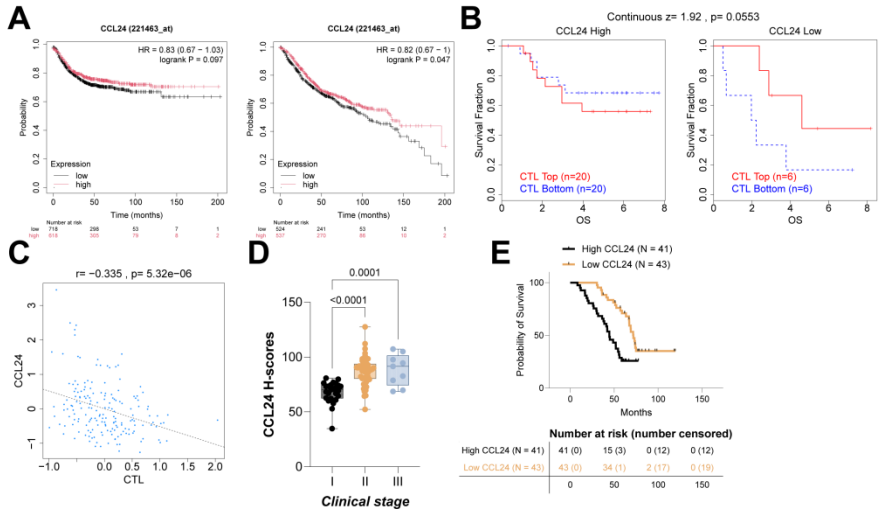


Fig S1. CCL24 is linked to unfavorable prognosis in CRC patients. A, Kaplan-Meier analysis of the correlation between CCL24 expression and overall survival and progression-free survival in the TCGA-COAD database. B-C, Correlation analysis of CCL24 with T cell dysfunction and CTL infiltration in the TIDE dataset. D-E, Analysis of CCL24 staining intensity correlation with clinical staging and overall survival in 84 CRC TMAs. TMA includes 84 pairs of tissue samples. Data are presented as bars and dots. $p < 0.05$ was considered statistically significant.

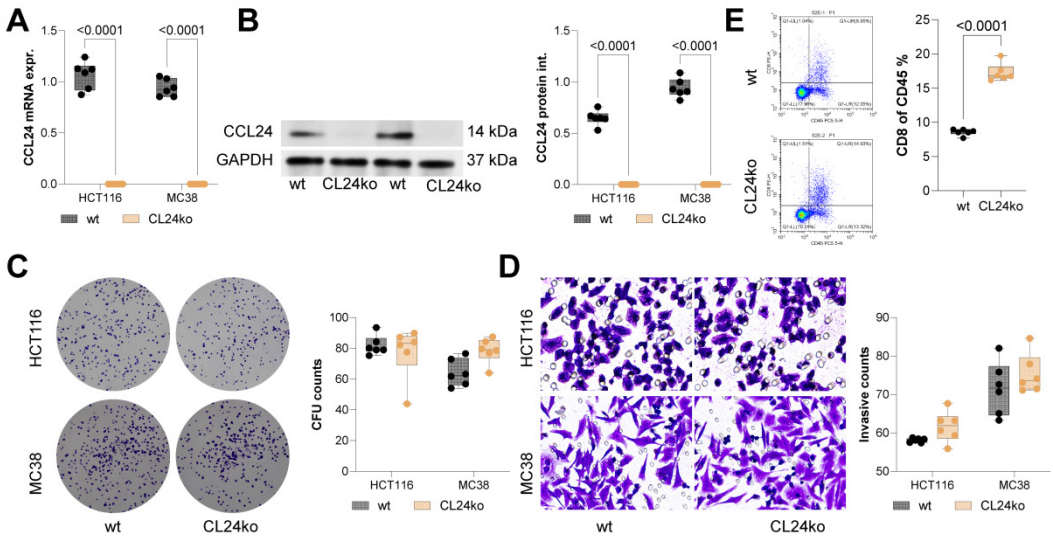


Fig S2. CCL24 knockout does not affect growth of CRC cells *in vitro*. CCL24^{ko} HCT116 and MC38 cell lines were generated using CRISPR/Cas9 technique. A-B, qPCR and WB analyses to detect CCL24 mRNA and protein levels in WT and CCL24^{ko} HCT116 and MC38 cells. C, Colony formation assays to analyze cell proliferation of HCT116 and MC38 cells. D, Transwell assays to analyze

invasion of HCT116 and MC38 cells. E, Number of $CD8^+$ T cells in tumors formed by $CCL24^{ko}$ MC38 cells in C57BL/6 mice determined using flow cytometry. Cell experiments repeated 6-8 times. Animal experiments include 6-8 mice per group. Data are presented as bars and dots. $p < 0.05$ was considered statistically significant.

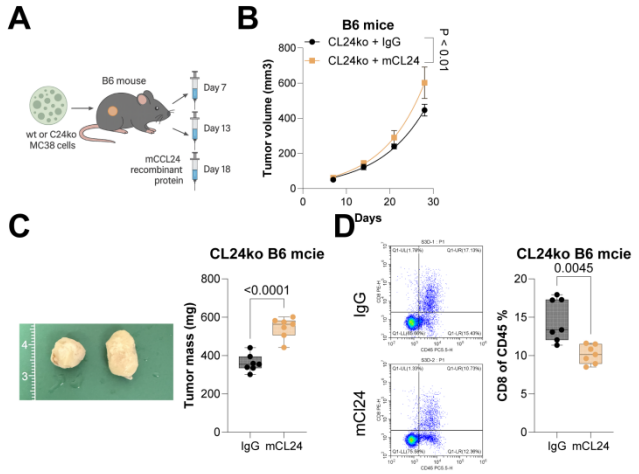


Fig S3. Mouse CCL24 recombinant protein restores tumorigenesis of $CCL24^{ko}$ MC38 cells in mice. A, C57BL/6 mice implanted $CCL24^{ko}$ MC38 cells were further treated with mCCL24. B-C, Analysis of MC38 cell growth rate and tumor weight in mice. D, Population of $CD8^+$ T cells in mice determined using flow cytometry. Each group includes 6-8 mice. Data are presented as bars and dots. $p < 0.05$ was considered statistically significant.

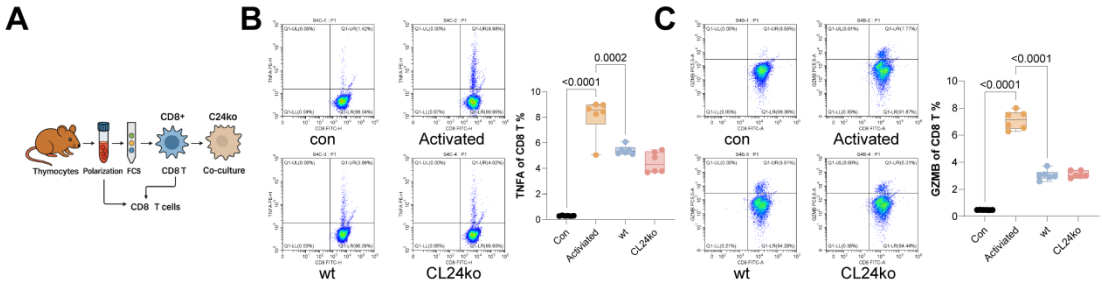


Fig S4. CCL24 expression in cancer cells does not directly affect T cell activity. A, $CD8^+$ T cells were extracted from thymocytes or C57BL/6 mice, activated with CD3/CD28 antibodies, sorted by flow cytometry, and co-cultured with WT or $CL24^{ko}$ MC38 cells *in vitro*. B-C, Populations of $TNFA^+$ (B) and $GZMB^+$ (C) $CD8^+$ T cells after co-culture with cancer cells determined using flow cytometry.

Cell experiments repeated 6-8 times. Data are presented as bars and dots. $p < 0.05$ was considered statistically significant.

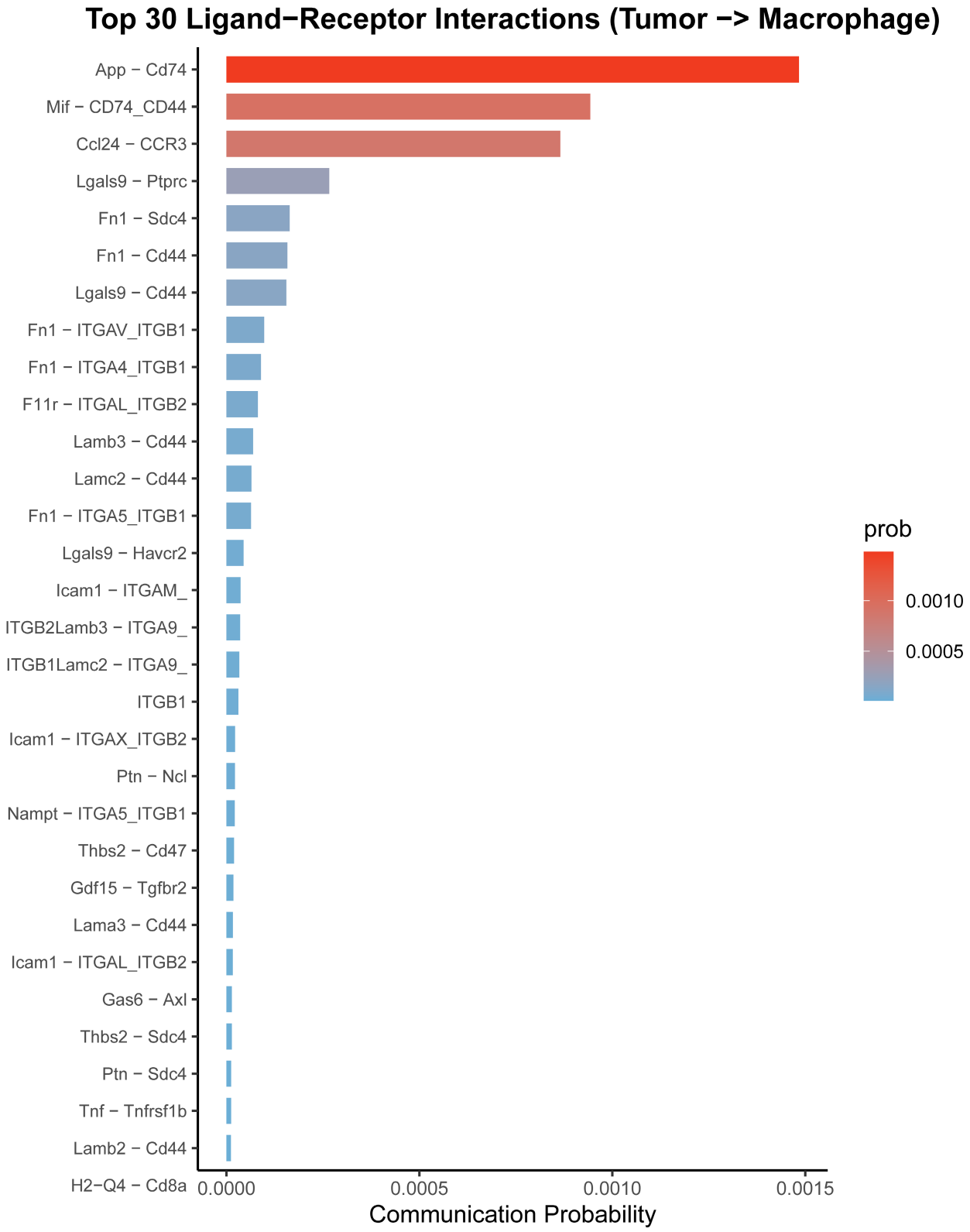


Fig S5. CellChat Analysis of the Top 30 signaling pathways between tumor cells and macrophages.

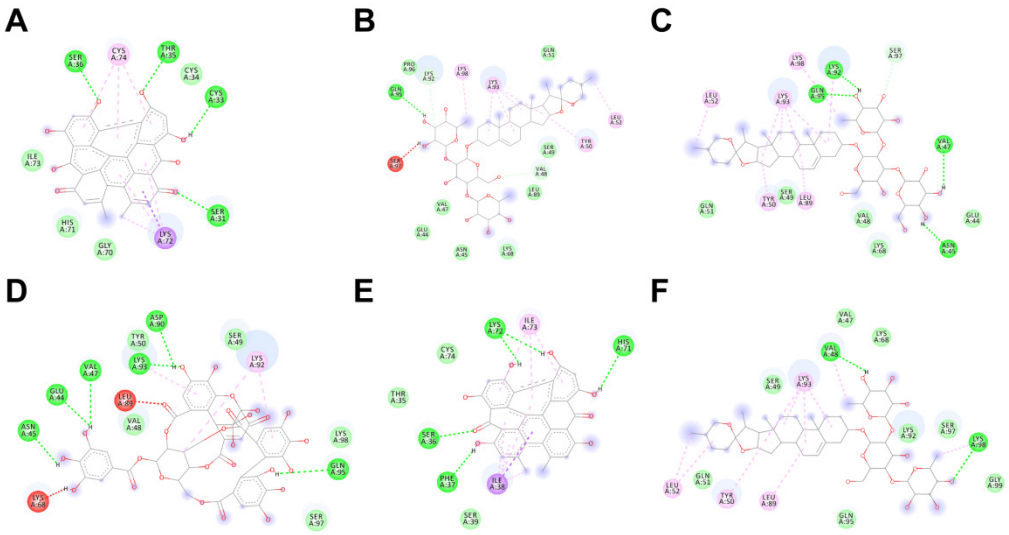


Fig S6. AutoDock Vina Analysis of Small Molecule Drugs Binding to CCL24. A, Hypericin (Binding Affinity, -8.3). B, Dioscin (Binding Affinity, -8.8). C, Gracillin (Binding Affinity, -8.9). D, Geraniin (Binding Affinity, -8.4). E, L-asparaginase (Binding Affinity, -8.2). F, CHEMBL2036082 (Binding Affinity, -8.5).

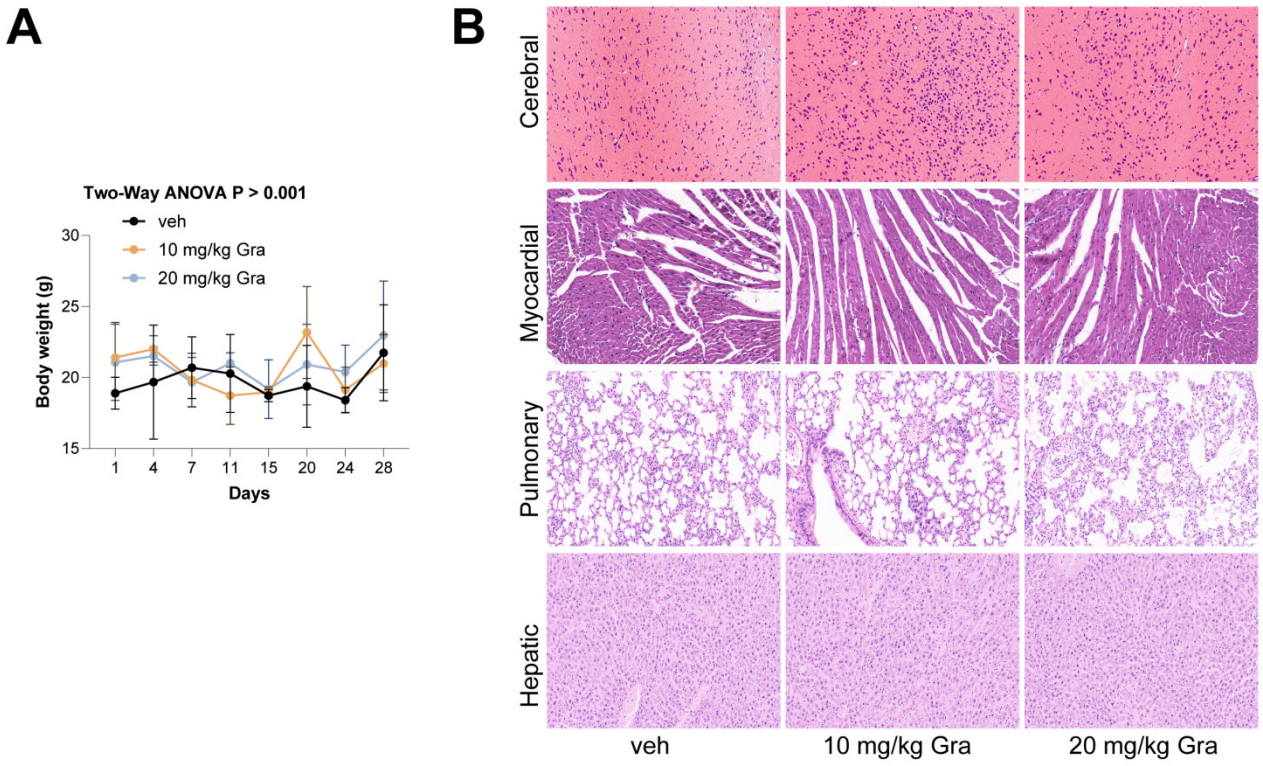
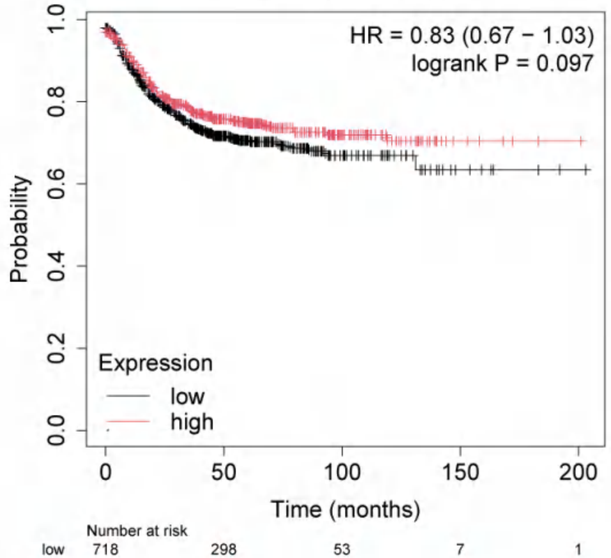
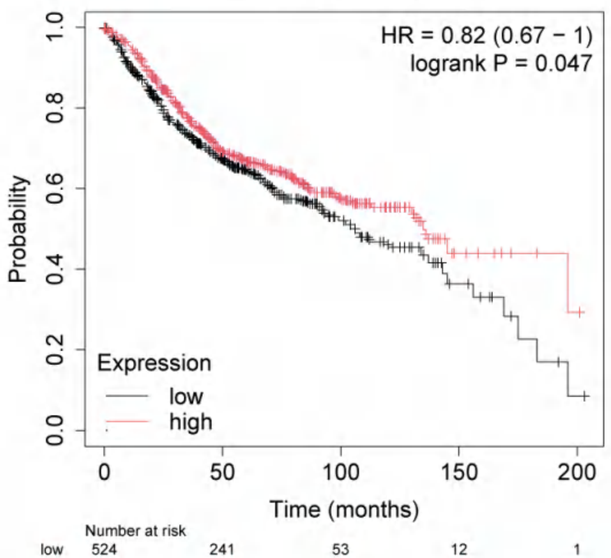


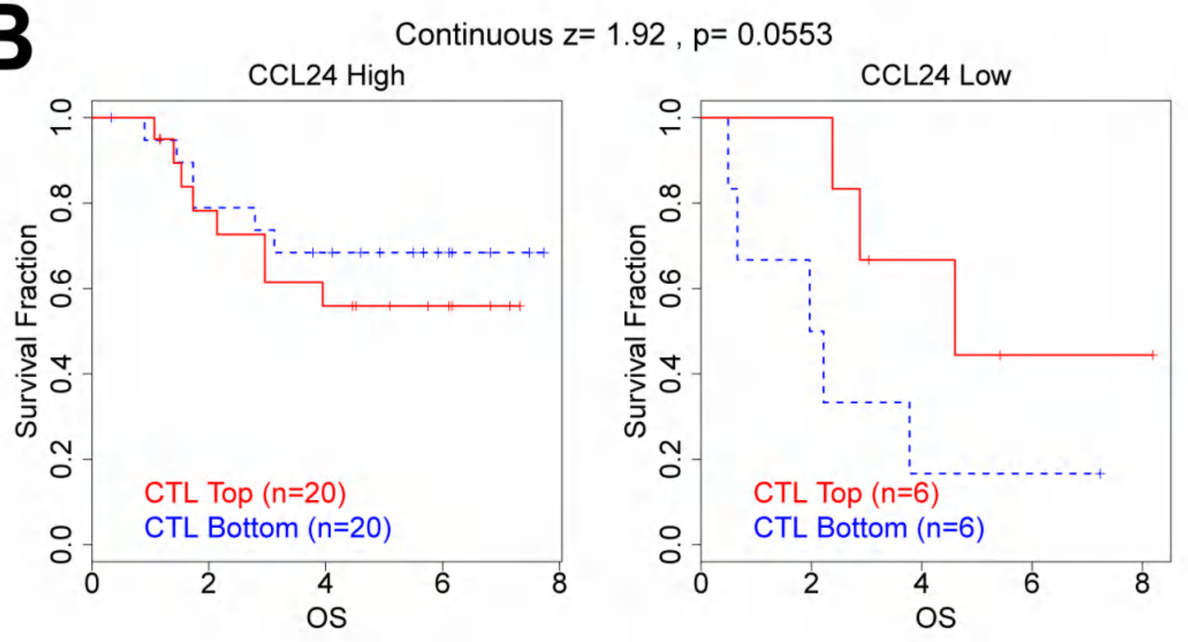
Fig S7. Safety evaluation of Gracillin in vivo. (A) Body weight changes of C57BL/6 mice during the course of Gracillin treatment. (B) Representative HE staining images of major organs (Heart, Liver, Lung, Kidney) harvested from vehicle- and Gracillin-treated mice at the endpoint. Scale bar = 100 μm. Note the absence of tissue damage or necrosis.

A

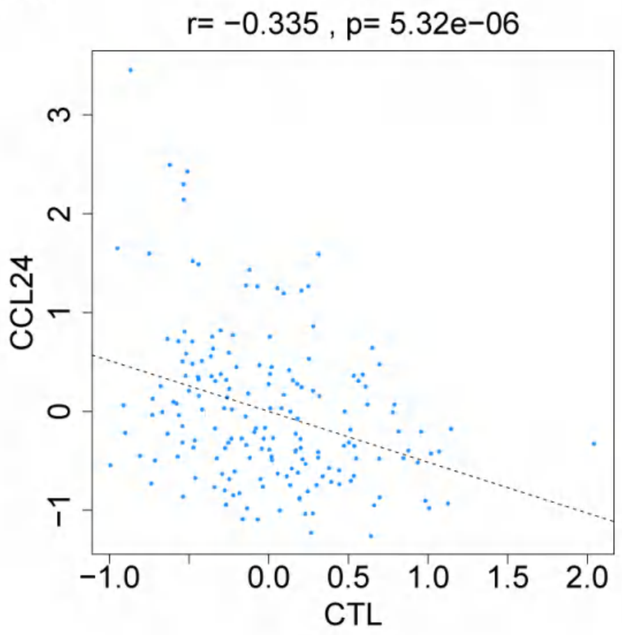




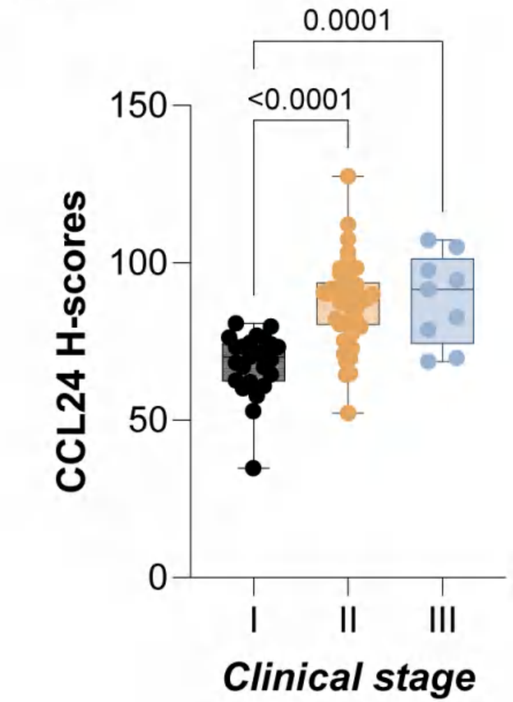
B



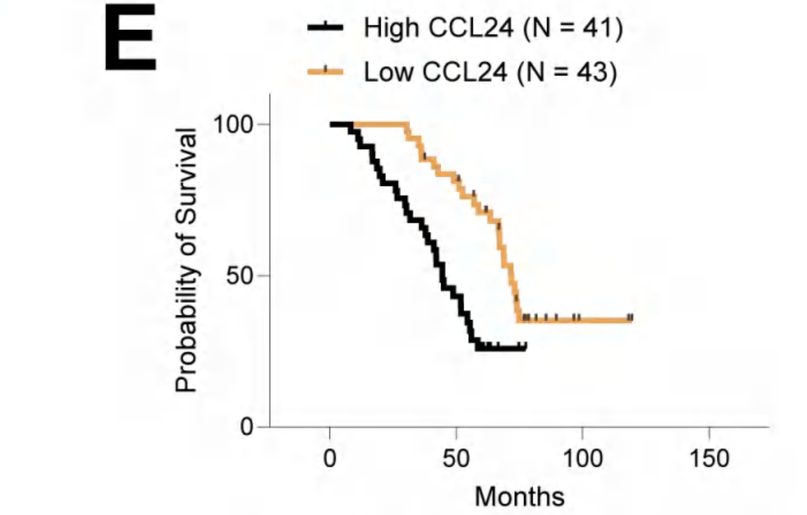
C



D

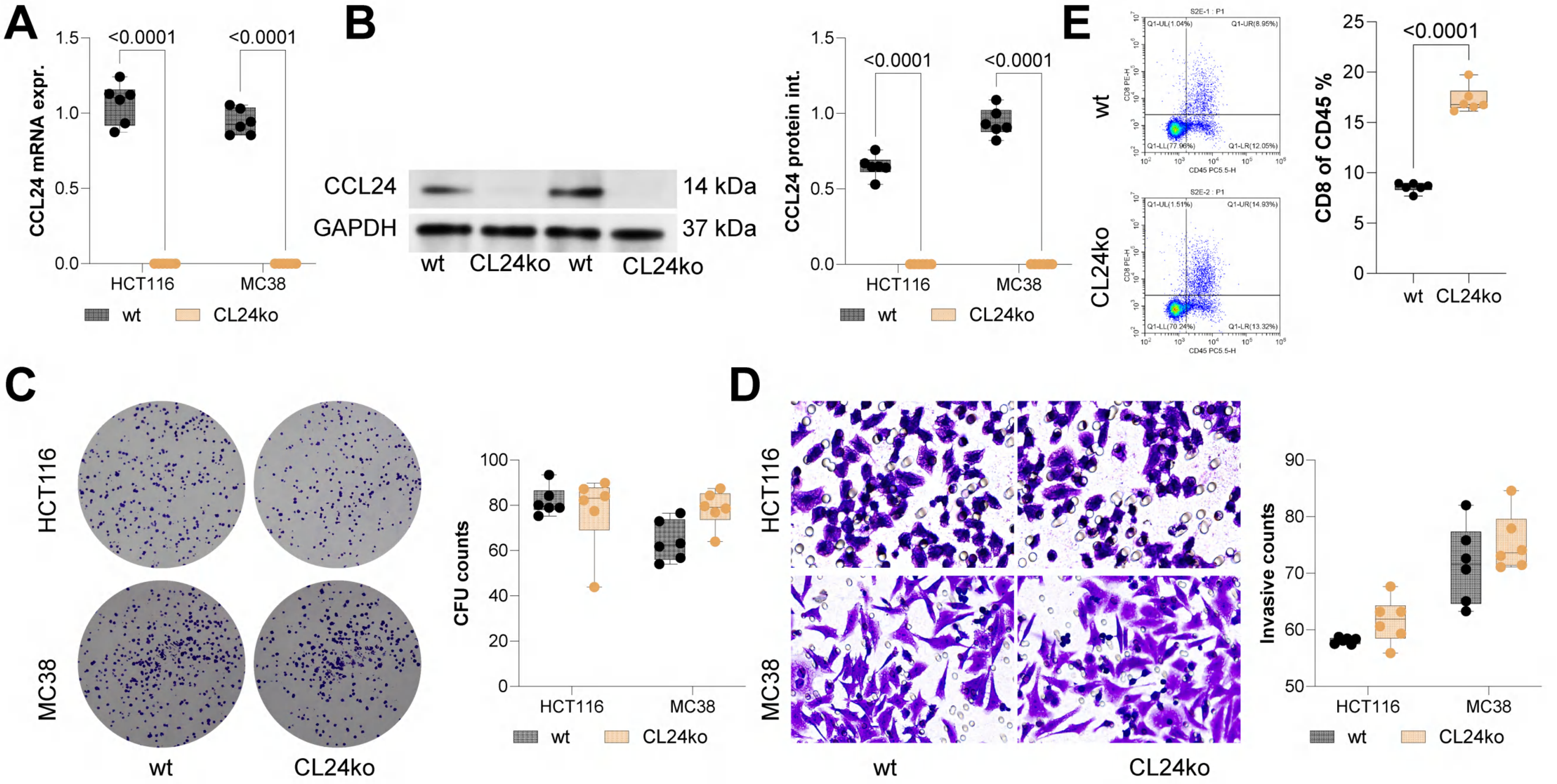


E

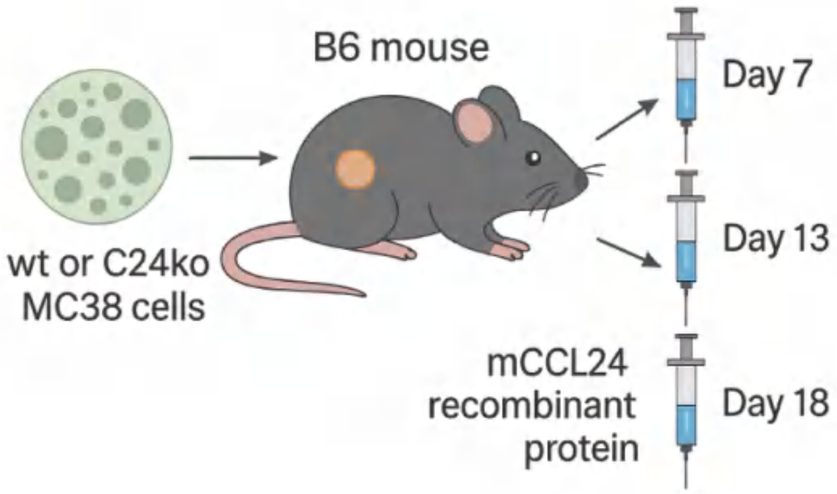


Number at risk (number censored)

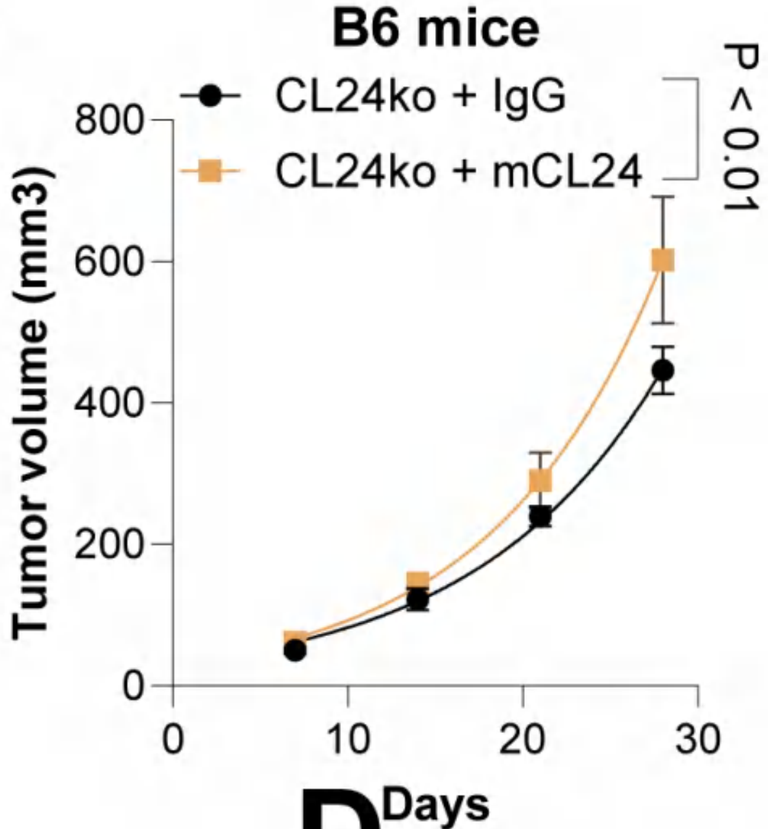
High CCL24 (N = 41)	41 (0)	15 (3)	0 (12)	0 (12)
Low CCL24 (N = 43)	43 (0)	34 (1)	2 (17)	0 (19)
	0	50	100	150


A
CCL24 mRNA expr.
1.5
1.0
0.5
0.0
<0.0001
<0.0001
HCT116
MC38
wt
CL24ko
B
CCL24
GAPDH
14 kDa
37 kDa
wt
CL24ko
wt
CL24ko
CCL24 protein int.
1.5
1.0
0.5
0.0
<0.0001
<0.0001
HCT116
MC38
wt
CL24ko
E
S2E-1 : P1
Q1-UL(1.04%)
Q1-UR(8.95%)
Q1-LL(77.96%)
Q1-LR(12.05%)
CD8 PE-H
CD45 PC5.5-H
wt
S2E-2 : P1
Q1-UL(1.51%)
Q1-UR(14.93%)
Q1-LL(70.24%)
Q1-LR(13.32%)
CD8 PE-H
CD45 PC5.5-H
CL24ko
CD8 of CD45 %
25
20
15
10
5
0
<0.0001
wt
CL24ko
C
HCT116
MC38
wt
CL24ko
CFU counts
100
80
60
40
20
0
HCT116
MC38
wt
CL24ko
D
HCT116
MC38
wt
CL24ko
Invasive counts
90
80
70
60
50
HCT116
MC38
wt
CL24ko

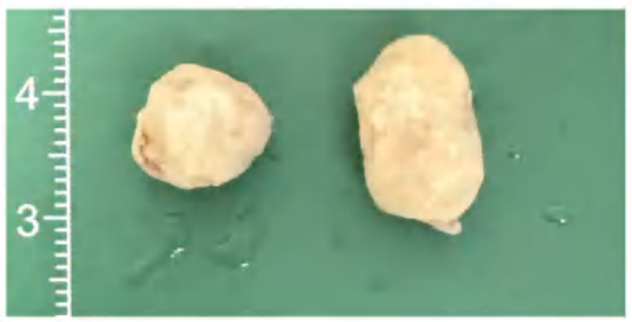
A


B6 mouse
Day 7
Day 13
Day 18
wt or C24ko
MC38 cells
mCCL24
recombinant
protein

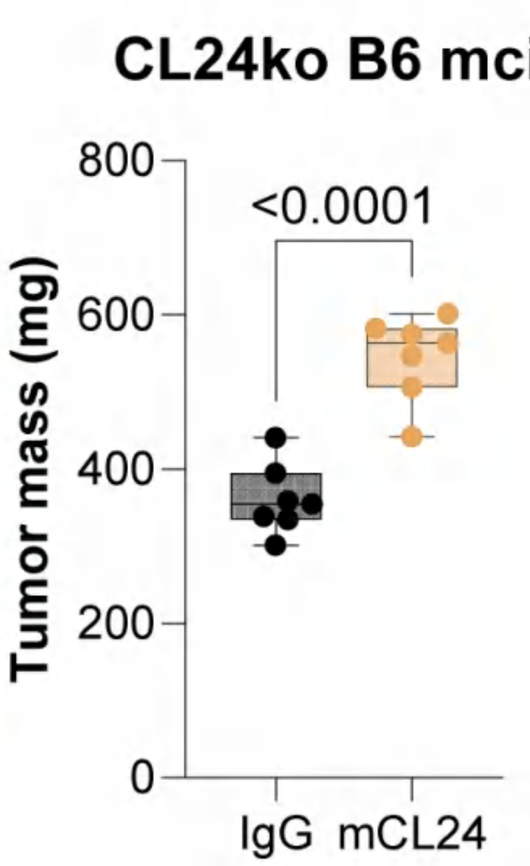
B

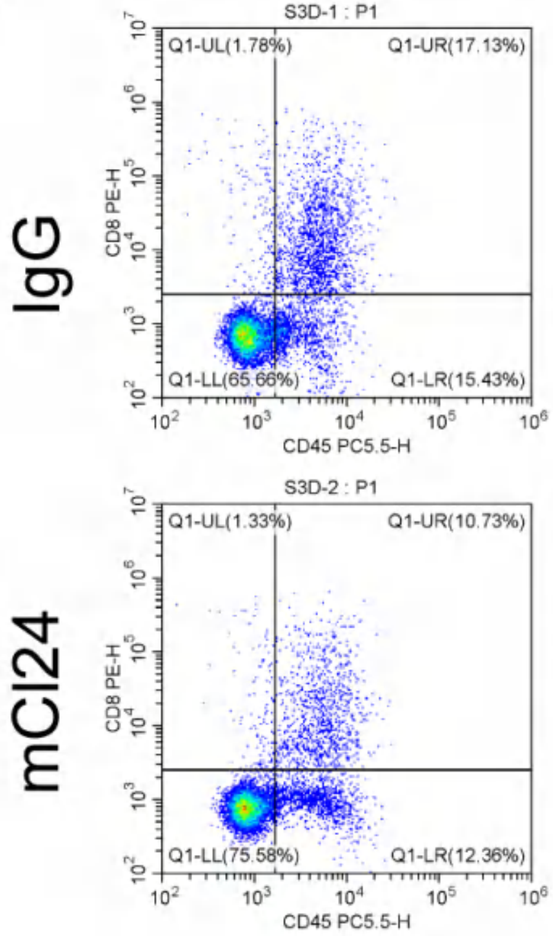

B6 mice
CL24ko + IgG
CL24ko + mCL24
P < 0.01
Tumor volume (mm3)
800
600
400
200
0
0
10
20
30
Days

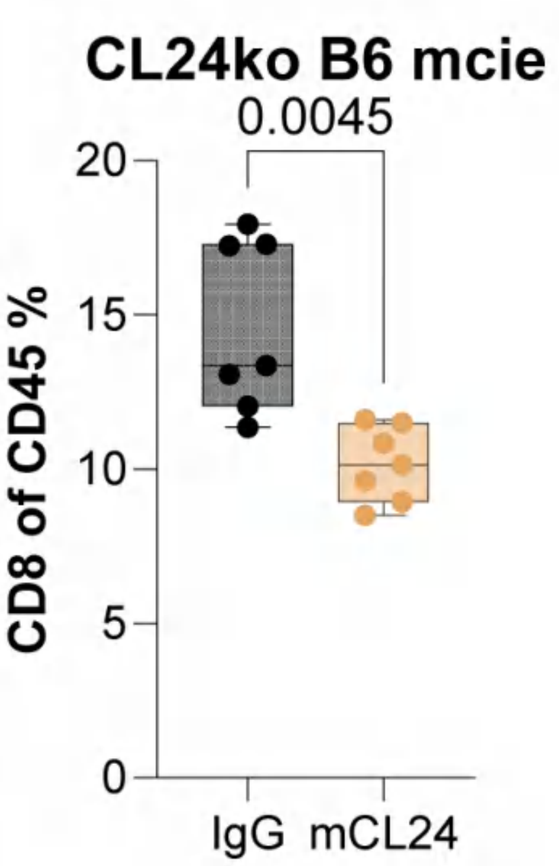
C

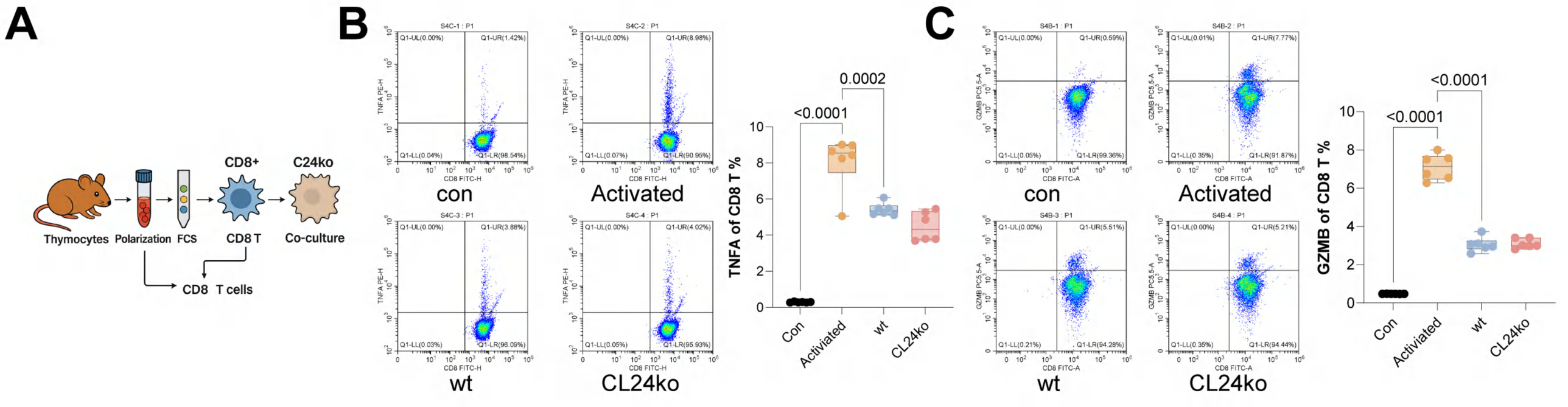

4
3

D


CL24ko B6 mcie
<0.0001
Tumor mass (mg)
800
600
400
200
0
IgG
mCL24


S3D-1 : P1
Q1-UL(1.78%)
Q1-UR(17.13%)
Q1-LL(65.66%)
Q1-LR(15.43%)
CD8 PE-H
CD45 PC5.5-H
IgG
S3D-2 : P1
Q1-UL(1.33%)
Q1-UR(10.73%)
Q1-LL(75.58%)
Q1-LR(12.36%)
mCl24


CL24ko B6 mcie
0.0045
CD8 of CD45 %
20
15
10
5
0
IgG
mCL24


A
Thymocytes
Polarization
FCS
CD8+
CD8 T
C24ko
Co-culture
CD8 T cells
B
S4C-1 : P1
Q1-UL(0.00%)
Q1-UR(1.42%)
Q1-LL(0.04%)
Q1-LR(98.54%)
con
S4C-2 : P1
Q1-UL(0.00%)
Q1-UR(8.98%)
Q1-LL(0.07%)
Q1-LR(90.95%)
Activated
S4C-3 : P1
Q1-UL(0.00%)
Q1-UR(3.88%)
Q1-LL(0.03%)
Q1-LR(96.09%)
wt
S4C-4 : P1
Q1-UL(0.00%)
Q1-UR(4.02%)
Q1-LL(0.05%)
Q1-LR(95.93%)
CL24ko
TNFA PE-H
CD8 FITC-H
TNFA of CD8 T %
<0.0001
0.0002
Con
Activiated
wt
CL24ko
C
S4B-1 : P1
Q1-UL(0.00%)
Q1-UR(0.59%)
Q1-LL(0.05%)
Q1-LR(99.36%)
con
S4B-2 : P1
Q1-UL(0.01%)
Q1-UR(7.77%)
Q1-LL(0.35%)
Q1-LR(91.87%)
Activated
S4B-3 : P1
Q1-UL(0.00%)
Q1-UR(5.51%)
Q1-LL(0.21%)
Q1-LR(94.28%)
wt
S4B-4 : P1
Q1-UL(0.00%)
Q1-UR(5.21%)
Q1-LL(0.35%)
Q1-LR(94.44%)
CL24ko
GZMB PC5.5-A
CD8 FITC-A
GZMB of CD8 T %
<0.0001
<0.0001
Con
Activiated
wt
CL24ko

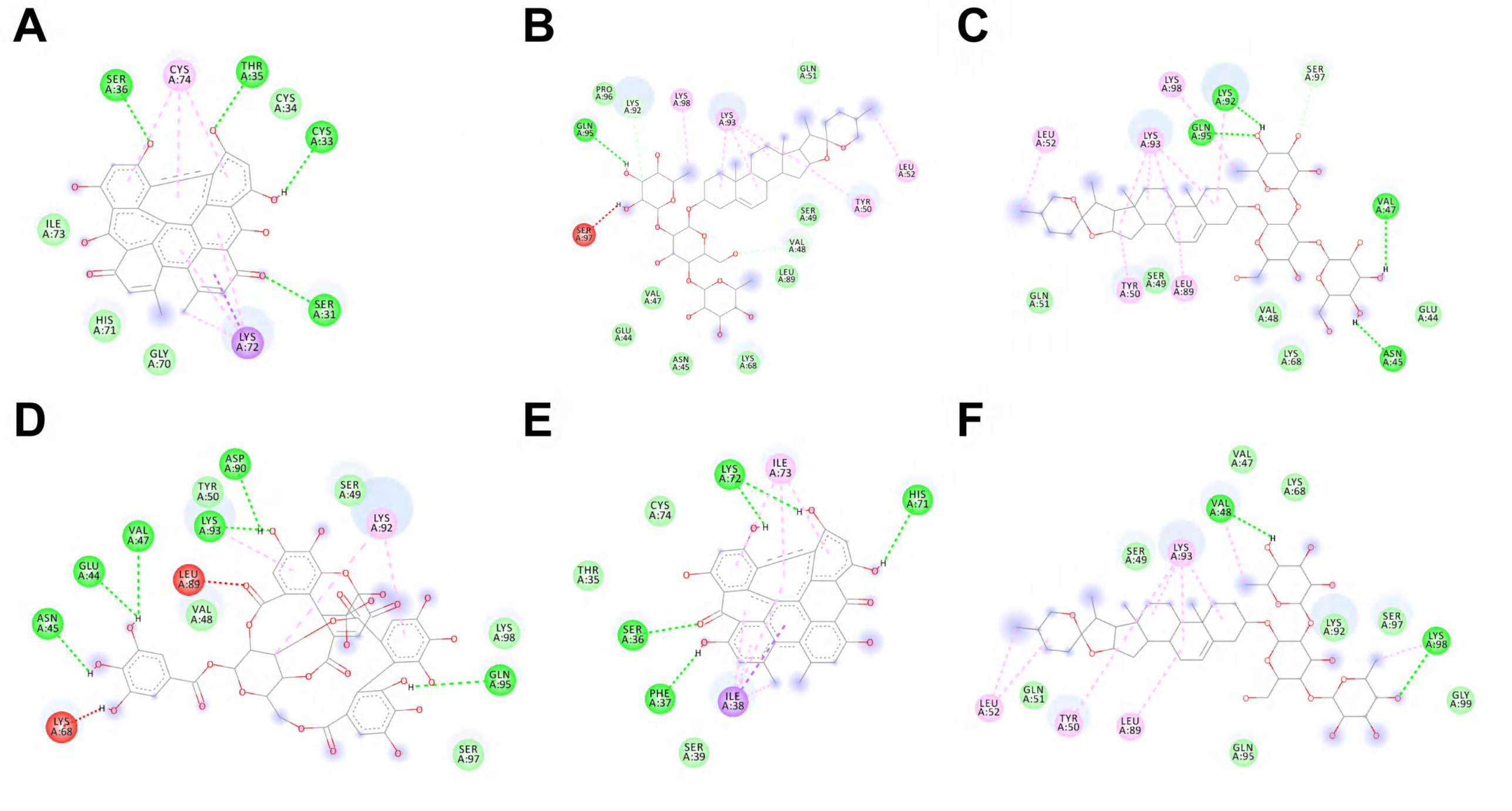
Top 30 Ligand−Receptor Interactions (Tumor −> Macrophage)

App − Cd74
Mif − CD74_CD44
Ccl24 − CCR3
Lgals9 − Ptprc
Fn1 − Sdc4
Fn1 − Cd44
Lgals9 − Cd44
Fn1 − ITGAV_ITGB1
Fn1 − ITGA4_ITGB1
F11r − ITGAL_ITGB2
Lamb3 − Cd44
Lamc2 − Cd44
Fn1 − ITGA5_ITGB1
Lgals9 − Havcr2
Icam1 − ITGAM_
ITGB2Lamb3 − ITGA9_
ITGB1Lamc2 − ITGA9_
ITGB1
Icam1 − ITGAX_ITGB2
Ptn − Ncl
Nampt − ITGA5_ITGB1
Thbs2 − Cd47
Gdf15 − Tgfbr2
Lama3 − Cd44
Icam1 − ITGAL_ITGB2
Gas6 − Axl
Thbs2 − Sdc4
Ptn − Sdc4
Tnf − Tnfrsf1b
Lamb2 − Cd44
H2−Q4 − Cd8a

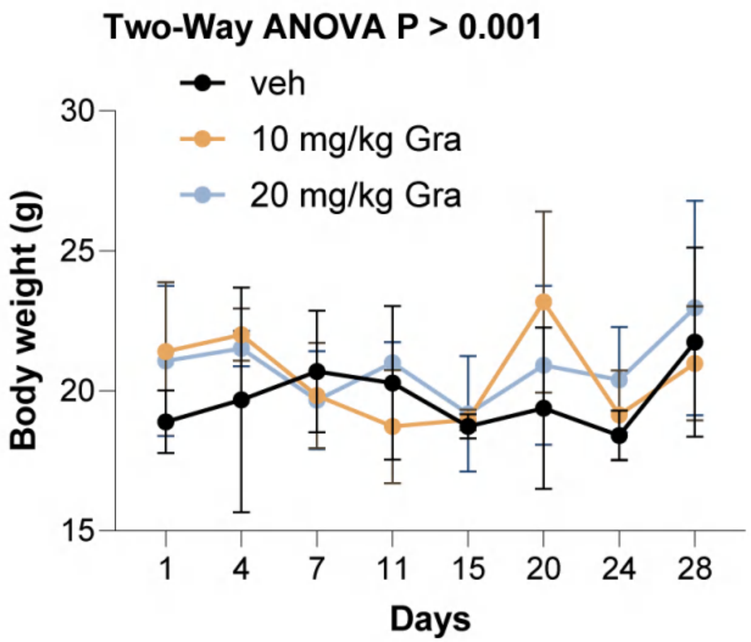
prob
0.0010
0.0005

0.0000
0.0005
0.0010
0.0015

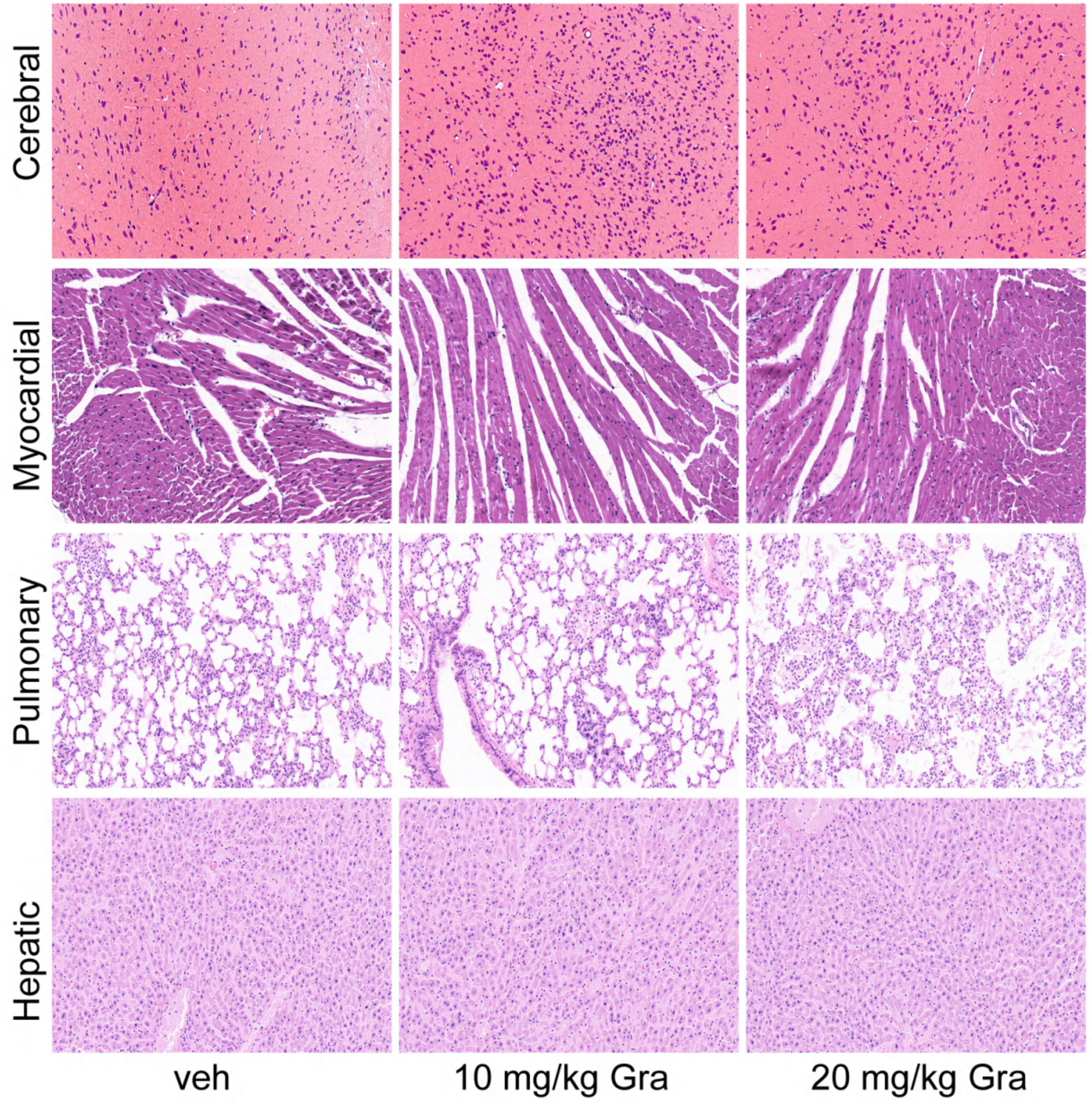
Communication Probability


A
SER A:36
CYS A:74
THR A:35
CYS A:34
CYS A:33
ILE A:73
SER A:31
HIS A:71
LYS A:72
GLY A:70
B
GLN A:51
PRO A:96
LYS A:92
LYS A:98
LYS A:93
GLN A:95
LEU A:52
TYR A:50
SER A:49
SER A:97
VAL A:48
LEU A:89
VAL A:47
GLU A:44
ASN A:45
LYS A:68
C
LYS A:98
SER A:97
LYS A:92
LEU A:52
LYS A:93
GLN A:95
VAL A:47
TYR A:50
SER A:49
LEU A:89
GLN A:51
VAL A:48
GLU A:44
LYS A:68
ASN A:45
D
ASP A:90
TYR A:50
SER A:49
LYS A:93
LYS A:92
VAL A:47
GLU A:44
LEU A:89
VAL A:48
ASN A:45
LYS A:98
GLN A:95
LYS A:68
SER A:97
E
LYS A:72
ILE A:73
HIS A:71
CYS A:74
THR A:35
SER A:36
PHE A:37
ILE A:38
SER A:39
F
VAL A:47
LYS A:68
VAL A:48
SER A:49
LYS A:93
LYS A:92
SER A:97
LYS A:98
GLN A:51
LEU A:52
TYR A:50
LEU A:89
GLY A:99
GLN A:95

A


Two-Way ANOVA P > 0.001
veh
10 mg/kg Gra
20 mg/kg Gra
Body weight (g)
30
25
20
15
1
4
7
11
15
20
24
28
Days

B


Cerebral
Myocardial
Pulmonary
Hepatic
veh
10 mg/kg Gra
20 mg/kg Gra