Supplementary Data

Figure S1

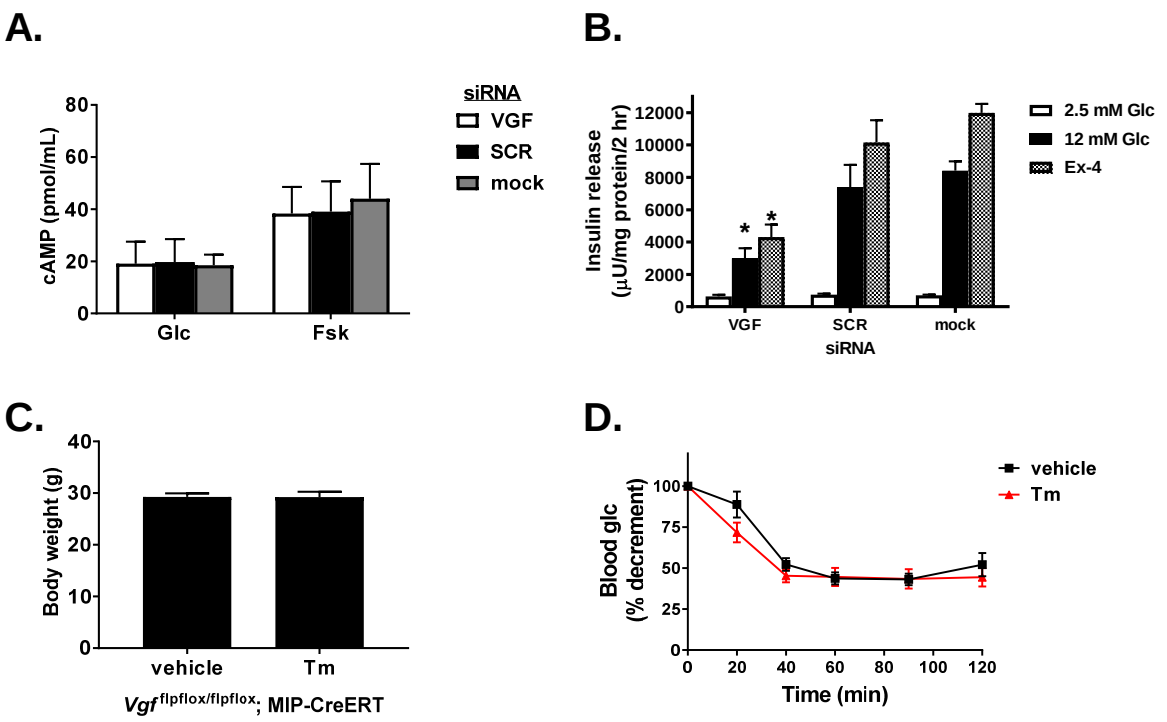

Figure S1. cAMP signaling and insulin sensitivity is not impaired in VGF loss of function models, related to Figures 1 and 2. (A, **B**) 832/3 cells were transfected with a mixture of VGF duplexes (VGF; SMARTpool), non-targeting control duplex (SCR) or mock transfected. (**A**) cAMP was measured in cell lysates following 15 min stimulation with glucose (12 mM) alone or with forskolin (0.5 µM Fsk). (**B**) GSIS was measured by static incubation in media containing 2.5 mM Glc followed by 12 mM Glc in the presence or absence of exendin-4 (20 nM) as indicated. (**C**, **D**) Male *Vgf* $^{flpflox/flpflox}$; MIP-CreERT mice (6-8 weeks of age) were injected (i.p.) with either vehicle (corn oil) or tamoxifen (Tm) as indicated and analyzed 4-6 weeks post-tamoxifen. (**C**) Body weights of 10-14 week old mice at time of GTT in Figure 2 C, D (n = 8-10 per group). (**D**) Fasted mice were injected (i.p.) with 1.0 mU/g bw Humulin-R (n = 8 per group). Blood glucose was measured at the indicated times and reported as the percentage decrement from starting glucose. (**A**, **B**) Data represent the mean ± S.E.M of at least 3 independent experiments. * $p \leq 0.05$ as compared to SCR siRNA- or mock-transfected cells. (**C**, **D**) Data represent the mean ± S.E.M.

Figure S2

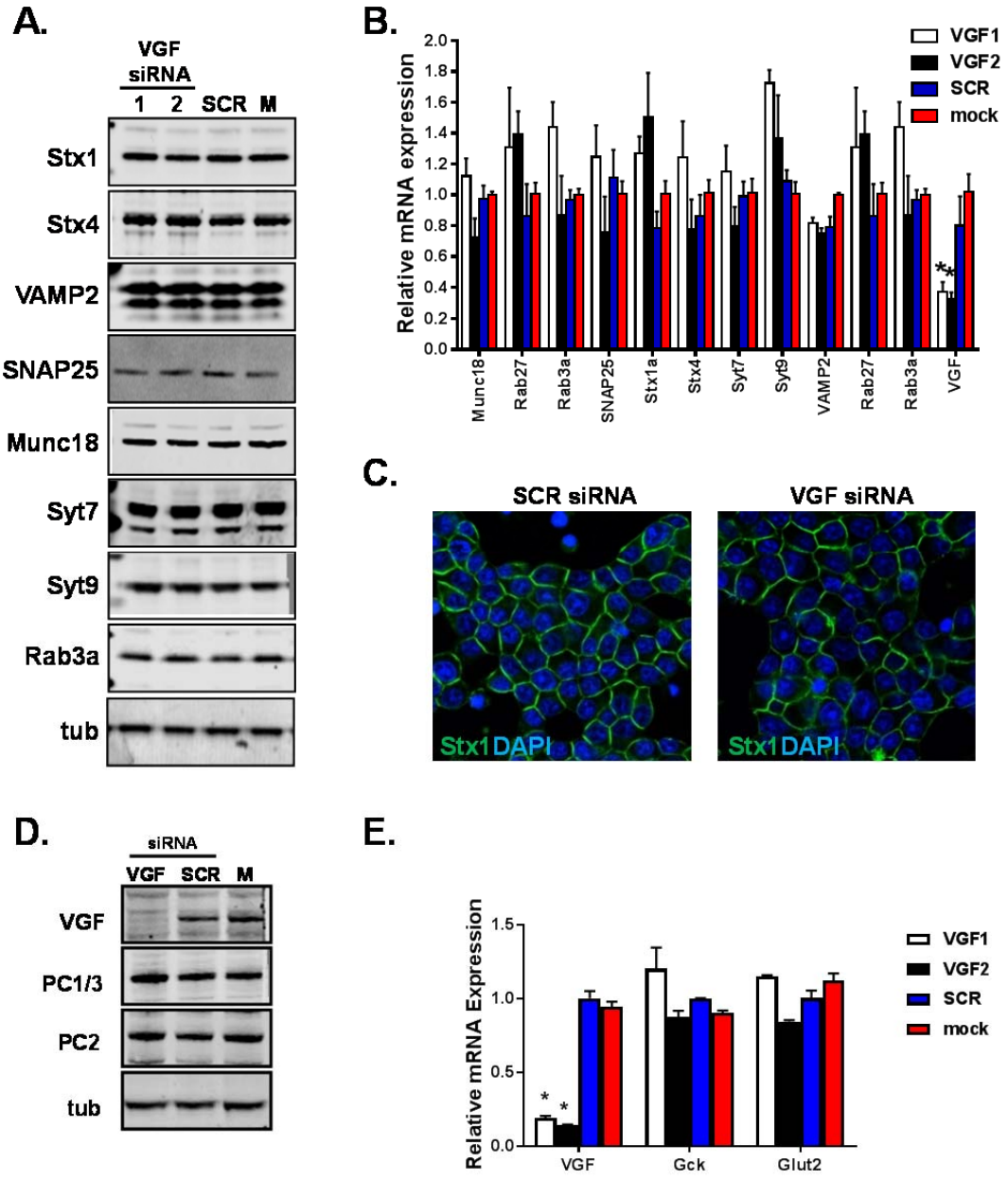

Figure S2. VGF suppression does not alter expression of exocytic machinery, related to Figure 1. 832/3 cells were transfected with rat VGF siRNA duplexes (SMARTpool), non-targeting control duplex (SCR) or mock transfected. (**A**, **D**) Immunoblot analysis of whole cell lysates. (**B**, **E**) Real time PCR analysis of mRNA expression. Data represent the mean ± S.E.M. * $p \leq 0.05$ as compared to SCR siRNA- or mock-transfected cells. (**C**) Immunostaining of syntaxin 1A (Stx1; green), counterstained with DAPI (blue).

Figure S3

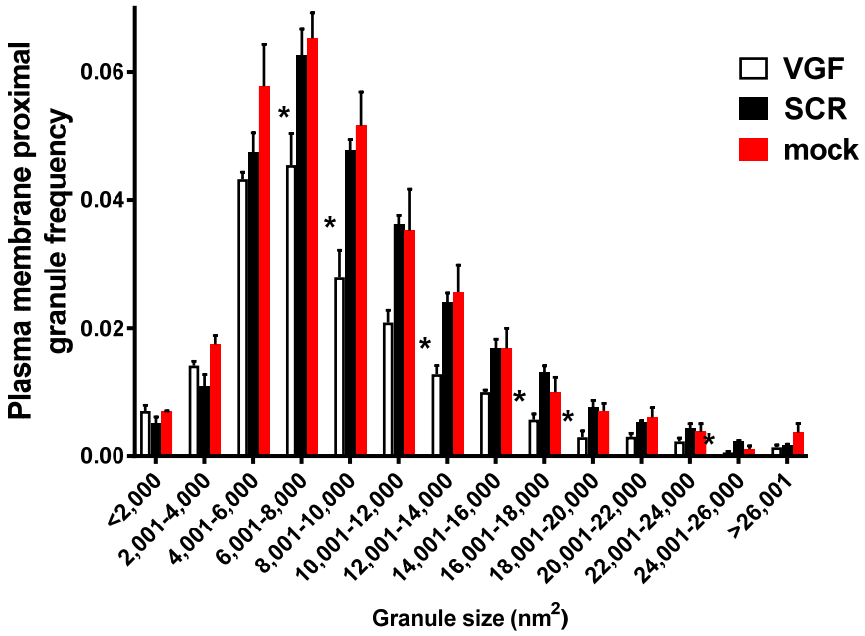

Figure S3. Frequency distribution of secretory granules at the plasma membrane, related to Figure 3. 832/3 cells were transfected with rat VGF siRNA duplexes (SMARTpool), non-targeting control duplex (SCR) or mock transfected. Frequency distribution of granule sizes binned within 200 nm of the plasma membrane. Data represent the mean ± S.E.M of 3 independent experiments. * $p \leq 0.05$ as compared to SCR siRNA- or mock-transfected cells.

Figure S4

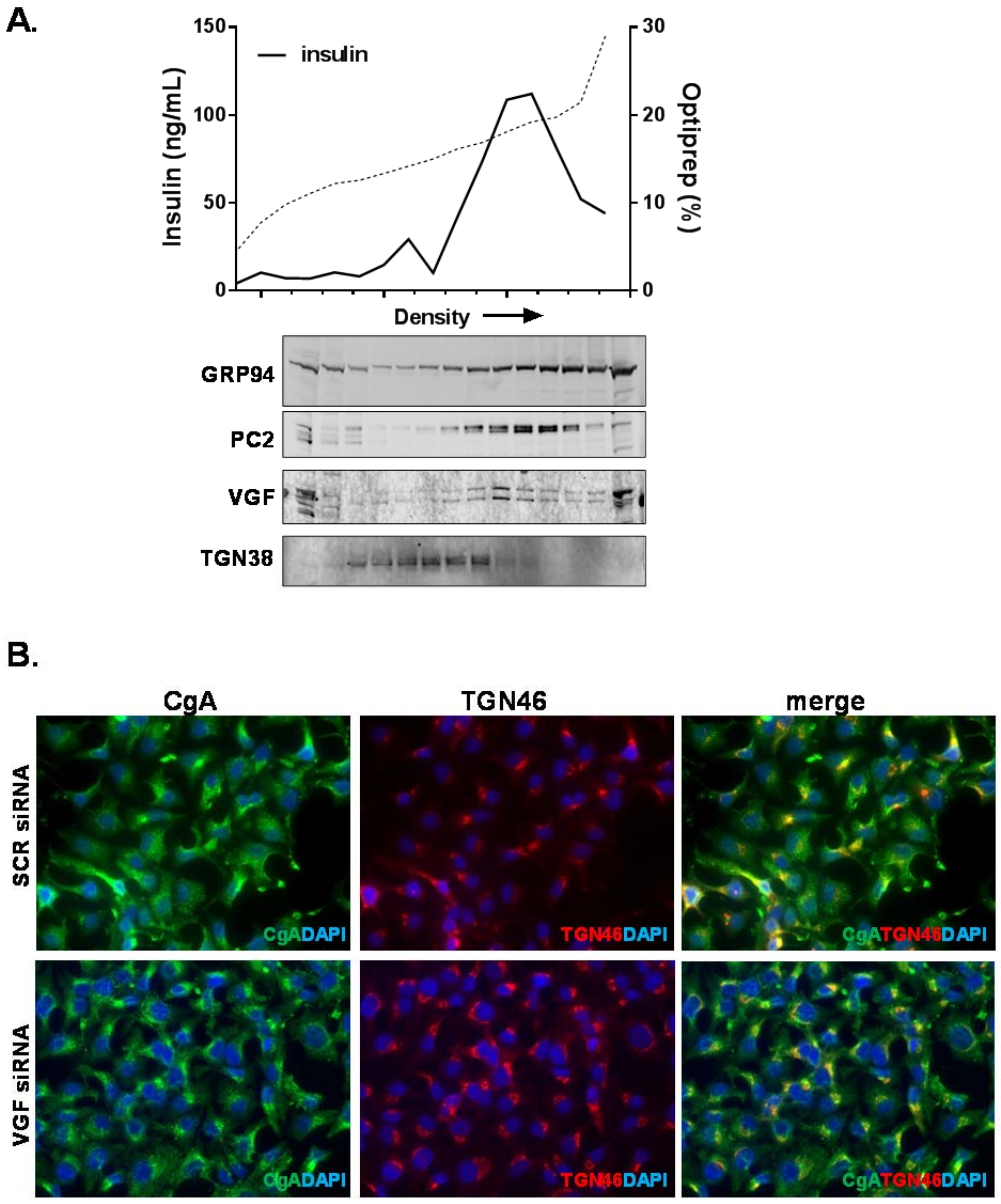

Figure S4. Purification of secretory granules using iodixanol gradients, related to Figure 4. (**A**) 832/3 cells were homogenized and post-nuclear supernatants loaded atop 8-20% linear iodixanol gradients. Fractions were manually collected and insulin determined by ELISA (bold line, dark circles). Gradients were verified by absorbance at 340 nm (dotted line). Immunoblot analysis of gradient fractions. (**B**) 832/3 cells were transfected with rat VGF siRNA duplexes (SMARTpool) or a non-targeting control duplex (SCR). Immunostaining of chromogranin A (CgA; green) relative to TGN46 (red) counterstained with DAPI (blue).

Figure S5

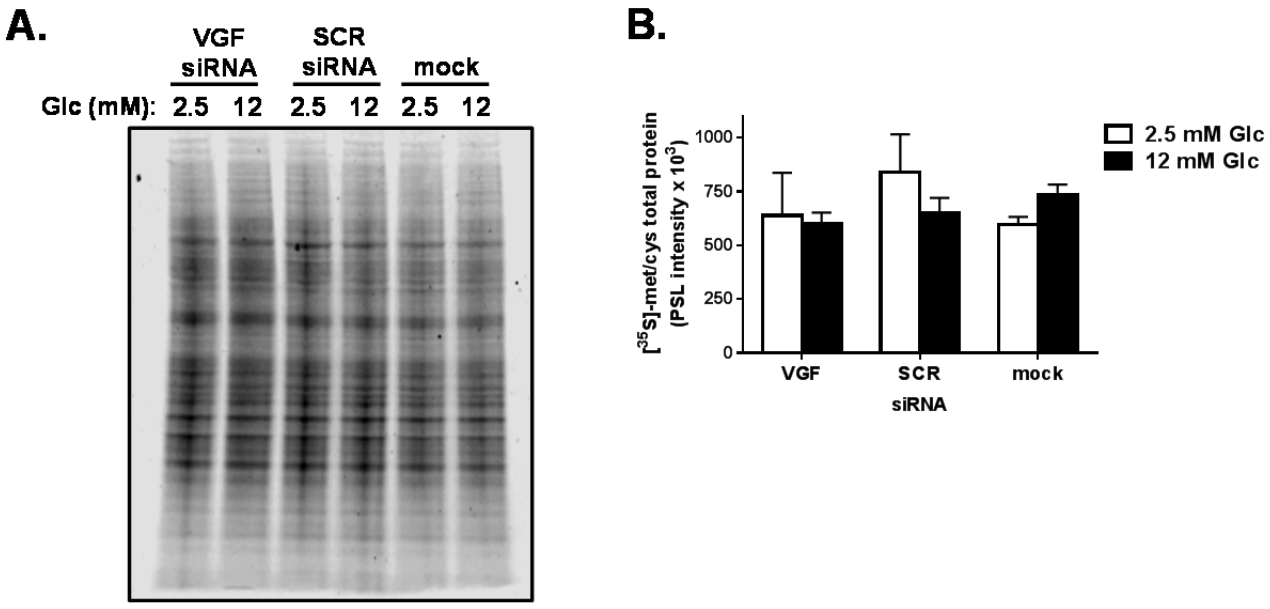

Figure S5. VGF suppression does not alter total protein synthesis, related to Figure 5. 832/3 cells were transfected with rat VGF siRNA duplexes (SMARTpool), non-targeting control duplex (SCR) or mock transfected as indicated. Cells were cultured in 2.5 mM or 12 mM Glc as indicated and pulse labeled with [^{35}S]-methionine/cysteine. Total protein synthesis was determined from phosphorimager analysis of whole cell lysates (**A**) quantitated (**B**). (**B**) Data represent the mean ± S.E.M. of three independent experiments.