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Supplemental Information

Clearance of heparan sulfate in the brain prevents neurodegeneration and neurocognitive impairment in MPS II mice

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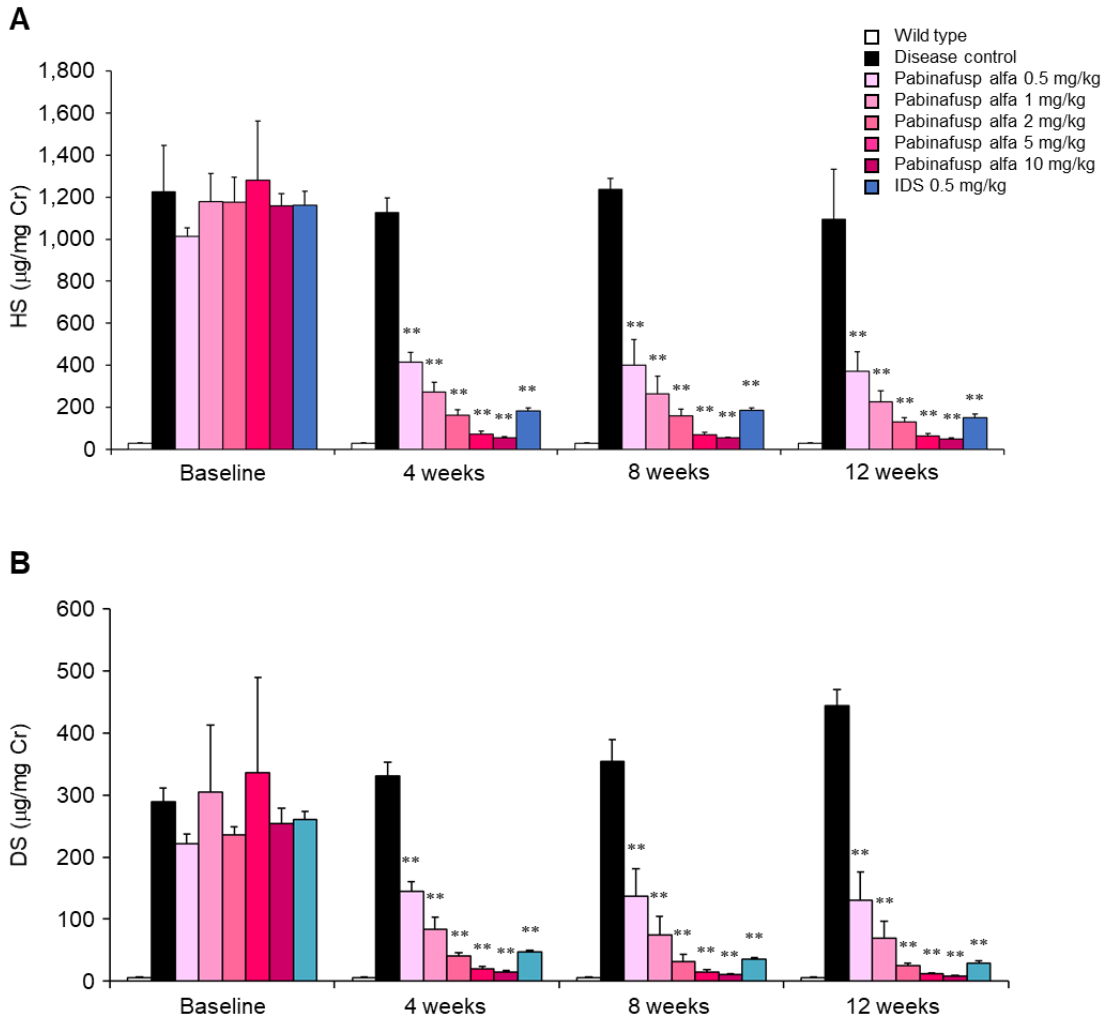

Figure S1. Concentrations of heparan sulfate and dermatan sulfate in the urine during a 12-week repeated dose study of pabinafusp alfa or IDS. Concentrations of heparan sulfate (HS, A) and dermatan sulfate (DS, B) are shown. Drugs were intravenously administered to 12-week-old MPS II mice once every week for 12 weeks. Values are expressed as mean with S.D. bars. Each group contains 5 animals. $^{**}P < 0.01$ (vs. Disease control group), Tukey-Kramer test.

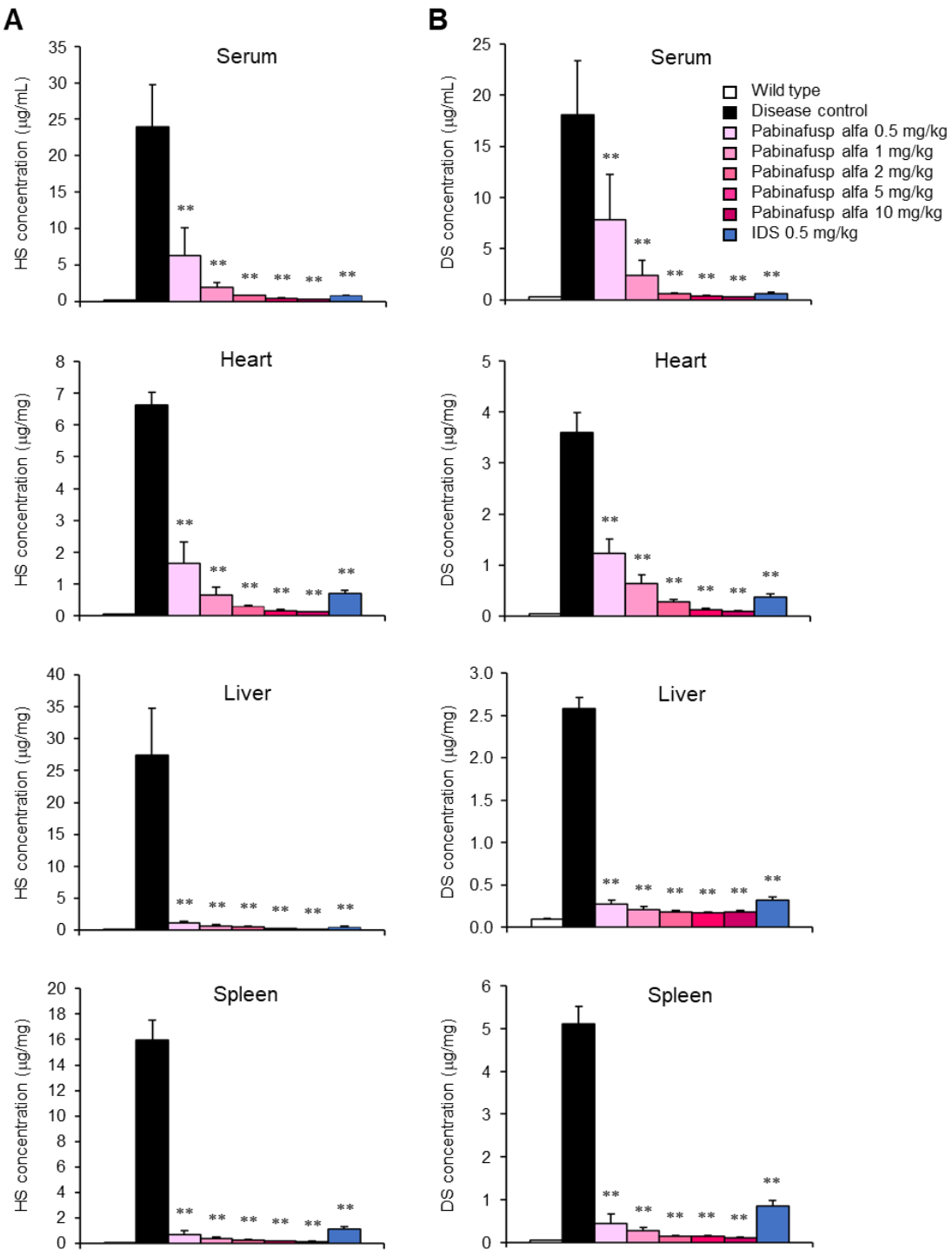

Figure S2. Concentrations of heparan sulfate and dermatan sulfate in peripheral tissues after a 12-week repeated dose of pabinafusp alfa. Concentrations of heparan sulfate (HS, A) and dermatan sulfate (DS, B) are shown. Drugs were intravenously administered to 12-week-old MPS II mice once every week for 12 weeks. Values are expressed as mean with S.D. bars. Each group contains 5 animals. $^{**}P < 0.01$ (vs. Disease control group), Tukey-Kramer test.

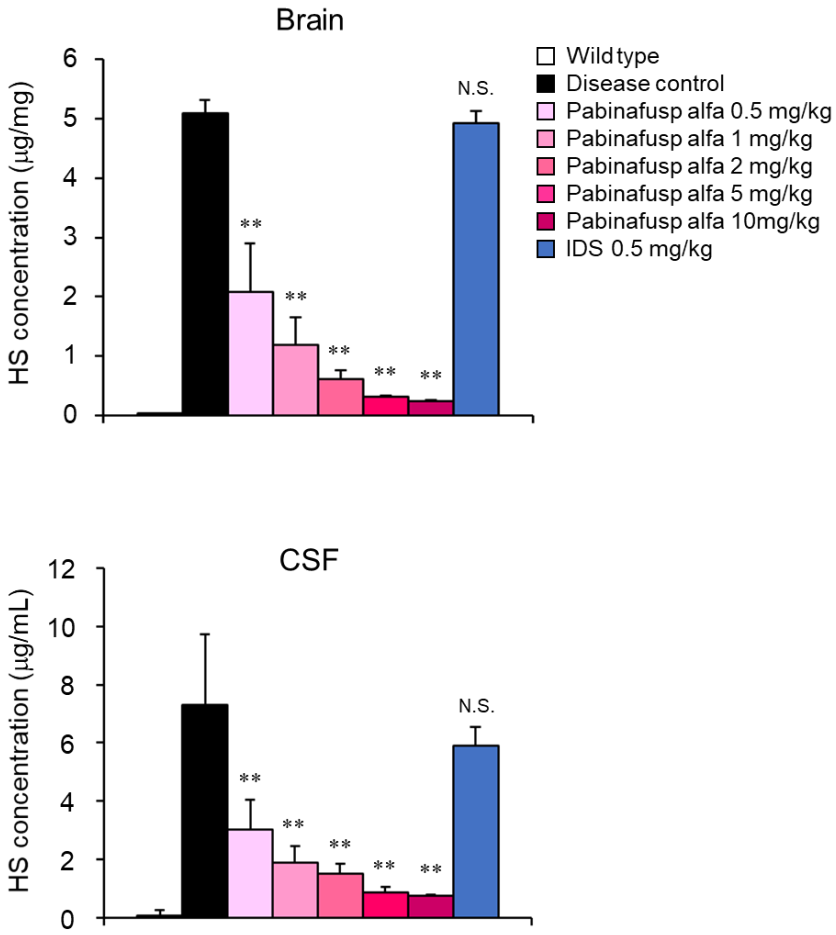

Figure S3. Concentrations of heparan sulfate in the brain and CSF after 12-week repeated dose of pabinafusp alfa. Drugs were intravenously administered to 12-week-old MPS II mice once every week for 12 weeks. Values are expressed as mean with S.D. bars. Each group contains 5 animals. $^{**}P < 0.01$; N.S., not significant (vs. Disease control group), Tukey-Kramer test.

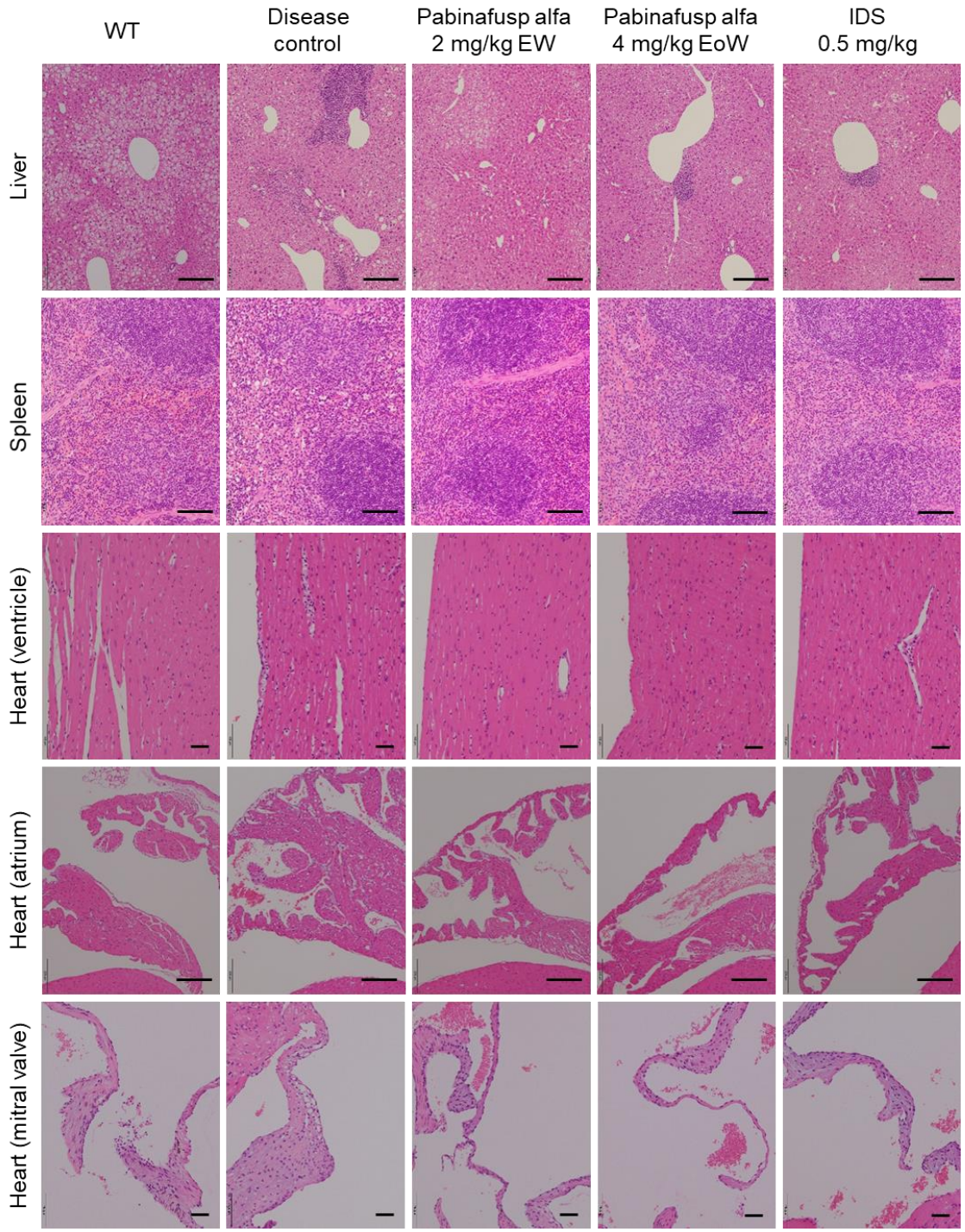

Figure S4. Histopathological observations in MPS II mice after 36 weeks of treatment with pabinafusp alfa. Drugs as indicated were intravenously administered to MPS II mice once every week, starting at the age of 10 weeks. Representative photomicrographs of HE staining are shown. Scale bars, 200 μm.

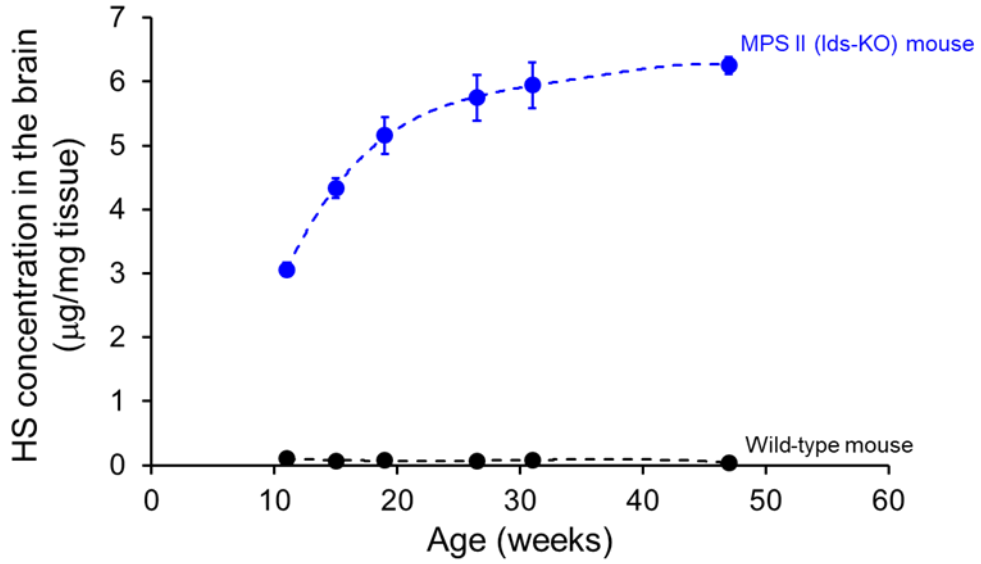

Figure S5. HS concentration in the brain of MPS II mice. HS concentrations in the brain were measured at 11, 15, 19, 27, 31, and 47 weeks of age. Values are presented as the mean with S.D. for each group (n = 3-5).

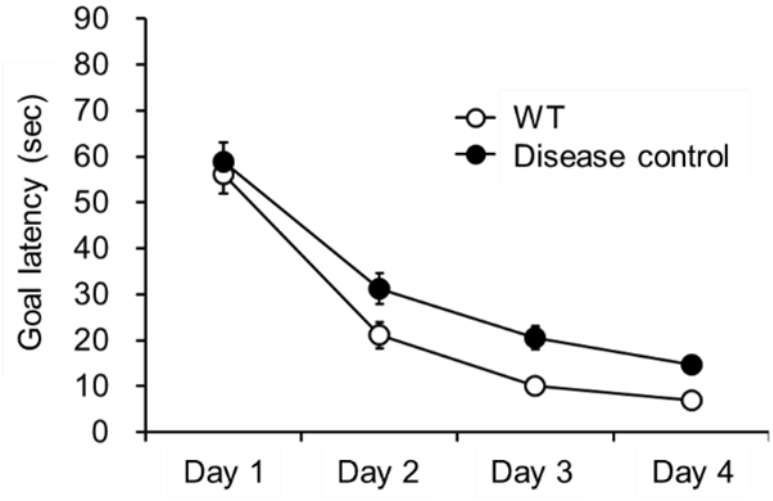

Figure S6. Spatial learning ability in younger MPS II mice. Goal latency in the Morris water maze test in 24-week-old MPS II mice. Values are presented as the mean with S.E. for each group (n = 15).

Table S1. Histopathological changes in peripheral organs and the brain

Organ	Finding	Wild type										Disease control*								Pabinafusp alfa, 2 mg/kg, EW										Pabinafusp alfa, 4 mg/kg, EoW										IDS, 0.5 mg/kg*						
		1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7
Liver	Swelling, macrophage	-	-	-	-	-	-	-	-	-	-	+	±	±	±	±	±	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	±	-	-	-	-
	Fatty change, centrilobular	++	++	++	++	++	++	+	++	++	++	-	±	-	+	-	-	±	+++	-	±	+	±	+	-	+	++	++	++	-	-	++	±	++	++	++	+	-	±	+	±	+	-	++	±	-
	Infiltration, mononuclear	-	-	-	-	+	-	+	-	+	-	±	-	±	-	±	-	++	++	++	-	-	+	-	-	-	-	±	±	++	-	+	±	-	-	±	++	-	±	-	+	++	++	++	-	-	-
Spleen	Swelling, macrophage, red pulp	-	-	-	-	-	-	-	-	-	-	++	++	++	++	++	++	++	++	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Hematopoiesis, extramedullar, increased	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	±	-	-	-	-
Heart	Vacuolization, myocardium, atrium	-	-	-	-	-	-	-	-	-	-	++	++	++	++	++	++	++	++	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Vacuolization, myocardium, ventricle	-	-	-	-	-	-	-	-	-	-	±	±	±	±	+	±	±	±	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Infiltration, foamy cell	-	-	-	-	-	-	-	-	-	-	+	+	±	+	++	+	±	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Infiltration, inflammatory cell	-	-	-	-	-	-	-	-	-	-	±	±	±	±	+	±	±	±	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Fibrosis	-	-	-	-	-	-	-	-	-	-	-	±	-	-	+	±	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Swelling, interstitial cell, aortic valve	-	-	-	-	-	-	-	-	-	-	±	±	±	±	±	±	±	±	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Swelling, interstitial cell, pulmonary valve	-	-	-	-	-	-	-	-	-	-	±	±	-	±	±	±	-	±	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Swelling, interstitial cell, tricuspid (right atrioventricular) valve	-	-	-	-	-	-	-	-	-	-	+	±	±	±	+	±	±	±	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Swelling, interstitial cell, mitral (left atrioventricular) valve	-	-	-	-	-	-	-	-	-	-	±	±	±	±	+	+	±	±	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Brain	Vacuolization, Purkinje cell, cerebellum	-	-	-	-	-	-	-	-	-	-	++	++	++	++	++	++	++	++	±	-	-	±	±	-	±	-	-	±	±	±	±	-	±	-	±	±	-	±	++	++	++	++	++	++	++
	Deposition, eosinophilic material, medulla oblongata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	±	-	-	-	-	-	-
	Mineralization	-	-	-	-	-	-	-	-	-	±	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	±	-	-	-	-	-	-	-	±	-	-	-	-	-	-	-	-	-	-

-: negative, ±: minimal, +: mild, ++: moderate, +++: severe.

*Two animals in disease control group and three animals in IDS group died during dosing period.

Table S2. Lamp1-positive scores in the brain

Region in the brain			Wild type										Disease control*								Pabinafusp alfa, 2 mg/kg, EW										Pabinafusp alfa, 4 mg/kg, EoW										IDS, 0.5 mg/kg*						
			1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7
Meninges and brain surface		Meninges and endothelium	–	–	–	–	–	–	–	–	–	–	+	±	+	±	+	±	+	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	+	±	±	±	±	±	±	±	±	±	±	±	±	±
Cerebrum	Frontoparietal cortex	Nerve and glial cells	–	–	–	–	–	–	–	–	–	–	±	±	±	±	±	±	±	±	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	±	±	±	±	–	±	±
		Endothelium	–	–	–	–	–	–	–	–	–	–	±	±	±	±	±	±	±	±	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	±	±	±	±	±	±	±
	Piriform cortex	Nerve and glial cells	–	–	–	–	–	–	–	–	–	–	+	±	+	+	+	+	±	+	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	+	+	+	+	±	+	+
		Endothelium	–	–	–	–	–	–	–	–	–	–	±	±	±	±	±	±	±	±	–	–	–	–	±	–	–	–	±	–	±	–	–	–	–	–	–	–	–	–	±	±	±	±	±	±	±
	White matter and ventricle	Ependyma	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+	+	+	+	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	+	+	+	+	+	+	+
Hippocampus and dentate gyrus		Nerve and glial cells	–	–	–	–	–	–	–	–	–	–	+	±	+	+	+	+	+	+	±	±	±	±	±	±	±	±	–	±	±	±	±	±	±	±	±	–	±	±	+	+	+	+	+	+	+
		Endothelium	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+	+	+	+	±	±	±	±	±	±	±	–	±	±	±	±	±	±	±	±	±	–	±	±	+	+	+	+	+	+	+
Interbrain		Nerve and glial cells	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+	+	+	+	–	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	+	+	+	+	±	+	+
		Endothelium	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+	+	+	+	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	+	+	+	+	+	+	+
Medulla oblongata		Nerve and glial cells	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+	+	+	+	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	–	±	±	+	+	+	+	+	+	+
		Endothelium	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+	+	+	+	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	–	±	±	+	+	+	+	+	+	+
Cerebellum	Molecular layer	Cell body	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+	+	+	+	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	+	±	+	+	+	+	+
	Purkinje and granular layer	Purkinje layer cells	±	±	±	±	±	±	±	±	±	±	++	++	++	++	++	++	++	++	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	++	+	++	++	++	++	++
	White matter	Cell body	–	–	–	–	–	–	–	–	–	–	±	±	±	±	±	±	±	±	–	–	–	±	±	±	±	–	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
		Endothelium	–	–	–	–	–	–	–	–	–	–	±	±	±	±	±	±	±	±	±	–	–	±	±	±	±	–	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±

-: negative, ±: minimal, +: mild, ++: moderate, +++: severe.
*Two animals in disease control group and three animals in IDS group died during dosing period.

Table S3. Histopathological changes in MPS II mice at different ages

Group	Age (weeks)	Number of animals	Findings																						
			Deposition, eosinophilic material, pons/medulla oblongata					Deposition, eosinophilic material, diencephalon					Vacuolization, Purkinje cell					Vacuolization, nerve cell, pons/cerebellar medulla					Vacuolization, nerve cell, diencephalon				
			-	±	+	++	+++	-	±	+	++	+++	-	±	+	++	+++	-	±	+	++	+++	-	±	+	++	+++
Wild type	8	5	5	0	0	0	0	5	0	0	0	0	5	0	0	0	0	5	0	0	0	0	5	0	0	0	0
Disease control		5	5	0	0	0	0	5	0	0	0	0	4	1	0	0	0	5	0	0	0	0	5	0	0	0	0
WT	31	5	5	0	0	0	0	5	0	0	0	0	5	0	0	0	0	5	0	0	0	0	5	0	0	0	0
Disease control		5	0	5	0	0	0	0	3	2	0	0	0	4	1	0	0	4	1	0	0	0	5	0	0	0	0
WT	55	10	10	0	0	0	0	10	0	0	0	0	10	0	0	0	0	10	0	0	0	0	10	0	0	0	0
Disease control		9	0	0	9	0	0	0	0	9	0	0	0	0	8	1	0	2	7	0	0	0	3	3	3	0	0

-: negative, ±: minimal, +: mild, ++: moderate, +++: severe