Electronic Supplementary Material (ESI) for Dalton Transactions.

Terbium-Based Time-Gated Förster Resonance Energy Transfer Imaging for Evaluating Protein-Protein Interactions on Cell Membranes

Stina Lindén, Manish Kumar Singh, K. David Wegner, Marie Regairaz, François Dautry, François Treussart, and Niko Hildebrandt

SUPPORTING INFORMATION

Cell membrane E- and N-cadherin expression for MCF-7 and A549 cell lines, measured by flow cytometry

Flow cytometry immunolabelling protocol. Culture flasks were washed once with PBS. Versene solution (ref. 15040-066, Life Technologies) was then used to dissociate cells at 4°C. Aliquots of 5-6 x 10^5 dissociated cells were prepared and washed twice with PBS + 2% FBS (washing buffer). Cells were then incubated with the washing buffer for 30 minutes at 4°C as a protein blocking step. After removing the washing buffer, the fluorophore-labelled primary antibodies APC anti-human CD324 E-Cadherin (ref. 324108, BioLegend, San Diego, USA) and PE anti-human CD325 N-Cadherin (ref. 350805, BioLegend) were added. For the control, we used the corresponding fluorophore conjugated isotype APC Mouse IgG1, κ Isotype Ctrl (ref. 400122, BioLegend) and antibody PE Mouse IgG1, κ Isotype Ctrl (ref. 400114, BioLegend) diluted in the washing buffer. Aliquots were then kept at 4°C for one hour. The samples were washed 3 times with the washing buffer before the flow cytometry measurements. The flow cytometer used is a guava easyCyte model (EMD Millipore, Massachusetts).

Results

MCF-7 cells (human breast cancer cell line) were used as a reference for strong expression of E-cadherin at the membrane. Figure S1 shows flow cytometry detection of E-cadherin in MCF-7 cells. The experiment was performed on living cells, and staining and blocking were performed at 4°C to keep metabolic activity low (see protocol above). Flow cytometry also confirmed the co-expression of E- and N-cadherins at the cell membrane in A549 cells. Moreover, two populations of A549 cells were observed: cells located in the first quadrant of Figure S2(a) are positive for both E- and N-cadherins (E^+/N^+), whereas cells in the second quadrant are positive for E-cadherin but negative for N-cadherin (E^+/N^-).

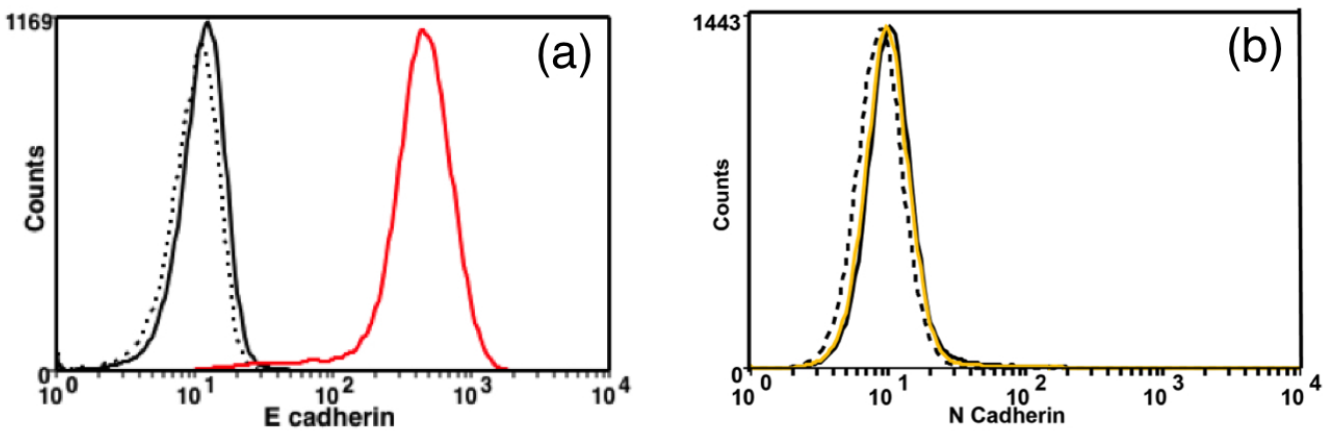

Figure S1: Flow cytometry measurement of (a) E-cadherin and (b) N-cadherin proteins membrane expression for MCF-7 cells. For E-cadherin labeling, MCF-7 cells were targeted with APC anti-human CD324 (E-Cadherin) Antibody (red line). Control sample was treated with APC Mouse IgG1, κ Isotype Ctrl (black regular line). For N-cadherin labeling, MCF-7 were treated with PE anti-human CD325 (N-Cadherin) antibody (yellow line). Control sample was treated with PE Mouse IgG1, κ Isotype Ctrl (black regular line). Autofluorescence samples for E- and N-cadherin experiments were exposed to the washing buffer (dashed lines). These results show that MCF-7 cells express E cadherin but not N-cadherin.

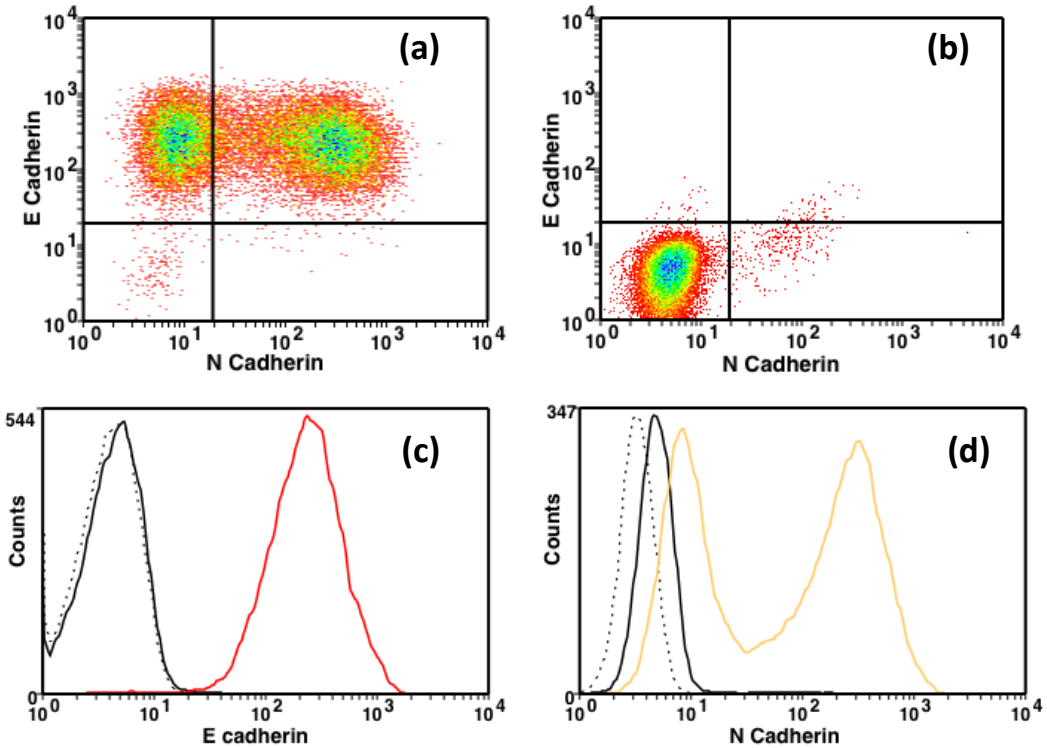

Figure S2: Flow cytometry measurement of E-cadherin and N cadherin expressions in A549 cells. (a) Dot plot showing A549 cells labelled simultaneously with APC anti-human CD324 (E-Cadherin) antibody and PE anti-human CD325 (N-Cadherin) antibody. (b) Control samples treated with APC Mouse IgG1, κ Isotype Ctrl and Antibody PE Mouse IgG1, κ Isotype Ctrl. Comparing the results (a) and (b), it can be concluded that the labelling of both proteins is highly specific. The observation of two cell populations indicates the presence of different phenotypes in the cell line. The major population, which represents approximately 55% of the total cells, expresses both proteins. (c) Histogram of E-cadherin expression, APC anti-human CD324 (E-Cadherin) Antibody (red line), control sample was treated with APC Mouse IgG1, κ Isotype Ctrl (black regular line) and autofluorescence sample was exposed to the washing buffer (dashed line) and (d) Histogram of N-cadherin expression, PE anti-human CD325 (N-Cadherin) Antibody (yellow line). Control sample was treated with PE Mouse IgG1, κ Isotype Ctrl (black regular line). Autofluorescence sample was exposed to the washing buffer (dashed line).

PL decay (and average decay times) of Tb-labelled antibodies

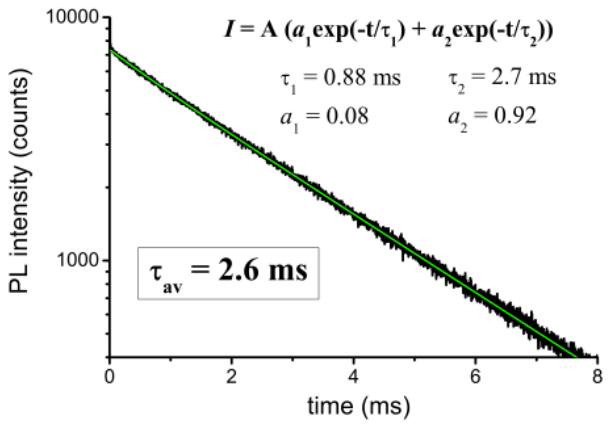

Figure S3: PL decay curve and determination of PL decay time of Tb-labelled antibodies

Additional TG imaging and control experiments

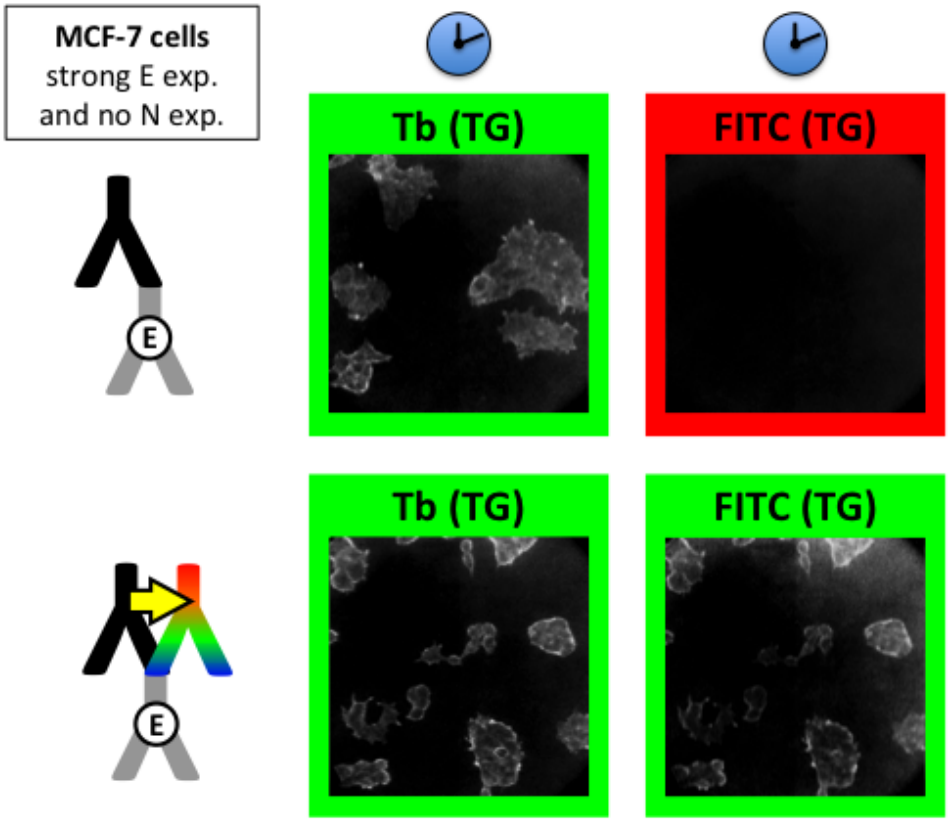

Figure S4: Time-gated FRET microscopy images of control experiments for E-cadherin expression on MCF-7 cells, using FITC-labelled acceptor secondary antibodies.

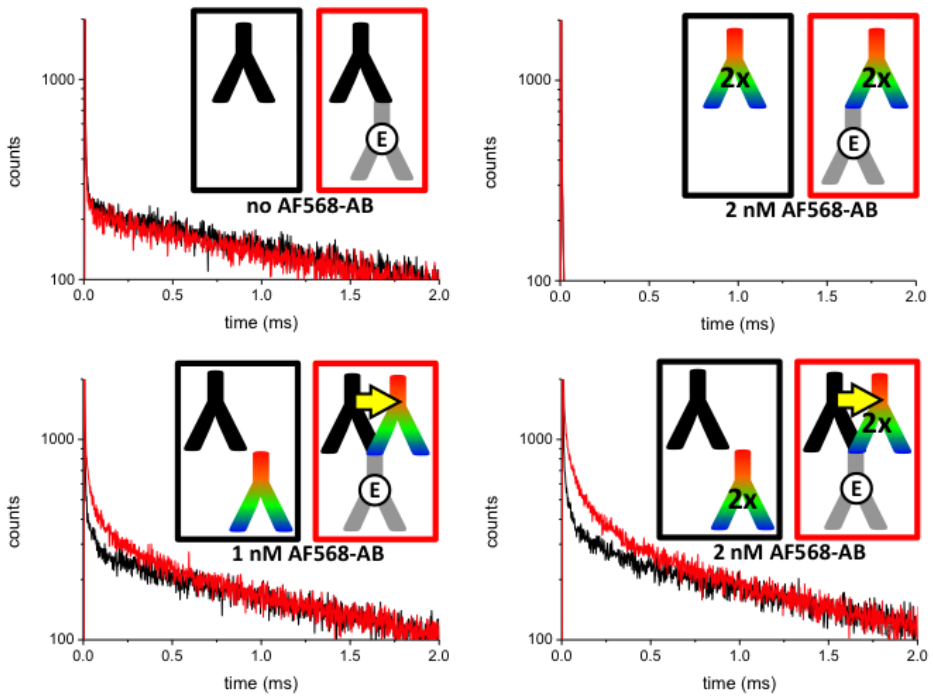

Figure S5: PL decay curves of solution-phase assays demonstrating Tb-antibody and AF568 dye-antibody binding to the same primary antibody.

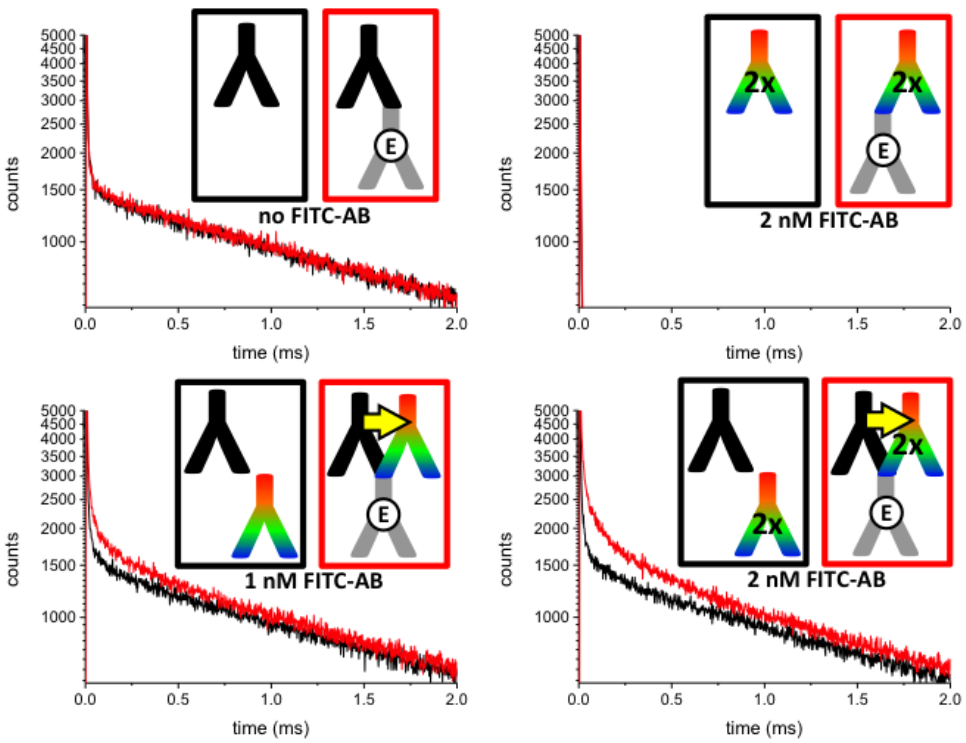

Figure S6: PL decay curves of solution-phase assays demonstrating Tb-antibody and FITC dye-antibody binding to the same primary antibody.

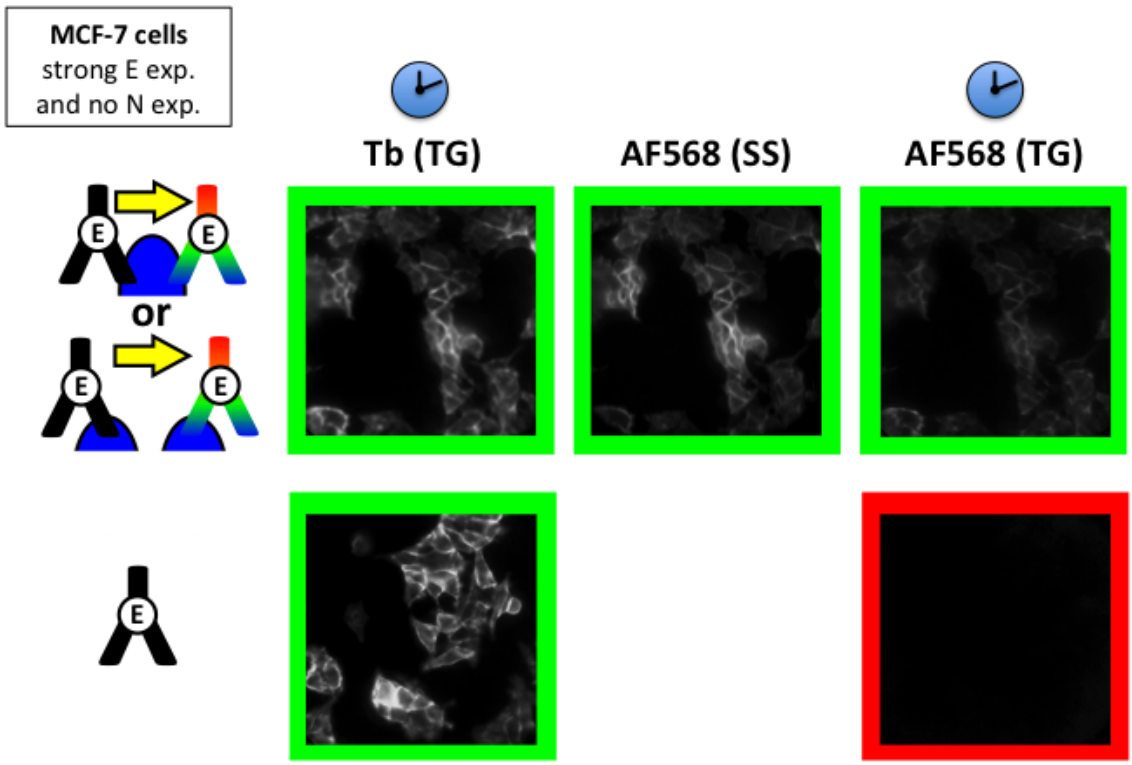

Figure S7: Control experiments showing that there is no crosstalk of Tb PL in the FRET channel (AF568) in the case of the E-cadherin cluster investigations using FRET and MCF-7 cells.

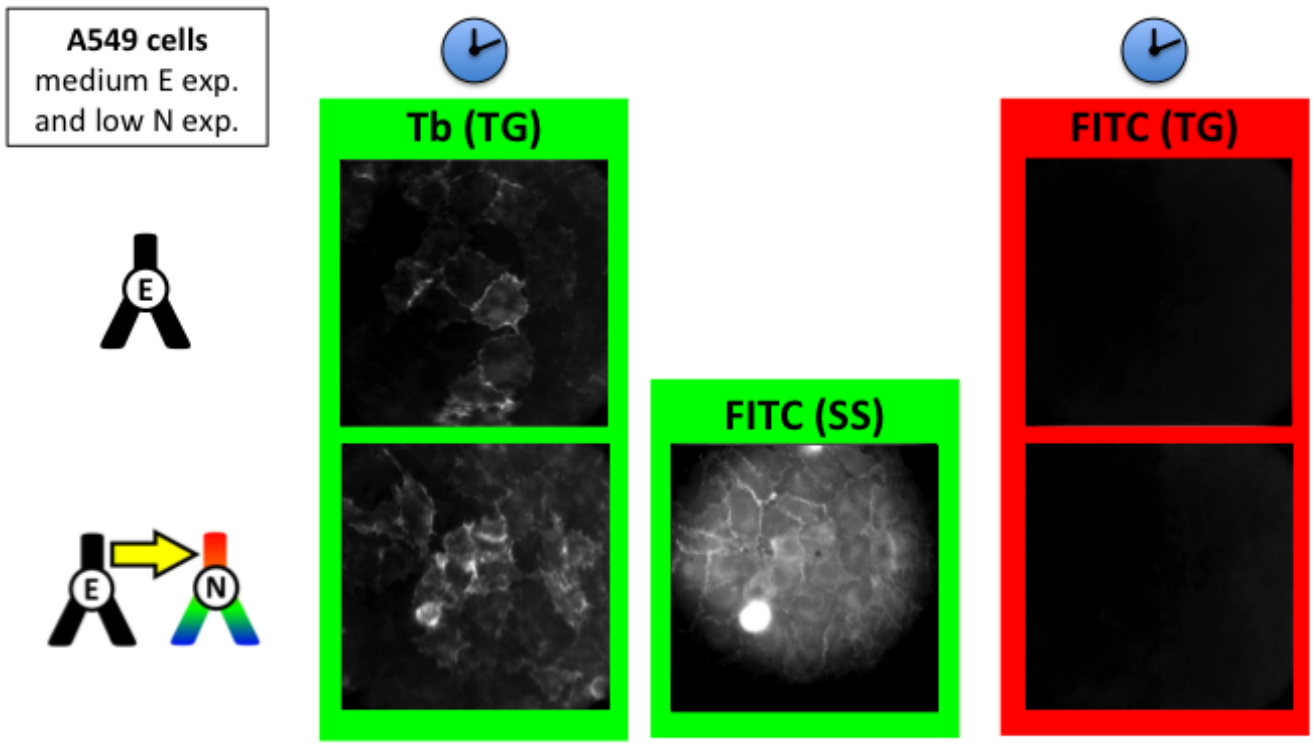

Figure S8: Time-gated and steady-state microscopy images of control experiments for E/N cadherin co-expression on A549 cells, using FITC-labelled N-cadherin primary antibodies.

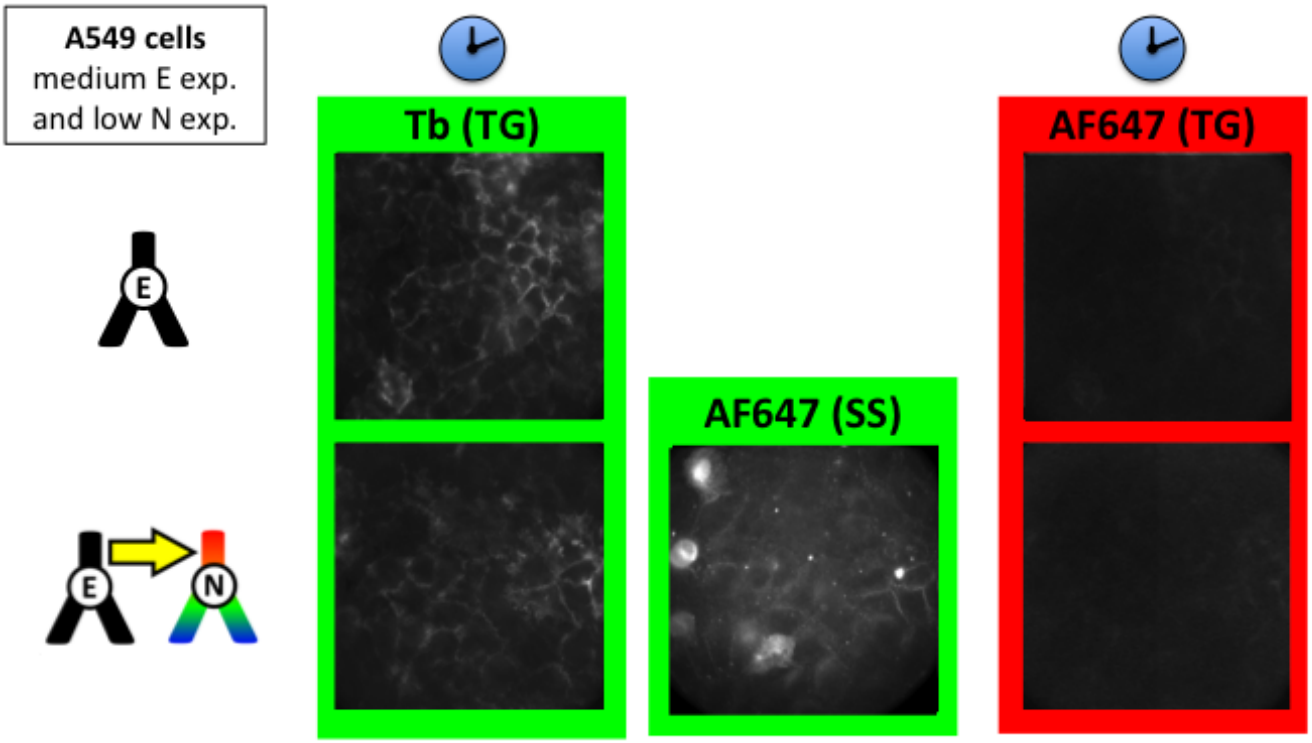

Figure S9: Time-gated and steady-state microscopy images of control experiments for E/N cadherin co-expression on A549 cells, using AF647-labelled N-cadherin primary antibodies.

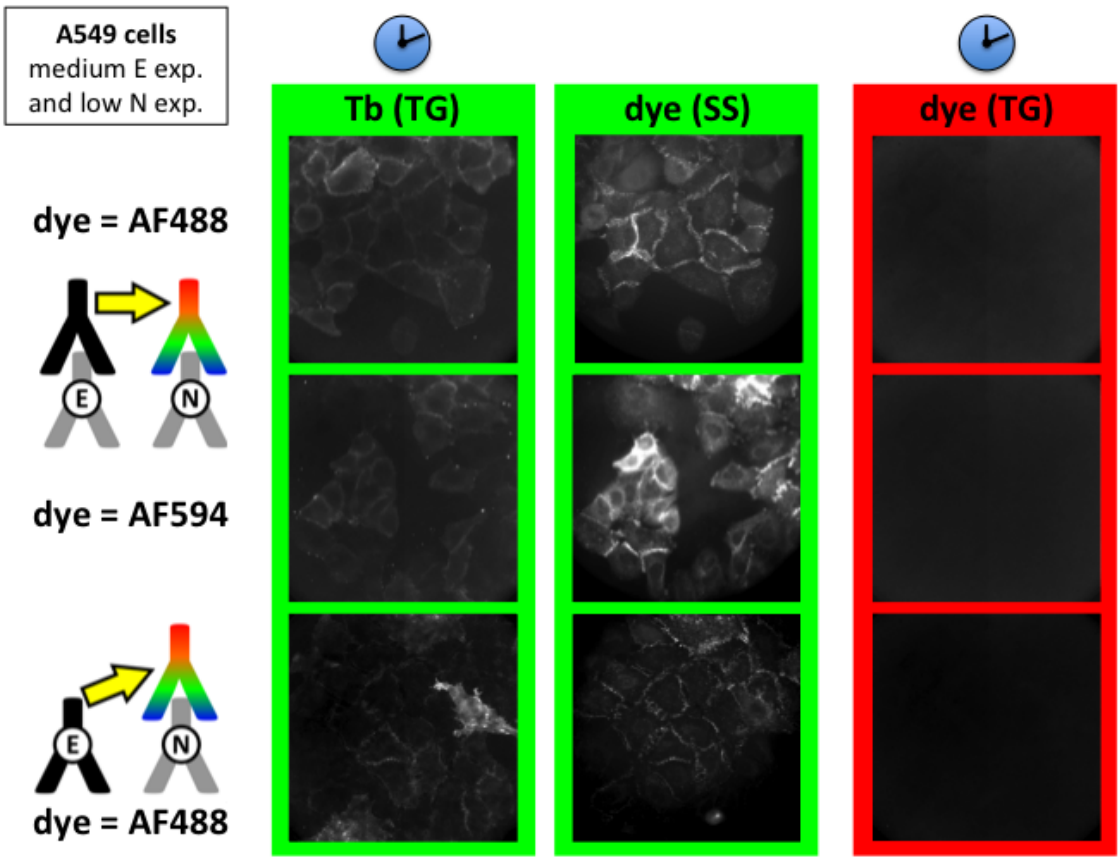

Figure S10: Time-gated and steady-state microscopy images of control experiments for E/N cadherin co-expression on A549 cells, using Tb- and AF488-labelled (or AF594-labelled) secondary antibodies against E- and N-cadherin primaries, respectively (two top lines). Bottom line: similar experiments but with Tb-labelled primary antibodies against E-cadherin and AF488-labelled secondaries against N-cadherin primaries. AF594 was measured with the AF568 transmission filter (607±4 nm) to suppress spectral crosstalk from Tb.

Additional images concerning photobleaching (Figure S11) and the order of image acquisition and successful co-labelling of N-cadherin with AF488 and AF568-labelled antibodies (Figure S12). In these Figures the identification of central transmission or reflection wavelengths of the filters and dichroic mirrors used is given by X/Y/Z, where X is the central transmission wavelength of the excitation filter, Y is the central wavelength between reflection and transmission of the dichroic mirror, and Z is the central transmission wavelength of the emission filter.

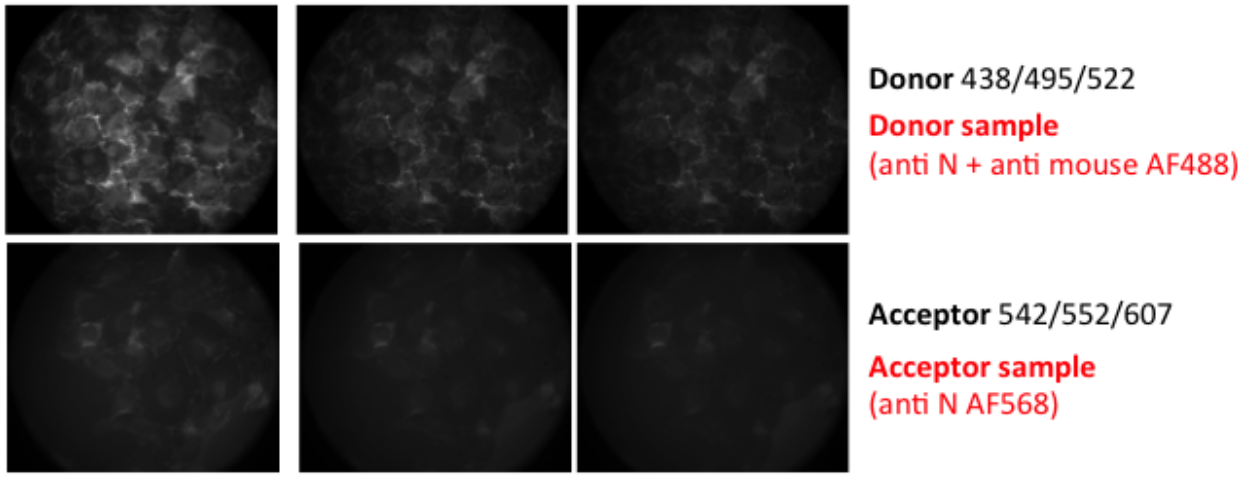

Figure S11: Photobleaching of acceptor and donor dyes using N-cadherin targeting in M4-T cells.

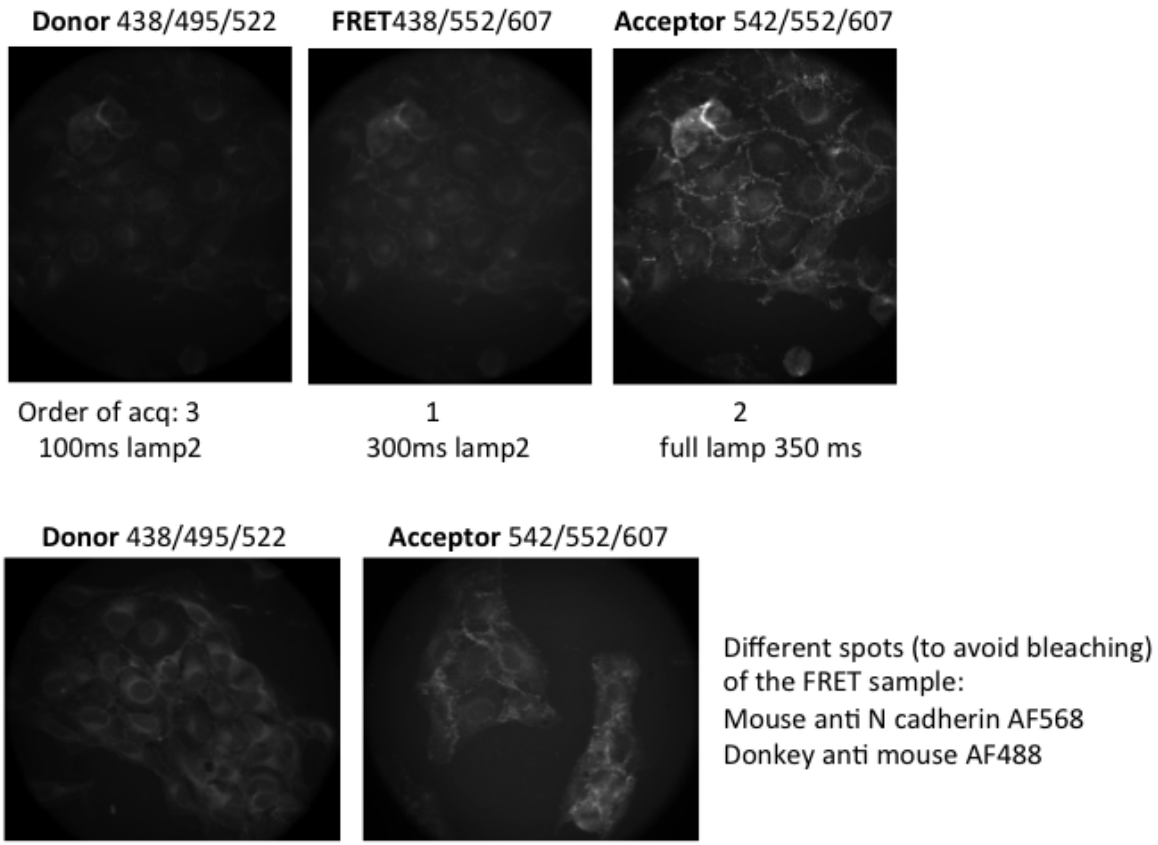

Figure S12: Top: The order of acquisition of the same imaging spot may change FRET interpretation because photobleaching is convoluted with donor quenching and acceptor sensitization. Bottom: Two images taken from the same sample at different cells to avoid photobleaching but to show co-staining of N-cadherins on the M4-T cells with AF488-labelled and AF568-labelled antibodies.