Expanded View Figures

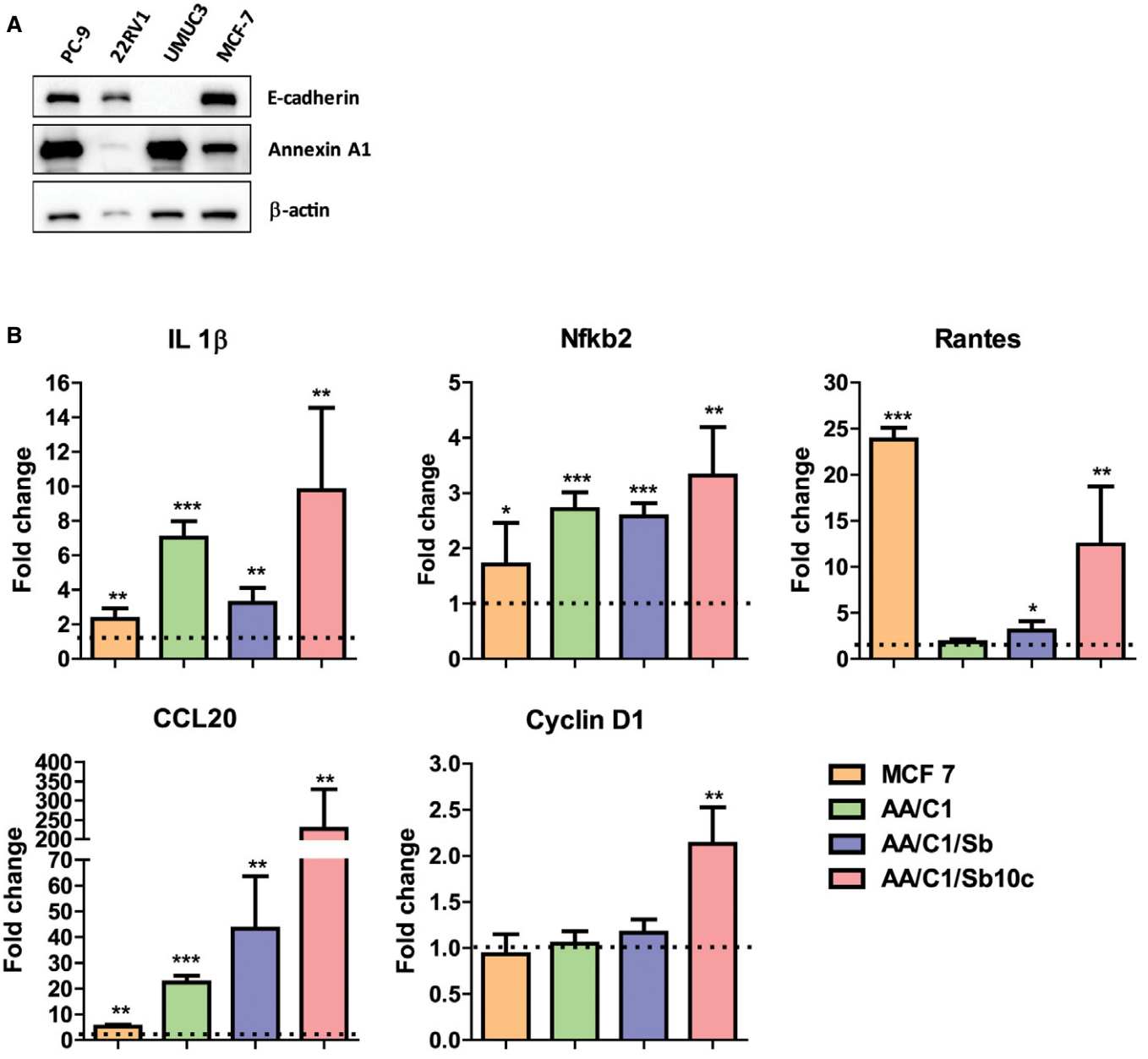

Figure EV1. Expression of E-cadherin, Annexin A1, inflammatory genes and oncogene Cyclin D1 in different cell lines.

A Western blot analysis of E-cadherin and Annexin A1 expression in lung cancer cells PC-9, prostate cancer cells 22RV1, bladder cancer cells UMUC3, and breast cancer cells MCF-7. β-Actin was included as an internal control.

B Real-time qPCR analysis of Il-1β, Nfkb2, Rantes, CCL20, and CCND1 mRNA in MCF-7, AA/C1, AA/C1/SB (aka SB), and AA/C1/SB/10C (aka 10C) either untreated or following incubation with wild-type *F. nucleatum* 12230. Results obtained from untreated controls were designated as 1. Data were mean values ± SD. The experiment was performed in duplicates and repeated twice. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ (Student's *t*-test).

Source data are available online for this figure.

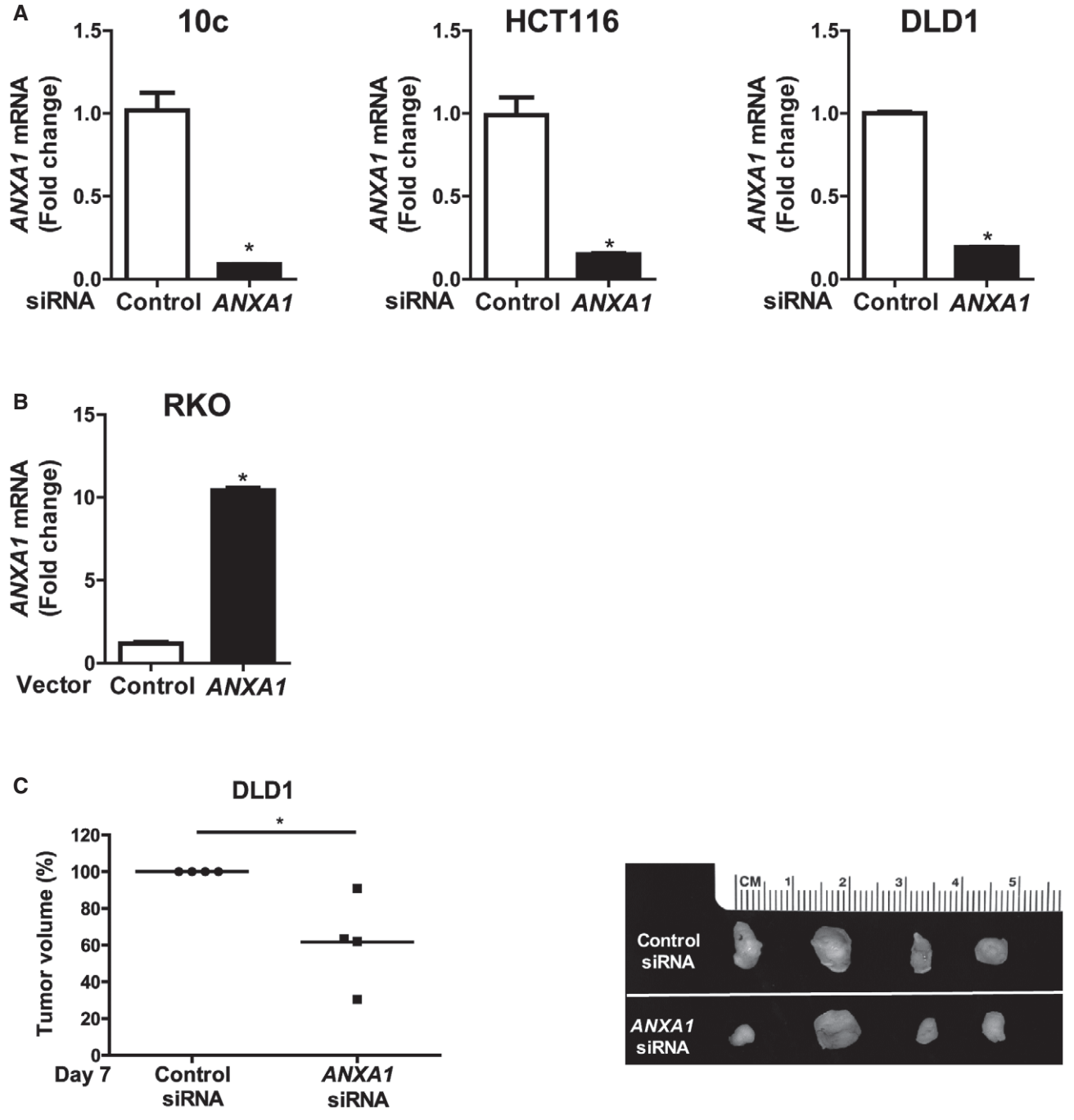

Figure EV2. Annexin A1 knockdown in 10C, HCT116 and DLD1 cells and knock-in in RKO cells, and the effect of knockdown in DLD1 xenograft tumor growth.

A qPCR analysis of *ANXA1* mRNA levels in 10C, HCT116, DLD1 cells transfected with control siRNA or *ANXA1*-specific siRNA, demonstrating knockdown of *ANXA1*. The experiment was performed in triplicates. Data are mean values ± SEM. *$P < 0.05$ (Student's *t*-test).

B qPCR analysis of *ANXA1* mRNA levels in RKO cells transfected with control vector or *ANXA1*, demonstrating knock-in of *ANXA1*. The experiment was performed in triplicates. Data are mean values ± SEM. *$P < 0.05$ (Student's *t*-test).

C Xenografted tumor growth in nude mice following subcutaneous and bilateral inoculation of DLD1 cells transfected with control siRNA or *ANXA1*-specific siRNA ($n = 4$). The tumor volumes were measured after 8 days postinjection (left panel). For each mouse, the tumor resulting from *ANXA1*-specific siRNA-treated cells was normalized to that from control siRNA-treated cells, which was designated as 100%. The line represents the average. *$P < 0.05$ (paired *t*-test). The individual tumor pairs are shown on the right panel: top, tumors arising from control siRNA treated cells; bottom, tumors arising from *ANXA1*-specific siRNA-treated cells.

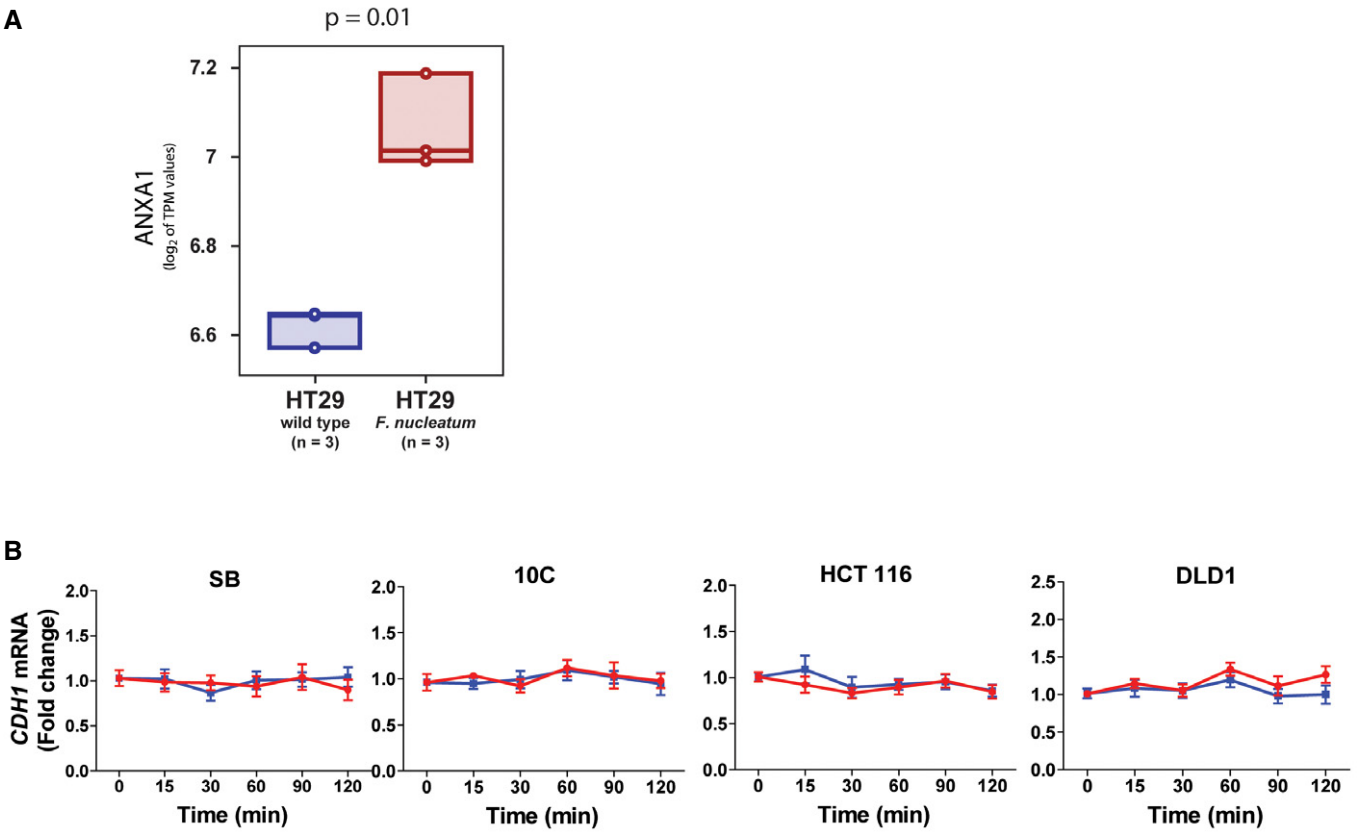

Figure EV3. Induction of Annexin A1 mRNA expression in HT29 cells by *F. nucleatum* 25586 and effect of *F. nucleatum* 12230 on E-cadherin mRNA expression in SB, 10C, HCT116 and DLD1 cells.

A Statistical analysis of associations between exposure to *F. nucleatum* and up-regulation of *ANXA1* mRNA expression levels in colon cancer cell HT29. The *ANXA1* mRNA levels in HT29 cells were analyzed in an RNA-sequencing (RNA-seq) dataset publicly available from the NCBI-GEO online repository (GSE90944) and containing global gene-expression measurements from HT29 cells, both at baseline and following incubation with *F. nucleatum* ATCC25586 in triplicates [14]. The distribution of *ANXA1* mRNA expression levels in the two sample groups (baseline versus infected) was visualized using boxplots, using the $\log_2$ of their TPM (*transcripts per million*) expression values as a metric. Individual data points were represented as circles, and box-plots were drawn to span the inter-quartile range (from the 25th to the 75th percentile) with an internal band to identify the median. Differences in mean $\log_2$ TPM values between HT29 cells at baseline (n = 3) and following incubation with *F. nucleatum* (n = 3) were tested for statistical significance using a two-tailed t-test for continuous variables. The analysis revealed that HT29 cells exposed to *F. nucleatum* were characterized by increased levels of *ANXA1* mRNA expression, as compared to HT29 cells at baseline (P = 0.01).

B Real-time qPCR analysis of E-cadherin (*CDH1*) mRNA levels in SB, 10C, HCT116, and DLD1 cells following incubation with *F. nucleatum* 12230 (*Fn*) and *fadA*-deletion mutant US1 (US1) for indicated time periods. All results were normalized to those of the untreated cells. Data are mean values ± SEM of three independent experiments each performed in triplicates.

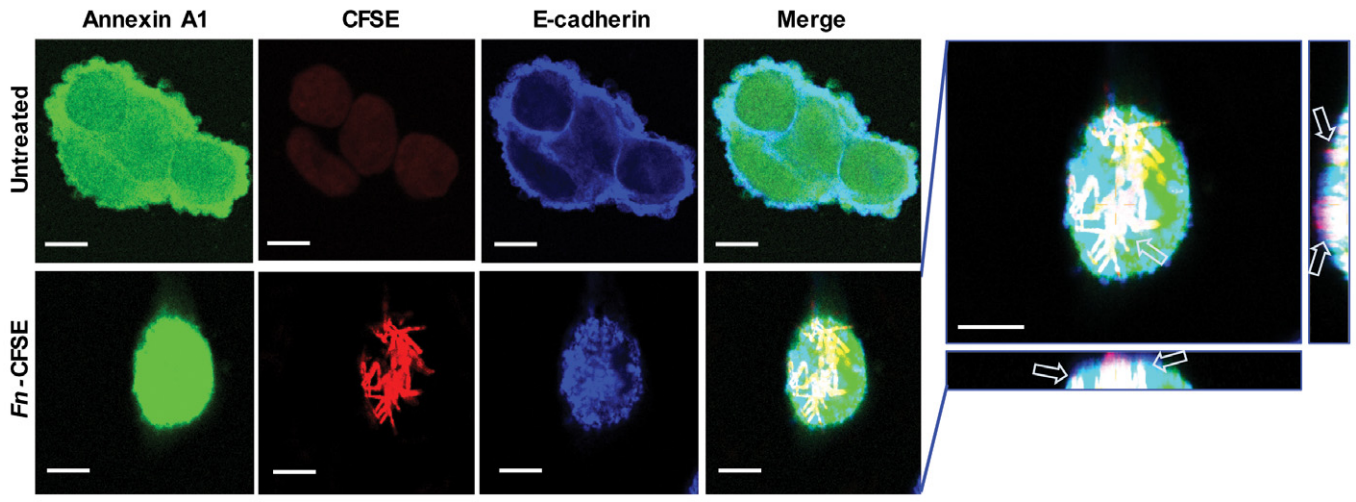

Figure EV4. Co-localization of *F. nucleatum* 12230, Annexin A1 and E-cadherin on DLD1 cells.

Confocal microscopy analysis of DLD1 cells either untreated (top panel) or following incubation with CFSE-labeled *F. nucleatum* 12230 (red, bottom panel) at MOI of ~5:1 for 3 h and immunostaining of Annexin A1 (green) and E-cadherin (blue). Images were 1,200× magnification. Arrows point to co-localization of Annexin A1, E-cadherin, and *F. nucleatum*. The side views are shown to the right and bottom of the enlarged image. Scale bar, 500 nm.

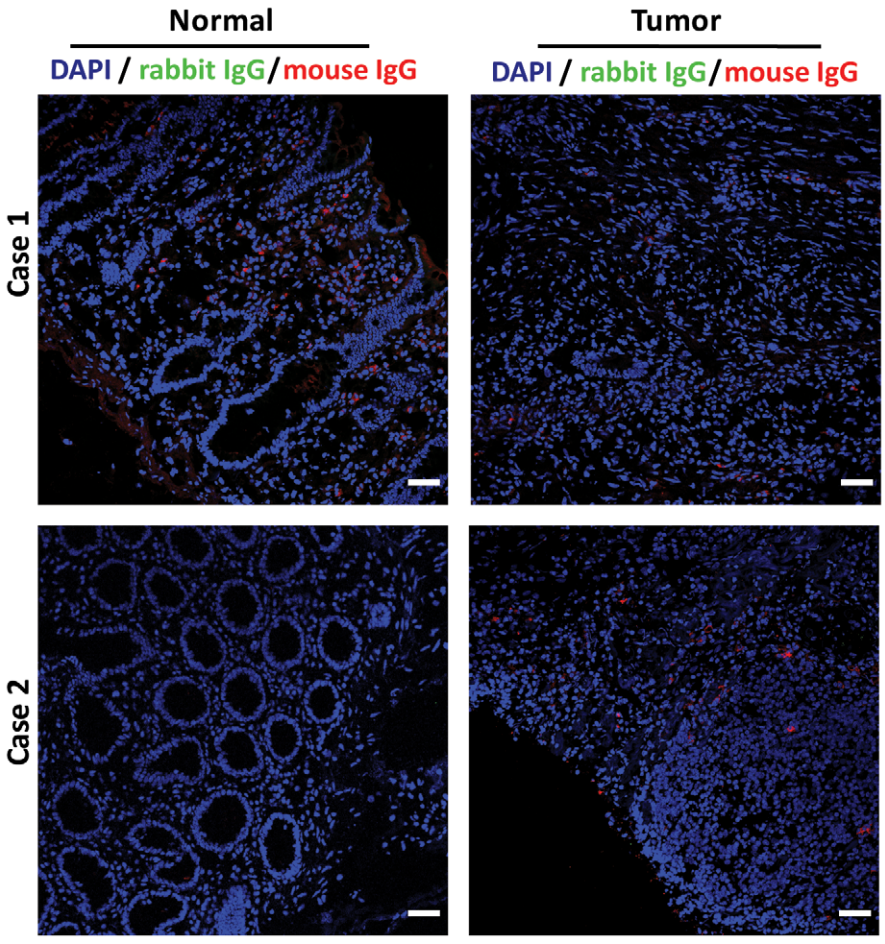

Figure EV5. Isotype controls of paired normal and carcinoma tissues from two colon cancer patients (see Fig 7G).

The frozen sections were incubated with rabbit and mouse IgG. The slides were then stained with Alexa Fluor® 680-conjugated donkey anti-rabbit and Alexa Fluor® 555-conjugated goat anti-mouse IgG antibodies, washed, and covered in mounting medium containing DAPI. The scanning confocal microscopy mages were taken with a Nikon Ti Eclipse inverted microscope at 200× magnification. Scale bar, 50 μm.