

Membrane damage and repair

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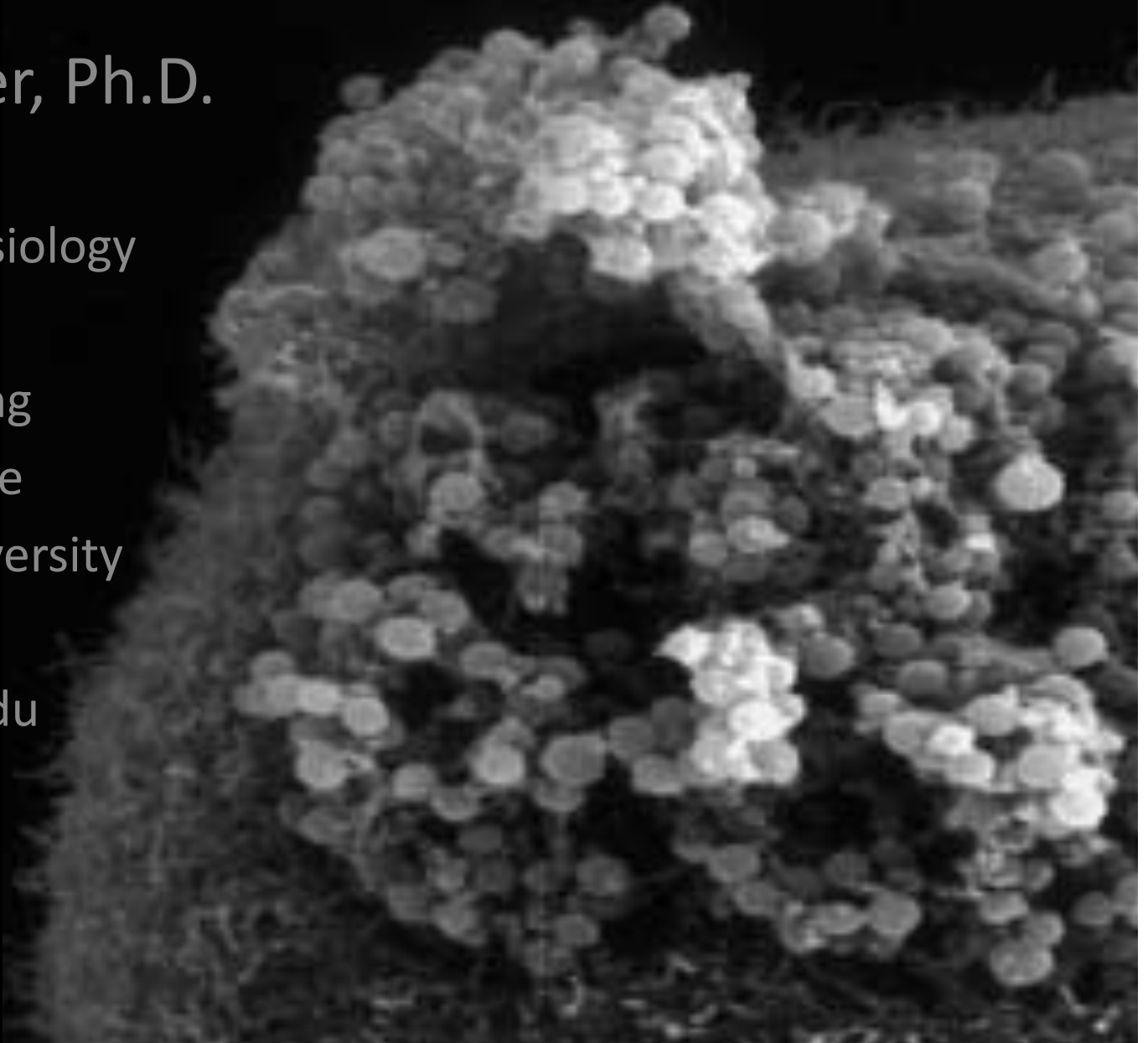
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UNIVERSITY



Topics for the class:

What is cell membrane repair?

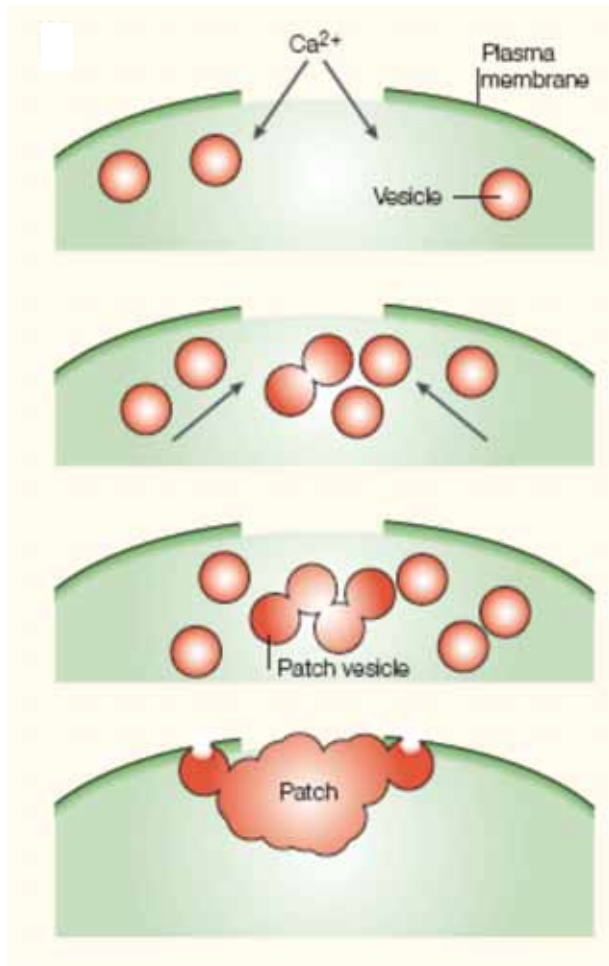
Why do cells need to actively repair their membranes?

How do cells repair the plasma membrane at the molecular level?

What are the pathologic consequences of defective membrane repair?

Can membrane repair be targeted as a therapeutic approach?

Ca²⁺-triggered patch model for membrane repair



Membrane disruption



Ca²⁺ entry through the injury site



Ca²⁺-triggering vesicle exocytosis

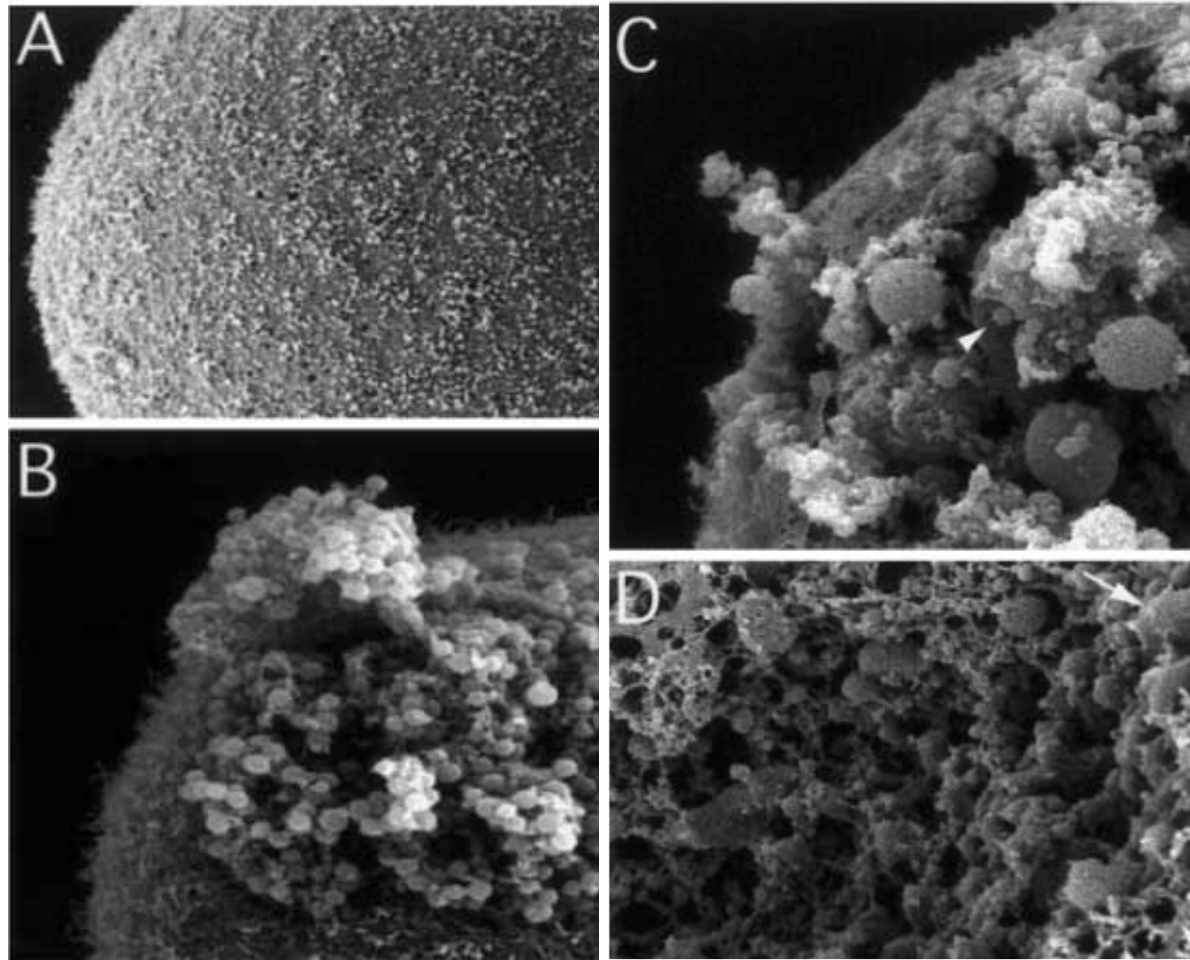


Vesicle fusion

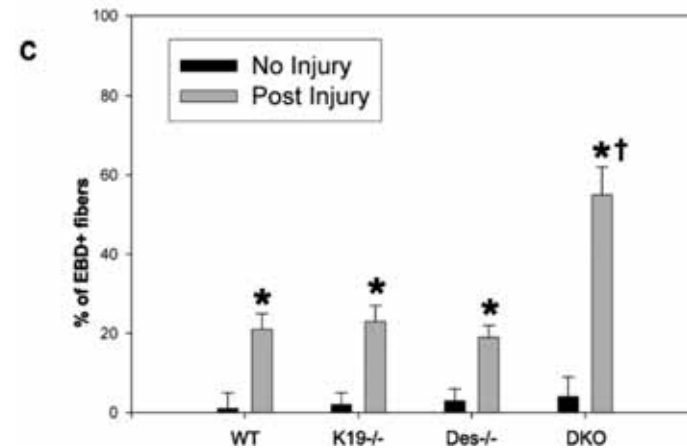
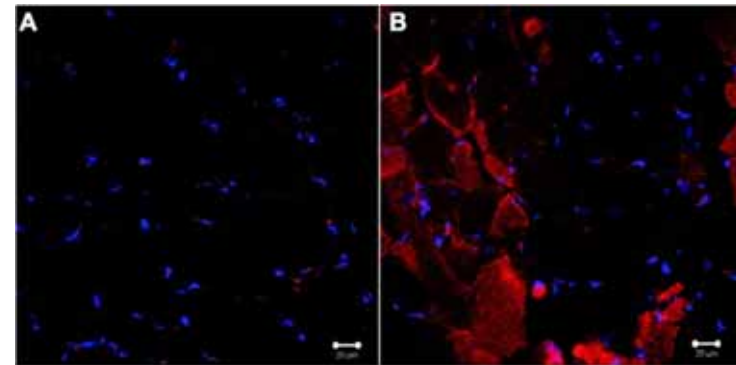
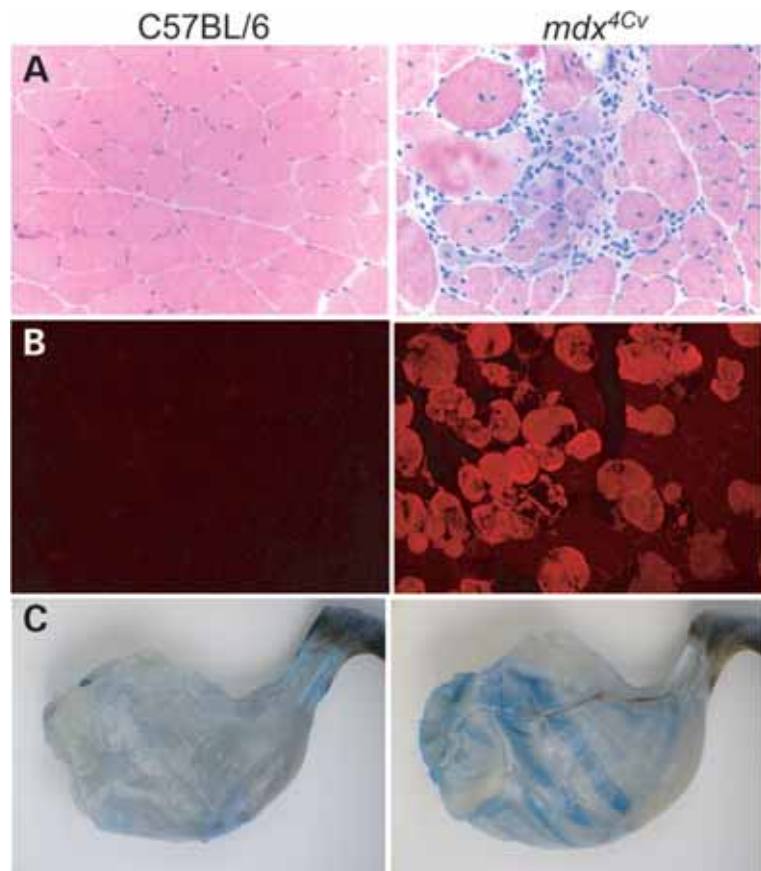


Patch formation; membrane resealing

Intracellular vesicles participate in membrane patching following damage



Evidence of membrane resealing in living mammalian cells



Methods to measure membrane repair

- Dye exclusion/entry into the cell
 - UV laser damage
 - Physical disruption of the membrane
 - Histological analysis of tissues (Evans blue, IgG entry)
- Leak of a biomarker out of the cell
 - Creatine kinase
 - Troponins
 - Lactate dehydrogenase
- Changes in membrane biophysical conditions

Membrane repair is a conserved physiologic process in multiple tissues

Organ	Type of mechanical force	Principal cells stressed	Cell wounding (proportion of cells involved)	Reference
Skeletal muscle	Aperiodic, highly variable intensity: shear, stretch	Skeletal muscle cells (myocytes)	Yes (3-20%)	McNeil and Khakee, 1992
Cardiac muscle	Cyclic: shear, stretch	Cardiac myocytes	Yes (25%)	Clarke et al., 1995
Skin	Aperiodic, highly variable intensity: shear, stretch, compression	Epidermal cells, Fibroblasts, etc.	Yes (3-6%)	McNeil and Ito, 1990
Gastrointestinal tract	Cyclic: shear, stretch	Epithelial cells, smooth muscle cells	Yes, epithelial cells (% not measured)	McNeil and Ito, 1989
Vascular (conducting)	Constant and cyclic: shear	Endothelial cells, smooth muscle cells	Yes, aortic endothelial cells (6.5%)	Yu and McNeil, 1992
Respiratory	Cyclic: stretch	Epithelial cells, endothelial cells, smooth muscle cells	Alveolar cells (2-30% in mechanically ventilated lung)	Gajic et al., 2003
Peripheral nervous	Aperiodic, highly variable intensity: shear	Inner ear cells	Yes, hair cells (% not measured)	Mulroy et al., 1998

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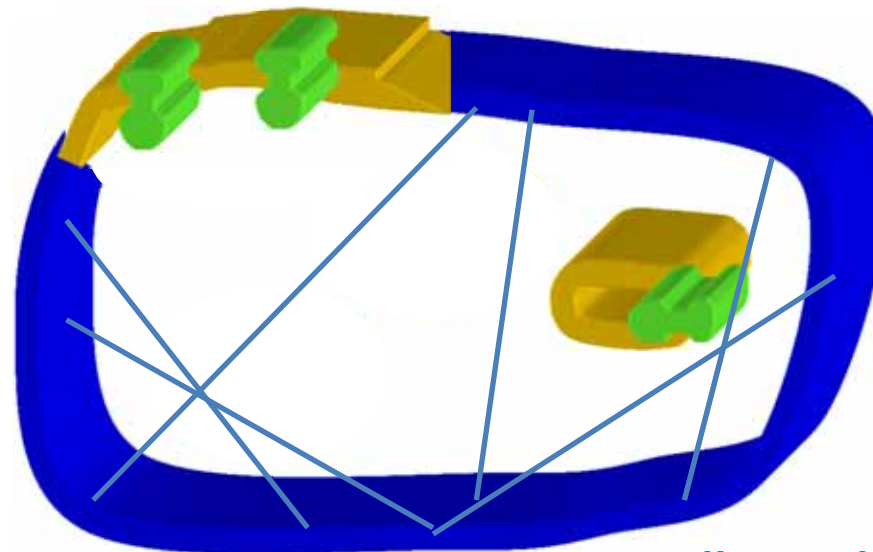
What are the pathologic consequences of defective membrane repair?

Can membrane repair be targeted as a therapeutic approach?

Necessity of membrane repair in eukaryotic cells

Membrane Patch = Cell Survival

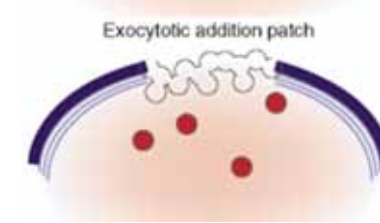
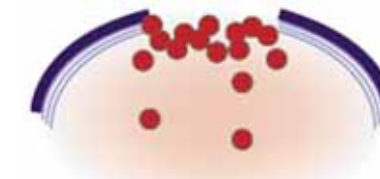
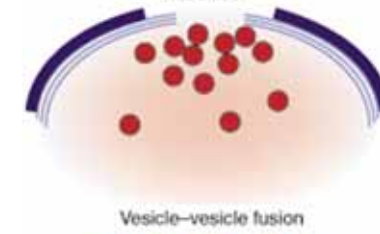
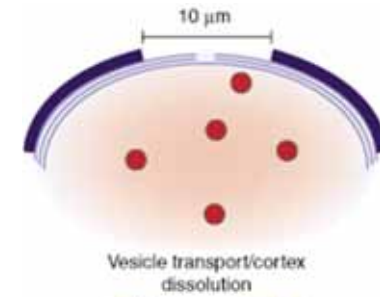
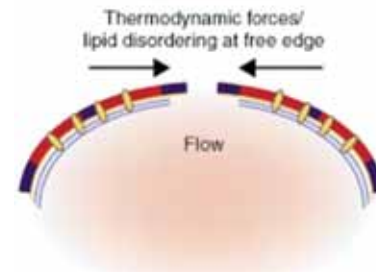
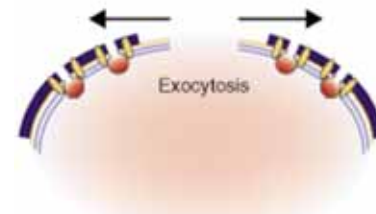
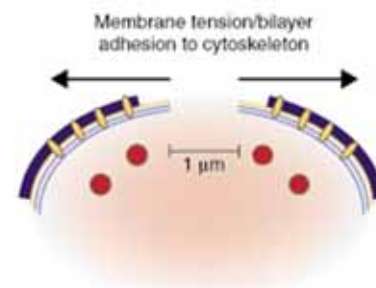
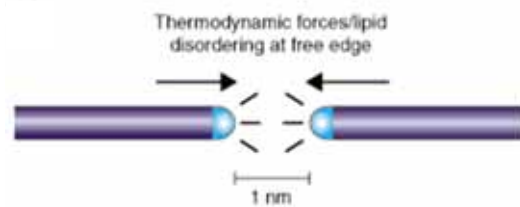
? drives
membrane repair



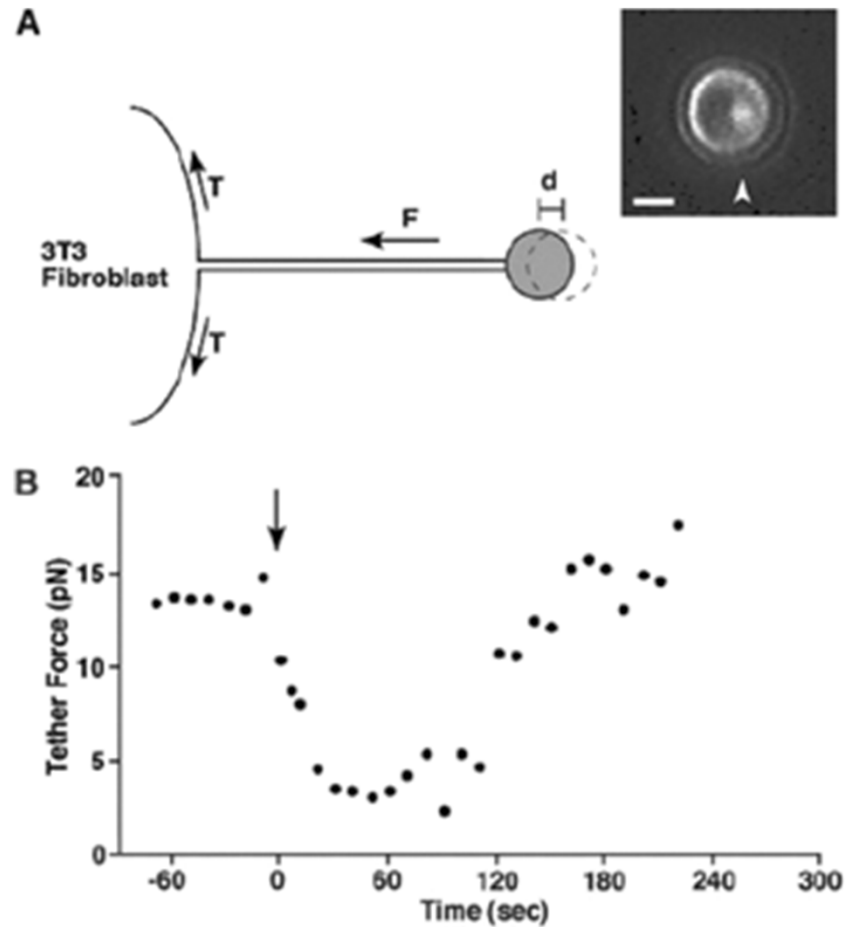
Cell Membrane

Resealing mechanism is dependent on size of membrane disruption

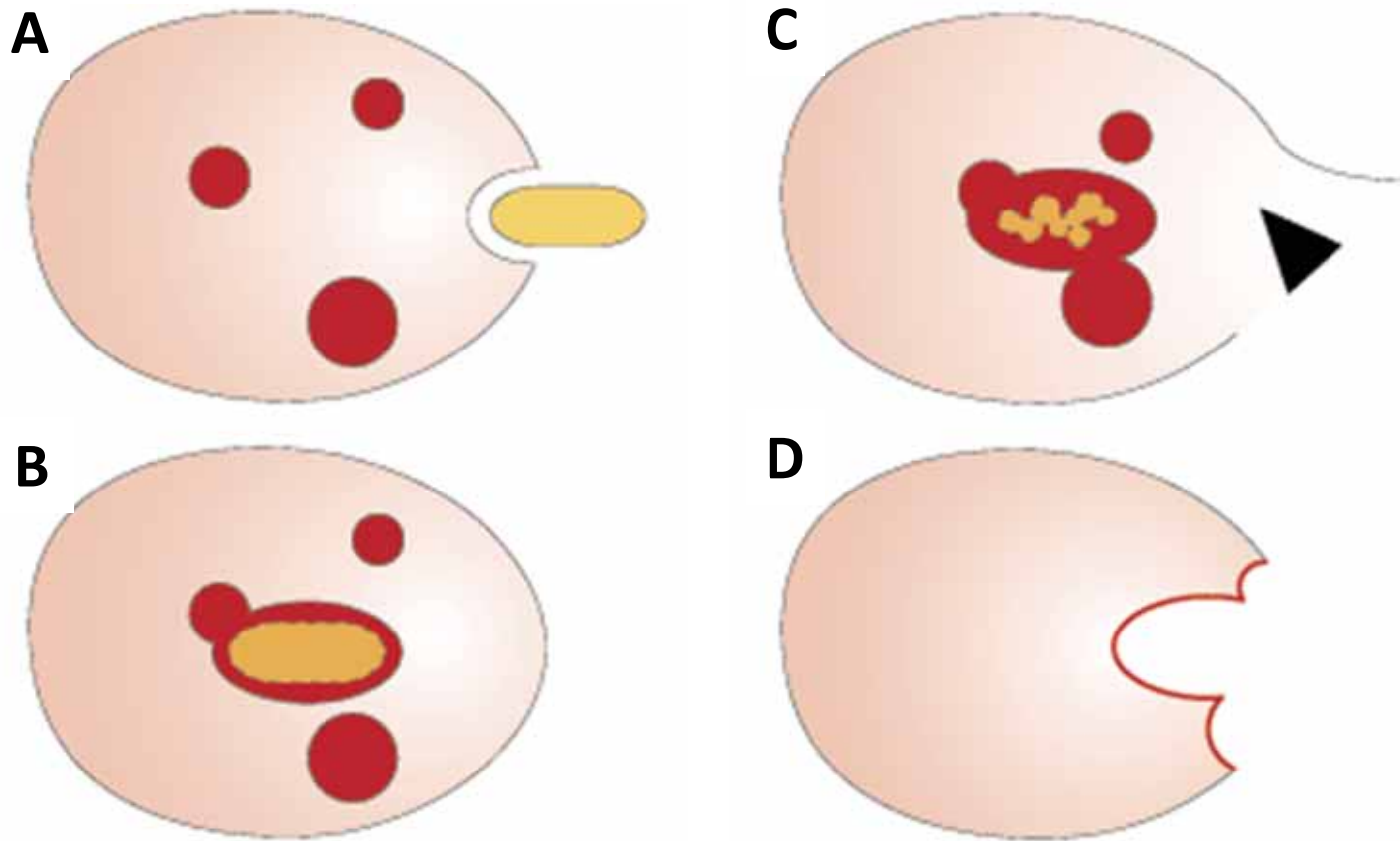
Increasing size of membrane disruption →



Membrane tension is a key determinate of cell membrane resealing



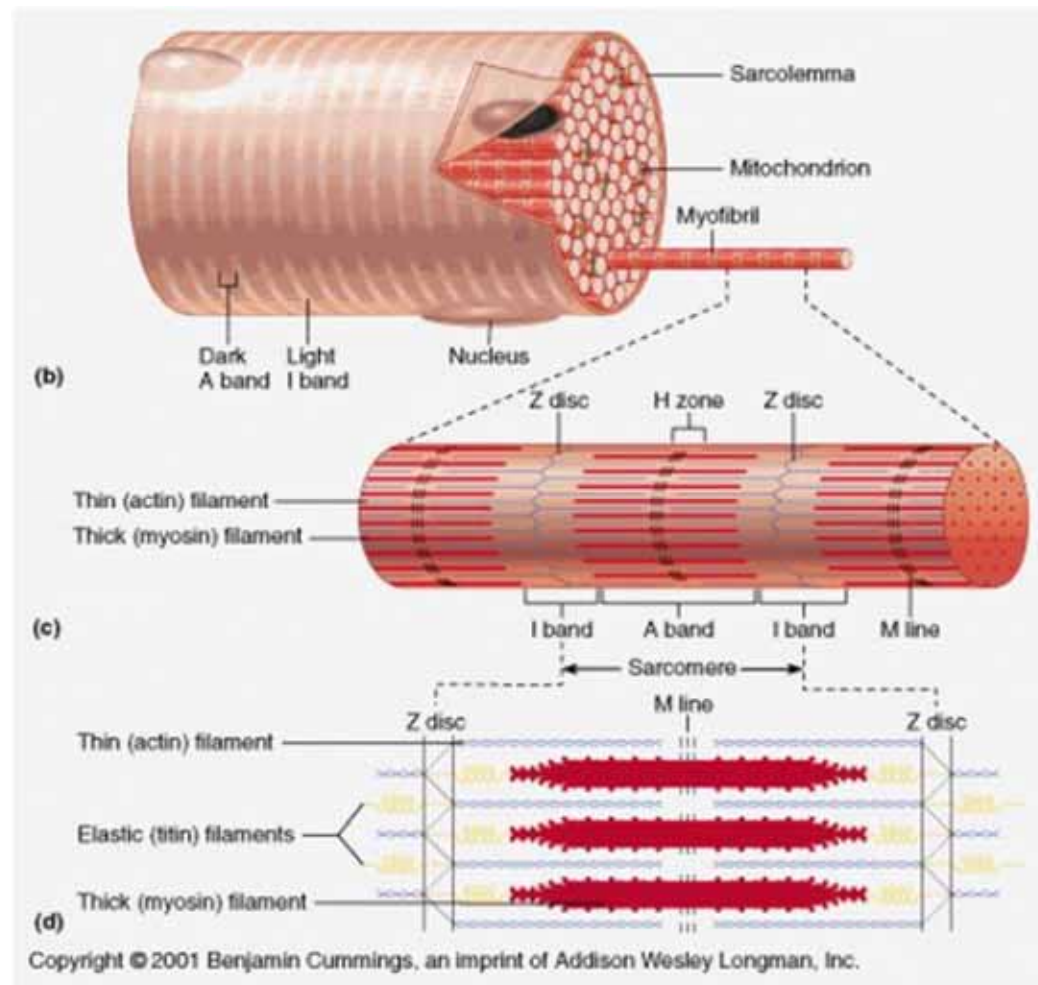
Evolution of fusion-based resealing following development of endocytotic apparatus



Skeletal muscle displays a substantial amount of membrane resealing

Organ	Type of mechanical force	Principal cells stressed	Cell wounding (proportion of cells involved)	Reference
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Contractile nature of skeletal muscles lead to extensive mechanical stress



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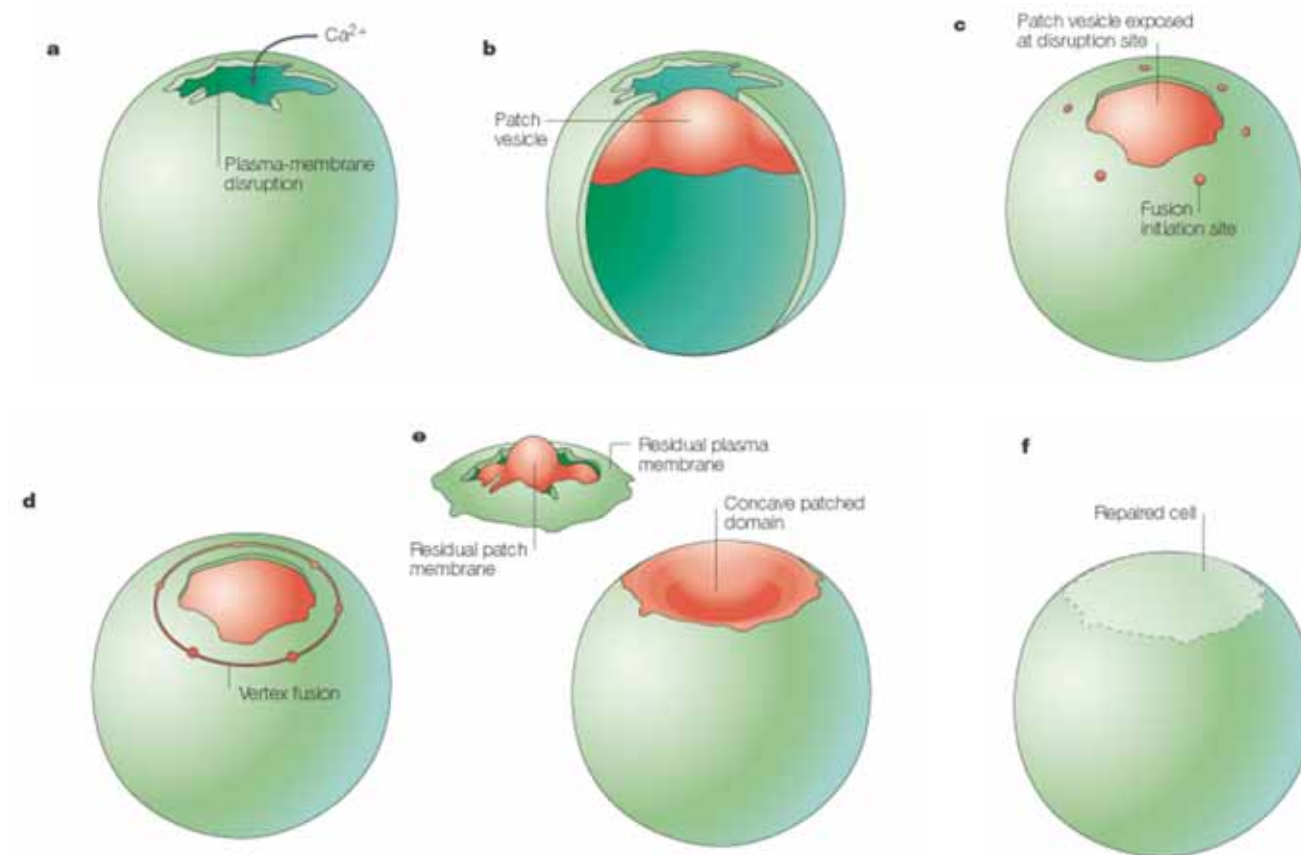
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How do cells repair the plasma membrane at the molecular level?

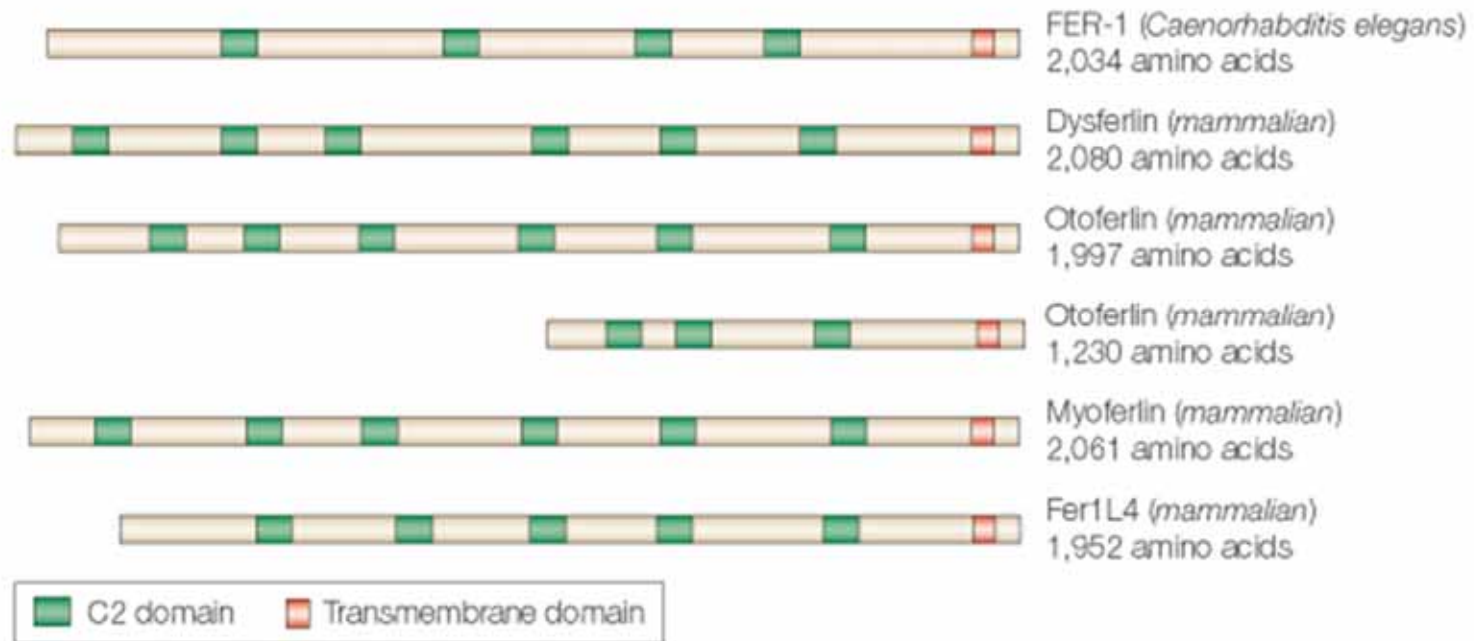
What are the pathologic consequences of defective membrane repair?

Can membrane repair be targeted as a therapeutic approach?

Cellular mechanism are better understood than the molecular mechanisms

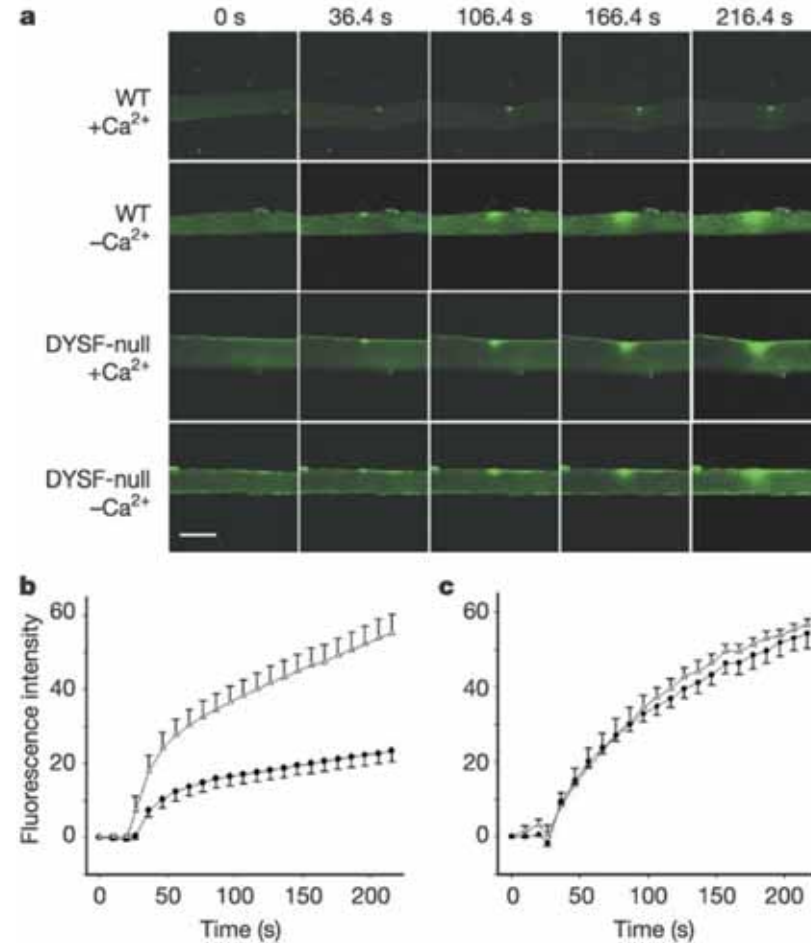
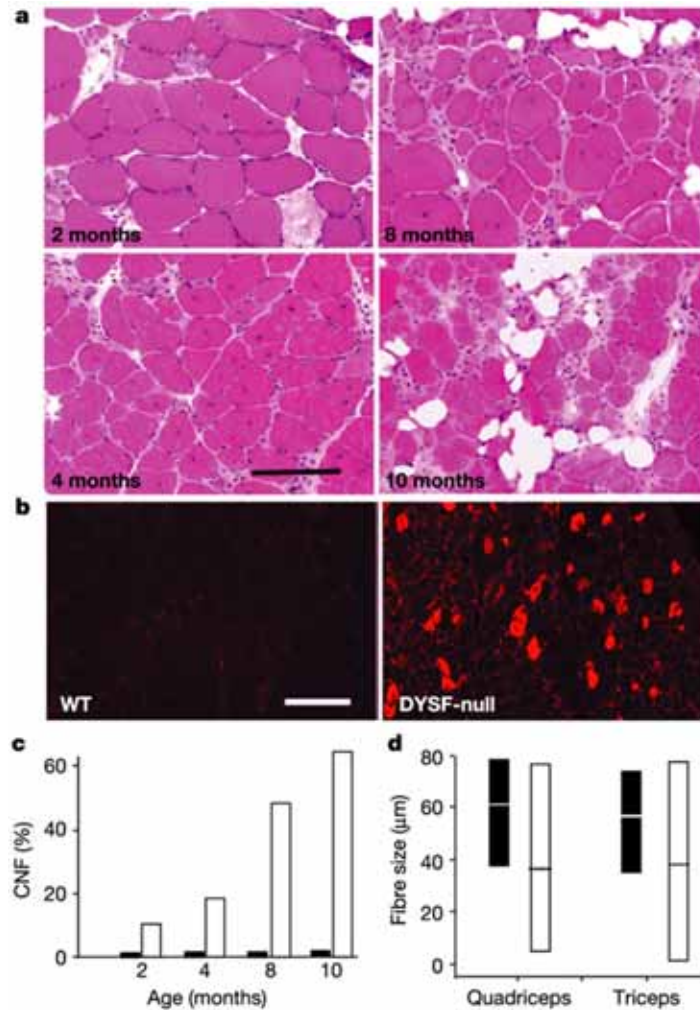


Dysferlin

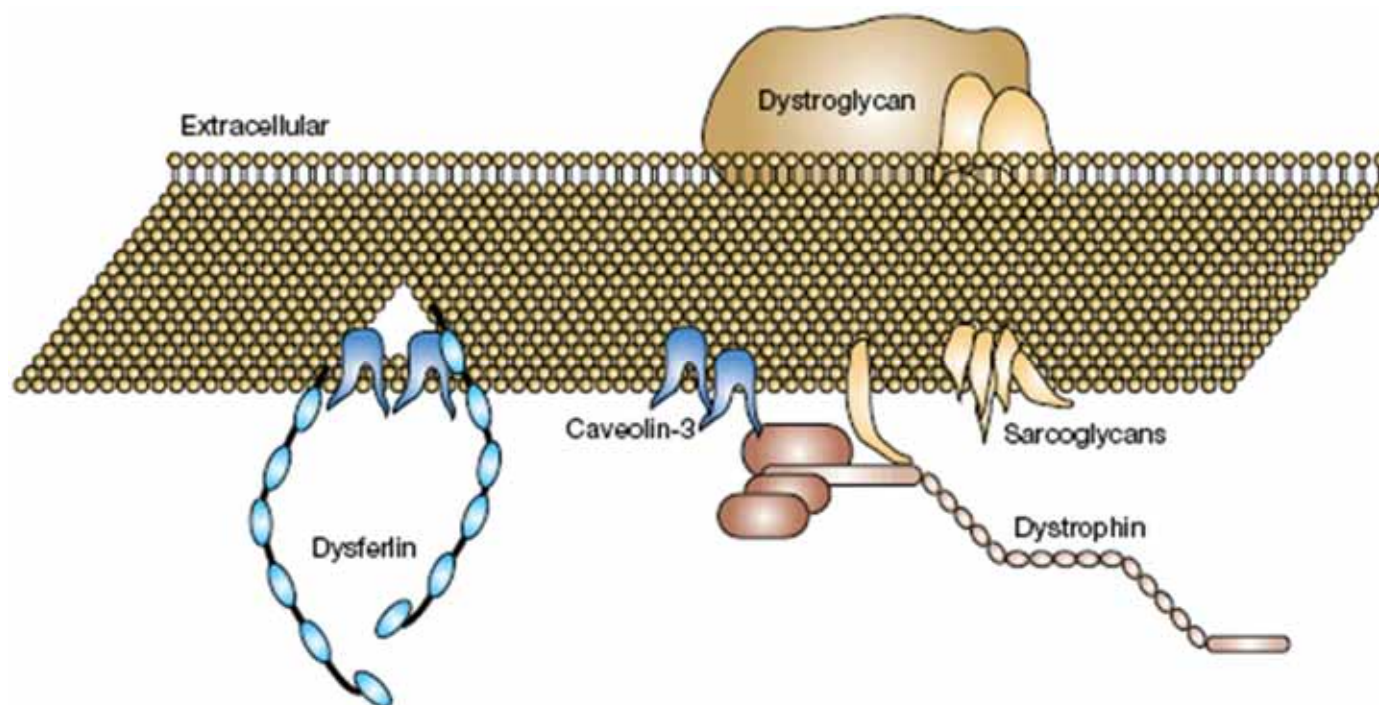


Originally determined to be a ferlin family protein that was known to be mutated in limb girdle muscular dystrophy (type 2B) and Myoshi myopathy patients, (Nat Genet. 1998 Sep;20(1):31-6).

Muscular dystrophy and compromised membrane repair in dysferlin null mice

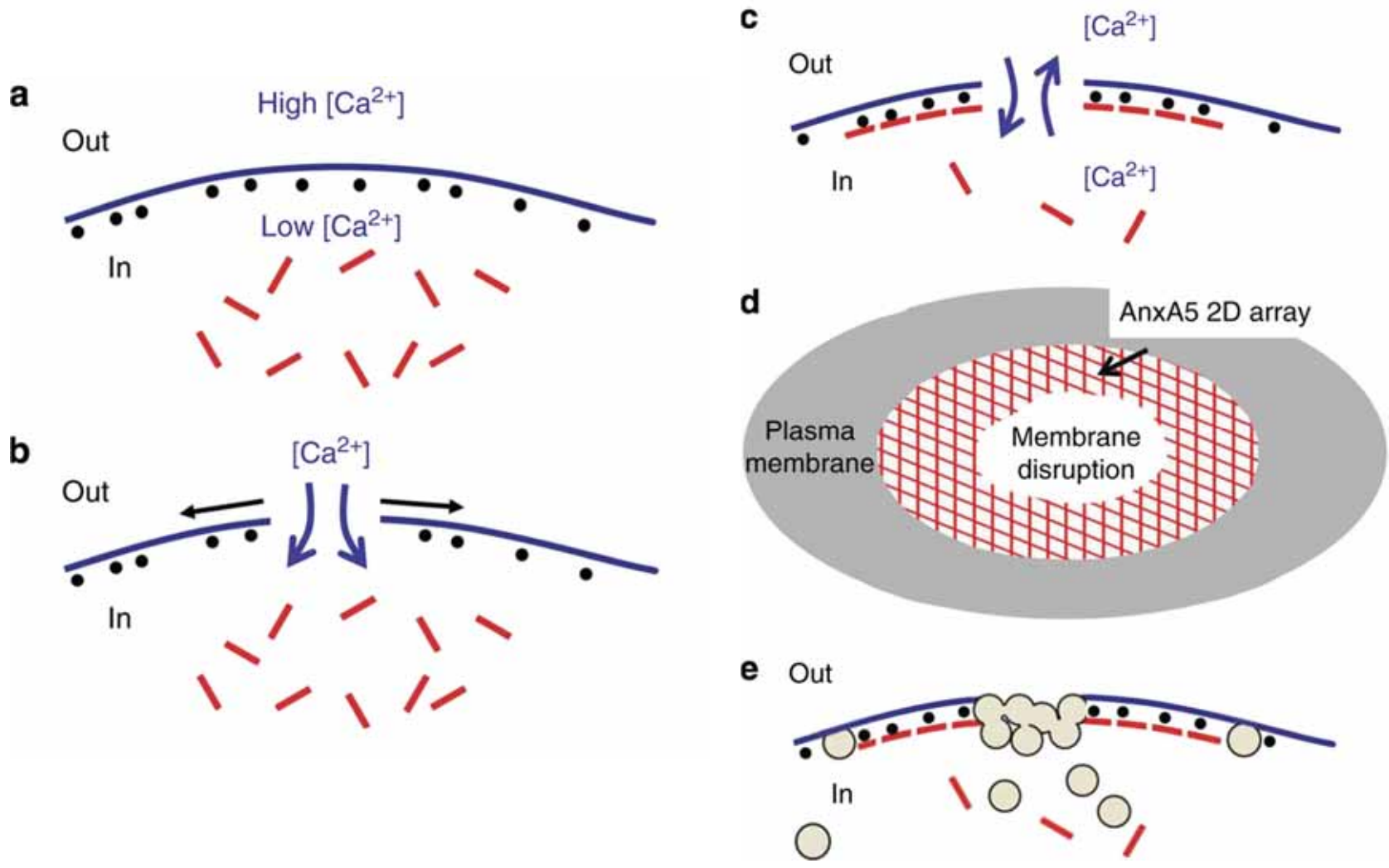


Dysferlin function in membrane repair is not clear

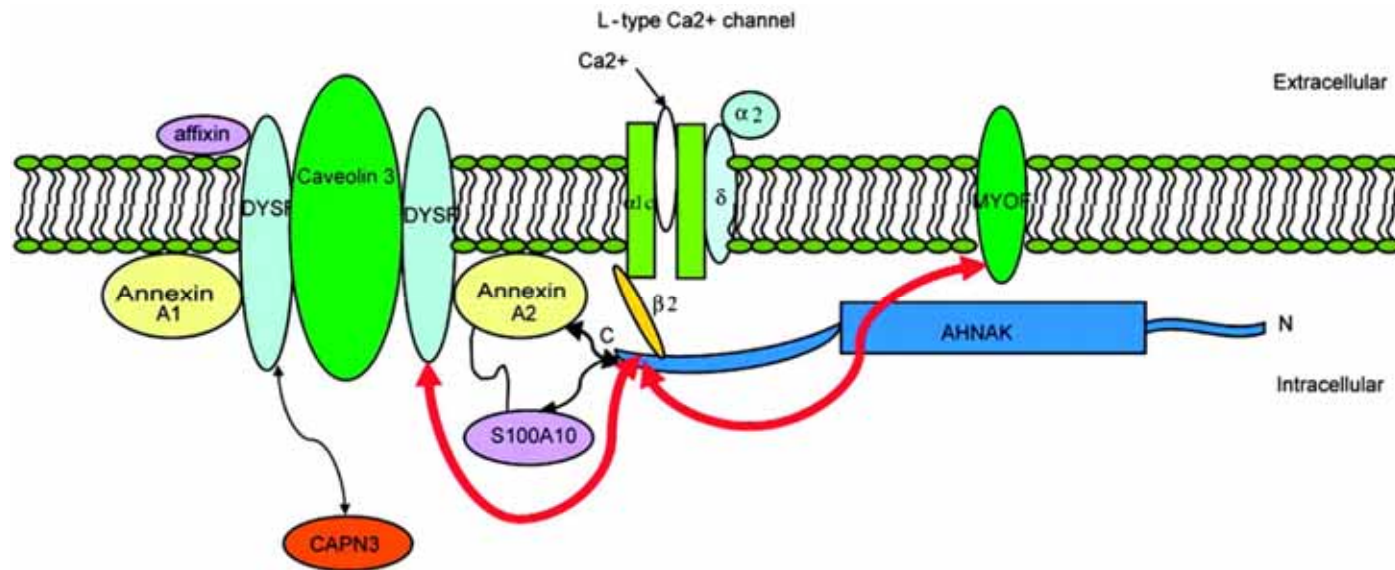


Molecular function of dysferlin in membrane repair is still not clear. Does it act as A fusogen for vesicles? Direct effects on remodeling of membrane? A platform to assemble other factors?

Annexin-A5 assembled into two-dimensional arrays promotes cell membrane repair

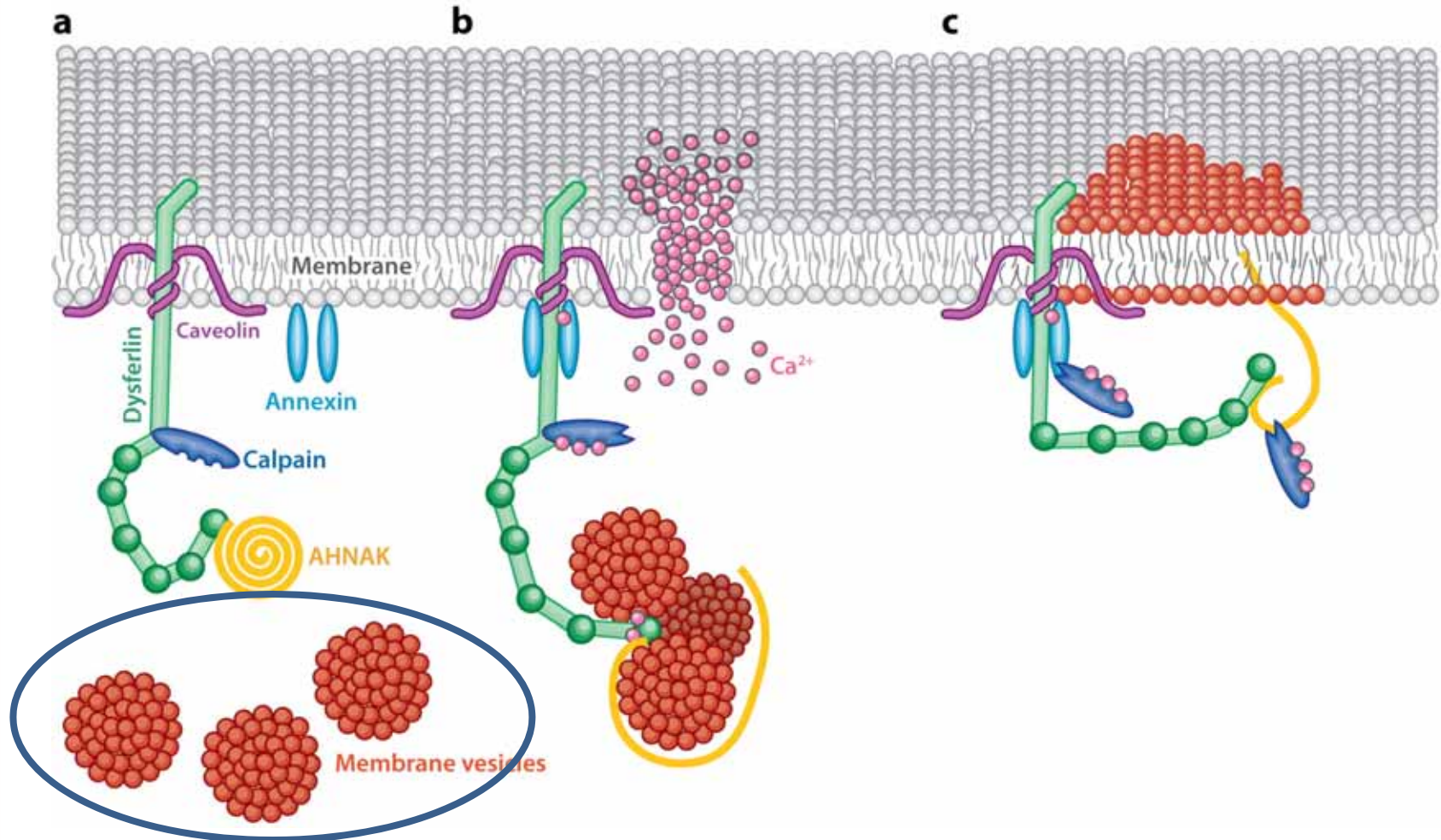


AHNAK and other proteins interact with dysferlin and modulate membrane repair capacity

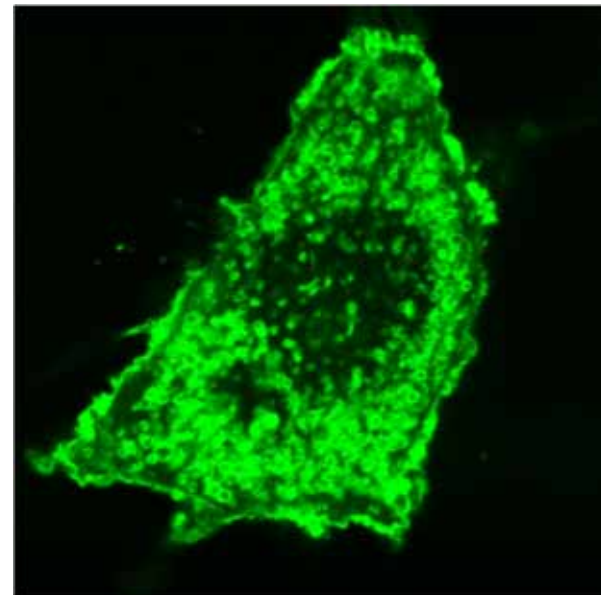
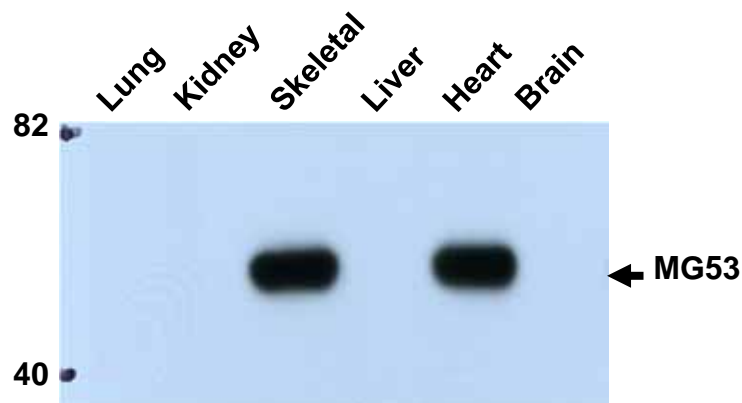
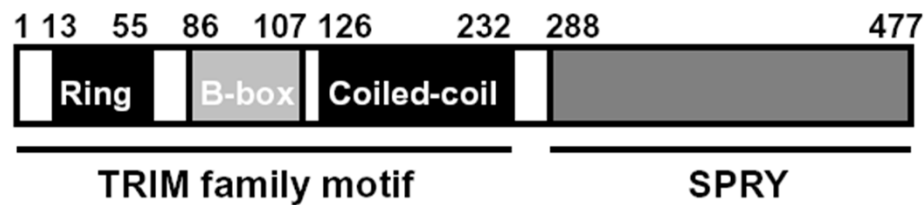


Multiple proteins have been shown to be important for the resealing of membranes, however the molecular function of these proteins is not clear.

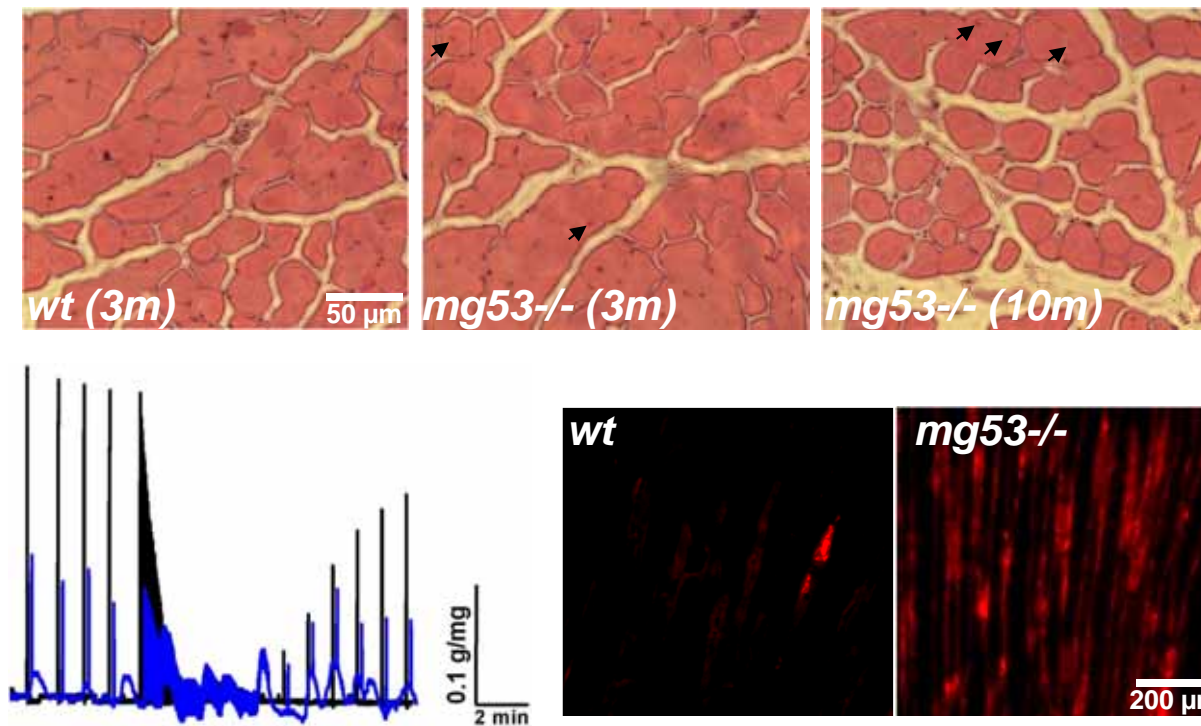
Other dysferlin interacting proteins linked to membrane repair



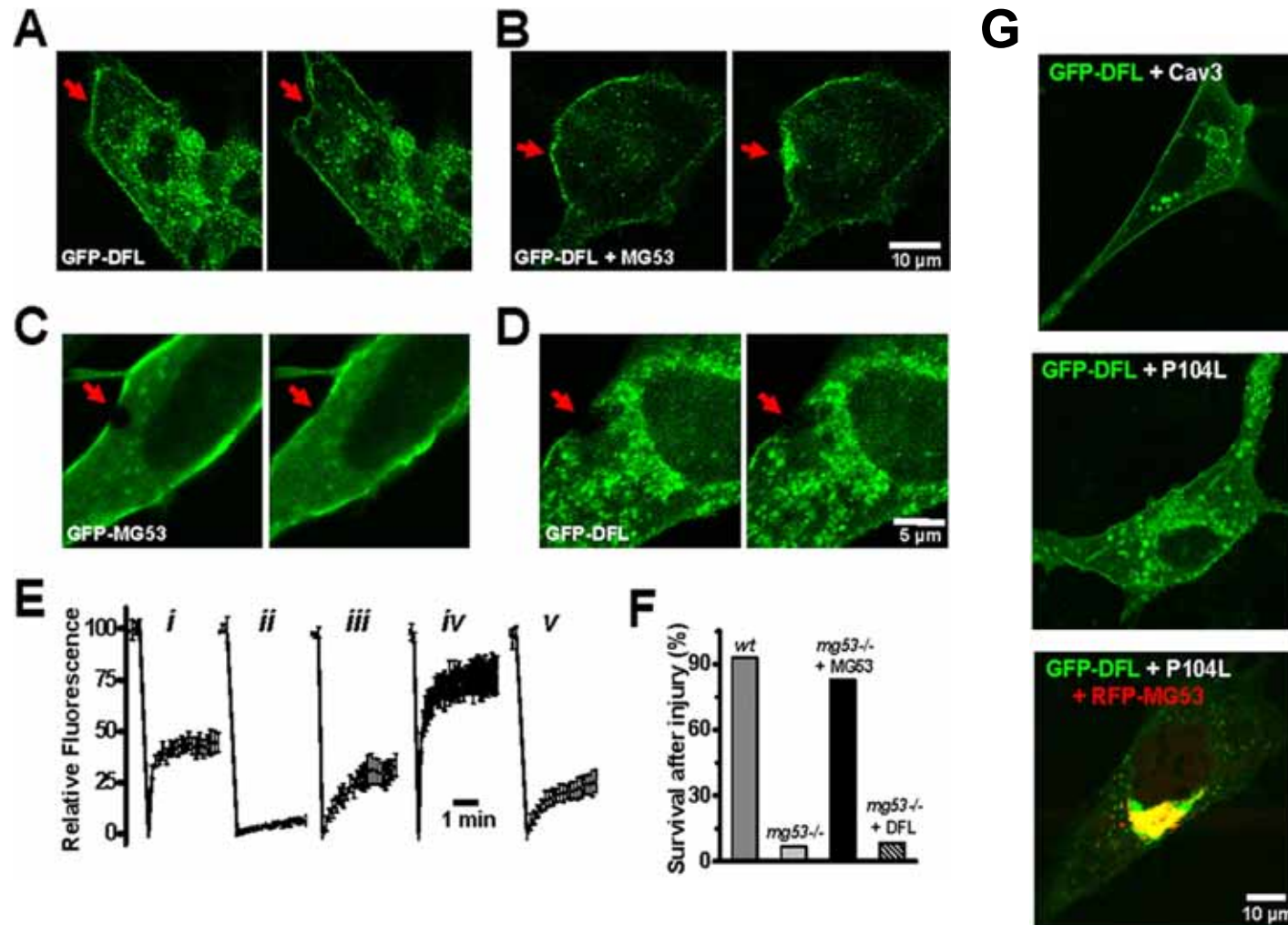
Mitsugumin 53 (MG53) is a tripartite motif family protein linked to membrane trafficking during membrane repair



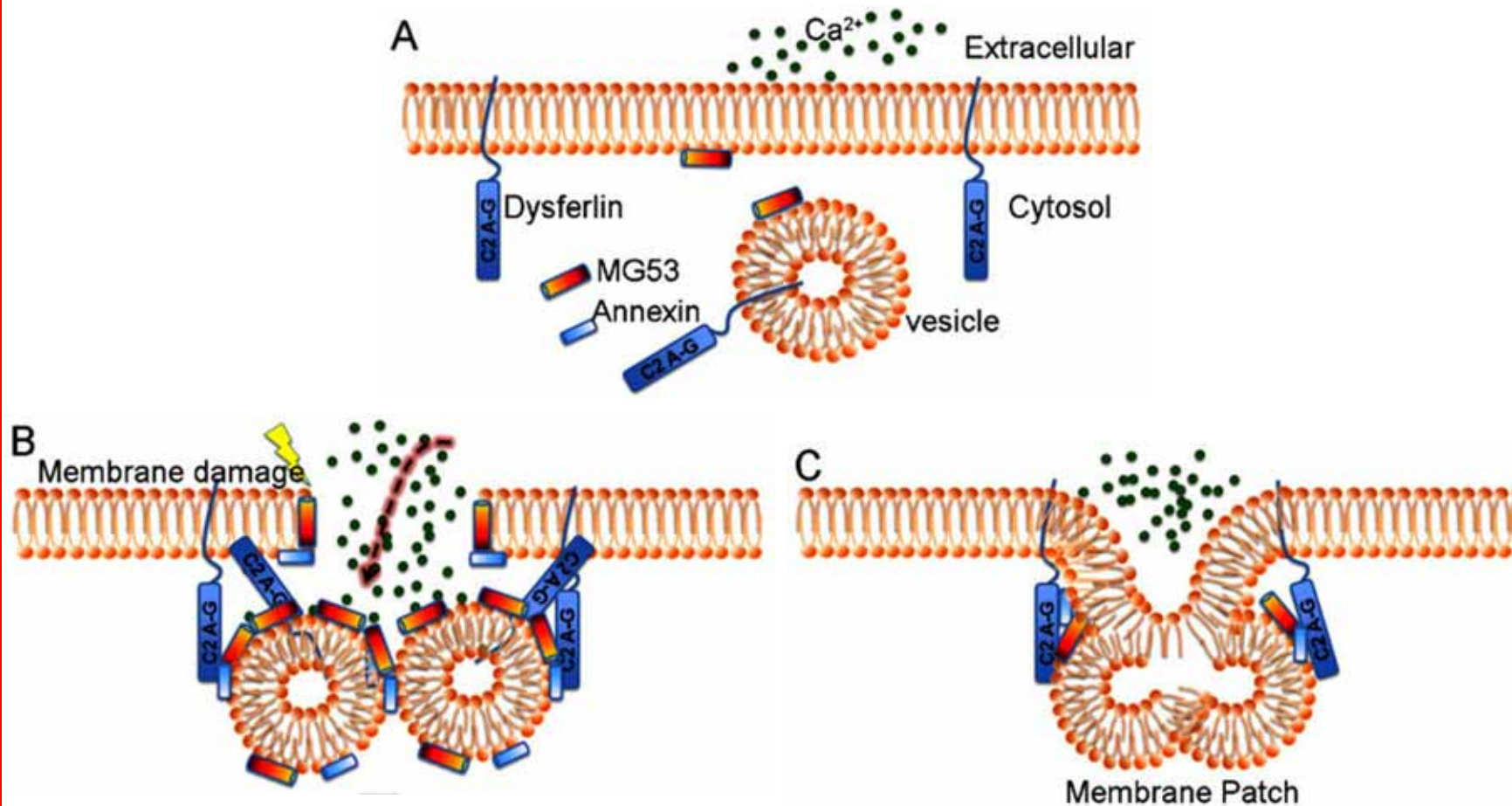
MG53 knockout mice display myopathy and breakdown of plasma membrane integrity



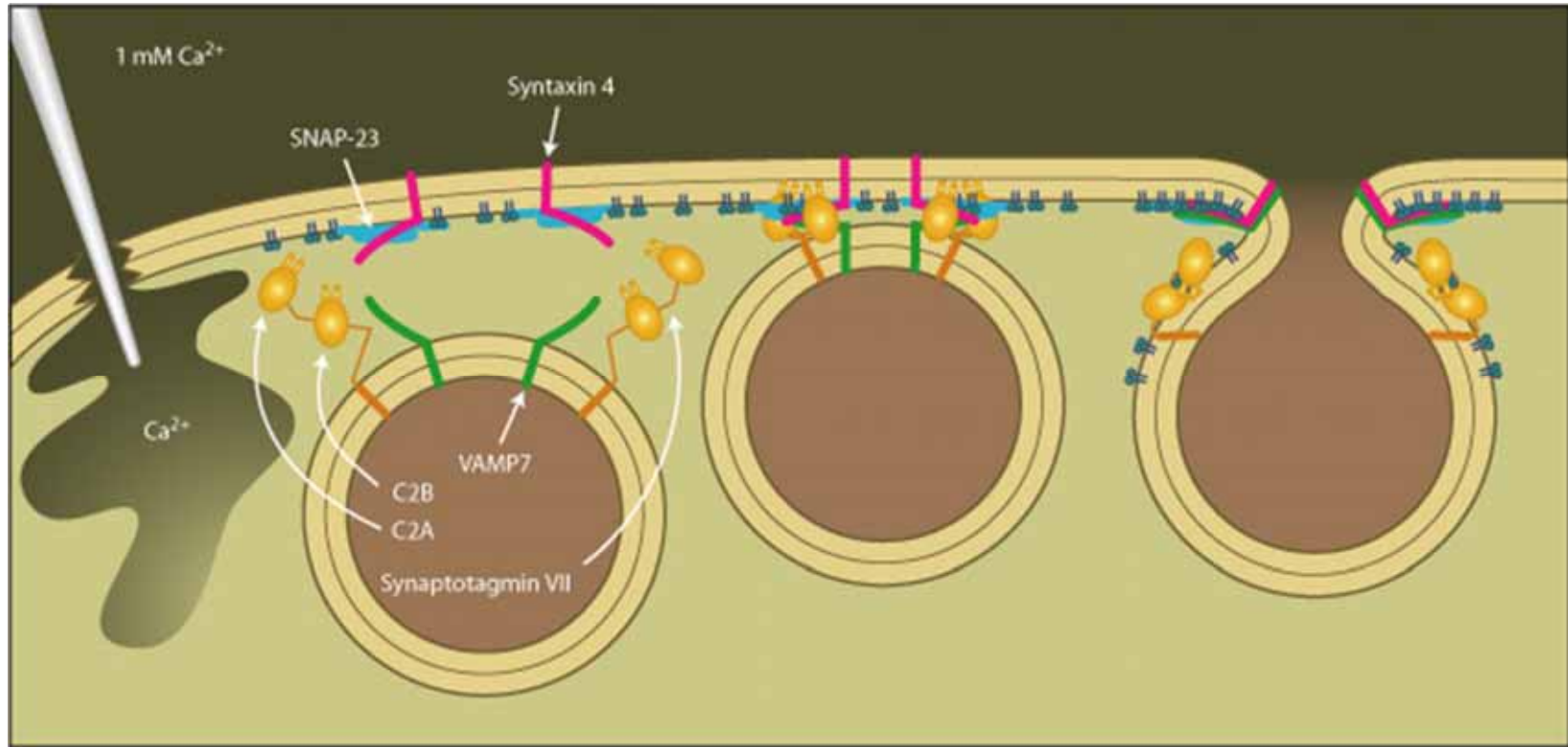
MG53 is required for translocation of dysferlin to injury sites on the plasma membrane



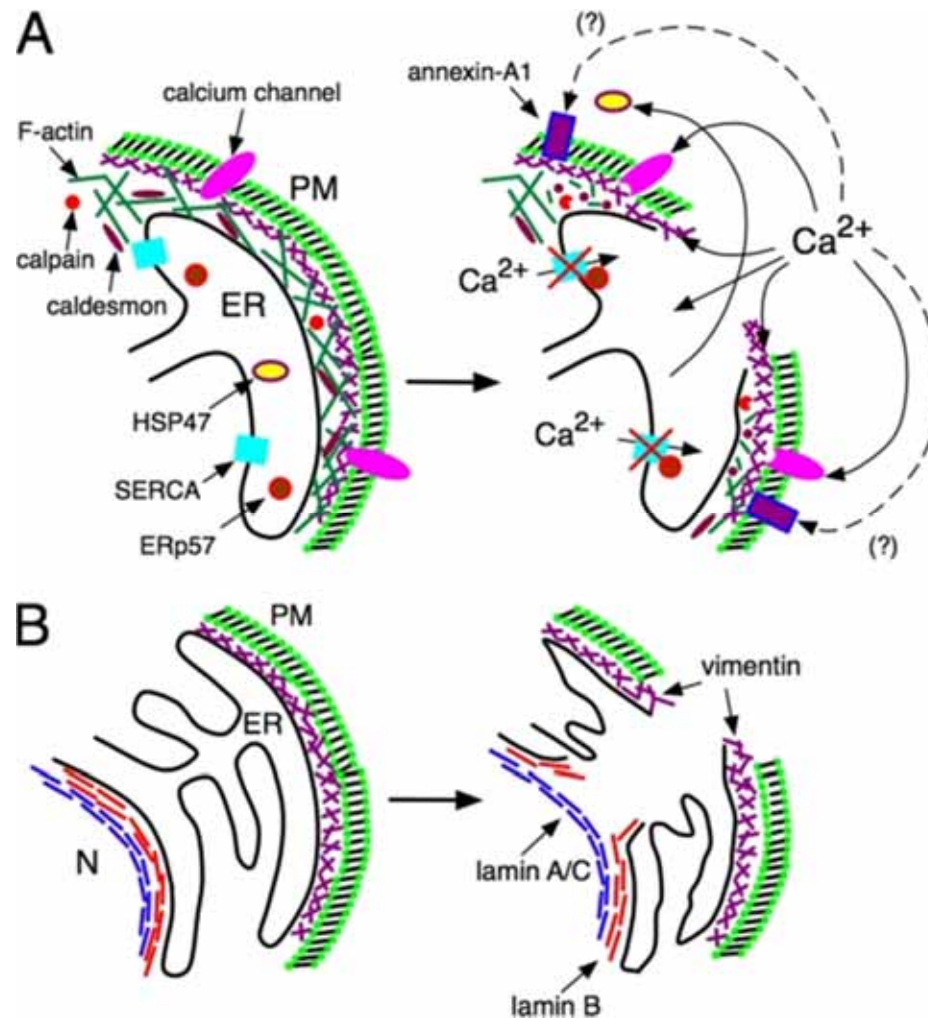
MG53 also associated with dysferlin to facilitate membrane repair



Proteins associated with vesicle fusion during endo/exocytosis are also involved in membrane repair



Reorganization of the cytoskeleton and organelles is required for effective membrane repair



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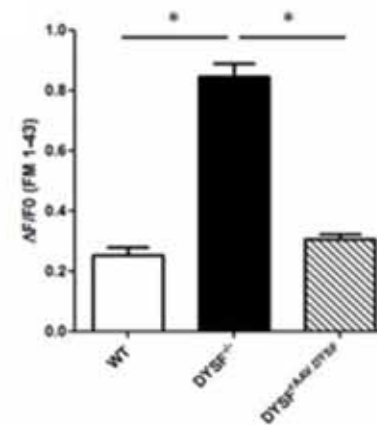
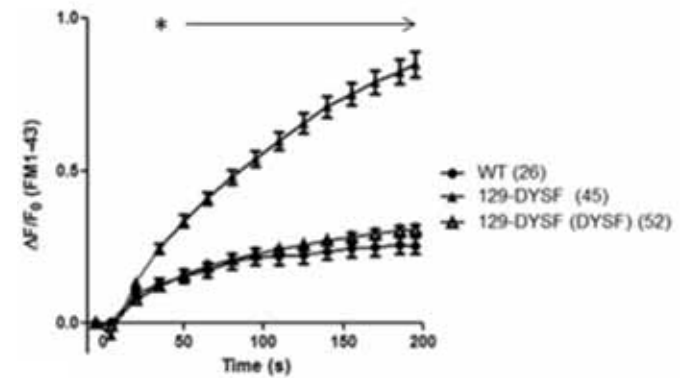
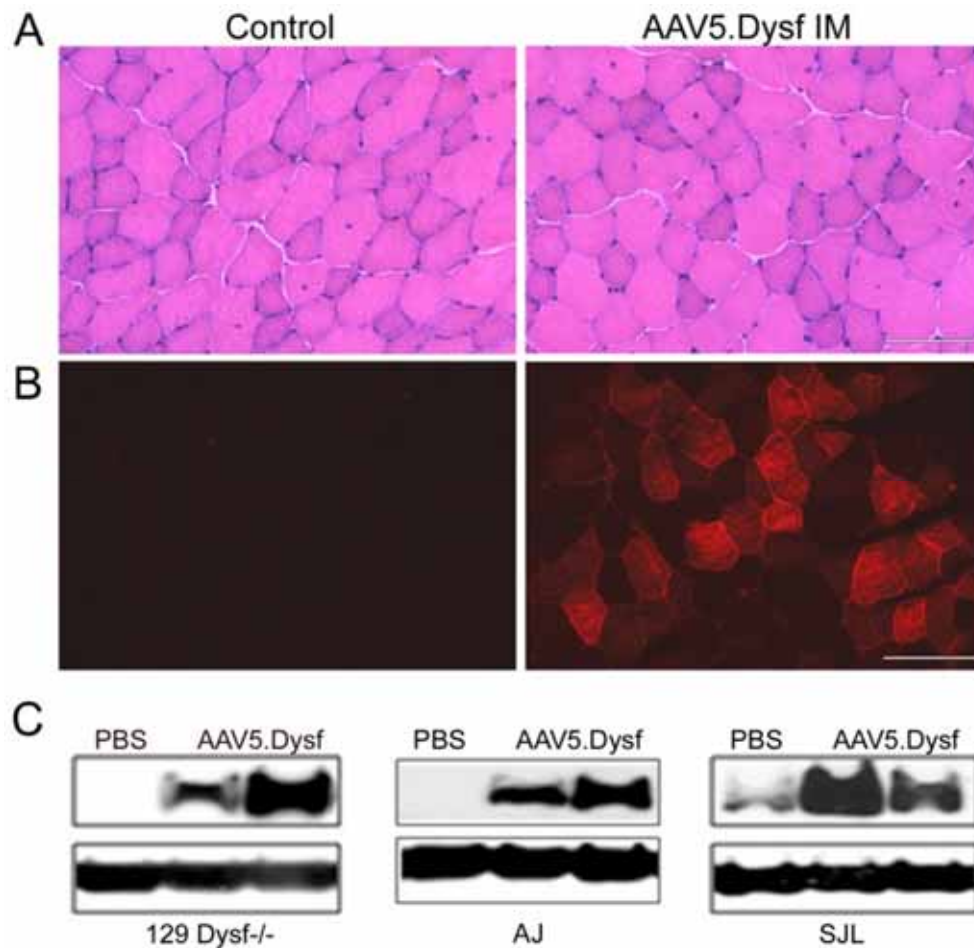
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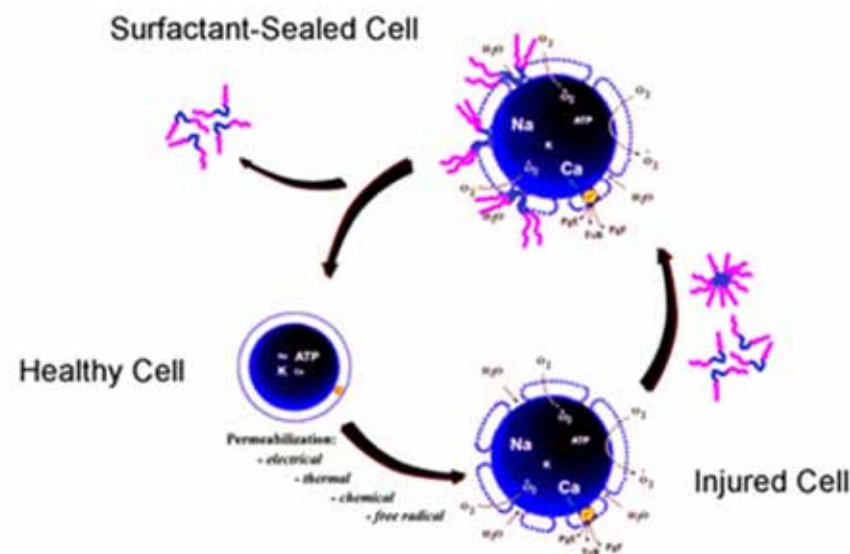
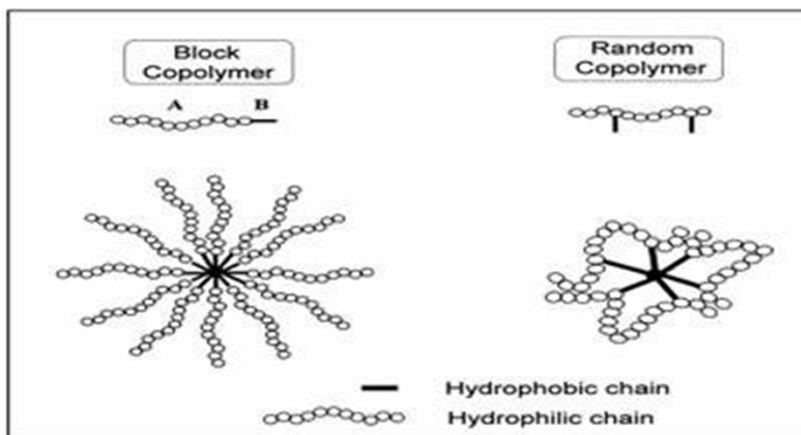
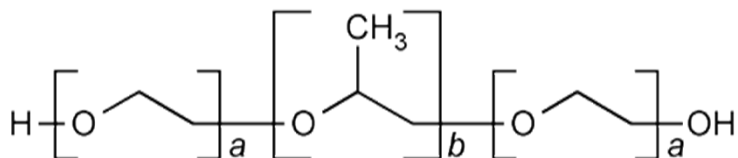
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Viral overexpression of target genes such as dysferlin

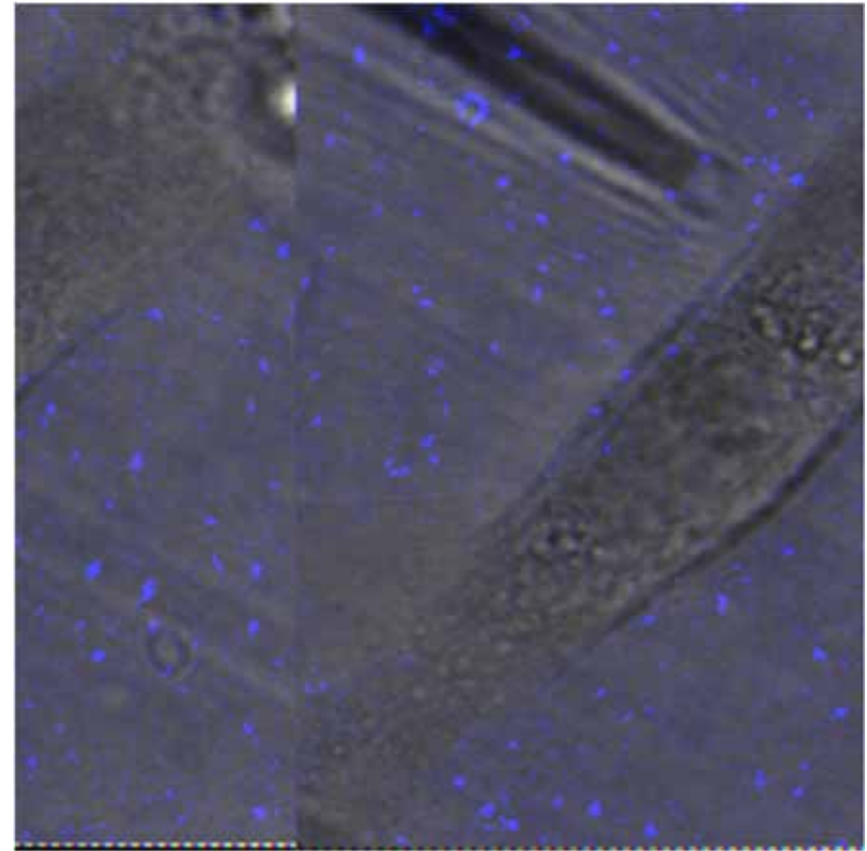
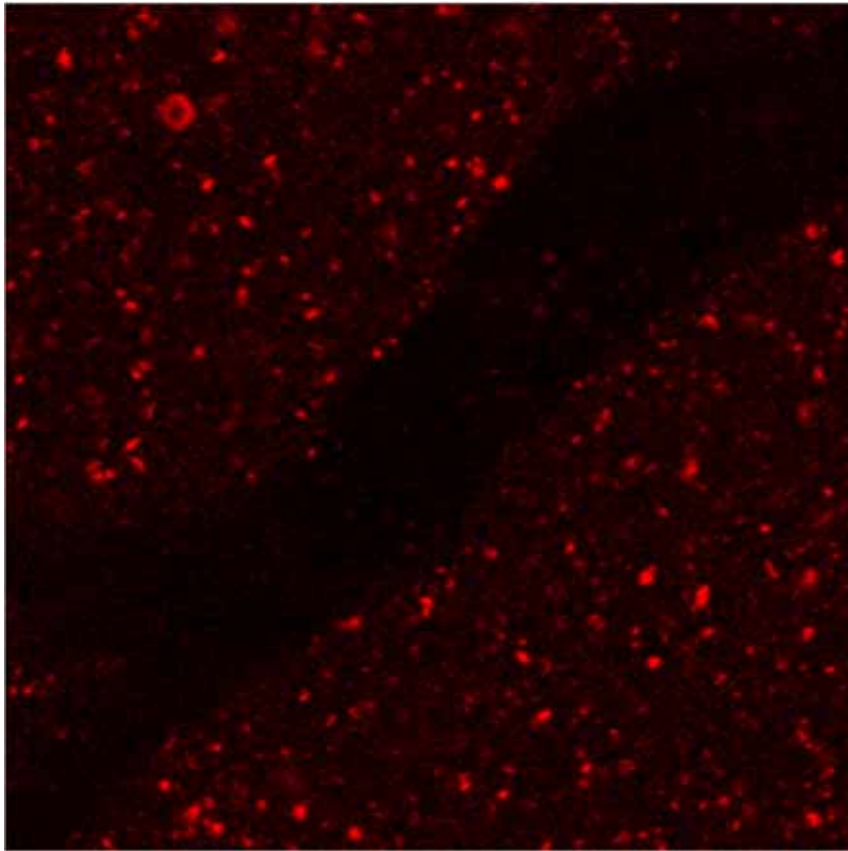


Poloxamer 188 treatments

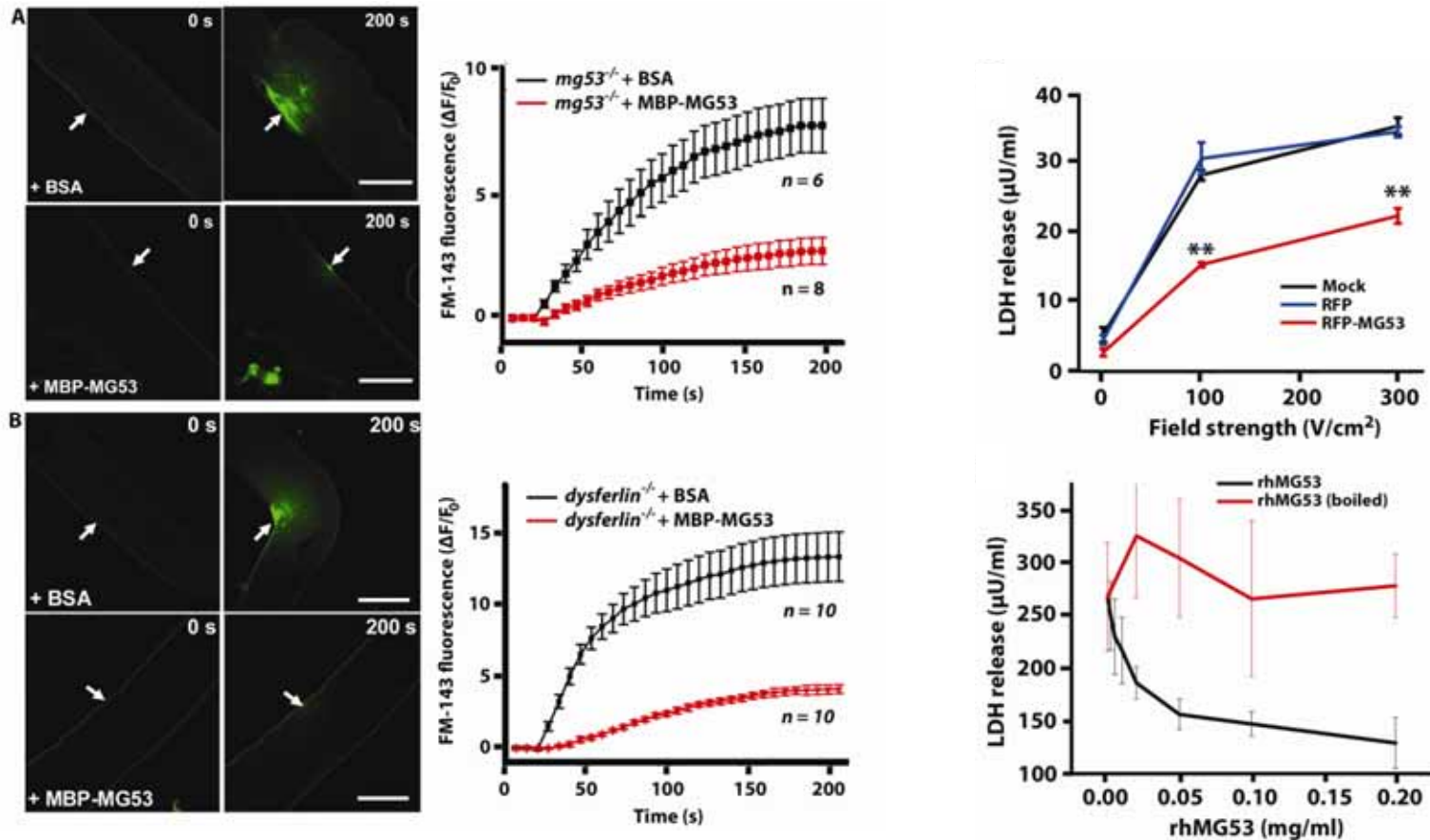


Poloxamer 188 is a long chain co-block polymer that can increase membrane resealing following a number of different types of membrane damage. Has been shown to decrease dystrophic pathology in cardiac muscle.

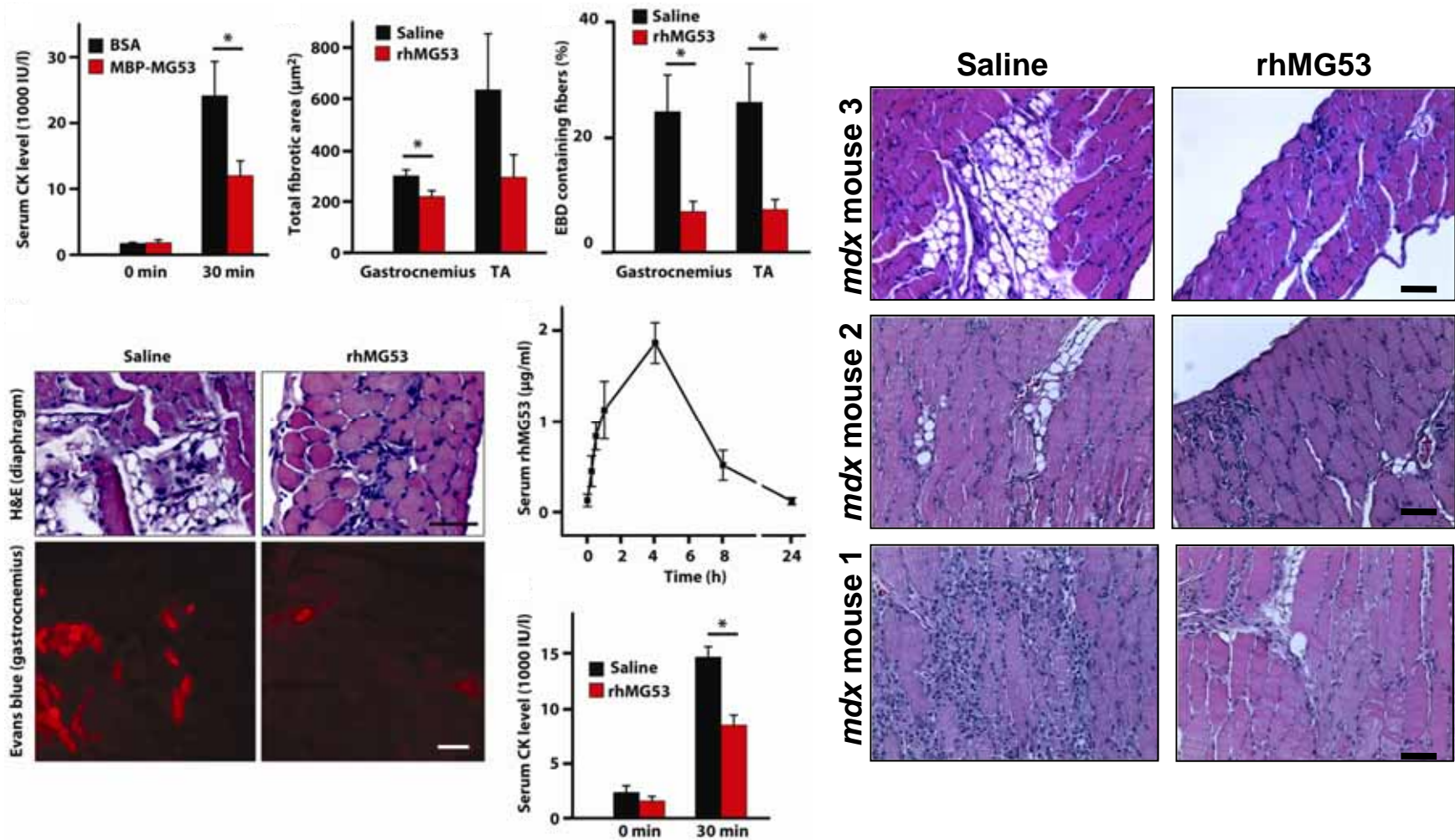
Recombinant MG53 (rhMG53) can locate to injury sites on the cell membrane



rhMG53 can increase the resealing of muscle and non-muscle cell membranes following various injuries



Injection of rhMG53 can improve pathology in *mdx* model of muscular dystrophy



Potential mechanism for rhMG53 in membrane resealing

