Chen, S et al.: Control of T_{reg} cell homeostasis and immune equilibrium by Lkb1 in dendritic cells Supplementary materials: 17 Figures; 5 tables





10² 10³ 10⁴ 10⁵ CFSE

10² 10³ 10⁴ 10⁵

b





Spl

LN





а













Supplementary Figure 11







← Lkb1^{f/f}Foxp3^{DTR}+E.coli

b

---- Cd11c^{Cre}Lkb1^{f/f}Foxp3^{DTR}+E.coli







b

а





Supplementary Table 1. Schematic description of conserved NF-κB binding site in Ox40l locus

■ NF _k B binding site ■ Negative control $Ox40/$ NC1 1 2 3 4 5 6 NC2 3 kb TSS +1							
ID	Start	End	Strand	Site sequence			
1	103	112	-1	GTGAATTACC			
2	771	780	-1	AGGGTTTTCC			
3	1821	1830	1	GAGACTTTCC			
4	2441	2450	-1	GGGAAATTCA			
5	2882	2891	-1	GTGGCATCCC			
6	2945	2954	1	GGGAAATTCA			
7	2967	2976	-1	GGGAACTTCT			

Supplementary Table 2. Oligonucleotide primers

Primer/Purpose	5'-Sequence-3'		
Or AOL/a PCR of mouse $Or AOL$ cDNA	F: GAAGAAGACGCTAAGGCTGGT		
oxtor/q-1 eK of mouse oxtor eDIVI	R: GAGCTGATGAATAGTTGCCCAT		
$\Omega r 40 / a_P C R$ of mouse $\Omega r 40 c D N A$	F: TACCTACCCCAGTGGTCACAA		
0x40/q-1 CK of mouse 0x40 CDNA	R: ACGGATGACATAGAGTATCCCTG		
Lkhl/a-PCR of mouse $Lkhl$ cDNA	F: GCCAACGTCAAGAAGGAGAT		
Ekon /q-1 CK of mouse Ekon CDNA	R: CGCAGTACTCCATCACCATATAC		
Crell 1/a PCP of mouse $Crell 1/a$ DNA	F: GGCTTCCTTATGTTCAAACAGGG		
CACHT/q-I CK of mouse CACHT CDNA	R: GCCGTTACTCGGGTAAATTACA		
III_a /a PCP of mouse III_a cDNA	F: CGAAGACTACAGTTCTGCCATT		
<i>illa</i> /q-FCK of mouse <i>illa</i> cDNA	R: GACGTTTCAGAGGTTCTCAGAG		
U27/a DCD of mouse U27 aDNA	F: CTGTTGCTGCTACCCTTGCTT		
<i>n27/q</i> -PCK of mouse <i>n27</i> cDNA	R: CACTCCTGGCAATCGAGATTC		
Cuelle /a DCD of manage Cuelle aDNA	F: TCCTTTTGGGCATCATCTTCC		
CXCI9/q-PCR of mouse CXCI9 CDINA	R: TTTGTAGTGGATCGTGCCTCG		
	F: ACATCCATCTCGTGCTACTTGT		
1115/q-PCR of mouse 1115 cDNA	R: GCCTCTGTTTTAGGGAGACCT		
	F: ACCCCCAACATAACTGAGTCT		
Cd80/q-PCR of mouse $Cd80$ cDNA	R: TTCCAACCAAGAGAAGCGAGG		
	F: CCAAGTGCTGCCGTCATTTTC		
<i>Cxcl10</i> /q-PCR of mouse <i>Cxcl10</i> cDNA	R: GGCTCGCAGGGATGATTTCAA		
	F: TCCTGACTTTGGGGGAGGTTTT		
<i>Illf9/</i> q-PCR of mouse <i>Illf9</i> cDNA	R: TCACGCTGACTGGGGGTTACT		
	F: GCTGCTTTGCCTACCTCTCC		
Ccl5/q-PCR of mouse Ccl5 cDNA	R: TCGAGTGACAAACACGACTGC		
	F: AGGTCGGTGTGAACGGATTTG		
Gapgh/q-PCR of mouse Gapgh cDNA	R: TGTAGACCATGTAGTTGAGGTCA		
	F: CTGGTCATGTGCTGATGAGT		
Ox401-NC1/ChIP-qPCR of mouse Ox401 gene	R: TGAAGTTGGCTATGGTGAGAAT		
	F: CCCACCAGAGTTGGTAATTCA		
Ox401-1/ChIP-qPCR of mouse Ox401 gene	R: ATTTCAGTCCTGGGCAGATG		
	F: CCACCATGAGGTTCTGAGTTT		
Ox401-2/ChIP-qPCR of mouse Ox401 gene	R: CCCGTTTCAGCTTCCATAGT		
	F: CTGTTGACGGCTCCATGATAAA		
<i>Ox401-3</i> /ChIP-qPCR of mouse <i>Ox401</i> gene	R: GCACAGGGATGCTGAACATAA		
	F: GCTGAATTTCCCTGAAATGAGAAA		
<i>Ox401</i> -4/ChIP-qPCR of mouse <i>Ox401</i> gene	R: GAGCAACTGCTATGTGTCAGA		
	F: TTCATAACTGGGATGCCACAA		
<i>Ox401-5</i> /ChIP-qPCR of mouse <i>Ox401</i> gene	R: GGGAACTTCTATGTGACTAATCTGAA		
	F: CAAGTGGAAGAAGACGCTAAGG		
<i>Ox401</i> -6/ChIP-qPCR of mouse <i>Ox401</i> gene	R: CTTACCGGAGAGGGAAGAGAGTT		
	F: ACTTCCAGTGCTTTCCTTACA		
<i>Ox401</i> -NC2/ChIP-qPCR of mouse <i>Ox401</i> gene	R: GCTGACCAAGATAGCCAATTTC		

Supplementary Table 3. Flow cytometry gating strategies.

Tissue/	Target	Gating Strategy	Figure number
cells	Population		
Spleen, lymph	CD4 ⁺ Foxp3 ⁺ T _{reg} cells	Immune cells/Singlets/CD4 ⁺ /Foxp3 ⁺	Supplementary Figure 16a-> Figure 1a,1c,1e,1g,6c,6e,7b,7d
nodes, thymus,			and Supplementary Figure 3c,4a,6a,8a
lung and	CD4 ⁺ Foxp3 ⁻ T	Immune	Supplementary Figure 16a->
brain	cells	cells/Singlets/CD4 ⁺ /Foxp3 ⁻	Figure 2b and Supplementary Figure 2a,2b,4c,6c,8a,14b
	CD8 ⁺ Foxp3 ⁻ T		Supplementary Figure 16a->
	cells	cells/Singlets/CD8 ⁺ /Foxp3 ⁻	Supplementary Figure 2a,2b,4c,6c
Co-cultured	CD4 ⁺ CD45.1 ⁺ T		Supplementary Figure 16b->
cells	cells	cells/Singlets/CD4 ⁺ CD45.1 ⁺	Figure 1h and Supplementary Figure 2e,10a
Spleen	CD4 ⁺ CD45.1 ⁺ T	Immune	Supplementary Figure 16c->
	cells	cells/Singlets/CD4 ⁺ CD45.1 ⁺	Figure 2d,2g
Co-cultured	CD4 ⁺ CD45.1 ⁺ Fox	Immune	Supplementary Figure 16d->
cells	$p3^+ T_{reg}$ cells	cells/Singlets/CD4 ⁺ CD45.1 ⁺ /Foxp3 ⁺	Figure 3a,3d,3g,4e,5e,6g and Supplementary Figure 9a
Spleen	CD4 ⁺ CD45.1 ⁺ Fox	Immune	Supplementary Figure 16e->
-1	$p3^+ T_{reg}$ cells	cells/Singlets/CD4 ⁺ CD45.1 ⁺ /Foxp3 ⁺	Figure 3h,4h,5h
Spleen and	MHCII ⁺ CD11c ⁺	Immune	Supplementary Figure 16f->
lymph	DCs	cells/Singlets/MHCII ⁺ CD11	Figure 4d,5d,8e and
nodes		c ⁺	Supplementary Figure 1a,1c,
	MHCII ⁺ CD11c ^{hi} C	Immune	Supplementary Figure 16f->
	D11b ⁺ cDCs	cells/Singlets/MHCII ⁺ CD11 c ^{hi} /CD8 ⁻ CD11b ⁺	Supplementary Figure 7a
	MHCII ⁺ CD11c ^{hi} C	Immune	Supplementary Figure 16f->
	D8 ⁺ cDCs	cells/Singlets/MHCII ⁺ CD11 c ^{hi} / CD8 ⁺ CD11b-	Supplementary Figure 7a
	MHCII ⁺ CD11c ^{low}	Immune	Supplementary Figure 16f->
	CD11b-B220 ⁺ LY6	cells/Singlets/MHCII ⁺ CD11	Supplementary Figure 7a
	C ⁺ pDCs	c ^{low} /CD11b-/ B220 ⁺ LY6C ⁺	
Spleen	$CD4^+CD25^+ T_{reg}$	Immune cells/Singlets/	Supplementary Figure 17a->
	cells	CD4'/CD25'	Supplementary Figure 3a
Co-cultured	CD4 ⁺ T cells		Supplementary Figure 1/b->
cells		cells/Singlets/CD4	Supplementary Figure 10d