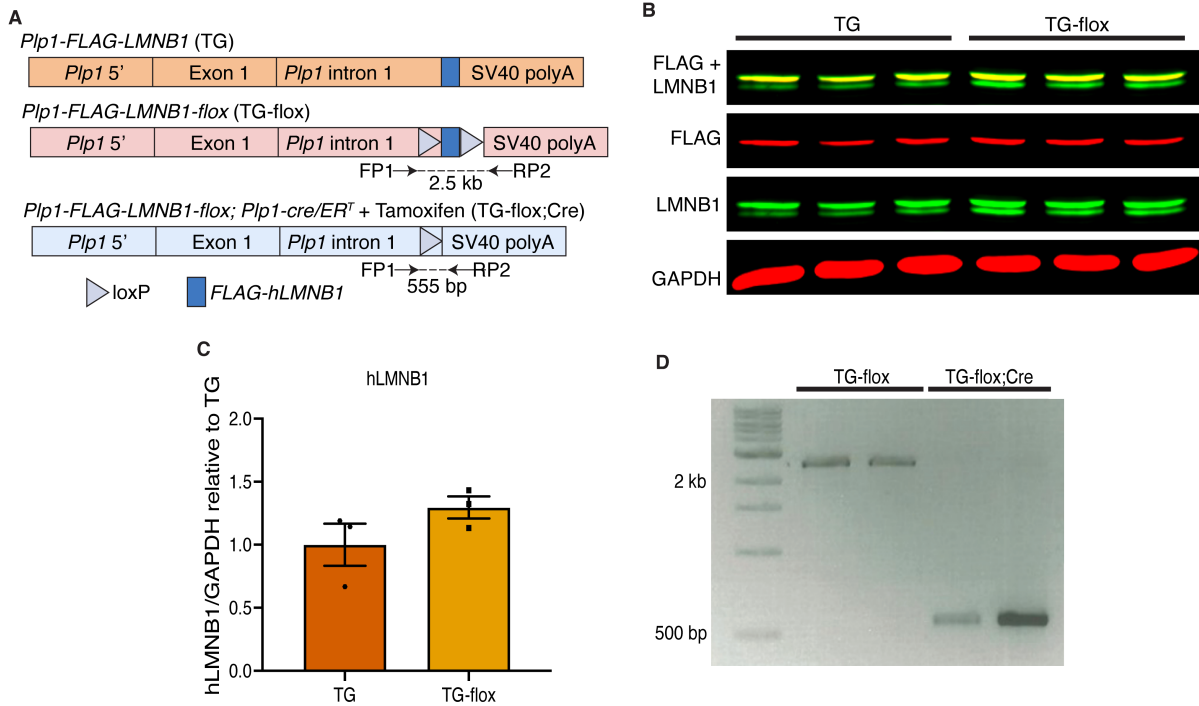
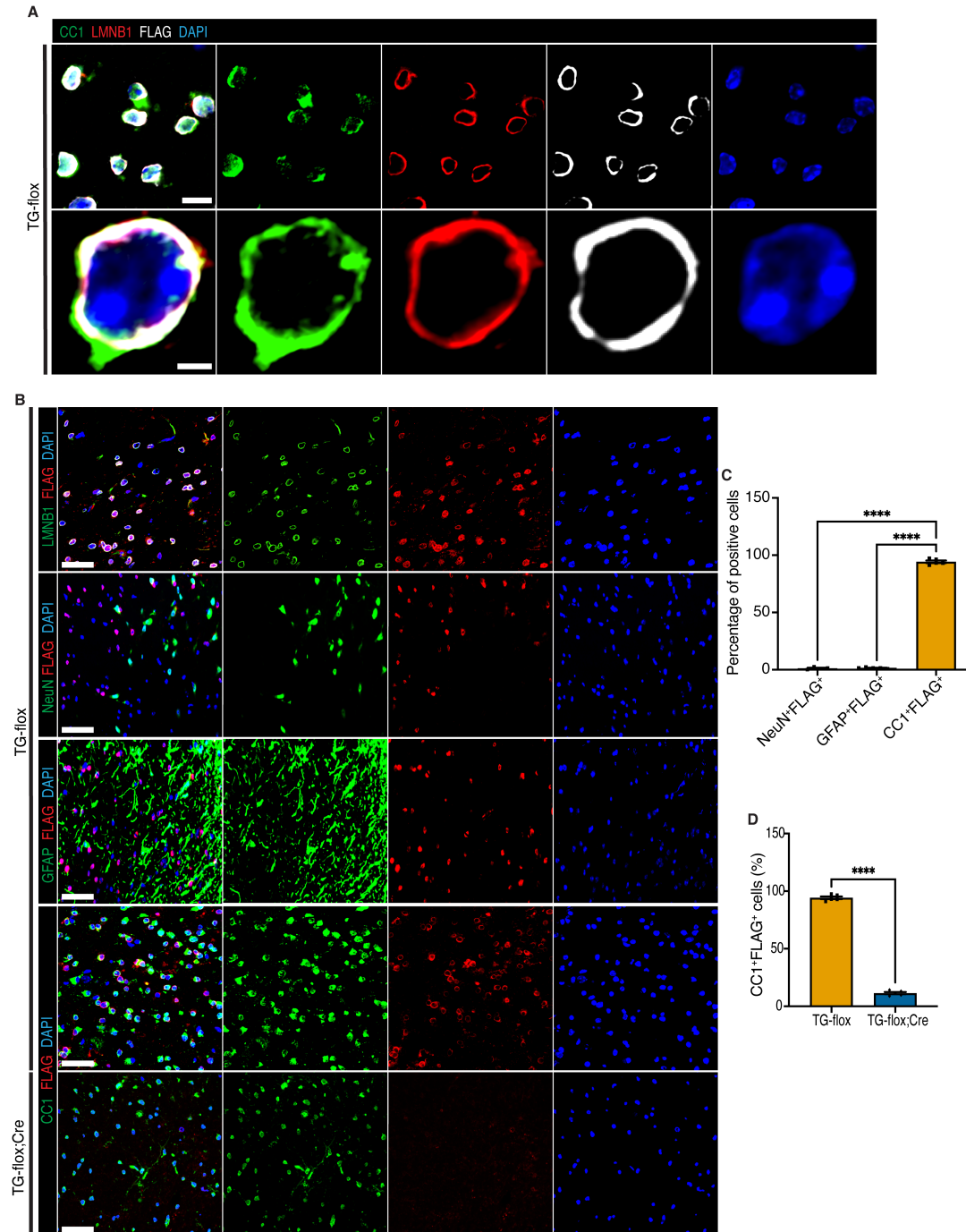




**Supplemental Figure 1: Protein expression between ADLD mouse lines is similar, and excision of hLMNB1 is confirmed by gDNA PCR, related to Figure 1.**

(A) Schematic of the *Plp1-FLAG-LMNB1* (TG) and *Plp1-FLAG-LMNB1-flox* cassette without (TG-flox) and with *Plp1-cre/ER<sup>T</sup>* (TG-flox;Cre) + tamoxifen (TAM) injections. Arrows indicate the binding sites of the forward (FP1) and reverse (RP2) primers, as well as the expected product sizes. (B-C) Representative western blot (B) and quantification (C) of ADLD mouse line spinal cord lysates probed for endogenous LMNB1, exogenous FLAG-tagged human LMNB1 (hLMNB1), and GAPDH. hLMNB1 migrates slightly higher than endogenous mouse LMNB1. hLMNB1 levels were normalized to GAPDH and further normalized to TG. No significant differences were found between TG and TG-flox.  $n = 3$  per group. (D) Spinal cord gDNA PCR using FP1 and RP2 from TAM-injected mice. (Statistical tests: B. Welch's t-test. Non-significant comparisons not shown.)



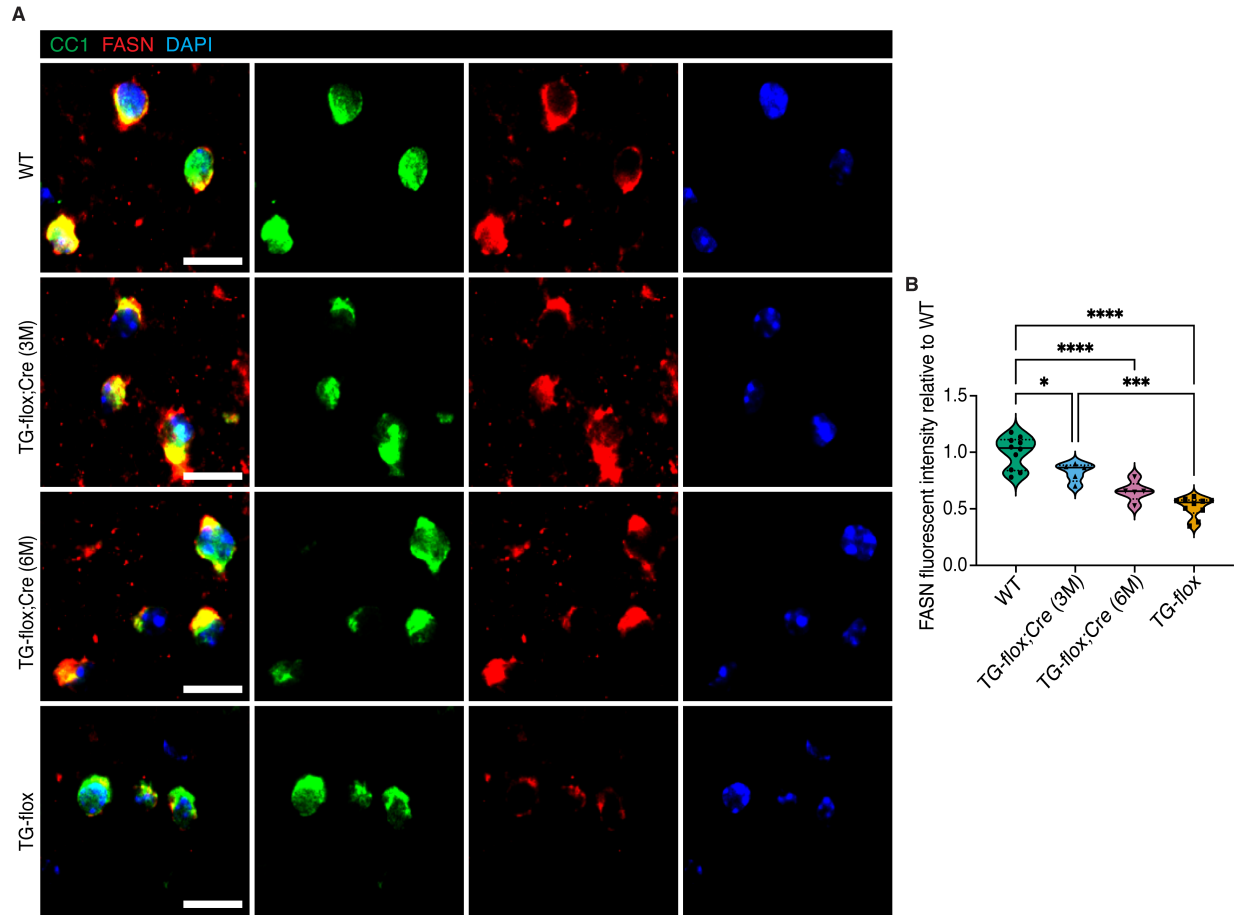
**Supplemental Figure 2: Validation of oligodendrocyte-specific expression of hLMNB1 and Cre-mediated ablation.**

(A) Representative Tg-flox spinal cord oligodendrocyte stained with CC1, LMNB1, FLAG, and DAPI. Scale bar = 10  $\mu$ m (top) and 2  $\mu$ m (bottom). (B) Representative Tg-flox or Tg-flox;Cre

spinal cord sections stained with LMNB1, NeuN, GFAP, or CC1 together with FLAG and DAPI. NeuN labels neurons, GFAP labels astrocytes, CC1 labels mature oligodendrocytes, and FLAG labels exogenous human LMNB1. Scale bar = 30  $\mu$ m. **(C)** Quantification of the percentage of NeuN<sup>+</sup>, GFAP<sup>+</sup>, or CC1<sup>+</sup> cells that were also FLAG<sup>+</sup> in Tg-flox mice.  $n \geq 4$  per group. **(D)** Quantification of the percentage of CC1<sup>+</sup> cells that were also FLAG<sup>+</sup> in Tg-flox and Tg-flox;Cre mice.  $n \geq 4$  per group. All mice were TAM-injected. Each point represents one independent field per mouse. (Statistical tests: **C.** Welch's ANOVA test with Dunnett's T3 post-hoc test; **D.** Welch's t-test. Data plotted as mean  $\pm$  SEM. \*\*\*\* $p < 0.0001$ , non-significant comparisons not shown.)



determination and visualization; C. Mixed effects test with Tukey's post-hoc test. Data plotted as mean  $\pm$  SEM. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ , non-significant comparisons not shown.)

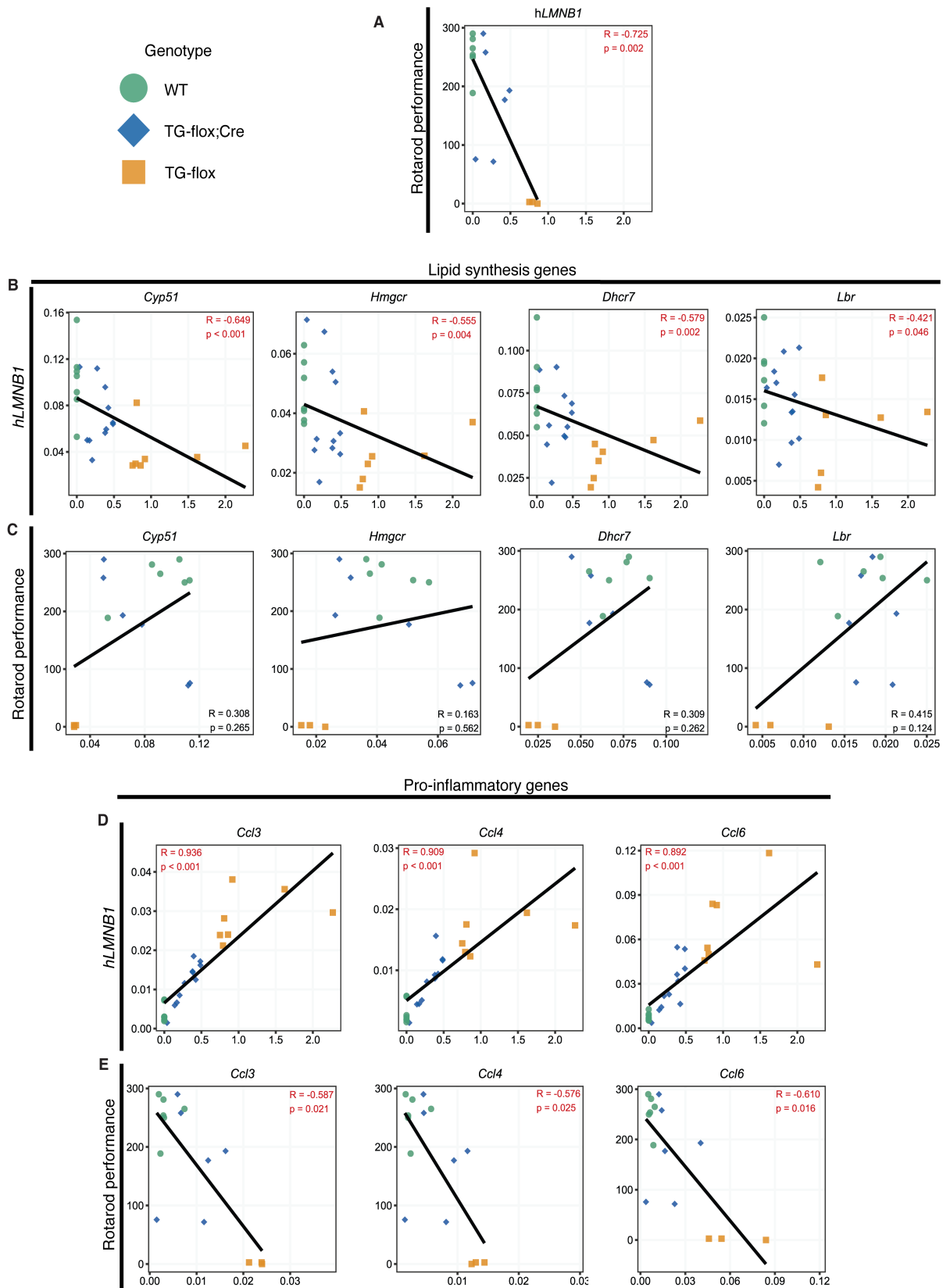


**Supplemental Figure 4: FASN intensity is reduced regardless of hLMNB1 reduction time point, related to Figure 6.**

(A) Representative spinal cord sections stained for CC1, FASN, and DAPI. CC1 labels mature oligodendrocytes, and FASN labels the fatty acid synthase protein. Scale bar = 20  $\mu$ m. (B)

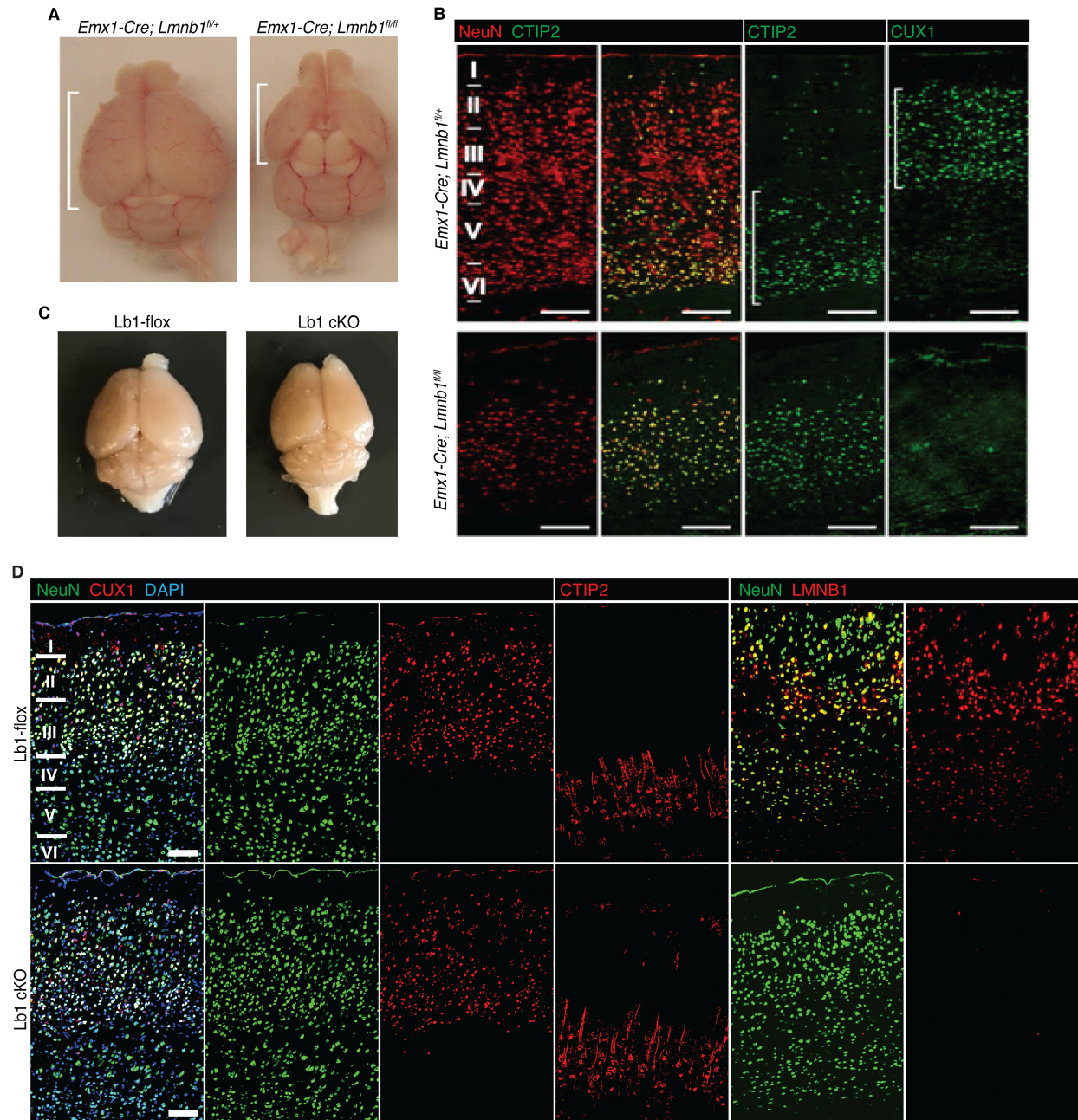
Quantification of the FASN fluorescence intensity normalized to Wt. Each point represents the average of at least 16 cells per sample.  $n \geq 5$  per group. (Statistical tests: B. one-way ANOVA test with Tukey's post-hoc test. Data plotted as mean  $\pm$  SEM.  $*p < 0.05$ ,  $***p < 0.001$ ,

$****p < 0.0001$ , non-significant comparisons not shown.)



**Supplemental Figure 5: Pro-inflammatory gene expression, but not lipid synthesis expression or neurodegeneration biomarkers, correlates with both *hLMNB1* levels and rotarod performance, related to Figure 6.**

(A) Correlation of *hLMNB1* mRNA expression with rotarod performance at 10 months of age.  $n = 15$  (WT:  $n = 6$ , TG-flox;Cre:  $n = 6$  [3M:6M = 6:0], TG-flox:  $n = 3$ ). (B-C) Correlation of lipid synthesis genes (*Cyp51*, *Hmgcr*, *Dhcr7*, and *Lbr*) with *hLMNB1* expression (B) or rotarod performance at 10 months of age (C).  $n = 25$  (WT:  $n = 7$ , TG-flox;Cre:  $n = 11$  [3M:6M = 6:5], TG-flox:  $n = 7$ ) (B),  $n = 15$  (WT:  $n = 6$ , TG-flox;Cre:  $n = 6$  [3M:6M = 6:0], TG-flox:  $n = 3$ ) (C). (D-E) Correlation of pro-inflammatory genes (*Ccl3*, *Ccl4*, *Ccl6*) with *hLMNB1* expression (D) or rotarod performance at 10 months of age (E).  $n = 25$  (WT:  $n = 7$ , TG-flox;Cre:  $n = 11$  [3M:6M = 6:5], TG-flox:  $n = 7$ ) (D),  $n = 15$  (WT:  $n = 6$ , TG-flox;Cre:  $n = 6$  [3M:6M = 6:0], TG-flox:  $n = 3$ ) (E). Solid lines indicate best-fit lines for visualization. Spearman's rank correlation coefficients ( $R$ ), and p-values are shown within each panel. Red comparisons indicate statistical significance.



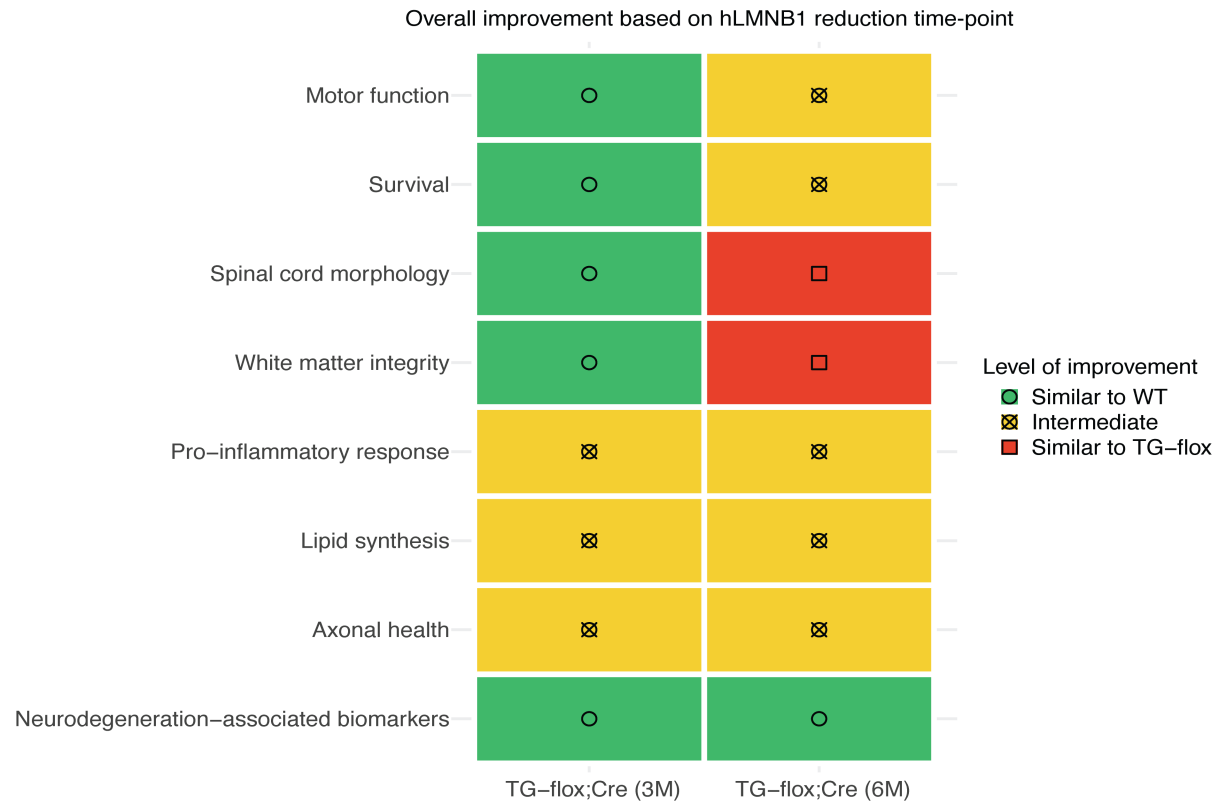
**Supplemental Figure 6: Embryonic forebrain-specific knockout of *Lmnbl1* results in decreased forebrain size and abnormal cortical neuron layering, related to Figure 8.**

**Adapted from PMID: 21976703.**

Panels **A** and **B** are adapted from previously published work using an *Emx1*-Cre mediated embryonic, forebrain-specific *Lmnbl1* knockout (PMID: 21976703). Panels **C-E** show data

generated in this study using an inducible, tissue-wide UBC-Cre-mediated *Lmnbl* knockout in adulthood. Panels C–E are reproduced from our Fig. 8J–K to facilitate direct comparison with the embryonic, forebrain-specific *Lmnbl* knockout.

(A) Representative images of a 1-month-old *Emx1-Cre* mouse forebrain, with the forebrain region indicated by brackets. (B) Representative cortical sections from 1-month-old *Emx1-Cre* mice stained with NeuN, CUX1, CTIP2, and DAPI. NeuN labels neurons, CUX1 labels layers II to upper IV, and CTIP2 labels lower IV to VI. Cortical layers are labeled with Roman numerals. Scale bar = 200  $\mu\text{m}$ . (C) Representative forebrain images from 12-month-old UBC-Cre mice. (D) Representative cortical sections stained with a combination of NeuN, CUX1, DAPI, CTIP2, or LMNB1 antibodies. Cortical neuron layers are labeled by roman numerals in Lb1-flox images. Scale bar = 50  $\mu\text{m}$ .



### Supplemental Figure 7: Early reduction of hLMNB1 leads to greater overall improvement compared to late reduction

Heatmap showing the level of improvement in TG-flox;Cre (3M) and TG-flox;Cre (6M) across assessed categories. “Similar to WT” or “Similar to TG-flox” indicates that the cohort was indistinguishable from them, while 'intermediate' indicates partial improvement over TG-flox or mixed results across measures.

**Supplemental Table 1: Functional observation battery (FOB) scoring criteria.**

| Assessment Criteria |                                  | 0                       | 1                          | 2                                 |
|---------------------|----------------------------------|-------------------------|----------------------------|-----------------------------------|
| Observation         | In cage motor activity/frequency | Moves frequently        | Limited mobility           | Immobile, even upon touch         |
|                     | Gait                             | Normal                  | Mild ataxia                | Severe ataxia or paralysis        |
|                     | Condition of fur                 | Smooth, shiny coat      | Ungroomed or patchy fur    | Matted fur, piloerection          |
|                     | Posture/body tone                | Normal, relaxed posture | Mild to moderate hunching  | Severe hunching                   |
|                     | Seizures                         | None observed           | Seizures upon handling     | Continuous seizures               |
|                     | Tremors                          | None observed           | Mild tremors               | Continuous tremors                |
|                     | Eyelids/ptosis                   | Normal                  | Partial ptosis             | Complete ptosis                   |
| Handling            | Tail flip test                   | Cannot flip mouse       | Flips, but reverts upright | Flips, but cannot regain position |
|                     | Tail pinch                       | Immediate response      | Delayed response           | No response                       |
|                     | Grip test                        | Strong grip on mesh     | Weak grip                  | No response                       |

**Supplemental Table 2: Sequences of oligonucleotides used in the study.**

| <b>Genotyping primers</b>         |                                         |                                         |
|-----------------------------------|-----------------------------------------|-----------------------------------------|
|                                   | <b>Primer sequences</b>                 | <b>Expected sizes</b>                   |
| <i>Plp-FLAG-LMNB1</i> -flox       | Forward: TAACATGGGCTGCATAACTTCG         | Transgenic: 550 bp                      |
|                                   | Reverse: TCTTAGCATAGTTGAGGAGCAGC        | Wild-type: 324 bp                       |
| <i>Plp1</i> -cre/ERT <sup>1</sup> | Forward: AGGTGGACCTGATCATGGAG           | Transgenic: 440 bp                      |
|                                   | Reverse: ATACCGGAGATCATGCAAGC           | Wild-type: 324 bp                       |
| UBC-Cre-ERT <sup>2</sup>          | Forward: AGGCGTTTTCTGAGCATACCTG         | Transgenic: 478 bp                      |
|                                   | Reverse: AACATTCTCCACCGTCAGTAC          | Wild-type: 324 bp                       |
| <i>Lmnbl</i> -flox                | Flox Forward: AACAACTTGGCCTCACCAG       | Transgenic: 450 bp<br>Wild-type: 300 bp |
|                                   | Wild-type Forward: CCACTTAGCTCGGGGAGTTT |                                         |
|                                   | Common Reverse: CTGTGGGACAAAGACCCAGT    |                                         |
| <i>Gapdh</i> (internal control)   | Forward: GGCCTGTTTAAGGGAAAGAAGT         | -                                       |
|                                   | Reverse: CCCCTTCATTGACCTCAACTAC         |                                         |

\* *Gapdh* was used with *Plp-FLAG-LMNB1*-flox, *PLP/creERT*, and *UBC-Cre-ERT2* primers as an internal control for genotyping

| <b>Knockout gDNA primers</b> |                                                 |                       |
|------------------------------|-------------------------------------------------|-----------------------|
|                              | <b>Primer sequences</b>                         | <b>Expected sizes</b> |
| <i>hLMNB1</i> knockout       | Forward Primer 1 (FP1): CCTTTACTTTTCCCATGGTCTCC | Intact: 2542 bp       |
|                              | Reverse Primer 2 (RP2): TTCCGAAGCCCAACCTTTCATA  | Knockout: 555 bp      |

| <b>mRNA expression qRT-PCR primers</b> |                             |
|----------------------------------------|-----------------------------|
|                                        | <b>Primer sequences</b>     |
| Mouse <i>β-Actin</i>                   | CCACTGCCGCATCCTCTTCC        |
|                                        | CTCGTTGCCAATAGTGATGACCTG    |
| Mouse <i>Lmnbl</i>                     | GCTGCTCAATTATGCCAAGAAG      |
|                                        | GCCGCATCCTTAGAGTTTAGT       |
| Human <i>LMNB1</i>                     | GATTGCCAGTTGGAAGCCT         |
|                                        | TGGTCTCGTTAATCTCCTCTTCATACA |
| Mouse <i>Cyp51</i>                     | GGCCTTAACATAGCCCACTT        |
|                                        | CACATTTCTTTCTCCGCTTCTC      |
| Mouse <i>Hmgcr</i>                     | CCAGAAGCTTTCGTCAGTAGAG      |
|                                        | CTCTGCTTGTAGTCTCTGCTTC      |
| Mouse <i>Dhcr7</i>                     | CCTGACTTCTGCCACAGATT        |
|                                        | CAGCCCATTACCTCATACTT        |
| Mouse <i>Lbr</i>                       | CCGAACACATGGAGAAGAA         |
|                                        | CGAAGGCTGTACTGTGTAAGT       |
| Mouse <i>Ccl3</i>                      | GAAGATTCCACGCCAATTCATC      |
|                                        | GATCTGCCGGTTTCTCTTAGTC      |
| Mouse <i>Ccl4</i>                      | CCACTTCCTGCTGTTTCTCTTA      |
|                                        | CAAAGACTGCTGGTCTCATAGT      |
| Mouse <i>Ccl6</i>                      | CGTCGCTATAACCCTCCAATAA      |
|                                        | TGGGATCTGTGTGGCATAAG        |

**Supplemental Table 3: List of antibodies used in the study.**

| <b>Western blot antibodies</b> |                         |                |                 |                     |                  |
|--------------------------------|-------------------------|----------------|-----------------|---------------------|------------------|
|                                | <b>Antibody</b>         | <b>Species</b> | <b>Dilution</b> | <b>Manufacturer</b> | <b>Catalog #</b> |
| Primary                        | LMNB1                   | Rabbit         | 1:1,500         | Abcam               | ab16048          |
|                                | FLAG                    | Mouse          | 1:1,500         | Sigma-Aldrich       | F1804            |
|                                | $\beta$ -ACTIN          | Mouse          | 1:3,000         | ThermoFisher        | MA5-15739        |
|                                | GAPDH                   | Mouse          | 1:5,000         | ThermoFisher        | MA5-15738        |
|                                | Anti-Mouse IRDye 680LT  | Goat           | 1:10,000        | LI-COR Biosciences  | 926-68020        |
| Secondary                      | Anti-Rabbit IRDye 800CW | Donkey         | 1:10,000        | LI-COR Biosciences  | 926-32213        |

| <b>Immunofluorescence antibodies</b> |                   |                |                 |                     |                  |
|--------------------------------------|-------------------|----------------|-----------------|---------------------|------------------|
|                                      | <b>Antibody</b>   | <b>Species</b> | <b>Dilution</b> | <b>Manufacturer</b> | <b>Catalog #</b> |
| Primary                              | LMNB1             | Rabbit         | 1:500           | Abcam               | ab16048          |
|                                      | FLAG              | Mouse          | 1:100           | Sigma-Aldrich       | F1804            |
|                                      | FLAG              | Rabbit         | 1:200           | Sigma-Aldrich       | F7425            |
|                                      | NeuN              | Mouse          | 1:100           | Millipore           | MAB377           |
|                                      | GFAP              | Mouse          | 1:250           | Millipore           | MAB360           |
|                                      | MBP               | Chicken        | 1:500           | EnCor               | CPCA-MBP         |
|                                      | PLP               | Rat            | 1:100           | Millipore           | MABN2620         |
|                                      | CC-1 (Anti-APC)   | Mouse          | 1:100           | Abcam               | AB16794-1002     |
|                                      | APP               | Rabbit         | 1:100           | Invitrogen          | 512700           |
|                                      | IBA1              | Rabbit         | 1:250           | Wako Chem           | 019-19741        |
|                                      | FASN              | Rabbit         | 1:200           | Abcam               | ab22759          |
|                                      | CUX1              | Rabbit         | 1:500           | ProteinTech         | 11733-1-AP       |
|                                      | CTIP2 (BCL11B)    | Rabbit         | 1:200           | ProteinTech         | 55414-1-AP       |
|                                      | Anti-Chicken FITC | Donkey         | 1:350           | Jackson IR          | 703-095-155      |
|                                      | Anti-Rat Cy3      | Donkey         | 1:350           | Jackson IR          | 712-165-150      |
| Secondary                            | Anti-Mouse FITC   | Donkey         | 1:350           | Jackson IR          | 715-096-150      |
|                                      | Anti-Mouse Cy3    | Donkey         | 1:350           | Jackson IR          | 715-165-150      |
|                                      | Anti-Rabbit Cy3   | Donkey         | 1:350           | Jackson IR          | 711-165-152      |
|                                      | Anti-Rabbit FITC  | Donkey         | 1:350           | Jackson IR          | 711-095-152      |