SUPPLEMENTARY DATA

Supplementary Table 1. Regression coefficients for GK activity: immunoreactivity plots: The slopes of each individual data set from Figure 2 were calculated using linear regression analysis. The regression coefficient (r) was calculated using the Fig.P programme. N.S. = not significant

Figure	GK	Regression Coefficient	P
2B	WT	0.897	0.001
	G72R	0.662	0.052
	M298K	0.758	0.018
2C	WT	0.897	0.001
	Y214C	0.912	0.001
	G264S	0.71	0.032
2D	WT	0.891	0.001
	W99R	0.711	0.032
	E300K	0.496	NS
2E	WT	0.813	0.008
	E70K	0.713	0.031
	R275C	0.877	0.002
2F	WT	0.871	0.002
	V62M	0.769	0.016
	S263P	0.594	NS
2G	WT	0.732	0.025
	A53S	0.099	NS
	H137R	0.935	0.0002
2H	WT	0.756	0.018
	V455M	0.932	0.0002
	A456V	0.644	0.061
2I	WT	0.805	0.009
	T65I	0.684	0.049
	K414E	0.435	NS

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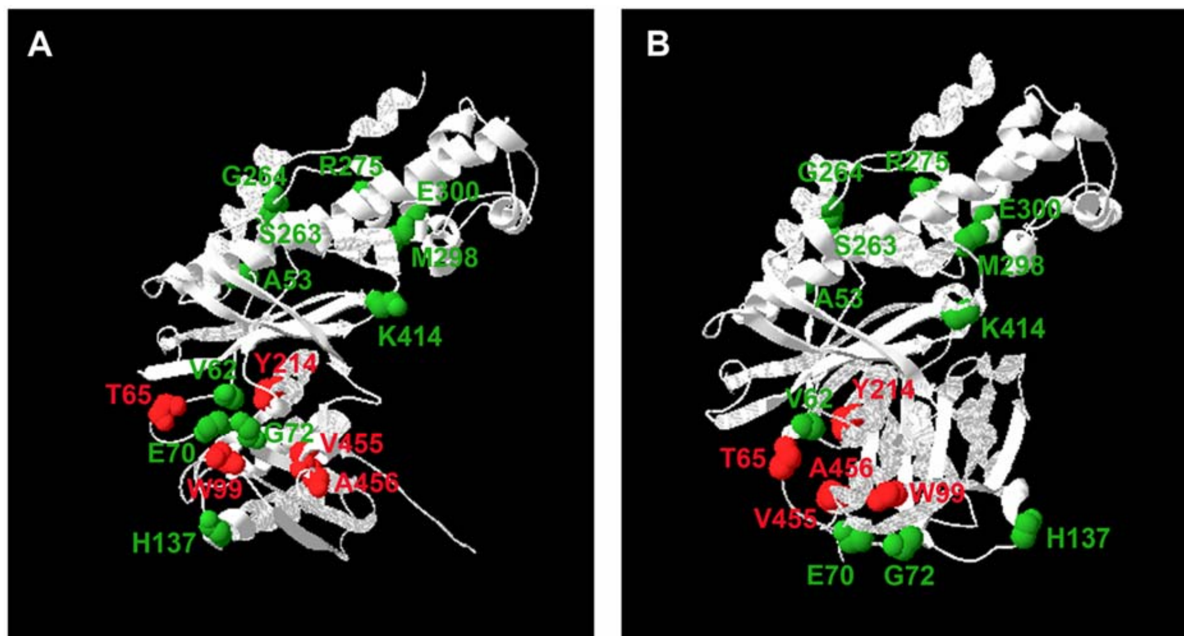
SUPPLEMENTARY DATA

Supplementary Figure 1. Localisation of GK-MODY and PHHI mutants on GK crystal structure: GK-MODY and PHHI mutants were localised on the crystalised structural model of either open WT-GK (**A**) or closed WT-GK co-crystallised with RO0283946 (**B**) (30). PHHI mutants are shown in red and GK-MODY mutants in green.



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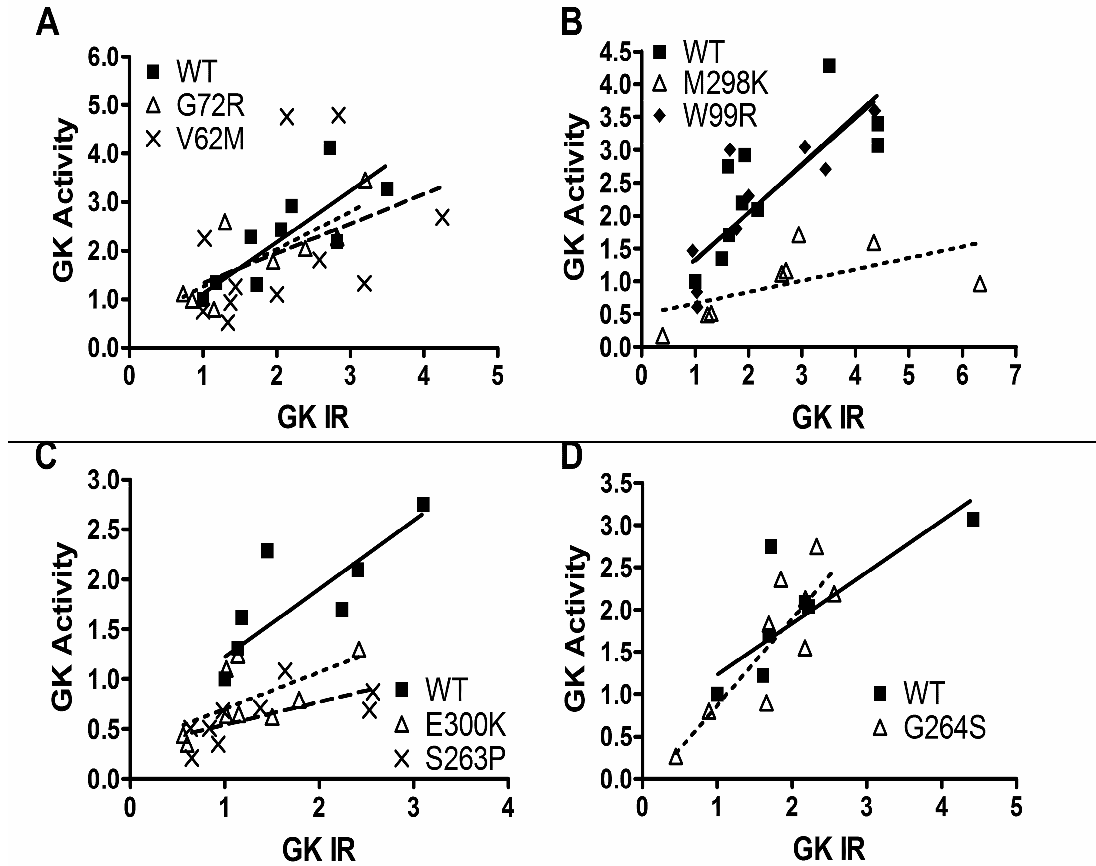
SUPPLEMENTARY DATA

Supplementary Figure 2. Cellular stability of wild-type and mutant GK in H411E cells: H4IIE cells were transiently transfected with wild-type or mutant GK at increasing cDNA titres (0.2, 0.4, 0.6µg/well) and cultured for 24h. GK activity was determined via a spectrophotometric method. Immunoreactivity was determined via western blotting and quantified by densitometry. GK activity is plotted against GK immunoreactivity. Results are expressed relative to myc-WT titre 1 (0.2µg). The slope of the graph was determined by linear regression analysis. n=3 plasmid titres from 3 independent experiments (G72R, W99R M298K, S263P, G264S, E300K) or 4 independent experiment (V62M).



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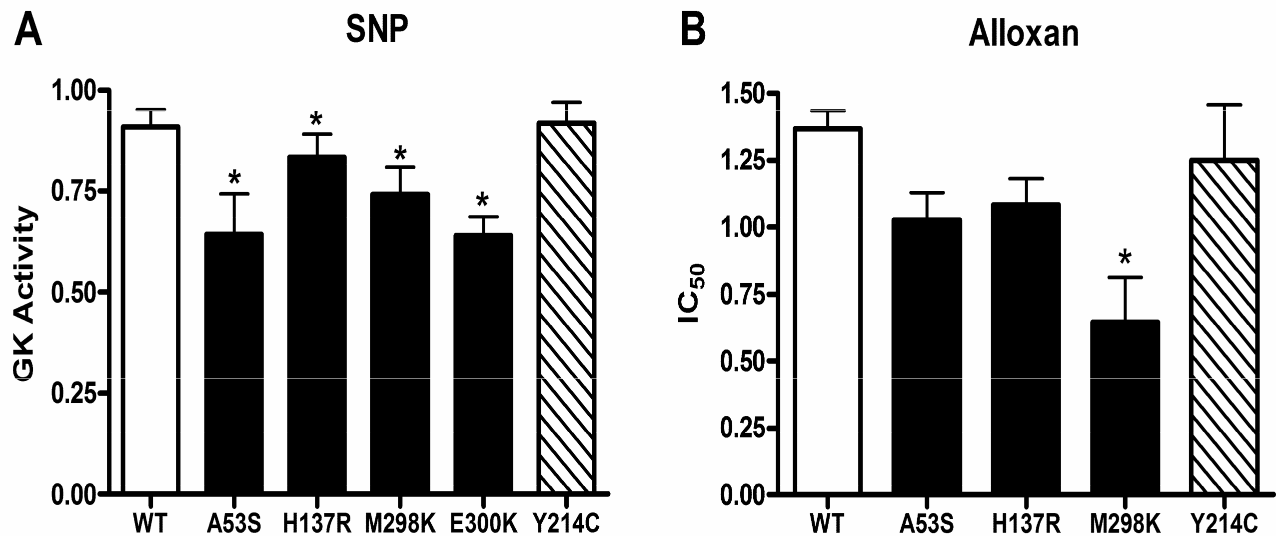
Supplementary Figure 3. Sensitivity of wild-type and mutant GK to oxidation: A, B. MIN6 cells were transiently transfected with 0.6µg/well (WT, Y214C) or 1.2µg/well (A53S, H137R, M298K, E300K) of myc-GK and cultured for 24h.
A. Cells were treated with 100µmol/l diamide and 100µmol/l sodium nitroprusside (SNP) at 5mmol/l glucose for 1h. GK activity was determined using a spectrophotometric method and expressed relative to absence of SNP. Means -/+ SEM of 4 (Y214C), 5 (H137R, M298K) or 6 (A53S, E300K) independent experiments. *P <0.05 relative to wild-type.
B. Cells were permeabilised with 0.04mg/ml digitonin and the cell lysate incubated with increasing concentrations of alloxan (0.25, 0.5, 0.75, 1, 2.5, 5, 10mmol/l) in the absence of glucose for 5min. GK activity was determined at 25mmol/l glucose, plotted against alloxan concentration on a log plot and the IC_{50} for alloxan calculated by nonlinear regression analysis (mmol/l). Means -/+ SEM of 3 independent experiments. P <0.05 relative to WT.



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