

Figure S1. (a) Chromium release assay (18 hour) using MC38-Ova cells as targets and OT-I T-cells as effectors (0.1:1), in the presence of the indicated concentrations of birinapant (b) Chromium release assay (18 hour) using MC38-Ova cells as targets and and treated with TNF (2 ng/ml), in the presence of the indicated concentrations of birinapant. Data representative of 3 individual experiments. (c) Chromium release assay (18 hour) using MC38-Ova cells as targets and OT-I T-cells as effectors, in the presence or absence of BV6 (1  $\mu$ M). (d) Chromium release assay (18 hour) using HeLa cells as targets and KHYG1 NK cells as effectors, in the presence or absence of BV6 (1  $\mu$ M). Error bars represent the mean ± S.E.M. of triplicate determinations from a representative experiment (n=3), \*p< 0.05 by unpaired Student's t test.



Figure S2. MC38-Ova cells were exposed to OT-I T-cells at the indicated effector to target (E:T) ratio. After either 6 hours or 18 hours, cytokines in supernatants were measured by CBA. Data is representative of three individual experiments.

Figure S3

CD8<sup>+</sup> T-Cells

Pooled experimental data (n=3)



**NK Cells** 



CD8<sup>+</sup> T-Cells



## CD8⁺ T-Cells



## **NK Cells**

Pooled experimental data (n=3)







Figure S3. Pooled data (n=3) from key representative experiments shown in main figures, see main figures for experimental details. \*p< 0.05 by unpaired Student's t test.

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## **Supplementary Movie**

Supplementary movie. MC38-Ova cells were seeded in chamber slides, then overlaid with perforin deficient OT-I T-cells. T-cells were labeled with cell trace violet (CTV). A cleaved caspase3/7 reporter and birinapant (1  $\mu$ M) was added to the co-culture media. Cells were incubated for 8 hours at 37°C/10% CO2. Optical sections were acquired through sequential scans or Brightfield/DIC on a confocal microscope using a 40X (NA 0.85) air objective.