Unbiased profiling of the human proinsulin biosynthetic interaction network reveals a role for peroxiredoxin 4 in proinsulin folding

Short running title: Human proinsulin biosynthetic interaction network

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Supplemental Figure Legends

Figure S1 - Specificity of monoclonal antibody 20G11 to proinsulin. (A) Lysates from human islets immunoprecipitated with in-house generated monoclonal antibody 20G11 to proinsulin or insulin antibody. Note negligible precipitation of insulin by 20G11. B) WT proinsulin, proinsulin MIDY mutants (A, B, C) or GFP (G) were overexpressed in COS1 cells and immunoprecipitated and immunoblotted with 20G11 antibody. The upper band in the lysate may be preproinsulin, recognized by 20G11 in reduced form (but not in native form in IP).

Figure S2 - Comparison of Label Free and TMT Mass Spectrometric analysis on one islet preparation identified common proteins but with different sensitivities.

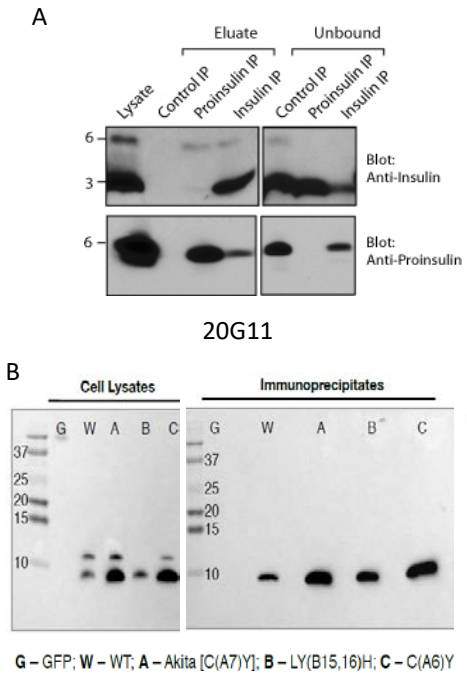
Figure S3- MS/MS Proinsulin Interactions Validated by IP-western blot. (A) Validation of MYO18A interaction with proinsulin was performed by proinsulin immunoprecipitation of lysates from human islets. 4% of lysate and supernatant, and 12% IP were analyzed by reducing SDS-PAGE and immunoblotted for MYO18A. (B) Validation of BiP, ERDJ5, GRP94 interaction with proinsulin was performed by immunoprecipitation of proinsulin from human islet lysate with proinsulin specific primary antibody (20G11) or mouse IgG. Lysates and Supernatants (Sup) 5%, and IP 12% were resolved on SDS-PAGE. Membranes were cut according to molecular weight markers and blotted for the indicated primary antibodies: BIP, ERDJ5, GRP94. (C) HEK293A cells were transfected with plasmids expressing WT human Proinsulin-MYC, human Akita mutant Proinsulin-MYC, human PRDX4-FLAG or pCDNA. Lysates were immunoprecipitated with anti-MYC (tag)-beads or anti-FLAG (tag)-beads. Lysates and supernatants 5%, and IPs 15% were immunoblotted for PRDX4, FLAG, human proinsulin (20G11) and MYC (tag).

Figure S4- PRDX4 mRNA expression in human beta cell single cell RNA-seq analysis is shown compared to insulin and the beta cell transcription factor PDX1 (22). Note that insulin is on a log scale.

Figure S5- PRDX4 immunoprecipitation controls for Figure 3A. (A) Briefly HEK293T cells were transfected with combinations of Proinsulin-MYC, PRDX4-FLAG and GFP. After 44 hours cells were treated with SubAb (2 µg/mL, 4 hrs), Mutant SubAb (2 µg/mL, 4 hrs), HA15 (10 µM, 4 hrs) or untreated, followed by immunoprecipitation with anti-FLAG magnetic beads. Non-reducing SDS-PAGE of PRDX4 immunoprecipitated with anti-FLAG beads immunoblotted with PRDX4-HRP direct conjugate antibody. The data shows that all PRDX4 conformations were immunoprecipitated (20% of IP analyzed). (B) The same experimental conditions described above except that control GFP was transfected in place of PRDX4-FLAG. Lysates were immunoprecipitated with anti-FLAG beads, showing that no proinsulin or BiP binds non-specifically to FLAG beads.

Figure S6- Menadione induces proinsulin misfolding and recruits PRDX4 into HMW complexes in human islets. One thousand human islet equivalents were treated with either 100 or 200 µM Menadione for 1 hour at 37ºC and lysed with 2X Laemmli without BME and analyzed on reducing and non-reducing SDS-PAGE. Left panel shows non-reducing SDS-PAGE immunoblotted for proinsulin and its accompanying reducing SDS-PAGE immunoblotted for loading control vinculin, PRDX4 and proinsulin. Right panel shows non-reducing SDS-PAGE immunoblotted for PRDX4 and its accompanying reducing SDS-PAGE for vinculin, PRDX4, and proinsulin.

Figure S1. Specificity of monoclonal antibody 20G11 to proinsulin.



G – GFP; **W** – WT; **A** – Akita [C(A7)Y]; **B** – LY(B15,16)H; **C** – C(A6)Y

Figure S2. Comparison of Label Free and TMT analysis in human islets.

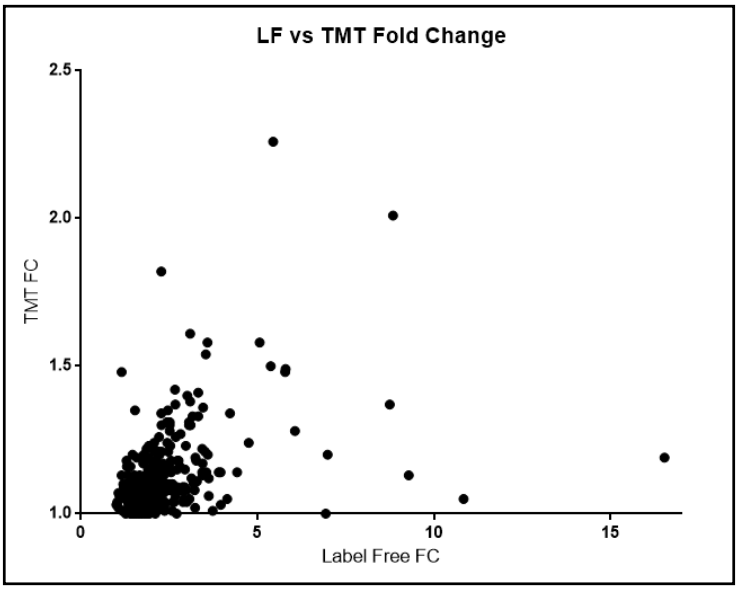
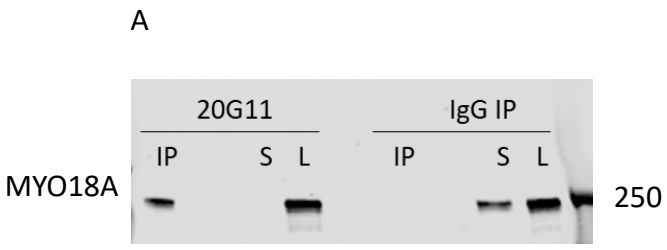
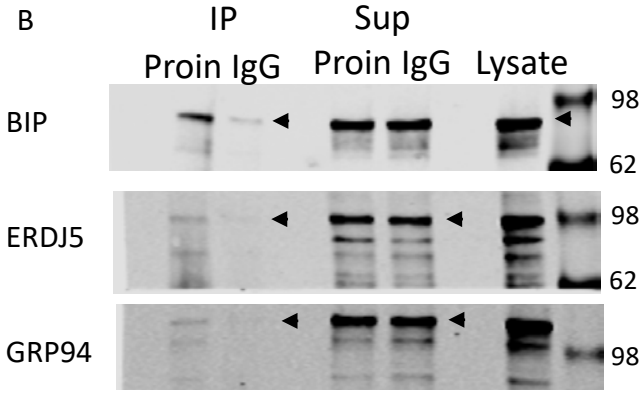


Figure S3. MS/MS Proinsulin Interactions Validated by IP-western blot.





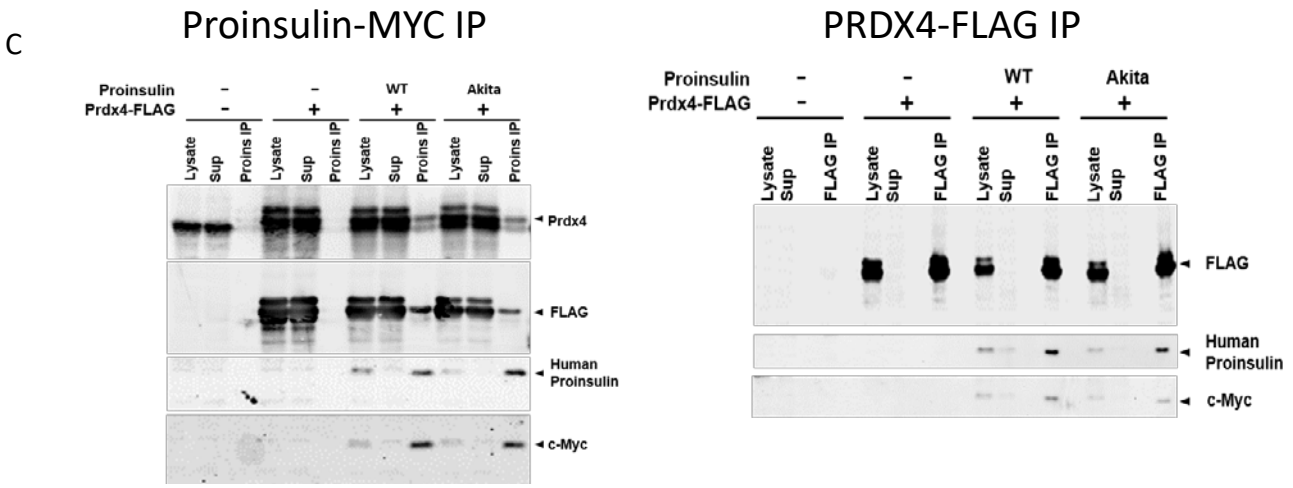


Figure S4. PRDX4 mRNA expression in human beta cell single cell RNA-seq analysis.

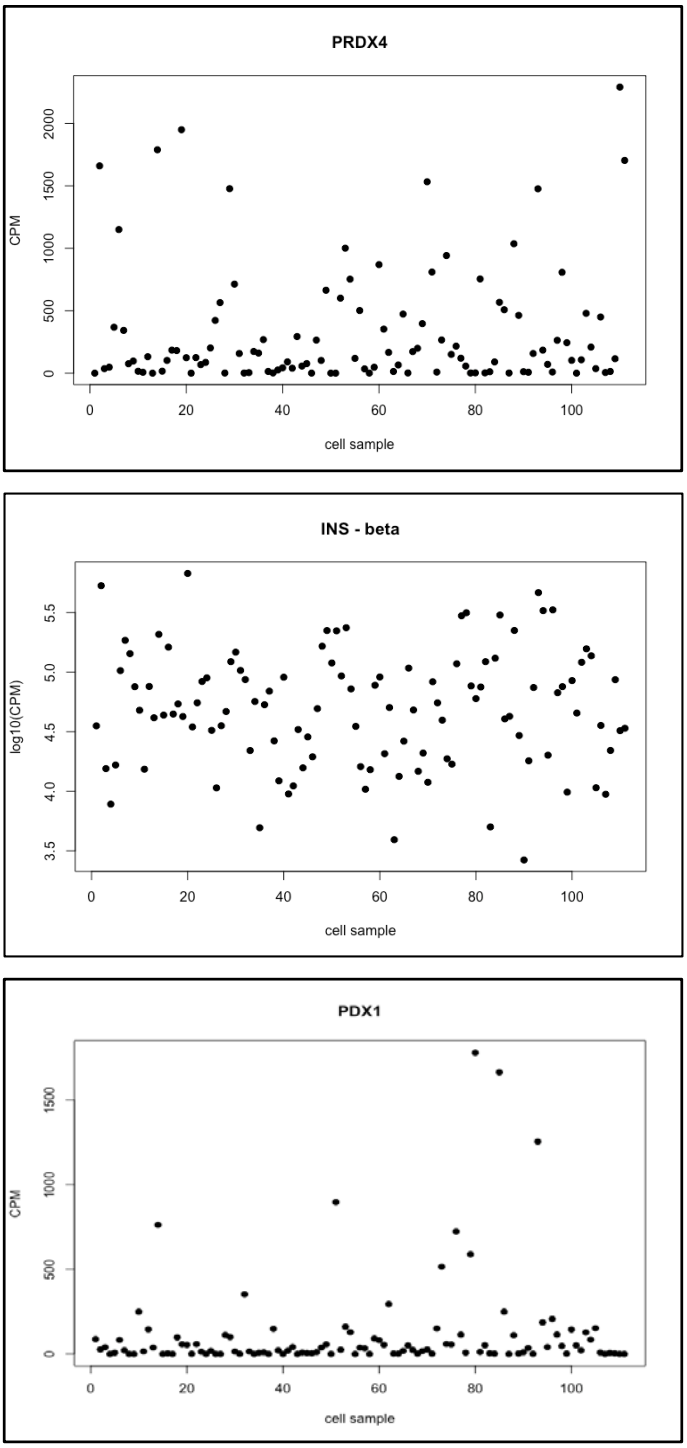
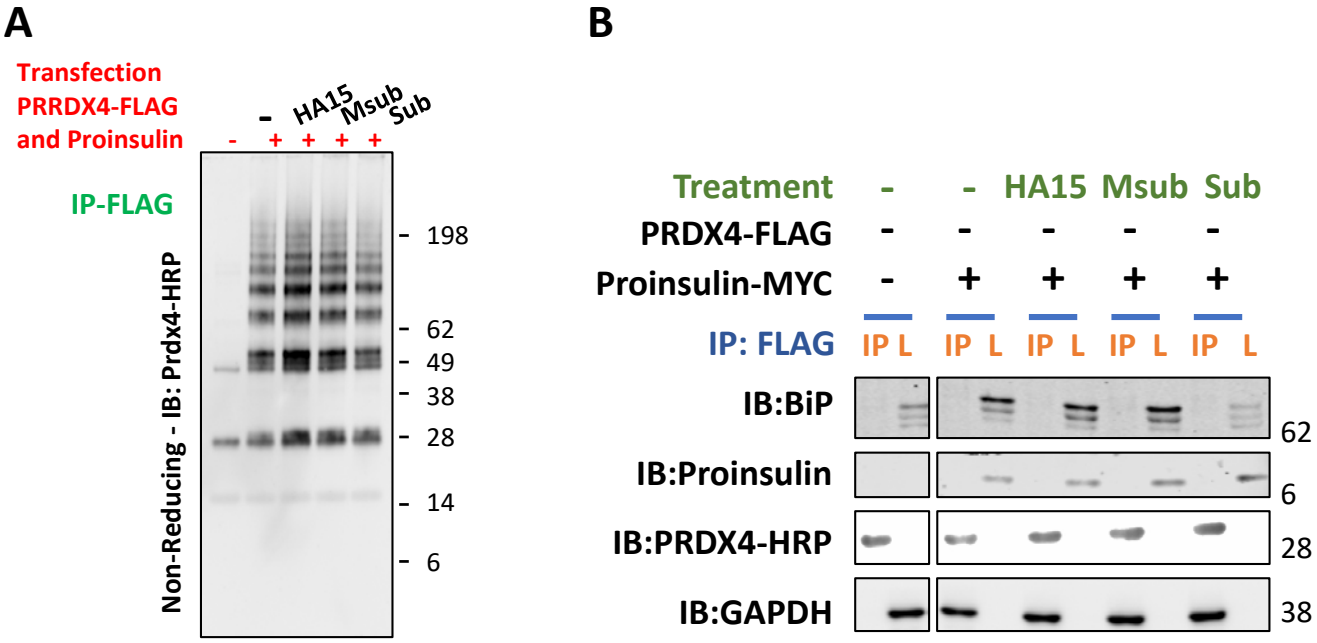


Figure S5. PRDX4 immunoprecipitation controls for Figure 3A.



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