

Age-dependent progression from clearance to vulnerability in the response of periventricular glia to α -synuclein toxic species

Supplementary information

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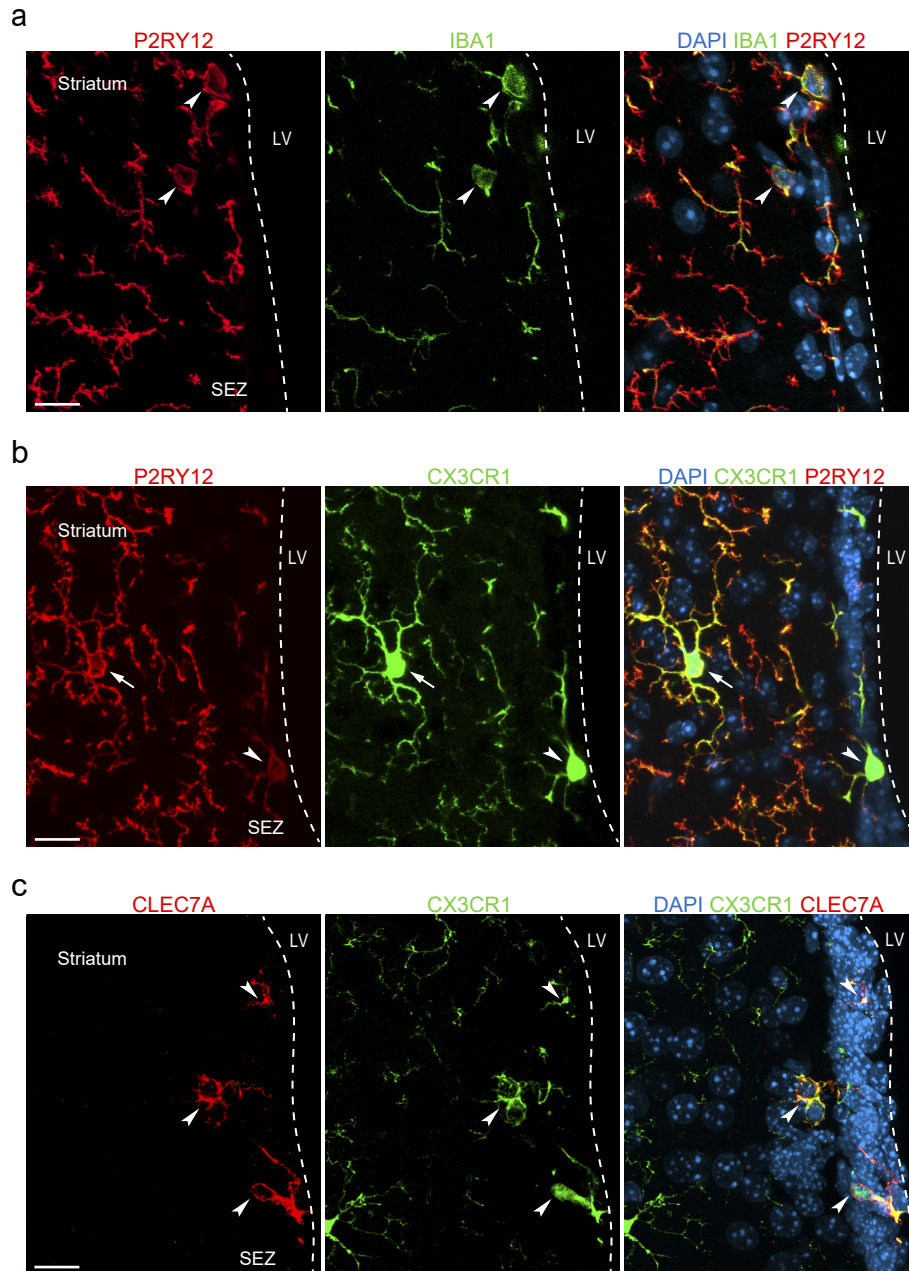
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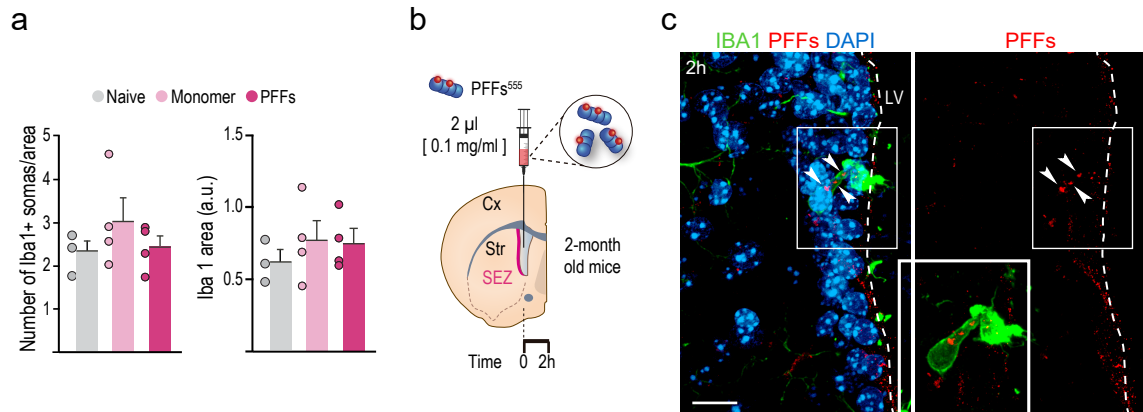
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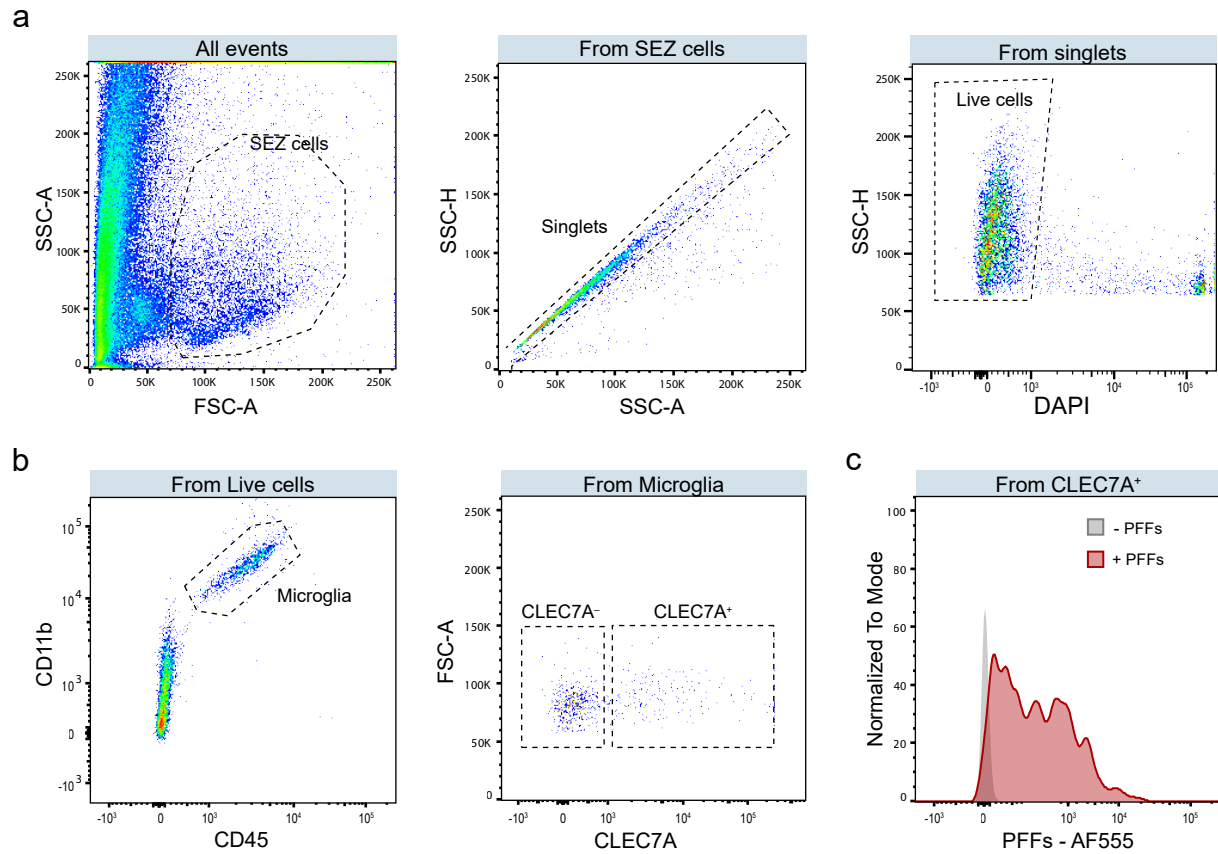
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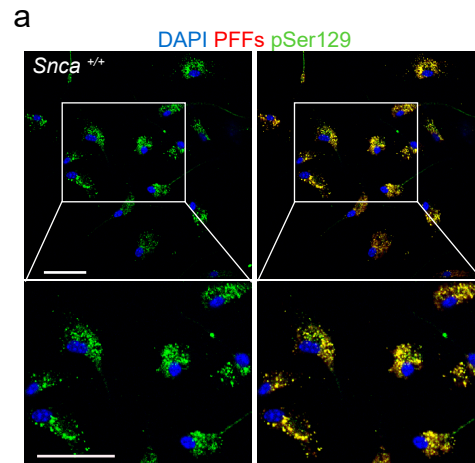
Suppl. Fig 1. Microglial specific stainings. **a.** Confocal images of coronal sections from the SEZ of 2-month-old mice, showing immunostainings for IBA1 (green) and P2YR12 (red). **b.** Confocal images of coronal sections from the SEZ of 2-month-old *Cx3cr1*^{+/GFP} knockin mice showing GFP fluorescence (green, CX3CR1) and immunostaining for P2YR12 (red). **c.** Confocal images of coronal sections from the SEZ of 2-month-old *Cx3cr1*^{+/GFP} knockin mice showing GFP fluorescence (green, CX3CR1) and immunostaining for CLEC7A (red). Microglial cells are indicated by arrows (at the striatum) and arrowheads (at the SEZ). DAPI: blue. Scale bar: 20 μ m.



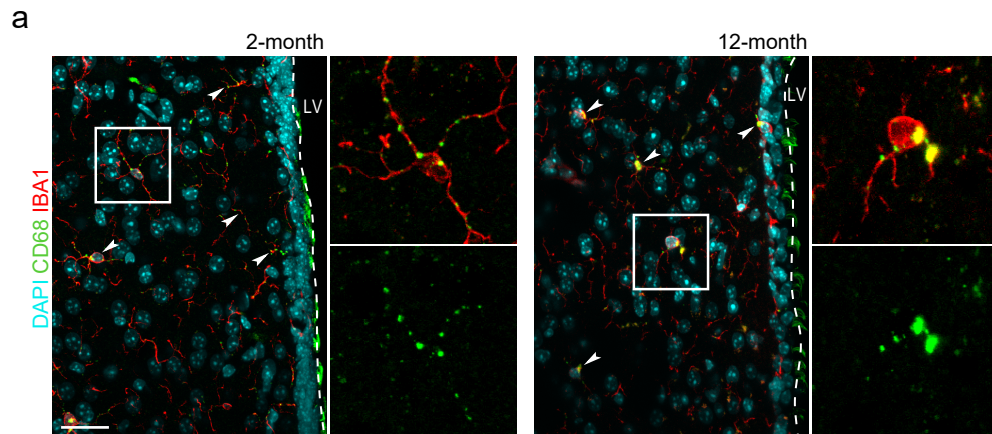
Suppl. Fig. 2. Microglial activation and short-term microglial uptake of α Syn PFFs. **a.** Quantification of the number of somas and the area occupied by microglia 15 days after the infusion of either monomeric α Syn or PFFs into the LV of 2-month-old ($n = 4$) or naive mice. Data are presented as mean values \pm SEM. **b.** Schematic representation of the in vivo experiment. α Syn PFFs were microinjected into the right LV and the animals were euthanized two hours afterwards. **c.** Confocal images of the SEZ showing PFFs (red) and immunostaining for IBA1 (green). Arrowheads point at PFFs inside a microglial cell. DAPI: blue. Scale bar: 20 μ m.



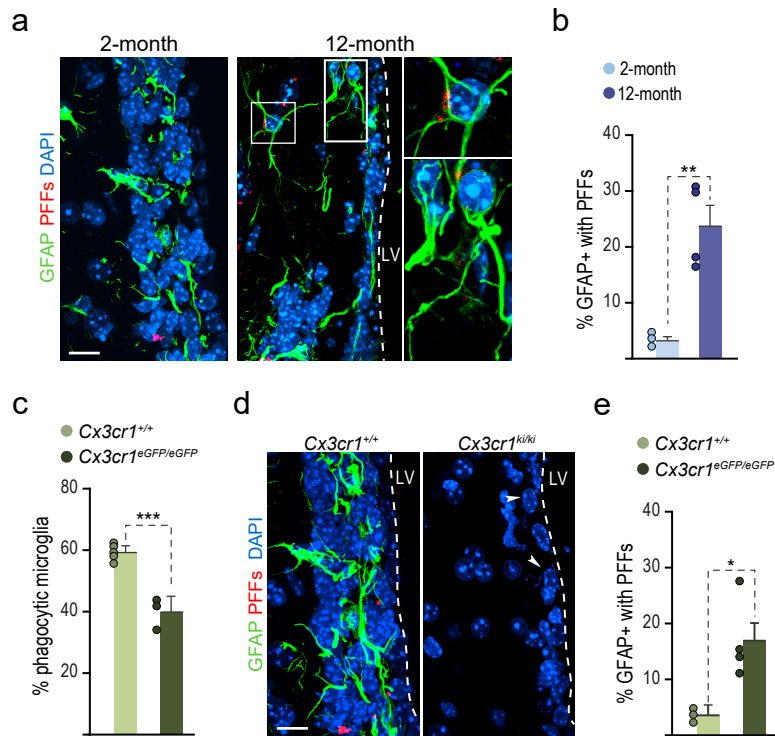
Suppl. Fig. 3. FACS gating strategy for microglia and ex vivo PFF phagocytosis by CLEC7A⁺ microglia. **a.** Representative flow cytometry dot plots depict the initial steps of the gating strategy for live cell selection. **b.** Representative flow cytometry dot plot depicting the gating strategy for the selection of CLEC7A⁺ and CLEC7A⁻ microglia. **c.** Representative flow cytometry histogram illustrating AF555 intensity in CLEC7A⁺ microglia incubated *ex vivo* with PFFs-AF555 (red) or PBS (grey).



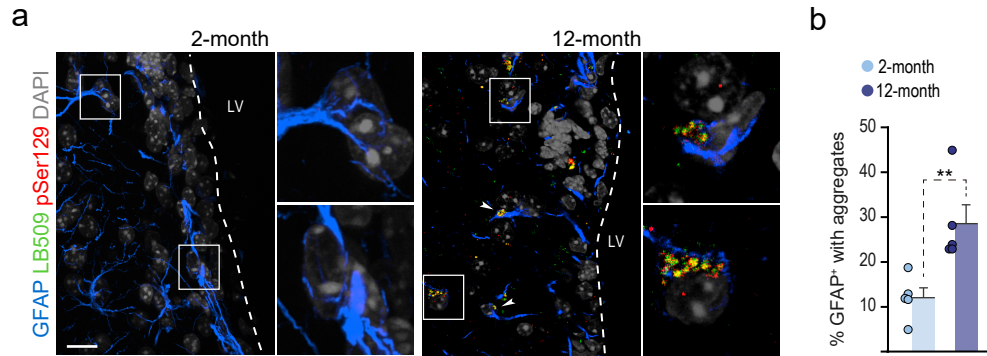
Suppl. Fig. 4. PFFs phosphorylation in *Snca*^{+/+} mice. a. Detection of PFFs (red) and αSyn pSer129 (green) in *Snca*^{+/+} mixed glial cultures. Scale bar: 40 μm.



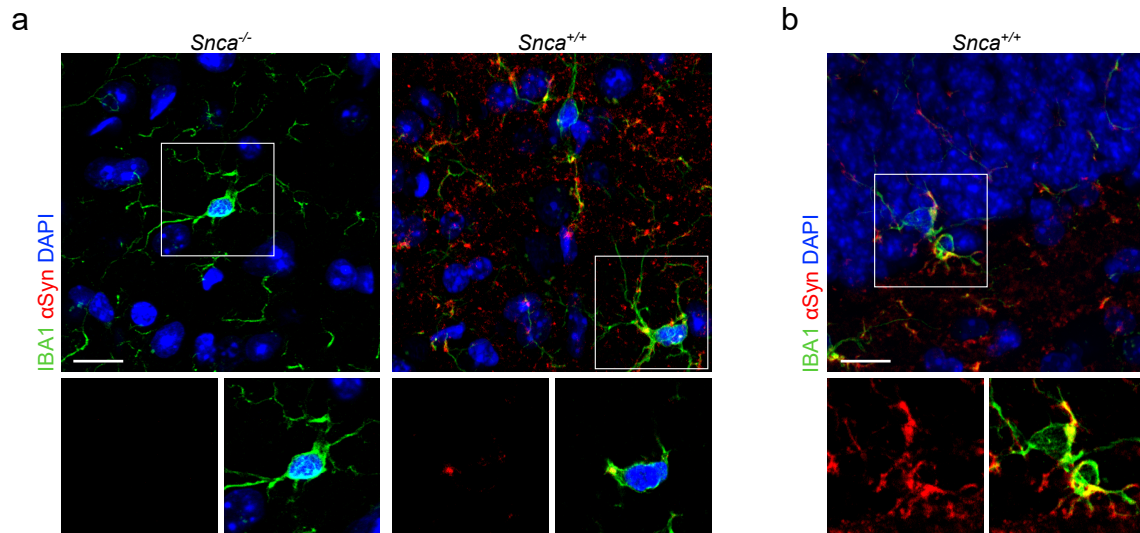
Suppl. Fig. 5. Age-associated lysosomal changes. a. Confocal images of the SEZ of 2- and 12-month-old mice showing immunostainings for CD68 (green) and IBA1 (red). Arrowheads point at microglial cells. DAPI: cyan. Scale bar: 30 μm.



Supp. Fig. 6. Microglial impairment is associated with increased astrocytic PFF uptake. a. Confocal images of the SEZ of 2- and 12-month-old mice showing PFFs (red) and immunostaining for GFAP (green) 15 days after PFF infusion. **b.** Quantification of the percentage of astrocytes containing PFFs in 2- (n=3) and 12-month-old (n=4) mice. Data are presented as mean values \pm SEM; Student's t test, $p < 0.01$. **c.** Flow cytometry analysis of PFF phagocytic capacity in *ex vivo* experiments with microglia isolated from the SEZ of *Cx3cr1*^{+/+} mice (n = 5) and *Cx3cr1*^{eGFP/eGFP} mice (n=3). Data are presented as mean values \pm SEM; Student's t-test, $p < 0.001$. **d.** Confocal images of the SEZ showing PFFs (red) and immunostaining for GFAP (green) in 2-month-old *Cx3cr1*^{+/+} and *Cx3cr1*^{eGFP/eGFP} mice. **e.** Quantification of the percentage of astrocytes containing PFFs in *Cx3cr1*^{+/+} (n=3) and *Cx3cr1*^{eGFP/eGFP} (n=4). Data are presented as mean values \pm SEM; Student's t test, $p < 0.05$. DAPI: blue. Scale bar: a and d, 20 μ m.

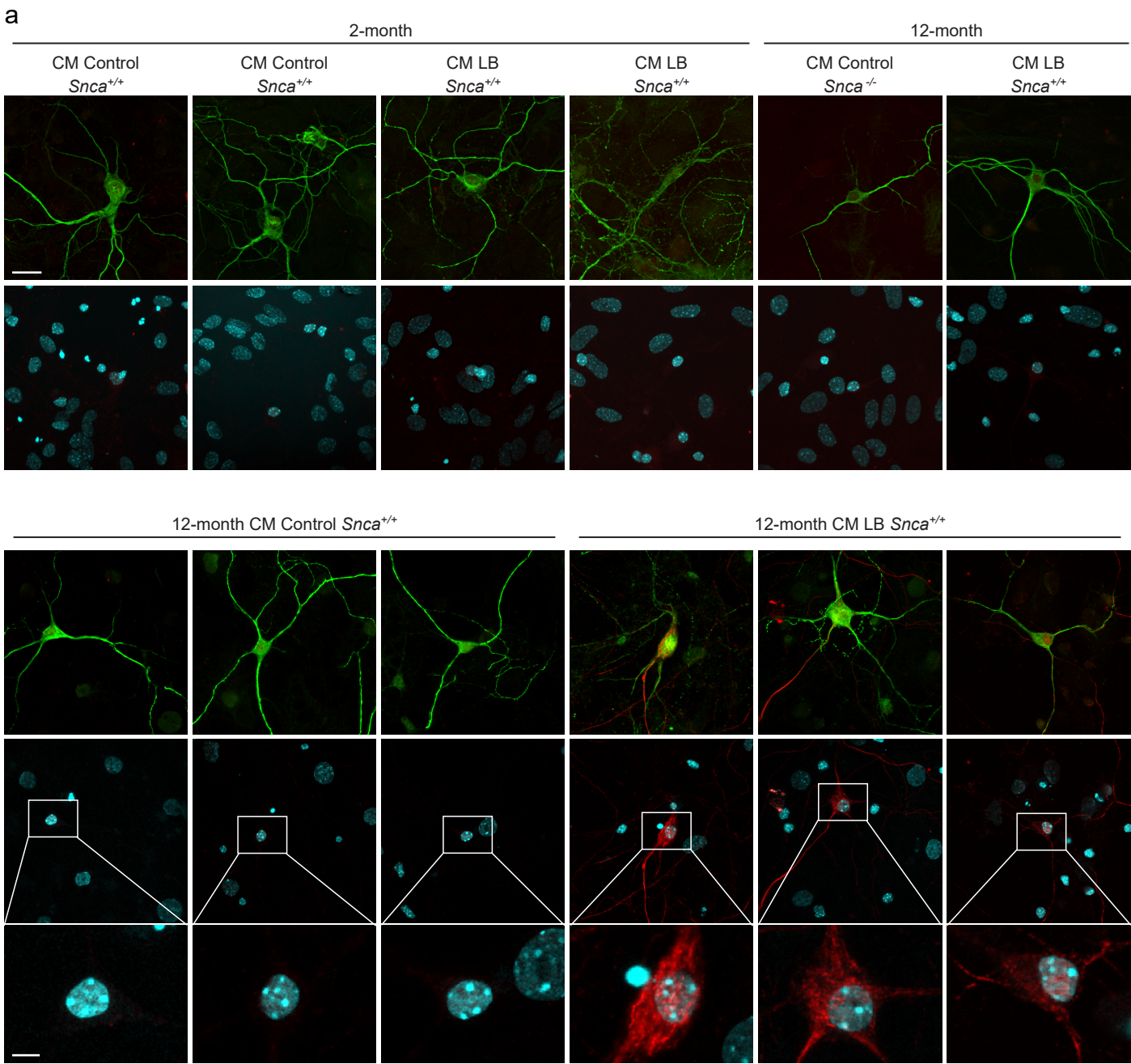


Suppl. Fig. 7. Increased astrocytic LB uptake in aged mice. **a.** Confocal images of the SEZ of 2- and 12-month-old mice showing immunostainings for human α Syn (LB509, green), pSer129 (red), and GFAP (blue) 15 days after LB-fraction infusion. Arrowheads point at astrocytes containing LB material. **b.** Quantification of the percentage of astrocytes containing LB material in 2- (n = 5) and 12-month-old (n = 5) mice. Data are presented as mean values \pm SEM; Student's t-test, $p < 0.01$. DAPI: grey. Scale bar: 20 μ m.



Suppl. Fig. 8. *αSyn* expression by microglia in cortex and dentate gyrus. **a.** Confocal images of the cerebral cortex showing immunostainings for IBA1 (green) and *αSyn* (red). *αSyn* staining in *Snca*^{-/-} mice is used as a control of antibody specificity. **b.** Confocal images of the dentate gyrus showing immunostainings for IBA1 (green) and *αSyn* (red). DAPI: blue. Scale bar: a and b, 20 μ m.

Suppl. Fig. 9



Suppl. Fig. 9. *α*Syn prion-like effects induced by conditioned media obtained from microglia treated with LBs. Representative fluorescence images of pSer129 (red) in hippocampal neuron (MAP2, green) cultures incubated with concentrated conditioned media (CM) obtained from mixed glial cells from 2-month *Snca*^{+/+} mice or 12-month-old *Snca*^{+/+} and *Snca*^{-/-} mice treated with either PBS (control) or LBs. DAPI: cyan. Scale bars: panoramic images, 20 μm; detail images, 5 μm.

Suppl. Table 1

	Age	Gender	Other pathologies
Controls	65	male	Hypertension, hyperlipidemia
	74	male	L4-L5 lumbar stenosis, fibromyalgia and depression
	82	male	Hypertension, type II diabetes
PD patients	67	male	Hyperlipidemia, type II diabetes
	72	male	Arthritis, depression
	80	female	Osteoporosis, depression
	84	male	Hypertension