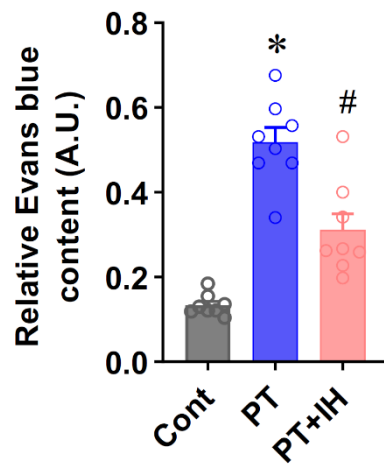
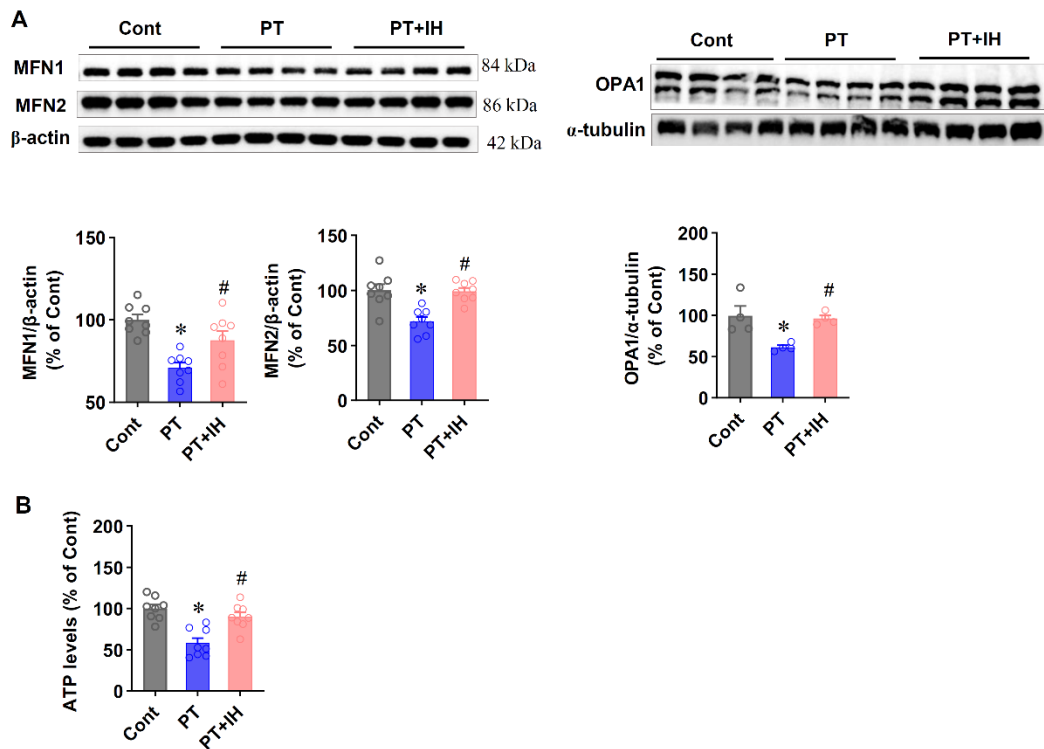


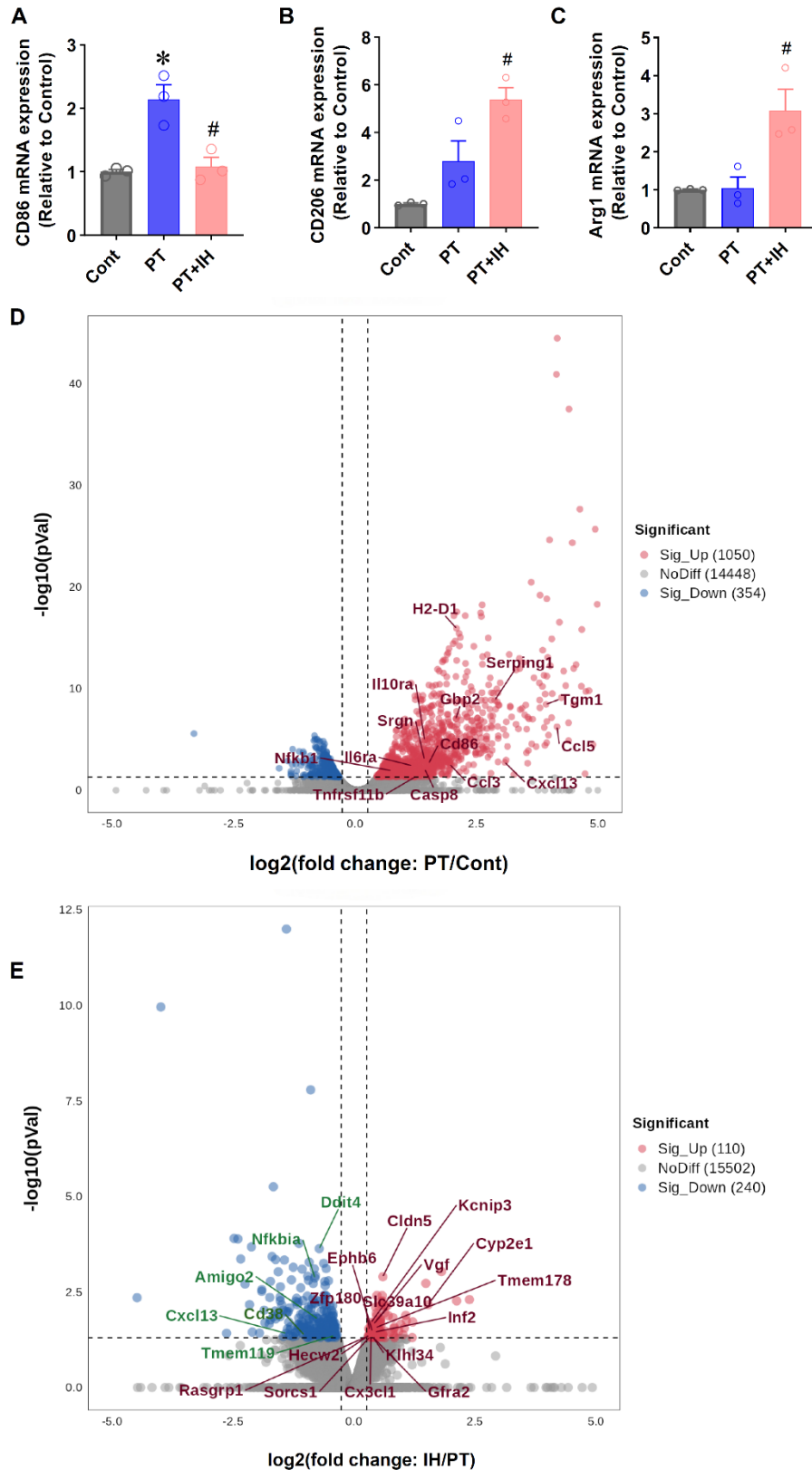
Supplementary Figures



Supplementary Figure 1. IH treatment attenuates Evans blue extravasation in PT stroke mice. Relative Evans blue content in the ipsilateral cerebral cortex of mice in the Cont, PT, and PT+IH groups. Data are presented as mean \pm SEM. $n=8$. * $P < 0.05$ vs. Cont group; # $P < 0.05$ vs. PT group. A.U., arbitrary units.

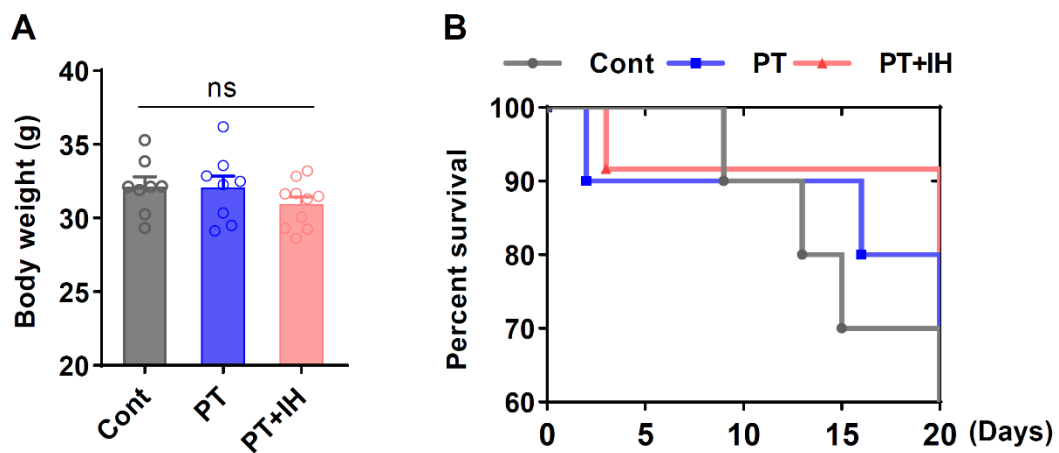


Supplementary Figure 2. IH treatment restores mitochondrial dynamics and ATP production in PT stroke mice. (A) Representative Western blot images and quantitative analysis of mitochondrial fusion proteins MFN1, MFN2, and OPA1 in the peri-infarct area. β -actin and α -tubulin were used as internal loading controls. (B) Quantitative analysis of ATP levels in the peri-infarct area of mice across the three groups. All data are normalized to the Cont group and presented as mean \pm SEM. * P < 0.05 vs. Cont group; # P < 0.05 vs. PT group.



Supplementary Figure 3. IH treatment modulates microglial polarization and transcriptomic profile in PT stroke mice. (A-C) RT-qPCR analysis of CD86, CD206, and Arg1 mRNA expression in the peri-infarct area of mice in the Cont, PT, and PT+IH groups, normalized to the Cont group. (D) Volcano plot showing

differentially expressed genes (DEGs) between the PT and Cont groups, with red dots indicating significantly upregulated genes, blue dots indicating significantly downregulated genes, and gray dots indicating no significant difference (NoDiff, 14448). Key pro-inflammatory and microglial activation-related genes (e.g., Cd86, Il6ra, Nfkb1) are labeled. (E) Volcano plot showing DEGs between the PT+IH and PT groups, with red dots indicating significantly upregulated genes, blue dots indicating significantly downregulated genes, and gray dots indicating no significant difference. Key genes involved in inflammation, barrier function, and metabolism are labeled. All RT-qPCR data are presented as mean \pm SEM. n=3. * $P < 0.05$ vs. Cont group; # $P < 0.05$ vs. PT group.



Supplementary Figure 4. IH treatment does not affect body weight and improves long-term survival in PT stroke mice. (A) Body weight of mice in the Cont, PT, and PT+IH groups at the endpoint of the experiment. No significant difference (ns) was observed among the three groups. Data are presented as mean \pm SEM. n=8-10. (B) Kaplan-Meier survival curve showing the percent survival of mice in the three groups over a 20-day observation period following PT stroke induction.

Fig. S1

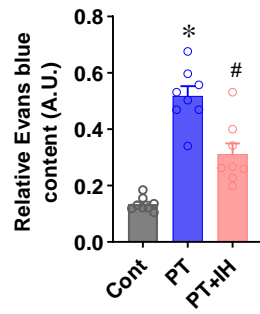


Fig. S2

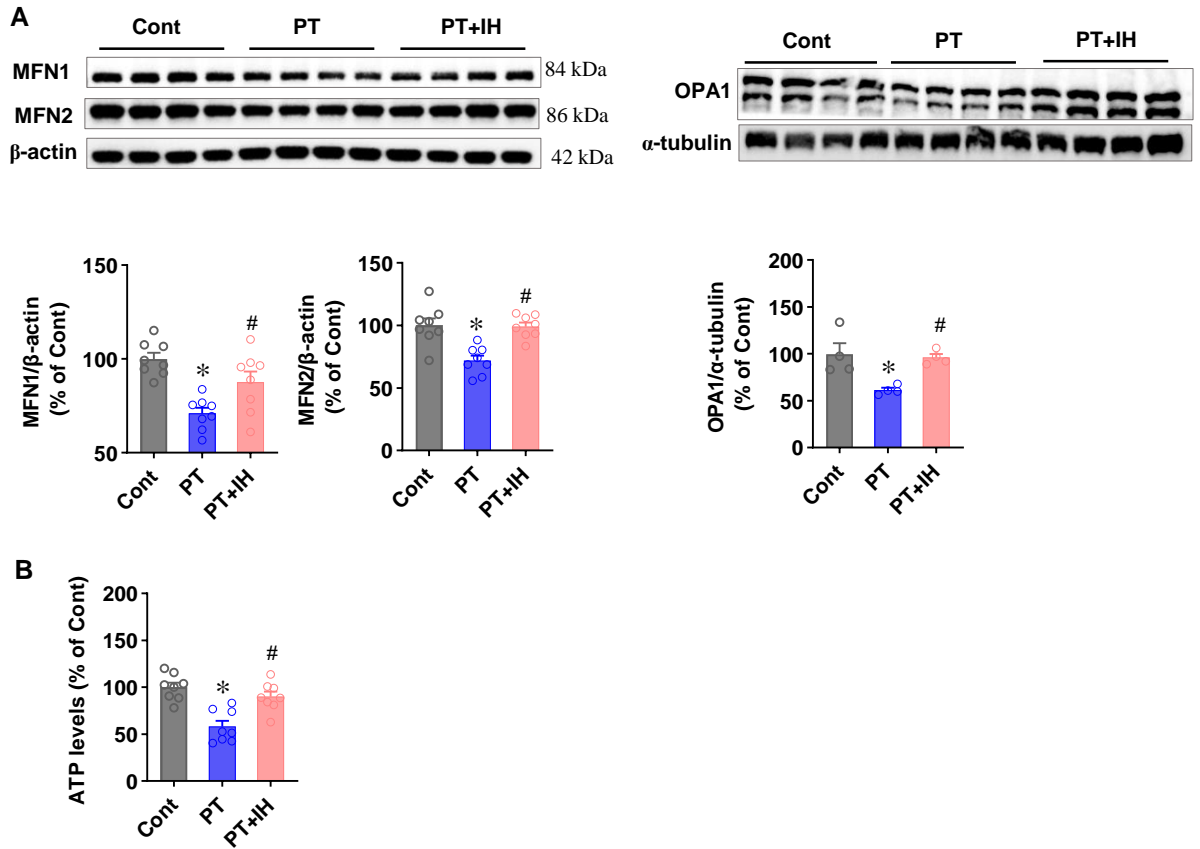


Fig. S3

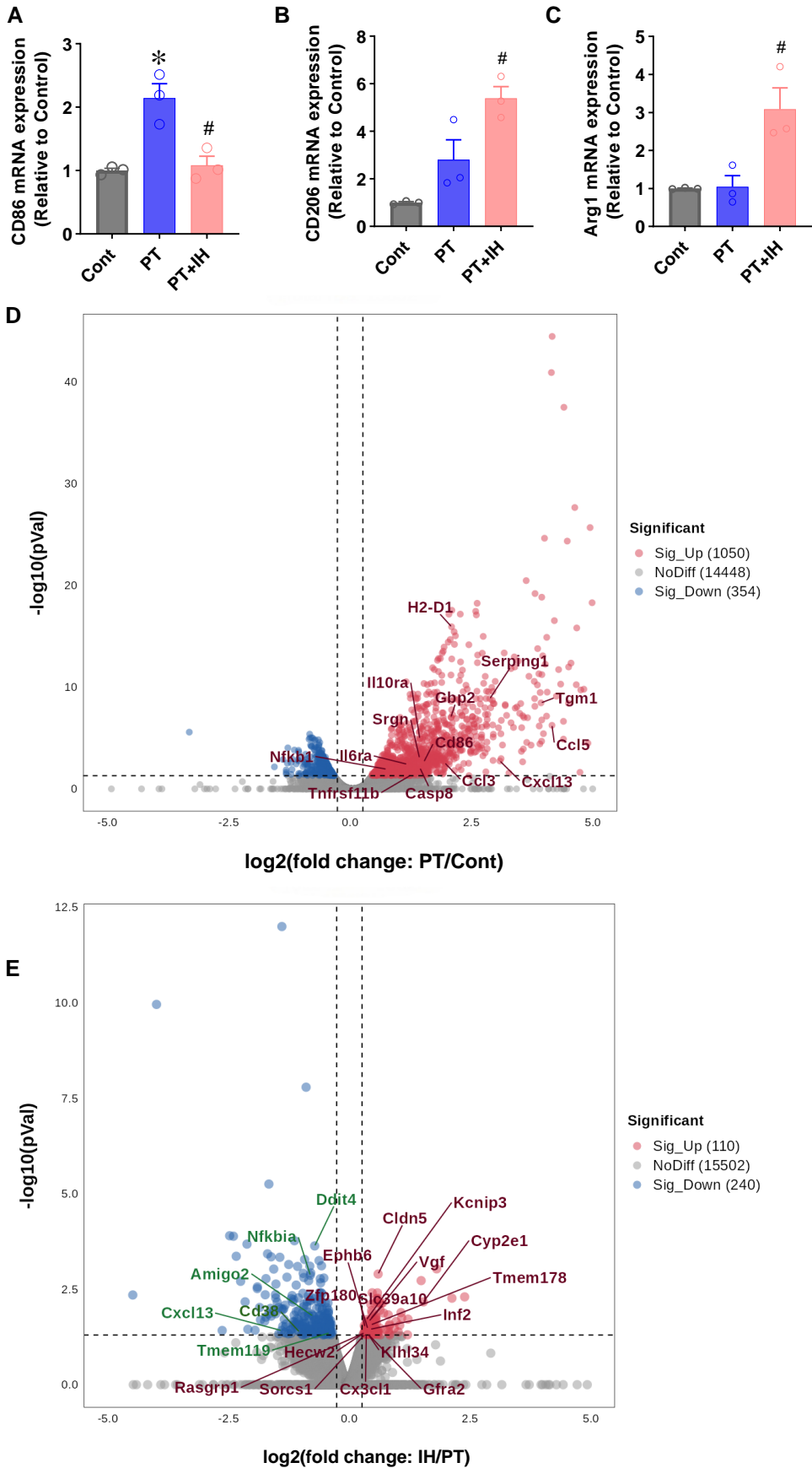
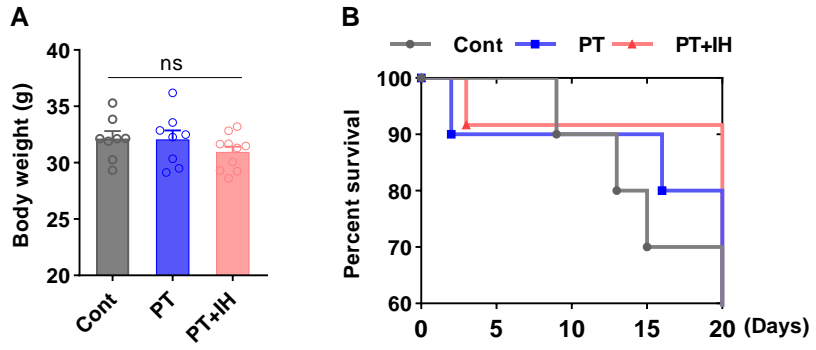


Fig. S4



Supplementary Table 1. Antibodies used in this study

| Antibody | Host | Catalog number | Vendor |
|-------------------|-------------|-----------------------|----------------------|
| NeuN | Rabbit | ET1602-12 | HUABIO |
| Cleaved-caspase 3 | Mouse | ARG66888 | Arigobio |
| MAP2 | Chicken | ab5392 | Abcam |
| MBP | Rabbit | ab218011 | Abcam |
| Synaptophysin | Mouse | S5768 | Sigma |
| Spinophilin | Rabbit | #14136 | CST |
| RECA1 | Mouse | MCA970R | Bio-Rad |
| GFAP | Chicken | ab4674 | Abcam |
| HIF-1 α | Rat | HA721997 | HUABIO |
| Tom20 | Rabbit | 11802 | Proteintech |
| Iba-1 | Rabbit | 019-19741 | FUJIFILM |
| CD86 | Mouse | ab213044 | Abcam |
| CD206 | Rabbit | HA722892 | HUABIO |
| S100A10 | Rabbit | PA5-120679 | Thermo Fisher |
| C3d | Goat | AF2655 | R&D Systems |
| BrdU | Mouse | MA3-071 | Thermo Fisher |
| DCX | Rabbit | 48-1200 | Thermo Fisher |
| Ki67 | Rabbit | ab16667 | Abcam |
| GBP2 | Rabbit | DF2499 | Affinity Biosciences |
| CLCF1 | Rabbit | ABIN4902021 | Antibodies-online |
| β -actin | Rabbit | 4967 | CST |
| MFN1 | Rabbit | 13798-1-AP | Proteintech |
| MFN2 | Rabbit | 12186-1-AP | Proteintech |
| OPA1 | Rabbit | 80471S | CST |
| α -Tubulin | Rabbit | 2144S | CST |

Supplementary Table 2. Primer sequences used for RT-qPCR.

| Gene | Forward primer (5'→3') | Reverse primer (5'→3') |
|--------------|-------------------------------|-------------------------------|
| 18S rRNA | CCCCTCGATGCTCTTAGCTG | GAACCGCGGTCCTATTCCAT |
| GAPDH | GCCTCCTCCAATTCAACCCTT | TGTCTACGGGACGAGGAAAC |
| CD86 | CTGGACTCTACGACTTCACAATG | AGTTGGCGATCACTGACAGTT |
| CD206 | CTCTG TTCAGCTATTGGACGC | TGGCACTCCCAAACATAATTTGA |
| Arg1 | TGGCTTGCGAGACGTAGAC | GCTCAGGTGAATCGGCCTTT |
| TNF α | CCTGTAGCCCACGTCGTAG | GGGAGTAGACAAGGTACAACCC |

Supplementary Table 3. Holm-Šídák multiple comparisons test results for all outcomes in aged PT stroke mice with IH treatment

| Outcome | Comparison | Effect Size (Mean Diff.) | 95% CI of Effect Size | Adjusted P Value |
|--|--------------|--------------------------|-----------------------|------------------|
| Infarct volume | PT vs.Cont | 98.28 | [76.57, 119.99] | <0.0001 |
| | PT+IH vs. PT | -58.93 | [-80.64, -37.22] | <0.0001 |
| Grip strength test | PT vs.Cont | -0.0108 | [-0.0174, -0.0042] | 0.0049 |
| | PT+IH vs. PT | 0.0122 | [0.0055, 0.0188] | 0.0024 |
| Beam Balance test (Score) | PT vs.Cont | -2.467 | [-3.154, -1.780] | <0.0001 |
| | PT+IH vs. PT | 2.121 | [1.449, 2.793] | <0.0001 |
| Beam Balance test (Average Speed) | PT vs.Cont | -0.0257 | [-0.0401, -0.0114] | 0.0021 |
| | PT+IH vs. PT | 0.0272 | [0.0136, 0.0409] | 0.0012 |
| Relative contralateral paw use (%) | PT vs.Cont | -29.03 | [-40.25, -17.81] | <0.0001 |
| | PT+IH vs. PT | 21.69 | [9.99, 33.39] | 0.0016 |
| Removal time (Sec) | PT vs.Cont | 107.7 | [66.56, 148.84] | <0.0001 |
| | PT+IH vs. PT | -84.86 | [-127, -42.72] | 0.0011 |
| No. of foot slips/total steps (% of cont) | PT vs.Cont | 399.5 | [324.37, 474.63] | <0.0001 |
| | PT+IH vs. PT | -342.6 | [-419.42, -265.78] | <0.0001 |
| Open field test Velocity (m/s) | PT vs.Cont | -0.0214 | [-0.0344, -0.0084] | 0.0046 |
| | PT+IH vs. PT | 0.026 | [0.0127, 0.0394] | 0.0013 |
| Open field test Line crossings | PT vs.Cont | -68.91 | [-109.68, -28.14] | 0.0041 |
| | PT+IH vs. PT | 71 | [30.23, 117.77] | 0.0041 |
| Cle-caspase 3 intensity (A.U.) | PT vs.Cont | 6.191 | [4.49, 7.90] | <0.0001 |
| | PT+IH vs. PT | -3.978 | [-5.68, -2.27] | 0.0002 |
| Cle-caspase 9 intensity (A.U.) | PT vs.Cont | 7.587 | [6.01, 9.16] | <0.0001 |
| | PT+IH vs. PT | -4.315 | [-5.89, -2.74] | <0.0001 |
| Surviving neurons/ROI | PT vs.Cont | -23.38 | [-31.05, -15.71] | <0.0001 |
| | PT+IH vs. PT | 11.88 | [4.21, 19.55] | 0.0081 |
| MAP2 intensity (% of Cont) | PT vs.Cont | -32.26 | [-48.06, -16.46] | 0.0011 |
| | PT+IH vs. PT | 26.39 | [10.59, 42.19] | 0.0045 |
| Area of continuous Structure (% of Cont) | PT vs.Cont | -26.46 | [-42.74, -10.18] | 0.0084 |
| | PT+IH vs. PT | 20.05 | [3.77, 36.33] | 0.036 |
| Number of small Particles (ROI) | PT vs.Cont | 1282 | [459.7, 2104.3] | 0.0117 |
| | PT+IH vs. PT | -960.8 | [-1781.1, -138.5] | 0.0479 |
| MBP intensity (% of Cont) | PT vs.Cont | -37.26 | [-52.53, -21.99] | 0.0002 |
| | PT+IH vs. PT | 17.36 | [2.09, 32.63] | 0.0278 |
| Dispersion (% of Cont) | PT vs.Cont | 25.66 | [10.36, 40.96] | 0.0067 |
| | PT+IH vs. PT | -19.15 | [-34.45, -3.85] | 0.0332 |
| SYP intensity (% of Cont) | PT vs.Cont | -38.56 | [-50.17, -26.95] | <0.0001 |
| | PT+IH vs. PT | 18.3 | [6.69, 29.91] | 0.0036 |
| Spin intensity (% of Cont) | PT vs.Cont | -28.5 | [-40.73, -16.27] | 0.0003 |
| | PT+IH vs. PT | 15.79 | [3.56, 28.02] | 0.0276 |
| Colocalized puncta | PT vs.Cont | -41.67 | [-58.01, -25.33] | <0.0001 |

| | | | | |
|--|--------------|----------|--------------------|---------|
| (% of Cont) | PT+IH vs. PT | 17.11 | [0.77, 33.45] | 0.041 |
| Vascular density (% area) | PT vs.Cont | -7.213 | [-8.91, -5.51] | <0.0001 |
| | PT+IH vs. PT | 3.613 | [1.91, 5.31] | 0.0005 |
| Vascular surface (μm^2) | PT vs.Cont | -33085 | [-38976, -27194] | <0.0001 |
| | PT+IH vs. PT | 22003 | [16112, 27894] | <0.0001 |
| Vascular length density (% area) | PT vs.Cont | -6.15 | [-8.69, -3.61] | 0.0002 |
| | PT+IH vs. PT | 2.675 | [0.13, 5.22] | 0.0399 |
| Capillary Vascular volume (% area) | PT vs.Cont | -4.825 | [-6.66, -2.99] | <0.0001 |
| | PT+IH vs. PT | 2.013 | [0.18, 3.85] | 0.0328 |
| Vascular astrocytes volume (% of Cont) | PT vs.Cont | 702.9 | [595.7, 810.1] | <0.0001 |
| | PT+IH vs. PT | -515.1 | [-622.3, -407.9] | <0.0001 |
| Vessel-associated astrocytes (% astrocytic volume) | PT vs.Cont | 9.492 | [6.92, 12.06] | <0.0001 |
| | PT+IH vs. PT | -4.725 | [-7.29, -2.16] | 0.0018 |
| HIF-1 α intensity (A.U.) (IF) | PT vs.Cont | 12.89 | [8.83, 16.95] | <0.0001 |
| | PT+IH vs. PT | 9.065 | [5, 13.13] | 0.0001 |
| Tom20 intensity (% of Cont) | PT vs.Cont | -33.4 | [-46.86, -19.94] | 0.0001 |
| | PT+IH vs. PT | 21.51 | [8.05, 34.97] | 0.0064 |
| Total fragmentation | PT vs.Cont | 0.5256 | [0.369, 0.682] | <0.0001 |
| | PT+IH vs. PT | -0.3044 | [-0.461, -0.148] | 0.0012 |
| Small fragmentation (<1.5 μm) | PT vs.Cont | 1.586 | [1.066, 2.106] | <0.0001 |
| | PT+IH vs. PT | -0.9520 | [-1.472, -0.432] | 0.0021 |
| Continuous fragmentation (2 μm to infinity) | PT vs.Cont | 0.13 | [0.0912, 0.1688] | <0.0001 |
| | PT+IH vs. PT | -0.07986 | [-0.1187, -0.0410] | 0.0007 |
| Microglial cell body diameter (μm) | PT vs.Cont | 6.071 | [4.61, 7.53] | <0.0001 |
| | PT+IH vs. PT | -3.828 | [-5.29, -2.37] | <0.0001 |
| Branch length (μm) | PT vs.Cont | -316.9 | [-385.6, -248.2] | <0.0001 |
| | PT+IH vs. PT | 179.7 | [111.0, 248.4] | <0.0001 |
| Number of branches /cell | PT vs.Cont | -28.38 | [-36.99, -19.77] | <0.0001 |
| | PT+IH vs. PT | 18.50 | [9.89, 27.11] | 0.0004 |
| GFAP intensity (A.U.) | PT vs.Cont | 29.18 | [22.34, 36.02] | <0.0001 |
| | PT+IH vs. PT | -20.66 | [-27.50, -13.82] | <0.0001 |
| Astrocyte volume/ROI (μm^3) | PT vs.Cont | 27095 | [19786, 34404] | <0.0001 |
| | PT+IH vs. PT | -16635 | [-23944, -9326] | 0.0002 |
| Iba-1 intensity (% of cont) | PT vs.Cont | 180.5 | [123.0, 238.0] | <0.0001 |
| | PT+IH vs. PT | -106.8 | [-164.3, -49.3] | 0.0018 |
| CD86 intensity (A.U.) | PT vs.Cont | 9.365 | [6.92, 11.81] | <0.0001 |
| | PT+IH vs. PT | -6.144 | [-8.59, -3.7] | <0.0001 |
| CD206 intensity | PT vs.Cont | 0.015 | [-0.775, 0.805] | 0.9689 |

| | | | | |
|---|--------------|---------|--------------------|---------|
| (A.U.) | PT+IH vs. PT | 4.788 | [3.998, 5.578] | <0.0001 |
| CD206/ β -actin (% of Cont) | PT vs.Cont | 18.04 | [-27.85, 63.93] | 0.539 |
| | PT+IH vs. PT | 107.4 | [61.47, 153.20] | 0.0003 |
| GBP2/ β -actin (% of Cont) | PT vs.Cont | 394.5 | [257.10, 531.90] | <0.0001 |
| | PT+IH vs. PT | -344.3 | [-481.70, -206.90] | 0.0002 |
| CLCF1/ β -actin (% of Cont) | PT vs.Cont | 91.54 | [29.43, 153.6] | 0.0066 |
| | PT+IH vs. PT | 134.5 | [72.44, 196.6] | 0.0005 |
| S100A10 intensity (A.U.) | PT vs.Cont | 1.212 | [-0.64, 3.06] | 0.1870 |
| | PT+IH vs. PT | 6.716 | [4.86, 8.57] | <0.0001 |
| C3d intensity (A.U.) | PT vs.Cont | 18.68 | [15.55, 21.81] | <0.0001 |
| | PT+IH vs. PT | -11.48 | [-14.61, -8.35] | <0.0001 |
| BrdU+NeuN+ Cells | PT vs.Cont | 3.000 | [1.66, 4.34] | 0.0011 |
| | PT+IH vs. PT | 6.000 | [3.68, 8.32] | 0.0010 |
| Ki67 ⁺ cells | PT vs.Cont | 2.0 | [0.72, 3.28] | 0.0039 |
| | PT+IH vs. PT | 5.5 | [4.22, 6.78] | <0.0001 |
| DCX ⁺ NeuN ⁺ Cells | PT vs.Cont | 1.750 | [0.12, 3.38] | 0.0364 |
| | PT+IH vs. PT | 7.250 | [5.62, 8.88] | <0.0001 |
| RECA1/Ki67 double labeled cells (ROI) | PT vs.Cont | 2.125 | [0.80, 3.45] | 0.0032 |
| | PT+IH vs. PT | 3.625 | [2.30, 4.95] | <0.0001 |
| ATP levels | PT vs.Cont | -41.76 | [-57.88, -25.64] | <0.0001 |
| | PT+IH vs. PT | 31.68 | [15.56, 47.80] | 0.0010 |
| Relative Evans blue content (A.U.) | PT vs.Cont | 0.3840 | [0.294, 0.474] | <0.0001 |
| | PT+IH vs. PT | -0.2069 | [-0.297, -0.117] | 0.0002 |
| MFN1/ β -actin (% of Cont) | PT vs.Cont | 29.07 | [16.08, 41.34] | 0.0002 |
| | PT+IH vs. PT | 16.66 | [4.39, 28.93] | 0.0203 |
| MFN2/ β -actin (% of Cont) | PT vs.Cont | -28.08 | [-40.85, -15.31] | 0.0005 |
| | PT+IH vs. PT | 27.22 | [14.45, 39.99] | 0.0005 |
| OPA1/ α -tubulin (% of Cont) | PT vs.Cont | -38.23 | [-61.60, -14.86] | 0.0147 |
| | PT+IH vs. PT | 34.74 | [11.37, 58.11] | 0.0167 |
| CD86 mRNA expression | PT vs.Cont | 1.143 | [0.601, 1.685] | 0.0063 |
| | PT+IH vs. PT | -1.062 | [-1.604, -0.520] | 0.0063 |
| CD206 mRNA expression | PT vs.Cont | 1.791 | [-0.183, 3.765] | 0.0682 |
| | PT+IH vs. PT | 2.593 | [0.619, 4.567] | 0.0362 |
| Arg1 mRNA expression | PT vs.Cont | 0.0421 | [-1.230, 1.314] | 0.9380 |
| | PT+IH vs. PT | 2.041 | [0.770, 3.312] | 0.0209 |
| Body weight (g) | PT vs.Cont | -0.0875 | [-2.07, 1.89] | 0.9270 |
| | PT+IH vs. PT | -1.108 | [-2.99, 0.77] | 0.4895 |

Supplementary Table 4. A priori power analysis for aged mouse stroke study

| Item | Value/Parameter | Notes/Calculation Basis |
|--|--|--|
| Software version | G*Power 3.1.9.7 | Gold-standard for statistical power analysis in biomedical research |
| Statistical test type | One-way fixed-effects omnibus ANOVA (between-subjects) | For 3-group comparison (Control/PT/PT+IH) |
| Effect size (f) | 0.80 (large) | Conservative choice accounting for high biological variability in aged mouse cohorts |
| Alpha level (α) | 0.05 (two-tailed) | International standard significance threshold for biomedical research |
| Statistical power ($1-\beta$) | 0.80 | International standard, ensuring 80% probability of detecting a true treatment effect |
| Number of groups (k) | 3 | Control, photothrombotic (PT) stroke, PT+intermittent hypoxia (IH) |
| Minimum sample size per group (biological replicates, n) | 8 mice | G*Power standard output for the above parameter combination |
| Total minimum sample size (N) | 24 mice | $k \times n = 3 \times 8$ |
| Noncentrality parameter (λ) | 15.3600 | $\lambda = N \times f^2 = 24 \times 0.80^2 = 15.36$ |
| Critical F value | 3.4668 | Derived from F-distribution table (df1=2, df2=21, $\alpha=0.05$) |
| Numerator degrees of freedom (df1) | 2 | $df1 = k-1 = 3-1$ |
| Denominator degrees of freedom (df2) | 21 | $df2 = N-k = 24-3$ |
| Actual sample size (behavioral assays) | 8-12 mice/group | Exceeds minimum to account for potential post-stroke attrition in aged mice |
| Actual sample size (histological/molecular analyses) | 8 mice/group | Matches the minimum required sample size (statistically sufficient) |
| Replication type definition | n = biological replicates (mice) | Technical replicates (brain sections/ROIs) averaged per mouse to avoid pseudoreplication |

Supplementary Table 5. GSEA of KEGG pathways in peri-infarct tissues

| Comparison | Pathway | NES | P-value | FDR q-value |
|-------------------|---|------------|----------------|--------------------|
| PT vs Cont | Apoptosis | 1.5780 | <0.0001 | 0.0118 |
| PT vs Cont | Ferroptosis | 1.6669 | <0.0001 | 0.0040 |
| PT vs Cont | Necroptosis | 1.6508 | <0.0001 | 0.0050 |
| PT vs Cont | NF- κ B signaling pathway | 1.5527 | <0.0001 | 0 |
| PT vs Cont | Toll-like receptor signaling pathway | 1.8462 | <0.0001 | 3.11E-04 |
| PT vs Cont | RIG-I-like receptor signaling pathway | 1.6710 | <0.0001 | 0.0040 |
| PT vs Cont | TGF- β signaling pathway | 1.6741 | <0.0001 | 0.0040 |
| PT vs Cont | JAK-STAT signaling pathway | 1.5855 | <0.0001 | 0 |
| PT vs Cont | Natural killer cell-mediated cytotoxicity | 1.5519 | <0.0001 | 0 |
| IH vs PT | Apoptosis | -1.6614 | <0.0001 | 0.0230 |
| IH vs PT | Ferroptosis | -1.6465 | 0.0090 | 0.0246 |
| IH vs PT | Necroptosis | -1.6369 | 0.0012 | 0.0248 |
| IH vs PT | NF- κ B signaling pathway | -1.6313 | 0.0012 | 0.0250 |
| IH vs PT | Toll-like receptor signaling pathway | -1.5671 | 0.0072 | 0.0426 |
| IH vs PT | RIG-I-like receptor signaling pathway | -1.3740 | 0.0733 | 0.1234 |
| IH vs PT | TGF- β signaling pathway | -1.6433 | <0.0001 | 0.0241 |
| IH vs PT | JAK-STAT signaling pathway | -1.7832 | <0.0001 | 0.0109 |
| IH vs PT | Natural killer cell-mediated cytotoxicity | -1.2305 | 0.1496 | 0.2670 |