Supplementary Data for

Loss of LAMP5 interneurons drives neuronal network dysfunction in Alzheimer’s disease

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### Supplementary Tables

#### Table S1. Oligonucleotide primers used for genotyping of mouse strains

| Strain   | Forward primer (5’-3’)          | Reverse primer (5’-3’)          |
|----------|---------------------------------|---------------------------------|
| APP23    | GTTCTGCTGCATCTTTGGACA           | GAATTCCGACATGACTCAGG            |
| TAU58    | AAGTCACCAGCAGGGAGGTG            | TGCTCCAATGCCTGTCTCTTC           |
| tau<sup>−/−</sup> | AAGTTCATCTGCACCACC              | TGCTCAGGTAGTGTTTGTCG            |
| Lamp5<sup>Δ/Δ</sup> | GCAGATACCATTGCAGAGCA         | TGTTGCTCTCTGGAGTTCT              |
| APPswe   | AGGACTGACCACCTCGACCAG           | CGGGGGTCTAGTTCTGCA              |
| PSEN1    | AATAGAGAACGGCAGGAGCA            | GCCATGAGGGCAGACTATCAT           |
| Group | Age (y) | Sex | PMI | Thal phase | CERAD score | Braak stage | TDP-43 | α-synuclein | Region |
|-------|---------|-----|-----|------------|-------------|-------------|--------|-------------|--------|
| Ctr   | 62      | M   | 19  | 0          | -           | 0           | no     | no          | no     |
| Ctr   | 44      | M   | 14  | 0          | -           | 0           | no     | no          | no     |
| Ctr   | 61      | M   | 20.5| 0          | -           | 0           | no     | no          | no     |
| Ctr   | 50      | M   | 36  | 0          | -           | 0           | no     | no          | no     |
| Ctr   | 56      | M   | 14  | 0          | -           | 0           | no     | no          | no     |
| Ctr   | 80      | F   | 36  | 2          | -           | 0           | no     | no          | CTX, HIP |
| Ctr   | 83      | M   | 10.5| 1          | -           | 1--2        | no     | no          | CTX    |
| Ctr   | 73      | M   | 8   | 1          | -           | 1           | no     | no          | CTX    |
| Ctr   | 75      | F   | 17  | 2          | -           | 2           | no     | no          | CTX    |
| Ctr   | 65      | M   | 29  | 0          | -           | 2           | no     | no          | CTX    |
| Ctr   | 70      | F   | 13  | 0          | -           | 1           | no     | no          | CTX    |
| Ctr   | 68      | F   | 23  | 0          | -           | 1           | no     | no          | CTX    |
| Ctr   | 66      | M   | 8   | 0          | -           | 2           | no     | no          | CTX, HIP |
| Ctr   | 65.6±3.1| M   | 19.1±2.7| 0.46±0.2 | 0.91±0.3   | no          | no     | no          | HIP    |
| Ctr   | 72.0±3.6| M   | 9.8±2.1| 0.3±0.3 | 0.8±0.3    | no          | no     | no          | CTX    |
| AD    | 78      | M   | 21  | 5          | -           | 6           | no     | no          | CTX, HIP, GP, PU |
| AD    | 64      | M   | 16  | 5          | -           | 6           | no     | no          | CTX, GP, PU |
| AD    | 80      | F   | 2   | 5          | -           | 5           | no     | no          | CTX, HIP, GP, PU |
| AD    | 56      | F   | 1   | 5          | -           | 5           | no     | no          | CTX, HIP, GP, PU |
| AD    | 79      | F   | 18  | 4          | -           | 5           | no     | no          | CTX, GP, PU |
| AD    | 80      | F   | 2   | 5          | -           | 6           | no     | no          | CTX, HIP |
| AD    | 79      | M   | 4   | 5          | -           | 6           | no     | no          | CTX, HIP |
| AD    | 77      | F   | 10  | 5          | -           | 6           | no     | no          | CTX, HIP |
| AD    | 62      | M   | -   | 5          | -           | 6           | no     | no          | CTX    |
| AD    | 66      | F   | -   | 5          | -           | 6           | no     | no          | CTX    |
| AD    | 78      | M   | -   | 5          | -           | 6           | no     | no          | CTX    |
| AD    | 60      | M   | -   | 5          | -           | 6           | no     | no          | CTX    |
| AD    | 64      | M   | -   | 5          | -           | 6           | no     | no          | CTX    |
| AD    | 62      | F   | -   | 5          | -           | 6           | no     | no          | CTX    |
| AD    | 59      | M   | -   | 5          | -           | 6           | no     | no          | CTX    |
| AD    | 69.6±2.4| M   | 4.9±0.1| 5.8±0.1 | -           | -           | -      | -           | -      |
| AD    | 78      | M   | 13  | -          | 3           | 6           | no     | no          | HIP    |
| AD    | 68      | M   | 23  | 3          | 3           | 5           | no     | no          | HIP    |
| AD    | 70      | M   | 6   | -          | 3           | 6           | no     | no          | HIP    |
| AD    | 72.0±3.1| M   | 14.0±4.9| 3    | 5.7±0.3   | no          | no     | no          | HIP    |
| FTLD-11a | 68     | F   | 6.5 | -          | -           | -           | no     | no          | CTX    |
| FTLD-11a | 73     | F   | 7.5 | -          | -           | -           | no     | no          | CTX    |
| FTLD-11a | 89     | F   | 11  | -          | -           | -           | no     | no          | CTX    |
| FTLD-11a | 75     | M   | 9   | -          | -           | -           | no     | no          | CTX    |
| FTLD-11a | 87     | F   | 36  | -          | -           | -           | no     | no          | CTX    |
| FTLD-11a | 71     | M   | 23  | -          | -           | -           | no     | no          | CTX    |
| FTLD-11a | 67     | F   | 18  | -          | -           | -           | no     | no          | CTX    |
| FTLD-11a | 75.7±3.3| F   | 15.9±4.1| -     | -          | no          | no     | no          | CTX    |
| FTLD-11a | 71     | F   | 6   | -          | 0           | 0           | no     | no          | HIP    |
| FTLD-11a | 65     | F   | 3   | -          | 0           | 4           | no     | no          | HIP    |
| FTLD-11a | 76     | F   | 9   | -          | 0           | 0           | no     | no          | HIP    |
| FTLD-11a | 62     | F   | 9   | -          | 0           | 0           | no     | no          | HIP    |
| FTLD-11a | 78     | M   | 26  | -          | 0           | 0           | no     | no          | HIP    |
| FTLD-11a | 76     | F   | 9   | -          | 0           | 0           | no     | no          | HIP    |

PMI, post-mortem interval; CTX, cortex; PU, Putamen; GP, Globus pallidus; HIP, hippocampus; Amy, amygdala ‘-‘, data not available; mean ± SEM of group in bold.
| Strain | Susceptible | Non Susceptible | Total Alleles | Odds Ratio | p-value (FDR) |
|--------|-------------|-----------------|---------------|------------|---------------|
| AJ     | 6           | 2               | 8             | 5.00       | ns            |
| BL6    | 2           | 26              | 28            | 0.08       | ***           |
| 129S1  | 2           | 10              | 12            | 0.27       | ns            |
| NOD    | 8           | 14              | 22            | 0.83       | ns            |
| NZO    | 9           | 16              | 25            | 0.81       | ns            |
| CAST   | 5           | 0               | 5             | infinite   | ns            |
| PWK    | 6           | 4               | 10            | 2.43       | ns            |
| WSB    | 10          | 0               | 10            | infinite   | ***           |

***, p<0.001; ns, not significant
Supplementary Figures

Supplementary Figure 1

*Lamp*<sup>5Δ/Δ</sup> *strain genotyping*. Representative genotyping PCR demonstrating 250bp deletions in mice derived from M1 founder. Homozygous deletion of Lamp5 (Δ/Δ), heterozygous deletion (Δ/+), and wild-type (+/+) mice are shown here.
The Collaborative Cross. (a) To establish the Collaborative Cross (CC) platform, 8 founder strains (different colour chromosomes) are crossed in random permutation to derive four G1 hemizygous strains. These are crossed to yield a G3 population where the inbreeding process begins for another 20 generations. (b) An exemplified chromosome arrangement from a CC strain. All murine chromosomes with colour denoting parental origin of genomic segments from (a). After 20 generations of inbreeding, the estimated residual hemizygosity is less than 10%.
**QLT mapping.** (a) Manhattan blot of QTL mapping using Mean Seizure Severity showing a locus on chromosome 2. (b) Haplotype of each strain contributing to the phenotype is shown. Haplotype calls are made based on contribution at 135.0 MB of the murine chromosome 2 (green line) given the frequency of recombination within this region. Animals with mean Seizure Severity Score of less than 3 are shown above the red line and greater or equals to 3 below the red line.
LAMP5 staining in the cortex of Alzheimer’s disease, FTLD-tau and control brains.

Representative staining of LAMP5 (brown) in the cortex from all human AD, FTLD-tau and control (CTR) brains. Scale bar, 50 µm.
LAMP5 staining in the hippocampus of Alzheimer’s disease, FTLD-tau and control brains. Representative staining of LAMP5 (brown) in the hippocampus (CA4) from all human AD, FTLD-tau and control (CTR) brains. Scale bar, 50 μm.
Supplementary Figure 6

(a) Representative staining of LAMP5 (brown) in the globus pallidus from human AD and control (CTR) brains. No globus pallidus tissue was available for case #1. Scale bar, 100 μm.

(b) Representative staining of LAMP5 (brown) in the putamen from human AD and control (CTR) brains. Scale bar, 100 μm.

(c) Quantification of LAMP5+ staining in the globus pallidum (*, p<0.05; Student t test).

(d) Quantification of LAMP5+ staining in the putamen (ns, not significant; Student t test).

LAMP5 staining in the basal ganglia of Alzheimer’s disease and control brains. (a) Representative staining of LAMP5 (brown) in the globus pallidus from human AD and control (CTR) brains. No globus pallidus tissue was available for case #1. Scale bar, 100 μm. (b) Representative staining of LAMP5 (brown) in the putamen from human AD and control (CTR) brains. Scale bar, 100 μm. (c) Quantification of LAMP5+ staining in the globus pallidum (*, p<0.05; Student t test). (d) Quantification of LAMP5+ staining in the putamen (ns, not significant; Student t test).
**Murine Lamp5 reporter.** (a) experimental design for Lamp5 reporter expression experiments. Newborn wild-type Lamp5+/+ and Lamp5Δ/Δ littermates were injected with adeno-associated virus (AAV) for expression of green fluorescence protein (GFP) under control of the murine Lamp5 promoter. At 1 and 3 months of age, GFP expression was imaged. (b) Staining of AAV-pLamp5-GFP brains at 1 months of age with antibodies to LAMP5 conformed expression of GFP (green) in LAMP5+ (red) cells in the brains of wild-type Lamp5+/+ mice. No GFP expression was observed in LAMP5-negative cells in the brains of wild-type Lamp5+/+ mice. Nuclei were visualized with DAPI (blue). Scale bar, 20 μm.
Sex-specific survival and body weights of APP23/Lamp5ΔΔ mice. (a) Survival of male Lamp5+/+ (n=23), Lamp5Δ+ (n=94), Lamp5ΔΔ (n=56), APP23/Lamp5+/+ (n=22), APP23/Lamp5Δ+ (n=60), APP23/Lamp5ΔΔ (n=29) mice (*, p<0.05; ****, p<0.0001; ns, not significant; Mantel-Cox test). (b) Survival of female Lamp5+/+ (n=16), Lamp5Δ+ (n=64), Lamp5ΔΔ (n=44), APP23/Lamp5+/+ (n=18), APP23/Lamp5Δ+ (n=58), APP23/Lamp5ΔΔ (n=30) mice (*, p<0.05; **, p<0.01; ****, p<0.0001; ns, not significant; Mantel-Cox test). (c-d) Body weights were not significantly different in male and female mice of the respective genotypes. Body weight tracing was limited by the premature mortality of APP23/Lamp5ΔΔ.
Supplementary Figure 9

Unchanged Aβ pathology in aged APP23/Lamp5^{+/+} mice. (a) Representative Thioflavin S (ThioS, green) staining of amyloid-β (Aβ) plaques in 12 months old APP23/Lamp5^{+/+} and APP23/Lamp5^{Δ/Δ} mice. Quantification of ThioS+ plaques (ns, not significant; Student t test). Scale bar, 500 μm. (b) Representative immunostaining of Aβ (6E10 antibody, red) in 12 months old APP23/Lamp5^{+/+} and APP23/Lamp5^{Δ/Δ} brains. Quantification of 6E10+ cells (ns, not significant; Student t test). Scale bar, 500 μm. (c) Aβ_{1-42} levels in the brains of control (Lamp5^{+/+}) and APP23/Lamp5^{+/+} and APP23/Lamp5^{Δ/Δ} brains (*, p<0.05; ns, not significant; repeated Student t test).
Supplementary Figure 10

Augmented behavioural deficits in APP23/Lamp5Δ mice. (a) Linear regression slopes of Morris water maze (MWM) escape latency curves shown in the main Fig. 3c (*, p<0.05; one-way ANOVA). (b) Average swim speed during MWM testing (ns, not significant; one-way ANOVA). (c) Time spent in target and opposing quadrant during MWM probe trials. (d-h) Open field locomotor testing at 2 months of age. (d) Example movement traces in the open field arena per genotype. (e) Time spent for inner zone exploration. (f) Total distance travelled in the open field arena. (g) Distance travelled per minute in the open field arena. (h) Linear regression slopes of minute-by-minute distance travelled shown in (h) (*, p<0.05; ***, p<0.001; ****, p<0.0001; ns, not significant; two-way ANOVA (Tukey post hoc) for minute-by-minute analysis; one-way ANOVA for other comparisons).
Neuronal network deficits in APP23/Lamp5Δ mice. (a) Example electroencephalography (EEG) traces, local field potential (LFP) and isolated theta and gamma waves for all indicated genotypes. (b) Spectral power of theta (left) and gamma (right) EEG wave frequencies for indicated genotypes together with area under the curve (AUC) analysis for indicated frequency ranges (broken boxes) (*, p<0.05; ****, p<0.0001; ns, not significant; one-way ANOVA).
Unchanged tau pathology in mature TAU58/Lamp5Δ/Δ mice. (a) Representative immunohistochemistry with antibodies to tau phosphorylated at Serine 422 (pS422; brown, arrows) in the cortex (CTX) hippocampus (Hip) and amygdala (Amy) of TAU58/Lamp5+/+, TAU58/Lamp5Δ/+ and TAU58/Lamp5Δ/Δ mice (ns, not significant; one-way ANOVA). Scale bars, 100 μm. (a) Representative immunohistochemistry with antibodies to tau phosphorylated at Serine 214 (pS214; brown) in the cortex (CTX) hippocampus (Hip) and amygdala (Amy) of TAU58/Lamp5+/+, TAU58/Lamp5Δ/+ and TAU58/Lamp5Δ/Δ mice (ns, not significant; one-way ANOVA). Scale bars, 100 μm.
Additional functional data from TAU58/Lamp5^5/Δ^ mice. (a) Survival of Lamp5^+/+ (n=21), Lamp5^Δ/+ (n=47), Lamp5^Δ/Δ (n=22), TAU58/Lamp5^+/+ (n=17), TAU58/Lamp5^Δ/+ (n=34), TAU58/Lamp5^Δ/Δ (n=21) mice (ns, not significant; Mantel-Cox test). (b) Linear regression slopes of Morris water maze (MWM) escape latency curves shown in the main Fig. 4c (*, p<0.05; one-way ANOVA). (c) Mean latency for male mice to find escape platform on individual days of the acquisition trials during MWM testing (**, p<0.01; two-way ANOVA (Tukey post hoc)). (d) Linear regression slopes of Morris water maze (MWM) escape latency curves shown in (c) (*, p<0.05; one-way ANOVA). (e) Average swim speed during MWM testing (ns, not significant; one-way ANOVA). (f) Time spent in target and opposing quadrant during MWM probe trials.
Neuronal network deficits in TAU58/Lamp5^Δ/Δ mice. (a) Example electroencephalography (EEG) traces, local field potential (LFP) and isolated theta and gamma waves for all indicated genotypes. (b) Spectral power of theta (left) and gamma (right) EEG wave frequencies for indicated genotypes together with area under the curve (AUC) analysis for indicated frequency ranges (broken boxes) (*, p<0.05; **, p<0.01; ****, p<0.0001; ns, not significant; one-way ANOVA).