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IBMC symposium
Rho GTPases and cell signalling
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Rac1 signalling modulates transcriptional regulation by BCL-6 and STAT5

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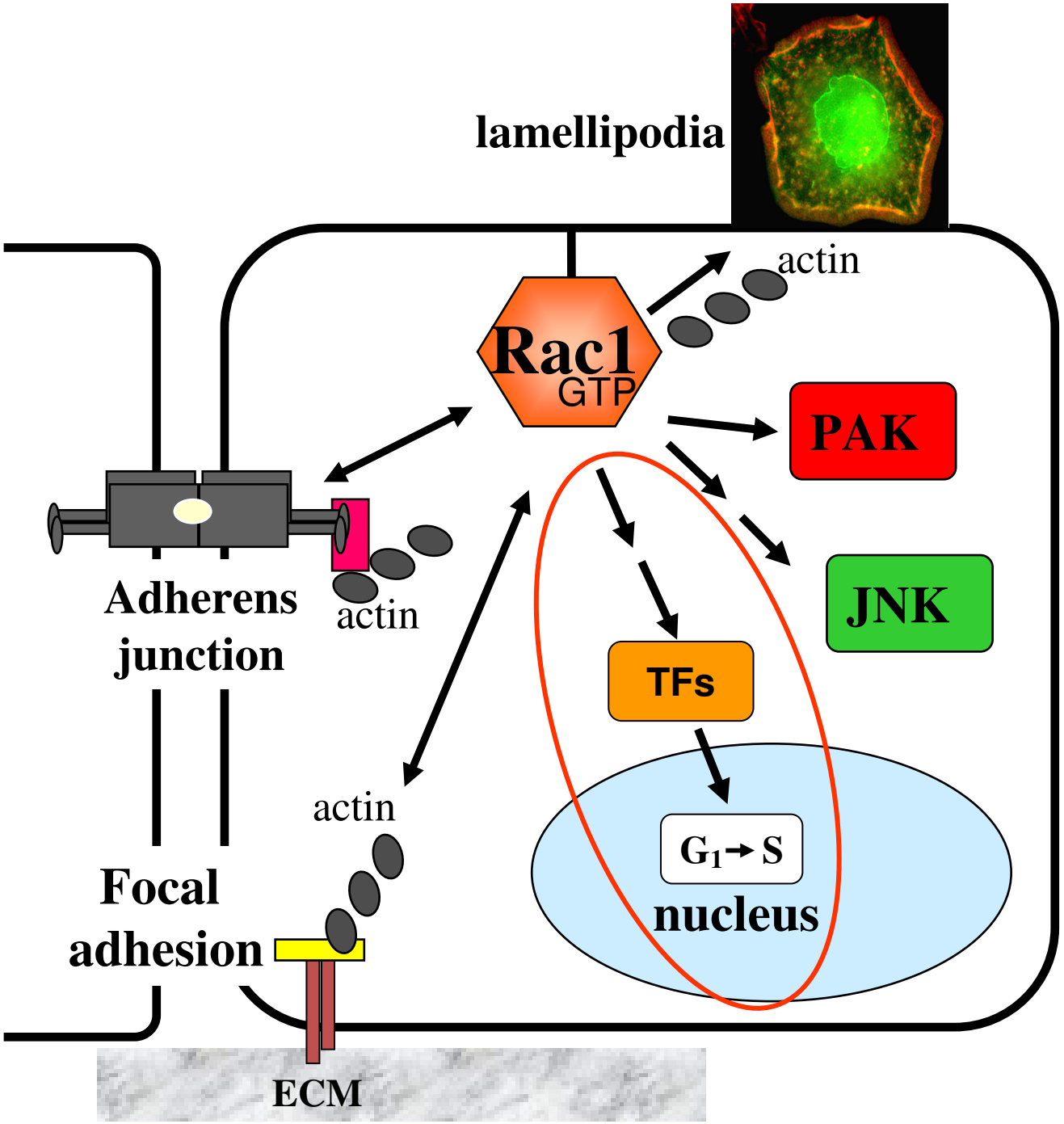
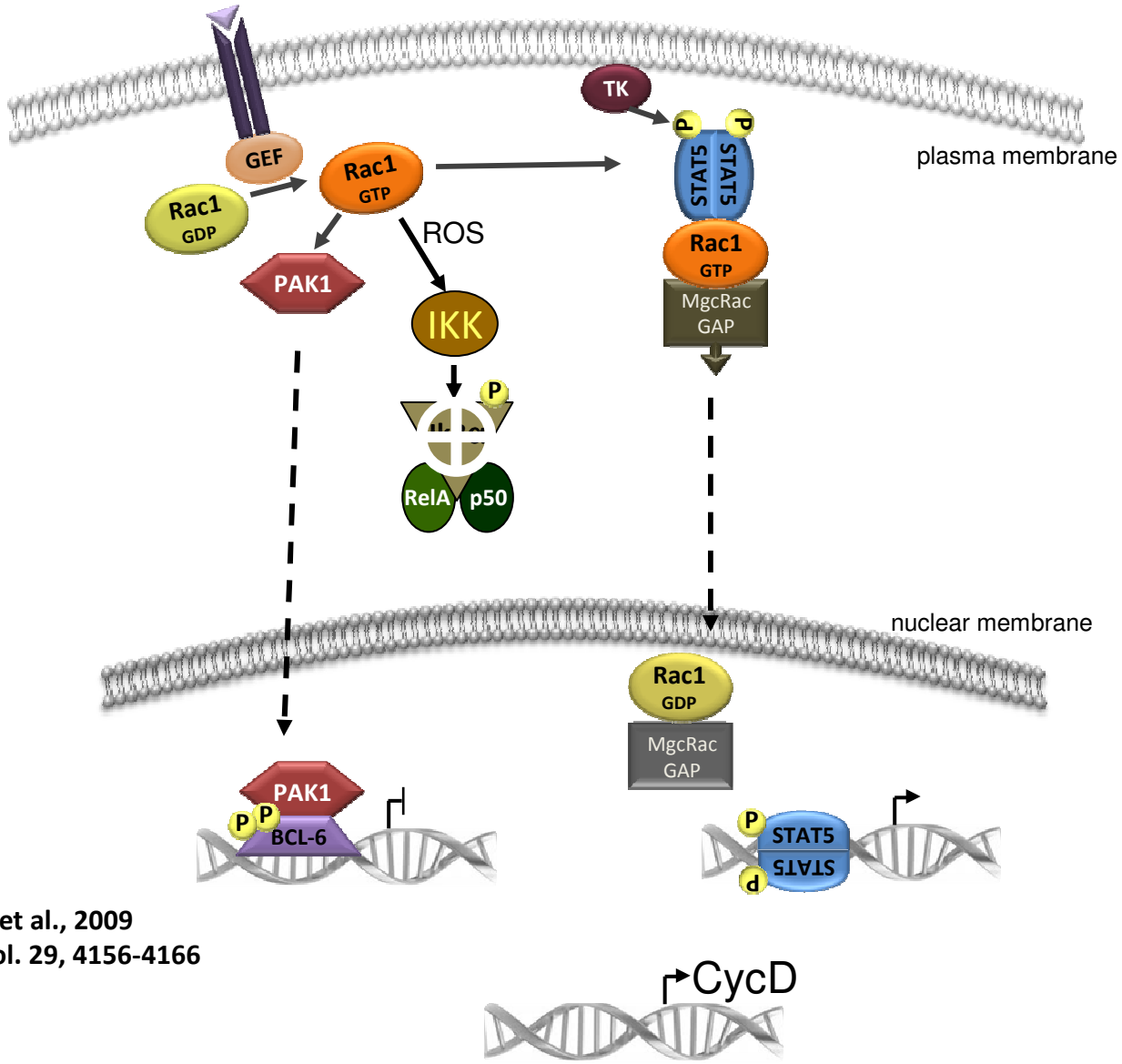


Rac1 signaling

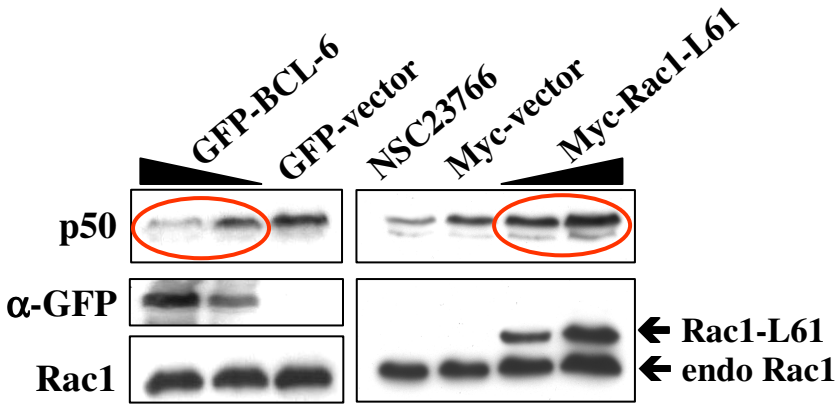
-adhesion
-motility
-gene expression/
proliferation

Rac1 signalling regulates gene transcription

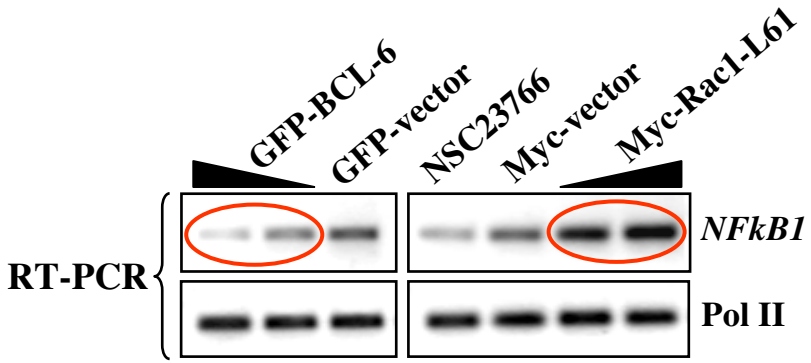


Barros et al., 2009
Mol. Cell. Biol. 29, 4156-4166

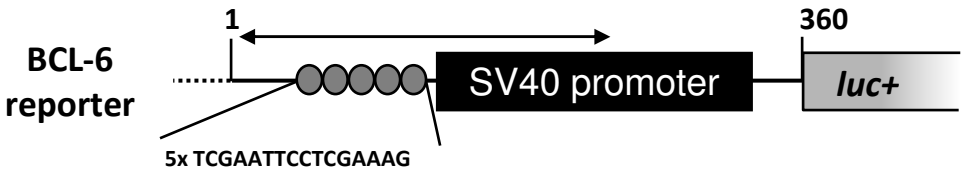
NFkB1/p50 expression is repressed by BCL-6

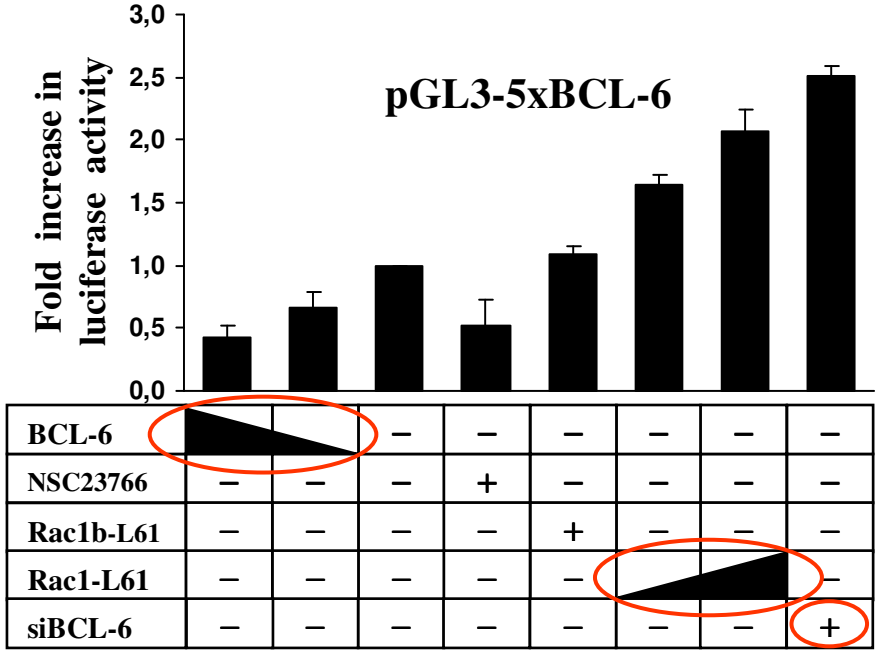


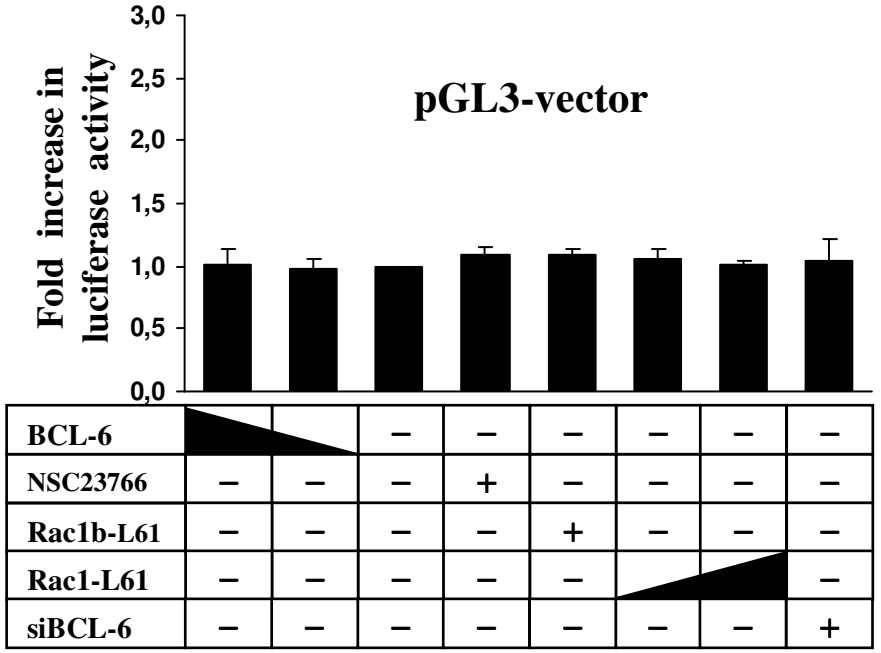
NFKB1/p50 protein

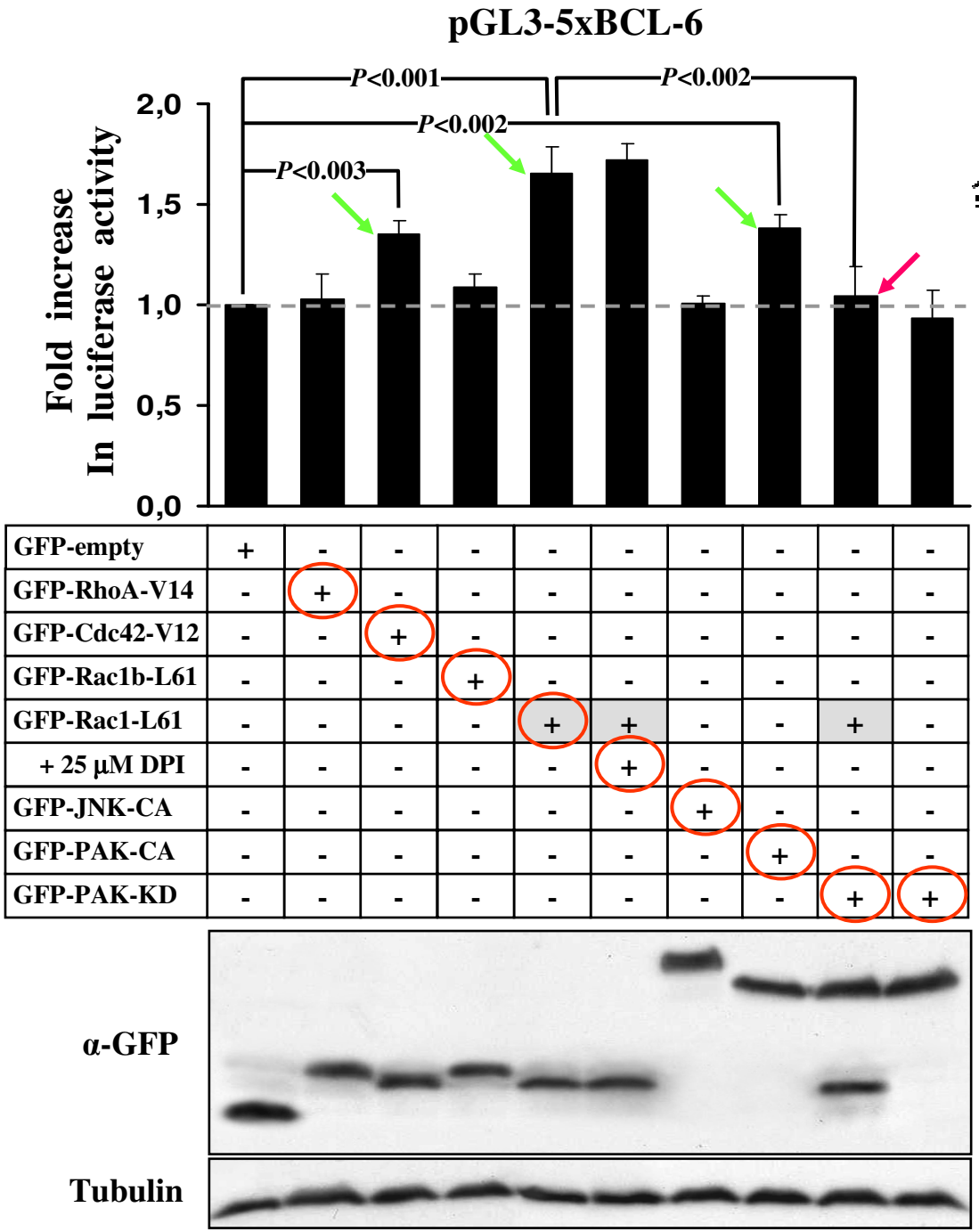


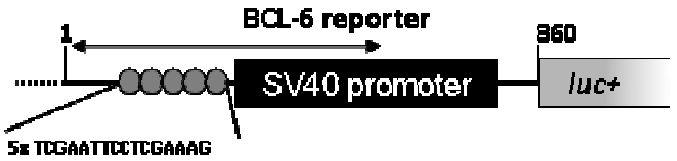
NFKB1 transcript


BCL-6
reporter
1
360
SV40 promoter
luc+
5x TCGAATTCCTCGAAAG


pGL3-5xBCL-6
Fold increase in luciferase activity
3,0
2,5
2,0
1,5
1,0
0,5
0,0
BCL-6 – – – – – – – –
NSC23766 – – – + – – – –
Rac1b-L61 – – – – + – – –
Rac1-L61 – – – – – – – –
siBCL-6 – – – – – – – +


pGL3-vector
Fold increase in luciferase activity
3,0
2,5
2,0
1,5
1,0
0,5
0,0
BCL-6 – – – – – – – –
NSC23766 – – – + – – – –
Rac1b-L61 – – – – + – – –
Rac1-L61 – – – – – – – –
siBCL-6 – – – – – – – +

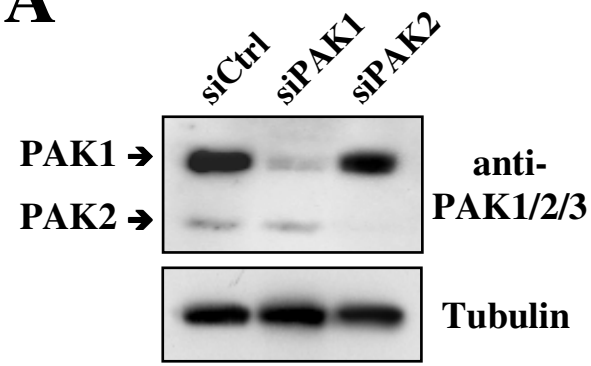




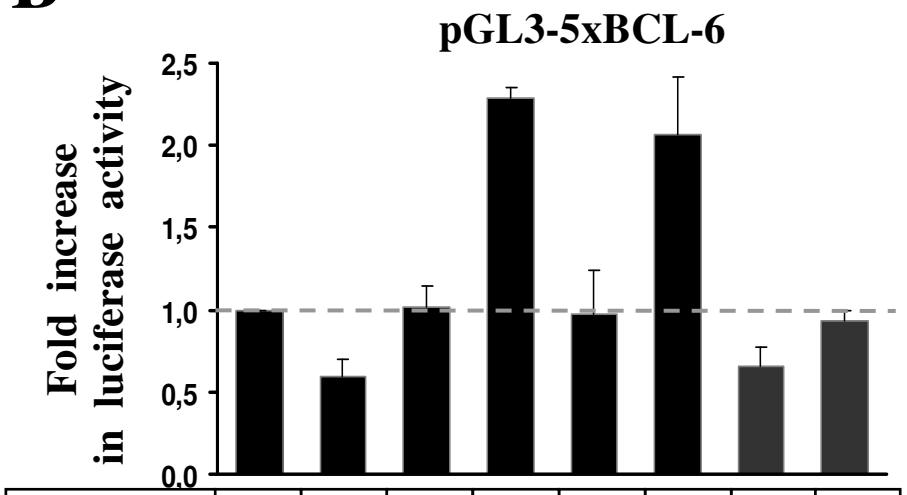
The mechanism downstream of Rac1

The mechanism downstream of Rac1 relies on PAK1

A



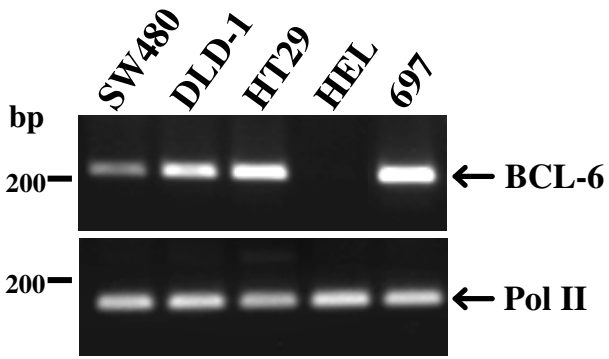
B

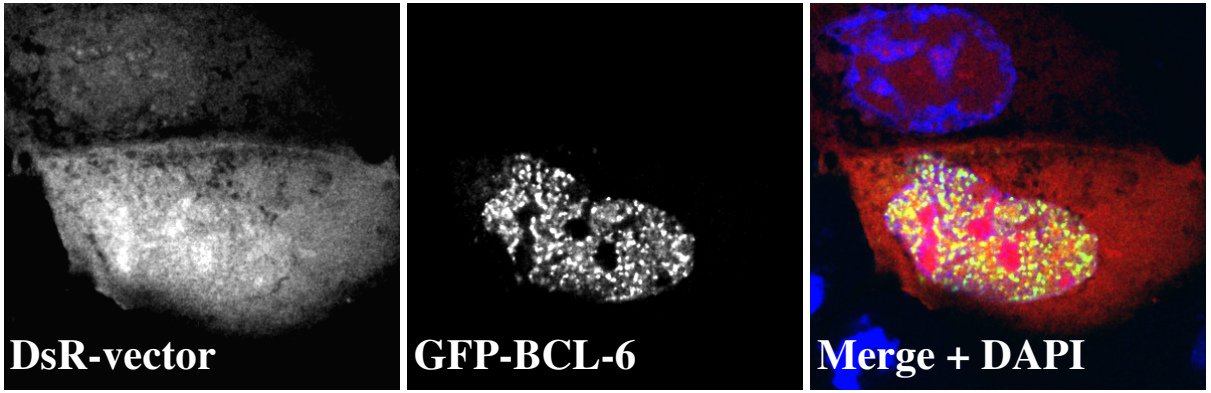


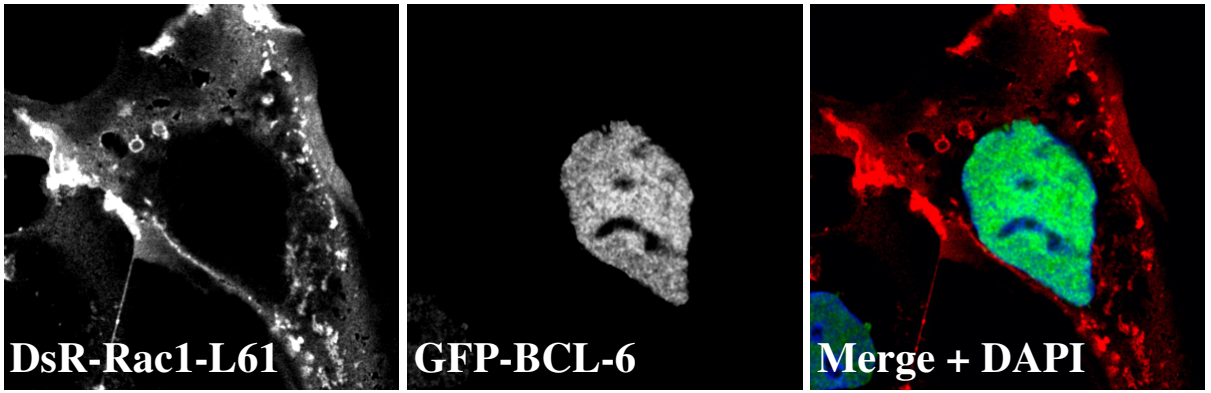
Ctrl	+	-	-	+	-	-	+	-
IPA-3 10µM	-	-	-	-	-	-	+	+
siPAK1	-	+	-	-	+	-	-	-
siPAK2	-	-	+	-	-	+	-	-
Rac1-L61	-	-	-	+	+	+	-	+

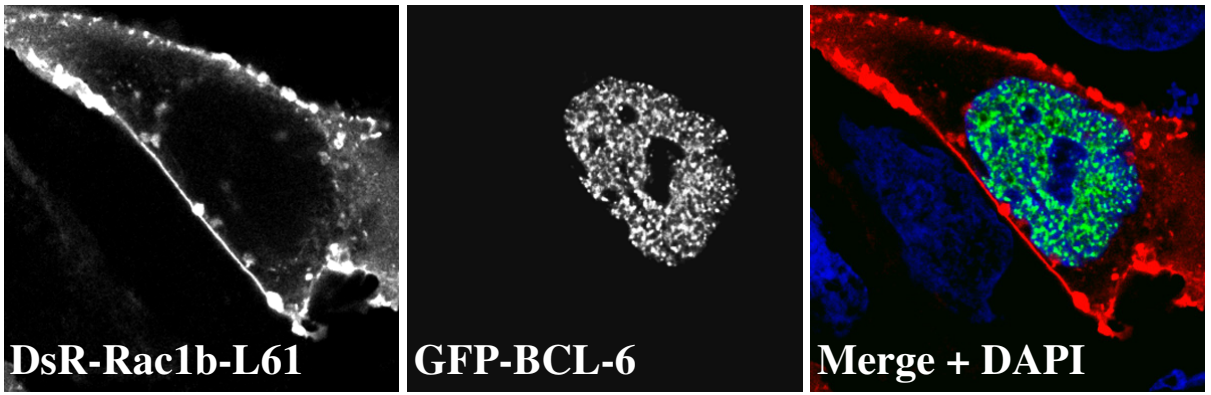
BCL-6 in colorectal cells

BCL= B-Cell lymphoma

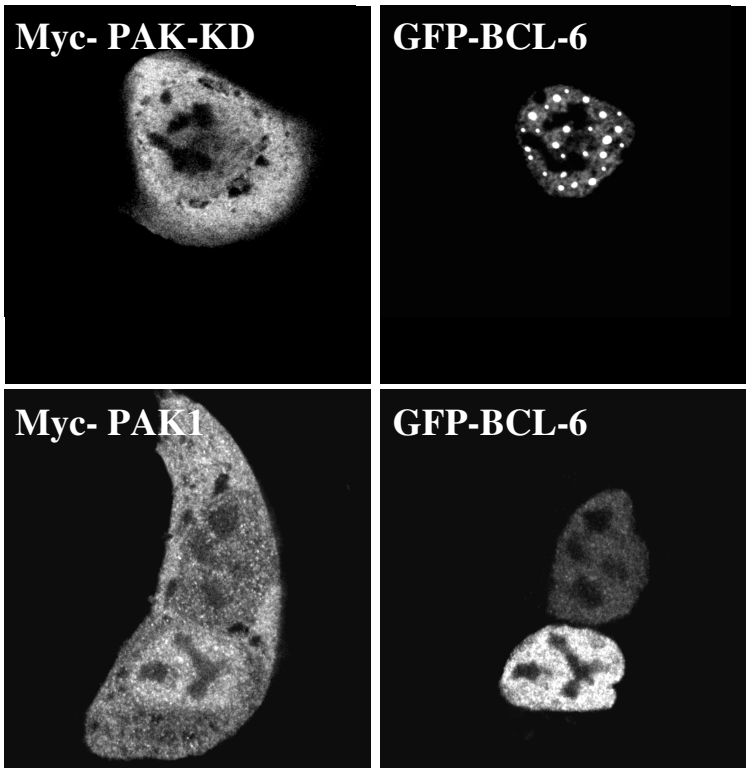




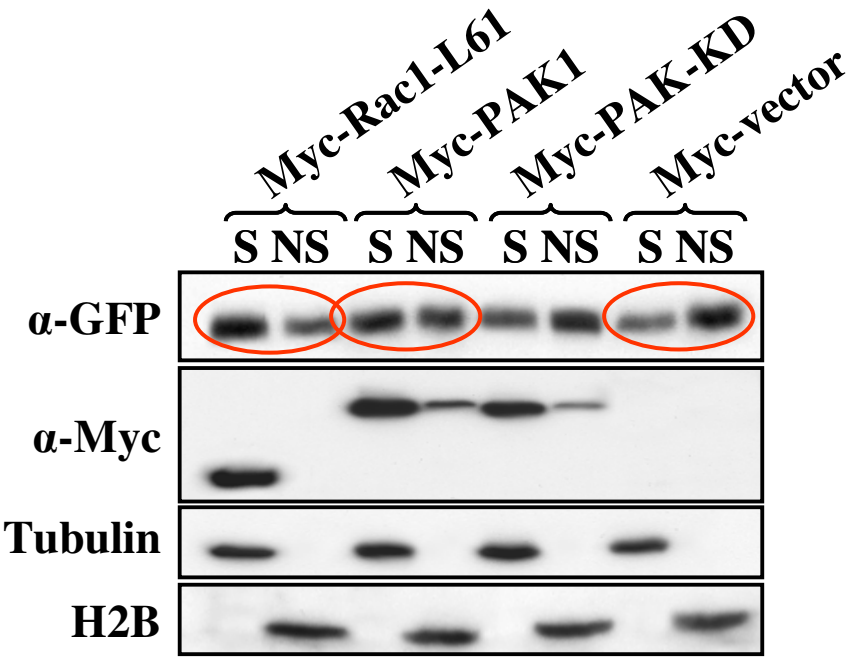




Effect of PAK1 on BCL-6 in colorectal cells

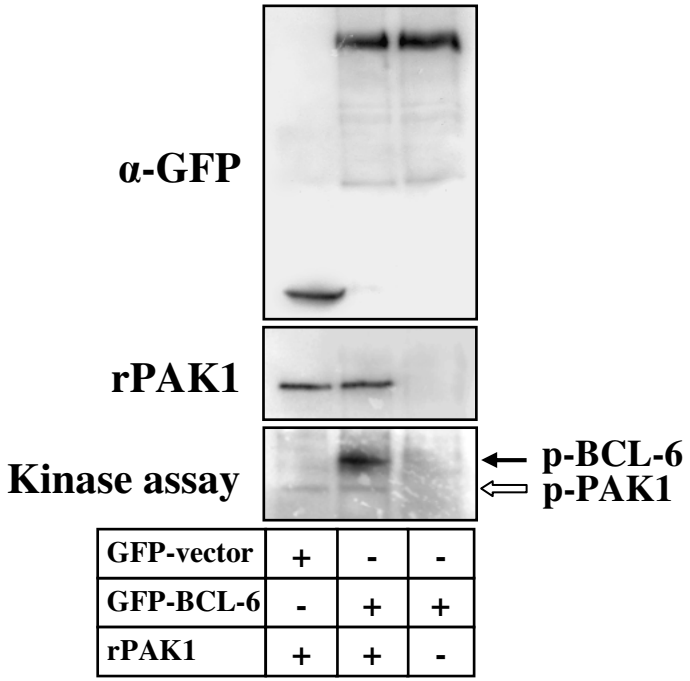


subcellular fractionation

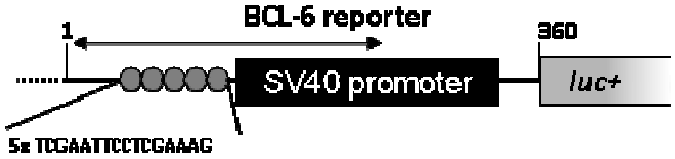


S= soluble
NS= non-soluble, chromatin-bound

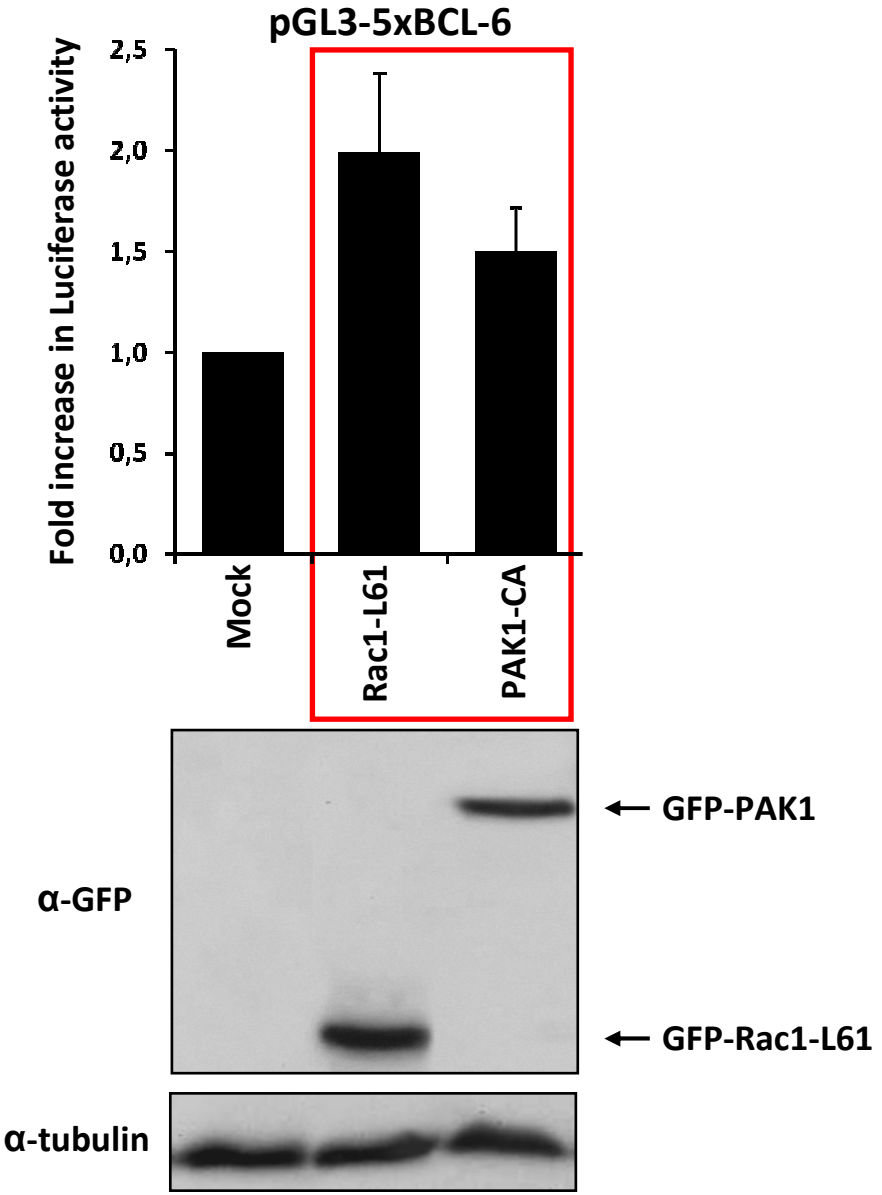
PAK1 phosphorylates BCL-6 *in vitro*



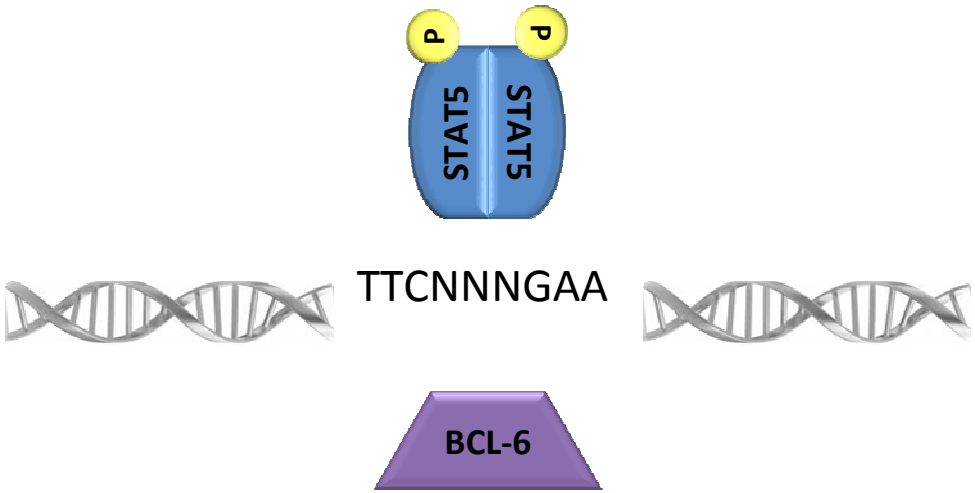
→ Rac1/PAK1 signalling promotes the release of repressor BCL-6 from chromatin.



.. Rac makes a difference



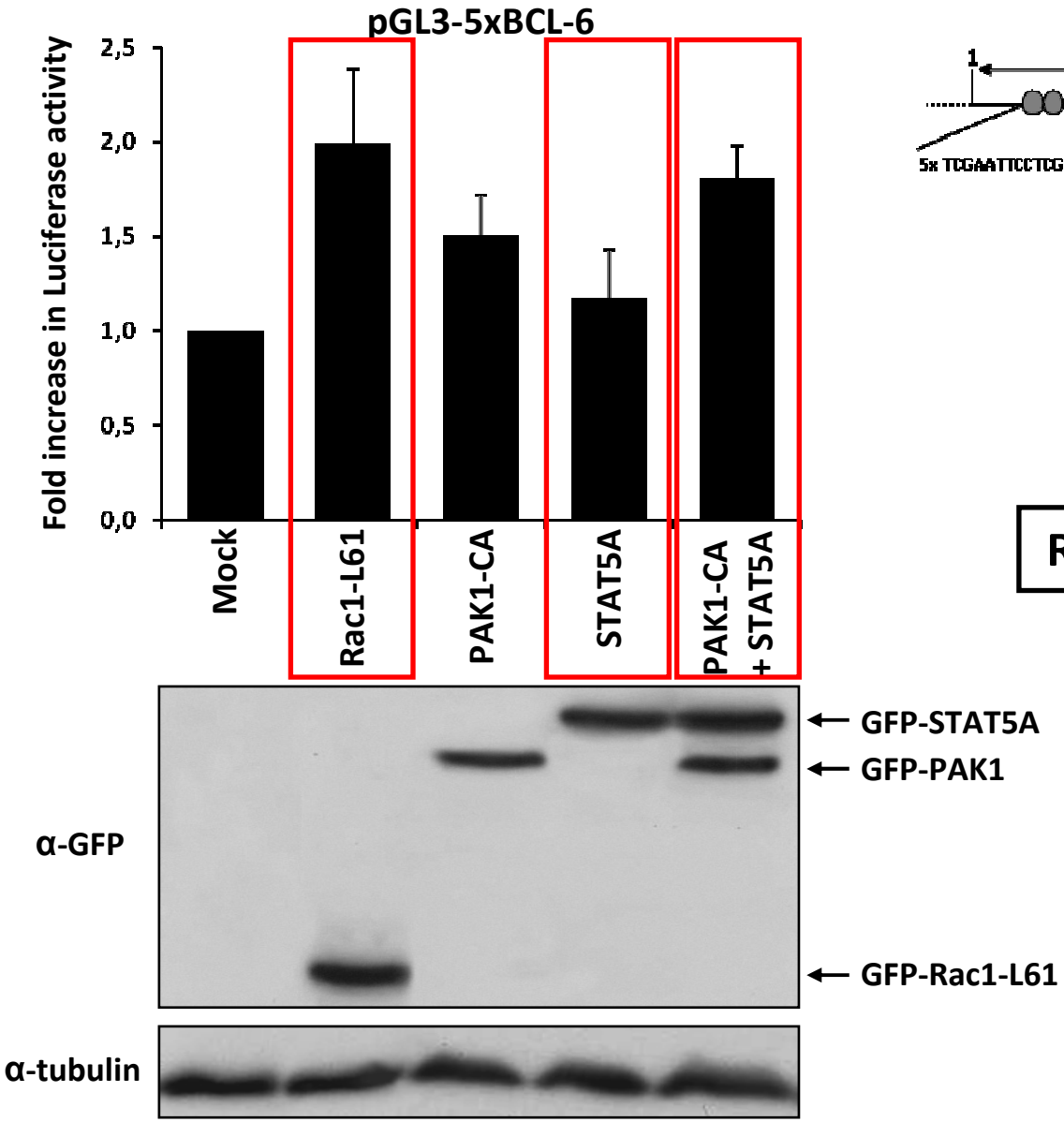
BCL-6 and STAT5 recognise a similar core binding motif

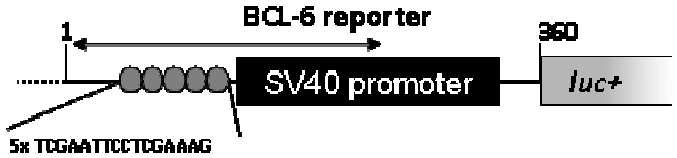


Genes reported to be regulated by both factors:

cyclin D2, blimp-1, BCL-XL, Socs2, prolactin

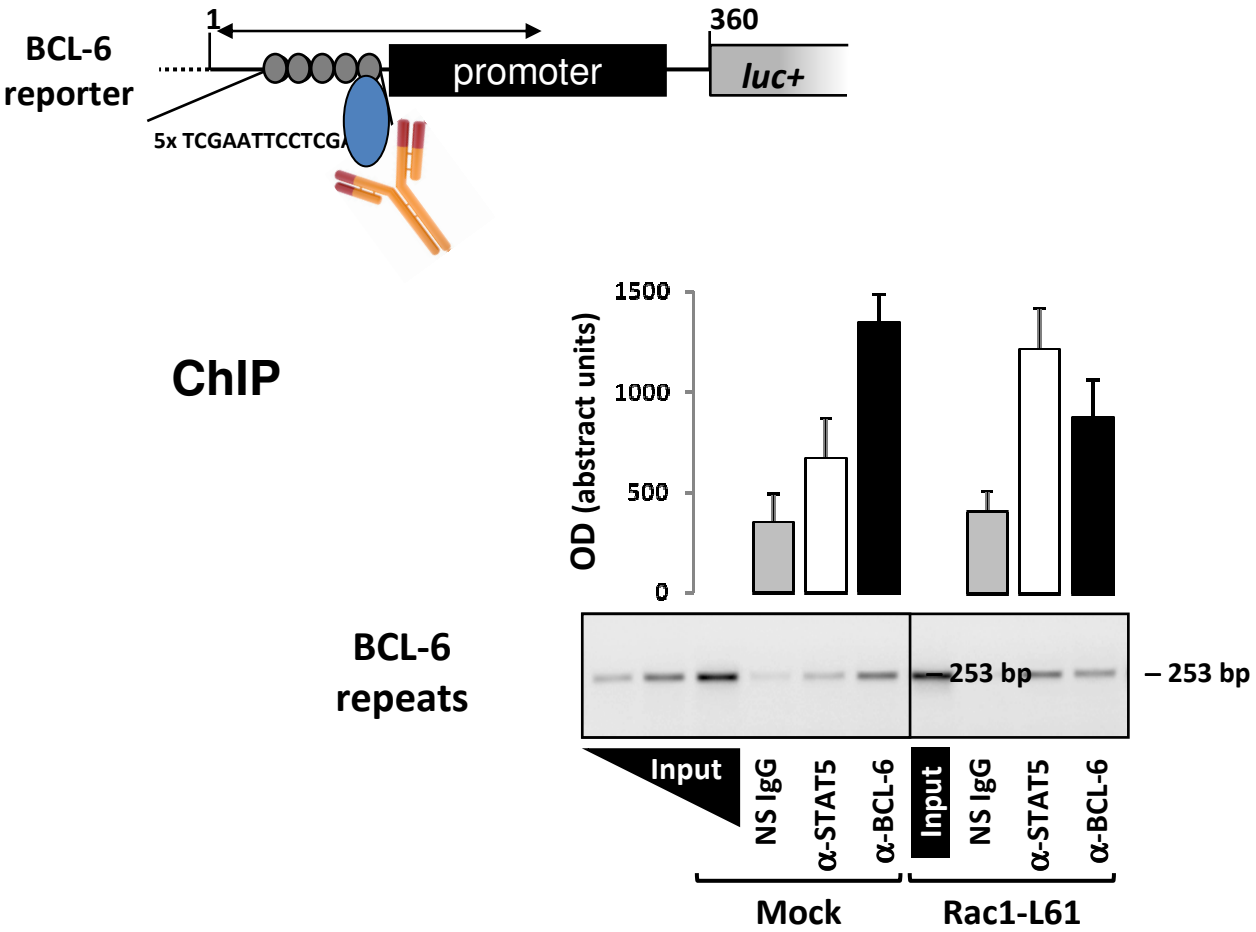
Does Rac1 signalling coordinate the activity of BCL-6 and STAT5?





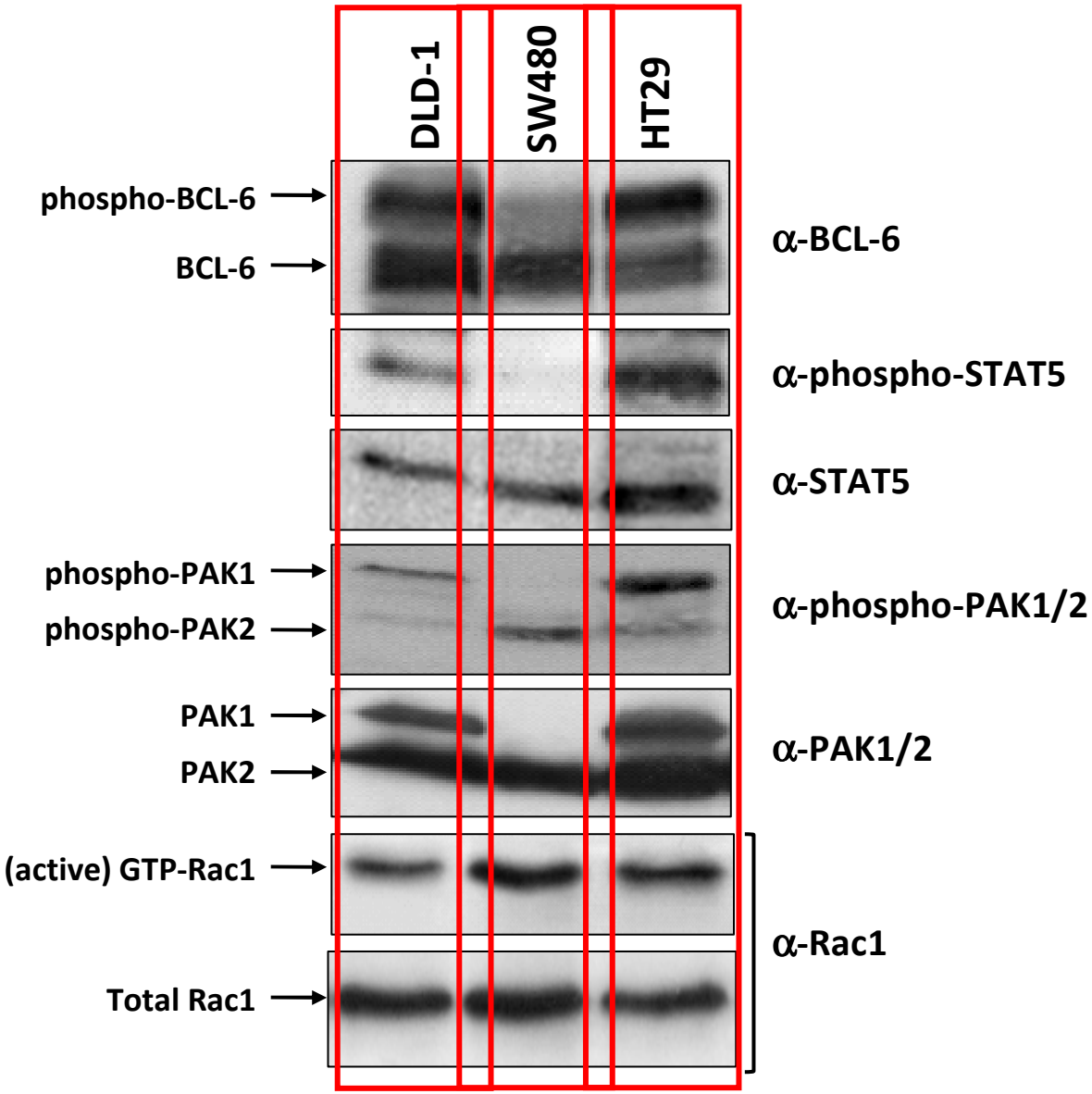
Rac1 = PAK1+STAT5

Does Rac1 signalling affect promoter binding?

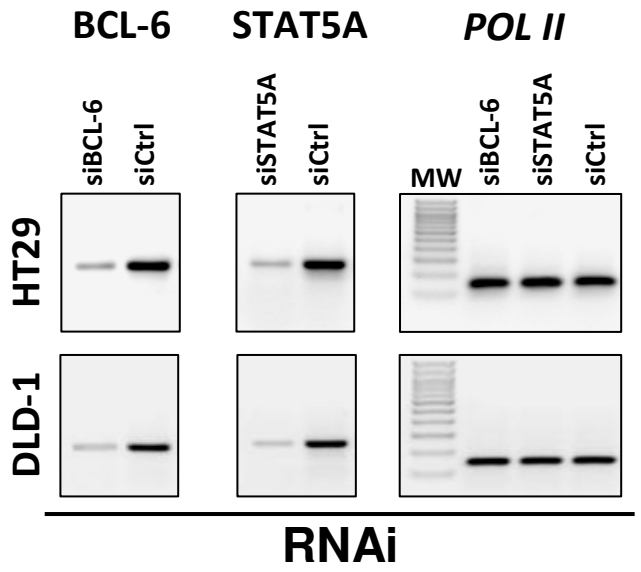


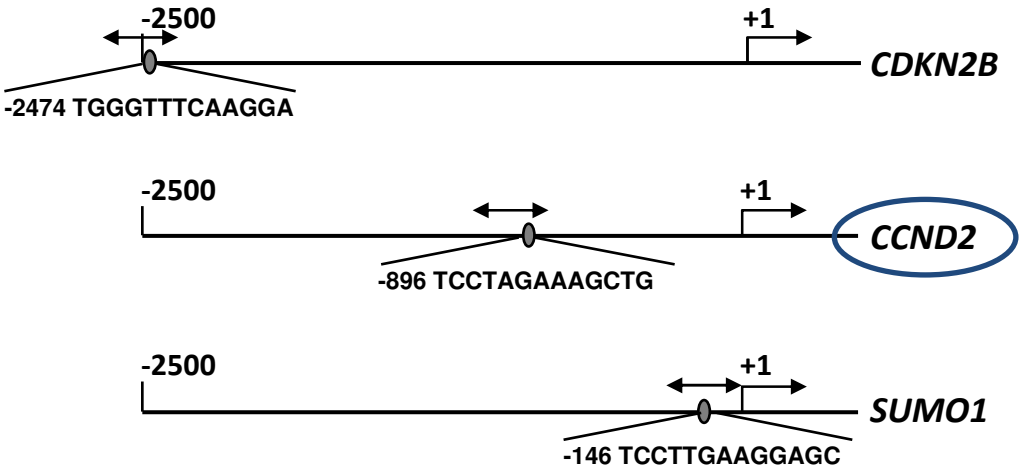
Active Rac1 promotes a switch from BCL-6 to STAT5 at the promoter.

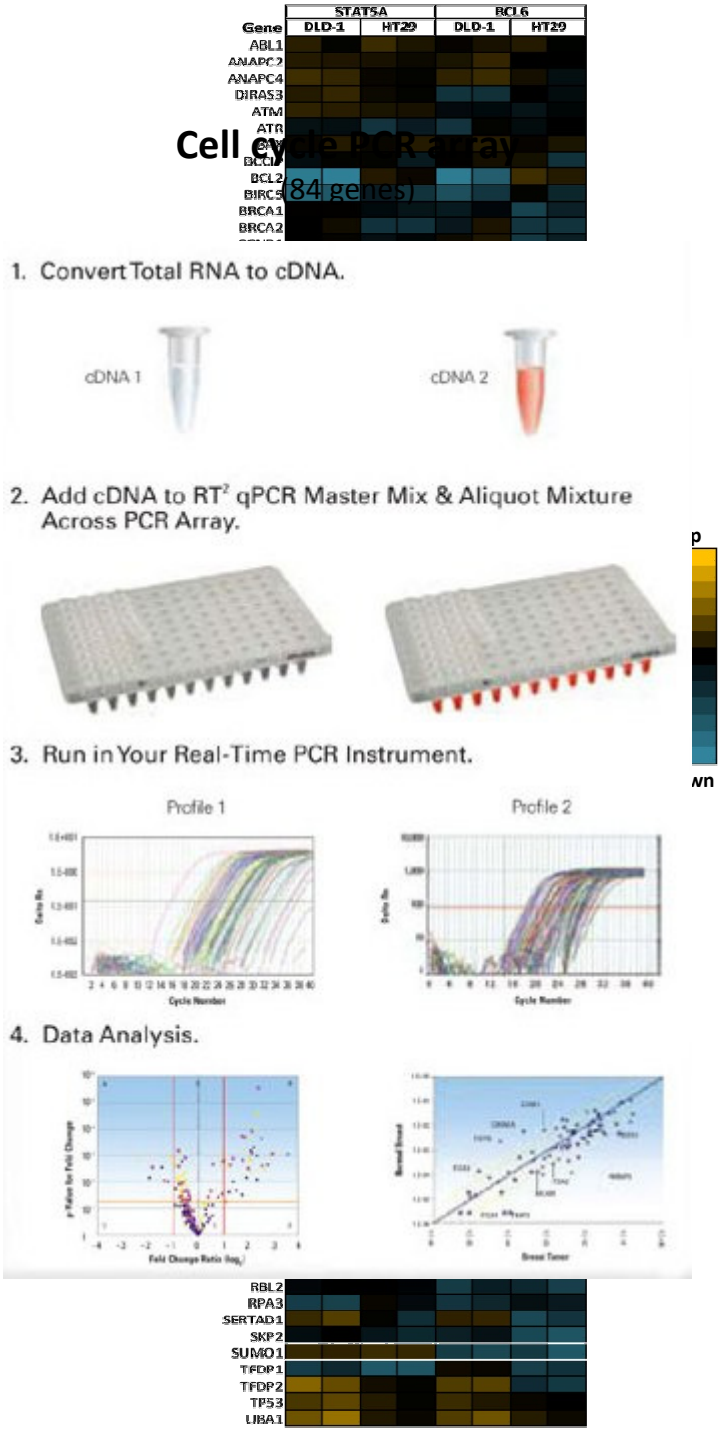
Does BCL-6/STAT5 transcription switching happen in cells?


DLD-1
SW480
HT29
phospho-BCL-6
BCL-6
α-BCL-6
α-phospho-STAT5
α-STAT5
phospho-PAK1
phospho-PAK2
α-phospho-PAK1/2
PAK1
PAK2
α-PAK1/2
(active) GTP-Rac1
Total Rac1
α-Rac1

Which endogenous genes might be targets of the transcription factor switch?

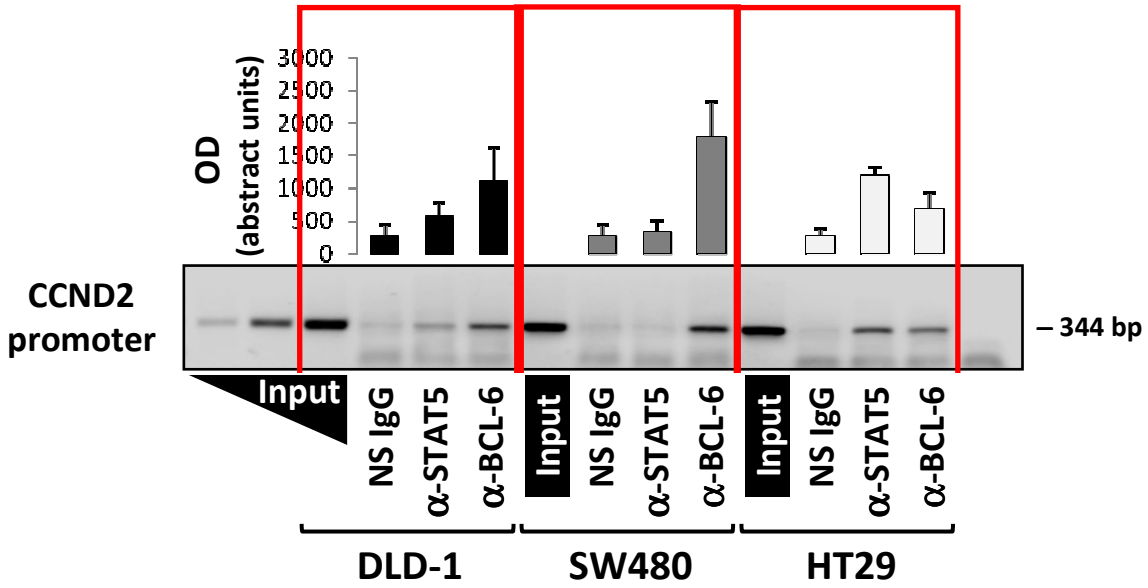

BCL-6
STAT5A
POL II
siBCL-6
siCtrl
siSTAT5A
siCtrl
MW
siBCL-6
siSTAT5A
siCtrl
HT29
DLD-1
RNAi


-2500
+1
CDKN2B
-2474 TGGGTTTCAAGGA
-2500
+1
CCND2
-896 TCCTAGAAAGCTG
-2500
+1
SUMO1
-146 TCCTTGAAGGAGC


STAT5A
BCL6
Gene
DLD-1
HT29
DLD-1
HT29
ABL1
ANAPC2
ANAPC4
DIRAS3
ATM
ATR
BCL2
BIRC5
BRCA1
BRCA2
Cell cycle PCR array
(84 genes)
1. Convert Total RNA to cDNA.
cDNA 1
cDNA 2
2. Add cDNA to RT² qPCR Master Mix & Aliquot Mixture Across PCR Array.
3. Run in Your Real-Time PCR Instrument.
Profile 1
Profile 2
4. Data Analysis.
RBL2
RPA3
SERTAD1
SKP2
SUMO1
TFDP1
TFDP2
TP53
UBA1

‘Steady state’ promoter occupancies in colorectal cell lines

ChIP



SW480

+++ Active Rac1
0 Active PAK1
+ Active STAT5
+ Active BCL-6

HT29

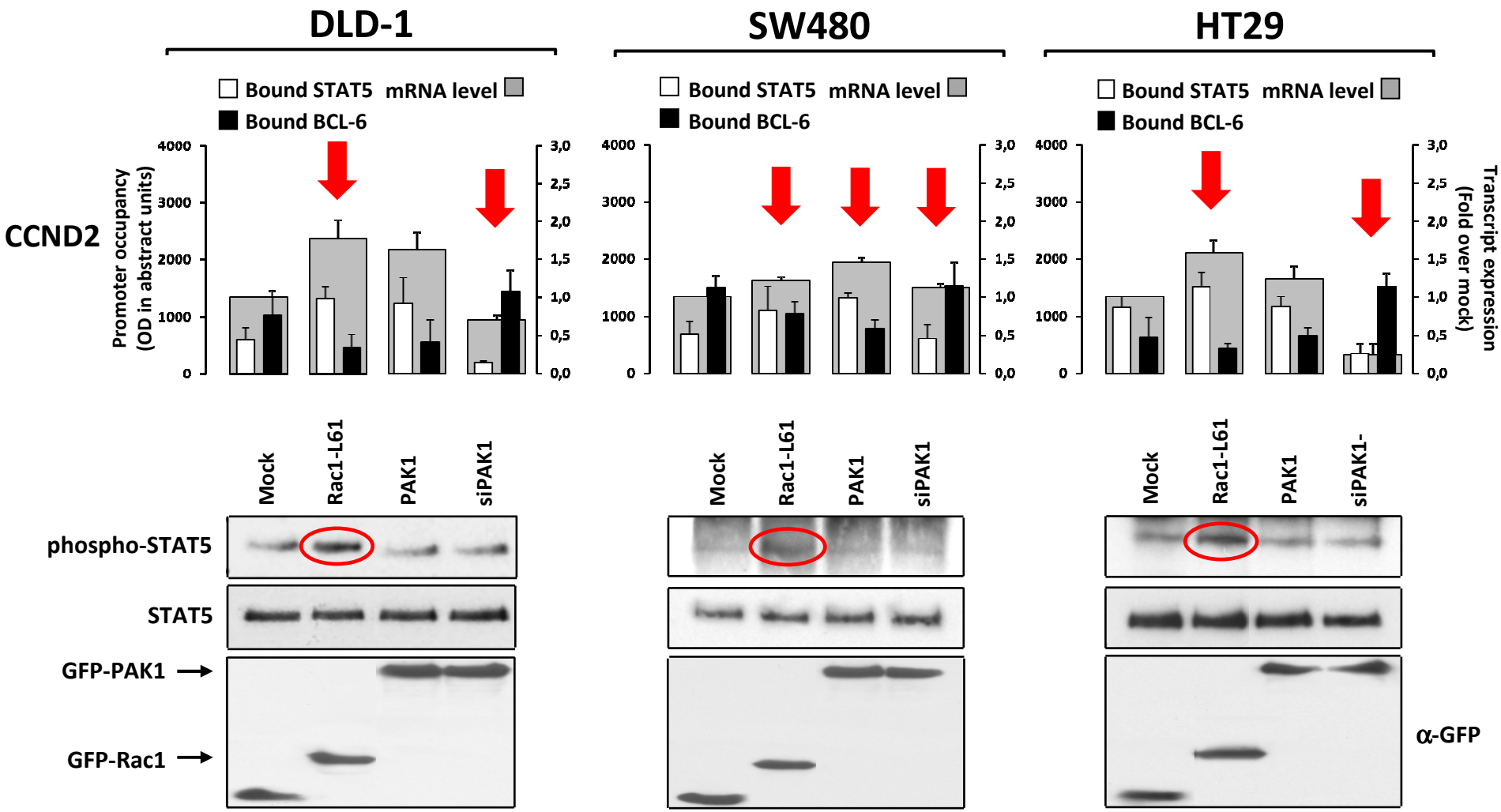
++ Active Rac1
++ Active PAK1
++ Active STAT5
++ Active BCL-6

DLD-1

+ Active Rac1
+ Active PAK1
+ Active STAT5
+ Active BCL-6

Do the observed promoter occupancy rates reflect changes in gene expression?

Do promoter occupancy and gene expression respond to Rac1 signalling?


DLD-1
SW480
HT29
CCND2
Bound STAT5 mRNA level
Bound BCL-6
Promoter occupancy (OD in abstract units)
Transcript expression (Fold over mock)
4000
3000
2000
1000
0
3,0
2,5
2,0
1,5
1,0
0,5
0,0
Mock
Rac1-L61
PAK1
siPAK1
siPAK1-
phospho-STAT5
STAT5
GFP-PAK1
GFP-Rac1
α-GFP

Summary

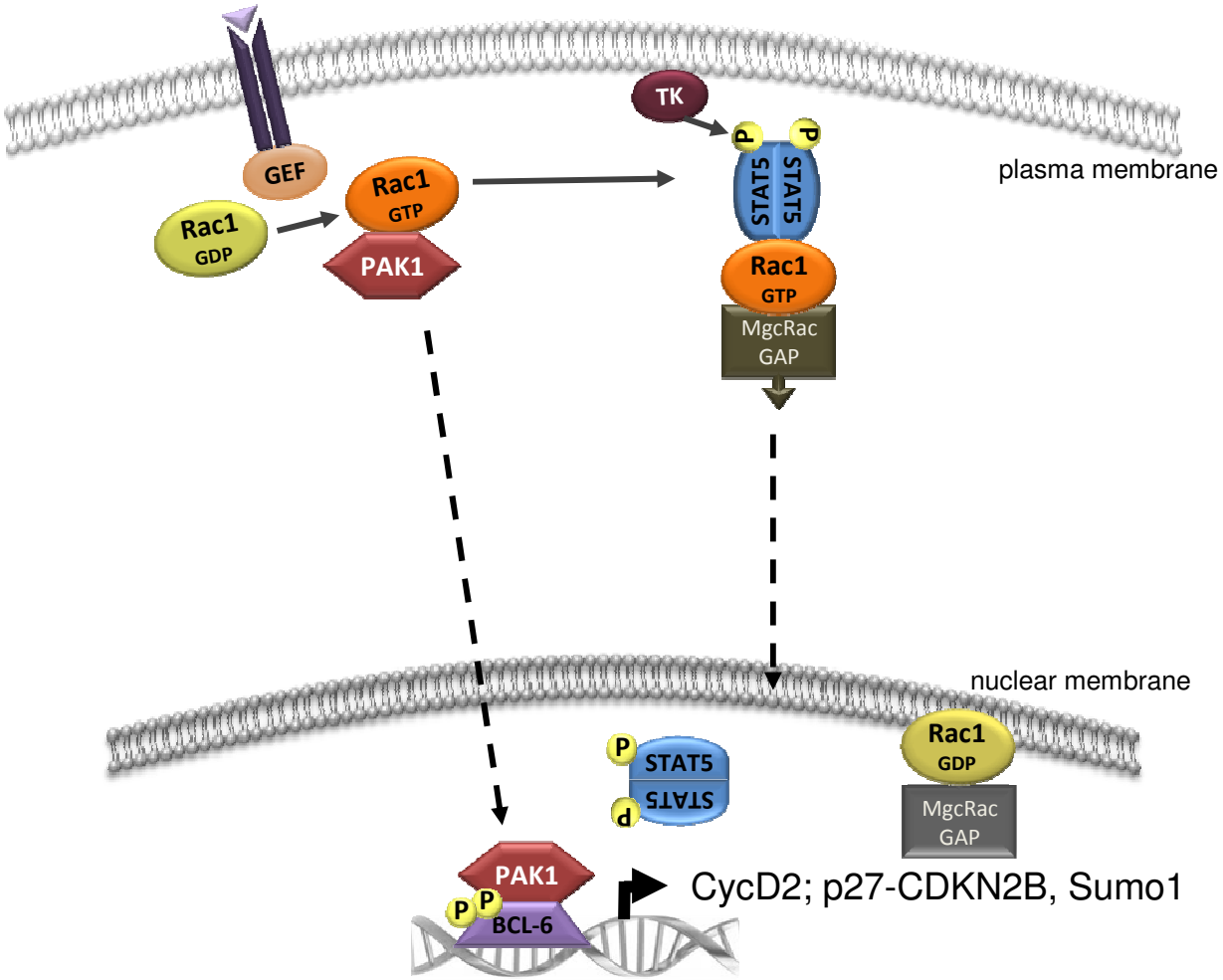
Novel insights into the modulation of gene transcription by Rac1 signalling.

- Rac1/PAK1 signalling promotes the release of repressor BCL-6 from chromatin.

- Rac1 signalling activates gene transcription by inducing a transcriptional switch from repressor BCL-6 to activator STAT5 at gene promoters.

- cyclin D2, CDKN2B and SUMO1 are endogenous genes, which are inversely regulated by BCL-6 and STAT5 in colorectal cells.

Proposed mechanism



Acknowledgements

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Patrícia Barros

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Funding

FCT Fundação para a Ciência e a Tecnologia
MINISTÉRIO DA CIÊNCIA, TECNOLOGIA E ENSINO SUPERIOR

BioFIG Center for Biodiversity, Functional and Integrative Genomics

EMBO STF

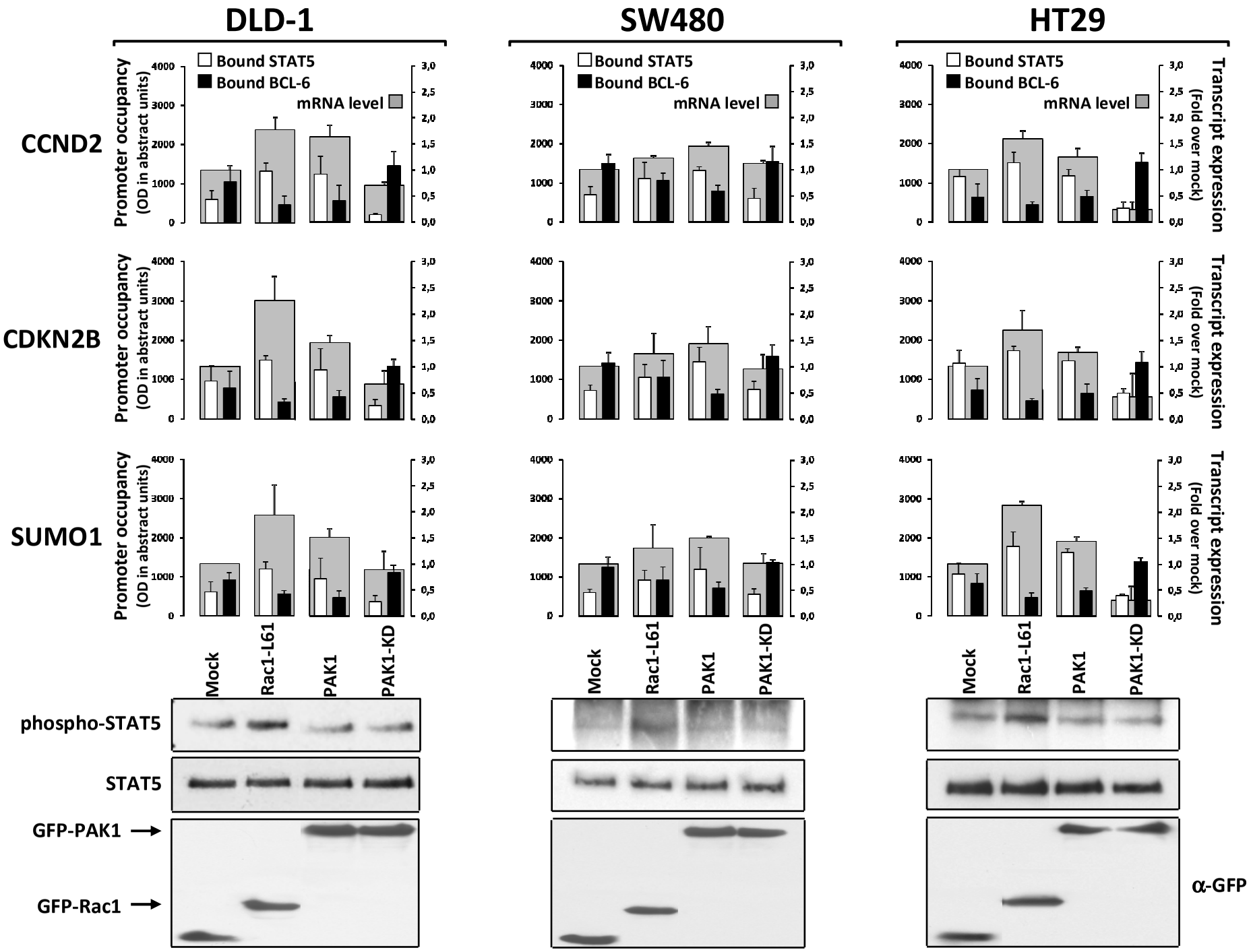
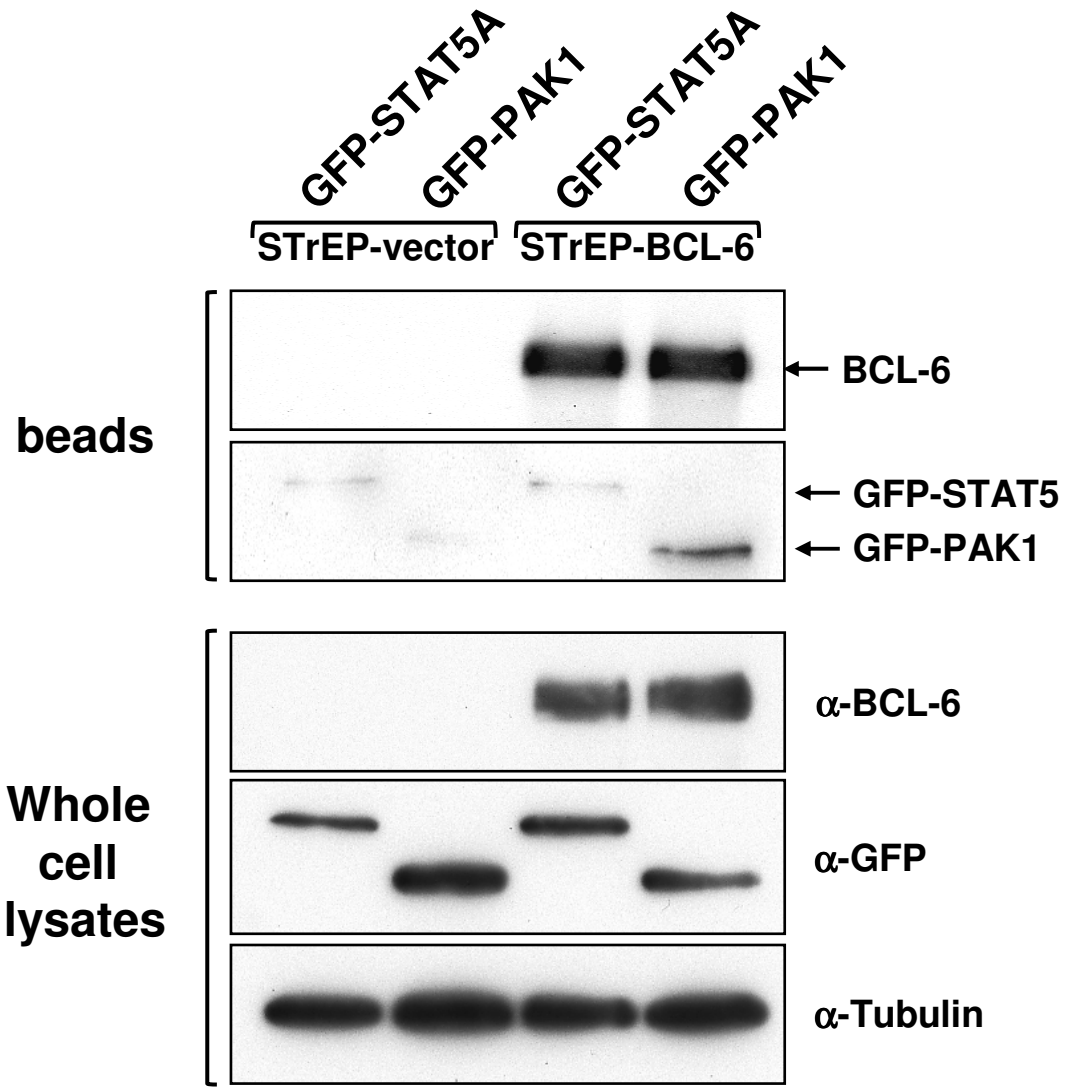

DLD-1
SW480
HT29
CCND2
CDKN2B
SUMO1
Bound STAT5
Bound BCL-6
mRNA level
Promoter occupancy (OD in abstract units)
Transcript expression (Fold over mock)
Mock
Rac1-L61
PAK1
PAK1-KD
phospho-STAT5
STAT5
GFP-PAK1
GFP-Rac1
α-GFP

Fig S1


GFP-STAT5A
GFP-PAK1
GFP-STAT5A
GFP-PAK1
STrEP-vector
STrEP-BCL-6
beads
BCL-6
GFP-STAT5
GFP-PAK1
Whole cell lysates
α-BCL-6
α-GFP
α-Tubulin

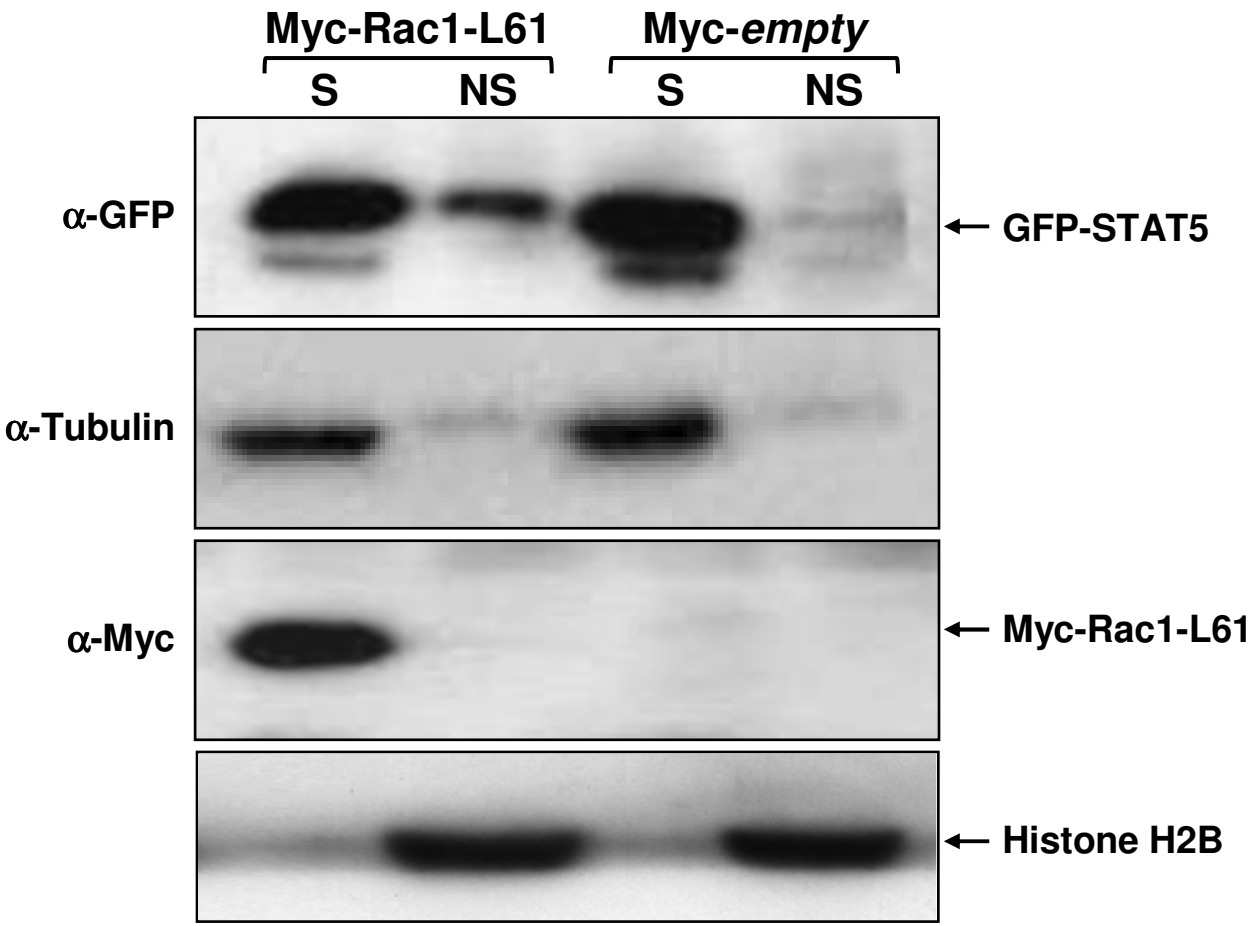
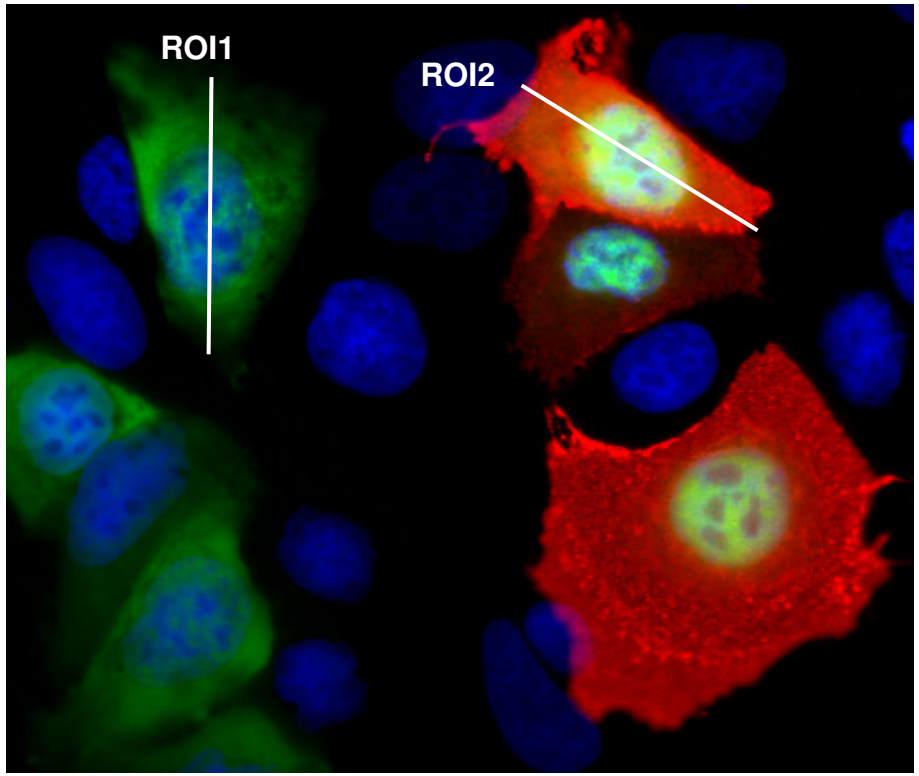
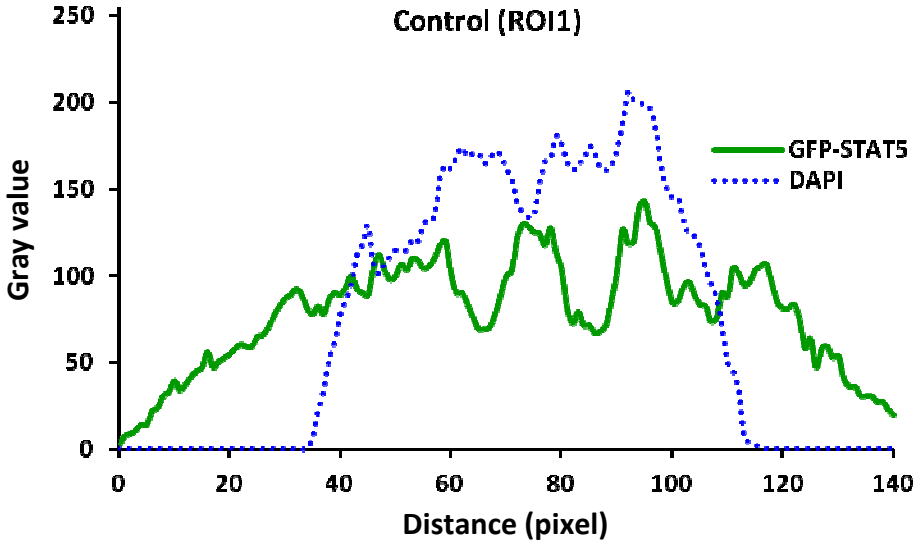
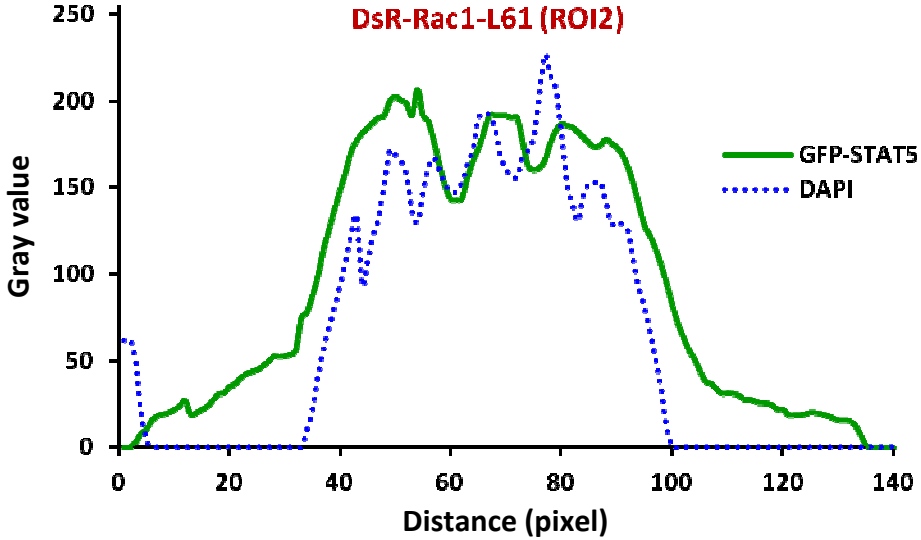
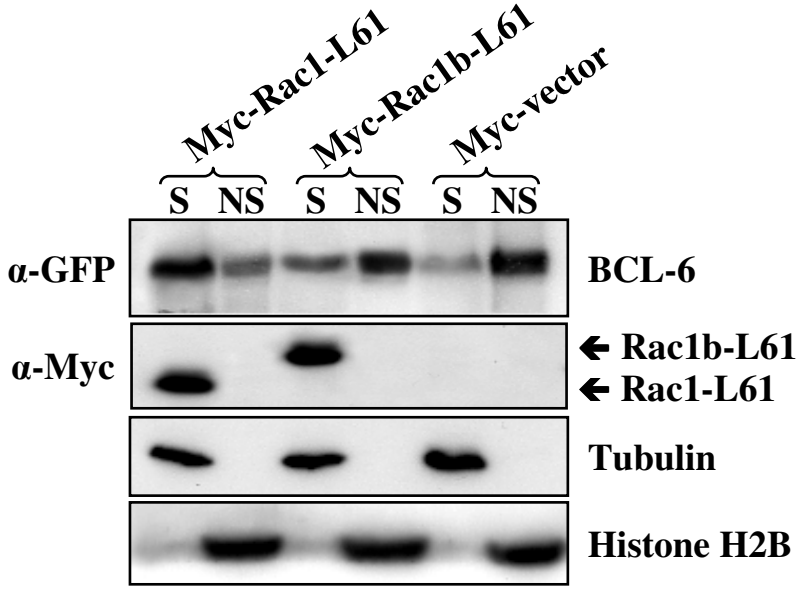

Myc-Rac1-L61
Myc-*empty*
S
NS
S
NS
α-GFP
GFP-STAT5
α-Tubulin
α-Myc
Myc-Rac1-L61
Histone H2B

Fig 2B


ROI1
ROI2


Control (ROI1)
Gray value
Distance (pixel)
GFP-STAT5
DAPI
0
50
100
150
200
250
0
20
40
60
80
100
120
140


DsR-Rac1-L61 (ROI2)
Gray value
Distance (pixel)
GFP-STAT5
DAPI
0
50
100
150
200
250
0
20
40
60
80
100
120
140


Myc-Rac1-L61
Myc-Rac1b-L61
Myc-vector
S NS S NS S NS
α-GFP
BCL-6
α-Myc
Rac1b-L61
Rac1-L61
Tubulin
Histone H2B

BCL-6 in colorectal cells

subcellular fractionation