Engineering a dual small molecule gated ZAP70 switch in T cells

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SUPPLEMENTARY FIGURES

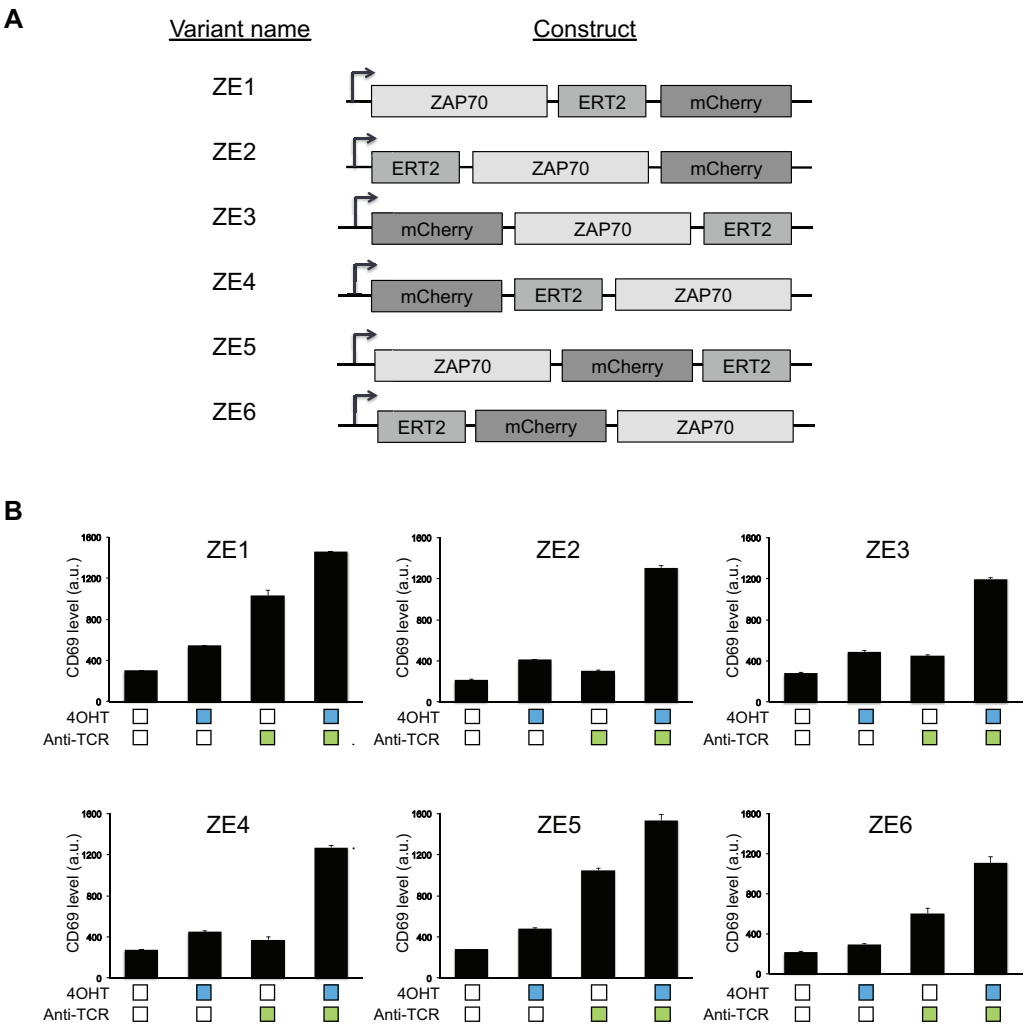

Figure S1. Various configurations of ZAP70 and ERT2 tested for 4OHT control. A) Diagram of constructs with different orderings of the ZAP70 gene, ERT2 domain, and mCherry fluorescent tag that were introduced into P116 cells. B) CD69 levels of cells expressing the constructs under various combinations of 4OHT and anti-TCR antibody.

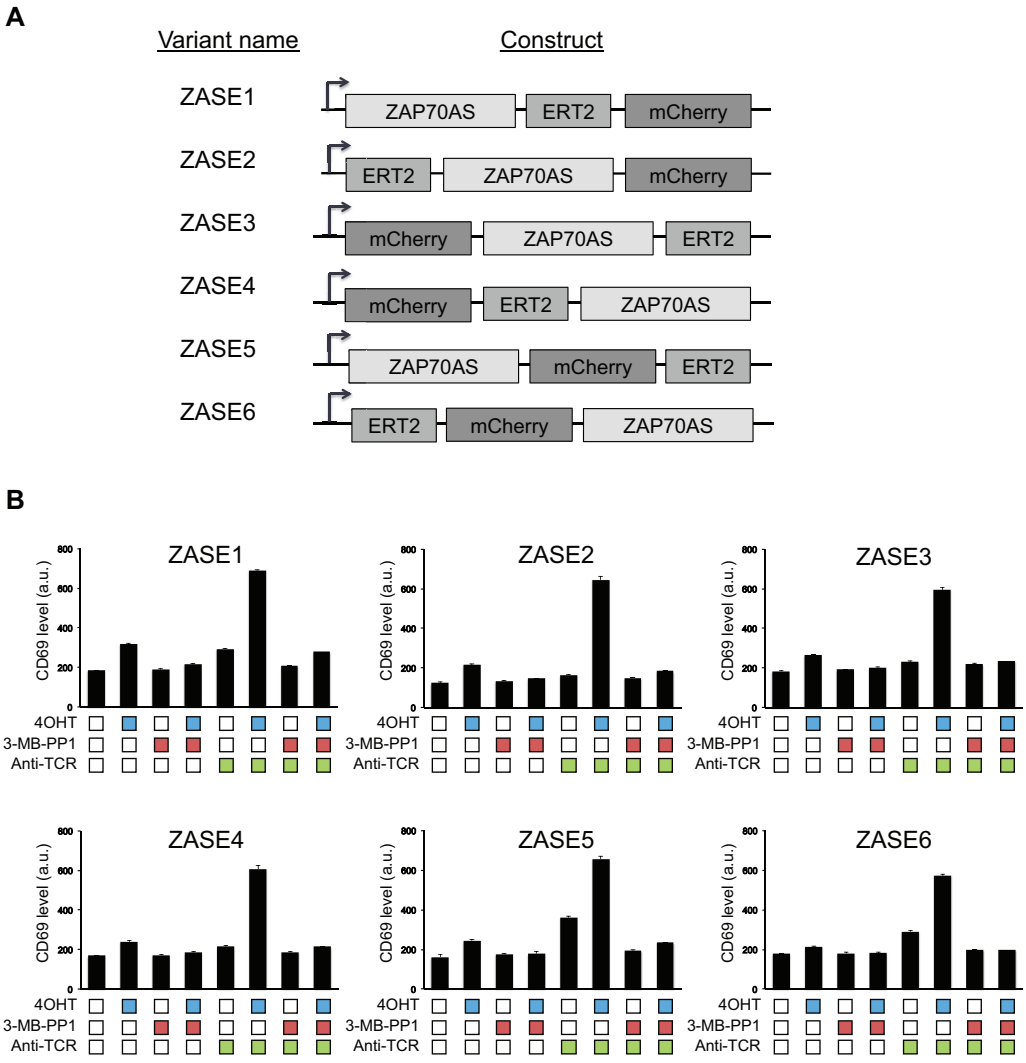

Figure S2. Different configurations of analog-sensitive ZAP70 and ERT2 tested for 4OHT and 3-MB-PP1 control. A) Constructs tested, with varying arrangements of the analog-sensitive ZAP70 gene, ERT2 domain, and mCherry fluorescent tag. B) CD69 levels of cells expressing the constructs under various combinations of 4OHT, 3-MB-PP1, and anti-TCR antibody.

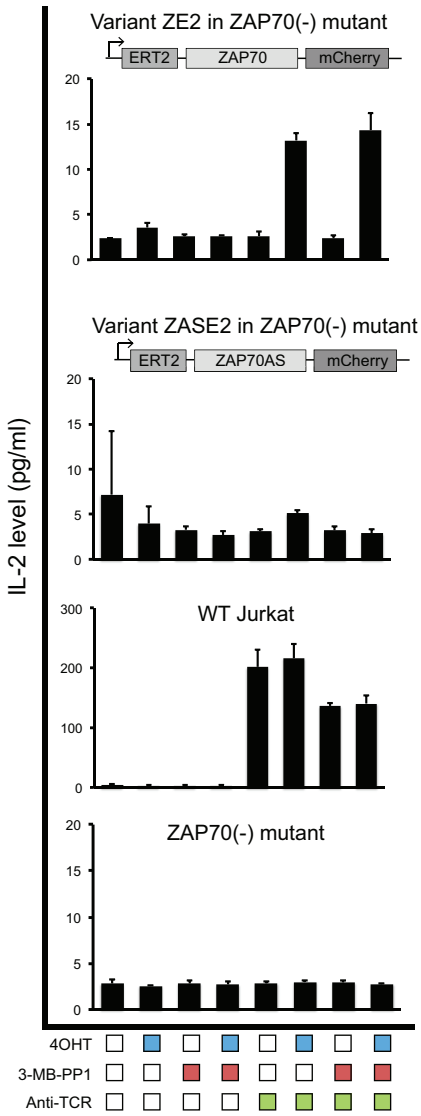

Figure S3. IL-2 release in cells treated with different combinations of small molecules.

ZE-expressing, ZASE-expressing, wild-type, and ZAP70-deficient Jurkat cells were treated with different combinations of 4OHT, 3-MB-PP1, and C305. The supernatant was tested in an ELISA to measure IL-2 levels (mean ± s.d., n = 3).

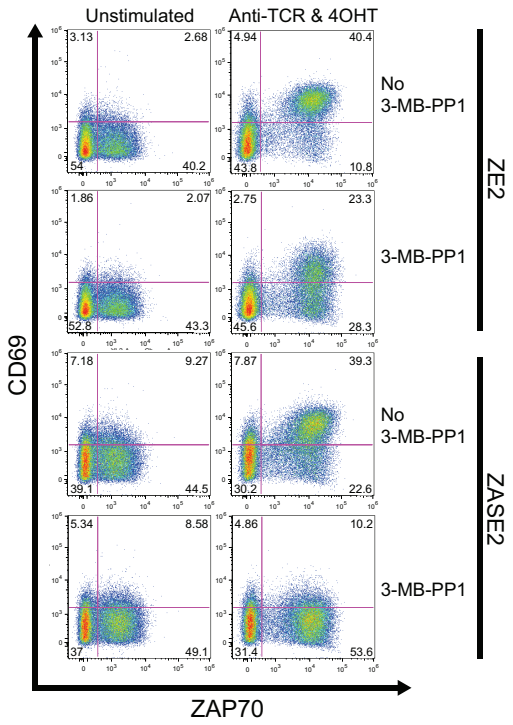

Figure S4. Nonspecific effects of 3-MB-PP1 on ERT2-ZAP70. P116 cells expressing either ERT2-ZAP70 or ZASE were stained for ZAP70 and CD69 and analyzed by flow cytometry. Conditions tested were T cell activation and inhibition by 3-MB-PP1.

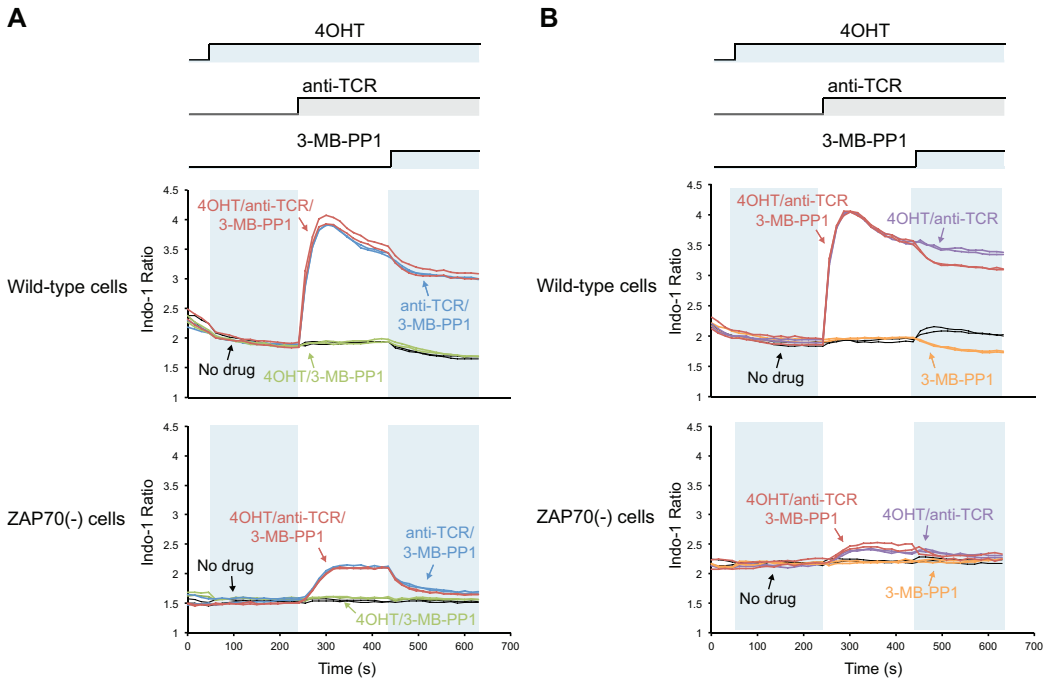

Figure S5. Wild-type Jurkat cells and ZAP70-deficient cells treated with various drug combinations. A) Cells were treated with various activation conditions (4OHT or anti-TCR antibody), then inhibited with 3-MB-PP1. B) Cells were treated with or without activation conditions (4OHT and anti-TCR antibody) and with or without inhibition using 3-MB-PP1.

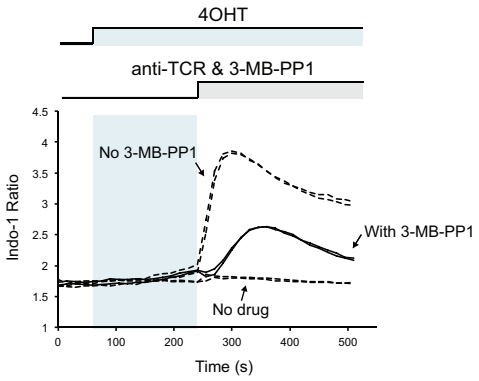

Figure S6. Intracellular calcium levels of ZASE-expressing cells upon simultaneous addition of anti-TCR antibody and inhibitor. Cells were treated with 4OHT, followed by the concurrent addition of anti-TCR antibody and 3-MB-PP1, and calcium levels were monitored over time.

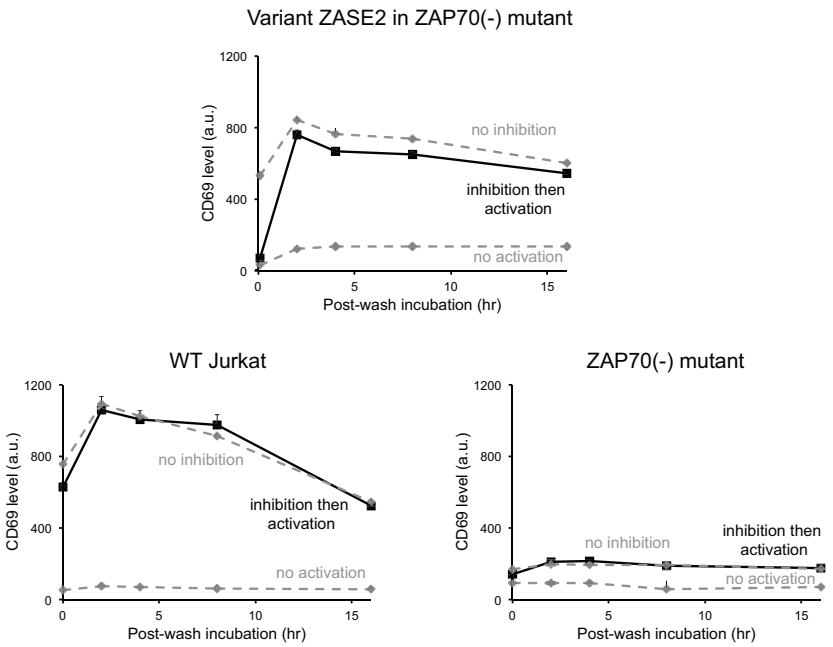

Figure S7. OFF-ON activity of ZAP70 switch. Cells were incubated with 4OHT and 3-MB-PP1, followed by a washout of 3-MB-PP1. They were then left for various time points (0hr, 2hr, 4hr, 8hr, 16hr) before activation. CD69 levels were measured by flow cytometry (mean ± s.d., n = 3). Control samples include cells that were not incubated with 3-MB-PP1 but were activated (no inhibition, positive control), and cells that were inhibited with 3-MB-PP1 but were not activated (no activation, negative control).

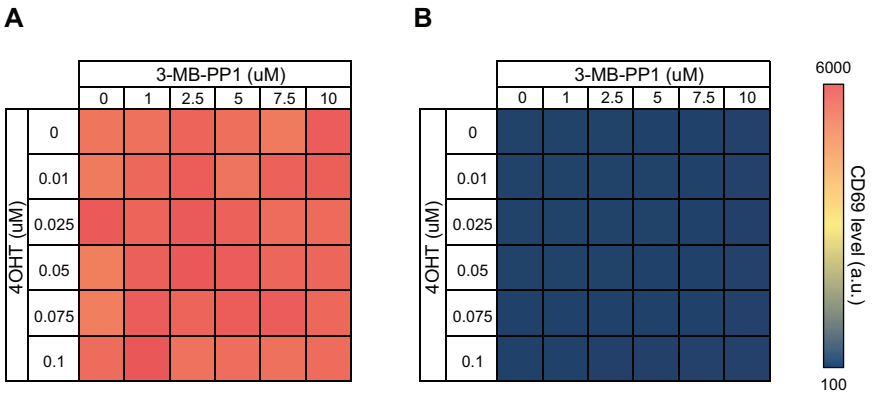

Figure S8. 2-D dose response under varying levels of activator and inhibitor. CD69 levels for A) wild-type Jurkat cells and B) P116 cells were quantified using flow cytometry after treatment with different concentrations of 4OHT and 3-MB-PP1.

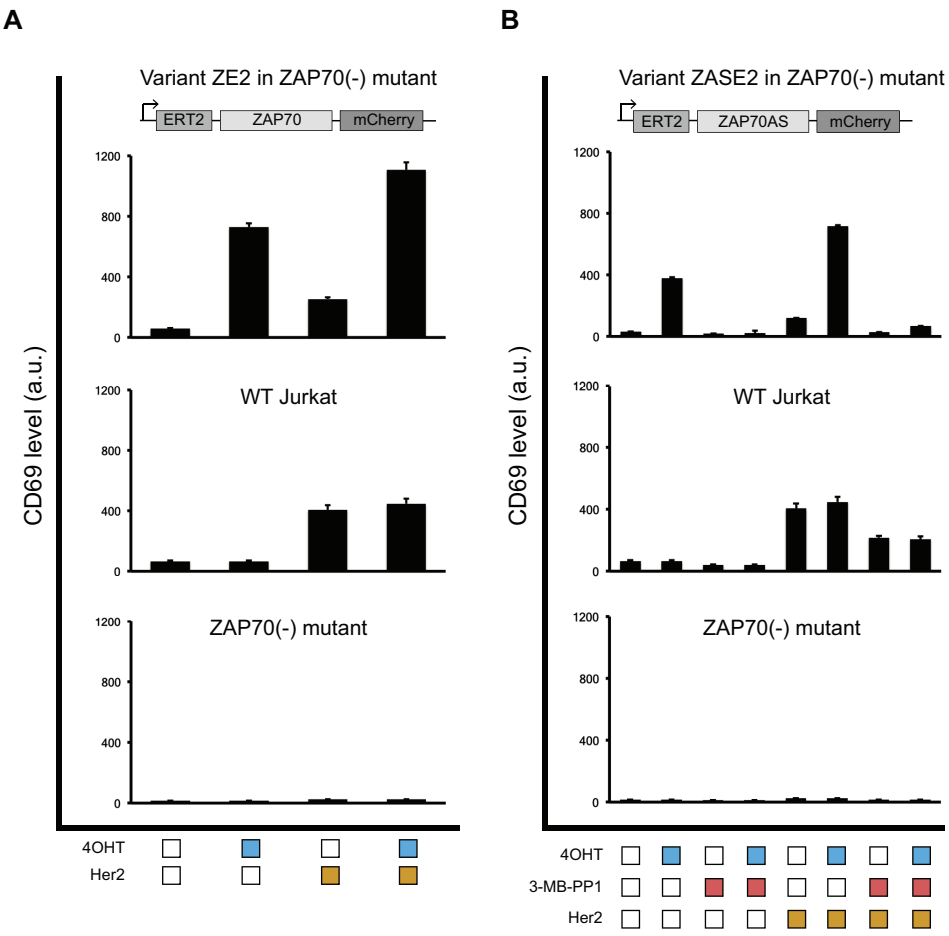

Figure S9. ZAP70 switch activity in Jurkat cells expressing a Her2 CAR. A) P116 cells expressing the ERT2-ZAP70 fusion protein and a CAR were stimulated with 4OHT and Her2 antigen, and CD69 levels measured. B) ZASE and CAR-expressing P116 cells were treated with combinations of 4OHT, Her2, and 3-MB-PP1 to observe whether ZASE is compatible with CARs. CD69 levels were measured as an indicator of T cell activity.