LetPub 结果：

Substrate characterization and baseline stiffness responses

The collagen I-coated gel system separated matrix mechanics from coating-related signals. The measured elastic modulus was higher in the stiff condition than in the soft condition ($P < 0.001$), whereas collagen I surface fluorescence did not differ significantly between the two collagen I-coated substrates (Figure 1A,B).

Both triple-negative breast cancer (TNBC) cell lines showed baseline stiffness-responsive phenotypes. Cell spreading area was greater on stiff than on soft substrates in BT-549 cells ($P < 0.01$) and MDA-MB-231 cells ($P < 0.001$; Figure 1C). Stress fiber number also increased on stiff substrates in both cell lines ($P < 0.01$ for each comparison; Figure 1D). Consistent with increased focal adhesion kinase (FAK) activation under stiff conditions, p-FAK Tyr397 normalized to total FAK and to the soft-control mean was higher on stiff substrates in both BT-549 and MDA-MB-231 cells ($P < 0.001$ for each comparison; Figure 1E). In contrast, total FAK abundance normalized to GAPDH did not differ significantly between soft and stiff substrates in either cell line (Figure 1F).

Compound X reduced FAK Tyr397 autophosphorylation under both stiffness conditions

准确识别两种细胞的剂量依赖性抑制趋势，未擅自推断图中未报告的IC_{50}参数。

Compound X reduced normalized p-FAK Tyr397 autophosphorylation in a concentration-dependent manner in both TNBC cell lines cultured on collagen I-coated soft and stiff gels. In MDA-MB-231 cells, relative p-FAK decreased progressively with increasing Compound X concentration on both soft and stiff substrates, with closely overlapping dose-response profiles and slightly lower mean values on stiff gels at higher concentrations (Figure 2A). BT-549 cells showed a similar graded reduction, with p-FAK levels declining across the concentration range under both stiffness conditions and comparable inhibition on soft and stiff gels (Figure 2B). Individual replicate values showed consistent downward trends with increasing Compound X concentration in both cell lines (Figure 2A,B).

Compound X preferentially reduced gap closure on stiff substrates

In 12-h gap-closure assays on collagen I-coated soft and stiff gels, 0.5 μM Compound X altered migration in a stiffness-dependent manner. In MDA-MB-231 cells, Compound X did not significantly change gap closure relative to vehicle on soft gels, whereas it reduced gap closure on stiff gels ($P < 0.001$ vs vehicle; Figure 3A). The same pattern was observed in BT-549 cells: the Compound X versus vehicle comparison was not significant on soft gels, but Compound X decreased gap closure on stiff gels ($P < 0.001$ vs vehicle; Figure 3B). Thus, in both TNBC cell lines,

准确区分软硬基质下的药物效应，并将显著性正确对应至Compound X与Vehicle的比较。

Compound X suppressed gap closure under stiff-gel conditions, in which vehicle-treated cells showed greater closure.

Compound X reduced vinculin-positive focal adhesion area

准确识别两种细胞在软、硬基质下的显著性差异，并完整保留相应统计结果（P＜0.05和P＜0.001）。

Compound X also reduced vinculin-positive focal adhesion area relative to vehicle in both TNBC cell lines cultured on collagen I-coated soft and stiff gels. In MDA-MB-231 cells, 0.5 μM Compound X decreased vinculin-positive area on soft gels ($P < 0.05$) and produced a larger reduction on stiff gels ($P < 0.001$), where vehicle-treated cells showed the greatest focal adhesion area (Figure 4A). BT-549 cells showed the same pattern, with lower vinculin-positive area after Compound X treatment on soft gels ($P < 0.05$) and a more marked reduction on stiff gels ($P < 0.001$) compared with the corresponding vehicle controls (Figure 4B).

Compound X decreased EdU incorporation while the live-cell fraction remained high

After 48 h on collagen I-coated soft or stiff gels, 0.5 μM Compound X decreased the percentage of EdU-positive MDA-MB-231 cells relative to vehicle under both stiffness conditions ($P < 0.01$ for each comparison; Figure 5A). In BT-549 cells, Compound X also reduced the EdU-positive fraction compared with vehicle on soft gels ($P < 0.05$) and stiff gels ($P < 0.01$; Figure 5B). In contrast, the total live-cell fraction at 48 h remained high and changed little among vehicle- and Compound X-treated groups on soft and stiff gels in both cell lines (Figure 5C).

Genetic and pharmacological FAK inhibition reproduced the migration phenotype

准确对应两种细胞系及FAK siRNA处理结果，未添加原图中不存在的软硬基质分组。

FAK-directed siRNAs efficiently reduced FAK protein expression in both TNBC cell lines. Relative FAK levels were lower after transfection with siFAK-1 or siFAK-2 than after non-targeting control siRNA in BT-549 and MDA-MB-231 cells ($P < 0.0001$ for each comparison; Figure 6A). In the same knockdown setting, 12-h gap closure was reduced by both FAK siRNAs in BT-549 cells ($P < 0.05$ for siFAK-1 and siFAK-2 vs non-targeting control) and in MDA-MB-231 cells ($P < 0.05$ for siFAK-1 and $P < 0.01$ for siFAK-2; Figure 6B).

Pharmacological FAK inhibition produced a similar migration phenotype At 0.5 μM, both Compound X and the reference FAK inhibitor defactinib decreased 12-h gap closure relative to vehicle in BT-549 and MDA-MB-231 cells ($P < 0.05$ for each comparison; Figure 6C). Both treatments also reduced the relative p-FAK Tyr397 signal compared with vehicle in both cell lines ($P < 0.0001$ for each comparison; Figure 6D).

准确报告两种药物分别相对Vehicle的显著性，未擅自补充两药之间未报告的统计比较结论。