Supporting Information

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SI Materials and Methods

Western Blot. HMEC-1 cells were treated with siRNA for 72 h, and the whole-cell extracts were detected by an anti-HYPB antibody (Abcam). Acid-extracted histones from embryos and HMEC-1 cells were subjected to histone methylation state analyses by anti-H3 (Abcam), H3K36me1 (Abcam), H3K36me2 (Upstate), and H3K36me3 (Abcam).

Tetraploid Complementation Assay. Wild-type, two-cell-stage embryos were obtained from the oviducts of superovulated ICR females at E1.5 and electrofused with CF-150B Cell Fusion Instrument (BLS Ltd). Two-cell-stage embryos were recovered from $Hypb^{+/-}$ crosses at E1.5. After zona removal, two four-cell-stage, tetraploid, wild-type embryos and a diploid, eight-cell-stage embryo were aggregated overnight, and blastocysts were transferred into the uteri of E2.5 pseudopregnant B6CBAF1 recipients. The fetuses and placentas were collected at E10.5–E16.5.

Microarray and RT-qPCR. Total RNA was isolated from yolk sacs ($n = 4$ per assay) with RNAzol (Campro Scientific). Microarray analysis was performed with the Affymetrix GeneChip Mouse Genome 430 2.0 Arrays. Statistical significance was analyzed with the Significance Analysis of Microarrays (SAM) tool with the delta value 0.59. The logged expression values were adjusted by subtracting the raw-wise median and subjected to hierarchical clustering. For RT-qPCR, total RNA was isolated from yolk sacs ($n = 3$ per assay) with TRIzol reagent (Invitrogen), followed by reverse transcription with the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Real-time PCR was performed with the Custom Plating TaqMan Array Plates and a 7300 Real-Time PCR system (Applied Biosystems). The relative gene expression was calculated with the ΔΔCt method.

ES Cell Culture and EB Differentiation. $Hypb^{+/-}$ and $Hypb^{-/-}$ ES cells were cultured in high-glucose DMEM (Invitrogen) with 15% FCS, nonessential amino acids (Invitrogen), β-mercaptoethanol (Invitrogen), L-glutamine (Invitrogen), leukemia inhibitory factor (LIF; Millipore), and penicillin–streptomycin. Trypsinized ES cells were plated onto Petri dishes and cultured in suspension without LIF for 3 days. For attached cultures, day 3 EBs were transferred on gelatin-coated cover glasses for another 23 days.

Endothelial Cell Culture. HMEC-1 cells were cultured in MCDB 131 medium (Invitrogen) containing 15% FCS (Invitrogen), 10 ng/mL EGF (Sigma-Aldrich), 1 ng/mL hydrocortisone (Sigma-Aldrich), and antibiotics.

siRNA Sequences. HYPB siRNA: sense 5′-GGAGUAUGCACGAAACAAATT-3′, antisense 5′-UUUGUUUCGUGCAUACUCCTT-3′; Scramble siRNA: sense 5′-UUCUCCGAACGUGUCACGUTT-3′, antisense 5′-ACGUGACACGUUCGGAGAATT-3′.

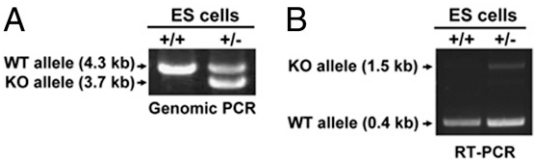

Fig. S1. PCR analyses of targeted ES cells. (*A*) Genomic PCR analysis of targeted ES cells with primers P1 and P2. For the wild-type (WT) allele and the Hypb knockout (KO) allele, the amplified fragments are 4.3 kb and 3.7 kb, respectively. (*B*) Determination of *Hypb* expression in ES cells by RT-PCR with primers P4 and P5. Amplified fragments of parental clones are 0.4 kb, whereas those of $Hypb^{+/-}$ clones include a new 1.5-kb fragment that contains the inserted TK-neo cassette.

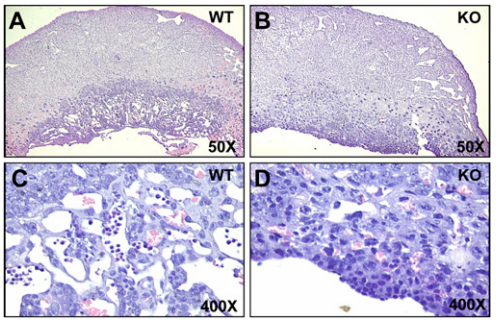

Fig. S2. Wild-type tetraploid extraembryonic trophoblast fails to rescue $Hypb^{-/-}$ phenotype. Representative histological sections of wild-type and $Hypb^{-/-}$ placentas after tetraploid complementation are shown. Note that the failure of allantois attachment to the chorion in $Hypb^{-/-}$ embryos leads to an undeveloped labyrinthine region (*B* and *D*), compared with that in a wild-type placenta (*A* and *C*).

Table S1. Genotyping statistics of *Hypb* heterozygous mouse intercrosses

	Number of survivals			
Stage	+/+	+/–	–/–	Total
E8.5	6	15	7	28
E9.5	12	20	9	41
E10	21	47	25	93
E10.5	51	103	43	197
E11	4	9	6*	19
E11.5	23	43	20*	86
E12.5	8	15	0	23
E15.5	5	12	0	17
3 Weeks	87	159	0	246

*Embryos found dead and necrotic.

Table S2. Genotyping statistics after tetraploid complementation assays

	Number of survivals			
Stage*	+/+	+/–	–/–	Total
E9.5	4	4	2	10
E10.5	7	10	9	26
E11.5	8	9	6	23
E12.5	11	15	14	40
E13.5	7	10	0	17
E14.5	4	5	0	9

*Because the aggregation experiments required an in vitro embryo culture and resulted in a delay in development, viable mutant embryos were observed up to E12.5.