Supplemental Materials

Molecular Biology of the Cell

Sébastien et al.

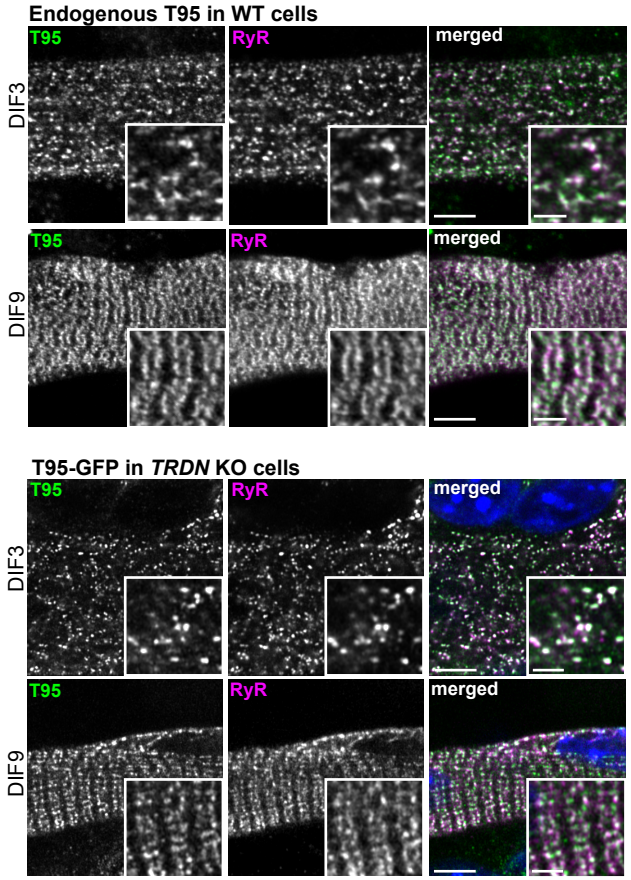


Figure S1: GFP-tagged version of T95 and endogenous T95 have the same localization in triads.

DIF3 and DIF9 WT (upper panels) and *TRDN* KO (lower panels) myotubes immunolabeled with anti-Triadin (T95) or anti-GFP (green) and anti-RyR1 (magenta) antibodies. Scale bars = 5 μm. Endogenous T95 and T95-GFP colocalize with RyR1 as scattered clusters at DIF3 and double rows of dots at DIF9 (insets, scale bars 2 μm).

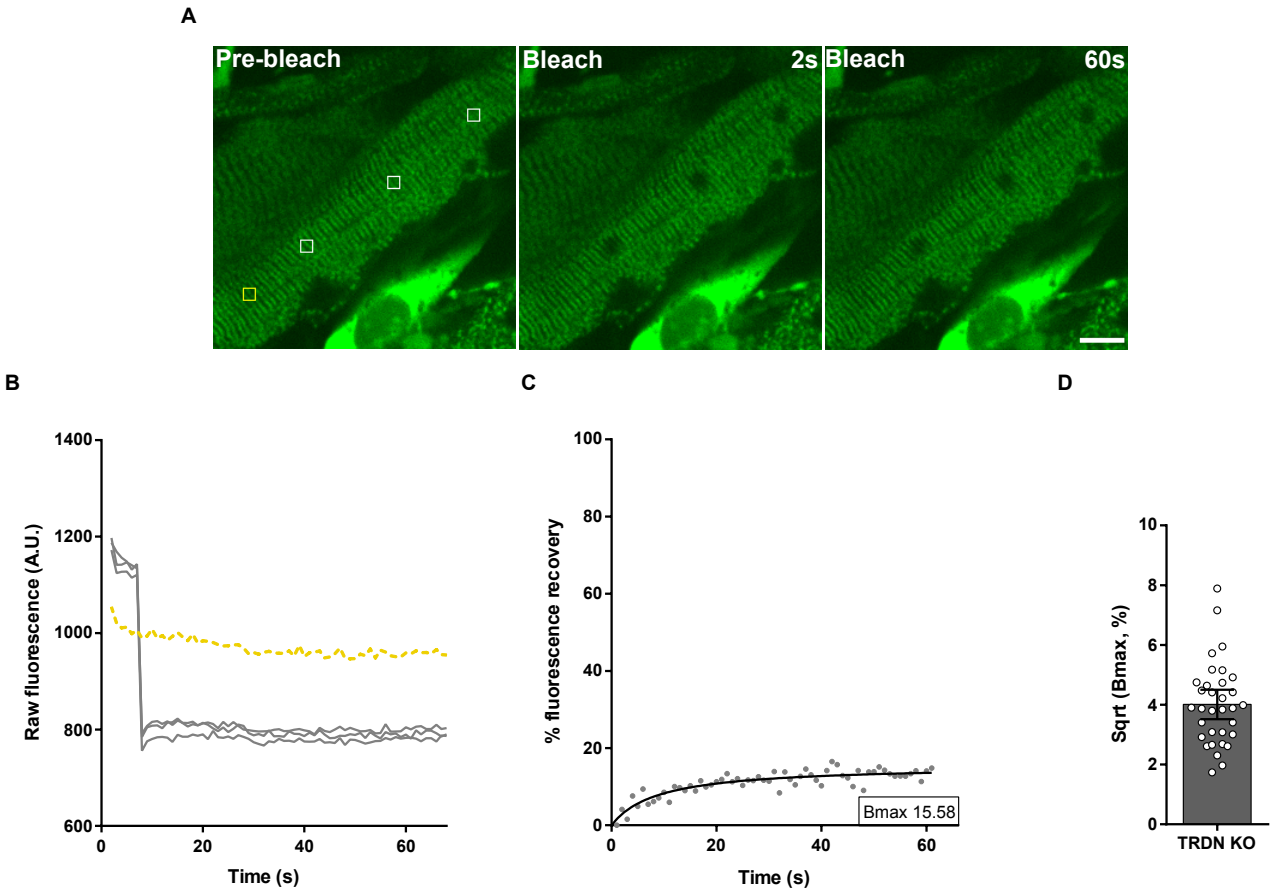


Figure S2: A fraction of T95 is still mobile at DIF9

A) Images of FRAP experiments in DIF9 *TRDN* KO cells expressing T95-GFP localized in triads. The three white squares represent the bleached areas while the yellow square was used to measure variations of unbleached fluorescence during the experiment. Shown are one image pre-bleach and two images post bleach (2 and 60 seconds). Scale bars = 10 µm. **B)** Raw fluorescence measurements from the white areas (gray curves) and the yellow area (yellow dotted curve), as indicated in A. **C)** Mean fluorescence measurements from the gray curves, corrected for the variations of unbleached fluorescence (yellow curve) as shown in B. Bmax is the percentage of fluorescence recovery as calculated (see Methods section). **D)** Histogram of Bmax values calculated as described in C for *n*=33 cells from 2 independent experiments. Note that distribution of "Square root (Bmax, %)" was preferred to obtain more accurate mean±c.i. values.

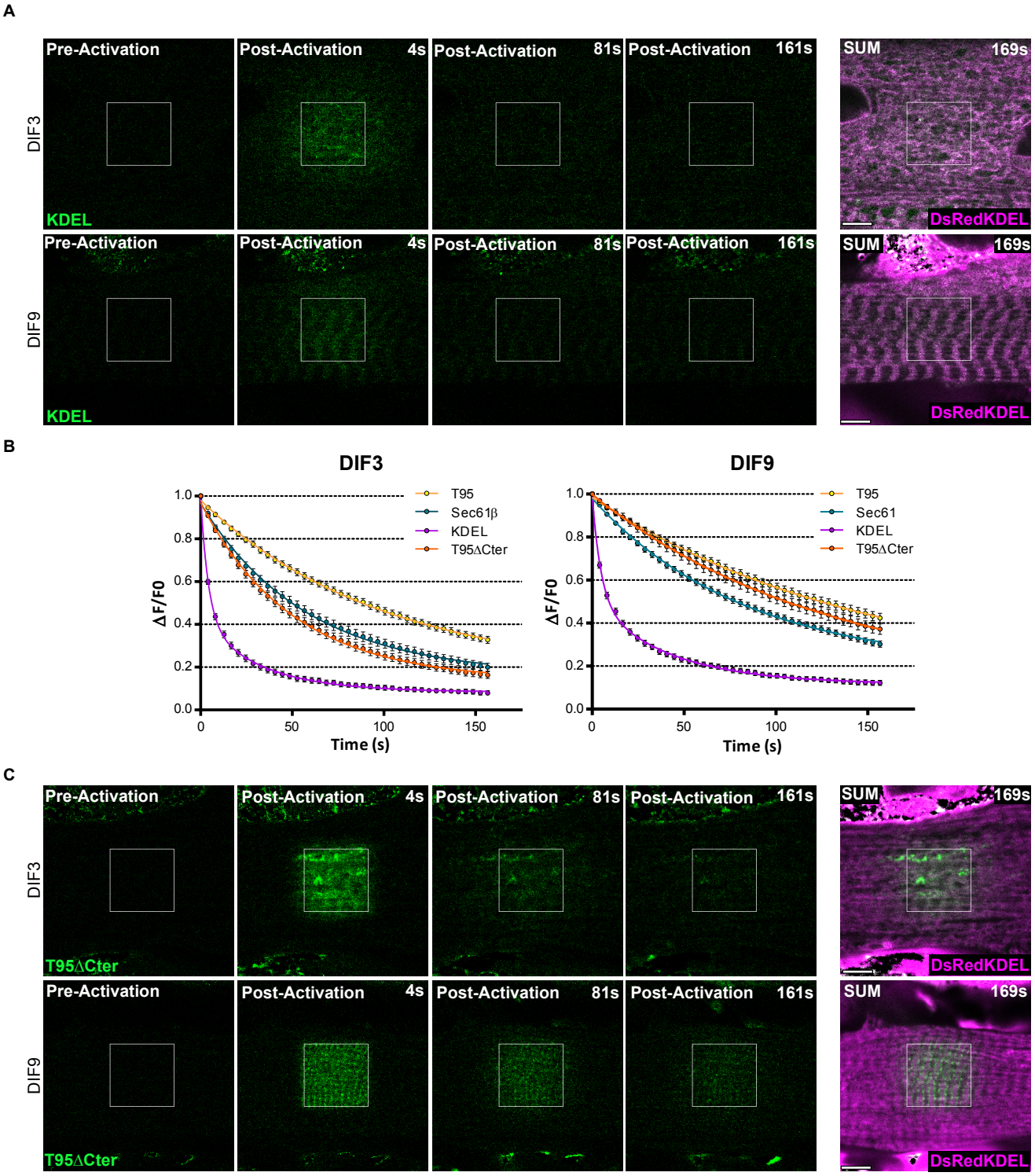
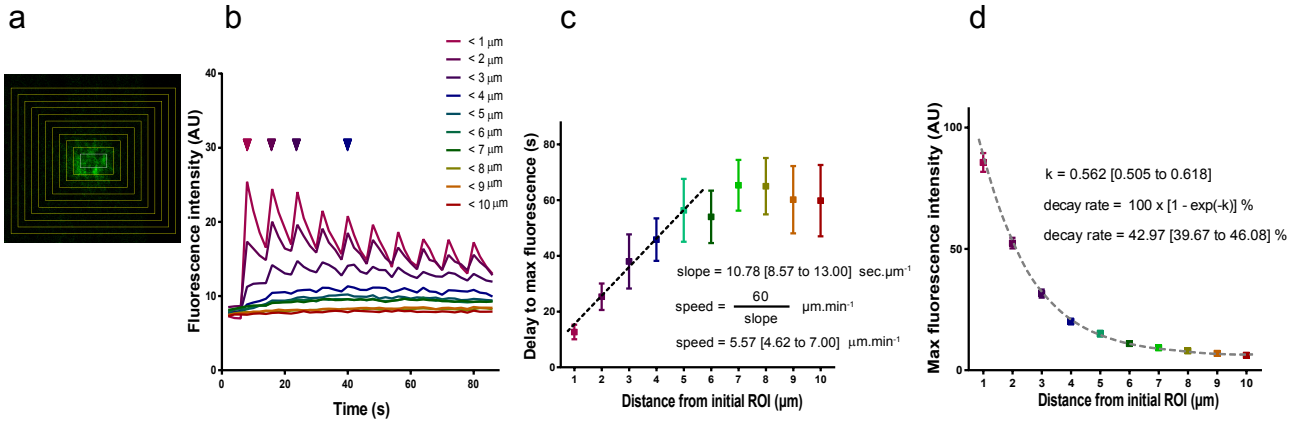

A
Pre-Activation
Post-Activation 4s
Post-Activation 81s
Post-Activation 161s
SUM 169s
DIF3
KDEL
DsRedKDEL
DIF9
B
DIF3
DIF9
T95
Sec61β
Sec61
KDEL
T95ΔCter
ΔF/F0
Time (s)
C
T95ΔCter

Fig S3.

Figure S3: Proteins dynamics at DIF3 and DIF9

A) Single photoactivation of DIF3 (upper panels) and DIF9 (lower panels) myotubes coexpressing PAGFP-KDEL (green) and DsRedKDEL (lumenal reticulum marker, magenta). Shown are one image pre-activation, and three images post-activation (4, 81, 161 seconds). SUM images are sum intensity projections of all post-activation frames (169 seconds). Scale bars = 5 µm. **B)** Curves representing the decrease in fluorescence intensity quantified for each protein-PAGFP in the activated ROI after a single photoactivation in DIF3 (left) and DIF9 (right) myotubes. Each image was normalized to 0 as the background intensity and to 1 as the fluorescence intensity of the first image post-activation. Values obtained were fitted to an exponential decay and mean±c.i. are represented. n = 32, 32, 36 and 34 cells for T95, Sec61β, T95ΔCter and KDEL at DIF3, respectively and n = 34, 40, 28 and 34 cells for T95, Sec61β, T95ΔCter and KDEL at DIF9, respectively from 4 independent experiments. **C)** Single photoactivation of DIF3 (upper panels) and DIF9 (lower panels) myotubes coexpressing T95ΔCter-PAGFP (green) and DsRedKDEL (lumenal reticulum marker, magenta). Shown are one image pre-activation, and three images post-activation (4, 81, 161 seconds). SUM images are sum intensity projections of all post-activation frames (169 seconds). Scale bars = 5 µm.

A


a
b
c
d
< 1 µm
< 2 µm
< 3 µm
< 4 µm
< 5 µm
< 6 µm
< 7 µm
< 8 µm
< 9 µm
< 10 µm
Fluorescence intensity (AU)
Time (s)
Delay to max fluorescence (s)
slope = 10.78 [8.57 to 13.00] sec.µm-1
speed = 60 / slope µm.min-1
speed = 5.57 [4.62 to 7.00] µm.min-1
Distance from initial ROI (µm)
Max fluorescence intensity (AU)
k = 0.562 [0.505 to 0.618]
decay rate = 100 x [1 - exp(-k)] %
decay rate = 42.97 [39.67 to 46.08] %
Distance from initial ROI (µm)

B

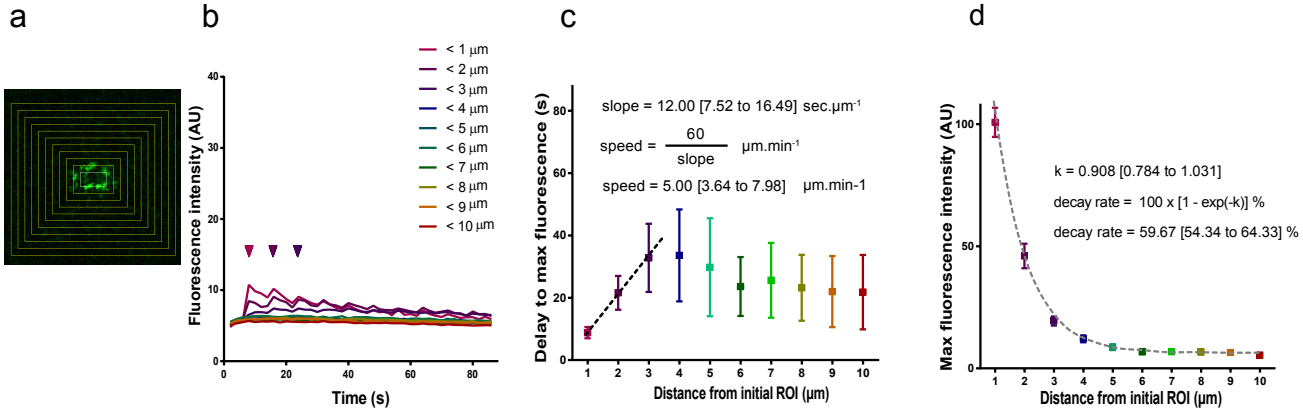

a
b
c
d
< 1 µm
< 2 µm
< 3 µm
< 4 µm
< 5 µm
< 6 µm
< 7 µm
< 8 µm
< 9 µm
< 10 µm
Fluorescence intensity (AU)
Time (s)
Delay to max fluorescence (s)
slope = 12.00 [7.52 to 16.49] sec.µm-1
speed = 60 / slope µm.min-1
speed = 5.00 [3.64 to 7.98] µm.min-1
Distance from initial ROI (µm)
Max fluorescence intensity (AU)
k = 0.908 [0.784 to 1.031]
decay rate = 100 x [1 - exp(-k)] %
decay rate = 59.67 [54.34 to 64.33] %
Distance from initial ROI (µm)

Fig S4.

Figure S4 : Quantification of T95 and Sec61β diffusion in SR membranes of cultured myotubes at DIF3

Cultured myotubes at DIF3 co-expressing Sec61β-PAGFP and DsRedKDEL (**A**) or T95-PAGFP and DsRedKDEL (**B**) were imaged and repeatedly photoactivated as described in M&M. **Aa)** and **Ba)** Concentric squares drawn around the initially photoactivated ROI allowed for measurements of fluorescence in increasingly distant "rings" as reported in **Ab)** and **Bb)**. Arrowheads indicate the time of maximum fluorescence in the curve of the corresponding color (red-violet, purple, dark purple, dark blue for 1 μm, 2 μm, 3 μm, 4 μm distances from the photoactivated ROI, respectively). **Ac)** and **Bc)** Delay to maximal fluorescence observed in each concentric "ring" (red-violet, purple, dark purple, dark blue, light blue, dark green, light green, yellow, orange and red for 1 μm, 2 μm, 3 μm, 4 μm, 5 μm, 6 μm, 7 μm, 8μm, 9 μm and 10 μm distances from the photoactivated ROI, respectively). On each graph, linear fitting of the aligned first points (dotted gray line) enabled the calculation of the slope and thus the speed of the fluorescence wave, as indicated. **Ad)** and **Bd)** Fluorescence values at the time of maximal fluorescence observed in each concentric "ring" (red-violet, purple, dark purple, dark blue, light blue, dark green, light green, yellow, orange and red for 1 μm, 2 μm, 3 μm, 4 μm, 5 μm, 6 μm, 7 μm, 8μm, 9 μm and 10 μm distances from the photoactivated ROI, respectively) and normalized to that observed in the first square, as 100%. On each graph, exponential decay fitting of all the points (dotted gray curve) enabled the calculation of the k parameter and thus the decay rate, as indicated. Values are means±c.i. n = 12 and 10 cells for Sec61β and T95 respectively at DIF3 from 3 independent experiments.

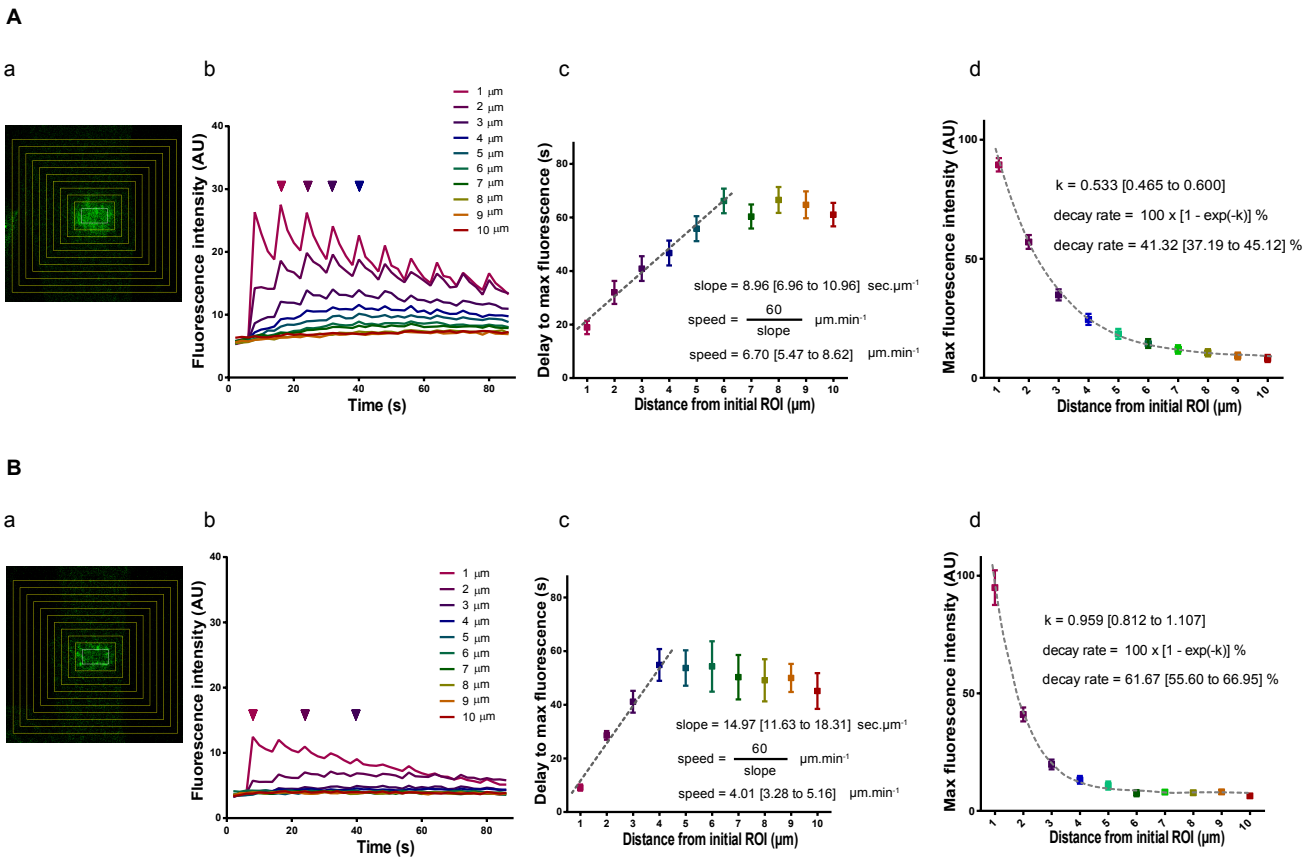

A
a
b
c
d
1 µm
2 µm
3 µm
4 µm
5 µm
6 µm
7 µm
8 µm
9 µm
10 µm
Fluorescence intensity (AU)
Time (s)
Delay to max fluorescence (s)
Distance from initial ROI (µm)
slope = 8.96 [6.96 to 10.96] sec.µm-1
speed = 60 / slope µm.min-1
speed = 6.70 [5.47 to 8.62] µm.min-1
Max fluorescence intensity (AU)
k = 0.533 [0.465 to 0.600]
decay rate = 100 x [1 - exp(-k)] %
decay rate = 41.32 [37.19 to 45.12] %
B
a
b
c
d
Fluorescence intensity (AU)
Time (s)
Delay to max fluorescence (s)
Distance from initial ROI (µm)
slope = 14.97 [11.63 to 18.31] sec.µm-1
speed = 60 / slope µm.min-1
speed = 4.01 [3.28 to 5.16] µm.min-1
Max fluorescence intensity (AU)
k = 0.959 [0.812 to 1.107]
decay rate = 100 x [1 - exp(-k)] %
decay rate = 61.67 [55.60 to 66.95] %

Fig S5.

Figure S5 : Quantification of T95 and Sec61β diffusion in SR membranes of cultured myotubes at DIF9

Cultured myotubes at DIF9 co-expressing Sec61β-PAGFP and DsRedKDEL (**A**) or T95-PAGFP and DsRedKDEL (**B**) were imaged and repeatedly photoactivated as described in M&M. **Aa)** and **Ba)** Concentric squares drawn around the initially photoactivated ROI allowed for measurements of fluorescence in increasingly distant "rings" as reported in **Ab)** and **Bb)**. Arrowheads indicate the time of maximum fluorescence in the curve of the corresponding color (red-violet, purple, dark purple, dark blue for 1 μm, 2 μm, 3 μm, 4 μm distances from the photoactivated ROI, respectively). **Ac)** and **Bc)** Delay to maximal fluorescence observed in each concentric "ring" (red-violet, purple, dark purple, dark blue, light blue, dark green, light green, yellow, orange and red for 1 μm, 2 μm, 3 μm, 4 μm, 5 μm, 6 μm, 7 μm, 8μm, 9 μm and 10 μm distances from the photoactivated ROI, respectively). On each graph, linear fitting of the aligned first points (dotted gray line) enabled for the calculation of the slope and thus the speed of fluorescence wave, as indicated. **Ad)** and **Bd)** Fluorescence values at the time of maximal fluorescence observed in each concentric "ring" (red-violet, purple, dark purple, dark blue, light blue, dark green, light green, yellow, orange and red for 1 μm, 2 μm, 3 μm, 4 μm, 5 μm, 6 μm, 7 μm, 8μm, 9 μm and 10 μm distances from the photoactivated ROI, respectively) and normalized to that observed in the first square, as 100%. On each graph, exponential decay fitting of all the points (dotted gray curve) enabled the calculation of the k parameter and thus the decay rate, as indicated. Values are means±c.i. n = 11 and 7 cells for Sec61β and T95 at DIF9 respectively, from 3 independent experiments.

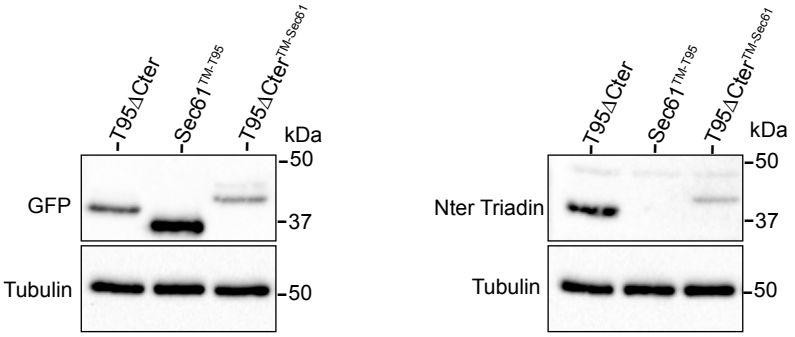


Figure S6 : Western blot analysis of mutant and hybrids proteins

Western blot analysis has been performed with antibodies against GFP (left) or against N-terminal part of triadin (right) on 20 µg *TRDN* KO cells lysates transduced with T95ΔCter-PAGFP, Sec61$^{TM\text{-}T95}$-PAGFP or T95ΔCter$^{TM\text{-}Sec61}$-PAGFP. Antibody against GFP detected bands at 40kDa for T95ΔCter-PAGFP and T95ΔCter$^{TM\text{-}Sec61}$-PAGFP, and at 37kDa for Sec61$^{TM\text{-}T95}$-PAGFP. As expected, antibody against the N-terminal part of triadin, detected only T95ΔCter-PAGFP and T95ΔCter$^{TM\text{-}Sec61}$-PAGFP. The β-tubulin has been used as a loading control (lower panel).

Fig S6.

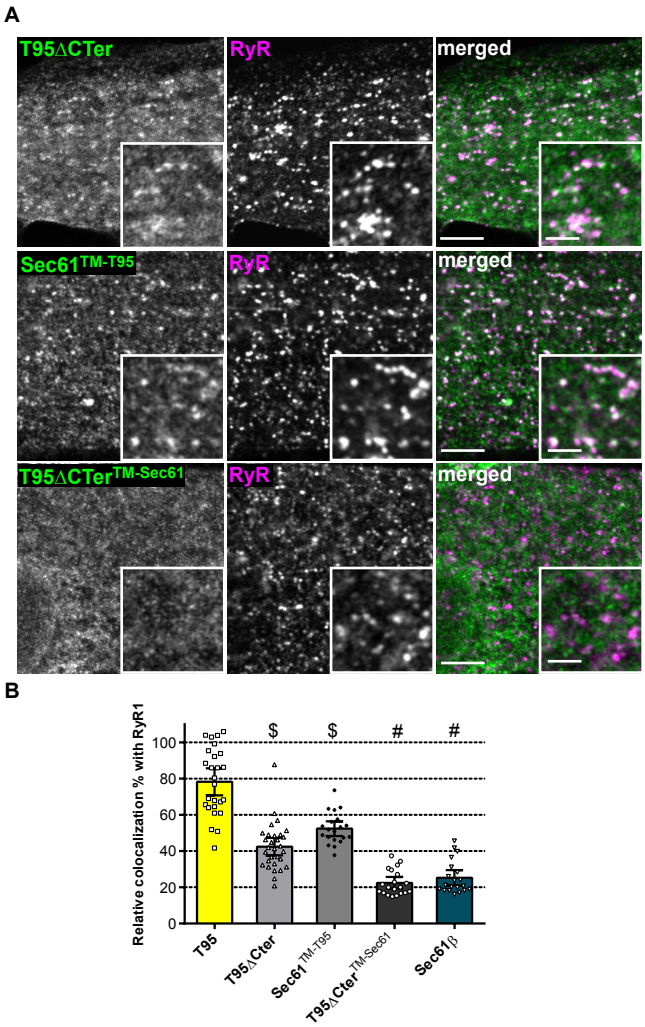


Figure S7 : Triadin transmembrane domain is necessary for localization at triads at DIF3

(A) DIF3 myotubes expressing T95ΔCter, Sec61$^{TM\text{-}T95}$ and T95ΔCter$^{TM\text{-}Sec61}$ immunolabeled with anti-GFP (green) and anti-RyR1 (magenta) antibodies. Scale bars = 5 µm. Insets of T95ΔCter, Sec61$^{TM\text{-}T95}$ or T95ΔCTer$^{TM\text{-}Sec61}$ immunolabelings with that of RyR1 are shown. Scale bars = 2µm. **B)** Percentage of PAGFP constructs colocalized with RyR1 labeling and normalized to that of DIF9 T95. Values are means±c.i., n = 26 cells from 3 independent experiments for T95, n = 20 cells from 2 independent experiments for Sec61β, n = 30 cells from 3 independent experiments for T95ΔCter, n = 20 cells from 2 independent experiments for T95ΔCter$^{TM\text{-}Sec61}$, n = 20 cells from 2 independent experiments for Sec61$^{TM\text{-}T95}$.

Using a non parametric Kuskal-Wallis test followed by Dunn's post-hoc comparisons: $ comparison with Sec61β: T95ΔCter ($p<0.05$) and Sec61$^{TM\text{-}T95}$ ($p<0.001$). # comparison with T95ΔCter : T95ΔCTer$^{TM\text{-}Sec61}$ ($p<0.01$) and Sec61β ($p<0.05$).

Fig S7.