**Generation of IL-6 reporter mKATE2 mice**

**Targeting plan**

A red fluorescent protein mKate2 was inserted into Il6 locus (Fig. S1), exactly

located after translation start site, ATG.

**Fig. S1.** Il6 mKate2 report mice targeting strategy: A red fluorescent protein mKate2 was

inserted into Il6 locus, and it is directly after translation start site, ATG.

**Targeting construct**

BAC clones (RP23-121M2 and RP24-172N24) containing mouse Il6 gene were obtained

from BACPAC Resources Center (Children's Hospital Oakland Research Institute,

Oakland (CHORI), California, USA). A 7550 bp fragment of Il6 gene containing exons 1,

2 and 3 was subcloned into the minimal vector, pACYC177 (New England Biolabs, MA,

USA) by Red/ET recombination method according to the manufacturer’s instructions

(Gene Bridges GmbH, Germany). PCR product used in subcloning was amplified from

pACYC177 plasmid using the primers

(A) 5’-CTCAGTTGGCACTGAATATACAGAATGACACTGCACCTTCACACGTGCAG

GCAGACCTCAGCGCTAG -3’and (B) 5’- GTACATGAAGAACAACTTAAAAGATA

ACAAGAAAGACAAAGCCAGAGTCTGAAGACGAAAGGGCCTC -3’ where homology arms to 5’ flanking region of exon 1 (primer A) and 3’ untranslated region (primer B) of the Il6 gene are indicated in bold, and PCR primers for an ampicillin resistance (amp) gene and ori in pACYC177-plasmid are in italics. A mammalian expression vector encoding for red fluorescent protein mKate2, pmKate2-N vector was purchased from Evrogen (Evrogen JSC, Moscow, Russia). Neo resistant gene flanked with two loxP sites (loxP-PGK-Neo-loxP cassette, Gene Bridges) was introduced into pmKate2-N vector with In-Fusion® HD cloning kit (Clontech, CA, USA) according to the manufacturer’s instructions. Finally, the replacement of partial exon 1, exons 2 and 3 and intron 1 and 2 of Il6 gene with mKate2 and Neo cassette containing 50 bp Il6 homology arms amplified by PCR was carried out by Red/ET recombination method. Primers were (C)

5’- GTAGCTCATTCTGCTCTGGAGCCCACCAAGAACGATAG

TCAATTCCAGAAACCGCTATGGTGAGCGAGCTGATTAAGG and (D) 5’-

ATAGCACTGGTTGGTAAACTTTCCCTCACCCTAGCAGCTGCTGAGGTACC

GCGGATTTGTCCTACTCAGG -3’ where homology arms to the Il6 gene are indicated in

bold and PCR primers for mKate2 and Neo cassette are shown in italics. Validity of final

targeting construct was confirmed by restriction enzyme digestion and sequencing.

**Gene targeting in ES cells**

G4 embryonic stem cells (derived from mouse 129S6/C57BL/6Ncr) were cultured on

neomycin-resistant primary embryonic fibroblast feeder layers, and 106 cells were

electroporated with 30 ug of linearized targeting construct. After electroporation, the cells

were plated on 100-mm culture dishes and exposed to G418 (300 ug/ml; Sigma). Colonies

(192) were picked up after 7-9 days selection and grown on 96-well plate. In order to

delete Neo cassette in the targeted ES cells, and targeted ES cells were re-electroporated

with plasmid, pCAGGS-Cre. After electroporation, the cells were plated on 100-mm

culture dishes and colonies were picked up after 3-5 days growth, and grown on 96-well

plate.

**Screening of targeted ES clones**

DNA isolated from ES cell clones was screened by long-range (LR) - PCR for both 5’ and

3’ homologous arms. PCR products with 5’ homologous arm, 3054 bp fragment was

generated with a primer pair corresponding to 5’ flanking region of 5’ homologous arm

(IL65HaUF1: TAGTAGAAGCTCAAGCTCTGGG) and to mKate2 gene (mKate2AntiSense2: GGTGTGGTTGATGAAGGTTT), and PCR product with 3 homologous arm, 3731 bp fragment was generated with primer pair corresponding to Neo gene (Neogenese1: CCTCGTGCTTTACGGTATCG), and 3’ flanking region of 3’ homologous arm (IL63HaDR2: GCTCTCATAATGGGTGACTATG).

Total 192 clones were picked up and screened by LR-PCR. Two clones contained both 5’

and 3’ homologous arm, 3054, and 3731 bp fragments in PCR analysis (Fig. S2). Correct

PCR products were verified by sequencing.

**Fig. S2.** Representative positive ES clone was found to contain homologous recombination of Il6 by LR-PCR screening using the primer pairs, Il6Arm5UF1 and mKate2Antisense2, Neogenese1 and Il6Arm3DR2.

In order to detect targeted ES clones with Neo deletion after Cre recombination, DNA

isolated from ES cells re-electroporated with plasmid, pCAGGS-Cre were screened by

PCR with several different primer pairs. Several correct clones were found from 48 clones.

The right clones were further confirmed by sequencing.

**Blastocyst injection**

The targeted ES cells with Neo deletion were injected into C57BL/N6 mouse blastocysts to

generate chimeric mice. Germline transmission was achieved by cross-breeding male

chimeras with C57BL/N6 females. C57BL/6N mice used as blastocyst donors were

obtained from Charles River Laboratories (Willmington, MA). The mice were maintained

in a specific pathogen free stage at Central Animal Laboratory at the University of Turku.

All studies carried out with the mice were approved by The Finnish ethical committee for experimental animals, complying with international guidelines on the care and use of

laboratory animals.

**DNA extraction and genotyping**

Genotyping of the mice was carried out with DNA extracted from the ear marks of 2-

week-old mice. Genotyping was performed as follows:

**Table S1.** Genotype Protocol of Il6-mKate KI Mice (TUKO62) (TCDM, ZHANG FP)

**Combined RNA Scope and immunohistochemistry**

In-situ hybridization (RNA Scope) was performed as described in Materials and Methods with the following changes: The mouse IL-6 mRNA probe (Mm-Il6 315891), positive- (Mm-Ppib 213911) and negative controls (DapB 310043) were stained with cy3. Brain slices from four heterozygous RedIL6 mice were used. Following the last step in this protocol, slides were incubated overnight with anti-RFPtag (Table 1) in normal goat serum. The following day slides were washed 3x5 min in TNT buffer, followed by 1h incubation with Alexa488 anti-mouse (Table 1). Slides were washed 3x5 min in TNT buffer and cell nuclei were stained with DAPI followed by another 3x5 min wash. Slides were then mounted with ProLong Diamond Antifade and coverslipped. Micrographs were developed using the lsm700 system.

**Figure S3.** Representative confocal micrograph of combined RNAscope and immunohistochemistry showing high co-localization between IL-6 mRNA (cy3, red) and RedIL6 (Alexa 488, green) fluorescence in PBNel (B). Cell nuclei stained by DAPI (blue). Examples of individual cells where IL-6 mRNA and RedIL6 is co-localized shown in (C).

**Figure S4.** IL-6 ab immunohistochemistry (green) in wt (A) and homozygous RedIL6 mice (B). Cell nuclei stained by DAPI (blue). The IL-6 ab used in this paper shows markedly less staining in homozygous RedIL6 “IL-6 -/-” mice. Scale bars – 100 μm. scp – superior cerebellar peduncle.

**S4A**

**RedIL6 and CGRP immunohistochemistry**

**Figure S5A.** RedIL6 (red) and CGRP (green) immunoreactivity partially overlaps in the PBNel. Cell nuclei stained by DAPI (blue). Examples of RedIL6 (red arrowheads), CGRP-immunoreactive cells (green arrowheads) and co-localization (yellow arrowheads). Scale bar – 10 μm.

**Figure S5B.** About 40% of all CGRP-immunoreactive cells also show RedIL6-fluorescence and vice versa.

**Figure S6.** Representative confocal micrographs of cells containing IL-6 in the PBNel as indicated by the mKATE2-IL-6 (RedIL6) reporter mouse (red) 60 min after intraperitoneal injection of Ex4 (A-B) or vehicle (C-D). Shown are overviews of PBN (A, C) and magnifications of the indicated parts of PBNel (B, D). Red arrowheads indicate examples of IL-6 containing cells (B, D). Cell counting shows that there is a marked increase in IL-6 containing cells in PBNel, as indicated by the mKATE2-IL-6 reporter mouse, after Ex4 injection as compared with vehicle (E). In contrast, there is no difference in IL-6 containing cells in the lateral PBN excluding the PBNel (E).

Confocal micrographs were obtained and cell counting was performed as described in Materials and Methods. Scale bars 80 μm (overview), 10 μm (zoom). scp - superior cerebellar peduncle