Isolated FDB muscle fibers for damage-repair assay

-The Han Lab

1. Dissect the FDB muscles tendon-to-tendon from the mouse feet.

2. Digest the muscle with 0.2% collagenase type I or type IV in PBS+1mM Ca2+ @ 37 C for 1 h with slow rotation.

3. Let the muscle sit down and decant the digestion solution, wash the muscle with fresh PBS+1mM Ca2+ twice.

- 4. Add 1ml PBS+1mM Ca2+ and gently triturate the muscles with a large open-mouth plastic pipette till you can see the muscle bundles are dissociated into single fibres.
- 5. Resuspend the fibres in fluorescein dextran (FDx, 10kDa, lysine fixable) solution.
- 6. Flush the fibres once using a 18G needle.
- 7. IMMEDIATELY put the fibre solution at 37 $\hat{A}^{\circ}C$ incubator for 2 min. (Timely)
- 8. Add equal amount of 4% PFA, at room temperature for 15 min.
- 9. Wash once with PBS

[The steps 10-11 are only needed certain antibodies to retrieve antigen]

- 10. Incubate with 100 mM Glycine in PBS for 10 min at room temperature.
- 11. Incuate in 0.05% SDS in PBS for 30 min at 50 $\hat{A}^{\circ}C$.
- 12. Wash once with PBS
- 13. Incuate with primary antibody overnight @ room temperature.
- 14. Wash three times 5 min each with PBS
- 15. Incuate with secondary antibody at room temperature for 30 min.
- 16. Wash three times 5 min each with PBS.
- 17. Confocal microscopic examination.

(The end)

