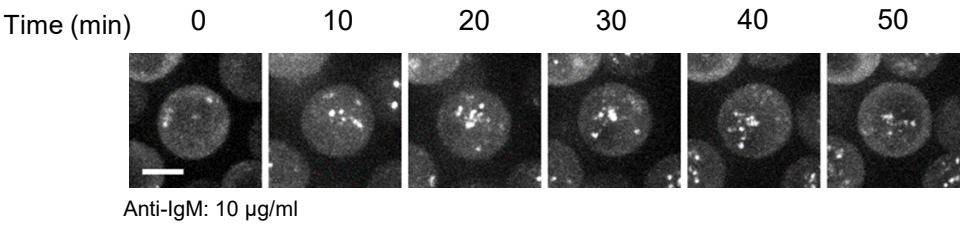
a



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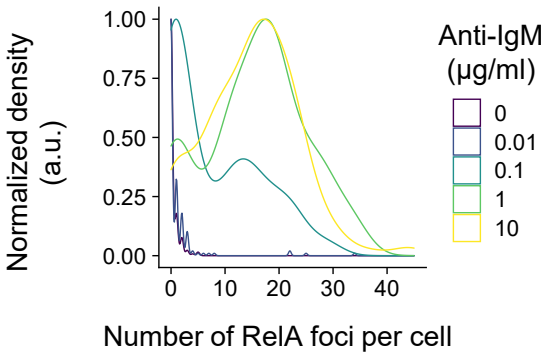
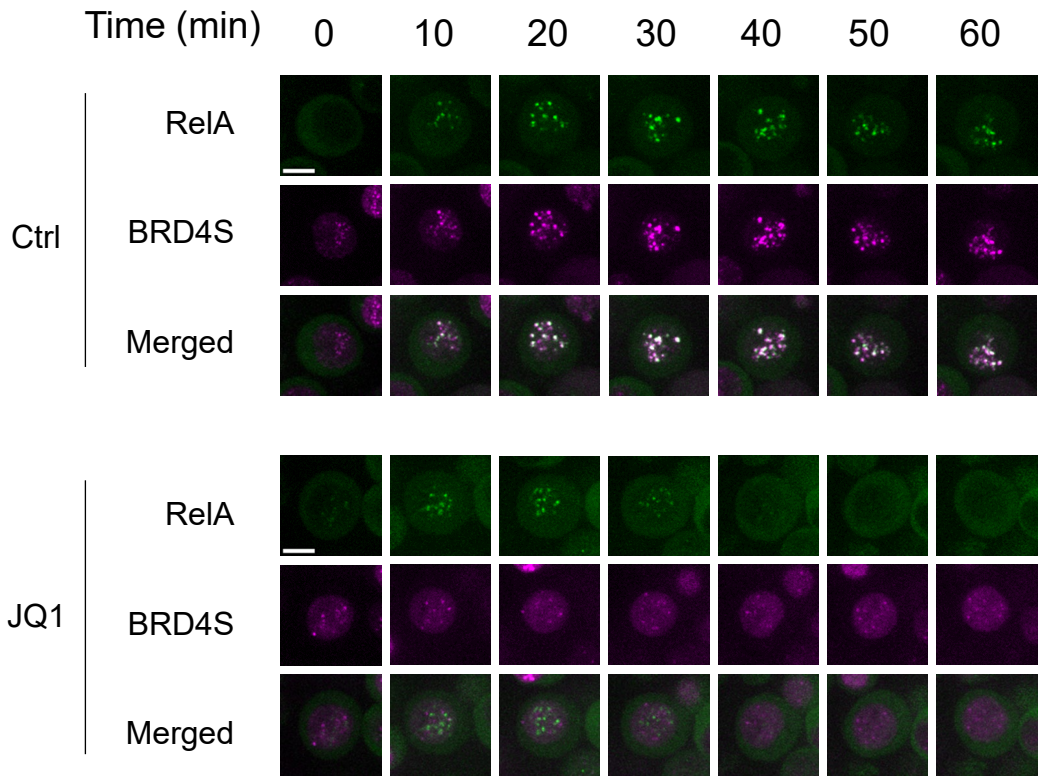


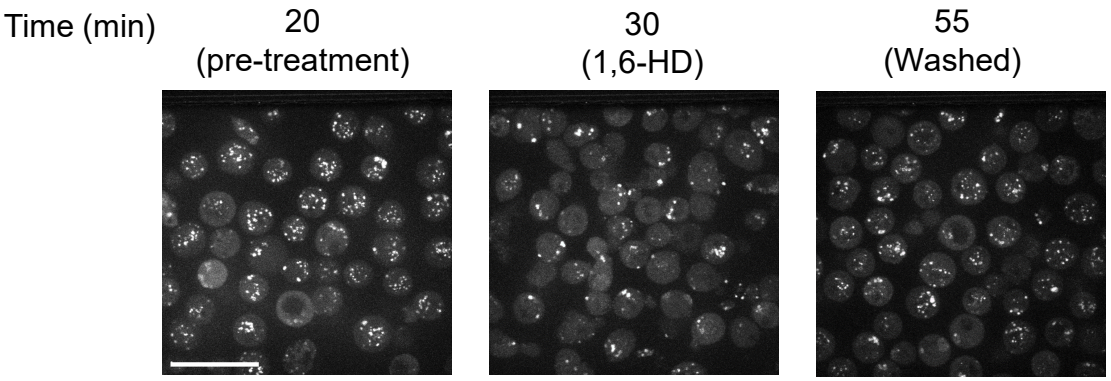
Supplementary figure 1. Dynamics of RelA nuclear translocation upon anti-IgM stimulation on DT40 cells. (a) Representative fluorescence micrographs of a single cell upon stimulation with 10 μg/mL anti-IgM (scale bar, 10 μm). (b) Distribution of RelA foci per cell upon stimulation with various doses of anti-IgM for 20 min.



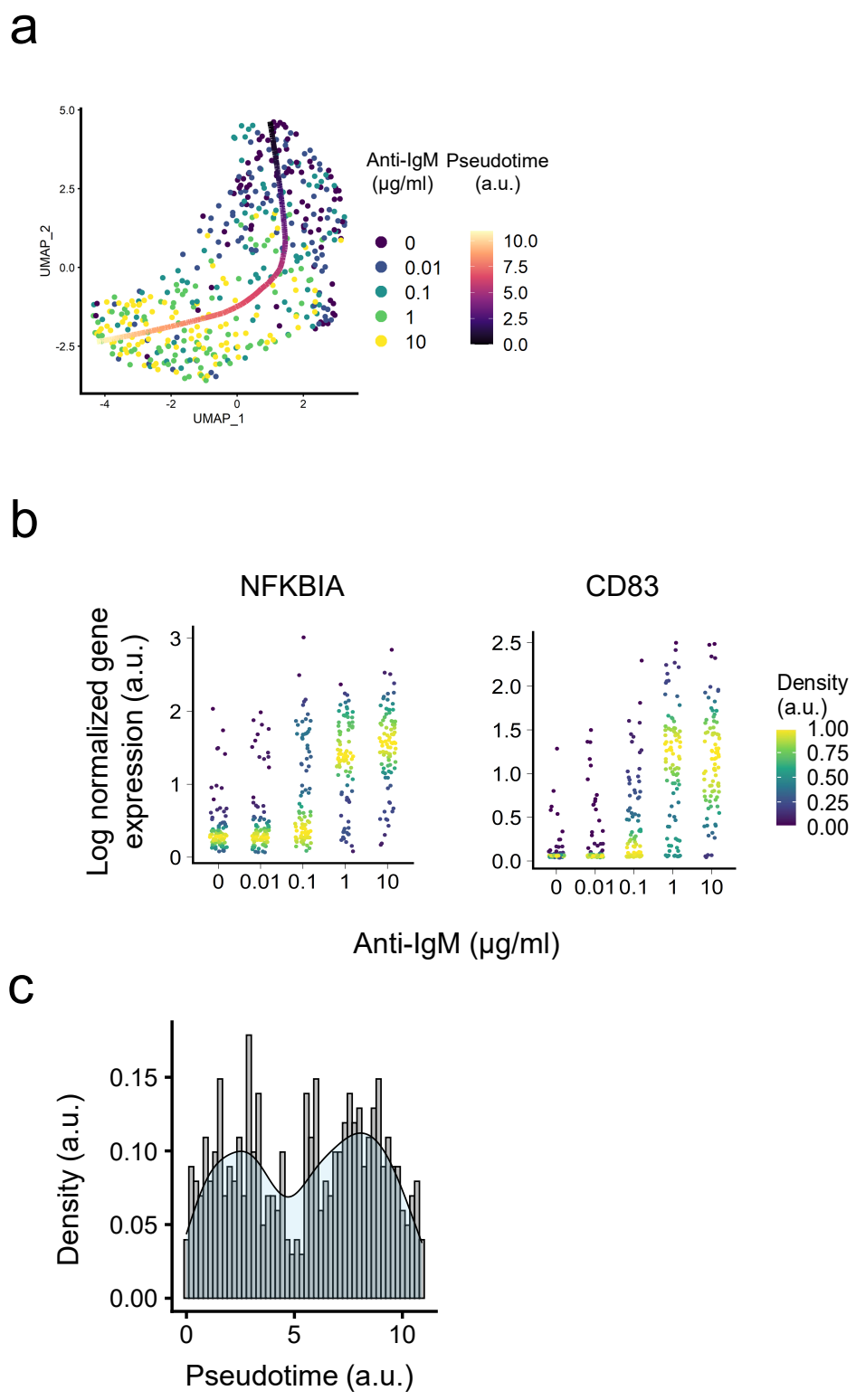
Supplementary figure 2. JQ1 treatment of mKate2-BRD4S and RelA-GFP coexpressing cells. Time-lapse fluorescence micrographs of DT40 cells co-expressing mKate2-BRD4S and RelA-GFP upon stimulation with 10 μg/mL anti-IgM and pre-treatment with JQ1 (5 μM) for 60 min (scale bar, 5 μm).

a

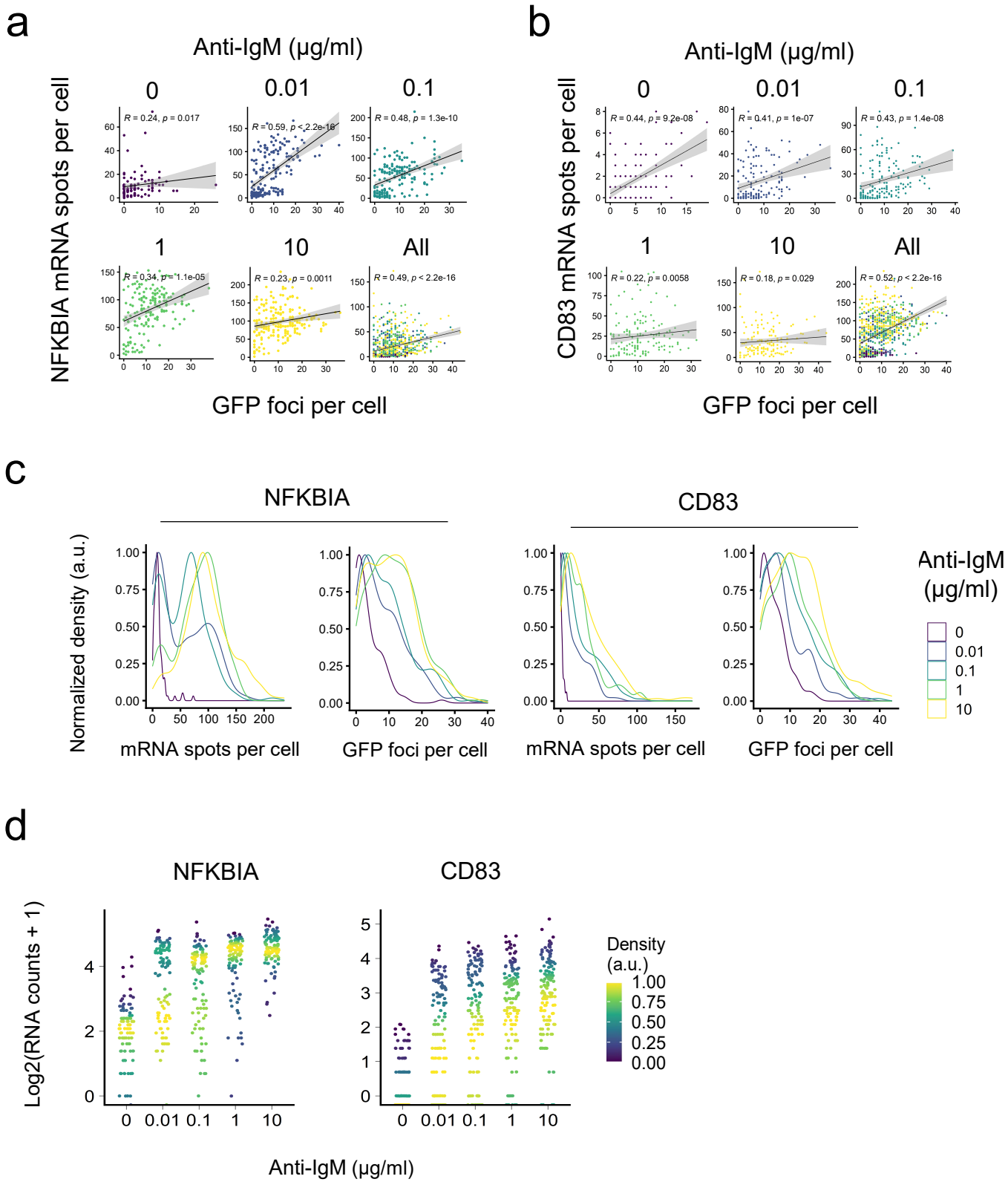
b



Supplementary figure 3. RelA-GFP foci demonstrate LLPS condensate-like biophysical properties. (a). PONDR VLXT disorder scores of Brd4 and RelA. (b). Representative fluorescence micrographs of a cell population stimulated with 10 µg/mL anti-IgM before treatment, after 1,6-hexanediol treatment, and upon washing (scale bar, 25 µm).

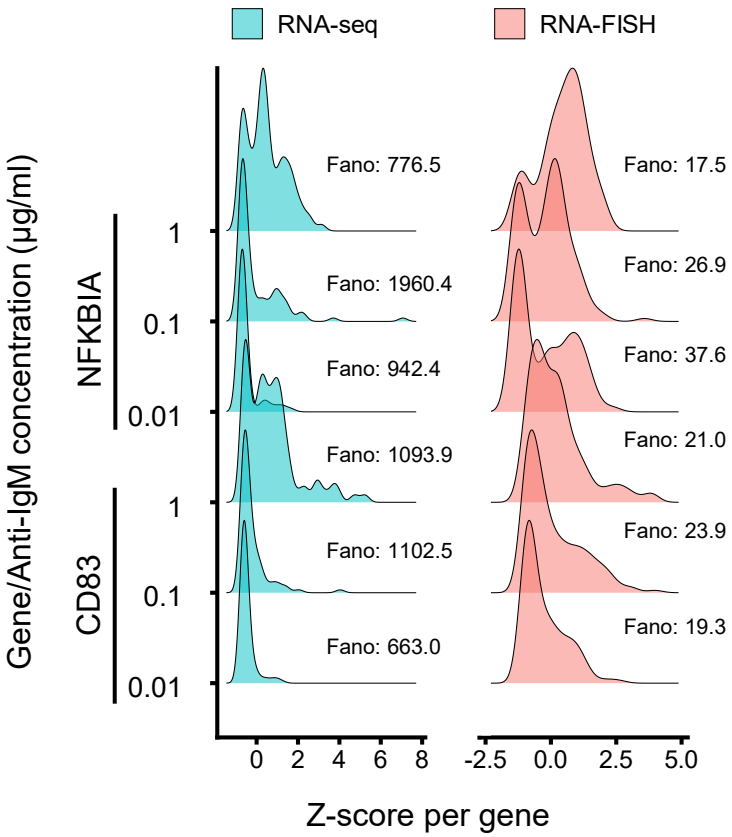


Supplementary figure 4. scRNA-seq analysis of DT40 cell stimulated with various doses of anti-IgM. (a) Pseudo-time axis taken using a principal curve over a UMAP projection showing the doses of anti-IgM. (b) RNA expression of CD83 and NFKBIA across dose points obtained from scRNA-seq. (c) Distribution of cells across pseudo-time.

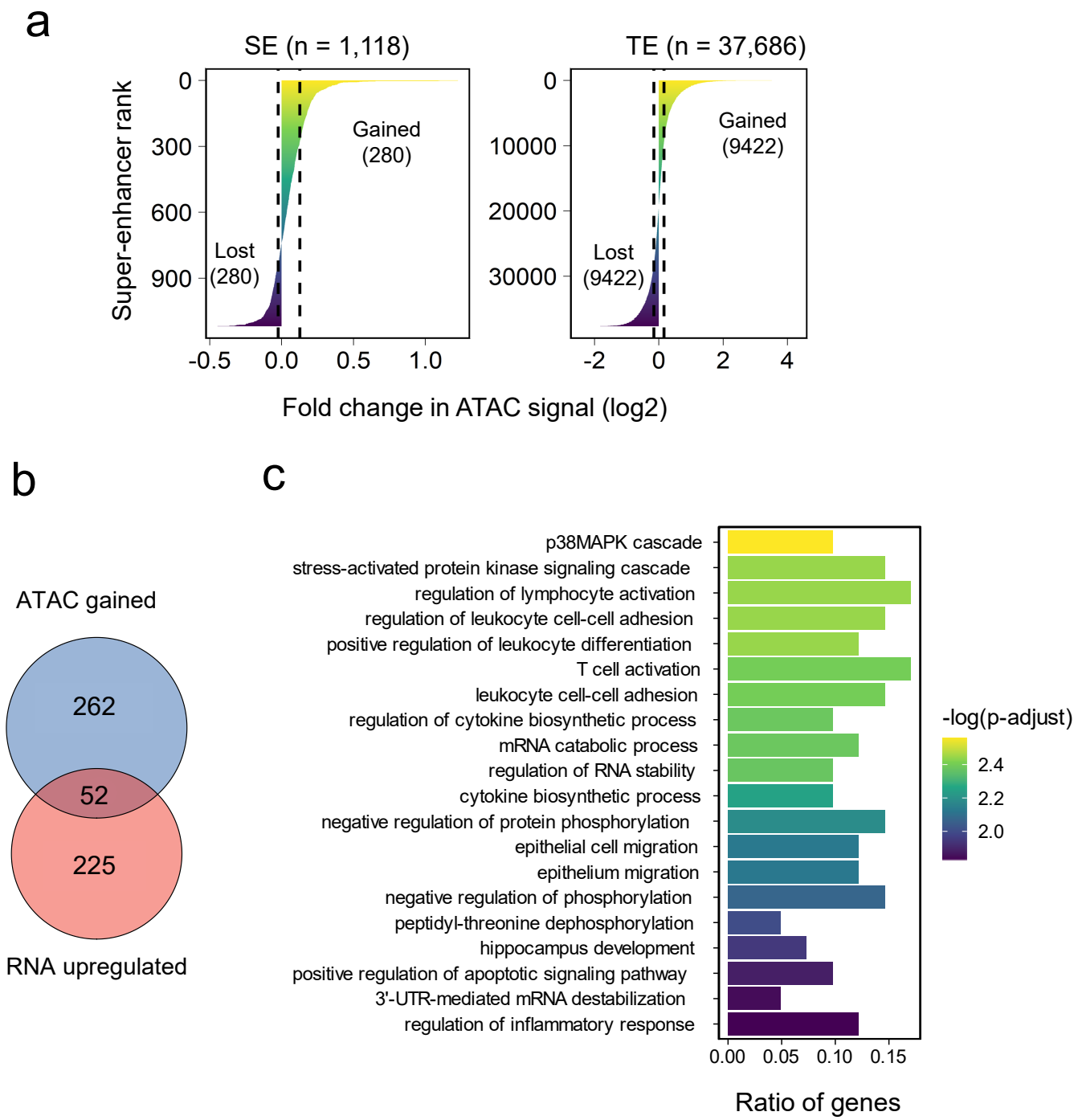


Supplementary figure 5. smRNA-FISH analysis of DT40 cells stimulated with various doses of anti-IgM.

(a–b) Correlation plot between GFP foci and mRNA spots per cell for various doses of anti-IgM. (c) Density plot of RNA spots and GFP foci at various anti-IgM doses from smRNA-FISH analysis. (d) RNA expression of CD83 and NFKBIA across dose points obtained from both scRNA-seq and smRNA-FISH.



Supplementary Figure 6. Gene expression distribution of *CD83* and *NFKBIA*. Single-cell expression of *CD83* (B cell activation marker) and *NFKBIA* (NF-κB target gene) obtained from scRNA-seq and smRNA-FISH at various doses of anti-IgM.



Supplementary figure 7. Biological functions of SEs.
(a) Regions with log2 fold changes more than the upper quantile were annotated as gained TEs/SEs and lower than the lower quantile were annotated as lost TEs/SEs. (b) Venn diagram of gained SEs (blue) and upregulated genes upon activation (red). (c) Gene ontology (GO) enrichment analysis of genes with both gained SE (upper quantile) and upregulated RNA (upper quantile).

Supplementary table 1. Number of single-cell RNA-seq data used in scRNA-seq analysis for each dose point of anti-IgM

Anti-IgM (μg/ml)	Number of cells
0	89
0.01	92
0.1	87
1	92
10	93

Supplementary table 2. Number of activated and inactivated cells

Anti-IgM	Activated	Inactivated	% Activated	Data source
0.00	2	87	2.25	RNA-seq
0.01	11	81	12.0	RNA-seq
0.10	24	63	27.6	RNA-seq
1.00	70	22	76.1	RNA-seq
10.00	75	18	80.6	RNA-seq
0.00	6	164	3.53	Imaging
0.01	20	151	11.7	Imaging
0.10	127	113	52.9	Imaging
1.00	220	36	85.9	Imaging
10.00	243	25	90.7	Imaging

Supplementary table 3. Primers used in qPCR

Oligonucleotide name	**Sequence (5’ to 3’)**
CD83 (forward)	ACCCTGTGCAATGTTTGGAG
CD83 (reverse)	CTGGTAGGCGATCGAGGAAT
NFKBIA (forward)	TTCACGAGGAAAAGGCCCTG
NFKBIA (reverse)	TCTGGCTGAGGTTGTTCTGG
GAPDH (forward)	AGGTGCTGAGTATGTTGTGGAGTC
GAPDH (reverse)	GTGGTGCACGATGCATTGCTGACAAT

Supplementary table 4. Primers used in mKate2-BRD4S cell line construction

Oligonucleotide name	**Sequence (5’ to 3’)**
attB1-mKate2 (forward)	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAGCCA CCATGGTGAGCGAGCTGATTA
attB2-*BRD4S* (reverse)	GGGGACCACTTTGTACAAGAAAGCTGGGTATTAGG CAGGACCTGTTTCGGAGTCTTCGCTGTCAGAG
Linker-*BRD4S* (forward)	GTGCTGGTAGTGCAGCAGGTTCTGGAGAATTTATGT CTGCGGAGAGCGGCCCTG
Linker-mKate2 (reverse)	CAGAACCTGCTGCACTACCAGCACTTCCTCTGTGC CCCAGTTTGCTAGGGAG