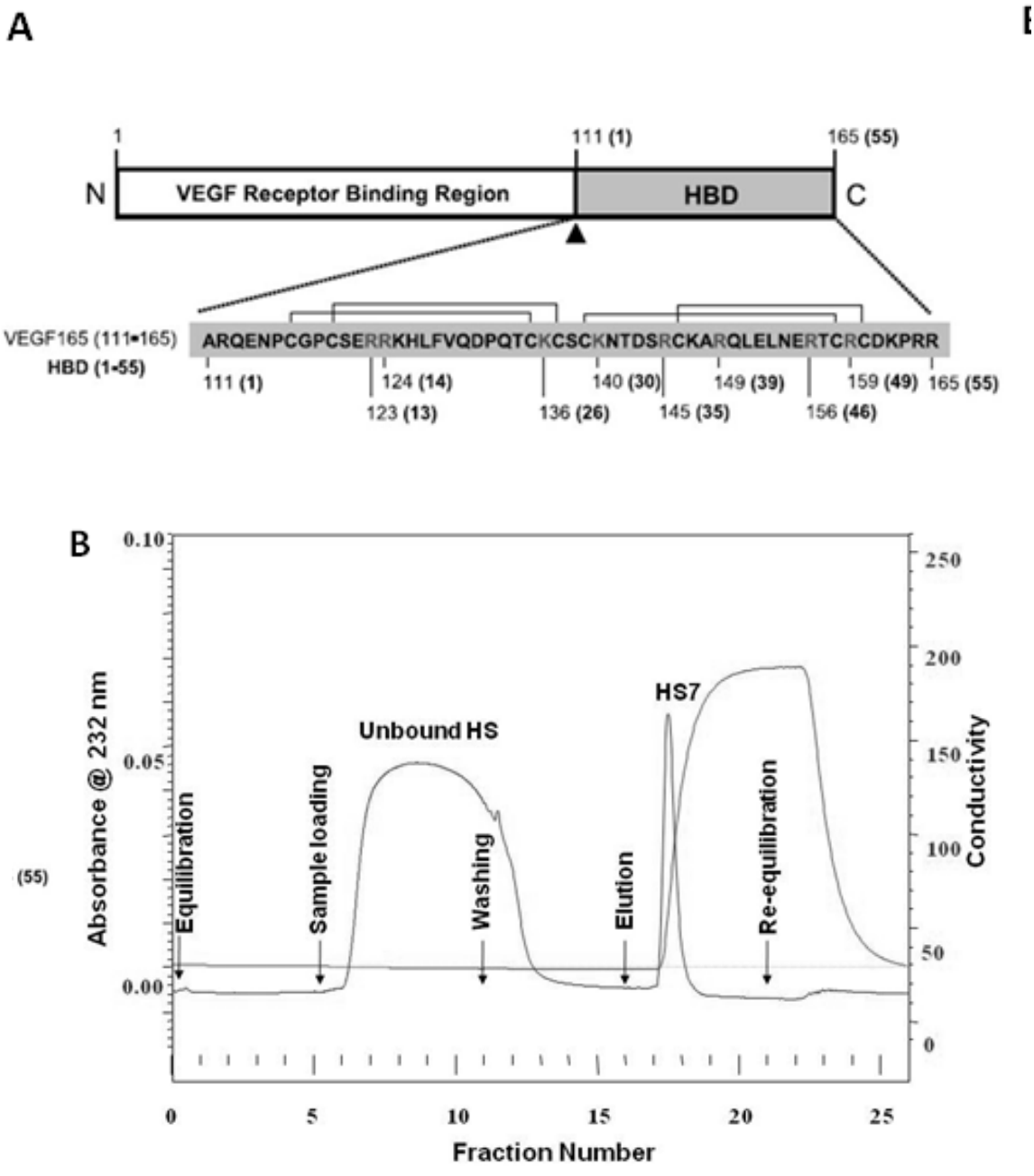

A
1
111 (1)
165 (55)
N
VEGF Receptor Binding Region
HBD
C
VEGF165 (111-165)
HBD (1-55)
ARQENPCGPCSERRKHLFVQDPQTCKCSCKNTDSRCKARQLELNERTCRCDKPRR
111 (1)
124 (14)
123 (13)
136 (26)
140 (30)
145 (35)
149 (39)
156 (46)
159 (49)
165 (55)
B
(55)
Absorbance @ 232 nm
Conductivity
Fraction Number
Equilibration
Sample loading
Unbound HS
Washing
Elution
HS7
Re-equilibration
0.10
0.05
0.00
250
200
150
100
50
0
0
5
10
15
20
25

FIGURE 1

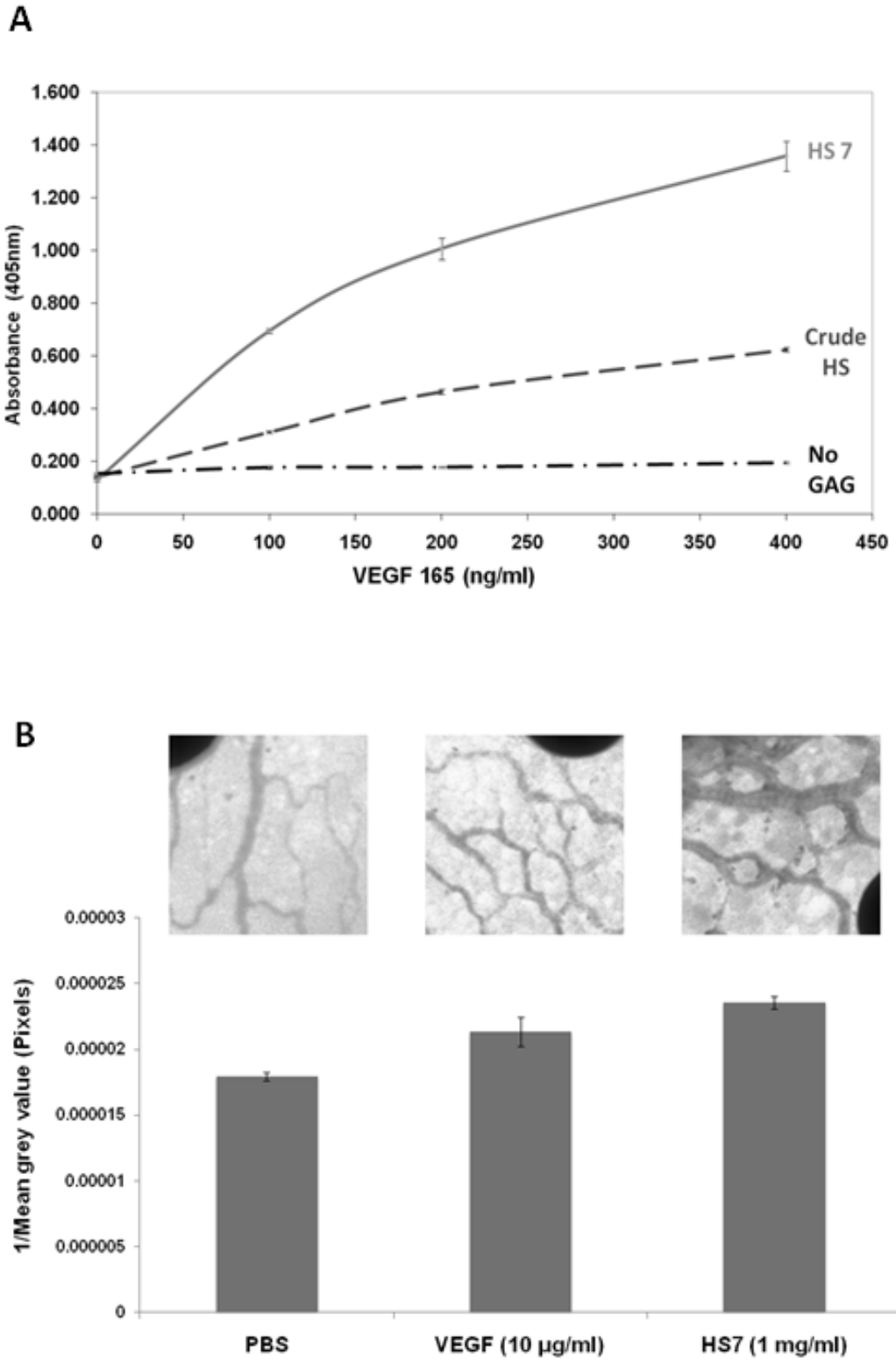

A
1.600
1.400
1.200
1.000
0.800
0.600
0.400
0.200
0.000
Absorbance (405nm)
0
50
100
150
200
250
300
350
400
450
VEGF 165 (ng/ml)
HS 7
Crude HS
No GAG
B
0.00003
0.000025
0.00002
0.000015
0.00001
0.000005
0
1/Mean grey value (Pixels)
PBS
VEGF (10 µg/ml)
HS7 (1 mg/ml)

FIGURE 2

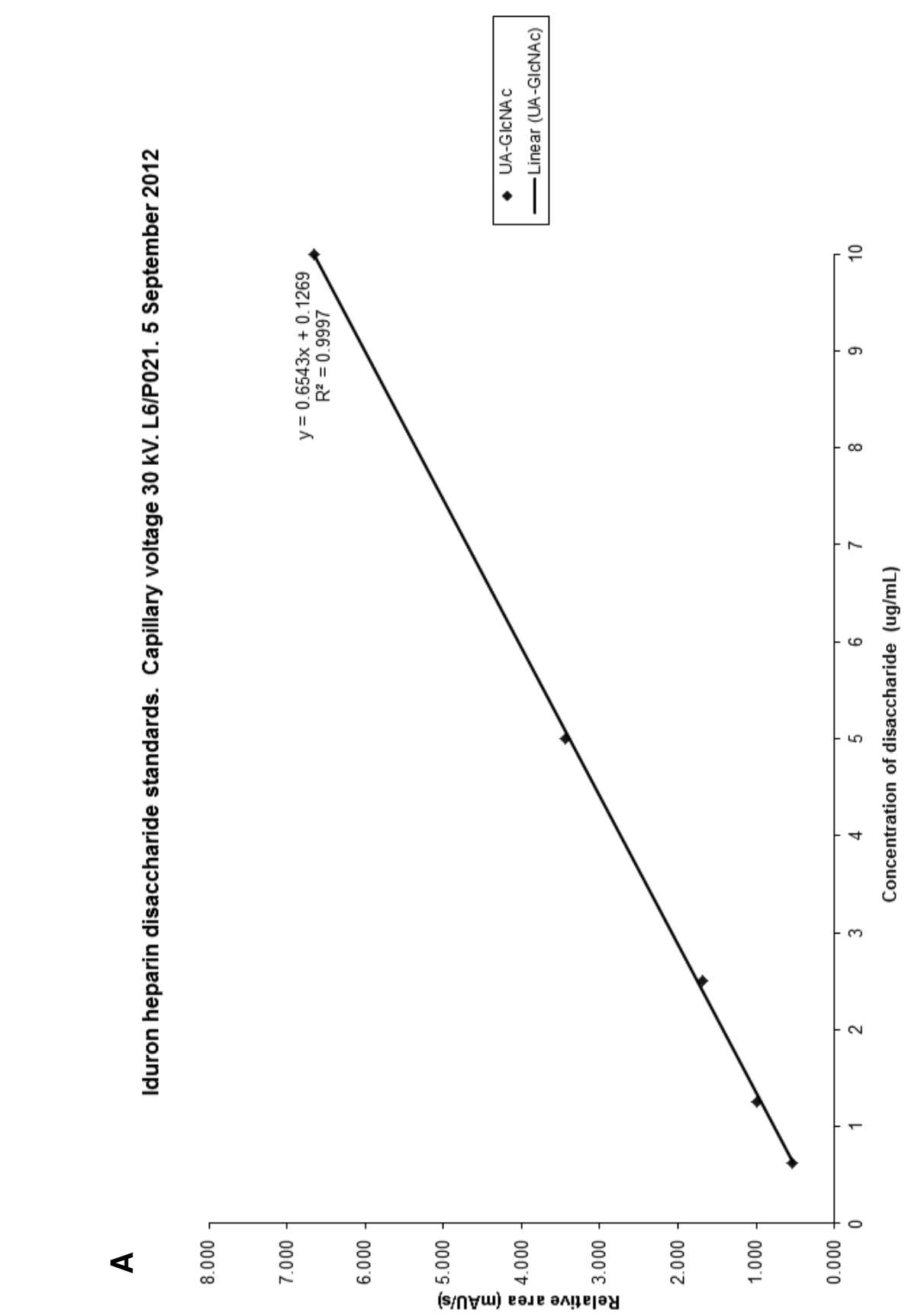

A
Iduron heparin disaccharide standards. Capillary voltage 30 kV. L6/P021. 5 September 2012
y = 0.6543x + 0.1269
R² = 0.9997
UA-GlcNAc
Linear (UA-GlcNAc)
Relative area (mAU/s)
Concentration of disaccharide (ug/mL)
0.000
1.000
2.000
3.000
4.000
5.000
6.000
7.000
8.000
0
1
2
3
4
5
6
7
8
9
10

FIGURE 3

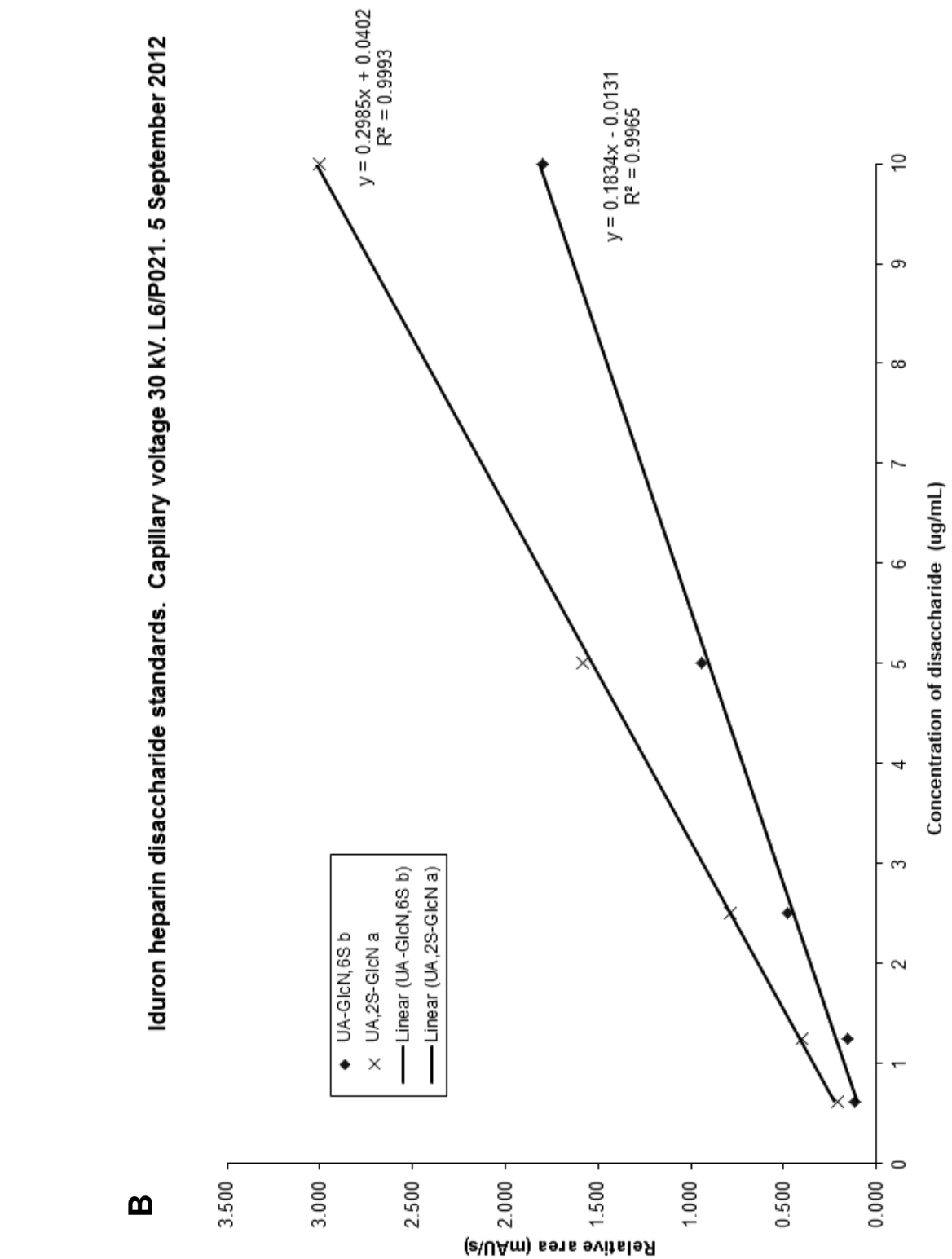

B
Iduron heparin disaccharide standards. Capillary voltage 30 kV. L6/P021. 5 September 2012
UA-GlcN,6S b
UA,2S-GlcN a
Linear (UA-GlcN,6S b)
Linear (UA,2S-GlcN a)
y = 0.2985x + 0.0402
R² = 0.9993
y = 0.1834x - 0.0131
R² = 0.9965
Relative area (mAU/s)
Concentration of disaccharide (ug/mL)
0.000
0.500
1.000
1.500
2.000
2.500
3.000
3.500
0
1
2
3
4
5
6
7
8
9
10

FIGURE 3

C

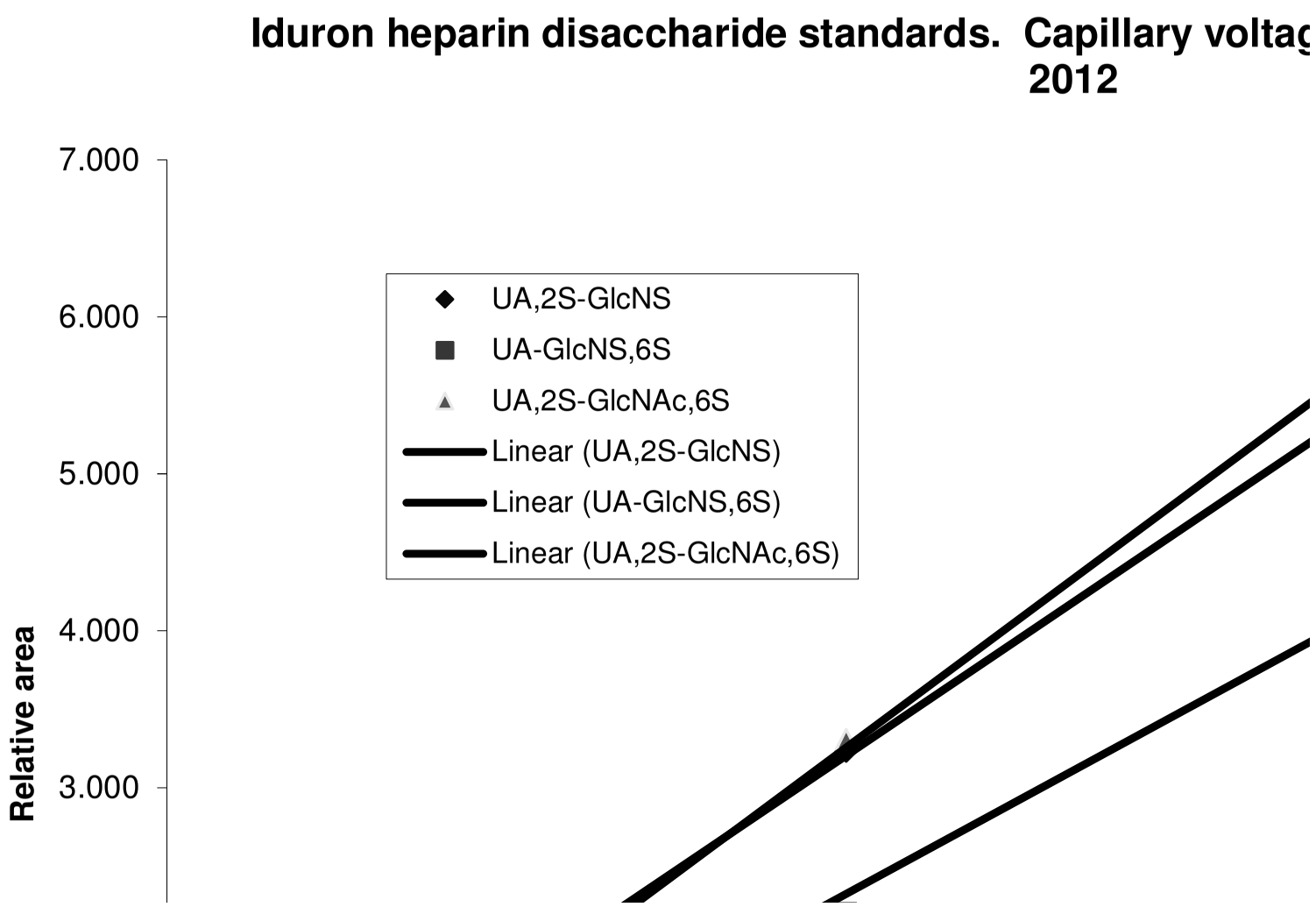

Iduron heparin disaccharide standards. Capillary voltag
2012
7.000
6.000
5.000
4.000
3.000
Relative area
UA,2S-GlcNS
UA-GlcNS,6S
UA,2S-GlcNAc,6S
Linear (UA,2S-GlcNS)
Linear (UA-GlcNS,6S)
Linear (UA,2S-GlcNAc,6S)

FIGURE 3

D

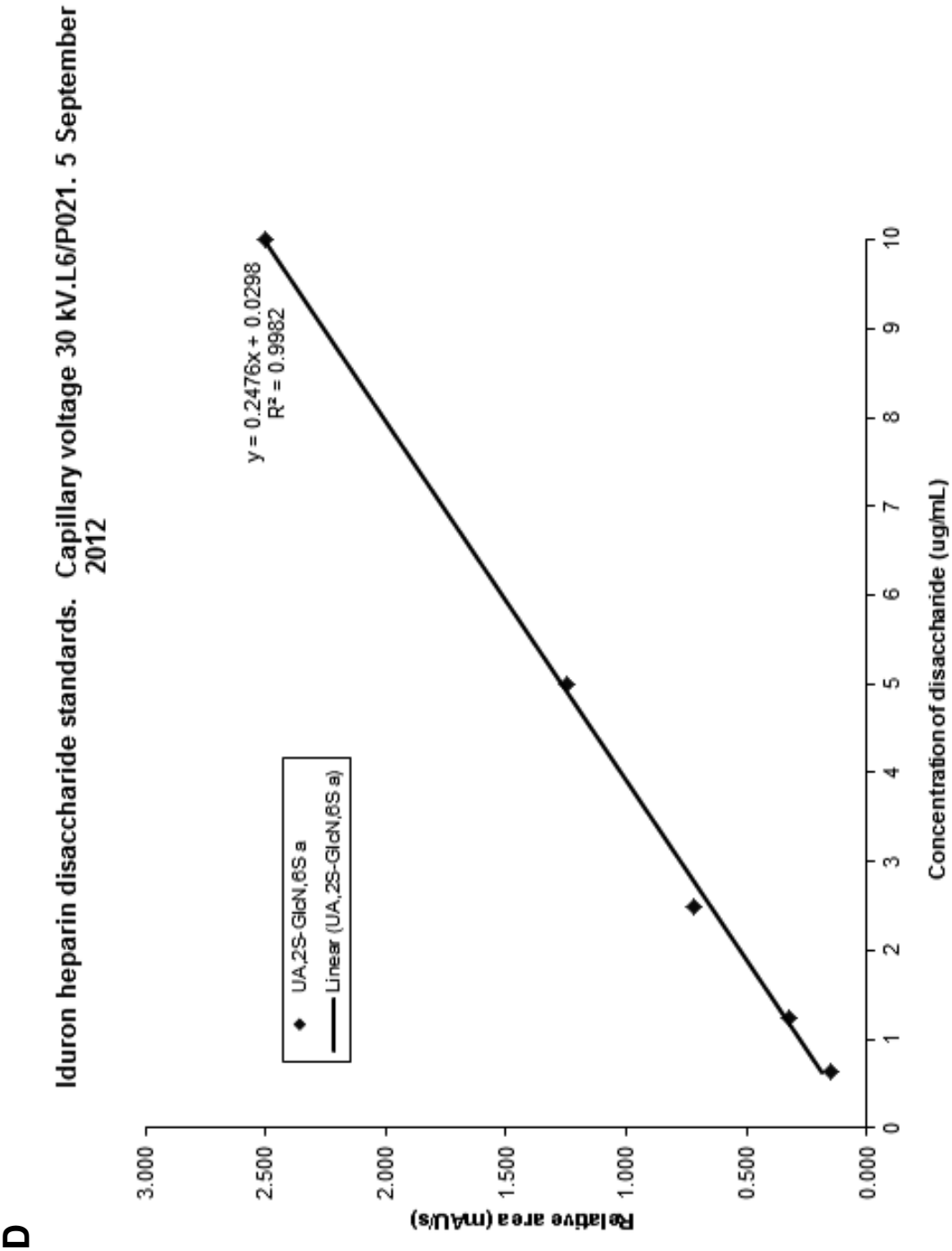

Iduron heparin disaccharide standards. Capillary voltage 30 kV.L6/P021. 5 September 2012
y = 0.2476x + 0.0298
R² = 0.9982
UA,2S-GlcN,6S a
Linear (UA,2S-GlcN,6S a)
Relative area (mAU/s)
Concentration of disacchanide (ug/mL)
0.000
0.500
1.000
1.500
2.000
2.500
3.000
0
1
2
3
4
5
6
7
8
9
10

FIGURE 3

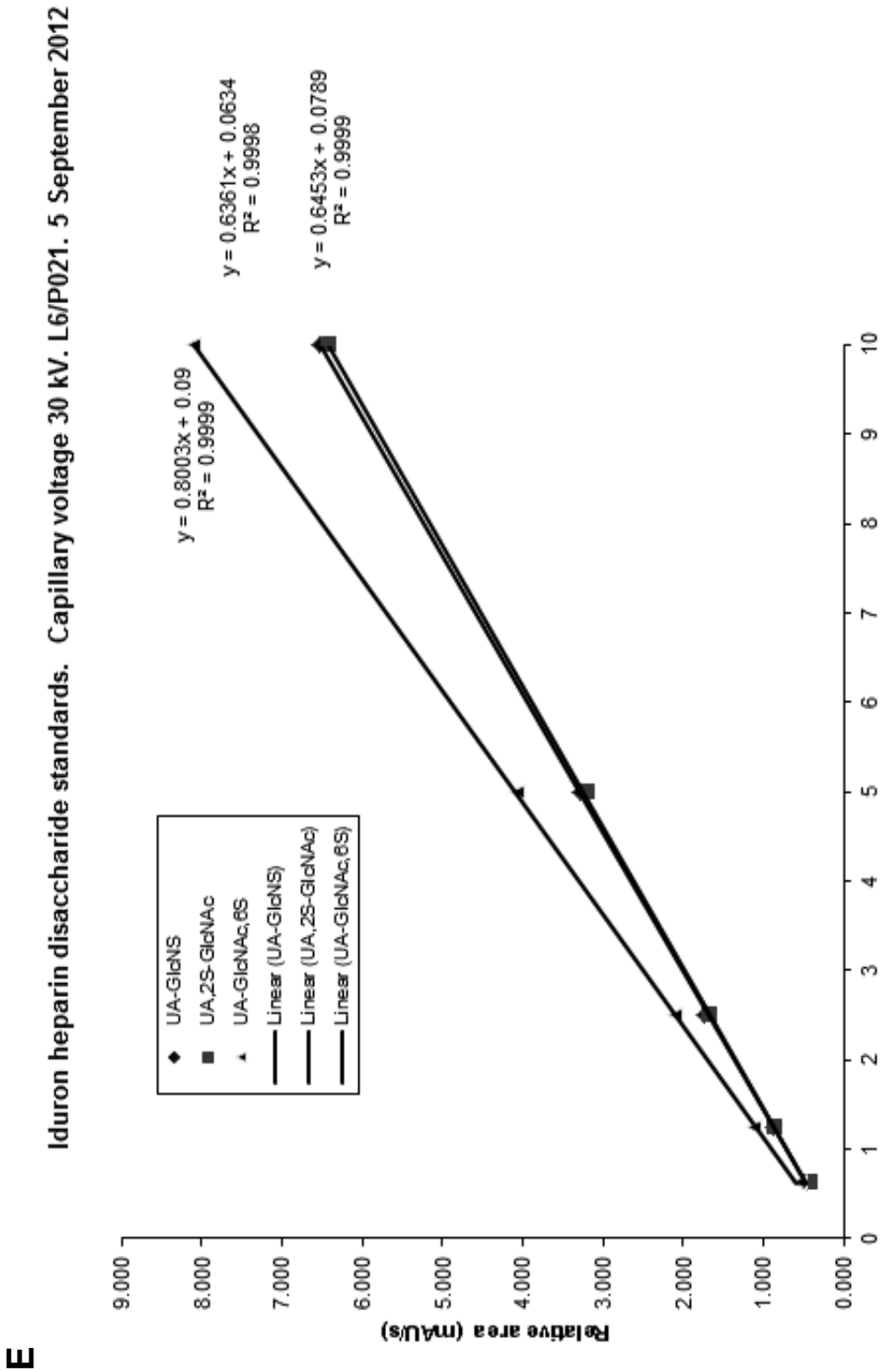

E
Iduron heparin disaccharide standards. Capillary voltage 30 kV. L6/P021. 5 September 2012
y = 0.8003x + 0.09
R² = 0.9999
y = 0.6361x + 0.0634
R² = 0.9998
y = 0.6453x + 0.0789
R² = 0.9999
UA-GlcNS
UA,2S-GlcNAc
UA-GlcNAc,6S
Linear (UA-GlcNS)
Linear (UA,2S-GlcNAc)
Linear (UA-GlcNAc,6S)
Relative area (mAU/s)
Concentration of disaccharide (ug/mL)
0.000
1.000
2.000
3.000
4.000
5.000
6.000
7.000
8.000
9.000
0
1
2
3
4
5
6
7
8
9
10

FIGURE 3

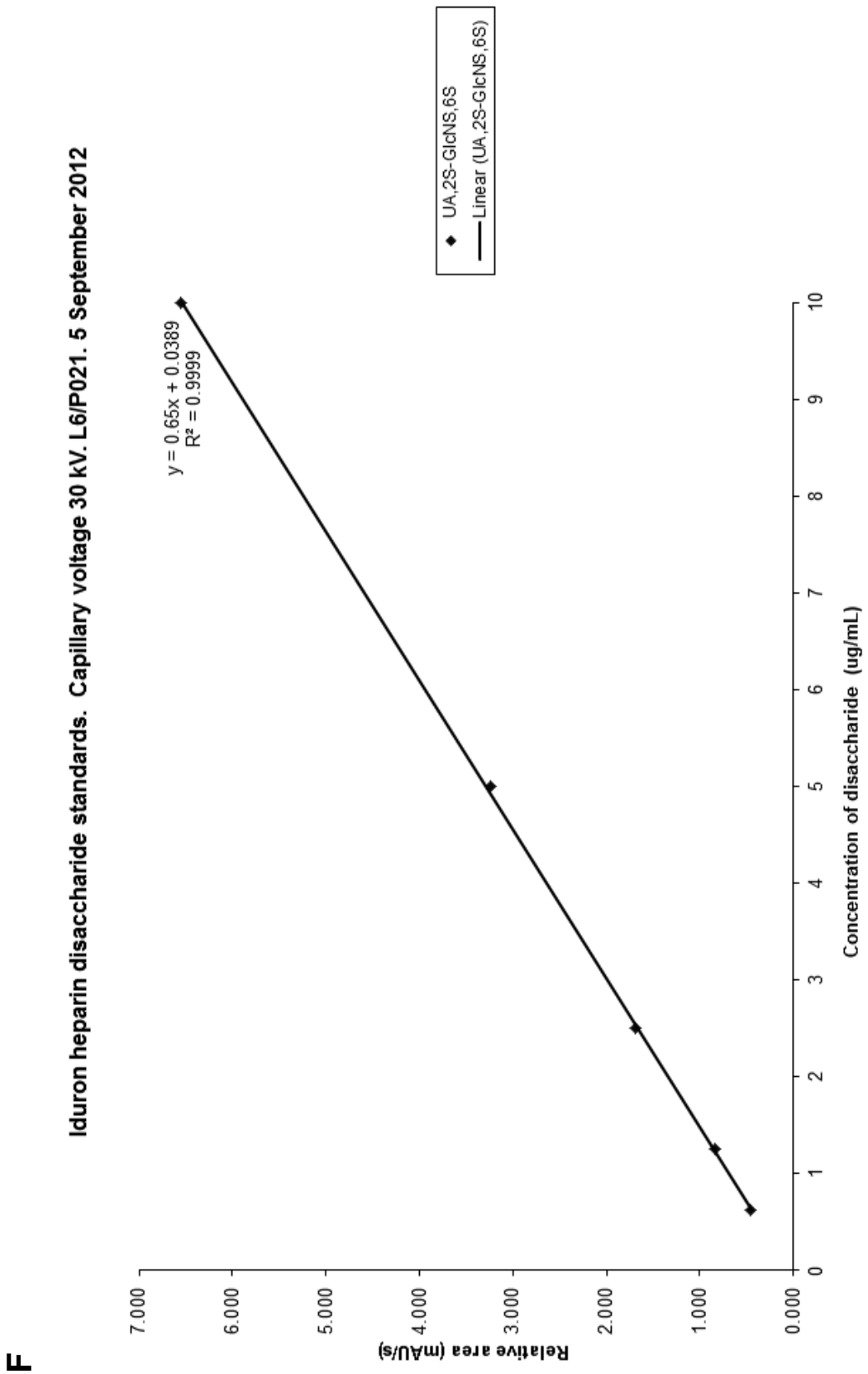

Iduron heparin disaccharide standards. Capillary voltage 30 kV. L6/P021. 5 September 2012
y = 0.65x + 0.0389
R² = 0.9999
UA,2S-GlcNS,6S
Linear (UA,2S-GlcNS,6S)
Relative area (mAU/s)
Concentration of disaccharide (ug/mL)
0.000
1.000
2.000
3.000
4.000
5.000
6.000
7.000
0
1
2
3
4
5
6
7
8
9
10

FIGURE 3

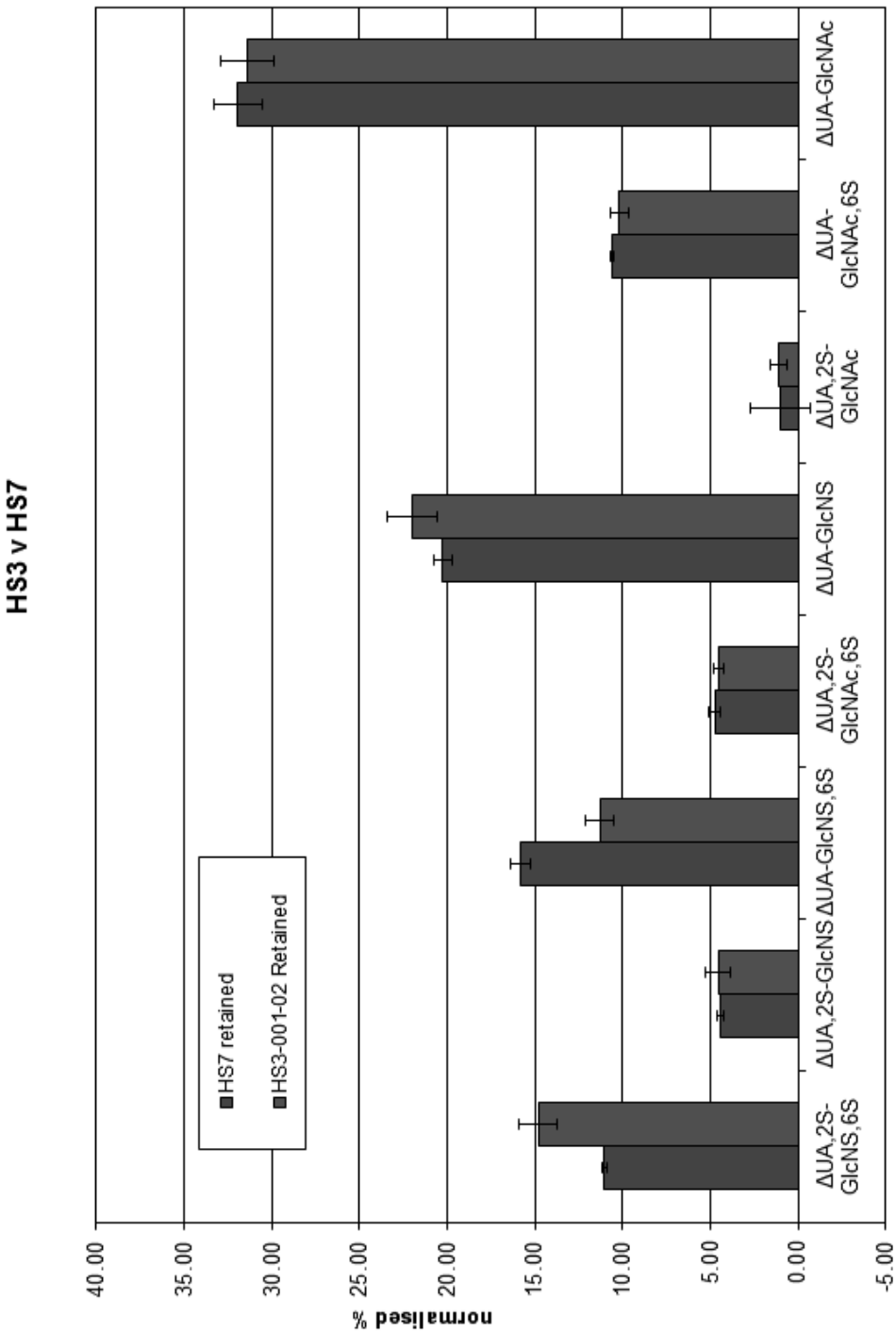

HS3 v HS7
HS7 retained
HS3-001-02 Retained
normalised %
40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
-5.00
ΔUA,2S-GlcNS,6S
ΔUA,2S-GlcNS
ΔUA-GlcNS,6S
ΔUA,2S-GlcNAc,6S
ΔUA-GlcNS
ΔUA,2S-GlcNAc
ΔUA-GlcNAc,6S
ΔUA-GlcNAc
Heparin disaccharide

FIGURE 4

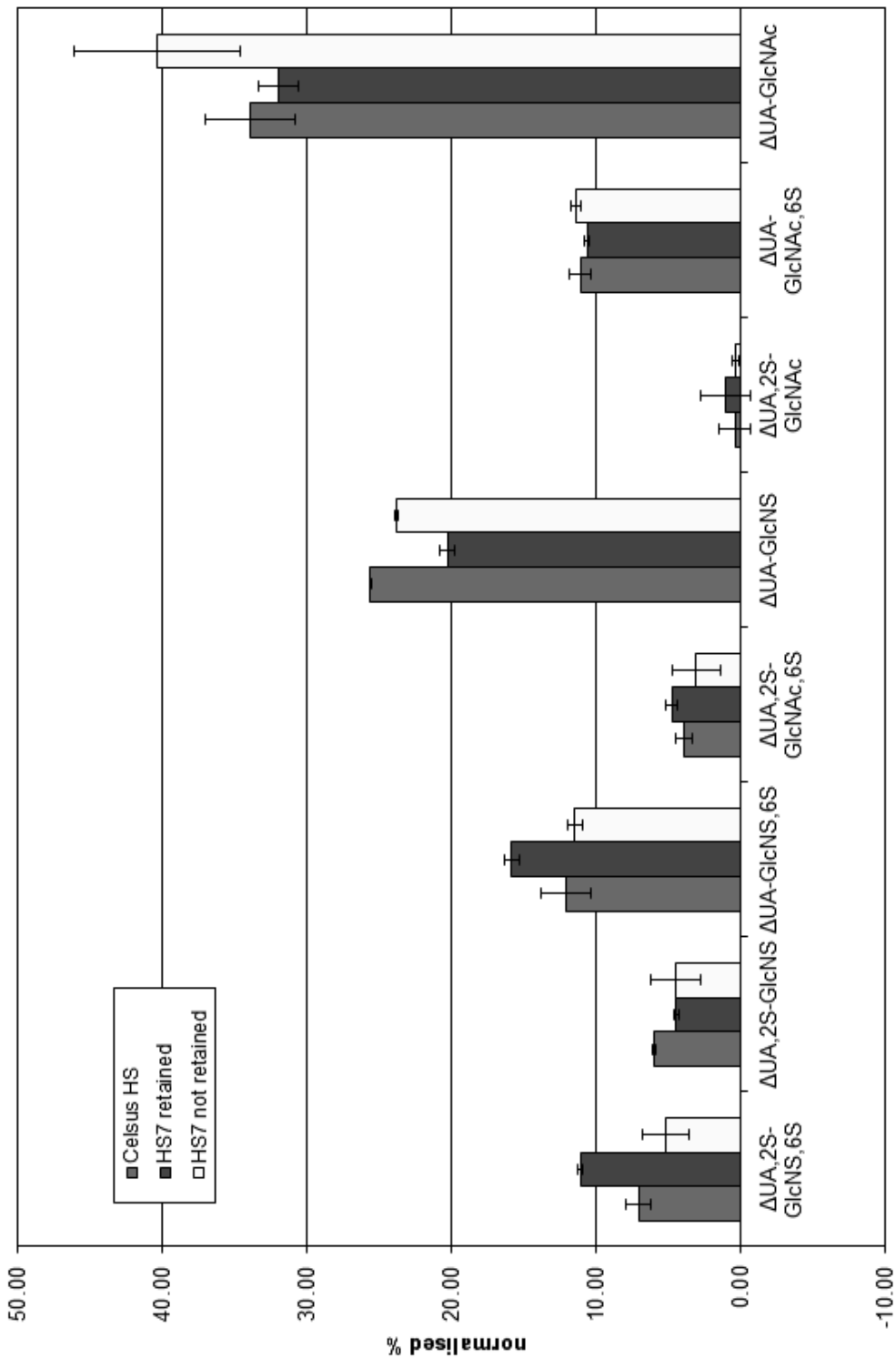
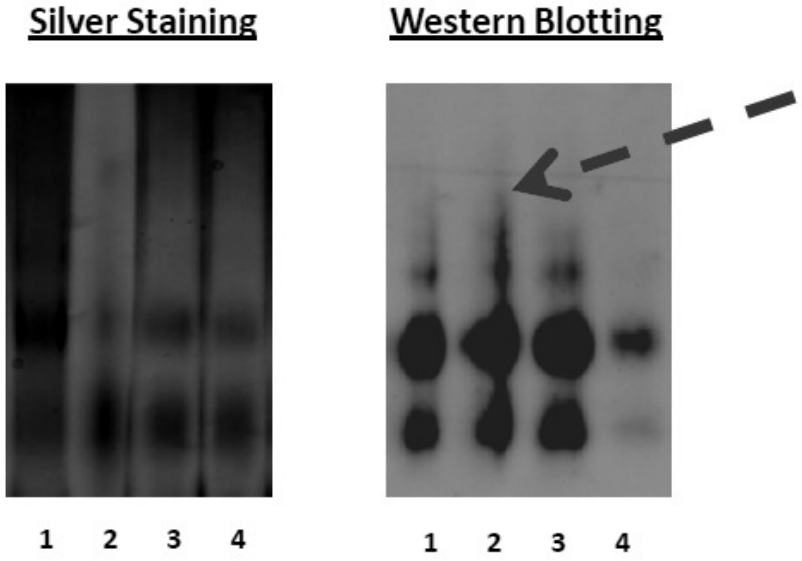

Celsus HS
HS7 retained
HS7 not retained
normalised %
50.00
40.00
30.00
20.00
10.00
0.00
-10.00
ΔUA,2S-GlcNS,6S
ΔUA,2S-GlcNS
ΔUA-GlcNS,6S
ΔUA,2S-GlcNAc,6S
ΔUA-GlcNS
ΔUA,2S-GlcNAc
ΔUA-GlcNAc,6S
ΔUA-GlcNAc
Heparin disaccharide

FIGURE 5

Alpha																	
0.05		L6P021															
			% normalised disaccharide									normalised %					
			ΔUA,2S-GlcNS,6S	ΔUA,2S-GlcNS	ΔUA-GlcNS,6S	ΔUA,2S-GlcNAc,6S	ΔUA-GlcNS	ΔUA,2S-GlcNAc	ΔUA-GlcNAc,6S	ΔUA-GlcNAc			A-NS	A-Nac	A-6S	UA-2S	
	Celsus HS #1067	tjb-Hsa-032-1	7.00	5.95	12.19	3.99	25.59	0.48	11.13	33.67		Celsus HS #1067	51	49	34	17	
		tjb-Hsa-032-1	7.13	5.98	11.92	3.90	25.61	0.30	11.01	34.16			51	49	34	17	
			7.06	**5.96**	**12.05**	**3.95**	**25.60**	**0.39**	**11.07**	**33.92**	**Mean**		**51**	**49**	**34**	**17**	**Mean**
			0.83	0.14	1.72	0.59	0.09	1.14	0.76	3.14							
												HS7 retained	52	48	42	21	
													51	49	42	23	
	HS7 retained	tjb-Hsa-032-3	11.08	4.60	15.82	5.01	20.71	0.45	10.53	31.79			52	48	42	20	
		tjb-Hsa-032-3	11.21	4.49	15.36	4.90	20.01	2.65	10.60	30.79			52	48	42	20	
		tjb-Hsa-032-4	11.03	4.41	16.13	4.58	20.03	0.40	10.69	32.74			**52**	**48**	**42**	**21**	**Mean**
		tjb-Hsa-032-4	11.01	4.35	16.03	4.56	20.35	0.53	10.69	32.49							
			11.08	**4.46**	**15.84**	**4.76**	**20.27**	**1.01**	**10.63**	**31.95**	**Mean**	**HS7 not retained**	43	57	29	10	
			0.14	0.17	0.54	0.36	0.52	1.74	0.13	1.39			43	57	29	11	
													47	53	33	16	
													47	53	33	16	
	HS7 not retained	tjb-Hsa-032-5	4.21	3.44	11.27	2.20	23.73	0.21	11.05	43.88			**45**	**55**	**31**	**13**	**Mean**
		tjb-Hsa-032-5	4.45	3.61	11.10	2.16	23.85	0.54	11.31	42.98							
		tjb-Hsa-032-6	6.10	5.32	11.75	3.99	23.83	0.25	11.48	37.28	**Mean**	**HS3-001-01 Retained**	52	48	39	24	
		tjb-Hsa-032-6	6.07	5.44	11.68	3.98	23.78	0.31	11.52	37.21		**from previous analysis**	52	48	39	24	
			5.21	**4.45**	**11.45**	**3.08**	**23.80**	**0.33**	**11.34**	**40.34**		**L5P161**	52	48	39	24	
			1.62	1.71	0.50	1.66	0.09	0.23	0.34	5.72			53	47	41	26	
													52	**48**	**40**	**25**	**Mean**
	HS3-001-01 Retained	L5P161-7 A	13.57	4.98	10.78	4.44	22.54	1.22	9.93	32.55	**Mean**	**HS3-001-02 Retained**	52	48	40	24	
	from previous analysis	L5P161-7 B	13.91	4.89	10.99	4.41	22.04	1.16	10.17	32.44		L5P161	52	48	41	25	
	L5P161	L5P161-8 A	13.67	4.73	10.72	4.51	22.73	1.22	10.51	31.91		**from previous analysis**	54	46	41	26	
		L5P161-8 B	14.60	5.47	11.41	4.75	21.60	1.00	9.90	31.27			**53**	**47**	**41**	**25**	**Mean**
			13.93	**5.02**	**10.97**	**4.53**	**22.23**	**1.15**	**10.13**	**32.04**							
			0.74	0.51	0.50	0.24	0.81	0.17	0.45	0.93	**Mean**						
	HS3-001-02 Retained	L5P161-9 A	14.33	4.31	10.98	4.40	22.53	1.34	10.38	31.73							
	L5P161	L5P161-9 B	15.01	4.56	11.36	4.61	21.38	1.04	10.27	31.76							
	from previous analysis	L5P161-10 A	15.17	4.88	11.60	4.59	22.12	0.96	9.98	30.71							
			14.84	**4.58**	**11.31**	**4.53**	**22.01**	**1.11**	**10.21**	**31.40**							
			1.11	0.71	0.78	0.28	1.45	0.49	0.52	1.49							

FIGURE 6

A



Lane 1: VEGF
Lane 2: VEGF+HS7
Lane 3: VEGF+ $HS7^{Neg}$
Labe 4: VEGF+ HS^{Celsus}

B

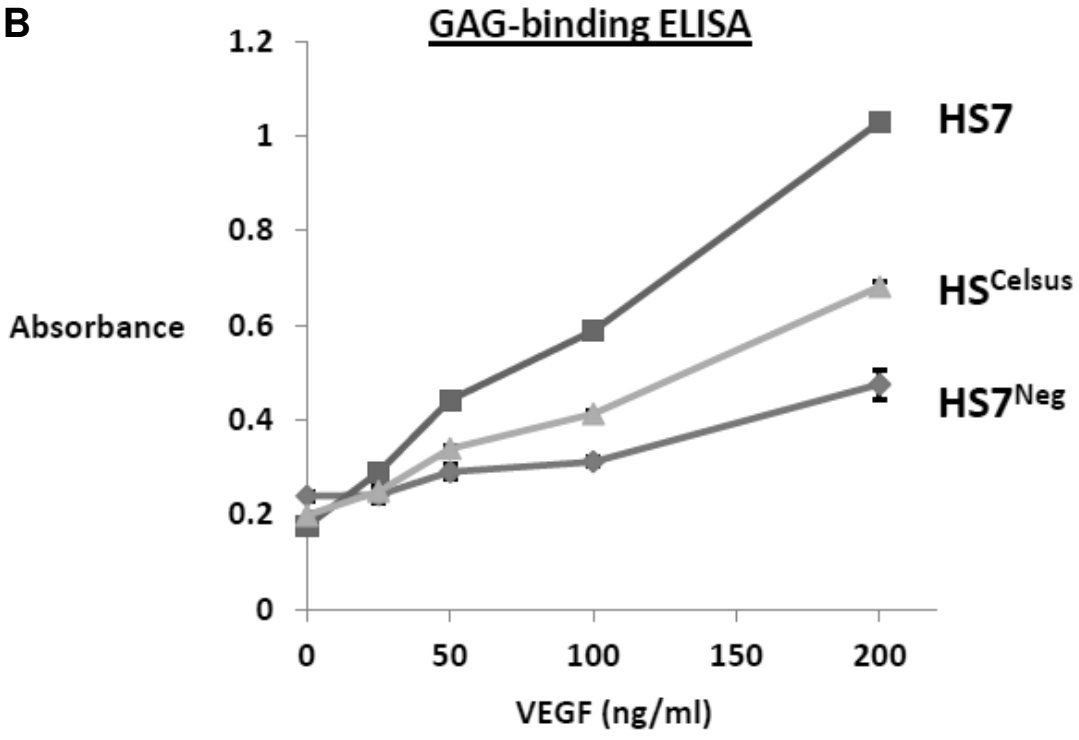
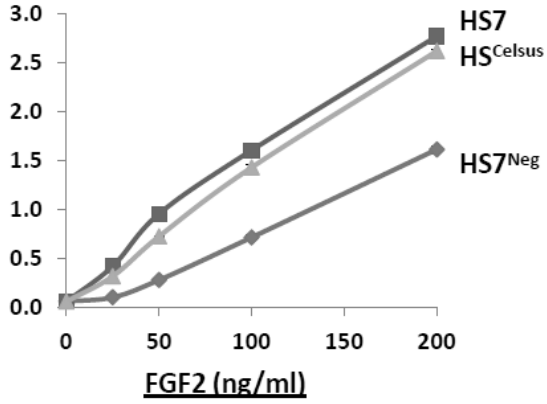
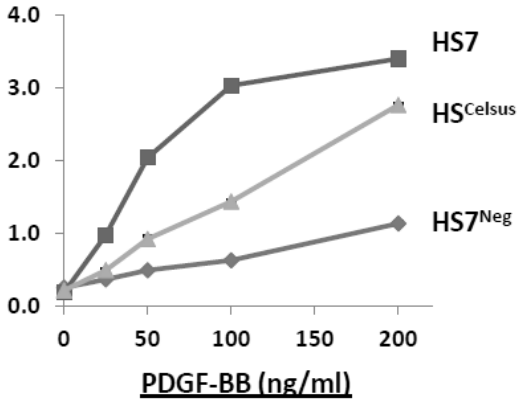


FIGURE 7

C


3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
50
100
150
200
FGF2 (ng/ml)
HS7
HSCelsus
HS7Neg


4.0
3.0
2.0
1.0
0.0
0
50
100
150
200
PDGF-BB (ng/ml)
HS7
HSCelsus
HS7Neg

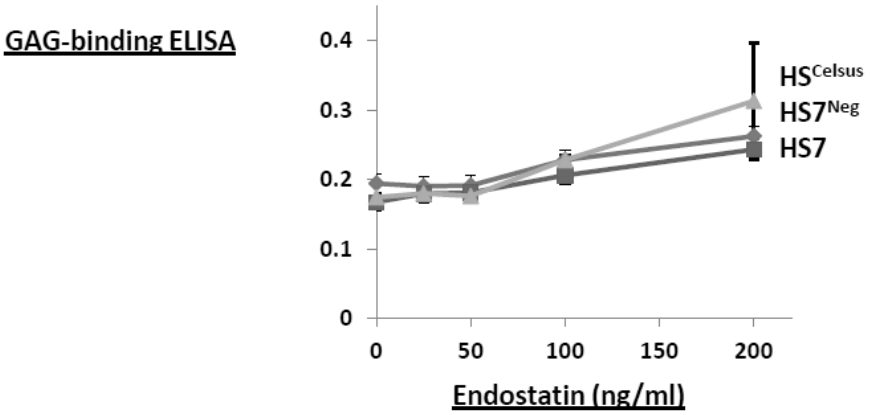

GAG-binding ELISA
0.4
0.3
0.2
0.1
0
0
50
100
150
200
Endostatin (ng/ml)
HSCelsus
HS7Neg
HS7

FIGURE 7

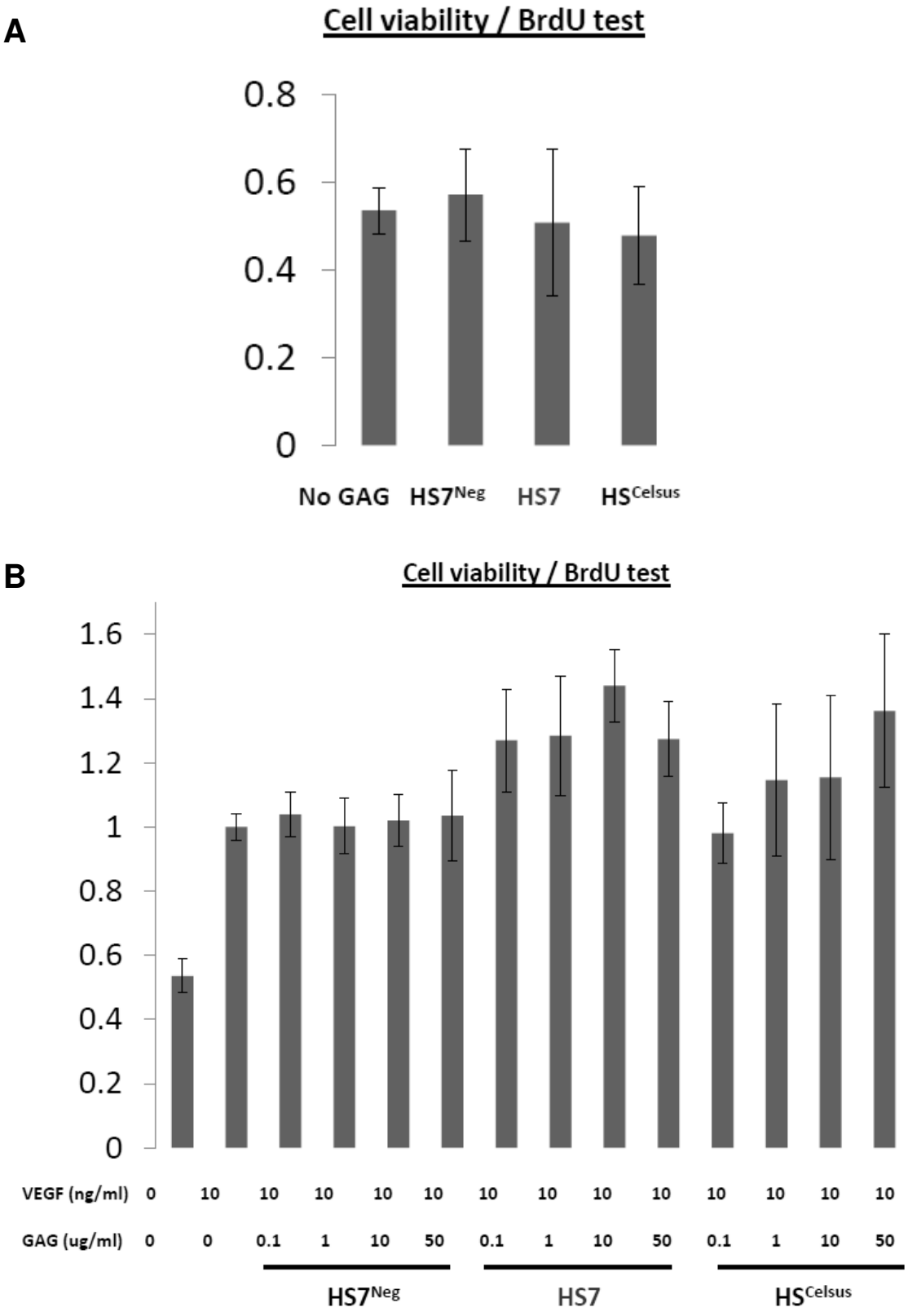

A
Cell viability / BrdU test
0.8
0.6
0.4
0.2
0
No GAG
HS7Neg
HS7
HSCelsus
B
Cell viability / BrdU test
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
VEGF (ng/ml) 0 10 10 10 10 10 10 10 10 10 10 10 10 10
GAG (ug/ml) 0 0 0.1 1 10 50 0.1 1 10 50 0.1 1 10 50
HS7Neg
HS7
HSCelsus

FIGURE 8

A

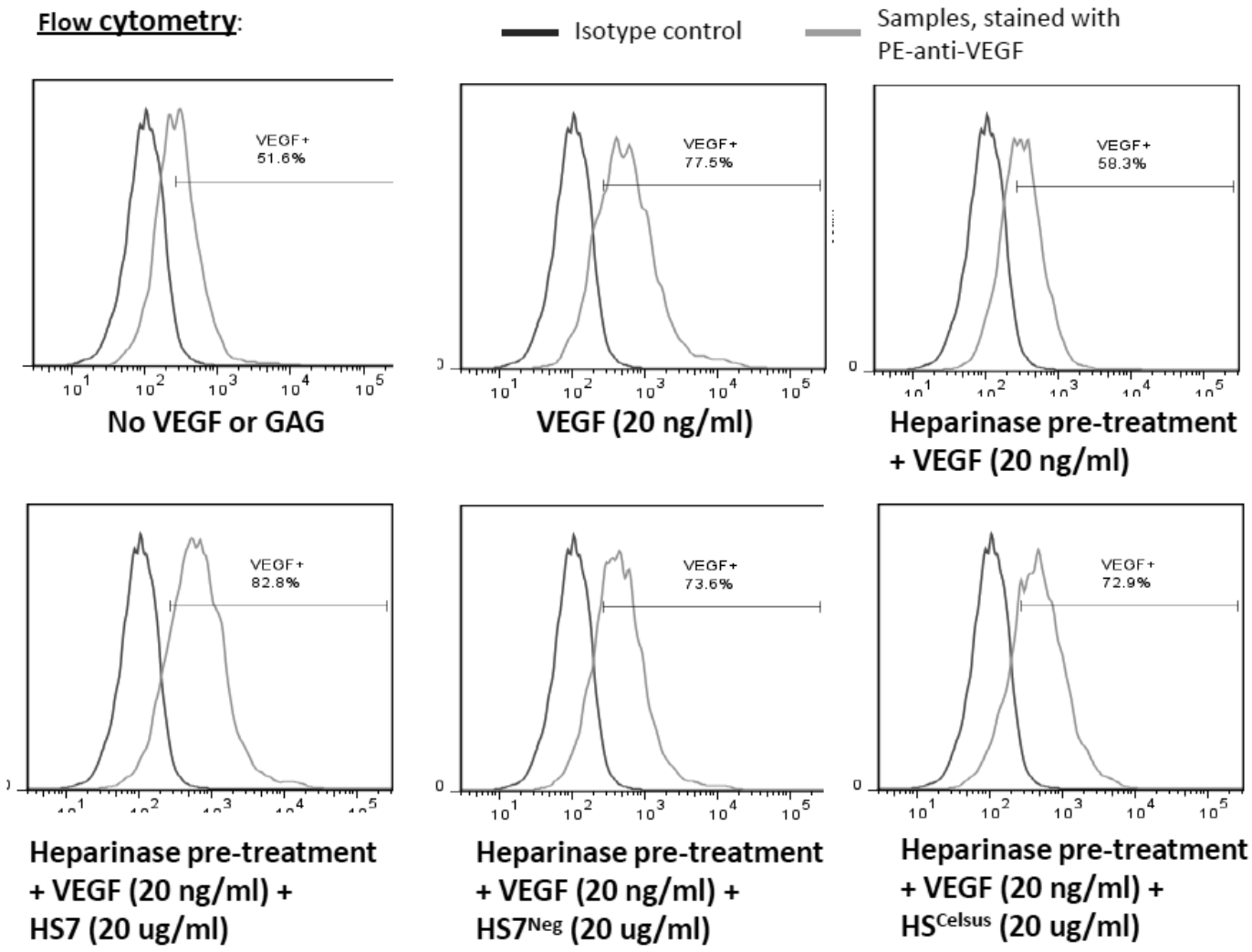
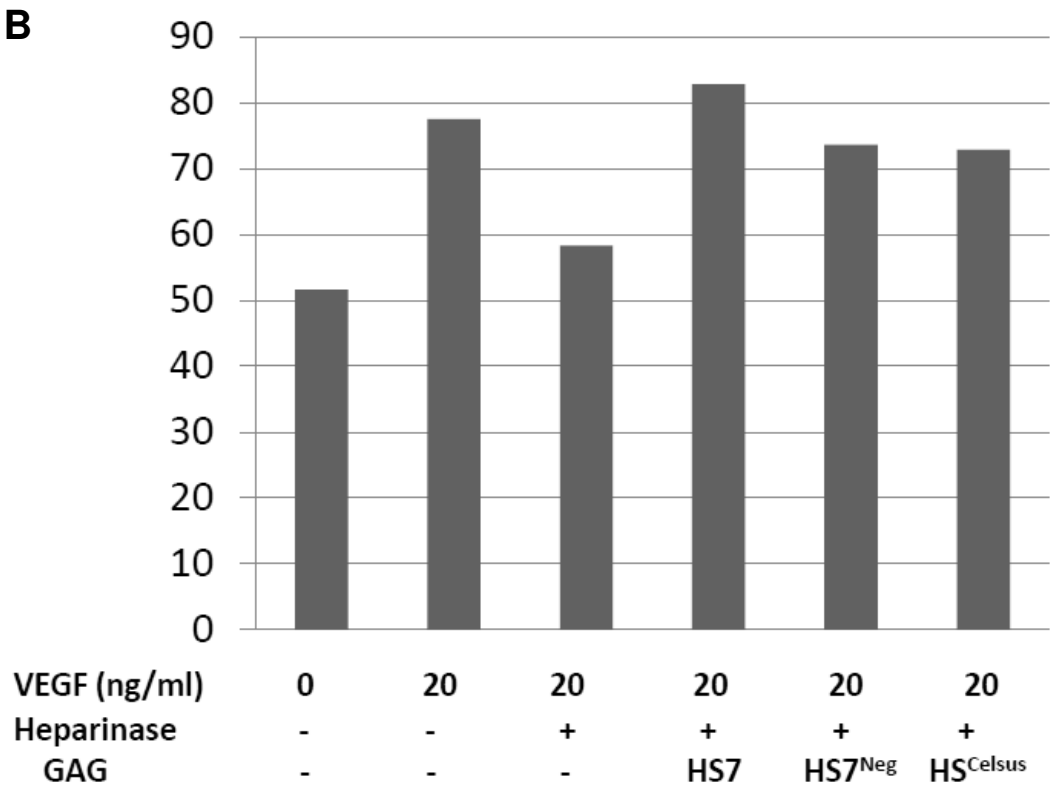

Flow cytometry:
Isotype control
Samples, stained with PE-anti-VEGF
VEGF+ 51.6%
No VEGF or GAG
VEGF+ 77.5%
VEGF (20 ng/ml)
VEGF+ 58.3%
Heparinase pre-treatment + VEGF (20 ng/ml)
VEGF+ 82.8%
Heparinase pre-treatment + VEGF (20 ng/ml) + HS7 (20 ug/ml)
VEGF+ 73.6%
Heparinase pre-treatment + VEGF (20 ng/ml) + HS7Neg (20 ug/ml)
VEGF+ 72.9%
Heparinase pre-treatment + VEGF (20 ng/ml) + HSCelsus (20 ug/ml)

FIGURE 9


B
90
80
70
60
50
40
30
20
10
0
VEGF (ng/ml) 0 20 20 20 20 20
Heparinase - - + + + +
GAG - - - HS7 HS7Neg HSCelsus

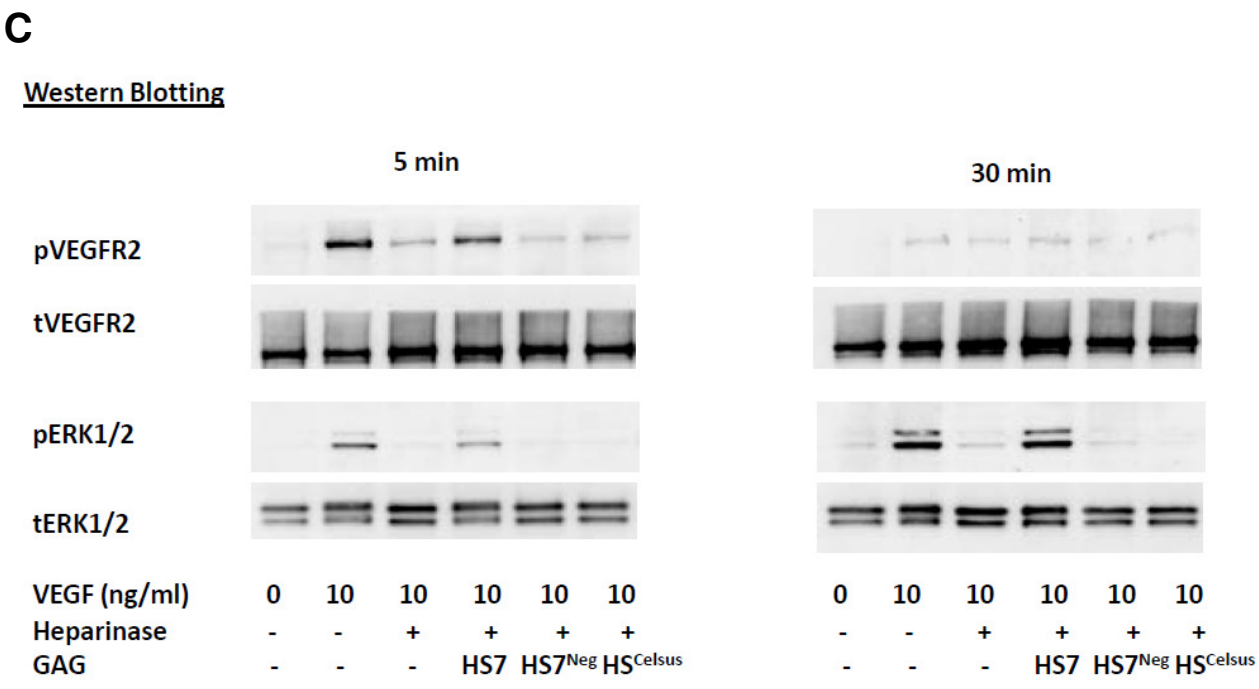
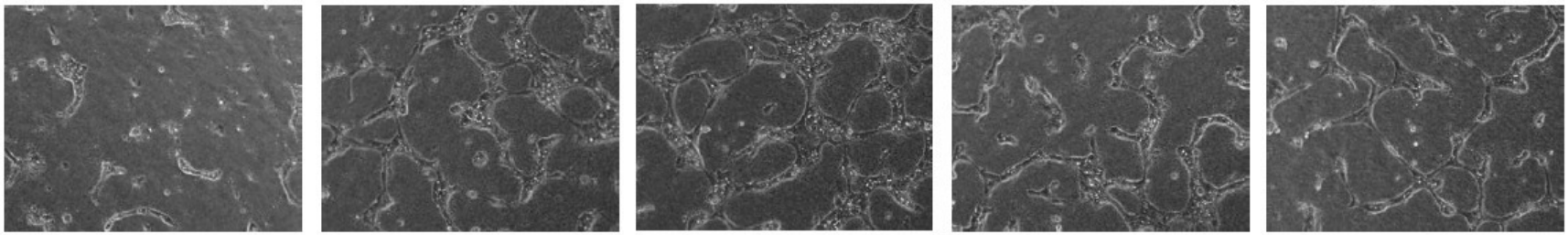
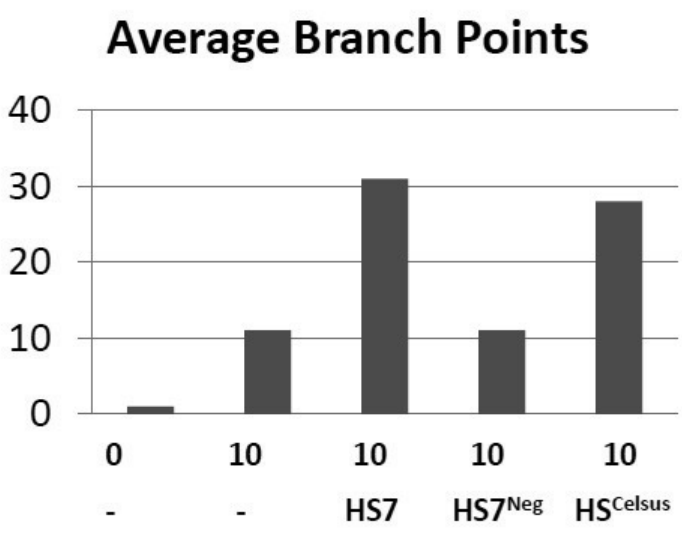

C
Western Blotting
5 min
30 min
pVEGFR2
tVEGFR2
pERK1/2
tERK1/2
VEGF (ng/ml) 0 10 10 10 10 10
Heparinase - - + + + +
GAG - - - HS7 HS7Neg HSCelsus
0 10 10 10 10 10
- - + + + +
- - - HS7 HS7Neg HSCelsus

FIGURE 9

A.



B



C

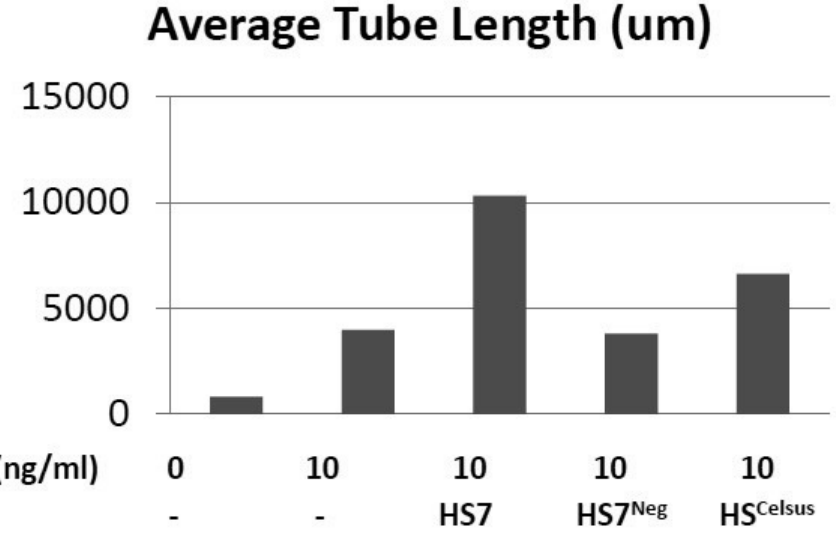


FIGURE 10

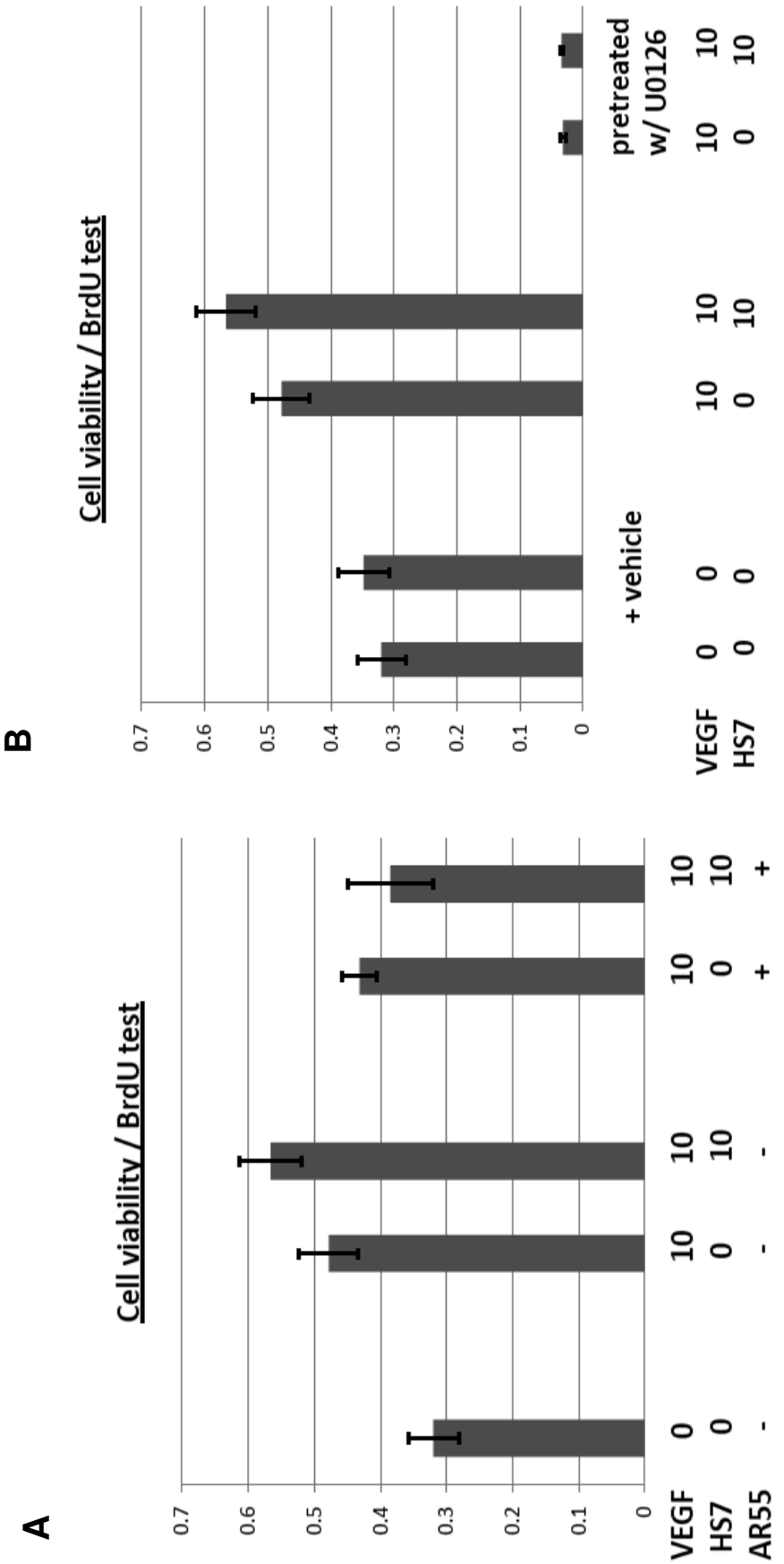

A
Cell viability / BrdU test
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
VEGF 0 10 10 10
HS7 0 0 10 0 10
AR55 - - - + +
B
Cell viability / BrdU test
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
VEGF 0 0 10 10
HS7 0 0 0 10
+ vehicle
pretreated w/ U0126 10 10
0 10

FIGURE 11

n=4 so far, and still ongoing…

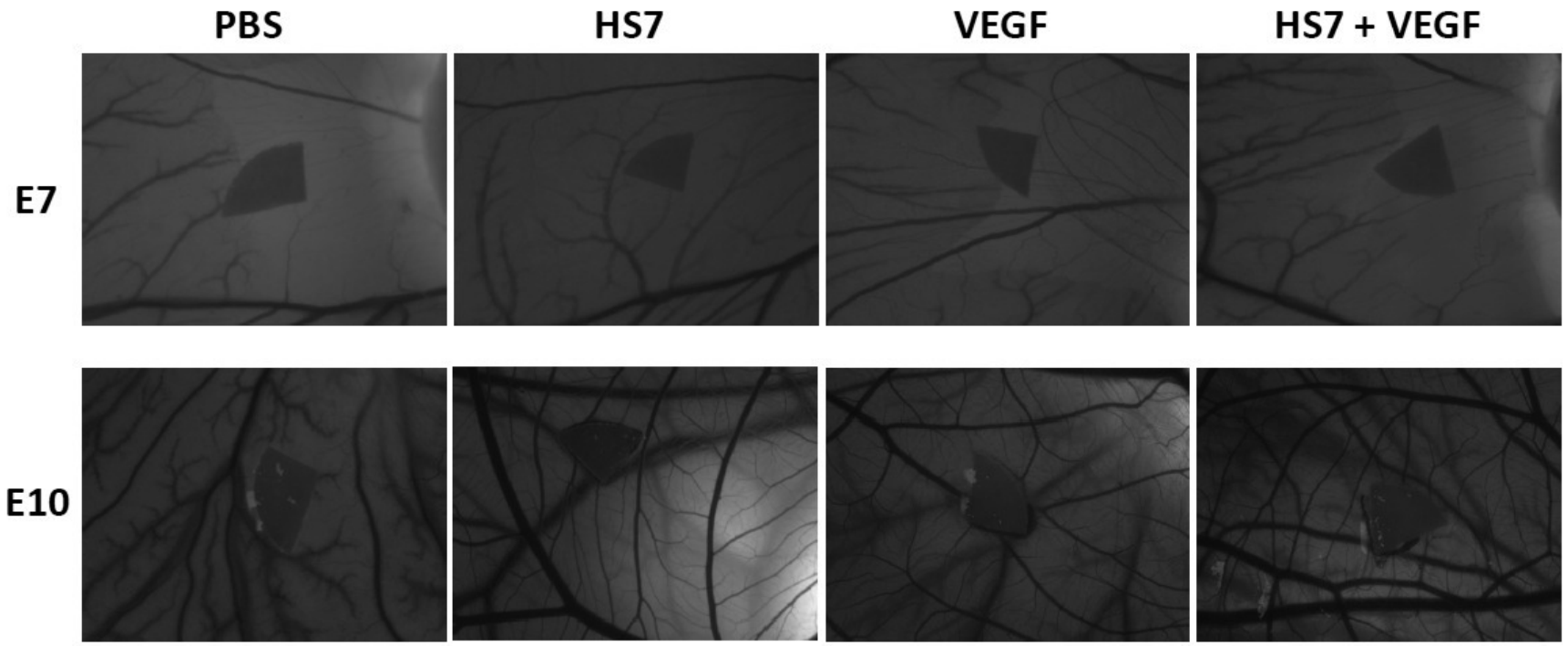


FIGURE 12

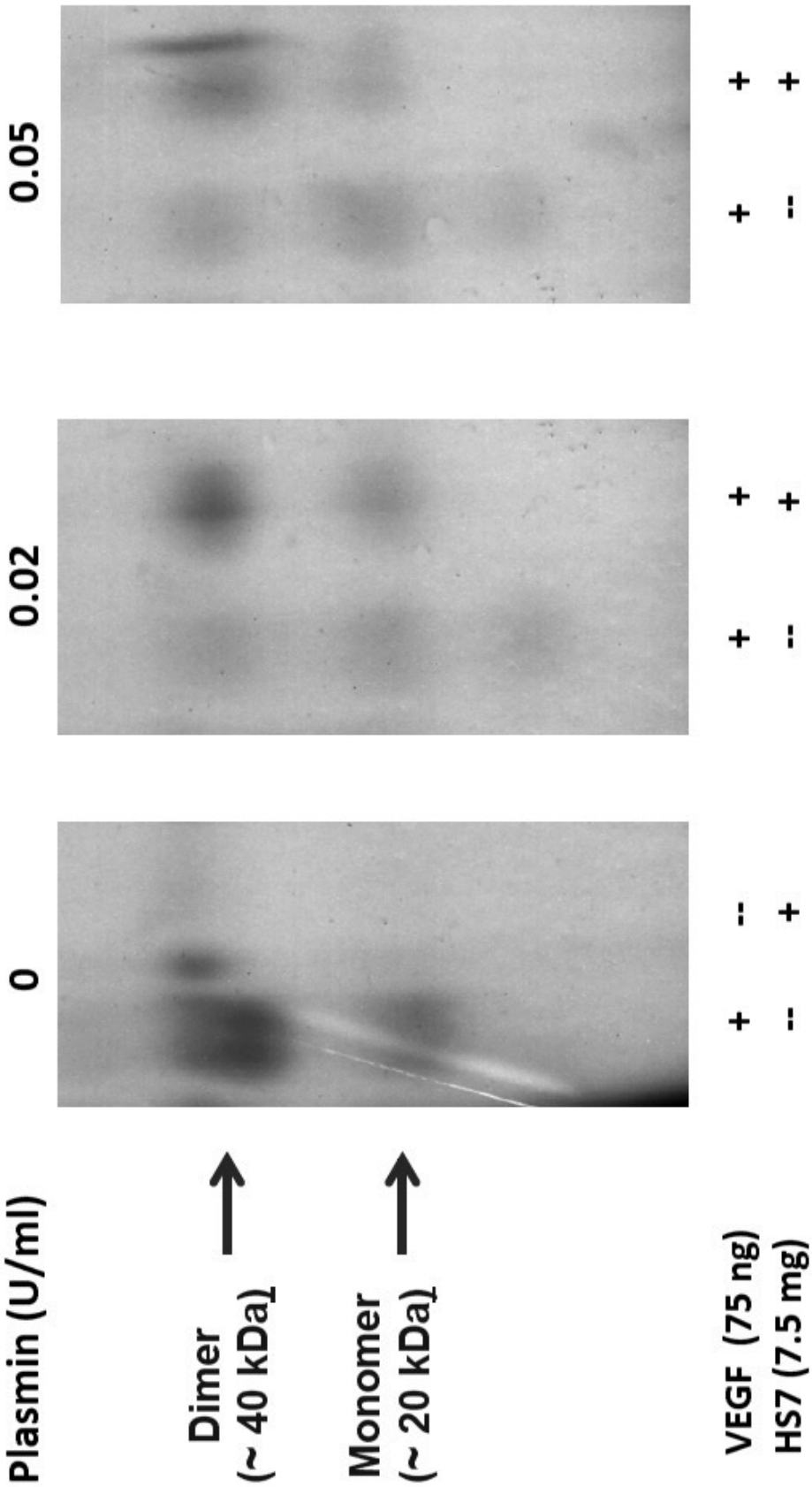

Plasmin (U/ml)
0
0.02
0.05
Dimer
(~ 40 kDa)
Monomer
(~ 20 kDa)
VEGF (75 ng)
HS7 (7.5 mg)
+ - - + + - - + + - - +

FIGURE 13