Supplementary Information

Single-cell intracellular epitope and transcript detection revealing signal transduction dynamics

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Contents

Supplementary Notes

I. Antibody labeling

All antibodies (Abs) were purchased purified (see **Supplementary Table 1** for a full list of the Abs used). Abs used in the QuRIE-seq experiment were first validated using flow cytometry.

QuRIE-seq requires the use of DNA-tagged Abs for staining cells prior to encapsulation to characterize the single-cell proteome. In the following is briefly detailed the DNA conjugation strategy to the Abs which made us of standard NHS chemistry. First, Abs were buffer exchanged into 0.2M $NaHCO_3$ (pH 8.3) (Sigma Aldrich, USA) using Zeba™ Spin Desalting Columns, 40K MWCO (Thermo Fisher Scientific, USA). Then, dibenzocyclooctyne-S-S-N-hydroxysuccinimidyl ester (Sigma Aldrich, USA) was resuspended in DMSO (Sigma Aldrich, USA), added in a 10x molar excess to the Abs and incubated for 2 hours at room temperature. Abs were then buffer exchanged into PBS (Thermo Fisher Scientific, USA) and excess linker molecules were removed using 30K amicon centrifuge units (Merck, USA). Subsequently, 5' Azide modified oligos (Biolegio, The Netherlands) were added in a 3x molar excess to the chemically modified Abs and incubated for 16 hours at 4°C in dark. After incubation, Abs were buffer exchanged into PBS (Thermo Fisher Scientific, USA) containing 0.05% sodium azide (Sigma Aldrich, USA) and 0.1mM EDTA (Sigma Aldrich, USA), and excess oligos were removed using 100K amicon centrifuge filters (Merck, USA). After labelling, DNA-tagged Abs were stored at 4°C. Labelling efficiency was determined by non-reducing SDS page gel analysis where 0.5 µg antibody was diluted in 1x Laemmli sample buffer (Bio-Rad, USA) and loaded on a mini-PROTEAN TGX stain-free gel (Bio-Rad, USA). Gels were imaged on a ChemiDoc Touch imaging system (Bio-Rad, USA).

II. Flow cytometry analysis

DSP and PDSP fixed and permeabilized BJAB cells were centrifuged at 800 g and resuspended in PBS (Thermo Fisher Scientific, USA) supplemented with 0.1% BSA (Thermo Fisher Scientific, USA) containing the indicated Abs, and incubated for one hour at room temperature in dark (**Supplementary Table 2** shows different Abs used for flow cytometry analysis). After incubation, cells were washed three times with PBS containing 0.1% BSA and resuspended in PBS with 0.1% BSA. Finally, cells were measured on a FACSverse (BD Bioscience, USA). Flow cytometry results were analysed using FlowJo software (BD Bioscience, USA).

III. ELISA

BJAB cells were cultured in round bottom 96-wells plates at a concentration of 10^5 cells/well in a volume of 200 µl. Cells were stimulated with 0-20 µg/ml F(ab') ₂ Fragment Goat Anti-Human IgA + IgG + IgM (H+L) (Jackson ImmunoResearch, USA) and incubated for 16 hours at 37 °C. The supernatant was collected and analysed for the presence of IL6, IL10 and CCL3 using uncoated ELISA kit (Thermo Fisher Scientific, USA). ELISA was performed according to the manufacturer's instructions.

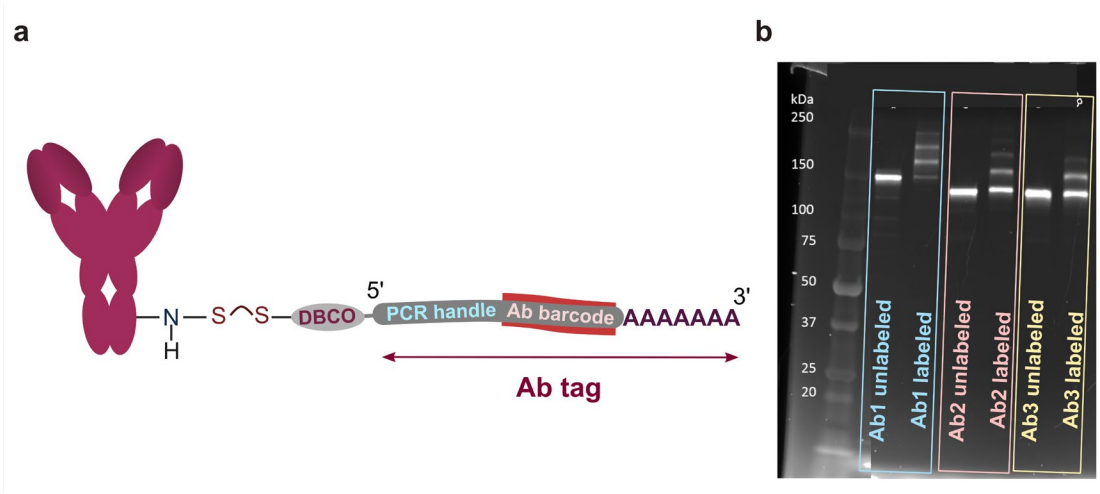
IV. Bulk mRNA Sequencing

BJAB cells were harvested, centrifuged and resuspended in complete medium at $0.5*10^6$ cells/ml. Thereafter, 4 ml of cells were seeded in T25 flasks (Thermo Fisher Scientific, USA), and cells were incubated for 1 hour at 37 °C. After incubation, the cells were stimulated with 10 µg/ml $F(ab')_2$ Fragment Goat Anti-Human IgA + IgG + IgM (H+L) (Jackson ImmunoResearch, USA) for the indicated time points. After stimulation, cells were immediately collected on ice and centrifuged at 200 g for 5 minutes at 4°C. Supernatant was collected and stored at -20°C for further analysis and the cell pellet was resuspended in the lysis buffer RLT (Qiagen, The Netherlands). Cell lysates were stored at -80°C until further use. Library preparation and sequencing was performed by Macrogen (Macrogen, Korea).

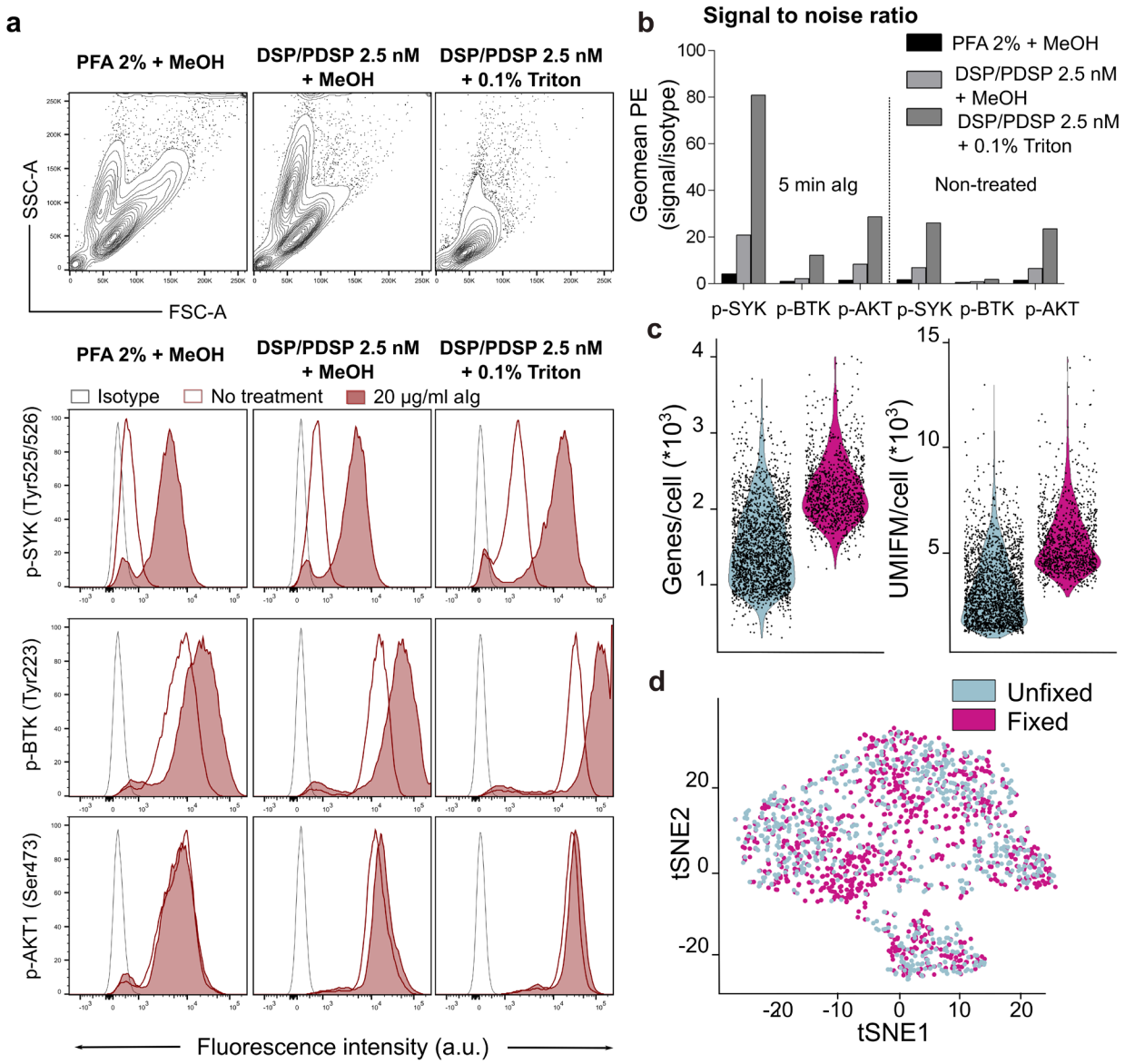
V. Bulk mRNA Sequencing data analysis

RNAseq fastq files of samples were aligned to the human genome GRCh38 using HISAT2 v2.1.0. Number of reads was assigned to genes by using featureCounts v1.6.1. Reads mapped to genes were normalized using count per million method (CPM) implemented in edgeR package in R Bioconductor. To analyze genes that have the highest fold change upon different durations of stimulation with aIg (60, and 180 min), genes were ranked by the difference between their CPM normalized values and mean of CPM normalized values of this gene in the unstimulated control sample.

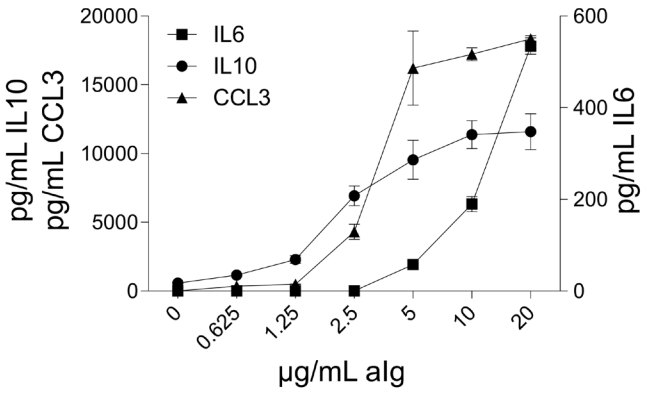
Supplementary Figures



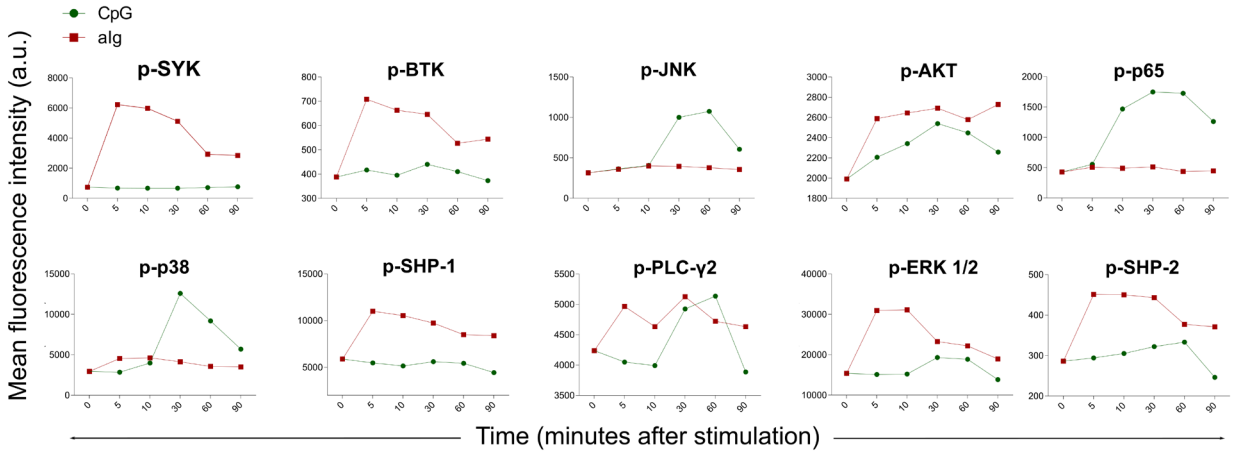
Supplementary Figure 1: Antibodies (Abs) labelling for single-cell proteome quantification in Quantification of RNA and Intracellular Epitopes (QuRIE-seq). a, Scheme of the Ab with a DNA tag. *DBCO = Dibenzocyclooctyne.* **b**, SDS page gel for the determination of Ab labelling efficiency where 0.5 µg Ab was diluted in non-reducing 1x Laemmli sample buffer (Bio-Rad) and loaded on a mini-PROTEAN TGX stain-free gel (Bio-Rad). Gels were imaged on a ChemiDoc Tough imaging system (Bio-Rad).



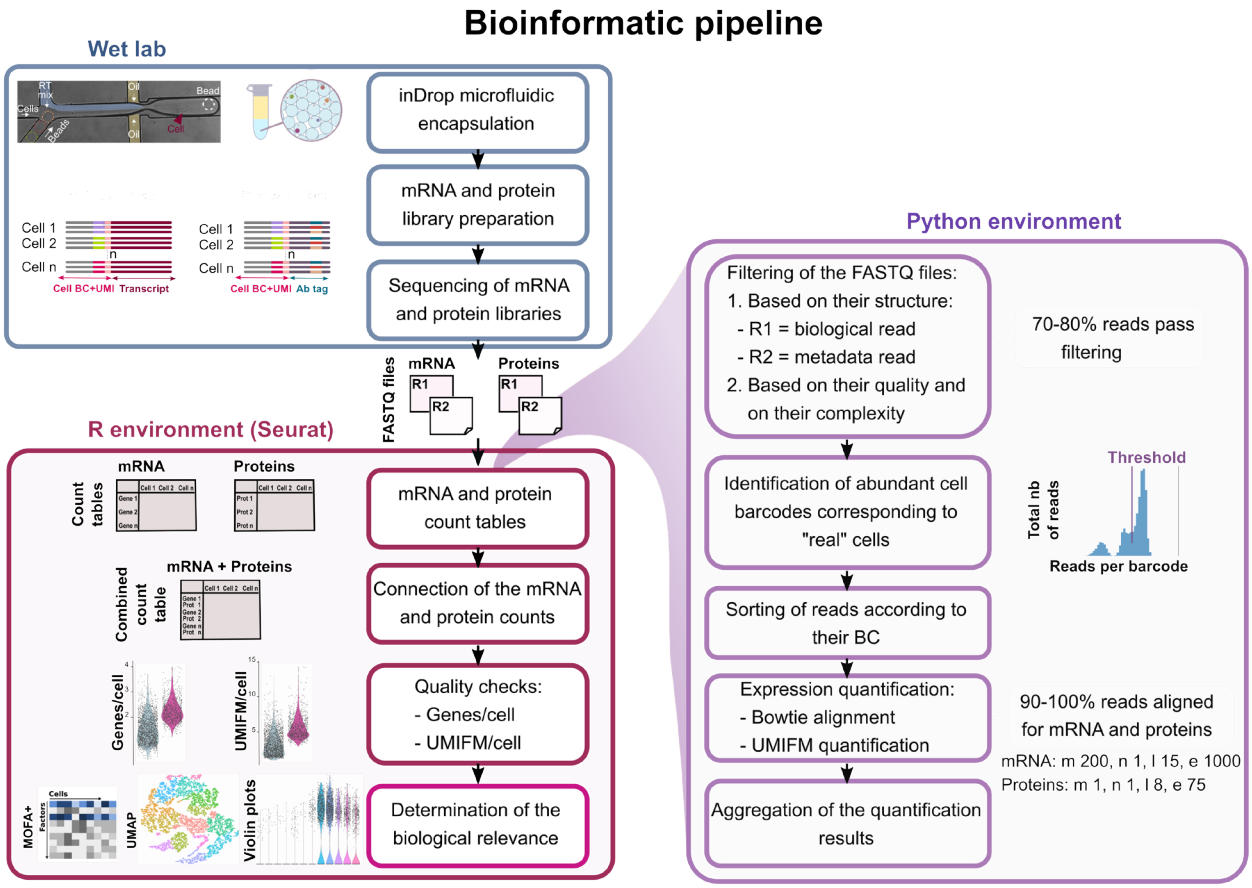
Supplementary Figure 2: Effect of the fixation procedure with BJAB cells on antibody binding, detection of phosphorylated proteins, and gene detection. a, Two different fixation methods were compared with flow cytometry: DSP/PDSP and PFA fixation both in combination with methanol (MeOH) permeabilization, and two permeabilization methods: MeOH and Triton-X100 in combination with DSP/PDSP fixation. The phosphorylation of SYK, BTK and AKT after 5 minute-stimulation with alg. **b**, The signal to noise ratio was compared with flow cytometry for two different fixation methods: DSP/PDSP and PFA in combination with MeOH permeabilization, and two permeabilization methods: MeOH and Triton-X100 in combination with DSP/PDSP fixation. **c**, Comparison of the number of genes and the number of UMIFM (UMI-filtered mapped) detected per cell between unfixed and fixed BJABs using QuRIE-seq technology. **d**, t-SNE visualization of the unfixed and fixed BJABs transcriptome expression using QuRIE-seq technology.



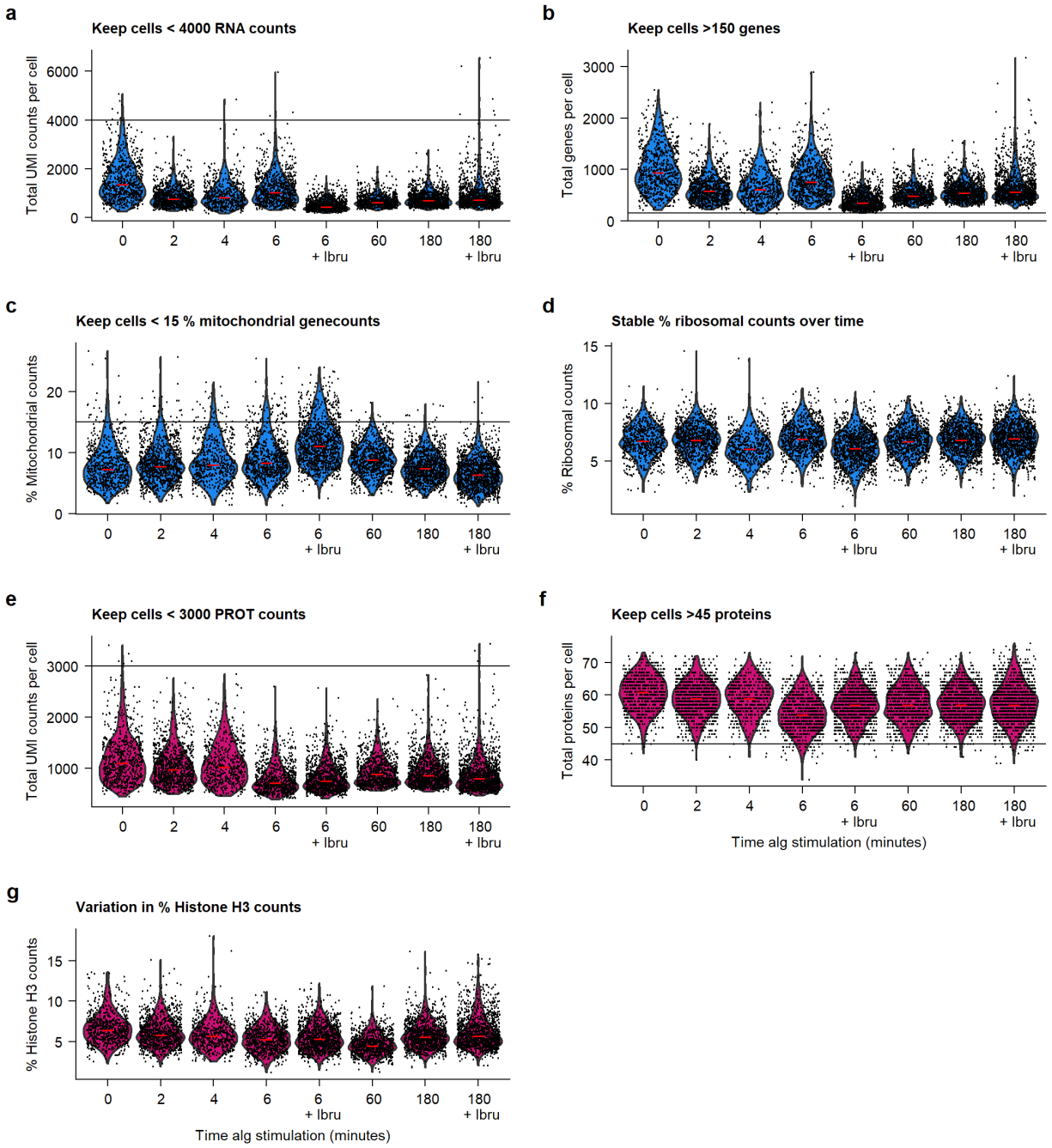
Supplementary Figure 3: Analysis of BJABs culture supernatant for cytokines upon overnight stimulation with polyclonal anti-immunoglobulin antibody (aIg). BJAB cells were stimulated for 16 hours with a titration of aIg after which the supernatant was analysed by ELISA for IL6, IL10 and CCL3.



Supplementary Figure 4: Validation by flow cytometry of stimulation by polyclonal anti-immunoglobulin antibody (aIg) of the BCR pathway. The phosphorylation of SYK, BTK, JNK, AKT, p65, p38, SHP-1, PLC-γ2, ERK 1/2, and SHP-2 was characterized upon different stimulation conditions: unstimulated cells (0 minutes); stimulation with aIg (red line) or CpG (green line) for: 5, 10, 30, 60 and 90 minutes. *CpG stimulation targeting the toll-like receptor (TLR9) was used as a control since it is expected to have different dynamics compared to aIg.*

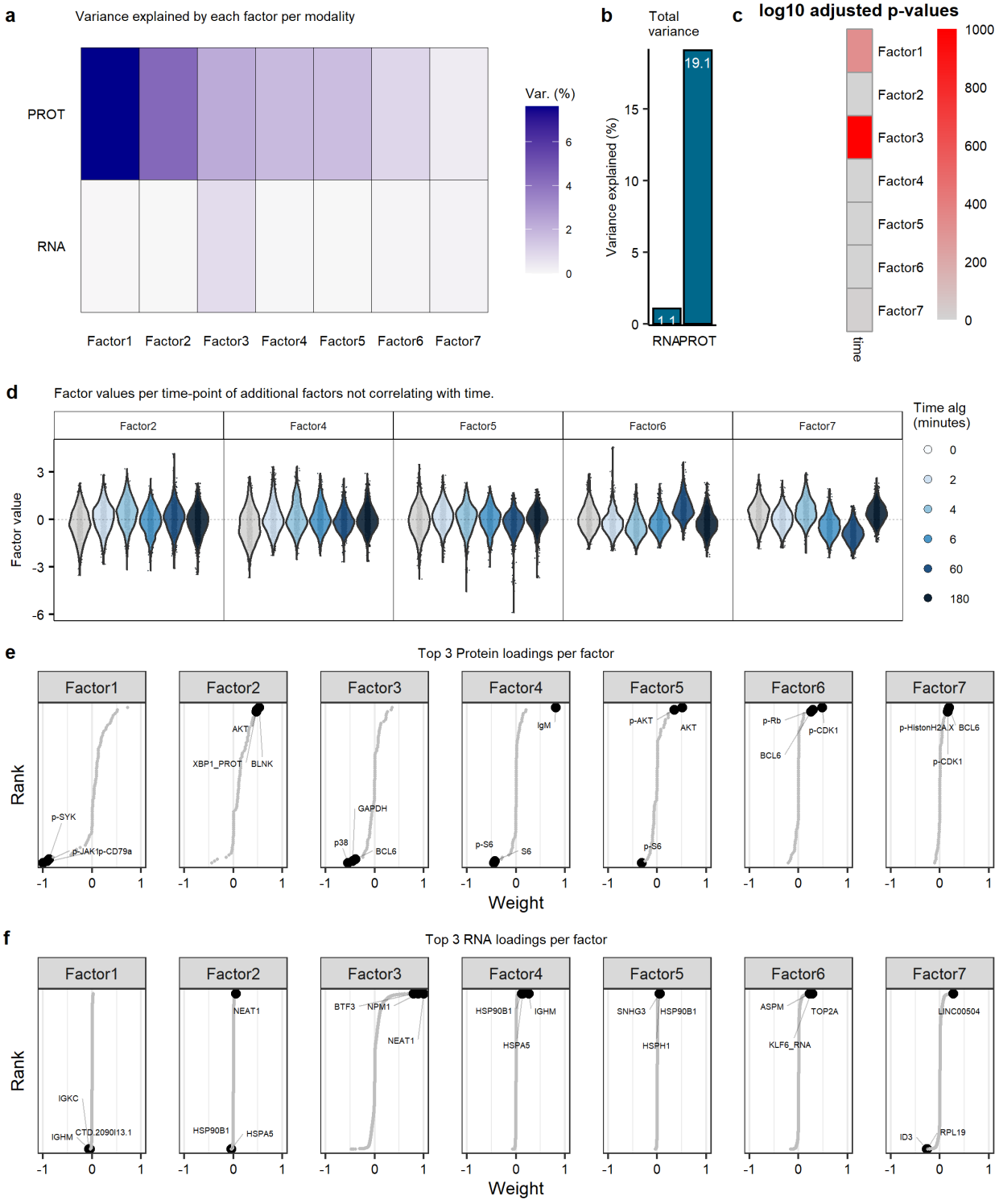


Supplementary Figure 5: Workflow and extended bioinformatics pipeline for the analysis of results obtained with QuRIE-seq platform. In the wet lab, cells are encapsulated together with barcoding beads using QuRIE-seq technology. Subsequently, an mRNA and a protein library are prepared in parallel and sequenced. **In the Python environment**, the FASTQ files are processed to generate mRNA and protein count tables. This processing comprehends: filtering of the reads, determination of the "real" cells, mapping of the reads to the reference genome using bowtie alignment, and quantification of UMIFM (UMI-filtered mapped) counts. With m = the maximum number of different alignments allowed per read, n = the number of mismatches allowed in the first l bases of the read and e = the maximum sum allowed of the quality values at all mismatched positions. **In the R environment**, the two mRNA and protein count tables are connected in one combined count table. These count tables are the basis for quality check, normalization and scaling, and MOFA+ analysis.



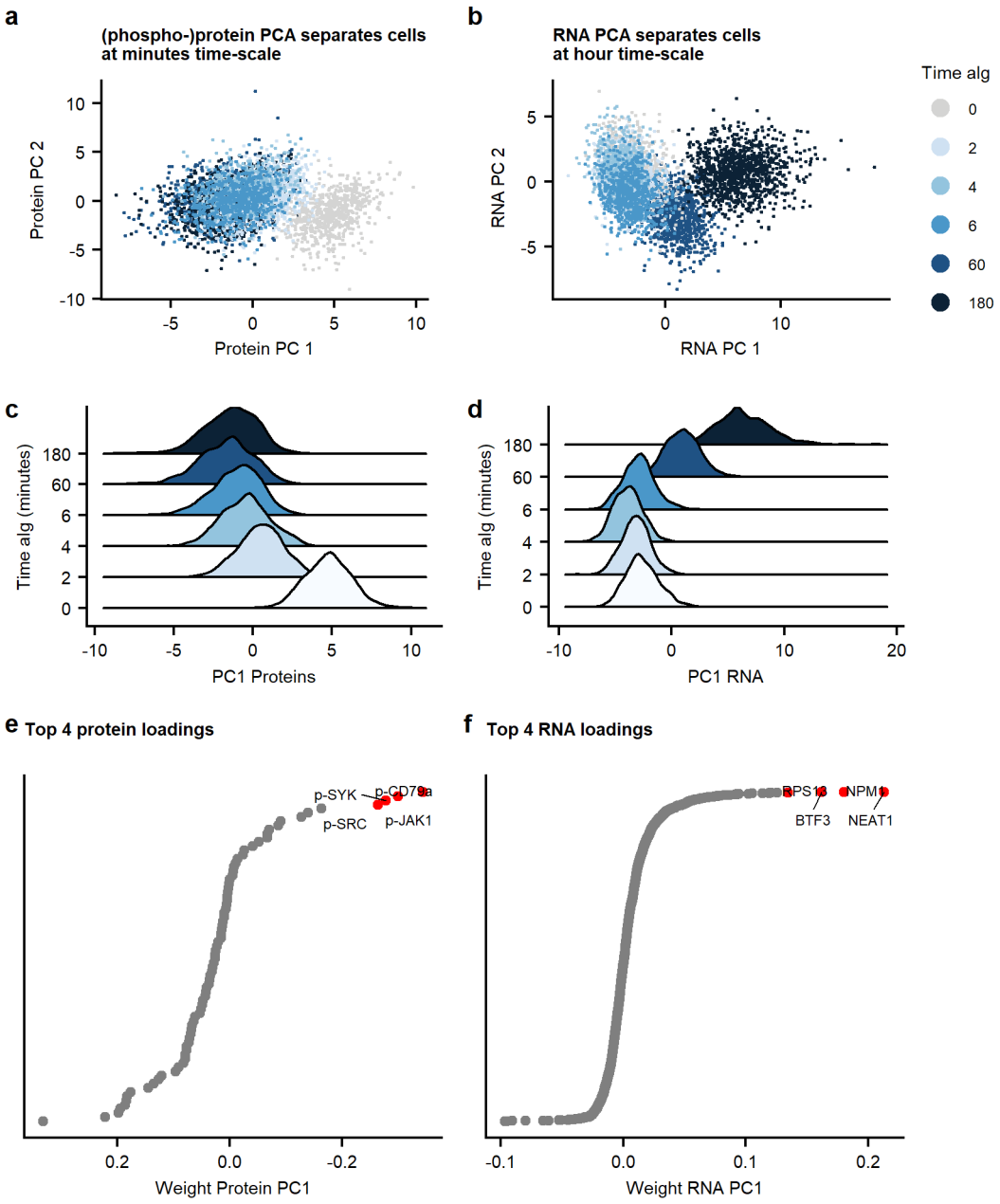
Supplementary Figure 6: Technical quality of the QuRIE-seq transcriptomic and proteomic libraries and quality checks filtering for fixed BJAB cells at eleven different stimulation and/or inhibition conditions: after stimulation with alg for: 0, 2, 4, 6, 60 and 180 minutes, with or without prior Ibrutinib inhibition resulted in a multimodal dataset of 6952 cells with matched gene and (phospho)protein expression levels. Violin plots for (**a-d, blue**) the transcriptomic and (**e-g, pink**) the proteomic libraries. **a**, The number of unique molecular identifiers filtered mapped (UMIFM) detected per cell. Cells with < 4000 UMIFM counts per cell were kept for further analysis. **b**, The number of different genes detected per cell. Cells with > 150 genes per cell were kept for further analysis. **c**, Percentage of mitochondrial UMIFM counts

out of the total number of UMIFM counts per cell. Cells with < 15% mitochondrial counts per cell were kept for further analysis. **d**, Percentage of ribosomal UMIFM counts out of the total number of UMIFM counts per cell. **e**, The number of protein counts per cell. Cells with < 3000 protein counts per cell were kept for further analysis. **f**, The number of different proteins detected per cell. Cells with > 45 different proteins detected were kept for further analysis. **g**, The percentage of Histone H3 counts out of the total number of protein counts per cell.

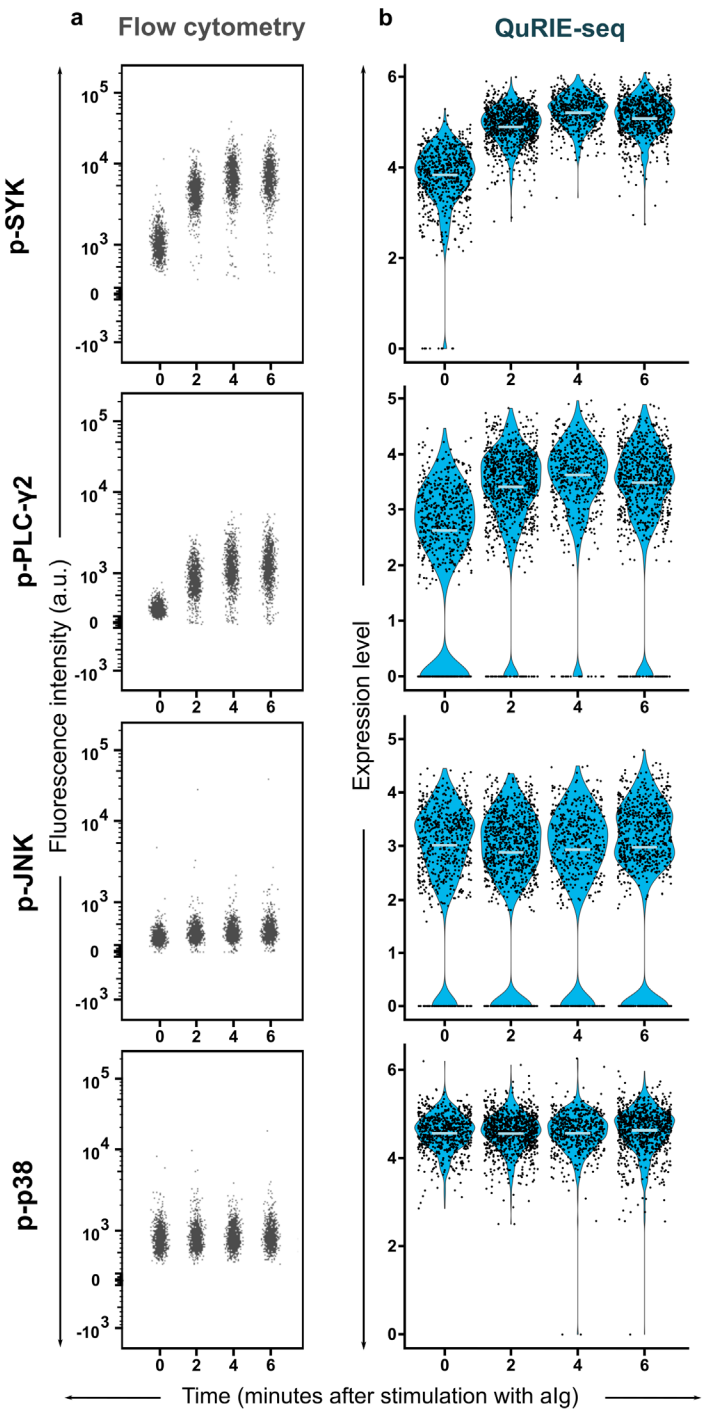


Supplementary Figure 7: Supplementary results on MOFA+ analysis Fig. 2. a, Percentage of variance explained by each factor per modality (proteins and RNA). **b**, Percentage of the total variance explained per modality. **c**, Pearson's correlation (p-value) between the MOFA+ factors and the duration of anti-immunoglobulin antibody (alg) stimulation. **d**, Violin plots of the factor values as a function of alg stimulation for: 0, 2, 4, 6, 60 and 180 minutes. Factor 1 and 3 are shown in Fig. 2a,e respectively. **e**, Protein

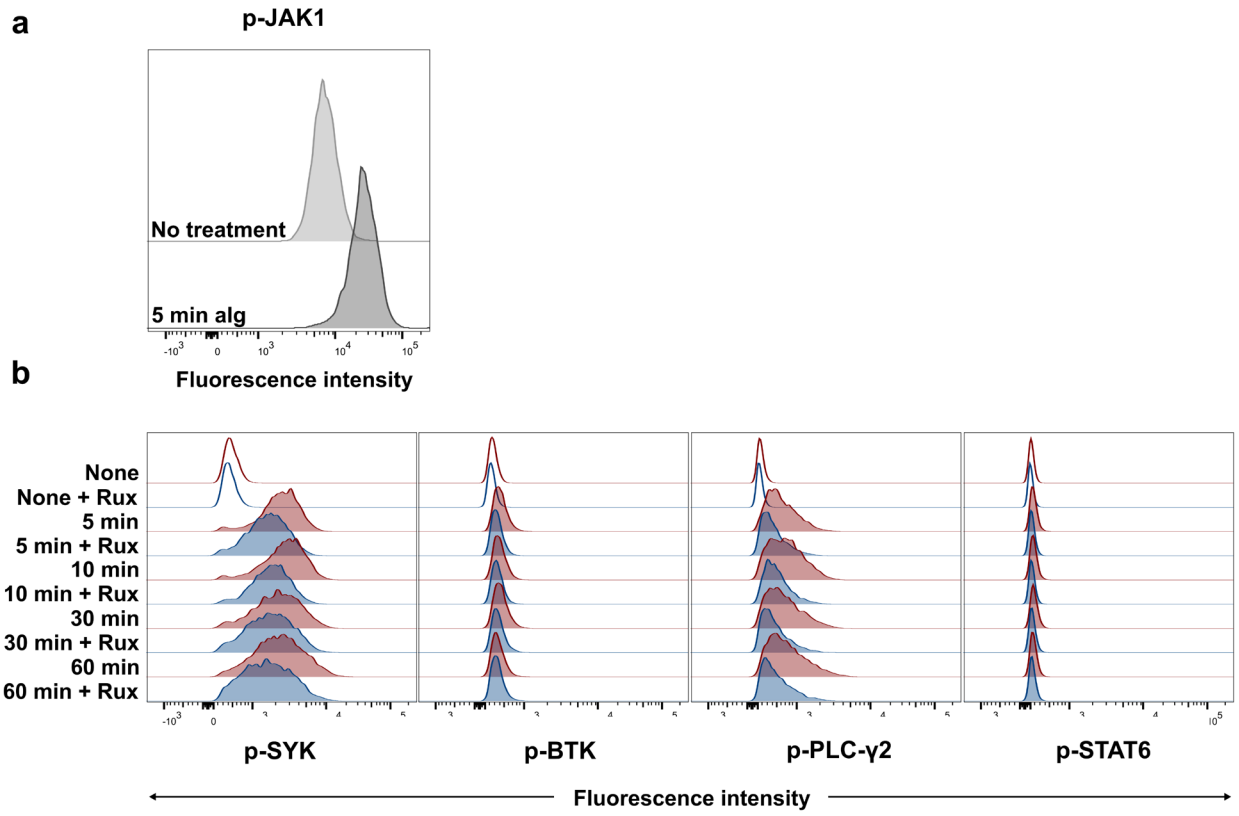
loadings per factor. The names of the top three proteins loading per factor are marked in the plots. **f**, RNA loadings per factor. The name of the top three RNA loadings per factor are marked in the plots.



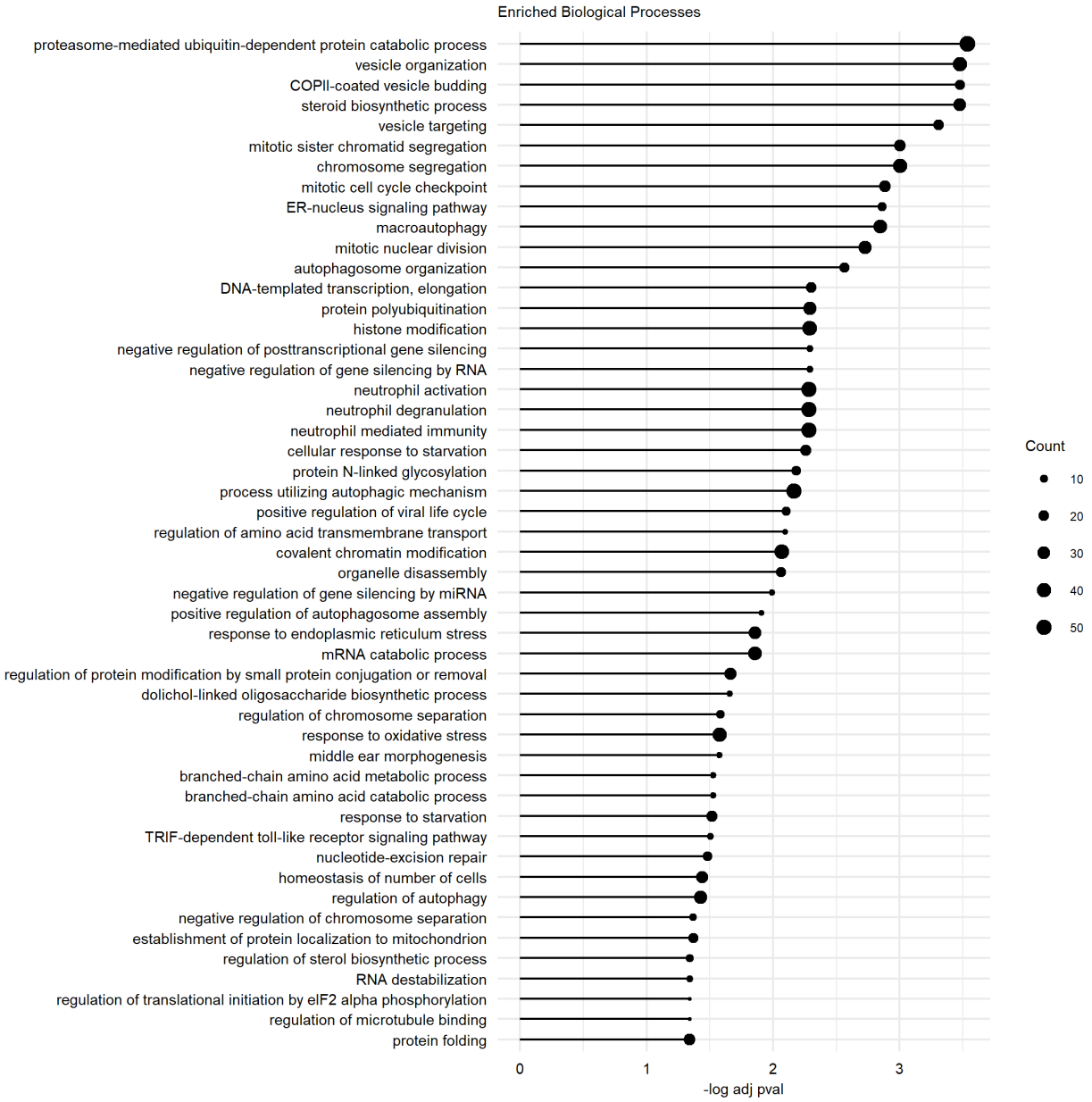
Supplementary Figure 8: PCA on protein and RNA dataset shows the equivalence of PC1 in separate modalities: proteomics and transcriptomics to factor 1 and factor 3 respectively in MOFA+ analysis. **a**, Principal component analysis (PCA) plot (PC1 and PC2) using the proteomic data. **b**, PCA plot (PC1 and PC2) using the transcriptomic data. **c**, PC1 as a function of the duration of alg stimulation for the proteomic data. **d,** PC1 as a function of the duration of alg stimulation for the transcriptomic data. **e,** Protein loadings contributing to PC1. The top 4 protein loadings are annotated. **f**, RNA loadings contributing to PC1. The top 4 RNA loadings are annotated. The results are equivalent to factors 1 and 3 in MOFA+ analysis, respectively.



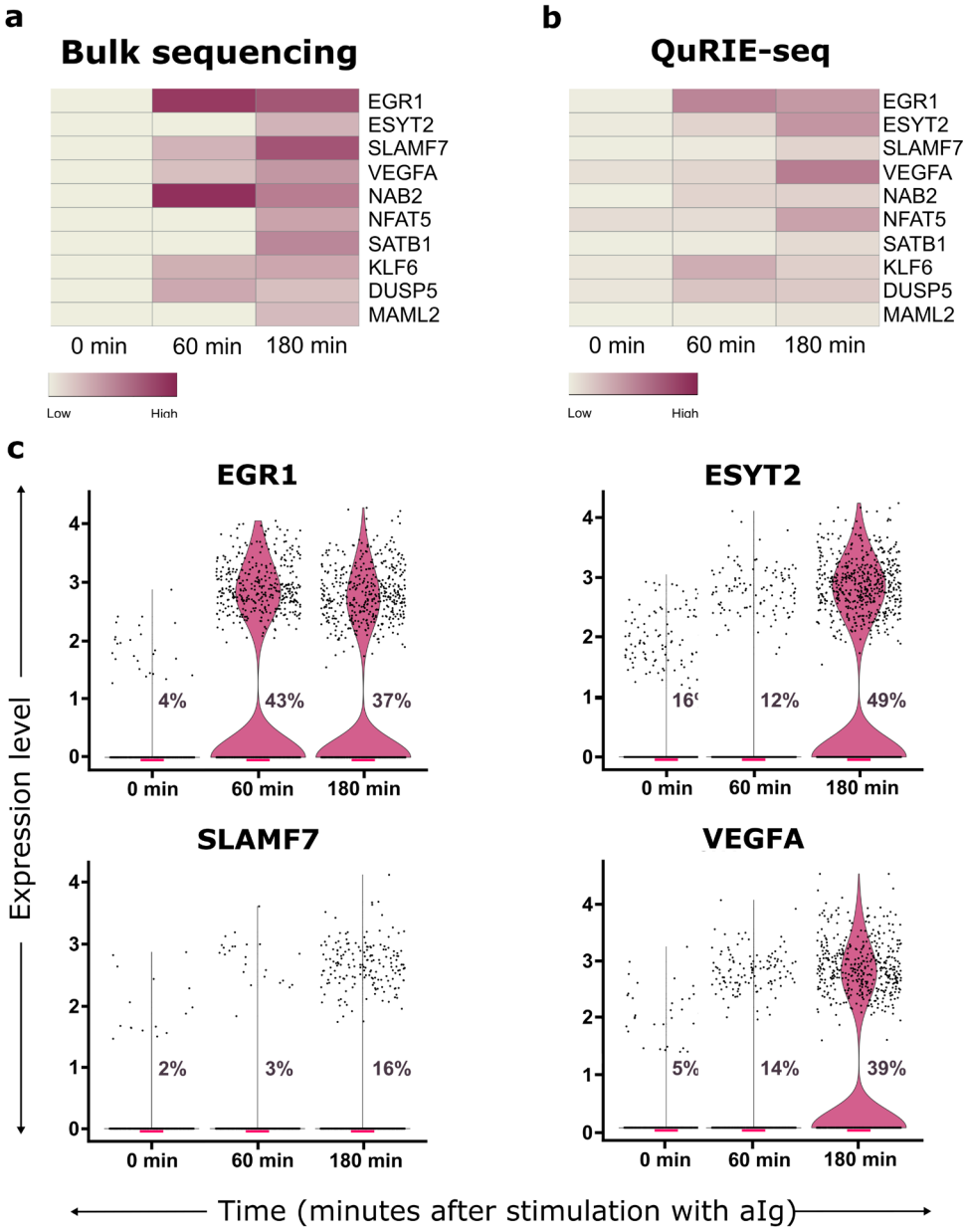
Supplementary Figure 9: **Regulation of p-SYK, p-PLC-γ2, p-JNK and p-p38 protein expression upon stimulation with alg in BJAB cells.** The phosphorylation level of four proteins was determined using: **a**, flow cytometry, and **b**, QuRIE-seq technology. Different durations of stimulation were compared: 0, 2, 4 and 6 minutes.



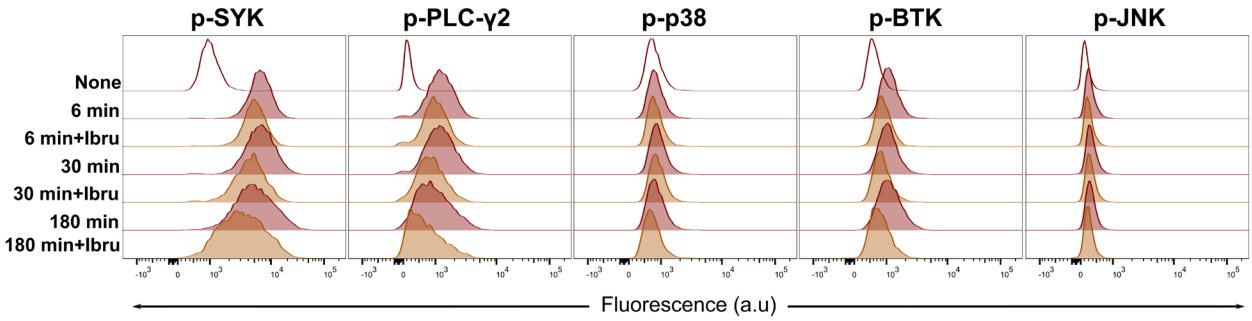
Supplementary Figure 10: The role of JAK1 in the BCR response to stimulation with aIg. a, JAK1 phosphorylation after 5 minutes stimulation with aIg characterized by flow cytometry. **b**, The phosphorylation level of four proteins: SYK, BTK, PLC-γ2, and STAT6 determined using flow cytometry for unstimulated BJAB cells, and stimulated with aIg for: 5, 10, 30, and 60 minutes with (blue curves) or without (red curves) previous Ruxolitinib (Rux) inhibition. *Rux is a JAK1/2 inhibitor.*



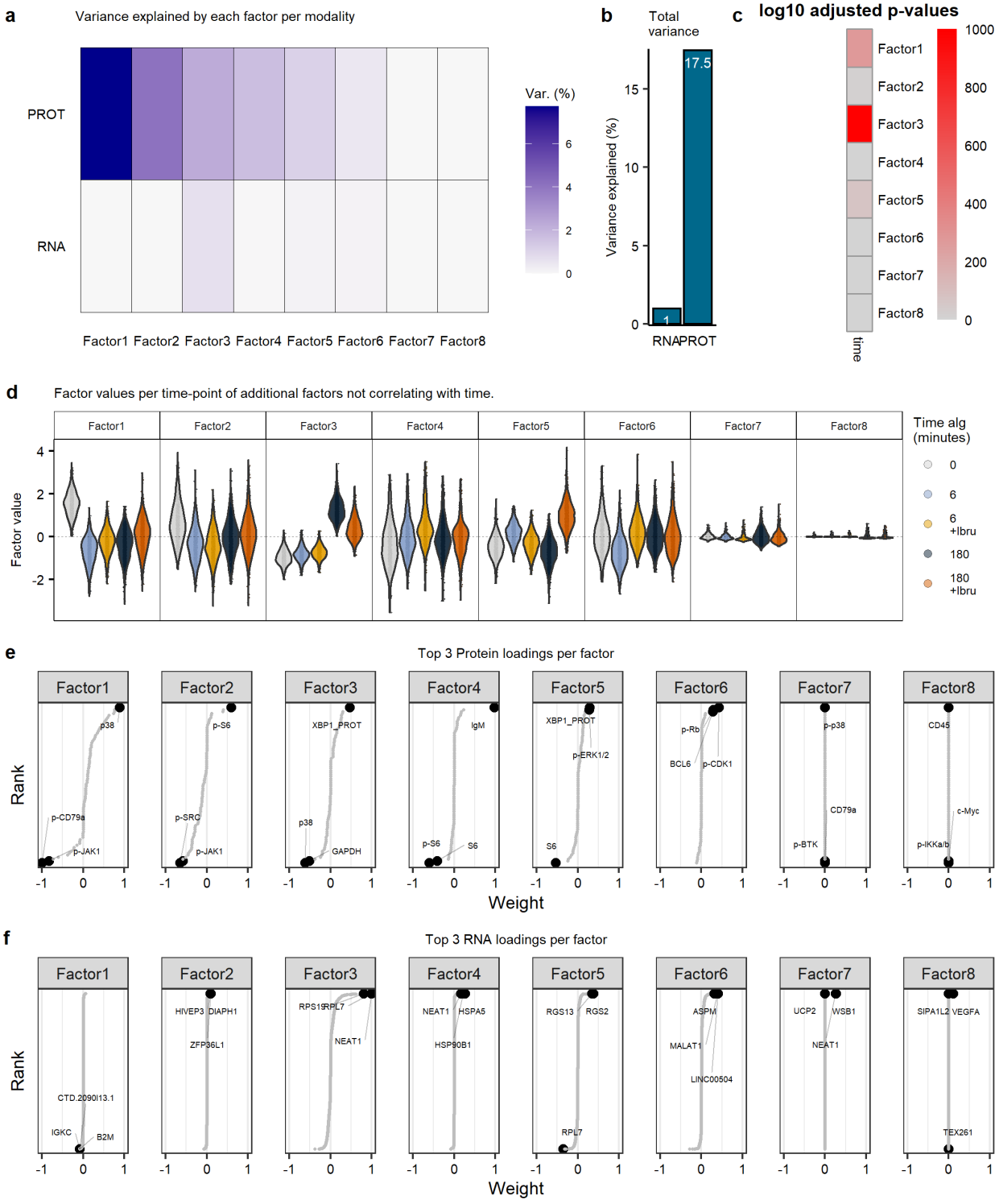
Supplementary Figure 11: Top 50 gene-set (biological processes) significantly enriched in genes with positive factor 3 loadings.



Supplementary Figure 12: **Regulation of gene expression in BJAB cells upon stimulation with aIg.** The expression of ten genes (*EGR1*, *ESYT2*, *SLAMF7*, *VEGFA*, *NAB2*, *NFAT5*, *SATB1*, *KLF6*, *DUSP5*, *MAML2*) was determined using: **a**, bulk sequencing, and **b**, the individual cell expression (determined by QuRIE-seq) averaged over the whole population, and plotted as a heatmap. **c**, The expression of each single-cell determined by QuRIE-seq measurement is shown as a violin plot for four different genes: *EGR1*, *ESYT2*, *SLAMF7*, and *VEGFA*; the percentages indicate the fraction of cells with the gene expression >0 for each stimulation time point (0, 60, 180 minutes).

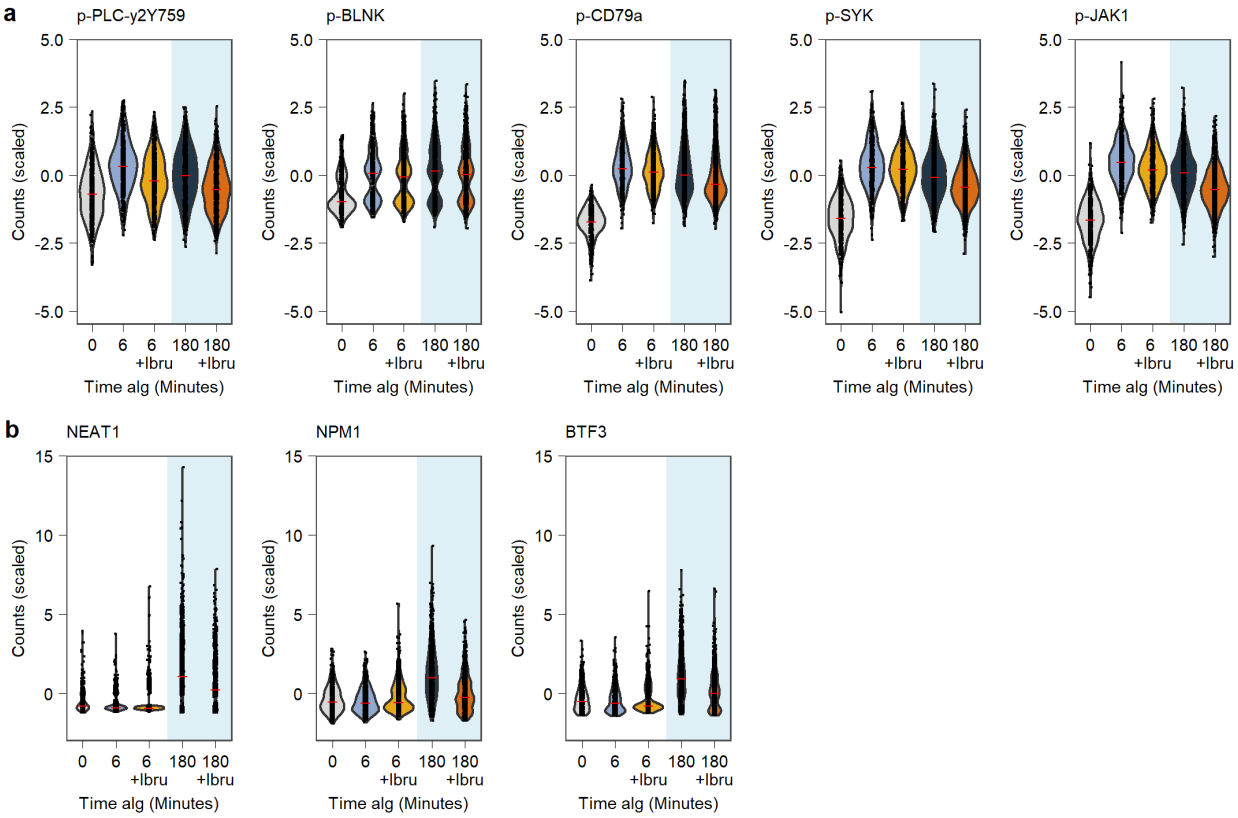


Supplementary Figure 13: Validation by flow cytometry of inhibition with Ibrutinib (Ibru) of the BCR pathway. The phosphorylation of SYK, PLC-γ2, p38, BTK, and JNK was characterized upon different stimulation conditions: unstimulated cells (0 minutes - red empty line); stimulation with aIg for: 6, 30, and 180 minutes with (orange filled line) and without (red filled line) prior Ibru inhibition.

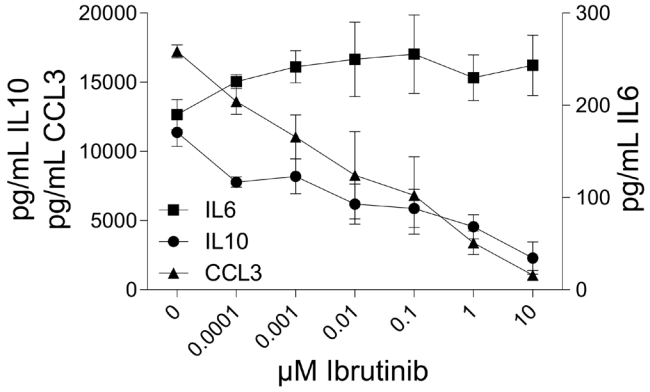


Supplementary Figure 14: Supplementary results on MOFA+ analysis Fig. 3. a, Percentage of variance explained by each factor per modality (proteins and RNA). **b**, Percentage of the total variance explained per modality. **c**, Pearson's correlation (p-value) between the MOFA+ factors and the duration of anti-immunoglobulin antibody (alg) stimulation. **d**, Violin plots of the factor values as a function of stimulation with alg for: 0, 6, and 180 minutes with and without Ibru. **e**, Protein loadings per factor. The names of the

top three proteins loading per factor are marked in the plots. **f**, RNA loadings per factor. The name of the top three RNA loadings per factor are marked in the plots.



Supplementary Figure 15: Phosphorylation up- and downstream of BTK, and downstream genes, dampened by Ibrutinib (Ibru) measured with QuRIE-seq. a, Phosphoprotein scaled counts of signaling components up- or downstream of BTK: PLC-y2, BLNK, CD79a, SYK, and JAK1 as a function of stimulation with aIg for: 0, 6, and 180 minutes with and without Ibru. **b**, Top 3 genes of factor 3 loadings: *NEAT1*, *NPM1*, and *BTF3*, scaled counts as a function of stimulation with aIg for: 0, 6, and 180 minutes with and without Ibru.



Supplementary Figure 16: Analysis of BJABs culture supernatant for cytokines upon overnight (16 hours) stimulation in presence of Ibrutinib. The BTK inhibitor (Ibrutinib) was used to block BTK (part of BCR signaling pathway), and titrated while cells were stimulated with 10 µg/ml aIg. The dose-dependent decrease of cytokine secretion was observed through ELISA for IL10 and CCL3, but not for IL6.

Supplementary Tables

Supplementary Table 1: Antibodies used for immunostaining of cells prior to microfluidic encapsulation.

	Target	Ab barcode sequence	Vendor	Clone	Used concentration
1	**Histone H3**	**CAATCCCT**	Cell Signaling 4499BF	D1H2	0.1 µg/ml
2	**p-p65**	**GTCCAGGC**	Cell Signaling 3033BF	93H1	1 µg/ml
3	**p-SYK**	**TGTGTATA**	Cell Signaling 2710BF	C87C1	1 µg/ml
4	**p-JNK**	**AGGATCGA**	Cell Signaling 9255BF	G9	1 µg/ml
5	**p-p38**	**CACGATTC**	Cell Signaling 4511BF	D3F9	1 µg/ml
6	**p-PLC-y2**	**GTATCGAG**	R&D Systems MAB37161	790623	1 µg/ml
7	**p-BTK**	**TCTCGACT**	Biolegend 601702	A16128B	1 µg/ml
8	**p-SHP-2**	**ACCCGCAC**	Cell Signaling 5431BFF	D66F10	1 µg/ml
9	**p-SHP-1**	**CATGCGTA**	Cell Signaling 8849BF	D11G5	1 µg/ml
10	**Cyclin E**	**GTGATAGT**	ThermoFisher 32-1600	HE12	1 µg/ml
11	**c-MYC**	**TGATATCG**	ThermoFisher 700648	27H46L35	1 µg/ml
12	**p-RB**	**AGGGCGTT**	ThermoFisher 701059	14H7L14	0.1 µg/ml
13	**p-c-JUN**	**CTATACGC**	ThermoFisher MA5-27760	GT653)	1 µg/ml
14	**active Caspase-3**	**GCTCGTCA**	BD Bioscience 559565	C92-605	1 µg/ml
15	**CD70**	**TACATAAG**	BD Bioscience 555833	Ki-24	1 µg/ml
16	**IgD**	**AATTGAAC**	BD Bioscience 555776	IA6-2	1 µg/ml
17	**CD86**	**CCAGTGGA**	BD Bioscience 555655	2331 (FUN-1)	1 µg/ml
18	**CD20**	**GTCCATTG**	BD Bioscience 555677	H1	0.1 µg/ml
19	**CD79a**	**TGGACCCT**	BD Bioscience 555934	HM47	0.1 µg/ml
20	**CD5**	**AGCAGTTA**	BD Bioscience 555350	UCHT2	1 µg/ml
21	**p-AKT**	**CTTGTACC**	Cell Signaling 4060BF	D9E	1 µg/ml
22	**S6**	**GAACCCGG**	Cell Signaling 2317B	54D2	1 µg/ml
23	**ERK 1/2**	**TCGTAGAT**	Cell Signaling 4696BF	L34F12	1 µg/ml
24	**p38**	**ACGCGGAA**	Cell Signaling 8690BF	D13E1	1 µg/ml
25	**AKT**	**CGCTATCC**	Cell Signaling 4685BF	11EE7	1 µg/ml
26	**SYK**	**GTTGCATG**	Cell Signaling 13198BF	D3Z1E	1 µg/ml
27	**BTK**	**TAAATCGT**	Cell Signaling 8547BF	D3H5	0.1 µg/ml
28	**p-S6**	**ATCGCCAT**	Cell Signaling 4858BF	D57.2.2E	0.1 µg/ml
29	**p-ERK 1/2**	**CATAAAGG**	Cell Signaling 5726BF	D1H6G	1 µg/ml
30	**p65**	**TCACGGTA**	Cell Signaling 8242BF	D14E12	0.1 µg/ml
31	**CD19**	**CACTCAAC**	SantaCruz sc-373897	F-3	2 µg/ml
32	**p-Histon H3**	**GCTGTGA**	Biolegend 650802	11D8	0.1 µg/ml
33	**IgM**	**TTGCGTCG**	Biolegend 314502	MHM-88	1 µg/ml
34	**Cyclin A**	**ATATGAGA**	Biolegend 644001	E23.1	0.1 µg/ml
35	**p-Histon H2A.X**	**CACCTCAG**	Biolegend 613402	2F3	0.1 µg/ml
36	**Cyclin B1**	**GCTACTTC**	Biolegend 647902	V152	0.1 µg/ml

37	**Ki-67**	**TGGGAGCT**	Biolegend 350523	Ki-67	0.1 µg/ml
38	**JNK**	**ATCCGGCA**	R&D Systems AF1387		1 µg/ml
39	**SHP-1**	**CCGTTATG**	R&D Systems MAB1878	255402	1 µg/ml
40	**p-SRC**	**GGTAATGT**	R&D Systems MAB2685	1246F	1 µg/ml
41	**p-TOR**	**TAAGCCAC**	R&D Systems MAB1665	834115	0.1 µg/ml
42	**GAPDH**	**ACCGAACA**	Biolegend 607902	W17079A	1 µg/ml
43	**BLNK**	**GTTTGTGG**	BD Biosciences 559930	2B11	1 µg/ml
44	**p-BLNK**	**TAGACGAC**	BD Biosciences 558366	J117-1278	0.1 µg/ml
45	**p-PKC-b1**	**ACGCTTGG**	ThermoFisher 702430	3H8L1	0.1 µg/ml
46	**CD53**	**CGCTACAT**	BD Biosciences 555506	HI29	0.1 µg/ml
47	**CD38**	**GAAAGACA**	Biolegend 303535	HIT2	0.1 µg/ml
48	**CD45**	**TTTGCGTC**	Biolegend 304045	HI30	0.1 µg/ml
49	**p-IRAK4**	**ATGGTCGC**	Cell Signaling 11927S	D6D7	1 µg/ml
50	**p-CD79a**	**CGACATAG**	Cell Signaling 14732BF	D1B9	0.1 µg/ml
51	**p-CDK1**	**GATTCGCT**	ThermoFisher 701808	17H29L7	1 µg/ml
52	**p-CDK4**	**TCCAGATA**	ThermoFisher 702556	9H2L7	1 µg/ml
53	**p-AMPK-a1/2**	**ACTACTGT**	ThermoFisher701068	10H2L20	0.1 µg/ml
54	**p-AMPK-b1**	**CGGGAACG**	Thermo Fisher 700241	9H26L42	0.1 µg/ml
55	**T-bet**	**GACCTCTC**	Biolegend 644825	4B10	1 µg/ml
56	**p-IKK a/b**	**TTATGGAA**	ThermoFisher 701643	7H17L17	1 µg/ml
57	**CD27**	**ACAGCAAC**	Abcam ab192336	EPR8569	0.1 µg/ml
58	**p-JAK1**	**CGCAATTT**	ThermoFisher 700028	59H4L5	1 µg/ml
59	**p-PLC-y2 (Y759)**	**GAGTTGCG**	R&D Systems MAB7377	744757	0.1 µg/ml
60	**IL10**	**TTTCGCGA**	ThermoFisher 16-7108-85	JES3-9D7	1 µg/ml
61	**CCL3/4**	**ACCAGTCC**	R&D Systems MAB2701-100	93342	1 µg/ml
62	**CD80**	**CTTTCCTT**	Biolegend 305212	2D10	1 µg/ml
63	**p-CDK6**	**CTGGACGT**	ThermoFisher	16HCLC	1 µg/ml
64	**p-STAT1**	**GAACGGTC**	ThermoFisher 33-3400	ST1P-11A5	1 µg/ml
65	**p-STAT3**	**TGTTCACG**	Biolegend 690402	A16002B	1 µg/ml
66	**p-STAT5**	**ATCTGATC**	ThermoFisher 701063	6H5L15	1 µg/ml
67	**p-STAT6**	**GAGAAGGG**	ThermoFisher 700247	46H1L12	1 µg/ml
68	**KLF6**	**TCACTCCT**	ThermoFisher 39-6900	9A2	1 µg/ml
69	**BCL6**	**AGATAACA**	BD Bioscneces 561520	K112-91	1 µg/ml
70	**AID**	**CTTATTTG**	BD Biosciences 565784	EK2-5G9	1 µg/ml
71	**IgG**	**GCGGGCAT**	BD Biosciences 555784	G18-145	0.1 µg/ml
72	**CD24**	**TACCCGGC**	RnD Systems MAB5247	ML5	1 µg/ml
73	**IgA**	**ATTGTTTC**	Biolegend 411502	HP6123	0.5 µg/ml
74	**IgE**	**CGCAGGAG**	Biolegend 325502	MHE-18	1 µg/ml
75	**CD23**	**GCACCAGT**	Abcam ab245732	SP163	0.1 µg/ml
76	**BLIMP1**	**TAGTACCA**	RnD Systems MAB36081	646702	1 µg/ml

77	**IRF8**	**ATTCGTGC**	Biolegend 656502	656502	1 µg/ml	
78	**IRF4**	**CGCGTGCA**	ThermoFisher 14-9858-82	3 E 4	1 µg/ml	
79	**BAFF-R**	**GAATACTG**	RnD Systems MAB1162	2403C	1 µg/ml	
80	**XBP1**	**TCGACAAT**	Abcam ab239954	EPR4086	1	µg/ml

Supplementary Table 2: Antibodies used for flow cytometry analysis of BJABs.

Target	Fluorophore	Vendor	Clone	Used concentration
p-SYK	PE	Cell Signaling 6485	C87C1	1/50
p-PLC-y2	Alexa 647	BD Bioscience 558498	K86-689.37	1/100
p-BTK	PE	Biolegend 601704	A16128B	1/50
p-p38	Alexa 488	Cell Signaling 41768S	3D7	1/50
p-AKT	PE	Cell Signaling 5315	D9E	1/50
p-ERK 1/2	PE	Cell Signaling 75765S	D1H6G	1/50
p-JNK	Alexa 647	Cell Signaling 9257S	G9	1/50
p-p65	Alexa 647	Cell Signaling 5733	93H1	1/50
p-S6	PE	Cell Signaling 5316	D57.2.2E	1/800
CD19	PerCP/Cy5.5	Biolegend 302230	HIB19	1/100
IgD	BV510	Biolegend 348220	IA6-2	1/50
CD27	FITC	BD Bioscience 555440	M-T271	1/25
aCD20	APC-H7	BD Bioscience 560853	2H7	1/25
CD38	PE-Cy7	eBioscience 250-388-41	HB7	1/2000
IgA	PE	Miltenyi 130-113-472	IS11-8E10	1/200

Supplementary Table 3: Sequences of all the primers used for library preparation. All primers were ordered from Biolegio (The Netherlands).

	Primer sequence 5’ -> 3’	Length (bp)
PE2-N6	TCGGCATTCCTGCTGAACCGCTCTTCCGATCT NNNNN	38
PE1	CAAGCAGAAGACGGCATACGAGAT [6-bp library index] CTCTTTCCCTACACGA	46
PE2	AATGATACGGCGACCACCGAGATCTACACGGTCTCGGCATTCCTGCTG AAC	51
PE2-NNNN-Next1	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNGATGTGTATAAG AGAC	52
PE2-NNNN-BioHash2	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNCGTGTGCTCTTCC GAT	52
PE2-Nextera2 (long)	AATGATACGGCGACCACCGAGATCTACACGGTCTCGTCGGCAGCGTCA GATG	52
Custom Read 1 primer	GGCATTCCTGCTGAACCGCTCTTCCGATCT	30
Custom Index Read primer	AGATCGGAAGAGCGTCGTGTAGGGAAAGAG	30
Custom Read 2 primer	CTCTTTCCCTACACGACGCTCTTCCGATCT	30