Supporting information for: *Preferential apelin-13 production by the proprotein convertase PCSK3 is implicated in obesity.* ***FEBS Open Bio.***

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In vitro assay optimization

Elution times of pure apelin isoform samples (apelin-12, apelin-13, Pyr-apelin-13, apelin-17, apelin-36, proapelin) were initially characterized to determine if various isoforms could be differentiated using RP-HPLC separation (Varian ProStar HPLC, Mississauga, Ontario) with an analytical column C18-AR-II (4.6 x 250 mm, Cosmosil). The mobile phase components were Type II water and acetonitrile, both with 0.1% trifluoroacetic acid (v/v). Using a method of 2-100% in 49 min, approximate elution time points were determined; then, using a method of 20-40% in 20 min with flow rate of 1 ml/min, a more accurate elution time for each apelin isoform was determined (Table S2). Spontaneous production of Pyr-apelin-13 was also observed during determination of apelin-13 elution time. Prior to studying the role of PCSKs in proapelin processing, stability of proapelin was determined over 5 hours, for which 90-97% stability in PCSK3 buffer was noted (Supp. Fig. 2).

To test for the potential that proapelin could be cleaved into multiple apelin isoforms or produce an unknown product that may elute outside the range of 20-40%, each enzyme reaction mixture was initially analyzed using an RP-HPLC method of 2-100%. The initial 20-40% in 20 min method was incorporated into 2-100% method, since the apelin isoforms eluted between 20-40% and could easily be identified at 1%/min rate. Once it was confirmed that no eluents were detected with >40% acetonitrile, a revised 2-40% method was used to decrease the time required for each RP-HPLC analysis.

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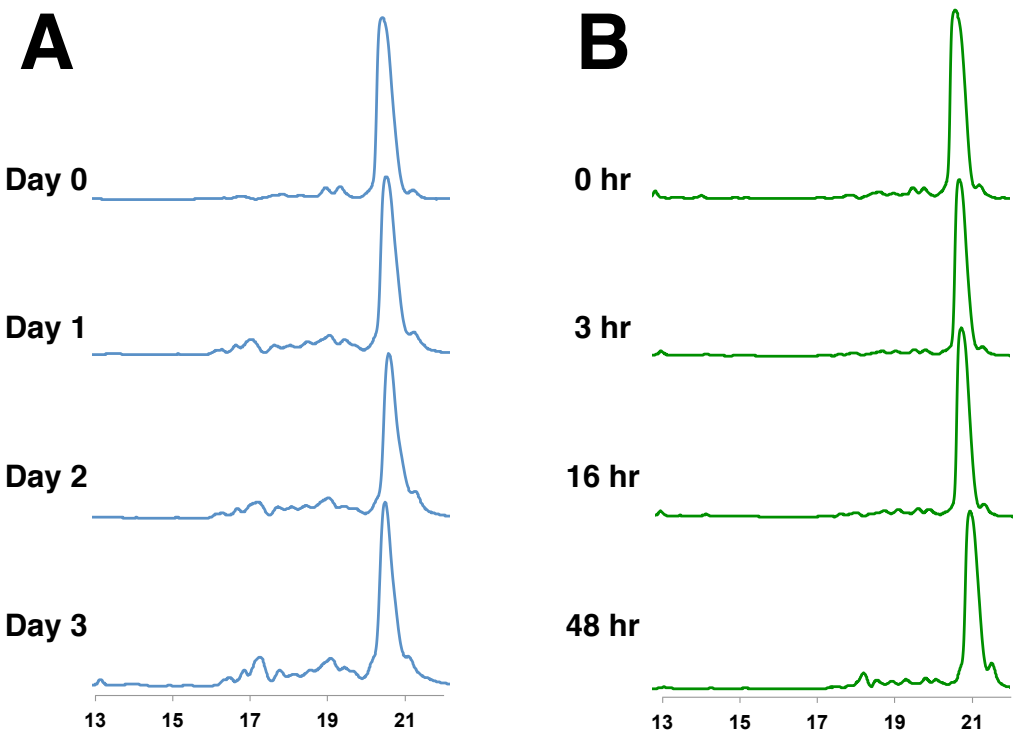
Supplementary Figures



Supplementary Figure S1: Proapelin is stable over 5 hours at A) pH 5 B) pH 6 C) pH 7. Proapelin was incubated in PCSK3 assay buffer without the enzyme and quantified at 0 and 5 hr incubation time points using RP-HPLC analysis. Approximately 90-97% stability was observed.

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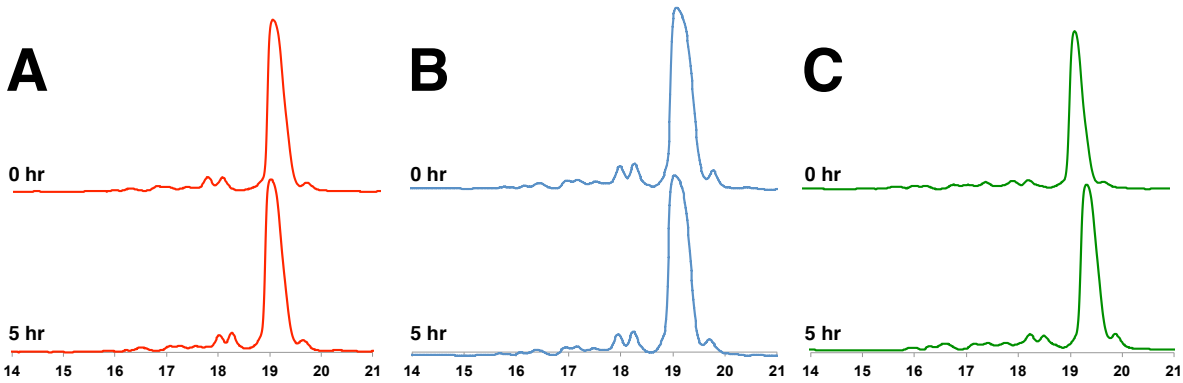

Supplementary Figure S2: Proapelin is not cleaved in over 48 hours by A) PCSK1 and B) PCSK7. Proapelin was incubated with PCSK1 or 7 in their respective buffer. The reactions were monitored at discrete time points over 48 hour period by RP-HPLC analysis.

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Supplementary Tables

Supplementary Table S1: Primers for RT-PCR.

Gene	Sequence 5'→3'
18S	For: TCAACTTTCGATGGTAGTCGCCGT
	Rev: TCCTTGGATGTGGTAGCCGTTTCT
Preproapelin	For: CCCAACCAATCAACCAACCAACCA
	Rev: TGCAGTGGCTCTAAGCAGGTTACT
PCSK1	For: CCAAAGTTGGAGGCATAAGAATG
	Rev: GTCTGTGTAGCCATCACAGTCA
PCSK3	For: TGTTAGCTGCCAGACCACATGACT
	Rev: ACTTGGTCAGCGTCCCATAGTTGT
PCSK7	For: GCTATGCCAACTCCATCTACAC
	Rev: AGTCAGTGGTCACAATGCTC

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Supplementary Table S2: Apelin isoform elution properties.

Apelin isoform	% Acetonitrile in H_2O	Elution time (min)
Proapelin	30.98	10.98
Apelin-12	27.87	7.87
Apelin-13	27.60	7.60
Pyroglutamate-apelin-13	28.35	8.35
Apelin-17	27.70	7.70
Apelin-36	28.08	8.08

Method: Linear gradient; 20-40% solvent B in 20 min