Supplementary Information

Hypoxia inducible factor-1β is essential for upregulation of the hypoxia-induced *FLT1* gene in placental trophoblasts

Tadashi Sasagawa[1], Takeshi Nagamatsu[2], Manami Yanagisawa[2], Tomoyuki Fujii[3,2], and Masabumi Shibuya[1]

[1] Institute of Physiology and Medicine, Jobu University, Gunma, 370-1393, Japan

[2] Department of Obstetrics and Gynecology, The University of Tokyo, Tokyo, 113-8655, Japan

[3] Present address: Sanno Hospital, Tokyo, 107-0052, Japan

Figure S1

Immunoprecipitation of HIF-1β protein using the antibody against HIF-2α in the nuclear extracts of the three hypoxic trophoblast-derived choriocarcinoma cell lines.

Figure S2

Silencing of HIF-1β inhibits the hypoxia-induced elevation in the secretion of the sFLT1 in the human primary trophoblasts derived from a different donor than that shown in Figure 2.

Figure S3

DMOG-induced upregulation *of the HIF-1β* mRNA in BeWo cells.

Figure S4

Uncropped images of the western blots shown in the main and supplementary figures.

Table S1

Oligonucleotide primer sequences for qRT-PCR.

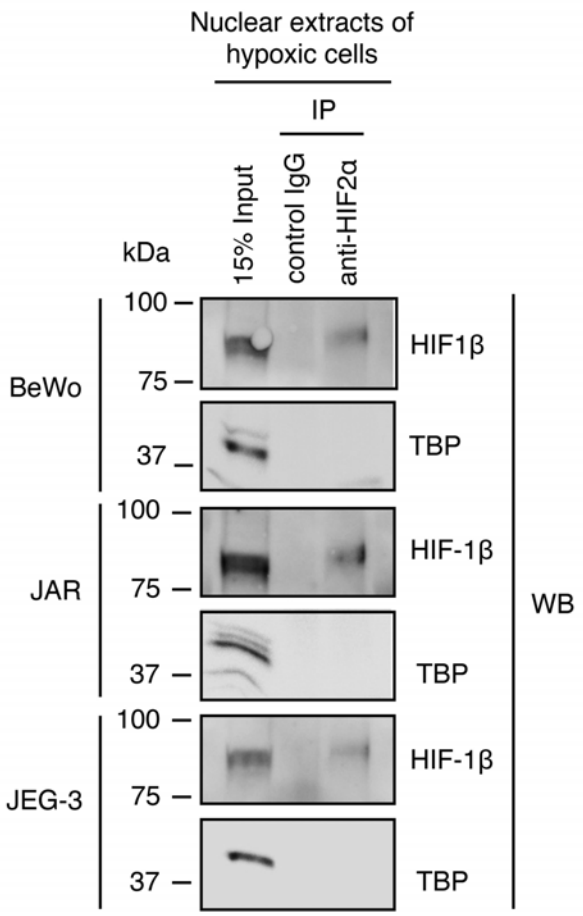


Figure S1. Immunoprecipitation of HIF-1β protein using the antibody against HIF-2α in the nuclear extracts of the three hypoxic trophoblast-derived choriocarcinoma cell lines.

The immunoprecipitates and nuclear extracts (input) were subjected to western blotting analysis with anti-HIF-1β and anti-TBP antibodies, respectively. Uncropped images of the western blots are presented in Supplementary Fig. S4.

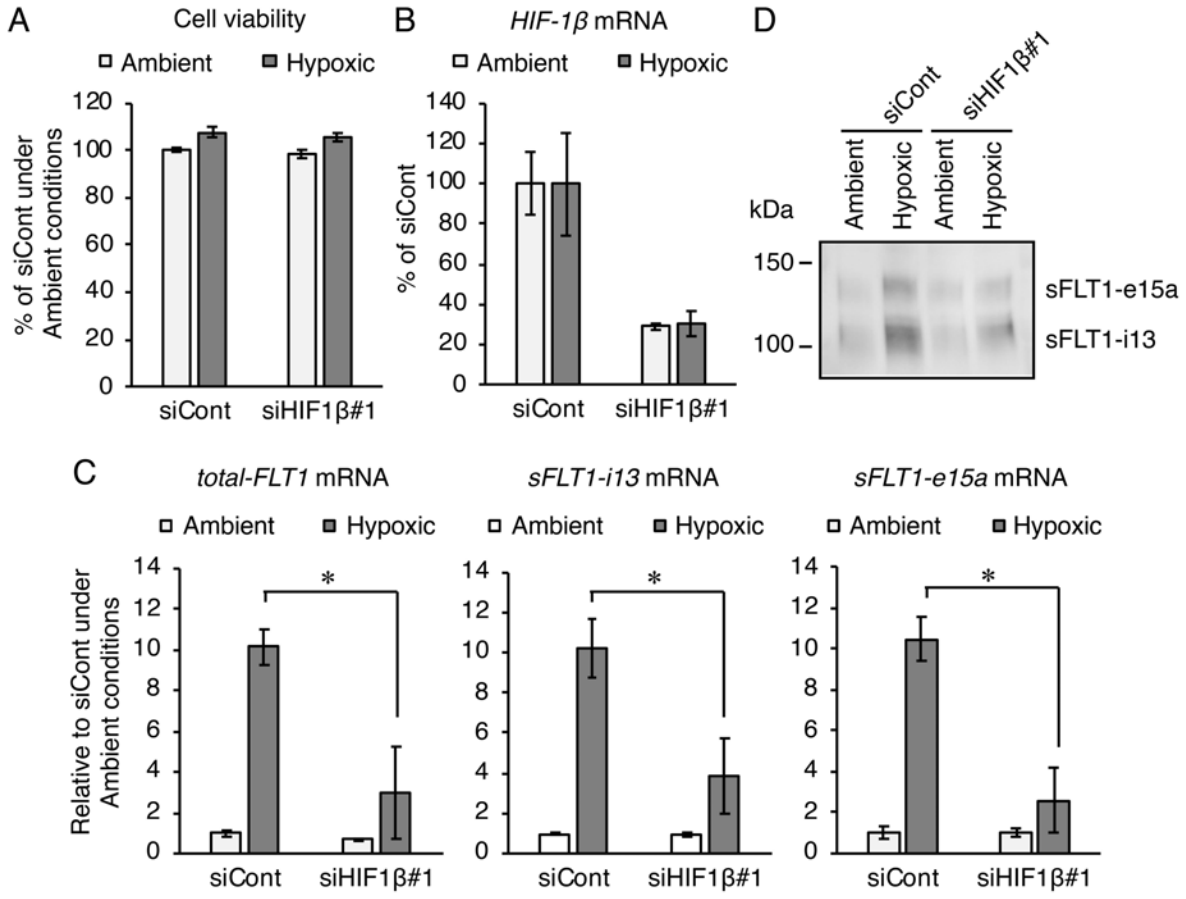


Figure S2. Silencing of HIF-1β inhibits the hypoxia-induced elevation in the secretion of the sFLT1 in the human primary trophoblasts derived from a different donor than that shown in Figure 2.

(A) Cell viability was assessed to determine the potential toxicity of each siRNA. Results are expressed as the percentage relative to the siCont-transfected cells under ambient conditions. (B) Evaluation of the knockdown of the *HIF-1β* mRNA via transfection with siRNA. Results are expressed as the percentage relative to the siCont-transfected cells under ambient or hypoxic conditions. (C) The mRNA expression levels of *total-FLT1*, *sFLT1-i13*, and *sFLT1-e15a* in the trophoblasts were measured by qRT-PCR using *β-actin* mRNA as a reference. Results are expressed as the change relative to the siCont-transfected cells under ambient conditions. (D) Western blotting of sFLT1 proteins secreted by the trophoblasts into the conditioned media. Uncropped image of the western blots is presented in Supplementary Fig. S4. All values are represented as the mean ± SD (n = 3). Asterisks indicate the significant difference ($P < 0.05$).

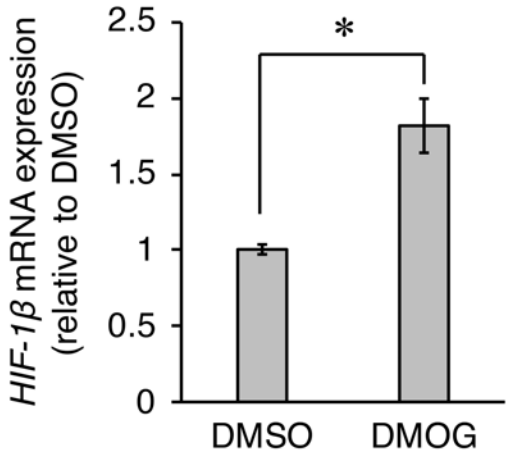


Figure S3. DMOG-induced upregulation *of the HIF-1β* mRNA in BeWo cells.

The cells were treated with 100 μM DMOG or 0.1% DMSO as a vehicle control for 24 h. The *HIF-1β* mRNA expression levels were measured by qRT-PCR using *β-actin* mRNA as a reference. Results are expressed as a fold change relative to the vehicle-treated cells. All values are represented as the mean ± SD (n = 3). Asterisks indicate the significant difference ($P < 0.05$).

Figure 1-B (Whole cell lysates)

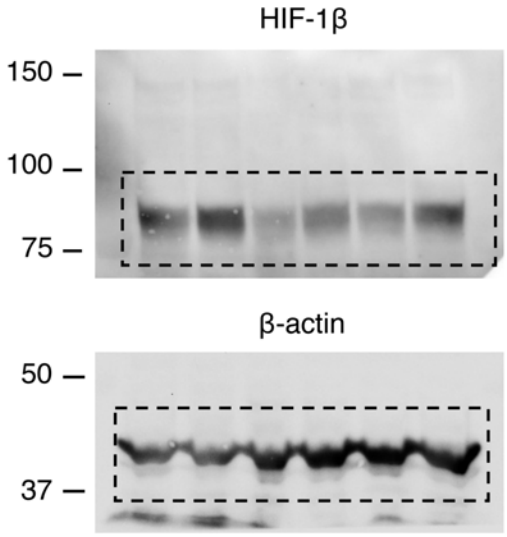


Figure 1-B (Nuclear extracts)

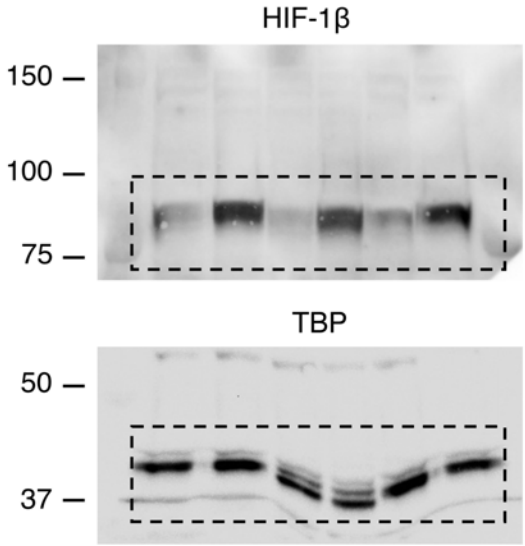


Figure 1-D : BeWo

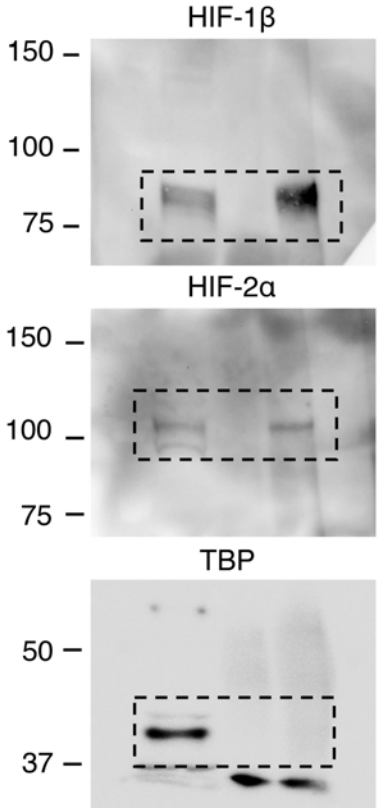


Figure 1-D : JAR

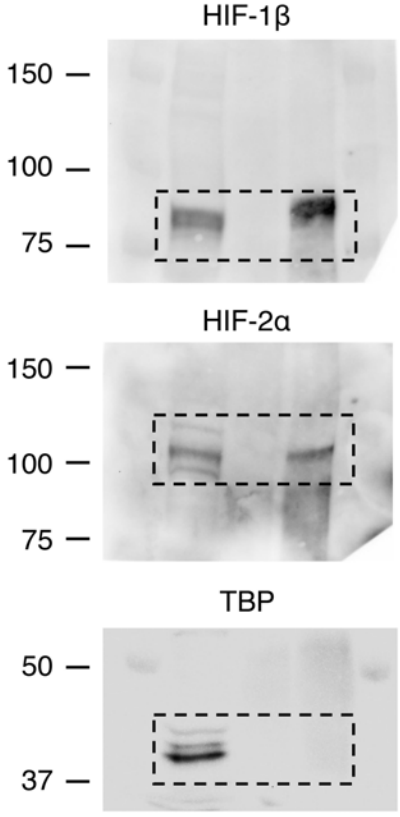


Figure 1-D : JEG-3

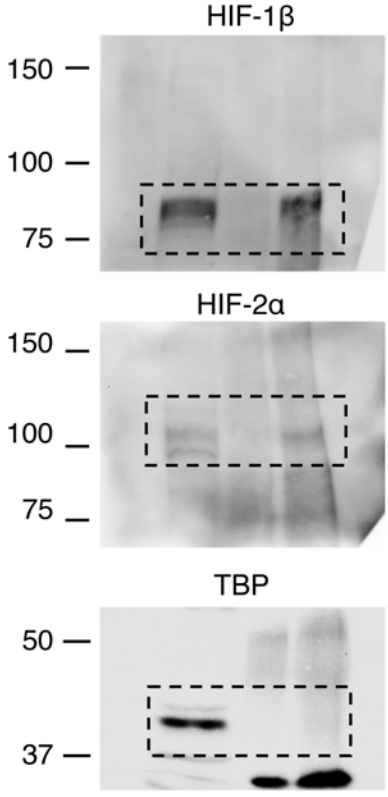


Figure 2-A

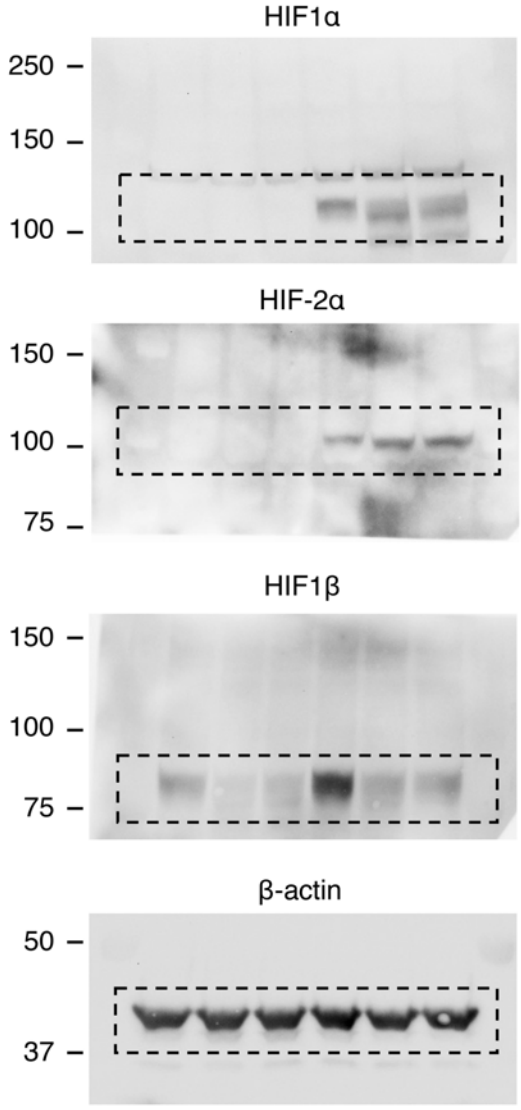


Figure 2-E

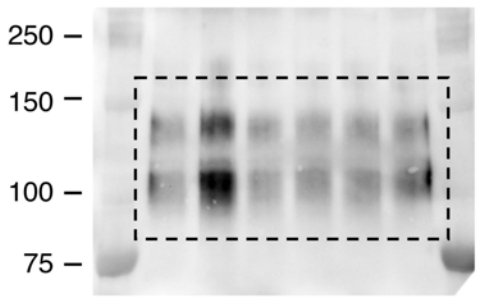


Figure 3-D

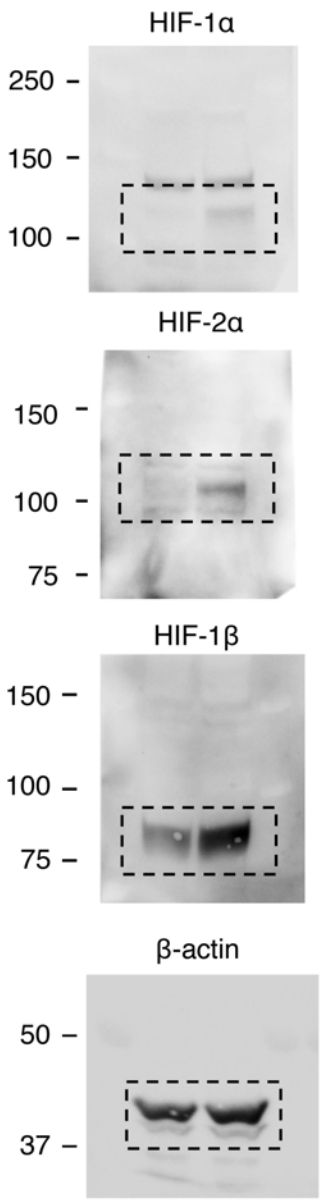
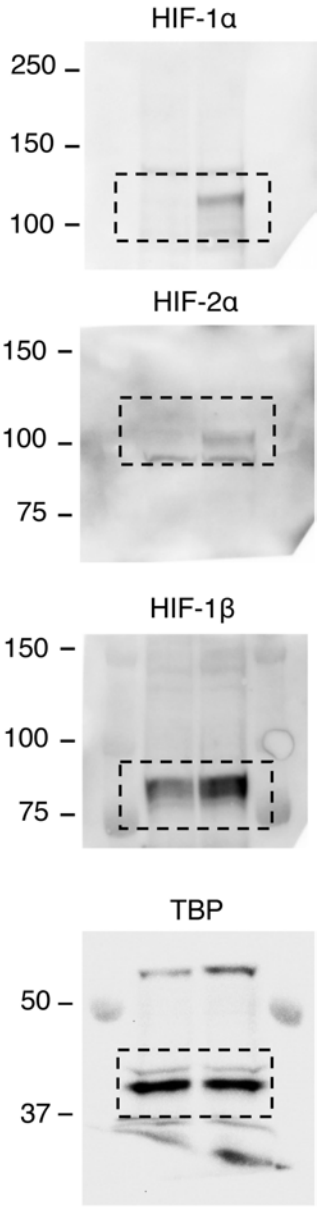


Figure 3-E



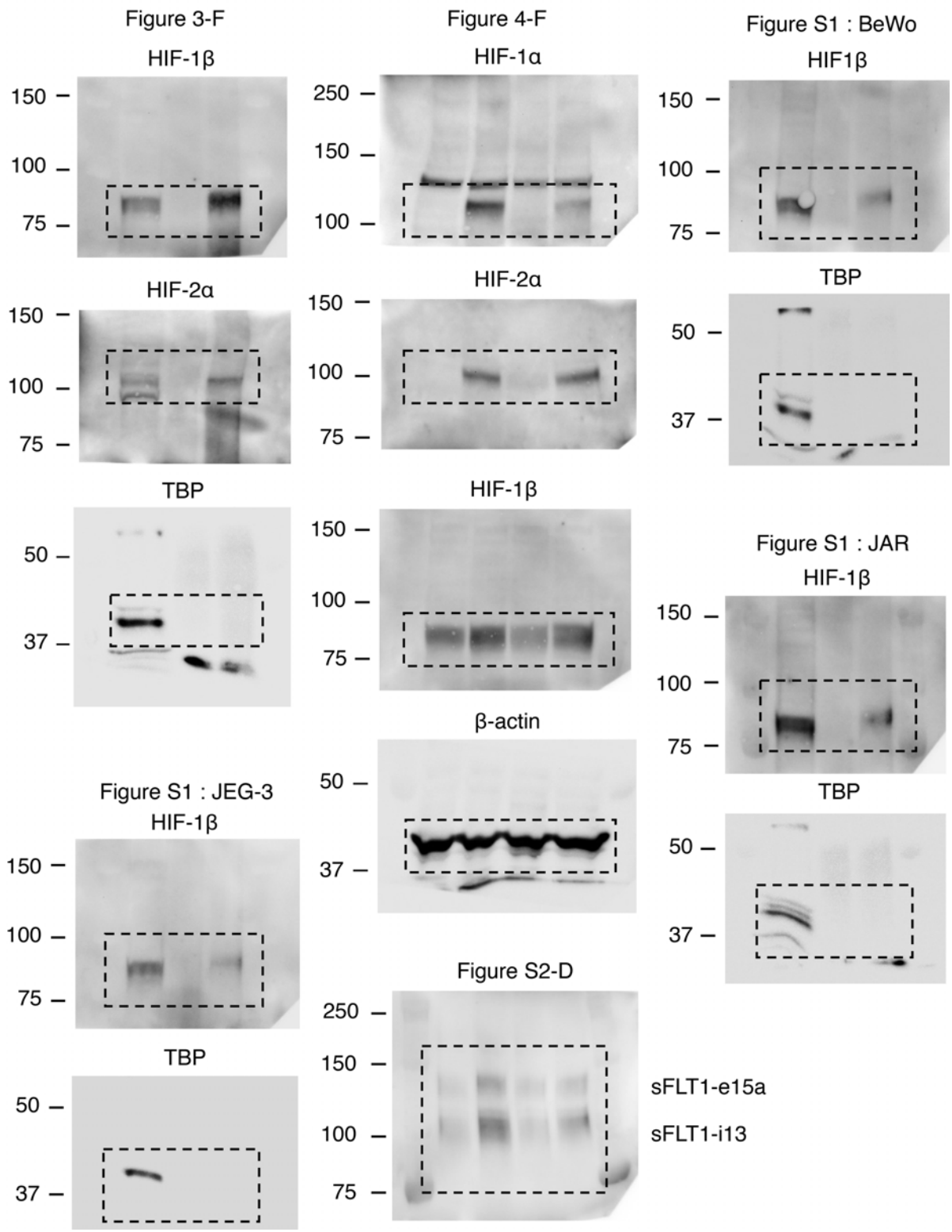


Figure S4. Uncropped images of the western blots shown in the main and supplementary figures.

Boxed areas indicate the cropped regions.

Table S1. Oligonucleotide primer sequences for qRT-PCR.

Gene		Primer sequence
HIF-1β	Forward	5'-TGAATGGTTTGGCAGCACAC-3'
	Reverse	5'-TGAAGTGGAAAGCTGCTCAC-3'
total-FLT1	Forward	5'-CCCTGTAACCATAATCATTCCGAAG-3'
	Reverse	5'-TCAGCCACAACCAAGGTGCTA-3'
tmFLT1	Forward	5'-AGAGATGGGACCGTCATCAG-3'
	Reverse	5'-CTGGCTCTAGCCTGCTTTTG-3'
sFLT1-i13	Forward	5'-ACTTGGTGCACGTTTGGATT-3'
	Reverse	5'-AGAGGTTGGCATCAAAATGG-3'
sFLT1-e15a	Forward	5'-AGTTGGAGAGCCAAGACAATC-3'
	Reverse	5'-CAGCATTTCACCATCTTGGTC -3'
β-actin	Forward	5'-AAATCTGGCACCACACCTTC-3'
	Reverse	5'-TGATCTGGGTCATCTTCTCG -3'