

## **Appendix**

### **The transcriptional coactivator Bob1 promotes the development of follicular T helper cells via Bcl6**

Dennis Stauss, Cornelia Brunner, Friederike Berberich-Siebelt, Uta E. Höpken,  
Martin Lipp and Gerd Müller

#### **Appendix Inventory**

##### **1. Supplemental Figures and Tables**

**Appendix Figure S1:    Sorting of murine CD4<sup>+</sup> T cell populations**

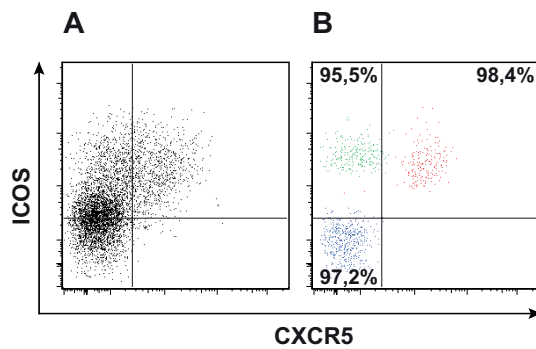
##### **2. Supplemental Experimental Procedures**

**Appendix Table S1:    Antibodies for flow cytometry,  
                                 immunohistology and ChIP**

**Appendix Table S2:    EMSA oligonucleotides**

**Appendix Table S3:    PCR primers for ChIP experiments**

## Appendix Figure S1 Sorting of murine CD4<sup>+</sup> T cell populations



Splenic CD4<sup>+</sup> T cells were enriched by magnetic cell sorting and subsequently sorted by FACS for CXCR5<sup>-</sup>ICOS<sup>-</sup> naïve CD4<sup>+</sup> T cells, CXCR5<sup>-</sup>ICOS<sup>+</sup> activated CD4<sup>+</sup> T cells and CXCR5<sup>hi</sup>ICOS<sup>+</sup> follicular helper CD4<sup>+</sup> T cells (Tfh cells). All CD4<sup>+</sup> T cell populations were sorted to >95% purity. Representative dot plots showing (A) unsorted CD4<sup>+</sup> T cells and (B) an overlay of the three sorted cell populations.

**Appendix Table S1: Antibodies**

Antigen	Fluorochrome	Clone	Isotype	Application	Company
B220	Horizont V500	RA3-6B2	Rat IgG2a	FACS	BD
B220	Pacific Blue	RA3-6B2	Rat IgG2a	FACS	Biolegend
B220	APC	RA3-6B2	Rat IgG2a	FACS	Biolegend
Bcl6	PE	K112-91	Mouse IgG1	FACS	BD
BTLA	PE	8F4	Mouse IgG1	FACS	eBioscience
BTLA	Alexa Fluor 647	8F4	Mouse IgG1	FACS	eBioscience
CD3	Horizont V500	500A2	Hamster IgG2	FACS	BD
CD4	PE	GK1.5	Rat IgG2b	FACS	BD
CD4	Horizont V500	RM4-5	Rat IgG2a	FACS	BD
CD4	Pacific Blue	GK1.5	Rat IgG2b	FACS	Biolegend
CD4	FITC	GK1.5	Rat IgG2b	FACS	Biolegend
CD4	APC	GK1.5	Rat IgG2b	FACS	Biolegend
CD45.2	Pacific Blue	104	Mouse IgG2a	FACS	Biolegend
CD45.2	APC-Cy7	104	Mouse IgG2a	FACS	Biolegend
CD90.2	PE-Cy7	30-H12	Rat IgG2b	FACS	Biolegend
CD90.2	APC	30-H12	Rat IgG2b	FACS	Biolegend
CD90.2	PerCP	30-H12	Rat IgG2b	FACS	Biolegend
CXCR5	BV 421	L138D7	Rat IgG2b	FACS	Biolegend
CXCR5	-	2G8	Rat IgG2a	FACS	BD
CXCR5	APC	2G8	Rat IgG2a	FACS	BD
FAS	Alexa Fluor 488	15A7	Mouse IgG1	FACS	eBioscience
GL7	PE	GL7	Rat IgM	FACS	BD
GL7	Alexa Fluor 647	GL7	Rat IgM	FACS	BD
ICOS	PE	7E.17G9	Rat IgG2b	FACS	Biolegend
ICOS	Alexa Fluor 488	C398.4A	Hamster IgG	FACS	Biolegend
IL21R	PE	4A9	Rat IgG2a	FACS	Biolegend
IL6R	PE	D7715A7	Rat IgG2b	FACS	Biolegend
PD1	PE	RMP1-30	Rat IgG2b	FACS	Biolegend
PD1	PE-Cy7	RMP1-30	Rat IgG2b	FACS	Biolegend
PD1	FITC	29F.1A12	Rat IgG2a	FACS	Biolegend
CD4	BV 421	GK1.5	Rat IgG2b	Histology	Biolegend
GL7	-	GL7	Rat IgM	Histology	eBioscience
IgD	FITC	11-26C.2A	Rat IgG2a	Histology	BD
CD3	-	145-2C11	Hamster IgG	<i>in vitro</i> activ.	Biolegend
CD28	-	37.51	Hamster IgG	<i>in vitro</i> activ.	Biolegend
Bob11	-	sc-955	Rabbit	ChIP	Santa Cruz
Oct1	-	sc-232	Rabbit	ChIP	Santa Cruz
Oct2	-	sc-233	Rabbit	ChIP	Santa Cruz

Secondary Antibodies	Fluorochrome	Application	Company
Goat anti-Rat IgG (H+L)	Alexa Fluor 568	Histology	Life Technologies
Mouse anti-Rat IgG	Alexa Fluor 488	FACS	Jackson ImmunoResearch
Mouse anti-Rat IgG	Alexa Fluor 647	FACS	Jackson ImmunoResearch

**Appendix Table S2: EMSA oligonucleotides**

	forward	reverse
BTLA M1	<u>ggcgTGTGAAGATAAATTTGCATT</u> <u>TTCCCAAATT</u>	ggcgAATTTGGGAAAATGCAAATT TATCTTCACA
BTLA M2	<u>ggcgTGGCCAATGGCATTGCAA</u> <u>CTGTGCAGGGA</u>	ggcgTCCCTGCACAGTTGCAAAT GCCATTGGCCA
BTLA M3	<u>ggcgAAAAAAAAAATAAAAGTAA</u> <u>GGGGCAGAAT</u>	ggcgATTCTGCCCTTACTTTTAT TTTTTTTTTT
BTLA M4	<u>ggcgCCTACATAAACATTTACATA</u> <u>CACCCACACA</u>	ggcgTGTGTGGGTGTATGTAAAT GTTTATGTAGG
Bcl6 M1	<u>ggcgAATCCAAATCCTTTTACATC</u> <u>TCTTTCAAGT</u>	ggcgACTTGAAAGAGATGTAAAA GGATTGATT
Bcl6 M2	<u>ggcgCAGGAAAGCTTCAGCAAAA</u> <u>GTCCAAGAGGA</u>	ggcgTCCTCTTGGACTTTTGCTGA AGCTTTCCTG
Bcl6 M3	<u>ggcgCAGTCCCGACGATTTACAT</u> <u>AACACCACAAA</u>	ggcgTTTGTGGTGTTATGTAAATC GTCGGGACTG
Bcl6 M4	<u>ggcgACACCACAACTTGCAAAA</u> <u>GGCAAAAATCA</u>	ggcgTGATTTTGCCTTTTGCAAG TTTGTGGTGT
Bcl6 M5	<u>ggcgAGACCCCGGGAAAGCAAA</u> <u>GCGCACTCTCCC</u>	ggcgGGGAGAGTGCGCTTTGCTT TCCCGGGGTCT
Bcl6 M6	<u>ggcgATGTCACCGAATAGCAAAT</u> <u>TAGTTCTCAGA</u>	ggcgTCTGAGAACTAATTTGCTAT TCGGTGACAT
Oct cons	gcggACCTGGGTAATTTGCATTTC TAAAAT	gcggATTTTAGAAATGCAAATTAC CCAGGT
NF- kB	gcggGCCTGGGAAAGTCCCCTCA ACT	gcggAGTTGAGGGGACTTTCCCA GGC

**Appendix Table S3: PCR primers used in ChIP experiments**

	forward	reverse
intergen_Ch8	AAGGGGCCTCTGCTTAAA AA	AGAGCTCCATGGCAGGTAG A
IFN- $\gamma$ prom M5	CACAAGAATGGCACAGGT GGGCAC	GATCGAAGGCTCCTCGGGA TTACG
BTLA prom M1	GCTGCTCTTCTGGTCACC TGG	GTCCATTGCCAGTTTCATGT TGAATGAC
BTLA prom M4	CCAGCAGAAAGCACACAG CATGTTC	CCACCCATCCTAAAGCTCGA GATATC
Bcl6 M1+M2	GGGGAATGTTCAAGACCT TAAGAAAATAGC	CTTTTGCTGAAGCTTTCCTG CACT
Bcl6 M3+M4	CAACGAATGACAGTCCCG ACG	GGCTTGGGATGCTCCTGTT G
Bcl6 M5+M6	GCTCGAGGAGCCGAGTTT ATGGG	GTCTTCGCTGTAGCAAAGCT CGGC