Expanded View Figures

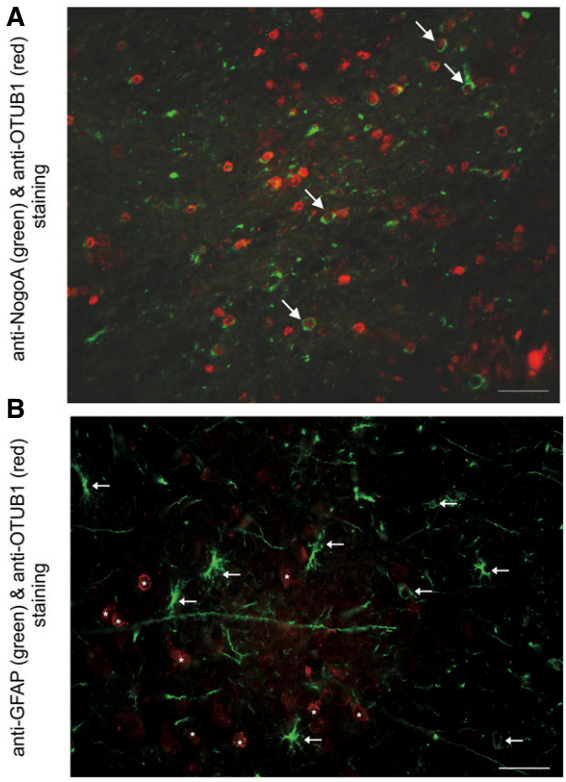

Figure EV1. Single oligodendrocytes express OTUB1 in MS, while astrocytes do not express OTUB1 in the peritumoral tissue of astrocytoma.

A NogoA$^+$ oligodendrocytes in a white matter MS lesion (case 5, Table EV1) with inflammatory infiltrates and demyelination express OTUB1 (arrows). This pattern is representative for all 10 patients analyzed. Double immunofluorescence with rabbit anti-OTUB1 (Cy3) and mouse anti-NogoA (Alexa Fluor 488); original magnification ×400; scale bar corresponds to 50 μm.

B In the gray matter and the subcortical region adjacent to an astrocytoma (WHO grade II), GFAP$^+$ astrocytes do not express OTUB1 (arrows). Neurons are OTUB1-positive (asterisks). Data are representative for three cases of peritumoral tissue of astrocytomas (WHO grade II). Double immunofluorescence with mouse anti-GFAP (FITC) and rabbit anti-OTUB1 (Cy3); original magnification ×400; scale bar corresponds to 50 μm.

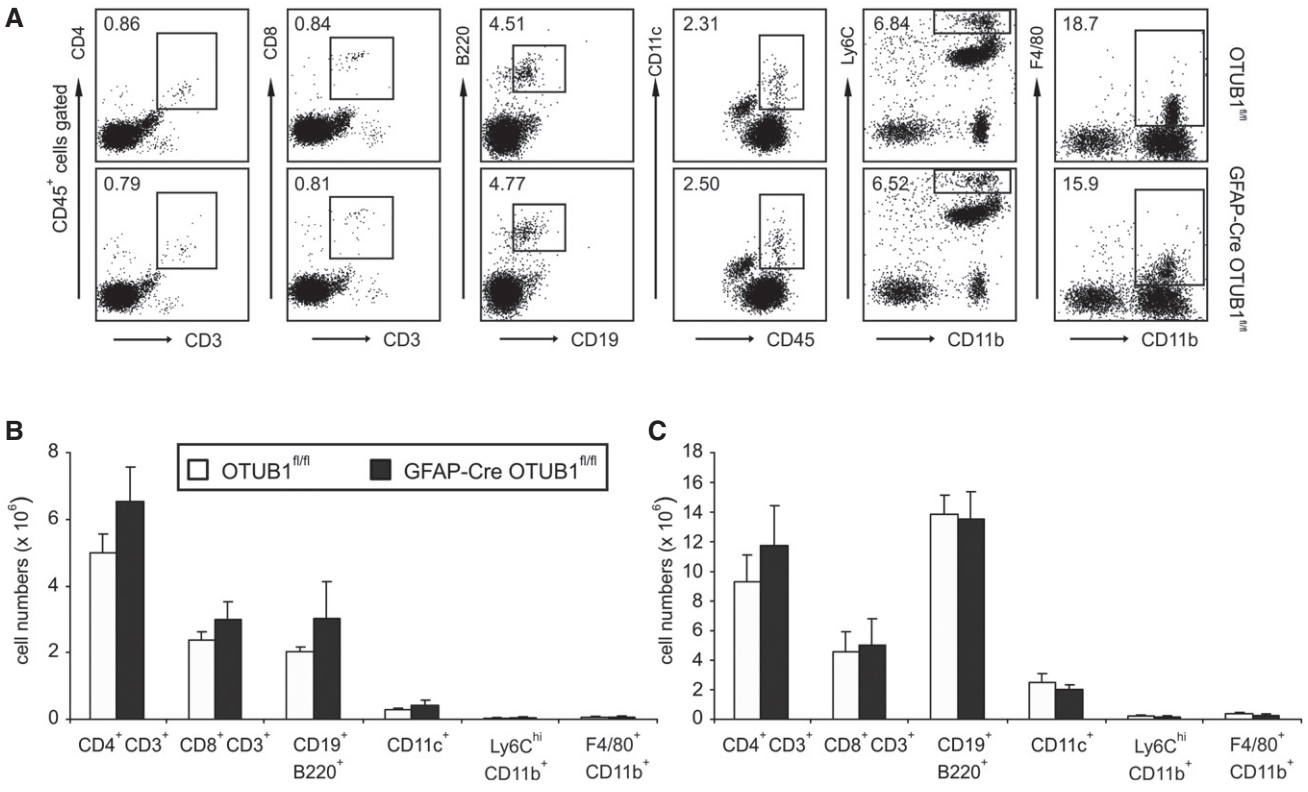

Figure EV2. Normal composition of leukocytes in GFAP-Cre OTUB1$^{fl/fl}$ mice.

A $CD45^+$ leukocytes were isolated from the spinal cord of GFAP-Cre $OTUB1^{fl/fl}$ mice and control mice by Percoll gradients. The percentages of $CD4^+$ T cells ($CD4^+$ $CD3^+$), $CD8^+$ T cells ($CD8^+$ $CD3^+$), B cells ($CD19^+$ $B220^+$), dendritic cells ($CD11c^+$), inflammatory monocytes ($Ly6C^{high}$ $CD11b^+$), and macrophages ($F4/80^+$ $CD11b^+$) were analyzed by flow cytometry. Representative dot plots are shown.

B, C $CD45^+$ leukocytes were isolated from the lymph node (B) and spleen (C) of GFAP-Cre $OTUB1^{fl/fl}$ mice (n = 4) and control mice (n = 4). Cells were counted with the hemocytometer and analyzed by flow cytometry. Data show the absolute number of indicated cell populations (mean + SEM).

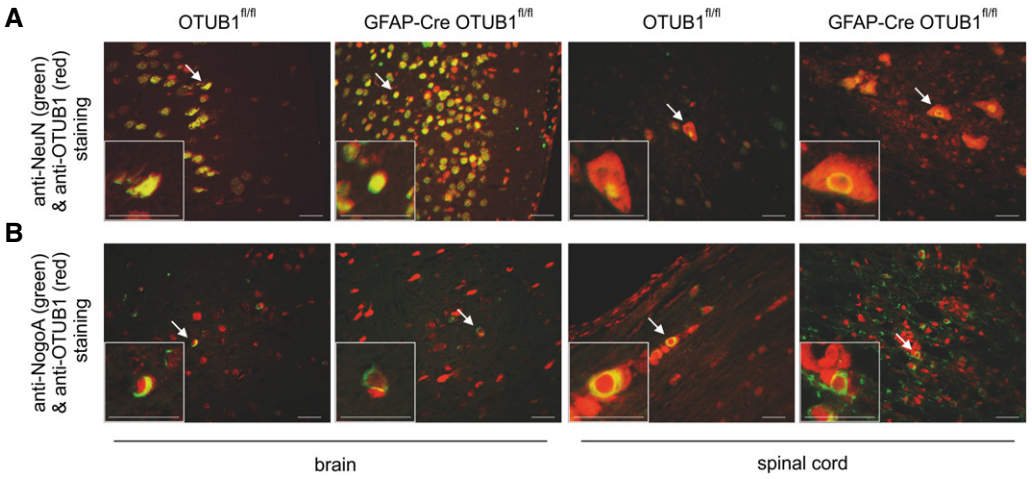

Figure EV3. OTUB1 expression in the brain and spinal cord of OTUB1$^{fl/fl}$ and GFAP-Cre OTUB1$^{fl/fl}$ mice at day 22 p.i.

A NeuN$^+$ neurons of an OTUB1$^{fl/fl}$ and a GFAP-Cre OTUB1$^{fl/fl}$ mouse equally express OTUB1 (arrows) in the brain and spinal cord. Double immunofluorescence with rabbit anti-OTUB1 (Cy3) and mouse anti-NeuN (FITC).

B Some NogoA$^+$ oligodendrocytes (arrows) in the brain and spinal cord express OTUB1 in an OTUB1$^{fl/fl}$ and a GFAP-Cre OTUB1$^{fl/fl}$ mouse. Double immunofluorescence with rabbit anti-OTUB1 (Cy3) and mouse anti-NogoA (Alexa Fluor 488).

Data information: All photographs are representative of three mice per group; original magnification ×400; the inserts show higher magnification of cells marked by an arrow (×1,200). Scale bars correspond to 50 μm.

Figure EV4. GFAP-Cre OTUB1$^{fl/fl}$ but not Synapsin-Cre OTUB1$^{fl/fl}$ mice develop more severe EAE.

A EAE was induced in Synapsin-Cre OTUB1$^{fl/fl}$ mice (n = 5) and OTUB1$^{fl/fl}$ control littermates (n = 5) by MOG_{35-55} peptide immunization with pertussis toxin. Graph shows the mean clinical scores ± SEM.

B, C Leukocytes were isolated from the lymph node (B) and spleen (C) of OTUB1$^{fl/fl}$ (n = 7) and GFAP-Cre OTUB1$^{fl/fl}$ (n = 7) mice at day 15 p.i. Cells were counted with the hemocytometer and analyzed with flow cytometry. Data show the absolute number of indicated cell populations (mean + SEM).

D, E Absolute numbers of GM-CSF-, IFN-γ-, and IL-17-producing CD4$^+$ T cells in the lymph node (D) and spleen (E) of OTUB1$^{fl/fl}$ (n = 7) and GFAP-Cre OTUB1$^{fl/fl}$ (n = 7) mice were calculated based on flow cytometry results. Data show the mean + SEM at day 15 p.i.

F Body weight of OTUB1$^{fl/fl}$ (n = 7) and GFAP-Cre OTUB1$^{fl/fl}$ (n = 7) mice was monitored daily after EAE induction. Data show the relative change in body weight normalized to that on day 0 (mean + SEM). Two-tailed Student's t-test was used, *$P < 0.05$.

G Astrocytes were selectively isolated by MACS from unimmunized and diseased (day 15 p.i.) OTUB1$^{fl/fl}$ and GFAP-Cre OTUB1$^{fl/fl}$ mice, respectively. Relative expression of IL-6 and CXCL1 mRNA was determined by quantitative real-time PCR. Data are presented as relative increase in genes at day 15 p.i. over gene expression of astrocytes from unimmunized control mice (n = 3 for all groups; mean + SEM).

H Primary astrocytes isolated from OTUB1$^{fl/fl}$ (n = 3) and GFAP-Cre OTUB1$^{fl/fl}$ (n = 3) mice were left untreated or stimulated with IFN-γ (10 ng/ml), IL-17 (50 ng/ml), and TNF (10 ng/ml), respectively, for 16 h. mRNA levels of IL-6 and CXCL1 were detected by quantitative real-time PCR. Data are shown as relative increase over untreated controls (mean + SEM).

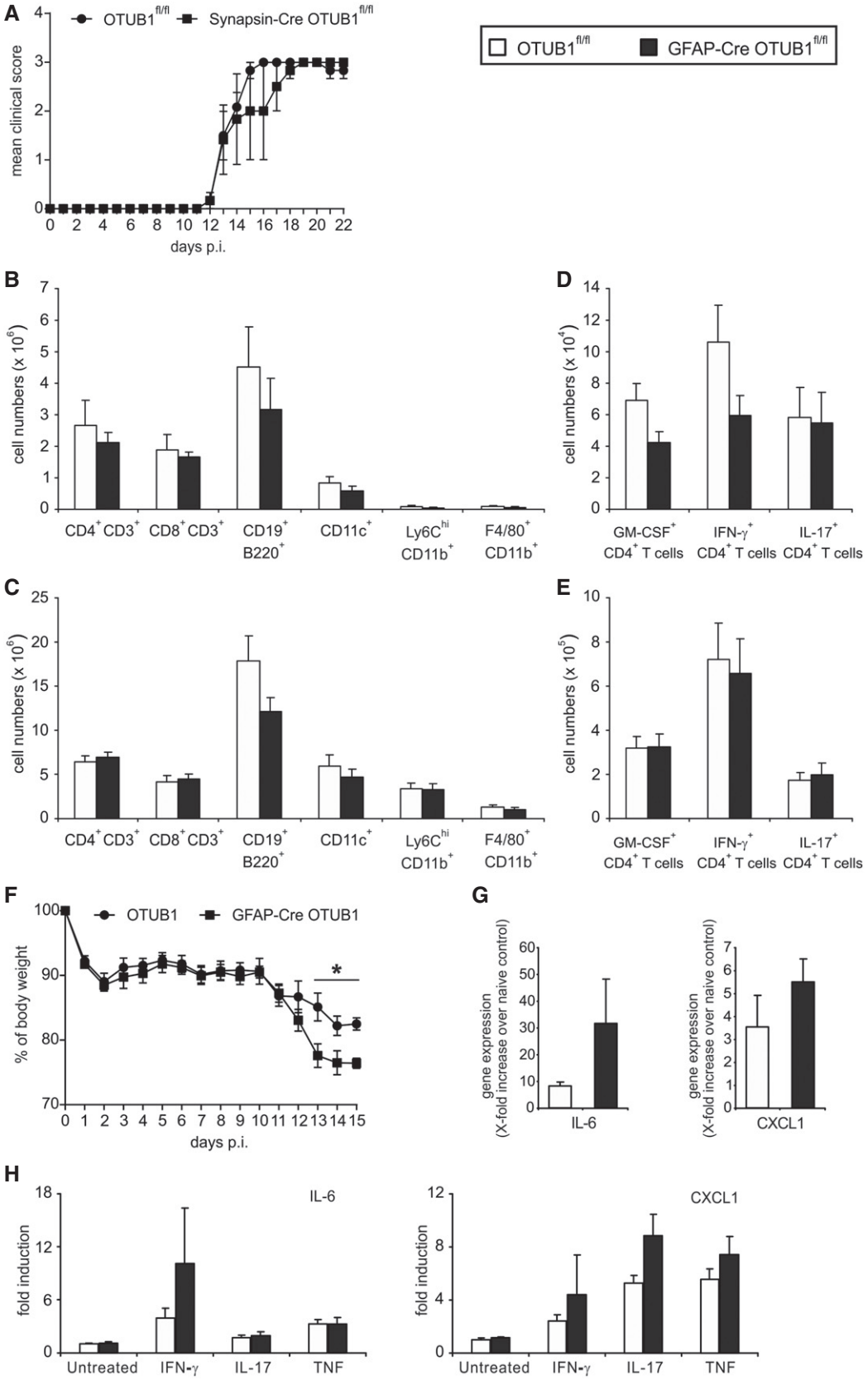
A
OTUB1fl/fl
Synapsin-Cre OTUB1fl/fl
mean clinical score
days p.i.
OTUB1fl/fl
GFAP-Cre OTUB1fl/fl
B
cell numbers (x 10^6)
CD4+ CD3+
CD8+ CD3+
CD19+ B220+
CD11c+
Ly6Chi CD11b+
F4/80+ CD11b+
D
cell numbers (x 10^4)
GM-CSF+ CD4+ T cells
IFN-γ+ CD4+ T cells
IL-17+ CD4+ T cells
C
cell numbers (x 10^6)
E
cell numbers (x 10^5)
F
OTUB1
GFAP-Cre OTUB1
% of body weight
days p.i.
G
gene expression (X-fold increase over naive control)
IL-6
CXCL1
H
fold induction
IL-6
CXCL1
Untreated
IFN-γ
IL-17
TNF

Figure EV4.

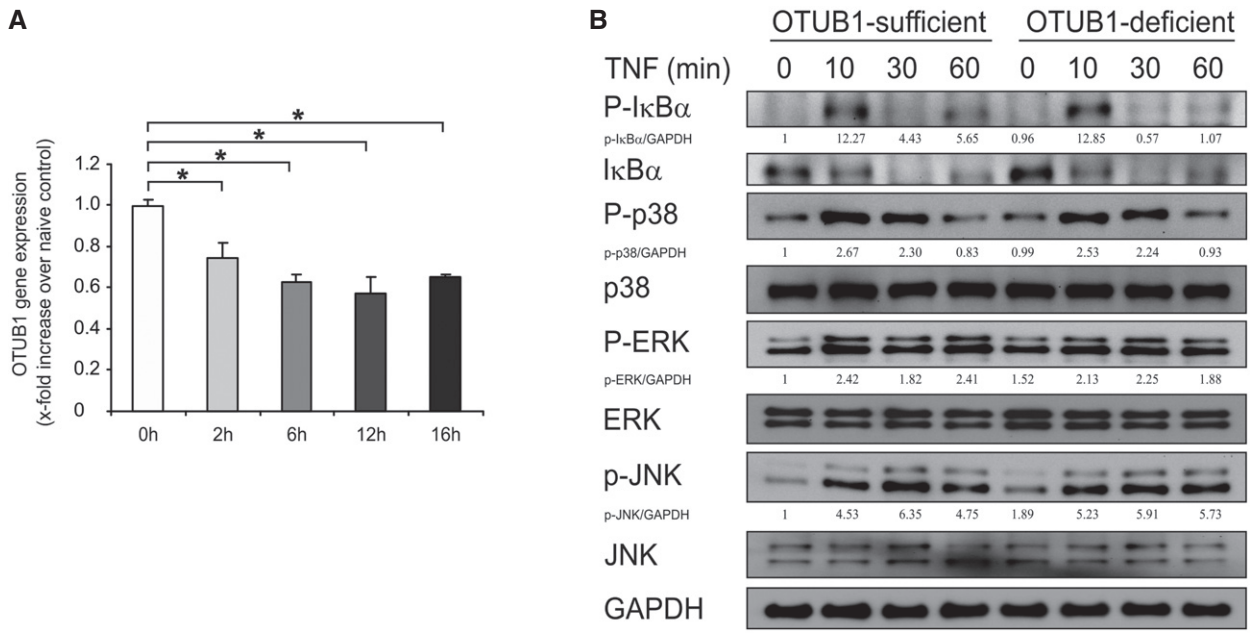

Figure EV5. OTUB1 mRNA levels are downregulated by IFN-γ treatment, and OTUB1 does not interfere with TNF-induced signaling pathways.

A Primary astrocytes from wild-type C57BL/6 mice were stimulated with IFN-γ (10 ng/ml) for indicated times. Thereafter, mRNA was isolated and analyzed by quantitative real-time PCR. Data show the relative expression of OTUB1 mRNA compared to untreated controls (n = 3 for all groups). Data represent mean + SEM. Two-tailed Student's t-test was used, $^*P < 0.05$.

B Primary astrocytes from OTUB1$^{fl/fl}$ and GFAP-Cre OTUB1$^{fl/fl}$ mice were stimulated with TNF (10 ng/ml) for indicated times. Whole cell lysates were analyzed by WB with indicated antibodies.

Source data are available online for this figure.