SUPPLEMENTARY FIGURES

Figure S1

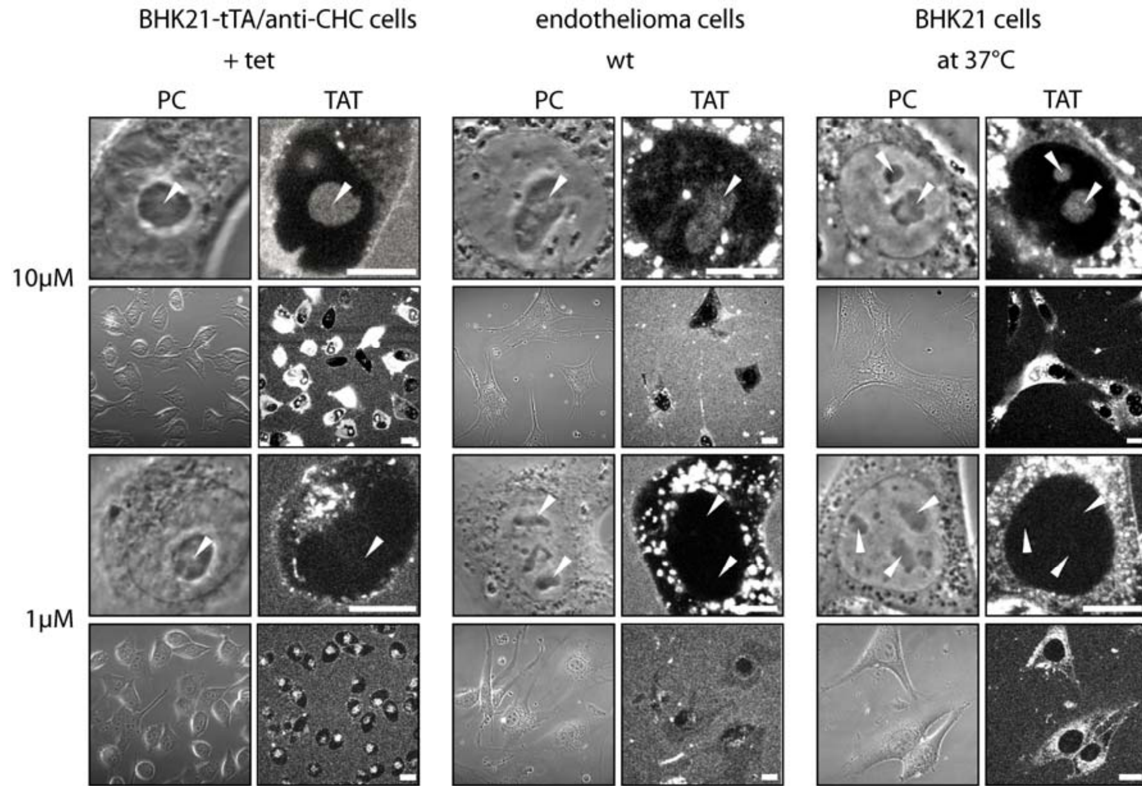

Fig. S1. Concentration-dependent mode of uptake of TAT CPP. TAT was incubated with the cells indicated at 1 and 10 μM concentration for one hour. Confocal optical sections of living cells are displayed with phase contrast (PC) and fluorescence images to show the uptake mode and intracellular distribution of TAT. Each panel contains high and low magnification images. Below a certain threshold concentration, transduction of TAT CPP, (visualized by the presence of peptide freely available in the cytoplasm and accumulation in the nucleolus (arrowheads) as shown in Fig. 1-5) does not occur whereas endocytosis of the TAT CPP persists as shown here. Scale bar 10µm for high and 20 µm for low magnification images.

Figure S2

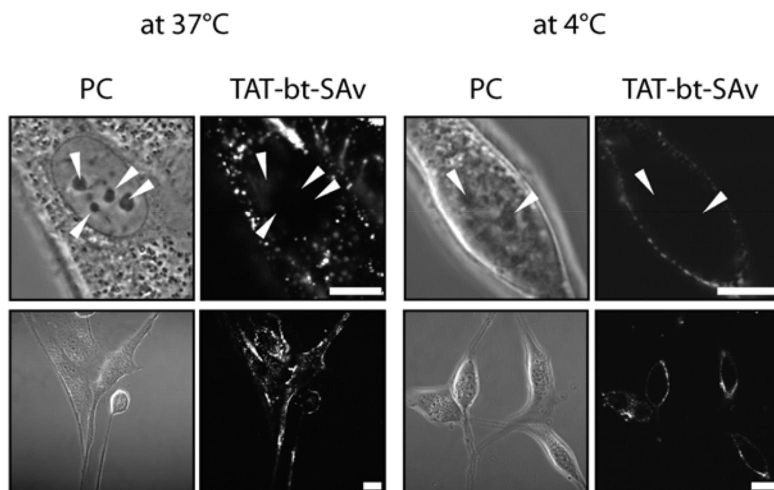

Fig. S2. Low temperature incubation abrogates all endocytic pathways. Mouse fibroblasts were incubated at 37°C and 4° C with the globular protein TAT-biotin-streptavidin-Cy5 (TAT-bt-SAv) as an additional fluid phase marker to the peptide PTD_4 and the fluorophore FITC* shown in Fig. 4. Confocal optical sections of living cells are shown as high and low magnifications after one hour incubation with the globular TAT-fusion protein. Arrowheads mark the nucleoli. Protein uptake was blocked at the level of the plasma membrane at a temperature of 4°C, whereas at 37°C the protein became rapidly internalized into vesicles. Scale bar 10µm for high and 20 µm for low magnification images.

Figure S3

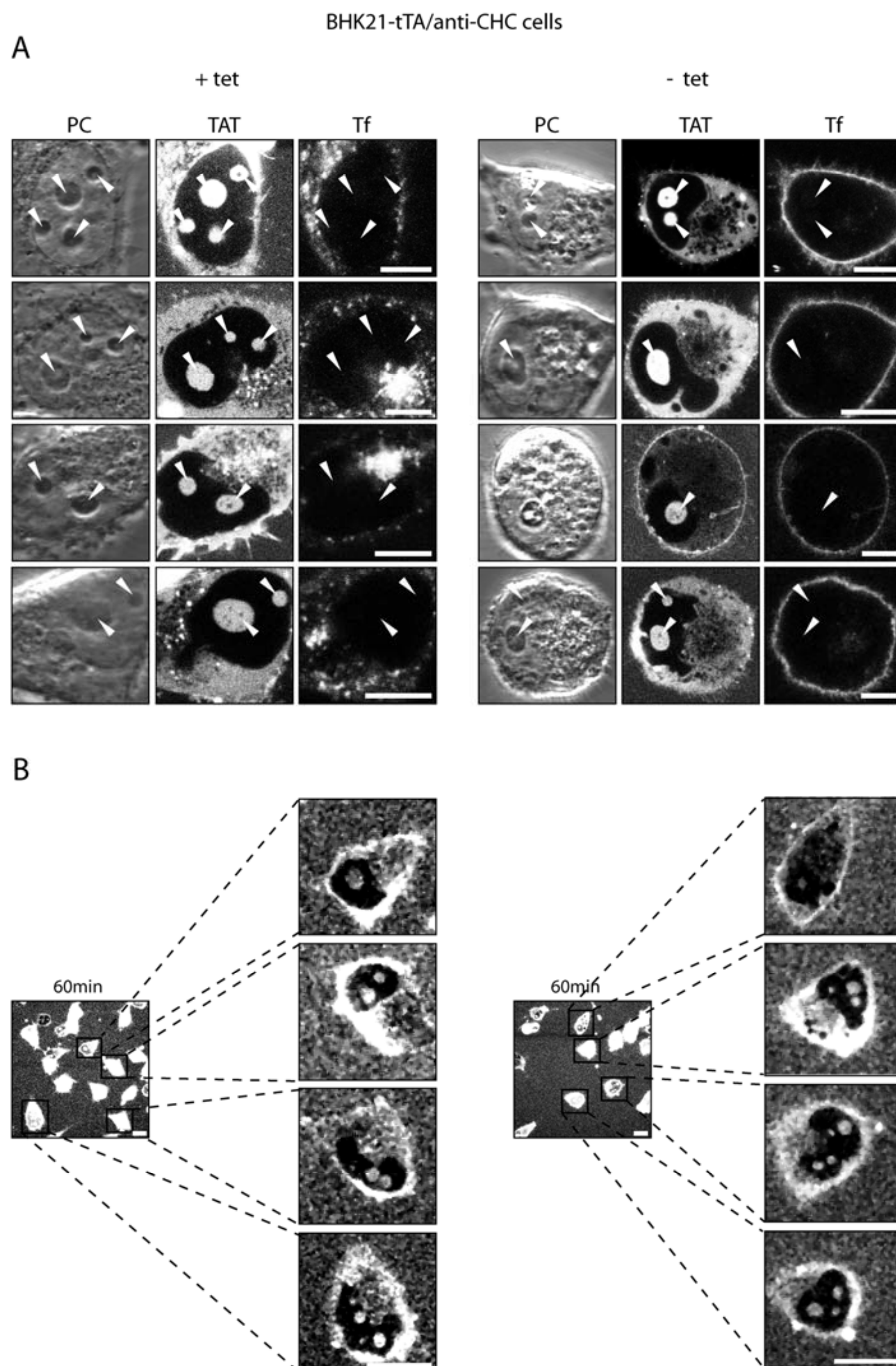

Fig. S3. Transduction of TAT is independent of clathrin-mediated endocytosis. Additional examples to the ones depicted in Fig. 2 are shown as high magnification images (*A*) and images magnified from the online supplementary movies S1 and S7 (*B*) after incubation with the TAT peptide for 60 minutes. Confocal optical sections of living cells during incubation with the fluorescent CPP TAT in the presence of transferrin (Tf) as a marker for clathrin-dependent endocytosis (only *A*). The control cells (left panel) and the cells after knockdown of the clathrin heavy chain (right panel) are shown as images of the phase contrast (PC) and the peptide (*A*) or of the peptide alone (*B*). Arrowheads mark the position of nucleoli (*A*). While uptake of Tf is nearly abolished after tetracycline removal over a period of six days (*A*) and vesicular uptake of TAT is reduced, the TAT CPP is still capable of reaching all intracellular compartments (non-vesicular, cytoplasmic and nucleolar fluorescence), indicating that this mode of uptake is not influenced by clathrin-dependent endocytosis. Scale bar 10 µm.

Figure S4

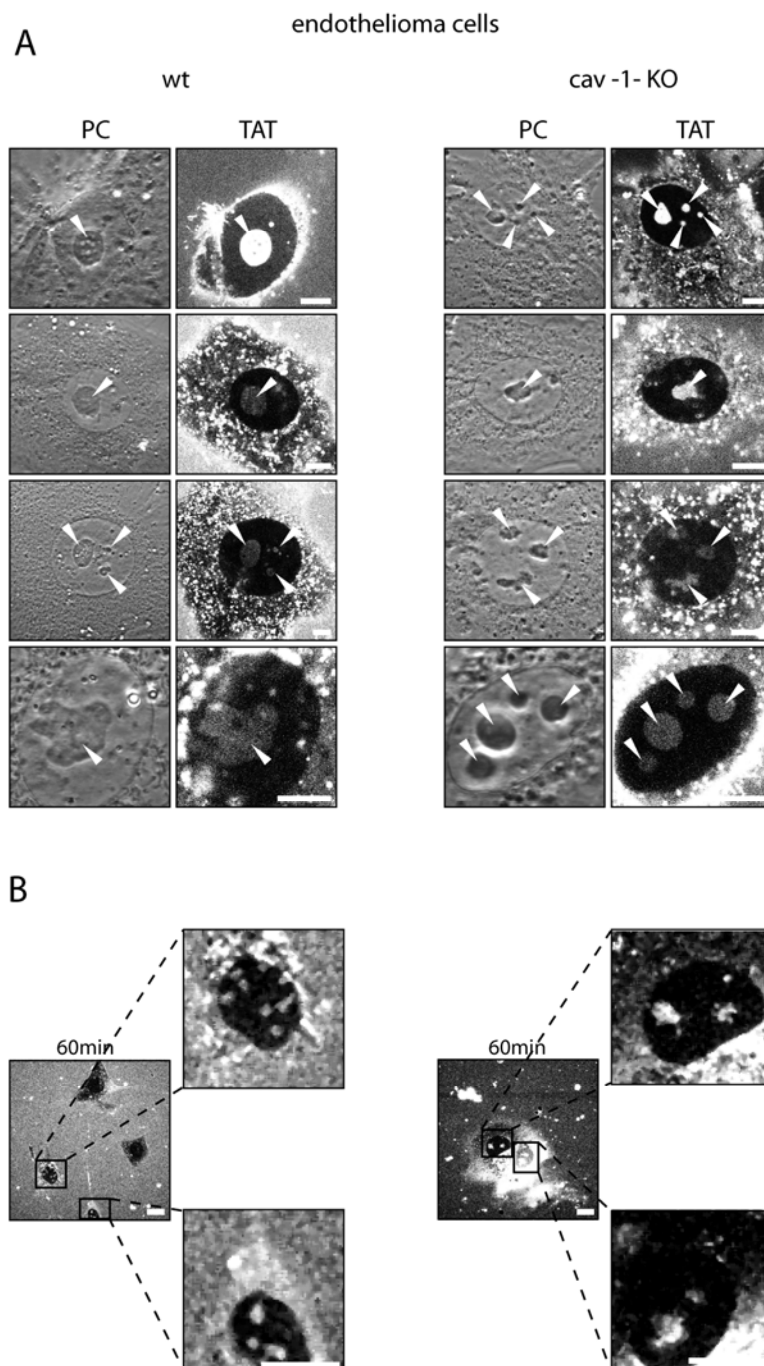

Fig. S4. Transduction of TAT is independent of caveolin-mediated endocytosis. Additional examples to the ones depicted in Fig. 3 are shown as high magnification images (*A*) and images magnified from the online supplementary movies S8 and S9 (*B)* after incubation with the TAT peptide for 60 minutes. Confocal optical sections of the wild type (left panel, wt) and caveolin-1 knockout (right panel, cav-1-KO) cells during incubation with the fluorescent CPP TAT. *A*-panels display images of the phase contrast (PC) and the peptide, *B*-panels only the peptide fluorescence images. Neither vesicular uptake of the CPP TAT nor the amount of TAT that was homogeneously distributed in the cytoplasm and accumulated inside the nucleolar compartment (marked by arrowheads), was different in both cases. Scale bar 10 µm.

Figure S5

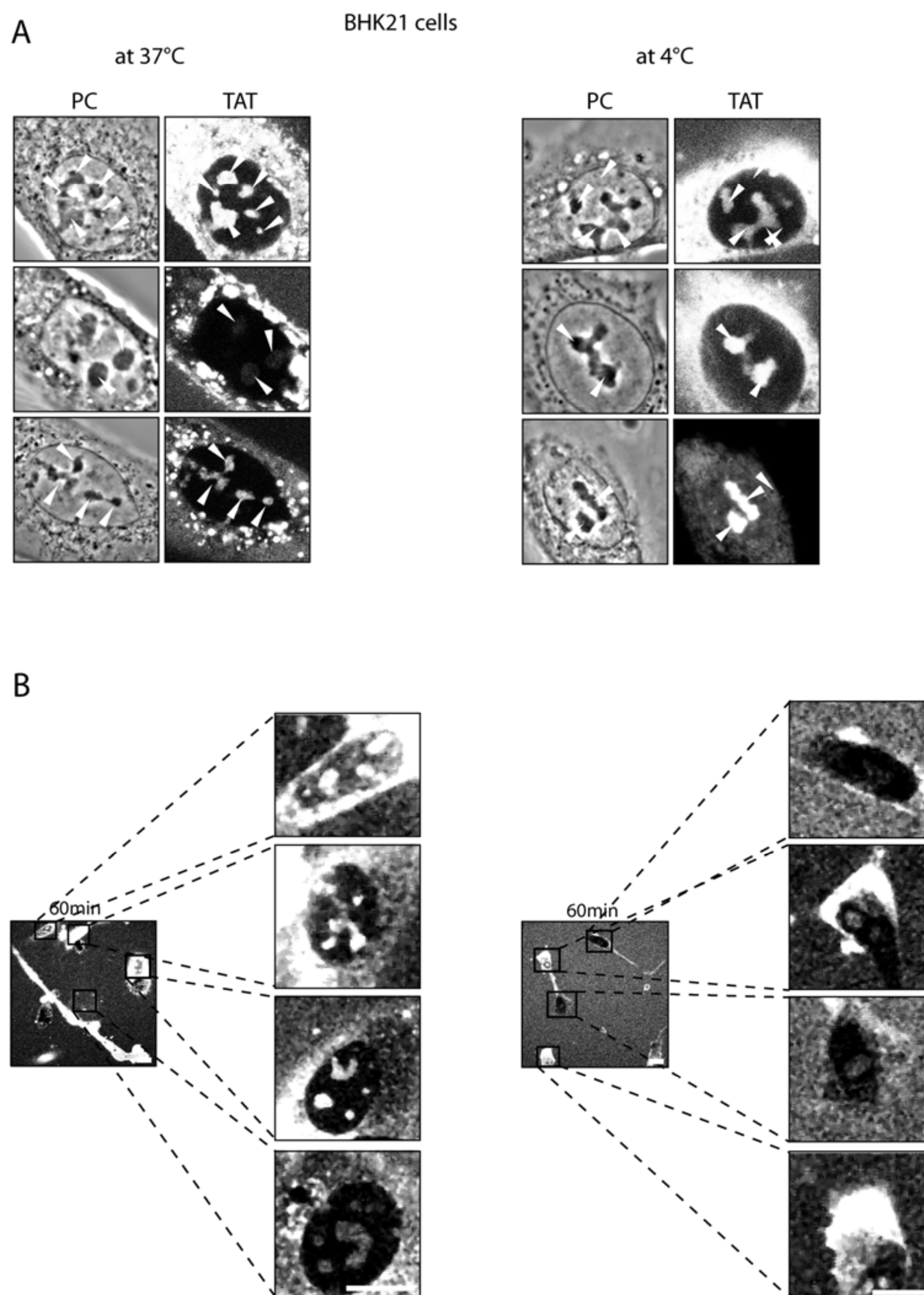

Fig. S5. Transduction of TAT is independent of endocytosis. Additional examples to the ones depicted in Fig. 4 are shown as high magnification images (*A*) and images magnified from the online supplementary movie S10 (*B)* after incubation with the TAT peptide for 60 minutes at 37°C (left panel) and at 4°C (right panel). In *A* each panel displays the phase contrast (PC) and the fluorescently labelled peptide. Arrowheads mark the position of nucleoli as unambiguous sign of freely diffusing intracellular TAT. In *B* the fluorescence of the labelled TAT-peptide is displayed. The transduction experiments were performed in BHK21 cells kept at the indicated temperatures before and during the experiment. Whereas there was no vesicle formation at 4°C (*A*) the transduction of TAT remained unchanged both at 37°C and 4°C (*A* and *B*). Scale bar 10 μm.