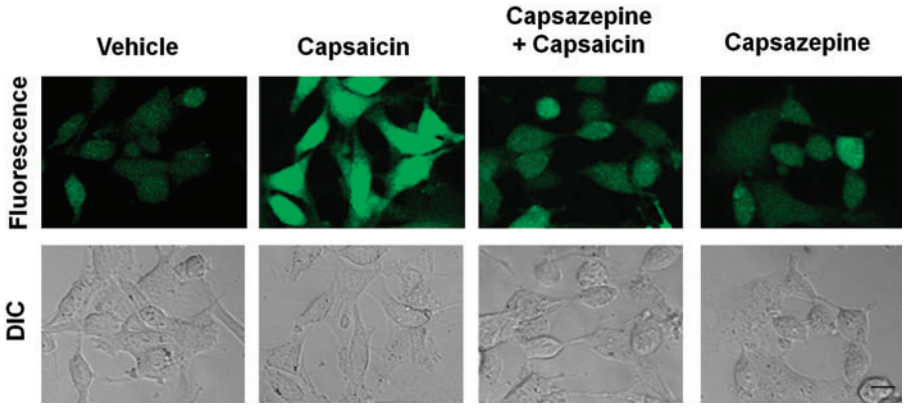
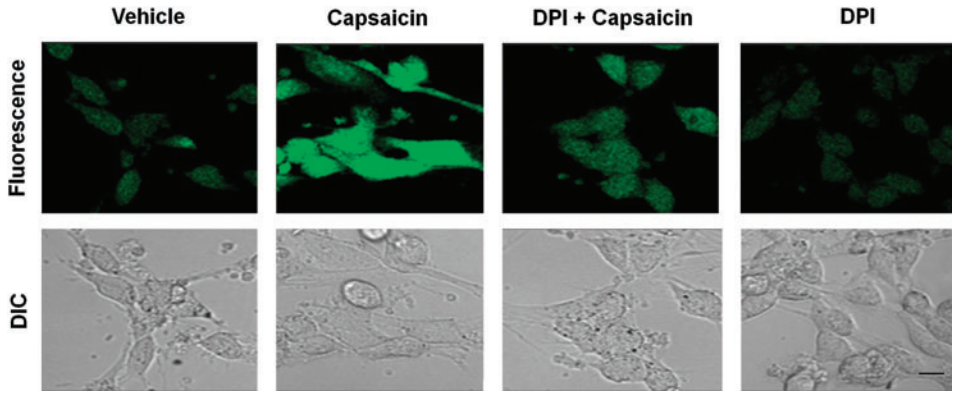
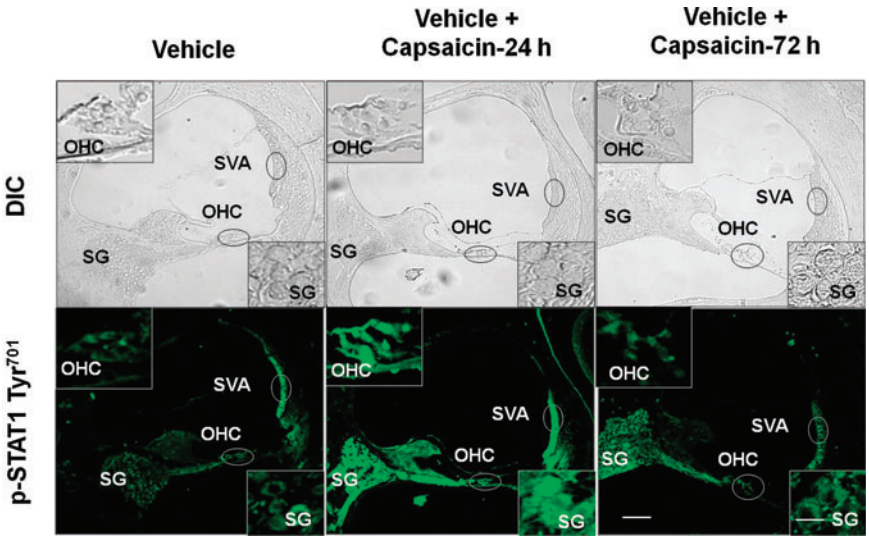
Supplementary Data



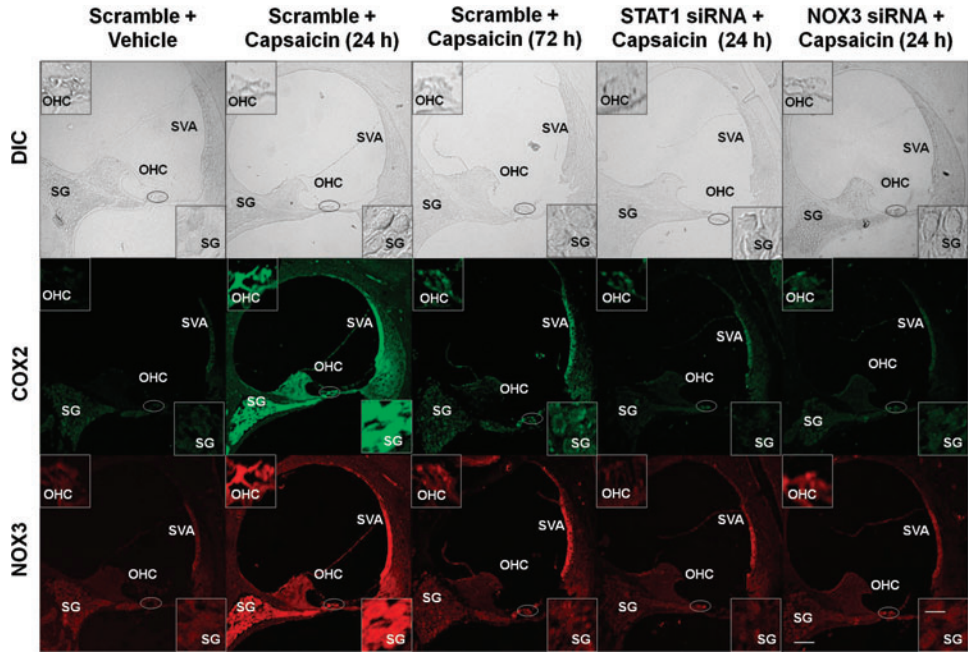
SUPPLEMENTARY FIG. S1. Transient receptor potential 1 antagonist inhibits capsaicin-induced ROS generation in UB/OC-1 cells. Cells were pretreated with 1 μM capsazepine for 30 min, followed by 15 min treatment with capsaicin (2.5 μM). ROS generation was determined by H_2DCFDA and fluorescence imaging was determined by confocal microscopy. The image shown is a representative of three independent experiments. Scale bar (*lower right*) represents 10 μm. DIC, differential interference contrast. H_2DCFDA, 2′,7′-dichlorodihydrofluorescein diacetate; ROS, reactive oxygen species.



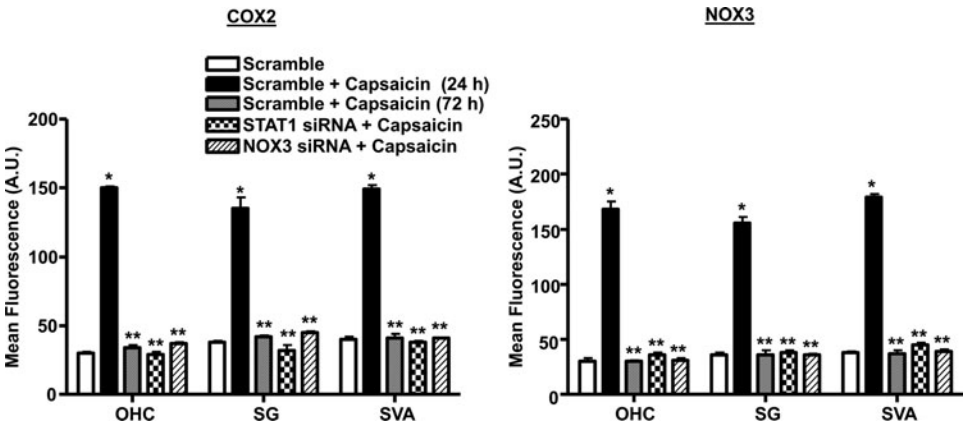
SUPPLEMENTARY FIG. S2. NADPH oxidase inhibitor suppresses capsaicin-induced ROS generation. UB/OC-1 cells were grown on glass coverslips and pretreated with DPI (5 μM) for 30 min. Cells were then exposed to capsaicin (2.5 μM) for 15 min and H_2DCFDA dye was used to determine the level of ROS generation. The image shown is a representative of three independent experiments. Scale bar (*lower right*) represents 10 μm. DPI, diphenyleneiodonium.



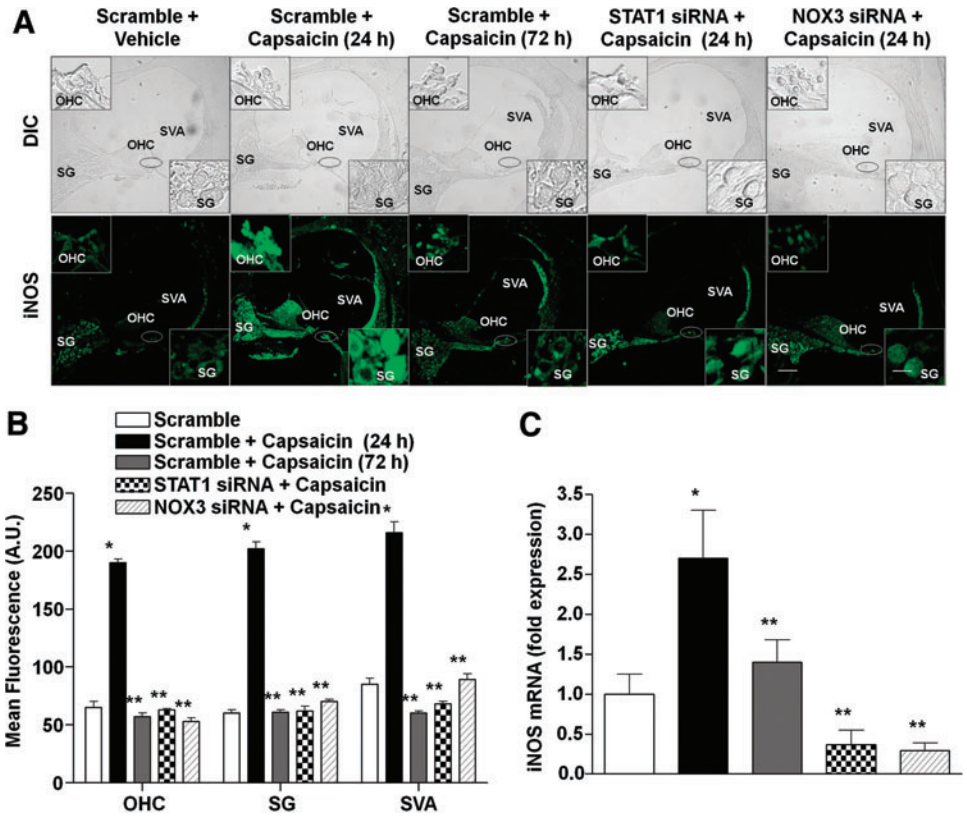
SUPPLEMENTARY FIG. S3. Capsaicin increases phosphorylation of Tyr^{701} residue of STAT1. Cochlear sections were prepared from the rats treated with capsaicin (trans-tympanically) for 24 or 72 h, and immunolabeled with p-STAT1 (Tyr^{701})-specific antibody. Activation of p-STAT1 Tyr^{701} is seen as bright green fluorescence at 24 h, which returned to almost basal levels after 72 h capsaicin treatment. Images were captured by confocal microscope. Insets are enlarged sections of OHCs and SGs. Scale bars (*lower right*) represent 50 and 10 μm (for *insets*). Data shown are a representative from four cochleae from four different rats. OHC, OHC, outer hair cell; SG, spiral ganglion; STAT1, signal transducer and activator of transcription 1; SVA, stria vascularis.



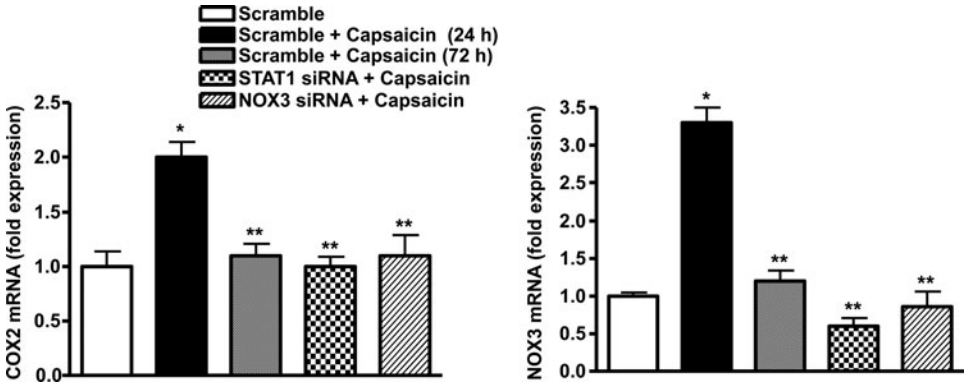
SUPPLEMENTARY FIG. S4. Capsaicin increases levels of COX-2 and NOX3 in rat cochlea *via* STAT1 activation. Rats were anesthetized and administered scrambled siRNA (scramble), STAT1 or NOX3 siRNAs by trans-tympanic injections. Two days later capsaicin was administered by trans-tympanic injections for 24 or 72 h. Cochlear sections were then used for COX-2 (green) and NOX3 (red) coimmunolabeling studies. Capsaicin increased expression of both these proteins at 24 h in SVA, OHC, and SG. However, these increases recovered to baseline levels by 72 h. Animals pretreated with STAT1 or NOX3 siRNA did not show any increase in COX-2 and NOX3 immunolabeling. Insets are enlarged sections of OHCs and SGs. Scale bars represent 50 and 10 μm (for *insets*). Data are a representative labeled section from one of four animals showing similar results. siRNA, short interfering RNA.



SUPPLEMENTARY FIG. S5. Quantitative analysis of COX-2 and NOX3 immunoreactivity shown in Supplementary Figure S4. *Statistically significant difference from the scramble group ($p < 0.05$, $n = 4$); **statistically significance difference from the scramble + capsaicin group (24 h) ($p < 0.05$, $n = 4$).



SUPPLEMENTARY FIG. S6. Capsaicin increases inducible nitric oxide synthase (iNOS) expression in a STAT1 and NOX3-dependent manner. (A) Immunohistochemistry for iNOS was performed on the cochlear sections isolated from the rats that were pretreated with scramble siRNA (scramble), STAT1 or NOX3 siRNA before 24 or 72 h treatment with capsaicin by trans-tympanic injections. **(B)** Quantitative analysis of fluorescent intensity from **(A)** shows statistically significant increase in iNOS immunoreactivity after 24 h capsaicin administration. This increase was reduced to baseline levels in presence of STAT1 and NOX3 siRNA. **(C)** A similar profile was seen for *iNOS* mRNA levels as determined by real-time RT-polymerase chain reaction studies on rat cochleae. Insets represent enlarged sections of OHCs and SGs. Scale bars shown on the *lower right panel* of **(A)** represent 50 and 10 μm (for *insets*). *Statistically significant difference from scramble siRNA-treated rats; **statistically significant difference from scramble + capsaicin-24 h treated rats ($p < 0.05$; $n = 4$).



SUPPLEMENTARY FIG. S7. Capsaicin increases COX-2 and NOX3 expression. Quantification of mRNA from rat cochleae by real-time polymerase chain reaction indicated significant increases in *COX-2* and *NOX3* expression at 24 h, which recovered to essentially baseline levels at 72 h. Knockdown of STAT1 or NOX3 by siRNAs abolished the increases in these transcripts. *Statistically significant difference from scramble siRNA-treated rats; **statistically significant difference from scramble + capsaicin-24 h treated rats ($p < 0.05$; $n = 4$).