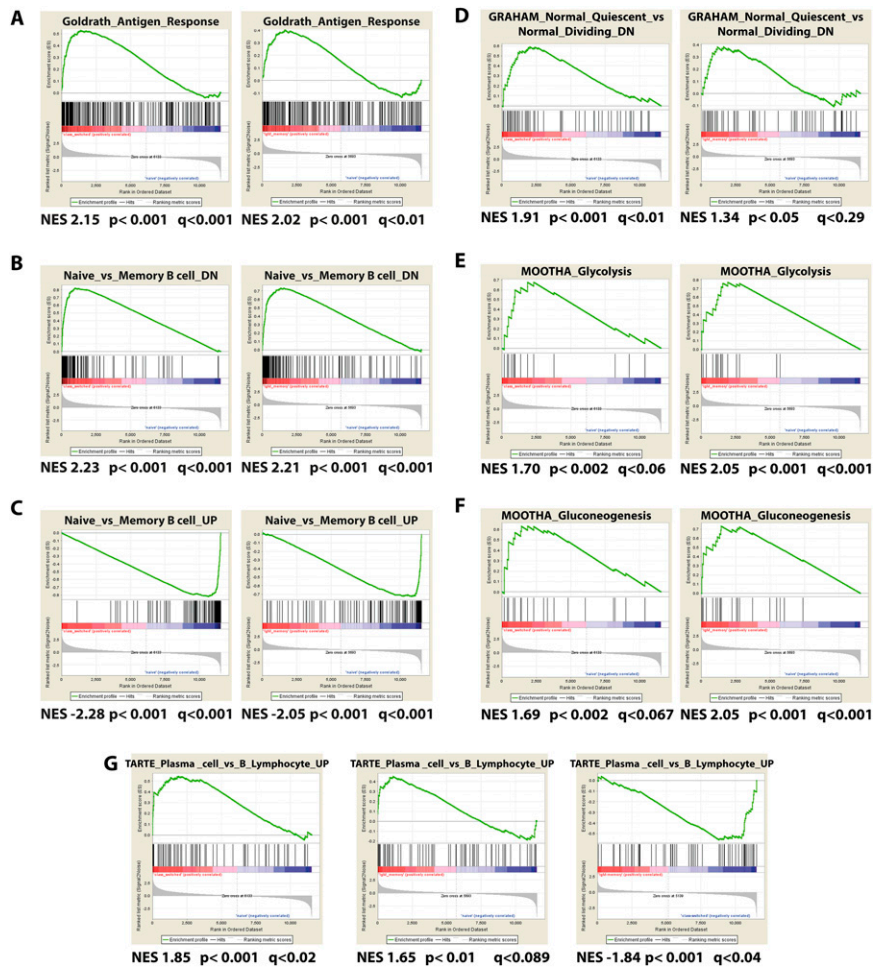


# Supporting Information

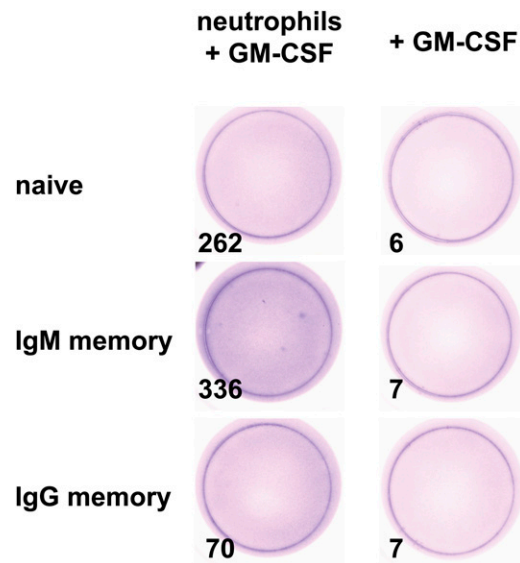
Seifert et al. 10.1073/pnas.1416276112



**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. S4.** Human neutrophil IFN- $\gamma$  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.







**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
<b>Cytoskeleton and ECM</b>			
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
<b>Lymphocyte signaling</b>			
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	NT5E
<b>Ca<sup>++</sup> signaling</b>			
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	EW5R1 /// FLI1
<b>GTP signaling</b>			
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	TGFB1
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 S2. Summary of fluorescence microscopic analysis of nuclear BACH2 expression as determined by a blinded evaluation**

Population	Stimulus	Cells analyzed <sup>a</sup>	Bright nuclear BACH2 <sup>a</sup>	Dim nuclear BACH2 <sup>a</sup>	Absent nuclear BACH2 <sup>a</sup>	Bright cytosolic BACH2 <sup>a</sup>	Dim cytosolic BACH2 <sup>a</sup>	Absent cytosolic BACH2 <sup>a</sup>
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.

<sup>a</sup>Total cell numbers from three donors are given; counting was performed in a blinded study (cell type and activation status masked).