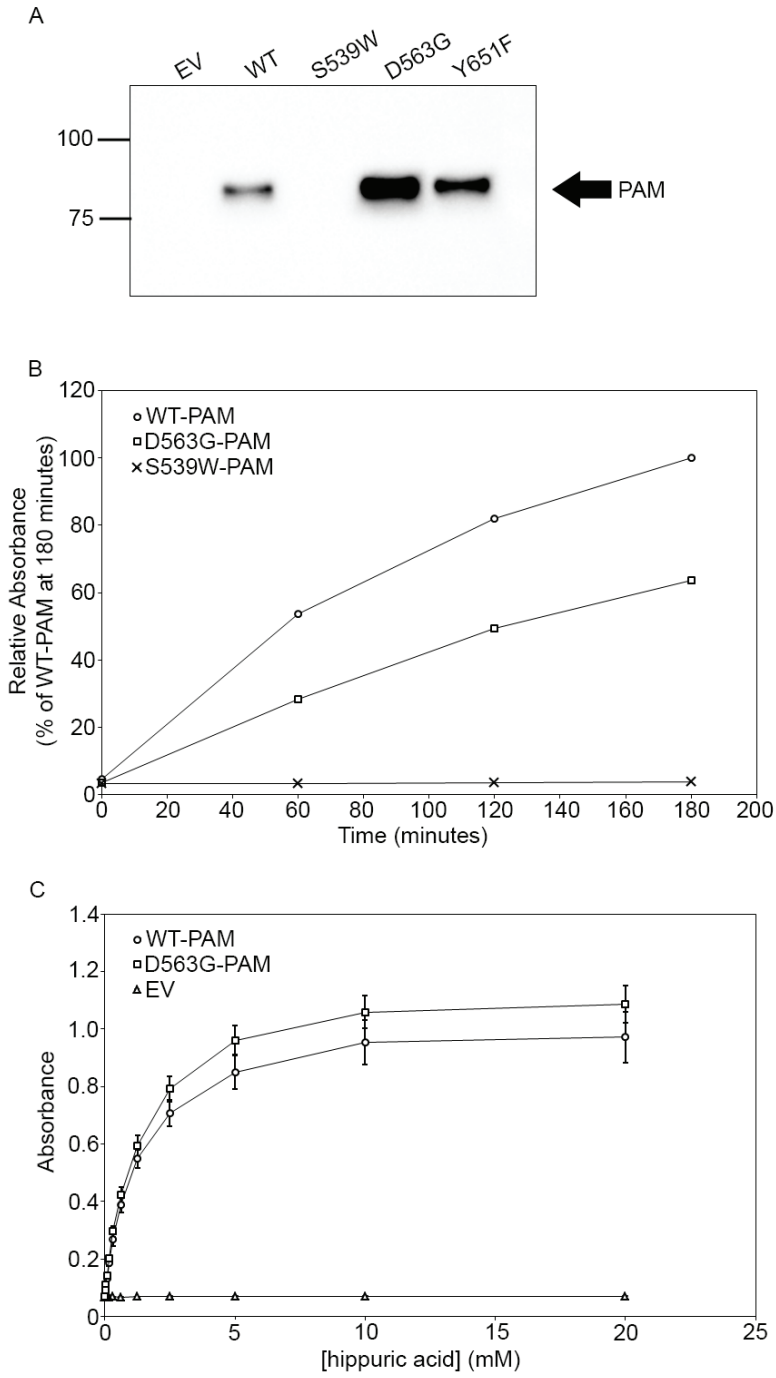
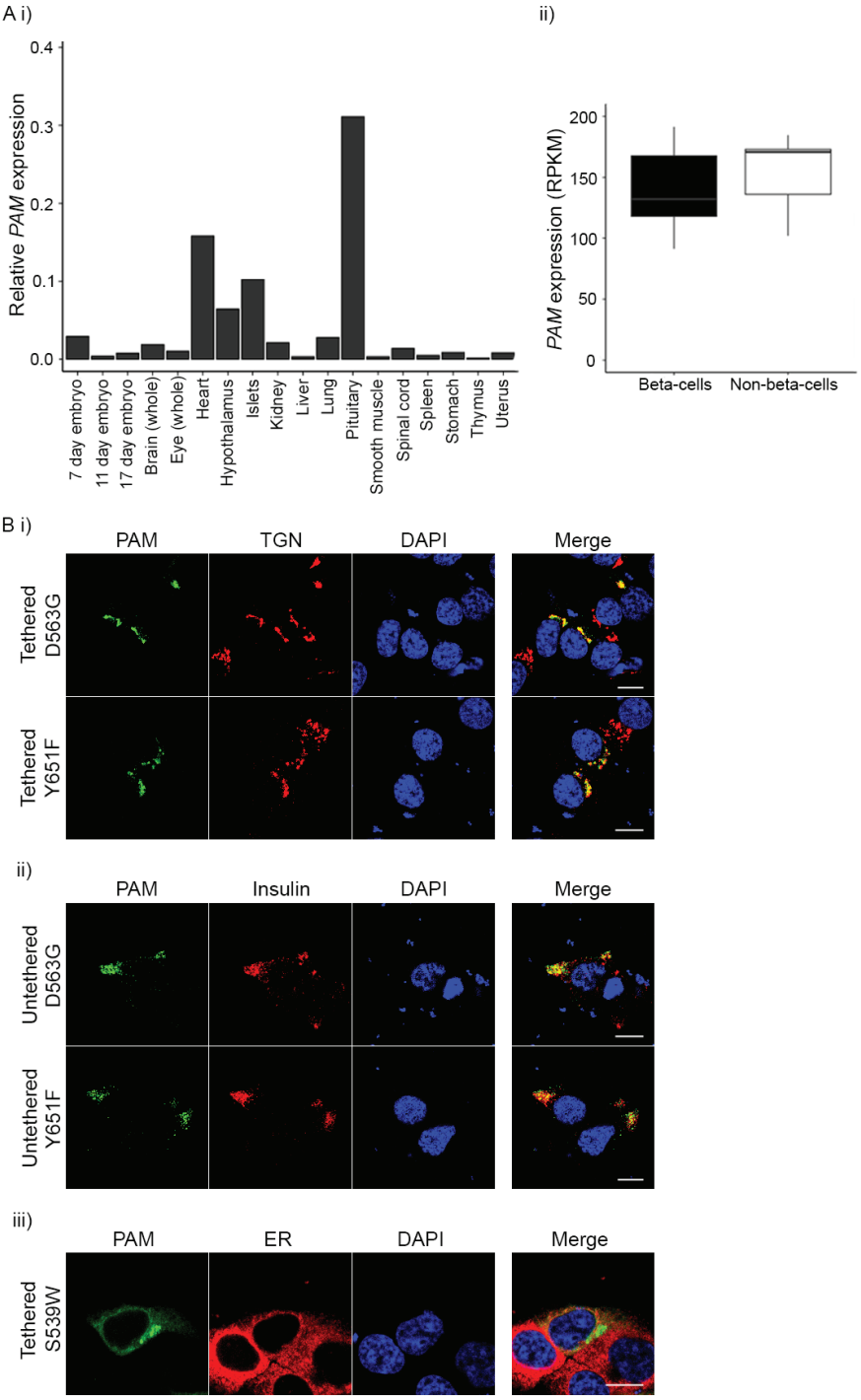
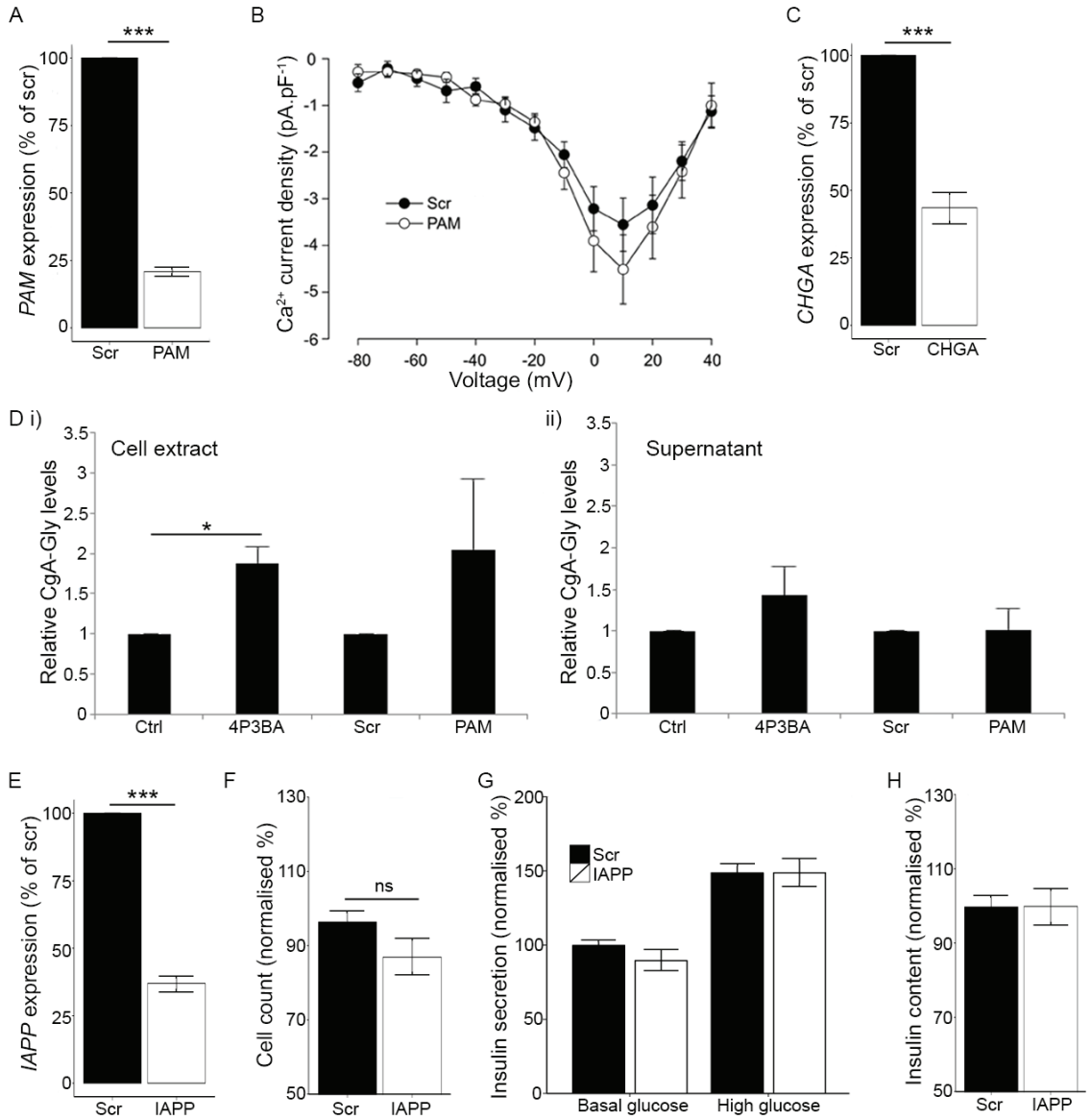


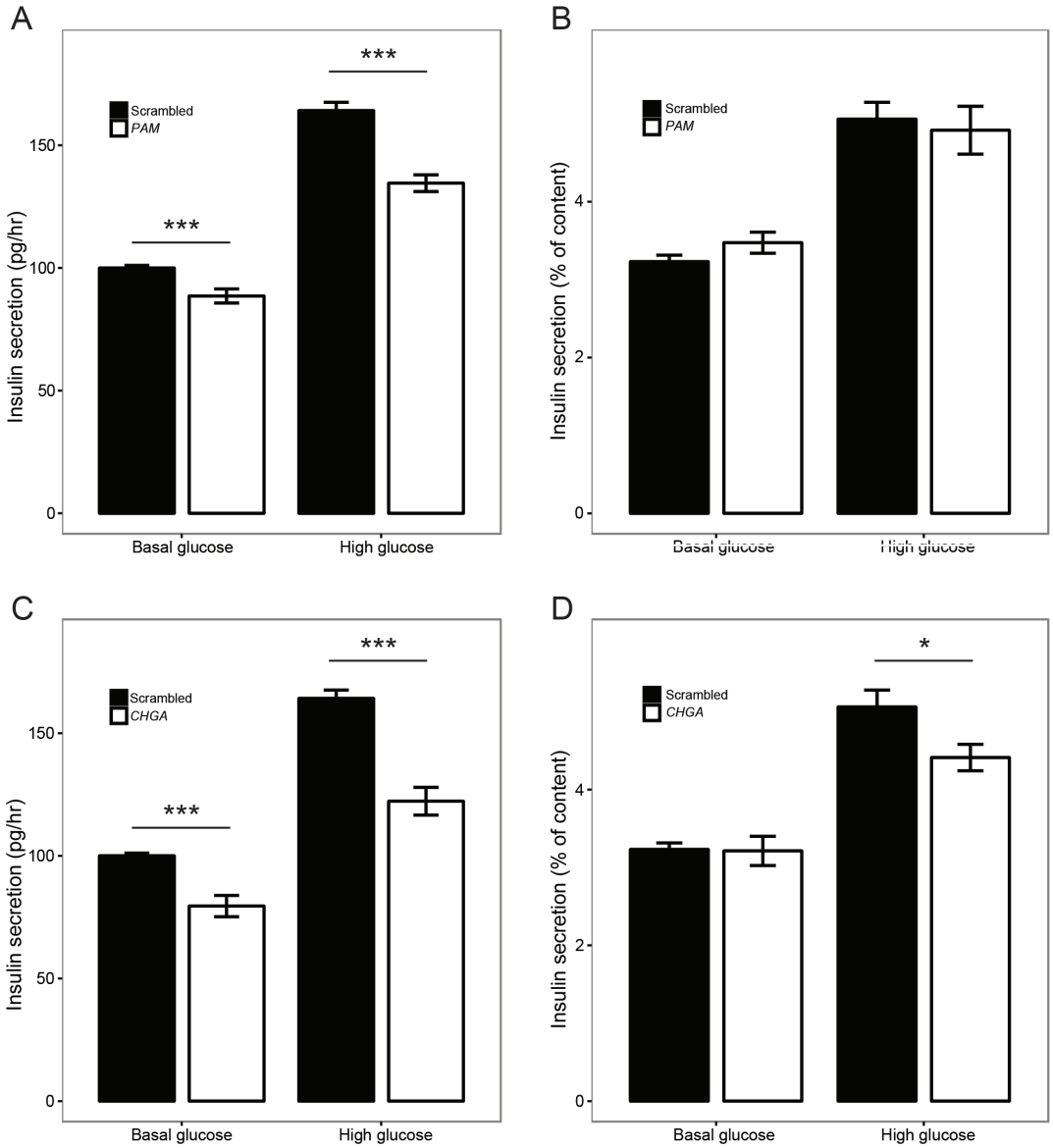
Supplementary Figure 1. Analysis of WT and variant PAM expression and function. A) Western analysis of recombinant PAM protein production in supernatant from HEK 293 stable cells. Size markers indicate protein mass in kilodaltons. Results shown are from a single representative experiment that is indicative of at least three independent experiments. B) Amidating activity of wild-type (WT)-PAM (circles), D563G-PAM (squares), and S539W-PAM (crosses) (n=1). C) Amidating activity of WT-PAM (circles), D563G-PAM (squares), and empty vector (EV) (triangles) over a substrate concentration range (n=5). Error bars are mean ±SEM.



Supplementary Figure 2. Analysis of WT and variant PAM expression. A) *PAM* expression was investigated in i) mouse tissues (n=1) and ii) FACS human beta-cells and non-beta-cells (n=11 and n=5 respectively). Box plots show the interquartile range for *PAM* expression, and whiskers indicate variability outside the upper and lower quartiles. RPKM, reads per kilobase of transcript per million mapped reads. B) EndoC-βH1 cells were transfected with i&iii) membrane-tethered or ii) untethered variant PAM expression vectors, then labelled for PAM (green), the trans-Golgi network (TGN) (red in i), insulin (red in ii) or calnexin (ER) (red in iii). DAPI (blue) was used as a nuclear marker. Scale bar, 2µm. Results shown are from a single representative experiment that is indicative of at least two independent experiments.



Supplementary Figure 3. Effects of endogenous *PAM*, *CHGA* and *IAPP* on beta-cell function and CgA amidation. EndoC-βH1 cells were either transfected with scrambled (Scr) or gene-specific siRNA or treated with vehicle (ctrl) or 4P3BA then measured for A,C&E) efficiency of gene knockdown (n=3), B) calcium current density (n=15), D) CgA amidation in i) cell extract and ii) cell supernatant via Western using antibodies against CgA-Gly and total CgA (n=2), F) cell viability (n=16), G) insulin secretion (n=8), or H) cellular insulin content (n=16). Error bars are mean ±SEM. P values * <0.05, *** <0.001. ns, not significant.



Supplementary figure 4. Insulin secretion measures following *PAM* and *CHGA* knockdown. The data presented in figures 2A and 4A have been processed using alternative normalisation methods to compare the direct impact of gene silencing on insulin secretion releative to effects on insulin content and cell numbers. (A & C) show un-normalised insulin secretion (pg/hr) and (B & D) show content-normalised secretion (% of content). P values ** <0.01, *** <0.001.