Supplemental Fig. 1. Characterization of IL-23 responsive Ba/F3 cells. *A.* Total RNA of stably transduced Ba/F3-gp130 cells expressing either murine IL-12Rβ1, murine IL-23R or both was extracted using the NucleoSpin® RNA II Kit according to the manufacturer's instructions (Macherey-Nagel, Düren, Germany). Resulting RNA was used for cDNA synthesis using M-MuLV Reverse Transcriptase (Fermentas, Thermo Scientific, St. Leon-Rot, Germany). cDNA was analyzed for expression of IL-12Rβ1 and IL-23R. Expression analysis of *GAPDH* was performed as control for cDNA synthesis. *B.* Representative histograms of IL-23R (upper) and IL-12Rβ1 (lower) surface expression of stably transduced Ba/F3-gp130 cell lines are shown. Gray-shaded areas indicate Ba/F3-gp130 cells (negative control) and dark solid lines are the respective Ba/F3 cell lines as indicated.

Supplemental Fig. 2. Expression and signaling of IL-23 receptors on co-transfected HeLa cells. *A.* HeLa cells were transiently transfected with cDNAs for murine IL-12Rβ1, IL-23R or both, and 30 h after transfection, cells were washed with PBS and starved over-night in serum-free medium. Afterwards, cells were stimulated with 0.2% HIL-23 for 10 min and whole cell lysates were prepared. Equal amounts of total protein were loaded on SDS gels followed by immunoblotting using specific antibodies for phospho-STAT3 and STAT3. Expression of cytokine receptors was verified by western blot with anti-c-myc antibody due to the presence of the C-terminal c-myc tag on both proteins. Because of the detection of a double band for the IL-12Rβ1 which complicates the visualization of the IL-23R. A biotinylated antibody was used for validation of IL-23R expression. *B.* HeLa cells were transiently transfected with either mIL-12Rβ1 or mIL-23R cDNA, and 48 h after transfection, cells were assessed for IL-23R and IL-12Rβ1 surface expression by flow cytometry. Representative histograms of IL-23R (left) and IL-12Rβ1 (right) surface expression of transfected and untransfected HeLa cells are shown. Gray-shaded areas indicate untransfected cells (negative control) and dark solid lines indicates transfected cells. *C* HeLa cells were transiently transfected with cDNAs for murine IL-12Rβ1, hIL-23(mET/hC), containing the cytoplasmic domain of the human receptor, or both. Cells were analyzed as described in Supplemental Fig. 2A.

Supplemental Fig. 3. Expression of IL-23R single mutant variants. *A.* Expression of IL-23R mutants (mouse: Y426F, Y504F, Y542F, Y626F, and human: Y397F, Y463F, Y611F) and mIL-12Rβ1 after co-transfection of HeLa cells was investigated by immunoblotting using anti-c-myc (mouse mutants) or biotinylated IL-12Rβ1 (human mutants) antibodies. For validation of IL-23R expression membranes were stripped and re-probed with a biotinylated antibody directed against IL-23R. *B.* Surface expression of stably transduced Ba/F3-gp130 cell lines was investigated by flow cytometry. Representative histograms are presented. Gray-shaded areas indicate Ba/F3-gp130 cells (negative control) and dark solid lines are the respective Ba/F3 cell lines as indicated.

Supplemental Fig 4. Expression of IL-23R variants with various mutations. *A.* Expression of IL-12Rβ1 and IL-23R mutants with two or three Y→F substitutions after co-transfection of HeLa cells was investigated by immunoblotting using anti-c-myc (mouse mutants) or biotinylated IL-12Rβ1 (human mutants) antibodies. For validation of IL-23R expression membranes were stripped and re-probed with a biotinylated antibody directed against IL-23R. *B.* Surface expression of stably transduced Ba/F3-gp130 cell lines was investigated by flow cytometry. Representative histograms are presented. Gray-shaded areas indicate Ba/F3-gp130 cells (negative control) and dark solid lines are the respective Ba/F3 cell lines as indicated.

Supplemental Fig. 5. Expression of IL-23R deletions and receptor variant with four mutations. *A.* Expression of IL-12Rβ1 and IL-23R deletions, as well as the mutant with four

or three Y→F substitutions (Y416F-Y504F-Y542F-Y626F) after co-transfection of HeLa cells was investigated by immunoblotting using anti-c-myc antibodies. For validation of IL-23R expression membranes were stripped and re-probed with a biotinylated antibody directed against IL-23R. *B.* Surface expression of stably transduced Ba/F3-gp130 cell lines was investigated by flow cytometry. Representative histograms are presented. Gray-shaded areas indicate Ba/F3-gp130 cells (negative control) and dark solid lines are the respective Ba/F3 cell lines as indicated.

Supplemental Fig. 6. The C-terminal part of the IL-23R. *A.* Surface expression of the cytokine receptors was verified by flow cytometry using specific antibodies. Representative histograms for IL-23R (upper) and IL-12Rβ1 (lower) are presented. Gray-shaded areas indicate Ba/F3-gp130 cells (negative control) and dark solid lines are the respective Ba/F3 cell lines as indicated. *B.* The amino acid sequences of IL-23R were aligned. Postulated binding motifs for STAT4 and STAT1/3, and the Y542 of murine IL-23R are underlined and respective tyrosines are shown in red. C-terminal deletion variants of the IL-23R are indicated by dashed lines. Residues that are important for unconventional binding of STAT3 are highlighted in blue. A putative CK1 phosphorylation site (SXXS/T) is shown in italic type. *C.* Surface expression of the cytokine receptors was verified by flow cytometry using specific antibodies as it was described in part A.