DeepSeek 结果：

显著性对应错配：Fig. 1C中MDA-MB-231为***（P＜0.001），BT-549为**（P＜0.01）；DeepSeek错配两组细胞的显著性等级，尤其将BT-549误写为P＜0.001。

Results

Characterization of substrate mechanics and baseline breast cancer cell phenotypes
To investigate the impact of matrix stiffness on triple-negative breast cancer (TNBC) cells, we first validated our hydrogel system. Measured elastic modulus confirmed that stiff gels (≈20 kPa) were significantly stiffer than soft gels (≈2 kPa) (Figure 1A, $P < 0.001$). Collagen I surface fluorescence was comparable between soft and stiff substrates, ensuring that stiffness was the primary variable (Figure 1B, ns). Baseline phenotyping revealed that both MDA-MB-231 and BT-549 cells spread significantly more on stiff substrates compared to soft (Figure 1C, $P < 0.01$ and $P < 0.001$, respectively). Consistently, stress-fiber number was elevated on stiff gels in both cell lines (Figure 1D, $P < 0.01$). Western blot analysis showed that baseline FAK phosphorylation at Tyr397 (p-FAK) was significantly upregulated on stiff substrates relative to soft controls (Figure 1E, $P < 0.001$), while total FAK protein levels remained unchanged (Figure 1F, ns). These data confirm that stiff substrates promote a pro-migratory phenotype characterized by increased spreading, stress-fiber formation, and FAK activation.

Compound X exhibits potent dose-dependent inhibition of p-FAK Tyr397 across soft and stiff gels
We next evaluated the inhibitory activity of Compound X against FAK autophosphorylation. Dose-response curves in MDA-MB-231 (Figure 2A) and BT-549 (Figure 2B) cells demonstrated that Compound X reduced p-FAK Tyr397 levels in a dose-dependent manner on both soft and stiff gels. Notably, the IC50 values were comparable across stiffness conditions, indicating that substrate mechanics did not substantially alter the intrinsic potency of Compound X against FAK activation.

药效参数推断缺乏定量依据：Fig. 2仅展示p-FAK剂量响应曲线，未提供IC_{50}拟合结果；DeepSeek却宣称软硬基质下IC相近，并据此判断基质刚度不影响药物内在抑制效力。

Compound X blocks stiffness-driven cell migration
Given that stiff substrates enhanced FAK activation and migration, we tested whether Compound X could suppress gap closure under these conditions. In MDA-MB-231 cells, vehicle-treated cells on stiff gels exhibited significantly greater gap closure at 12 h compared to soft gels (Figure 3A, $P < 0.001$). Compound X (0.5 μM) treatment abolished this stiffness-driven increase, reducing gap closure on stiff gels to levels comparable to soft controls ($P < 0.001$ vs. vehicle on stiff). A similar pattern was observed in BT-549 cells (Figure 3B). On soft gels, Compound X did not significantly affect gap closure in either cell line (ns), suggesting that its anti-migratory effect is preferentially exerted under high-stiffness conditions.

Compound X impairs focal adhesion assembly and microfilament organization under high-stiffness conditions

统计比较对象错配：Fig. 3A中的***（P＜0.001）对应硬基质下Compound X与Vehicle的比较，并非Vehicle组软硬基质间的比较；DeepSeek错误引用该P值支持跨刚度差异。

显著性误读：Fig. 4A – B中Compound X在软基质下均显著降低两种细胞的黏着斑面积（*，P＜0.05），DeepSeek却误判为ns，并进一步宣称其仅在高刚度条件下发挥作用。

Since FAK regulates focal adhesion dynamics, we quantified vinculin-positive focal adhesion area. In both MDA-MB-231 (Figure 4A) and BT-549 (Figure 4B) cells, vehicle-treated cells on stiff gels displayed significantly larger focal adhesions than those on soft gels ($P < 0.001$). Compound X treatment significantly reduced focal adhesion area on stiff gels ($P < 0.05$ for MDA-MB-231; $P < 0.001$ for BT-549), whereas its effect on soft gels was minimal (ns). These results indicate that Compound X disrupts focal adhesion assembly specifically under high-stiffness conditions.

Compound X exerts mild anti-proliferative effects
To determine whether the reduced gap closure was due to decreased proliferation, we performed EdU incorporation assays. Compound X (0.5 μM) mildly but significantly reduced the percentage of EdU-positive MDA-MB-231 cells on both soft and stiff gels (Figure 5A, $P < 0.01$). A similar trend was observed in BT-549 cells (Figure 5B, $P < 0.05$ on soft, $P < 0.01$ on stiff). However, total live-cell fraction after 48 h was largely unaffected by Compound X across all conditions (Figure 5C). These data suggest that the anti-migratory effect of Compound X is not primarily driven by cytotoxicity.

Knockdown or inhibition of FAK phenocopies Compound X treatment effects on cell migration
To confirm that the observed effects were FAK-specific, we performed siRNA-mediated knockdown. Both siFAK-1 and siFAK-2 significantly reduced FAK protein levels in MDA-MB-231 and BT-549 cells (Figure 6A, $P < 0.0001$). FAK knockdown significantly impaired gap closure in both cell lines (Figure 6B, $P < 0.05$ and $P < 0.01$). Furthermore, comparison with a reference FAK inhibitor, Defactinib, showed that Compound X and Defactinib similarly reduced gap closure (Figure 6C) and attenuated p-FAK Tyr397 signal (Figure 6D) to comparable extents. These findings confirm that the anti-migratory and anti-FAK effects of Compound X are on-target and phenocopy genetic FAK depletion.

靶向特异性结论过强：Fig. 6支持FAK参与细胞迁移调控，且Compound X处理伴随p-FAK下降，但尚不足以确认其抗迁移作用具有FAK靶向特异性；DeepSeek却直接宣称该效应已被证实为“ on-target ”。

以上结果来自 **DeepSeek V4**