

Supplementary Material

Blimp-1 Contributes to the Development and Function of Regulatory B Cells

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SUPPLEMENTARY FIGURE 1. Proliferation and apoptosis rates in Ctrl and Cko B10

cells after stimulation.

SUPPLEMENTARY FIGURE 2. Gross pathology of kidneys from Ctrl and Cko mice infected with *C. albicans*.

SUPPLEMENTARY FIGURE 3. B10 cells differentiated into CD138⁺ plasmablasts after LPS stimulation.

SUPPLEMENTARY FIGURE 4. Adoptive transfer of serum from *C. albicans* infected mice reduced the mortality caused by *C. albicans* infection in Cko mice.

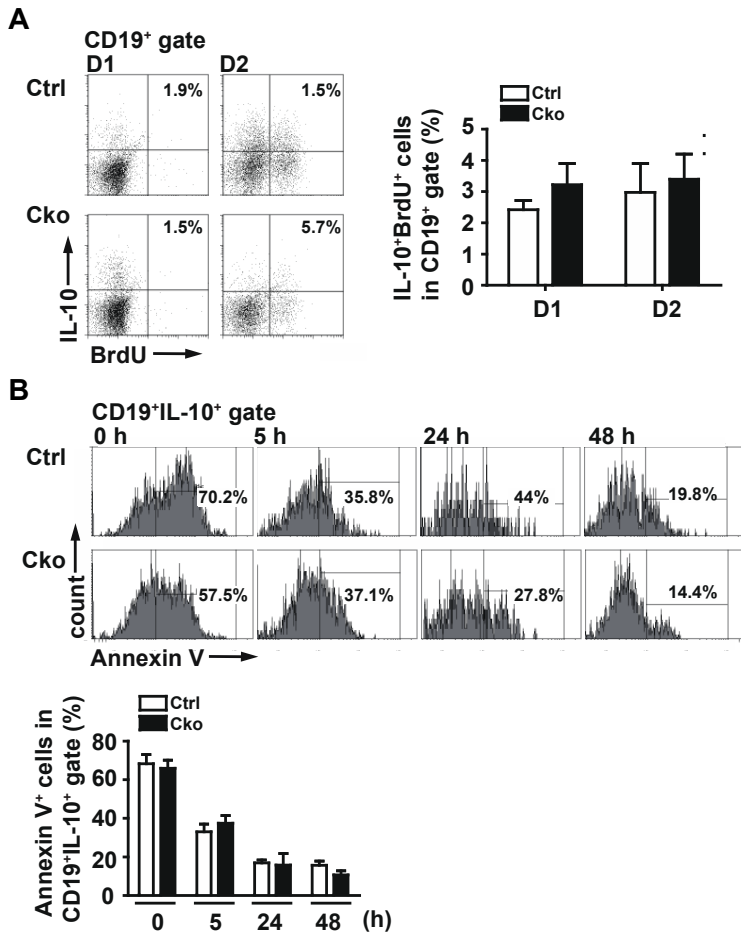
SUPPLEMENTARY TABLE 1. Differentially expressed genes in IL-10⁺ and IL-10⁻ splenic B cells stimulated with LPS or anti-CD40 antibody. Microarray analysis revealed the up- and down-regulated genes in IL-10⁺ splenic B cells after stimulation, as compared with IL-10⁻ splenic B cells. Thirty-four and four genes were up- and down-regulated, respectively (fold changes of ≥ 1.8), in IL-10⁺ B cells as compared with IL-10⁻ B cells. Two independent experiments were performed.

Probe Set ID	Experiment 1		Experiment 1		Experiment 2		Experiment 2		Gene	Entrez Gene	Alignments
	LPS		anti-CD40 antibody		LPS		anti-CD40 antibody				
	Fold Change	Regulation	Fold Change	Regulation	Fold Change	Regulation	Fold Change	Regulation			
1450330_at	5.29521	up	12.714221	up	4.292656	up	10.32317	up	<i>Il10</i>	16153	chr1:131019846-131024970 (+) // 99.09 // qE4
1422707_at	4.3830457	up	2.0213315	up	3.1081445	up	3.0763001	up	<i>Pik3cg</i>	30955	chr12:32173132-32208470 (-) // 88.11 // qA3
1439897_at	4.304222	up	5.0168533	up	3.3988318	up	3.5492852	up	<i>Nebl</i>	74103	chr2:17375018-17390982 (-) // 99.87 // qA3
1420425_at	4.285112	up	11.076121	up	3.0637705	up	6.451456	up	<i>Prdm1</i>	12142	chr10:44437174-44458748 (-) // 94.56 // qB2
1417995_at	3.292895	up	3.5207381	up	2.2172718	up	2.0241675	up	<i>Ptpn22</i>	19260	chr3:103860291-103912247 (+) // 94.29 // qF2.2
1417392_a_at	3.2892392	up	4.4193306	up	2.0447004	up	2.2043543	up	<i>Slc7a7</i>	20540	chr14:54369373-54409051 (-) // 90.25 // qC2
1429775_a_at	2.9582744	up	2.026032	up	1.8286815	up	4.024682	up	<i>Gpr137b</i>	83924	chr13:12616893-12650380 (-) // 99.48 // qA1
1428942_at	2.9540799	up	8.978006	up	2.6136837	up	6.330001	up	<i>Mt2</i>	17750	chr8:94172781-94173564 (+) // 93.29 // qC5
1436649_at	2.94711	up	2.548358	up	2.0841537	up	2.1637824	up	<i>Ikzf3</i>	22780	chr11:98464895-98466043 (-) // 84.31 // qD
1434252_at	2.945844	up	5.3405695	up	2.3646157	up	2.11213	up	<i>Tmcc3</i>	319880	chr10:94588022-94590098 (+) // 89.71 // qC2
1455660_at	2.8591983	up	4.974766	up	2.3839257	up	4.3152986	up	<i>Csf2rb</i>	12983	chr15:78350198-78350997 (+) // 97.78 // qE1
1422557_s_at	2.7599635	up	5.329776	up	2.2777429	up	3.9664156	up	<i>Mt1</i>	17748	chr8:94179141-94180327 (+) // 55.44 // qC5
1450297_at	2.7019446	up	2.4143407	up	3.1647139	up	1.9088011	up	<i>Il6</i>	16193	chr5:30013143-30019968 (+) // 90.85 // qB1

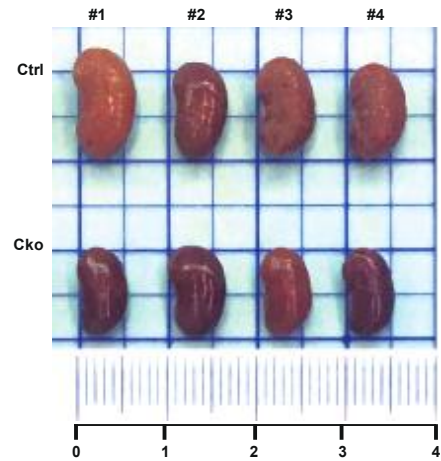
Probe Set ID	Experiment 1		Experiment 1		Experiment 2		Experiment 2		Gene	Entrez Gene	Alignments
	LPS		anti-CD40 antibody		LPS		anti-CD40 antibody				
	Fold Change	Regulation	Fold Change	Regulation	Fold Change	Regulation	Fold Change	Regulation			
1427577_x_at	2.6779115	up	2.2451954	up	2.065782	up	2.1219835	up	<i>Igk-V21</i>	16071	chr6:70371872-70726738 (+) // 50.13 // qC1
1450140_a_at	2.674659	up	5.1833787	up	2.5587728	up	3.4741762	up	<i>Cdkn2a</i>	12578	chr4:89274544-89294599 (-) // 89.22 // qC4
1416808_at	2.6027803	up	3.7149382	up	3.221675	up	2.3177993	up	<i>Nid1</i>	18073	chr13:13437601-13512275 (+) // 92.63 // qA1
1416066_at	2.5760148	up	2.4849408	up	1.9680418	up	2.3939195	up	<i>Cd9</i>	12527	chr6:125460265-125494765 (-) // 92.74 // qF3
1449152_at	2.5496545	up	5.7169304	up	2.9264941	up	2.2581322	up	<i>Cdkn2b</i>	12579	chr4:89306286-89310993 (-) // 99.2 // qC4
1420450_at	2.5078306	up	2.4896743	up	2.8194594	up	1.8753654	up	<i>Mmp10</i>	17384	chr9:7502341-7510238 (+) // 98.52 // qA1
1419298_at	2.4851675	up	3.0290012	up	2.3365417	up	3.9852161	up	<i>Pon3</i>	269823	chr6:5221350-5256278 (-) // 98.08 // qA1
1451951_at	2.362497	up	4.441163	up	2.8539584	up	1.9155523	up	<i>Igk</i>	243469	chr6:68256581-68256866 (+) // 38.61 // qC1
1419249_at	2.345807	up	2.1448216	up	2.2939608	up	5.3039994	up	<i>Cdk14</i>	18647	chr5:4803384-5380251 (-) // 97.47 // qA1
1424927_at	2.2323787	up	2.299978	up	2.0620165	up	1.8619931	up	<i>Glipr1</i>	73690	chr10:111985454-111997264 (-) // 91.1 // qD2
1427837_at	2.20593	up	4.8589444	up	2.3319187	up	3.8068845	up	<i>Igkv15-103</i>	692169	chr6:68437642-68437928 (+) // 52.31 // qC1
1427860_at	2.1379027	up	2.278748	up	1.9711353	up	2.2426953	up	<i>Igkv6-15</i>	108022	chr6:70406465-70406736 (-) // 86.73 // qC1
1420679_a_at	2.0804083	up	1.8736295	up	2.2257009	up	2.1644626	up	<i>Aigl</i>	66253	chr10:13652450-13868957 (-) // 96.23 // qA2
1455854_a_at	2.0626478	up	2.6412814	up	1.9874107	up	2.2677712	up	<i>Ssh1</i>	231637	chr5:113939764-113941100 (-) // 71.58 // qF
1423311_s_at	1.985859	up	3.9583142	up	2.306383	up	2.8652906	up	<i>Tpbp</i>	21983	chr9:85842379-85847055 (+) // 93.8 // qE3.1
1430523_s_at	1.9613429	up	2.916712	up	1.8423766	up	3.700013	up	<i>Iglv1</i>	16142	chr16:19084852-19085498 (-) // 99.82 // qA3
1416431_at	1.936019	up	2.074861	up	1.9593635	up	1.8447341	up	<i>Tubb6</i>	67951	chr18:67390729-67402752 (+) // 94.69 // qE1
1419665_a_at	1.8736795	up	3.4004576	up	3.0027506	up	2.764955	up	<i>Nupr1</i>	56312	chr7:126623247-126625475 (-) // 93.05 // qF3

Probe Set ID	Experiment 1		Experiment 1		Experiment 2		Experiment 2		Gene	Entrez Gene	Alignments
	LPS		anti-CD40 antibody		LPS		anti-CD40 antibody				
	Fold Change	Regulation	Fold Change	Regulation	Fold Change	Regulation	Fold Change	Regulation			
1447918_x_at	1.8658237	up	2.0910437	up	1.8439312	up	2.1277769	up	<i>Iglc2</i>	110786	chr16:19198400-19198554 (-) // 86.63 // qA3
1418340_at	1.8390578	up	2.1524723	up	3.4976294	up	9.314293	up	<i>Fcer1g</i>	14127	chr1:171229576-171234309 (-) // 88.6 // qH3
1424931_s_at	1.8093346	up	2.8896892	up	1.8624929	up	3.18194	up	<i>Iglc1</i>	16142	chr16:19061618-19085499 (-) // 99.77 // qA3
1455530_at	5.299976	down	3.403353	down	5.44376	down	2.872428	down	<i>Ighd</i>	380797	chr12:113406604-113416235 (-) // 71.12 // qF1
1422122_at	2.6667225	down	2.4160252	down	3.0335176	down	6.074062	down	<i>Fcer2a</i>	14128	chr8:3681805-3694174 (-) // 72.11 // qA1.1
1430558_at	2.506817	down	2.268199	down	2.5393598	down	1.9812241	down	<i>Zfp318</i>	57908	chr17:46413583-46414817 (+) // 100.0 // qC
1442544_at	2.0500302	down	2.530094	down	2.5675843	down	3.9537458	down	<i>Ighg1</i>	16017	chr12:113325242-113325950 (-) // 96.82 // qF1

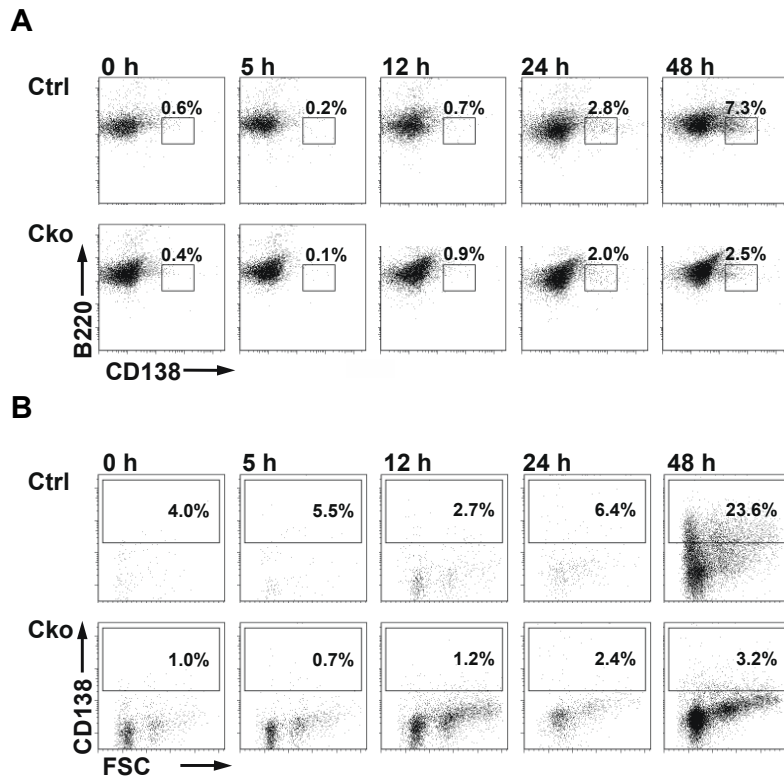
SUPPLEMENTARY TABLE 2. Microarray analysis of the expression of genes with more than 1.5-fold changes in Cko B10 cells before and 48 h after anti-CD40 stimulation. B10 cells were sorted from Ctrl or Cko mice and subjected for anti-CD40 stimulation. Genes whose expression was changed for more than 1.5-fold in Cko B10 cells were listed in a separate Excel data sheet (Supplementary Table 2).



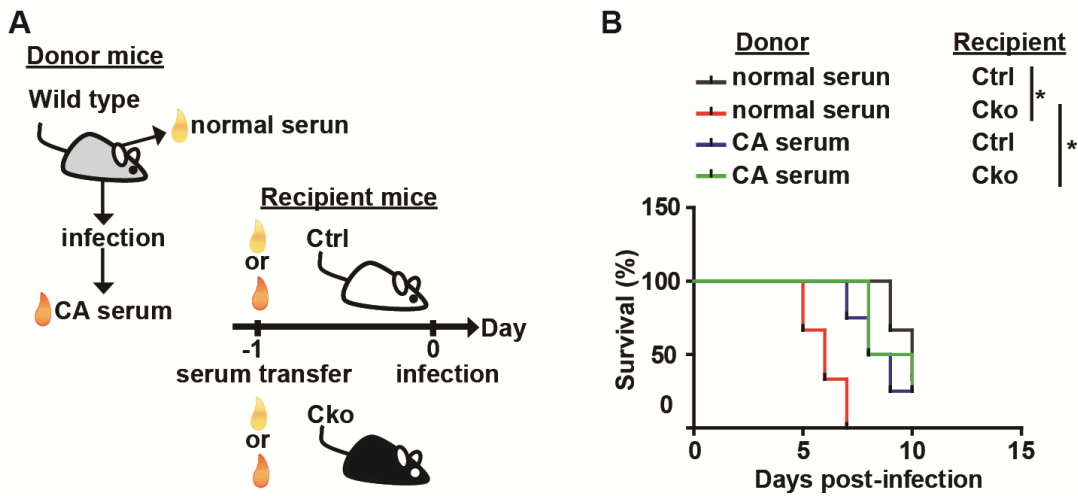
SUPPLEMENTARY FIGURE 1. Proliferation and apoptosis rates in Ctrl and Cko B10 cells after stimulation. (A) Splenic B cells from Ctrl or Cko mice were stimulated with LPS (10 $\mu\text{g}/\text{ml}$) for the indicated lengths of time as described previously. Cells were treated with 30 μM 5-bromo-2'-deoxyuridine (BrdU) for 2 h before being harvested. Data represent the BrdU⁺ cells in the group of CD19⁺IL-10⁺ cells. (B) Cells were collected at the indicated timepoints and subjected to Annexin V staining. All antibodies and reagents are from BD Bioscience, and the staining procedure was performed in accordance with the manufacturer's instructions. Data are the mean \pm SEM ($n = 3\text{--}5$ mice per group). At least two independent experiments were performed.



SUPPLEMENTARY FIGURE 2. Gross pathology of kidneys from Ctrl and Cko mice infected with *C. albicans*. Ctrl and Cko mice were infected with 5×10^5 CFU of *C. albicans* by intravenous injection. Three days after infection, kidneys from infected mice were removed for comparison (n = 4 mice per group).



SUPPLEMENTARY FIGURE 3. B10 cells differentiated into CD138⁺ plasmablasts after LPS stimulation. CD19⁺CD1d^{hi}CD5⁺ B10 cells were sorted from Ctrl × *tiger* or Cko × *tiger* mice and then stimulated with LPS (10 μg/ml) for the indicated lengths of time. **(A)** FACS analysis showing the frequency of CD138⁺ plasmablasts. **(B)** FACS showing the frequency of CD138⁺ in the IL-10⁺ gate. Data are the mean ± SEM (n = 4 mice per group). Two independent experiments were performed.



SUPPLEMENTARY FIGURE 4. Adoptive transfer of serum from *C. albicans* infected mice reduced the mortality caused by *C. albicans* infection in Cko mice. (A) Schematic design for transfer of serum collected from day 14 *C. albicans* infected (CA serum) or uninfected (normal serum) wildtype C57BL/6 mice into Ctrl or Cko mice, followed by challenge with 5×10^5 CFU of *C. albicans*. (B) The survival rate of Ctrl and Cko mice in (A) was recorded (n = 3–4 mice per group). The differences in survival rates were analyzed by a log-rank (Mantel-Cox) test. * $p < 0.05$.