Method.

Flt1, sFlt1, sFlt1_v3, and sFlt1_v4 were amplified from a variety of cells using primers shown in Table. Real-time PCRs were done using Brilliant SYBR Green QPCR master mix (Stratagene) with the following cycling conditions: one cycle for 10 min at 95°C followed by 35-40 cycles (10-15 cycles for 18S rRNA) of 30 sec denaturation at 95°C, 1-min annealing at 56–60°C, and 1-min extension at 72°C. The number of cycles, annealing temperature, and extension time were varied as appropriate for the abundance of transcripts, the melting temperature of primers, and the size of amplicons. All reactions were performed in an Mx3000p Multiplex quantitative PCR system (Stratagene) and dissociation curves were generated for all samples.

Table

	Forward	**Reverse**
sFlt1_v3	5′-GTC GAC ACA GTG GCC ATC AGC AGT T-3′	5′-GCG AAT AAG GCA GGA CAC ACA-3′
Flt1	Same as above	5′-TTC CAC AGA GCC CTT CTG GTT-3′
sFlt1_v4	5′-TGG CCA TCA CTA AGG AGC ACT-3′	5′-CCC AGT GTT TGG GGC TCT ATC-3′
sFlt1	Same as above	5′-TCC GAG CCT GAA AGT TAG CAA-3′

Figure legends

Figure 1. Alternate transcripts of sFlt1. A: Schematic of the sFlt1 transcripts compared to Flt1. The genomic organization of FLT1 between exons 13 and 15 are shown above with exons and introns drawn to different scales. Below are shown each of the 5 described transcripts for FLT1 using the nomenclature of Thomas *et al.,* and Heydarian *et al*.

Figure 2. Abundance of sFlt1 and Flt1 transcripts in CTB and HUVEC. A: Real time RT-PCR in CTB (panels A and C) and HUVEC (panels B and D). In panels A and B, sFlt1_v3 and Flt1 were amplified with the same forward primer but distinct reverse primers (exon 15b and exon 15 respectively). The expression of Flt1 is expressed as fold difference compared to sFlt1_v3. In panels C and D, sFlt1 and sFlt1_v4 were amplified with the same forward primer but distinct reverse primers (intron 13 and 14 respectively). The expression of sFlt1 is expressed as fold difference compared to sFlt1_v4. *$p < 0.001$ for all panels by 1 tailed student's *t*-test.

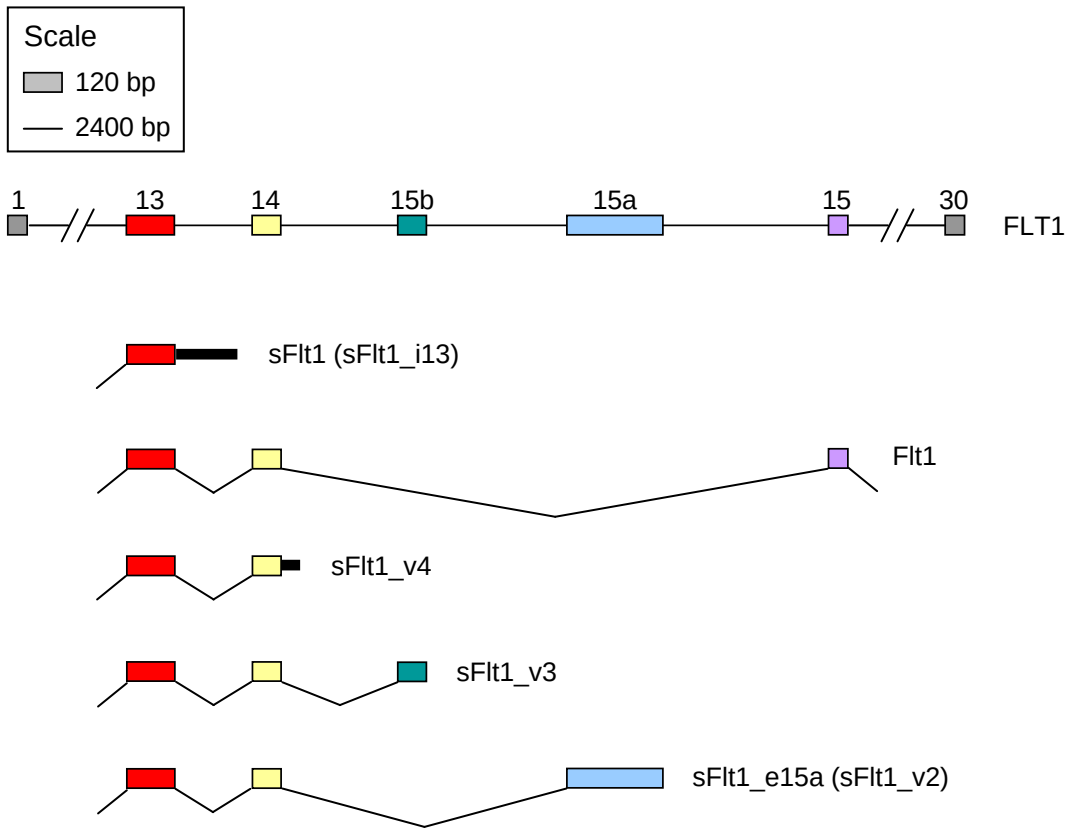
Scale
120 bp
2400 bp
1
13
14
15b
15a
15
30
FLT1
sFlt1 (sFlt1_i13)
Flt1
sFlt1_v4
sFlt1_v3
sFlt1_e15a (sFlt1_v2)

Figure 1

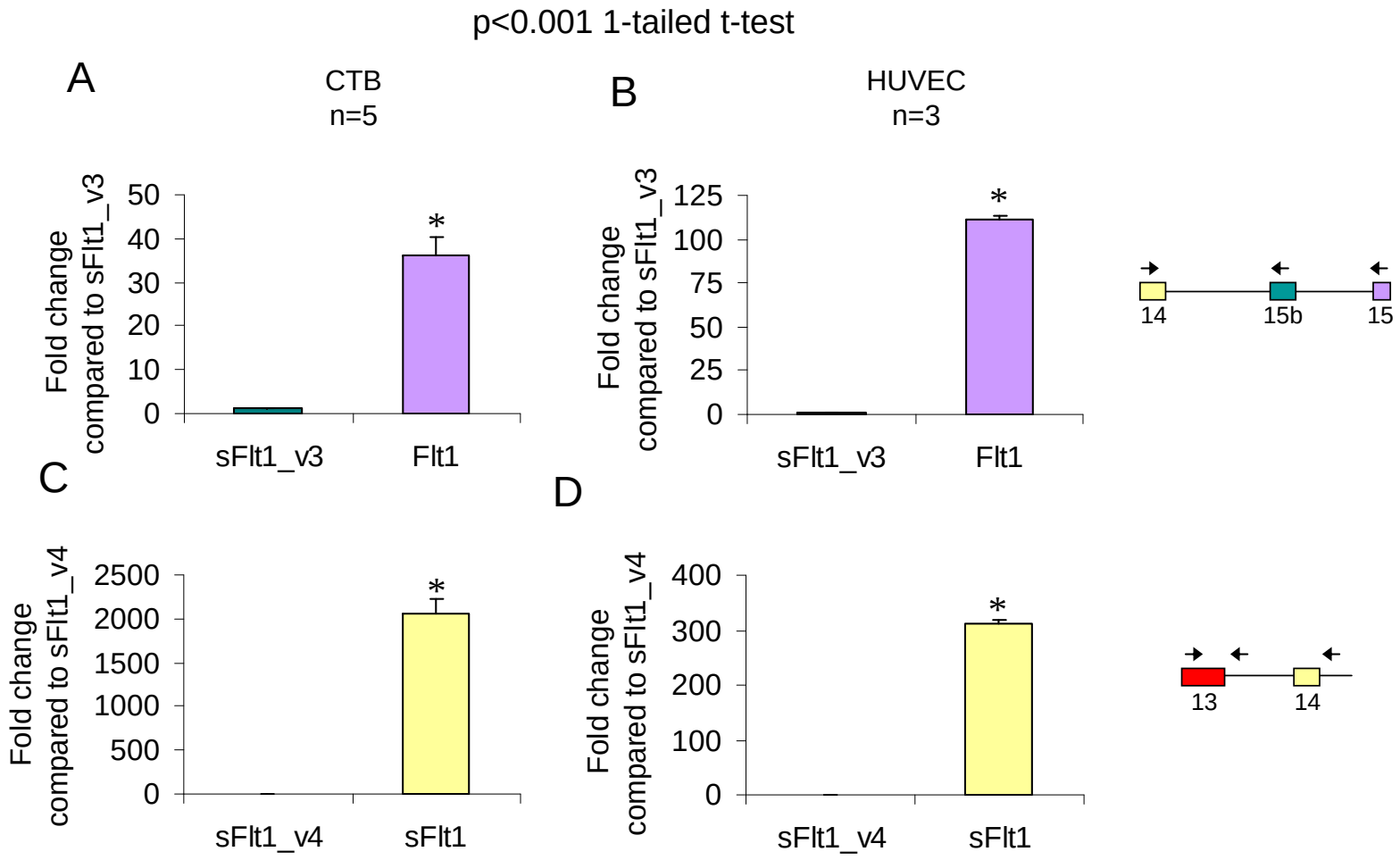
p<0.001 1-tailed t-test
A
CTB
n=5
Fold change compared to sFlt1_v3
50
40
30
20
10
0
*
sFlt1_v3
Flt1
B
HUVEC
n=3
Fold change compared to sFlt1_v3
125
100
75
50
25
0
*
sFlt1_v3
Flt1
14
15b
15
C
Fold change compared to sFlt1_v4
2500
2000
1500
1000
500
0
*
sFlt1_v4
sFlt1
D
Fold change compared to sFlt1_v4
400
300
200
100
0
*
sFlt1_v4
sFlt1
13
14

Figure 2