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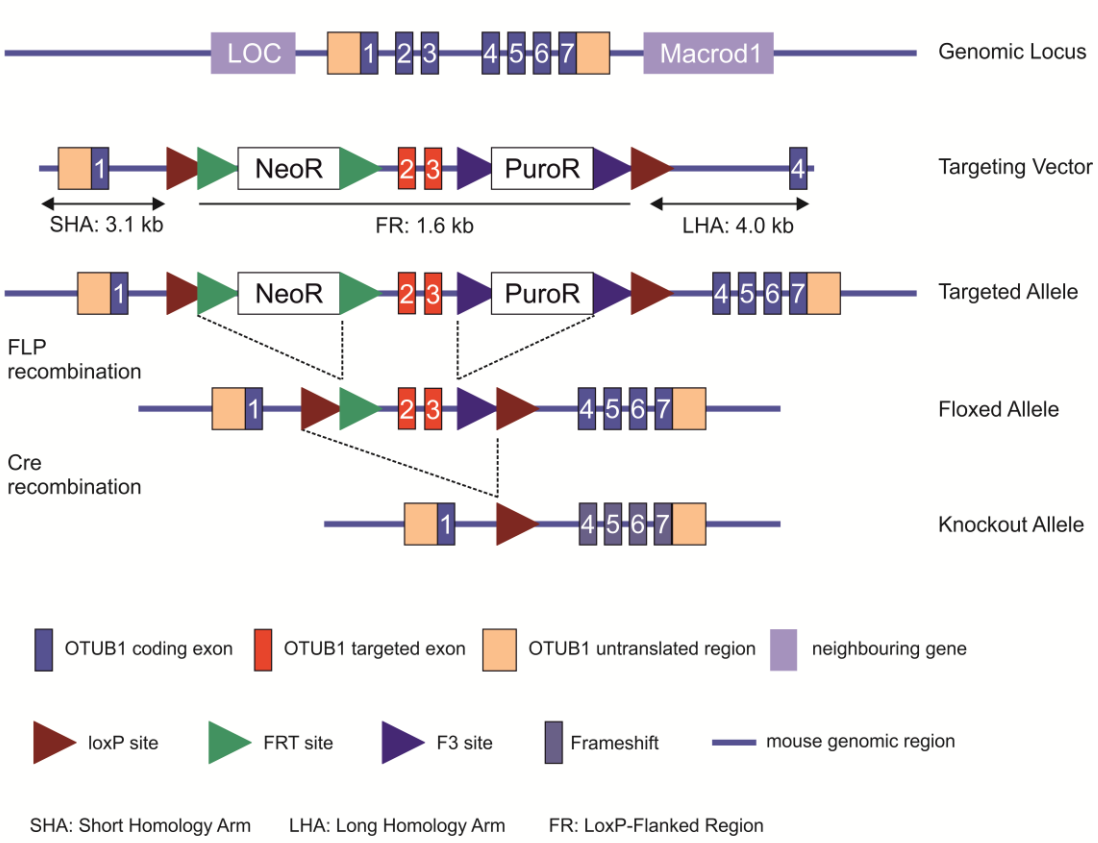
Appendix Figure S1

A



B

Breeding: $OTUB1^{wt/\Delta}$ X $OTUB1^{wt/\Delta}$

mice	$OTUB1^{wt/wt}$	$OTUB1^{wt/\Delta}$	$OTUB1^{\Delta/\Delta}$
number	11	18	0
percentage	38%	60%	0%

C

Breeding: Nestin-Cre $OTUB1^{wt/fl}$ X $OTUB1^{wt/fl}$

Cre / OTUB1	Nestin-Cre^{-} (number/percentage)	Nestin-Cre^{+} (number/percentage)
$OTUB1^{wt/wt}$	19 (11.9%)	24 (15%)
$OTUB1^{wt/fl}$	42 (26.3%)	50 (31.3%)
$OTUB1^{fl/fl}$	25 (15.6%)	0 (0%)

Appendix Figure S1. Generation of $OTUB1^{fl/fl}$ mice and phenotypes of $OTUB1^{wt/\Delta}$, and Nestin-Cre $OTUB1^{wt/fl}$ mice

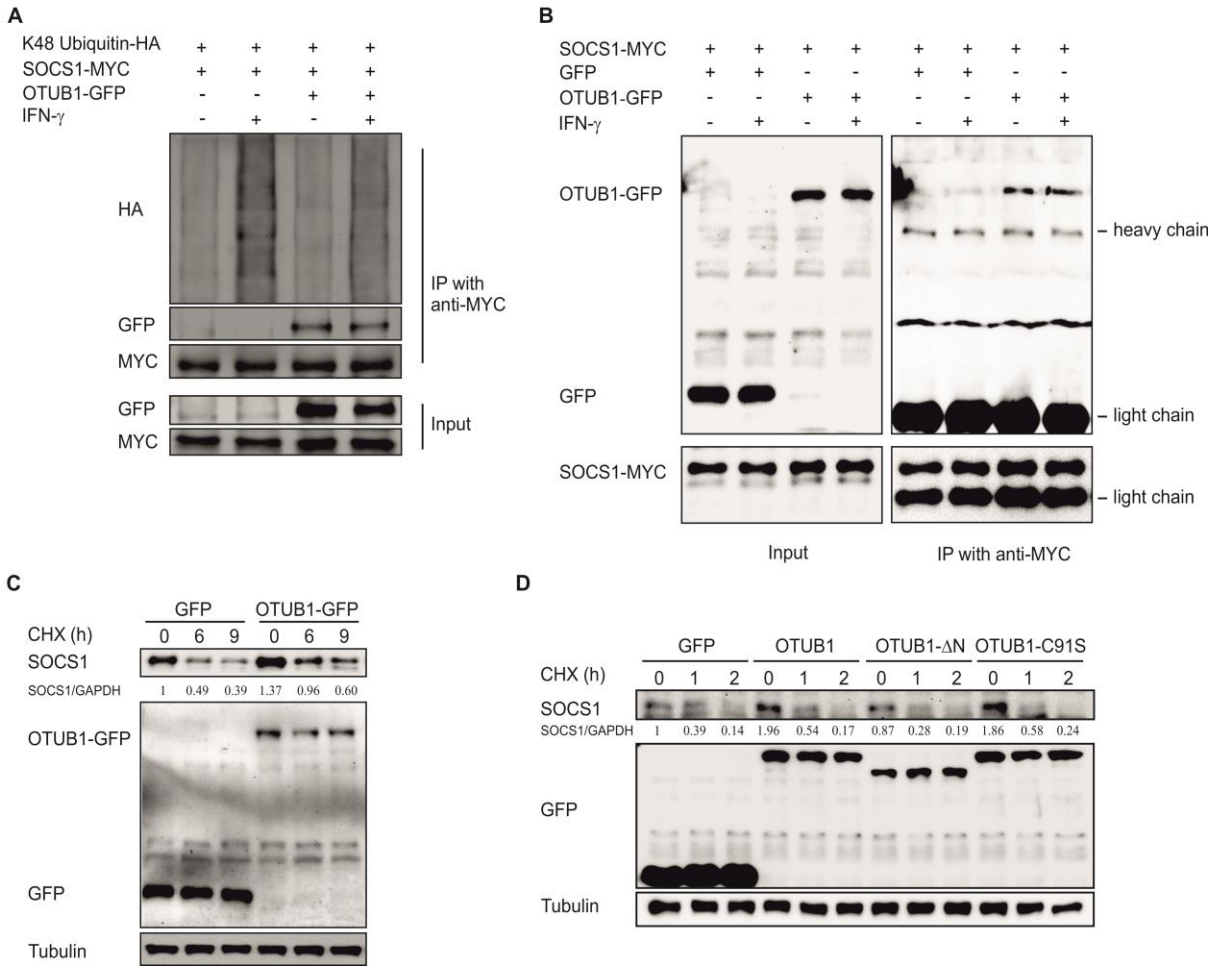
(A) Schematic representation of the gene targeting strategy for the generation of $OTUB1^{fl/fl}$ mice. The neomycin and puromycin resistance markers were removed from the conditional

allele by FLP-mediated gene recombination. The resulting OTUB1$^{fl/fl}$ mice were further crossed with mice containing different Cre transgenes to generate global/conditional OTUB1 knockout mice.

(B) OTUB1$^{wt/fl}$ mice were crossed with Rosa 26-Cre mice to delete exons 2 and 3 of OTUB1, resulting in out-of-frame translation and germline inactivation of OTUB1. Rosa 26-Cre OTUB1$^{wt/fl}$ mice were crossed with C57BL/6 mice to remove the Rosa 26-Cre transgene, resulting in the heterogeneous OTUB1$^{wt/\Delta}$ mice, in which one OTUB1 allel was inactivated. For the generation of OTUB1$^{\Delta/\Delta}$ mice, OTUB1$^{wt/\Delta}$ mice were crossed with OTUB1$^{wt/\Delta}$ mice.

(C) Nestin-Cre OTUB1$^{wt/fl}$ mice were generated by crossing OTUB1$^{fl/fl}$ mice with Nestin-Cre mice.

Appendix Figure S2



Appendix Figure S2. OTUB1 directly interacts with and stabilizes SOCS1

(A) Primary astrocytes from GFAP-Cre OTUB1$^{fl/fl}$ mice were cotransfected with SOCS1-MYC and K48 ubiquitin-HA plasmids or a combination of SOCS1-MYC, OTUB1-GFP, and K48 ubiquitin-HA plasmids. Twenty-four hours after transfection, cells were left untreated or stimulated with IFN-γ (10 ng/ml) for 30 min before lysation. Proteins from whole cell lysates were immunoprecipitated with anti-MYC antibody and analyzed by WB for MYC, GFP, and HA.

(B) Primary astrocytes from GFAP-Cre OTUB1$^{fl/fl}$ mice were cotransfected with SOCS1-MYC and GFP plasmids or SOCS1-MYC and OTUB1-GFP plasmids. Twenty-four hours after transfection, cells were left untreated or stimulated with IFN-γ (10 ng/ml) for 30 min

before lysation. Proteins from whole cell lysates were immunoprecipitated with anti-MYC antibody and analyzed by WB for MYC and GFP.

(C) Primary astrocytes from GFAP-Cre OTUB1$^{fl/fl}$ mice were cotransfected with SOCS1-MYC and GFP plasmids or SOCS1-MYC and OTUB1-GFP plasmids. Twenty-four hours after transfection, cells were treated with CHX (100 μg/ml) for indicated times. SOCS1 protein levels were analyzed by WB.

(D) Primary astrocytes from GFAP-Cre OTUB1$^{fl/fl}$ mice were transfected with GFP, OTUB1-GFP, OTUB1-ΔN-GFP, and OTUB1-C91S-GFP plasmids, respectively. Twenty-four hours after transfection, cells were stimulated with IFN-γ (10 ng/ml) for 4 h followed by treatment with CHX (100 μg/ml) for indicated times. SOCS1 protein abundance in the whole cell lysates was analyzed by WB.