



Supplemental Material to:

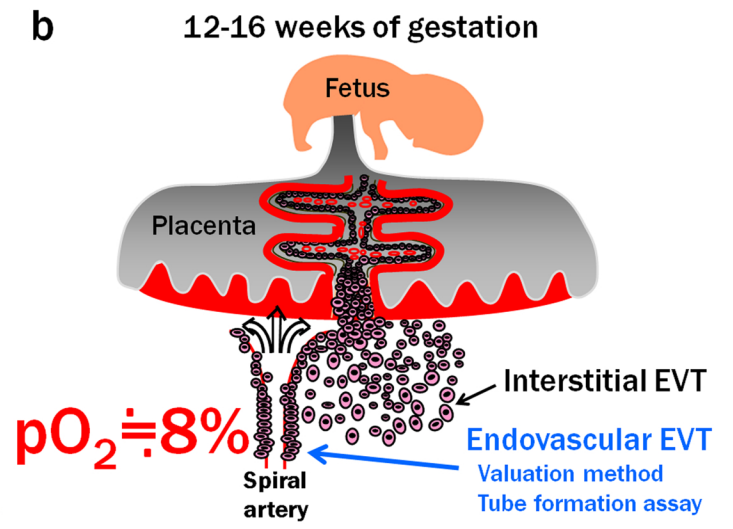
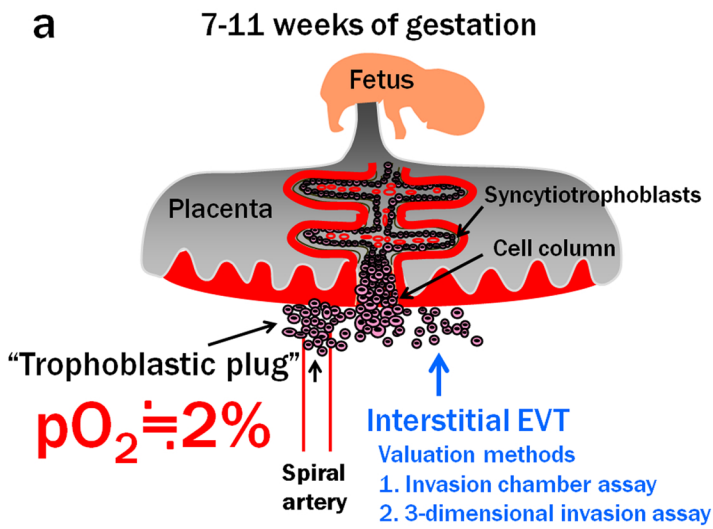
**Akitoshi Nakashima, Mikiko Yamanaka-Tatematsu,
Naonobu Fujita, Keiichi Koizumi, Tomoko Shima,
Toshiko Yoshida, Toshio Nikaido, Aikou Okamoto,
Tamotsu Yoshimori and Shigeru Saito**

**Impaired autophagy by soluble endoglin,
under physiological hypoxia in early pregnant period,
is involved in poor placentation in preeclampsia**

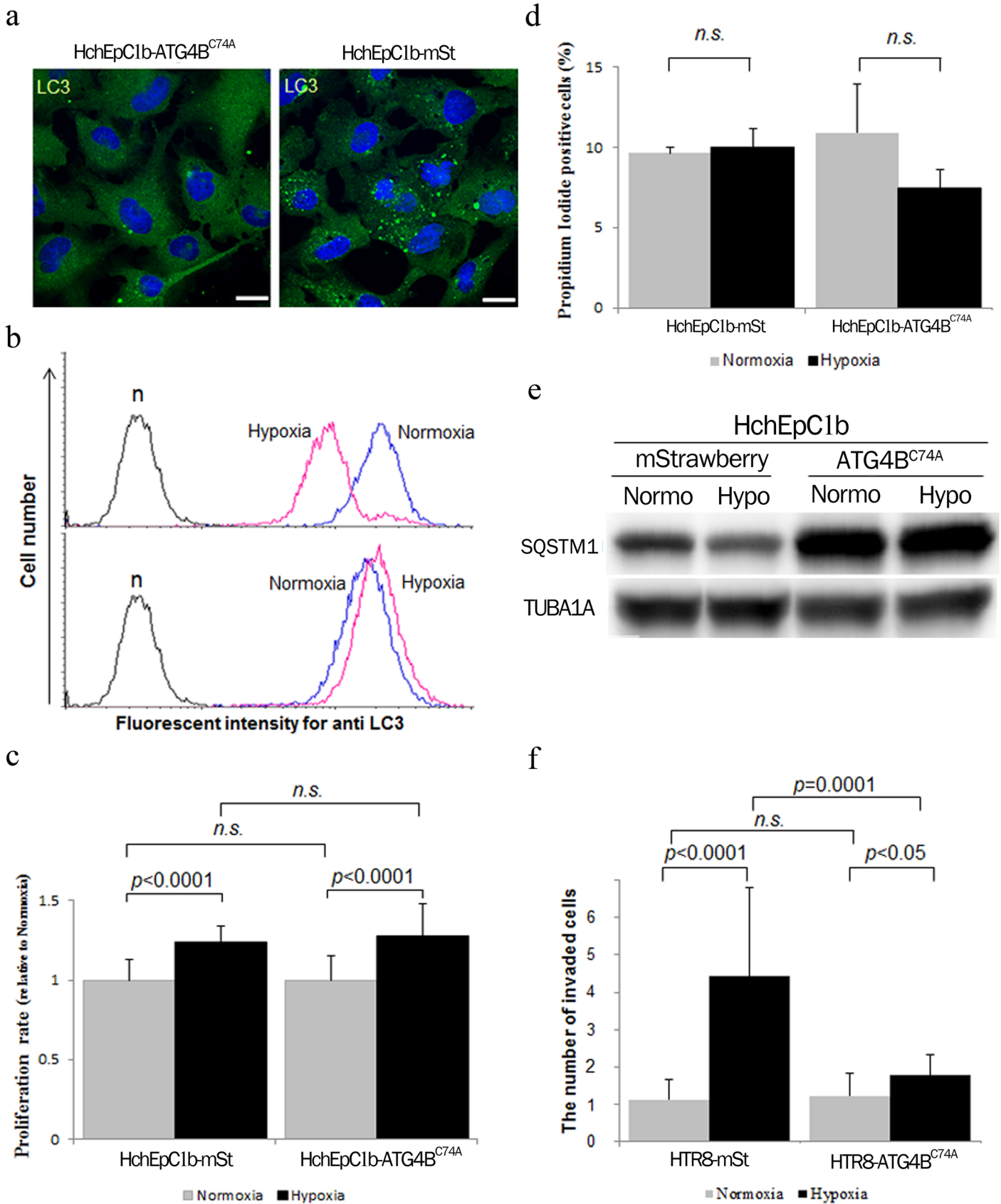
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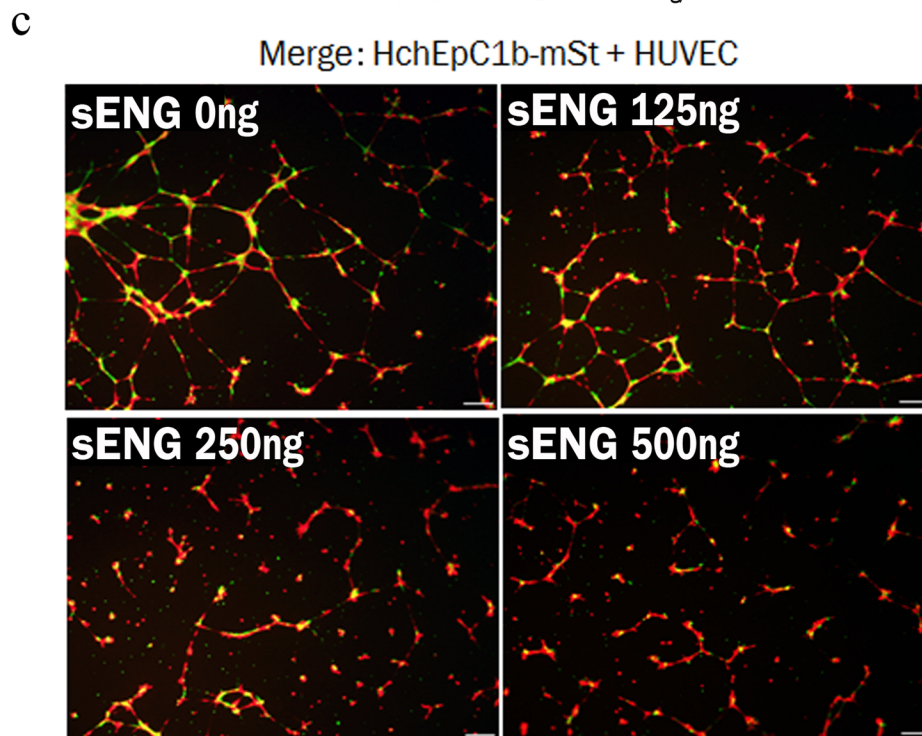
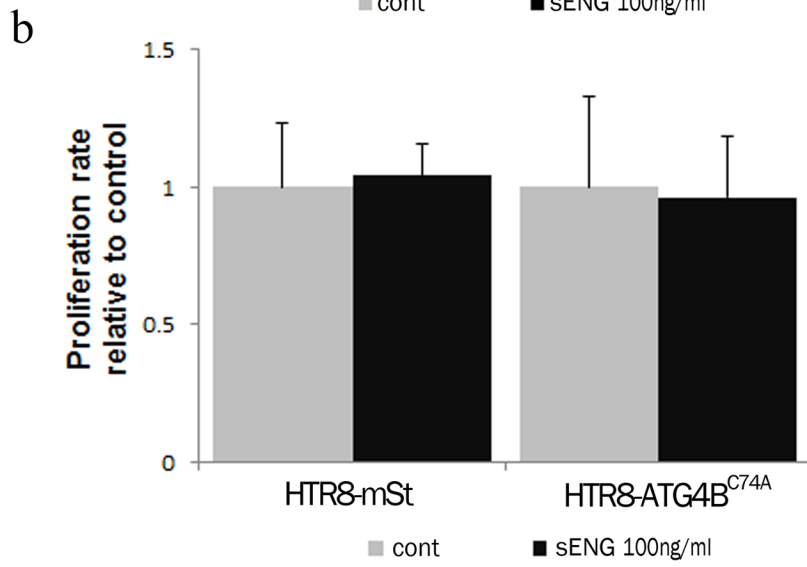
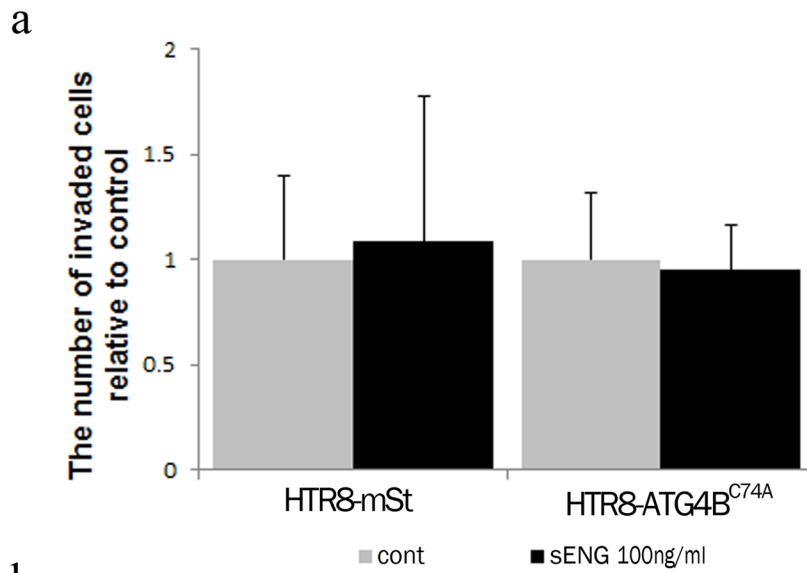
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supplemental figure 1



supplemental figure 2



supplemental figure 3

	PE (n=9)	NP (n=17)	Pl. previa (n=3)
Maternal age (years)	35 ± 4.5	32.6 ± 4.0	34.3 ± 5.0
Gestational age (weeks)	33.6 ± 3.0	37.8 ± 1.1[*]	34.3 ± 0.6
Primiparous	44%	43%	67%
Systolic blood pressure (mmHg)	168 ± 28	118 ± 11^{**}	104 ± 10[*]
Diastolic blood pressure (mmHg)	105 ± 15	73 ± 6^{**}	63 ± 4[*]
Proteinuria (g protein / g creatinine)	2.4 ± 1.6	N.D.[@]	N.D.[@]
Body weight of neonate (g)	1823 ± 667	2977 ± 540^{**}	2140 ± 142

Data is presented as Mean ± SEM, * p<0.05, ** p<0.01 vs PE.

N. D.: not detected, @: The quantification of urine protein was not performed in NP group, but all the patients in NP group had urine dipstick negative for proteinuria.

PE: preeclampsia NP: normal pregnancy Pl. previa: placenta previa

supplemental table 1

1 **Supplemental Figure 1. EVT functions during 7 to 11 weeks of gestation (a) and 12 to 16**
2 **weeks of gestation (b).**

3 (a) No blood flow was observed in the intervillous space before 12 weeks of gestation
4 (2% O₂). Interstitial EVTs can invade the maternal decidua under harsh conditions before 12
5 weeks of gestation. In this study, the invasion chamber assay and three-dimensional invasion
6 assay were used as a model of interstitial EVT invasion. (b) Subsequently, loss of the
7 trophoblast plug in the spiral arteries after 12 weeks of gestation allowed maternal blood to
8 perfuse the intervillous space, resulting in a marked increase in the pO₂ level in the placenta
9 (8% O₂). After 12 weeks of gestation, endovascular EVTs started to invade the uterine spiral
10 arteries, and replace endothelial cells. The tube formation assay was used as a model of vascular
11 remodeling by endovascular EVTs.

12 **Supplemental Figure 2. Autophagy induction (a and b), cell proliferation (c) and cell death**
13 **(d), intracytoplasmic SQSTM1 expression (e) and cell invasion (f) in autophagy-deficient**
14 **EVT cells.**

15 (a) Representative panels show the images with anti-MAP1LC3B (LC3) (green) and
16 nuclear staining (Hoechst33342, blue) merged in HchEpC1b-ATG4B^{C74A}, an autophagy-
17 deficient EVT cell line, and HchEpC1b-mStrawberry cells under 2% oxygen tension for 24 h.

1 Scale bar: 20 μ m. (b) Amount of MAP1LC3B in HchEpC1b-mStrawberry cells (upper Figure in
2 b) and -ATG4B^{C74A} cells (lower figure in b). The amount of MAP1LC3B was estimated at 24 h
3 under 20% oxygen tension (normoxia) and 2% oxygen tension (hypoxia). n: normal rabbit
4 serum used as a negative control. (c) Cell proliferation rates were estimated by WST-1 assay in
5 HchEpC1b-ATG4B^{C74A} and -mStrawberry cells for 48 h. (d) Analysis of dead cell percentages
6 in HchEpC1b-ATG4B^{C74A} and -mStrawberry cells under normoxia or hypoxia (2% oxygen
7 tension) for 48 h analyzed by propidium iodide staining. (e) Western blot analysis revealed that
8 SQSTM1 expression under hypoxia (2% oxygen tension) for 24 h decreased in HchEpC1b-
9 mStrawberry cells, but was not changed in HchEpC1b-ATG4B^{C74A} cells. (f) Invasion assays
10 were performed under normoxia (gray bars) or hypoxia (black bars) for 48 h. In HTR8-
11 mStrawberry cells, EVT invasion was increased under hypoxic conditions, but this increase was
12 not observed in HTR8-ATG4B^{C74A} cells. Data were normalized to 1 for normoxia at 24 h.

13 **Supplemental Figure 3. sENG inhibited tube formation, but not EVT invasion under**
14 **normoxia.**

15 (a) Invasion assays were performed with HTR8-ATG4B^{C74A}, an autophagy-deficient
16 EVT cell line, and -mStrawberry cells in the presence (black bars) or absence (gray bars) of 100
17 ng/ml sENG under normoxia for 48 h. The Y-axis indicates the number of invading cells. Data
18 were normalized to 1 for the control at 48 h. (b) Cell proliferation rates were estimated by WST-

1 1 assay in HTR8-ATG4B^{C74A} and -mStrawberry cells in the presence (black bars) or absence
2 (gray bars) of 100 ng/ml sENG under hypoxia for 48 h. Data were normalized to 1 for the
3 control at 48 h. (c) Tube formation assays by HUVECs (labeled with green) with HchEpC1b-
4 mStrawberry cells (labeled with red) were performed under 8% oxygen tension for 24 h in the
5 presence of 0, 125, 250 or 500 ng/ml sENG. Representative figures show the images with
6 HchEpC1b and HUVECs merged. Scale bar: 300 μ m.