Nitric Oxide and Cellular Signaling

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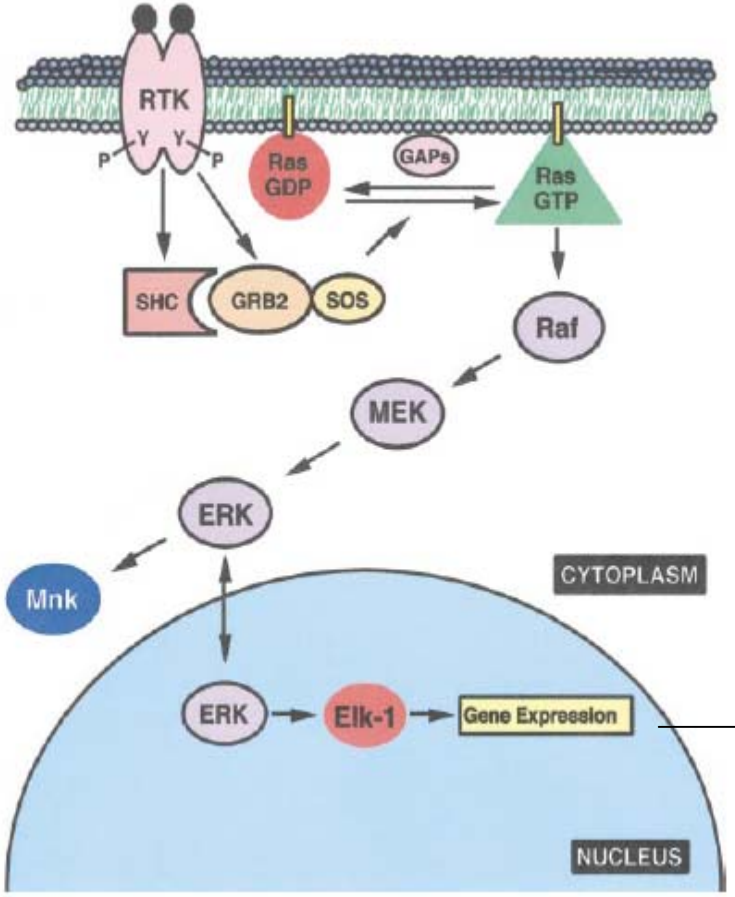
•Most aspects of cellular physiology are influenced by TWO SIGNALING SYSTEMS which are based on the principle of post-translational modification of proteins.

• One is protein phosphorylation .

•The other is the redox-based modification of proteins.

•They govern many of the same signal transduction pathways through overlapping sets of cellular targets.

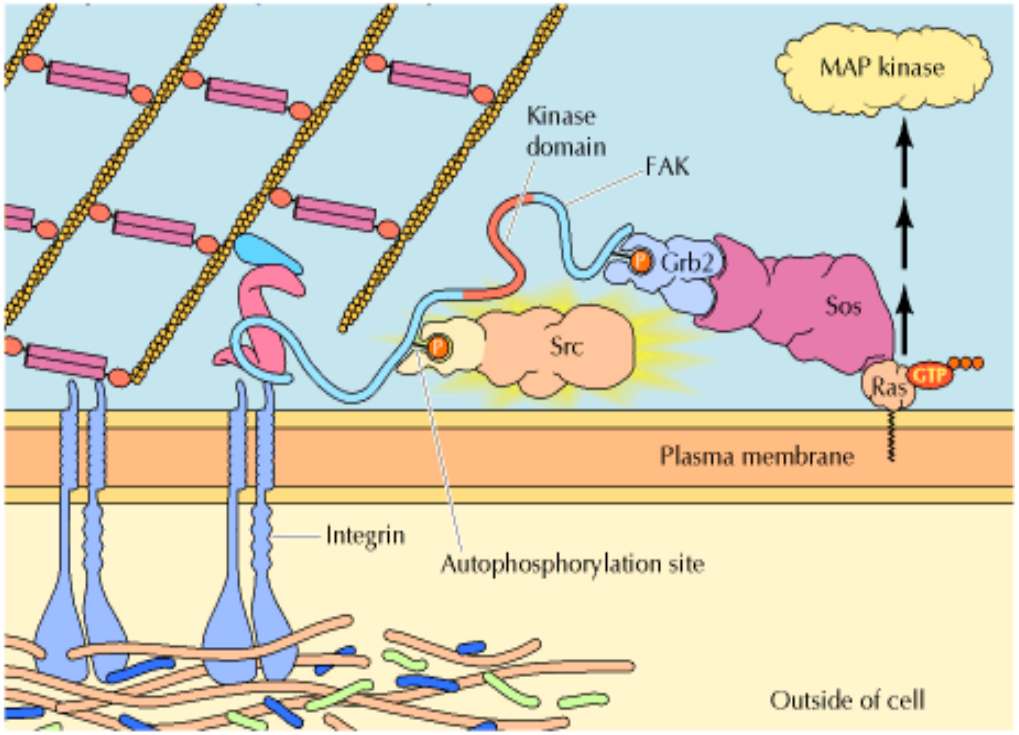
Growth Factor receptors and p21Ras regulate a cascade of kinases



Cell proliferation and cell survival

Vojtek & Der (1998) JBC 273; 19925-19928

ADHESION PROCESSES MEDIATED SIGNALING PATHWAYS



Cooper (2000) The Cell a Molecular Approach (2nd. Edition)

GROWTH FACTOR RECEPTORS AND INTEGRINS COOPERATE TO RELAY SIGNALS ASSOCIATED WITH CELL SURVIVAL , PROLIFERATION AND MIGRATION

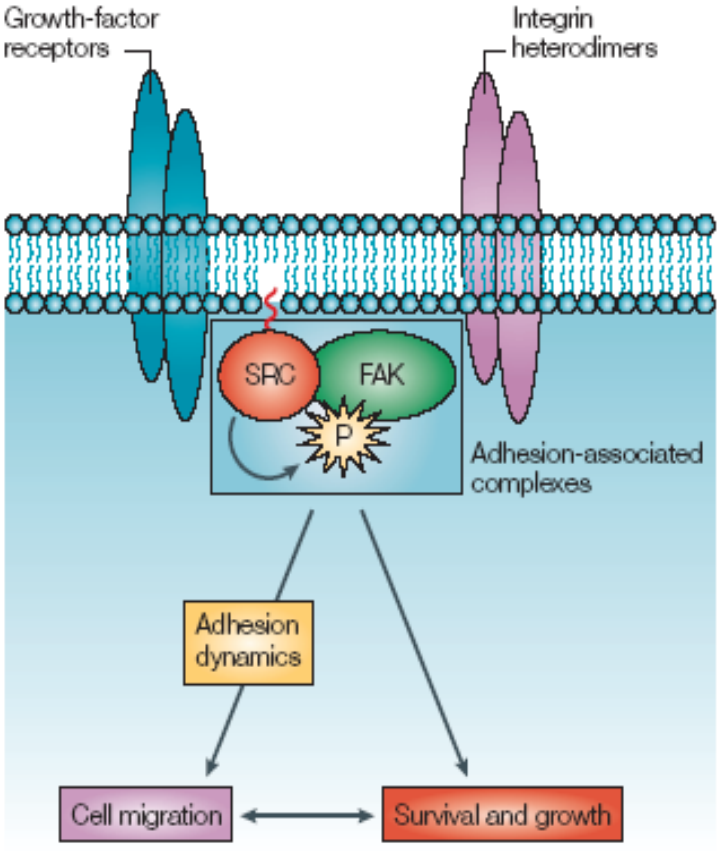


Figure 1 | **Focal-adhesion kinase as a signal integrator.**

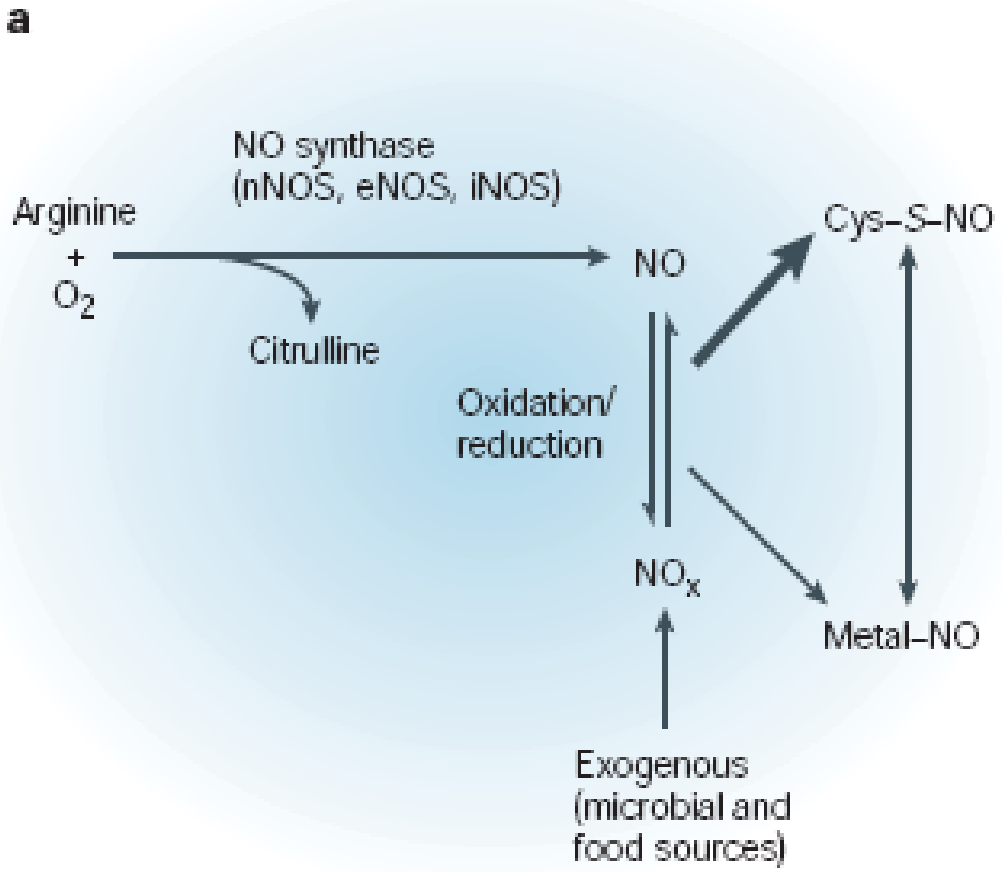
McLean et al (2005) Nat Rev Cancer 5; 505-515

•In addition to protein phosphorylation/dephosphorylation, redox-based modifications in proteins introduced by oxidizing agents, reactive species and free radicals play a major role in signal transduction pathways. Redox-based modifications on proteins could compete or cooperate with phosphorylation.

•(Monteiro et al., (1991) FEBS Lett. 295; 146-148; Monteiro & Stern (1996) FRBM 21; 323-333).

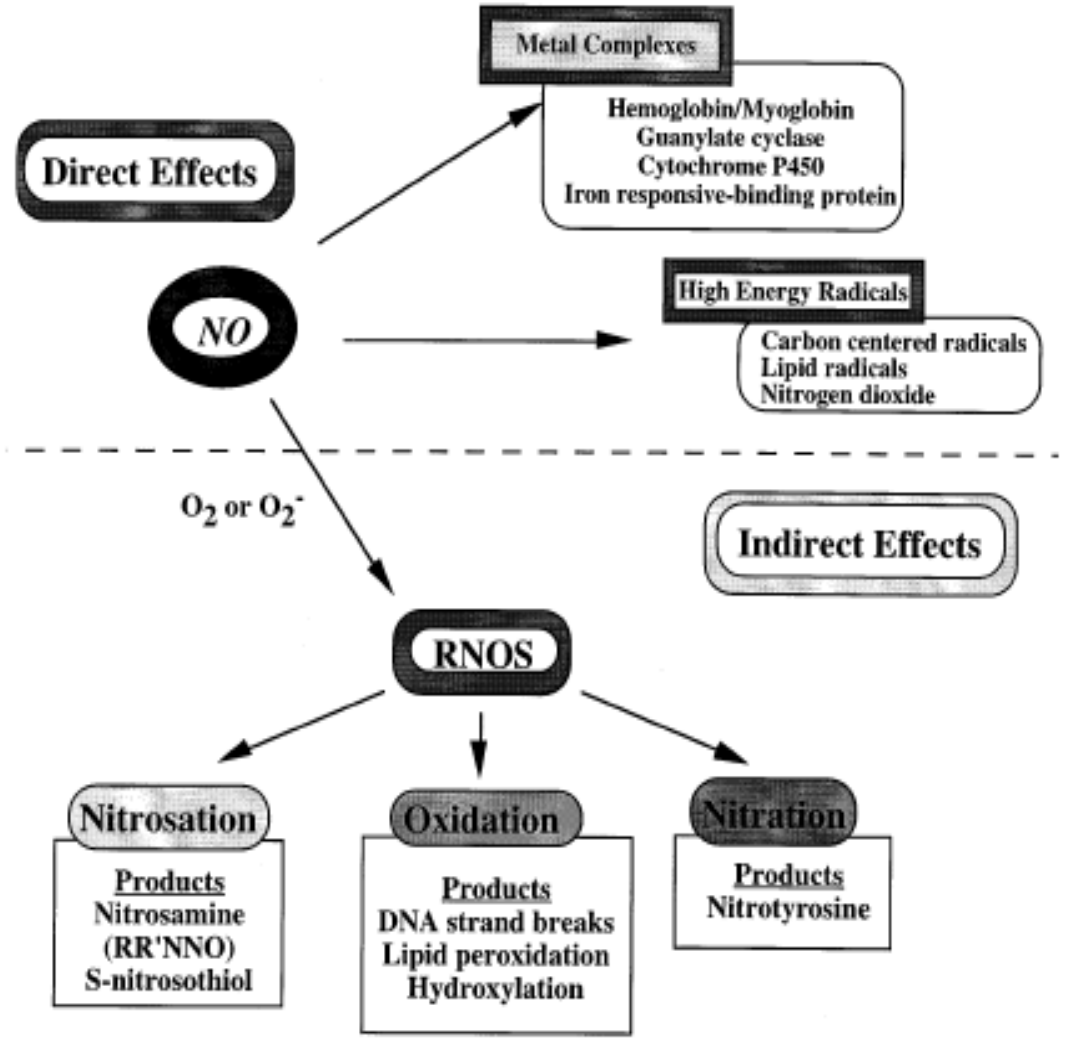
•In particular, the gaseous free radical NO is a signaling free radical. Unlike other biological mediators, the chemistry of NO determines its biological properties.

ENDOGENOUS AND EXOGENOUS SOURCES OF NITRIC OXIDE

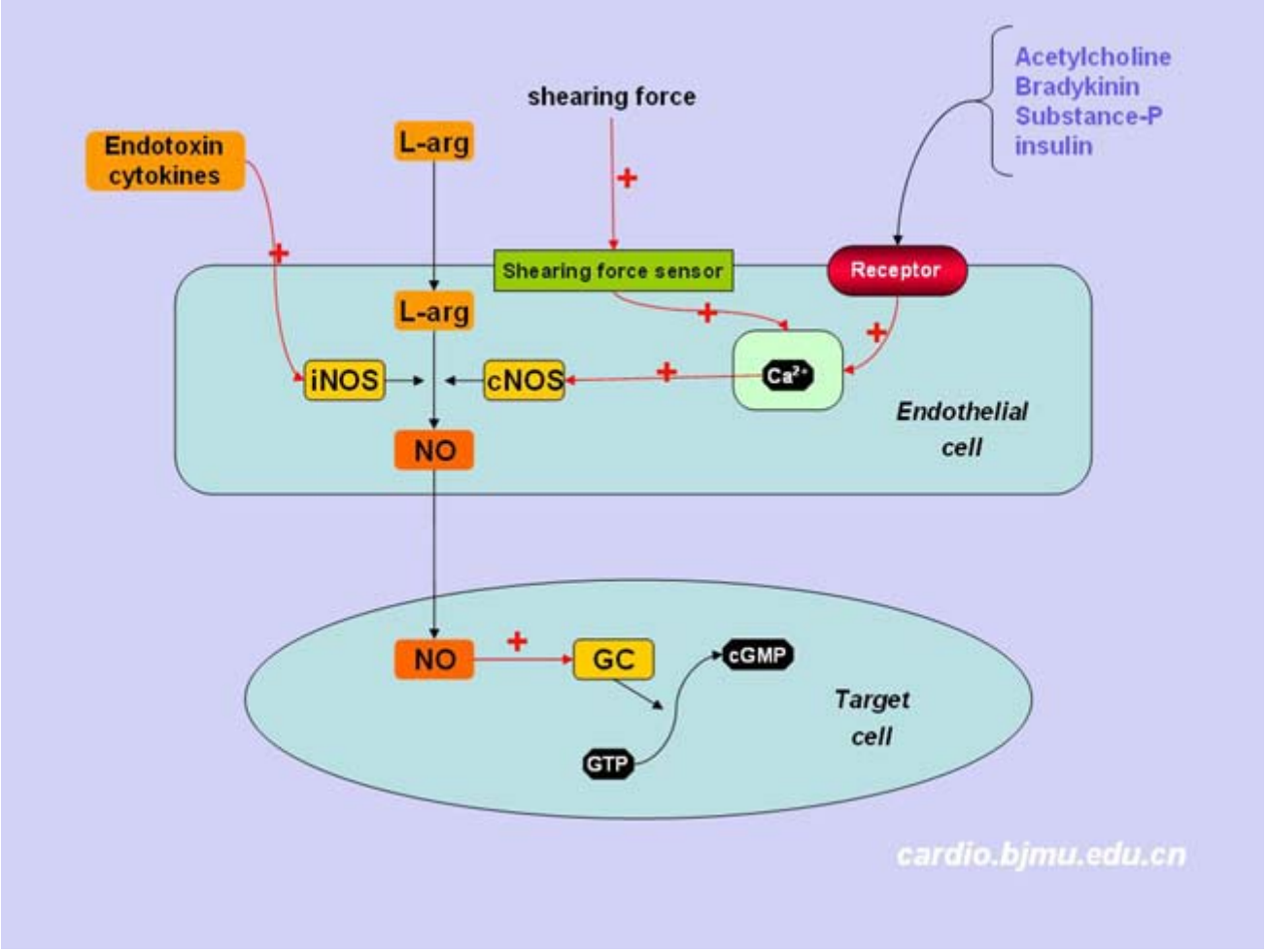


- Additional exogenous sources
  * NO-donors - S-Nitrosoglutathione

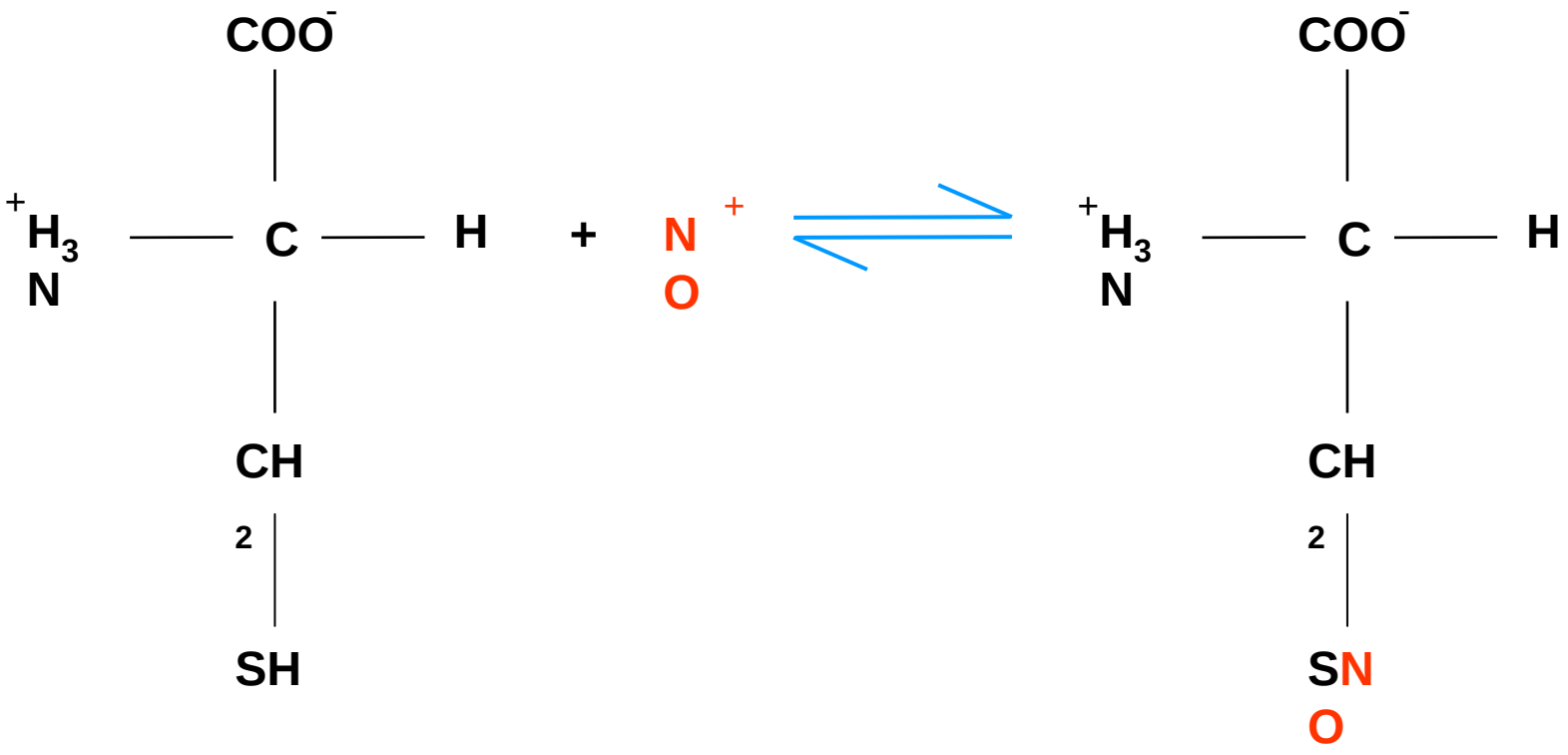
THE CHEMICAL BIOLOGY OF NITRIC OXIDE



Signaling events classically mediated by NO

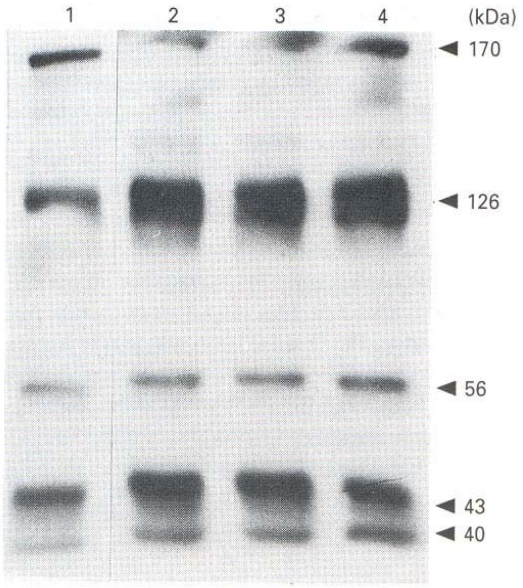


S-nitrosylation reaction

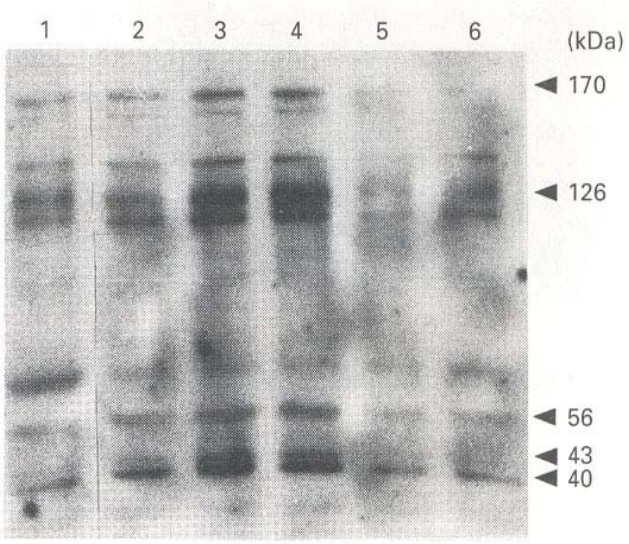


COOPERATION BETWEEN PROTEIN PHOSPHORYLATION AND PROTEIN NITROSILATION?

PARTICIPATION OF SRC KINASE

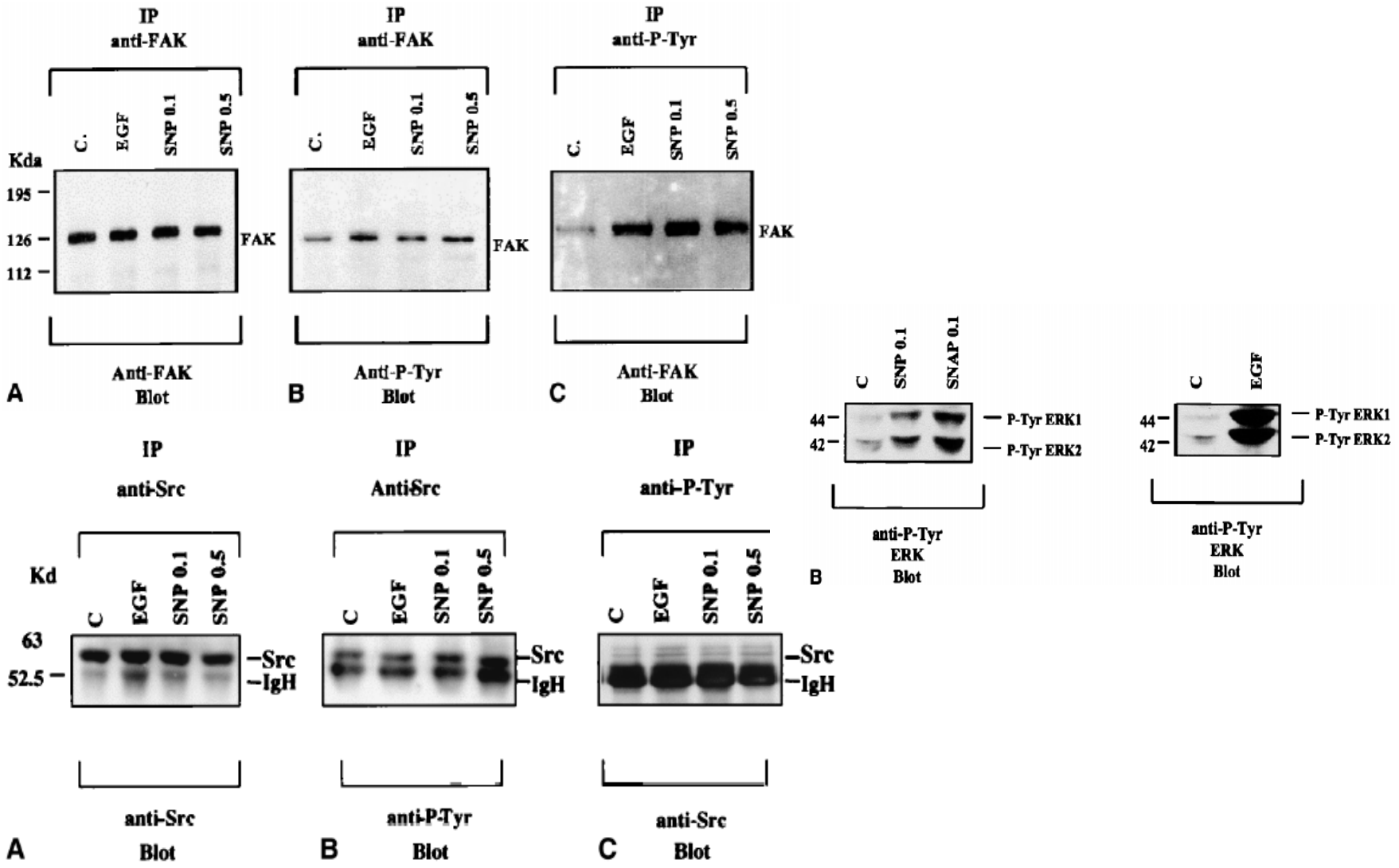


Effects of increasing concentrations of the nitrosothiol SNAP on protein tyrosine phosphorylation in murine fibroblasts



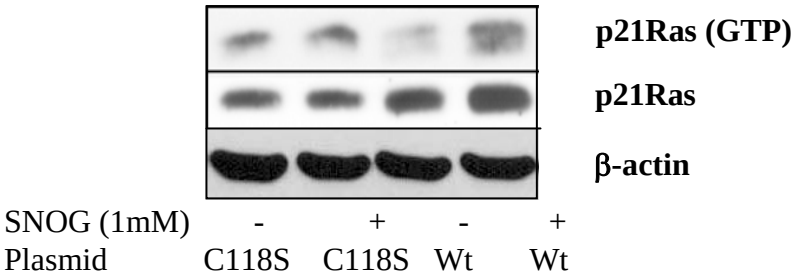
Effects of pre-incubation with Hemoglobin on SNP-stimulated tyrosine phosphorylation in murine fibroblasts

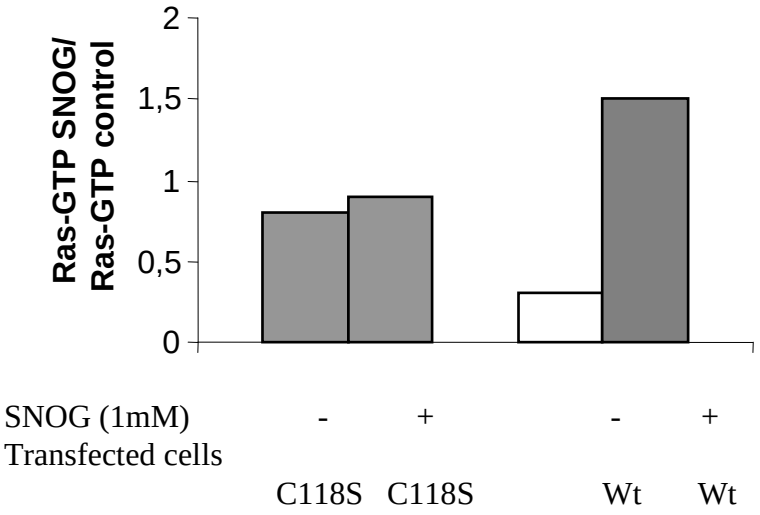
Peranovich et al. (1995) Biochem J. 305 ,613-619.



Monteiro et al. (2000) FRBM 28; 174-182

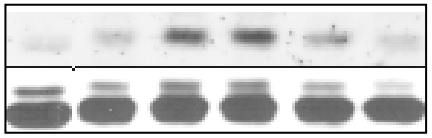
Assay for NO-stimulated activation of p21Ras in p12Ras^{C118S} and p21Ras^{Wt} transfected cells





Taujita et al. (2007) Manuscript to be submitted

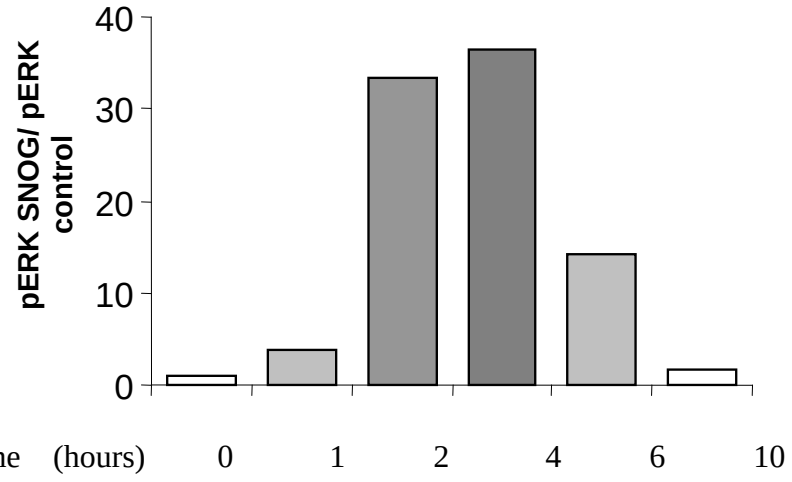
Nitric oxide activates ERK1/2 (44/42kDa) MAP Kinases in parental cells



pERK (42 kDa)

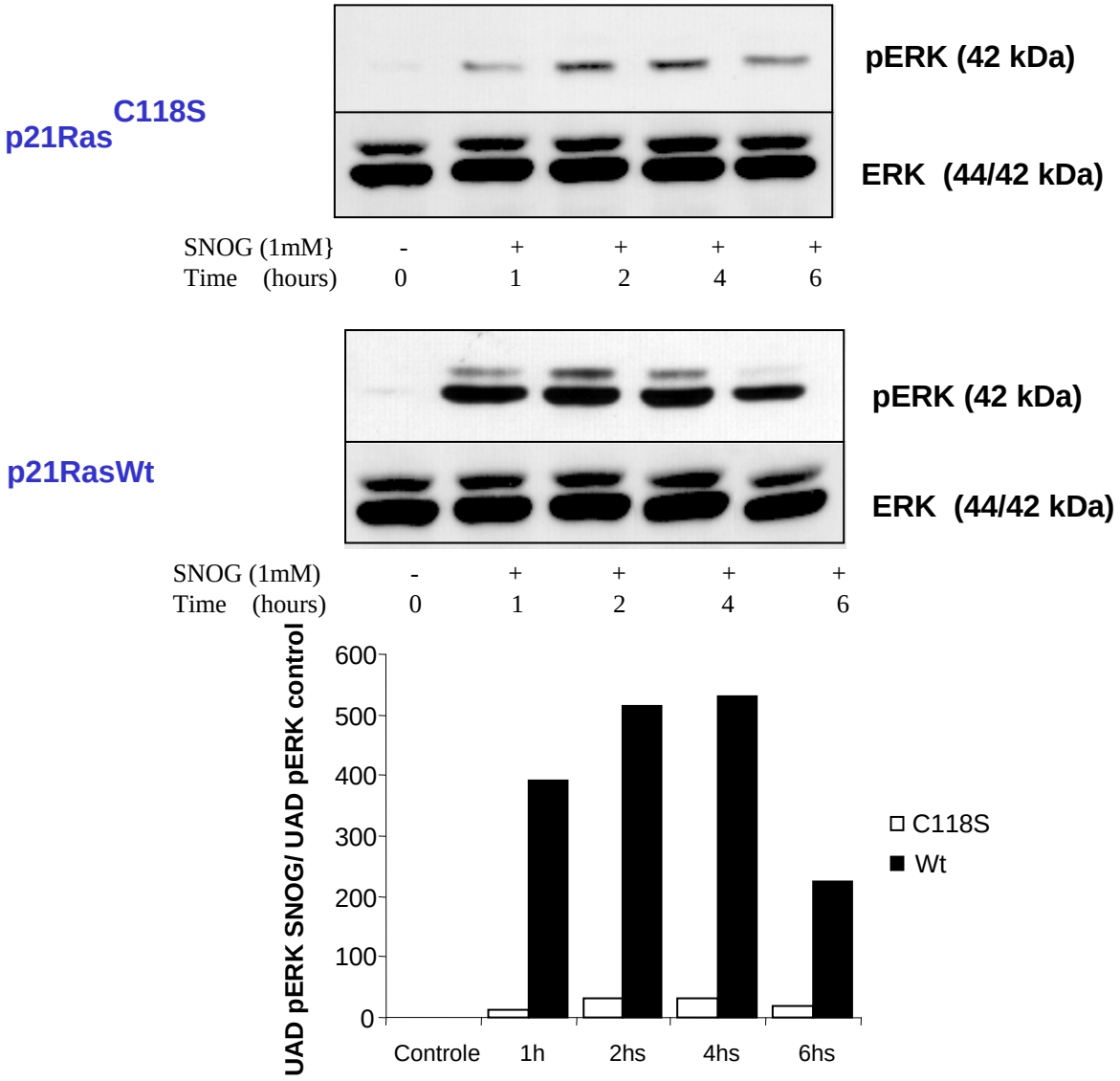
ERK total (44/42 kDa)

SNOG 1mM	-	+	+	+	+	+
Tempo (horas)	0	1	2	4	6	10



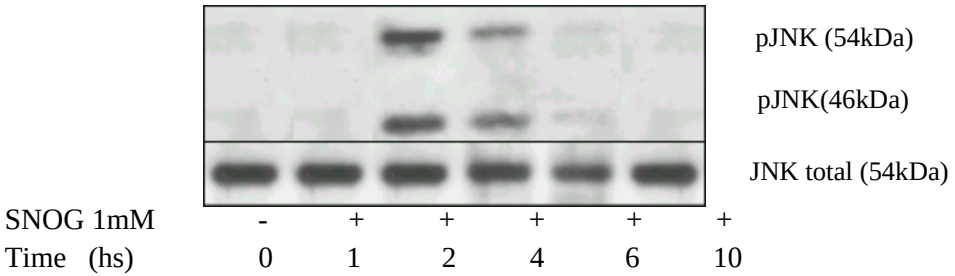
Taujita et al. (2007) Manuscript to be submitted

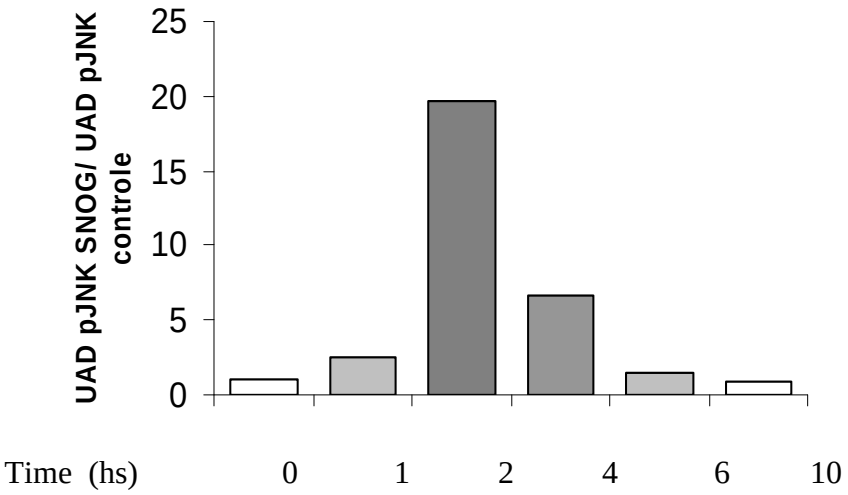
Differential activation of ERK1/2 MAP Kinases in p21Ras^{C118S} and p21Ras^{Wt} transfected THP-1 cells



Taujita et al. (2007) Manuscript to be submitted

Nitric oxide activates JNK in parental cells





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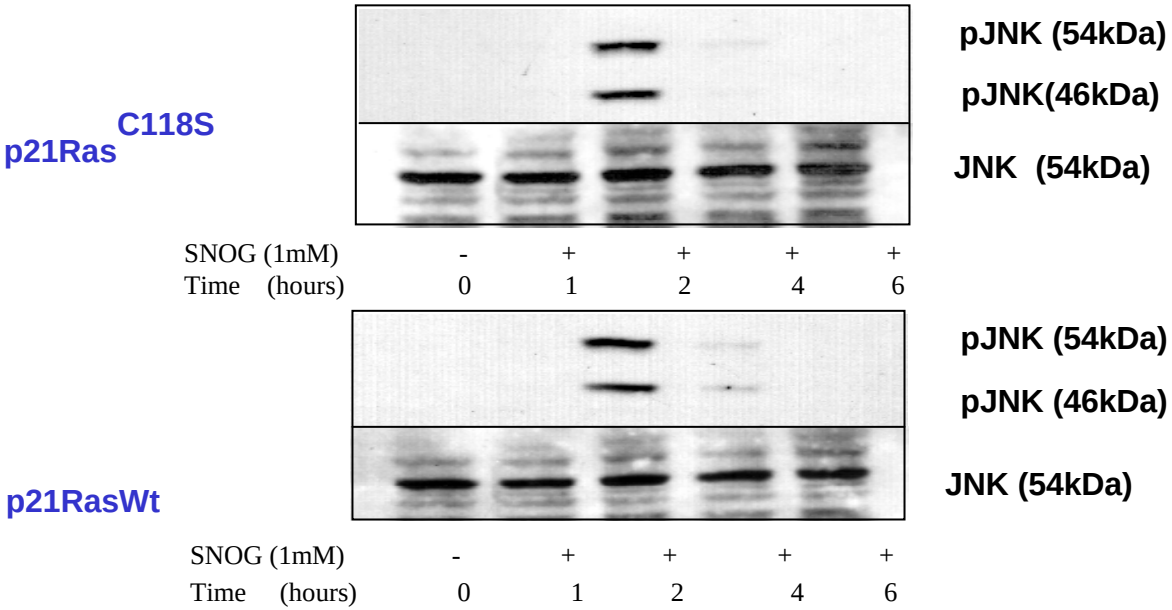
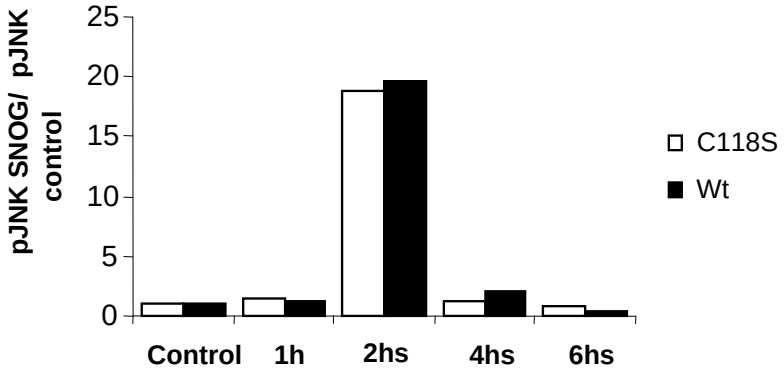
Differential activation of JNK in p21Ras^{C118S} and p21Ras^{Wt} transfected THP-1 cells





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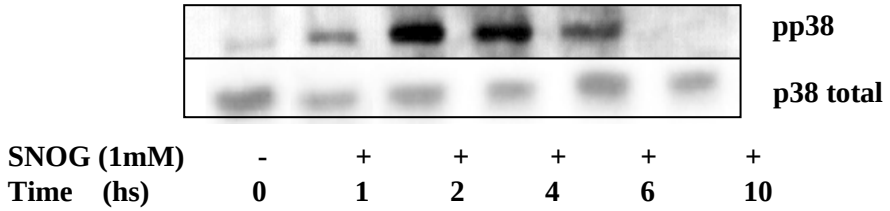
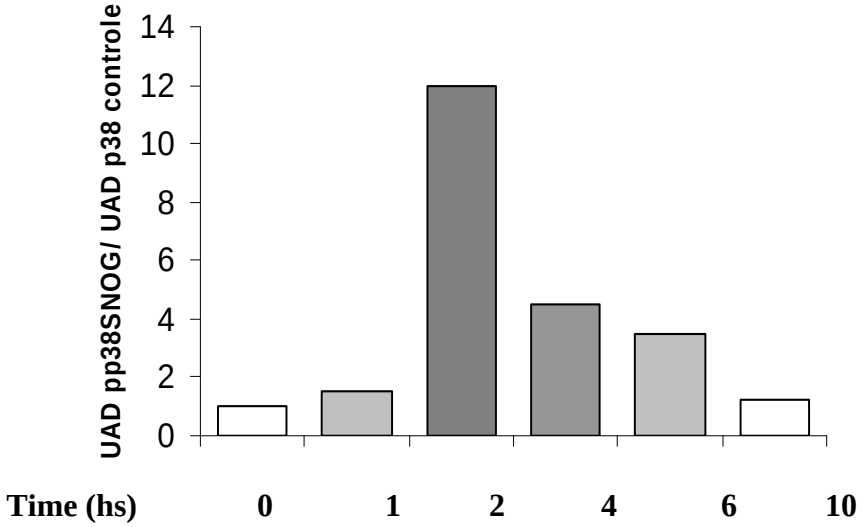
Activation of p38 in THP1 parental cells

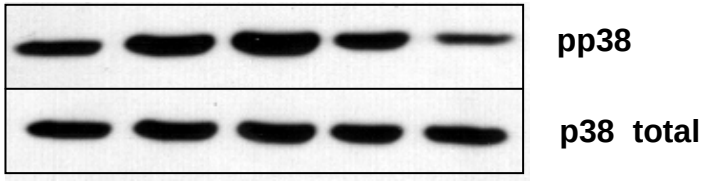




Taujita et al. (2005/2006) Unpublished observations

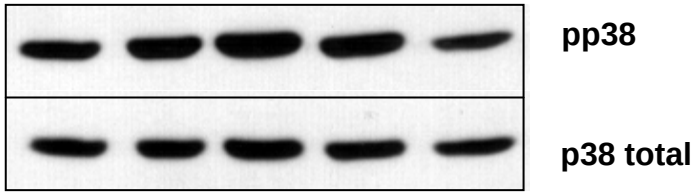
Differential activation of p38 MAP Kinase in p21Ras^{C118S} and p21Ras^{Wt} transfected THP-1 cells

p21Ras^{C118S}

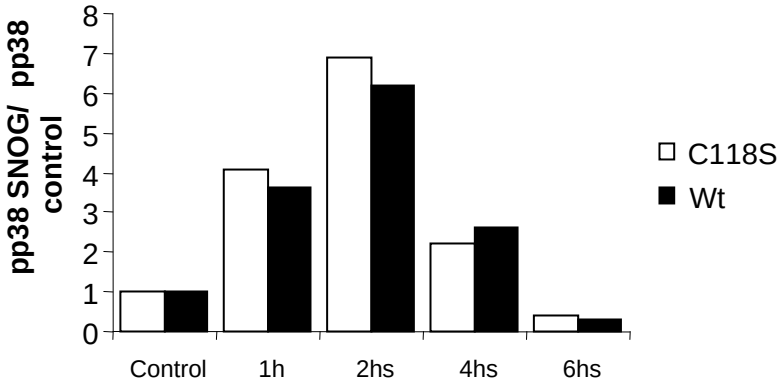


SNOG (1mM) - + + + +
Time (hours) 0 1 2 4 6

p21RasWt



SNOG (1mM) - + + + +
Time (hours) 0 1 2 4 6



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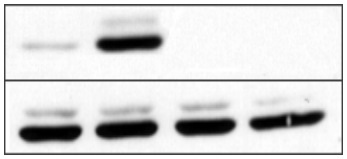
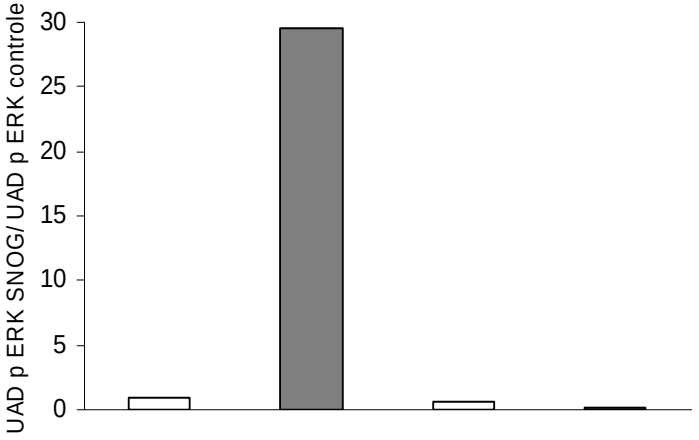
PD98059 inhibits NO-mediated activation of ERK1/2



pERK1/2 (44/42 kDa)

ERK1/2 (44/42kDa)

PD098059 (50µM)	-	-	+	+
SNOG 1mM	-	+	-	+



PD098059 (50µM)	-	-	+	+
SNOG (1mM)	-	+	-	+

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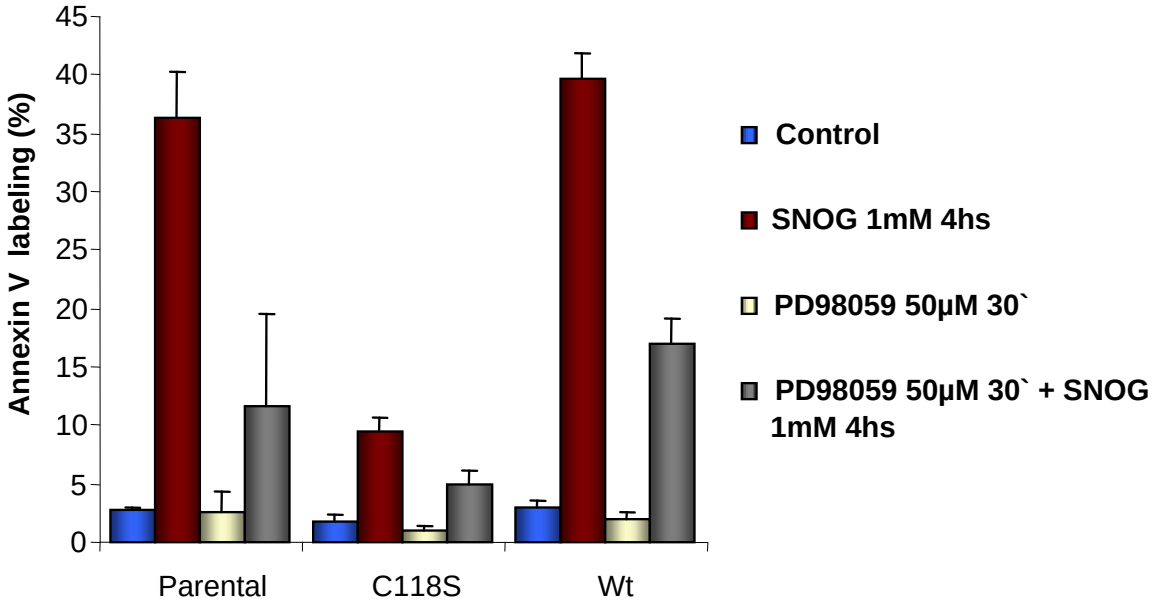
NO-induced apoptosis depends on the activation of ERK1/2



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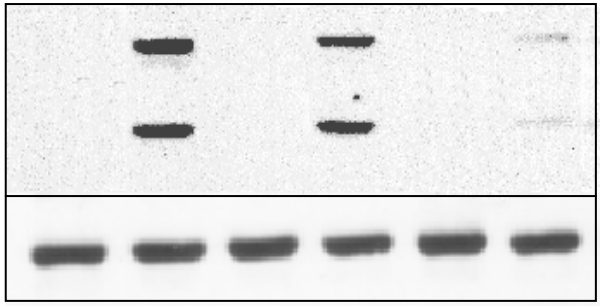
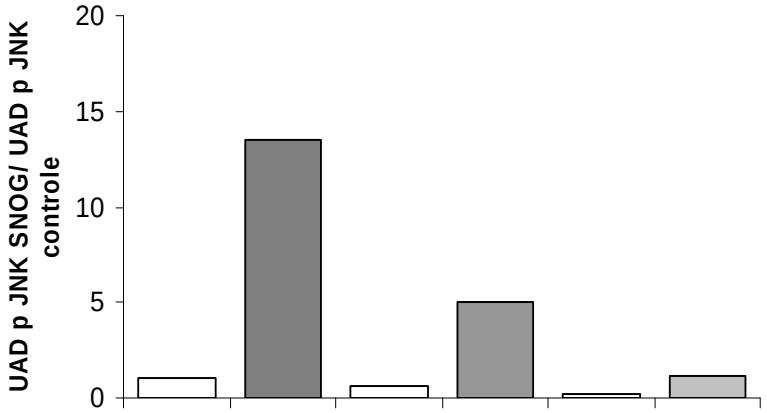
CEP inhibits the NO-mediated activation of JNK



CEP 11004 (nM)	-	-	100	100	300	300
SNOG 1mM	-	+	-	+	-	+



CEP 11004 (nM)	-	-	100	100	300	300
SNOG 1mM	-	+	-	+	-	+

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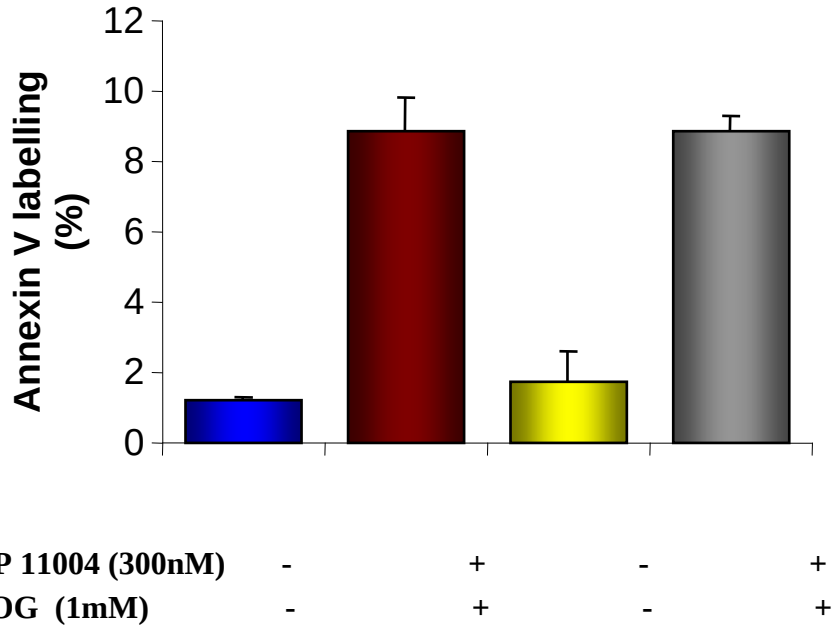
NO-induced apoptosis is not dependent on the activation of JNK



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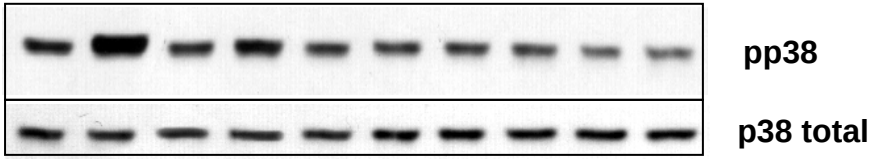
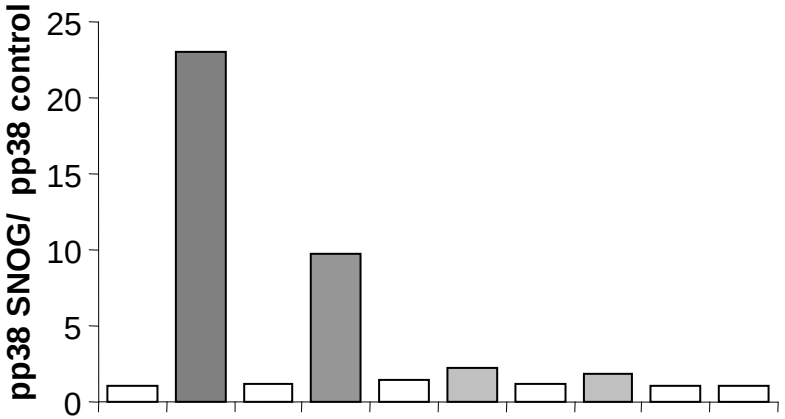
SB220025 inhibits NO-mediated activation of p38 MAP Kinase



SB220025 (μM)	-	-	0.25	0.25	0.5	0.5	1	1	2	2
SNOG (1mM)	-	+	-	+	-	+	-	+	-	+



SB220025 (μM)	-	-	0.25	0.25	0.5	0.5	1	1	2	2
SNOG (1mM)	-	+	-	+	-	+	-	+	-	+

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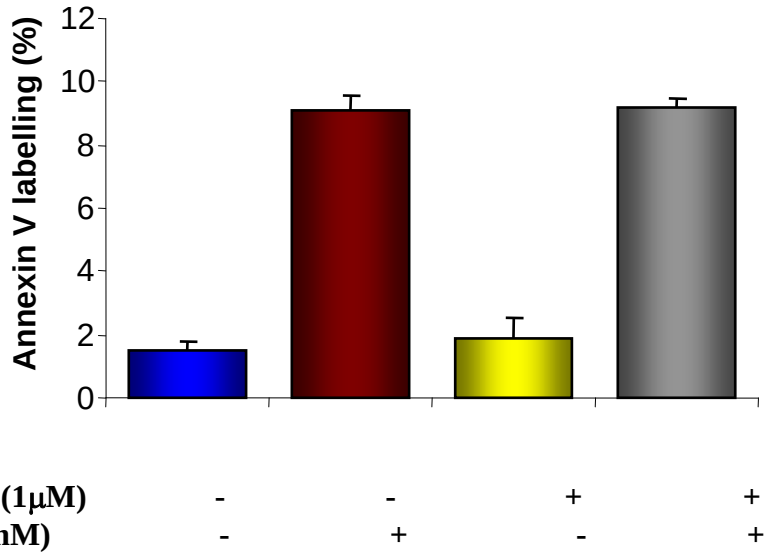
NO-induced apoptosis is not dependent on the activation of p38 MAP Kinase



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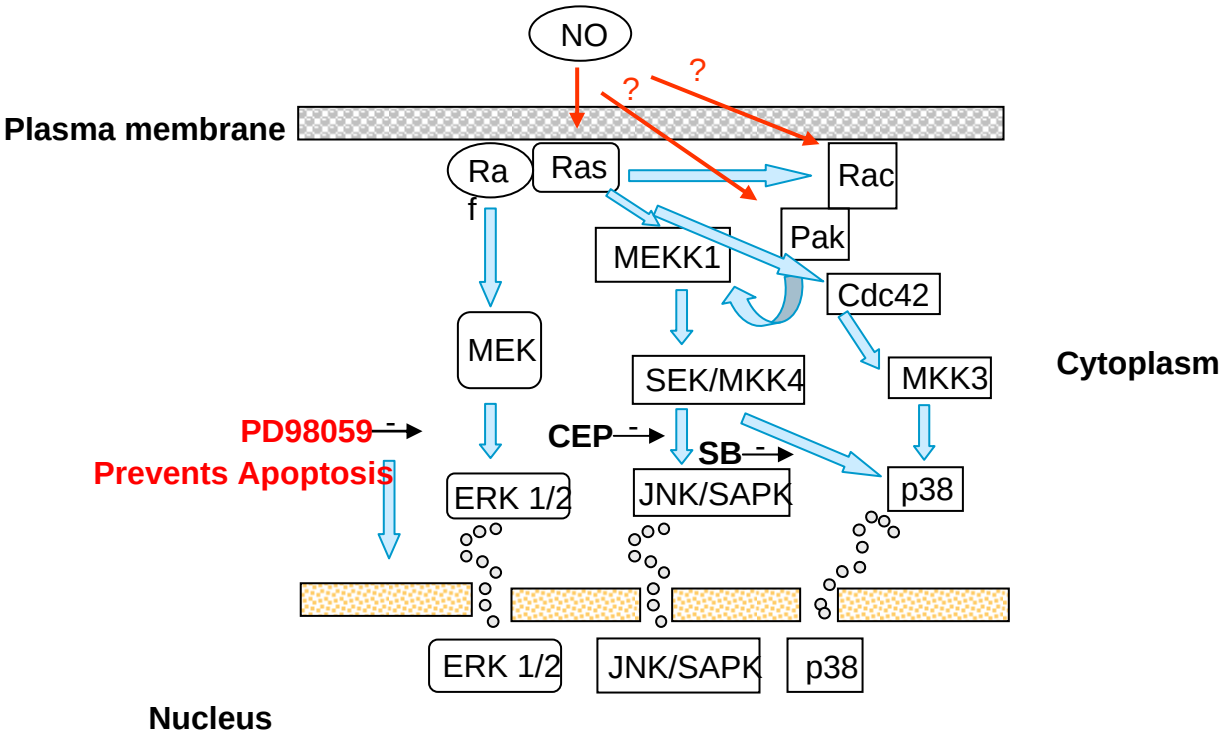
SUMMARY



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