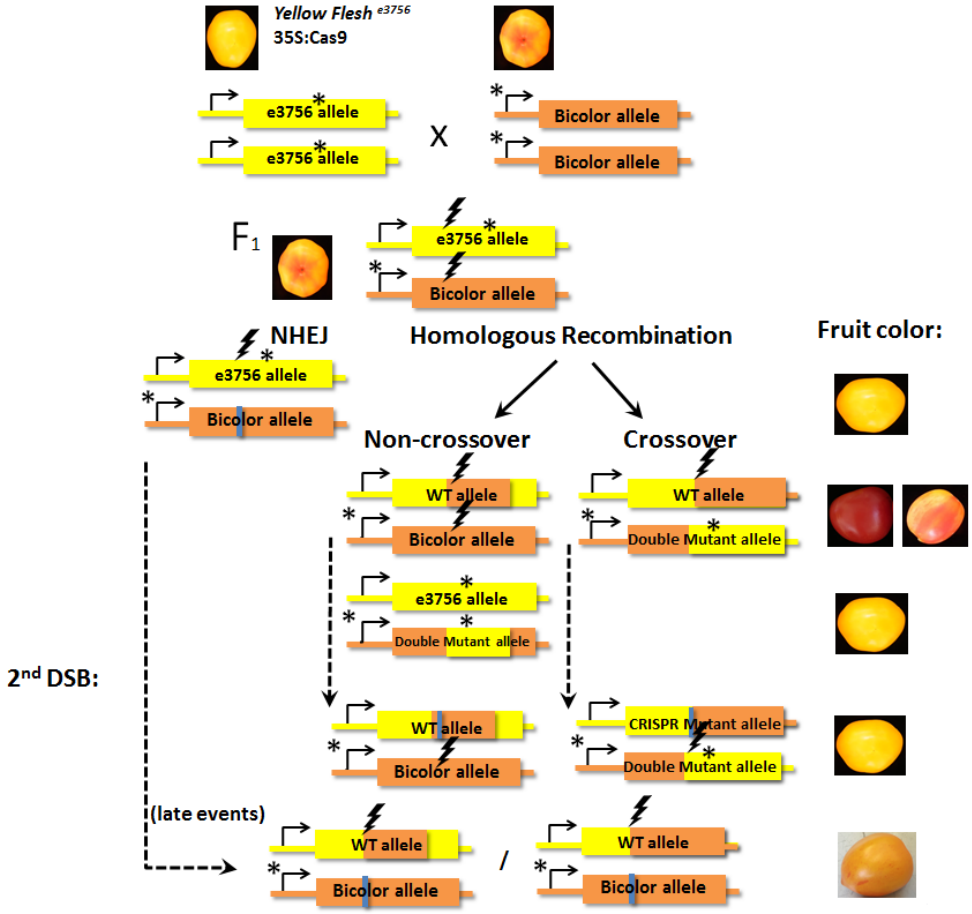
Supplementary Information:



Supplementary Figure 1 - Tomato fruit color assay and molecular analysis for possible outcomes of DNA double strand break (DSB) repair events (Extended Fig 1A). The cross of *yellow flesh*^{e3756} 35S:Cas9 and *bicolor*^{cc383} u6-26:Ps#1-sgRNA is expected to give F_1 plants with a pale Bicolor fruit phenotype. F_1 plants expressing both Cas9 and gRNA were selected. The gRNA was designed for DSB induction (shown as black lightning) in both alleles between the *yellow flesh*^{e3756} and *bicolor*^{cc383} mutations (*) 1086 bp from the deletion in bicolor and 556 bp from the *yellow flesh*^{e3756} mutation. In case of NHEJ repair of the *bicolor*^{cc383} allele, or of both alleles, fruit color is expected to be yellow following error-prone repair leaving indel footprints (blue line). In some of the cases of non-crossover or crossover, fruit color is expected to be red or bicolor with red spots in case of late event. Note that red fruits are shown as expected recombination products but were not found. Rather, fruits with red spots in a yellow or *bicolor* background were found and are shown (together with various putative products of HR-induced repair). In case that only one allele experienced DNA DSB, the second DNA DSB event (in dashed arrow) may be repaired independently. For example, NHEJ in the *bicolor*^{cc383} allele can

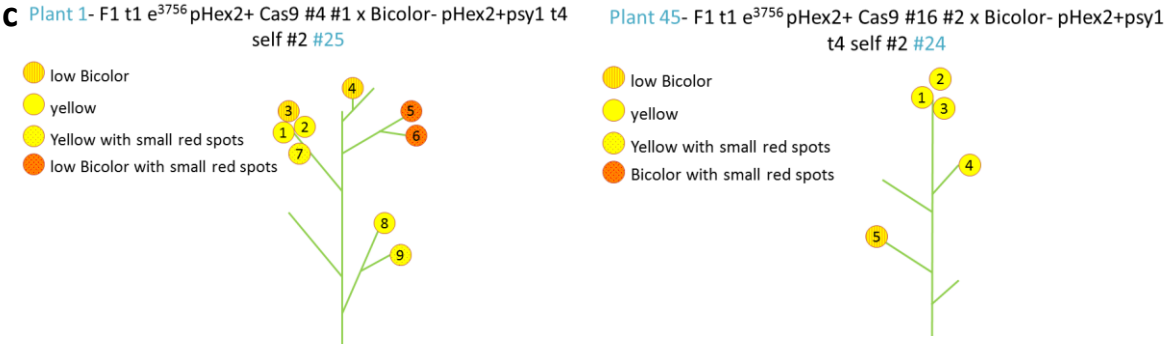
be followed by a late event of HR-mediated DSB repair of *yellow flesh*^{e3756}. This would give rise to yellow fruits with red spots—a phenotype that was observed. Generally, when repair via HR restored the WT allele, the CRISPR target site is still available for subsequent DSB induction. Therefore, yellow fruits may be the result of different repair mechanisms. Additional scenarios can be obtained depending on both timing and mechanism of DSB repair in each allele.

a

b

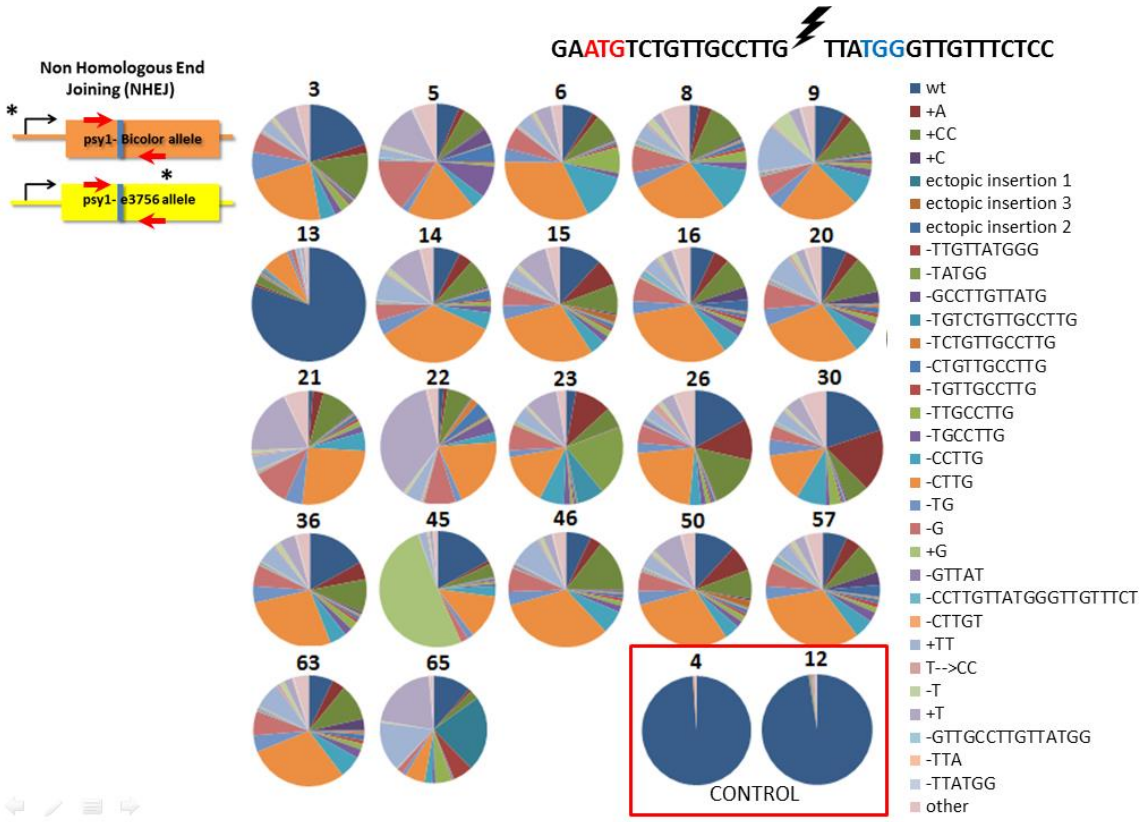
F1 plant	Fruit number	F2 plant	Cas9	gRNA	*Yellow flesh* allele (3756)	*Bicolor* allele (Bic)
1	1	11	-	+		Bic/4bp del
16	1	1	+	+	3756/4bp del	T insertion
16	1	2	-	-	3756	T insertion
16	2	3	+	-	3756	T insertion
16	2	4	-	+	3756	T insertion
16	3	1	+	-	3756	T insertion
16	3	2	+	-		T insertion
18	1	1	+	+		4bp del
18	1	4	+	+	T insertion	4bp del
18	2	2	+	+		4bp del
45	1	1	+	-	3756	G insertion
45	1	6	+	-	3756	G insertion
45	3	1	+	+	3756	G insertion
45	3	4	+	-	3756	G insertion
45	3	5	+	-	3756	G insertion

c

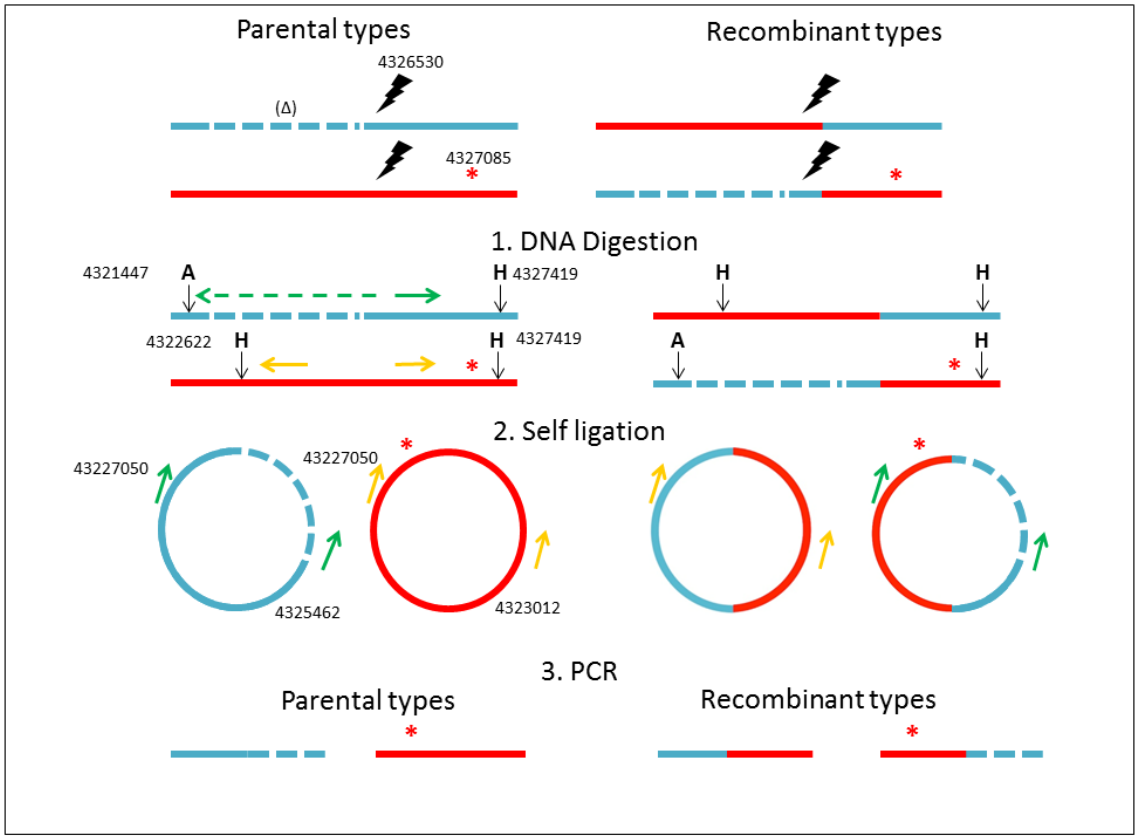


Supplementary Figure 2 - NHEJ germinal events derived from yellow fruits. A. *bicolor*^{cc383} allele in blue, *yellow flesh* e^{3756} in red, purple arrows represents primers that flanking the *bicolor* mutation and amplifying 2 PCR products in different sizes. Each product extracted from gel and then sequenced by Sanger. B. Allele specific PCR products of F_2 plants derived from yellow fruits of F_1 *yellow flesh* e^{3756} 35S:Cas9 x *bicolor*^{cc383} u6-26:Ps#1-sgRNA were sequenced by Sanger sequencing. All the plants

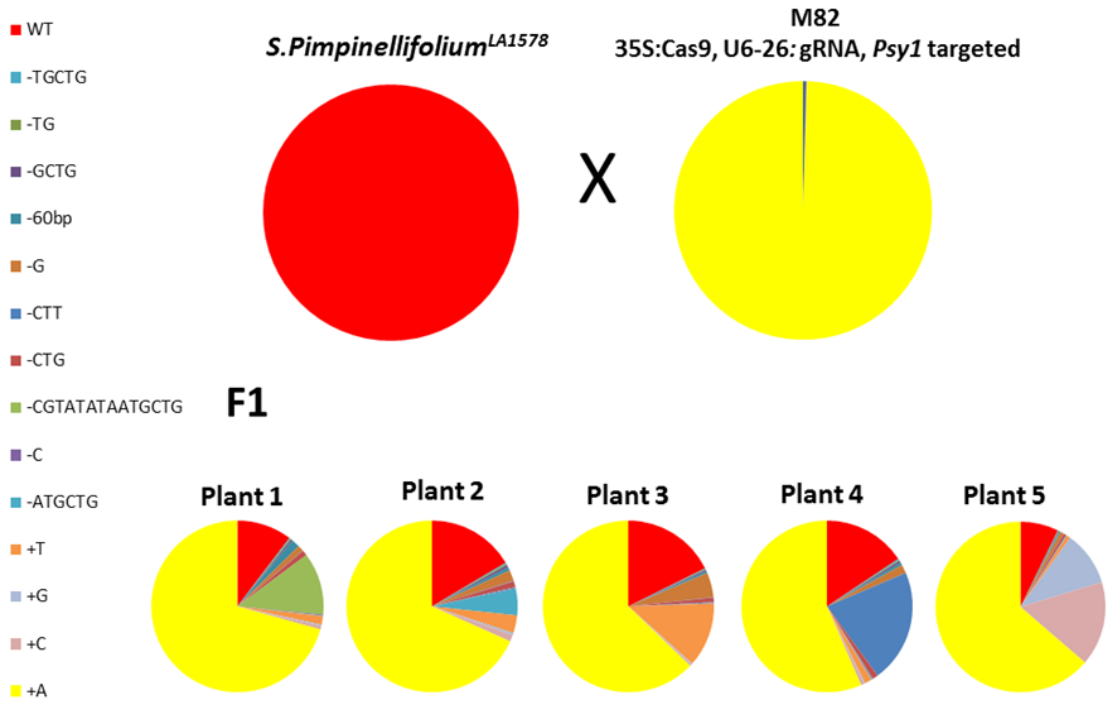
contained indels at the *bicolor* allele. 2 out of 11 contained indels in the *yellow flesh* allele as well. In some cases, the *yellow flesh* allele was not amplified due to homozygosity for *bicolor*. For some of the alleles we saw two bicolor alleles: the $bicolor^{cc383}$ allele and a $bicolor^{cc383}$ allele with a 4bp deletion mutation (e.g. Plant F2#11 derived from fruit#1 in F1). This apparent discrepancy between the F1 yellow fruit color and this *bicolor* genotype can be explained by independent events that occurred during the development of F1 flowers & fruits (different mutations in the maternal pericarp and ovule versus the non-mutated pollen cells as in F1 plant#1 fruit#1). C. In support of this, the fruits on F2 plant #11 were all *bicolor*. Moreover, the schematic map of F1 plant#1 shows that within some fruit clusters (as in F1 plant #1 fruit cluster 1,2,3,7) the DSB repair event seems to have occurred late, resulting in different fruit colors on the same fruit cluster. While in other clusters (as in F1 plant #16 fruit cluster 1,2,3) the DSB event seems to be early and characterized by uniform fruit phenotype and genotype. The illumina Hiseq result in Fig S3 are also supports early G insertion in F1 plant #45. . In plant F2#1 derived from F1#16 we saw 3 different alleles. This can be explained by the presence of both Cas9 and gRNA in this plant.



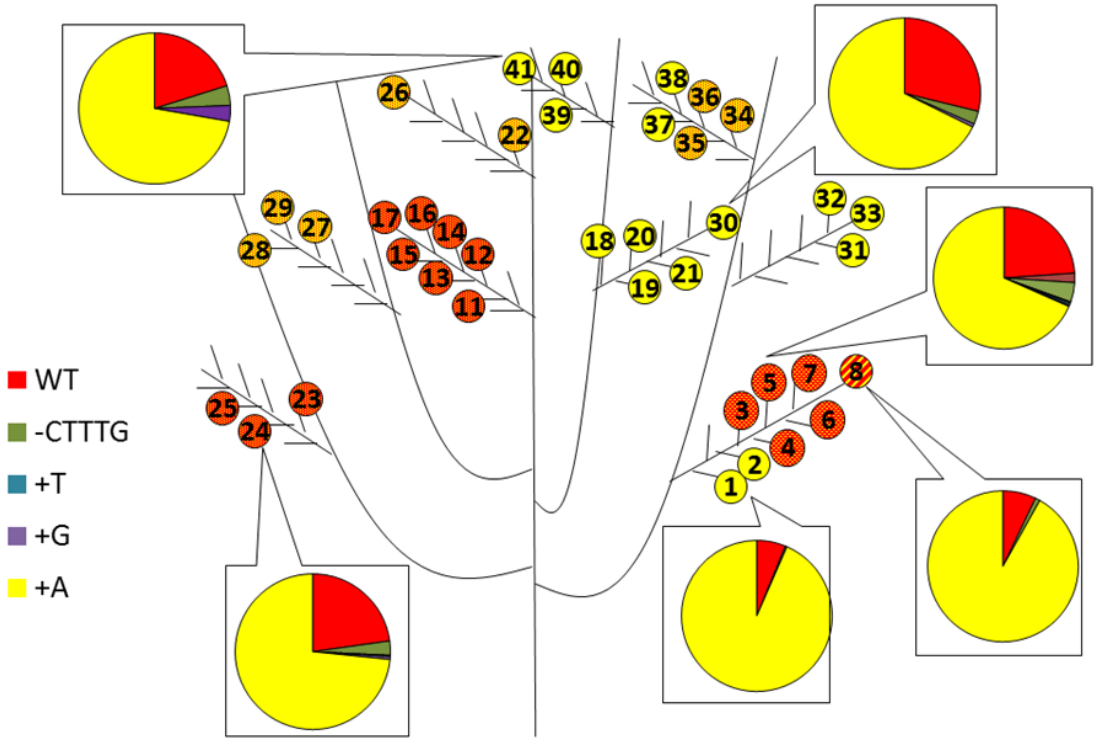
Supplementary Figure 3 - NHEJ repair in somatic cells. NHEJ footprints distribution in individual F_1 plants and in control plants (*yellow flesh* e^{3756} x *bicolor*^{cc383}) obtained by sequencing of PCR products amplified around the CRISPR-Cas9 induced DSB (lightning) with primers shown as red arrows. Each pie represents the total illumina Hiseq reads for single plant (250,000-850,000 reads per plant).



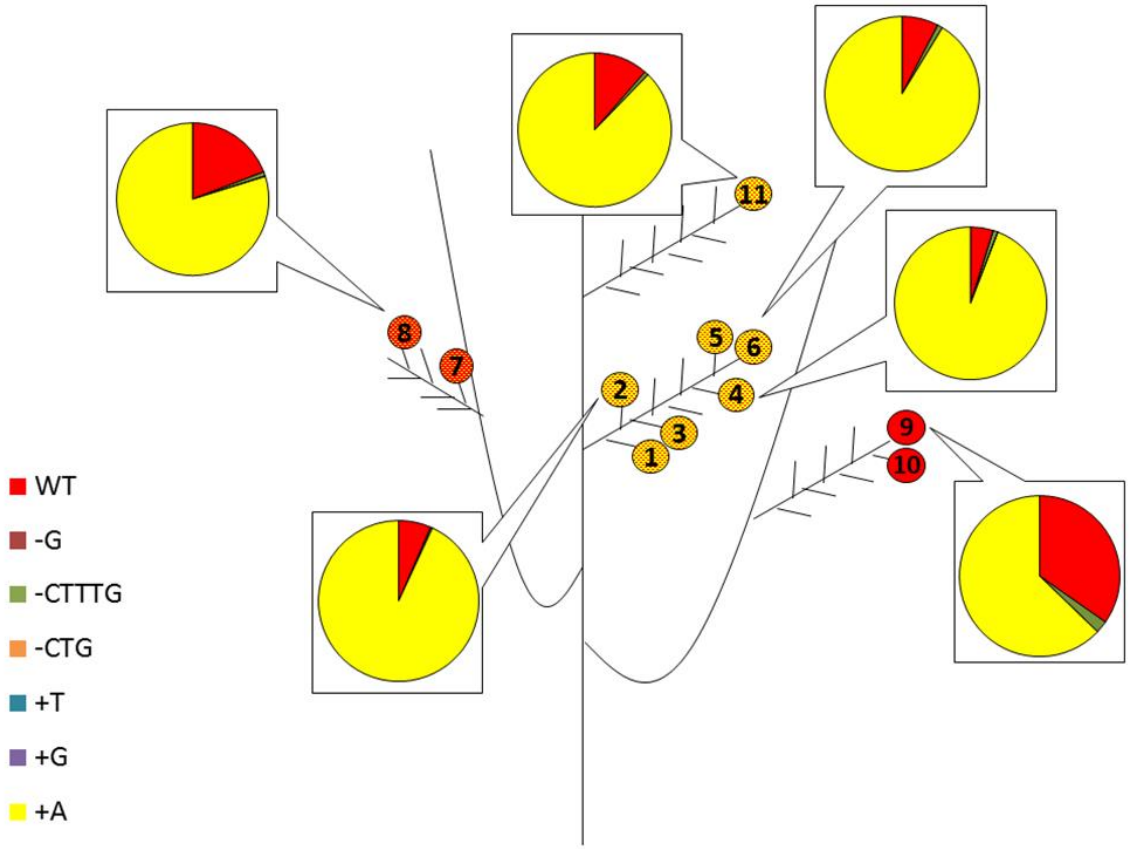
Supplementary Figure 4 - Inverse PCR detailed scheme for identification of recombinant DNA fragments. The specific coordinates of *bicolor*^{cc383} and *yellow flesh* e^{3756} mutation and of DNA DSB site on tomato chromosome 3 are listed above the *bicolor*^{cc383} and *yellow flesh* e^{3756} parental types alleles. (1) DNA from separate leaves samples was first digested with ApaI(A) and HindIII(H) and then blunted. The coordinates of ApaI(A) and HindIII(H) are listed next to the relevant restriction sites. (2) Each sample was self-ligated. (3) Each sample was amplified by two different primer sets shown as green and yellow arrows. Primers coordinates are listed next to each primer; for primer sequences see primers list). The reverse primers are designed specifically for each allele: The green dashed arrow represents a bicolor-specific primer that spans both sides of the deletion (hence the dashed line), The Yellow reverse primer is specific to the *yellow flesh* e^{3756} allele because it is inside the region that corresponds to the deletion in bicolor. Blue- *Bicolor* allele; red- *Yellow flesh* allele; Dashed blue line- *Bicolor* deletion, *- *Yellow flesh* mutation, lightning- DSB site.



Supplementary Figure 5 - Tomato SNPs assay for allele-specific DNA DSB repair- DNA was extracted from 4 leaves of M82 35S:Cas9 u6-26:Ps#2-sgRNA *psy1*$^{+A}$/ *psy1*$^{+A}$, *S. pimpinellifolium*^{LA1578} and 5 plants of their F_1 inbred. illumina sequencing was preformed and each pie represent a summary of 600,000-900,000 reads per plant.



Supplementary Figure 6 - A schematic map of Fruit color phenotypes throughout development and sequencing of DNA DSB repair footprints from fruit pericarp tissues using illumina sequencing. An example is shown for plant#1, which is a F_1 plant of M82 35S:Cas9 u6-26:Ps#2-sgRNA *psy1*$^{+A}$/ *psy1*$^{+A}$ x *S. pimpinellifolium*^{LA1578} . Fruit color phenotype was variable from red through red with small or big yellow sectors to yellow. Each pie was built out of 15,000-50,000 illumina sequencing reads per fruit.



Supplementary Figure 7 - DNA DSB repair event followed by fruit phenotype and pericarp specific illumina sequencing- plant#2. All details are similar to figure S6. This plant showed high level of *psy1*$^{+A}$. The conversion products in figure 2B are the progeny of this plant.

Supplementary Table 1- Primers list:

Primers for sgRNA targets:	
Ps#1 sgRNA F	attgGAATGTCTGTTGCCTTGTTA
Ps#1 sgRNA R	aaacTAACAAGGCAACAGACATT
Ps#2 sgRNA F	attgGAGCGTATATAATGCTGCTT
Ps#2 sgRNA R	aaacAAGCAGCATTATATACGCT
Primers for inverse PCR HR detection (allele specific primers):	
a 3756 bic hr f	tcagCTATGCTAATGACTCCCGAG
a bic hr r	agtcCATTCTCTATTCCGCATAGTGA
a 3756 r	tgacAACCGACCTAAATCGATCCG
b bic hr r	actgCATTCTCTATTCCGCATAGTGA
b 3756 r	gactactgAACCGACCTAAATCGATCCG
Primers for synthetic crossover - control plasmids :	
pupd_y1_f	gcgccgtctcgctcgtactCGAACGAGGGTCATC
pupd_y1_r	gcgccgtctcgctcgagcgCCATAATTGGAACACTCATCAA
pupd_ps_f	gcgccgtctcgctcgggagCAACCTTATTTTGTACTT
pupd_ps_r	gcgccgtctcgctcgagtaCAACATATCAAAATAGGTAT
Primers for NHEJ High throughput sequencing	
psy1 t1 htp f	GGTTTGCCTGTCTGTGGTCT
psy1 t1 htp r1	agtcCCATGAAACTTGTCCCATTTG
psy1 t1 htp r2	tcagCCATGAAACTTGTCCCATTTG
psy1 t1 htp r3	actgCCATGAAACTTGTCCCATTTG
psy1 t1 htp r4	tgacCCATGAAACTTGTCCCATTTG
psy1 t1 htp r5	gactCCATGAAACTTGTCCCATTTG
psy1 t1 htp r6	ctgaCCATGAAACTTGTCCCATTTG
nhej_psy1_t2_r	GCCTAAATACGGCACTTCCA
a_nhej_psy1_t2_f	agtcGTATCGCCCCTGAATCAAAG
b_nhej_psy1_t2_f	tcagGTATCGCCCCTGAATCAAAG
c_nhej_psy1_t2_f	tgacGTATCGCCCCTGAATCAAAG
d_nhej_psy1_t2_f	actgGTATCGCCCCTGAATCAAAG
e_nhej_psy1_t2_f	gactGTATCGCCCCTGAATCAAAG
f_nhej_psy1_t2_f	ctgaGTATCGCCCCTGAATCAAAG
nnn_a_nhej_psy1_t2_f	nnnnnnagtcGTATCGCCCCTGAATCAAAG
nnn_b_nhej_psy1_t2_f	nnnnnntcagGTATCGCCCCTGAATCAAAG
nnn_nhej_psy1_t2_r	nnnnnngcctAAATACGGCACTTCCA
Primers for Sanger sequencing of psy1 allele (SNPs detection)	
PSY1_1_F	tttgcagaagtcaagaaacagg
PSY1_t4_ident_R	gatgtcatcgtccgttctcc
PSY1 psnps F	acggtatcttcccaccttca
PSY1 psnps R2	atagtgttaattgtgtaggctcctt

PSY1 psnps F2	cgacgaggagtaaggtttgc
PSY1 psnps R	tcagtccatttcgttttcgt
psy1_t123_f	atgttgcagccattcagaga
psy1_t123_r	tgatcatggctcgtcactgt
psy1 term f	acaagtaccctgggttggag
psy1_term_r2	gcagtttttgtaggaggcaca
psy1_term_f2	tgtgcctcctacaaaaactgc
psy1 term r	tggattgaatcgaatttggataa
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20kb_up_t2_r	cccttaggctccgaagttgt
40kb_up_t2_f	cacataagaggacacgtttattca
40kb_up_t2_r	gccacggagaaaatagttga
Primers for NHEJ germinal events estimation (allele specific bends*):	
JF_F	tgcaaagtgctacgtgtcct
PSY1_1_R	aatgtgaacagcaacgcaaa

* This set of primers give 2 bends. We extracted each of them from gel and sequence.

Supplementary Note 1 - Plasmids list:

Plasmids for Tomato fruit color assay:

35S:Cas9 plasmid:

CaMV35S, atCas9, OCS terminator

>
GGGGACAAGTTTGTACAAAAAAGCAGGCTATGGATCCCCGGGATCATCTACTTCTGAAGACTCAGACTCAGACTAAGCAGGTGAC
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U6-26*:Ps#1* gRNA plasmid

U6-26 promoter, *Ps#1* gRNA

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GTCGGTGCTTTTTTTCTAGACCCAGCTTTCTTGTACAAAGTTGGCATT

Plasmids for Allele-specific DSB induction and allele dependent repair:

We used similar plasmids as for Tomato fruit color assay with different gRNA molecule:

GAGCGTATATAATGCTGCTTGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACC
GAGTCGGTGCTTTTTTT

Plasmids for synthetic crossover control (inverse PCR assay):

>R1

GGAGCAACCTTATTTTGTACTTTAAAAAATTCATTTTTTTTATTTTTTCGACTTTAAAGCCAAAATTATCCTTTATTTATGAAAGTGGA
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