Supplementary figure legends

Figure 1. Western Blot analysis of the two polymorphic variants of IL-12 p40.
Supernatants from non-transfected cells or from transfected cell lines expressing only the
SJL/J derived IL-12p40 or the C57BL/6 derived IL-12p40 were run on 4-12% gradient gels
under non-denaturing conditions. After transfer to 0.45 μm nitrocellulose the blotted bands
were immunodetected with a specific rat anti-mouse IL-12p40 Mab and subsequently
visualized with peroxidase labeled rabbit anti-rat IgG antibodies.
Lane 1: MW marker, lane 2: Recombinant mouse IL-12p40, lane 3: Recombinant Mouse
IL12- p40 Homodimer (IL-12p80), lanes 4 & 7: Supernatants of nontransfected cells, lane 5
& 6: Supernatants of cells transfected with C57BL/6 derived Il-12p40, lane 8 & 9:
Supernatants of cells transfected with SJL/J derived IL-12p40

Figure 2. Assessment of inhibition of N-Glycosylation by kifunensine and BGN. Shown is
the shift in MFI with Con A (□) and GNA (■) (figure 2A) or with HPA (□) and PNA (■)
(figure 2B) after incubation of IL-12 p40/p35 transfected cells with kifunensine or BGN
respectively. MFI’s were normalized against the MFI of cells grown without inhibition which
was set at 100.
The increased MFI’s of HPA and PNA with Benzyl-α-GalNAc incubation indicate blocked
O-glycosylation while the increased MFI’s of Con A and GNA with kifunensine incubation
indicate blocked N-Glycosylation

Figure 3. IL-12p40 transcription in colon after intrarectal administration of TNBS.
Control: nontransgenic SJL/J WT mice (n=5). SJL/type: C57Bl/6 mice carrying the SJL/J
derived p40 variant (n=10). C57Bl/6 type): C57Bl/6 mice carrying the C57Bl/6 derived p40
variant (n=10). Non Tg littermates: non transgenic C57Bl/6 littermates (n=12). Four days
after the induction of colitis, mice were sacrificed and IL-12p40 transcription was determined in the colon by means of Quantitative-PCR in an ABI 7900HT sequence detection system (Applied Biosystems, USA). Since the p40 chain is not constitutively expressed, one would expect no basal expression of this gene, neither in the wild type nor in the transgenic animals. Indeed, no measurable expression of p40 was found in colon homogenates from untreated mice (not shown). In addition, after TNBS challenge, no differences in expression were observed between the transgenic strains, the control WT SJL/J mice or the nontransgenic littermates. Relative amounts of IL-12p40 mRNA are given in arbitrary units. Bars represent mean ± SD.

The following primersets were used:

Forward Murine IL-12p40 primer: 5’-AGA CCC TGC CCA TTG AAC TG-3’
Reverse Murine IL-12p40 primer: 5’-CGG GTC TGG TTT GAT GAT GTC-3’
Forward Murine GAPDH primer: 5’- GAC AAC TCA TCA AGA TTG TCA GCA -3’
Reverse Murine GAPDH primer: 5’- TTC ATG AGC CCT TCC ACA ATG -3’

Figure 4. Histological analysis of the colons of transgenic, nontransgenic littermates and SJL/J WT mice after TNBS colitis induction. Representative H&E-stained cross sections of colon specimens are shown.

A: represents the normal appearing colon from resistant mice predominantly found in mice carrying the C57Bl/6 derived p40 variant and non transgenic littermates. B and C are examples of the mild (B) to moderate (C) forms of colitis predominantly found in mice carrying the SJL/J derived p40 variant. D shows the severe colitis found in the colitis susceptible SJL/J WT control group.
Supplementary Figures.

Figure 1
Figure 2

(A) Comparison of normalized MFRs with and without kifunensin, showing a significant difference (P < 0.0001) and a trend (P < 0.05).

(B) Comparison of normalized MFRs with and without BGN, showing a significant difference (P < 0.0001) and a trend (P < 0.05).
Figure 3