1 Supplementary Figures and Tables

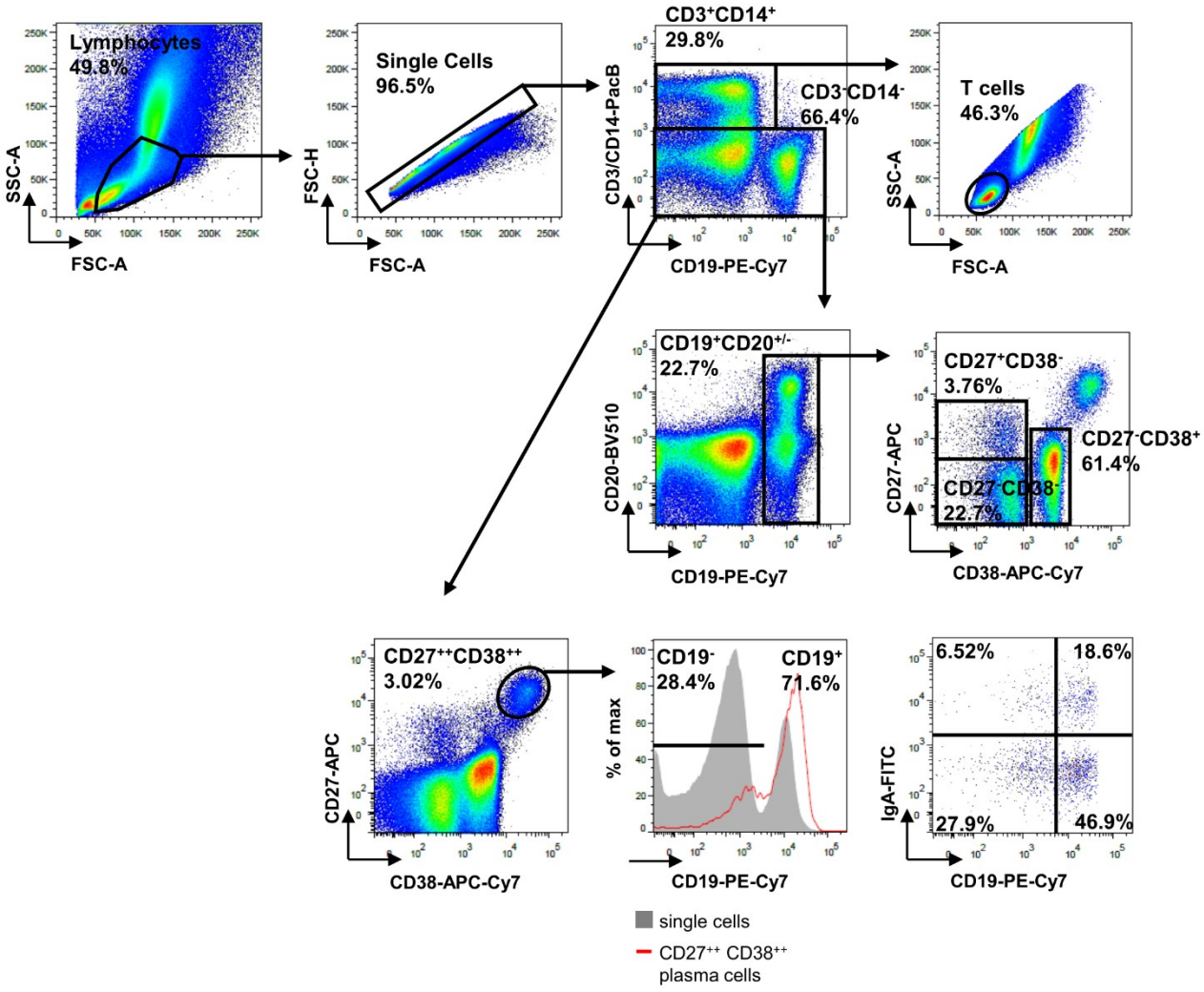

Figure S1. Gating of BMPC and BMBC subsets. After gating on lymphocytes and single cells, $CD3^+$ and $CD14^+$ cells were excluded. In some experiments, T cells were identified by their scatter properties ($SSC\text{-}A^{lo}$ and $FSC\text{-}A^{lo}$) among $CD3^+CD14^+$ cells. BMBC were discriminated among $CD3^-CD14^-$ cells by expression of CD19, in some experiments together with CD20. BC subsets were discriminated by the expression of CD27 and CD38. BMPC were gated by their high expression of CD27 and CD38 out of the $CD3^-CD14^-$ cells and CD19 expression was assessed in a histogram. Arrows indicate sequential gating.

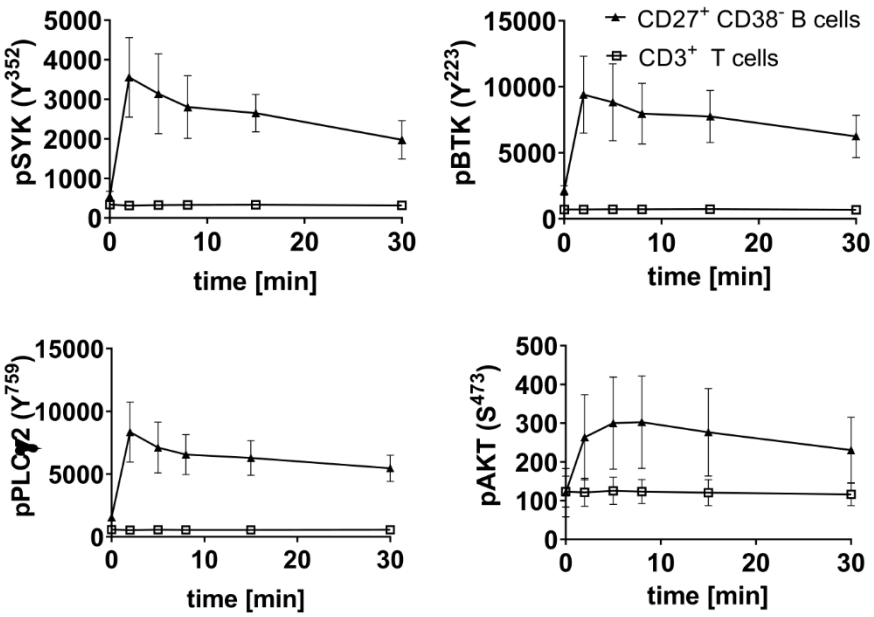

Figure S2. Stimulation of CD19^{+} and CD19^{-} BM MNCs. MNCs isolated from the BM were stimulated with anti-BCR F(ab)$_2$ IgM/IgG/IgA at 37°C and stained intracellularly for the respective phosphosites of different PTKs. The MFI of the respective pPTK is shown for CD27^{+}CD38^{-} BMBC (positive control) and T cells (negative control), respectively. Data are presented as mean±SD.

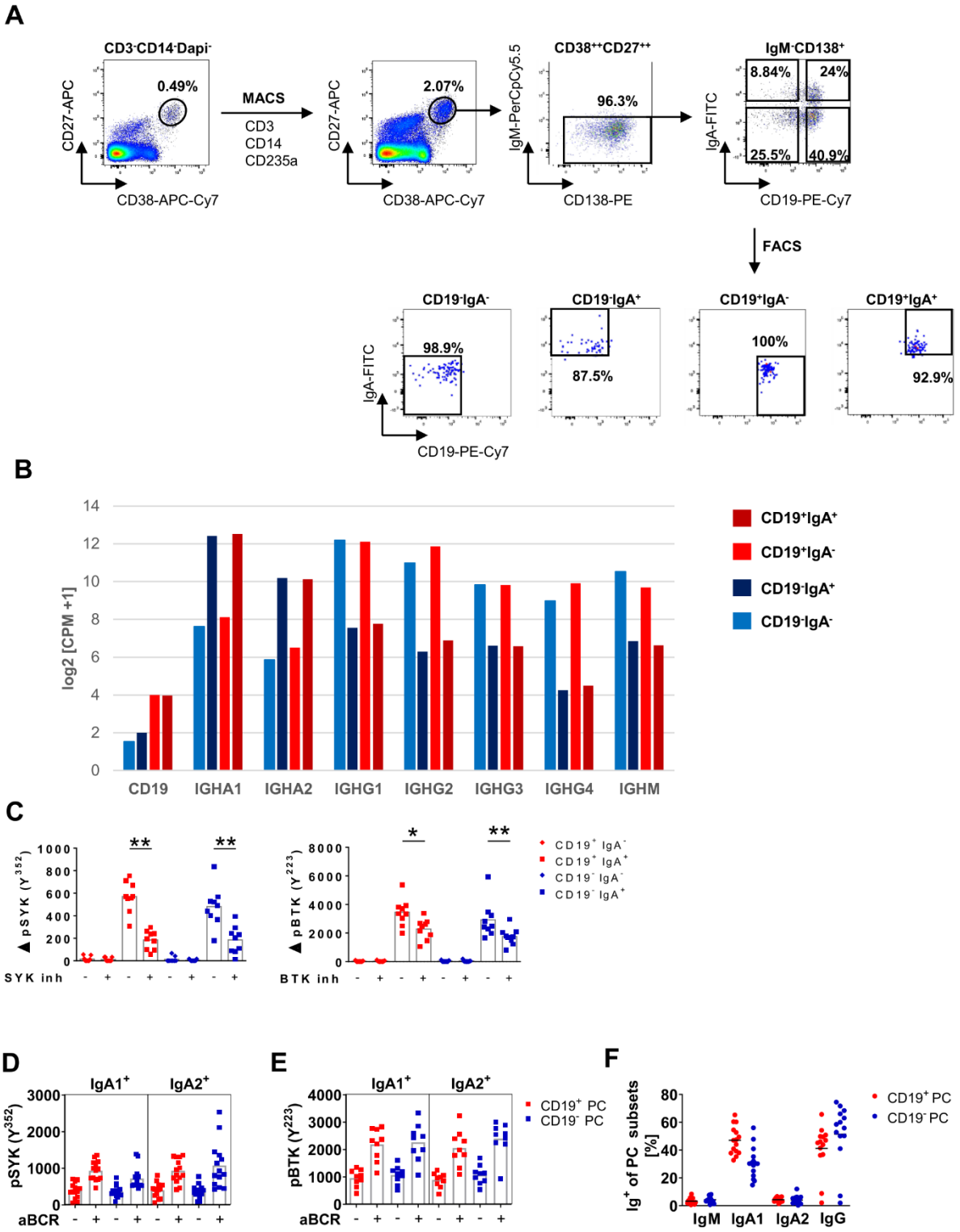

Figure S3. Ig isotype expression determines BCR responsiveness. (**A**) Magnetically sorted CD3$^-$ CD14$^-$CD235a$^-$ cells were subjected to FACS analysis. BMPC subsets were sorted as CD3$^-$CD14$^-$Dapi$^-$ IgM$^-$CD27^{++}CD38^{++}CD19$^+$IgA$^+$/CD19$^+$IgA$^-$/CD19$^-$IgA$^+$/CD19$^-$IgA$^-$ for mRNA expression analysis

(mRNA Seq); purity of sorted populations was checked by flow cytometry. (**B**) Ig isotype expression on mRNA level in both sorted BM samples. For $CD19^{+}IgA^{-}$, only one sample was analyzed. (**C**) BM MNCs were incubated with SYK inhibitor entospletinib (**left**, n=9) or BTK inhibitor acalabrutinib (**right**, n=9) prior to stimulation with anti-IgA (aIgA) and stained for pSYK Y^{352} or pBTK Y^{223} together with CD19 and IgA. MFI of pSYK Y^{352} and pBTK Y^{223} from a stimulated sample minus unstimulated control (ΔMFI) for the BMPC subsets defined by CD19 and IgA, bar indicates mean, **p<0.01, *p<0.05 by Friedman-Test with Dunn‘s correction for multiple comparisons. (**D, E**) BM MNCs were stimulated with 30 µg/ml anti-BCR $F(ab)_2$ IgM/IgG/IgA (aBCR) for 5 min and $IgA1^{+}$ and $IgA2^{+}$ cells among $CD19^{+}$ and $CD19^{-}$ BMPC were analyzed for the MFI of pSYK Y^{352} (**D**, n=14) and pBTK Y^{223} (**E,** n=9). Bar indicates mean. Statistical significance was tested with the Friedman Test and Dunn’s correction for multiple comparisons, p>0.05. (**F**) Frequency of IgM-, IgA1-, IgA2- and IgG-expressing cells among $CD19^{+}$ and $CD19^{-}$ BMPC as determined by intracellular staining. n=15. Statistical significance was tested with the Friedman Test and Dunn’s correction for multiple comparisons, p>0.05.

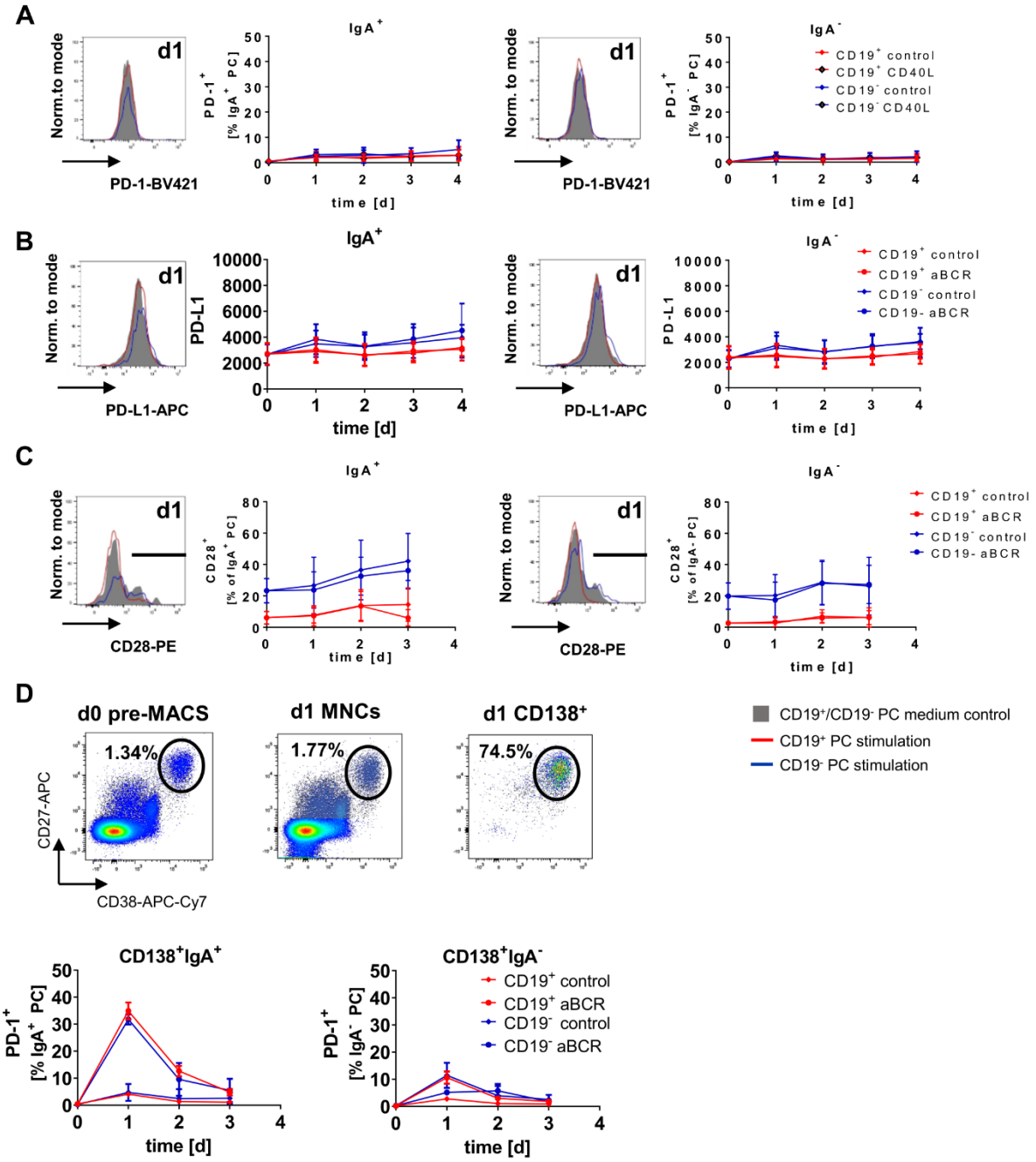

Figure S4. Stimulation of the BCR does not change the expression of PD-L1, BTLA and CD28 in BMPC. BM MNCs were cultured for up to four days with either medium (control), 1 µg/ml anti-BCR F(ab)$_2$ IgM/IgG/IgA (aBCR) or 0.5 µg/ml CD40L. Data are presented as mean±SD. (**A**) Representative histogram (d1 control and CD40L) of PD-1 and cumulative PD-1 data of IgA$^+$ (**left**) and IgA$^-$ (**right**) CD19$^{+/-}$ BMPC after CD40L stimulation. n=4-6 for each timepoint. (**B, C**) Representative histogram (d1 control and aBCR) of PD-L1 (MFI, **B**) and CD28$^+$ cells (frequency, **C**) and cumulative data of

IgA^+ and IgA^- $CD19^{+/-}$ BMPC after BCR stimulation. n=3-16 for each timepoint. (**D**) Dotplots showing CD27 and CD38 expression of living $CD3^-CD14^-$ cells in MNCs on d0, in MNCs on d1 after incubation with medium and in CD138-enriched BMPC at d1 after incubation with medium (top). CD138-enriched BMPC were cultured for up to three days with medium alone or 1 µg/ml aBCR IgM/IgG/IgA (bottom).

Table S1. Antibodies used for flow-cytometry and sorting. PacB: Pacific Blue, BUV: Brilliant UV, PE: Phycoerythrin, PE-Cy7: PE-Cyanine 7, APC: Allophycocyanine, APC-Cy7: APC-Cyanine 7, FITC: Fluorescein isothiocyanate, BV: Brilliant Violet, PerCPCy5.5: Peridinin-chlorophyll protein cyanine 5.5, AF: Alexa Fluor.

target of antibody	clone	fluorochrome	manufacturer	RRID
CD3	UCHT1	PacB, BUV395	BD Biosciences, Heidelberg, Germany	AB_397038, AB_2744387
CD14	M5E2	PacB, BUV395	BD Biosciences, Heidelberg, Germany	AB_397041, AB_2740025
CD19	SJ25C1	PE-Cy7, APC-H7, BV711	BD Biosciences, Heidelberg, Germany	AB_396893, AB_1645728, AB_2737970
	HIB19	PE-Cy7	ThermoFisher Scientific, Waltham, USA	AB_1582278
CD20	HI47	PacO	Invitrogen ThermoFisher Scientific, Waltham, USA	AB_1500441
	H1FB1	PerCPCy5.5	BD Biosciences, Heidelberg, Germany	AB_396990
	2H7	BV510	Biolegend, San Diego, USA	AB_2561941
CD27	L128	APC, BV786	BD Biosciences, Heidelberg, Germany	AB_647368, AB_2744353
CD38	HIT2	APC-Cy7, APC, PE	BD Biosciences, Heidelberg, Germany	AB_2561605, AB_398599, AB_395853
CD138	B-B4	PE	Miltenyi Biotech, Bergisch Gladbach, Germany	AB_244212
	MI15	BUV737	BD Biosciences, Heidelberg, Germany	AB_2738786
SYK	2D10	FITC	BD Biosciences, Heidelberg, Germany	AB_394399
	4D10.2	PE	Biolegend, San Diego, USA	AB_2565304
pSYK Y^{352}	17A/P-ZAP70	PE, AF647	BD Biosciences, Heidelberg, Germany	AB_396919, AB_396884
PLC-γ2	K86-1161	PE	BD Biosciences, Heidelberg, Germany	AB_1645527
PLC-γ2 Y^{759}	K86-689.37	PE, AF647	BD Biosciences, Heidelberg, Germany	AB_647226, AB_647139
BTK	53/Btk	PE	BD Biosciences, Heidelberg, Germany	AB_647183

pBTK Y^{223}	N35-86	AF488, PE	BD Biosciences, Heidelberg, Germany	AB_2738981, AB_2721028
panAKT	REA676	PE	Miltenyi Biotech, Bergisch Gladbach, Germany	AB_2651206
pAKT S^{473}	M89-61	PE	BD Biosciences, Heidelberg, Germany	AB_1645328
	D9E	APC	Cell Signaling, Cambridge, UK	AB_2797780
IgA	M24A	FITC	Chemicon ThermoFisher Scientific, Waltham, USA	AB_92852
IgA1	B3506B4	FITC	Southern Biotech, Birmingham, USA	AB_2796652
IgA2	IS11-21E11	PE	Miltenyi Biotech, Bergisch Gladbach, Germany	AB_1036168
IgG	G18-145	FITC, PE-Cy7	BD Biosciences, Heidelberg, Germany	AB_396121, AB_10611712
IgM	G20-127	PerCPCy5.5, BV421	BD Biosciences, Heidelberg, Germany	AB_10611998, AB_2737681
PD-1	EH12.1	BV421	BD Biosciences, Heidelberg, Germany	AB_11153482
PD-L1	29E.2A3	APC	Biolegend, San Diego, USA	AB_940360
BTLA	MIH26	PE	Biolegend, San Diego, USA	AB_2043945
LAG-3	3DS223H	APC	ThermoFisher Scientific, Waltham, USA	AB_2573186
TIM-3	F38-2E2	PE	Biolegend, San Diego, USA	AB_2116576