

OMTM, Volume 20

Supplemental Information

Deferoxamine mesylate improves splicing and GAA activity of the common c.-32-13T>G allele in late-onset PD patient fibroblasts

Emanuele Buratti, Paolo Peruzzo, Luca Braga, Irene Zanin, Cristiana Stuani, Elisa Goina, Maurizio Romano, Mauro Giacca, and Andrea Dardis

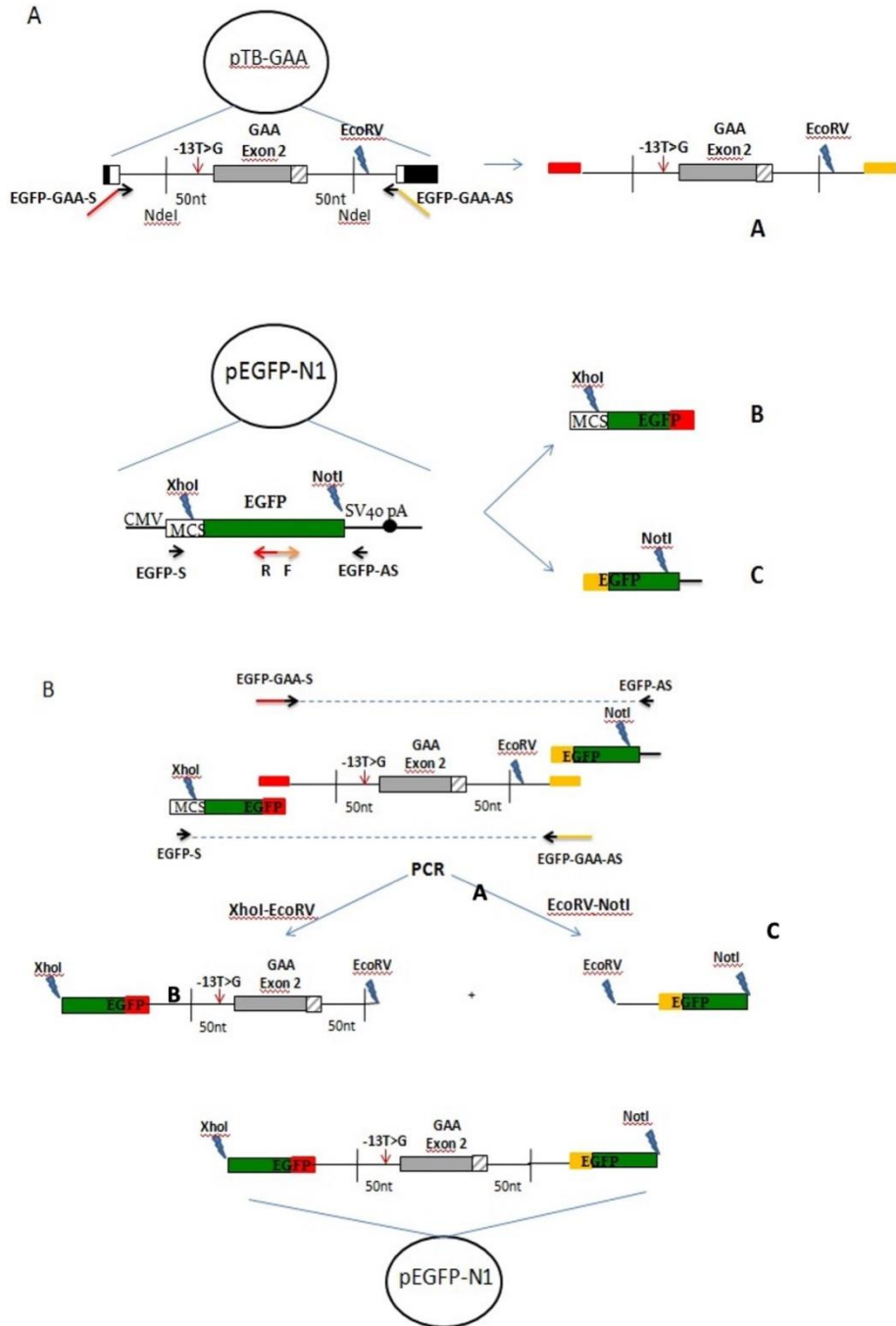


Figure S1: Schematic representation of the hybrid EGFP-GAA mutant minigene construction.

The GAA exon 2, 50 nt of the flanking introns and additional intronic sequences of the pTB-GAA MUT minigene (20) were PCR amplified using primers EGFP-GAA-S and EGFP-GAA-AS (Suppl. Table 2) to generate fragment A. These primers contained an extra sequence complementary to the central region of the EGFP. In parallel, the entire coding sequence of the EGFP cDNA was PCR amplified in 2 fragments using primers EGFP-S/R and EGFP-F/AS. Then, two additional fragments were generated by PCR using primers EGFP-S/EGFP-GAA-AS (Suppl. Table 2) using the commercial pEGFP-N1 plasmid as template to generate fragments B and C, respectively. Then, using fragments A-B and A-C as template two additional PCR fragments were generated using primers EGFP-S/EGFP-GAA-AS and EGFP-GAA-S/EGFP-AS, respectively. These obtained sequences were then digested with XhoI/EcoRV and EcoRV/NotI, respectively, clones in the pBluescript KS+ vector, sequences, and the entire sequence sub-cloned into the XhoI/NotI sites of the pEGFP-N1 vector.

TOP 100 downregulated genes

#	Gene	Gene Symbol	Fold Change	value	padj
1	ENSG0000011739	CCDC20	0.01	1.82E-123	1.95E-119
2	ENSG0000011296	KIF20A	0.02	4.75E-71	8.04E-68
3	ENSG0000011765	NEK2	0.02	1.36E-63	1.95E-60
4	ENSG0000012876	DLGAP5	0.02	4.54E-19	4.63E-17
5	ENSG0000011819	SAPC2C	0.02	1.23E-86	3.12E-83
6	ENSG0000011403	GSTM5	0.03	4.97E-21	5.18E-19
7	ENSG0000010144	FAM83D	0.03	3.43E-109	1.39E-105
8	ENSG0000011540	TROAP	0.03	6.30E-17	4.91E-15
9	ENSG0000011519	CENPA	0.03	1.45E-87	4.21E-84
10	ENSG0000010480	DEPDC1	0.04	2.99E-09	8.30E-08
11	ENSG00000102967	RP11-343B15.2	0.04	9.74E-07	1.61E-05
12	ENSG0000011825	ACTC1	0.04	0.000749009	0.00530843
13	ENSG0000011634	LMOD1	0.04	6.26E-19	6.17E-17
14	ENSG0000011540	CCDC5C	0.04	1.96E-35	4.74E-33
15	ENSG0000010902	CDKN3	0.04	1.43E-54	1.21E-51
16	ENSG0000011819	KIF4	0.04	1.62E-51	1.44E-48
17	ENSG0000010449	DEPDC3	0.04	3.72E-48	2.24E-45
18	ENSG0000011885	PLK1	0.04	1.05E-100	3.54E-97
19	ENSG0000011779	CDC20	0.04	1.94E-113	8.86E-110
20	ENSG0000011805	GASL2	0.05	8.42E-34	2.14E-31
21	ENSG0000011540	MYO21	0.05	2.43E-05	0.00278629
22	ENSG0000011877	MK167	0.05	4.31E-12	1.94E-10
23	ENSG0000011274	TTK	0.05	3.30E-45	1.56E-42
24	ENSG0000010757	HMMR	0.05	1.89E-45	8.41E-43
25	ENSG0000010989	BIRC5	0.05	1.26E-36	4.28E-34
26	ENSG0000010627	ASPM	0.05	8.37E-17	6.37E-15
27	ENSG0000012919	FAM64A	0.05	1.47E-76	2.98E-73
28	ENSG0000011863	RP11-920D10.1	0.06	2.90E-08	5.65E-07
29	ENSG0000011869	BUB1B	0.06	7.17E-06	9.40E-05
30	ENSG0000010297	RP11-920D10.1	0.06	3.06E-05	0.00041603
31	ENSG0000010916	EEF1A1P22	0.06	0.003397467	0.02032694
32	ENSG0000011742	HGF1	0.06	2.73E-23	3.82E-21
33	ENSG0000011574	TOP2A	0.06	6.32E-14	3.51E-12
34	ENSG0000013469	CDC40A	0.06	6.36E-51	4.62E-48
35	ENSG0000010948	PRH11	0.06	4.96E-47	2.17E-44
36	ENSG0000014322	NUP2	0.06	7.69E-34	1.97E-31
37	ENSG0000010241	CTD-2510F.4	0.06	2.94E-38	1.11E-35
38	ENSG0000011545	CNBE2	0.06	8.90E-53	7.23E-50
39	ENSG0000011867	LIP3	0.06	1.12E-36	3.81E-34
40	ENSG0000011115	NCAPH1	0.07	5.11E-11	1.96E-09
41	ENSG0000011249	HUJRP1	0.07	4.58E-07	8.16E-06
42	ENSG0000011981	SIXL1	0.07	6.43E-06	8.96E-05
43	ENSG0000013877	CENPE	0.07	1.32E-38	5.13E-36
44	ENSG0000010758	AURKA	0.07	2.02E-48	1.14E-45
45	ENSG0000011781	CASC3	0.07	1.92E-10	6.74E-09
46	ENSG0000011506	UBE2C	0.07	5.46E-39	2.23E-36
47	ENSG0000011885	HGAP3	0.07	9.73E-38	3.95E-35
48	ENSG0000010982	TPK2	0.07	2.96E-41	1.31E-38
49	ENSG0000011819	CEP350	0.07	4.73E-11	1.82E-09
50	ENSG0000010988	KIF4A	0.07	3.45E-12	1.98E-10

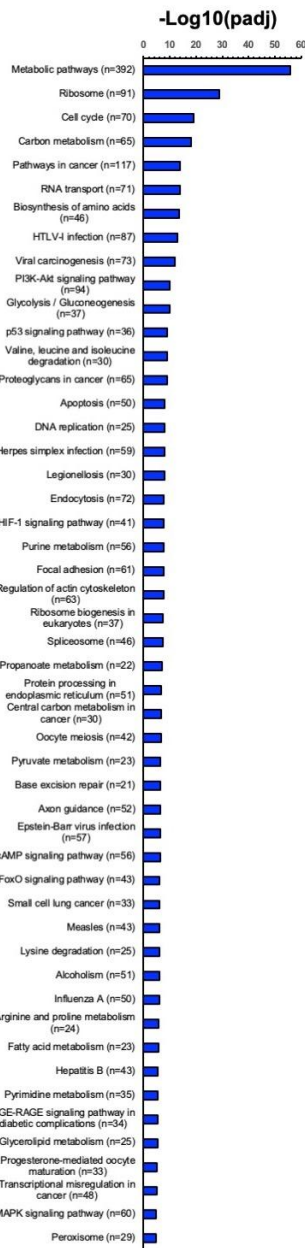
TOP 100 upregulated genes

#	Gene	Gene Symbol	Fold Change	value	padj
1	ENSG0000020412	C2orf72	825.33	4.19E-20	6.46E-23
2	ENSG00000209725	MT3	368.66	1.34E-20	1.47E-18
3	ENSG0000010559	ISM2	240.15	6.42E-20	1.15E-20
4	ENSG0000011875	ASCL2	172.87	2.35E-15	1.23E-11
5	ENSG0000011827	AL450307.1	165.33	1.75E-15	1.14E-13
6	ENSG00000114237	BEND5	99.91	9.75E-16	4.82E-14
7	ENSG0000011738	APLN	68.44	4.06E-16	2.84E-14
8	ENSG00000202363	KB-1905E3.3	67.73	2.27E-34	6.14E-32
9	ENSG0000011899	LICAM	60.67	1.07E-46	1.55E-43
10	ENSG0000011032	AREG	58.67	5.09E-20	8.54E-08
11	ENSG0000011938	RHBB	56.07	1.61E-12	7.65E-11
12	ENSG00000204339	LCE1F	54.50	2.14E-11	8.78E-10
13	ENSG0000011748	BARX1	51.71	1.84E-35	5.50E-33
14	ENSG0000011933	FLJ4304D	51.67	8.09E-09	2.23E-07
15	ENSG00000203982	AC00982.5	41.73	2.89E-16	2.69E-16
16	ENSG0000011801	KISS1R	41.50	1.91E-08	4.42E-07
17	ENSG0000011677	ANGPTL4	41.27	5.96E-70	9.31E-67
18	ENSG0000011928	CCDC64B	40.37	1.49E-22	2.06E-20
19	ENSG0000010566	POE4C	38.45	5.85E-10	1.86E-08
20	ENSG00000203496	KIFL2	38.25	5.99E-10	1.93E-08
21	ENSG0000011339	KCNK3	38.04	2.64E-40	1.12E-37
22	ENSG00000112628	MGAT3	35.67	9.19E-07	1.53E-06
23	ENSG00000103176	SEC14L5	34.67	8.19E-07	1.07E-05
24	ENSG00000202053	RP11-180C1.1	33.50	8.65E-16	5.90E-16
25	ENSG00000203609	RP6-191P20.4	33.50	1.04E-07	2.11E-06
26	ENSG0000011428	PHF294	32.48	8.89E-112	8.19E-112
27	ENSG0000011726	CEB3	31.92	2.31E-21	2.76E-19
28	ENSG00000114162	RNF165	31.00	1.01E-05	0.000127029
29	ENSG0000011404	UCN2	29.45	9.16E-17	6.28E-15
30	ENSG00000208834	FER1L4	28.53	9.81E-79	1.31E-75
31	ENSG0000011421	PURL4	28.43	2.42E-16	1.73E-14
32	ENSG00000119116	STC1	27.81	3.54E-139	3.54E-139
33	ENSG0000020304	RP11-415M8.1	27.33	4.69E-26	6.52E-25
34	ENSG0000011438	PPP1A4	27.31	3.64E-45	4.92E-42
35	ENSG00000202367	RP11-400V13.3	26.58	5.03E-10	1.61E-13
36	ENSG00000203584	RP11-399G2.2	26.29	1.25E-10	4.52E-09
37	ENSG0000011529	JAKMIP1	26.00	1.69E-05	0.000200661
38	ENSG0000010981	HES2	25.88	8.86E-10	2.15E-08
39	ENSG00000114620	ANG7	25.54	3.66E-56	3.86E-53
40	ENSG00000116484	GPR146	25.48	8.62E-40	1.06E-61
41	ENSG00000118165	ATG16L1	24.36	8.12E-27	1.03E-24
42	ENSG00000114258	SLC2A5	24.17	1.07E-09	3.26E-08
43	ENSG00000118652	CYP4F3	23.83	2.24E-09	6.34E-08
44	ENSG00000112115	GASL2	22.73	1.99E-06	2.99E-05
45	ENSG00000204062	HLA-J	22.67	4.03E-22	5.22E-20
46	ENSG00000202006	RP11-133D20.1	22.50	2.23E-06	3.38E-05
47	ENSG00000109817	PNP1	22.17	1.62E-06	3.70E-07
48	ENSG00000101194	VSIG1	22.00	6.31E-07	1.09E-05
49	ENSG00000209724	AC00982.4	21.73	4.86E-14	2.62E-12
50	ENSG0000011518	CCL28	21.63	1.62E-58	1.73E-55

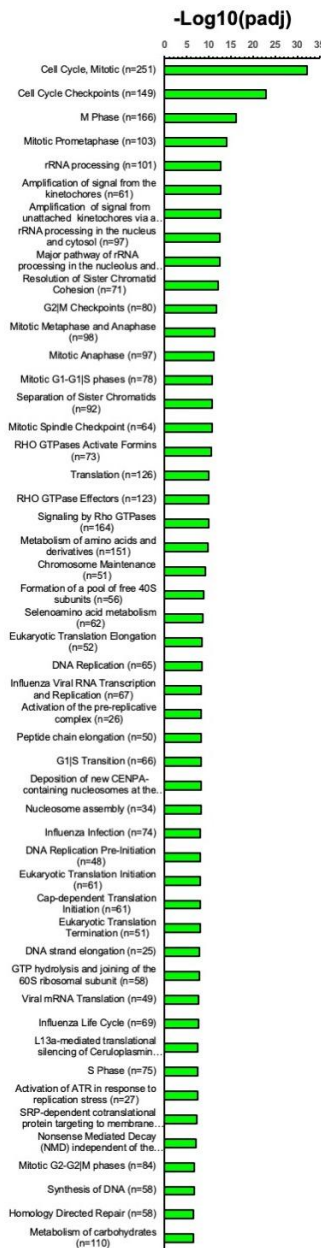
#	Gene	Gene Symbol	Fold Change	value	padj
51	ENSG0000021985	RP11-21.50	21.50	6.16E-08	1.30E-06
52	ENSG0000010087	SLC3A3	21.00	1.24E-07	2.48E-06
53	ENSG0000011955	CACNA1H	20.71	1.99E-08	5.72E-08
54	ENSG0000011862	HMOX13	20.42	5.12E-20	5.44E-18
55	ENSG00000222784	RP15-102G15.3	19.67	0.000150537	0.001342337
56	ENSG00000202687	KIF11P1	19.50	4.47E-05	0.000466795
57	ENSG0000010794	PODZA	19.27	1.43E-16	1.03E-14
58	ENSG0000011234	ITIH6	18.43	6.12E-08	1.29E-06
59	ENSG0000011495	ZP1	18.33	0.000127374	0.00156631
60	ENSG00000202428	RP3-326F13.1	17.68	3.18E-14	1.83E-12
61	ENSG0000011781	FOXO1	17.27	1.67E-09	4.86E-08
62	ENSG00000201943	CTC-379422.2	17.00	2.44E-05	0.00219232
63	ENSG0000011914	DPYSL4	16.63	1.75E-28	3.29E-26
64	ENSG00000203092	G51-308P.4	16.59	7.87E-32	1.88E-29
65	ENSG0000010687	CYP3A7	16.44	1.68E-25	2.68E-23
66	ENSG00000203798	AP00246.5	16.43	4.75E-07	8.40E-06
67	ENSG00000201118	LINC00589	16.33	0.00041832	0.00114046
68	ENSG0000011354	SULT1A1	16.25	5.99E-05	0.00094466
69	ENSG0000011167	ENO2	16.15	4.95E-64	5.91E-61
70	ENSG0000011508	ODF1	15.33	0.0004281	0.00299187
71	ENSG0000011916	FQF11	15.25	0.000186522	0.00149361
72	ENSG00000110632	TFR2	14.66	3.85E-19	3.97E-17
73	ENSG00000118612	HLA-DQB1	14.33	0.003812246	0.01953999
74	ENSG0000011868	RTT18	14.28	1.18E-16	8.88E-15
75	ENSG00000202859	NBEA1P1	14.00	0.000420657	0.00324066
76	ENSG00000202497	PCDH41	14.00	0.0198889	0.00716124
77	ENSG00000201168	ADAMTS-AS2	13.80	2.54E-05	0.00027936
78	ENSG0000011916	SLC3A1	13.63	3.33E-07	6.11E-06
79	ENSG0000011869	KANK3	13.63	6.72E-10	2.13E-08
80	ENSG0000011916	SPAG4	13.61	6.32E-49	4.02E-46
81	ENSG00000202547	hsa-mi-210	13.20	6.11E-13	3.07E-11
82	ENSG00000118414	CNTN2	13.19	1.74E-06	2.69E-05
83	ENSG00000116658	RNAD	13.08	8.11E-30	1.66E-27
84	ENSG0000011970	OCK	13.00	0.00028518	0.00214446
85	ENSG0000011032	QALN18	12.96	1.78E-05	0.000299663
86	ENSG0000011745	NMI1	12.93	1.29E-48	7.49E-46
87	ENSG0000011916	HBA1	12.75	0.000430463	0.00307686
88	ENSG0000011916	GPRC5A	12.59	2.82E-30	8.31E-28
89	ENSG00000202888	CTD-2550B.7	12.33	0.000361788	0.01374201
90	ENSG00000202720	IMPCH4	12.33	0.00447195	0.02322895
91	ENSG0000011920	KIF1A	12.25	0.00045484	0.003136739
92	ENSG00000201655	TPS22	12.22	4.05E-08	1.06E-07
93	ENSG00000118620	GRB3	12.19	0.00270389	0.015428705
94	ENSG00000202484	RRRT4	12.00	0.000870389	0.006041702
95	ENSG00000202962	AC00982.1	12.00	0.003436905	0.011828464
96	ENSG00000202503	RP11-46C20.1	11.92	6.48E-12	2.89E-10
97	ENSG0000011784	LINC0036	11.73	9.24E-08	0.00117788
98	ENSG00000117877	C5orf46	11.75	0.000732602	0.00532051
99	ENSG0000011963	RAB23	11.73	1.99E-50	1.32E-47
100	ENSG0000010979	PKD3	11.69	2.52E-37	8.98E-35

Figure S2. List of top 100 up- and down-regulated genes. List of the top 100 significant differentially expressed genes after exposure of fibroblasts from patients carrying the c.-32-13T>G variant to Deferoxamine. Red, upregulation; Green, downregulation.

KEGG Pathways



Reactome



Panther Pathways

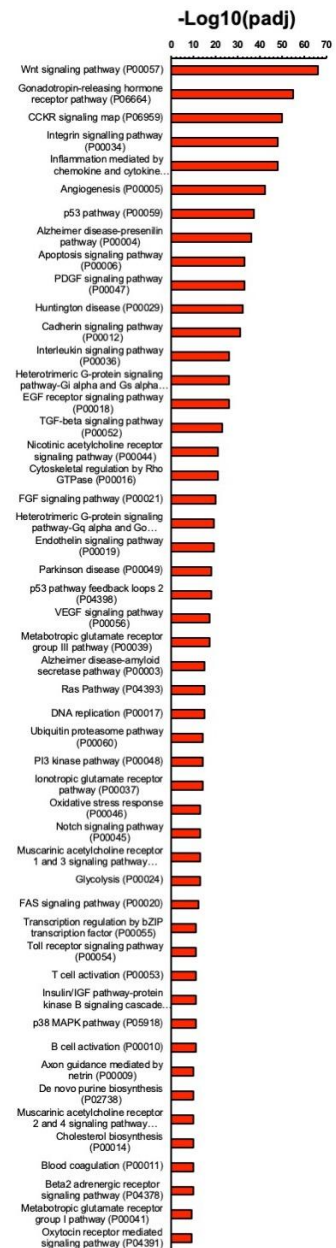
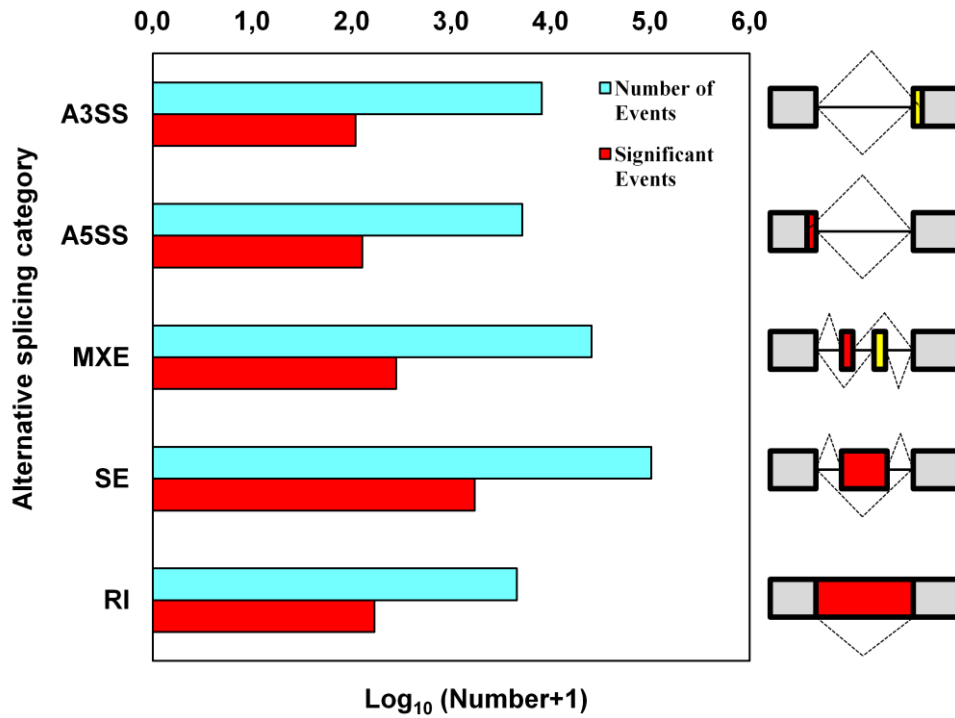


Figure S3: KEGG, Reactome and Panther pathways of DEGs after Defe treatment of c.-32-13T>G fibroblasts. Full list of the top 50 KEGG (left panel), Reactome (middle panel) and Panther (right panel) pathways differentially regulated after Defe treatment. Over-represented pathways have been ranked on the basis of their statistical significance.



Event Type	Number of Events	Significant Events
A3SS	8126	109
A5SS	5201	127
MXE	25842	280
SE	103099	1720
RI	4570	169

Figure S4: Alternative splicing events associated to Defe treatment. Chart (upper panel) and Table (lower panel) showing the number of total (■) and significant (■) alternative splicing events induced by treatment with Defe. SE: Skipped exon; MXE: Mutually exclusive exon; A5SS: Alternative 5' splice site; A3SS: Alternative 3' splice site; RI: Retained intron. In the chart, the Y-axis illustrates the 5 types of AS events, and the X-axis illustrates the counts for each type of AS events, respectively.

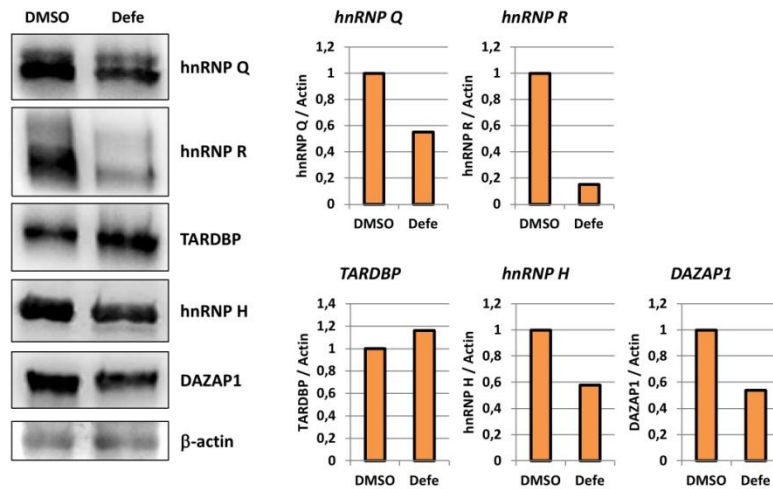


Figure S5: Protein expression levels of some of the hnRNPs identified by the RNAseq analysis modulated by Defe treatment in c.-32-13T>G fibroblasts.

c.-32-13T>G fibroblasts, treated for 7 days with 10 mM Defe or 0,1% DMSO, were lysed in TNN buffer (50 mM Tris-HCl, 250 mM NaCl, 5 mM EDTA, 0,5% NP-40) and the expression of hnRNP Q, hnRNP R, hnRNP H, TARDBP and DAZAP1 was determined by Western blot analysis using the following primary antibodies: anti-hnRNP Q (HPA041275 – Sigma-Aldrich); anti-hnRNP R (ab30930 – Abcam); anti-hnRNP H (home-made); anti-TARDBP (10782-2-AP – Proteintech); anti-DAZAP1 (HPA004201 – Sigma-Aldrich). A representative immunoblot and its quantification using Uvitec software is shown.

Table S3: Primer sequences.

Primer	Sequence (5'-3')
EGFP-GAA-S	CCCGAAGGCTACGTCCAGGTAAGTATGCATTAGCGTTATGGCCA
EGFP-GAA-AS	GAAGAAGATGGTGCCTCTGAAAAAGAAAAAGAAAAAATGAAGCCTCATTGATATAT TTAAAAAGG
EGFP-S	CCGGACTCAGATCTCGAGCTCAA
EGFP-AS	TAAAGCAAGTAAACCTCTAC
PDK1 for	AGTTCCTGGACTTCGGATCA
PDK1 rev	TGGTGCCTGAGAAGATTATCTG
VEGFA for	CTTCAAGCCATCCTGTGTGC
VEGFA rev	GAGGTTTGATCCGCATAATCTG
EGLN3 for	CAATGGTGGCTTGCTATCCG
EGLN3 rev	CCATGTAGCTTGGCATCCC
P4HA1 for	GGGTAATCTCCAGGAGTGAAAC
P4HA1 rev	TAGGGCTTGTTCCATCCACA