Supplementary Material for the Manuscript

A novel multi-modal RAGE-specific inhibitor controls amyloid-β-mediated brain disorder in mice

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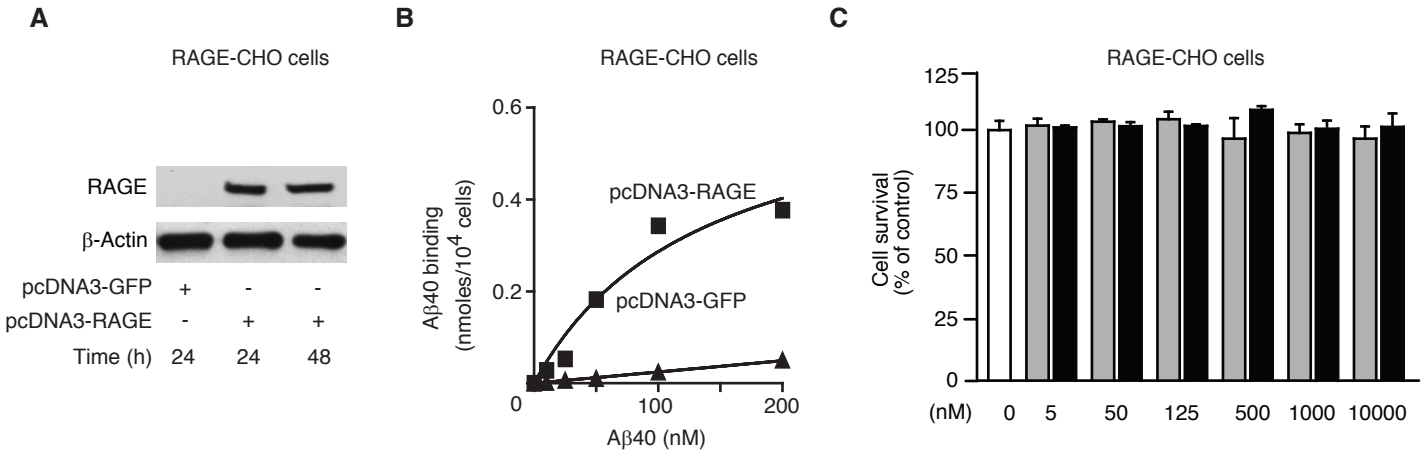
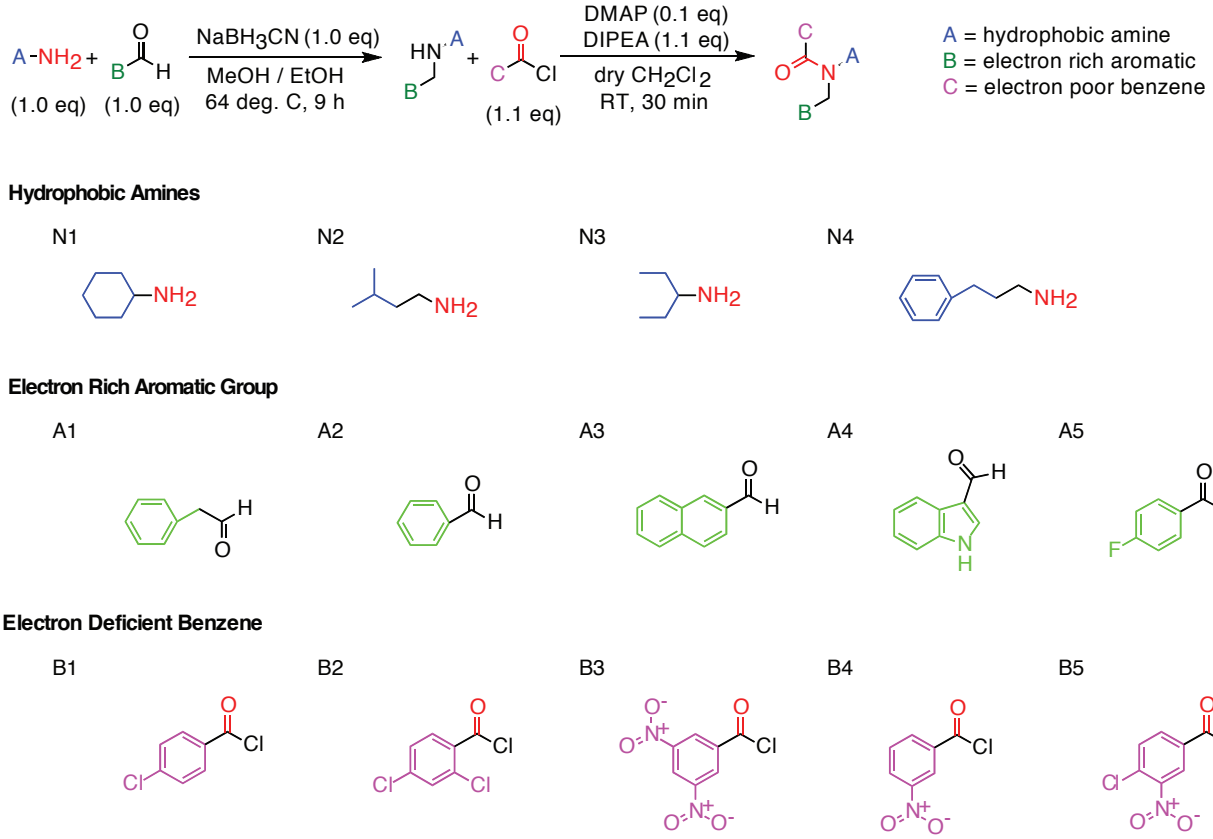
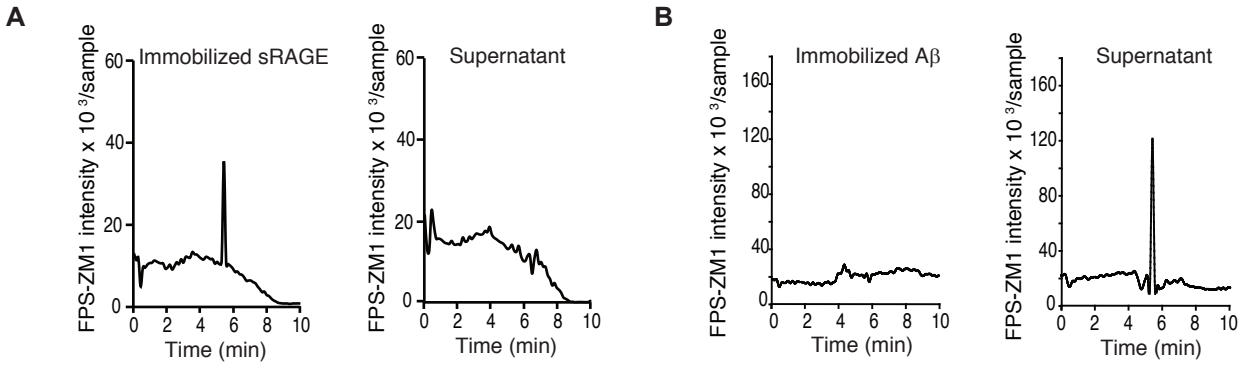


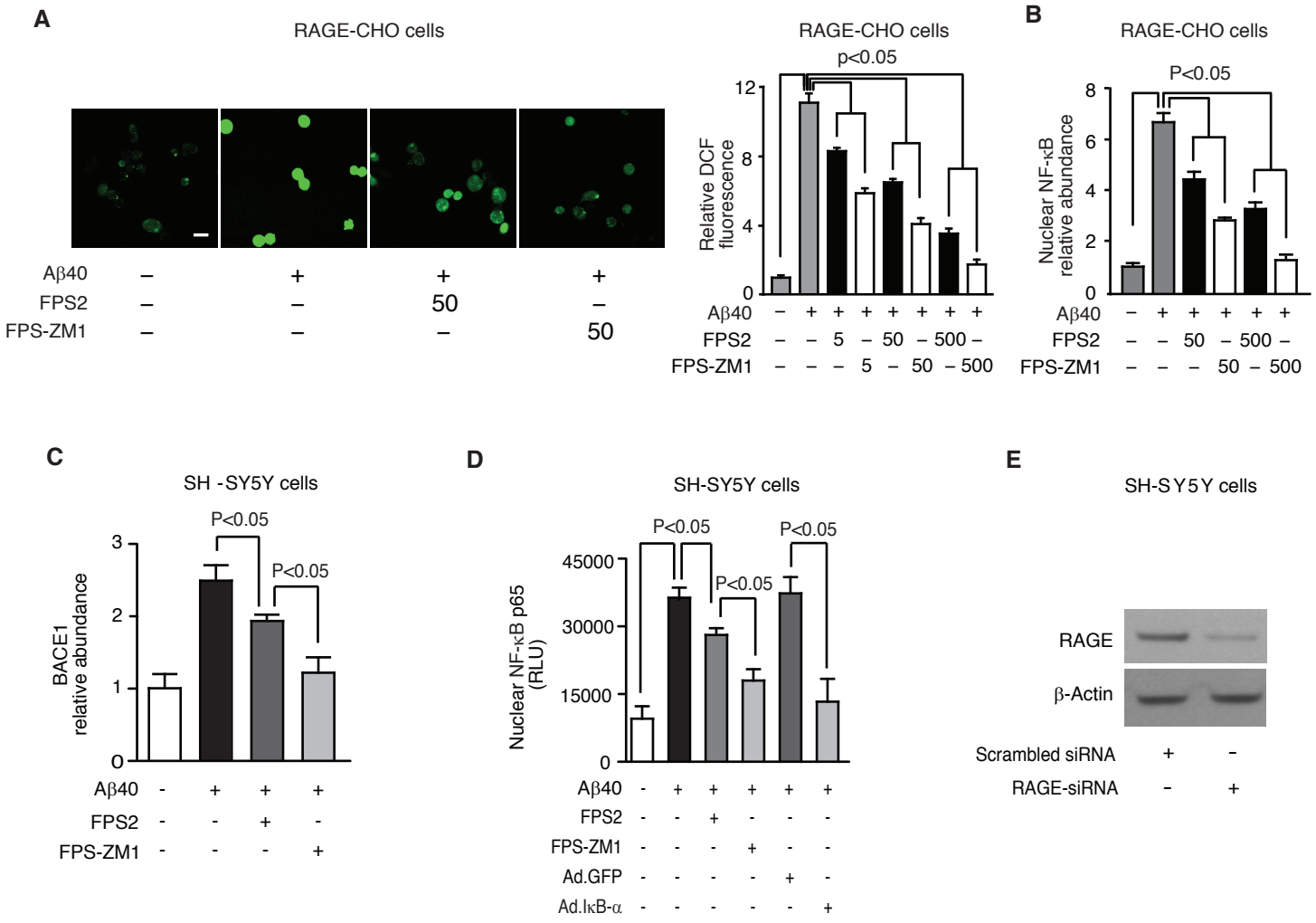
Supplementary Figure 1. **Saturable Aβ binding to RAGE-CHO cells.** (**A**) Western blot analysis of RAGE levels in RAGE-transfected CHO cells (pcDNA3-RAGE; RAGE-CHO cells) and mock-transfected CHO cells (pcDNA3-GFP, green fluorescent protein)**.** (**B**) ^{125}I-Aβ40 binding to RAGE-CHO cells (closed squares) and mock-transfected CHO cells (closed triangles) in the presence of various concentrations of unlabeled Aβ40. (**C**) CHO cell survival with and without the indicated concentrations of FPS2 (gray column) or FPS-ZM1 (black column). Values are mean ± s.e.m., n = 3 independent experiments.



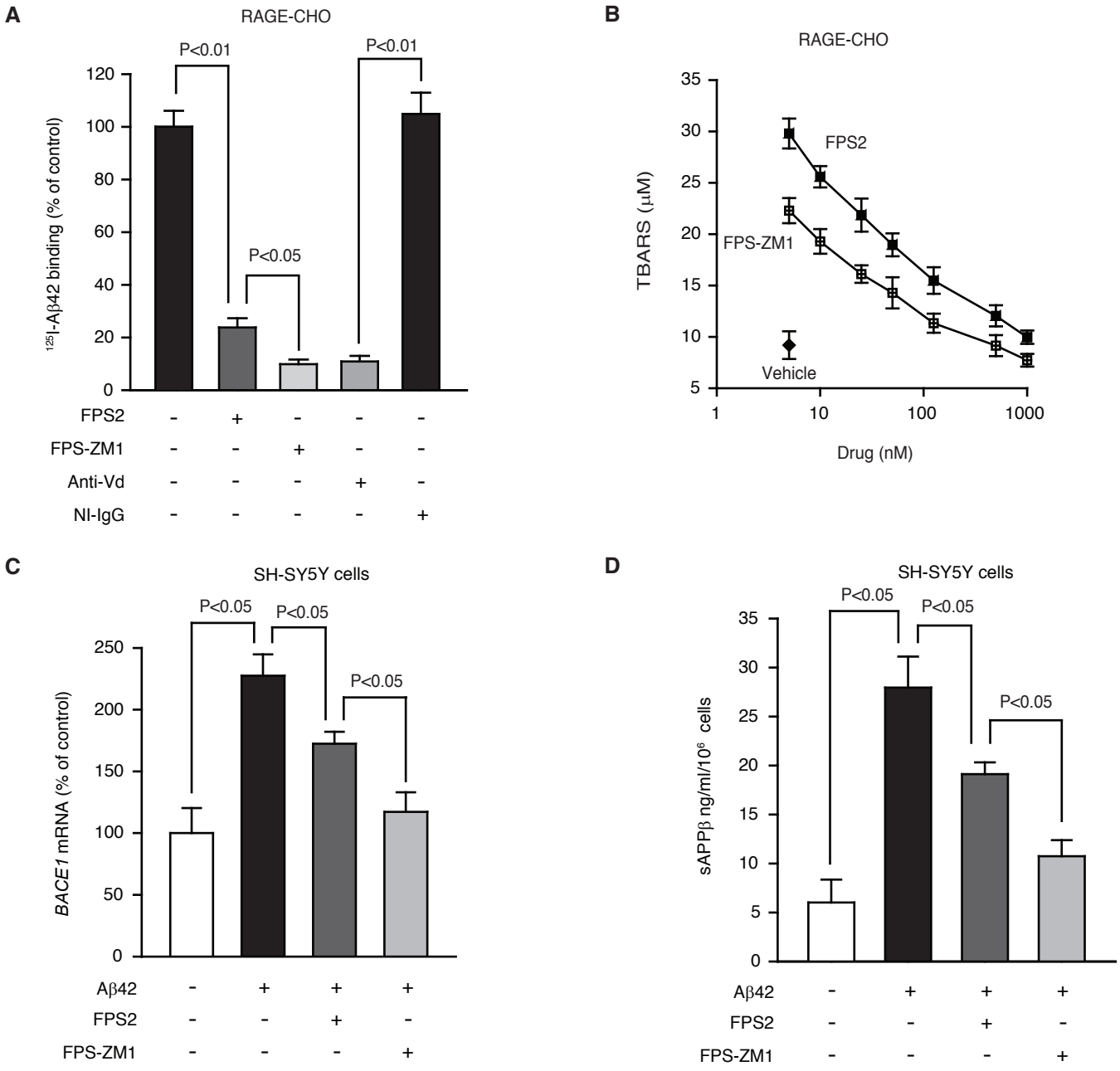
Supplementary Figure 2. Diagram for the synthesis of the second generation library of compounds. The overall reaction equation is shown at the top. The various substitutes of hydrophobic amines (N1 to N4), electron aromatic groups (A1 to A5) and electron deficient benzene (B1 to B5) are shown below the overall reaction equation. DMAP (4-N,N-dimethylpyridine); DIPEA (diisopropylethylamine). For details see the Results section.



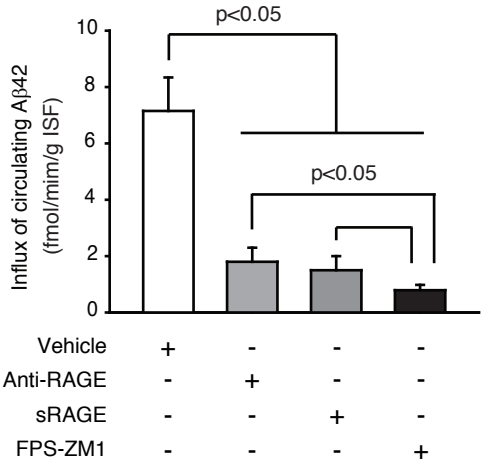
Supplementary Figure 3. FPS-ZM1 binds to immobilized sRAGE but not to immobilized Aβ40. (**A**) FPS-ZM1 was detected in the pellet containing immobilized sRAGE (left), but not in the supernatant containing no sRAGE (right). (**B**) FPS-ZM1 was absent in the pellet containing immobilized Aβ40 (left) but present in the supernatant containing no Aβ40 (right).



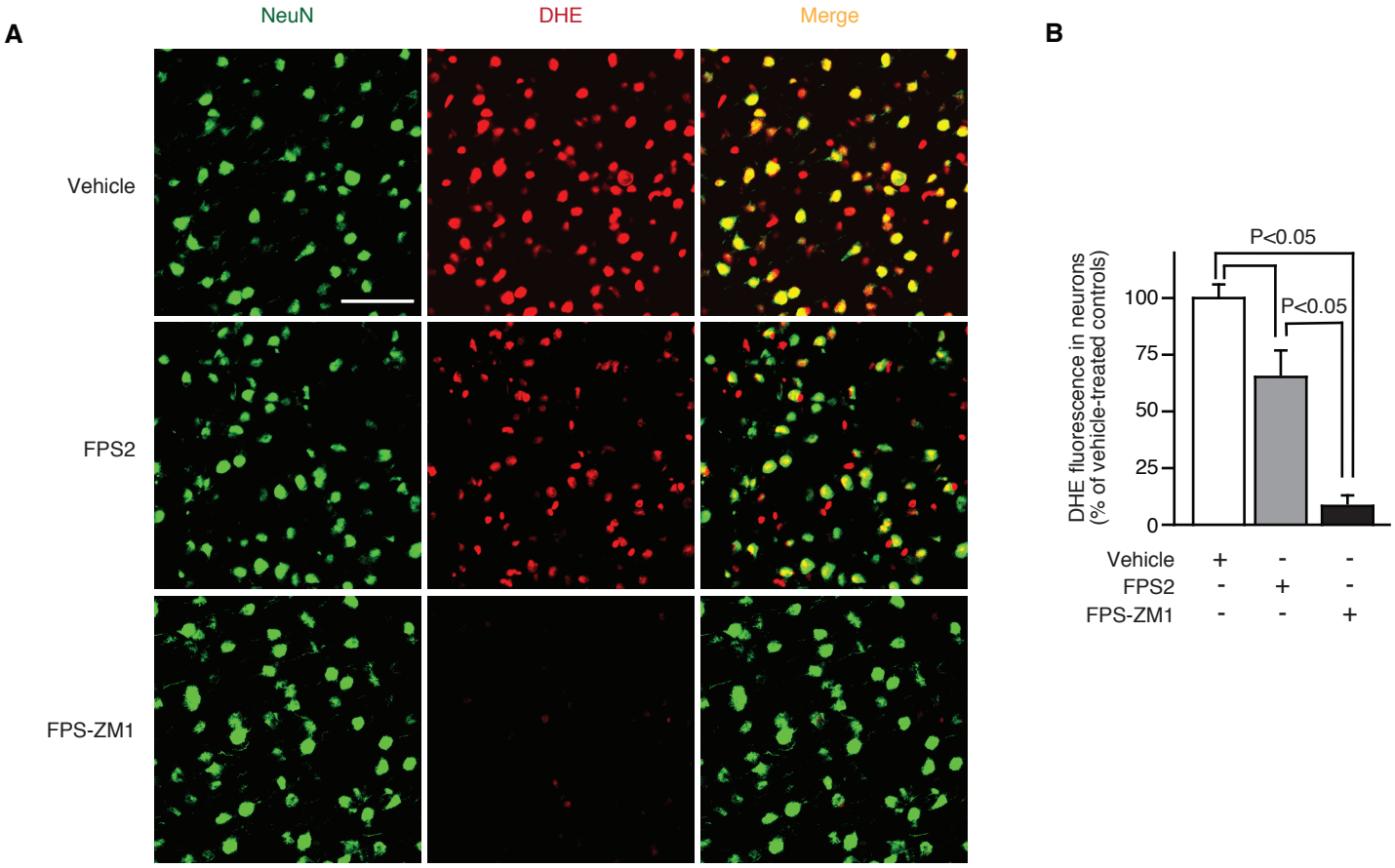
Supplementary Figure 4. **FPS-ZM1 and FPS2 block oxidative stress and inhibit BACE1 in RAGE-expressing cells.** (**A**) Confocal microscopy analysis of Aβ40 (1μM)-induced oxidative stress in RAGE-CHO cells (left, bar = 10 μm) and the relative DCF (dihydrofluorescein diacetate) fluorescence in the absence (grey column) and presence of different concentrations (nM) of FPS2 (black column) or FPS-ZM1 (clear column) (right). (**B**) Quantitative densitometry of NF-κB nuclear levels in RAGE-CHO cells treated with vehicle or Aβ40 (1μM) and different concentrations of FPS-ZM1 and FPS2 from data as in Figure 2E. (**C**) Quantification of BACE1 protein levels by scanning densitometry in SH-SY5Y cells treated with vehicle or Aβ40 (1μM) and FPS2 or FPS-ZM1 (50 nM) from data as in Figure 2G. (**D**) Nuclear NF-κB p65 levels in SH-SY5Y cells treated vehicle or Aβ40 (1μM) with and without FPS2 or FPS-ZM1 (50 nM) and transduced with Ad.GFP or mutant Ad.IκB-α (S32, 36A). RLU, relative light units. (**E**) Western blot analysis of RAGE in SH-SY5Y cells after transfection with scrambled siRNA or RAGE-siRNA. β-actin was used as a loading control. All values are means ± s.e.m. n=3-5 independent experiments.



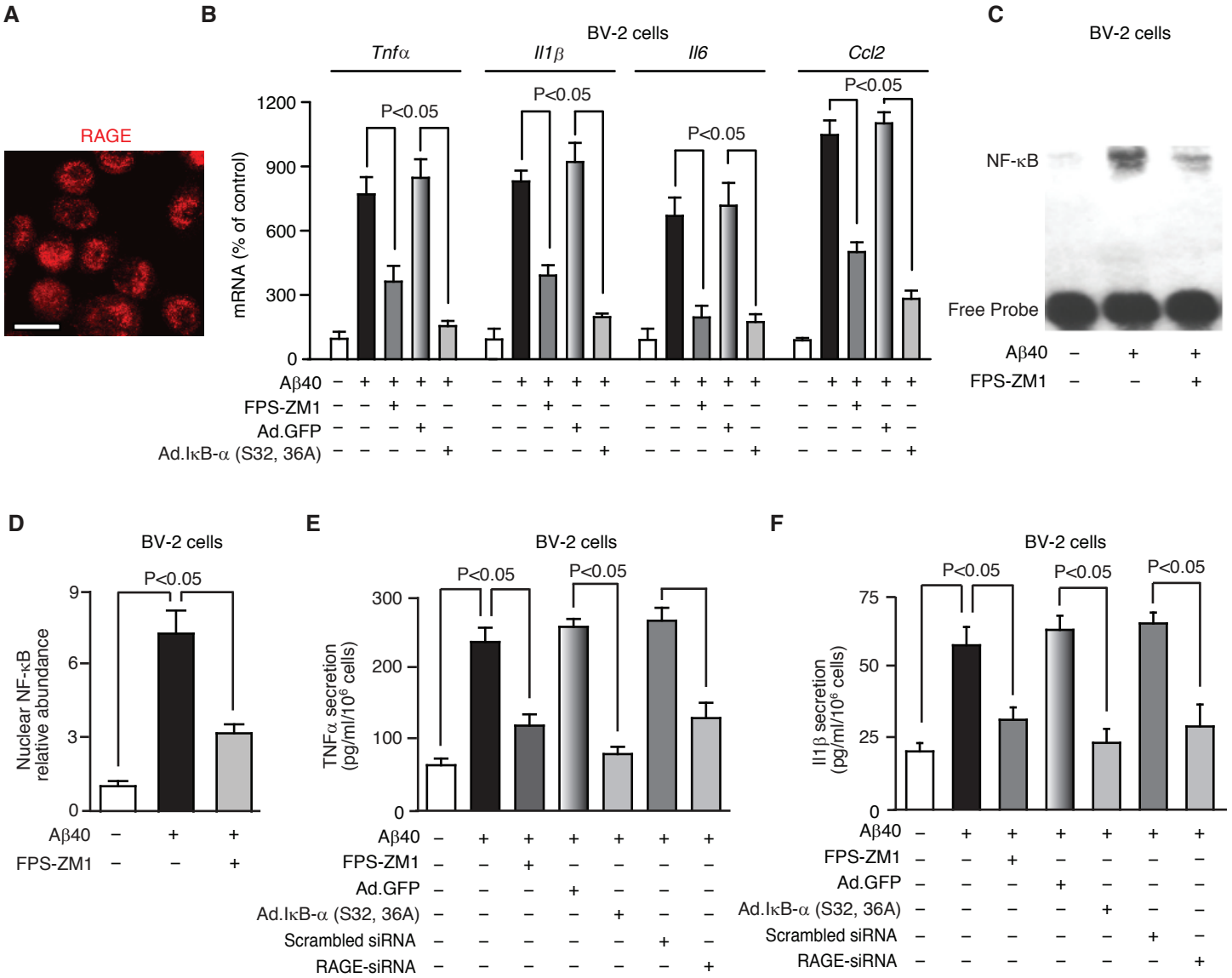
Supplementary Figure 5. FPS and FPS-ZM1 block Aβ42/RAGE binding in cell-based assays. (**A**) ^{125}I-Aβ42 (5 nM) binding to RAGE-transfected CHO cells (RAGE-CHO cells) in the presence of vehicle, FPS (100 nM), FPS-ZM1 (100 nM), RAGE-specific V-domain (anti-Vd) antibodies (20 μg/ml) or non-immune IgG (NI-IgG, 20 μg/ml). (**B**) Aβ42 (1 μM) induced thiobarbituric acid-reactive substances (TBARS) in RAGE-CHO cells in the presence of vehicle or various concentrations of FPS2 or FPS-ZM1 drugs. (**C-D**) *BACE1* mRNA (**C**) and secreted sAPPβ (**D**) levels determined by RT-QPCR and ELISA, respectively, in SH-SY5Y cells treated with vehicle or Aβ42 (1 μM) with or without FPS2 (50 nM) or FPS-ZM1 (50 nM). All values are means ± s.e.m. n=4-5 independent experiments.



Supplementary Figure 6. FPS-ZM1 blocks RAGE-mediated Aβ42 transport across the blood-brain barrier in old *APP$^{sw/0}$* mice. Influx of circulating ^{125}I-Aβ42 (1.5 nM) into brain with and without anti-RAGE antibody (αRAGE, 40 μg/ml), soluble RAGE (sRAGE, 20 μg/ml) or FPS-ZM1 (200 nM) in 15-17 months old *APP$^{sw/0}$* mice. Values are mean ± s.e.m., n=4 mice per group.



Supplementary Figure 7. FPS-ZM1 and FPS2 effects on oxidative stress in brains of 17-month old *APP*$^{sw/0}$ mice. (**A**) Confocal microscopy analysis of dihydroethidine hyderchloride (DHE, red) and neurons (NeuN, green) and merged images in the cortex (bar = 50 μm). (**B**),Relative intensities of DHE fluorescence-positive neurons in the cortex using data as in panels A. *APP*$^{sw/0}$ mice were treated daily with vehicle and/or FPS2 or FPS-ZM1 for 2 months beginning at 15 months of age. Values are mean ± s.e.m., n = 4 mice per group.



Supplementary Figure 8. FPS-ZM1 reduces expression of proinflammatory cytokines in cultured BV-2 microglia. (**A**) RAGE immunostaining (red; bar = 10 μm) in BV-2 cells (**B**) Relative mRNA expression levels of *Tnf-α*, *Il1β*, *Il6* and *Ccl2* in BV-2 cells treated with and without Aβ40 (1 μM) in the absence and presence of FPS-ZM1 (50 nM) and after transduction with Ad.GFP or Ad.IκB-α (S32,36A)**.** (**C-D**) NF-κB activation (**C**) and nuclear NF-κB relative abundance (**D**) in cells treated with vehicle or Aβ40 (1μM) and in the presence and absence of FPS-ZM1 (50 nM)**.** (**E-F**) TNFα (**E**) and IL1β (**F**) protein levels in the conditioned medium of cells treated with vehicle or Aβ40 (1μM) with and without FPS-ZM1 (50 nM), after transduction with Ad.GFP or Ad.IκB-α (S32,36A) or transfection with scrambled siRNA or RAGE-siRNA. Values are means ± s.e.m., n = 4 independent experiments.