

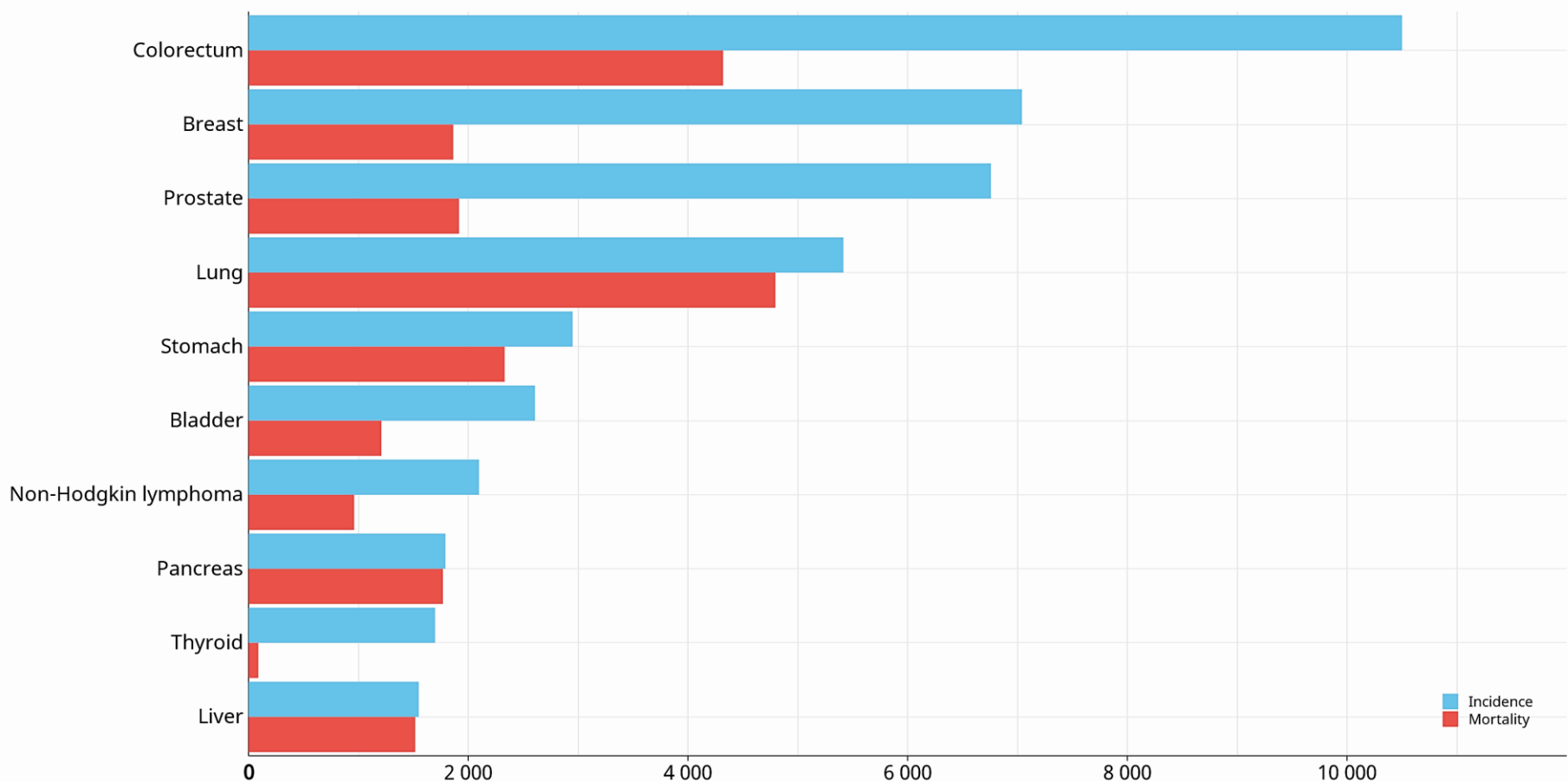


Ibuprofen inhibits alternative splicing of tumor-related RAC1B in *BRAF*-mutant colon cancer: a role in adjuvant therapy?

Peter Jordan

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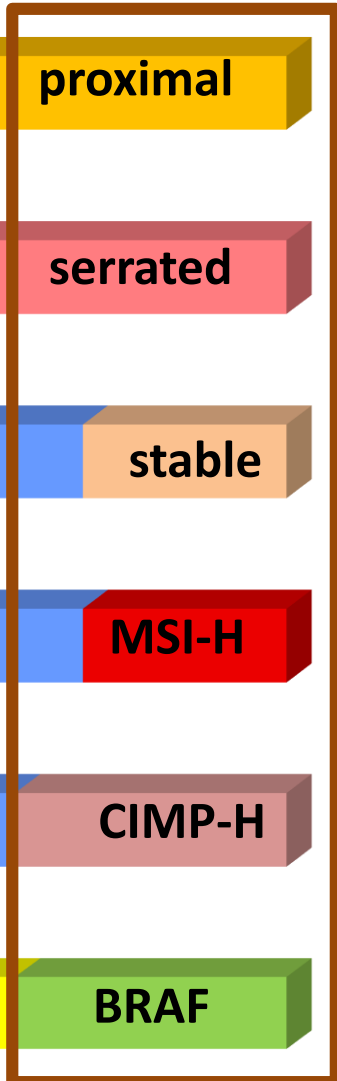
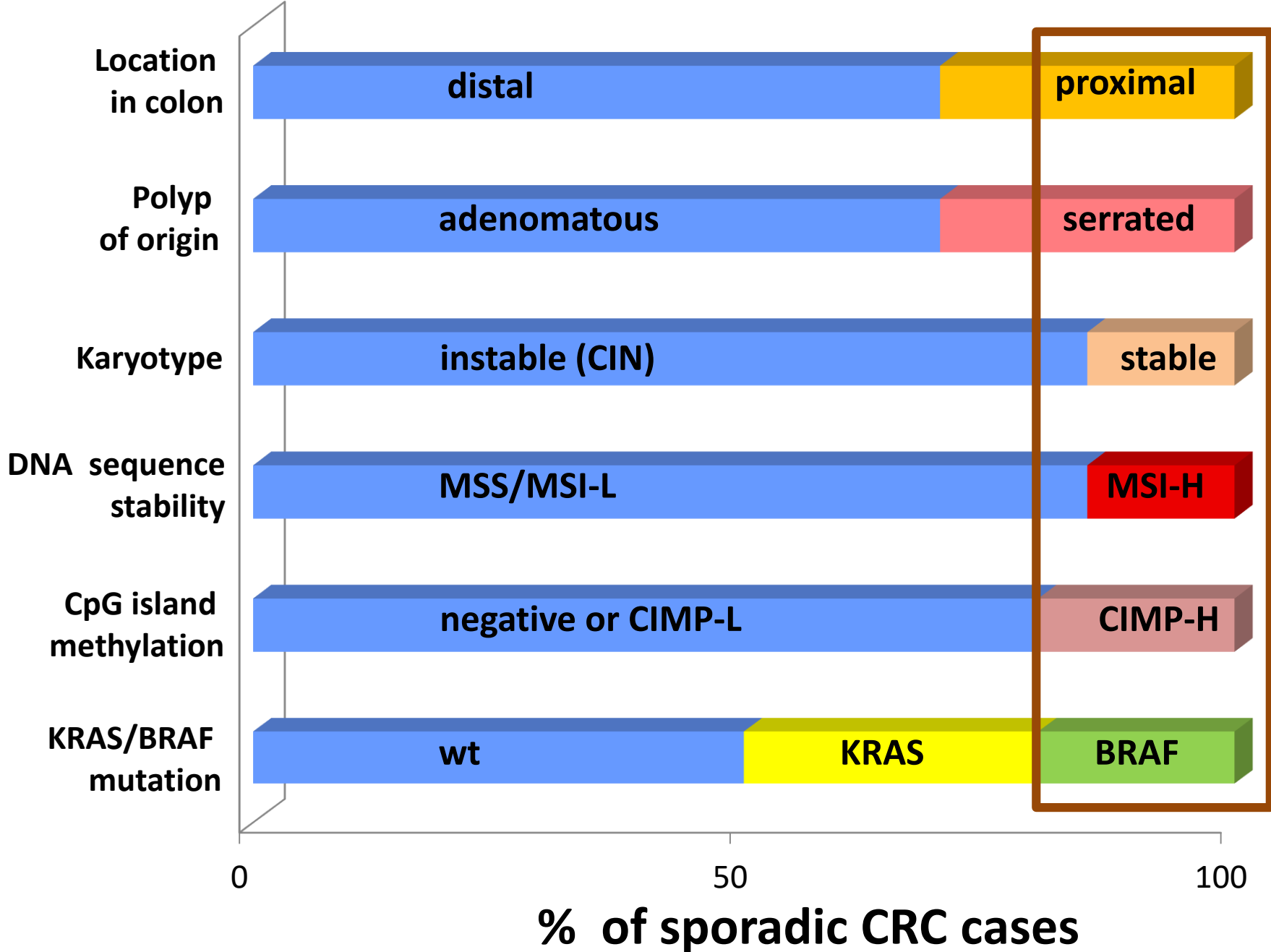
2020 Estimated number of incident cases and deaths Portugal, both sexes, all ages



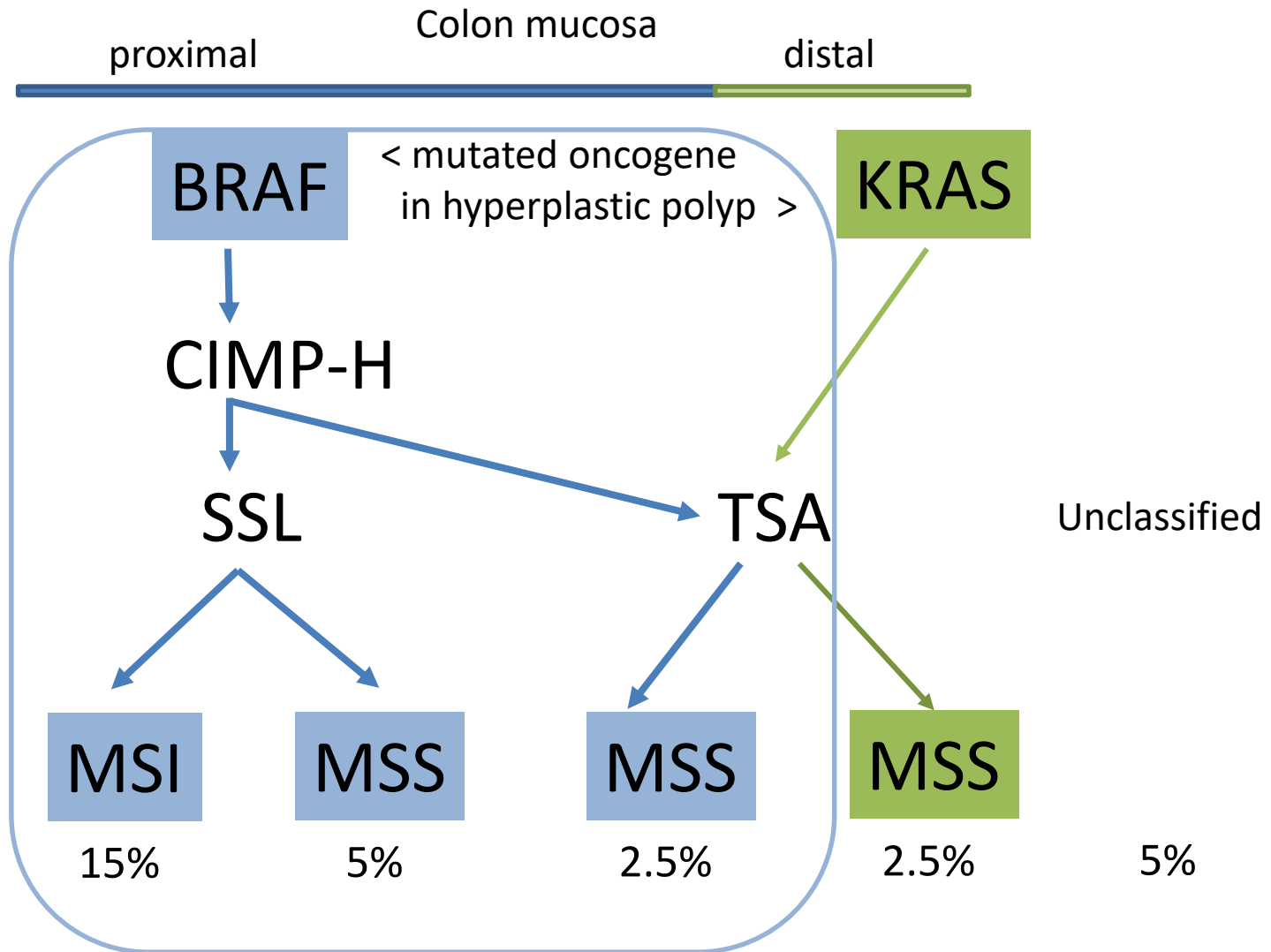
Data source: Globocan 2020
 Graph production: Global Cancer
 Observatory (<http://gco.iarc.fr>)

International Agency for Research on
 World Health
 Organization

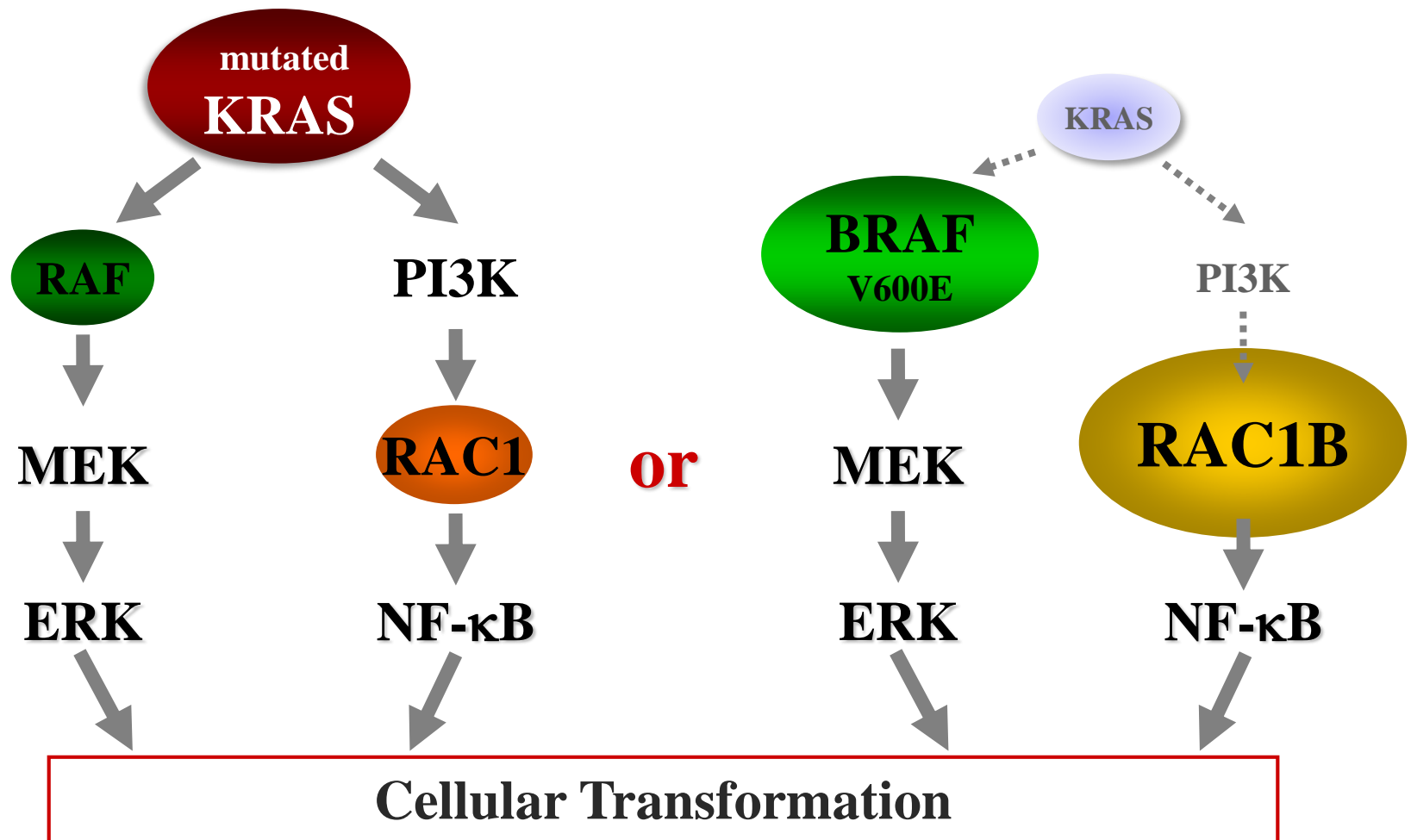
https://gco.iarc.fr/today/online-analysis-multi-bars?v=2020&mode=cancer&mode_population=countries&population=900&populations=620&key=total&sex=0&cancer=39&type=0&statistic=5&prevalence=0&population_group=0&ages_group%5B%5D=0&ages_group%5B%5D=17&nb_items=10&group_cancer=1&include_nmsc=1&include_nmsc_other=1&type_multiple=%257B%2522inc%2522%253Atrue%252C%2522mort%2522%253Atrue%252C%2522prev%2522%253Afalse%257D&orientation=horizontal&type_sort=0&type_nb_items=%257B%2522top%2522%253Atrue%252C%2522bottom%2522%253Afalse%257D



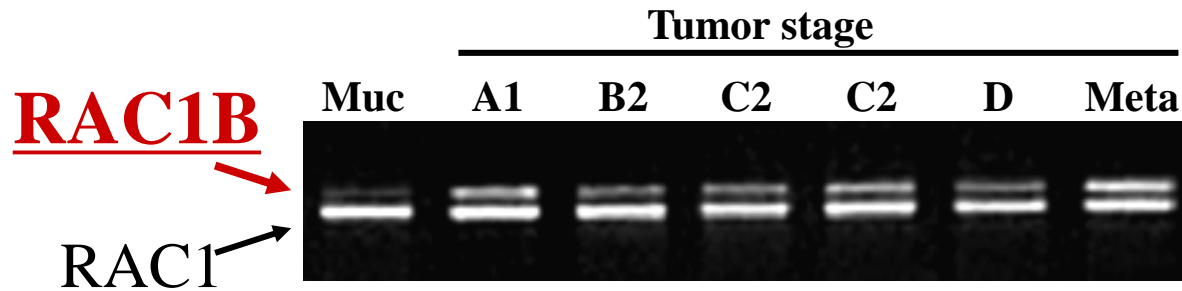
The Serrated pathway subtypes account for ~30% of all CRCs



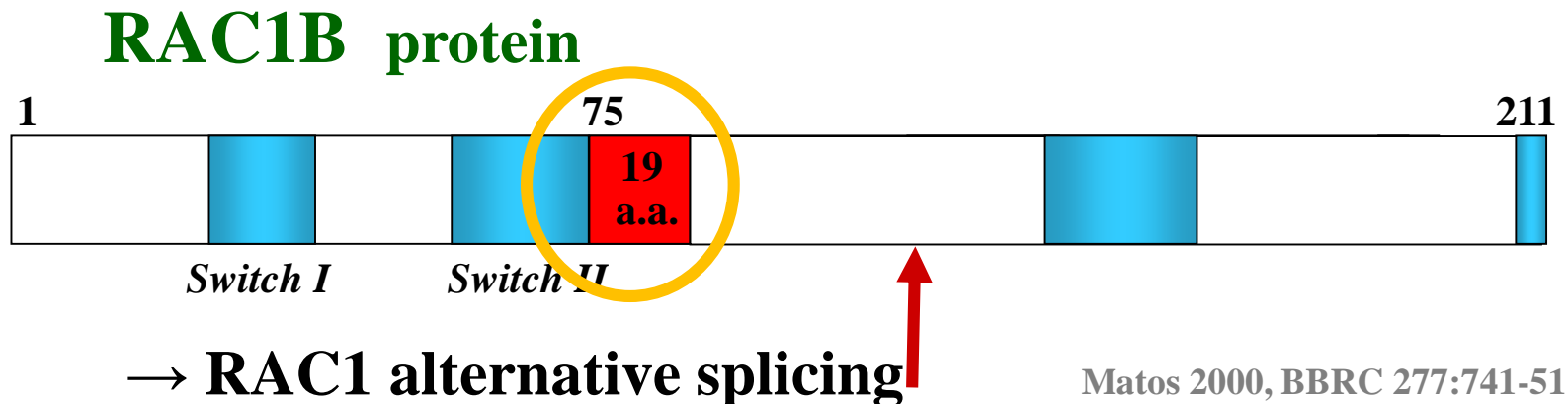
An alternative oncogenic pathway in CRC



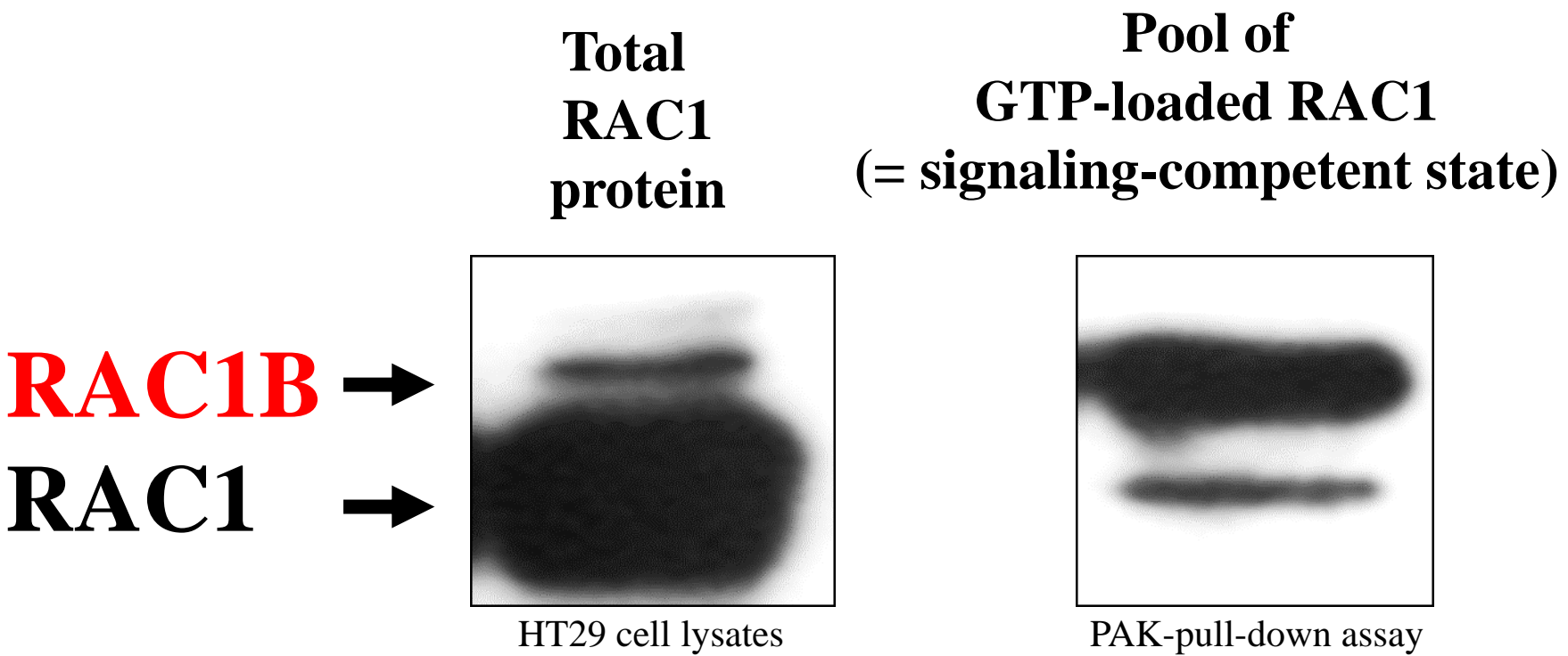
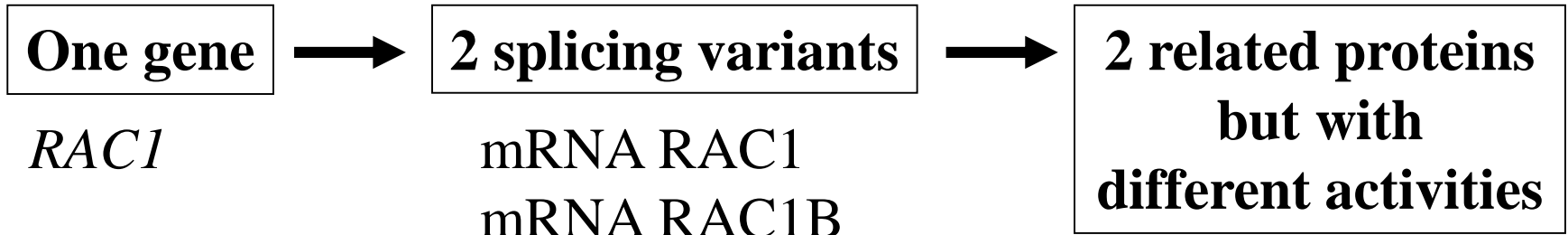
RAC1 expression in colorectal cancer (CRC): discovery of RAC1B



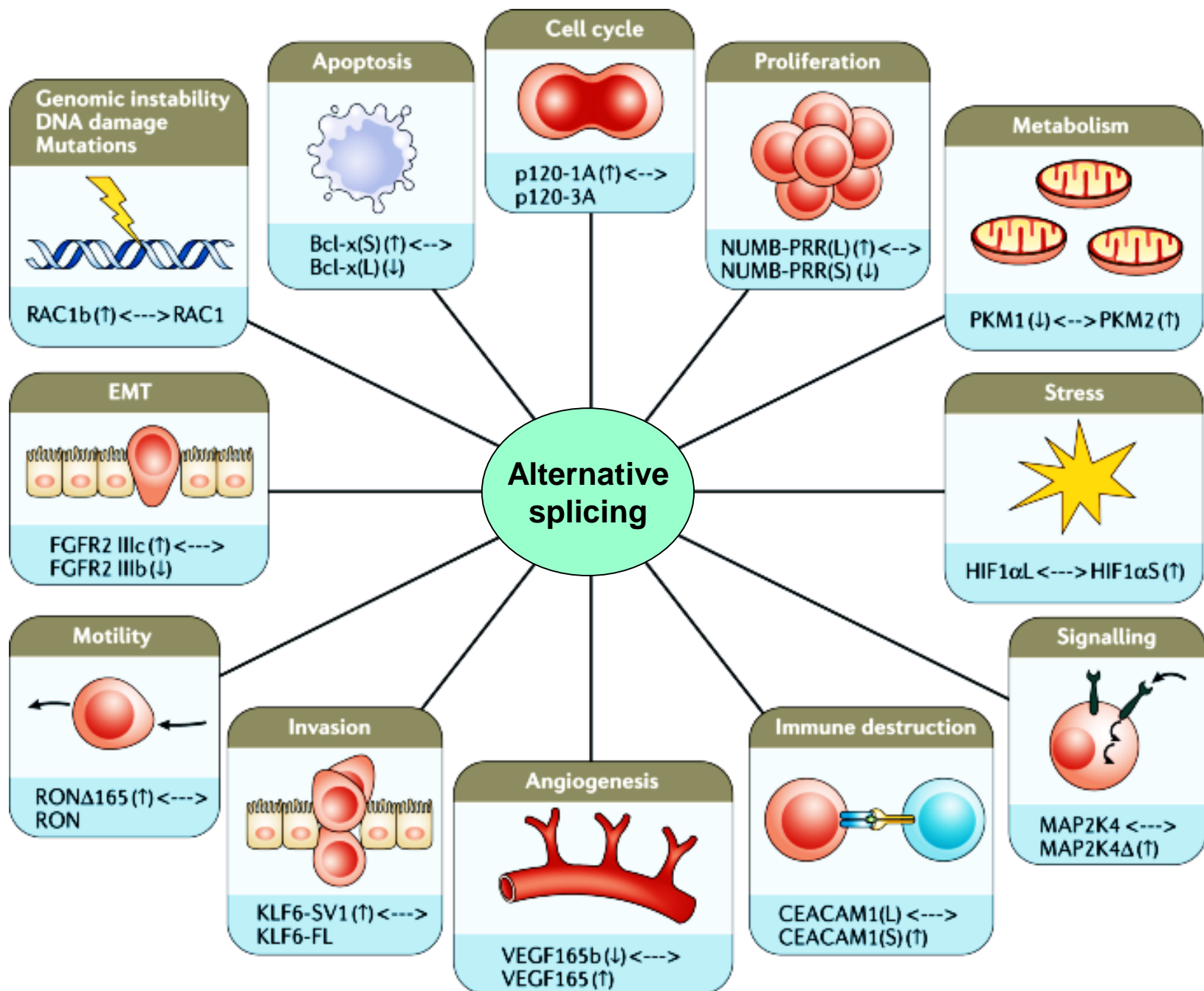
Jordan 1999, *Oncogene* 18:6835-39



Matos 2000, *BBRC* 277:741-51



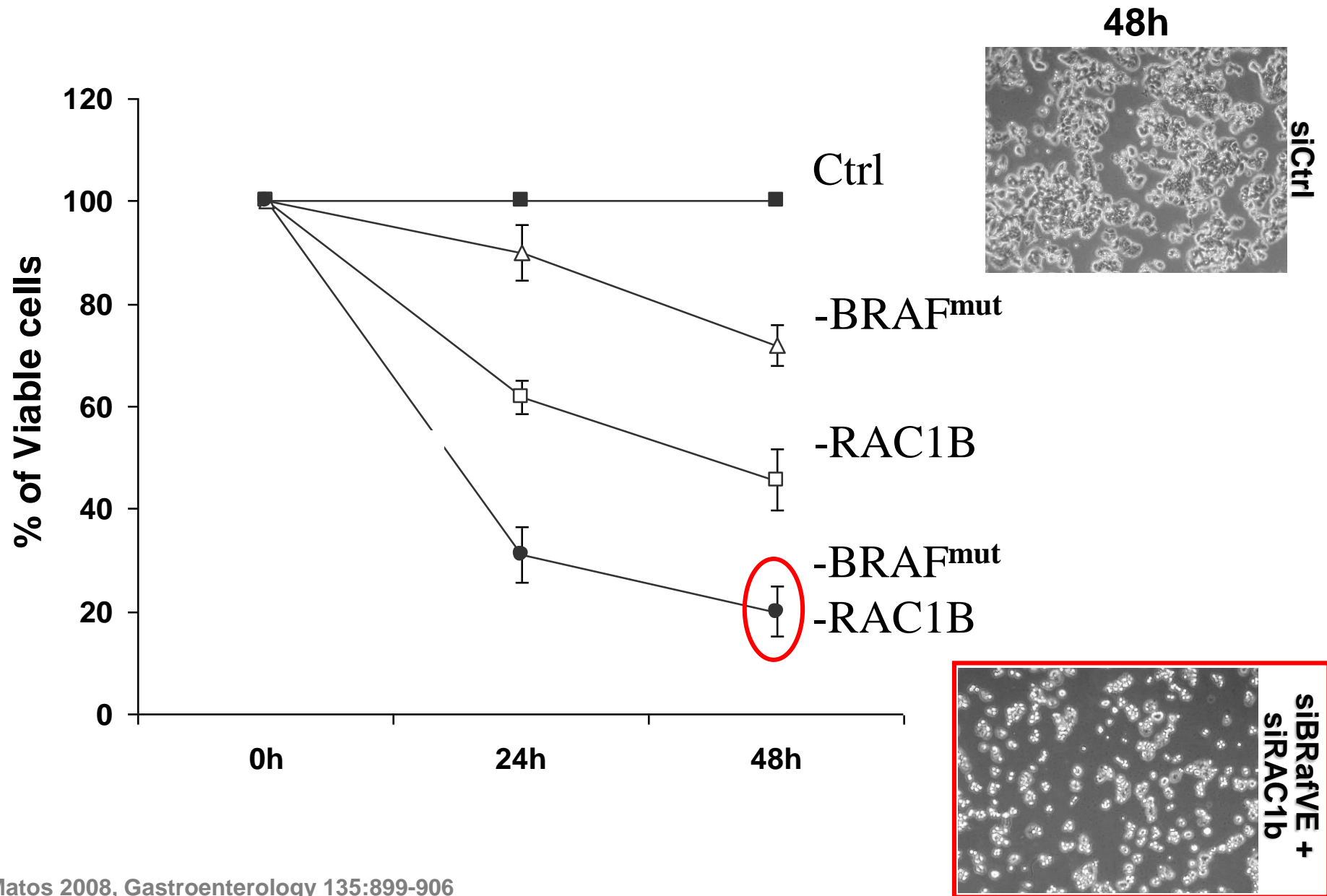
RAC1B predominantly in signaling-competent state



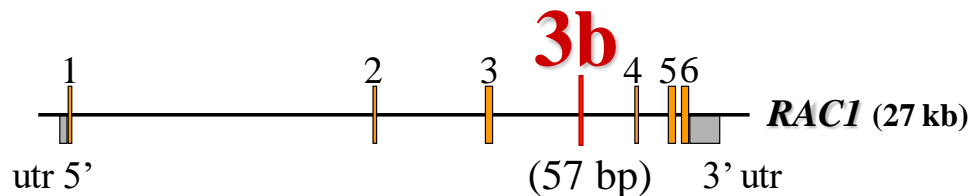
Main findings so far...

- **Transcription of the *RAC1* gene can generate two mRNAs by alternative splicing: RAC1 and RAC1B**
- **Overexpression of RAC1B occurs in ~80% of CRC tumors with mutation in *BRAF* (~10-15% of cases)**
- **RAC1B protein differs in regulation and signaling**
- **Overexpression of RAC1B cooperates with mutant BRAF to sustain the survival of colorectal tumor cells**

Cell viability after suppressing RAC1B and BRAF in HT29 cells

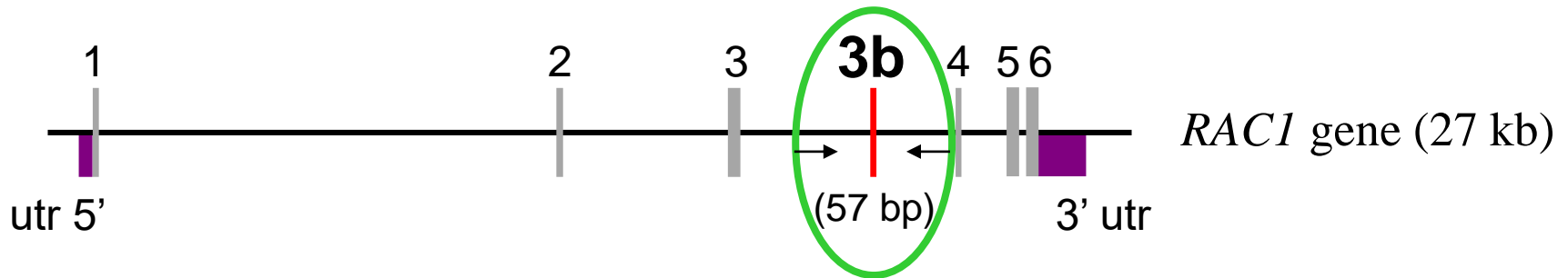
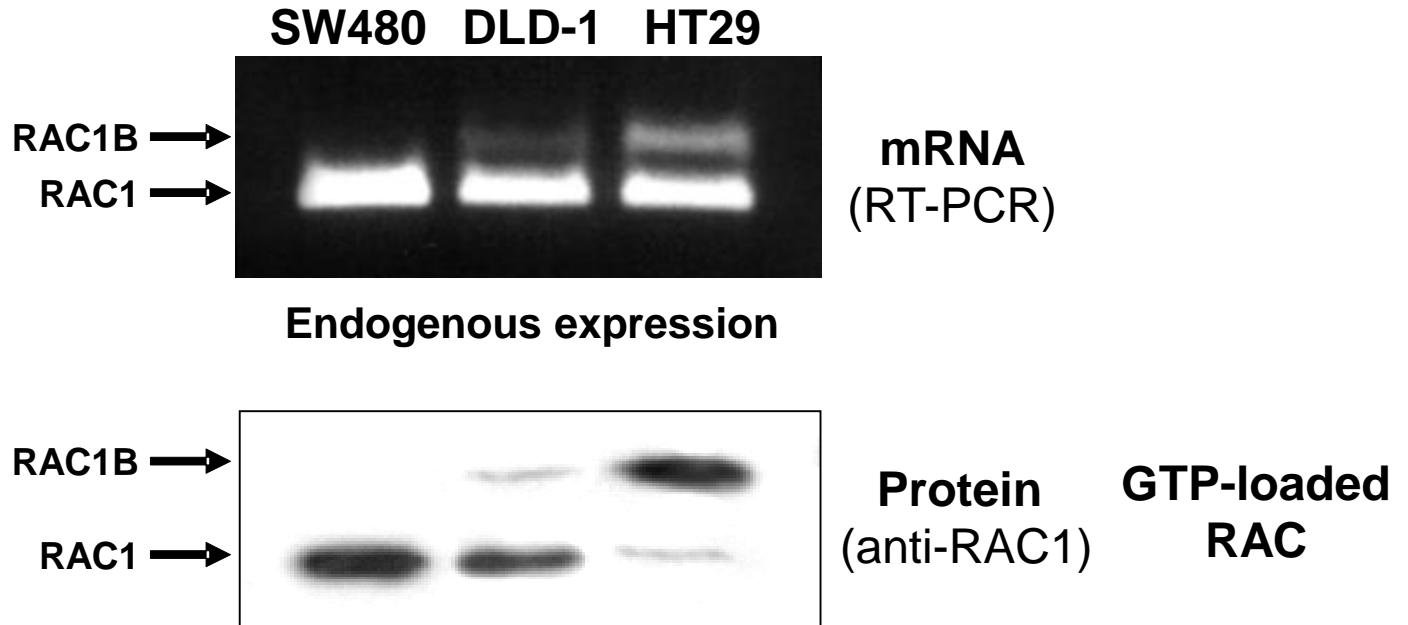


What leads to overexpression of RAC1B in colorectal tumor cells ?



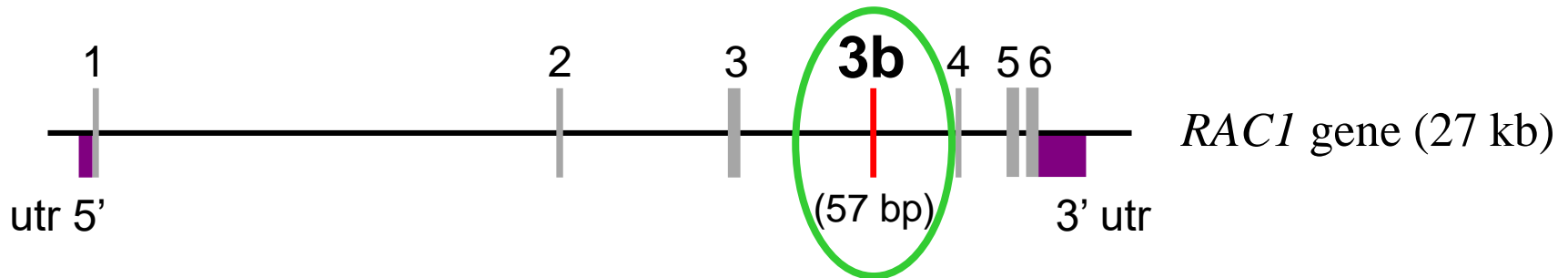
alternative splicing

Do genomic mutations cause increased exon 3b inclusion?

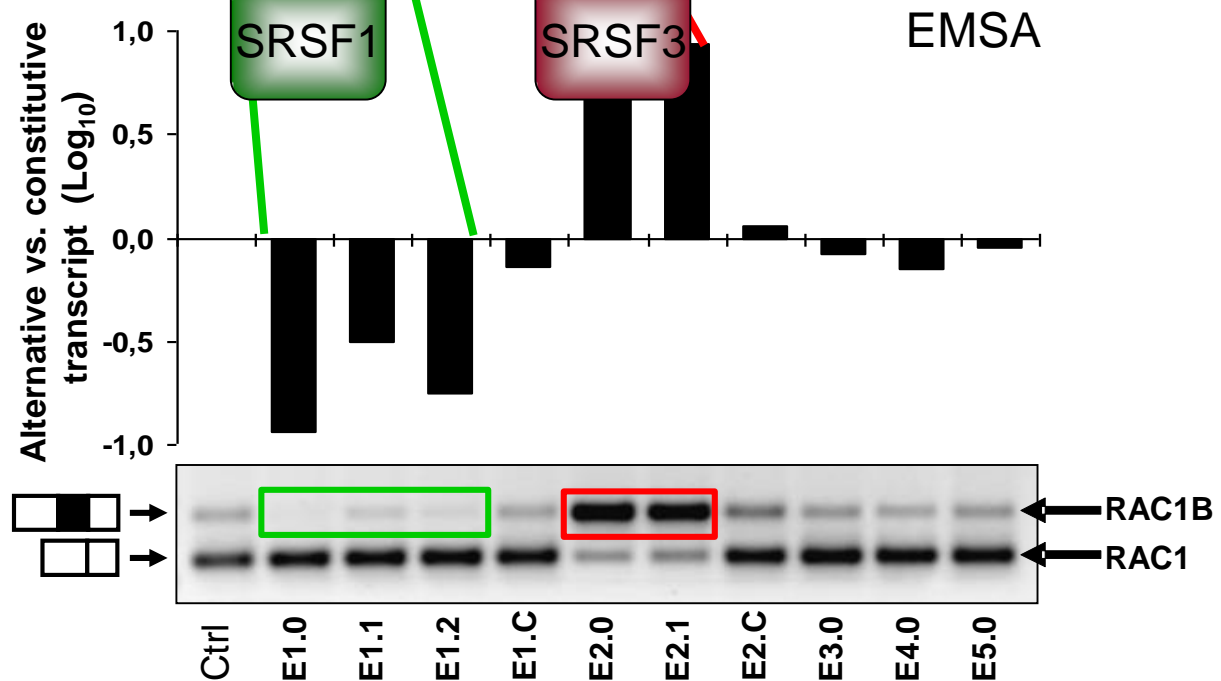
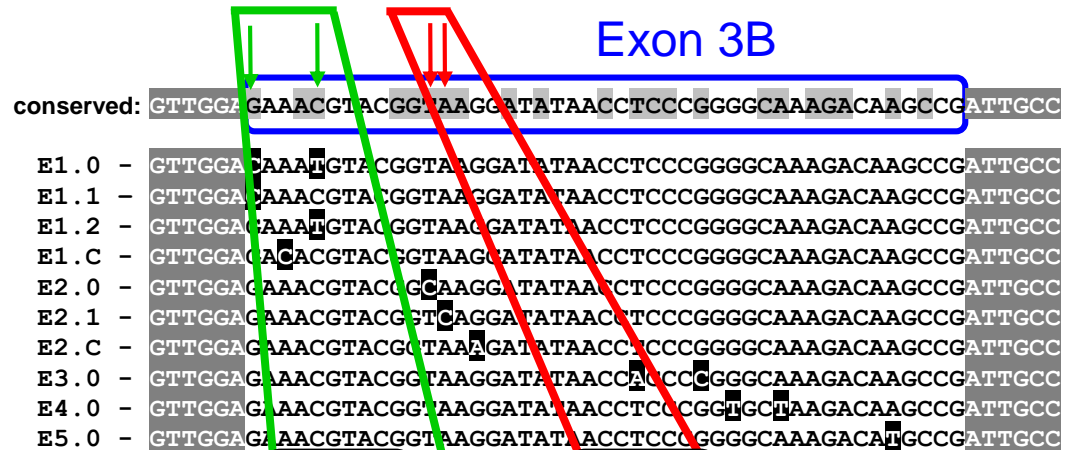
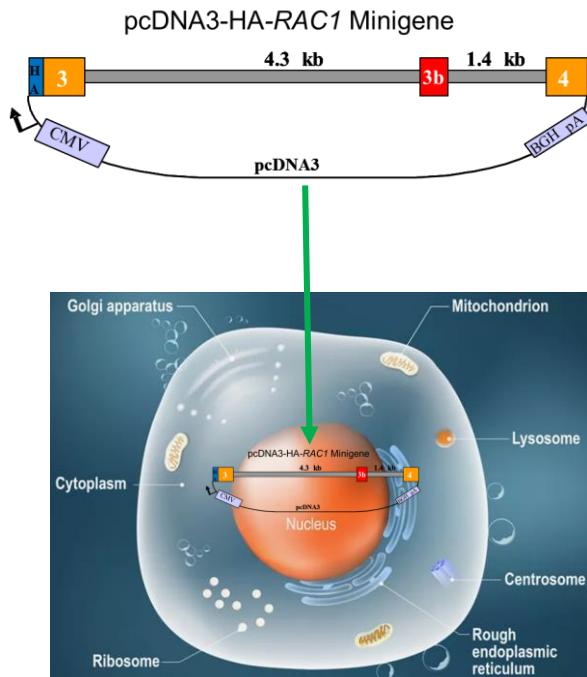


➔ Cell lines have identical genomic *RAC1* sequence

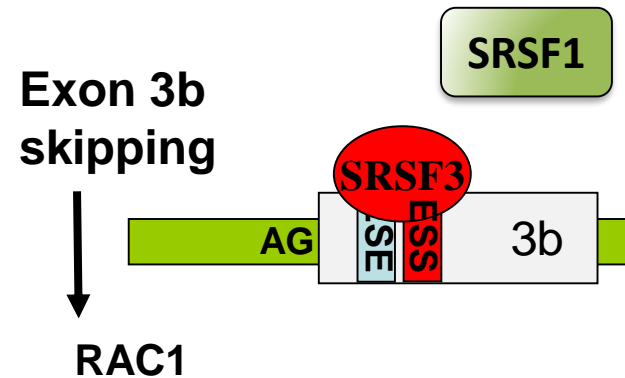
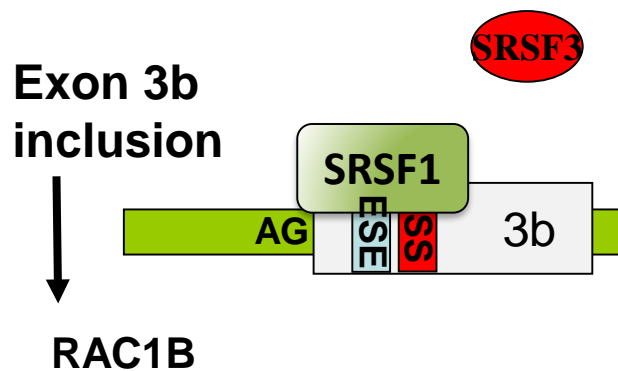
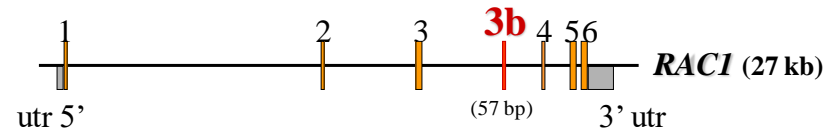
Instead,
alternative splicing of RAC1B
is regulated by
altered signalling mechanisms
in colorectal cancer cells



Two regulatory sequence elements determine exon 3b inclusion or skipping



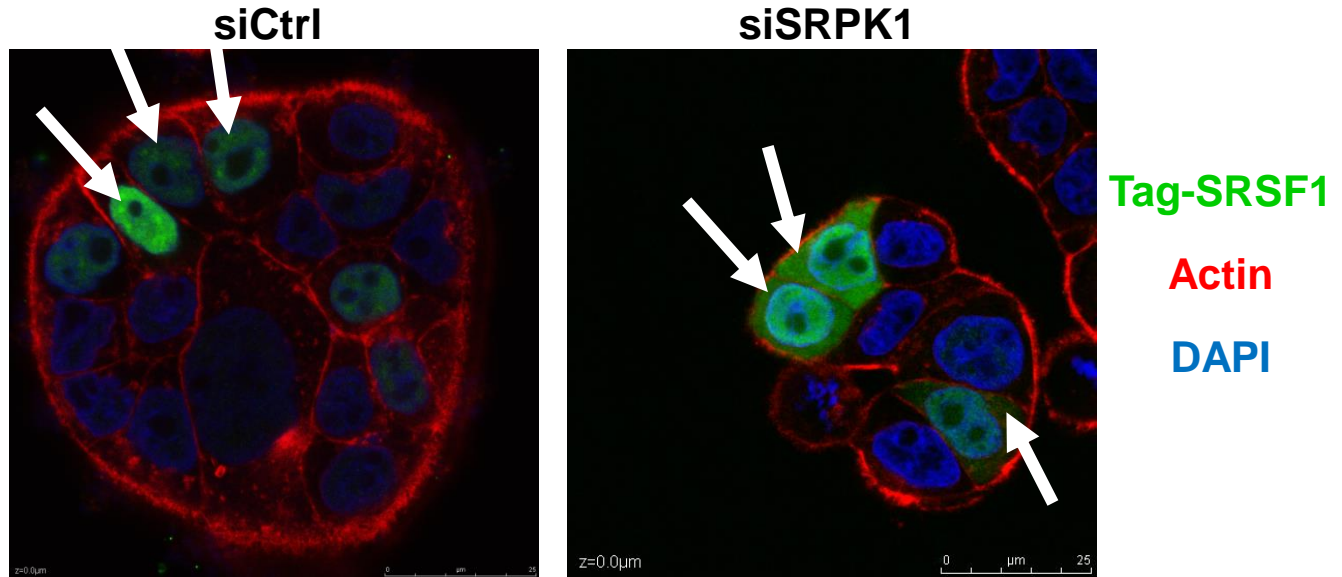
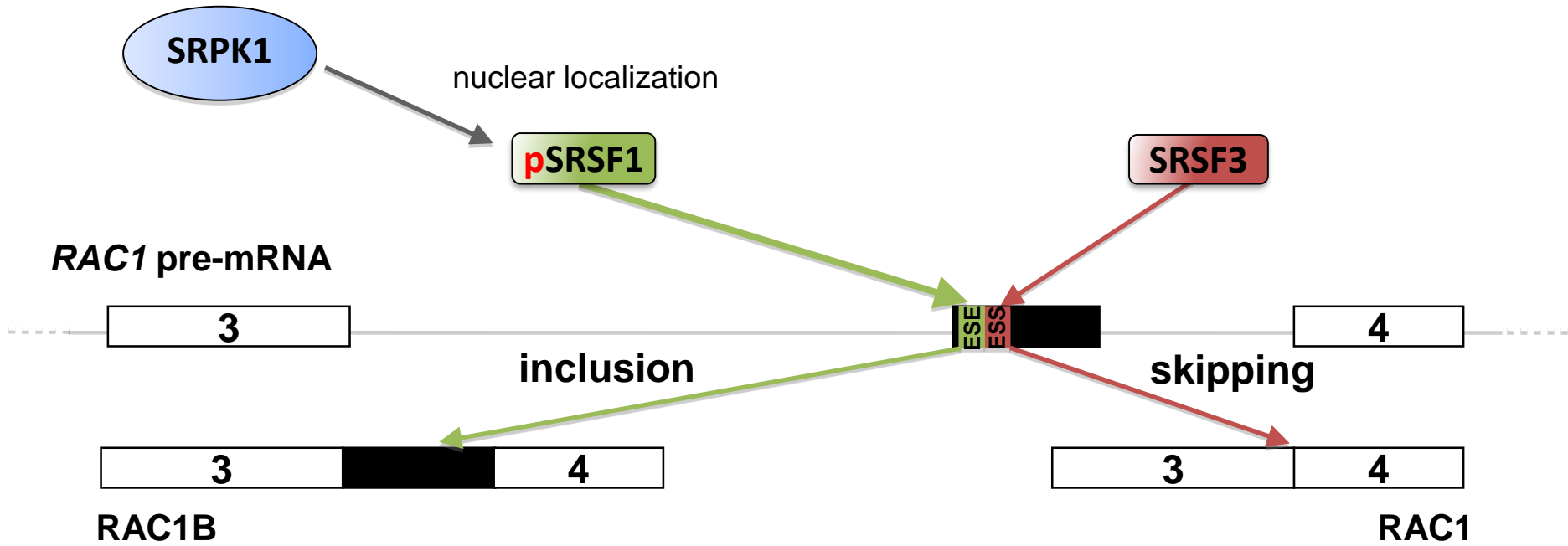
Model for regulation of *RAC1* alternative splicing in CRC



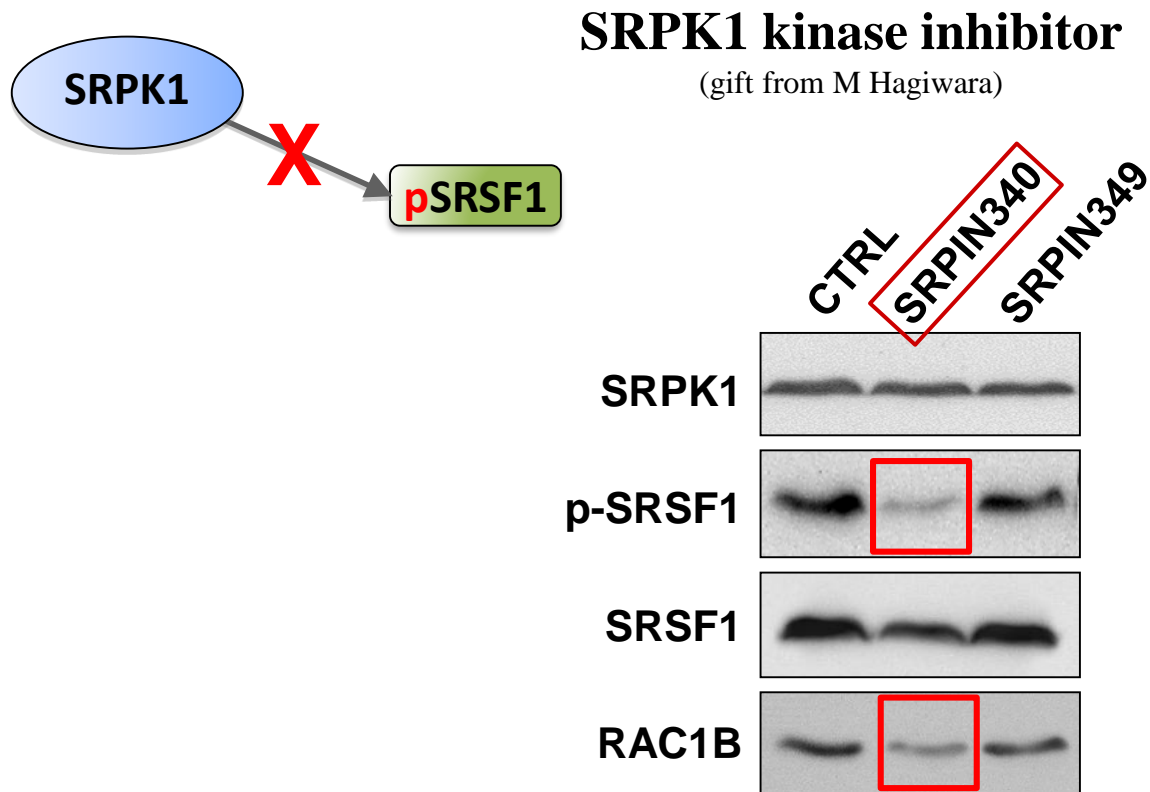
How is the binding of SRSF1 and SRSF3 regulated?

What is different in colorectal tumor cells?

Role of protein kinase SRPK1 in RAC1B overexpression

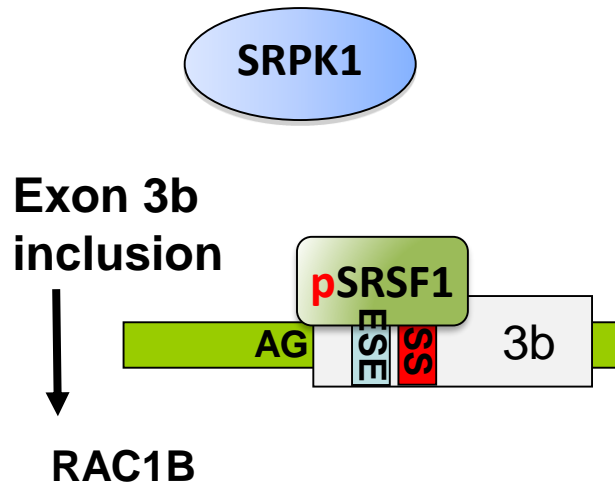
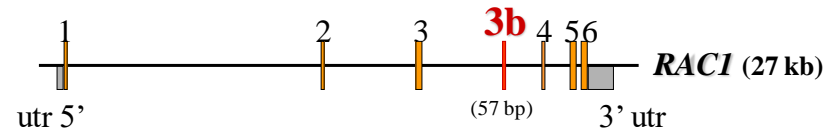


Role of protein kinase SRPK1 in RAC1B overexpression



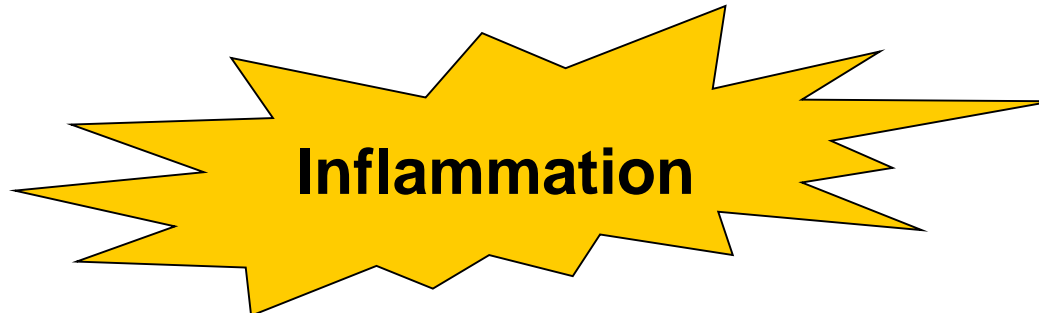
Inhibition of SRPK1 activity with small-molecule drugs decreased phosphorylation of SRSF1 and reversed the overexpression of RAC1B

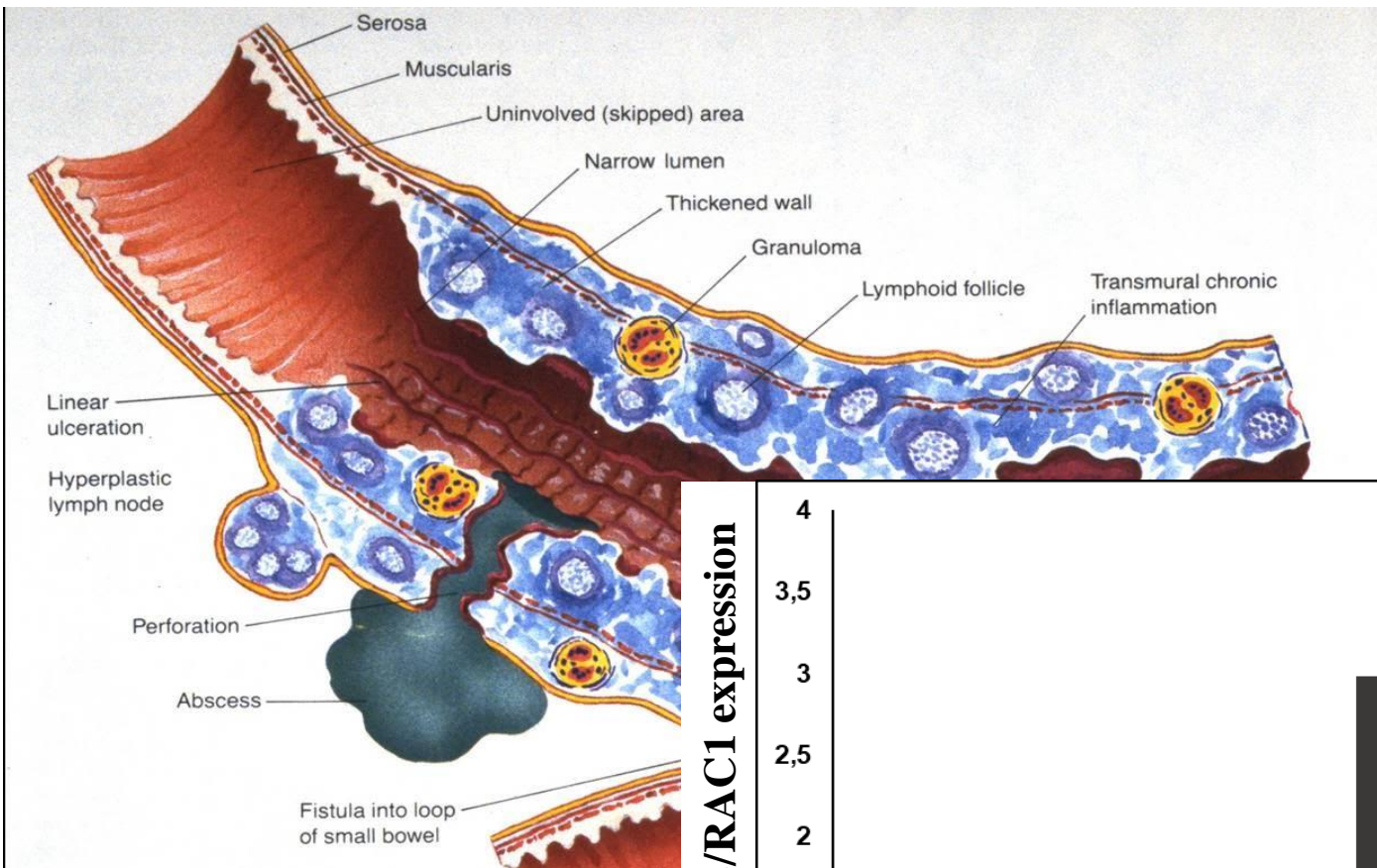
Model for regulation of *RAC1* alternative splicing in CRC



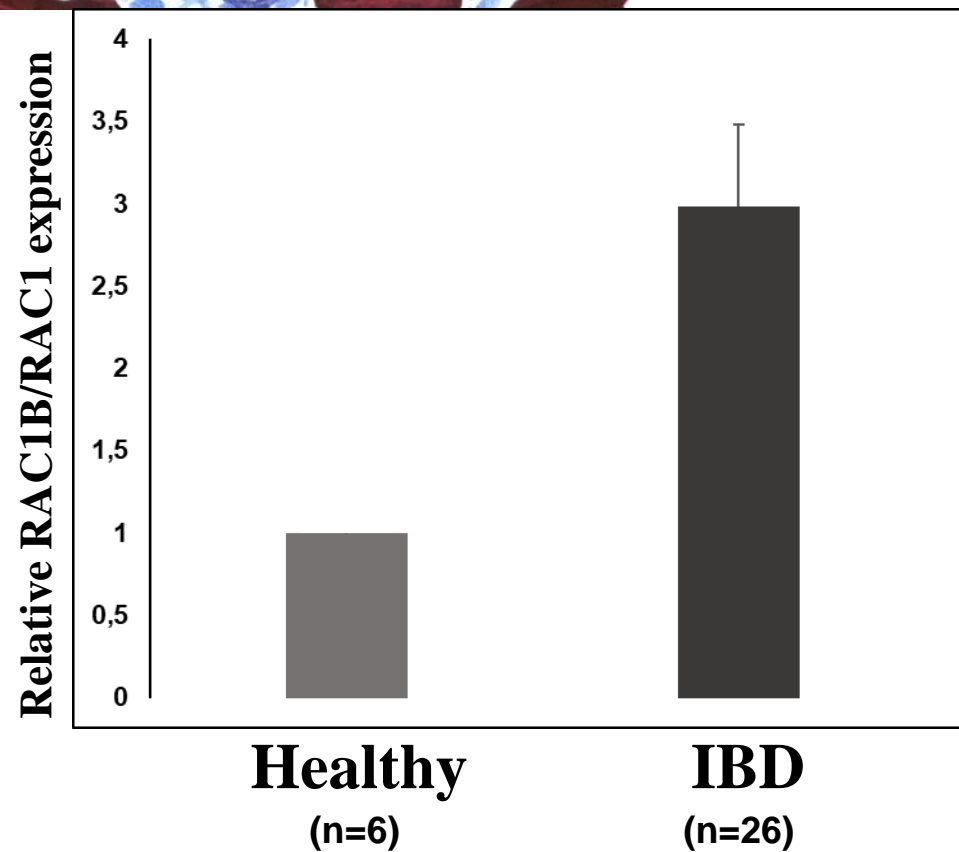
What determines the phosphorylation of SRSF1?

What is different in colorectal tumor cells?

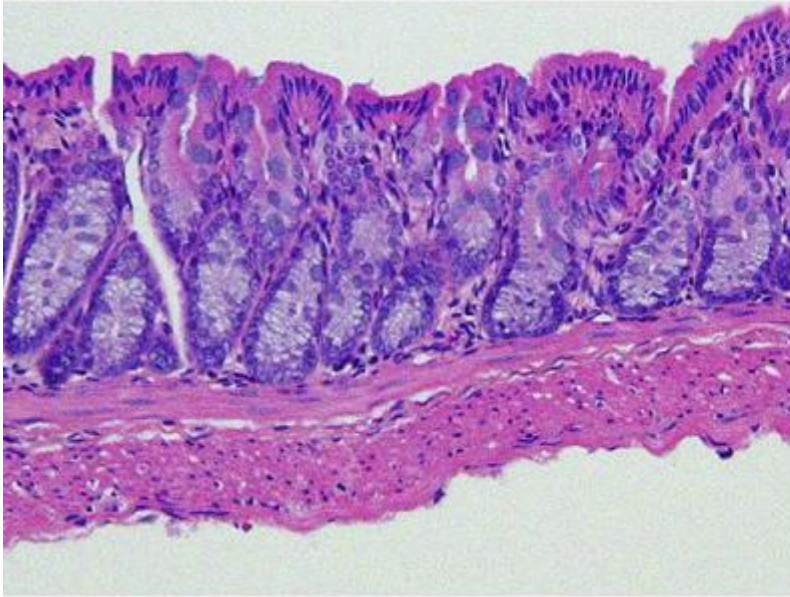




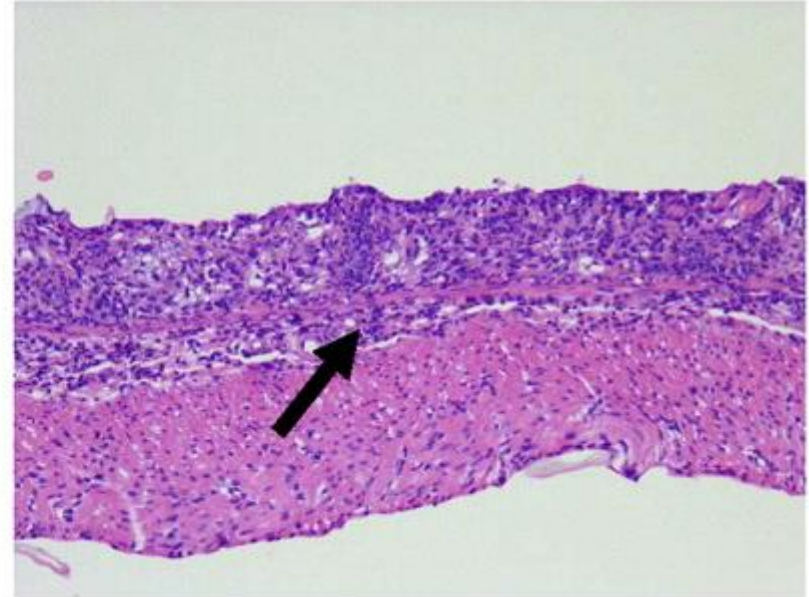
**RNA extracted
from 32
human IBD
samples**



Untreated Control



WT DSS

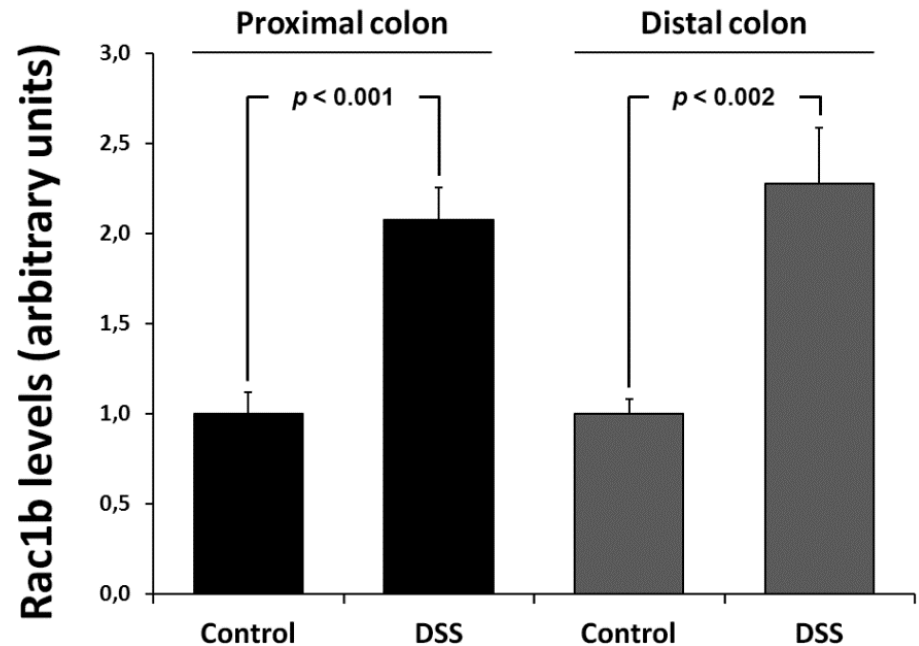


Colitis mouse model



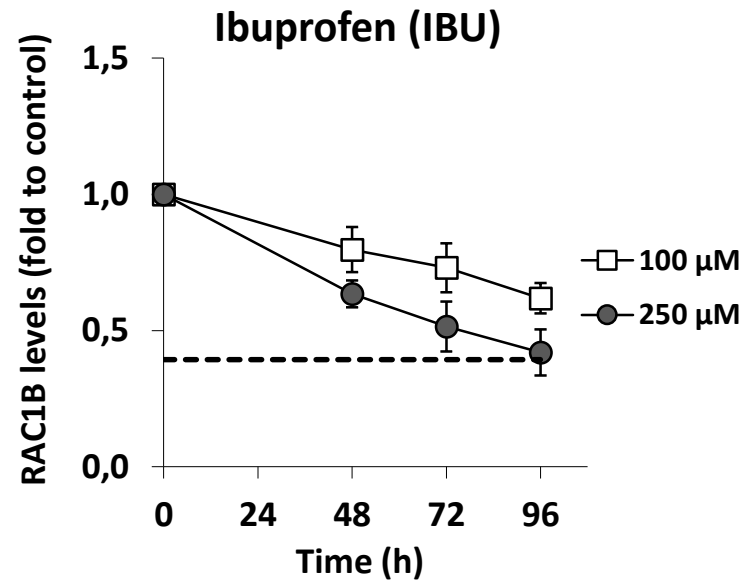
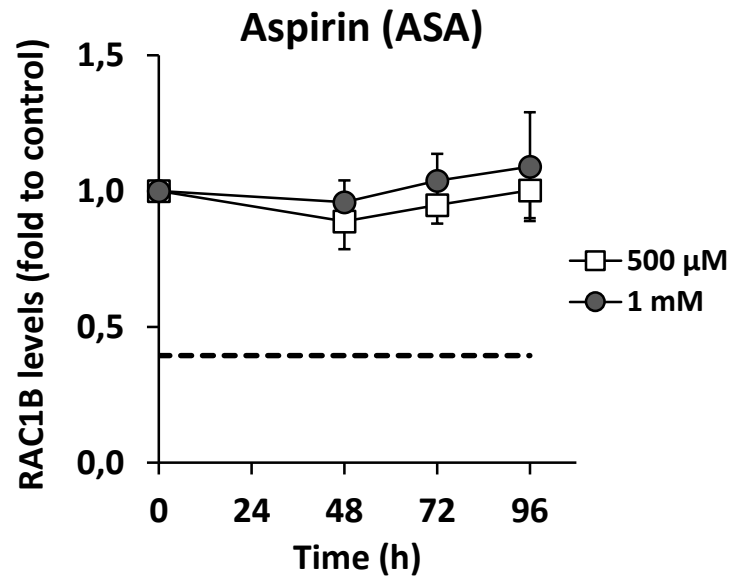
(mechanical mucosa irritation by DSS (dextran sulfate) in drinking water for 5 days)

(Collaboration Eric Chastre, INSERM Paris)



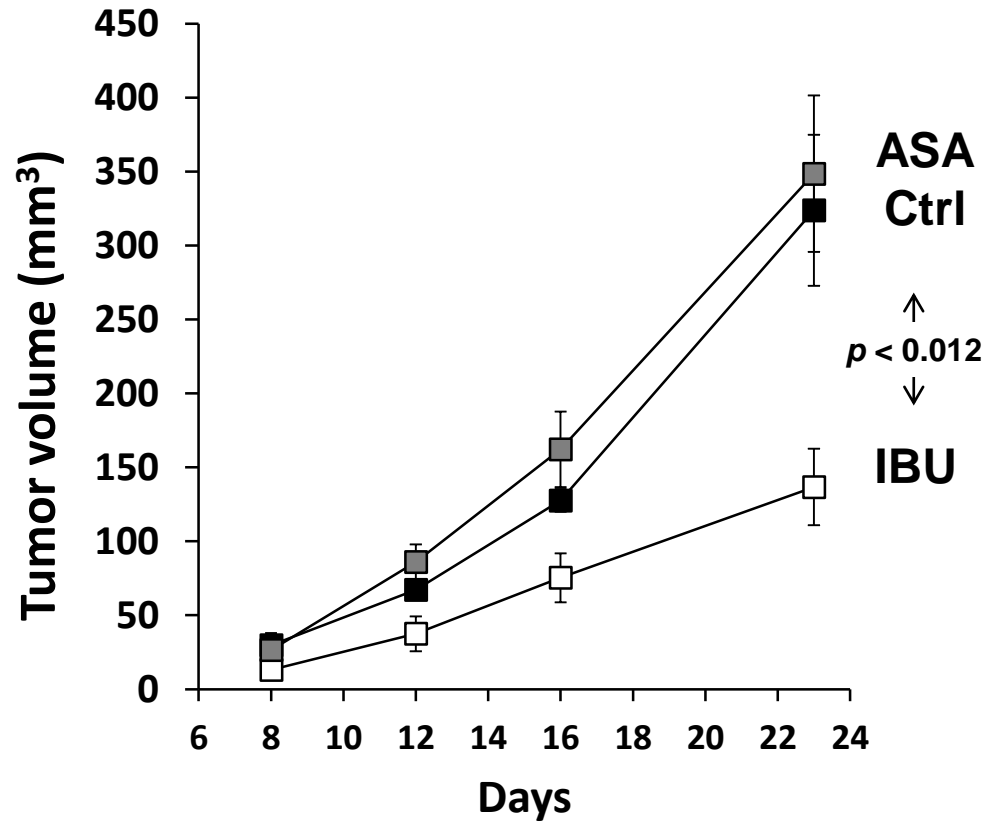
Effect of NSAIDs on RAC1B levels in HT29 cells

Neoplasia (2013) 15, 102–111

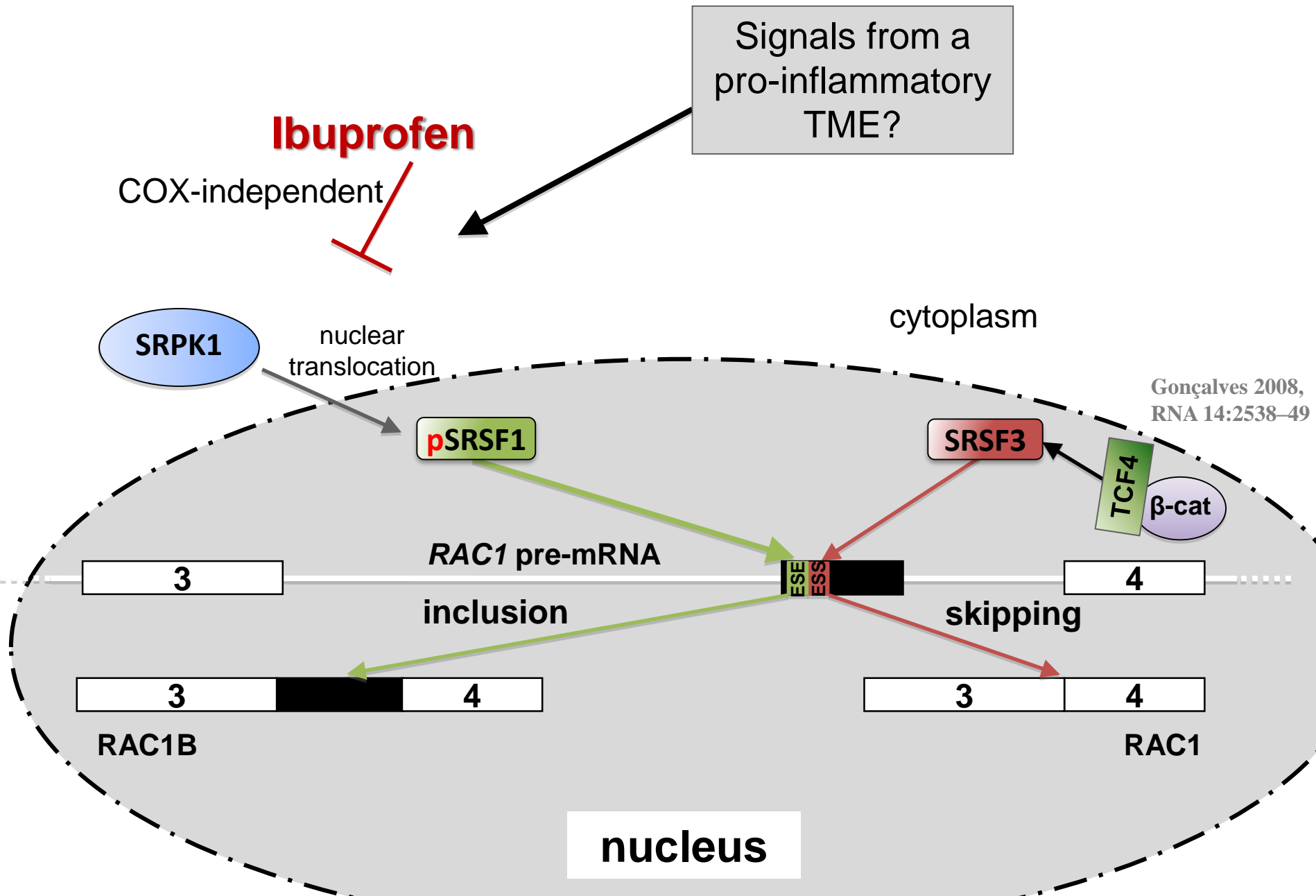


Tumors from xenografted HT29 cells*

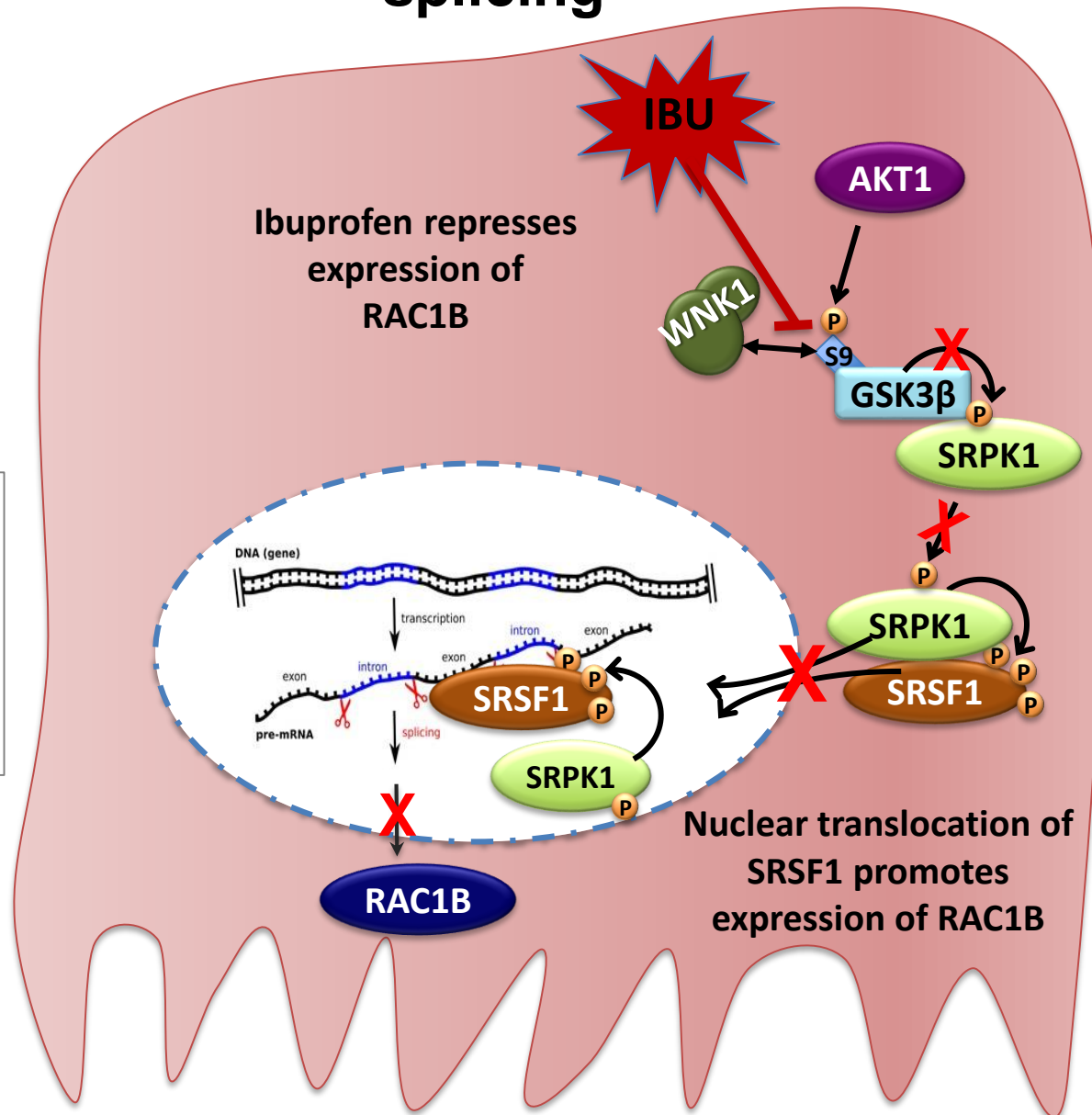
* BRAF mutant
RAC1B high



Signaling pathways regulating RAC1B splicing



Proposed model for how Ibuprofen interferes with RAC1B splicing



Current Pharmaceutical Design, 2015, 21, 2978-2982

Beyond Cox-Inhibition: 'Side-Effects' of Ibuprofen on Neoplastic Development and Progression

Summary

- **Ibuprofen inhibits the production of tumor-related RAC1B in colorectal cells by acting via splicing factor SRSF1**
- **A protein kinase complex of WNK1, GSK3 β and SRPK1 regulates SRSF1 and is the molecular target of Ibuprofen**
- **Ibuprofen could serve as adjuvant therapy to treat BRAF mutant and RAC1B overexpressing CRC (~10%)**
- **Pro-inflammatory signals from the microenvironment may induce RAC1B overexpression during tumorigenesis**

Acknowledgements

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