Supporting Information

Modulating and measuring intracellular H_2O_2 using genetically-encoded tools to study its toxicity to human cells

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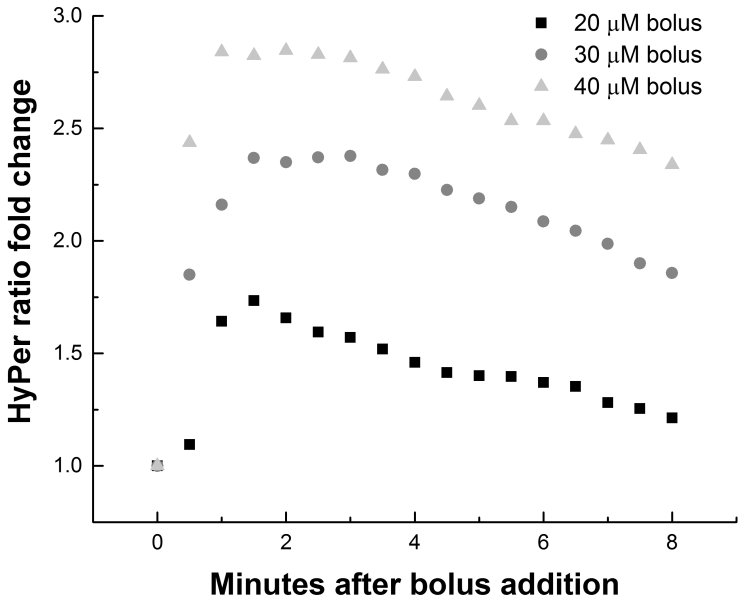
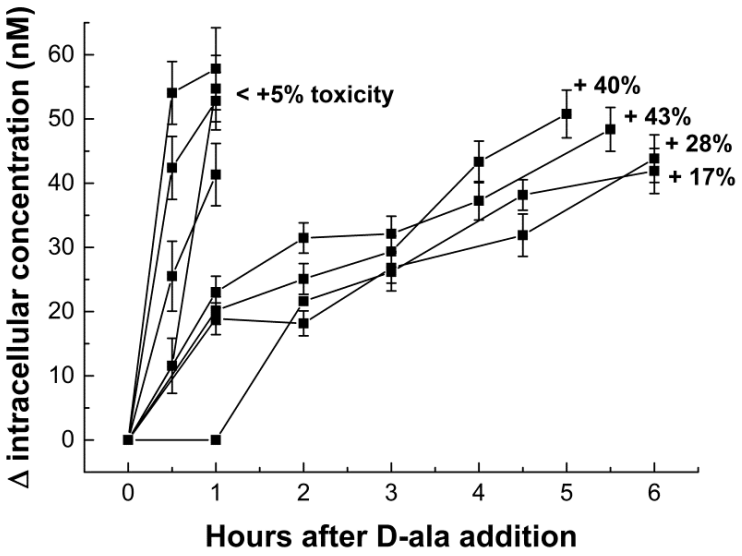
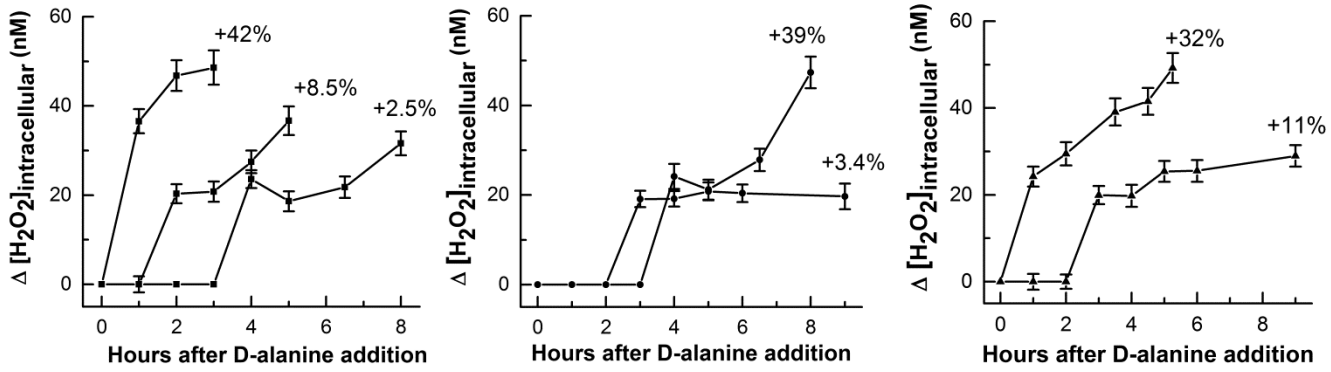


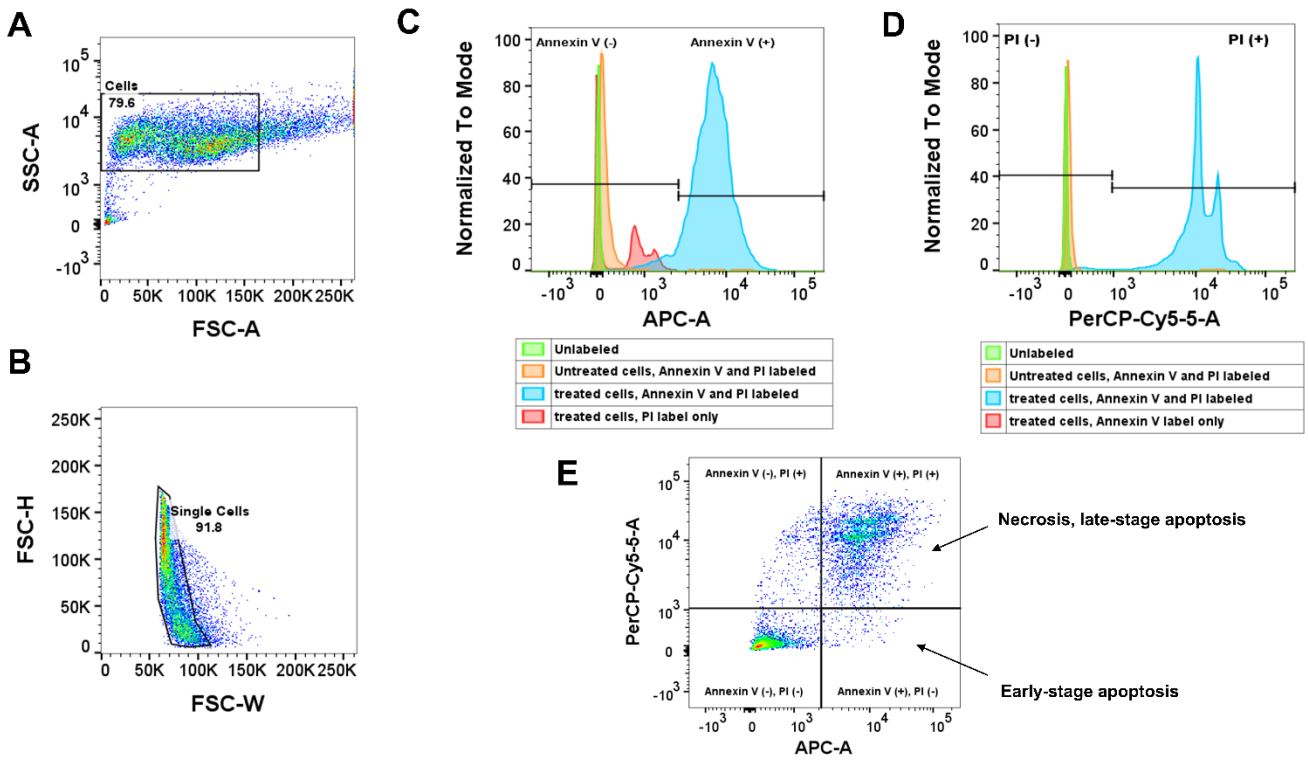
Supplementary Figure 1: Intracellular HyPer fluorescence fold change in response to H_2O_2 bolus added externally. The HyPer signal reaches its peak signal within 2 minutes of the bolus addition, but it takes a much longer time for HyPer to be reduced to its original form. The kinetics of the oxidation and reduction of HyPer are driven by the slow conformational change of oxidized HyPer and the limited reduction of oxidized HyPer by glutaredoxin.



Supplementary Figure 2: Example kinetic curves that had similar end-point intracellular H_2O_2 concentrations but very different toxicity. The kinetic curves for short generation times (1 hour) reached high end-point concentrations but exhibited low toxicity in comparison with longer generation times with similarly high end-point concentrations.



Supplementary Figure 3: Example kinetic curves that had similar cumulative area (nM x hr) but very different toxicity. Kinetic curves that had low end-point concentration exhibited low toxicity, suggesting the existence of a threshold intracellular concentration that has to be reached before H_2O_2-mediated cell death can occur.



Supplementary Figure 4: Flow cytometry gating design for measuring cell toxicity via Annexin V and PI. A) Separating cells from debris. B) Finding singlet cells. C) Fluorescence gating for Annexin V-APC. Both unlabeled cells and cells that are untreated with H_2O_2 but labeled with Annexin V and PI showed two orders of magnitude lower signal than the cells that are undergoing H_2O_2 mediated cell death. We also controlled for PerCP-Cy5-5 bleed over to APC channel with dead cells that are labeled with PI alone, there is a small fraction of bleed-over from the PI signal, and we set our gate to the right of the bleed-over. D) Fluorescence gating for PerCP-Cy5-5. Signal for the unlabeled cells, untreated but labeled cells and treated but labeled with Annexin V alone are more than two orders of magnitude lower than treated cells. There is minimal bleed over from the APC channel. E) Example data of cells with H_2O_2 generator undergoing cell death due to peroxide production. Cells that are double stained with Annexin V and PI are in their late-stage apoptosis/necrosis; cells that are only stained with Annexin V are in early stage apoptosis.