Novel Neuroprotective Therapy with NeuroHeal by Autophagy Induction for
Damaged Neonatal Motoneurons

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SUPPLEMENTARY INFORMATION

- Supplementary Tables

**Table S1.** Primary antibodies for immunohistochemistry

| Antibody name                          | Reference                      |
|----------------------------------------|--------------------------------|
| rabbit anti-Iba1                       | 1:1000, Wako, ref 1919741      |
| rabbit anti-SIRT1                      | 1:100, Millipore, ref 07-131   |
| rabbit anti-H3K9Ac                     | 1:50, Millipore, ref 06-942    |
| rabbit anti-p53-K373Ac                 | 1:500, Millipore, ref 06-916   |
| rabbit anti-cleaved Casp3              | 1:200; Cell Signaling, ref 96615|
| rabbit anti-LAMP1                      | 1:200, Antibodies Online, ref 3187789|
| mouse anti-ATG5                        | 1:200, Nanotool, ref 0263-100  |
| mouse anti-p62                         | 1:100; BD Transduction Laboratories, ref 610833|
| rabbit anti-phospho-FOXO3a (Ser253)   | pFOXO3a; 1:500, Abcam, ref ab 31109-100|
| rabbit anti-FOXO3a                     | Novus Biologicals, 1:200, ref NBP2-16521|
| rabbit anti-phospho-S6 kinase (Thr389)| pP70S6k; 1:1000, Antibodies Online, ref 863186|
| rabbit anti-PARP1/2                    | 1:200; Santa Cruz, ref sc-7150 |
| anti-β-III-tubulin                     | 1:500, Covance, ref MMS-435P/801202|
| anti SMI32                             | 1:1000; Biolegend, ref 801701  |

**Table S2.** Primary antibodies for Western Blot

| Antibody name                          | Reference                      |
|----------------------------------------|--------------------------------|
| rabbit anti-PARP1/2                    | 1:500; Santa Cruz Biotechnology, ref sc-7150|
| rabbit anti-phospho-Ulk1 (Ser 555)     | pUlk1; 1:1000; Millipore, ref ABC124|
| mouse anti-Atg5                        | 1:1000; Nanotools, ref 0263-100 |
| rabbit anti-LC3                        | 1:1000; Abcam, ref ab48394     |
| rabbit anti-pFOXO3a                    | 1:1000, Abcam, ref ab31109-100 |
| rabbit anti-FOXO3a                     | Novus Biologicals, 1:500, ref NBP2-16521|
| rabbit anti-phospho-AKT                | pAKT; 1:1000; Cell Signaling, ref 4060|
### Table S3. Primers used for RT-PCR

| Primer Name | Sequence (5' to 3') |
|-------------|---------------------|
| rBNIP3-F    | CAGAGCGGCGAGGAGAACCTGCAG (24) |
| rBNIP3-R    | GCTGCTCCCATCCCATTGCTGAAG (25) |
| rBeclin-F   | GGTTACCTTTCTGGACTGTGTGCAGCAG (28) |
| rBeclin-R   | GTCTTCATTCTTGCTTTCTCCACGTCC (28) |
| rLC3-F      | ACCCTCCCTGATGCAGCTGTCC (23) |
| rLC3-R      | ACCAGGGACATGACGACGTACACAACC (27) |
| rGADD45-R   | TGAGGGTGAAATGGATCTG (19) |
| rGADD45-F   | GAAGATCGAAAGGATGGAC (19) |
| rBax2-F     | CTGCAGAGGATGATTGCTGA (20) |
| rBax2-R     | GATCAGCTCGGCACCTTTAG (20) |
| rActin-F    | TCAGGTGATCACTACTGG (18) |
| rActin-R    | CAGTAATCTCCTCTGCATC (20) |

- **Supplementary Figures and Legends**
Supplementary Figure S1. **Top,** Representative confocal microphotographs of the immunolabelling of PARP1/2 (red), and FluoroNissl (green), and DAPI (blue) in merged pictures of MNs from different experimental conditions at 10 dpi. Scale bar 20 µm.

**Bottom,** Quantification of the integrated density of PARP1/2 in nuclei of MNs (n=4 per group, ANOVA, post hoc Bonferroni, *p<0.05).
**Supplementary Figure S2.** (A) Representative images from two independent experiments of immunoblots to detect FOXO3a (top) or BIM (down) from NSC34 cells either untransfected (CTL) or nucleofected with either GFP, double negative (DN) or nonphosphorylatable triple mutant (TM) plasmids. Note that FOXO3a corresponding band is normally detected above 80 Kda (band a) except when expression of the delected form of DN mutant (<50KDa, band b). (B) Graph showing the percentage survival (± SEM) of transfected NSC-34 cells with respect to cells transfected with control vector analysed at 4 DIV.
Supplementary Figure S3. Confocal microphotographs of samples stained for LAMP1 (red), and counterstained with FluoroNissl (blue) from a representative contralateral side and injured side of vehicle-treated and NH-treated injured animals at 10 dpi. Scale bar 25 µm.
Supplementary Figure S4. Confocal microphotographs of SOCs immunolabelled for SIRT1, p53Ac, or H3K9Ac (red), SMI3-32 (green), and DAPI (blue) at 15 DIV after the treatment with vehicle, NeuroHeal (NH), or NH+SIRT1 inhibitors. Scale bar 25 µm.
Supplementary Figure S5. Analysis of cell survival using MTT assay in different conditions: 100 μM Bafilomycin A1 (a V-ATPase inhibitor, Baf) to apoptotic cells induced by staurosporine (St) and treated with NeuroHeal (NH). Ctrl, control; veh1, 0.0002% DMSO; veh2, 0.01% EtOH.*p<0.05 vs Control;# p<0.05 vs St; $ p<0.05 vs St+NH.
Supplementary Material

Extra Immunoblot Membranes for Fig 2B_ Casp3