Supplementary Information

Supplementary Figures

Figure S1



Figure S1. cDNT transfer prevented NASH development in MCD-fed mice.

C57BL/6 mice received single CD45.1-positive cDNT adoptive transfer were fed with an MCD for 4 weeks. a Body weight of mice from each group with or without cDNT transfer. NCD, black; MCD, red; MCD + cDNT, blue. Actual p values (left → right): 6.07e-9, 0.77. b Plasma ALT, AST, and TG levels were measured in NCD- and MCDfed mice. Actual p values (left-right): 0.000018, 0.0045, 4.26e-8, 0.00056, 0.0065, 0.0058. c Representative H&E staining, oil red o staining, Sirius red staining and α -SMA staining in liver paraffin sections. Quantification of liver histology staining. Scale bars, 100 μ m. (n = 6 biologically independent samples per group). Actual p values (left \rightarrow right): for NAS score, 0.0017, 0.010; for oil red o staining, 5.81e-9, 5.69e-7; for sirius red staining, 4.03e-7, 0.000092; for α -SMA staining, 2.62e-7, 0.000034. d Western blot analysis of α -SMA in liver tissues of MCD-fed mice. Actual p values (left-right): 4.16e-7, 0.000061. e Fibrosis-related genes in the mouse liver were detected via quantitative real-time PCR. Actual p values (left \rightarrow right): 1.37e-7, 0.00022, 0.00036, 0.044, 0.000001, 0.00014, 7.57e-7, 0.0024. f Hydroxyproline levels in liver tissues from each group. Actual p values (left-right): 0.000038, 0.000078. g Inflammatory cytokine levels were measured in plasma from NCD- and MCD-fed mice. Actual p values (left -> right): 0.00042, 0.029, 0.037, 0.000004, 5.80e-9, 6.84e-9, 0.036, 0.013, 0.019, 1.61e-8, 0.024, 0.0035, 0.000015, 7.26e-7, 0.0084. Data are presented as the mean \pm SD; n = 6 mice/group. Statistical analysis for the first right figure in c (NAS score group) was performed by Kruskal-Wallis multiple comparisons test, and others were performed by one-way ANOVA with post-hoc multiple comparisons test. Twosided p values < 0.05 were considered significant. *p < 0.05; **p < 0.01; NS, not significant. Source data, including exact p values, are provided as a Source data file.





Figure S2. cDNT and Rapamycin had similar therapeutic effects on NASH.

a Plasma ALT, AST, and TG levels were measured (n = 6 mice/group). Actual p values (left→right): 4.05e-10, 2.05e-8, 1.90e-9, 1.32e-10, 8.82e-8, 1.2e-8, 2.76e-8, 1.47e-7,

2.42e-7. b Statistical analysis of NAS score and positive area of oil red o staining in each group (n = 6 mice/group). Actual p values (left \rightarrow right): 0.0053, 0.0095, 0.0086, 8.46e-13, 3.79e-9, 7.66e-12. c Representative H&E staining and oil red o staining in liver paraffin sections. Scale bars, $100 \mu m$. (n = 6 biologically independent samples per group). d and e Representative flow cytometry plots (d) and statistical analysis (e) of the percentages of IL-17⁺ cells among liver resident DNT after cDNT or rapamycin treatment (n = 6 mice/group). Actual p values (left \rightarrow right): e 0.000002, 0.000012. f and g Representative flow cytometry plots (f) and statistical analysis (g) of the percentages of IL-17⁺ cells among liver resident DNT and cDNT in MCD-fed mice (n = 5mice/group). For g: **p = 0.000002. Data are presented as the mean \pm SD. Statistical analysis for the first right figure in b (NAS score group) was performed by Kruskal-Wallis multiple comparisons test, statistical analysis for g was performed by Student's t test, and others were performed by one-way ANOVA with post-hoc multiple comparisons test. Two-sided p values < 0.05 were considered significant. **p < 0.01; NS, not significant. Source data, including exact p values, are provided as a Source data file.



Figure S3. cDNT transfer prevented NASH development in CD-HFD-fed mice.

C57BL/6 mice were fed a CD-HFD for 12 weeks, received a transfer of cDNT, and were then continuously fed the CD-HFD for another 4 weeks. a Body weight of mice from each group before (12 weeks) and after (16 weeks) cDNT transfer. NCD, black; CD-HFD, red; CD-HFD + cDNT, blue. Actual p values (left \rightarrow right): 0.00012, 0.97, 0.000003, 0.043. b Fasting plasma glucose levels were examined in mice fed a CD-HFD for 12 or 16 weeks. Actual p values (left→right): 0.000017, 0.99, 4.08e-7, 0.00066. c Representative and statistical analysis of H&E staining, oil red o staining, Sirius red staining and α -SMA staining in liver paraffin sections. Quantification of liver histology staining. Scale bars, 100 μ m. Actual p values (left \rightarrow right): for NAS score, 0.00617, 0.046; for oil red o staining, 1.62e-8, 0.000038; for Sirius red staining, 0.000055, 0.00050; for α-SMA staining, 0.0020, 0.0073. d Plasma ALT, AST, and TG levels were measured in NCD- and CD-HFD-fed mice. Actual p values (left-right): 0.000003, 0.00024, 0.000016, 0.00011, 0.020. e Hydroxyproline levels in liver tissues of each group. Actual p values (left \rightarrow right): 0.00026, 0.018. Data are presented as the mean \pm SD. NCD group, n = 5 mice/group; CD-HFD group, n = 4 mice/group; CD-HFD + cDNT group, n = 4 mice/group. Statistical analysis for the first right figure in c (NAS) score group) was performed by Kruskal-Wallis multiple comparisons test, and others were performed by one-way ANOVA with post-hoc multiple comparisons test. Twosided p values < 0.05 were considered significant. *p < 0.05; **p < 0.01; NS, not significant. Source data, including exact p values, are provided as a Source data file.



Figure S4. Representative flow cytometry images of the gating strategy used for flow cytometry analysis.

a Typical flow cytometry gating strategy to distinguish hepatic lymphocytes. **b** Typical flow cytometry gating strategy to distinguish hepatic macrophages and Kupffer cells.



Figure S5. The tissue distribution of transferred CD45.1⁺ cDNT in MCD-fed mice.

a and **b** Representative flow cytometry plots (**a**) and statistical analysis (**b**) of the percentages of CD45.1⁺ cDNT in blood, spleen, liver and different lymph nodes of MCD-fed mice every week. Actual p values (left \rightarrow right): **b** 0.0057, 0.000008, 0.000032, 0.015, 0.029, 0.019. **c** Relative mRNA levels of *CXCL9* and *CXCL10* in liver and adipose tissues of NCD-, HFD-, MCD-, and CD-HFD-fed mice. Actual p values (left \rightarrow right): 0.00018, 4.78e-7, 0.00034, 0.000003, 0.037, 1.21e-8, 0.000088, 0.043, 0.00042. Data are presented as the mean \pm SD; n \geq 5 mice/group. Statistical analysis was performed by one-way ANOVA with post-hoc multiple comparisons test. Two-sided p values < 0.05 were considered significant. *p < 0.05; **p < 0.01. Source data, including exact p values, are provided as a Source data file.



Figure S6. The cytokines secretion and survival of cDNT in vitro and in vivo.

a-c The cytokine secretion and immunoregulatory molecules expression profile and statistical analysis of activated CD4⁺ T cells (red) and cDNT (blue) *in vitro*. ($n \ge 7$

biologically independent samples per group). Actual p values (left \rightarrow right): **b** 0.000002, 0.012, 0.00023, 0.000021, 0.0022, 0.00060, 0.0084; **c** 1.11e-10. **d** Representative flow cytometry plots and statistical analysis of the cytokine secretion and Granzyme B (GZMB) expression in transferred cDNT *in vivo*. (**n** = 5 biologically independent samples per group). **e** and **f** The Annexin V and Ki67 staining of transferred cDNT in liver tissues (**n** = 5 biologically independent samples per group). **g** CD4 and CD8 staining of cDNT after transfer at 3 and 4 weeks (**n** = 5 biologically independent samples per group). Data are presented as the mean ± SD. Statistical analysis was performed by one-way ANOVA with post-hoc multiple comparisons test. Two-sided p values < 0.05 were considered significant. *p < 0.05; **p < 0.01. Source data, including exact p values, are provided as a Source data file.



Figure S7. The proportions of lymphocytes in different tissues of HFD-fed mice and the suppression of Th17 cells by cDNT *in vitro*.

a-c Percentages of CD4⁺ T-cells, CD8⁺ T-cells, DNT, NK cells, and NKT cells relative to the total number of CD45.2⁺ cells in spleen, blood and draining lymph nodes (n = 6 mice/group). NCD, black; HFD, red; HFD + cDNT, blue. Actual p values (left→right): **a** 0.024, 0.022, 0.017; **c** 0.0023, 0.025, 0.014. **d** and **e** Representative images (**e**) and statistical analysis (**d**) of CD45, CD3, CD20 staining in liver paraffin sections of HFDfed mice in each group (n = 6 mice/group). Scale bars, 100 µm. Actual p values (left→right): **d** 0.000011, 0.012, 3.43e-7, 0.000022. **f** and **g** Naïve CD45.1⁺ CD4⁺ T cells were induced to Th17 for 3 days and cocultured with CD45.2⁺ cDNT for 24 hours. Representative flow cytometry plots (**f**) and statistical analysis (**g**) of Annexin V⁺ cells and IL-17⁺ cells relative to the total CD45.1⁺CD4⁺ T cells in each group (n = 5 biologically independent samples per group). Actual p values (left→right): **g** 0.046, 0.0064, 0.024, 0.00055. Data are presented as the mean ± SD. Statistical analysis was performed by one-way ANOVA with post-hoc multiple comparisons test. Two-sided p values < 0.05 were considered significant. *p < 0.05; **p < 0.01. Source data, including exact p values, are provided as a Source data file.



Figure S8. The immunohistochemistry of different immune cells in liver and VAT of NASH models.

a Representative images and statistical analysis of CD11b and F4/80 staining in liver paraffin sections of HFD-fed mice in each group. Scale bars, 100 µm. Actual p values (left→right): 3.62e-7, 0.00015, 0.000004, 0.000038. **b** Quantification of CD45, CD3, CD20, CD11b and F4/80 staining in VAT paraffin sections of each group. Actual p values (left→right): 5.80e-9, 5.80e-9, 1.40e-7, 6.30e-7, 0.00048, 0.0015, 5.89e-9, 6.25e-9, 5.29e-8, 3.17e-7. Data are presented as the mean \pm SD; n = 6 mice/group. Statistical analysis was performed by one-way ANOVA with post-hoc multiple comparisons test. Two-sided p values < 0.05 were considered significant. *p < 0.05; **p < 0.01. Source data, including exact p values, are provided as a Source data file.



Figure S9. cDNT decreased the proportion of infiltrating macrophages and the secretion of proinflammatory cytokines in MCD-fed mice.

a Representative flow cytometry plots (left) and statistical analysis (right) of macrophages, Kupffer cells, neutrophils, and pDCs relative to intrahepatic CD45.2⁺

cells in each group. NCD, black; MCD, red; MCD + cDNT, blue. Actual p values (left \rightarrow right): 1.55e-7, 0.0030, 0.012, 0.000003. b Statistical analysis of M1 and M2 macrophages and Ly6C^{high} cells (%CD45.2⁺ cells) in mouse livers from each group, as determined by flow cytometry. Actual p values (left→right): 1.63e-7, 0.00060, 1.41e-8, 0.018. c Absolute numbers of M1 and M2 macrophages and Ly6C^{high} cells in mouse livers from each group. Actual p values (left \rightarrow right): 0.00019, 0.023, 0.00012, 0.046. **d-g** Representative flow cytometry plots (**d**) and statistical analysis of the percentages of TNF- α^+ (e), IL-6⁺ (f), and IL-10⁺ (g) cells among macrophages and Kupffer cells from livers of NCD- and MCD-fed mice with or without cDNT. Actual p values (left→right): e 3.18e-8, 8.22e-7, 0.0059; f 0.0051, 0.049; g 0.0059, 0.00052. h Western blot analysis of TNF- α , IL-10 in liver tissues of MCD-fed mice. Actual p values (left-right): 0.0024, 0.0056, 0.0012, 0.66. i Relative mRNA levels of the indicated genes in livers from NCD- or MCD-fed mice. Actual p values (left→right): 0.000019, 0.000659, 0.000002, 0.000032, 0.00060, 0.0045, 0.000010, 0.036, 0.0068, 0.000034, 0.00038, 0.0092, 0.0037, 9.24e-8, 0.00049. Data are presented as the mean \pm SD; n \geq 5 mice/group. Statistical analysis was performed by one-way ANOVA with post-hoc multiple comparisons test. Two-sided p values < 0.05 were considered significant. *p < 0.05; **p < 0.01; NS, not significant. Source data, including exact p values, are provided as a Source data file.



Figure S10. cDNT transfer decreased the proportion of infiltrating macrophages and the secretion of proinflammatory cytokines in CD-HFD-fed mice.

a Statistical analysis of the percentages of intrahepatic macrophages and Kupffer cells among CD45.2⁺ cells in each group. NCD, black; CD-HFD, red; CD-HFD + cDNT, blue. Actual p values (left \rightarrow right): 0.000516, 0.021. **b** Statistical analysis of M1 and M2 macrophages (%CD45.2⁺ cells) in the livers of mice from each group, as determined by flow cytometry. Actual p values (left \rightarrow right): 0.000007, 0.00087. **c** Absolute numbers of M1 and M2 macrophages in mouse livers from each group. Actual p values (left \rightarrow right): 1.42e-7, 0.000011. **d** The secretion of TNF- α , IL-6, and IL-10 by infiltrating macrophages was determined by flow cytometry. Data are presented as the mean \pm SD; n = 5 mice/group. Statistical analysis was performed by one-way ANOVA with post-hoc multiple comparisons test. Two-sided p values < 0.05 were considered significant. *p < 0.05; **p < 0.01. Source data, including exact p values, are provided as a Source data file.



Figure S11. The expression of mTOR pathway after IL-10 stimulation and the roles of mTOR pathway in cDNT cytokine secretion.

a and **b** Representative flow cytometry plots (**a**) and statistical analysis (**b**) of pi-mTOR expression in cDNT with or without IL-10 stimulation (n = 8 biologically independent samples per group). **c** The cytokines secretion from cDNT cells after IL-10 (red) or rapamycin (blue) stimulation (n = 11 biologically independent samples per group). *p = 0.032. Statistical analysis was performed by one-way ANOVA with post-hoc multiple comparisons test. Two-sided p values < 0.05 were considered significant. Source data, including exact p values, are provided as a Source data file.

Gene	Strand	Primer sequence (5'-3')
a-SMA	Sense	ATCGTCCACCGCAAATGC
	Antisense	AAGGAACTGGAGGCGCTG
Collal	Sense	CTGCTGGCAAAGATGGAGA
	Antisense	ACCAGGAAGACCCTGGAATC
Col3a1	Sense	CAAATGGCATCCCAGGAG
	Antisense	CATCTCGGCCAGGTTCTC
TGFb	Sense	GAGGTCACCCGCGTGCTA
	Antisense	TGTGTGAGATGTCTTTGGTTTTCTC
Tbx21	Sense	GTTCCCATTCCTGTCCTTCA
	Antisense	ACCCACTTGCCGCTCTG
Gata3	Sense	GGGTTCGGATGTAAGTCGAG
	Antisense	CCACAGTGGGGTAGAGGTTG
RORYT	Sense	TACCTTGGCCAAAACAGAGG
	Antisense	ATGCCTGGTTTCCTCAAAA
Foxp3	Sense	CACCCAGGAAAGACAGCAACC
	Antisense	GCAAGAGCTCTTGTCCATTGA
IL-4	Sense	AGC AGT TCC ACA GGC ACA AG
	Antisense	AGC AGT TCC ACA GGC ACA AG
IL-15	Sense	CATCCATCTCGTGCTACTTGTG
	Antisense	GCCTCTGTTTTAGGGAGACCT
IL-17	Sense	GCTCCAGAAGGCCCTCAGACT
	Antisense	CCAGCTTTCCCTCCGCATTGA
Ifng	Sense	GGCCATCAGCAACAACATAAGCGT
	Antisense	TGGGTTGTTGACCTCAAACTTGGC
F4/80	Sense	CTTTGGCTATGGGCTTCCAGTC
	Antisense	GCAAGGAGGACAGAGTTTATCGTG
Inos	Sense	ATCTTTGCCACCAAGATGGCCTGG
	Antisense	TTCCTGTGCTGTGCTACAGTTCCG
Arg-1	Sense	TGACTGAAGTAGACAAGCTGGGGAT
	Antisense	CGACATCAAAGCTCAGGTGAATCGG
H2-Dma	Sense	TGAAGGTCAAATCCCAGTGTCC
	Antisense	AGCGGTCAATCTCGTGTGTCAC
H2-DMb1/2	Sense	CAACAAGGAGAAGACGGCTCA
	Antisense	CGCTGTGCTGAACCACG
H2-IA-a	Sense	AGGCTTCCTGAGTTTGG
	Antisense	AGGGTGTTGGGCTGAC
TLR4	Sense	ACCTGGCTGGTTTACACGTC
	Antisense	CTGCCAGAGACATTGCAGAA
CCR2	Sense	TTTGTTTTTGCAGATGATTCAA
	Antisense	TGCCATCATAAAGGAGCCAT
Tnfa	Sense	TCCCAGGTTCTCTTCAAGGGA

Supplementary Table S1. Primer sequences used for real-time PCR

	Antisense	GGTGAGGAGCACGTAGTCGG
IL-10	Sense	AGAAAAGAGAGCTCCATCATGC
	Antisense	TTATTGTCTTCCCGGCTGTACT
IL4Ra	Sense	AGCGGGCACGATAACT
	Antisense	CTACCACCTGACCACCAA
IL6Ra	Sense	CCCTTGTCAACGCCATCT
	Antisense	AAACAGCACAGCCTTCG
IL10Ra	Sense	GCCTCACGACTTCTTCC
	Antisense	CCTCCAGGTCCACGAT
IL17Ra	Sense	CAGCATCACCGTAAGCG
	Antisense	CCTCACAGTCAGGCACAA
Ifngr1	Sense	TTGACGAGCACTGAGGA
	Antisense	AGGAACCCGAATACACC
TNFR1	Sense	GACCGGGAGAAGAGGGATAG
	Antisense	GTTCCTTTGTGGCACTTGGT
TNFR2	Sense	GTCCAGAATCTCCCTCCTT
	Antisense	CAGCCTGCCTGTAACCT
Bcl-2	Sense	GGAAGGTAGTGTGTGTGGG
	Antisense	ACTCCACTCTCTGGGTTCTTGG
Bcl-xl	Sense	AACATCCCAGCTTCACATAACCCC
	Antisense	GCGACCCCAGTTTACTCCATCC
Cytc	Sense	CACGCTTTACCCTTCGTTCT
	Antisense	CTCATTTCCCTGCCATTCTCTA
EpCAM	Sense	AGGGGCGATCCAGAACAACG
	Antisense	ATGGTCGTAGGGGCTTTCTC
Cflar	Sense	TGGAATACCGTGACAGTC
	Antisense	CTTGCATATCGGCGAAC
Spi1	Sense	TCAGATGAGGAGGAGGGGGG
	Antisense	TTGGACGAGAACTGGAAGG
NKG2A	Sense	ACTCATTGCTGGTACCCTGGG
	Antisense	GAGGACAAGGCTGTGCTGAAG
NKG2D	Sense	ACGTTTCAGCCAGTATTGTGC
	Antisense	GGAAGCTTGGCTCTGGTTC
Prf1	Sense	CTGCCACTCGGTCAGAATG
	Antisense	CGGAGGGTAGTCACATCCAT
NKp46	Sense	CACAGGAGGTGTTGAGAA
	Antisense	AAGAAGTAGGGTCGGTAG
KLRG1	Sense	TCTCATCCCTTCCTCTGC
-	Antisense	TTGCGTCTTTCTGTCTTGT
Slamf6	Sense	CAGCTAATGAATGGCGTTCTAGG
	Antisense	CTTAGGTTGATAACGAGGGCAG
Sh2d1b2	Sense	ATGGTGGTTCATCTTTCA
	Antisense	TTCAGTCTTCTCGCTCC
CD94	Sense	GGCAGTTTCTAGGATCACTCG
	Antisense	CTTCCTGGAATTCTACAGTGGT

CD107a	Sense	AGGCTCCACTGATTTGACT
	Antisense	TGCTCCCGTTTGCTTC
CD226	Sense	ACCACATGGCTTTCTTGCTC
	Antisense	CAGCATGAGAGTTGGACCAG
Fasl	Sense	TGAATTACCCATGTCCCCAG
	Antisense	AAACTGACCCTGGAGGAGCC
Lair1	Sense	GCTGGTTTGCCTTATC
	Antisense	AGTGGTCCTCTGGGTAT
GAPDH	Sense	AAGGTCATCCCAGAGCTGAA
	Antisense	CTGCTTCACCACCTTCTTGA
CXCL9	Sense	TGTGGAGTTCGAGGAACCCT
	Antisense	TGCCTCGGCTGGTGCTG
CXCL10	Sense	ATCCCTGCGAGCCTATC
	Antisense	GCCATCCACTGGGTAAA

Supplementary Materials and Methods

Reagents and antibodies

The LEGENDplex Mouse Th Cytokine Panel was purchased from BioLegend (CA, USA). Mouse T-cell enrichment columns were obtained from R&D Systems (MN, USA). Anti-PE microbeads and magnetic bead separation columns were obtained from Miltenyi Biotec (Bergisch Gladbach, Germany). ALT, AST, and TG detection kits were purchased from NanJing JianCheng Biochemical Institute (Jiang Su, China). Collagenase IV and bovine serum albumin (BSA) were obtained from Sigma (MO, USA). Percoll was purchased from GE Healthcare (USA). Fluorochrome-conjugated antibodies against mouse CD3, CD4, CD8, CD11b, CD11C, CD25, CD45.1, CD45.2, CD69, CD206, Ter119, B220, Ly6C, Ly6G, MHC II, NK1.1, TCRαβ, Foxp3, IL-6, IL-10, IL-17, IFN-γ, TNF-α, PD-1, Tim3, and CTLA4 were obtained from BioLegend. Antibodies against B-cell lymphoma 2 (Bcl-2) and B-cell lymphoma-extra large (BclxL) were purchased from Cell Signaling Technology (MA, USA). Recombinant mouse IL-2, IL-4, IL-6, IL-10, IL-17, IFN- γ , TNF- α , and granulocyte-macrophage colonystimulating factor (GM-CSF) were obtained from Peprotech (NJ, USA). Mouse IL-10 neutralizing antibody was obtained from R&D Systems, anti-mouse CD210 (IL-10R) neutralizing antibody was obtained from BioLegend, and mouse Qa-1b neutralizing antibody was purchased from BD Biosciences (CA, USA). The Annexin V-PE kit was purchased from BD Biosciences, the EdU staining kit was purchased from RiboBio Corporation (Guangzhou, China), and the Caspase-3 staining kit was purchased from Beyotime Biotechnology (Shanghai, China).