

Supplemental information for

UDP-Glucose: A Cereblon-Dependent Glucokinase Protein Degradar.

This pdf file includes:

Abbreviations

Supplementary Figures S1 to S6

Abbreviations

BSP, bromosulphophthalein

cIAP1, cellular inhibitor of apoptosis protein 1

CRBN, cereblon

CRBN-F, FLAG-tagged cereblon

CRL4, cullin ring ubiquitin ligase 4

CUL4, cullin 4

DDB1, damage-specific DNA binding protein 1

2DG, 2-deoxy-ad-glucose

ER, endoplasmic reticulum

GCK, glucokinase

GCK-HA, HA-tagged glucokinase

GLUT, glucose transporter

GSIS, glucose-induced insulin secretion

IC₅₀, 50% inhibitory concentration

IMiDs, Immunomodulatory drugs

IP, immunoprecipitation

MDM2, murine double minute 2

MODY, maturity-onset diabetes of the young

PROTACs, proteolysis-targeting chimeras

TCA cycle, tricarboxylic acid cycle

UGGT, UDP-glucose:glycoprotein glucosyltransferase

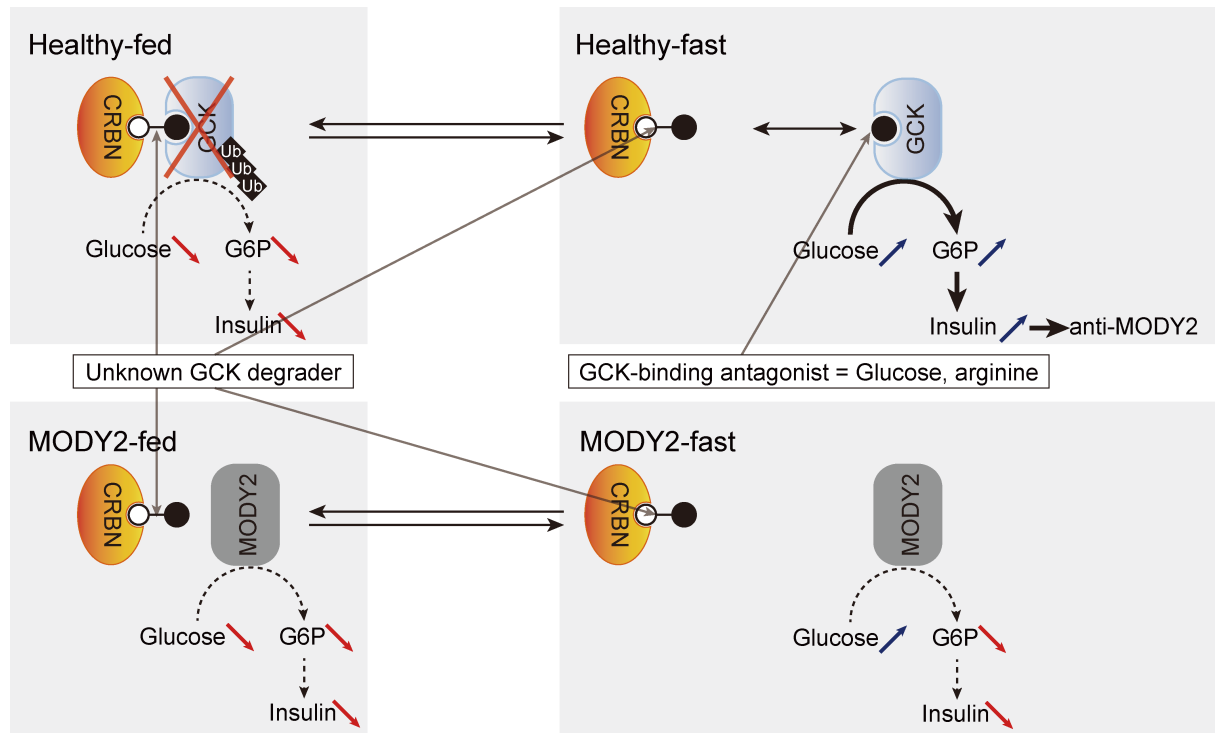
UGP, UDP-glucose pyrophosphorylase

UPR, unfolded protein response

VHL, von Hippel-Lindau

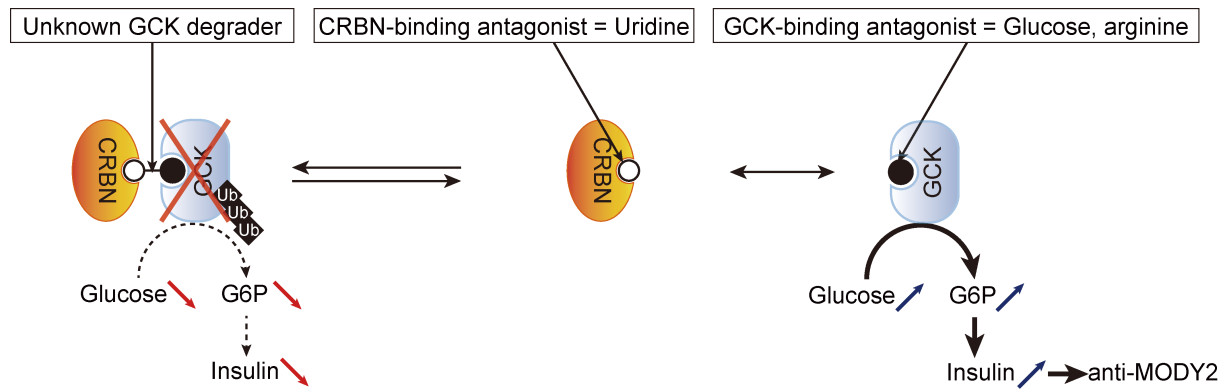
WB, Western blot

WT, wild type



Supplementary Figure S1. Glucose inhibits glucokinase ubiquitination and degradation.
(Supplementary data for Fig. 1).

(S1a) Summary of Fig 1. During fasting, the unknown glucokinase protein degrader X degrades glucokinase, and G6P production is reduced. After feeding, glucose and arginine bind to glucokinase and repress X-dependent glucokinase degradation, leading to induction of G6P production. In the case of glucokinase variants of MODY2, glucokinase variants less bound to glucose, and less G6P production.



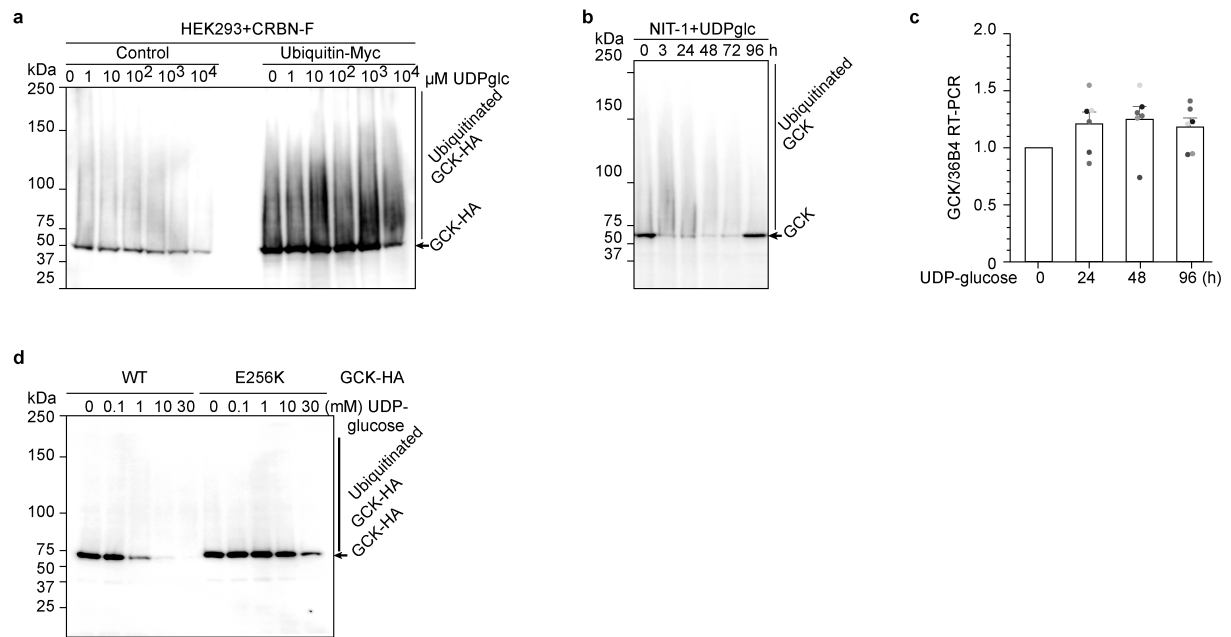
Supplementary Figure S2. Uridine binds to cereblon (CRBN). (Supplementary data for Figure 2).

Possible model of glucokinase cereblon-dependent ubiquitination and degradation by endogenous ligands.

Agonist; unknown

CRBN-binding antagonist; uridine

Glucokinase-binding antagonist; glucose, arginine



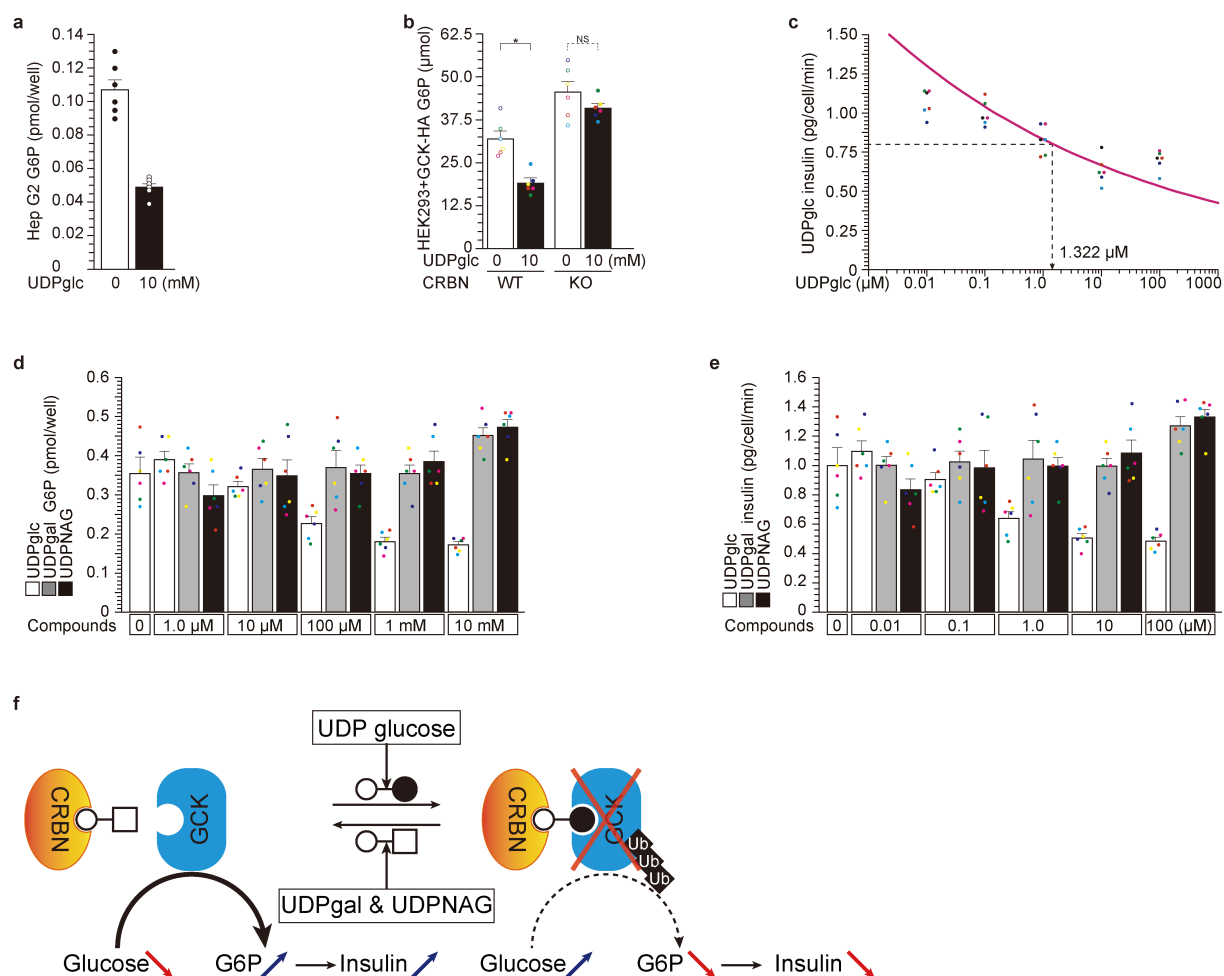
Supplementary Figure S3. UDP-glucose induces glucokinase ubiquitination and degradation. (Supplementary data for Fig. 3).

(S3a) UDP-glucose induces glucokinase ubiquitination in a dose-dependent manner. The HEK 293 cells were transfected with HA-tagged glucokinase (GCK-HA), FLAG-tagged cereblon (CRBN-F), and Myc-tagged ubiquitin (ubi-Myc) expression vectors for one day, and then UDP-glucose was added for 3 h. The cells were harvested and intracellular GCK-HA was detected by WB.

(S3b) UDP-glucose administration induced glucokinase degradation from 3 h to 72 h. NIT-1 cells were treated with UDP-glucose for the indicated times (0, 3, 24, 48, 72, and 96 h). Ubiquitinated and non-ubiquitinated glucokinase were detected with anti-glucokinase WB.

(S3c) UDP-glucose administration does not change glucokinase transcription. NIT-1 cells were treated with UDP-glucose for 0, 24, 48, and 96 h. Total RNA was extracted, and RT-PCR was performed.

(S3d) One mM UDP-glucose administration induced glucokinase^{WT} ubiquitination and degradation, although thirty mM UDP-glucose induced glucokinase^{E256K} mutated protein.



Supplementary Figure S4. UDP-glucose induces glucokinase degradation, and reduces insulin secretion from 3 h to 24 h. (Supplementary data for Fig. 4).

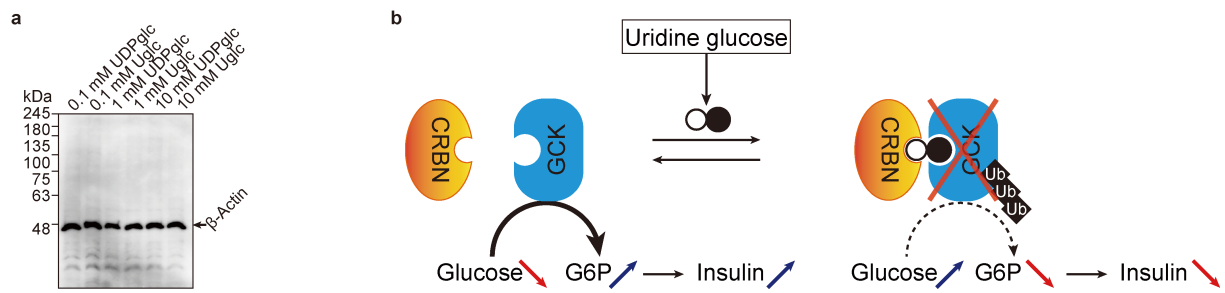
(S4a) UDP-glucose administration reduced G6P production in Hep G2 cells for 3 h, and then the cells were extracted. Intracellular G6P was analyzed using commercial kit.

(S4b) UDP-glucose did not reduce G6P production in CRBN KO cells for 3 h, and then cells were extracted. Intracellular G6P was analyzed using commercial kit. *, $p < 0.05$, NS; $p > 0.05$.

(S4c) UDP-glucose degrades proinsulin at $IC_{50} = 1.322 \mu M$

(S4d and e) UDP-monosaccharide regulates G6P production and insulin secretion.

(S4f) Possible model of UDP-monosaccharide regulating glucokinase ubiquitination.



Supplementary Figure S5 Uridine-glucose degraded glucokinase, and reduced insulin secretion. (Supplementary data for Fig. 5).

(S5a) WB of control β -actin in Fig 5b.

(S5b) Possible model of uridine-glucose-degraded glucokinase protein.

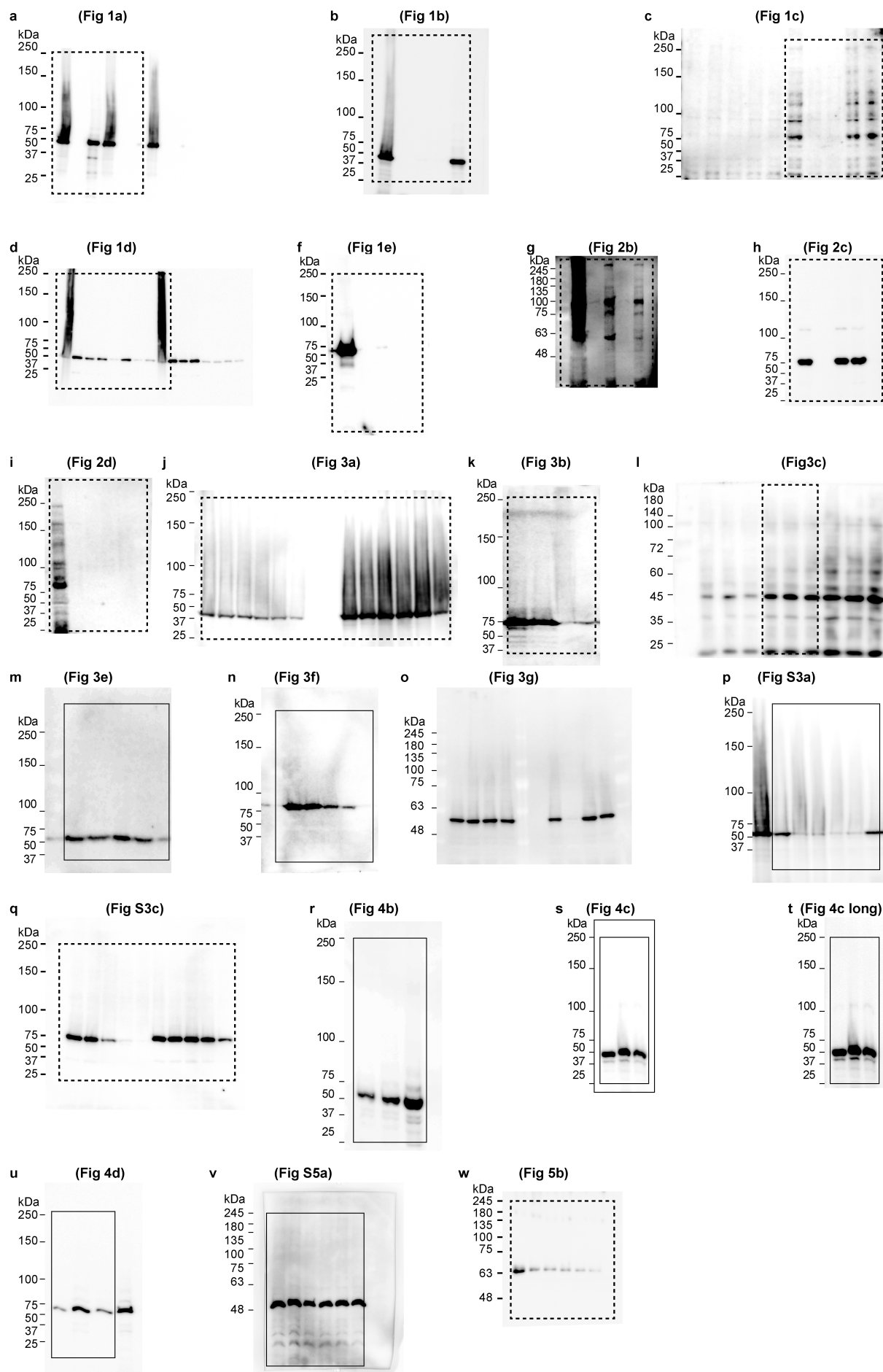


Figure S6 Source of Data