

Supplementary Figures

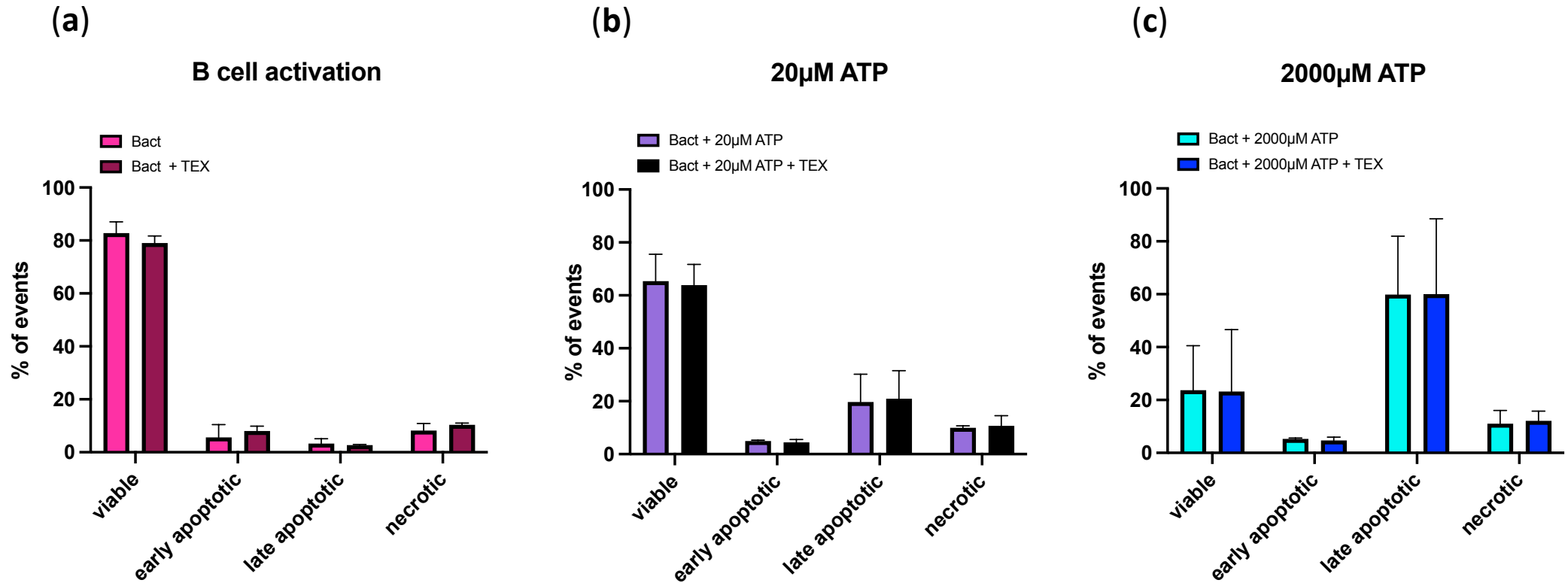
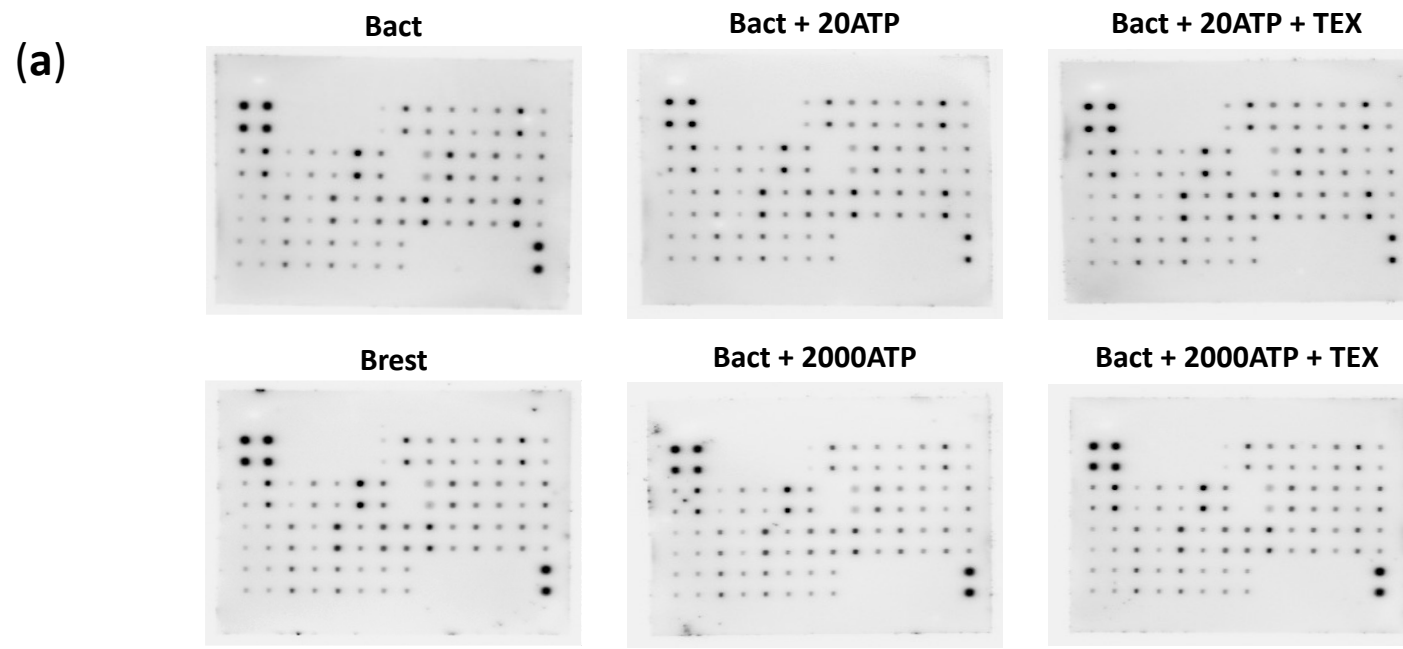


Figure S1. Bar charts showing the effect of TEX on cell death in activated B cells (Bact) with or without ATP based on flow cytometry data of Annexin V and Propidium Iodide (PI) staining as seen in figure 5a. **(a)** Bar charts showing no significant changes by TEX on the rates of viable or dead cells in Bact. **(b)** Bar charts showing no significant changes by TEX on the rates of viable or dead cells in Bact co-incubated with 20μM ATP. **(c)** Bar charts showing no significant changes by TEX on the rates of viable or dead cells in Bact co-incubated with 2000μM ATP. Statistical significance was determined using a 2-way ANOVA Tukey's multiple comparisons test. The mean of four independent experiments with standard deviation are presented.



(b)

Pos	Pos	Neg	Neg	BLANK	BLANK	Bad	Bax	Bcl-2	Bcl-w	BID	BIM	Caspase 3	Caspase 8
Pos	Pos	Neg	Neg	BLANK	BLANK	Bad	Bax	Bcl-2	Bcl-w	BID	BIM	Caspase 3	Caspase 8
CD40	CD40L	clAP-2	cytoC	DR6	Fas	FasL	BLANK	HSP27	HSP60	HSP70	HTRA	IGF-I	IGF-II
CD40	CD40L	clAP-2	cytoC	DR6	Fas	FasL	BLANK	HSP27	HSP60	HSP70	HTRA	IGF-I	IGF-II
IGFBP-1	IGFBP-2	IGFBP-3	IGFBP-4	IGFBP-5	IGFBP-6	IGF-1sR	Survivin	p21	p27	p53	SMAC	Survivin	sTNF-R1
IGFBP-1	IGFBP-2	IGFBP-3	IGFBP-4	IGFBP-5	IGFBP-6	IGF-1sR	Survivin	p21	p27	p53	SMAC	Survivin	sTNF-R1
sTNF-R2	TNF- α	TNF- β	TRAILR-1	TRAILR-2	TRAILR-3	TRAILR-4	XIAP	BLANK	BLANK	Neg	Neg	Neg	Pos
sTNF-R2	TNF- α	TNF- β	TRAILR-1	TRAILR-2	TRAILR-3	TRAILR-4	XIAP	BLANK	BLANK	Neg	Neg	Neg	Pos

Figure S2. Dot blots of 43 apoptosis-associated proteins and controls. **(a)** Representative apoptosis dot blot results of the experiments with isolated B cells in six different conditions (Brest: resting B cells; Bact: activated B cells; Bact with 20 μ M or 2000 μ M ATP, and Bact with 20 μ M or 2000 μ M ATP and TEX). **(b)** Array map for the 43 apoptosis markers and controls.

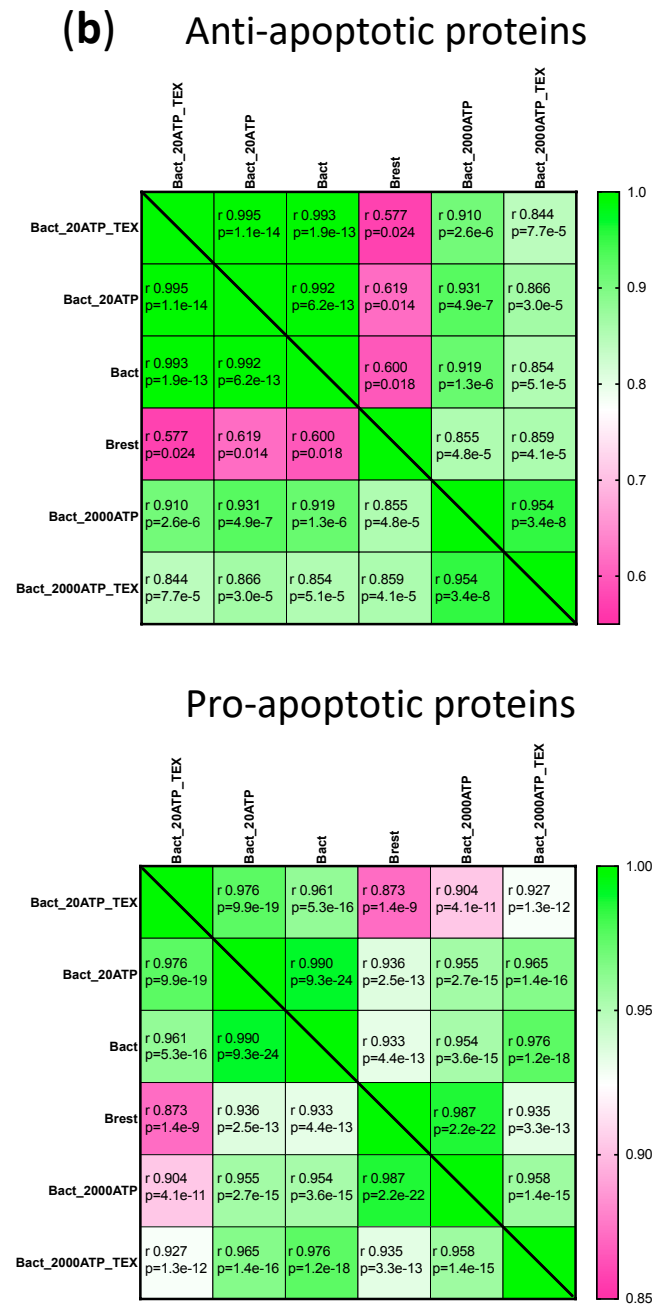
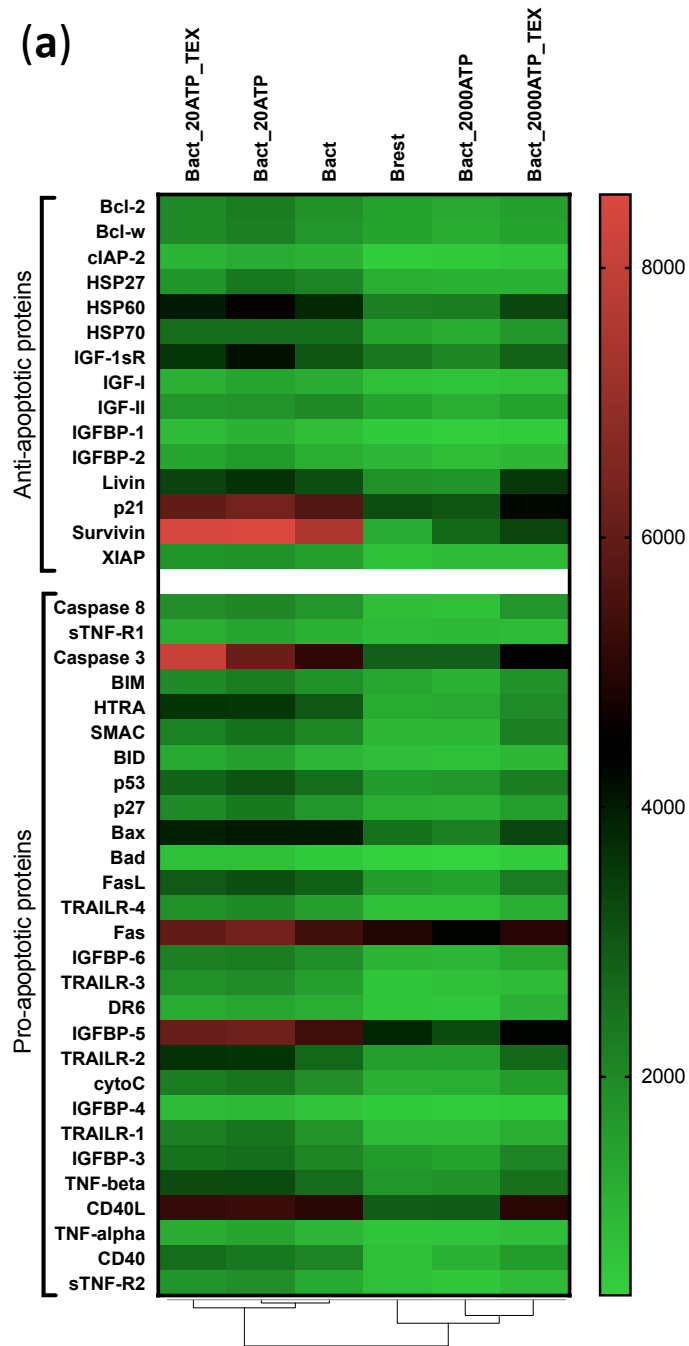


Figure S3. Heatmaps showing the expression of the 43 apoptosis-associated proteins separated as pro- and anti-apoptotic proteins as a mean of four independent experiments in isolated B cells in six different conditions (Brest: resting B cells; Bact: activated B cells; Bact with 20 μ M or 2000 μ M ATP, and Bact with 20 μ M or 2000 μ M ATP and TEX). **(a)** Semi-supervised clustering uncovered 2 groups: Bact and Bact with 20 μ M ATP with or without TEX versus Brest and Bact with 2000 μ M ATP with or without TEX. Distance measuring method: Pearson. Clustering method: Average Linkage. **(b)** Heatmap showing the protein expression as an overall mean. Differences between each group were highly significant. For the anti-apoptotic proteins, the biggest differences were seen between Brest and the Bact or Bact + 20ATP +/- TEX. For the pro-apoptotic proteins, these differences were less pronounced. Instead, the differences were greater between Bact + 20ATP + TEX and Brest or Bact + 2000ATP +/- TEX. The matrix of the anti-apoptotic proteins showed lower r-values than the one of the pro-apoptotic (see legend bar). Pearson correlation matrix with their respective r-values and statistical significances (p-values).

Apoptotic effect of TEX in low ATP concentration

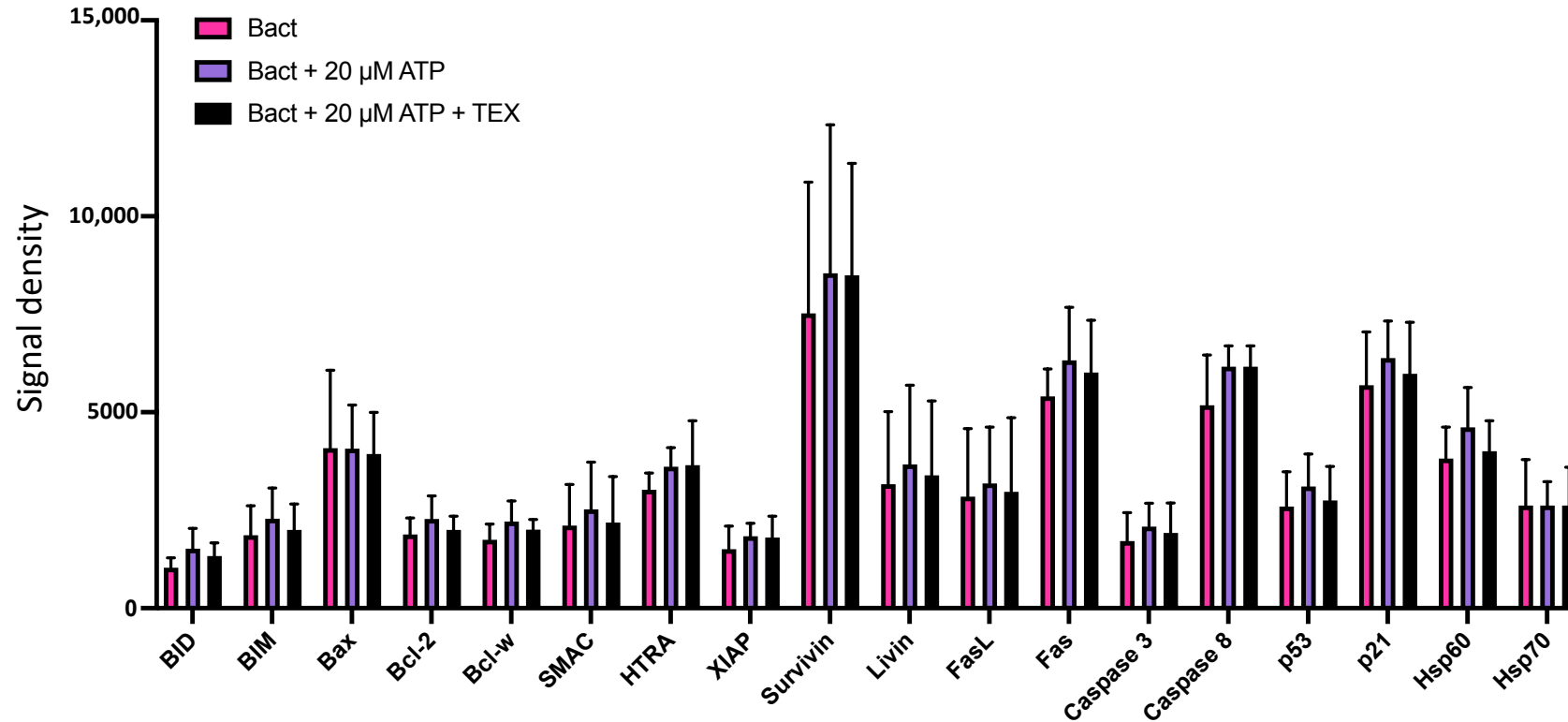
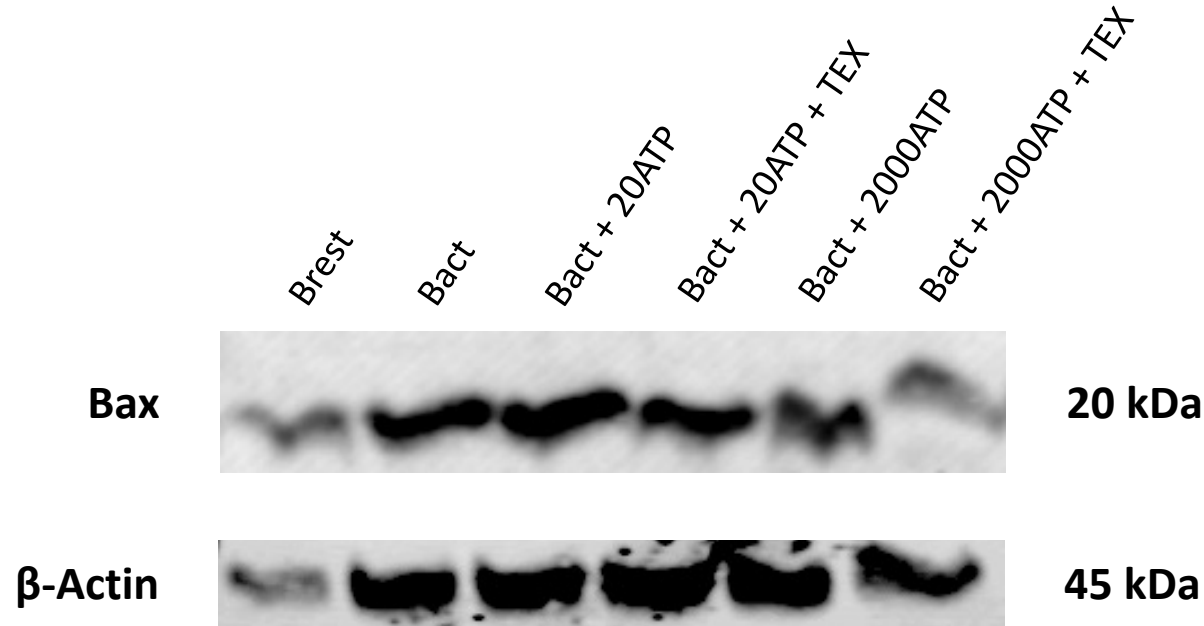


Figure S4. Bar charts comparing the expression of 18 of the pro- and anti-apoptotic proteins shown in figure 6b in activated B cells (Bact), Bact + 20 μ M ATP and Bact + 20 μ M ATP + TEX. No significant changes detected. Significance determined by a 2-way ANOVA Tukey's multiple comparisons test.

(a)



(b)

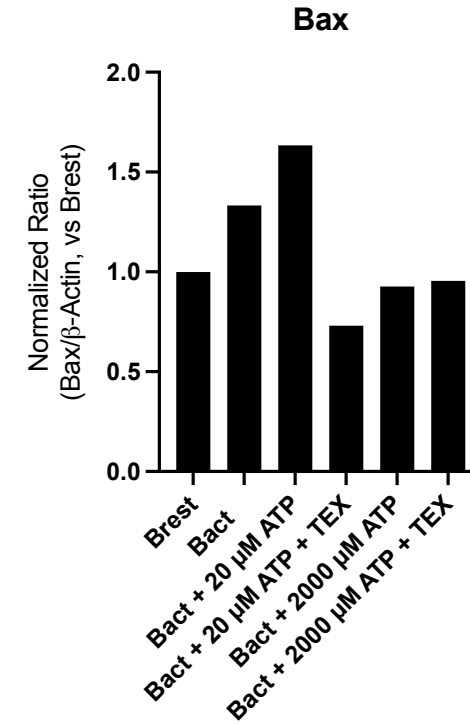


Figure S5. (a) Western Blot analysis of the pro-apoptotic protein Bax in resting B cells (Brest) or activated B cells (Bact), including different culture interventions. **(b)** Quantification of western blot analysis of Bax in Brest or Bact, including different culture interventions. Activation of B cells increased Bax expression. Low amounts of ATP (20 μ M ATP) further increased Bax expression. However, high amounts of ATP (2000 μ M ATP) strongly decreased Bax expression. Whereas TEX addition to low amounts of ATP starkly decreased Bax expression, TEX addition to high amounts of ATP maintained expression levels with a possible minor increase.

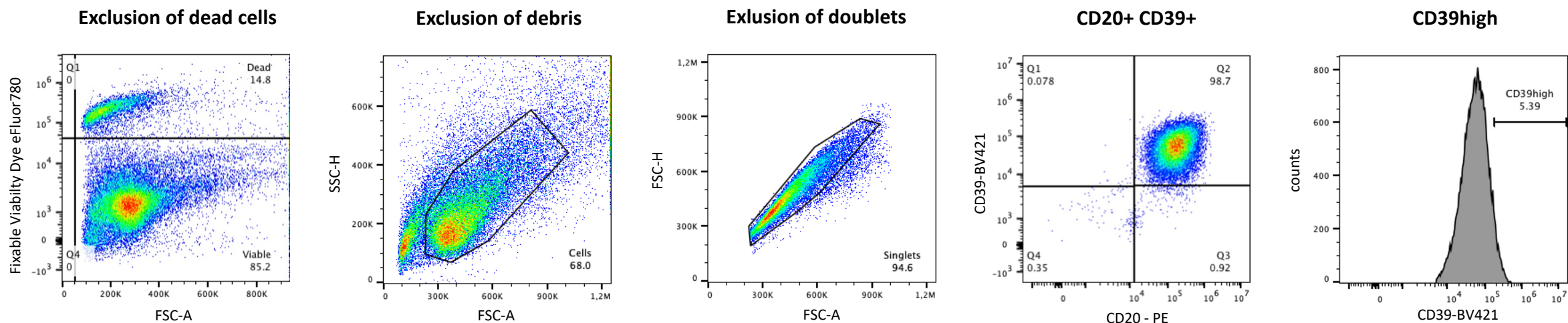


Figure S6. Flow cytometry gating strategy for CD39+ B cells and CD39high B cell subset in anti-CD19 magnetically separated B cells. CD39high B cells were defined as the 5% of CD39+ B cells with the highest fluorescence intensity for CD39 following B cell activation [22].