Online Supplementary Methods

Online Supplementary Methods 1: P446L-variant site-directed mutagenesis primers (mismatches underlined)

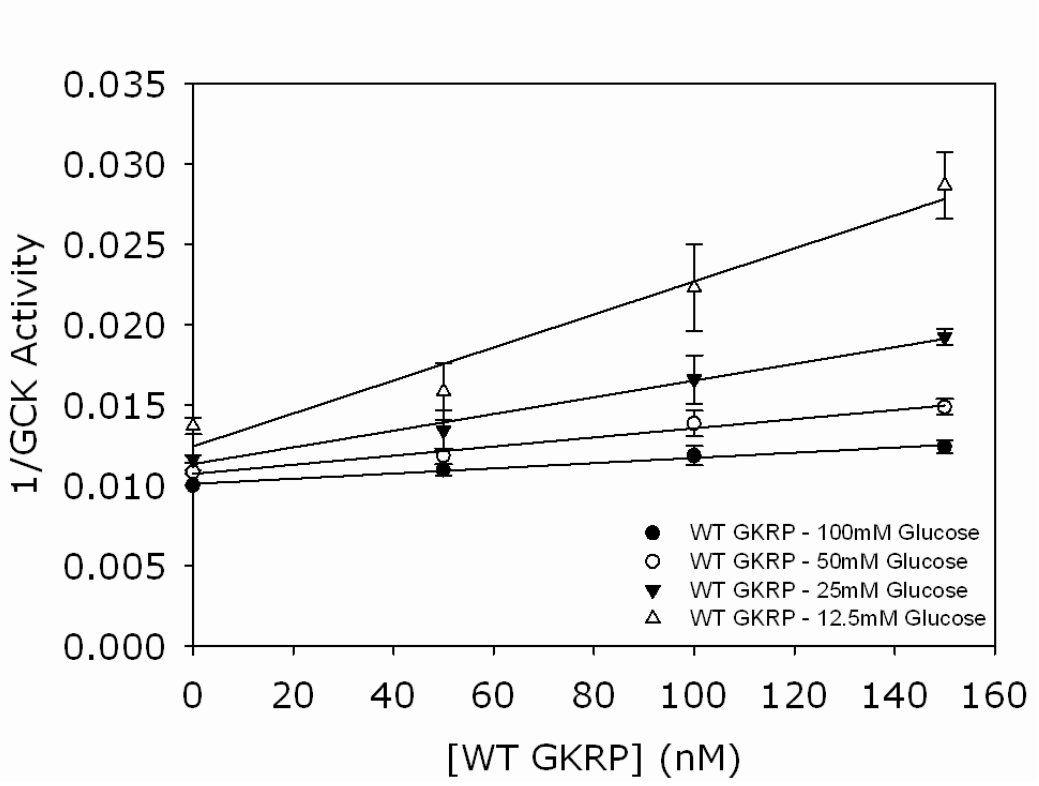
Forward:	5′ CAG ACC TTG C<u>T</u>G ATC CCT CTG AAG AAG CTC TTT CCC TCC 3′
Reverse:	5′ GGA GGG AAA GAG CTT CTT CAG AGG GAT C<u>A</u>G CAA GGT CTG 3′

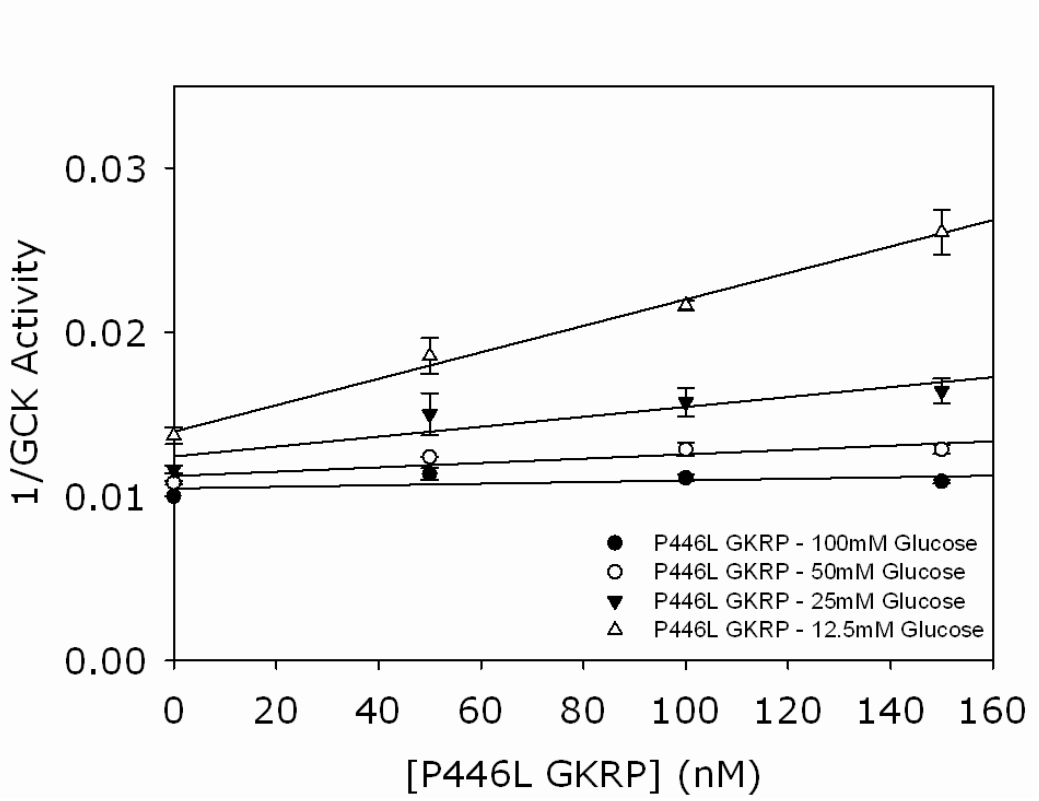
Online Supplementary Methods 2:

Primer ID	**Sequence (5'- 3')**
Vector† 5'_F	GGA AGC TGT GGT ATG GCT GT
Vector† 5'_R	GGT CAG AAT GGA TTC GCT GT
A_F	GAT GCT GAG ATC TTC CAG GAG
A2_F	GTG CCA ATC ACG GAG AAG TC
A/A2_R	TGT CGG AAT GTT GAA CTC CA
B_F	AGG TGG TGA CAG GTC TGT GG
B_R	AAT GTC CGC AAG ATT TCC AG
C_F	ATT CCG ACA AGT AGC AGA GC
C_R	ATG AGA AAG CCA CGG ACA TC
D_F	ATT TGA GCG AGC TCA TCA GG
D_R	TCT TCT CTT TCA CCT GCT CCA
E_F	TGC TGA TTT CCG AGA TGT CC
E_R	TGC TAA TCC GAA GGT CCA AC
F_F	TGG AGC AGG TGA AAG AGA AGA
F_R	CCT GTT CCT TCT CAT GTG CA
Vector† 3'_F	ACC ACA TGT TGG ACC TTC G
Vector† 3' B_F	TCC AGG TTG CAC ATG AGA AG
Vector† 3'/B_R	CGC TTC TGC GTT CTG ATT TA

† Vector containing human GKRP coding sequence was p-FLAG CTC (Sigma Aldrich Ltd). All primers contained M13-tail attachments at start (Forward: 5'-TGT AAA ACG ACG GCC AGT-3'; Reverse: 5'-CAG GAA ACA GCT ATG ACC-3'; '_F' denotes forward primer, '_R' denotes reverse).

Online Supplementary Results





Online Figures 1a and 1b: Dixon plots using equimolar concentrations of WT and P446L-GKRP (n=3)

GCK activity was assayed at varying glucose concentrations (black circles=100mM, white circles = 50mM, black triangle=25mM, white triangles=12.5mM) in the presence of 0nM, 50nM, 100nM and 150nM WT and P446L-GKRP. Dixon plots of 1/GCK activity vs. [GKRP] were used to determine the inhibitor dissociation constant ('Ki'), and these were found to be 24.0 ± 40.7nM for WT vs. 23.1 ± 40.8nM for P446L-GKRP, and thus was not significantly different between the two (means ± SEM; P=1).