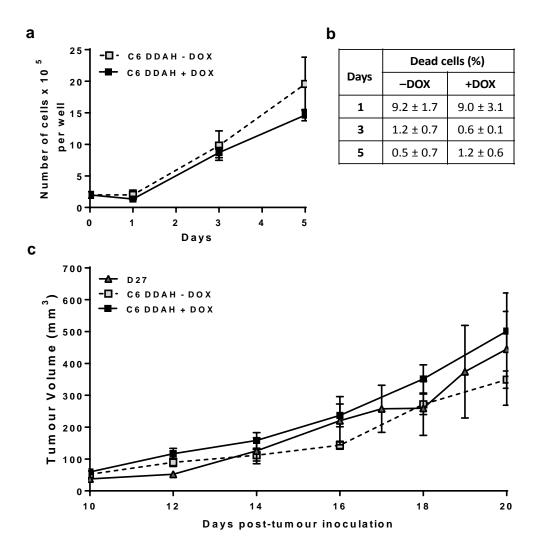**Assessment of DDAH I NO-independent effects on the vascular phenotype of C6 gliomas *in vivo***
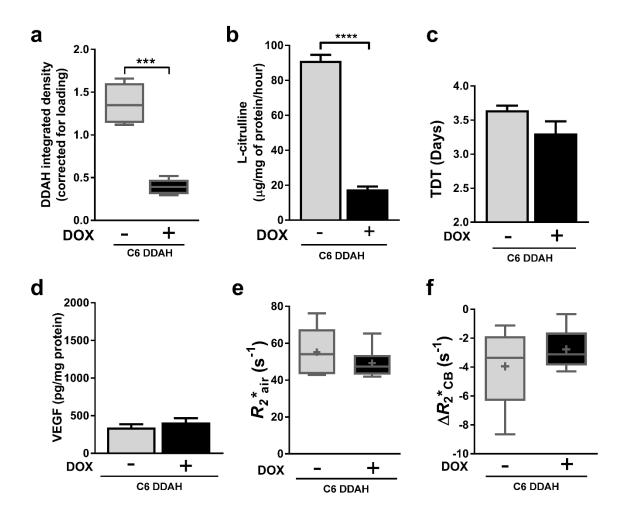
Angiogenesis

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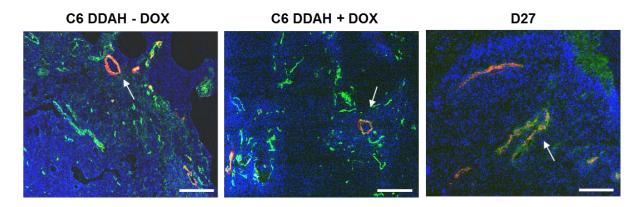
Electronic Supplementary Material

Supplementary Fig. 1 DDAH I effect on cell proliferation and tumour growth in C6 DDAH cells and xenografts (group A). (a,b) Cells seeded at a density of 2×10^5 cells / well, were treated with $2 \, \mu$g/ml doxycycline. At days 1, 3 & 5 cells were trypsinised, collected and mixed with eosin, which was taken up by dead cells, and counted. (a) Cell proliferation and (b) percentage of dead cells. Results are the mean ± 1 SEM of three separate experiments performed in duplicate. (c) Mean tumour growth curves for C6 DDAH $\pm$ DOX ($n = 6$ per group) and D27 ($n = 4$) tumours up to day 20 post-tumour inoculation, when all mice were still alive.

Supplementary Fig. 2 *In vivo* **characterisation of C6 DDAH tumour xenografts (group B).** C6 DDAH cells were grown in normal medium without doxycycline (group B). Mice with C6 DDAH tumours were given 0.2 mg/ml doxycycline in 5% (w/v) sucrose or 5% (w/v) sucrose alone ($n = 6$ per group) in the drinking water *ad libitum*. (a) Mean box plot distributions of the integrated densities of the individual protein bands for DDAH I corrected using α-tubulin determined by western blot. (b) L-citrulline production. (c) Tumour doubling times in days. (d) VEGF expression in tumour homogenates. (e,f) Box plot distributions of the median R_2*_{air} (e) and ΔR_2*_{CB} (f) values measured by MRI over the whole tumour volume of C6 DDAH ± DOX (group B) ($n = 6$) xenografts. Results are the mean ± 1 SEM of measurements made from all tumours in each group (***P <0.001, ****P < 0.0001, Unpaired student's t-test).

Supplementary Fig. 3 Representative images of areas of tumour sections derived from C6 DDAH ± DOX (group A) and D27 xenografts stained for CD31 and α-SMA. Composite images from C6 DDAH ± DOX and D27 tumour sections stained with the endothelial cell marker CD31 (green fluorescence), the perivascular cell marker α-SMA (red fluorescence) and the nuclear counterstain DAPI (blue fluorescence). White arrows indicate large vessels were the perivascular cells surround the endothelial cell layer. Scale bar is 100 μm.