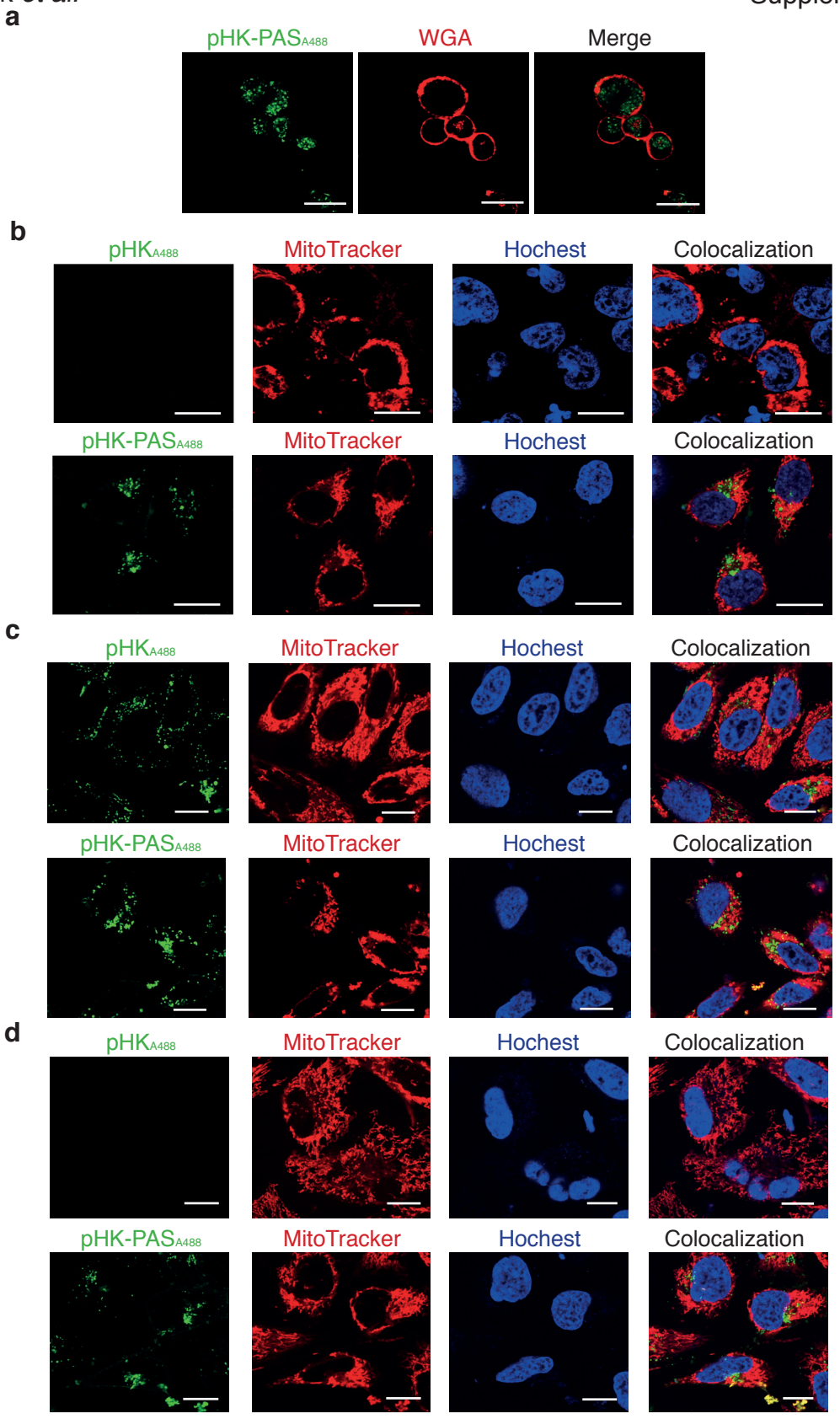
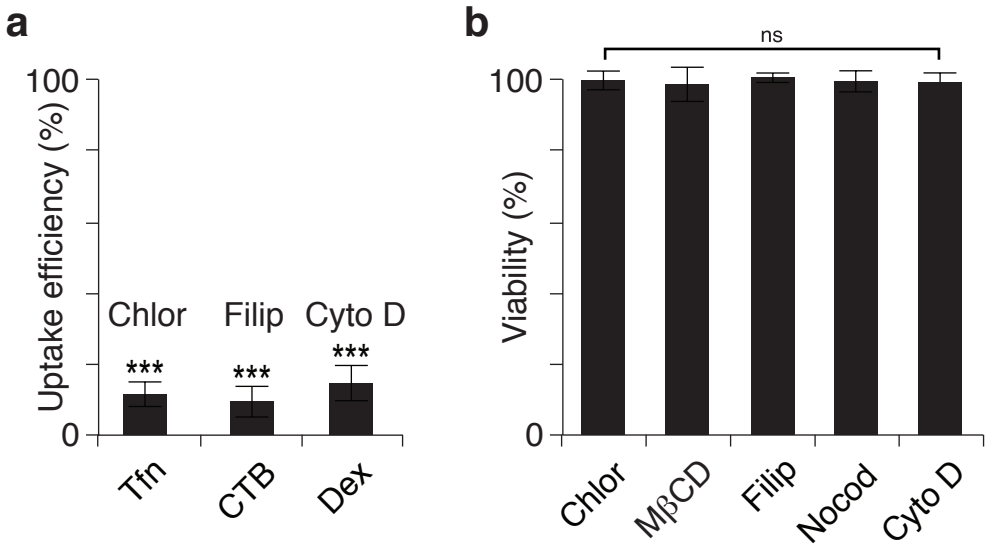
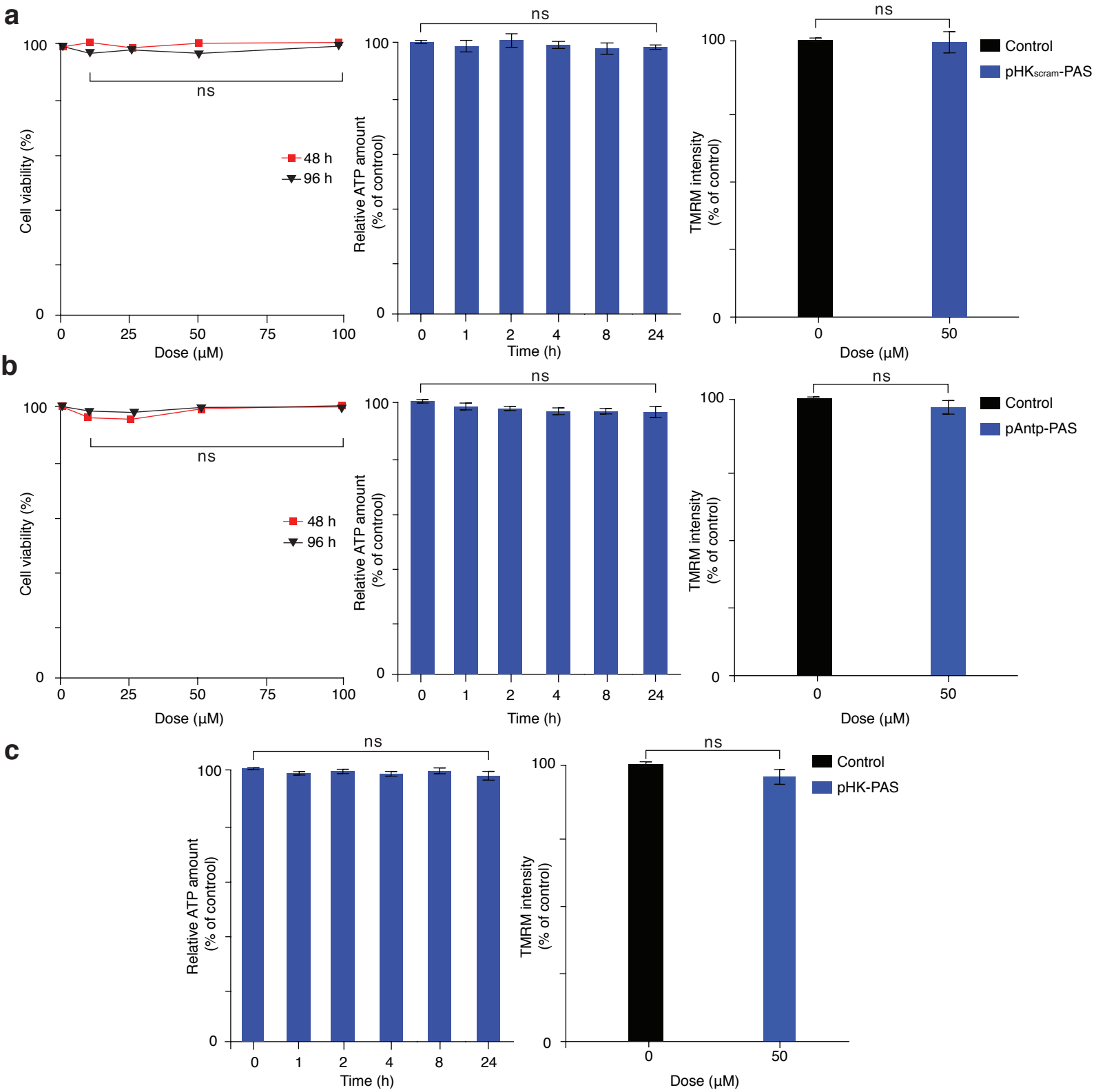


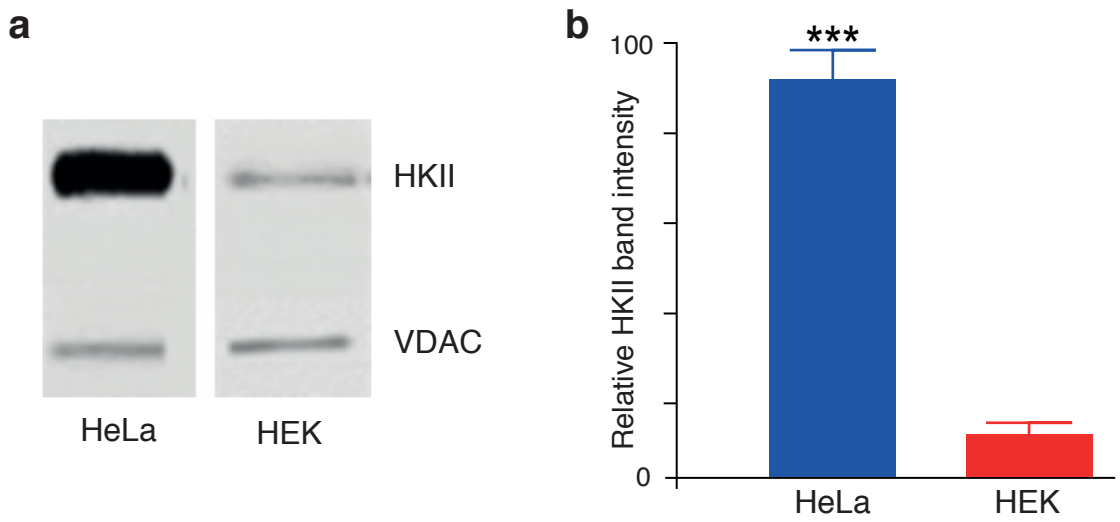

**Supplemental Figure S1. Confocal microscopy control experiments.** (**a**) Verification of removal of membrane-bound peptide. Confocal fluorescence microscopy image of HeLa cells following trypsinization. The image shows intact cells with no colocalization between pHK-PASA488 (*green*) and the cell membrane (WGA-Alexa 594; *red*), clearly illustrating that the trypsin treatment removes all membrane-bound peptide. The round shape is due to the fact that cells have not yet adhered to the substrate. Scale bar = 10 µm. (**b – d**) Confocal imaging of peptide uptake at low temperature, in ATP-depleted cells, or in the presence of endocytosis inhibitor. HeLa cells were pre-incubated for 1 h at 4 °C in serum-free DMEM (**b**), pre-treated for 1 h at 37 °C with 10 mM sodium azide/6 mM 2-deoxy-D-glucose in serum- and glucose-free DMEM (**c**), or pre-treated for 30 min at 37 °C with 10 µM cytochalasin D in serum-free DMEM (**d**). 25 µM pHKA488 (*top panels*) or pHK-PASA488 (*lower panels*) was then added, and the cells were maintained for 2 h at 4 °C (**b**), or 2 h at 37 °C (**c, d**). Finally, the cells were stained with organelle markers (5 µg/mL Hoechst 33342 and 50 nM MitoTracker Red) for 30 min prior to confocal imaging. Scale bar = 10 µm.




**Supplemental Figure S2. Effects of the endocytosis inhibitors on uptake of endocytosis markers and cell viability.** (**a**) HeLa cells were pretreated for 30 min at 37 °C in serum-free DMEM with the inhibitors: 10 µM chlorpromazine (Chlor), 4 µM filipin (Filip) or 10 µM cytochalasin D (Cyto D). Subsequently, the following endocytosis markers were added: 25 µg/ml transferrin-Alexa 546 (Tfn; clathrin-mediated endocytosis), 1 µg/ml cholera toxin B subunit-Alexa 555 (CTB; caveolae-dependent endocytosis), or 100 µg/ml 70 kDa neutral dextran-tetramethylrhodamine (Dex; macropinocytosis). The cells were then maintained for 2 h at 37 °C before the fluorescence was measured by FACS. (**b**) HeLa cells were treated for 2.5 h with the inhibitors: 10 µM chlorpromazine (Chlor), 5 mM methyl-β-cyclodextrin (MβCD), 4 µM filipin (Filip), 10 µM nocodazole (Nocod), or 10 µM cytochalasin D (Cyto D). 20 µL MTS reagent was then added and the cells were incubated for 4 h at 37 °C. The absorbance of the soluble formazan product ($\lambda$ = 490 nm) of MTS reduction was measured on a Synergy H1MF Multi-Mode microplate-reader, with a reference wavelength of 650 nm to subtract background. Cells treated with peptide-free carrier were used as control. The % viability was determined form the ratio of the absorbance of the treated cells to the control cells. ***, $p < 0.0001$; non-significant (ns), $p > 0.05$ compared with controls.




**Supplemental Fig. S3. Peptide control experiments.** (**a,b**) Cytotoxic effects of the pHK-PAS variants in HeLa cells. Effects of pHK$_{scram}$-PAS (**a**) and pAntp-PAS (**b**) on MTS reduction (*left panels*), intracellular ATP levels (*middle panels*) and mitochondrial inner membrane potential ($\Delta\Psi_m$) (*right panels*). HeLa cells were treated with the indicated concentrations of peptides for the indicated durations (*left panels*), or 50 μM peptide for the indicated durations (*middle panels*) or 24 h (*right panels*). Cells treated with peptide-free carrier were used as control. The % cell viability (MTS reduction), intracellular ATP, or tetramethylrhodamine methyl ester (TMRM) signal ($\Delta\Psi_m$) was determined form the ratio of the absorbance, luminescence or fluorescence, respectively, of the treated cells to the control cells. (**c**) Effects of pHK-PAS on intracellular ATP levels (*left panel*) and $\Delta\Psi_m$ (*right panel*) in HEK-293 cells. HEK-293 cells were treated with 50 μM pHK-PAS for the indicated durations (*left panel*) or 24 h (*right panel*). Cells treated with peptide-free carrier were used as control. The % intracellular ATP or TMRM signal ($\Delta\Psi_m$) was determined form the ratio of the luminescence or fluorescence, respectively, of the treated cells to the control cells. Note: for some data points, the error bars lie within the symbol. Non-significant (ns), $p > 0.05$ compared with controls.




**Supplemental Fig. S4. Comparison of the expression levels of HKII in HeLa and HEK-293 cells**. HeLa and HEK-293 cells were harvested and the mitochondria-enriched fractions were isolated. Samples were normalized for protein content and electrophoresed, then transferred to a nitrocellulose membrane and incubated overnight with mouse anti-human HKII antibody, followed by 3 h with horseradish peroxidase-conjugated mouse IgG antibody, and finally visualized using SuperSignal West Pico Chemiluminescent Substrate. (**a**) Immunoblots of HKII in the mitochondria-enriched fractions of HeLa and HEK-293 cells. (**b**) Densitometric quantification of the HKII band intensities normalized against the VDAC control. (**a**). ***, $p < 0.0001$ compared with HEK-293 cells.