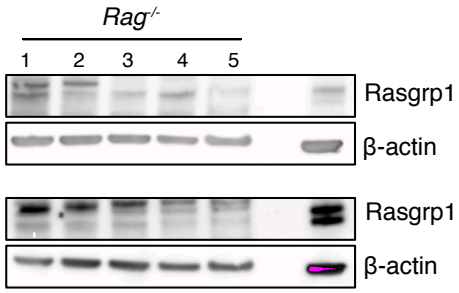
Gbenedio et al., Supplemental Figures and legends

Gbenedio et al., Supplemental Figure 1

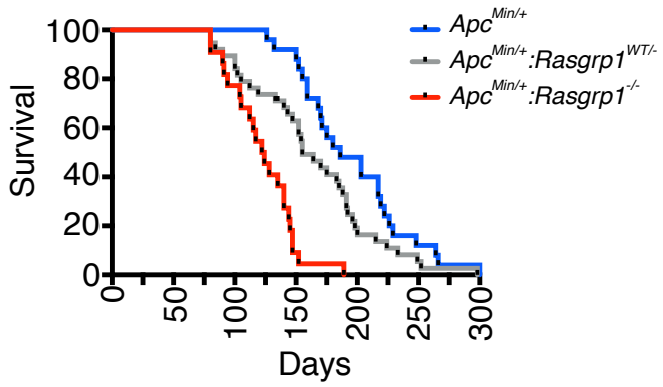


Supplemental Figure 1: Detection of Rasgrp1 in intestinal epithelial cells in different part of small intestine and colon.

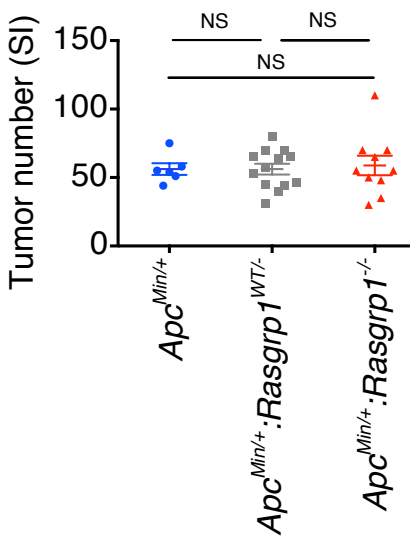
Detection of RasGRP1 expression by western blot in different sections of small intestine (duodenum (1), Jejunum (2) and ileum (3)) and colon (proximal (4) and distal (5)) of a *Rag1*$^{-/-}$ mouse. Western blot panels are independent experiments and extraction confirming the observation in the experiment presented in Figure 1C. β-Actin serves as protein loading control. Protein lysate from CD4-positive mouse T cells (C57Bl6 mouse) is used as positive control for Rasgrp1.

Gbenedio et al., Supplemental Figure 2

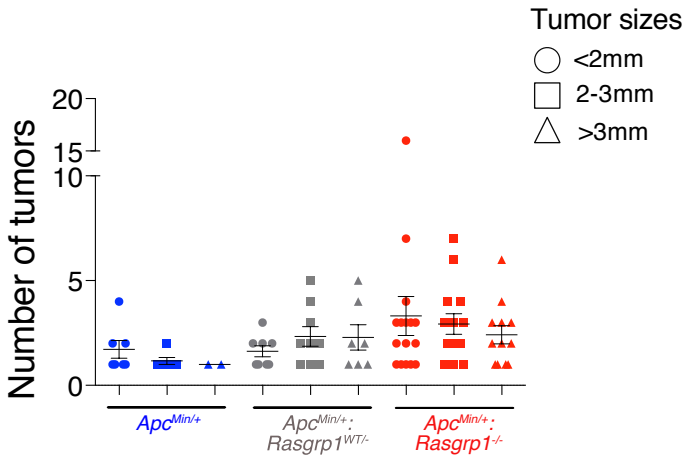
Supplemental Figure 2A



Supplemental Figure 2B



Supplemental Figure 2C



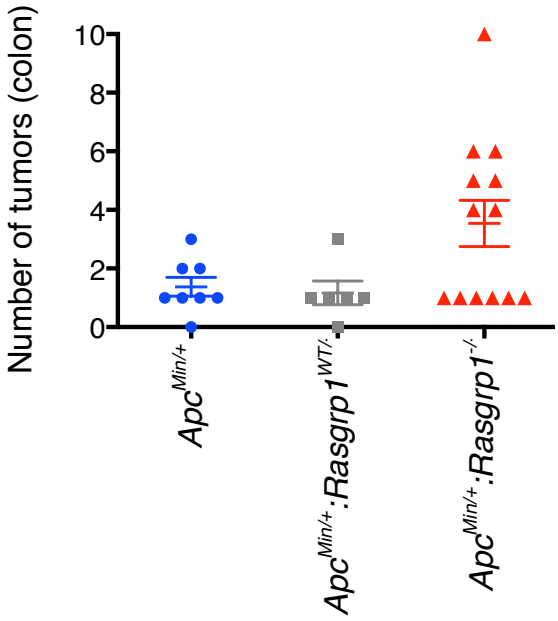
Supplemental Figure 2: RasGRP1 acts as a tumor suppressor in *Apc^{Min}* mice.

(A) Kaplan–Meier curves of *Apc$^{Min/+}$* mouse survival with different copies of *Rasgrp1* alleles. Statistical analysis was performed on *Apc$^{Min/+}$:Rasgrp1$^{WT/-}$* (n = 37) and *Apc$^{Min/+}$:Rasgrp1$^{-/-}$* (n = 22) compared to *Apc$^{Min/+}$* mice (n = 25). **** $p < 0.0001$, Log-

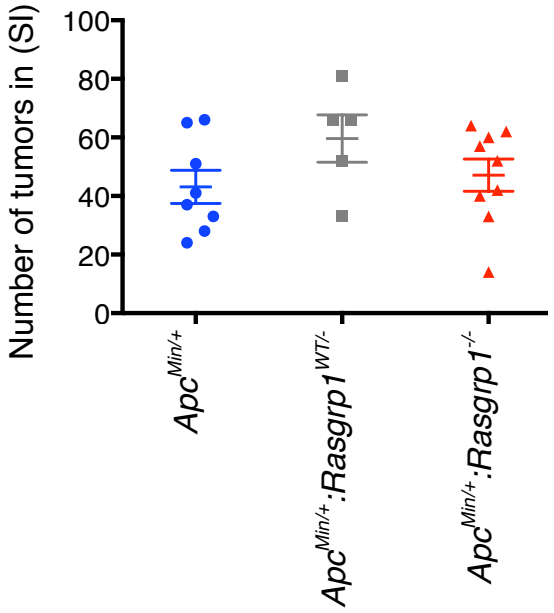
rank (Mantel-Cox) Test, which means that the three groups are significantly different from each other. Over the course of the studies we had to sacrifice n = 23 *Apc*$^{Min/+}$*Rasgrp1*$^{-/-}$ mice with rectal prolapse in compliance with IACUC regulation. Note that these early time point euthanizations were not counted towards Figure 1F. (**B**) Quantification of tumor numbers in the small intestine (SI) of *Apc*$^{Min/+}$ (n = 6), *Apc*$^{Min/+}$*:Rasgrp1*$^{WT/-}$ (n = 13) and *Apc*$^{Min/+}$*:Rasgrp1*$^{-/-}$ (n = 9) mice. NS = not significant. (**C**) Quantification of colonic tumor number grouped by sizes (circle < 2mm; square 2-3mm and triangle >3mm) in *Apc*$^{Min/+}$ (blue symbols), *Apc*$^{Min/+}$*:Rasgrp1*$^{WT/-}$ (gray symbols) and *Apc*$^{Min/+}$*:Rasgrp1*$^{-/-}$ (red symbols) mice (n = 10, 15 and 19 respectively). Depicted are colonic tumors in individual mice that correspond to the percentages in figure 1I. Note that we decided not to perform statistical analyses here. *Apc*$^{Min/+}$ mice infrequently develop colonic tumors so we have few data points here. Furthermore, *Apc*$^{Min/+}$*:Rasgrp1*$^{WT/-}$ and *Apc*$^{Min/+}$*:Rasgrp1*$^{-/-}$ mice succumb from disease at earlier age, so these tumor numbers do not correspond to mice of the same ages.

Gbenedio et al., Supplemental Figure 3

Supplemental Figure 3A



Supplemental Figure 3B

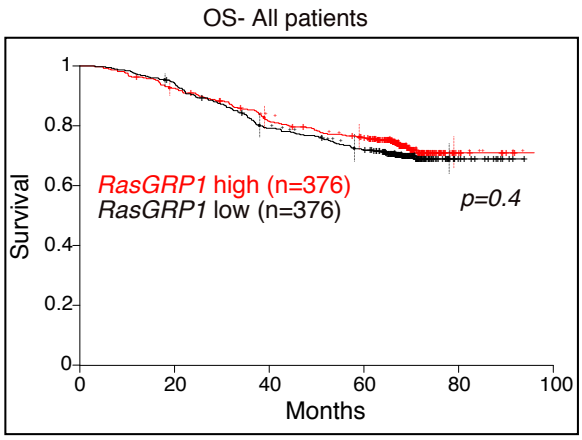


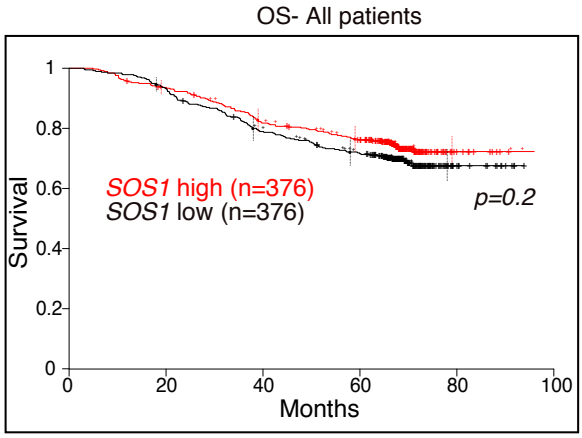
Gbenedio et al. Supplemental Figure 3

Supplemental Figure 3: Bone marrow reconstitutions do not diminish colonic tumors in *Apc$^{Min/+}$:Rasgrp1$^{-/-}$* mice. (**A**) Quantification of colonic tumor incidence in *Apc$^{Min/+}$*, *Apc$^{Min/+}$:Rasgrp1$^{WT/-}$* and *Apc$^{Min/+}$:Rasgrp1$^{-/-}$* mice that were reconstituted with bone marrow from wildtype mice (n = 8, 6 and 12 respectively). (**B**) Quantification of tumor numbers in the small intestine (SI) of *Apc$^{Min/+}$* (n = 8), *Apc$^{Min/+}$:Rasgrp1$^{WT/-}$* (n = 5) and *Apc$^{Min/+}$:Rasgrp1$^{-/-}$* (n = 9) mice that were reconstituted with bone marrow from wildtype mice. NS = not significant.

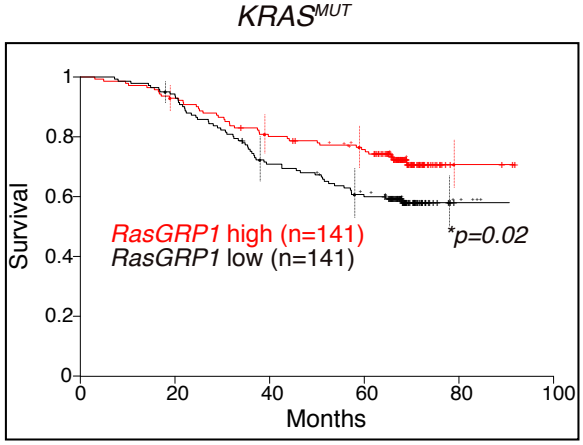
Gbenedio et al., Supplemental Figure 4

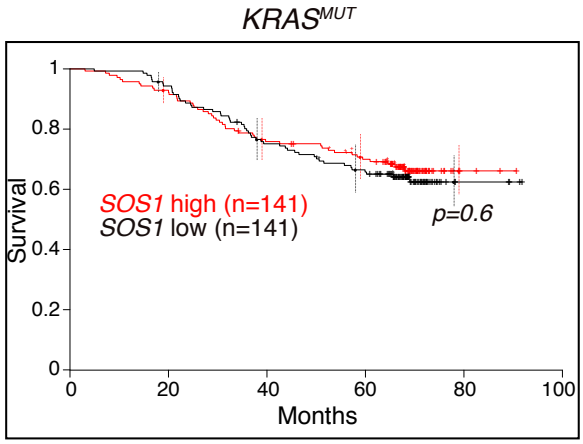
Supplemental figure 4A



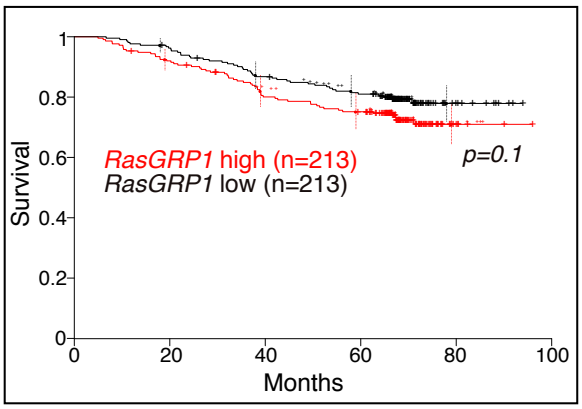


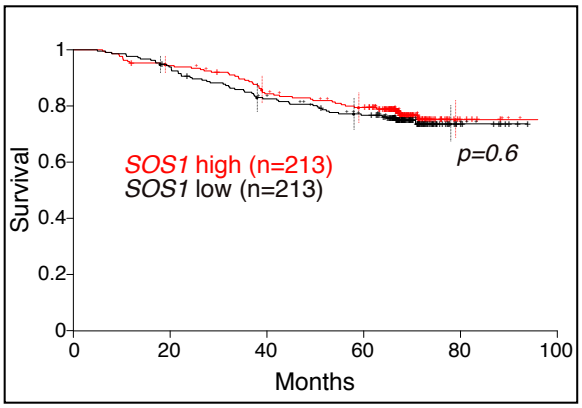
Supplemental figure 4B





Supplemental figure 4C





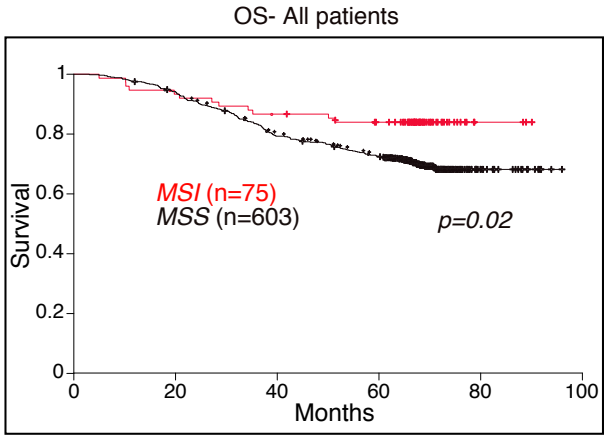
Gbenedio et al. Supplemental Figure 4

Supplemental Figure 4: Kaplan Meier curve of CRC patients with low and high *RasGRP1* or *SOS1* in PETACC3.

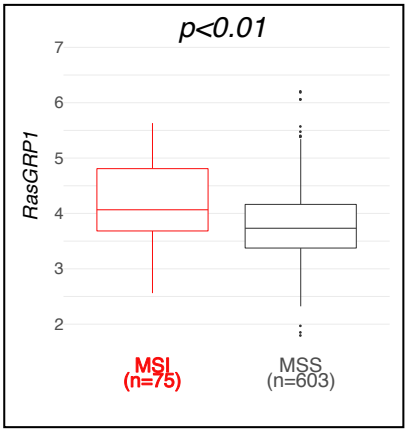
Kaplan–Meier survival curve for stage II and III PETACC3 CRC patients (n=752). For each analysis patients were divided in two equal groups. Panel a presents overall survival for all patients (n=376 per group high and low expression). Panel b and c represent either $KRAS^{MUT}$ (n=141/group)- or $KRAS^{WT}$ (n=213/group)-carrying patients, respectively. All left panels show *RasGRP1* expression level plotted against patient survival and all right panels SOS1 expression plotted against patient survival. All red lines represent high expression of gene of interest black lines low expression level in patient samples. All statistical analysis were done using *Log-rank (Mantel-Cox) Test*. $*p < 0.05$.

Gbenedio et al., Supplemental Figure 5

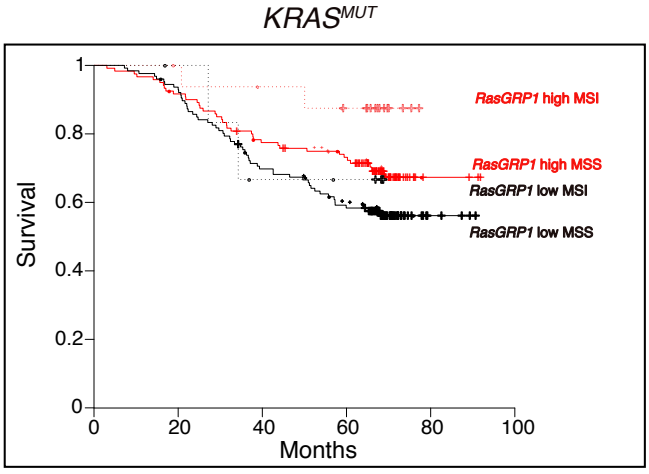
Supplemental figure 5A



Supplemental figure 5B



Supplemental figure 5C



RasGRP1 high, MSI vs RasGRP1 high, MSS
p = 0.2

RasGRP1 high, MSI vs RasGRP1 low, MSS
p = 0.05

RasGRP1 high, MSS vs RasGRP1 low, MSI
p = 0.9

RasGRP1 high, MSS vs RasGRP1 low, MSS
p = 0.06

RasGRP1 high, MSI vs RasGRP1 low, MSI
p = 0.3

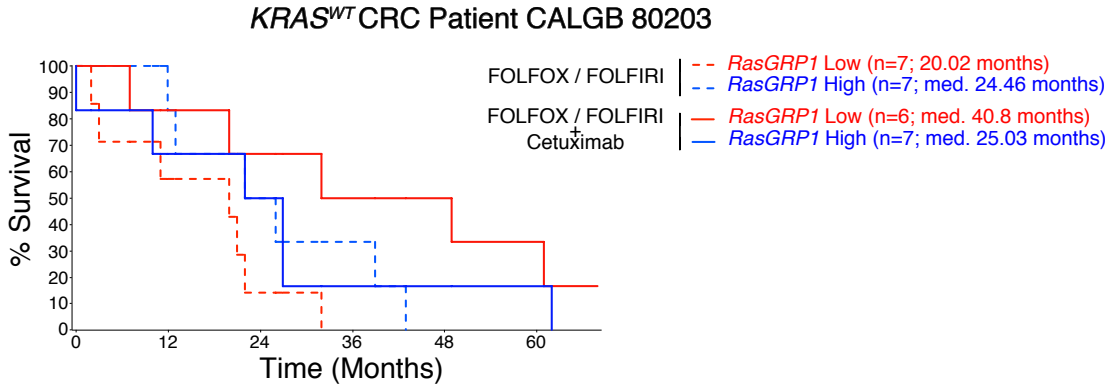
Gbenedio et al. Supplemental Figure 5

Supplemental Figure 5: Impact of RasGRP1 level in CRC patients with microsatellite instability (MSI) versus microsatellite stable (MSS).

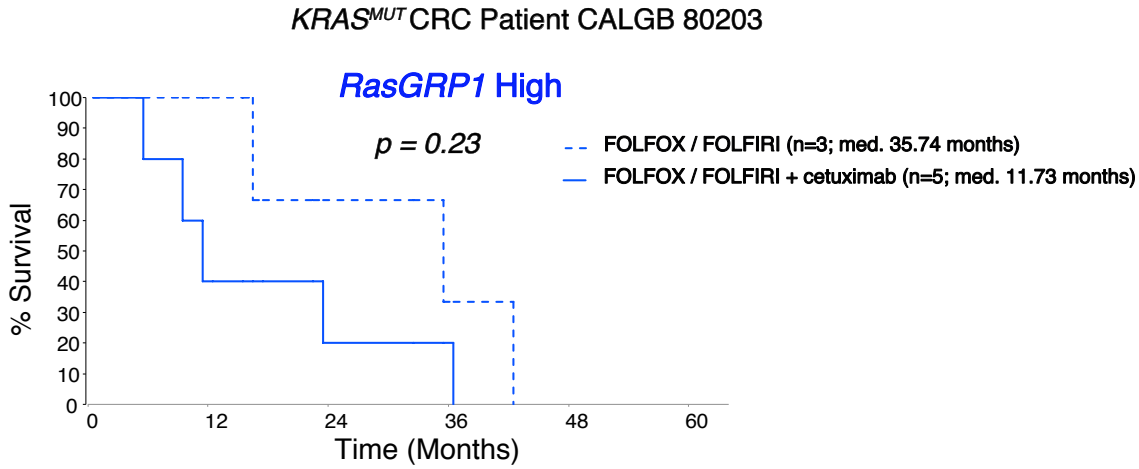
(A) Kaplan–Meier survival curve for stage II and III PETACC3 CRC patients (n=678). Patients were divided in two groups of MSI (n=75) versus MSS (n=603). **(B)** Patients from panel A in Box plot comparing *RasGRP1* expression level in patients MSS and patients MSI.). Statistical analysis was performed using unpaired t-test and the p-value < 0.05 was considered significant. (**C**) Kaplan-Meier survival curve of $KRAS^{MUT}$ (n=268)-carrying patients from panel a. Patients were then split into four different groups. *RasGRP1* high; MSI (n=15) (red dash curve) *RasGRP1* high; MSS (171) (solid red curve), *RasGRP1* low; MSI (n=7) (black dash curve) and *RasGRP1* low; MSS (n=132) (solid black curve). All statistical analyses for Kaplan-Meier survival were done using *Log-rank (Mantel-Cox) Test*. *$p < 0.05$.

Gbenedio et al., Supplemental Figure 6

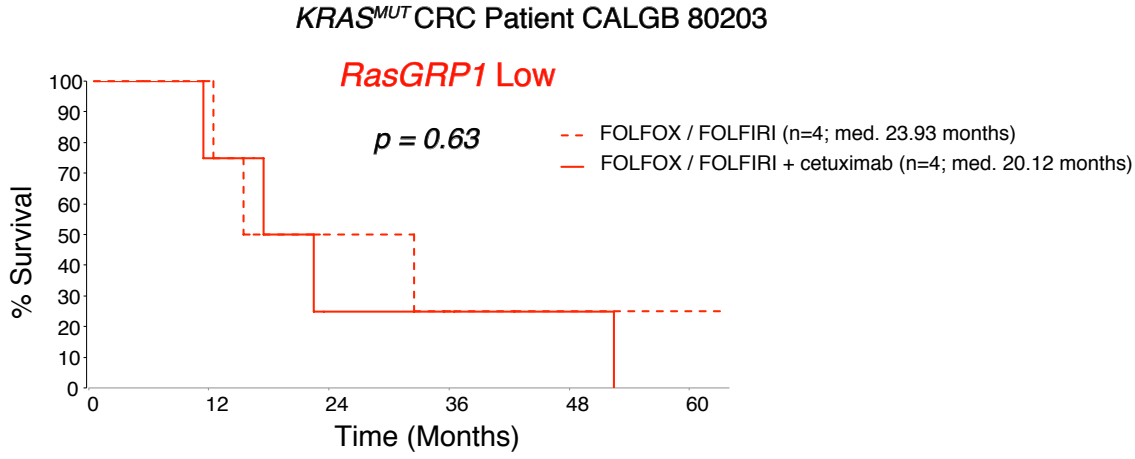
Supplemental Figure 6A



Supplemental Figure 6B



Supplemental Figure 6C



Gbenedio et al. Supplemental Figure 6

Supplemental Figure 6: Patient survival data accompanying Figures 5E and 5F.

(**A**) Summary of all the data in figs 5E and 5F. Comparison of FOLFOX/FOLFIRI chemotherapy (dashed lines) versus FOLFOX/FOLFIRI with cetuximab (solid lines) for the quartile of patients with highest *RasGRP1* expression in their tumors ("*RasGRP1* High" in blue) as well as the quartile of patients with lowest *RasGRP1* expression in their tumors ("*RasGRP1* Low" in red). Overall survival was graphed in a retrospective analysis of $KRAS^{WT}$ colorectal cancer patients enrolled in the CALG80203 trial.

(**B** and **C**) Comparison of FOLFOX/FOLFIRI chemotherapy (dashed lines) versus FOLFOX/FOLFIRI with cetuximab (solid lines) in colorectal cancer patients with $KRAS^{MUT}$ status who were enrolled in the CALG80203 trial. The quartile of patients with highest *RasGRP1* expression in their tumors ("*RasGRP1* High" in blue in **B**) as well as the quartile of patients with lowest *RasGRP1* expression in their tumors ("*RasGRP1* Low" in red in **C**) were analyzed. The p values indicate that there were no significant differences between the groups of $KRAS^{MUT}$ CRC patients.