Assessing Changes in Mechanosensitivity in Fibroblast-like Synoviocytes in response to siRNA-mediated IFT88 knockdown

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Introduction

- The synovium is a connective tissue that lines the interior of the joint capsule and maintains a lubricating environment for the articulating surfaces.[1]
- Fibroblast-like synoviocytes (FLS) reside in the synovial lining, where they are exposed to the dynamic fluid environment within the knee joint.
- Physical stimuli (e.g. fluid shear) have been shown to affect FLS function; calcium signaling is a useful indicator of responses to mechanical stimuli.[2]
- Length and incidence of primary cilia, which are microtubule-based cellular organelles that extend from the cell surface, are implicated in cell mechanosensitivity.[3]
- Intraflagellar Transport Protein 88 (IFT88) is involved in cilia homeostasis, and knockdown of IFT88 has been shown to reduce primary cilia incidence.[4]

We hypothesize that IFT88 siRNA knockdown will modulate FLS mechanosensitivity to fluid shear by altering primary cilia incidence or length.

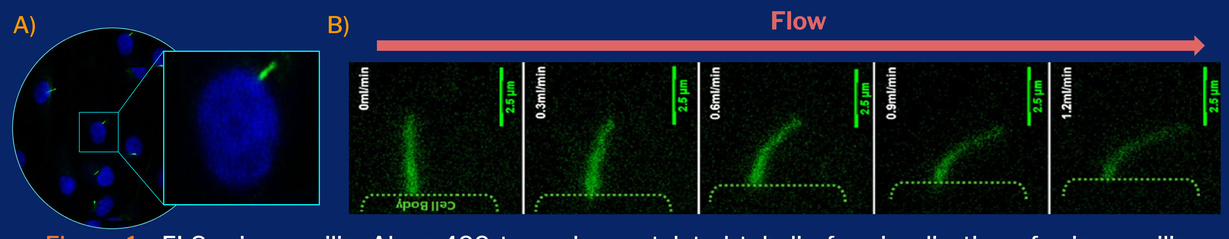


Figure 1. FLS primary cilia. Alexa-488-tagged α-acetylated tubulin for visualization of primary cilia (green, arrows), with nuclear counterstaining (blue)[5]

Methods

Cell Source: Young FLS were obtained by pooling cells from 3 cadavers (age 28.3 ± 8.3 y.o.; MTF Biologics, Edison, NJ).

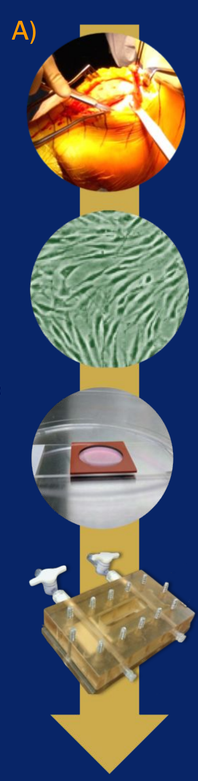
IFT88 Primer Optimization: qPCR was run on a dilution series of RNA pooled from several different cell monolayers.

IFT88 siRNA Knockdown Optimization

- FLS were plated and transfected according to the manufacturer's suggested protocol.
- IFT88 and Scramble siRNA products were transfected at 0.5, 2.5, 5, and 10 pmol siRNA per well; untransfected negative controls were also assessed.
- RNA was isolated and cDNA was synthesized prior to performing qPCR; data were analyzed via the comparative Ct method (ΔΔCt).

Fluid Shear Calcium Imaging

- Transfected and negative control FLS were plated into silicone wells on collagen-coated glass slides.
- FLS on slides were loaded with 5 µM Fura Red-AM for 40 min at 37°C.



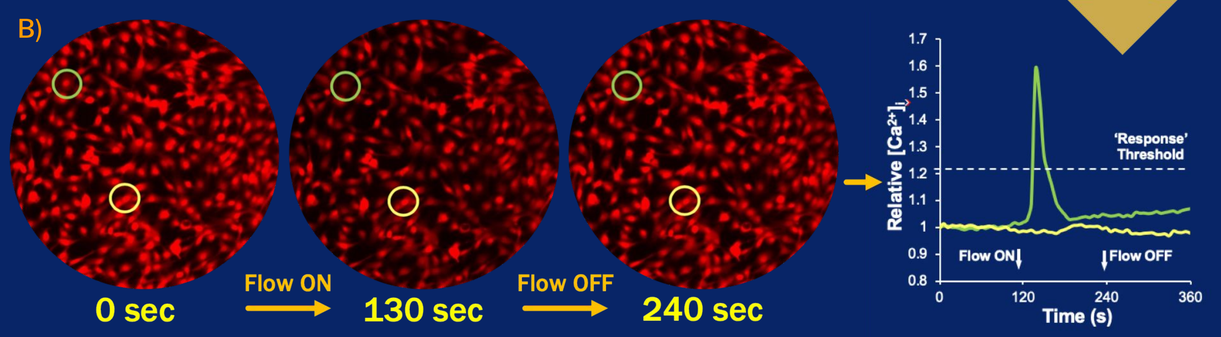


Figure 2. A) Overview of calcium imaging analysis with fluid shear B) Representative calcium measurements for responders and non-responders.[5]

Results

qPCR Standard Curves validated IFT88 Primer Two as most efficient.

- Primer one was determined to have an efficiency of 106.44%, whereas primer two was found to be 99.87% efficient.

$$Efficiency\ (\%) = (10^{\frac{-1}{Slope}} - 1) \times 100$$

Equation 1. Primer Efficiency Equation.

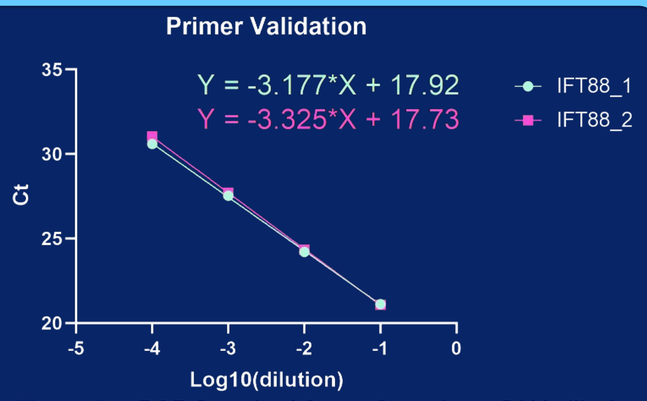
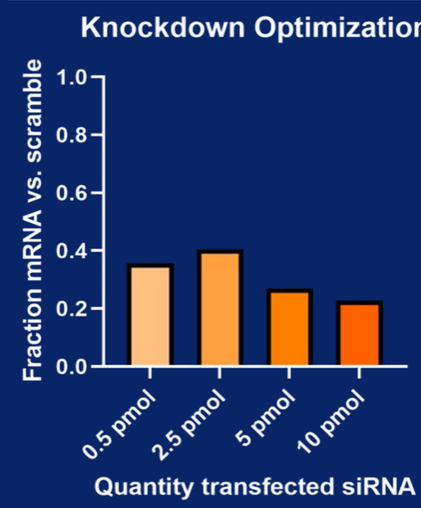


Figure 3. qPCR Standard Curve Ct vs. Log cDNA dilution of IFT88 Primer One and IFT88 Primer Two.



An optimal transfected RNA quantity of 10 pmol was found to reduce IFT88 mRNA by 77%.

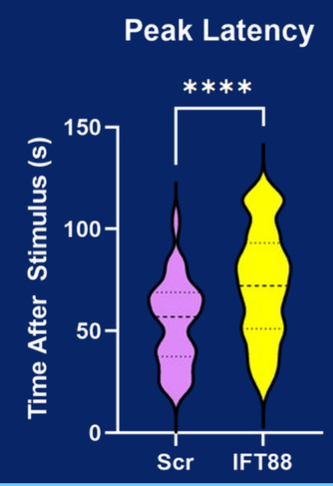
- Transfection quantities of 0.5, 2.5, and 5 pmol reduced mRNA content by 66%, 60%, and 73%, respectively.

Figure 4. Proportion of IFT88 mRNA remaining for various siRNA transfection quantities.

Peak Latency was significantly higher among IFT88 Knockdown FLS.

- Peak Latency between Scramble siRNA and IFT88 siRNA KD FLS differed by 29.66% (54.22s vs. 73.11s, $p < 0.0001$).
- There was no significant difference in peak magnitude or percent responders between Scramble siRNA and IFT88 siRNA KD FLS.

Figure 5. Peak Latency (s) seen in response to fluid shear in IFT88 siRNA knockdown FLS and Scramble siRNA FLS.



Conclusions

- Mechanosensation is mediated by primary cilia[3], and IFT88 plays an essential role in generation of the primary cilia[4].
- IFT88 knockdown has been used to decrease the expression of primary cilia, and we have demonstrated substantial knockdown of IFT88 in our study.
- IFT88 knockdown in FLS alters mechanosensitivity, specifically by increasing the time required for stimulated cells to produce a calcium transient (peak latency).
- It is likely that the altered mechanosensitivity is due to changes in cilia incidence or length, though that cannot be concluded directly from this study.
- Future studies will aim to correlate cilia properties with mechanosensitivity in FLS.
- Understanding how the synovium senses and responds to its mechanical environment can inform future studies of joint diseases and potential therapies.

References

[1]Hui + *Wiley Interdiscip Rev Syst Biol Med*, 2011; [2]Estell + *J Biomech*, 2017; [3]Oh + *Cell Metabolism*, 2015; [4]Huangfu + *Nature*, 2003; [5]Sakhrani + *Front. Bioeng. Biotechnol*, 2022

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