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Supplemental Data

Pathogenic Variants in Fucokinase Cause a Congenital Disorder of Glycosylation

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Supplemental Data

Figure S1 – Determination of newly synthesized [^{3}H]- GDP-Fucose and [^{3}H]-Fuc1p and lentiviral complementation of primary fibroblasts.

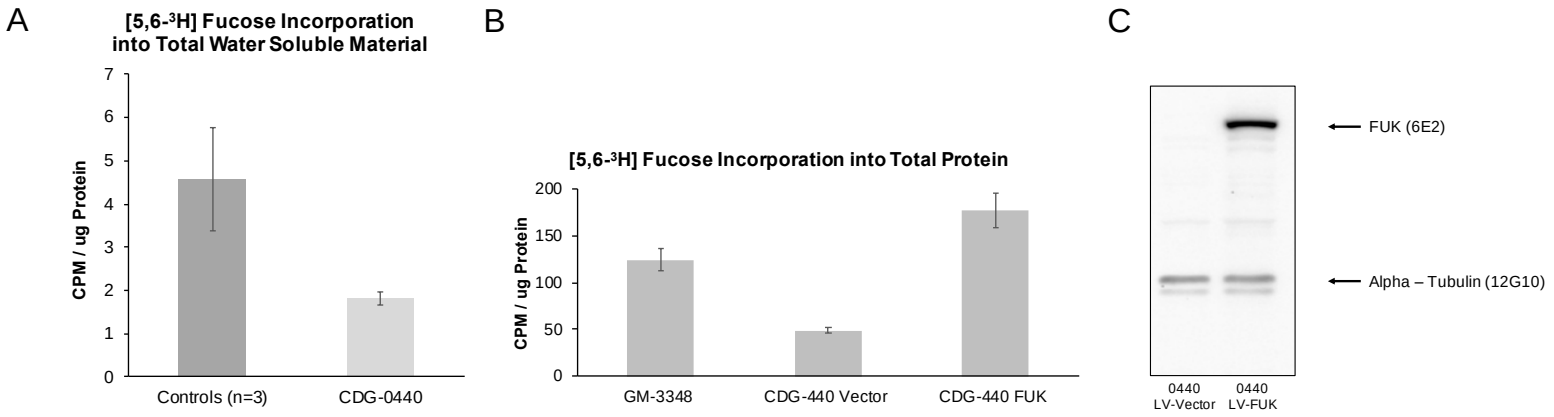

Figure S1 – Determination of newly synthesized [^{3}H]- GDP-Fucose and [^{3}H]-Fuc1p and lentiviral complementation of primary fibroblasts.

(A) Primary fibroblasts (3-controls and CDG-0440 all in triplicates) were metabolically labeled with 2uCi/mL [5,6-^{3}H]-fucose for 24hrs to determining the newly synthesized [^{3}H]- GDP-Fucose and [^{3}H]-Fuc1p, which are purified from a water-soluble extraction. After the extraction of water-soluble metabolites and normalizing samples total protein content, CDG-0440 had a 60% reduction in both [^{3}H]- GDP-Fucose and [^{3}H]-Fuc1p, when compared to the three controls. (B) Lentiviral transduction into fibroblast from CDG-0440 with either an empty vector carrying GFP or human FUK showing complementation of the [5,6-^{3}H]-fucose incorporation into cell associate protein deficiency. (C) Western blot analysis of FUK in lentiviral transduced fibroblast from CDG-0440. The mAb to FUK (6E2) (Thermo Fisher MA5-15847) was used at a 1:1000

dilution. In panel Figure S1A and S1B, experiments were performed in biological triplicates and error bars were calculated as a standard deviation of the group.

Figure S2 – Characterization of a HAP1-FUK knockout cell line.

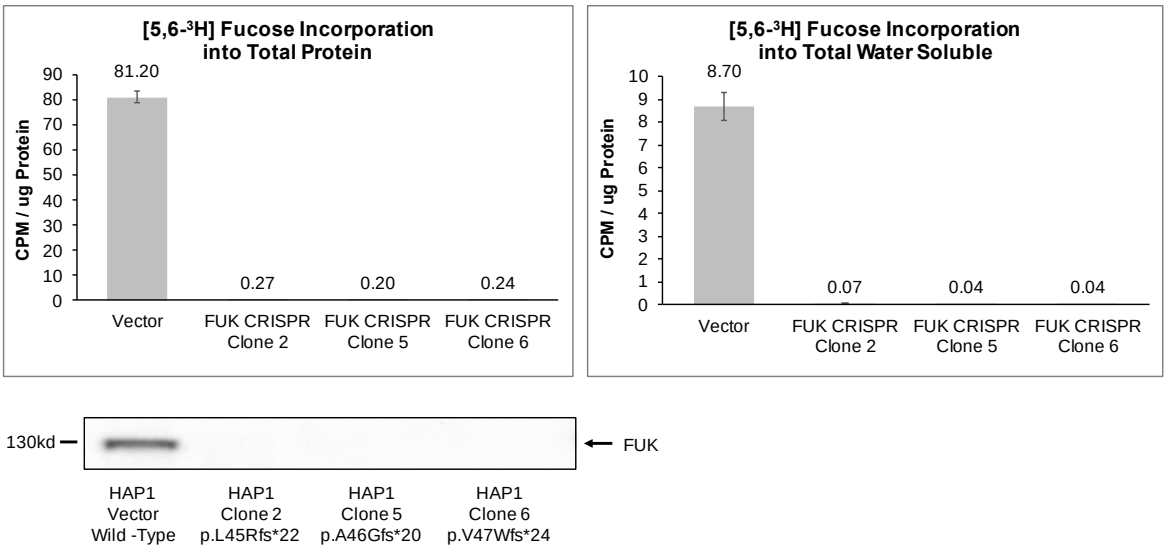

Figure S2 – Characterization of a HAP1-FUK knockout cell line.

Metabolic labeling HAP1 cells with 2uCi/mL [5,6-^{3}H]-fucose for 24hrs and determining [^{3}H]-fucose incorporation into either cell associate proteins (*left panel*) or water-soluble metabolites (*right panel*). Both showing no incorporation of [5,6-^{3}H]-fucose into either set. Western blot analysis of the three individual FUK KO clones, confirming the loss of FUK protein (*lower panel*). In panel Figure S2A, experiments were performed in biological triplicates and error bars were calculated as a standard deviation of the group.