

**Silibinin overcomes EMT-driven
lung cancer resistance to new-generation ALK inhibitors**

SUPPLEMENTARY INFORMATION

Table S1. Comparison of ALK-TKIs IC₅₀ values (in μmol/L) in ALK-rearranged NSCLC cells cultured in the absence/presence of SBN

	H2228	H2228/CR	H3122	H3122/CR
crizotinib	2.1 ± 0.8 (1.0)	10.6 ± 1.7 (1.0)	0.29 ± 0.06 (1.0)	1.08 ± 0.40 (1.0)
+ 100 μmol/L SBN	1.3 ± 0.7 (1.6)	3.7 ± 0.7 (2.9) ^a	0.10 ± 0.00 (2.9)	0.17 ± 0.03 (6.3)
brigatinib	0.5 ± 0.08 (1.0)	3.8 ± 0.4 (1.0)	0.02 ± 0.00 (1.0)	0.12 ± 0.03 (1.0)
+ 100 μmol/L SBN	0.4 ± 0.07 (1.2)	0.6 ± 0.1 (6.3)	0.01 ± 0.00 (2.0)	0.07 ± 0.01 (1.7)
lorlatinib	1.0 ± 0.11 (1.0)	83 ± 5.1 (1.0)	0.004 ± 0.00 (1.0)	0.02 ± 0.00 (1.0)
+ 100 μmol/L SBN	9.0 ± 2.2 (0.1)	19.3 ± 3.2 (4.3)	0.004 ± 0.00 (1.0)	0.003 ± 0.00 (6.7)

^a Number in parentheses represent the silibinin (SBN) extent of sensitization expressed as fold-change compared to untreated control cells

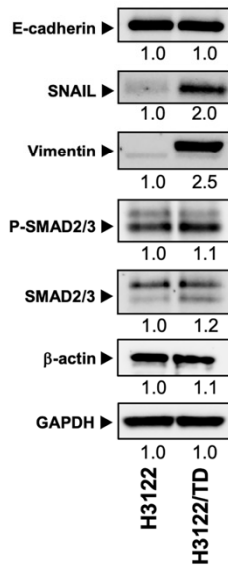
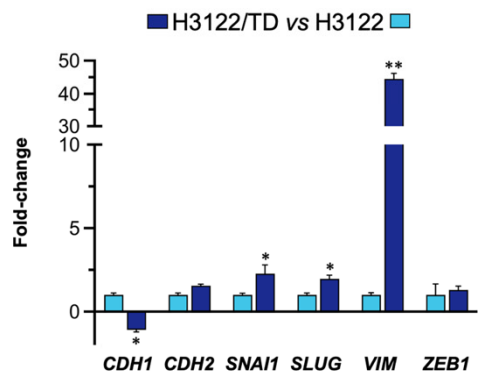
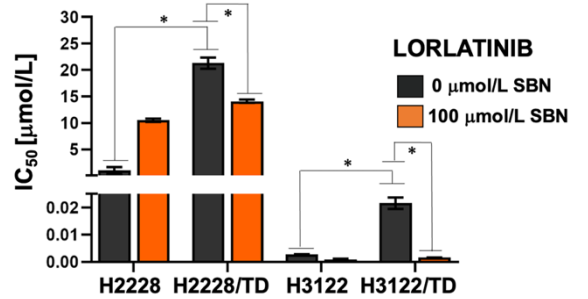
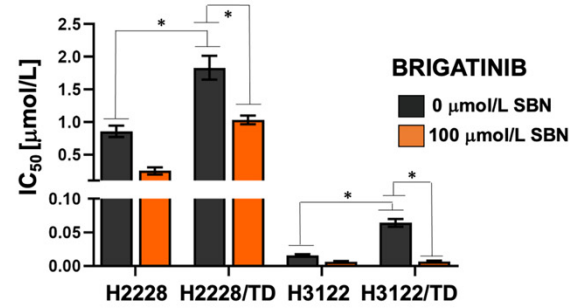
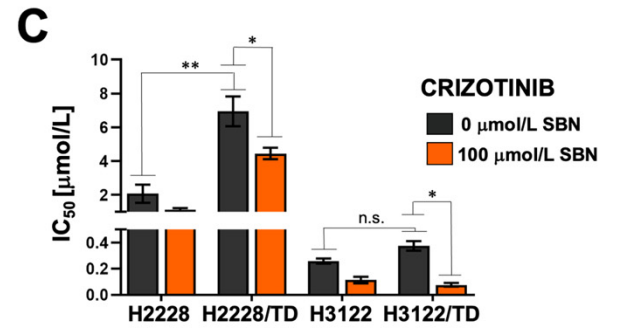
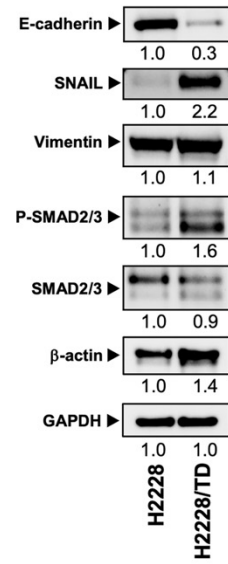
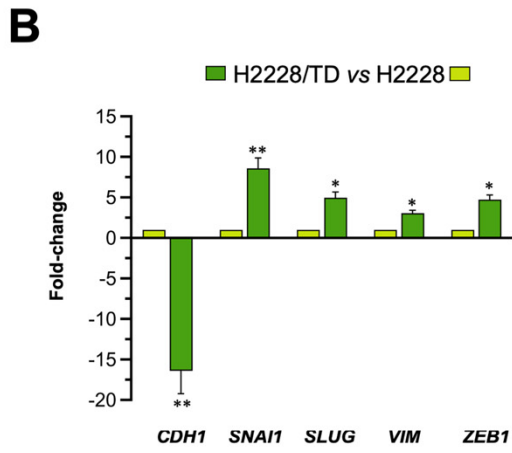
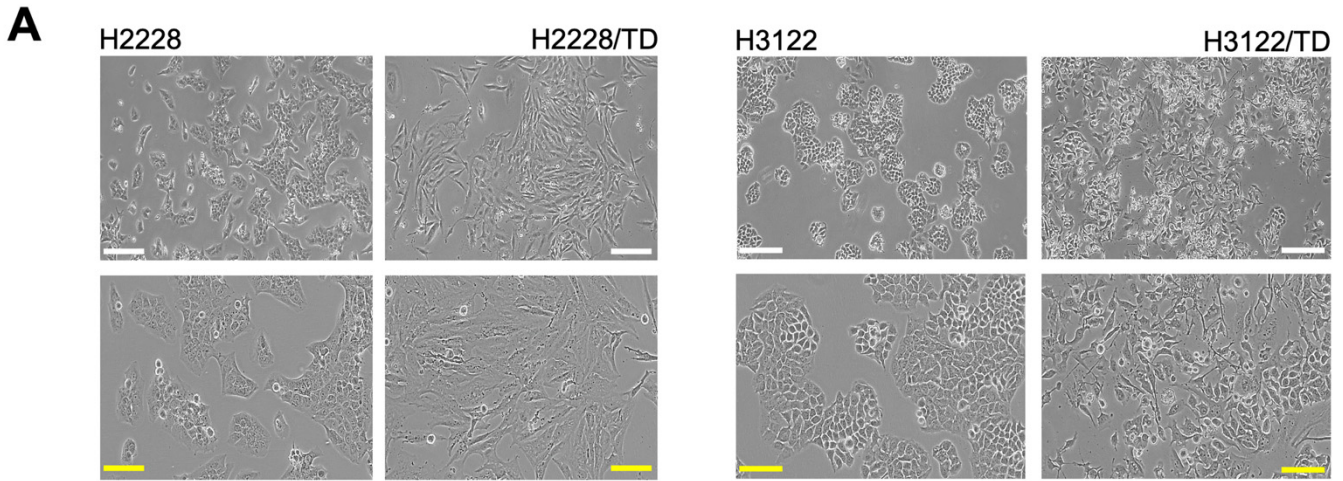
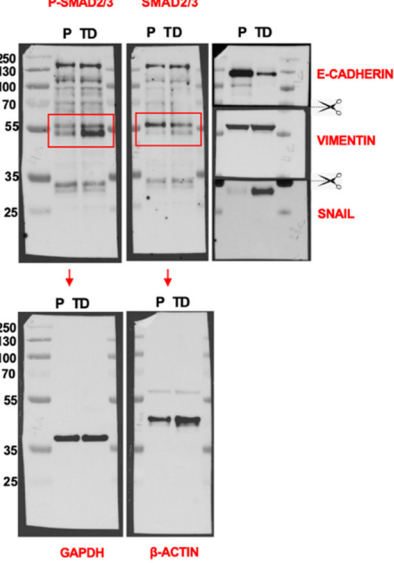
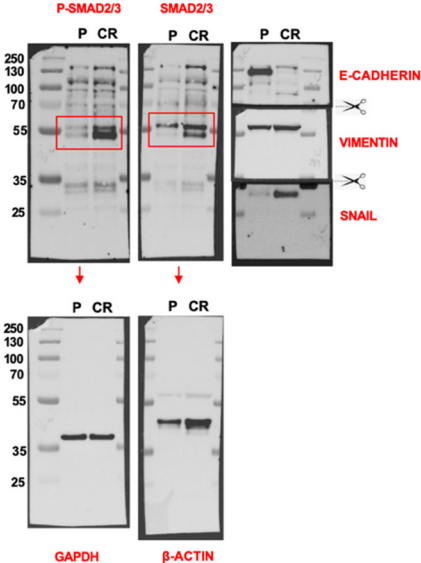


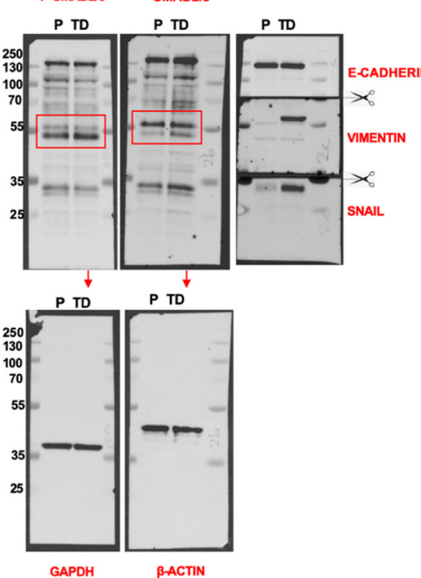
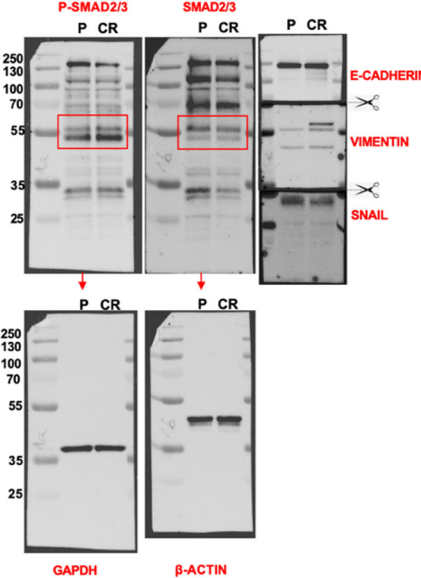
Figure S1. EMT caused by chronic TGF β stimulation drives cross-resistance to multiple-generation ALK-TKIs. **A.** Representative phase contrast microphotographs of H2228/H2228TD and H3122/H3122TD ALK-rearranged NSCLC cell line pairs. TD: transdifferentiated; Scale bar, 100 μ m. **B.** *Left.* The transcript abundance of *CDH1*, *CDH2*, *SNAI1*, *SLUG*, *VIM*, and *ZEB1* was calculated using the ΔC_t method and presented as fold-change in H2228/H2228TD and H3122/H3122TD cells; * $p < 0.05$ and ** $p < 0.005$, statistically significant differences. *Right.* Expression levels of E-cadherin, SNAIL, vimentin, phospho-SMAD2/3, total SMAD2/3 were detected by immunoblotting in H2228/H2228TD and H3122/H3122TD cells using specific antibodies. The intensity of the bands was measured using the ImageJ software. Fold-change of each protein relative to parental cells was calculated using GAPDH as a loading control. Figure shows representative immunoblots of multiple ($n=3$) independent experiments. **C.** Bar graphs showing the MTT-based IC₅₀ values of crizotinib, brigatinib, and lorlatinib for H2228/H2228TD and H3122/H3122TD cells calculated in the absence or presence of 100 μ mol/L silibinin. The results are presented as the means (*columns*) \pm S.D (*bars*) ($n=3$, in triplicate). * $p < 0.05$ and ** $p < 0.005$, statistically significant differences; n.s. not significant.

Figure S2. Uncropped western blots

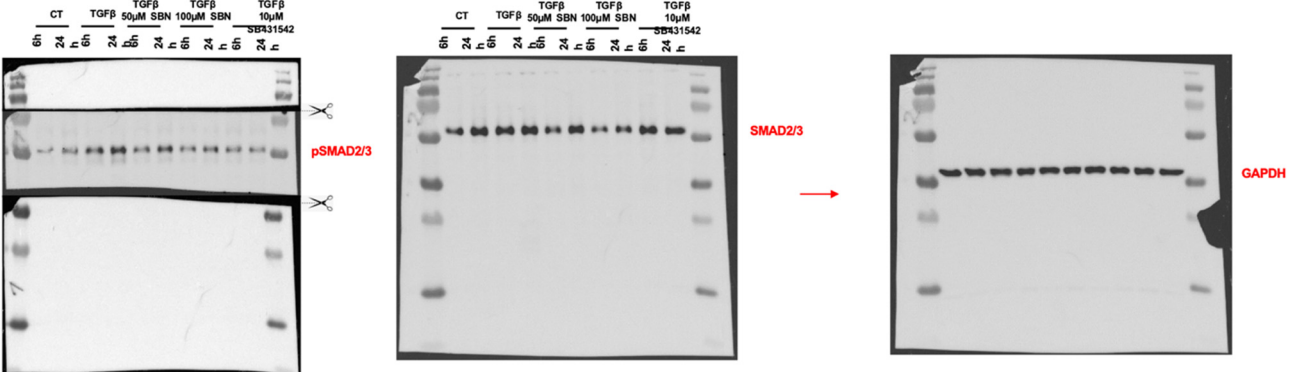
H2228



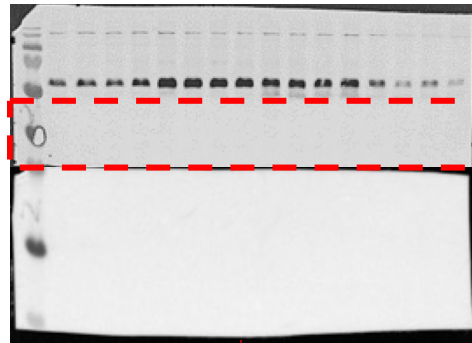
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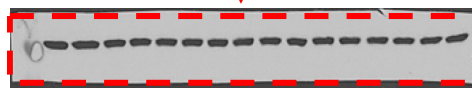
HEK



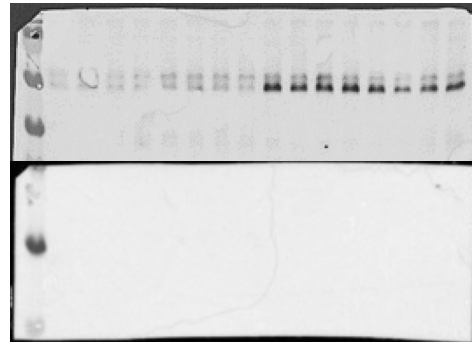
H2228



SMAD2/3

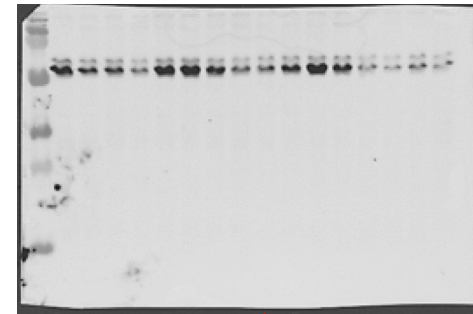


GAPDH

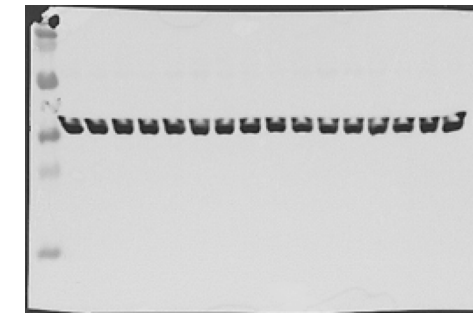


pSMAD2/3

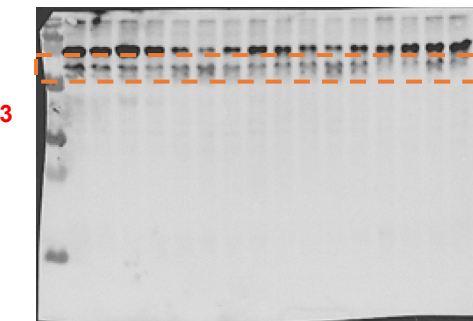
H3122



pSMAD2/3



GAPDH



SMAD2/3