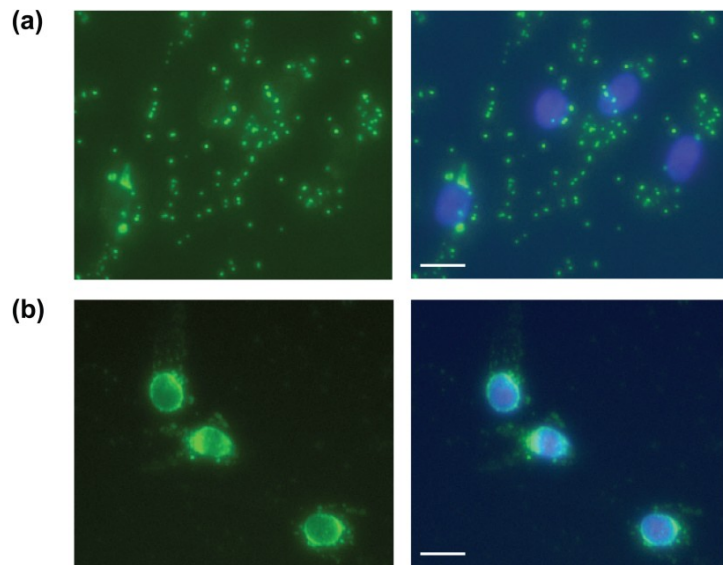
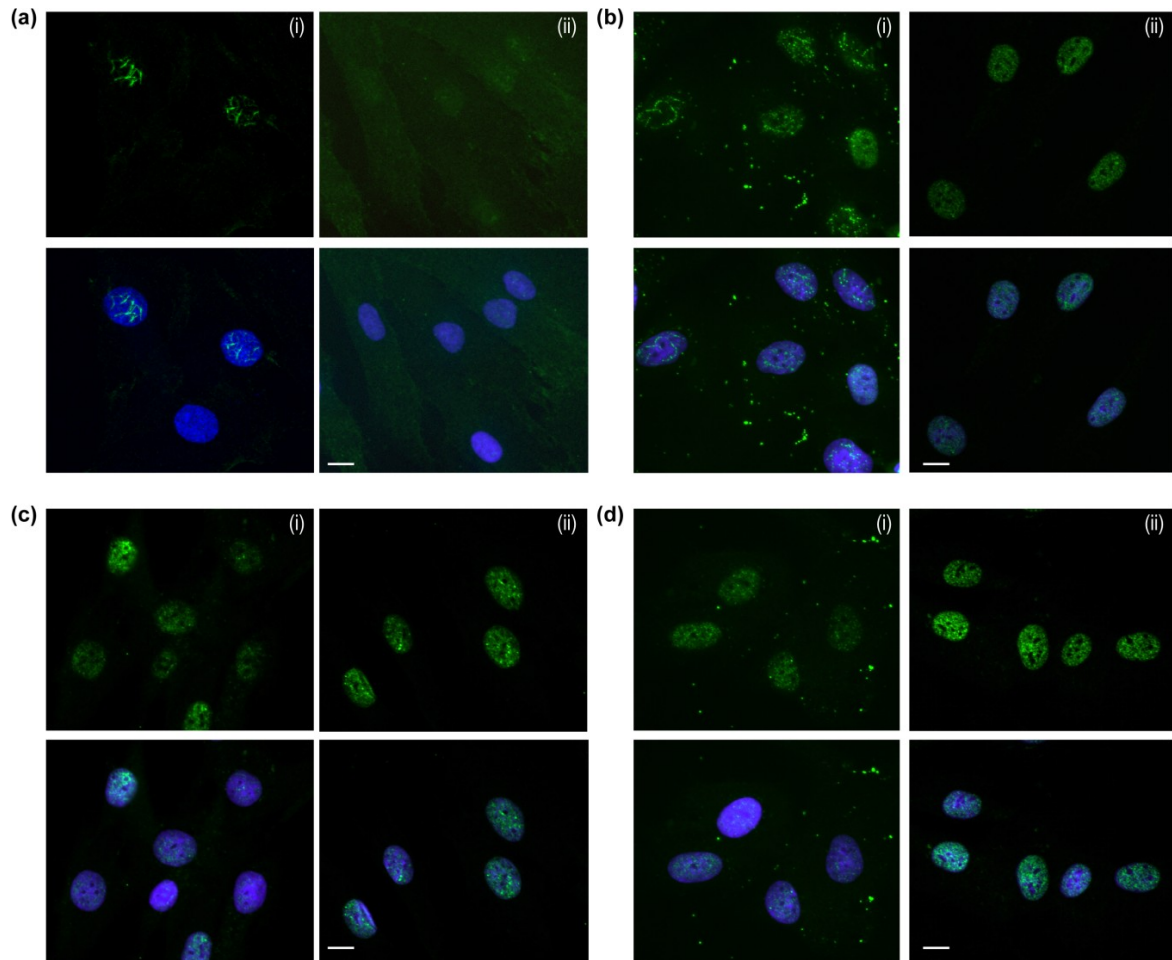


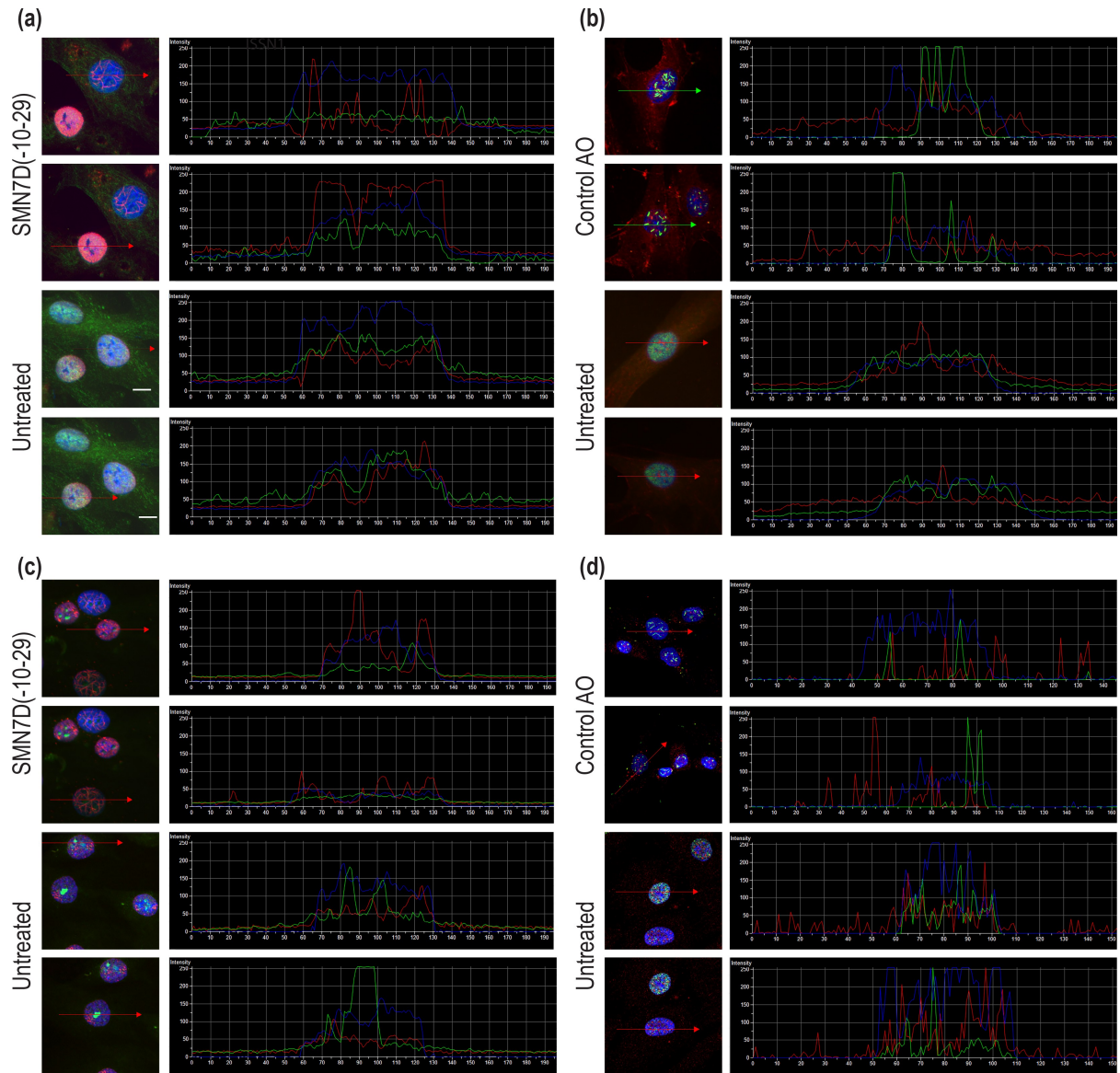
**Supplementary Figure 1.** Fluorescence staining of paraspeckle proteins in additional cell types, following transfection with (a) (i) 2' O-methyl phosphorothioate *SMN7D(-10-29)* in primary human myogenic cells; (b) (i) 2' O-methyl phosphorothioate AO targeting *Smn* in mouse myogenic cells; and (c) (i) the 2' O-methyl phosphorothioate control AO in SH-SY5Y neuroblastoma cells. (i) 2' O-methyl phosphorothioate-transfected and (ii) untreated shown in each panel. NONO was immunostained in human cells and SFPQ in mouse cells. Scale bar = 10  $\mu$ m.



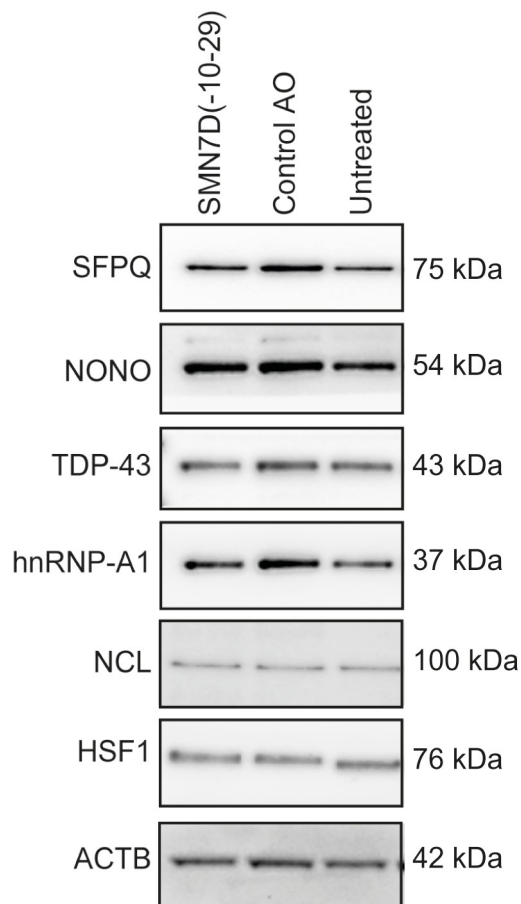
**Supplementary Figure 2.** Overlay of Hoechst and SFPQ staining in fibroblasts following 2'-O-methyl phosphorothioate transfection (AO 43, Supplementary Table 3 (100 nM, 24 hours), showing **(a)** cytoplasmic SFPQ staining in cells without nuclear inclusions after transfection with AO 43 (Table 3); and **(b)** staining of SFPQ around the nuclear envelope in cells without nuclear inclusions, transfected with AO 90 (Table 3). Scale bar = 10  $\mu$ m.



**Supplementary Figure 3.** Immunofluorescent staining of paraspeckle proteins following 2' O-methyl phosphorothioate *SMN7D(-10-29)* transfection (100 nM for 24 hours), showing **(a)** paraspeckle protein component 1 (PSPC1); **(b)** fused in sarcoma (FUS); **(c)** TAR-DNA binding protein 43 (TDP43); and **(d)** heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1); in (i) 2' O-methyl transfected and (ii) untreated cells shown in each panel. Scale bar = 10  $\mu$ m.



**Supplementary Figure 4.** Immunofluorescent staining of paraspeckle proteins following transfection of the 2' O-methyl phosphorothioate *SMN7D(-10-29)* (**a, c**) and Control AOs (**b, d**) (100 nM for 24 hours) and intensity profiling of selected cells showing (**a**) SFPQ (red) and SC35 (green); (**b**) NONO (green) and FBL (red); (**c**) SFPQ (red) and NCL (green); (**d**) NONO (green) and coilin (red).



**Supplementary Figure 5** Western blots of extracts from cells transfected with the 2' O-methyl phosphorothioate *SMN7D(-10-29)* and Control AOs (100 nM for 24 hours), probed with antibodies that recognize SFPQ, NONO, TDP-43, hnRNP-A1, NCL, HSF1 and ACTB (Supplementary Table 2).

**Supplementary Tables:**
**Supplementary Table 1** primer sequences and PCR conditions used in this study.

Primers	Concentration/amount of primer per reaction	Sequence 5'-3'	Temperature profile
<i>SMN</i>	25 ng	AGGTCTCCTGGAATAAATCAG TGGTGTCATTTAGTGCTGCTCT	55°C 30 min 94°C 2 min 25 cycles: 94°C 40 sec 56°C 30 sec 68°C 1 min
<i>BCL2</i>	25 ng	AAT GTC TCA GAG CAA CCG GG GGG AGG GTA GAG TGG ATG GT	55°C 30 min 94°C 2 min 25 cycles: 94°C 40 sec 60°C 30 sec 68°C 1 min
<i>5S</i>	50 nM	GGC CAT ACC ACC CTG AAC GC CAG CAC CCG GTA TTC CCA GG	95 °C 20 sec 40 cycles 95 °C 3 sec 60 °C 30 sec
<i>18S</i>	250 nM	GTA ACC CGT TGA ACC CCA TT CCA TCC AAT CGG TAG TAG CG	
<i>45S</i>	100 nM	TGT CAG GCG TTC TCG TCT C AGC ACG ACG TCA CCA CAT C	
<i>TBP</i>	500 nM	TCAGGCGTTCGGTGGATCGAGT AGTGATGCTGGGCACTGCGGAGAA	
<i>TUBB</i>	100 nM	CTT CGG CCA GAT CTT CAG AC AGA GAG TGG GTC AGC TGG AA	

**Supplementary Table 2** Antibodies used in this study, indicating dilutions used and detection method.

Protein	Protein name	Supplier	Catalogue #	Dilution		Conjugate
				IF	WB	
NONO	Non-POU domain containing octamer	Made in house	NA	1:1000	1:10000	mouse
SFPQ	Splicing factor proline & glutamine rich	Abcam	Ab38148	1:1000	1:8000	rabbit
PSPC1	paraspeckle protein component 1	Merck Millipore	HPA038904	1:50	NA	mouse
FUS	Fused in sarcoma	Santa Cruz	Sc-47711	1:500	NA	mouse
TDP-43	TAR-DNA binding protein 43	Protein Tech	10782-2-AP	1:300	1:3000	rabbit
hnRNP-A1	Heterogeneous nuclear ribonucleoprotein A1	Thermo Scientific	PA5-19431	1:700	1:2000	rabbit
NCL	Nucleolin	Thermo Scientific	39-6400	1:250	1:2000	mouse
FBL	Fibrillarin	Cell Signalling	2639	1:400	NA	rabbit
COIL	Coilin	Sapphire Bioscience	GTX1125710	1:250	NA	rabbit

LMNB1	Lamin B1	Protein Tech	12987-1-AP	1:500	NA	rabbit
SC35	Splicing factor SC35	Merk Millipore	04-1550	1:1000	NA	mouse
HSF1	Heat shock factor 1	Cell Signalling	12972	1:400	1:500	rabbit
ACTB	Beta actin	Sigma	A5441	NA	1:60000	mouse
P53	Tumor Protein 53	Novocasta	NCL-Lp53-D07	NA	1:1000	mouse

**Supplementary Table 3** Results of SFPQ immunostaining following transfection of fibroblasts with 2' O-methyl phosphorothioate AOs (100 nM, 24 hours). The nucleotide composition and length of each AO is indicated. Cells were stained for SFPQ and the percentages of cells with nuclear inclusions recorded, as was the percentage of cells showing cytoplasmic SFPQ aggregation.

AO #	Nucleotide composition				Length	Cells with nuclear inclusions	Total cell number	SFPQ-positive nuclear inclusions	Cytoplasmic SFPQ staining	Comments
	A	C	G	U						
1	4	9	4	8	25	56	56	100.0		
2	5	9	3	8	25	60	60	100.0		
3	3	8	3	11	25	107	109	98.2		
4	4	10	3	8	25	87	89	97.8		
5	4	8	7	6	25	118	121	97.5	>90%	
6	5	11	4	5	25	95	98	96.9		
7	6	7	8	4	25	29	30	96.7		
8	4	9	3	9	25	112	116	96.6		
9	4	7	3	11	25	76	79	96.2		
10	7	2	7	9	25	70	73	95.9		
11	6	2	3	14	25	128	134	95.5		
12	7	8	3	7	25	63	66	95.5		
13	7	7	3	8	25	55	58	94.8		
14	10	10	5	2	27	78	84	92.9		
15	10	7	2	6	25	130	140	92.9		
16	4	9	2	9	24	122	132	92.4		
17	11	3	9	7	30	101	110	91.8		
18	5	10	4	6	25	52	57	91.2		
19	4	6	8	7	25	29	32	90.6		
20	1	12	0	12	25	33	37	89.2		
21	15	2	3	2	22	44	50	88.0		
22	11	3	10	2	26	109	124	87.9	>90%	
23	6	6	4	4	20	95	111	85.6		
24	7	8	2	8	25	53	63	84.1		
25	4	9	2	11	26	118	142	83.1		
26	7	8	3	7	25	90	109	82.6		
27	5	7	8	6	26	98	119	82.4		
28	3	9	8	5	25	98	120	81.7		
29	3	14	6	3	26	40	50	80.0		
30	3	7	7	8	25	57	72	79.2		
31	0	8	3	14	25	88	113	77.9		
32	6	9	5	5	25	109	140	77.9		
33	18	2	6	3	29	100	129	77.5		
34	6	7	5	7	25	62	81	76.5		
35	3	10	3	12	28	104	136	76.5		
36	14	5	5	4	28	26	35	74.3		



37	3	9	6	7	25	56	77	<b>72.7</b>		
38	10	7	4	4	25	52	72	<b>72.2</b>		
39	9	8	4	4	25	114	159	<b>71.7</b>	>90%	
40	5	7	5	8	25	77	108	<b>71.3</b>		
41	1	9	4	4	18	72	109	<b>66.1</b>		
42	8	4	9	4	25	83	126	<b>65.9</b>	>90%	
43	3	10	8	4	25	86	131	<b>65.6</b>	>90%	
44	9	3	10	3	25	75	115	<b>65.2</b>	>90%	
45	8	4	7	6	25	47	74	<b>63.5</b>		
46	6	7	2	10	25	100	158	<b>63.3</b>		
47	10	3	5	7	25	65	103	<b>63.1</b>		
48	7	6	10	2	25	72	115	<b>62.6</b>	>90%	
49	7	7	8	11	33	25	40	<b>62.5</b>		
50	6	4	8	2	20	83	137	<b>60.6</b>	>90%	
51	4	8	6	7	25	54	93	<b>58.1</b>		
52	1	10	11	8	30	36	62	<b>58.1</b>		
53	2	7	8	8	25	78	138	<b>56.5</b>		
54	6	8	6	5	25	55	100	<b>55.0</b>		
55	7	5	8	4	24	68	125	<b>54.4</b>		
56	8	4	7	6	25	60	112	<b>53.6</b>		
57	5	7	6	7	25	40	77	<b>51.9</b>		
58	6	9	6	5	26	95	187	<b>50.8</b>		
59	8	4	5	8	25	34	67	<b>50.7</b>		
60	6	5	6	8	25	99	207	<b>47.8</b>		
61	6	3	13	3	25	23	49	<b>46.9</b>	>90%	
62	9	4	9	3	25	41	89	<b>46.1</b>	>90%	
63	8	7	7	3	25	55	121	<b>45.5</b>		
64	6	1	6	11	24	65	146	<b>44.5</b>		
65	3	7	8	7	25	57	134	<b>42.5</b>		
66	11	9	3	8	31	44	104	<b>42.3</b>		
67	3	10	7	5	25	22	55	<b>40.0</b>		
68	6	7	9	3	25	48	122	<b>39.3</b>	>90%	
69	2	9	13	1	25	43	114	<b>37.7</b>	>90%	
70	2	7	6	4	19	45	123	<b>36.6</b>		
71	6	7	9	3	25	36	104	<b>34.6</b>		
72	5	6	3	0	14	45	132	<b>34.1</b>		
73	9	7	5	4	25	30	90	<b>33.3</b>		
74	6	3	7	9	25	29	88	<b>33.0</b>	>90%	
75	5	10	8	2	25	52	160	<b>32.5</b>		
76	5	6	4	5	20	17	59	<b>28.8</b>		
77	6	6	8	1	21	33	115	<b>28.7</b>		
78	5	6	7	7	25	33	117	<b>28.2</b>		
79	3	8	5	9	25	39	143	<b>27.3</b>		
80	3	5	12	5	25	26	96	<b>27.1</b>	>90%	
81	7	8	7	3	25	21	78	<b>26.9</b>		
82	6	4	9	6	25	32	122	<b>26.2</b>	>90%	

83	4	10	6	5	25	40	156	<b>25.6</b>		
84	4	8	10	2	24	30	131	<b>22.9</b>		
85	4	5	9	5	23	15	134	<b>11.2</b>	>90%	
86	4	3	8	4	19	10	138	<b>7.2</b>	>90%	
87	2	4	11	8	25	8	146	<b>5.5</b>	>90%	
88	6	6	12	3	27	3	169	<b>1.8</b>	>90%	
89	9	4	11	1	25	2	161	<b>1.2</b>	>90%	
90	3	4	12	6	25	0	85	<b>0.0</b>		25% of cells show SFPQ localised to nuclear envelope
UT 1						0	122	<b>0.0</b>		
UT 2						0	85	<b>0.0</b>		