A pro-inflammatory microenvironment triggers overexpression of tumor-related RAC1B in polarized colorectal cancer cells

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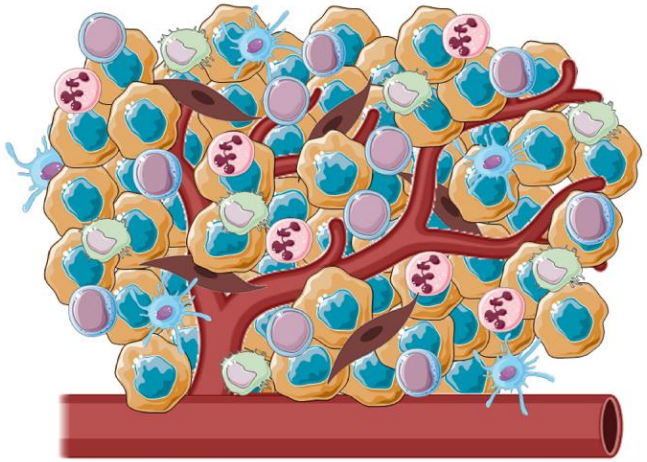
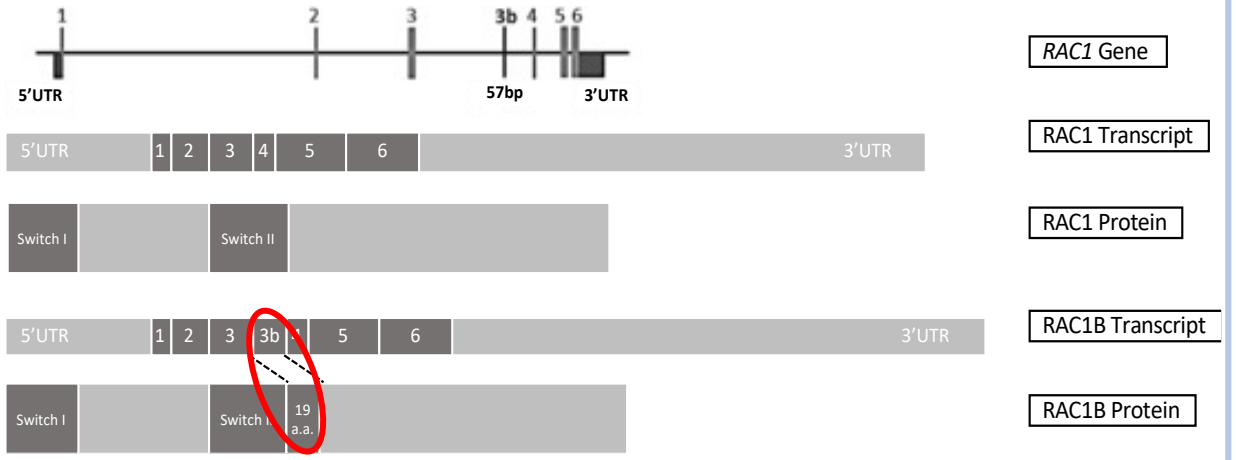
Introduction

Inflammatory tumor cell microenvironment



- Tumor-promoting condition;
- Provides survival signals to tumor cells, which respond with changes in gene expression.

Alternative splicing



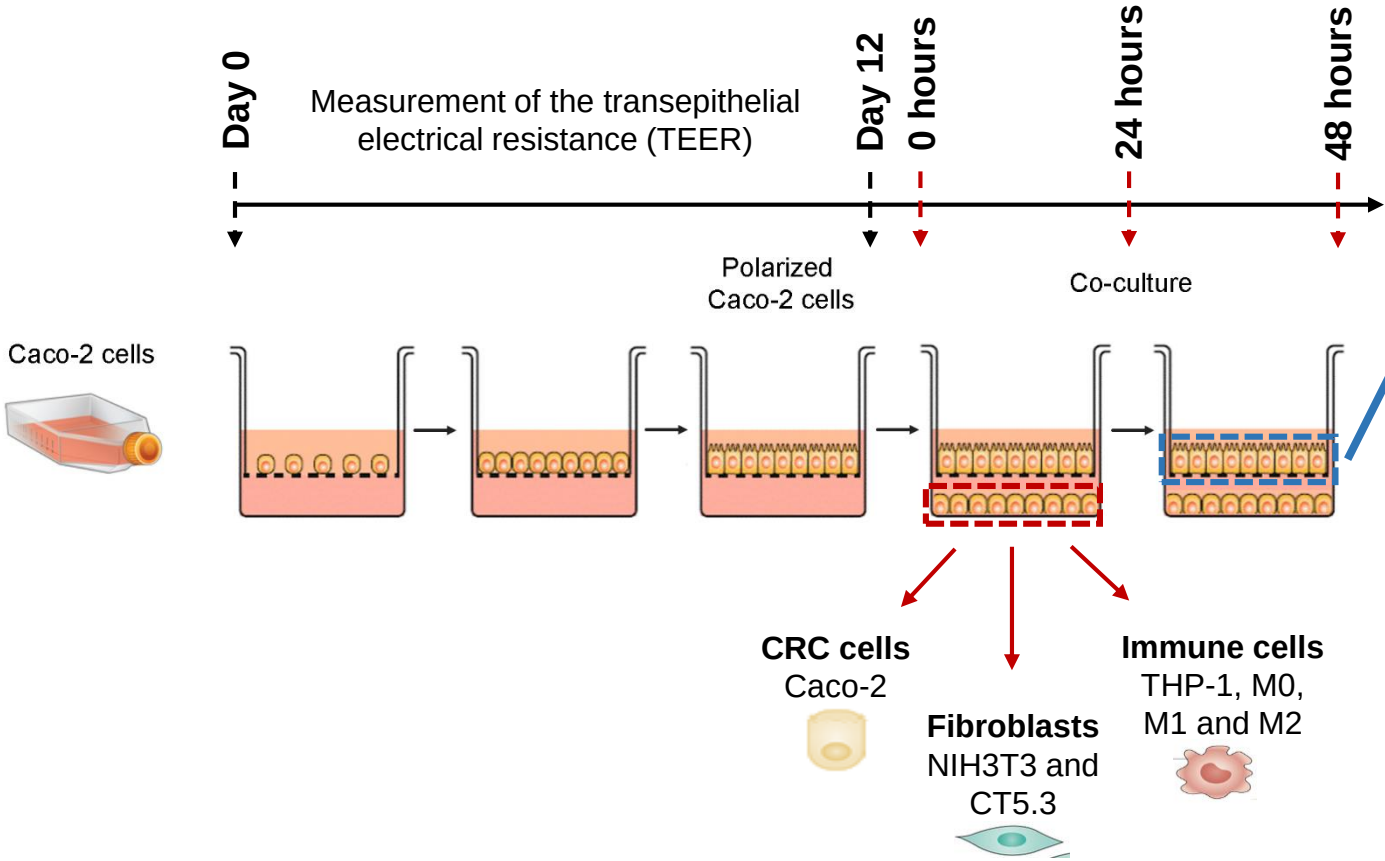
- RAC1B stimulates cell survival *via* NF-κB
- Overexpressed in a subgroup of colorectal tumors;
- Induced by inflammatory conditions.

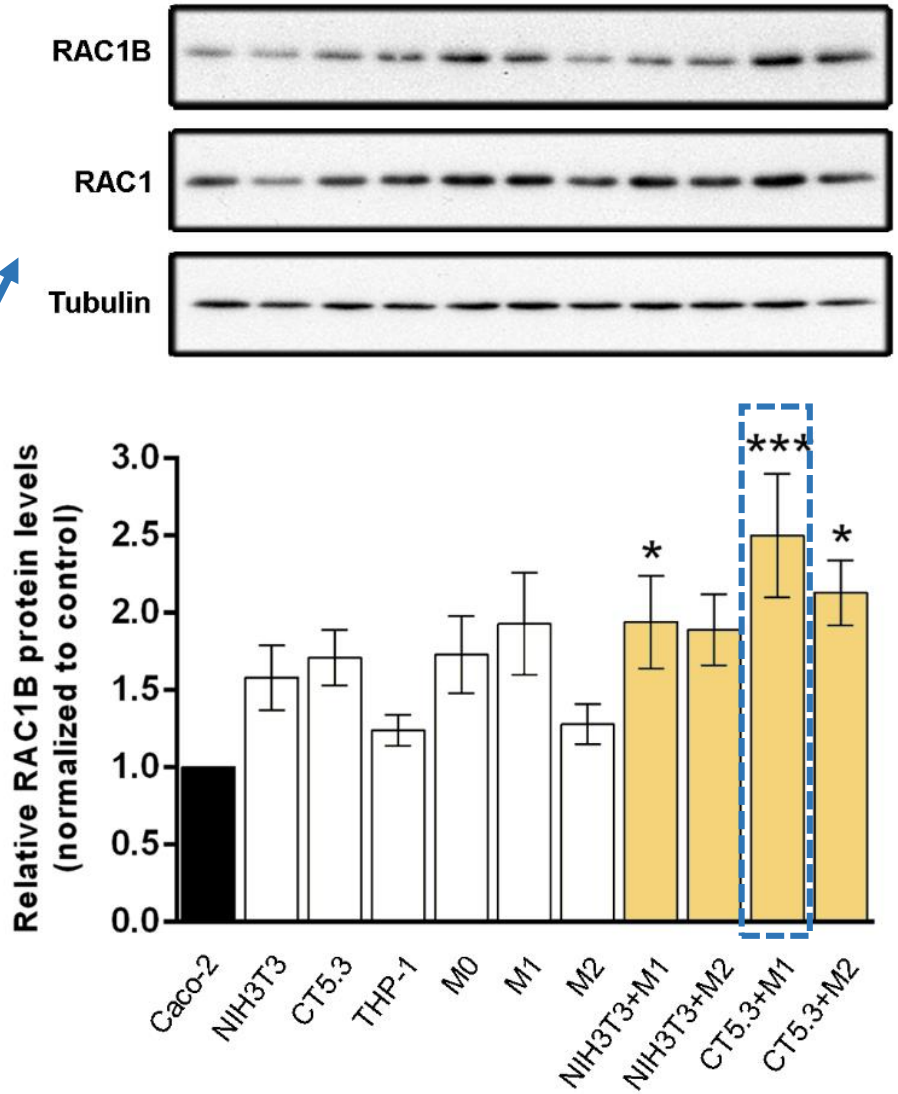
Understand how tumor cells respond to a pro-inflammatory microenvironment with changes in the alternative splicing of RAC1B

Coussens & Werb, *Nature*, 2002, 420, 860-867; Jordan et al., *Oncogene*, 1999, 18, 6835-6839; Matos et al., *Gastroenterology*, 2008, 135, 899-906; Matos et al., *Neoplasia*, 2013, 15, 102-111

Results: *Establishment of a co-culture model between polarized Caco-2 and stromal cells*

Experimental procedure:

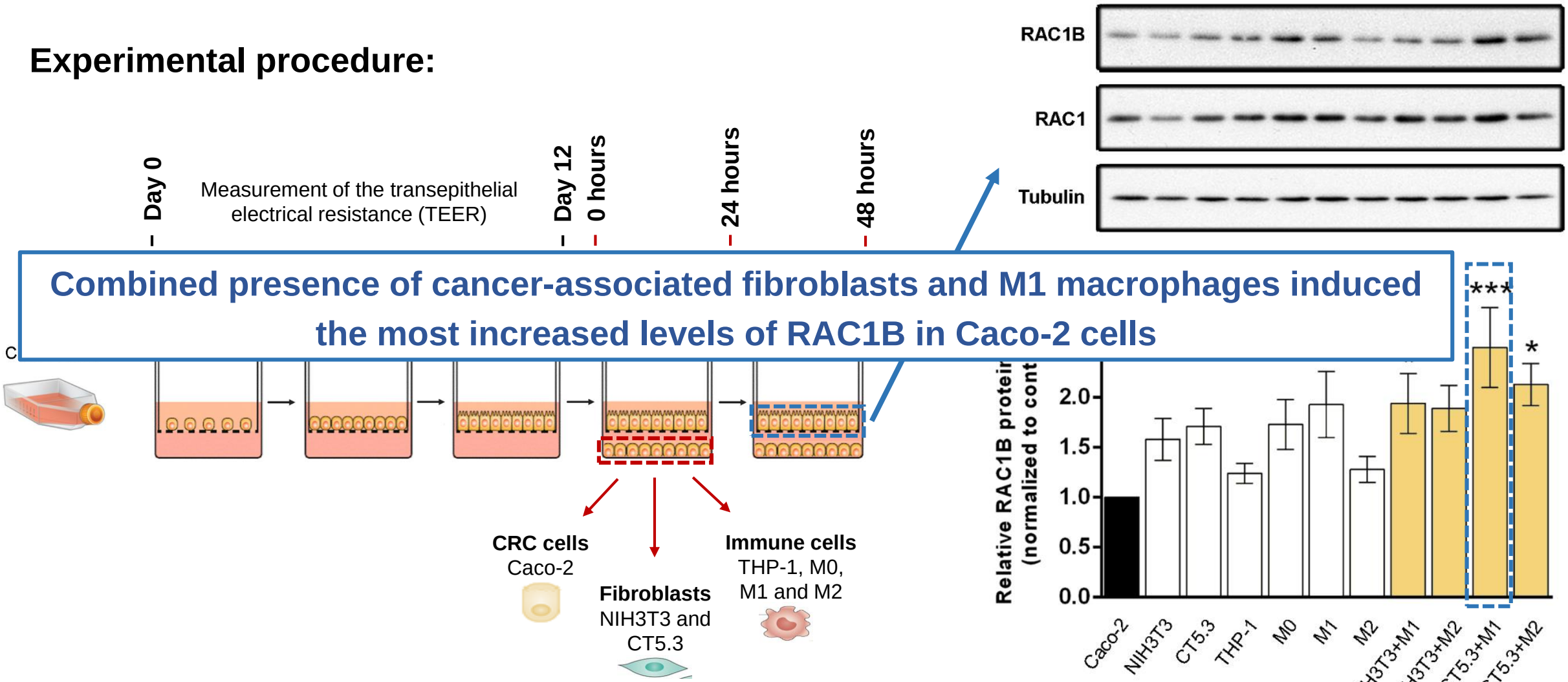




Immunoblot representative of the protein levels of RAC1B and RAC1 in Caco-2 cell lysates after 48h of co-culture, and the graph corresponds to the respective quantification of relative RAC1B protein levels. Quantification of RAC1B protein levels was presented as fold change relative to control (Caco2+Caco-2). Data are means±SEM of 8 experiments; values differing with statistic significance from control: *p<0.05, ***p<0.001; one-way ANOVA with Dunnett's multiple comparison test.

Results: *Establishment of a co-culture model between polarized Caco-2 and stromal cells*

Experimental procedure:

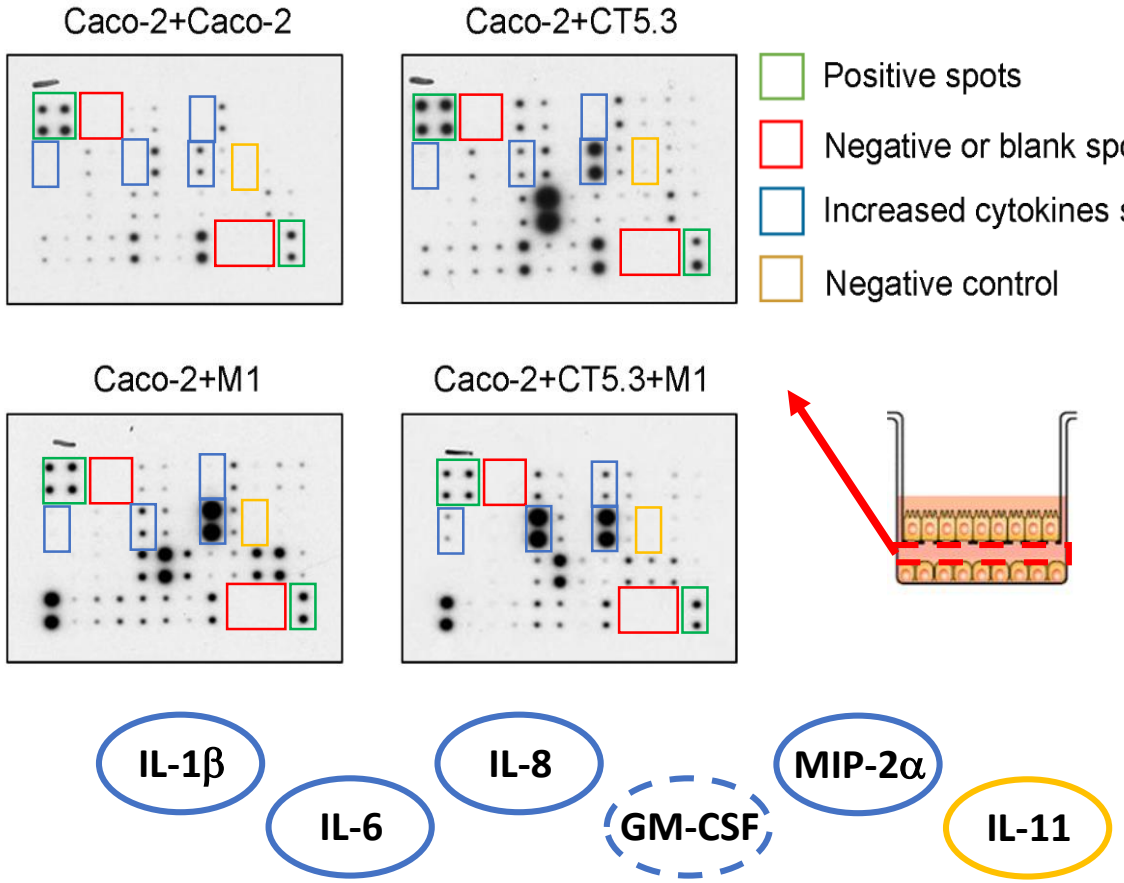


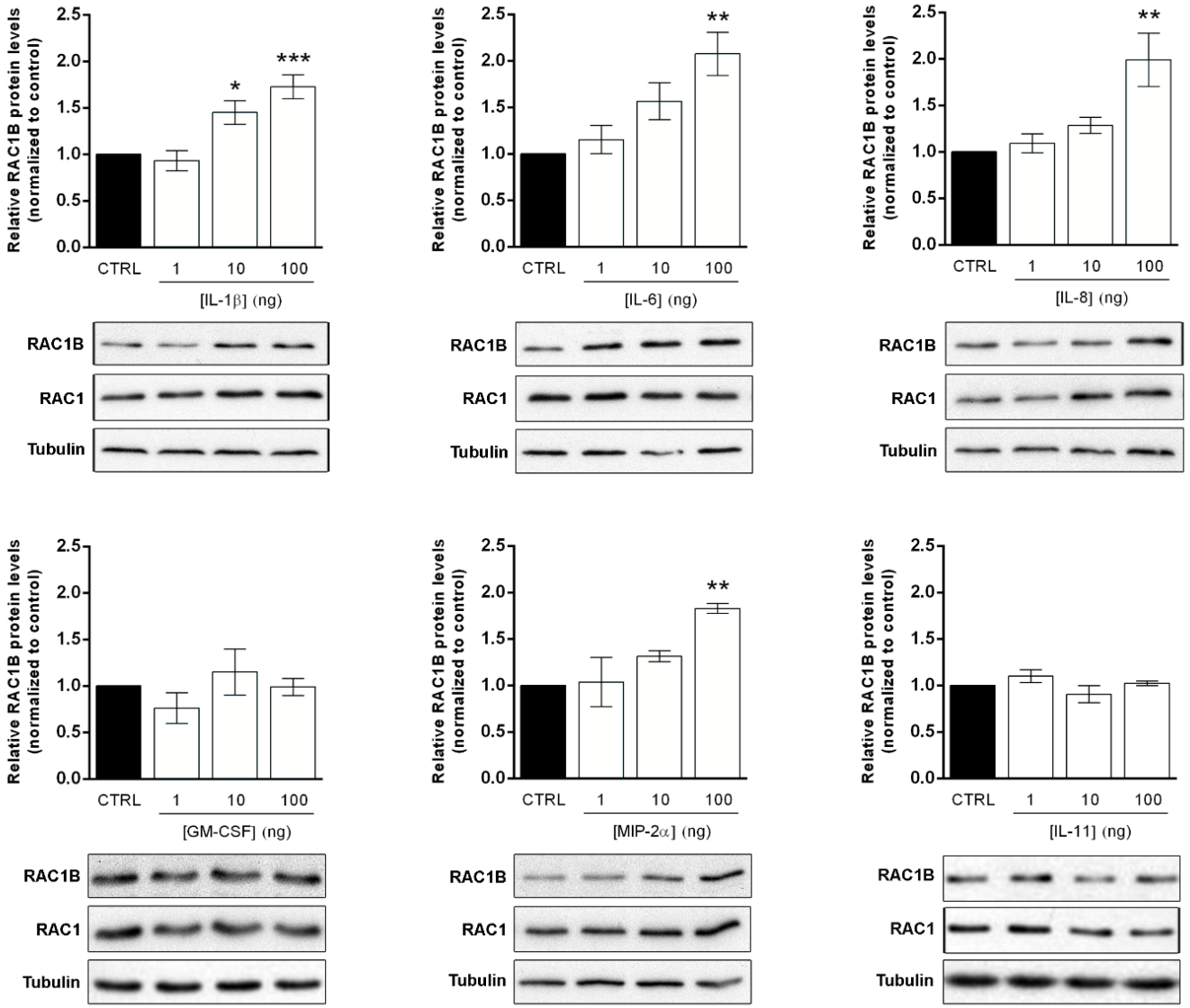
Combined presence of cancer-associated fibroblasts and M1 macrophages induced the most increased levels of RAC1B in Caco-2 cells

Immunoblot representative of the protein levels of RAC1B and RAC1 in Caco-2 cell lysates after 48h of co-culture, and the graph corresponds to the respective quantification of relative RAC1B protein levels. Quantification of RAC1B protein levels was presented as fold change relative to control (Caco2+Caco-2). Data are means±SEM of 8 experiments; values differing with statistic significance from control: *p<0.05, ***p<0.001; one-way ANOVA with Dunnett's multiple comparison test.

Results: *Increased levels of cytokines secreted by co-cultured cells associate with increased RAC1B*

Human Inflammation Antibody Array C3:





Human Inflammation Antibody Array C3. Each spot correspond to the presence of one cytokine in the basolateral medium after 48h of co-culture. Immunoblots are representative of the protein levels of RAC1B and RAC1 in Caco-2 cell lysates after 48h of treatment with purified cytokines, and the graphs correpond to the quantification of relative RAC1B protein levels. Quantification of RAC1B protein levels was presented as fold change relative to control (Caco-2 treated with PBS). Data are means±SEM of 5 experiments; values differing with statistic significance from control: *p<0.05, ***p<0.001; one-way ANOVA with Dunnett's multiple comparison test.

Conclusions

- Pro-inflammatory signals from the microenvironment modulate RAC1B alternative splicing in Caco-2 colon epithelial cells;
- Cytokines IL-1β, IL-6 and IL-8 are increased in conditioned co-culture media of Caco-2+CT5.3+M1, and increased RAC1B levels when added in purified form.

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