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Supplementary Materials for

Ponatinib (AP24534) inhibits MEKK3-KLF signaling and prevents formation and progression of cerebral cavernous malformations

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Published 7 November 2018, *Sci. Adv.* **4**, eaau0731 (2018)
DOI: 10.1126/sciadv.aau0731

This PDF file includes:

Supplementary Materials

Supplementary Figure S1

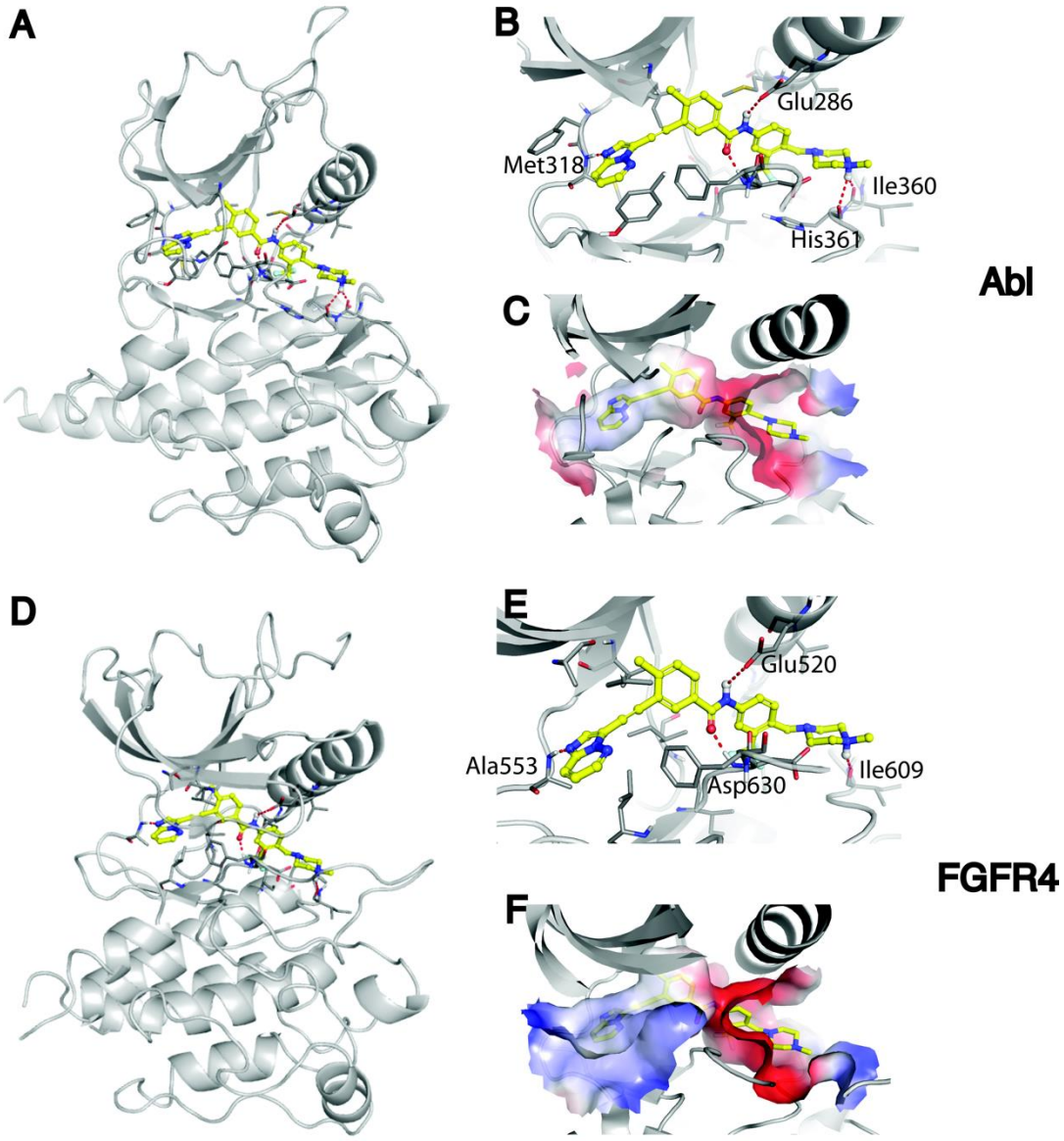

Fig. S1. Three-dimensional structural comparison of the binding between ponatinib and Abl, FGFR4, and MEKK3. (**A-C**) Ribbon and surface plot of Ponatinib binding to Abl. (**D-F**) Ribbon and surface plot of Ponatinib binding to FGFR4.

Supplementary Figure S2

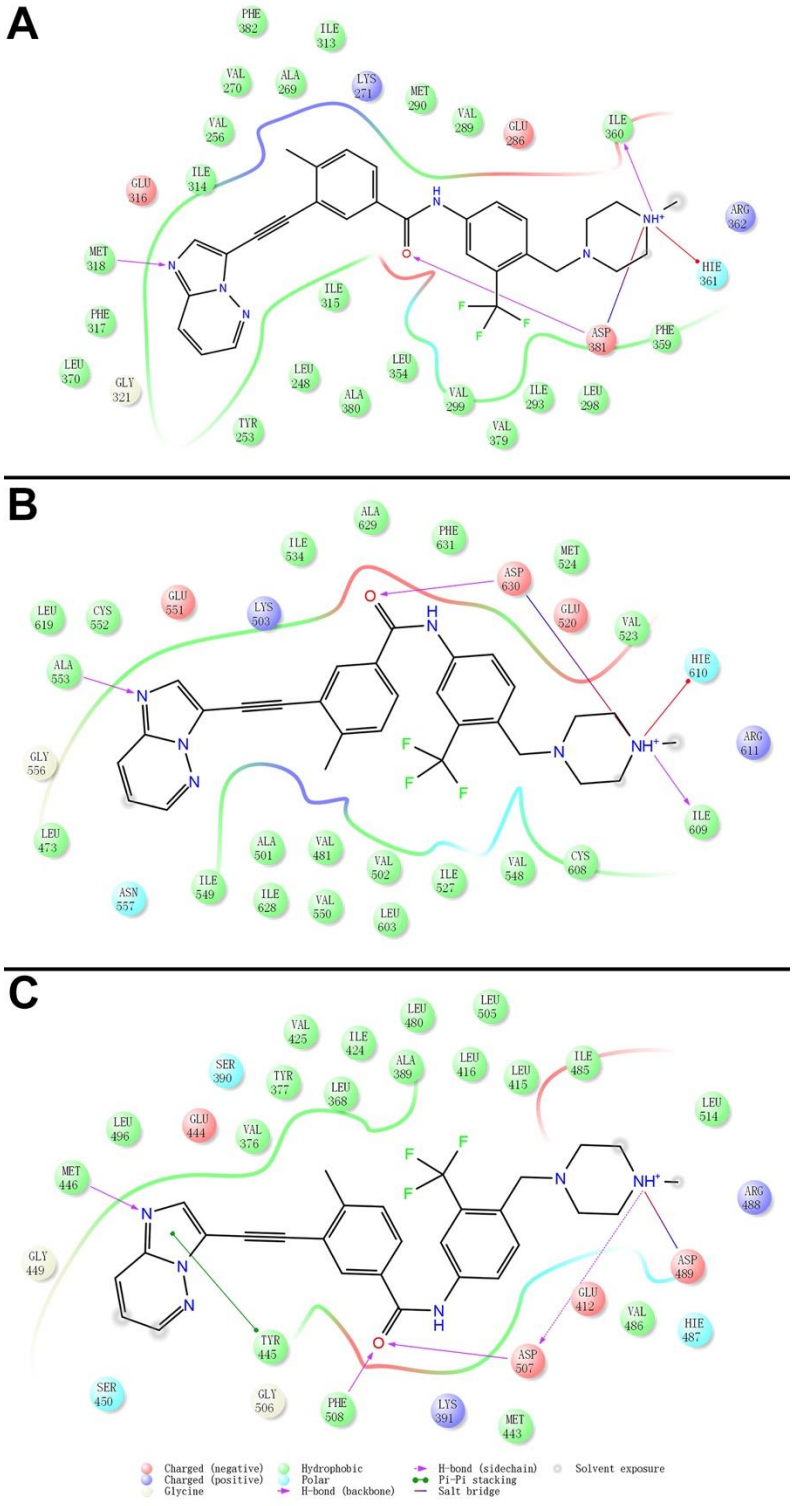

Fig. S2. Two-dimensional mapping of ponatinib interaction with Abl, FGFR4, and MEKK3. (**A**) Abl, (**B**) FGFR4, and (**C**) MEKK3.

Supplementary Figure S3

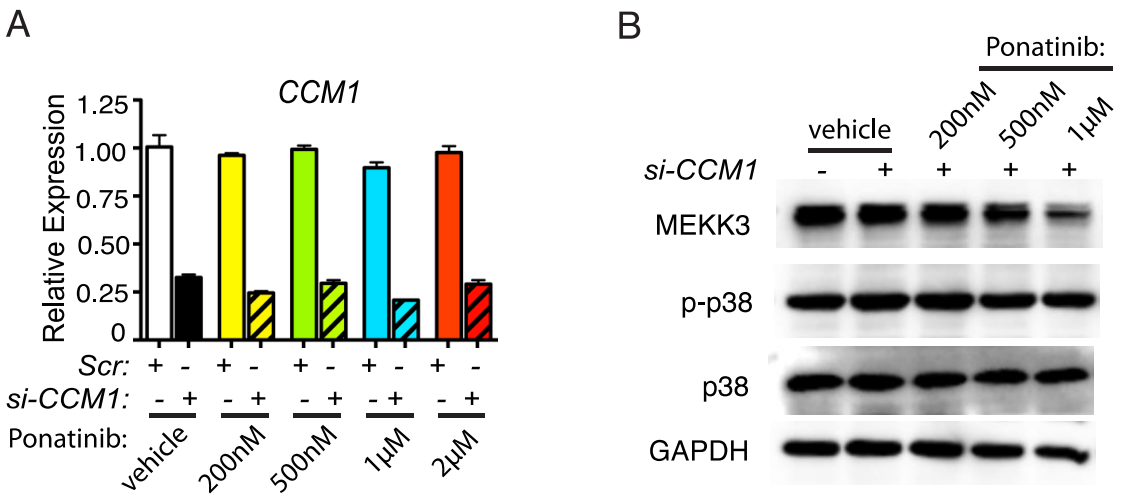

Fig. S3. CCM1, p38, and MEKK3 expression in cultured HUVECs. (**A**) qPCR analysis demonstrating efficient knockdown of *CCM1* with siRNA compared to Scr controls. Ponatinib did not alter *CCM1* expression. Error bars shown as SEM (*n*=4). (**B**) Western blots show the expression level of MEKK3, p38 and p-p38 in si-*CCM1* HUVECs treated with vehicle or varying doses of Ponatinib. Blots are representatives of three repeats.

Supplementary Figure S4

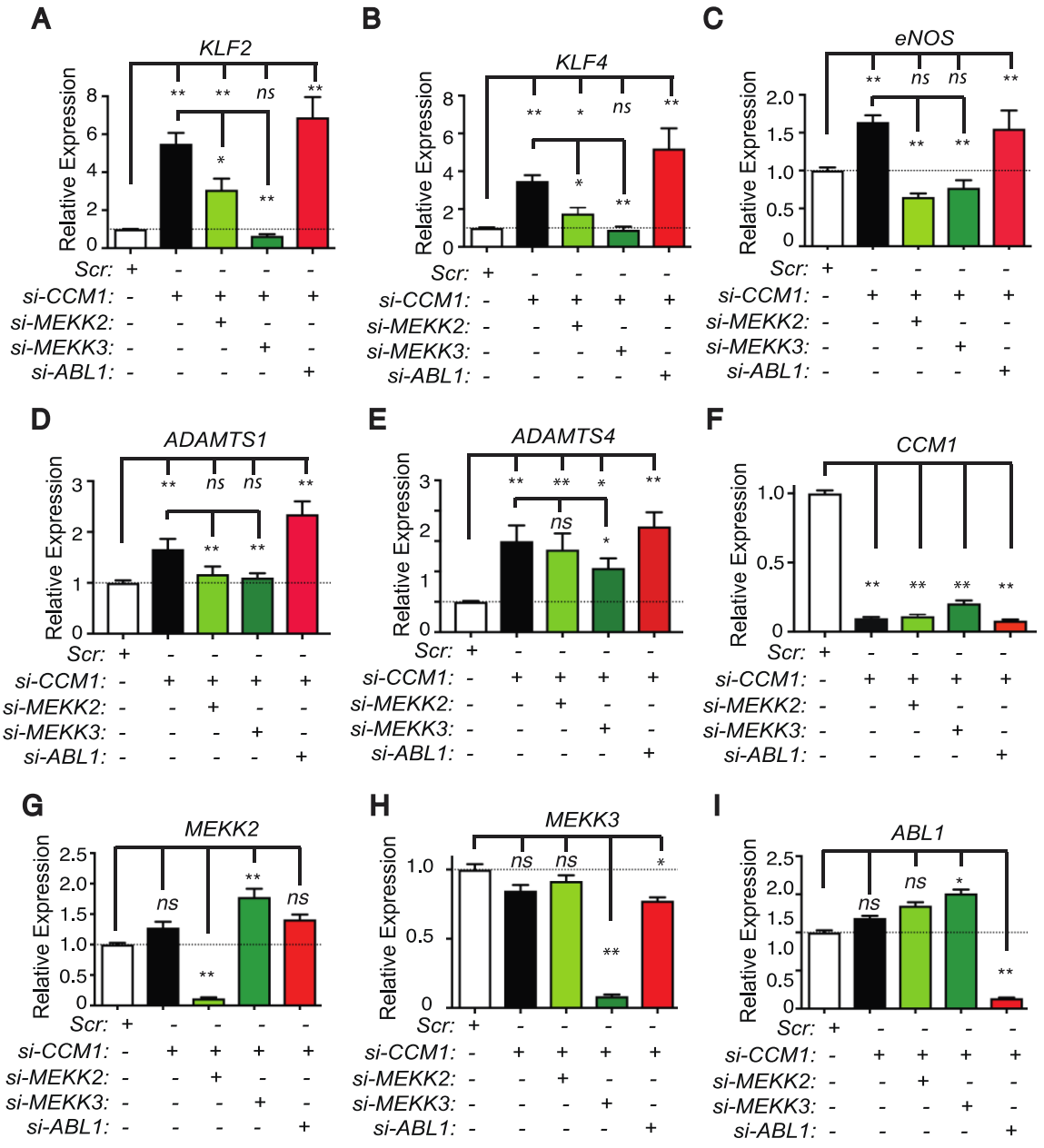

Fig. S4. MEKK3 is the dominant regulator of KLF signaling. (**A-I**) Gene expression analyses show *MEKK3* knockdown completely normalized *KLF* and its target gene expressions. *MEKK2* knockdown can also partially normalize gene expression changes. In contrast, *ABL* knockdown rather increases these gene expressions. Error bars shown as SEM and significance determined

by one-way ANOVA for multiple comparisons (n=4). * $p<0.05$; ** $p<0.001$; NS indicates $p>0.05$.

Supplementary Figure S5

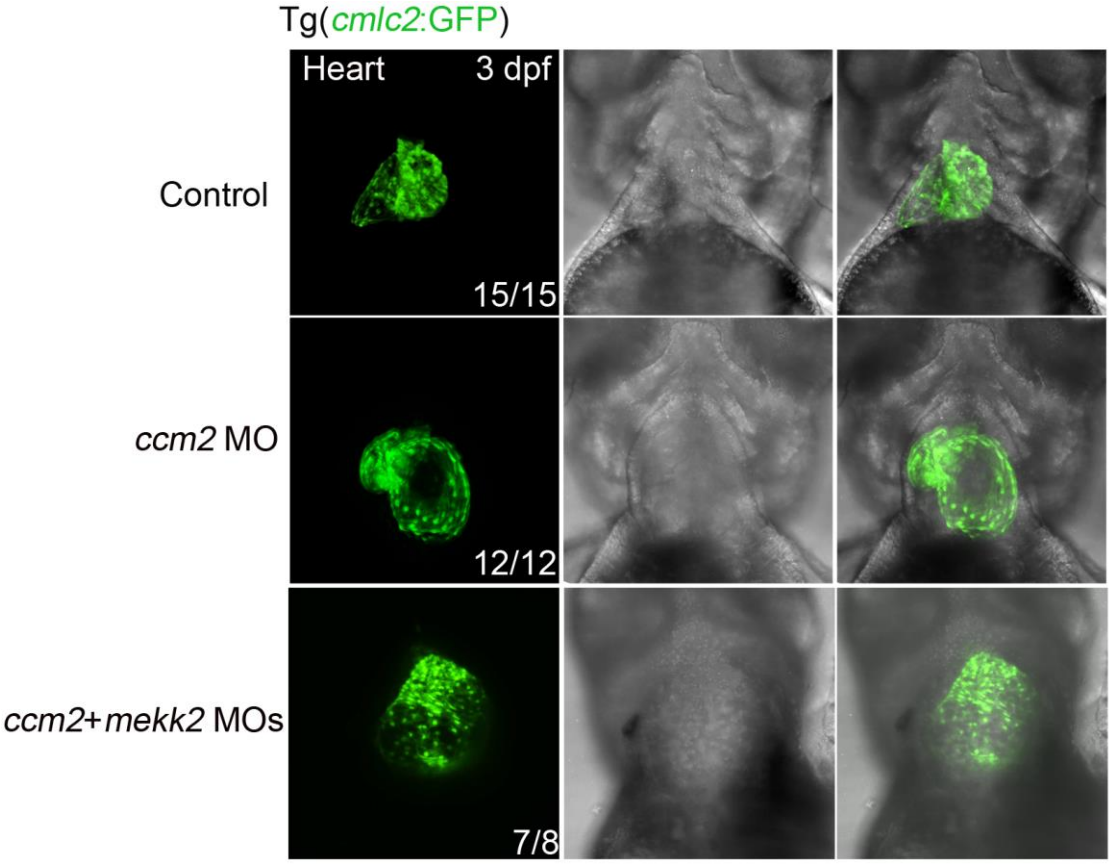

Fig. S5. Confocal images of Tg(*cmlc2:*EGFP) embryos injected with control, *ccm2*, or *ccm2* + *mekk2* morpholinos. *ccm2* morphants exhibited dilated heart phenotype that was not rescued with *mekk2* morpholino injection.

Supplementary Figure S6

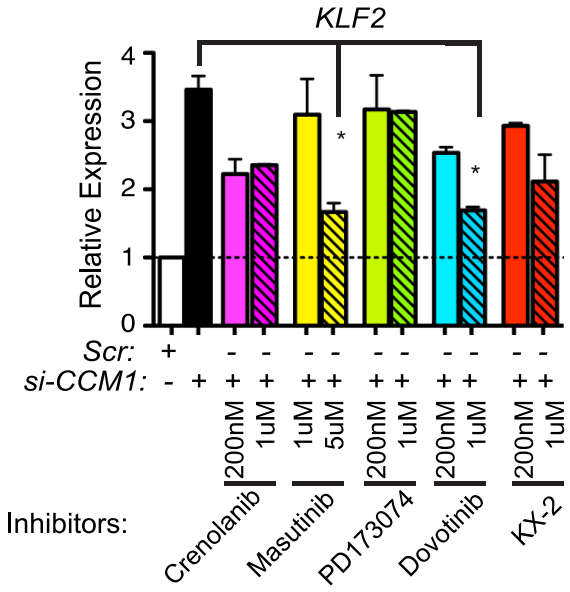

Fig. S6. Effects of ponatinib and other kinase inhibitors on KLF2 expression. *si-CCM1* knockdown increases KLF2 expression in HUVECs, Masutinib and Dovotinib, but not PD173074, can partially reverse the increased KLF2 expression in a dosage dependent manner. Results are representative of three independent experiments.

Supplementary Figure S7

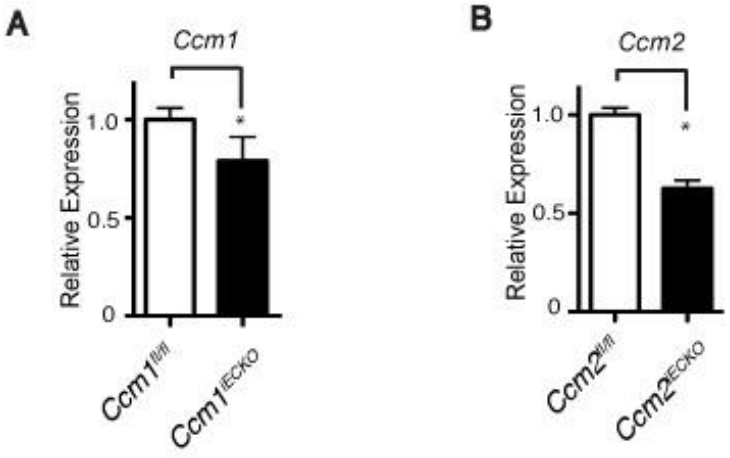

Fig. S7. qPCR quantification of CCM gene expression in brain endothelial cells isolated from 4-HT–induced p6 pups. (**A**) CCM1 (n =5) and (**B**) CCM2 (n= 3 gene expression analysed by qPCR. Error bars shown as SEM and significance determined by t-test. *p<0.05.

Supplementary Figure 8

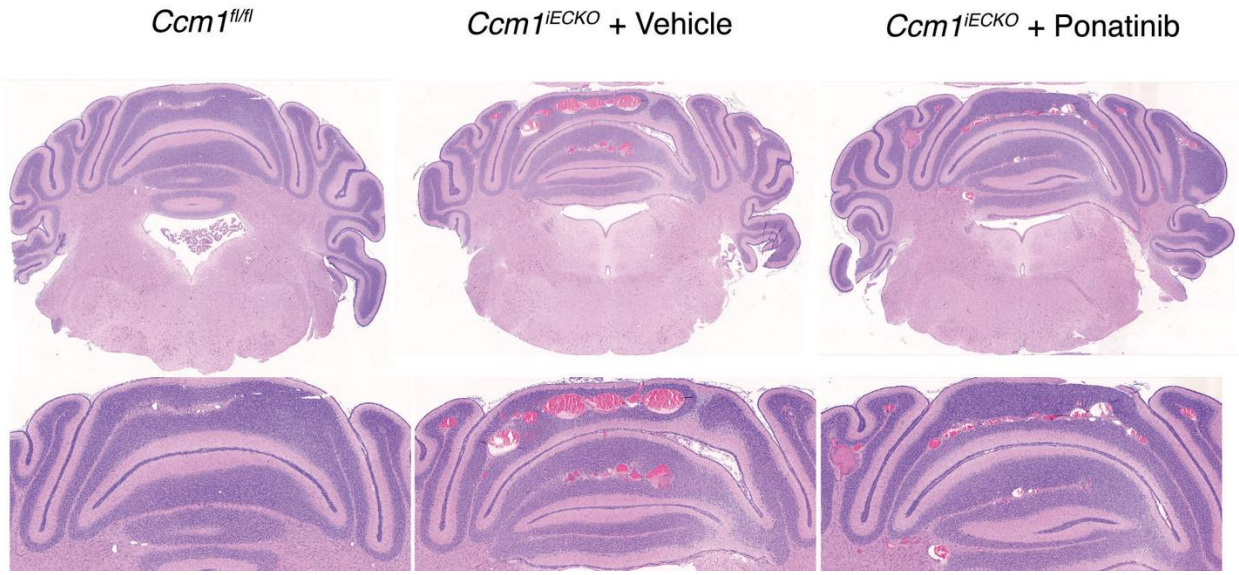

Fig. S8. Hematoxylin and eosin staining of CCM lesions at P13. Lesion formation in the *Ccm1*^{iECKO} littermates given vehicle or Ponatinib treatment. *Ccm1*$^{fl/fl}$ mice were also littermates injected with 4-HT.

Supplementary Figure 9

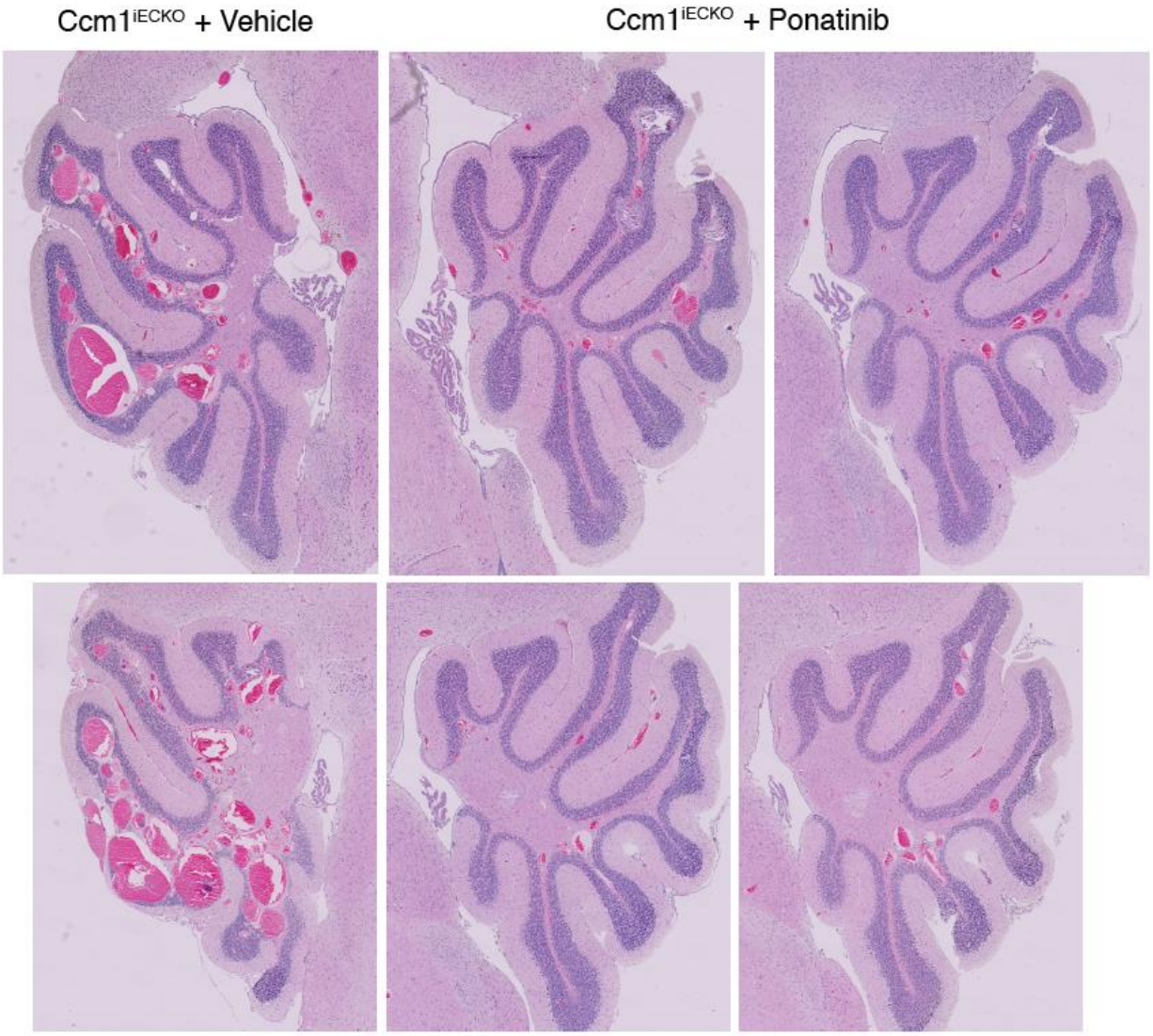

Fig. S9. Hematoxylin and eosin staining of CCM lesions at P30. Histology of midbrains sections from *Ccm1*^{iECKO} littermates given vehicle or Ponatinib treatment.