SUPPLEMENTARY INFORMATION

Supplementary Figure 1: Phenotype of splenic RTX-PC.

(A) Flow cytometry analysis of splenic mononuclear cells from a RTX-treated patient. RTX-PC ($CD19^{+}CD27^{+}CD24^{-}CD38^{high}$) were labeled with antibodies to CD45, CD20, CD138, CD86, CXCR4, CCR2, CD79a (intracellular staining), HLA-DR, CD40, CD9, Ki67 (intracellular staining), CD11a, CD54. Patient sample is shown in red and isotype control in green. (B) Distribution of IgM, IgG, and IgA isotype Ig secretion among RTX-PC. Flow cytometry analysis of RTX-PC labeled by intracellular staining with antibodies to IgM, IgG, IgA (left). In vitro Ig-secretion detectable after 6 hours of after 5 days of culture of $5x10^{5}$ spleen cells in triplicate, estimated by ELISPOT (right).

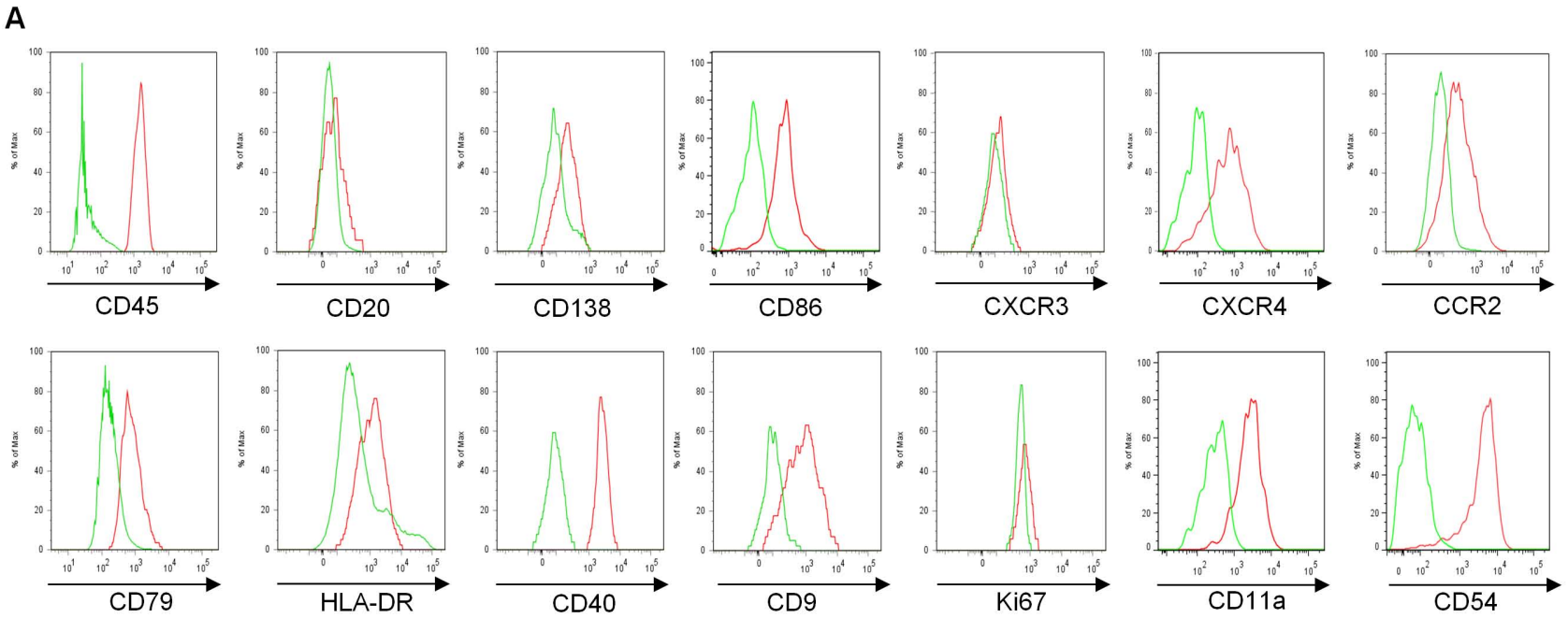
Supplementary Figure 2: Gating strategy for cell sorting.

(A) Flow cytometry analysis of splenic mononuclear cells from an ITP patient and a HD with a FACS Aria cell sorter (Becton Dickinson). Cells were labeled with anti-CD3, CD19, CD27, CD20, CD38, HLA-DR antibodies. Using side and forward scatters, non-lymphoid cells and *doublets were excluded.* After gating on $CD3^{-}CD19^{+}CD27^{+}$ cells, ASC were identified as $CD20^{-}$ $CD38^{high}$. Plasmablasts and plasma cells were sorted as $HLA\text{-}DR^{high}$ and $HLA\text{-}DR^{low}$, respectively. (B) Flow cytometry analysis of splenic mononuclear cells from a RTX-treated patient with a FACS Aria cell sorter (Becton Dickinson). Cells were labeled with anti-CD3, CD16, CD19, CD24, CD27, CD38 antibodies. After gating on $CD3^{-}CD16^{-}CD24^{-}CD19^{+/low}$ cells, RTX-PC were identified as $CD27^{+}CD38^{high}$.

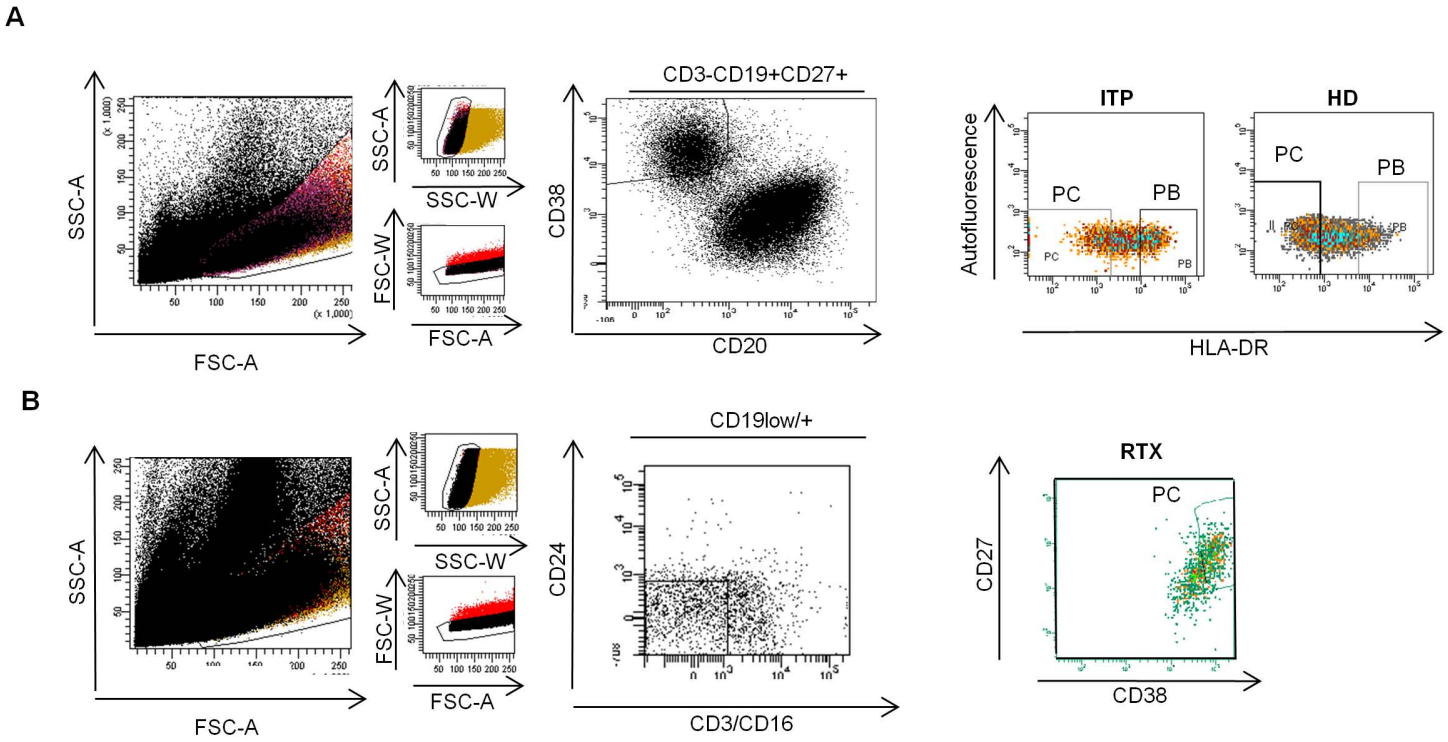
Supplementary Figure 3: HD-PC and ITP-PC showed an intermediate gene expression profile at the single cell level, compared to RTX-PC and ITP-PB

Heat map representation of multiplex single-cell RT-PCR, performed with the Fluidigm Dynamic Array on RTX-PC, HD-PC, ITP-PB and ITP-PC, using 15 genes showing differential expression between RTX-PC and ITP-PB (*BIRC5, BUB1, MKI67, ZWINT, CDC6, CCND2, CENPF, KLF6, KLF9, FOS, TNFAIP3, BIRC3, ATF3, CD9, SOCS1*, with *B2M* as control). Columns represent individual gene probes, rows single cells, with color relative to the Ct expression value (scale bar at the right).

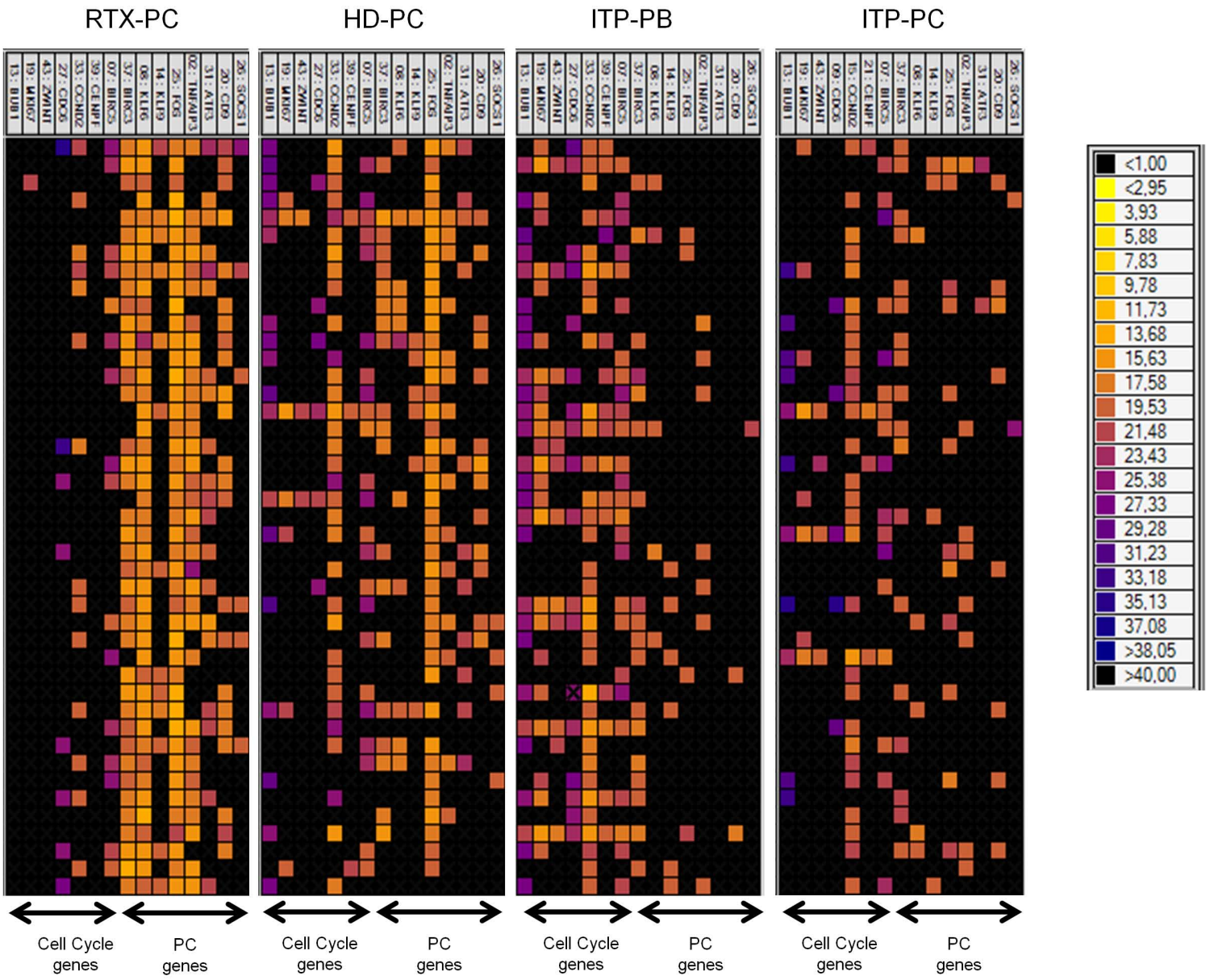
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



Supplementary Table 1

Clinical characteristics of splenectomized ITP patients.

Patients	Age / Gender	Duration of ITP before RTX (months)	Previous treatments received before splenectomy and RTX	Interval between RTX and splenectomy (months)	Iv Ig before splenectomy (Yes/no, time)	Response to splenectomy	Follow-up since splenectomy (months)
RTX 1	28/F	6	CTX[a]	3	Yes, 3 weeks before	CR[c]	18
RTX 2	51/F	3	CTX, Iv Ig[b], danatrol, vincristine (before RTX)	3	no	CR	21
RTX 3	54/M	2	CTX, disulone	4	no	NR[d]	12
RTX 4	48/F	48	CTX, Iv Ig, imurel	4	no	NR	12
RTX 5	74/F	3	CTX, Iv Ig, disulone	6	no	NR	8
RTX 6	32/F	10	CTX, disulone	6	no	CR	24
RTX 7	27/M	8	CTX, Iv Ig, plaquenil, disulone	6	no	CR	16
RTX 8	28/F	36	CTX, Iv Ig, disulone	6	no	CR	9
RTX 9	53/F	5	CTX, plaquenil, Iv Ig	6	Yes, 2 weeks before	CR	8
RTX 10	26/M	3	CTX, danatrol, disulone	6	no	CR	12
ITP 1	17/F	_	CTX	_	no	CR	23
ITP 2	66/F	_	CTX, disulone	_	no	CR	30
ITP 3	51/F	_	CTX, Iv Ig	_	Yes, 2 weeks before	CR	28
ITP 4	45/F	_	CTX	_	no	CR	24
ITP 5	60/H	_	CTX, Iv Ig	_	Yes, 1 weeks before	CR	18

a: Corticosteroids, b:Intravenous immunoglobulins, c: Complete response, d: No Response

Supplementary Table 2

Spleen ASC and Anti-GpIIbIIIa secretion in splenectomized ITP patients and healthy

	Age / gender	ASC[a] / $CD19^+$	Plasmablasts[b] / $CD19^+$	Plasma cells[c] / CD19+	Germinal center B cells[d] / $CD19^+$	Absolute numbers of IgG-secreting cells / 10^6 cells	Anti-GPIIbIIIa ASC / total IgG (ELISPOT assay)
ITP patients							
ITP 1	17/F	5.1%	4.3%	0.77%	5.5%	4608	0.51%
ITP 2	66/F	6.0%	3.5%	2.5%	5.2%	7424	< 0.05 %
ITP 3	51/F	6.9%	4.7%	2.2%	2.8%	6912	0.57%
ITP 4	45/F	4.7%	1.8%	3.0%	2.3%	3840	1.0%
ITP 5	60/M	7.2%	3.9%	3.4%	1.5%	2900	1.2%
Healthly Donors							
HD 1	49/F	2.9%	0.43%	2.4%	0.80%	720	<0.1 %
HD 2	41/F	2.8%	0.10%	2.7%	0.70%	1400	<0.1 %
HD 3	51/M	0.77%	0.05%	0.72%	0.90%	700	<0.1 %
HD 4	48/M	0.19%	0.02%	0.17%	0.30%	800	<0.1 %
HD 5	30/F	0.92%	0.16%	0.76%	0.81%	_	_
HD 6	48/M	1.0%	0.12%	0.88%	0.20%	_	_
HD 7	32/M	0.58%	0.09%	0.49%	0.10%	_	_
HD 8	39/F	1.4%	0.03%	1.4%	0.50%	_	_

a: Antibody secreting cells ($CD19^+CD20^-CD24^-CD27^+CD38^{high}$), b: plasmablasts ($CD19^+CD20^-CD24^-CD27^+CD38^{high}Ki67^{+)}$

c: plasma cells ($CD19^+CD20^-CD24^-CD27^+CD38^{high}Ki67^-$), d: Germinal center B cells ($CD19^+CD20^+CD24^-CD38^{int/+}$)

Supplementary Table 3

Flow cytometry	Clone	Origine
Primary antibodies		
CD9-Alexa647	MRP-1	AbB serothec
CD20-APCH7	L27	BD
CD86-PE	2331(FUN-1)	BD
CD27-APC	L128	BD
CD24-PE	ML5	BD
CD62L-PE	DREG-56	BD
HLA-DR-FITC	G46-6	BD
CD3-PE	UCHT1	BD
CD3-BIOTIN	III 471	BD
CD14-BIOTIN	3-C39	BD
CD138-PE	MI15	BD
CXCR3-PE	1C6	BD
CD3-APCH7	5k7	BD
Ki67-Alexa 488	B56	BD
CD14-APCH7	(MφP9)	BD
CXCR5-Alexa 647	RF8B2	BD
CD54-FITC	3 e 2	BD
CD16-APCH7	IV N409	BD
CD123	6H6	Biolegend
CD38-PerCP-Cy5-5	HIT2	Biolegend
CD15-fitc	HI98	Biolegend
CD79a-PE	HM47	Biolegend
CD43-FITC	NEM9	Biolegend
CD11a	Hi111	Biolegend
CD184-PE	12G5	Biolegend
IgA-FITC	Goat F $(ab')_2$	DAKO
IgG-FITC	Goat F $(ab')_2$	DAKO
CD20-FITC	2H7	ebioscience
CD9-PE	SN4 C3-3A2	ebioscience
FCeRIa-Biotin	AER-37	ebioscience
IgD-FITC	Goat F $(ab')_2$	Invitrogen
CD4-FITC	13B8.2	IOTest
CD45-FITC	J33	IOTest
CCR2-PE	48607	RD

Isotype		
Mouse IgG1k-PE	MOPC-21	BD
IG2ak-PE	G155-178	BD
IgG2ak-FITC	G155-178	BD
IgG2b-PE	LHG14	RD
Ig2b-Alexa647	27-35	BD
IgG1κ-Alexa 488	MOPC-21	BD
IgG1k-FITC	MOPC-21	BD
IgG2b-FITC	eBMG2b	ebioscience
Secondary antibody		
Streptavidin-APC	_	Invitrogen
Immunofluorescence	**Clone**	**Origine**
Primary antibodies		
CD20 mouse IgG2a	Clone L26	DAKO
CD3 rabbit polyclonal	_	DAKO
CD3 IgG2b	OKT3	ebioscience
CD3 mouseIgG1k	F7.2.38	DAKO
CD4 mouse IgG1k	RPA-T4	BD
CD8 mouse IgG1k	RPA-T8	BD
Kappa light chain rabbit	_	DAKO
Kappa light rabbit	_	DAKO
Ki67 mouse IgG1k	MIB-1	DAKO
CD14 Biotin mouse	3-c39	BD
Secondary antibody		
Goat anti-rabbit IgG-Alexa 488	_	Invitrogen
Goat anti-mouse IgG1-Cy5	_	Southern Biotech
Goat anti-mouse IgG1-Alexa 555	_	Invitrogen
Rat anti-mouse IgG2a-biotin	R19-15	BD
Conjugated streptavidin-CY3	_	Jackson immunoreasearch

Supplementary Table 4

Gene Name	Assay ID
Control genes (4):	
CD3g molecule, gamma (CD3-TCR complex)	Hs00962186_m1
major histocompatibility complex, class II, DR alpha	Hs00219575_m1
CD38 molecule	Hs01120071_m1
CD27 molecule	Hs00386811_m1
Diagnostic genes (15):	
suppressor of cytokine signaling 1	Hs00705164_s1
CD9 molecule	Hs00233521_m1
Kruppel-like factor 9	Hs00230918_m1
Kruppel-like factor 6	Hs00810569_m1
tumor necrosis factor, alpha-induced protein 3	Hs00234713_m1
FBJ murine osteosarcoma viral oncogene homolog	Hs00170630_m1
antigen identified by monoclonal antibody Ki-67	Hs01032443_m1
budding uninhibited by benzimidazoles 1 homolog (yeast)	Hs00177821_m1
activating transcription factor 3	Hs00231069_m1
baculoviral IAP repeat containing 3	Hs00154109_m1
baculoviral IAP repeat containing 5	Hs04194392_s1
ZW10 interactor	Hs00199952_m1
centromere protein F, 350/400kDa (mitosin)	Hs01118845_m1
cell division cycle 6 homolog (S. cerevisiae)	Hs00154374_m1
cyclin D2	Hs00153380_m1
Housekeeping gene (1):	
beta-2-microglobulin	Hs00984230_m1