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Supplementary Materials for

The Nonphagocytic NADPH Oxidase Duox1 Mediates a Positive Feedback Loop During T Cell Receptor Signaling

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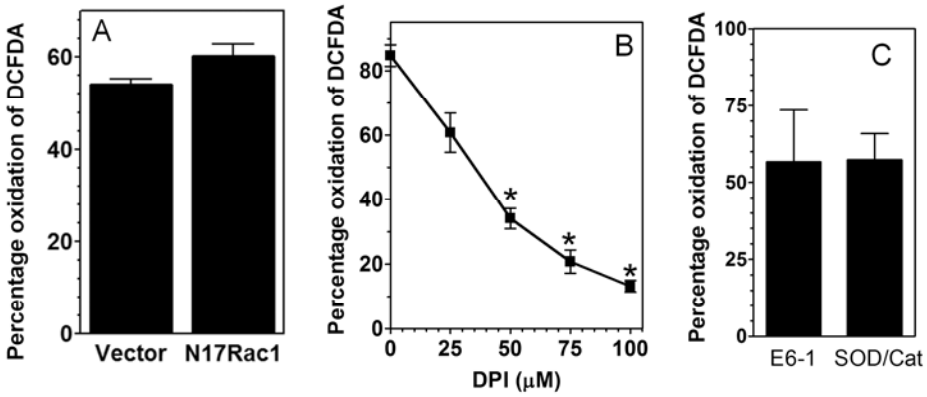

Fig. S1. TCR-induced generation of ROS in Jurkat cells. TCR-induced oxidation of DCFDA was measured after 10 min of stimulation in Jurkat cells. (**A**) Cells were transiently transfected with empty vector or with plasmid encoding N17Rac1 in the presence of a plasmid encoding RFP. The data represent 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 ± SEM of four separate experiments. *, $P < 0.05$.

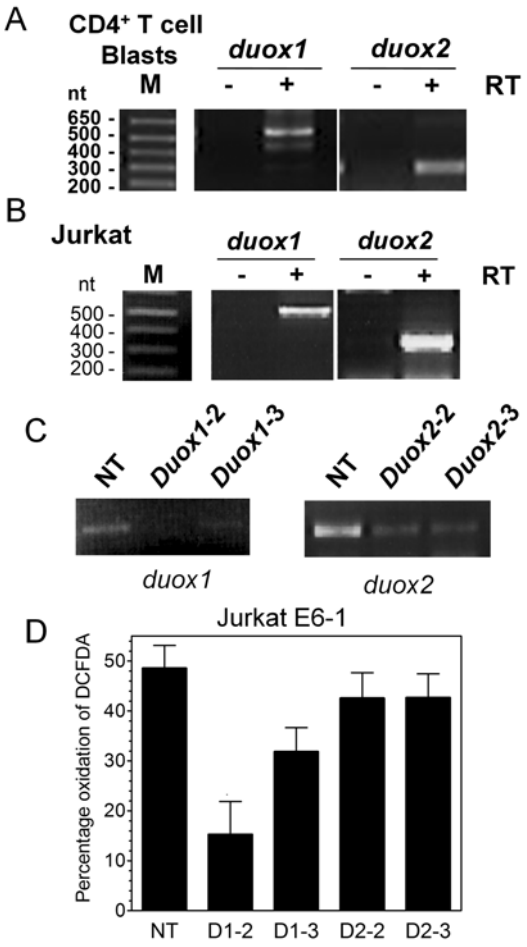

Fig. S2. Expression and function of Duox1 in human T cells. mRNA was isolated from (**A**) primary human $CD4^+$ T cell blasts or (**B**) Jurkat cells, and the expression of the Ca^{2+}-dependent NADPH oxidases Duox1 and Duox2 was determined by RT-PCR performed in the presence or absence of reverse transcriptase. 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 ± SEM relative to that of unstimulated control cells and represent the average of at least three separate experiments. *, $P < 0.05$.