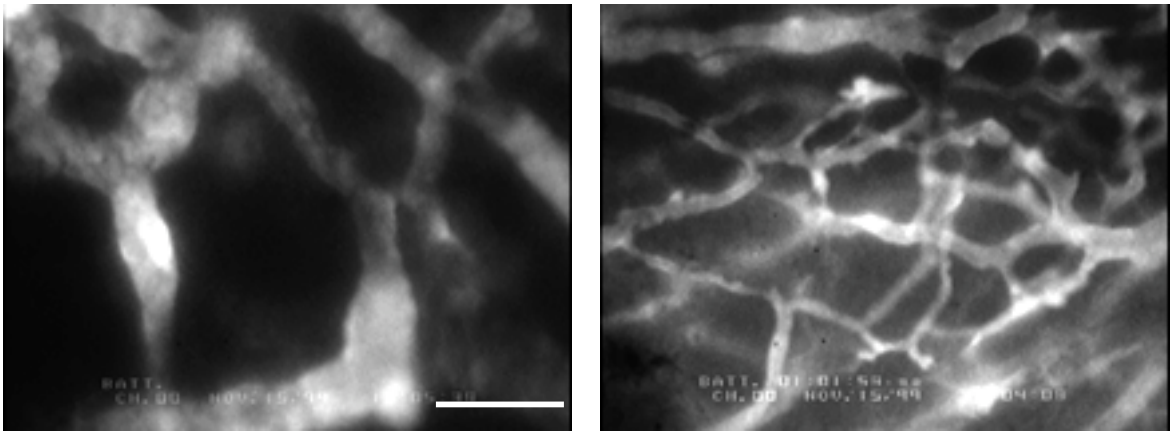
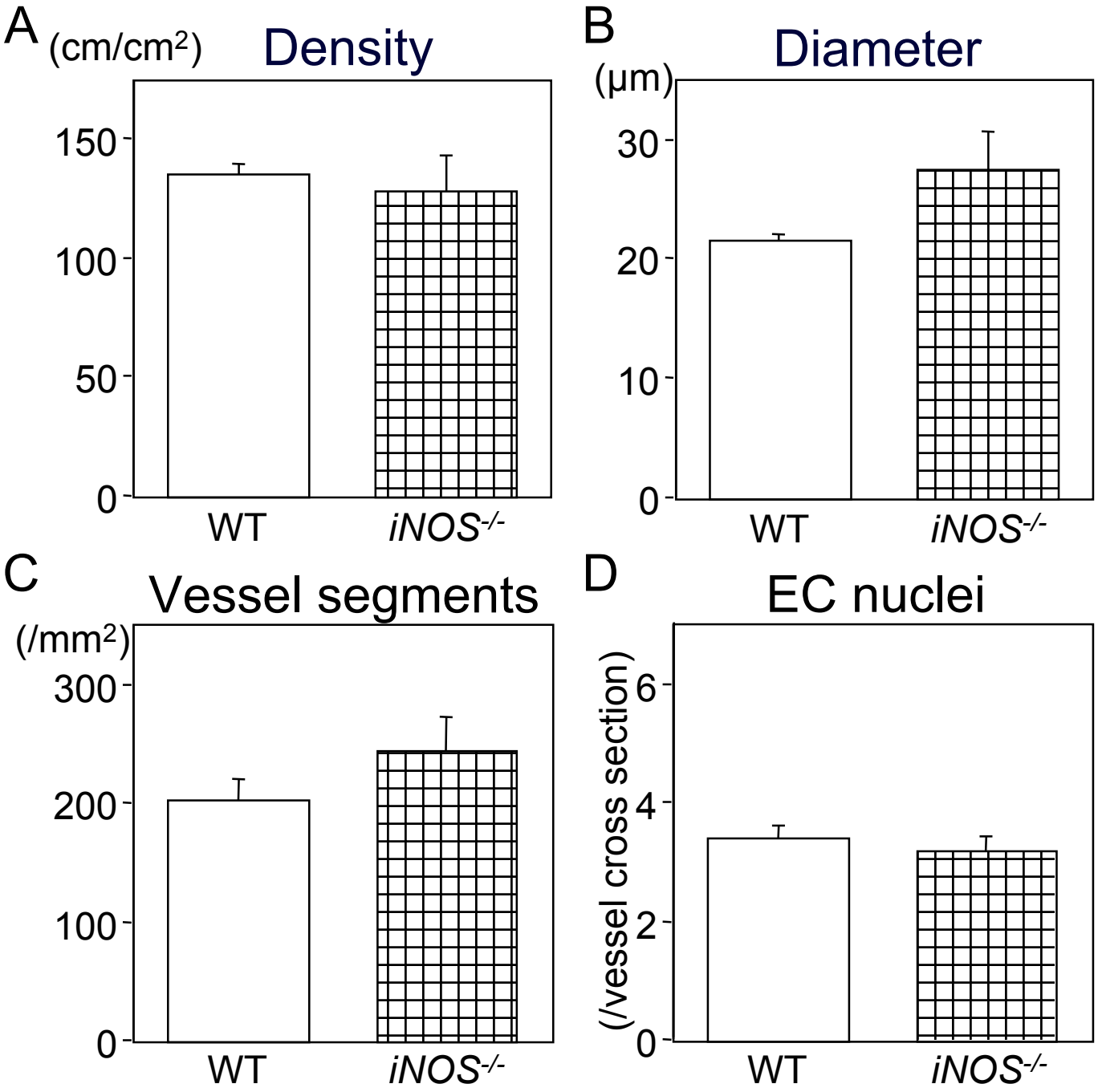


**B16F1** **B16F10**

**Supplemental Figure S1**

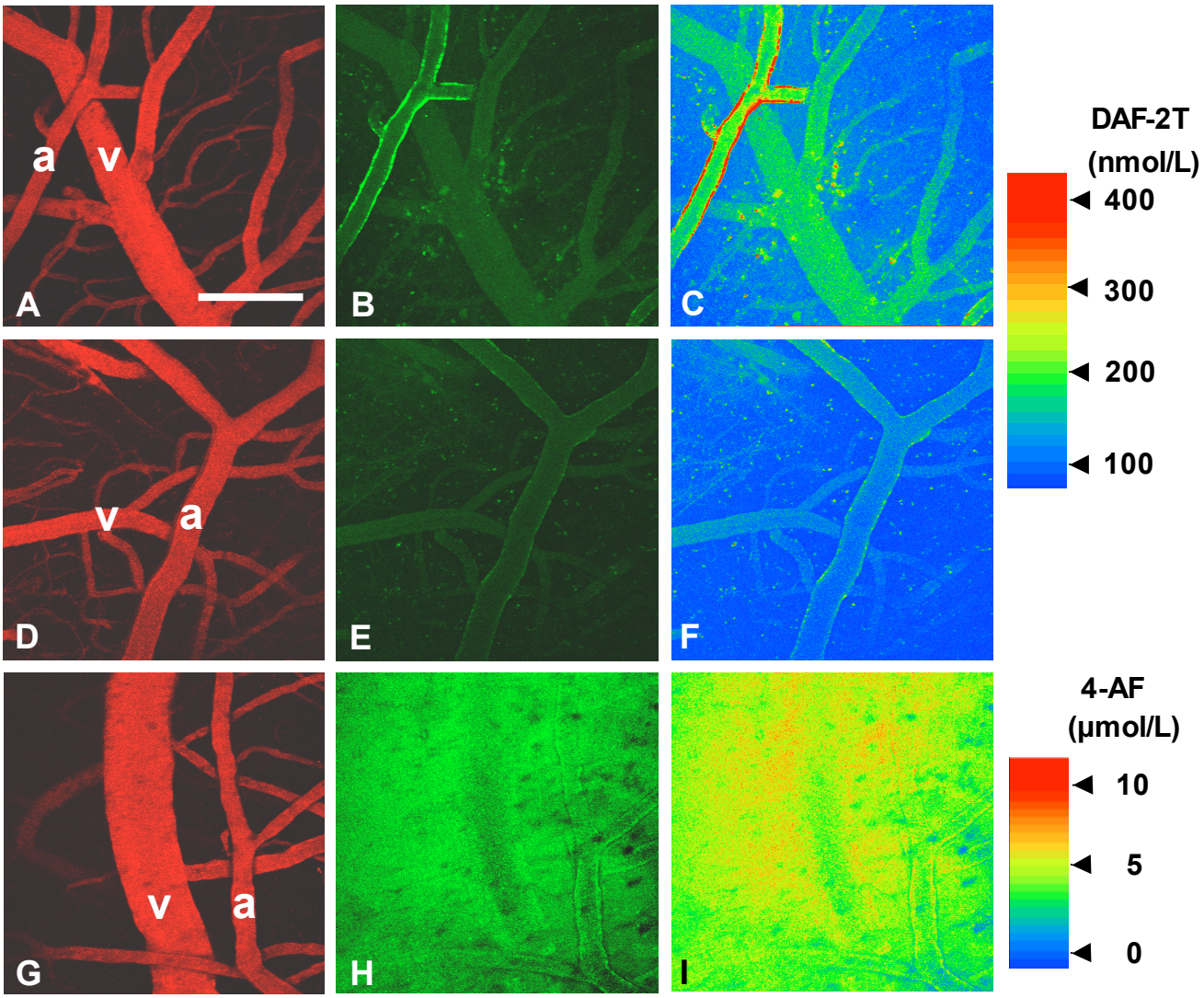
Representative microangiography images of B16F1 and B16F10 tumors grown in the cranial windows. FITC-dextran (2 million MW) was injected systemically to visualize blood vessels. Bar = 100 μm.




**Supplemental Figure S2**

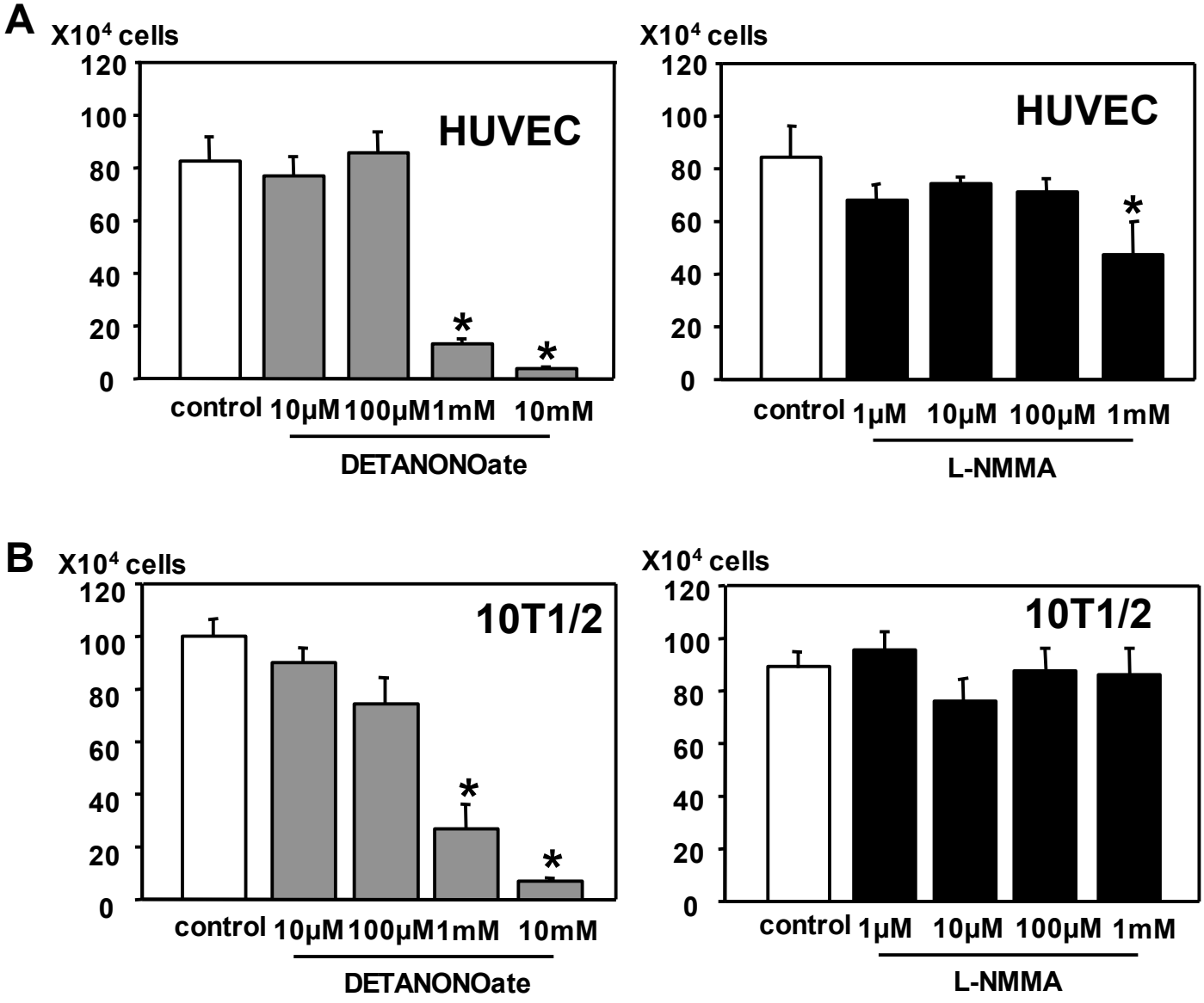
Effect of host iNOS deletion on B16F10 tumor vessels in the cranial window.

(**A**-**C**) Vessel parameters quantified by off line analyses of the digitized microangiography images. There was no significant difference between wild-type C57BL/6 (n=5) and *iNOS*$^{-/-}$ mice (n=5) in vascular density (***A***), vessel diameter (***B***) and branching (***C***). (**D**) Histological quantification of endothelial cell nuclei per vessel cross-section in B16F10 tumors grown in C57BL/6 (132 vessels in 3 tumors, 5 locations in each) or *iNOS*$^{-/-}$ mice (134 vessels in 3 tumors, 5 locations in each).




**Supplemental Figure S3**

Effects of the acute (24hrs) application of L-NMMA on the DAF-2 associated fluorescence in the pial microvessels. (**A, C, D**), microangiography using tetramethylrhodamine-dextran (2 million MW); (**B, E**), Representative microfluorography captured after the 60-minute loading of DAF-2; (**C, F**), pseudocolor representation of DAF-2 microfluorographs. The animals were treated with D-NMMA (***A-C***) or L-NMMA (***D-F***). Note that DAF-2 associated fluorescence mainly distributed along the arteriole and was abolished with L-NMMA treatment. Color bars in the top right represent calibration of the pseudocolor with known concentrations of DAF-2T. (**G-I**), microangiograph (***G***), microfluorograph captured at 60 minutes after injection of 4-AF (***H***), an NO-insensitive control fluorochrome, and its pseudocolor representation (***I***). Color bars in the bottom right shows calibration with known concentrations of 4-AF. ***a***, arteriole; ***v***, venule, Bar = 100 $\mu$m.




**Supplemental Figure S4**

Effects of exogenous NO and NO suppression on HUVECs and 10T1/2 cell proliferation. HUVECs or 10T1/2 cells were plated in uncoated 6-well plates at $5x10^4$ cells per well in EGM with 2% FBS or BME with 10% FBS, respectively. Twenty-four hours later, the culture medium was changed and the cells were challenged with 1 μM, 10 μM, 100 μM and 1 mM of L-NMMA or with 10 μM, 100 μM, 1mM and 10mM of DETA NONOate in their respective media. Seventy-two hours after the treatment, cells were suspended and number of cells was determined using a hemocytometer. All proliferation assays were conducted in triplicate and each set of experiments was performed at least 5 times.

**Supplemental Table S1.**

Vessel morphology of B16 melanomas in paraffin histological specimens.

| | B16F10 | B16F1 | *eNOS*$^{-/-}$ | *iNOS*$^{-/-}$ | D-NMMA | L-NMMA |
|---|---|---|---|---|---|---|
| Vessel Density (/mm$^2$) | 165 ± 17 | 91± 21* | 87± 9* | 140± 6 | 139± 17 | 85 ± 16# |
| Vessel perimeter ($\mu$m) | 67 ± 5 | 93± 8* | 87± 8* | 57± 6 | 63 ± 5 | 113 ± 11# |

* # $p<0.05$ as compared with corresponding B16F10 and D-NMMA treated tumors, respectively. Five random locations per tumor were examined in 3 tumors per group, respectively. The numbers of vessels examined (from the left to the right) are 127, 91, 105, 114, 109, 77.

## Supplemental Table S2.

Angiogenic gene array analysis of B16F10 malanoma.

| gene | D-NMMA | L-NMMA | gene | D-NMMA | L-NMMA | gene | D-NMMA | L-NMMA |
|---|---|---|---|---|---|---|---|---|
| Adamts1 | 0.881 | 0.647 | Figf | 0.015 | 0.027 | Plau | 0.242 | 0.192 |
| Adamts8 | 0.019 | 0.015 | Kdr | 0.172 | 0.183 | Ptgs1 | 0.048 | 0.018 |
| Angpt1 | 0.015 | 0.008 | Flt1 | 0.978 | 0.800 | Ptgs2 | 0.014 | 0.020 |
| Angpt2 | 0.277 | 0.235 | Fn1 | 0.471 | 0.391 | Rsn | 0.945 | 0.881 |
| Ang | 0.312 | 0.253 | Cxcl1 | 0.039 | 0.032 | Ccl2 | 0.072 | 0.040 |
| Cd36 | 0.067 | 0.088 | Hgf | 0.221 | 0.245 | Serpinb5 | 0.017 | 0.030 |
| Cdh5 | 0.503 | 0.435 | Hif1a | 0.949 | 0.885 | Serpine1 | 0.473 | 0.310 |
| Chga | 0.697 | 0.525 | Idb1 | 0.056 | 0.037 | Serpinb2 | 0.013 | 0.023 |
| Col18a1 | 0.051 | 0.030 | Ifna1 | 0.410 | 0.222 | Serpinf1 | 0.008 | 0.011 |
| Csf3 | 0.080 | 0.051 | Ifnb1 | 0.039 | 0.059 | Sparc | 0.849 | 0.755 |
| Ctgf | 0.575 | 0.630 | Ifng | 0.065 | 0.091 | Spp1 | 0.798 | 0.716 |
| Edg1 | 0.744 | 0.536 | Igf1 | 0.070 | 0.078 | Tek | 0.423 | 0.418 |
| Efna5 | 0.639 | 0.576 | Il10 | 0.050 | 0.043 | Tgfa | 0.457 | 0.255 |
| Efnb2 | 0.196 | 0.164 | Il12a | 0.011 | 0.018 | Tgfb1 | 0.402 | 0.346 |
| Egf | 0.268 | 0.142 | Itga5 | 0.375 | 0.320 | Tgfb2 | 0.558 | 0.560 |
| Egfr | 0.027 | 0.030 | Itgav | 0.797 | 0.701 | Tgfb3 | 0.457 | 0.555 |
| Eng | 0.710 | 0.561 | Itgb3 | 0.205 | 0.295 | Tgfbr1 | 0.810 | 0.659 |
| Ephb4 | 0.216 | 0.246 | Smad1 | 0.033 | 0.060 | Tgfbr2 | 0.608 | 0.417 |
| Erbb2 | 0.037 | 0.071 | Mdk | 0.007 | 0.014 | Tgfbr3 | 0.180 | 0.131 |
| Ets1 | 0.661 | 0.646 | Mmp2 | 0.030 | 0.043 | Thbs1 | 0.123 | 0.214 |
| F2 | 0.011 | 0.014 | Mmp9 | 0.017 | 0.029 | Thbs2 | 0.551 | 0.553 |
| Fgf1 | 0.265 | 0.193 | Msr1 | 0.131 | 0.167 | Thbs3 | 0.020 | 0.021 |
| Fgf16 | 0.545 | 0.438 | Nos3 | 0.459 | 0.286 | Tie1 | 0.194 | 0.153 |
| Fgf2 | 0.019 | 0.012 | Nrp1 | 0.598 | 0.341 | Timp1 | 0.259 | 0.169 |
| Fgf4 | 0.191 | 0.104 | Pdgfa | 0.443 | 0.305 | Timp2 | 0.686 | 0.578 |
| Fgf6 | 0.051 | 0.033 | Pdgfb | 0.011 | 0.009 | Tnc | 0.029 | 0.019 |
| Fgf7 | 0.767 | 0.756 | Pdgfra | 0.020 | 0.036 | Vcam1 | 0.039 | 0.051 |
| Fgfr1 | 0.938 | 1.027 | Pdgfrb | 0.035 | 0.026 | Vegfa | 0.517 | 0.479 |
| Fgfr3 | 0.126 | 0.144 | Pecam1 | 0.227 | 0.129 | Vegfb | 0.448 | 0.621 |
| Fgfr4 | 0.243 | 0.127 | Pgf | 0.276 | 0.146 | Vegfc | 0.237 | 0.347 |

Three tumors of each treatment group were examined. Values represent relative gene expression normalized to β−actin.

**Supplemental Table S3.**

Realtime PCR analysis of endogenous angiogenic and vessel maturation factors.

| gene | PDGF-B | Angiopoietin-1 | Angiopoietin-2 | Sphingosine kinase type1 | VEGF-A |
|---|---|---|---|---|---|
| D-NMMA | 1.34 ± 0.33 | 1.15 ± 0.28 | 1.22 ± 0.04 | 1.10 ± 0.21 | 1.67 ± 0.53 |
| L-NMMA | 2.08±0.26 | 1.67±0.59 | 1.33±0.19 | 1.74±0.32 | 1.46 ± 0.28 |
| gene | PDGFRβ | Tie-2 | EDG-1 | VEGFR-1 | VEGFR-2 |
| D-NMMA | 0.90 ± 0.05 | 0.70 ± 0.02 | 1.46 ± 0.36 | 1.08 ± 0.17 | 1.16 ± 0.05 |
| L-NMMA | 1.19 ± 0.24 | 0.80 ± 0.10 | 2.47 ± 0.34 | 1.63 ± 0.28 | 1.20 ± 0.20 |

Three tumors per each group were independently analyzed. Values represent relative gene expression normalized to β−actin. There was no statistical significant difference between D-NMMA and L-NMMA treated tumors in any factors.

**Supplemental Table S4.**

Realtime PCR primers

| Gene | Fam-labeled primer (5'-3') | Unlabeled primer (5'-3') |
|---|---|---|
| PDGF-B | GACCCTCAGACTTGGGCTTGGAGG[FAM]C | CTGCCTGCTGGCTTAGCTT |
| Angiopoietin-1 | CAACCTCGTGCTGGGTCTGG[FAM]TG | GCAGGGTGAGGTTAGGCTTC |
| Angiopoietin-2 | CACCAGATCACTTCTACCTCGCTGG[FAM]G | CCGTGAGTCCTGTAAGGTGAA |
| Sphingosine kinase type1 | GAACCACAGCTCCCTGGCATGG[FAM]TC | CAGAGTGCTGGTGCTGCTGA |
| VEGF-A | CACTCCTAACGATGAAGCCCTGGAG[FAM]G | TTGGTCTGCATTCACATCTGC |
| PDGFRβ | CAACATCCATTCTCGATCACAGAGATG[FAM]TG | TGAGTGATTCGGGCACCTAT |
| Tie-2 | GACCATTGTTGACGCATCTTCATGG[FAM]C | GCCTTAATGAACCAGCACCAA |
| EDG-1 | CACAAAGCGGGAAGGGAGTATGTTTG[FAM]G | AGCTGTTGCTCCCGTTGTGT |
| VEGFR-1 | CGGAAGAGGTAGTTGGACAGGTTTC[FAM]G | CAAGGAGGGCCTCTGATGGT |
| VEGFR-2 | CGGACACAGTGGGCAGTCAAGTC[FAM]G | GGGCCTTCCATTTCTGTACCAT |
| β-actin | CACAGGTCTTGGGTATGGAATCCTG[JOE]G | TCTTTACGGATGTCAACGTCA |

Note that β-actin was JOE-labeled and the genes of interest were FAM-labeled.