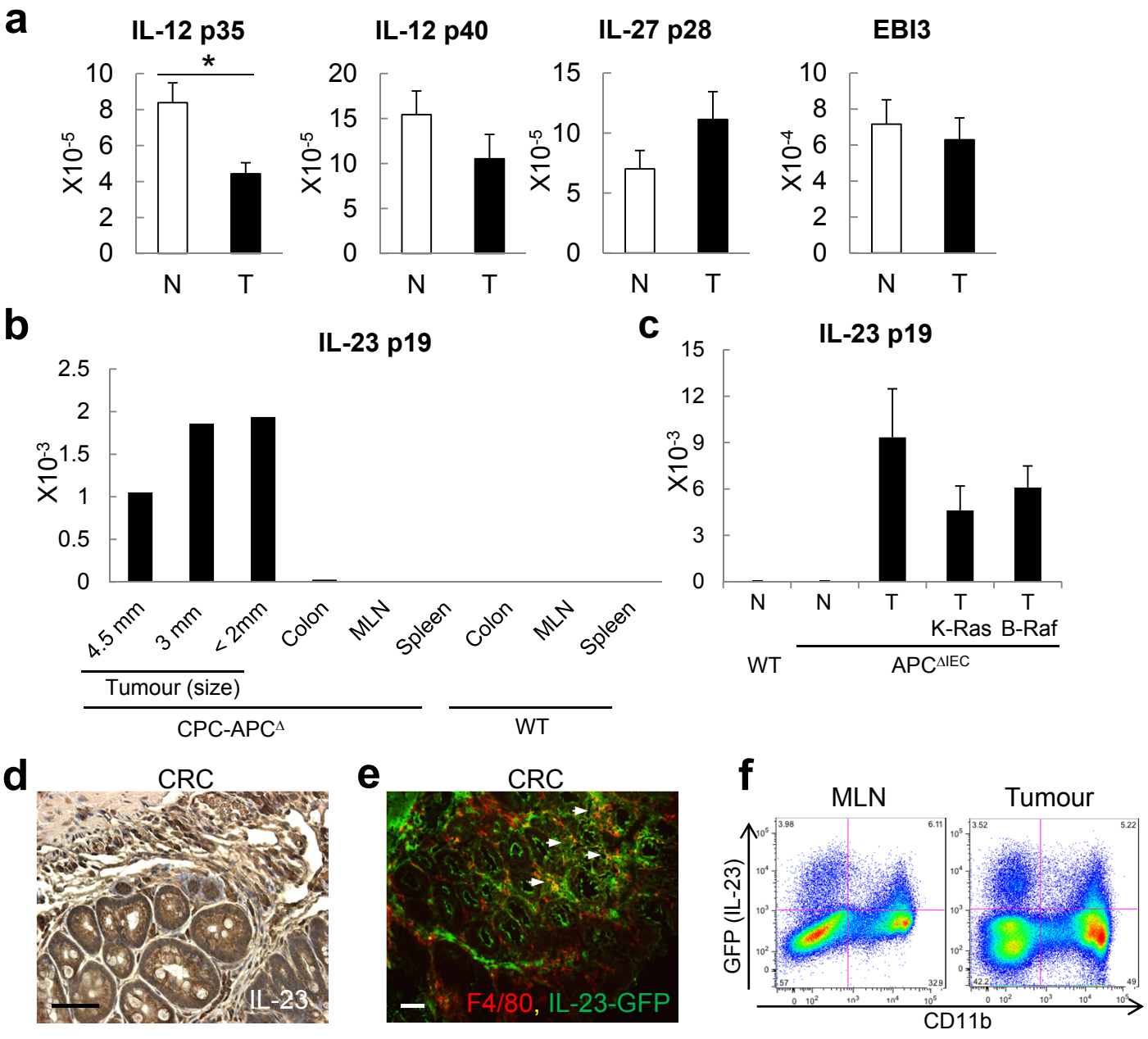
Supplementary Table 1: List of primers used for Q-RT-PCR.

Human Q-RT-PCR

Claudin 4	5'-TAACTGCTCAACCTGTCCCC-3'	5'-ATAAAGCCAGTCCTGATGCG-3'
Claudin 5	5'-GCAGCCAATCACAGAGCC-3'	5'-AGTGGTGTCACCTGAACTGG-3'
Claudin 7	5'-GCAAAATGTACGACTCGGTG-3'	5'-CACAAACATGGCCAGGAAG-3'
IL-17A	5'-ACCAATCCCAAAAGGTCCTC-3'	5'-CACTTTGCCTCCCAGATCAC-3'
IL-23 p19	5'-AGAAGCTCTGCACACTGGC-3'	5'-CCACACTGGATATGGGGAAC-3'
JAM-A	5'-CCTCTTCATATTGGCGATCC-3'	5'-CCAGTTGGCAAGAAGGTCAC-3'
MUC2	5'-GACACCATCTACCTCACCCG-3'	5'-TGTAGGCATCGCTCTTCTCA-3'

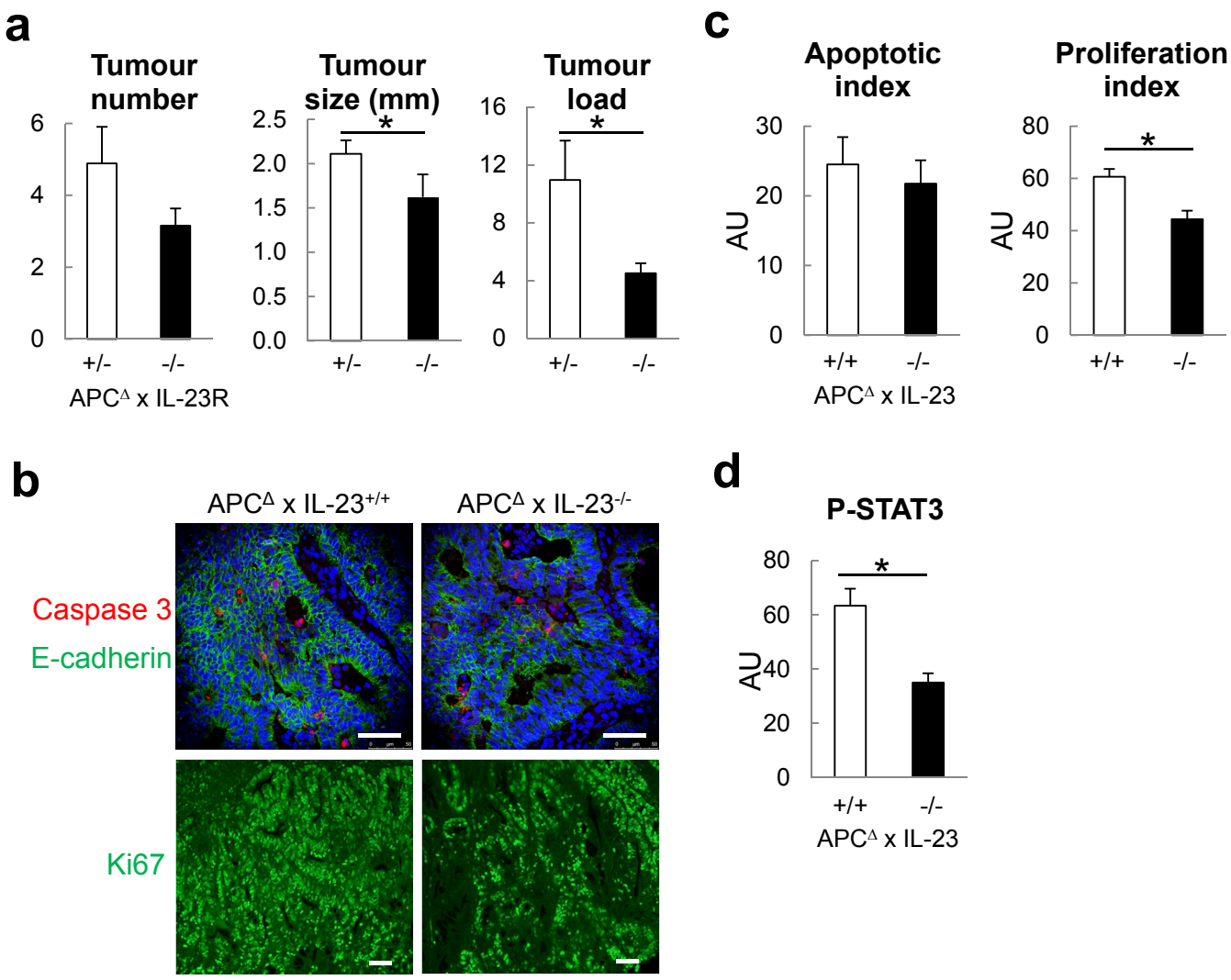
Mouse Q-RT-PCR

Claudin 4	5'-GGCGTAATGGCAAGAGTAGC-3'	5'-CTCGGAGTGGATGTCCTCAT-3'
EBI3	5'-GAGAAGATGTCCGGGAAGG-3'	5'-TCCTAGCCTTTGTGGCTGAG-3'
IL-6	5'-ACCAGAGGAAATTTTCAATAGGC-3'	5'-TGATGCACTTGCAGAAAACA-3'
IL-12 p35	5'-GAGGACTTGAAGATGTACCAG-3'	5'-CTATCTGTGTGAGGAGGGC-3'
IL-12 p40	5'-GACCCTGCCCATTGAACTGGC-3'	5'-CAACGTTGCATCCTAGGATCG-3'
IL-17A	5'-GCCCTCAGACTACCTCAACC-3'	5'-ACACCCACCAGCATCTTCTC-3'
IL-17F	5'-AATTCCAGAACCGCTCCAGT-3'	5'-TTGATGCAGCCTGAGTGTCT-3'
IL-22	5'-CAGGAGGTGGTACCTTTCCTG-3'	5'-TCTGGTCGTCACCGCTGAT-3'
IL-23 p19	5'-CCAGCGGGACATATGAATCT-3'	5'-AGGCTCCCCTTTGAAGATGT-3'
IL-27 p28	5'-CTGGCAAGGTACAGGCTGA-3'	5'-CAGGTGACAGGAGACCTTGG-3'
JAM-C	5'-CTGCCTGACTTCTTCCTGCT-3'	5'-ATGTACCACTGGGTTTCGGT-3'
MUC2	5'-GTAGTTTCCGTTGGAACAGTG-3'	5'-ATGCCCACCTCCTCAAAGAC-3'

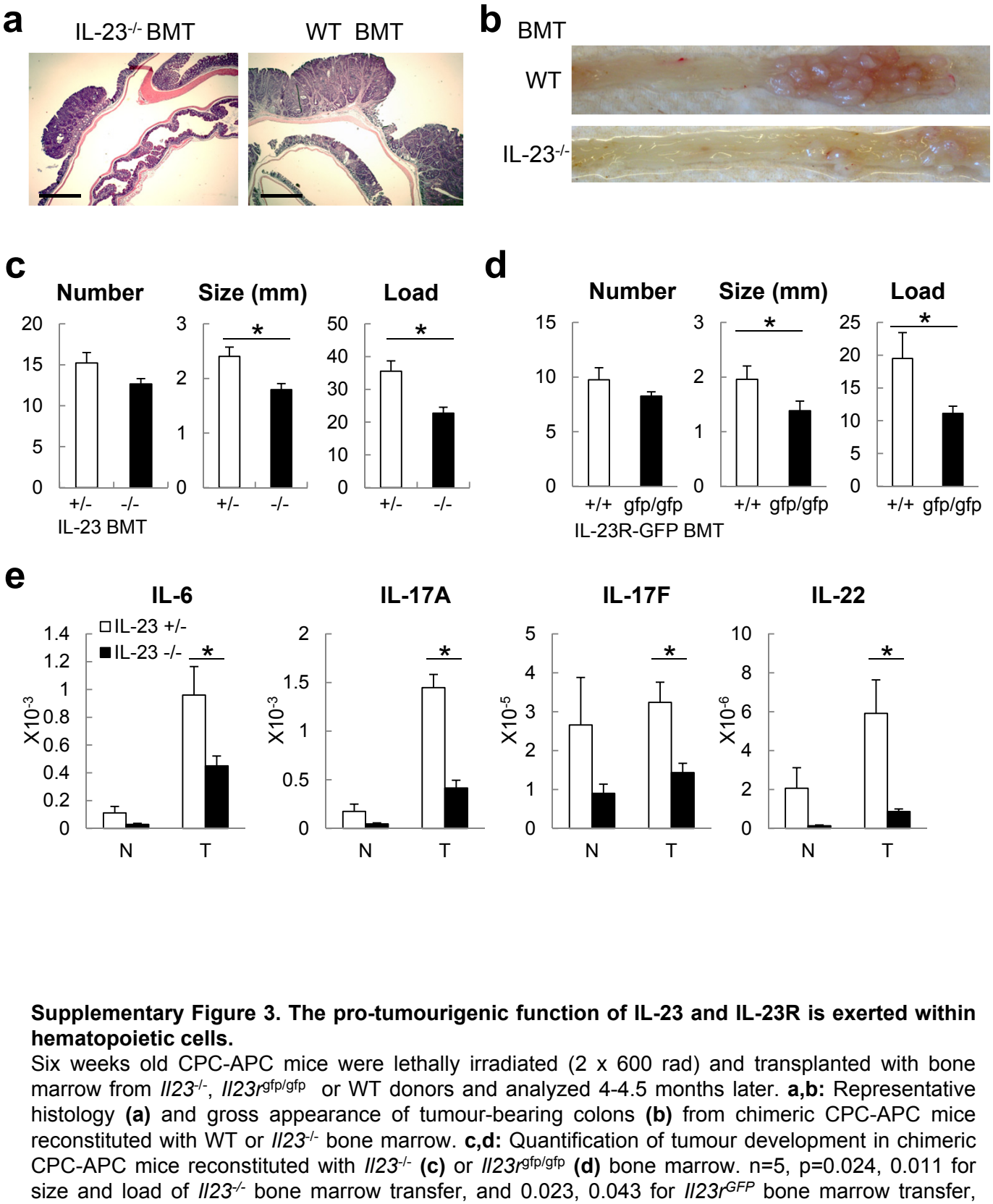


Supplementary Figure 1. Expression of IL-12 family members in normal and tumour tissue of CPC-APC mice.

a-c: RT-qPCR analysis of cytokine mRNA expression in normal colon (N), tumours (T) and mesenteric lymph nodes (MLN) of 5 months old CPC-APC or WT mice **(a, b)** or in tumours of *Apc*$^{\Delta IEC}$ mice without or in combination with *Kras*^{G12D} (K-Ras) or *Braf*^{V600E} (B-Raf) alleles, activated by intra-colonic administration of Adenovirus-Cre into *Apc*$^{F/F}$, *Apc*$^{F/F}$/*K-ras-LSL*^{G12D} or *Apc*$^{F/F}$/*Braf*^{V600E} mice **(c)**. **d,e:** Immunostaining for IL-23 **(d)** or IL-23-driven GFP in CRC, and co-staining of F4/80^{+} cells **(e)**. Arrows indicate positive cells. **f:** Flow cytometric analysis of single cell suspensions isolated from mesenteric lymph nodes (MLN) or colorectal tumours of *Il23*$^{-/-}$ CPC-APC mice stained with anti-CD11b antibody. Live/Dead^{-}CD45^{+} are shown. Data represent averages ± s.e.m., n=6 for **a** and **b**, n=3 for **c**. p=0.006 for IL-12p35 in **a**. Differences between the different tumour models in **c** are not significant. * p<0.05. Scale bars, 50 μm.

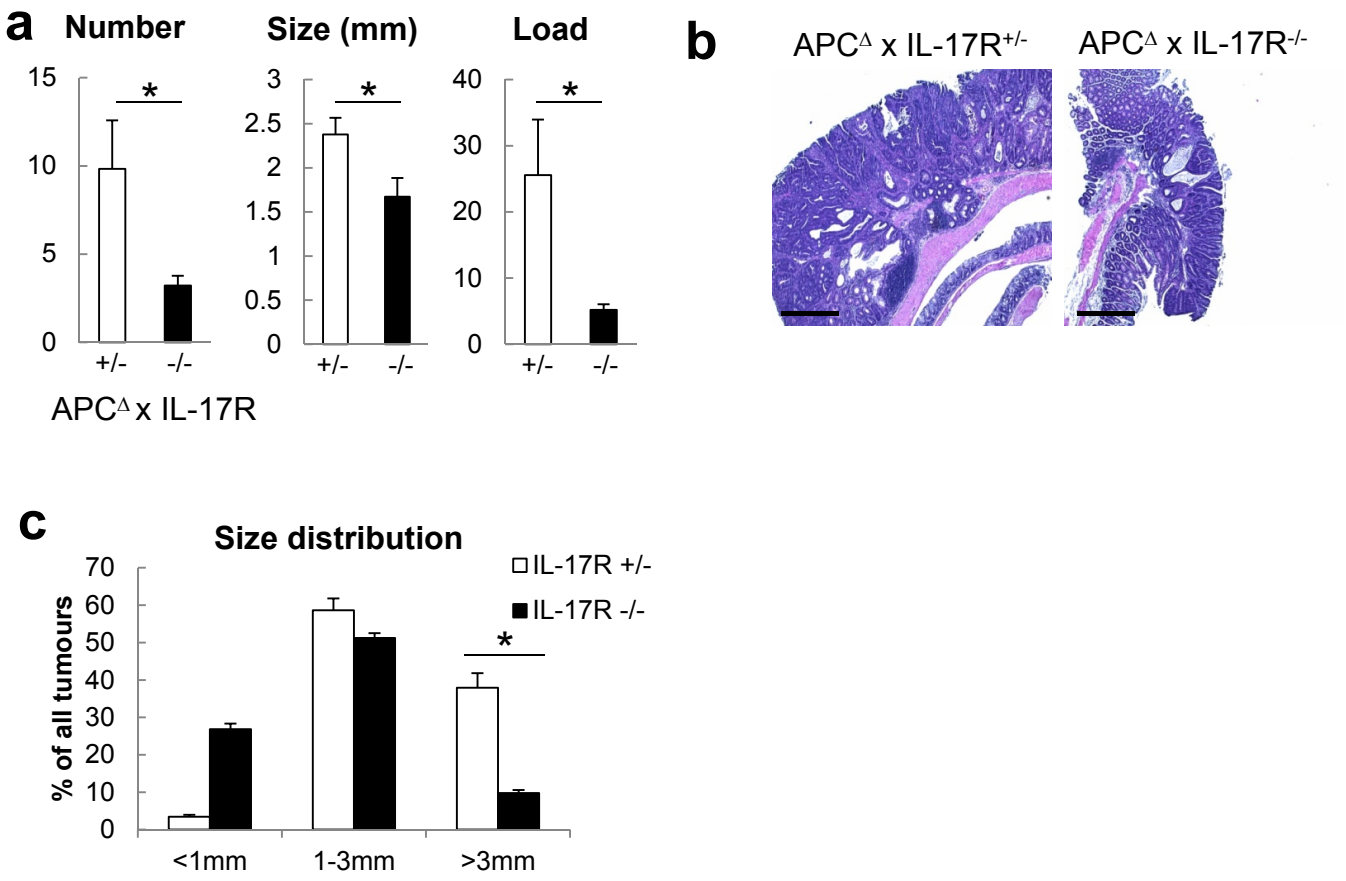


Supplementary Figure 2. IL-23 signaling promotes colorectal tumour development and growth.
a: Colonic tumours from 4.5 months old *Il23r*$^{-/-}$/CPC-APC and control CPC-APC mice were enumerated and measured. n=8, p=0.081, 0.041, 0.024, respectively. **b,c:** Paraffin sections of colons from *Il23*$^{-/-}$/CPC-APC or control CPC-APC mice were stained with active caspase 3 and E-cadherin antibodies, TUNEL kit or a Ki67 antibody and TUNEL-positive apoptotic or Ki67 positive proliferating cells were quantified. n=12, p=0.001 for proliferation index **(c)**. **d:** Quantification of phospho-STAT3^{high} cells in colonic tumours of *Il23*$^{-/-}$/CPC-APC or control CPC-APC mice (see Fig. 1g). 4 fields from different sections of 5 independent tumours were counted. p=0.0045. Data represent averages ± s.e.m. *p<0.05. Scale bars, 50 μm.

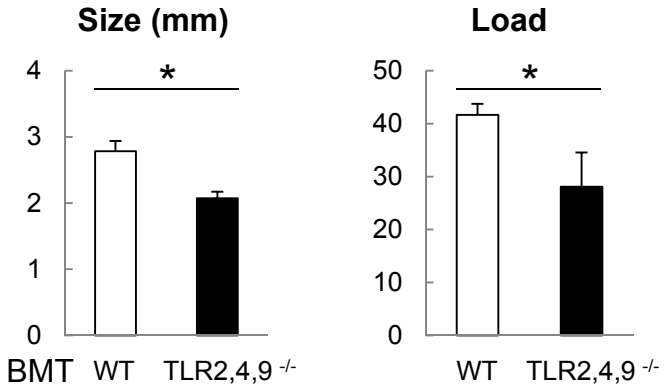


Supplementary Figure 3. The pro-tumourigenic function of IL-23 and IL-23R is exerted within hematopoietic cells.

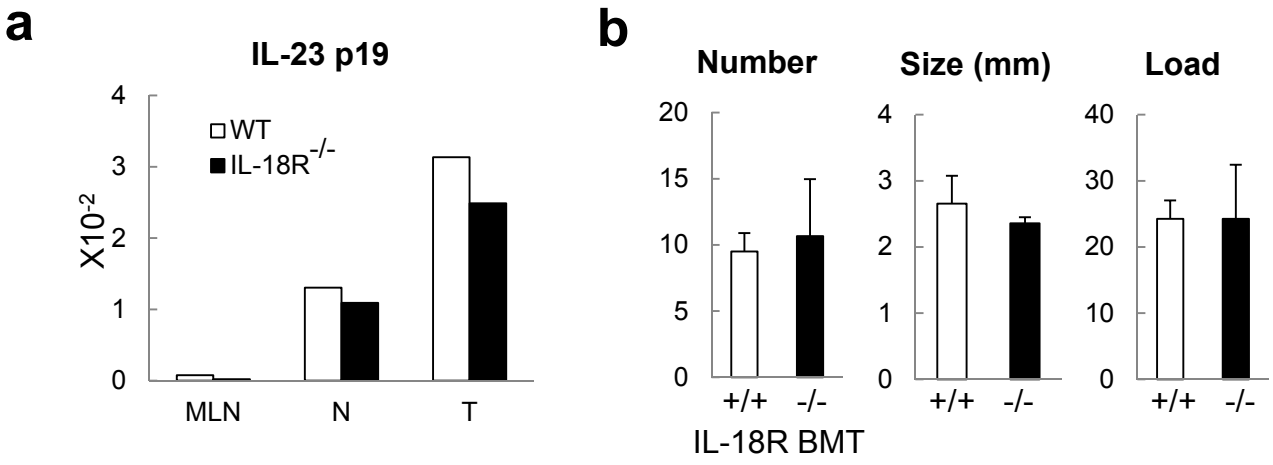
Six weeks old CPC-APC mice were lethally irradiated (2 x 600 rad) and transplanted with bone marrow from *Il23*$^{-/-}$, *Il23r*$^{gfp/gfp}$ or WT donors and analyzed 4-4.5 months later. **a,b:** Representative histology **(a)** and gross appearance of tumour-bearing colons **(b)** from chimeric CPC-APC mice reconstituted with WT or *Il23*$^{-/-}$ bone marrow. **c,d:** Quantification of tumour development in chimeric CPC-APC mice reconstituted with *Il23*$^{-/-}$ **(c)** or *Il23r*$^{gfp/gfp}$ **(d)** bone marrow. n=5, p=0.024, 0.011 for size and load of *Il23*$^{-/-}$ bone marrow transfer, and 0.023, 0.043 for *Il23r*^{GFP} bone marrow transfer, respectively. **e:** RT-qPCR analysis of cytokine mRNAs in tumours (T) and adjacent normal colon (N) of chimeric CPC-APC mice reconstituted with control or *Il23*$^{-/-}$ bone marrow. n=6, p=0.0066, 2.3x10^{-5}, 0.014 and 0.0032, respectively. Data represent averages ± s.e.m. *p<0.05. Scale bars, 500 μm.



Supplementary Figure 4. IL-17R signaling promotes growth and progression of colorectal tumours.
Tumours from 5 months old *Il17ra*$^{-/-}$/CPC-APC and control mice were analyzed. **a:** Tumour number, size and load. n=7, p=0.014, 0.045, 0.011, respectively. **b:** H&E staining of colon sections from CPC-APC mice of the indicated genotypes. **c:** Distribution of tumour sizes among all detectable tumours. n=7, p=0.045 for tumours bigger than 3 mm. Data represent averages ± s.e.m. *p<0.05. Scale bars, 200 μm.

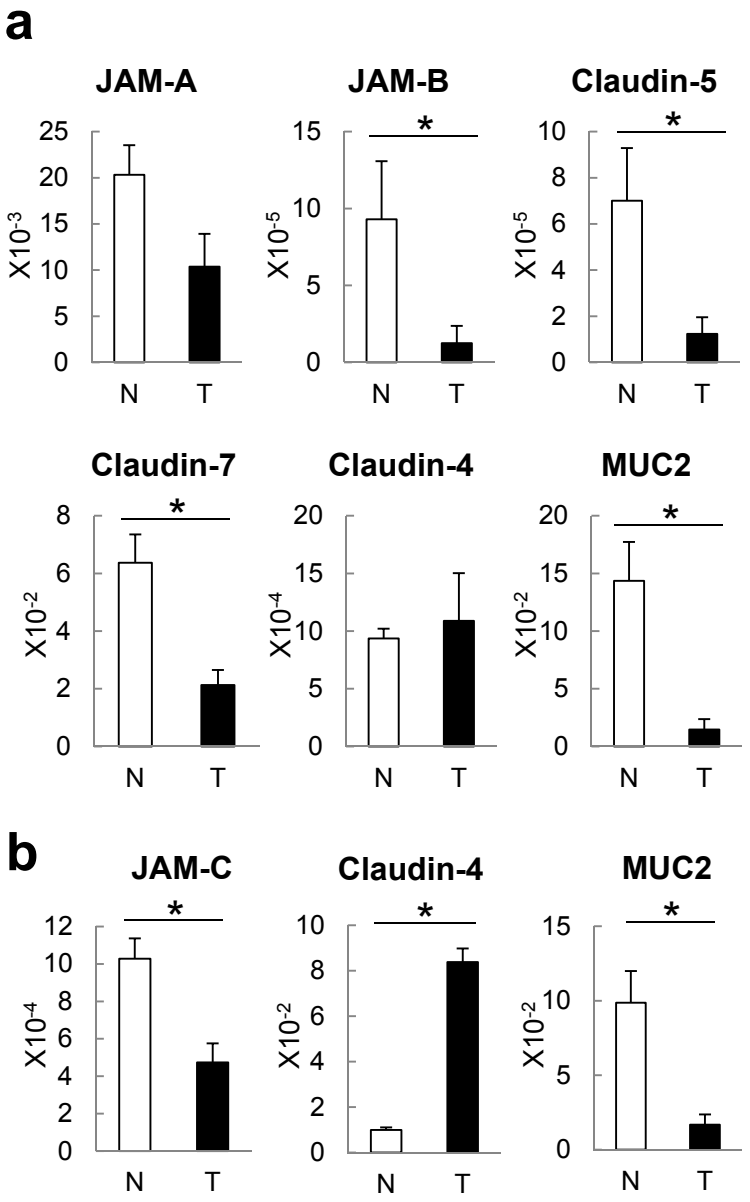


Supplementary Figure 5. Signaling through TLRs is important for CRC tumour growth.
Six to seven weeks old CPC-APC mice were lethally irradiated (2 x 600 rad) and transplanted with bone marrow from *Tlr2,4,9*$^{-/-}$ or WT donors and analyzed 4.5 months later. Quantification of tumour size and load in chimeric CPC-APC mice is presented. n=5, p=0.025, 0.049 respectively. Data represent averages ± s.e.m. *p<0.05.



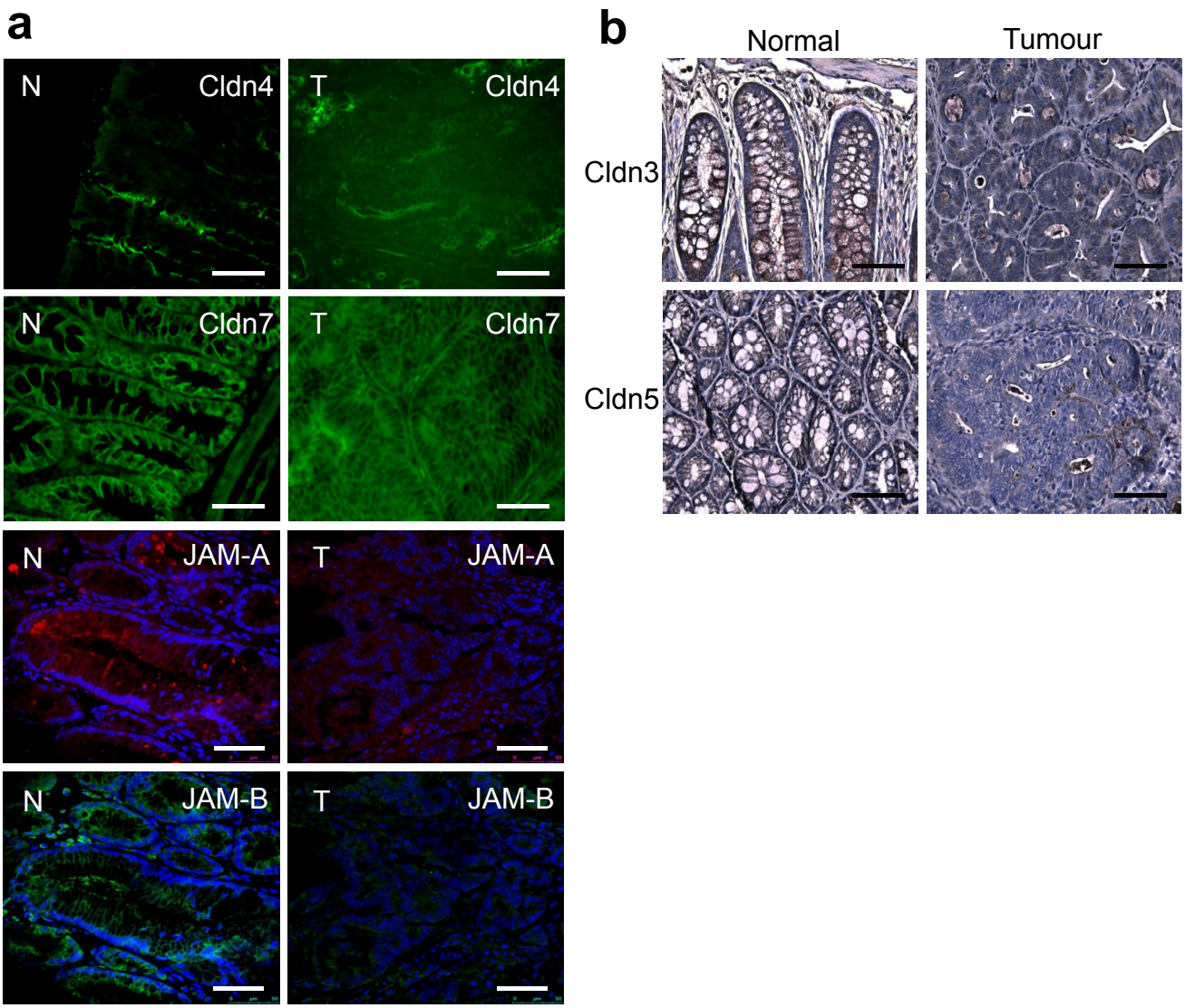
Supplementary Figure 6. IL-18R signaling in hematopoietic cells is not required for IL-23 induction and growth of colorectal tumours.

Six weeks old CPC-APC mice were lethally irradiated and transplanted with *Il18r*$^{-/-}$ or WT bone marrow and sacrificed for analysis 4 months later. **a:** Single cell suspensions from normal colons, tumours and MLN were prepared and CD11b^{+} cells were sorted by positive selection on magnetic beads. IL-23p19 mRNA expression was measured by RT-qPCR. **b:** Tumour number, size and load were determined. n=5. None of the differences (number, size and load) are significant. Data represent averages ± s.e.m.

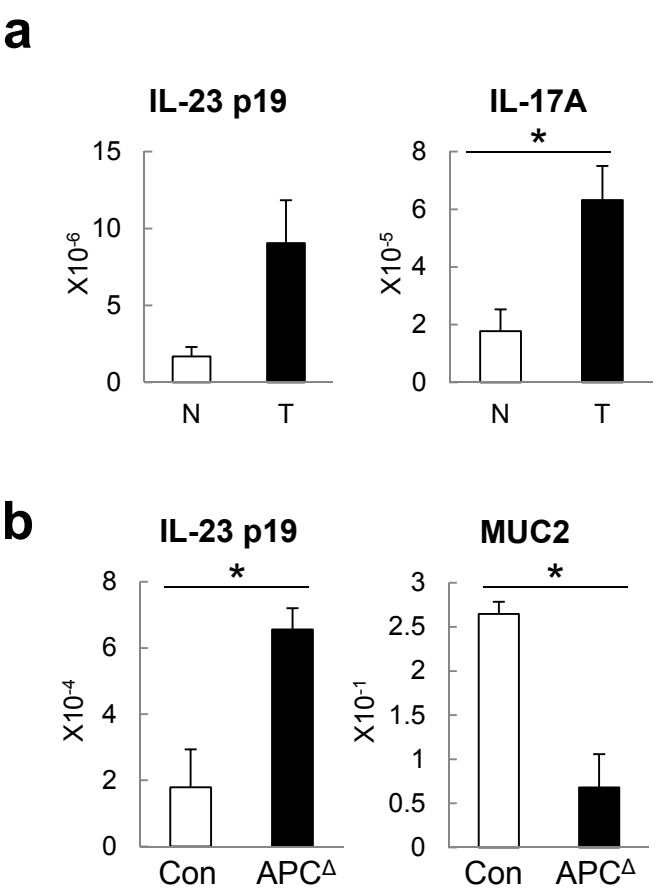


Supplementary Figure 7. Human CRC and mouse colorectal tumours display dysregulation of mRNAs encoding barrier and junctional proteins.

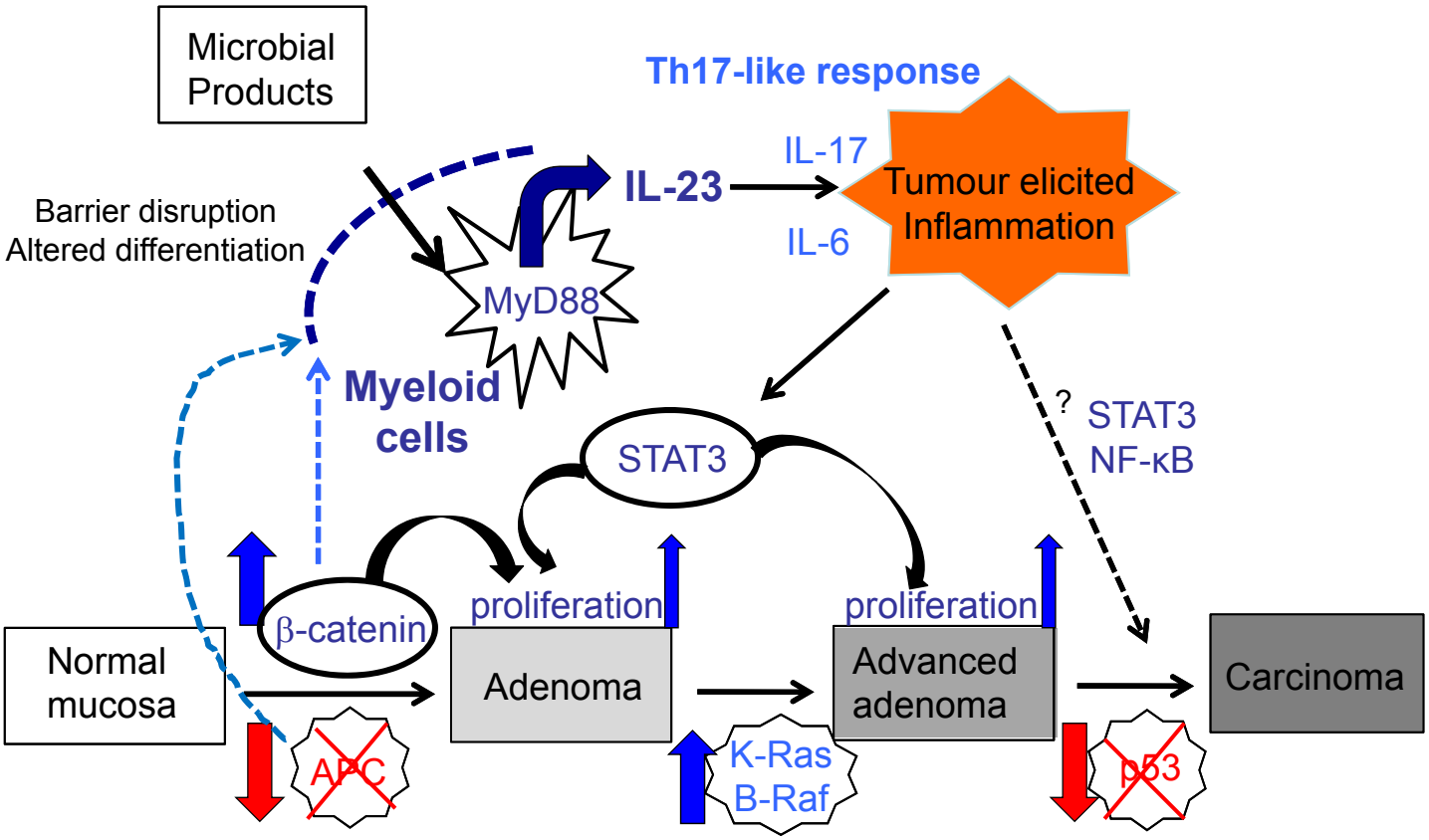
a,b: Total RNA was isolated from frozen tumour (T) and tumour-free (N) colon tissue of CRC patients **(a)** or tumour bearing CPC-APC mice **(b)** and subjected to RT-qPCR analysis of indicated mRNAs. Human: n=7, p=0.078, 0.025, 0.016, 0.025, 0.0065 for JAM-A, JAM-B, claudin-5, claudin-7, and Muc2, respectively; Mouse: n=12, p=0.0012, 0.0035 and 0.0027 for JAM-C, claudin-4 and Muc2, respectively. Data represent averages ± s.e.m. *p<0.05.



Supplementary Figure 8. Mouse colorectal tumours display dysregulation of barrier and junctional proteins.
Paraffin-embedded colon sections from CPC-APC mice were stained with antibodies to claudin 4, claudin 7, JAM-A or JAM-B and analyzed by fluorescent microscopy **(a)** or with claudin 3 and claudin 5 specific antibodies **(b)**. Normal (N) and Tumour (T) areas are shown. Scale bars, 50 μm.

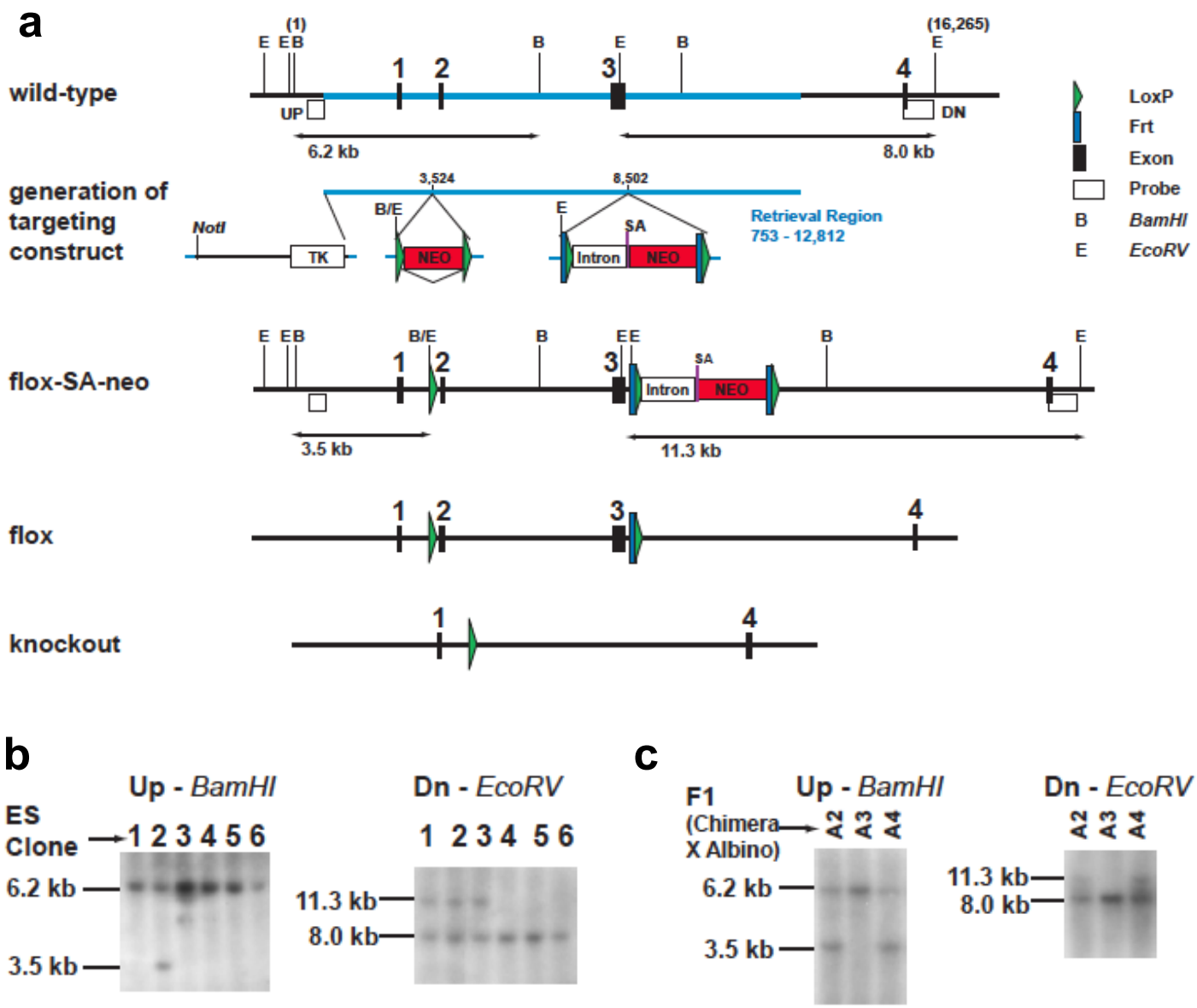


Supplementary Figure 9. Early human adenomas and mouse rapidly transformed colons display upregulation of cytokines and dysregulation of barrier proteins.
Total RNA was isolated from frozen human adenoma tissue removed during colonoscopy (T) and matching tumour-free (N) colon tissue **(a)** or from *Cdx2*$^{ERT-Cre}$ x *Apc*$^{F/F}$ and control mice injected 3 times with tamoxifen and left for 20 days for rapid colonic transformation **(b)**. RNA was subjected to RT-qPCR analysis of the indicated transcripts. n=5, p=0.056, 0.024 for IL-23p19 and IL-17A in **a**. n=5, p=0.018, 0.014 for IL-23p19 and MUC2 in **b**. Data represent averages ± s.e.m. * p<0.05.



Supplementary Figure 10. Scheme: "Tumour elicited inflammation" is triggered by the classical genetic pathway that drives colorectal tumourigenesis and can further stimulate tumour progression.

Inactivation of tumour suppressor APC is the first step in the classical genetic pathway of colorectal tumourigenesis, leading to persistent activation of β-catenin signaling. We propose that β-catenin signaling in early neoplastic lesions induces altered tissue homeostasis and disruption of the epithelial barrier, including downregulation of mucin 2 expression due to failed goblet cell differentiation and disruption of tight junctions. Tumour-specific deterioration of barrier function allows commensal microflora and microbial products to penetrate the adenomas, activate TLR-MyD88 signaling and induce IL-23 in tumour associated macrophages (TAM). IL-23 regulates tumour elicited inflammation and the Th17-like response, particularly its effector cytokines that include IL-17A and IL-6, to activate transcription factor STAT3, which together with β-catenin enhances the proliferation and growth of adenoma cells. Tumour elicited inflammation further contributes to tumour progression and malignant conversion by multiple mechanisms, some of which many also involve STAT3 activation. Tumour promoting events are in 'blue", tumour suppressors are in 'red'.



Supplementary Figure 11. Generation of *Il23r*$^{F/F}$ and *Il23r*$^{-/-}$ mice.
a: Targeting strategy. Two *LoxP* sites were introduced into introns 1 and 3 of the *Il23r* gene, such that Cre-mediated deletion results in excision of exon2 and exon3, which encode the ligand-binding extracellular portion of the receptor. The targeting construct was introduced into Bruce 4 C57Bl6 ES cells and homologous integrants were used to generate *Il23r*$^{F/F}$ mice. **b:** Southern blot screening of Bruce 4 ES clones. Representative clones are shown. **c:** Selected clones were injected into C57BL/6-Albino blastocysts and transferred to pseudopregnant females. Chimeric offspring were bred with C57BL/6-Albino partners to generate F1 mice that were screened for integration by Southern analysis (for more details see Methods). Resulting mice with the *Il23r*$^{SA\text{-}flox\text{-}neo}$ allele were crossed with C57BL/6-FLPe mice to facilitate recombination between *Frt* sequences and generate the *Il23r*^{flox} allele. The null allele was generated by crossing *Il23r*$^{F/F}$ mice with *CPC-Cre* transgenic mice (for more details see Methods). Complete ablation of IL-23R was confirmed by Q-RT-PCR of spleen and tumour-derived cells.