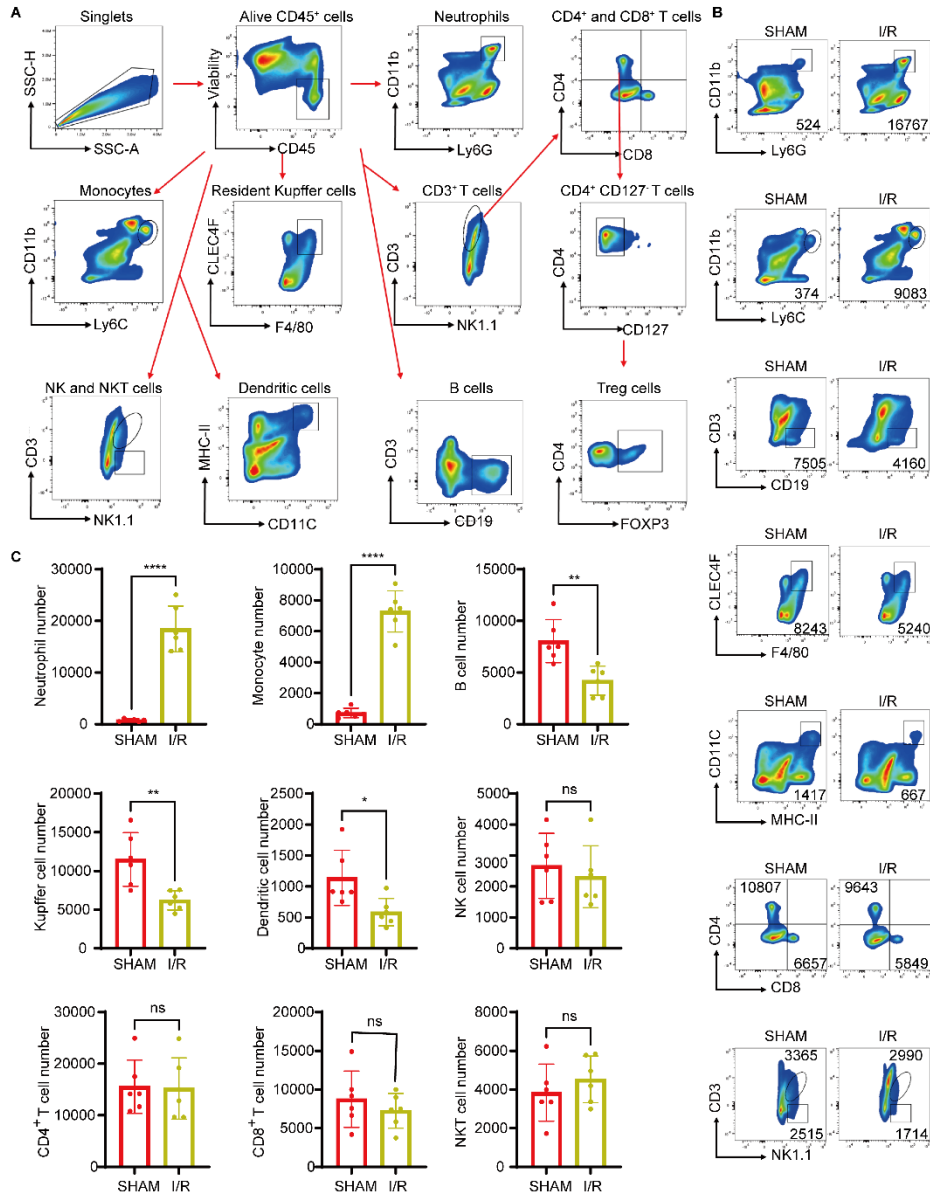


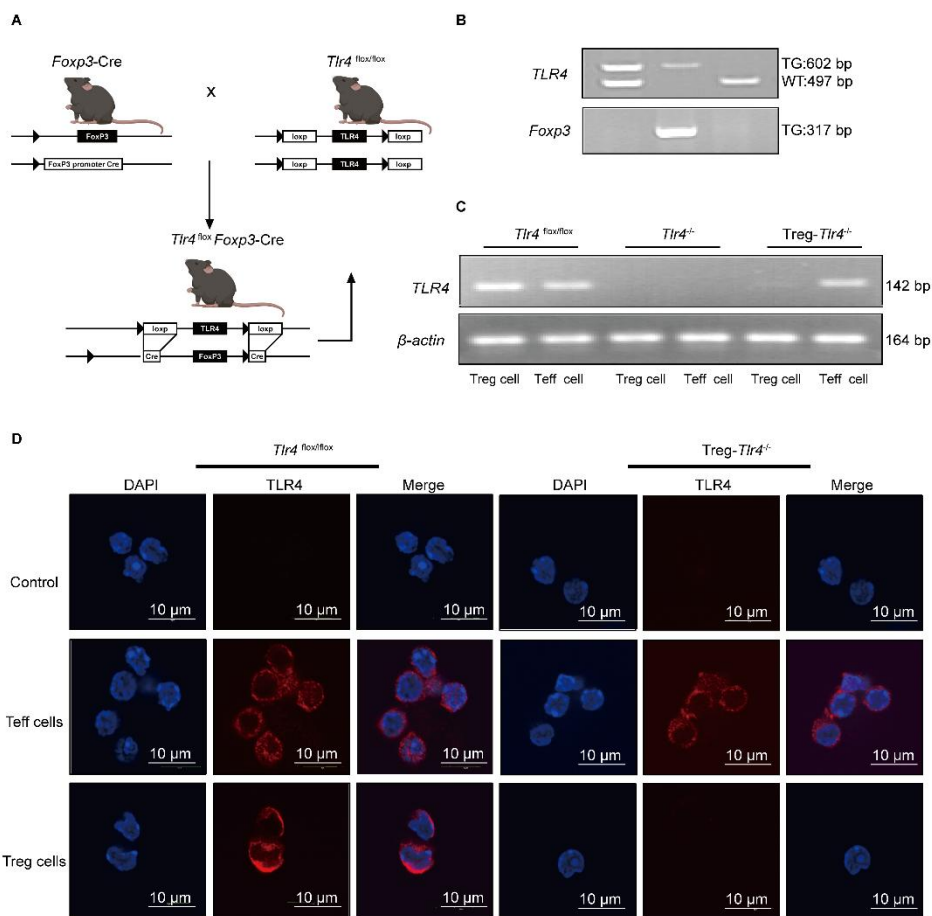
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Supplemental figures and tables



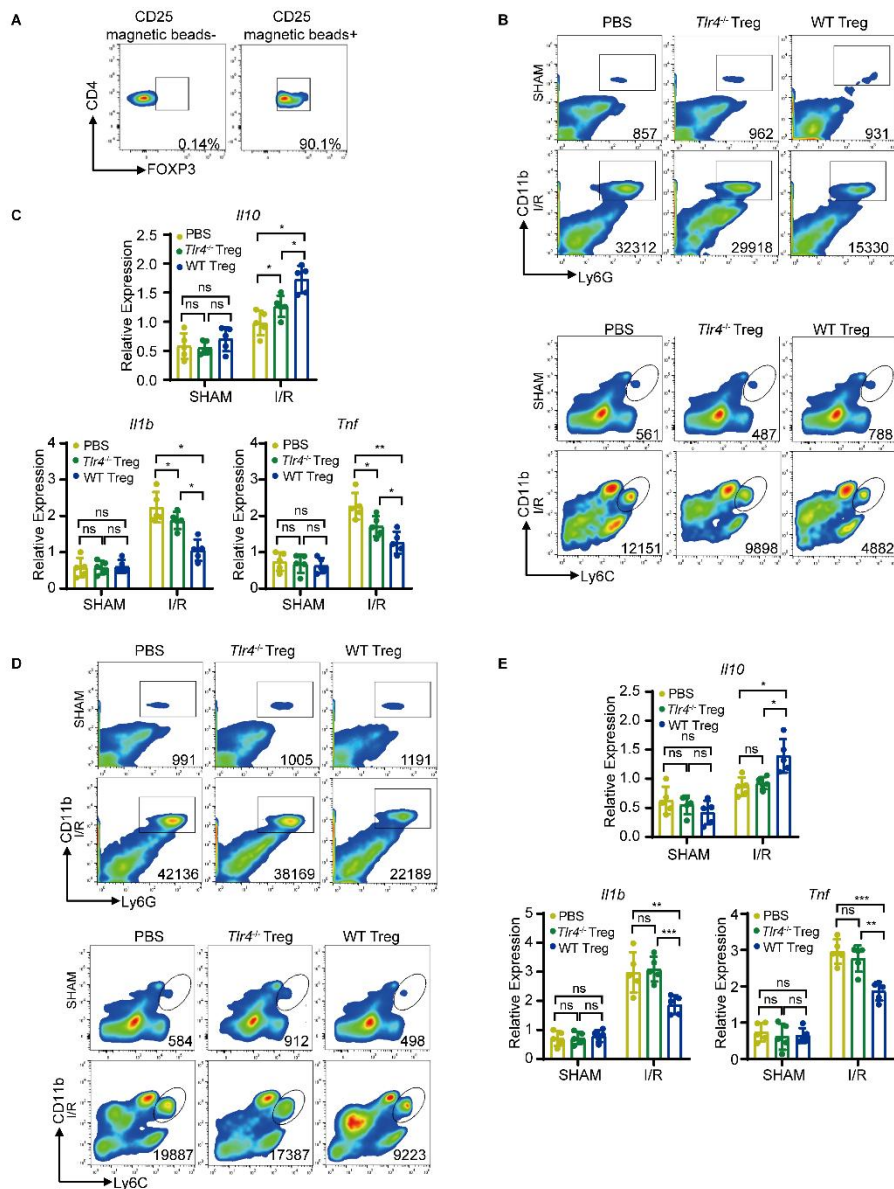
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Supplemental Figure 1. Quantification of hepatic immune cells from sham and I/R WT mice. (A) Gating strategy for the identification of hepatic CD45⁺ immune cell populations by flow cytometry in sham and I/R WT mice. **(B-C)** Representative FACS plots and absolute counts of neutrophils, monocytes, dendritic cells, B cells, resident Kupffer cells, CD4⁺ T cells, CD8⁺ T cells, NK cells, and NKT cells per 1,000,000 hepatic non-parenchymal cells in sham and I/R WT mice (n=6 per group). Statistical analyses were performed using unpaired, two-tailed t-tests **(C)**. *p < 0.05, **p < 0.01, ***p < 0.001; ****p < 0.0001; ns, no significant. I/R: ischemia/reperfusion.



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Supplemental Figure 2. Generation and identification of Treg-*Tlr4*^{-/-} mice. (A) Schematic diagram illustrating the generation of Treg-*Tlr4*^{-/-} mice. (B) PCR-based identification of DNA expression for *loxP*-flanked *TLR4* alleles and *Cre* recombinase expression to distinguish heterozygous *Tlr4*^{flx/flx} mice, homozygous *Tlr4*^{flx/flx} mice, and Treg-*Tlr4*^{-/-} mice. (C) PCR-based analysis of *Tlr4* gene expression in Teffs and Tregs isolated from *Tlr4*^{flx/flx} mice, *Tlr4*^{-/-} mice, and Treg-*Tlr4*^{-/-} mice. (D) Representative immunofluorescence images showing TLR4 expression (red) in Teffs and Tregs isolated from *Tlr4*^{flx/flx} mice (left panel) and Treg-*Tlr4*^{-/-} mice (right panel) through magnetic bead sorting. Scale bars, 10 μ m. Tregs: regulatory T cells; Teffs: effector T cells.



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23 **Supplemental Figure 3. *Tlr4* expression in Tregs alleviates liver I/R injury in Treg-depleted mice.** (A)

24 Representative flow cytometry plots showing the proportion of CD4⁺FOXP3⁺ Tregs in isolated Treg cells and control

25 using MACS-sorting. (B) Representative flow cytometry plots of CD11b⁺Ly6G⁺ neutrophils and CD11b⁺Ly6C⁺

26 monocytes in Treg-depleted *Foxp3* DTR mice administered PBS, *Tlr4*^{-/-} Tregs, or WT Tregs before and after liver I/R

27 (n=5 per group). (C) Quantitative PCR analysis of *Il1b*, *Tnf*, and *Il10* mRNA expression in ischemic liver from Treg-

28 depleted *Foxp3* DTR mice administered PBS, *Tlr4*^{-/-} Tregs, or WT Tregs before and after liver I/R (n=5 per group). (D)

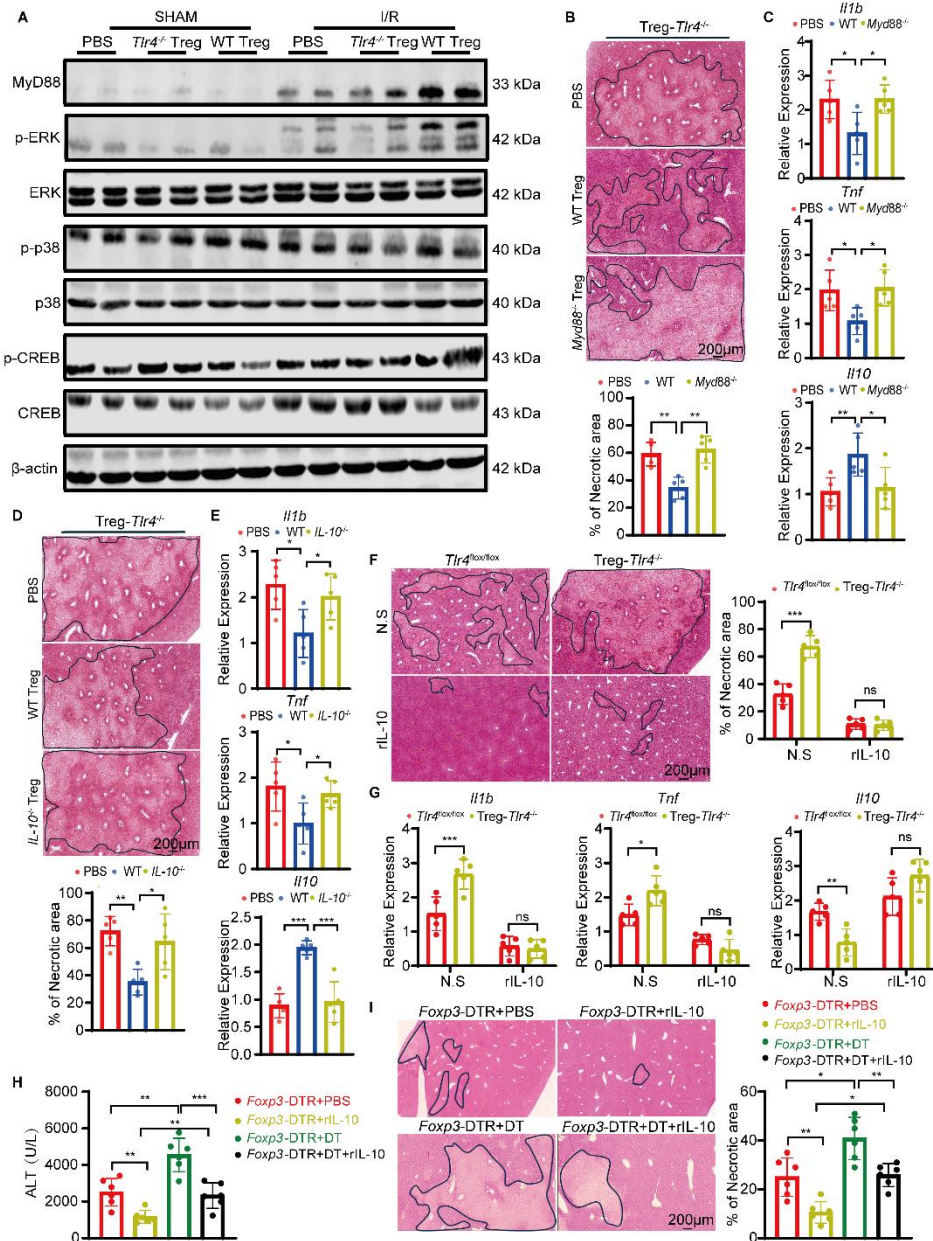
29 Representative flow cytometry plots of CD11b⁺Ly6G⁺ neutrophils and CD11b⁺Ly6C⁺ monocytes in Treg-depleted WT

30 mice through anti-CD25 antibody and then administered PBS, *Tlr4*^{-/-} Tregs, or WT Tregs before and after liver I/R (n=5

31 per group). (E) Quantitative PCR analysis of *Il1b*, *Tnf*, and *Il10* mRNA expression in ischemic liver from Treg-depleted

32 WT mice adoptively transferred with PBS, *Tlr4*^{-/-} Tregs, or WT Tregs after I/R (n=5 per group). Statistical analyses were

33 performed using one-way ANOVA with Tukey's post test (C and E). I/R: ischemia/reperfusion.



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36 **Supplemental Figure 4. TLR4/MyD88/ERK/CREB-mediated IL-10 production in Tregs alleviates liver I/R injury.**
 37 (A) Western blot analysis of MyD88, p-ERK, p-p38, and p-CREB protein expression levels in Tregs from Treg-*Tlr4*^{-/-}
 38 mice adoptively transferred with PBS, *Tlr4*^{-/-} Tregs or WT Tregs before and after I/R. (B) Representative H&E staining
 39 images of ischemia liver from Treg-*Tlr4*^{-/-} mice adoptively transferred with PBS, *Myd88*^{-/-} Tregs or WT Tregs after liver
 40 I/R (n=5 per group). (C) Quantitative PCR analysis of *Il1b*, *Tnf*, and *Il10* mRNA expression in ischemia liver from Treg-
 41 *Tlr4*^{-/-} mice adoptively transferred with PBS, *Myd88*^{-/-} Tregs or WT Tregs after liver I/R (n=5 per group). (D)
 42 Representative H&E staining images of ischemia liver from Treg-*Tlr4*^{-/-} mice adoptively transferred with PBS, *IL-10*^{-/-}
 43 Tregs or WT Tregs after liver I/R (n=5 per group). (E) Quantitative PCR analysis of *Il1b*, *Tnf*, and *Il10* mRNA expression
 44 in ischemia liver from Treg-*Tlr4*^{-/-} mice adoptively transferred with PBS, *IL-10*^{-/-} Tregs or WT Tregs after liver I/R (n=5
 45 per group). (F) Representative H&E staining images of ischemia liver lobe from *Tlr4*^{lox/lox} mice and Treg-*Tlr4*^{-/-} mice
 46 administered recombinant IL-10 or its control after I/R (n=5 per group). (G) Quantitative PCR analysis of *Il1b*, *Tnf*, and
 47 *Il10* mRNA expression in ischemia liver from *Tlr4*^{lox/lox} mice and Treg-*Tlr4*^{-/-} mice administered recombinant IL-10 or its
 48 control after I/R (n=5 per group). (H) Serum ALT levels in I/R *Foxp3-DTR* mice following Treg depletion or not, and
 49 treatment with recombinant IL-10 or vehicle (n=6 per group). (I) Representative H&E staining images of ischemia liver
 50 from these mice (n=6 per group); Scale bars, 200 μm. Statistical analyses were performed using one-way ANOVA with
 51 Tukey's post test (B-E), two-way ANOVA with Sidak's post-test (F-G) and one-way ANOVA (H-I). *p < 0.05, **p < 0.01,
 52 ***p < 0.001; ns, not significant; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; I/R:
 53 ischemia/reperfusion; rIL-10: recombinant IL-10.

54 **Supplemental Table 1. Preoperative and Postoperative clinical**
55 **characteristics of patients with hepatic hemangioma (p values were**
56 **determined using ANOVA test)**

	No hepatic portal block (N=6)	Hepatic portal block (<8.4min) (N=5)	Hepatic portal Block (>8.4min) (N=5)	p-value
Preoperative				
Age – median (IQR)	49.5 (39-62)	48 (36-57)	50.2 (41-63)	0.92
Female gender – n (%)	3 (50%)	2 (40%)	3 (60%)	0.72
Hypertension– n (%)	0(0%)	0(0%)	1(20%)	0.46
Hyperlipidemia– n (%)	0(0%)	1(20%)	0 (0%)	0.46
Body mass index – median (IQR)	23.30 (19.92- 30.67)	23.2 (19.31- 24.8)	24.11 (21.65- 25.39)	0.43
AST – median (IQR)	19.67 (15-27)	20 (17-22)	21(19-25)	0.89
ALT – median (IQR)	18.33 (9-28)	19.1 (8-25)	17.5 (11-28)	0.68
Total bilirubin – median (IQR)	11.38(5.3- 18.8)	13.75 (7.2-19.6)	14.3 (9.2-20.5)	0.17
INR – median (IQR)	1.68(0.87-4.2)	1.70 (0.94-3.2)	1.74 (0.98-3.5)	0.94
Albumin – median (IQR)	40.35 (36.2-43.1)	41.78 (38.9-43.6)	44.1 (41.2-48.6)	0.17
platelet count– median (IQR)	178.5 (117-244) *10 ⁹ /L	179 (104-225) *10 ⁹ /L	176.4 (111-243) *10 ⁹ /L	0.93
PT–median (IQR)	12.8 (11.7-13.9)	11.85 (12.9-15.1)	11.85 (13-14.8)	0.57
APTT–median (IQR)	39.82 (29.9-42.9)	39.9 (33.1-40.8)	38.28 (32.5-41.4)	0.26
Hepatic pedicle block–median (IQR)	0(0) min	3.6 (1-8.4) min	10.2 (8.4-15) min	<0.01
Postoperative				

AST – median (IQR)	327.5 (91-655)	471.8 (268-694)	1015.6 (830-1284)	0.002
ALT – median (IQR)	323.67 (73-705)	597.2 (196-746)	1250 (933-1910)	0.005
Total bilirubin – median (IQR)	16.75(7.7-26)	18.36 (11- 28.7)	21.7 (11.7-26)	0.47
INR – median (IQR)	1.58 (0.97-3.2)	1.77 (0.89-3.42)	1.84 (0.86-3.98)	0.06
Albumin – median (IQR)	34.45 (30.7-38)	37.26 (22.1-39.6)	35.71 (25.1-40.6)	0.85
platelet count– median (IQR)	191 (102-261) *10 ⁹ /L	183 (89-277) *10 ⁹ /L	195 (121-281) *10 ⁹ /L	0.71
PT–median (IQR)	13.1 (12.4-16.2)	16.5 (14.9-18.5)	19.5 (17.7-20.5)	0.04
APTT–median (IQR)	39.97 (29.9-41.9)	38.28 (32.5-38.4)	41.1 (38.5-43.4)	0.14

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83 **Supplemental Table 2. Antibodies**

Name	Supplier	Cat no.	Clone no.
anti-CD45-FITC	BioLegend	103108	103108
anti-CD11b- Brilliant Violet 421™	BioLegend	101251	M1/70
anti-F4/80 Spark YG™ 593	BioLegend	101251	QA17A29
anti-CLEC4F Alexa Fluor® 647	BioLegend	156804	3E3F9
anti-CD19 BUV563	BD Biosciences	749028	1D3
anti-Ly6G- Alexa Fluor® 700	BioLegend	127622	1A8
anti-Ly6C- Brilliant Violet 510™	BioLegend	128033	HK1.4
anti-NK1.1- Brilliant Violet 570™	BioLegend	108733	PK136
anti-CD8a BUV496	BD Biosciences	750024	53-6.7
anti-CD4- BUV615	BD Biosciences	613006	GK1.5
anti-CD3-BUV661	BD Biosciences	741562	17A2
anti-CD11c Brilliant Violet 650™	BioLegend	117339	N418
anti-I-A/I-E Brilliant Violet 711™	BioLegend	107643	M5/114.15.2
anti-IL-10- Brilliant Violet 421™	BioLegend	505021	JES5-16E3
anti-CD127- PE-cy7	eBioscience™	25-1271-82	A7R34
anti-mouse FOXP3-PE	BioLegend	126404	MF-14
anti-mouse FOXP3-APC	eBioscience	17-5773-82	FJK-16s
anti-TLR1-BV510	BD Biosciences	743134	TR23
anti-TLR2- PE/Cyanine7	BioLegend	153012	QA16A01
anti-TLR3- Alexa Fluor® 700	Novus	NBP227405	27N3D4
anti-TLR4- BV786	BD Biosciences	741015	MTS510
anti-TLR5- Alexa Fluor® 647	BioLegend	148104	ACT5
anti-TLR6- BV650	BD Biosciences	743269	C1N2
anti-TLR7- PE/Cy5.5	Novus	N104349PEC5	4F4
anti-TLR8	Novus	DDX0481P100	307D3.01
anti-TLR9- PerCP	Novus	NB371746	26C593R
anti-TLR11-mFluor Violet 450	Novus	NB346482	786404
Anti-FOXP3 antibody	Abclonal	A5760	Polyclonal
anti-TLR4 antibody	eBioscience™	HTA125	14991782
Cy3-conjugated anti-mouse	Beyotime	Polyclonal	A0521
Cy5-conjugated anti-mouse	Sigma	Polyclonal	AC111S
anti-mouse CD25 antibody	Bio X cell	PC61	BE0012
anti-mouse CD16/32 Antibody	BioLegend	93	101302
Recombinant Mouse IL-10	R&D	417-ML-025/CF	/
Anti-MyD88 antibody	Abcam	ab2064	Polyclonal
Phospho-ERK1/2 antibody	Abclonal	AP0472	Polyclonal
ERK1/2 antibody	Abclonal	A16686	Polyclonal
Phospho-p38 MAPK-antibody	Abclonal	AP0526	Polyclonal
p38 MAPK-antibody	Abclonal	A5049	Polyclonal

Phosph- CREB antibody	Abclonal	AP0019	Polyclona
CREB antibody	Abclonal	A10826	Polyclonal
β -actin antibody	Cell Signaling Technology	4970	Polyclonal

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Supplemental Table 3. Primers for genotyping and RT-qPCR (mouse)

Name	Sequence	Supplier
<i>Tlr4</i> ^{flox/flox} forward A	AGGGAGATGTGTGTGAAGAAG CC	Sangon Biotech
<i>Tlr4</i> ^{flox/flox} reverse B	TGACTTTCTCAGTTTGGTCCTGG	Sangon Biotech
<i>Tlr4</i> ^{flox/flox} reverse C	TTCCAGCTATGGCCCAGATGAACT	Sangon Biotech
<i>Foxp3</i> -Cre forward	CGGGTCAGAAAGAATGGTGT	Sangon Biotech
<i>Foxp3</i> -Cre reverse	CAGTTTCAGTCCCCATCCTC	Sangon Biotech
<i>Tlr4</i> forward	CAAGAACATAGATCTGAGCTTCAA CCC	Sangon Biotech
<i>Tlr4</i> reverse	GCTGTCCAATAGGGAAGCTTTCTAGAG	Sangon Biotech
<i>Il1b</i> forward	GGATGAGGACATGAGCACC	Sangon Biotech
<i>Il1b</i> reverse	GGAGCCTGTAGTGCAGTTGT	Sangon Biotech
<i>Tnf</i> forward	ACTGAACTTCGGGGTGATCG	Sangon Biotech
<i>Tnf</i> reverse	GGCTACAGGCTTGTCACTCG	Sangon Biotech
<i>Il10</i> forward	CCCTTTGCTATGGTGCCTT	Sangon Biotech
<i>Il10</i> reverse	TGGTTTCTCTTCCAAGACC	Sangon Biotech
<i>Foxp3</i> forward	TGACCAAGGCTTCATCTGTG	Sangon Biotech
<i>Foxp3</i> reverse	GAGGAACTCTGGGAATGTGC	Sangon Biotech
<i>Ikzf2</i> forward	CAGCATGCACCAAGCACAAT	Sangon Biotech
<i>Ikzf2</i> reverse	ATGGGGCCATCCATGTTGTT	Sangon Biotech

<i>Ii2ra</i> forward	GCGTTGCTTAGGAAACTCCTGG	Sangon Biotech
<i>Ii2ra</i> reverse	GCATAGACTGTGTTGGCTTCTGC	Sangon Biotech
<i>Ctla4</i> forward	CTCAGCTGAACCTGGCTACC	Sangon Biotech
<i>Ctla4</i> reverse	CCACTTGCAGACACCATTG	Sangon Biotech
<i>Myd88</i> forward	CATGTTCTCCATACCCTTGGT	Sangon Biotech
<i>Myd88</i> reverse	AAACTGCGAGTGGGGTCAG	Sangon Biotech
<i>Mapk1</i> forward	ACCCAAGTGATGAGCCCATTG	Sangon Biotech
<i>Mapk1</i> reverse	TGCATTGAAAGTGCACACTGC	Sangon Biotech
<i>Creb1</i> forward	GCTGCCTCTGGAGACGTACAA	Sangon Biotech
<i>Creb1</i> reverse	GCTAGTGGGTGCTGTGCGA	Sangon Biotech
<i>Mapk14</i> forward	GCATCGTGTGGCAGTTAAGA	Sangon Biotech
<i>Mapk14</i> reverse	GTCCTTTTGGCGTGAATGAT	Sangon Biotech
β - <i>actin</i> forward	TGTTACCAACTGGGACGACA	Sangon Biotech
β - <i>actin</i> reverse	GGGGTGTGAAGGTCTCAA	Sangon Biotech

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Supplemental Table 4. Deposited data

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Name of repository	Identifier	Link
Bulk RNA-Seq for murine liver Treg cells before and after liver I/R	PRJNA1206100	https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1206100/
Bulk RNA-Seq for murine liver Treg cells in <i>Tlr4</i> ^{flox/flox} and Treg- <i>Tlr4</i> ^{-/-} after liver I/R	PRJNA1206185	https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1206185/

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