

Supplemental Table 1

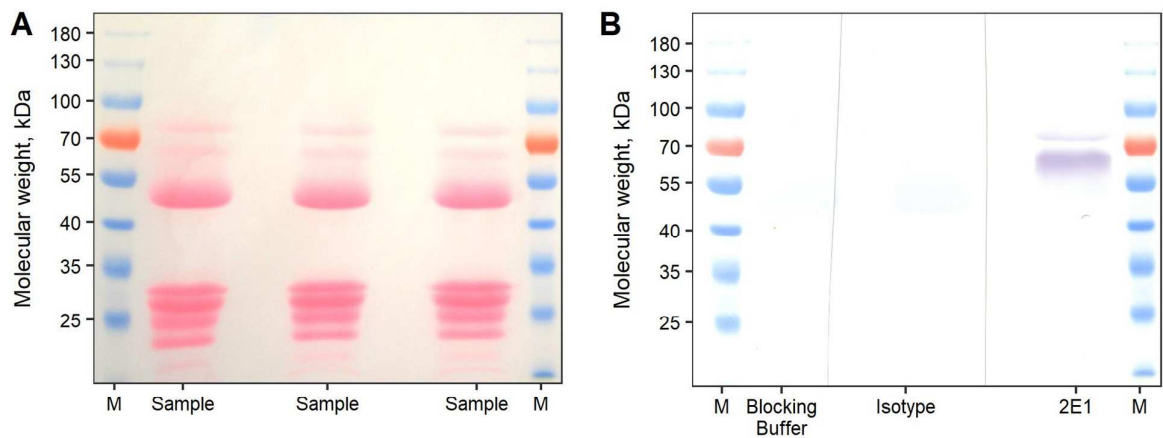
Individual levels of sEng (ng/ml) in various biological fluids measured with two in-house sandwich ELISAs and three commercial kits.

Types of samples	#	ELISA				Aviscera Bioscience
		4E4-4C9	SN6h-4E4	R&D Systems	RayBiotech	
Blood plasma from healthy women	1	334	306	4.0	5.0	1.12
	2	375	333	5.2	4.4	0.88
	3	412	392	5.4	6.3	2.92
	4	349	304	4.3	5.1	1.05
	5	380	381	5.2	8.2	1.28
Blood plasma from women with PE	6	1145	1299	31.2	24.1	4.15
	7	1162	1127	32.6	26.1	1.17
	8	644	648	9.5	7.6	0.45
	9	2187	1989	62.6	18.0	0.87
	10	1289	1120	43.5	18.0	1.19
Urine from healthy volunteers	11	3.5	1.2	0	0	0
	12	6.0	3.5	0	0	0
	13	1.3	0.6	0	0	0
	14	3.3	1.2	0	0	0
	15	2.1	1.1	0.3	0	0.04
Cerebrospinal fluid	16	6.4	2.2	0	0	0
	17	3.4	1.7	0	0	0
	18	3.2	1.3	0	0.1	0
	19	1.5	0.6	0.1	0.1	0
	20	2.3	1.3	0	0	0.15
EA.hy926 medium	21	11.4	4.6	1.5	0.2	0.04
	22	12.0	4.7	1.3	0.1	0
	23	15.8	7.6	2.1	0.3	0.03
	24	6.8	2.1	1.5	0.1	0.03
	25	7.3	2.5	1.7	0.2	0
JEG-3 medium	26	6.5	2.4	5.7	0.5	0.08
	27	10.8	3.8	3.9	0.3	0.24
	28	3.7	1.7	5.0	0.4	0.50
	29	6.1	2.3	6.0	0.4	0.15
	30	1.6	0.6	1.1	0	0.05

Supplemental Table 2

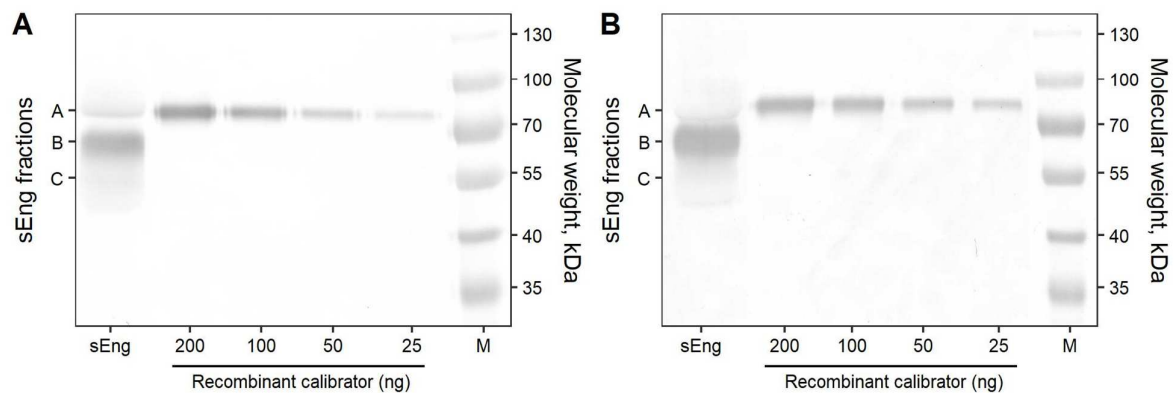
Analytical recovery of recombinant endoglin in the 4E4-4C9 ELISA.

Sample type	#	Added, ng/ml	Measured, ng/ml	Increase, ng/ml	Recovery, %	Mean recovery $\pm$ CI, %
Blood plasma	1	None	480.3			
		240	701.0	220.7	92.0	
		500	848.8	368.5	73.7	
		1000	1541.5	1061.2	106.1	
	2	None	449.8			
		240	702.8	253	105.4	99.3 $\pm$ 10.5
		500	966.5	516.7	103.3	
		1000	1641.2	1191.4	119.1	
	3	None	530.8			
		240	758.2	227.4	94.8	
		500	970.6	439.8	88.0	
		1000	1643.0	1112.2	111.2	
Urine	4	None	13.9			
		5	18.9	5.0	100.0	
		10	22.0	8.1	81.0	
		15	31.4	17.5	116.7	
	5	None	9.4			
		5	13.1	3.7	74.0	92.4 $\pm$ 11.8
		10	18.4	9.0	90.0	
		15	24.4	15.0	100.0	
	6	None	7.9			
		5	11.9	4.0	80.0	
		10	15.8	7.9	79.0	
		15	24.6	16.7	111.3	
	7	None	1.8			
		5	6.1	4.4	87.2	
		10	11.1	9.4	93.6	
		15	15.7	14.0	93.1	
	8	None	3.5			
		5	7.9	4.4	88.0	92.7 $\pm$ 2.5
		10	12.7	9.3	92.5	
		15	18.0	14.5	96.7	
	9	None	1.9			
		5	6.5	4.6	92.6	
		10	11.3	9.4	94.3	
		15	16.4	14.5	96.7	
DMEM + 10% fetal calf serum	10	None	0			
		5	5.1	5.1	101.0	102.1 $\pm$ 6.9
		10	10.0	10.0	100.0	
		15	15.8	15.8	105.2	



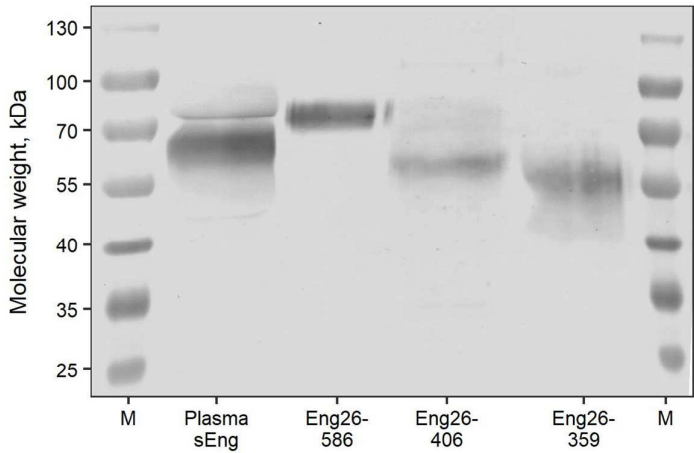
Supplemental Figure 1

Control experiment confirms the specificity of endoglin detection in Western Blot assay. Three identical blood plasma sEng samples were separated by SDS-PAGE and transferred onto a nitrocellulose membrane. (A) Co-precipitating proteins stained by Ponceau S. (B) The membrane pieces were left in the blocking buffer or incubated with IgG1 isotype control MAbs or anti-human endoglin MAb 2E1. Next, all the pieces were developed using goat anti-mouse Ig polyclonal antibodies conjugated with horseradish peroxidase.



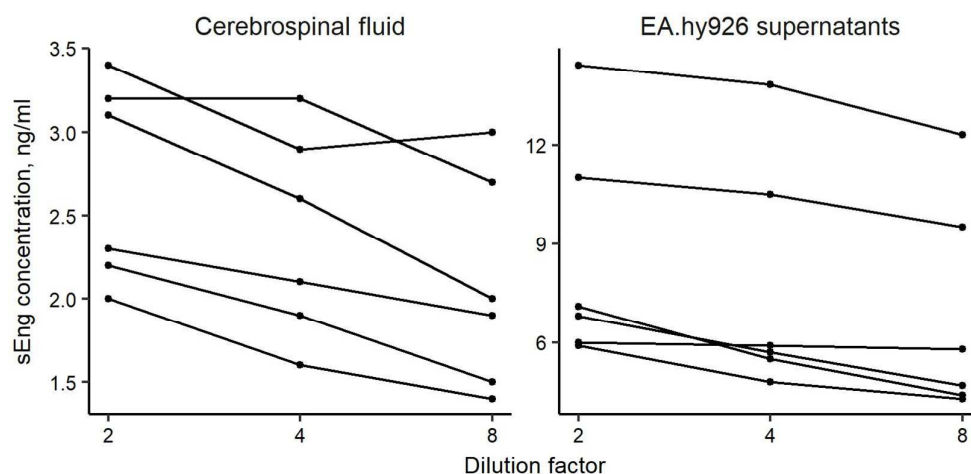
Supplemental Figure 2

Quantification (Western Blot) of sEng isolated from two individual blood plasma samples (A and B). The samples were prepared to contain 200 of sEng according to the 4E4-4C9 ELISA. Antigen content estimates were made with the R&D Systems kit and equaled 2.1 and 3.2 ng (A and B, respectively). MAb 2E1 was used as a primary reagent. The densitometry-based quantification indicated that the samples contained ~504 and ~1294 ng of the antigen, respectively.



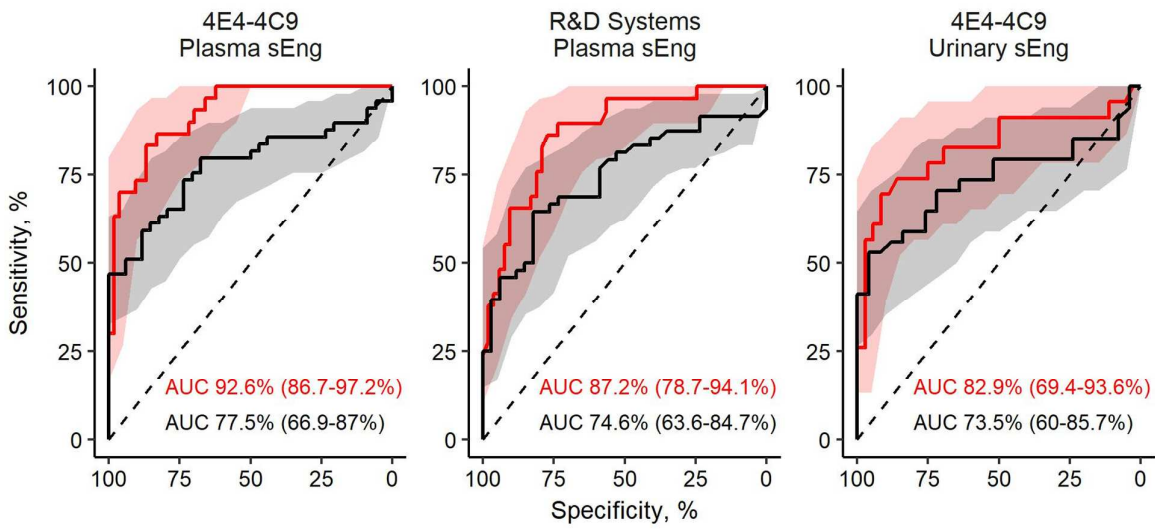
Supplemental Figure 3

Western blot analysis of blood plasma sEng and recombinant endoglin fragment expressed in HEK293 cells. In contrast to commercial endoglin fragment expressed in mouse myeloma cells NS0 similar fragment (Eng26-586) expressed in HEK293 cells migrated faster than the largest fragment of sEng of natural origin.



Supplemental Figure 4

Dilution-response curves for sEng quantified with the 4E4-4C9 ELISA in cerebrospinal fluid and EA.hy926 endothelial cell supernatants (cells were grown in DMEM medium supplemented with 10% fetal calf serum).



Supplemental Figure 5

ROC curves predicting PE (black) and severe PE (red) based on the sEng contents in blood plasma and urine. Antigen concentrations were estimated with the 4E4-4C9 ELISA and R&D Systems kit. Filled areas indicate 95% CI. Estimates of areas under the curves (AUC) and 95% CIs are shown at the bottom.

Patient ID	Group	Age of patients	Week of gestation	Goecke score	Systolic blood pressure
1	Severe PE	21	27	15	150-170
2	Mild PE	27	39	9	<130
3	Severe PE	34	32	15	150-170
4	Mild PE	39	30	10	<130
5	Control	28	39	2	<130
6	Control	34	39	2	<130
7	Mild PE	30	39	8	130-150
8	Control	27	38	1	<130
9	Severe PE	37	29	14	130-150
10	Mild PE	37	36	11	<130
11	Mild PE	35	39	10	130-150
12	Control	37	39	3	<130
13	Mild PE	39	40	9	<130
14	Severe PE	39	32	12	<130
15	Control	26	39	0	<130
16	Control	25	37	0	<130
17	Control	32	40	2	<130
18	Control	25	39	1	<130
19	Control	30	34	1	<130
20	Control	27	38	2	<130
21	Severe PE	38	32	16	>170
22	Mild PE	36	33	9	<130
23	Severe PE	31	34	14	130-150
24	Mild PE	24	40	9	130-150
25	Severe PE	37	32	17	130-150
26	Control	32	38	1	<130
27	Control	28	39	1	<130
28	Control	33	36	1	<130
29	Mild PE	28	40	9	<130
30	Control	25	39	0	<130
31	Control	34	37	2	<130
32	Mild PE	26	27	10	<130
33	Severe PE	34	28	13	130-150
34	Mild PE	33	38	8	<130
35	Control	31	38	1	<130
36	Control	37	38	2	<130
37	Control	32	38	0	<130
38	Control	34	38	1	<130
39	Mild PE	23	39	9	<130
40	Mild PE	32	33	10	<130
41	Mild PE	39	37	9	<130
42	Control	28	33	0	<130
43	Severe PE	34	35	12	130-150
44	Mild PE	26	40	10	<130
45	Control	34	41	1	<130
46	Mild PE	34	36	11	130-150
47	Mild PE	30	41	9	<130
48	Severe PE	30	38	12	<130
49	Severe PE	30	27	16	>170

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2	50 Severe PE	39	36	15 130-150
3	51 Control	26	38	0 <130
4	52 Mild PE	32	36	11 150-170
5	53 Mild PE	22	40	9 <130
6	54 Control	28	39	1 <130
7	55 Control	21	39	0 <130
8	56 Control	28	40	2 <130
9	57 Control	38	38	2 <130
10	58 Control	29	40	1 <130
11	59 Control	35	37	3 <130
12	60 Control	32	39	1 <130
13	61 Severe PE	42	28	15 >170
14	62 Control	32	40	0 <130
15	63 Control	30	40	1 <130
16	64 Control	24	40	2 <130
17	65 Control	28	40	0 <130
18	66 Severe PE	34	32	17 >170
19	67 Control	31	41	1 <130
20	68 Severe PE	32	37	12 150-170
21	69 Severe PE	25	41	13 >170
22	70 Severe PE	28	33	12 >170
23	71 Severe PE	32	38	14 150-170
24	72 Severe PE	26	35	13 >170
25	73 Severe PE	39	34	19 >170
26	74 Severe PE	28	39	12 >170
27	75 Severe PE	30	35	13 >170
28	76 Severe PE	41	34	12 150-170
29	77 Severe PE	27	34	15 >170
30	78 Severe PE	40	37	12 150-170
31	79 Severe PE	38	27	13 >170
32	80 Severe PE	41	25	18 >170
33	81 Severe PE	32	40	14 >170
34	82 Severe PE	34	38	15 150-170
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	Diastolic blood pressure	Proteinuria	Edema	Plasma sEng (4E4-4C9)
1				
2	90-110	1-4.99	no	800.2
3	90-110	<0.3	feet	665.5
4	90-110	1-4.99	feet and abdominal	2271.7
5	85-90	<0.3	feet and abdominal	454.8
6	<85	<0.3	feet	676.6
7	85-90	<0.3	no	717.2
8	90-110	0.3-0.99	feet and abdominal	1775.1
9	<85	<0.3	feet	680.3
10	90-110	0.3-0.99	feet and abdominal	2014.2
11	90-110	<0.3	feet and abdominal	537.5
12	90-110	0.3-0.99	feet	746.4
13	<85	<0.3	feet	1449.7
14	90-110	<0.3	feet	521.2
15	90-110	<0.3	feet	1832.7
16	<85	<0.3	no	700
17	<85	<0.3	no	576.3
18	<85	<0.3	feet	674.1
19	<85	<0.3	feet	537.8
20	<85	<0.3	feet	522.9
21	85-90	<0.3	no	512.9
22	90-110	>5	feet	2975.9
23	90-110	<0.3	no	312
24	90-110	0.3-0.99	feet and abdominal	17383.4
25	90-110	0.3-0.99	feet and abdominal	891.5
26	>110	0.3-0.99	feet	2754.7
27	<85	<0.3	feet	477.1
28	<85	<0.3	feet	1048
29	<85	<0.3	no	1377.3
30	90-110	<0.3	feet and abdominal	383.1
31	<85	<0.3	no	746.5
32	<85	<0.3	feet	1212.4
33	85-90	<0.3	feet and abdominal	212.3
34	90-110	0.3-0.99	feet and abdominal	1984.1
35	90-110	<0.3	feet and abdominal	842.1
36	<85	<0.3	feet	888.9
37	85-90	<0.3	no	355.3
38	<85	<0.3	no	326.7
39	<85	<0.3	feet	469.5
40	90-110	<0.3	feet and abdominal	674.2
41	90-110	<0.3	feet and abdominal	448.2
42	90-110	<0.3	feet and abdominal	1219
43	<85	<0.3	no	619.8
44	90-110	0.3-0.99	feet and abdominal	1311.3
45	90-110	<0.3	feet	1449.3
46	<85	<0.3	feet	688.2
47	90-110	0.3-0.99	feet and abdominal	1031.6
48	90-110	<0.3	feet and abdominal	621.2
49	>110	<0.3	feet and abdominal	1655.4
50	90-110	>5	general	3183.7
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2	>110	0.3-0.99	feet and abdominal	2277.4
3	<85	<0.3	no	443.9
4	90-110	1-4.99	feet	2438
5	90-110	<0.3	feet	784
6	<85	<0.3	feet	893.2
7	<85	<0.3	no	891.3
8	<85	<0.3	feet	1540
9	<85	<0.3	no	775
10	<85	<0.3	feet	581.7
11	85-90	<0.3	feet	466.1
12	<85	<0.3	feet	616.7
13	90-110	>5	general	1845
14	<85	<0.3	no	636
15	<85	<0.3	feet	1437.2
16	85-90	<0.3	no	599.1
17	<85	<0.3	no	703.3
18	>110	>5	feet and abdominal	1786.9
19	<85	<0.3	feet	552.8
20	90-110	1-4.99	feet and abdominal	3115.6
21	90-110	>5	feet and abdominal	1565.3
22	90-110	>5	feet and abdominal	2073
23	90-110	1-4.99	feet and abdominal	850.8
24	90-110	>5	general	1355.4
25	>110	>5	feet and abdominal	3139.7
26	90-110	>5	feet and abdominal	1167.5
27	90-110	>5	feet and abdominal	770.2
28	90-110	1-4.99	general	4294.2
29	90-110	>5	general	1958.5
30	90-110	1-4.99	general	1447.2
31	90-110	>5	feet and abdominal	4351.9
32	90-110	>5	general	2072.3
33	90-110	>5	feet and abdominal	1302.7
34	90-110	1-4.99	general	725.2
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Plasma sEng (R&D Systems)	Urinary sEng (4E4-4C9)
16.3 NA	
14.3 NA	
45.1 NA	
5.5 NA	
10.5 NA	
15.7	11.9
45.6 NA	
11.9 NA	
53 NA	
12.8 NA	
12.2 NA	
29 NA	
14.2 NA	
34.7 NA	
11.1 NA	
11.8 NA	
15.8 NA	
10.8 NA	
7.5 NA	
8.5 NA	
84.8 NA	
5.3 NA	
681.5 NA	
15.9 NA	
84.8	9.6
7.7	3
31	1
36.9	15.9
8.8	2.8
16	7.6
17	5
3.6	3
50.8	33.7
19.9	7.1
17.4	12.9
5.9	7.8
5.8	12.3
9.5	8
11	29.9
4.8	4.8
30.6	12.2
15	7.9
36.6	42
43.3	19.3
13.1	31.2
21.2	38.9
11.6	14.2
34.5	9.6
63.6	226.3

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2	19.9	98.7
3	6.7	8.1
4	63.6	136.4
5	13.1	7.3
6	14.5	7.8
7	15.3	10.3
8	30	45.9
9	12.9	24.8
10	12.5	9.9
11	8.7	9.3
12	12.2	5.8
13	62.2	1353.5
14	10.8	6.1
15	48.2	27.8
16	32.9	6.3
17	11	32.9
18	37.4	91.3
19	5.5	31
20	80.2	222.7
21	27.9	72
22	41.6	55.2
23	29.4	73.2
24	21.1	81
25	129	31.2
26	14.2	15.7
27	13.2	96.7
28	110.8	3.3
29	43.9	329.5
30	20.7	38.2
31	121.3	261.9
32	47.7	208.3
33	28.6	21.4
34	9	2.8
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