

Supplemental Material

XRCC1 Deficiency Sensitizes Human Lung Epithelial Cells to Genotoxicity by
Crocidolite Asbestos and Libby Amphibole

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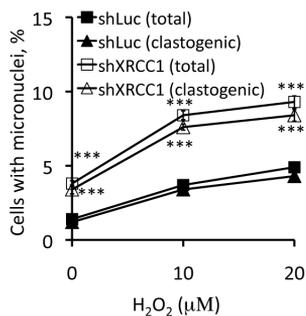
Supplemental Material, Table 1. Size distribution of UICC crocidolite and Libby amphibole

Length (μm)	% of fibers in size range		Diameter (μm)	% of fibers in size range	
	crocidolite asbestos	Libby amphibole		crocidolite asbestos	Libby amphibole
0.1-1.0	46.4	12.6	0.03-0.10	12.4	5.3
1.1-5.0	44.8	38.5	0.11-0.25	56.6	21.1
5.1-8.0	3.8	23.1	0.26-0.50	18.6	36.1
8.1-10.0	0.9	10.4	0.51-1.00	9.7	26.3
10.1-20.0	2.4	11.6	1.01-1.50	0.9	9.0
≥ 20.1	1.7	3.6	1.51-2.5	1.8	2.3

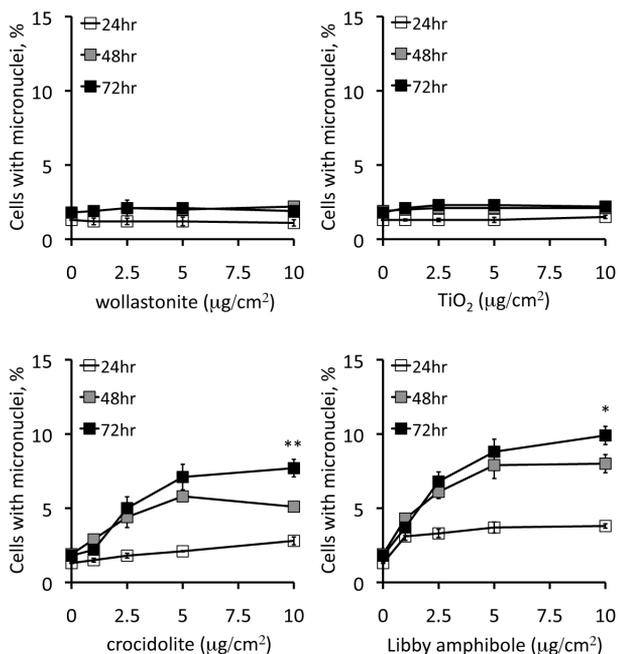
Fiber size distributions were determined by transmission electron microscopy using the methods described in (Moalli 1987).

Supplemental Material, Figure 1: Spontaneous and damage-induced micronuclei in H460 cells

A



B



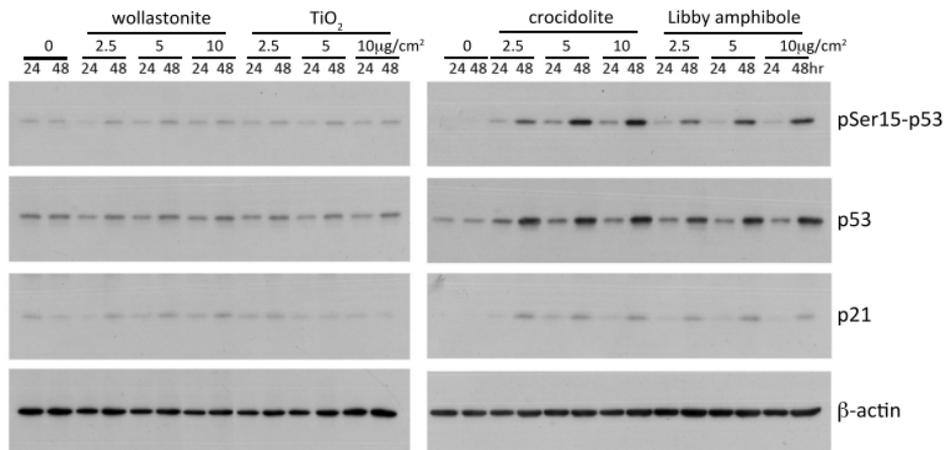
(A) XRCC1 deficiency increases frequencies of both spontaneous and H₂O₂-induced micronuclei in H460 cells.

Micronuclei in shLuc and shXRCC1 were scored 48 hours after a 1-hour exposure to H₂O₂. Filled symbols, shLuc; open symbols, shXRCC1; squares, total micronuclei; triangles, clastogenic micronuclei. Means \pm SE of three replicates are shown. *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$ compared with shLuc, one-way ANOVA.

(B) Formation of micronuclei by carcinogenic fibers is maximal at 5 $\mu\text{g}/\text{cm}^2$.

H460 cells were exposed to 0-10 $\mu\text{g}/\text{cm}^2$ particles or fibers for 24-72 hours and total micronuclei were scored in 1000 interphase cells per replicate. Means \pm SE of three replicates are shown, *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$ compared to 48hr, one-way ANOVA.

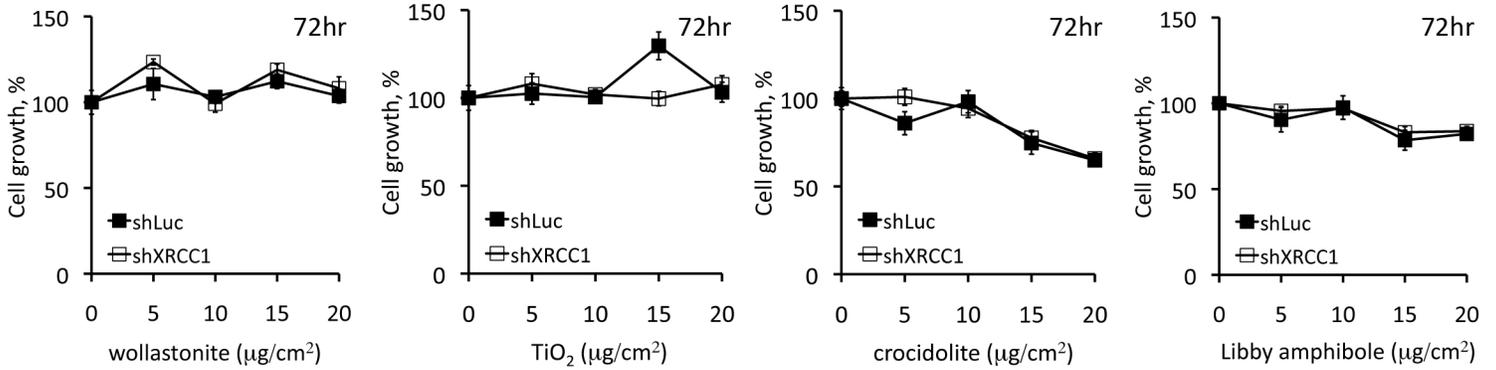
Supplemental Material, Figure 2. Carcinogenic fibers activate the p53 pathway



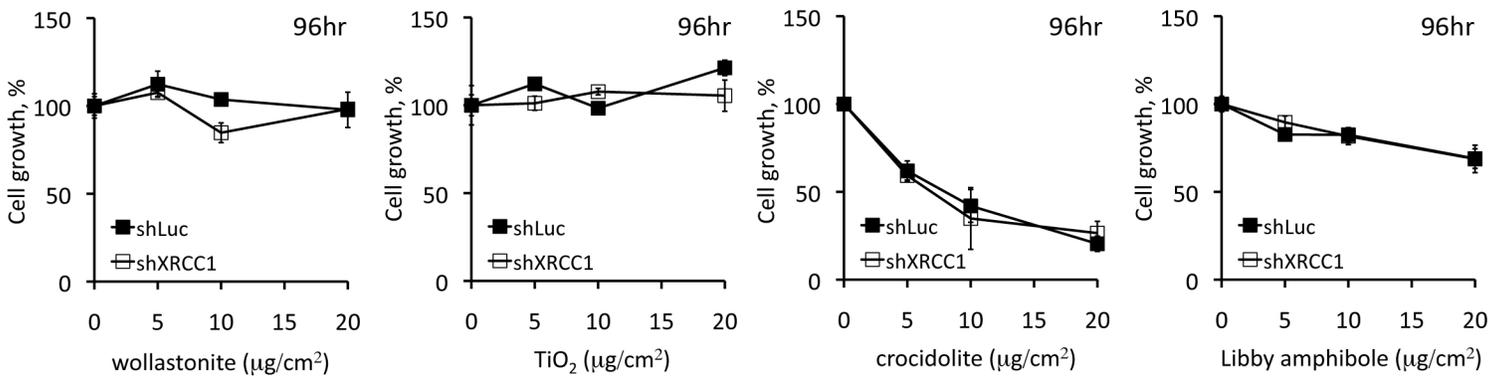
Western blot of shLuc cells exposed to 0-10 μg/cm² wollastonite, TiO₂, crocidolite, or Libby amphibole for 24 hours or 48 hours. Exposure to crocidolite asbestos or Libby amphibole, but not wollastonite or TiO₂, induces p53 stabilization and upregulation of p21. β-actin was included as a loading control.

Supplemental Material, Figure 3: XRCC1 deficiency does not sensitize H460 cells to the growth inhibitory effects of fibers

A

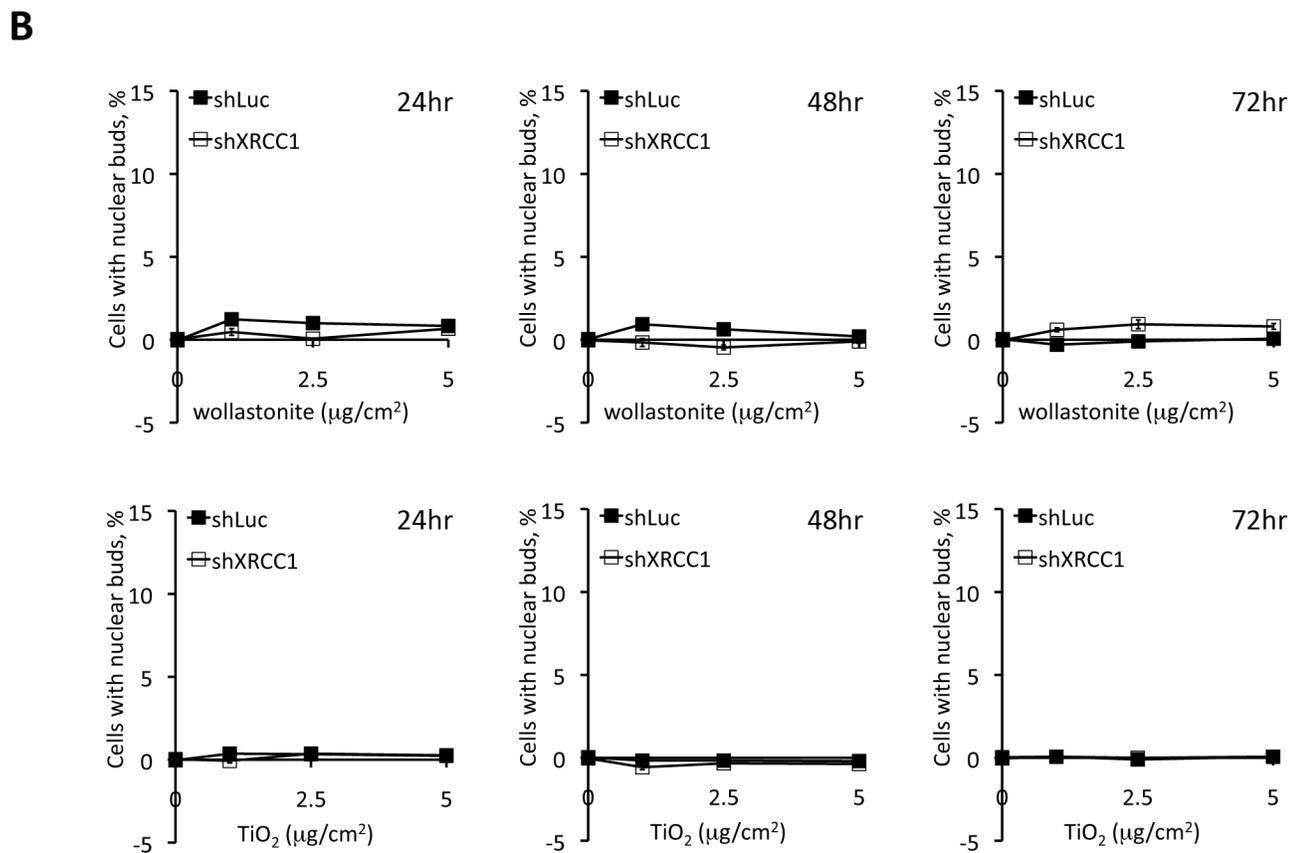
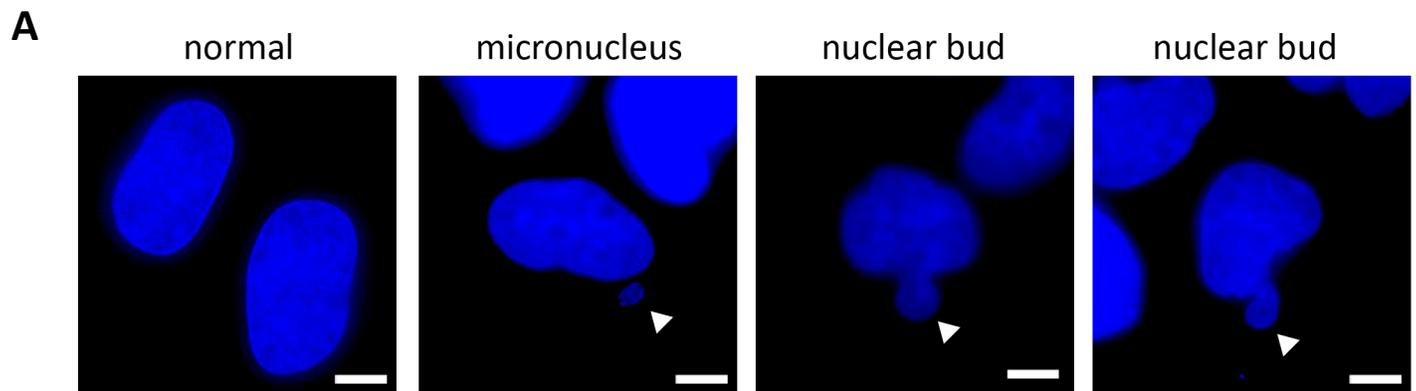


B



XRCC1 deficiency does not enhance growth inhibition by carcinogenic fibers at doses greater than 5 µg/cm². shLuc (filled squares) and shXRCC1 (open squares) were exposed to 0-20 µg/cm² fibers for (A) 72 hours or (B) 96 hours. Means ± SE of four replicates are shown.

Supplemental Material, Figure 4: Nongenotoxic fibers and particulates do not induce nuclear bud formation



(A) Representative nuclear staining of H460 cells with no nuclear aberrations (normal), a micronucleus, or a nuclear bud. Scale bar, 20µm.

(B) Nuclear buds were scored in shLuc (filled squares) and shXRCC1 (open squares) exposed for 24, 48, or 72 hours to wollastonite or TiO₂. Means ± SE of three replicates are shown, in which the percentage of spontaneous nuclear buds has been subtracted. *, P<0.05, **, P<0.01, ***, P<0.001 compared with shLuc, one-way ANOVA.

References

Moalli PA, MacDonald JL, Goodglick LA, Kane AB. 1987. Acute injury and regeneration of the mesothelium in response to asbestos fibers. *American Journal of Pathology* 128(3):4426-4445.