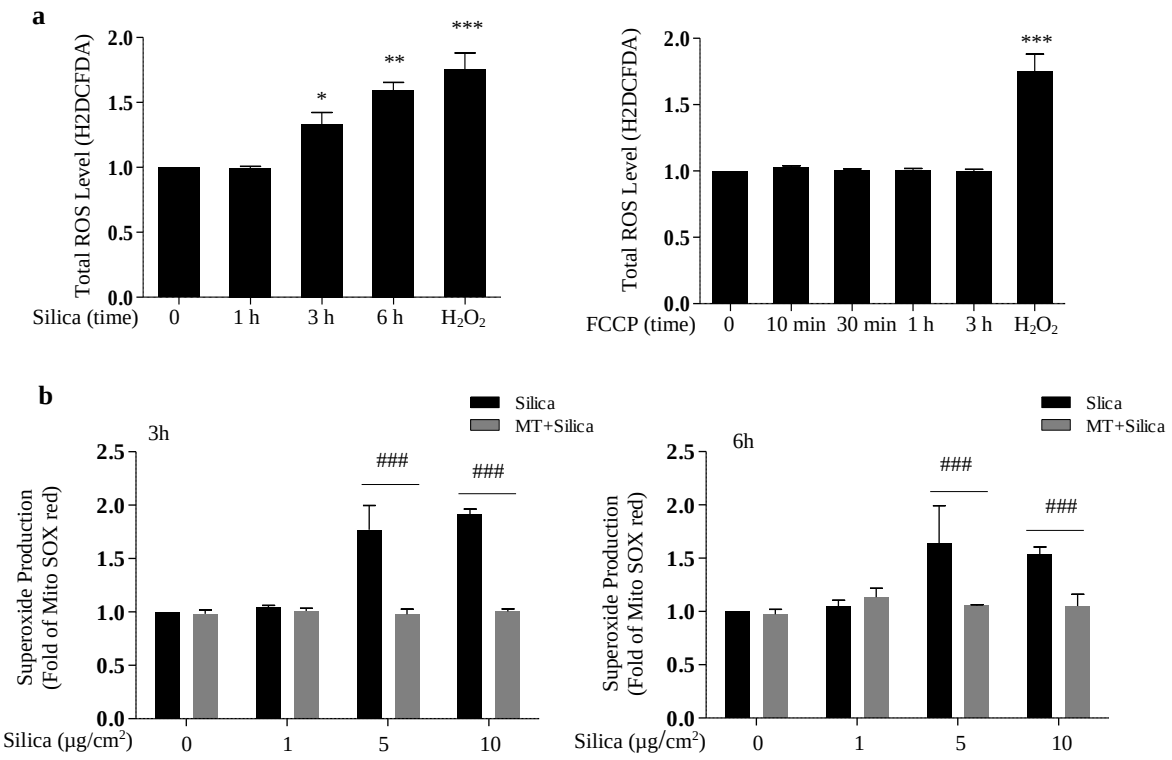
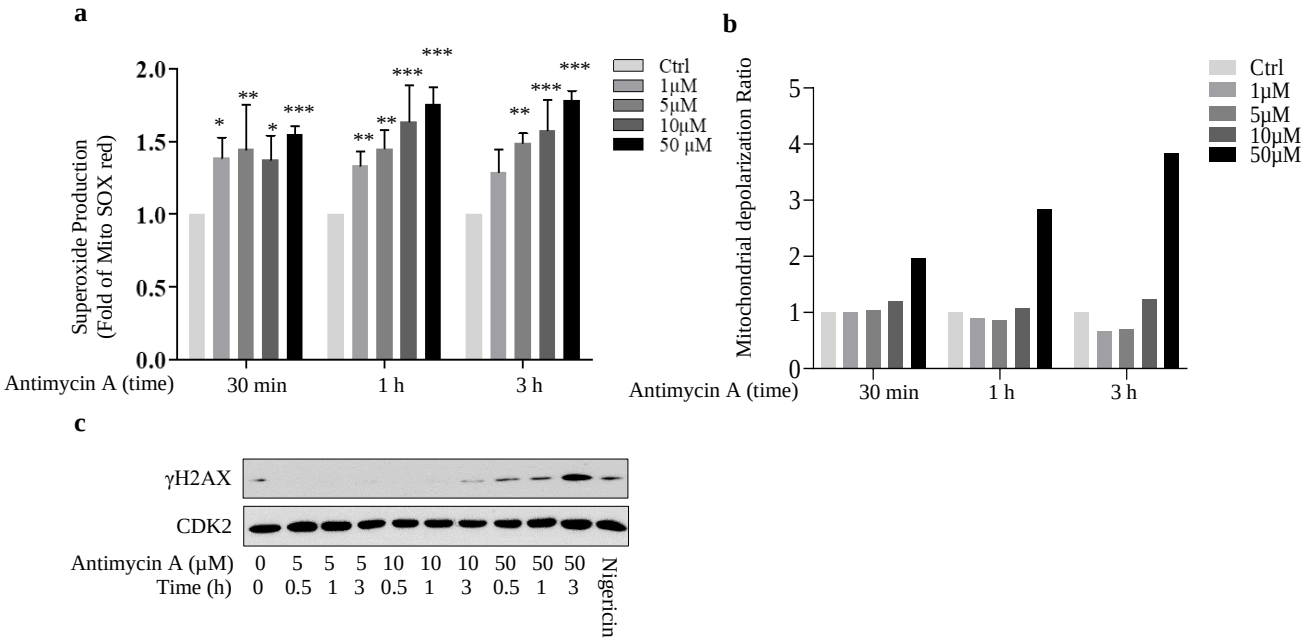
Supplementary Fig. 1



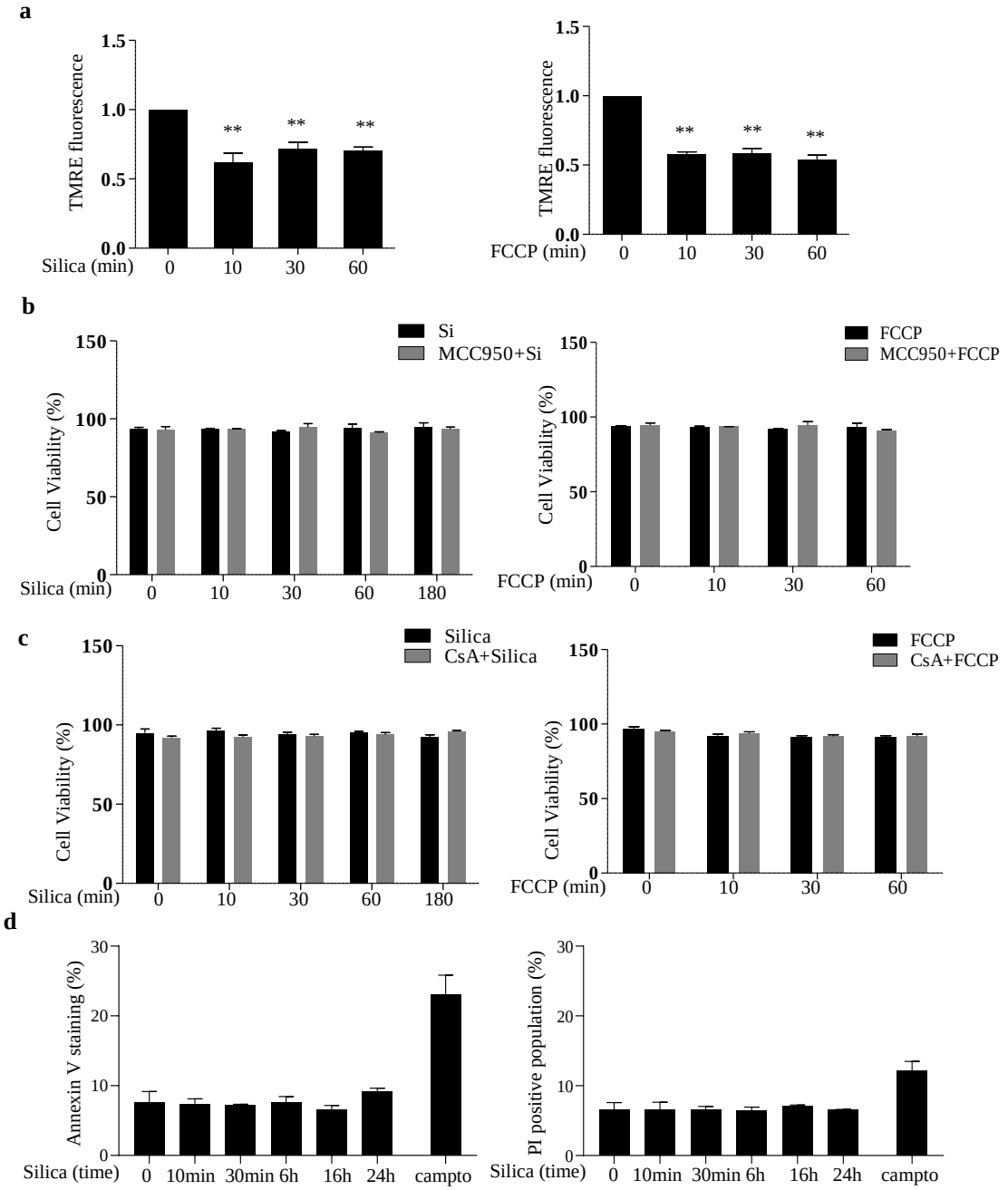
Supplementary Fig. 1 Silica-induced ROS production does not increase until 3 h in 16HBE. **a** Cellular ROS production was analyzed using H2DCFDA in cells treated with silica (5 µg/cm^2) or FCCP. H_2O_2 (1µM, 10min) was used as a positive control. **b** Mitochondrial ROS was measured in cells pre-incubated with MitoTEMPO (500 nM) for 1 h and thereafter treated with silica. Bars show means ± SD from at least three independent experiments. All experiments were performed at least in triplicate. *$p < 0.05$, **$p < 0.01$,***$p < 0.001$compared to untreated cells, or #$p < 0.05$, ##$p < 0.01$ compared to cells not exposed to inhibitor, as determined by ANOVA.

Supplementary Fig. 2



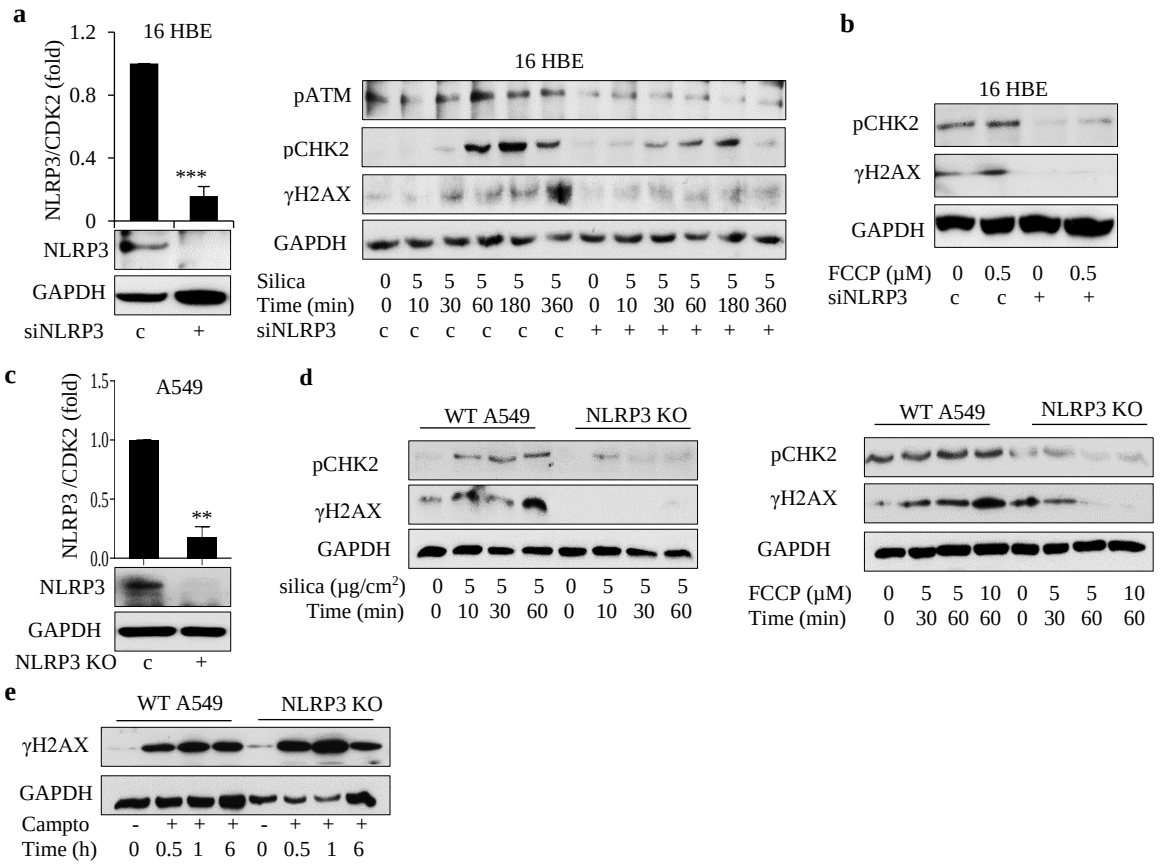
Supplementary Fig. 2 Antimycin A increases mtROS but does not induce NLRP3. **a** Mitochondrial ROS production was analyzed in 16HBE cells treated with antimycin A for times indicated. **b** Mitochondrial membrane potential was analyzed using JC-1. **c** Western blot analysis of γH2AX in cell lysates. Cells treated with Nigericin (6 μM) for 30 min was used as a positive control. CDK2 was used as loading control. Bars show means ± SD. *p < 0.05, **p < 0.01, ***p < 0.001 compared to untreated cells as determined by one-way repeated measures ANOVA.

Supplementary Fig.3



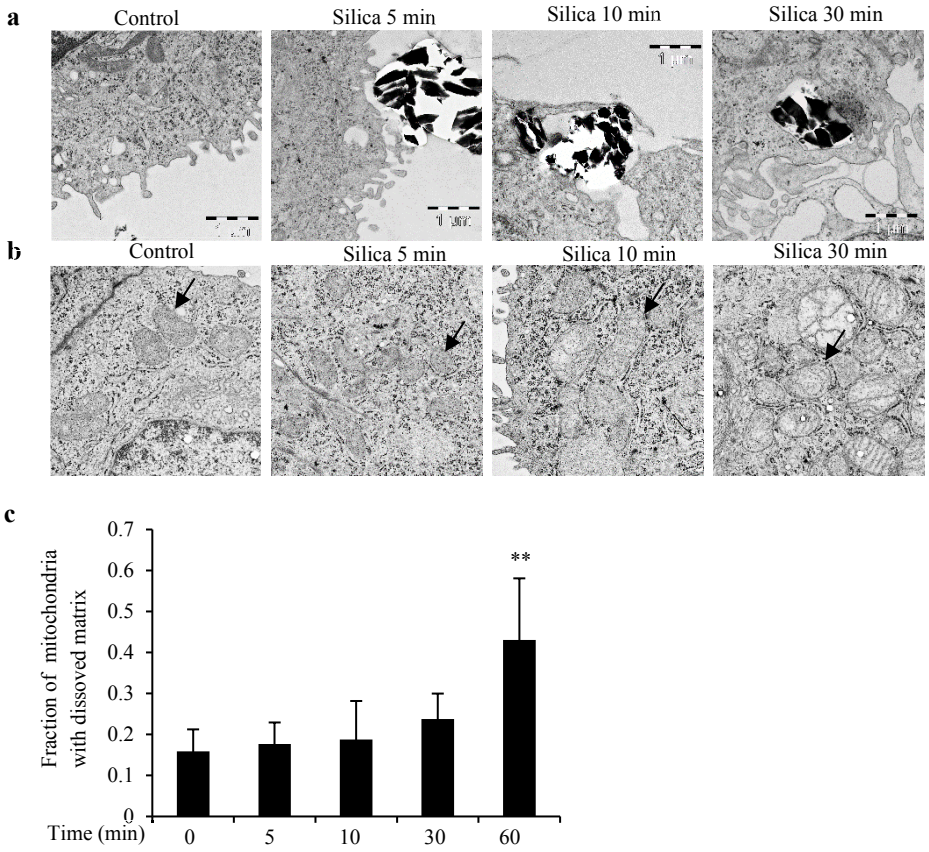
Supplementary Fig. 3 Silica and FCCP decrease mitochondrial membrane potential. **a** Mitochondrial membrane potential was analyzed using TMRE in 16HBE cells treated with silica (5 μg/cm^2) or FCCP (500 nM). **b, c** LDH assay was measured for cell viability in 16HBE cells pretreated with MCC950 (100 nM) (**b**) or with cyclosporine A (10 μM) (**c**) as indicated and thereafter treated with silica (5 μg/cm^2) or FCCP (500 nM). **d** Apoptosis assay by Annexin V and PI staining in 16HBE cells that treated with silica (5 μg/cm^2) from 10 min to 24 hrs. Treatment of Camptothecin (10uM) for 12hrs was used as positive control. Bars show means ± SD from at least three independent experiments. **$p < 0.01$ compared to untreated cells as determined by ANOVA.

Supplementary Fig. 4



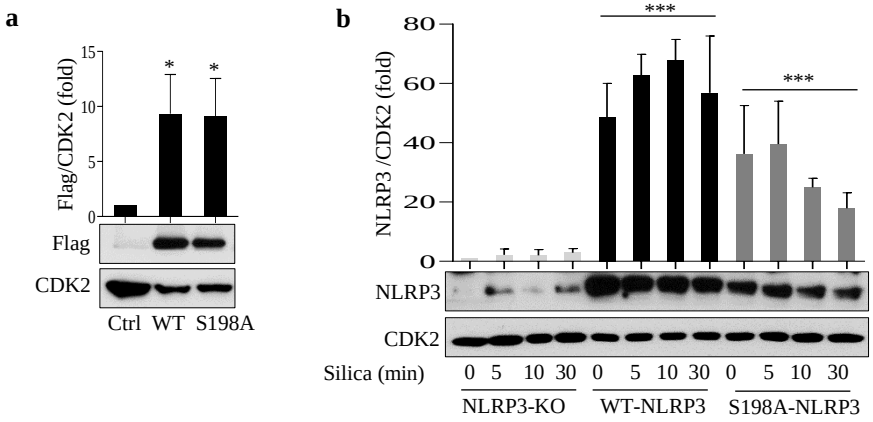
Supplementary Fig. 4 NLRP3 is essential for silica- and FCCP-induced DNA damage. **a, b** Western blot analysis of NLRP3, pATM, pCHK2, and γH2AX in cell lysates from 16HBE cells transfected with siRNA NLRP3 (+) or control siRNA (c) for 72 h and thereafter treated with silica (5 μg/cm^2) (**a**) or FCCP (500 nM, 30 min) (**b**). **c** Western blot analysis of NLRP3 in cell lysates from A549 cells transfected with gNLRP3 CRISPER CAS9 plasmids**. d** Western blot analysis of pCHK2, and γH2AX in cell lysates from A549 WT or NLRP3- KO cells treated with silica (5 μg/cm^2) or FCCP (500 nM). **e** Western blot analysis of γH2AX and pCHK2 in cell lysates from A549 WT or NLRP3-KO cells treated with Camptothecin (10 μM) for time indicated.

Supplementary Fig. 5



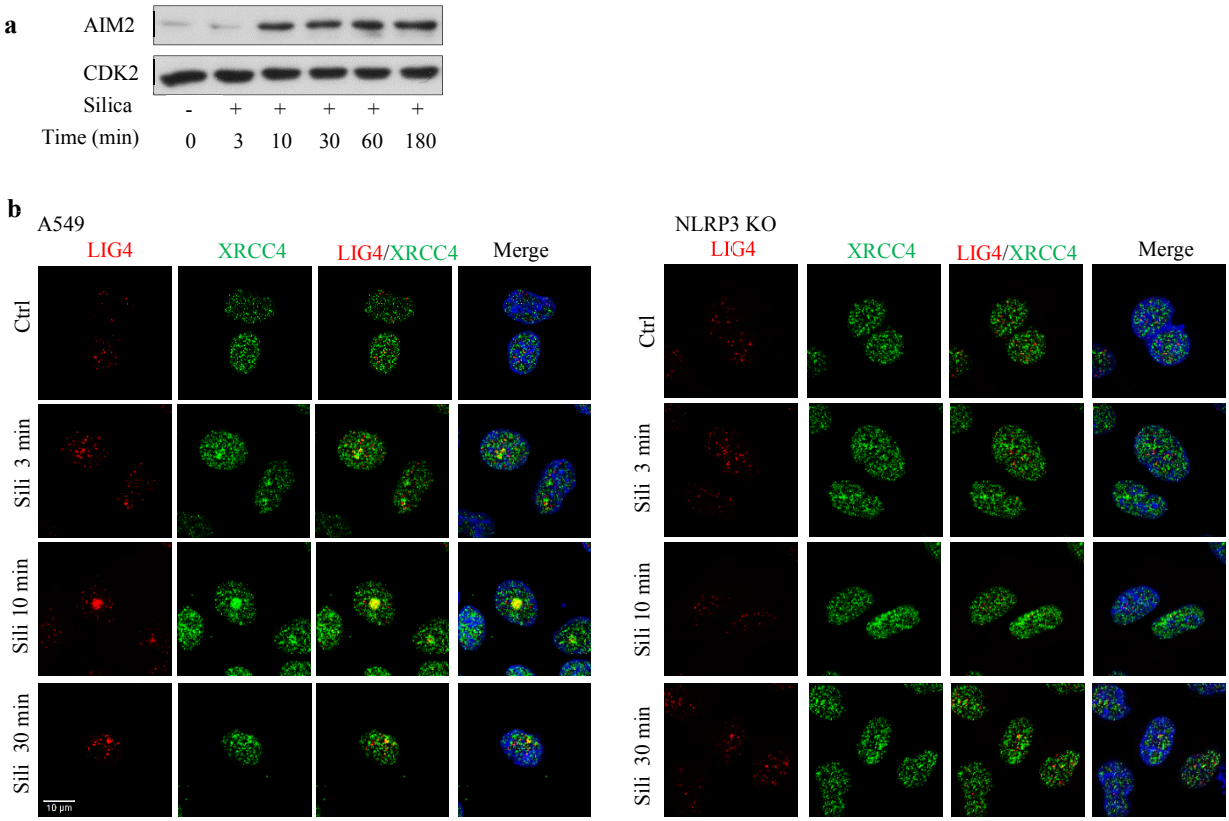
Supplementary Fig. 5 Silica activates cell membrane and mitochondrial alterations. **a** Transmission electron microscopy showed particle contact and particle uptake in 16HBE cells exposed to silica (10 μg/cm^2) for times indicated. **b** Mitochondrial morphological changes are indicated with the black arrows. **c** Bars show the fraction of mitochondria with dissolved or dark matrix. 200 mitochondria were analyzed at each time point. **$p < 0.01$ compared to untreated cells, as determined by ANOVA.

Supplementary Fig. 6



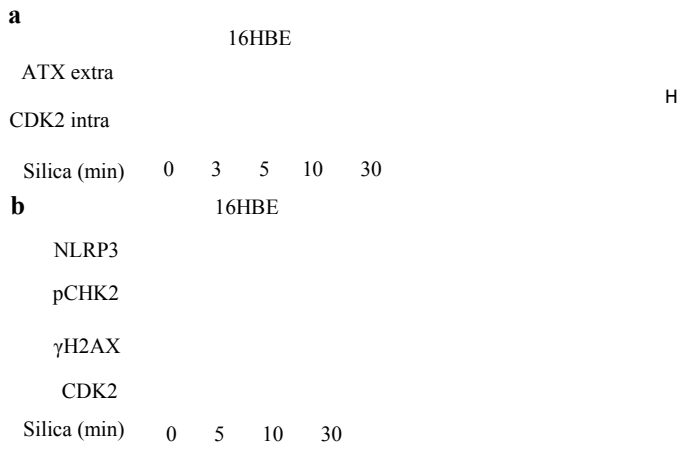
Supplementary Fig. 6 Transfection efficiency of WT NLRP3 and mutant NLRP3 in 16 HBE cells or A549 KO cells. **a** Western blot analysis of Flag in cell lysate from 16HBE cells transfected with WT Flag- NLRP3 or S198A Flag- NLRP3 plasmids for 24 hours. **b** Western blot analysis of NLRPJ3 in cell lysate from A549 KO cells transfected with WT Flag-NLRP3 or S198A Flag-NLRP3 plasmids for 24 hours. All experiments were performed at least in triplicate. *$p < 0.05$, ***$p < 0.001$ compared to control group, as determined by ANOVA.

Supplementary Fig. 7



Supplementary Fig. 7 Silica induced AIM2 in 16HBE cells and NLRP3 KO prevents silica-induced co-localization of NHEJ repair proteins. **a** Western blot analysis of AIM2 in cell lysate from 16HBE cells treated with silica (5 μg/cm^2) for times indicated. **b** Confocal microscopy analysis of co-localization of XRCC4 and LIG4 in A549 WT and 549 NLRP3 KO cells treated with silica. Quantification of data is shown in Fig. 9**c**. Scale bar is 10 μm.

Supplementary Fig. 8



Supplementary Fig. 8 Silica rapidly induces DNA damage in 16HBE cells. **a**, **b** Western blot analysis of indicated proteins in supernatants (**a**) and cell lysates (**b**) from 16HBE cells exposed to silica (5 μg/cm^2) for times indicated.