Supplemental Methods

APPswe/PS1ΔE9 and Wildtype mice. Male and female mice heterozygous for the APPswe/PS1ΔE9 mutation (19) (APP/PS1) or littermate controls (Wt) on a B6C3 background at 3 and 18 months were used in these studies. Mice were given food and water *ad libitum* and maintained on a 12:12 light/dark cycle.

Glucose clamp combined in vivo microdialysis. Five-days prior to glucose clamps (28), 3-month or 18-month APP/PS1 mice (n=6-8 mice/group) were anesthetized via isofluorane and tapered catheters (MRE025 tubing, Braintree Scientific) inserted into both the jugular vein and the femoral artery, and sutured into place. The catheter lines were filled with polyvynilporolidone (PVP), the ends double knotted, and a suture affixed to the ends. A small incision was made between the scapulae and the lines tunneled to this area for externalization prior to glucose clamps. Two days prior to glucose clamps, guide cannulas (BR-style, BIoanalytical Systems) were stereotaxically implanted into the hippocampus (from bregma, A/P: -3.1mm, M/L: -2.5mm, D/V: -1.2mm at 12˚ angle) and secured into place with dental cement. Cresyl violet staining was used to validate probe placement post-mortem. One day prior to glucose clamps, the mice were transferred to Raturn sampling cages (Bioanalytical Systems) and microdialysis probes (2 mm; 38 kDa molecular weight cut-off; BR-style, Bioanalytical Systems) inserted into the guide cannula, connected to a syringe pump and infused with artificial cerebrospinal fluid (aCSF; 1.3mM $CaCl_2$, 1.2mM $MgSO_4$, 3mM KCl, 0.4mM KH_2PO_4, 25mM $NaHCO_3$ and 122mM NaCl; pH=7.35) at a flow rate of 1 µl/minute. At this time, the jugular and femoral lines were externalized and the PVP removed. The femoral and jugular lines were flushed, connected to a syringe pump and slowly infused with 0.9% sodium chloride at a flow rate of 1µl/min overnight to prevent clots. Hourly collection of hippocampal ISF began. The following morning, mice were fasted 4-5 hours prior to and during the 4-hour glucose clamps. For the duration of the clamp, the jugular vein was infused with a 12.5% dextrose solution in PBS (control mice received PBS alone) at a variable flow rate. Every 10 minutes,

blood was sampled via the femoral artery and blood glucose concentration assessed using a hand held glucometer (Contour, Bayer). The concentration of blood glucose was targeted to 150-200 mg/dL and the flow rate of the dextrose solution was adjusted accordingly. After the 4-hour clamp, the dextrose solution was stopped, the lines flushed, euglycemia restored, and food returned to the bowls. Hourly ISF collection continued for the duration of the clamp and for 10-15 hours post-clamp.

Drug administration via reverse microdialysis. Guide cannula implantation, microdialysis, and glucose clamps were performed as described above. Glibenclamide (0.5, 1, 10, 50, and 100μM; Sigma-Aldrich), diazoxide (30, 100, and 300μM; Sigma-Aldrich), and pinacidil (30, 100, and 300μM; Sigma-Aldrich) were infused directly in the APP/PS1 brain (n=5-7) via reverse microdialysis for 2 or 3 hours, respectively, under euglycemic conditions. During hyperglycemia, diazoxide (300μM) was infused into the APP/PS1 hippocampus (n=5) 3 hours prior to and 4 hours during glucose clamps. Both drugs were first diluted into DMSO due to solubility issues, and subsequently in 1X aCSF to the appropriate working concentration. To determine the clearance rate of ISF Aβ and its half-life in the ISF, Compound E (200nM; Enzo Life Sciences) was infused during the 4-hour clamp via reverse microdialysis as previously described (18). Statistical significance was determined using a one-way ANOVA and Dunnett's multiple comparison post-hoc test. Data is represented by means +/- SEM.

Quantification of soluble and insoluble $A\beta_{40}$ and $A\beta_{42}$ in brain. 3-month old APP/PS1 mice mice were sacrificed at 3 hours into either a hyperglycemic or PBS clamp (n=5-6 mice/group). Tissue from the cortex was used to determine soluble and insoluble Aβ40 and 42 as previously described (29).

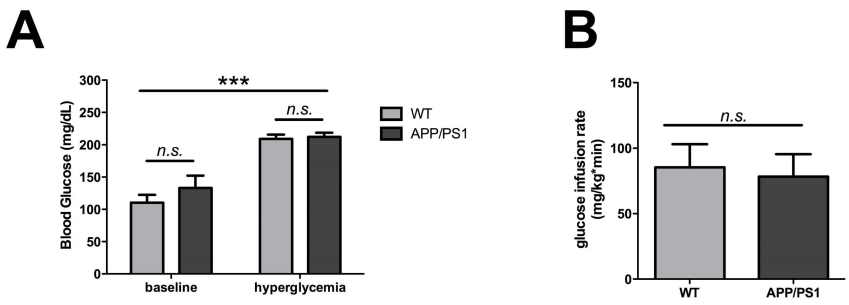
Quantification of Aβ deposition. 3-month and 18-month APP/PS1 mice (n=4/group) were anesthetized with an overdose of sodium pentobarbital and transcardially perfused with ice-cold phosphate buffered saline (PBS). The brains were removed and fixed in 4% paraformaldehyde for 48 hrs at 4°C. Prior to sectioning, the brains were cryoprotected in 30% sucrose and then

sectioned on a freezing microtome at 50µm. Serial sections (300µm apart) through the anterior-posterior aspect of the hippocampus were immunostained for Aβ deposition using a biotinylated, HJ3.4 antibody (anti-$A\beta_{1-13}$, mouse monoclonal antibody generated in-house) and developed using a Vectastain ABC kit and DAB reaction. The brain sections were imaged using a NanoZoomer slide scanner (Hamamatsu Photonics) and the percent area occupied by HJ3.4 was quantified by a blinded researcher as previously described (13, 22). Statistical significance was determined using a two-tailed, unpaired t-test. Data is represented by means +/- SEM.

Glucose and lactate measurements. Glucose and lactate concentrations (glucose- and lactate- oxidase method) were measured in each ISF sample from 3-month and 18-month APP/PS1 mice (n=5-7/group) using the YSI 2700 analyzer (YSI incorporated) per the manufacturer's instructions. Statistical significance was determined using a two-tailed, unpaired t-test. Data is represented by means +/- SEM.

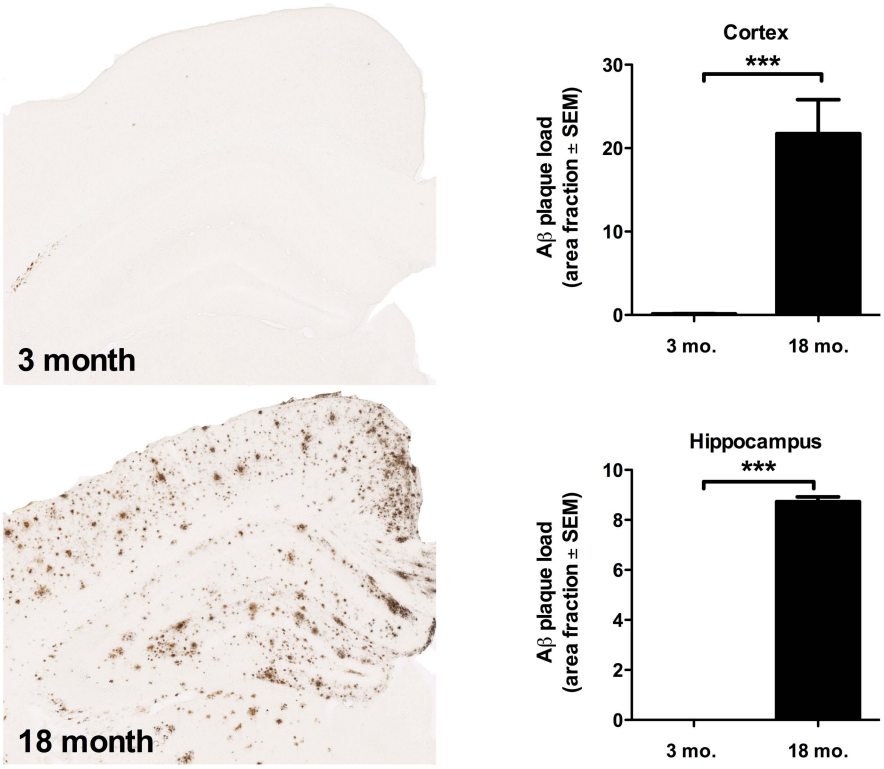
$A\beta_{1-X}$ ELISA. ISF samples from 3-month and 18-month APP/PS1 mice (n=5-7/group) collected from microdialysis experiments were analyzed for $A\beta_{1-x}$ using sandwich ELISAs as previously described(13, 22). Briefly, $A\beta_{1-x}$ was quantified using a monoclonal capture antibody targeted against Aβ13-28 (m266) and a biotinylated detection antibody targeted against Aβ1-5 (3D6), both generous gifts from Dr. Ron DeMattos, Eli Lilly and Co., Indianapolis, IN. After incubation with streptavidin-poly-HRP-20, the assay was developed using Super Slow TMB (Sigma) and the plates read on a Bio-Tek Synergy 2 plate reader at 650nm. Statistical significance was determined using a 2way ANOVA. Data is represented by means +/- SEM.

Supplemental Figure 1



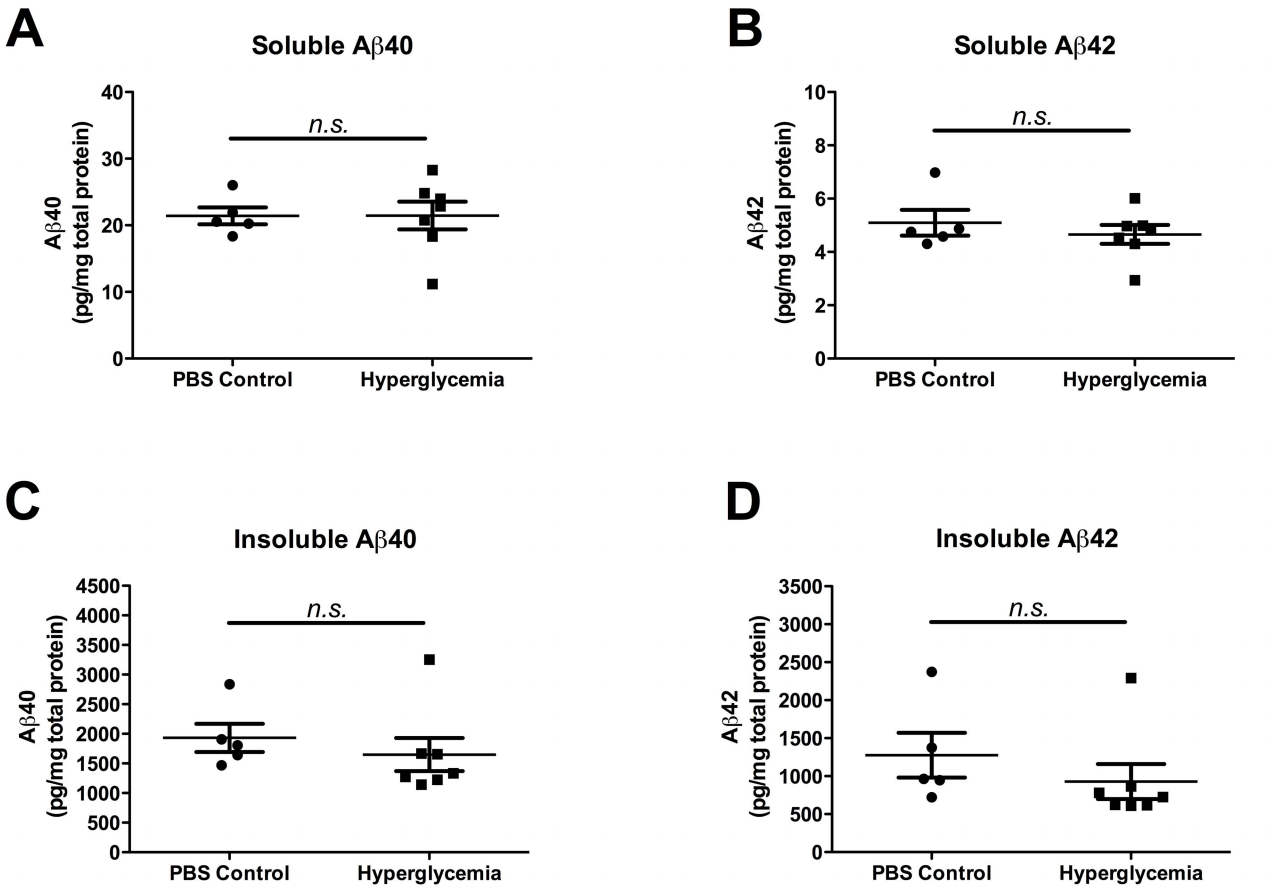
Supplemental Figure 1. Comparable metabolic response to glucose clamps in 3-month APP/PS1 and WT mice. (A) There was no difference in blood glucose levels in 3-month APP/PS1 and WT mice (n=5-6 mice/group) at fasted baseline or during hyperglycemia. (B) No difference in glucose infusion rate, a test of insulin responsiveness, was observed in APP/PS1 and WT mice. Data represent mean ± SEM. For all analyses, n=5-6 mice/group, ****P*<0.001 using 2-way ANOVA in (A) and *n.s.* indicates not significant using t-tests in (B).

Supplemental Figure 2



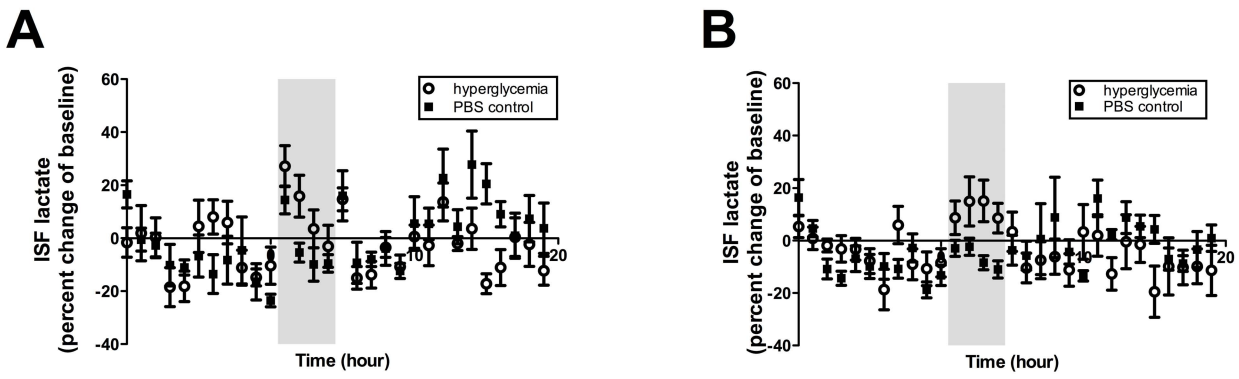
Supplemental Figure 2. Aβ deposition in the cortex and hippocampus of 3 and 18 month old APP/PS1 mice. Increased Aβ deposition in both the cortex and hippocampus of 18-month APP/PS1 mice (n=4 mice/group). Data represent mean ± SEM. For all analyses, n=4 mice/group, ****P*<0.001 using t-tests.

Supplemental Figure 3



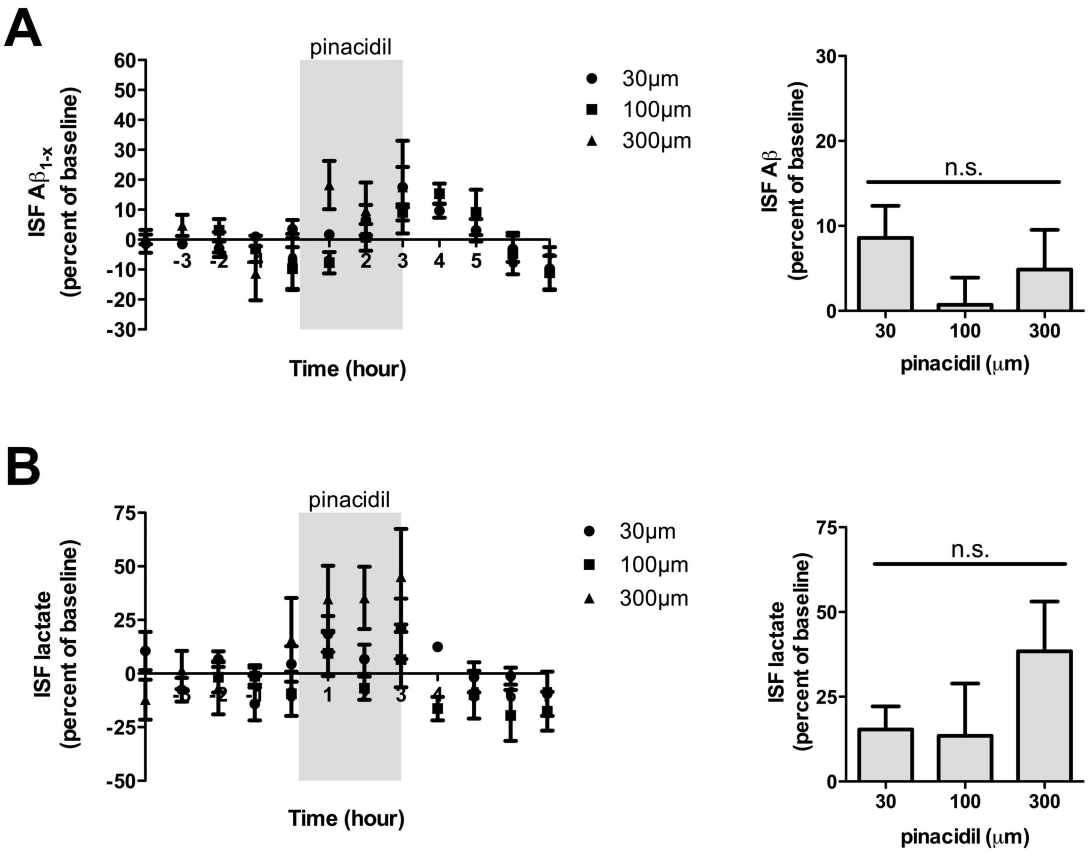
Supplemental Figure 3. Total amount of soluble and insoluble $A\beta_{40}$ and $A\beta_{42}$ in the brain during hyperglycemia. There is no difference between the total amount of (A) soluble $A\beta_{40}$, (B) soluble $A\beta_{42}$, (C) insoluble $A\beta_{40}$, and (D) insoluble $A\beta_{42}$ between hyperglycemia and PBS controls during the 3rd hour of the clamp. Data represent mean ± SEM. For all analyses, n=5-7 mice/group, *n.s.* denotes no statistical significance was obtained using t-tests.

Supplemental Figure 4



Supplemental Figure 4. Time course of ISF lactate during 3- and 18-month APP/PS1 mice during glucose clamps. (A) Levels of ISF lactate at baseline, during clamp, and post-clamp in 3-month APP/PS1 mice (n=6-7 mice/group). (B) Levels of ISF lactate at baseline, during clamp, and post-clamp in 18-month APP/PS1 mice (n=6-8 mice/group). Data represent mean ± SEM.

Supplemental Figure 5



Supplemental Figure 5. The effects of pinacidil, a K_{ATP} channel agonist, on ISF Aβ and lactate. (A) There is no significant difference in ISF Aβ, at any dose tested, following pinacidil administration. (B) At all doses tested, there is no significant difference in ISF lactate following pinacidil infusion. Data represent mean ± SEM. For all analysis, n=4 mice/group/dose, no statistical significance was found using one-way ANOVAs and denoted by *n.s.*