Supplementary Information

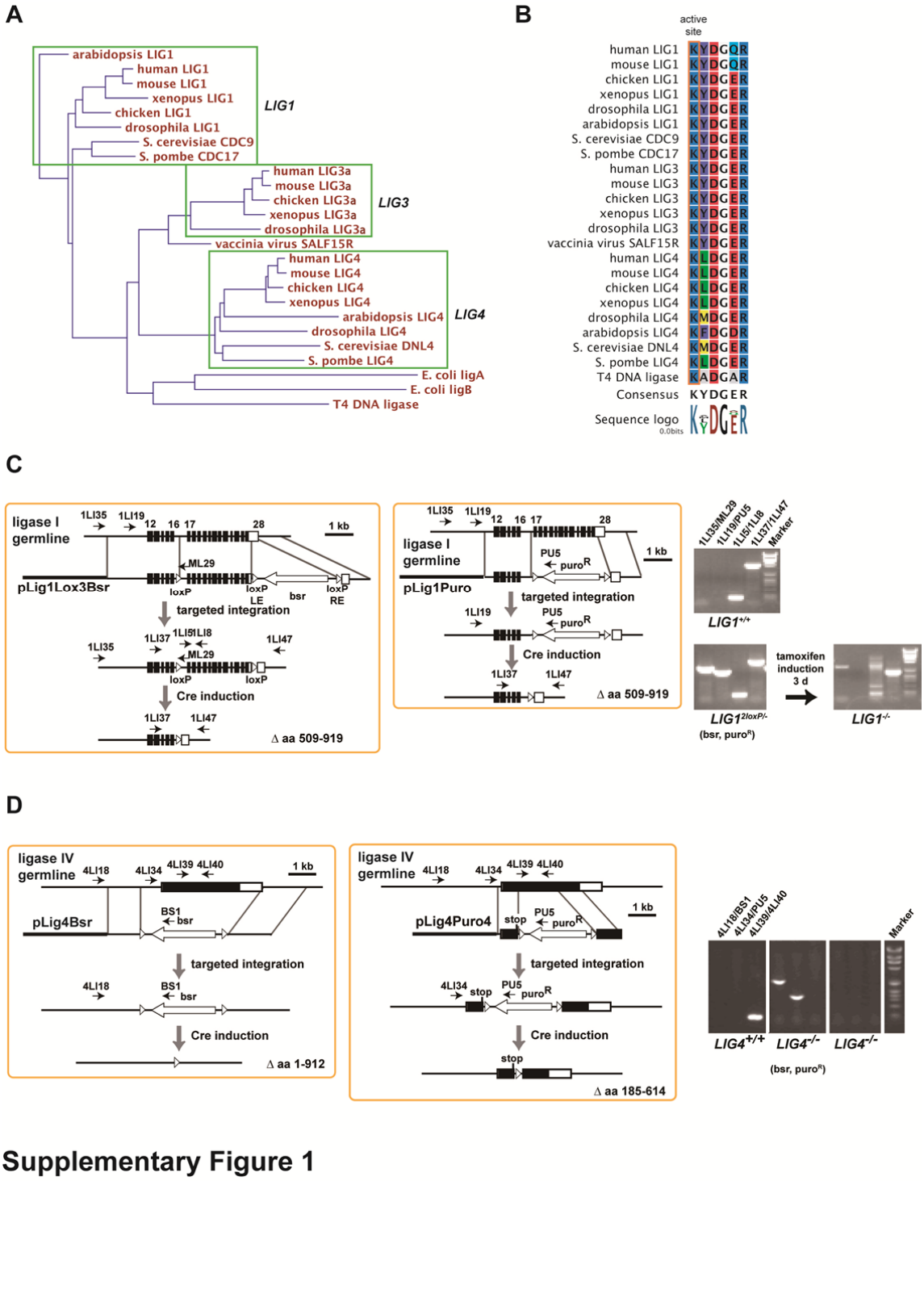
One Supplementary Table

Three Supplementary Figures 1- 3 and Legends

Supplementary Table 1

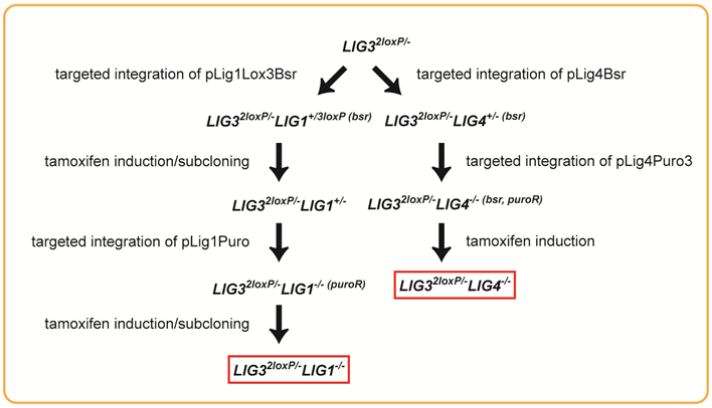
List of primer sequences used for genotyping PCR reactions. See text for more details.

oligo nucleotide	Sequence
1LI19	CCCCCTTCTACCCCAAATCCTGATAATTTT
1LI35	GACAGTGTTGGTGGGATGACACAGGGATCC
1LI37	TTTGTAGTCTGCTAGTAAGAAGATTGACAT
1LI47	GGGACTAGTTGGTAGGGGCAGTTAGTGCTAGGCT
1LI5	TCCCGGTGCTGCTGGAGCATGGCCTGGA
1LI8	TTGTACTCGCAGGTGAATGCAGCCTCCTCA
3LI32	GGTTCATGTCTGCAATTAAGTAAAAGTAGC
3LI34	TTAGCACCAGAATCAGACTTGGAGAGAAAT
3LI41	CAACTGCAACATCTAAACTAGTGGAT
3LI44	CATCCCTCTGCCTGTGCACACGTGGCTGTG
3LI62	GGACAGCAAGCAGCCTCCAAGCGGACTGAG
4LI18	TGCTTCATCTCTGCCTGAAAGACAATTTCA
4LI34	CGGCTGCGCGCGGCGGTTCTTTTCCGACTC
4LI39	CTGGATGGTGAACGTATGCAGATGCACAAA
4LI40	AATACATCAAACACACAGAAGCAGGTCTGC
ML29	GGCTAGCGAATTCATAACTTCGTATAGCAT
BS1	CGATTGAAGAACTCATTCCACTCAAATATACCC
GP1	TGTTGATATCCCGCAAGATACCTGGATTGA
PU5	CCCACCGACTCTAGAGGATCATAATCAGCC



Supplementary Figure 1

E



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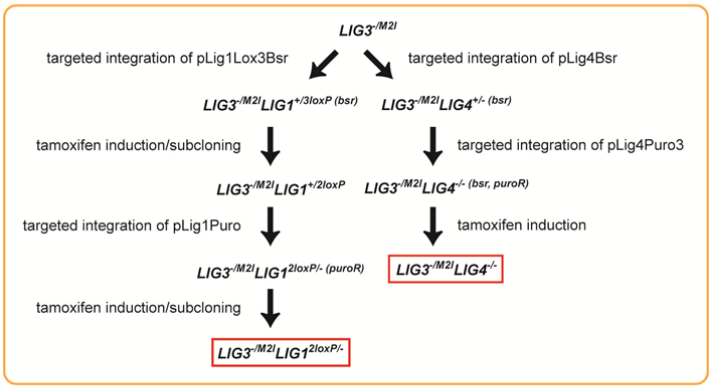

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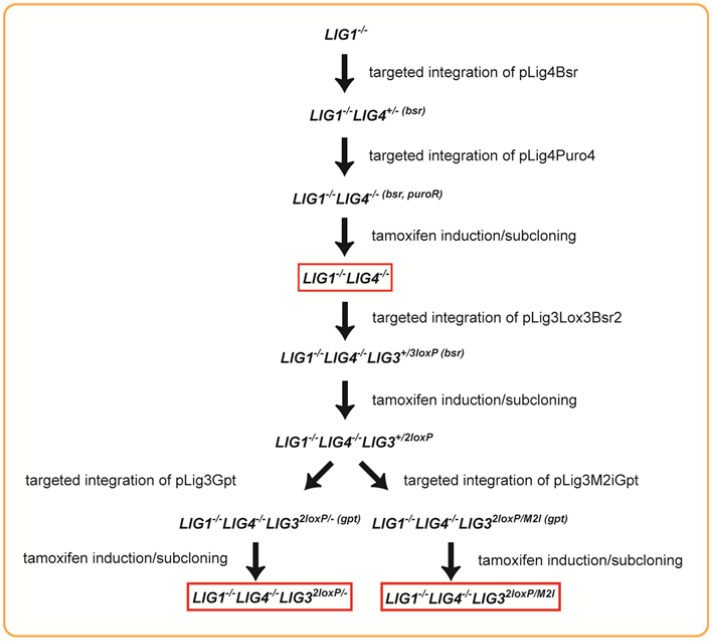
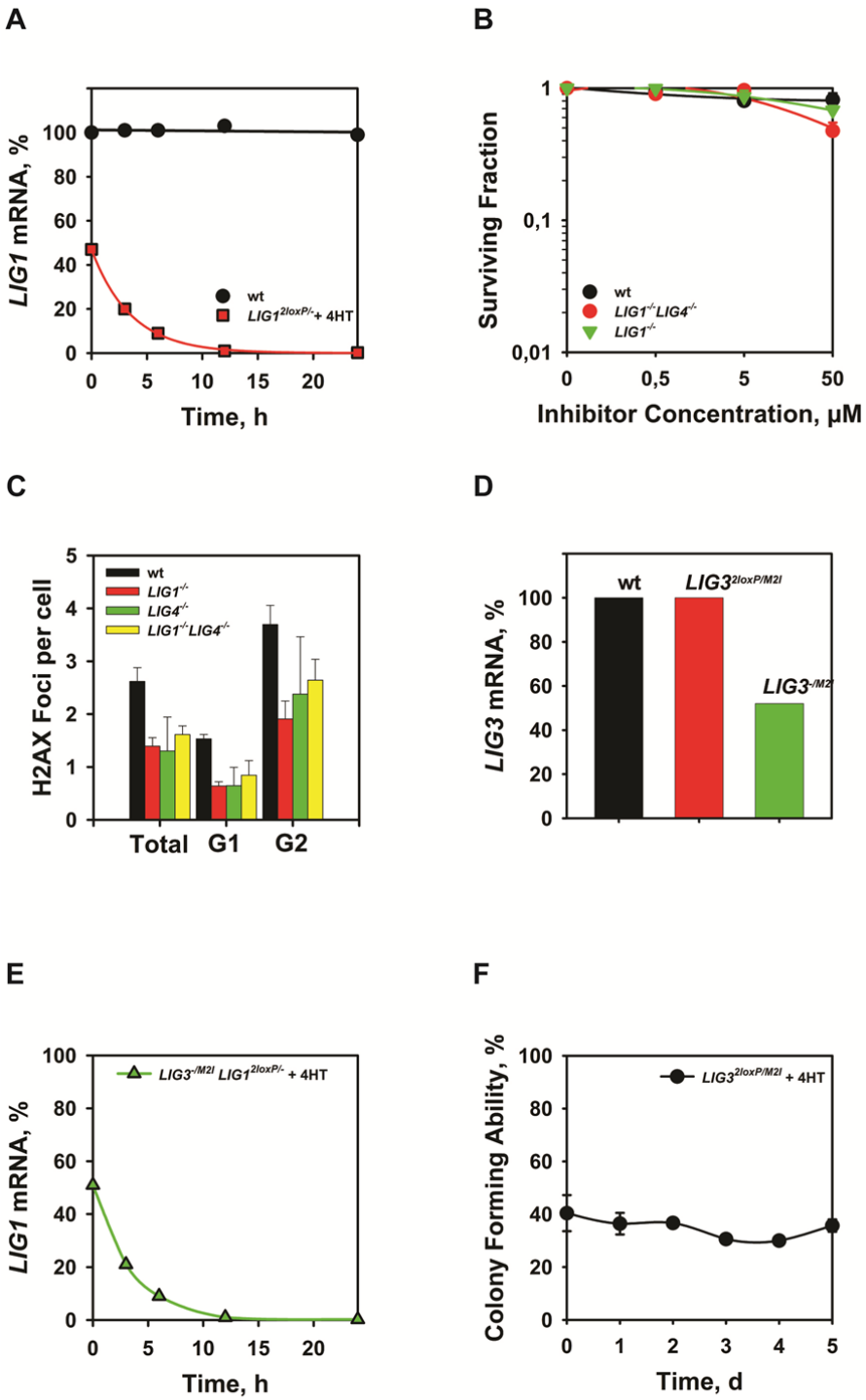


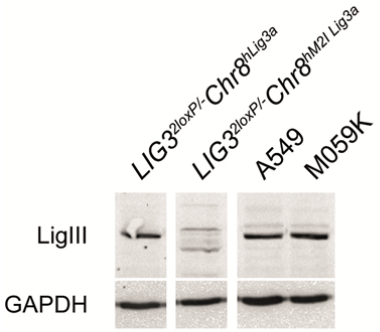
Supplementary Figure 1 (continued)

Supplementary Figure 1: A) Phylogenetic tree analysis of eukaryotic DNA ligases. **B)** Alignment of the six amino acids comprising the ligase active site in different species. **C)** Approach to generate conditional and constitutive knockout alleles of *LIG1* in DT40. Gels show PCR products obtained at different stages of targeting. The first *LIG1* allele was targeted using the vector *pLig1Lox3Bsr* containing the *LIG1* exons 12-28 and the *bsr* drug resistance cassette, in addition to the three loxP sites (left panel). This allowed the generation of the conditional mutant $LIG1^{+/2loxP}$. The second *LIG1* allele was disrupted with the *pLig1Puro* targeting vector (middle panel), which deletes the same *LIG1* region and generates the $LIG1^{2loxP/-}$ mutant. Treatment of this mutant with 4HT deletes exons 17-28 and generates a truncated LigI lacking amino acids 509-919, which cover the entire catalytic core including the enzyme active site (red bar in Figure 1a). **D)** Strategy adopted for the targeting of *LIG4* in DT40. The vector *pLig4Bsr* was designed to delete the entire coding sequence of one *LIG4* allele, while allowing at the same time recycling of the selection marker. Vector *pLig4Puro4* was designed to delete in the second allele of $LIG4^{+/-}$ amino acids 185-614 (red bar in Figure 1a) and to insert an in-frame stop-codon at position 184, generating thus $LIG4^{-/-}$ cells. The latter vector also allowed recycling of the selection marker. The PCR reactions on the right panel confirm the successive targeting and the selection-marker-recycling steps. **E)** Steps taken for the generation of the indicated double DNA ligase mutants. **F)** Steps taken for the generation of mitochondria specific double DNA ligase mutants. G**)** Steps taken for the generation of triple DNA ligase mutants.



Supplementary Figure 2

Supplementary Figure 2: A) *LIG1* mRNA level, measured by Real-time PCR in wild type (wt) and $LIG1^{2loxP/-}$ cells at different times after treatment with 4HT. **B)** Surviving fraction of wt, $LIG1^{-/-}$ and the double mutant $LIG1^{-/-}LIG4^{-/-}$ in the presence of different PARP inhibitor concentrations. **C)** γ-H2AX foci scored in non-irradiated wt, $LIG1^{-/-}$, $LIG4^{-/-}$ and $LIG1^{-/-}LIG4^{-/-}$ cells. Shown are results obtained by analyzing the entire cell population (total) or by separately analyzing cells in the G1 and G2 phase of the cell cycle. Allocation of cells in G1 and G2 phase of the cell cycle was based on the DAPI signal measured in approximately 2,500 nuclei. Values shown are means and standard errors. The higher level of γ-H2AX foci in wt cells is attributed to experimental variation. The results indicate that the tested mutants do not show higher levels of γ-H2AX foci than the wt, which would be indicative of replication stress from inefficient ligation of Okazaki fragments. **D)** *Lig3* mRNA level in wt, $LIG3^{2loxP/M2I}$ and $LIG3^{-/M2I}$ cells. The mRNA level was measured by Real-time PCR and the values plotted as a percentage of control. **E)** *LIG1* mRNA levels measured by Real-time PCR in $LIG3^{-/M2I}LIG1^{2loxP/-}$ cells at different times after incubation with 4HT. **F)** Colony forming ability of $LIG3^{2loxP/M2I}$ cells in the presence of 4HT.



Supplementary Figure 3

Supplementary Figure 3: Western blot analysis of LigIII protein in the indicated mutants. The signal measured in A549 and M059K cells in the same blot is shown for comparison. The results demonstrate expression of hLig3α and h-mitLig3α from the targeted site on chromosome 8. DT40 Lig3α expression was much lower in this blot (not shown), demonstrating thus the high expression levels of the transgenes.