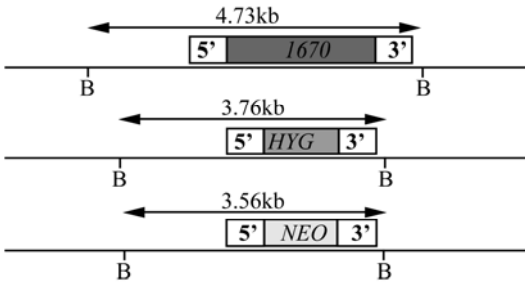
SUPPLEMENTARY MATERIALS

Figure S1. *LinJ18_V3.1670* inactivation in *Leishmania infantum*. (A) Schematic representation of the *LinJ18_V3.1670* locus in *L. infantum* before and after integration of the inactivation cassettes (hygromyin phosphotransferase B, *HYG*; neomycin phosphotransferase, *NEO*); and the relevant BglI restriction sites (B). (B) Southern blot analysis with genomic DNA digested with BglI from the WT and knock outs and hybridization with a 549bp probe from the 5' flank *LinJ18_V3.1670*. Molecular weights are indicated on the left, the various alleles are pinpointed on the right. 1, *L. infantum LinJ18_V3.1670* $^{\text{wild type}}$; 2, *LinJ18_V3.1670/HYG* ; 3, *LinJ18_V3.1670* null mutant *HYG/NEO*

A



B

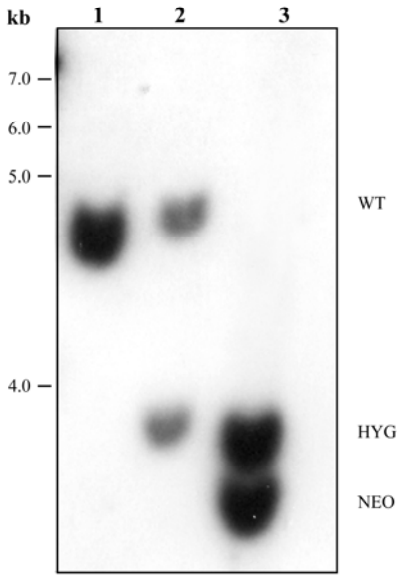


Figure S2. Events of BIR in the absence of repeated sequence in the targeting construct. (I) Double cross over of the *GSH1* locus with the inactivation cassette (M) would lead to integration of the cassette and disruption of *GSH1*. (II and III) However, if the inactivation cassette integrates into the homologous position by a single cross over to survive the selection pressure, it creates a double strand break (DSB) on the chromosome. (IV) After 5'-3' degradation of broken DNA, single strand DNA could initiate pairing and strand invasion of homologous duplex DNA at the level of 3' flank of *GSH1*. The invading strand is then extended by DNA synthesis till the 466bp direct repeats (yellow). This structure is unstable and can undergo dissociation (V) and reinvasion with homologous DNA (green) if it fails to pair with right side of the break. Second round of strand invasion and DNA synthesis takes place mediated by direct repeats (yellow and blue) but this time with misaligned sister chromatid or homologous chromosome (green) and synthesis can continue till end of chromosome (VI) resulting in the tandem duplication in one chromosomal homolog, keeping the other one intact .

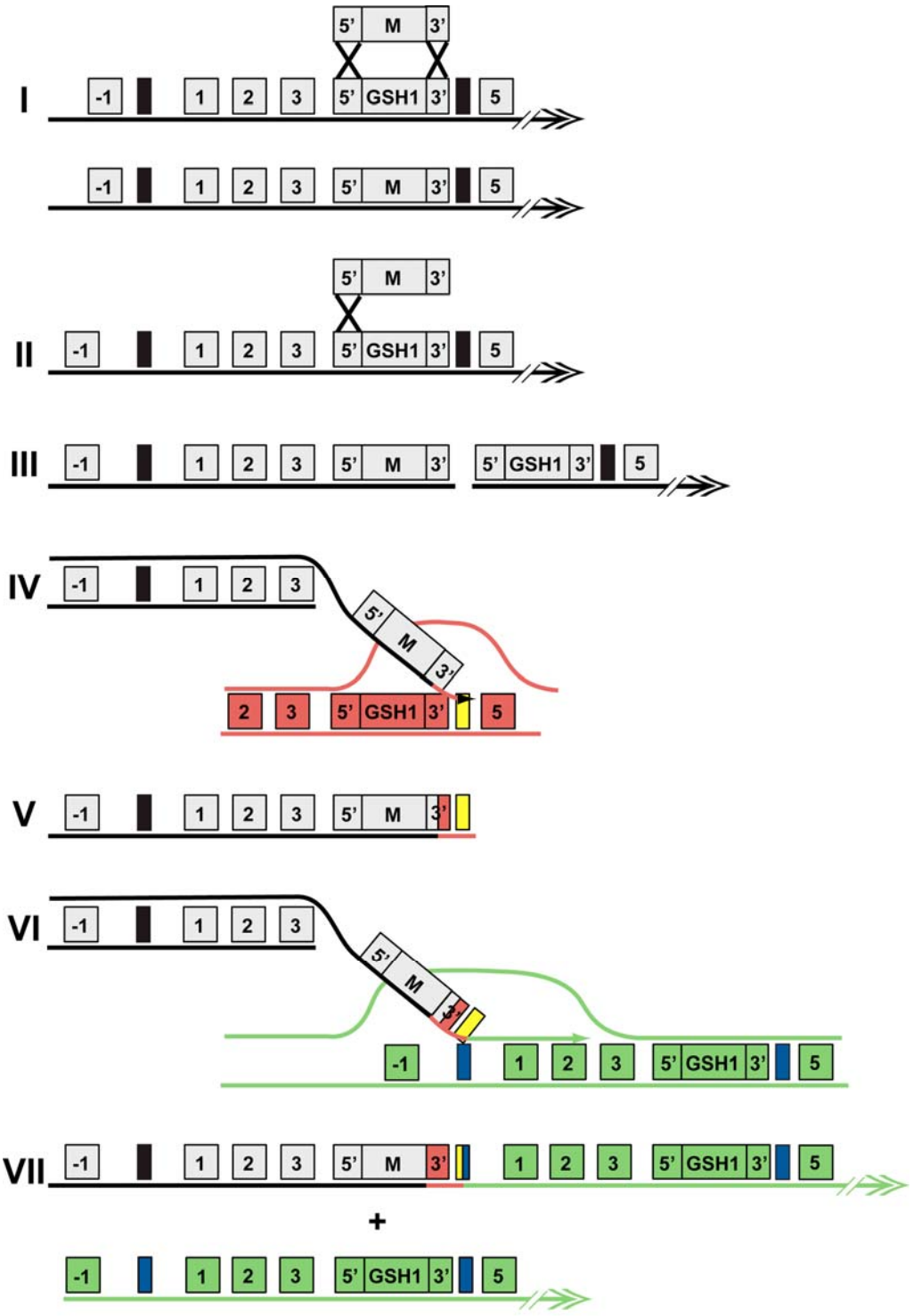

I
II
III
IV
V
VI
VII
-1
1
2
3
5'
M
3'
GSH1
5
+

Figure S3. Tandem duplication of gene clusters by homologous recombination via direct repeated sequences. (A) Tandem duplication of four genes on chromosome 2. *LmjF02.0220* and *LmjF02.0180* are the *SCA1* and *SCA2* genes respectively while the others are *SCGR* genes. The colour code relates to identities. The 412bp direct repeated sequences are indicated by the black arrows. Double arrowhead represents the telomere. (B) Tandem duplication of a two gene locus on chromosome 30. The genes *LmjF30.3500* and *LmjF30.3510* (*METK1*) are duplicated in tandem to form *LmjF30.3510* and *LmjF30.3520* (*METK2*). Black arrows represent the direct repeated sequences of 212bp (99% identity) through which this duplication is proposed to occur. Double arrowhead represents the telomere end.