Table S1: Full chemical names of the tested analogues.

cGMP	Guanosine- 3', 5'- cyclic monophosphate
cAMP	Adenosine- 3', 5'- cyclic monophosphate
2-NH_2-cPuMP	2- Aminopurine riboside- 3', 5'- monophosphate
8-AET-cGMP	8- (2- Aminoethylthio) guanosine- 3', 5'- cyclic monophosphate
8-APT-cGMP	8- (2- Aminophenylthio) guanosine- 3', 5'- cyclic monophosphate
1-NH_2-cGMP	N^1- Aminoguanosine- 3', 5'- cyclic monophosphate
2'-AHC-cGMP	2'- O- (6- Aminohexylcarbamoyl) guanosine- 3', 5'- cyclic monophosphate
Sp-2'AHC-cGMPS	2'- O- (6- Aminohexylcarbamoyl) guanosine- 3', 5'- cyclic monophosphorothioate, Sp- isomer
8-Br-cGMP	8- Bromoguanosine- 3', 5'- cyclic monophosphate
Rp-8-Br-cGMPS	8- Bromoguanosine- 3', 5'- cyclic monophosphorothioate, Rp- isomer
Sp-8-Br-cGMPS	8- Bromoguanosine- 3', 5'- cyclic monophosphorothioate, Sp- isomer
8-pCPT-cGMP	8- (4- Chlorophenylthio) guanosine- 3', 5'- cyclic monophosphate
Rp-8-pCPT-cGMPS	8- (4- Chlorophenylthio) guanosine- 3', 5'- cyclic monophosphorothioate, Rp- isomer
Sp-8-pCPT-cGMPS	8- (4- Chlorophenylthio) guanosine- 3', 5'- cyclic monophosphorothioate, Sp- isomer
Rp-8-pCPT-PET-cGMPS	8- (4- Chlorophenylthio)- β- phenyl- 1, N^2- ethenoguanosine- 3', 5'- cyclic monophosphorothioate, Rp- isomer
Sp-8-pCPT-PET-cGMPS	8- (4- Chlorophenylthio)- β- phenyl- 1, N^2- ethenoguanosine- 3', 5'- cyclic monophosphorothioate, Sp- isomer
5,6-DM-cBIMP	5, 6- Dimethylbenzimidazole riboside- 3', 5'- cyclic monophosphate
DB-cGMP	N^2, 2'- O- Dibutyrylguanosine- 3', 5'- cyclic monophosphate
5,6-DCl-cBIMP	5, 6- Dichlorobenzimidazole riboside- 3', 5'- cyclic monophosphate
Sp-5,6-DCl-cBIMPS	5, 6- Dichlorobenzimidazole riboside- 3', 5' - cyclic monophosphorothioate, Sp- isomer
2'-dcGMP	2'- Deoxyguanosine- 3', 5'- cyclic monophosphate
Rp-cGMPS	Guanosine- 3', 5'- cyclic monophosphorothioate, Rp-isomer
Sp-cGMPS	Guanosine- 3', 5'- cyclic monophosphorothioate, Sp- isomer
cIMP	Inosine- 3', 5'- cyclic monophosphate
MANT-cGMP	2'- O- (N- Methylanthraniloyl) guanosine- 3', 5'- cyclic monophosphate
2'O-MS-cGMP	2'- O- Monosuccinylguanosine- 3', 5'- cyclic monophosphate
2'O-MS-TME-cGMP	2'- O- Monosuccinylguanosine- 3', 5'- cyclic monophosphate, tyrosylmethyl ester
2'O-ME-cGMP	2'- O- Methylguanosine- 3', 5'- cyclic monophosphate
PET-cGMP	β- Phenyl- 1, N^2- ethenoguanosine- 3', 5'- cyclic monophosphate
8-Br-PET-cGMP	8 - Bromo- β- phenyl- 1, N^2- ethenoguanosine- 3', 5'-cyclic monophosphate
Rp-8-Br-PET-cGMPS	8- Bromo- β- phenyl- 1, N^2- ethenoguanosine- 3', 5'- cyclic monophosphorothioate, Rp- isomer
Sp-8-Br-PET-cGMPS	8- Bromo- β -phenyl- 1, N^2- ethenoguanosine- 3', 5'- cyclic monophosphorothioate, Sp- isomer
cPuMP	Purine riboside- 3', 5'- cyclic monophosphate
cXMP	Xanthosine- 3', 5'- cyclic monophosphate

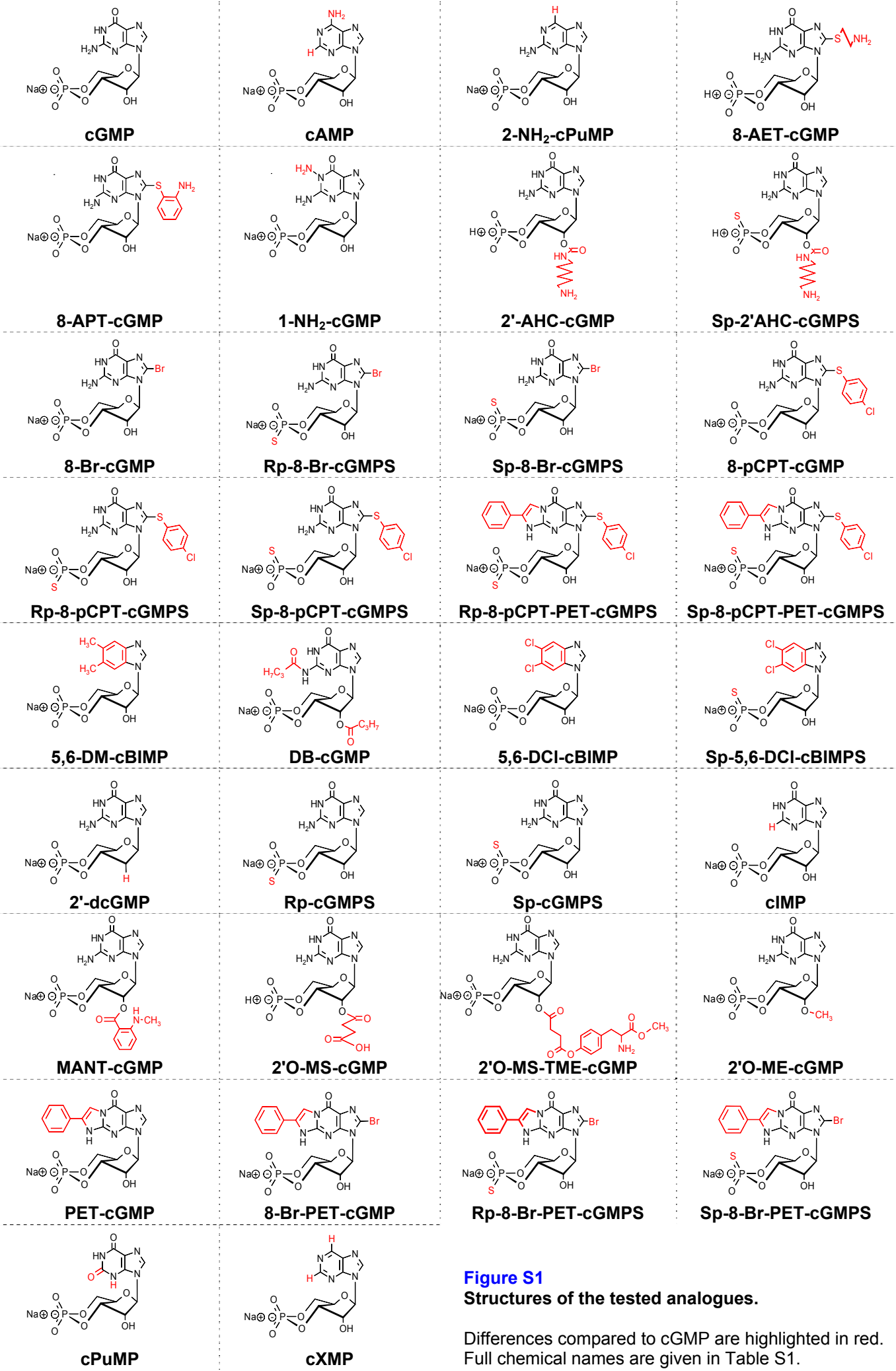

Figure S1
Structures of the tested analogues.

Differences compared to cGMP are highlighted in red.
Full chemical names are given in Table S1.

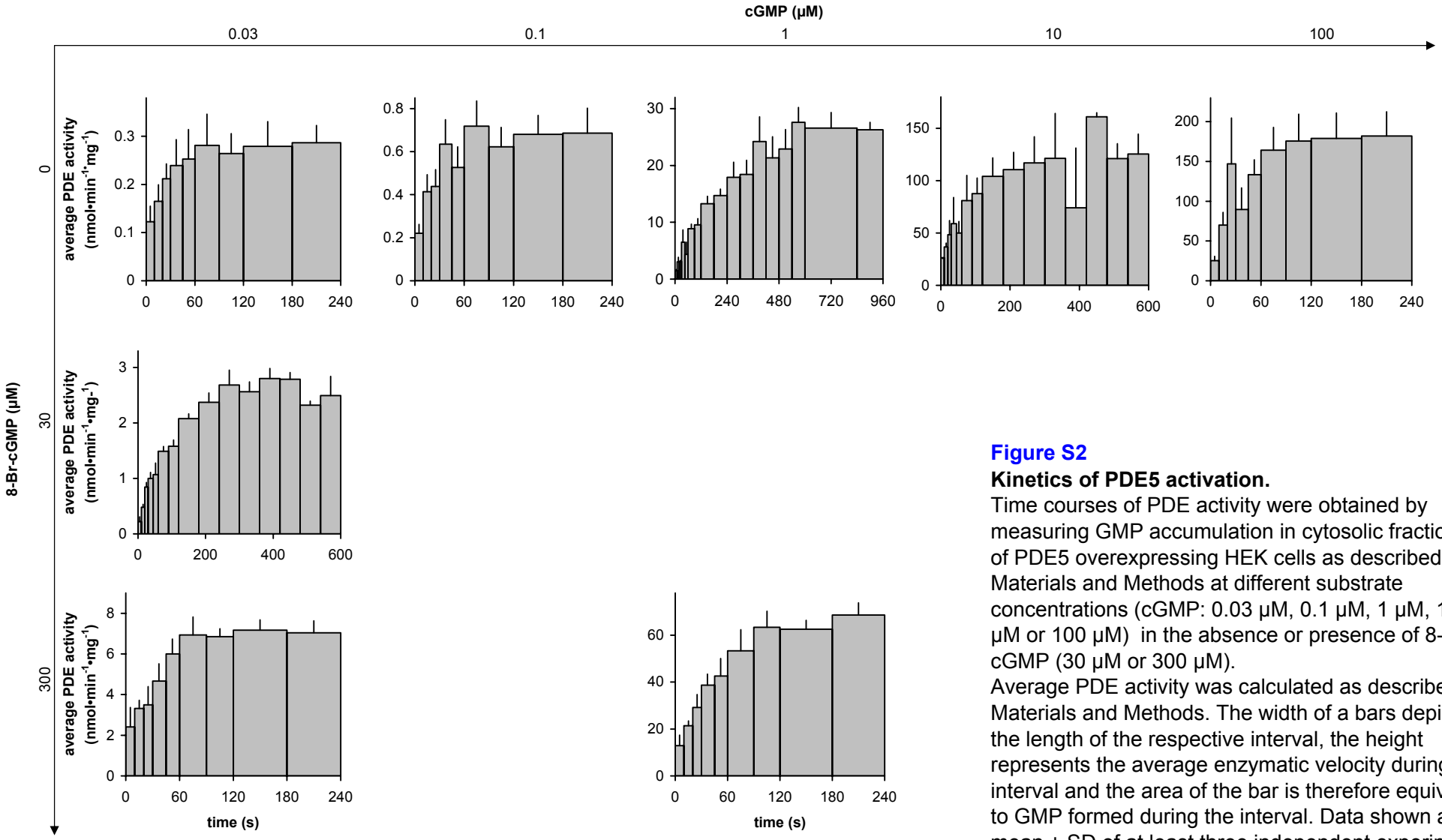

Figure S2

Kinetics of PDE5 activation.

Time courses of PDE activity were obtained by measuring GMP accumulation in cytosolic fractions of PDE5 overexpressing HEK cells as described in Materials and Methods at different substrate concentrations (cGMP: 0.03 µM, 0.1 µM, 1 µM, 10 µM or 100 µM) in the absence or presence of 8-Br-cGMP (30 µM or 300 µM).

Average PDE activity was calculated as described in Materials and Methods. The width of a bars depicts the length of the respective interval, the height represents the average enzymatic velocity during the interval and the area of the bar is therefore equivalent to GMP formed during the interval. Data shown are mean ± SD of at least three independent experiments performed in duplicates.

Time courses at 0.03, 1 and 100 µM are replicated from Figure 4 to allow for side-by-side comparison of the traces.

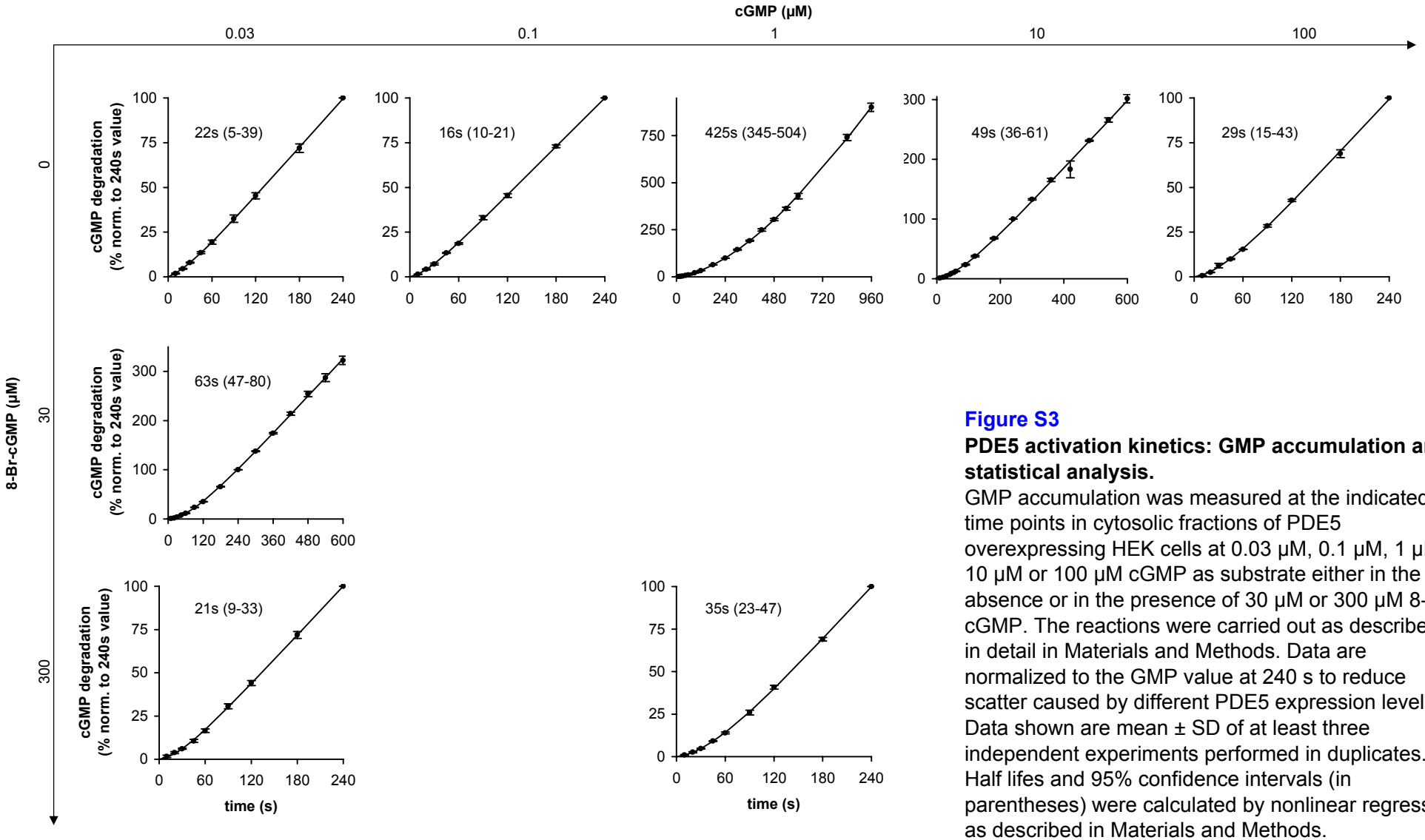

Figure S3

PDE5 activation kinetics: GMP accumulation and statistical analysis.

GMP accumulation was measured at the indicated time points in cytosolic fractions of PDE5 overexpressing HEK cells at 0.03 µM, 0.1 µM, 1 µM, 10 µM or 100 µM cGMP as substrate either in the absence or in the presence of 30 µM or 300 µM 8-Br-cGMP. The reactions were carried out as described in detail in Materials and Methods. Data are normalized to the GMP value at 240 s to reduce scatter caused by different PDE5 expression levels. Data shown are mean ± SD of at least three independent experiments performed in duplicates. Half lifes and 95% confidence intervals (in parentheses) were calculated by nonlinear regression as described in Materials and Methods.

Time courses at 0.03, 1 and 100 µM are replicated from Figure 5 to allow for side-by-side comparison of the traces.

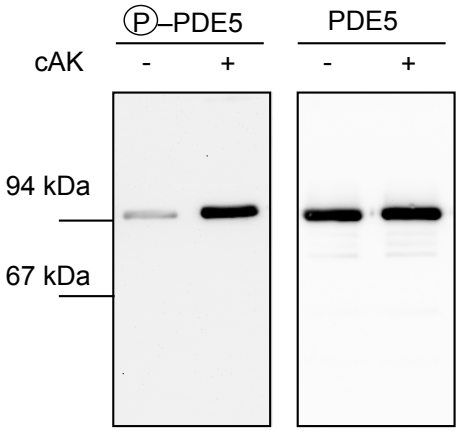

Figure S4

Complete lanes of the Western blot shown in Figure 7B.