Supplementary figures and legends.

Figure S1,

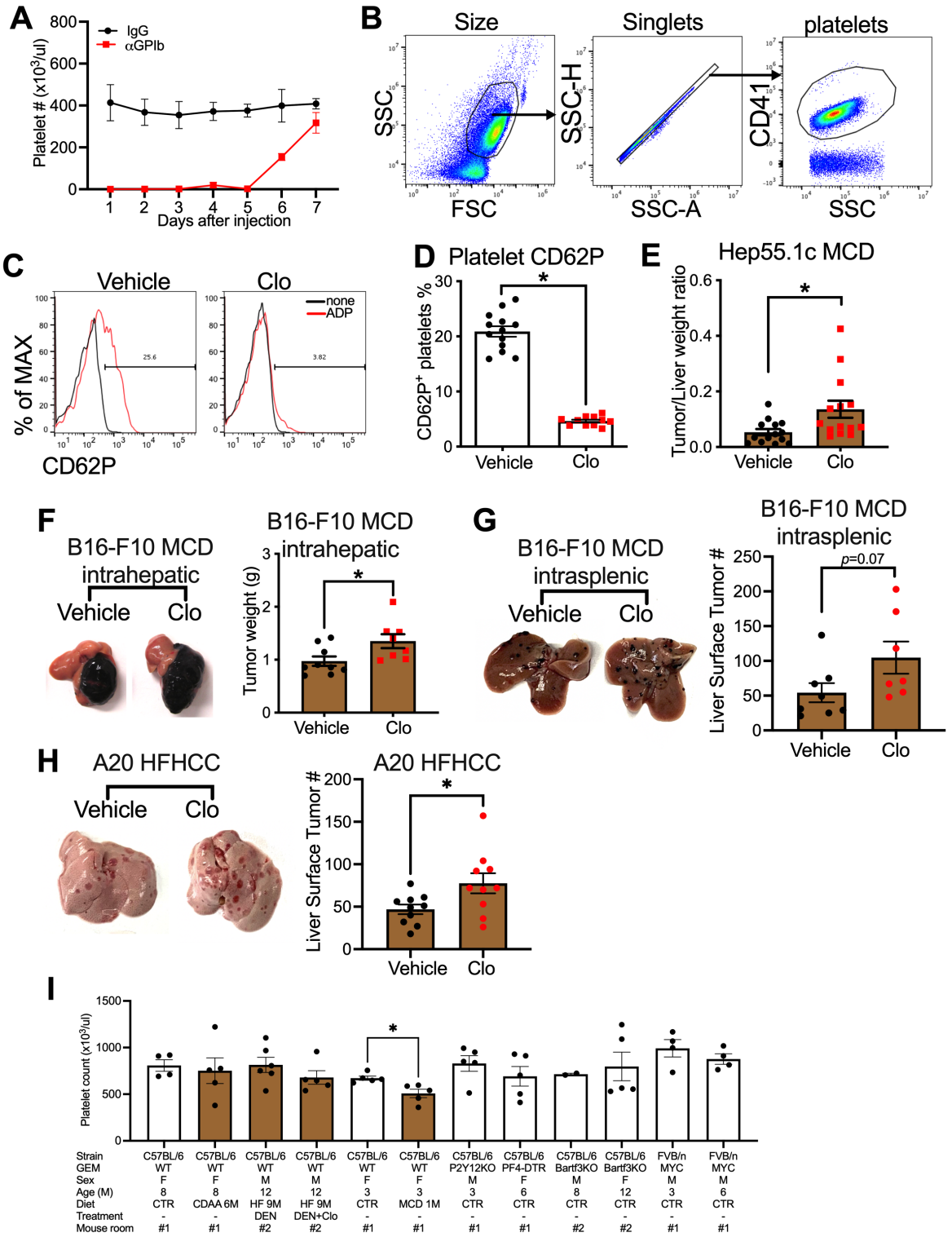

Figure S1, P2Y12 inhibiton or platelet depletion accelerates HCC in NAFLD. Related to Figure 1

(A) Confirmation of platelet depletion by αGPIb antibody. Blood platelet count was measured by flow cytometry following *i.p.* injection of 50 μg platelet depleting αGPIb antibody. n=5 for IgG, 9 for αGPIb.

(B-D) Confirmation of efficacy of *in vivo* clopidogrel treatment to block platelet function. Freshly isolated platelets from mice treated with clopidogrel or vehicle were subjected to *ex vivo* ADP stimulation. Platelet surface CD62P, an activation marker, was measured by flow cytometry. Platelet gating strategy is shown in **B**. Representative CD62P histogram plots are shown in **C**. Cumulative data are presented as mean±s.e.m in **D**. n=13 for vehicle, 11 for Clo. **P*<0.05, Student's *t*-test.

(E) Mice treatment was described in **Fig.1C**. Tumor to liver weight ratio was calculated. Data are presented as mean±s.e.m. from two independent experiments. n=15, **P* <0.05, Student's *t*-test.

(F) B16-F10 tumor cells were intrahepatic injected into MCD diet-fed mice treated with clopidogrel or vehicle. Liver tumor weight were measured. Data are presented as mean± s.e.m. of two independent experiments. n=9 for vehicle, 8 for Clopidogrel, **P* <0.05, Student's *t*-test.

(G) B16-F10 tumor cells were intrahepatic injected into MCD diet fed C57BL/6 mice. Then mice were treated with clopidogrel or vehicle for 3 weeks. Liver surface tumors were counted. Data are presented as mean ± s.e.m. of two independent experiments. n=8 for vehicle, 7 for Clopidogrel, Student's *t*-test.

(H) A20 tumor cells were injected *i.v*. into HFHCC diet fed BALB/c mice. Then mice were treated with clopidogrel or vehicle for 3 weeks. Liver surface tumors were counted. Data are presented as mean ± s.e.m. of two independent experiments. n=10**P* <0.05, Student's *t*-test.

(I) Circulating platelet levels of different mouse strains with different genetic modifications or feeding and housing conditions were measured by flow cytometry. *P* <0.05, Student's *t*-test.

Figure S2

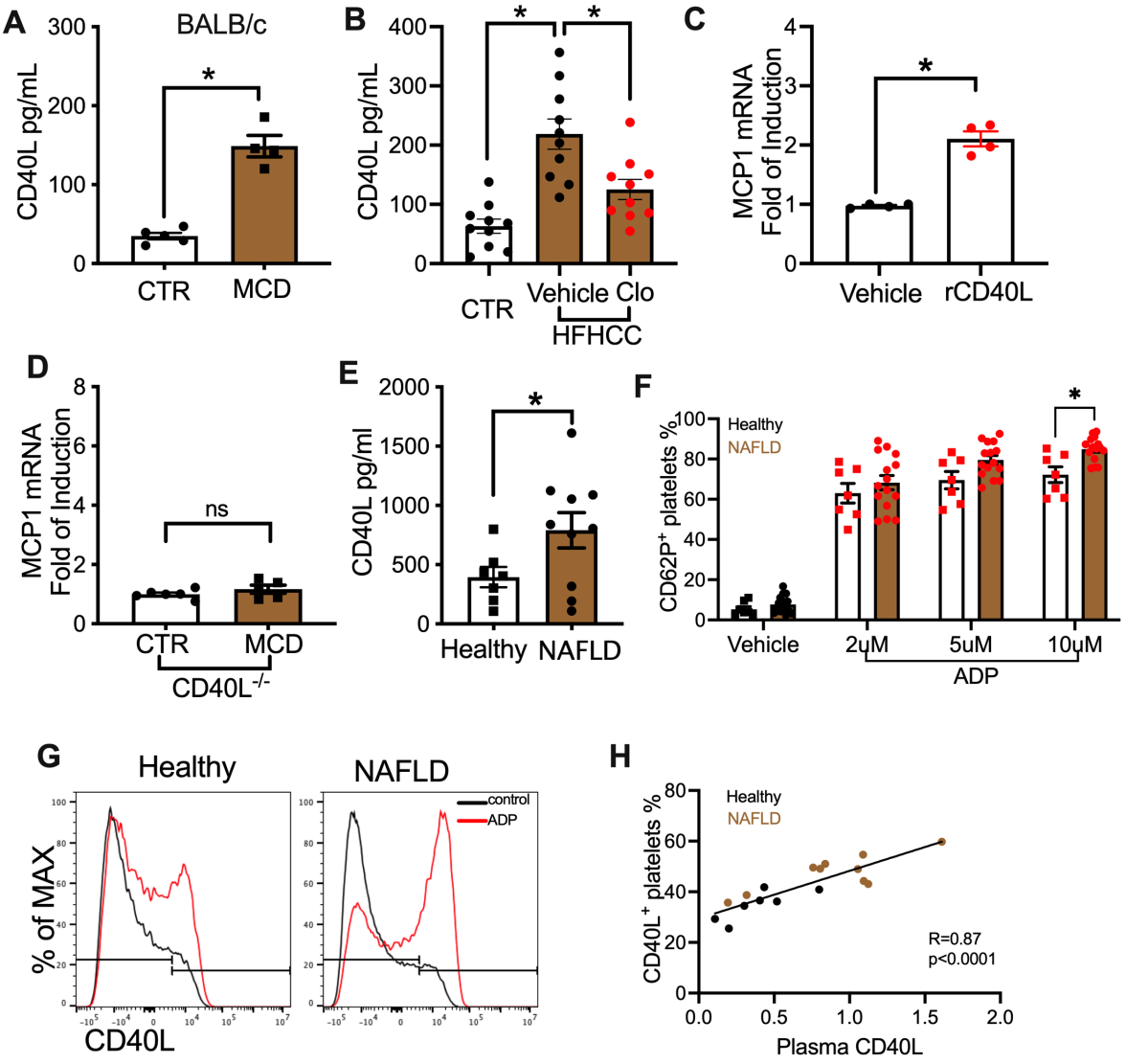

Figure S2, Platelets release more CD40L in NAFLD. Related to Figure 2

(A) Plasma CD40L level of BALB/c mice fed with MCD diet or control diet for 4 weeks. Cumulative data are presented as mean±s.e.m. n=5 for control, 4 for MCD. **P* <0.05, Student's *t*-test.

(B) C57BL/6 mice were kept on control diet or HFHCC diet with or without clopidogrel treatment for 3 weeks. Plasma CD40L level was measured. Data are presented as mean ± s.e.m. n=10, **P* < 0.05, one-way ANOVA.

(C) MS-1 endothelia cells were incubated with or without recombinant mouse CD40L. MCP1 mRNA levels were measured by real-time PCR. Data are presented as mean±s.e.m. n=4, **P* <0.05, Student's *t*-test.

(D) Plasma samples from control diet- or MCD diet-fed $CD40L^{-/-}$ mice were incubated with MS-1 cells. MCP1 mRNA level of MS-1 cells was measured by real-time PCR. Data are presented as mean± s.e.m. of two independent experiments. n=6 for control, 5 for MCD.

(E) Plasma CD40L levels of NAFLD patients or healthy controls were measured. Cumulative data are presented as mean± s.e.m. n=10 for NAFLD, 7 for healthy. *P <0.05, Student's t-test.

(F-H) Platelet surface CD62P levels were measured by flow cytometry **F**. Cumulative data are presented as mean±s.e.m. n=15 for NAFLD patients, 7 for healthy donors. Representative CD40L histogram plots are shown in **G**. Correlation of plasma CD40L with platelet surface CD40L expression after *ex vivo* ADP stimulation was shown in **H**.

Figure S3

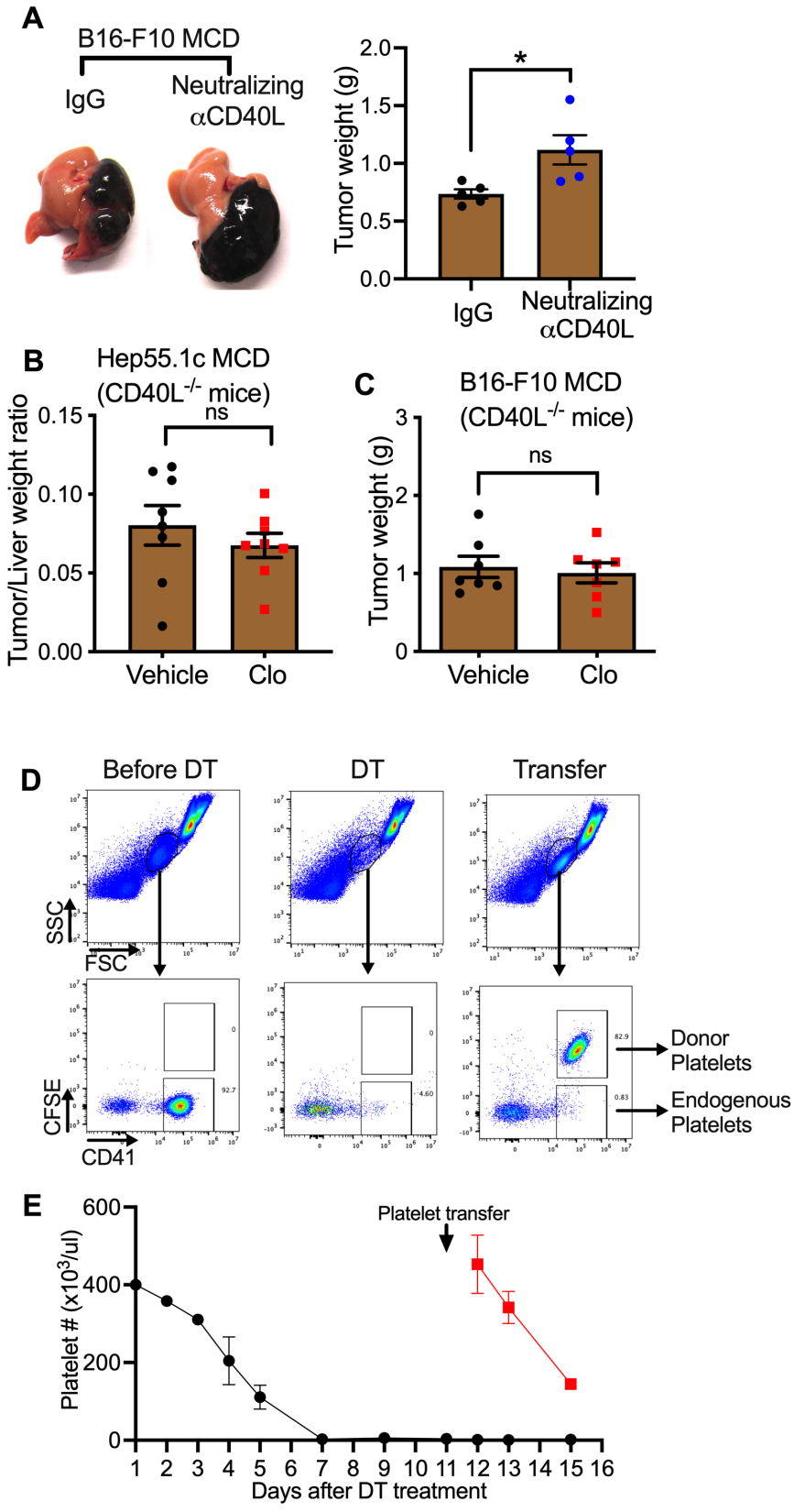
A
B16-F10 MCD
IgG
Neutralizing αCD40L
Tumor weight (g)
2.0
1.5
1.0
0.5
0.0
*
IgG
Neutralizing αCD40L
B
Hep55.1c MCD (CD40L-/- mice)
Tumor/Liver weight ratio
0.15
0.10
0.05
0.00
ns
Vehicle
Clo
C
B16-F10 MCD (CD40L-/- mice)
Tumor weight (g)
3
2
1
0
ns
Vehicle
Clo
D
Before DT
DT
Transfer
SSC
FSC
CFSE
CD41
92.7
4.60
82.9
0.83
Donor Platelets
Endogenous Platelets
E
Platelet transfer
Platelet # (x10³/ul)
600
400
200
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Days after DT treatment

Figure S3, Platelet-derived CD40L inhibits HCC in NAFLD. Related to Figure 3

(A) B16-F10 tumor cells were intra-hepaticly injected into livers of C57BL/6 mice fed with MCD diet. Then mice were given neutralizing CD40L antibody or control IgG. Tumor weight was measured at experimental end point. Data are presented as mea ± s.e.m. n=5, **P* <0.05, Student's *t*-test.

(B) Mice treatment was described in **Fig.3D**. Tumor to liver weight ratio was calculated. Data are presented as mean± s.e.m. from two independent experiments.

(C) B16-F10 tumor cells were intrahepatic injected into MCD diet-fed $CD40L^{-/-}$ mice treated with clopidogrel or vehicle. Liver tumor weight were measured. Data are presented as mean± s.e.m. of two independent experiments. n=7. **P* <0.05, Student's *t*-test.

(D, **E)** Confirmation of endogenous platelet depletion and platelet transfer in MCD diet fed PF4-DTR mice. Diphtheria toxin (DT) were injected s.c. as described in the methods. CFSE-labelled donor platelets were given by tail vein injection. Endogenous and $CFSE^{+}$ donor platelet levels were monitored by flow cytometry analysis of peripheral blood. Representative of platelet levels before DT treatment, 7 days after DT treatment, and one day after platelet transfer were shown.

Figure S4

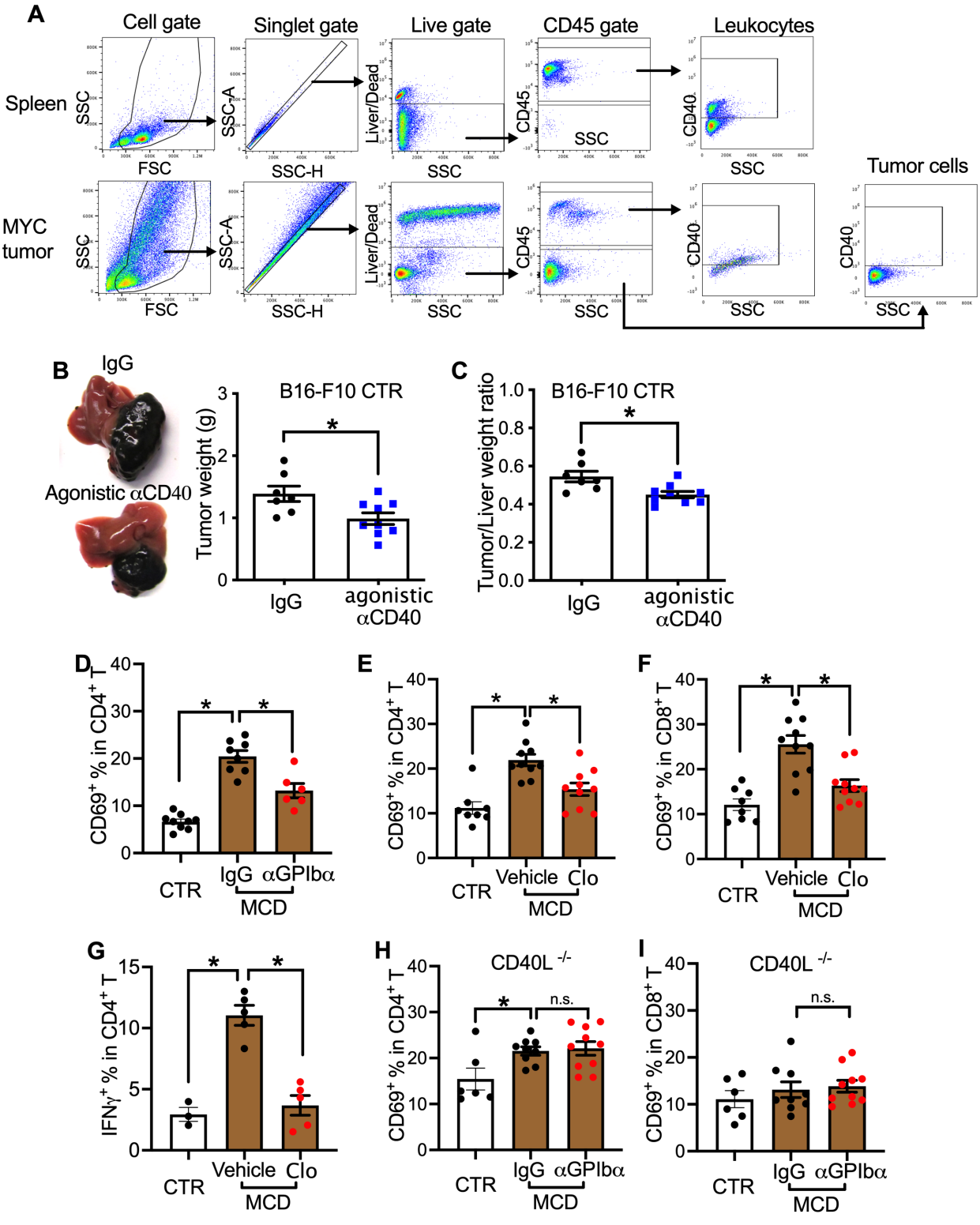
A
Cell gate
Singlet gate
Live gate
CD45 gate
Leukocytes
Tumor cells
Spleen
MYC tumor
SSC
FSC
SSC-A
SSC-H
Liver/Dead
CD45
CD40
B
IgG
Agonistic αCD40
B16-F10 CTR
Tumor weight (g)
IgG
agonistic αCD40
C
Tumor/Liver weight ratio
D
CD69+ % in CD4+ T
CTR
IgG αGPIbα
MCD
E
Vehicle Clo
F
CD69+ % in CD8+ T
G
IFNγ+ % in CD4+ T
H
CD40L -/-
n.s.
I

Figure S4, CD40 and CD8$^+$T cells mediate the platelet dependent tumor inhibition. Related to Figure 4

(A) Spleen cells or single cell suspension of MYC tumor were subject to flow cytometry analysis. CD40 staining in tumor cells or tumor infiltrating immune cells are shown.

(B, **C)** Mice bearing intrahepatic B16-F10 tumors were treated with agonistic CD40 antibody or control IgG. Representative liver images are shown in **B**. Liver tumor weight (**B**) and tumor to liver weight ratio (**C**) were measured. Data are presented as mean± s.e.m. from two independent experiments. n=7 for IgG, 9 for αCD40. *P <0.05, Student's t-test.

(D) CD69 expression on intrahepatic CD4$^+$ T cells from MCD diet-fed mice injected *i.p.* with α-GPIbα was measured by flow cytometry. Data are presented as mean± s.e.m. from two independent experiments. n=9 for control, 8 for MCD IgG, and 6 for MCD α-GPIbα.*P < 0.05, one-way ANOVA.

(E, **F)** CD69 expression on intrahepatic CD4$^+$ T cells (**E**) or CD8$^+$ T cells (**F**) from MCD diet-fed mice treated with or without clopidogrel was measured by flow cytometry. N=8 for CTR, 10 for MCD vehicle, and 10 for MCD Clo. *P < 0.05, one-way ANOVA.

(G) IFNγ expression on intrahepatic CD4$^+$ T cells from MCD diet-fed mice treated with or without clopidogrel. Data are presented as mean± s.e.m. from two independent experiments. n=3 for control, 5 for MCD vehicle and MCD Clo. *P < 0.05, one-way ANOVA.

(H, **I)** CD40L$^{-/-}$ mice fed with MCD diet were given *i.p.* injection of α-GPIbα or IgG control. CD69 expression of intrahepatic CD4$^+$ T cells (**H**) or or CD8$^+$ T cells (**I**) was measured. Data are presented as mean± s.e.m. from two independent experiments. n=6 for control, 9 for MCD IgG, and 10 for MCD α-GPIbα.

Figure S5

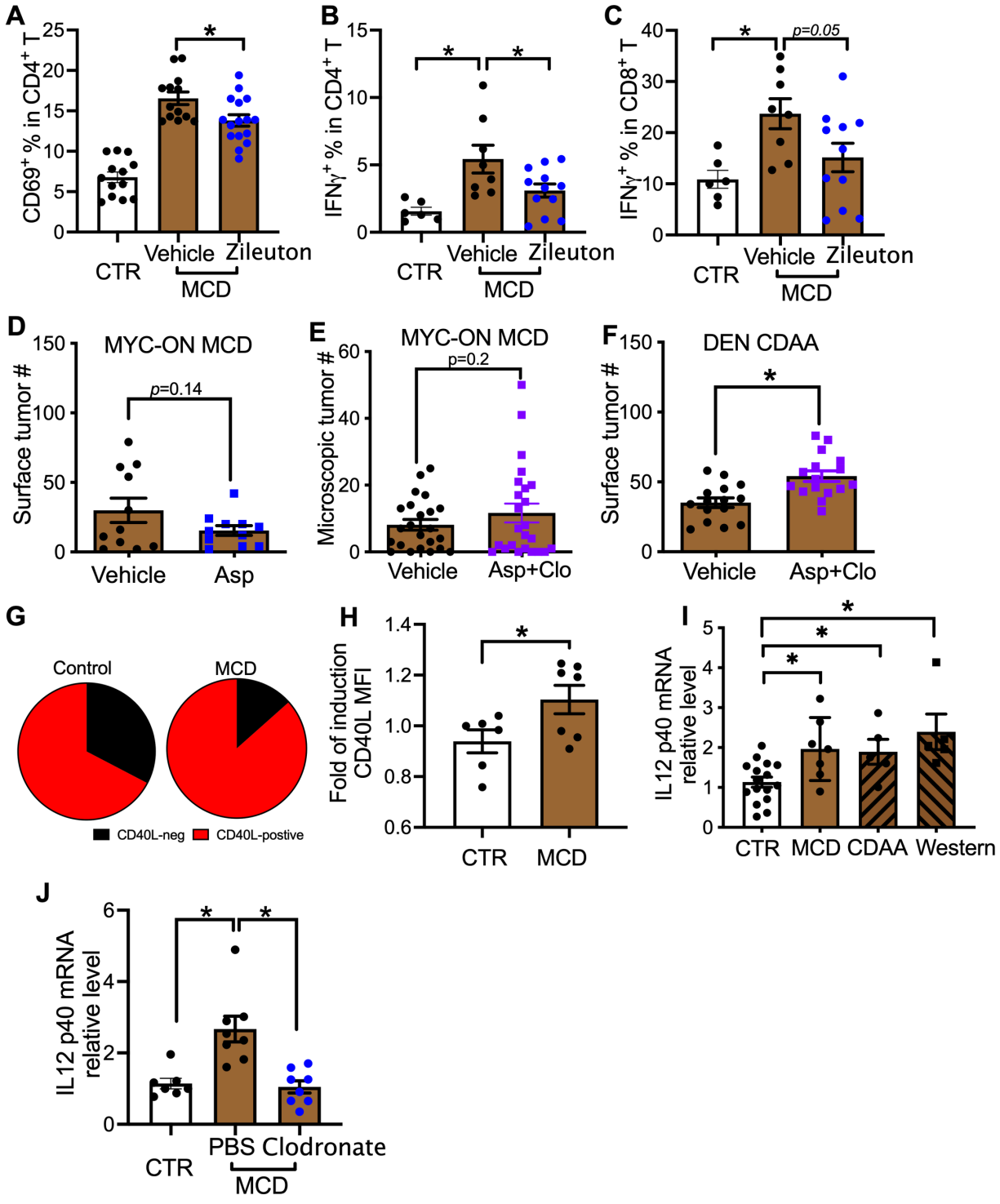
A
CD69+ % in CD4+ T
CTR
Vehicle
Zileuton
MCD
B
IFNγ+ % in CD4+ T
C
IFNγ+ % in CD8+ T
p=0.05
D
MYC-ON MCD
Surface tumor #
p=0.14
Vehicle
Asp
E
MYC-ON MCD
Microscopic tumor #
p=0.2
Asp+Clo
F
DEN CDAA
G
Control
MCD
CD40L-neg
CD40L-postive
H
Fold of induction CD40L MFI
I
IL12 p40 mRNA relative level
CDAA
Western
J
PBS Clodronate

Figure S5, P2Y12 controls platelet CD40L release and HCC growth. Related to Figure 5

(A-C) MCD diet-fed mice were treated with or without zileuton. CD69 (**A**) or IFNγ expression (**B**) of Intrahepatic $CD4^+$ T cells, and IFNγ expression of intrahepatic $CD8^+$ T cells (**C**) were measured by flow cytometry. Data are presented as mean± s.e.m. **P* < 0.05, one-way ANOVA.

(D), Surface tumor nodule counts of MYC-ON MCD mice treated with aspirin (Asp) or vehicle. Cumulative data are presented as mean± s.e.m. n=11, *P*=0.14, Student's *t*-test.

(E) Microscopic tumor lesion counts of MYC-ON MCD mice treated with the combination of aspirin and clopidogrel (Asp+Clo) or vehicle. Cumulative data are presented as mean± s.e.m. n=23 for vehicle, 24 for Asp+Clo, *P*=0.2, Student's *t*-test.

(F) DEN CDAA mice were treated with clopidogrel/aspirin combine treatment(Asp+Clo) or vehicle for 6 months. Mice were euthanized and liver surface tumor nodules were counted. Representative liver images were shown. Cumulative data are presented as mean± s.e.m. n=15 for vehicle, 16 for Asp+Clo, **P* <0.05, Student's *t*-test.

(G) Quantification of CD40L negative or positive of $CD41^+$ megakaryocytes in Fig.5M. More than 250 $CD41^+$ megakaryocytes were counted in each condition.

(H) Cultured megakaryocytes derived from mouse fetal liver were incubated with plasma from mice fed with MCD diet or control diet. CD40L expression of megakaryocytes was measured by intracellular flow cytometry analysis. Data are presented as mean± s.e.m. of two independent experiments. **P* <0.05, Student's *t*-test.

(I) PCR analysis of IL-12p40 mRNA levels in liver tissues from mice fed with MCD, CDAA, western or control diet. Data are presented as mean± s.e.m. n=16 for CTR, n=5 for CDAA or Western, 7 for MCD. **P* < 0.05, one-way ANOVA.

(J) PCR analysis of IL-12p40 mRNA levels in liver tissues from control mice or MCD diet fed mice treated with clodronate liposome or PBS liposome. Data are presented as mean ± s.e.m. n=8, **P* < 0.05, one-way ANOVA.

Supplementary Table S1

Human Subject Characteristics. Related to Figure 2 and Figure S2.

	NAFLD (n=15)	**Healthy (n=7)**
Age [years]	50.1±13.1[1]	38.5±9.2
Male sex [n (%)]	5 (33%)	5(71%)
Body Mass Index [Kg/m^2]	35.2±7.9	23.2±1.6
Platelet count [X1000/µl]	243±35	287±65
NASH[2]	9 (90%)	n/a
Advanced fibrosis[3]	4 (27%)	n/a

[1] Data presented as mean ± standard deviation or n (%).

[2] Liver biopsies to assess presence or absence of NASH were available in 10 subjects

[3] Advanced fibrosis defined as bridging fibrosis or cirrhosis and assessed using histology, imaging or elastography