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Supplemental information

Grabody B, an IGF1 receptor-based shuttle, mediates efficient delivery of biologics across the blood-brain barrier

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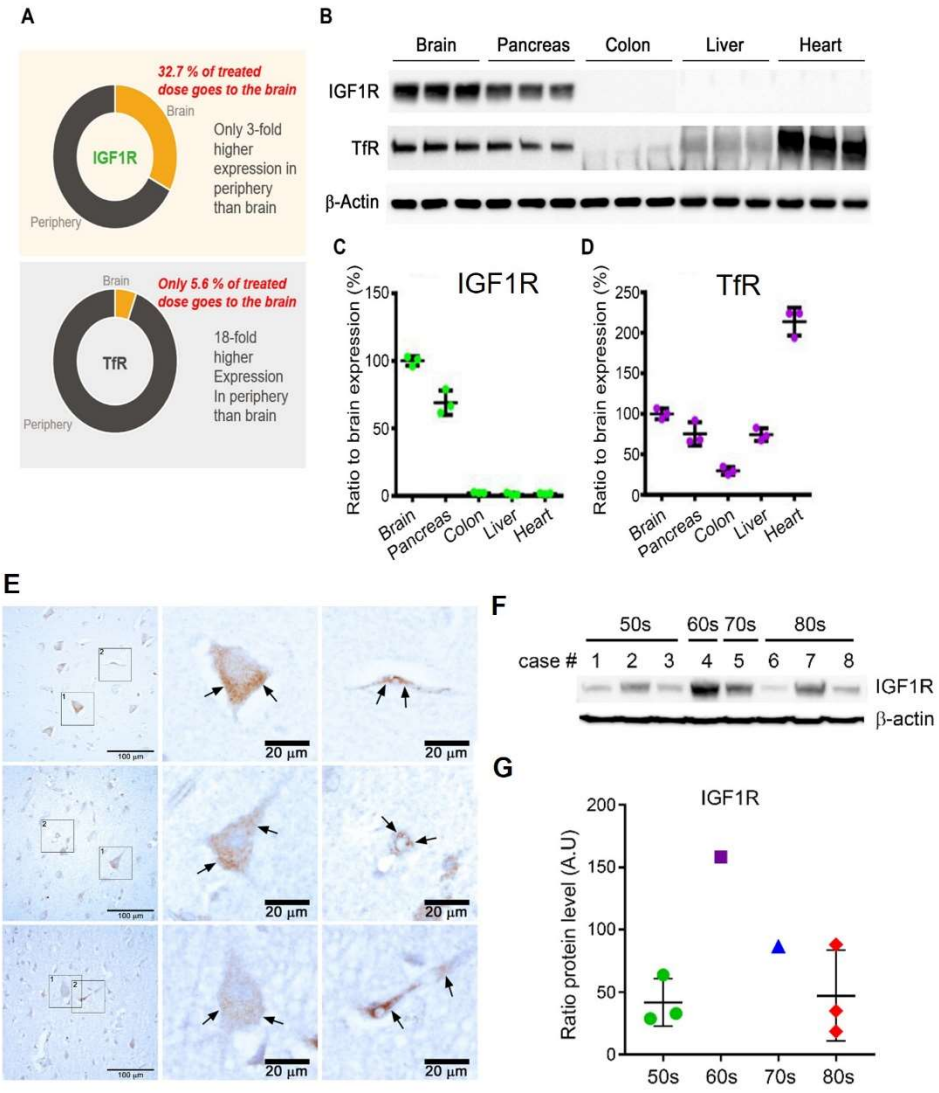
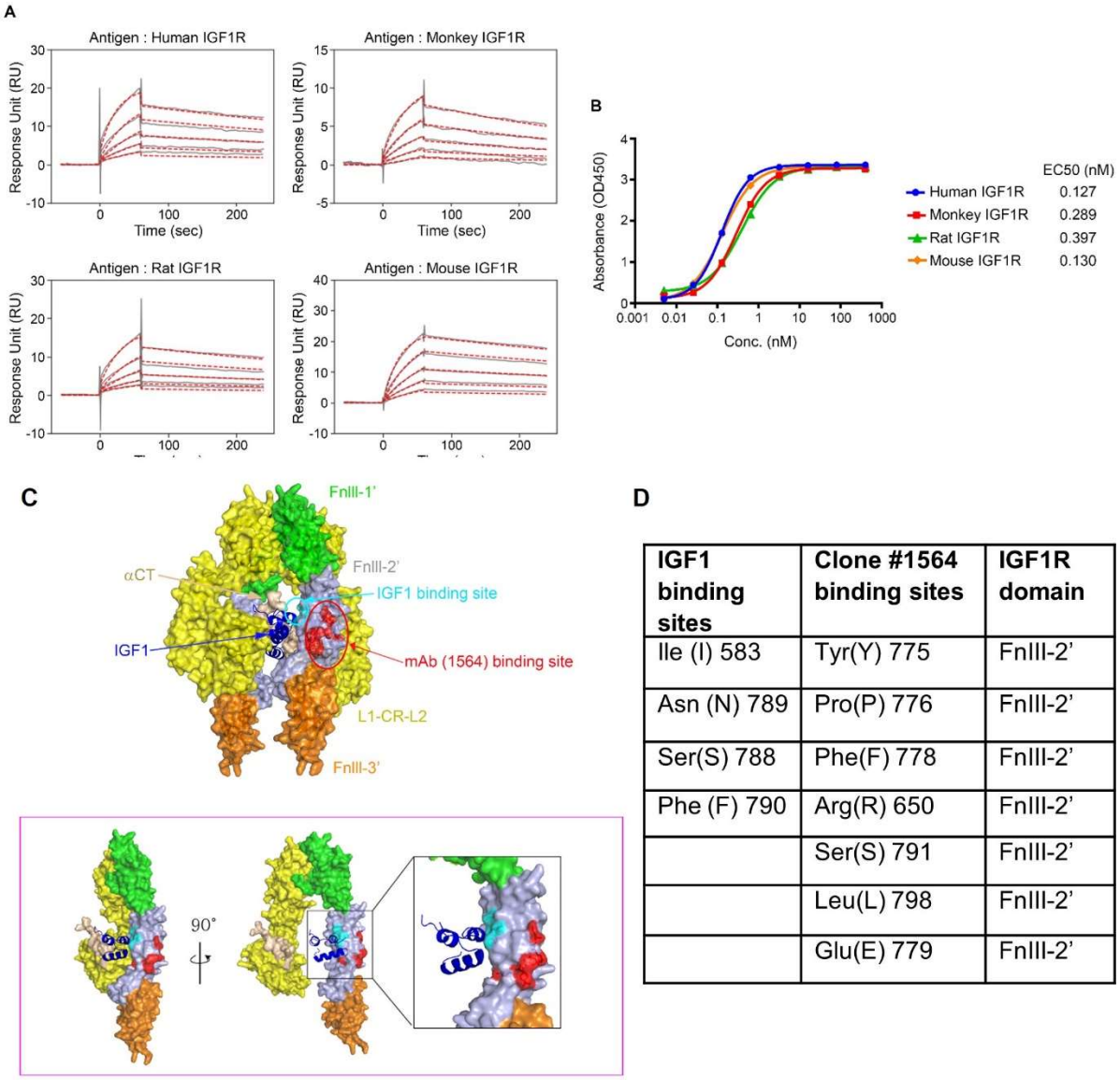


Figure S1. Brain IGF1R expression is higher than its peripheral expression, in contrast to the higher peripheral expression of TfR, Related to Figure 1.

(A) The absolute expression level of IGF1R and TfR in various tissues was obtained from a public database (https://pax-db.org) (Wang et al., 2012). PaxDB organized the expression level of targets as an absolute value called 'parts per million' in order to enable a direct comparison between targets. The expression levels in the brain, liver, lung, colon, esophagus, pancreas, and heart were summed together and compared to the target's expression in the brain (IGF1R_total: 9.02 ppm vs. IGF1R_brain: 2.22 ppm; TfR_total: 266.67 ppm vs. TfR_brain: 14 ppm). Due to high peripheral TfR expression, up to 18-fold more than its brain expression, a significant amount of therapeutic mAb fused with the anti-TfR shuttle may be systemically sunk. In contrast, brain IGF1R expression is approximately one-third the IGF1R expression in a system; thus, a bispecific antibody with a therapeutic mAb and anti-IGF1R shuttle may reach the CNS without losing too much in the periphery after intravenous dosing. (B) Immunoblot of lysates from five mouse organs (brain, pancreas, colon, liver, and heart) with either anti-IGF1R antibody or anti-TfR antibody. Notably, a minimal or absent IGF1R-positive band was observed in the colon, liver, and heart, whereas strong immunoreactivity to TfR was found in the three organs, especially the heart. (C, D)

Quantification of the band intensity in (B). An average intensity of three wells of brain samples was normalized to 100, and the relative band intensity of other organs was obtained. Data are presented as mean ± SEM. n = 3 mice. (E) Postmortem brains from three subjects (one in each row) showed clear IGF1R-immunoreactivity in brain cells (#1) and the brain microvessels (#2) (arrows). The middle and right columns are enlarged images of #1 and #2, respectively. Scale bar = 20 μm. (F) Western blot bands for IGF1R in postmortem brain tissues from different ages (50s: $n = 3$; 60s: $n = 1$; 70s: $n = 1$; 80s: $n = 3$; total $n = 8$). β-actin was used as a loading control. None of the 8 subjects had neurodegenerative diseases. Subject 4 (age: 60s) had a history of diabetes and related medication for years. (G) Quantification of the IGF1R bands after normalization to β-actin. The optical density of each band was averaged with the same group. Overall, three age groups showed similar IGF1R expression levels, implying that brain IGF1R levels are maintained (50s: green; 60s: purple; 70s: blue; 80s: red; One-way ANOVA, $p > 0.05$). Subject 4 was an outlier possibly because of the history of diabetes and medication, which could have increased the IGF1R expression. The line and whiskers indicate mean ± SD.



IGF1 binding sites	Clone #1564 binding sites	IGF1R domain
Ile (I) 583	Tyr(Y) 775	FnIII-2'
Asn (N) 789	Pro(P) 776	FnIII-2'
Ser(S) 788	Phe(F) 778	FnIII-2'
Phe (F) 790	Arg(R) 650	FnIII-2'
	Ser(S) 791	FnIII-2'
	Leu(L) 798	FnIII-2'
	Glu(E) 779	FnIII-2'

Figure S2. Grabody B is an IGF1R binder that has broad cross-reactivity to IGF1R across species, and its parental antibody (Clone #1564)'s IGF1R binding site indicates no interference with IGF1 binding site, Related to Figure 2.

(A) SPR sensorgram plot of B30104 (Grabody B-fused anti-α-Syn antibody) for the extracellular domain (ECD) of human, mouse, rat, and monkey IGF1R. Each panel shows an overlay plot of the measured response (grey color) with the global fit (red color). B30104 showed fairly similar binding kinetics to four different IGF1R proteins. (B) B30104 binding to the IGF1R ECD proteins from the four species in ELISA. B30104 showed similar binding profiles (EC50) between human (blue) and mouse (orange) IGF1R, and between monkey (red) and rat (green) IGF1R. (C) The structure of homo-dimeric IGF1Rs was obtained using PDB: 5U8Q (Xu et al., 2018) with each domain marked in different colors. The binding of IGF1 (dark blue) to its binding site on IGF1R (sky blue) is clearly visible. In contrast, the binding site of clone #1564 (red) is shown apart from the IGF1 binding site. Pink box: Monomeric IGF1R with the same color code. The enlarged image (inset) clearly describes the physical distance between the IGF1 binding site (sky blue) and the 1564 binding site (red), as it is consistent with the distance between the two binding sites (D).

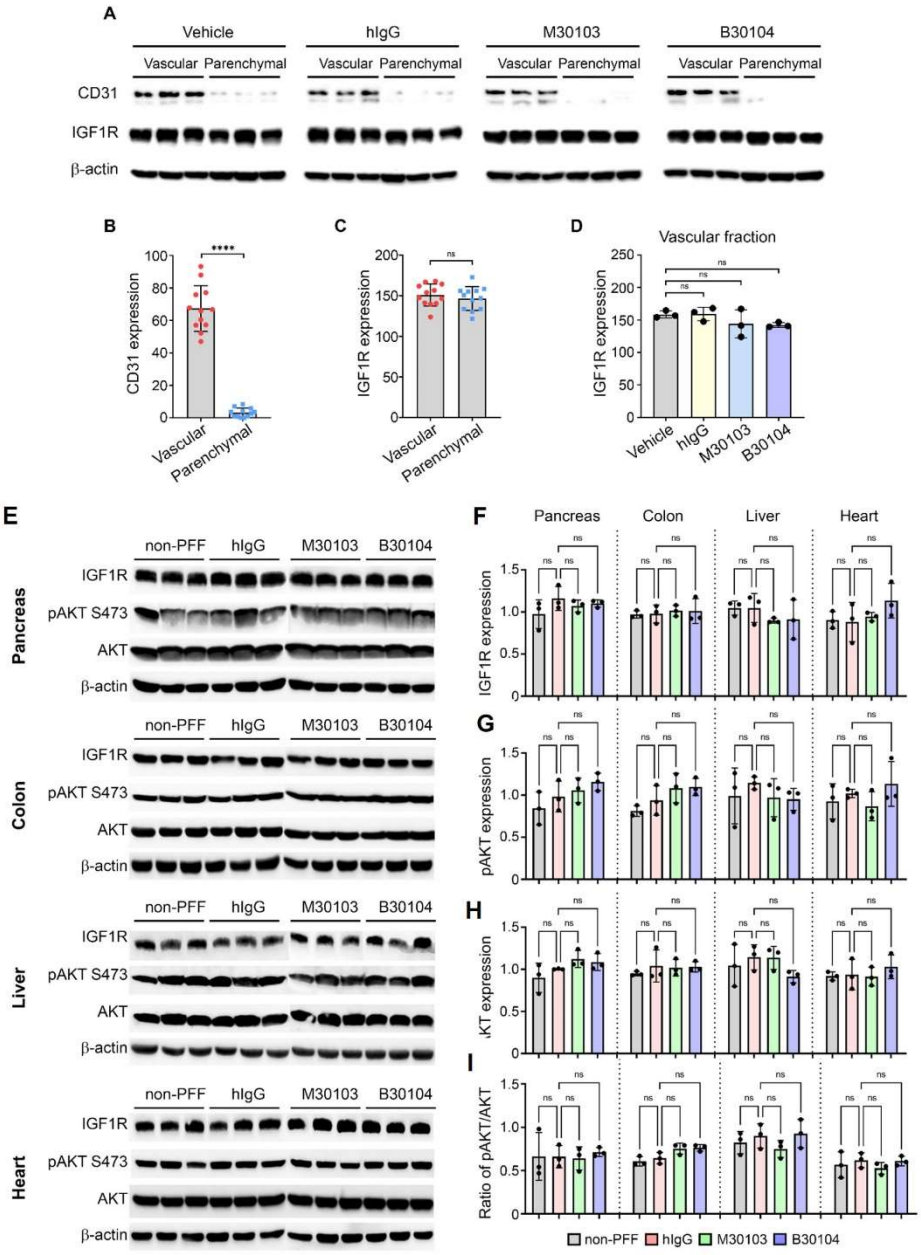


Figure S3. No significant changes in brain and peripheral IGF1R after repeated dosing with B30104, Related to Figure 2.

(A) Immunoblot bands for mouse brain vascular or parenchymal fractions treated with 30 mg/kg hIgG or M30103 or 35.1 mg/kg B30104 for 3 months. The IGF1R and CD31 levels in each fraction are shown as an immunoblot. β-actin was used as a loading control. (B) Quantification of the band intensity for CD31in all treated animals in (A). Note the lack of a band for CD31 in parenchymal fractions. (C) Quantification of the band intensity for brain IGF1R in all treated animals and (D) in individual treatment groups. Note the similar vascular IGF1R level between groups. Data are presented as mean ± SEM. B,C: paired t-test; D: ordinary one-way ANOVA, **** $p < 0.0001$, ns = not significant. (E) Immunoblot bands for total IGF1R, pAKT, and AKT in the pancreas, colon, liver, and heart of mice treated with weekly intraperitoneal doses of 30 mg/kg hIgG or M30103 or 35.1 mg/kg B30104 for 3 months. The mice, except the non-PFF control group, were injected in the striatum with PFF 3 months prior to the antibody dosing. (F-H) Quantification of the band intensity in (E). Data are presented as mean ± SD; n = 3 per treatment. ns = not significant; ordinary one-way ANOVA. (I) Ratio of the pAKT- and AKT-immunoreactive band intensity. Data are presented as mean ± SD; n = 3 per treatment. ns = not significant, ordinary one-way ANOVA.

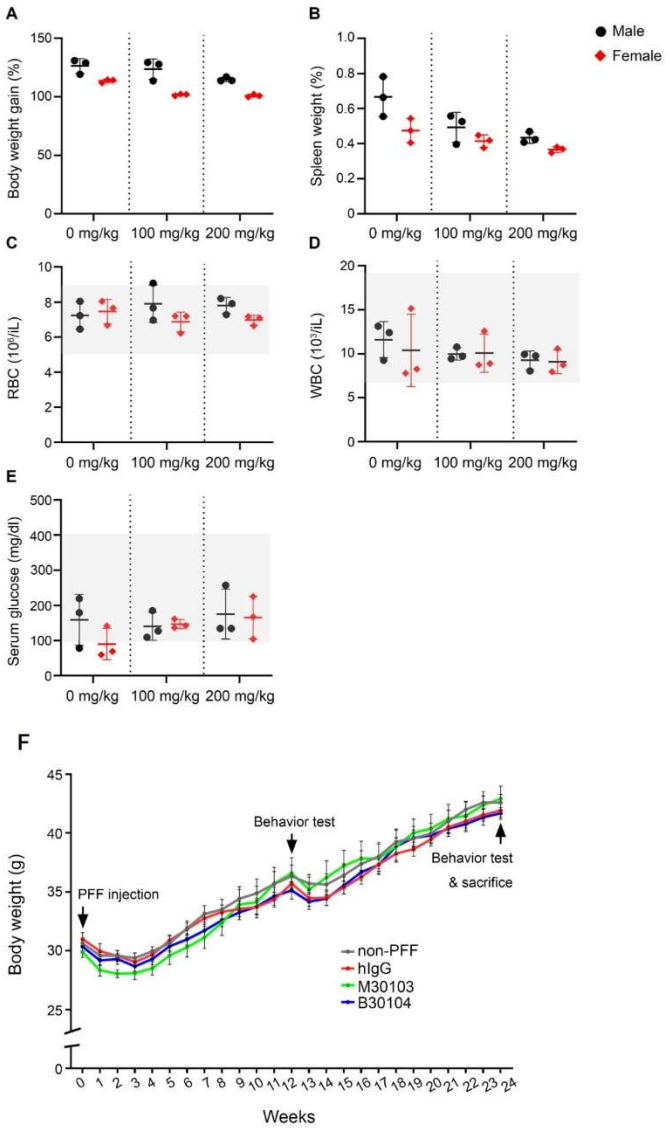


Figure S4. B30104 induces no drug-related adverse events in rats and PFF-injected mice with repeated dosing, Related to Figure 2.

Six SD rats (3 males, black circles; and 3 females, red diamonds) received vehicle or 100 mg/kg or 200 mg/kg of B30104 weekly for 2 weeks. The body weight (A), spleen weight (B), red blood cells (C), white blood cells (D), and serum glucose levels (E) of the two groups treated with B30104 were comparable to the vehicle group (0 mg/kg). Data are presented as mean ± SD; n = 6. (F) The weight of the PFF-injected mice in Figure 7-9 was monitored during 24 weeks with weekly intraperitoneal administration of 15 mg/kg hIgG or M30103 or 17.55 mg/kg B30104. The four groups, including the non-PFF control, had overall similar weight over the 24 weeks. Data are presented as mean ± SEM; n = 12 per treatment. ns = not significant; ordinary one-way ANOVA.

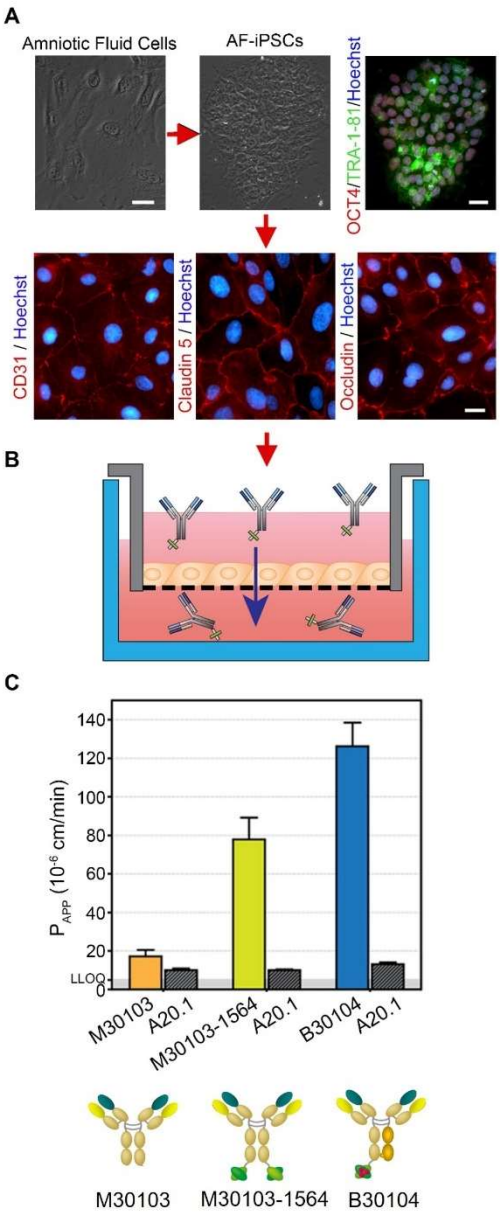
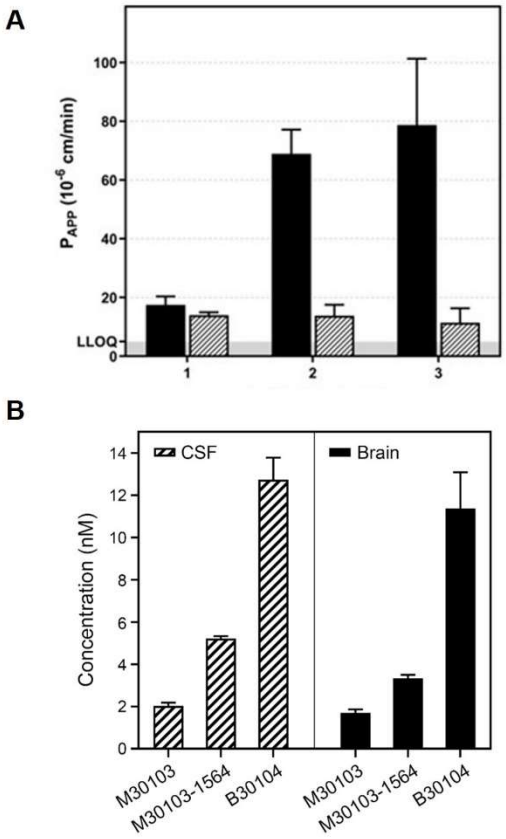


Figure S5. Grabody B mediates efficient transcytosis in a trans-well system, Related to Figure 3.
(A) Schematic of the induced brain endothelial cells (iBECs) differentiated from human amniotic fluid-derived induced pluripotent stem cells (AF-iPSCs). Scale bar = 20 μm. (B) Illustration of the trans-well assay using the AF-iPSCs. (C) Apparent permeability coefficient (Papp) of three antibodies (M30103, yellow; M30103-1564 bivalent, green; and B30104, blue) in the iBEC in vitro BBB model. Single-domain antibody A20.1 (hashed bars, negative control) against a non-mammalian target was used to confirm the trans-well's selective permeability. Data are presented as mean ± SD for at least three independent trans-well inserts.



C

Parameters	Units	M30103	B30104
Serum AUC_{0-T}	hr*nmol/ml	301.8	306.6
CSF AUC_{0-T}	hr*nmol/ml	0.159	0.918
Brain AUC_{0-T}	hr*nmol/ml	0.134	1.116
CSF AUC_{0-T}/Serum AUC_{0-T}	%	0.053	0.299
Brain AUC_{0-T}/Serum AUC_{0-T}	%	0.044	0.364

Figure S6. *In vitro* and *in vivo* assessment of BBB penetration by Grabody B using SV-ARBECs and rats, Related to Figure 3 and 4.

(A) Apparent permeability coefficient (Papp) of three antibodies in the rat SV-ARBEC *in vitro* BBB model. The antibodies (filled bars) refer to M30103 (#1), M30103-1564 bivalent (#2), and B30104 (#3). Single-domain antibody A20.1 (hashed bars) was used in each trans-well insert as an 'in-experiment' control for endothelial monolayer permeability. Data are presented as mean ± SD of at least three independent trans-well inserts. (B) CSF and brain hIgG levels measured by LC-SRM 24 hours after a single intravenous dose of 30 mg/kg M30103 or the molar equivalent dose of M30103-1564 bivalent, B30104 to rats. Data are presented as mean ± SD; n = 3 rats per treatment. (C) Rat PK profile of M30103 and B30104 shown in Figure 4.

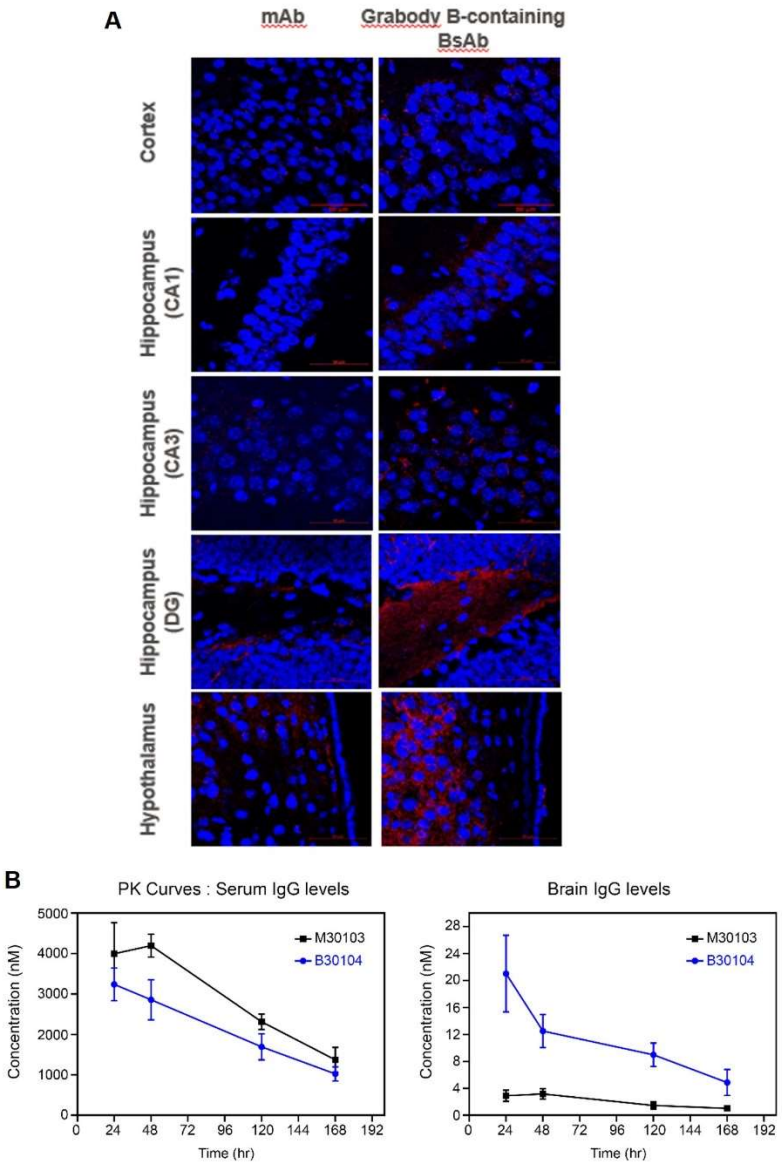


Figure S7. Increased brain level of Grabody B-fused antibody compared to monoclonal antibody in mice, Related to Figure 4-7.

(A) Immunostaining of mouse brain sections after a single intravenous injection of monoclonal antibody targeting BACE1 at 50 mg/kg or the same molar concentration of Grabody B-containing anti-BACE1 bispecific antibody. Note the higher intensity signal of Grabody B-fused anti-BACE1 antibody from 72 hours post-injection compared to anti-BACE1 antibody in four different brain areas. (B) Serum and brain levels of M30103 and B30104 in PFF-injected mice. M30103 at a dose of 30 mg/kg or the molar equivalent of B30104 was administered intraperitoneally to the PFF-injected mice weekly for 3 months. The dosing was initiated 3 months after PFF injection. Serum and brains were collected from mice at four different time points (24, 48, 120, and 168 hours) after the last dosing, and their hIgG level was analyzed using LC-SRM. B30104 was present at comparable or slightly lower levels in serum than M30103. In the brain, the B30104 level was significantly higher at all four time points than the M30103 level, suggesting that the better efficacy of B30104's could potentially be driven by the elevated brain exposure to B30104 (Time points: $p = 0.0079$; M30103 vs. B30104: $p < 0.0001$; Two-way ANOVA). Data are presented as mean ± SD.