Supporting Information

Figure S1
PKG Iα oxidation by ambient air. Ambient air oxidation of reduced PKG Iα was achieved through four rounds of buffer exchange into 50 mM MES, 150 mM NaCl, pH 6.9 to remove the TCEP. Oxidation was visualized by denaturing, non-reducing SDS-PAGE in the absence of maleimide. M - monomer, D - dimer, O - oligomer

Figure S2
Sequence alignment of mammalian PKG Iα and Iβ isoforms denoting the locations of cysteine residues confirmed to form disulfide bonds.Sequence alignment colored by BLOSUM62 score denoting **A)** the placement of C42 within the dimerization domain and **B)** the location of C117 and C195 in relation to nearby structural features within the CNB A-site of PKG Iα, including the A helix, the B/C helix, and the phosphate-binding cassette.

Figure S3
Oxidation-dependent activation of PKG Iβ. A) The cGMP-dependent activation of PKG Iα (black) and Iβ (yellow) under reducing (solid circles and line) and oxidizing (open circles and dashed line conditions by an *in vitro* phosphotranspherase assay. **B)** Non-reducing, denaturing SDS-PAGE of PKG Iβ treated with increasing concentrations of H_2O_2 (0-2 mM). The dashed line indicates the point at which the concentration of H_2O_2 overcomes the concentration of TCEP in the buffer. H_2O_2 values corrected for the presence of TCEP are shown in red. M - monomer, D - dimer

Figure S4
Comparison of median channel open times when exposed to PKG Iα WT and C42S. K_{Ca}1.1 channel open dwell times corresponding to patches in **Figure 4** were analyzed using non-parametric Mann-Whitney statistics for conditions where patches were exposed to control (-kinase, -cGMP) or experimental (+kinase, +cGMP) conditions. Individual measurements (scatter) are shown overlaid with the mean ± SEM are depicted for **Aa)** PKG Iα WT (N=13) and **Ba)** PKG Iα C42S (N=6). Channel open times were plotted as histograms for representative patches (0.1 ms bins) when exposed to **Ab)** PKG Iα WT and **Bb)** PKG Iα C42S (solid fill) in the presence of 5 μM cGMP. Control conditions (dashed fill) in the absence of kinase and cGMP are also depicted. (n.s. - not significant, *$p<0.02$)

Figure S5
Activity of K_{Ca}1.1 treated with C42S (0 cGMP and ATP) by patch clamp in the inside-out configuration. K_{Ca}1.1 channel recordings under reducing conditions when exposed to PKG Iα C42S in the absence of cGMP and ATP. Activity was measured using symmetrical K^+ at +40, 0, and -40 mV.

Figure S1

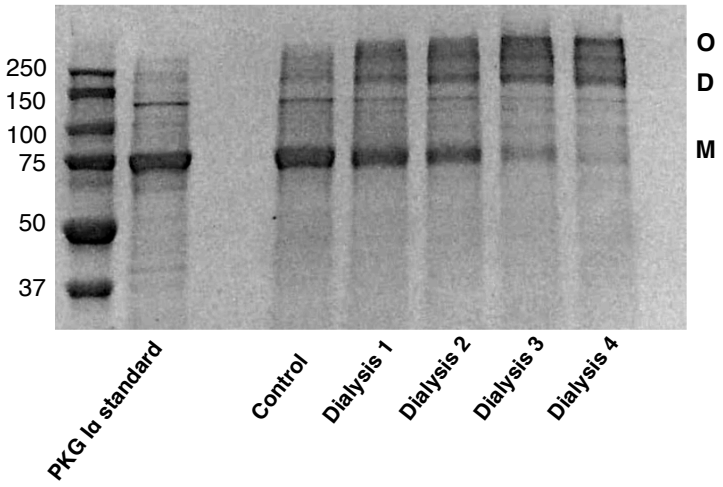

Figure S2

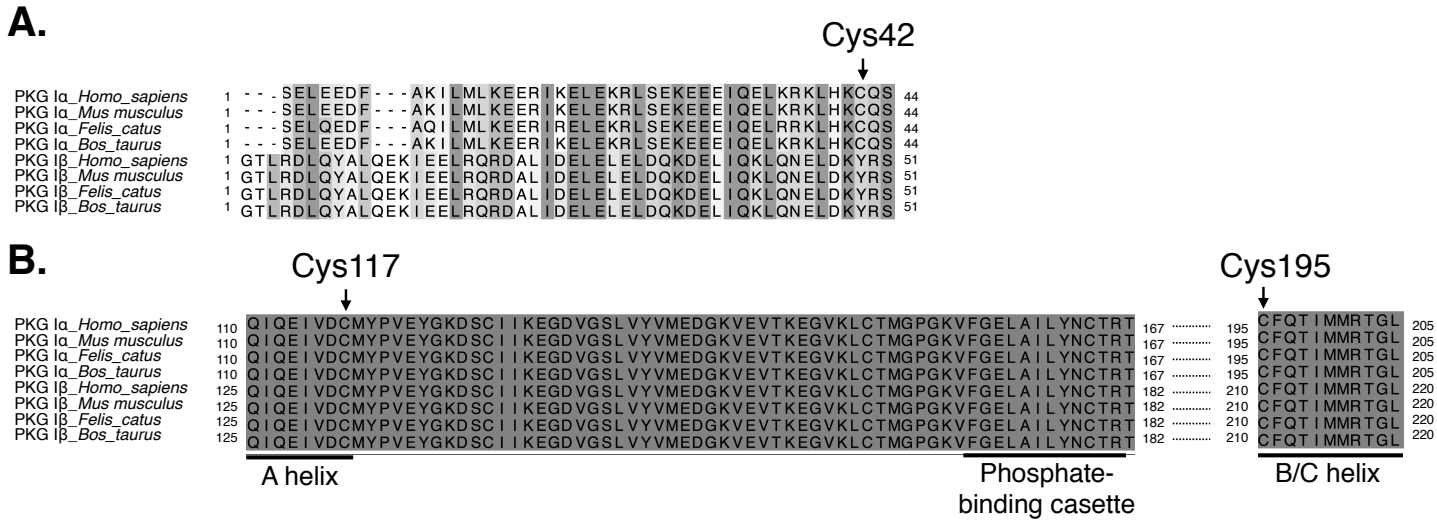

Figure S3

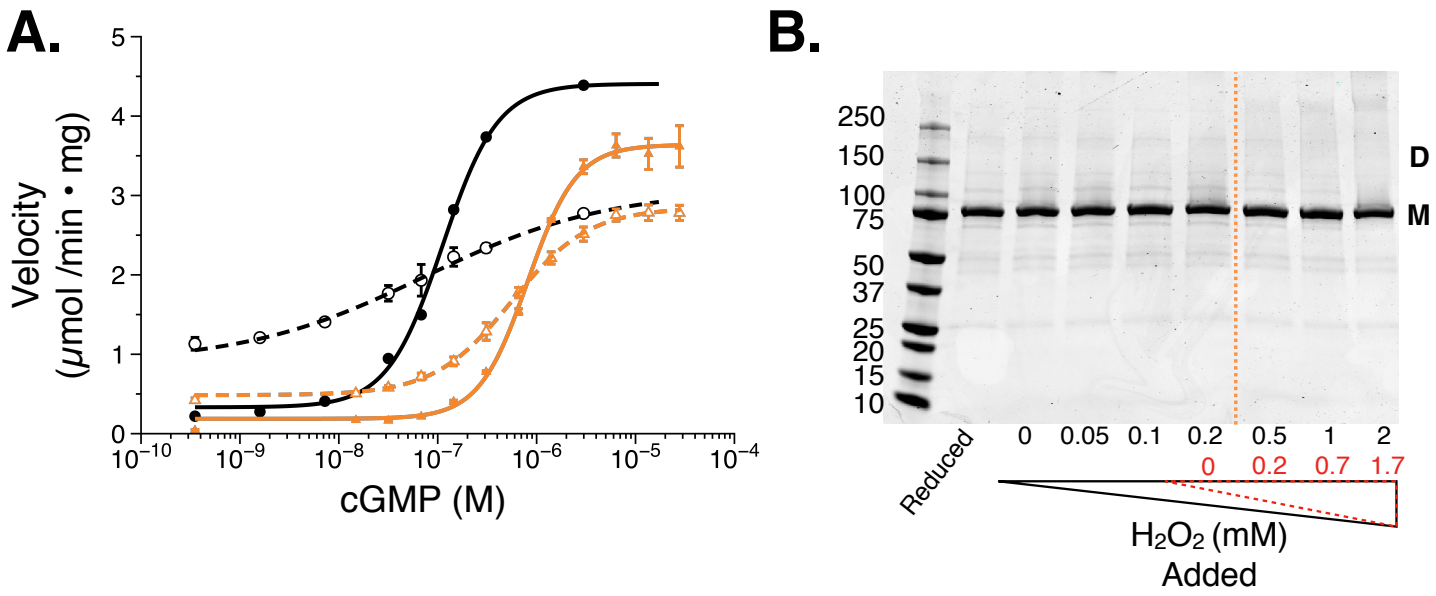
A.
Velocity
(μmol /min • mg)
cGMP (M)
B.
250
150
100
75
50
37
25
20
15
10
D
M
Reduced
0
0.05
0.1
0.2
0.5
1
2
0
0.2
0.7
1.7
H2O2 (mM)
Added

Figure S4

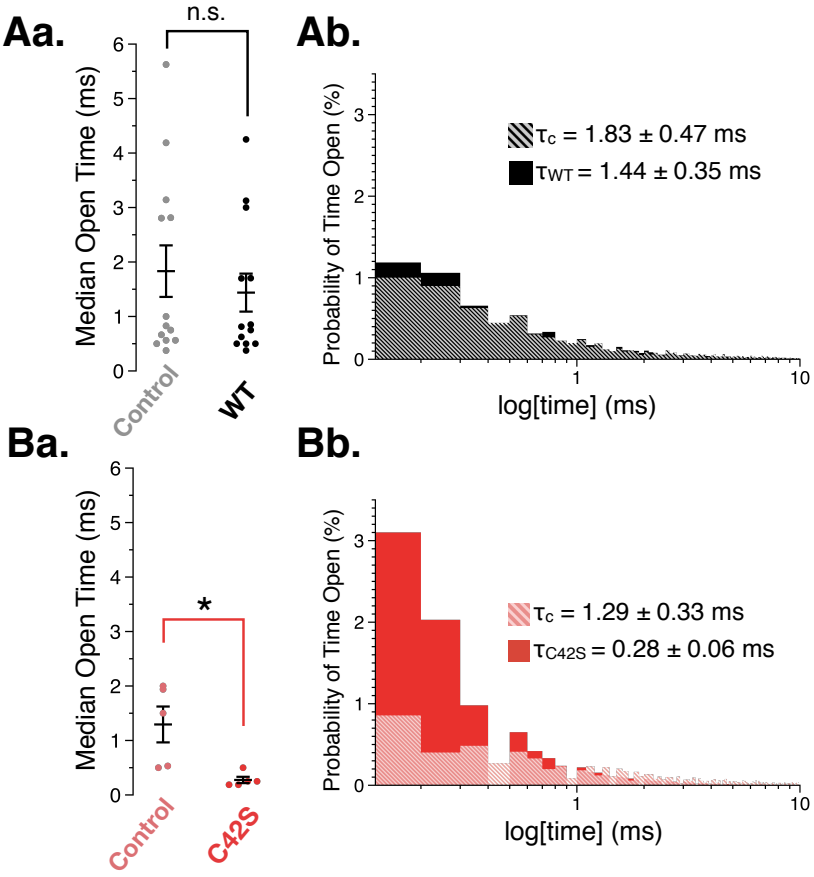

Figure S5