UNDER PRESSURE: MECHANICAL LOAD INHIBITS CANCER CELL PROLIFERATION IN THE HEART

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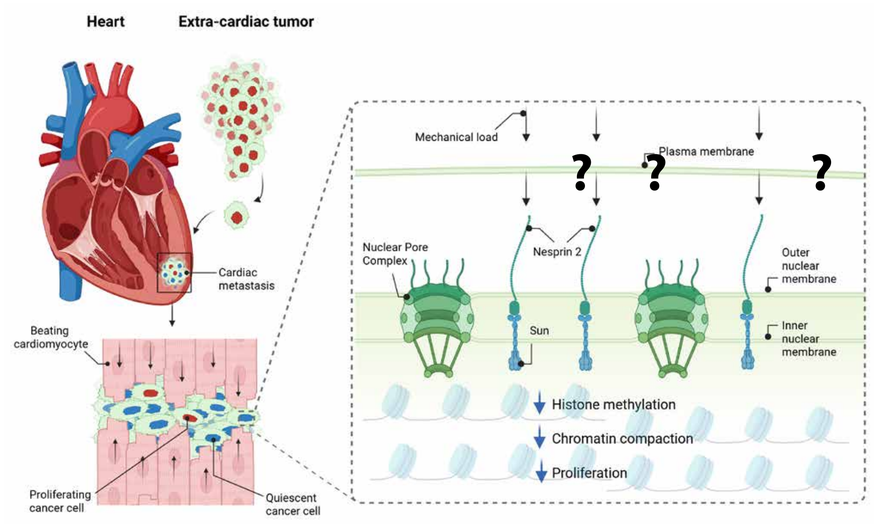
BACKGROUND

- Cardiomyocytes (CMs) actively divide during embryonic development, whereas the adult mammalian heart retains a limited regenerative potential[1-2]. Among the mechanisms potentially responsible for the loss of CM proliferative capacity CM at birth is a sudden increase in mechanical load.
- Primary cardiac tumors are rare, with a reported prevalence of 0.0017-0.028%[3].
- Cardiac metastases are also rare, with a post-mortem incidence of 1.5–20% in cancer patients[4].

PROPOSED HYPOTHESIS AND OBJECTIVE



Mechanical load suppress tumor cell growth in the heart, as it does for CMs, thereby protecting the heart from cancer.
Mechanical load induces nuclear compression via Nesprin-2, altering chromatin condensation. Here, we aim to identify the mechanotransducers transmitting this signal from the plasma membrane to the LINC complex using a pooled library screening approach.

MECHANICAL LOAD INHIBITS CANCER GROWTH IN MOUSE AND HUMAN HEARTS

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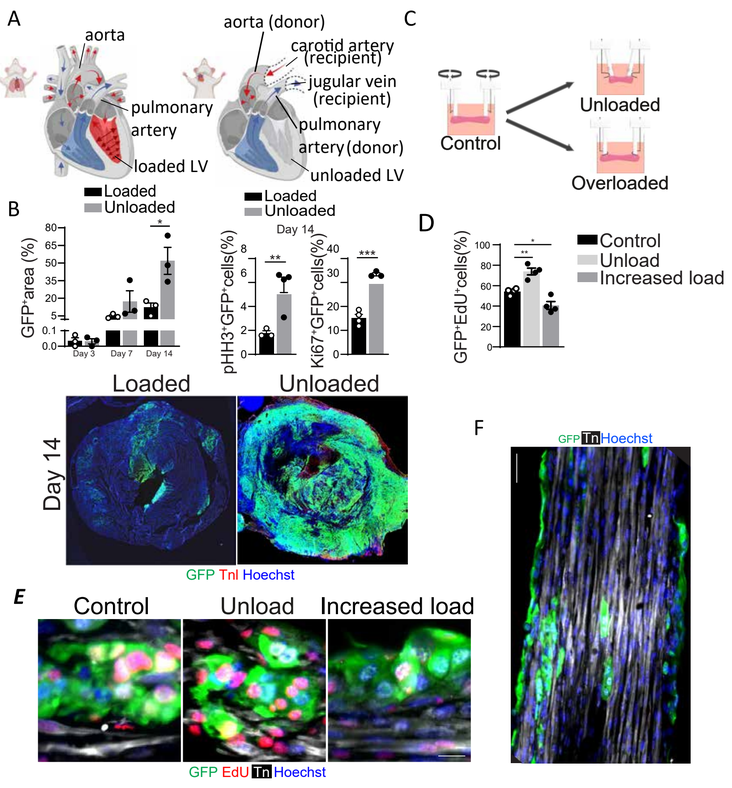
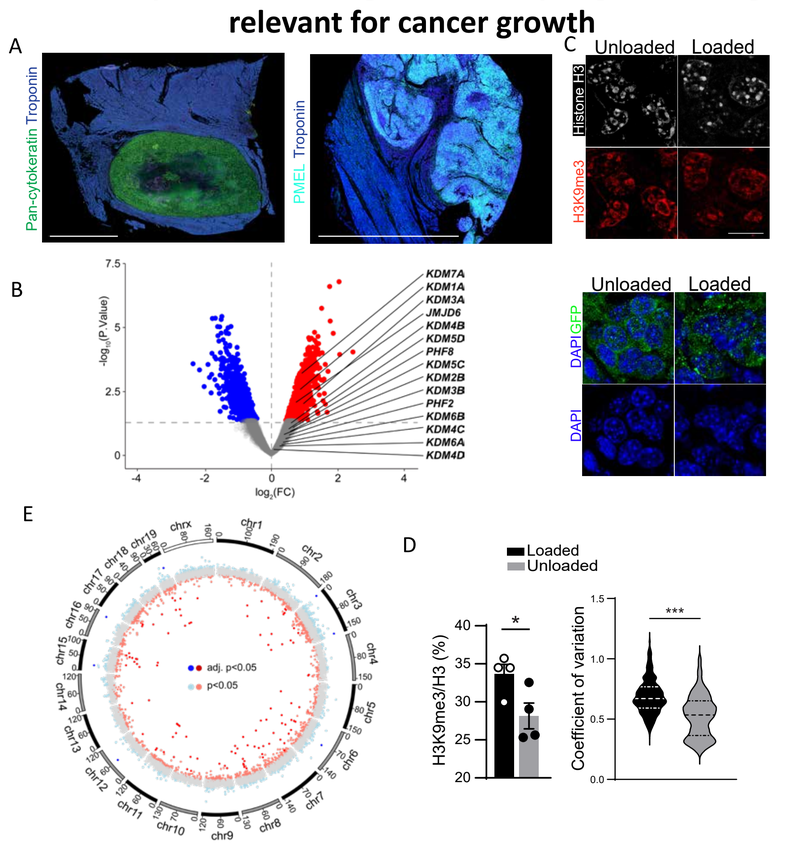
1. Cardiac mechanical load inhibits cancer growth



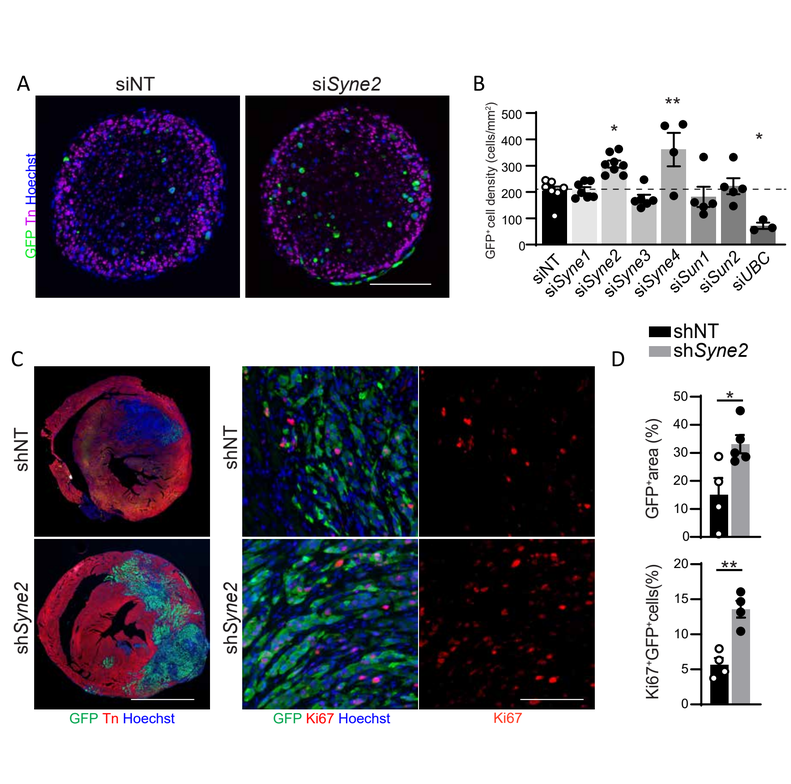
A. Heterotopic heart transplantation model[5]. **B**. GFP+ cancer cell area and proliferation in loaded vs loaded hearts at day 14. **C**. EHT loading configurations[6]. **D-F**. Cancer cell proliferation in EHTs.

2. Mechanical load reduces histone methylation and chromatin compaction, altering accessibility in genomic regions relevant for cancer growth



A. Immunofluorescence of cardiac and extra-cardiac metastases. **B.** Volcano plot derived from spatial transcriptomic of differential gene expression between cardiac and extra-cardiac metastases, highlighting upregulated histone demethylation genes. **C-D.** H3K9me3 and chromatin compaction in GFP+ cancer cells. **E.** ChIP-seq H3K9me3 peak intensity.

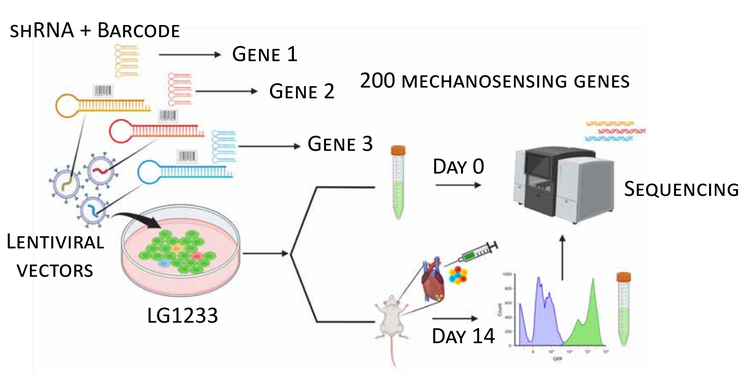
3. Nesprin-2 translates mechanical load into reduced cancer cell proliferation



A. EHT cross-sections with siSYNE2-treated GFP+ cancer cells. **B.** GFP+ cancer cell density in EHTs upon siRNA treatment. **C-D.** GFP+ cancer cell area and proliferation in hearts following shRNA-mediated knockdown.

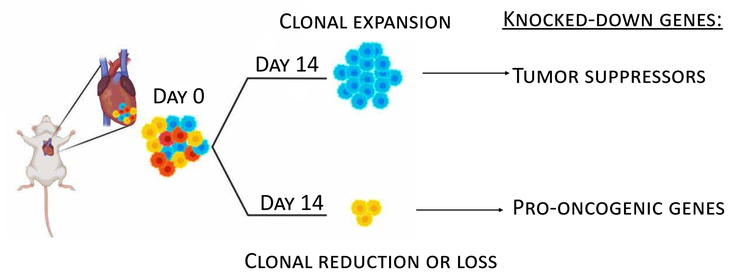
IN VIVO SCREENING OF MECHANOSENSING GENES IN THE HEART

1. Pooled genetic screening

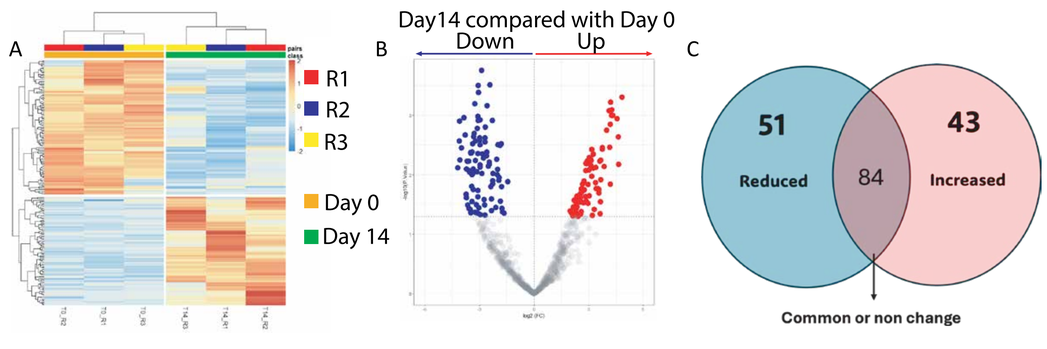


Flow chart of the experimental approach to identify molecular mechanisms responsible for the antiproliferative effect of mechanical therapy.
A **barcoded shRNA/gRNA library targeting 200 mechanotransduction genes** will be used to transduce cancer cells which will be implanted in loaded heart.

The idea behind this screen is that the downregulation of the molecules that sense mechanical stimulation and translate it into a cell proliferation arrest should make the cell unresponsive to mechanical loading .



2. Identification of mechanosensitive genes differentially enriched in cancer cells in the heart



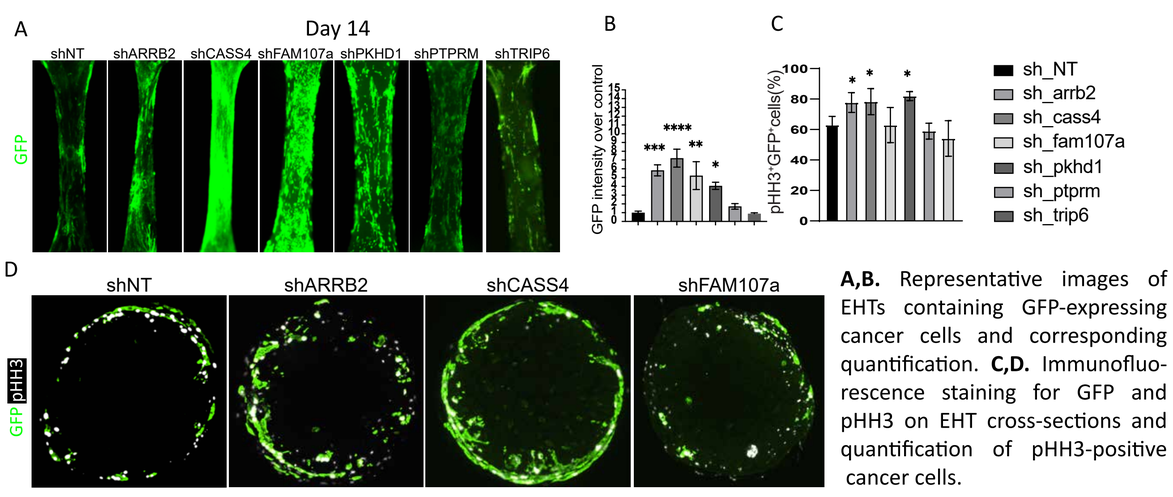
A-B. Heatmap and vulcano plot of differentially expressed genes between day 0 and day 14. **C**. Of the 200 genes included in the library, 51 were found to be downregulated and 43 upregulated at day 14 compared to day 0.

CONCLUSION

Overall, these data indicate that variations in mechanical load have a dramatic effect on the proliferation of cancer cells. Pooled shRNA library screening proves an effective strategy for identifying mechanotransducers under mechanical stimulation. Among the top hits, ARRB2, CASS4, and FAM107A are known regulators of tumor growth and invasiveness, suggesting a mechanobiological control of malignant progression. Ongoing experiments aim to dissect the molecular mechanisms by which these candidates modulate tumor cell proliferation and invasiveness in response to mechanical cues.

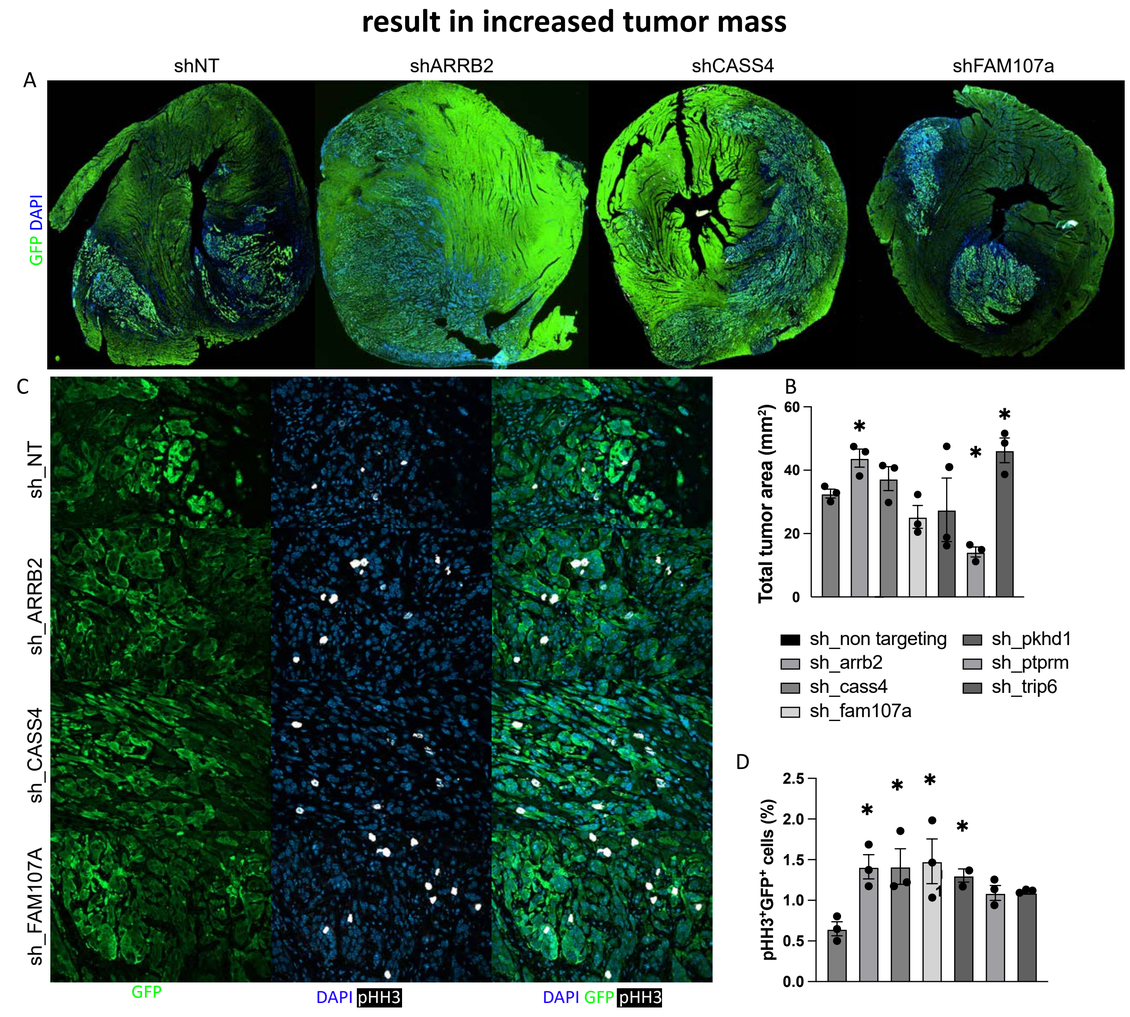
IN VITRO AND IN VIVO VALIDATION OF CANDIDATE GENES

1. Silencing of ARRB2, CASS4, FAM107A and PKHD1 promotes tumor growth in EHTs



A,B. Representative images of EHTs containing GFP-expressing cancer cells and corresponding quantification. **C,D.** Immunofluorescence staining for GFP and pHH3 on EHT cross-sections and quantification of pHH3-positive cancer cells.

2. The selected genes promote cancer cell proliferation in vivo, yet not all of them result in increased tumor mass



A, B. Representative cross-sectional images of hearts bearing tumor masses and quantification of the corresponding tumor area. **C,D**. Immunofluorescence staining for GFP and pHH3 on tumor masse in the heart and quantification of pHH3-positive cancer cells.

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