

Supplementary Materials for
**The Nonphagocytic NADPH Oxidase Duox1 Mediates a Positive
Feedback Loop During T Cell Receptor Signaling**

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- Fig. S1. TCR-induced generation of ROS in Jurkat cells.
- Fig. S2. Expression and function of Duox1 in human T cells.

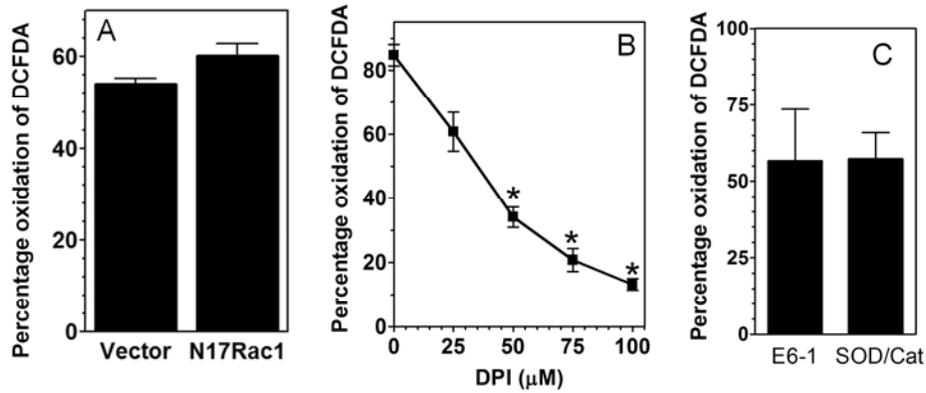


Fig. S1. TCR-induced generation of ROS in Jurkat cells. TCR-induced oxidation of DCFDA was measured after 10 min of stimulation in Jurkat cells. **(A)** Cells were transiently transfected with empty vector or with plasmid encoding N17Rac1 in the presence of a plasmid encoding RFP. The data represent the percentage of TCR-stimulated oxidation of DCFDA in RFP⁺ cells. **(B)** Effect of titrated concentrations of DPI on TCR-induced oxidation of DCFDA. **(C)** TCR-induced oxidation of DCFDA in Jurkat cells in the absence or presence of extracellular superoxide dismutase (SOD) and catalase (Cat). All graphs represent the mean \pm SEM of four separate experiments. *, $P < 0.05$.

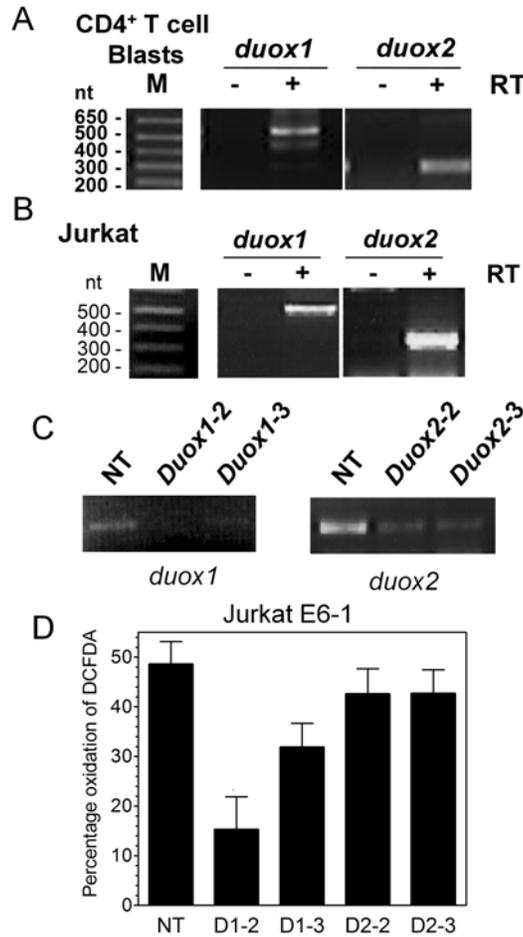


Fig. S2. Expression and function of Duox1 in human T cells. mRNA was isolated from (A) primary human CD4⁺ T cell blasts or (B) Jurkat cells, and the expression of the Ca²⁺-dependent NADPH oxidases Duox1 and Duox2 was determined by RT-PCR performed in the presence or absence of reverse transcriptase. PCR products were cloned and sequenced to verify their identities. (C) Knockdown of Duox1 (left) or Duox2 (right) in Jurkat cells by specific siRNAs. (D) TCR-induced oxidation of DCFDA in Jurkat cells transfected with the siRNAs shown in (C). The data are expressed as the percentage of TCR-stimulated DCFDA mean channel fluorescence \pm SEM relative to that of unstimulated control cells and represent the average of at least three separate experiments. *, $P < 0.05$.