2017.09.13. Revise

Journal: Scientific Reports

Title (19 / 20 words)

Dysregulated gene expressions of *MEX3D*, *FOS* and *BCL2* in human induced-neuronal (iN) cells from NF1 patients: a pilot study

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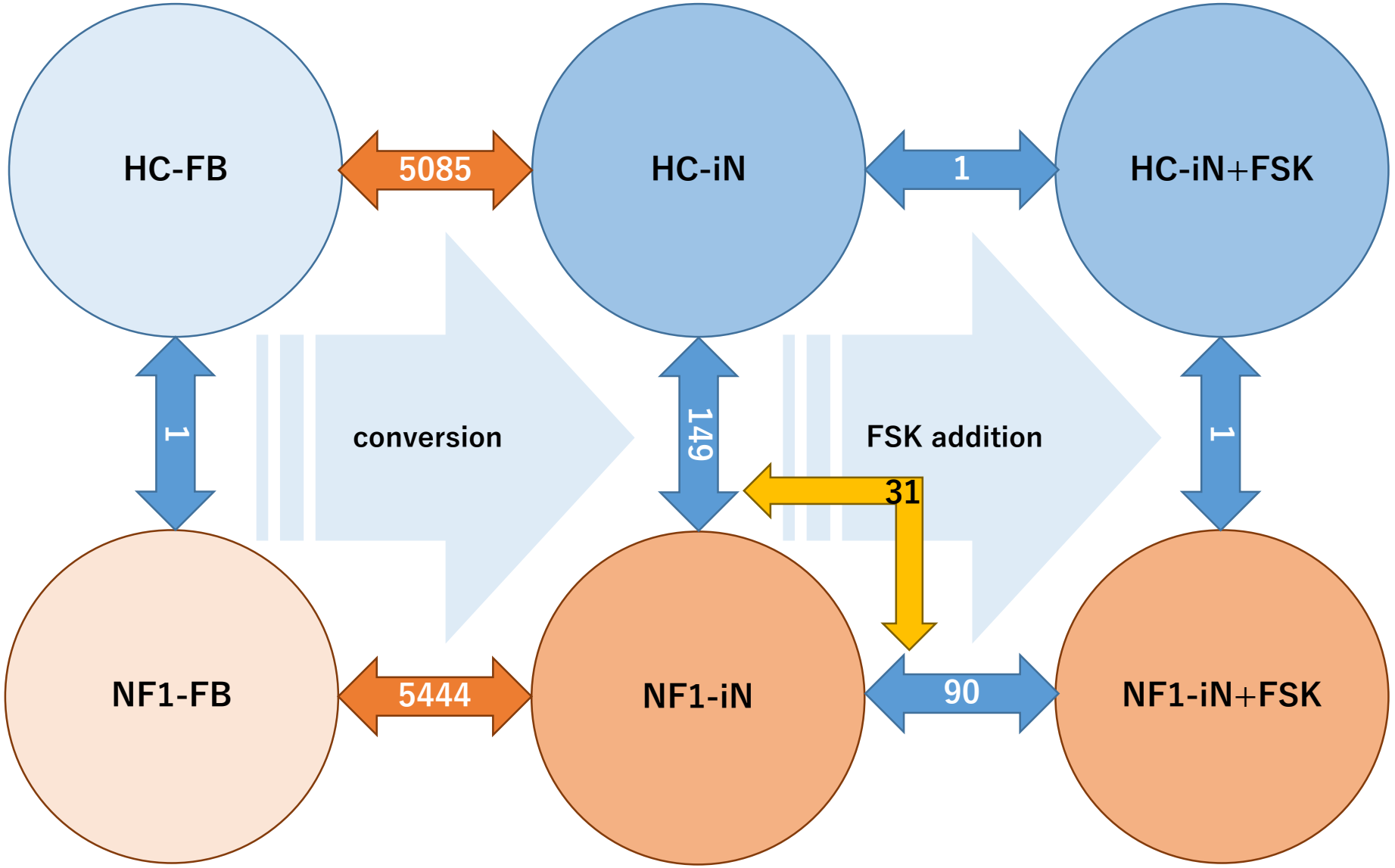
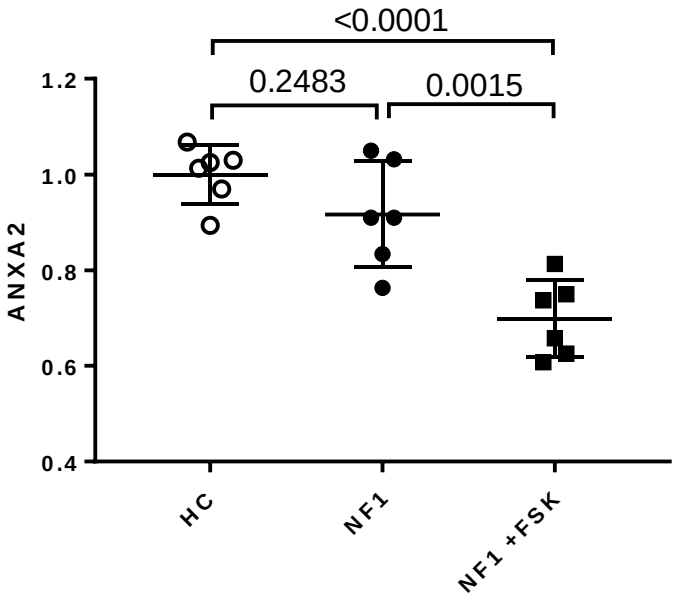
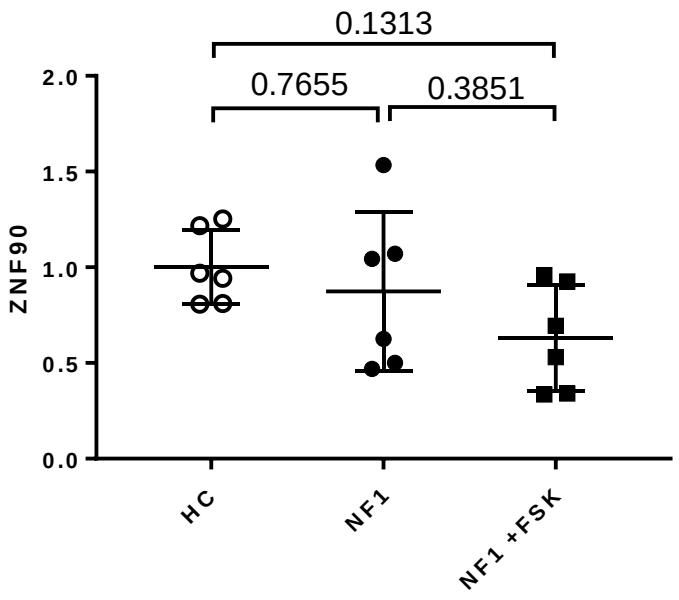
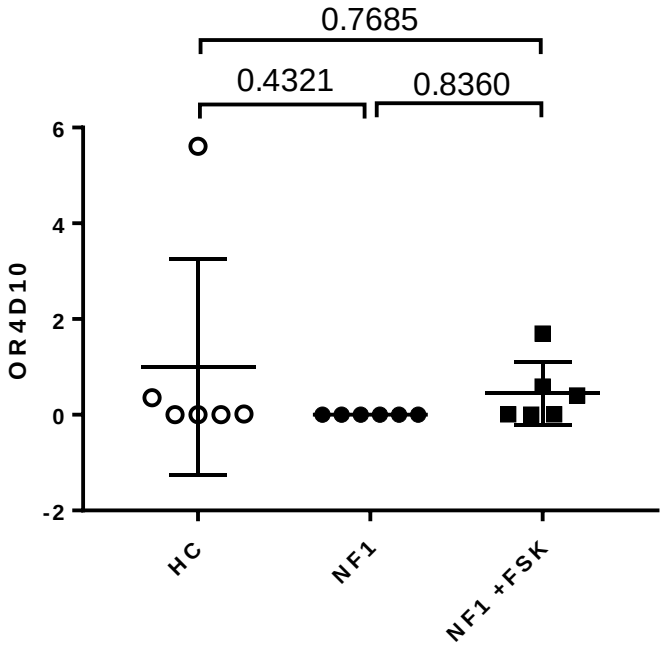
Supplemental information: 4 Figures and 4 Tables (These are included in the following files: Supplemental information (Sagata et al.).pdf, NF1 iN – Supplementary Table 3.xlsx, and NF1 iN – Supplementary Table 4.xlsx.)

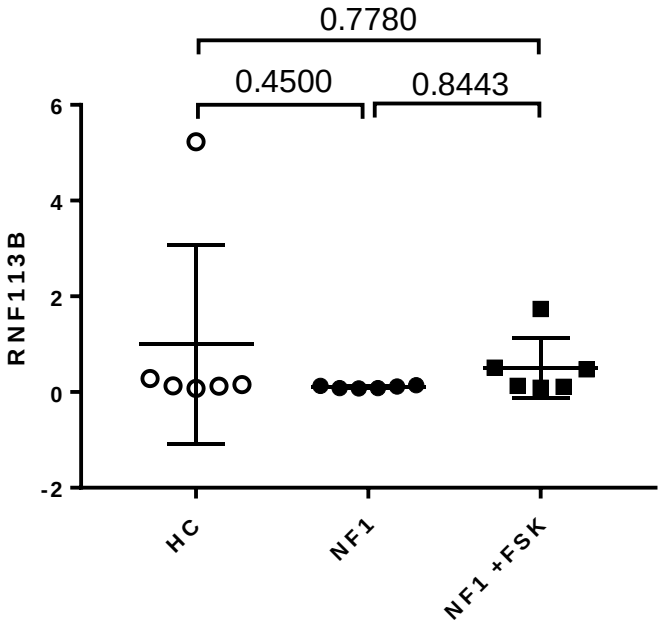


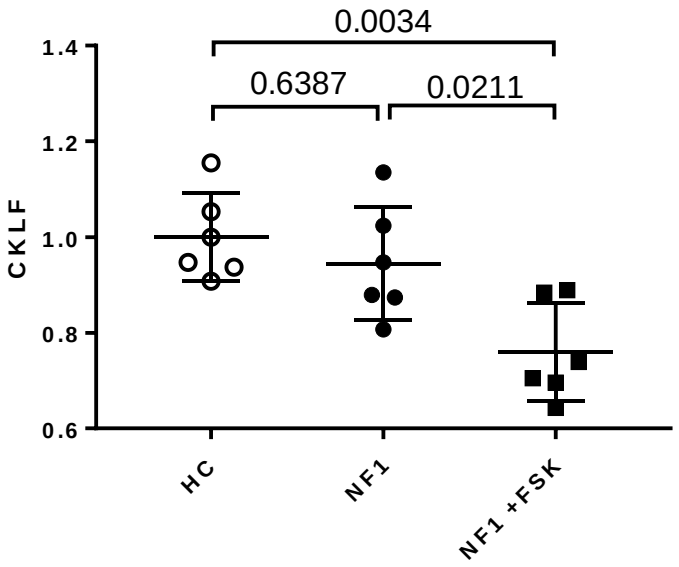
Supplementary Figure 1

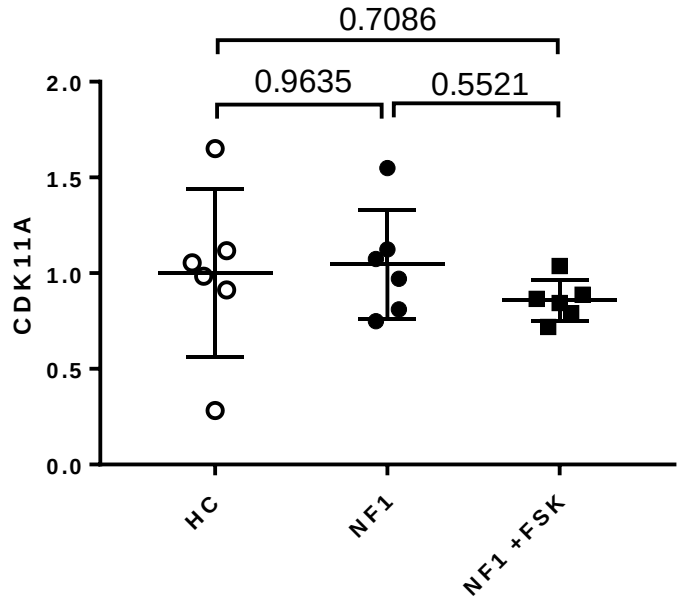




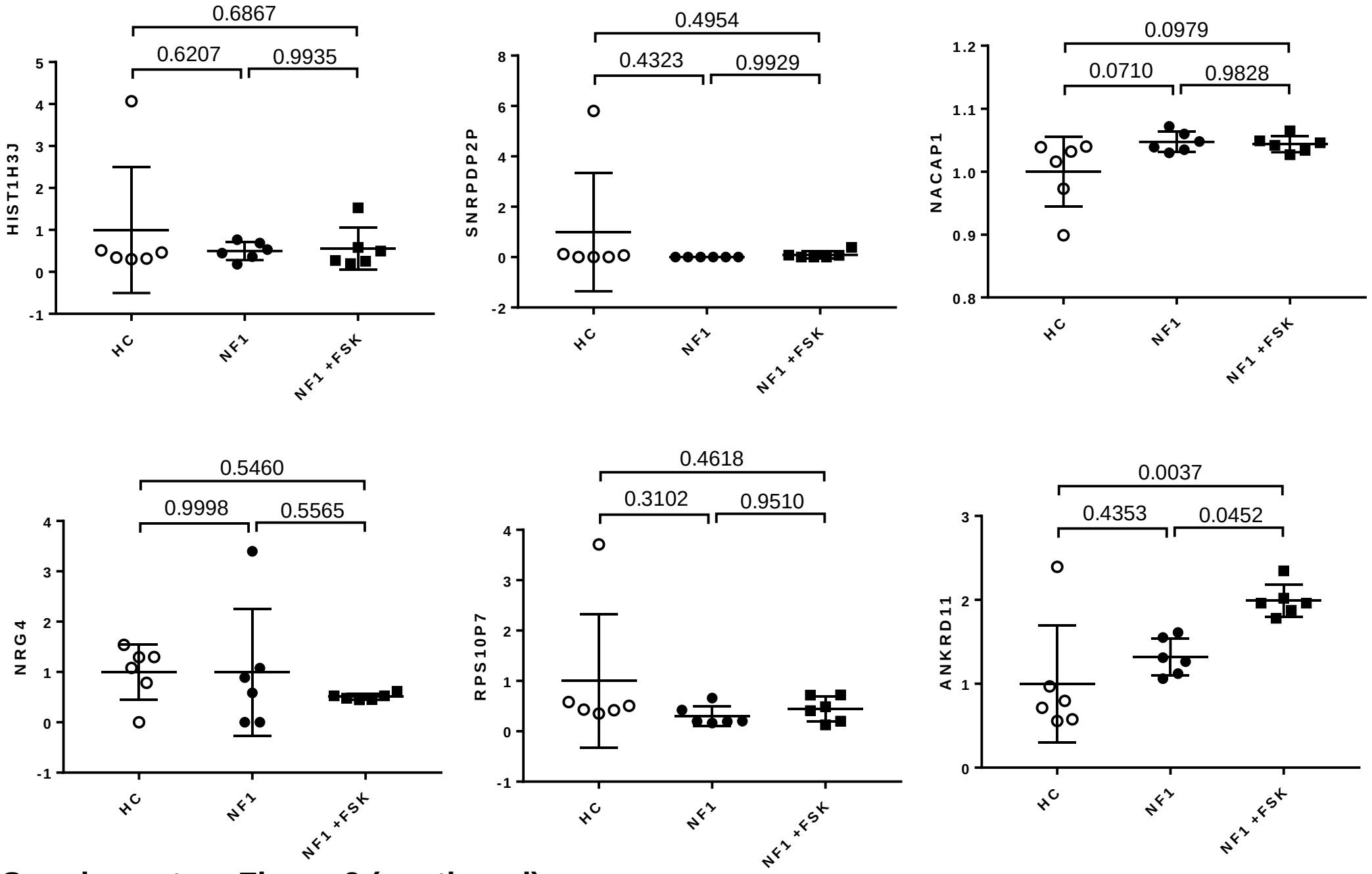








Supplementary Figure 2



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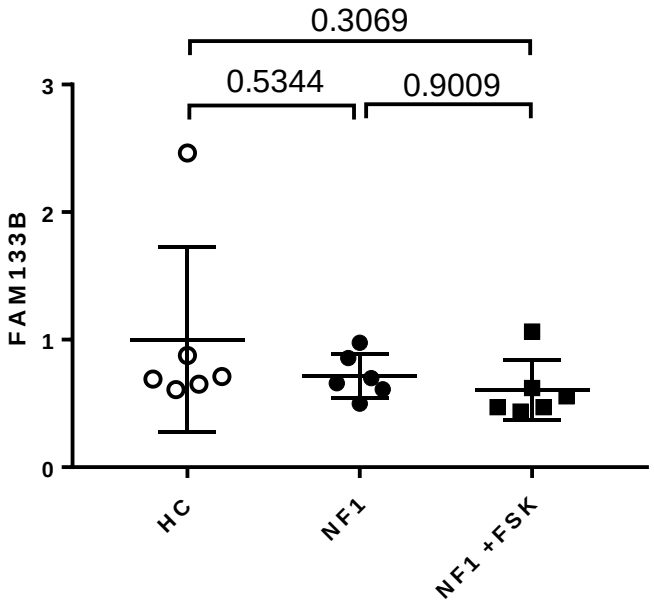

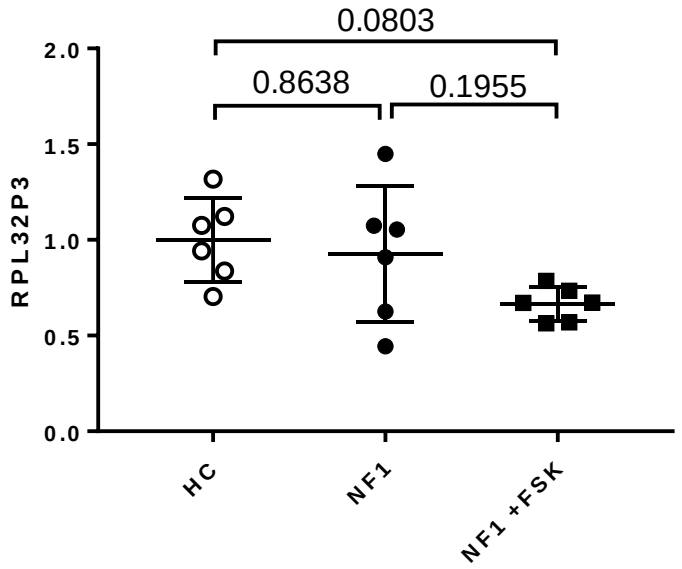

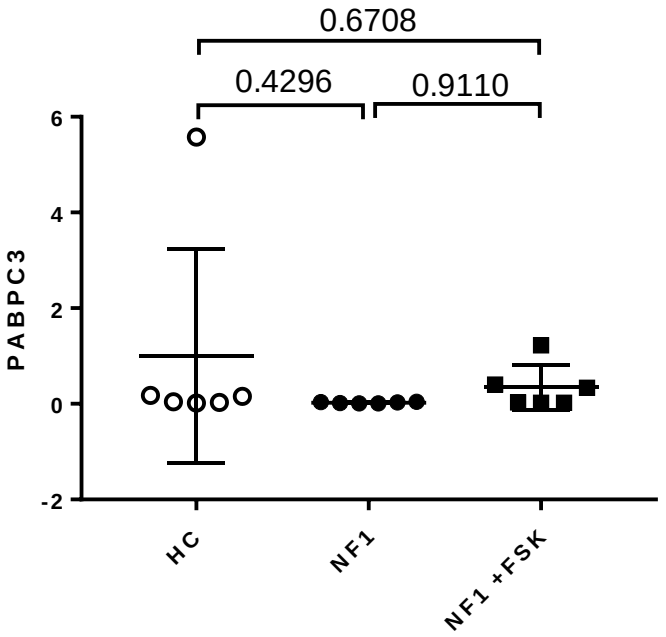

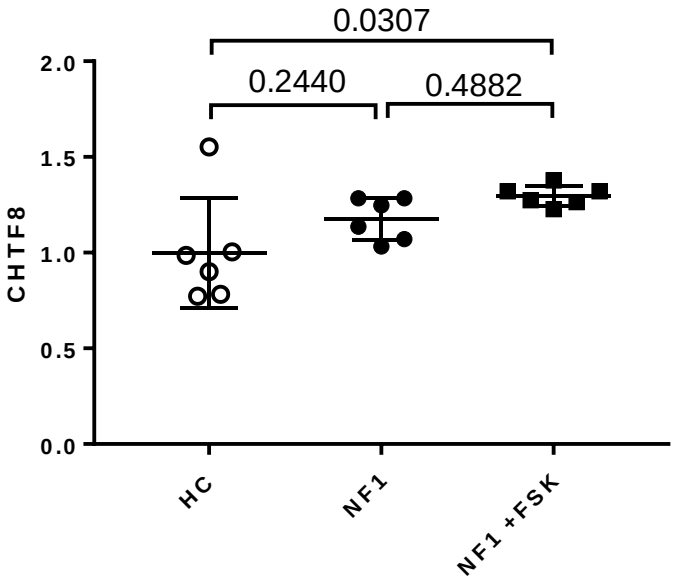

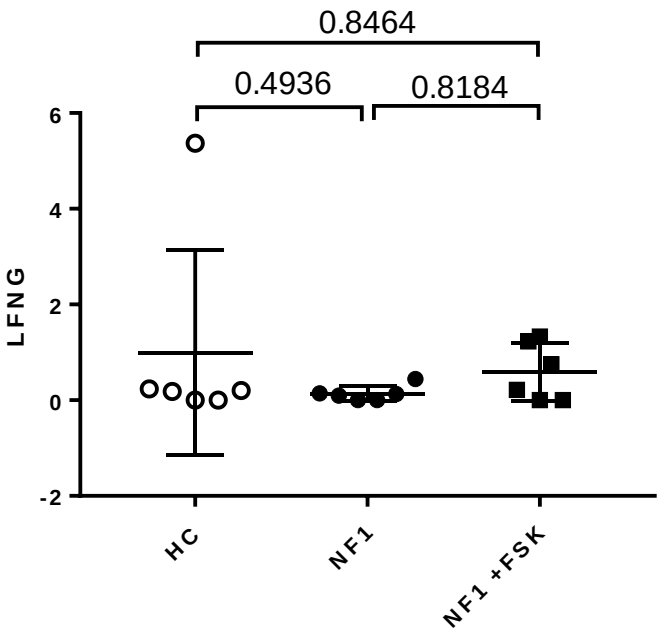

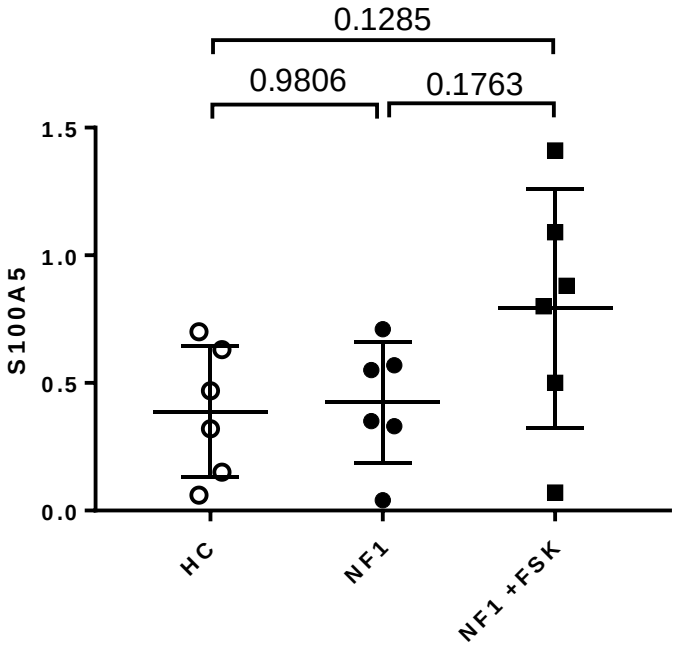

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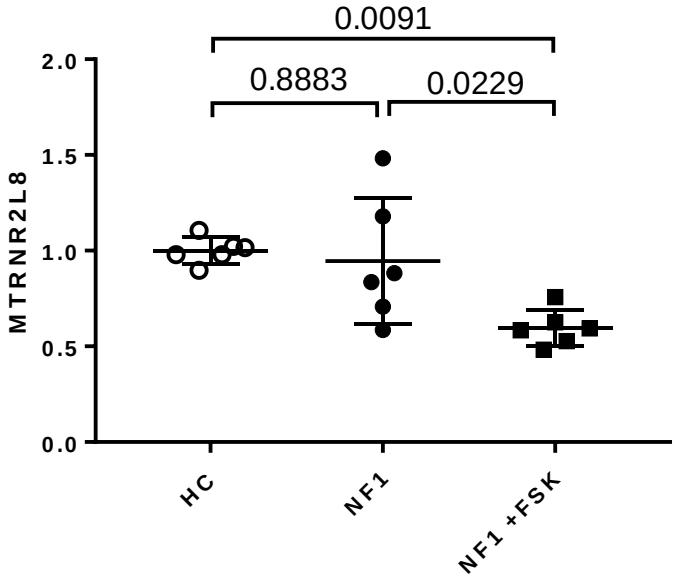

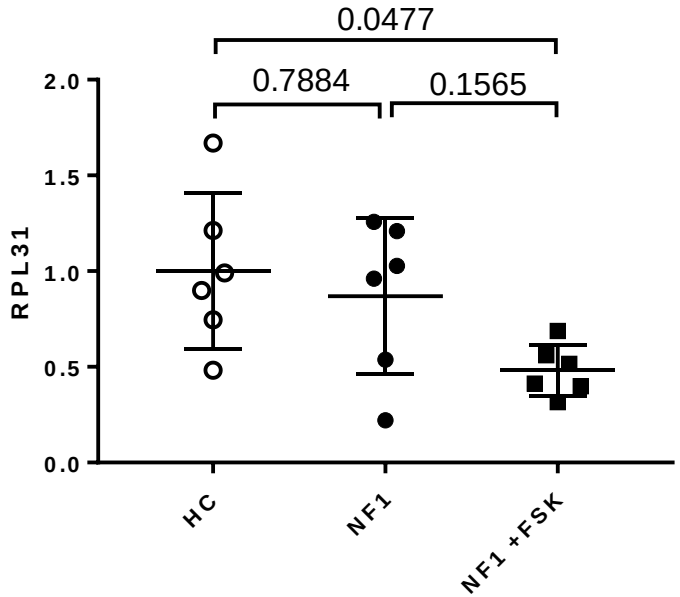

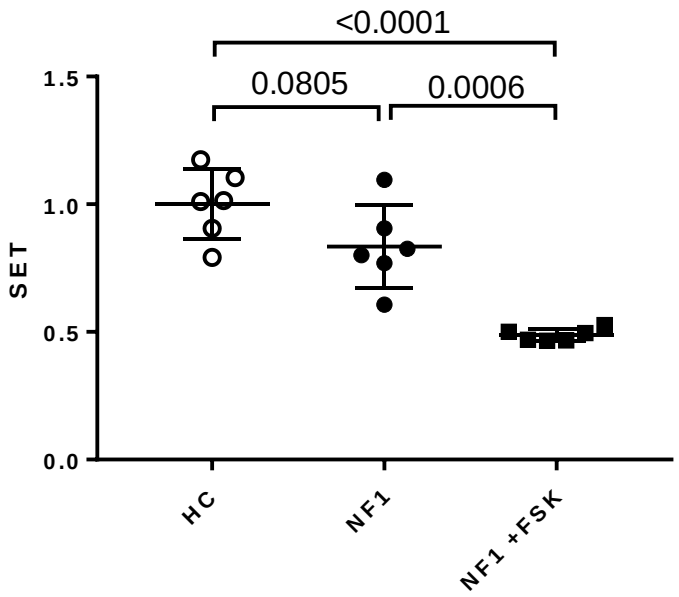

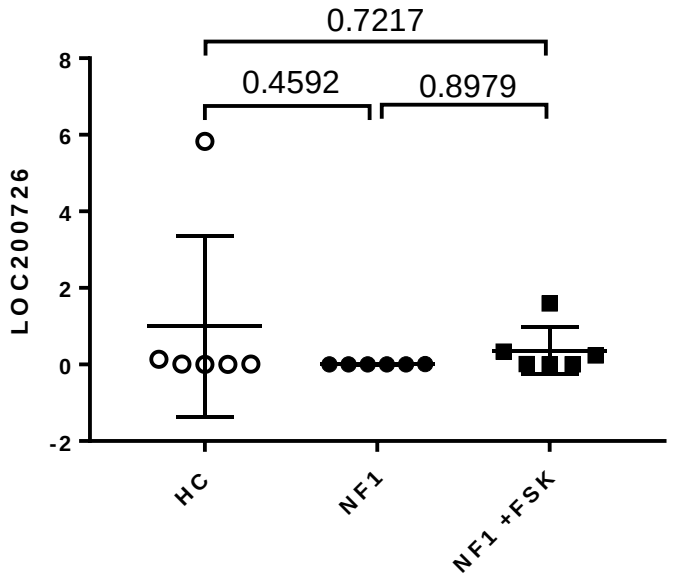

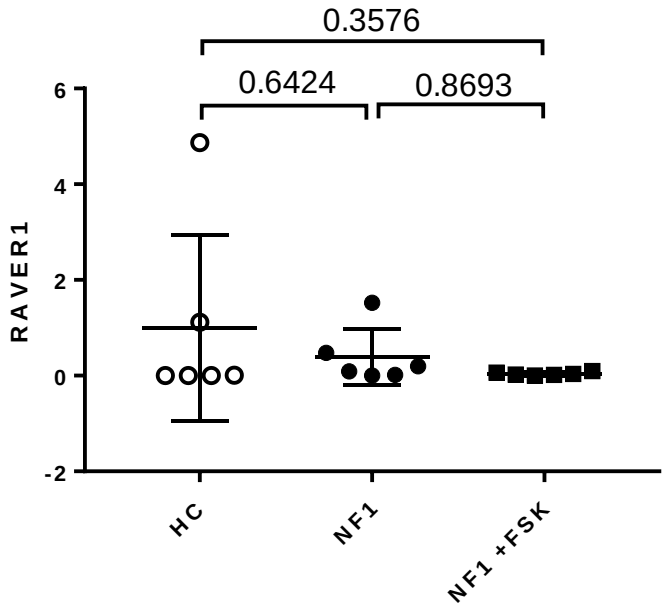

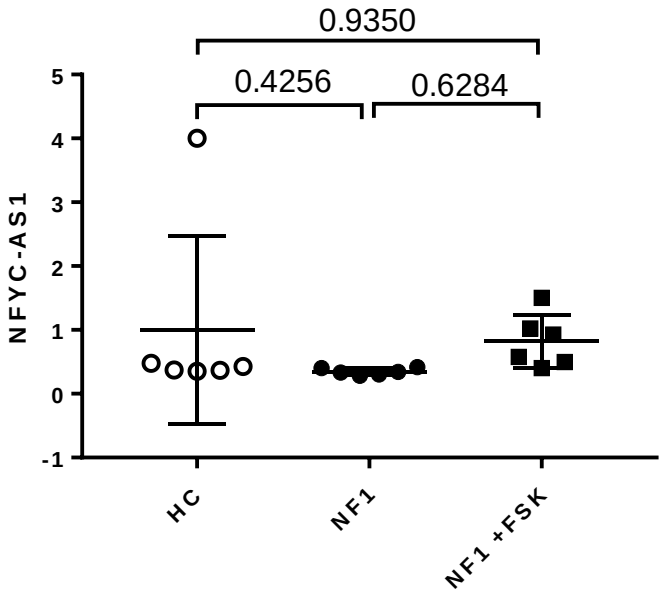

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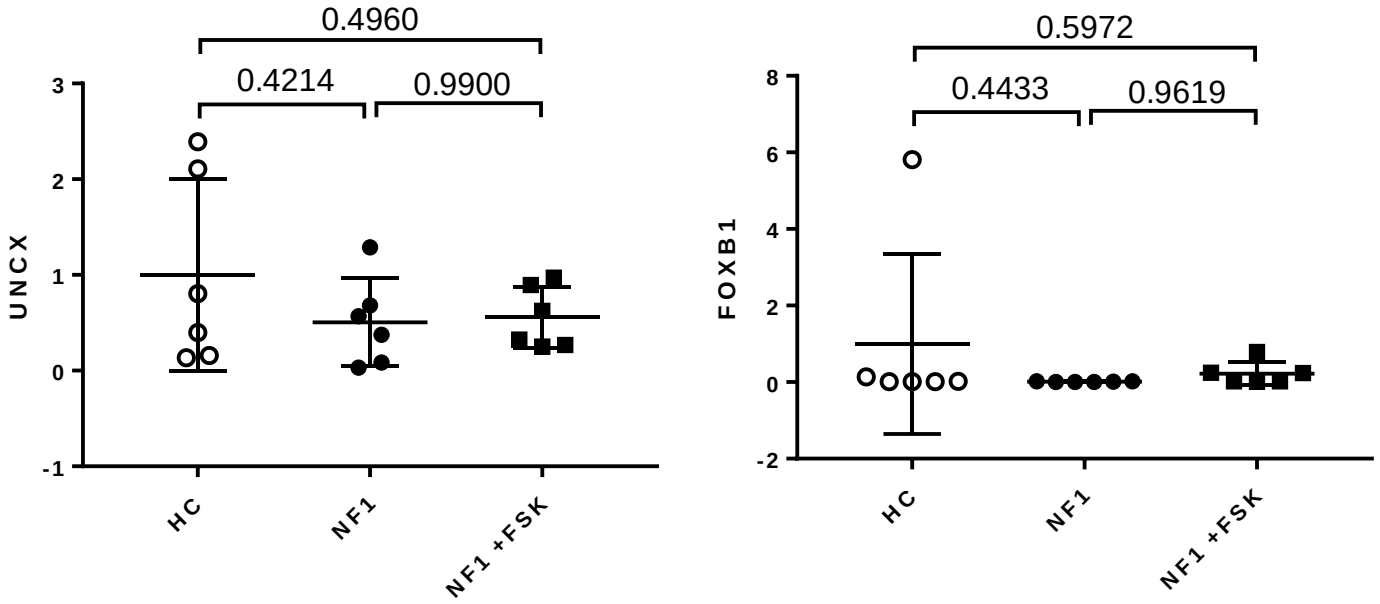

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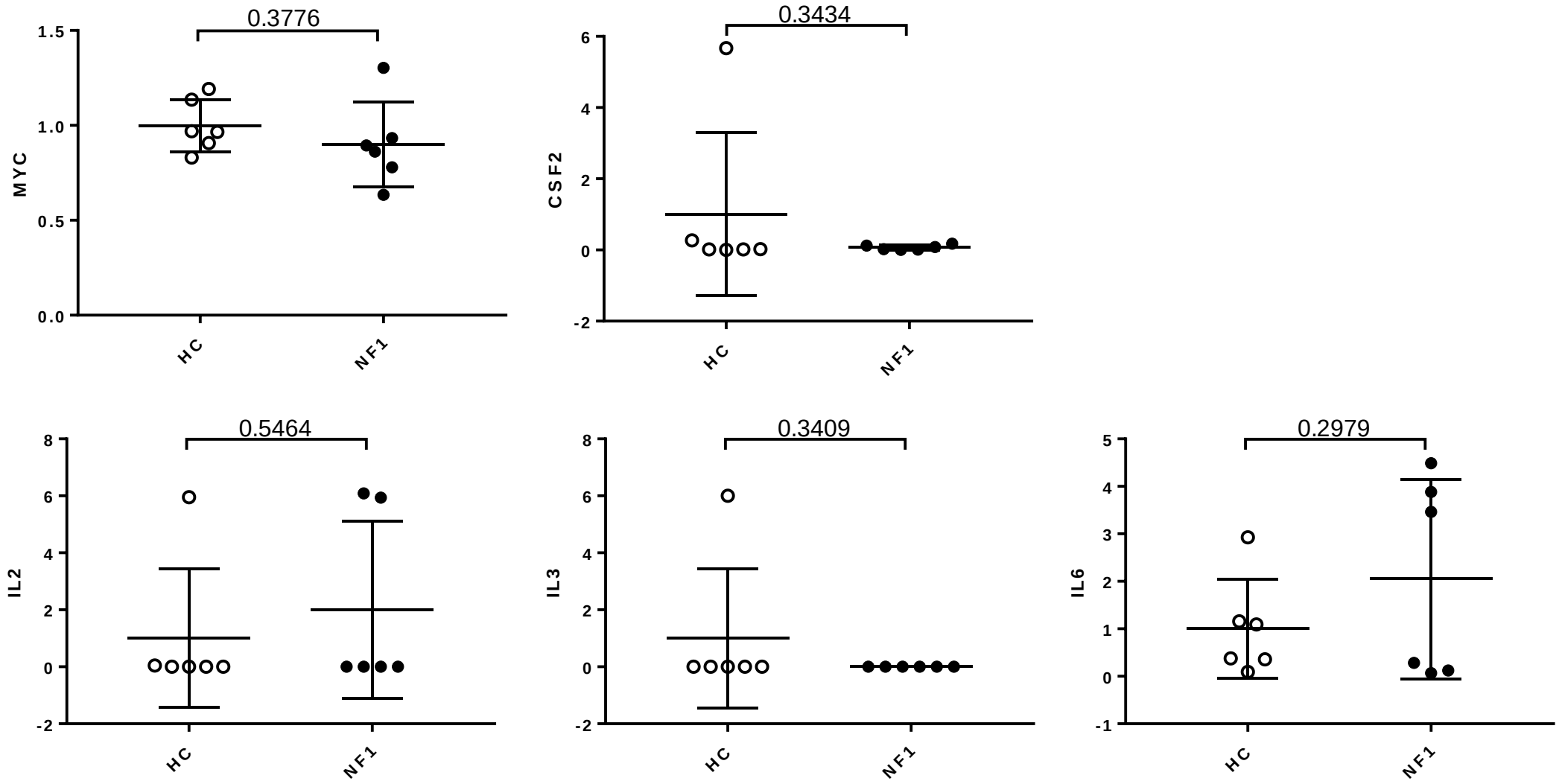
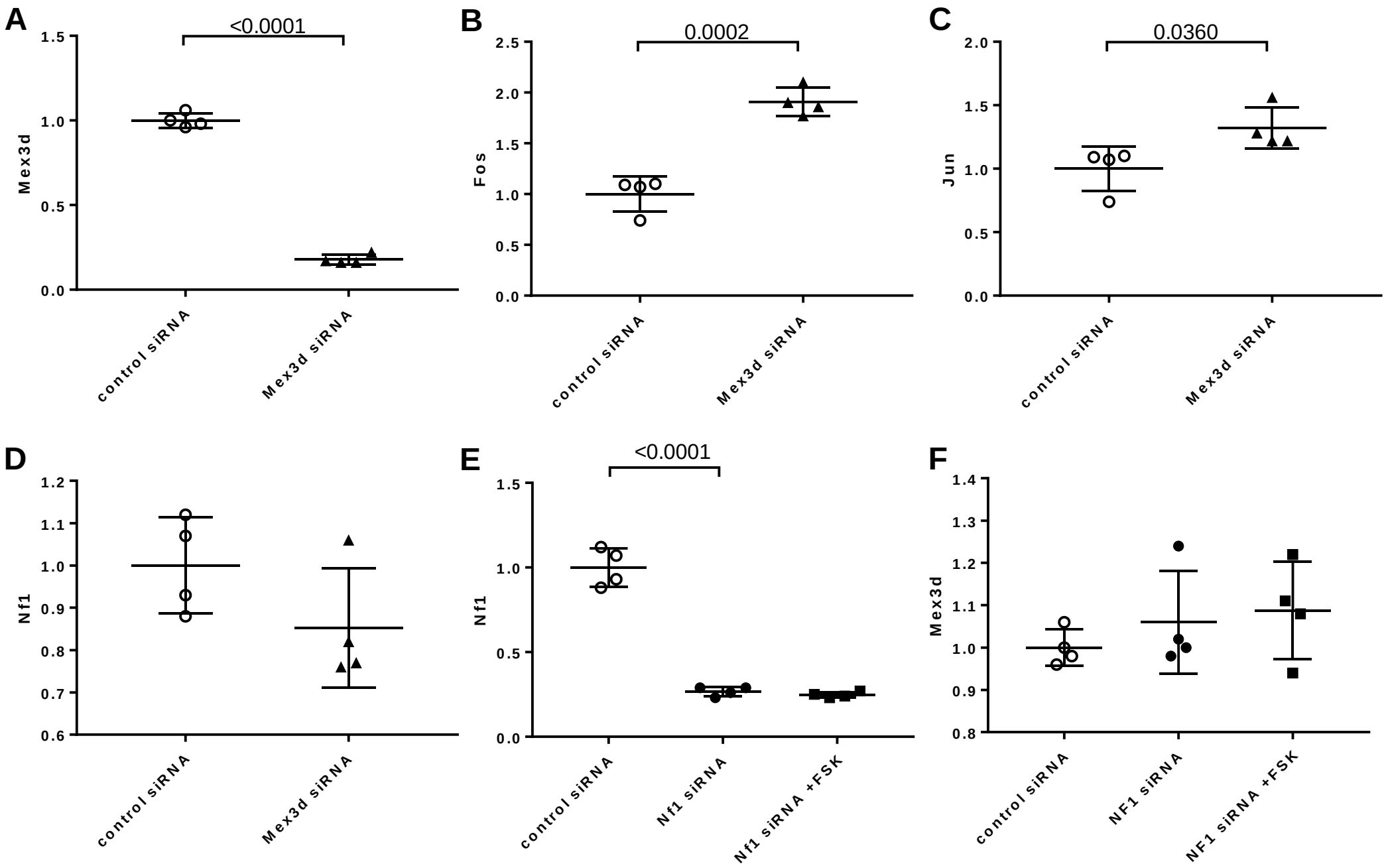


Supplementary Figure 3



Supplementary Figure 4

Supplementary Table 1: Information of fibroblasts for generating iN cells.

Description	ID	Sex	Age
Healthy control	GM03440*	Male	20
	GM05399	Male	1
	KYU-165*	Male	21
	KYU-166	Male	22
	KYU-168*	Male	61
	KYU-176	Male	49
NF1	GM00622*	Male	8
	GM01633*	Male	61
	GM01639	Female	19
	GM01859*	Male	41
	KYU-101	Female	67
	KYU-177	Female	35

(*Samples for microarray analysis)

Supplementary Table 2: Information of the primer and probe number for quantitative real-time PCR.

Species	Target	Position	Tm	%GC	Sequence	Probe#
human	ANKRD11	5202 - 5220	60	58	accagcttcgagaggatgc	85
		5255 - 5272	60	61	ctctccttgtgccgcttg	
human	ANXA2	859 - 879	59	48	ggaaagcatcaggaaagaggt	85
		913 - 934	59	45	cttgttctgaatgcactgaacc	
human	BCL2	2335 - 2354	59	60	agagcagtagaggggtgtgg	57
		2380 - 2397	59	56	tgaaaacgggcctacctg	
human	CDK11A	2462 - 2479	59	61	accacaggcgtggctact	19
		2512 - 2533	60	45	catggagcacaaagtaagacga	
human	CHTF8	1448 - 1468	60	57	caaactcgggtcctagctctc	81
		1497 - 1515	59	53	agcccttgacatgggagat	
human	CKLF	442 - 462	59	48	ttggcactgataccagaaacc	22
		487 - 507	60	48	tgctgtcacaagtgcaaacac	
human	CSF2	326 - 345	59	55	cagccactacaagcagcact	3
		456 - 473	59	67	ccggtctcactcctggac	
human	FAM133B	1139 - 1162	59	33	ttttctcttccagtgcttgataa	14
		1185 - 1211	59	41	cccaaatatcttaaccactagtacgac	
human	FOS	292 - 311	60	60	ctaccactcacccgcagact	67
		345 - 363	59	58	aggtccgtgcagaagtcct	
human	FOXB1	784 - 801	60	56	atccccgtgcccattaag	27
		852 - 870	59	58	cgagttcgagagcaaggtg	
human	HIST1H3J	342 - 361	59	50	cgccaagcgtgtcactatta	42
		404 - 425	59	50	gatagagcaggggattatgctc	
human	IL2	261 - 281	59	48	aggccacagaactgaaacatc	65
		332 - 354	59	35	aagtgaaagtttttgctttgagc	
human	IL3	164 - 190	59	30	catgatcgatgaaattataacacactt	83
		271 - 288	60	61	cctccaggtttggccttc	
human	IL6	109 - 128	59	55	caggagcccagctatgaact	7
		182 - 199	60	61	gaaggcagcaggcaacac	
human	JUN	3012 - 3032	59	43	ccaaaggatagtgcgatgttt	19
		3054 - 3073	60	60	ctgtccctctccactgcaac	
human	LFNG	1280 - 1298	60	63	cctggagagcaggagcagt	23
		1333 - 1351	59	58	caagcgtctggatctctgc	
human	LOC200726	4-21	60	61	aagctggggacagccact	55
		56 - 76	59	52	tggagagagttccctcgagta	
human	MAP2	188 - 214	59	33	cgaactttatattttaccacttccttg	2
		244 - 263	60	50	ccgttcatctgccattcttc	
human	MEX3D	663 - 680	60	61	gccggtcttcatcgtgac	12
		707 - 725	59	58	gacaggatctcacgcttgg	
human	MTRNR2L8	423 - 444	59	50	gaggaacagctctttggacact	83
		513 - 532	60	45	tcgagcttgaacgctttctt	
human	MYC	514 - 533	59	50	gctgcttagacgctggattt	66
		569 - 586	60	56	taacgttgaggggcatcg	
human	NACAP1	344 - 363	60	55	tgtctacaagagcccgcttc	60
		409 - 429	60	52	gctgctagttgtgcttcctga	
human	NF1	2314 - 2338	60	40	ccatggatcatgaagaattactacg	1
		2385 - 2404	59	55	ctgcatcctgctgcactatc	
human	NFYC-AS1	300 - 319	59	55	aagtgaggtctctggcgaag	70
		355 - 373	59	58	tctgtgcgagggtgatagg	
human	NRG4	242 - 263	59	45	ggggctttgttatgtgatacct	23
		284 - 307	59	42	cctgtatagttttcaacgcaccta	
human	OR4D10	418 - 435	60	50	cattgcattgggctcaca	27
		463 - 482	59	50	gaaatctgcacgatggagtg	
human	PABPC3	1407 - 1425	59	63	gcccaggtgctcctagagt	54
		1456 - 1476	60	52	gacatgactcgtggaacctgt	
human	PSPHP1	347 - 365	60	58	ctcctgggttcaggcagtt	10
		406 - 425	60	45	tcctccaaagccaatgaaag	
human	RAVER1	2816 - 2834	59	58	ccccagctctgattccttc	49
		2865 - 2883	60	47	tgtttcaaaccgcaaggtg	
human	RBFOX3	751 - 768	60	61	ggggaacccctacaccaa	11
		798 - 817	59	55	gaattcaggcccgtagactg	
human	RNF113B	1035 - 1056	59	50	gcagcctgttgagtaagatgag	4
		1081 - 1102	59	45	ttggttacatactcaggcatgg	
human	RPL31	4-23	60	55	cgacccggaagttgtacttg	7
		98 - 115	59	56	tctcgccaccttctttg	
human	RPL32P3	43 - 64	59	45	ccagtcagaccgatatgtcaaa	31
		134 - 153	60	45	tgctgggcatcatatcaaga	
human	RPS10P7	104 - 123	59	50	gaaagaacgtgcctttccag	83
		154 - 176	60	43	ccagttcttcttaggcatcaaca	
human	S100A4	138 - 158	60	48	gggtgacaagttcaagctcaa	17
		211 - 232	59	41	aagcagcttcatctgtcctttt	
human	S100A5	180 - 200	59	48	agggacagagatcagggaaat	8
		278 - 298	59	57	ccagaggagtctccatcacag	
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		982 - 1002	59	43	agggctcgtttccataaaaag	
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		2415 - 2434	60	55	gagcaccaggtctgttggat	
human	SNRPD2P2	532 - 551	60	60	gcagggactcagtcatcacc	10
		605 - 625	59	52	ccccataacaaagggtaggtc	
human	UNCX	1850 - 1875	59	35	aagagtaaacgaaagtgctgtatgaa	27
		1896 - 1914	59	63	gtctcaccccagtcctgt	
human	ZNF90	349 - 366	59	50	gattgccaaatccccaga	19
		399 - 417	59	53	acacagagattgcgctgct	
mouse	Fos	210 - 230	59	57	gggacagcctttcctactacc	67
		278 - 297	60	50	agatctgcgcaaaagtcctg	
mouse	Jun	154 - 173	59	45	tttctcaccaactgcttgga	92
		199 - 218	59	45	ccaaatgctccccaaaatac	
mouse	Mex3d	1562 - 1579	60	61	gccttcgaggggataacg	71
		1607 - 1624	59	61	ggctgcccacatagaagg	
mouse	Nf1	10872 - 10892	59	48	tcccccaaaggagactctaat	68
		10920 - 10941	59	36	ccaaattgtgtaaaatgggaca	

Supplementary Figure 1: Simplified schematic of microarray analysis. Circles show 6 sample groups; healthy control fibroblast (HC-FB), NF1 patient fibroblast (NF1-FB), healthy control iN cells (HC-iN), NF1 patient iN cells (NF1-iN), healthy control iN cells with forskolin (HC-iN+FSK), and NF1 patient iN cells with forskolin (NF1-iN+FSK). Blue and orange double arrows indicate the number of aberrant genes between two groups (circles). A yellow double arrow indicates the number of overlapping genes between two blue double arrows.

Supplementary Figure 2: Quantification of mRNA expressions by real-time PCR. There were no significant changes on the expressions of 26 genes of Day-14 iN cells between HC and NF1 group. One-way ANOVA / Tukey's test, n = 6 each group. Open circles show healthy controls and filled circles and squares show NF1 patients.

Supplementary Figure 3: Quantification of AREs-including mRNA expressions by real-time PCR. There were no significant differences on the expressions of 5 genes of Day-14 iN cells between HC and NF1 group. Student's t-test, n = 6 each group. Open circles show healthy controls and filled circles show NF1 patients.

Supplementary Figure 4: Quantification of mRNA expressions of Neuro2A cells by real-time PCR. (**A-D**) *Mex3d*, *Fos*, *Jun*, and *Nf1* mRNA expression level, respectively. Student's t-test, n = 4 each group. (**E-F**) *Nf1* and *Mex3d* mRNA expression level, respectively. One-way ANOVA / Tukey's test, n = 4 each group. Open circles show control siRNA, filled triangles show *Mex3d* knockdown using siRNA, and filled circles and squares show *Nf1* knockdown using siRNA.

Supplementary Table 1: Supplementary Table 1. Information of fibroblasts for generating iN cells.

Supplementary Table 2: Information of the primer and probe number for quantitative real-time PCR.

Supplelmentary Table 3: Significantly different 149 gene expressions in NF1-iN cells compared to HC-iN cells determined by microarray analysis.

Supplelmentary Table 4: Significantly different 90 gene expressions in NF1-iN cells compared to NF1-iN cells with forskolin determined by microarray analysis.