

Supplemental Data

B cell c-Maf signaling promotes tumor progression in tumor models of pancreatic cancer and melanoma

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Supplemental Table 1. Antibodies used in Flow cytometry and Western blot

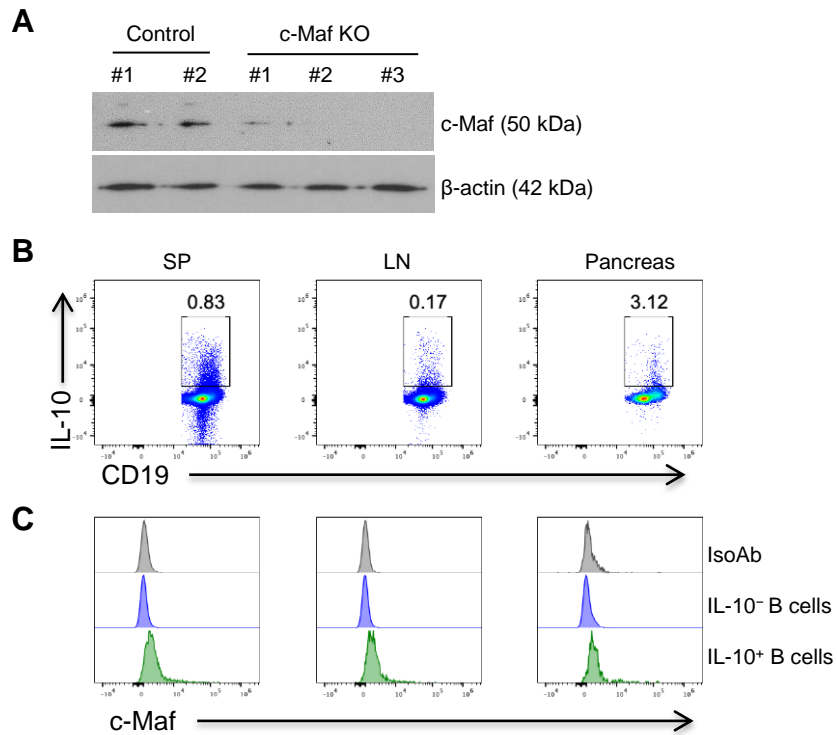
Antibodies	Company and catalog number
PerCP/Cyanine5.5 anti-mouse CD45 (clone S18009F)	BioLegend Cat # 157208
PE/Cyanine7 anti-mouse CD45 (clone S18009F)	BioLegend Cat # 157206
PerCP/Cyanine5.5 anti-mouse IgM (clone RMM-1)	BioLegend Cat # 406512
FITC anti-mouse CD21/CD35 (clone 7E9)	BioLegend Cat # 123408
APC anti-mouse CD19 (clone 6D5)	BioLegend Cat # 115512
PE anti-mouse IgM (clone RMM-1)	BioLegend Cat # 406508
PerCP/Cyanine5.5 anti-mouse IgD (clone 11-26c.2a)	BioLegend Cat # 405709
PE/Cyanine7 anti-mouse CD5 (clone 53-7.3)	BioLegend Cat # 100621
FITC Anti-Mouse CD43 (clone S7)	BD Bioscience Cat # 553270
APC anti-mouse CD4 (clone GK1.5)	BioLegend Cat # 100412
FITC anti-mouse CD8a (clone 53-6.7)	BioLegend Cat # 100706
APC anti-mouse CD19 (clone 6D5)	BioLegend Cat # 115512
PE anti-mouse IL-10 (clone JES5-16E3)	BioLegend Cat # 505007
PE anti-mouse IFN- γ (clone XMG1.2)	BioLegend Cat # 505808
PE anti-mouse IgG1 (clone RMG1-1)	BioLegend Cat # 406608
PE anti-mouse IgG2a (clone RMG2a-62)	BioLegend Cat # 407108
PE anti-mouse IgG2b (clone RMG2b-1)	BioLegend Cat # 406708
PE anti-human CD19 (clone HIB19)	BioLegend Cat # 302208
PerCP/Cyanine5.5 anti-human IgD (clone IA6-2)	BioLegend Cat # 348208
PE/Cyanine7 anti-mouse/rat/human CD27 (clone LG.3A10)	BioLegend Cat # 124215
APC anti-human IL-10 (clone JES3-19F1)	BioLegend Cat # 506807
Alexa Fluor 700 anti-human CD11c (clone Bu15)	BioLegend Cat # 337220
PerCP-eFluor™ 710 c-MAF monoclonal antibody (sym0F1)	Invitrogen Cat # 46-9855-42
PE c-MAF monoclonal antibody (sym0F1)	Invitrogen Cat # 12-9855-42
c-Maf Antibody (M-153) for Western blotting	Santa Cruz Cat # sc-7866
Fixable Viability Dye eFluor™ 780	Thermo Fisher Scientific Cat # 65-0865-14

Supplemental Table 2. Anti-mouse antibodies for CyTOF

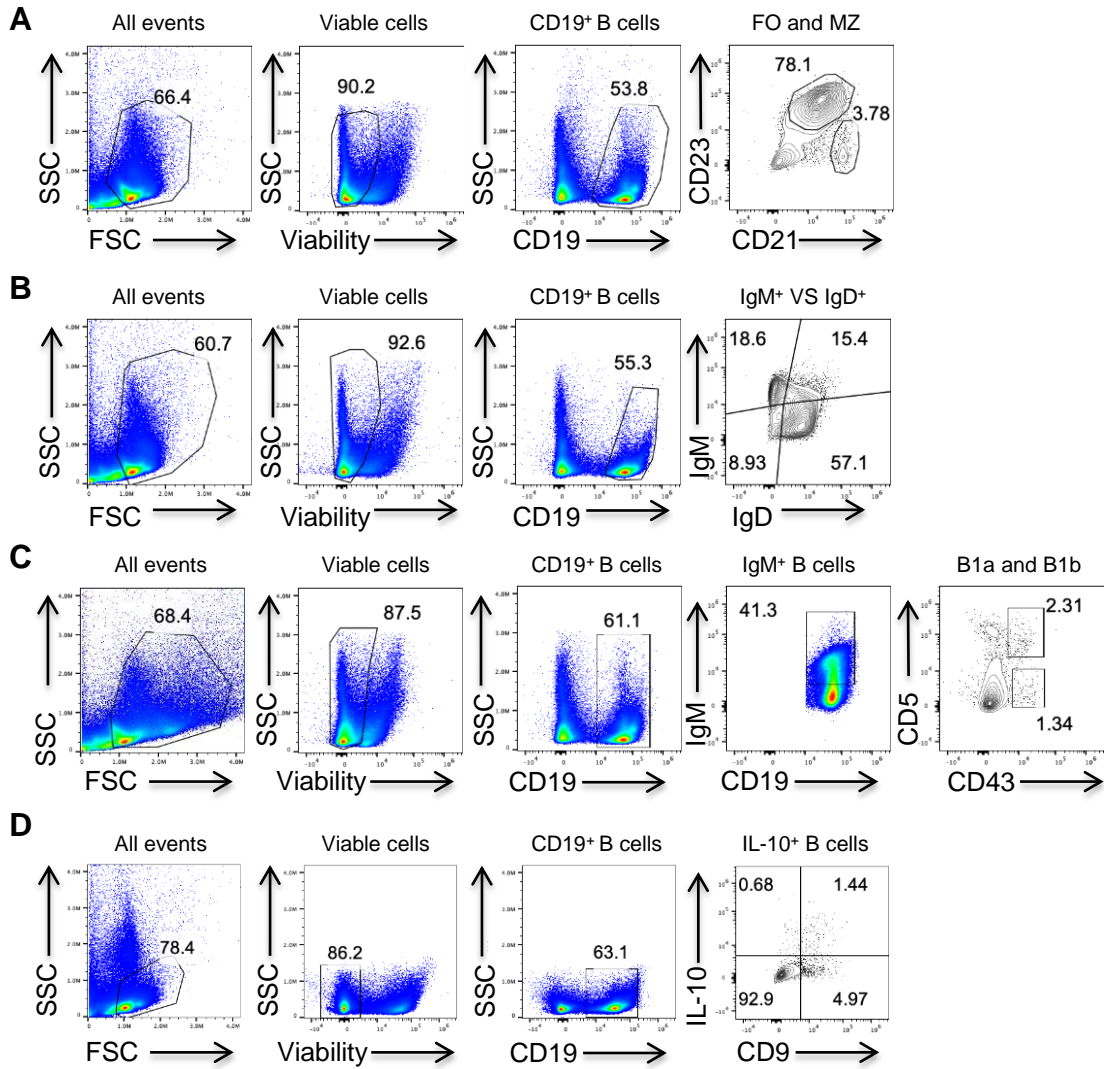
	Tag	Target	Clone	Company and catalog number
1	89Y	CD45	30-F11	Fluidigm Cat # 3089005B
2	141Pr	TNF- α	MP6-XT22	Fluidigm Cat # 3141013B
3	142Nd	CD11c	N418	Fluidigm Cat # 3142003B
4	143Nd	CD69	H1.2F3	Fluidigm Cat # 3143004B
5	144Nd	IL-2	JES6-5H4	Fluidigm Cat # 3144002B
6	145Nd	CD4	RM4-5	Fluidigm Cat # 3145002B
7	146Nd	F4/80	BM8	Fluidigm Cat # 3146008B
8	148Nd	CD103	2E7	BioLegend Cat # 121402
9	149Sm	CD19	6D5	Fluidigm Cat # 3149002B
10	150Nd	Ly-6C	NK1.4	Fluidigm Cat # 3150010B
11	151Eu	CD25	3C7	Fluidigm Cat # 3151007B
12	152Sm	CD3e	145-2C11	Fluidigm Cat # 3152004B
13	153Eu	CD274/PD-L1	MIH5	Fluidigm Cat # 3153031B
14	155Gd	IL-10	JES5-16E3	BioLegend Cat # 505029
15	156Gd	CCR2	475301R	R&D System Cat # MAB55381R
16	158Gd	Foxp3	FJK-16s	Fluidigm Cat # 3158003A
17	159Tb	PD-1	29F.1A12	Fluidigm Cat # 3159024B
18	160Gd	CD62L	MEL-14	Fluidigm Cat # 3160008B
19	161Dy	iNOS	CXNFT	Fluidigm Cat # 3161011B
20	162Dy	CD44	IM7	Fluidigm Cat # 3162030B
21	164Dy	CX3CR1	SA011F11	Fluidigm Cat # 3164023B
22	165Ho	IFN- γ	XMG1.2	Fluidigm Cat # 3165003B
23	167Er	IL-6	MP5-20F3	Fluidigm Cat # 3167003B
24	168Er	CD8a	53-6.7	Fluidigm Cat # 3168003B
25	169Tm	CD206	C068C2	Fluidigm Cat # 3169021B
26	170Er	NK1.1	PK136	Fluidigm Cat # 3170002B
27	172Yb	CD11b	M1/70	Fluidigm Cat # 3172012B
28	174Yb	CD223/LAG3	C9B7W	Fluidigm Cat # 3174019B
29	175Yb	CD127/IL7Ra	A7R34	Fluidigm Cat # 3175006B
30	176Yb	CD45R/B220	RA3-6B2	Fluidigm Cat # 3176002B
31	209Bi	I-A/I-E	M5/114.15.2	Fluidigm Cat # 3209006B

Supplemental Table 3. Anti-human antibodies for CyTOF

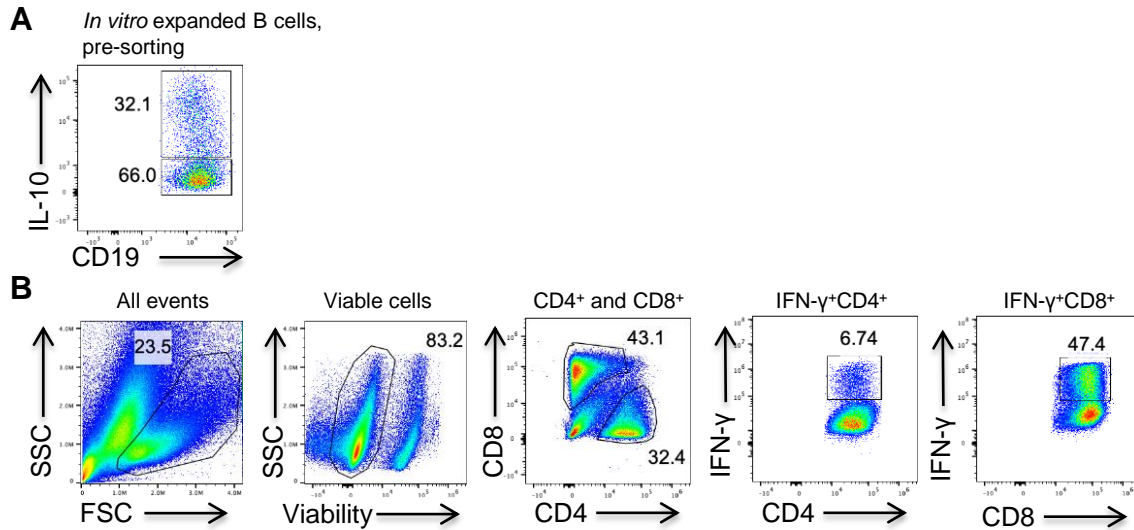
	Tag	Target	Clone	Company and catalog number
1	89Y	CD45	HI30	Fluidigm Cat # 3089003B
2	114Cd	CD20	2H7	BioLegend Cat # 302343
3	116Cd	CD19	HIB19	BioLegend Cat # 302247
4	141Pr	CD196/CCR6	G034E4(11A9)	Fluidigm Cat # 3141003A
5	142Nd	CD40	5C3	Fluidigm Cat # 3142010B
6	143Nd	CD123	6H6	Fluidigm Cat # 3143014B
7	144Nd	CD69	FN50	Fluidigm Cat # 3144018B
8	146Nd	IgD	IA6-2	Fluidigm Cat # 3146005B
9	147Sm	CD11c	Bu15	Fluidigm Cat # 3147008B
10	149Sm	CD45RO	UCHL1	Fluidigm Cat # 3149001B
11	152Sm	CD21	BL13	Fluidigm Cat # 3152010B
12	154Sm	TIM-3	F38-2E2	Fluidigm Cat # 3154010B
13	156Gd	CD86	IT2.2	Fluidigm Cat # 3156008B
14	158Gd	CD284	HTA125	Fluidigm Cat # 3158024B
15	159Tb	CD197/CCR7	G043H7	Fluidigm Cat # 3159003A
16	161Dy	CD80	2D10.4	Fluidigm Cat # 3161023B
17	162Dy	CD79b	CB3-1	Fluidigm Cat # 3162008B
18	163Dy	CXCR3	G025H7	Fluidigm Cat # 3163004B
19	164Dy	CXCR5	RF8B2	Fluidigm Cat # 3164029B
20	165Ho	CD45RA	HI100	BioLegend Cat # 304143
21	167Er	CD27	L128	Fluidigm Cat # 3167006B
22	169Tm	CD25	2A3	Fluidigm Cat # 3169003B
23	172Yb	CD38	HIT2	Fluidigm Cat # 3172007B
24	173Yb	HLA-Dr	L243	Fluidigm Cat # 3173005B
25	174Yb	CD279/PD1	EH12.2H7	Fluidigm Cat # 3174020B
26	175Lu	CD274/PD-L1	29E.2A3	Fluidigm Cat # 3175017B
27	176Yb	c-Maf	Polyclone	Thermo Fisher Cat # PA5-23179



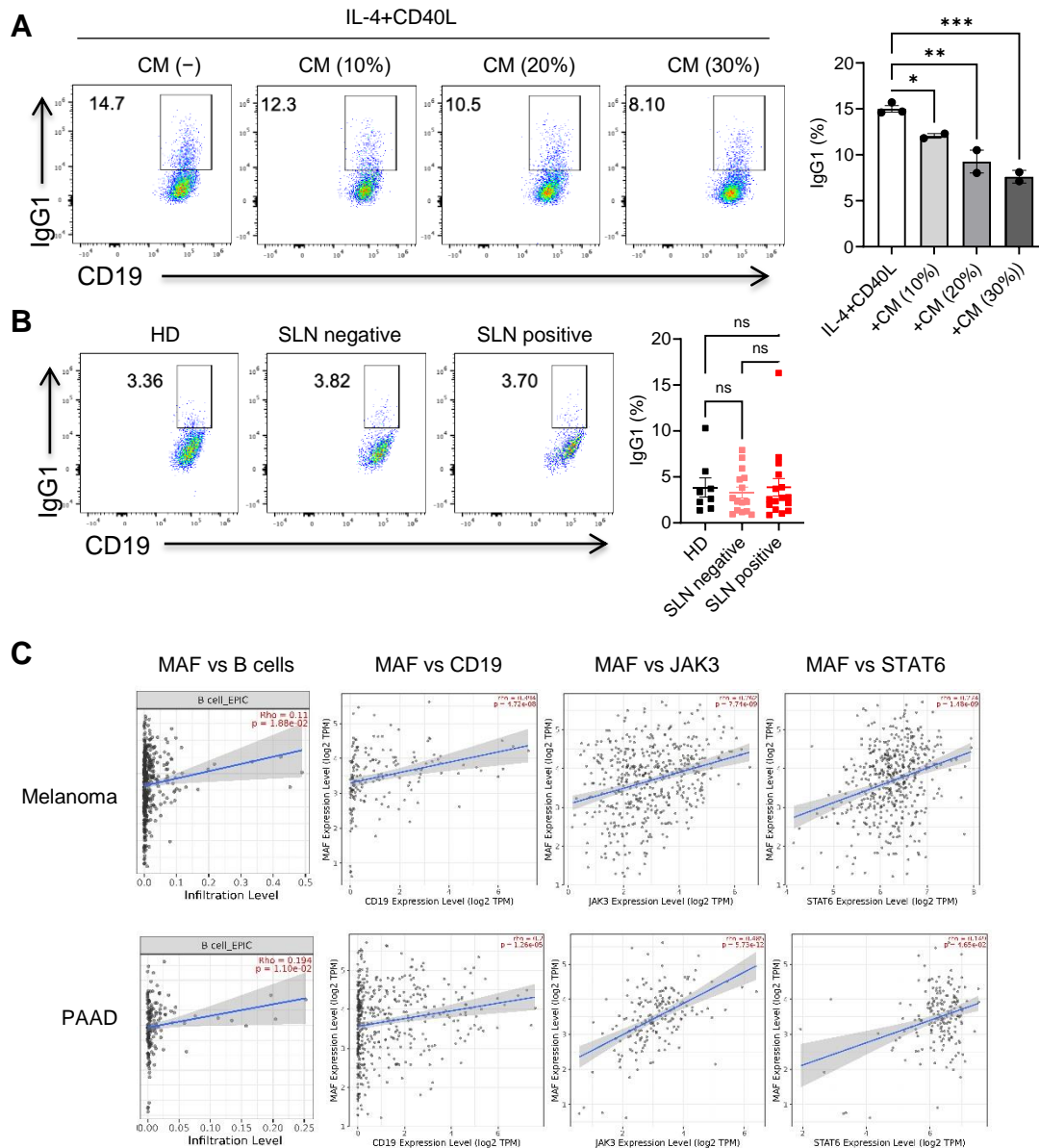
Supplemental Figure 1. (A) CD9⁺CD19⁺ B cells were sorted from spleen of control (n=2) and conditional B cell c-Maf KO mice (n=3). c-Maf expression was determined by using Western blotting. (B) Spleen, lymph node, and pancreas were collected from naïve IL-10^{GFP} reporter mice, and IL-10 expression was determined by measuring GFP fluorescence. (C) c-Maf expression in naïve IL-10⁻ and IL-10⁺ B cells was determined by intracellular staining and Flow cytometry.



Supplemental Figure 2. Gating strategy used (A) for identification of follicular B cells (FO) and marginal zone (MZ) B cells; (B) for identification of IgM⁺ and IgD⁺ B cells; (C) for identification of B1a and B1b; (D) for identification of CD9⁺IL-10⁺ B cells in figure 1.



Supplemental Figure 3. Gating strategy used for IL-10⁺ B cell sorting and coculture with CD4, CD8 T cells. (A) Splenocytes of IL-10^{9fp} reporter mice were cultured with CD40L- and BAFF-expressing feeder cells (CD40LB) in the presence of IL-4 for 4 days followed by additional 3 days culture in the presence of IL-21. IL-10 positive versus negative was determined by measuring GFP fluorescence in the *in vitro* expanded B cells. (B) Gating strategy of co-culture of B cells and T cells. IL-10⁺ and IL-10⁻ B cells were sorted and cocultured with anti-CD3 mAb activated CD4⁺ and CD8⁺ T cells for 3 days. The IFN- γ production by CD4⁺ and CD8⁺ T cells was evaluated by intracellular cytokine staining and Flow cytometry.



Supplemental Figure 4. (A) PBMCs from healthy donors were cultured in the presence of IL-4 and recombinant CD40L with or without A375 conditioned medium for 3 days. IgG1 positive B cells were determined by using Flow cytometry. (B) PBMCs from healthy donors, SLN negative and SLN positive melanoma patients were cultured in the presence of IL-4 and recombinant CD40L for 3 days. IgG1 positive B cells were determined by using Flow cytometry. Summarized data were shown. Each dot represents one person. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ by Ordinary one-way ANOVA test. (C) The correlation of MAF and B cell infiltration as well as CD19, JAK3, STAT6 gene expression in melanoma and PAAD patients from The Cancer Genome Atlas (TCGA) using publicly available web platform TIMER2.0.