Supplementary information for:

A reversible formation of S-D-3-phosphoglyceroyl glutathione contributes to cellular protection from acylation by cyclic 3-phosphoglyceric anhydride

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Supplementary information table of content:

Supplementary methods

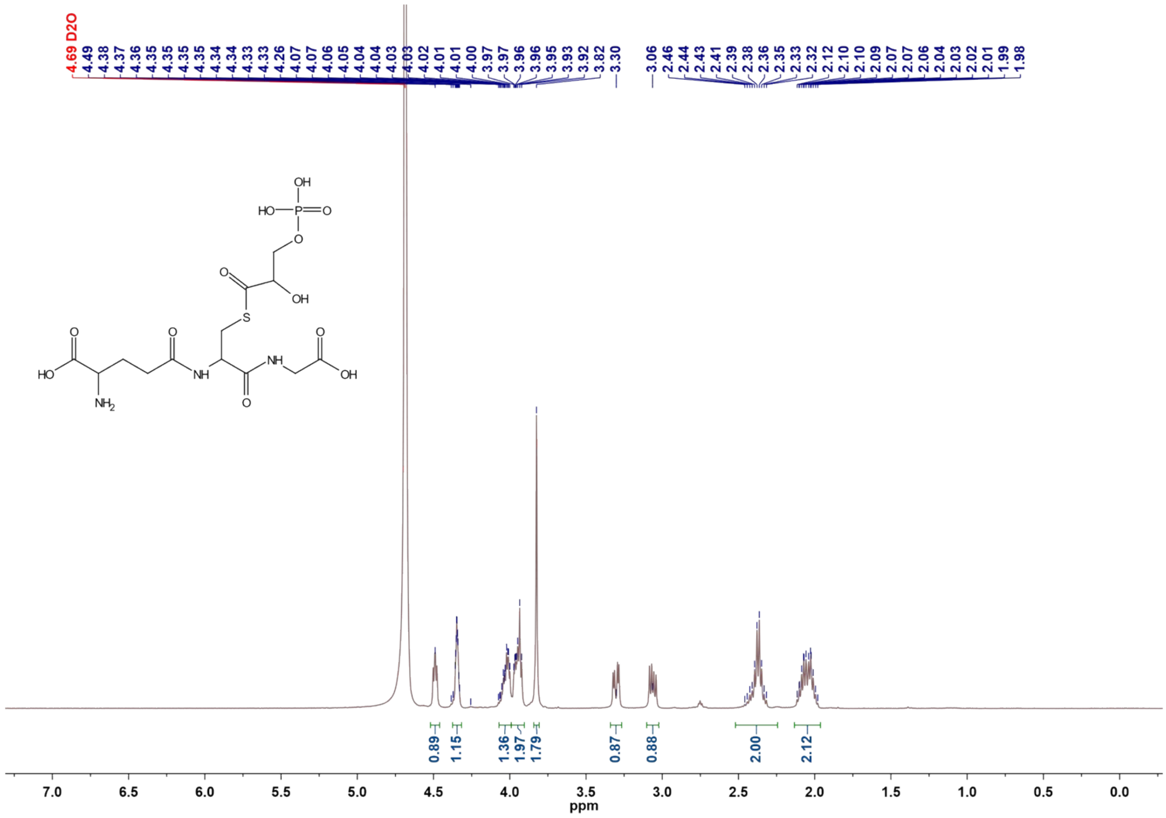
Affinity purification of α-gK/α-pgK antibodies.

NHS-activated Sepharose (1.5 mL) was washed with 5 mL of 1 mM HCl and incubated overnight with 20 mg/mL BSA in 0.2 M sodium phosphate, 0.5 M NaCl, pH 8 with gentle mixing. BSA-modified Sepharose was packed into a small gravity column and washed with 15 mL of 0.2 M sodium phosphate pH 8 with 0.5 M NaCl. Unreacted sites were blocked by incubation with 1.5 M Tris, pH 8.8 for three hours after which BSA-modified Sepharose was washed with 5 ml of water and re-suspended in 3 mL of 100 mM KH_2PO_4. To modify immobilized BSA, the resin was quickly mixed with equal volume (3 mL) of freshly prepared 50 mM cPGA, incubated for 10 minutes with mixing, washed with water and equilibrated with 20 mM Tris pH 8 with 0.5 M NaCl. Approximately one-third of the resulting affinity media was removed and dephosphorylated with 10 U of alkaline phosphatase for 10 minutes at room temperature. Afterwards, phosphatase-treated Sepharose was thoroughly washed, added back to the column and mixed well to obtain affinity media with immobilized BSA bearing both gK and pgK modifications.

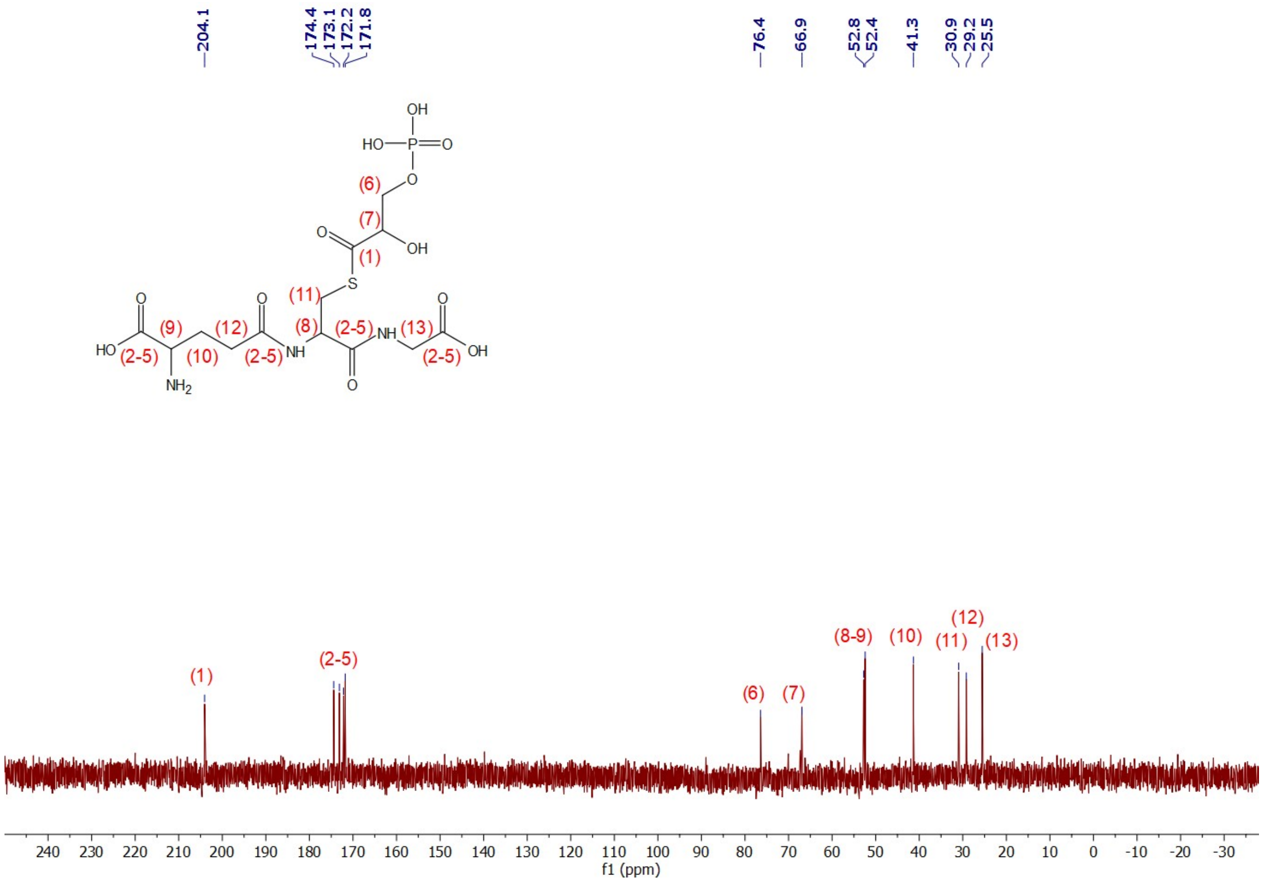
Precipitated serum immunoglobulins from rabbit immunized with glyceroyl-modified GAPDH were dissolved in 5 ml of 20 mM Tris pH 8.0, 0.5 M NaCl. The resulting solution was passed through the affinity column three times. Next, immunoglobulins from the rabbit immunized with phosphoglyceroyl-modified GAPDH were bound to the same affinity column in the same manner. The column was washed with 20 ml of 20 mM Tris pH 8, 0.5 M NaCl. Bound antibodies were eluted with 0.1 M glycine, pH 2.8 (300 µL fractions were collected into tubes with 150 µL of 0.5 M M Tris pH 8.5 to achieve immediate neutralization). Approximately 2 mg of affinity-purified α-gK/α-pgK antibodies were obtained.

Synthesis of *N*-3-phosphoglyceroyl glutamine and *N*-3-glyceroyl glutamine.

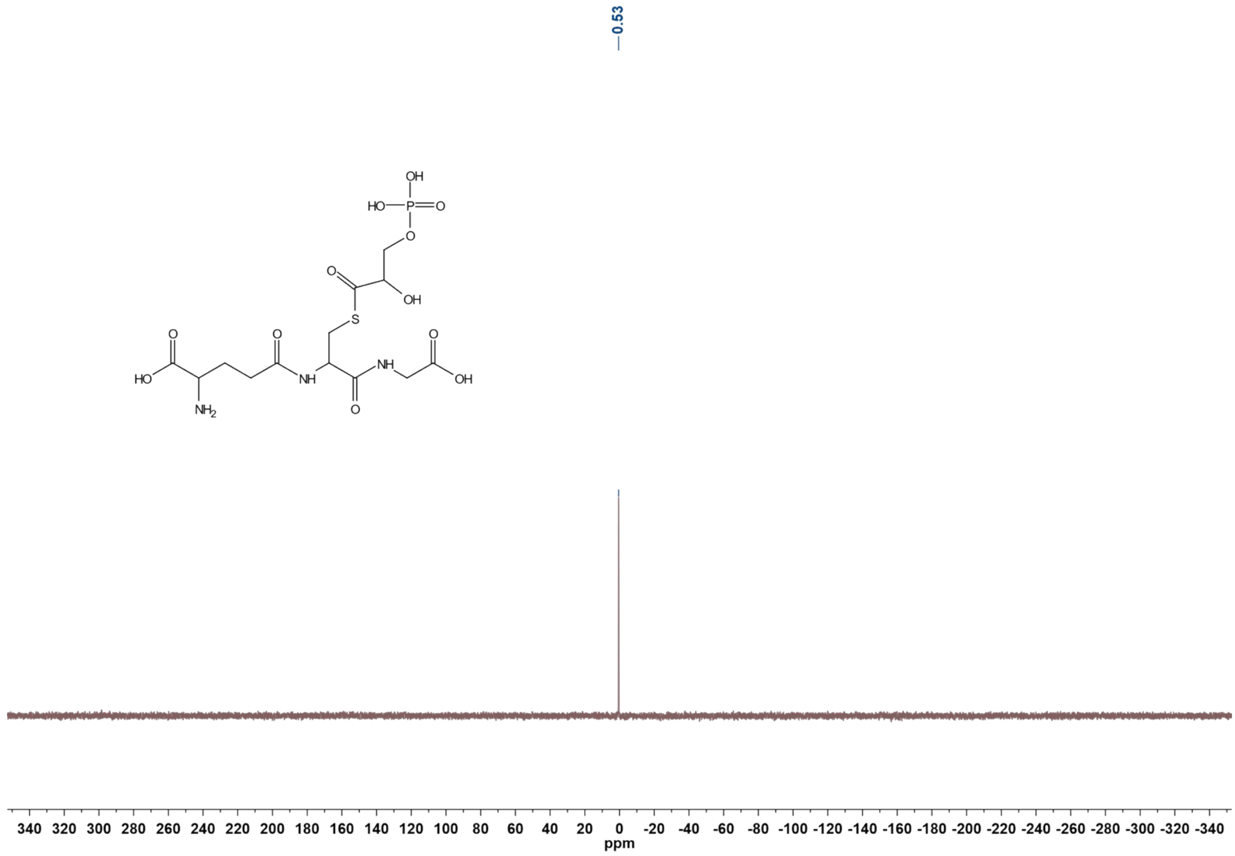
1 ml of 50 mM of Glutamine was mixed with equimolar amount of cPGA at pH=8 and allowed to react for 15 minutes at room temperature. The product was purified using Acta Pure FPLC with Resource Q column and recovered in 60 mM HCl solution. The product was diluted with 20 mM of Tris-HCl, pH =7.7 and mixed with 1 µl of CIAP (alkaline phosphatase) to obtain 100 µM of *N*-glyceroyl glutamine. The product was diluted to 1 µM and analyzed using LC-MS.



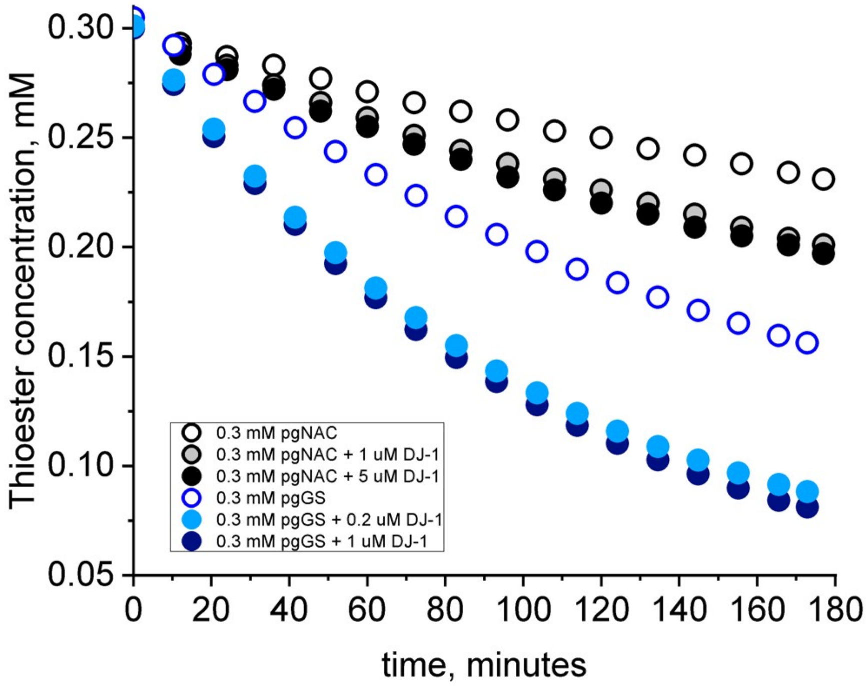
Supplementary Figure S1. [1]H NMR spectrum of glutathione thioester of 3-phosphoglyceric acid (pgGS)



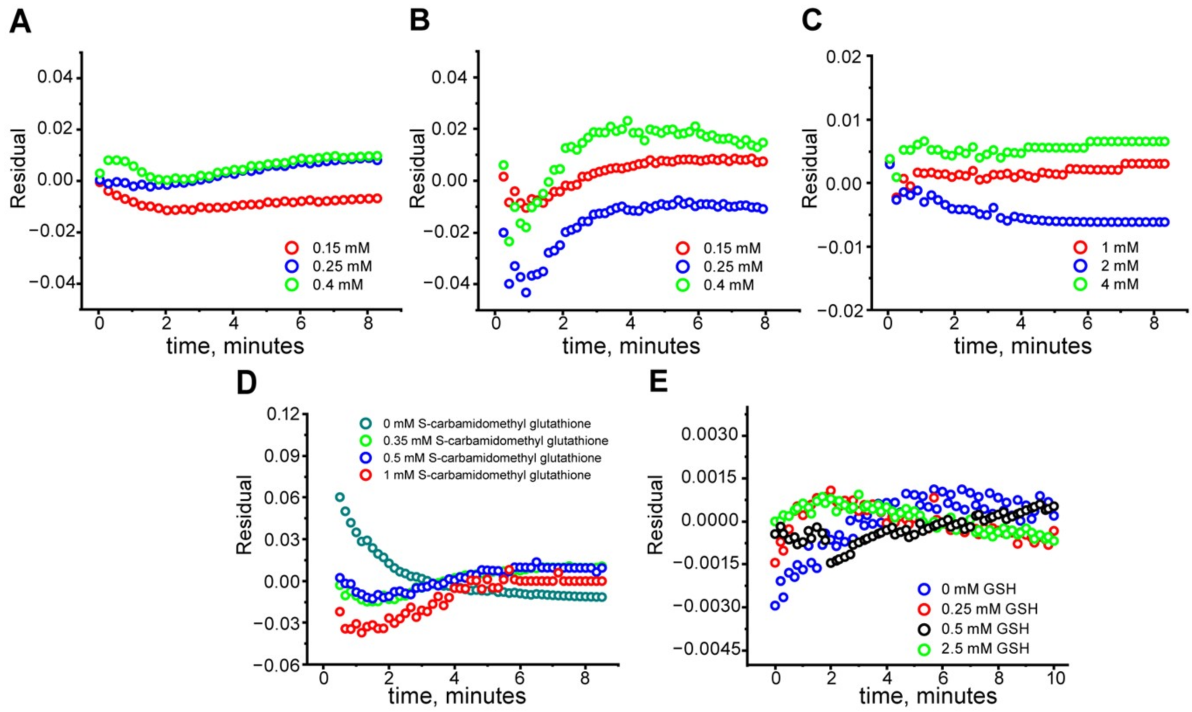
Supplementary Figure S2. ^{13}C NMR spectrum glutathione thioester of 3-phosphoglyceric acid (pgGS).



Supplementary Figure S3. ^{31}P NMR spectrum of glutathione thioester of 3-phosphoglyceric acid (pgGS)



Supplementary Figure S4. Decomposition of NAC and GSH thioesters of 3-phosphoglyceric acid in the presence and absence of DJ-1. Compared to pgGS, NAC thioester decomposes at much slower rate, but decomposition is also strongly accelerated by DJ-1 indicating the release of cPGA.



Supplementary Figure S5. Residuals from the global kinetic fit. *A*, Residuals from the global fit shown in Fig. 1A. *B*, Residuals from the global fit shown in Fig. 1B. *C*, Residuals from the global fit shown in Fig. 1D. *D*, Residuals from the global fit shown in Fig. 1E. *E*, Residuals from the global fit shown in Fig. 2E.

Uncropped images:

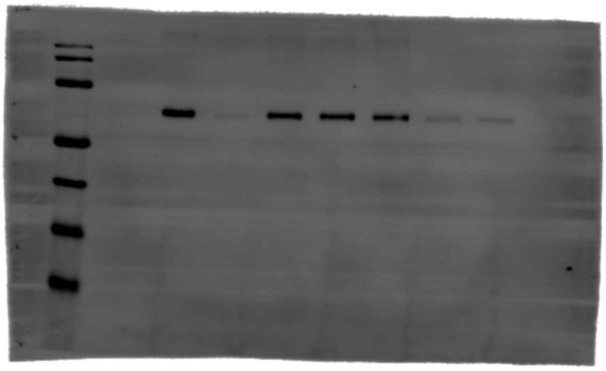


Fig. 1G top

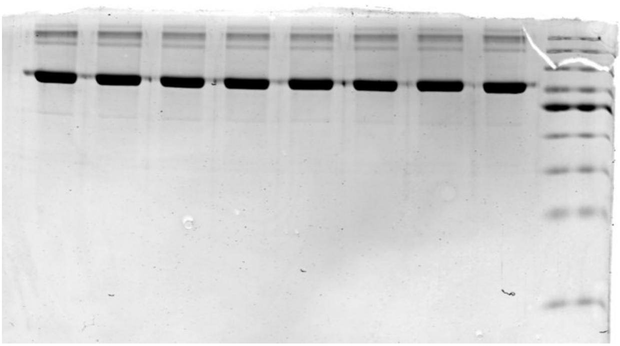


Fig1G bottom

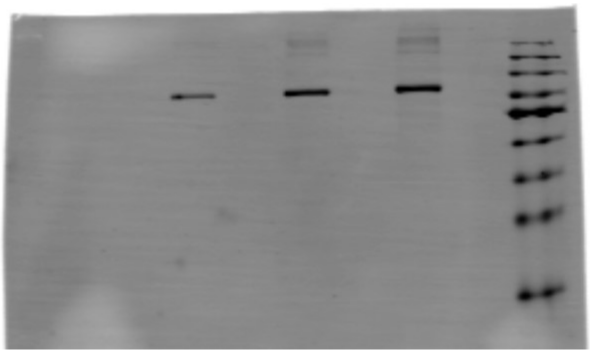


Fig. 3C top

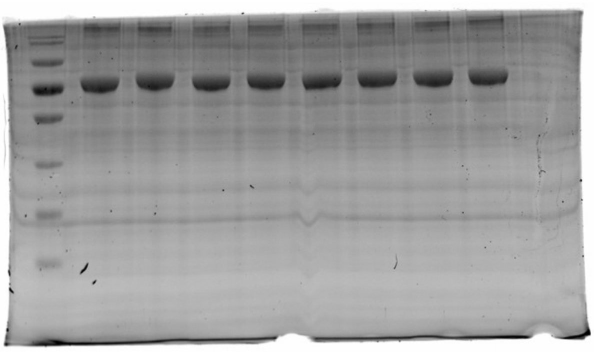


Fig 3C bottom