SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Primers and PCR conditions to amplify LIG gene – Primers used to amplify through the 46BR mutation (Arg-771 to Trp mutation) were: *46BR-F* (5'-CCTTCTTCATCTCTTGCCTCC-3') and *46BR-R* (5'-CCTCCTGCTTCTGCCATCAG-3'). Likewise, the four pair primers used to amplify over all the cDNA *LIGI* gene were from 5' to 3': *LIG8-F* (5'-ATGCAGCGAAGTATCATG-3') and *CLIG1.1R* (5'-GCTCCTCTTCCTTCACTTCT-3'); *CLIG1.2F* (5'-AAGACGCTCAGCAGCTTCTT-3') and *CLIG1.2R* (5'-TTCTGGCCTCTGCTGTCTTG-3'); *CLIG1.3F* (5'-CAAGACAGCAGAGGCCAGAA-3') and *CLIG1.3R* (5'-TTCACTGACTGCTCCAGGAA-3'); and *CLIG1.4F* (5'-CTGGACACCAAGGACATCGA-3') and *CLIG1.4R* (5'-GAGTCCTCGCCTTGTTGGTT-3'). DNA sequence changes that replace adjacent phenylalnine residues with alanine residues within the conserved PCNA interaction motif encoded by the mutant hLigI cDNA from 46BRLigI$^{m/m;wt\text{-}PCNA}$ cell line (8,9 aa location) were checked in the *LIG8-F/CLIG1.1R* fragment sequencing that enclose the 5' sequence of the *LIGI* gene. All PCRs were performed with a 50°C primer annealing temperature.

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Fig. 1. Magnitude of repeat tract length changes following replication by different hLigI backgrounds.

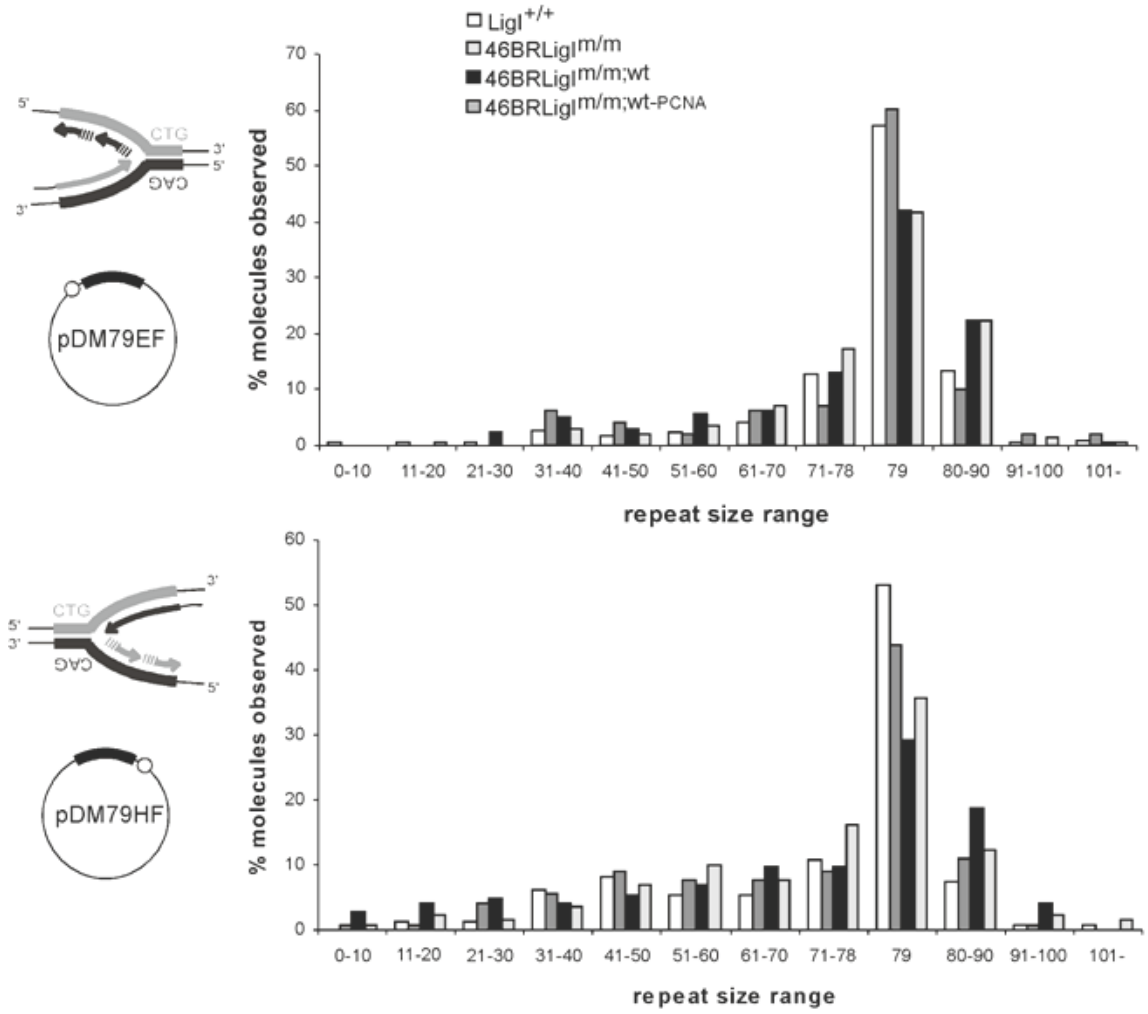
Supplemental Fig. 2. Schematic of slipped-strand substrates. Contraction intermediates (slip-outs in the continuous strand) and expansion intermediates (slip-outs in the nicked strand), with slipped $(CTG)_{30}$•$(CAG)_{50}$ or $(CTG)_{50}$•$(CAG)_{30}$ repeats, having an excess of CAG or CTG repeats and a unique nick at the *Eco*RI (*E*) or *Hin*dIII (*H*) site. As indicated, nicks are located 5' or 3' of the slip-out (43).

Supplemental Fig 3. Repair tract size differences for each slipped-DNA substrate when repaired by each of the various hLigI backgrounds. Repair products were processed as outlined in **Fig. 4.** Briefly, following repair products were digested with *Eco*RI and *Dde*I enzymes and resolved on native acrylamide gels. The relative incorporation/bp into each fragment, normalized to the 540-bp fragment (opposite the repeat), was determined by densitometric analysis. The relative repair-incorporation into the fragments upstream (1,124 bp) and downstream (709 bp) of the repeat tract is shown. Results reproducibility was performed only for substrates 3 and 8 in combination with all the different hLigI extracts used (average of three independent set of repair reactions); only one repair reaction was performed for the rest of the repair substrates.

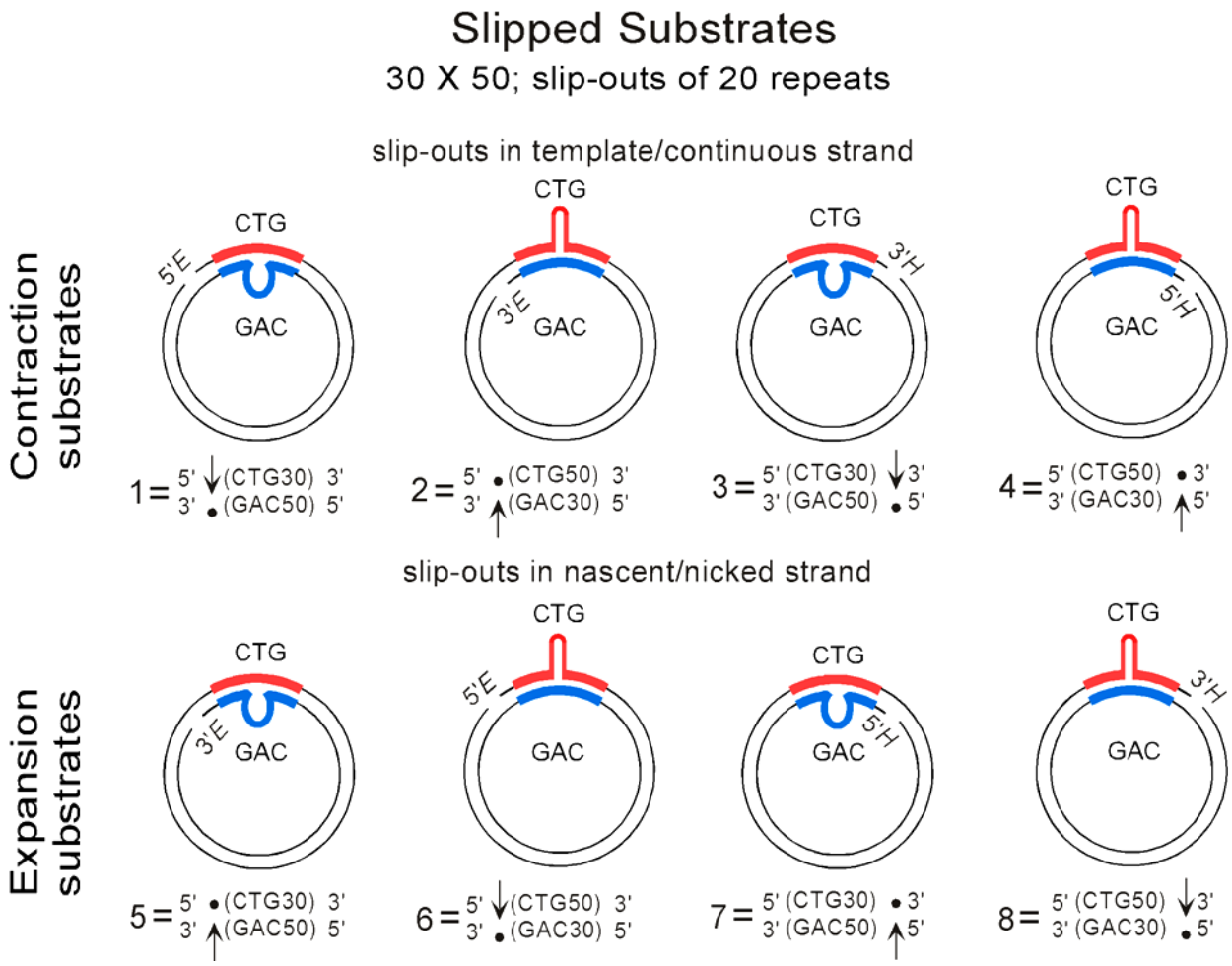
Supplemental Fig. 4. Repair levels for each slipped-DNA by the various hLigI extracts. Three independent set of repair assays were performed to each substrate and hLigI extracts.

Supplemental Fig. 5. Replication-mediated instability and repair efficiencies after specific F-Ara-ATP inhibition of hLigI activity (56). (A) 46BRLigI$^{m/m}$ *in vitro* replication analysis with and without F-ara-ATP (200 μM) using trinucleotide repeat templates pDM79EF and pDM79HF. Reduced replication levels were observed after inhibitor treatment. (B) Molecule length distributions were not significantly different with the inclusion of F-ara-ATP (ns, Fisher's test, $p<0.05$). (C) Repair efficiencies after F-ara-ATP inhibitor treatment on 46BRLigI extracts and substrate 1. Equal or decreased repair levels were observed. Results were normalized against 46BRLigI$^{m/m}$ extract repair level. In contrast, experiments using the hLigI diluted extract (46BRLigI$^{m/m;wt}$: 46BRLigI$^{m/m}$ extract, 1:16 mixture) showed increased repair efficiency.

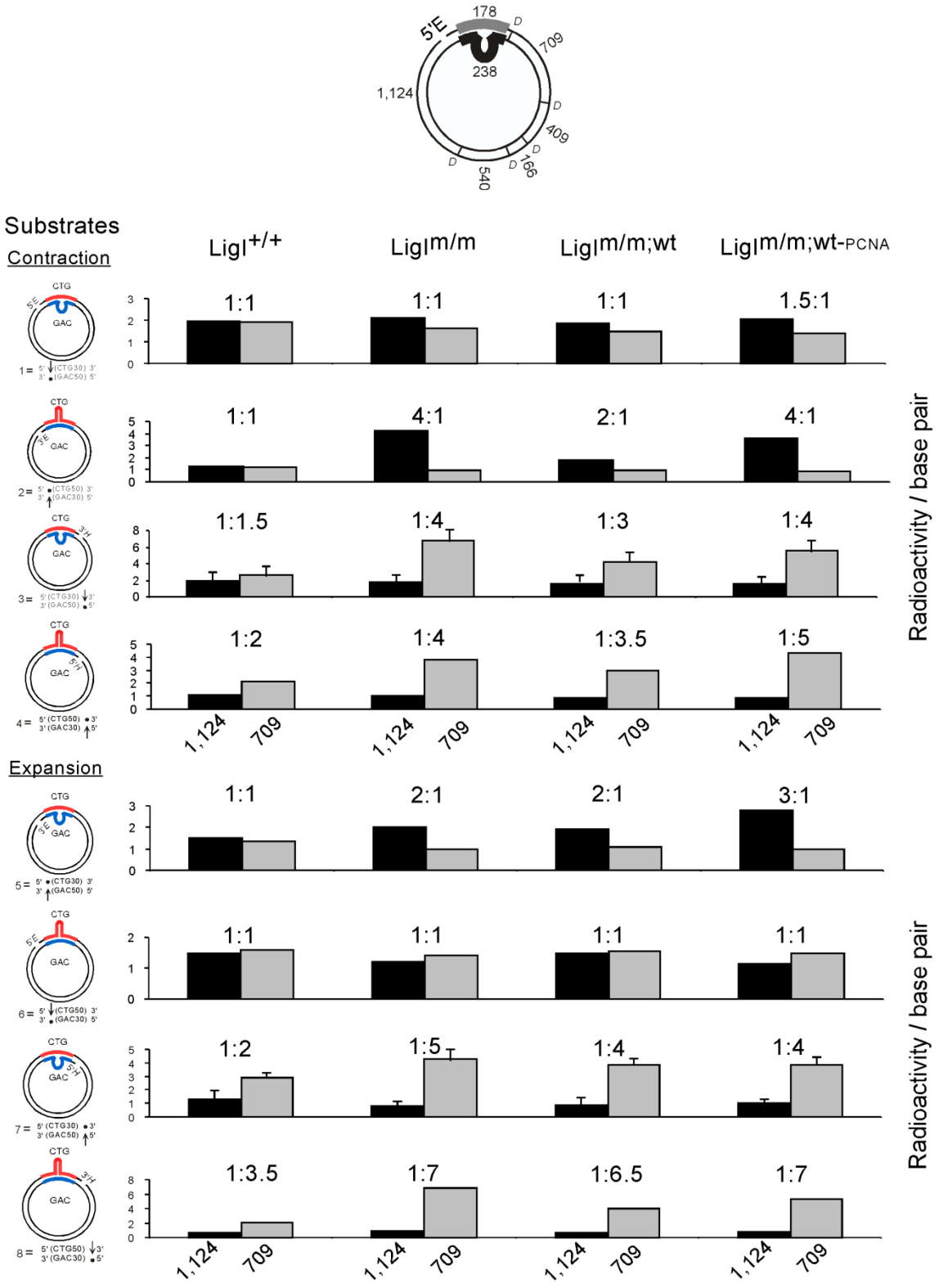
Supplemental Fig. 1 López et al.



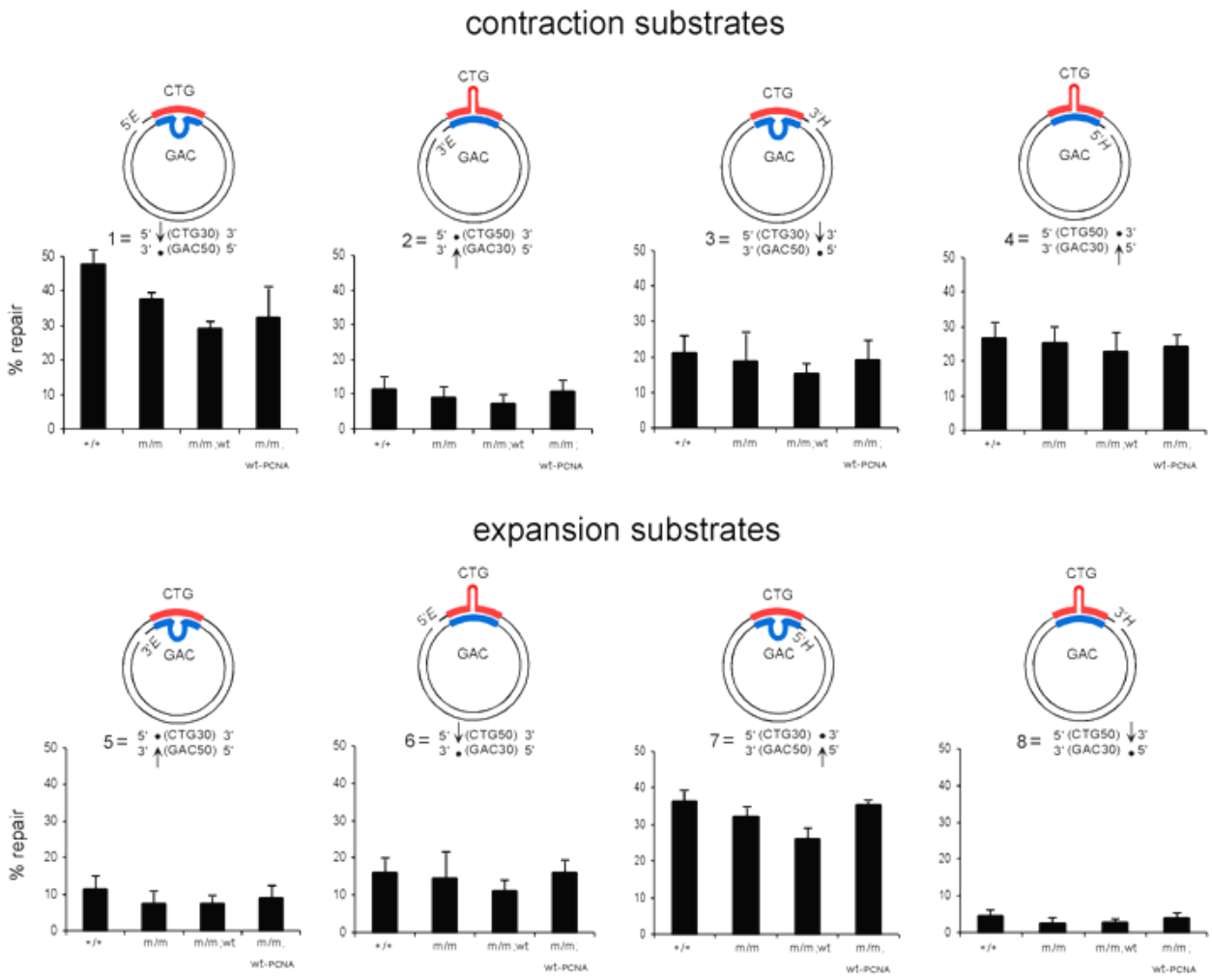
Supplemental Fig. 2 López et al.



Supplemental Fig. 3 López et al.



Supplemental Fig. 4 López et al.



Supplemental Fig. 5 López et al.