

В

#### LUHMES







**Supplementary Figure S1.** Differentiation status of neuronal cell lines (LUHMES and SH-SY5Y) were validated by examining neuronal marker gene expression by immunocytochemistry (ICC) (**A**) and Western blotting analyses (**B**). (**A**) Each cells were prepared with the same culture method used for the uptake assays and cultured for 6 (LUHMES) or 7 (SH-SY5Y) days. Targeted genes are  $\beta$ III tubulin, microtubule-associated protein 2 (MAP2), and tyrosine hydrolase (TH). The images of the cells only treated by 2<sup>nd</sup> antibody are also shown as negative control. (**B**) Cell lysates from LUHMES cells (on day 0/2/4/6 of culture) and SH-SY5Y cells (on day 0/3/7 of culture) were prepared. Targets are the same with that used in ICC, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as a loading control. The results obtained from a single experiment (N=1) are shown, and red arrows indicates the position where the bands would appear.



**Supplementary Figure S2.** Full, uncropped images of Western blotting analyses (Figure 2) detecting TfR, M6PR, and GAPDH proteins are shown.

## A HBMEC/ci18



+M6P	+M6P		
Pabinafusp alfa	IDS		





**Supplementary Figure S4.** Results from three separate experiments (N=3) conducted for investigating *in vitro* BBB

permeability of pabinafusp alfa and IDS in HBMEC/ci18 monocultured BBB models (BBB model) or the models without the cells (blank) (Figure 3C) are shown. Permeability-surface area product values (PS,  $\mu$ L/min) obtained from the BBB model and the blank are indicated in as PS<sub>BBB</sub> and PS<sub>blank</sub>, respectively.

A SH-SY5Y



Ctri	+NUTIK AD	+IVIOP	+huttr Ab +M6P	Ctri	+NUTIK AD	+IVI6P	+MGP	
	Pabinafusp alfa				[	DS		

for investigating uptake profiles of pabinafusp alfa and IDS in SH-SY5Y cells (Figures 4A and 4B) are shown. (B) Results of inhibition assays using huTfR, M6P and these negative controls (IgG for huTfR and G6P for M6P) for pabinafusp alfa or IDS uptake in SH-SY5Y cells are shown. Data are shown as the mean  $\pm$  S.E. of values obtained from three separate experiments (N=3), each performed in duplicate (\*, P < 0.05; \*\*, P < 0.01vs. each of the Ctrl; ##, P < 0.01 vs. pabinafusp alfa

#### **A** LUHMES



	Pahinafusn alfa							
Ctrl	+huTfR Ab	+M6P	+huTfR Ab +M6P	Ctrl	+huTfR Ab	+M6P	+huTfR Ab +M6P	

separate experiments (N=3) conducted for investigating uptake profiles of pabinafusp alfa and IDS in LUHMES shown. (B) Results of inhibition assays using huTfR, M6P and these negative controls (IgG and G6P, respectively) for pabinafusp alfa or IDS uptake in LUHMES cells are shown. Data are shown as the mean  $\pm$  S.E. of values obtained from three separate experiments (N=3), each performed in duplicate (\*, P < 0.05; \*\*, P < 0.01 vs. each of the Ctrl; #, P < 0.05 vs. pabinafusp alfa Ctrl; one-way



Pabinafusp alfa			IDS					
			+M6P				+M6P	
Ctri	+NUTIK AD		+NUTIK AD	Ctri	+NUTIK AD		+NUTIK AD	

#### A HBPC/ci37



Time



Pabinafusp alfa

**Supplementary Figure S8.** (A) Results from three separate experiments (N=3) conducted for investigating uptake profiles of pabinafusp alfa and IDS in HBPC/ci37 cells (Figures 4G and 4H) are shown. (B) Results of inhibition assays using huTfR or its isotype control IgG for pabinafusp alfa uptake in HBPC/ci37 cells are shown. Data are shown as the mean  $\pm$  S.E. of values obtained from three separate experiments (N=3), each performed in duplicate (\*, P < 0.05 *vs.* Ctrl; Student's t-test).

IDS