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# Supplementary Materials for

## Radiation inhibits salivary gland function by promoting STIM1 cleavage by caspase-3 and loss of SOCE through a TRPM2-dependent pathway

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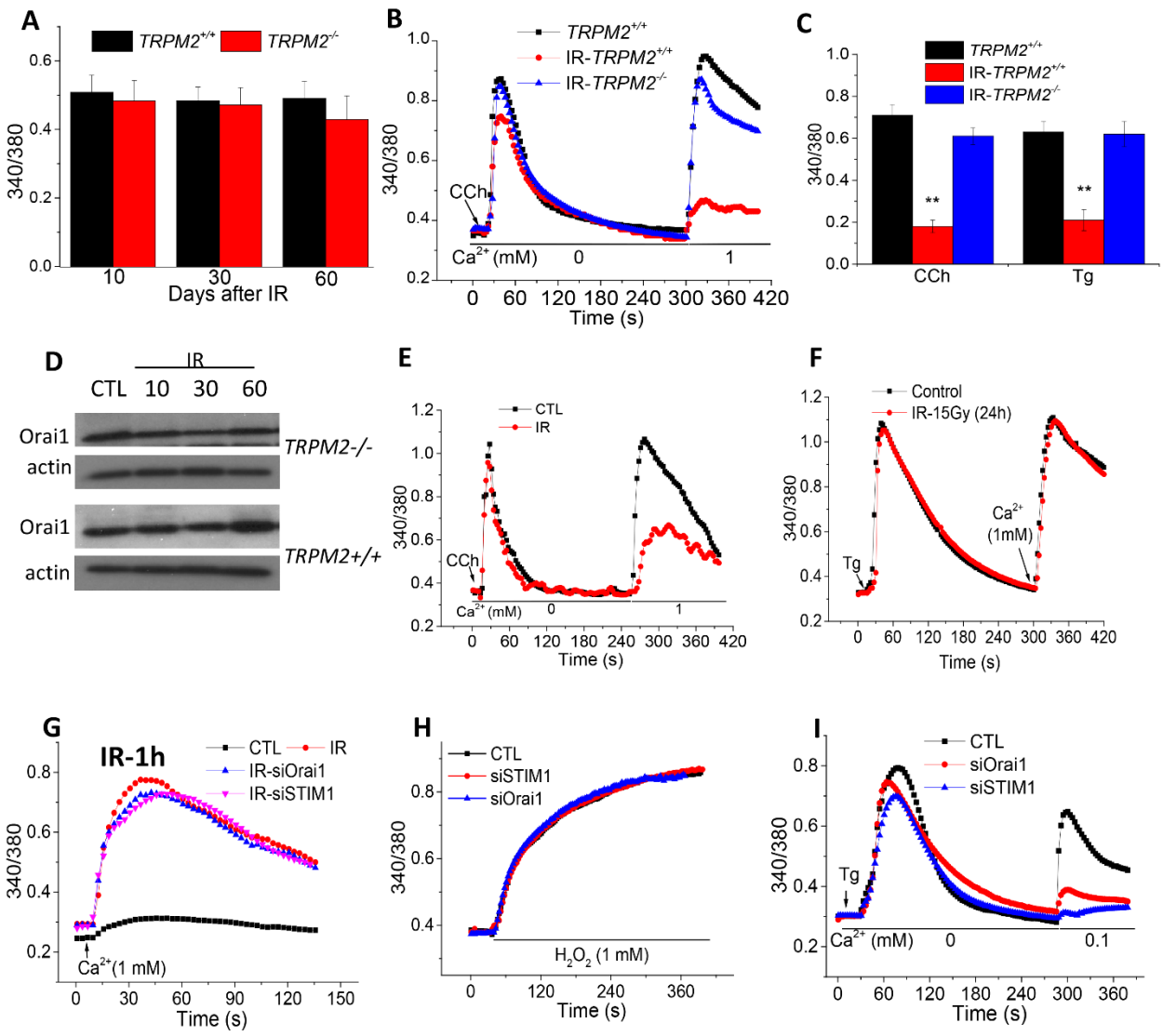


**Fig. S1. Effect of irradiation on SOCE in dispersed salivary gland acinar cells from *TRPM2*$^{+/+}$ and *TRPM2*$^{-/-}$ mice and HSG cells.** A. Tg-induced $Ca^{2+}$ release was measured in non-irradiated CTL mice (226 acini from 3 *TRPM2*$^{+/+}$ mice, 206 acini from 3 *TRPM*$^{-/-}$ mice) and after irradiation; 10 days (216 acini from 4 *TRPM2*$^{+/+}$ mice; 210 acini from 4 *TRPM2*$^{-/-}$ mice), 30 days (206 acini from 4 *TRPM2*$^{+/+}$ mice; 202 acini from 4 *TRPM2*$^{-/-}$ mice), and 60 days (186 acini from 4 *TRPM2*$^{+/+}$ mice; 192 acini from 4 *TRPM2*$^{-/-}$ mice). B. CCh-stimulated $[Ca^{2+}]_i$ changes (monitored by Fura 2 fluorescence measurements) in *TRPM2*$^{+/+}$ (180 acini from 3

mice), IR-*TRPM2*$^{+/+}$ (186 acini from 3 mice) and *TRPM2*$^{-/-}$ (182 acini from 3 mice) mice. C. Average data and statistical evaluation of B are shown. **P<0.01, unpaired *t* test comparing IR-*TRPM2*$^{+/+}$ mice to IR-*TRPM2*$^{-/-}$ mice. D. Western blots showing Orai1 in submandibular gland samples from non-irradiated and irradiated *TRPM2*$^{+/+}$ and *TRPM2*$^{-/-}$ mice. Results are representative of three independent experiments with 3 mice per time point. E. CCh-stimulated $[Ca^{2+}]_i$ changes in control (CTL) and IR-HSG cells (72 hours after IR) 180 or 188 cells from 3 independent experiments, respectively. F. Tg-stimulated $[Ca^{2+}]_i$ changes in control (CTL) and IR-HSG cells (24 hours after IR), 188 or 200 cells from 3 independent experiments, respectively. G. Effect of siOrai1 and siSTIM1 on IR-induced $Ca^{2+}$ entry in HSG cells measured 1h after IR. 160 to 220 cells from 3 independent experiments for each set. H**.** Effect of siSTIM1 and siOrai1 on $H_2O_2$-stimualated $Ca^{2+}$ influx in HSG cells. 170 to 210 cells from 3 independent experiments. I**.** Effect of siSTIM1 and siOrai1 on Tg-stimulated $[Ca^{2+}]_i$ changes in HSG cells. 120 to 160 cells from 3 independent experiments.

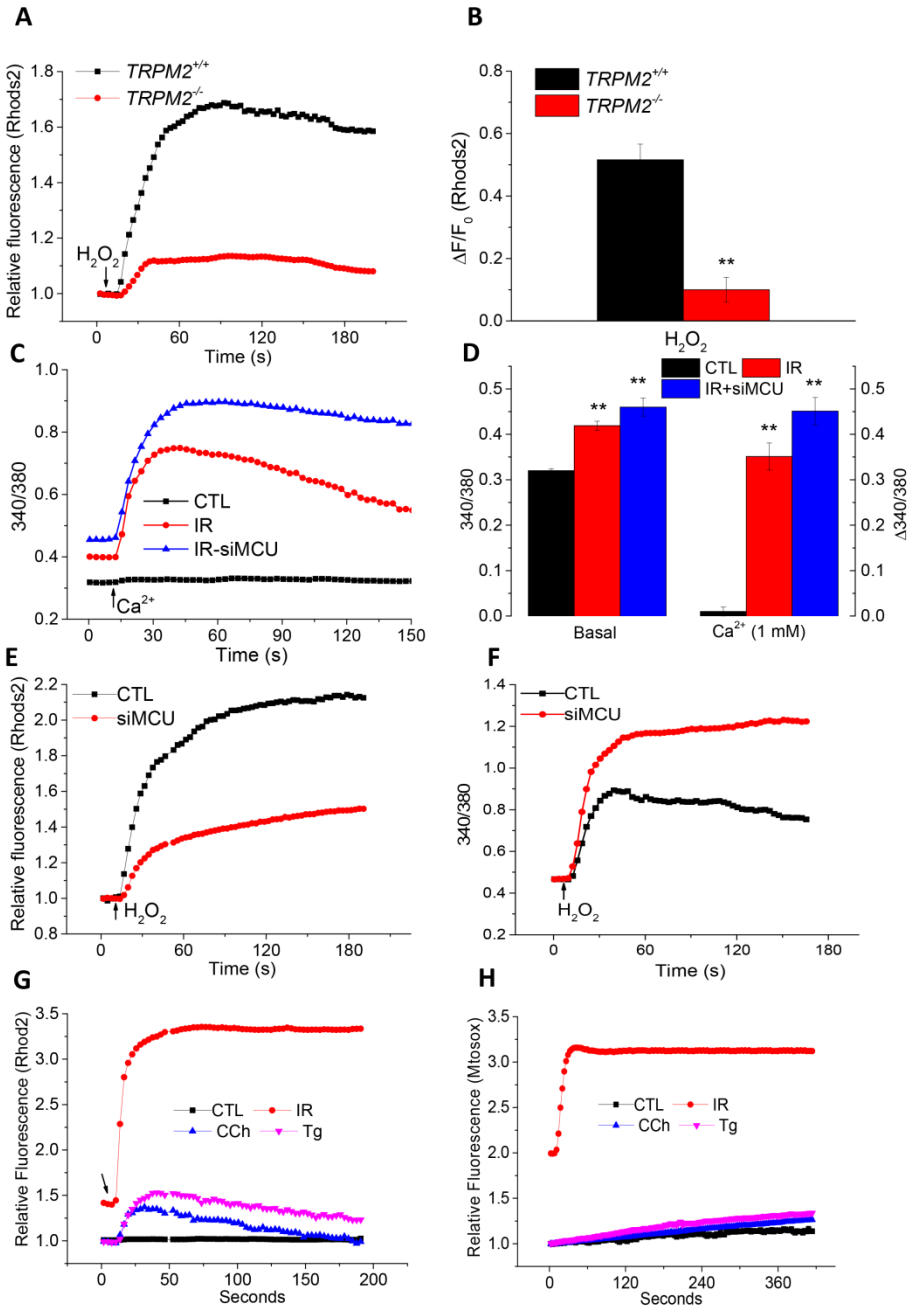


**Fig. S2. $H_2O_2$-induced changes in $[Ca^{2+}]_{mt}$ in dispersed salivary gland acinar cells from *TRPM2*$^{+/+}$ and *TRPM2*$^{-/-}$ mice and role of MCU in enhancement of $[Ca^{2+}]_{mt}$.** A, B. $H_2O_2$-induced $[Ca^{2+}]$ increase in mitochondria of acinar cells from *TRPM2*$^{+/+}$ (122 acini from 3 mice) and *TRPM2*$^{-/-}$ (132 acini from 3 mice) mice, **P<0.01, unpaired *t* test. C, D. Effect of siMCU on

IR-induced $[Ca^{2+}]_i$ increase in HSG cells. 120 to 180 cells from 3 independent experiments. **P<0.01, unpaired *t* test comparing IR or IR-siMCU to CTL, respectively. E, F. Effect of siMCU on $H_2O_2$-induced $[Ca^{2+}]_i$ increase in HSG cells. 100 to 120 cells from 3 independent experiments. G, H. Effect of $Ca^{2+}$ entry induced by IR or CCh- or Tg-stimulation on $[Ca^{2+}]_m$ and mitochondrial ROS. 110 to 150 cells from 3 independent experiments for each experimental condition.

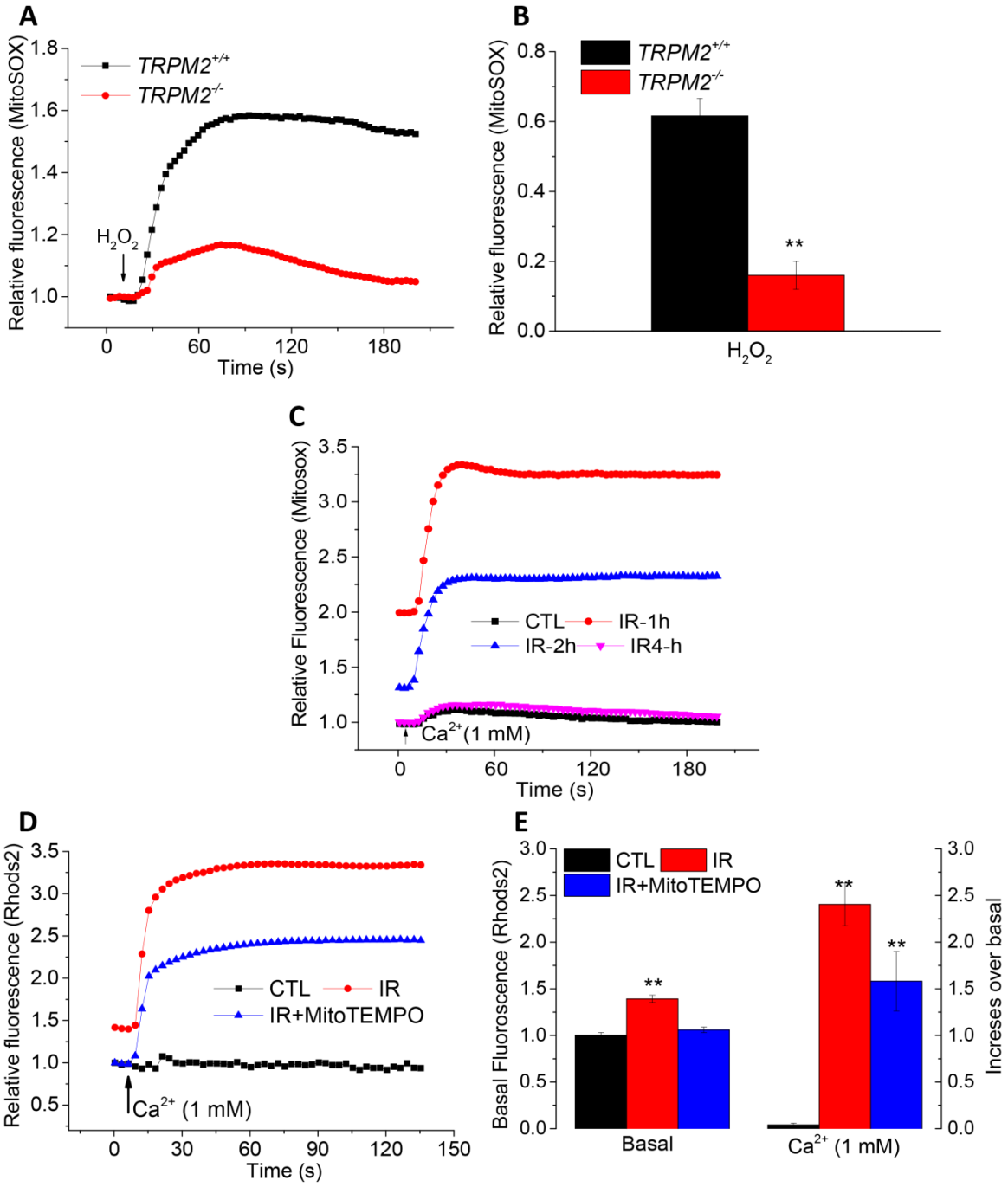


**Fig. S3. $H_2O_2$-induced changes in mitochondrial ROS in dispersed salivary gland acinar cells from *TRPM2*$^{+/+}$ and *TRPM2*$^{-/-}$ mice and effect of MitoTEMPO on $[Ca^{2+}]_{mt}$ and mitochondrial ROS.** A, B. Mitochondrial ROS measurements in $H_2O_2$-stimulated acinar cells from *TRPM2*$^{+/+}$ (112 acini from 3 mice) and *TRPM2*$^{-/-}$ (122 acini from 3 mice). **P<0.01,

unpaired *t* test. C. Time dependent changes in mitochondria ROS 1 to 4 hours after radiation treatment of HSG cells. 180-226 cells from 3 independent experiments. D, E. Mitochondrial ROS in CTL and irradiated HSG cells with or without treatment with mitoTEMPO. 130 to 150 cells from 3 independent experiments. Average data shown in E. **P<0.01, unpaired *t* test comparing CTL to IR or IR-mitoTEMPO group.

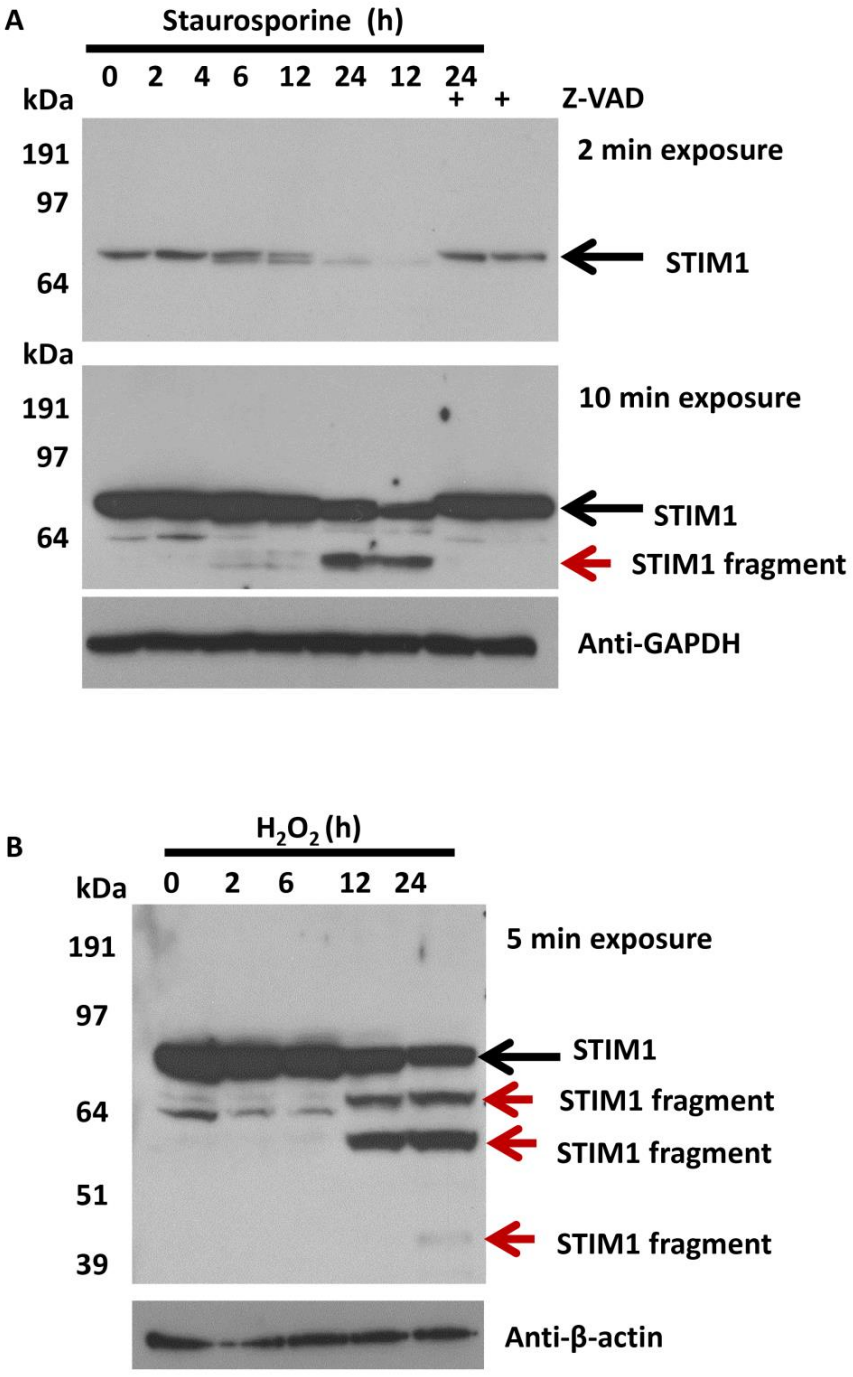


**Fig. S4. STIM1 cleavage in staurosporine- and $H_2O_2$-treated cells is mediated by caspase-3.** A. Western blot showing STIM1 in HSG cells treated with staurosporine for the indicated times and cells pre-treated with z-VAD before staurosporine. Lower molecular weight fragments of STIM1 were detected at longer time points after staurosporine but were not present in z-VAD treated cells. 3 independent experiments. B. STIM1 protein in HSG cells treated with $H_2O_2$, 3 independent experiments.

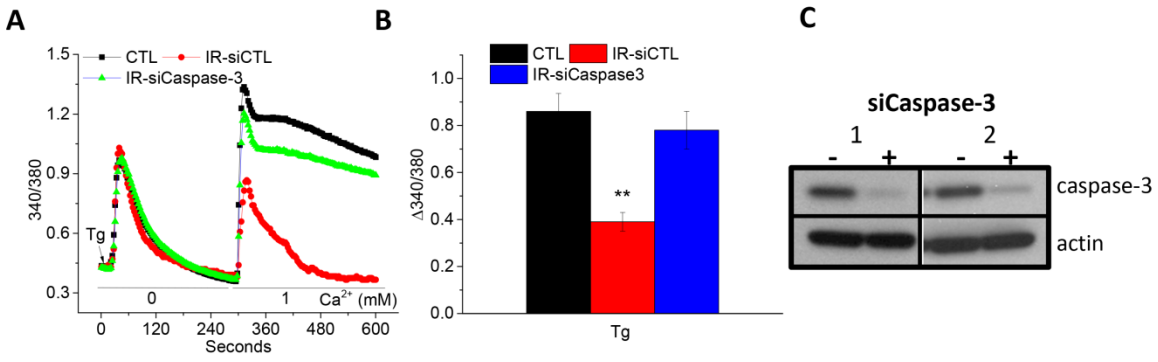


**Fig. S5. Knockdown of caspase-3 protects against radiation-induced loss of SOCE.** A. Tg-induced $Ca^{2+}$ influx measured in CTL, irradiated HSG cells pretreated with control siRNA, and irradiated HSG cells pre-treated with sicaspase-3 (source #2), 72 hours after irradiation. B. Quantitation of data in A. 230, 216 and 204 HSG cells for control, siCaspase3 and siControl, respectively, 3 independent experiments. **P<0.01, unpaired *t* test, compared to CTL. C. Western blot showing Caspase3 in cells treated with two different siCaspase-3 (siRNA-#1 from Dharmacon, Lafayette, CO, USA; siRNA-#2 from Invitrogen, Waltham, MA, USA, respectively). 3 independent experiments.