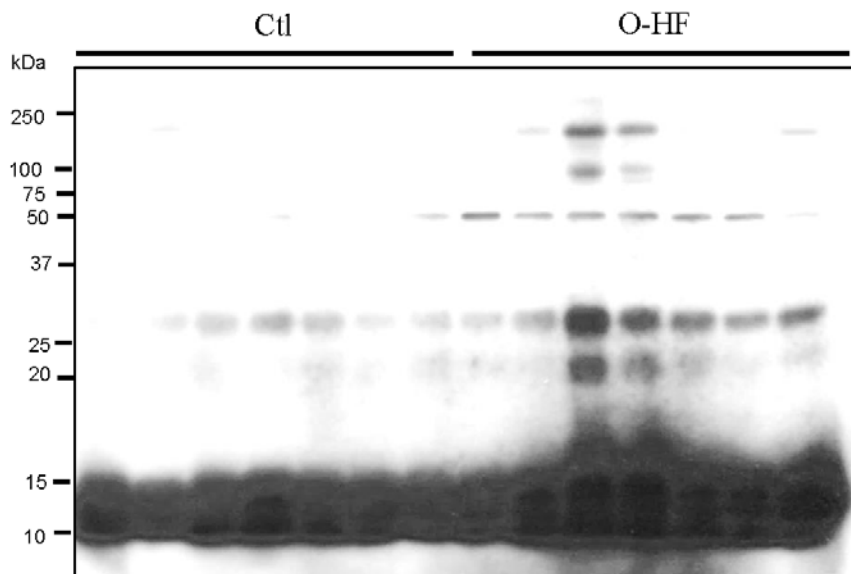


SUPPLEMENTARY DATA

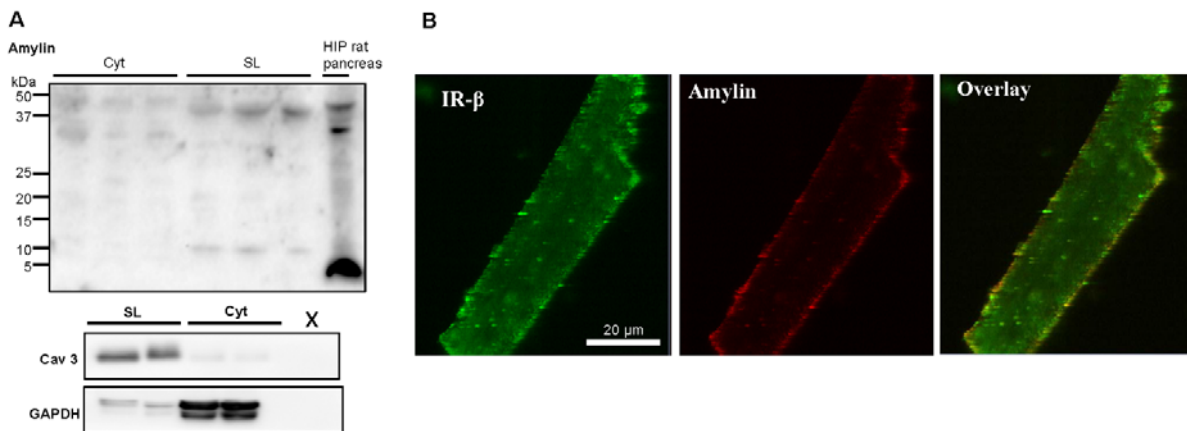
Supplementary Figure S1. The blood level of aggregated amylin is elevated in patients with obesity and heart failure.



The size distribution of aggregated amylin in blood samples from obese patients with heart failure (O-HF) vs. lean non-failing individuals (Ctl) was assessed by Western blot. Compared to controls, blood specimens from O-HF show a wider distribution of amylin aggregates and overall increased amylin immunoreactivity. A representative blot from 2 independent experiments is shown.

SUPPLEMENTARY DATA

Supplementary Figure S2. Anatomical localization of amylin with respect to the sarcolemma.



(A) Western blot analysis of amylin in HIP rat myocytes cytosol (Cyt) fraction and sarcolemma (SL) fraction (top panel). HIP rat pancreas (HIP pan) was used as a positive control. There is more amylin in the sarcolemma fraction. A representative blot from 2 independent experiments is shown. To separate the SL, myocyte lysates were centrifuged at 1,000 g for 5 min to generate a postnuclear supernatant. The supernatant was further centrifuged at 27,000 g for 35 min and plasma membrane pellet was re-suspended in Laemmli buffer. The protocol results in efficient separation of sarcolemmal (SL) and soluble fractions (Cyt) (bottom panel), i.e. the sarcolemmal protein Caveolin3 is found almost exclusively in the SL fraction while the cytosolic protein GAPDH is highly enriched in the soluble fraction.

(B) Dual immunofluorescence staining of amylin (red) and insulin receptor (green) in isolated HIP rat cardiac myocytes. Consistent with Western blot analysis showing more amylin in SL fraction (see, A), amylin deposits are identified at the sarcolemma in HIP rat cardiac myocytes (Scale bar, 20 μm). The primary antibodies were IR-β antibody (anti-mouse, SC-25103, Santa Cruz biotech; TX) and anti-human amylin antibody (anti-rabbit, T4149, Bachem-Peninsula). The secondary antibodies were Alexa Fluor 488 conjugated anti-mouse IgG (A11029; Invitrogen; NY) and Texas red conjugated anti-rabbit IgG (SC-2780; Santa Cruz biotech; TX).