SUPPLEMENTAL MATERIAL

Supplemental Table 1: Primer pairs utilized for gene expression analysis via qPCR, where (HK) denotes housekeeping gene

Gene (NCBI mRNA accession ID)	Forward	Reverse
ACTB (NM_001101) (HK)	5'-AGTCCCTTGCCATCCTAAAAG-3'	5'- CAATGCTATCACCTCCCCTG-3'
HIF1A (NM_001530)	5'-AACATAAAGTCTGCAACATGGAAG-3'	5'- TTTGATGGGTGAGGAATGGG -3'
VEGFA (NM_001025366)	5'-AGTCCAACATCACCATGCAG-3'	5'-TTCCCTTTCCTCGAACTGATTT-3'
MET (NM_000245)	5'- GCCCAAACCATTTCAACTGAG -3'	5'-ACCTGTTATTGTGCTCCCAC -3'
MMP2 (NM_004530)	5'-ACCCATTTACACCTACACCAAG -3'	5'- TGTTTGCAGATCTCAGGAGTG -3'
MMP9 (NM_004994)	5'-TTGGTCCACCTGGTTCAACT-3'	5'-ACGACGTCTTCCAGTACCGA-3'
CTNNB1 (NM_001098209)	5'-CCACAAGATTACAAGAAACGGC-3'	5'-CATCCTGGCGATATCCAGG-3'
MYC (NM_002467)	5'-CGACGACGAGACCTTCATCAA-3'	5'- GCCGCTCCACATACACAGTCCT-3'
FOS (NM_005252)	5'-TTGTGAAGACCATGACAGGAG-3	5'- CCATCTTATTCCTTTCCCTTCGG-3'
TIMP3 (NM_000362)	5'-TGATGGCAAGATGTACACGG-3	5'- GAAGTCACAAAGCAAGGCAG-3
THBS1 (NM_003246)	5'-CTCCCCTATGCTATCACAACG-3'	5' -AGGAACTGTGGCATTGGAG-3'
ITGAV (NM_002210)	5'-AGAATCAAGGAGAAGGTGCC -3'	5' -GGCGAGTTTGGTTTTCTGTC-3'
FGF2 (NM_ 002006)	5'-ACCCTCACATCAAGCTACAAC-3'	5'-AAAAGAAACACTCATCCGTAACAC-3'
ENG (NM_000118)	5'- ATAGGACTGTCTTCATGCGC-3'	5' -GTAGATGTACCAGAGTGCAGC -3'
SP1 (NM_138473.2)	5'-CAGATGCCCAACCCCAAG-3'	5' –TGCCATACACTTTCCCACAG-3'
MMP14 (NM_004995)	5'-TGCCTACCGACAAGATTGATG-3'	5'-ATCCCTTCCCAGACTTTGATG-3'

Legends to Supplemental Figures

FIG. S1. **Decorin does not affect expression of *ACTB* nor promote mRNA decay.** *A*, quantitative RT-PCR (qPCR) of *ACTB* expression following a 24-h treatment with decorin (500 nM). *B*, total RNA was harvested from HeLa cells at the indicated time points following decorin (500 nM) exposure and reverse transcribed into cDNA. qPCR analysis was carried to evaluate *ACTB* expression changes. *C-E*, determination of mRNA stability for *VEGFA, HIF1A, and THBS1*, respectively. Actinomycin D, which binds to RNA-primed DNA and inhibits DNA dependent RNA synthesis, was used at a concentration of 5 μg/ml (~4 μM) for 1 h prior to treatment either alone or in combination with the indicated concentrations of decorin protein core for 4 hours. Note: control Ct values (cells received PBS) were used to normalize all succeeding treatment groups; thereafter, the Actinomycin D only group was used as the calibrator sample to calculate the ΔΔCt values and subsequent fold changes for the combined treatment (i.e. 4 μM Actinomycin D + 100 nM decorin and 4 μM Actinomycin D + 500 nM decorin). Expression data are representative of at least 2 independent trials run in a minimum of triplicate and presented as the fold change ± SEM in accordance with the ΔΔCt method. All experiments were found to have $p > 0.05$ (not significant, N.S.).

FIG. S2. **Decorin evokes a reduction of VEGFA in tumor cell lines at 100 nM and in a time-dependent manner.** *A*, representative slot blots (top panels) of tumor conditioned media from HeLa *(left panel)* and MDA-231 *(right panel)* in the absence or presence of decorin protein core (100 nM, 24 h). The middle panels are representative immunoblottings of the corresponding cell lysates. Membranes were probed with a primary antibody specific for VEGFA and developed using enhanced chemiluminscence (ECL) on a GE ImageQuant4000. The Coomassie blue-stained gel (bottom panels) were used for normalization. *B*, quantification of cellular VEGFA levels in HeLa and MDA-231. *C*, time course quantification of cellular VEGFA levels in HeLa cells. *D*, time course analysis of *VEGFA* mRNA expression in HeLa cells following treatment with decorin protein core (500 nM) at the indicated time points. Data are representative of at least 2 independent trials run in duplicate and displayed as mean ± SEM (**$p < 0.01$; ***$p < 0.001$). *E*, immunofluorescence staining of VEGFA in HeLa cells ± decorin for 4 h (500 nM). Bar = 12 μm.

FIG. S3. **Decorin induces pVHL coincident with a reduction of HIF-1α protein under normoxic conditions in tumor cell lines.** *A,* quantification of pVHL by immunoblotting from MDA-231wtHIF-1α *(left panel)* and MDA-231mutHIF-1α *(right panel)* cell lysates following increasing concentrations of decorin protein core as indicated after 4h. *B*, quantification of pVHL and HIF-1α by immunoblotting from parental MDA-231 treated with or without decorin for 4h. All membranes were quantified according to densitiometry utilizing ImageJ software. Raw values were corrected for background noise and normalized to the corresponding Coomassie-blue stained gel. Data are representative of two independent experiments perfomed in duplicate and presented as mean ± SEM (*$p < 0.05$; **$p < 0.01$)

FIG. S4. **Application of exogenous or systemic decorin induces TIMP3 in a cell model and within orthotopic mammary tumor xenografts.** *A,* HeLa cell conditioned media in the absence or presence of decorin protein core (500 nM) was harvested and filtered following the 24h incubation. Media was subjected to slot blot analysis and probed with an anti-TIMP3 antibody. Membrane was quantified via densitometry with ImageJ software and corrected for background noise. Data are representative of two independent trials performed in duplicate and represented as the average ± SEM (***, $p < 0.001$). *B,* TIMP3 detection via immunofluorescence of frozen sections of control MDA-231(GFP+) tumor xenografts or decorin-treated tumors. Mice with MDA-231(GFP+) xenografts were treated with intraperitoneal injections of decorin (5 mg/Kg) every other day over 23 days. Three-dimensional surface plots, which appear at right, were generated with ImageJ software and represent TIMP3 expression corresponding to the immunofluorescence signal intensity. Scale bars depict signal intensity and appear to the right of each surface plot.

FIG. S5. **Systemic administration of decorin inhibits VEGFA levels within HeLa tumor xenografts.** Detection of VEGFA via immunofluorescence of frozen sections of control or decorin-treated HeLa tumor xenografts. All micrographs were imaged using the same exposure and gain. Mice bearing HeLa xenografts were treated with intraperitoneal injections of decorin (5 mg/kg) every other day over 23 days. 3D surface plots, which appear at right, were generated with ImageJ software and represent VEGFA expression corresponding to the signal intensity obtained by the immunofluorescence. Scale bars, which depict signal intensity, appear to the right of each surface plot. Bar = 10 μm.

FIG. S6. **Systemic administration of decorin induces thrombospondin-1 and concurrently inhibits VEGFA levels within orthotopic mammary tumor xenografts**. *A,B,* detection of thrombospondin-1 and VEGFA via immunofluorescence of frozen sections of control MDA-231(GFP+) tumor xenografts or decorin-treated tumors. All micrographs were imaged using the same exposure and gain. Mice bearing MDA-231(GFP+) xenografts were treated with intraperitoneal injections of decorin (5 mg/kg) every other day over 23 days. 3D surface plots, which appear at right, were generated with ImageJ software and represent VEGFA expression corresponding to the signal intensity obtained by the immunofluorescence. Scale bars, which depict signal intensity, appear to the right of each surface plot. Bar = 10 μm.

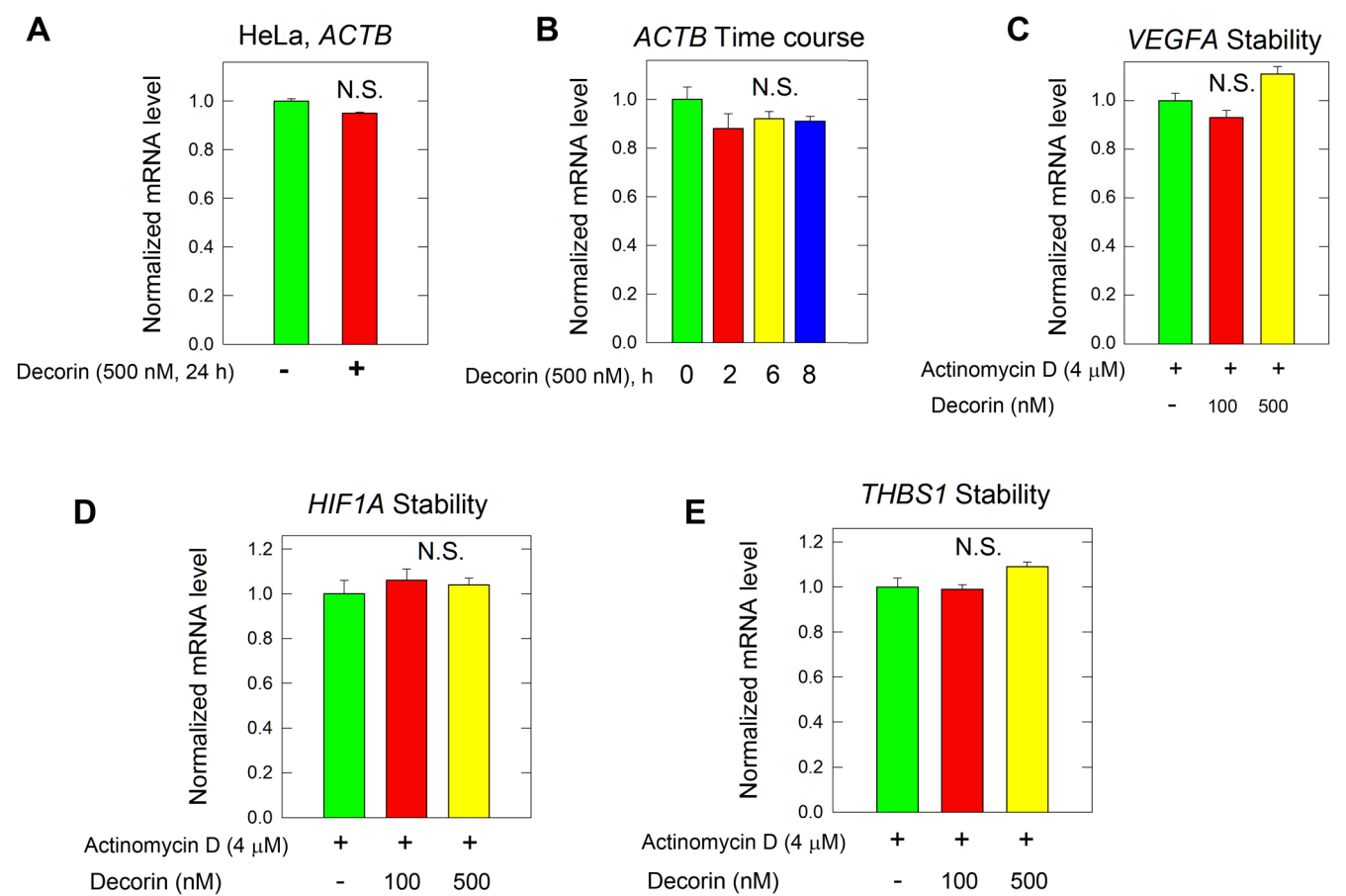
A
HeLa, ACTB
Normalized mRNA level
N.S.
Decorin (500 nM, 24 h)
-
+
B
ACTB Time course
Normalized mRNA level
N.S.
Decorin (500 nM), h
0 2 6 8
C
VEGFA Stability
Normalized mRNA level
N.S.
Actinomycin D (4 μM)
Decorin (nM)
- 100 500
D
HIF1A Stability
Normalized mRNA level
N.S.
Actinomycin D (4 μM)
Decorin (nM)
- 100 500
E
THBS1 Stability
Normalized mRNA level
N.S.
Actinomycin D (4 μM)
Decorin (nM)
- 100 500

Figure S1

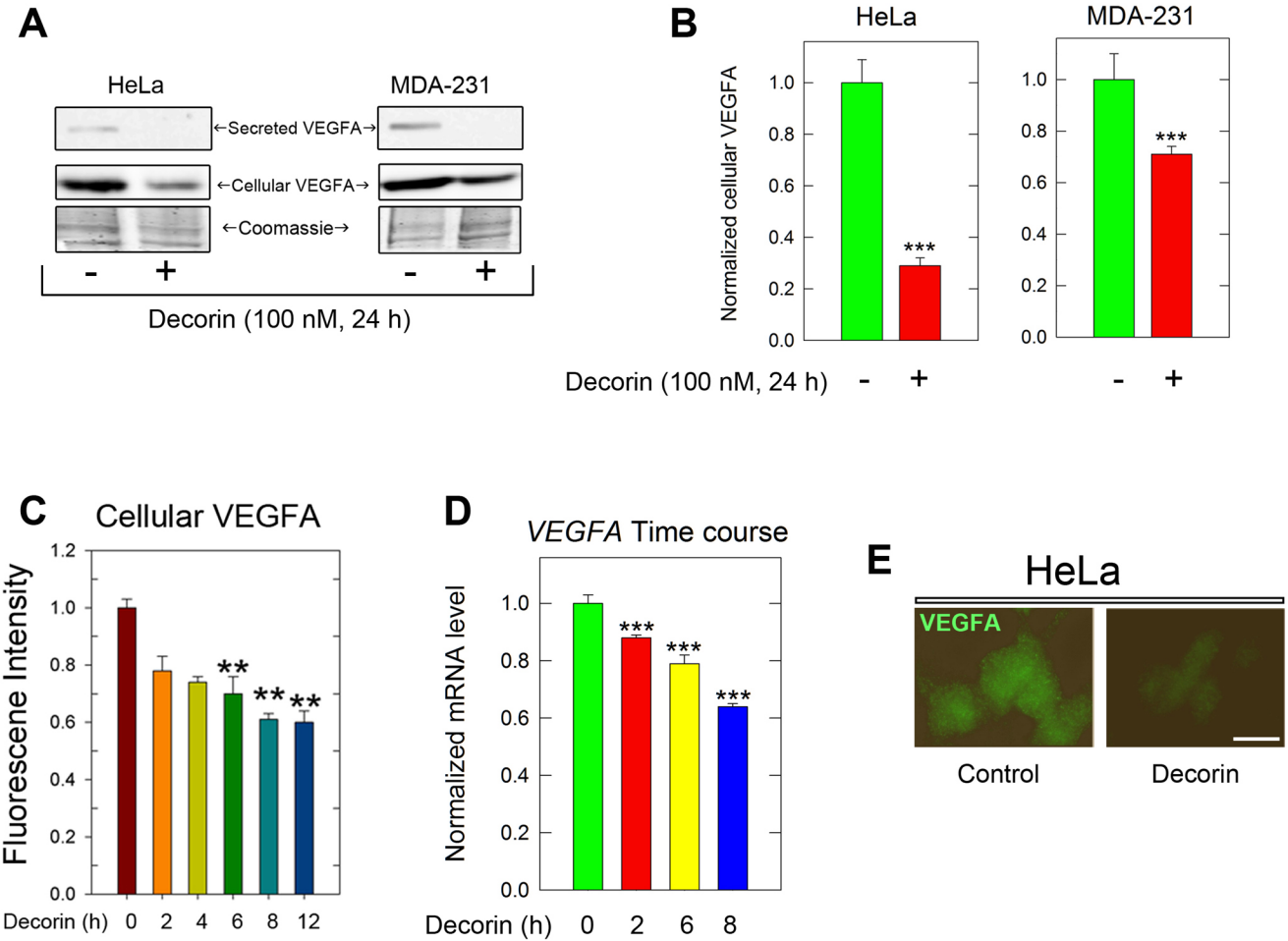
A
HeLa
MDA-231
←Secreted VEGFA→
←Cellular VEGFA→
←Coomassie→
- + - +
Decorin (100 nM, 24 h)
B
HeLa
MDA-231
Normalized cellular VEGFA
1.0
0.8
0.6
0.4
0.2
0.0
***
***
Decorin (100 nM, 24 h) - + - +
C
Cellular VEGFA
Fluorescene Intensity
1.2
1.0
0.8
0.6
0.4
0.2
0.0
**
**
**
Decorin (h) 0 2 4 6 8 12
D
VEGFA Time course
Normalized mRNA level
1.0
0.8
0.6
0.4
0.2
0.0
***
***
***
Decorin (h) 0 2 6 8
E
HeLa
VEGFA
Control
Decorin

Figure S2

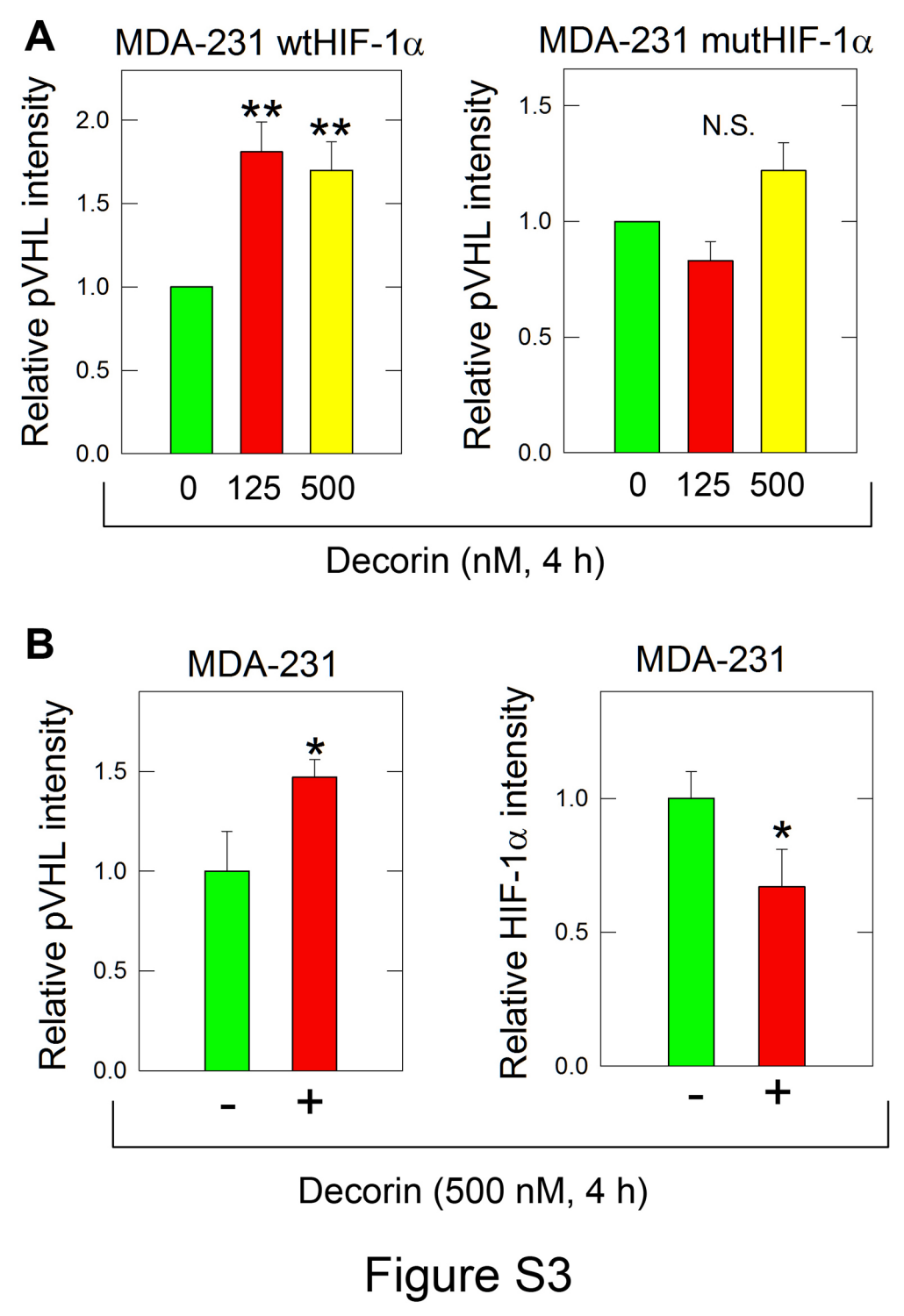
A
MDA-231 wtHIF-1α
MDA-231 mutHIF-1α
Relative pVHL intensity
2.0
1.5
1.0
0.5
0.0
**
**
N.S.
0
125
500
Decorin (nM, 4 h)
B
MDA-231
MDA-231
Relative pVHL intensity
Relative HIF-1α intensity
*
*
-
+
Decorin (500 nM, 4 h)

Figure S3

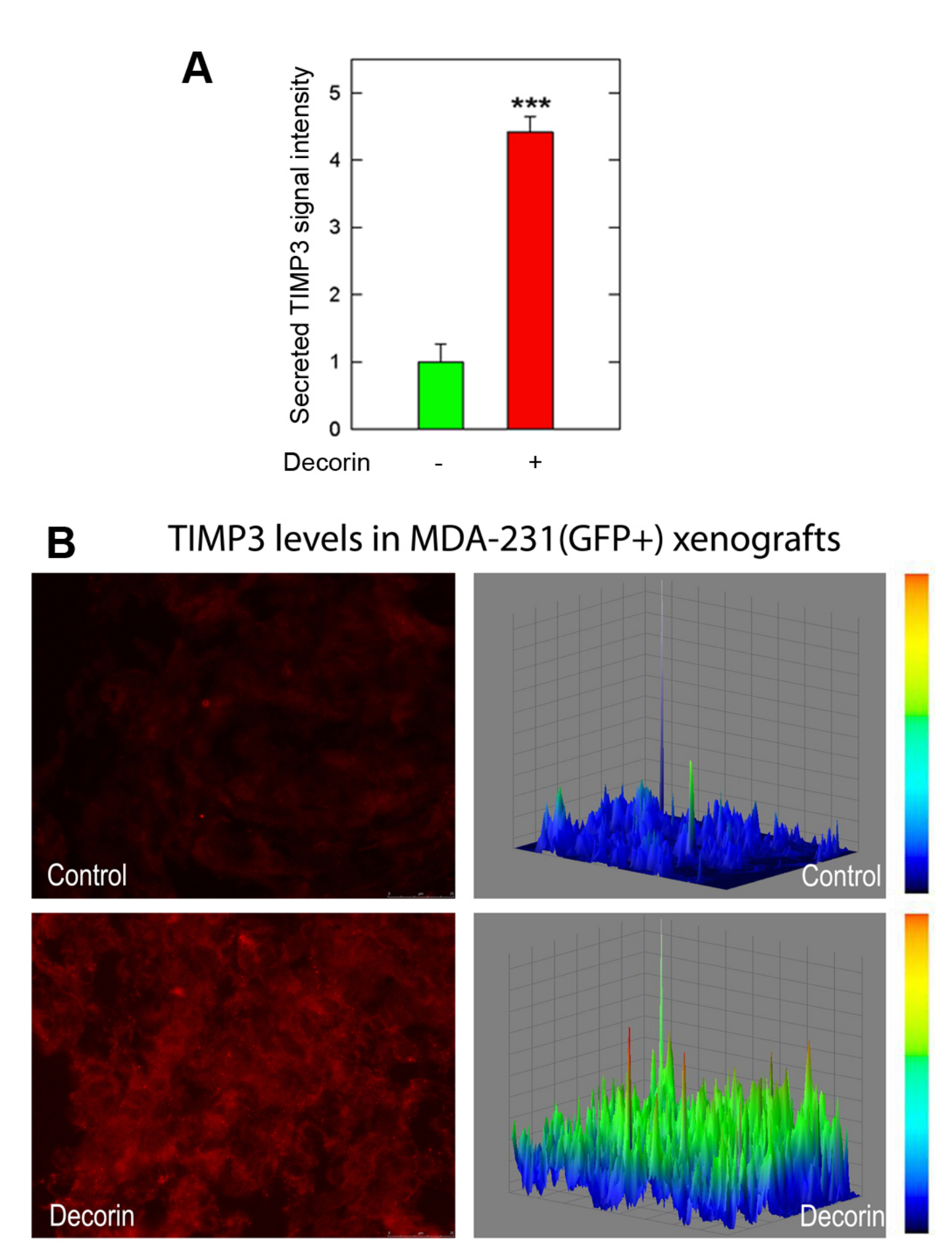
A
Secreted TIMP3 signal intensity
0
1
2
3
4
5
***
Decorin
-
+
B
TIMP3 levels in MDA-231(GFP+) xenografts
Control
Control
Decorin
Decorin

Figure S4

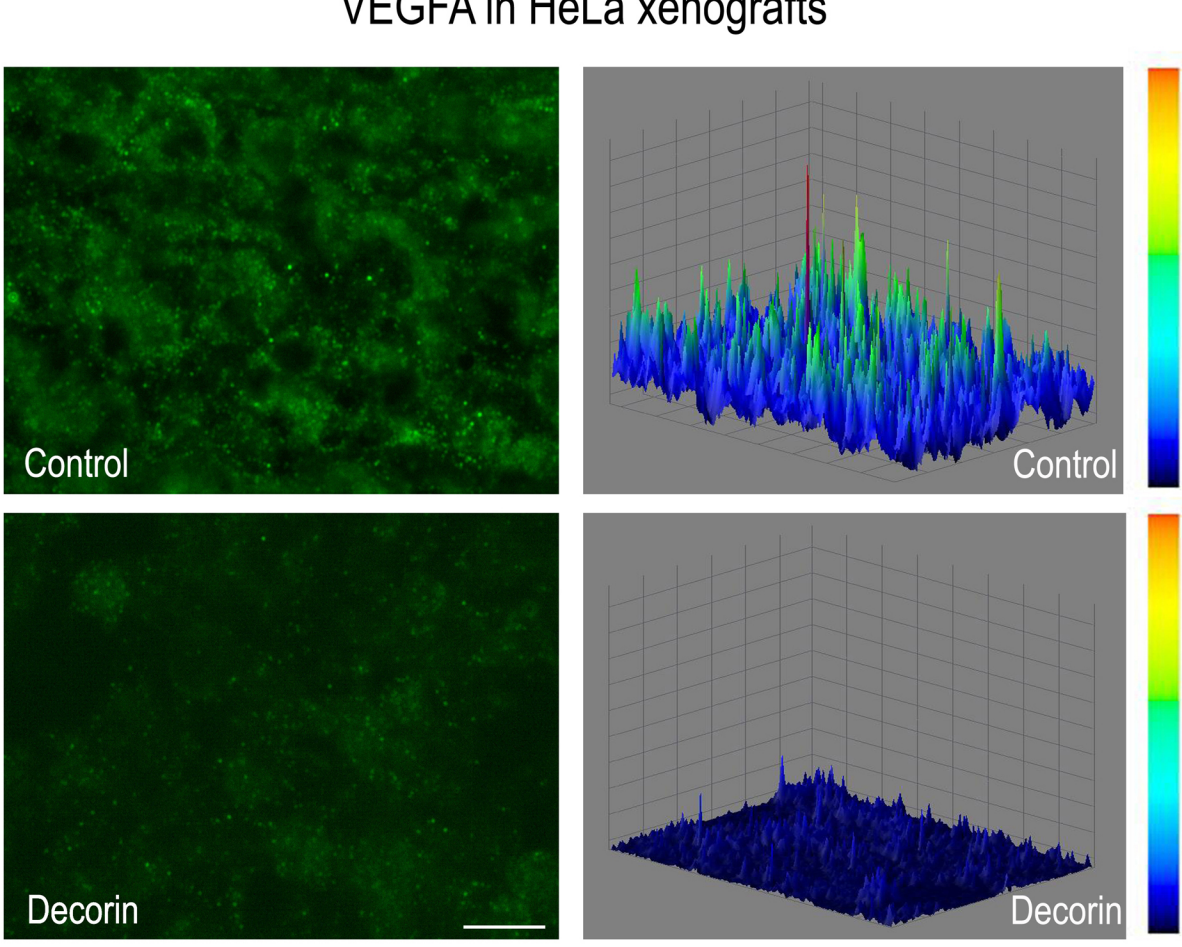

Figure S5

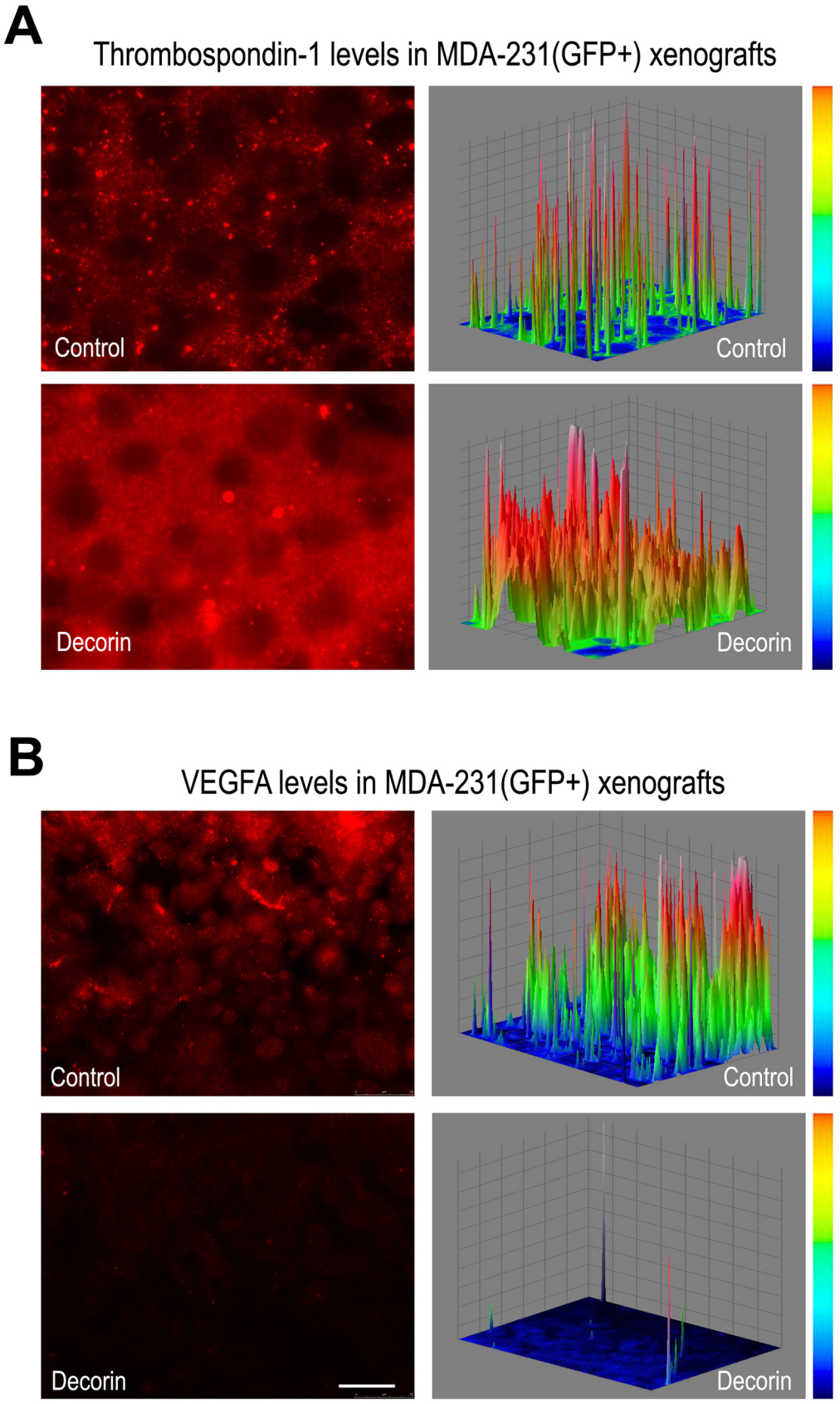
A
Thrombospondin-1 levels in MDA-231(GFP+) xenografts
Control
Control
Decorin
Decorin
B
VEGFA levels in MDA-231(GFP+) xenografts
Control
Control
Decorin
Decorin

Figure S6