SUPPLEMENTARY MATERIAL

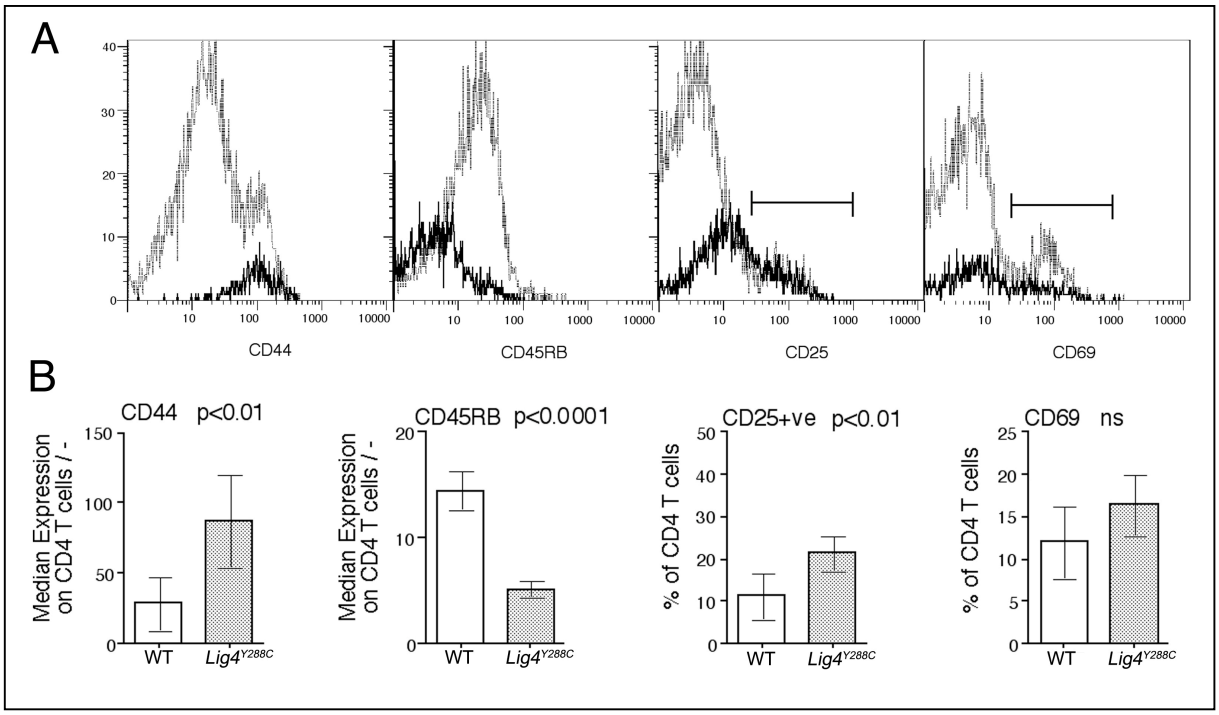
Supplementary Table 1.

Overview of the human Ligase IV syndrome: mutations and pathology.

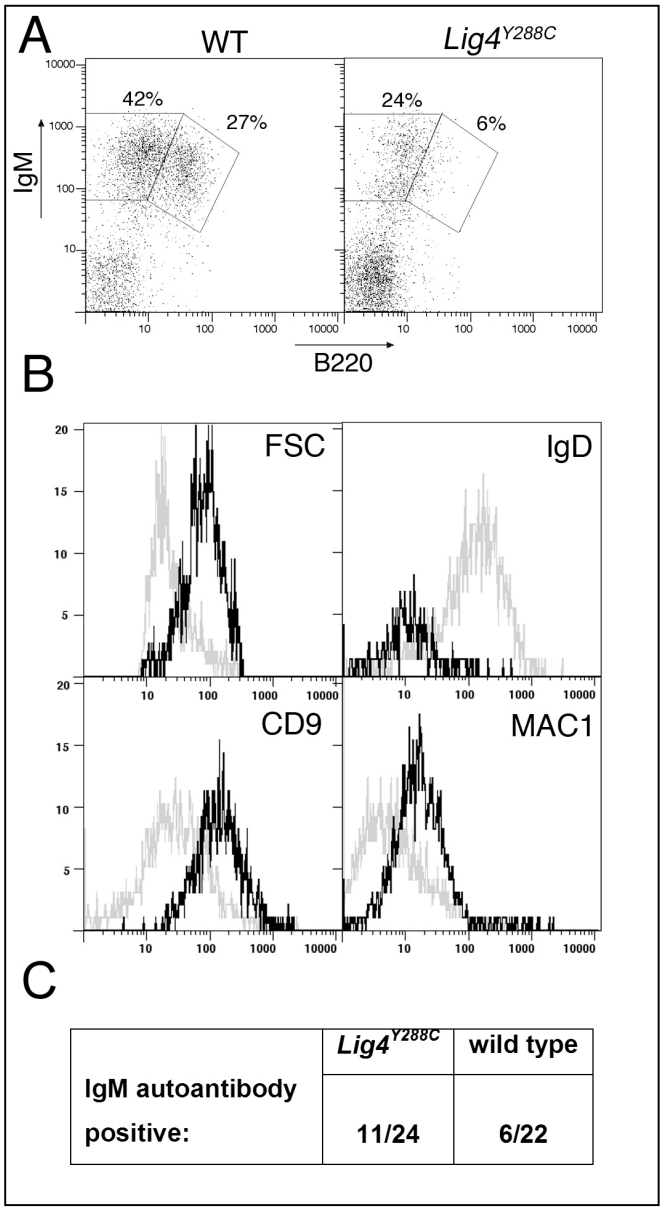
Patient	LigIV Mutations	Effects on LigIV Activity	Pathology	Refs.
180BR	R278H substitution in the active site, homozygous;	5-10% of wild type activity, normal stability and XRCC4-binding	Radiosensitivity, T cell leukaemia;	(11)
411BR	R278H, A3Vand T9I substitutions, all homozygous;	1% wild type activity, normal stability and XRCC4-binding	Growth retardation, radiosensitivity, lymphopenia;	(4)
2303, 2304	R580X and R814X truncations, compound heterozygous, loss of BRCT domains;	R580X - a null allele, no XRCC4 binding, cytosolic localisation;	Growth retardation, microcephaly, lymphopenia;	
99P0149	R814X truncation and G469E substitution, compound heterozygous;	R814X - 10-fold reduced stability, <1% of wild type activity in vivo;		
-	R814X truncation, homozygous;	G469E, <1% activity in vivo;	Growth retardation, microcephaly, lymphopenia, T cell leukaemia;	(5)
SC2	Q433 deletion in the catalytic domain, homozygous;	Reduced protein stability, undetectable levels;	Lymphopenia, no developmental defects;	(6)
P1, P2	Q280R substitution close to the active site, and a frame shift at K424, causing termination 20-residues downstream; compound heterozygous;	K424FS - a null allele, loss of BRCT domains and XRCC4-binding region, Q280R - normal in vitro activity, reduced levels in vivo;	Microcephaly, severe combined immunodeficiency, EBV-associated lymphoproliferative syndrome;	(7)

P#1, P#2	H282L substitution in catalytic domain, and a frame-shift at K424, causing termination 20 residues downstream; compound heterozygous;	H282L - unknown, likely a hypomorphic allele; K424FS - a null allele, loss of BRCT domains and XRCC4-binding region;	Growth retardation, microcephaly, lymphopenia, impaired humoral immunity, autoimmune thrombocytopenia, EBV-associated lymphoma;	(8)
-	M249V substitution, and a deletion at K424, causing a frame-shift; compound heterozygous;	M249V – unknown; K424FS – null allele, see above;	Growth retardation, microcephaly, lymphopenia, EBV-associated lymphoma;	(9)
-	R814X truncation and G469E substitution, compound heterozygous;	See above;	Growth retardation, microcephaly, pancytopenia and bone marrow failure.	(10)

Supplementary Figure 1. Activation of peripheral CD4 T cells in *Lig4*^{Y288C} mice. (**A**) Histograms of the expression of CD44, CD45RB, CD25, and CD69 on CD4 T cells in the spleen of wild type (grey line) and *Lig4*^{Y288C} (black line) mice, the plots are representative of n≥4. (**B**) Median expression levels of CD44 and CD45RB on CD4 T cells, and the percentages of CD25^{+} and CD69^{+} cells in the CD4 T cells gate in the spleens of wild type and *Lig4*^{Y288C} mice. Bars represent means and 95% confidence limits, n≥4.



Supplementary Figure 2. Relative preservation of B1 cells in the peritoneal cavity of *Lig4*^{Y288C} mice. (A) Flow cytometry profiles of the peritoneum of wild-type (WT) and *Lig4*^{Y288C} mice stained for B220 and IgM and gated on lymphocytes, representative of n=6 per group. Numbers represent the percentages of cells in the plot that fall within the B1 (IgM^{+} B220^{low}) and B2 (IgM^{+} B220^{high}) gates. **(B)** Forward scatter (FSC) and expression of IgD, CD9, and MAC1 on B2 cells in WT (grey line) and B1 cells in *Lig4*^{Y288C} (black line) mice. The plots are gated on IgM^{+} B220^{low} or IgM^{+}IgD^{low} for B1 cells, and on IgM^{+} B220^{high} or IgM^{+}IgD^{high} for B2 cells, and are representative of n=3. **(C)** Serum IgM autoantibodies were detected in 11/24 *Lig4*^{Y288C}, compared to 6/22 wild-type mice ($p<0.05$, χ^2-test, with the wild-type measurements used as the "expected" parameter versus *Lig4*^{Y288C} as the "observed"). From the staining pattern (data not shown), the IgM autoantibodies targeted cytosolic proteins, similar to autoantibodies previously seen in the *scid*$^{DNA\text{-}PKcs}$ (49).



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	Lig4^{Y288C}	wild type
IgM autoantibody positive:	**11/24**	**6/22**