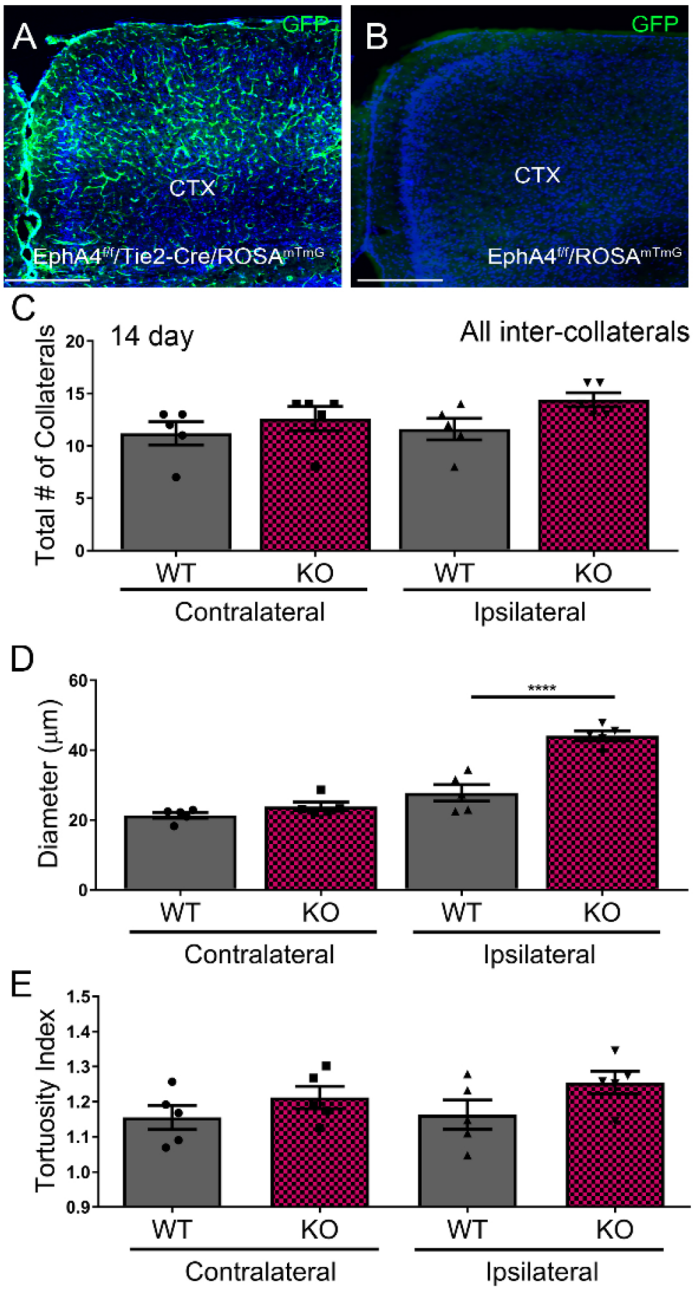
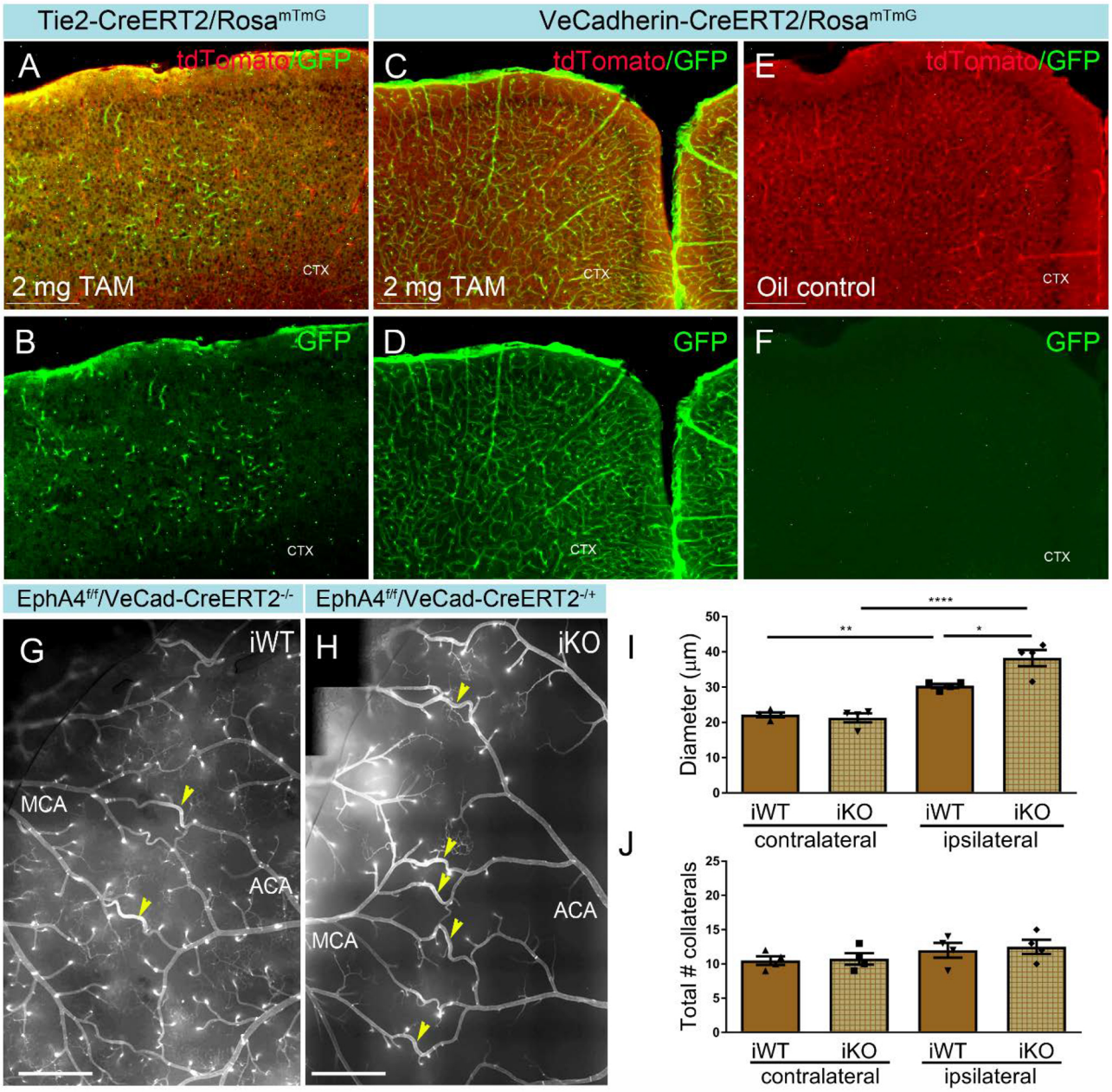
Supp Figure 1



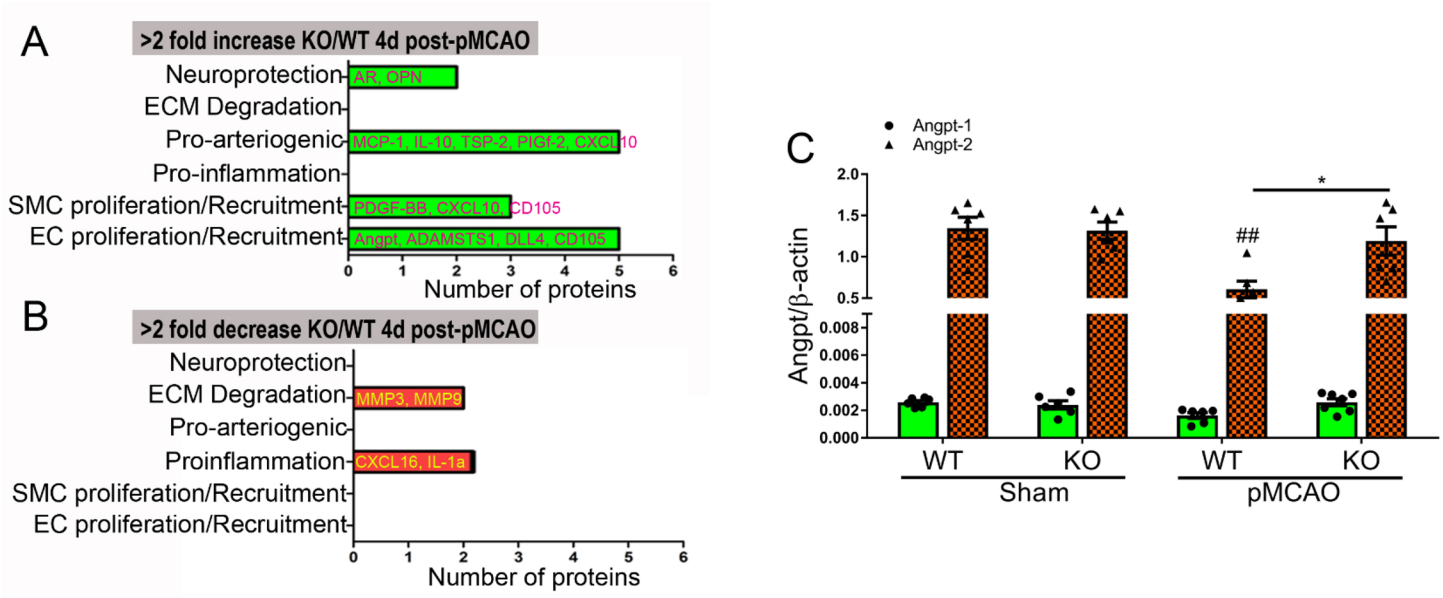
Supplemental Figure 1. Genetic deletion of EphA4 using Tie2-Cre mice. (A) EphA4$^{f/f}$/Tie2-Cre mice were bred to ROSA^{mTmG} mice. GFP expression in the adult EphA4$^{f/f}$/ Tie2-Cre/ROSA^{mTmG} brain is restricted to blood vessels compared to lack of GFP expression in (B) EphA4$^{f/f}$/ ROSA^{mTmG} mice. (C-E) Quantitative analysis of collateral number, size and tortuosity index at 14d post-pMCAO in WT and KO mice, respectively. A-B Scale Bar=500µM. n=5 mice/group.

Supp Figure 2



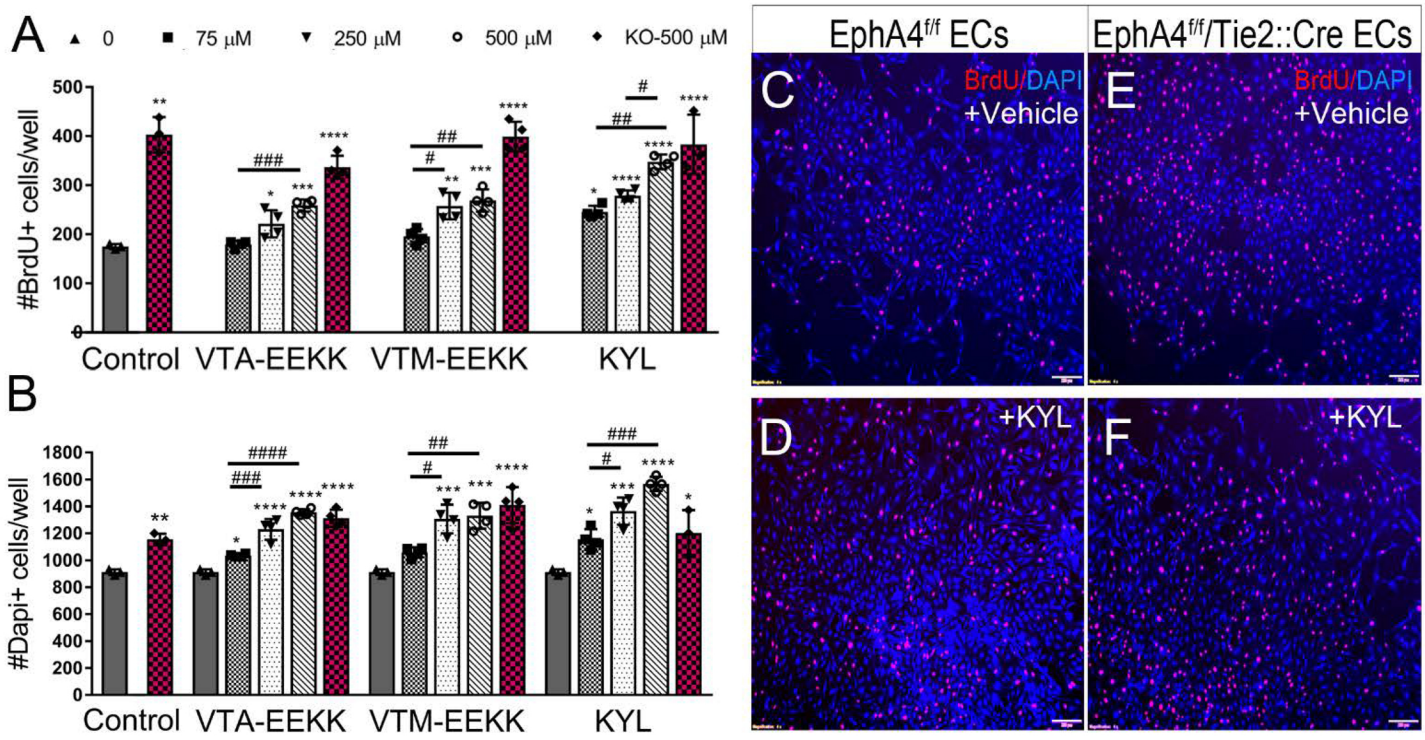
Supplemental Figure 2. Inducible knockout of EphA4 using Vecdherin-CreERT2 mice enhances pial collateral remodeling after pMCAO. Tie2-CreERT2 and Vecadherin-CreERT2 mice were bred to ROSA^{mTmG} mice. GFP expression in the brain of Tie2-CreERT2/ROSA^{mTmG} showed incomplete recombination 2-weeks following 2 mg/kg/day injections of tamoxifen for 5 consecutive days (A, B). Complete recombination was, however, seen in VeCadherin-CreERT2/ROSA^{mTmG} mice (C, D) following tamoxifen treatment compared vehicle oil control (E, F). EphA4$^{f/f}$/VeCadherin-CreERT2$^{+/-}$ mice (iKO) (G-I) subjected to pMCAO 2-weeks post-tamoxifen injections showed a significant increase in pial collateral diameter (µm) at 1d compared to EphA4$^{f/f}$/VeCadherin-CreERT2$^{-/-}$ mice (iWT) (G). No difference was seen in the total number of collaterals (J). All scale bars= 500 µm. n=4-5 mice per group.

Supp Figure 3



Supplemental Figure 3. Angiogenic profile analysis and quantified Angpt-1 and -2 protein expression in WT and KO mice. Cortical protein lysate analysis of angiogenic expression using R&D angiogenic proteome profiler at 4d post-pMCAO. (A) Increased expression >2-fold and (B) decreased expression <2-fold in KO-injured compared to WT-injured mice. Quantified expression of Angpt-1 (green) and -2 (orange) relative to β-actin, following 1d post-pMCAO using western blot analysis from WT and KO ipsilateral cortical protein lysate. $^{*}P<0.05$ WT pMCAO compared to KO pMCAO. $^{\#\#}P<0.01$ compared to WT sham. n=6-7 mice per group.

Supp Figure 4



Supplemental Figure 4. EphA4 peptide inhibitors KYL and VTM-EEKK enhance brain-derived EC proliferation. (A) Quantified total number of BrdU-positive WT and EphA4-null ECs at 24 hrs following a dose dependent treatment of VTA-EEKK, VTM-EEKK and KYL. A significant increase in BrdU-positive cells were observed in untreated KO ECs and in all treatment groups at 250µM and 500µM compared to untreated WT ECs (*). (B) The number of DAPI-positive cells was also increased in untreated KO ECs and following 250µM and 500µM peptide treatment compared to untreated WT ECs. (C-F) Representative immunofluorescence images of BrdU/DAPI stained WT and KO ECs treated with 500µm KYL. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 compared to vehicle control treated WT ECs. #P<0.05, ##P<0.01, ###P<0.001, ####P<0.0001 compared to corresponding treatment. Scale in C-F =200µm; 4x magnification. One-way ANOVA with Bonferroni post-hoc was performed per treatment. Each experiment was performed in triplicate and repeated 4 times.