

Microbiota-derived H₂S induces c-kit⁺ cDC1 autophagic cell death and liver inflammation in metabolic dysfunction-associated steatohepatitis

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Supplementary Fig. 1. Gating strategy and antibody availability of mass cytometry.

Supplementary Fig. 2. Changes in hepatic myeloid composition in MASH mice and the change of c-kit⁺ cDC1.

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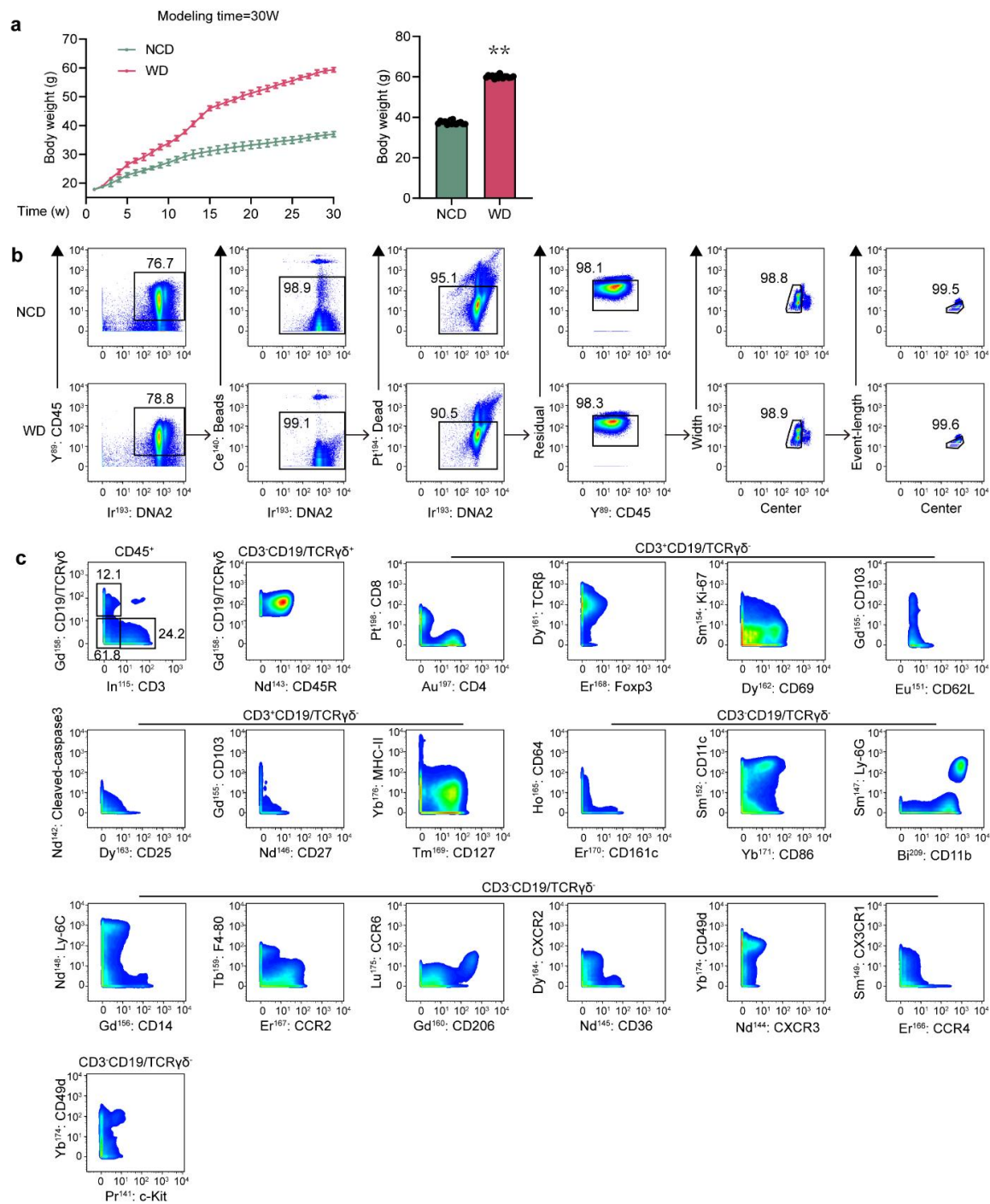
Supplementary Fig. 10. The efficiency of inducing c-kit⁺ DCs from mouse BM stem cells *in vitro*.

Supplementary Table 1. Baseline characteristics of patients.

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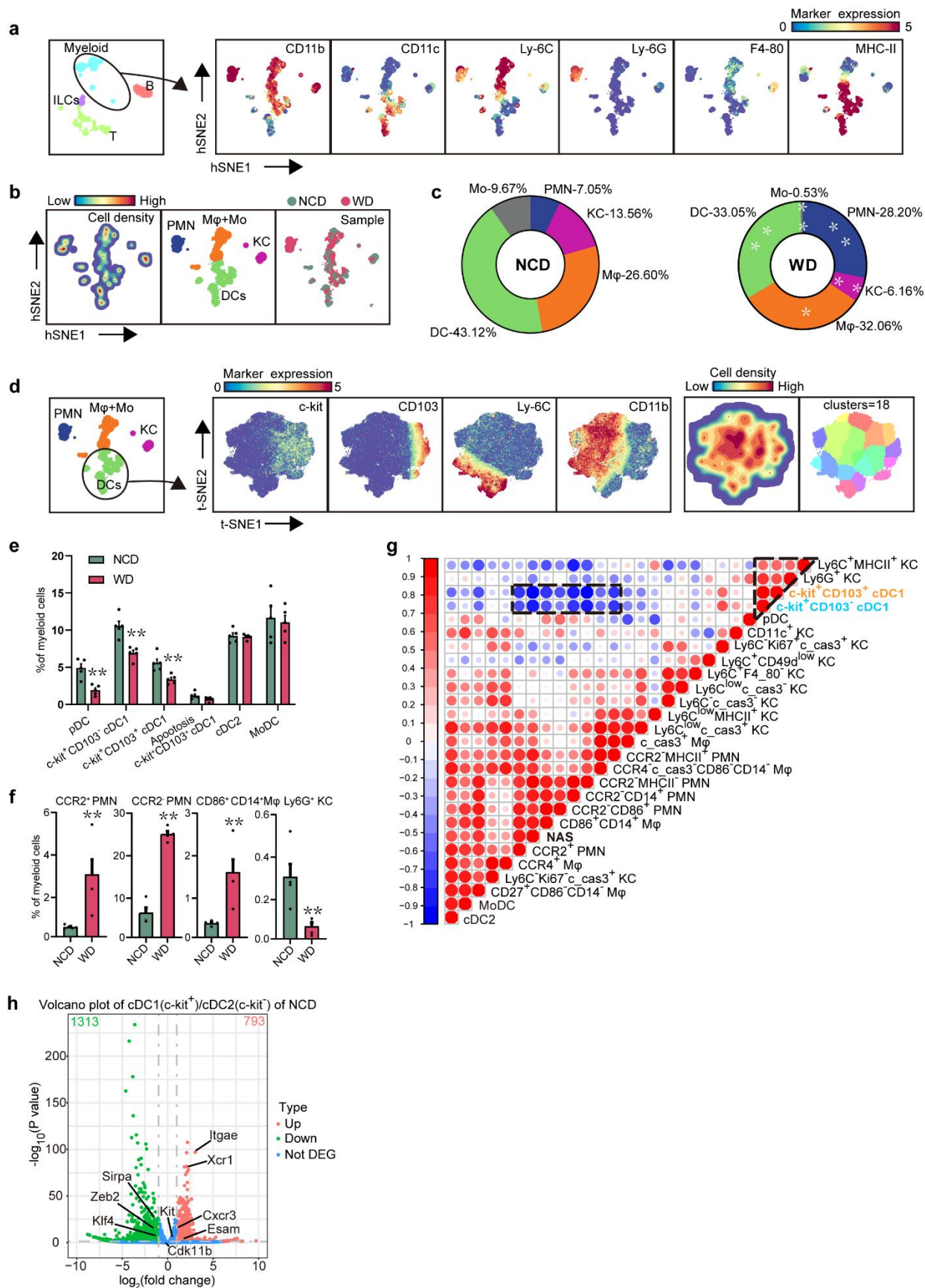
Supplementary Table 3. Antibodies used for cell suspension mass cytometry.

Supplementary Table 4. Antibodies used in flow cytometry.

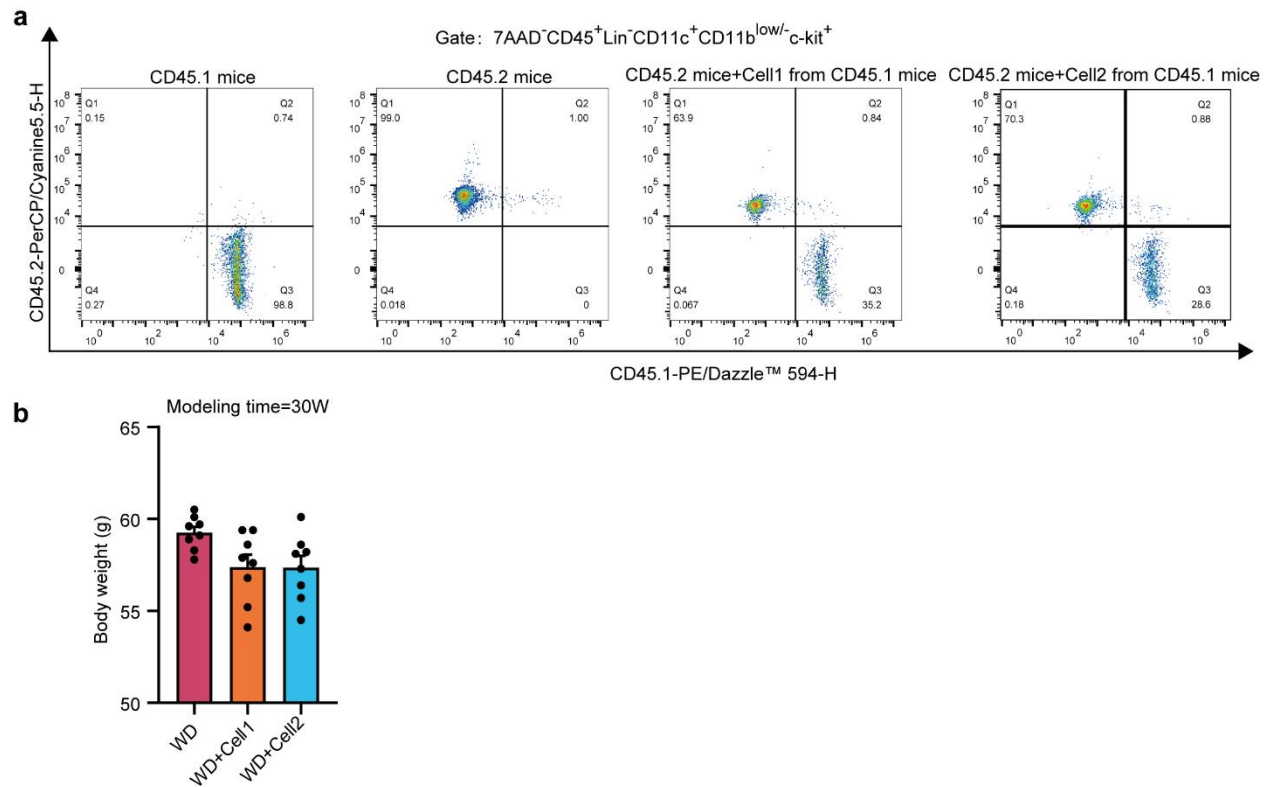


Supplementary Fig. 1. Gating strategy and antibody availability of mass cytometry.

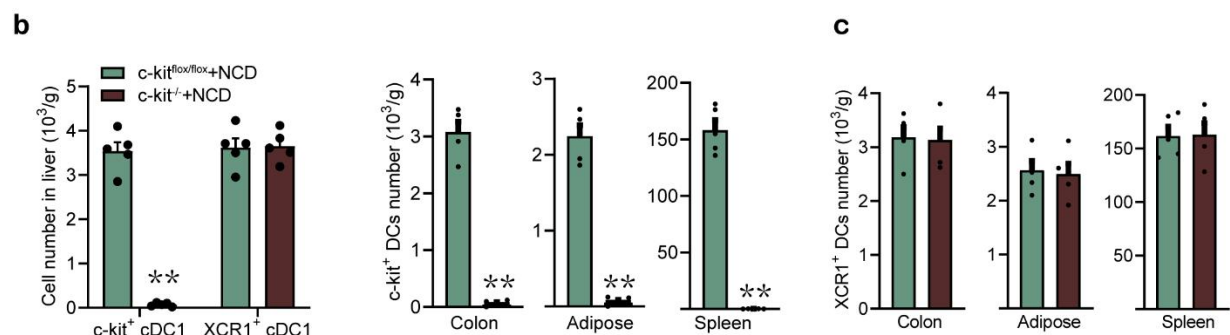
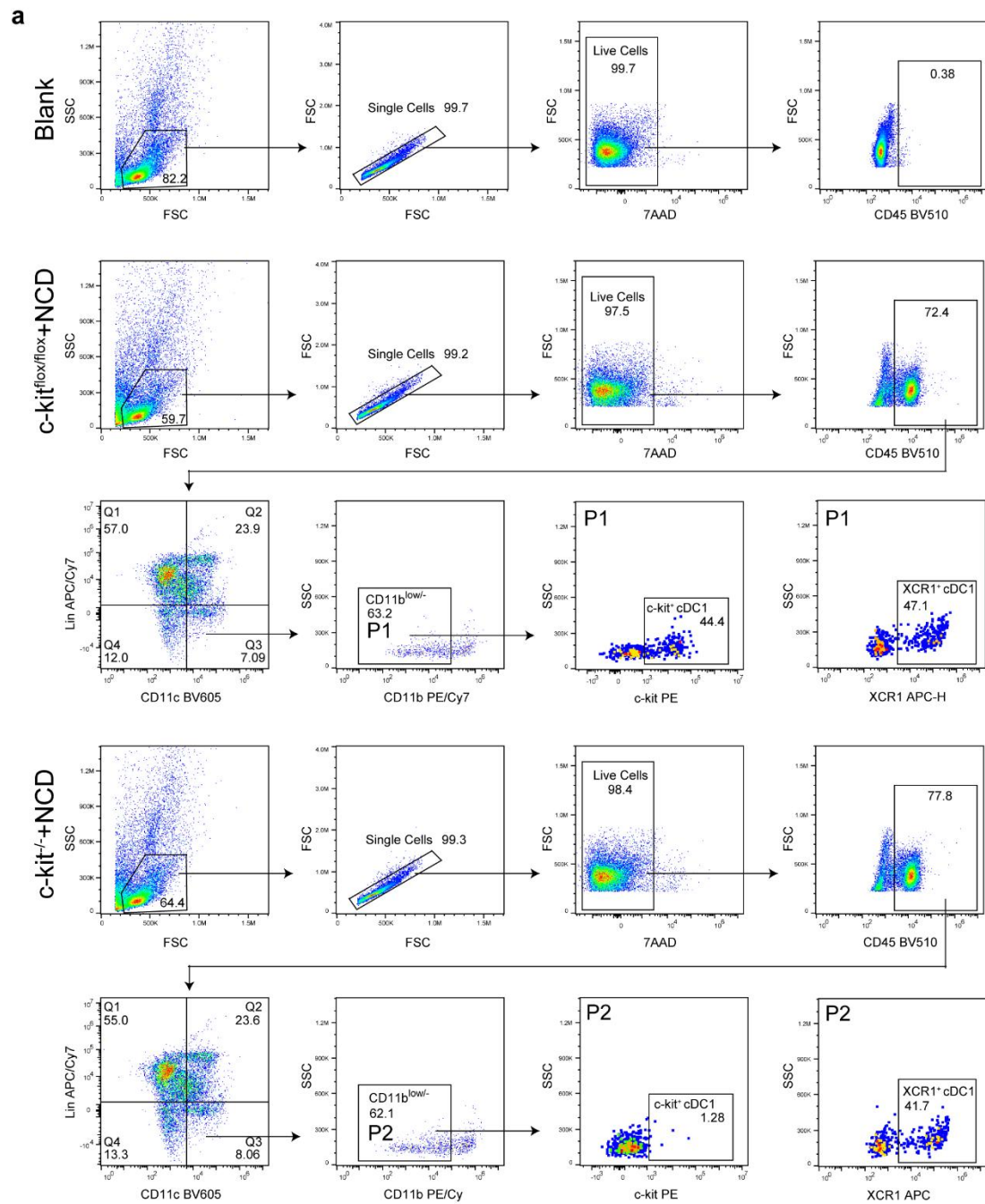
(a) Body weight of NCD and WD mice. $n = 15/\text{group}$. Data were shown as Mean $\pm$ SD and analyzed using a two-tailed unpaired Student's t-test (a). Significance levels were reported as $**P < 0.01$. (b) Representative gating strategy. The obtained mass cytometry data were processed with immune cells, bead-free cells, live cells, single cells 1, single cells 2, and single cells 3, respectively. (c) Antibody availability. The availability of antibodies was shown in different immune cell lineages. The cell lineage name can be found above the density map of flow cytometry. Source data were provided as a Source Data file.



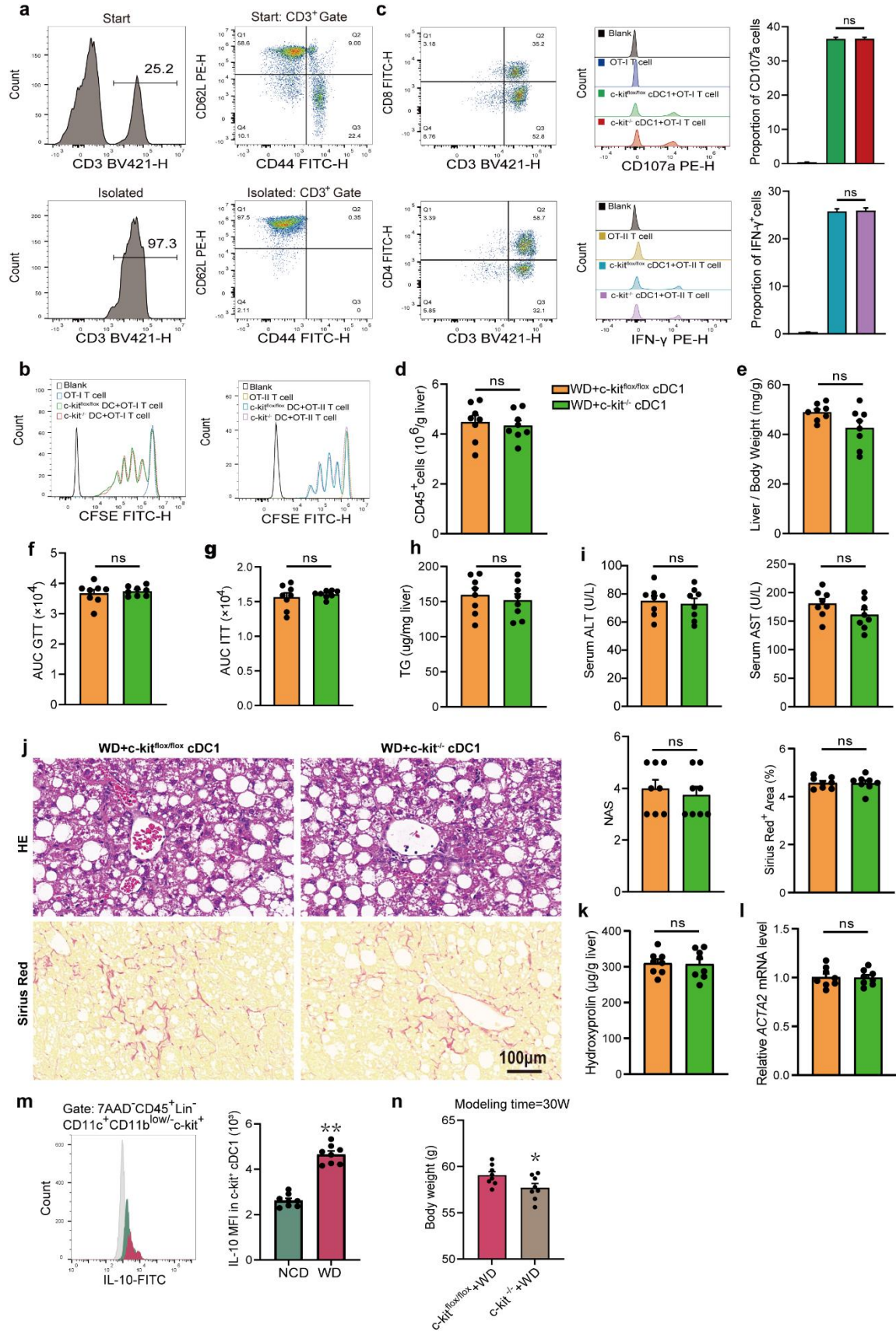
Supplementary Fig. 2. Changes in hepatic myeloid composition in MASH mice and the change of c-kit⁺ cDC1. Six-week-old male C57BL/6 mice were fed with a NCD or WD diet for a duration of 30 W. (a) hSNE plots showing protein expression in hepatic myeloid cells. (b) hSNE plots showing major populations of hepatic myeloid cells. n = 5/group. (c) Percentage of each population identified in b. (d) t-SNE plots showing c-kit, CD103, Ly-6C, and CD11b expression in DCs. (e) The proportion of different clusters of DCs in myeloid cells. (f) The proportion of CCR2⁺ PMN, CCR2⁻ PMN, CD86⁺CD14⁺ Mφ, and Ly6G⁺ KC in myeloid cells. (g) Correlation coefficients among the myeloid clusters proportion and NAS. Spearman's rank correlation coefficient test was performed. Red for positive correlation, blue for negative correlation, and the depth of color represented the strength of the correlation. The absolute value of correlation coefficient (r value) below 0.3 was weak or no correlation, between 0.3 and 0.5 was weak, between 0.5 and 0.8 was moderate, and above 0.8 was strong correlation. (h) Volcano plot of mRNA abundance in hepatic c-kit⁺ vs c-kit⁻ cDC1 of mice fed with NCD for 30 W. Red for up-regulated and green for up-regulated. Data were analyzed using a two-tailed unpaired Student's t-test (c, e, f). Data were represented as mean ± SEM. Significance levels were reported as **P* < 0.05, ***P* < 0.01. Source data were provided as a Source Data file.



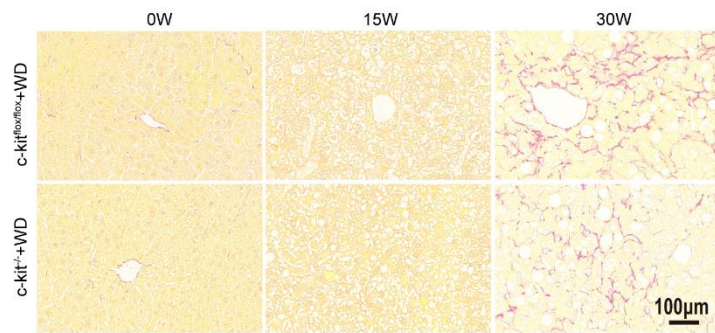
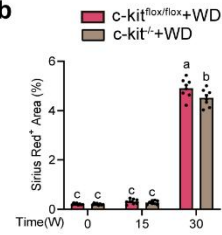
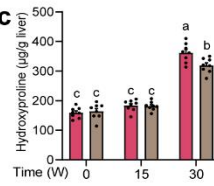
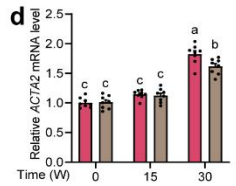
Supplementary Fig. 3. Adoption transfer experiment. (a) Validation of adoption transfer experiment. Six-week-old male C57BL/6 (CD45.2) mice were received weekly injections of the cell 1 and cell 2 via the tail vein for a total of 4 W. Cell 1 (c-kit⁺CD103⁺) and Cell 2 (c-kit⁺CD103⁻) were isolated from the liver and spleen of 6-week-old male CD45.1 mice. n = 3/group. (b) Body weight of WD, WD + Cell 1 and WD + Cell 2 mice at 30 W. n = 8/group. Data were represented as mean $\pm$ SEM. *P* values were calculated by one-way ANOVA with Tukey's post hoc test.



Supplementary Fig. 4. Flow cytometric analysis showing the knockout efficiency of c-kit in cDC. (a) Gating strategy and representative flow cytometry dot plots of XCR1⁺ cDC1 in the liver. n = 5/group. (b) The number of the c-kit⁺ cDC1 in the liver, colon, adipose, and spleen of flow cytometry data. n = 5/group. (c) The number of the cDC1 (labeled with XCR1) in the liver, colon, adipose, and spleen of flow cytometry data. Data were represented as mean $\pm$ SEM and analyzed using a two-tailed unpaired Student's t-test (b, c). $**P < 0.01$. Source data were provided as a Source Data file.

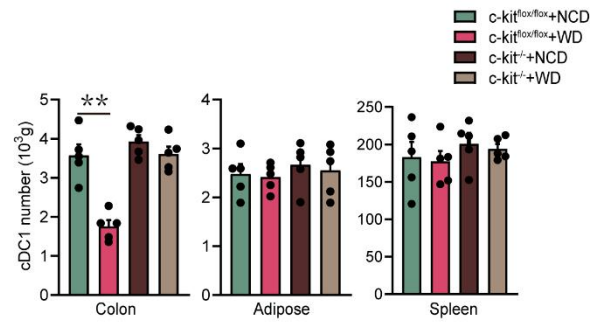
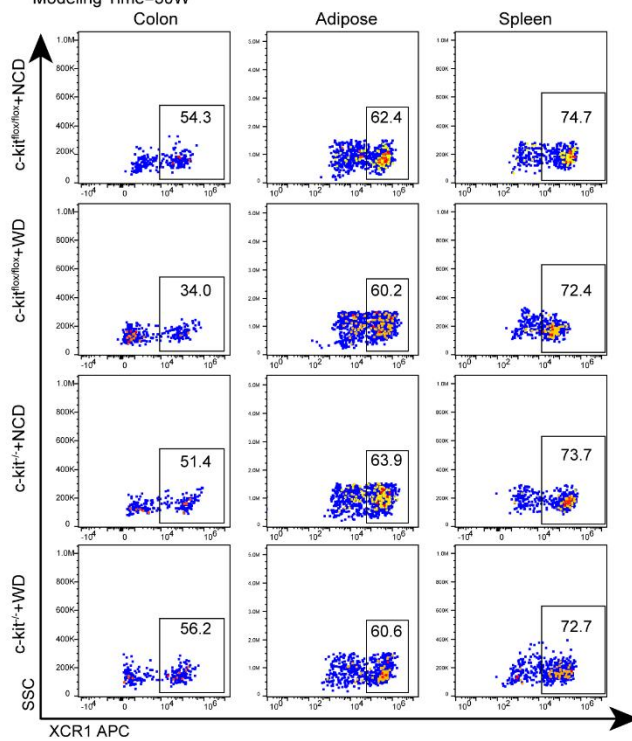


Supplementary Fig. 5. C-kit⁺ cDC1 function detection. (a) Purity detection of T cells sorted by magnetic beads. (b) T cell proliferation level after co-culture with cDC1. Each well contains a total of 20,000 cells, with a DC to T cell ratio of 1:4. (c) Activation of CD8⁺ T cells from OT-I or CD4⁺ T cells from OT-II after co-culture with cDC1. (d) Quantity of liver immune cells after adoptive transfer. (e) Liver cytokines content after adoptive transfer. (f) AUC of GTT. (g) AUC of ITT. (h) Liver TG content (i) Serum ALT and AST activity. (j) H&E, Sirius red staining and NAS. Magnification $\times 40$, scale bar = 100 μm . (k) Liver hydroxyproline content. (l) Liver *ACTA2* mRNA level. (m) The level of IL-10 expression in c-kit⁺ cDC1. (n) Body weight of c-kit^{flox/flox}+WD and c-kit^{-/-}+WD mice at 30 W. n = 8/group. Data were represented as mean $\pm$ SEM. *P* values were calculated by a two-tailed unpaired Student's t-test (d-n) or one-way ANOVA with Tukey's post hoc test (c). ns, not significant, $**P < 0.01$. TG, triglyceride; AUC, area under curve; GTT, glucose tolerance test; ITT, insulin tolerance test; H&E, hematoxylin and eosin; NAS, NAFLD activity score; ALT, alanine aminotransferase; AST, aspartate aminotransferase. Source data were provided as a Source Data file.

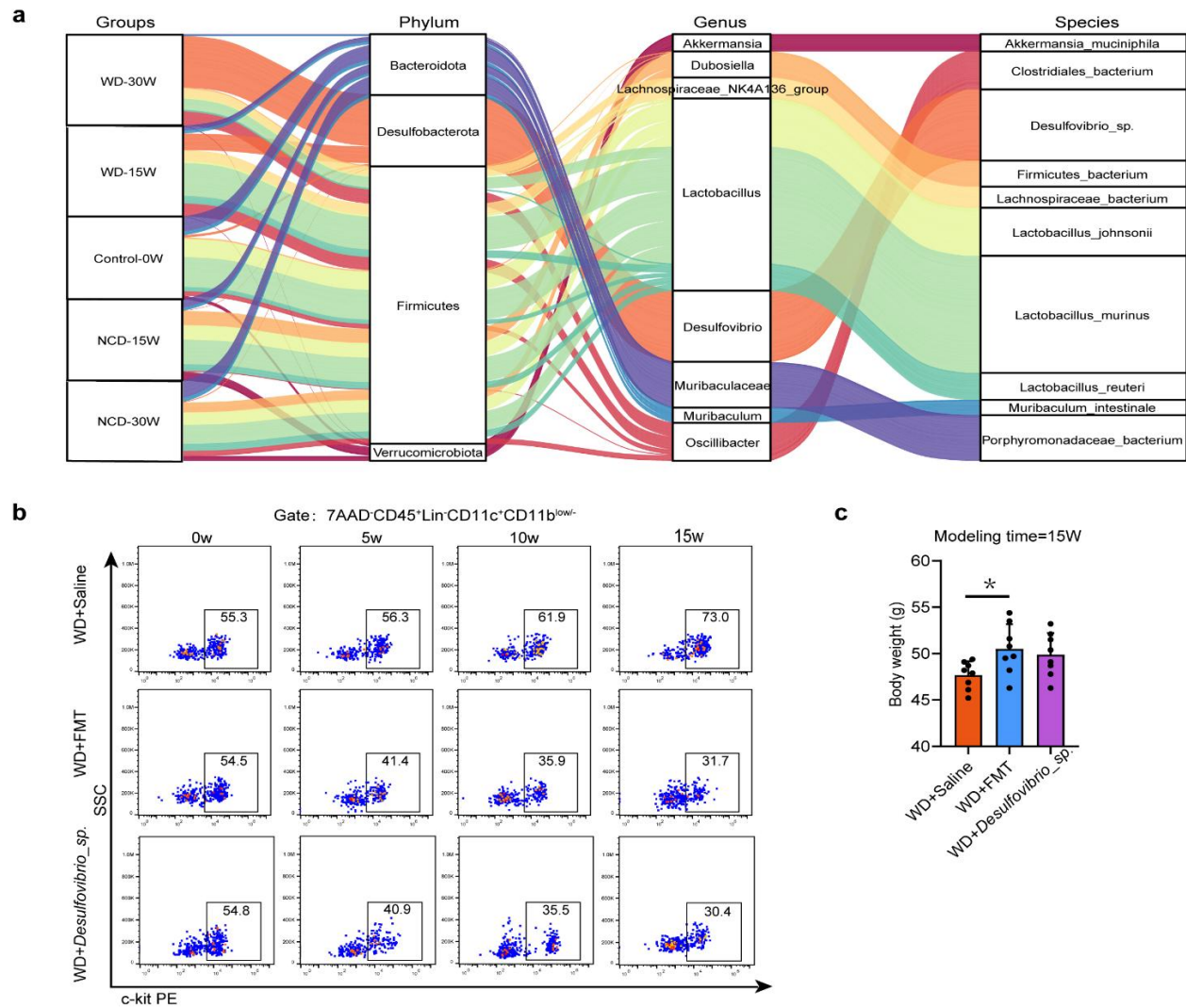
a**b****c****d****e**

Model Gate: 7AAD⁺CD45⁺Lin⁺CD11c⁺CD11b^{low/-}

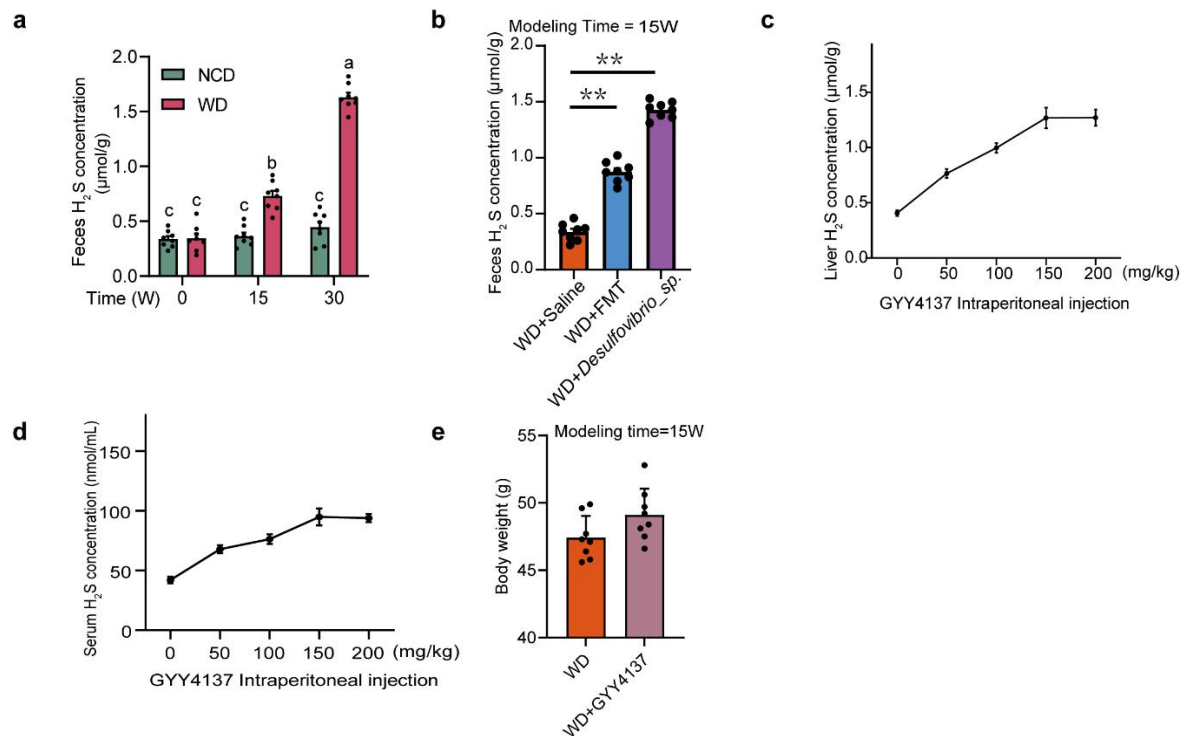
Modeling Time=30W



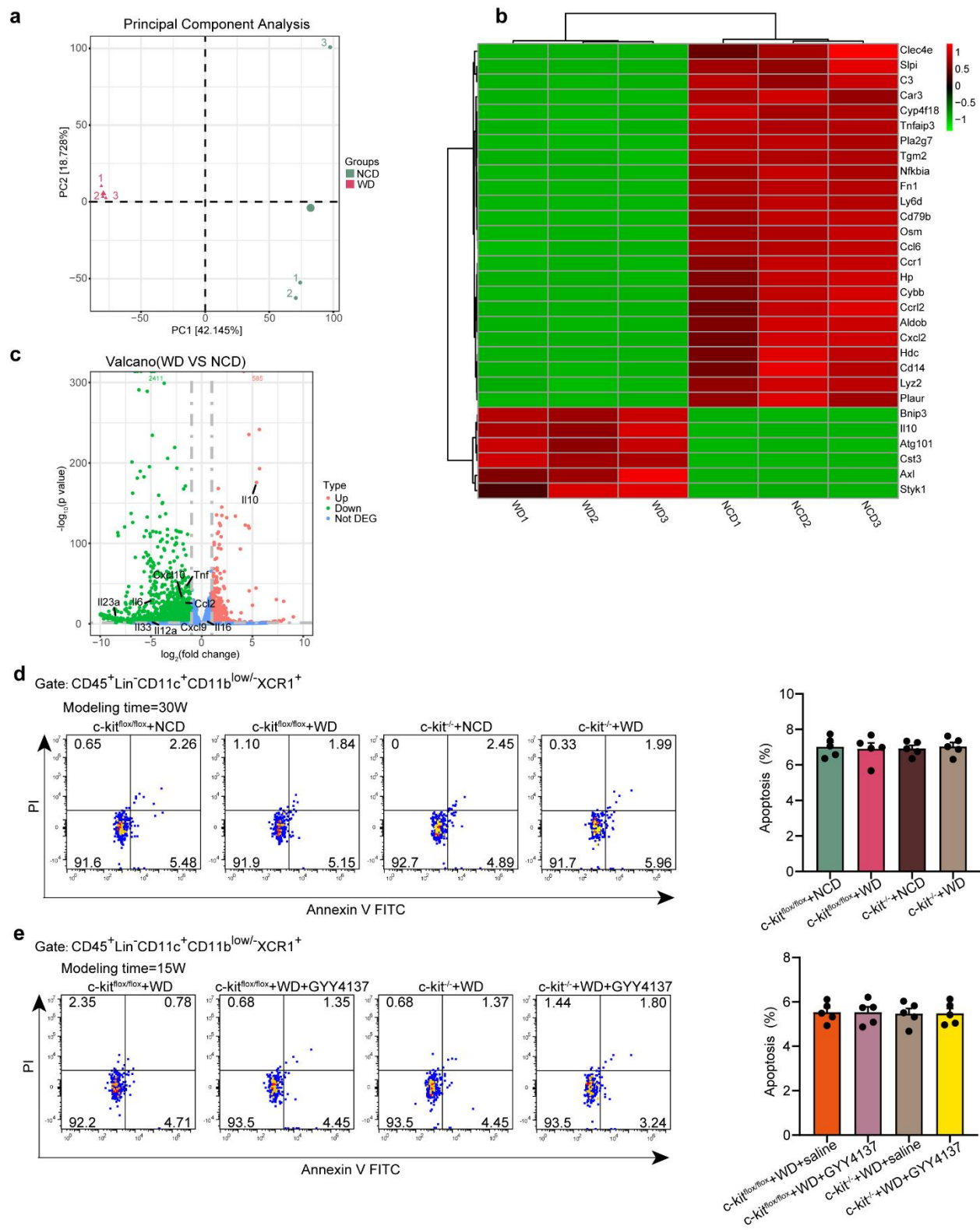
Supplementary Fig. 6. The effect of cDC1 specific knockout of c-kit on liver fibrosis and the quantity of cDC1 in various tissues of MASLD/MASH mice. (a-b) Sirius red staining. Magnification $\times 40$, scale bar = 100 μm . n = 8/group. (c) Liver hydroxyproline content. n = 8/group. (d) Liver *ACTA2* mRNA level. n = 8/group. (e) Quantity of XCR1⁺ cDC1 in different tissues. n = 5/group. Data were represented as mean $\pm$ SEM. *P* values were calculated by one-way ANOVA with Tukey's post hoc test (e) or two-way ANOVA with Tukey's post hoc test (b-d). Significance levels were reported as $^{**}P < 0.01$. a–c, groups sharing no common letters indicate statistically significant differences ($P < 0.05$), groups sharing partial letters indicate no significant difference ($P > 0.05$). Source data were provided as a Source Data file.



Supplementary Fig. 7. Changes in gut microbiota of mice fed with WD and the effects of microbiota transplantation on liver fibrosis index and cDC1 quantity (a) Top 10 abundance at the phylum level, genus level, and species level in mice fed with NCD or WD for different durations. (b) Quantity of hepatic c-kit⁺ cDC1. n = 5/group. (c) Body weight of WD+Saline, WD+FMT and WD+*Desulfovibrio_sp.* mice at 15 W. Data were represented as mean $\pm$ SEM. *P* values were calculated by one-way ANOVA with Tukey's post hoc test. Significance levels were reported as **P* < 0.05.

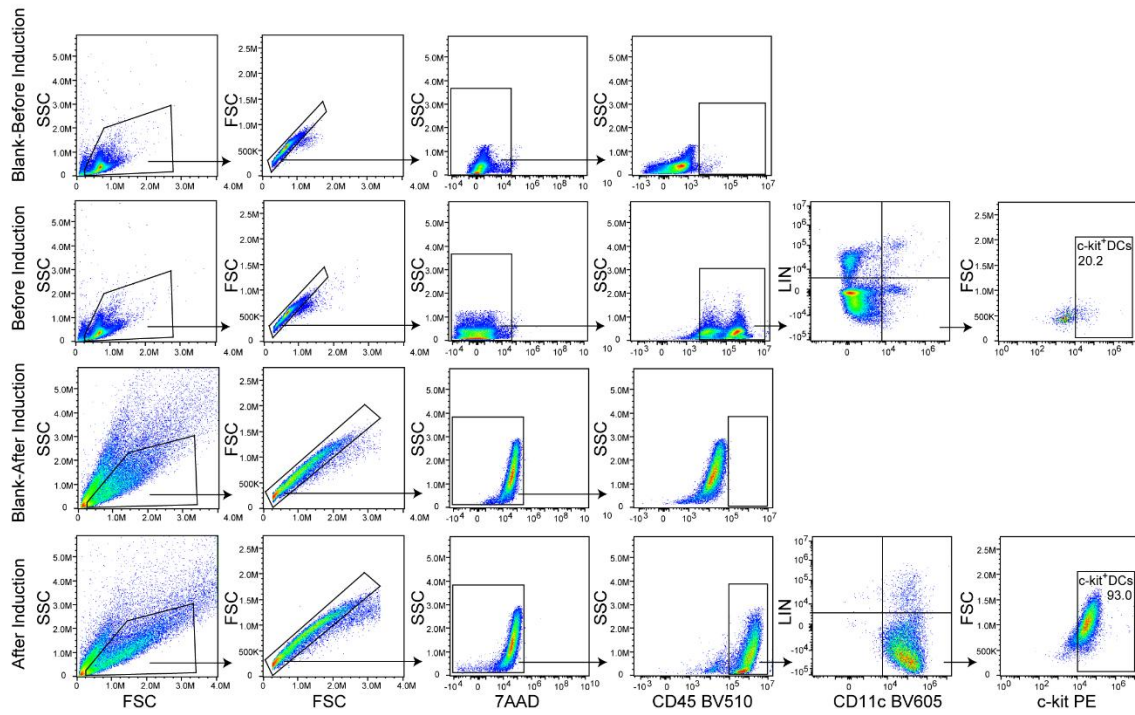


Supplementary Fig. 8. Feces H₂S concentration and selection of injection concentration for GYY4137. (a) Feces H₂S concentration of WT mice fed with NCD or WD for 0, 15, and 30 W. (b) Feces H₂S concentration of WT mice after microbial transplantation. (c) Liver H₂S concentration after GYY4137 administration. (d) Serum H₂S concentration after GYY4137 administration. *n* = 3/group. Data were represented as mean ± SEM. *P* values were calculated by two-way ANOVA with Tukey's post hoc test (a), one-way ANOVA with Tukey's post hoc test (b), or two-tailed unpaired Student's *t*-test (e). Significance levels were reported as ***P* < 0.01. a–c, groups sharing no common letters indicate statistically significant differences (*P* < 0.05), groups sharing partial letters indicate no significant difference (*P* > 0.05). Source data were provided as a Source Data file.

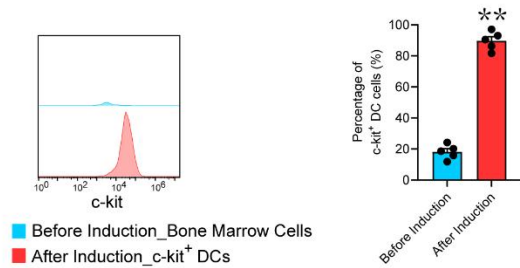


Supplementary Fig. 9. H₂S reduces hepatic anti-inflammatory c-kit⁺ cDC1 through an ACD mechanism rather than apoptosis in MASH mice. (a-c) Six-week-old male C57BL/6 mice were fed with a NCD or WD diet for a duration of 30 W. (a) PCA plot of transcriptome sequencing analysis. n = 3/group. (b) Top 30 gene. (c) Volcano plot showing expression of DC-related cytokines of hepatic c-kit⁺ cDC1. Red for up-regulated and green for up-regulated. (d and e) Apoptotic detection of XCR1⁺ cDC1. (d) Apoptotic ratio of XCR1⁺ cDC1 from NCD and WD mice for 30 W. n = 5/group. (e) Apoptotic ratio of XCR1⁺ cDC1 from NCD and WD mice for 15 W with or without GYY4137 treatment. Data were represented as mean ± SEM. *P* values were calculated by one-way ANOVA with Tukey's post hoc test (d, e). Source data were provided as a Source Data file.

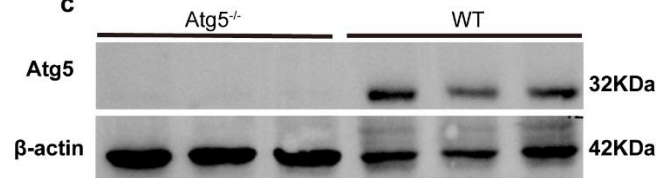
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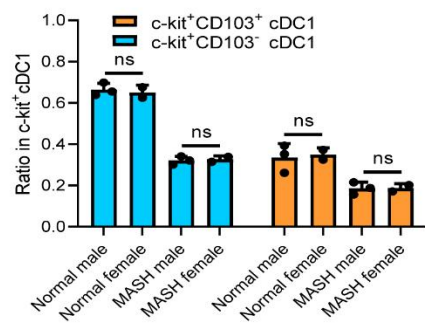
b



c



d



Supplementary Fig. 10. The efficiency of inducing c-kit⁺ DCs from mouse BM stem cells *in vitro*. (a) Gating strategy and representative flow cytometry dot plots of c-kit⁺ DCs. (b) Representative flow cytometry histograms showing the expression of c-kit (left) and the percentage of c-kit⁺ DCs before and after induction (right). (c) The knockout efficiency of Atg5 in DCs. n=3/group. Data were represented as mean $\pm$ SEM. *P* values were calculated by a two-tailed unpaired Student's t-test (b). Significance levels were reported as, ns, not significant, ***P* < 0.01. Source data were provided as a Source Data file.

Supplementary Table 1. Baseline characteristics of patients.

	Normal (n = 5)	MASH (n = 5)	<i>P</i> value
Age (y)	50.40 ± 3.12	47.40 ± 5.84	0.6624
Gender			
Male	3	3	NA
Female	2	2	NA
BMI (kg/m ²)	21.34 ± 1.12	31.02 ± 0.97	0.0002
NAS	0.40 ± 0.24	6.60 ± 0.24	< 0.0001
Steatosis	0.00 ± 0.00	2.80 ± 0.20	< 0.0001
Ballooning	0.00 ± 0.00	1.60 ± 0.24	0.0002
Lobular inflammation	0.40 ± 0.24	2.20 ± 0.37	0.0038

Values were shown as mean ± SEM. *P* values were calculated by a two-tailed unpaired Student's t-test. MASH, metabolic dysfunction-associated steatohepatitis; BMI, body mass index; NAS, NAFLD activity score. Source data were provided as a Source Data file.

Supplementary Table 2. Primer sequences.

Name	Primer sequences (5'-3')
β -actin	FOR: CGTTCAATACCCCAGCCATG; REV: GACCCCGTCACCAGAGTCC
<i>ACTA2</i> (α -SMA)	FOR: CCCTGAAGAGCATCCGACA REV: CATCTCCAGAGTCCAGCACAA

Supplementary Table 3. Antibodies used for cell suspension mass cytometry.

Marker	Metal	Clone	Vendor	Cat	Staining
CD45	89Y	30-F11	BioLegend	103102	surface
CD3e	115In	145-2C11	BioLegend	100302	surface
CD117(c-kit)	141Pr	2B8	BioLegend	105829	surface
cleaved caspase 3	142Nd	D3E9	CST	9664S	Intracellular
B220(CD45R)	143Nd	RA3-6B2	BioLegend	103202	surface
CD183(CXCR3)	144Nd	CXCR3-173	BioLegend	126502	surface
CD36	145Nd	HM36	BioLegend	102602	surface
CD27	146Nd	LG.3A10	BioLegend	124202	surface
Ly-6G	147Sm	IA8	BioLegend	127602	surface
Ly-6C	148Nd	HK1.4	BioLegend	128002	surface
CX3CR1	149Sm	SA011F11	BioLegend	149002	surface
CD62L	151Eu	MEL-14	BioLegend	104402	surface
CD11c	152Sm	N418	BioLegend	117302	surface
Ki67	154Sm	SolA15	eBioscience	14-5698-82	Intracellular
CD103	155Gd	2E7	BioLegend	121402	surface
CD14	156Gd	Sa14-2	BioLegend	123302	surface
CD19	158Gd	6D5	BioLegend	115502	surface

TCR $\gamma\delta$	158Gd	GL3	Homemade	NA	surface
F4/80	159Tb	C1:A3-1	Bio-RAD	MCA497G	surface
CD206	160Gd	C068C2	BioLegend	141702	Intracellular
TCR β	161Dy	H57-597	BioLegend	109235	surface
CD69	162Dy	H1.2F3	BioLegend	104502	surface
CD25(IL-2R)	163Dy	3C7	BioLegend	101902	surface
CD182(CXCR2)	164Dy	SA044G4	BioLegend	149302	surface
CD64(Fc γ RI)	165Ho	X54-5/7.1	BioLegend	139301	surface
CD194(CCR4)	166Er	2G12	BioLegend	131202	surface
CD192(CCR2)	167Er	475301	R&D	MAB55381	surface
FoxP3	168Er	FJK-16s	eBioscience	14-5773-82	Intracellular
CD127(IL-7Ra)	169Tm	A7R34	BioLegend	135002	surface
CD161c(NK1.1)	170Er	PK136	BioLegend	108701	surface
CD86	171Yb	GL-1	BioLegend	105002	surface
CD71	172Yb	R17217	BioLegend	113802	surface
CD49d	174Yb	R1-2	BioLegend	103629	surface
CD196(CCR6)	175Lu	29-2L17	BioLegend	129802	surface
MHC II	176Yb	M5/114.15.2	Bio-Xcell	BE-0178	surface
CD4	197Au	RM4-5	BioLegend	100520	surface
CD8	198Pt	53-6.7	BioLegend	100716	surface
CD11b	209Bi	M1/70	Homemade	NA	surface

Supplementary Table 4. Antibodies used in flow cytometry.

Antibody	Fluorescence dye	Vendor	Clone	Cat.	Dilution ratio
Fc Block	Unconjugated	BioLegend	93	101319	1:50
-	7-AAD	BioLegend	-	420403	1:100
CD45	Brilliant Violet 510™	BioLegend	30-F11	103137	1:100
CD11c	Brilliant Violet 605™	BioLegend	N418	117333	1:200
CD3	APC/Cyanine7	BioLegend	17A2	100221	1:200
CD19	APC/Cyanine7	BioLegend	1D3/CD19	152411	1:200
NK1.1	APC/Cyanine7	BioLegend	S17016D	156509	1:100
CD11b	PE/Cyanine7	BioLegend	M1/70	101215	1:200
c-kit	PE	BioLegend	2B8	105807	1:100
CD103	Brilliant Violet 421™	BioLegend	QA17A24	156915	1:100
CD172a	FITC	BioLegend	P84	144005	1:100
XCR1	APC	BioLegend	ZET	148205	1:100
CD3	Brilliant Violet 421™	BioLegend	17A2	100227	1:200
CD44	FITC	BioLegend	NIM-R8	156007	1:100
CD62L	PE	BioLegend	W18021D	161211	1:100
CD4	FITC	BioLegend	GK1.5	100405	1:200
CD8	FITC	BioLegend	53-6.7	100705	1:200
CD107a	PE	BioLegend	1D4B	121611	1:100
IFN- γ	PE	BioLegend	XMG1.2	505807	1:100
IL-10	FITC	BioLegend	JES5-16E3	505005	1:100
CD45.1	PE/Dazzle™ 594	BioLegend	A20	110745	1:100
CD45.2	PerCP/Cyanine5.5	BioLegend	104	109827	1:100