

Mechanism of action of RXC004, a Wnt pathway inhibitor, in genetically-defined models of cancer



Caroline Phillips, Inder Bhamra, Clifford Jones, Catherine Eagle and Simon Woodcock

Introduction

Signalling through the Wnt pathway is highly regulated at the level of ligand (Wnt), receptor (Fzd/LRP) and downstream components (e.g. destruction complex - APC/Axin/GSK3 β). Post-translational modification of Wnt ligands via porcupine (PORCN; a membrane bound O-acyltransferase) is essential for secretion of active Wnt¹. Activity of RNF43/ZNRF3 (E3-ubiquitin ligases) results in ubiquitination and membrane clearance of Fzd, whilst RNF43/ZNRF3 levels are kept in check via LGR and secreted RPSO ligands² (Fig. 1).

The potent and selective PORCN inhibitor RXC004 is being investigated in a Phase 1 clinical trial (NCT03447470)³, and has the potential to treat tumours dependent on Wnt ligand. Upstream Wnt pathway aberrations, including RNF43/ZNRF3 mutations and RPSO-fusions, result in high levels of surface Fzd receptors and increased Wnt-ligand dependent signalling⁴ (Fig. 1). These aberrations are implicated in pancreatic and colorectal cancer (CRC). Dysregulated Wnt signalling initiates oncogenic pathways involved in tumour initiation, growth and metastasis⁵, and is more recently linked to tumour immune evasion^{6,7}.

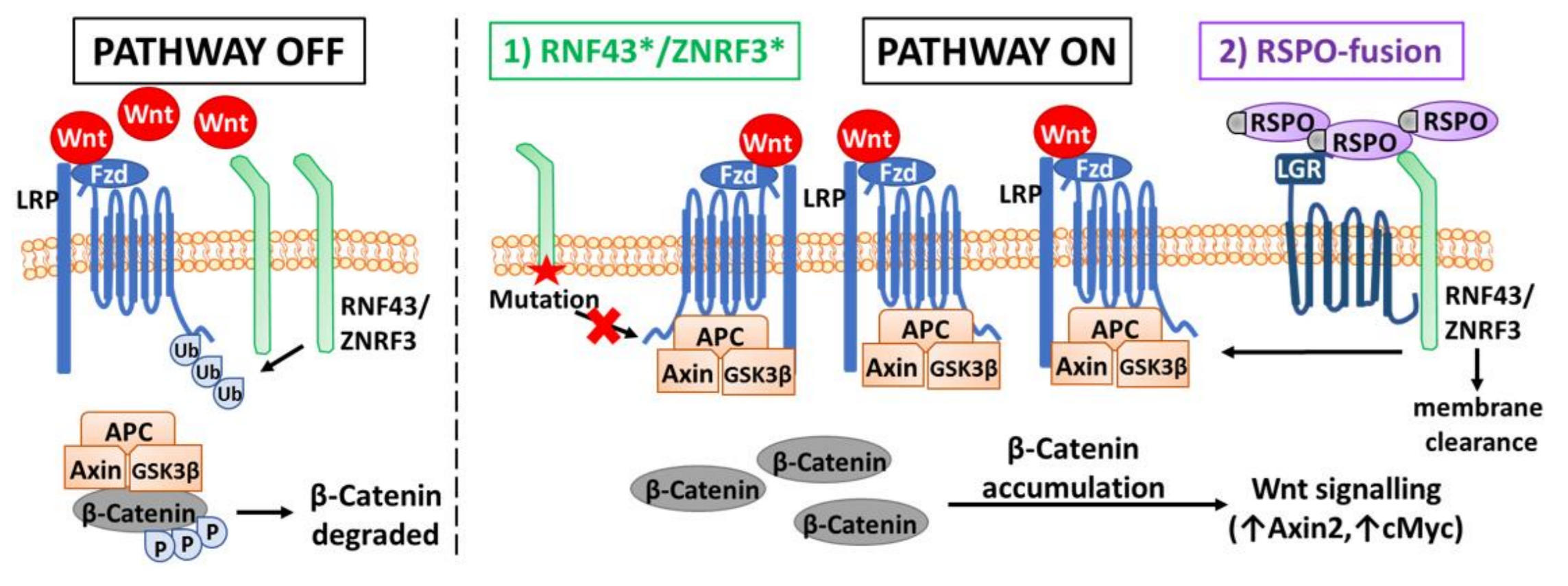


Figure 1: Upstream alterations trigger aberrant Wnt ligand-dependent signalling
RNF43/ZNRF3 keep surface Fzd in check, allowing the destruction complex to phosphorylate and degrade β -catenin - Wnt pathway 'OFF'. Loss-of-function (LOF) RNF43/ZNRF3 mutations (1), or high RPSO expression through gene fusion (2), ultimately leads to accumulation of β -catenin - Wnt pathway 'ON'.

Results

Anti-proliferative effects of RXC004 in genetically-defined tumour cell lines

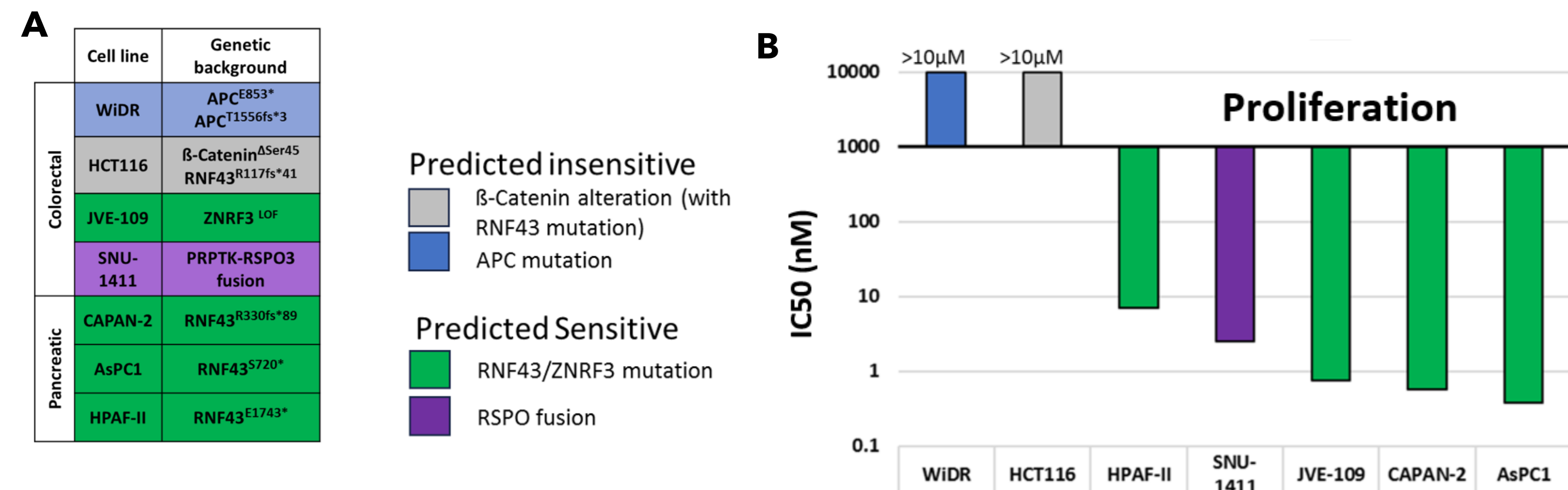


Figure 2: RXC004 anti-proliferative potency in genetically-defined pancreatic and CRC cell lines.
(A) RXC004 was evaluated across a panel of 7 genetically-defined tumour cell lines. (B) Indicated cell lines were treated with a dose response of RXC004 for 5 days, proliferation was measured using an ATP-lite assay. N \geq 3 throughout. Cell lines harbouring RNF43/ZNRF3 mutations or RPSO-fusions are sensitive to RXC004 as predicted, with anti-proliferative effects ranging from 0.3nM to 7nM.

RXC004 arrests at the G1/S and G2/M cell cycle checkpoints

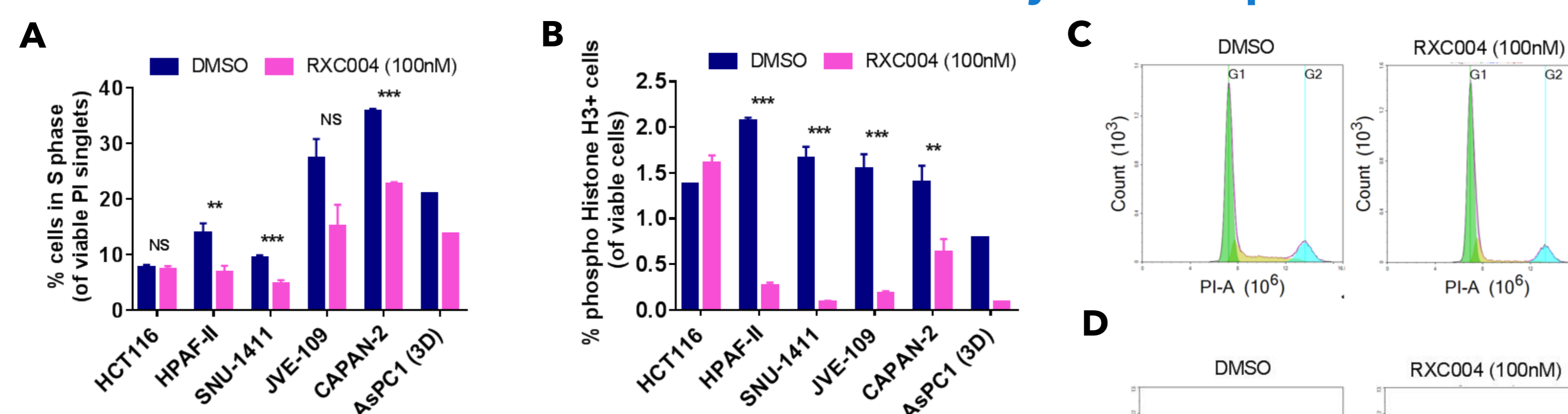


Figure 3: Effects of RXC004 treatment on the cell cycle

Cells were treated with RXC004 (100nM) or vehicle (0.1% DMSO) for 72h. Cell cycle profiles were determined using propidium iodide (A, C) and phospho-Ser10-Histone H3 (mitotic marker; B, D) staining by flow cytometry. Data are N \geq 3 except for HCT116 and AsPC1 (N=1). Representative flow plots for cell cycle (C) and phospho-Histone H3 (D) in HPAF-II cells treated as indicated. T-test p values.

RXC004 reduces tumour volume, eliminates proliferation and differentiates RPSO fusion tumour cells

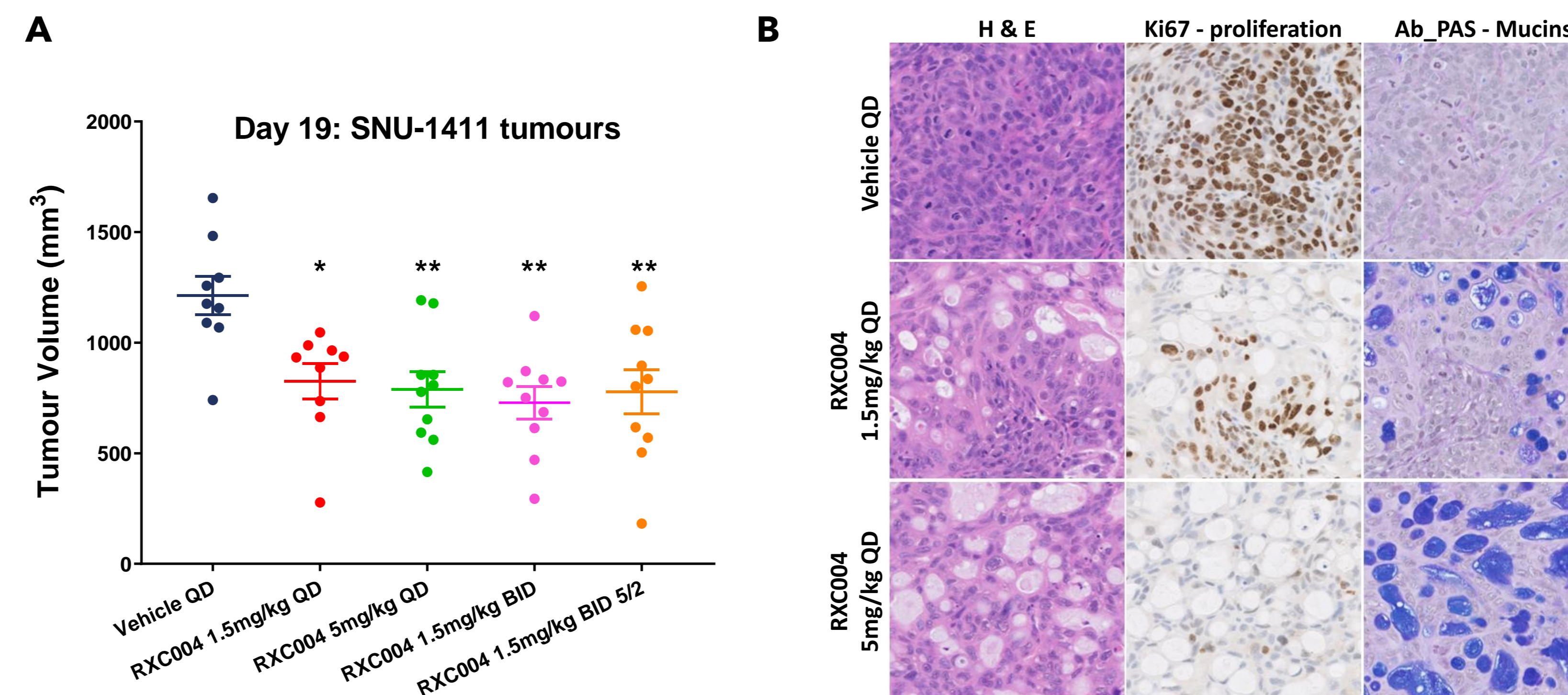


Figure 4. RXC004 displays efficacy and cell differentiation effects in the RPSO fusion SNU-1411 colorectal xenograft model
SNU-1411 (1x10⁷ cells; NOC-SCID mice) were implanted subcutaneously and treatment with RXC004 at indicated doses was initiated when tumours reached an average size of 200mm³. (A) tumour volume measurements at day 19 show significant reduction in tumour volume at all doses. N \geq 9 per group. Ordinary one-way ANOVA p values. (B) tumour PD histology after 7 days dosing with RXC004 at indicated doses. Tumours were FFPE and sections stained with H&E, anti-Ki67 as a marker of proliferation or Ab_PAS for Mucins, as indicated. A dose dependent reduction in Ki67 staining and increase in Mucin staining in response to RXC004 compared to vehicle control shown.

RXC004 pre-treatment results in continued growth inhibition even in the absence of continued treatment

Study 1A

SNU-1411 cells were implanted into mice, selected at ~350mm³ and randomised into two groups:

Group 1: Vehicle treated
Group 2: RXC004 5mg/kg QD

Animals treated for 9 days, tumours were then removed from each group and fragments re-implanted into new, tumour and treatment naïve mice in Study 1B

Study 1B

Viable tumour fragments were implanted into tumour naïve mice and tumour growth monitored:

Group 1b: tumour fragments from previously vehicle treated (group 1) mice
Group 2b: tumour fragments from previously RXC004 treated (group 2) mice.

No drug treatment was given to either group in Study 1B

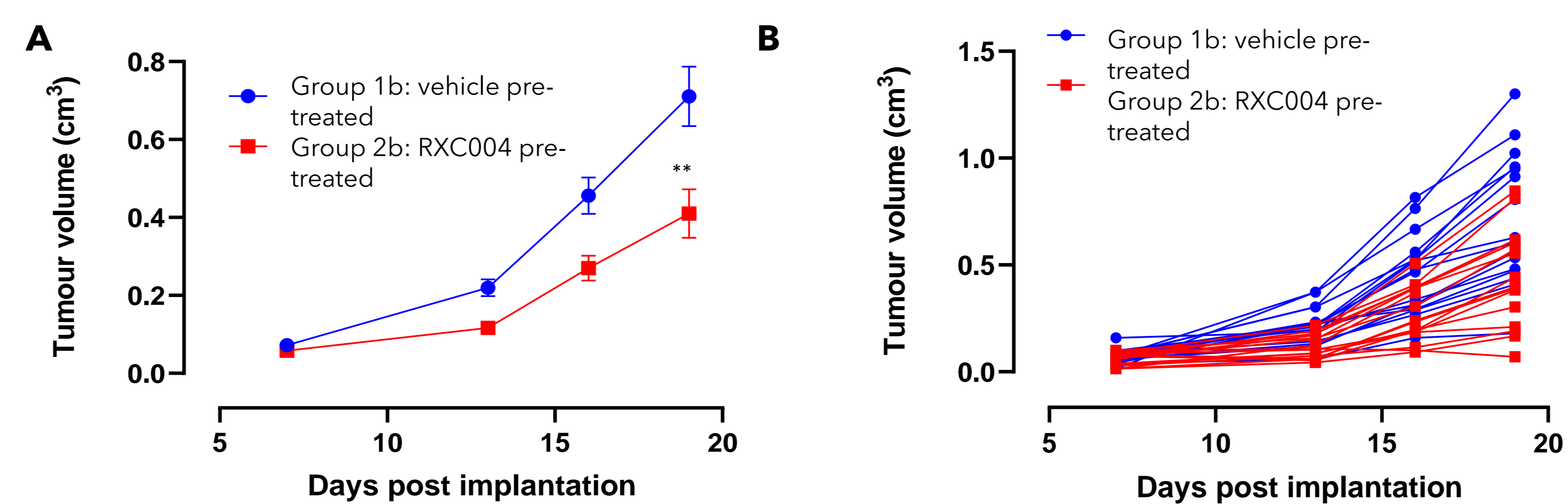
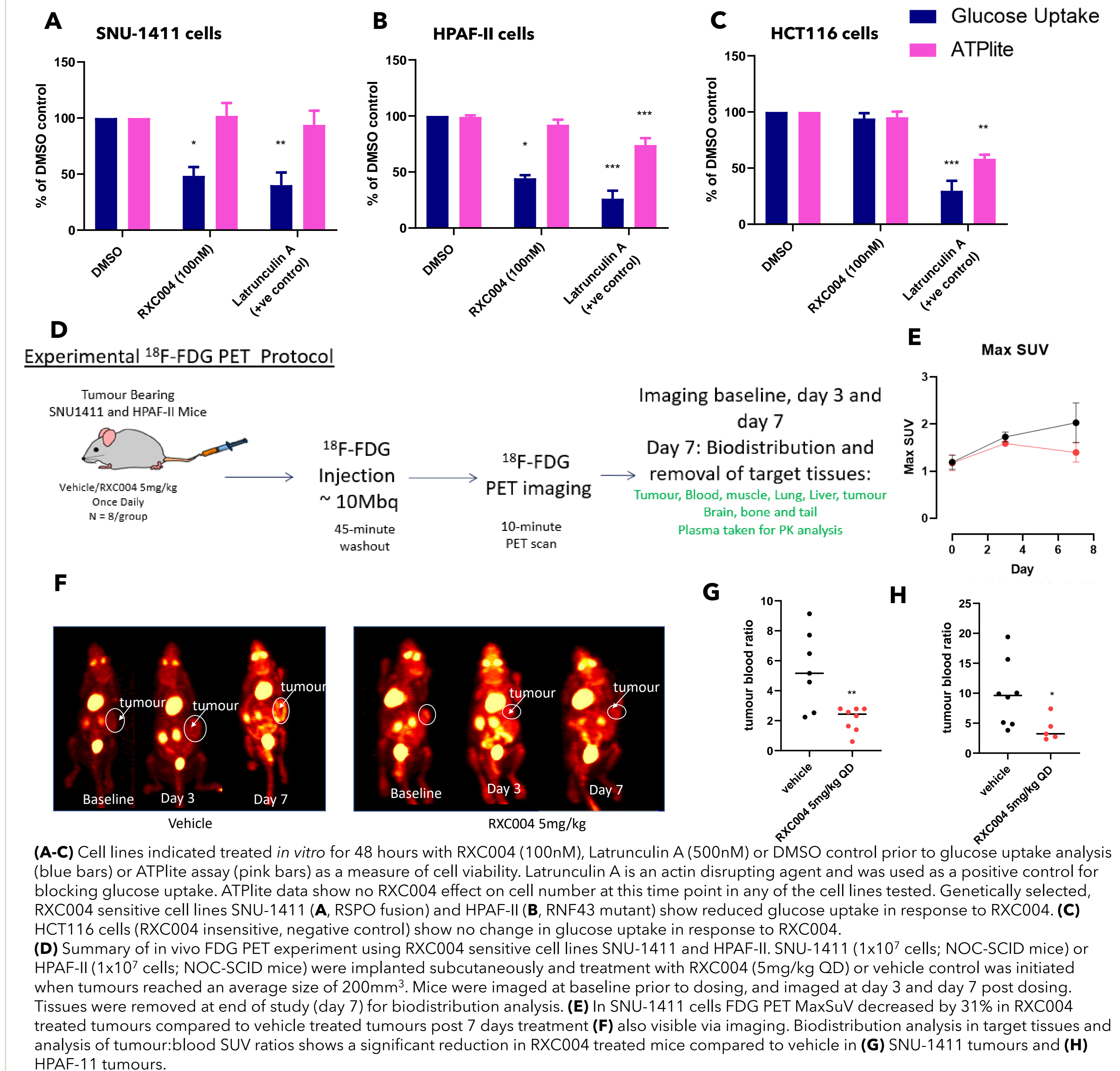


Figure 5. RXC004 treated RPSO fusion SNU-1411 colorectal tumours show slower growth compared to control tumours when re-implanted in tumour and treatment naïve mice
SNU-1411 (1x10⁷ cells; NOC-SCID mice) were implanted subcutaneously and treatment with RXC004 (5mg/kg QD) was initiated when tumours reached an average size of 350mm³. After 9 days treatment, tumours were resected for the re-implantation study, N=8 per group. Viable tumour fragments from study 1A were re-implanted in tumour and treatment naïve mice. RXC004 pre-treated tumours showed slower growth after re-implantation (group 2b) compared to re-implanted vehicle pre-treated tumours (group 1b). Mean data (A) and individual animal data (B) shown. N=16 per group. Ordinary one-way ANOVA p values.

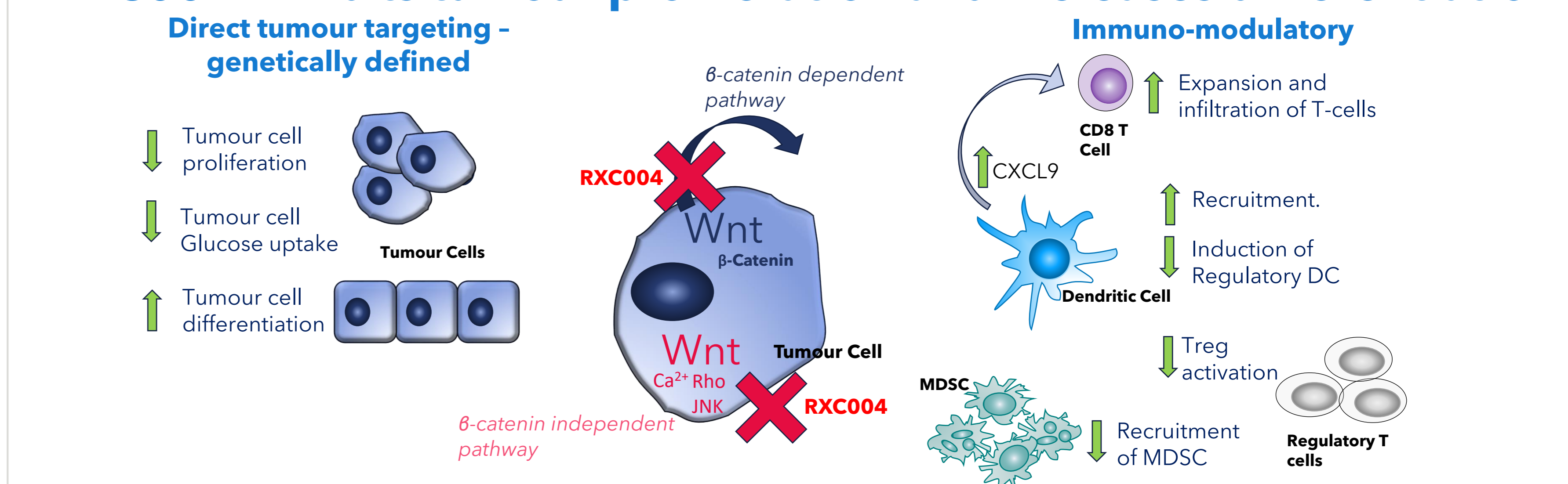
RXC004 treatment alters metabolic activity of sensitive cells *in vitro* and *in vivo*



(A-C) Cell lines indicated treated *in vitro* for 48 hours with RXC004 (100nM), Latrunculin A (500nM) or DMSO control prior to glucose uptake analysis (blue bars) or ATP-lite assay (pink bars) as a measure of cell viability. Latrunculin A is an actin disrupting agent and was used as a positive control for blocking glucose uptake. ATP-lite data show no RXC004 effect on cell number at this time point in any of the cell lines tested. Genetically selected, RXC004 sensitive cell lines SNU-1411 (A, RPSO fusion) and HPAF-II (B, RNF43 mutant) show reduced glucose uptake in response to RXC004. (C) HCT116 cells (RXC004 insensitive, negative control) show no change in glucose uptake in response to RXC004. (D) Summary of *in vivo* FDG PET experiment using RXC004 sensitive cell lines SNU-1411 and HPAF-II (1x10⁷ cells; NOC-SCID mice) or HPAF-II (1x10⁷ cells; NOC-SCID mice) were implanted subcutaneously and treatment with RXC004 (5mg/kg QD) or vehicle control was initiated when tumours reached an average size of 200mm³. Mice were imaged at baseline prior to dosing, and imaged at day 3 and day 7 post dosing. Tissues were removed at end of study (day 7) for biodistribution analysis. (E) In SNU-1411 cells FDG PET MaxSUV decreased by 31% in RXC004 treated tumours compared to vehicle treated tumours post 7 days treatment (F) also visible by imaging. Biodistribution analysis in target tissues and analysis of tumour:blood SUV ratios shows a significant reduction in RXC004 treated mice compared to vehicle in (G) SNU-1411 tumours and (H) HPAF-II tumours.

Summary

RXC004 inhibits tumour proliferation and increases differentiation



References

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