

SUPPLEMENTARY MATERIAL 1

Regulator of G-protein signaling 5 (RGS5) protein: a novel marker of cancer vasculature elicited and sustained by the tumor's proangiogenic microenvironment.

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Figure 1S

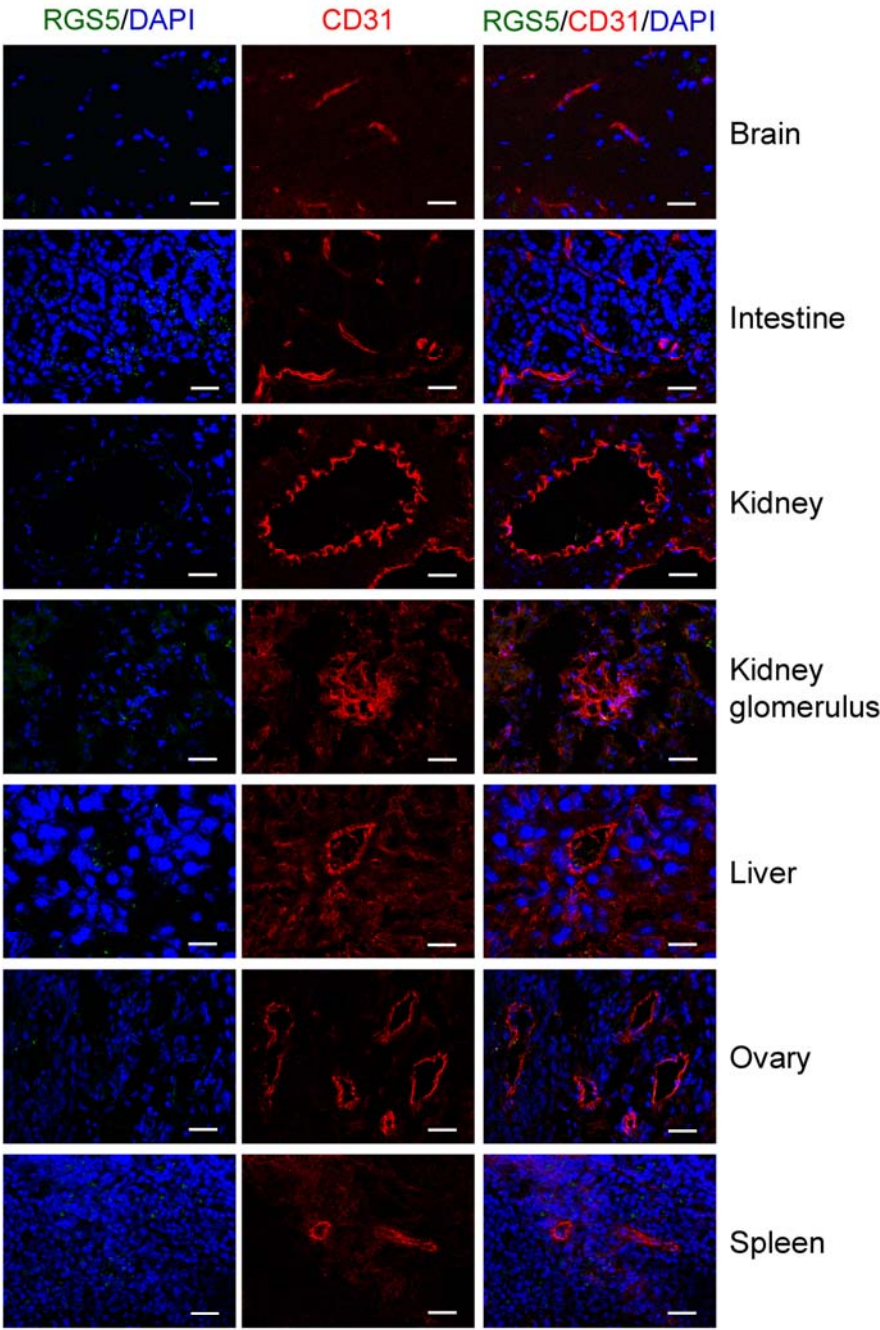


Figure 1S. RGS5 expression is not detectable in the endothelium lining the blood vessels of normal mouse organs. Representative confocal microscopy images (scale bars=20µm) illustrating tissue sections from normal mouse organs stained with RGS5 (green), CD31/PECAM (red) and counterstained with DAPI (blue). The staining clearly shows CD31/PECAM expression (marker of endothelial cells) with a pattern reminiscent of the different organs' vasculature. Conversely, no clearly visible signal for RGS5 protein is detectable in any of the mouse organs investigated (brain, intestine, kidney, liver, ovary, spleen).

SUPPLEMENTARY METHODS

Organs from healthy NCr-*nu/nu* mice (Harlan) were harvested and immediately snap frozen in OCT. Frozen tissues were briefly examined by Hematoxylin (not shown) prior to assess the vasculature and the expression of RGS5 protein by immunofluorescence. Briefly, eight micron cryostat sections were thawed, fixed (acetone), blocked (3-5% rabbit serum) and stained with polyclonal goat-anti- RGS5 (1:250, Santa Cruz Biotechnology) and rat-anti-mouse CD31 cloneMEC13.3 (1:50, BD Pharmingen) for 1-2 hours at Room Temperature. Rinsed sections were incubated with Alexa Fluor 488 rabbit-anti-goat (1:800, Molecular Probes) and TRITC rabbit-anti-rat (1:200, Sigma).

Stained sections were briefly analyzed with an Olympus BX61 epifluorescence microscope, selected specimens were then explored more in depth with the Zeiss LSM 510 Meta confocal microscope. The images were acquired with a 40X objective lens magnification.