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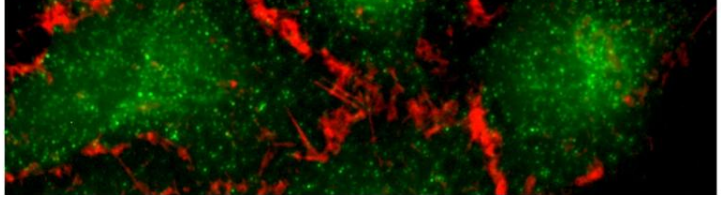

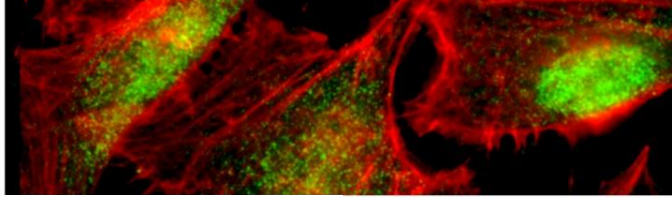

NON-SMALL CELL LUNG CANCER P53 HOTSPOT MUTATION CELL PANEL

p53 is a tumor suppressor protein encoded by the TP53 gene that responds to DNA damage by regulating cell-cycle arrest, apoptosis and senescence. At least 50 % of human tumors contain mutations or deletions of the TP53 gene. **Non-Small Cell Lung Cancer p53 Hotspot Mutation Cell Panel (ATCC® TCP-2030™)** is comprised of6 select cell lines derived from the lung that have been sequenced and validated by ATCC. The panel includes WT p53 or null p53 cell lines as well as cultures with p53 hotspot mutations at codons 245, 248, or 273. The panel is useful for anti-cancer drug targeting or reactivation of mutant p53, as well as studies related to p53 molecular mechanisms.

ATCC® No.	Name	Tissue	Histology	Tumor Source	TP53 status	Zygosity	CDS mutation	AA mutation
CRL-9609™	BEAS-2B	lung	normal tissue,SV-40 immortalized	NA	WT	-	-	-
CCL-185™	A549	lung	non-small cell lung carcinoma	primary	WT	-	-	-
CRL-5803™	NCI-H1299	lung	non-small cell lung carcinoma	metastasis (lymph node)	NULL	homozygous	c.(del)	-
HTB-178™	NCI-H596	lung	adenosquamous carcinoma	primary	MUT	homozygous	c.733G>T	p.G245C
CRL-5893™	NCI-H1770	lung	non-small cell lung carcinoma	metastasis (lymph node)	MUT	homozygous	c.741 742CC>TT	p.R248W
CRL-5908™	NCI-H1975	lung	adenocarcinoma	primary	MUT	homozygous	c.818G>A	p.R273H

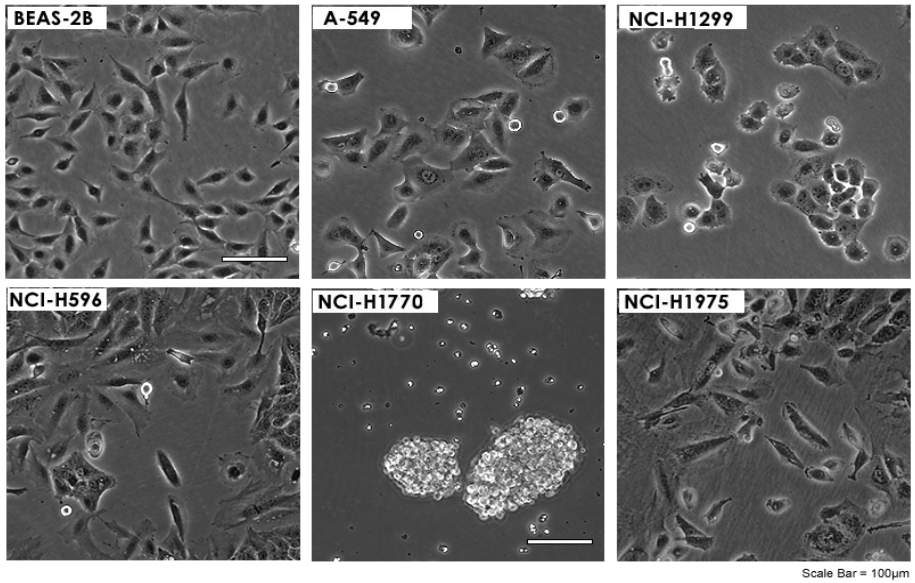

Figure 1. Cell morphology of six cell lines in the Non-Small Cell Lung Cancer p53 Hotspot Mutation Cell Panel. Two p53 wild-type lung cell lines, BEAS-2B and A549, one p53 null cell line, NCI-H1299, and three p53 hotspot mutation lung cancer cell lines, NCI-H594, NCI-H1770, and NCI-H1975, were maintained in ATCC recommended culture conditions. Cell morphology was observed under Nikon™ microscopy, and images of the indicated cell lines were captured by Olympus® digital camera. Scale bar represents 100µm.

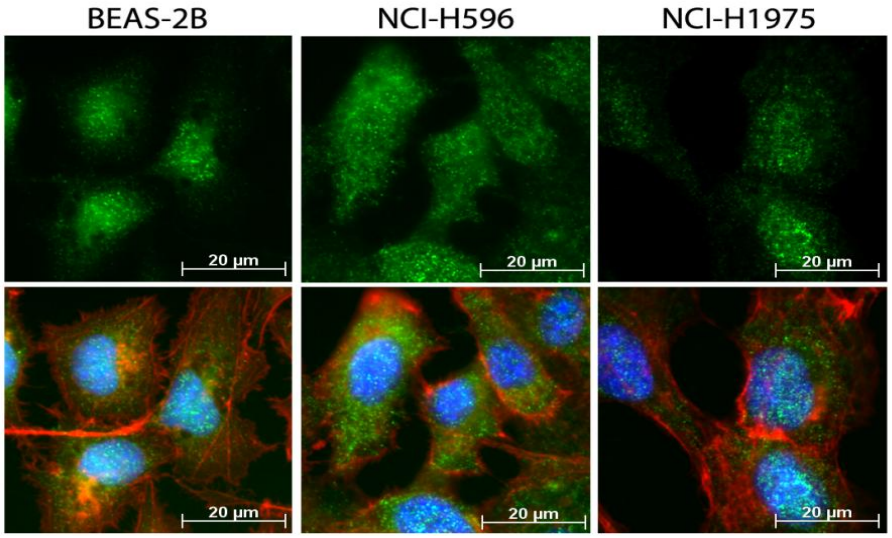

Figure 2. Immunofluorescence staining of p53. The indicated p53 wild-type and p53 mutation cells were grow on collagen-coated coverslips. Cells were fixed with 4% paraformaldehyde. p53 was stained with p53 primary antibody and Alexa Fluor 488 secondary antibody (green). F-actin was visualized with phalloidin Alexa Fluor 594 (red). Nuclei of the cells were visualized with Hoechst 33342 (blue). Single florescence channel images of p53 staining are shown in the upper row, and multichannel merged images are shown in the bottom row.

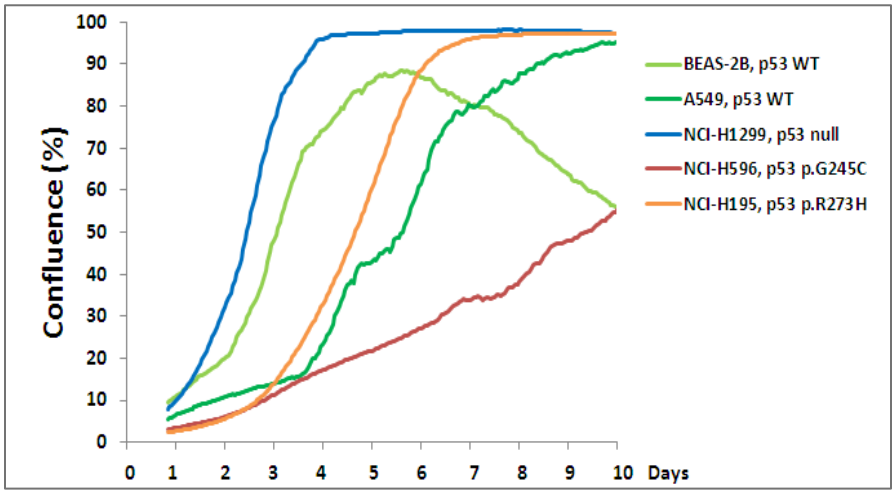

Figure 3. Cell growth kinetics. The indicated p53 wild-type and p53 mutation cells were cultured in ATCC recommended media, and plated at 3000 cells/well in 96-well plates. The cell growth kinetics were constantly monitored for 10 days using a label-free automated IncuCyte® live-cell imaging system (Essen Bioscience).

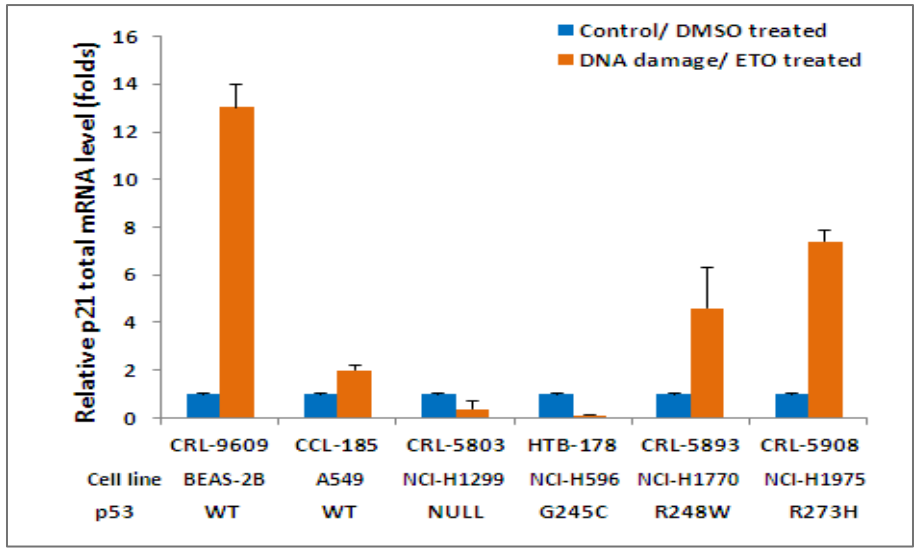

Figure 4. Real time PCR analysis of total mRNA levels of p21, a downstream target of p53, in the indicated p53 wild-type and p53 mutation cell lines. Cells were treated with 20 µM etoposide (ETO) for 6 hours to induce DNA damage, or treated with DMSO as a control. Total mRNA level of p21 and 36B4 were determined by real time quantitative PCR. Relative p21 total mRNA changes were normalized to the housekeeping gene 36B4.

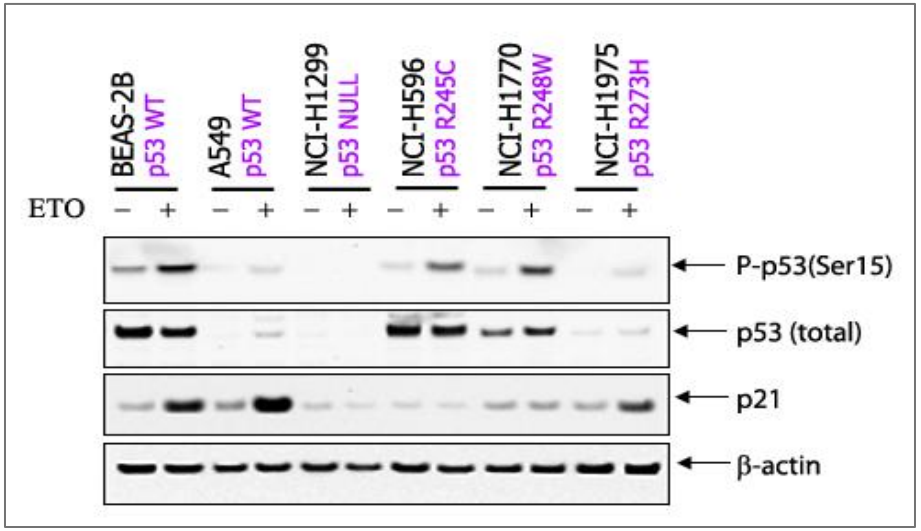

Figure 5. The indicated p53 wild-type and p53 mutation cells were treated with 20 µM etoposide (ETO) for 8 hours to induce DNA damage, or treated with DMSO as a control. Western blotting assay was used to examine phosphorylation of p53 at Serine 15, total protein expression of p53, and expression of p21, a downstream target of p53. β-actin protein was also examined as a control.