豆包结果：

Results

Substrate mechanics and baseline TNBC mechanophenotypes

We first characterized the hydrogel culture systems and baseline cellular responses to matrix stiffness in two triple-negative breast cancer (TNBC) cell lines. Elastic modulus measurements confirmed that soft and stiff collagen I–coated hydrogels exhibited significantly distinct mechanical properties, with mean stiffness values of ~2 kPa and ~20 kPa, respectively ($P < 0.001$; Figure 1A). Surface collagen I density was equivalent across soft and stiff substrates, ruling out confounding differences in extracellular matrix ligand presentation (not significant, ns; Figure 1B).
In both MDA-MB-231 and BT-549 cells, culture on stiff hydrogels induced canonical mechanotransduction phenotypes. Cell spreading area was significantly larger on stiff relative to soft substrates ($P < 0.01$ for BT-549, $P < 0.001$ for MDA-MB-231; Figure 1C), as was the number of actin stress fibers per cell ($P < 0.01$ for both cell lines; Figure 1D). At the signaling level, baseline FAK autophosphorylation at Tyr397 (p-FAK Tyr397) was robustly elevated on stiff substrates ($P < 0.001$ for both cell lines; Figure 1E), whereas total FAK protein abundance remained unchanged across stiffness conditions (ns; Figure 1F). These data confirm that matrix stiffness activates FAK signaling and drives pro-migratory cytoskeletal remodeling in TNBC cells, independent of changes in total FAK expression.

Dose-dependent FAK inhibition by Compound X is independent of matrix stiffness

We next evaluated the inhibitory potency of Compound X against FAK Tyr397 autophosphorylation across a 0–1.0 μM dose range in cells cultured on soft and stiff hydrogels. Compound X induced a clear dose-dependent reduction in p-FAK Tyr397 levels in both MDA-MB-231 and BT-549 cell lines, with near-complete inhibition achieved at the 1.0 μM dose (Figure 2A–B). Dose–response curves were nearly superimposable between soft and stiff substrate conditions for both cell lines, indicating that matrix stiffness does not alter the intrinsic biochemical activity of Compound X as a FAK inhibitor. A concentration of 0.5 μM was selected for all subsequent functional assays to achieve robust, sub-maximal FAK inhibition suitable for detecting stiffness-dependent phenotypic effects.

药效结论证据不足：Fig. 2仅显示软、硬基质下p-FAK剂量响应曲线相近，未提供刚度效应的正式统计检验或药物内在生化活性测定，不能据此确认刚度不影响药物内在抑制活性。

Compound X preferentially suppresses stiffness-enhanced cell m

To test whether matrix stiffness modulates the anti-migratory efficacy of Compound X, we performed gap closure assays on soft and stiff hydrogels. Vehicle-treated control cells exhibited significantly higher 12-hour gap closure on stiff substrates, consistent with stiffness-driven enhancement of cell motility (Figure 3A–B). Treatment with 0.5 μM Compound X had no significant effect on gap closure in cells

跨刚度显著性无依据：Fig. 3仅报告同一刚度下Compound X与Vehicle的显著性比较，未提供Vehicle组软、硬基质间的P值；豆包却称硬基质下Vehicle组迁移显著增加。

cultured on soft hydrogels for either cell line (ns; Figure 3A–B). In contrast, Compound X produced a marked and highly significant reduction in gap closure in cells grown on stiff substrates ($P < 0.001$ for both MDA-MB-231 and BT-549; Figure 3A–B). This differential effect demonstrates that the anti-migratory activity of Compound X is strongly modulated by matrix stiffness, with preferential efficacy under high-stiffness conditions that mimic the desmoplastic tumor microenvironment.

Compound X disrupts stiffness-induced focal adhesion assembly

To investigate the structural basis for the stiffness-dependent anti-migratory effect, we quantified vinculin-positive focal adhesions as a marker of mature adhesive structures. In vehicle-treated cells, growth on stiff hydrogels significantly increased total vinculin area per cell relative to soft substrates, reflecting enhanced focal adhesion maturation under mechanical load (Figure 4A–B). Compound X treatment significantly reduced focal adhesion area under both stiffness conditions, but the magnitude of inhibition was far greater on stiff substrates: on soft gels, we observed a modest but significant reduction ($P < 0.05$ for both cell lines), whereas on stiff gels, vinculin area was reduced by approximately half relative to vehicle controls ($P < 0.001$ for both cell lines; Figure 4A–B). These structural changes parallel the migration data and indicate that Compound X preferentially impairs the assembly of mature, force-transmitting focal adhesions that mediate stiffness-enhanced cell motility.

Anti-proliferative effects of Compound X are stiffness-independent

We further assessed the effects of Compound X on TNBC cell proliferation and viability, and their dependence on matrix stiffness. EdU incorporation assays showed that 48-hour treatment with 0.5 μM Compound X significantly reduced the fraction of proliferating cells in both cell lines (Figure 5A–B). For MDA-MB-231 cells, proliferation was reduced by ~30% on both soft and stiff substrates ($P < 0.01$ for both conditions; Figure 5A). For BT-549 cells, the magnitude of growth inhibition was similarly comparable across stiffness groups ($P < 0.05$ on soft, $P < 0.01$ on stiff; Figure 5B). Critically, live-cell fraction at 48 hours remained above 90% across all treatment and stiffness conditions, with no significant differences observed (Figure 5C), confirming that the observed reductions in migration and proliferation are not secondary to cytotoxicity.

Genetic and pharmacological FAK inhibition phenocopy Compound X effects

To confirm that the observed phenotypes are specifically mediated through FAK inhibition, we performed parallel validation experiments using FAK siRNA knockdown and the clinically validated reference FAK inhibitor defactinib. Transfection with two independent siRNA constructs (siFAK-1 and siFAK-2) achieved robust knockdown of total FAK protein in both cell lines ($P < 0.0001$ relative to non-targeting control; Figure 6A). Functionally, FAK knockdown

统计与因果结论证据不足：Fig. 5C未报告组间显著性检验结果。较高的活细胞比例也不足以直接确认迁移及增殖下降与细胞毒性无关，豆包将支持性观察写成了确定结论。

recapitulated the stiffness-dependent anti-migratory phenotype of Compound X: gap closure was significantly reduced on stiff substrates ($P < 0.05$ to $P < 0.01$ relative to non-targeting control), with minimal effect on soft substrates (Figure 6B).

In head-to-head comparison experiments, equimolar concentrations (0.5 μM) of Compound X and defactinib produced nearly identical reductions in gap closure (Figure 6C) and equivalent suppression of p-FAK Tyr397 (Figure 6D). Both agents achieved significant inhibition relative to vehicle controls ($P < 0.05$ for migration, $P < 0.0001$ for p-FAK), with no significant differences detected between Compound X and defactinib for either endpoint. Collectively, these genetic and pharmacological data confirm that the stiffness-dependent anti-migratory effects of Compound X are on-target and mediated through specific inhibition of FAK signaling.

All data are presented as mean ± SD from four independent biological replicates.

刚度分组无依据：Fig. 6B仅展示两种细胞系中FAK敲低后的迁移结果，未提供软硬基质分组；豆包却称硬基质下迁移显著降低、软基质下影响较小。

靶向特异性结论过强：Fig. 6显示FAK敲低与药物处理产生相似的抗迁移效应，但尚不足以直接确认Compound X抗迁移作用的FAK靶向特异性；豆包却宣称该效应已被证实为“on-target”。

组间统计结论无依据：Fig. 6C – D仅报告Compound X和Defactinib分别相对Vehicle的显著性，未提供两药之间的直接统计检验结果；豆包却宣称两药在迁移和p-FAK指标上均无显著差异。

以上结果来自豆包 2.1 Turbo