

Supporting Information

Seifert et al. 10.1073/pnas.1416276112

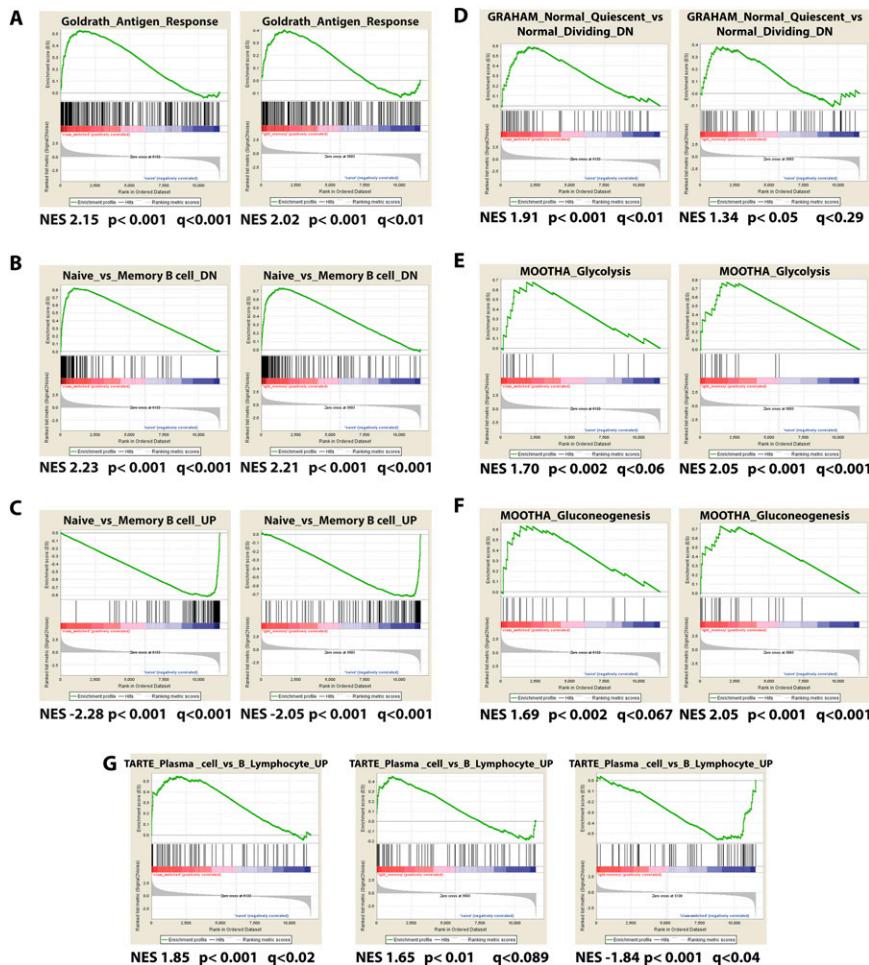


Fig. S1. GSEA of PB B-cell subsets reveals a memory B-cell phenotype of IgM⁺IgD⁺CD27⁺ B cells. (A–F) Selected plots from a GSEA based on 29,969 probe sets from gene expression profiling (GEP) of five IgG⁺CD27⁺ (Left, red), five IgM⁺IgD⁺CD27⁺ (Right, red), and five naive (blue) B-cell samples, respectively. (G) PC-related gene sets are expressed at a higher level in IgG memory (red) vs. IgM⁺IgD⁺CD27⁺ (blue) B cells. The normalized enrichment score (NES), nominal P value (p), and FDR (q) are given for each plot.

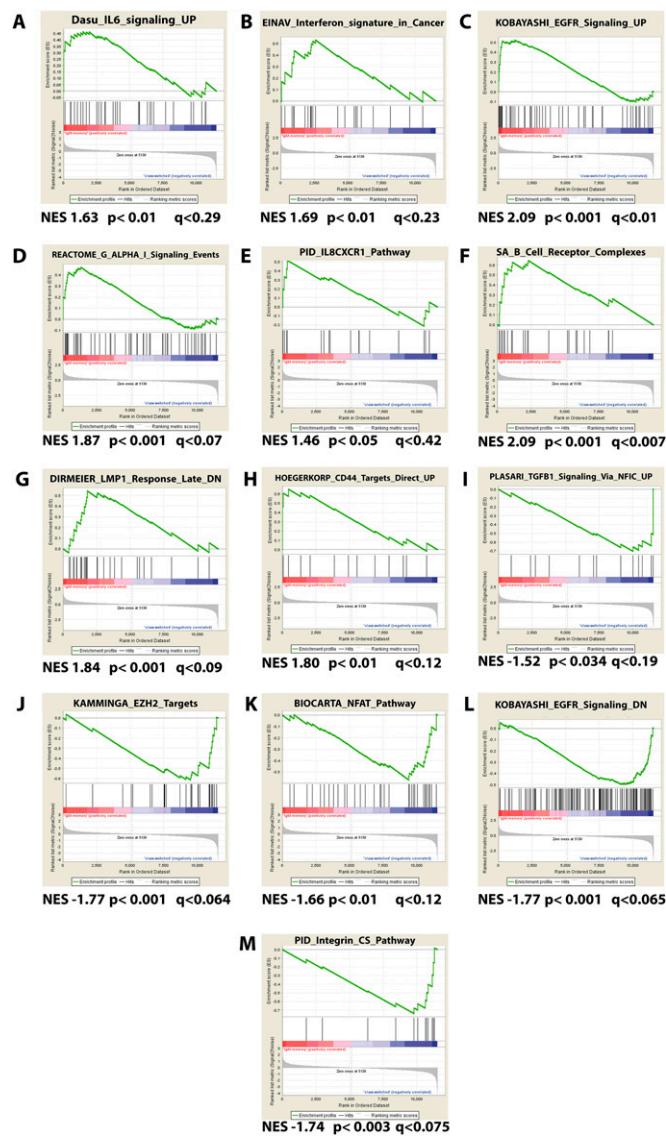


Fig. S2. GSEA of IgM vs. IgG memory B cells. (A–M) Selected plots from a GSEA based on 29,969 probe sets of GEP from five IgM memory (red) and five IgG memory (blue) B-cell samples. IgM memory B cells carry specific cytokine-, growth factor- and BCR-signaling signatures, as well as cell adhesion or cell contact patterns. The NES, nominal *P* value (*p*), and FDR (*q*) are given for each plot.

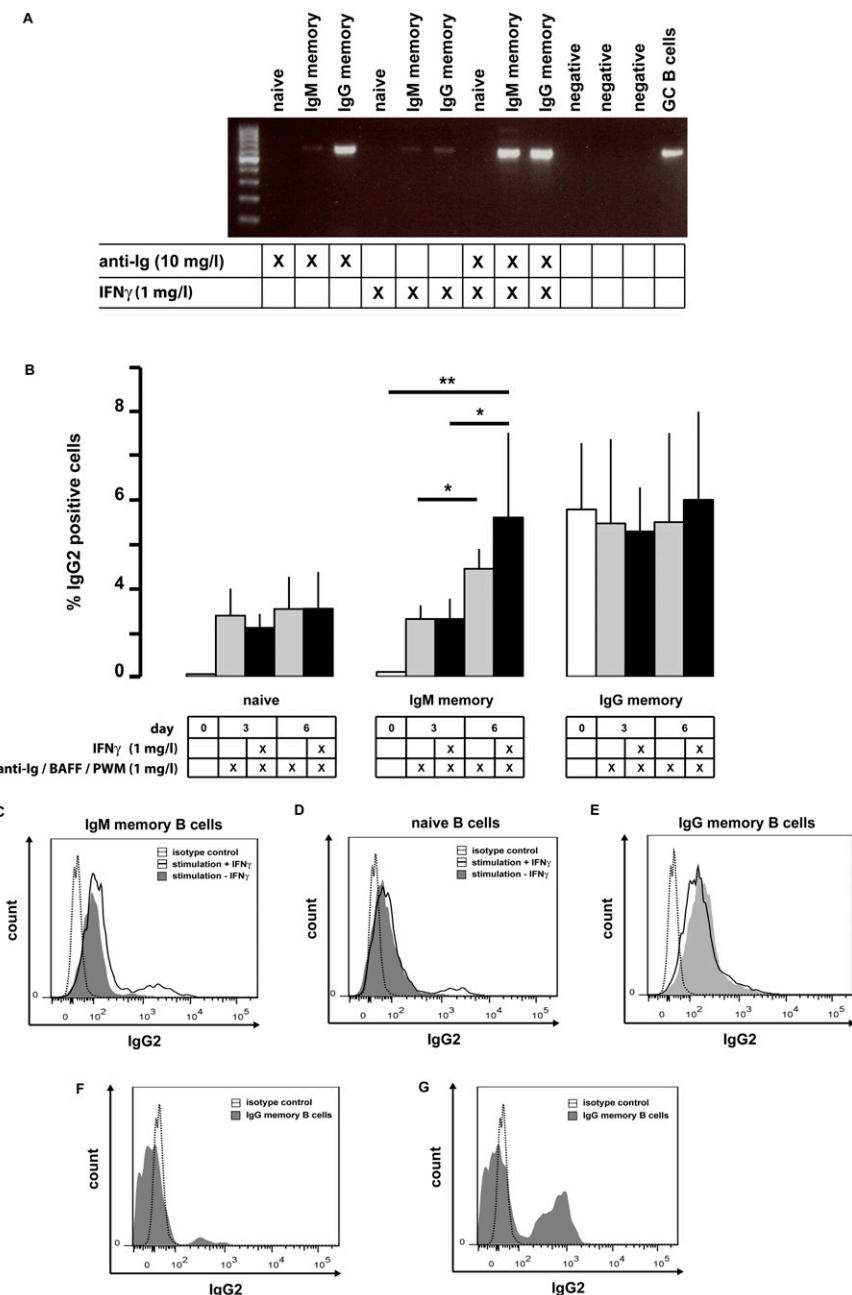


Fig. S3. Induction of C γ 2 switch transcription and IgG $_2$ CSR in IFN γ -stimulated B-cell subsets. (A) Representative RT-PCR result of sort-purified B-cell subsets, incubated with 1 mg/L IFN γ and/or 10 mg/L anti-Ig antibody overnight. Data are representative of three donors showing C γ 2 switch transcript induction out of six donors analyzed. (B) Fraction of IgG $_2$ -positive B cells per population, as determined by flow cytometry after sort purification (day 0) and after 3 and 6 d of incubation with anti-Ig, PWM, and BAFF (1 mg/L each) with or without 1 mg/L IFN γ . Bars show the mean and SD of four independent donors for naive and IgM memory B-cell samples and two donors for IgG memory B-cell samples. * $P < 0.05$; ** $P < 0.01$. (C-E) FACS histograms of sort-purified naive, IgM memory, and IgG memory B cells at day 6 of in vitro stimulation with BAFF, PWM, and anti-Ig antibody (1 mg/L each) with (black line) or without (gray shade) 1 mg/L IFN γ . The dashed line represents an isotype control. Data are representative of four donors analyzed. FACS histograms of two independent healthy adult human PB CD20 $^+$ IgG $^+$ B cells show the variable proportions of 5% (F) and 26% (G) IgG $_2$ -positive B cells of donors 1 and 2, respectively.

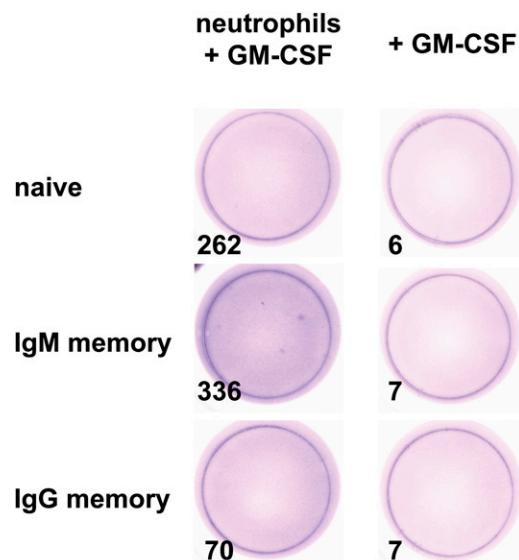


Fig. S4. Human neutrophil IFN- γ secretion capacity. Representative ELISpot analysis out of two experiments with cocultures of sort-purified B-cell subsets and neutrophils, stimulated with 1 mg/L GM-CSF. Numbers in the lower left corners give the total number of spots counted by the ELISpot reader.

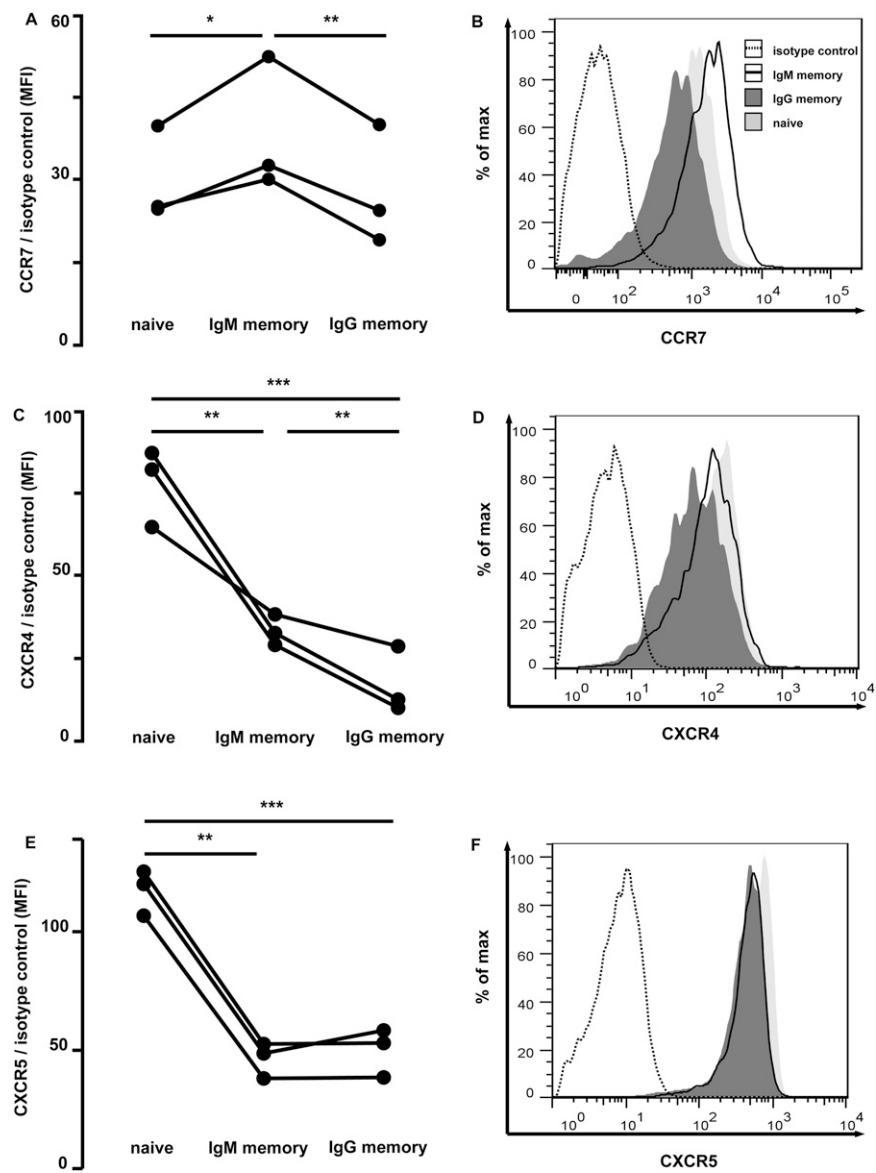


Fig. S5. (A, C, and E) Surface expression of CXCR4, CXCR5, and CCR7 on human PB B-cell subsets as determined by flow cytometry of three healthy donors. (B, D, and F) Representative FACS histograms are given. The histogram legend is as in B (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). max, maximum.

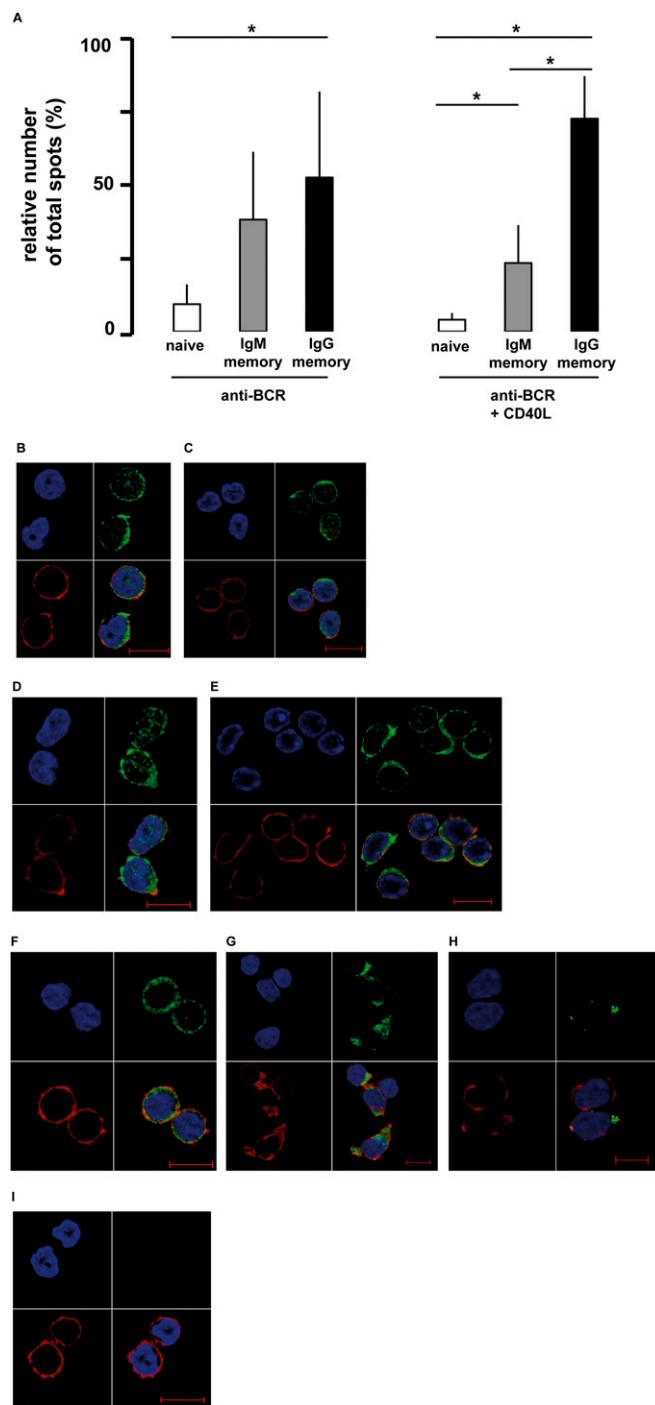


Fig. S6. GC B-cell differentiation capacity of human IgM memory B cells. (A) Summary of ELISpot analysis from three experiments with sort-purified B-cell subsets (25,000 B cells each), stimulated with 1 mg/L anti-Ig antibody, 2 mg/L recombinant human CD40L-HA, and 1 mg/L anti-HA (TD) and anti-Ig antibody alone (TI). Spots were counted by an ELISpot reader and summarized per donor, and the relative fraction of spots per population was calculated. * $P < 0.05$. (B–I) BACH2 fluorescence microscopy before and after 16 h of anti-Ig treatment. No difference in BACH2 nuclear or cytosolic expression was detectable between unstimulated (B) and stimulated (C) naive B cells. A similar picture was observed for unstimulated (D) but not stimulated (E) IgM memory B cells, which showed BACH2 nuclear export in about 50% of the cells. In contrast, IgG memory B cells (F) show quick and efficient nuclear export of BACH2 upon stimulation (G) and cytosolic degradation at the PC differentiation stage (H). (I) Isotype control staining. Data are representative of three donors tested (Table S2). Photographs show the fluorescence of each channel and overlay pictures: green, BACH2; blue, DAPI; red, phalloidin. (Scale bars: 10 μ m.)

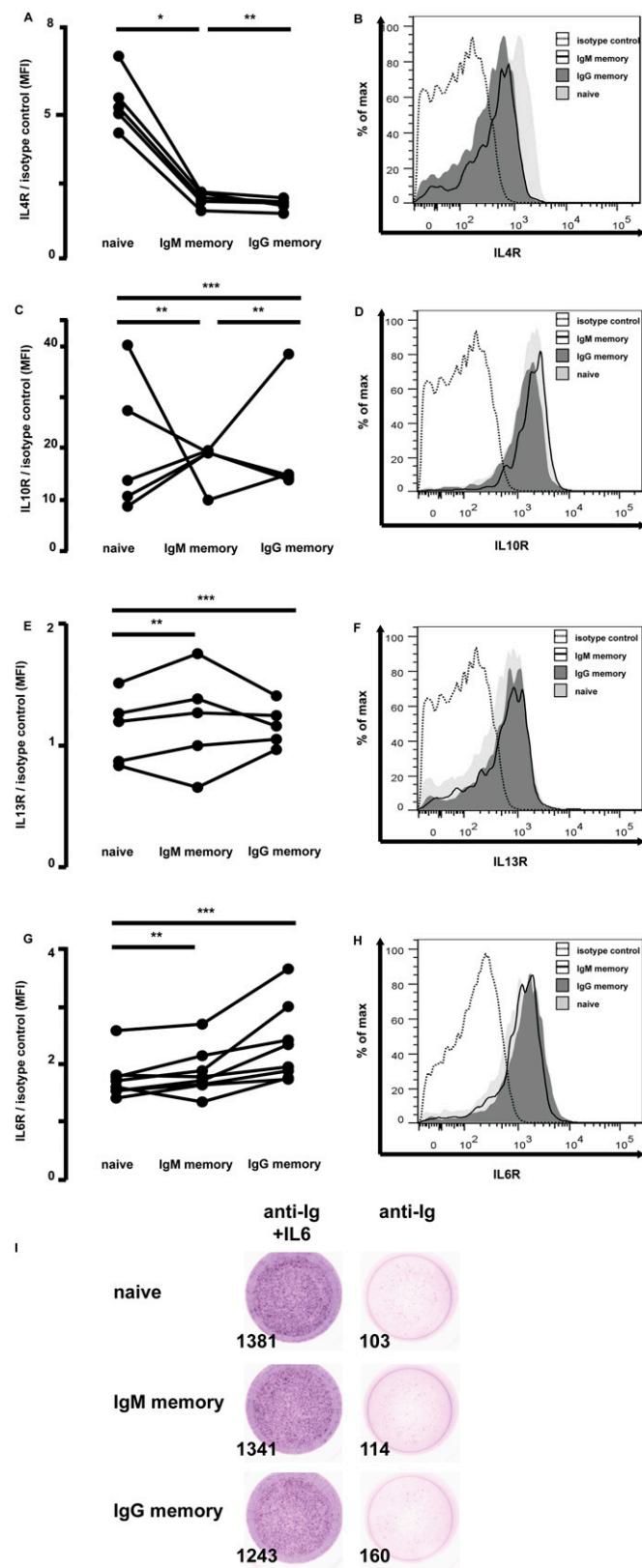


Fig. S7. Surface expression of IL receptors on human PB B-cell subsets as determined by flow cytometry of at least five healthy donors. Summaries and representative FACS histograms are given for IL-4R (A and B), IL-10R (C and D), IL-13R (E and F), and IL-6R (G and H). *P < 0.05; **P < 0.01; ***P < 0.001. (I) Representative ELISpot analysis out of three experiments with sort-purified B-cell subsets stimulated with 10 mg/L anti-Ig antibody and 100 IU of IL-6 or anti-Ig antibody alone. Numbers in the lower left corners give the total number of spots counted by the ELISpot reader.

Table S1. Two hundred fourteen annotated transcripts with at least twofold differential expression between IgM memory, IgG memory, and naive B cells

Probe set	IgM memory/naive	IgM memory/IgG memory	Gene symbol
Cytoskeleton and ECM			
204834_at	14.4	2.0	FGL2
212992_at	2.8	2.9	AHNAK2
209191_at	2.6	3.7	TUBB6
240777_at	2.4	3.5	SYNE2
225613_at	30.1	3.8	MAST4
216699_s_at	7.3	3.5	KLK1
228673_s_at	2.2	2.1	EML4
224374_s_at	4.1	5.0	EMILIN2
229566_at	3.1	4.7	LOC645638
203881_s_at	2.3	3.3	DMD
212993_at	3.8	2.6	BTBD14A
204964_s_at	14.5	-6.6	SSPN
202890_at	2.4	-16.7	MAP7
200696_s_at	3.0	-4.1	GSN
214602_at	18.5	-2.4	COL4A4
206167_s_at	3.2	-7.4	ARHGAP6
235670_at	2.4	-2.5	STX11
200974_at	2.5	-3.7	ACTA2
213638_at	-2.5	2.7	PHACTR1
212365_at	-5.3	3.2	MYO1B
201718_s_at	-5.6	4.3	EPB41L2
222234_s_at	-33.3	2.1	DBNDD1
219259_at	-10.8	-2.6	SEMA4A
218330_s_at	-2.2	-3.0	NAV2
225540_at	-3.9	-2.5	MAP2
205268_s_at	-2.6	-4.1	ADD2
Lymphocyte signaling			
227900_at	2.1	2.7	CBLB
210313_at	9.0	20.2	LILRA4
224192_at	2.9	2.9	FCRL2
205789_at	2.1	2.6	CD1D
205987_at	7.3	4.9	CD1C
1553678_a_at	13.5	-4.2	ITGB1
204647_at	7.2	-9.7	HOMER3
1554519_at	5.5	-2.1	CD80
210031_at	7.9	-9.3	CD247
209583_s_at	-9.3	5.6	CD200
205790_at	-22.2	2.4	SKAP1
210644_s_at	-3.2	2.5	LAIR1
206759_at	-14.7	2.5	FCER2
215925_s_at	-2.1	6.6	CD72
210325_at	-14.1	13.5	CD1A
210972_x_at	-2.7	-7.0	TRAC
212646_at	-35.7	-22.4	RFTN1
205269_at	-2.3	-10.8	LCP2
204891_s_at	-4.6	-2.1	LCK
235310_at	-6.7	-6.7	GCET2
205692_s_at	-10.3	-5.3	CD38
1553995_a_at	-4.1	-3.4	NTSE
Ca ⁺⁺ signaling			
209469_at	5.8	5.1	GPM6A
218872_at	17.4	-2.8	TESC
225019_at	-2.0	3.3	CAMK2D
206637_at	-27.0	-2.2	P2RY14
211323_s_at	-4.5	-2.2	ITPR1
210786_s_at	-2.4	-2.0	FLI1
211825_s_at	-3.1	-2.1	EWSR1 /// FLI1
GTP signaling			
202975_s_at	17.6	16.9	RHOBTB3
206290_s_at	3.1	10.7	RGS7
204115_at	9.3	10.0	GNG11

Table S1. Cont.

Probe set	IgM memory/naive	IgM memory/IgG memory	Gene symbol
202748_at	2.4	2.1	GBP2
222942_s_at	4.6	-10.9	TIAM2
217762_s_at	3.1	-2.1	RAB31
209581_at	2.0	-6.0	HRASLS3
218807_at	-2.3	2.0	VAV3
203431_s_at	-23.6	2.2	RICS
Insulin signaling			
209185_s_at	-2.3	2.5	IRS2
213792_s_at	-3.8	-2.7	INSR
MAPK signaling			
204068_at	4.4	3.5	STK3
208892_s_at	-4.4	6.8	DUSP6
PI3K signaling			
219358_s_at	2.0	2.6	CENTA2
210612_s_at	2.4	-2.0	SYNJ2
209504_s_at	2.1	-2.3	PLEKHB1
1558770_a_at	2.1	-3.1	PIK3R6
217677_at	-2.5	2.2	PLEKHA2
219024_at	-3.0	2.3	PLEKHA1
219155_at	-2.0	-7.2	PITPNC1
225688_s_at	-29.2	-5.4	PHLDB2
Wingless-type MMTV integration site family member/Hedgehog/Notch signaling			
229103_at	2.7	2.6	WNT3
224022_x_at	2.4	2.4	WNT16
1553873_at	3.2	2.3	KLHL34
209815_at	3.2	-2.7	PTCH1
226666_at	4.7	-2.3	DAAM1
223709_s_at	-2.6	2.5	WNT10A
212611_at	-2.9	2.1	DTX4
227336_at	-2.2	5.4	DTX1
203987_at	-6.0	-2.1	FZD6
Signaling lymphocytic activation molecule signaling			
211192_s_at	2.6	2.8	CD84
222838_at	19.9	-2.2	SLAMF7
Transcription factors			
215767_at	17.2	2.0	ZNF804A
225802_at	3.9	2.6	TOP1MT
228698_at	36.3	11.2	SOX7
219387_at	4.7	2.4	CCDC88A
228994_at	3.9	2.8	CCDC24
219221_at	6.5	-3.0	ZBTB38
219836_at	9.9	-14.0	ZBED2
204529_s_at	5.1	-22.9	TOX
205255_x_at	6.0	-5.6	TCF7
228964_at	3.1	-4.8	PRDM1
202393_s_at	5.1	-5.5	KLF10
236635_at	-5.9	2.2	ZNF667
218793_s_at	-2.5	3.2	SCML1
203140_at	-4.4	2.4	BCL6
208703_s_at	-8.8	10.0	APLP2
210829_s_at	-4.9	-2.1	SSBP2
204900_x_at	-2.5	-3.4	SAP30
219396_s_at	-7.5	-4.8	NEIL1
1555420_a_at	-5.4	-4.6	KLF7
211597_s_at	-55.2	-224.2	HOPX
236442_at	-3.4	-2.7	DPF3
230807_at	-5.9	-3.9	CCDC151
Cell adhesion molecules			
221004_s_at	2.6	2.2	ITM2C
209498_at	18.3	5.0	CEACAM1
212813_at	3.2	2.2	JAM3
206869_at	7.7	-9.8	CHAD

Table S1. Cont.

Probe set	IgM memory/naive	IgM memory/IgG memory	Gene symbol
208949_s_at	4.8	-38.3	LGALS3
201506_at	5.9	-2.5	TGFBI
219737_s_at	-7.0	7.2	PCDH9
1555691_a_at	-2.1	4.5	KLRC4
200606_at	-69.0	4.0	DSP
204591_at	-2.9	2.9	CHL1
201005_at	-31.9	-3.7	CD9
215145_s_at	-17.5	-9.7	CNTNAP2
225673_at	-2.9	-3.3	MYADM
Cytokines, hormones, growth factors, and their receptors			
206170_at	4.5	2.8	ADRB2
209392_at	41.3	11.3	ENPP2
217853_at	4.4	2.8	TNS3
205207_at	2.2	12.6	IL-6
204912_at	4.1	2.1	IL-10RA
242903_at	2.5	2.3	IFNGR1
200678_x_at	3.4	2.1	GRN
205225_at	4.4	2.9	ESR1
206978_at	41.1	2.9	CCR2
205098_at	5.2	-16.0	CCR1
226333_at	3.4	-35.5	IL-6R
216176_at	2.5	-8.5	HCRP1
209499_x_at	-2.4	4.0	TNFSF12-TNFSF13
205114_s_at	-7.8	4.4	CCL3
201161_s_at	-10.3	3.5	CSDA
204897_at	-2.2	-2.1	PTGER4
203233_at	-105.0	-4.5	IL-4R
211612_s_at	-6.3	-7.4	IL-13RA1
216615_s_at	-2.1	-7.6	HTR3A
209524_at	-4.0	-2.3	HDGFRP3
228948_at	-5.0	-106.7	EPHA4
Igs			
215118_s_at	13.3	-13.9	IGHG/IGHM
228518_at	7.7	-2.1	IGHG/IGHM
217022_s_at	15.0	-7.4	IGHA1/IGHA2
216491_x_at	-2.4	2.7	IGHM
213674_x_at	-3.6	32.6	IGHD
1558438_a_at	-2.4	-13.3	IGHE
Channels and transporters			
230748_at	5.1	-2.5	SLC16A6
205234_at	9.9	4.8	SLC16A4
212192_at	2.9	-2.9	KCTD12
205262_at	2.0	-2.3	KCNH2
39248_at	3.2	-9.1	AQP3
214595_at	-34.5	3.0	KCNG1
202124_s_at	-4.1	-2.4	TRAK2
210432_s_at	-43.1	-16.4	SCN3A
219287_at	-2.4	-2.1	KCNMB4
223727_at	-3.5	-2.5	KCNIP2
1552742_at	-23.8	-16.6	KCNH8
Metabolism			
237180_at	2.1	2.4	PSME4
224839_s_at	3.1	2.2	GPT2
45288_at	2.5	2.7	ABHD6
202589_at	3.8	-9.8	TYMS
223062_s_at	2.6	-2.0	PSAT1
32502_at	2.4	-3.1	GDPD5
223952_x_at	2.3	-2.3	DHRS9
226517_at	9.6	-2.2	BCAT1
218844_at	2.5	-2.6	ACSF2
213725_x_at	-2.4	2.1	XYLT1
225207_at	-10.6	2.2	PDK4

Table S1. Cont.

Probe set	IgM memory/naive	IgM memory/IgG memory	Gene symbol
204646_at	-2.0	4.8	DPYD
207819_s_at	-2.7	2.6	ABCB4
209994_s_at	-2.5	2.4	ABCB1 // ABCB4
237465_at	-2.6	-2.5	USP53
207245_at	-72.5	-7.3	UGT2B17
218217_at	-3.5	-3.0	SCPEP1
227417_at	-13.7	-54.1	MOSC2
209512_at	-2.4	-3.0	HSDL2
202967_at	-4.1	-2.0	GSTA4
221253_s_at	-3.7	-7.4	MUTED
Regulation of apoptosis			
204780_s_at	31.1	-4.2	FAS
210609_s_at	2.9	-2.4	TP53I3
205780_at	2.3	-3.9	BIK
217963_s_at	2.2	-2.6	NGFRAP1
209995_s_at	-48.5	10.8	TCL1A
203795_s_at	-2.7	2.5	BCL7A
Cell cycle			
212912_at	2.4	4.5	RPS6KA2
224428_s_at	9.7	-4.8	CDCA7
216176_at	2.5	-8.5	HCRP1
202503_s_at	2.6	-12.0	KIAA0101
212560_at	-29.2	-3.8	SORL1
204249_s_at	-7.0	-8.3	LMO2
224847_at	-5.0	-7.1	CDK6
Transcription			
213273_at	3.7	3.8	ODZ4
226425_at	3.3	-6.8	CLIP4
204207_s_at	-5.4	-10.2	RNGTT
203865_s_at	-18.3	-2.8	ADARB1
225941_at	-3.3	-2.4	EIF4E3
218696_at	-3.5	-3.4	EIF2AK3
Miscellaneous signaling molecules			
206907_at	19.5	2.3	TNFSF9
205805_s_at	2.0	2.1	ROR1
227396_at	2.9	3.7	PTPRJ
212230_at	15.8	3.3	PPAP2B
218888_s_at	7.4	2.1	NETO2
223159_s_at	4.5	-3.0	NEK6
207395_at	3.4	-6.0	BTN1A1
208923_at	6.0	-2.5	CYFIP1
225116_at	7.2	-6.3	HIPK2
228568_at	-5.7	2.0	FCER3
228494_at	-10.2	-25.1	PPP1R9A
209371_s_at	-10.6	-2.1	SH3BP2
227013_at	-5.1	-9.1	LATS2
204491_at	-3.8	-17.6	PDE4D
204119_s_at	-4.1	-3.4	ADK

For transcripts up-regulated in IgM memory B cells, fold changes are given in red; for down-regulated transcripts, fold changes are given in green.

Table S2. Summary of fluorescence microscopic analysis of nuclear BACH2 expression as determined by a blinded evaluation

Population	Stimulus	Cells analyzed ^a	Bright nuclear BACH2 ^a	Dim nuclear BACH2 ^a	Absent nuclear BACH2 ^a	Bright cytosolic BACH2 ^a	Dim cytosolic BACH2 ^a	Absent cytosolic BACH2 ^a
Naive	None	80	20	47	13	17	55	8
	16 h anti-Ig	109	25	39	45	50	55	4
IgM memory	None	40	8	19	12	15	25	0
	16 h anti-Ig	143	12**	54	77*	80	59	4
IgG memory	None	59	11	30	18	37	22**	0*
	16 h anti-Ig	138	8***	48	82**	53	66	19**

Numbers are significantly different (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$) from the corresponding naive B-cell activation condition as calculated by Fisher's exact test.

^aTotal cell numbers from three donors are given; counting was performed in a blinded study (cell type and activation status masked).