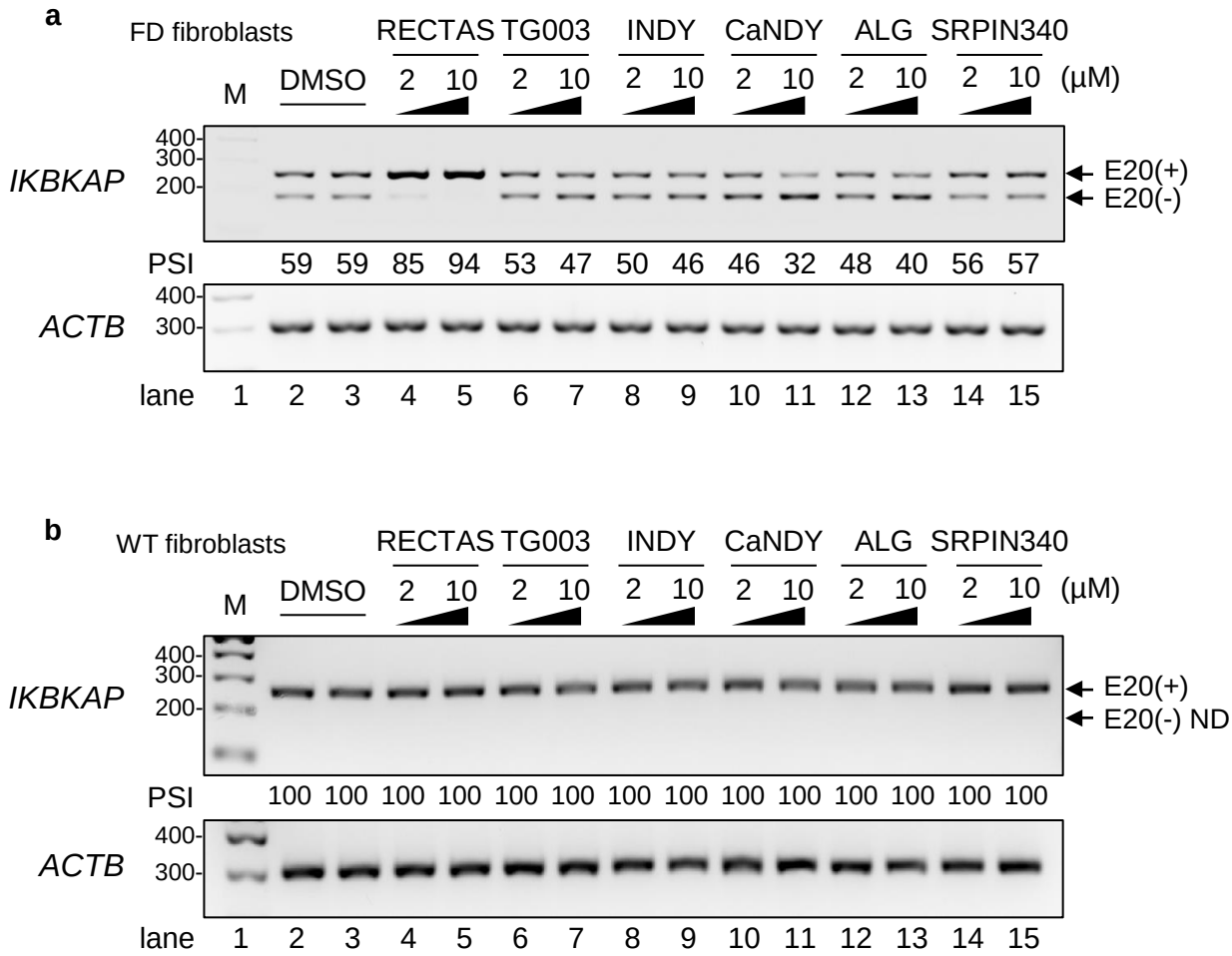
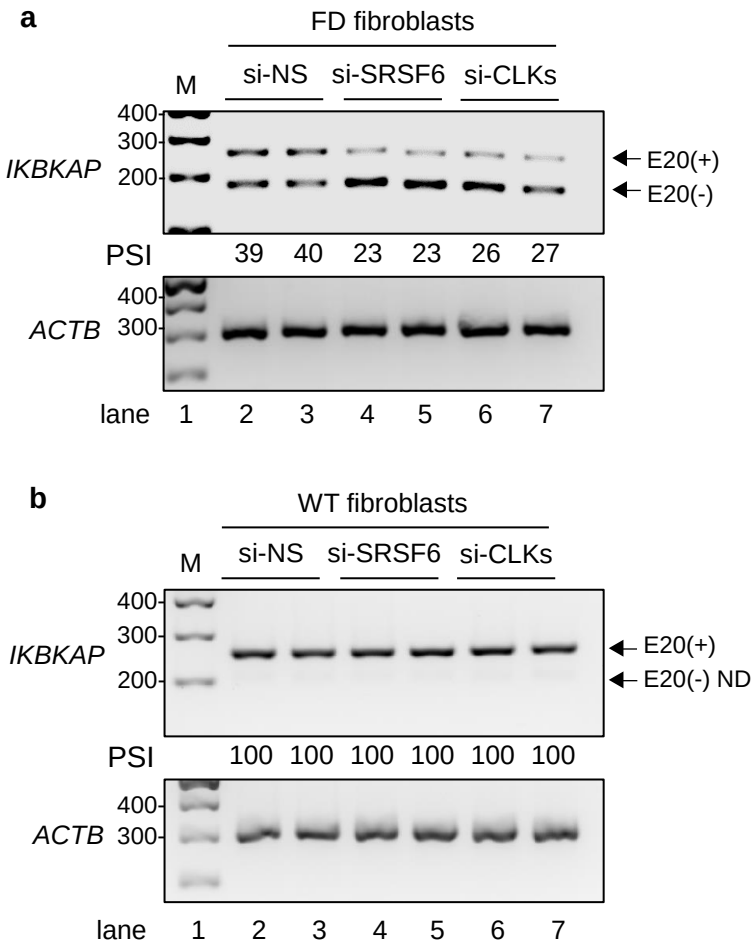
Supplementary Information

Therapeutic manipulation of *IKBKAP* mis-splicing with a small molecule to cure familial dysautonomia



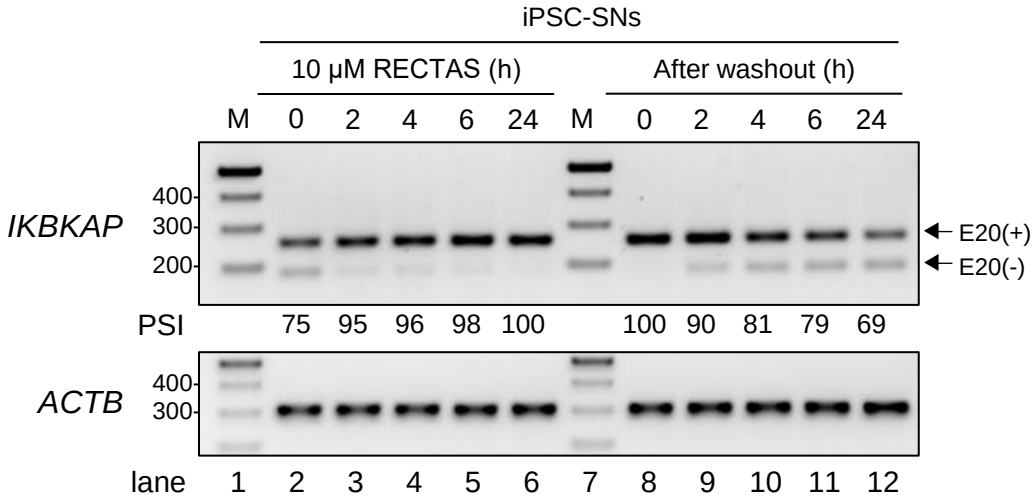
Supplementary Fig. 1. Treatment with RECTAS, CLK inhibitors, and SRPK inhibitors in primary fibroblasts from FD patient and healthy donor.

Compound response (24 h treatment at 2 or 10 μM) of endogenous *IKBKAP*-FD exon 20 was shown for primary fibroblasts from FD patient (P1) (a) and healthy donor (C2) (b). Solvent only (0.1% DMSO) served as a negative control, and *ACTB* served as a loading control. Representative data from two replicates are shown in (a) and (b). PSI, percent spliced-in; E20 (+) and E20 (-), exon 20 inclusion and skipping products, respectively. *IKBKAP* and *ACTB* were detected with primers oAM138+oAM139 and oAM13+oAM14, respectively.



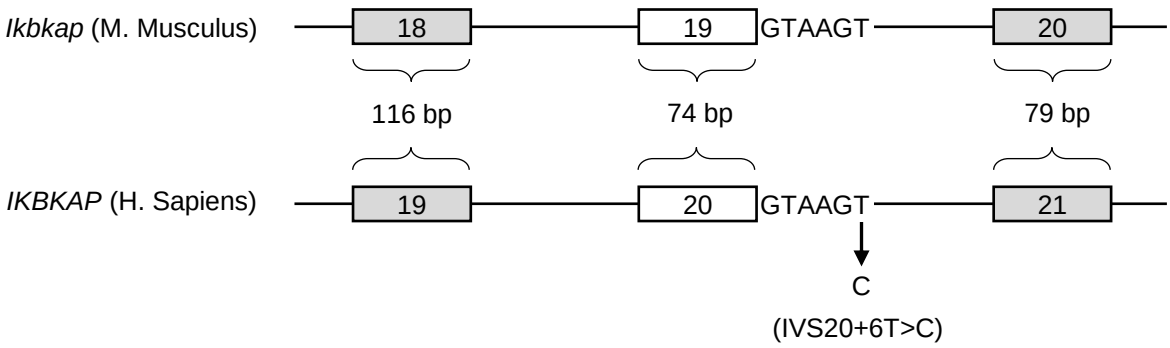
Supplementary Fig. 2. Knockdown effect of SRSF6 and CLK isoforms on *IKBKAP* exon 20 splicing in primary fibroblasts from FD patient and healthy donor.

Knockdown effect of SRSF6 (si-SRSF6) and CLK isoforms (si-CLKs) for endogenous *IKBKAP*-FD exon 20 was shown for primary fibroblasts from FD patient (P1) (a) and healthy donor (C2) (b). Non-targeting siRNA (si-NS) served as a negative control, and *ACTB* served as a loading control. Representative data from two replicates are shown in (a) and (b). PSI, percent spliced-in; E20 (+) and E20 (-), exon 20 inclusion and skipping products, respectively. *IKBKAP* and *ACTB* were detected with primers oAM138+oAM139 and oAM13+oAM14, respectively.



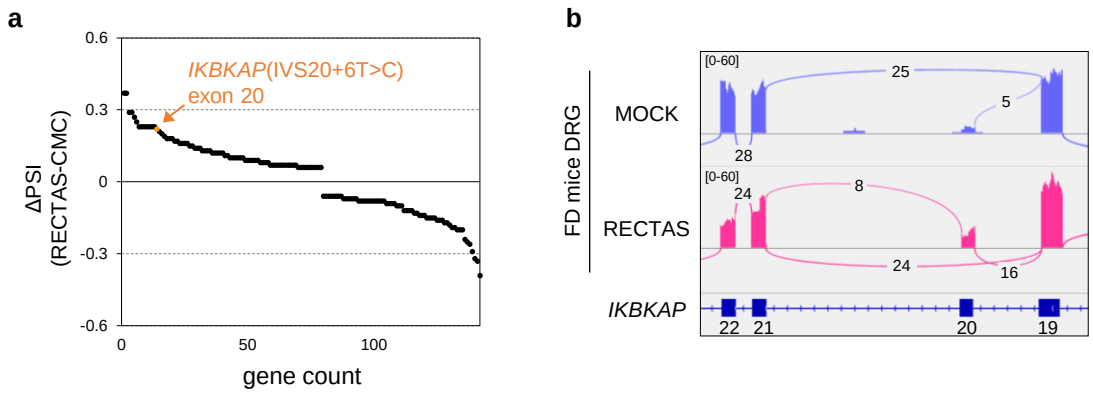
Supplementary Fig. 3. Time-course experiment for exon 20 inclusion promoting activity by RECTAS.

FD patient cell-derived iPSC-SNs at 12 days of induced differentiation were analyzed for *IKBKAP* exon 20 splicing at 0, 2, 4, 6, and 24 h after the RECTAS treatment (10 μM). 24 h-RECTAS treated iPSC-SNs were also subjected to washout by PBS (-) and kept in culture media without RECTAS, and analyzed at 0, 2, 4, 6, and 24 h after the washout. *ACTB* served as a loading control. Representative data from three replicates are shown. PSI, percent spliced-in; E20 (+) and E20 (-), exon 20 inclusion and skipping products, respectively. *IKBKAP* and *ACTB* were detected with primers oAM138+oAM139 and oAM13+oAM14, respectively.



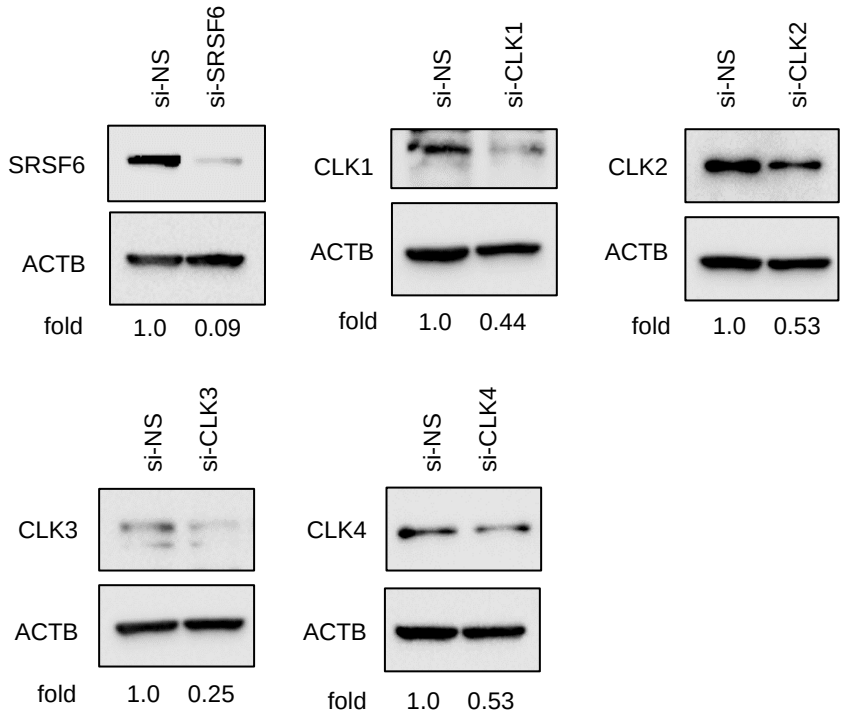
Supplementary Fig. 4. Diagram for human *IKBKAP* and mouse *Ikbkap*.

Exon 18, 19, and 20 of mouse *Ikbkap* is homologous to exon 19, 20, and 21 of human *IKBKAP* with matched exon length and surrounding intronic sequences.



Supplementary Fig. 5. RNA-Seq analysis for DRG from *IKBKAP*-FD transgenic mice treated with MOCK or RECTAS (300 mg/kg BW).

(a) Differential splice events with >0.05 of |ΔPSI (PSI_{RECTAS}-PSI_{CMC})| in RECTAS (300 mg/kg BW)-administered mice over CMC-administered mice were plotted. Vertical, ΔPSI; Horizontal, gene count. Arrow (orange) indicates *IKBKAP*-FD exon 20 inclusion. (b) Representative sashimi plot with junction read number was shown for MOCK and RECTAS groups. *IKBKAP* gene structure is shown on the bottom. Displayed read-count range was set to 0-60.



Supplementary Fig. 6. Western blot analysis for knockdown efficiency.

HeLa cells were transfected with non-targeting siRNA (si-NS) or siRNA targeting SRFS6, CLK1, CLK2, CLK3, and CLK4. Forty-eight hours after the transfection, each protein expression was analyzed by Western blot. Representative data from two experiments are shown. ACTB served as a loading control.

Supplementary Table 1. Summary of pre-clinical study of RECTAS.

Toxicity test		Species	Dose	Route[#1]	Result[#2]
General toxicity	Clinical sign or mortality Body weight change Necropsy check	Rat (n=5)	0, 100, 300, 1,000 mg/kg BW, single dose[#3]	p.o.	n/d
	Clinical sign or mortality Body weight change Necropsy check Food consumption Hematology test[#4] (RBC, HGB, HCT, PLT, MCV, MCH, MCHC, WBC, RET, PT, APTT, Fbg, AST, ALT, ALP, T-Cho, TG, TP, IN, CRE, T-Bil, Glu, IP, Ca, Na, K, Cl) Body weight, food consumption	Rat (n=3)	0, 40, 200, 1,000 mg/kg BW/day, 14 days	p.o.	n/d
Genotoxicity	Ames test			in vitro	negative
	Micronucleus test			in vitro	negative
Cell permiability	Cacl-2		10 μM	in vitro	95.5 ± 0.02%
Metabolic stability	Human liver microsome (0.5 mg/mg)		1 μM	in vitro	$T_{1/2}$ = 98.36 min

[#1]RECTAS was resuspended in 0.5% carboxymethyl cellulose for p.o.

[#2]n/d, no severe toxicity attributable to RECAS administration was detected.

[#3]observation was conducted for 14 days following the administration.

[#4]RBC, red blood cell count; HGB, hemoglobin; HCT, hematocrit; PLT, platelet; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; WBC, white blood cell count; RET, reticulocyte count; PT, prothrombin time; APTT, activated partial thromboplastin time; Fbg, fasting blood glucose; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkalne phosphatase; T-Cho, total cholesterol; TG, triglycerides; TP, total protein; UN, urea nitrogen; CRE, creatinine; T-Bil, total bilirubin; Glu, glucose; IP, inorganic phosphorus; Ca, calcium; Na, sodium; K, potassium; Cl, chloride.

Supplementary Table 2. List of primers used in this study.

Name	Target gene	Species	orientation	sequence
oAM13	*ACTB*	*H. Sapiens*	forward	5′-CCAACCGCGAGAAGATGACC-3′
oAM14	*ACTB*	*H. Sapiens*	reverse	5′-AGCTTCTCCTTAATGTCACG-3′
oAM138	*IKBKAP*	*H. Sapiens*	forward	5′-GGATTGTCACTGTTGTGC-3′
oAM139	*IKBKAP*	*H. Sapiens*	reverse	5′-CAAGTTAATATGATTCACAGAATCT-3′
HsIKAPRT18F	*IKBKAP*	*H. Sapiens*	forward	5’-TGTTTTTGCCTGAGGGATGC-3’
HsIKAPRT21R	*IKBKAP*	*H. Sapiens*	reverse	5’-AATGAAGGTTTCCACATTTCCA-3’
oAM669	*RBM24*	*H. Sapiens*	forward	5′-GTCTTCGGCGAGATCGAGGA-3′
oAM671	*RBM24*	*H. Sapiens*	reverse	5′-TGATCCTTGGTTTTGCTCCT-3′
oAM365	*Actb*	*H. Sapiens*	forward	5′-CCCAGAGCAAGAGAGGTATC-3′
oAM366	*Actb*	*H. Sapiens*	reverse	5′-GACGCAGGATGGCGTGAGG-3′
oAM666	*Ikbkap*	*M. Musculus*	forward	5′-TCACGGATCGTGACAGTTGT-3′
oAM667	*Ikbkap*	*M. Musculus*	reverse	5′-GGGTTATGGTCATGAATCAG-3′
Rbm24-F	*Rbm24*	*M. Musculus*	forward	5′-CTGCGCAAGTACTTTGAGGTCTTCG-3′
Rbm24-R	*Rbm24*	*M. Musculus*	reverse	5′-GTAGACGTAGTGGGCGGGG-3′
oAM124	GST of reporter vector	-	forward	5′-TCCAGCAAGTATATAGCAT-3′
oAM126	RFP of reporter vector	-	reverse	5′-CTCCTTGATGACGTCCTCG-3′