Supplemental Figure Legends

Supplemental Figure 1. Microvascular TNFR1 shedding. Abbreviations are as for Figure 1. (A) Concentration dependence of sTNFα-induced TNFR1 shedding in single lung microvessels determined 10 min after sTNFα (1 ng/mL) infusion. (B) Images show time-course of sTNFα-488 fluorescence in a microvessel following 10 min infusion of sTNFα-488 infusion. (C) Group data were determined by line-scan analyses of endothelial fluorescence. $^*p<0.05$ compared with zero time point, mean±SEM, n=3 for each point.

Supplemental Figure 2. Expression of roGFP in lung microvessels. *GFP*, green fluorescence protein; *DTT*, dithiothreitol (1 mM) and *DCF*, dichlorofluorescein (2.5 µM). (A) Immunoblot is representative for three lungs transfected with the roGFP plasmid, or vector alone. (B,C) Confocal images and plot shows microvascular roGFP fluorescence from a single experiment. H_2O_2 was microinfused by micropuncture. DTT was added to the perfusate. Bar=25 µm. (D) Data from a single lung, shows responses in microvessels co-expressing fluorescence of roGFP and DCF. Each point is from a different microvessel. Lines were drawn by linear regression analysis.

Supplemental Figure 3. Endothelial Ca^{2+} responses in lung microvessels following antioxidant treatment. *Veh,* vehicle; *mCAT-OX,* mitochondrial catalase overexpression; *BL,* baseline data prior to infusion of agents. All other abbreviations are as for Figure 1. Responses are shown for treatments given as for Figure 5. *$p<0.05$ compared with baseline Data are mean±SEM. *n*=3 each bar.

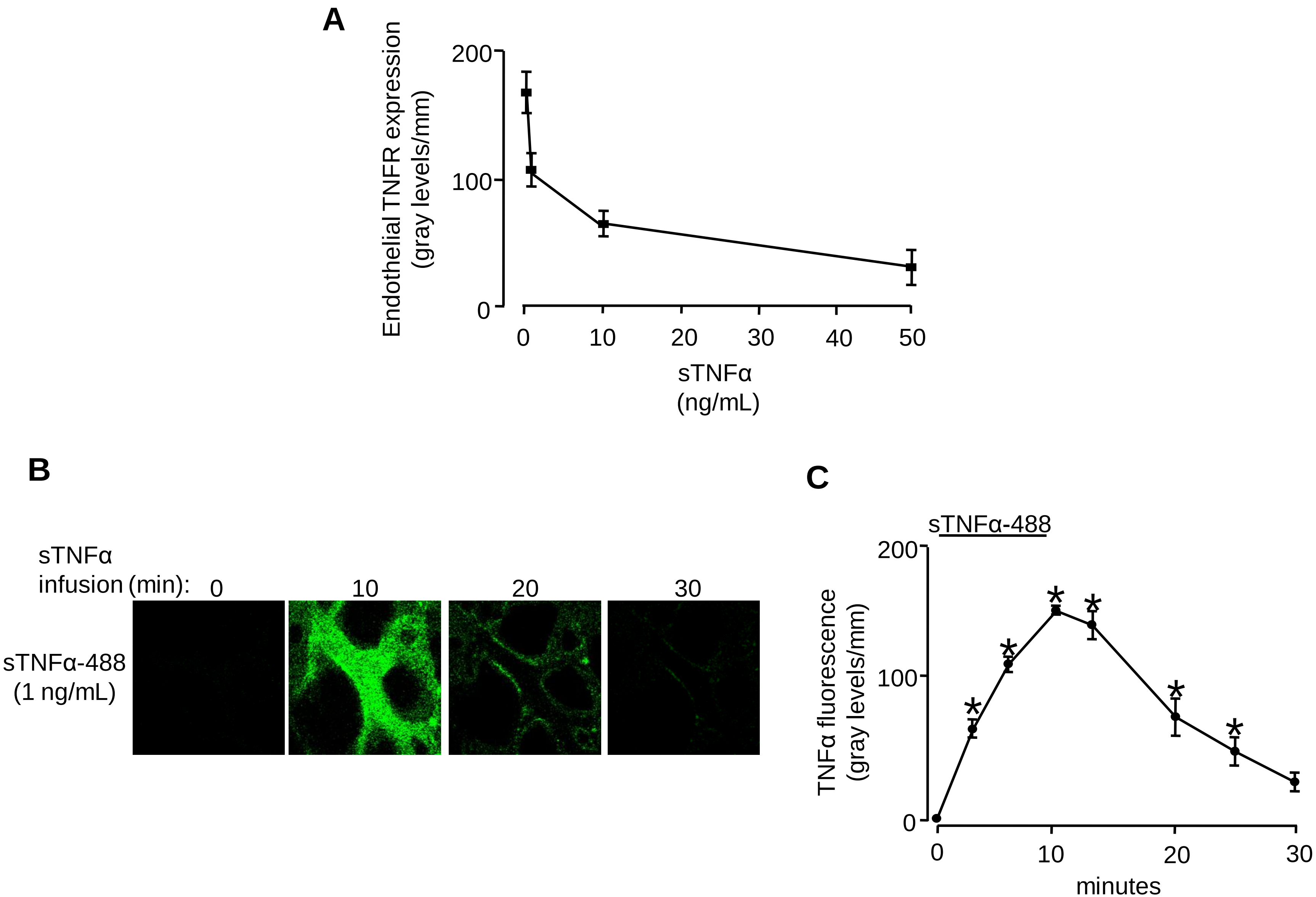
Supplemental Figure 4. sTNFα-induced endothelial ROS generation detected by DCF. *DCF,* dichlorofluorescein (2.5 µM); *DPI*, diphenylene iodonium (10 µM); *LN*, L-NAME (100 µM) and *ROT*, rotenone (1 µM). (A) Color-coded endothelial DCF fluorescence (arrows) in lung microvessels following sTNFα (1 ng/mL) infusion in the presence or absence of ROT. (B) Time-course of DCF response to sTNFα in the presence and absence of ROT. H_2O_2 (10 µM) was infused at end of experiment for positive control. (C) Group data of DCF response to sTNFα in the presence and absence of ROT, DPI, LN or in lungs from mice carrying a *Nox2* deletion ($Nox2^{-/-}$). *$p<0.05$ compared with BL, #$p<0.05$ compared with sTNFα, mean±SEM.

Supplemental Figure 5. Expression of cCAT and mCAT in lung endothelium. (A) Images show dsRed fluorescence in lung microvessels from mice transfected with cCAT or mCAT constructs that encode dsRed, bar=25 µm. (B-D) Immunoblots for catalase in cytosolic (B) and mitochondrial fractions (C) of vascular cells isolated from lungs transfected with empty vector, cCAT or mCAT constructs. VDAC and α-actin serve as mitochondrial- and cytosol-specific loading controls (D).

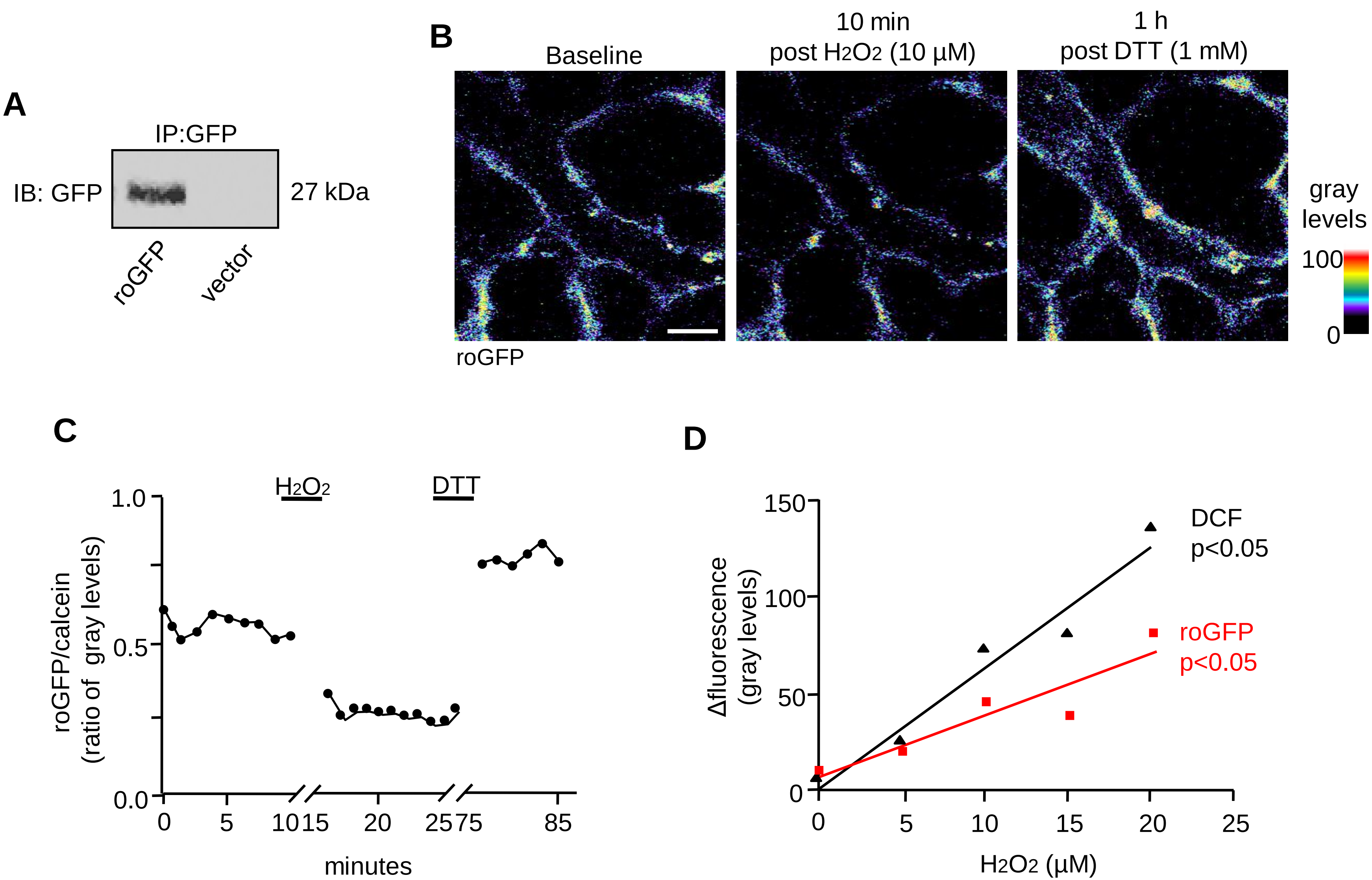
Supplemental Figure 6. Effects of siRNA. *BAL*, broncho-alveolar lavage; *Mito*, respiration buffer containing isolated lung mitochondria; *ADP*, adenosine diphosphate and *DNP*, 2'3'-dinotrophenol. Other abbreviations are as in Figure 6. All data mean±SEM; *n*=4 each bar. (A) Densitometry for blots in Figure 6. *EC*, primary endothelial isolates from lung; *lung*, lung lysate. *p<0.05 compared with untreated. (B,C) Data are for samples from mice obtained 48 h after scRNA or siRNA treatment. (D) Tracings form single experiments show oxygen determinations in mitochondrial buffer. (E) Bars show uptake of safranin O in lung mitochondria. The mitochondrial potential induces the uptake, which was determined as the decrease of buffer fluorescence after addition of isolated mitochondria. Similar uptakes for the two siRNAs indicate that RISP knockdown did not alter potential. FCCP, which abolishes the potential, blocked safranin uptake (positive control). *p<0.05 compared with scRNA.

Supplemental Figure 7. Mitochondrial RISP is required for TNFR1 shedding in lung microvessels. *RISP*, Rieske iron sulphur protein; *siRISP*, microvessels transfected with siRNA against RISP; *scRNA*, microvessels transfected with scrambled siRNA; other abbreviations are as for Figure 1. (A,B) Line-scan analysis of siRISP and TNFR1 fluorescence at baseline and 20 min post-sTNFα infusion and analyzed by linear regression, data from a single experiment shown.

Supplemental Figure 1



Supplemental Figure 2



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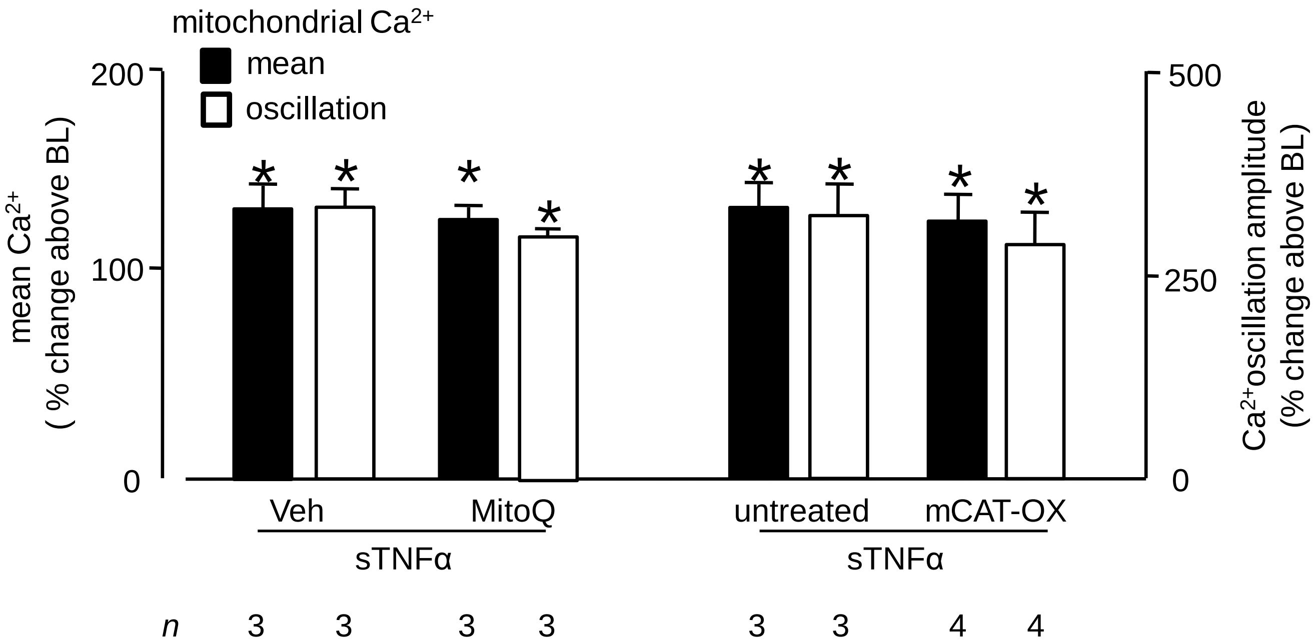

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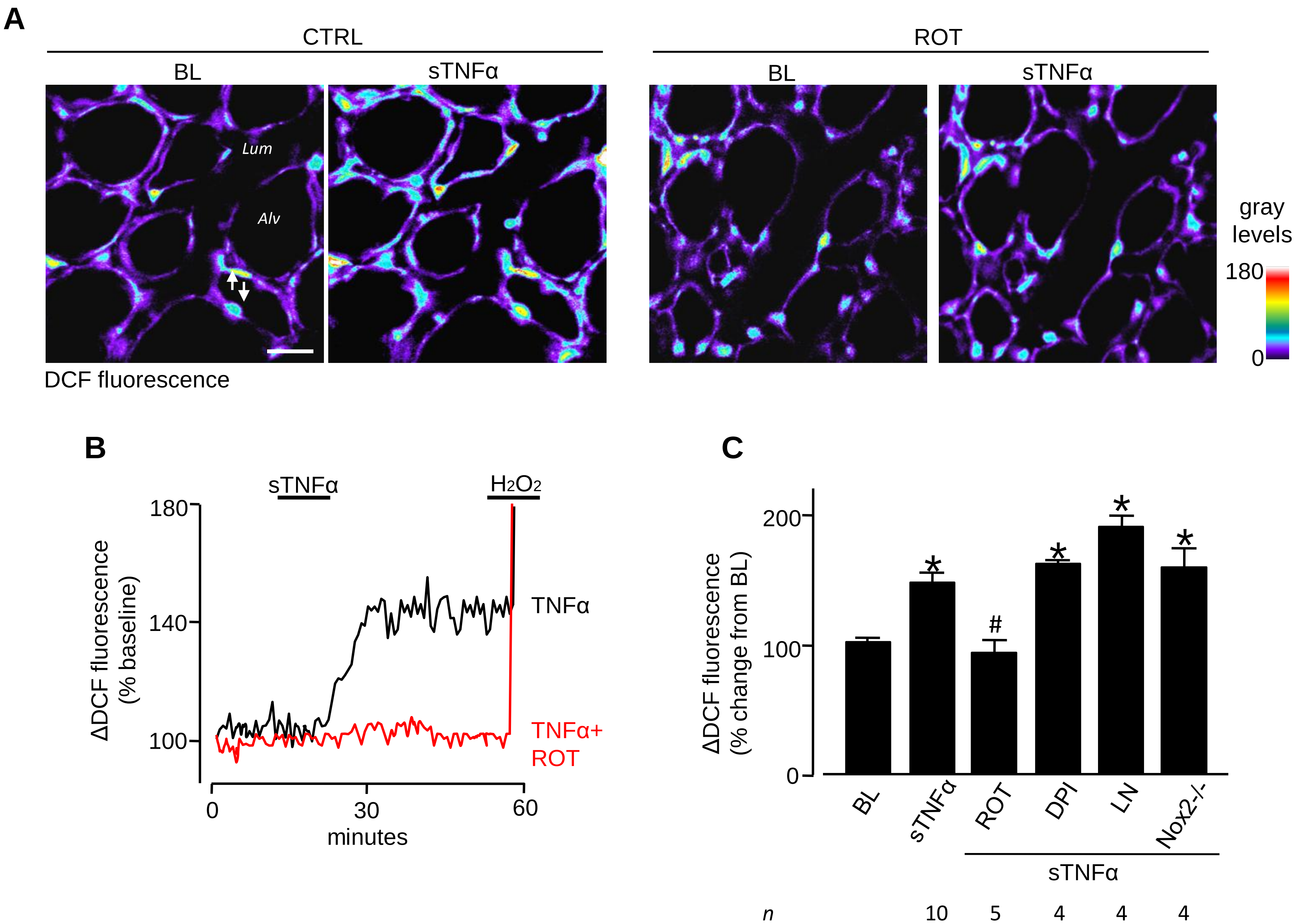
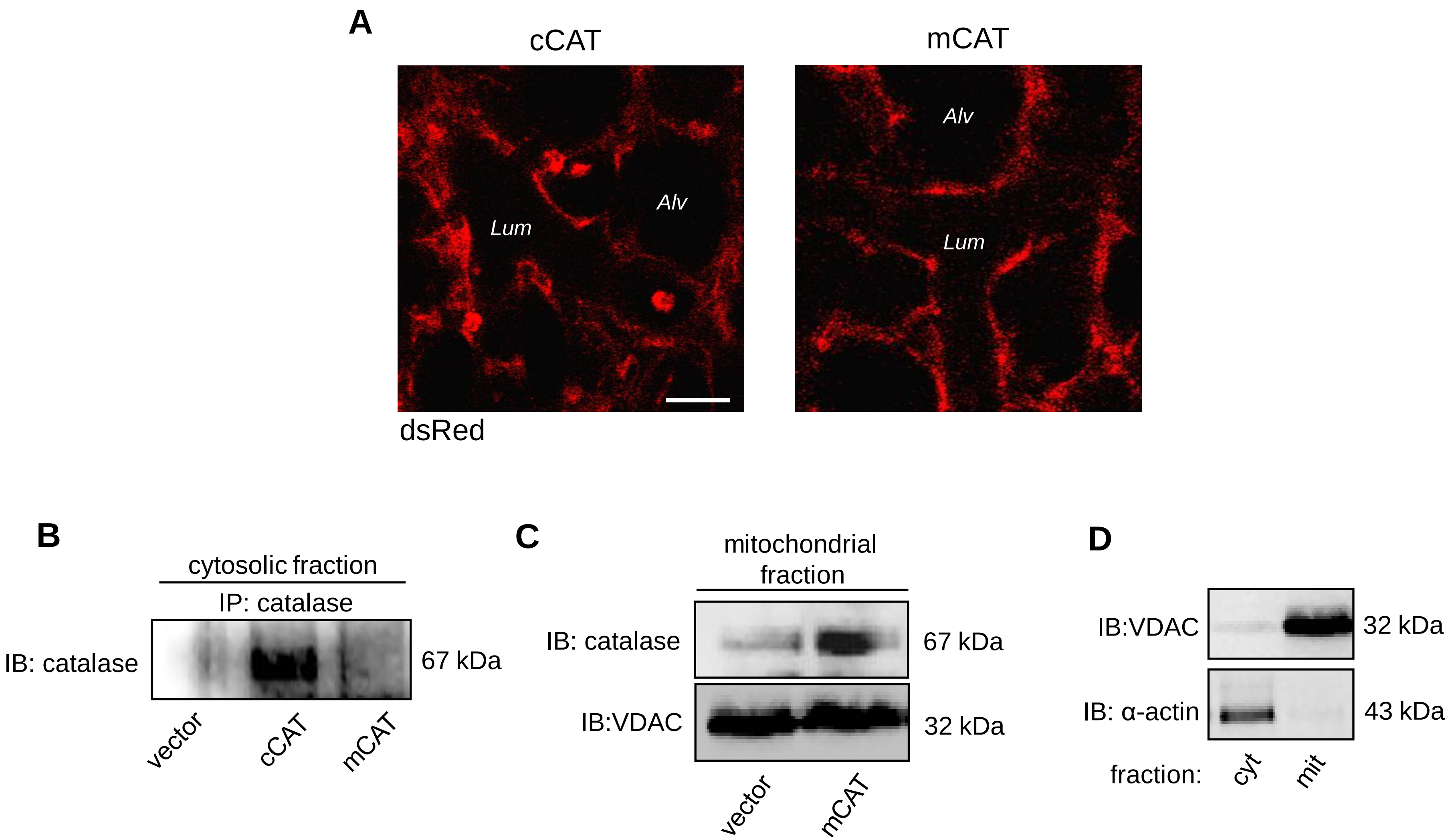


Supplemental Figure 5



Supplemental Figure 6

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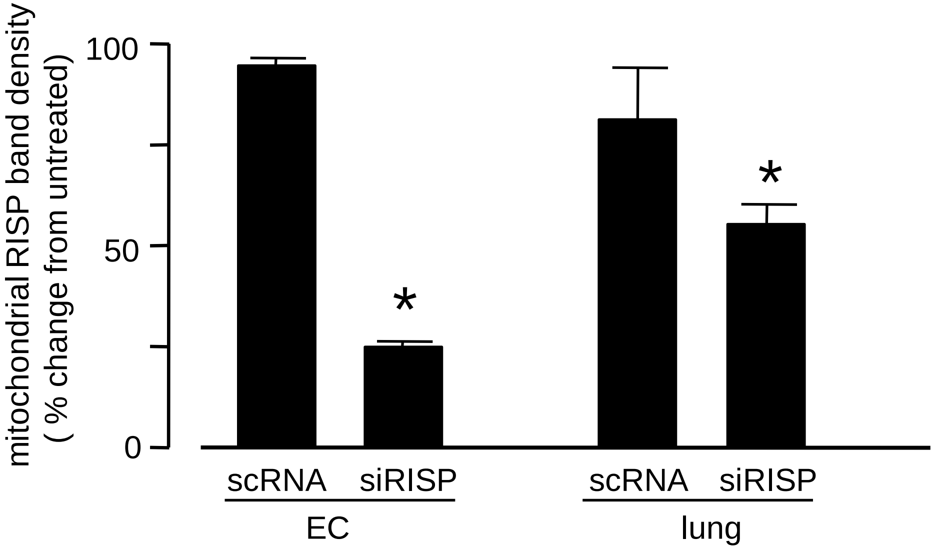
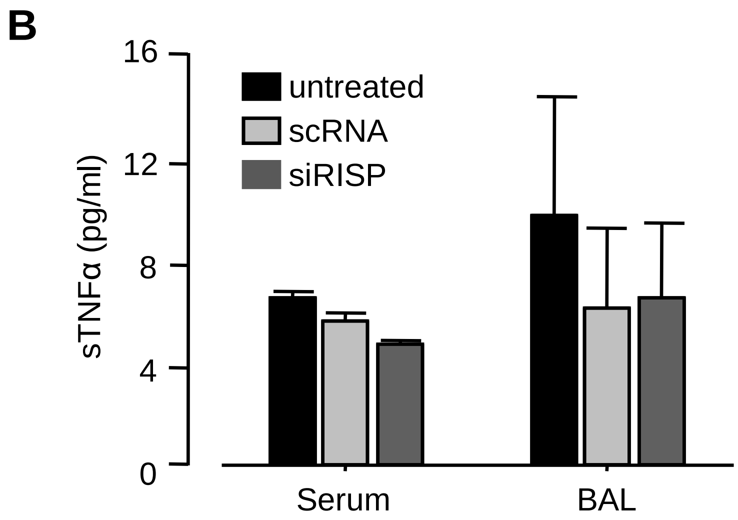
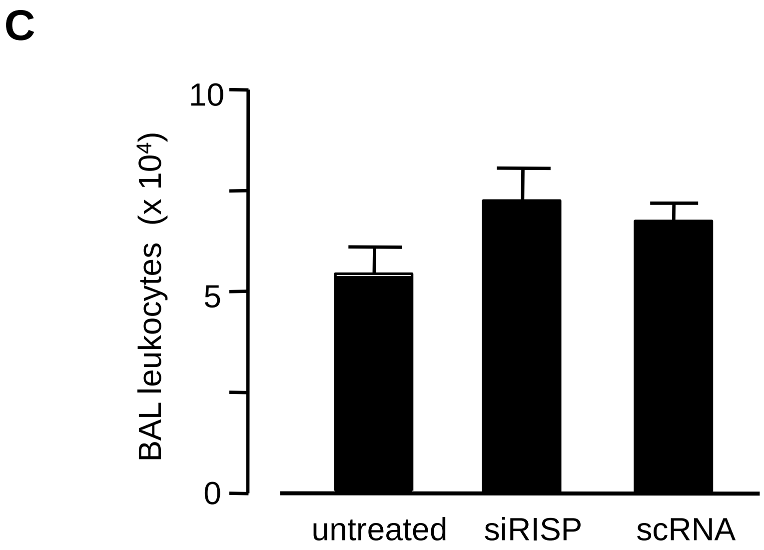


Supplemental Figure 6 (contd.)





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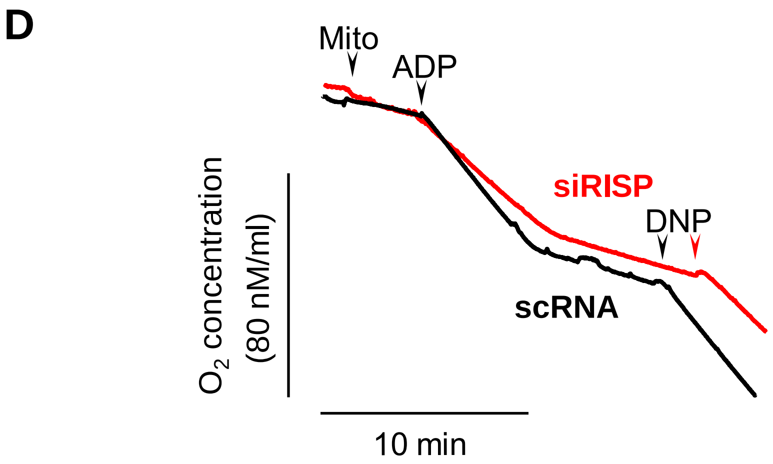

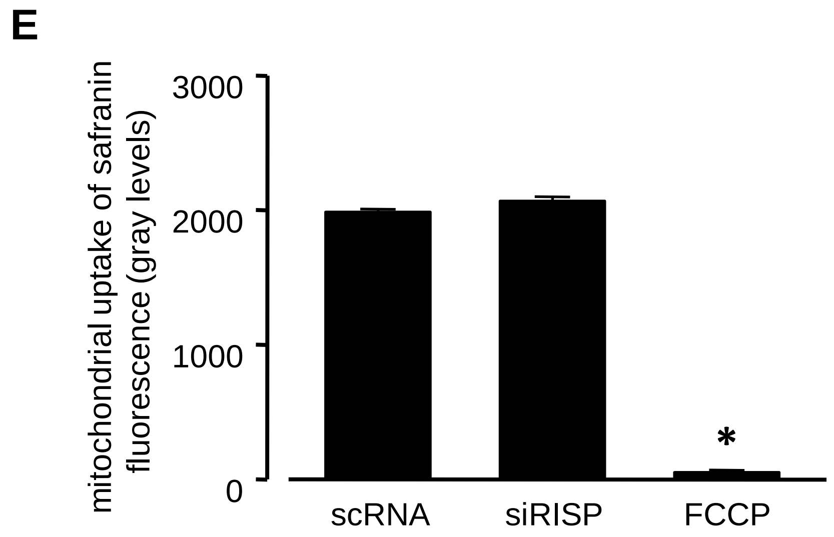

Supplemental Figure 7