

BAF mobility tunes nuclear mechanics to enable confined invasion in glioblastoma

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Normal Tumor core Infiltrative edge

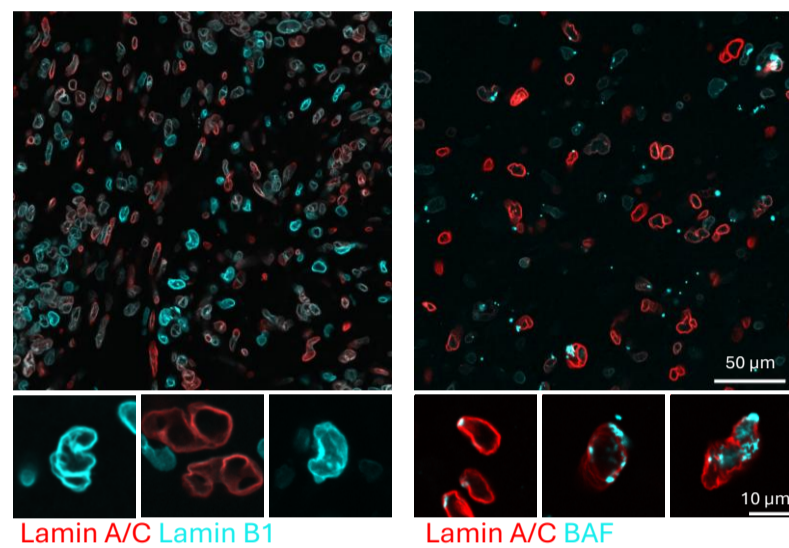
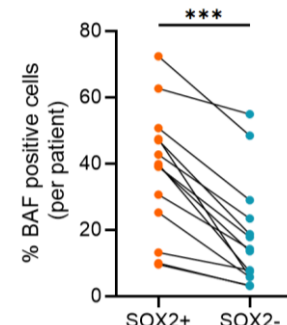
- Glioblastoma (GBM) is the most common and **aggressive primary brain tumor** in adults
- GBM cells are exposed to **extensive mechanical forces**.
- As a central mechanosensor, the **nucleus integrates mechanical inputs** into cell fate decisions.
- **Excessive mechanical forces** can lead to severe nuclear deformation and nuclear envelope (NE) rupture, promoting DNA damage and **compromising cell fitness**.

Hypothesis
Invasive GBM cells adapt their intrinsic nuclear mechanical properties to cope with the biophysical challenges of the tumour microenvironment

1. Dysmorphic GBM nuclei are prone to NE damage

Patient cryosections

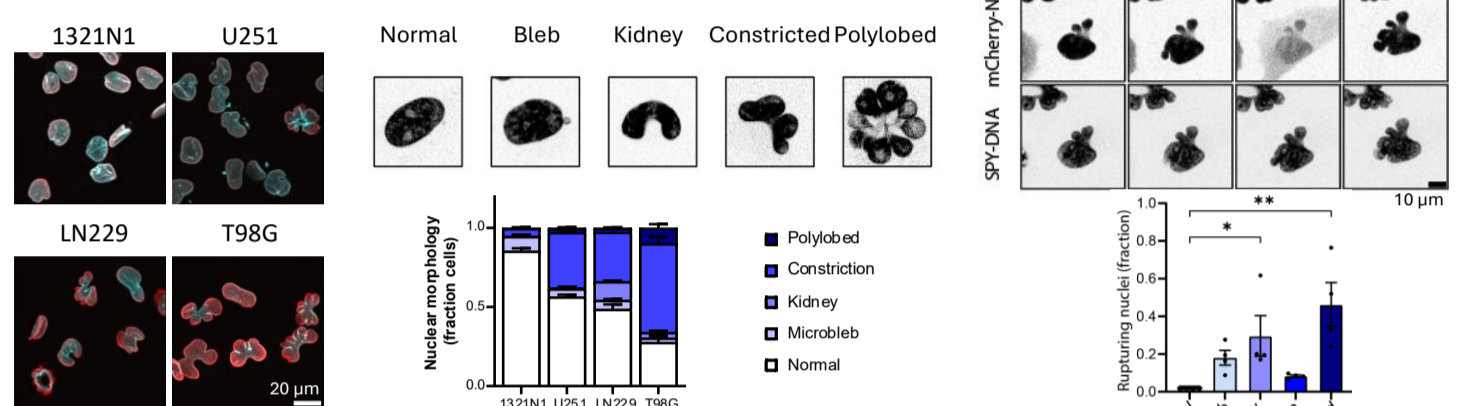
Prominent nuclear deformation and NE rupture in patient cryosections reveal **extensive mechanical stress in vivo**.



GBM cell lines

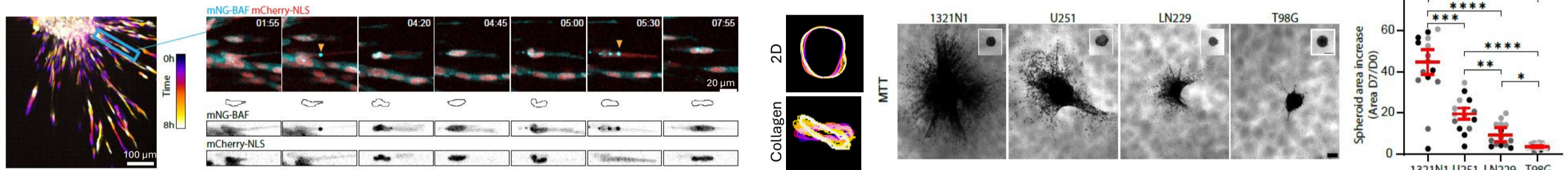
GBM cell lines have range of nuclear morphologies. Dysmorphic GBM nuclei are prone to rupture.

→ Differences in nuclear mechano-resilience between cell lines.

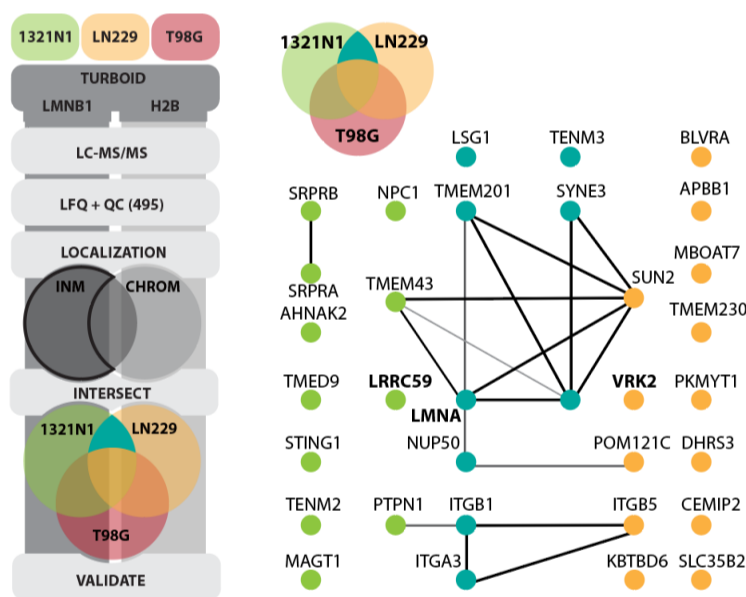


2. Mechano-resilient GBM cell lines are more invasive

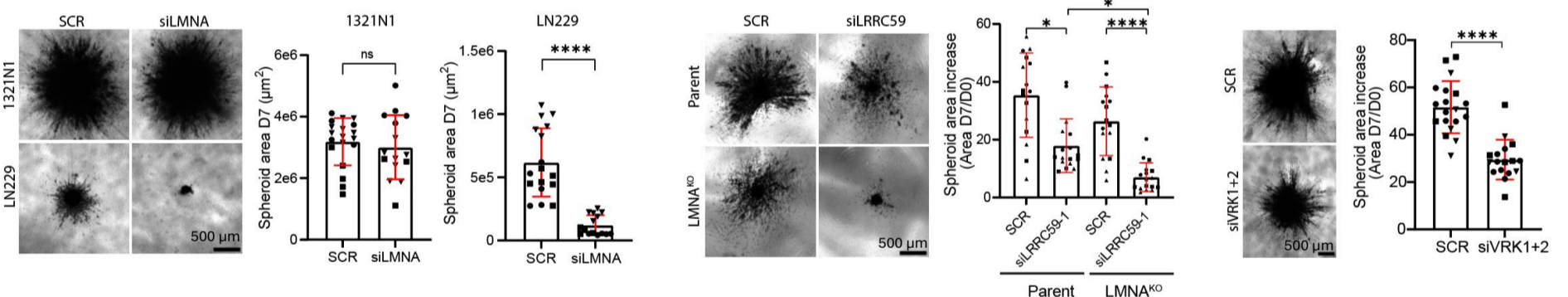
During **invasion**, nuclei are exposed to mechanical forces. Indeed, we saw that 1321N1 nuclei often adopted an **arrowhead-like shape**, accompanied by substantial **dynamic nuclear deformation**. When comparing different GBM cell lines, cell lines exhibiting the **highest mechano-resilience** (1321N1, U251) could **invade more efficiently** than cell lines with the lowest resilience (T98G).



3. Proximity proteomics identifies a NE-localized hub associated with invasive GBM



Through proximity proteomics (**TurboID**), we identified a hub of **NE-associated proteins enriched in invasive GBM cell lines**. This mechano-regulatory hub was comprised of **lamin A/C**, Leucine-Rich Repeat-Containing protein 59 (**LRRC59**), and Vaccinia-Related Kinase 2 (**VRK2**). These regulators converge on barrier-to-autointegration factor (**BAF**), a critical chromatin-NE tether. **Depletion** of these factors could indeed **decrease invasion into collagen**.



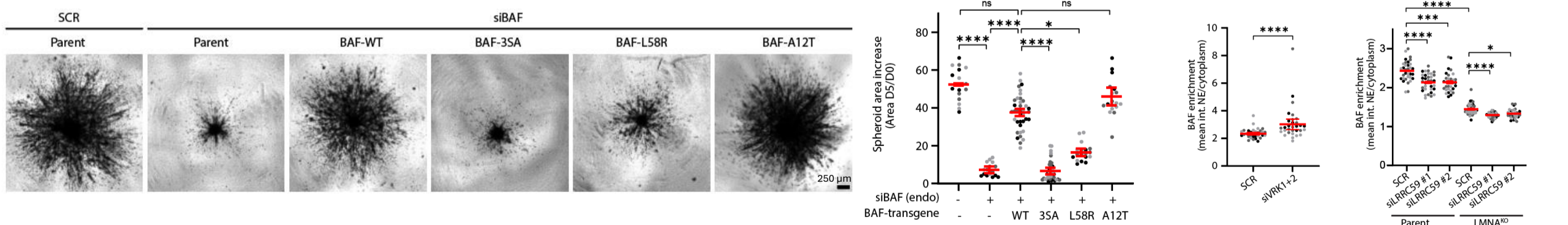
4. The invasive behaviour of GBM cells is determined by modifiers of BAF mobility

As our proteomics pointed to **BAF as a central node in GBM invasion**, we tested whether BAF itself is required for invasion. Indeed, **BAF-depletion abolished the ability** of 1321N1 cells to invade.

Next, we created **mEOS4b-BAF mutants to mimic the depletion effects of the identified regulators**:

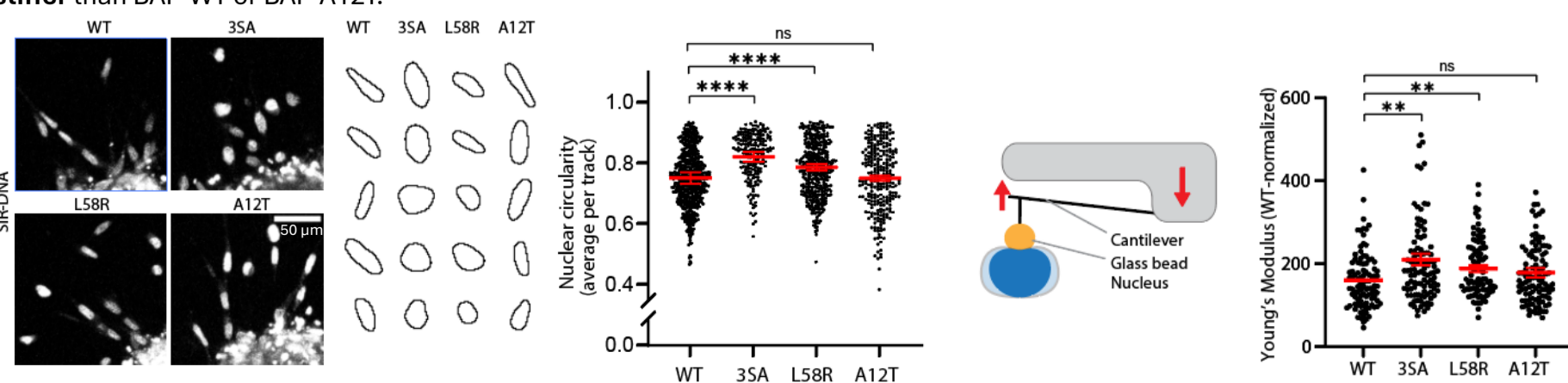
- BAF-3SA (defective in phosphorylation; mimicking VRK1/2 depletion)
- BAF-L58R (defective in binding LEM domain proteins; mimicking LRRC59 depletion)
- BAF-A12T (defective in binding lamin A/C; mimicking lamin A/C depletion)

Invasion was **strongly reduced in BAF-3SA cells**, and to a lesser extent in **BAF-L58R**. BAF-A12T cells retained their invasive capacity, similar to lamin A/C-depleted 1321N1 cells



5. Controlled BAF turnover at the NE governs nuclear resilience during GBM invasion

To see how BAF mobility impacts invasion, we examined **nuclear dynamics** during invasion. While BAF-WT cells predominantly had arrowhead or ellipsoid shapes, **BAF-3SA and BAF-L58R cells displayed more rigid, circular nuclei**. This indicates BAF-regulation is needed to control nuclear shape and deformability. **Nanoindentation** experiments revealed that BAF-3SA and BAF-L58R cells are **stiffer** than BAF-WT or BAF-A12T.



6. Conclusions

With these findings, we show:

- pervasive nuclear mechanical stress in GBM patient samples
- a direct connection between nuclear mechano-resilience and invasive capacity in a 3D collagen matrix,
- an invasion-associated, mechano-adaptive NE hub composed of LRRC59, lamin A/C, and VRK1/2, converging on the BAF NE-chromatin tether,
- BAF mobility as a key determinant of nuclear stiffness and deformability,
- dynamic BAF interactions as a crucial factor for GBM invasiveness.