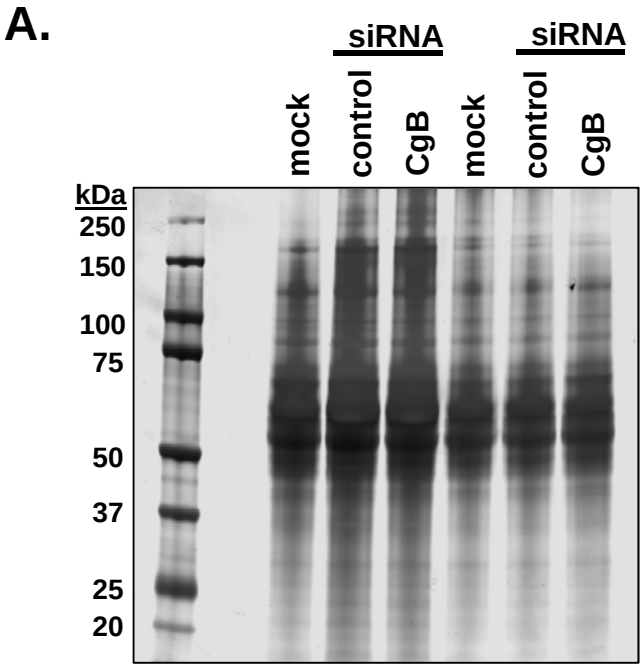

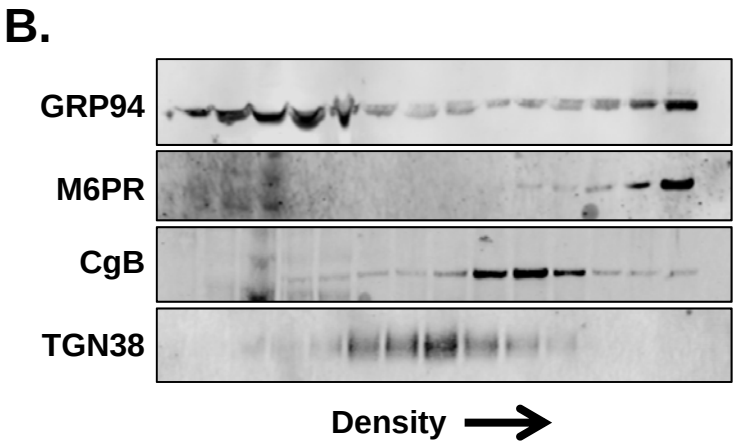

Figure S1. Basal exocytosis in CgB knockdown cells and density sedimentation markers. (**A**) 832/3 insulinoma cells were transfected with pooled siRNA duplexes targeting chromogranin B (CgB), a non-targeting control siRNA, or mock (no siRNA) as indicated. Media was collected following a 3 h incubation at 2.5 mM glucose. Proteins were TCA precipitated, resolved on 4-12% NuPAGE gels, and visualized by silver staining. (**B**) 832/3 cells were homogenized and post-nuclear supernatants loaded atop 8-23% linear iodixanol gradients. Lysates were resolved by ultracentrifugation at 110,000 xg's for 16-18 h. Immunoblot analysis of gradient fractions is shown. GRP94 is an ER resident chaperone; cation-dependent mannose-6 phosphate receptor (M6PR) is a lysosomal trafficking receptor; chromogranin B (CgB) is a granule marker; TGN38 is a Golgi marker.

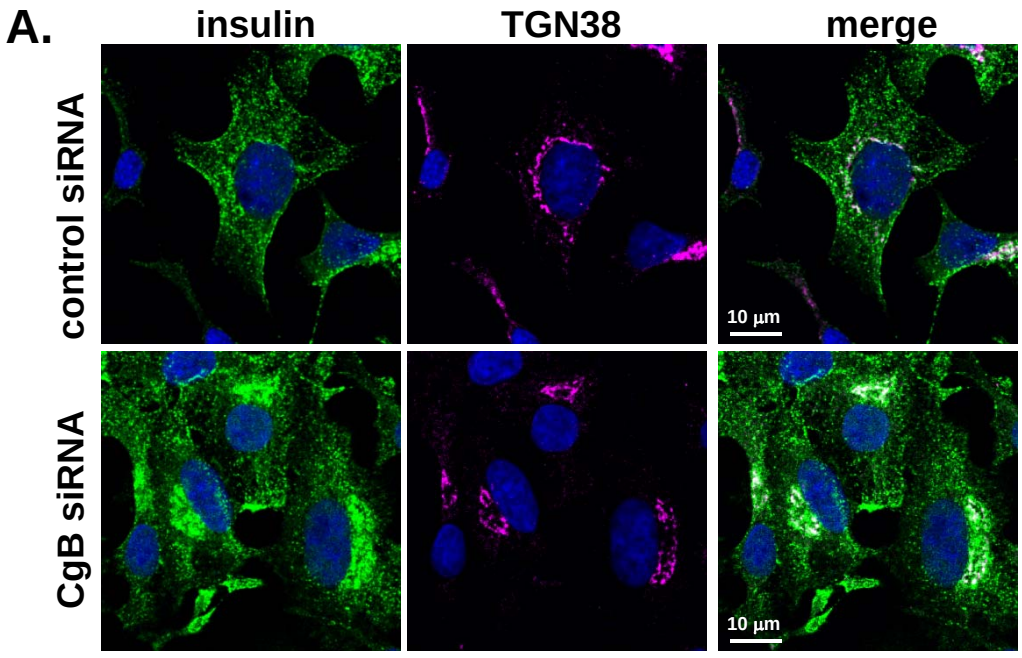

Figure S2. Loss of CgB is accompanied by increased TGN staining of insulin. 832/3 insulinoma cells were transfected with pooled siRNA duplexes targeting chromogranin B (CgB), a non-targeting control siRNA, or mock (no siRNA) as indicated. Cells were immunostained for insulin (green), TGN (magenta) and counterstained with DAPI (blue). Representative images are shown.

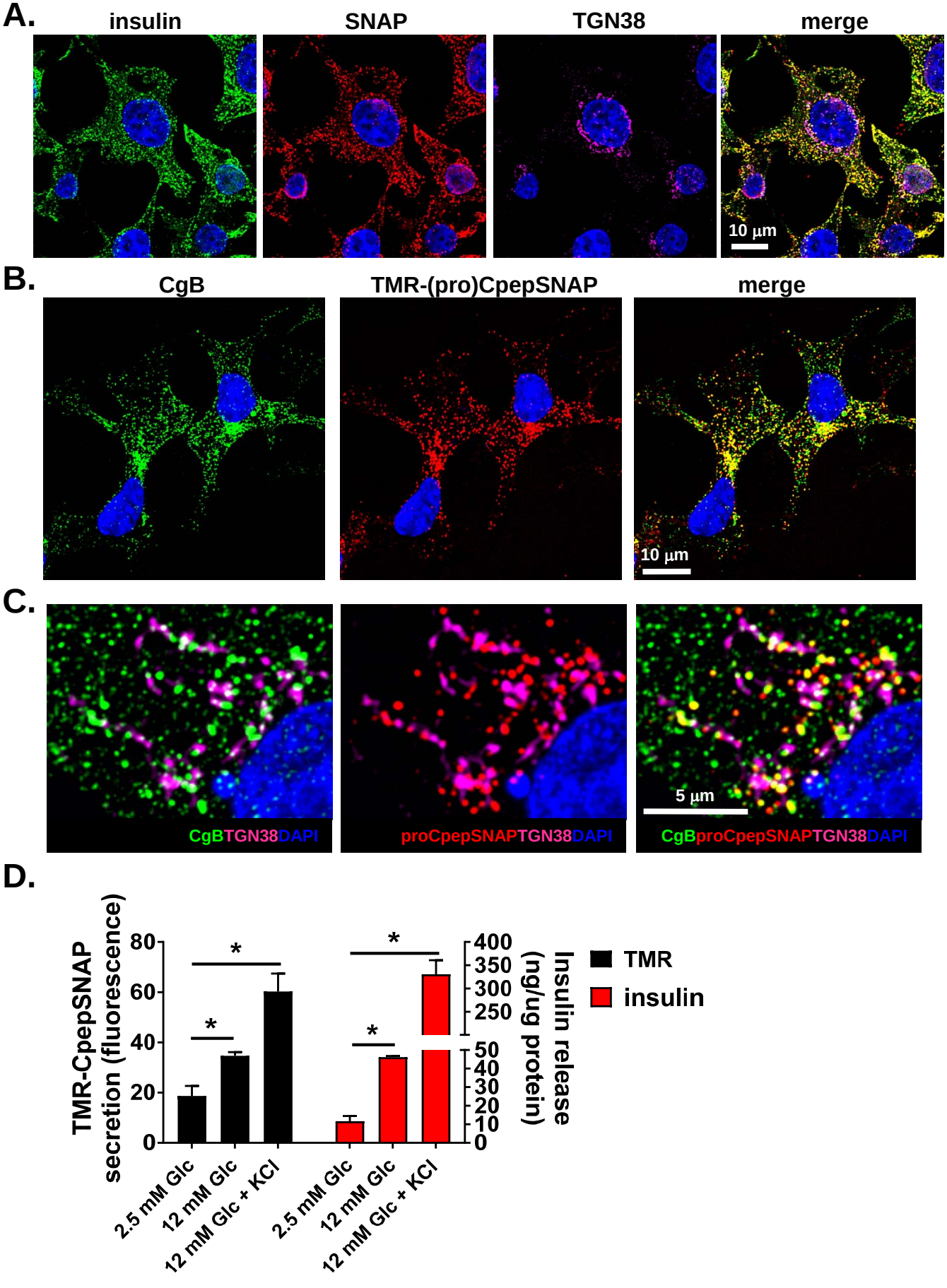

Figure S3. Subcellular localization and release of proCpepSNAP. 832/3 insulinoma cells stably expressing proCpepSNAP were evaluated for expression. (**A**) 832/3 cells were immunostained for insulin (green), SNAP (red), and TGN38 (magenta) and counterstained with DAPI (blue). Representative images are shown (max project from 5 z-stacks). (**B**) Cells were pulse-labeled with SNAP-TMR (red) for 20 min without prior non-fluorescent (block) labeling, washed and fixed. Cells were immunostained for CgB (green) and TGN (magenta) and counterstained with DAPI (blue). Representative images are shown (max project from 5 z-stacks). (**C**) Cells were pulse-labeled with SNAP-TMR (red) for 20 min with prior non-fluorescent (block) labeling, washed and fixed at the beginning of the chase period (t=0 h). Cells were immunostained for CgB (green) and TGN (magenta) and counterstained with DAPI (blue). Representative images are shown (max project from 5 z-stacks). (**D**) Cells were pulse-labeled with SNAP-TMR for 40 min, chased for 4 h, followed by static 1 h incubations with glucose and KCl as indicated. Fluorescence was measured from the cell media (n=3). Data represent the mean ± S.E.M. * $p < 0.05$ by one-way ANOVA as compared to basal (2.5 mM) Glc.

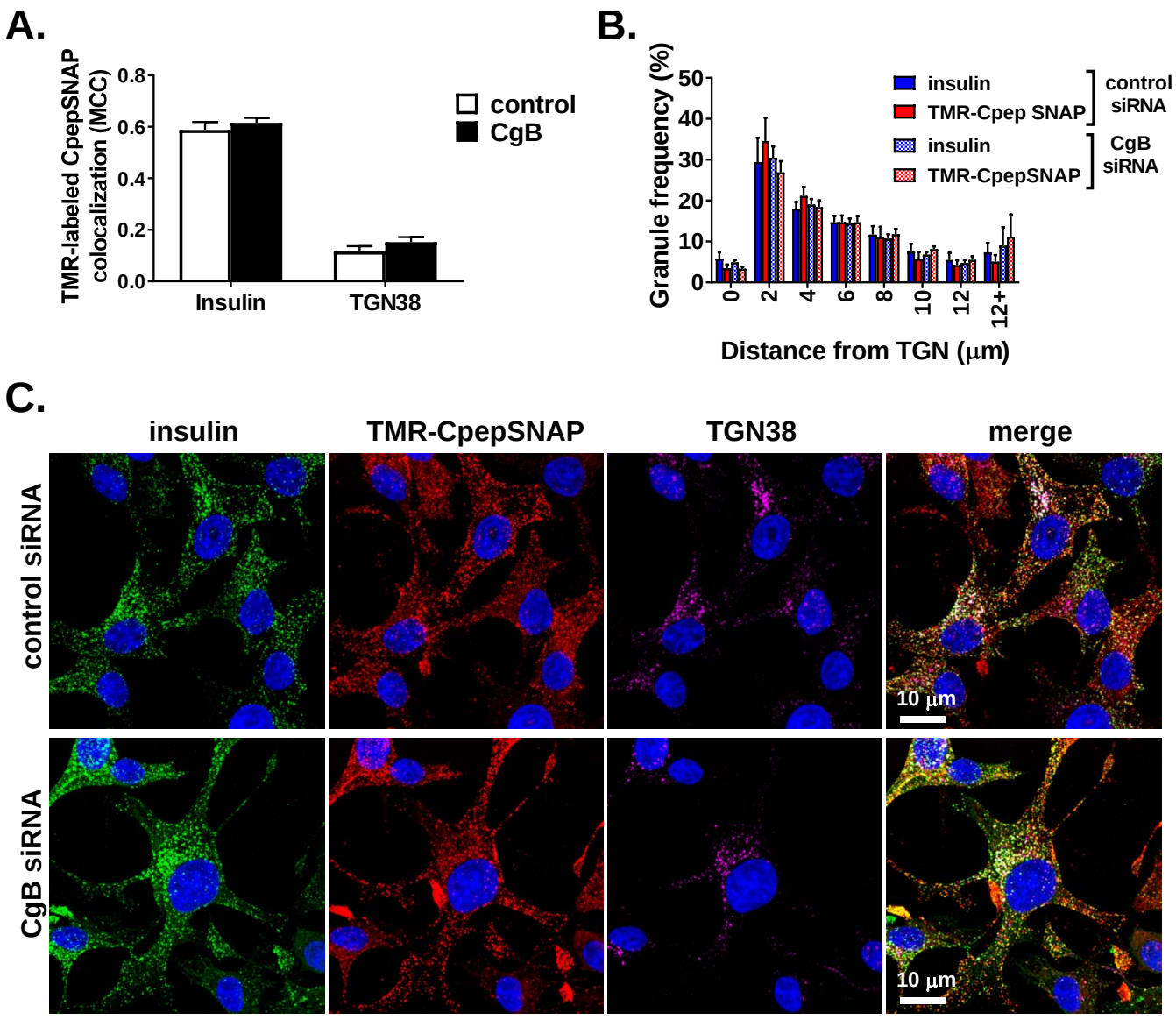

Figure S4. Long-term pulse-chase labeling of CgB knockdown cells have normal granule distribution. 832/3 cells stably expressing proCpepSNAP were transfected with pool of siRNA duplexes targeting CgB or a non-targeting control. Cells were pulse-labeled with SNAP-TMR (red) for 20 min, chased for 18 h, and fixed. Cells were immunostained for insulin (green), TGN38 (magenta), and counterstained with DAPI (blue). (**A**) Colocalization was determined by Mander's correlation coefficient (MCC). (**B**) Frequency distributions of binned granule distances from the TGN are shown. (**C**) Representative images are shown (max project from 5 z-stacks). (**A**, **B**) Data represent the mean ± S.E.M. of 73-90 imaged cells per condition from n=3 independent experiments.