Supplement Figures

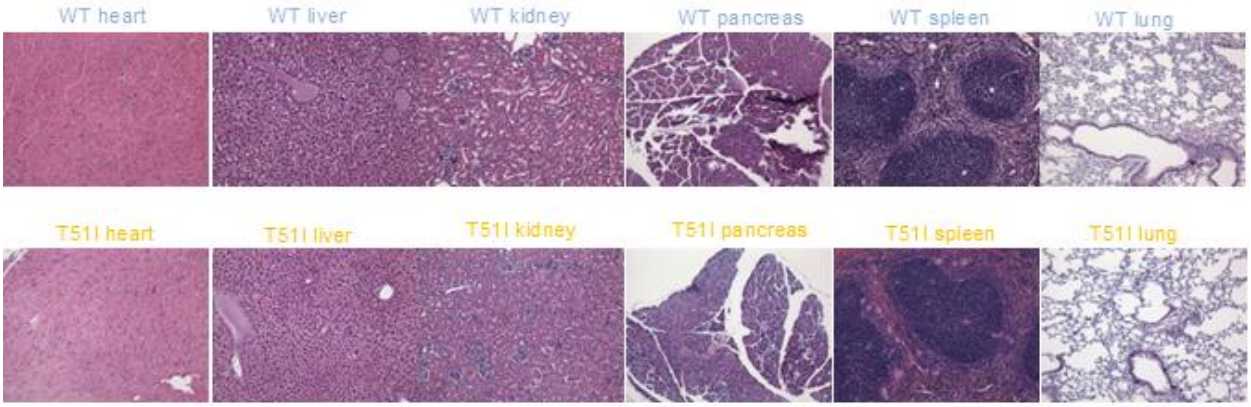


Figure S1. Histology analysis in peripheral organs of female *SptssaT51I/+* mice.

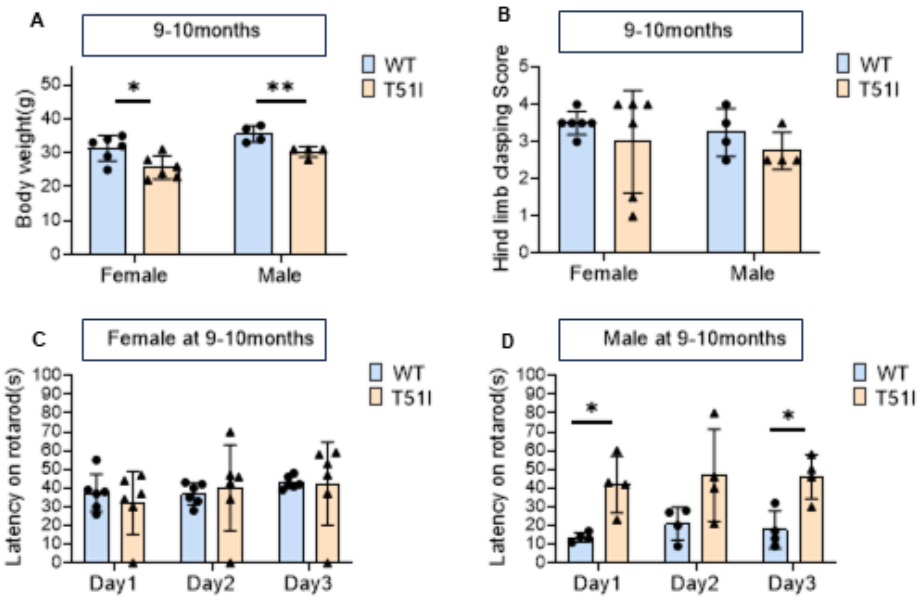


Figure S2. Body weight and behavioral test in aged *SptssaT51I/+* mice. Body weight(**A**) hind limb reflex extension score(**B**) in aged (9-10months) *SptssaT51I/+* mice compared to control(n=4-6). Rotarod test in female(**C**) and male(**D**) *SptssaT51I/+* mice at 9-10month of age(n=4-6). *p<0.05 and **p<0.01 as compared between indicated groups.

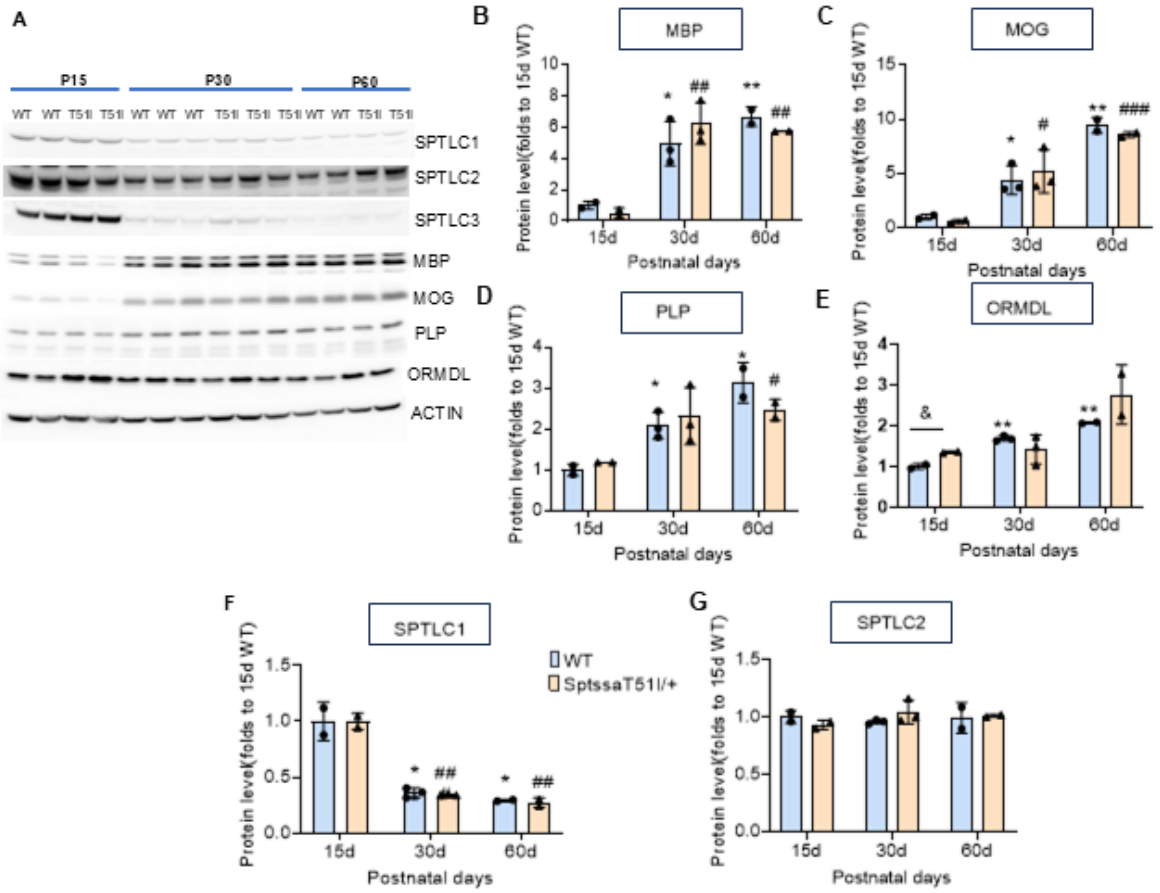


Figure S3. Expression myelin-related protein and SPT complex during postnatal development in male mouse brain. (**A**) Representative western blot images showing postnatal expression of myelin-related proteins and SPT complex at postnatal day 15 (15d), day 30 (30d) and day 60 (60d) in male mice. Protein quantification of myelin-related proteins including MBP (**B**), MOG (**C**), PLP **(D**) in postnatal mouse brain of male WT and *SptssaT51I/+* mice (n=2-3 samples per group). ORMDL(**E**) as well as SPT complex subunits including SPTLC1(**F**), SPTLC2 (**G**) protein quantification in brain of postnatal male WT and *SptssaT51I/+* mice (n=2-3 samples per group). *, # $p<0.05$, **,##$p<0.01$, ***, ###$p<0.001$ as compared to WT at 15d and *SptssaT51I/+* at 15d respectively. & $p<0.05$ as compared between indicated groups.

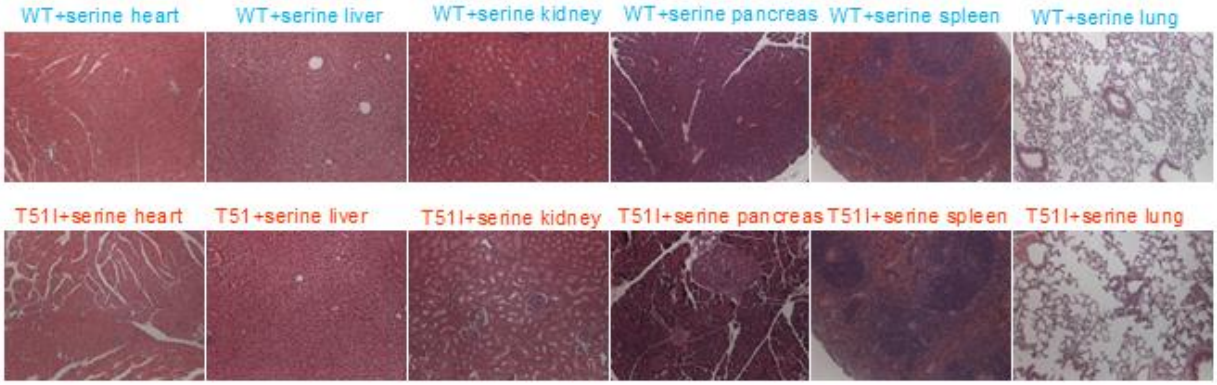


Figure S4. Histology analysis in peripheral organs of female *SptssaT51I/+* mice after 10% serine treatment for 3months, initiated at 2-3 months of age.

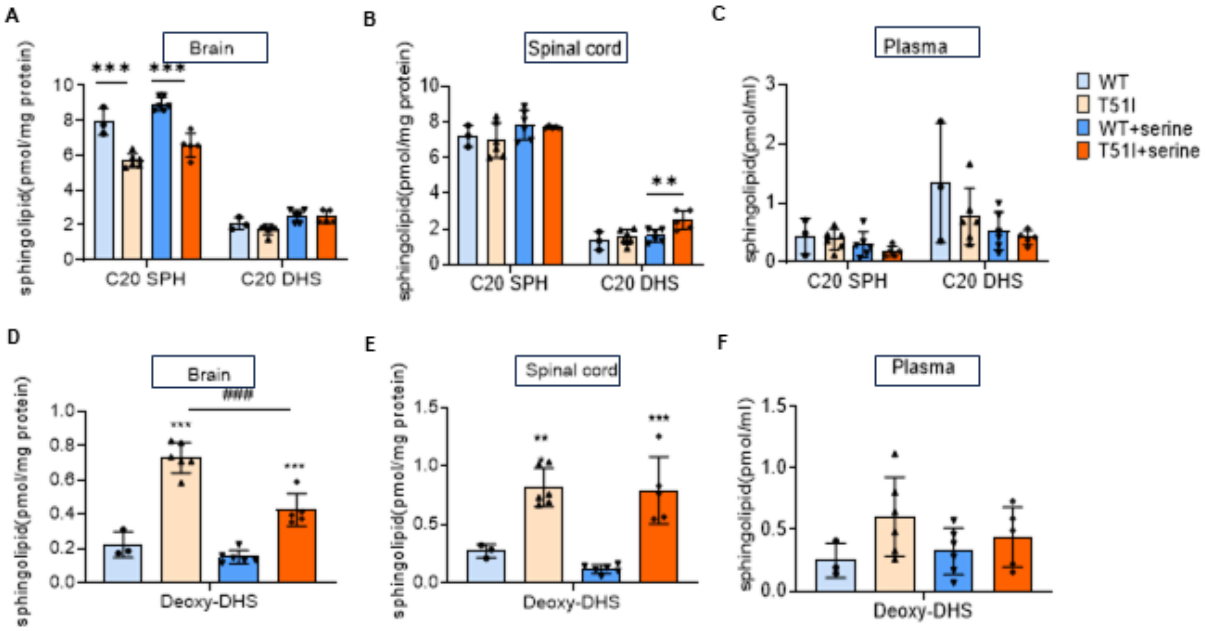


Figure S5. D20 sphingolipids (SLs) in brain(**A**), spinal cord(**B**) and plasma(**C**) of *SptssaT51/+* mice with and without 3 months of 10% serine supplementation, initiated at 2-3 months of age(n=3-6). Deoxy-SLs in brain(**D**), spinal cord(**E**) and plasma(**F**) of *SptssaT51/+* mice with and without 3 months of 10% serine supplementation, initiated at 2-3 months of age(n=3-6). Results were expressed as means ± SD., **p<0.01,***p<0.001 as compared between indicated groups.

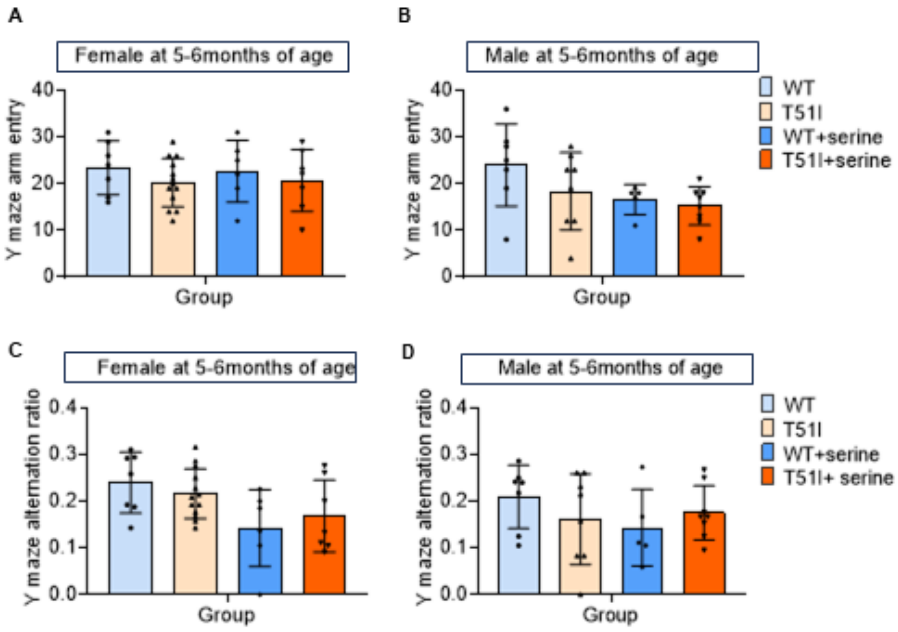


Figure S6. Cognitive behavioral test in *SptssaT51I/+* mice with and without 3 months of 10% serine supplementation, initiated at 2-3 months of age. Arm entry of female(**A**) and male mice(**B**) as well as alternation analysis in female(**C**) (n=6-13) and male(**D**) (n=5-8) were quantified. Results were expressed as means ± SD.

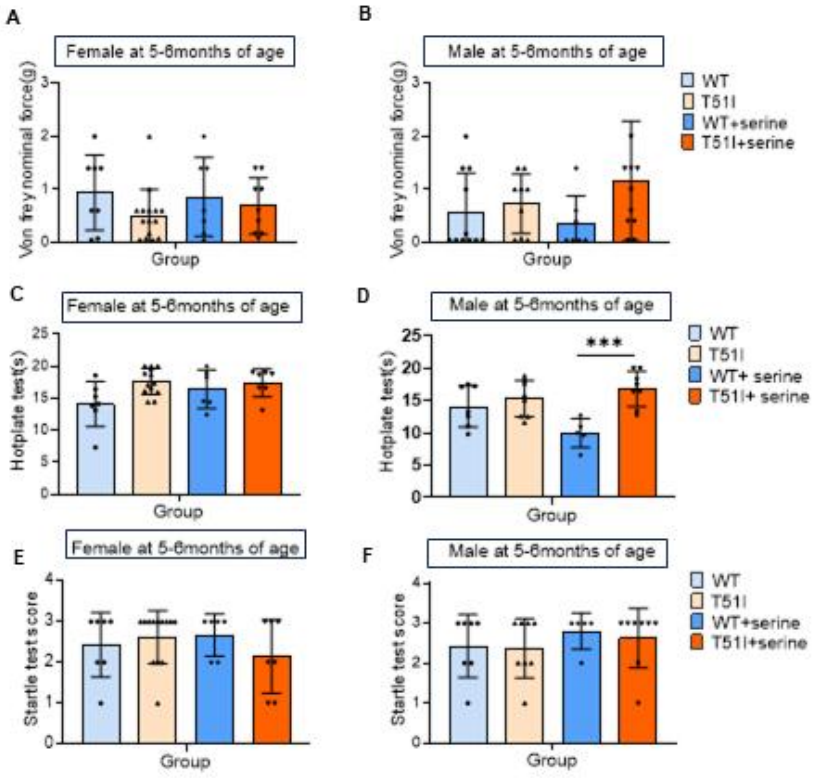


Figure S7. Sensory behavioral test by von frey in *SptssaT51I/+* mice with and without 3 months of 10% serine supplementation, initiated at 2-3 months of age, in female (**A**)(n=7-15) and male(**B**)(n=7-11), hot plate in female(n=6-13) (**C**) and male(**D**) (n=5-8) as well as startle response assay in female (**E**)(n=6-13) and male (**F**)(n=5-8) mice. Results were expressed as means ± SD. ***p<0.001 as compared between indicated groups.

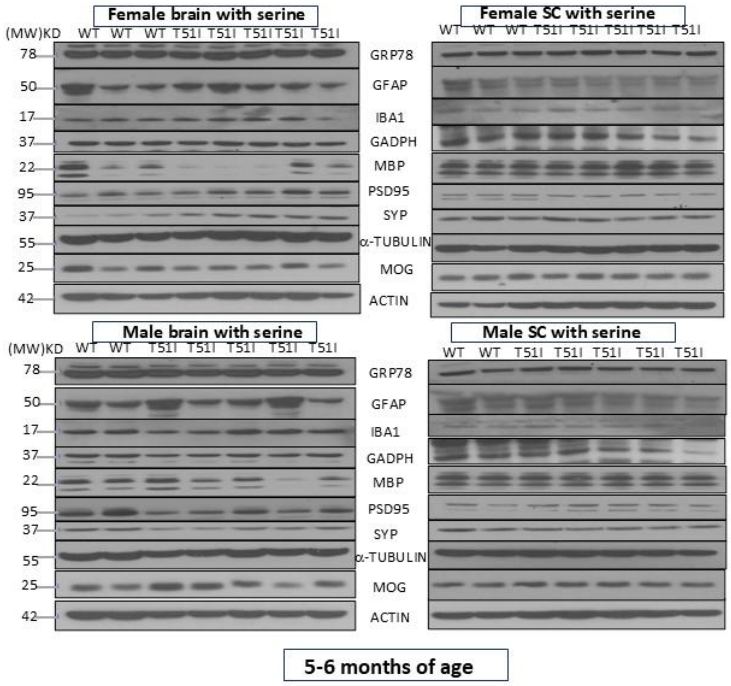


Figure S8. Representative western blot images showing cell marker expression in brain and spinal cord of WT and *SptssaT51/+* mice with 3 months of 10% serine supplementation, initiated at 2-3 months of age.

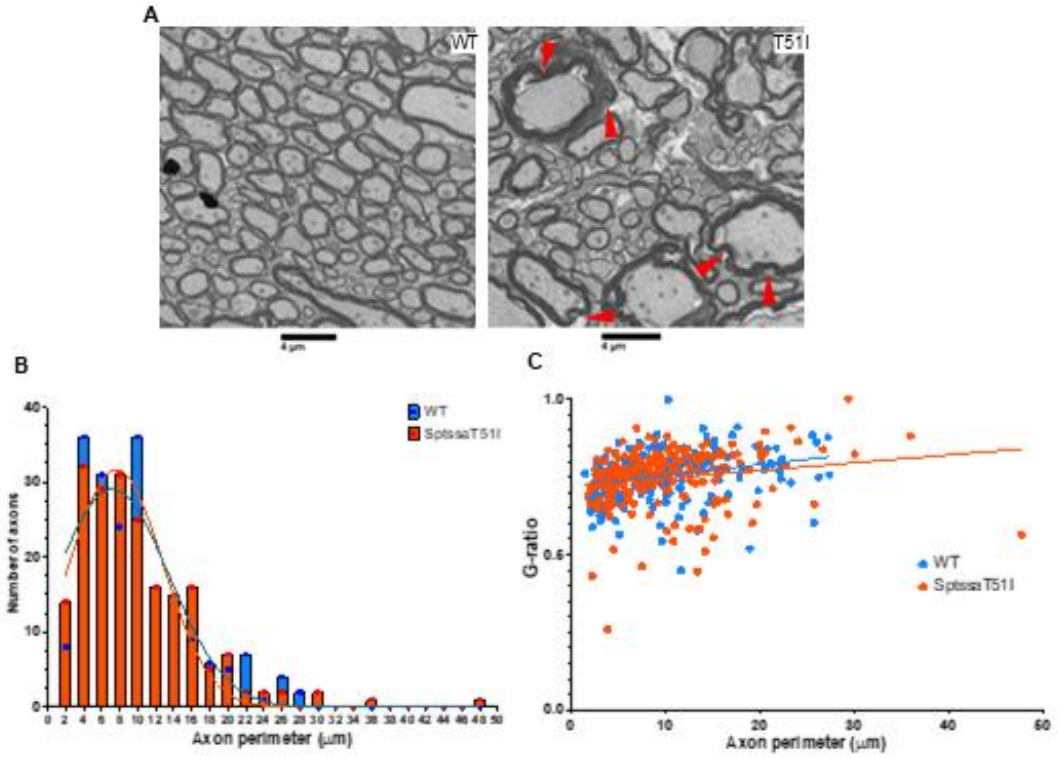


Figure S9. Myelin abnormalities in spinal cord (corticospinal tract) of *SptssaT51/+* mice after 3 months of 10% serine supplementation, initiated at 2-3 months of age. (**A**) Representative EM images show myelin abnormalities including myelin folding and redundancy (red arrows) in spinal cord. (**B**) Histogram of axon perimeter reveals a wider and flatter distribution of axon sizes in spinal cord of *SptssaT51/+* mice. (**C**) G-ratio decreases in large axons in *SptssaT51/+* mice compared to WT, suggesting hypermyelination.

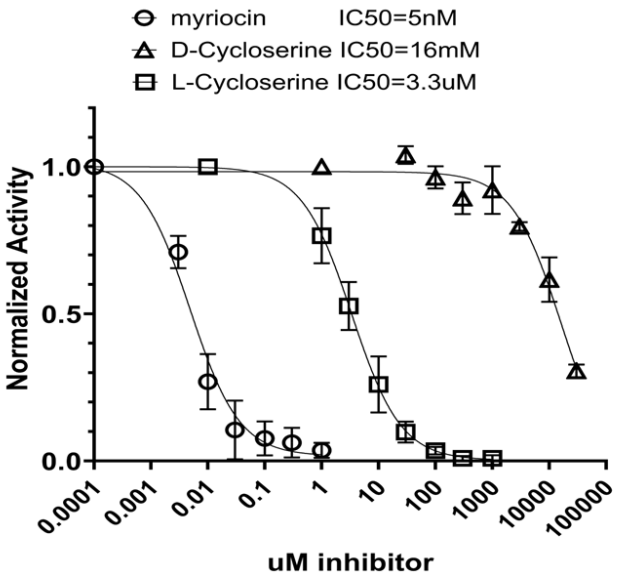


Figure S10 Inhibition of SPT by myriocin, L-cycloserine and D-cycloserine. Purified SPT was incubated with the indicated concentration of inhibitor and activity was measured as described in Materials and Methods. The IC_{50}s, calculated using Prism, were myriocin [○], 5 nM, L-cycloserine [□], 3.3 μM and D-cycloserine [Δ], 16 mM. (n=3-6). Results were expressed as means ± SD.

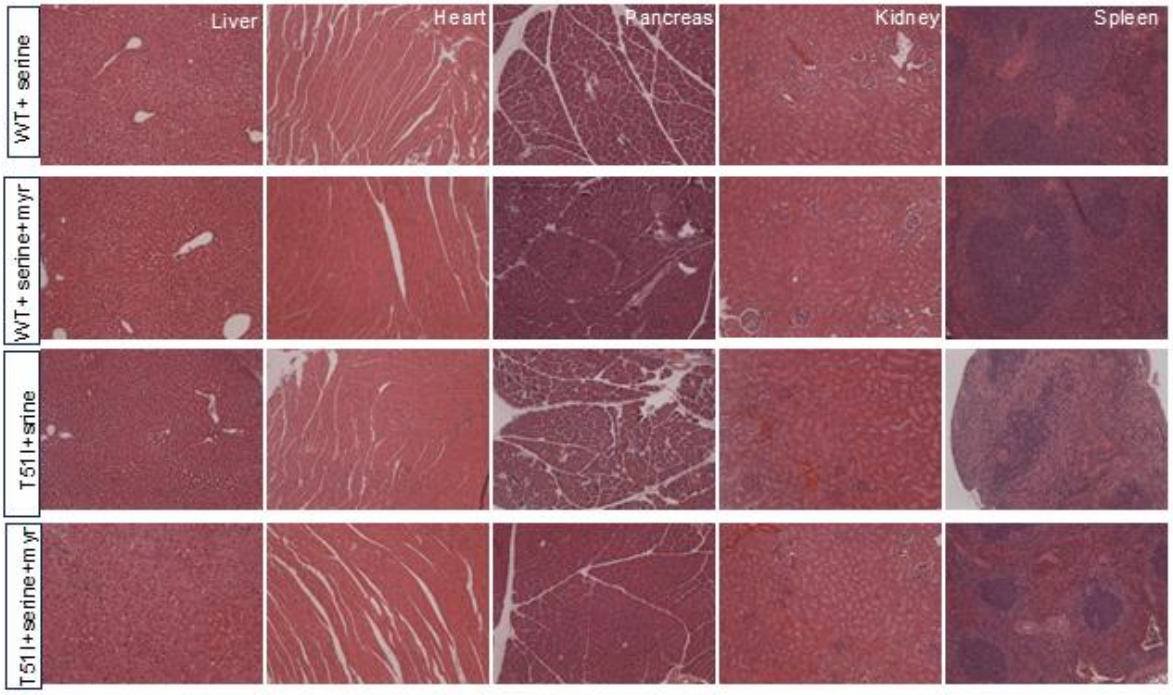


Figure S11. Histology analysis in other peripheral organs of serine dosed *SptssaT51/+* mice. Two months of 10% serine and myriocin treatment (2.2mg/kg diet) were initiated at 1 month of age.

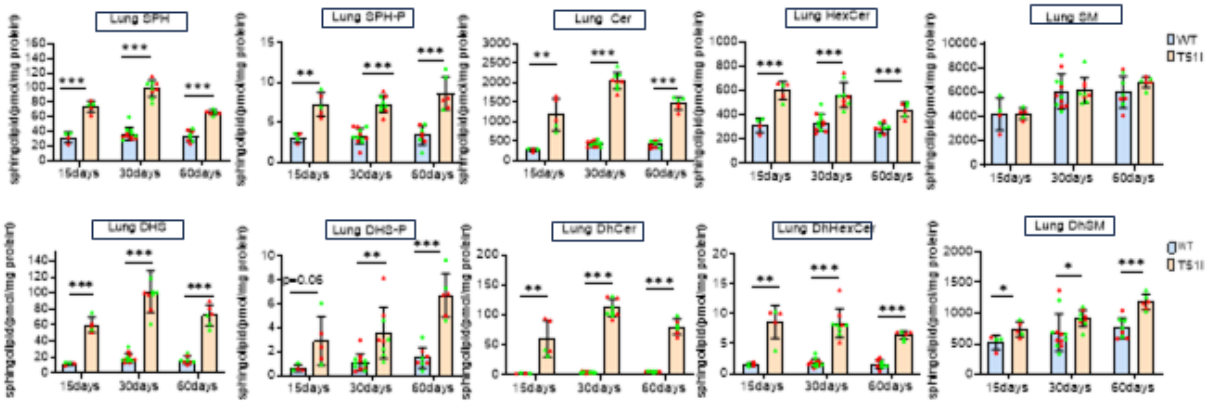


Figure S12. Postnatal developmental changes of sphingolipids (SLs) in lung tissues of WT and *SptssaT51/+* mice (n=4-12). Results were expressed as means ± SD. *p<0.05, **p<0.01 ,***p<0.001 as compared between indicated groups.

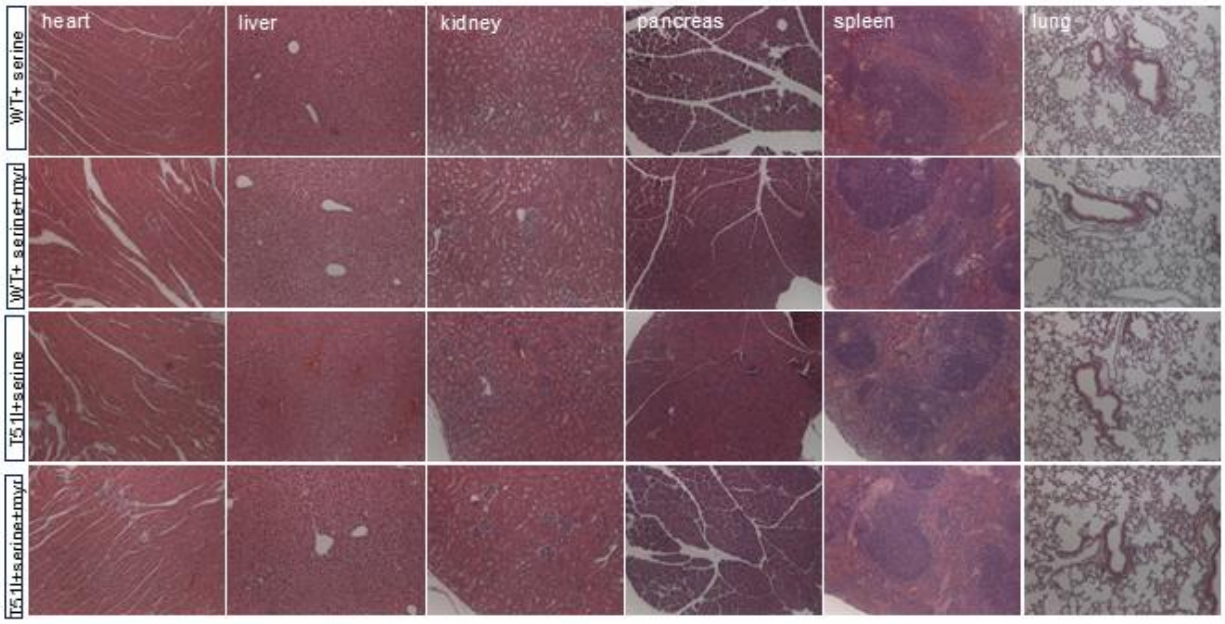
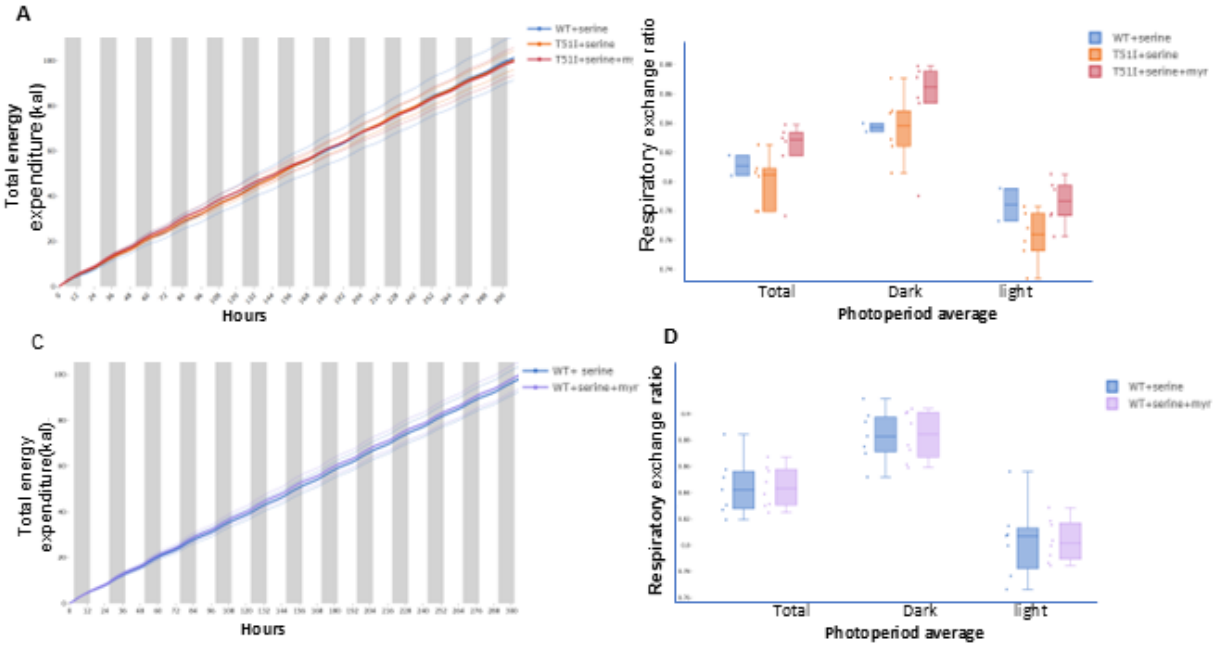
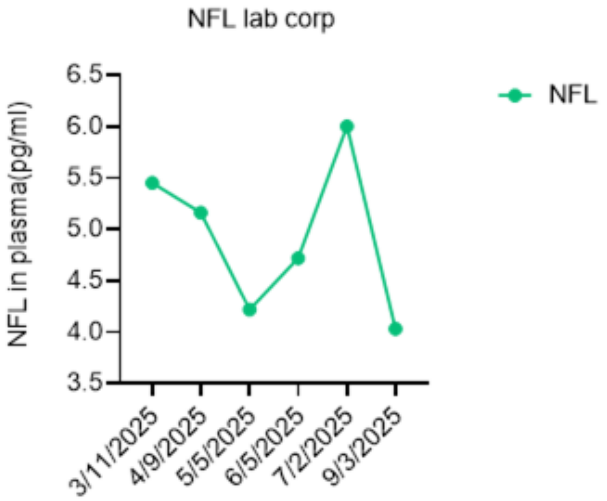


Figure S13. Histology analysis in other peripheral organs of serine dosed *SptssaT51/+* mice. Four months of 10% serine and myriocin treatment (2.2mg/kg diet) were initiated at 2-3 months of age.



FigureS14. Metabolic cage analysis of respiratory exchange ratio and energy expenditure in *SptssaT51/+* mice. Four months of 10% serine and myriocin treatment (2.2mg/kg diet) were initiated at 2-3 months of age (n=2-8).



FigureS15 After January 2025, per new institutional policies, NFL levels were sent to Labcorp instead of Mayo Clinic. Given that different reference ranges are used in each lab, NFL levels from Labcorp could not be meaningfully compared to those from May Clinic and are therefore included in the supplementary materials.

Supplement Table S1 Antibody List

Antibody	Catalogue	Purpose
GFAP	Abcam, Ab7260	Western blot
IBA1	Wako, 016-20001	Western blot
MBP	Santa cruz, sc-271524	Western blot
MOG	Millipore, MAB5680	Western blot
PLP	Abcam, Ab28486	Western blot
ORMDL1/2/3	Millipore, ABN417	Western blot
GRP78	Abcam, Ab21685	Western blot
SPTLC1	Santa cruz, sc-374143	Western blot
SPTLC2	Proteintech, 51012-2-AP	Western blot
GRP78	Abcam, Ab21685	Western blot
GADD153	Santa cruz, sc-166682	Western blot
c-caspase3	cell signaling, 9661	Western blot
Synaptophysin	Cell signaling, 5461P	Western blot
PSD95	Abcam, Ab2723	Western blot
b-ACTIN	Santa Cruz, sc47778	Western blot
GADPH	Santa Cruz, sc32233	Western blot
a-TUBULIN	sc-5286	Western blot
HA	Cell signaling, 3724	IF
HA	Abcam, ab130275	IF
IBA1	Wako, 019-19741	IF
Neurofilament (SMI32)	Biolegend, 801701	IF

NeuN	Abcam, ab104224	IF
MBP	Abcam, Ab40390	IF

Supplement Table S2 D-cycloserine leve in blood after dosing.

Date	D-Cycloserine Level	Unit	How Long After 7:00am Morning Dose (Rounded to the nearest half hour)
10/6/2025	27.33	mcg/mL	6.5 hours (07:00 to 13:21)
9/3/2025	19.39	mcg/mL	7.5 hours (07:00 to 14:33)
7/2/2025	19.21	mcg/mL	6 hours (08:15 to 14:13)
6/4/2025	22.49	mcg/mL	6.5 hours (07:00 to 13:22)
5/5/2025	29.97	mcg/mL	6.5 hours (07:00 to 13:37)
4/8/2025	21.67	mcg/mL	5.5 hours (08:00 to 13:32)
3/11/2025	27.52	mcg/mL	5.5 hours (08:00 to 13:35)
2/13/2025	1.12	mcg/mL	Several days - report details last dose 02/09/2024 at 20:00
1/6/2025	40.58	mcg/mL	6 hours (07:00 to 13:10)
11/25/2024	38.03	mcg/mL	6 hours (07:00 to 12:55)
11/13/2024	36.05	mcg/mL	5.5 hours (07:45 to 13:00)
10/28/2024	36.78	mcg/mL	6 hours (07:00 to 13:02)
10/15/2024	33.67	mcg/mL	6 hours (07:00 to 12:59)
10/1/2024	20.14	mcg/mL	2 hours (07:00 to 09:01)
9/23/2024	27.92	mcg/mL	2 hours (09:00 to 11:00)
9/16/2024	13.83	mcg/mL	2 hours (09:00 to 11:09)
9/9/2024	15.77	mcg/mL	2 hours (08:00 to 10:12)
9/3/2024	40.79	mcg/mL	6 hours (07:00 to 13:14)
8/26/2024	43.96	mcg/mL	7 hours (07:00 to 13:56)
8/19/2024	35.51	mcg/mL	6 hours (07:00 to 12:53)