SUPPLEMENTAL INFORMATION

The Ligand Binding Landscape of Diacylglycerol Kinases

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SUPPLEMENTAL FIGURES

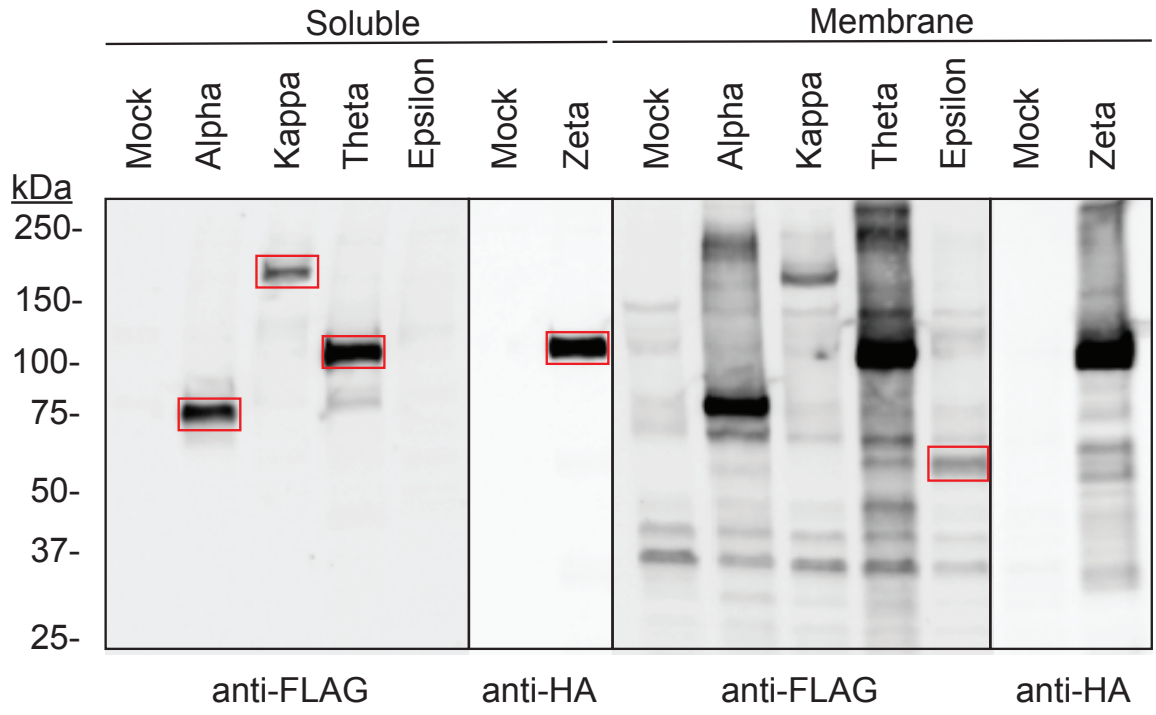

Figure S1. Related to Figure 5 - 7. Western blot analysis of recombinantly overexpressed DGK isoforms. DGK isoforms were recombinantly expressed in HEK293T cells and proteomes subjected to western blot analysis (anti-FLAG, 0.8 μg/mL; anti-HA, 0.1 μg/mL). Soluble fractions were used for analysis with the exception of epsilon, which is expressed predominantly in the membrane fraction (highlighted by red boxes).

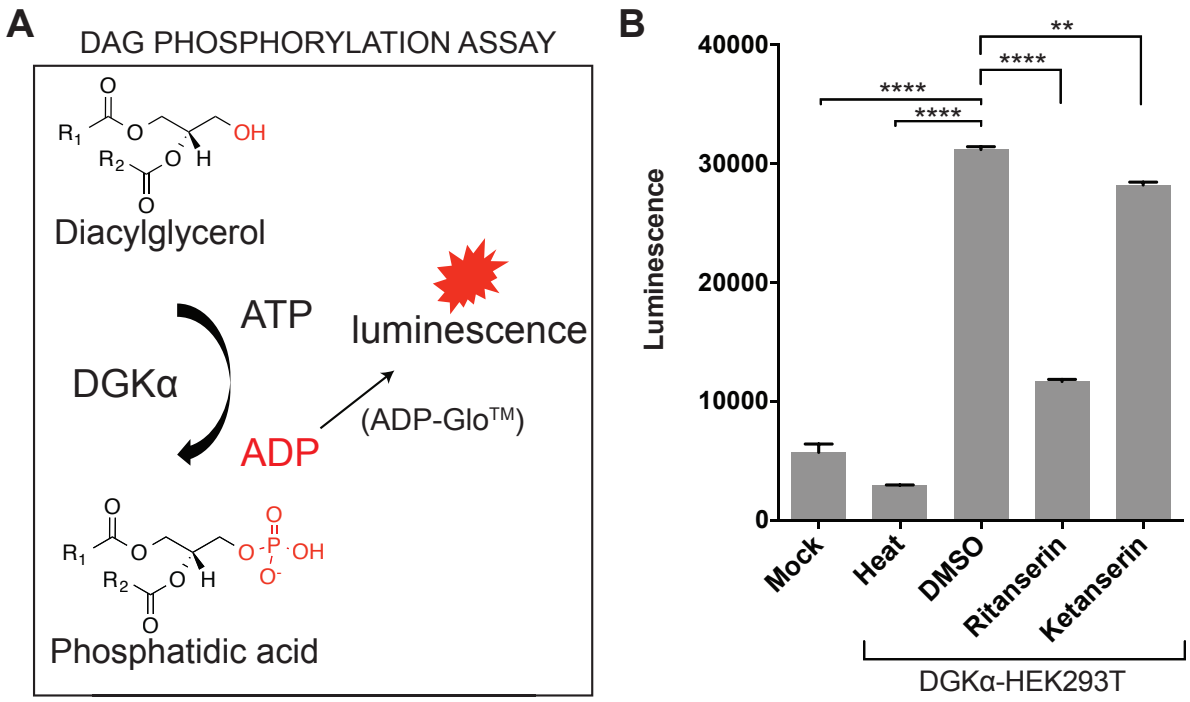

Figure S2. Related to Figure 3 and 7. Measuring activity and inhibition of recombinant DGKα in HEK293T cells by substrate assay. (A) The ADP-glo assay measures ATP that has been converted to ADP by the action of kinases in the cell lysate though production of luminescent signal in proportion to ADP produced. (B) Production of active recombinant DGKα was determined by enhanced activity in DGKα-HEK293T versus mock-transfected soluble proteomes as measured using ADP-glo assay. The lack of activity with heat-denatured (95° C for 5 min) DGKα-HEK293T proteomes supports that activity was specific for recombinant DGKα. Pretreatment with ritanserin but not ketanserin (100 μM compounds) resulted in ~80% blockade of recombinant DGKα activity. Data shown are mean + s.e.m. for two independent biological replicates; $n = 2$ per group. ****$P \leq 0.0001$ for mock versus DGKα overexpressed group (DMSO); **$P \leq 0.01$, ****$P \leq 0.0001$ for vehicle-treated versus heat-denatured or inhibitor-treated DGKα overexpressed groups (DMSO).

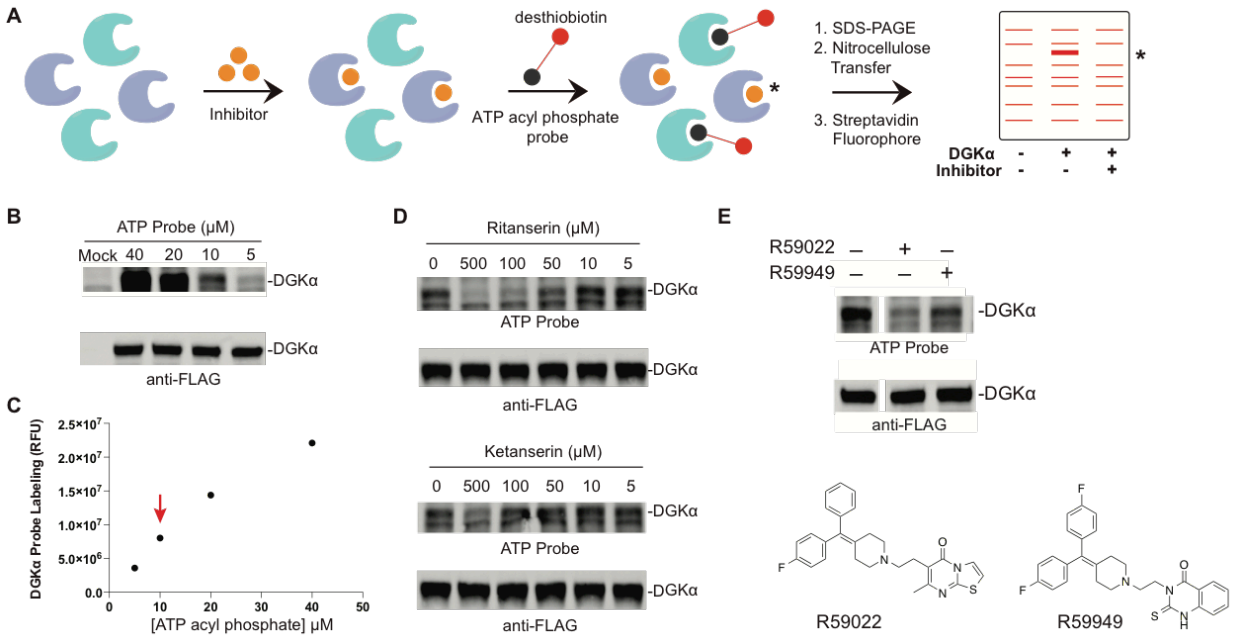

Figure S3. Related to Figure 3. Optimization of gel-based ATP acyl phosphate assay for profiling DGK inhibitors. (A) Schematic of gel-based chemical proteomic analysis of recombinant DGKα activity. DGKα-HEK293T soluble proteomes were labeled with ATP acyl phosphate, proteins separated by SDS-PAGE, transferred to nitrocellulose, and desthiobiotin-modified proteins detected by streptavidin fluorophore and in-blot fluorescence. Pretreatment of proteomes with inhibitors blocks labeling at ATP probe binding sites, resulting in reductions in fluorescence signals to profile on- and off-targets of recombinant DGKα. (B) DGKα-HEK293T soluble proteomes were treated with ATP probe at the indicated concentrations for 30 min, quenched with gel loading buffer, and subjected to gel-based analysis as described above. (C) Integrated band intensities from these studies were plotted as a function of ATP probe concentrations to identify a suitable treatment condition for profiling of reversible inhibitors (10 μM ATP probe, 30 min; labeling reaction was ~40% complete). (D) DGKα-HEK293T soluble proteomes were pretreated with ritanserin or ketanserin for 30 min at the indicated concentrations followed by ATP probe labeling (10 μM, 30 min) and gel-based chemical proteomics analysis as described above. Ritanserin but not ketanserin showed concentration dependent blockade of ATP probe labeling. (E) Pretreatment with the widely used DGK inhibitors R59022 and R59949 (100 μM compounds) also blocked DGKα probe labeling as measured by gel-based chemical proteomics. For all chemical proteomics studies, western blots (anti-FLAG, 0.8 μg/mL) were included to confirm that changes in fluorescence were not due to variations in recombinant protein expression (bottom panels).

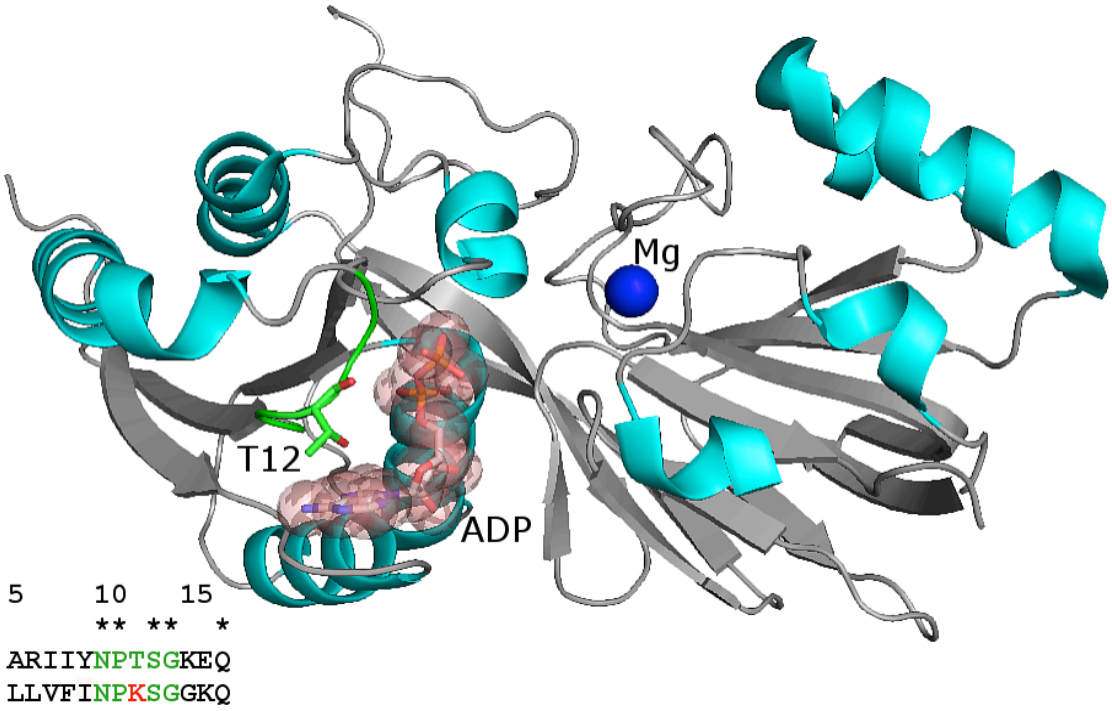

Figure S4. **Related to Figure 4**. **Proximity of *S. aureus* DgkB residue, with homology to DGKα K377, to phosphate groups of ADP**. Cartoon Diagram of DgkB monomer (PDB code: 2QV7): α helices are cyan, β sheets and loops are grey, ADP is transparent spheres with a stick model, Mg is blue, the aligned region is green and the homologous residue (threonine 12) is depicted as a green stick model. Partial Structure-Aided Sequence Alignment of *S. aureus* DgkB and rat DGKα: aligned region is green and ATP acyl phosphate probe-modified DGKα residue is red (K377). Note the threonine residue homologous to the probe-modified lysine of DGKα is in proximity to phosphate groups of ADP.

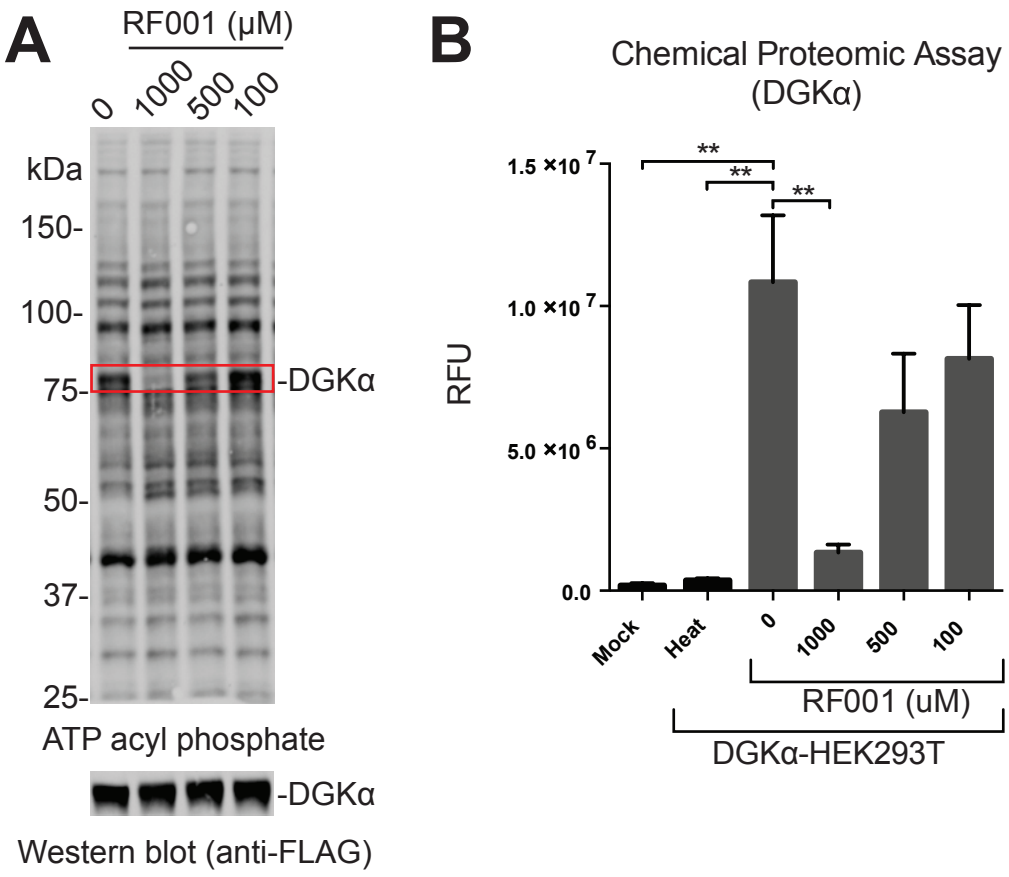

Figure S5. **Related to Figure 7**. **Chemical proteomic analysis of RF001 activity against recombinant DGKα in HEK293T proteomes**. (A) DGKα-HEK293T soluble proteomes were pretreated with RF001 at the indicated concentrations for 30 min prior to labeling with ATP acyl phosphate probe (10 μM, 30 min). After probe labeling, proteomes were subjected to gel-based analyses as described in **Figure S3A**. RF001 blocked probe labeling in a concentration-dependent manner and the decrease in fluorescence signals was not due to differences in recombinant protein expression as confirmed by western blot (bottom panel, anti-FLAG, 0.8 μg/mL). Integrated band intensities from these gel-based ATP acyl phosphate assays (B) were used to quantify DGKα inhibition by RF001. Mock-transfected and heat-denatured (95° C for 2 min) recombinant DGKα lysates were included as additional controls. Data shown are mean + s.e.m. for three biological replicates. $^{**}P \leq 0.01$ for mock versus DGKα overexpressed group (0 μM); $^{**}P \leq 0.01$ for vehicle-treated (0 μM) versus heat-denatured or inhibitor-treated DGKα overexpressed groups.

DAGKc subdomain

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P51556 DGKA  347 -TTDVTSLCTPEAFRIEPVSNTHPLLVFINPKSGGKQGQSVLWKFQYILNPRQVFNLK-D  404
Q5KSL6 DGKK  481 -----------N--LDWSSACSCPLLIFINSKSGDHQGIVFLRKFKQYLNPSQVFDLLKG  527
P52429 DGKE  201 -----DKKTDYEVLASKLGKQWTPLIILANSRSGTNMGEGLLGEFRILLNPVQVFDVTKT  255
Q13574 DGKZ  462 -KGPEEGRWRPFIIRPTPSPLMKPLLVFVNPKSGGNQGAKIIQSFLWYLNPRQVFDLSQG  520
P52824 DGKQ  565 RGRLLTALVLPDLLHAKLPPDSCPLLVFVNPKSGGLKGRDLLCSFRKLLNPHQVFDLTNG  624
                                      **::: * :**   *  .: .*   *** ***::
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DAGKa subdomain

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P51556 DGKA  503 --------------------------EKSDPVPSQIINNYFSIGVDASIAHRFHLMREKY  536
Q5KSL6 DGKK  822 GTLSSISSLKSEDLDNLNLDHLHFTPESIRFKEKCVMNNYFGIGLDAKISLDFNTRRDEH  881
P52429 DGKE  360 --------------------------YNLRKPKEFTMNNYFSVGPDALMALNFHAHREKA  393
Q13574 DGKZ  626 --------------------------GATDRLPLDVFNNYFSLGFDAHVTLEFHESREAN  659
P52824 DGKQ  732 --------------------------ADAEPPKIVQMSNYCGIGIDAELSLDFHQAREEE  765
                                                :.** .:* ** ::  *:  *:

P51556 DGKA  537 PEKFNSRMKNKLWYLEFATSESIFSTCKKLEESVTVEICGKLLD--LSDLSLEGIAVLNI  594
Q5KSL6 DGKK  882 PGQYNSRLKNKMWYGLLGTKELLQRSYRKLEERVHLECDGETIS--LP--NLQGIVVLNI  937
P52429 DGKE  394 PSLFSSRILNKAVYLFYGTKDCLVQECKDLNKKVELELDGERVA--LP--SLEGIIVLNI  449
Q13574 DGKZ  660 PEKFNSRFRNKMFYAGTAFSDFLMGSSKDLAKHIRVVCDGMDLTPKIQDLKPQCVVFLNI  719
P52824 DGKQ  766 PGKFTSRLHNKGVYVRVGLQKI--SHSRSLHKQIRLQVERQEVE--LP--SIEGLIFINI  819
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Figure S6. Related to Figure 5. Sequence similarity in ATP binding sites of DGK isoforms. Multiple sequence alignment of DGK isoforms were performed with Clustal Omega (Goujon et al., 2010; Sievers et al., 2011) and ATP-sensitive probe-modified peptides found in DAGKc and DAGKa subdomains are shown (highlighted by a red box). Site of probe labeling for ATP acyl phosphate is denoted by a red underline. An asterisk denotes single, fully conserved residue; colon denotes indicates conservation between groups of strongly similar properties; a period signifies conservation between groups of weakly similar properties (Goujon et al., 2010; Sievers et al., 2011).