Modifiers of prion protein biogenesis and recycling identified by a highly-parallel endocytosis kinetics assay

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Supporting Information

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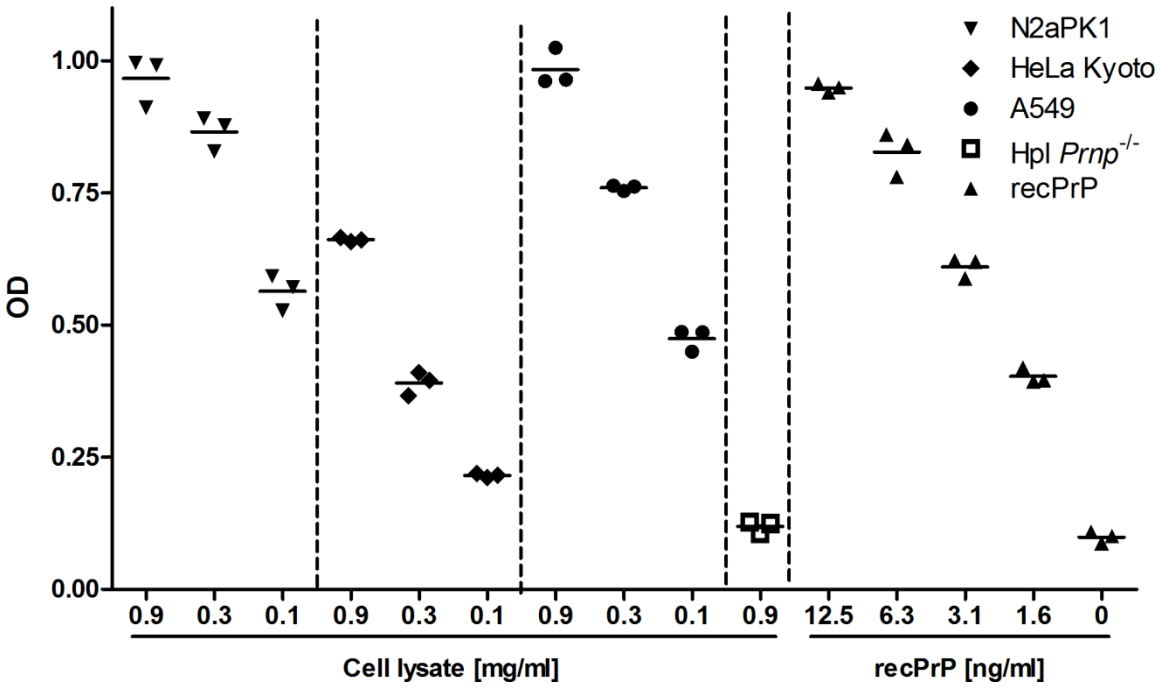

Figure S1. Cell line selection for the FRET assays by sandwich ELISA.

PrP^C levels from crude cell lysates were detected by POM2/POM2bio ELISA. Plates were coated with POM2 antibody and detected with biotinylated (bio) POM2.

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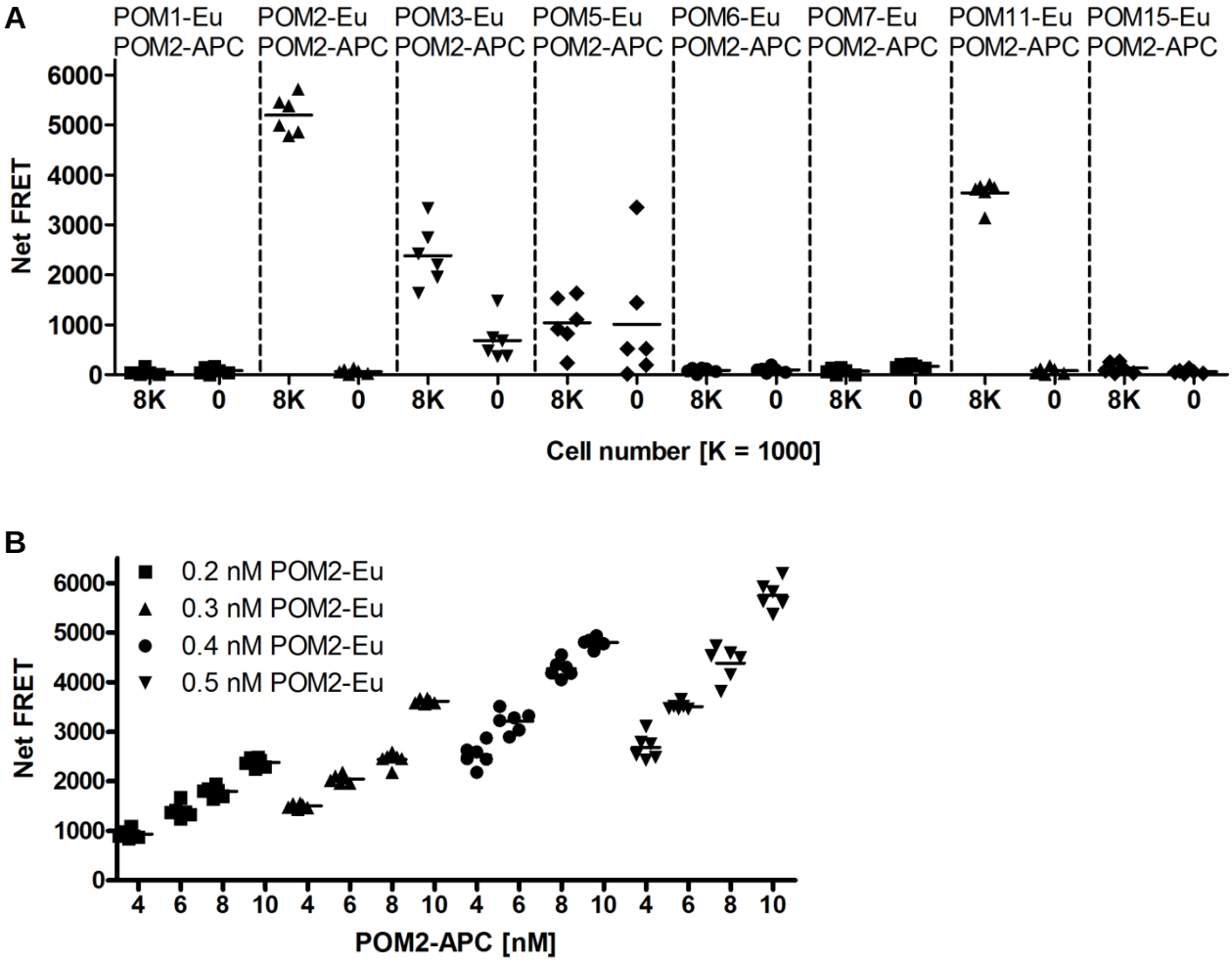

Figure S2. Selection of best POM antibody pair for the detection of cell surface PrP^C by FRET. (A) Different FRET antibody combinations were tested for the detection of PrP^C on the surface of living A549 cells. APC-coupled POM2 antibody (POM2-APC) was used as FRET acceptor for all experiments. Various antibodies (POM1, 2, 3, 5, 6, 7, 11, 15, 3F4) were coupled to Eu and tested. (B) Titration of the POM2-Eu and POM2-APC FRET antibody pair in HeLa cells (K = 8000). Shown are results from hexaplicates ± SD.

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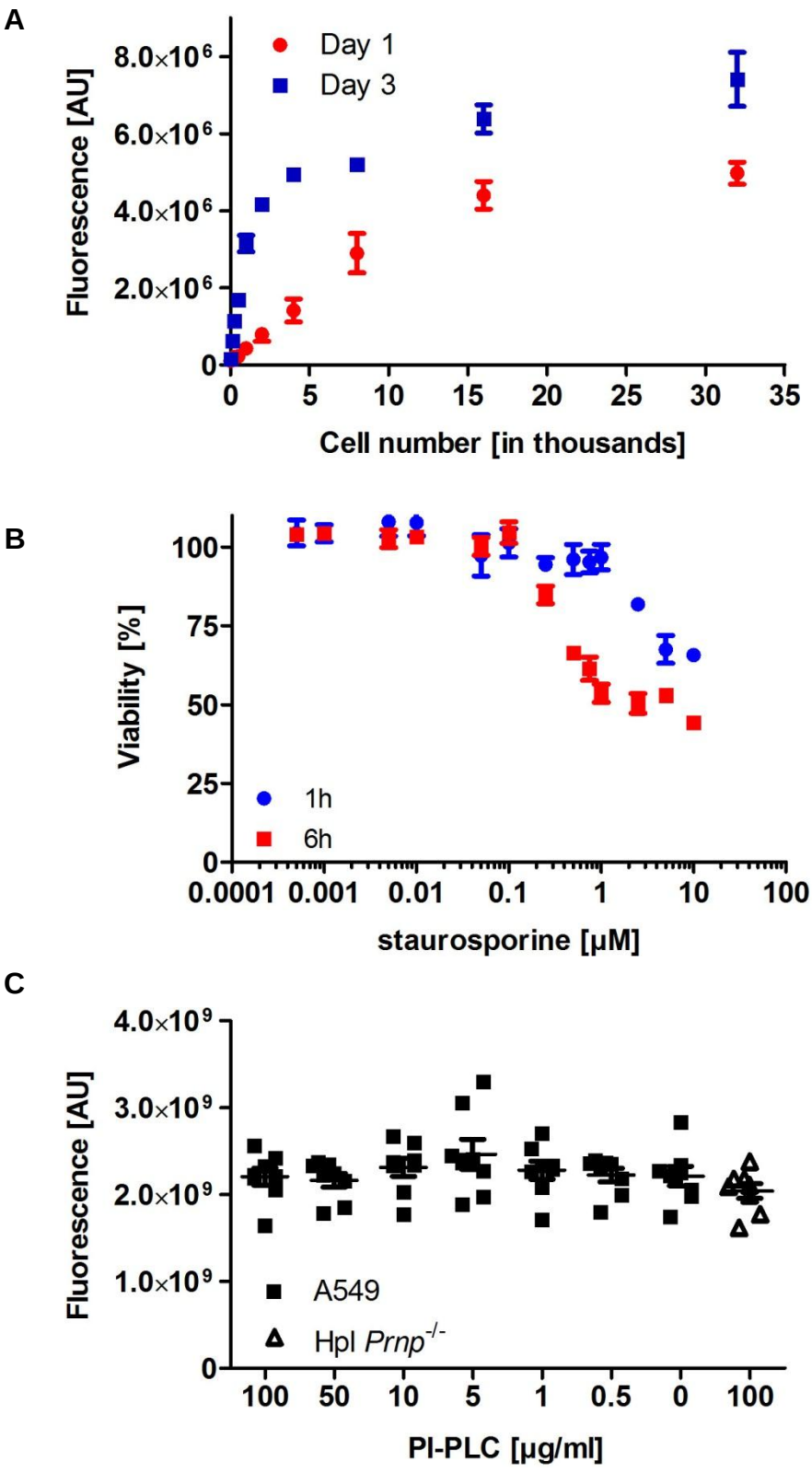

Figure S3. Optimization of the alamarBlue cell viability assay.

(A) Viability of HeLa cells was monitored over three days in a titration experiment, day one (red) and day three (blue). AU: Arbitrary Units. (B) Measurement of cell death induced by Staurosporine treatment for one or six hours. AU: Arbitrary Units. (C) Effect of PI-PLC treatment on cell viability of A549 cells. AU: Arbitrary Units.

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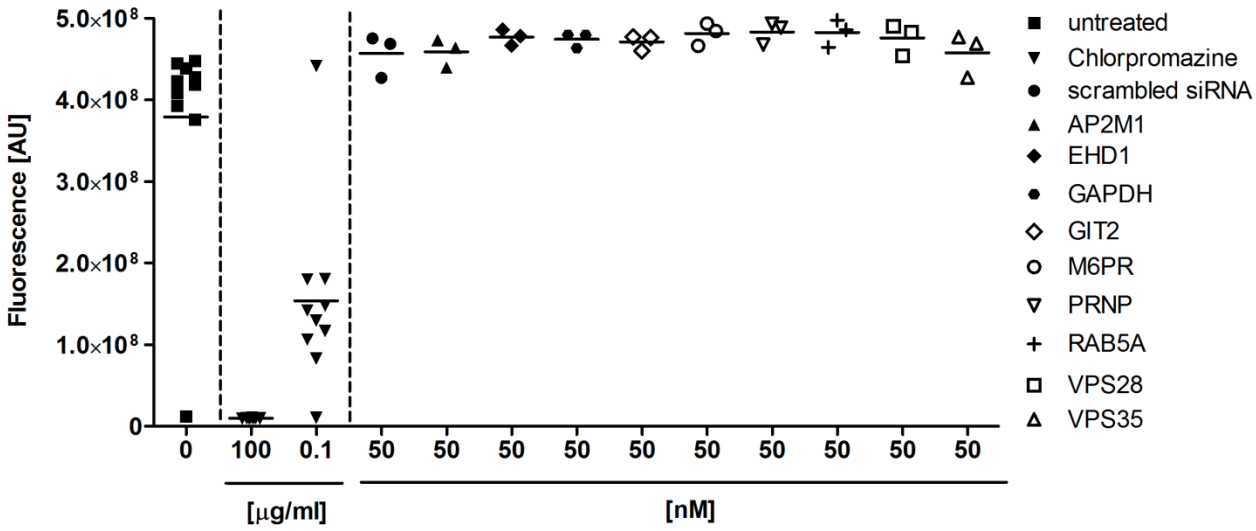

Figure S4. Viability of siRNA-treated A549 cells.

A549 cells were treated for three days with 50 nM siRNA. Cell viability was assessed by the alamarBlue assay. Chlorpromazine (CPZ) was used as a positive control for cell death. Shown are biological triplicates of siRNA treated cells.

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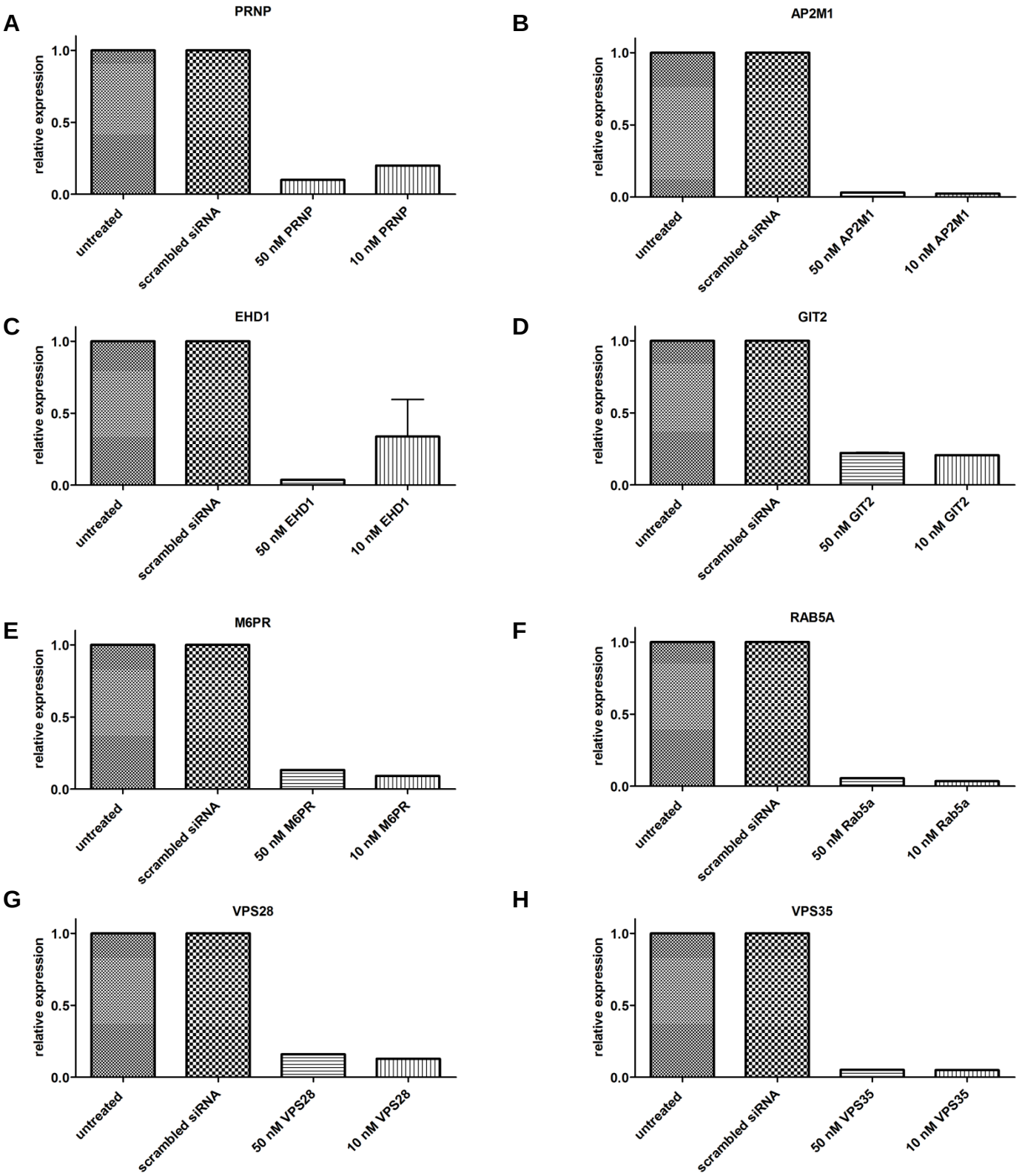

Figure S5. Functionality test of used siRNAs by qPCR in A549 cells.

The gene-silencing activity in A549 cells treated for three days with different siRNAs was quantified by real-time PCR. mRNA levels were normalized by using GAPDH mRNA. Shown are technical triplicates ± standard deviation.

Ballmer et al., Figure S6

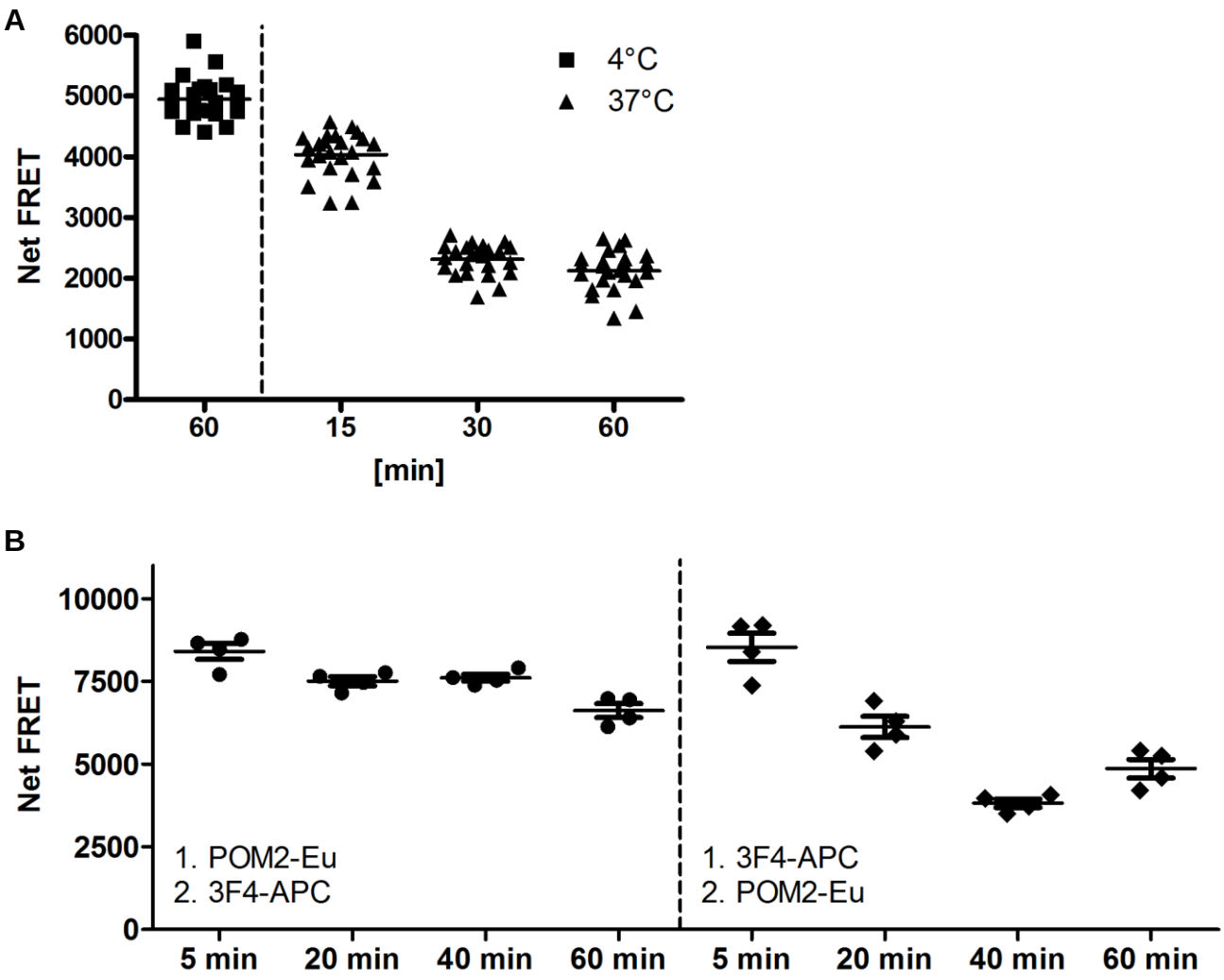

Figure S6. Optimization of PrP^C endocytosis assay

(A) PrP^C endocytosis assay in HeLa cells. Endocytosis rate was measured at 15, 30, and after 60 minutes. In controls, endocytosis assay was performed at 4°C. (B) PrP^C endocytosis assay in A549 cells. Endocytosis rate was measured at different time points. Cells were either labeled first with POM2-Eu and then with 3F4-APC or first with 3F4-APC and then with POM2-Eu.