

Confined Migration-Derived Breast Cancer Sublines Reveal Subtype-Specific Responses to Arp2/3 Inhibition

Zain Nofal, Margarita Pustovalova, and Sergey Leonov.

Institute of Future Biophysics, Moscow Institute of Physics and Technology (MIPT) 141701 Dolgoprudny, Russia

INTRODUCTION & AIM

Metastasis is the lethal hallmark of breast cancer, driven by tumor cell migration through microenvironments. This process of Confined Migration (CM) promotes aggressive, treatment-resistant phenotypes. The **Arp2/3 complex** is a critical molecular machine that powers cell movement by generating branched actin networks, making it a compelling target for anti-metastatic therapy with drugs like CK-666. While the role of Arp2/3 in migration is established, the impact of confined migration on cellular biomechanics and subsequent drug response remains unclear. Our study directly investigates how CM induces biomechanical adaptations in different breast cancer subtypes and how these physical changes correlate with a functional response to Arp2/3 inhibition.

METHODS

CM-selected sublines were established from parental triple-negative MDA-MB-231 and luminal MCF7 breast cancer cells by subjecting them to three rounds of confined migration as shown in Figure 1. Cellular biomechanics were quantified by measuring Young's modulus using Scanning Ion-Conductance Microscopy (SICM). The response to CK-666 treatment was evaluated on three fronts:

1. Cell viability was measured via SRB assay
2. Ki67+ cell percentage (proliferation)
3. Collective migration in wound healing assay

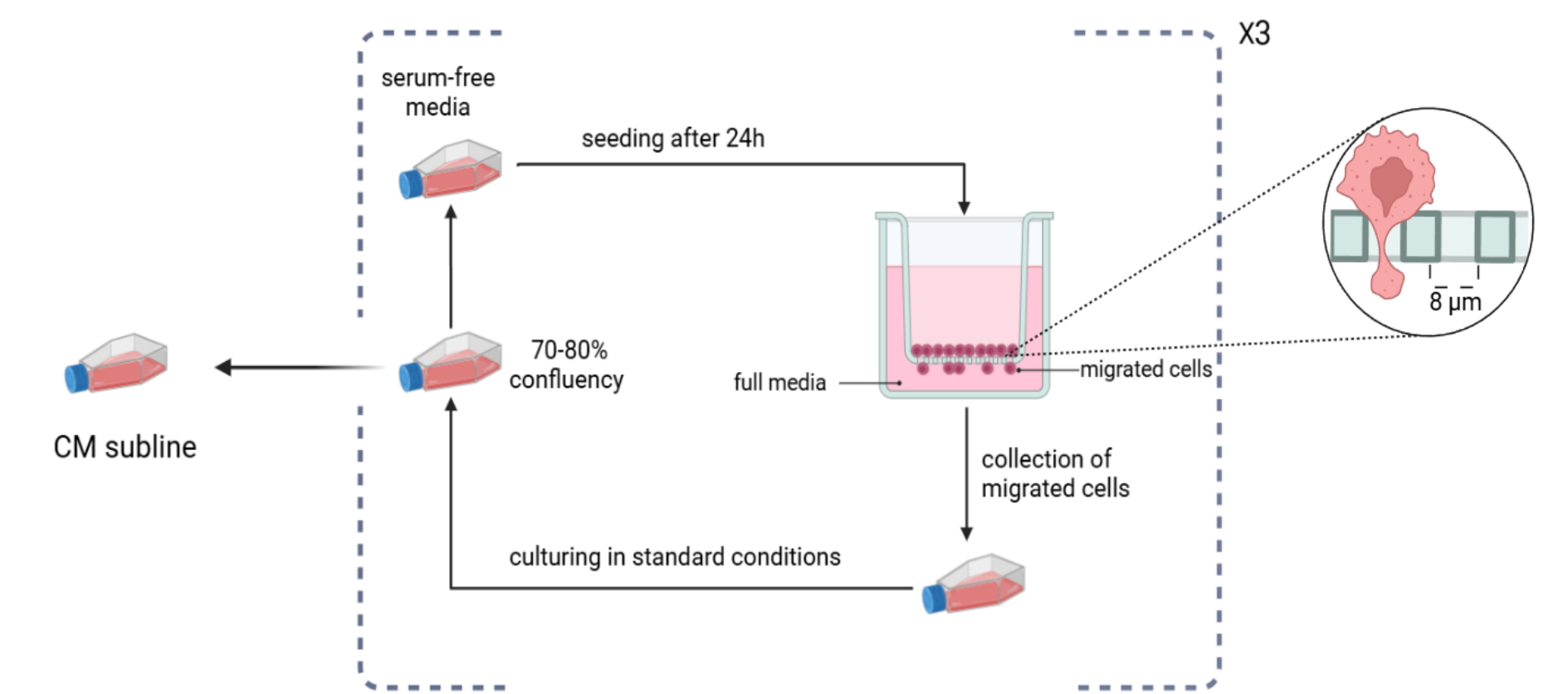


Figure 1. a schematic representation of the process of obtaining the Confined Migration (CM) sublines (adapted from Nofal et al., Sci Rep 2025)."

RESULTS & DISCUSSION

Confined Migration Induces Universal Membrane Softening

Quantification of cell mechanics via SICM revealed that confined migration significantly softened the plasma membrane in both breast cancer subtypes. CM MDA-MB-231 subline had exhibited a significant decrease in relative Young's modulus ($786.4 \text{ Pa} \pm 531 \text{ Pa}$) compared to parental cells ($1051 \text{ Pa} \pm 624.2 \text{ Pa}$). For the MCF7 subline, CM cells demonstrated significantly softened membranes ($1614 \text{ Pa} \pm 1198 \text{ Pa}$) relative to parental cells ($2446 \text{ Pa} \pm 1391 \text{ Pa}$) (Figure 2A). This indicates that biomechanical softening is a shared adaptation to CM-induced migratory stress in both subtypes.

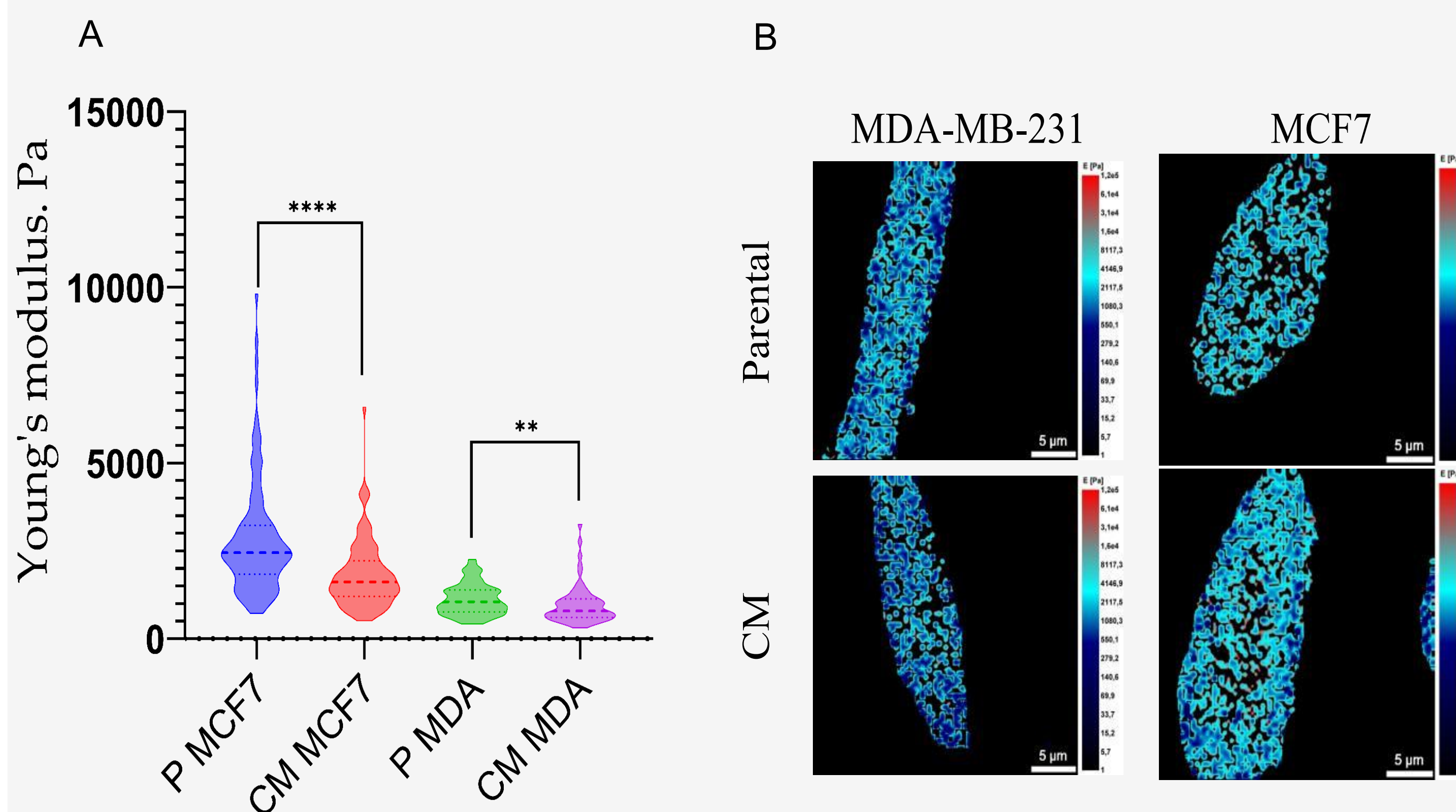


Figure 2. Violin graph showing the relative Young's modulus (A) and representative images of stiffness mapping of Parental and CM sublines of MDA-MB-231 and MCF7, measured by SICM. ** $p < 0.01$, **** $p < 0.0001$

Arp2/3 Dependence of Collective Migration Diverges Between Breast Cancer Subtypes After Confined Migration

In wound healing assay, Inhibition of Arp2/3 via CK-666 had strikingly different effects on collective migration across the CM sublines. In MDA-MB-231, CK-666 reduced scratch closure in parental cells after 24 h from $34.3\% \pm 6.3\%$ to $17.8\% \pm 5.25\%$ and completely hindered wound closure in CM cells. In contrast, CM MCF7 cells retained substantial collective mobility under CK-666 ($10.9\% \pm 1.37\%$ vs. $22.9\% \pm 3.2\%$ in control), while parental cells' wound closure decreased to $5.28\% \pm 3.8\%$ with CK-666 treatment compared to $18.3\% \pm 1.1\%$ in control. These results are consistent with a more Arp2/3-independent phenotype in CM MCF7, which supports the notion that MCF7 tend toward a drug-tolerant motile phenotype following confined migration, whereas CM MDA-MB-231 cells are shifted by Arp2/3 inhibition into a less-motile low-proliferative state.

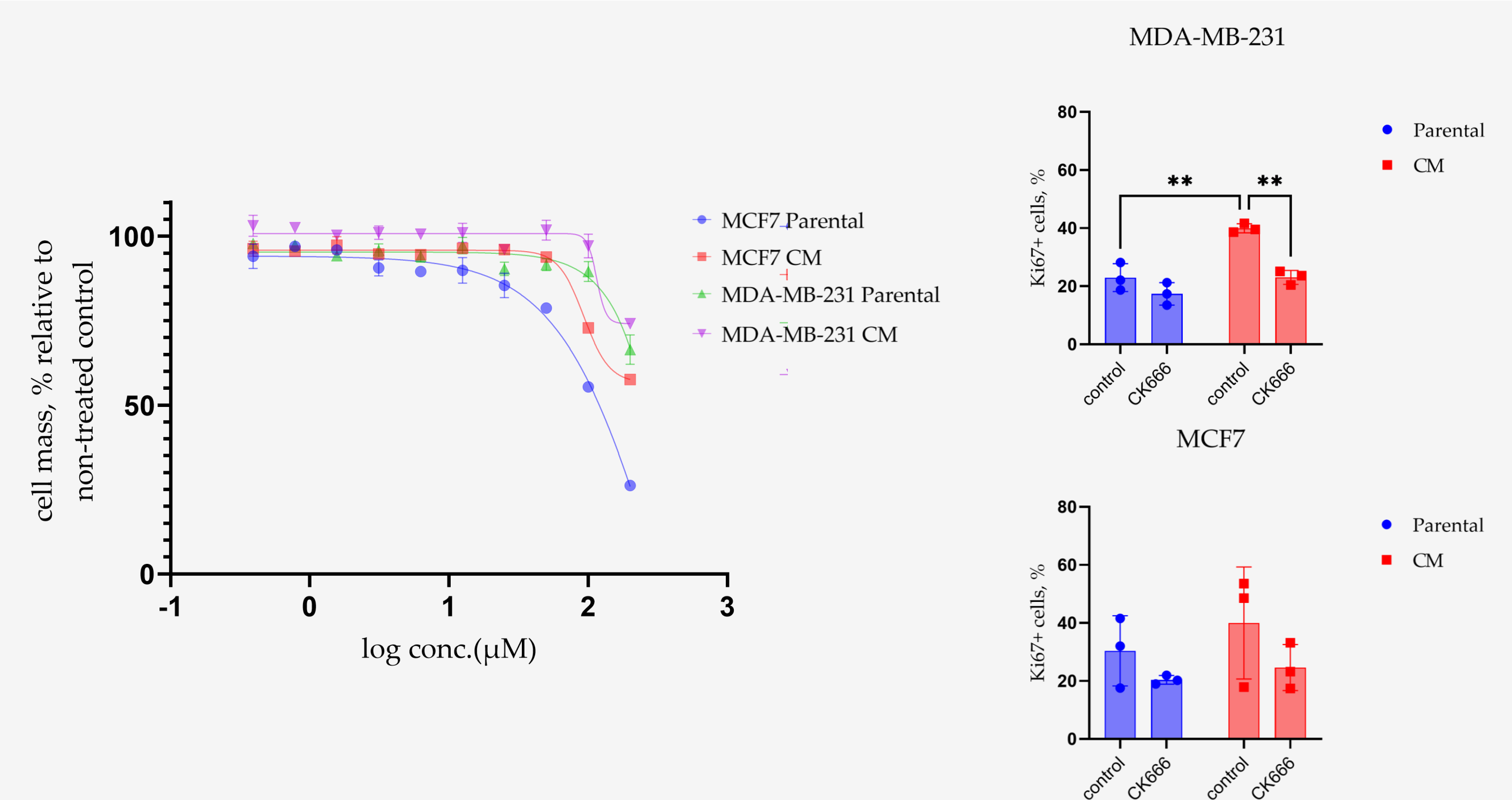


Figure 3. (left) Residual cell mass was determined by the SRB assay after 48h of exposure to CK-666. (right) Ki67+ cells fraction detected by immunofluorescent staining of parental and CM cells after 24 h of treatment with $100 \mu\text{M}$ CK-666

Response to Arp2/3 Inhibition Diverges by Subtype After Confined Migration

Viability assays following CK-666 treatment uncovered a clear divergence in response. While MDA-MB-231 cells (both parental and CM) were inherently resistant, CM-selected MCF7 cells developed significant resistance, showing a 17.5% increase in residual cell mass compared to parental MCF7 cells at a $100 \mu\text{M}$ dose (Figure 3, left).

CK-666 treatment revealed that CM MDA-MB-231, but not CM MCF7, are functionally dependent on Arp2/3 for active cycling. In CM MDA-MB-231 cells, CK-666 markedly reduced the Ki67+ fraction to $23.0\% \pm 2.4\%$ ($p = 0.0018$) vs. $39.9\% \pm 1.54\%$ in control (Figure 3, right), shifting the population towards a low-proliferative, possibly protective, state. whereas parental MDA-MB-231 cells showed little change in Ki67 under the same conditions. Meanwhile, neither parental nor CM MCF7 cells showed no marked change in the fraction of Ki67+ cells, tolerating Arp2/3 inhibition without exiting the cell cycle.

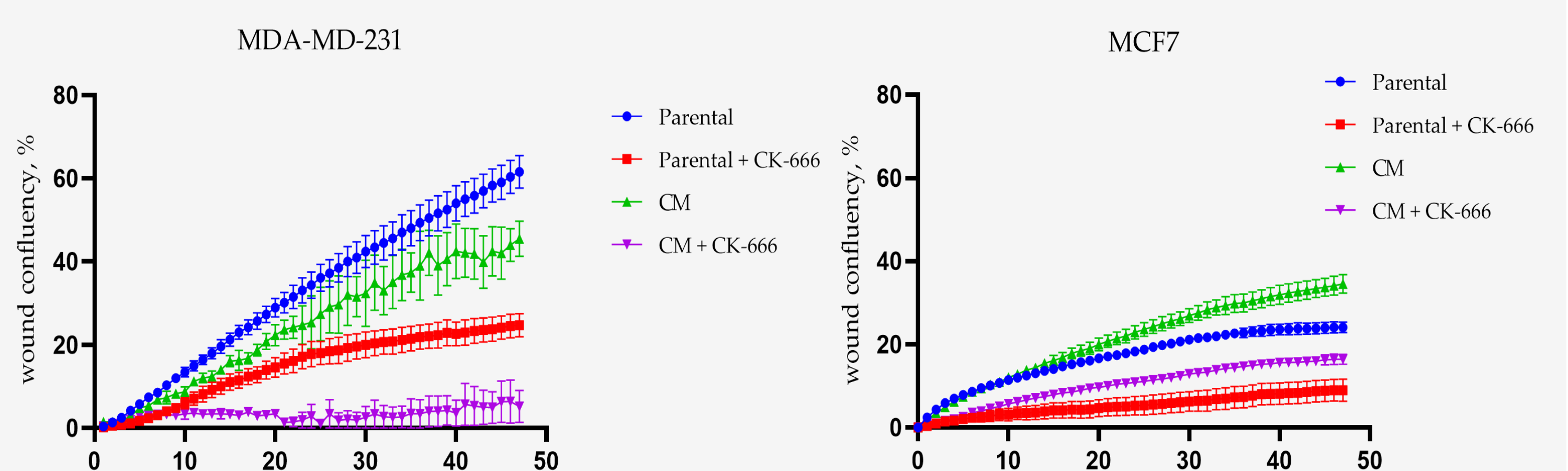


Figure 4. Wound closure percentage relative to the initial wound area with and without CK-666 treatment in serum-free media, images were taken each hour for 48 h using JuLi Stage real-time live cell imaging system placed inside a standard 37°C , $5\% \text{ CO}_2$ incubator.

Table 1. Divergent Arp2/3 dependence of CM-derived cell lines in response to CK-666.

Metric	MDA-MB-231 (CM)	MCF7 (CM)
Sensitivity to CK-666	Inherently resistant	Acquired partial resistance
Proliferation (Ki67)	Strongly reduced	unaffected
Collective Migration	Near abolished	Partly preserved

CONCLUSIONS

CM-related softening is accompanied by subtype-specific functional responses to Arp2/3 inhibition. In high metastatic MDA MB 231, CM and Arp2/3 blockade together shift cells into a viable but low-proliferative, migration-defective state. In contrast, in low metastatic MCF7, the softened CM subline preserves higher proliferation and substantial motility under CK-666, suggesting more Arp2/3 independent, mechanically adapted migration and a more drug tolerant phenotype.

The functional consequences of Arp2/3 inhibition are subtype-specific and may help guide rational selection of patients for Arp2/3-targeted anti-metastatic strategies.