

Paulo Matos¹, Larissa Kotevelets², Vânia Gonçalves¹, Andreia Henriques¹, Philippe Zerbib³, Eric Chastre² and Peter Jordan¹

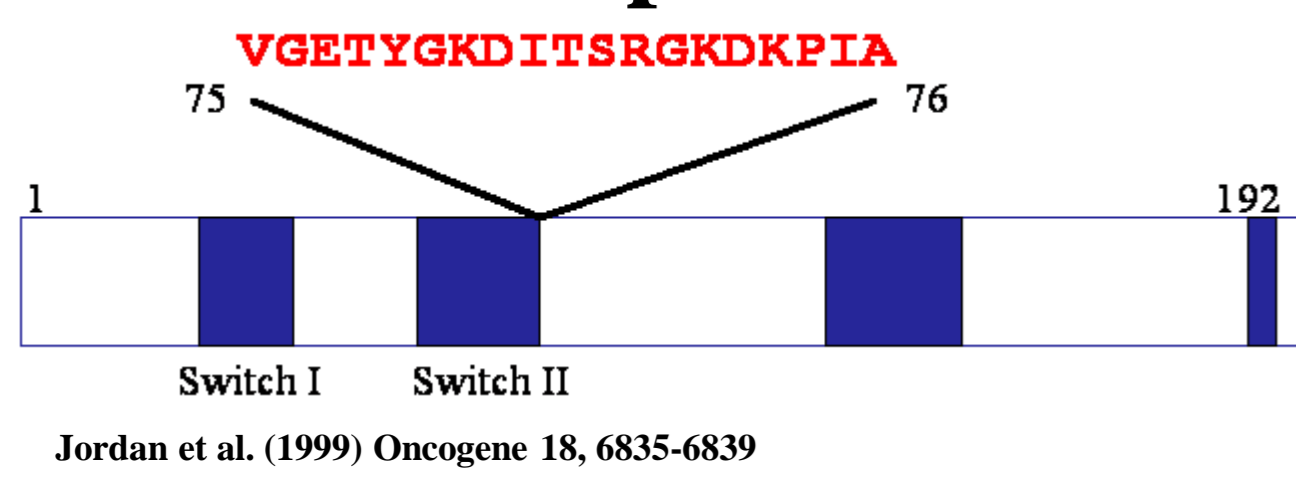
¹Department of Human Genetics, National Health Institute 'Doutor Ricardo Jorge', Lisbon, Portugal;

²INSERM U 773, Centre de Recherche Biomédicale Bichat Beaujon CRB3, Paris; ³Hôpital Claude Huriez, CHU Lille

SUMMARY The serrated pathway to colorectal tumor formation involves oncogenic mutations in the BRAF gene, which are sufficient for initiation of hyperplastic growth but not for tumour progression. The analysis of colorectal tumours revealed that overexpression of splice variant Rac1b occurs in around 80% of tumours with mutant B-Raf and both events were shown to cooperate in tumour cell survival. Here we provide evidence for increased expression of Rac1b in samples from inflammatory bowel disease patients as well as following experimentally induced colitis in mice. The increase of Rac1b in the mouse model was specifically prevented by the non-steroidal anti-inflammatory drug ibuprofen, which also inhibited Rac1b expression in cultured HT29 colorectal tumour cells through a cyclooxygenase inhibition-independent mechanism. Accordingly, the presence of ibuprofen led to a reduction of HT29 cell survival *in vitro* and inhibited Rac1b-dependent tumour growth of HT29 xenografts. Together, our results suggest that inflammation can trigger changes in Rac1b expression in the colon and identify ibuprofen as a highly specific and efficient inhibitor of Rac1b overexpression in colorectal tumours. Our data suggest that the use of ibuprofen may be beneficial in the treatment of patients with serrated colorectal tumours and in cancer prophylaxis for colon inflammation disorders.

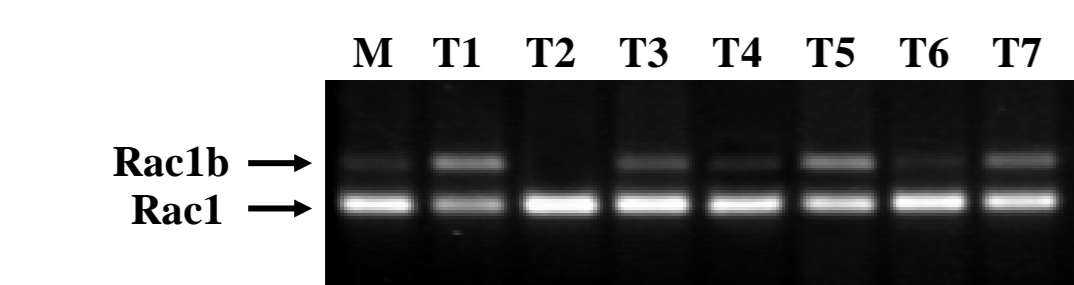
Introduction to previous work on Rac1b

Rac1b is a splice variant..



Jordan et al. (1999) *Oncogene* 18, 6835-6839

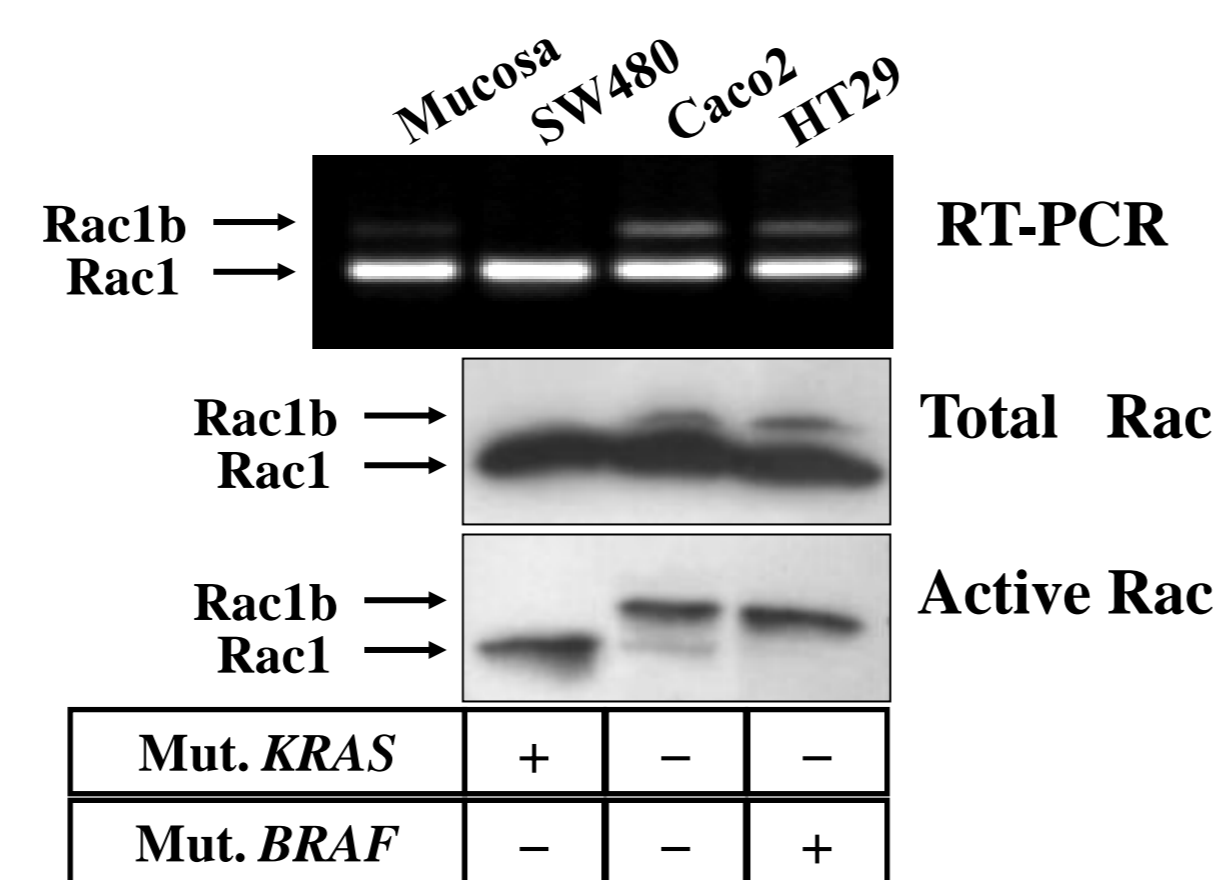
.. overexpressed in a subgroup of colorectal tumors



Mut. KRAS	-	-	+	-	-	-	+	-
Mut. BRAF	-	+	-	-	-	+	-	+

Matos et al. (2008) *Gastroenterology* 135, 899-906

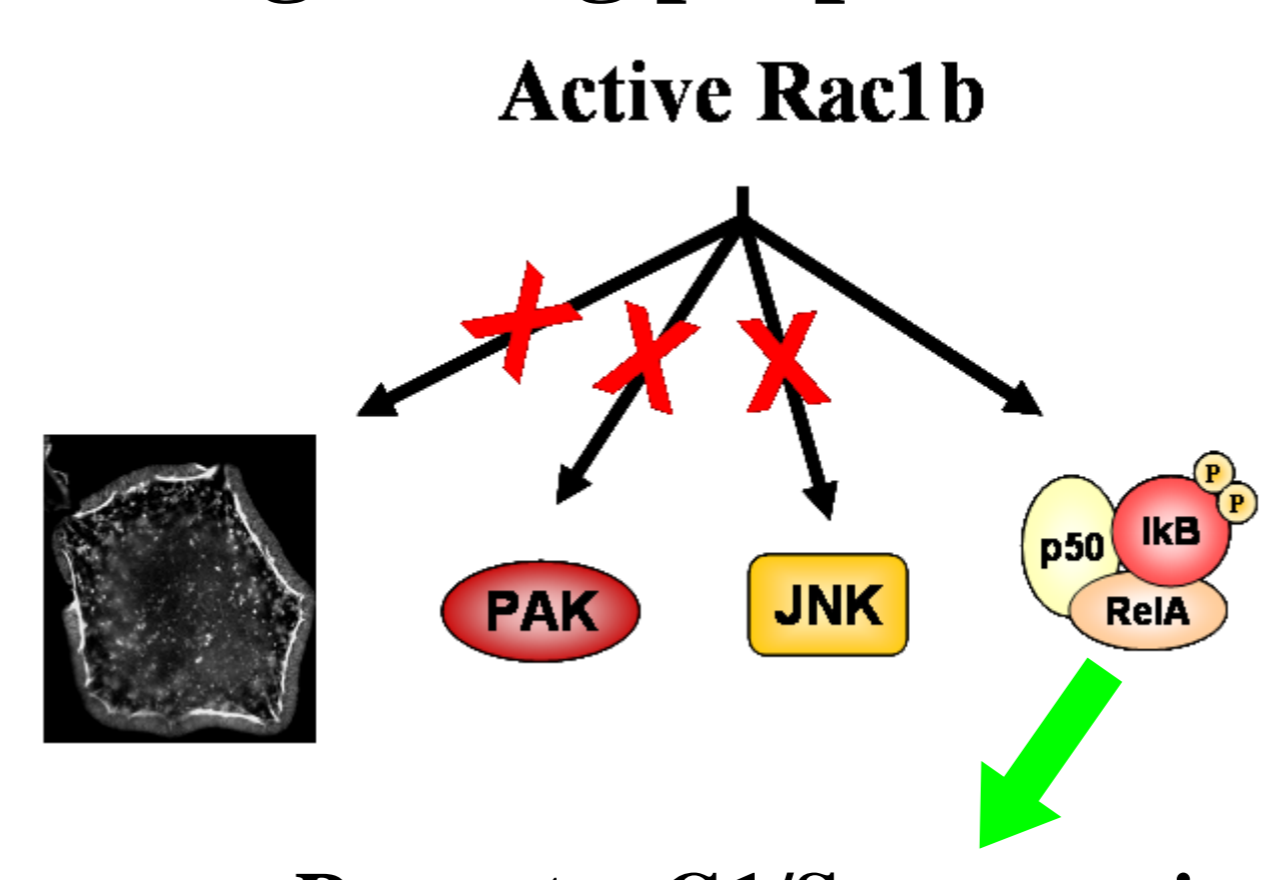
Endogenous Rac1b: little protein but highly active



Distinct properties of Rac1b:

- Increased GDP exchange rate *in vitro*
- Rho-GDI cannot downregulate Rac1b *in vivo*

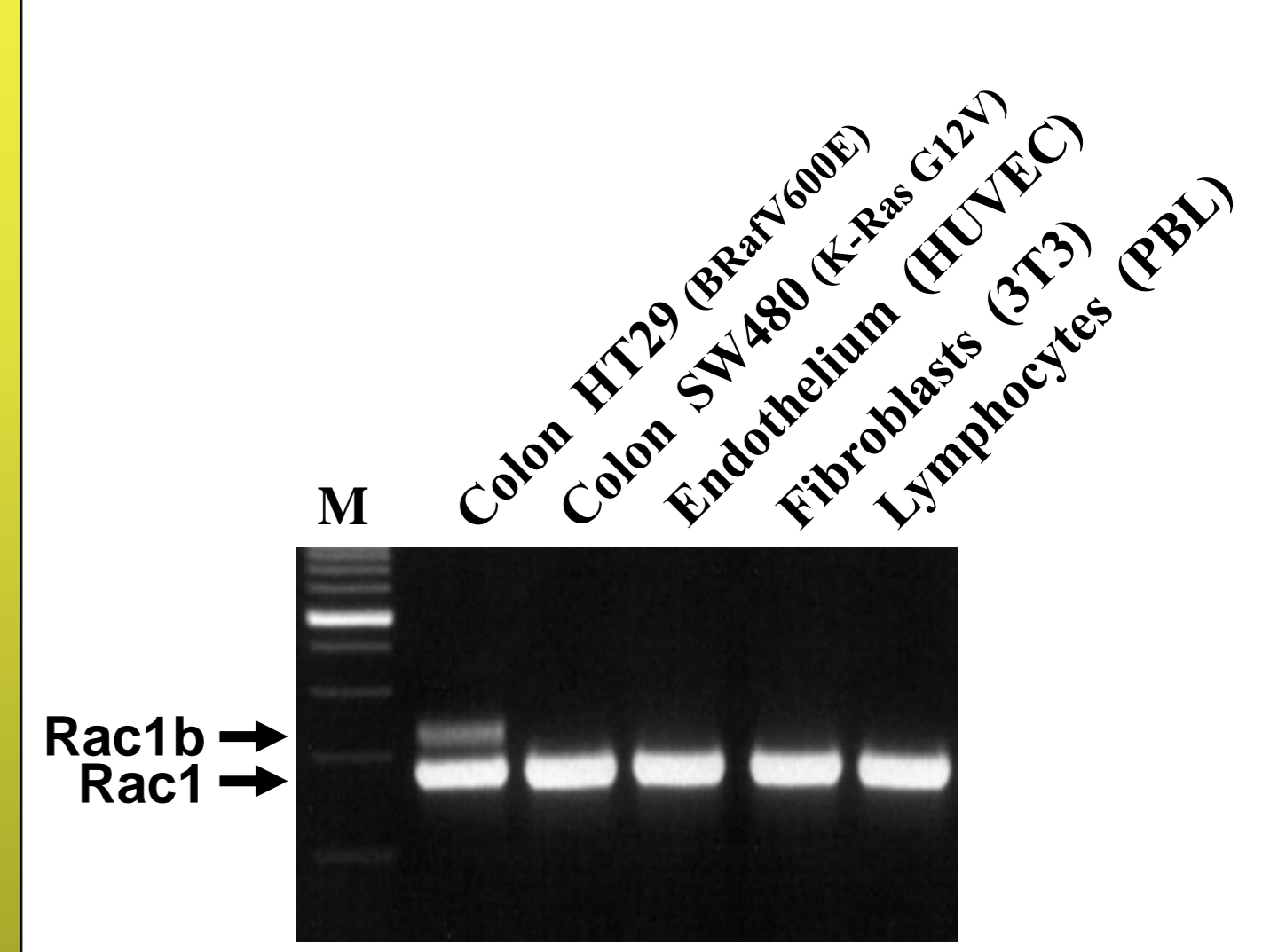
Selective downstream signalling properties



Promotes G1/S progression and cell survival

Matos & Jordan (2005) *Exp Cell Res* 305, 292-299;
 Matos & Jordan (2008) *Mol Cancer Res* 6, 1178-84

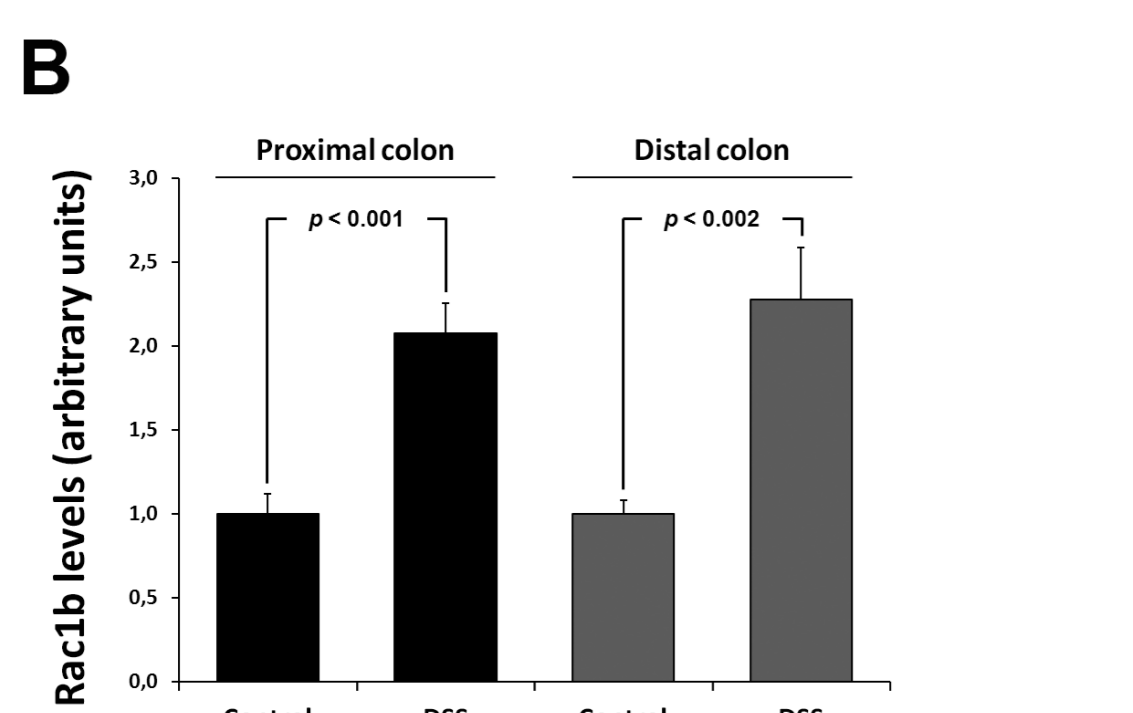
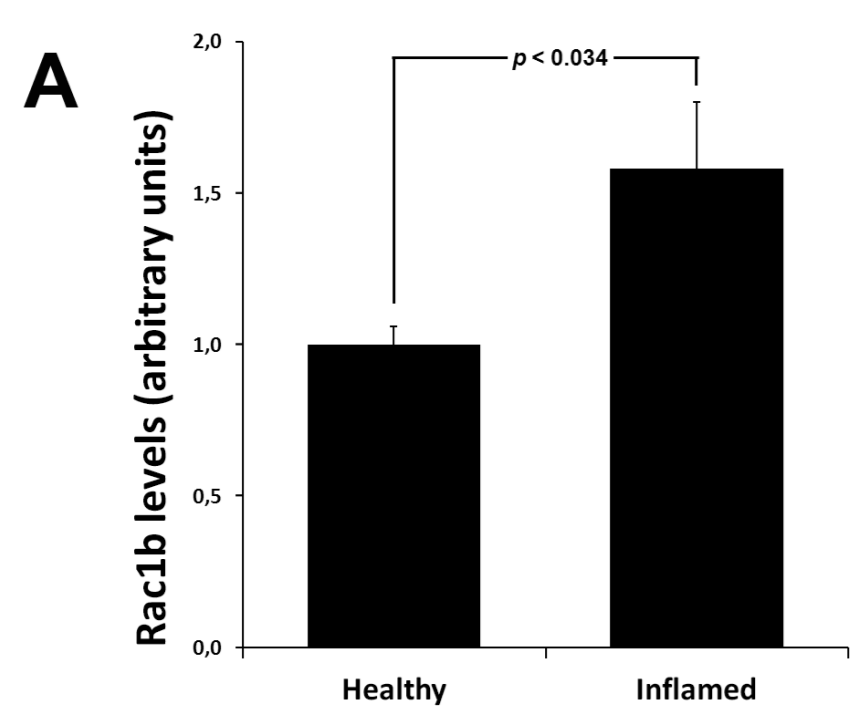
Rac1b is expressed in colon epithelium, not stroma



New data: colon inflammation and increased expression of Rac1b

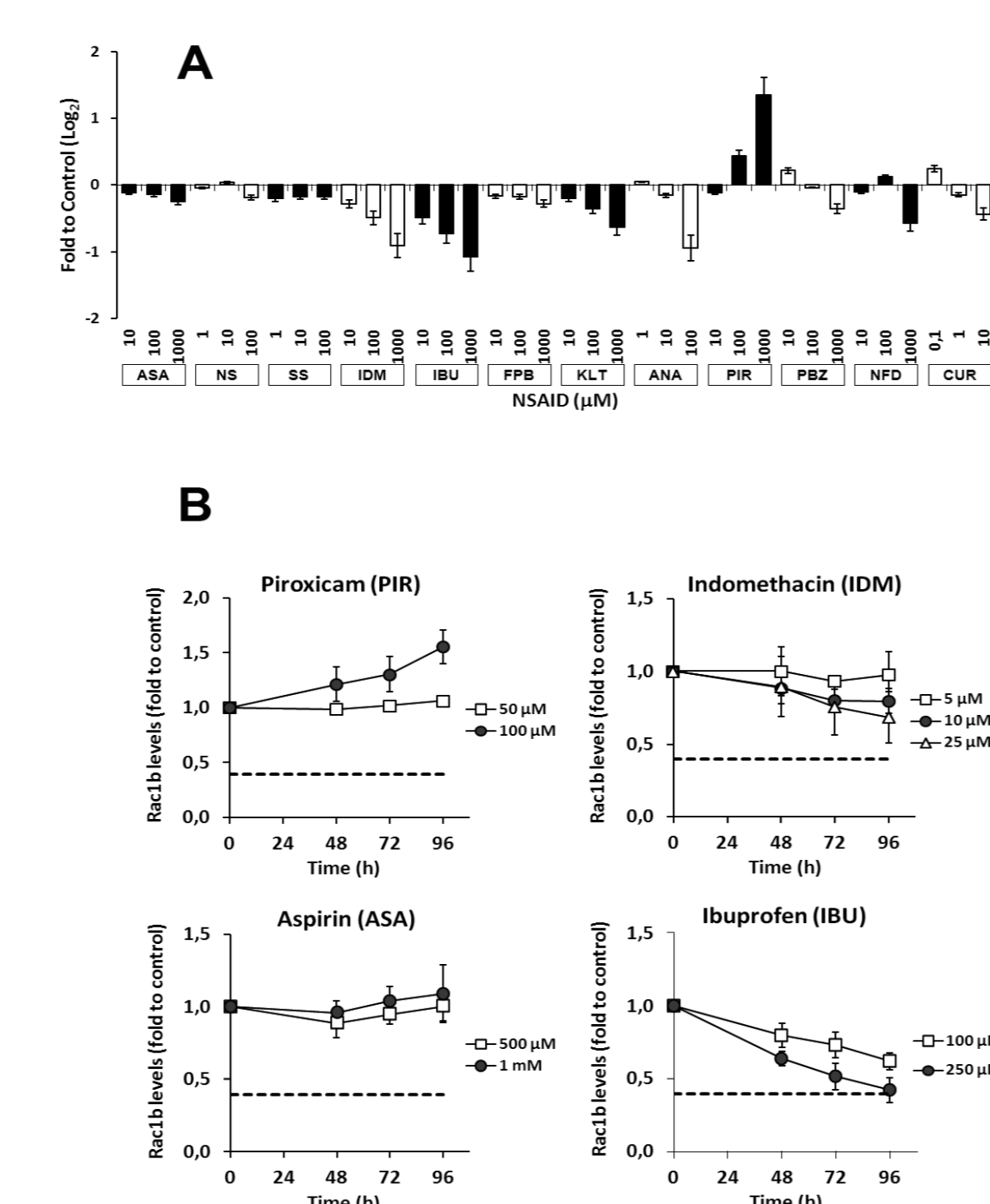
Inflammation leads to increased expression of Rac1b

Rac1b levels found by qPCR quantification in:
 A) samples from patients with inflammatory bowel disorders;
 B) mouse colon samples following dextran sulfate (DSS)-induced acute intestinal inflammation



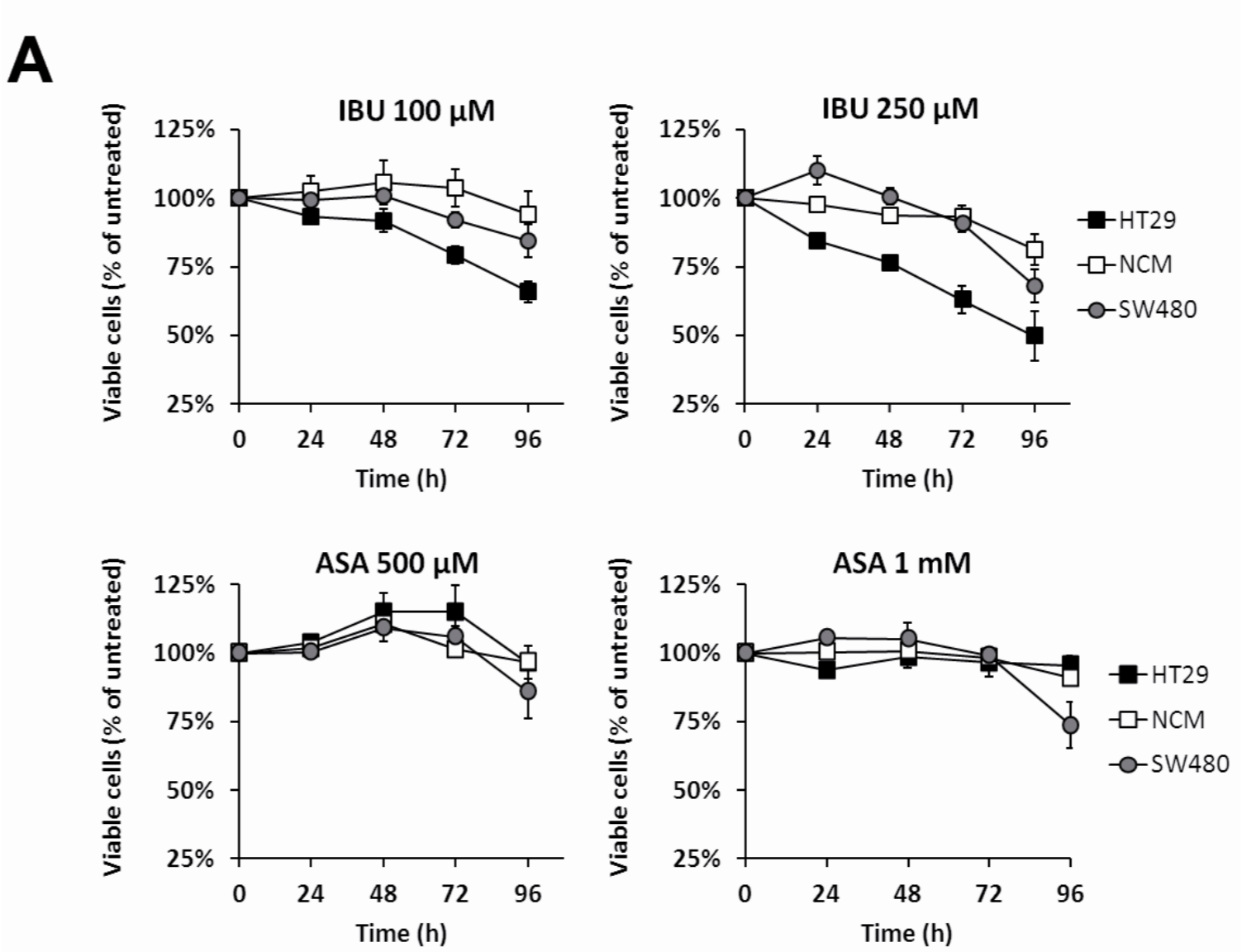
Ibuprofen inhibits endogenous Rac1b expression in HT29 cells

Rac1b levels were quantified by qPCR in HT29 cells after treatment with
 A) the indicated NSAID concentrations for 48 h;
 B) clinically relevant concentrations of selected NSAIDs for up to 96 h



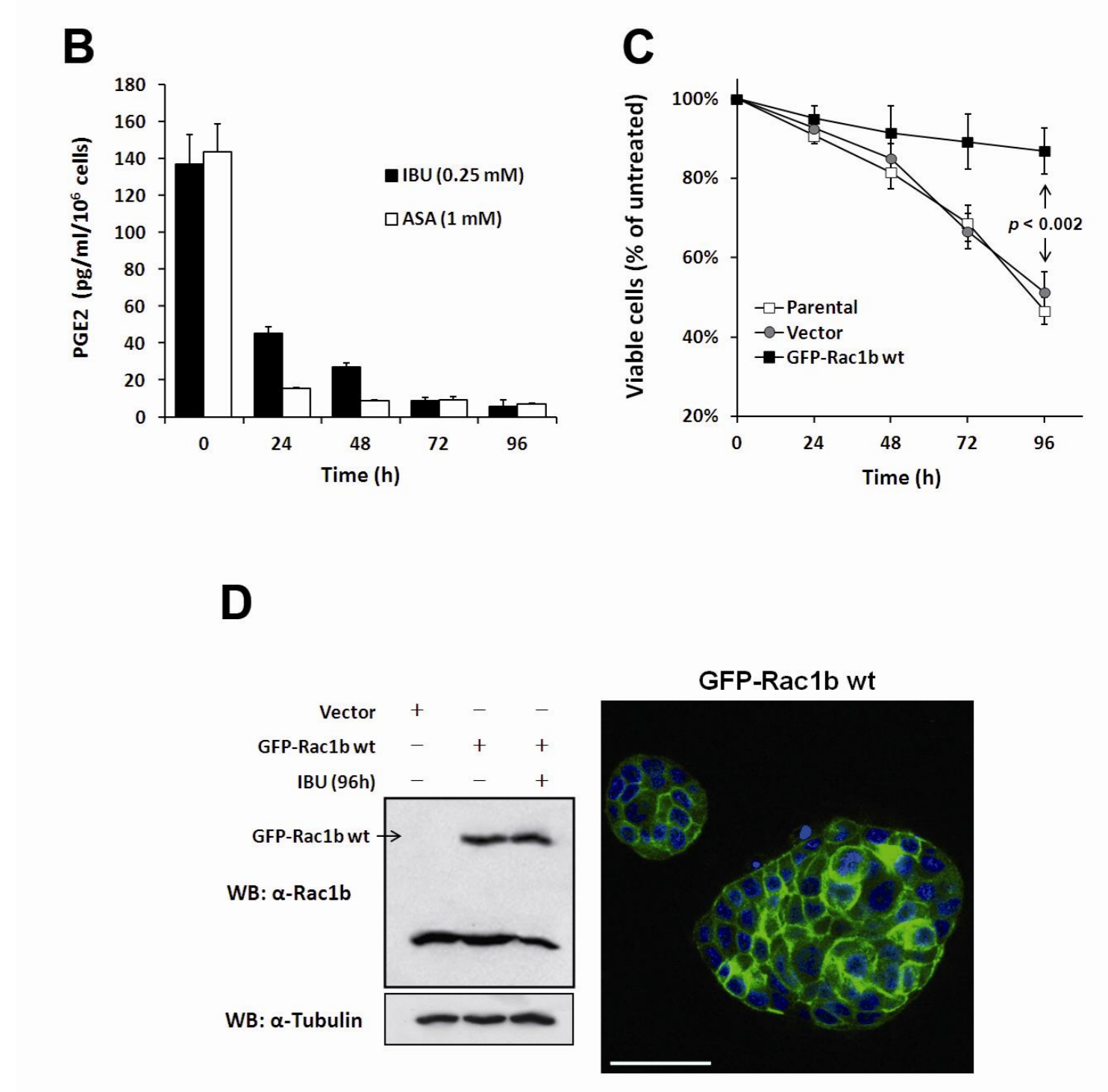
Ibuprofen but not aspirin decreases cell survival of Rac1b expressing HT29 cells

Rac1b expressing cells were previously shown to require Rac1b for sustaining their cell survival. Ibuprofen is shown not only to decrease Rac1b levels but also to inhibit the survival of Rac1b-overexpressing HT29 cells, but not of the Rac1b-lacking SW480 cells. Aspirin had no effect.

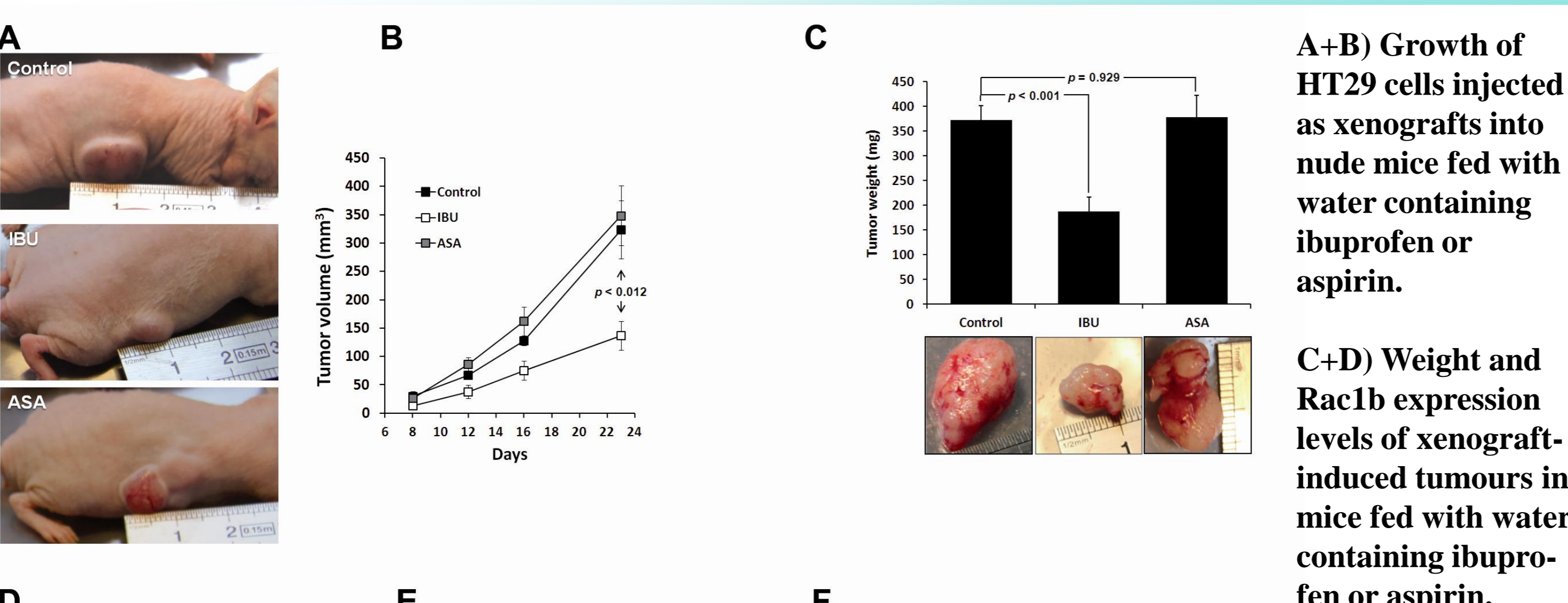


Ibuprofen inhibits cell survival via Rac1b and independent of Cox-2

B) Aspirin and ibuprofen cause comparable Cox-2 inhibition in HT29 cells;
 C+D) Transfection with GFP-Rac1b rescues HT29 cell survival in the presence of ibuprofen

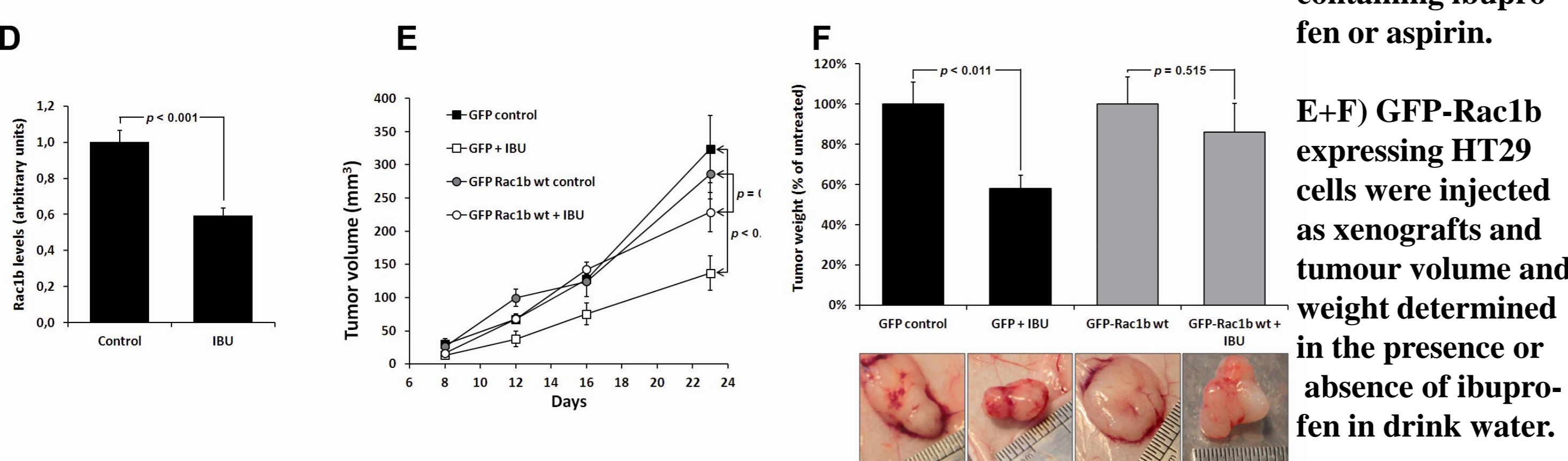


Ibuprofen inhibits growth of Rac1b-associated tumours



A+B) Growth of HT29 cells injected as xenografts into nude mice fed with water containing ibuprofen or aspirin.

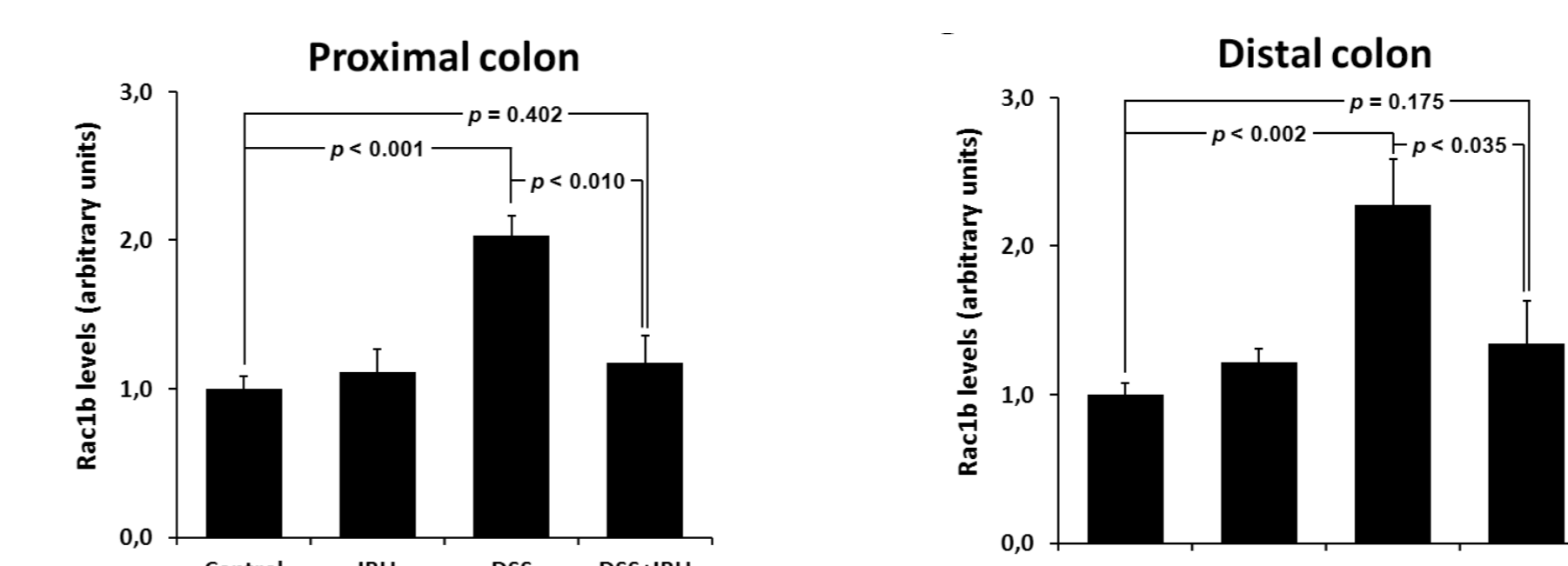
C+D) Weight and Rac1b expression levels of xenograft-induced tumours in mice fed with water containing ibuprofen or aspirin.



E+F) GFP-Rac1b expressing HT29 cells were injected as xenografts and tumour volume and weight determined in the presence or absence of ibuprofen in drink water.

Colitis-induced Rac1b expression in DSS-treated mice is prevented by ibuprofen

Ibuprofen fed in drinking water prevents the increase in Rac1b expression during DSS-induced acute colitis in mice.



Rac1b expression levels were determined by qPCR in the proximal and distal colon segments of mice sacrificed at day 7.

Conclusions:

- Inflammatory conditions trigger increased expression of splice variant Rac1b;
- Ibuprofen has a specific and Cox2-independent inhibitory effect on Rac1b expression and Rac1b-dependent survival of HT29 tumour cells;
- Ibuprofen inhibits growth of Rac1b-mediated tumour xenografts;
- Ibuprofen prevents Rac1b overexpression during acute inflammation in mice.