SUPPLEMENT MATERIAL

ONLINE MATERIALS & METHODS

Identification and cloning of full length zebrafish AGM cDNA

To identify the zebrafish orthologue of AGM a NCBI-TBLASTN search was performed using human and mouse AGM (NCBI Accession # NM_001553 & NM_008048, respectively) against the zebrafish genome. The matching stretch of genomic DNA was compared to zebrafish cDNA database. The I.M.A.G.E. clone 6969595 was identified and subsequently obtained from ATCC (Manassus, VA).

Online Table I. ***Homology of zebrafish angiomodulin to predicted and reported orthologues in other species.***

Species	NCBI Accession #	Homology to zebrafish angiomodulin
Mouse	NP_032074	50%
Human	NP_001544	54%
Rat	NP_001013066	52%
Dog	XP_850270	53%
Chicken	XP_420577	51%
Rainbow Trout	ABA33953	65%

Online Table II. ***Homology of angiomodulin to reported members of the IGFBP family in zebrafish at the amino acid level.***

Zebrafish IGFBP family member	NCBI Accession #	Homology to angiomodulin*	Homology within IGFBP domain*	Conserved cysteine residues
IGFBP-1	NP_775390	9%	29%	10
IGFBP-2	NP_571533	15%	38%	12
IGFBP-3	NP_991314	10%	33%	10
IGFBP-5	AAM51549	11%	35%	12
IGFBP-rP2	NP_001015041	14%	31%	10
IGFBP-rP4	NP_001074456	7%	25%	9
IGFBP-rP5	NP_001002219	22%	44%	16
IGFBP-rP9	XP_695243	15%	34%	8

** Sequences were aligned using the NCBI BLAST alignment tool and the Prosite database of protein domains, families and functional sites.*

Online Table III. ***Comparison of phenotypes induced by translational vs. splice morpholinos targeting AGM at optimal doses.***

Phenotype	ATG-MO 10 ng	ATG-MO 5 ng	SPL-MO 10 ng
Absent heartbeat (28 hpf)	32%	2%	8%
Defective hematopoiesis (28 hpf)	77%	36%	24%
Yolk extension defect (28 hpf)	97%	76%	3%
Cardiac edema (28 hpf)	49%	60%	89%
Axial circulation defect (48 hpf)	60%	18%	24%
ISV circulation defect (48 hpf)	98%	80%	86%

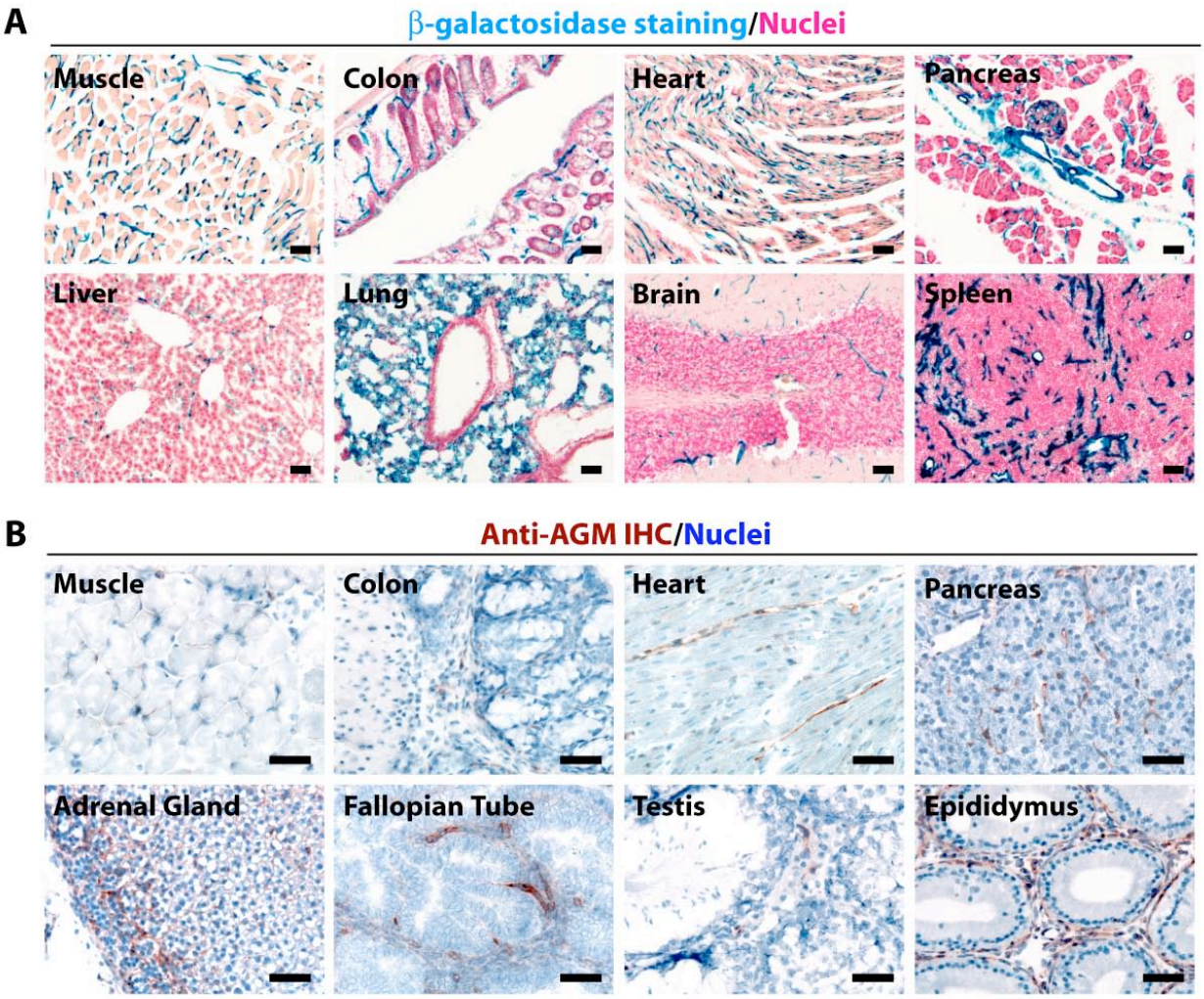
Online Table IV. ***Comparison of angiogenic phenotypes in fli1:EGFP embryos induced by translational vs. splice morpholinos targeting AGM at optimal doses (as compared to control).***

Phenotype	ATG-MO 10 ng	ATG-MO 5 ng	SPL-MO 10 ng
Reduced sprout length (28 hpf)	88%	70%	77%
Reduced protrusive activity (28 hpf)	72%	50%	16%
Complete inhibition of sprouting (28 hpf)	11%	13%	0%
Angiogenesis defect (28 hpf)	99%	83%	77%
Incomplete DLAV (48 hpf)	95%	75%	14%
Angiogenesis defect (48 hpf)	98%	82%	86%

ONLINE FIGURES & LEGENDS

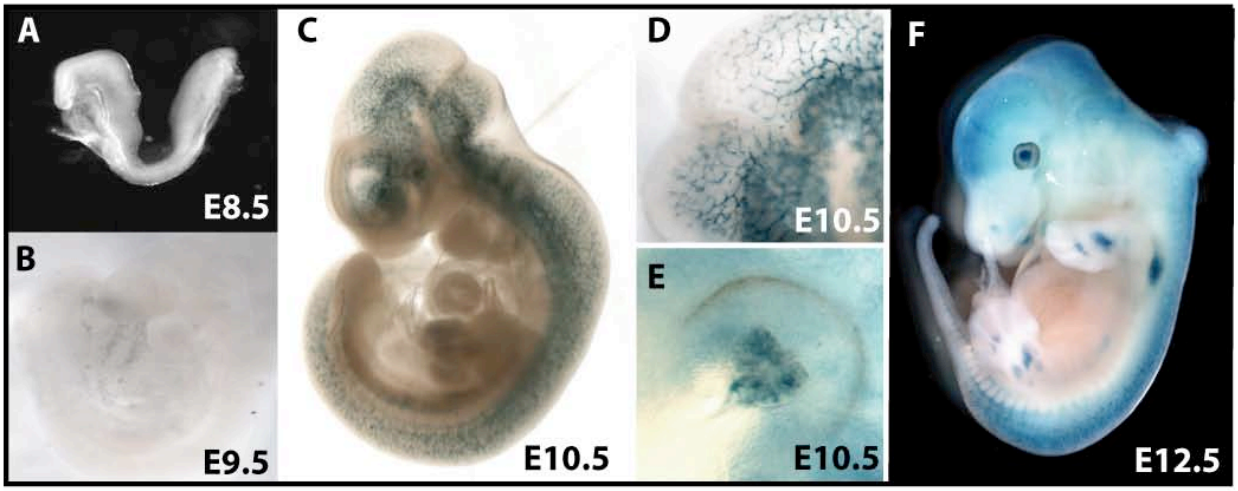
Online Figure I. Expression of AGM in normal mouse tissues. A) Tissues from AGM$^{lacZ/+}$ mice were processed for X-gal staining for the detection of β-gal activity using the enhanced method of detection, whereby animals were perfused with 0.2% GA. As shown, AGM, as measured by β-gal reporter activity, is expressed in the vasculature, both SMC-invested large vessels and capillaries, in all organs, except for liver where it is expressed at low level only in selected vessel structures. AGM$^{lacZ/+}$ mice can, therefore, be used as a vascular reporter model. *Micron bar = 50 μm.* B) Tissue from WT mice were snap-frozen and immunostained with anti-AGM Ab. AGM protein is weakly to moderately expressed in the vasculature and vascular rich tissues, corroborating the data from the AGM$^{lacZ/+}$ reporter mouse model. *Micron bar = 50 μm.*

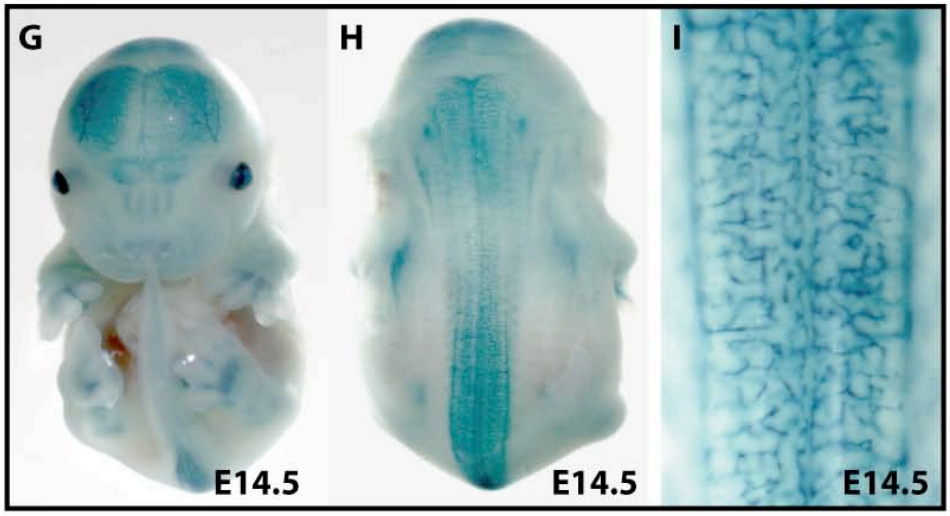
Online Figure I.



Online Figure II. AGM is expressed in the vasculature during embryogenesis. AGM expression was assessed during embryogenesis in AGM$^{lacZ/+}$ mice. Embryos were harvested at various time points post-coitus as follows: E8.5 (A), E9.5 (B), E10.5 (C-E), E12.5 (F), E14.5 (G-I). Whole mounts were stained for β-gal activity. Note strong AGM expression localized to the vasculature during development.

Online Figure II.





Online Figure III. Molecular characterization of zebrafish AGM. A) Amino acid alignment of zebrafish (Danio rerio) AGM with mouse (Mus musculus) and human (Homo sapiens) AGM demonstrating 50 & 54% homology at the amino acid level, respectively. Identical residues are shaded. B) Phylogenetic tree analysis of zebrafish AGM in relation to AGM published and predicted amino acid sequences from mouse, rat, dog, human, chicken and rainbow trout.

Online Figure III.

A

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Dr.   M----LCVLVLA------LLASASAQRLSRGCGSCDRTQCAPLPAQGCSSGTLRD
Mm.   MERPP-RALLLGAAGLLLLLLPLSSSSSSDACGPCVPASCPALPRLGCPLGETRD
Hs.   MERPSLRALLLGAAGLLLLLLPLSSSSSSDTCGPCEPASCPPLPPLGCLLGETRD

Dr.   SCGCCELCAAAEGEFCGGRGVSARRCGSGLECVKPDKKSKS----------GI
Mm.   ACGCCPVCARGEGEPCGGGAAGGGHCAPGMECVKSRKRRRGKAGAAAGGPATLAV
Hs.   ACGCCPMCARGEGEPCGGGGAGRGYCAPGMECVKSRKRRKGKAGAAAGGPGVSGV

Dr.   CVCKSGVPVCGTDGRNYNSGCALRAASARALQEKQPEIRVHNTGRCAQGPEIVTP
Mm.   CVCKSRYPVCGSNGITYPSGCQLRAASLRAESRGEKAITQVSKGTCEQGPSIVTP
Hs.   CVCKSRYPVCGSDGTTYPSGCQLRAASQRAESRGEKAITQVSKGTCEQGPSIVTP

Dr.   PGEVWNITGSQVFLSCEAIGVPTPVLTWRKVS---GGSEKPVPLPGDKDNLAVQT
Mm.   PKDIWNVTGAKVFLSCEVIGIPTPVLIWNKVKRDHSGVQRTELLPGDRENLAIQT
Hs.   PKDIWNVTGAQVYLSCEVIGIPTPVLIWNKVKRGHYGVQRTELLPGDRDNLAIQT

Dr.   RGGPEKHQVTGWVLISPLTKAEDGSYECHASNTQGEASAVGSIHVVESISDIPVQ
Mm.   RGGPEKHEVTGWVLVSPLSKEDAGEYECHASNSQGQASAAAKITVVDALHEIPLK
Hs.   RGGPEKHEVTGWVLVSPLSKEDAGEYECHASNSQGQASASAKITVVDALHEIPVK

Dr.   MVKDSEL
Mm.   KGEGAQL
Hs.   KGEGAEL
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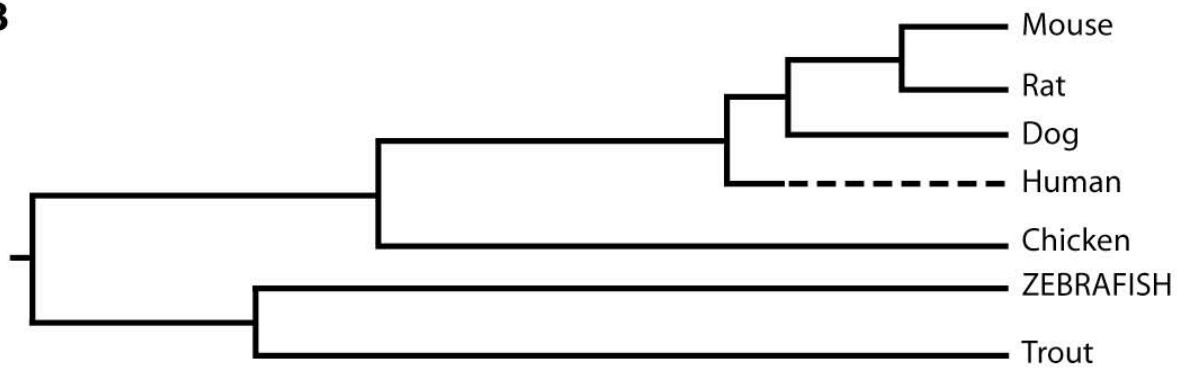
B



ONLINE MOVIE LEGENDS

Online Movie I. Angiogenesis is impaired in AGM morphants. Angiogenic sprouting of ISV from the DA is severely impaired in embryos injected with either ATG-MO or SPL-MO. Embryo shown here was injected with 20ng SPL-MO and was imaged at 48 hpf. Note that the ISV are mispatterned.

Online Movie II. AGM and VEGF interact in the same pathway. At a subeffective dose of either ATG-MO or VEGF-MO alone, there was minimal effect on angiogenesis; however, simultaneous knockdown of both VEGF-A and AGM significantly disrupted angiogenic sprouting of ISV, suggesting that AGM and VEGF interact in the same genetic pathway. The sprouts that did form were grossly mispatterned and the DLAV was not formed (arrows). Left side: VEGF-MO alone, 0.5 ng. Right side: VEGF-MO and ATG-MO, 0.5 ng each injected simultaneously. Imaged at 48 hpf.