EXTENDED EXPERIMENTAL PROCEDURES

Antibodies

Polyclonal anti-VEGF-Ax antibody was generated by injecting into rabbits synthetic KLH-conjugated peptide, AGLEEGASLRVSGTR; the same peptide was used for affinity purification. Anti-hnRNP A2/B1 was from Abcam (for immunofluorescence microscopy) or from Santa Cruz (clone EF-67, for immunoprecipitation and immunoblot analysis). Anti-VEGF-A (N-terminus), neutralizing anti-VEGF-A (clone JH121), and horseradish peroxidase-conjugated secondary antibodies were from Thermo Fisher; anti-VEGF-Ab was from R&D; anti-Myc-tag, anti-phospho VEGFR2 and anti-VEGFR2 antibodies were from Cell Signaling; anti-GAPDH and anti-α-tubulin were from Sigma; anti-HDAC-1 was from BioVision; IgG from Santa Cruz, and Alexa Fluor-conjugated secondary antibody was from Molecular Probes.

Cell migration

Cell migration was measured by razor-wound method. Confluent bovine aortic EC cultures maintained in serum-free medium for 24 h were wounded by gently pressing a razor through the cell layer to mark the wound line and then drawn through the monolayer to remove cells on one side of the line. Migration was allowed for 24 h in serum-free medium containing 1 mg/ml of bovine serum albumin and IgG or anti-VEGF-A or -VEGF-Ax antibodies (5 μg/ml) or His-VEGF-Ax^{Ala} (50 ng/ml). Following fixation and staining with Giemsa-Wright (Sigma), cells crossing the wound line at two randomly chosen regions 1.5 mm in length were counted by a semi-automated, computer-assisted procedure. To calculate root-mean-square displacement and speed, cells were imaged every 5 min for 1000 min by a phase-contrast microscope (Leica) equipped with temperature-controlled humidified chamber and motorized x-y stage. Migrating cells were subjected to automatic tracking using “track objects” function in Metamorph and *x*-

and *y*-coordinates were acquired. All cell migration experiments were analyzed by a person blinded to the treatments.

Cell proliferation

Bovine aortic ECs were allowed to attach for 4 h in 96-well plates (~10,000 cells/well) and medium replaced with Opti-MEM (Invitrogen) containing IgG, or anti-VEGF-A or anti-VEGF-Ax antibodies (5 μg/ml), recombinant VEGF-A (R&D Systems, 20 ng/ml), or His-VEGF-Ax^{Ala} (50 ng/ml). Total cell DNA was determined fluorometrically using CyQUANT NF Cell Proliferation Assay Kit (Invitrogen) with excitation at 485 nm and emission at 538 nm (Spectramax Gemini EM).

Immunoblot analysis

Cell lysates or conditioned media were denatured and resolved on 4-20% gradient SDS-PAGE. To differentiate canonical VEGF-A and VEGF-Ax on same gel, lysates or conditioned media were subjected to protein deglycosylation mix (New England Biolabs) and resolved on 16% Tricine gel (Invitrogen). After transfer, the blots were probed with specific primary antibody followed by HRP-conjugated secondary antibody, and developed using ECL or ECL plus reagent (Amersham). Nuclear and cytoplasmic fractions were isolated using NE-PER reagent (Thermo Scientific).

Mass spectrometry

Bovine *VEGFA$_{164}$* cDNA and the Ax element were cloned in a construct containing an in-frame polyHis-tag. The downstream stop codon was excluded so that readthrough at the canonical stop codon generated a polyHis-tagged chimeric protein. Serum-free conditioned medium was obtained from stably transfected HEK293 cells. His-tagged readthrough product was purified using Ni-NTA agarose (Qiagen) and subjected to SDS-PAGE electrophoresis. An about 28 kDa band was cut from the gel stained with Imperial protein stain (Thermo), digested with trypsin, and analyzed by capillary column

LC-MS/MS (LTQ-Orbitrap Elite system coupled to a Dionex Ultimate 3000 HPLC fitted with a 15 cm x 75 μm i.d. Acclaim Pepmap C18 reverse-phase capillary column). Peptides were eluted using 0.5% formic acid and 95% acetonitrile mobile phases at a flow rate of 0.3 μl/min. The digest was analyzed in survey and targeted modes. Survey experiments were done using the data-dependent multitask capability of the instrument acquiring full scan mass spectra to interrogate peptide molecular weights and product ion spectra to determine amino acid sequence in successive instrument scans. The data were analyzed by Mascot using all collected CID spectra to search the bovine reference sequence database. The targeted selective reaction monitoring (SRM) experiments involved fragmentation of specific readthrough peptides over the entire course of the LC experiment.

siRNAs

VEGF-A-specific siRNAs target the following sequences in bovine *VEGFA* mRNA:

siRNA 1, 5' GCTTCCTACAGCATAACAAATGTGA 3'

siRNA 2, 5' GGAGTACCCAGATGAGATT 3'

siRNA 3, 5' ATGTGAATGCAGACCAAAG 3'

hnRNP A2/B1-specific siRNA targets the following sequence in bovine *HNRNPA2/B1* mRNA: 5' GGCTTTGTCTAGACAAGAAATGCAG 3'.

All siRNAs were transfected using Lipofectamine 2000 and expression of target genes determined after 3 days by immunoblot analysis.

Immunoprecipitation and RNA-binding protein immunoprecipitation (RIP)

Cell extracts were pre-cleared with protein A-Sepharose beads and IgG for 1 h at 4 °C. Antibody was added and samples tumbled overnight at 4 °C. Immune complexes bound to protein A-Sepharose beads were isolated by centrifugation followed by extensive washing. For immunoprecipitation, protein was extracted using Laemmli buffer. For RIP analysis, RNA bound to immune complexes was isolated by RNeasy Mini Kit (Qiagen).

VEGFA sequencing

Total RNA was isolated from bovine ECs using RNeasy Mini Kit (Qiagen). *VEGFA* cDNA was generated by reverse transcription followed by PCR using SuperScript III One-Step RT-PCR System (Invitrogen). Primers used were:

Forward, 5' ATGCAAGCTTATGAACTTTCTGCTCTCTTGGG 3'

Reverse, 5' ATGCGGATCCGTCTTTCCTGGTGAGACGTCT 3'

cDNAs were cloned in pGEM-T vector and subjected to capillary sequencing using T7 Universal-R1 primer: 5' TAATACGACTCACTATAGG 3'.

RT-PCR analysis

Total bovine EC RNA, or RNA isolated from RIP samples, were subjected to reverse transcription and real-time PCR using AgPath-ID One-Step RT-PCR reagent (Ambion). Firefly *luciferase (FLuc)*-, bovine *VEGFA*- and *GAPDH*-specific TaqMan probes (Applied Biosystems) were used. *FLuc* and *VEGFA* mRNA levels were normalized by *GAPDH* mRNA. In RIP experiments, hnRNP A2/B1-bound *FLuc* mRNA was quantified relative to the *FLuc* mRNA in input samples. Following *VEGFA*-specific primers were used for PCR to differentiate between anti-angiogenic *VEGF-Ab* isoforms and pro-angiogenic *VEGF-A* isoforms:

Forward, 5' ATGCGGATCAAACCTCACC 3'

Reverse, 5' GTCTTTCCTGGTGAGACG 3'

Plasmid construction

pcDNA 3.1 (Invitrogen) was the backbone vector for all constructs. Bovine $VEGFA_{164}$ cDNA from ECs was cloned with the Ax element in HindIII and BamHI sites. The canonical stop codon separating VEGF-A_{164} coding sequence from Ax element was retained, but the downstream in-frame stop codon was omitted. Firefly luciferase (*FLuc*) was cloned without its start codon (ATG) between BamHI and NotI. The following linker sequence was added at the 5' end of *FLuc*: 5' GGCGGCTCCGGCGGCTCCCTC-

GTGCTCGAG 3'. In a separate construct, *Myc* replaced VEGF-A_{164}. In all constructs, *FLuc* was in-frame with *$VEGFA_{164}$* or *Myc*. Mutations were done using GeneArt Site-Directed Mutagenesis System (Invitrogen). To test candidate genes for translational readthrough, ~700 nt at the 3' end of human coding sequences (*TOX,* 684 nt; *ADAMTS4*, 741 nt; *AGO1*, 696 nt; *NR1D1*, 669 nt; *MTCH2*, 732 nt) were cloned with inter-stop codon regions upstream to and in-frame with *FLuc*. Authenticity of all constructs was confirmed by sequencing.

Surface plasmon resonance analysis of protein-RNA binding

Biotinylated RNA (5' Bio-UGUGACAAGCCGAGGCGGUGAGCCGGGCAGGAGGAAG-GAGCCUC 3') containing the putative A2RE of *VEGFA* mRNA (Dharmacon) was immobilized on a streptavidin sensor chip in a BIAcore 3000 (GE Health). Recombinant human hnRNP A2/B1 (Abnova) was injected at 20 μl/min for 3 min in a running buffer of 10 mM HEPES (pH 7.4), 150 mM NaCl and 0.005% Surfactant P20, and cells were regenerated using 50 mM NaOH. K_D were calculated using Biaevaluation software.

Expression and purification of His-VEGF-Ax^{Ala}

HEK293-6E cells were cultured in serum-free Freestyle 293 expression medium (Invitrogen) and transfected with vector pTT5 expressing His-VEGF-Ax^{Ala} using PEI (Polysciences); the canonical TGA stop codon was mutated to GCA to ensure robust synthesis of VEGF-Ax-like protein. His-VEGF-Ax^{Ala} was purified from conditioned medium collected 6 days after transfection using HisTrap FF crude column (GE healthcare). Purified His-VEGF-Ax^{Ala} was detected with anti-VEGF-Ax, anti-VEGF-Ab and anti-VEGFA antibodies.

Solid phase enzyme-linked receptor-binding assay

Maxisorp 96-well plates (Nunc) were coated with 3 μg/ml of VEGFR2 (R&D Systems) in Tris-buffered saline (TBS) at 4 °C overnight. Wells were blocked in 5% milk in TBS for

1 h followed by incubation with serial dilutions of ligands for 2 h, and then with anti-VEGF-A antibody for 2 h. HRP-conjugated secondary antibody was added for 1 h and color developed with 3,3',5,5'-tetramethylbenzidine (TMB, Pierce). The reaction was stopped by addition of 1 N HCl, and absorption at 450 nm was measured using a microplate reader (SpectraMax 190, Molecular Devices). All washes were done with TBS containing 0.05% Tween 20. For binding to neuropilin-1, a similar protocol was followed except phosphate-buffered saline (PBS) was used in place of TBS, 0.5% BSA was used in place of 5% milk, and 2 μg/ml of heparin was added with ligand.

VEGFR2 phosphorylation studies

HUVECs were cultured in serum-free OptiMem medium for 4 h, followed by treatment with VEGF-A (20 ng/ml) or VEGF-Ax (50 ng/ml) for up to 30 min. Cells were lysed using PhosphoSafe extraction reagent (EMD Millipore) and resolved on 7.5% SDS-PAGE. After transfer, blots were probed with anti-phospho-VEGFR2 and anti-VEGFR2 antibodies.

Fluorescence microscopy

Tissue arrays contained 1.5-mm by 5-μm thick samples (US Biomax). The adenocarcinoma array contained samples from 30 cases and 5 healthy individuals. Sections were deparaffinized, rehydrated, and subjected to antigen retrieval using Target retrieval solution (Dako) at 95 °C for 25 min. Sections were blocked with 5% horse serum for 2 h, incubated with anti-VEGF-A or -VEGF-Ax antibodies, or IgG overnight at 4 °C, and then stained with Alexa Fluor 488-conjugated secondary antibody at room temperature for 1 h. Fluorescence intensity was quantified using NIH ImageJ software, and background fluorescence from IgG samples was subtracted. Frozen human tissue sections were fixed with 4% paraformaldehyde in PBS, permeabilized with 0.1% Triton X-100, and incubated with anti-VEGF-Ax antibody followed by Alexa Fluor 488-conjugated secondary antibody. The same protocol was used to image hnRNP A2/B1 in

cultured ECs. Single-plane, confocal images were obtained with a 1.0 Airy pinhole (Leica DMRXE with confocal TCS SP2 unit).

Endothelial cell tube formation

Bovine aortic ECs were pretreated with 50 ng/ml of His-VEGF-Ax^{Ala} for 6 h after which they were (10^5 cells/cm^2) seeded on growth factor-reduced LDEV-free Matrigel (BD Biosciences) on μ-slides developed for angiogenesis assays (Ibidi). Incubation with His-VEGF-Ax^{Ala} was continued for 10 h. Cells were then fixed with 4% paraformaldehyde, stained with CyQUANT dye and imaged. Total tube length was quantified using ImageJ.

Genome-wide analysis of readthrough

3'UTRs from *H. sapiens*, *M. mulatta, B. taurus, M. musculus,* and *R. norvegicus* were retrieved from a UTR database (http://utrdb.ba.itb.cnr.it/). 3'UTR sequences from 6357 genes common to the five species were translated *in silico* using BioPerl translate module. The 5'-most 60-amino acid sequence of each 3'UTR were aligned and scored. Using a cut-off of 1400, heuristically chosen to include scores > 70% of *VEGFA* score, 539 genes were selected for manual screening; mRNAs that undergo alternative splicing at the 3'UTR, lack downstream, in-frame stop codons, or exhibit conservation after the downstream stop codon were removed.

Animal studies

Nu/Nu athymic nude male mice were inoculated subcutaneously on both flanks with HCT116 human colon carcinoma cells. Mice were injected subcutaneously with His-VEGF-Ax (10 μg/mouse) obtained from HEK293 cells stably expressing bovine VEGF-Ax with Ser in place of the canonical stop codon. HiTrap Heparin HP Columns (GE Healthcare) were used for purification. Control mice were injected with buffer containing 25 mM Tris and 150 mM NaCl (pH 8). Injections were given every third day starting from the 4th day after tumor inoculation. Tumor progression was monitored by caliper

determination of volume [(shortest diameter)^2 x (longest diameter) x 0.525]. For determination of tumor angiogenesis, treatment was started on the second day after tumor inoculation, and blood vessels feeding directly into day 6 tumors were counted under a dissecting microscope at 12.5x magnification. Images were captured using PSMT5 operating microscope (World Precision) with 12.5x objective lens; every visible vessel touching the circumference of the tumor nodule was scored as a single vessel by an observer blinded to the treatment.

Statistics

Unless mentioned otherwise Student's t-test was employed to test the significance of differences we observed in various experiments. Mann-Whitney test was used for the analysis of VEGFA isoform expression in colon tissues.

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. *VEGFA* Isoform Sequences and Conservation, Related to Figure 1

(A,B) VEGF-A_{164} (A) and (B) VEGF-A_{120} nucleotide sequences expressed in bovine aortic ECs. Cleaved signal peptide is indicated (gray background).

(C) Electropherogram of *VEGFA* cDNA sequence from bovine aortic EC. Distal part of coding sequence and region between the canonical stop codon (in box) and downstream stop codon.

(D) Absence of VEGF-Ab mRNA isoforms in ECs. RNA isolated from bovine aortic ECs was subjected to reverse transcription followed by PCR (35 cycles). Primers were designed so that alternatively spliced VEGF-Ab isoforms would generate 273- and 141-bp products, and isoforms not spliced in this region (exon 8) would give 321- and 189-bp products; non-specific band (*).

(E) Multiple sequence alignment of amino acids potentially encoded by the region between two in-frame stop codons in mammalian *VEGFA* 3'UTR. Conserved sequences are highlighted (gray background).

(F) Comparison of sequence conservation. Sequence conservation (amino acid identity, %) in the coding sequence, the peptide extension between the two stop codons (Ax element), and the region 66 nt downstream of the distal stop codon is shown.

Figure S2. Specificity of Anti-VEGF-Ax Antibody, Related to Figure 2

(A) Anti-VEGF-Ax antibody recognizes VEGF-Ax, but not VEGF-A. HEK293 cells were transfected with Myc-tagged *VEGFA* cDNA in which the canonical stop codon is mutated to GCA (VEGF-Ax^{Ala}), or with Myc-tagged *VEGFA* cDNA, and conditioned media subjected to immunoblot analysis with anti-VEGF-Ax and -VEGF-A antibodies.

(B) Anti-VEGF-Ax antibody does not recognize VEGF-A or VEGF-Ab. Recombinant His-VEGF-Ax^{Ala}, VEGF-A, and VEGF-Ab were immunoblotted with anti-VEGF-Ax, anti-VEGF-Ab and anti-VEGF-A antibodies.

(C) Confirmation of anti-VEGF-Ax specificity. EC lysates were pre-adsorbed with AGLEEGASLRVSGTR peptide (1 μg/ml overnight at 4° C) and immunoblotted with anti-VEGF-Ax antibody.

(D) VEGF-Ax is expressed in multiple cell types. Lysates from multiple cell types were immunoblotted with anti-VEGF-Ax and anti-GAPDH antibodies.

Figure S3. Ax-dependent Readthrough *in vitro* using Rabbit Reticulocyte Lysate and Readthrough of Heterologous Transcript, Related to Figure 3

(A) Ax-dependent readthrough *in vitro*. Plasmids containing in-frame *VEGF*-Ax-*FLuc*, and variants with TGA-to-GCA substitution, no Ax element, and Ax replaced by a non-specific sequence (ns), were subjected to *in vitro* coupled transcription-translation using rabbit reticulocyte lysate, and FLuc activity of the products measured.

(B) Mass spectrometric analysis of readthrough of heterologous transcript. ECs were transfected with plasmid containing *Myc-Ax-FLuc* and lysate subjected to immuno-precipitation using anti-Myc-tag antibody. The precipitate was resolved in SDS-PAGE, the ~66 kDa band subjected to MS analysis; identified FLuc peptides are highlighted (underline).

(C) Ax-dependent readthrough of heterologous transcript *in vitro*. Chimeric plasmids containing *Myc*-Ax-*FLuc* and *Myc-FLuc* were subjected to *in vitro* coupled transcription-translation using rabbit reticulocyte lysate, and FLuc activity measured.

Figure S4. VEGF-Ax in BAEC Conditioned Medium and Migration of hnRNP A2/B1 Knockdown BAECs, Related to Figure 5.

(A) Conditioned medium derived from BAECs grown in serum-free medium without any growth factors was concentrated and subjected to immunodepletion using anti-VEGF-Ax antibody. Immunodepleted samples and immunopreciptants were resolved on SDS-PAGE and probed with anti-VEGF-A antibody.

(B) Effect of VEGF-Ax inhibition on EC proliferation. ECs were treated with antibodies

indicated and cell proliferation quantified using CyQUANT NF Cell Proliferation Assay Kit (*, $P < 0.01$, 2-way ANOVA).

(C) Migration of hnRNP A2/B1 knockdown BAECs.

Figure S5. *In Vivo* Expression of VEGF-Ax, Related to Figure 6

(A) VEGF-Ax expression in human tissues. Microarray sections of frozen human tissues were subjected to immunofluorescence with anti-VEGF-Ax antibody followed by Alexa Fluor 488-conjugated secondary antibody, and DAPI stain, and imaged by confocal microscopy.

(B) VEGF-Ax expression in human serum. Serum from healthy subjects (n=4) was subjected to immunoblot analysis with anti-VEGF-Ax antibody.

(C) Validation of anti-VEGF-Ax antibody for immunofluorescence. Frozen colon tissue sections were stained with anti-VEGF-Ax antibody and DAPI before and after preadsorption with AGLEEGASLRVSGTR (1 μg/ml overnight at 4 °C), and imaged by immunofluorescence microscope.

Figure S6. Genome-wide analysis of translational readthrough targets, Related to Figure 7

Flow chart of bioinformatic screen employed to identify readthrough candidates.

Figure S1

A

ATGAACTTTCTGCTCTCTTGGGTACATTGGAGCCTTGCCTTGCTGCTCTACCTTCACCATGCCAAGTGGTCCCAGGCTGCA
CCCATGGCAGAAGGAGGGCAGAAACCCCACGAAGTGGTGAAGTTCATGGATGTCTACCAGCGCAGCTTCTGCCGTCCCATC
GAGACCCTGGTGGACATCTTCCAGGAGTACCCAGATGAGATTGAGTTCATTTTCAAGCCGTCCTGTGTGCCCCTGATGCGG
TGCGGGGGCTGCTGTAATGACGAAAGTCTGGAGTGTGTGCCCACTGAGGAGTTCAACATCACCATGCAGATTATGCGGATC
AAACCTCACCAAAGCCAGCACATAGGAGAGATGAGCTTCCTACAGCATAACAAATGTGAATGCAGACCAAAGAAAGATAAA
GCAAGGCAAGAAAATCCCTGTGGGCCTTGCTCAGAGCGGAGAAAGCATTTGTTTGTACAAGATCCGCAGACGTGTAAATGT
TCCTGCAAAAACACAGACTCGCGTTGCAAGGCGAGGCAGCTTGAGTTAAACGAACGTACTTGCAGATGTGACAAGCCGAGG
CGGTGAGCCGGGCTGGAGGAAGGAGCCTCCCTCAGGGTTTCGGGAACCAGACGTCTCACCAGGAAAGACTGA

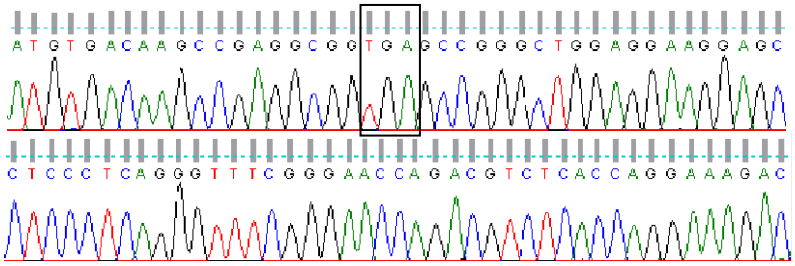
Expected MW of readthrough product: 20.46 kDa (after signal peptide cleavage)

B

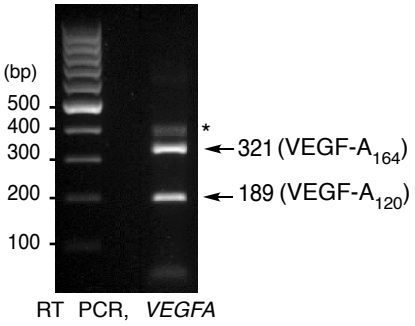
ATGAACTTTCTGCTCTCTTGGGTACATTGGAGCCTTGCCTTGCTGCTCTACCTTCACCATGCCAAGTGGTCCCAGGCTGCA
CCCATGGCAGAAGGAGGGCAGAAACCCCACGAAGTGGTGAAGTTCATGGATGTCTACCAGCGCAGCTTCTGCCGTCCCATC
GAGACCCTGGTGGACATCTTCCAGGAGTACCCAGATGAGATTGAGTTCATTTTCAAGCCGTCCTGTGTGCCCCTGATGCGG
TGCGGGGGCTGCTGTAATGACGAAAGTCTGGAGTGTGTGCCCACTGAGGAGTTCAACATCACCATGCAGATTATGCGGATC
AAACCTCACCAAAGCCAGCACATAGGAGAGATGAGCTTCCTACAGCATAACAAATGTGAATGCAGACCAAAGAAAGATAAA
GCAAGGCAAGAAAAATGTGACAAGCCGAGGCGGTGAGCCGGGCTGGAGGAAGGAGCCTCCCTCAGGGTTTCGGGAACCAGA
CGTCTCACCAGGAAAGACTGA

Expected MW of readthrough product: 15.62 kDa (after signal peptide cleavage)

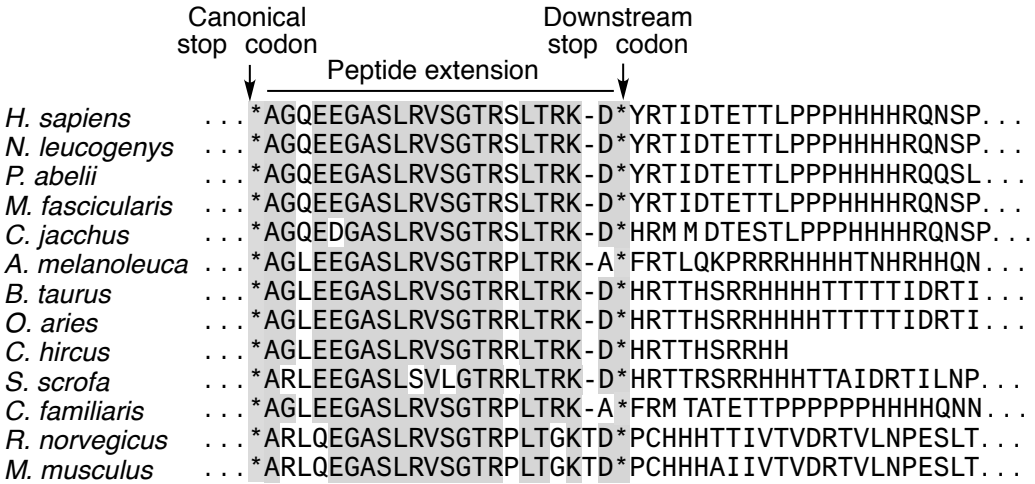
C



D



E



F

	Sequence conservation		
Species comparison	Coding sequence	Peptide extension	Downstream 3' UTR (66 nt after distal stop codon)
H. sapiens vs. *B. taurus*	95%	91%	9%
B. taurus vs. *M. musculus*	89%	78%	9%
H. sapiens vs. *M. musculus*	90%	74%	5%

Figure S2

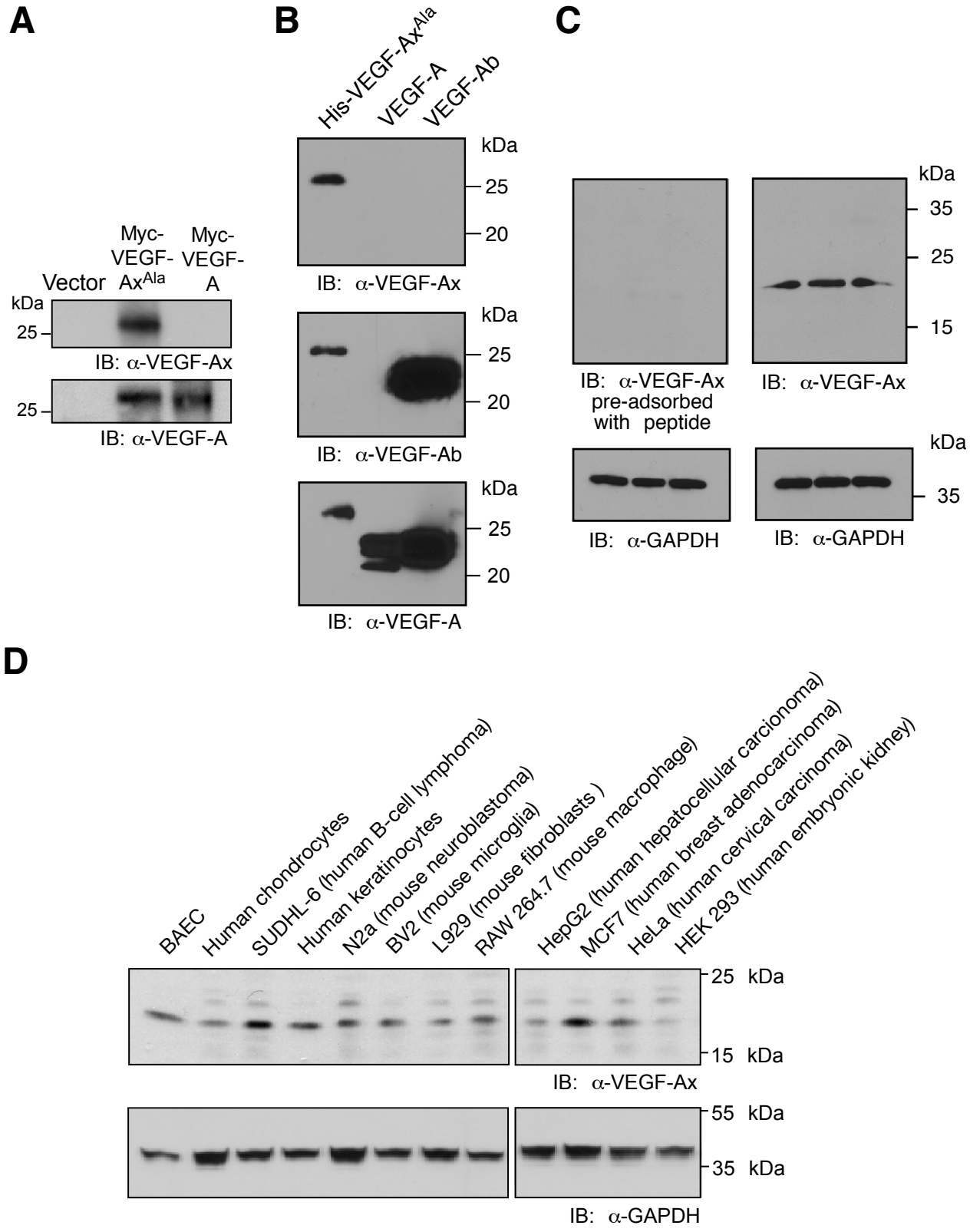

A
Vector
Myc-VEGF-Ax^{Ala}
Myc-VEGF-A
kDa
25
IB: α-VEGF-Ax
25
IB: α-VEGF-A
B
His-VEGF-Ax^{Ala}
VEGF-A
VEGF-Ab
kDa
25
20
IB: α-VEGF-Ax
kDa
25
20
IB: α-VEGF-Ab
kDa
25
20
IB: α-VEGF-A
C
IB: α-VEGF-Ax pre-adsorbed with peptide
kDa
35
25
15
IB: α-VEGF-Ax
IB: α-GAPDH
kDa
35
IB: α-GAPDH
D
BAEC
Human chondrocytes
SUDHL-6 (human B-cell lymphoma)
Human keratinocytes
N2a (mouse neuroblastoma)
BV2 (mouse microglia)
L929 (mouse fibroblasts)
RAW 264.7 (mouse macrophage)
HepG2 (human hepatocellular carcionoma)
MCF7 (human breast adenocarcinoma)
HeLa (human cervical carcinoma)
HEK 293 (human embryonic kidney)
25 kDa
15 kDa
IB: α-VEGF-Ax
55 kDa
35 kDa
IB: α-GAPDH

Figure S3

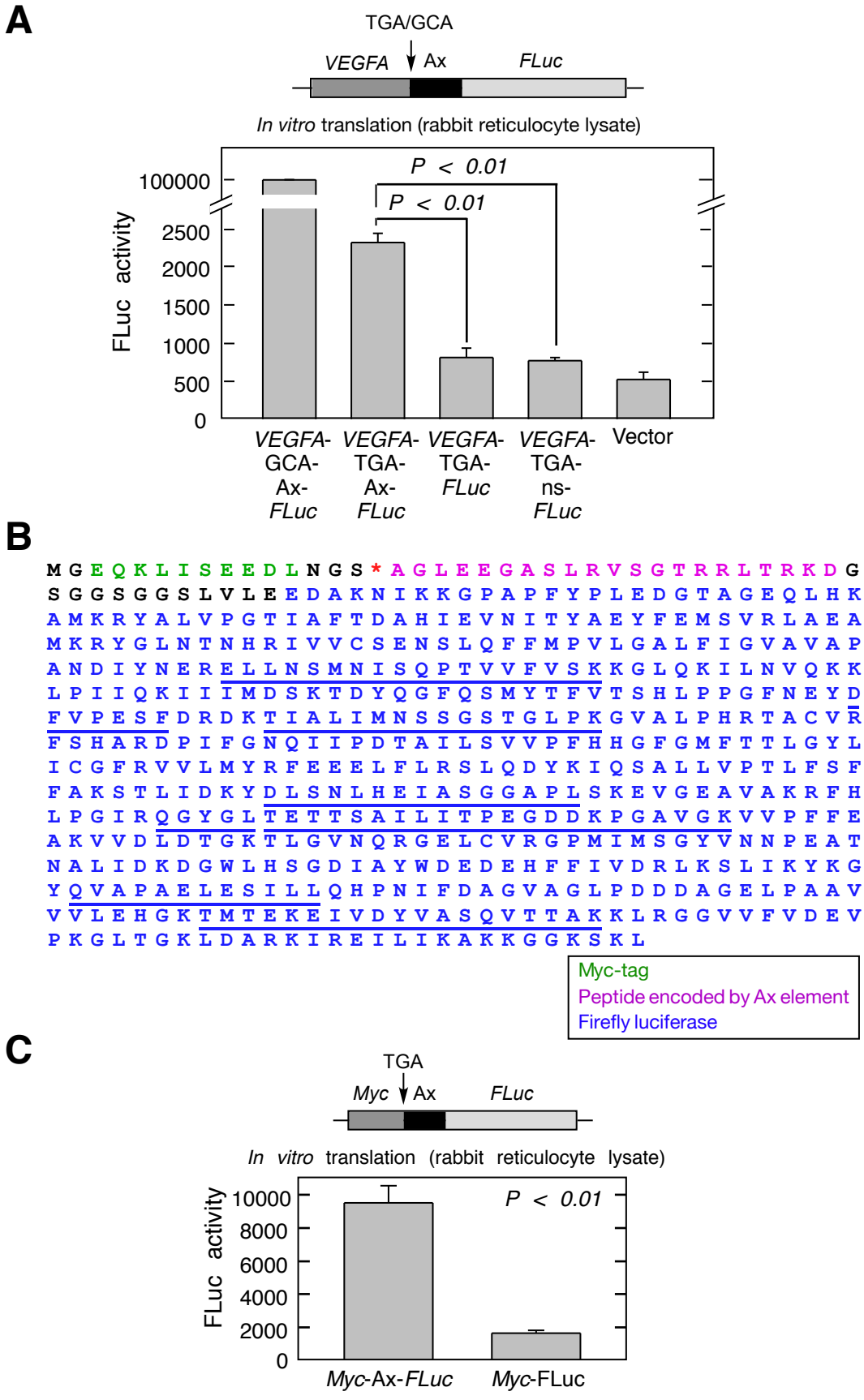
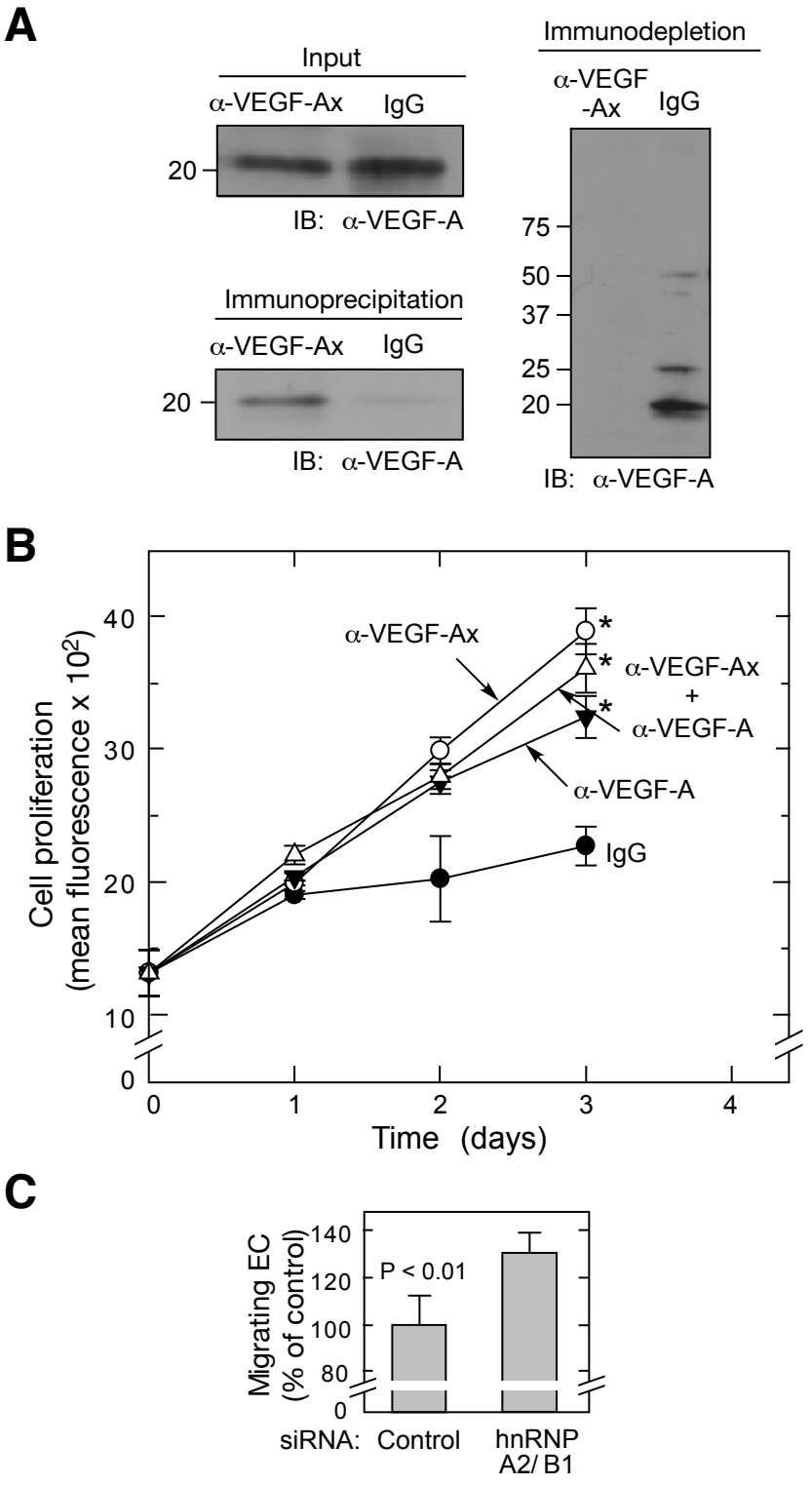

A
TGA/GCA
VEGFA
Ax
FLuc
In vitro translation (rabbit reticulocyte lysate)
FLuc activity
100000
2500
2000
1500
1000
500
0
P < 0.01
P < 0.01
VEGFA-GCA-Ax-FLuc
VEGFA-TGA-Ax-FLuc
VEGFA-TGA-FLuc
VEGFA-TGA-ns-FLuc
Vector
B
MGEQKLISEEDLNGS*AGLEEGASLRVSGTRRLTRKDG
SGGSGGSLVLEEDAKNIKKGPAPFYPLEDGTAGEQLHK
AMKRYALVPGTIAFTDAHIEVNITYAEYFEMSVRLAEA
MKRYGLNTNHRIVVCSENSLQFFMPVLGALFIGVAVAP
ANDIYNERELLNSMNISQPTVVFVSKKGLQKILNVQKK
LPIIQKIIIMDSKTDYQGFQSMYTFVTSHLPPGFNEYD
FVPESFDRDKTIALIMNSSGSTGLPKGVALPHRTACVR
FSHARDPIFGNQIIPDTAILSVVPFHHGFGMFTTLGYL
ICGFRVVLMYRFEEELFLRSLQDYKIQSALLVPTLFSF
FAKSTLIDKYDLSNLHEIASGGAPLSKEVGEAVAKRFH
LPGIRQGYGLTETTSAILITPEGDDKPGAVGKVVPFFE
AKVVDLDTGKTLGVNQRGELCVRGPMIMSGYVNNPEAT
NALIDKDGWLHSGDIAYWDEDEHFFIVDRLKSLIKYKG
YQVAPAELESILLQHPNIFDAGVAGLPDDDAGELPAAV
VVLEHGKTMTEKEIVDYVASQVTTAKKLRGGVVFVDEV
PKGLTGKLDARKIREILIKAKKGGKSKL
Myc-tag
Peptide encoded by Ax element
Firefly luciferase
C
TGA
Myc
Ax
FLuc
In vitro translation (rabbit reticulocyte lysate)
FLuc activity
10000
8000
6000
4000
2000
0
P < 0.01
Myc-Ax-FLuc
Myc-FLuc

Figure S4


A
Input
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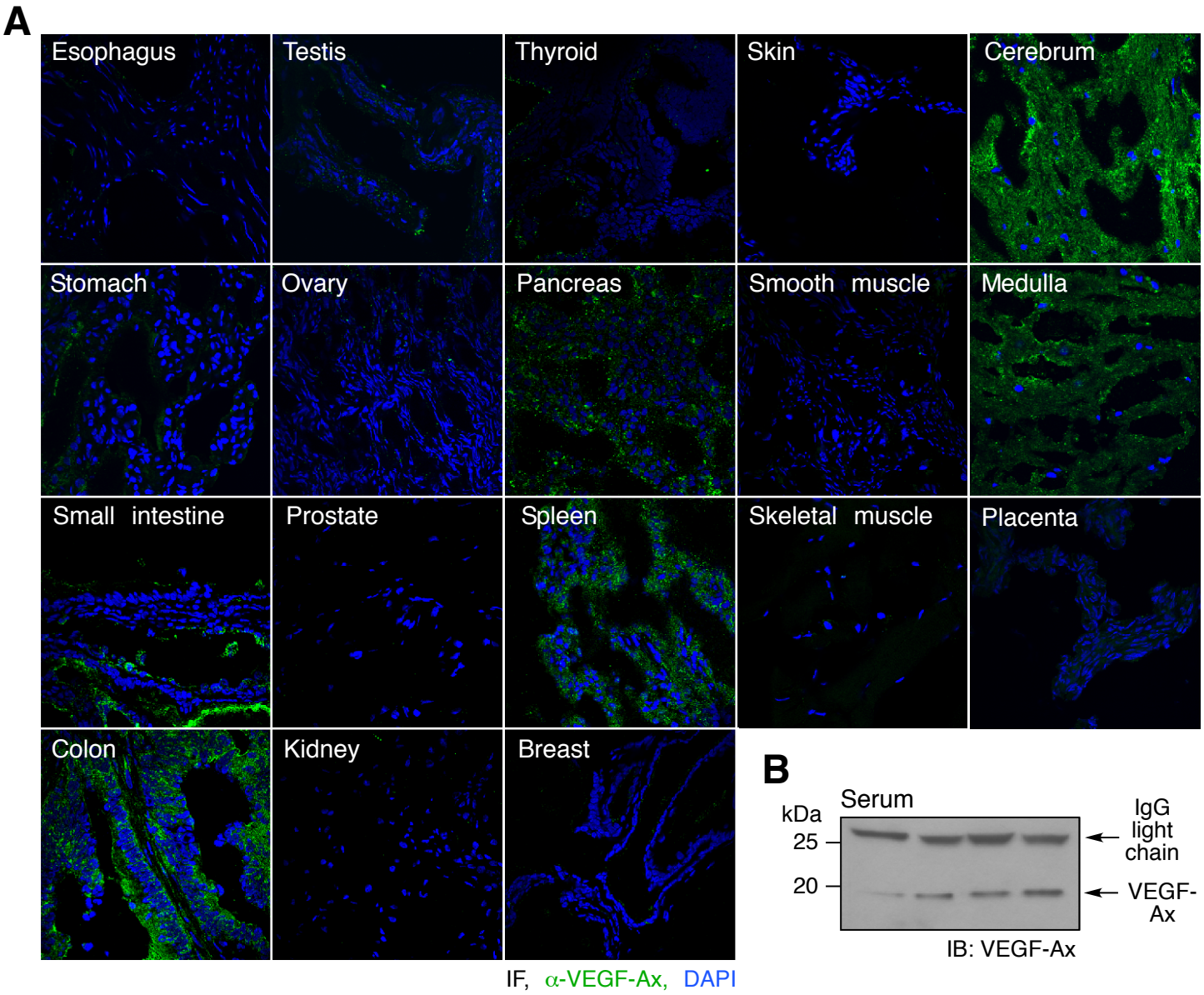
IgG
20
IB: α-VEGF-A
Immunoprecipitation
α-VEGF-Ax
IgG
20
IB: α-VEGF-A
Immunodepletion
α-VEGF -Ax
IgG
75
50
37
25
20
IB: α-VEGF-A
B
Cell proliferation (mean fluorescence x 10²)
40
30
20
10
0
0
1
2
3
4
Time (days)
α-VEGF-Ax
α-VEGF-Ax + α-VEGF-A
α-VEGF-A
IgG
*
C
Migrating EC (% of control)
140
120
100
80
0
P < 0.01
siRNA: Control
hnRNP A2/ B1

Figure S5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Esophagus
Testis
Thyroid
Skin
Cerebrum
Stomach
Ovary
Pancreas
Smooth muscle
Medulla
Small intestine
Prostate
Spleen
Skeletal muscle
Placenta
Colon
Kidney
Breast
IF, α-VEGF-Ax, DAPI
B
Serum
kDa
25
20
IgG light chain
VEGF-Ax
IB: VEGF-Ax
C
Control
Pre-absorbed, Ax peptide
Colon
IF, α-VEGF-Ax,
DAPI

Supplemental Figure6

Figure S6