The motif ExExxxL in the cytosolic tail of the secretory human proprotein convertase PC7 regulates its trafficking and cleavage activity

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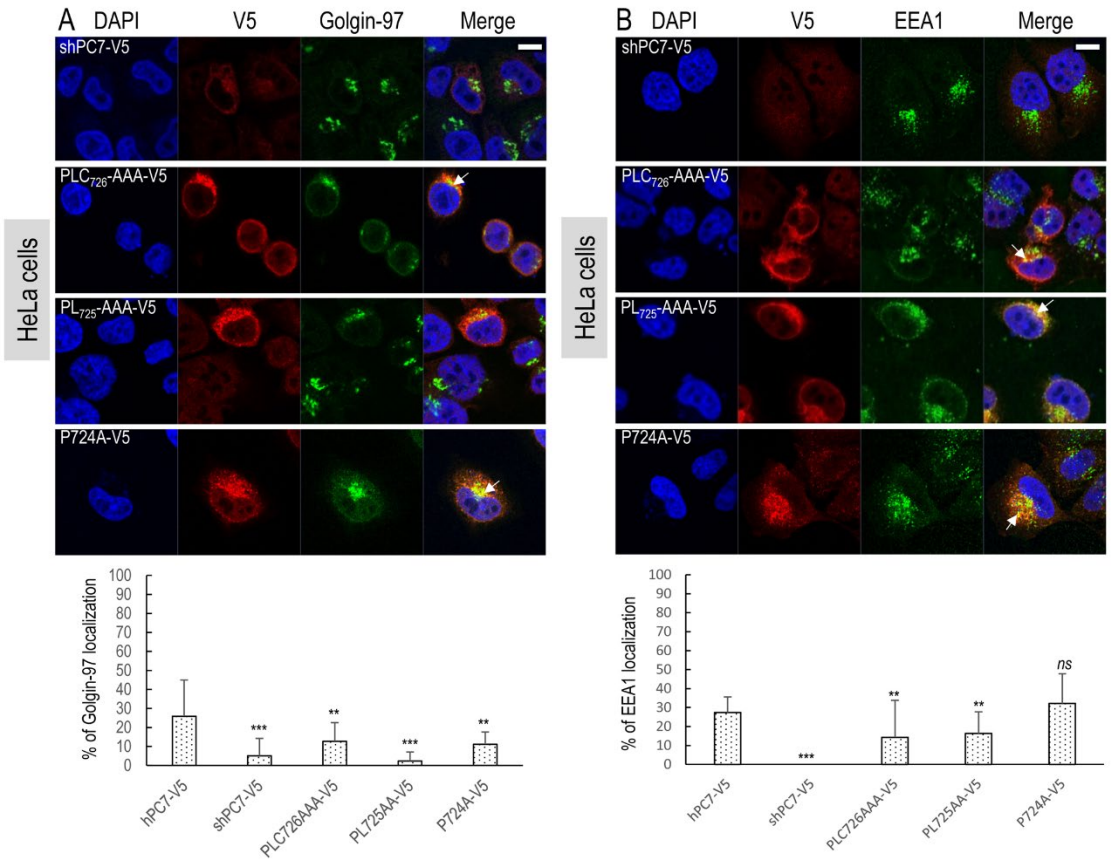
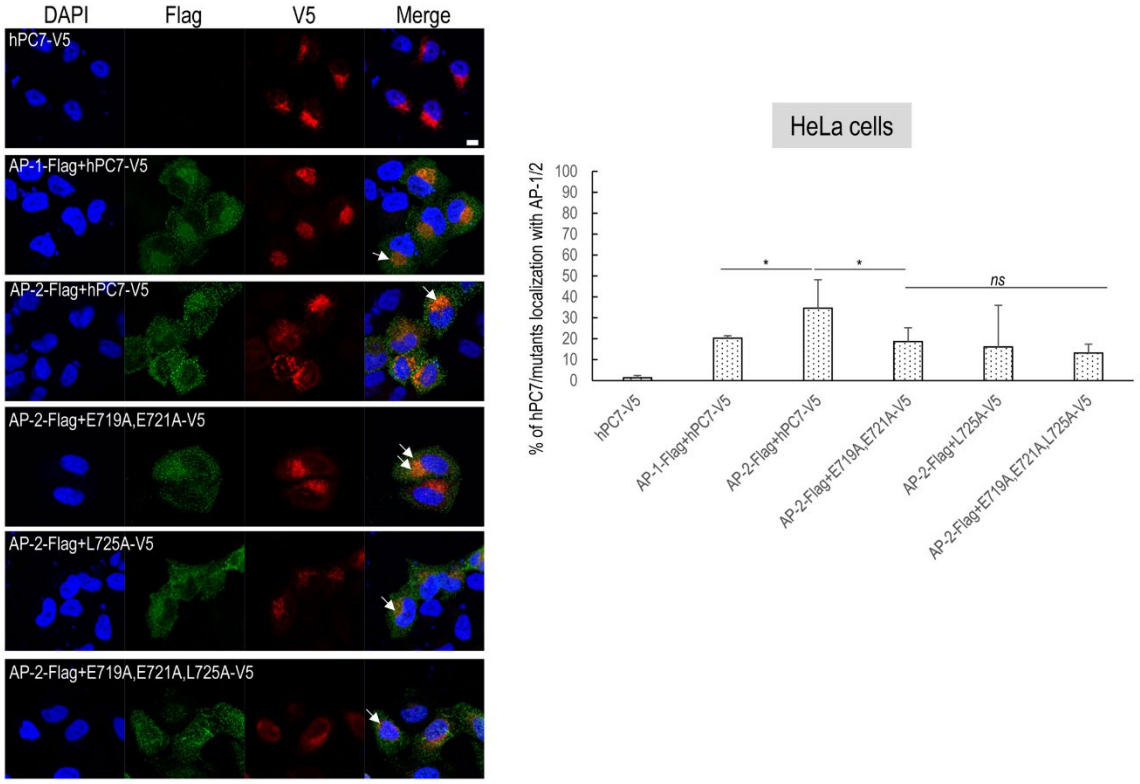
SUPPORTING INFORMATION

Supporting information Figure S1. Localization of human PC7-CT mutants in the Trans-Golgi network or early endosomes. (A)(B) Immunofluorescence of CT mutants (red) on permeabilized HeLa cells in the presence of **(A)** cell compartments Golgin-97 marker or **(B)** Early Endosomal marker are labeled in green. Cell nuclei are marked by DAPI (blue). Quantification of co-localization between hPC7 mutants and Golgin-97 **(A)** or with EEA1 **(D)** was performed using IMARIS software. These results are representative of minimum three independent experiments and quantification representative of N=15 cells. Error bars indicate averaged values ± standard error of the mean (SEM). ~~*, P < 0.1,~~ **, P <0.01, ***, P <0.001, *ns*: not significant (Student's *t*-test). Bar = 1 µm.



Supporting information Figure S2. Overexpression and co-localization of adaptor-proteins (AP) and human PC7 or its CT mutants. Immunofluorescence of hPC7 or its CT mutants (red) in the presence of AP proteins (green), on permeabilized HeLa cells. Cell nuclei are marked by DAPI (blue). Quantification, using IMARIS software, of the co-localization between hPC7 or its mutants with of overexpressed AP-1μ or AP-2μ.These results are representative of minimum three independent experiments and quantification is representative of N=15 cells. Error bars indicate averaged values ± SD. *, P < 0.1, **, P <0.01, ***, P <0.001, *ns*: not significant (Student's *t*-test). Bar = 1 μm.



Supporting information Figure S3. Overexpression and co-localization of adaptor-proteins (AP) and human TfR1. Immunofluorescence of overexpressed hTfR1 (green) and **(A)** endogenous EEA1 (red) or **(B)** Golgin-97 (red) in permeabilized HeLa cells following 48 hours incubation with an AP-2 siRNA or its control (scrambled siRNA). Cell nuclei are marked by DAPI (blue). Quantification, using IMARIS software, of the co-localization between hPC7 and EEA1 or Golgin-97. These results are representative of minimum three independent experiments and quantification is derived from N=15 cells per condition. Error bars indicate averaged values ± SD. *, P < 0.1, ~~**, P <0.01, ***, P <0.001,~~ *ns*: not significant (Student's *t*-test). Bar = 1 µm

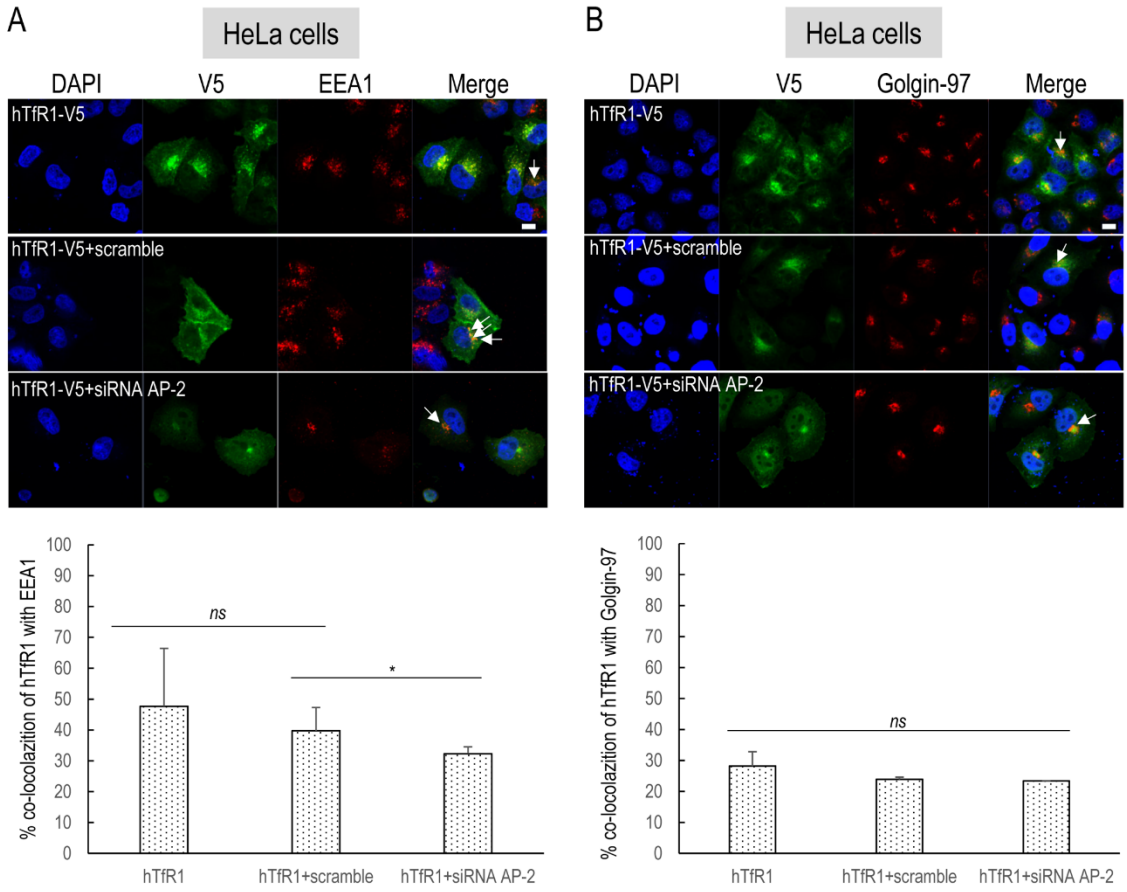


TABLE S1. Oligonucleotides used in PCR for site-directed mutagenesis of hPC7 and its mutants		
mutants or chimeras	forward	reverse
hPC7+xTMCT	ACCCTCAAGACCTTGTTGCTGTTGGGCTG	GCAACAAGGTCTTGAGGGTGTTGGGGG
hPC7+xCT	GGAAGTATATTTGTCTCAGGGTTCTAGCGACGG	CCCTGAGACAAATATACTTCCAGCATGTAGTAAAC
xPC7+hTMCT	CACCCTTCGGACACTGGTGCTGGTAGGCTGTTTC	GAAACAGCCTACCAGCACCAGTGTCCGAAGGGTG
xPC7+hCT	CACATTGGAGTTGTACTGGAGCCAGAGGAATGTGGC	GCCACATTCCTCTGGCTCCAGTACAACTCCAATGTG
L668X (soluble)	GCCACCCGGTATGAGGATCG	GACACCGGTGGTCTTGAGGGTGTTGGGGG
Q690X (ΔCT)	GCCACCCGGTATGAGGATCG	GCGTCGACTCAGCTCAAATATACTTCCAGCATG
G717X	GCCACCCGGTATGAGGATCG	CCCGTCGACTCACTTCCTCCTTGGCTTTCC
L753X	GCCACCCGGTATGAGGATCG	CCCGTCGACTCACAGCAGGTCTGGGGC
PLC726-AAA	CTGGCAGCTGCCACTGATTCTAGC	GTGGCAGCTGCCAGCAGCAAGG
P724A	GAATCAGTGGCACTTTGCAG	GCTGCTGCAAAGTGCCACTG
L725A	GAATCAGTGCCAGCTTGCAGC	GCTGCTGCAAGCTGGCACTG
C726A	GCCACTTGCCAGCAGCAAGG	CCTTGCTGCTGGCAAGTGGC
C726F	CCACTTTTCAGCAGCAAGGATCC	GGATCCTTGCTGCTGAAAAGTGGCACTG
E719A	AAGGGACAGCGCTAGAATCAGTGCC	CTGATTCTAGCGCTGTCCCTTCCTCCTTGGC
E721A	CAGAGCTAGCATCAGTGCCAC	GTGGCACTGATGCTAGCTCTG
E719A,E721A	CACTGATGCTAGCGCTGTCCCTTCC	GACAGCGCTAGCATCAGTGCCACTTTG
E733A,E735A	CTGTTGCCACTGCGTCTGGATCC	CCAGACGCAGTGGCAACAGAGAGC
ELE721-AAA	CCAAGGAGGAAGGGACAGCGGCAGCATCAGTGCCACTTTGCAGC	GCTGCAAAGTGGCACTGATGCTGCCGCTGTCCCTTCCTCCTTGG
E719A,E721A,L725A	GAAGGGACAGCGCTAGCATCAGTGCCAGCTTGCAG	CTGCAAGCTGGCACTGATGCTAGCGCTGTCCCTTC
ESV723-AAA	CAGAGCTAGCAGCAGCGCCACTTTGC	GCAAAGTGGCGCTGCTGCTAG
S722A	CAGAGCTAGAAGCAGTGCCACTTTGCAGC	GCTGCAAAGTGGCACTGCTTCTAGCTCTG
V723A	CAGAGCTAGAATCAGCGCCACTTTGCAGC	GCTGCAAAGTGGCGCTGATTCTAGCTCTG