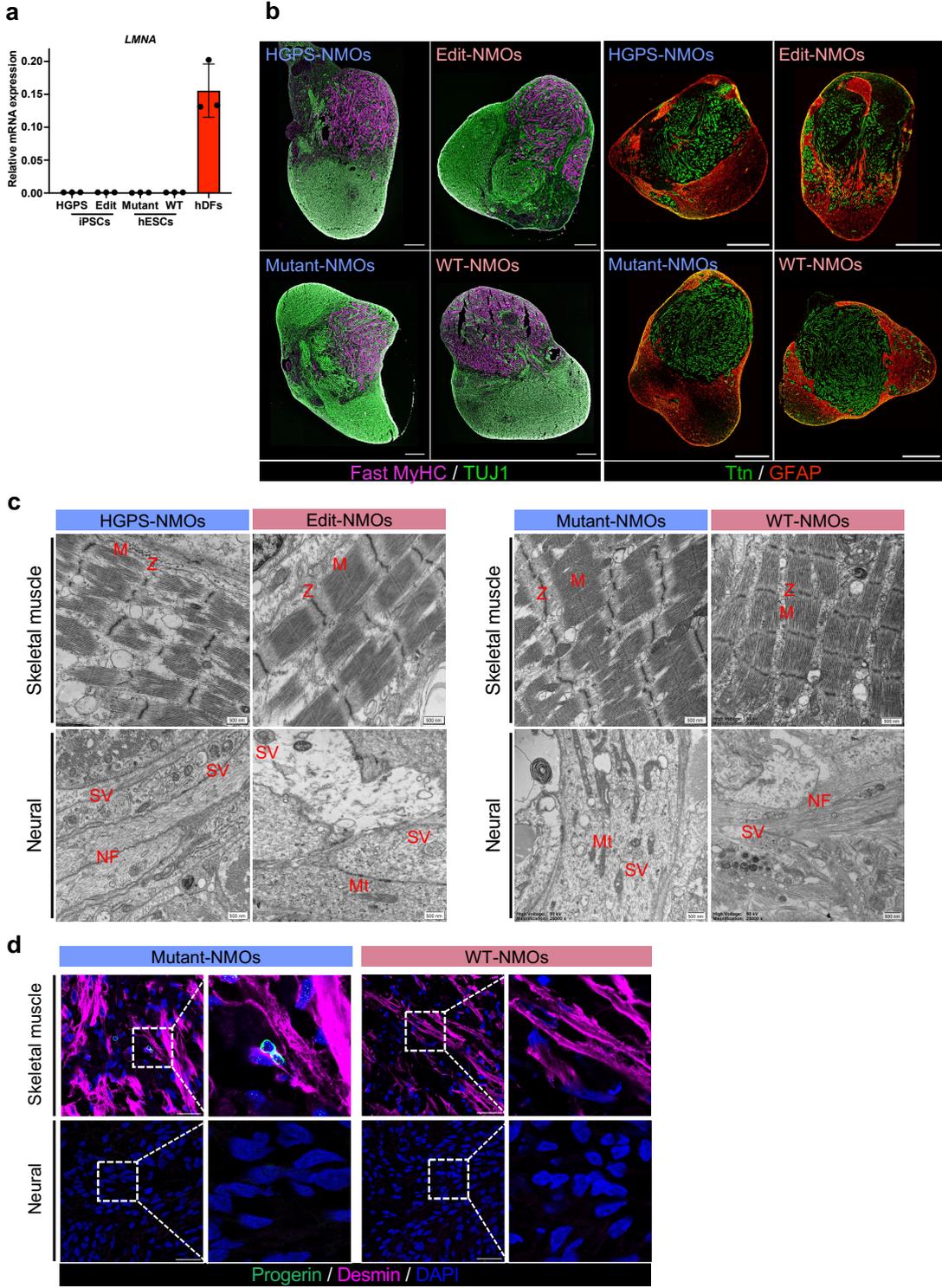


1 **Mutation-Agnostic Base Editing of the Progerin Farnesylation Site Rescues Hutchinson-**  
2 **Gilford Progeria Syndrome Phenotypes in Neuromuscular Organoids**  
3 **Running Title:** Mutation-Agnostic Editing of Lamin A Farnesylation in Progeria  
4 Dong-Woo Kim<sup>\*</sup>, Eun-Ji Kwon<sup>\*</sup>, Beom-Jin Jeon, Dong-Hyeok Kwon, Goo Jang, Youngyoon  
5 Yoon, Yuna Hwang, Hyukjin Lee and Hyuk-Jin Cha<sup>#</sup>  
6

# Extended Data Figure 1

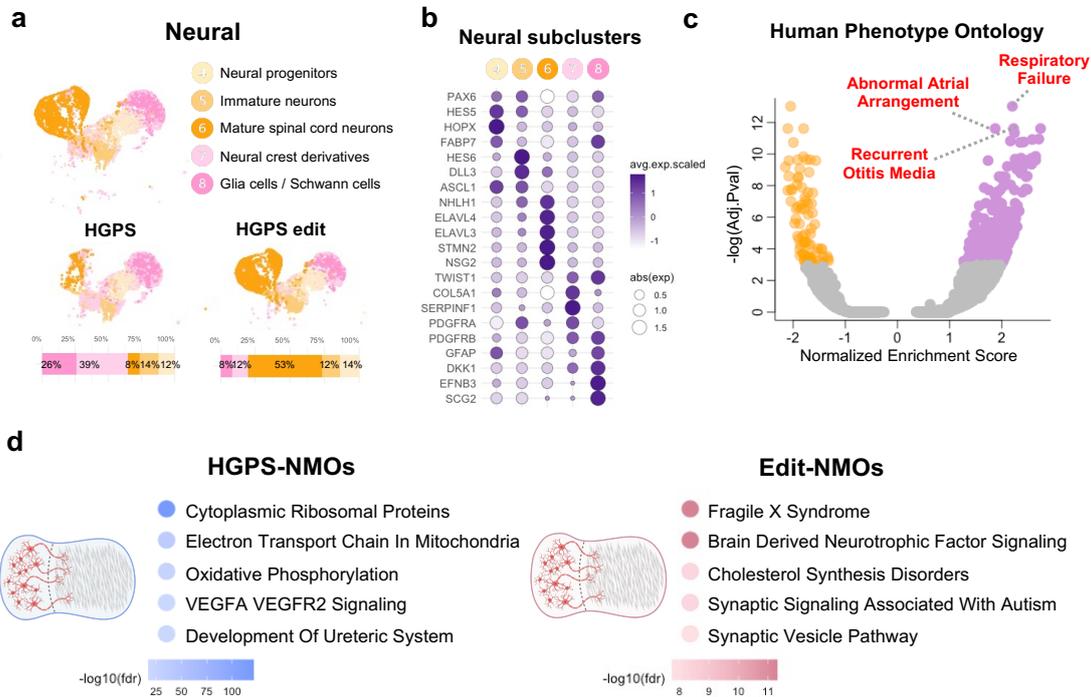


1 **Extended Data Fig. 1: Lamin A expression in two sets of hPSCs and establishment of two**  
2 **sets of isogenic NMO pairs.**

3 **a**, Real-time PCR analysis of LMNA (Lamin A), GAPDH as an internal control. **b**,  
4 Immunofluorescence images showing established NMOs derived from two sets of isogenic  
5 hPSCs at day 40 and day 70. At day 40, fast myosin heavy chain (magenta, muscle marker)  
6 and Tuj1 (green, neuron marker) are shown. At day 70, Ttn (green, muscle marker) and GFAP  
7 (red, glial marker) are shown. Scale bars, 200  $\mu\text{m}$  (day 40) and 500  $\mu\text{m}$  (day 70). **c**,  
8 Transmission electron microscopy images of day 70 HGPS-, Edit-, Mutant-, and WT-NMOs  
9 showing two major functional compartments. The muscle region displays advanced sarcomeric  
10 organization, including aligned myofibrils with distinct Z-lines (Z) and M-bands (M). The  
11 neural region reveals neurofilaments (NF), mitochondria (Mi), and synaptic vesicles (SV),  
12 indicative of mature synaptic structures. Scale bars are indicated in each panel. **d**,  
13 Immunofluorescence images of day 40 Mutant- and WT-NMOs stained with Progerin (green),  
14 Desmin (magenta), and DAPI (blue). Scale bar, 25  $\mu\text{m}$ .

15

## Extended Data Figure 2



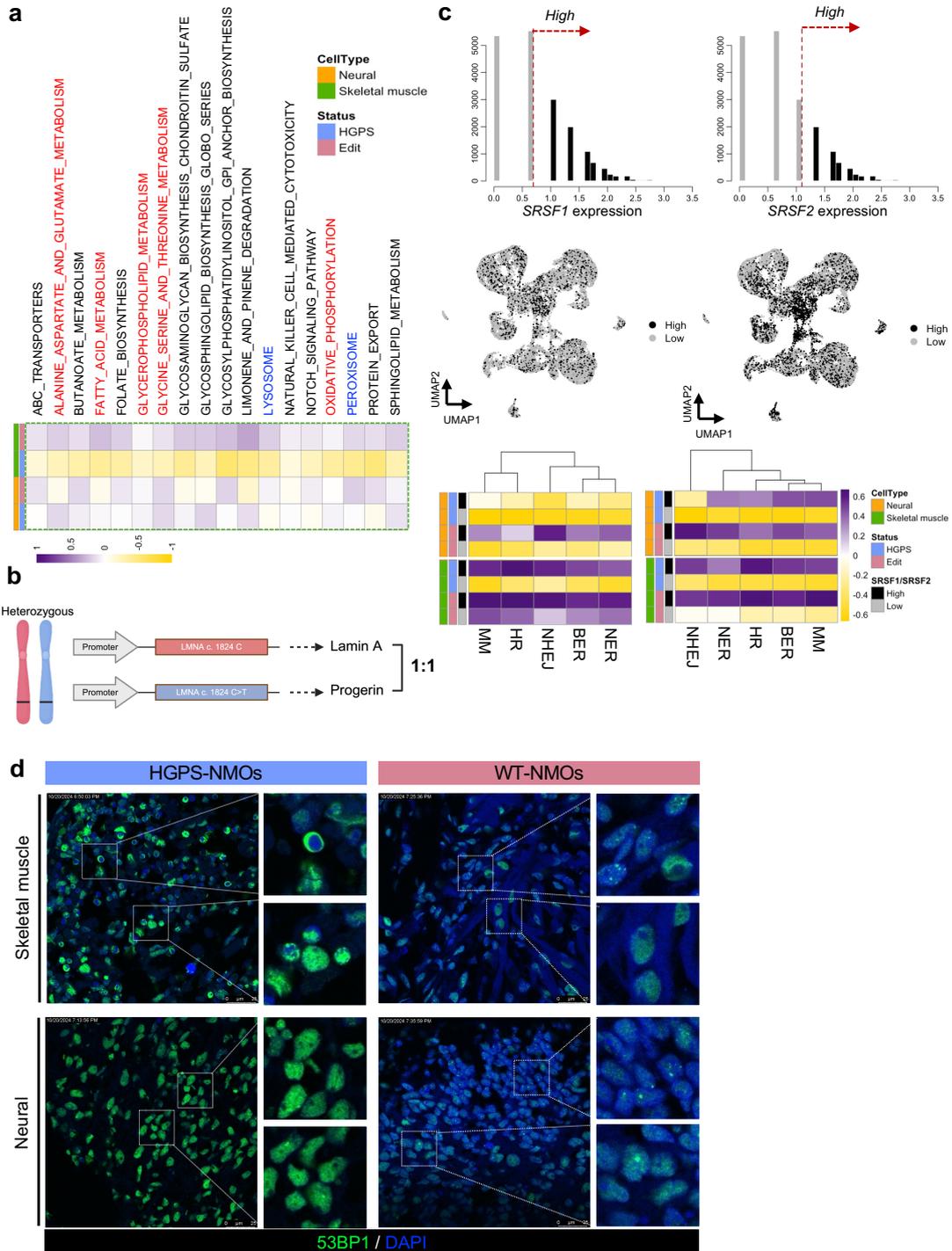
1

2 **Extended Data Fig. 2: Single-cell analysis of neural cellular composition and**  
 3 **developmental trajectories in HGPS and Edit NMOs.**

4 **a**, Sub-clustering of the neural trajectories reveals 5 distinct subpopulations. Shades of orange  
 5 and pink indicate neural subclusters. Relative proportions of each subcluster in HGPS  
 6 organoids are shown below. **b**, Dot plot showing representative gene expression across three  
 7 neural subclusters. **c**, Human Phenotype Ontology terms enriched in HGPS neural cells  
 8 compared to Edit controls. **d**, Pathway enrichment analysis of neuronal compartments in  
 9 HGPS- and Edit-NMOs.

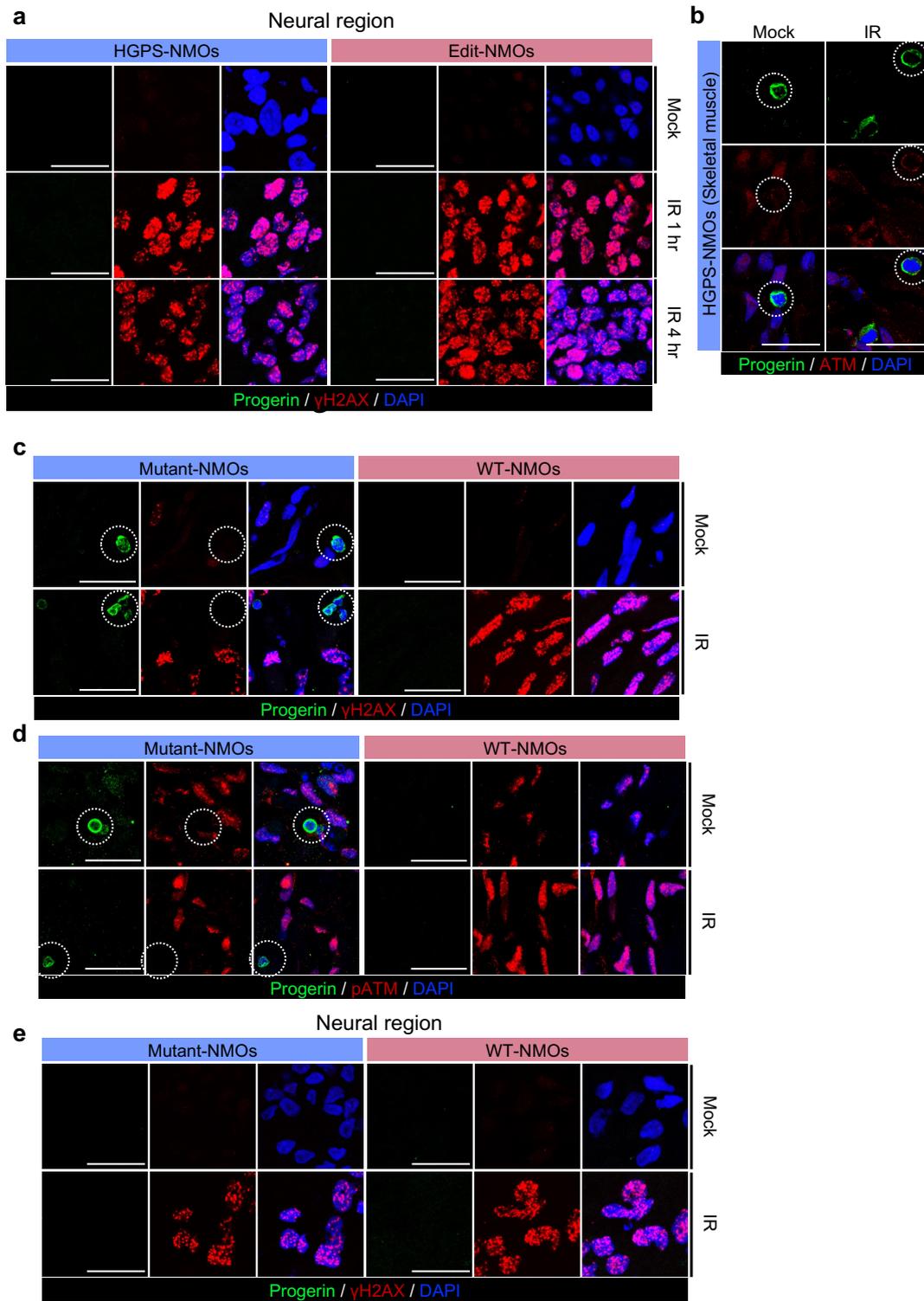
10

### Extended Data Figure 3



1 **Extended Data Fig. 3: Single-cell analysis and altered 53BP1 localization in HGPS-NMOs**  
2 **a**, Heatmap showing GSVA-based KEGG pathway enrichment in neuronal and muscle  
3 compartments of HGPS- and Edit-NMOs. Gene sets related to metabolism are highlighted in  
4 red, and those related to autophagy are highlighted in blue. **b**, Schematic illustrating the  
5 heterozygous expression of LMNA and Progerin in HGPS iPSCs, driven by a shared promoter  
6 resulting in a 1:1 expression ratio. **c**, Cells with SRSF1 and SRSF2 expression in the top 25%  
7 were designated as the “SRSF1/ SRSF2-high” group (top). UMAP visualization shows the  
8 distribution of SRSF1/ SRSF2-high and SRSF1/ SRSF2-low groups across organoids (middle).  
9 Heatmap displaying enrichment of DNA repair–related gene sets in SRSF1/ SRSF2-high and  
10 SRSF1/ SRSF2-low groups within the neuronal and muscle compartments of HGPS and Edit  
11 organoids (bottom). **d**, Immunofluorescence images of day 50 HGPS and WT NMOs stained  
12 with 53BP1 (green), and DAPI (blue), showing both neuronal and muscle regions. Scale bar,  
13 25  $\mu\text{m}$ .  
14

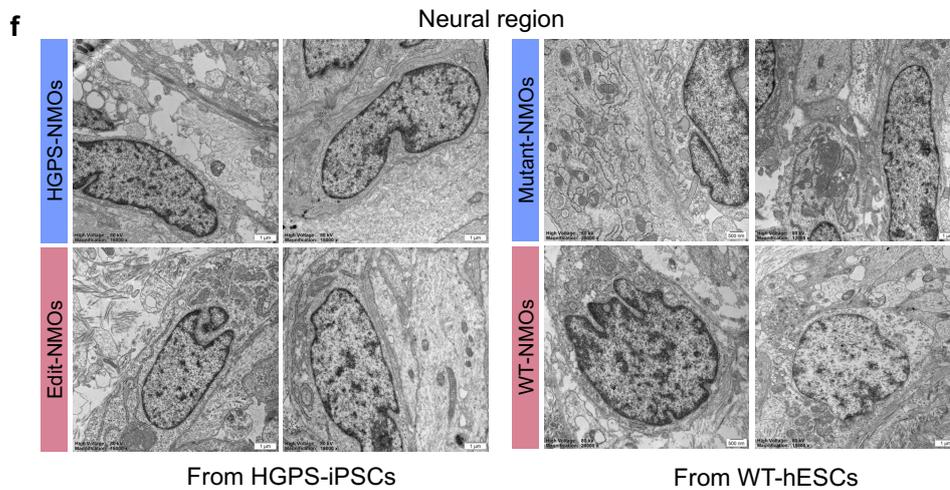
### Extended Data Figure 4



1

2

## Extended Data Figure 4



1

2 **Extended Data Fig. 4: Impaired foci formation of DNA damage repair machinery**  
3 **specifically in the muscular compartment of HGPS and mutant NMOs, and**  
4 **heterochromatin organization in the neural compartment.**

5 **a**, Immunofluorescence images of day 70 HGPS and Edit NMOs stained with Progerin (green),

6  $\gamma$ H2AX (red), and DAPI (blue), showing the neuronal region under mock, 1-hour, and 4-hour

7 conditions following 5 Gy X-ray irradiation. Scale bar, 25  $\mu$ m. **b**, Immunofluorescence images

8 of day 70 HGPS and Edit NMOs stained with Progerin (green), pATM (red), and DAPI (blue),

9 showing the muscle region under mock and 1-hour conditions following 5 Gy X-ray irradiation.

10 Scale bar, 25  $\mu$ m. **c, d**, Immunofluorescence images of day 70 Mutant and WT NMOs stained

11 with Progerin (green),  $\gamma$ H2AX (red), and DAPI (blue), showing both muscle and neuronal

12 regions under mock and 1-hour conditions following 5 Gy X-ray irradiation. Scale bar, 25  $\mu$ m.

13 **e**, Immunofluorescence images of day 70 mutant and WT NMOs stained with Progerin (green),

14 pATM (S1981) (red), and DAPI (blue), showing the muscle region under mock and 1-hour

15 conditions following 5 Gy X-ray irradiation. Scale bar, 25  $\mu$ m. **f**, Transmission electron

16 microscopy (TEM) images showing the nuclear heterochromatin structure in the neural region

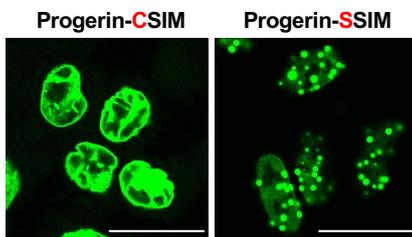
17 of neuromuscular organoids (NMOs) derived from two isogenic HGPS hPSC pairs.

## Extended Data Figure 5

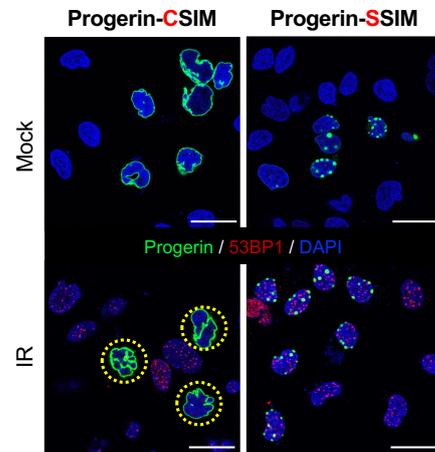
**a**

Class	Variant	Protospacer sequence (example)	Editing tool	Concern
Classical	c. 1824C>T (antisense G>A)	GGTCCACCCACCTGGGCTCC	ABE	-
	c. 1821G>A	GTAGGCGGACCCATCTCCTC	ABE	Bystander 'A', Suboptimal
	c. 1822G>A	GTGAGCGGACCCATCTCCTC	ABE	Bystander 'A'
	c. 1868C>G (antisense G>C)	GCTGCGACTGACCGTGACAC	PE, CGBE	Transversion mutation
Non-classical	c. 1940T>G (antisense A>C)	GCTGGAGTTGCCAGGCGGT	PE	Transversion mutation
	c. 1968G>A	GAACCAAGTGAGTTGTCTC	ABE	Bystander 'A'
	c. 1968+1 G>A	CAGATGAGTTGTCTCTGCTT	ABE	Bystander 'A'
	c. 1968+5 G>A	CAGGTGAATTGTCTCTGCTT	ABE	Bystander 'A'

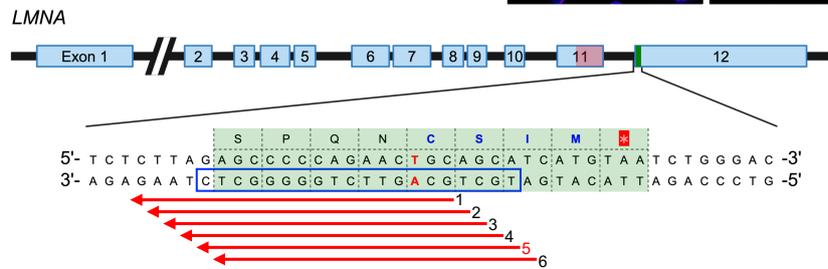
**b**



**c**



**d**



No	sgRNA Sequence	PAM	ABE version
1	GCA GTTCTGGGGCTCTAAG	AGA	SpRY ABE8e / NG-ABEmax
2	TGCA GTTCTGGGGCTCTAA	GAG	SpRY ABE8e
3	CTGCA GTTCTGGGGCTCTA	AGA	SpRY ABE8e / NG-ABEmax
4	GCTGCA GTTCTGGGGCTCT	AAG	SpRY ABE8e
5	TGCTGCA GTTCTGGGGCTC	TAA	SpRY ABE8e
6	ATGCTGCA GTTCTGGGGCT	CTA	SpRY ABE8e

1

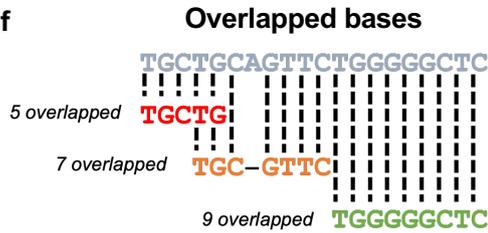
2

## Extended Data Figure 5

e

Region	Name	Target	Chromosome	Mismatch	Conversion Ratio
Exon	LMNA	TGCTGCAGTTCTGGGGGCTC	chr1:156139092	0	Mock: P(14):48.3% Treated: P(14):0%
Exon	MYO1E	TGCTGgAGTTCTGGGtGCTC	chr15:59173819	2	Mock: P(14):0% Treated: P(14):0%
Exon	SLC4A3	TGCTGgAGTgCTGGGGGCTC	chr2:219629688	2	Mock: P(14):0% Treated: P(14):0%
Exon	TARS2	TtCTGgAGTTCTGGGGGCTC	chr1:150507161 chr1:150507162	2	Mock: P(14):0% Treated: P(14):0% Mock: P(15):0% Treated: P(15):0.22%
Exon	TSKU	TGCTGCAGTTCTGGgGcCTC	chr11:76797647 chr11:76797655	2	Mock: P(6):0% Treated: P(6):0% Mock: P(14):0% Treated: P(14):0%

f



REF	ALT	DP	Info	Ratio	Ref_values	Alt_values	Overlapped (5'→3')	Overlapped (3'→5')	Gene	SNP
<b>WT</b>										
0	421	421	3;195782558	1	A	G	5	3	MUC4	rs28542401
0	642	642	6;32580950	1	A	G	5	6	HLA-DRB1	rs9269768
361	406	767	13;113816318	0.5293351	A	G	6	5	TMEM255B	rs67559311
1	230	231	1;39413694	0.995671	T	C	7	7	MACF1	rs604316
0	350	350	5;711867	1	T	C	7	9	ZDHHC11B	rs74987061
451	482	933	5;796162	0.5166131	T	C	7	9	ZDHHC11	rs386219
199	289	488	11;1095004	0.5922131	T	C	4	5	MUC2	rs1273413249
165	248	413	11;1095015	0.6004843	T	C	4	5	MUC2	rs1355085893
<b>Plasmid</b>										
109	132	241	1;201211020	0.5477178	A	G	9	4	IGFN1	rs145517342
436	1009	1445	3;195783103	0.6982699	A	G	3	4	MUC4	rs139925940
4	715	719	3;195784719	0.9944367	A	G	5	4	MUC4	rs3103955
42	1064	1106	3;195785122	0.9620253	A	G	4	4	MUC4	rs3107749
4	510	514	3;195784654	0.9922179	T	C	5	3	MUC4	rs199709753
196	181	377	1;156139092	0.4801061	T	C	6	20	LMNA	-

g

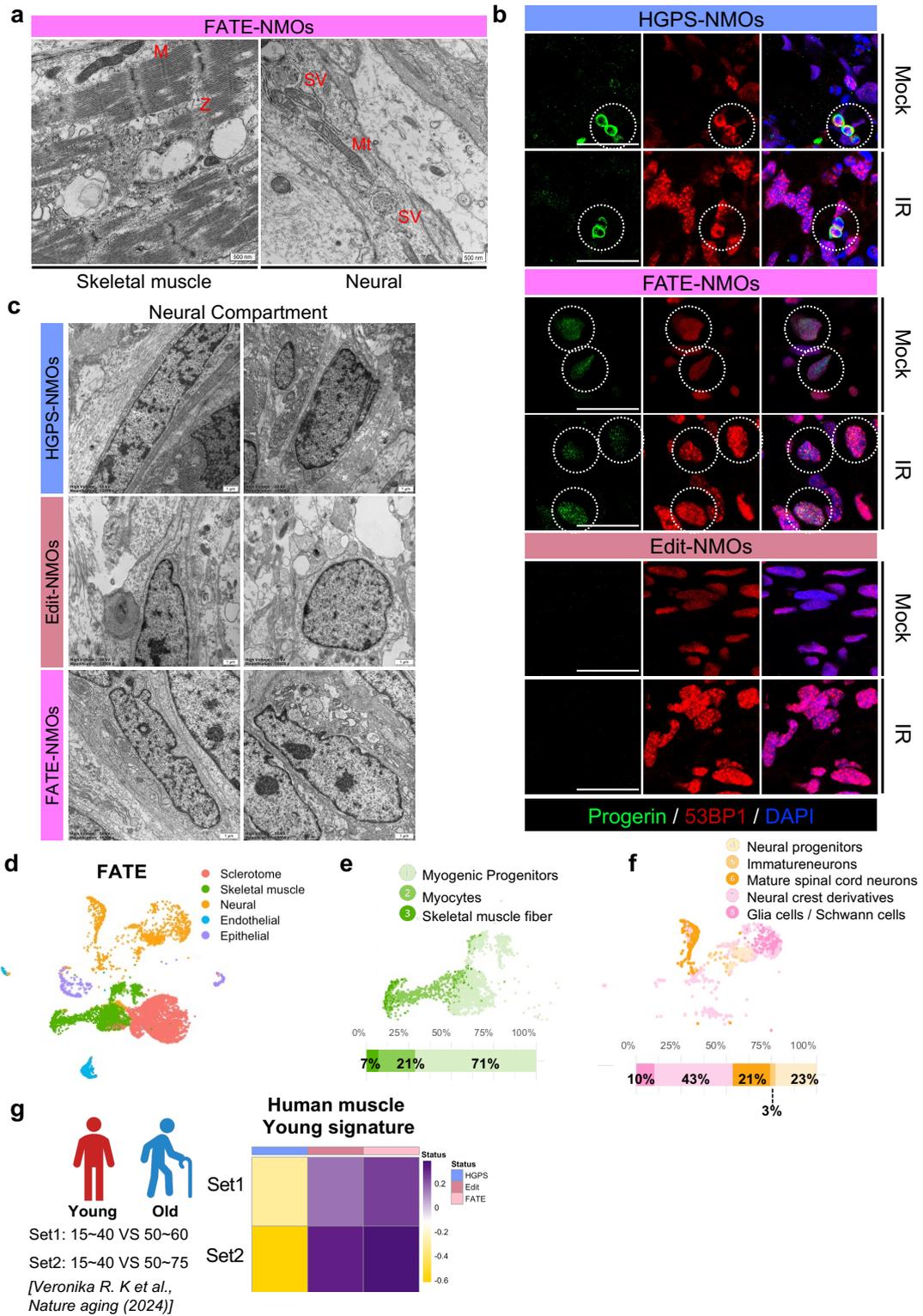
Region	Name	Target	Chromosome	Mismatch	Conversion Ratio	Note
Exon	LMNA	TGCTGCAGTTCTGGGGGCTC	chr1:156139092	0	Mock: P(14):0.06% Treated: P(14):42.6%	LMNA, Positive control
Intergenic	Off-Target1	TGCTGCAGTTCTGGGGGCTC	chr13:98581985	0	Mock: P(14):0.01% Treated: P(14):0.09%	STK24, SLC15A1 intergenic
Intergenic	Off-Target2	TGCTGCAGgTCTGGGGGCTC	chr12:128780232	1	Mock: P(14):0.01% Treated: P(14):0.01%	TMEM132C, SLC15A4 intergenic
Intergenic	Off-Target3	TGCTGCAGTTCTGGtGGCTC	chr11:76166363	1	Mock: P(14):0.22% Treated: P(14):18.2%	UVRAG, WNT11 intergenic

1 **Extended Data Fig. 5: Atypical HGPS variants, technical challenges, and FATE strategy**  
2 **including gRNA design and off-target site identification.**

3 **a**, Table summarizing classical and non-classical HGPS mutation types, including the mutation  
4 position, example protospacer sequences for sgRNA design, the corresponding genome editing  
5 tools (ABE or PE or CGBE), and associated concerns such as bystander editing or transversion  
6 mutations. **b**, Immunofluorescence images of cells overexpressing Progerin-GFP, Progerin  
7 (SSIM)-GFP with a C661S mutation. **c**, Immunofluorescence images of cells overexpressing  
8 Progerin-GFP or Progerin (SSIM)-GFP with a C661S mutation after 5 Gy X-ray irradiation,  
9 stained with 53BP1 (red) and DAPI (blue). Scale bar, 25  $\mu$ m. **d**, Table showing candidate  
10 sgRNA sequences, PAMs, and compatible ABE versions for targeting the CSIM motif in exon  
11 12 of the LMNA gene for FATE. The schematic above depicts the partial LMNA locus  
12 sequence and the relative binding positions of each sgRNA. **e**, Table showing genes, target  
13 sequences, genomic locations, mismatch numbers (0, 1, or 2), and A-to-G conversion ratios for  
14 candidate sites with high sequence similarity to the FATE gRNA identified by WES in mock-  
15 and FATE plasmid-treated groups. **f**, Top : Schematic illustrating the method for identifying  
16 the number of overlapping bases with nucleotide substitutions. Bottom : Table showing  
17 genomic locations of variants called from WES with  $\geq 200$  reads, A-to-G conversion ratios,  
18 REF and ALT nucleotide information (A, T, G, C), and sequence similarity to the FATE gRNA  
19 in both 5'→3' and 3'→5' orientations. **g**, Table showing names, target sequences, genomic  
20 locations, mismatch numbers (0 or 1), and A-to-G conversion ratios for candidate sites in non-  
21 coding regions, along with the genomic features of each location.

22

## Extended Data Figure 6



1

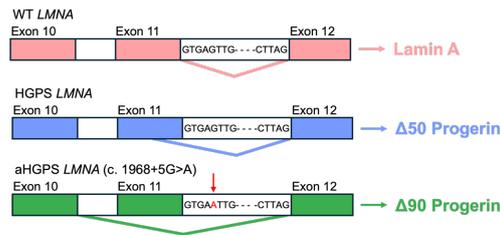
2

## Extended Data Figure 6

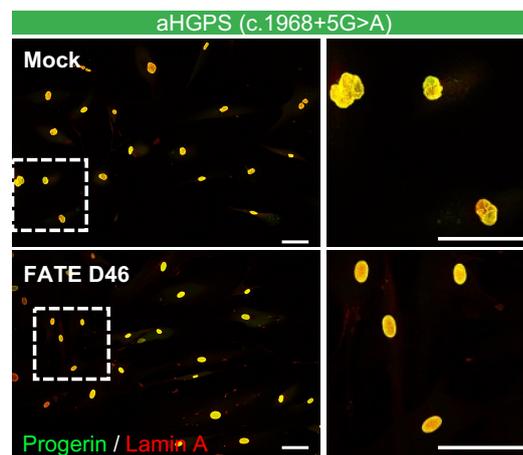
**h**

Cell Line #	Relation to Proband	Age at Donation	Gender	Mutational Analysis	Progerin Type
PSADFN576	Proband	34 yrs 11 mos	Female	LMNA Exon 11/Intron 11 junction, heterozygous c. 1968+5G>A	$\Delta 90$

**i**



**j**



1

2 **Extended Data Fig. 6: Integrated characterization of FATE-NMOs by single-cell and**  
 3 **molecular profiling, and assessment of FATE treatment in non-classical  $\Delta 90$  progerin**  
 4 **HGPS fibroblasts.**

5 **a**, Transmission electron microscopy images of day 70 FATE-NMOs showing two major  
 6 functional compartments. The muscle region displays advanced sarcomeric organization,  
 7 including aligned myofibrils with distinct Z-lines (Z) and M-bands (M). The neuronal region  
 8 reveals neurofilaments (NF), mitochondria (Mi), and synaptic vesicles (SV), indicative of  
 9 mature synaptic structures. Scale bars are indicated in each panel. **b**, Immunofluorescence  
 10 images of HGPS, Edit, and FATE NMOs stained with Progerin (green), 53BP1 (red), and DAPI  
 11 (blue). Scale bar, 25  $\mu$ m. **c**, Transmission electron microscopy (TEM) images showing the  
 12 nuclear heterochromatin structure in the neural region of neuromuscular organoids (NMOs)  
 13 derived from two isogenic HGPS hPSC pairs, including FATE-NMOs. **d**, Mapping of single-  
 14 cell transcriptomic data from FATE-treated neural-muscular organoids. **e**, Relative proportions  
 15 of sub-populations within FATE skeletal muscle cells, visualized in shades of green. **f**, Relative

1 proportions of sub-populations within FATE neural cells, visualized in shades of orange and  
2 pink. **g**, Heatmap showing enrichment of a “Young” gene expression signature—derived from  
3 transcriptomic profiles of young versus old human skeletal muscle—in HGPS, Edit, and FATE  
4 organoids. **h**, Table summarizing clinical and genetic characteristics of the non-classical HGPS  
5 fibroblast line (PSADFN576) obtained from the Progeria Research Foundation. Key details  
6 include the heterozygous *LMNA* mutation (c.1968+5G>A) at the exon 11/intron 11 junction,  
7 which results in the  $\Delta 90$  Progerin isoform. **i**, Schematic comparing pre-mRNA splicing patterns  
8 of WT *LMNA* (Lamin A), classical HGPS ( $\Delta 50$  Progerin), and atypical HGPS (aHGPS). The  
9 red arrow indicates the c.1968+5G>A mutation, which activates a cryptic splice site to generate  
10  $\Delta 90$  Progerin. **j**, Low-magnification immunofluorescence images of aHGPS fibroblasts at day  
11 46 post-transfection with Mock (top) or FATE plasmids (bottom). Cells were stained for  
12 Progerin (green) and Lamin A (red). Dashed boxes indicate regions shown in higher  
13 magnification. FATE treatment markedly reduced  $\Delta 90$  Progerin and restored Lamin A  
14 expression. Scale bars are indicated in each panel.

15

16

1 **Supplementary Information**

2 **Supplementary Video 1: 3D confocal imaging of HGPS-NMOs without X-ray irradiation**

3 Three-dimensional confocal imaging shows progerin (green), 53BP1 (red), and lamin A (grey)  
4 in HGPS-NMOs without X-ray irradiation.

5 **Supplementary Video 2: 3D confocal imaging of HGPS-NMOs with X-ray irradiation**

6 Three-dimensional confocal imaging shows progerin (green), 53BP1 (red), and lamin A (grey)  
7 in HGPS-NMOs 1 hour after 5Gy X-ray irradiation.

8 **Supplementary Video 3: 3D confocal imaging of Edit-NMOs without X-ray irradiation**

9 Three-dimensional confocal imaging shows progerin (green), 53BP1 (red), and lamin A (grey)  
10 in Edit-NMOs without X-ray irradiation.

11 **Supplementary Video 4: 3D confocal imaging of Edit-NMOs with X-ray irradiation**

12 Three-dimensional confocal imaging shows progerin (green), 53BP1 (red), and lamin A (grey)  
13 in Edit-NMOs 1 hour after 5Gy X-ray irradiation.

14 **Supplementary Video 5: 3D confocal imaging of FATE-NMOs without X-ray irradiation**

15 Three-dimensional confocal imaging shows progerin (green), 53BP1 (red), and lamin A (grey)  
16 in FATE-NMOs without X-ray irradiation.

17 **Supplementary Video 6: 3D confocal imaging of FATE-NMOs with X-ray irradiation**

18 Three-dimensional confocal imaging shows progerin (green), 53BP1 (red), and lamin A (grey)  
19 in FATE-NMOs 1 hour after 5Gy X-ray irradiation.

20

21

22

23

24

25

1 **Supplementary Table**

2

Supplementary Table 1: RT-PCR primers	
	Sequence (5'→3')
LMNA Fwd	TCTTCTGCCTCCAGTGTCACG
LMNA Rvs	AGTTCTGGGGGCTCTGGGT

3

Supplementary Table 2: PCR primers	
	Sequence (5'→3')
LMNA_1824_Fwd	TGGGCACAGAACCACACCTTC
LMNA_1824_Rvs	AGACAAAGCAGAGACAACCTCACCT
LMNA_1981_Fwd	CTCCTACCTCCTGGGCAACT
LMNA_1981_Rvs	GGCAGATGTGGAGTTTCCTG
Off-Target1_Fwd	CTGCCAGAAAAGGGTTCAGA
Off-Target1_Rvs	GCAAATCCAGCTTCAAGAGG
Off-Target2_Fwd	ATTCTCCCCTGATGCTGGAC
Off-Target2_Rvs	GTGGGCGCCTATAATTCCAG
Off-Target3_Fwd	GAGATGGAGTAGGGCTGGAG
Off-Target3_Rvs	CTCACGCTAAGGACTGAGGG

4