

OMTM, Volume 23

Supplemental information

**Adeno-associated viral vector
serotype 9-based gene replacement
therapy for *SURF1*-related Leigh syndrome**

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Supplementary Information

Supplementary item #1: Figures

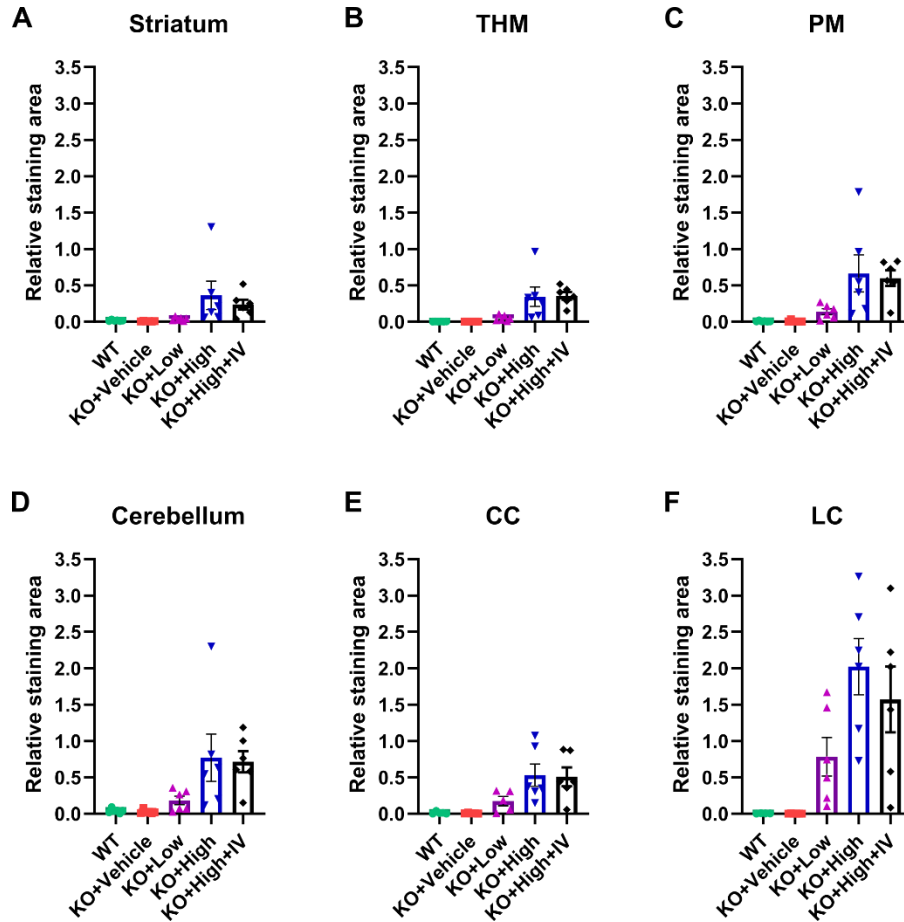


Figure S1. hSURF1opt mRNA expression in brain regions and spinal cord. Data represent percent area staining positive for hSURF1opt mRNA. THM: Thalamus, hypothalamus, and midbrain; PM: pons and medulla; CC: cervical cord; LC: lumbar cord. Each data point represents measurement from an individual animal, with bars representing the Mean ± SEM.

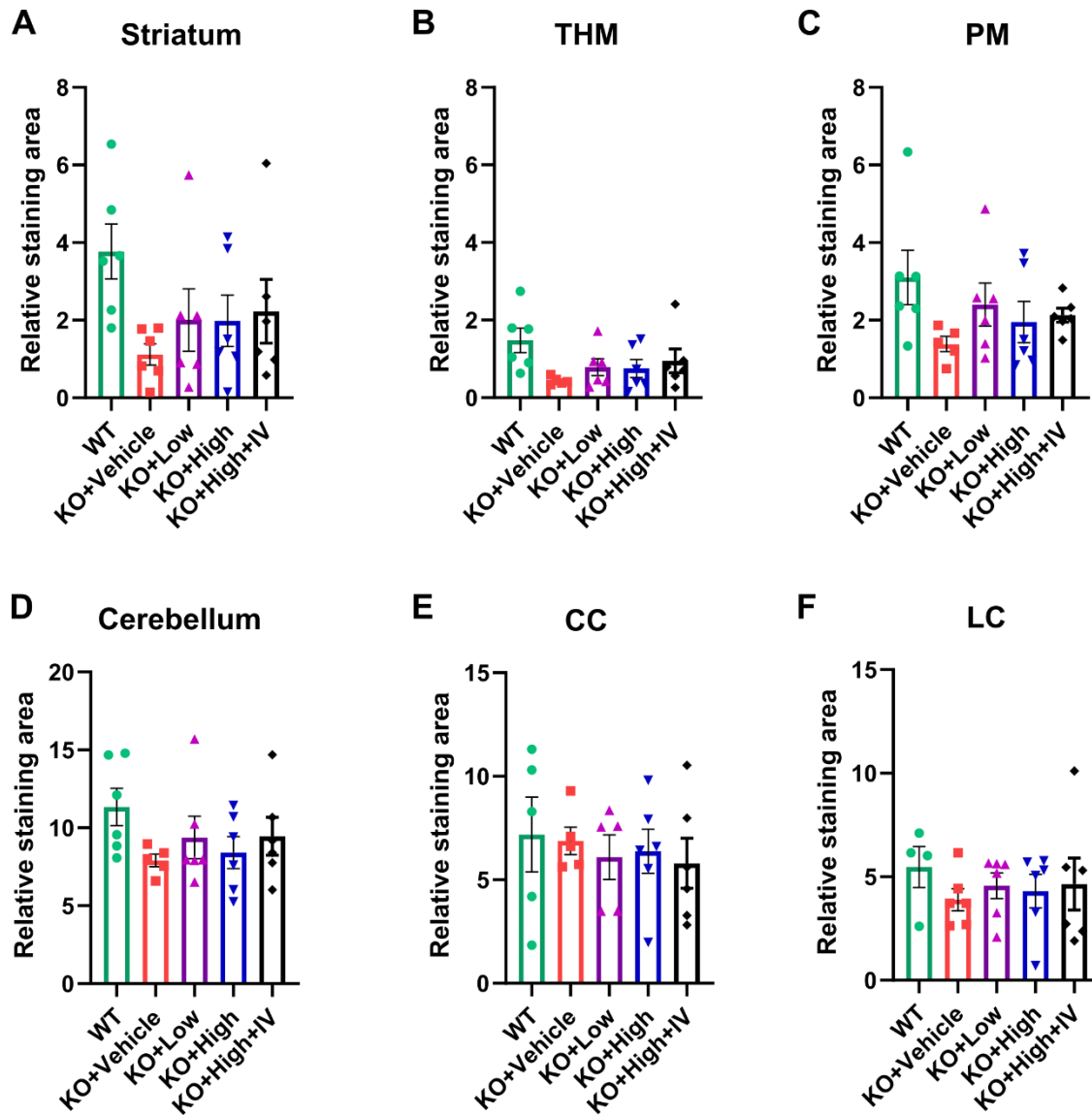


Figure S2. COX1 protein expression in brain regions and spinal cord. Data represent percent area staining positive for COX1 protein expression. THM: Thalamus, hypothalamus, and midbrain; PM: pons and medulla; CC: cervical cord; LC: lumbar cord. Each data point represents measurement from an individual animal, with bars representing the Mean ± SEM.

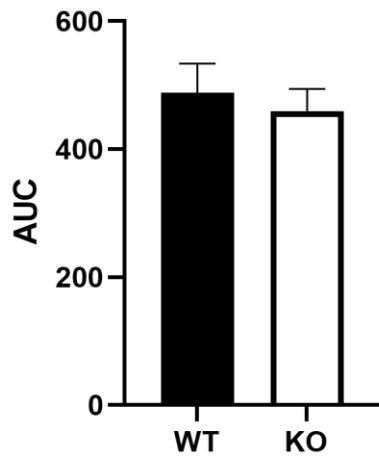


Figure S3. Rotarod performance of 12-months old mice. AUC: area under the curve. Bars representing the Mean \pm SEM.

Supplementary item #2: Tables

Table S1. Individual body weight and statistics

Mouse Number	Sex	Genotype	Weight 8/7/19	Treatment	Weight on Dose Date	Dose Date	Weight 1 wk.	Weight 2 wk.	Weight 3 wk.	Weight 4 wk.	Weight 2 mo.	Weight 3 mo.	Weight 4 mo.	Weight 5 mo.	Weight 6 Mo.	Weight 7 Mo.	Weight 8 Mo.	Weight 9 Mo.	Weight 10 Mo.	Weight 11 Mo.	Weight 12 Mo.
1.1	F	WT	10.5	Vehicle	13.4	8/15/2019	15.5	16.8	16.9	17.5	20.4	21.2	25.0	27.0	25.2	26.8	26.3	32.0	30.6	33.5	32.0
1.4	F	WT	9.7	Vehicle	15.8	8/15/2019	18.5	17.9	19.3	19.1	20.6	21.9	23.4	24.7	24.0	24.0	24.5	23.6	26.5	29.1	28.0
2.2	F	WT	8.7	Vehicle	12.5	8/13/2019	15.0	17.0	17.3	17.2	19.5	20.0	20.5	21.7	19.7	20.1	21.1	20.4	21.5	20.9	19.0
2.5	F	WT	8.5	Vehicle	12.6	8/13/2019	16.1	17.8	18.4	19.3	24.5	24.6	24.2	25.4	24.8	24.2	25.6	26.5	27.0	27.7	28.0
3.3	F	WT	9.0	Vehicle	14.2	8/16/2019	17.2	17.8	17.8	17.9	21.1	21.8	22.1	22.5	22.6	22.9	24.6	25.2	26.2	28.4	27.0
Mean			9.3		13.7		16.5	17.5	17.9	18.2	21.2	21.9	23.0	24.3	23.3	23.6	24.4	25.5	26.4	27.9	26.8
SD			0.8		1.4		1.4	0.5	0.9	0.9	1.9	1.7	1.8	2.2	2.2	2.4	2.0	4.3	3.2	4.5	4.8
5.1	M	WT	10.2	Vehicle	15.9	8/15/2019	18.4	19.3	20.2	20.3	23.3	26.4	27.5	27.6	28.7	29.4	30.4	29.9	30.8	30.5	30.0
5.4	M	WT	12.2	Vehicle	18.4	8/15/2019	20.0	21.5	23.0	24.7	27.6	29.6	31.5	32.4	34.2	34.6	35.0	35.4	36.4	36.8	34.0
6.2	M	WT	9.5	Vehicle	14.6	8/13/2019	18.7	21.1	22.6	24.0	26.9	28.3	30.1	31.0	32.1	34.4	35.6	35.8	38.5	39.8	39.0
6.5	M	WT	11.9	Vehicle	16.3	8/13/2019	18.8	20.5	22.8	24.4	28.0	30.1	32.2	32.3	33.7	35.0	37.0	37.7	38.8	40.4	39.0
7.3	M	WT	8.8	Vehicle	16.3	8/16/2019	20.8	22.5	23.2	23.8	27.8	30.2	31.9	33.2	34.6	36.1	38.1	40.0	39.8	41.2	41.0
Mean			10.5		16.3		19.3	21.0	22.4	23.4	26.7	28.9	30.6	31.3	32.7	33.9	35.2	35.8	36.9	37.7	36.6
SD			1.5		1.4		1.0	1.2	1.2	1.8	2.0	1.6	1.9	2.2	2.4	2.6	3.0	3.7	3.6	4.4	4.5
1.3	F	WT	10.2	2 x 10 ¹¹ vg	13.4	8/15/2019	16.9	17.0	17.4	17.5	19.0	22.1	24.3	25.1	23.6	26.0	26.4	28.8	30.0	30.5	29.0
2.1	F	WT	10.1	2 x 10 ¹¹ vg	14.3	8/13/2019	17.4	17.6	18.0	19.0	22.1	23.5	23.0	23.5	23.8	25.5	28.4	29.3	29.4	30.8	34.0
2.4	F	WT	8.2	2 x 10 ¹¹ vg	11.9	8/13/2019	14.7	15.7	16.7	16.9	18.8	19.0	21.2	22.3	21.2	21.7	23.2	23.8	26.2	26.7	27.0
3.2	F	WT	9.3	2 x 10 ¹¹ vg	15.3	8/16/2019	17.8	18.5	19.2	19.5	21.1	22.1	22.7	23.6	24.7	20.0	24.3	24.4	24.6	26.4	25.0
3.5	F	WT	9.6	2 x 10 ¹¹ vg	14.5	8/16/2019	18.2	18.7	19.3	18.6	21.3	21.9	22.5	23.5	23.5	24.6	25.1	25.0	26.6	25.0	28.0
Mean			9.5		13.9		17.0	17.5	18.1	18.3	20.5	21.7	22.7	23.6	23.4	23.6	25.5	26.3	27.4	27.9	28.6
SD			0.8		1.3		1.4	1.2	1.1	1.1	1.5	1.6	1.1	1.0	1.3	2.6	2.0	2.6	2.3	2.6	3.4
5.3	M	WT	10.8	2 x 10 ¹¹ vg	15.8	8/15/2019	18.1	18.7	20.5	21.0	23.2	25.0	27.3	27.5	29.1	30.2	30.9	31.2	33.2	34.0	32.0
6.1	M	WT	11.4	2 x 10 ¹¹ vg	17.0	8/13/2019	20.0	22.1	23.8	25.1	28.2	29.8	30.8	31.8	32.2	34.0	36.0	35.6	38.0	39.8	39.0
6.4	M	WT	9.5	2 x 10 ¹¹ vg	14.0	8/13/2019	18.6	21.1	22.9	24.6	28.9	30.8	32.4	33.5	32.8	34.8	36.9	36.6	38.0	38.3	35.0
7.2	M	WT	11.9	2 x 10 ¹¹ vg	17.9	8/16/2019	19.8	22.7	23.6	25.0	26.4	28.1	28.9	30.5	30.4	32.4	33.3	34.5	35.5	37.1	36.0
7.5	M	WT	11.4	2 x 10 ¹¹ vg	17.2	8/16/2019	19.4	22.1	23.1	24.1	26.9	29.2	30.0	31.1	31.2	31.5	32.9	34.5	33.7	36.0	32.0
Mean			11.0		16.4		19.2	21.3	22.8	24.0	26.7	28.6	29.9	30.9	31.1	32.6	34.0	34.5	35.7	37.0	34.8
SD			0.9		1.5		0.8	1.6	1.3	1.7	2.2	2.2	1.9	2.2	1.5	1.9	2.4	2.0	2.3	2.2	2.9
1.2	F	WT	11.7	8 x 10 ¹¹ vg	13.9	8/15/2019	14.2	14.6	15.3	15.8	18.5	19.4	20.1	21.1	20.9	21.1	21.4	24.5	22.8	23.9	23
1.5	F	WT	9.8	8 x 10 ¹¹ vg	14.0	8/15/2019	16.0	16.4	17.5	17.1	20.9	20.4	22.4	21.1	21.6	22.2	23.2	23.3	24.7	25.1	24.0
2.3	F	WT	9.0	8 x 10 ¹¹ vg	13.2	8/13/2019	16.0	17.4	18.4	19.5	22.3	22.8	24.1	27.3	25.3	26.9	30.1	30.5	34.2	32.8	35.0
3.1	F	WT	10.2	8 x 10 ¹¹ vg	14.7	8/16/2019	16.2	17.6	17.4	18.0	20.7	21.8	22.2	23.7	24.1	24.6	25.7	26.7	29.5	34.5	35.0
3.4	F	WT	9.5	8 x 10 ¹¹ vg	14.9	8/16/2019	16.9	17.3	18.3	19.3	21.6	22.2	22.8	23.7	25.2	27.0	29.1	28.5	28.8	29.8	32.0
Mean			8.9		12.7		14.2	15.2	15.9	16.5	19.0	19.6	20.5	21.4	21.4	22.3	23.7	24.3	25.4	26.5	26.7
SD			1.0		0.7		1.0	1.2	1.2	1.5	1.4	1.4	1.4	1.4	2.5	2.1	2.7	3.7	2.9	4.5	4.6
5.2	M	WT	12.0	8 x 10 ¹¹ vg	18.6	8/15/2019	19.4	20.3	21.0	21.8	24.2	25.8	27.9	28.3	29.5	30.8	32.6	32.8	34.8	34.3	35.0
5.5	M	WT	11.4	8 x 10 ¹¹ vg	16.3	8/15/2019	16.9	17.0	18.7	20.2	22.4	24.9	26.5	26.8	28.2	28.9	30.1	30.5	31.6	33.0	29.0
6.3	M	WT	11.3	8 x 10 ¹¹ vg	16.2	8/13/2019	17.4	19.3	21.2	22.5	25.5	26.0	27.6	28.7	28.5	29.9	29.2	30.7	30.9	31.8	30.0
7.1	M	WT	8.7	8 x 10 ¹¹ vg	17.4	8/16/2019	20.9	22.8	24.4	25.0	28.7	30.6	31.7	33.0	32.3	34.0	34.3	36.0	35.0	36.0	34.0
7.4	M	WT	10.5	8 x 10 ¹¹ vg	20.0	8/16/2019	22.3	24.3	26.2	27.7	31.4	32.9	34.1	34.8	35.2	36.9	37.8	39.2	40.0	41.1	39.0
Mean			10.8		17.7		19.4	20.7	22.3	23.4	26.4	28.0	29.6	30.3	30.7	32.1	32.8	33.8	34.5	35.2	33.4
SD			1.3		1.6		2.3	2.9	3.0	2.9	3.6	3.5	3.2	3.4	3.0	3.3	3.4	3.7	3.6	3.6	4.0

Table S2. Histopathology of mice injected with AAV9/hSURF1opt vectors

See file "Table S2.xlsx"

- "x" indicates the described abnormalities do not present
- "Mild, Moderate, Mild to moderate, Severe" indicates the degree of damage
- "Absent" indicates the histologist could not find the tissue
- "NA" indicates the tissue was not collected
- Mass on left horn of uterus. This is uncommon stromal change found in older mice
- No abnormalities were found in the lumbar spine however many sections were too caudal and represented the cauda equina instead of lumbar spinal cord.

Supplementary item #3: AAV9/hSURF1 certification of analysis



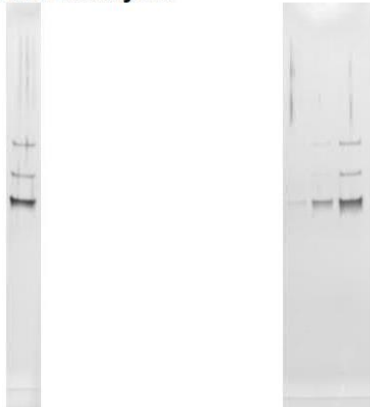
Quality Control Summary

Lot #	LAV93-final	Name	SC-CBh-hSURF1opt-BGH
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Test by qPCR

Test #	Titer, vg/mL	Analyst	Date	File
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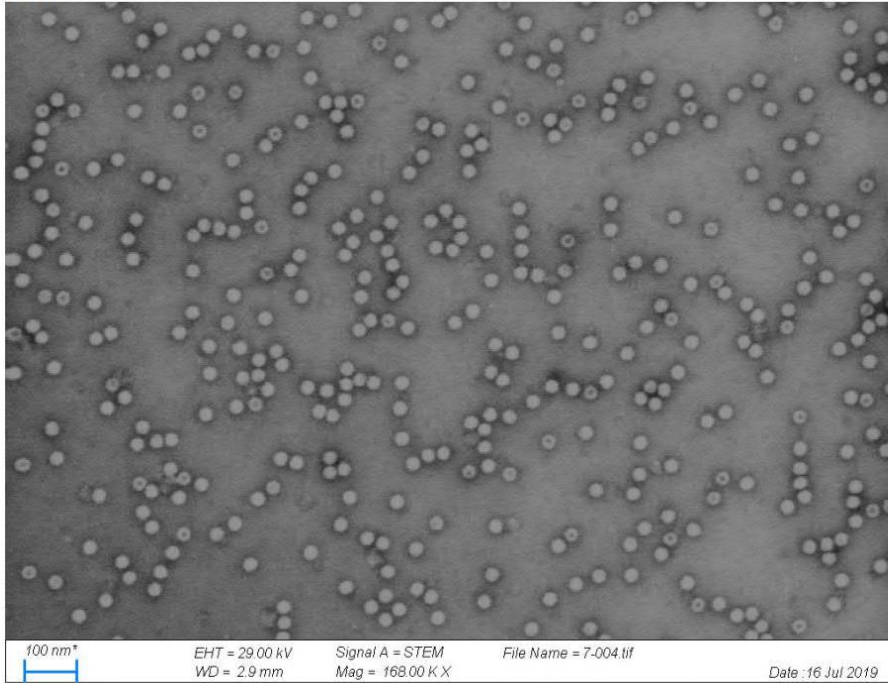
PAGE analysis



Loaded 5.00E+09 vg 4980E std 2e9vg 5e9vg 1e10vg
Calculated 5.70E+09 vg

Analyst	Ping Zhang
Date	07/02/2019
Reference #	20190702-silver

SEM



88% full

Analyst	Ping Zhang
Date	07/16/2019
Reference #	20190716-7-004