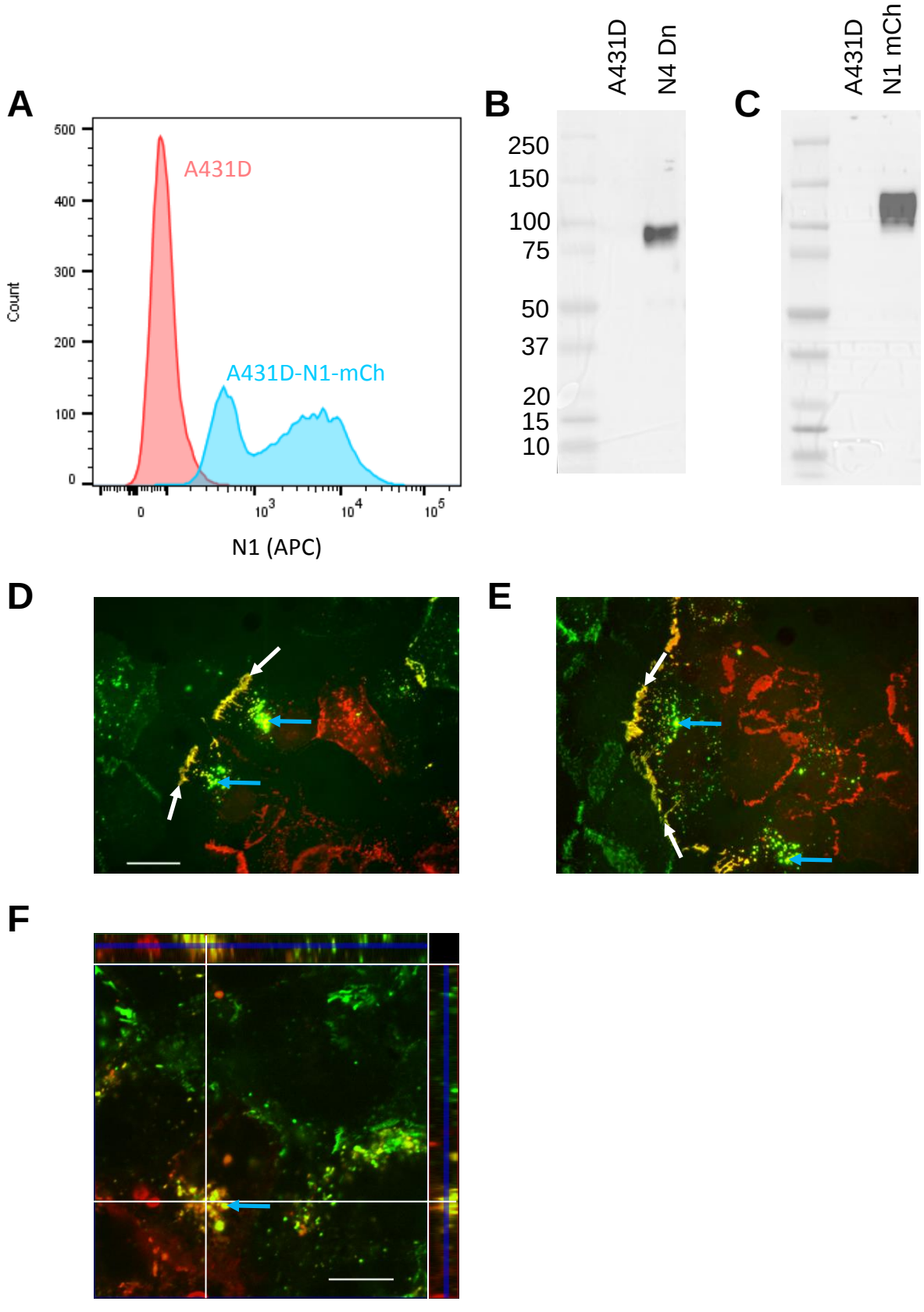
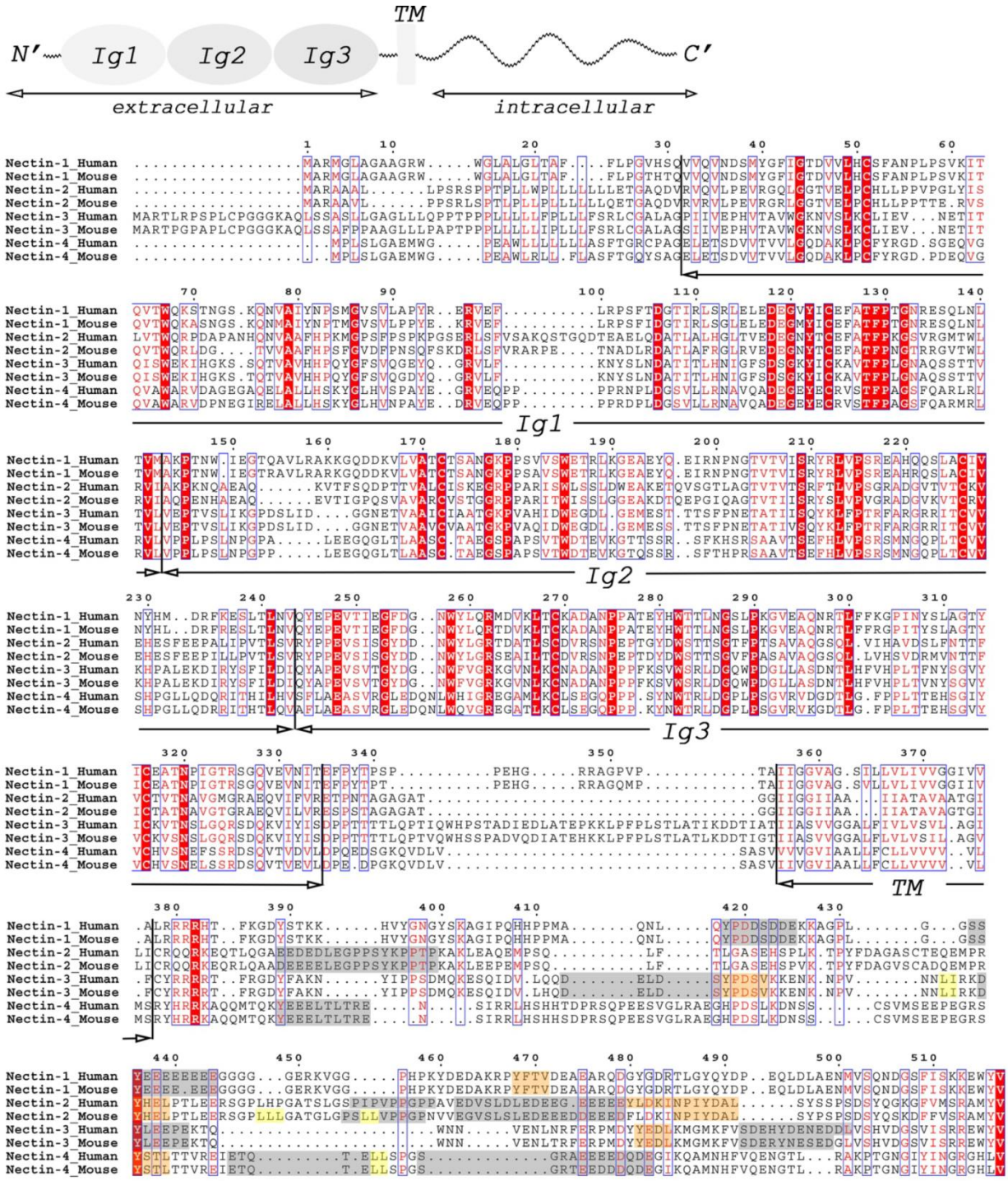


Figure S1. Related to Figure 1. Control experiments for Figure 1. (A) A431D cells do not express N1. Anti-N1 antibody (R1.302, R&D Systems) surface staining of A431D (red line) cells and A431D-N1-mCherry (N1 mCh) cells. (B, C) Western blot analysis of the expression of chimeric N4 and N1 protein by different cell lines. (B) Anti-Dendra2 was used to detect chimeric N4-Dendra2 in control A431D and A431D-N4-Dendra2 (N4 Dn) cells. (C) Anti-mCherry was used to detect chimeric N1-mCherry in control A431D and A431D-N1-mCherry (N1 mCh) cells. (D and E) Immunofluorescence analysis of co-cultures of A431-N4-Dendra2 (green) and A431-N1-mCherry (red) cells. The two panels show different fields of view. The parental A431 cells express cadherins, while A431D cells do not. Merged red and green signals are shown in yellow. White arrows point at co-localization signals at cell adhesion sites, blue arrows at internal co-localization signals. Scale bar = 40 µm. In panels (D-F) yellow signal is sometimes surrounded by green signal. This is expected because these cells express endogenous N1, so they will internalize N4-Dendra2 that does not co-localize with N1-mCherry. (F) Confocal analysis of the same cells with z-dimension shown on the top and right hand side bars. Scale bar = 10 µm.



Dileucine motifs:

LL,LI,IL,II
recognized by AP2 complex
function:internalization

Tyrosine based motifs:

YxxΦ, YxxxΦN (Φ = F,M,L,I,or V)
recognized by AP2 complex

NPxY
recognized by AP2 complex and Dab2
function:internalization

Φ - a bulky hydrophobic residue: F,M,L,I,or V
x - any residue

Other signals:

Acidic clusters
near phosphorylation sites
recognized by PACS-1
function:transport from
trans-Golgi network to endosomes

Proline-rich signals
recognized by TIP47
transport from
trans-Golgi network to endosomes

DDL signal
function:internalization

Figure S2. Related to Figure 2. Potential endocytic motifs in the cytoplasmic regions of nectins. Alignment was created using Clustal Omega[1], colored using %Equivalent similarity coloring scheme as implemented in ESPript 3.0[2]; red color denotes conservation. Potential endocytic signals found in nectin alignment: 1) dileucine based motifs - LL, LI[3]; 2) tyrosine based motifs - YxxΦ[3], YxxxΦN[4], NPxY[5]; 3) other signals - acidic clusters with proximity to a phosphorylation site, FW- or P-rich motif[5], DDL signal[6].

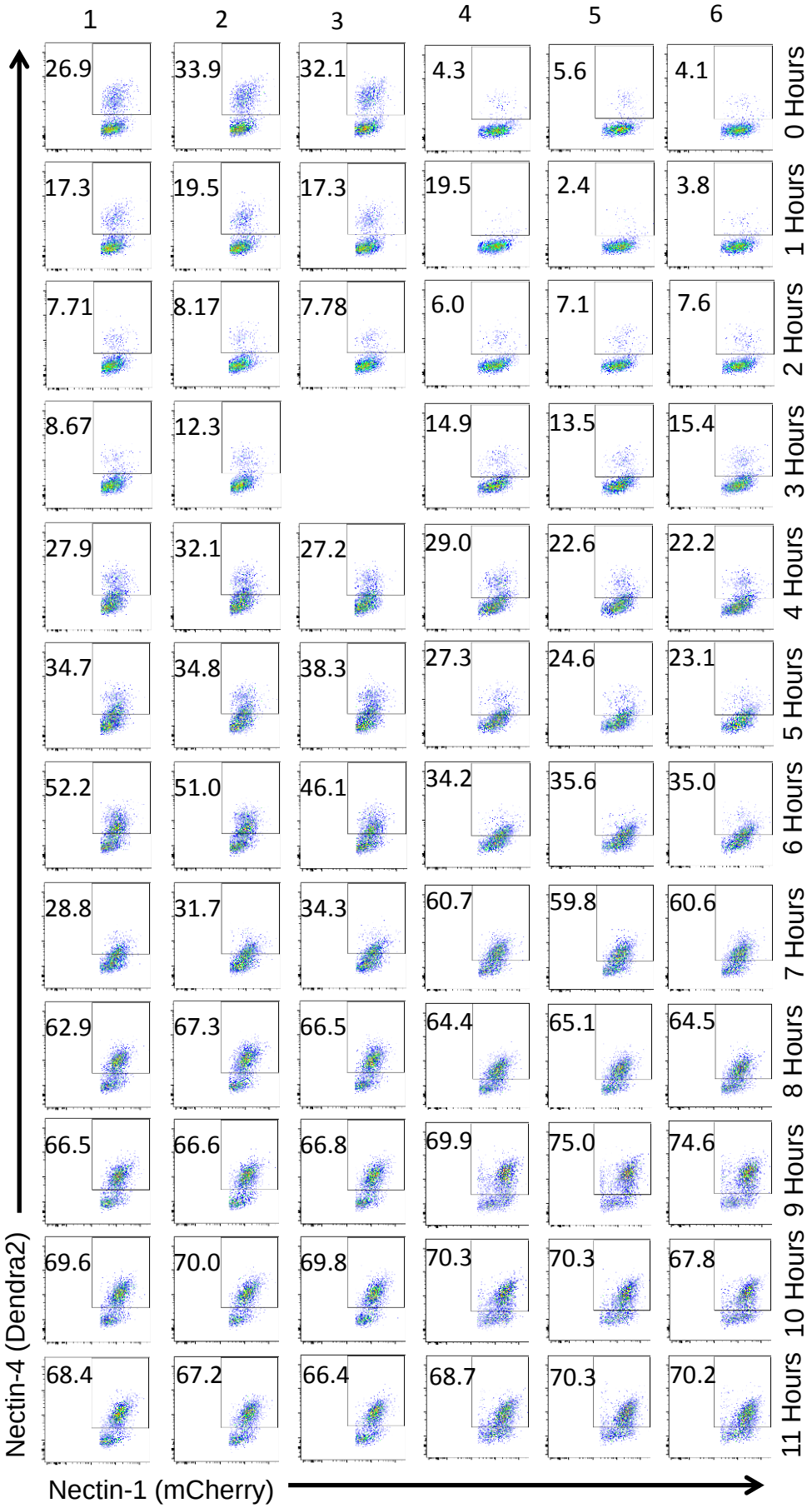


Figure S3. Related to Figure 3. FACS analyses of the kinetics of N4 uptake by N1-expressing cells. Results of two experiments used to generate the graph in Figure 3b are shown. Incubation times are indicated on the right. Columns 1-3 and 4-6: three technical replicates for each experiment. Vertical axis: N4-Dendra2 expression levels. Horizontal axis: N1-mCherry expression levels.

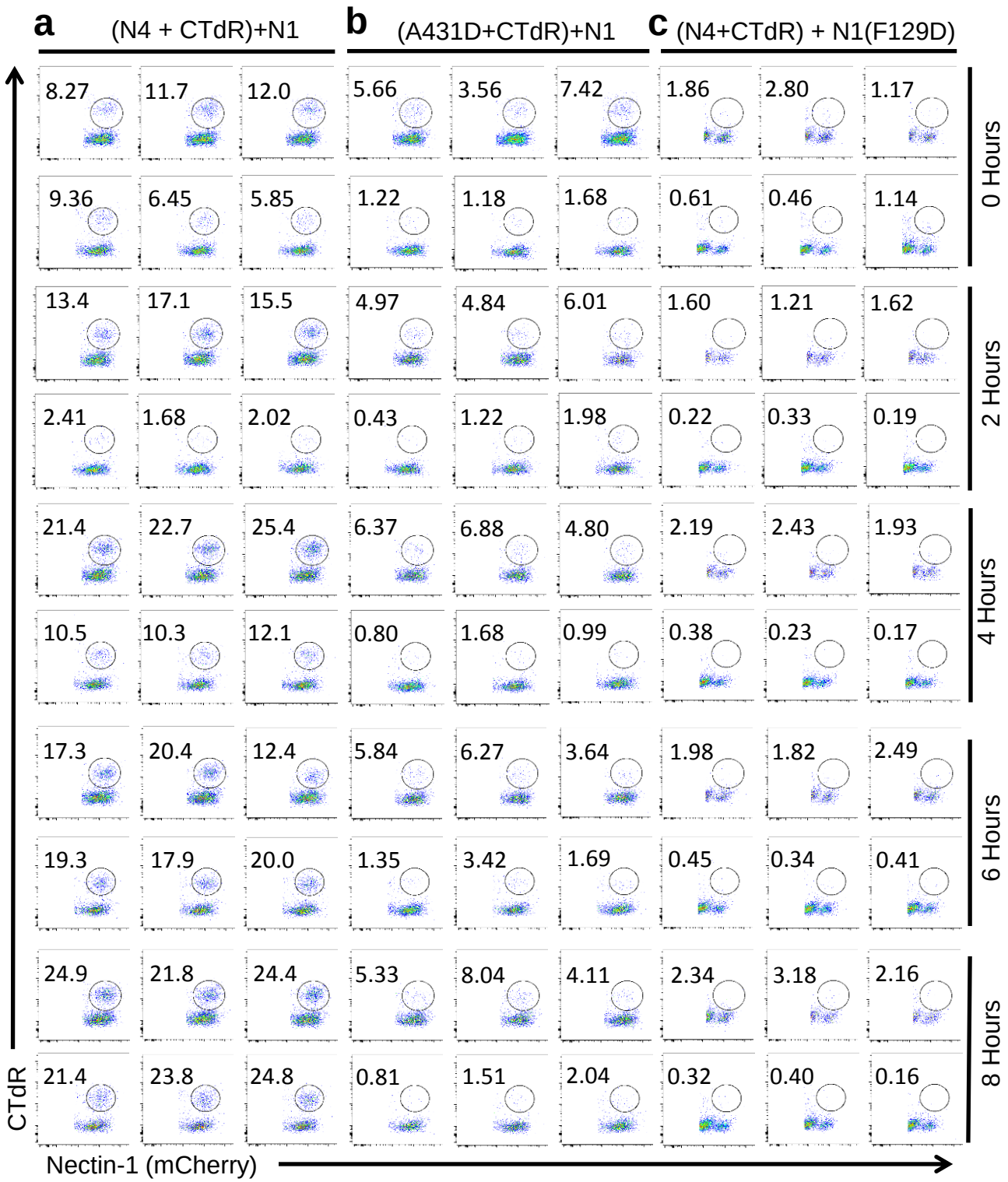


Figure S4. Related to Figure 3. FACS analyses of the kinetics of CTdR uptake by N1-expressing cells. Results of two experiments used to generate the graph in Figure 3d are shown. Incubation times are indicated on the right. The top and bottom rows of panels in each time point are two independent experiments. Three technical replicates are shown within each independent experiment. (a) CTdR-stained N4-expressing cells co-cultured with N1-expressing cells. (b) Negative control for donor cells: CTdR-stained A431D cells co-cultured with N1-expressing cells. (c) Negative control for acceptor cells: CTdR-stained N4-expressing cells co-cultured with N1(F129D)-expressing cells. Vertical axis: CTdR levels. Horizontal axis: N1-mCherry expression levels.

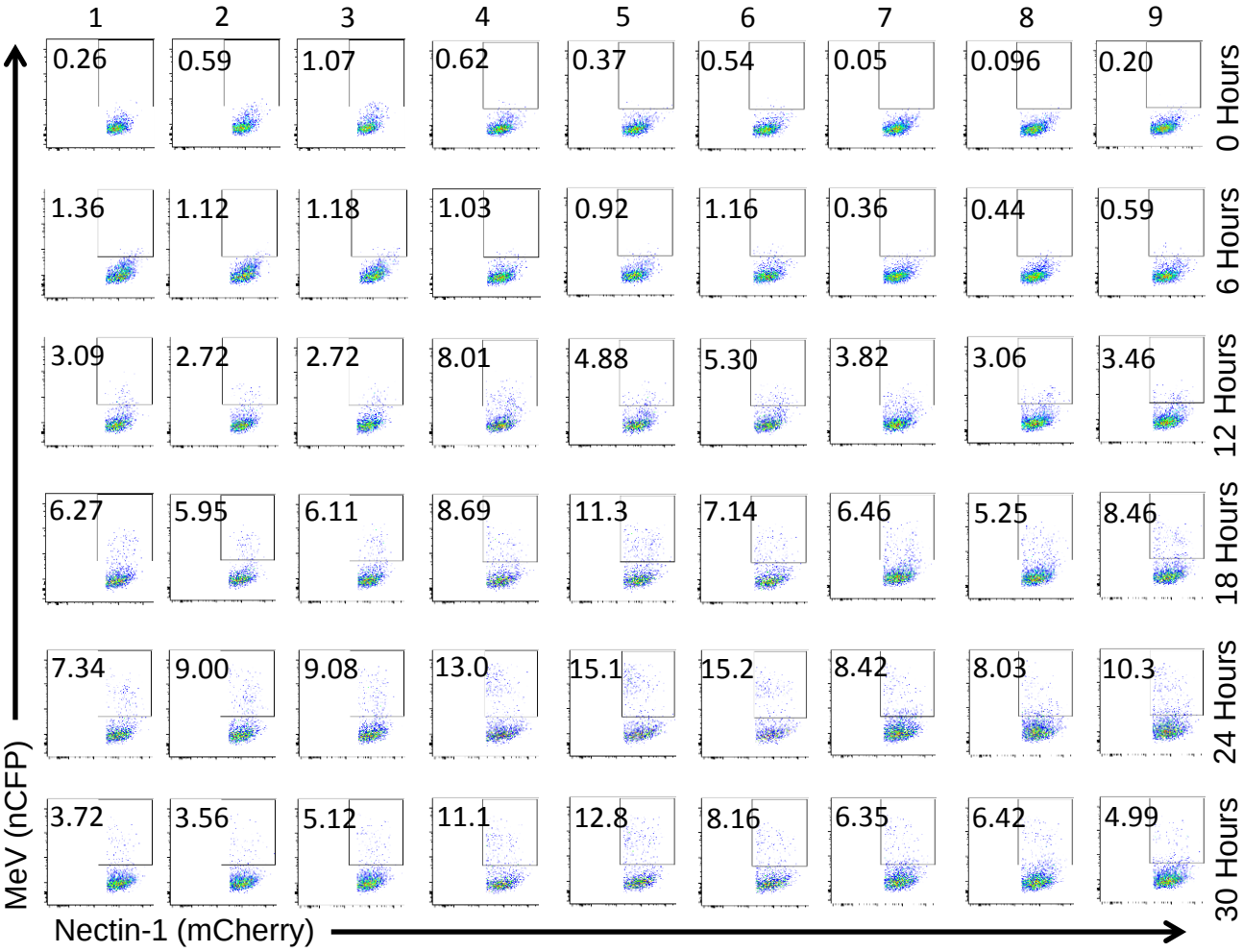


Figure S5. Related to Figure 4. Time course analyses of reporter gene expression after MeV uptake. Results of three experiments used to generate the graph in Figure 4f are shown. N1-expressing cells were overlaid on infected N4-expressing cells for up to 30 hours, as indicated on the right. Columns 1-3, 4-6 and 7-9: technical replicates for the three experiments. Vertical axis: nCFP expression levels. Horizontal axis: N1-mCherry expression levels.

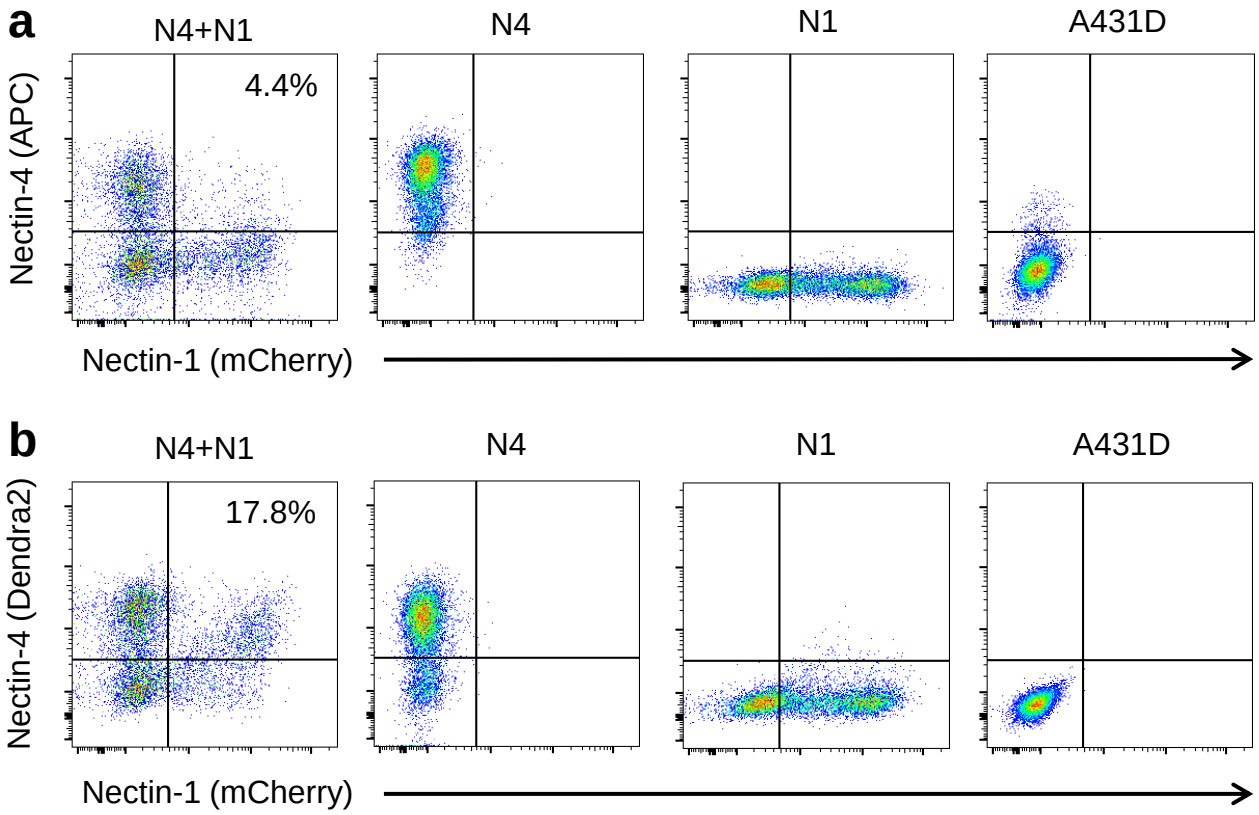


Figure S6. Related to Figure 5. FACS analysis shows efficient N4 internalization by N1-expressing cells. All data were collected 12 hours after seeding the cells indicated above each panel. From left to right: N4 and N1 cells co-cultured for 12 hours, N4 cells alone, N1 cells alone, or A431D cells alone. (a) Cell surface N4, as detected with antibodies (4.4%, first panel, upper right quadrant). (b) Total cellular N4, as detected by Dendra2 fluorescence (17.8%, first panel, upper right quadrant). Vertical axis: N4 detection levels. Horizontal axis: N1-mCherry detection levels.

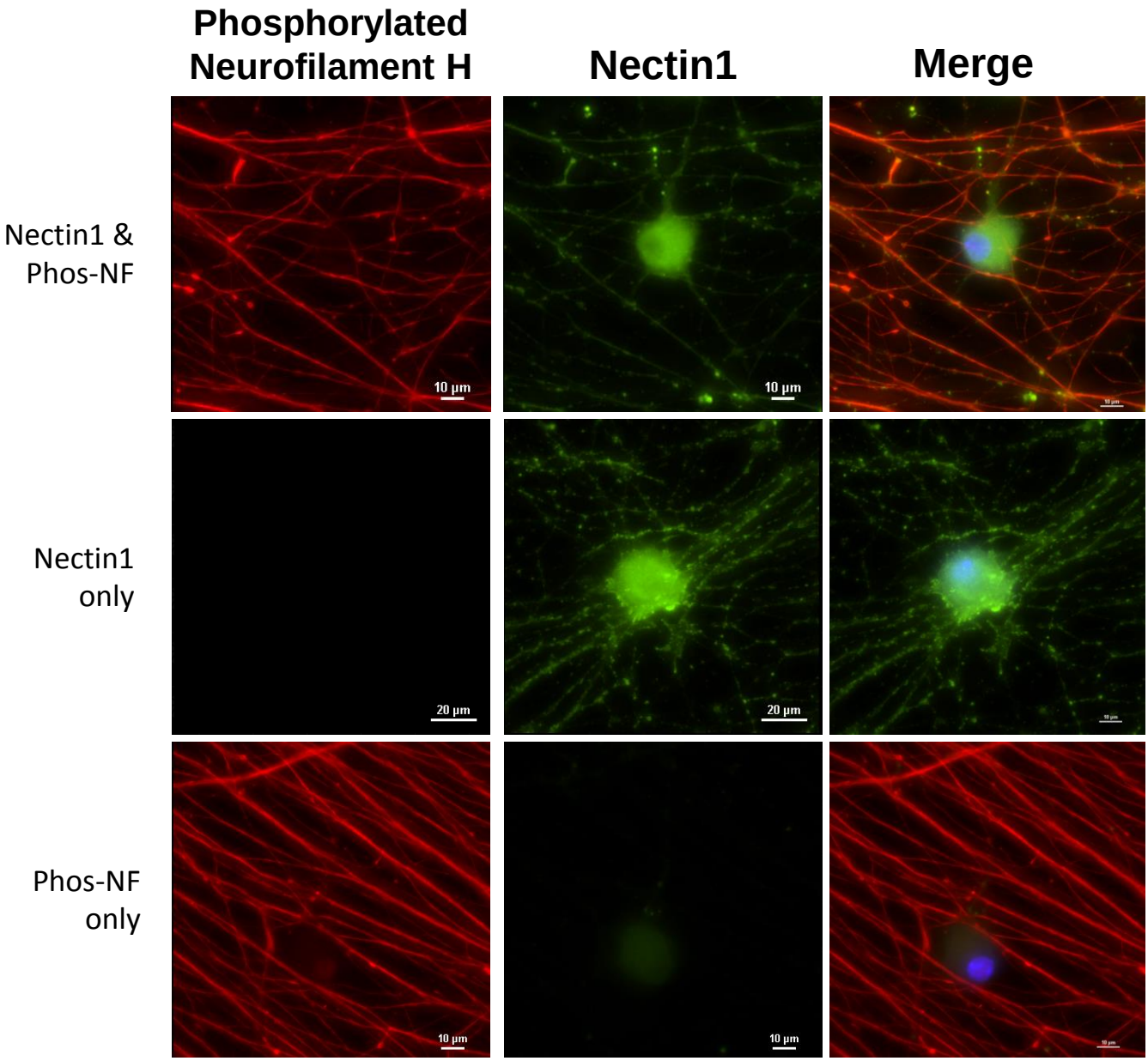
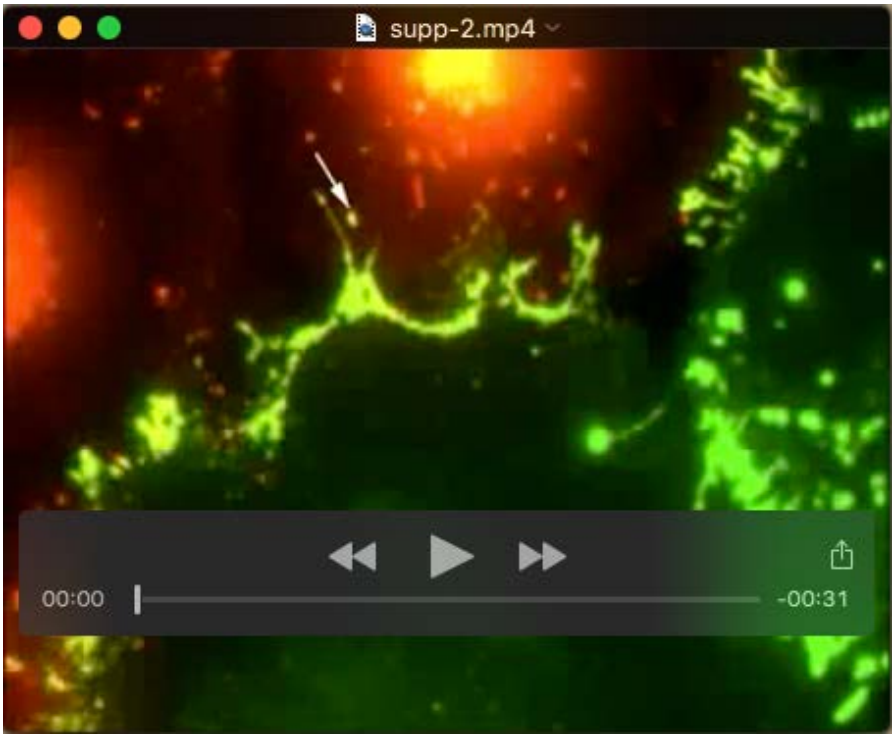


Figure S7. Related to Figure 6. Controls for autofluorescence of neurons and N1 distribution. Neurons were fixed, permeabilized and antibody stained with rabbit polyclonal N1-specific antibody (green) and mouse monoclonal phosphorylated neurofilament H-specific antibody (red) as indicated. All neurons were then stained with species specific, labeled secondary antibodies. Nuclei are contrasted with Hoechst staining (Blue). While phosphorylated neurofilament H is only present in axons, N1 is observed in all neuronal structures, with the highest signal present in the cell body.



Movie 1. Time-lapse microscopy analysis of N4-Dendra2 uptake by N1-mCherry-expressing cells. A431D cells expressing N1-mCherry (red, endosomal localization) were co-cultured with A431D cells expressing N4-Dendra2 (green, plasma membrane localization). Cells were imaged on the second day after plating. Green and red images were taken simultaneously using an image splitter in 1 min intervals using halogen light that minimized phototoxicity and photobleaching and are shown at 2 images per second. White arrows indicate green fluorescence moving from the plasma membrane of N4 cells into N1 cells. Yellow color visualizes co-localization of green and red signals.

SI REFERENCES

Bonifacino, J. S. and Traub, L. M. (2003). Signals for sorting of transmembrane proteins to endosomes and lysosomes. *Annual review of biochemistry* **72**, 395-447. doi:10.1146/annurev.biochem.72.121801.161800.

Kelly, B. T., McCoy, A. J., Spate, K., Miller, S. E., Evans, P. R., Honing, S. and Owen, D. J. (2008). A structural explanation for the binding of endocytic dileucine motifs by the AP2 complex. *Nature* **456**, 976-979. doi:10.1038/nature07422.

Kozik, P., Francis, R. W., Seaman, M. N. and Robinson, M. S. (2010). A screen for endocytic motifs. *Traffic* **11**, 843-55. doi:10.1111/j.1600-0854.2010.01056.x.

Robert, X. and Gouet, P. (2014). Deciphering key features in protein structures with the new ENDscript server. *Nucleic acids research* **42**, W320-4. doi:10.1093/nar/gku316.

Sievers, F., Wilm, A., Dineen, D., Gibson, T. J., Karplus, K., Li, W., Lopez, R., McWilliam, H., Remmert, M., Soding, J. et al. (2011). Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Molecular systems biology* **7**, 539. doi:10.1038/msb.2011.75.

Vohra, R. S., Walker, J. H., Howell, G. J., Homer-Vanniasinkam, S. and Ponnambalam, S. (2009). The LOX-1 scavenger receptor cytoplasmic domain contains a transplantable endocytic motif. *Biochemical and biophysical research communications* **383**, 269-74. doi:10.1016/j.bbrc.2009.04.012.