Contributions of inflammation and tumor microenvironment to neurofibroma tumorigenesis

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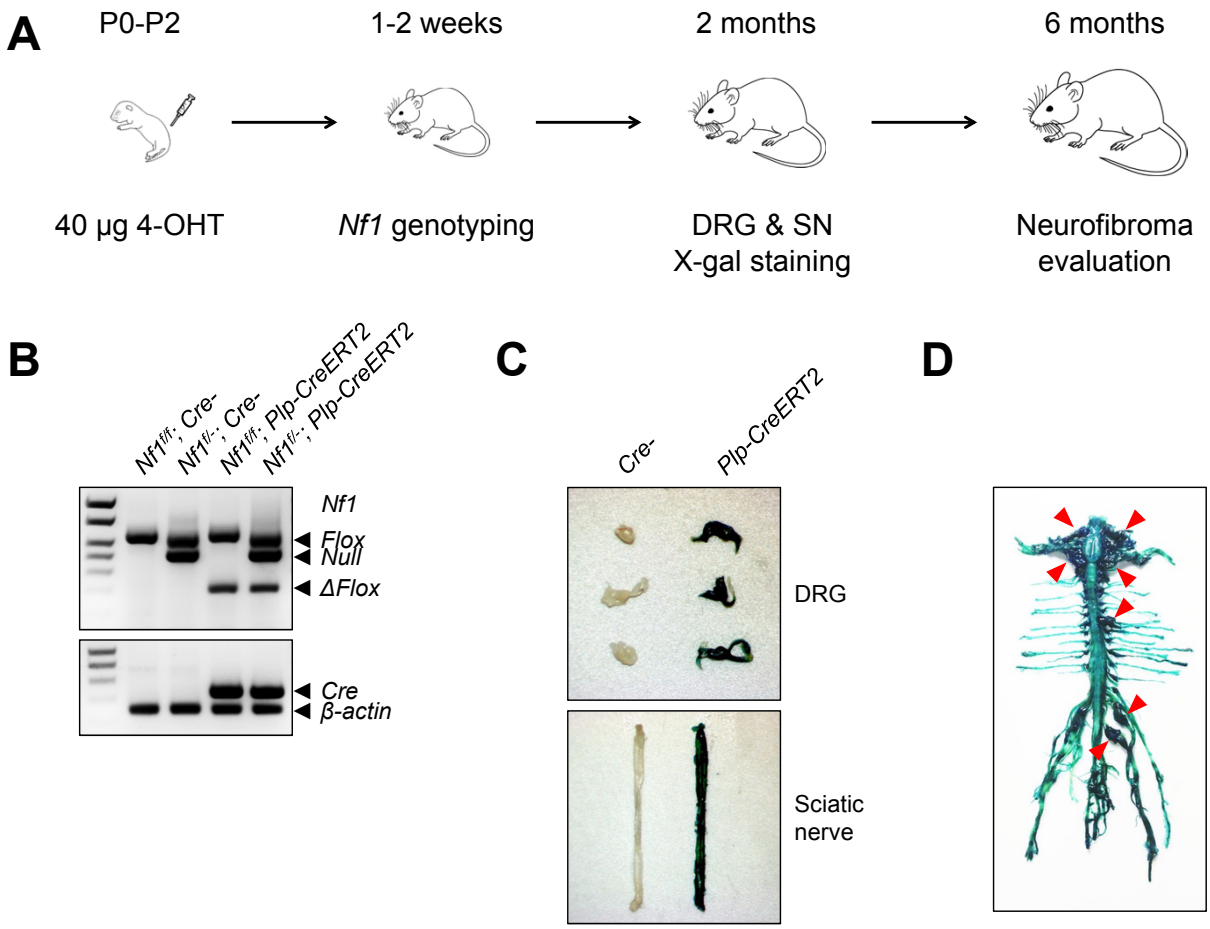
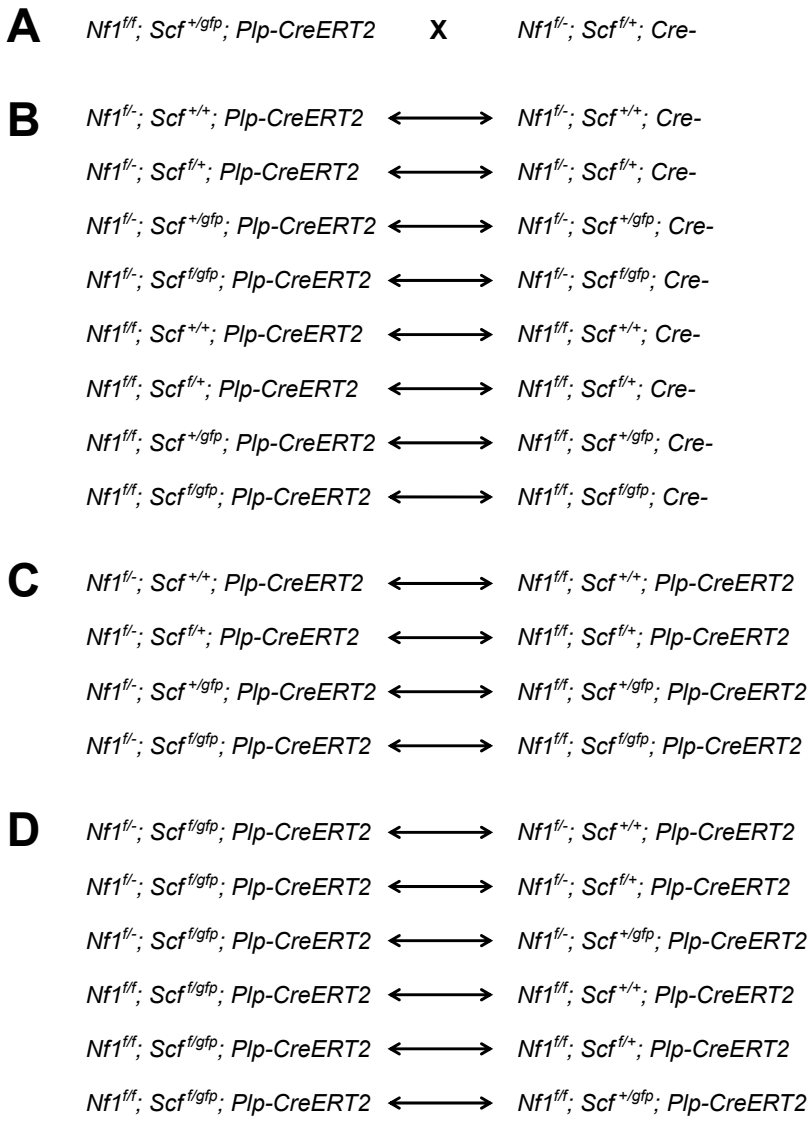
Supplemental Figure 1



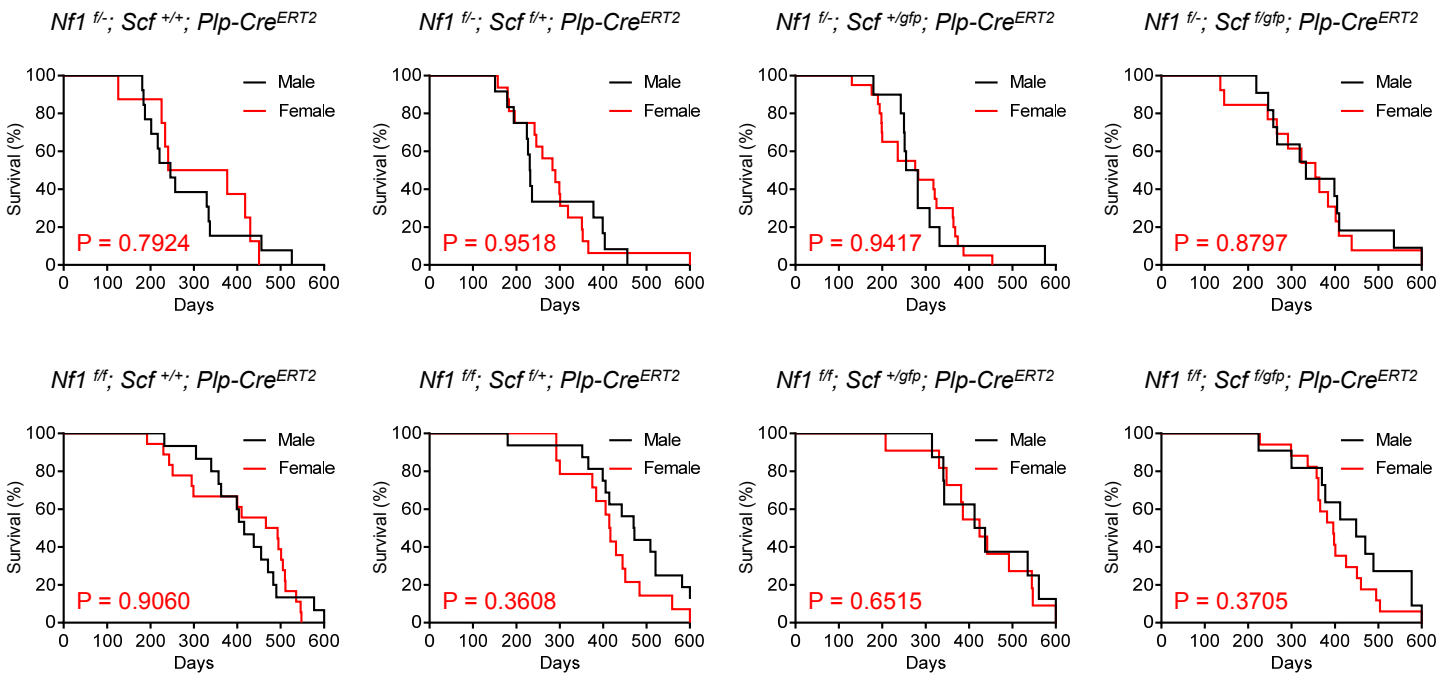
Supplemental Figure 1. Tamoxifen-inducible plexiform neurofibroma mouse model.
(**A**) The flow to generate and to evaluate tamoxifen-inducible plexiform neurofibroma mouse model by using *Plp-CreERT2*. (**B**) In 1-2 weeks old pups, genotyping was performed to determine mouse genotypes and to evaluate *Nf1* ablation in pups with *Plp-CreERT2*. (**C**) In a pilot experiment performed in 2 months old mice, DRG and sciatic nerve were isolated and then subjected to X-gal staining. This was to demonstrate that *R26-LacZ* was specifically and efficiently induced in nerve tissues to mark *Plp*-lineage cells in *Plp-CreERT2* mice. (**D**) In a 6 months old $Nf1^{f/-}$*; Plp-CreERT2* mouse, the whole spinal cord was extracted followed by X-gal staining. Multiple plexiform neurofibromas (arrow head) were identified and shown as aberrantly enlarged nerve tissues. This representative spinal cord was from mouse #11673 which is also shown in Supplemental Figure 4 that comprising all the spinal cords with the same genotype ($Nf1^{f/-}$*; Plp-CreERT2*).

Supplemental Figure 2



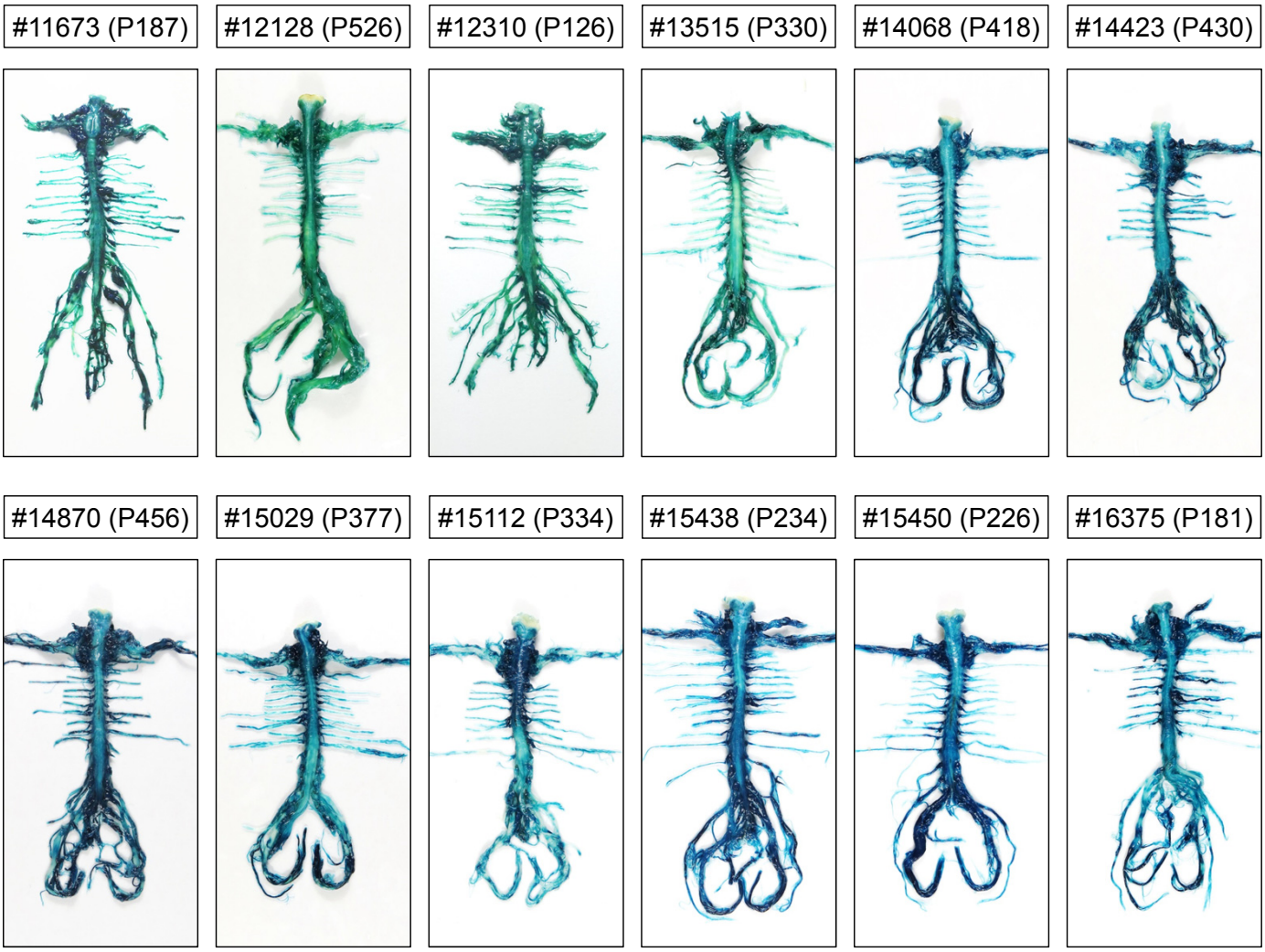
Supplemental Figure 2. Genetically-engineered mouse breeding strategy and study approaches.
(**A**) The genotypes of mouse breeding pairs. All pups were subjected to 4-hydroxytamoxifen treatment at P0-P2. (**B**) Summary of all possible genotypes in the progeny and the comparisons between potential tumor-bearing *Plp-CreERT2*(+) groups and their corresponding *Cre*(-) groups to determine the Cre recombinase induced neurofibroma tumorigenicity. (**C**) The comparisons between $Nf1^{f/-}$ and $Nf1^{f/f}$ in *Plp-CreERT2*(+) groups to determine the role of *Nf1* heterozygosity. (**D**) The comparisons between *Scf* knockout ($Scf^{f/gfp}$) and *Scf* wild-type ($Scf^{+/+}$) or partially depleted controls ($Scf^{f/+}$ and $Scf^{+/gfp}$) in *Plp-CreERT2*(+) groups to determine the contribution of SCF expression in Schwann cells during neurofibroma development. In addition, *R26-LacZ* lineage tracing reporter was included in all above mice to identify the *Plp*-lineage Schwann cells.

Supplemental Figure 3



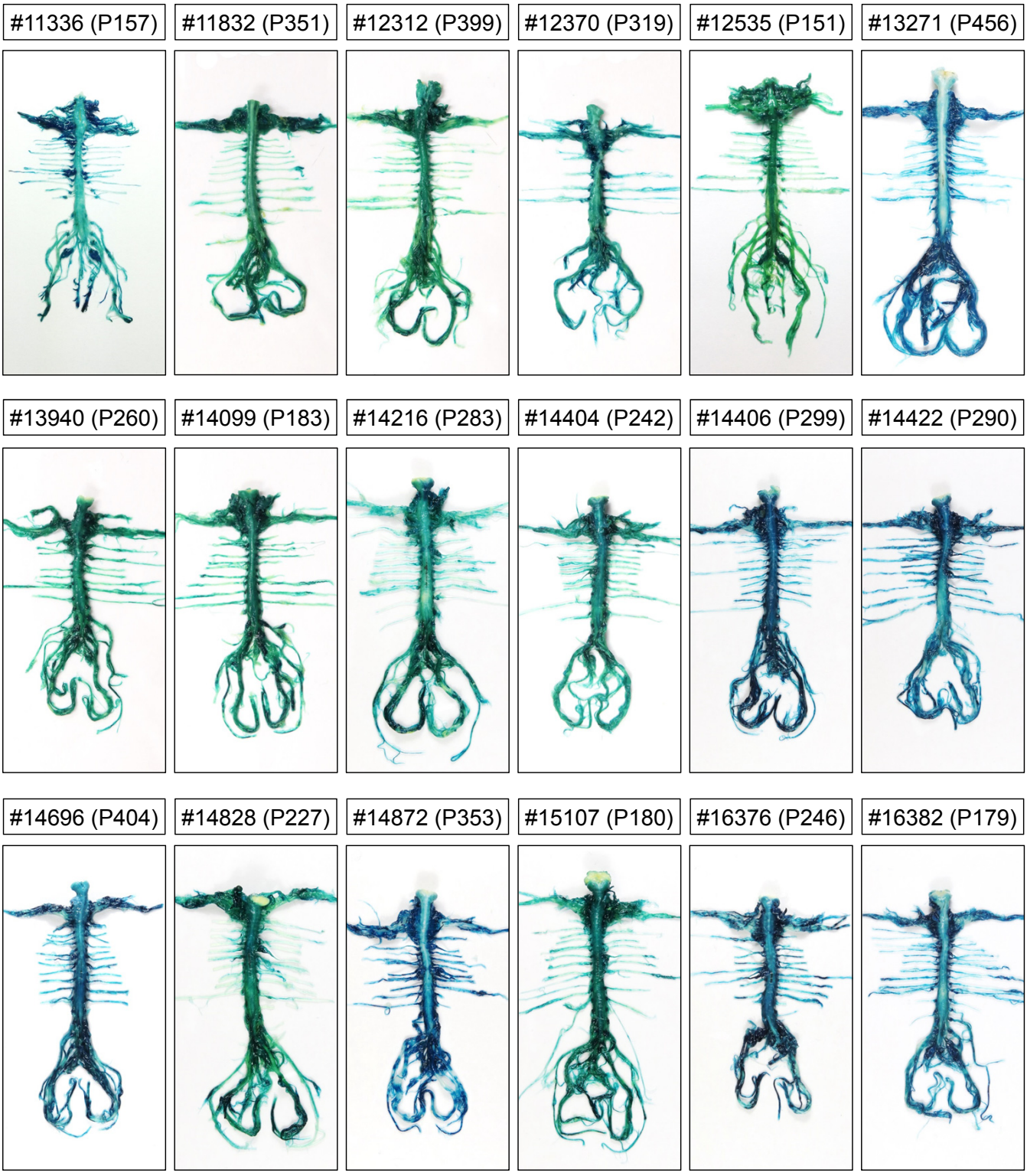
Supplemental Figure 3. Neurofibroma progression in different sex of mice.
The survivals of neurofibroma-bearing mice with the same genotype were compared between male and female mice. No significant difference was noticed in any comparison. The statistics was performed by Kaplan-Meier estimator with log-rank test.

Supplemental Figure 4



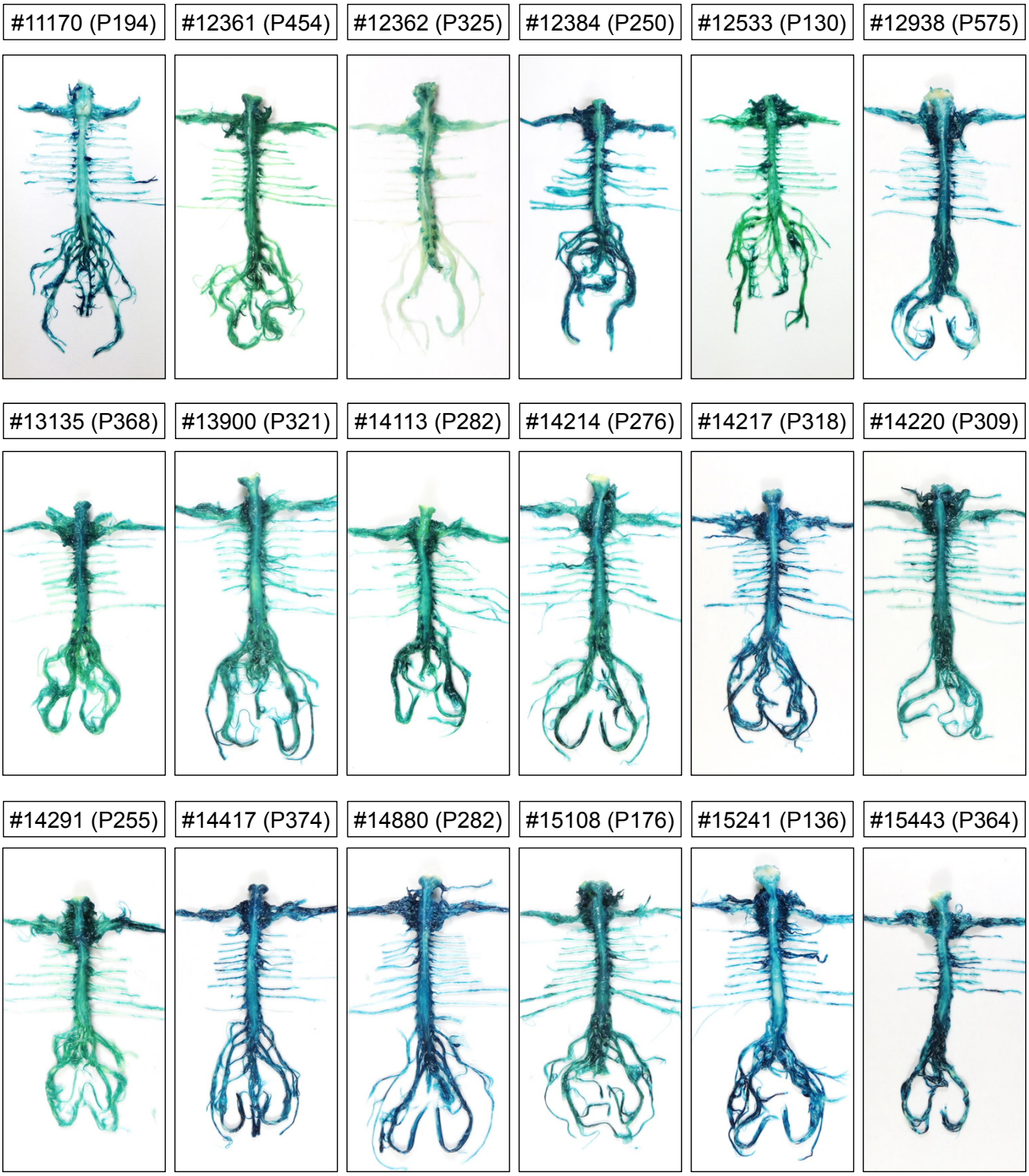
Supplemental Figure 4.
Whole spinal cord analysis for neurofibroma development in *Nf1$^{f/-}$; Scf$^{+/+}$; Plp-CreERT2* mice.

Supplemental Figure 5



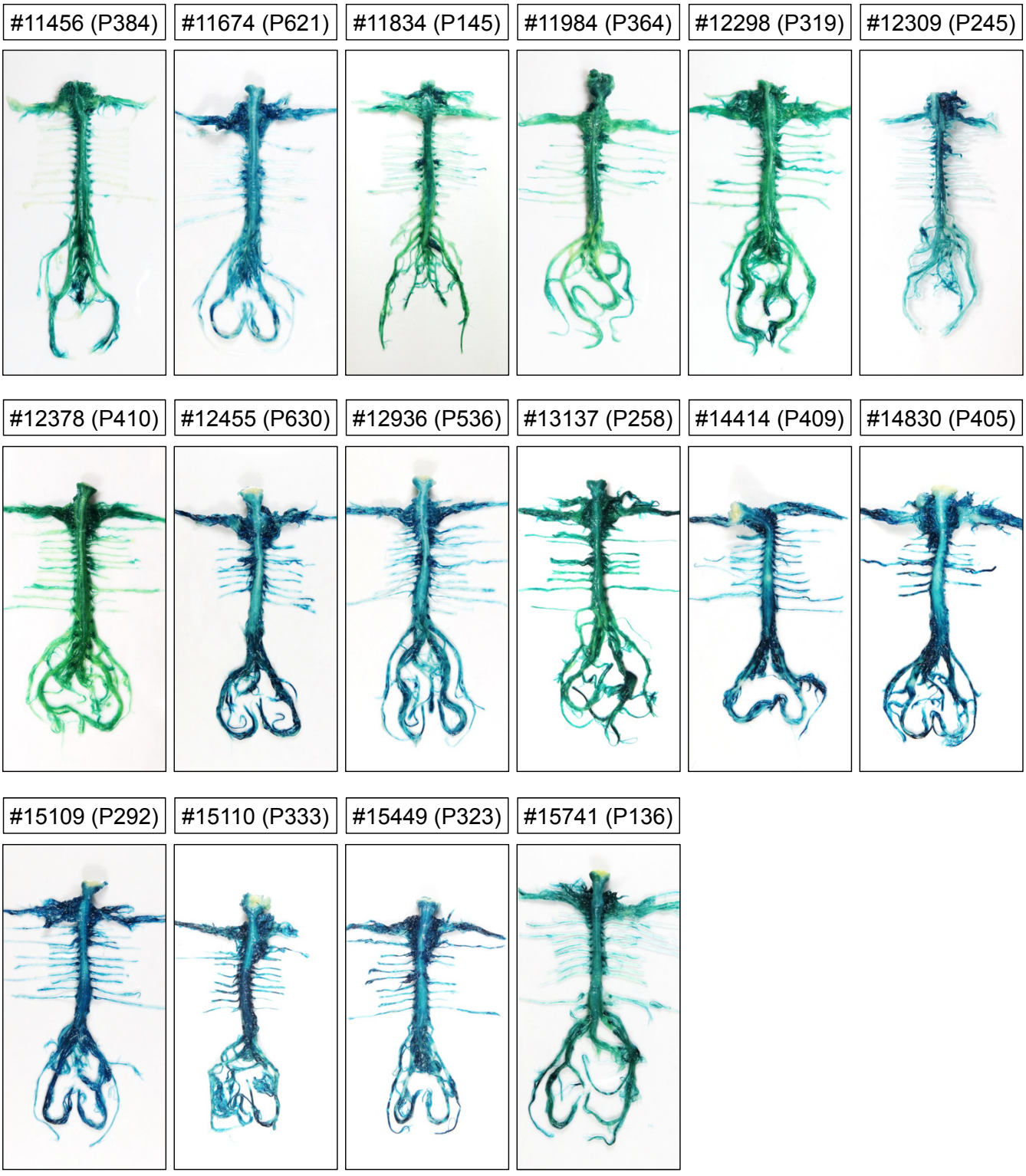
Supplemental Figure 5.
Whole spinal cord analysis for neurofibroma development in *Nf1$^{f/-}$; Scf$^{f/+}$; Plp-CreERT2* mice.

Supplemental Figure 6



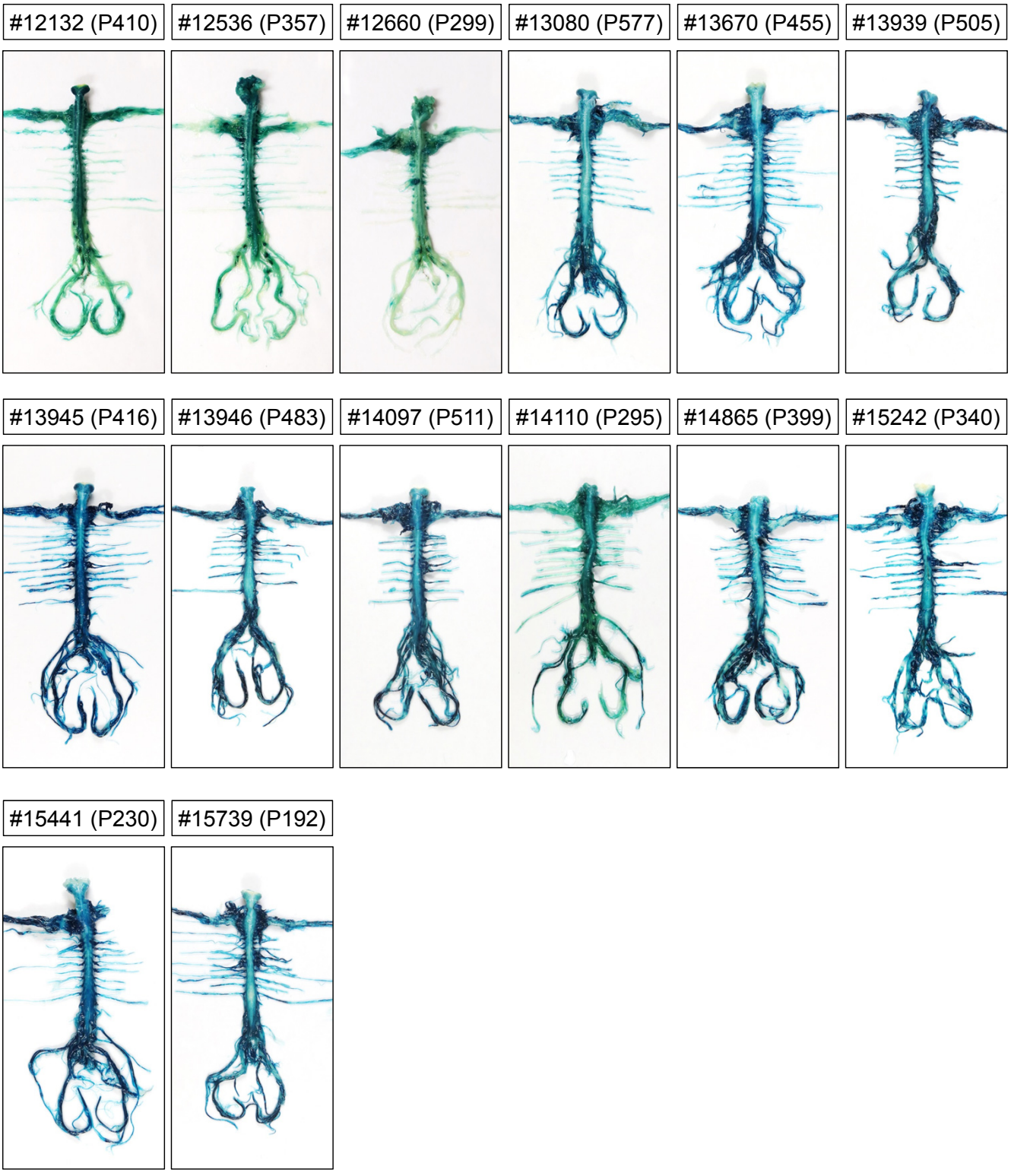
Supplemental Figure 6.
Whole spinal cord analysis for neurofibroma development in *Nf1$^{f/-}$; Scf$^{+/gfp}$; Plp-CreERT2* mice.

Supplemental Figure 7



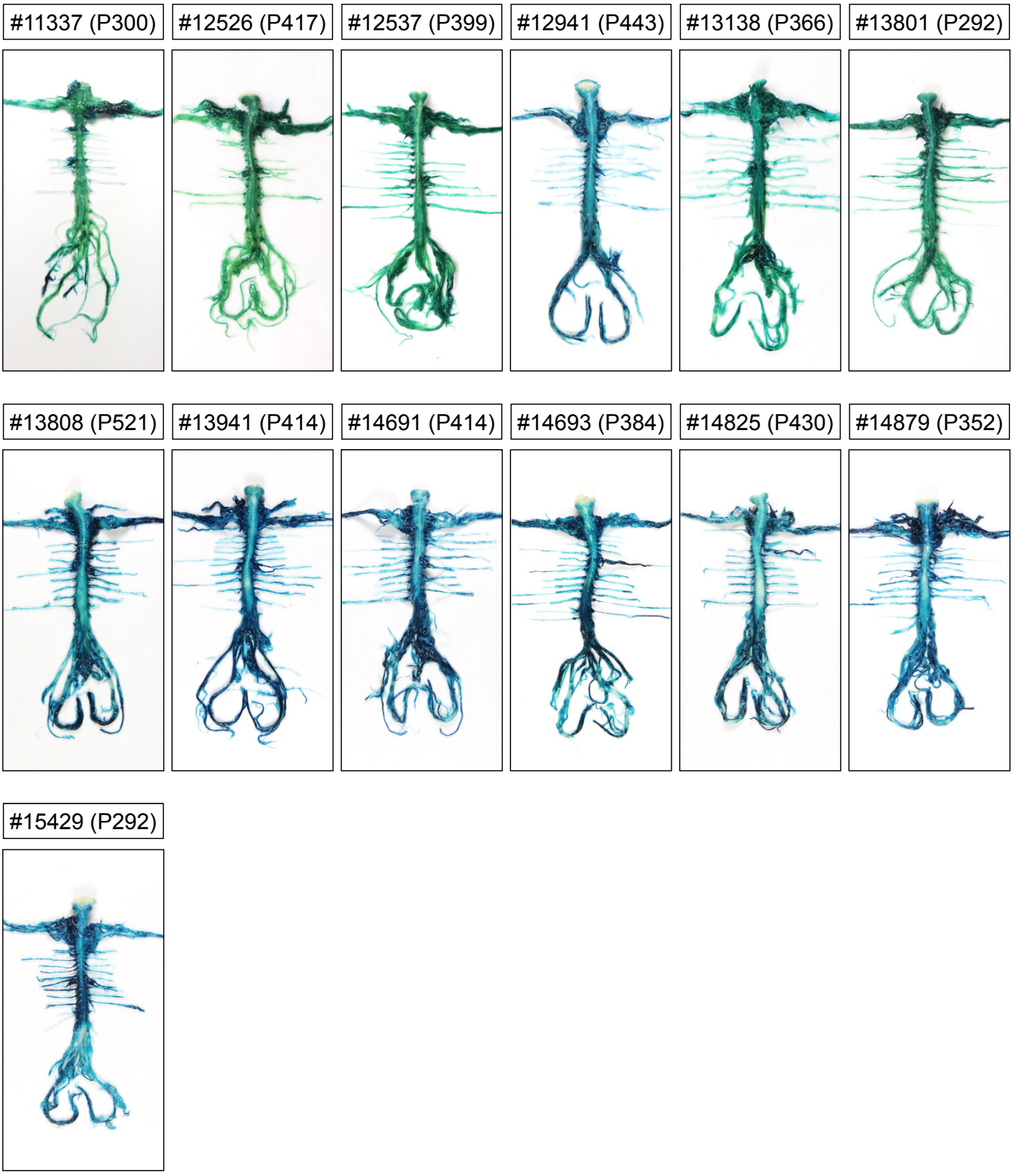
Supplemental Figure 7.
Whole spinal cord analysis for neurofibroma development in *Nf1$^{f/-}$; Scf$^{f/gfp}$; Plp-CreERT2* mice.

Supplemental Figure 8



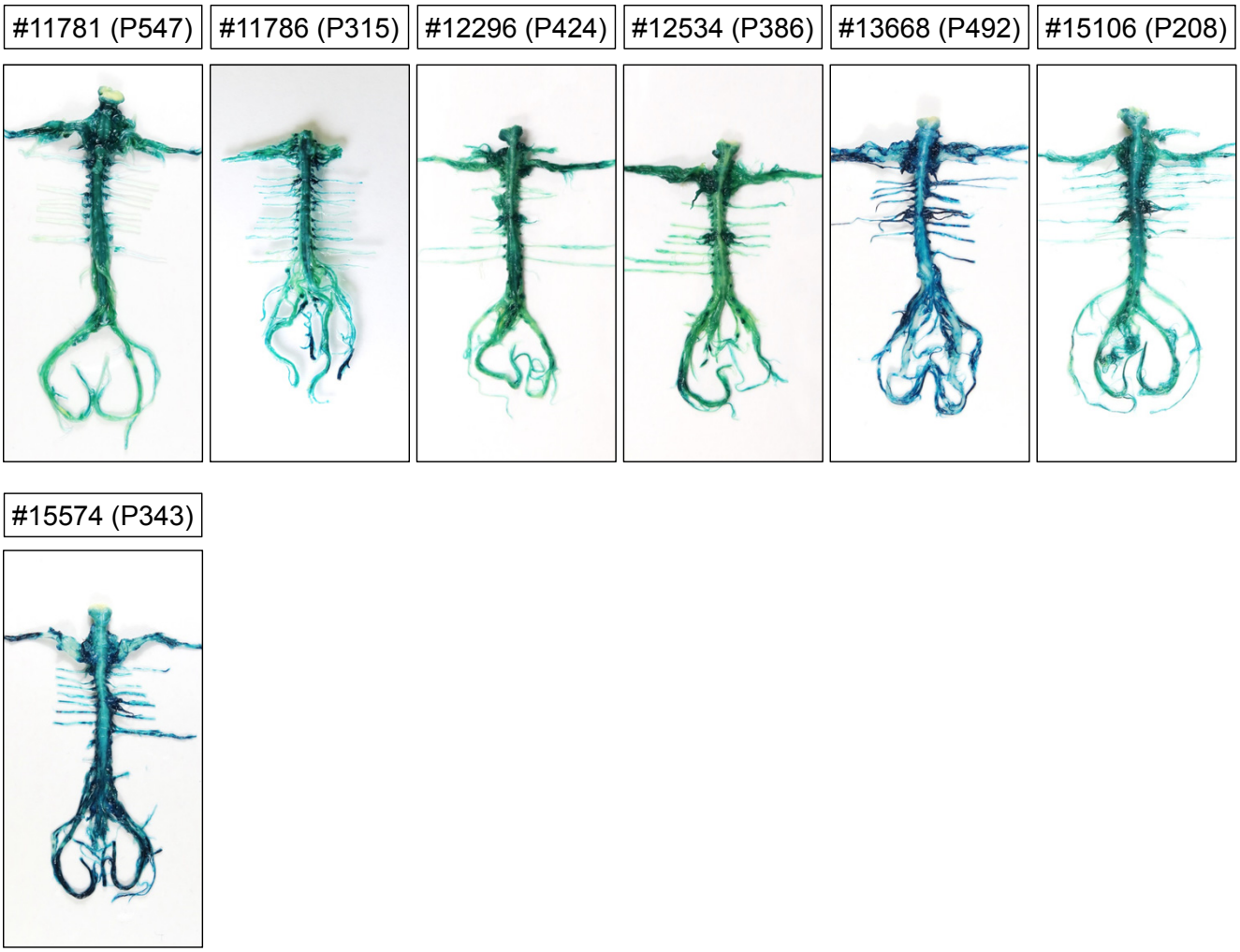
Supplemental Figure 8.
Whole spinal cord analysis for neurofibroma development in *Nf1$^{f/f}$; Scf$^{+/+}$; Plp-CreERT2* mice.

Supplemental Figure 9



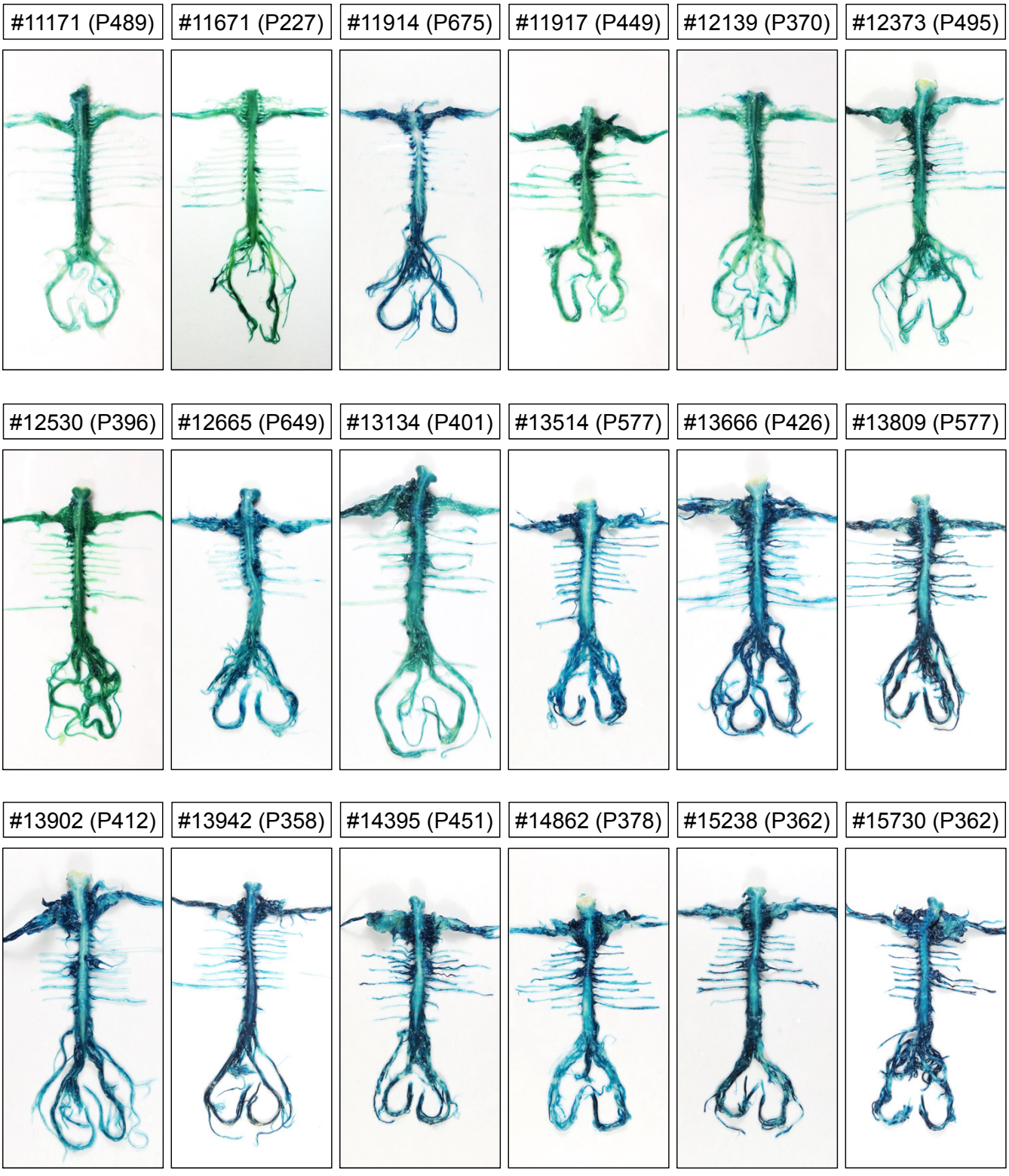
Supplemental Figure 9.
Whole spinal cord analysis for neurofibroma development in *Nf1$^{f/f}$; Scf$^{f/+}$; Plp-CreERT2* mice.

Supplemental Figure 10



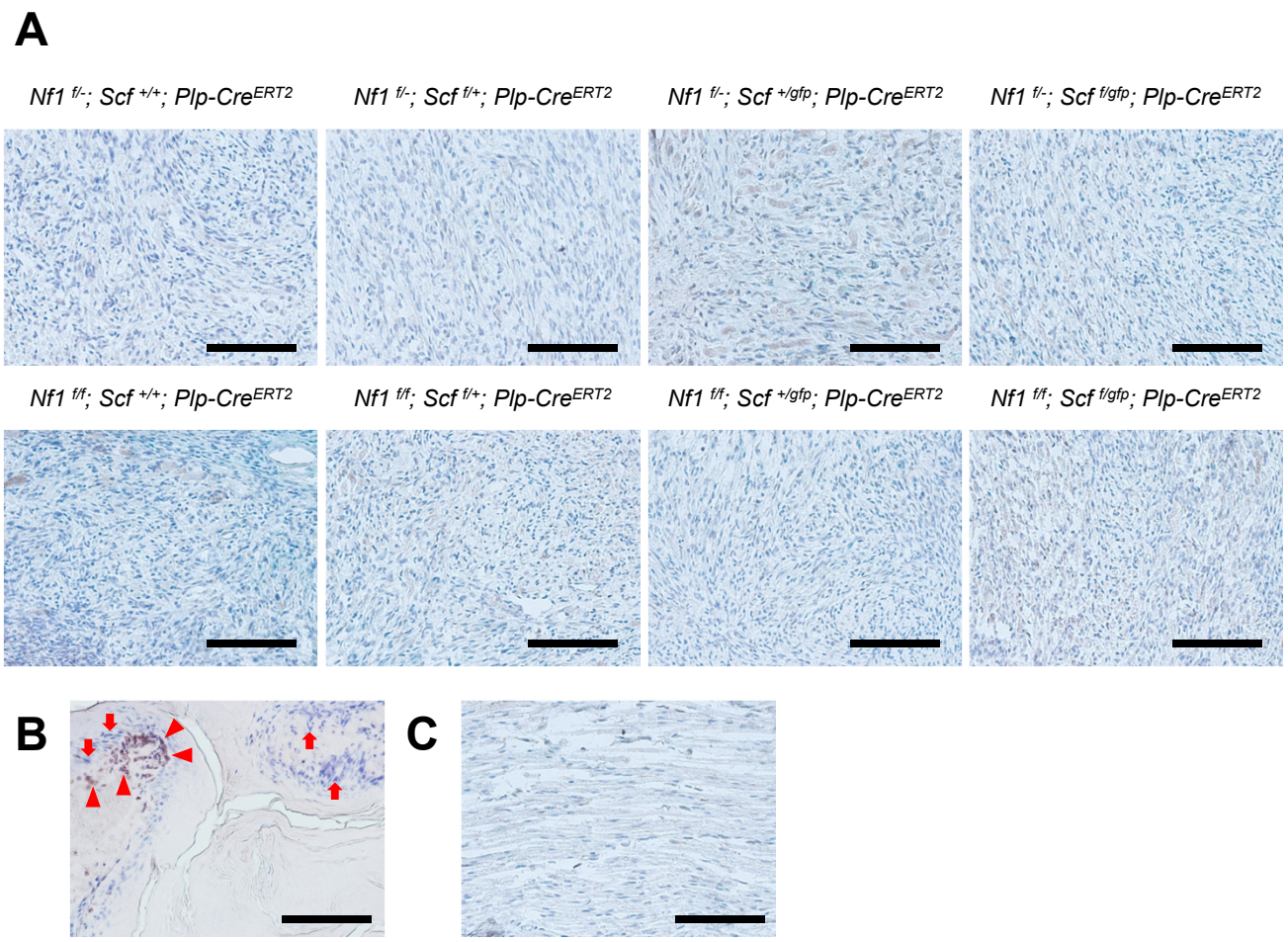
Supplemental Figure 10.
Whole spinal cord analysis for neurofibroma development in *Nf1$^{f/f}$; Scf$^{+/gfp}$; Plp-CreERT2* mice.

Supplemental Figure 11



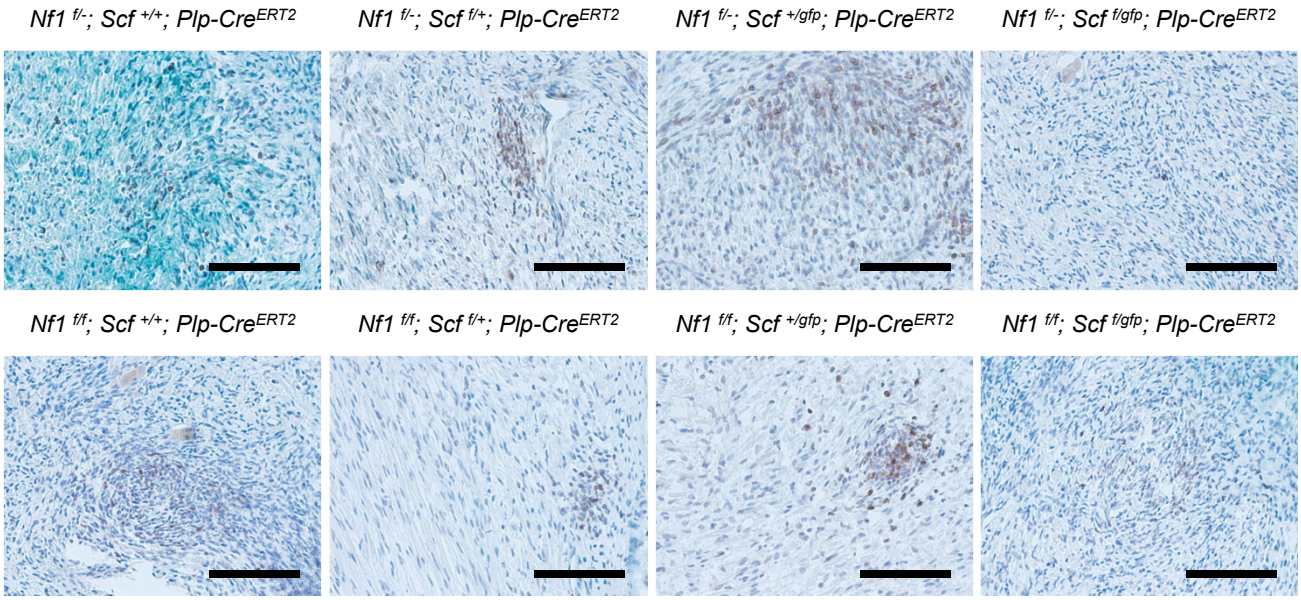
Supplemental Figure 11.
Whole spinal cord analysis for neurofibroma development in *Nf1$^{f/f}$; Scf$^{f/gfp}$; Plp-CreERT2* mice.

Supplemental Figure 12



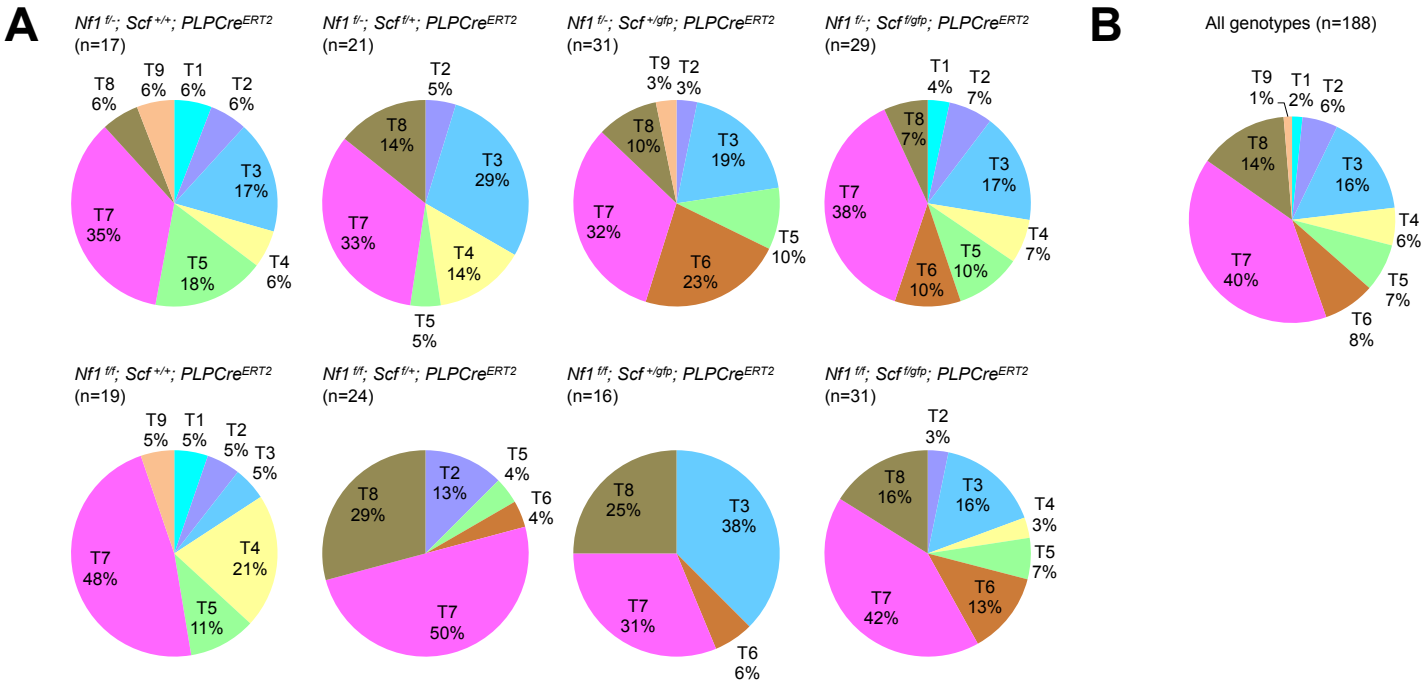
Supplemental Figure 12. M2 macrophage in neurofibroma tumor microenvironment.
(A) Mannose receptor immunohistochemical staining for M2 macrophage in the cervical plexiform neurofibroma tumor. M2 macrophage was not detected in tumor. Representative images were shown from mice with similar age (10 -12 months) in each group. (B) Mouse squamous cell carcinoma was stained for mannose receptor under the same staining condition in (A) as a positive control. M2 macrophages (arrow head) were highlighted but not in nearby keratinocytes (arrow). (C) Mouse nerve was also stained for mannose receptor as a negative control. Scale bar represents 100 μm.

Supplemental Figure 13



Supplemental Figure 13. T lymphocyte in neurofibroma tumor microenvironment.
CD3 immunohistochemical staining for T lymphocyte in the cervical plexiform neurofibroma tumor. T lymphocyte was detected in a few small areas in a tumor and they are usually found in cluster. Representative images were shown from mice with similar age (10 -12 months) in each group. Scale bar represents 100 μm.

Supplemental Figure 14



Supplemental Figure 14. The prevalence of thoracic neurofibroma in different thoracic nerves.
(**A**) The percentages of thoracic neurofibromas in different thoracic nerves by groups. The numbers of tumors ranged from 16 to 31 in each group as labeled in the figures. (**B**) The percentages of thoracic neurofibromas in different thoracic nerves from a total of 188 tumors in (A).

Supplemental Table 1. Summary of plexiform neurofibroma mice

Genotype	Mouse ID	Sex	Survival (days)	Plexiform neurofibroma		
				Cervical (grade)	Thoracic (location and size (mm^3))	Cauda equina (number)
Nf1f/-; Scf+/+; Plp-CreERT2	11673	M	187	5.0	T1 (0.5), T2 (0.5), T3 (0.5), T7 (10)	2
Nf1f/-; Scf+/+; Plp-CreERT2	12128	M	526	2.7	T7 (1), T9 (0.5)	3
Nf1f/-; Scf+/+; Plp-CreERT2	12310	F	126	4.3	T5 (4), T5 (4)	1
Nf1f/-; Scf+/+; Plp-CreERT2	13515	M	330	3.0		0
Nf1f/-; Scf+/+; Plp-CreERT2	14068	F	418	2.3		3
Nf1f/-; Scf+/+; Plp-CreERT2	14423	F	430	3.3	T3 (0.5)	0
Nf1f/-; Scf+/+; Plp-CreERT2	14870	M	456	3.0		4
Nf1f/-; Scf+/+; Plp-CreERT2	15029	F	377	4.3	T7 (0.5)	4
Nf1f/-; Scf+/+; Plp-CreERT2	15112	M	334	4.7	T3 (4), T7 (1)	0
Nf1f/-; Scf+/+; Plp-CreERT2	15438	F	234	2.3	T4 (0.5), T7 (4), T8 (4)	6
Nf1f/-; Scf+/+; Plp-CreERT2	15450	F	226	2.7		2
Nf1f/-; Scf+/+; Plp-CreERT2	16375	M	181	4.3	T5 (1.5), T7 (6)	0
Nf1f/-; Scff/+; Plp-CreERT2	11336	F	157	3.7	T2 (2), T3 (6), T8 (0.5), T8 (8)	2
Nf1f/-; Scff/+; Plp-CreERT2	11832	F	351	2.3	T3 (0.5)	2
Nf1f/-; Scff/+; Plp-CreERT2	12312	M	399	3.3		0
Nf1f/-; Scff/+; Plp-CreERT2	12370	F	319	2.0	T3 (4), T7 (4)	2
Nf1f/-; Scff/+; Plp-CreERT2	12535	M	151	4.0	T3 (0.5), T7 (1)	2
Nf1f/-; Scff/+; Plp-CreERT2	13271	M	456	4.0	T3 (0.5), T7 (0.5)	2
Nf1f/-; Scff/+; Plp-CreERT2	13940	F	260	2.3	T3 (1)	3
Nf1f/-; Scff/+; Plp-CreERT2	14099	F	183	3.3		1
Nf1f/-; Scff/+; Plp-CreERT2	14216	F	283	2.3	T4 (1.5)	2
Nf1f/-; Scff/+; Plp-CreERT2	14404	F	242	2.7	T7 (4)	0
Nf1f/-; Scff/+; Plp-CreERT2	14406	F	299	2.3		1
Nf1f/-; Scff/+; Plp-CreERT2	14422	F	290	2.0		0
Nf1f/-; Scff/+; Plp-CreERT2	14696	M	404	1.7	T7 (1)	1
Nf1f/-; Scff/+; Plp-CreERT2	14828	M	227	2.0	T4 (4), T5 (8)	0
Nf1f/-; Scff/+; Plp-CreERT2	14872	F	353	3.7	T3 (4), T7 (4), T7 (6)	0
Nf1f/-; Scff/+; Plp-CreERT2	15107	F	180	1.7	T7 (0.5)	0
Nf1f/-; Scff/+; Plp-CreERT2	16376	F	246	3.7	T7 (1), T8 (6)	3
Nf1f/-; Scff/+; Plp-CreERT2	16382	M	179	3.3		2
Nf1f/-; Scf+/gfp; Plp-CreERT2	11170	F	194	1.3	T3(1), T7 (1), T8 (0.5)	0
Nf1f/-; Scf+/gfp; Plp-CreERT2	12361	F	454	2.0	T3 (0.5), T3 (0.5)	1
Nf1f/-; Scf+/gfp; Plp-CreERT2	12362	F	325	1.3	T7 (4)	0
Nf1f/-; Scf+/gfp; Plp-CreERT2	12384	M	250	4.0	T3 (0.5), T3 (0.5), T5 (0.5), T5 (4)	2
Nf1f/-; Scf+/gfp; Plp-CreERT2	12533	F	130	4.0	T7 (6)	1
Nf1f/-; Scf+/gfp; Plp-CreERT2	12938	M	575	3.7		3
Nf1f/-; Scf+/gfp; Plp-CreERT2	13135	F	368	2.3	T6 (1), T6 (1), T7 (0.5), T7 (1)	0
Nf1f/-; Scf+/gfp; Plp-CreERT2	13900	F	321	1.3	T6 (0.5), T7 (0.5)	0
Nf1f/-; Scf+/gfp; Plp-CreERT2	14113	M	282	1.7		0
Nf1f/-; Scf+/gfp; Plp-CreERT2	14214	F	276	2.7	T5 (6)	0
Nf1f/-; Scf+/gfp; Plp-CreERT2	14217	F	318	3.3	T6 (1.5)	0
Nf1f/-; Scf+/gfp; Plp-CreERT2	14220	M	309	3.7		0
Nf1f/-; Scf+/gfp; Plp-CreERT2	14291	M	255	4.0	T6 (1)	0
Nf1f/-; Scf+/gfp; Plp-CreERT2	14417	F	374	2.0	T7 (6)	0
Nf1f/-; Scf+/gfp; Plp-CreERT2	14880	M	282	3.3	T7 (0.5)	0
Nf1f/-; Scf+/gfp; Plp-CreERT2	15108	F	176	2.3	T7 (1), T8 (0.5), T9 (0.5)	0
Nf1f/-; Scf+/gfp; Plp-CreERT2	15241	F	236	4.7	T2 (1.5), T3 (4), T6 (8), T8 (1)	0
Nf1f/-; Scf+/gfp; Plp-CreERT2	15443	F	364	2.7	T6 (0.5), T7 (1)	0

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Nf1f/-; Scff/gfp; Plp-CreERT2	11456	F	384	2.3	T1 (0.5), T2 (0.5), T3 (0.5)	0
Nf1f/-; Scff/gfp; Plp-CreERT2	11674	M	621	3.7		2
Nf1f/-; Scff/gfp; Plp-CreERT2	11834	F	145	2.0	T7 (1), T7 (4)	1
Nf1f/-; Scff/gfp; Plp-CreERT2	11984	F	364	1.3	T3 (1), T7 (4)	0
Nf1f/-; Scff/gfp; Plp-CreERT2	12298	M	319	2.7	T3 (1), T7 (4), T7 (6)	3
Nf1f/-; Scff/gfp; Plp-CreERT2	12309	F	245	3.7	T5 (1), T5 (6)	0
Nf1f/-; Scff/gfp; Plp-CreERT2	12378	M	410	1.3	T6 (1), T7 (1)	0
Nf1f/-; Scff/gfp; Plp-CreERT2	12455	F	630	4.0	T5 (10)	0
Nf1f/-; Scff/gfp; Plp-CreERT2	12936	M	536	3.0	T2 (0.5), T6 (0.5), T7 (0.5), T7 (4)	0
Nf1f/-; Scff/gfp; Plp-CreERT2	13137	M	258	3.0	T7 (1), T7 (1), T8 (0.5)	0
Nf1f/-; Scff/gfp; Plp-CreERT2	14414	F	409	1.3		0
Nf1f/-; Scff/gfp; Plp-CreERT2	14830	M	405	3.3	T6 (0.5), T7 (0.5)	0
Nf1f/-; Scff/gfp; Plp-CreERT2	15109	F	292	3.3	T3 (0.5), T3 (0.5)	2
Nf1f/-; Scff/gfp; Plp-CreERT2	15110	M	333	4.3		0
Nf1f/-; Scff/gfp; Plp-CreERT2	15449	F	323	4.3	T4 (1), T4 (4), T8 (6)	2
Nf1f/-; Scff/gfp; Plp-CreERT2	15741	F	136	2.7		0
Nf1f/f; Scf+/+; Plp-CreERT2	12132	F	410	0.7		0
Nf1f/f; Scf+/+; Plp-CreERT2	12536	M	357	1.3		0
Nf1f/f; Scf+/+; Plp-CreERT2	12660	F	299	2.0	T7 (4)	0
Nf1f/f; Scf+/+; Plp-CreERT2	13080	M	577	2.7	T5 (0.5), T5 (1)	2
Nf1f/f; Scf+/+; Plp-CreERT2	13670	M	455	4.0	T4 (4)	0
Nf1f/f; Scf+/+; Plp-CreERT2	13939	F	505	4.0		0
Nf1f/f; Scf+/+; Plp-CreERT2	13945	M	416	1.7	T7 (1), T7 (4)	0
Nf1f/f; Scf+/+; Plp-CreERT2	13946	M	483	2.3	T4 (0.5)	0
Nf1f/f; Scf+/+; Plp-CreERT2	14097	F	511	2.3	T7 (4)	1
Nf1f/f; Scf+/+; Plp-CreERT2	14110	F	295	1.3	T7 (4)	0
Nf1f/f; Scf+/+; Plp-CreERT2	14865	M	399	2.3	T1 (0.5), T2 (0.5), T3 (0.5), T7 (0.5), T7 (4)	2
Nf1f/f; Scf+/+; Plp-CreERT2	15242	M	340	2.7	T7 (6)	0
Nf1f/f; Scf+/+; Plp-CreERT2	15441	F	230	2.3	T4 (0.5), T4 (0.5)	2
Nf1f/f; Scf+/+; Plp-CreERT2	15739	F	192	3.7	T7 (4), T7 (4), T9 (1)	0
Nf1f/f; Scff/+; Plp-CreERT2	11337	F	300	3.0	T2 (1), T2 (1), T7 (6)	0
Nf1f/f; Scff/+; Plp-CreERT2	12526	F	417	3.0	T8 (1), T8 (4)	0
Nf1f/f; Scff/+; Plp-CreERT2	12537	M	399	2.0	T8 (0.5), T8 (4)	0
Nf1f/f; Scff/+; Plp-CreERT2	12941	M	443	1.7	T6 (1)	0
Nf1f/f; Scff/+; Plp-CreERT2	13138	M	366	3.7	T7 (1), T7 (4)	1
Nf1f/f; Scff/+; Plp-CreERT2	13801	F	292	3.0	T7 (1), T7 (1)	0
Nf1f/f; Scff/+; Plp-CreERT2	13808	M	521	3.3	T7 (0.5), T7 (4)	3
Nf1f/f; Scff/+; Plp-CreERT2	13941	F	414	3.0	T7 (0.5), T7 (4)	0
Nf1f/f; Scff/+; Plp-CreERT2	14691	M	414	2.3	T7 (4), T8 (1)	0
Nf1f/f; Scff/+; Plp-CreERT2	14693	F	384	2.7	T5 (2.5)	0
Nf1f/f; Scff/+; Plp-CreERT2	14825	F	430	2.7		0
Nf1f/f; Scff/+; Plp-CreERT2	14879	M	352	3.7	T7 (4), T7 (4)	0
Nf1f/f; Scff/+; Plp-CreERT2	15429	F	292	3.7	T8 (6), T8 (6)	0
Nf1f/f; Scf+/gfp; Plp-CreERT2	11781	F	547	3.3	T3(0.5), T3 (0.5)	0
Nf1f/f; Scf+/gfp; Plp-CreERT2	11786	M	315	2.0	T3 (0.5), T3 (1)	0
Nf1f/f; Scf+/gfp; Plp-CreERT2	12296	F	424	2.7	T7 (4), T7 (6)	0
Nf1f/f; Scf+/gfp; Plp-CreERT2	12534	F	386	2.7	T8 (4), T8 (6)	0
Nf1f/f; Scf+/gfp; Plp-CreERT2	13668	F	492	4.3	T8 (4), T8 (4)	0
Nf1f/f; Scf+/gfp; Plp-CreERT2	15106	F	208	2.0	T3 (0.5), T3 (0.5), T6 (4), T7 (0.5), T7 (6)	0
Nf1f/f; Scf+/gfp; Plp-CreERT2	15574	M	343	2.3	T7 (13.5)	2

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Nf1f/f; Scff/gfp; Plp-CreERT2	11171	M	489	0.0		0
Nf1f/f; Scff/gfp; Plp-CreERT2	11671	F	227	1.0	T7 (1)	0
Nf1f/f; Scff/gfp; Plp-CreERT2	11914	F	675	2.3		0
Nf1f/f; Scff/gfp; Plp-CreERT2	11917	M	449	3.7	T3 (1), T7 (1), T7 (6)	0
Nf1f/f; Scff/gfp; Plp-CreERT2	12139	M	370	0.7		0
Nf1f/f; Scff/gfp; Plp-CreERT2	12373	F	495	3.0	T3 (1.5), T4 (1), T8 (4), T8 (6)	0
Nf1f/f; Scff/gfp; Plp-CreERT2	12530	F	396	2.0		0
Nf1f/f; Scff/gfp; Plp-CreERT2	12665	M	649	1.7	T6 (4), T8 (4)	0
Nf1f/f; Scff/gfp; Plp-CreERT2	13134	F	401	4.0	T2 (0.5), T3 (1), T7 (4), T7 (6)	0
Nf1f/f; Scff/gfp; Plp-CreERT2	13514	M	577	3.0		0
Nf1f/f; Scff/gfp; Plp-CreERT2	13666	F	426	4.7	T3 (0.5), T3 (0.5), T7 (1), T7 (1)	2
Nf1f/f; Scff/gfp; Plp-CreERT2	13809	M	577	4.0	T8 (0.5)	3
Nf1f/f; Scff/gfp; Plp-CreERT2	13902	M	412	4.3	T7 (6), T7 (6)	0
Nf1f/f; Scff/gfp; Plp-CreERT2	13942	F	358	2.7	T7 (0.5)	0
Nf1f/f; Scff/gfp; Plp-CreERT2	14395	F	451	4.7	T5 (0.5), T5 (6), T6 (0.5), T6 (4)	1
Nf1f/f; Scff/gfp; Plp-CreERT2	14862	M	378	3.3	T6 (1), T7 (4)	0
Nf1f/f; Scff/gfp; Plp-CreERT2	15238	F	362	3.0	T7 (4), T7 (4)	0
Nf1f/f; Scff/gfp; Plp-CreERT2	15730	F	362	3.7	T8 (13.5)	0

In the column of sex , M = male, F = female. Plexiform neurofibroma was defined by DRG size equal or greater than 1 mm x 1 mm. The grading of cervial tumor was the average of blind grading by three neurofibroma research scientists based on the reference in Figure 3D. The tumor size of thoracic tumor was determined by by the formula (length) × (width) ^{2} × 0.5.