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Supplemental information

Targeted exon skipping of *NF1* exon 17

as a therapeutic for neurofibromatosis type I

André Leier, Marc Moore, Hui Liu, Michael Daniel, Alexis M. Hyde, Ludwine Messiaen, Bruce R. Korf, Jamuna Selvakumaran, Lukasz Ciszewski, Laura Lambert, Jeremy Foote, Margaret R. Wallace, Robert A. Kesterson, George Dickson, Linda Popplewell, and Deeann Wallis

Supplemental Table 1A: Exon Deletions and Reported Phenotypes

Reported phenotypes

Exon No. (Original)	Exon No. (LOVD)	RNA change	Protein change	Publications (reporting exon deletion but not necessarily details on phenotype)	Phenotype
1	1	r.1_60del	Start Codon at End of Exon 2		
2	2	r.61_204del	p.Leu21_Met68del	Fahsold et al. 2000; Ars et al. 2003; Hsiao et al. 2015	CALs, "symptomatic paravertebral masses from cervical to sacral region", "one symptomatic internal neurofibroma infiltrating the kidney", cutaneous neurofibromas, congenital tibial dysplasia (Hsiao et al.)
3	3	r.205_288del	p.Arg69_Gly96del	Ars et al. 2003; Liu et al. 2003; Pros et al. 2008; Sabbagh et al. 2013; Xu et al. 2014; Hsiao et al. 2015; Zhang et al. 2015	"typical phenotypes of NF1" (Liu et al.); CALs, subcutaneous neurofibromas, plexiform neurofibromas, Lisch nodules, speaking problems (Zhang et al.)
4a	4	r.289_479del	p.Gln97fs	De Luca et al. 2007; Wimmer et al. 2007; Pros et al. 2008; Sabbagh et al. 2013; Hsiao et al. 2015	CALs; Lisch nodules; neurofibromas; gangliofibromas; optical pathway gliomas
4b	5	r.480_586del	p.Leu161fs	Ars et al. 2000; Fahsold et al. 2000; Ars et al. 2003; Zatkova et al. 2004; Hsiao et al. 2015	CALs, cutaneous neurofibromas, optic nerve glioma, juvenile pilocytic astrocytoma (Hsiao et al. 2015)
4c	6	r.587_654del	p.Glu196fs	Sabbagh et al. 2013	reported as pathogenic
5	7	r.655_730del	p.Ala219fs	Sabbagh et al. 2013	reported as pathogenic
6	8	r.731_888del	p.Cys245fs	Fahsold et al. 2000	reported as pathogenic
7	9	r.889_1062del	p.Lys297_Lys354del	Hoffmeyer et al. 1998; Messiaen et al. 2000; Fahsold et al. 2000; Ars et al. 2003; Zatkova et al. 2004; Xu et al. 2014	Scoliosis (IVS7+1G>A); CALs and dermal neurofibromas (c.910C>T)
8	10	r.1063_1185del	p.Asn355_Lys395del	Fahsold et al. 2000; Ars et al. 2003; Sabbagh et al. 2013; Xu et al. 2014	reported as pathogenic
9	11	r.1186_1260del	p.Ile396_Asn420del	Ars et al. 2003	reported as pathogenic
10a	12	r.1261_1392del	p.Ser421_Pro464del	De Luca et al. 2004; De Luca et al. 2007; Sabbagh et al. 2013	CALs, nodular neurofibromas, cutaneous neurofibromas, plexiform neurofibromas, Lisch nodules, scoliosis, schwannoma, thyroid nodules, Becker naevus
10b	13	r.1393_1527del	p.Ser465_Cys509del	Ars et al. 1999; Ars et al. 2003; Wimmer et al. 2007; Pros et al. 2008; Sabbagh et al. 2013; Hsiao et al. 2015; Zhang et al. 2015	Plexiform neurofibromas (IVS10b+1G>A); Scoliosis (IVS10b+1G>A)
10c	14	r.1528_1641del	p.Asn510_Glu547del	Hsiao et al. 2015; Zhang et al. 2015	CALs
11	15	r.1642_1721del	p.Ala548fs	Purandare et al. 1994; Fahsold et al. 2000; Ars et al. 2003; De Luca et al. 2007; Pros et al. 2008; Sabbagh et al. 2013; Xu et al. 2014	Learning disability, cutaneous neurofibromas, CALs, acoustic neurinoma, pectus excavatum
12a	16	r.1722_1845del	Skipping this exon leads to a frameshift.		
12b	17	r.1846_2001del			
13	18	r.2002_2251del	p.?	Ars et al. 2003	reported as pathogenic
14	19	r.2252_2325del	p.Arg752fs	Maynard et al. 1997; Origone et al. 2003; Sabbagh et al. 2013; Nemethova et al. 2013	premature sceletal development; hamartomas in the brain; mental retardation; CALs; Lisch nodules; optical pathway glioma
15	20	r.2326_2409del		Ars et al. 2003; Xu et al. 2014	reported as pathogenic
16	21	r.2410_2850del	p.Ala804_Gln950del	Xu et al. 2014; Hsiao et al. 2015	reported as pathogenic
17	22	r.2851_2990del	p.Leu952fs	Ars et al. 2003; Jeong et al. 2006; Wimmer et al. 2007; Sabbagh et al. 2013	Plexiform neurofibromas (IVS17+2insT)
18	23	r.2991_3113del	p.Tyr998_Arg1038del	Purandare et al. 1995; Osborn and Upadhyaya 1999; Ars et al. 2000; Kluwe et al., 2002; Ars et al. 2003; Pros et al. 2008; Sabbagh et al. 2013; Zhang et al. 2015	Plexiform neurofibromas; Hamartomas in the brain; CALs; (IVS18+5G>C)
19a	24	r.3114_3197del	p.?	Ars et al. 2003; Xu et al. 2014	reported as pathogenic
19b	25	r.3198_3314del			
20	26	r.3315_3496del	p.Tyr1106fs	Sabbagh et al. 2013; Xu et al. 2014	reported as pathogenic
21	27	r.3497_3708del	p.Gly1166fs	Sabbagh et al. 2013; Xu et al. 2014	reported as pathogenic
22	28	r.3709_3870del	p.Asp1237Valfs	Hsiao et al. 2015	reported as pathogenic
23.1	29	r.3871_3974del	p.Tyr1292fs	Upadhyaya 1997	reported as pathogenic
23.2	30	r.3975_4110del	p.Leu1326fs	Hsiao et al. 2015	reported as pathogenic

24	32	r.4111_4269del	p.Val1371_Lys1423del	Nemethova et al. 2013	CAL, mental retardation, deafness (c.4268A>G)
25	33	r.4270_4367del	p.Ile1424fs	Ars et al. 2003; Pros et al. 2008; Sabbagh et al. 2013; Xu et al. 2014	reported as pathogenic
26	34	r.4368_4514del	p.Phe1457_Arg1505del	Hsiao et al. 2015	CALs, scoliosis, cutaneous neurofibromas
27a	35	r.4515_4661del	p.Asp1506_Arg1554del	Ars et al. 2003; Pros et al. 2008	reported as pathogenic
27b	36	r.4662_4772del	p.His1555_Arg1591del	Hsiao et al. 2015	CALs, Lisch nodules, cutaneous neurofibromas, spinal neurofibroma
28	37	r.4773_5205del	p.Phe1592fs	Sabbagh et al. 2013; Zhang et al. 2015	Zhang et al.: Various types of neurofibromas in one patient and CALs (but no NFs) in another patient
29	38	r.5206_5546del	p.Gly1737fs	Peters et al. 1999; Fahsold et al. 2000; Girondon-Boulandet et al. 2000; Messiaen et al. 2000; Ars et al. 2003; Sabbagh et al. 2013; Xu et al. 2014; Zhang et al. 2015	CALs and dermal neurofibromas (c.5546G>A); CALs, Lisch nodules (c.5206-2A>G); Spinal tumors
30	39	r.5547_5749del	p.Ser1850fs	Purandare et al. 1994; Zatkova et al. 2004; Sabbagh et al. 2013; Hsiao et al. 2015	reported as pathogenic
31	40	r.5750_5943del	p.Ser1917fs	Sabbagh et al. 2013; Hsiao et al. 2015	reported as pathogenic
32	41	r.5944_6084del			
33	42	r.6085_6364del	p.Val2029fs	Sabbagh et al. 2013; Zhang et al. 2015	CALs, Gastrointestinal stromal tumor, cutaneous and subcutaneous neurofibromas (Zhang et al.)
34	43	r.6365_6579del	p.?	Ars et al. 2003; De Luca et al. 2007; Xu et al. 2014	CALS, cutaneous neurofibromas
35	44	r.6580_6641del	p.Ala2194fs	Fahsold et al. 2000; Ars et al. 2003; De Luca et al. 2004; De Luca et al. 2007; Nemethova et al. 2013; Sabbagh et al. 2013	CALs; Lisch nodules; cutaneous neurofibromas; optic glioma
36	45	r.6642_6756del	p.Phe2215fs	Fahsold et al. 2000; Ars et al. 2003; De Luca et al. 2003; De Luca et al. 2007; Pros et al. 2008; Sabbagh	CALs, dermal neurofibromas, nodular neurofibromas, plexiform neurofibromas
37	46	r.6757_6858del	p.Ala2253_Lys2286del	Robinson et al. 1995; Upadhyaya et al. 1996; Messiaen et al. 1997; Hoffmeyer et al. 1998; Fahsold et al. 2000; Messiaen et al. 2000; Ars et al. 2003; Zatkova et al. 2004; Wimmer et al. 2007; Skoko et al. 2008; Sabbagh et al. 2013; Xu et al. 2014	CALs and dermal neurofibromas (c.6792C>A); Scoliosis (IVS37+2T>G)
38	47	r.6859_6999del		Pros et al. 2008	reported as pathogenic
39	48	r.7000_7126del	p.Ser2334fs	Sabbagh et al. 2013	reported as pathogenic
40	49	r.7127_7258del	p.Gly2376_Ala2419del	Griffiths et al. 2007; Pros et al. 2008; Sabbagh et al. 2013	"met NIH [diagnostic] criteria for NF1"
41	50	r.7259_7394del	Skipping this exon leads to a frameshift.		
42	51	r.7395_7552del	p.Thr2466fs	De Smet et al. 2002; Brems et al. 2009; Hsiao et al. 2015	NF1-associated glomus tumor; CALs, cutaneous neurofibromas, pseudarthrosis (Hsiao et al.)
43	52	r.7553_7675del			
44	53	r.7676_7806del	p.?	Ars et al. 2003; Xu et al. 2014	reported as pathogenic
45	54	r.7807_7907del	p.Thr2604fs	De Luca et al. 2004; Sabbagh et al. 2013	CALs (reported for c.7907+5G>A); all others reported as pathogenic
46	55	r.7908_8050del	p.Val2636fs	Sabbagh et al. 2013; Hsiao et al. 2015	reported as pathogenic
47	56	r.8051_8097del	Skipping this exon leads to a frameshift.		
48	57	r.8098_8314del	p.?	Xu et al. 2014	reported as pathogenic
49	58	r.8315_8454del	Skipping this exon leads to a frameshift.		

Supplemental Table 1B: Exon Deletions and LOVD entries

LOVD 3.0 Build 21 November 2018

Exon deleted	RNA change	Protein change	DB-ID	Origin	LOVD Individual ID	LOVD link	Publication
2	r.61_204del	p.Leu21_Met68del	NF1_001689	Germline	131376	https://databases.lovd.nl/shared/variants/0000221390#00014502	
			NF1_001740	Germline	131428	https://databases.lovd.nl/shared/variants/0000221442#00014502	Hsiao et al. 2015
			NF1_001740	De Novo	131429	https://databases.lovd.nl/shared/variants/0000221443#00014502	Hsiao et al. 2015
			NF1_001740	Germline	131430	https://databases.lovd.nl/shared/variants/0000221444#00014502	Hsiao et al. 2015
			NF1_001741	Germline	131431	https://databases.lovd.nl/shared/variants/0000221445#00014502	Hsiao et al. 2015
			NF1_002236	Unknown	64581	https://databases.lovd.nl/shared/variants/0000096129#00014502	
			NF1_002237	Unknown	64580	https://databases.lovd.nl/shared/variants/0000096128#00014502	
3	r.205_288del	p.Arg69_Gly96del	NF1_000267	Germline	129034	https://databases.lovd.nl/shared/variants/0000219046#00014502	
			NF1_000267	Unknown	130383	https://databases.lovd.nl/shared/variants/0000220395#00014502	Liu et al. 2003
			NF1_000267	Unknown	130981	https://databases.lovd.nl/shared/variants/0000220995#00014502	
			NF1_001745	De Novo	131435	https://databases.lovd.nl/shared/variants/0000221449#00014502	Hsiao et al. 2015
			NF1_001690	Germline	131377	https://databases.lovd.nl/shared/variants/0000221391#00014502	
			NF1_001484	Unknown	131042	https://databases.lovd.nl/shared/variants/0000221056#00014502	Pros et al. 2008
			NF1_001484	Germline	132905	https://databases.lovd.nl/shared/variants/0000223167#00014502	Pros et al. 2008
4	r.289_479del	p.Gln97fs	NF1_001747	De Novo	131437	https://databases.lovd.nl/shared/variants/0000221451#00014502	Hsiao et al. 2015
			NF1_001489	Unknown	131047	https://databases.lovd.nl/shared/variants/0000221061#00014502	Pros et al. 2008
			NF1_001143	Unknown	130384	https://databases.lovd.nl/shared/variants/0000220396#00014502	Sabbagh et al. 2013
			NF1_001147	Unknown	130388	https://databases.lovd.nl/shared/variants/0000220400#00014502	Wimmer et al. 2007
5	r.480_586del	p.Leu161fs	NF1_001751	De Novo	131441	https://databases.lovd.nl/shared/variants/0000221455#00014502	Hsiao et al. 2015
			NF1_000717	Germline	129793	https://databases.lovd.nl/shared/variants/0000219805#00014502	Fahsold et al. 2000
			NF1_000717	Unknown	130394	https://databases.lovd.nl/shared/variants/0000220406#00014502	Fahsold et al. 2000
			NF1_000718	Unknown	64318	https://databases.lovd.nl/shared/variants/0000095856#00014502	
			NF1_000718	Unknown	129794	https://databases.lovd.nl/shared/variants/0000219806#00014502	Ars et al. 2000
			NF1_000718	Unknown	129795	https://databases.lovd.nl/shared/variants/0000219807#00014502	Ars et al. 2000
			NF1_000718	Unknown	130391	https://databases.lovd.nl/shared/variants/0000220403#00014502	Ars et al. 2000
6	r.587_654del	p.Glu196fs	NF1_001149	Unknown	130413	https://databases.lovd.nl/shared/variants/0000220425#00014502	Sabbagh et al. 2013
			NF1_000722	Unknown	129799	https://databases.lovd.nl/shared/variants/0000219811#00014502	
			NF1_000722	Unknown	131049	https://databases.lovd.nl/shared/variants/0000221063#00014502	
			NF1_001707	De Novo	131394	https://databases.lovd.nl/shared/variants/0000221408#00014502	
7	r.655_730del	p.Ala219fs	NF1_001150	Unknown	130414	https://databases.lovd.nl/shared/variants/0000220426#00014502	Sabbagh et al. 2013
8	r.731_888del	p.Cys245fs	NF1_000941	De Novo	130151	https://databases.lovd.nl/shared/variants/0000220163#00014502	
			NF1_000941	Unknown	130152	https://databases.lovd.nl/shared/variants/0000220164#00014502	
			NF1_000941	Unknown	130422	https://databases.lovd.nl/shared/variants/0000220434#00014502	Fahsold et al. 2000
9	r.889_1062del	No entries in LOVD 3.0					
10	r.1063_1185del	p.Asn355_Lys395del	NF1_001166	Unknown	130441	https://databases.lovd.nl/shared/variants/0000220453#00014502	Sabbagh et al. 2013
11	r.1186_1260del	No entries in LOVD 3.0					
12	r.1261_1392del	p.Ser421_Pro464del	NF1_001176	Unknown	130458	https://databases.lovd.nl/shared/variants/0000220470#00014502	De Luca et al. 2004
			NF1_001176	Unknown	131076	https://databases.lovd.nl/shared/variants/0000221090#00014502	De Luca et al. 2004
13	r.1393_1527del	p.Ser465_Cys509del	NF1_001770	Unknown	131460	https://databases.lovd.nl/shared/variants/0000221474#00014502	Hsiao et al. 2015
			NF1_002081	Unknown	64519	https://databases.lovd.nl/shared/variants/0000096065#00014502	
			NF1_000067	Unknown	128708	https://databases.lovd.nl/shared/variants/0000218720#00014502	
			NF1_000067	Unknown	128709	https://databases.lovd.nl/shared/variants/0000218721#00014502	
			NF1_000067	Unknown	128710	https://databases.lovd.nl/shared/variants/0000218722#00014502	
			NF1_000067	Unknown	130469	https://databases.lovd.nl/shared/variants/0000220481#00014502	Ars et al. 1999
			NF1_001180	Unknown	130476	https://databases.lovd.nl/shared/variants/0000220488#00014502	Pros et al. 2008
			NF1_001180	Unknown	130477	https://databases.lovd.nl/shared/variants/0000220489#00014502	Pros et al. 2008
			NF1_001180	Unknown	130478	https://databases.lovd.nl/shared/variants/0000220490#00014502	Pros et al. 2008
			NF1_001506	De Novo	64324	https://databases.lovd.nl/shared/variants/0000095864#00014502	
			NF1_001506	De Novo	131081	https://databases.lovd.nl/shared/variants/0000221095#00014502	
			NF1_001181	Unknown	130479	https://databases.lovd.nl/shared/variants/0000220491#00014502	Wimmer et al. 2007
14	r.1528_1641del	p.Asn510_Glu547del	NF1_001773	Unknown	131463	https://databases.lovd.nl/shared/variants/0000221477#00014502	Hsiao et al. 2015
15	r.1642_1721del	p.Ala548fs	NF1_001187	Unknown	130492	https://databases.lovd.nl/shared/variants/0000220504#00014502	Sabbagh et al. 2013
			NF1_000099	Unknown	64334	https://databases.lovd.nl/shared/variants/0000095874#00014502	
			NF1_000099	Unknown	128768	https://databases.lovd.nl/shared/variants/0000218780#00014502	
			NF1_000099	Unknown	130497	https://databases.lovd.nl/shared/variants/0000220509#00014502	Pros et al. 2008
			NF1_001188	Unknown	130493	https://databases.lovd.nl/shared/variants/0000220505#00014502	Sabbagh et al. 2013
			NF1_001189	Unknown	130494	https://databases.lovd.nl/shared/variants/0000220506#00014502	Sabbagh et al. 2013
			NF1_001190	De Novo	130498	https://databases.lovd.nl/shared/variants/0000220510#00014502	Pros et al. 2008
			NF1_001190	Unknown	131863	https://databases.lovd.nl/shared/variants/0000221878#00014502	Cali et al. ??

			NF1_000101	Unknown	128770	https://databases.lovd.nl/shared/variants/0000218782#00014502	Purandare et al. 1994
			NF1_000101	Unknown	130499	https://databases.lovd.nl/shared/variants/0000220511#00014502	Purandare et al. 1994
			NF1_000101	Unknown	130500	https://databases.lovd.nl/shared/variants/0000220512#00014502	Purandare et al. 1994
			NF1_000101	Unknown	130501	https://databases.lovd.nl/shared/variants/0000220513#00014502	Purandare et al. 1994
			NF1_000101	De Novo	130879	https://databases.lovd.nl/shared/variants/0000220893#00014502	Purandare et al. 1994
			NF1_000101	Unknown	131086	https://databases.lovd.nl/shared/variants/0000221100#00014502	Purandare et al. 1994
			NF1_000101	Germline	132872	https://databases.lovd.nl/shared/variants/0000223134#00014502	Purandare et al. 1994
16	r.1722_1845del	No entries in LOVD 3.0					
17	r.1846_2001del	No entries in LOVD 3.0					
18	r.2002_2251del	No entries in LOVD 3.0					
19	r.2252_2325del	p.Arg752fs	NF1_000183	De Novo	64300	https://databases.lovd.nl/shared/variants/0000095838#00014502	
			NF1_000183	De Novo	128919	https://databases.lovd.nl/shared/variants/0000218931#00014502	
			NF1_000183	Germline	177090	https://databases.lovd.nl/shared/variants/0000401383#00014502	
			NF1_000180	De Novo	128915	https://databases.lovd.nl/shared/variants/0000218927#00014502	
			NF1_000180	Unknown	130543	https://databases.lovd.nl/shared/variants/0000220555#00014502	Maynard et al. 1997
			NF1_000180	Unknown	131101	https://databases.lovd.nl/shared/variants/0000221115#00014502	Maynard et al. 1997
			NF1_001209	Unknown	130541	https://databases.lovd.nl/shared/variants/0000220553#00014502	Origone et al. 2003
			NF1_001713	De Novo	131400	https://databases.lovd.nl/shared/variants/0000221414#00014502	
			NF1_001208	De Novo	64312	https://databases.lovd.nl/shared/variants/0000095850#00014502	
			NF1_001208	Unknown	130539	https://databases.lovd.nl/shared/variants/0000220551#00014502	Sabbagh et al. 2013
			NF1_001208	Unknown	130540	https://databases.lovd.nl/shared/variants/0000220552#00014502	Sabbagh et al. 2013
			NF1_001208	Germline	132889	https://databases.lovd.nl/shared/variants/0000223151#00014502	Sabbagh et al. 2013
20	r.2326_2409del	No entries in LOVD 3.0					
21	r.2410_2850del	p.Ala804_Gln950del	NF1_001781	Unknown	131471	https://databases.lovd.nl/shared/variants/0000221485#00014502	Hsiao et al. 2015
22	r.2851_2990del	p.Leu952fs	NF1_001524	Unknown	131112	https://databases.lovd.nl/shared/variants/0000221126#00014502	
			NF1_001008	Unknown	64315	https://databases.lovd.nl/shared/variants/0000095853#00014502	
			NF1_001008	Unknown	130235	https://databases.lovd.nl/shared/variants/0000220247#00014502	
			NF1_001008	Unknown	130574	https://databases.lovd.nl/shared/variants/0000220586#00014502	Sabbagh et al. 2013
			NF1_001008	Unknown	131113	https://databases.lovd.nl/shared/variants/0000221127#00014502	
			NF1_001008	Unknown	131815	https://databases.lovd.nl/shared/variants/0000221830#00014502	Cali et al. ??
			NF1_001234	Unknown	130579	https://databases.lovd.nl/shared/variants/0000220591#00014502	Jeong et al. 2006
			NF1_001231	Unknown	130575	https://databases.lovd.nl/shared/variants/0000220587#00014502	Sabbagh et al. 2013
			NF1_001232	Unknown	130577	https://databases.lovd.nl/shared/variants/0000220589#00014502	Sabbagh et al. 2013
			NF1_000281	De Novo	129065	https://databases.lovd.nl/shared/variants/0000219077#00014502	
			NF1_000281	Unknown	130576	https://databases.lovd.nl/shared/variants/0000220588#00014502	Sabbagh et al. 2013
			NF1_000285	De Novo	129070	https://databases.lovd.nl/shared/variants/0000219082#00014502	
			NF1_000285	Unknown	130580	https://databases.lovd.nl/shared/variants/0000220592#00014502	Wimmer et al. 2007
			NF1_001716	Germline	131403	https://databases.lovd.nl/shared/variants/0000221417#00014502	
23	r.2991_3113del	p.Tyr998_Arg1038del	NF1_000289	De Novo	129074	https://databases.lovd.nl/shared/variants/0000219086#00014502	
			NF1_000289	Unknown	129075	https://databases.lovd.nl/shared/variants/0000219087#00014502	
			NF1_000289	Unknown	129076	https://databases.lovd.nl/shared/variants/0000219088#00014502	
			NF1_000289	De Novo	130583	https://databases.lovd.nl/shared/variants/0000220595#00014502	Osborn and Upadhyaya 1999
			NF1_000289	De Novo	131605	https://databases.lovd.nl/shared/variants/0000221619#00014502	
			NF1_001235	Unknown	130581	https://databases.lovd.nl/shared/variants/0000220593#00014502	Sabbagh et al. 2013
			NF1_001238	Germline	130585	https://databases.lovd.nl/shared/variants/0000220597#00014502	Ars et al. 2000
			NF1_001238	Unknown	131122	https://databases.lovd.nl/shared/variants/0000221136#00014502	Pros et al. 2008
24	r.3114_3197del	No entries in LOVD 3.0					
25	r.3198_3314del	No entries in LOVD 3.0					
26	r.3315_3496del	p.Tyr1106fs	NF1_002197	De novo	64321	https://databases.lovd.nl/shared/variants/0000095860#00014502	
			NF1_002197	Unknown	64339	https://databases.lovd.nl/shared/variants/0000095879#00014502	
			NF1_001242	Unknown	130593	https://databases.lovd.nl/shared/variants/0000220605#00014502	Sabbagh et al. 2013
27	r.3497_3708del	p.Gly1166fs	NF1_001244	Unknown	130600	https://databases.lovd.nl/shared/variants/0000220612#00014502	Sabbagh et al. 2013
28	r.3709_3870del	No entries in LOVD 3.0					
29	r.3871_3974del	p.Tyr1292fs	NF1_000413	Unknown	129277	https://databases.lovd.nl/shared/variants/0000219289#00014502	Upadhyaya 1997
			NF1_000413	Unknown	130633	https://databases.lovd.nl/shared/variants/0000220645#00014502	Upadhyaya 1997
			NF1_000413	Unknown	130634	https://databases.lovd.nl/shared/variants/0000220646#00014502	Upadhyaya 1997
			NF1_000413	Unknown	130932	https://databases.lovd.nl/shared/variants/0000220946#00014502	Upadhyaya 1997
			NF1_000414	Unknown	132944	https://databases.lovd.nl/shared/variants/0000223206#00014502	Upadhyaya 1997
30	r.3975_4110del	p.Leu1326fs	NF1_001696	Germline	131383	https://databases.lovd.nl/shared/variants/0000221397#00014502	
			NF1_001785	De Novo	131475	https://databases.lovd.nl/shared/variants/0000221489#00014502	Hsiao et al. 2015
31	r.4111_4269del	No entries in LOVD 3.0					
32	r.4270_4367del	p.Ile1424fs	NF1_001273	Unknown	130657	https://databases.lovd.nl/shared/variants/0000220669#00014502	Sabbagh et al. 2013

			NF1_001420	Unknown	131169	https://databases.lovd.nl/shared/variants/0000221183#00014502	Pros et al. 2008
			NF1_001421	Unknown	130922	https://databases.lovd.nl/shared/variants/0000220936#00014502	
			NF1_001420	Somatic	177019	https://databases.lovd.nl/shared/variants/0000400792#00014502	
33	r.4368_4514del	p.Phe1457_Arg1505del49	NF1_001787	De Novo	131477	https://databases.lovd.nl/shared/variants/0000221491#00014502	Hsiao et al. 2015
34	r.4515_4661del	p.Asp1506fs	NF1_002054	Unknown	64579	https://databases.lovd.nl/shared/variants/0000096127#00014502	
			NF1_001557	Unknown	131183	https://databases.lovd.nl/shared/variants/0000221197#00014502	Pros et al. 2008
35	r.4662_4772del	p.His1555_Arg1591del37	NF1_001790	Germline	131480	https://databases.lovd.nl/shared/variants/0000221494#00014502	Hsiao et al. 2015
			NF1_001788	De novo	131478	https://databases.lovd.nl/shared/variants/0000221492#00014502	Hsiao et al. 2015
			NF1_001789	De novo	131479	https://databases.lovd.nl/shared/variants/0000221493#00014502	Hsiao et al. 2015
36	r.4773_5205del	No entries in LOVD 3.0					
37	r.5206_5546del	p.Gly1737fs	NF1_001384	De novo	130855	https://databases.lovd.nl/shared/variants/0000220867#00014502	Sabbagh et al. 2013
			NF1_001313	Unknown	130727	https://databases.lovd.nl/shared/variants/0000220739#00014502	Peters et al. 1999
			NF1_000677	Unknown	64521	https://databases.lovd.nl/shared/variants/0000096067#00014502	
			NF1_000677	Unknown	129696	https://databases.lovd.nl/shared/variants/0000219708#00014502	Ars et al. 2003
			NF1_000677	De Novo	129697	https://databases.lovd.nl/shared/variants/0000219709#00014502	Ars et al. 2003
			NF1_000677	De Novo	129698	https://databases.lovd.nl/shared/variants/0000219710#00014502	Ars et al. 2003
			NF1_000677	De Novo	129699	https://databases.lovd.nl/shared/variants/0000219711#00014502	Ars et al. 2003
			NF1_000677	Unknown	129700	https://databases.lovd.nl/shared/variants/0000219712#00014502	Ars et al. 2003
			NF1_000677	Unknown	129701	https://databases.lovd.nl/shared/variants/0000219713#00014502	Ars et al. 2003
			NF1_000677	Unknown	129702	https://databases.lovd.nl/shared/variants/0000219714#00014502	Ars et al. 2003
			NF1_000677	Unknown	129703	https://databases.lovd.nl/shared/variants/0000219715#00014502	Ars et al. 2003
			NF1_000677	Unknown	129704	https://databases.lovd.nl/shared/variants/0000219716#00014502	Ars et al. 2003
			NF1_000677	Unknown	129705	https://databases.lovd.nl/shared/variants/0000219717#00014502	Ars et al. 2003
			NF1_000677	Unknown	130712	https://databases.lovd.nl/shared/variants/0000220724#00014502	Ars et al. 2003
			NF1_000677	Unknown	130713	https://databases.lovd.nl/shared/variants/0000220725#00014502	Ars et al. 2003
			NF1_000677	Unknown	130891	https://databases.lovd.nl/shared/variants/0000220905#00014502	Ars et al. 2003
			NF1_000677	Unknown	131213	https://databases.lovd.nl/shared/variants/0000221227#00014502	Ars et al. 2000
			NF1_000677	Unknown	131214	https://databases.lovd.nl/shared/variants/0000221228#00014502	Ars et al. 2000
			NF1_001312	Unknown	130721	https://databases.lovd.nl/shared/variants/0000220733#00014502	Ars et al. 2000; Kluwe et al. 2003
			NF1_000678	De novo	129706	https://databases.lovd.nl/shared/variants/0000219718#00014502	Ars et al. 2000
			NF1_000678	Unknown	130725	https://databases.lovd.nl/shared/variants/0000220737#00014502	Ars et al. 2000
			NF1_000673	Unknown	129690	https://databases.lovd.nl/shared/variants/0000219702#00014502	Fahsold et al. 2000
			NF1_000673	Unknown	130726	https://databases.lovd.nl/shared/variants/0000220738#00014502	Fahsold et al. 2000
			NF1_000673	Unknown	131215	https://databases.lovd.nl/shared/variants/0000221229#00014502	Fahsold et al. 2000
			NF1_001573	Unknown	131216	https://databases.lovd.nl/shared/variants/0000221230#00014502	
38	r.5547_5749del	p.Ser1850fs	NF1_001314	Unknown	130728	https://databases.lovd.nl/shared/variants/0000220740#00014502	Sabbagh et al. 2013
			NF1_000700	Unknown	129733	https://databases.lovd.nl/shared/variants/0000219745#00014502	Purandare et al. 1994
			NF1_000700	Unknown	130731	https://databases.lovd.nl/shared/variants/0000220743#00014502	Purandare et al. 1994
39	r.5750_5943del	p.Ser1917fs	NF1_001799	De Novo	131489	https://databases.lovd.nl/shared/variants/0000221503#00014502	Hsiao et al. 2015
			NF1_001800	De Novo	131490	https://databases.lovd.nl/shared/variants/0000221504#00014502	Hsiao et al. 2015
			NF1_001798	Somatic	131488	https://databases.lovd.nl/shared/variants/0000221502#00014502	Hsiao et al. 2015
			NF1_001801	De Novo	131491	https://databases.lovd.nl/shared/variants/0000221505#00014502	Hsiao et al. 2015
			NF1_001316	Unknown	130732	https://databases.lovd.nl/shared/variants/0000220744#00014502	Sabbagh et al. 2013
40	r.5944_6084del		NF1_002132	De Novo	64524	https://databases.lovd.nl/shared/variants/0000096071#00014502	
41	r.6085_6364del	p.Val2029fs	NF1_002139	De Novo	64515	https://databases.lovd.nl/shared/variants/0000096061#00014502	
			NF1_002139	Unknown	64611	https://databases.lovd.nl/shared/variants/0000096160#00014502	
			NF1_001322	Unknown	130747	https://databases.lovd.nl/shared/variants/0000220759#00014502	Sabbagh et al. 2013
			NF1_001322	Unknown	131234	https://databases.lovd.nl/shared/variants/0000221248#00014502	Sabbagh et al. 2013
			NF1_001323	Unknown	130748	https://databases.lovd.nl/shared/variants/0000220760#00014502	Sabbagh et al. 2013
42	r.6365_6579del	No entries in LOVD 3.0					
43	r.6580_6641del	p.Ala2194fs	NF1_000795	Unknown	129884	https://databases.lovd.nl/shared/variants/0000219896#00014502	De Luca et al. 2004
			NF1_000795	Unknown	129885	https://databases.lovd.nl/shared/variants/0000219897#00014502	De Luca et al. 2004
			NF1_000795	Unknown	130774	https://databases.lovd.nl/shared/variants/0000220786#00014502	De Luca et al. 2004
			NF1_000796	Germline	129886	https://databases.lovd.nl/shared/variants/0000219898#00014502	
			NF1_000796	Unknown	130765	https://databases.lovd.nl/shared/variants/0000220777#00014502	Sabbagh et al. 2013
			NF1_000796	Unknown	130766	https://databases.lovd.nl/shared/variants/0000220778#00014502	Sabbagh et al. 2013
			NF1_000796	Unknown	131239	https://databases.lovd.nl/shared/variants/0000221253#00014502	Sabbagh et al. 2013
			NF1_001341	Unknown	130776	https://databases.lovd.nl/shared/variants/0000220787#00014502	Fahsold et al. 2000
			NF1_001341	Unknown	130775	https://databases.lovd.nl/shared/variants/0000220788#00014502	Fahsold et al. 2000
44	r.6642_6756del	NF1_001346	NF1_001346	Unknown	130787	https://databases.lovd.nl/shared/variants/0000220799#00014502	Fahsold et al. 2000; Pros et al. 2008
			NF1_001343	Unknown	130778	https://databases.lovd.nl/shared/variants/0000220790#00014502	Sabbagh et al. 2013
			NF1_001342	Unknown	130777	https://databases.lovd.nl/shared/variants/0000220789#00014502	Sabbagh et al. 2013

			NF1_000809	De novo	129921	https://databases.lovd.nl/shared/variants/0000219932#00014502	Pros et al. 2008
			NF1_000809	Unknown	129920	https://databases.lovd.nl/shared/variants/0000219933#00014502	Pros et al. 2008
			NF1_000809	Unknown	129922	https://databases.lovd.nl/shared/variants/0000219934#00014502	Pros et al. 2008
			NF1_000809	Unknown	130788	https://databases.lovd.nl/shared/variants/0000220800#00014502	Pros et al. 2008
			NF1_000809	Unknown	131022	https://databases.lovd.nl/shared/variants/0000221036#00014502	Pros et al. 2008
45	r.6757_6858del	p.Ala2253_Lys2286del	NF1_002150	Unknown	64518	https://databases.lovd.nl/shared/variants/0000096064#00014502	
			NF1_001347	Germline	130789	https://databases.lovd.nl/shared/variants/0000220801#00014502	Sabbagh et al. 2013
			NF1_001348	Unknown	130790	https://databases.lovd.nl/shared/variants/0000220802#00014502	Sabbagh et al. 2013
			NF1_000816	Germline	129948	https://databases.lovd.nl/shared/variants/0000219960#00014502	Robinson et al. 1995
			NF1_000816	Germline	129949	https://databases.lovd.nl/shared/variants/0000219961#00014502	Robinson et al. 1995
			NF1_000816	Germline	129950	https://databases.lovd.nl/shared/variants/0000219962#00014502	Robinson et al. 1995
			NF1_000816	De novo	129951	https://databases.lovd.nl/shared/variants/0000219963#00014502	Robinson et al. 1995
			NF1_000816	Unknown	129952	https://databases.lovd.nl/shared/variants/0000219964#00014502	Robinson et al. 1995
			NF1_000816	Unknown	129953	https://databases.lovd.nl/shared/variants/0000219965#00014502	Robinson et al. 1995
			NF1_000816	Unknown	129954	https://databases.lovd.nl/shared/variants/0000219966#00014502	Robinson et al. 1995
			NF1_000816	Unknown	129955	https://databases.lovd.nl/shared/variants/0000219967#00014502	Robinson et al. 1995
			NF1_000816	Unknown	129956	https://databases.lovd.nl/shared/variants/0000219968#00014502	Robinson et al. 1995
			NF1_000816	Unknown	129957	https://databases.lovd.nl/shared/variants/0000219969#00014502	Robinson et al. 1995
			NF1_000816	Unknown	129958	https://databases.lovd.nl/shared/variants/0000219970#00014502	Robinson et al. 1995
			NF1_000816	Unknown	129959	https://databases.lovd.nl/shared/variants/0000219971#00014502	Robinson et al. 1995
			NF1_000816	Unknown	129960	https://databases.lovd.nl/shared/variants/0000219972#00014502	Robinson et al. 1995
			NF1_000816	Unknown	129961	https://databases.lovd.nl/shared/variants/0000219973#00014502	Robinson et al. 1995
			NF1_000816	De novo	130794	https://databases.lovd.nl/shared/variants/0000220806#00014502	Robinson et al. 1995
			NF1_000816	De novo	130795	https://databases.lovd.nl/shared/variants/0000220807#00014502	Robinson et al. 1995
			NF1_000816	De novo	130796	https://databases.lovd.nl/shared/variants/0000220808#00014502	Robinson et al. 1995
			NF1_000816	De novo	130797	https://databases.lovd.nl/shared/variants/0000220809#00014502	Robinson et al. 1995
			NF1_000816	De novo	130798	https://databases.lovd.nl/shared/variants/0000220810#00014502	Robinson et al. 1995
			NF1_000816	De novo	130799	https://databases.lovd.nl/shared/variants/0000220811#00014502	Robinson et al. 1995
			NF1_000816	De novo	130800	https://databases.lovd.nl/shared/variants/0000220812#00014502	Robinson et al. 1995
			NF1_000816	Unknown	131245	https://databases.lovd.nl/shared/variants/0000221259#00014502	Robinson et al. 1995
			NF1_000816	Unknown	131246	https://databases.lovd.nl/shared/variants/0000221260#00014502	Robinson et al. 1995
			NF1_000816	De Novo	131850	https://databases.lovd.nl/shared/variants/0000221865#00014502	Robinson et al. 1995
			NF1_000816	Unknown	131851	https://databases.lovd.nl/shared/variants/0000221866#00014502	Robinson et al. 1995
			NF1_000816	De Novo	64516	https://databases.lovd.nl/shared/variants/0000096062#00014502	
			NF1_000816	De Novo	64517	https://databases.lovd.nl/shared/variants/0000096063#00014502	
			NF1_000816	Unknown	64537	https://databases.lovd.nl/shared/variants/0000096084#00014502	
			NF1_000816	De Novo	64538	https://databases.lovd.nl/shared/variants/0000096085#00014502	
			NF1_000816	Unknown	64539	https://databases.lovd.nl/shared/variants/0000096086#00014502	
			NF1_000816	Unknown	131744	https://databases.lovd.nl/shared/variants/0000221758#00014502	
			NF1_000816	Unknown	131745	https://databases.lovd.nl/shared/variants/0000221759#00014502	
			NF1_000816	Germline	131746	https://databases.lovd.nl/shared/variants/0000221760#00014502	
			NF1_000817	De novo	129962	https://databases.lovd.nl/shared/variants/0000219974#00014502	Messiaen et al. 1997, Wimmer et al. 2007, Skoko et al. 2008
			NF1_000817	Unknown	129963	https://databases.lovd.nl/shared/variants/0000219975#00014502	Messiaen et al. 1997, Wimmer et al. 2007, Skoko et al. 2008
			NF1_000817	Unknown	129964	https://databases.lovd.nl/shared/variants/0000219976#00014502	Messiaen et al. 1997, Wimmer et al. 2007, Skoko et al. 2008
			NF1_000817	Unknown	129965	https://databases.lovd.nl/shared/variants/0000219977#00014502	Messiaen et al. 1997, Wimmer et al. 2007, Skoko et al. 2008
			NF1_000817	De novo	130803	https://databases.lovd.nl/shared/variants/0000220815#00014502	Messiaen et al. 1997, Wimmer et al. 2007, Skoko et al. 2008
			NF1_000817	Unknown	130869	https://databases.lovd.nl/shared/variants/0000220883#00014502	Messiaen et al. 1997, Wimmer et al. 2007, Skoko et al. 2008
			NF1_000821	Unknown	129969	https://databases.lovd.nl/shared/variants/0000219981#00014502	Upadhyaya et al. 1996
			NF1_000821	Unknown	129970	https://databases.lovd.nl/shared/variants/0000219982#00014502	Upadhyaya et al. 1996
			NF1_000821	Unknown	130801	https://databases.lovd.nl/shared/variants/0000220813#00014502	Upadhyaya et al. 1996
			NF1_000821	Unknown	130802	https://databases.lovd.nl/shared/variants/0000220814#00014502	Upadhyaya et al. 1996
			NF1_000823	Unknown	64530	https://databases.lovd.nl/shared/variants/0000096077#00014502	
			NF1_000823	Unknown	64527	https://databases.lovd.nl/shared/variants/0000096074#00014502	
			NF1_000823	Unknown	129972	https://databases.lovd.nl/shared/variants/0000219984#00014502	
46	r.6859_6999del	No entries in LOVD 3.0					
47	r.7000_7126del	p.Ser2334fs	NF1_001355	Unknown	130812	https://databases.lovd.nl/shared/variants/0000220824#00014502	Sabbagh et al. 2013
			NF1_001356	Unknown	130813	https://databases.lovd.nl/shared/variants/0000220825#00014502	Sabbagh et al. 2013
48	r.7127_7258del	p.Gly2376_Ala2419del	NF1_001366	Germline	130825	https://databases.lovd.nl/shared/variants/0000220837#00014502	Griffiths et al. 2007
			NF1_000865	Unknown	64423	https://databases.lovd.nl/shared/variants/0000095965#00014502	
			NF1_000865	De novo	130031	https://databases.lovd.nl/shared/variants/0000220043#00014502	
			NF1_000865	Unknown	131253	https://databases.lovd.nl/shared/variants/0000221267#00014502	
			NF1_001587	Unknown	131254	https://databases.lovd.nl/shared/variants/0000221268#00014502	Pros et al. 2008

49	r.7259_7394del	No entries in LOVD 3.0					
50	r.7395_7552del	p.Thr2466fs	NF1_001809	De novo	131499	https://databases.lovd.nl/shared/variants/0000221513#00014502	Hsiao et al. 2015
			NF1_001370	Unknown	130830	https://databases.lovd.nl/shared/variants/0000220842#00014502	Brems et al. 2009
51	r.7553_7675del	No entries in LOVD 3.0					
52	r.7676_7806del	No entries in LOVD 3.0					
53	r.7807_7907del	p.Thr2604fs	NF1_001378	Unknown	130846	https://databases.lovd.nl/shared/variants/0000220858#00014502	Sabbagh et al. 2013
			NF1_000909	Unknown	130847	https://databases.lovd.nl/shared/variants/0000220859#00014502	Sabbagh et al. 2013
			NF1_000909	Unknown	131266	https://databases.lovd.nl/shared/variants/0000221280#00014502	Sabbagh et al. 2013
			NF1_000909	Unknown	130100	https://databases.lovd.nl/shared/variants/0000220112#00014502	
			NF1_000909	Unknown	130099	https://databases.lovd.nl/shared/variants/0000220111#00014502	
			NF1_001598	Unknown	131271	https://databases.lovd.nl/shared/variants/0000221285#00014502	De Luca et al. 2004
			NF1_001599	Unknown	131272	https://databases.lovd.nl/shared/variants/0000221286#00014502	
54	r.7908_8050del	p.Val2636fs	NF1_001111	Unknown	130849	https://databases.lovd.nl/shared/variants/0000220861#00014502	Sabbagh et al. 2013
			NF1_001111	Unknown	130343	https://databases.lovd.nl/shared/variants/0000220355#00014502	
55	r.8051_8097del	No entries in LOVD 3.0					
56	r.8098_8314del	No entries in LOVD 3.0					
57	r.8315_8454del	No entries in LOVD 3.0					

Supplemental Table 1C: Exon Deletions References

Reference	Link
Ars et al., Prenat Diagn, 19(8), 1999	https://www.ncbi.nlm.nih.gov/pubmed/10451518
Ars et al., Hum Mol Genet, 9(2), 2000	http://www.ncbi.nlm.nih.gov/pubmed/10607834
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Purandare et al., Hum Mol Genet, 4, 1995	http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.870.5910&rep=rep1&type=pdf
Robinson et al., Hum Genet, 96(1), 1995	https://www.ncbi.nlm.nih.gov/pubmed/7607663
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Skoko et al., FEBS Lett, 582(15), 2008	https://www.ncbi.nlm.nih.gov/pubmed/18503770
Upadhyaya et al., Am J Hum Genet, 67(4), 1996	https://www.ncbi.nlm.nih.gov/pubmed/8837715
Upadhyaya et al., Hum Genet, 99, 1997	https://www.ncbi.nlm.nih.gov/pubmed/9003501
Wimmer et al., Hum Mutat, 28, 2007	http://www.ncbi.nlm.nih.gov/pubmed/17311297
Xu et al, Int J Mol Med, 34, 2014	https://www.ncbi.nlm.nih.gov/pubmed/24789688
Zatkova et al., Hum Mutat, 24, 2004	https://www.ncbi.nlm.nih.gov/pubmed/15523642

Supplementary Table 2: Assessment of selected exons as targets for therapeutic exon skipping

Exon	Details	Conclusion
9	Predictions suggest that deleting exon 9 impacts the secondary structure of at most 4% and the solvent accessibility of at most 13% of remaining residues, if any, as reliability for these predictions are very low or low. Solvent accessibility of NF1delE9 is likely reduced beyond what can be attributed to the deleted exon. 11 out of 13 residues at positions 801-813 are predicted to be ordered (from disordered in full-length NF1). This suggests that the exon deletion could potentially reduce the protein's flexibility in this area, possibly affecting protein function. However, with the exception of one such prediction that has medium reliability, predictions have very low to low reliability. Average Conservation score for this exon is rather moderate at 5.9.	Deletion could potentially reduce the protein's flexibility and function
12	Predictions suggest that deleting Exon 12 impacts the secondary structure of at most 3% and the solvent accessibility of at most 8% of remaining residues, if any, as reliability for these predictions are very low or low. Solvent accessibility of NF1delE12 may be somewhat reduced beyond what can be attributed to the deleted exon. A 19 residue long sequence, starting with the residue produced by the first codon after the deleted exon, is now predicted to be ordered (from disordered in full-length NF1) - some of these individual predictions have medium reliability. This suggests that the exon deletion could potentially reduce the protein's flexibility in this area, possibly affecting protein function. Average Conservation score for this exon is rather moderate at 4.9.	Deletion could potentially reduce the protein's flexibility, possibly affecting protein function.
17	Predictions suggest that deleting Exon 17 impacts the secondary structure by at most 2% and the solvent accessibility of at most 7% of remaining residues, if any, as reliability for these predictions are very low or low. NF1delE17's solvent accessibility seems somewhat similar to that of neurofibromin minus the solvent accessibility attributed to amino acids translated from exon 17. Differences in predicted residue order/disorder state are minimal, and all have the lowest reliability score. Average Conservation score for this exon is the lowest of all exons at 1.2.	This suggests that the deletion of exon 17 seems to affect the protein's function very little, if at all.
18/19	Predictions suggest that deleting Exons 18/19 impacts the secondary structure of at most 3% of residues. While most predictions have very low or low reliability, four predicted changes have medium (4 or 5) and one has high (6) reliability, out of which those with reliability 5 or higher are found in the CSRD, suggesting that deleting Exons 18/19 affects the structure of the CSRD. It is further predicted that the solvent accessibility of at most 9% of remaining residues changes. Again, the reliabilities of most of these predictions are very low or low, with the exception of one predicted residue, which changed from intermediate to buried and is located in the CSRD. That said, the average solvent accessibility change per residue is roughly zero. Out of 27 residues predicted to change from ordered to disordered, 17 are in a 20-residue long sequence within the CSRD (pos. 796-815 in NF1fl), but prediction reliabilities are all very low or low. Average Conservation scores for both exons are rather moderate at 6.4.	Overall, it appears that the CSRD could be affected by E18/19 deletion. Deletion is predicted to alter secondary structure

20	Only 3% of residues are predicted to change their secondary structure. While the majority of predictions has very low or low reliability, 11 predicted changes of residue secondary structure have high or very high reliability. All these residues are located in a 14 residue long subsequence (pos. 804-817) of the CSRD. The same sequence has predicted changes to solvent accessibility and order/disorder status with medium to very high and medium to high reliability, respectively (in total, at most 8% of residues are predicted to have an altered solvent accessibility status). Interestingly, the change of average solvent accessibility per residue is negative, when compared to neurofibromin, implying an increase of solvent accessibility for the remaining residues in NF1del20. Average Conservation score for this exon is rather moderate at 4.5.	In conclusion, the protein structure and conformation could be significantly altered by the deletion of exon 20.
21	5% of residues have a different predicted secondary structure, albeit with low or very low reliability, while for 14% a changed solvent accessibility is predicted, again with low or very low reliability - with one exception: a residue in the CSRD, which is adjacent to the deleted exon sequence. NF1delE21 has likely a reduced overall solvent accessibility, beyond what is attributed to the loss of exon 21. A cluster of eight consecutive residues located in the CSRD at positions 796-803, adjacent to the deleted exon in NF1fl, is predicted to be disordered (compared to ordered in full-length NF1) with medium reliability. Average Conservation score for this exon is rather moderate at 5.2.	This indicates a potential increase in flexibility in this region of the protein, possibly affecting function.
25	4% of NF1delE25's residues have a different predicted secondary structure, mostly with low or very low reliability (for 3 residues, predicted changes have medium reliability). For 12% of residues a changed solvent accessibility is predicted. The protein's solvent accessibility seems reduced as indicated by the relative high loss of average solvent accessibility per residue, the second highest of all tested proteins with deleted exons. Also, there is a cluster of 11 residues at position 801-813 (in full-length NF1) that is predicted to be ordered, indicating a loss of flexibility. However, prediction reliabilities are very low, which limits the interpretability of these results. Conservation of this exon is quite high with a score of 8.1 suggesting this exon might be essential.	Conservation of this exon is quite high with a score of 8.1 suggesting this exon might be essential.
28	NF1 structure is likely significantly affected by exon 28 deletion: 7 predicted changes have medium, 3 have high, and another 8 have very high reliability; and 7 out of those 8 are related to residues in the GRD, while the 8th is for a residue within the Nex-GRDmin-Ces region. We hypothesize that this has a direct negative impact on Ras/Spred1 binding. Moreover, 5 residues are predicted, with medium reliability, to have changed solvent accessibility status, out of which 3 are found in the GRD, one is right outside the GRD and another is in the Tubulin binding domain. NF1delE28's overall solvent accessibility seems slightly reduced, but residues in the GRD seem to be affected the most. Conservation of this exon is high with a score of 8.4 suggesting this exon might be essential.	We fully anticipate that loss of this exon will result in loss of GRD activity as this exon codes for a known essential portion of the GRD.
36	All predictions, i.e. changes to secondary structure (4% of residues), solvent accessibility (12% of residues), disorder/order status, and protein binding have low or very low reliability, limiting the interpretability of the data. That said, NF1delE36 has an overall solvent accessibility that is reduced beyond what can be attributed to the loss of exon 36. Also, 11 resides in the CSRD (pos. 801-813) may have changed from disordered to ordered, which would potentially reduce the protein's flexibility. Conservation of this exon is quite high with a score of 7.4 suggesting this exon might be essential.	Conservation of this exon is quite high with a score of 7.4 suggesting this exon might be essential.

41	Only 3% of residues are predicted to have changed their secondary structure. While the majority of predictions have very low or low reliability, 3 predicted changes of residue secondary structure have medium or high reliability (all outside known domains). Also, 8% of residues have been predicted to change their solvent accessibility state, but all predictions have low or very low reliability. Total solvent accessibility of NF1delE41 seems similar to that of neurofibromin, if we disregard the lost solvent accessibility due to the deleted exon. Predictions of changes to the order/disorder status of residues have low or very low reliability and only occur in isolated single or pairs or residues. Conservation of this exon is high with a score of 7.5 suggesting this exon might be essential.	Overall, structural changes are likely and conservation of this exon is high with a score of 7.5 suggesting this exon might be essential.
47	3% of residues are predicted to have changed their secondary structure. While most predictions have low or very low reliability, 6 predictions have medium and 3 predictions have high reliability (but all these residues are outside any known domain). 10% of residues are predicted to have changed their solvent accessibility state. All predictions except one have low or very low reliability. The average loss of solvent accessibility per residue is the highest of all tested proteins with deleted exon(s). 19 residues within a 30 residue long sequence are predicted to have a change from disordered to ordered, albeit with low reliability. Conservation of this exon is moderate with a score of 6.8	Overall, structural changes are indicated and could in principle impact proper function.
52	Only 3% of residues are predicted to have changed their secondary structure. All predictions has very low or low reliability. For 12% of residues a changed solvent accessibility is predicted, but reliabilities are low or very low. There is only a small additional loss of solvent accessibility (on top of the loss due to exon 52 deletion). Predictions of changes to the order/disorder status of residues have low or very low reliability and mostly occur in isolated single or pairs or residues. Conservation of this exon is low with a score of 2.2. Overall, structural changes do not appear to be dramatic. Of concern is the loss of phosphorylation sites as mentioned in the main text: Exon 52 is phosphorylated at T2554 by PKA, which was shown to regulate interaction with 14-3-3 beta (human)[24], and at Y2556[25]. Loss of NLS is also a concern.	Overall, structural changes do not appear to be dramatic, but PTMs and NLS will be lost.

Supplementary Table 3: Aggregated phenotypic features in probands carrying a truncating NF1 mutation in exon

	N		
NF1 feature	**Exon 17[12b]**	**Exon 25[19b]**	**Exon 52[43]**
> 5 CALMs	64/66	53/55	17/21
Skinfold freckling	51/66	46/56	10/20 [a]
Lisch nodules [A]	8/17	11/15	1/4
externally visible plexiform neurofibromas [A]	4/28	4//27	1/6
externally visible and/or known internal plexiform neurofibromas [A]	8/28	8/27	1/6
Cutaneous neurofibromas [B]	18/19	10/14	4/5
Subcutaneous neurofibromas [B]	2/8	5/12	0/1
symptomatic spinal neurofibromas by MRI	1/6	2/7	2/6
osseous lesions	15/64 (including scoliosis, pseudarthrosis, pectus abnormality, pseudarthrosis)	10/64 (including bone cysts, pseudarthrosis and scoliosis)	1/21 (scoliosis)
OPGs [C]	3/16	4/14	1/7 [b]
Other malignant neoplasms [D]	1/49 [c]	1/49 [d]	0/18

A probands aged ≥9 years

B probands aged ≥19 years

C by clinical exam or MRI (symptomatic or asymptomatic)

D not taking into account neurofibromas or optic pathway gliomas

[a] 4/10 individuals with reportedly no skinfold freckling were ≤5 years old

[b] all 7 patients had an MRI and 1 *asymptomatic* OPG was identified

[c] MPNST

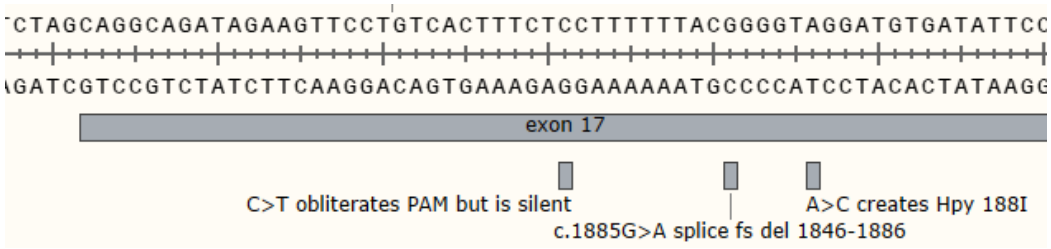
[d] adrenal ganglioneuroblastoma

Supplementary Table 4: Additional Notable Variants in exons 17, 25, and 52 reported in MGL Data set

Exon 17	In addition to 74 unrelated truncating pathogenic variants, eight different missense variants were observed in exon 17, with three classified as likely benign (p.Ile634Thr, p.His647Tyr and p.Arg659Gln) and five of uncertain significance (p.Asp646Tyr; p.Pro654Ser; p.Leu658Phe; p.Lys662Arg and p.Gly663Arg). Four pathogenic variants in exon 17 are recurrent: c.1885G>A, which results in out of frame missplicing r.1846_1886del; p.Gln616GlyfrTer4 and is found in 40 unrelated probands, and c.1846C>T, p.Gln616Ter, which was found in three unrelated probands. Furthermore, c.1882delT and c.1882dupT were both observed twice as well (recurrence due to presence of a stretch of 6 T's).
Exon 25	Splicing of exon 25 is complex, as multiple exonic substitutions affect splicing, and substitutions at the canonical AG/GT splice sites lead to activation of cryptic exonic splice donor/acceptor sites and out of frame missplicing. Six probands carry one of two different observed exonic splice variants mimicking a missense variant but leading to out-of-frame missplicing, i.e,c.3277G >A (r.3275_3314del) and c.3304T>G (r.3304_3314del); two probands carried an exonic splice variant mimicking a nonsense variant but leading to out-of-frame missplicing, i.e., c.3313A>T (r.3275_3314del); three probands carried an exonic splice variant mimicking a missense variant but leading to in-frame missplicing, i.e., c.3212C>T (r.3211_3314del). Fifteen probands carry one of four different splice variants affecting the AG/GT splice sites flanking the exon leading to out-of-frame missplicing (and *not* just in-frame skipping of the exon 25 and therefore also considered truncating variants), i.e., c.3198-2A>G (r.3198_3199del); c.3198-2A>T (r.3198_3199del); c.33314+1G>A (r.3275_3314del) and c.3314+2T>A (r.3275_3314del).
Exon 52	c.7606C>T, identified in two probands, mimicks a nonsense; and c.7610A>T mimicks a missense, but both lead to out of frame missplicing (r.7605_7675del71); and c.7675+1del results in out of frame skipping of the last nucleotide (G) of the exon. Another two probands carry the c.7675+1G>A pathogenic variant which results in both in-frame skipping of exon 52 in the majority of transcripts, *and* in-frame insertion of 39 nts from the intron 52, incorporating a premature termination codon (PTC) in a small fraction of the transcripts (r.7675_7676ins7675+1_7675+39). (The presence of a PTC suggests that the longer transcript is prone to nonsense mediated decay and may explain why there is less of this transcript.) One >14-<18-year-old proband carrying the c.7675+1G>A presented with <6 CALMs and symptomatic spinal neurofibromas; the second proband presented with ~10 CALMs at the time of testing (<24-months) and his mother (>26-year) had >6 CALMs and bilateral inguinal freckling only.

Suppl Figure 1: Characterization of Precision Cell Line Models for c.1885G>A in NF1 Exon 17

A

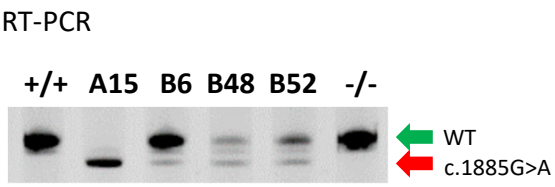


B

Sub-cloning results

Clone	WT Allele	Mut allele
A15	0	20
B6	17	3
B48	10	10
B52	6	14

C



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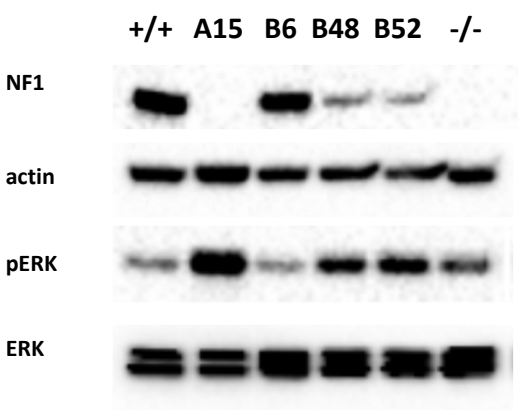

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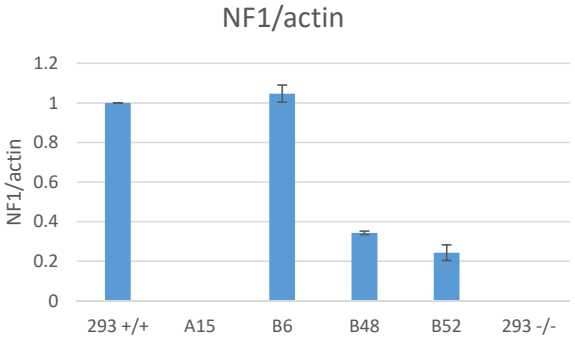

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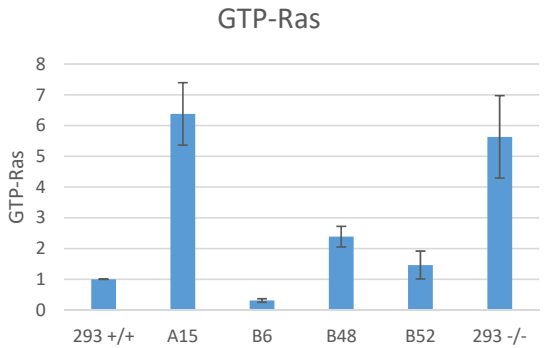
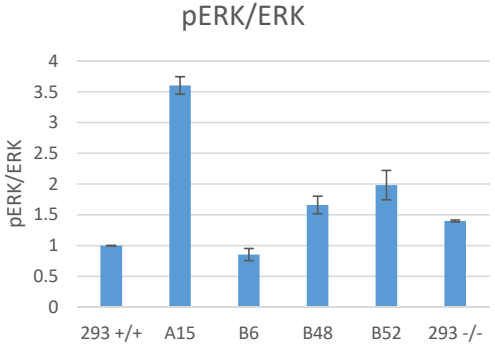
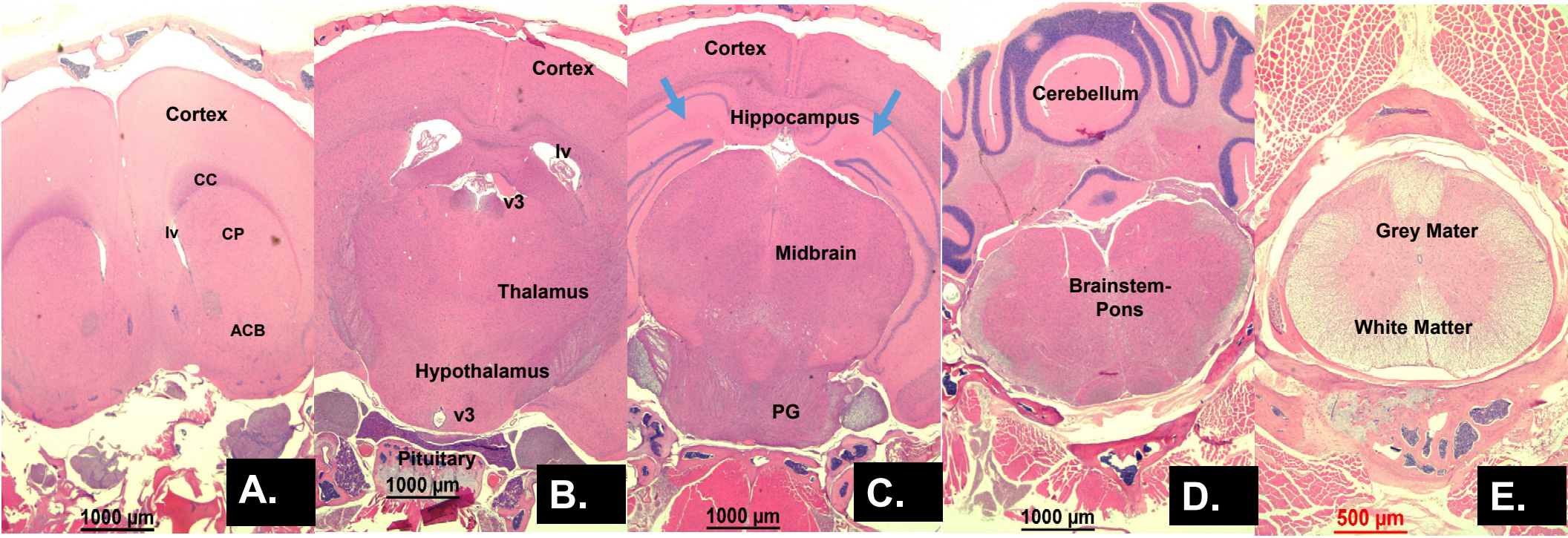


G



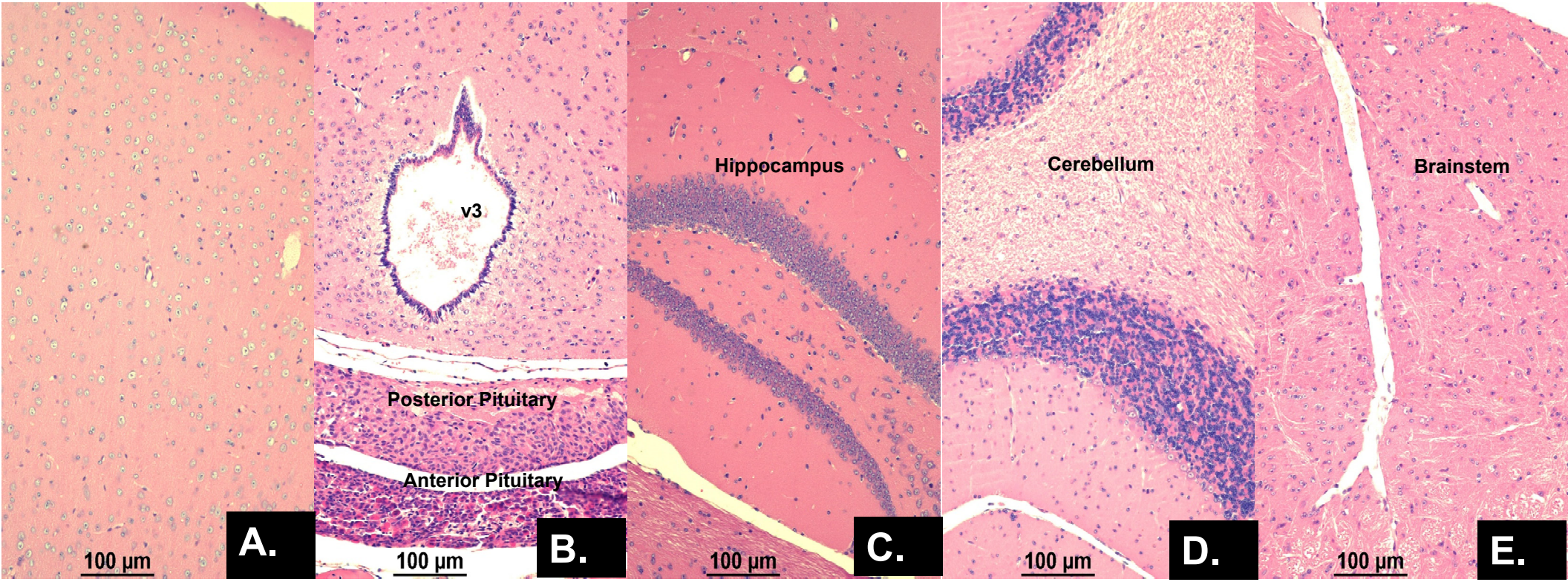
- **Supplementary Figure 1:** Characterization of Precision Cell Line Models for c.1885G>A in NF1 Exon 17 in HEK293 cell lines. A. Diagram depicting wild type genomic sequence surrounding 5' region of NF1 exon 17. Exon 17 is denoted with a gray bar and alterations are indicated with gray rectangles. B. Table summarizing sequencing results from subclones of four different single cell isolates containing c.1885G>A: A15, B6, B48, and B52 indicating various ratios of WT and null alleles. C. RT-PCR products of each cell line displaying both WT allele (denoted with green arrow) and mutant deletion allele (denoted with red arrow) indicating the anticipated altered splicing in variant cell lines in comparison to WT. D. Example Western blots showing representative neurofibromin, actin, pERK, and total ERK expression for each variant cell line indicated in comparison to WT HEK293 cells (+/+) and null HEK293 cells (-/-) where exon 2 has been targeted. E. Quantitation of neurofibromin/actin ratios for the various cell lines. For comparison between cells, WT cells were set at 1 and variant cell lines were normalized based on WT. N=3; error bars represent SEM. F. Quantitation of GTP-Ras level via Ras-GLISA for the various cell lines N=3; error bars represent SEM. For comparison between cells, WT cells were set at 1 and variant cell lines were normalized based on WT GTP-Ras levels. G. Quantitation of pERK/ERK ratios for the various cell lines N=3; error bars represent SEM. Again, WT pERK/ERK ratios were set at 1 and all other lines normalized to WT levels.

Suppl Figure 2: Low magnification (2x) coronal Sections of Brain including (A) Cerebral cortex, (B) Thalamus/hypothalamus, (C) Hippocampus, (D) Cerebellum and brainstem, and (E) Spinal cord (2x magnification) with further definition of subanatomic regions



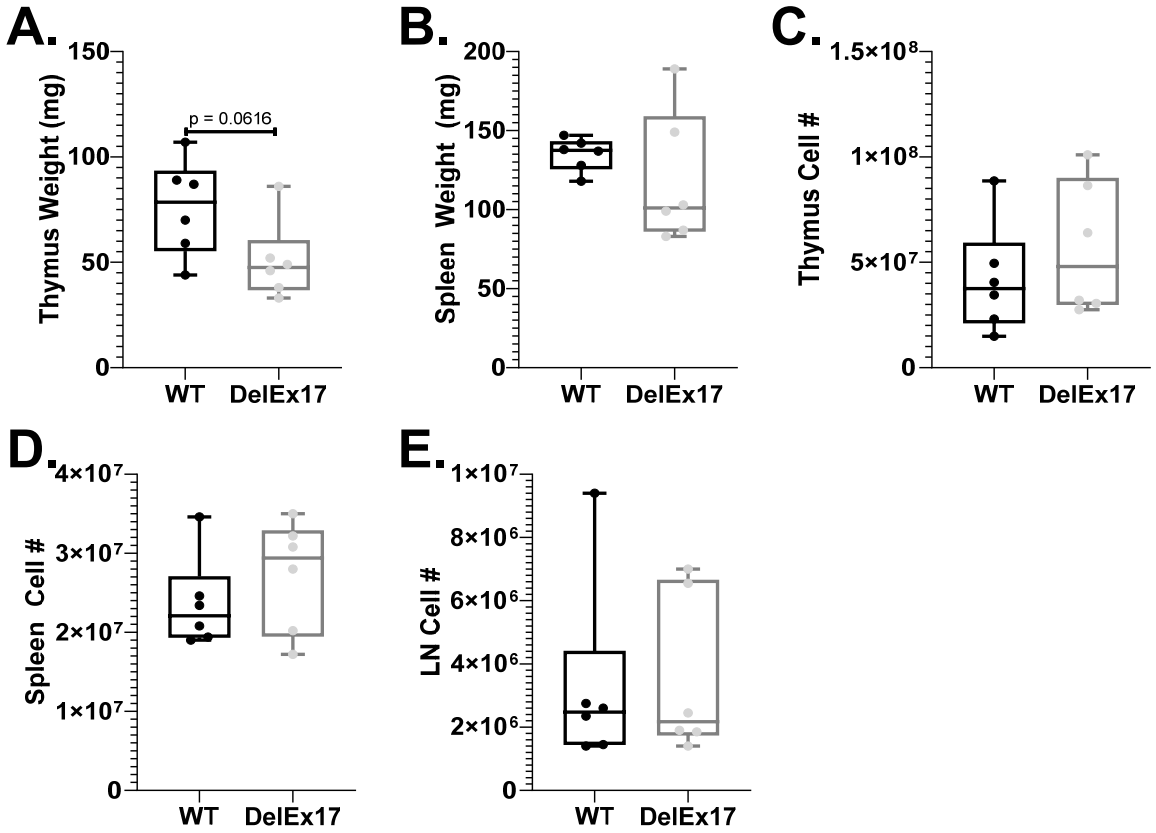
- **Supplementary Figure 2:** Lower magnification coronal sections through the brain reveal no discernable tumor formation of pathology in DelE17 mice. **A-E.** Coronal sections through the (**A**) cerebrum, (**B**) thalamus and hypothalamus, (**C**) Hippocampus, thalamus, and hypothalamus, (**D**) cerebellum and brainstem, and (**E**) spinal cord. All images are 2x magnification with the exception of spinal cord (4x magnification). All sections are representative of CNS histology seen in all 6 mice evaluated.

Suppl Figure 3: Higher magnification (20x) of coronal Sections of (A) Cerebral cortex, (B) Hypothalamus,3rd ventricle, pituitary, (C)Hippocampus, (D) Cerebellum, and (E) Brainstem (20x magnification)



Supplementary Figure 3: Higher magnification of coronal sections through the brain reveal no discernable tumor formation of pathology in DelE17 mice. **A-E.** Coronal sections through the (**A**) cerebrum, (**B**) hypothalamus and pituitary, (**C**) Hippocampus, (**D**) cerebellum, and (**E**) brainstem. All images are 20x magnification. All sections are representative of CNS histology seen in all 6 mice evaluated.

Suppl Figure 4: Gross and cellular assessment of thymus, spleen, and lymph nodes in WT and DelE17 mice



Supplementary Figure 4: Gross and cellular assessment of thymus, spleen, and lymph nodes in WT and DelE17 mice. **A.** Thymus and **B.** Spleen weights (mg). **C.** Thymus, **D.** Spleen, **E.** Lymph node cellularity represented as absolute cell numbers.