**IMMUNOFLUORESCENCE STAINING OF MOUSE MUSCLE SECTIONS**

1. Hydrate sections (slides straight from cryosection or -80°C) in PBS for 10 min.

2. Fix in 1.5% paraformaldehyde in PBS for 10 min;  
   *Note: 1.5% paraformaldehyde is dissolved in PBS in 60°C water bath; if staining for Pax7, acetone fixation is required instead. (DSHB Pax7 antibody does not always work, the one from Dennis Guttridge Lab works according to Marni Boppart, only with acetone fixation.)*

3. Block with 5% BSA/PBS for 20 min.

4. Block with goat anti-mouse IgG Fab fragment for 30 min.  
   *Note: use final conc. ~0.13 mg/mL; this is important to block off the mouse tissue Ig (very abundant in tissue sections).*

5. Wash with 1% BSA/PBS for 2 min each, 3 times.

6. Incubate in primary eMHC Ab (clone F1.652 from DSHB) for 1hr.  
   *Note: use 1: 1000 dilution of 250 µg/mL Ig stock*

7. Wash with 1% BSA/PBS for 2 min each, 3 times.


9. Wash with 1% BSA/PBS for 2 min each, 3 times;

10. Mount with coverslips with Fluorosave.