YMTHE, Volume *26*

Supplemental Information

A Blood-Brain-Barrier-Penetrating Anti-human Transferrin Receptor Antibody Fusion Protein for Neuronopathic Mucopolysaccharidosis II

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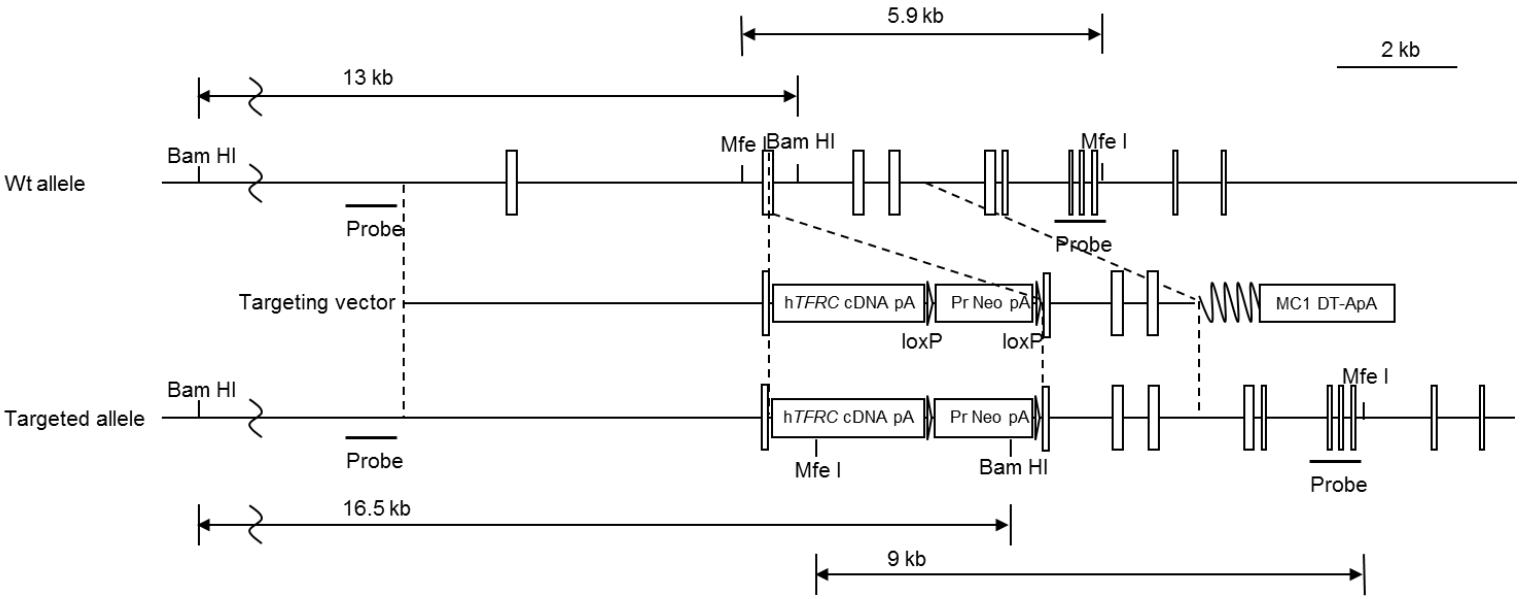


Figure S1. Schematic representation of mouse *Tfrc* gene, targeting vector, and targeted allele
A human *TFRC* cDNA cassette was inserted into exon 2 of the mouse *Tfrc* gene. Positive and negative clones can be discriminated by the DNA fragment size in Southern blot analysis after digestion with Bam HI or Mfe I.

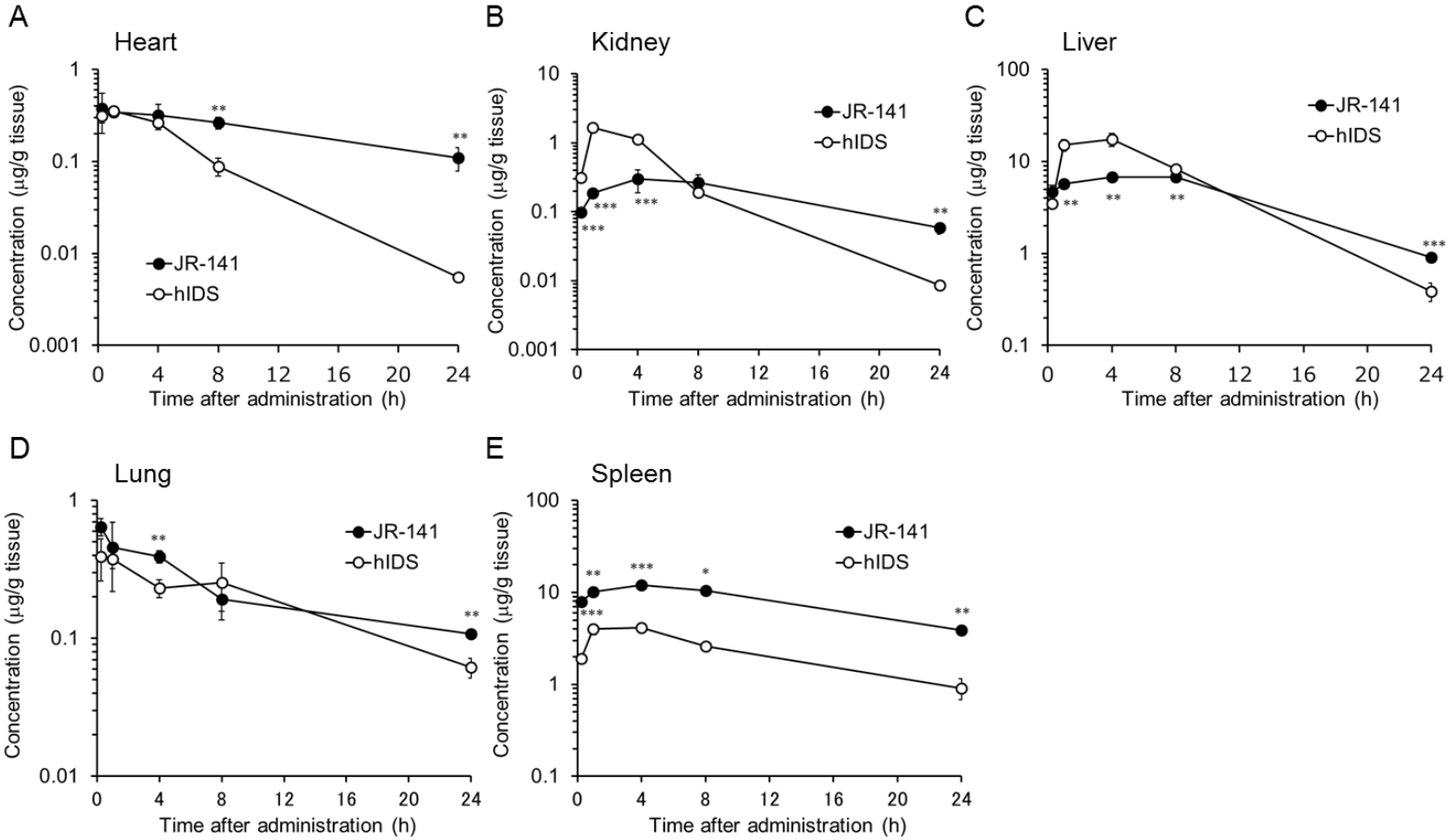


Figure S2. Pharmacokinetics of JR-141 and hIDS in peripheral tissues of *TFRC*-KI mice
Concentration of JR-141 was measured in the heart (A), kidney (B), liver (C), lung (D), and spleen (E) by electrochemiluminescent immunoassay (n = 3; **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *t*-test). Data are plotted as mean ± SD bars.

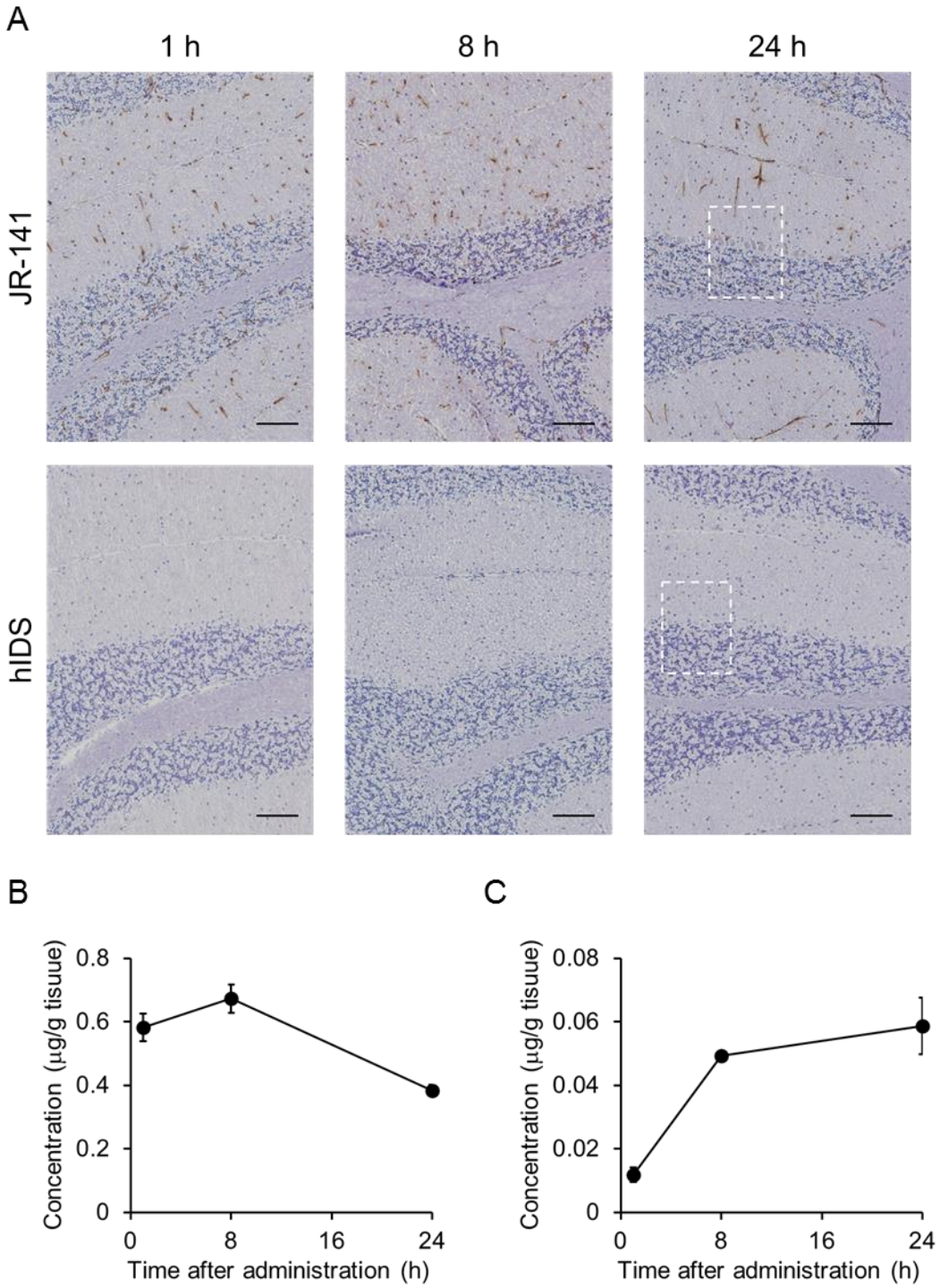


Figure S3. Immunohistochemistry in the brain of *TFRC*-KI mice
(A) Low magnification microphotographs of the cerebellum at 1, 8, 24 h after the administration of 1 mg/kg of JR-141 or naked IDS. White dashed rectangles indicate the highly magnified areas shown in Figure 3C. Scale bars, 100 μm. (B and C) Concentrations of JR-141 in brain capillaries (B) and parenchyma (C). Data are plotted as mean ± SD bars (n = 3).

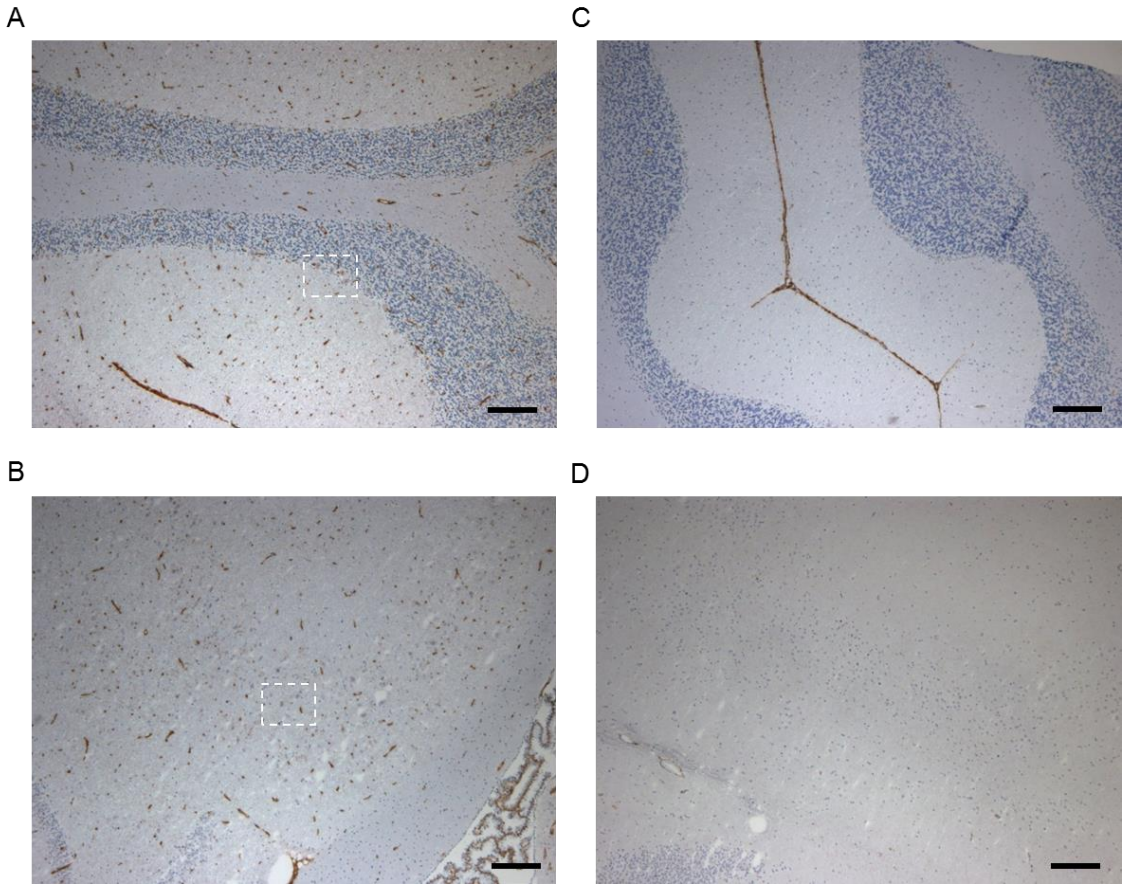
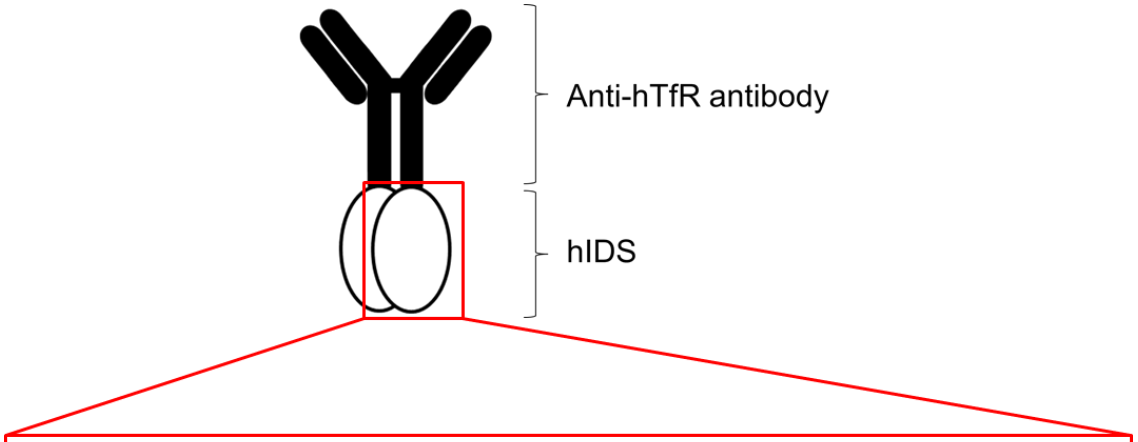


Figure S4. Immunohistochemistry in the brain of cynomolgus monkey
Representative brain sections from the JR-141 (5 mg/kg) group (A and B) and control (no administration) group (C and D). Low magnification microphotographs of the cerebellum (A and C) and the hippocampus (B and D) are shown. Non-specific staining is observed in the pia mater (C). White dashed rectangles indicate the highly magnified areas shown in Figures 4D and 4E. Scale bars, 200 μm.



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GELHANLLCP VDVLDVPEGT LPDKQSTEQA IQLLEKMKTS ASPFFLAVGY
HKPHIPFRYP KEFQKLYPLE NITLAPDPEV PDGLPPVAYN PWMDIRQRED
VQALNISVPY GPIPVDFQRK IRQSYFASVS YLDTQVGRLL SALDDLQLAN
STIIAFTSDH GWALGEHGEW AKYSNFDVAT HVPLIFYVPG RTASLPEAGE
KLFPYLDPFD SASQLMEPGR QSMDLVELVS LFPTLAGLAG LQVPPRCPVP
SFHVELCREG KNLLKHFRFR DLEEDPYLPG NPRELIAYSQ YPRPSDIPQW
NSDKPSLKDI KIMGYSIRTI DYRYTVWVGF NPDEFLANFS DIHAGELYFV
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Figure S5. The amino acid sequence of the hIDS moiety of JR-141 predicted from the cDNA sequence
N: N-glycosylation site

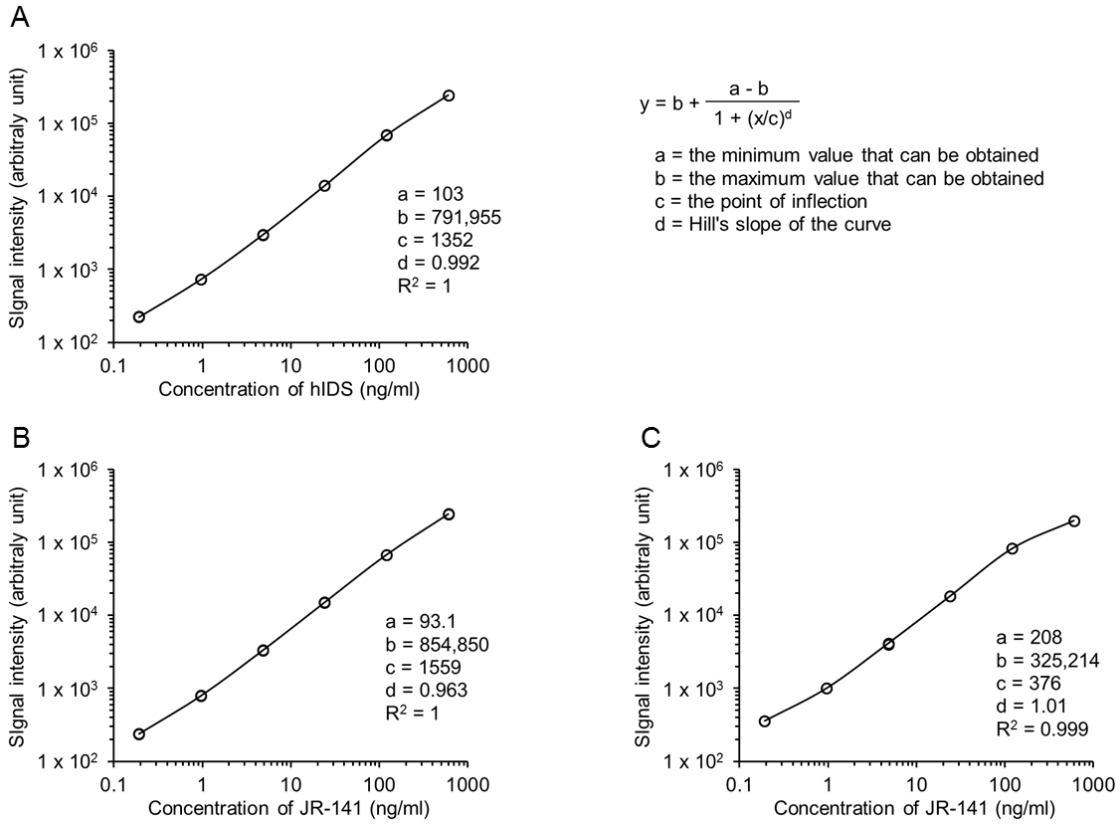

Figure S6. Validity of electrochemiluminescent immunoassay of hIDS and JR-141
(A) A representative standard curve for hIDS measurement. Defined concentrations of hIDS were added to the mixture of the sulfo-labeled anti-hIDS antibody and the biotinylated anti-hIDS antibody, and the reactions were then added to pre-blocked Streptavidin Gold plate (Meso Scale Diagnostics, Gaithersburg, MD). The intensity of electrochemiluminescence was quantified by Sector Imager 6000 (Meso Scale Diagnostics). (B and C) Representative standard curves for JR-141 measurement. Defined concentrations of JR-141 were mixed with biotinylated light chain of anti-human IgG antibody (B) or biotinylated anti-hIDS antibody (C) and SULFO-TAG-labeled anti-hIDS antibody. The subsequent procedure was similar to that described in (A). Four-parameter logistic regression was applied.

Table S1. Pharmacokinetic parameters of JR-141 and naked hIDS after intravenous administration to *TFRC*-KI mice

	Drug	C_{max} (μg/ml or g)	$AUC_{0\text{-}t}$ (μg·h/ml or g)	$AUC_{0\text{-}inf}$ (μg·h/ml or g)	$t_{1/2}$ (h)	MRT (h)	CL (ml/h/kg)	V_{ss} (ml/kg)
Plasma	JR-141	15.3	25.4	25.6	4.37	1.78	39.1	69.5
	hIDS	21.2	17.9	17.9	4.89	0.718	55.8	40.1
Brain	JR-141	0.440	8.14	16.6	23.9	34.9	60.4	2,110
	hIDS	-	-	-	-	-	-	-
Heart	JR-141	0.370	5.51	7.55	12.9	17.8	132	2,350
	hIDS	0.356	2.68	2.71	3.69	4.95	369	1,830
Kidney	JR-141	0.297	4.54	5.24	8.13	11.8	191	2,250
	hIDS	1.66	9.17	9.20	3.02	3.99	109	433
Liver	JR-141	6.82	112	120	5.51	8.85	8.36	74.0
	hIDS	17.4	177	179	3.64	5.97	5.58	33.3
Lung	JR-141	0.588	5.40	7.21	12.3	16.4	139	2,270
	hIDS	0.399	4.79	5.67	9.52	12.2	176	2,150
Spleen	JR-141	11.9	201	269	12.0	17.1	3.72	63.5
	hIDS	4.14	55.9	68.2	9.54	13.3	14.7	196

C_{max}; maximum concentration, $AUC_{0\text{-}t}$: area under the concentration-time curve from zero to the time of the last measurable drug concentration, $AUC_{0\text{-}inf}$: area under the concentration-time curve from zero to infinity, $t_{1/2}$: half-life, MRT: mean residence time, CL: clearance, V_{SS}: steady-state distribution volume.

Table S2. Pharmacokinetic parameters of JR-141 in plasma after intravenous administration to cynomolgus monkeys

C_{max} (μg/mL)	$AUC_{0\text{-}t}$ (μg·h/ml)	$AUC_{0\text{-}inf}$ (μg·h/ml)	$t_{1/2\beta}$ (h)	MRT (h)	CL (m1/h/kg)	V_{ss} (ml/kg)
75.4	446	424	4.69	6.25	12.2	74.1

C_{max}; maximum concentration, $AUC_{0\text{-}t}$: area under the concentration-time curve from zero to the time of the last measurable drug concentration, $AUC_{0\text{-}inf}$: area under the concentration-time curve from zero to infinity, $t_{1/2}$: half-life of β phase, MRT: mean residence time, CL: clearance, V_{SS}: steady-state distribution volume.

Table S3. Absolute values of concentration of GAG levels in the brain and peripheral tissues

		GAG (μg/mg tissue)																					
		Brain				Heart				Kidney				Liver				Lung				Spleen			
			mean	SD	*P* value		mean	SD	*P* value		mean	SD	*P* value		mean	SD	*P* value		mean	SD	*P* value		mean	SD	*P* value
Wilde-type		2.15 2.37 2.33 2.29	2.29	0.09	-	0.97 0.95 0.78 0.88	0.89	0.08	-	2.00 1.96 1.99 1.96	1.98	0.02	-	0.98 1.10 1.05 1.15	1.07	0.07	-	1.09 1.06 1.08 1.15	1.09	0.04	-	0.64 0.55 0.51 0.65	0.59	0.07	-
Ids-KO (Control)		3.14 3.05 3.01 3.24	3.11	0.10	-	4.89 6.22 5.57 5.83	5.63	0.56	-	11.65 11.98 12.43 11.82	11.97	0.33	-	14.95 19.16 15.67 13.00	15.69	2.57	-	7.40 7.28 7.02 6.67	7.09	0.32	-	4.65 4.54 3.91 5.82	4.73	0.79	-
JR-141	1 mg/kg	2.71 2.71 2.81 2.63	2.72	0.07	0.000053	2.00 2.05 2.26 1.82	2.03	0.18	4.54.E-08	8.67 8.09 7.79 6.06	7.65	1.13	0.000004	3.60 3.47 3.58 2.60	3.31	0.48	0.000028	5.83 6.16 6.33 5.66	5.99	0.31	0.005	1.76 1.52 1.51 1.50	1.57	0.13	0.000001
	3 mg/kg	2.50 2.39 2.40	2.43	0.06	5.77.E-07	1.66 1.62 1.65	1.64	0.02	4.26.E-08	5.07 4.17 4.15	4.46	0.52	6.78.E-08	3.69 2.68 3.19	3.19	0.50	4.33.E-07	4.94 3.98 3.93	4.28	0.57	0.000003	1.35 1.29 1.34	1.33	0.03	0.000001
	10 mg/kg	2.40 2.54 2.52 2.51	2.49	0.06	6.54.E-07	1.28 1.23 1.30 1.19	1.25	0.05	3.49.E-08	3.03 3.39 3.40 3.12	3.23	0.19	3.64.E-08	2.20 2.27 2.88 2.38	2.43	0.31	1.32.E-07	2.17 2.35 3.00 2.33	2.46	0.36	4.23.E-08	1.00 1.27 1.31 1.18	1.19	0.14	4.46.E-07
hIDS	0.5 mg/kg	3.11 3.07 2.94 2.80	2.98	0.14	0.179	1.50 1.54 1.36 1.64	1.51	0.11	0.000007	7.36 8.40 8.96 8.03	8.19	0.67	0.000055	4.27 3.50 3.61 3.79	3.79	0.34	0.000094	5.12 5.29 3.75 5.55	4.93	0.80	0.002	2.14 1.97 1.99 1.86	1.99	0.12	0.00049

Differences between *Ids*-KO control and JR-141 groups, and *Ids*-KO control and hIDS groups were analyzed with Dunnett's test and Student *t*-test, respectively.

SUPPLEMENTAL MATERIALS AND METHODS

Generation of *TFRC*-KI mice

TFRC-KI mice in a C57BL/6 background were generated by homologous recombination of the *TFRC* gene with the mouse *Tfrc* gene. A cDNA coding for a chimeric protein of the extracellular domain of human TFRC and the transmembrane and intracellular domain of mouse Tfrc was inserted into exon 2 of the mouse *Tfrc* gene (Figure S1). The nucleotide sequence of the cDNA with loxP-neo-loxP is shown below:

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ggtccctcgaagaggttcactagttctagagcatttaaatacgtgctagc

Boxed letters indicate the cDNA for the chimeric receptor and underlines indicate loxP sequences. The expression pattern of TfR in *TFRC*-KI mice was qualitatively similar to that in WT mice.