

Figure S1. Related to Figure 2: *Peptide coverage map of PLCβ2 and zoom in on differences in exchange in dynamic regions.*

(A) The coverage map of PLCβ2 used for HDX-MS analysis is shown, with black bars indicating peptides that were analyzed for all conditions and time points.

(B) Zoom in on differences in exchange for indicated peptides from Fig. 2 showing differences in deuterium incorporation.

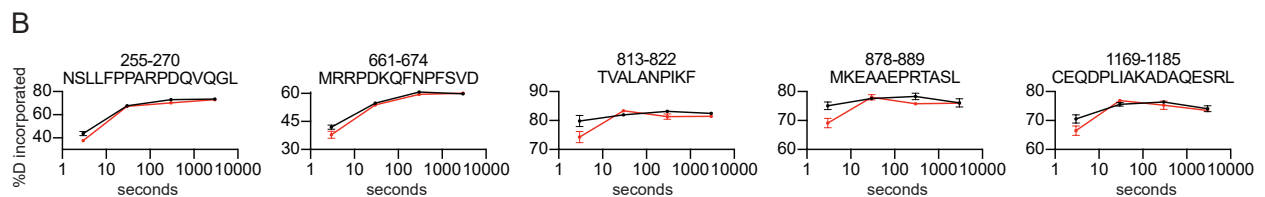


Figure S2. Related to Figure 4: *PLC β 2 is activatable by $G\beta\gamma$ under HDX conditions.*

Reconstituted assay of PLC enzymatic activity as described in in methods section with the exception that reaction was only allowed to proceed for 30 seconds under HDX conditions (200nM PLC, 600nM free Ca^{2+} and 600nM purified $G\beta\gamma$ subunits) Experiments were performed at least in duplicate. The data shown are mean \pm S.D

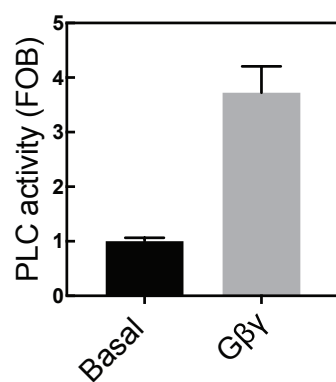


Figure S3. Related to Figure 5: *Full length PLC β 2 and PLC β 2- Δ CTD have similar basal activities.* Reconstituted assay of PLC enzymatic activity unstimulated activity for the PLC β - Δ CTD and Full length PLC β 2 in the absence of any activators. Experiments performed at least 3 times in duplicate. The data shown are mean \pm S.E.

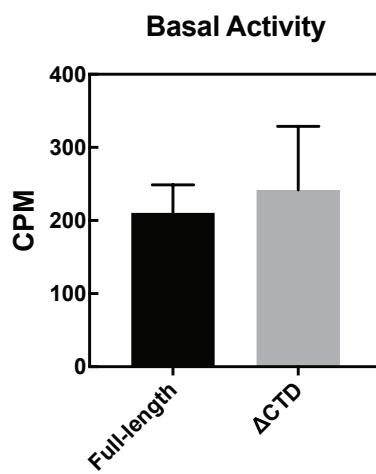


Table S1. Related to Figures 2-4. Full statistics on all hydrogen deuterium exchange experiments according to the guidelines from the International Conference on HDX-MS.

Data Set	PLC β 2	PLC β 2 membrane	PLC β 2 membrane G $\beta\gamma$	PLC β 2 membrane G α_q
HDX reaction details	%D ₂ O=69% pH _(read) = 7.5 Temp= 16°C	%D ₂ O=69% pH _(read) = 7.5 Temp= 16°C	%D ₂ O=69% pH _(read) = 7.5 Temp= 16°C	%D ₂ O=69% pH _(read) = 7.5 Temp= 16°C
HDX time course	3, 30, 300 and 3000 sec at 23°C	3, 30, 300 and 3000 sec at 23°C	3, 30, 300 and 3000 sec at 23°C	3, 30, 300 and 3000 sec at 23°C
HDX controls	N/A	N/A	N/A	N/A
Back-exchange				
Number of peptides	192	192	192	192
Sequence coverage	81.2%	81.2%	81.2%	81.2%
Average peptide length/ redundancy	Length = 12.6 Redundancy = 2.0	Length = 12.6 Redundancy = 2.0	Length = 12.6 Redundancy = 2.0	Length = 12.6 Redundancy = 2.0
Biological replicates	3	3	3	3
Repeatability	Average StDev = 0.7%	Average StDev = 0.7%	Average StDev = 0.6%	Average StDev = 0.6%
Significant differences in HDX	>4% and >0.4 Da and unpaired t-test <0.05	>4% and >0.4 Da and unpaired t-test <0.05	>4% and >0.4 Da and unpaired t-test <0.05	