

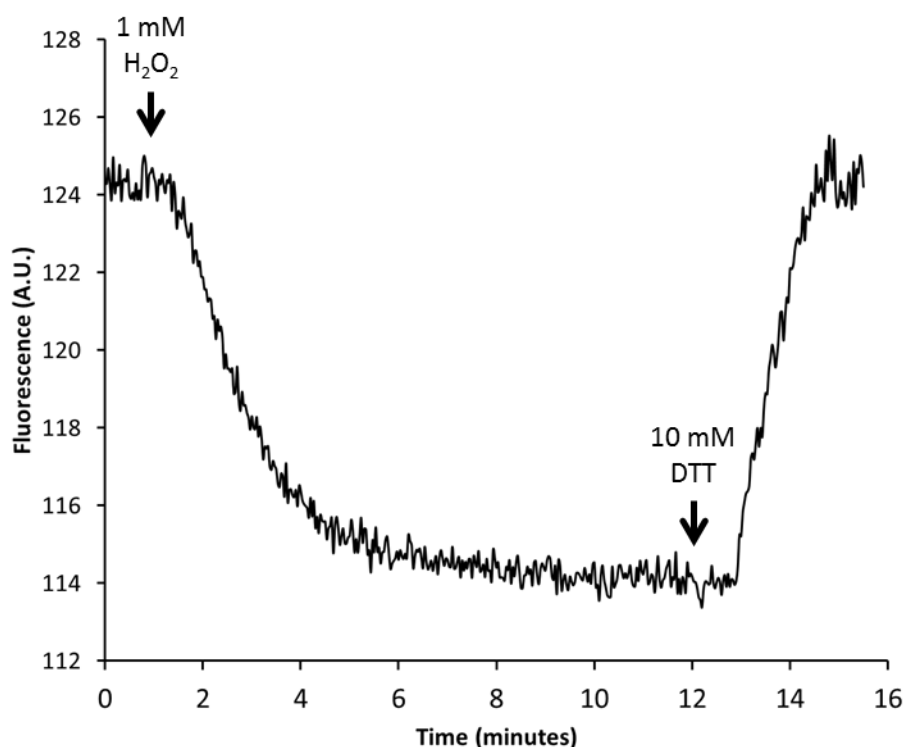
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**Supplemental Information**

**Oxidation of RyR2 Has a Biphasic Effect on the Threshold for Store  
Overload-Induced Calcium Release**

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## Supplementary Material



**Supplementary Figure 1. Effect of H<sub>2</sub>O<sub>2</sub> and DTT on protein oxidation in HEK293 cells**  
HEK293 cells stably expressing RyR2 were transfected with the H<sub>2</sub>O<sub>2</sub> sensitive fluorescent protein roGFP2-Orp1. The cells were then transiently superfused with KRH containing 1 mM Ca<sup>2+</sup> followed by KRH containing 1 mM H<sub>2</sub>O<sub>2</sub> or 10 mM DTT. Trace is representative of 69 cells.

### Supplementary Methods

Stable, inducible HEK293 cells expressing RyR2 were used with the addition of transfection with roGFP2-Orp1 cDNA (1). Transfection took place 24 h before imaging. The cells were perfused continuously at room temperature with KRH containing 1 mM Ca<sup>2+</sup>, 1 mM H<sub>2</sub>O<sub>2</sub> or 10 mM DTT. Fluorescence images of HEK293 cells were acquired every 2 s with an exposure time of 100 ms and excitation at 470 nm (40 nm bandwidth) using a CoolLED system (Coherent Scientific Pty. Ltd, Australia). Fluorescence was detected through a long pass dichroic mirror (495 nm) and a long pass emission filter (>515 nm) by a CoolSNAP HQ2 CCD camera (Photometrics, AZ).

### Supporting Reference

1. Gutscher, M., M. C. Sobotta, G. H. Wabnitz, S. Ballikaya, A. J. Meyer, Y. Samstag and T. P. Dick. 2009. Proximity-based Protein Thiol Oxidation by H<sub>2</sub>O<sub>2</sub>-scavenging Peroxidases. *J. Biol. Chem.* 284:31532-31540.