Supplementary Methods

Intracellular Staining

Cells were activated with Phorbol 12-myristate 13-acetate (PMA) (50ng/mL, SIGMA), Ionomicyn (1μg/mL, SIGMA), GolgiStop (1X, BD) and Brefeldin A (1X, BD) for 5hrs at 37°C 5% CO_2. Cells were stained with Live/Dead dye (Invitrogen), anti-CD20-AlexaFluor780 and anti-CD3-PerCP-Cy5.5 (all eBioscience) for 30min at 4°C. Samples were then fixed and permeabilized with $FoxP_3$ Fix and Perm Kit (BD) and stained with anti-IL10-PE (BD) and TNF-α (eBioscience) for 30min at 4°C. Cells were acquired on LSRFortessa (BD).

ELISA

ELISA (eBioscience: IL-10) (R&D: TNF-α) was performed according to manufacturer's instructions. Briefly, 96-well plates (Nunc™, Thermo Scientific) were incubated overnight with capture antibody at 4°C. The next day, plates were blocked for 1hr with 1%BSA, and standards and supernatant were added for 2hrs at room temperature. Detection antibody was added for 2hrs at room temperature and streptavidin-HRP was added for 20min at room temperature. Finally, plates were incubated with TMB (eBioscience) for 10min at room temperature and reaction was stopped using 0.5 M 2N H_2SO_4. Optical density at 405nm was measured using Sunrise ELISA Reader (TECAN, UK). The concentration of each cytokine was calculated from standard curves using Magellan data analysis Software. For IL-10: the sensitivity of the kit was 2 pg/mL and the standard curve range was from 2 to 300 pg/mL. For TNF-α: the sensitivity of the kit was 15.6 pg/mL and the standard curve range was from 15.6 to 1000 pg/mL.

B-cell subsets from peripheral blood cones

RosetteSep™ Human B cell enrichment cocktail (STEM CELL, UK) was used to obtain purified $CD20^+$B-cells from leukocytes retained filtering cones from healthy volunteers blood donations (NHSBT Tooting blood bank, UK). B-cell subsets were sorted with ARIA II (BD). Memory B-cells were $CD20^+CD27^+$ (purity >95%), naïve B-cells $CD20^+CD27^-CD24^+CD38^+$ (purity >95%) and transitional B-cells $CD20^+CD27^-CD24^{hi}CD38^{hi}$ (purity >95%). For IL-10 measurements in different B-cell subsets: $0.5x10^6$ B-cell subsets were cultured in complete media supplemented with IL-2 (100ng/ml, R&D) with or without $1.0x10^4$ plate bound CD40L-transfected and non-transfected mouse L fibroblast cells (X-ray irradiated for 30min 9,045 cGy), and CpG – a TLR9 agonist - (1μM; ODN 2006, InvivoGen) for 72hrs. After 3 days of activation, cells were stained with anti-CD20-AlexaFluor780 (eBioscience) and Live/Dead fixable yellow dead cell staining kit (Invitrogen) by surface staining and with IL-10-PE (BD) by intracellular staining for 30min at 4°C. For BCR signalling assays: $0.5x10^6$ non-activated or anti-IgM (20μg/ml)/anti-IgG (20μg/ml)-activated B-cell subsets were co-cultured with $0.5x10^5$ CD40L-transfected or non-transfected L-cells for 3 days. IL-10-PE (BD) was detected by intracellular staining. Cells were acquired on an LSRFortessa (BD). Data was analysed using FlowJo (Tree Star, Inc. Ashland, OR 97520. USA).

IL-10 production by transitional B-cells from patients and healthy controls

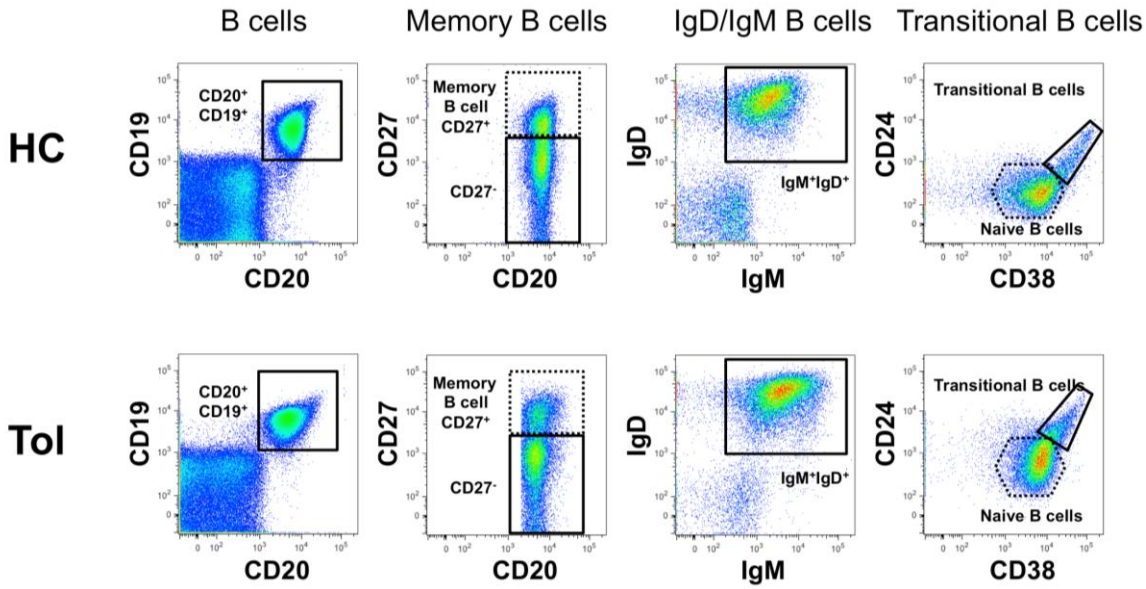
For IL-10 measurements in transitional B-cell subsets: $10x10^4$ sorted transitional B-cells from 3 healthy controls and 3 tolerant recipients were activated with $1x10^3$ non-transfected or CD40L-transfected L-cells, with or without BCR activation anti-IgM (20μg/ml)/anti-IgG (20μg/ml) for 3 days. IL-10 in the supernatants was measured using Cytometric Beads Array human Th1/Th2/Th17 kit (BD). For IL-10: the sensitivity of the

kit was 4.5 pg/mL and the standard curve range was from 4.5 to 5000 pg/mL. Beads were acquired on LSRFortessa (BD).

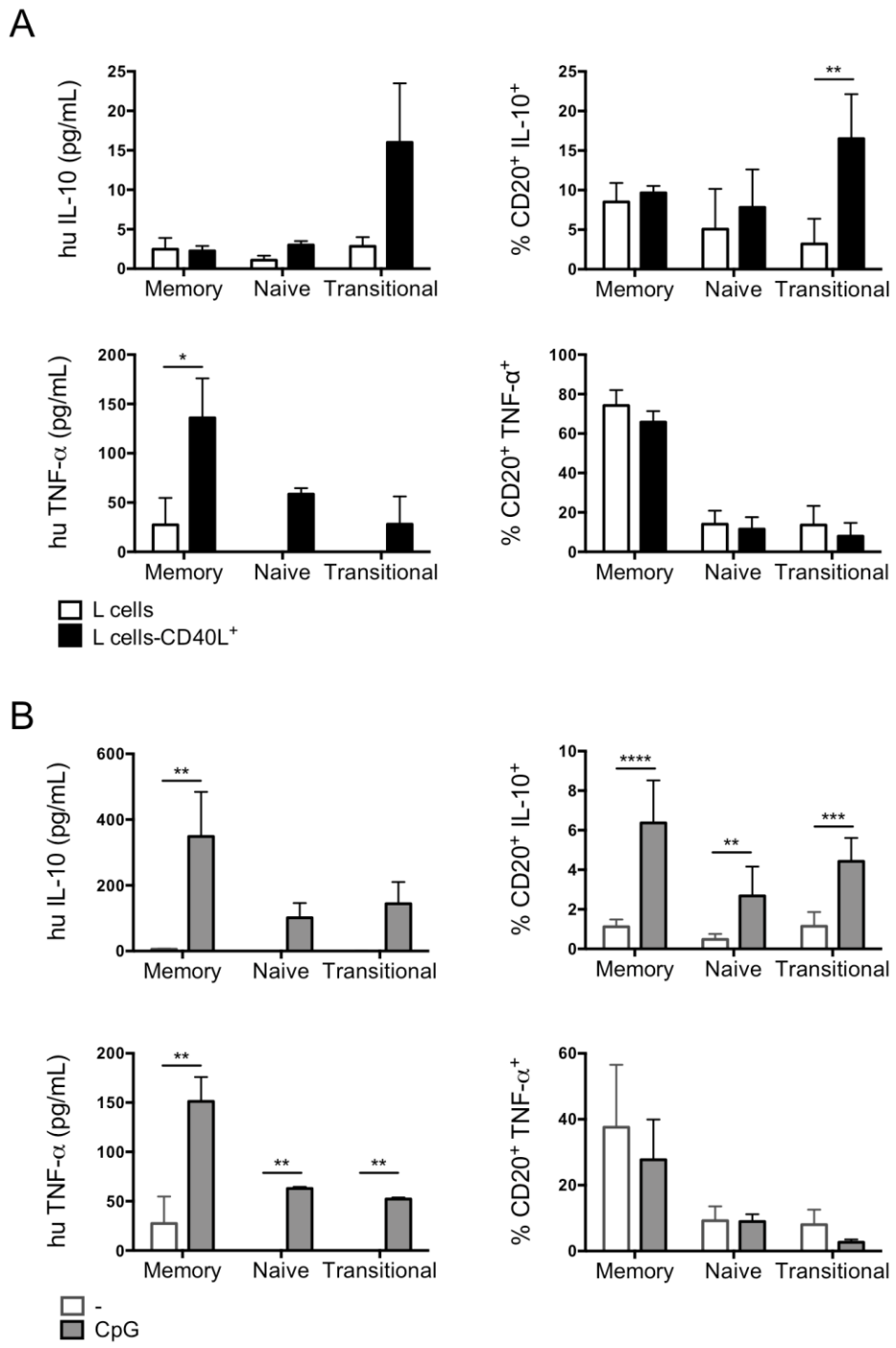
Flow Cytometry settings were used to monitor staining panels in healthy volunteers and patients samples

Flow cytometry panels in this study were monitored with CS&T (Cytometer Setup and Tracking, BD) beads, as a quality control for the Flow Cytometer; Application settings, as a quality control of the flow cytometry staining; and Inter and Intra-assay controls, as a quality control of the flow cytometry staining and the quality of the samples.

Supplementary Figures

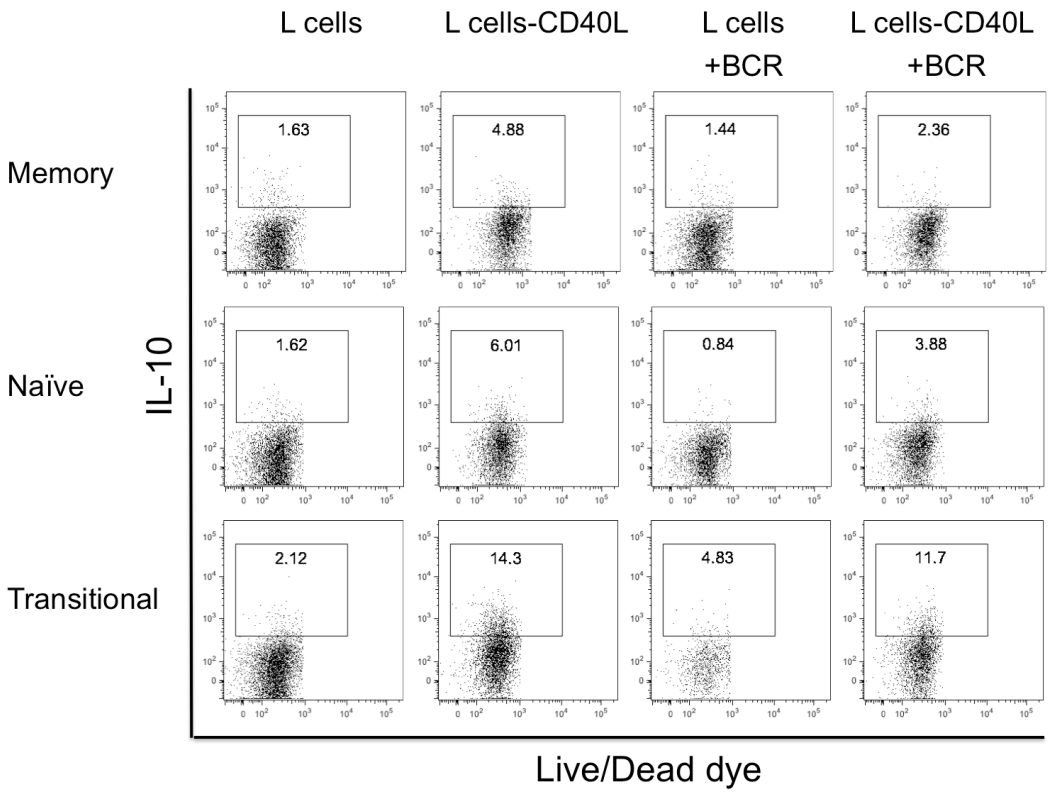


Supplementary Figure 1: B-cell subsets flow cytometry gating strategy. B-cells were identified as $CD20^{+}CD19^{+}$ B-cells. Within the B-cell population, memory B-cells were identified as $CD27^{+}$ B-cells. $IgM^{+}IgD^{+}$ cells were gated from the $CD27^{-}$ B-cell population and from the $IgM^{+}IgD^{+}$ B-cells, naive B-cells were identified as $CD24^{+}CD38^{+}$ cells and transitional B-cells were identified as $CD24^{hi}CD38^{hi}$ cells. Representative dot-plots of total, memory, $IgD^{+}IgM^{+}$, naïve and transitional B-cells were obtained from PBMCs from healthy controls (HC) and tolerant (Tol) patients by flow cytometry.



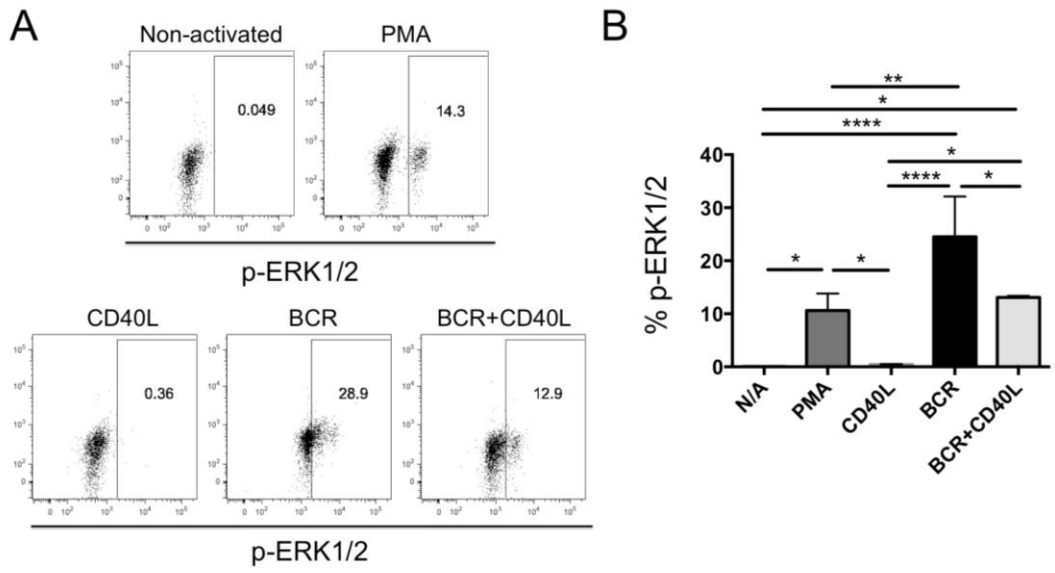
Supplementary Figure 2: IL-10 production by isolated B-cell subsets after CD40L and CpG activation. A) Sorted memory, naïve and transitional B-cells were obtained from healthy leukocytes retained in filtering cones. $0.5x10^6$ sorted B-cell subsets were activated with $0.1x10^5$ non-transfected L-cells or human-CD40L-transfected L-cells B) and with or without CpG (1μM) for 72hrs. ELISA and intracellular

staining were used to measure IL-10 and TNF-α. Two-way RM ANOVA test with a Sidak's multiple comparisons test was used. For all statistical tests **** $P<0.0001$, *** $P<0.001$ and ** $P<0.01$ were considered significant.



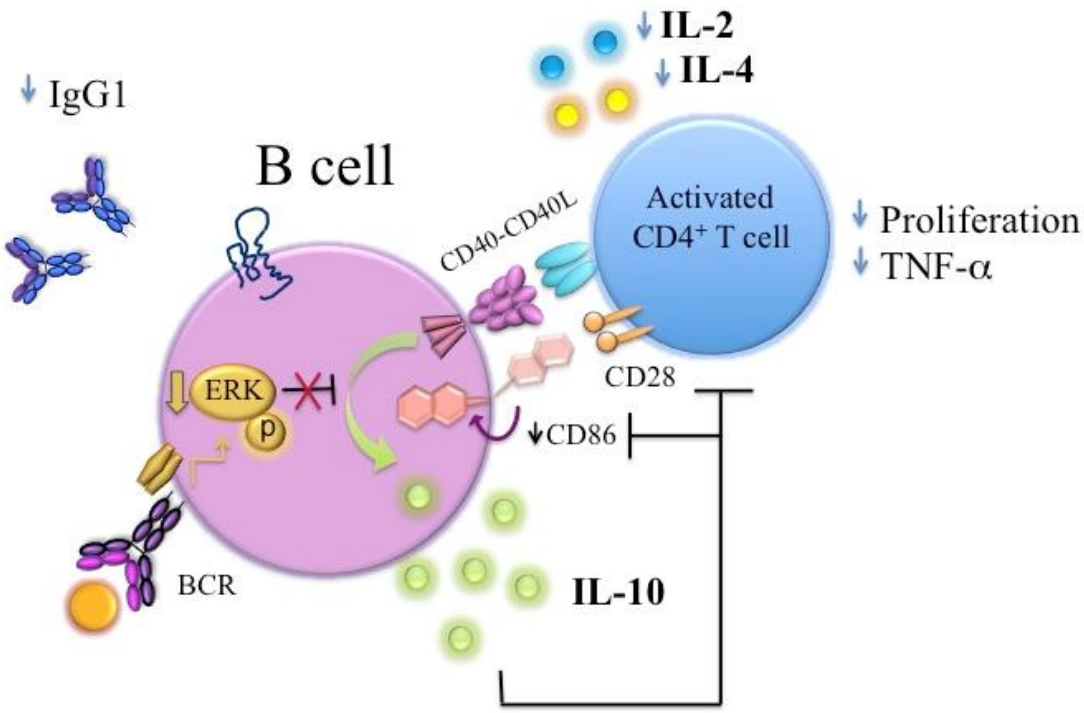
Supplementary Figure 3: IL-10 production by isolated B-cell subsets after CD40L, BCR and CD40/BCR activation.

IL-10 production after CD40, BCR and CD40/BCR activation was measured by adding $0.5x10^5$ non-transfected or CD40L-transfected L-cells in $0.5x10^6$ non-activated or BCR-activated sorted memory, naïve and transitional B-cells obtained from healthy leukocytes retained filtering cones. B-cell subsets were activated for 72hrs and IL-10 was detected with intracellular staining.



Supplementary Figure 4: ERK phosphorylation after BCR and CD40L activation.

A) Phosphorylation of ERK was assessed by phospho-flow using non-activated samples as negative controls and PMA-activated samples as positive controls. Phosphorylation ERK after CD40L, BCR, or BCR/CD40 activation was measured by adding $0.5x10^5$ non-transfected or CD40L-transfected L-cells in $0.5x10^6$ non-activated or BCR-activated B-cells from healthy volunteers after 3 days of culture by phospho-flow. B) ERK phosphorylation between different activating conditions was compared. Ordinary one-way ANOVA with a Tukey's multiple comparisons test was used, **** $P<0.0001$, *** $P<0.001$, ** $P<0.01$ and * $P<0.05$ were considered significant.



Supplementary Figure 5: Schema of B-cell from tolerant recipients.

B-cells from tolerant patients exhibit higher percentages of IL-10-producing B-cells after CD40 activation with limited evidence of concomitant pro-inflammatory cytokines in parallel and reduced BCR signalling all when compared to B-cells from healthy controls. Reduction in the BCR signalling favoured IL-10 production in the transitional B-cell population of tolerant recipients. In addition, reduced IL-2 and IL-4 production by T-cells, may contribute to the low IgG1 levels observed in the serum. *Schema based on data from this manuscript and Nova-Lamperti et al., 2016[14].*

Tolerant	Cause of end-stage renal disease	
Tol 1	Glomerular Disease	-Mesangial GN and Malignant Hypertension
Tol 2	Genetic Disorder	-Polycystic Kidney Disease
Tol 3	Other	-Unknown, possibly B-cell lymphoproliferative disorder
Tol 4	Other	-Traumatic loss of Kidney after Road accident
Tol 5	Other	-Unknown
Tol 6	Diabetes	-Diabetes type 2. Biopsy proven Nephropathy. Patient has Marfan Syndrome
Tol 7	Glomerular Disease	-Membranoproliferative GN Type 1
Tol 8	Pyelonephritis	-Pyelonephritis/Reflux Nephropathy
Tol 10	Glomerular Disease	-Membranous GN
Tol 11	Pyelonephritis	-Unknown, possibly chronic pyelonephritis
Tol 12	Genetic Disorder	-Congenital Renal Dysplasia
Tol 13	Glomerular Disease	-Membranoproliferative GN
Tol 14	Glomerular Disease	-Mesangial GN and Malignant Hypertension
Tol 15	Other	-Drug Induced ESRF post-leukemia
Tol 16	Glomerular Disease	-GN

Supplementary Table 1: Causes of Kidney failure or end-stage renal disease (ESRD) in tolerant kidney transplant recipients.

Tol: Tolerant recipient. GN: Glomerulonephritis. ESRF: End-stage Renal Failure.