

Supplemental Figures 1-6

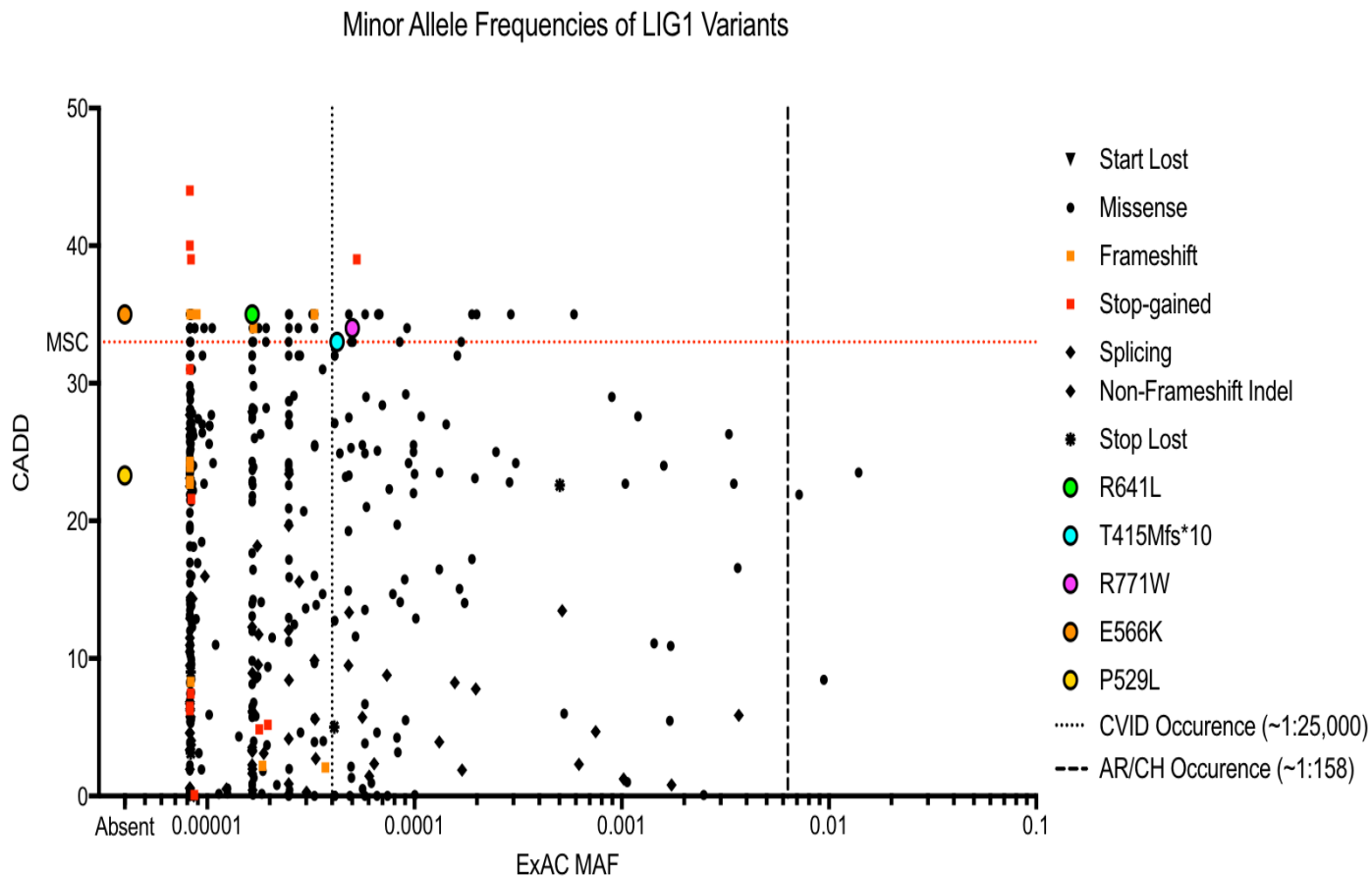


Figure S1. Minor Allele Frequencies of *LIG1* Variants: E566K and P529L have not been reported in ExAC or 1KG; the T415Mfs*10, R641L, and R771W variants occur at frequencies consistent with the estimated occurrence of CVID (1:20,000 to 1:50,000) and the recessive model. CADD = Combined Annotation Dependent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

<u>LIG1 - p.T415</u>		<u>LIG1 - p.P529</u>		<u>LIG1 - p.E566</u>	
H.sapiens	GVFSKFRDIARL T GSASTAKKIDII	H.sapiens	IPVLLHGLERL L PEHCKLSPGIPLK	H.sapiens	VLKRFEEAAFT C EYKYDGQRAQIHA
P.troglodytes	GVFSKFRDIARL T GSASTAKKIDII	P.Troglodytes	IPVLLHGLERL L PEHCKLSPGIPLK	P.Troglodytes	VLKRFEEAAFT C EYKYDGQRAQIHA
M.mulatta	GVFSKFRDIARL T GSASTAKKIDII	M.mulatta	IPVLLHGLERL L PEHCKLSPGIPLK	M.mulatta	VLKRFEEAAFT C EYKYDGQRAQIHA
G.gorilla	GVFSKFRDIARL T GSASTAKKIDII	G.gorilla	IPVLLHGLERL L PEHCKLSPGIPLK	G.gorilla	VLKRFEEAAFT C EYKYDGQRAQIHA
C.lupus	GVFAKFRDIARL A GSASTAKKIDVI	C.lupus	IPVLLHGLEHL L PEHCRLSPGVPLK	C.lupus	VLKRFEEAAFT C EYKYDGQRAQIHV
M.musculus	GVFTKFCDIARL T GSASMAKKMDII	M.musculus	IPVLLHGLERL L PEHCKLSPGVPLK	M.musculus	VLKRFEEVDFT C EYKYDGQRAQIHV
C.jacchus	GVFTKFRDIARL T GSASTAKKIDII	C.jacchus	VPVLLHGLERL L PEHCKIIPGIPLK	C.jacchus	VLKRFEEAAFT C EYKYDGQRAQIHA
X.tropicalis	GVFSKLDIARM T GNASMNKKIDII	X.tropicalis	IPILLEHGIDDL L PKHCRLTPGIPLK	X.tropicalis	VLKRFDEAAFT C EYKYDGERAQIHI
D.rerio	GVFNKLKEIAH M SGNSAMNKKIDII	D.rerio	IPVLLKEGIDEL L PNHCKLTPGVPLR	D.rerio	VMKRFDEASFT C EYKYDGERAQIHI
G.gallus	GVLGKLEMAAM S GNASASKKIDII	G.gallus	VPVLLHGLEQL L PKHCGITPGVPLK	G.gallus	LLKRFEEAAFT C EYKYDGERTQIHV
A.thaliana	KVFDTRQIAKE S GKDSNEKKKNRM	A.thaliana	VPALLSGGVWNL L PKTCNFTLGVPIG	A.thaliana	ILNKFQDIVFT C EYKYDGERAQIHF

<u>LIG1 - p.R641</u>		<u>LIG1 - p.R771</u>	
H.sapiens	KKQIQPFQVL T TRKRKEVDASEIQV	H.sapiens	LVVIGAYLGRGK R AGRYGGFLLASY
P.Troglodytes	KKQIQPFQVL T TRKRKEVDASEIQV	P.Troglodytes	LVVIGAYLGRGK R AGRYGGFLLASY
M.mulatta	KKQIQPFQVL T TRKRKEVDASEIQV	M.mulatta	LVVIGAYLGRGK R AGRYGGFLLASY
G.gorilla	KKQIQPFQVL T TRKRKEVDASEIQV	G.gorilla	LVVIGAYLGRGK R AGRYGGFLLASY
C.lupus	KKQIQPFQVL T TRKRKEVDAAEIQV	C.lupus	LVVIGAYLGRGK R AGRYGGFLLAAY
M.musculus	KKQIQPFQVL T TRKRKEVDASEIQV	M.musculus	LVVIGAYLGRGK R AGRYGGFLLAAY
C.jacchus	KKQIQPFQVL T TRKRKEVDASEIQV	C.jacchus	LVVIGAYLGRGK R AGRYGGFLLACY
X.tropicalis	KKQIQPFQVL T TRKRKDVDASEIKV	X.tropicalis	LVVIGAYLGK G RTGIYGGFLLASY
D.rerio	KKQIQPFQVL T TRKRKDVDASEIKV	D.rerio	LCVIGAYLGK G RAGGYGGFLLACY
G.gallus	TKQLQPFQVL T TRKRKDVDAAAIKV	G.gallus	LVVIGAYLGK G RAGIYGGFLLACY
A.thaliana	KKKILPFQIL S TARKNNVNDIKV	A.thaliana	LVPIAAFHGRGK R TGVYGAFLACY

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

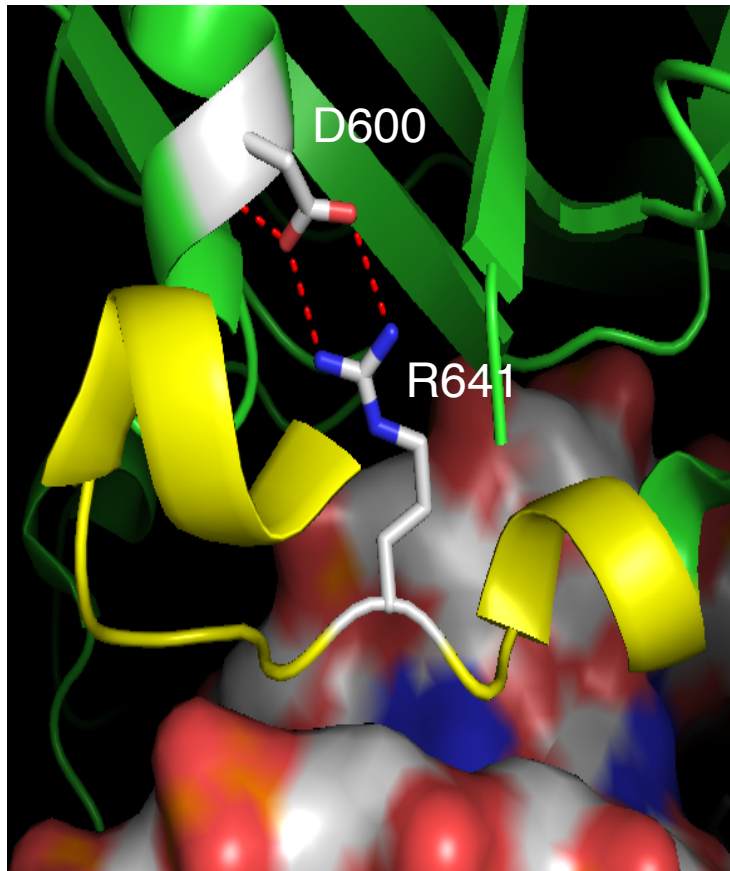


Figure S3. Structure of the DNA-binding loop containing R641. The residues D600 and R641 of LIG1 form a salt bridge. This interaction stabilizes LIG1 (in yellow) that interacts with the DNA substrate (red, white, and blue.) Structural modeling was performed with the crystal structure of LIG1 in complex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

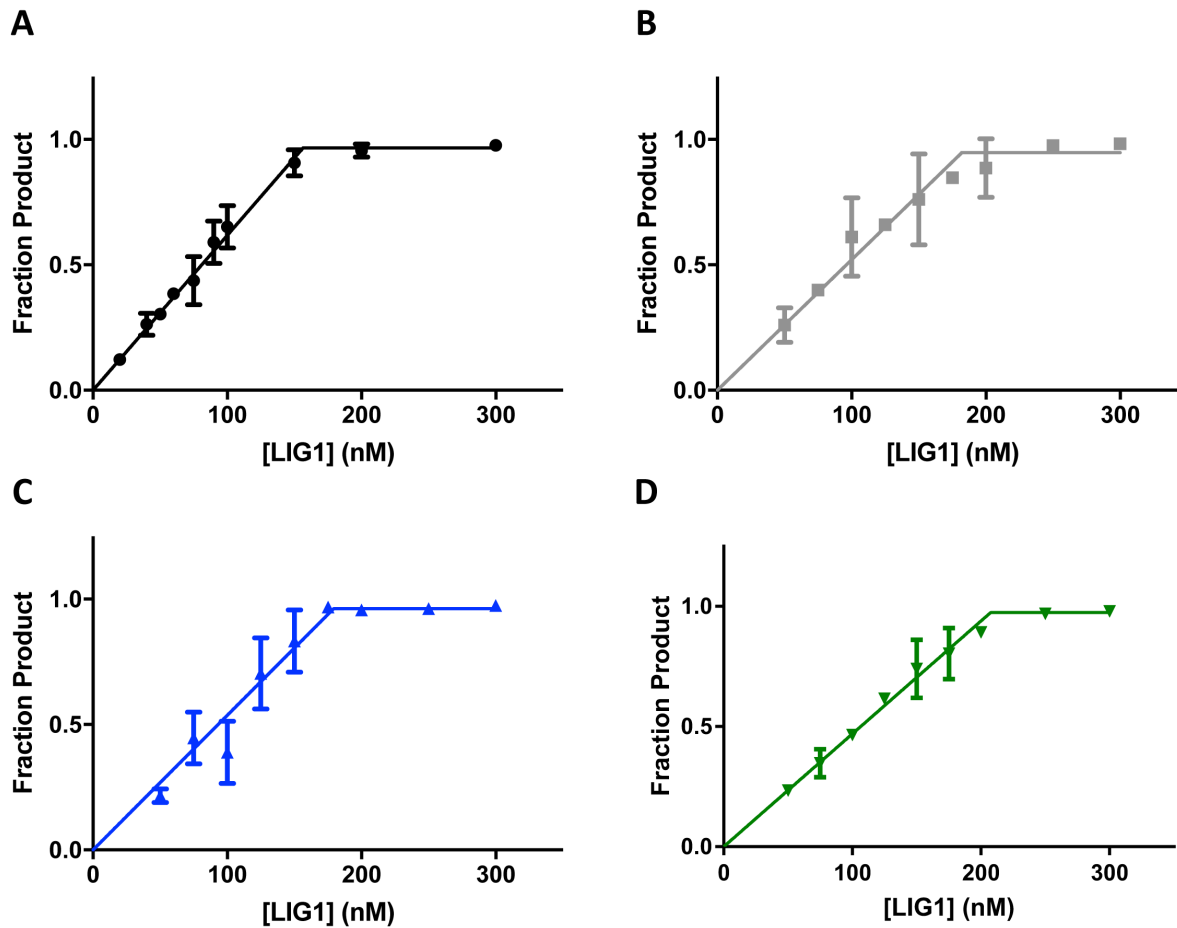


Figure S4. Determination of active concentrations of $\Delta 232$ LIG1 constructs. The concentration of active, adenylylated $\Delta 232$ LIG1 was determined by quantifying the amount of ligated DNA in the absence of added ATP. The nicked DNA substrate was fixed at 150 nM and the nominal concentration of LIG1 protein was varied from 0-300 nM. The mean \pm SD ($N \geq 3$) 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.

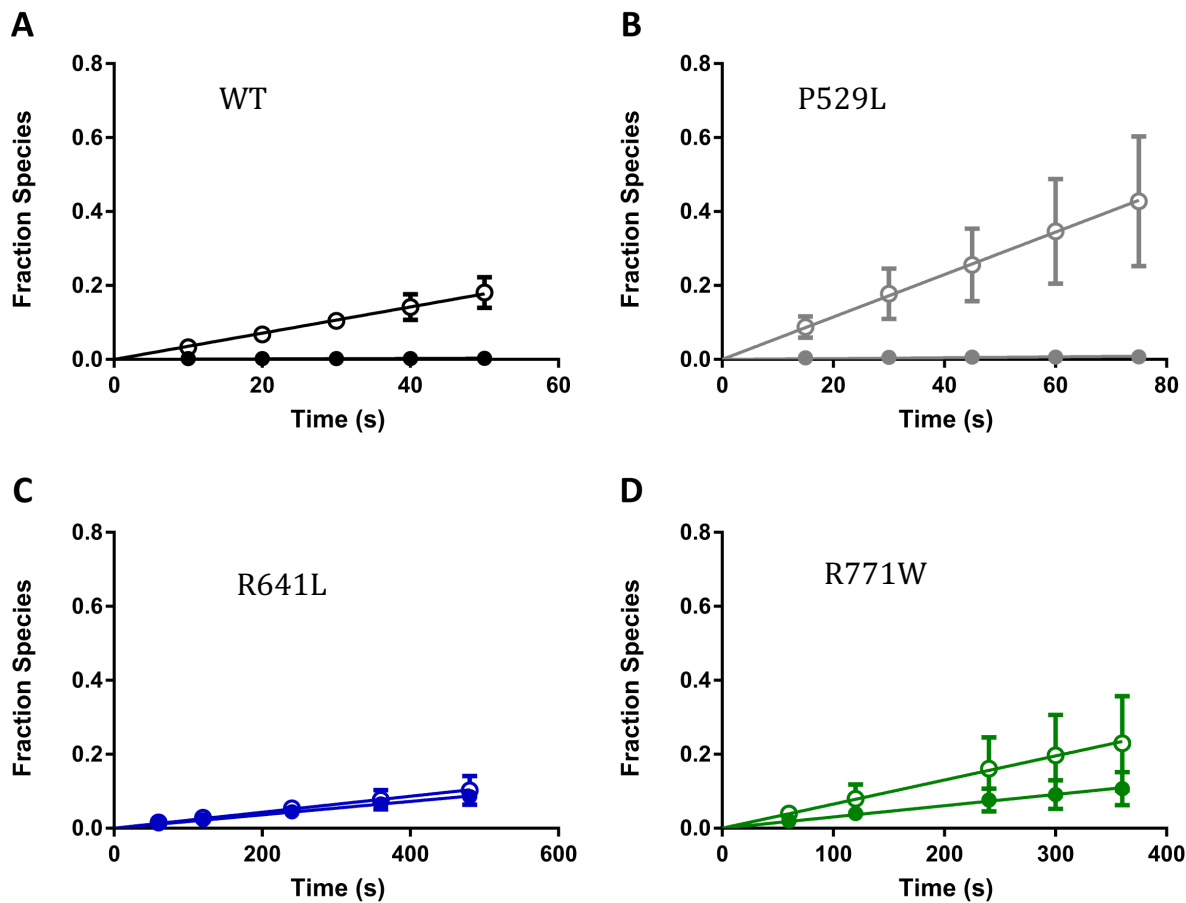


Figure S5. Multiple-turnover ligation by WT and mutant $\Delta 232$ LIG1 under conditions of physiological Mg^{2+} and ATP concentrations. Reactions contained 5 nM of each of the $\Delta 232$ LIG1 enzymes, 500 nM nicked DNA substrate, 1 mM ATP and 2 mM $MgCl_2$. See Figure 7A for a representative gel. Multiple experiments were quantified and the mean \pm SD for ligated product (open circles) and build-up of AMP-DNA (closed circles) is shown ($N \geq 3$). 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.

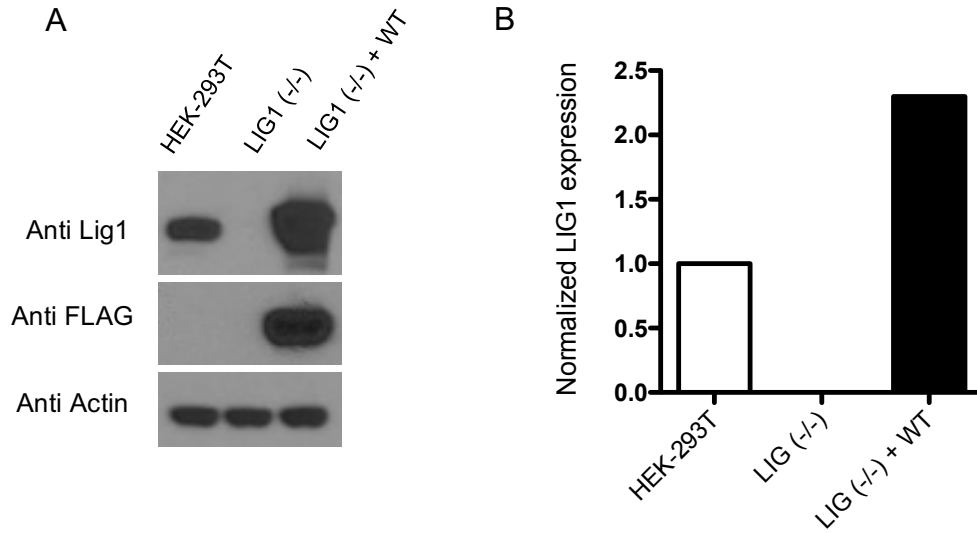


Figure S6. Western blot of native and mutant cells. A. Immunoblot of clone 37 HEK293T cells, LIG1 $-/-$ cells and LIG1 $-/-$ cells transduced with Flag tagged-WT LIG1 used in the comet assay. B. Densitometry showing relative enzyme expression is absent in LIG $-/-$ (using rabbit polyclonal LIG1) but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