Figure S1

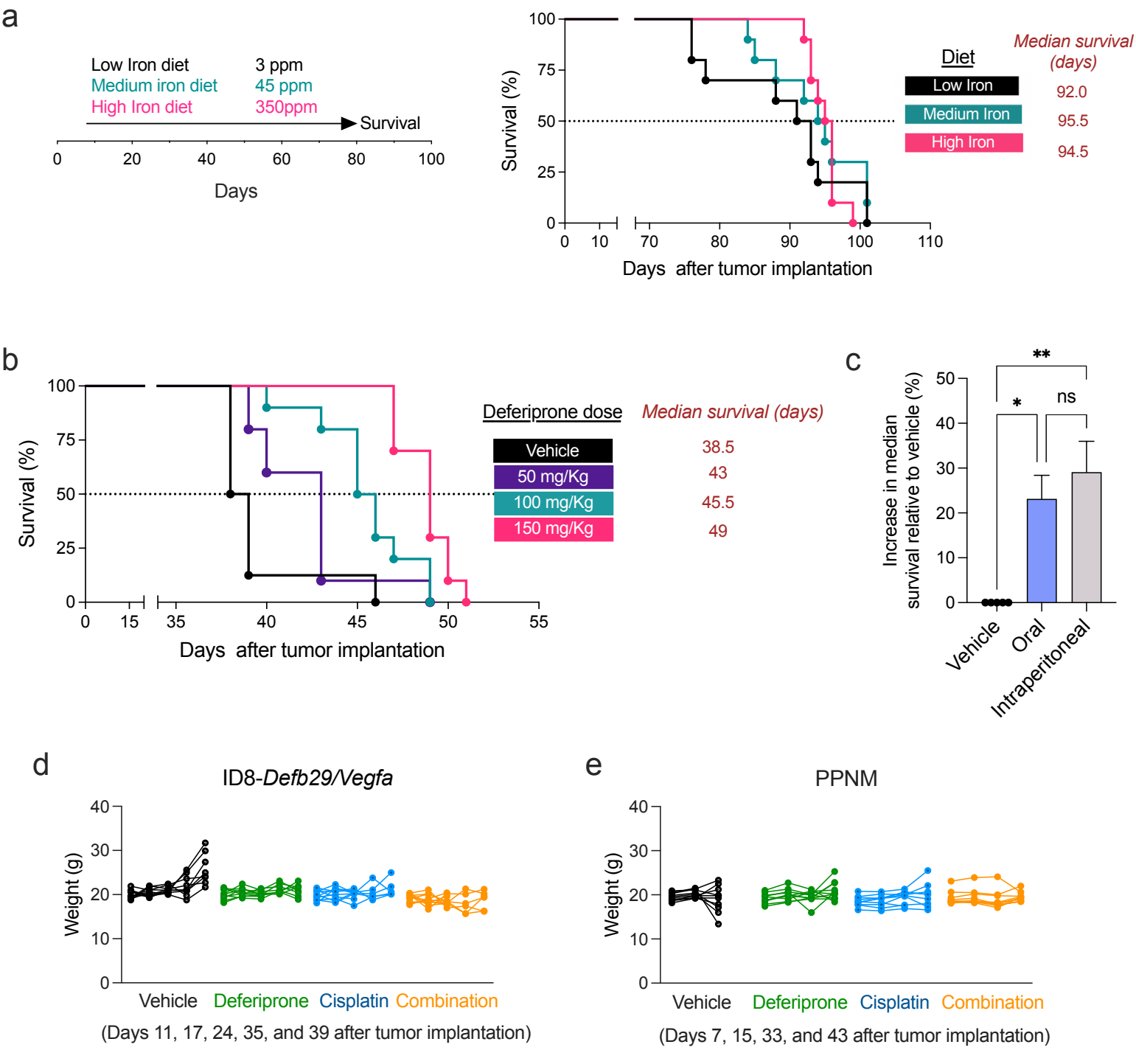
a
Low Iron diet 3 ppm
Medium iron diet 45 ppm
High Iron diet 350ppm
Survival
Days
Survival (%)
Days after tumor implantation
Diet
Median survival (days)
Low Iron 92.0
Medium Iron 95.5
High Iron 94.5
b
Deferiprone dose
Median survival (days)
Vehicle 38.5
50 mg/Kg 43
100 mg/Kg 45.5
150 mg/Kg 49
c
Increase in median survival relative to vehicle (%)
**
*
ns
Vehicle
Oral
Intraperitoneal
d
ID8-Defb29/Vegfa
Weight (g)
Vehicle Deferiprone Cisplatin Combination
(Days 11, 17, 24, 35, and 39 after tumor implantation)
e
PPNM
Weight (g)
Vehicle Deferiprone Cisplatin Combination
(Days 7, 15, 33, and 43 after tumor implantation)

Figure S1. (**a**) Effect of dietary iron levels on ovarian cancer progression. Isocaloric diets containing the indicated levels of iron were administered *ad libitum*, and overall survival was monitored. Kaplan-Meier survival curve comparison for each group of treatment (n = 8 mice/group). (**b**) Mice were implanted with ID8-*Defb29/Vegfa* ovarian cancer cells and 7 days later, mice were treated with increasing doses of deferiprone (50, 100, or 150 mg/Kg). Kaplan-Meier survival curves are shown (n = 8-10 mice/group). (**c**) Mice bearing ID8-*Defb29/Vegfa* tumors for 7 days were treated with 150 mg/kg of deferiprone administered either orally or intraperitoneally. The percentage increase in median survival compared with the vehicle group is presented as bar plots +/- SEM. Significance levels are indicated: *p<0.05, **p<0.01, ns: not significant. (**d-e**) Weight of mice bearing ID8-*Defb29/Vegfa*- or PPNM-based ovarian cancer and treated as indicated.

Figure S2

Flow cytometry gating strategy

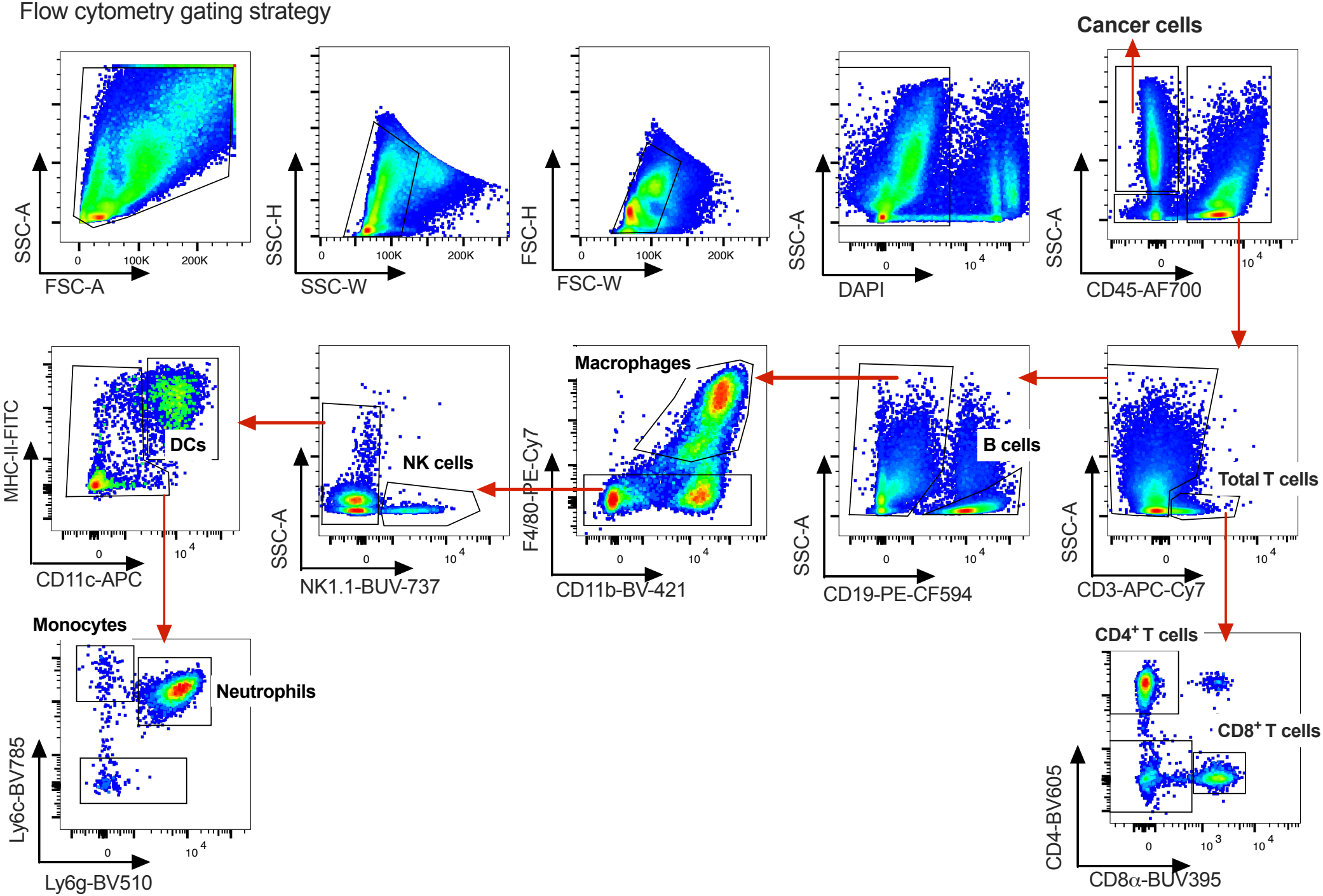

Figure S2. Flow cytometry gating strategy for the analysis of peritoneal cells from mice bearing ID8-*Defb29/Vegfa* tumors that were treated with deferiprone as single agent or in combination with cisplatin.

Figure S3

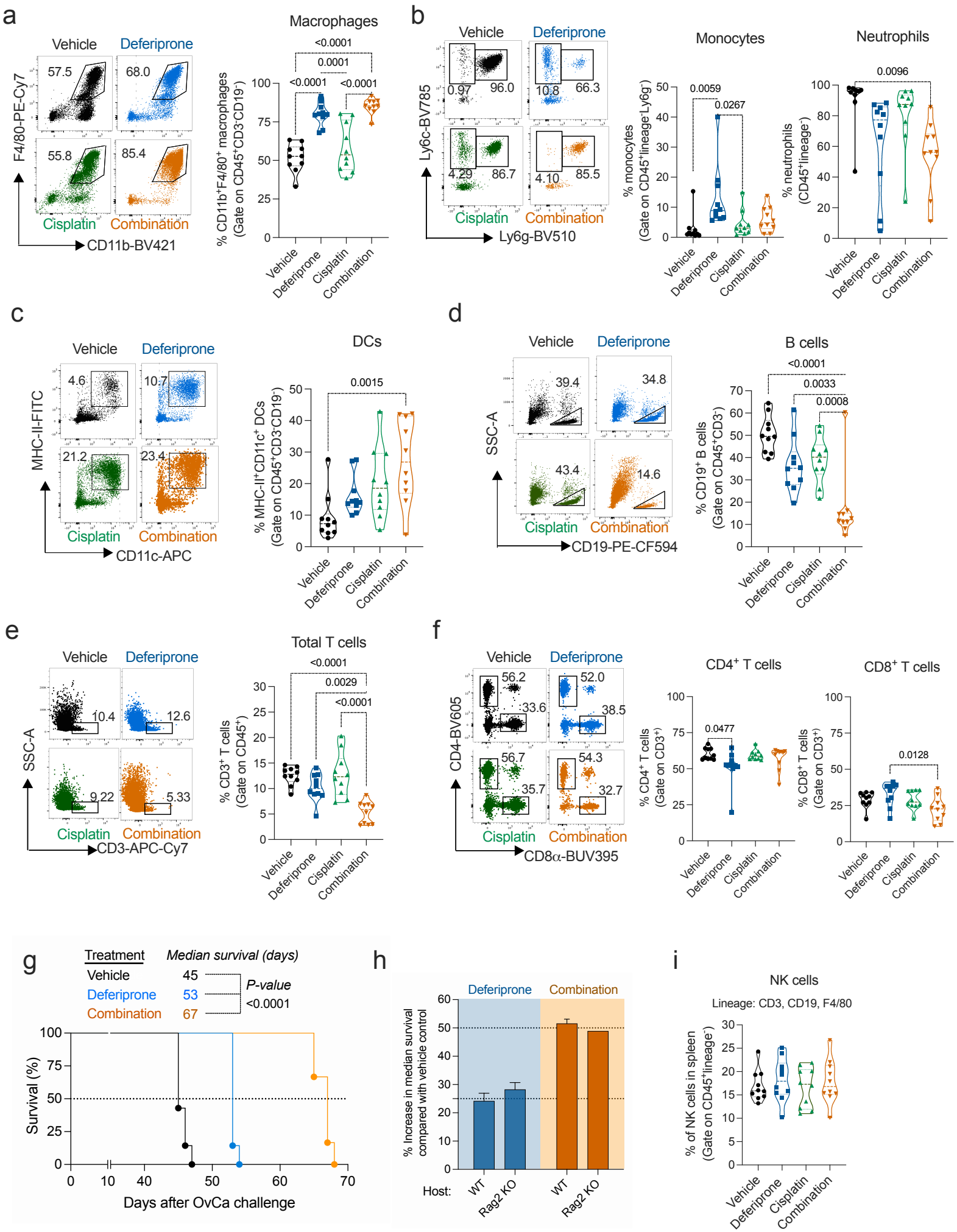
a
Vehicle
Deferiprone
57.5
68.0
55.8
85.4
Cisplatin
Combination
F4/80-PE-Cy7
CD11b-BV421
Macrophages
<0.0001
0.0001
<0.0001
<0.0001
% CD11b+F4/80+ macrophages (Gate on CD45+CD3-CD19-)
Vehicle
Deferiprone
Cisplatin
Combination
b
Vehicle
Deferiprone
0.97
96.0
10.8
66.3
4.29
86.7
4.10
85.5
Cisplatin
Combination
Ly6c-BV785
Ly6g-BV510
Monocytes
0.0059
0.0267
% monocytes (Gate on CD45+lineage-Ly6g-)
Neutrophils
0.0096
% neutrophils (CD45+lineage-)
c
Vehicle
Deferiprone
4.6
10.7
21.2
23.4
Cisplatin
Combination
MHC-II-FITC
CD11c-APC
DCs
0.0015
% MHC-II+CD11c+ DCs (Gate on CD45+CD3-CD19-)
d
Vehicle
Deferiprone
39.4
34.8
43.4
14.6
Cisplatin
Combination
SSC-A
CD19-PE-CF594
B cells
<0.0001
0.0033
0.0008
% CD19+ B cells (Gate on CD45+CD3-)
e
Vehicle
Deferiprone
10.4
12.6
9.22
5.33
Cisplatin
Combination
SSC-A
CD3-APC-Cy7
Total T cells
<0.0001
0.0029
<0.0001
% CD3+ T cells (Gate on CD45+)
f
Vehicle
Deferiprone
56.2
52.0
33.6
38.5
56.7
54.3
35.7
32.7
Cisplatin
Combination
CD4-BV605
CD8α-BUV395
CD4+ T cells
0.0477
% CD4+ T cells (Gate on CD3+)
CD8+ T cells
0.0128
% CD8+ T cells (Gate on CD3+)
g
Treatment
Median survival (days)
Vehicle 45
Deferiprone 53
Combination 67
P-value
<0.0001
Survival (%)
Days after OvCa challenge
h
Deferiprone
Combination
% Increase in median survival compared with vehicle control
Host:
WT
Rag2 KO
WT
Rag2 KO
i
NK cells
Lineage: CD3, CD19, F4/80
% of NK cells in spleen (Gate on CD45+lineage-)

Figure S3. (a-f) Wild type female mice were implanted with ID8-*Defb29/Vegfa* tumors and treated as described in Fig 2e. At day 30, peritoneal lavage samples were obtained, and the cellular fraction was analyzed by FACS. Representative FACS plots and the proportion of the indicated immune cell types in all groups are shown. Violin plots display the distribution of all data points, including median and quartiles, with each point representing an independent mouse. Statistical comparisons were done using one-way ANOVA with Tukey's multiple comparison, and exact *P*-values are shown. (**g**) Kaplan-Meier survival curve for Rag2-deficient mice implanted with ID8-*Defb29/Vegfa* tumors and treated as described in Fig 2e. (**h**) Effects of treatment in wild type (WT) vs. Rag2-deficient mice, expressed as percent increase in median survival compared with intrinsic control groups. Log-Rank (Mantel-Cox) test was used and *P*-values are shown. (**i**) Splenic NK cell proportion in tumor-bearing mice receiving the indicated treatments.

Figure S4

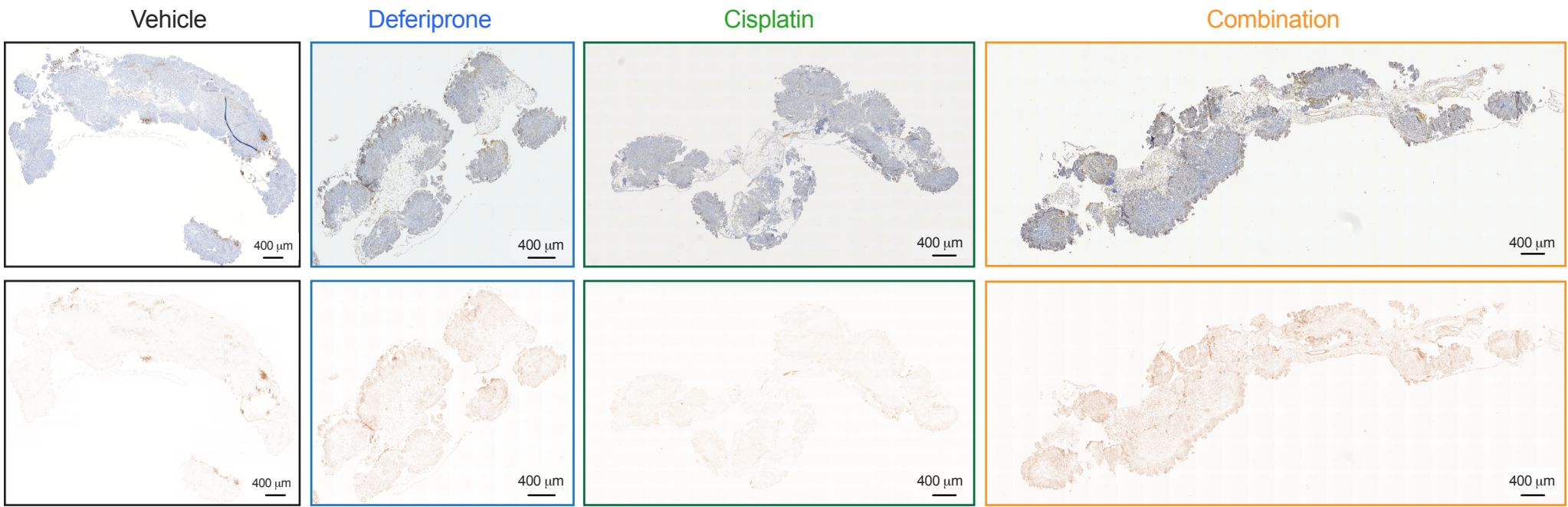
Vehicle
Deferiprone
Cisplatin
Combination
400 μm
400 μm
400 μm
400 μm
400 μm
400 μm
400 μm
400 μm

Figure S4. Representative images of NKp46 staining on the entire omentum. The top sections illustrate an overlay of all channels, while the bottom sections show the deconvoluted NKp46 signal. Scale bar represents 400 μm.

Figure S5

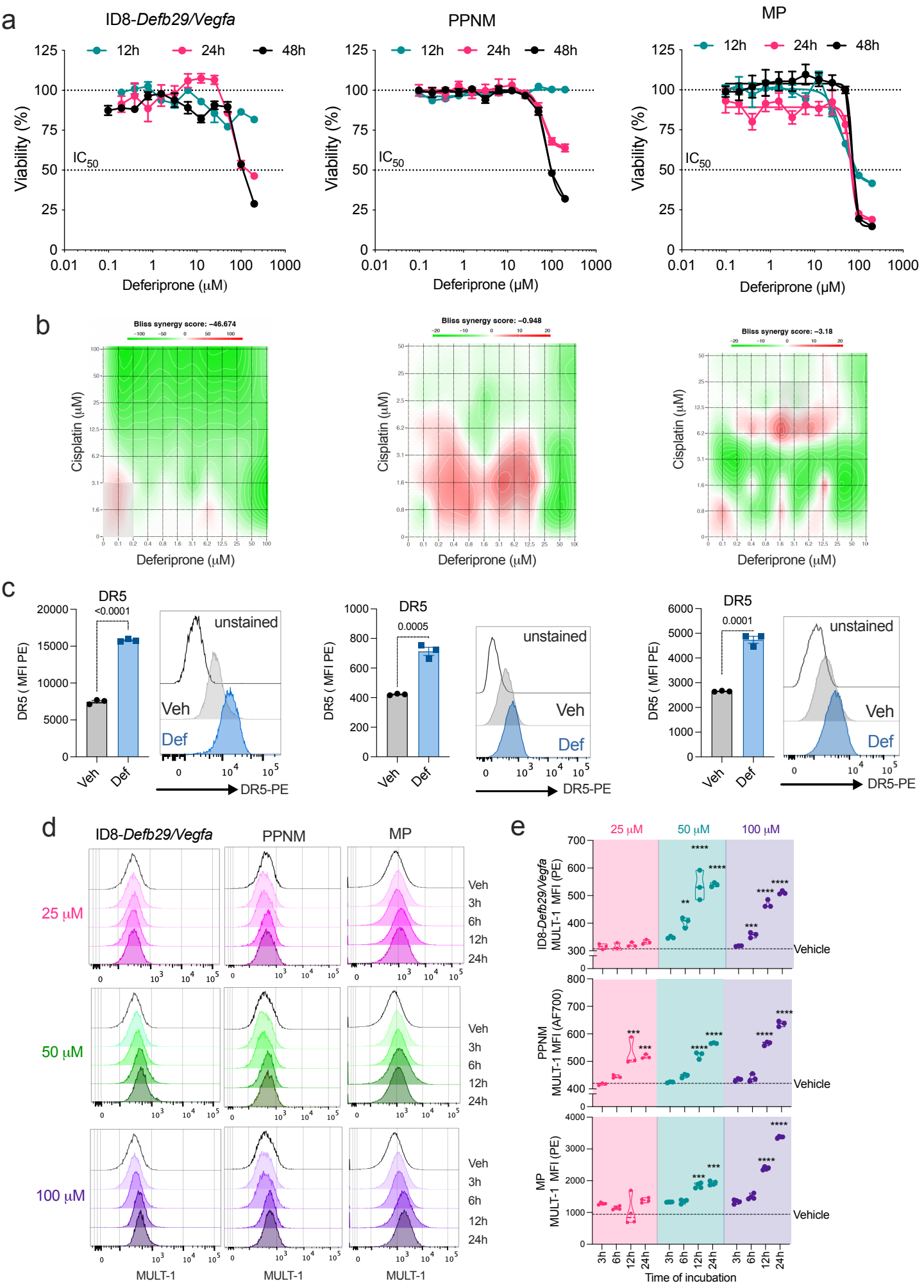
a
ID8-Defb29/Vegfa
PPNM
MP
12h
24h
48h
Viability (%)
IC50
Deferiprone (μM)
b
Bliss synergy score: −46.674
Bliss synergy score: −0.948
Bliss synergy score: −3.18
Cisplatin (μM)
Deferiprone (μM)
c
DR5
<0.0001
0.0005
0.0001
DR5 (MFI PE)
unstained
Veh
Def
DR5-PE
d
ID8-Defb29/Vegfa
PPNM
MP
25 μM
50 μM
100 μM
Veh
3h
6h
12h
24h
MULT-1
e
25 μM
50 μM
100 μM
ID8-Defb29/Vegfa MULT-1 MFI (PE)
PPNM MULT-1 MFI (AF700)
MP MULT-1 MFI (PE)
Vehicle
Time of incubation

Figure S5. (**a**) MTT assays for ID8-*Defb29/Vegfa*, MP, and PPNM cells treated with different concentrations of deferiprone for 12, 24, and 48 hours. (**b**) Bliss synergy scores for in vitro combinations of cisplatin and deferiprone across different concentrations for ID8-*Defb29/Vegfa*, PPNM, and MP cells. (**c**) ID8-*Defb29/Vegfa*, PPNM, and MP ovarian cancer cells were treated with 100 μM deferiprone for 24 hours and surface expression of DR5 was assessed by FACS. Bar plots of mean fluorescence intensity (NFI) and representative histograms are shown. Unpaired Student's *t*-test was used, and exact *P*-values are shown ($n = 3$ technical replicates). (**d** and **e**) ID8-*Defb29/Vegfa*, PPNM, and MP cells were treated in vitro with increasing doses of deferiprone for the indicated times and MULT1 surface expression was analyzed by FACS. Representative histograms (**d**) and violin plots (**e**) are shown. ($n = 3$ technical replicates). (**e**) One-way ANOVA with Dunnet's comparison test against vehicle was applied. All experiments were repeated at least three independent times with similar results.

Figure S6

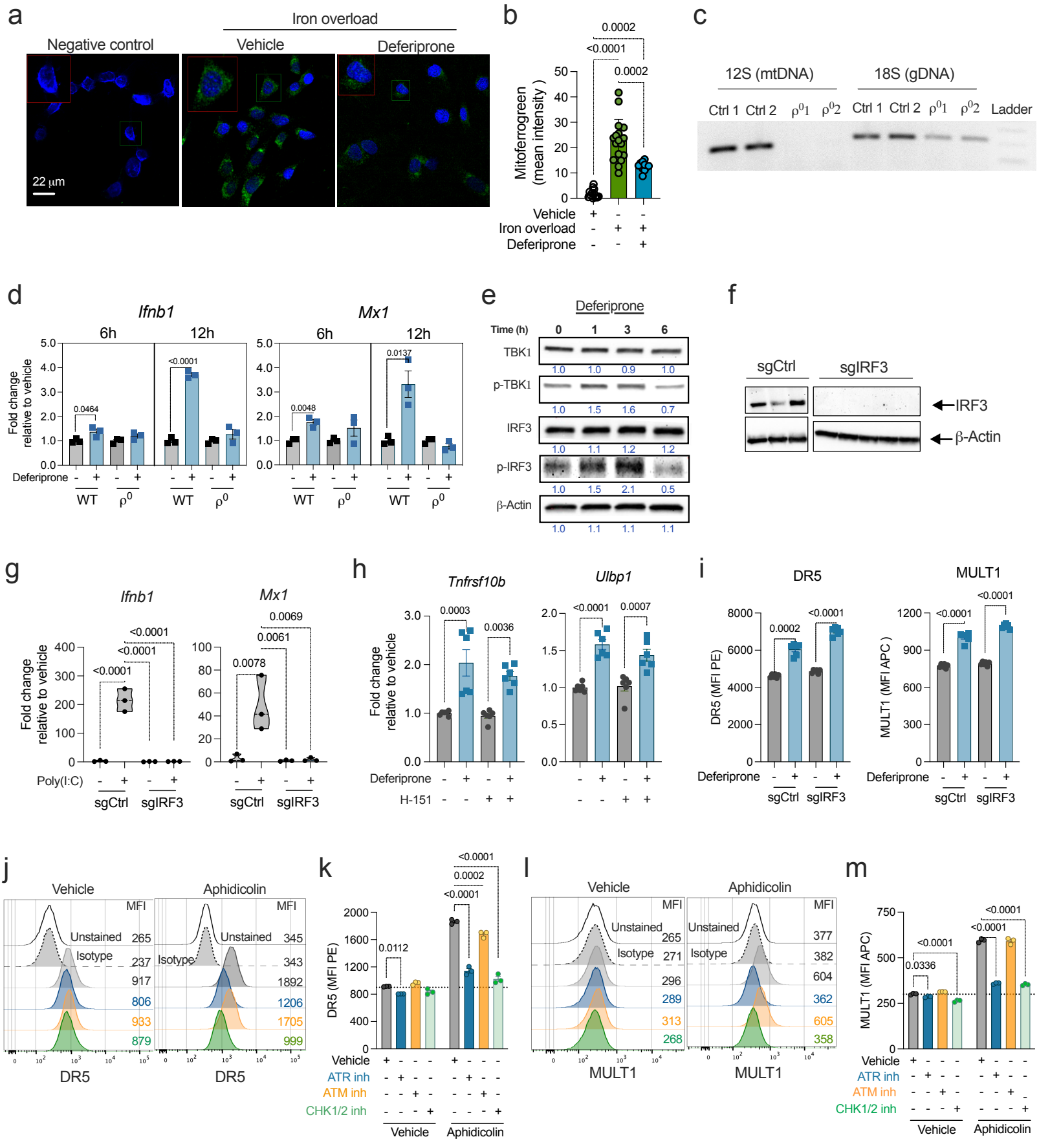
a
Iron overload
Negative control
Vehicle
Deferiprone
22 µm
b
Mitoferrogreen (mean intensity)
0.0002
<0.0001
0.0002
Vehicle
Iron overload
Deferiprone
c
12S (mtDNA)
18S (gDNA)
Ctrl 1
Ctrl 2
ρ0 1
ρ0 2
Ctrl 1
Ctrl 2
ρ0 1
ρ0 2
Ladder
d
Ifnb1
Mx1
6h
12h
Fold change relative to vehicle
0.0464
<0.0001
0.0048
0.0137
Deferiprone
WT
ρ0
e
Deferiprone
Time (h)
TBK1
p-TBK1
IRF3
p-IRF3
β-Actin
f
sgCtrl
sgIRF3
IRF3
β-Actin
g
Ifnb1
Mx1
Fold change relative to vehicle
Poly(I:C)
sgCtrl
sgIRF3
h
Tnfrsf10b
Ulbp1
Fold change relative to vehicle
0.0003
0.0036
<0.0001
0.0007
Deferiprone
H-151
i
DR5
MULT1
DR5 (MFI PE)
MULT1 (MFI APC)
0.0002
Deferiprone
sgCtrl
sgIRF3
j
Vehicle
Aphidicolin
MFI
Unstained
Isotype
DR5
k
DR5 (MFI PE)
0.0112
0.0002
Vehicle
ATR inh
ATM inh
CHK1/2 inh
Vehicle
Aphidicolin
l
Vehicle
Aphidicolin
MFI
Unstained
Isotype
MULT1
m
MULT1 (MFI APC)
0.0336
Vehicle
ATR inh
ATM inh
CHK1/2 inh
Vehicle
Aphidicolin

Figure S6. (**a-b**) Mitochondrial iron was traced using mito-ferrogreen in iron-overloaded ID8-*Defb29/Vegfa* cells that were exposed to deferiprone (100 μM) or vehicle control. (**a**) Representative confocal microscopy images showing mito-ferrogreen (green) and nuclear DAPI (blue) staining. (**b**) Mean intensity of mito-ferrogreen quantified on a per cell basis. (**c**) Agarose gel image showing the presence of 12S mtDNA or 18S gDNA in parental (WT) or mtDNA-deficient (ρ^0) ID8-*Defb29/Vegfa* cancer cells. (**d**) WT and ρ^0 ID8-*Defb29/Vegfa* cells were treated with vehicle or deferiprone (100 μM) for 6 and 12 hours, and the expression of *Ifnb1* and *Mx1* was assessed by RT-qPCR (n = 3 technical replicates per condition). (**e**) ID8-*Defb29/Vegfa* cells were treated with deferiprone (100 μM) or vehicle for 0, 1, 3 or 6 hours, and the levels of the indicated proteins were analyzed by western blot. Representative images are shown, and protein loading was controlled by β-actin. (**f**) Western blot showing IRF3 levels in ID8-*Defb29/Vegfa* cells carrying control non-targeting sgRNA (sgCtrl, 3 clones), or IRF3-targeting sgRNA (sgIRF3, 5 clones). (**g**) sgCtrl or sgIRF3 ID8-*Defb29/Vegfa* cells were transfected with poly (I:C) and the expression of *Ifnb1* and *Mx1* was assessed (n = 3 technical replicates per condition). (**h**) Expression of the indicated genes in deferiprone-exposed ID8-*Defb29/Vegfa* cells pretreated with the STING inhibitor H-151 (n = 6 technical replicates per condition). (**i**) Surface expression of DR5 and MULT1 in deferiprone-exposed sgCtrl or sgIRF3 ID8-*Defb29/Vegfa* cells (n = 6 technical replicates per condition). (**j-m**) Role of the DDR in the induction of DR5 and MULT1 on ovarian cancer cells. ID8-*Defb29/Vegfa* cells were pre-treated for 1 hour with an ATR inhibitor (AZD6738, 1 μM), ATM inhibitor (AZD0156, 100 nM), or CHK1/2 inhibitor (AZD7762, 300 nM). Then, aphidicolin or vehicle were added, and expression of DR5 (**j-k**) and MULT1 (**l-m**) was measured by FACS (n = 3 technical replicates per condition). Experiments were repeated at least three independent times with similar results. (**b, d, h, i, k, m)** Bar plots with mean ± SEM are shown. (**d**) unpaired Student's *t*-test for each time point, comparing vehicle vs deferiprone; (**b, g, h, i**) one-way ANOVA with Tukey's multiple comparison test. (**k, m**) two-way ANOVA with Šídák's multiple comparisons test.

Figure S7

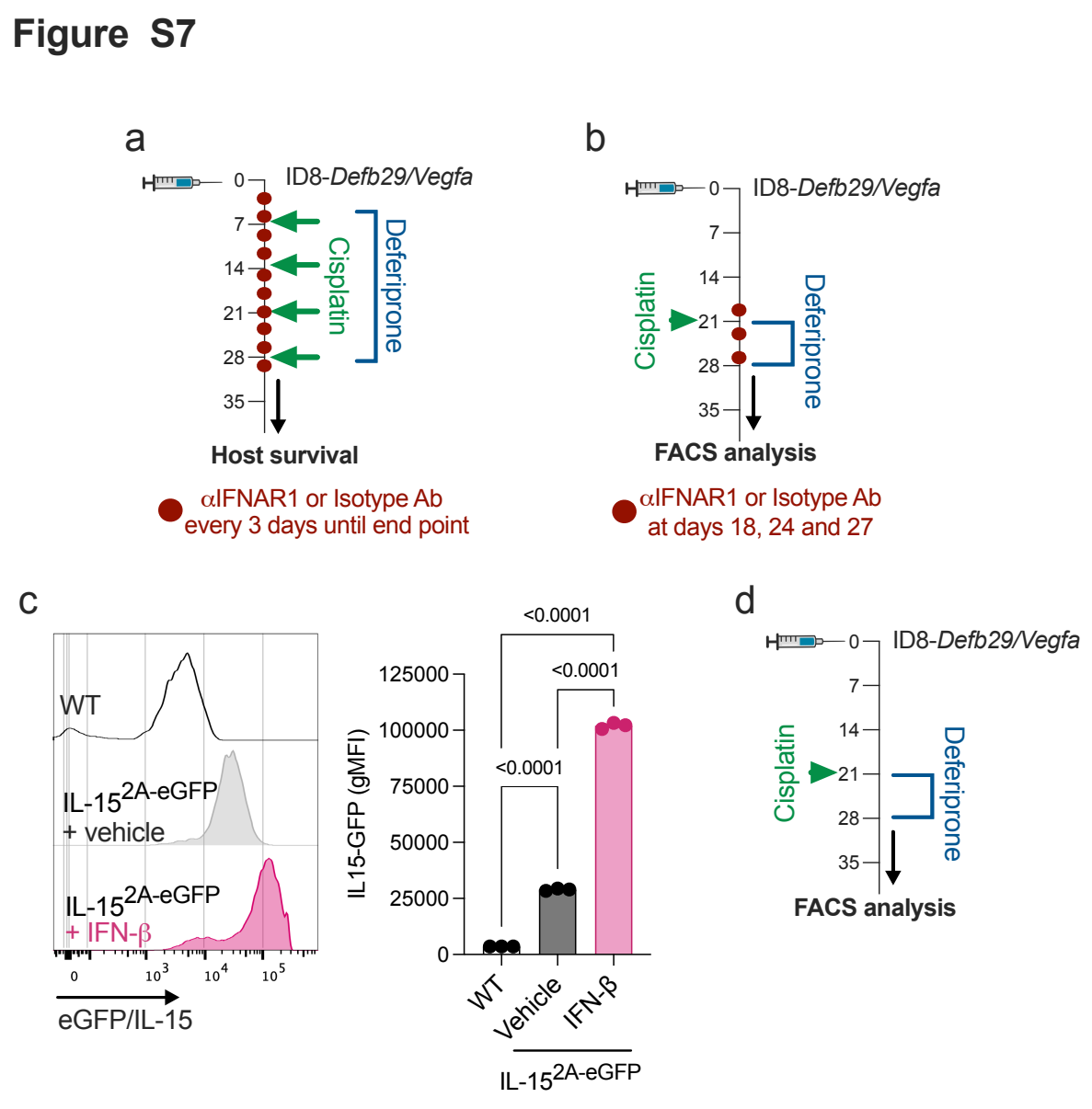
a
ID8-Defb29/Vegfa
0
7
14
21
28
35
Cisplatin
Deferiprone
Host survival
αIFNAR1 or Isotype Ab
every 3 days until end point
b
ID8-Defb29/Vegfa
0
7
14
21
28
35
Cisplatin
Deferiprone
FACS analysis
αIFNAR1 or Isotype Ab
at days 18, 24 and 27
c
WT
IL-15^2A-eGFP
+ vehicle
IL-15^2A-eGFP
+ IFN-β
0
10^3
10^4
10^5
eGFP/IL-15
<0.0001
<0.0001
<0.0001
125000
100000
75000
50000
25000
0
IL15-GFP (gMFI)
WT
Vehicle
IFN-β
IL-15^2A-eGFP
d
ID8-Defb29/Vegfa
0
7
14
21
28
35
Cisplatin
Deferiprone
FACS analysis

Figure S7. (**a** and **b**) Schematic representation of treatment regimens under IFNAR1 blockade related to Fig. 6. (**c**) Bone marrow-derived dendritic cells (BMDCs) generated from IL-15$^{2A\text{-}eGFP}$ female mice were stimulated with 100 pg/ml (500 U/ml) of recombinant IFN-β for 24 hours, and cells were then analyzed by FACS. (**c, left**) Representative histograms of eGFP/IL-15 expression. (**c, right**) Results presented as bar plots +/- SEM (n = 3 technical replicates per condition). Experiments were repeated twice with similar results. (**d**) Schematic representation of treatment regimens related to Fig. 6, f-k.