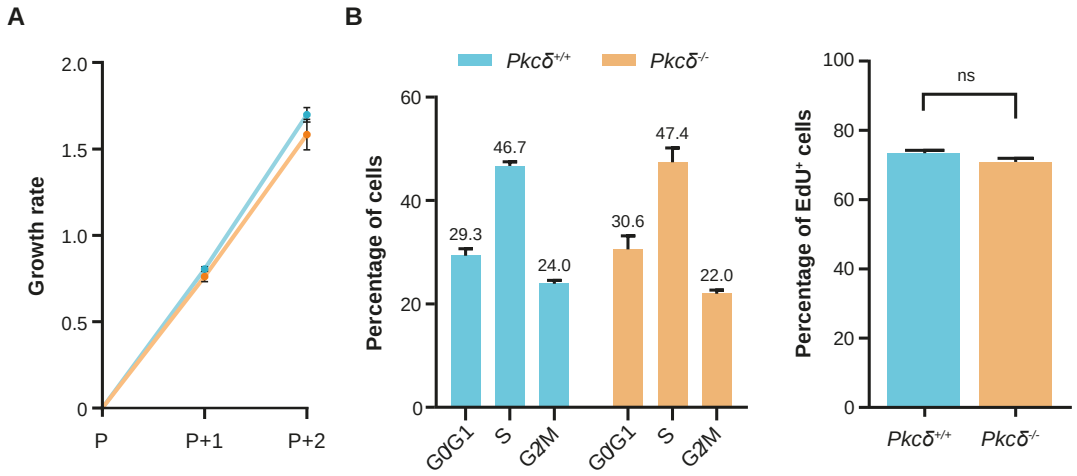

Figure S1. Analysis of cell growth and cell cycle dynamics of mESCs deficient for PKCδ. A) The growth of the *Pkcδ*$^{-/-}$ and *Pkcδ*$^{+/+}$ mESC lines was analyzed along two consecutive cell passages. To obtain the growth rate, the natural logarithm of the expansion factor (cell population divided by the number of cells initially plated) was in turn divided by the number of days. **B)** *Left panel:* Murine *Pkcδ*$^{-/-}$ and *Pkcδ*$^{+/+}$ mESCs were dissociated and stained with 25 µg/ml propidium iodide (Sigma Cat#P4864) with 25 µg/ml RNase (Roche Cat#10109142001) and their cell cycle distribution was analyzed using a BD FACSVerse flow cytometer. *Right panel*: Murine *Pkcδ*$^{-/-}$ and *Pkcδ*$^{+/+}$ ESCs received 10 µM EdU pulse for 1h before being dissociated and processed for EdU detection with the Click-iT™ Plus EdU Alexa Fluor™ 555 Flow Cytometry Assay Kit (ThermoFisher Cat#C10638). The samples were analyzed in a LSR-Fortessa flow cytometer to obtain the percentage of EdU^{+} cells.

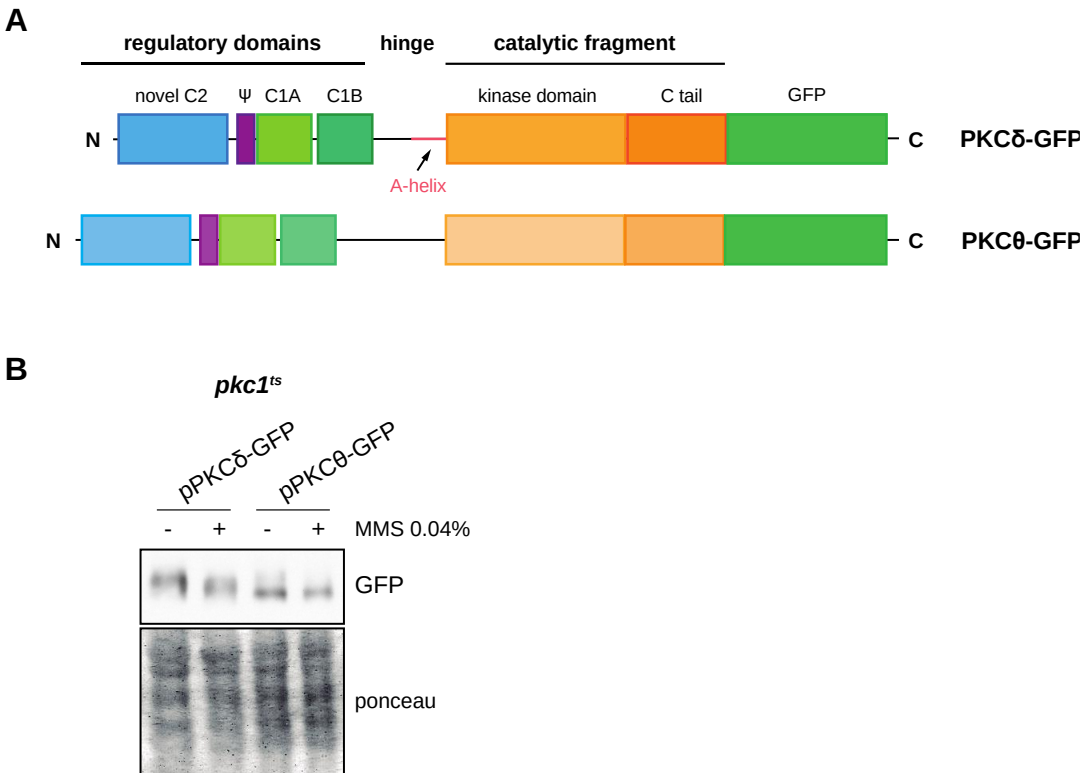

Figure S2. Protein level analysis of PKCδ and PKCθ. **A)** Schematic representation of GFP-tagged PKCδ and PKCθ proteins. **B)** Exponentially growing cultures of the *pkc1*^{ts} (JC6-3a) strain transformed with plasmids pPKCδ-GFP and pPKCθ-GFP were transferred to 37°C for 3 hours and then incubated in the absence or presence of MMS 0.04% for 1 hour. Protein levels of GFP-tagged PKCδ and PKCθ were detected by western blot. Ponceau staining of the membrane is shown as loading control.

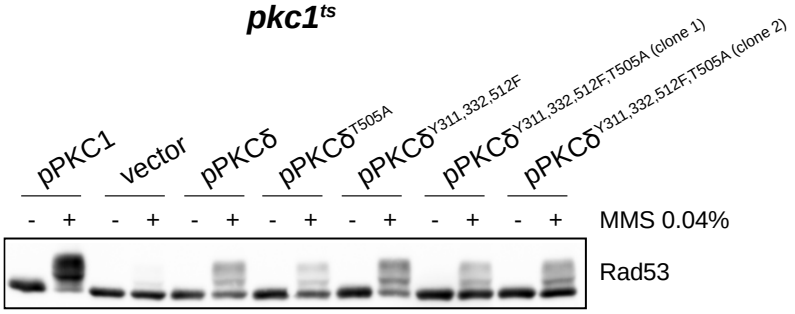

Figure S3. Analysis of DNA integrity checkpoint activation in key Tyr mutants in PKCδ oxidative stress response. Exponentially growing cultures of the *pkc1*^{ts} (JC6-3a) strain transformed with plasmids pPKC1, pPKCδ, pPKCδ$^{Y311F,\ Y332F,\ Y512F}$, pPKCδ$^{Y311F,\ Y332F,\ Y512F,\ T505A}$ or with an empty vector were transferred to 37°C for 3 hours and then incubated in the absence or presence of MMS 0.04% for 1 hour. The activation of Rad53 was analyzed by western blot.

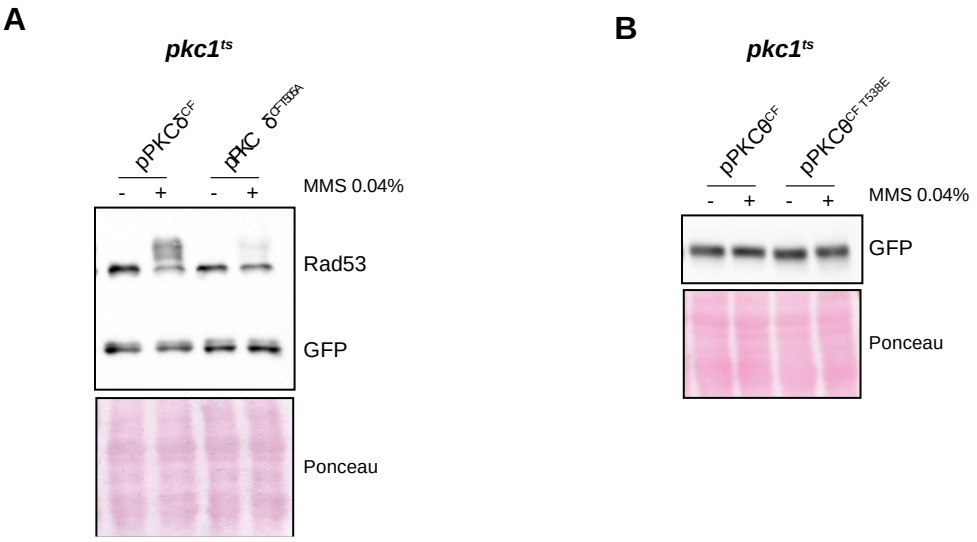

Figure S4. Protein level analysis of PKCδ^{CF} and PKCθ^{CF} mutants. Exponentially growing cultures of the *pkc1^{ts}* (JC6-3a) strain transformed with plasmids **A)** pPKCδ^{CF}-GFP and pPKCδ^{CFT505A}-GFP or **B)** pPKCθ^{CF}-GFP and pPKCθ^{CFT538E}-GFP were transferred to 37°C for 3 hours and then incubated in the absence or presence of MMS 0.04% for 1 hour. The activation of Rad53 and protein levels of GFP-tagged proteins were detected by western blot. Ponceau staining of the membrane is shown as loading control.

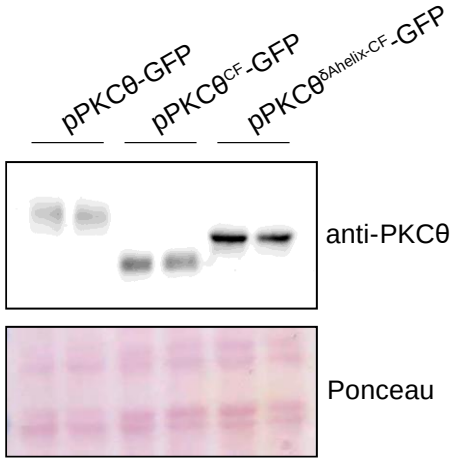

Figure S5. Protein level analysis of PKCθ truncated versions. Exponentially growing cultures of the *pkc1^{ts}* (JC6-3a) strain transformed with plasmids pPKCθ-GFP, pPKCθ^{CF}-GFP and pPKCθ^{CFT538E}-GFP were transferred to 37°C for 3 hours and then incubated in the absence or presence of MMS 0.04% for 1 hour. Protein levels were detected by western blot with a rabbit mAb anti-PKCθ antibody (Cell Signaling Cat#13643). Ponceau staining of the membrane is shown as loading control.

Table S1. Comparison of amino acids between novel PKCs. PKCδ residues involved in the A-helix dependent mechanism of activation loop stabilization and the corresponding residues of the PKCθ and PKCε isoforms.

nPKC	Contact 1		Contact 2		Contact 3	
δ	**Tyr^{332}**	**Phe^{498}**	**Trp^{336}**	**Arg^{397}**	**Ile^{497}**	**Phe^{525}**
θ	Gln	Leu	Trp	Arg	Met	His
ε	Asn	Met	Arg	Arg	Ile	Pro

Table S2. Mutant versions of PKCδ and PKCθ proteins.

Protein	Mutant version
PKCδ	PKCδ^{T505A}
	PKCδ^{E500G}
	PKCδ^{T505A,E500G}
	PKCδ^{Y311,332,512F}
	PKCδ^{Y311,332,512F,T505A}
PKCδ^{CF}	PKCδ$^{CF\ T505A}$
	PKCδ$^{CF\ E500G}$
	PKCδ$^{CF\ T505A,E500G}$
PKCδ$^{Ahelix\text{-}CF}$	PKCδ$^{Ahelix\text{-}CF\ T505A}$
	PKCδ$^{Ahelix\text{-}CF\ E500G}$
	PKCδ$^{Ahelix\text{-}CF\ T505A,E500G}$
	PKCδ$^{Ahelix\text{-}CF\ T505A,E500G,Y332Q,W336R,I497M,F498L,F525H}$
	PKCδ$^{Ahelix\text{-}CF\ T505A,I497A,F498A,F525A}$
PKCθ	PKCθ^{T538A}
PKCθ^{CF}	PKCθ$^{CF\ T538E}$

Table S3. Oligonucleotides used for site-directed mutagenesis. Mutated nucleotides are highlighted in bold.

Point mutation	Oligonucleotide	Sequence (5'-3')
PKCδ Y311F	forward	CTGTCGGAATAT**TT**CAGGGATTTGAGAAG
	reverse	CTTCTCAAATCCCTG**AA**ATATTCCGACAG
PKCδ Y332F	forward	CAACGGGACCT**T**TGGCAAGATCTGGG
	reverse	CCCAGATCTTGCCA**A**AGGTCCCGTTG
PKCδ Y332Q	forward	CTAGACAACAACGGGACC**CAA**GGCAAGATCTGGG
	reverse	CCCAGATCTTGCC**TTG**GGTCCCGTTGTTGTCTAG
PKCδ W336R	forward	GACCTATGGCAAGATC**C**GGGAGGGGAGCAC
	reverse	CGGGTGCTCCCCTCCC**G**GATCTTGCCATAG
PKCδ I497M	forward	CAAAGAGAATAT**G**TTTGGGGAG
	reverse	CTCCCCAAA**C**ATATTCTCTTTG
PKCδ I497A	forward	GTGCAAAGAGAAT**GCT**TTTGGGGAGG
	reverse	CCTCCCCAAA**AGC**ATTCTCTTTGCAC
PKCδ F498L	forward	GAATATATT**G**GGGGAGGGCCGG
	reverse	CCGGCCCTCCCC**C**AATATATTC
PKCδ F498A	forward	GTGCAAAGAGAATATA**GCT**GGGGAGGGC
	reverse	GCCCTCCCC**AGC**TATATTCTCTTTGCAC
PKCδ E500G	forward	GAATATATTTGGGG**GT**GGCCGGGCCAG
	reverse	CTGGCCCGGCC**AC**CCCCAAATATATTC
PKCδ T505A	forward	GGCCGGGCCAGC**GCT**TTCTGCGGC
	reverse	GCCGCAGAA**AGC**GCTGGCCCGGCC
PKCδ Y512F	forward	GGCACTCCTGACT**TT**ATCGCCCCTG
	reverse	CAGGGGCGAT**AA**AGTCAGGAGTGCC
PKCδ F525H	forward	GGCCTGAAGTACTCC**CAT**TCGGTGGACTG
	reverse	GTCCACCGA**ATG**GGAGTACTTCAG
PKCδ F525A	forward	GGCCTGAAGTACTCC**GCT**TCGGTGGACTG
	reverse	CAGTCCACCGA**AGC**GGAGTACTTCAGGCC
PKCθ T538A	forward	GCGAAGACAAAT**G**CTTTCTGTG
	reverse	CACAGAAAG**C**ATTTGTCTTCGC
PKCθ T538E	forward	GAAGACAAAT**GAA**TTCTGTGGAACTC
	reverse	GAGTTCCACAGAA**TTC**ATTTGTCTTC