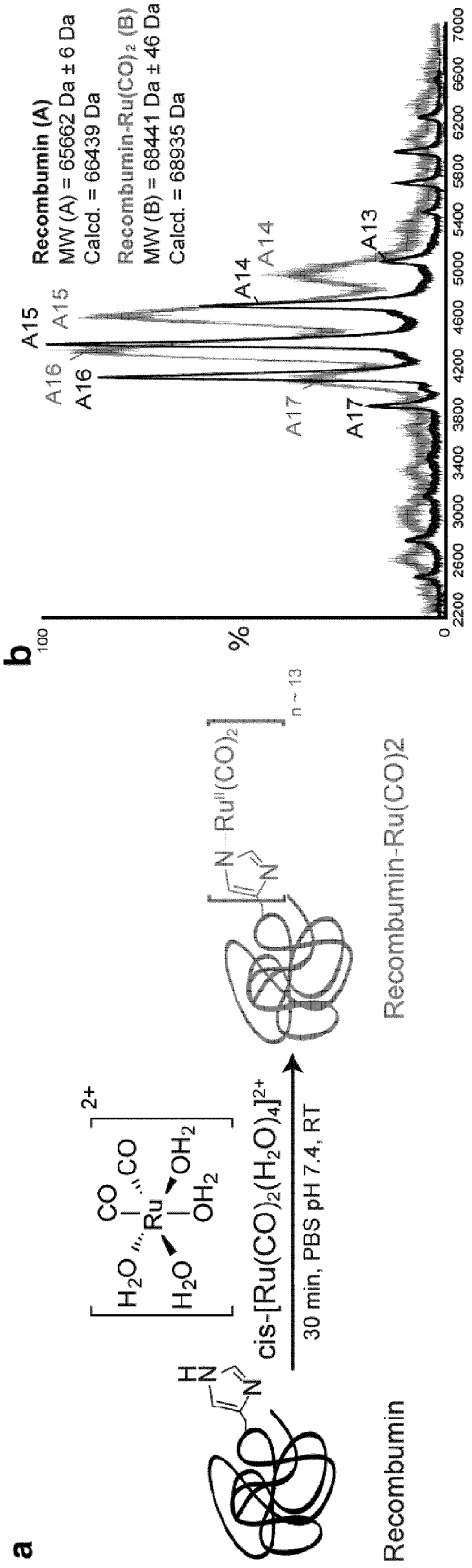

a
Recombumin
cis-[Ru(CO)2(H2O)4]2+
30 min, PBS pH 7.4, RT
Recombumin-Ru(CO)2
n ~ 13
b
Recombumin (A)
MW (A) = 65662 Da ± 6 Da
Calcd. = 66439 Da
Recombumin-Ru(CO)2 (B)
MW (B) = 68441 Da ± 46 Da
Calcd. = 68935 Da
A13
A14
A15
A16
A17
%
100
0
2200 2600 3000 3400 3800 4200 4600 5000 5400 5800 6200 6600 7000

FIGURE 1

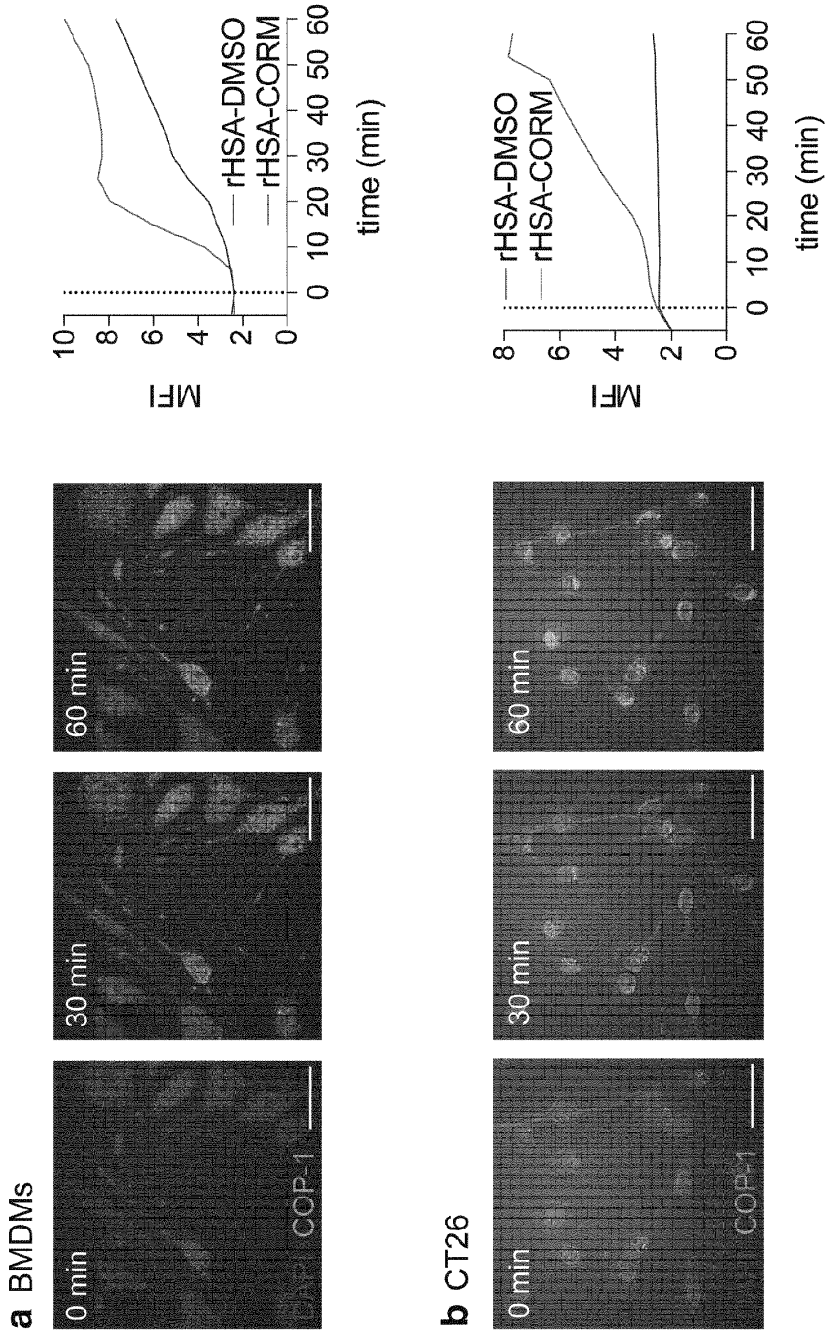
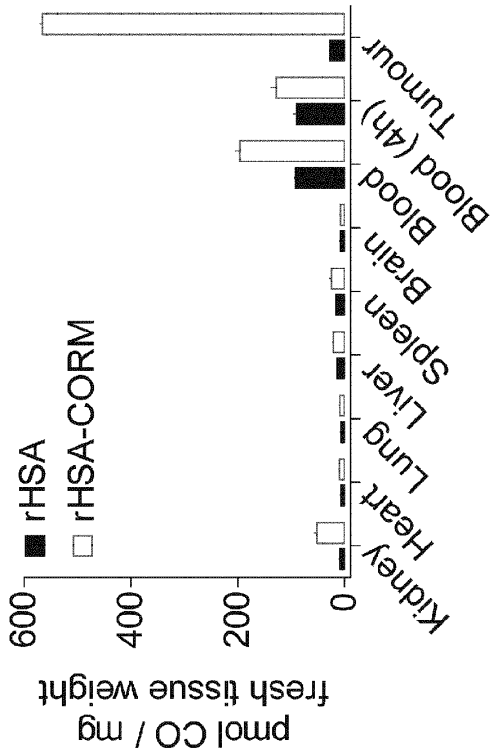

a BMDMs
0 min
30 min
60 min
DAPI COP-1
MFI
time (min)
rHSA-DMSO
rHSA-CORM
b CT26
0 min
30 min
60 min
COP-1
MFI
time (min)
rHSA-DMSO
rHSA-CORM

FIGURE 2


pmol CO / mg
fresh tissue weight
600
400
200
0
rHSA
rHSA-CORM
Kidney
Heart
Lung
Liver
Spleen
Brain
Blood
Blood (4h)
Tumour

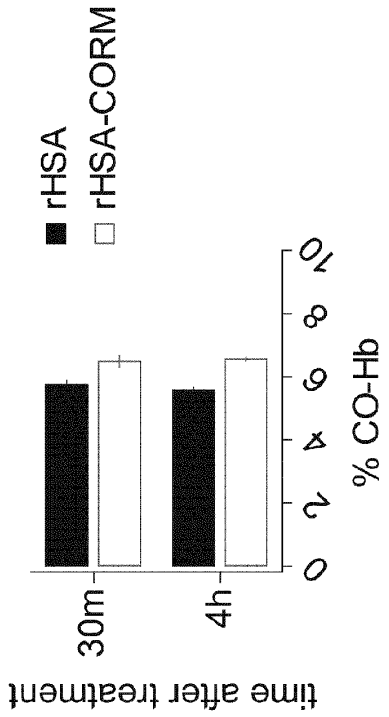

time after treatment
30m
4h
rHSA
rHSA-CORM
0
2
4
6
8
10
% CO-Hb

FIGURE 3

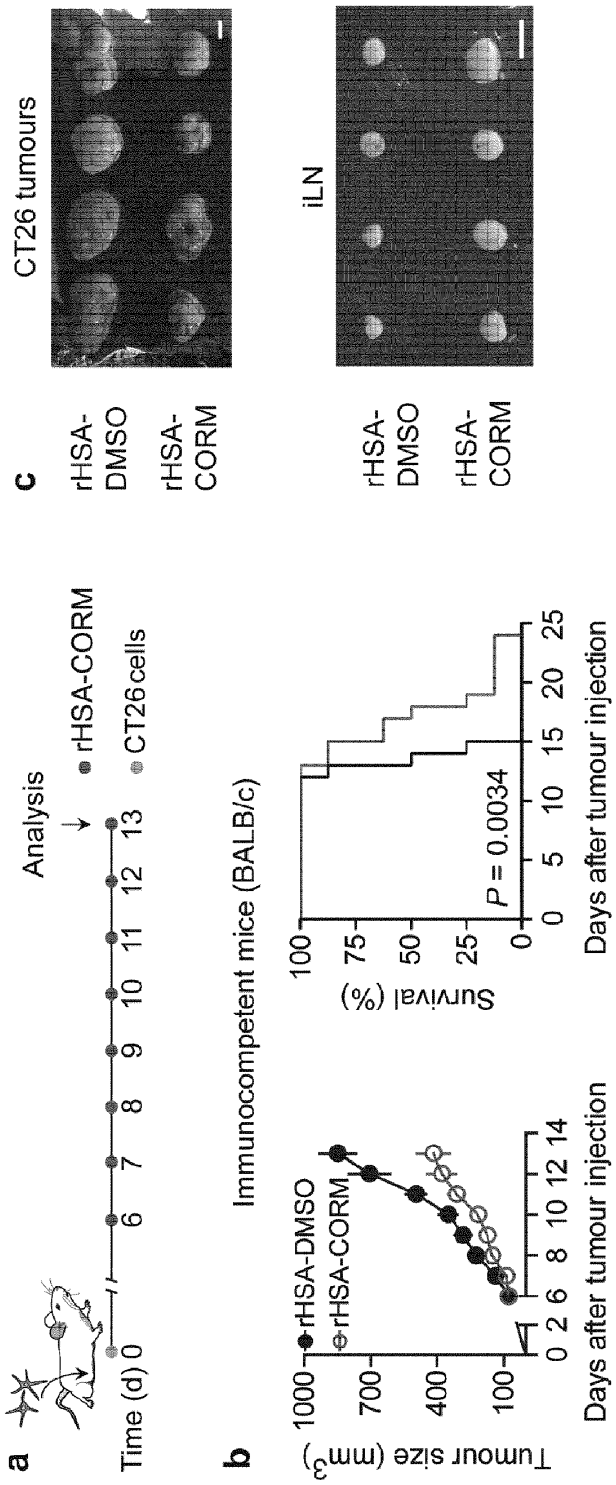
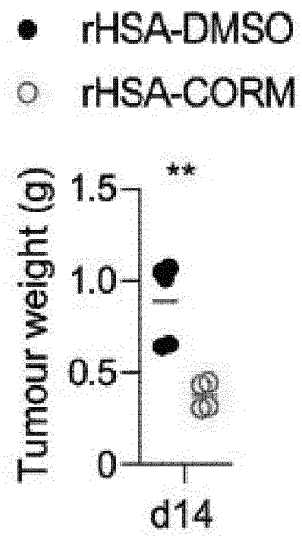

a
Analysis
rHSA-CORM
CT26 cells
Time (d) 0 6 7 8 9 10 11 12 13
b
Immunocompetent mice (BALB/c)
rHSA-DMSO
rHSA-CORM
Tumour size (mm3)
1000
700
400
100
0 2 6 8 10 12 14
Days after tumour injection
Survival (%)
100
75
50
25
0
P = 0.0034
0 5 10 15 20 25
Days after tumour injection
c
CT26 tumours
rHSA-DMSO
rHSA-CORM
iLN
rHSA-DMSO
rHSA-CORM

FIGURE 4(a-c)

FIGURE 4(d)



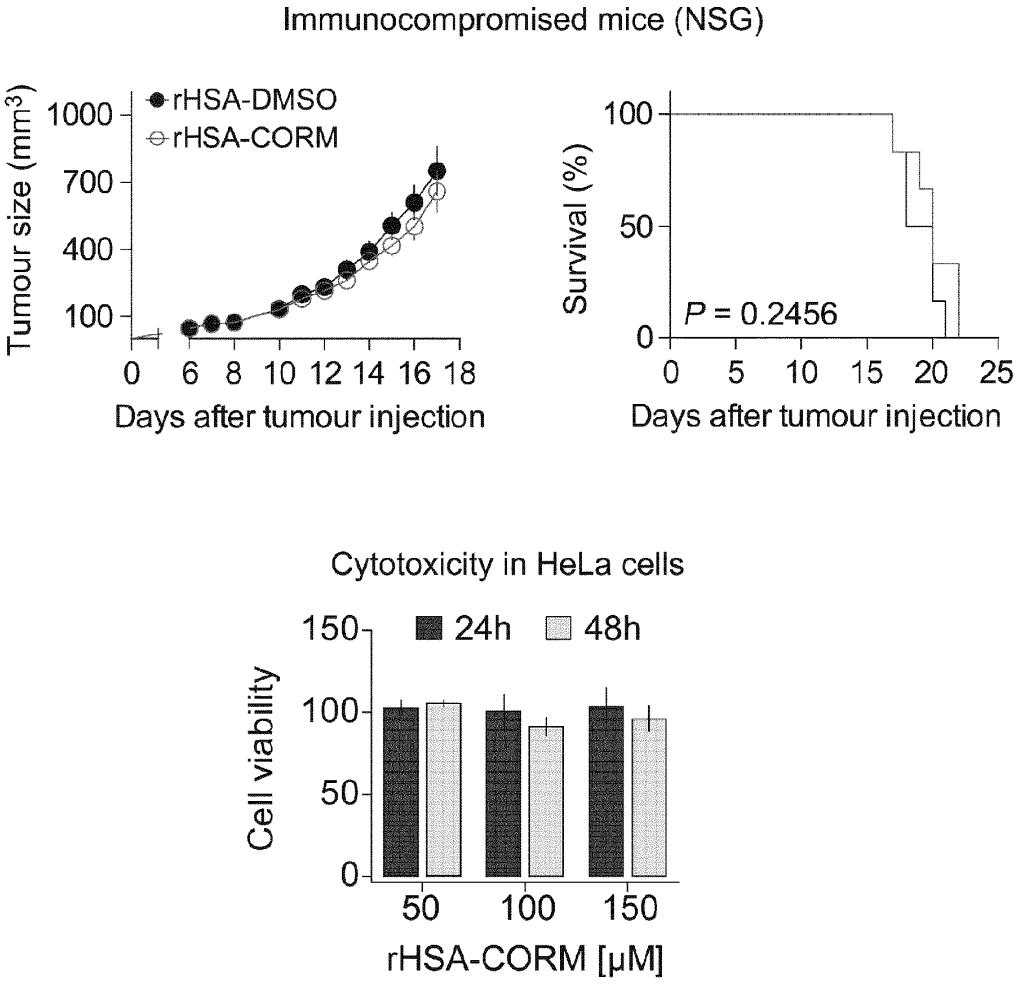
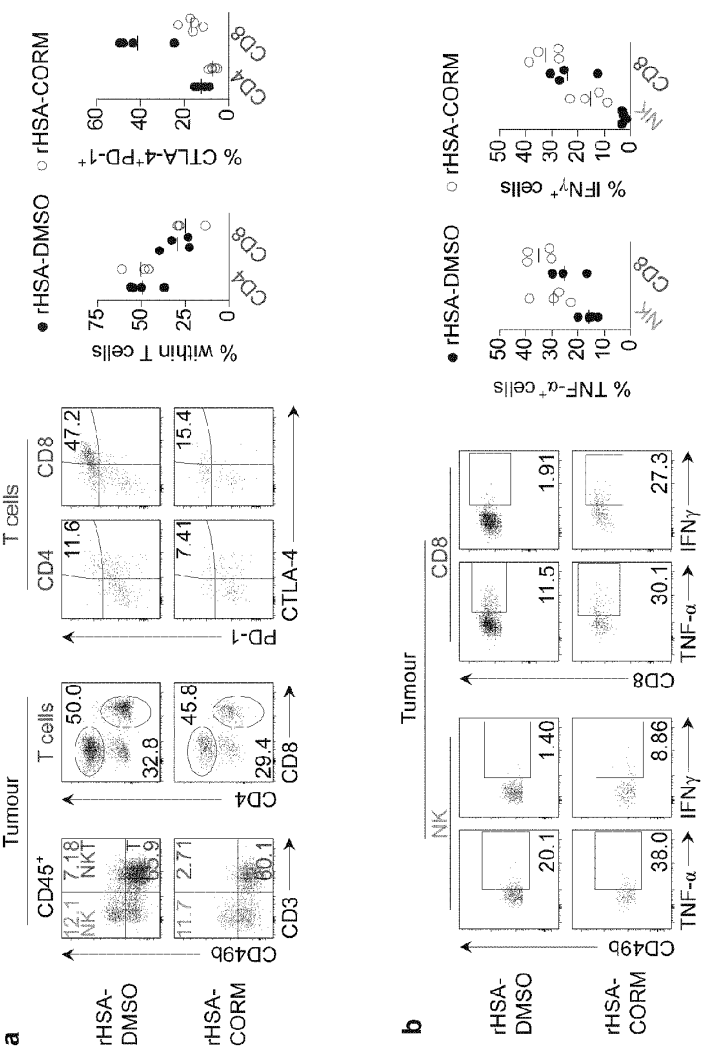

Immunocompromised mice (NSG)
Tumour size (mm3)
1000
700
400
100
rHSA-DMSO
rHSA-CORM
0 6 8 10 12 14 16 18
Days after tumour injection
Survival (%)
100
50
0
P = 0.2456
0 5 10 15 20 25
Days after tumour injection
Cytotoxicity in HeLa cells
Cell viability
150
100
50
0
24h
48h
50 100 150
rHSA-CORM [µM]

FIGURE 5

FIGURE 6


a
Tumour
T cells
CD45+
CD4
CD8
rHSA-DMSO
rHSA-CORM
CD49b
CD3
CD4
CD8
PD-1
CTLA-4
12.1
NK
7.18
NKT
T
15.9
11.7
2.71
60.1
50.0
32.8
45.8
29.4
11.6
47.2
7.41
15.4
● rHSA-DMSO ○ rHSA-CORM
% within T cells
% CTLA-4+PD-1+
b
Tumour
NK
CD8
TNF-α
IFNγ
20.1
1.40
38.0
8.86
11.5
1.91
30.1
27.3
% TNF-α+ cells
% IFNγ+ cells

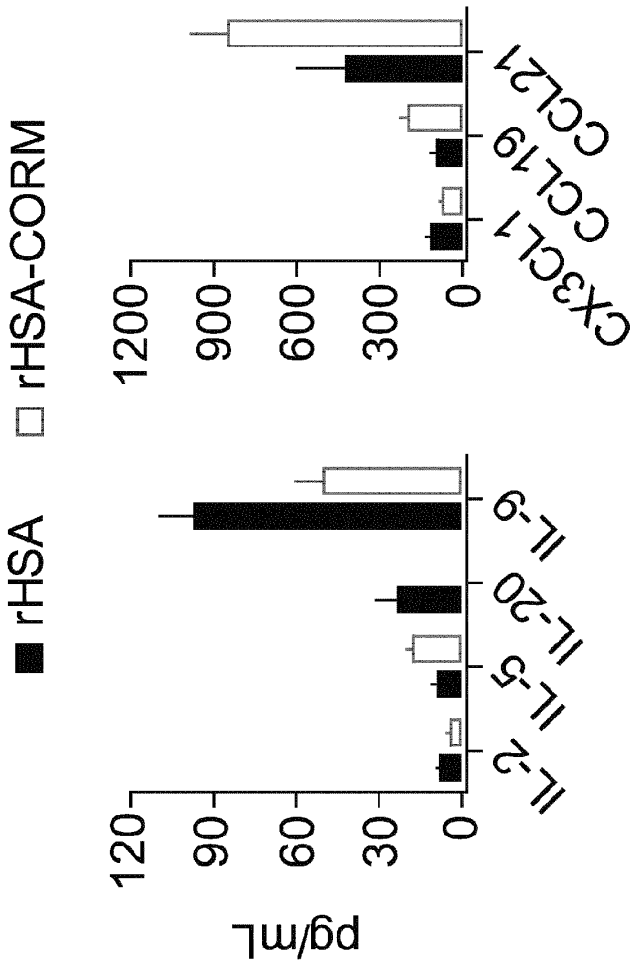

rHSA
rHSA-CORM
pg/mL
120
90
60
30
0
IL-2
IL-5
IL-20
IL-9
1200
900
600
300
0
CX3CL1
CCL19
CCL21

FIGURE 7(a)

FIGURE 7(b,c)

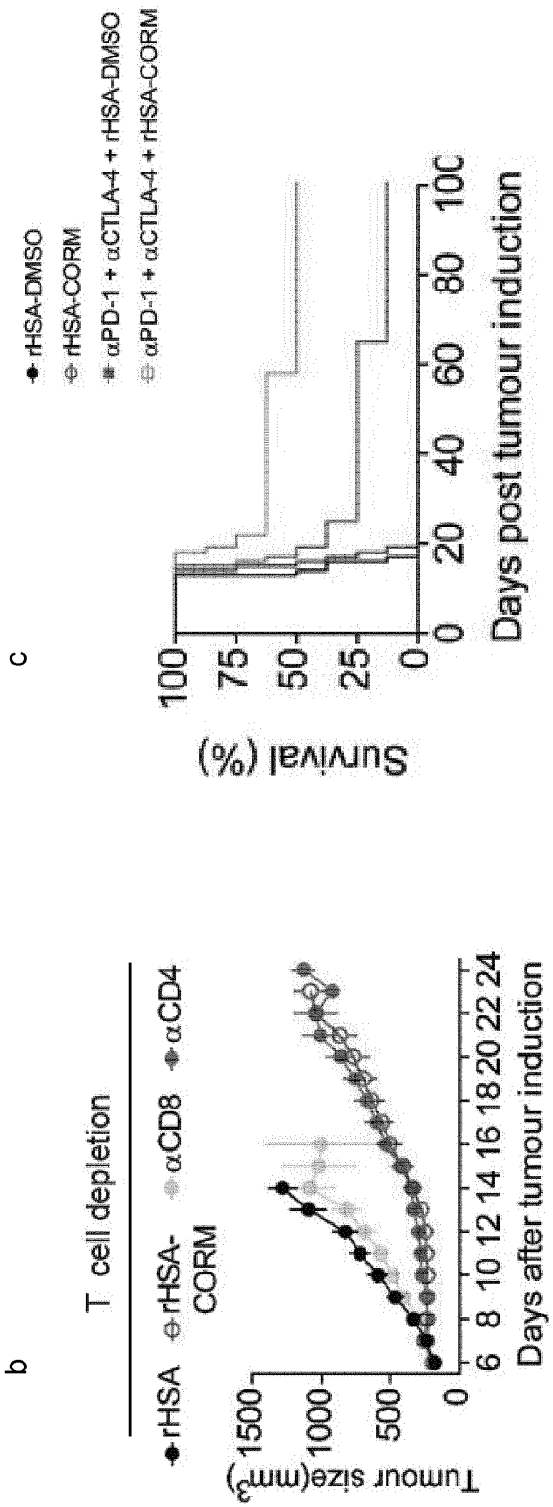
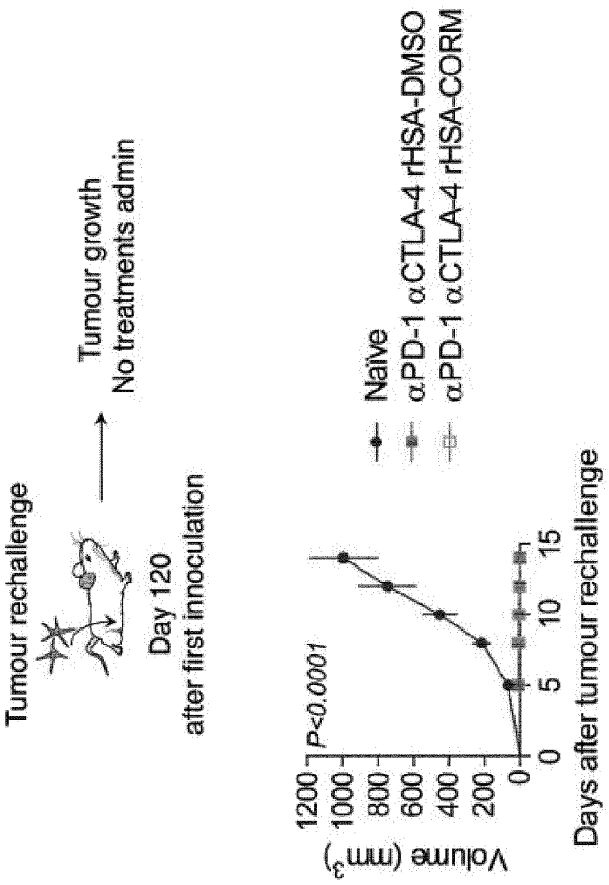

b
T cell depletion
rHSA
rHSA-CORM
αCD8
αCD4
Tumour size(mm³)
1500
1000
500
0
6 8 10 12 14 16 18 20 22 24
Days after tumour induction
c
rHSA-DMSO
rHSA-CORM
αPD-1 + αCTLA-4 + rHSA-DMSO
αPD-1 + αCTLA-4 + rHSA-CORM
Survival (%)
100
75
50
25
0
0 20 40 60 80 100
Days post tumour induction

FIGURE 7(d)


Tumour rechallenge
Day 120
after first innoculation
Tumour growth
No treatments admin
Naïve
αPD-1 αCTLA-4 rHSA-DMSO
αPD-1 αCTLA-4 rHSA-CORM
P<0.0001
Volume (mm3)
1200
1000
800
600
400
200
0
0
5
10
15
Days after tumour rechallenge

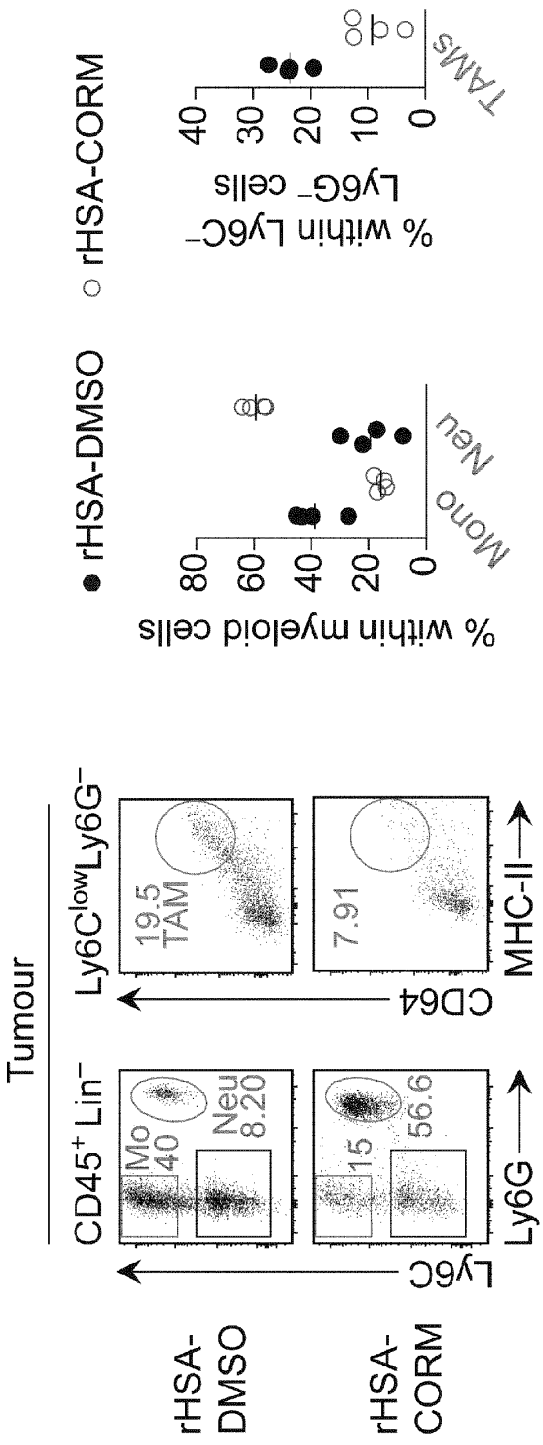

Tumour
CD45+ Lin−
Ly6C
Ly6G
Mo
40
Neu
8.20
15
56.6
rHSA-DMSO
rHSA-CORM
Ly6Clow Ly6G−
CD64
MHC-II
19.5
TAM
7.91
● rHSA-DMSO ○ rHSA-CORM
% within myeloid cells
Mono
Neu
% within Ly6C− Ly6G− cells
TAMs

FIGURE 8

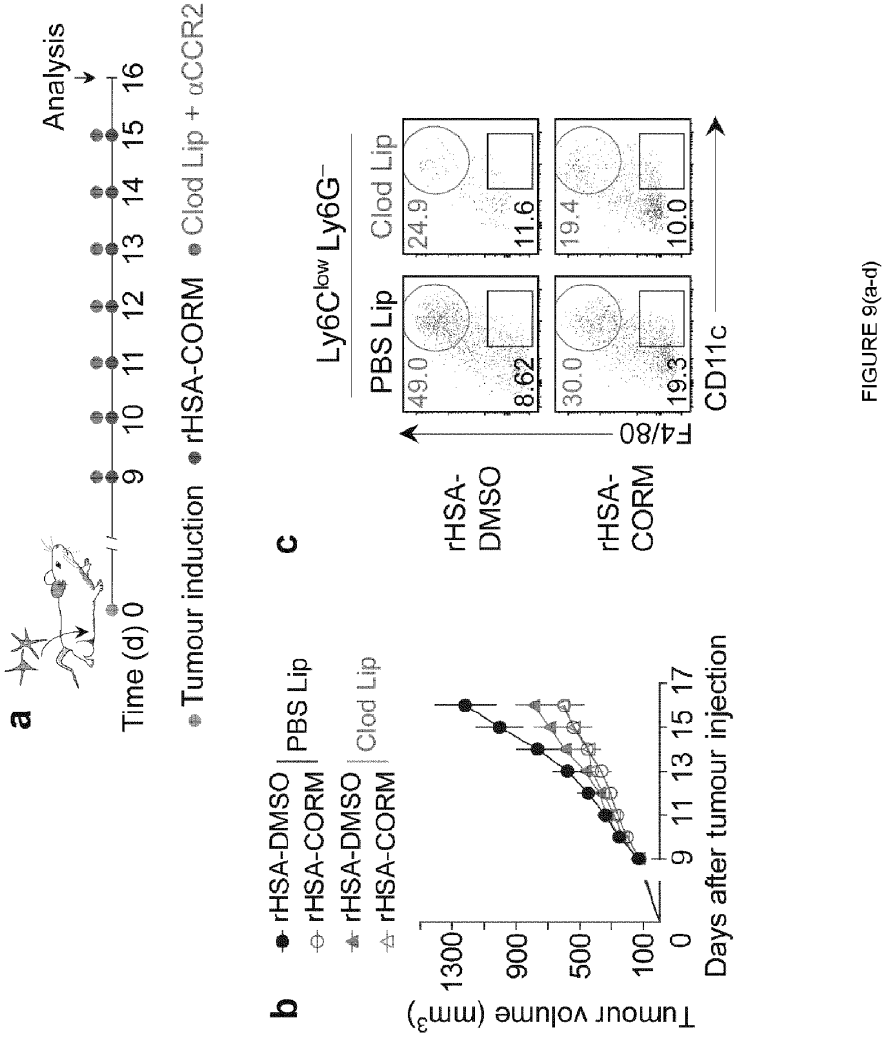
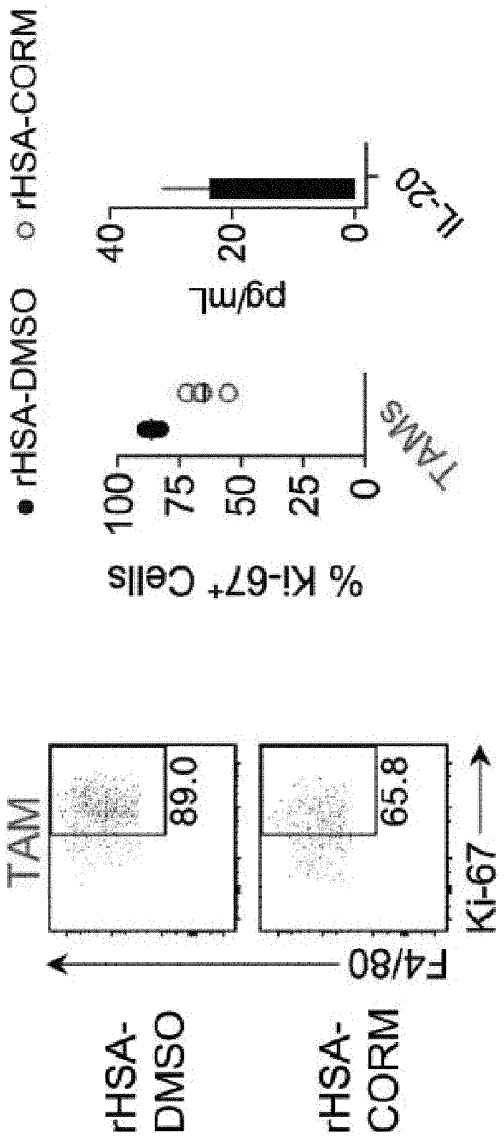

a
Analysis
Time (d) 0 9 10 11 12 13 14 15 16
Tumour induction
rHSA-CORM
Clod Lip + αCCR2
b
rHSA-DMSO | PBS Lip
rHSA-CORM | PBS Lip
rHSA-DMSO | Clod Lip
rHSA-CORM | Clod Lip
Tumour volume (mm3)
1300
900
500
100
0 9 11 13 15 17
Days after tumour injection
c
Ly6Clow Ly6G−
PBS Lip
Clod Lip
rHSA-DMSO
rHSA-CORM
49.0
8.62
24.9
11.6
30.0
19.3
19.4
10.0
F4/80
CD11c
d
rHSA-DMSO | PBS Lip
rHSA-CORM | PBS Lip
rHSA-DMSO | Clod Lip
rHSA-CORM | Clod Lip
% within Ly6Clow Ly6G−
60
40
20
0
DC
TAM

FIGURE 9(a-d)

FIGURE 9(e)


rHSA-DMSO
rHSA-CORM
% Ki-67+ Cells
100
75
50
25
0
TAMs
pg/mL
40
20
0
IL-20
TAM
89.0
65.8
rHSA-DMSO
rHSA-CORM
F4/80
Ki-67

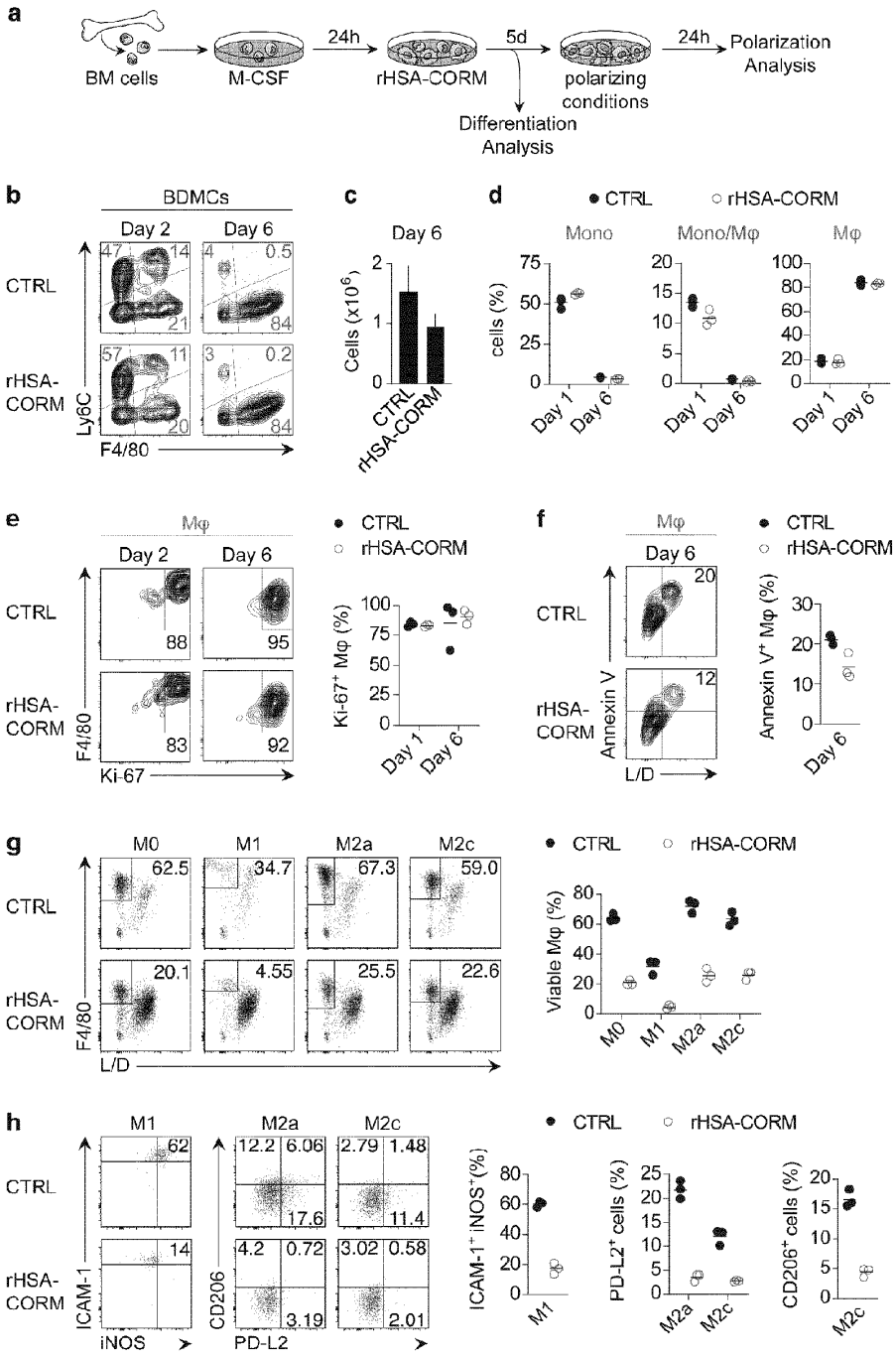
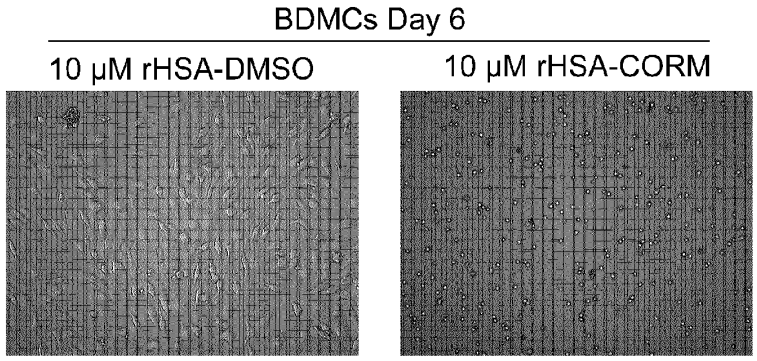

a
BM cells
M-CSF
24h
rHSA-CORM
5d
polarizing conditions
24h
Polarization Analysis
Differentiation Analysis
b
BDMCs
Day 2
Day 6
CTRL
rHSA-CORM
Ly6C
F4/80
c
Day 6
Cells (x10^6)
CTRL
rHSA-CORM
d
CTRL
rHSA-CORM
Mono
Mono/Mφ
Mφ
cells (%)
Day 1
Day 6
e
Mφ
Day 2
Day 6
CTRL
rHSA-CORM
F4/80
Ki-67
Ki-67+ Mφ (%)
f
Mφ
Day 6
Annexin V
L/D
Annexin V+ Mφ (%)
g
M0
M1
M2a
M2c
F4/80
L/D
Viable Mφ (%)
h
M1
M2a
M2c
ICAM-1
iNOS
CD206
PD-L2
ICAM-1+ iNOS+(%)
PD-L2+ cells (%)
CD206+ cells (%)

FIGURE 10(a-h)

a Compounds added **at the same time** as BM cells



b Compounds added **after** differentiation

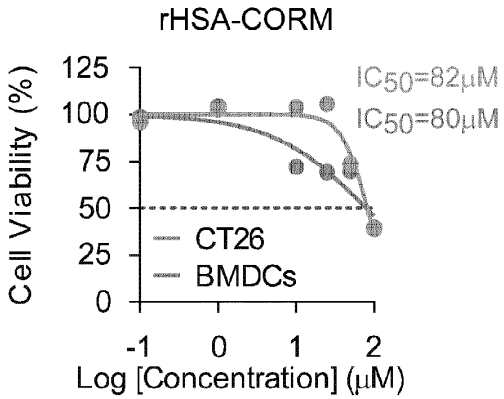
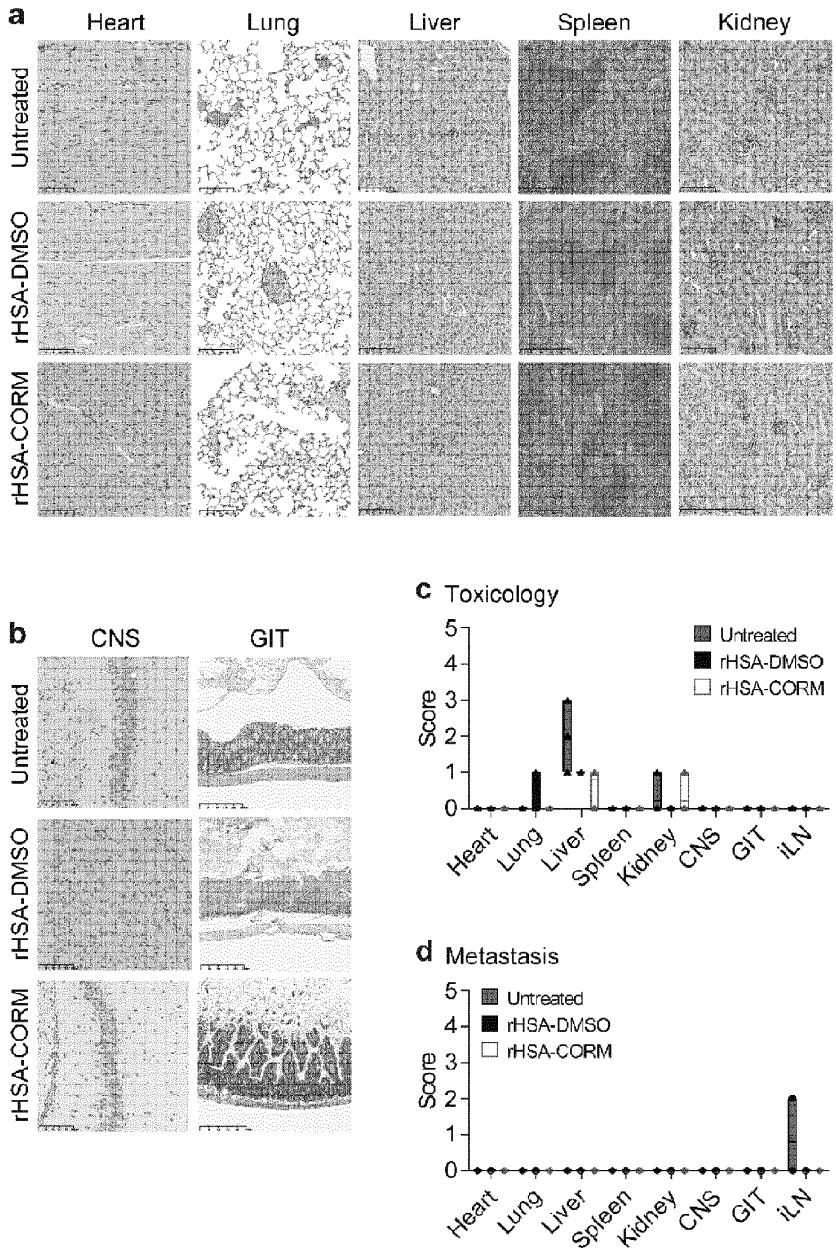


FIGURE 11(a,b)

FIGURE 12 (a-d)


a
Heart
Lung
Liver
Spleen
Kidney
Untreated
rHSA-DMSO
rHSA-CORM
b
CNS
GIT
Untreated
rHSA-DMSO
rHSA-CORM
c Toxicology
Untreated
rHSA-DMSO
rHSA-CORM
Score
0
1
2
3
4
5
Heart
Lung
Liver
Spleen
Kidney
CNS
GIT
iLN
d Metastasis
Untreated
rHSA-DMSO
rHSA-CORM
Score
0
1
2
3
4
5
Heart
Lung
Liver
Spleen
Kidney
CNS
GIT
iLN