Online Supplemental figures and tables for:

Red Pulp Macrophages in the Human Spleen are a Distinct Cell Population with a Unique Expression of Fc-gamma Receptors

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Figure S1

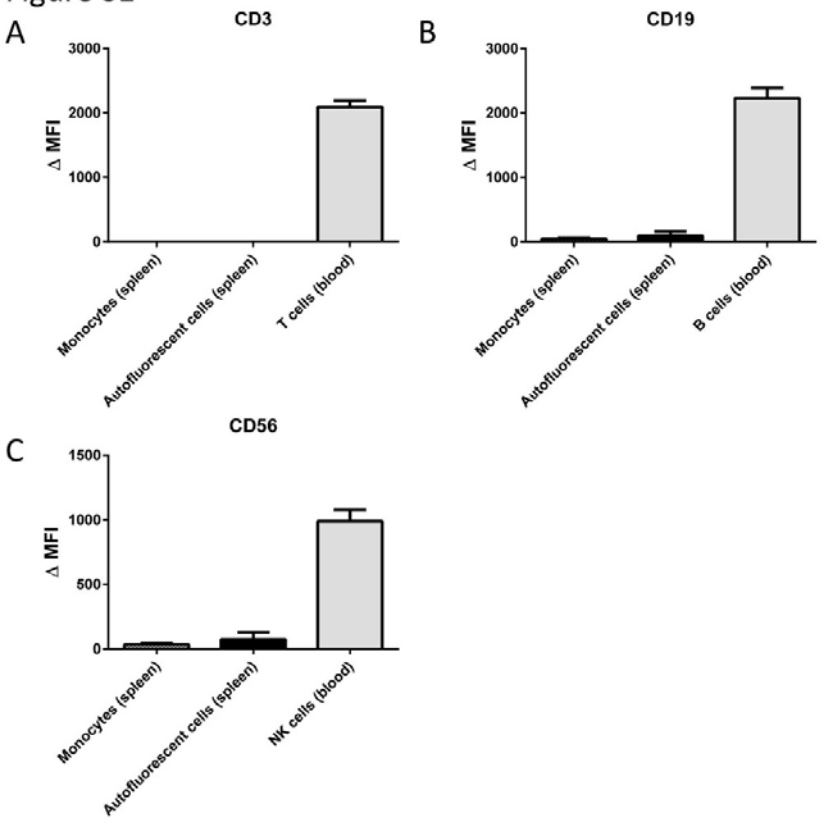


Figure S1. Spleen autofluorescent cells are negative for canonical lymphocyte markers
A CD3 staining on monocytes in the spleen (n=10), spleen autofluorescent cells (n=10), and circulating blood T cells (n=23). **B** CD19 staining on monocytes in the spleen (n=8), spleen autofluorescent cells (n=8), and circulating blood B cells (n=23). **C** CD56 staining on monocytes in the spleen (n=8), spleen autofluorescent cells (n=8), and circulating blood NK cells (n=23). MFI: median fluorescence intensity, Δ MFI: MFI corrected for staining with an isotype control. Means + s.e.m. are shown for each group.

Figure S2

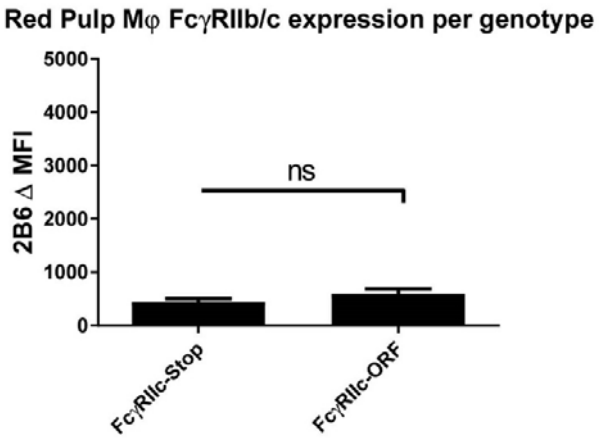


Figure S2. No evidence of expression of FcγRIIc on Red Pulp Macrophages

Comparison of staining of MoAb 2B6, which detects the identical extracellular domain of both FcγRIIb and FcγRIIc, on red pulp macrophages. MoAb 2B6 recognizes an extracellular epitope of both FcγRIIb and FcγRIIc, but since *FCGR2C*-Stop individuals cannot express FcγRIIc, the difference in MFI between *FCGR2C*-Stop and *FCGR2C*-ORF individuals can be assumed to derive from expression of FcγRIIc. We compared 2B6 staining on red pulp macrophages of FcγRIIc-Stop individuals (n=36) with individuals with 1 copy of FcγRIIc-ORF (n=10).

Individuals that carried other genotypes that are known to result in ectopic FcγRIIb expression, i.e. the 2B.4 promoter haplotype in *FCGR2B* [1] and/or a deletion of Copy Number Region 1 (CNR1, resulting in ectopic expression of FcγRIIb on NK Cells)[1;2] were excluded from the analysis to avoid accidental positive staining of FcγRIIb.

MFI: median fluorescence intensity, Δ MFI: MFI corrected for staining with an isotype control. Statistics by unpaired t test. ns: non-significant. Means + s.e.m. are shown for each group.

Figure S3

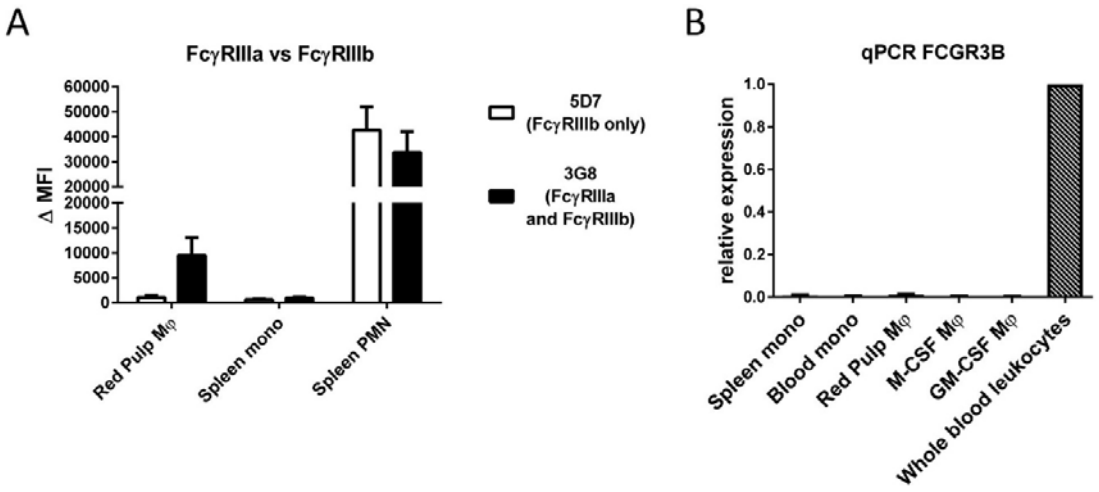
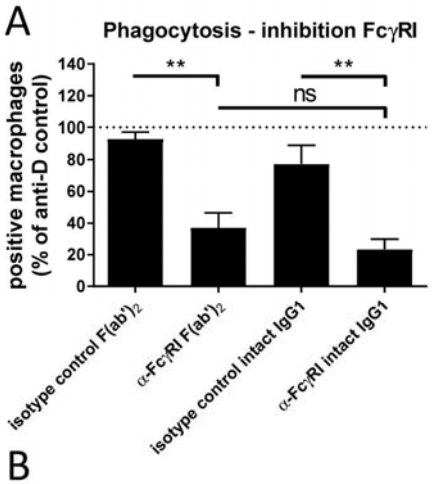
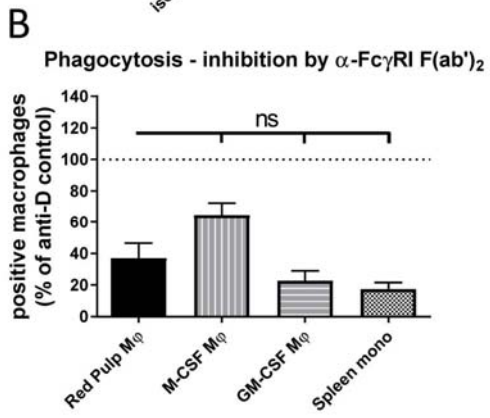


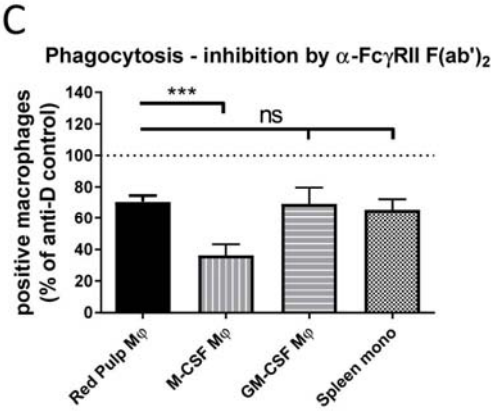
Figure S3. Red Pulp Macrophages express FcγRIIIa, not FγRIIIb

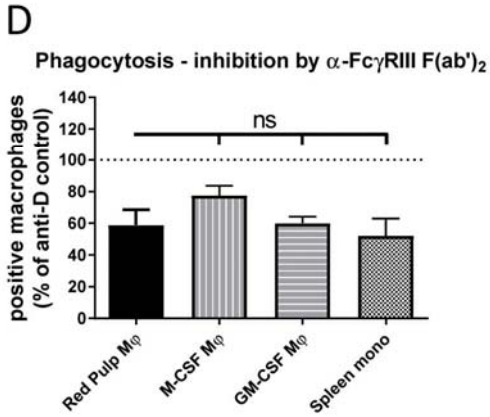
A Staining with different MoAbs for FcγRIII. FcγRIIIb staining by MoAb 5D7 (also known as CLBgran11), which specifically binds FcγRIIIb (white bars), is compared to staining by MoAb 3G8 (black bars), which binds both FcγRIIIa and FcγRIIIb. Stainings are on monocytes in the spleen, Red Pulp Mφ and neutrophils in the same spleen. This shows that FcγRIIIb is not expressed on Red Pulp Mφ, and therefore FcγRIII expression on these cells must be FcγRIIIa, for which no specific MoAbs exist. MFI: median fluorescence intensity, Δ MFI: MFI corrected for staining with an isotype control. Means + s.e.m. of n=7 are shown for each group. **B** quantitative mRNA analysis of the FCGR3B transcript encoding FcγRIIIb, which is expressed in high numbers by neutrophils. The relative expression compared with the expression in pooled whole blood leukocytes, corrected for housekeeping genes, is shown. The reference value of 1 for pooled whole blood leukocytes is shown as a positive control. Means + s.e.m. of n=3 are shown for each group.

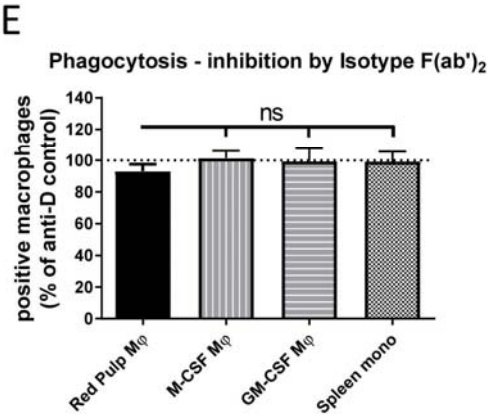
Figure S4

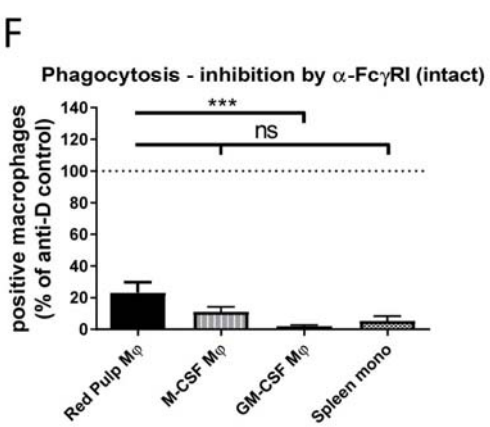

A
Phagocytosis - inhibition FcγRI
positive macrophages (% of anti-D control)
**
**
ns
isotype control F(ab')2
α-FcγRI F(ab')2
isotype control intact IgG1
α-FcγRI intact IgG1


B
Phagocytosis - inhibition by α-FcγRI F(ab')2
positive macrophages (% of anti-D control)
ns
Red Pulp Mφ
M-CSF Mφ
GM-CSF Mφ
Spleen mono


C
Phagocytosis - inhibition by α-FcγRII F(ab')2
positive macrophages (% of anti-D control)
***
ns
Red Pulp Mφ
M-CSF Mφ
GM-CSF Mφ
Spleen mono


D
Phagocytosis - inhibition by α-FcγRIII F(ab')2
positive macrophages (% of anti-D control)
ns
Red Pulp Mφ
M-CSF Mφ
GM-CSF Mφ
Spleen mono


E
Phagocytosis - inhibition by Isotype F(ab')2
positive macrophages (% of anti-D control)
ns
Red Pulp Mφ
M-CSF Mφ
GM-CSF Mφ
Spleen mono


F
Phagocytosis - inhibition by α-FcγRI (intact)
positive macrophages (% of anti-D control)
***
ns
Red Pulp Mφ
M-CSF Mφ
GM-CSF Mφ
Spleen mono

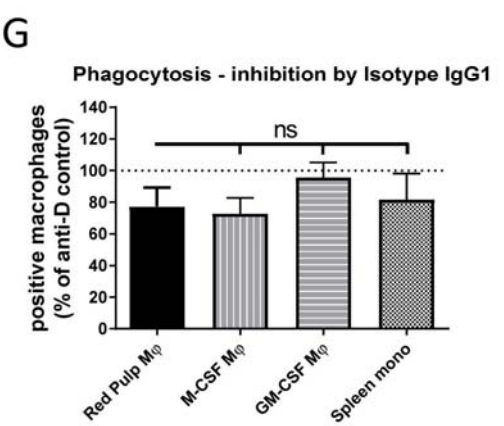

G
Phagocytosis - inhibition by Isotype IgG1
positive macrophages (% of anti-D control)
ns
Red Pulp Mφ
M-CSF Mφ
GM-CSF Mφ
Spleen mono

Figure S4. Comparison of FcγR usage between different types of macrophages

A comparison of F(ab')$_2$ fragments and intact IgG of MoAb 10.1 (blocking FcγRI) on phagocytosis of anti-D opsonized erythrocytes by red pulp macrophages. Isotype control F(ab')$_2$ n=9, 10.1 F(ab')$_2$ n=3, isotype control intact mouse IgG1 n=6, intact 10.1 n=7.

B-G comparison of different cell types for inhibition of phagocytosis by specific monoclonal antibodies against FcγRs. **B** comparison of α-FcγRI F(ab')$_2$ fragments; red pulp Mφ n=3, spleen monocytes n=2, M-CSF Mφ n=8, GM-CSF Mφ n=6.

C comparison of α-FcγRII F(ab')$_2$ fragments; red pulp macrophages n=10, spleen monocytes n=8, M-CSF Mφ n=12, GM-CSF Mφ n=8.

D comparison of α-FcγRIII F(ab')$_2$ fragments; red pulp macrophages n=10, spleen monocytes n=8, M-CSF Mφ n=8, GM-CSF Mφ n=6.

E comparison of isotype control F(ab')$_2$ fragments; red pulp macrophages n=9, spleen monocytes n=7, M-CSF Mφ n=9, GM-CSF Mφ n=9.

F comparison of α-FcγRI intact IgG; red pulp macrophages n=7, spleen monocytes n=5, M-CSF Mφ n=17, GM-CSF Mφ n=15.

G comparison of isotype control intact IgG; red pulp macrophages n=6, spleen monocytes n=4, M-CSF Mφ n=7, GM-CSF Mφ n=7.

Data are normalized against phagocytosis by unblocked macrophages.

Means + s.e.m. are shown for each group.

Table S1. List of Monoclonal antibodies

Antigen	Label	Isotype	Clone	Manufacturer	Use
CD3	PE-Cy7	Mouse IgG1	SK7	BD Pharmingen	FC
CD11a	FITC	Mouse IgG2a	CLB-LFA-1/2 (TB133)	PeliCluster	FC
CD11b	FITC	Mouse IgM	CLB-mon-gran/1 (B2)	PeliCluster	FC
CD11c	PE	Mouse IgG2b	S-HCL-3	BD Biosciences	FC
CD14	PE-Cy7	Mouse IgG2a	M5E2	BD Pharmingen	FC
CD16a,b	FITC	Mouse IgG1	3G8	BD Pharmingen	FC
CD16a,b	AF647	Mouse IgG1	3G8	BioLegend	IF
CD16b	Unstained	Mouse IgG2a	5D7 (CLBgran11)	a generous gift from Marion Kleijer, Sanquin, the Netherlands	FC
CD18	FITC	Mouse IgG1	L130	BD BioSciences	FC
CD19	APC	Mouse IgG1	HIB19	BD Pharmingen	FC
CD19	AF488	Mouse IgG1	HIB19	BioLegend	IF
CD32a,b,c	FITC	Mouse IgG1	AT10	AbD Serotec	FC
CD32a,b,c	AF647	Mouse IgG1	AT10	BioLegend	IF
CD32a	FITC	Mouse IgG2b	IV.3	Stem Cell	FC
CD32b,c	AF488	Humanized IgG, N297Q mutation	2B6	a generous gift from MacroGenics, Rockville, MD	FC, IF
CD33	PE	Mouse IgG1	WM53	BD Pharmingen	FC
CD36	FITC	Mouse IgG1	CLB-IVC7	PeliCluster	FC
CD56	APC	Mouse IgG1	B159	BD Pharmingen	FC
CD64	FITC	Mouse IgG1	10.1	BD Pharmingen	FC
CD64	AF647	Mouse IgG1	10.1	BioLegend	IF
CD68	PE	Mouse IgG2b	Y1/82A	BD Pharmingen	FC
CD89	FITC	Mouse IgG1	MIP8a	Novus Biologicals	FC
CD163	PE	Mouse IgG1	MAC2-158	Trillium	FC, IF

CD169	PE	Mouse IgG1	7-239	BioLegend	FC, IF
CD200R	AF647	Mouse IgG1	OX108	AbD Serotec	FC
HLA-DR	FITC	Mouse IgG2a	G46-6	BD Biosciences	FC
gp91PHOX	FITC	Mouse IgG1	7D5	MBL	FC
TLR4	APC	Mouse IgG2a	HTA125	eBioscience	FC
IgG	AF647	Goat anti human IgG	Polyclonal	Life Technologies	FC
Isotype	FITC	Mouse IgG1	CLB-203	PeliCluster	FC
Isotype	PE	Mouse IgG1	CLB-203	PeliCluster	FC
Isotype	FITC	Mouse IgG2a	CLB-713	PeliCluster	FC
Isotype	PE-Cy7	Mouse IgG2a	ICIGG2A	Abcam	FC
Isotype	FITC	Mouse IgG2b	B-Z40	Diaclone	FC
Isotype	PE	Mouse IgG2b	27-35	BD Pharmingen	FC
Isotype	APC	Mouse IgG1	MOPC-21	BD Pharmingen	FC
Isotype	Unstained	Mouse IgG2a	713	PeliCluster	FC
conjugate	AF633	$F(ab')_2$ Goat anti mouse IgG	Polyclonal	Invitrogen	FC

FC: flow cytometry, IF: immunofluorescence

Table S2. Primers used in RT-qPCR analysis

Transcript	Primers	Sequence	Product size
GUS	Forward primer	5'-GAAAATATGTGGTTGGAGAGCTCATT-3'	100 bp
	Reverse primer	5'-CCGAGTGAAGATCCCCTTTTTA-3'	
GAPDH	Forward primer	5'–TGCACCACCAACTGCTTAGC–3'	87 bp
	Reverse primer	5'–GGCATGGACTGTGGTCATGAG–3'	
FCGR1A1	Forward primer	5'–CTCCATCTGCCTGGGAGCAGCTCT–3'	437 bp
	Reverse primer	5'–GCTGGAAATAGCTCTTTCACCGTGACA–3'	
FCGR2A	Forward primer	5'-ATCATTGTGGCTGTGGTCATTGC-3'	275 bp
	Reverse primer	5'-TCAGGTAGATGTTTTTATCATCG-3'	
FCGR2B2	Forward primer	5'-GGAAAAAGCGCATTTGAGCCAATC-3'	193 bp
	Reverse primer	5'-GGAAATACGAGATCTTCCCTCTCTG-3'	
FCGR3A	Forward primer	5'-CACATATTTACAGAATGGCACAGG-3'	170 bp
	Reverse primer	5'-ACACTGCCAAACCTTGAGTGATGG-3' *	
FCGR3B	Forward primer	5'-CACATATTTACAGAATGGCAAGGA-3'	170 bp
	Reverse primer	5'-ACACTGCCAAACCTTGAGTGATGG-3' *	

* The same reverse primer is used for FCGR3A and FCGR3B, specificity derives from the forward primer

Supplemental Reference List

1. Tsang-A-Sjoe MWP, Nagelkerke SQ, Bultink IE et al. Fc-gamma receptor polymorphisms differentially influence susceptibility to systemic lupus erythematosus and lupus nephritis. Rheumatology (Oxford) 2016;55:939-948.

2. van der Heijden J, Breunis WB, Geissler J et al. Phenotypic variation in IgG receptors by nonclassical FCGR2C alleles. J.Immunol. 2012;188:1318-1324.