B-Cell Depletion Reduces the Development of Cerebral Cavernous

Malformations in Murine Models

Changbin Shi^{1,4}, Robert Shenkar¹, Hussein A. Zeineddine¹, Romuald Girard¹,

Maged D. Fam¹, Cecilia Austin¹, Thomas Moore¹, Rhonda Lightle¹, Lingjiao Zhang¹,

Meijing Wu¹, Ying Cao¹, Murat Gunel², Angeliki Louvi², Autumn Rorrer³, Carol Gallione³,

Douglas A. Marchuk³, Issam A. Awad¹

¹ Neurovascular Surgery Program, Section of Neurosurgery, Biological Sciences Division, University of Chicago, USA

² Department of Neurosurgery, Yale University School of Medicine, USA

³ Molecular Genetics and Microbiology Department, Duke University Medical Center, USA

⁴ Present affiliation: Department of Neurosurgery, The First Affiliated Hospital of Harbin Medical University, Harbin, Heilongjiang Province, China.

Changbin Shi and Robert Shenkar contributed equally to this work.

Douglas A. Marchuk and Issam A. Awad contributed equally to this work.

Corresponding author: Issam A. Awad, MD, Section of Neurosurgery, The University of Chicago Medicine, 5841 S. Maryland Ave. MC3026/Room J341, Chicago, IL 60637, USA. Tel.: +1 773 702 4452; fax: +1 773 702 3518. *E-mail address:* iawad@uchicago.edu (I.A. Awad).

SUPPLEMENTARY FIGURES AND LEGENDS

Supplementary Figure 1

а



B LYMPHOCYTES





Untreated control

Anti-BR3 treatment

Supplementary Fig. 1 Depletion of B lymphocytes in a CCM murine model. **a**) The numbers of CD45R/B220 positive B cells were reduced in both peripheral blood and spleen from 5 anti-BR3 antibody treated $Pdcd10^{+/-}Trp53^{-/-}$ mice (M1-M5) compared to 4 similar mice that were not treated (control), while the amounts CD3 positive T cells were not affected. **b**) Immunohistochemistry showed similar results in the spleen. Scale bars are 50 µm

Supplementary Figure 2

ANGIOSARCOMA





Hematoxylin and eosin

CD31

LYMPHOMA



Hematoxylin and eosin

CD3

Supplementary Fig. 2 Tumors from a sensitized murine model after B cell depletion. Formalin fixed paraffin embedded tumors from 2 $Pdcd10^{+/-}Trp53^{-/-}$ mice treated with anti-BR3 were stained with hematoxylin and eosin (left panels), anti-CD31 antibody (upper right) or anti-CD3 antibody (lower right) to identify them as an angiosarcoma (top panels) or a T-cell lymphoma (lower panels). Scale bars are 200 µm