Supporting Information

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SI Methods

Colony Formation in Soft Agar. For anchorage-independent growth, 10^4 cells were resuspended in 1.5 mL of growth medium containing 0.4% agarose and plated on 6-cm plates on a solidified bottom layer made of 0.6% agarose in medium. 50 μL of growth medium was added daily. Colonies were counted after 20 days and imaged at 100x magnification. Each experiment was performed in duplicates.

Cell Fractionation. Nuclei were isolated in 0.1% Nonidet P-40/PBS for 5min on ice, followed by centrifugation at 720*g*, washed twice with PBS and lysed for 30min in 50 mM Tris·HCl (pH7.5), 150 mM NaCl, 1% Nonidet P-40, 5 mM EGTA, 5 mM EDTA, 20 mM NaF, and complete protease inhibitor mixture (Roche). The supernatant was the cytoplasmic fraction.

Microarrays. Total RNA was isolated from Ls174T/dnTCF4 cells with different treatments: DMSO, DAPT, doxycycline, DAPT plus doxycycline, using RNA extraction kit (Qiagen). Samples were labeled with Cy3 and Cy5 by cDNA synthesis with Agilent low RNA input fluorescent linear amplification kits. Samples were hybridized on Whole Human Genome Oligo Microarrays (G4122A). Raw data were processed using a web implementation of Limma (1) and normalized by robust correction using the global lowess algorithm with scaling (2). For determining significant hits, the Significance Analysis of Microarray (SAM) statistic was performed with the normalized log2 ratios of the 2 replicate microarray hybridizations, using one class and 2 class unpaired tests for the standard *t* test, running on the R based implementation of SAM (samr) (3) on a web based implementation (SAMi) (Lozano et al., unpublished data).

Functional Enrichment Analysis. Functional annotation of genes based on Gene Ontology (4) were extracted from Ensembl v.47 (5). *Z* score analysis: $Z_x = X - \mu_x)/\sigma_{\bar{x}}$ (where μ_x = mean, $\sigma_{\bar{x}}$ = standard error). We display matrices of *Z* score values in which each cell is represented by a color-coded scale. Significance levels were corrected for multiple comparisons using false discovery rate (FDR) correction.

1. Smyth GK, Michaud J, Scott HS (2005) Use of within-array replicate spots for assessing differential expression in microarray experiments. *Bioinformatics* 21:2067–2075.
2. Smyth GK, Speed T (2003) Normalization of cDNA microarray data. *Methods* 31:265–273.
3. Tusher VG, Tibshirani R, Chu G (2001) Significance analysis of microarrays applied to the ionizing radiation response. *Proc Natl Acad Sci USA* 98:5116–5121.
4. Consortium GO (2006) The Gene Ontology (GO) project in 2006. *Nucleic Acids Res* 34:D322–D326.
5. Hubbard TJ, et al. (2007) Ensembl 2007. *Nucleic Acids Res* 35:D610–D617.

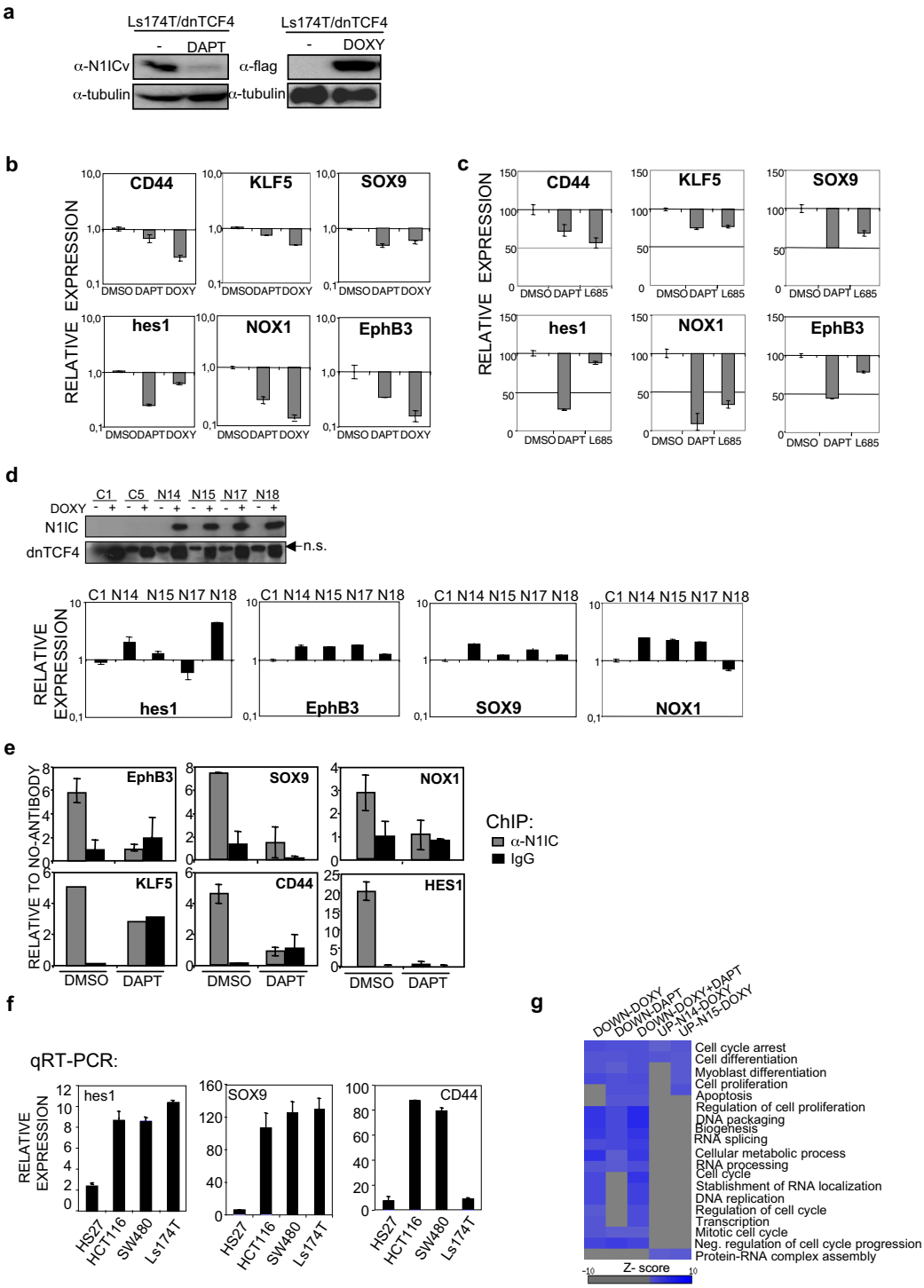


Fig. S1. (*a*) Down-regulation of Notch and β-catenin activities in response to DAPT and doxycycline treatments respectively. Western blot of Ls174T/dnTCF4 cells showing the blockage of Notch1 activation by DAPT (50 μM) and the expression levels of inducible dnTCF4 after doxycycline treatment in the conditions used for the microarray experiments in Fig. 1*a*. (*b*) Confirmation by qRT-PCR of different genes identified in the microarray screening from Fig. 1*a*. (*c*) Inhibition of different genes identified in the microarray screening with 2 different Notch inhibitors (DAPT and L685,458). (*d*) Confirmation by qRT-PCR of different genes identified in the microarray screening from Fig. 1*b*. (*Upper*) The inducible expression of N1IC and dnTCF4 by Western blot. (*e*) ChIP with anti-cleaved Notch antibody and qPCR analysis on the indicated promoters in Ls174T cells untreated or treated with DAPT. (*f*) (*Upper*) ChIP with the α-Notch1 antibody and qPCR analysis of the indicated promoters in different CRC cell lines. (*Lower*) Expression levels of these genes determined by qRT-PCR. (*g*) Functional annotation of genes down-regulated after inhibition of Wnt, Notch or both pathways or up-regulated in 2 N1IC-expressing clones (N14 and N15) based on Gene Ontology. Blue signifies over-representation of genes for the indicated groups. Gray means no significant difference from expected.

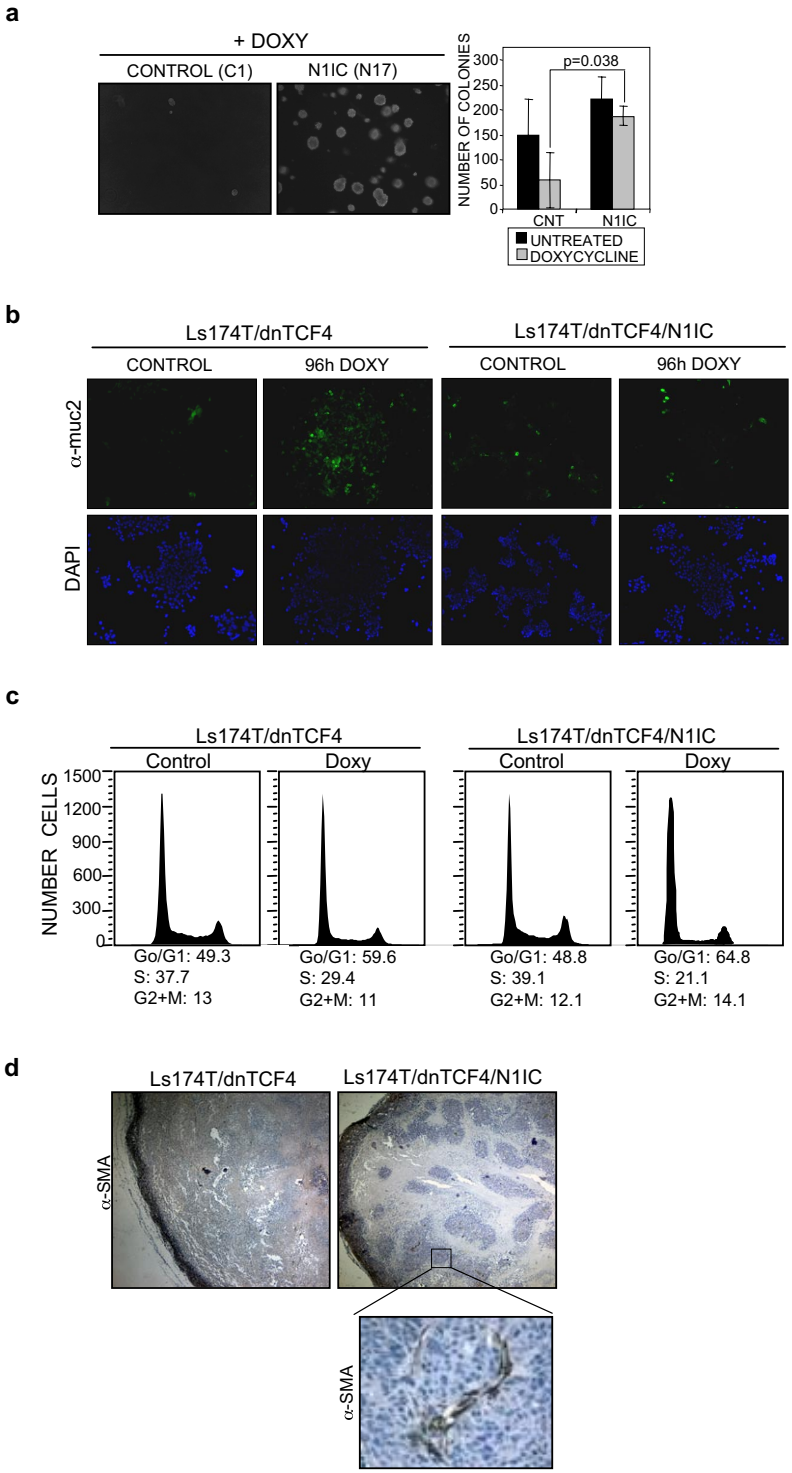


Fig. S2. (*a*) N1IC expression promotes colony formation in soft agar in the absence of Wnt signaling. Ls174T/dnTCF4 (CONTROL) and Ls174T/dnTCF4/N1IC (N1IC) were seeded in soft agar in the absence or presence of doxycycline. Colonies were counted after 7 days and representative images were obtained in an Olympus IX-10 at 40×. Average number of colonies of 2 independent experiments is represented. Error bars represent s.e.m. and P value is based on 2-sided Student's *t* test. (*b*) Immunostaining with α-muc2 antibody of the indicated cell lines untreated or treated with doxycycline. Representative images were obtained in an Olympus IX-10 at 200×. (*c*) Cell cycle profiles of untreated or doxycycline-treated (24h) Ls174T/dnTCF4 and Ls174T/dnTCF4/N1IC clones. Results from 1 representative of 3 different analyzed clones is shown. (*d*) α-SMA staining including a detail of vascularized tumor area (in the box) of representative tumors at 100x

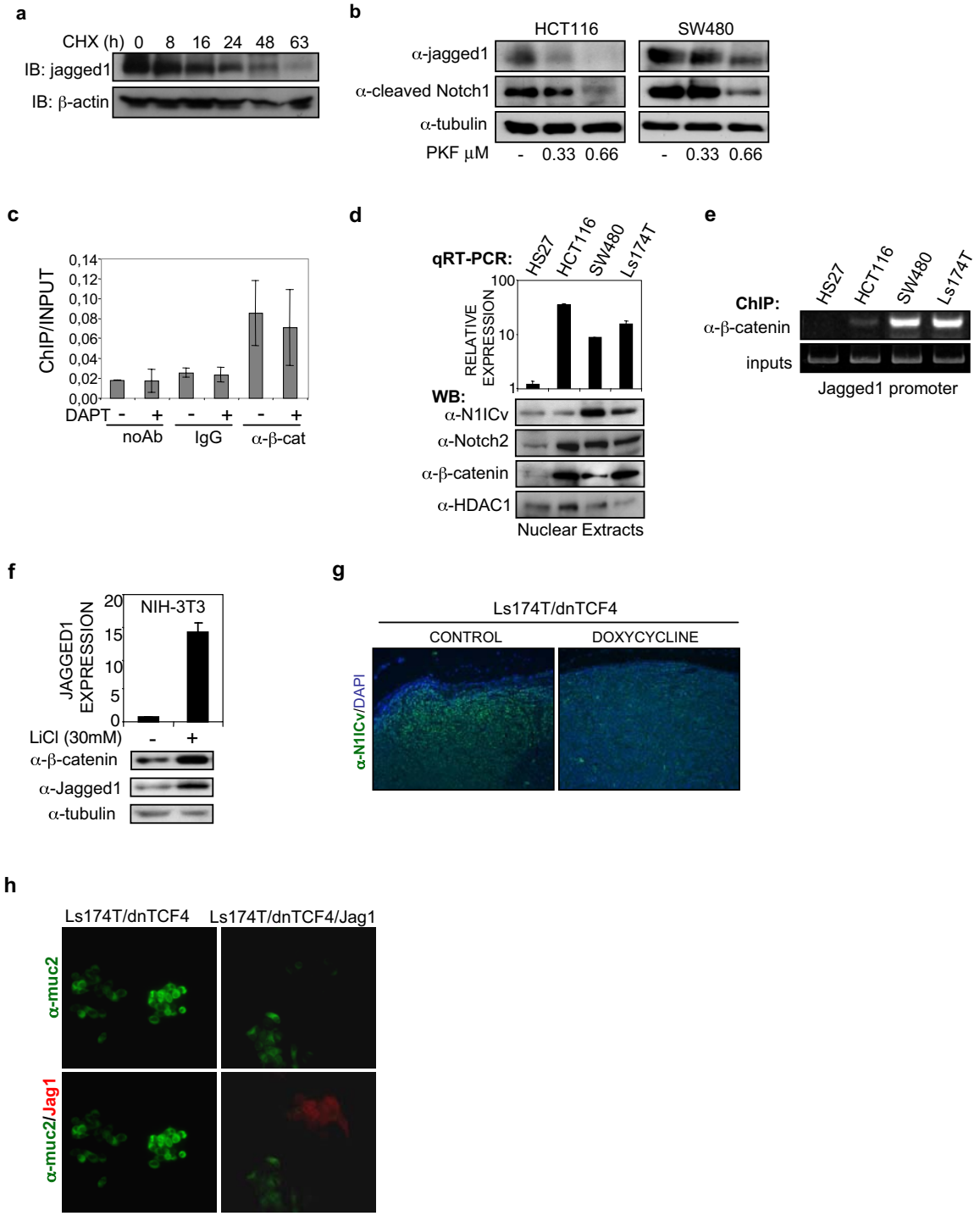


Fig. S3. (*a*) Half-life of the Jagged1 protein was determined by Western blot after incubation of Ls174T Cells with cycloheximide (CHX). (*b*) Pharmacological inhibition of β-catenin activity by PKF115–584 results in a dose dependent reduction of Jagged1 levels and inhibition of Notch activation as shown by Western blot analysis. (*c*) Recruitment of β-catenin to the Jagged1 promoter. qPCR to quantify the recruitment of β-catenin to the Jagged1 promoter in the absence or presence of the Notch inhibitor DAPT. (*d*) qRT-PCR was used to determine the expression of Jagged1 in CRC cell lines compared with nontransformed HS27 cells (*Upper*). Western blot analysis of CRC nuclear extracts with α-N1ICv, α-Notch2 and α-β-catenin antibodies (*Lower*). (*e*) Chromatin immunoprecipitation with α-β-catenin antibody from different cell lines compared with nontransformed HS27 cells. The presence of Jagged1 promoter in the precipitates was determined by semiquantitative PCR. (*f*) qRT-PCR and Western blot analysis to compare the mRNA and protein levels of Jagged1 in NIH 3T3 cells treated or untreated with the GSK3β-inhibitor LiCl. β-catenin and tubulin are shown as controls. (*g*) α-cleaved Notch staining of representative tumors at 100×. (*h*) Immunostaining with α-muc2 and α-Jagged1 antibodies of Ls174T/dnTCF4 cells transfected with mock or with Jagged1 plasmids, treated with doxycycline for 48 h. Representative images were obtained in an Olympus IX-10 at 400X.

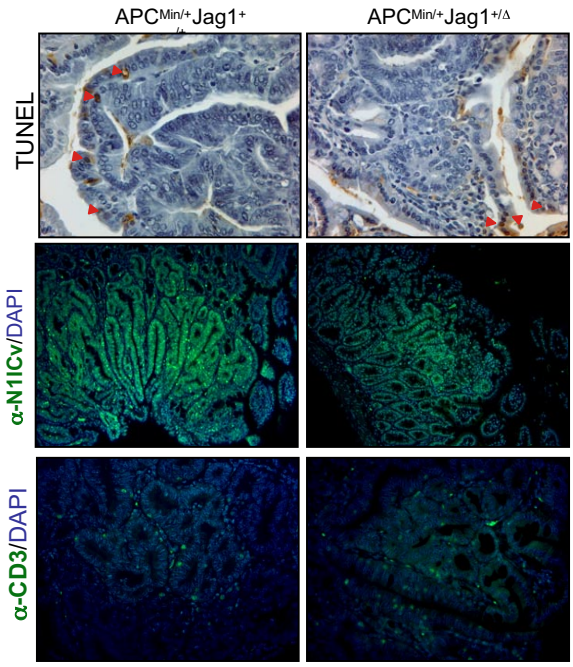
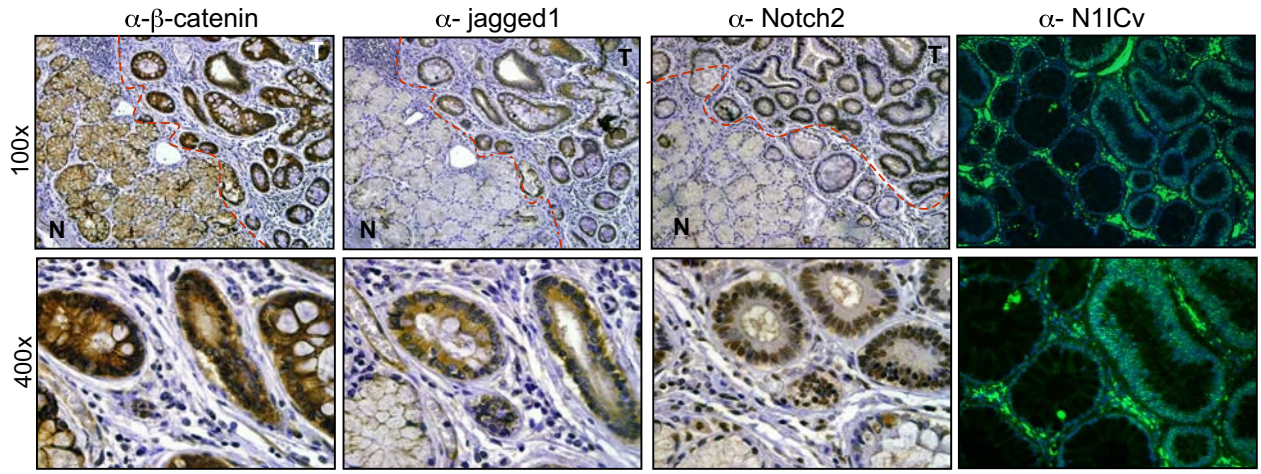


Fig. S4. TUNEL assay of representative tumors from different genotypes. Red arrows indicate apoptotic cells. Immunostaining with α-cleaved Notch1 (*Upper*) and α-CD3 (*Lower*) antibodies of representative tumors from $APC^{Min/+}$ or $APC^{Min/+}Jag1^{+/\Delta}$ mice. Representative images were obtained in an Olympus IX-10 at 200X.

a



b

FAP PATIENTS	SAMPLE NUMBERS	GERM LINE MUTATION
FAP1	5, 11, 15	c.1958 G>A; exon 14 skipping
FAP3	9, 17	c.1958+3A>G +c.1959G>A; exon 14 skipping
FAP4	8, 16, 20	c.4175C>A; p.Ser1392X
FAP6	7, 14, 19	c. 4612_4613delGA; p.Glu1538IlefsX5
FAP8	6, 10, 12	c.1262G>A; c.1263G>A; W421X

Fig. S5. Serial sections of an adenoma sample from a different FAP patient stained with the indicated antibodies. Images were obtained with an Olympus BX-61 at 2 different magnifications (100× and 400×). (*b*) List of germ line mutations in the APC gene in patients included in the qRT-PCR analysis.

Other Supporting Information Files

Table S1
Table S2
Table S3
Table S4