Figure S1

a

<table>
<thead>
<tr>
<th>Diet</th>
<th>Iron Content (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Iron</td>
<td>3 ppm</td>
</tr>
<tr>
<td>Medium Iron</td>
<td>45 ppm</td>
</tr>
<tr>
<td>High Iron</td>
<td>350 ppm</td>
</tr>
</tbody>
</table>

Survival (%)

Days after tumor implantation

Deferiprone dose

Vehicle
50 mg/Kg
100 mg/Kg
150 mg/Kg

Median survival (days)

- Low Iron: 92.0
- Medium Iron: 95.5
- High Iron: 94.5

b

Survival (%)

Days after tumor implantation

Deferiprone dose

Vehicle
Oral
Intraperitoneal

Median survival (days)

- Vehicle: 38.5
- Oral: 43
- Intraperitoneal: 45.5

Increase in median survival relative to vehicle (%)

C

- Vehicle: **
- Oral: *
- Intraperitoneal: ns

ID8-Defb29/Vegfa

(days 11, 17, 24, 35, and 39 after tumor implantation)

PPNM

(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

- **Cancer cells**
  - CD45-AF700

- **Total T cells**
  - CD3-APC-Cy7

- **B cells**
  - CD19-PE-CF594

- **Macrophages**
  - CD11b-BV421
  - F4/80-PE-Cy7

- **Monocytes**
  - CD11c-APC
  - NK1.1-BUV-737

- **Neutrophils**
  - Ly6g-BV510
  - Ly6c-BV785

- **DCs**
  - NK cells
  - CD11c-APC

- **NK cells**
  - MHC-II-FITC
  - CD11b-BV421

- **Cancer cells**
  - DAPI
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 vs Deferiprone vs Combination

b) Macrophages

b) Monocytes

b) Neutrophils

c) DCs

d) B cells

e) Total T cells

f) CD4+ T cells

f) CD8+ T cells

g) Treatment vs Median survival (days)

h) Deferiprone vs Combination

i) NK cells
**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
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) Viability (%) of ID8-Defb29/Vegfa, PPNM, and MP cells treated with different concentrations of Deferiprone over time. The IC50 values are indicated for each condition.

(b) Heatmaps showing the combination of Cisplatin and Deferiprone treatment with ID8-Defb29/Vegfa, PPNM, and MP cells. Bias synergy scores are indicated for each combination.

(c) DR5 MFI (AF700) and PPNM MFI (AF700) for Veh and Def treatments in ID8-Defb29/Vegfa, PPNM, and MP cells. Unstained samples are also included.

(d) Flow cytometry histograms for ID8-Defb29/Vegfa, PPNM, and MP cells treated with 25, 50, and 100 µM of Deferiprone at different time points (3h, 6h, 12h, 24h).

(e) Statistical analysis of MFI (AF700) for ID8-Defb29/Vegfa, PPNM, and MP cells treated with different concentrations of Deferiprone over time.
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 (MFI) 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-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 (ρ0) ID8-Defb29/Vegfa cancer cells. (d) WT and ρ0 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 Šidák’s multiple comparisons test.
Figure S7

(a) Host survival

- ID8-Defb29/Vegfa
- Cisplatin
- Deferiprone

- αIFNAR1 or Isotype Ab
  every 3 days until end point

(b) FACS analysis

- ID8-Defb29/Vegfa
- Cisplatin
- Deferiprone

- αIFNAR1 or Isotype Ab
  at days 18, 24 and 27

(c) FACS analysis

- WT
- IL-15^{2A-eGFP}
- IL-15^{2A-eGFP} + IFN-β

(d) FACS analysis

- ID8-Defb29/Vegfa
- Cisplatin
- Deferiprone

- IL-15^{2A-eGFP}

- IL-15-GFP (gMFI)
**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-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.