**Supplementary Figure S1.** Proinsulin keep mutants and lose mutants are synthesized at rates comparable to that of wild-type proinsulin. INS1 cells were transiently transfected to express myc-tagged proinsulin (‘wt’), or keep mutants or lose mutants as indicated. The methionine content of each of these constructs is identical. At 48 h, cells were labeled with pure $^{35}$S-Met for 15 min, lysed, immunoprecipitated with anti-myc, and analyzed by reducing Bis-Tris 4-12% polyacrylamide gradient gel electrophoresis and phosphorimaging (upper panel). The synthesis of proinsulin was quantified by scanning densitometry and ImageJ; the numbers in white reflect independent replicates, and no statistically significant differences were observed.

**Supplementary Figure S2.** Schematic of potential cysteine interlopers that may lure natural disulfide partners into disulfide mispairing.

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Supplementary Figure S3. Effect of MIDY mutations on the secretion of proinsulin lose-A6/A11. 293T cells were transfected with myc-tagged proinsulin (‘WT’), or the indicated constructs, or untransfected (‘Control’). At 30 h post-transfection, both cells and overnight culture media were collected and analyzed by Western blotting as in Fig. 2A.