

Supplementary Fig 1: Preferential access of rhod-2 to the longitudinal tubules. Images of 500 kDa fluorescein dextran (A) and rhod-2 (B) fluorescence signal from the t-system of a skinned fibre bathed in the standard internal solution. An overlay of A and B is shown in C. Note the rhod-2 only has access to the longitudinal tubules and the signal throughout the transverse and longitudinal tubules appear much more uniform, compared to Fig 1. *SL* ~ 2.8 µm. ID: 121407d. xy pixel size: 207 nm.



Supplementary Fig 2: Stretch-induced vacuolation occurs in the longitudinal tubules. Images of 500 kDa fluorescein dextran (A) and rhod-2 (B) fluorescence signal from the t-system of a skinned fibre in standard solution following stretch. An overlay of A and B is shown in C. Note that the transverse tubules in A are not damaged, in contrast to Fig 3. *SL* ~ 3.4 μ m. ID: 121407z. xy pixel size: 414nm.



Supplementary Fig 3: Colocalisation of 3kD dextran and rhod 2 throughout the t system network. Images of 3 kDa fluorescein dextran (A) and rhod-2 (B) fluorescence signal from the t-system of a skinned fibre in standard solution. An overlay of A and B is shown in C. Note that the 3 kD dextran and rhod 2 have identical access to all elements of the t system network. *SL* ~ 2.7µm. ID: 082708a. xy pixel size: 137nm.