DNA ligase I variants fail in the ligation of mutagenic repair intermediates with mismatches and oxidative DNA damage

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Supplementary Figures 1-14

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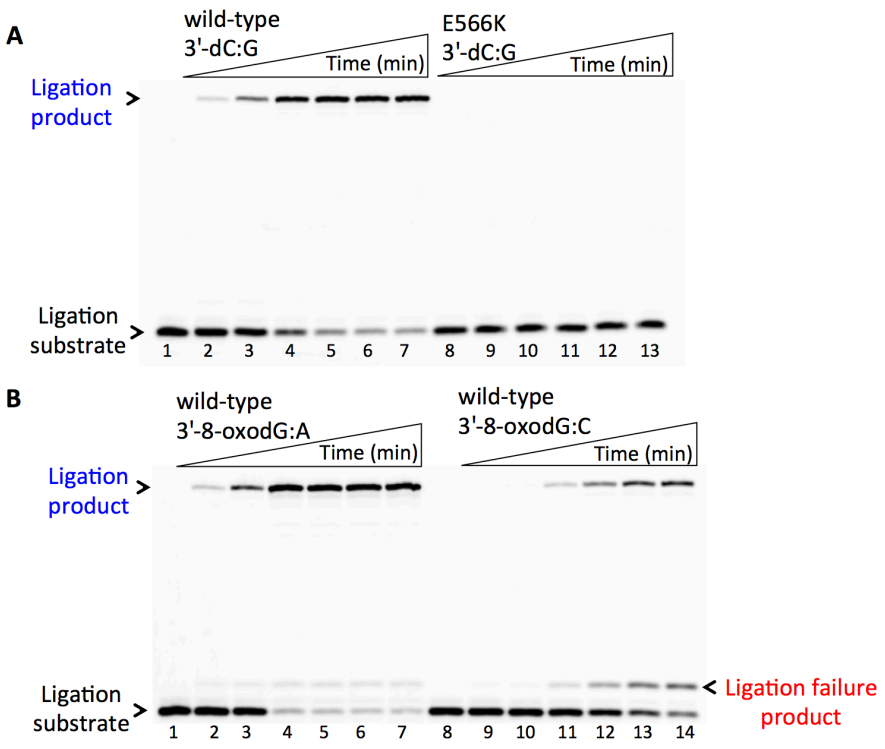
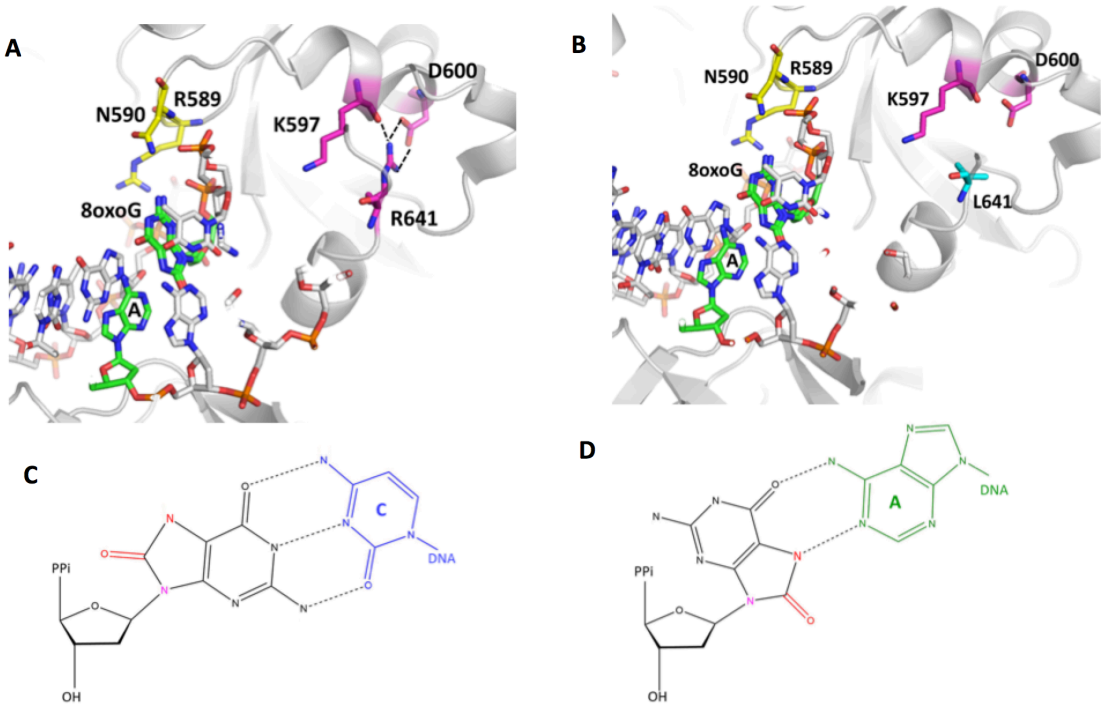
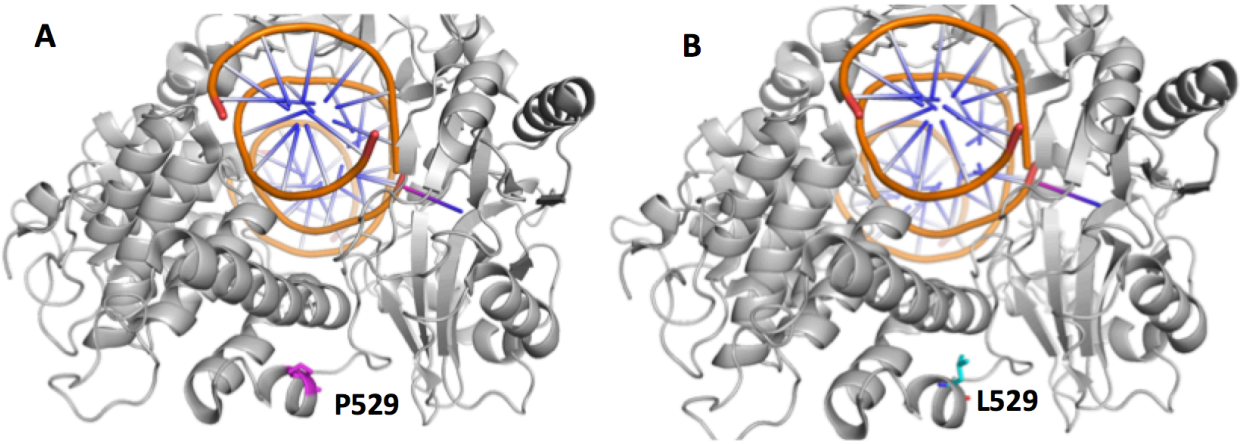


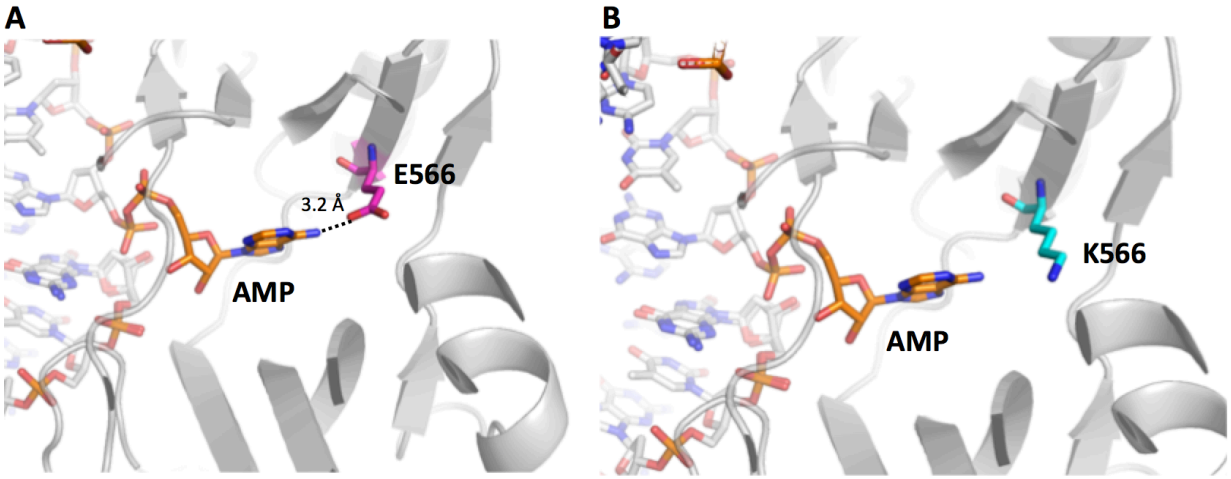
Supplementary Figure 1. Ligation of the repair intermediates by wild-type and LIG1 variant E566K. (**A**) Ligation of the repair intermediates with 3'-dC by wild-type and E566K. Lanes 1 is the negative enzyme control of the nicked DNA substrates with 3'-dC opposite template G. Lanes 2-7 and 8-13 show the reaction products by wild-type and E566K obtained at the time points 10, 30, 45, 60, 180, and 240 sec and 0.5, 1, 3, 5, 8, and 10 min, respectively. (**B**) Ligation of the repair intermediates with 3'-8-oxodG by wild-type LIG1. Lanes 1 and 8 are the negative enzyme controls of the nicked DNA substrates with 3'-8-oxodG opposite template A and C, respectively. Lanes 2-7 and 9-14 show the reaction products obtained for 3'-8-oxodG:A and 3'-8-oxodG:C substrates, respectively, at the time points 0.5, 1, 3, 5, 8, and 10 min.



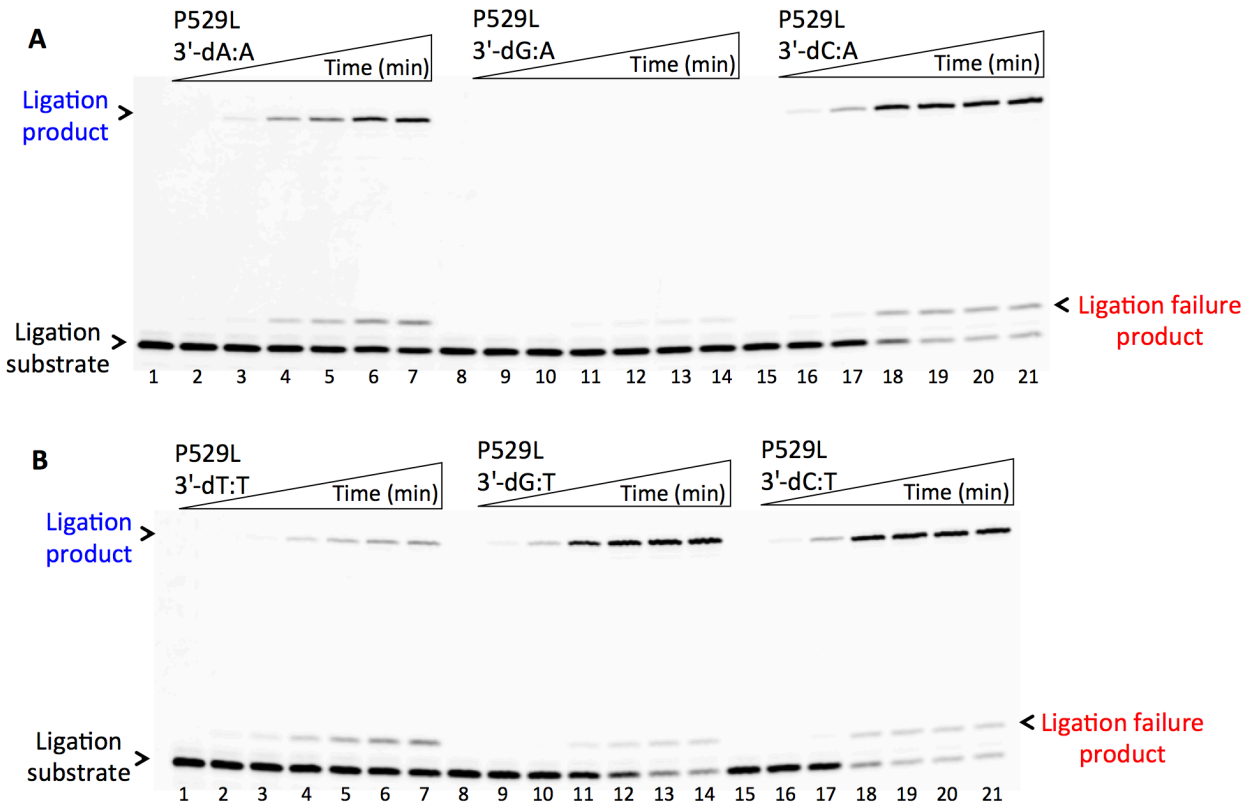
Supplementary Figure 2. The catalytic architecture of LIG1 in complex with the nicked DNA containing 8-oxoG. (**A**) The positions of the residues locating in LIG1 catalytic region and indicated in the patients with LIG1 deficiency syndrome. Active site residues N590 are R589 (yellow) are shown as sticks. The disease-associated residue R641 and its interacting partners K597 and D600 (magenta) are also presented in sticks. (**B**) Replacement of R641 with Leu (cyan, L641) compromises the interactions. **(C,D)** 8-oxodGTP base pairing with Cytosine (C) and Adenine (D).



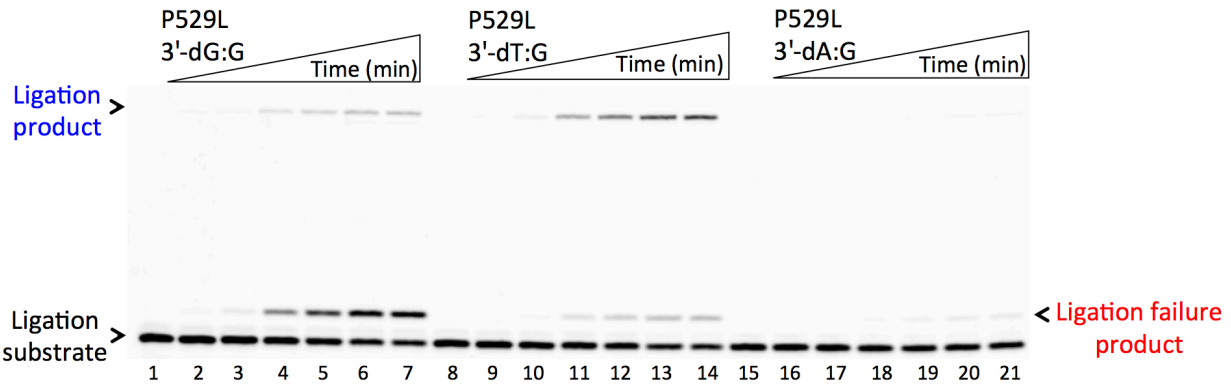
Supplementary Figure 3. Structure model of LIG1 with P529L identified in LIG1 deficiency syndrome. (**A**) Ribbon diagram showing LIG1 encircling a nicked DNA duplex (orange). P529 (magenta) shown as sticks does not interact with DNA, and the replacement of Pro641 with Leu (cyan, L529) is shown in panel B.



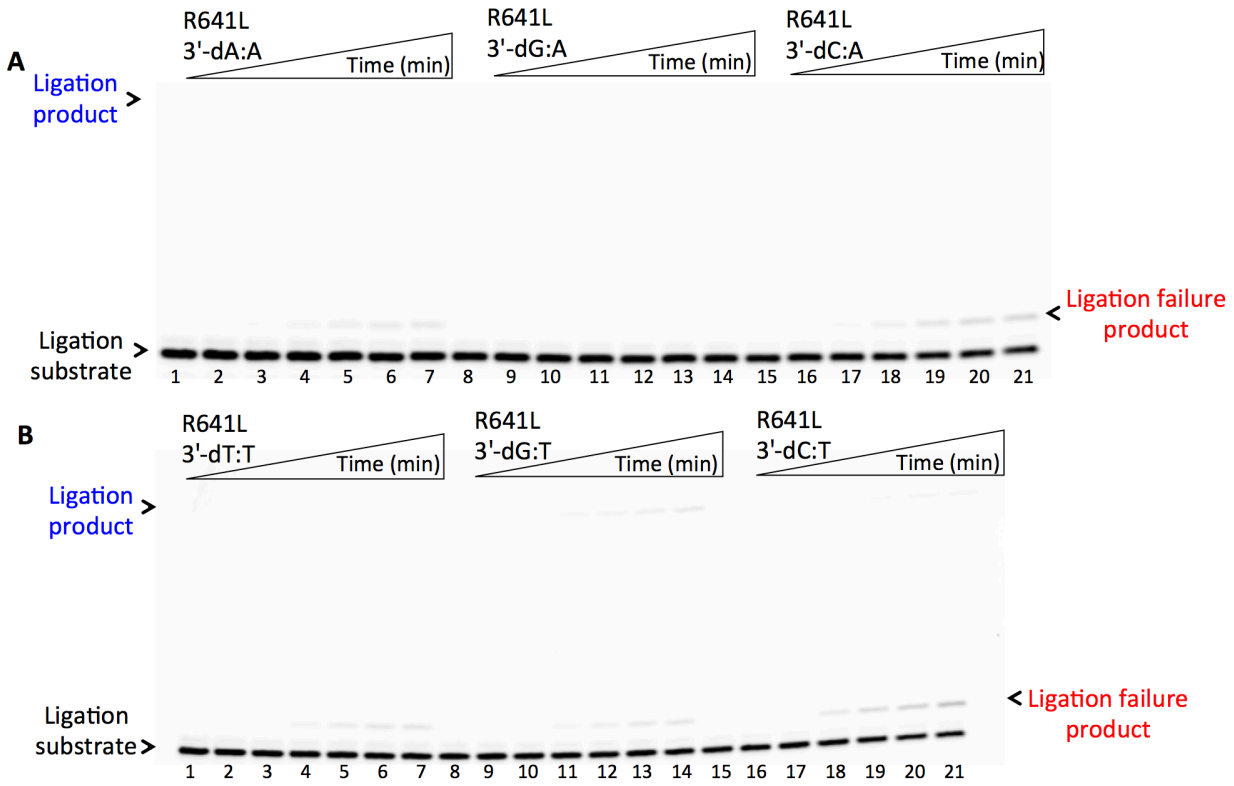
Supplementary Figure 4. Structure model of LIG1 with E566K identified in LIG1 deficiency syndrome. (**A**) The interaction between E566 (magenta) and N6 of adenine (AMP) shown as sticks is represented on LIG1 model (gray, ribbon). (**B**) Replacement of Glu566 (E566) with Lys (cyan, K566) results in the disruption of this interaction. Positive and negative potentials are shown in blue and red, respectively.



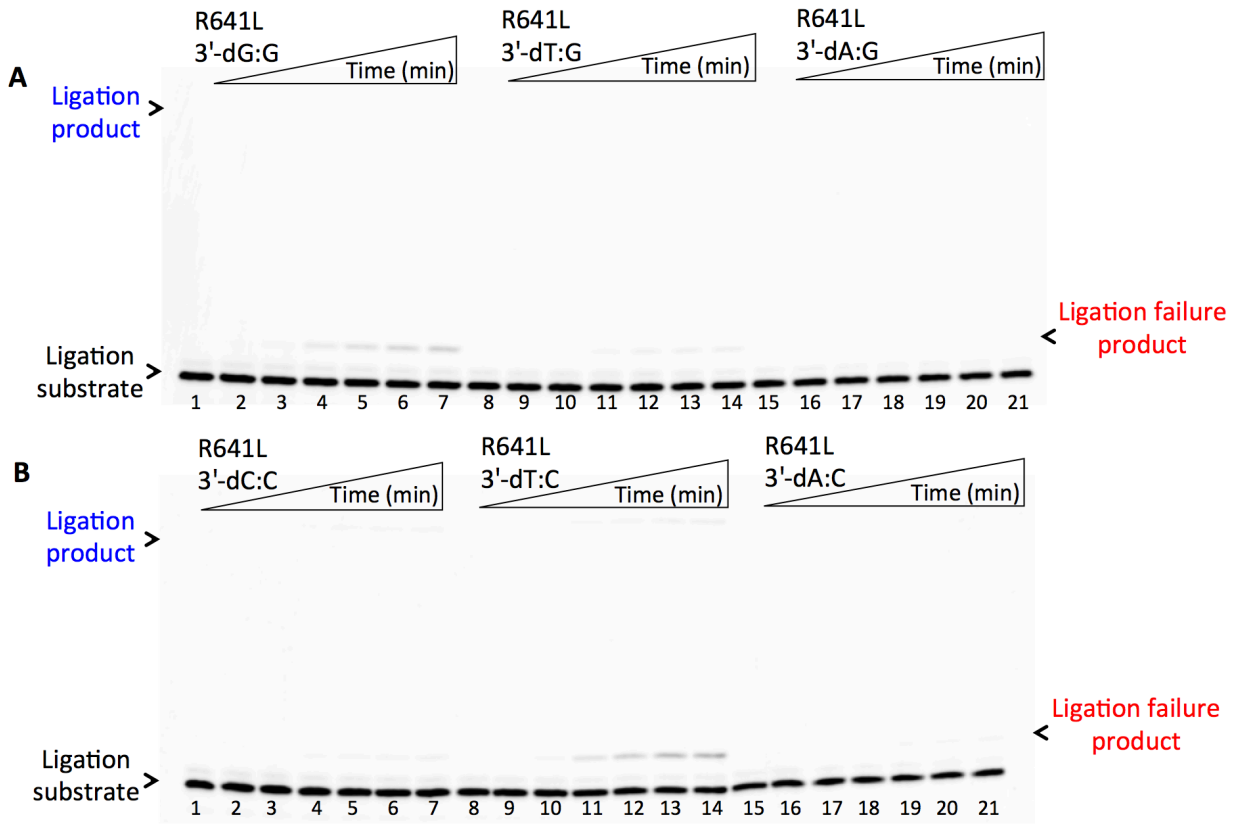
Supplementary Figure 5. Ligation of the repair intermediates with 3'-preinserted mismatches opposite template A and T by LIG1 P529L. (**A**) Lanes 1, 8, and 15 are the negative enzyme controls of the nicked DNA substrates with 3'-dA:A, 3'-dG:A, and 3'-dC:A, respectively. Lanes 2-7, 9-14, and 16-21 show the reaction products obtained at the time points 0.5, 1, 3, 5, 8, and 10 min. (**B**) Lanes 1, 8, and 15 are the negative enzyme controls of the nicked DNA substrates with 3'-dT:T, 3'-dG:T, and 3'-dC:T, respectively. Lanes 2-7, 9-14, and 16-21 show the reaction products obtained at the time points 0.5, 1, 3, 5, 8, and 10 min. The bar graphs are presented in Figure 10.



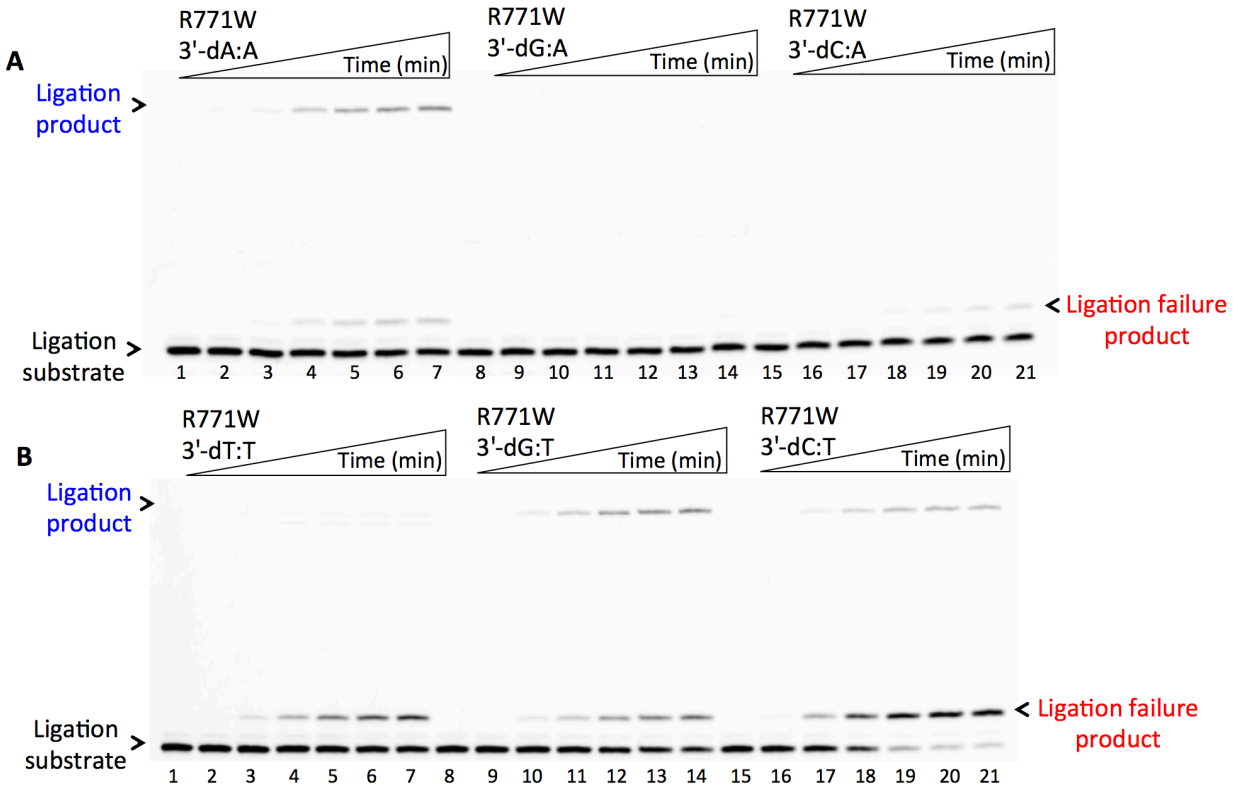
Supplementary Figure 6. Ligation of the repair intermediate with 3'-preinserted mismatches opposite template G by LIG1 variant P529L. (**A**) Lanes 1, 8, and 15 are the negative enzyme controls of the nicked DNA substrates with 3'-dG:G, 3'-dT:G, and 3'-dA:G, respectively. Lanes 2-7, 9-14, and 16-21 show the reaction products obtained at the time points 0.5, 1, 3, 5, 8, and 10 min. The bar graphs are presented in Figure 11.



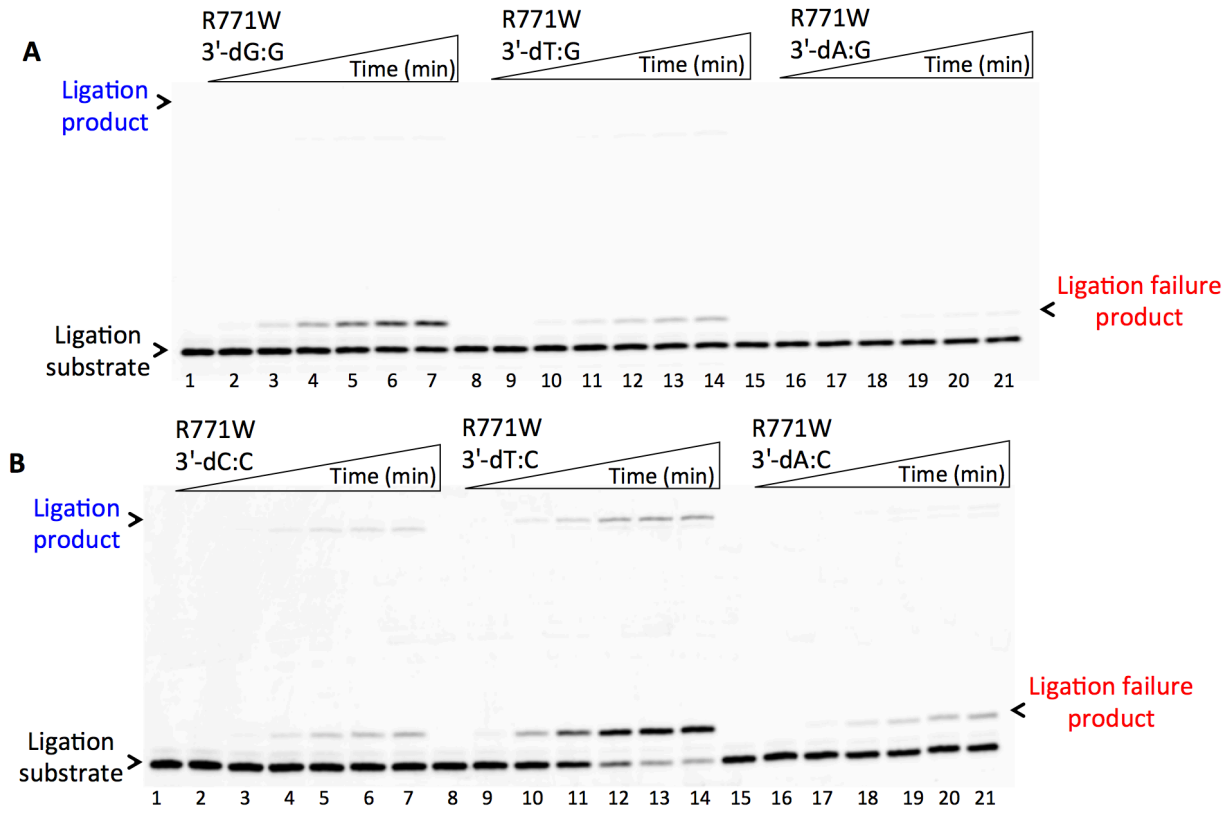
Supplementary Figure 7. Ligation of the repair intermediates with 3'-preinserted mismatches opposite template A and T by LIG1 variant R641L. (**A**) Lanes 1, 8, and 15 are the negative enzyme controls of the nicked DNA substrates with 3'-dA:A, 3'-dG:A, and 3'-dC:A, respectively. Lanes 2-7, 9-14, and 16-21 show the reaction products obtained at the time points 0.5, 1, 3, 5, 8, and 10 min. (**B**) Lanes 1, 8, and 15 are the negative enzyme controls of the nicked DNA substrates with 3'-dT:T, 3'-dG:T, and 3'-dC:T, respectively. Lanes 2-7, 9-14, and 16-21 show the reaction products obtained at the time points 0.5, 1, 3, 5, 8, and 10 min. The bar graphs are presented in Figure 12.



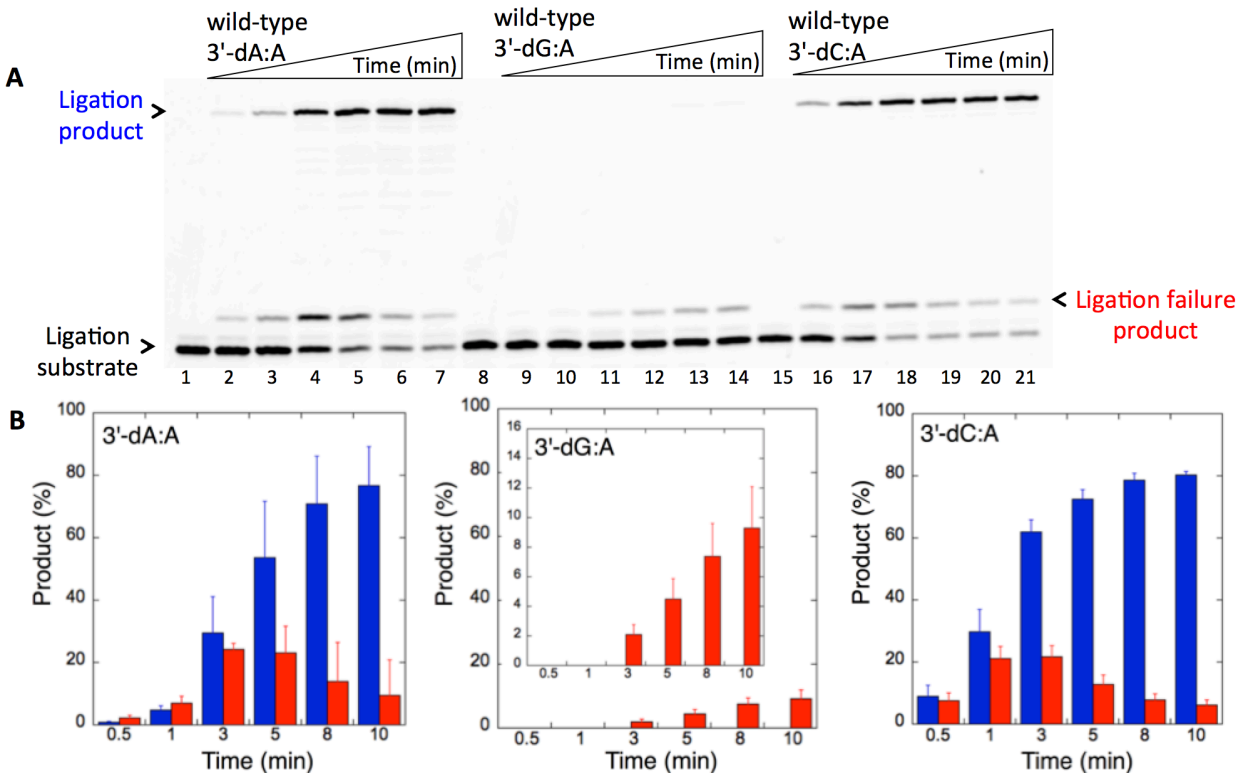
Supplementary Figure 8. Ligation of the repair intermediates with 3'-preinserted mismatches opposite template G and C by LIG1 variant R641L. (**A**) Lanes 1, 8, and 15 are the negative enzyme controls of the nicked DNA substrates with 3'-dG:G, 3'-dT:G, and 3'-dA:G, respectively. Lanes 2-7, 9-14, and 16-21 show the reaction products obtained at the time points 0.5, 1, 3, 5, 8, and 10 min. (**B**) Lanes 1, 8, and 15 are the negative enzyme controls of the nicked DNA substrates with 3'-dC:C, 3'-dT:C, and 3'-dA:C, respectively. Lanes 2-7, 9-14, and 16-21 show the reaction products obtained at the time points 0.5, 1, 3, 5, 8, and 10 min. The bar graphs are presented in Figure 12.



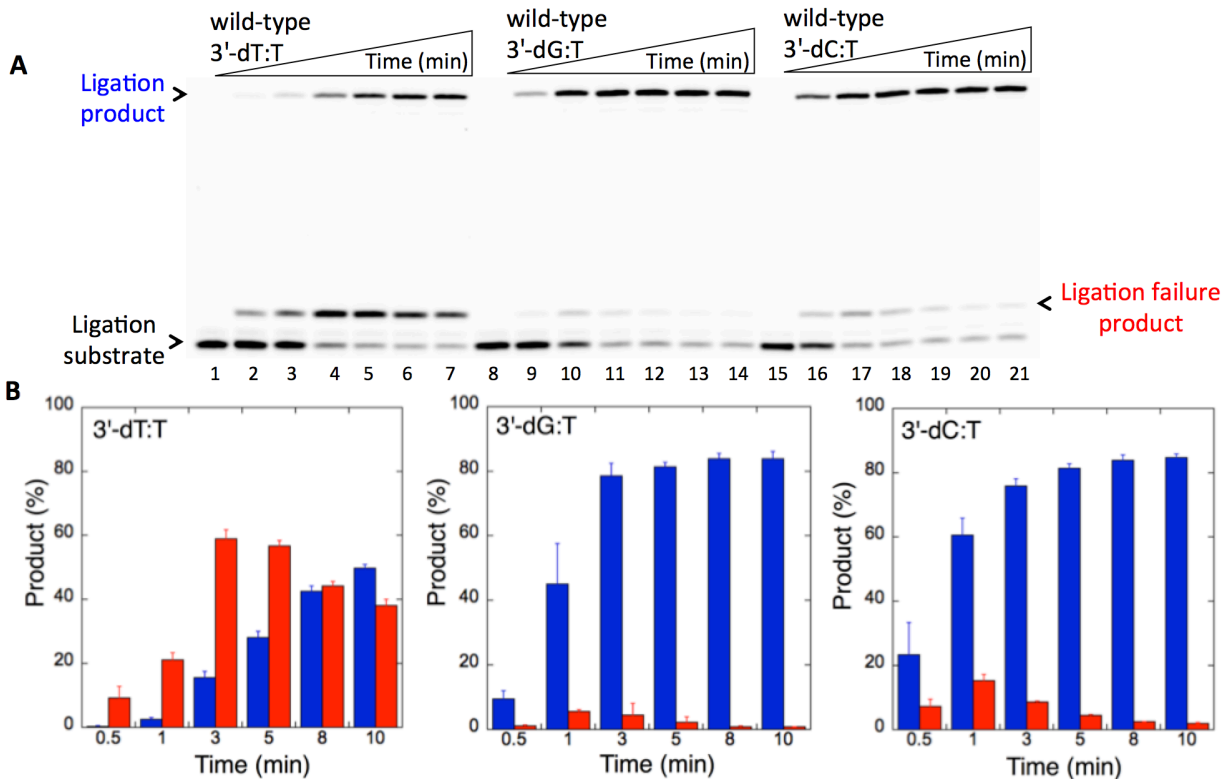
Supplementary Figure 9. Ligation of the repair intermediates with 3'-preinserted mismatches opposite template A and T by LIG1 variant R771W. (**A**) Lanes 1, 8, and 15 are the negative enzyme controls of the nicked DNA substrates with 3'-dA:A, 3'-dG:A, and 3'-dC:A, respectively. Lanes 2-7, 9-14, and 16-21 show the reaction products obtained at the time points 0.5, 1, 3, 5, 8, and 10 min. (**B**) Lanes 1, 8, and 15 are the negative enzyme controls of the nicked DNA substrates with 3'-dT:T, 3'-dG:T, and 3'-dC:T, respectively. Lanes 2-7, 9-14, and 16-21 show the reaction products obtained at the time points 0.5, 1, 3, 5, 8, and 10 min. The bar graphs are presented in Figure 13.



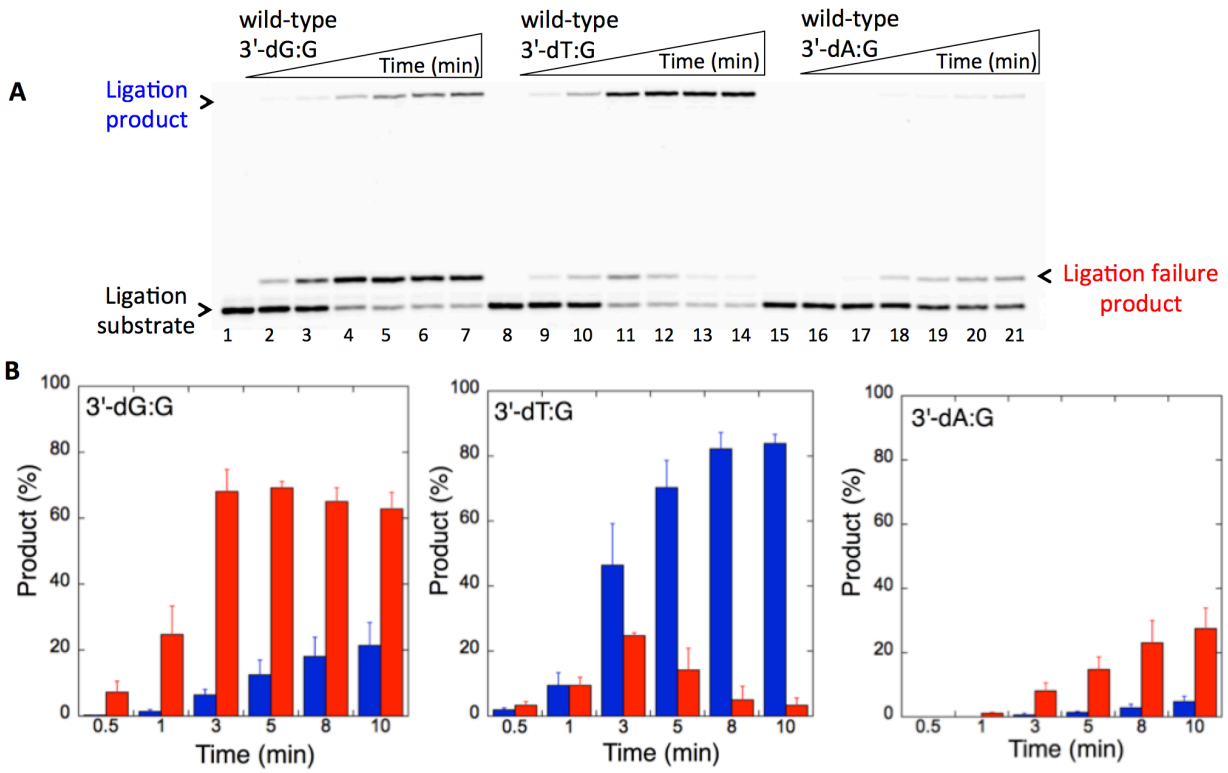
Supplementary Figure 10. Ligation of the repair intermediates with 3'-preinserted mismatches opposite template G and C by LIG1 R771W. (**A**) Lanes 1, 8, and 15 are the negative enzyme controls of the nicked DNA substrates with 3'-dG:G, 3'-dT:G, and 3'-dA:G, respectively. Lanes 2-7, 9-14, and 16-21 show the reaction products obtained at the time points 0.5, 1, 3, 5, 8, and 10 min. (**B**) Lanes 1, 8, and 15 are the negative enzyme controls of the nicked DNA substrates with 3'-dC:C, 3'-dT:C, and 3'-dA:C, respectively. Lanes 2-7, 9-14, and 16-21 show the reaction products obtained at the time points 0.5, 1, 3, 5, 8, and 10 min. The bar graphs are presented in Figure 14.



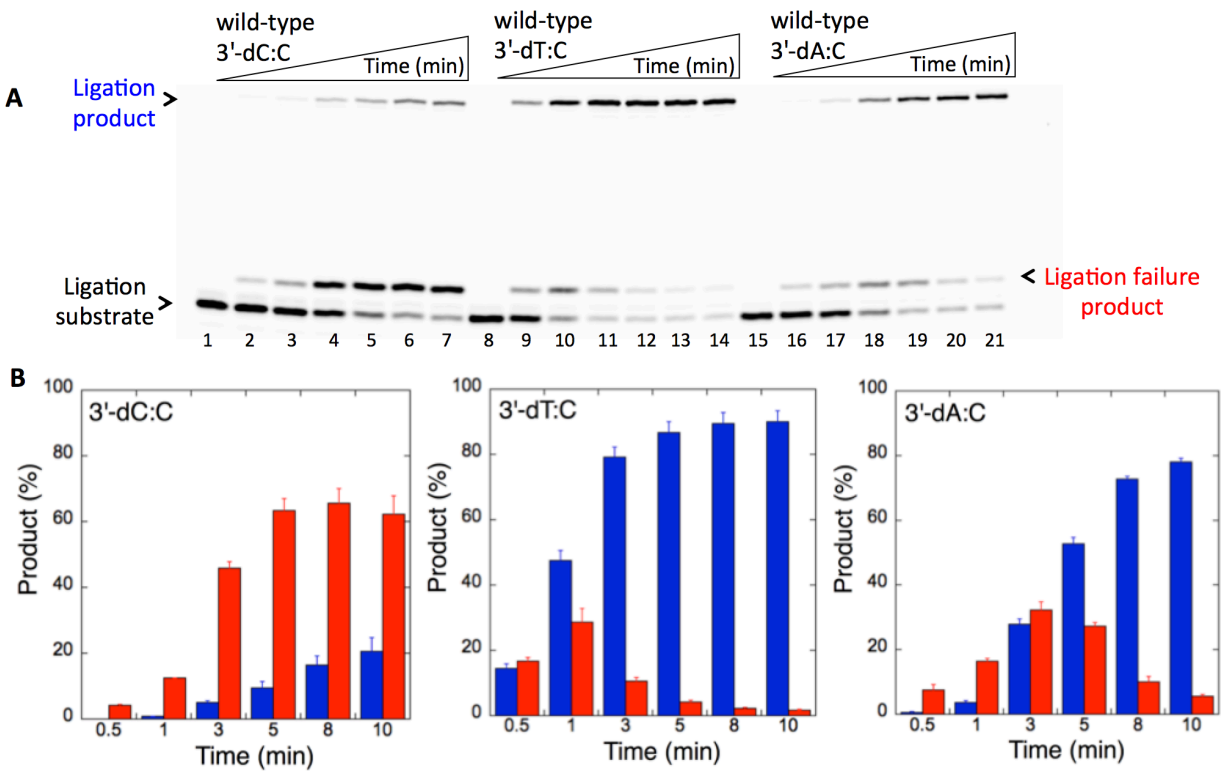
Supplementary Figure 11. Ligation of the repair intermediates with 3'-preinserted mismatches opposite template A by wild-type LIG1. (**A**) Lanes 1, 8, and 15 are the negative enzyme controls of the nicked DNA substrates with 3'-dA:A, 3'-dG:A, and 3'-dC:A, respectively. Lanes 2-7, 9-14, and 16-21 show the reaction products obtained at the time points 0.5, 1, 3, 5, 8, and 10 min. (**B**) The graphs show the time-dependent changes in the ligation (blue) and ligation failure (red) products, and the data are presented as the averages from three independent experiments ± SDs.



Supplementary Figure 12. Ligation of the repair intermediates with 3'-preinserted mismatches opposite template T by wild-type LIG1. (**A**) Lanes 1, 8, and 15 are the negative enzyme controls of the nicked DNA substrates with 3'-dT:T, 3'-dG:T, and 3'-dC:T, respectively. Lanes 2-7, 9-14, and 16-21 show the reaction products obtained at the time points 0.5, 1, 3, 5, 8, and 10 min. (**B**) The graphs show the time-dependent changes in the ligation (blue) and ligation failure (red) products, and the data are presented as the averages from three independent experiments ± SDs.



Supplementary Figure 13. Ligation of the repair intermediates with 3'-preinserted mismatches opposite template G by wild-type LIG1. (**A**) Lanes 1, 8, and 15 are the negative enzyme controls of the nicked DNA substrates with 3'-dG:G, 3'-dT:G, and 3'-dA:G, respectively. Lanes 2-7, 9-14, and 16-21 show the reaction products obtained at the time points 0.5, 1, 3, 5, 8, and 10 min. (**B**) The graphs show the time-dependent changes in the ligation (blue) and ligation failure (red) products, and the data are presented as the averages from three independent experiments ± SDs.



Supplementary Figure 14. Ligation of the repair intermediates with 3'-preinserted mismatches opposite template C by wild-type LIG1. (**A**) Lanes 1, 8, and 15 are the negative enzyme controls of the nicked DNA substrates with 3'-dC:C, 3'-dT:C, and 3'-dA:C, respectively. Lanes 2-7, 9-14, and 16-21 show the reaction products obtained at the time points 0.5, 1, 3, 5, 8, and 10 min. (**B**) The graphs show the time-dependent changes in the ligation (blue) and ligation failure (red) products, and the data are presented as the averages from three independent experiments ± SDs.

Patients/ Reported time	Onset age/Sex	Ethnicity	Zygous	Variants	Phenotype characteristics
Original patient (died)/1992	2 years/F	White	Heterozygous Heterozygous	E566K R771W	Hepatosplenomegaly, red cell macrocytosis, bronchiectasis, ocular telangiectasia, sun sensitivity, pneumonia, herpes zoster, lymphoma, growth retardation, normal mental activity
Patient 1/2018	2 years/M	White	Heterozygous Heterozygous	T415Mfs*10 R641L	Hypogammaglobulinemia, red cell macrocytosis, lymphocytic and an early history of diarrhea, normal mental activity
Patient 2/2018	2 years/M	White	Heterozygous Heterozygous	T415Mfs*10 R641L	Hypogammaglobulinemia, red cell macrocytosis, early viral infection, normal mental activity
Patient 3/2006	2 months/M	Sudanese	Homozygous Homozygous	P529L R771W	Severe eczema, severe anemia, lymphopenia with low B and T cells, adenovirus, rhinovirus, metapneumovirus, rotavirus, oral candidiasis, urinary tract infections, multicystic dysplastic kidney, normal mental activity
Patient 4/2006	1 month/M	Sudanese	Homozygous Homozygous	P529L R771W	Severe anemia, hypogammaglobulinemia, lymphopenia with low B and T cells, rhinovirus, metapneumovirus, ectopic multicystic dysplastic kidneys, normal mental activity
Patient 5/2006	1 month/F	Sudanese	Homozygous Homozygous	P529L R771W	Hypogammaglobulinemia, chronic respiratory tract infections, early growth failure macrocytosis and low numbers of B cells, normal mental activity

Supplementary Table 1. The LIG1 variants mutations, clinical and phenotypic characteristics of the patients with LIG1-deficiency disease. The information provided in this Table is combined from references 20-27.

Oligonucleotide	Sequence (5'-3')
Template C	GGTGAGGATGGGCCTCCGGTTCATGCCGCCCATG
Template T	GGTGAGGATGGGCCTCTGGTTCATGCCGCCCATG
Template A	GGTGAGGATGGGCCTCAGGTTCATGCCGCCCATG
Template G	GGTGAGGATGGGCCTCGGGTTCATGCCGCCCATG
Downstream primer	(P)GAGGCCCATCCTCACC-FAM
Upstream primer^{dC}	CATGGGCGGCATGAACCC
Upstream primer^{dG}	CATGGGCGGCATGAACCG
Upstream primer^{dA}	CATGGGCGGCATGAACCA
Upstream primer^{dT}	CATGGGCGGCATGAACCT
Upstream primer^{XdG}	CATGGGCGGCATGAACCXdG

Supplementary Table 2. The sequence of the oligonucleotides used to prepare the nicked DNA substrates for DNA ligation assays. The substrates include 3'-preinserted bases (dA, dT, dG, or dC) or 8-oxodG opposite template base A, T, G, or C. FAM denotes a flourescence tag and P is for a phosphate group. The bases at template base position are underlined.