Supplemental Figures 1-6

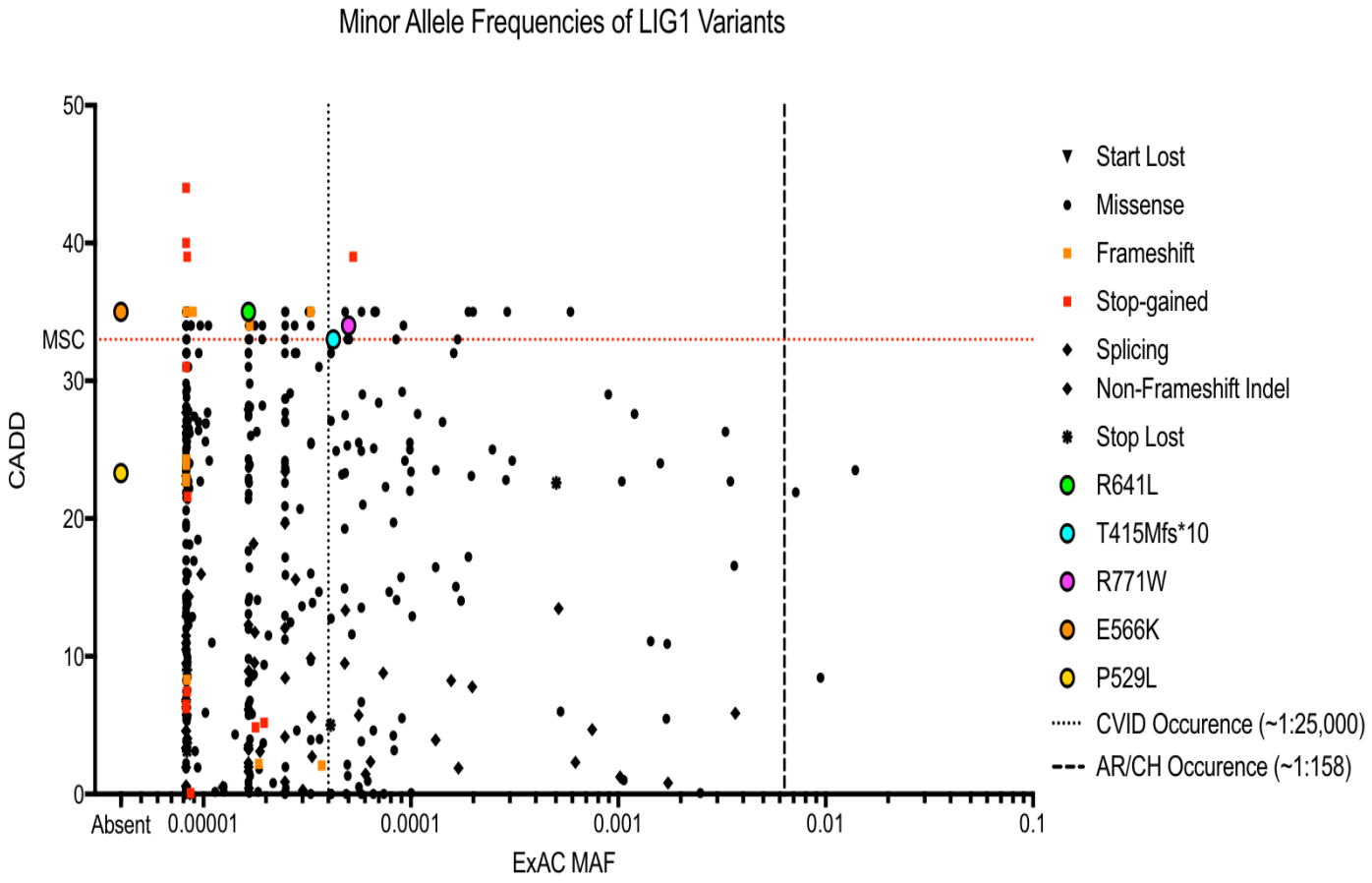

Figure S1. Minor Allele Frequencies of *LIG1* Variants: E566K and P529L have not been reported in ExAC or 1KG; the T415Mfs*10, R641L, and R771W variants occur at frequencies consistent with the estimated occurrence of CVID (1:20,000 to 1:50,000) and the recessive model. CADD = Combined Annotation Dependent Depletion; MSC=mutation significance cut-off; AR= autosomal recessive; CH= compound heterozygous; CVID= Common variable immune deficiency.

Figure S2. Evolutionary conservation of amino acids surrounding the identified *LIG1* mutations.

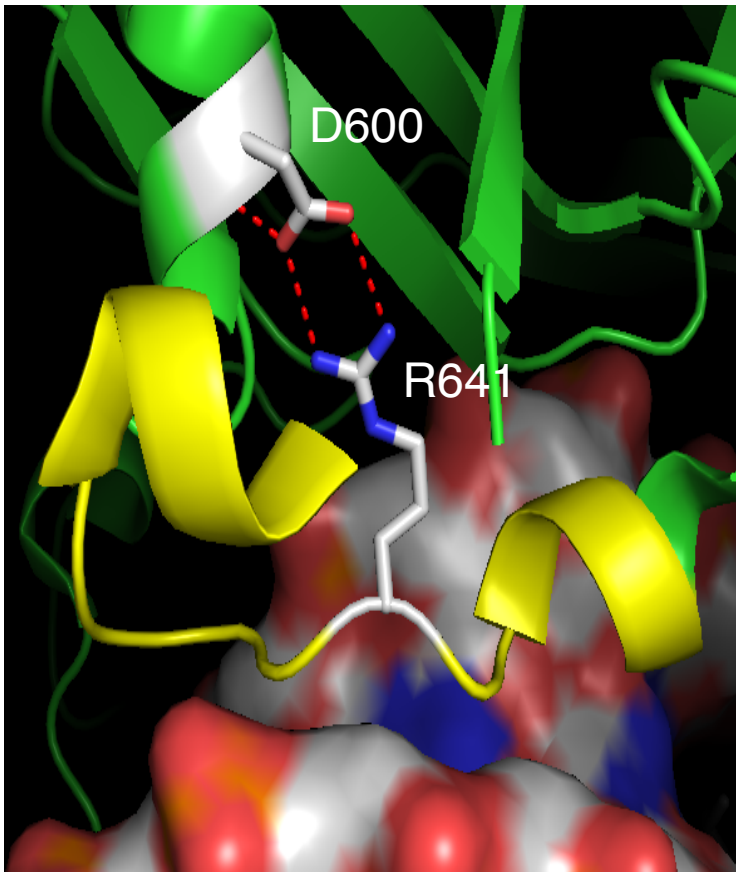

Figure S3. Structure of the DNA-binding loop containing R641. The residues D600 and R641 of LIG1 form a salt bridge. This interaction stabilizes LIG1 (in yellow) that interacts with the DNA substrate (red, white, and blue.) Structural modeling was performed with the crystal structure of LIG1 in complex with adenylylated nicked DNA (PDB# 1X9N) (14) using PyMol (https://www.pymol.org/) and UCSF Chimera (https://www.cgl.ucsf.edu/chimera/).

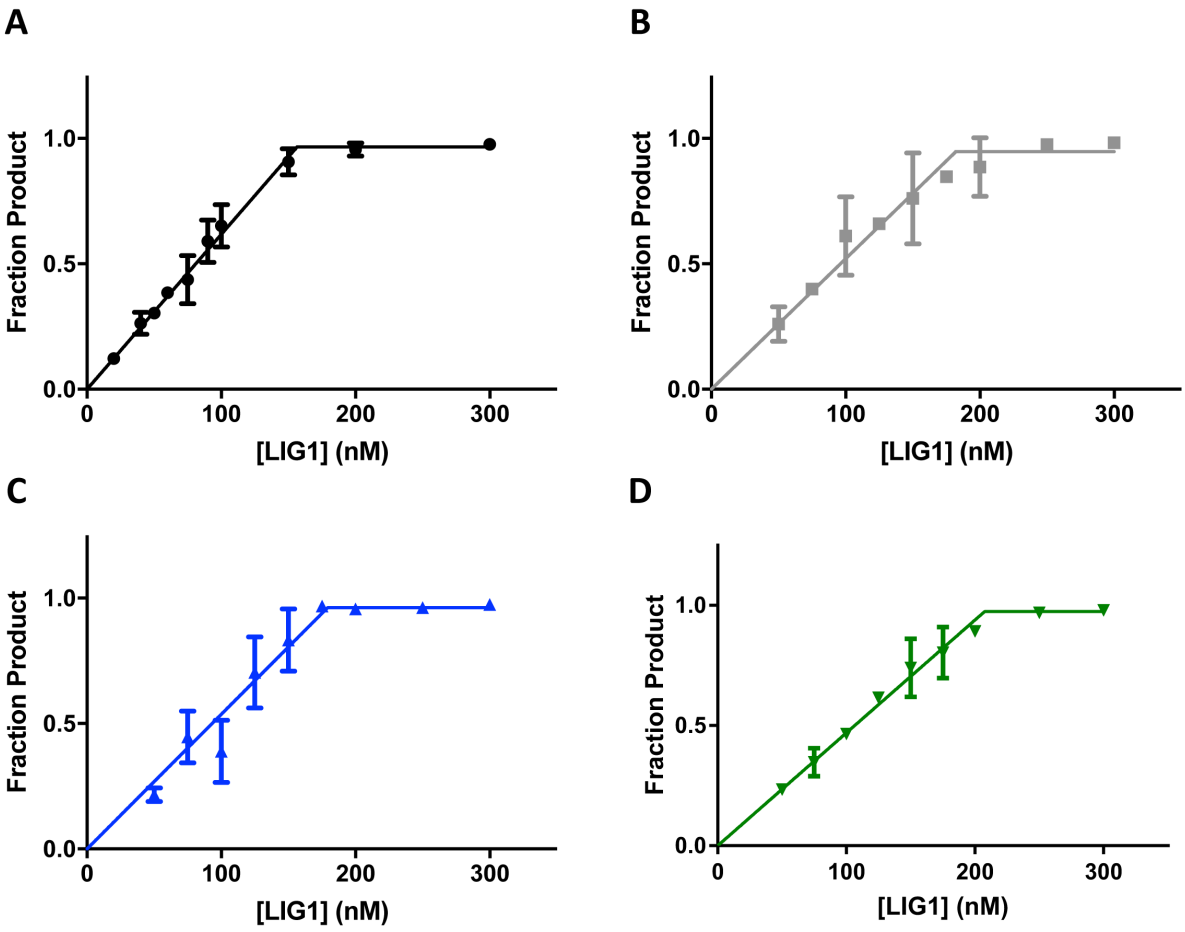

Figure S4. Determination of active concentrations of Δ232 LIG1 constructs. The concentration of active, adenylylated Δ232 LIG1 was determined by quantifying the amount of ligated DNA in the absence of added ATP. The nicked DNA substrate was fixed at 150 nM and the nominal concentration of LIG1 protein was varied from 0-300 nM. The mean ± SD (N≥3) is plotted for WT (A), P529L (B), R641L (C) and R771W (D). These data gave active concentration of 96%, 82%, 85%, and 84%, respectively. The corrected concentration is reported in the main text for all of the ligation kinetics.

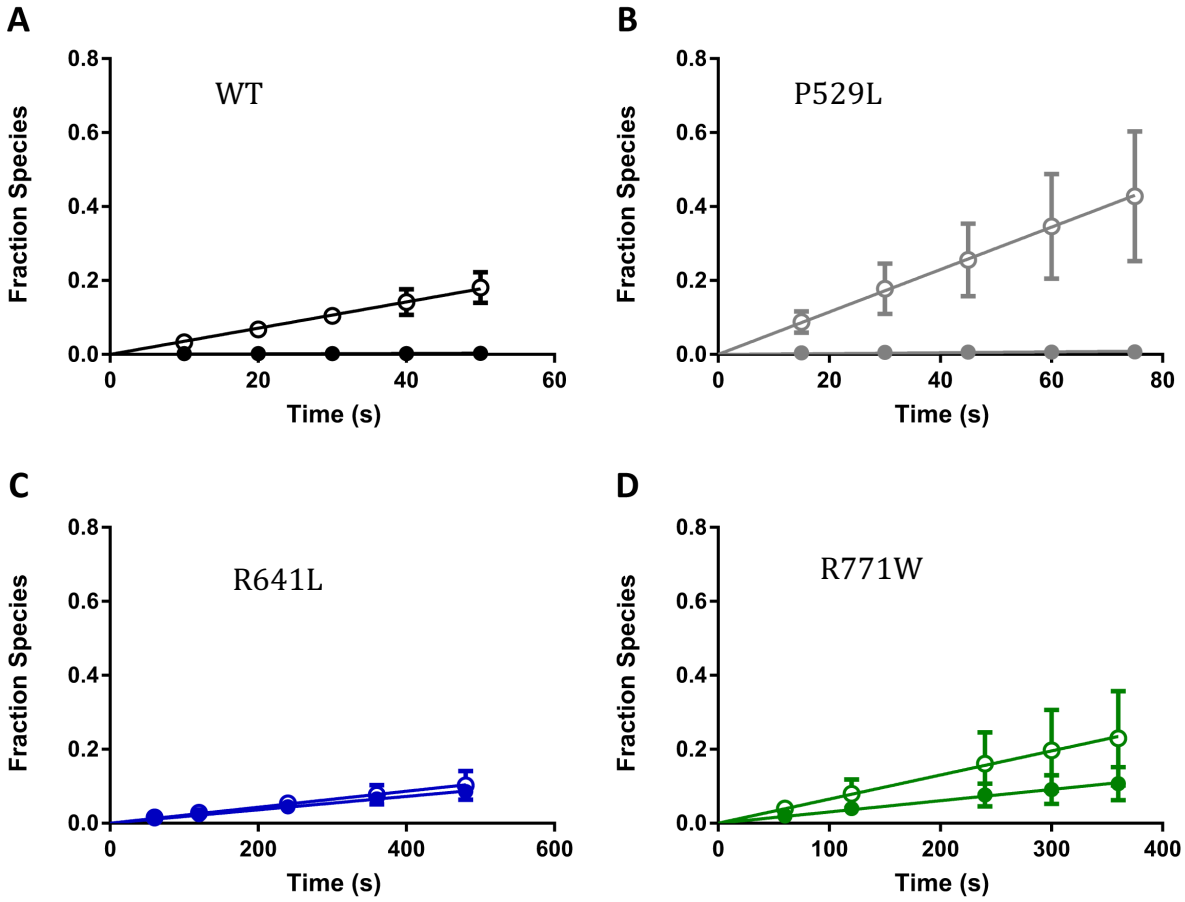

Figure S5. Multiple-turnover ligation by WT and mutant Δ232 LIG1 under conditions of physiological Mg^{2+} and ATP concentrations. Reactions contained 5 nM of each of the Δ232 LIG1 enzymes, 500 nM nicked DNA substrate, 1 mM ATP and 2 mM $MgCl_2$. See Figure 7A for a representative gel. Multiple experiments were quantified and the mean ± SD for ligated product (open circles) and build-up of AMP-DNA (closed circles) is shown (N≥3). These initial rates were used to calculate the fraction abortive ligation plotted in Figure 7C. WT (**A**) and P529L (**B**) LIG1 release less than 1% adenylylated DNA, whereas R641L (**C**) and R771W (**D**) LIG1 released substantial adenylylated DNA under these conditions.

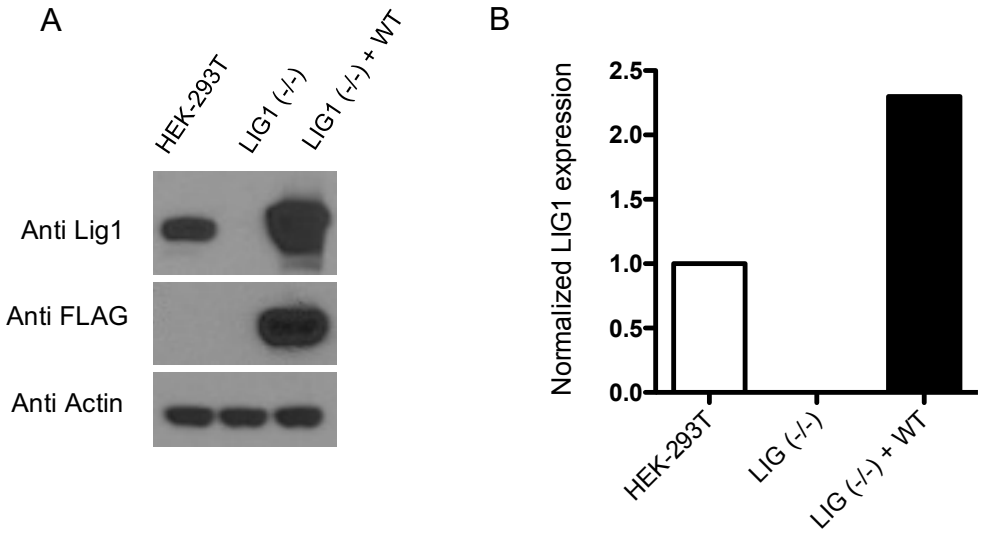

Figure S6. Western blot of native and mutant cells. A. Immunoblot of clone 37 HEK293T cells, LIG1 -/- cells and LIG1 -/- cells transduced with Flag tagged-WT LIG1 used in the comet assay. B. Densitometry showing relative enzyme expression is absent in LIG -/- (using rabbit polyclonal LIG1) but enhanced in transduced cells over baseline HEK-239T cells.