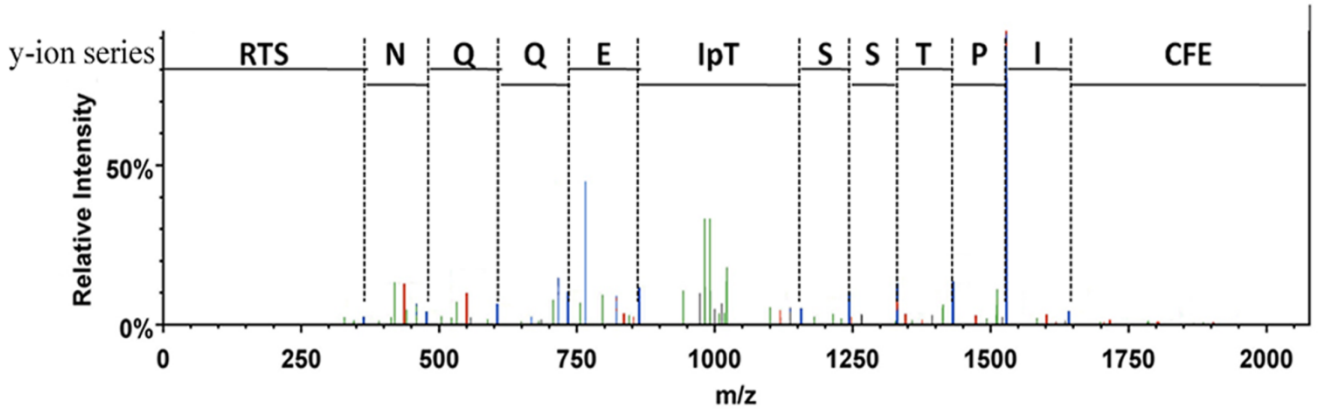
Sup. Fig. 1

A

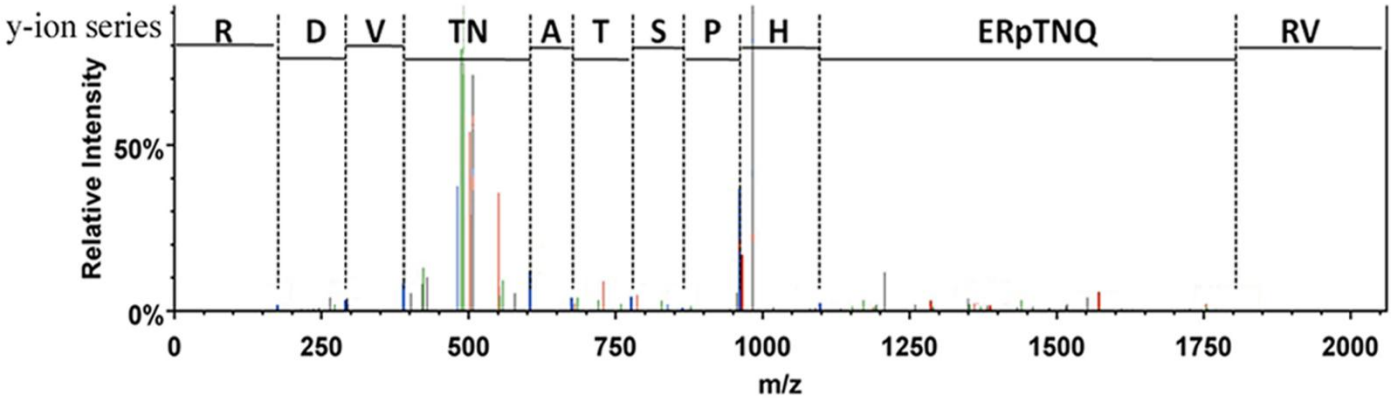


B Ions		Y Ion
	E	
	F	
437.1	C+57	
550.2	I	1641.7
	P	1528.7
	T	1431.6
835.4	S	1330.6
	S	1243.5
	T+80	1156.5
	I	
1345.5	E	862.4
1473.6	Q	733.4
1601.7	Q	605.3
1715.7	N	477.2
1802.7	S	363.2
1903.8	T	
	R	

Supplementary Fig. 1A-E. Mass spectrometry of the C-terminal tail of MOPr. Examples of mass spectrum of the tryptic digest derived from MOPr expressed in cells incubated in the presence or absence of morphine or DAMGO. The pSerine363 and pThreonine370 spectra were from non-stimulated cells.

B

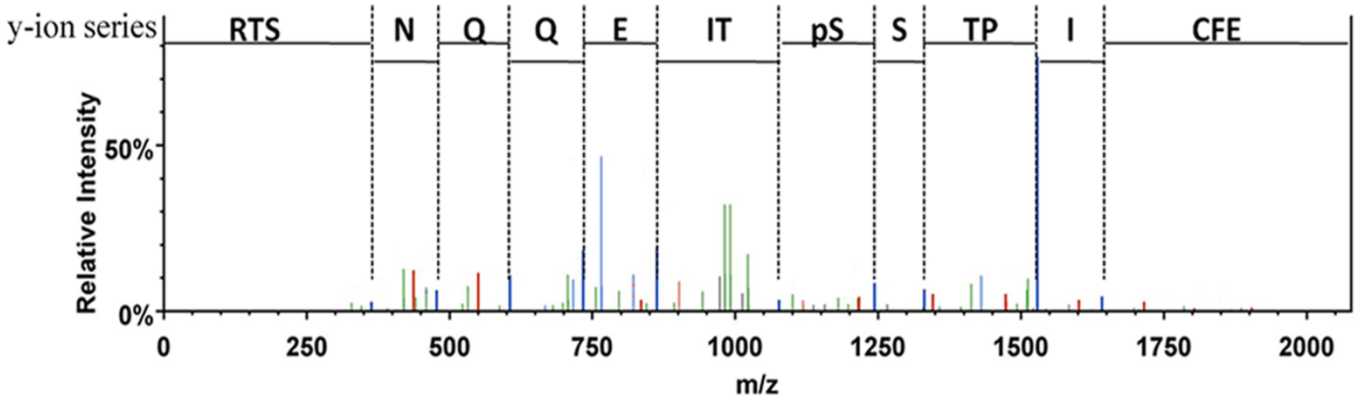
pThreonine 370
VRQNpTREHPSTANTVDR



B Ions		Y Ions
	V	
	R	
	Q	1805.8
	N	
679.3	T+80	
	R	
964.4	E	
	H	1097.5
	P	960.5
1285.6	S	863.4
1386.6	T	776.4
	A	675.3
1571.7	N	604.3
	T	
1771.8	V	389.2
	D	290.1
	R	175.1

C

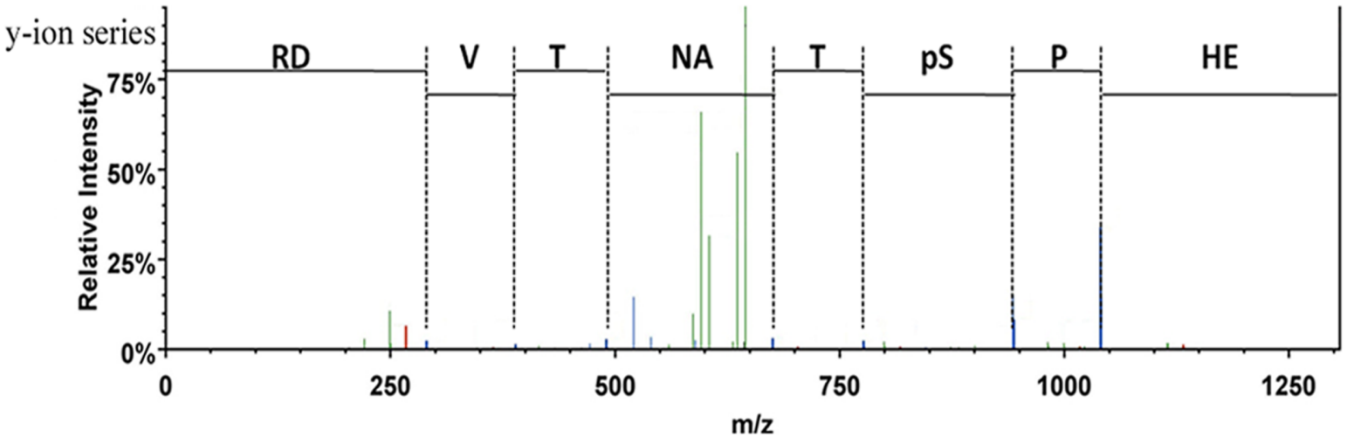
pSerine 356
EFCIPTSpSTIEQQNSTR



B Ions		Y Ions
	E	
	F	
437.1	C+57	
550.2	I	1641.7
	P	1528.7
	T	
835.4	S	1330.6
	S+80	1243.5
	T	1076.5
1216.5	I	
1345.5	E	862.4
1473.6	Q	733.4
1601.7	Q	605.3
1715.7	N	477.2
1802.7	S	363.2
1903.8	T	
	R	

D

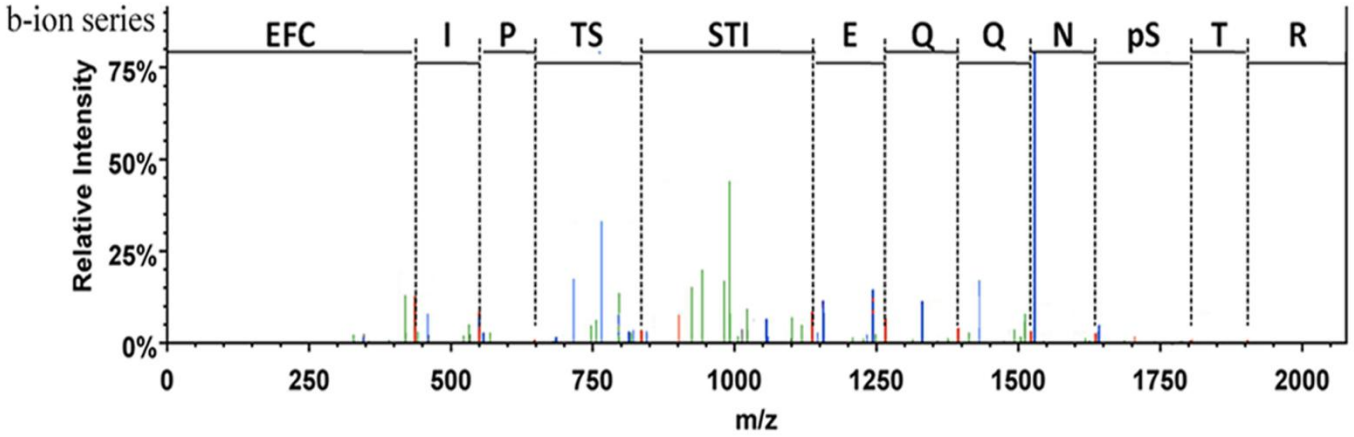
pSerine 375
EHPpSTANTVDR



B Ions		Y Ions
	E	
267.1	H	
364.2	P	1040.4
	S+80	943.4
	T	776.4
703.2	A	675.3
817.3	N	
	T	490.3
1017.4	V	389.2
1132.4	D	290.1
1306.5	R	

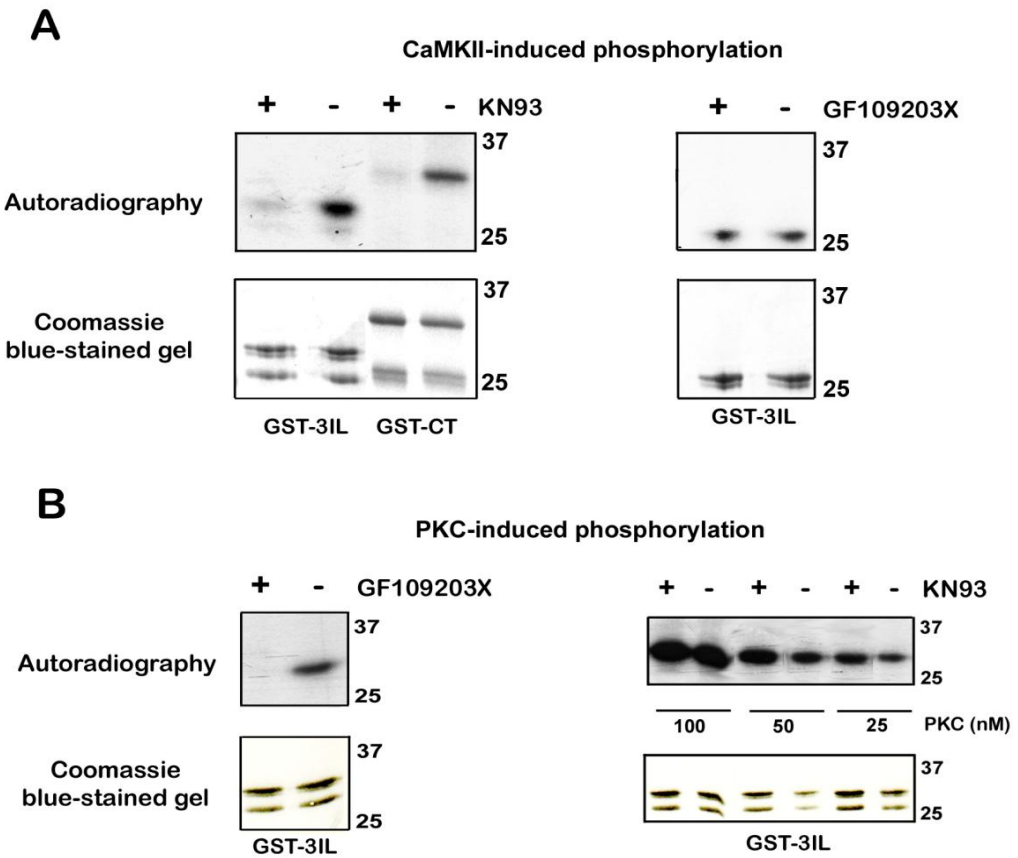
E

pSerine 363
EFCIPTSSTIEQQNpSTR



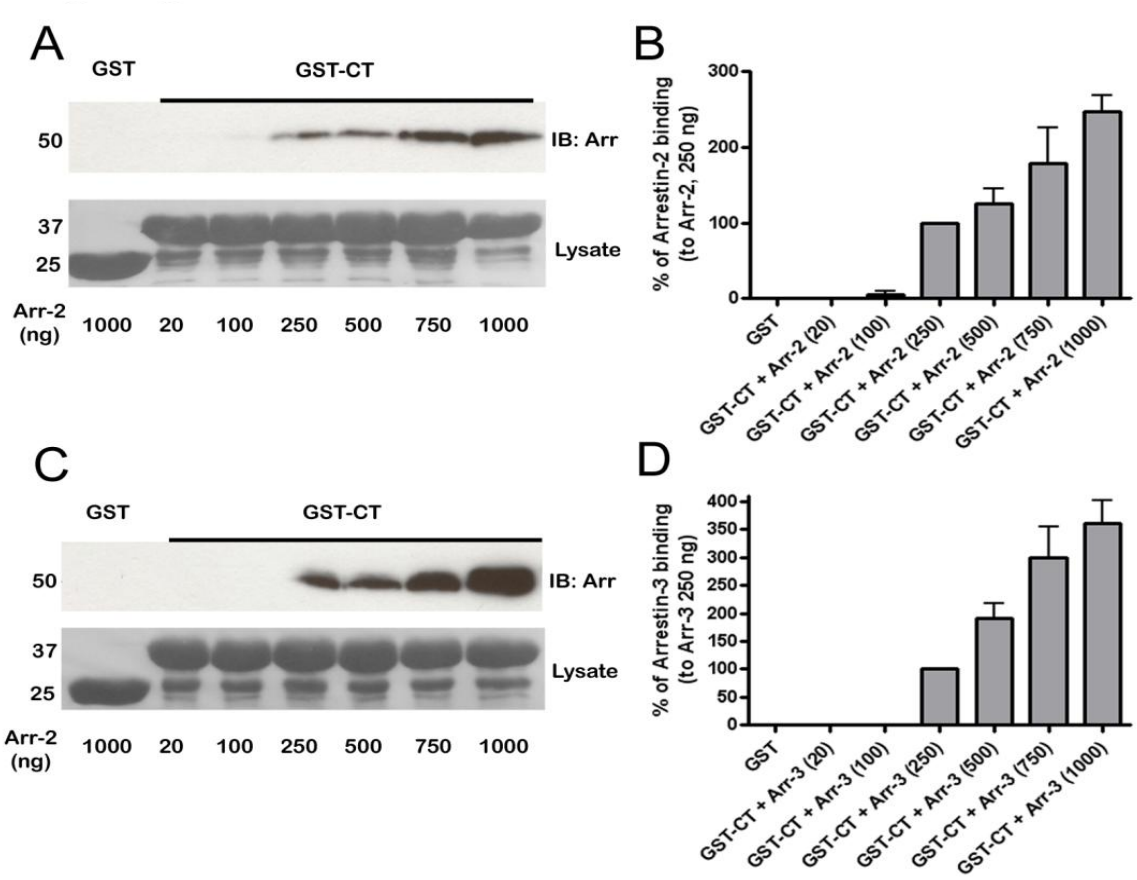
B Ions		Y Ions
	E	
	F	
437.15	C+57	1,801.78
550.23	I	1,641.75
647.29	P	1,528.66
	T	
835.37	S	1,330.56
	S	1,243.53
	T	1,156.50
1,136.53	I	1,055.45
1,265.57	E	
1,393.63	Q	813.33
1,521.69	Q	685.27
1,635.73	N	557.21
1,802.73	S+80	
1,903.78	T	
	R	

Sup. Fig. 2



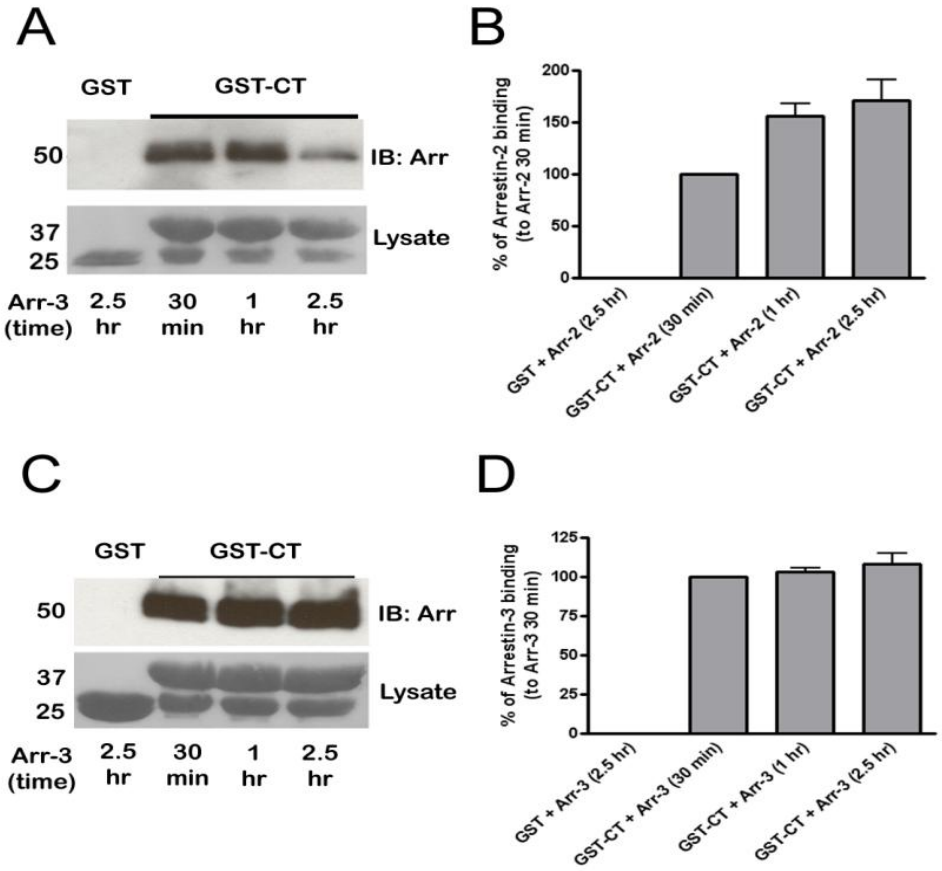
Supplementary Fig. 2. Inhibition of (A) CaMKII- or (B) PKC-induced phosphorylation of GST fusion proteins of MOPr with the selective CaMKII inhibitor KN93 or the selective PKC inhibitor GF109203X (each 10 μM), respectively. For (A) and (B) the top panels are autoradiographs of phosphorylation whilst the lower panels show the amount of protein loaded. Note that GF109203X was unable to inhibit CaMKII-induced phosphorylation whilst KN93 was unable to inhibit PKC-induced phosphorylation of the fusion proteins. The experiment was repeated on one further occasion with the same result

Sup. Fig. 3



Supplementary Fig. 3. Concentration-dependent interaction of arrestin-2 and -3 with a GST fusion protein of the COOH terminus of MOPr *in vitro*. Different amounts of (A) purified arrestin-2 (n=3) or (B) purified arrestin-3 (n=4) were incubated for 2.5 h with GST alone or with the GST fusion protein of the COOH terminus of MOPr. The results are shown as a % of 250 ng arrestin binding to the MOPr fusion protein. Left-hand panels show representative experiments whilst right-hand panels show bar graphs for data from 3 or 4 independent experiments. Values are means +/- S.E.M.

Sup. Fig. 4



Supplementary Fig. 4. Time-dependent interaction of arrestin-2 and -3 with a GST fusion protein of the COOH terminus of MOPr *in vitro*. (A, B) Purified arrestin-2 (250 ng) and (C, D) purified arrestin-3 (250 ng) were incubated for varying lengths of time with GST alone or with the GST fusion protein of the COOH terminus of MOPr. The results are shown as a % of arrestin binding to the MOPr fusion protein after 30 min. Left-hand panels show representative experiments whilst right-hand panels show bar graphs for data from 3 or 4 independent experiments. Values are means +/- S.E.M.