Midori Green Advance DNA Stain (Geneflow) to visualize full-length and deletion products. The latter were excised and

1 extracted from an agarose gel and subject to Sanger sequencing. Deletion products that could
2 not be resolved by agarose gels were cloned into pGem-T-easy vector (Promega) following
3 the manufacturer's protocol and individual clones were subject to Sanger sequencing.
4 CRISPR/Cas9-induced mutations in *CLE10*, *CLV3* and *GL1* destroyed *Bsu36I*, *BspHI* and
5 *DdeI* restriction endonuclease sites, respectively. To detect CRISPR/Cas9-induced mutations
6 in these genes, DNA fragments harbouring gRNA target sequences were PCR-amplified and
7 digested with the above restriction endonucleases. The resulting products were separated on
8 1% agarose gels and stained with Midori Green Advance DNA Stain (Geneflow). T7
9 endonuclease I (New England Biolabs) assays were used to detect CRISPR/Cas9-induced
10 mutations in *CLE9*, *FWA* and *eIF(iso)4E* as described (Pyott et al. 2016).

11 **Seed fluorescent measurement of crossovers**

12 Seed fluorescent measurements of crossovers in *CTL 2.10* and *CTL 5.1* intervals was
13 performed as described (Yelina et al. 2015), using CellProfiler (Carpenter et al. 2006).

14 **Pollen typing**

15 Pollen typing for 3a crossover hotspot was performed as previously described (Yelina et al.
16 2015).

17 **RT-PCR detection of gRNA transcripts**

18 RNA was extracted from closed buds of two independent pools of F₁ individuals used for
19 'pollen typing' using PureZOL™ RNA Isolation Reagent (Bio-Rad) according to the
20 manufacturer's protocol. 10 µg of total RNA was treated with TURBO DNase (ThermoFisher
21 Scientific) and reverse-transcribed in the presence or absence (negative control) of
22 SuperScript IV enzyme (ThermoFisher) using random hexamer primers, according to the
23 manufacturers' protocols. A 1:20 dilution of the resulting cDNA was PCR-amplified using
24 oligonucleotides listed in Supplemental table 13. The resulting products were resolved on a
25 2% agarose gel stained with Midori Green Advance DNA Stain (Geneflow).

26 **Kompetitive Allele-Specific PCR (KASP) Assay**

27 Arabidopsis genomic DNA was extracted as described (Edwards et al. 1991). Kompetitive
28 Allele-Specific PCR (KASP) Assay was performed following the manufacturer's protocol using
29 KASP master mix (BioResearch Technologies) and oligonucleotides are listed in Supplemental
30 table 13. Reactions were run on a CFX real-time PCR system (Bio-Rad), allele discrimination
31 was performed using the manufacturer's software.

32 **Data availability**

33 All plasmids, reagents and Arabidopsis transgenic lines generated in this study are available
34 upon request. Supplemental Tables S1-S13 contain raw data used for fertility and crossover
35 frequency scoring, as well as genomic positions of 3a crossover hotspot, guide RNAs, SNPs
36 and oligonucleotides used in this study. Supplemental figures S1-S3 contain CRISPR/Cas9
37 gene editing analysis and supplemental figure S4 contains confirmation of gRNA expression.

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References

- Acquaviva L, Székvölgyi L, Dichtl BSB, Dichtl BSB, de La Roche Saint André C, Nicolas A, Géli V, de La Roche Saint Andre C, Nicolas A, Geli V, et al. 2013. The COMPASS Subunit Spp1 Links Histone Methylation to Initiation of Meiotic Recombination. *Science* **339**: 215–218.
- Armstrong SJ, Caryl AP, Jones GH, Franklin FCH. 2002. Asy1, a protein required for meiotic chromosome synapsis, localizes to axis-associated chromatin in Arabidopsis and Brassica. *J Cell Sci* **115**: 3645–55.
- Bergerat A, de Massy B, Gadelle D, Varoutas PC, Nicolas A, Forterre P. 1997. An atypical topoisomerase II from Archaea with implications for meiotic recombination. *Nature* **386**: 414–417.
- Börner GV, Kleckner N, Hunter N. 2004. Crossover/noncrossover differentiation, synaptonemal complex formation, and regulatory surveillance at the leptotene/zygotene transition of meiosis. *Cell* **117**: 29–45.
- Bouuaert CC, Keeney S. 2016. Breaking DNA. *Science* **351**: 916–917.
- Cai X, Dong F, Edelmann RE, Makaroff CA. 2003. The Arabidopsis SYN1 cohesin protein is required for sister chromatid arm cohesion and homologous chromosome pairing. *J Cell Sci* **116**: 2999–3007.
- Carpenter AE, Jones TR, Lamprecht MR, Clarke C, Kang IH, Friman O, Guertin DA, Chang JH, Lindquist RA, Moffat J, et al. 2006. CellProfiler: Image analysis software for identifying and quantifying cell phenotypes. *Genome Biol* **7**: R100.
- Chambon A, West A, Vezon D, Horlow C, De Muyt A, Chelysheva L, Ronceret A, Darbyshire AR, Osman K, Heckmann S, et al. 2018. Identification of ASYNAPTIC4, a component of the meiotic chromosome axis. *Plant Physiol* **178**: 233–246.
- Chavez A, Tuttle M, Pruitt BW, Ewen-campen B, Ter-ovanesyan D, Haque SJ, Cecchi RJ, Kowal EJK, Buchthal J, Housden BE, et al. 2016. Comparative Analysis of Cas9 Activators Across Multiple Species. *Nat Methods* **13**: 563–567.
- Chelysheva L, Diallo S, Vezon D, Gendrot G, Vrielynck N, Belcram K, Rocques N, Márquez-Lema A, Bhatt AM, Horlow C, et al. 2005. AtREC8 and AtSCC3 are essential to the monopolar orientation of the kinetochores during meiosis. *J Cell Sci* **118**: 4621–4632.
- Chelysheva L, Gendrot G, Vezon D, Doutriaux MP, Mercier R, Grelon M. 2007. Zip4/Spo22 is required for class I CO formation but not for synapsis completion in Arabidopsis thaliana. *PLoS Genet* **3**: 802–813.
- Chelysheva L, Vezon D, Chambon A, Gendrot G, Pereira L, Lemhemdi A, Vrielynck N, Le Guin S, Novatchkova M, Grelon M. 2012. The Arabidopsis HEI10 is a new ZMM protein related to Zip3. *PLoS Genet* **8**: e1002799.
- Choi K, Henderson IR. 2015. Meiotic recombination hotspots - A comparative view. *Plant J* **83**: 52–61.
- Choi K, Yelina NE, Serra H, Henderson IR. 2017. Quantification and Sequencing of Crossover Recombinant Molecules from Arabidopsis Pollen DNA. *Methods Mol Biol* **1551**: 23–57.
- Choi K, Zhao X, Kelly K a, Venn O, Higgins JD, Yelina NE, Hardcastle TJ, Ziolkowski P a, Copenhaver GP, Franklin FCH, et al. 2013. Arabidopsis meiotic crossover hot spots overlap with H2A.Z nucleosomes at gene promoters. *Nat Genet* **45**: 1327–1338.
- Choi K, Zhao X, Tock AJ, Lambing C, Underwood CJ, Hardcastle TJ, Serra H, Kim J, Cho HS, Kim J, et al. 2018. Nucleosomes and DNA methylation shape meiotic DSB frequency in Arabidopsis thaliana transposons and gene regulatory regions. *Genome Res* **28**: 532–546.
- Cifuentes M, Rivard M, Pereira L, Chelysheva L, Mercier R. 2013. Haploid Meiosis in Arabidopsis: Double-Strand Breaks Are Formed and Repaired but Without Synapsis and Crossovers. *PLoS One* **8**: 1–12.
- Copenhaver GP, Browne WE, Preuss D. 1998. Assaying genome-wide recombination and centromere functions with Arabidopsis tetrads. *PNAS* **95**: 247–252.

- Crismani W, Girard C, Froger N, Pradillo M, Santos JL, Chelysheva L, Copenhaver GP, Horlow C, Mercier R. 2012. FANCM limits meiotic crossovers. *Science* **336**: 1588–1590.
- Darrier B, Rimbert H, Balfourier F, Pingault L, Josselin AA, Servin B, Navarro J, Choulet F, Paux E, Sourdille P. 2017. High-resolution mapping of crossover events in the hexaploid wheat genome suggests a universal recombination mechanism. *Genetics* **206**: 1373–1388.
- Drouaud J, Khademian H, Giraut L, Zanni V, Bellalou S, Henderson IR, Falque M, Mezard C. 2013. Contrasted Patterns of Crossover and Non-crossover at Arabidopsis thaliana Meiotic Recombination Hotspots. *PLoS Genet* **9**: e1003922.
- Drouaud J, Mézard C. 2011. Chapter 14 Characterization of Meiotic Crossovers in Pollen from Arabidopsis thaliana. *Methods Mol Biol* **745**: 223–249.
- Edwards K, Johnstone C, Thompson C. 1991. A simple and rapid method for preparation of rhizopus oryzae genomic DNA for PCR analysis. *Nucleic Acids Res* **19**: 1349.
- Feng Z, Zhang B, Ding W, Lui X, Yang D, Wei P, Cao F, Zhu S, Zhang F, Mao Y, et al. 2013. Efficient genome editing in plants using a CRISPR/Cas system. *Cell Res* **23**: 1229–1232.
- Ferdous M, Higgins JD, Osman K, Lambing C, Roitinger E, Mechtler K, Armstrong SJ, Perry R, Pradillo M, Cuñado N, et al. 2012. Inter-homolog crossing-over and synapsis in Arabidopsis meiosis are dependent on the chromosome axis protein AtASY3. *PLoS Genet* **8**: e1002507.
- Fernandes JB, Duhamel M, Seguéra-Arnaud M, Froger N, Girard C, Choinard S, Solier V, De Winne N, De Jaeger G, Gevaert K, et al. 2018. FIGL1 and its novel partner FLIP form a conserved complex that regulates homologous recombination. *PLoS Genet* **14**: e1007317.
- Fu M, Wang C, Xue F, Higgins J, Chen M, Zhang D, Liang W. 2016. The DNA Topoisomerase VI-B Subunit OsMTOPVIB Is Essential for Meiotic Recombination Initiation in Rice. *Mol Plant* **9**: 1539–1541.
- Gallego-Bartolomé J, Gardiner J, Liu W, Papikian A, Ghoshal B, Kuo HY, Zhao JM-C, Segal DJ, Jacobsen SE. 2018. Targeted DNA demethylation of the Arabidopsis genome using the human TET1 catalytic domain. *PNAS* **115**: E2125–E2134.
- Girard C, Chelysheva L, Choinard S, Froger N, Macaisne N, Lehmemdi A, Mazel J, Crismani W, Mercier R. 2015. AAA-ATPase FIDGETIN-LIKE 1 and Helicase FANCM Antagonize Meiotic Crossovers by Distinct Mechanisms. *PLoS Genet* **11**: 1–22.
- Giraut L, Falque M, Drouaud J, Pereira L, Martin OC, Mézard C. 2011. Genome-wide crossover distribution in Arabidopsis thaliana meiosis reveals sex-specific patterns along chromosomes. *PLoS Genet* **7**: e1002354.
- Grelon M, Vezon D, Gendrot G, Pelletier G. 2001. AtSPO11-1 is necessary for efficient meiotic recombination in plants. *EMBO J* **20**: 589–600.
- Hahn F, Mantegazza O, Greiner A, Hegemann P, Eisenhut M, Weber APM. 2017. An Efficient Visual Screen for CRISPR / Cas9 Activity in Arabidopsis thaliana. *Front Plant Sci* **8**: 39.
- Hartung F, Wurz-Wildersinn R, Fuchs J, Schubert I, Suer S, Puchta H. 2007. The Catalytically Active Tyrosine Residues of Both SPO11-1 and SPO11-2 Are Required for Meiotic Double-Strand Break Induction in Arabidopsis. *Plant Cell* **19**: 3090–3099.
- He Y, Wang M, Dukowic-Schulze S, Zhou A, Tiang C-L, Shilo S, Sidhu GK, Eichten S, Bradbury P, Springer NM, et al. 2017. Genomic features shaping the landscape of meiotic double-strand-break hotspots in maize. *PNAS* **114**: 12231–12236.
- Heigwer F, Kerr G, Boutros M. 2014. E-CRISP: Fast CRISPR target site identification. *Nat Methods* **11**: 122–123.
- Higgins JD, Armstrong SJ, Franklin FCH, Jones GH. 2004. The Arabidopsis MutS homolog AtMSH4 functions at an early step in recombination: Evidence for two classes of recombination in Arabidopsis. *Genes Dev* **18**: 2557–2570.
- Higgins JD, Perry RM, Barakate A, Ramsay L, Waugh R, Halpin C, Armstrong SJ, Franklin FCH. 2012. Spatiotemporal Asymmetry of the Meiotic Program Underlies the Predominantly Distal Distribution of Meiotic Crossovers in Barley. *Plant Cell* **24**: 4096–4109.
- Higgins JD, Vignard J, Mercier R, Pugh AG, Franklin FCH, Jones GH. 2008. AtMSH5 partners

AtMSH4 in the class I meiotic crossover pathway in *Arabidopsis thaliana*, but is not required for synapsis. *Plant J* **55**: 28–39.

Hui L, Zhao M, He J, Hu Y, Huo Y, Hao H, Hao Y, Zhu W, Xu M, Fu A. 2019. A simple and reliable method for creating PCR - detectable mutants in *Arabidopsis* with the polycistronic tRNA – gRNA CRISPR / Cas9 system. *Acta Physiol Plant* **41**: 170.

Hunter N. 2015. Meiotic Recombination: The Essence of Heredity. *Cold Spring Harb Perspect Biol* **7**: a016618.

Ito M, Kugou K, Fawcett JA, Mura S, Ikeda S, Innan H, Ohta K. 2014. Meiotic recombination cold spots in chromosomal cohesion sites. *Genes to Cells* **19**: 359–373.

Jackson N, Sanchez-Moran E, Buckling E, Armstrong SJ, Jones GH, Franklin FCH. 2006. Reduced meiotic crossovers and delayed prophase I progression in AtMLH3-deficient *Arabidopsis*. *EMBO J* **25**: 1315–1323.

Keeney S, Giroux CN, Kleckner N. 1997. Meiosis-specific DNA double-strand breaks are catalyzed by Spo11, a member of a widely conserved protein family. *Cell* **88**: 375–84.

Lei Y, Lu L, Liu HY, Li S, Xing F, Chen LL. 2014. CRISPR-P: A web tool for synthetic single-guide RNA design of CRISPR-system in plants. *Mol Plant* **7**: 1494–1496.

Lu P, Han X, Qi J, Yang J, Wijeratne AJ, Li T, Ma H. 2012. Analysis of *Arabidopsis* genome-wide variations before and after meiosis and meiotic recombination by resequencing *Landsberg erecta* and all four products of a single meiosis. *Genome Res* **22**: 508–518.

Macaisne N, Novatchkova M, Peirera L, Vezon D, Jolivet S, Froger N, Chelysheva L, Grelon M, Mercier R. 2008. SHOC1, an XPF Endonuclease-Related Protein, Is Essential for the Formation of Class I Meiotic Crossovers. *Curr Biol* **18**: 1432–1437.

Macaisne N, Vignard J, Mercier R. 2011. SHOC1 and PTD form an XPF-ERCC1-like complex that is required for formation of class I crossovers. *J Cell Sci* **124**: 2687–2691.

Mancera E, Bourgon R, Brozzi A, Huber W, Steinmetz M. 2008. High-resolution mapping of meiotic crossovers and noncrossovers in yeast. *Nature* **454**: 479–485.

Manhart CM, Alani E. 2016. Roles for mismatch repair family proteins in promoting meiotic crossing over. *DNA Repair* **38**: 84–93.

Mascher M, Gundlach H, Himmelbach A, Beier S, Twardziok SO, Wicker T, Radchuk V, Dockter C, Hedley PE, Russell J, et al. 2017. A chromosome conformation capture ordered sequence of the barley genome. *Nature* **544**: 427–433.

Mercier R, Mezard C, Jenczewski E, Macaisne N, Grelon M. 2015. The Molecular Biology of Meiosis in Plants. *Annu Rev Plant Biol* **66**: 297–327.

Neale MJ, Pan J, Keeney S. 2005. Endonucleolytic processing of covalent protein-linked DNA double-strand breaks. *Nature* **436**: 1053–1057.

Pan J, Sasaki M, Kniewel R, Murakami H, Blitzblau HG, Tischfield SE, Zhu X, Neale MJ, Jasin M, Socci ND, et al. 2011. A hierarchical combination of factors shapes the genome-wide topography of yeast meiotic recombination initiation. *Cell* **144**: 719–731.

Panizza S, Mendoza MA, Berlinger M, Huang L, Nicolas A, Shirahige K. 2011. Spo11-Accessory Proteins Link Double-Strand Break Sites to the Chromosome Axis in Early Meiotic Recombination. *Cell* **146**: 372–383.

Pecina A, Smith KN, Mézard C, Murakami H, Ohta K, Nicolas A. 2002. Targeted stimulation of meiotic recombination. *Cell* **111**: 173–184.

Phizicky EM, Hopper AK. 2010. tRNA biology charges to the front. *Genes Dev* **24**: 1832–1860.

Pyott DE, Sheehan E, Molnar A. 2016. Engineering of CRISPR/Cas9-mediated potyvirus resistance in transgene-free *Arabidopsis* plants. *Mol Plant Pathol* **17**: 1276–1288.

Qi LS, Larson MH, Gilbert LA, Doudna JA, Weissman JS, Arkin AP, Lim WA. 2013. Resource Repurposing CRISPR as an RNA-Guided Platform for Sequence-Specific Control of Gene Expression. *Cell* **152**: 1173–1183.

Robert T, Nore A, Brun C, Maffre C, Crimi B, Bourbon H-M, de Massy B. 2016. The TopoVIB-Like protein family is required for meiotic DNA double-strand break formation. *Science* **351**: 943–949.

Robine N, Uematsu N, Amiot F, Gidrol X, Barillot E, Nicolas A, Borde V. 2007. Genome-Wide Redistribution of Meiotic Double-Strand Breaks in *Saccharomyces cerevisiae*. *Mol Cell Biol* **27**: 1868–1880.

- Rodgers-Melnick E, Bradbury PJ, Elshire RJ, Glaubitz JC, Acharya CB, Mitchell SE, Li C, Li Y, Buckler ES. 2015. Recombination in diverse maize is stable, predictable, and associated with genetic load. *PNAS* **112**: 3823–3828.
- Rowan BA, Heavens D, Feuerborn TR, Tock AJ, Henderson IR, Weigel D. 2019. An ultra high-density arabidopsis thaliana crossover map that refines the influences of structural variation and epigenetic features. *Genetics* **213**: 771–787.
- Salomé PA, Bomblies K, Fitz J, Laitinen RAE, Warthmann N, Yant L, Weigel D. 2012. The recombination landscape in Arabidopsis thaliana F2populations. *Heredity (Edinb)* **108**: 447–455.
- Sanchez-Moran E, Santos J-L, Jones GH, Franklin FCH. 2007. ASY1 mediates AtDMC1-dependent interhomolog recombination during meiosis in Arabidopsis. *Genes Dev* **21**: 2220–33.
- Sarno R, Vicq Y, Uematsu N, Luka M, Lapierre C, Carroll D, Bastianelli G, Serero A, Nicolas A. 2017. Programming sites of meiotic crossovers using Spo11 fusion proteins. *Nucleic Acids Res* **45**: e164.
- Schimpl S, Fauser F, Puchta H. 2016. CRISPR/Cas-Mediated Site-Specific Mutagenesis in Arabidopsis thaliana Using Cas9 Nucleases and Paired Nickases. In *Methods in Molecular Biology*, Vol. 1469 of, pp. 111–122.
- Séguéla-Arnaud M, Crismani W, Larchevêque C, Mazel J, Froger N, Choinard S, Lemhemdi A, Macaisne N, Van Leene J, Gevaert K, et al. 2015. Multiple mechanisms limit meiotic crossovers: TOP3 α and two BLM homologs antagonize crossovers in parallel to FANCM. *PNAS* **112**: 4713–4718.
- Serrentino ME, Borde V. 2012. The spatial regulation of meiotic recombination hotspots: Are all DSB hotspots crossover hotspots? *Exp Cell Res* **318**: 1347–1352.
- Stacey NJ, Kuromori T, Azumi Y, Roberts G, Breuer C, Wada T, Maxwell A, Roberts K, Sugimoto-Shirasu K. 2006. Arabidopsis SPO11-2 functions with SPO11-1 in meiotic recombination. *Plant J* **48**: 206–216.
- Taagen E, Bogdanove AJ, Sorrells ME. 2020. Counting on Crossovers: Controlled Recombination for Plant Breeding. *Trends Plant Sci* **25**: 455–465.
- Villeneuve AM, Hillers KJ. 2001. Whence meiosis? *Cell* **106**: 647–50.
- Vrielynck N, Chambon A, Vezon D, Pereira L, Chelysheva L, De Muyt A, Mézard C, Mayer C, Grelon M, Patterson PH, et al. 2016. A DNA topoisomerase VI – like complex initiates meiotic recombination. *Science* **351**: 939–944.
- Wang W, Pan Q, He F, Akhunova A, Chao S, Trick H, Akhunov E. 2018. Transgenerational CRISPR-Cas9 Activity Facilitates Multiplex Gene Editing in Allopolyploid Wheat. *Cris J* **1**: 65–74.
- Wijeratne AJ, Chen C, Wei Z, Timofejeva L, Ma H. 2006. The Arabidopsis thaliana PARTING DANCERS Gene Encoding a Novel Protein Is Required for Normal Meiotic Homologous Recombination. *Mol Biol Cell* **17**: 1331–1343.
- Wijnker E, James GV, Ding J, Becker F, Klasen JR, Rawat V, Rowan BA, de Jong DF, de Snoo CB, Zapata L, et al. 2013. The genomic landscape of meiotic crossovers and gene conversions in Arabidopsis thaliana. *Elife* **2**: e01426.
- Wu G, Rossivito G, Hu T, Berlyand Y, Poethig RS. 2015. Traffic lines: New tools for genetic analysis in Arabidopsis thaliana. *Genetics* **200**: 35–45.
- Xie K, Minkenberg B, Yang Y. 2015. Boosting CRISPR / Cas9 multiplex editing capability with the endogenous tRNA-processing system. *PNAS* **112**: 3570–3575.
- Yamaguchi YL, Ishida T, Yoshimura M, Imamura Y, Shimaoka C, Sawa S. 2017. A Collection of Mutants for CLE-Peptide-Encoding Genes in Arabidopsis Generated by CRISPR / Cas9-Mediated Gene Targeting. *Plant Cell Physiol* **58**: 1848–1856.
- Yao Y, Li X, Chen W, Liu H, Mi L, Ren D, Mo A, Lu P. 2020. ATM Promotes RAD51-Mediated Meiotic DSB Repair by Inter-Sister-Chromatid Recombination in Arabidopsis. *Front Plant Sci* **11**: 1–17.
- Yelina NE, Choi K, Chelysheva L, Macaulay M, de Snoo B, Wijnker E, Miller N, Drouaud J, Grelon M, Copenhaver GP, et al. 2012. Epigenetic remodeling of meiotic crossover frequency in Arabidopsis thaliana DNA methyltransferase mutants. *PLoS Genet* **8**:

- 1 e1002844.
- 2 Yelina NE, Lambing C, Hardcastle TJ, Zhao X, Santos B, Henderson IR. 2015. DNA
3 methylation epigenetically silences crossover hot spots and controls chromosomal
4 domains of meiotic recombination in Arabidopsis. *Genes Dev* **29**: 2183–2202.
- 5 Zhang X, Henriques R, Lin SS, Niu QW, Chua NH. 2006. Agrobacterium-mediated
6 transformation of Arabidopsis thaliana using the floral dip method. *Nat Protoc* **1**: 641–
7 646.
- 8 Zickler D, Kleckner N. 1999. Meiotic chromosomes: Integrating structure and function. *Annu*
9 *Rev Genet* **33**: 603–754.

10

Figure legends

Figure 1. Complementation of Arabidopsis *mtopvib* with MTOPVIB fused to catalytically inactive Cas9 (MTOPVIB-dCas9). (A) Wild type and synthetic pathways to generate meiotic double-strand breaks. Homologous chromosomes shown as red and blue lines, MTOPVIB as grey ovals, SPO11 homologues as black ovals, CRISPR/dCas9 shown in blue, guide RNA paired to a genomic locus in yellow. (B) Arabidopsis inflorescences showing long fruit (siliques) in wild type and complementing lines (MTOPVIB-dCas9 in *mtopvib* background) and short fruit (siliques) in *mtopvib*. (C) Average seed count per silique and standard deviation for each genotype. (D) Seed-based reporter systems to measure crossovers in two tester intervals, interstitial *CTL 2.10* on chromosome 2 and sub-telomeric *CTL 5.1* on chromosome 5. Five Arabidopsis chromosomes are shown as black lines, reporter transgenes, *eGFP* and *dsRED*, represented by green and red triangles, respectively. (E) Fluorescent micrographs showing *CTL 2.10* (*GFP RFP/++*) seed using green or red fluorescent filters. (F) Genetic distances of *CTL 2.10* and *CTL 5.1* in wild type and MTOPVIB-dCas9 *mtopvib*. Each black dot represents crossover frequency in an individual plant, red dots denote mean crossover frequencies. Whitney-Mann test showed that mean crossover frequencies in *CTL 2.10* and *CTL 5.1* were not significantly different between wild type and complementing lines (*P* values of 0.54 and 0.68, respectively).

Figure 2. Testing gRNAs targeting 3a meiotic recombination hotspot via a catalytically active Cas9. (A) Histograms for the chromosome 3 sub-telomeric region showing library size normalized coverage values for SPO11-1-oligonucleotides (red), nucleosome occupancy (blue, MNase-seq), H3K4me3 (pink, ChIP-seq), RNA-seq (lilac) and crossovers (purple). Positions of CRISPR target regions are shown as red rectangles and individual gRNA target loci as red ticks. TAIR 10 gene annotations are shown in green and single nucleotide polymorphisms between Col and Ler as blue ticks. (B) 3a crossover profile, red line (centimorgans per megabase, cM/Mb), in Col/Ws MTOPVIB-dCas9 *mtopvib* F₁s. Black vertical lines delineate borders of the 3a hotspot, ticks on the x-axis represent polymorphisms between Col and Ws. Black arrows represent genes, dashed horizontal line - male chromosome 3 average crossover frequency. Six gRNAs were designed to target each of the three regions within 3a, 3a-P, 3a-B and 3a-I, shaded in blue. gRNA target sites are shown as black ticks within the blue shaded areas. (C) Multiplexing six gRNAs via endogenous tRNA-processing system. Schematic representation of a gRNA-tRNA transgene containing tandemly arranged tRNAs and gRNAs. Pol III promoter - grey arrow, terminator - grey rectangle, guide RNA-specific spacers are shown as diamonds of different colour (blue, green or pink), conserved gRNA scaffold shown as black rectangles, tRNA as red rectangles. The primary transcript is cleaved by endogenous RNase P and RNase Z (red arrows) to release mature tRNA (red cloverleaf structure). Processed mature gRNAs guide catalytically active Cas9 (orange) to specific targets. gRNAs 3-5 and their targets are not shown. (D) CRISPR/Cas9-induced deletions in 3a-P. 3a-P is shown as blue rectangle, six gRNAs as black squares. Wild-type and deleted regions within 3a-P are shown by black and dashed lines, respectively. Midori-green-stained agarose gel image shows PCR-amplified 3a-P in wild type (WT) and representative individual T₁s. Lower than wild type molecular weight products result from CRISPR/Cas9-mediated deletions in 3a-P. Percentage of T₁s with CRISPR/Cas9 induced deletions and the total number of T₁s analysed are indicated under the agarose gel image. (E) As in (D) but for 3a-I region. (F) As in (D) but for 3a-B region.

Figure 3. 3a crossover rates in targeted and wild type F₁ hybrids. (A) Generation of F₁ populations for fine-scale crossover analysis via 'pollen typing'. Col *mtopvib* lines complemented with MTOPVIB-dCas9 were crossed to Ws MTOPVIB/*mtopvib* carrying a guide RNA transgene targeting 3a crossover hotspot (gRNA@3a). The resulting Col/Ws F₁

populations were selected for the presence of *MTOPVIB-dCas9* transgene, absence of wild-type *MTOPVIB* (*mtopvib*) and presence of gRNA transgene. A similar crossing scheme was performed for negative controls, 'no gRNA' and 'non-3a gRNA', not shown. **(B)** Schematic representation of 'pollen typing'. Genomic DNA extracted from F₁ pollen is subject to PCR amplification with allele specific oligonucleotides (ASO) to determine the concentration of recombinant crossover molecules relative to parentals. Recombinant molecules are then subject to Sanger sequencing to determine crossover distribution within the hotspot. DNA molecules shown as black lines. Yellow and blue circles represent Col- and Ws-specific polymorphisms, respectively. **(C)** 3a crossover frequencies in centimorgans (cM) measured by pollen typing in five different F₁ populations. Error bars represent standard deviation. **(D)** Fine-scale 3a crossover profiles in the presence or absence of *gRNA-P* gRNAs targeting 3a-*P*. 3a recombination rates in centimorgans per megabase (cM/Mb) were analysed by pollen typing. Black vertical lines delineate borders of 3a hotspot, ticks on the x-axis represent polymorphisms between Col and Ws. Black arrows represent genes, dashed horizontal line - male chromosome 3 average crossover frequency. Blue shaded area (3a-*P*) marks guide RNA target region with black ticks representing individual guide RNA target sites. Recombination rates in Col/Ws *MTOPVIB-dCas9 mtopvib* F₁s in the absence of guide RNAs are shown in red and in the presence of *gRNA-P* gRNAs - in blue. **(E)** As in **(D)**, but for 3a-*B*. **(F)** As in **(D)** but for 3a-*I*.

Figure 4. Gene conversions in 3a **(A)** Generation of F₂ populations for gene conversion detection via Kompetitive Allele-Specific PCR (KASP). **(B)** An example plot showing allele discrimination via KASP assay for one single nucleotide polymorphism (SNP) between Col and Ws. Each dot represents an F₂ individual. Different colours - yellow, blue, green and black - represent Col, Ws, heterozygous and a 'no DNA' control, respectively. **(C)** 3a fine-scale crossover profile, red line in centimorgan per megabase (cM/Mb) in Col/Ws *MTOPVIB-dCas9 mtopvib* F₁ population. Black vertical lines delineate borders of 3a hotspot, ticks on the x-axis represent polymorphisms between Col and Ws. Black arrows represent genes, dashed horizontal line - male chromosome 3 average crossover frequency. 3a-*P* target region is shaded in blue and positions of individual guide RNAs are shown as black ticks. **(D)** As in **(C)** but in the presence of *gRNA-P* gRNAs. **(E)** Gene conversion events detected in Col/Ws *MTOPVIB-dCas9 gRNA-P* F₂ population. DNA sequence polymorphisms (SNPs and InDels) are shown as black ticks at the top of the plot. Maximum and minimum gene conversion tracts shown as black lines and blue rectangles, respectively.

Supplemental Figures S1-S2. Sanger sequencing analysis of gene editing events in 3a crossover hotspot. Nucleotide sequences of each of the target regions (3a-*P*, 3a-*B* and 3a-*I*) are shown, gRNA target sites highlighted in grey and protospacer adjacent motif, PAM, in red. To distinguish between overlapping gRNA-B-1 and gRNA-B-2, in addition to highlighting them in grey, one is shown bold and the other is underlined. Dots indicate wild type genomic sequence not shown due to space constraints, dashes indicate sequences deleted via CRISPR/Cas9-mediated gene editing. In the case where CRISPR/Cas9 editing causes an 8 bp insertion, dashes indicate a corresponding missing sequence in the wild type.

Supplemental Figure S3. Testing the efficiency of guide RNAs targeting outside 3a crossover hotspot. **(A)** Schematic representation of the five Arabidopsis chromosomes and gRNA positions. Chromosomes - green bars, blue rectangles - centromeres, red ticks - genes targeted with CRISPR/Cas9, star - 3a hotspot (shown for reference). **(B)** Constructs harbouring guide RNAs targeting six Arabidopsis genes (*CLE9*, *CLE10*, *CLV3*, *GL1*, *FWA* and *eIF(iso)4E*) and a catalytically active Cas9 were transformed into wild type Col. T₁ progenies were selected for the presence of *gRNA-Cas9* transgenes and tested for gene editing events. Representative Midori-green-stained agarose gel analyses of CRISPR/Cas9-mediated gene

editing in T₁ leaf tissue. Mutations introduced by CRISPR/Cas9 either destroy a restriction endonuclease recognition site (*Bsu36I* in *CLE10*, *BspHI* in *CLV3* and *DdeI* in *GL1*) resulting in higher molecular weight products compared to wild type control (untransformed Col) or introduce a mismatch that is recognised by T7 Endonuclease I resulting in endonucleolytic cleavage and lower molecular weight product(s) compared to untransformed Col control (in *CLE9*, *eIF(iso)4E* and *FWA*). Different leaf sectors of the analysed T₁ plants were mosaic for CRISPR/Cas9-mediated gene editing events and had one ('het'), two ('ko') or neither ('wt') gene edited allele(s). This was visually demonstrated for *GL1* responsible for trichome development. Leaf segments showing absence of trichomes (white arrows) had both *GL1* alleles mutated by CRISPR/Cas9. **(C)** Summary table showing number and percentage of T₁s affected by CRISPR/Cas9 gene editing.

Supplemental Figure S4. Detection of mature gRNAs via RT-PCR. **(A)** Schematic representation of a mature gRNA. An (RT-)PCR assay to detect mature gRNAs uses a 'universal' reverse primer complementary to the 3' end of the gRNA scaffold (black arrow) and a guide-RNA-spacer-specific forward primer, light blue arrow. **(B)** Detection of one of the six mature gRNAs targeting *3a-B* by RT-PCR in Col/Ws *mtopvib* *MTOPVIB-dCas9 gRNA-P* F₁ population. Black arrow points to the mature gRNA-specific PCR product resolved on a Midori-green-strained 2% agarose gel. Col and 'no gRNA' are negative controls. Two biological and two technical replicates were done for each genotype. Higher than 96-bp molecular weight bands are non-specific products. **(C)** As in **(B)** but for *gRNA-P*. **(D)** As in **(B)** but for *gRNA-I*. Two to three out of the six multiplexed gRNAs for each of the target regions was tested, but data for one gRNA is shown. **(E)** Ubiquitin was used as a '+RT' and '-RT' controls.

Figure 1

A

B

C

E

D

F

Figure 2

Figure 3

Figure 4

3a-P

ATGAATACCACTTCTTAAGTTCTCTGTTGACCATTCTATTACCTTCCTTCAAATTGATACAACAAATACATAAAAAAA

gRNA-P-1 gRNA-P-2
AAAAAAAATGTCTATGGTACAA ACTTATATCAGAAATAAAAA CCGTGTCTGGAGAGGCTAATCTTTGTTTGTCAA CCGTA
GCTCAGACACATTTGACAGACCTGGCATGTCTTTGGTCACACTCAACCTGCAAAAAAAAAAACATAAAAGAAACAAAAC
gRNA-P-3
GATAACGACCATTAATTTTTTAATTAGACCTAATTTATTGATCTAAT AAAAAAGAAAGGAAAAACAGAGGTCGTTTAAAC
GTCTACAAAAACATTACTGCAGTGATTTATTGTCATTTATGTAACGCAATATAGTTGACTCTGATAAAATTGTTACAA
CTAGTATTCATAAGTCATAACCTTATAATTAATTAATTATCTTTCTGTAAATTAGCTGTCAAATTAATACATAAGATTC
gRNA-P-4
TCTTTTAACTTTTTTGTCAAAAAGGAAATATTCCAATTAGTAACCTTTCTTTTTT CCTTTTTCTCTGCAATTATGACC A
TTTTCTCTCTCTTAGCCGGCTCTAGAGAGAGAAGAAAGACAGAGCTGAAACTCTTGTAACCAAAAAACACAAAAGTCTC
gRNA-P-5
TTTGTTCAAACACTCGTTTACGCGTAG CCGAAATAAATTTTCCAAAAACC CATATCC GAAAAAGTCGATATTTATTGA
gRNA-P-6
ATTTTTGGAA CCAAGGAGACGAAGAATTACAC GGTTTTTGAAGAAGAAGAAGATGAAGTATAAGCGTAAGCTAAGT
CTCTCTGTTGTCTTCTCTTTGTCTTCTATCTCGCTGCGGTGACTTCAGATCTAGAGT

436-bp deletion

gRNA-P-3 gRNA-P-6
Wt GATCTAAT AAAAAAGAAAGGAAAAACAGAGGTCG.....GGAA CCAAGGAGACGAAGAATTACAC GGTTTTTG
Mut GATCTAAT AAAAAAGAAAGGAA-----GACGAAGAATTACAC GGTTTTTG

238-bp deletion

gRNA-P-3 gRNA-P-4
Wt GATCTAAT AAAAAAGAAAGGAAAAACAGAGGTCG....TTTT CCTTTTTCTCTGCAATTATGACC ATTTTCTC
Mut GATCTAAT AAAAAAGAAAGGAAAA-----TCTCTGCAATTATGACC ATTTTCTC

423-bp deletion

gRNA-P-1 gRNA-P-4
Wt GTCTATGGTACAA ACTTATATCAGAAATAAAAA CCGTGT.....TTT CCTTTTTCTCTGCAATTATGACC ATTTTCTCTCT
Mut GTCTATGGTACAA ACTTATATCAGAAATA-----TCTCTGCAATTATGACC ATTTTCTCTCT

63-bp deletion

gRNA-P-5 gRNA-P-6
Wt CGCGTAG CCGAAATAAATTTTCCAAAAACC CATAGGAA CCAAGGAGACGAAGAATTACAC GGTTTTTGA
Mut CGCGTAG CCGAAA-----AGACGAAGAATTACAC GGTTTTTGA

3a-B

gRNA-B-1 and gRNA-B-2
TGTCTTCTATCTCGCTGCGGTGACTTCAGATCTAGAGTCTGA CCGGAGAGCTTTACTCGCTGTTTCGTAACAGTGTCCGT
gRNA-B-3
GGCCGTCCTTTGCTATGGAACATGAGTGCTTCTTCTCCTTGTAATTG GCACGGAGTCCACTGCGATG CCGGTCTGGGTG
gRNA-B-4
ACGGCTCTCCGATTACCCGGATCTGGTTTATTCGGT TCTTTACCAATCGGTGGTAT TGGTAATCTAACCCAGCTTAAGA
CTCTTTCTCTCCGGTTCAATTCTCTCTGTCCTATCCCTTCGGATTTCTCCAACCTTGTTCTCCTCCGTTACTTGTATCTT
CAAGGTAATGCCTTTCCGGTGAGATTCCGTGCTTCTCTTACGCTTCCGAGCATAATCAGAATCAATCTAGGGGAGA
gRNA-B-5
ATAAATTCTCGGGTCGGATCCCGGATAATGTCAATTCTGCGAC CCGGTTGGTTACTCTGTATT TGGAGAGGAATCAAC
gRNA-B-6
TCTCTGGTCCGATCCCTGAGATCACGCTTCTCTTACGAATTCAAT GTTTCTTCTAATCAGTTAAA CCGGTCTATTCCG
AGTTCGTTGTCTGCTTGGCCTCGAAGTCTTTGAAGGTAACACTCTCTGTGGGAAGCCTTTAGACACTTGTGAGGCAG

464-bp deletion

gRNA-B-1 and gRNA-B-2 gRNA-B-6
Wt TGA CCGGAGAGCTTTACTCGCTGTTTCGTAACAGTGTCCGT TGGCCGT...TTCAAT GTTTCTTCTAATCAGTTAAA CCGGTCT
Mut TGA CCGGAGAGCTTTACTCGCTGTTTCGTAACAGTGT-----AAA CCGGTCT

208-bp deletion

gRNA-B-1 and gRNA-B-2 gRNA-B-5
Wt TGA CCGGAGAGCTTTACTCGCTGTTTCGTAACAGTGTCCGT TGGCCGT...GCGACCCGGTTGGTTACTCTGTATT TGGAGA
Mut TGA CCGGAGAGCTTTACTCGCTGTTTCGTAACAGTGT-----TTGGAGA

161-bp deletion

gRNA-B-1 and gRNA-B-2 gRNA-B-4
Wt TGA CCGGAGAGCTTTACTCGCTGTTTCGTAACAGTGTCCGT TGGCCGT...TCGGTTCTTTACCAATCGGTGGTAT TGGTAAT
Mut TGA CCGGAG-----GTAT TGGTAAT

500-bp deletion

gRNA-B-3
Wt TTCTCCTTGTAATTG GCACGGAGTCCACTGCGATG CCGGTCTG....TGTGGGAAGCCTTTAGACACTTGTGAGGCAG
Mut TTCTCCTTGTAATTG GCACGGAGTCC-----TTAGACACTTGTGAGGCAG

3a-l

gRNA-I-1
TGGGGTCAAGTTGCAATTTCTTGAATAGTGTGAC CCCACTTCCCGCCTTTTTTCTACTTCTTCTTTGTTCTTGTTCTAGTTTGTTCGTTAATTTCTTGAGCCTCAAATTTGAGATGAGACCTATCAGTTTCTTTTTTCTTTCTTTCTACTTTTCTTTATTTCCAAAGTTTAAAAATGTCTATAAACTATTGCTTTTGCTAATTTTGTATAAATATAGTTGATTGCGTGTGAA
TAAATTGGTTTTTGTCTTTTCTTTACACATCTTATCCCAGAATCCTAGTCAACGATTCAGCCAAAAACAATGTCAACG
gRNA-I-2
AGTTTTATTTTTATCGCCGTCCAAAATTTTGTAGCCAAA AACAAAGTCAACGACAAAAGTGGTCATGAATATCG TCTCACGATTGACTGACAAATACTTCACGATTTGATTAGATATGAAGTTTTATAATGTGAAGTTGGTAACCACTAACTAATAACCC TACTTCACAGGAGAAAGAAAAACATTTTAGATATGAAAAACAACATTGACCCAGAAAGATATATATTTGCTTAGAGAT
gRNA-I-3
TTTGATGAAAGAATTACTAGTGCTGATAA ACTATTTAGTTCAAGCTTAA AGGGAATCGATTAGATAATTT TTCGAATT
gRNA-I-4
ATTT GAAGAAAACATATTGTGATC TGGACTTATAATTTTTCATGTGATATACCATTTACACCGAGTCATGGGCAGTTGCC
gRNA-I-5
ACAACCTTTATCTCAT GTTCCAACTCGTACCAGACT GGGTACATATGCACATGGGGTTCAATAAATTTATTAGTGGGATAC TCTTTGACATAAAACACTATTAGGAAAAAATGGAATTAAACCTATTCTATAAATT CATATAATTACGCACGTAAATAATG
gRNA-I-6
TTTTTCACAGTAATATTAGA ATTTGACCTTAGCAAGTTGA TGGTTTTTAAACCGTGAAGCAAATAGAAC

376-bp deletion

gRNA-I-2 gRNA-I-5
Wt GCCAAAAACAAAGTCAACGACAAAAGTGGTC.....CTCAT GTTCCAACTCGTACCAGACT GGGTACATATGC
Mut GCCAAAAACAAAGTCAACGACAA-----ACT GGGTACATATGC

8-bp insertion

gRNA-I-5
Wt GCAGTTGCCACAACCTTTATCTCAT GTTCCAACTCGTACCAG-----ACT GGGTACAT
Mut GCAGTTGCCACAACCTTTATCTCAT GTTCCAACTCGTACCAGTTGCCACAAC TGGTACAT

285-bp deletion

gRNA-I-2 gRNA-I-4
Wt GCCAAAAACAAAGTCAACGACAAAAGTGGTC.....ATTT GAAGAAAACATATTGTGATC TGGACTTATAA
Mut GCCAAAAACAAAGTCAACGACAA-----ATC TGGACTTATAA

335 bp deletion and 1 bp insertion

gRNA-I-1 gRNA-I-2
Wt TCGACCCCACTTCCCGCCTTTTTTCTACTTCTTCTC.....GCCAAAAACAAAGTCAACGACAAAAGTGGTCAT
Mut TCGACCCCACT-----AAAGTGGTCAT

164 bp deletion and 1 bp insertion

gRNA-I-5 gRNA-I-6
Wt TCTCAT GTTCCAACTCGTACCAGACT GGGTACAT.....TAGAATTTGACCTTAGCAAGTTGA TGGTTTTTAAACC
Mut TCTCAT GTTCCAACTCGTACCAG-----TTGATGGTTTTTAAACC

541 bp deletion

gRNA-I-2 gRNA-I-6
Wt CAAAAACAAAGTCAACGACAAAAGTGGTCAAGAAATTTGACCTTAGCAAGTTGA TGGTTTTT
Mut CAAAAACAAAGTCAACGACAA-----TGATGGTTTTT

Supplemental Figure S3

Supplemental Figure S4

SUPPLEMENTAL TABLES

Table 1. Seed sets in Col, *mtopvib* and complementing *MTOPVIB-dCas9 mtopvib* lines. Two-tailed t-test was used to calculate the *P* values

Genotype	Total siliques scored	Total seeds scored	Average	Standard Deviation	<i>P</i> value
Col	10	645	64.5	8.2	
<i>mtopvib</i>	56	105	1.9	1.7	<0.00001
<i>MTOPVIB-dCas9 mtopvib</i>	7	463	66.1	3.3	0.63

Table 2. Genetic distances of *CTL2.10* in wild type and *MTOPVIB-dCas9 mtopvib*. cM were calculated using the formula: $cM = 100 \times (1 - [1 - 2(N_G + N_R)/N_T]^{1/2})$, where N_G is a number of green-alone fluorescent seeds, N_R is a number of red-alone fluorescent seed and N_T is the total number of seeds counted.

Genotype	Green-alone	Red-alone	Both red and green	Neither red or green	Total	CTL 2.10, cM
Wild type	140	152	1744	467	2503	12.44
Wild type	133	129	1611	453	2326	11.98
Wild type	131	147	1649	447	2374	12.49
Wild type	131	166	1727	440	2464	12.88
Wild type	134	161	1734	477	2506	12.56
Wild type	145	163	1621	437	2366	14.00
Wild type	109	169	1695	449	2422	12.23
Wild type	136	139	1648	445	2368	12.38
<i>MTOPVIB-dCas9 mtopvib</i>	120	153	1665	449	2387	12.18

<i>MTOPVIB-dCas9 mtopvib</i>	138	174	1678	423	2413	13.90
<i>MTOPVIB-dCas9 mtopvib</i>	124	143	1597	413	2277	12.51
<i>MTOPVIB-dCas9 mtopvib</i>	131	146	1588	416	2281	12.99
<i>MTOPVIB-dCas9 mtopvib</i>	127	157	1681	463	2428	12.47
<i>MTOPVIB-dCas9 mtopvib</i>	145	169	1675	405	2394	14.11
<i>MTOPVIB-dCas9 mtopvib</i>	148	159	1648	431	2386	13.82
<i>MTOPVIB-dCas9 mtopvib</i>	147	174	1656	425	2402	14.40
<i>MTOPVIB-dCas9 mtopvib</i>	112	143	1627	417	2299	11.79
<i>MTOPVIB-dCas9 mtopvib</i>	133	164	1656	493	2446	12.99
<i>MTOPVIB-dCas9 mtopvib</i>	127	141	1599	433	2300	12.42

Table 3. Genetic distances of *CTL5.1* in wild type and *MTOPVIB-dCas9 mtopvib*. cM were calculated using the formula: $cM = 100 \times (1 - [1 - 2(N_G + N_R)/N_T]^{1/2})$, where N_G is a number of green-alone fluorescent seeds, N_R is a number of red-alone fluorescent seed and N_T is the total number of seeds counted.

Genotype	Green-alone	Red-alone	Both red and green	Neither red or green	Total	CTL 5.1, cM
Wild type	220	233	1455	353	2261	22.59
Wild type	276	264	1477	364	2381	26.08
Wild type	242	246	1532	332	2352	23.51

Wild type	264	275	1586	356	2481	24.80
Wild type	250	251	1661	379	2541	22.18
Wild type	280	237	1633	343	2493	23.50
Wild type	239	257	1648	355	2499	22.34
Wild type	193	225	1217	254	1889	25.34
Wild type	264	241	1559	363	2427	23.59
<i>MTOPVIB-dCas9 mtopvib</i>	229	234	1479	334	2276	22.98
<i>MTOPVIB-dCas9 mtopvib</i>	271	248	1562	306	2387	24.82
<i>MTOPVIB-dCas9 mtopvib</i>	273	272	1544	349	2438	25.64
<i>MTOPVIB-dCas9 mtopvib</i>	251	204	1354	339	2148	24.08
<i>MTOPVIB-dCas9 mtopvib</i>	269	247	1551	376	2443	24.00
<i>MTOPVIB-dCas9 mtopvib</i>	249	212	1489	327	2277	22.86
<i>MTOPVIB-dCas9 mtopvib</i>	259	241	1515	382	2397	23.66
<i>MTOPVIB-dCas9 mtopvib</i>	280	251	1621	360	2512	24.02
<i>MTOPVIB-dCas9 mtopvib</i>	286	228	1482	343	2339	25.13
<i>MTOPVIB-dCas9 mtopvib</i>	288	250	1542	364	2444	25.18
<i>MTOPVIB-dCas9 mtopvib</i>	250	209	1555	345	2359	21.84
<i>MTOPVIB-dCas9 mtopvib</i>	319	240	1566	388	2513	25.49

<i>MTOPVIB-dCas9</i> <i>mtopvib</i>	254	221	1639	358	2472	21.53
<i>MTOPVIB-dCas9</i> <i>mtopvib</i>	230	219	1457	334	2240	22.60

Table 4. Coordinates of 3a crossover hotspot and genes within 3a

Locus	Chromosome	Start coordinate	End coordinate
3a crossover hotspot	3	634,109	639,934
AT3G02880	3	634,465	637,284
AT3G02885	3	637,921	639,178

Table 5. Sequences and coordinates of guide RNAs used in this study

Guide RNA sequence	Target site	Genome coordinates	Target locus
GGTTTTTGAAAATTTATTT	AT3G02880 promoter and 5' end	Chr3: 634,713-634,732	<i>3a-P</i>
GTGTAAATTCTTCGTCTCCT	AT3G02880 promoter and 5' end	Chr3: 634,776-634,795	<i>3a-P</i>
GGTCATAATTGCAGAGAAAA	AT3G02880 promoter and 5' end	Chr3: 634,582-634,601	<i>3a-P</i>
ACAAAAGAAAGGAAAAACAG	AT3G02880 promoter and 5' end	Chr3: 634,330-634,349	<i>3a-P</i>
ACTTATATCAGAAATAAAAA	AT3G02880 promoter and 5' end	Chr3: 634,146-634,165	<i>3a-P</i>
GGCTAATCTTTGTTTGTCAA	AT3G02880 promoter and 5' end	Chr3: 643,178-643,197	<i>3a-P</i>
GCTGTTTCGTAACAGTGTCCTG	AT3G02880 gene body	Chr3: 634,921-634,940	<i>3a-B</i>
GAACAGCGAGTAAAGCTCTC	AT3G02880 gene body	Chr3: 634,908-634,927	<i>3a-B</i>

TCTTTACCAATCGGTGGTAT	AT3G02880 gene body	Chr3: 635,056-635,075	3a-B
CCGGTTGGTTACTCTGTATT	AT3G02880 gene body	Chr3: 635,304-635,323	3a-B
GCACGGAGTCCACTGCGATG	AT3G02880 gene body	Chr3: 634,989-635,008	3a-B
GTTTCTTCTAATCAGTTAAA	AT3G02880 gene body	Chr3: 635,386-635,405	3a-B
GTAGAAAAAAGGCGGGAAGT	Intergenic between AT3G02880 and AT3G02885	Chr3: 637,059-637,078	3a-I
GTTCCAACCTCGTACCAGACT	Intergenic between AT3G02880 and AT3G02885	Chr3: 637,756-637,775	3a-I
GAAGAAAACATATTGTGATC	Intergenic between AT3G02880 and AT3G02885	Chr3: 637,665-637,684	3a-I
AACAAAGTCAACGACAAAAG	Intergenic between AT3G02880 and AT3G02885	Chr3: 637,380-637,399	3a-I
ACTATTTAGTTCAAGCTTAA	Intergenic between AT3G02880 and AT3G02885	Chr3: 637,611-637,630	3a-I
ATTTGACCTTAGCAAGTTGA	Intergenic between AT3G02880 and AT3G02885	Chr3: 637,921-637,940	3a-I
AACTCACCGAACCGTCCCTA	AT1G69320, <i>CLE10</i>	Chr1: 26,062,017-26,062,036	non-3a gRNAs
ACGAACGGTGAGGAGTCACG	AT1G26600, <i>CLE9</i>	Chr1: 9,191,857-9,191,876	non-3a gRNAs
TGAGACCAGAAGCATCATGA	AT2G27250, <i>CLV3</i>	Chr2: 11,665,672-11,665,691	non-3a gRNAs
GGAAAAGTTGTAGACTGAGA	AT3G27920, <i>GL1</i>	Chr3: 10,363,224-10,363,243	non-3a gRNAs

ACGGAAAGATGTATGGGCTT	AT4G25530, <i>FWA</i>	Chr4: 13,038,251-13,038,270 and Chr4: 13,038,296-13,038,315	non-3a gRNAs
TGTGAACGAGCCTCTCCCGG	AT5G35620, <i>eIF(iso)4A</i>	Chr5: 13,826,298-13,826,317	non-3a gRNAs

Table 6. CRISPR/Cas9 gene editing efficiencies in 3a. Number and percentage of *Cas9-gRNA-P*, *Cas9-gRNA-B* and *Cas9-gRNA-I* T₁ progeny with gene editing events and gene editing efficiencies for individual guide RNAs.

Transgenic line (grey) or guide RNA	Total number of T ₁ s analysed	Number of T ₁ s with mutation	% T ₁ s with gene editing
<i>Cas9-gRNA-P</i>	90	8	8.9
gRNA-P-1	90	3	3.3
gRNA-P-2	90	0	0
gRNA-P-3	90	2	2.2
gRNA-P-4	90	4	4.4
gRNA-P-5	90	2	2.2
gRNA-P-6	90	4	4.4
<i>Cas9-gRNA-B</i>	33	4	12.1
gRNA-B-1	33	3	9.1
gRNA-B-2	33	2	6.1
gRNA-B-3	33	1	3.0
gRNA-B-4	33	1	3.0
gRNA-B-5	33	2	6.1
gRNA-B-6	33	2	6.1
<i>Cas9-gRNA-I</i>	66	17	25.8
gRNA-I-1	66	4	6.1
gRNA-I-2	66	13	19.7
gRNA-I-3	66	1	1.5
gRNA-I-4	66	4	6.1

gRNA-I-5	66	14	21.2
gRNA-I-6	66	15	22.8

Table 7. CRISPR/Cas9 gene editing efficiencies outside of 3a hotspot.

Guide RNA target site	Wild type	One allele affected	Both alleles affected	% T ₁ s with mutation
<i>CLE9</i>	46	2	0	4.2
<i>CLE10</i>	45	3	0	6.3
<i>CLV3</i>	32	14	2	33.3
<i>GL1</i>	22	24	2	50.0
<i>FWA</i>	47	1	0	2.1
<i>eIF(iso)4E</i>	30	18	0	37.5

Table 8. 3a crossover frequencies in Col/Ws *MTOPVIB-dCas9 mtopvib* F₁ populations in the presence or absence of guide RNAs targeting 3a. Chi-square test was used to calculate *P*-values.

Genotype	Crossovers per microliter	Parentals per microliter	3a	Standard Deviation	3a	<i>P</i> -value
non-3a gRNA	26.9	17412	0.155 cM	0.014	26.4 cM/Mb	
no gRNA	32.5	24767	0.131 cM	0.011 cM	22.3 cM/Mb	0.56
<i>gRNA-P</i>	45.6	30059	0.152 cM	0.014 cM	25.9 cM/Mb	0.96
<i>gRNA-B</i>	42.3	22375	0.189 cM	0.021 cM	32.2 cM/Mb	0.44
<i>gRNA-I</i>	41.2	23522	0.175 cM	0.020 cM	29.8 cM/Mb	0.64

Table 9. Fine-scale mapping of 3a crossover frequencies in Col/Ws *MTOPVIB-dCas9 mtopvib* F₁ populations in the presence or absence of guide RNAs targeting 3a.

Coordinate	Interval Length	CO 'no gRNA'	cM/Mb 'no gRNA'	CO 'non-3a gRNA'	cM/Mb 'non-3a gRNA'	CO <i>gRNA-P</i>	cM/Mb <i>gRNA-P</i>	CO <i>gRNA-B</i>	cM/Mb <i>gRNA-B</i>	CO <i>gRNA-I</i>	cM/Mb <i>gRNA-I</i>
634109											
634938	829	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
635457	519	5	15.8	2	7.8	0	0.0	2	8.4	2	7.7
635520	63	0	0.0	1	32.0	0	0.0	1	34.5	0	0.0
636119	599	3	8.2	3	10.1	2	5.6	3	10.9	0	0.0
637283	1164	6	8.4	9	15.6	13	18.9	14	26.1	13	22.2
637754	471	8	27.8	6	25.6	8	28.7	11	50.7	13	54.9
637900	146	3	33.6	0	0.0	0	0.0	0	0.0	3	40.9
637952	52	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
637974	22	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
637988	14	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
638139	151	4	43.4	3	40.0	6	67.1	3	43.2	2	26.3
638196	57	3	86.2	1	35.3	1	29.6	0	0.0	1	34.9
638483	287	7	39.9	8	56.1	17	100.0	12	90.8	20	138.6
638786	303	14	75.7	13	86.4	20	111.5	10	71.7	12	78.8
638818	32	1	51.2	0	0.0	1	52.8	1	67.9	1	62.1
639370	552	13	38.6	25	91.2	16	49.0	20	78.7	12	43.2
639538	168	13	126.7	6	71.9	6	60.3	10	129.3	9	106.5
639934	462	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Total	5825	80	22.49	77	26.61	90	26.09	87	32.45	88	30.04

Table 10. Genomic positions of single nucleotide polymorphisms (SNPs), in red, used for KASP assays. Assays highlighted in grey were used for initial screening of the F₂ population. Assays in white were used only on individual F₂ plants to determine gene conversion tract lengths.

KASP assay	Position in the genome	Distance between SNPs, bp	Sequence in Col	Sequence in Ws
SNP1v3	628865		ACTCCAACAACAGGGACTTTT T	ACTCCAACAACAGGGACTTTT C
SNP1	630828	1963	CCTTTCTCTCGTTCCAAGACAGC G	CCTTTCTCTCGTTCCAAGACAGC A
SNP2v3	631531	703	AAGAACCCAATGGCCTATATGTCA A	AAGAACCCAATGGCCTATATGTCA T
SNP3	632767	1236	TTGTTAGTGTGTGTGTCTTATGAG A	TTGTTAGTGTGTGTGTCTTATGAG G
SNP4	633695	928	AAGGCTGCGATTTTCGTGCTGGGG G	AAGGCTGCGATTTTCGTGCTGGGG A
SNP5	634938	1243	GCTGTTTCGTAACAGTGT C	GCTGTTTCGTAACAGTGT T
SNP5v3	635457	519	CGAACTGCTTTTGAAGG T	CGAACTGCTTTTGAAGG C
SNP5v4	635520	63	CCCAATGGTGGTGACGCT T	CCCAATGGTGGTGACGCA A
SNP6	636119	599	ATTGCACGGTATAGAAT A	ATTGCACGGTATAGAAT C
SNP7	637283	1164	AATAAATTGGTTTTTGTCTTCT T	AATAAATTGGTTTTTGTCTTCT A
SNP6v2	637754	471	AGTTGCCACAACCTTTATCTC A	AGTTGCCACAACCTTTATCTC G
SNP6v3	638139	385	TTTCATAAATATGTAACCAT A	TTTCATAAATATGTAACCAT T
SNP8	638483	344	GTGATGTTGCTGAACAACGG A	GTGATGTTGCTGAACAACGG T
SNP8v2	638818	335	TCAATATCAGAAAATAAGCC A	TCAATATCAGAAAATAAGCC G
SNP8v3/8v4	639370	552	ATTATATTGTGTAGTTGACTAGT G	ATTATATTGTGTAGTTGACTAGT T
SNP9v3	640000	1517	TAATAATGTAATTCTAAATT A	TAATAATGTAATTCTAAATT T
SNP10	641219	1219	TTTTCCGGTTCAAAGTCAGAT T	TTTTCCGGTTCAAAGTCAGAC C
SNP11v2	642469	1250	TACTCATCTTTCAATAGCCAC T	TACTCATCTTTCAATAGCCAC G
SNP11v3	642671	202	AAGTCCAATTCGTTTAATTCTT T	AAGTCCAATTCGTTTAATTCTT C
SNP11v4	642735	64	GACAGAAGACTGGGA T	GACAGAAGACTGGGA C
SNP12	644083	1348	TAATTGTGTTTTGTTTCACATG C	TAATTGTGTTTTGTTTCACATG G

Table 11. Gene conversion events observed in F₂ progenies of Col/Ws *MTOPVIB-dCas9 mtopvib* in the presence or absence of guide RNAs targeting 3a.

Genotype	Total F ₂ s analysed	Non-crossovers observed	Non-crossover frequency (%)
no gRNA	473	0	0
<i>gRNA-P</i>	469	2	0.43
<i>gRNA-B</i>	96	0	0
<i>gRNA-I</i>	96	0	0

Table 12. Gene conversion events observed in two individuals (#519 and #343) of F₂ progeny of Col/Ws *MTOPVIB-dCas9 gRNA-P mtopvib*.

Polymorphism Coordinate	Interval Length	Genotype	
		<i>gRNA-P</i> , F ₂ #519	<i>gRNA-P</i> , F ₂ #343
634109		Het	Ws
634938	829	Het	Ws
635457	519	Het	Ws
635520	63	Het	Ws
636119	599	Het	Het
637283	1164	Het	Ws
637754	471	Ws	Not tested
637900	146	Ws	Not tested
637952	52	Ws	Not tested
637974	22	Ws	Not tested
637988	14	Ws	Not tested
638139	151	Ws	Not tested
638196	57	Ws	Not tested
638483	287	Ws	Ws
638786	303	Het	Not tested
638818	32	Not tested	Not tested
639370	552	Not tested	Not tested

639538	168	Not tested	Not tested
640000	462	Het	Ws

Table 13 - Oligonucleotides used in this study

Name	Sequence	Description
MTOPVI-Prom-Sall-F	ATATATAGTCGACTTTGCGCCAAAGGAAAATG	Cloning MTOPVIB-dCas9
MTOPVI-Term-NotI-R	ATATATATATATGCGGCCGCGATATCTTGCAGGGAAGTCAC	Cloning MTOPVIB-dCas9
MTOPVI-NheI-F	TCTTAGCTAGCTTAAGTATTTAAAGTTATATC	Cloning MTOPVIB-dCas9
MTOPVI-Ascl-R	ATATACCATGGGGCGCGCCCAGAGATTCTCCGCTTTTCG	Cloning MTOPVIB-dCas9
MTOPVIB-C-HA-top	GGAAGGCGGCAGCGGAGGCTCCCCTAAGAAAAAGCGCAAAGTCT ATCCATATGACGTTCCAGATTACGCTTACCCTTACGATGTGCCTGA CTACGCTTCTAGAGCTGGATCCTAAGTGCA	Cloning MTOPVIB-dCas9
MTOPVIB-C-HA-bottom	CTTAGGATCCAGCTCTAGAAGCGTAGTCAGGCACATCGTAAGGGT AAGCGTAATCTGGAACGTCATATGGATAGACTTTGCGCTTTTTCTT AGGGGAGCCTCCGCTGCCGCCTTCCTGCA	Cloning MTOPVIB-dCas9
dCas9-Xba-F	GGAAGATCGGTTCAACGCCTCCTCG	Cloning MTOPVIB-dCas9
dCas9-BamHI-R	ATATATAGGATCCTCACTTTTTCTTTTTTGCCTGGC	Cloning MTOPVIB-dCas9, genotyping <i>MTOPVIB-dCas9</i> transgene
dCas9-1stMut-F	GACAAGAAGTACAGCATCGGCCTGGCCATCGGCACCAACTCTGTG GGCTG	dCas9 mutagenesis
dCas9-1stMut-R	GCCCAGCCCACAGAGTTGGTGCCGATGGCCAGGCCGATGC	dCas9 mutagenesis
dCas9-2ndMut-R	CCGGCTGTCCGACTACGATGTGGACGCTATCGTGCCTCAG	dCas9 mutagenesis
dCas9-2ndMut-R	CAGAAAGCTCTGAGGCACGATAGCGTCCACATCGTAGTCG	dCas9 mutagenesis
MTOPVIB-seq3-F	GTGCTTGAGGATTTGGAAT	Genotyping <i>MTOPVIB-dCas9</i> transgene
MTOPVIB-end-F	TGTCTGGCGACTATGCTG	Genotyping <i>MTOPVIB-dCas9</i> transgene
MTOPVIB-stop-R	CTATTCCTGCAGCATAGTCG	Genotyping <i>MTOPVIB-dCas9</i> transgene
MTOP-genot-compl-F	AGAATGGTGCAAAGTTTAGG	Genotyping <i>mtopvib-2</i> complemented with <i>MTOPVIB-dCas9</i>
MTOP-genot-compl-R	AAATGACCTACTTTCCTGCTC	Genotyping <i>mtopvib-2</i> complemented with <i>MTOPVIB-dCas9</i>

DNA_template_02880_prom-R	TCTAGATCTGAAGTCACCGC	Target DNA amplification for <i>in vitro</i> and <i>in planta</i> gRNA testing
DNA_template_02880_body-F	GTCACGAGAAACCAACCACT	Target DNA amplification for <i>in vitro</i> and <i>in planta</i> gRNA testing
DNA_template_02880_body-R	GAGAAGTTGTCCCATCACG	Target DNA amplification for <i>in vitro</i> and <i>in planta</i> gRNA testing
DNA_template_interlg-F	GCTACCTACATTGCGGTG	Target DNA amplification for <i>in vitro</i> and <i>in planta</i> gRNA testing
DNA_template_interlg_R	TGTAGCTTCCGTTGTTTCA	Target DNA amplification for <i>in vitro</i> and <i>in planta</i> gRNA testing
In_vitro_R	AAAAGCACCGACTCGGTGCCAC	<i>in vitro</i> gRNA testing
gRNA-P1	GAAATTAATACGACTCACTATAGG GGTTTTTGGAAAATTTATTT GTTTTAGAGCTAGAAATAGC	<i>in vitro</i> gRNA testing
gRNA-P2	GAAATTAATACGACTCACTATAGG GTGTAAATTCTTCGTCTCCT GTTTTAGAGCTAGAAATAGC	<i>in vitro</i> gRNA testing
gRNA-P3	GAAATTAATACGACTCACTATAGG GGTCATAATTGCAGAGAAAA GTTTTAGAGCTAGAAATAGC	<i>in vitro</i> gRNA testing
gRNA-P4	GAAATTAATACGACTCACTATAGG ACAAAGAAAGGAAAAACAG GTTTTAGAGCTAGAAATAGC	<i>in vitro</i> gRNA testing
gRNA-P5	GAAATTAATACGACTCACTATAGG ACTTATATCAGAAATAAAAA GTTTTAGAGCTAGAAATAGC	<i>in vitro</i> gRNA testing
gRNA-P6	GAAATTAATACGACTCACTATAGG GGCTAATCTTTGTTTGTCAA GTTTTAGAGCTAGAAATAGC	<i>in vitro</i> gRNA testing
gRNA-B1	GAAATTAATACGACTCACTATAGG GCTGTTCGTAACAGTGTCCG GTTTTAGAGCTAGAAATAGC	<i>in vitro</i> gRNA testing
gRNA-B2	GAAATTAATACGACTCACTATAGG GAACAGCGAGTAAAGCTCTC GTTTTAGAGCTAGAAATAGC	<i>in vitro</i> gRNA testing
gRNA-B3	GAAATTAATACGACTCACTATAGG TCTTTACCAATCGGTGGTAT GTTTTAGAGCTAGAAATAGC	<i>in vitro</i> gRNA testing
gRNA-B4	GAAATTAATACGACTCACTATAGG CCGGTTGGTTACTCTGTATT GTTTTAGAGCTAGAAATAGC	<i>in vitro</i> gRNA testing
gRNA-B5	GAAATTAATACGACTCACTATAGG GCACGGAGTCCACTGCGATG GTTTTAGAGCTAGAAATAGC	<i>in vitro</i> gRNA testing
gRNA-B6	GAAATTAATACGACTCACTATAGG GTTTCTTCTAATCAGTTAAA	<i>in vitro</i> gRNA testing

	GTTTTAGAGCTAGAAATAGC	
gRNA-I1	GAAATTAATACGACTCACTATAGG GTAGAAAAAAGGCGGGAAGT GTTTTAGAGCTAGAAATAGC	<i>in vitro</i> gRNA testing
gRNA-I2	GAAATTAATACGACTCACTATAGG GTTCCAACCTCGTACCAGACT GTTTTAGAGCTAGAAATAGC	<i>in vitro</i> gRNA testing
gRNA-I3	GAAATTAATACGACTCACTATAGG GAAGAAAACATATTGTGATC GTTTTAGAGCTAGAAATAGC	<i>in vitro</i> gRNA testing
gRNA-I4	GAAATTAATACGACTCACTATAGG AACAAAGTCAACGACAAAAG GTTTTAGAGCTAGAAATAGC	<i>in vitro</i> gRNA testing
gRNA-I5	GAAATTAATACGACTCACTATAGG ACTATTTAGTTCAAGCTTAA GTTTTAGAGCTAGAAATAGC	<i>in vitro</i> gRNA testing
gRNA-I6	GAAATTAATACGACTCACTATAGG ATTTGACCTTAGCAAGTTGA GTTTTAGAGCTAGAAATAGC	<i>in vitro</i> gRNA testing
L5AD5-F-pChimera	CGGGTCTCAGGCAGGATGGGCAGTCTGATTGAACAAAGCACCAGT GG	Cloning 6×(pre-tRNA-gRNA) into pEnChimera or pChimera
L3AD5-R	TAGGTCTCCAAACGGATGAGCGACAGCAAACAAAAAAAAAAGCAC CGACTCG	Cloning 6×(pre-tRNA-gRNA) into pEnChimera or pChimera
S5AD5-F-pChimera	CGGGTCTCAGGCAGGATGGGCAGTCTGATTG	Cloning 6×(pre-tRNA-gRNA) into pEnChimera or pChimera
S3AD5-R	TAGGTCTCCAAACGGATGAGCGACAGCAAAC	Cloning 6×(pre-tRNA-gRNA) into pEnChimera or pChimera
Sg-F-Pr6	TAGGTCTCCGAAAATTTATTTGTTTTAGAGCTAGAA	<i>gRNA-P</i> 6×(pre-tRNA-gRNA) assembly
Sg-R-Pr6	ATGGTCTCATTTCCAAAAACCTGCACCAGCCGGGAA	<i>gRNA-P</i> 6×(pre-tRNA-gRNA) assembly
Sg-F-Pr5	TAGGTCTCCTCTTCGTCTCCTGTTTTAGAGCTAGAA	<i>gRNA-P</i> 6×(pre-tRNA-gRNA) assembly
Sg-R-Pr5	ATGGTCTCAAAGAATTTACACTGCACCAGCCGGGAA	<i>gRNA-P</i> 6×(pre-tRNA-gRNA) assembly
Sg-F-Pr4	TAGGTCTCCTTGCAGAGAAAAGTTTTAGAGCTAGAA	<i>gRNA-P</i> 6×(pre-tRNA-gRNA) assembly
Sg-R-Pr4	ATGGTCTCAGCAATTATGACCTGCACCAGCCGGGAA	<i>gRNA-P</i> 6×(pre-tRNA-gRNA) assembly
Sg-F-Pr3	TAGGTCTCCAAGGAAAAACAGTTTTAGAGCTAGAA	<i>gRNA-P</i> 6×(pre-tRNA-gRNA) assembly
Sg-R-Pr3	ATGGTCTCACCTTTCTTTTGTTCACCAGCCGGGAA	<i>gRNA-P</i> 6×(pre-tRNA-gRNA) assembly
Sg-F-Pr1	TAGGTCTCCCAGAAATAAAAAGTTTTAGAGCTAGAA	<i>gRNA-P</i> 6×(pre-tRNA-gRNA) assembly
Sg-R-Pr1	ATGGTCTCATCTGATATAAGTTGCACCAGCCGGGAA	<i>gRNA-P</i> 6×(pre-tRNA-gRNA) assembly
Sg-F-Pr2	TAGGTCTCCTTTGTTTGTCAAGTTTTAGAGCTAGAA	<i>gRNA-P</i> 6×(pre-tRNA-gRNA) assembly
Sg-r-Pr2	ATGGTCTCACAAAGATTAGCCTGCACCAGCCGGGAA	<i>gRNA-P</i> 6×(pre-tRNA-gRNA) assembly
Sg-F-B1	TAGGTCTCCTAACAGTGTCCGGTTTTAGAGCTAGAA	<i>gRNA-B</i> 6×(pre-tRNA-gRNA) assembly

Sg-R-B1	ATGGTCTCAGTTACGAACAGCTGCACCAGCCGGGAA	<i>gRNA-B 6×(pre-tRNA-gRNA) assembly</i>
Sg-F-B2	TAGGTCTCCAGTAAAGCTCTCGTTTTAGAGCTAGAA	<i>gRNA-B 6×(pre-tRNA-gRNA) assembly</i>
Sg-R-B2	ATGGTCTCA TACTCGCTGTTCTGCACCAGCCGGGAA	<i>gRNA-B 6×(pre-tRNA-gRNA) assembly</i>
Sg-F-B3	TAGGTCTCCAATCGGTGGTATGTTTTAGAGCTAGAA	<i>gRNA-B 6×(pre-tRNA-gRNA) assembly</i>
Sg-R-B3	ATGGTCTCAGATTGGTAAAGATGCACCAGCCGGGAA	<i>gRNA-B 6×(pre-tRNA-gRNA) assembly</i>
Sg-F-B4	TAGGTCTCCTTACTCTGTATTGTTTTAGAGCTAGAA	<i>gRNA-B 6×(pre-tRNA-gRNA) assembly</i>
Sg-R-B4	ATGGTCTCAGTAACCAACCGGTGCACCAGCCGGGAA	<i>gRNA-B 6×(pre-tRNA-gRNA) assembly</i>
Sg-F-B5	TAGGTCTCCTCCACTGCGATGGTTTTAGAGCTAGAA	<i>gRNA-B 6×(pre-tRNA-gRNA) assembly</i>
Sg-R-B5	ATGGTCTCATGGACTCCGTGCTGCACCAGCCGGGAA	<i>gRNA-B 6×(pre-tRNA-gRNA) assembly</i>
Sg-F-B6	TAGGTCTCCTAATCAGTTAAAGTTTTAGAGCTAGAA	<i>gRNA-B 6×(pre-tRNA-gRNA) assembly</i>
Sg-R-B6	ATGGTCTCAATTAGAAGAACTGCACCAGCCGGGAA	<i>gRNA-B 6×(pre-tRNA-gRNA) assembly</i>
Sg-F-Int1	TAGGTCTCCAAGCGGGAAGTGTTTTAGAGCTAGAA	<i>gRNA-I 6×(pre-tRNA-gRNA) assembly</i>
Sg-R-Int1	ATGGTCTCACCTTTTTTCTACTGCACCAGCCGGGAA	<i>gRNA-I 6×(pre-tRNA-gRNA) assembly</i>
Sg-F-Int2	TAGGTCTCCTCGTACCAGACTGTTTTAGAGCTAGAA	<i>gRNA-I 6×(pre-tRNA-gRNA) assembly</i>
Sg-R-Int2	ATGGTCTCAACGAGTTGGAAGTGCACCAGCCGGGAA	<i>gRNA-I 6×(pre-tRNA-gRNA) assembly</i>
Sg-F-Int3	TAGGTCTCCCATATTGTGATCGTTTTAGAGCTAGAA	<i>gRNA-I 6×(pre-tRNA-gRNA) assembly</i>
Sg-R-Int3	ATGGTCTCATATGTTTTCTTCTGCACCAGCCGGGAA	<i>gRNA-I 6×(pre-tRNA-gRNA) assembly</i>
Sg-F-Int4	TAGGTCTCCCAACGACAAAAGGTTTTAGAGCTAGAA	<i>gRNA-I 6×(pre-tRNA-gRNA) assembly</i>
Sg-R-Int4	ATGGTCTCAGTTGACTTTGTTTGCACCAGCCGGGAA	<i>gRNA-I 6×(pre-tRNA-gRNA) assembly</i>
Sg-F-Int5	TAGGTCTCCGTTCAAGCTTAAGTTTTAGAGCTAGAA	<i>gRNA-I 6×(pre-tRNA-gRNA) assembly</i>
Sg-R-Int5	ATGGTCTCAGAACTAAATAGTTGCACCAGCCGGGAA	<i>gRNA-I 6×(pre-tRNA-gRNA) assembly</i>
Sg-F-Int6	TAGGTCTCCTTAGCAAGTTGAGTTTTAGAGCTAGAA	<i>gRNA-I 6×(pre-tRNA-gRNA) assembly</i>
Sg-R-Int6	ATGGTCTCACTAAGGTCAAATTGCACCAGCCGGGAA	<i>gRNA-I 6×(pre-tRNA-gRNA) assembly</i>
DNA_template_02880_body-F	TGTCTTCTATCTCGCTGCG	<i>in planta gRNA testing</i>
DNA_template_02880_body-R	ACAGGAGCTTCAACATTCC	<i>in planta gRNA testing</i>
3a-int-templ-fw-v3	TGGGGTCAAGTTGCAATTTCT	<i>in planta gRNA testing</i>
3a-int-templ-rev-v3	GTTCTATTTGCTTCCACGGTTTA	<i>in planta gRNA testing</i>
eIF(iso)4E _Fw	AAGAGTTAAATGCTCTGATGGAC	<i>in planta gRNA testing</i>
eIF(iso)4E _Rv	ACAAGTGAATTTGAGCATTCT	<i>in planta gRNA testing</i>
CLE10-fw	CGTCTCAATTGATGTAGCAGCA	<i>in planta gRNA testing</i>
CLE10-rev	GACGCCGTACCTTTGATCAA	<i>in planta gRNA testing</i>
CLE9-fw	TGTGCTTCTTCTGTGTTGCA	<i>in planta gRNA testing</i>
CLE9-rev	GGTTTTAGTTGTGAAGCGGGT	<i>in planta gRNA testing</i>
GLBR-fw	TCAACTTAACCGGCCAAATCT	<i>in planta gRNA testing</i>

GLRB-rev	GCACTGGCCAATGGAACC	<i>in planta gRNA testing</i>
FWA-fw	AGCGTCTACCAAATCTACACTTT	<i>in planta gRNA testing</i>
FWA-rev	GCGCTCGTATGAATGTTGAATG	<i>in planta gRNA testing</i>
CLV3-F A00954	TCTCGCCCTTGTAGGCTTACG	<i>in planta gRNA testing</i>
CLV3-R A02289	GCTGAAAGTTGTTTCTTGGCTG	<i>in planta gRNA testing</i>
Pr-RT-F1	ACTTATATCAGAAATAAAAA	RT-PCR
Pr-RT-F2	GGCTAATCTTTGTTTGTCAA	RT-PCR
Pr-RT-F3	ACAAAAGAAAGGAAAAACAG	RT-PCR
Pr-RT-F4	GGTCATAATTGCAGAGAAAA	RT-PCR
Pr-RT-F5	GTGTAAATTCTTCGTCTCCT	RT-PCR
Pr-RT-F6	GGTTTTTGAAAATTTATTT	RT-PCR
AG-RT-F1	GCTGTTTCGTAACAGTGTCCG	RT-PCR
AG-RT-F2	GAACAGCGAGTAAAGCTCTC	RT-PCR
AG-RT-F3	TCTTTACCAATCGGTGGTAT	RT-PCR
AG-RT-F5	GCACGGAGTCCACTGCGATG	RT-PCR
AG-RT-F6	GTTTCTTCTAATCAGTTAAA	RT-PCR
Interg-RT-F1	GTAGAAAAAAGGCGGGAAGT	RT-PCR
Interg-RT-F2	GTTCCAACCTCGTACCAGACT	RT-PCR
Interg-RT-F3	GAAGAAAACATATTGTGATC	RT-PCR
Interg-RT-F4	AACAAAGTCAACGACAAAAG	RT-PCR
Interg-RT-F5	ACTATTTAGTTCAAGCTTAA	RT-PCR
Interg-RT-F6	ATTTGACCTTAGCAAGTTGA	RT-PCR
Ub-RT-F	CAGATCGATAGCAGCACCTTGG	RT-PCR
Ub-RT-R	GGAAATGGCATCGAAACGG	RT-PCR
gRNA-R	GCACCGACTCGGTGCCAC	RT-PCR
SNP1v3-KASP-Rev-A	GAAGGTGACCAAGTTCATGCTCCATTGGACTTACGATTGGA	KASP assay
SNP1v3-KASP-Rev-B	GAAGGTCGGAGTCAACGGATTCCATTGGACTTACGATTGGG	KASP assay
SNP1v3-KASP-Fw	AACTTGAAACAATGGAATACACAATC	KASP assay
SNP1-KASP-Fw-A	GAAGGTGACCAAGTTCATGCTTTTCTCTCGTTCCAAGACAGCG	KASP assay
SNP1-KASP-Fw-B	GAAGGTCGGAGTCAACGGATTTTCTCTCGTTCCAAGACAGCA	KASP assay
SNP1-KASP-Rev	TTCAAAGTCCATGTACGTTGG	KASP assay
SNP2v3-KASP-Fw-A	GAAGGTGACCAAGTTCATGCTCCCAATGGCCTATATGTCAA	KASP assay
SNP2v3-KASP-Fw-B	GAAGGTCGGAGTCAACGGATTCCCAATGGCCTATATGTCAT	KASP assay
SNP2v3-KASP-Rev	ATCACATGTAGGCCTTTAAGGTTT	KASP assay

SNP3-KASP-Rev-A	GAAGGTGACCAAGTTCATGCTACCCTCATAAGATTCACCAAACCT	KASP assay
SNP3-KASP-Rev-B	GAAGGTCGGAGTCAACGGATTACCCTCATAAGATTCACCAAACC	KASP assay
SNP3-KASP-Fw	TGTCTTTGATTGCTAATTGTTATGTG	KASP assay
SNP4-KASP-Fw-A	GAAGGTGACCAAGTTCATGCTGATTTTCGTGCTGGGGG	KASP assay
SNP4-KASP-Fw-B	GAAGGTCGGAGTCAACGGATTGATTTTCGTGCTGGGGA	KASP assay
SNP4-KASP-Rev	TCAACAAAGAAGTAAGGCGAG	KASP assay
SNP5-KASP-Fw-A	GAAGGTGACCAAGTTCATGCTTCGCTGTTTCGTAACAGTGTC	KASP assay
SNP5-KASP-Fw-B	GAAGGTCGGAGTCAACGGATTTCGCTGTTTCGTAACAGTGTT	KASP assay
SNP5-KASP-Rev	TGGACTCCGTGCCAATTA	KASP assay
SNP5v3-KASP-Fw-A	GAAGGTGACCAAGTTCATGCTCCTCGAACTGCTTTTGAAGGT	KASP assay
SNP5v3-KASP-Fw-B	GAAGGTCGGAGTCAACGGATTTCGCTGTTTCGTAACAGTGTT	KASP assay
SNP5v3-KASP-Rev	CTCTCTGCCTCACAAGTGTCT	KASP assay
SNP5v4-KASP-Rev-A	GAAGGTGACCAAGTTCATGCTGGCGTATTGGGTCCTCCA	KASP assay
SNP5v4-KASP-Rev-B	GAAGGTCGGAGTCAACGGATTGGCGTATTGGGTCCTCCT	KASP assay
SNP5v4-KASP-Fw	ACACTCTCTGTGGGAAGCC	KASP assay
SNP6-KASP-Fw-A	GAAGGTGACCAAGTTCATGCTGCGATATTGCACGGTATAGAATA	KASP assay
SNP6-KASP-Fw-B	GAAGGTCGGAGTCAACGGATTGCGATATTGCACGGTATAGAATC	KASP assay
SNP6-KASP-Rev	CAAGATCACAACTAGCATCAGC	KASP assay
SNP7-KASP-Rev-A	GAAGGTGACCAAGTTCATGCTGGATTCTGGGATAAGATGTGTAAA	KASP assay
SNP7-KASP-Rev-B	GAAGGTCGGAGTCAACGGATTGGATTCTGGGATAAGATGTGTAA	KASP assay
SNP7-KASP-Fw	AAAATGTCTATAAACTATTGCTTTTGC	KASP assay
SNP6v2-KASP-Rev-A	GAAGGTGACCAAGTTCATGCTAGTCTGGTACGAGTTGGAACAT	KASP assay
SNP6v2-KASP-Rev-B	GAAGGTCGGAGTCAACGGATTAGTCTGGTACGAGTTGGAACAC	KASP assay
SNP6v2-KASP-Fw	TCATGTGATATACCATTTACACCG	KASP assay
SNP6v3-KASP-Rev-A	GAAGGTGACCAAGTTCATGCTTGTGTGCATGGCCGAACCTTAT	KASP assay
SNP6v3-KASP-Rev-B	GAAGGTCGGAGTCAACGGATTGTGTGCATGGCCGAACCTTAA	KASP assay
SNP6v3-KASP-Fw	ATTTTTGTAGGCTCAAATCAATTG	KASP assay
SNP8-KASP-Fw	CCAGGAGGAACACAAAGACA	KASP assay
SNP8-KASP-Rev-A	GAAGGTGACCAAGTTCATGCTGAGTGCAACTCAAAGTGTAGCTT	KASP assay
SNP8-KASP-Rev-B	GAAGGTCGGAGTCAACGGATTGAGTGCAACTCAAAGTGTAGCTA	KASP assay
SNP8v2-KASP-Fw-A	GAAGGTGACCAAGTTCATGCTACTTCAATATCAGAAAATAATAGC CA	KASP assay
SNP8v2-KASP-Fw-B	GAAGGTCGGAGTCAACGGATTACTTCAATATCAGAAAATAATAGC CG	KASP assay

SNP8v2-KASP-Rev	CTCGTGGAGGTAAAAATGACC	KASP assay
SNP8v3-KASP-Rev-A	GAAGGTGACCAAGTTCATGCTATTTTCCAAACAGAGCGTTTAGAC	KASP assay
SNP8v3-KASP-Rev-B	GAAGGTCGGAGTCAACGGATTATTTTCCAAACAGAGCGTTTAGAA	KASP assay
SNP8v3-KASP-Fw	AACATTTCTCTCAGTGAAAATTGG	KASP assay
SNP8v4-KASP-Fw-A	GAAGGTGACCAAGTTCATGCTTGGAATAATTATATTGTGTAGTTGAC TAGTG	KASP assay
SNP8v4-KASP-Fw-B	GAAGGTCGGAGTCAACGGATTTGGAATAATTATATTGTGTAGTTGAC TAGTT	KASP assay
SNP8v4-KASP-Rev	ACTCGCTTATTTTCCCCTTTC	KASP assay
SNP9v3-KASP-Rev-A	GAAGGTGACCAAGTTCATGCTAAATATTTAAGTTTTCTTCTTAAGCC T	KASP assay
SNP9v3-KASP-Rev-B	GAAGGTCGGAGTCAACGGATTAAATATTTAAGTTTTCTTCTTAAGC CA	KASP assay
SNP9v3-KASP-Fw	TTAAAATTAACCTTCCAATGAAATTACC	KASP assay
SNP10-KASP-Fw-A	GAAGGTGACCAAGTTCATGCTTTTTCCGGTTCAAAGTCAGAT	KASP assay
SNP10-KASP-Fw-B	GAAGGTCGGAGTCAACGGATTTTTTCCGGTTCAAAGTCAGAC	KASP assay
SNP10-KASP-Rev	CAGAAGAACGCAAAAGATTGTC	KASP assay
SNP11v2-KASP-Fw-A	GAAGGTGACCAAGTTCATGCTCTCATTACTCATCTTTCAATAGCCA CT	KASP assay
SNP11v2-KASP-Fw-B	GAAGGTCGGAGTCAACGGATTCTCATTACTCATCTTTCAATAGCCA CG	KASP assay
SNP11v2-KASP-Rev	TACCTTTAGGGGACTGGAGCT	KASP assay
SNP11v3-KASP-Fw-A	GAAGGTGACCAAGTTCATGCTTTGAAGTCCAATTCGTTTAATTCTTT	KASP assay
SNP11v3-KASP-Fw-B	GAAGGTCGGAGTCAACGGATTTTGAAGTCCAATTCGTTTAATTCTT C	KASP assay
SNP11v3-KASP-Rev	GGAAGCATATCATCATCCACTG	KASP assay
SNP11v4-KASP-Fw-A	GAAGGTGACCAAGTTCATGCTCACGACAGAAGACTGGGAAT	KASP assay
SNP11v4-KASP-Fw-B	GAAGGTCGGAGTCAACGGATTACGACAGAAGACTGGGAAC	KASP assay
SNP11v4-KASP-Rev	CATCTACTTGACATTGATTTGCCTA	KASP assay
SNP12-KASP-Fw-A	GAAGGTGACCAAGTTCATGCTGTATATAATTGTGTTTTGTTTCACAT GC	KASP assay
SNP12-KASP-Fw-B	GAAGGTCGGAGTCAACGGATTGTATATAATTGTGTTTTGTTTCACA TGG	KASP assay
SNP12-KASP-Rev	CATTTAATATATTTTCGTCAGTGGATTAGG	KASP assay