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Additional Declarations:

Yes there is potential Competing Interest. MuseCell Innovations PTE Ltd (MCI, Singapore) holds exclusive licensing and patents for Muse Cells.

A statement of ethics approval: while this is not a human study, our study was approved by the Ethics Committees of Toyama University (No. A2021med-252 and No. G2021med-60) and Tohoku University (No. 2025NdA-024) for the animal study, as mentioned in the Method file, not in the main text. A statement on participant consent: Since our study is neither a human study nor a clinical study, participant consent is not required.

Intravenous and intranasal Muse cell administration delivers cognitive and histologic improvements in an Alzheimer's disease model

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Abstract

Mesenchymal stromal cells (MSCs) contain several percentages of SSEA-3+ pluripotent-like Muse cells. We compared human bone marrow-derived Muse cells with MSC controls delivered intravenously (IV) or intranasally (IN) in 3-month-old 5xFAD mice without immunosuppression. Light-sheet imaging of mCherry-labeled cells showed markedly greater brain retention of Muse cells at day 7 after IN delivery compared with MSCs. At 16 weeks, human cells, detected by anti-human mitochondria, human nuclear Ku80, and Alu ddPCR, were highly persistent in both Muse groups with neural and microglial marker expression. Muse treatment reduced amyloid- β plaque burden and preserved pre-onset Y-maze and Morris water maze performance through 16 weeks, with similar outcomes after IV and IN delivery. These findings support further evaluation of IN Muse cell delivery for AD cell therapy.

Stem cell-based approaches hold therapeutic potential for Alzheimer's disease (AD). Muse cells are non-tumorigenic, endogenous, stress-tolerant, reparative stem cells with macrophage-like/pluripotent-like properties, collectable as pluripotent surface marker SSEA-3(+) from the bone marrow (BM), peripheral blood, and organ connective tissues.¹⁻³ Although they comprise several percent of mesenchymal stem cells (MSCs), they are biologically distinct from non-Muse/non-pluripotent MSCs and show superior therapeutic efficacy.^{1,3} Intravenously injected Muse cells sense the universal tissue damage signal sphingosine-1-P (S1P), home accurately to injured tissues, phagocytose apoptotic cells, utilize factors (e.g., transcription factors) obtained by phagocytosis to differentiate into the same lineage, and reconstruct the tissue by cell replacement.^{2,4} In stroke models, Muse cell-derived neurites incorporate into the pyramidal and sensory tracts and form synapses with host neurons, leading to a significant recovery of motor function and somatosensory evoked potentials.⁵ Notably, Muse cells do not require pre-differentiation or surgical implantation, enabling systemically delivered cells to participate directly in tissue reconstruction.³ Due to their intrinsic immunotolerance, allogeneic Muse cells have been administered intravenously without HLA matching or immunosuppressants in clinical trials for ischemic stroke, spinal cord injury, neonatal hypoxic-ischemic encephalopathy, and amyotrophic lateral sclerosis (ALS), demonstrating safety and therapeutic efficacy over 1 to 1.5 years without signs of immune rejection.⁶

Recently, intranasally administered molecules and cells were shown to reach the brain via olfactory pathways.⁷ In models of cognitive impairment, however, MSCs disappear within 12–72 h and show minimal distribution in the brain.⁸ Here, we compared the therapeutic effects of intravenous injection (IV) and intranasal administration (IN) of human BM-derived-MSCs (hMSCs) and hMuse cells in 3-month-old AD model (5xFAD) mice.⁹

Three-dimensional light sheet imaging showed that, of 50,000 IN cells, 62.3±4.5% of mCherry-hMSCs and 69.6±9.1% of mCherry-hMuse cells were detected in the olfactory bulb by day 1. By day 7, 1.2±0.1% of hMSCs and 28.9±3.3% of hMuse cells were detected in the whole brain (Fig. 1A-1B, Supplementary Movies 1-4, Extended Data Fig. 1a). Neither mCherry-hMSCs nor hMuse cells were detectable in the peripheral blood at days 1, 3, and 5. Muse cells migrate to S1P released from injured tissues, but non-Muse MSCs do not.⁴ Although mCherry-hMSCs and -hMuse cells were administered intranasally 1 day after liver damage, both of them remained undetectable in the peripheral blood at days 1, 3, and 5 (Extended Data Fig. 1b).

Consequently, for IN, a 5×10⁴ hMuse cell group was set (Muse-IN). For IV, 5×10⁴ hMSCs (MSC-Low), 5×10⁶ hMSCs (MSC-High, contains ~5×10⁴ hMuse cells), and 5×10⁴ hMuse cells (Muse-IV) were set. All groups (n=10) were examined at 16 weeks.

Administered cells were detected by human-mitochondria (h-mitochondria) and human-Ku80 (h-Ku80) antibodies. Across whole coronal sections, the density of h-mitochondria-positive and h-Ku80-positive cells was significantly higher in both Muse groups than in either MSC group (Fig.1C-1F, Extended Data Fig. 2a). Droplet digital-PCR (ddPCR) for *Alu* sequence supported these results (Fig. 1G, Extended Data Fig.3).

The number of h-mitochondria-positive cells between -3 to +2 mm from bregma was significantly higher in both Muse groups than in either MSC group throughout the region (Fig. 1H). The estimated whole-brain engraftment rate was significantly higher in the Muse-IV ($16.7 \pm 0.3\%$) and Muse-IN ($16.5 \pm 0.6\%$) groups than in the MSC-Low ($0.9 \pm 0.2\%$) and MSC-High ($0.9 \pm 0.1\%$) groups, and within Muse cell-treated brains, h-mitochondria-positive cells were more abundant in the cerebral cortex than in the hippocampus (Extended data Fig. 2b-2c), likely correlated with a tendency for greater neuronal death in the cortex than in the hippocampus in 5xFAD mice.¹⁰

In AD, including 5xFAD, the brain contains neurotoxic amyloid- β (A β) plaques and phosphorylated tau (p-tau).^{9,10} hMuse cells retained neuronal differentiation capacity even after phagocytosing pTau-positive neuronal debris, and naïve-Muse and neuro-differentiated Muse cells showed less apoptosis under the A β exposure than positive control, PC12 cells, in vitro (Extended data Fig. 4).

In both Muse groups, h-mitochondria-positive cells that expressed NeuN (neuronal marker) were the highest, followed by APC (oligodendrocyte), and Iba1 (microglia), while few GFAP (astrocyte)-positive cells were detected in the cortex (Fig. 2A-2D, Extended Data Fig. 2a, 5a-5b). h-Ku80-positive cells showed a similar trend (Extended data Fig. 5c). Tumor formation was not observed in the brain and other organs in any of the five groups, similar to previous preclinical and clinical studies (Fig. 1C-1E, Extended data Fig. 6).³⁻⁶

The mean number of A β plaques per unit area and the mean area occupied by A β plaques in the cortex and hippocampus were largest in the vehicle group, intermediate in the MSC groups, and significantly smaller in the Muse groups, with no significant difference between the Muse-IV and Muse-IN groups. The A β aggregate size in the cortex or hippocampus did not differ significantly among the five groups (Fig. 2E-2G, Extended Data Fig. 7).

Muse cells differentiated into Iba1-positive cells, and microglia are known to clear A β plaques.¹¹ Interestingly, a strong correlation between Iba-1(+) cells/mm² and A β plaque % area was seen in the cortex (Extended data Fig. 8). Fe and Cu, known to aggregate with A β plaques,¹² were substantially reduced in the cortex of the Muse-IN group than that of the vehicle group in Scanning X-ray fluorescence microscopy (Extended data Fig. 9). Gliosis was ameliorated in both Muse groups than other three groups (Extended data Fig. 10).

In the spontaneous alternation Y-maze test, the Muse groups maintained spontaneous alterations within the 1-week pre-administration range through 16 weeks, with no significant difference between them. In contrast, performance in the vehicle and MSC groups gradually declined beginning 2 to 4 weeks after treatment, became significantly below that of the Muse groups by 6 weeks in the MSC-High group, and by 12 weeks in the vehicle and MSC-Low groups (Fig. 2H). In the Morris water maze test, both the Muse groups exhibited significantly shorter escape latencies than the other three groups on day 4, with no significant differences between the two Muse groups from day 1 to day 4 (Fig. 2I).

Several factors may contribute to the improved performance of the Muse groups compared with the MSC groups. First, very few MSCs were detected in the brain at 16 weeks, consistent with previous reports, while Muse cells remain in the tissue after cell replacement for a long-term

period.^{5,6} After IV delivery, MSCs do not reach damaged organs, but rather become trapped in the lungs, where they produce temporal bystander effects because they disappear from the body within several weeks.⁶ Second, although MSCs contain ~1% Muse cells, the remaining ~99% of non-Muse MSCs can interfere with Muse cell activity, partly by reducing S1P through their anti-inflammatory effects, explaining the stronger effects in the Muse-IV (5×10^4 cells) group compared with the MSC-High (5×10^6 cells) group that contained the same number of Muse cells.¹³

As reported, Muse cells remained in the damaged tissue as functional components by IV.³ This study newly showed IN Muse cells remained in the brain comparable to IV. In IN, ~30% of Muse cells remained in the brain at day 7, with no detectable leakage into the systemic circulation. Approximately 17% of the total administered hMuse cells engrafted in the brain by 16 weeks in both the Muse groups. This finding is similar to that in a rabbit acute myocardial infarction model, in which ~15% of IV allogenic-Muse cells homed into the post-infarct tissue and remained as cardiac-lineage cells for up to 6 months without immunosuppressants.⁴

Following delivery, Muse cells differentiated into neural-lineage and microglial cells. Microglial differentiation is particularly relevant in AD, as microglia scavenge A β , Fe, and Cu, thereby improving the brain microenvironment and potentially supporting neuronal survival and function.¹¹ Besides differentiation, Muse cells also provide anti-apoptosis, anti-fibrosis, neovascular, and tissue protection effects.³ These synergistic effects likely contributed to the sustained improvements in cognitive functions observed in the Muse groups.

Unlike induced pluripotent stem cells, which require genetic manipulation to induce pluripotency and differentiation, pluripotent-like Muse cells rely on a phagocytosis-dependent mechanism for in vivo differentiation.² Furthermore, they express stress-tolerance factors and are resistant to stress.¹⁴ This intrinsic stress resilience may partly enable their survival and function within the hostile AD brain microenvironment. Long-term neural functionality promoted by Muse cells is a future research topic.

Considering the chronic course of AD, repeated dosing at regular intervals is likely to be more effective than a single administration, similar to the previous clinical trial in ALS, which suggested the safety and efficacy of once-monthly infusions over 6 months.⁶ Intranasal Muse cell delivery might be a more practical option than IV delivery because of its simplicity. The clinically optimal Muse cell dose and dosing schedule must be established in clinical trials.

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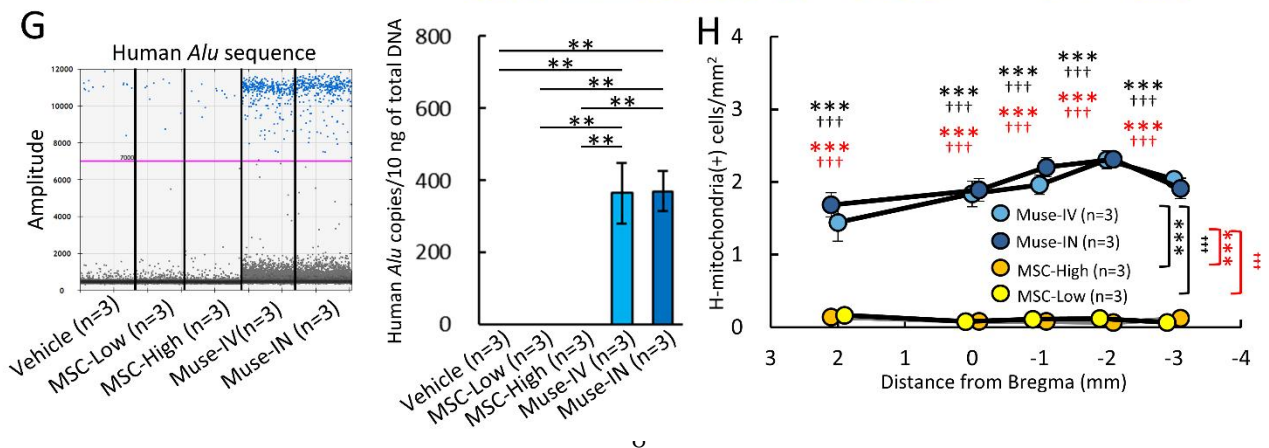
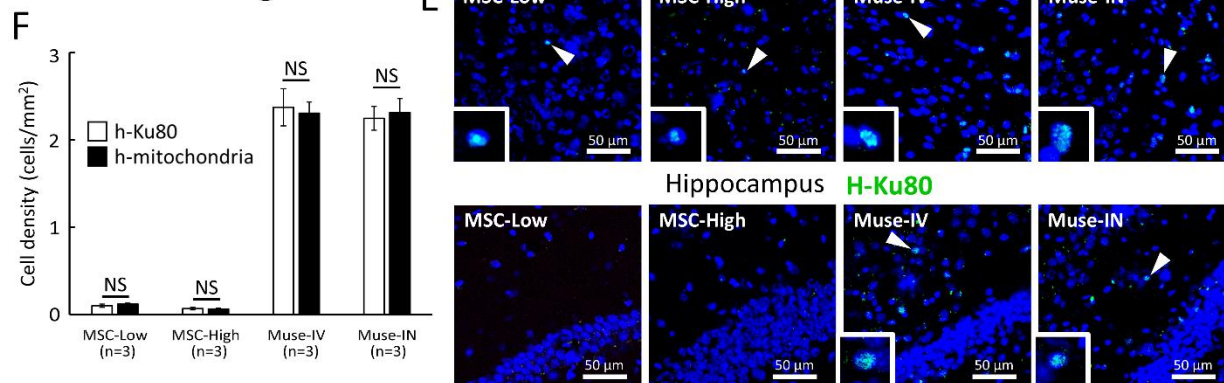
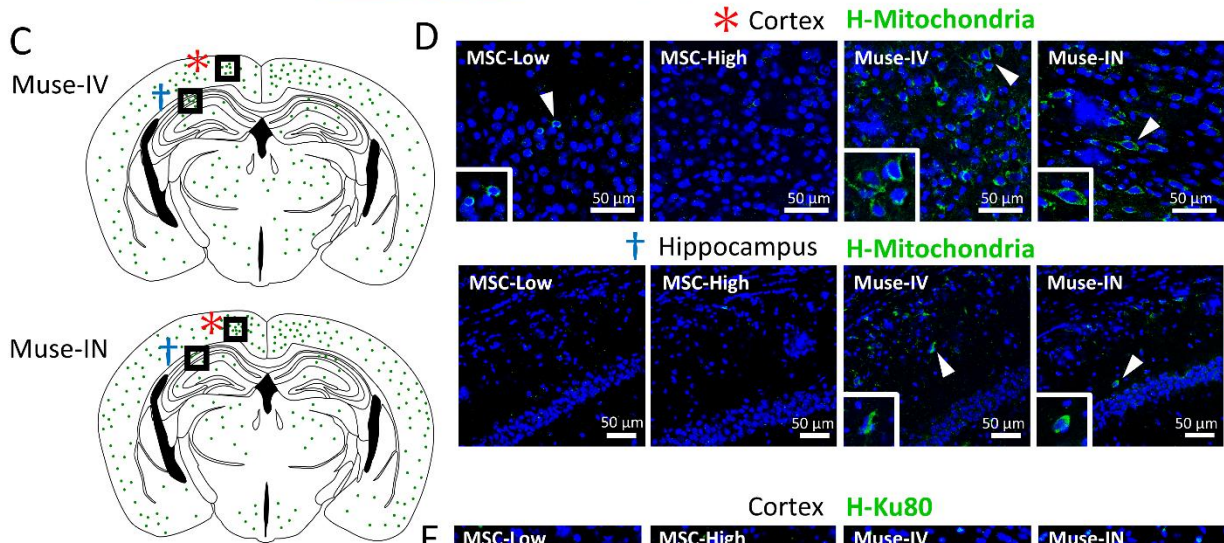
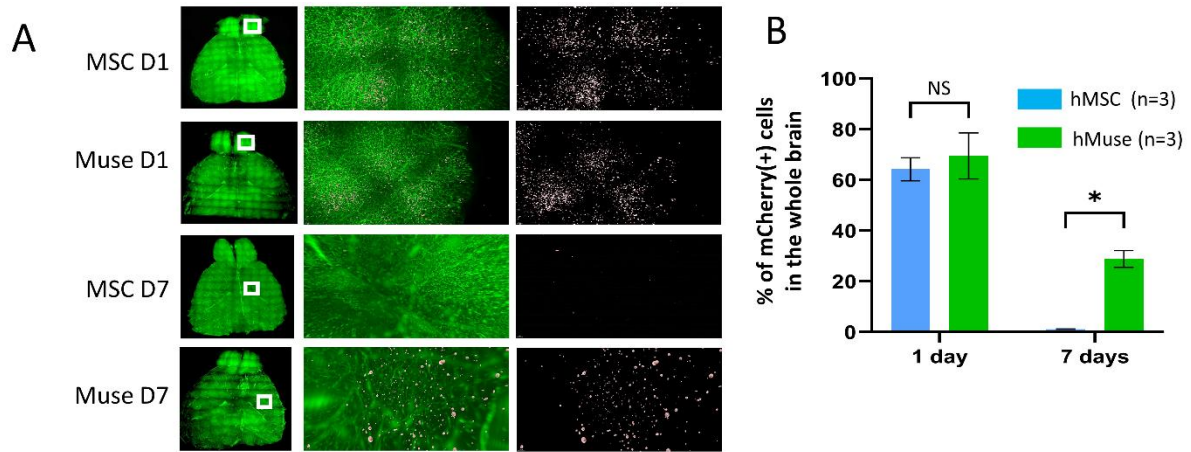
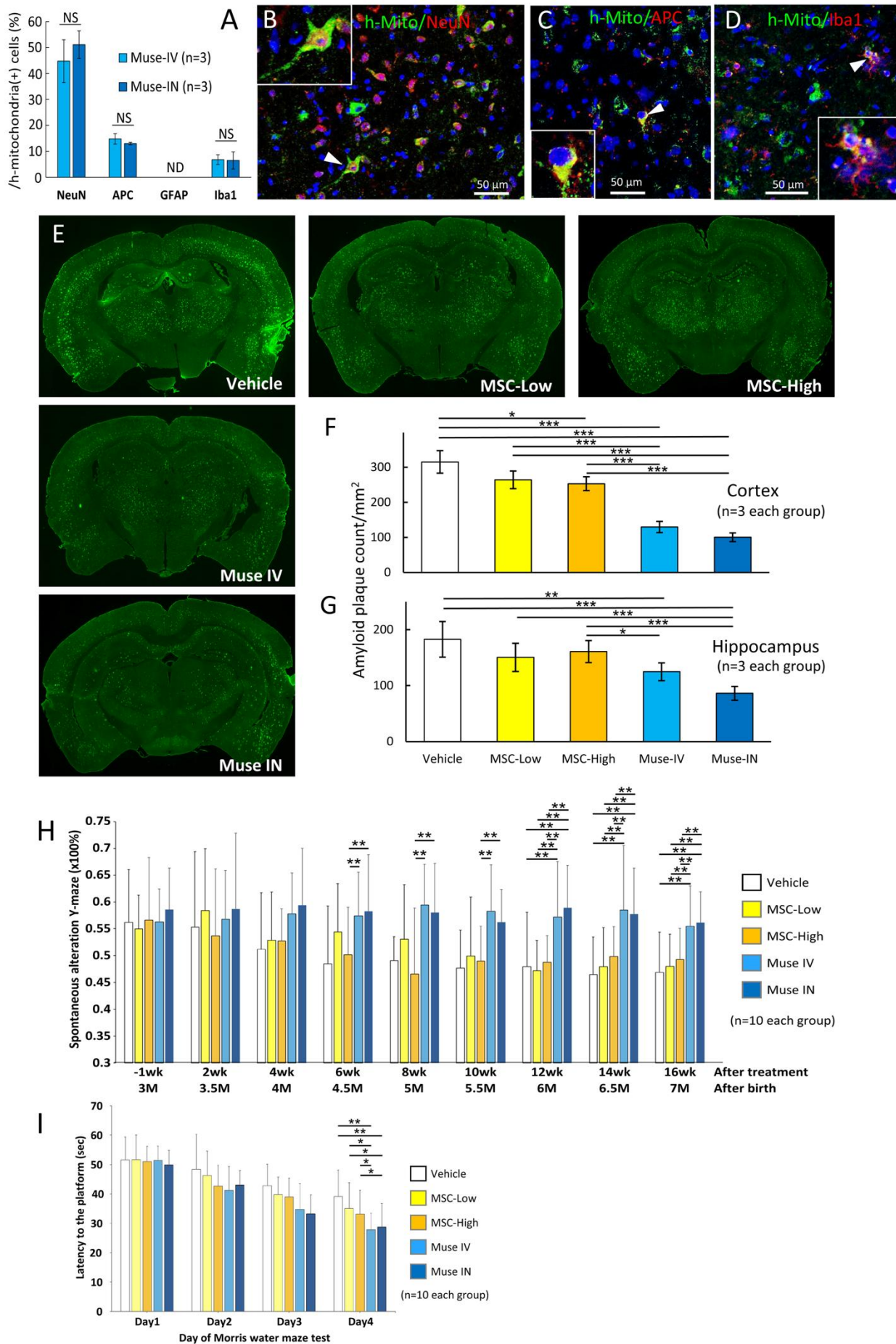


Figure 1. Distribution of administered cells in the brain.

Light sheet imaging of IN mCherry-hMSCs/-hMuse cells at days 1 and 7 (A). Percent of mCherry-positive cells detected in the whole brain (B), analyzed by two-way ANOVA followed by Sidak's multiple comparisons test (mean $\pm$ s.e.m). n=3 for each group. NS: not significant. *: p<0.05. Representative h-mitochondria-positive cell plots in the Muse groups (C). H-mitochondria-positive (D) and h-Ku80-positive (E) cells in the cortex (*) and hippocampus (†). The density of h-mitochondria- and h-Ku80-positive cells (cells/mm²) across the whole coronal section [mean $\pm$ standard deviation (SD)] was similar among the four groups (F). Representative ddPCR plots and human *Alu* sequence copy number (mean $\pm$ SD) (G). H-mitochondria-positive cells/mm² (mean $\pm$ SD) in whole coronal sections from -3 to +2 mm from the bregma (H). (C)-(H) from 16 weeks, analyzed by one-way ANOVA followed by Tukey-Kramer HSD test, except (F) analyzed by unpaired Student's t-test. n=3 for each group. **: p<0.01. p<0.001 with *** and †††. NS: not significant.



201 **Figure 2. Histologic and cognitive analysis (16 weeks).**
202 Marker positivity in h-mitochondria-positive cells in the cortex of the Muse groups (A). (B-D): Muse-IN
203 group.
204 A β -positive plaques (E). A β plaque count/mm² in the whole regions of the cortex (F) and hippocampus
205 (G) from the both hemispheres.
206 Spontaneous alternation Y-maze test (H) and Morris water maze performance (I) from 1 week pre-
207 administration to 16 weeks after treatment.
208 A, F-I; mean $\pm$ SD. A was analyzed by unpaired Student's t-test and F-I by one-way ANOVA followed
209 by Tukey-Kramer HSD test. A, F, G; n=3 for each group. H and I; n=10 for each group. *: p<0.05, **:
210 p<0.01, ***: p<0.001. NS: not significant.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SuppleMov2Muse1dayC.mp4](#)
- [SuppleMovMSC1dayC.mp4](#)
- [ExtendedDataFVXXX.pdf](#)
- [SuppeMov3MSC7daysC.mp4](#)
- [SuppleMov4Muse7daysC.mp4](#)