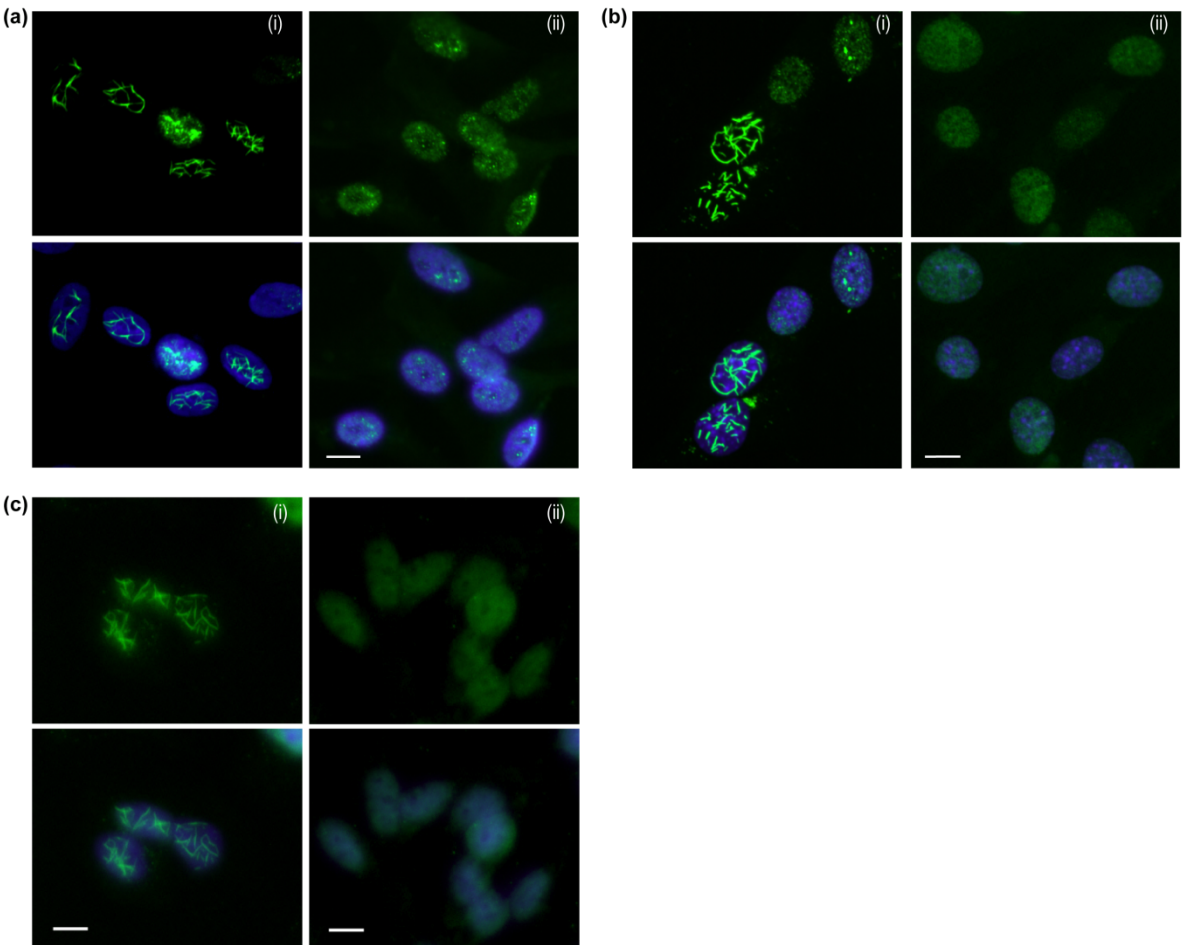
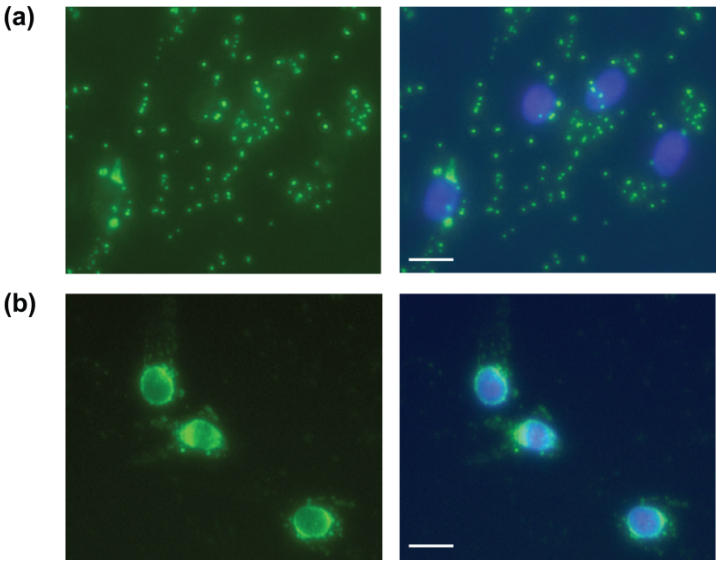
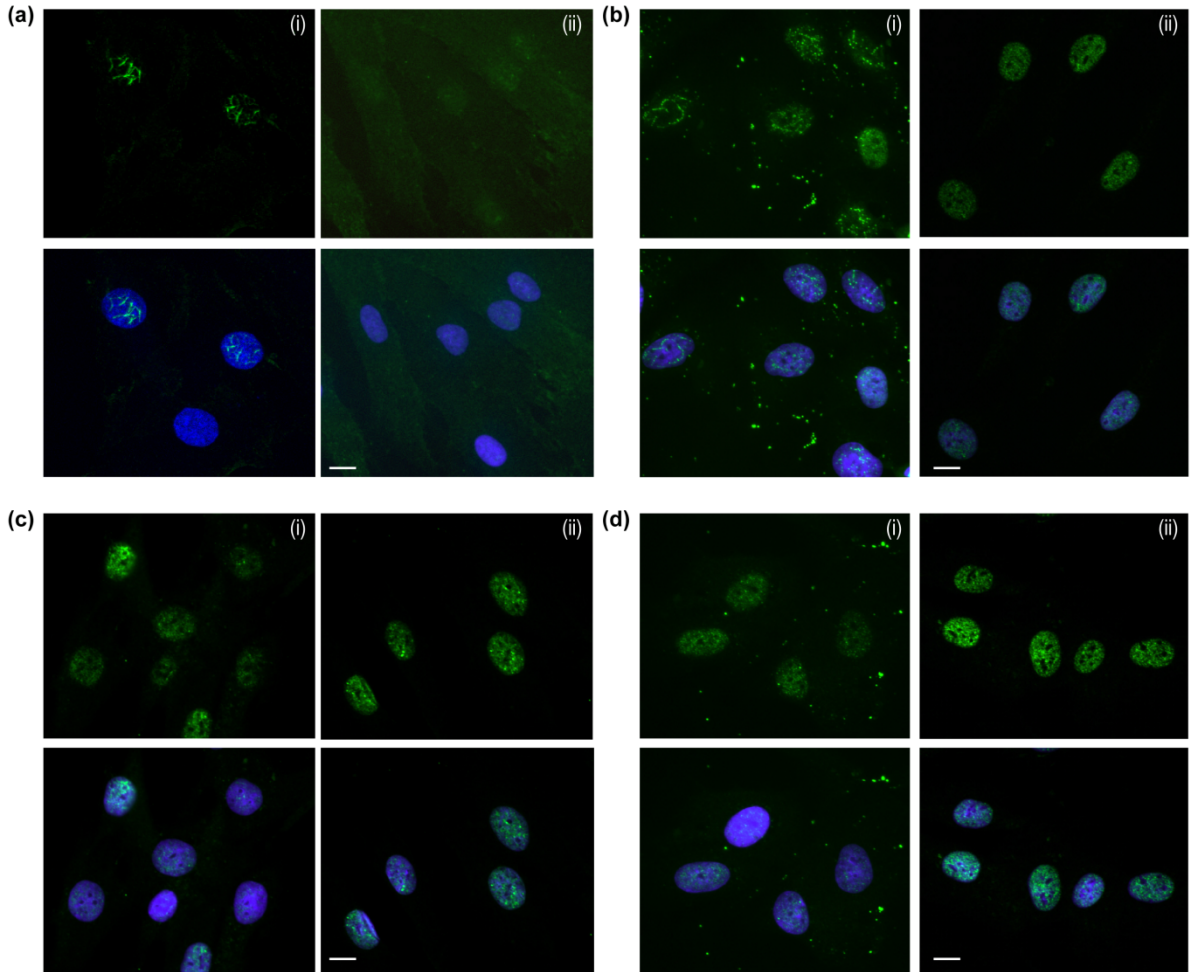


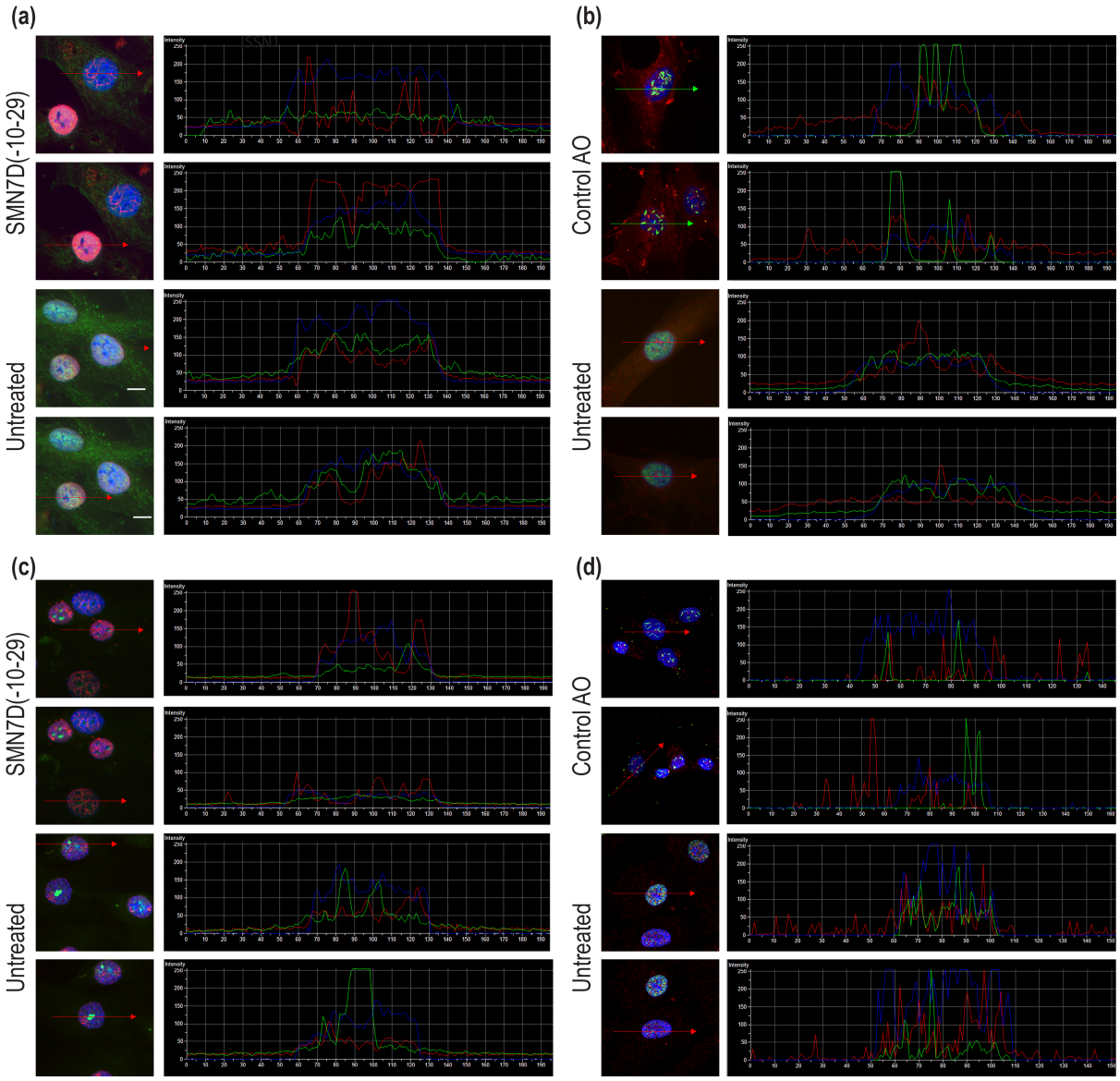
Supplementary Figure 1 fluorescence staining of paraspeckle proteins following 2´ O-methyl phosphorothioate AO transfection in additional cell types, including **(a)** primary human myogenic cells; **(b)** mouse myogenic cells; and **(c)** the SH-SY5Y neuroblastoma cell line; with (i) 2´ O-methyl phosphorothioate-transfected and (ii) untreated shown in each panel. NONO was immunostained in human cells and SFPQ in mouse cells. Scale bar = 10 μm.



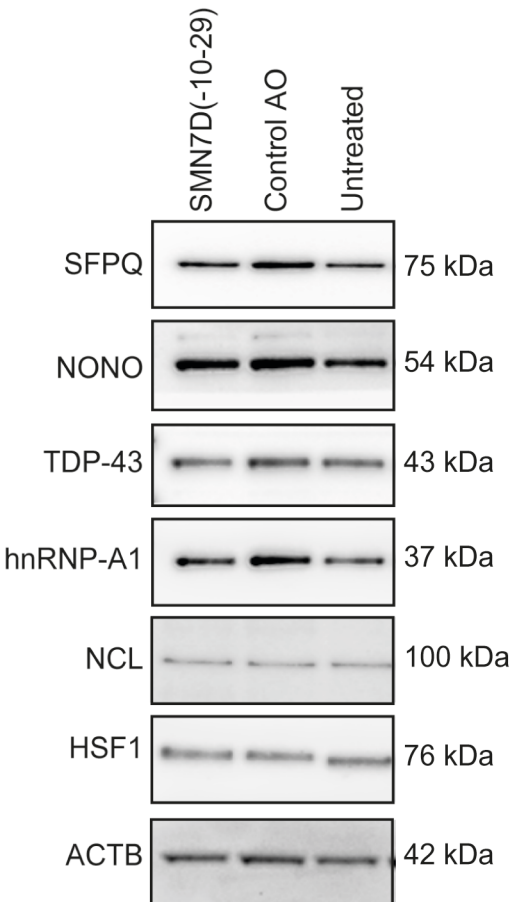
Supplementary Figure 2 Overlayed hoechst and SFPQ staining of fibroblasts following 2′O-methyl phosphorothioate transfection (100 nM, 24 hours), showing **(a)** cytoplasmic SFPQ staining in cells without nuclear inclusions; and **(b)** staining of SFPQ around the nuclear envelope in cells without nuclear inclusions. Scale bar = 10 µm.



Supplementary Figure 3 Immunofluorescent staining of paraspeckle proteins following 2′ O-methyl phosphorothioate 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 µm.



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
SMN	25 ng	AGGTCTCCTGGAAATAAATCAG TGGTGTCATTTAGTGCTGCTCT	55°C 30 min 94°C 2 min 25 cycles: 94°C 40 sec 56°C 30 sec 68°C 1 min
BCL2	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
5S	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
18S	250 nM	GTA ACC CGT TGA ACC CCA TT CCA TCC AAT CGG TAG TAG CG	
45S	100 nM	TGT CAG GCG TTC TCG TCT C AGC ACG ACG TCA CCA CAT C	
TBP	500 nM	TCAGGCGTTCGGTGGATCGAGT AGTGATGCTGGGCACTGCGGAGAA	
TUBB	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	**72.7**		
38	10	7	4	4	25	52	72	**72.2**		
39	9	8	4	4	25	114	159	**71.7**	>90%	
40	5	7	5	8	25	77	108	**71.3**		
41	1	9	4	4	18	72	109	**66.1**		
42	8	4	9	4	25	83	126	**65.9**	>90%	
43	3	10	8	4	25	86	131	**65.6**	>90%	
44	9	3	10	3	25	75	115	**65.2**	>90%	
45	8	4	7	6	25	47	74	**63.5**		
46	6	7	2	10	25	100	158	**63.3**		
47	10	3	5	7	25	65	103	**63.1**		
48	7	6	10	2	25	72	115	**62.6**	>90%	
49	7	7	8	11	33	25	40	**62.5**		
50	6	4	8	2	20	83	137	**60.6**	>90%	
51	4	8	6	7	25	54	93	**58.1**		
52	1	10	11	8	30	36	62	**58.1**		
53	2	7	8	8	25	78	138	**56.5**		
54	6	8	6	5	25	55	100	**55.0**		
55	7	5	8	4	24	68	125	**54.4**		
56	8	4	7	6	25	60	112	**53.6**		
57	5	7	6	7	25	40	77	**51.9**		
58	6	9	6	5	26	95	187	**50.8**		
59	8	4	5	8	25	34	67	**50.7**		
60	6	5	6	8	25	99	207	**47.8**		
61	6	3	13	3	25	23	49	**46.9**	>90%	
62	9	4	9	3	25	41	89	**46.1**	>90%	
63	8	7	7	3	25	55	121	**45.5**		
64	6	1	6	11	24	65	146	**44.5**		
65	3	7	8	7	25	57	134	**42.5**		
66	11	9	3	8	31	44	104	**42.3**		
67	3	10	7	5	25	22	55	**40.0**		
68	6	7	9	3	25	48	122	**39.3**	>90%	
69	2	9	13	1	25	43	114	**37.7**	>90%	
70	2	7	6	4	19	45	123	**36.6**		
71	6	7	9	3	25	36	104	**34.6**		
72	5	6	3	0	14	45	132	**34.1**		
73	9	7	5	4	25	30	90	**33.3**		
74	6	3	7	9	25	29	88	**33.0**	>90%	
75	5	10	8	2	25	52	160	**32.5**		
76	5	6	4	5	20	17	59	**28.8**		
77	6	6	8	1	21	33	115	**28.7**		
78	5	6	7	7	25	33	117	**28.2**		
79	3	8	5	9	25	39	143	**27.3**		
80	3	5	12	5	25	26	96	**27.1**	>90%	
81	7	8	7	3	25	21	78	**26.9**		
82	6	4	9	6	25	32	122	**26.2**	>90%	

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

Supplementary File 1 – intensity profiling analysis

Supplementary File 2 – All raw RNAseq data

Supplementary File 3 / Supplementary table 4 – GO network terms for Figure 6b