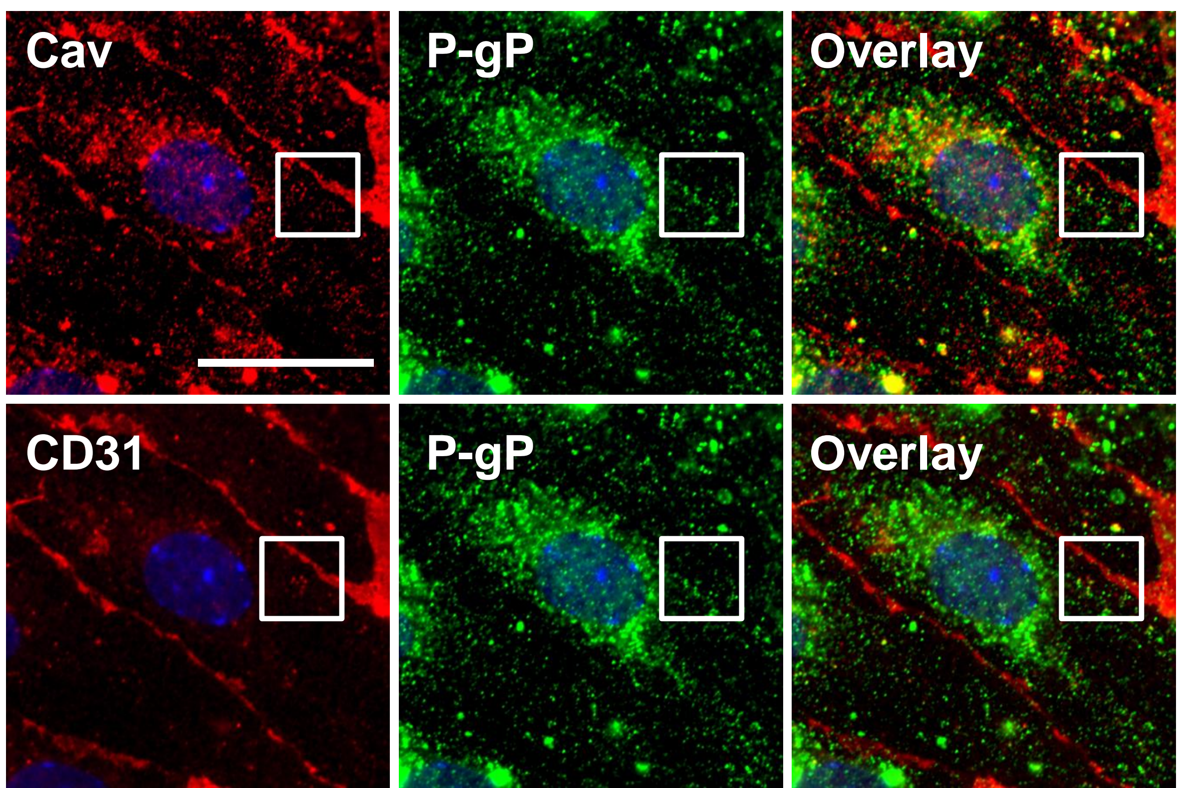
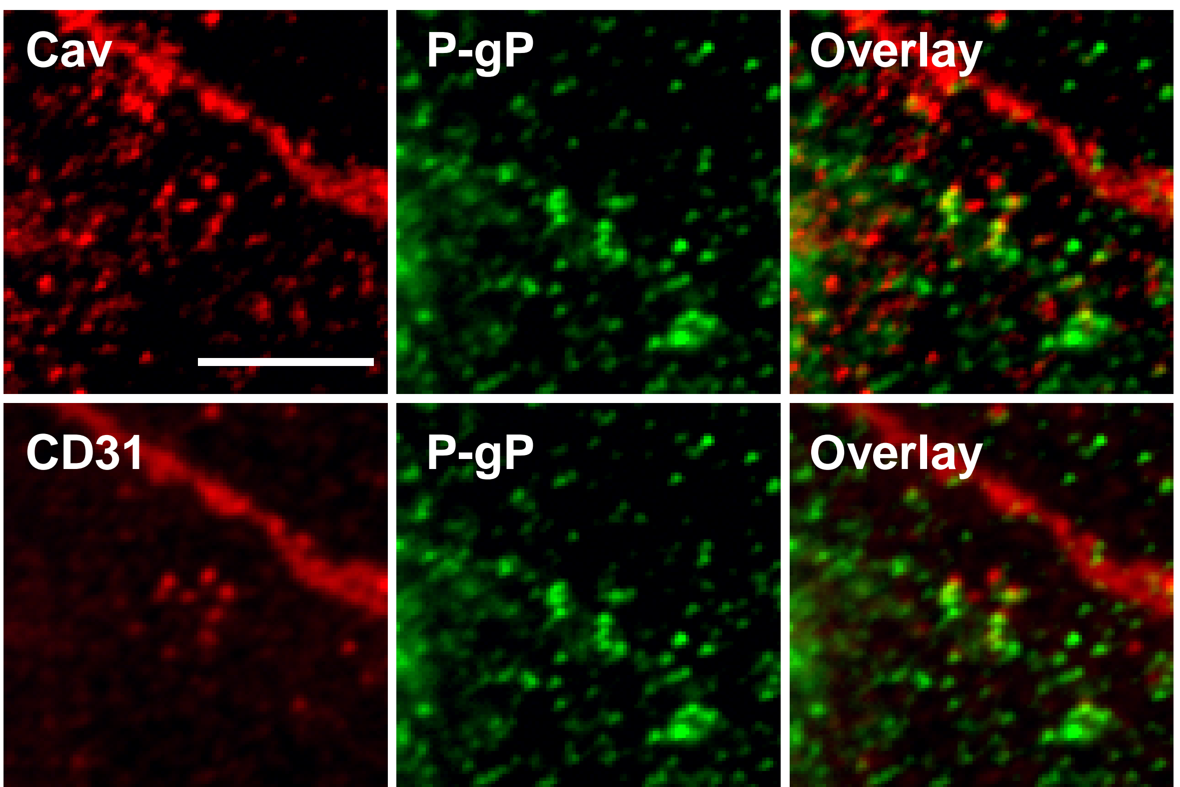
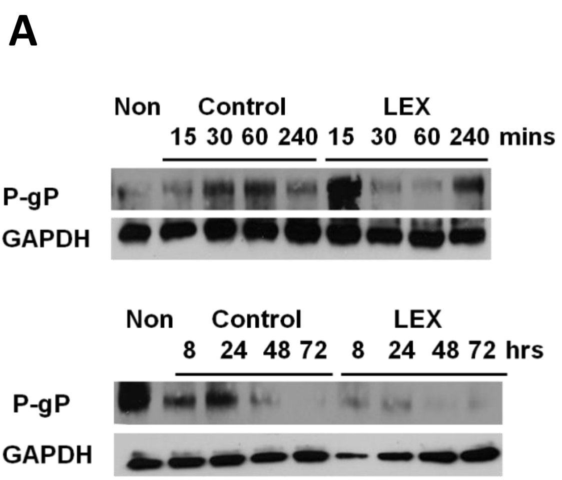
Supplementary Figure 1.

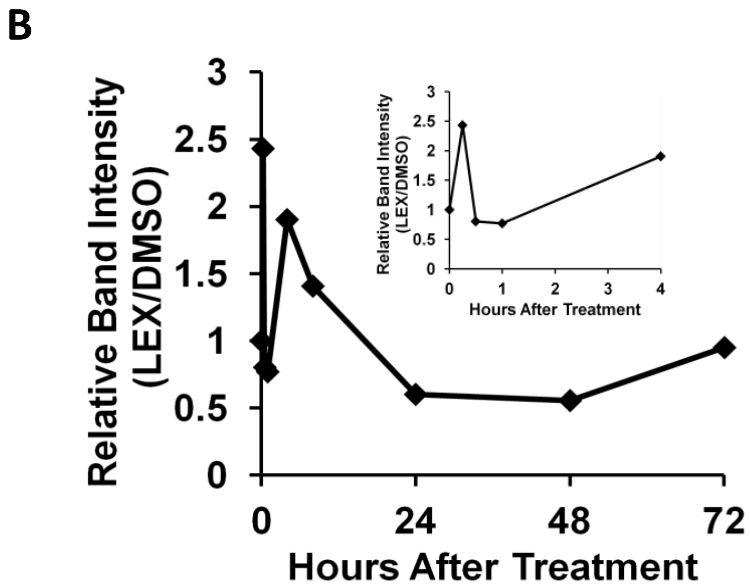


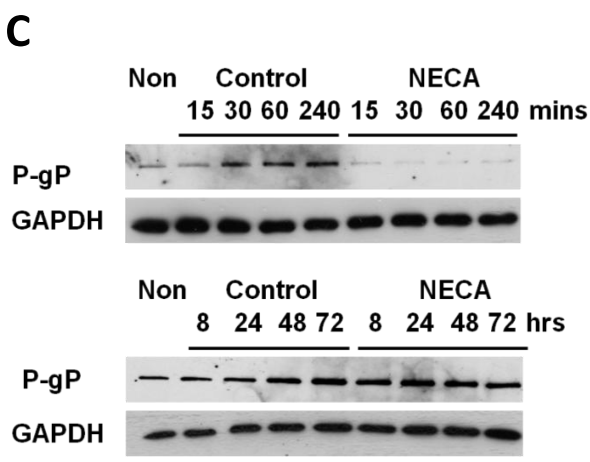


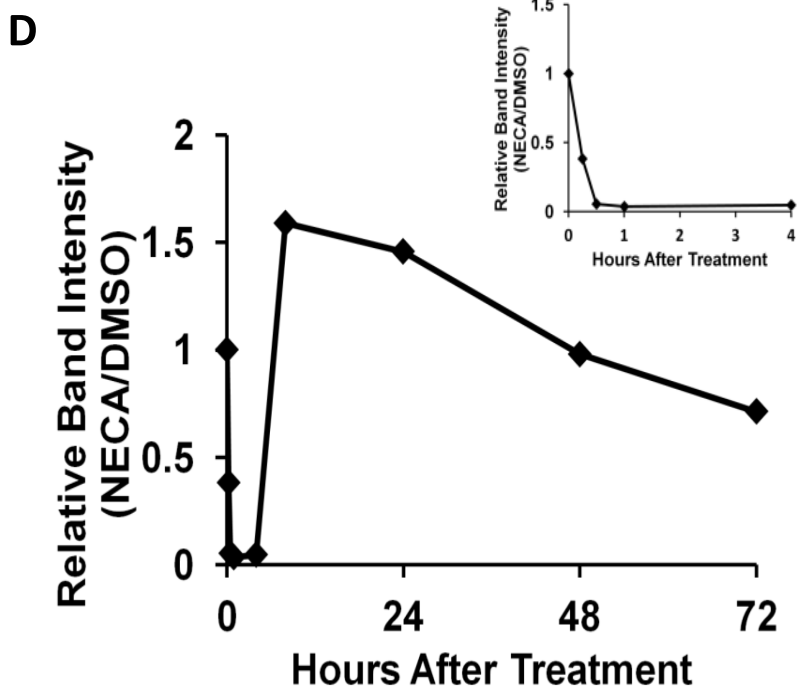
Supplementary Figure 1. P-gP is co-localized with Caveolin-1 in primary mouse brain endothelial cell. (A) Primary mouse brain endothelial cells were isolated and cultured on coverslips until they reached confluency. Cells were fixed with 4 % PFA and permeablized and stained with anti-Caveolin (Red, top panel), anti-P-gP (Green), or anti-CD31 (Red, bottom panel). Scale bar indicates 50 um. (**B**) Magnified images of boxed region from (A). Scale bar indicates 10 um.

Supplementary Figure 2.



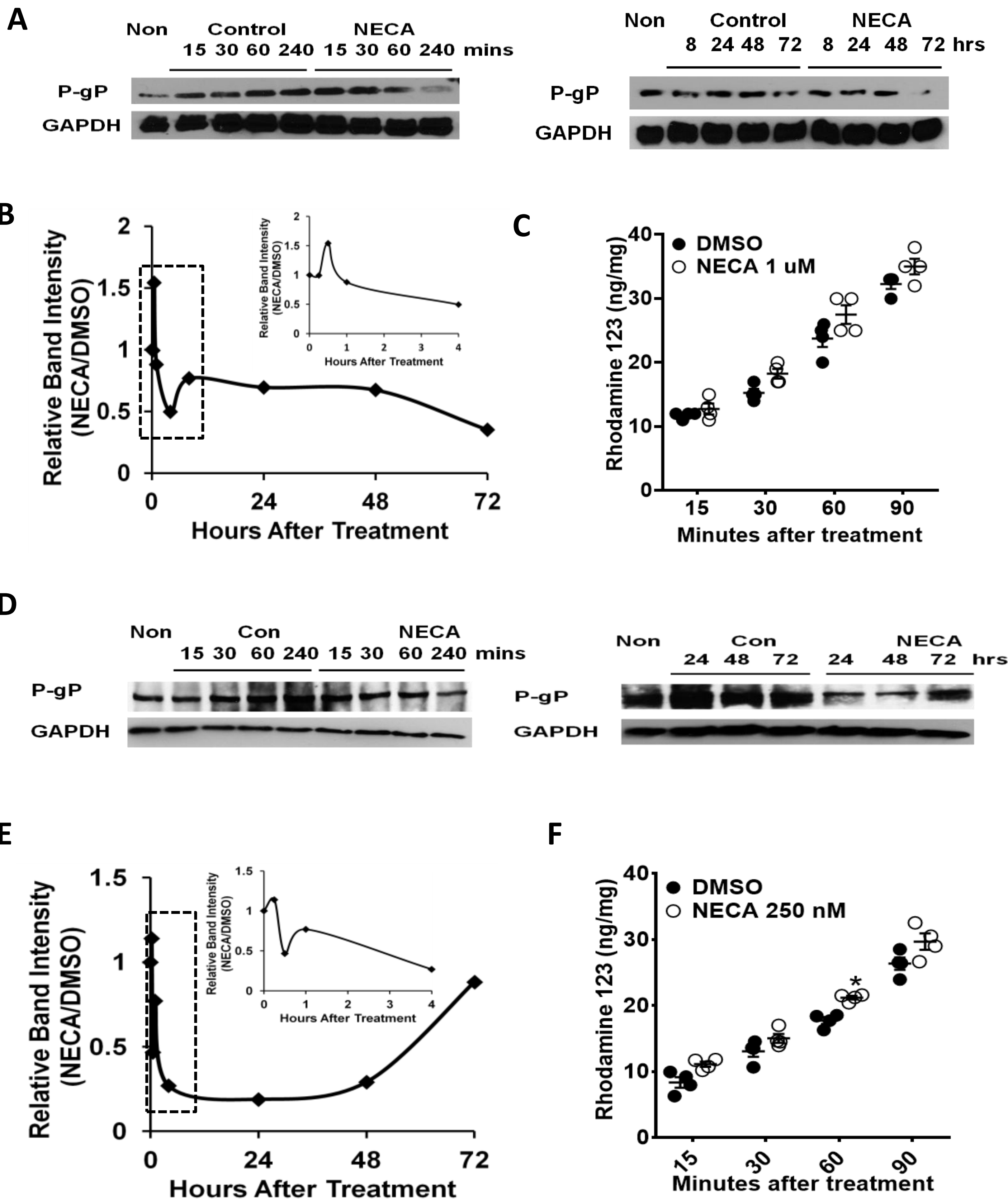






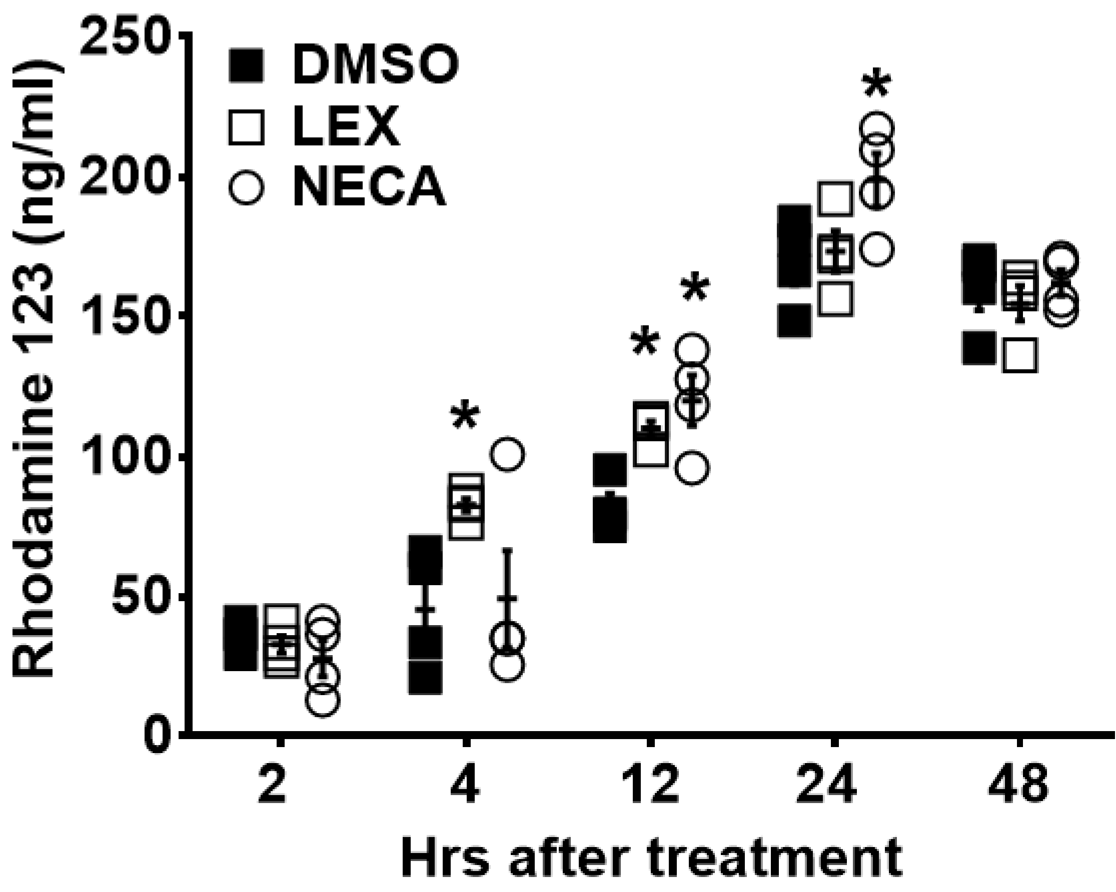
Supplementary Figure 2. A2A AR activation decreases expression of P-gp in primary mouse brain endothelial cell. (**A**) Western blot analysis of P-gp expression in primary brain endothelial cells treated with Lexiscan (1 uM) up to 72 hrs. GAPDH was used as a loading control. (**B**) Intensity of P-gp from western blot (A) was measured and divided by that of GAPDH for each time point and graphed. Short time points (up to 4 hrs) were plotted separately and depicted as inset (smaller graph). (**C**) Western blot analysis of P-gp expression on primary brain endothelial cells were treated with NECA (1 uM) up to 72 hrs. GAPDH was used as loading control. (**D**) Intensity of P-gp from western blot (C) was measured and divided by that of GAPDH from each time point and graphed. Short time points (up to 4 hrs) were plotted separately and depicted as inset (smaller graph).

Supplementary Figure 3



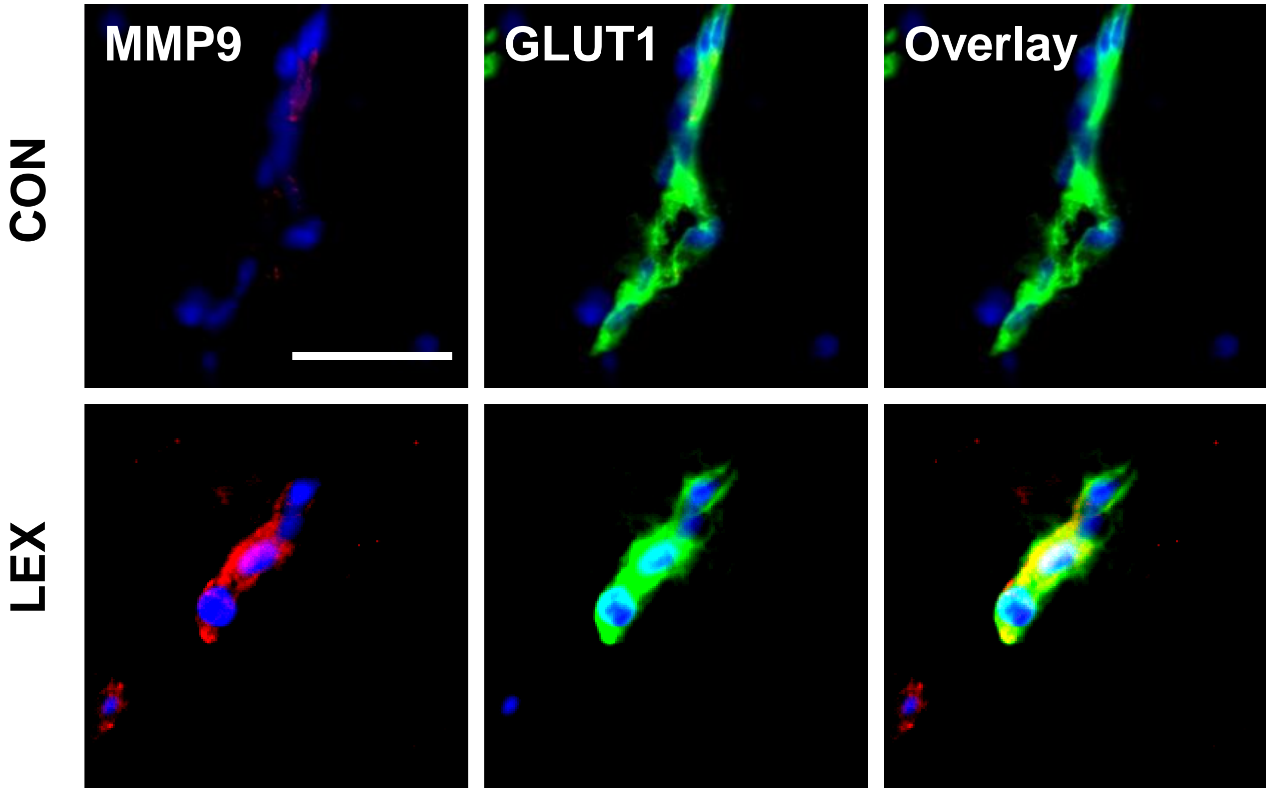
Supplementary Figure 3. Activation of A2A AR down-modulates P-gp expression and function in brain endothelial cells. (**A)** Western blot analysis of P-gp expression in HCMEC-D3 cells treated with NECA (1 uM) for up to 72 hrs. GAPDH was used as loading control. (**B)** For densitometric analysis, the intensity of P-gp with NECA treatment was divided by that of DMSO control and graphed. Densitometric analysis of short-time point (dotted box) is depicted as an inset (smaller graph). (**C**) Rho123 uptake assay of HCMEC-D3 cells was treated with NECA (1 uM) for up to 90 min. The concentration of Rho123 accumulation in brain endothelial cells was analyzed by Fluorimetry with excitation at 488 nm and emission at 523 nm. *, ** Indicates $P<0.05$ and $P<0.01$, respectively (n=4, two tailed student t-test, one representative result from three different experiments). (**D**) Western blot analysis depicting P-gp expression in human primary brain endothelial cells after treatment with NECA (1 uM) for up to 72 hrs. GAPDH was used as loading control. (**E**) For densitometric analysis, intensity of P-gp with Lexiscan or NECA treatment was divided by that of DMSO control and plotted as graph. (**F**) Densitometric analysis of short-time point (dotted box) is depicted as inset. (**G**) Rho123 uptake assay of human primary brain endothelial cells treated with NECA (1 uM). Concentration of Rho123 accumulation in endothelial cells was quantified by fluometry with excitation at 488 nm and emission at 523 nm. *, ** Indicate $P<0.05$ and $P<0.01$, respectively (n=4, two tailed student t-test, one representative result from three different experiments).

Supplementary Figure 4



Supplemantary Figure 4. Broad spectrum AR activation increases transmigration of Rho123 in primary human brain endothelial cells at later time point. *In vitro* blood brain barrier models were generated using primary human brain endothelial cells cultured on a porous membrane to evaluate Rho123 migration cross the BBB. Cells grown on porous membrane were treated with Lexiscan (0.25 uM) or NECA (0.25 uM) concomitantly with 2.5 uM of Rho123 and concentration of Rho123 at the bottom chambers was analyzed at 2, 4, 12, 24, and 48 hrs after treatment by fluometry, with excitation at 488 nm and emission at 523 nm * Indicates values where $P<0.05$ (n=4, two tailed student t-test, one representative result of three different experiments).

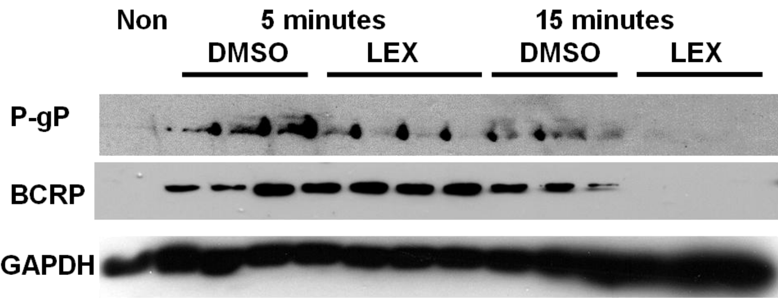
Supplementary Figure 5

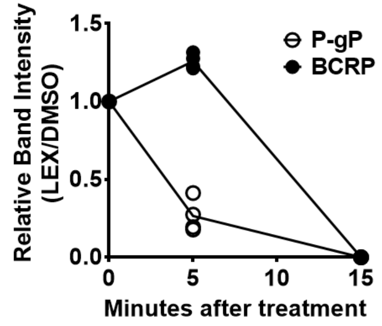


Supplemantary Figure 5. A2A AR activation induces rapid increase of MMP9 in mouse brain endothelial cells. Vehicle control (DMSO) or 0.05 mg/kg of Lexiscan was injected i.v. into mice and 5 minutes later mice were sacrificed and frozen section of the brain were stained with anti-MMP9 (Red) or anti-GLUT-1 (Green) antibodies. Nucleus was counterstained with DAPI (Blue). Scale bar indicates 50 um.

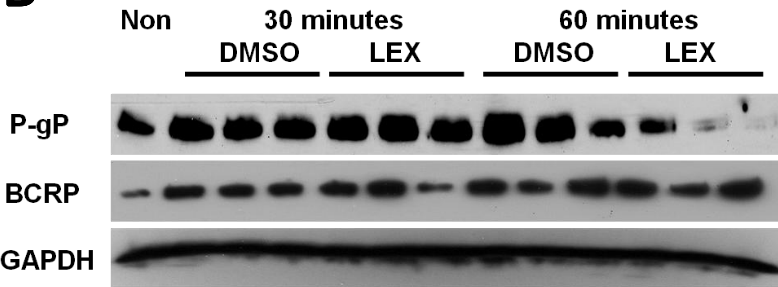
Supplementary Figure 6

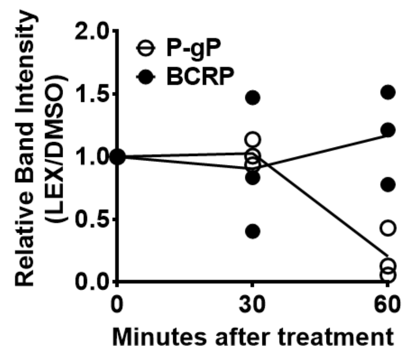
A





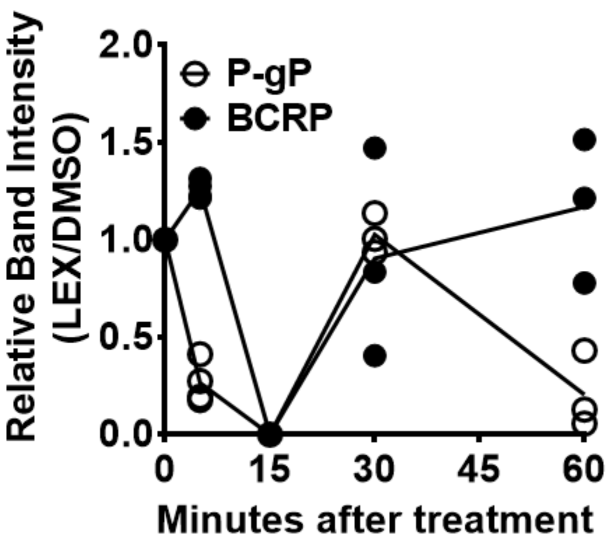
B





C

Summary of Supplementary Figure 6 A and B



Supplementary Figure 6. A2A AR activation by Lexiscan induces rapid and reversible down-modulation of P-gp and BCRP1 expression. (A) Western blot analysis of P-gP and BCRP1 expression in brains of Lexiscan treated WT mice for 5 and 15 minutes. GAPDH was used as loading control. Densitometric analysis of P-gp and BCRP1 expression were normalized using GAPDH and depicted as a graph (right). Intensity of bands from Lexiscan treated mice brains were divided by that of DMSO control brains. Each dot indicates each replicate at different time points. (B) Western blot analysis of P-gP and BCRP1 expression in brains of Lexiscan treated WT mice for 30 and 60 minutes. GAPDH was used as loading control. Densitometric analysis of P-gP and BCRP1 expression level normalized by GAPDH was depicted as a graph (right). Intensity of bands from Lexiscan treated brains were divided by that of DMSO control brains. Each dot indicates each replicate of at different time points. (C) The combined time course of Lexiscan treatment and measurement of P-gp and BCRP1 gene expression is depicted.