Supplementary information, Data S1

Material and methods

RT-PCR

Total RNA was extracted using the Absolutely RNA Miniprep Kit (Stratagene). For isolation of RNAs from paraffin-embedded tissues, each sample contained microdissected 30 pieces of 6 μm-thick paraffin-embedded colorectal continuous slices. Following treatment with xylene at 55°C, it was washed in absolute ethanol, dried and resuspended in the lysis buffer (Stratagene). After homogenizing thoroughly, it was digested with Protein K (0.4 mg/ml) at 45°C overnight and total RNA was extracted as above. They were reverse transcribed and the resulting cDNA was used for PCR using the SYBR-Green Master PCR Mix (Applied Biosystem) in triplicates. All RT² qPCR Primer pairs were purchased from SABiosciences. PCR and data collection were performed on the Mx3000 qPCR System (Stratagene). All quantitations were normalized to an endogenous β-actin control. The relative quantitation value for each target gene compared to the calibrator for that target is expressed as 2-(Ct-Cc) (Ct and Cc are the mean threshold cycle differences after normalizing to β-actin). The relative expression levels of samples are presented using a semi-log plot.

Construction and expression of recombinant hSlit2 and hRobo1-Fc

For construction of hSlit2, the forward primer 5'-ACCTTCTAGAATGCGCGGCGTTGGCTGG C-3' and the reverse primer 5'-GCAGCGGCCGCTCAGTGATGATGATGATGATGATCTGCCATTTCT CCAGGACC-3' were used to amplify the insert (1-2670 bp) from human *Slit2* cDNA. After restriction enzyme digestion with Xbal/BgIII, it was ligated into the pVL1393 vector (BD Pharmingen), encoding the 1-890 amino acid residues of human *Slit2*, including the first to fourth leucine-rich repeats and first

to sixth epidermal growth factor-like domains. For construction of hRobo1-Fc, the first to fifth immunoglobulin-like segment (1-1760 bp) was amplified from human Robo1 cDNA (Origene) using the forward primer 5'-GGCCAAGCTTATGAAATGGAAACATGTTCC-3' and the reverse primer 5'-TCCACGGAATTCAAATTTGGTTGCC-3'. The PCR product was digested with HindIII and EcoRI, which was subcloned into the pEGFP-N1 vector (BD Pharmingen) to generate pEGFP-N1-hRobo1. The human immunoglobulin heavy chain (714 bp) was amplified from pAc-k-CH3 (US Biologicals) using the forward primer 5'-AACCGTGAATTCCGTGGACAAGAGA GTTGAGCC-3' and the reverse primer 5'-TACGGGTCGACTCATTTACCCGGAGACAGGG-3'. The PCR product was digested with EcoRI and SalI and subcloned into pEGFP-N1-hRobo1 to generate pEGFP-N1-hRobo1-Fc, which was used as the template to PCR the hRobo1-Fc segment using the forward primer 5'-AGGCGGCCTCTAGAATGAAATGGAAACATGTTCC-3' and the reverse primer 5'-TACGGGCGGCCGCTCATTTACCCGGAGACAG-3'. The PCR product was digested by XbaI and NotI and ligated to the pVL1393 vector (BD Pharmaingen) for pVL1393-hRobo-Fc. The authenticity of these constructs was confirmed by DNA sequencing. Recombinant hSlit2 and hRobo1-Fc were expressed in Sf9 cells and purified as previously described [1].

Determination of promoter activities

For measurements of E-cadherin promoter activity, the plasmid encoding the E-cadherin promoter luciferase reporter was co-transfected with the β-galactosidase plasmid into HCT116 cells using Lipofectamine 2000 (Invitrogen) [2].

References

1 Wang H-B, Wang J-T, Zhang L, *et al.* P-selectin primes leukocyte integrin activation during inflammation. *Nat. Immunol.* 2007; **8**:882-892.

2 Xia Y-F, Ye B-Q, Li Y-D, *et al.*) Andrographolide attenuates inflammation by inhibition of NF- B activation through covalent modification of reduced cysteine[62] of p50. *J. Immunol.* 2004; **173**:4207-4217.