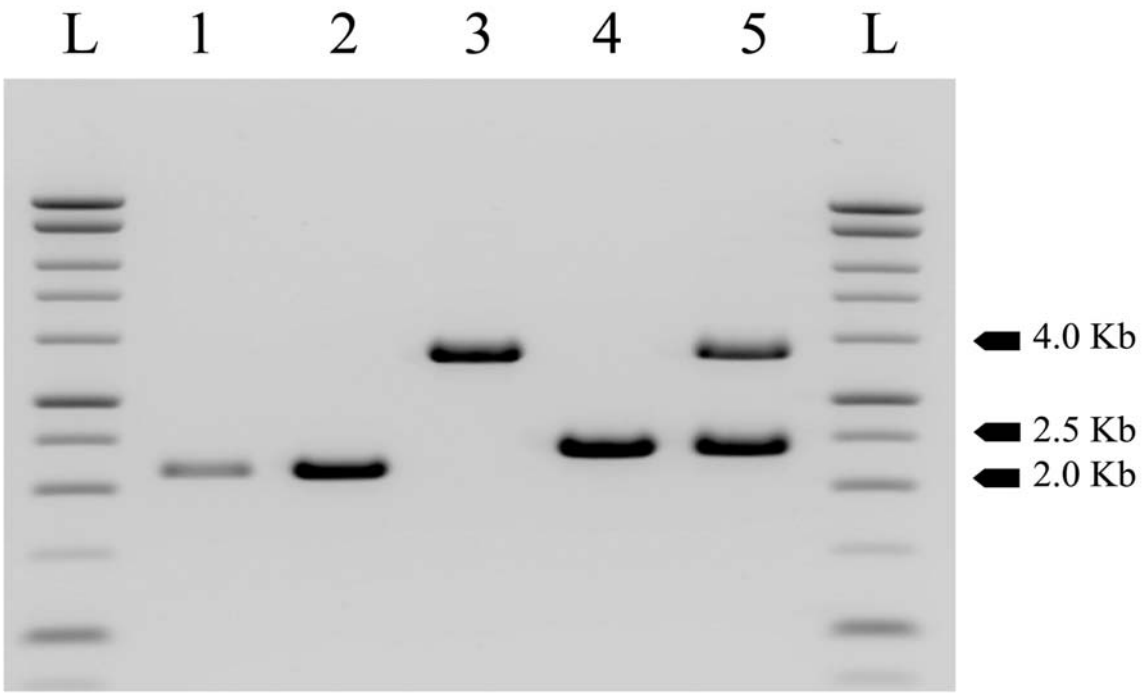
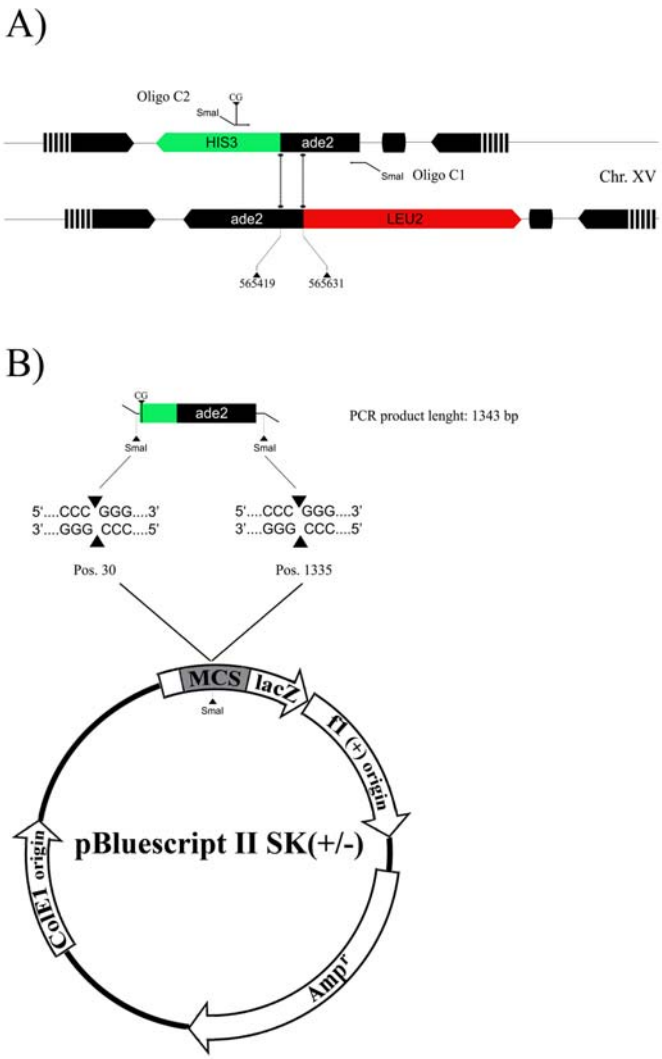
Supplementary Fig.1



PCR-based verification of the chromosome XV organization.
Colony-PCR were performed using two primers A (pos. 564332, 5'-ccttgcttcttgttactggata-3'), and H (pos. 566502, 5'-gtgagaagccgagaattttgta-3') that map apart from the stop and the start codons. Both ADE^{+} haploids, BY4741 and BY4742 (lane 1 and 2), give a product of 2171 bp. As consequence of the selectable markers integrations, the ADE^{-} haploids give different profiles: BY4741 (ade2Δ1-561, lane 3) a 3750 bp product; BY4742 (ade2Δ773-1716, lane 4) a 2380 bp band. The diploid strain 27.6.1 shows the expected dual-band profile of 2380 and 3750 bp fragments (lane 5) therefore excluding any event of mitotic crossover. As ladder, 1Kb Promega (Madison, USA) was used.

Supplementary Fig.2



Construction of the plasmid pMM-25.

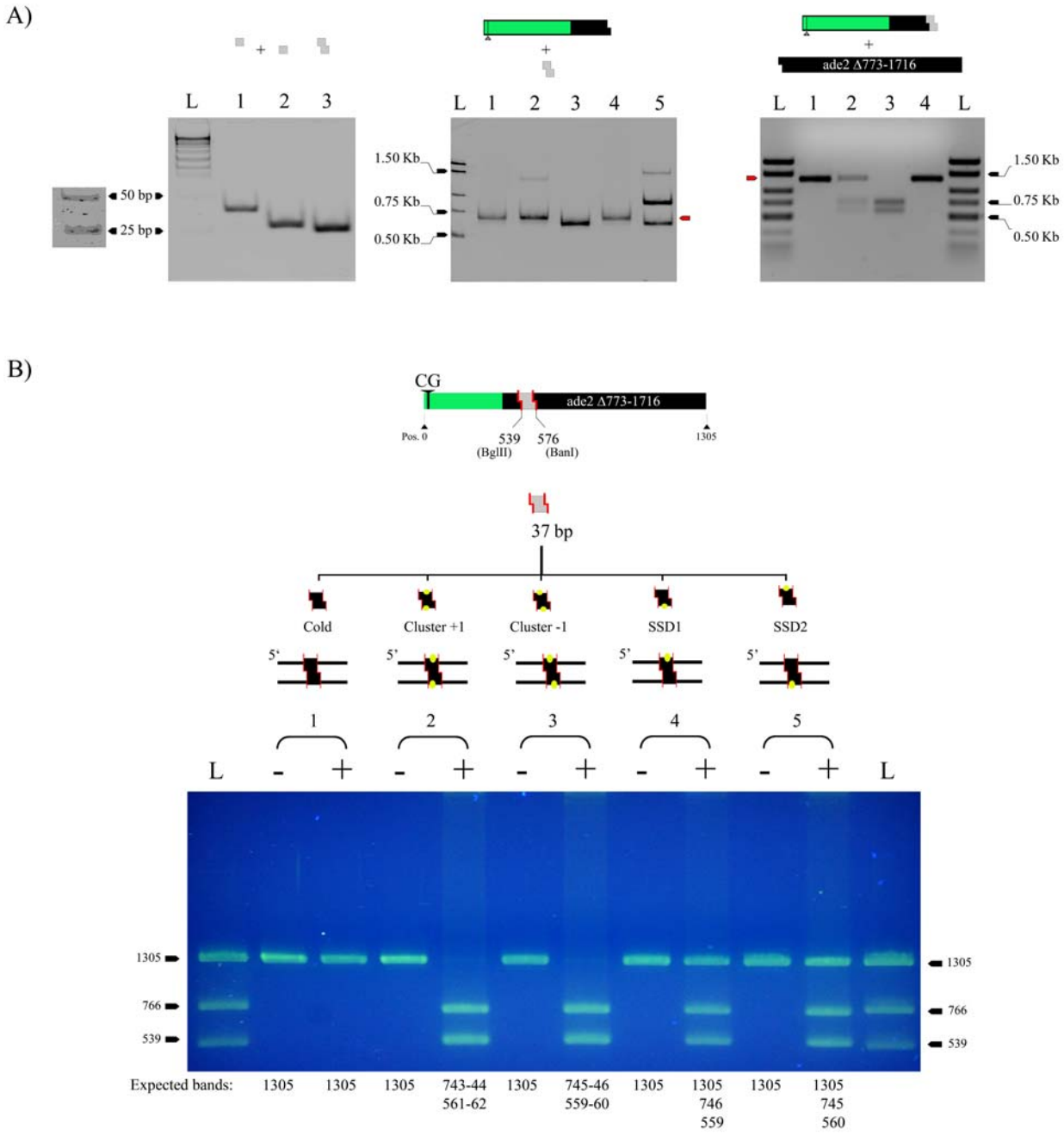
A) Chromosomal DNA amplification.

A selective PCR reaction amplified a DNA piece (hereafter named "cassette") on the chromosome XV with the ade2(Δ773-1716) allele. The diploid strain 27.6.1 was used as template in a reaction with adapter primers C1 and C2. The 1343 bp fragment is edged by two SmaI restriction sites used in the cloning strategy.

B) Cloning in pBluescript II SK+/-.

Both the vector pBluescript II SK (+/-) and the 1343 bp PCR fragment were SmaI-digested and purified by agarose gel electrophoresis using NucleoSpin ExtractII columns (Machery Nagel, Bethlehem, USA). The cassette (whose length dropped from 1343 to 1305 bp after SmaI digestion) was inserted in the Multi Cloning Site (MCS) of pBluescript SK II (+/-) generating a 4266 bp product named pMM-25. Note that the insert cassette has two additional bases (CG) that map in the HIS3 ORF.

Supplementary Fig.3



Synthesis and control of the uracil-containing cassettes.

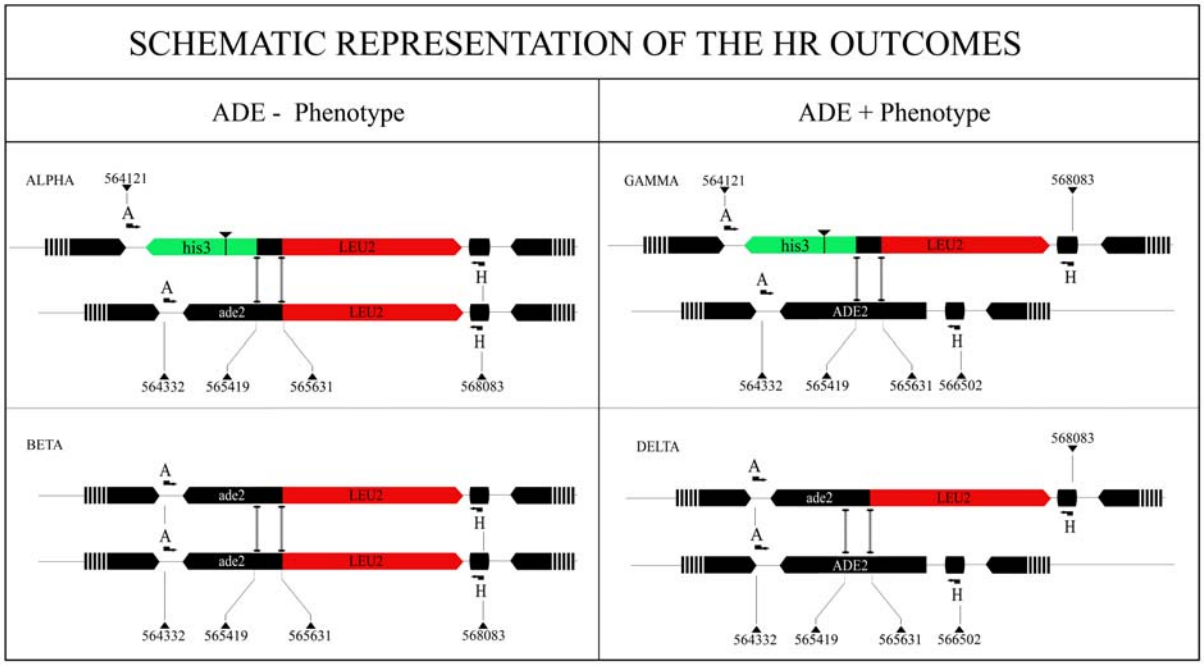
A) Ligase reactions.

The 37 bp fragment removed from the BglII/BanI digestion (described in Fig. 2) was replaced by DNA duplex molecules with identical sequence obtained by annealing the oligos listed in Supplementary Table 3.

(Left) The 12% polyacrylamide gel shows an example of the 37 bp annealing product. In lane L was loaded the Promega 50 bp DNA ladder (on the left side is shown a contrasted detail). 5 pmoles of the annealing reaction were loaded in

lane 1, and in lane 2 and 3 the equivalent amount of the single-strand oligos used.
(Center) The 5% polyacrylamide gel shows an example of the ligase reaction between the 539 bp fragment and the 37 bp duplex obtained by annealing the complementary oligos. A specific marker control was obtained by purifying the 576 bp band from BanI digestion of the 1.3 Kb cassette (lanes 1 and 4). 10% of the ligase reaction was loaded in lane 2 and the control (no T4 DNA ligase) in lane 3. As further marker control, a partial BglII digestion of the 1.3 Kb cassette was loaded in lane 5 (539, 766, and 1305 bp bands).
(Right) The 1.1% agarose gel shows the final ligase reaction between the 576 and 729 bp fragments to reconstitute the 1.3 Kb cassette.
As specific marker control, an aliquot of the 1.3 Kb fragment purified by the SmaI digestion of pMM-25 was loaded in lanes 1 and 4. 10% of the ligase reaction was loaded in lane 2, and the control (no T4 DNA ligase) in lane 3.
The red arrows on the side of gels indicate the height of the expected ligase product. The 1 Kb DNA ladder (Promega, Madison, USA) was loaded in lane L.
All gels were ethidium bromide stained.
B) UDG/hApe1-treatment of the cassettes.
Each 1305 bp cassette differs for the 37 bp central portion (not scaled). The one indicated as "Cold" has no damages, Cluster+1 and Cluster-1 have two uracil residues on opposite strands, while SSD1 and SSD2 have only one uracil residue.
About 100 ng of DNA were loaded in each lane of a denaturing 1.5% agarose gel. The symbols (-) or (+) indicate absence/presence of enzymatic treatment, respectively. The Cold cassette (no damages) was loaded in lane set 1 as the negative control. Cluster+1 and Cluster –1 were loaded in lane sets 2 and 3, while the single uracil containing cassettes, SSD1 and SSD2, were respectively loaded in lane sets 4 and 5. As molecular weight marker a partial BglII digestion of the 1305 cassette, was loaded on the side wells (L). The numbers depicted on the bottom of the gel indicate the expected length of the digestion products; black arrows on the sides the marker bands' height.

Supplementary Fig.4



Schematic representation of the HR outcomes.
The homologous recombination repair pathway involves two DNA molecules that have stretches of similar base sequences. In our system, the two chromosomes XV share a region of homology in the ADE2 locus (see Fig. 1B, pos. 565419-565631) where a DSB lesion was induced by repairing a cluster of uracil residues. Both ends of the DSB are engaged, either by independent strand invasion, or by second end capture, leading to the formation of a double Holliday junction. The junction can be processed by a resolvase either into non-crossover or crossover products. The figure describes the predicted outcomes grouped in two phenotypic classes: His^-/Ade^- (α and β); and, His^-/Ade^+ (γ and δ). Chromosome coordinates are indicated to explain the length of the amplification products with oligo mix A/H.

Supplementary Table 1

Oligo name	Sequence	Template	Amplified Marker	Description (3' OH)
U-Leu2	5'-ccgttaacagatctcacaatcatgactgctaattctttagtaaat**ctgtgcggtatttcacaccg**-3'	pRS305	LEU2 (2412 bp)	
L-Leu2	5'-attgattattacagctatgctgacaaatgactcttgttgcatggc**agattgtactgagagtgcac**-3'			
U-His3	5'-ggacacctgtaagcgttgatttctatgtatgaagtccacatttga**ctgtgcggtatttcacaccg**-3'	pRS303	HIS3 (1323 bp)	
L-His3	5'-atcaaatcttttcccggttgtggtatatttggtgtggaaatgttc**agattgtactgagagtgcac**-3'			
C1	5'- ttggtaagttacacgttc*ccggg*taatactaagtgattg -3'			Pos. 566346
C2	5'- tgatcgtatgaatca*cccggg*ctttctgctctgt**cg**catctttgccttcgtttatctt -3'			Pos. 565092
I	5'- agagatcgcaatctgaatcttg-3'			Pos. 565030
L	5'- gctttggaagtactgaaggat-3'			Pos. 565656

List of primers used in PCR reactions for the diploid strain construction, for the yeast integrative fragment (cassette) cloning in pMM-25 and for sequencing.
U/L Leu2 and U/L His3 primers: The underlined portion of the primer is the one that maps on the template vector, the extra 45 nucleotides form the flanking tail with sequence identity to the chromosomal target region.
C1 and C2 primers: The reaction with oligos C1 and C2 introduces two additional bases (CG in bold) in the HIS3 ORF, and two external SmaI endonuclease cut-sites (in italic) on the 1343 bp amplification product (see Supplementary Fig. 2A).
I and L primers: The reaction amplifies a part of the target region on chromosome XV. The 669 bp amplification product is then cloned in the vector pCR®4Blunt-TOPO (Invitrogen, Carlsbad, USA) for sequencing. That region spans the CG insertion and the position of the uracil residue (see Fig. 3A).

Supplementary Table 2

37 bp Duplex	5’ Phos (half BglII) 3’ OH (half BanI)	Name
Oligo No U-1 Oligo No U-2	gatctcacaatcatgactgctaattctttagtaaatg agtgttagtactgacgattaagaaatcatttaccacg	Cold Control Cassette (CCC)
Oligo U-24 Oligo U-19	gatctcacaatcatgactgctaa**u**tctttagtaaatg agtgttagtactgacgat**u**aagaaatcatttaccacg	Cluster Damage +1 (Clu +1)
Oligo U-21 Oligo U-20	gatctcacaatcatgactgc**u**aattctttagtaaatg agtgttagtactgacga**u**taagaaatcatttaccacg	Cluster Damage -1 (Clu -1)
Oligo U-24 Oligo No U-2	gatctcacaatcatgactgctaa**u**tctttagtaaatg agtgttagtactgacgattaagaaatcatttaccacg	Single Strand Damage 1 (SSD1)
Oligo No U-1 Oligo U-19	gatctcacaatcatgactgctaattctttagtaaatg agtgttagtactgacgat**u**aagaaatcatttaccacg	Single Strand Damage 2 (SSD2)

Polyacrylamide gel-purified 5’ end phosphate oligonucleotides used to generate double-stranded DNA molecules. U- followed by a number indicates the position of the uracil residue from 5’ phosphate termini.

Different combinations of the oligos generated 37 bp duplex containing no damage (CCC), a single uracil (SSD1 and SSD2) or two closely opposed uracil residues (Clu+1 and Clu-1). The DNA duplex sticky-ends have half restriction site of BglII and BanI endonucleases with ligase-active groups 5’ phosphate and 3’OH.