Membrane-associated epithelial cell adhesion molecule is slowly cleaved by γ-secretase prior to efficient proteasomal degradation of its intracellular domain

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Running title: *Cleavage of EpCAM CTF is a slow process*

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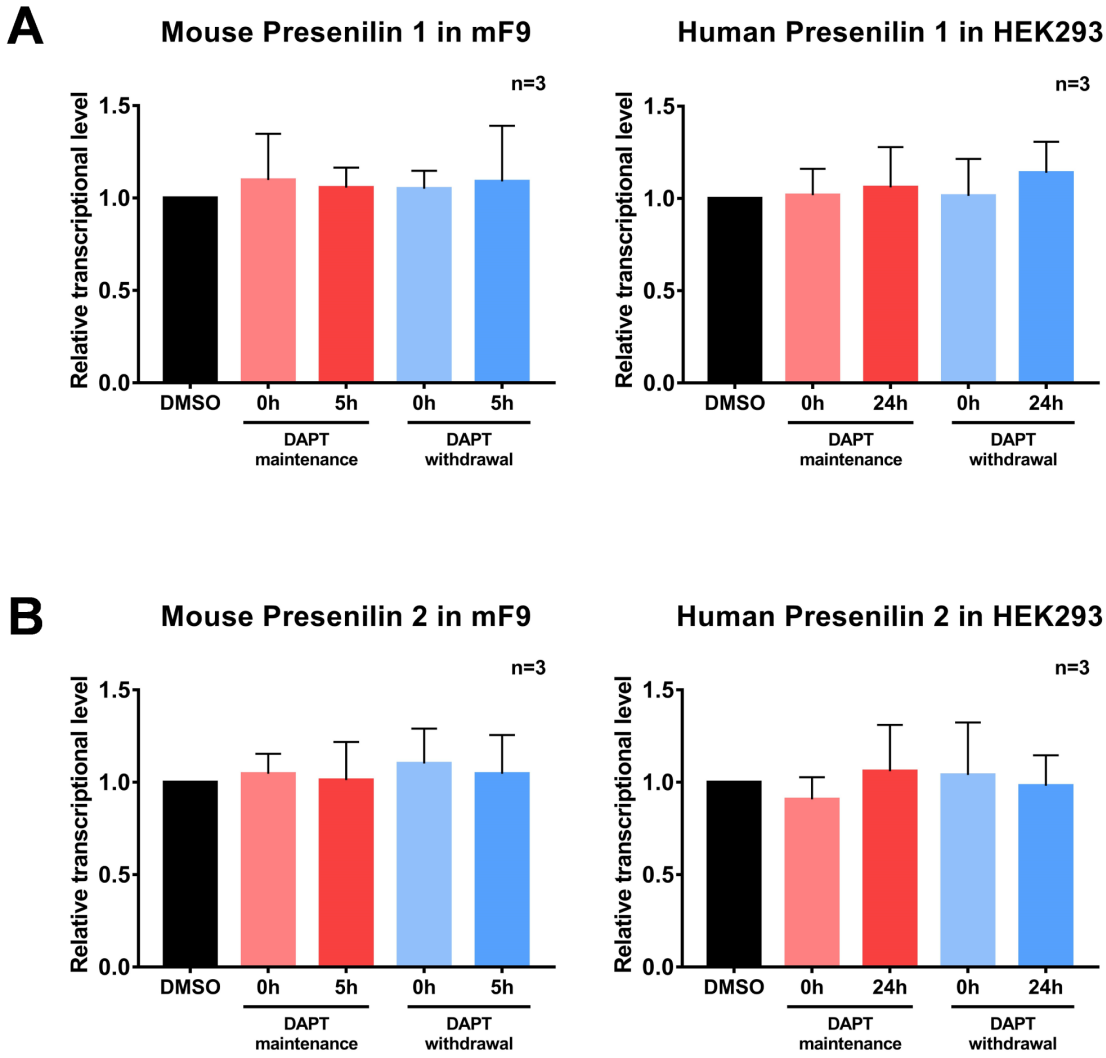
SUPPORTING INFORMATION

Supplementary Figure 1: Amount of Presenilin 1 and 2 at initial and final time-points of imaging experiment are equal in both mF9 and HEK293 cells

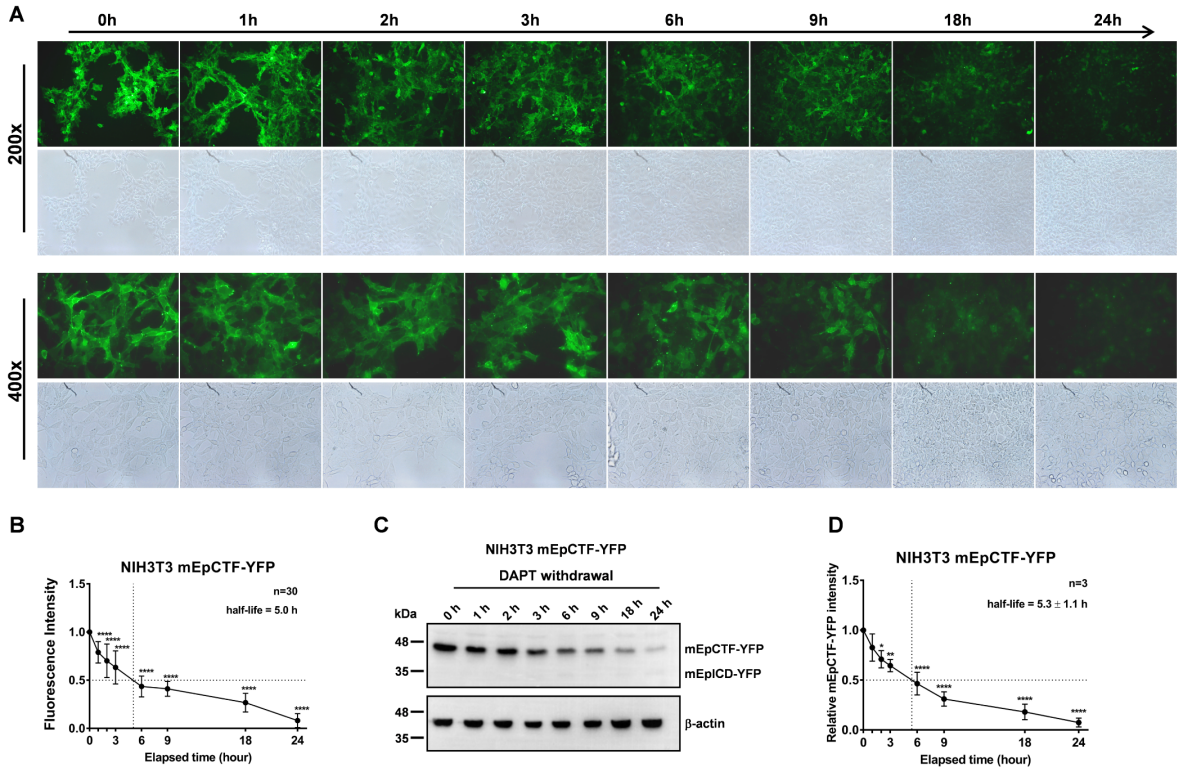
Supplementary Figure 2: Cleavage murine EpCTF in murine NIH3T3 fibroblast cells

Supplementary Figure 3: Cleavage human EpCTF in human FaDu carcinoma cells

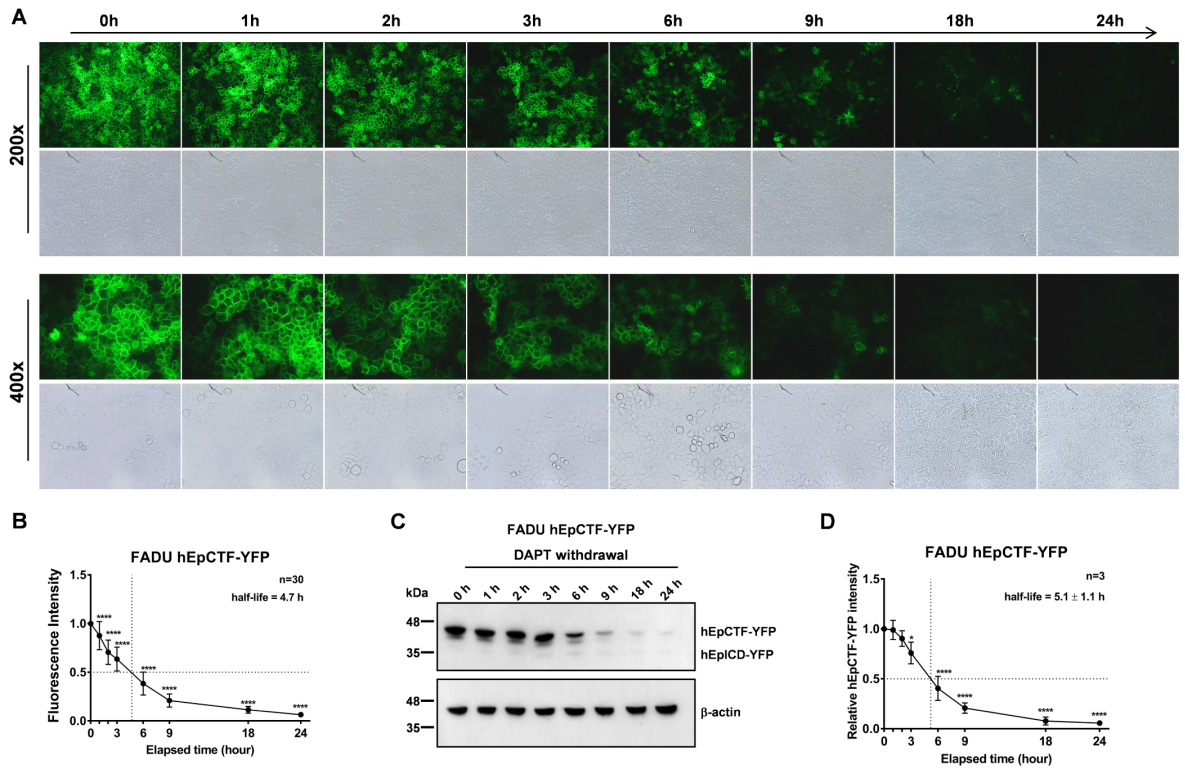
Supplementary Videos 1-6: Videos showing time-lapse confocal microscopy assessment of murine and human EpCTF-YFP fluorescence in mF9 and HEK293 cells treated with DAPT (Videos 1 and 4), released from DAPT treatment (Videos 2 and 5), and released from DAPT but treated with β-lactone (Videos 3 and 6)



Supplementary Figure 1 Amount of Presenilin 1 and 2 at initial and final time-points of imaging experiment are equal in both mF9 and HEK293 cells. Shown are mean relative transcript levels of murine and murine presenilin 1 and 2 with SEM from $n = 3$ independent quantitative PCR measurements performed in triplicates. Gusb and GAPDH served as standards. DMSO and DAPT treatments are indicated.



Supplementary Figure 2 Cleavage murine EpCTF in murine NIH3T3 fibroblast cells. *A*, mEpCTF-YFP was stably expressed in NIH3T3 cells. Following pretreatment with DAPT (see Materials and Methods), cells were maintained in normal medium for further 24 hours. Cells were monitored at the indicated time-points by immunofluorescence microscopy. Shown are representative pictures at the indicated time points from *n* = 3 independent experiments in 200x and 400x magnification. *B*, Immunofluorescence microscopy results shown in A were quantified from *n* = 30 cells from *n* = 3 independent experiments. Shown are mean values +/- SEM. P-values were calculated with One-way ANOVA. **** 0.00001. *C*, Visualization of mEpCTF-YFP and mEpICD-YFP expression was performed by immunoblotting. Shown are representative results from *n* = 3 independent experiments. *D*, Calculation of mEpCTF 50% protein turnover was performed from *n* = 3 independent immunoblot experiments. Shown are mean with SEM. P-values were calculated with One-way ANOVA. * 0.05; ** <0.001; *** <0.0001; **** 0.00001.



Supplementary Figure 3: Cleavage human EpCTF in human FaDu carcinoma cells. *A*, hEpCTF-YFP was stably expressed in FaDu cells. Following pretreatment with DAPT (see Materials and Methods), cells were maintained in normal medium for further 24 hours. Cells were monitored at the indicated time-points by immunofluorescence microscopy. Shown are representative pictures at the indicated time points from *n* = 3 independent experiments in 200x and 400x magnification. *B*, Immunofluorescence microscopy results shown in A were quantified from *n* = 30 cells from *n* = 3 independent experiments. Shown are mean values +/- SEM. P-values were calculated with One-way ANOVA. **** 0.00001. *C*, Visualization of hEpCTF-YFP and hEpICD-YFP expression was performed by immunoblotting. Shown are representative results from *n* = 3 independent experiments. *D*, Calculation of hEpCTF 50% protein turnover was performed from *n* = 3 independent immunoblot experiments. Shown are mean values with SEM. P-values were calculated with One-way ANOVA. * 0.05; ** <0.001; *** <0.0001; **** 0.00001

Supplementary Video 1: Shown is a time-lapse confocal microscopy assessment of murine EpCTF-YFP fluorescence in mF9 treated with DAPT and recorded over a time period of 5 hours with pictures taken every 5 min

Supplementary Video 2: Shown is a time-lapse confocal microscopy assessment of murine EpCTF-YFP fluorescence in mF9 treated released from DAPT treatment and recorded over a time period of 5 hours with pictures taken every 5 min

Supplementary Video 3: Shown is a time-lapse confocal microscopy assessment of murine EpCTF-YFP fluorescence in mF9 treated released from DAPT treatment but treated with β-lactone, and recorded over a time period of 5 hours with pictures taken every 5 min

Supplementary Video 4: Shown is a time-lapse confocal microscopy assessment of humane EpCTF-YFP fluorescence in HEK293 treated with DAPT and recorded over a time period of 24 hours with pictures taken every 7.5 min

Supplementary Video 5 Shown is a time-lapse confocal microscopy assessment of human EpCTF-YFP fluorescence in HEK293 treated released from DAPT treatment and recorded over a time period of 24 hours with pictures taken every 7.5 min

Supplementary Video 6: Shown is a time-lapse confocal microscopy assessment of human EpCTF-YFP fluorescence in HEK293 treated released from DAPT treatment but treated with β-lactone, and recorded over a time period of 24 hours with pictures taken every 7.5 min