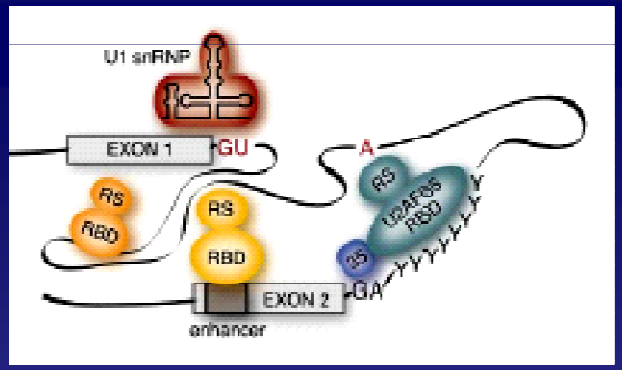
Alternative splicing of tumour-related Rac1b is regulated by upstream signalling pathways

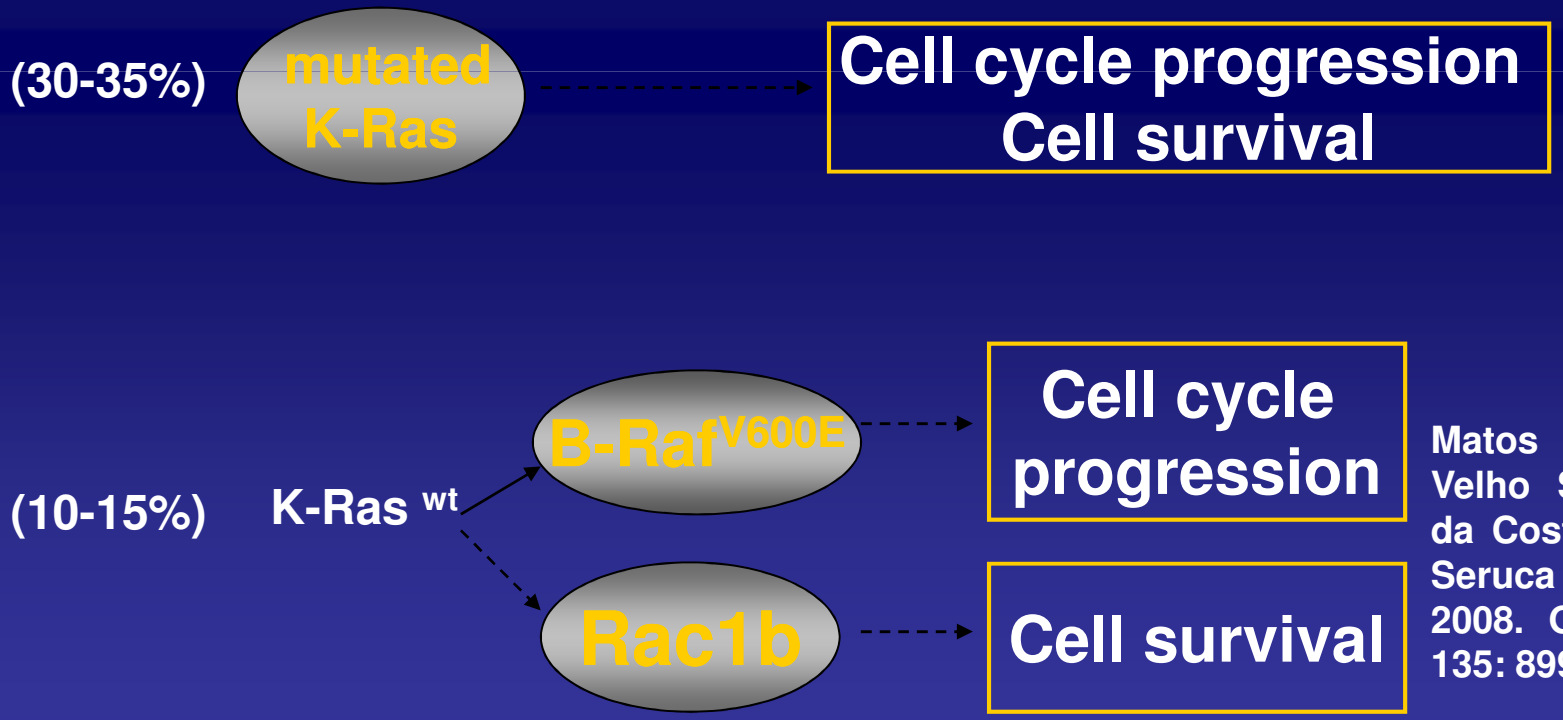


Vânia Gonçalves, Paulo Matos and Peter Jordan

November 2011

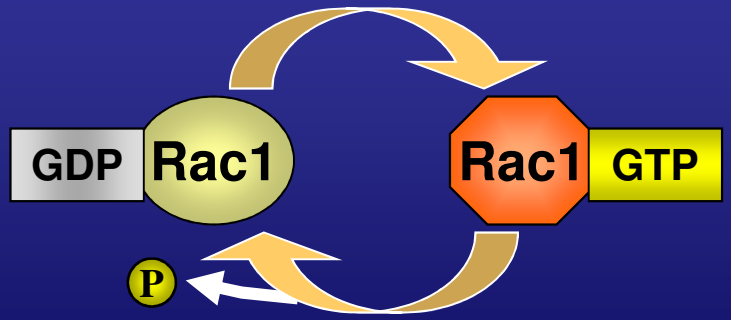
Colorectal tumorigenesis

The second most frequent cancer in European countries



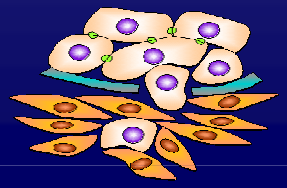
Matos P, Oliveira C, Velho S, Gonçalves V, da Costa LT, Moyer MP, Seruca R and Jordan P. 2008. Gastroenterology. 135: 899-906

Rac1 is a member of the Rho-GTPases family (Rho: *Ras homologue*)



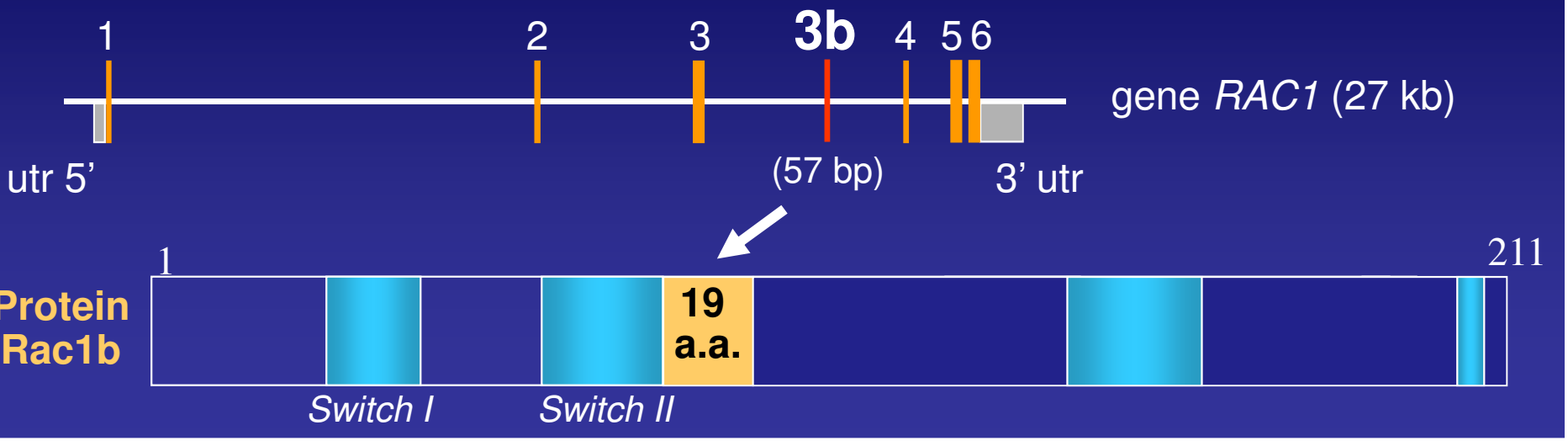
Cell motility, adhesion and proliferation

Deregulated activity

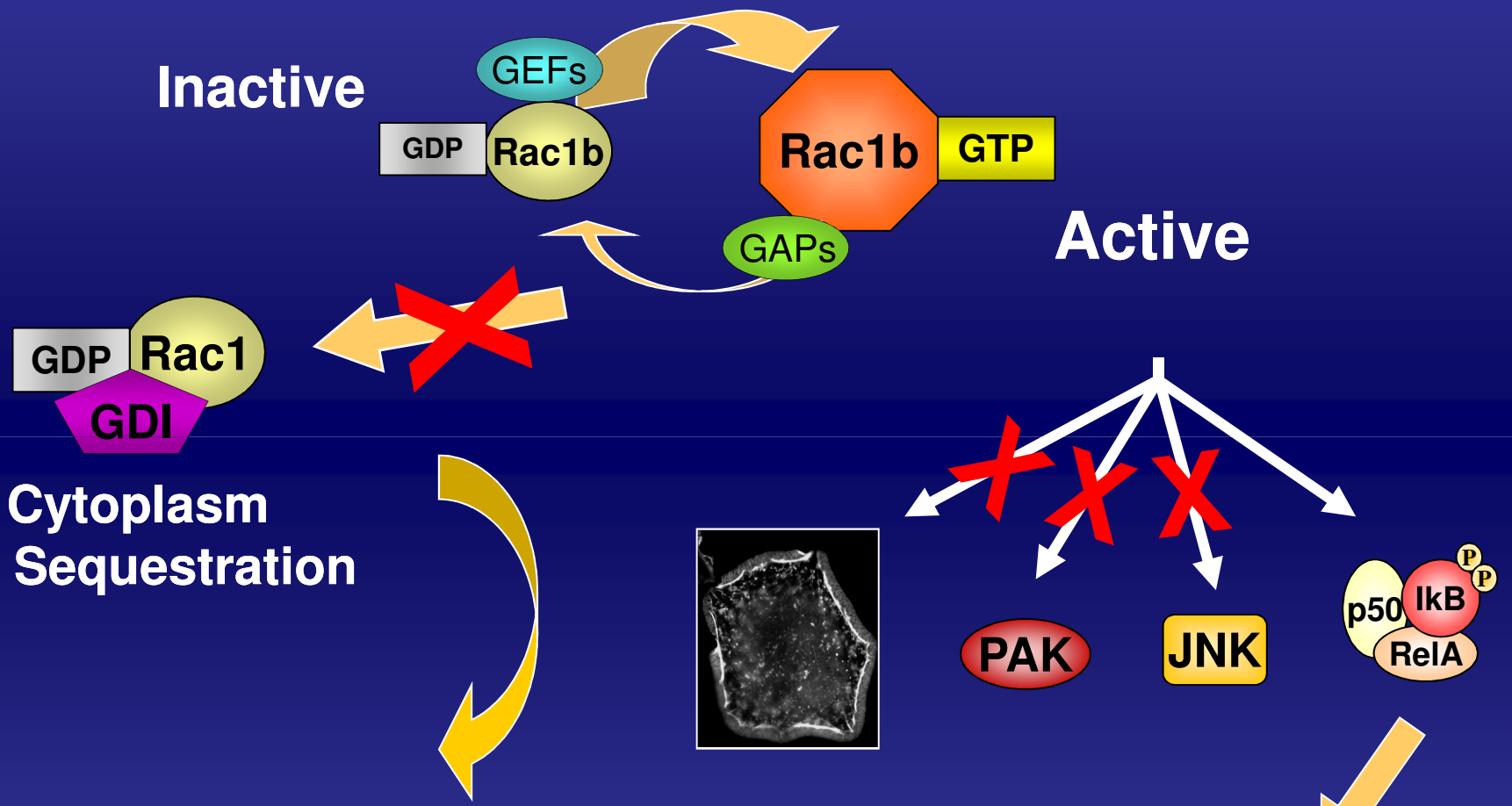


Tumour progression

Rac1b – a Rac1 splice variant



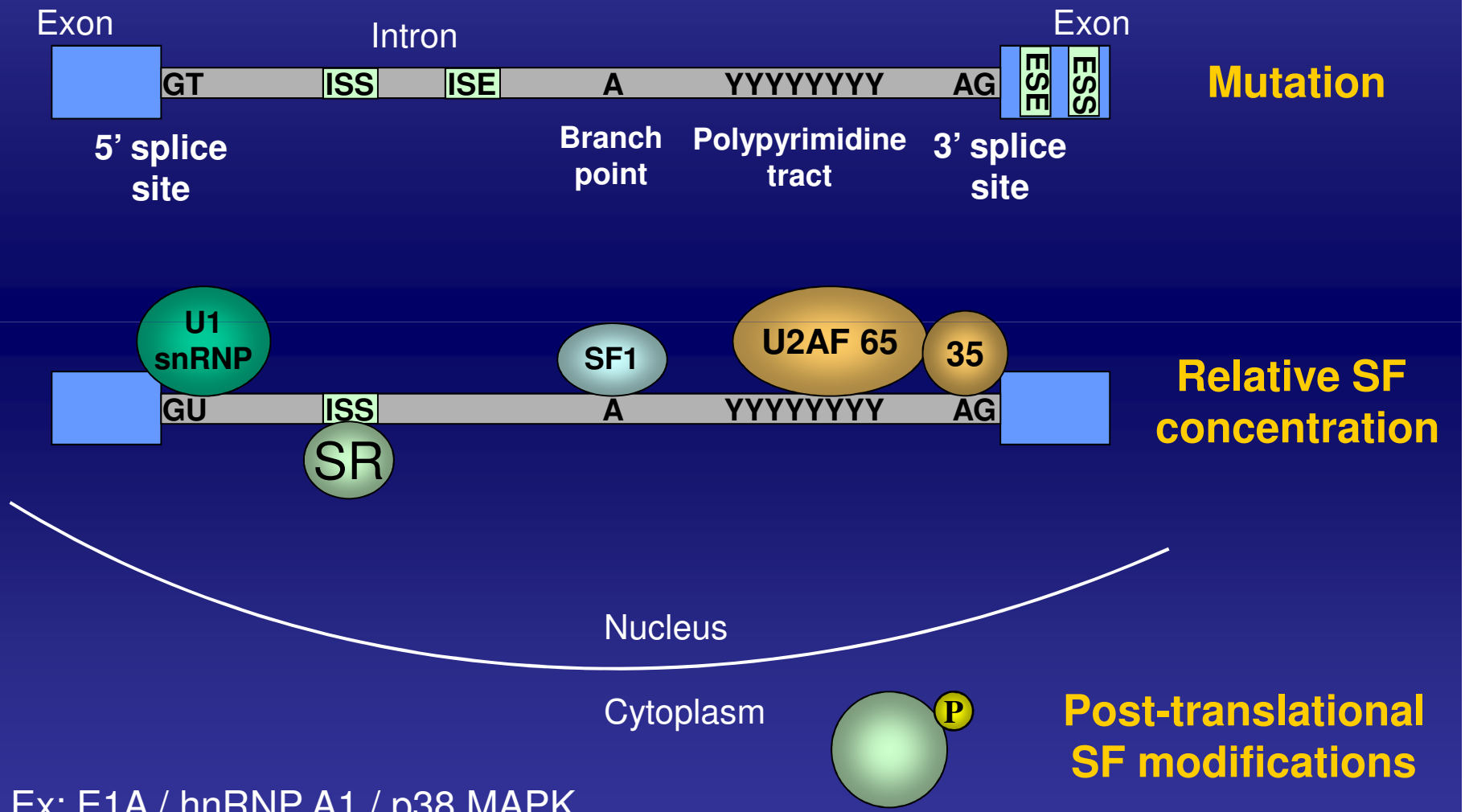
Rac1b differs in regulation and signalling



Rac1b is activated *in vivo* to much higher levels than Rac1

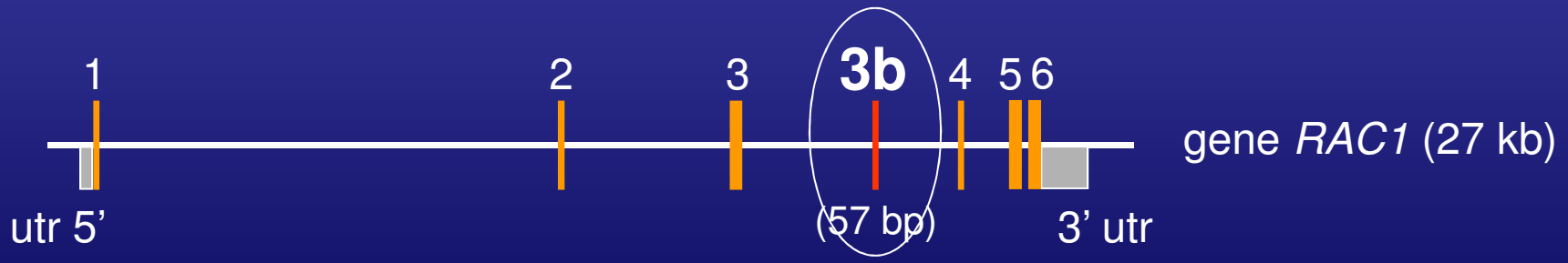
Cell survival
Cell cycle progression

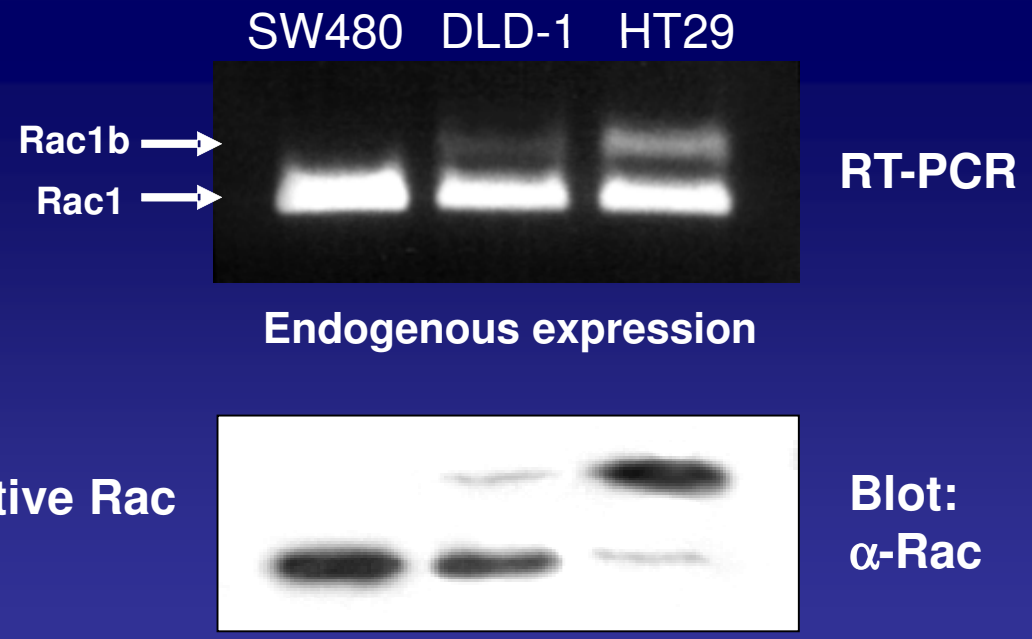
How is Rac1b splice site recognition regulated in colorectal tumour cells?



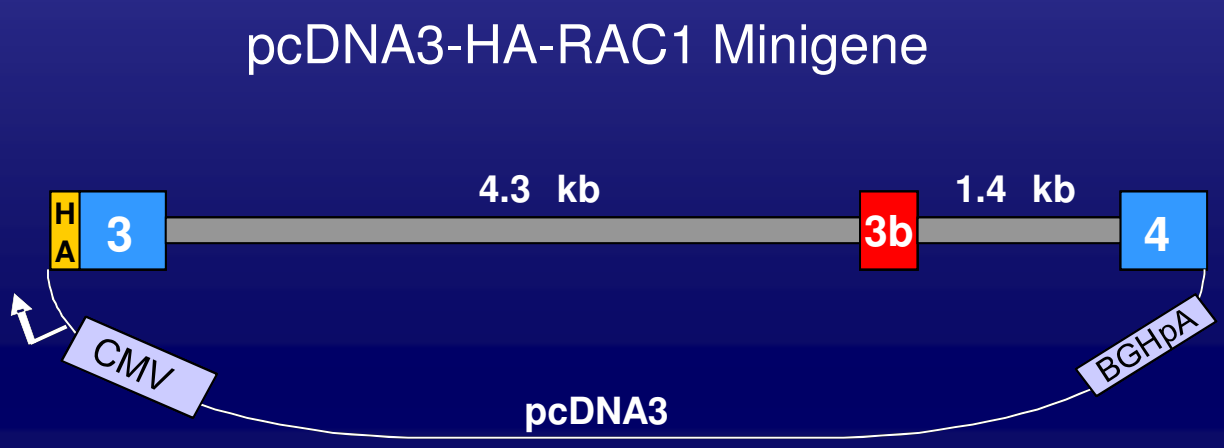
Ex: E1A / hnRNP A1 / p38 MAPK
CD44 / Sam 68 / ERK MAPK

Genomic mutations determine exon 3b inclusion/exclusion?





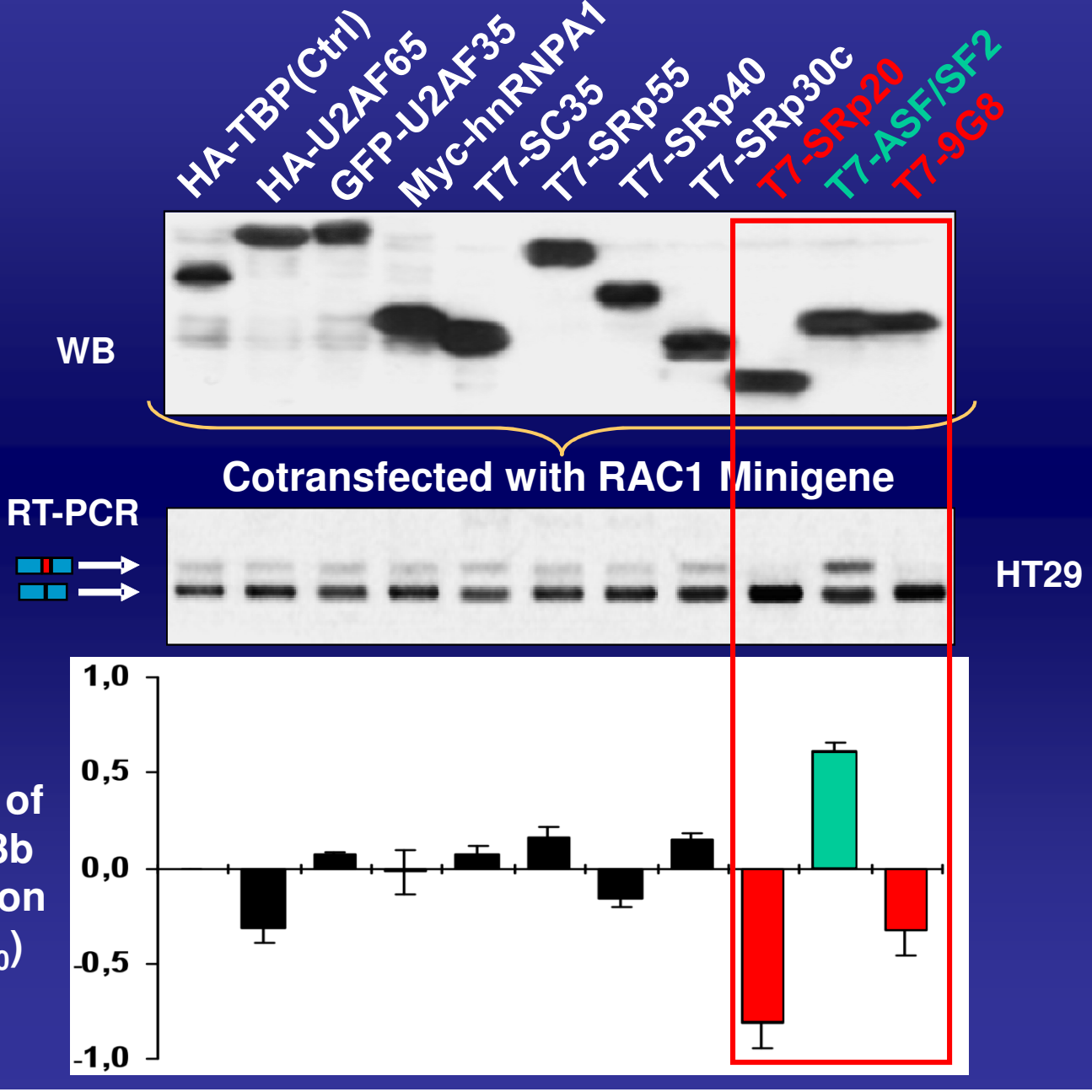
Which are the regulatory sequence elements that determine exon 3b inclusion/exclusion?



Endogenous expression

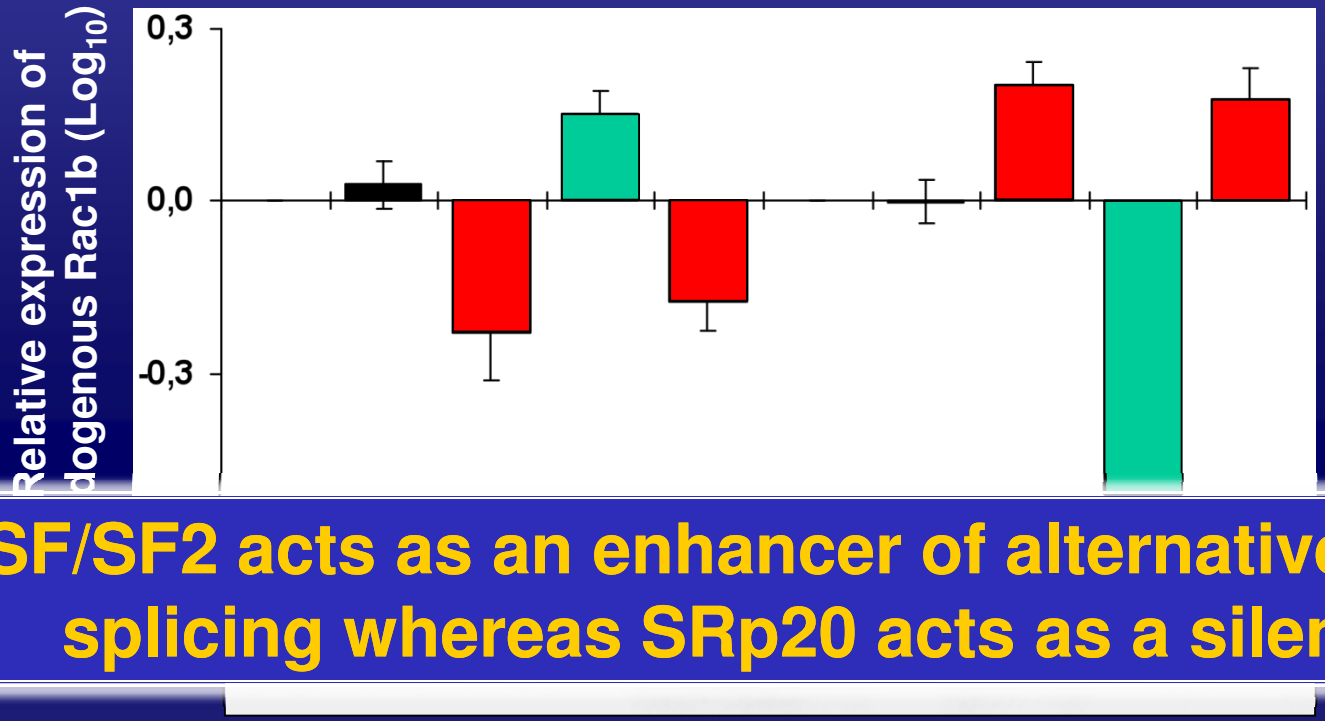
Transfected RAC1 minigene

Which splicing factors could be involved?



Hypothesis:

Antagonistic splicing factor activities regulate Rac1b splicing

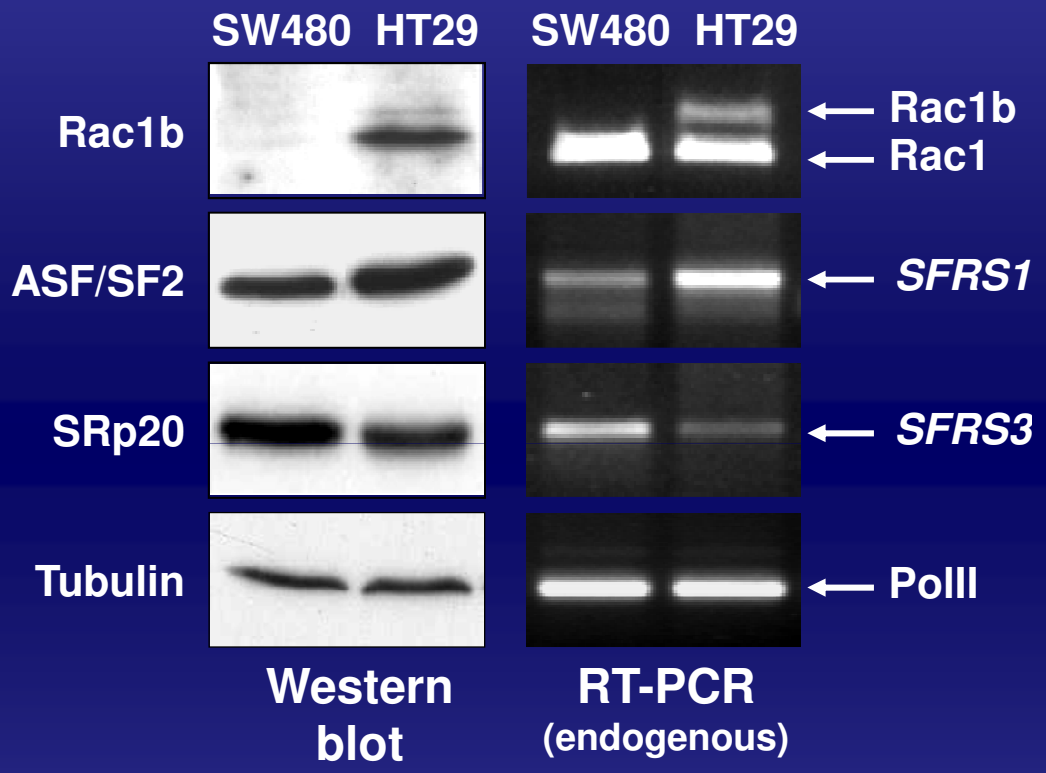


Real-Time PCR

ASF/SF2 acts as an enhancer of alternative Rac1b splicing whereas SRp20 acts as a silencer

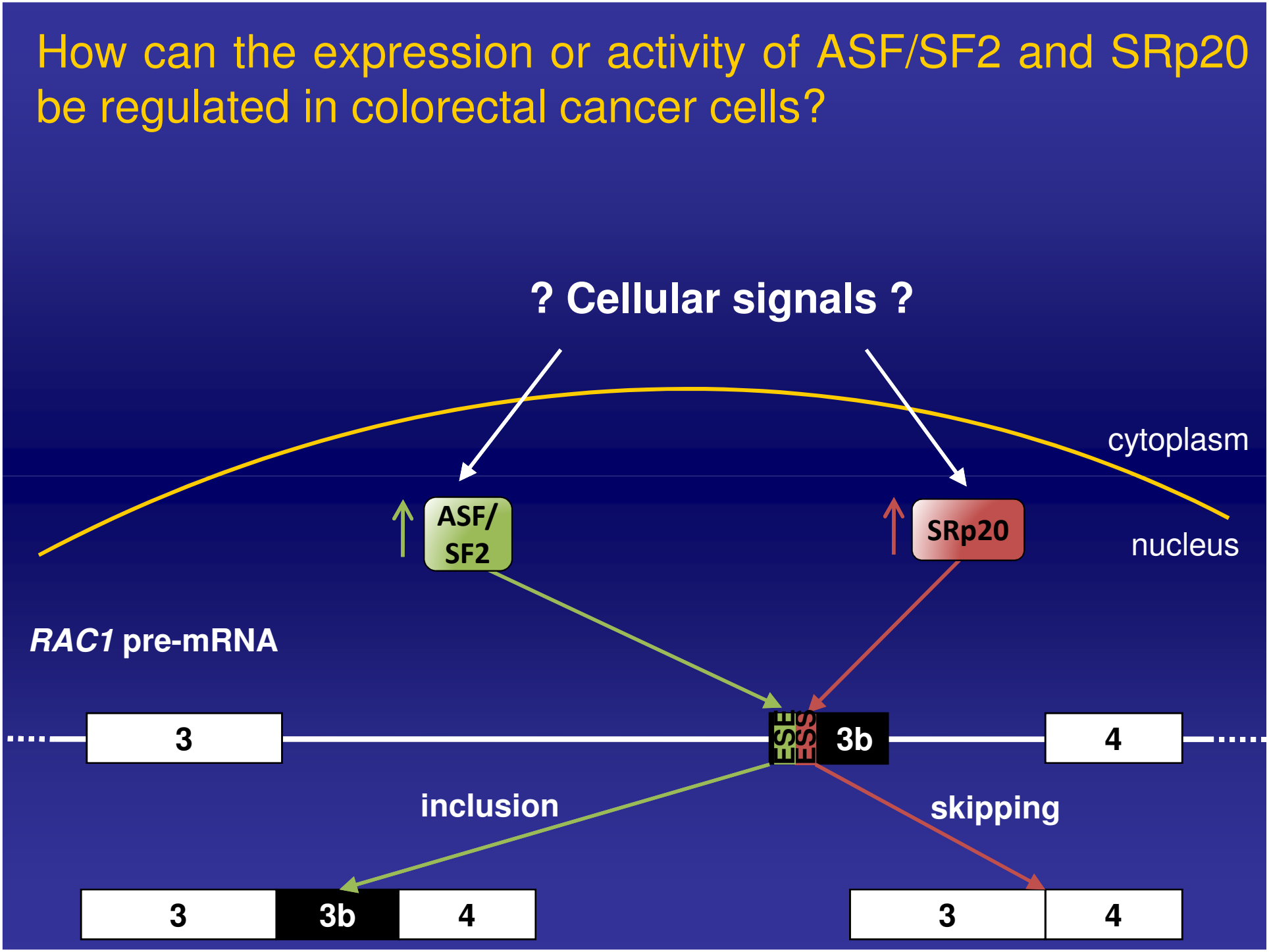
ASF/SF2 activates exon 3b recognition **SRp20/9G8 inhibit** exon 3b recognition

Correlation of endogenous expression levels

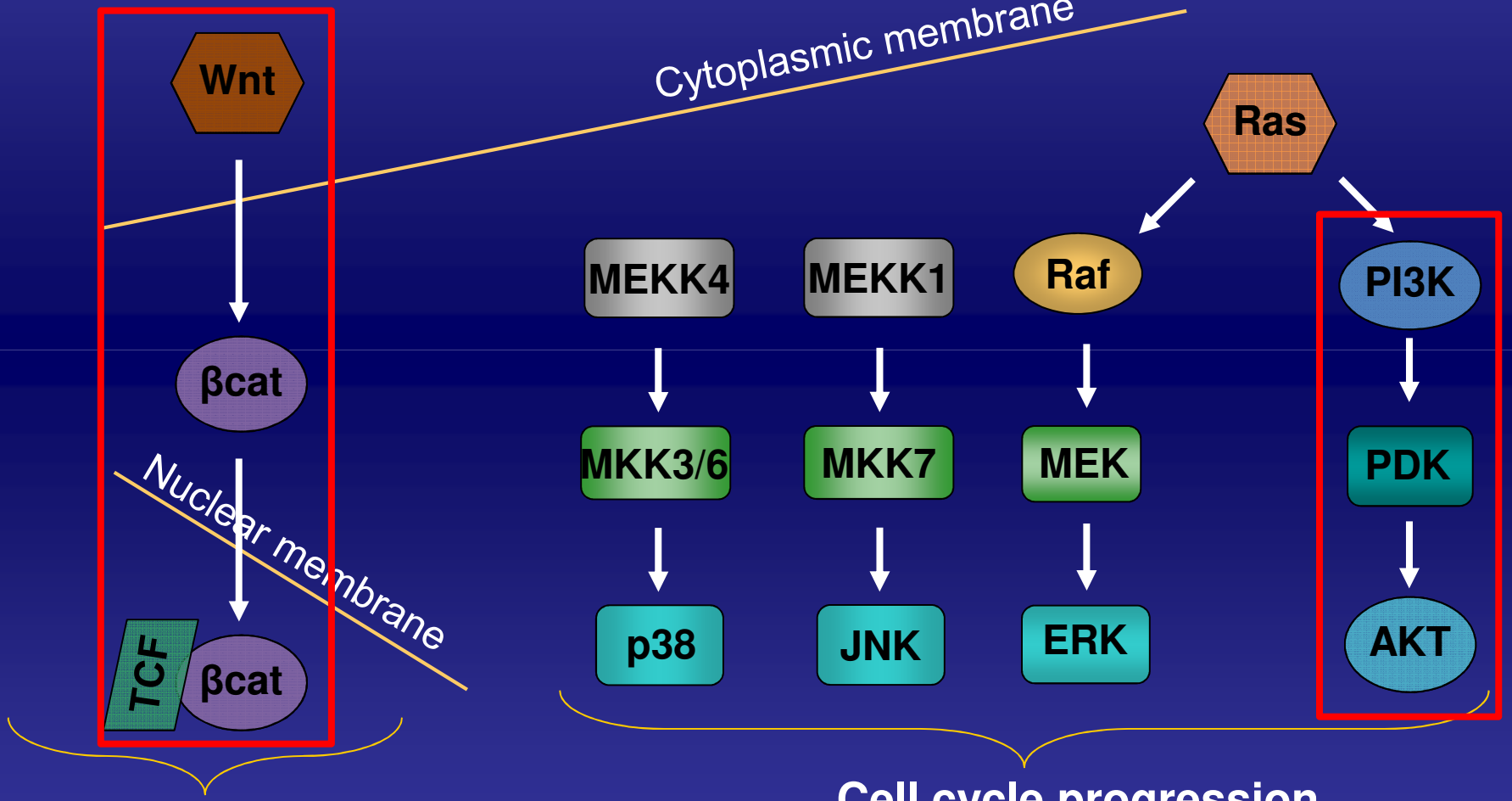


Endogenous levels of ASF/SF2 and SRp20 transcripts and proteins correlate in two different colorectal cell lines with the different Rac1b levels they express

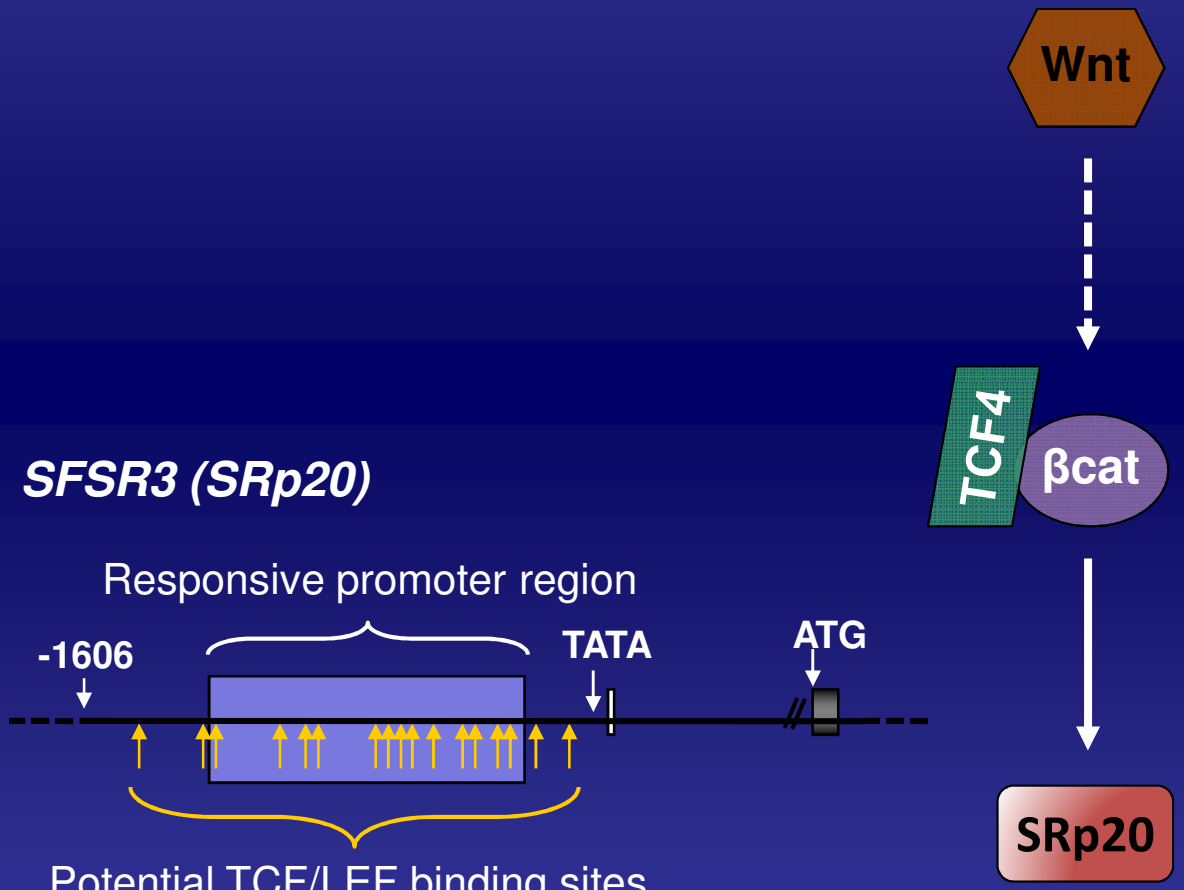
How can the expression or activity of ASF/SF2 and SRp20 be regulated in colorectal cancer cells?



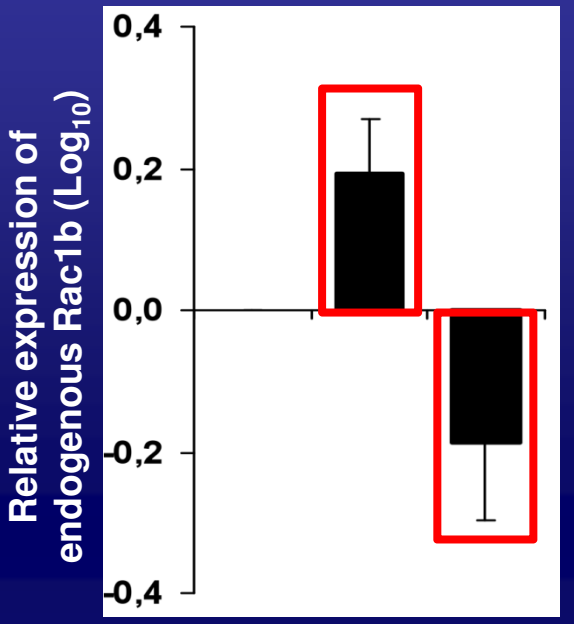
How can the expression or activity of ASF/SF2 and SRp20 be regulated in colorectal cancer cells?

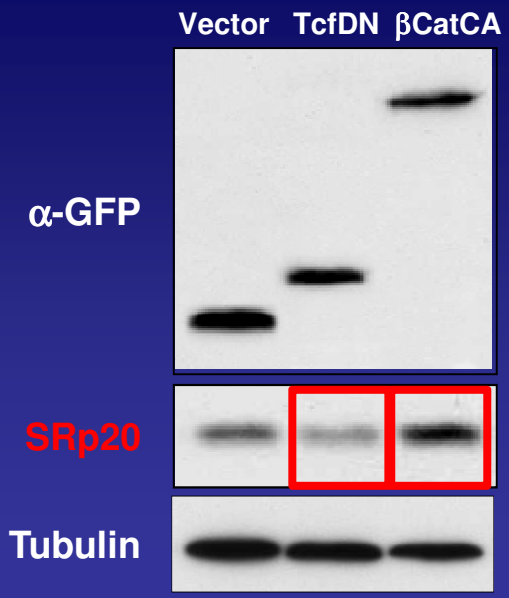


Wnt signalling pathway affects Rac1b expression



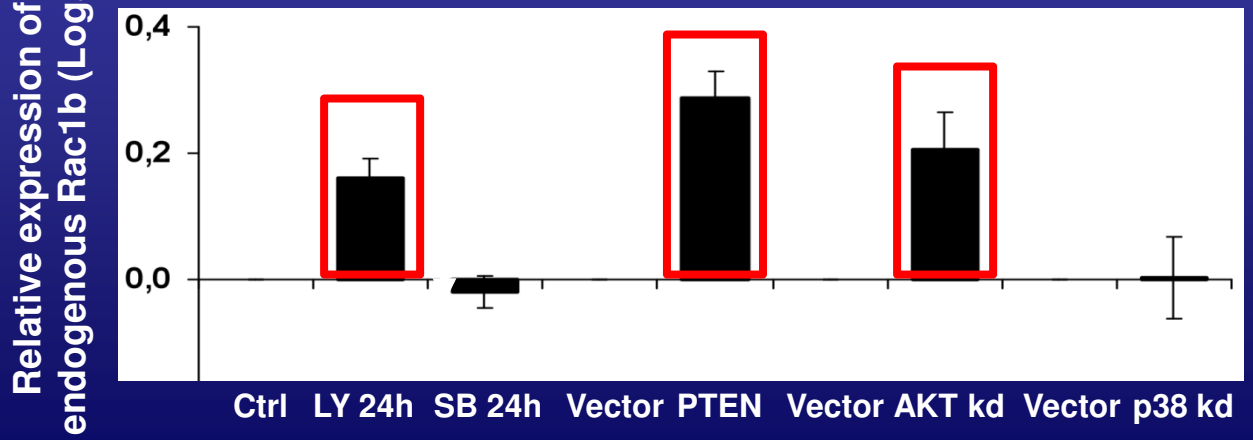
SFSR3 (SRp20)

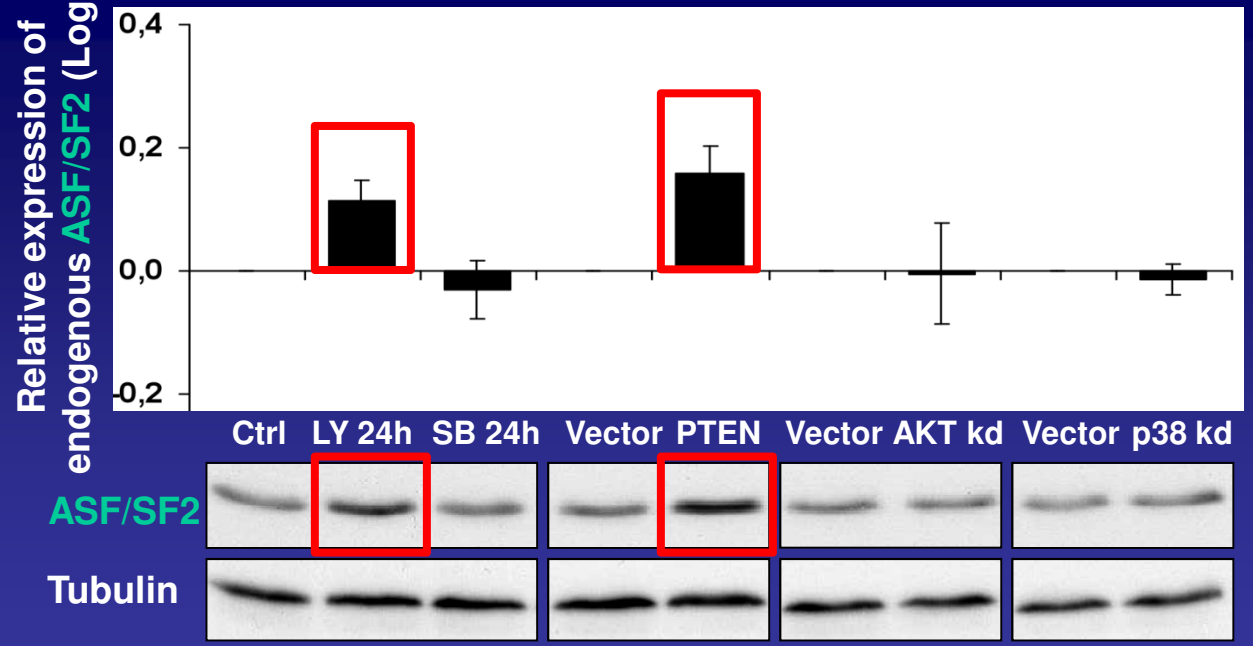


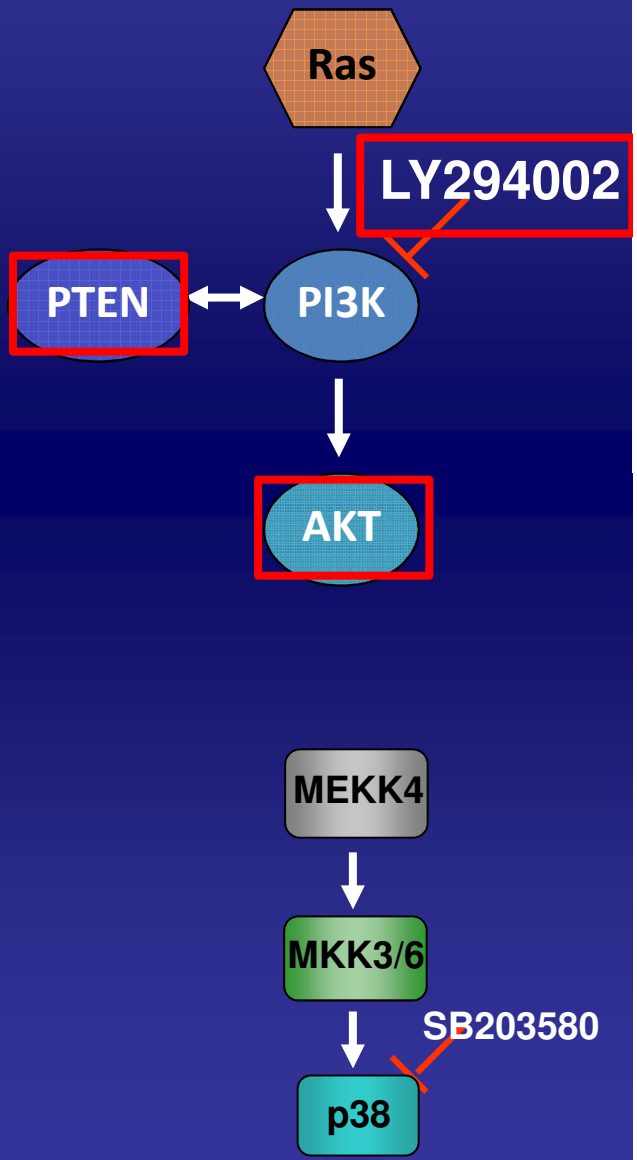


Gonçalves V, Matos P, Jordan P. 2008. RNA 14: 2538-2549

PI3-kinase signalling pathway affects Rac1b expression

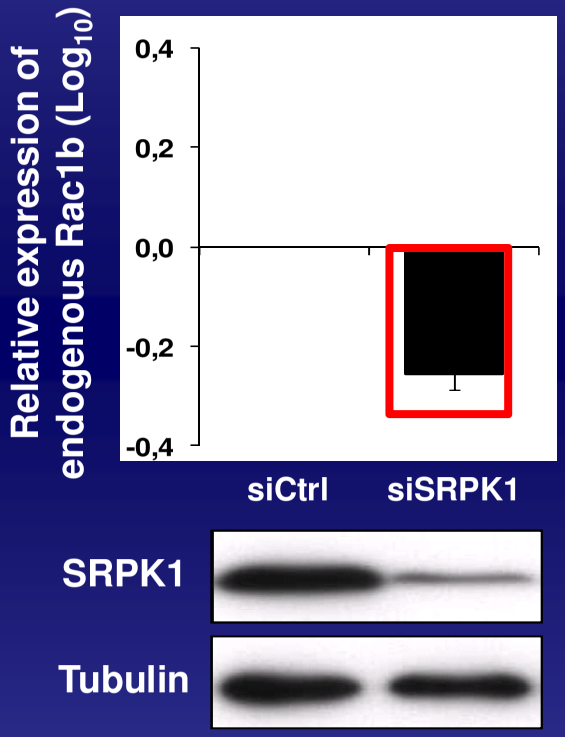


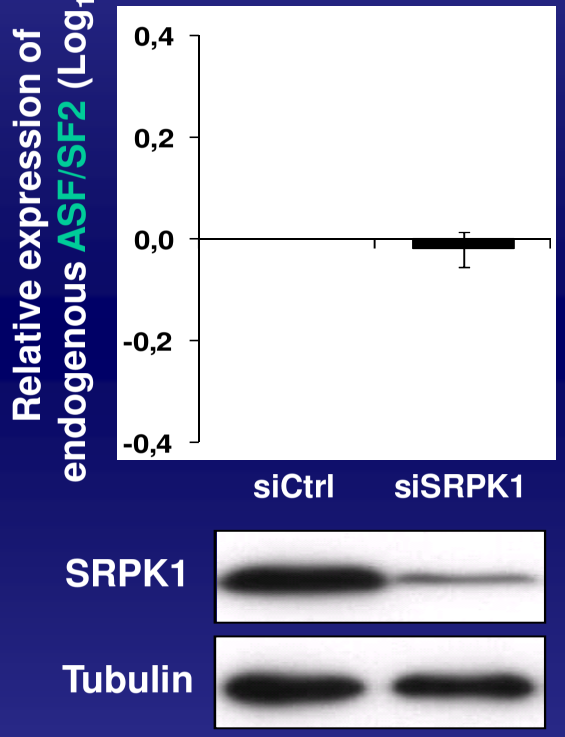




Gonçalves V, Matos P, Jordan P. 2009. Hum Mol Genet 18(19): 3696-3707

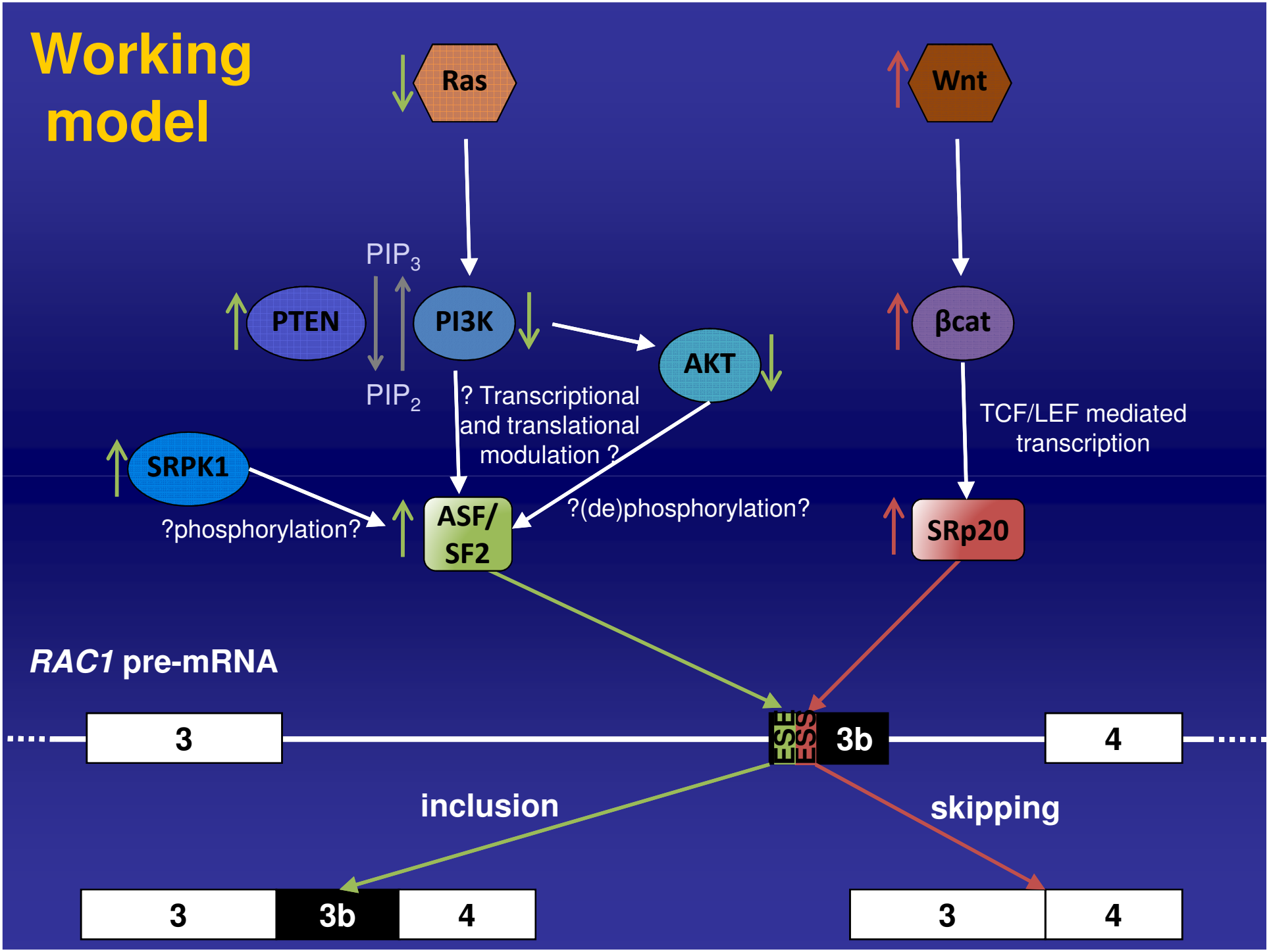
Depletion of SRPK1 also affects Rac1b expression





Conclusions:

- ✓ Splicing factor ASF/SF2 acts as an enhancer of alternative Rac1b splicing whereas SRp20 acts as a silencer.
- ✓ The levels of ASF/SF2 and SRp20 transcript and protein correlate with Rac1b expression in colorectal cells.
- ✓ PI3-kinase and Wnt signalling pathways synchronize the expression of ASF/SF2 and SRp20, respectively, and together regulate whether alternative exon 3b is included or skipped during splicing of the Rac1 pre-mRNA.


Working model
Ras
Wnt
PIP3
PTEN
PI3K
AKT
βcat
PIP2
? Transcriptional and translational modulation ?
TCF/LEF mediated transcription
SRPK1
?phosphorylation?
ASF/ SF2
?(de)phosphorylation?
SRp20
RAC1 pre-mRNA
3
ESE
ESS
3b
4
inclusion
skipping
3
3b
4
3
4

Acknowledgements:

- Margarida Gama-Carvalho and Maria Carmo Fonseca, Harald König, Javier Cáceres, Donald Tindall, BM Burgering, Stefan Ludwig and Ulf Rapp, Chris Smith and Juan Valcárcel for providing plasmids used in this study
- FCT for the funding of this PhD
- Peter Jordan and the Onco lab for everything

Instituto_Nacional de Saúde Doutor Ricardo Jorge

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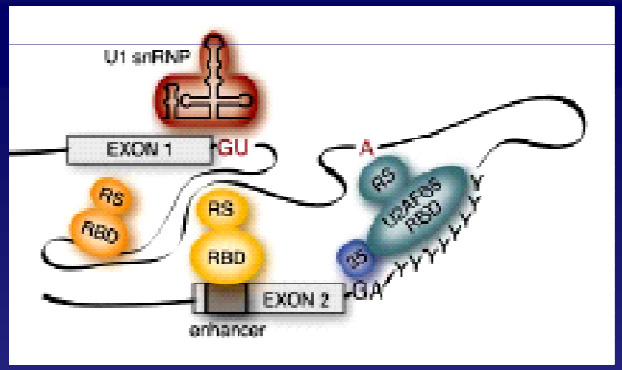
Alternative splicing of tumour-related Rac1b is regulated by upstream signalling pathways



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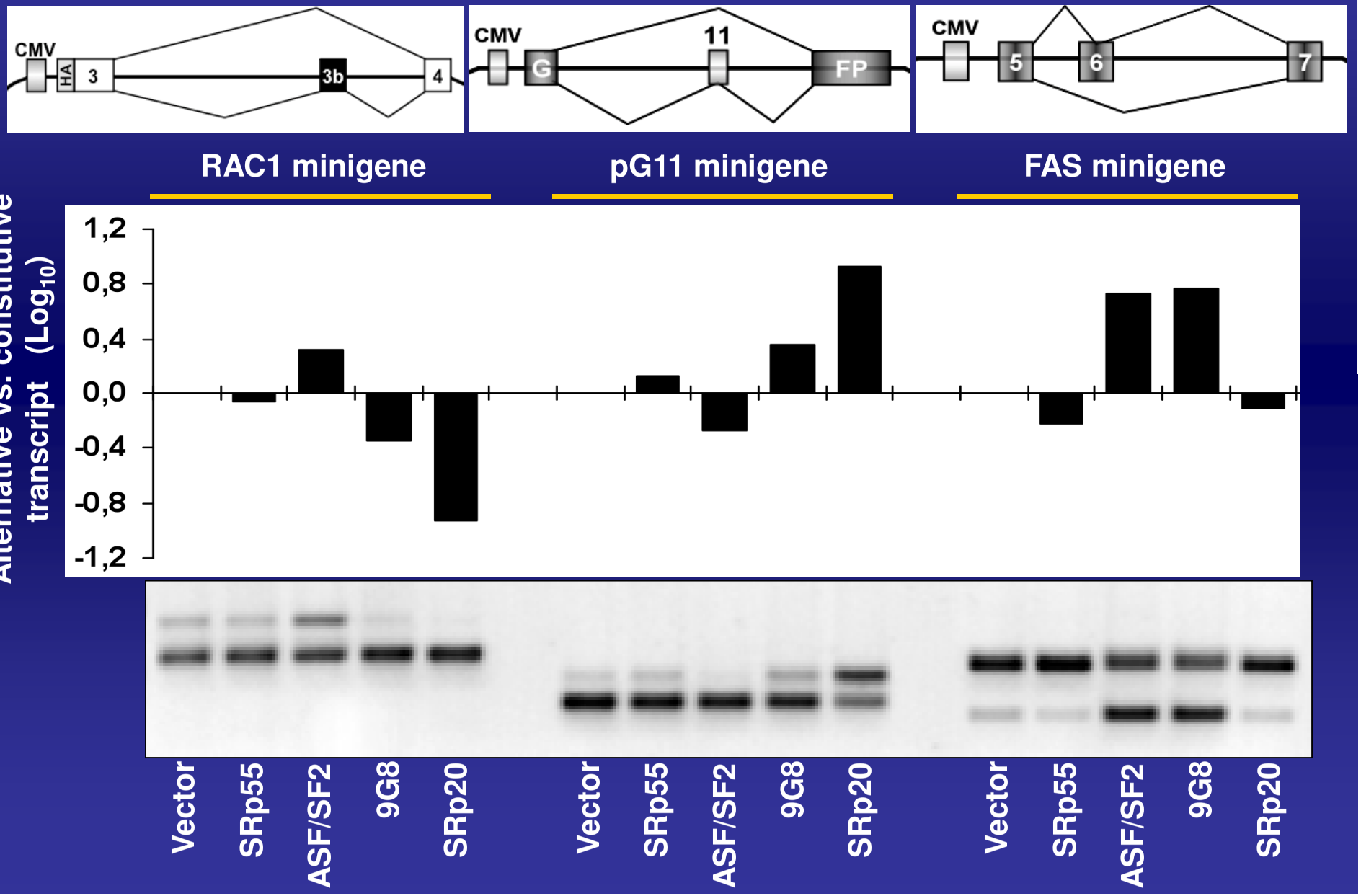
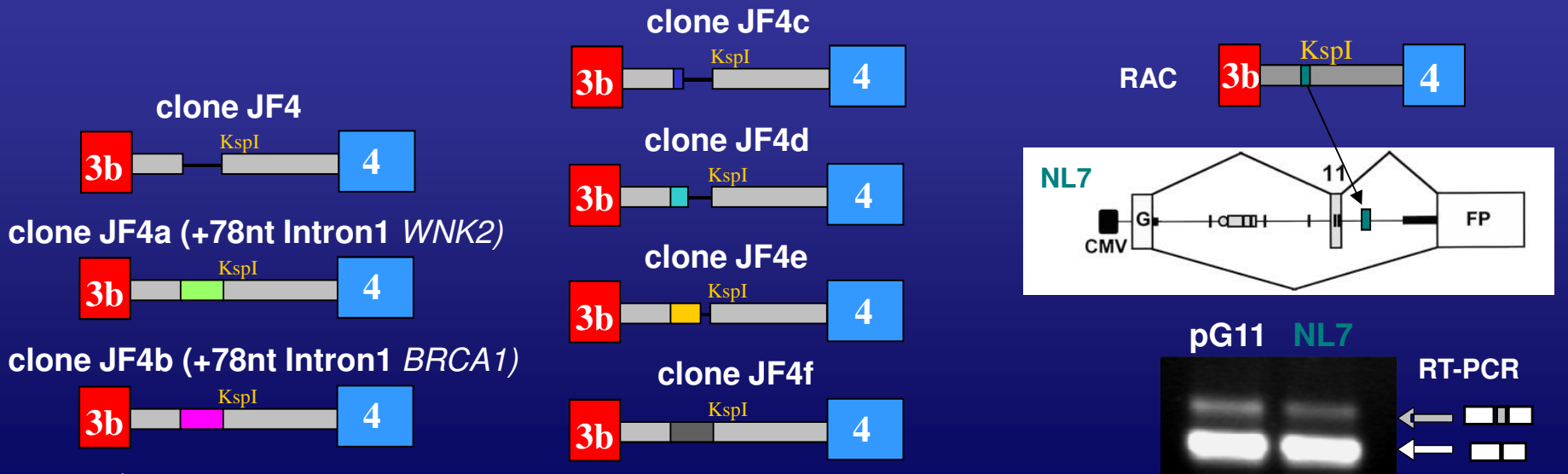
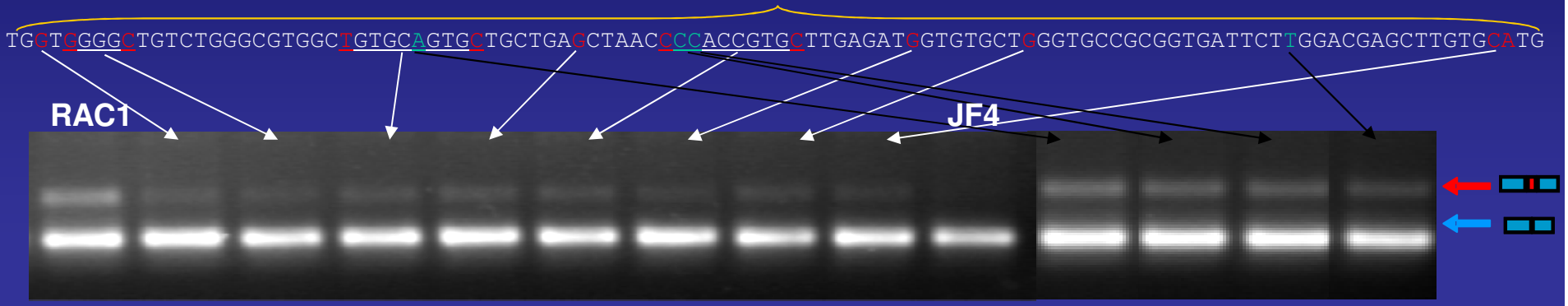
Effect of SF2 and SRp20 on splicing of different minigenes



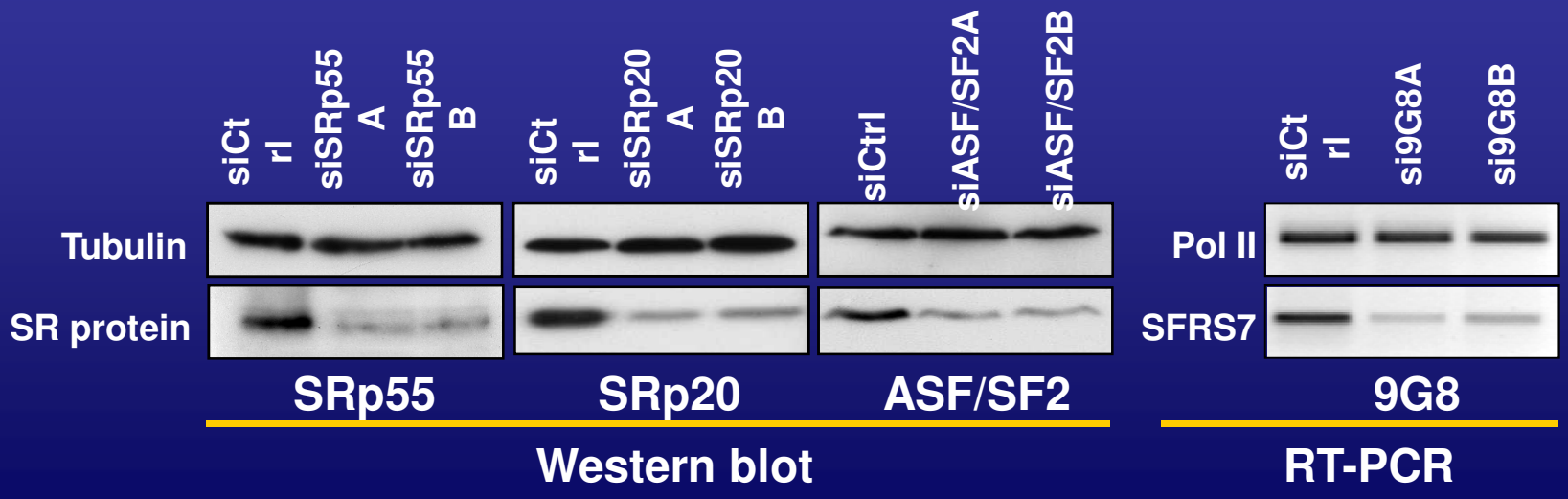
Contribution of intronic regulatory elements

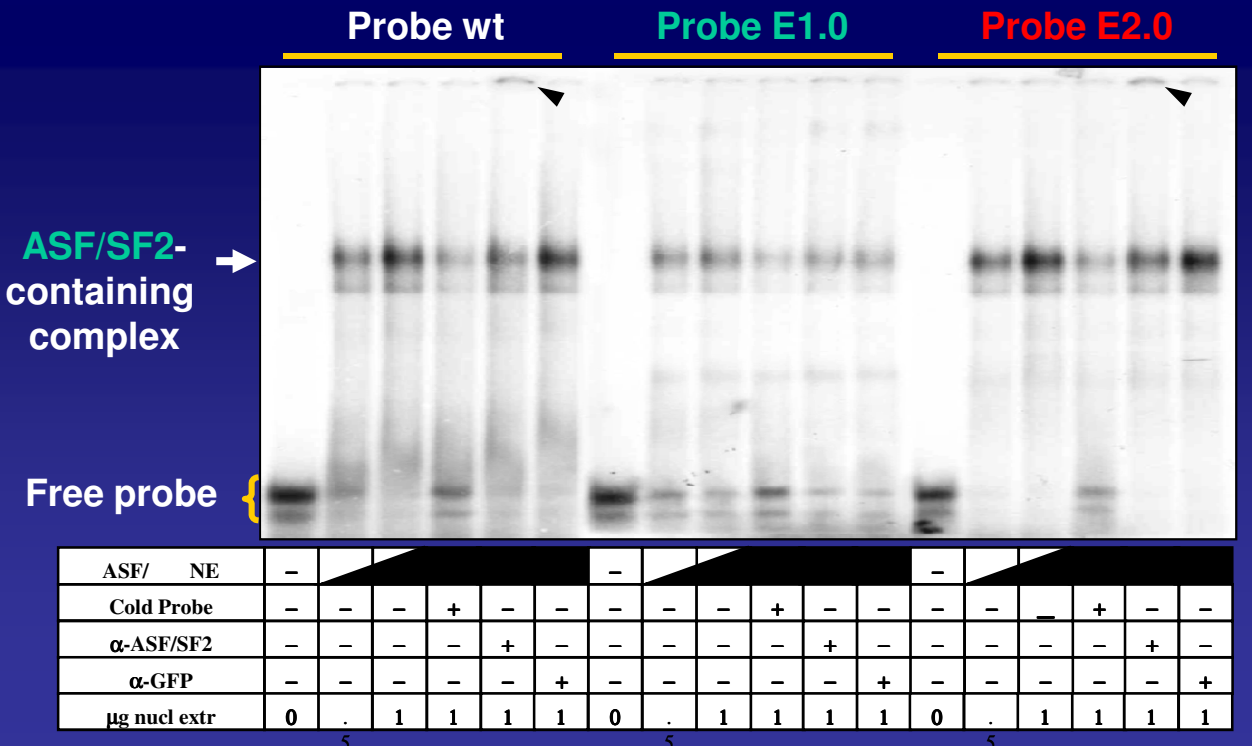


Intron 3b mutations



SFs endogenous depletion by specific siRNAs / ASF/SF2 EMSA





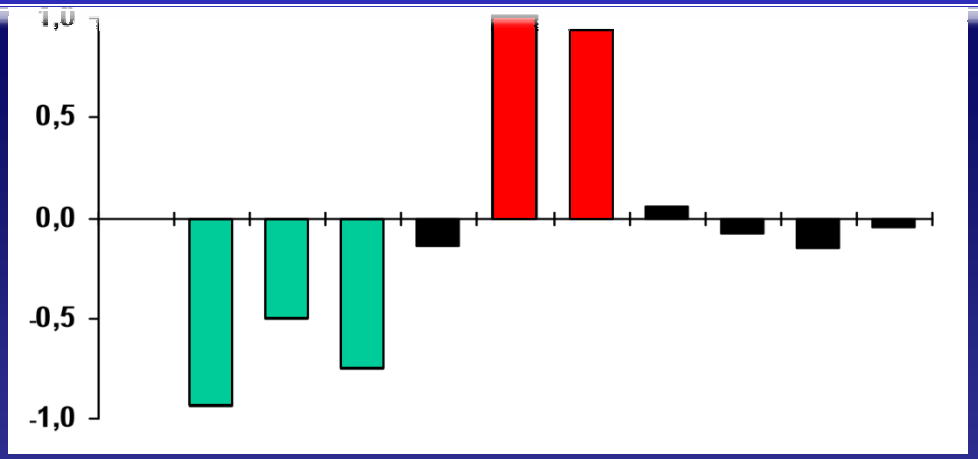
Which are the regulatory sequence elements that determine exon 3b inclusion/exclusion?

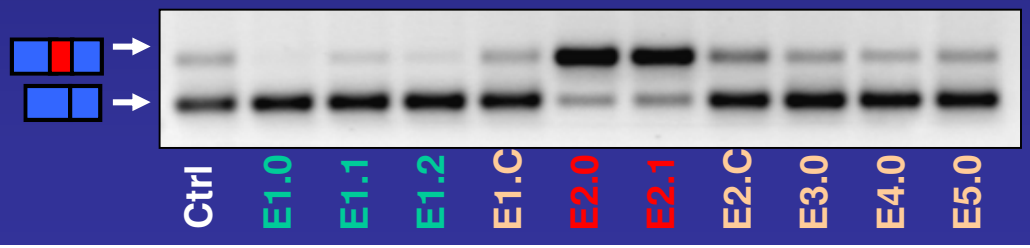
Exon 3b (Conserved nt)

RAC1(Ctrl) - GTTGGAGAAACGTACGGTAAGGATATAACCTCCCGGGGCAAAGACAAGCCGATTGCC
Mut E1.0 - GTTGGACAAATGTACGGTAAGGATATAACCTCCCGGGGCAAAGACAAGCCGATTGCC
Mut E1.1 - GTTGGACAAACGTACGGTAAGGATATAACCTCCCGGGGCAAAGACAAGCCGATTGCC
Mut E1.2 - GTTGGAGAAATGTACGGTAAGGATATAACCTCCCGGGGCAAAGACAAGCCGATTGCC
Mut E1.C - GTTGGAGACACGTACGGTAAGGATATAACCTCCCGGGGCAAAGACAAGCCGATTGCC
Mut E2.0 - GTTGGAGAAACGTACGGCAAGGATATAACCTCCCGGGGCAAAGACAAGCCGATTGCC
Mut E2.1 - GTTGGAGAAACGTACGGTCAGGATATAACCTCCCGGGGCAAAGACAAGCCGATTGCC
Mut E2.C - GTTGGAGAAACGTACGGCAAAGATATAACCTCCCGGGGCAAAGACAAGCCGATTGCC

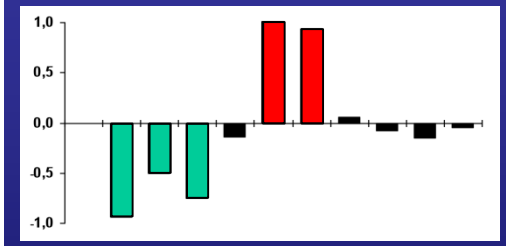
Alternative exon 3b contains an exonic splice enhancer element followed by a silencer element

Alternative *vs* constitutive transcript (Log_{10})





Rac1 exon 3b contains binding sites for ASF/SF2 and SRp20



EMSA
(electrophoretic mobility shift assay)

Probe wt | **Probe E1.0** | **Probe E2.0**

Alternative exon 3b is recognized by ASF/SF2 and SRp20

anti-ASF/SF2

250

75

rASF/SF2	–				
Probe wt	+	+	+	+	–

75

Free probe

rASF/SF2	–					–					–			
Cold Probe	–	–	–	+		–	–	–	+		–	–	–	+

Implications for colorectal tumorigenesis