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online data

SUPPLEMENTARY ONLINE DATA

Targeting of inositol 1,4,5-trisphosphate receptor to the endoplasmic reticulum by its first transmembrane domain

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Biochemical Journal

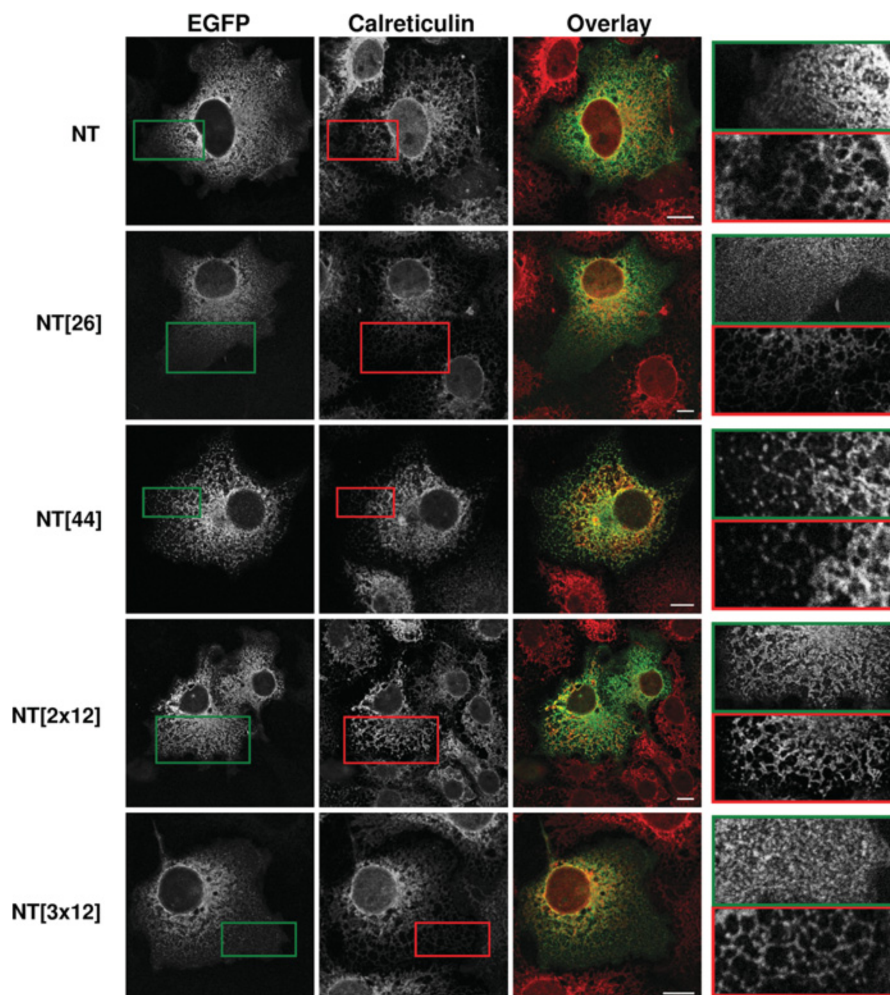

Figure S1 Localization of representative IP_3R1 fragments is not dependent on the fixative used

Cells transfected with the indicated constructs were fixed with methanol/acetone (1:1, v/v). They are shown in the same format as in Figure 2. Bars, 10 μm.

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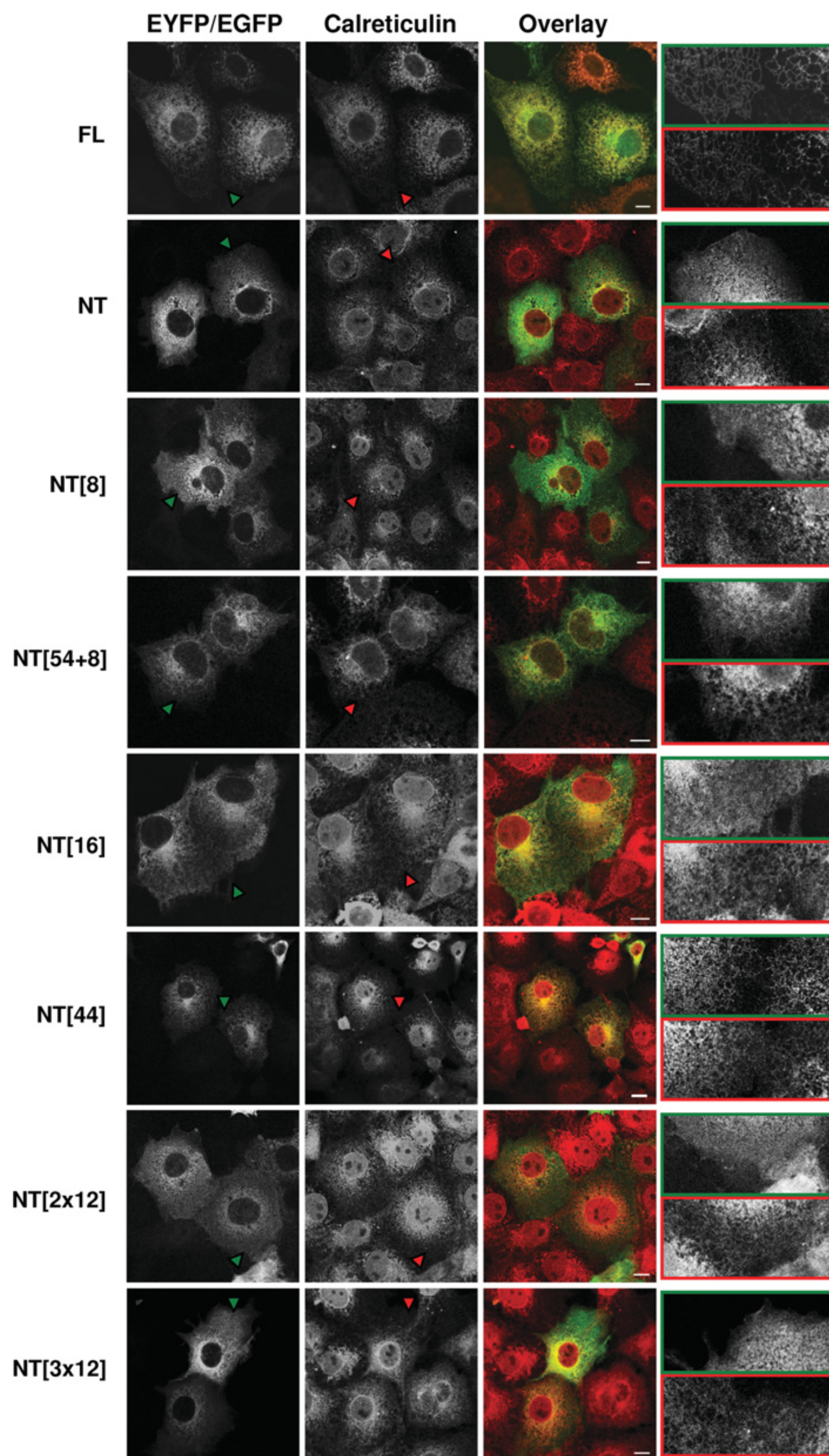

Figure S2 Representative fields of cells expressing IP_3R1 fragments

Cells transfected with the indicated constructs are shown in the same format as in Figure 2, but at lower magnification to allow a field of cells to be viewed. Bars, 10 μm.

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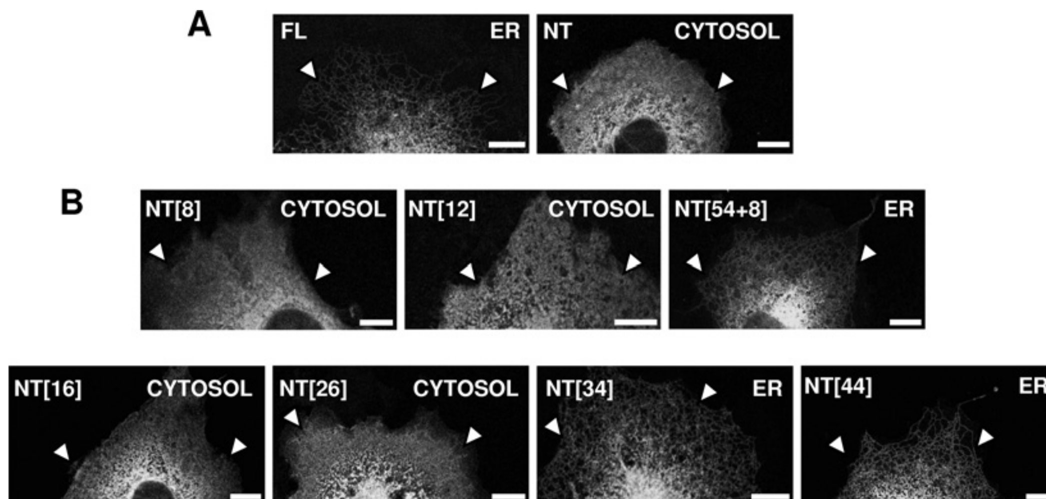

Figure S3 Higher magnification images of cells expressing IP_3R1 fragments

(**A**) Images of constructs expressed in the cytosol (NT) or ER (FL), shown with the cell boundaries highlighted to illustrate the clear difference between the two patterns of expression. (**B**) Typical magnified images demonstrating distributions of the indicated constructs. Arrow heads denote the cell boundaries. Bars, 10 μm.

Table S1 Primers used

Forward (F) and reverse (R) primers are shown, restriction sites are underlined, and start and stop codons are shown in bold. The sequence encoding FLAG is shown double-underlined.

Construct			Primer (5′ to 3′)
NT[8]	F	1	AGGAATTCGCCACC**TTG**TCTGACAAAATG
	R	2	CATGCGTCGAC**TTA**TAGTGTTCCTCCTCTCAC
NT[12]	F	3	TATCGAATTCAGAGAGGATCCTGTACAAC
	R	4	CATGCGTCGAC**TTA**CCAGTGCGGCTCTAGTGTTCCTCCTCTCAC
NT[16]	F	3	
	R	5	GAGCATGTCGAC**TTA**CAGGAGGCCGGACCAGTGCGG
NT[26]	F	3	
	R	6	GAGCATGTCGAC**TTA**AATTGCCAGAGAGATGAGC
NT[2×12]	F	3	
	R	7	AGCATGTCGAC**TTA**CCAATGAGGTTCCAGAGTGCCTCCACGAACTCCTTTCCAGTGCGGCTCTAGTGTTCCTCCTCTCAC
NT[34]	F	3	
	R	8	GAGCATGTCGAC**TTA**ATGGGGCTTGGGCAGCGCAAT
NT[3×12]	F	3	
	R	9	AGCATGTCGAC**TTA**CCAGTGTGGCTCTAGAGTACCCCCCCTGACAGGCTTCCAATGAGGTTCCAGAGTGCCTCCACGAACTCCTTTCCAGTGCGGCTCTAGTGTTCCTCCTCTCAC
NT[44]	F	3	
	R	10	GAGCATGTCGAC**TTA**GATTGTAGAAGCAATTAAGGC
NT[54 + 8]	F	3	
	R	11	CATGCGTCGAC**TTA**CTTATCGTCATCGTCTTTGTAGTCCTGTAATCCAACTGAAAATATCAGTCGTAGG
TMD1	F	12	CGGAATTCTGAATTTCTGACTAAGG
	R	13	GCTCTAGATAGTGTTCCTCCTCTCAC
TMD1-2	F	12	
	R	14	GCGGATCCGCTGTAATCCAACTGAA
NT	F	15	AGGAATTCGCCACC**ATG**TCTGACAAAATG
	R	16	CTGGAATTCTCAAGACATGTTTCGGGCGCACCAGTACAAGAC
FL	F	17	GTCGACGACATGTCTGACAAAATG
	R	18	GGTACCAATCTTCAGCATGACAGG

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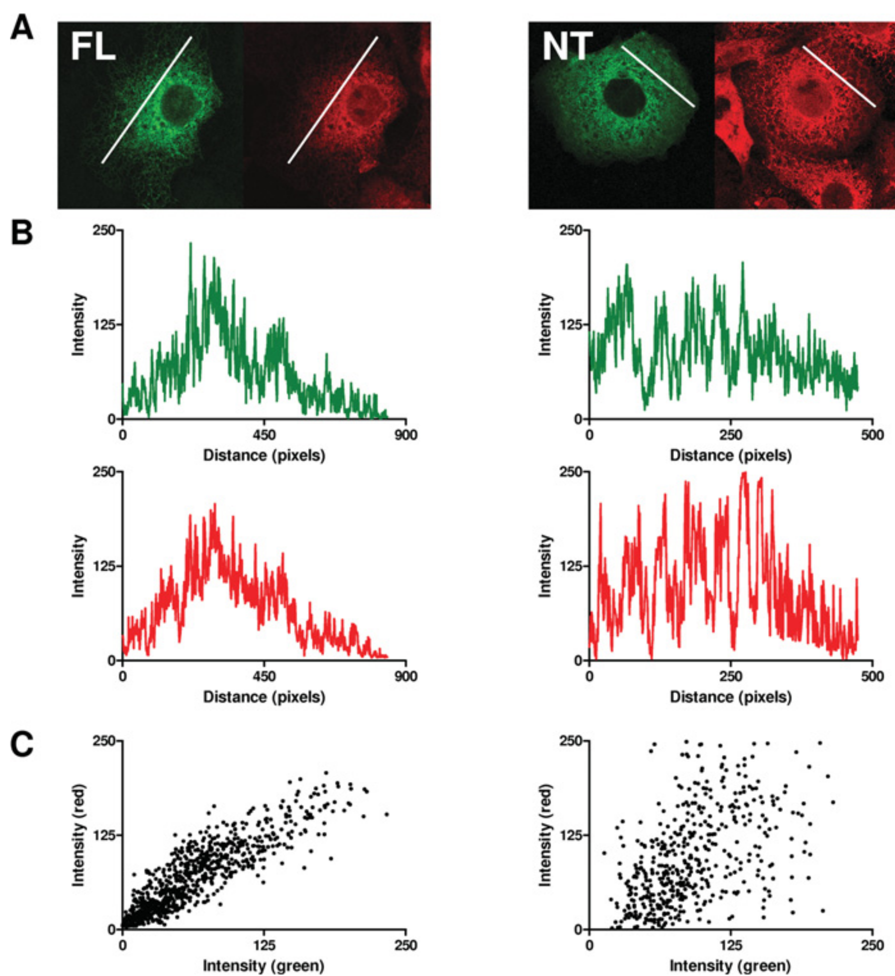

Figure S4 Quantitative analysis of the co-localization of calreticulin and IP_3R fragments

Several routines allow a Pearson's correlation coefficient (r) to be calculated for the distribution of two fluorescent markers using a pixel-by-pixel comparison of the fluorescence intensities of each fluorophore. There are, however, some pitfalls that are particularly pertinent to our analysis. First, pixels within which there is no fluorescence in either channel (e.g. a region just beyond the cell boundary or the nucleus of many cells) provide a spurious perfect correlation ($r = 1$). This problem can be corrected by imposing a threshold, but that requires selection of an arbitrary threshold. Secondly, the calreticulin antibody sometimes stains the nucleoplasm (see the Experimental section in the main text), from which IP_3R fragments are invariably excluded. Pixels that include the nucleoplasm might therefore contribute to a spuriously low measure of r. To circumvent these problems, we adopted the following simple analysis for estimating r. For each cell, we selected three straight lines across different regions of the entire cell that excluded the nucleoplasm. Typical examples for cells expressing FL or NT are shown in (**A**). The fluorescence intensity in each channel (green for IP_3R fragment; red for calreticulin immunostaining) was then plotted for each transect (**B**), and the relationship between the two was plotted (**C**). Finally, r was calculated from these scatter plots. The results are shown in Table 1 in the main text, which shows data derived from at least three cells for each construct, with three lines used to measure r in each cell.

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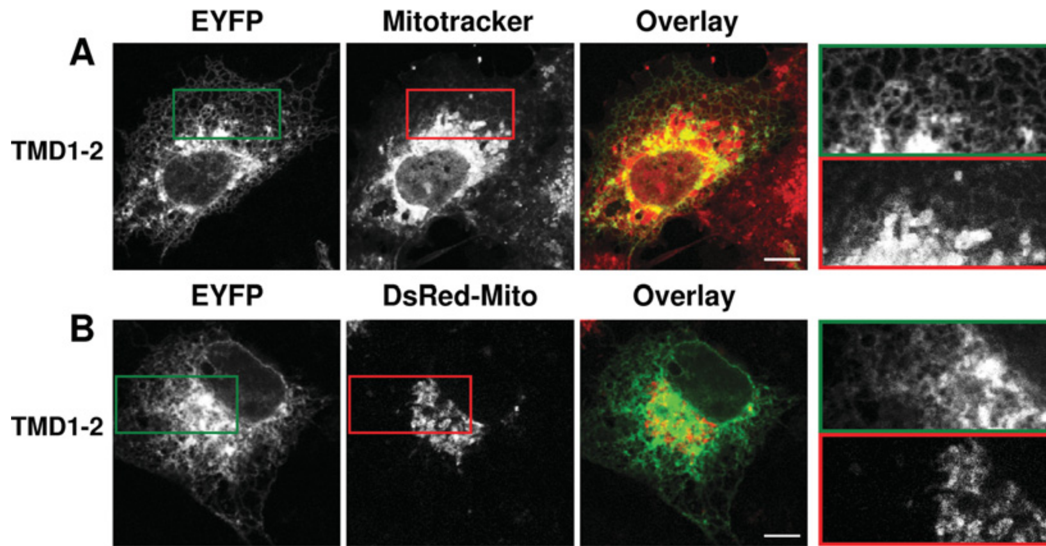

Figure S5 TMD1-2 does not co-localize with mitochondria

Cells transiently transfected with the TMD1-2 construct are shown in the same format as in Figure 2. Bars, 10 μm.

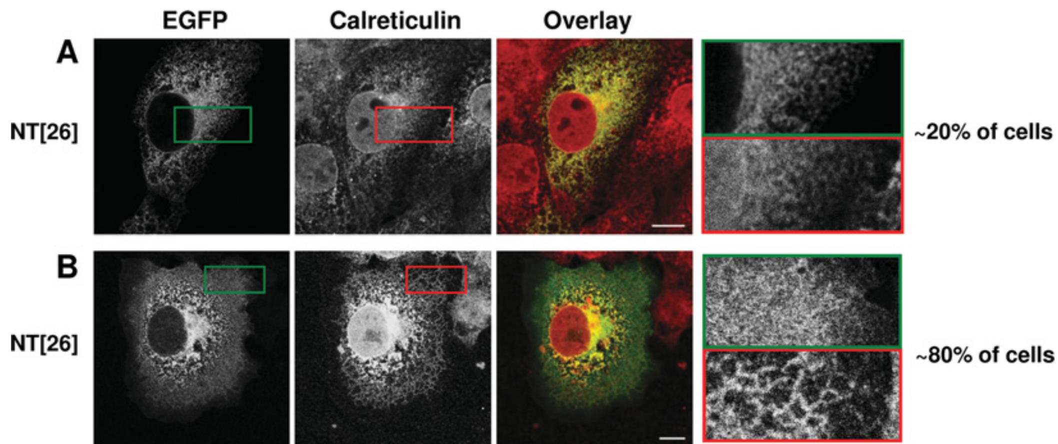

Figure S6 Heterogeneous distribution of NT[26]

Cells transiently transfected with the NT[26] construct are shown in the same format as in Figure 2. Bars, 10 μm. In approx. 20% of cells (**A**), NT[26] was localized to the ER, but in the remainder (**B**) it was cytosolic.

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