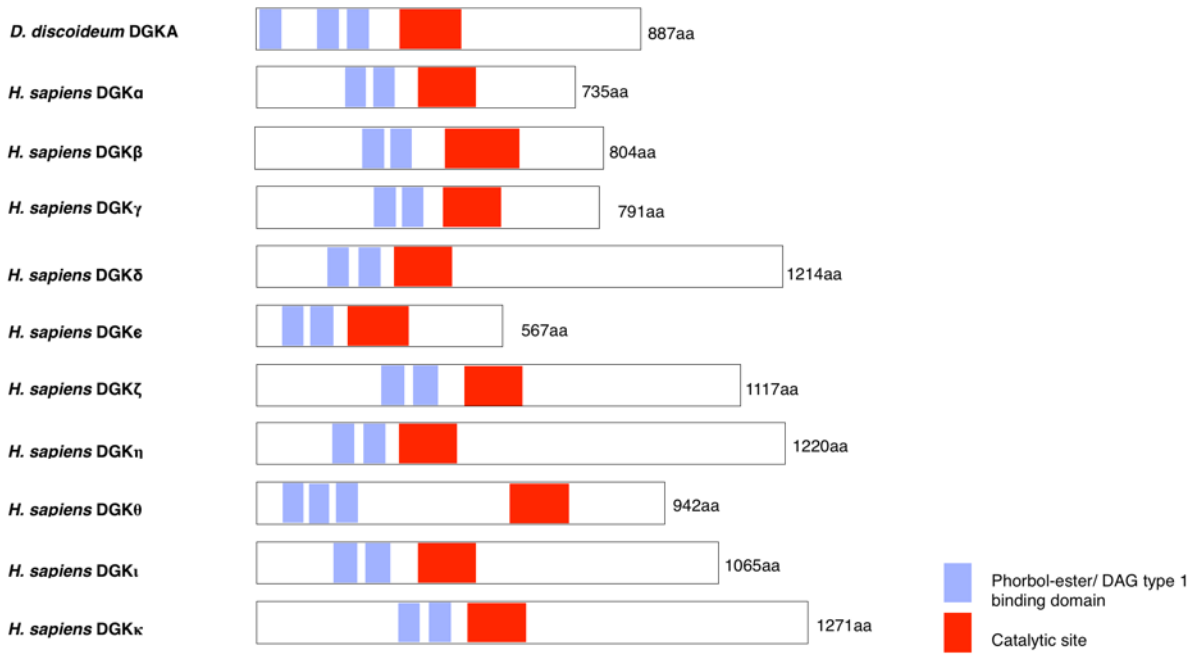


Fig. S1. Schematic comparison of domain structures for the *Dictyostelium* DGKA and the 10 Human DGK isoforms. The *Dictyostelium* DGKA and the 10 human DGK isoforms are shown with amino acid number, the number and position of the phorbol-ester/ DAG type 1 binding domains (purple) and the catalytic site.

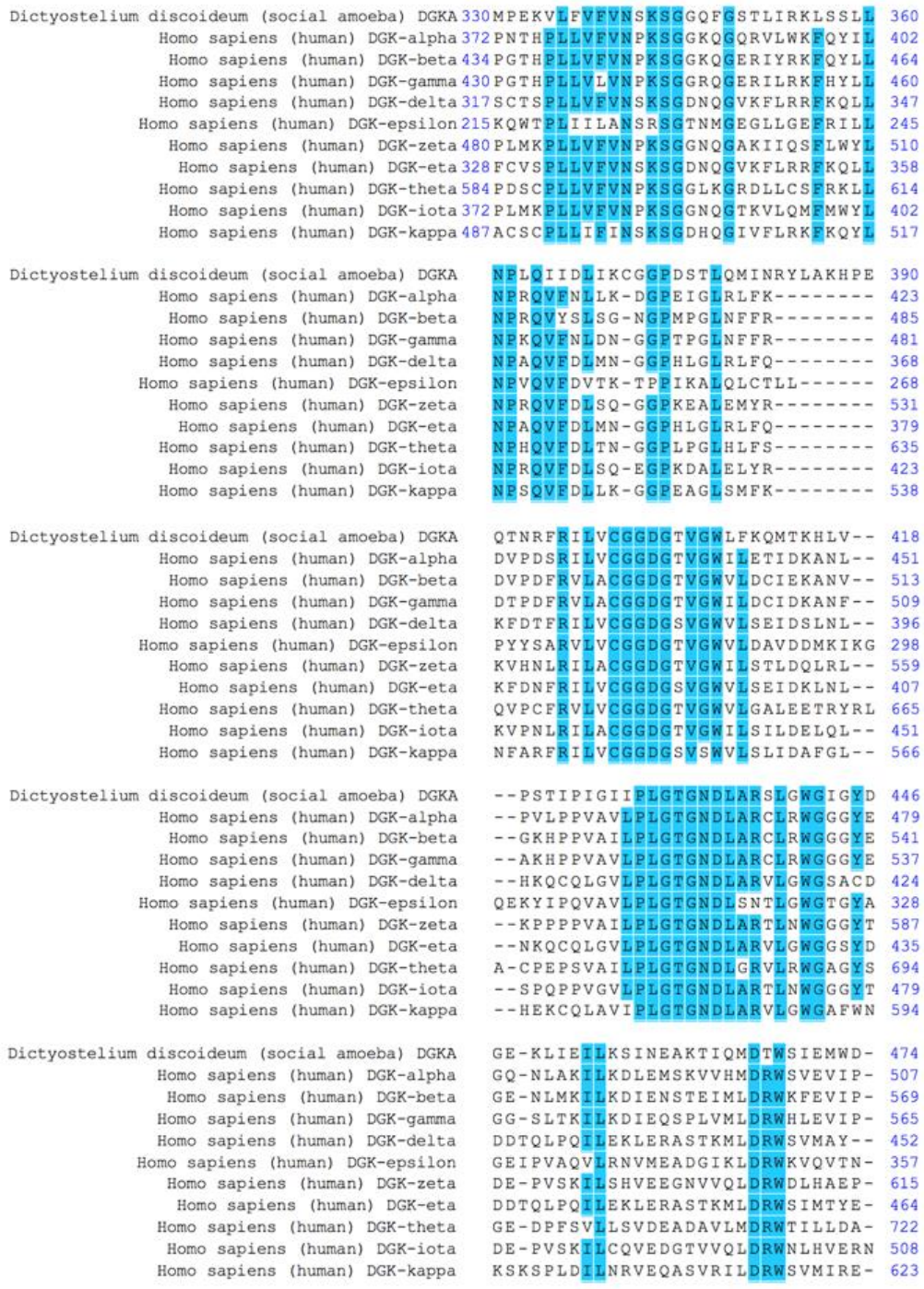


Fig. S2. Conservation of the DGK catalytic region between *D. discoideum* and all *H. sapiens* isoforms. Sequence alignment of the catalytic site of *D. discoideum* DGKA and the 10 *H. sapiens* DGK isoforms. The names of the species and DGK isoforms used for comparison in the alignment are shown to the left with the amino acid start and end position in the protein shown at the start and end of the sequence respectively. Identical amino acids are highlighted in blue.

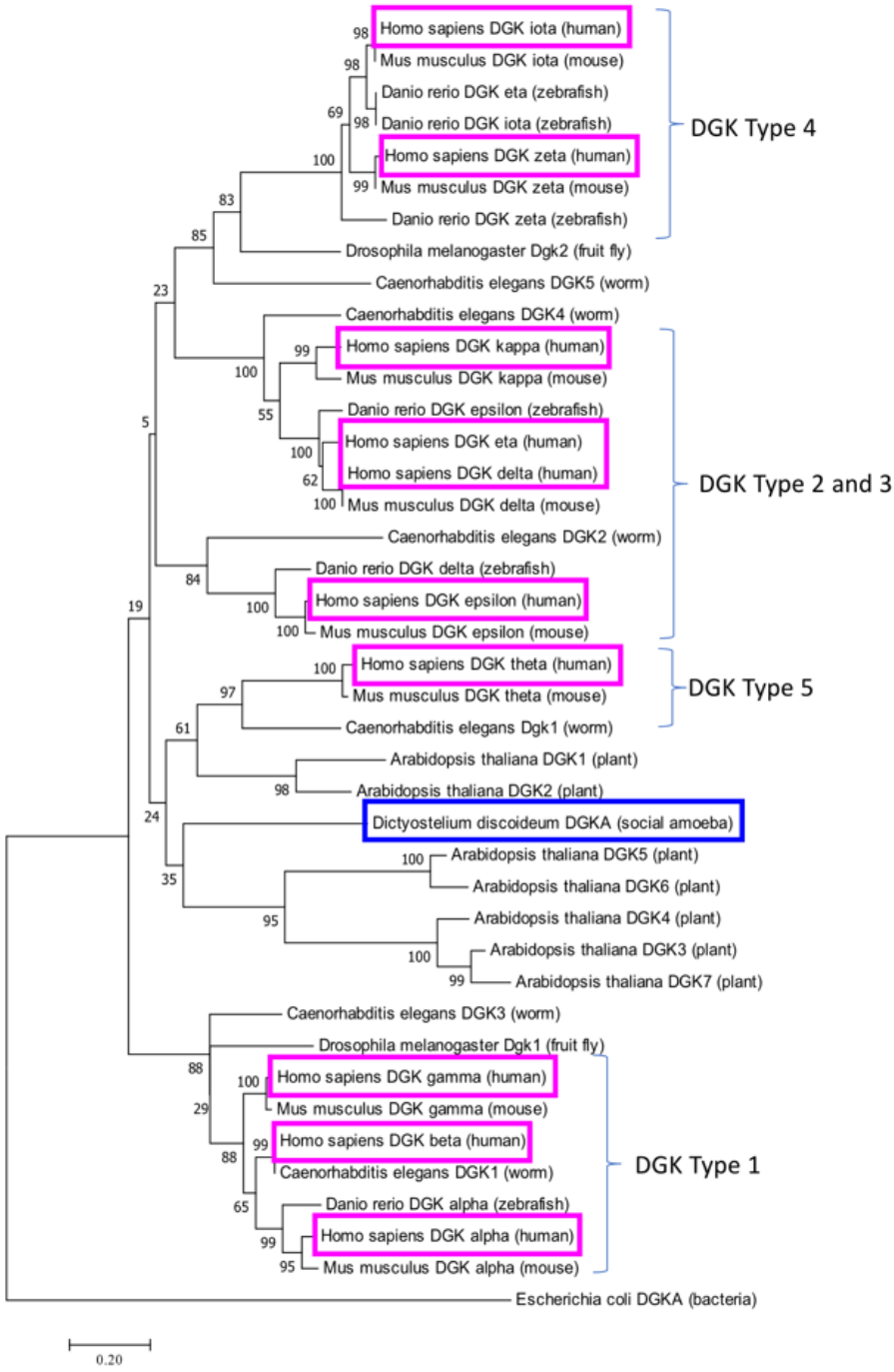


Fig. S3. Phylogenetic analysis of DGK proteins within a range of kingdoms. A Bootstrap consensus tree showing the evolutionarily conservation of DGK generated using the Molecular Evolutionary Genetic Analysis (MEGA) 7 software neighbour- joining method with Bootstrap test (500 replicates) and the Poisson correction method to compute the evolutionary distances. Numbers on the tree represent the percentage of replicate trees in which the associated taxa are clustered together. *E. coli* DGKA represents the root of the tree. Human isoforms are highlighted in pink, with the Dictyostelium protein highlighted in blue.

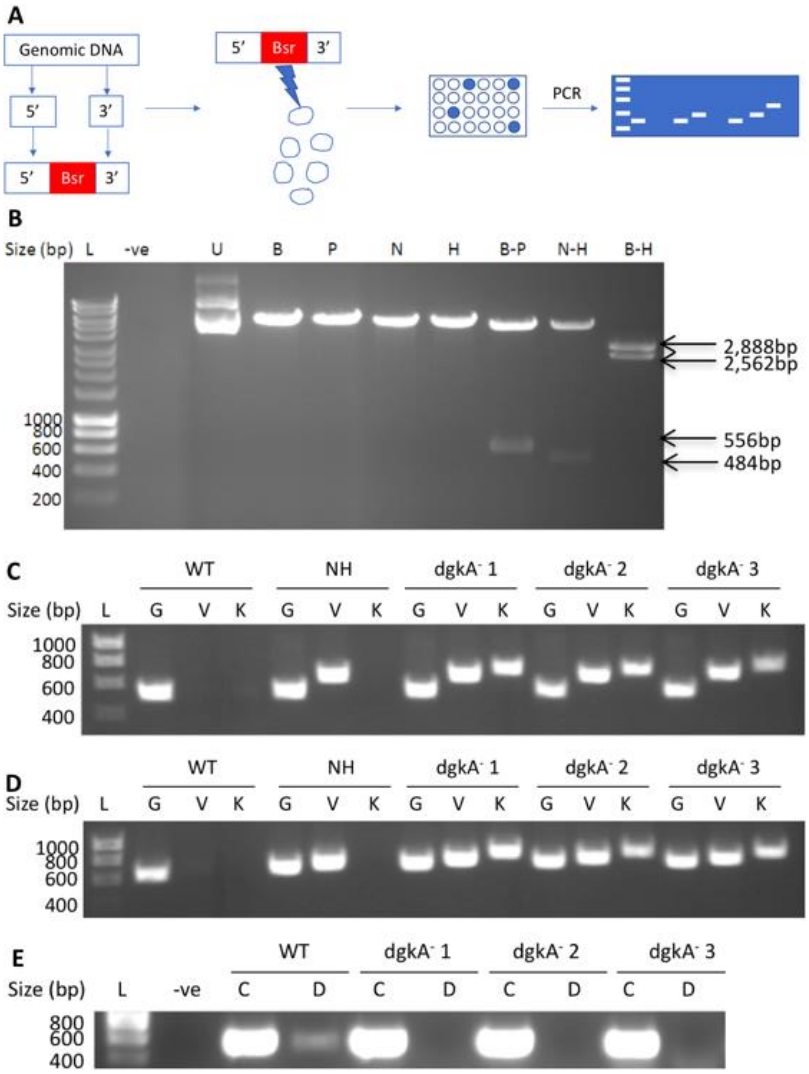
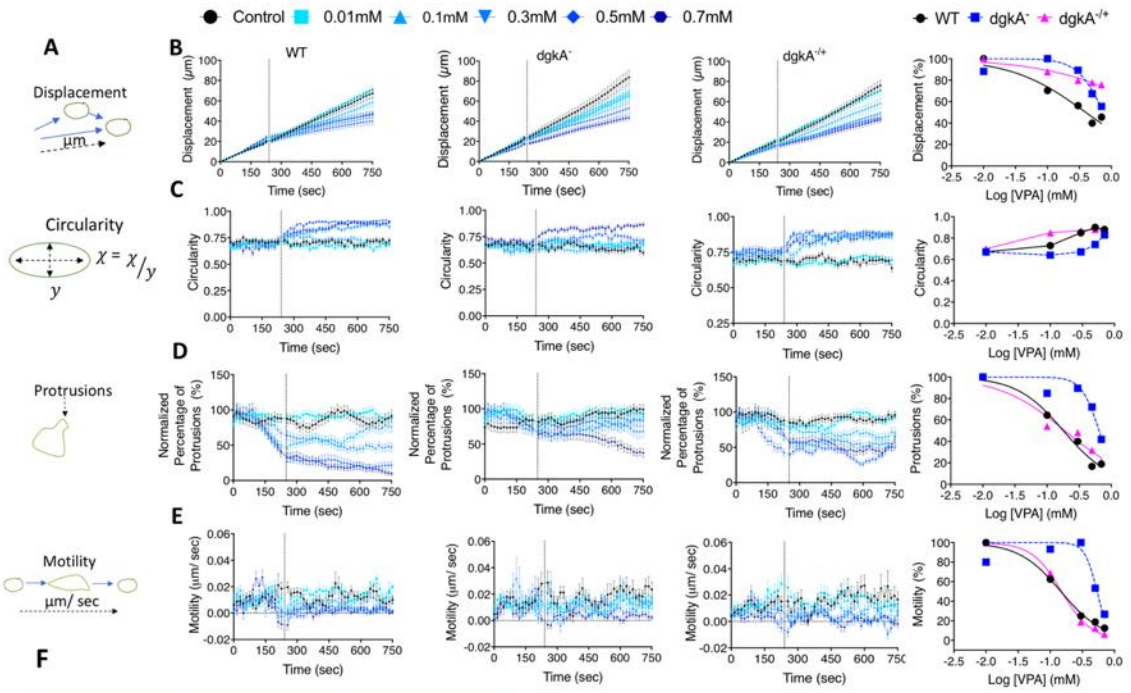


Fig. S4. Creation of *dgkA* knockout cassette and *dgkA*⁻ *Dictyostelium* cell lines. (A) Schematic of creating *dgkA* knockout cell lines where two fragments of genomic DNA sequence were PCR amplified and inserted either side of a blasticidin resistance gene, creating a knockout cassette which was electroporated into WT Ax2 *Dictyostelium* cells where transformant cell lines were selected by blasticidin and surviving knockout cell lines were confirmed by PCR. **(B)** Confirmation of the two *dgkA* genomic DNA inserts in the pLPBLP vector by a series of restriction digests run on a 1% agarose gel by electrophoresis using a 1kb DNA hyperladder (L), uncut knockout vector (U), single digests to confirm individual enzyme activity of BamHI (B), PstI (P), NcoI (N) and HindIII (H) and double digests of BamHI-PstI (B-P) and NcoI-HindIII (N-H) to confirm the presence of the 5' and 3' fragments and BamHI- HindIII (B-H) which excise the liniearised knockout fragment used for electroporation. Homologous intergration screening PCR gel electrophoresis of the genomic control (G), vector control (V) and knockout diagnostic (KO) PCR products where WT would only have the genomic control, non-homologous intergrants (NH) the genomic and vector control bands and homologous intergants

(*dgkA*$^{-}$*)* would have all three bands at the N (**C)** and C **(D)** terminal regions. **(E)** RT-PCR gel electrophoresis confirming the presence of the housekeeping gene Ig7 (C) in both WT and *dgkA*$^{-}$ cell lines and the absence of deleted region (D) only in the *dgkA*$^{-}$ cell lines.



Cell Line	Protrusions IC_{50} (mM)	Motility IC_{50} (mM)
WT	0.18 CI: 0.12-0.27	0.15 CI: 0.11-0.19
dgkA⁻	0.68 CI: 0.47-0.99	0.53 CI: 0.39-0.72
dgkA⁻/⁺	0.19 CI: 0.11-0.38	0.16 CI: 0.07-0.36

Fig. S5. *dgkA*$^{-/+}$ has restored acute cell response phenotype when exposed to VPA. (A) Quantification of cell displacement, circularity, percentage of protrusions and motility in the absence and presence of a range of VPA concentrations (0.01mM to 0.7mM) in WT Ax2, *dgkA*$^{-}$ and *dgkA*$^{-/+}$ cells. **(B)** Calculated IC_{50} values for WT, *dgkA*$^{-}$ and *dgkA*$^{-/+}$ cell lines for protrusions and motility. Data presented as mean (+/- SEM), *n = 30 cells*. A Kruskall- Wallis with Dunns post hoc test was used to compare WT, *dgkA*$^{-}$ and *dgkA*$^{-/+}$ cell lines where there was significant differences in WT cell and *dgkA*$^{-}$ cell (black starts) displacement (0.1mM, 0.3mM and 0.5mM VPA), circularity (0.3mM VPA), protrusions (0.3mM and 0.5mM VPA) and motility (0.3mM VPA); WT cell and *dgkA*$^{-/+}$ (pink stars) displacement (0.5mM); and between *dgkA*$^{-}$ and *dgkA*$^{-/+}$ (blue stars) displacement (0.1mM), circularity (0.1mM, 0.3mM and 0.5mM), protrusions (0.3mM) and motility (0.3mM). *P value: * P value = <0.05, ** P value = <0.01, *** P value = 0.01 and **** P value =< 0.001.*