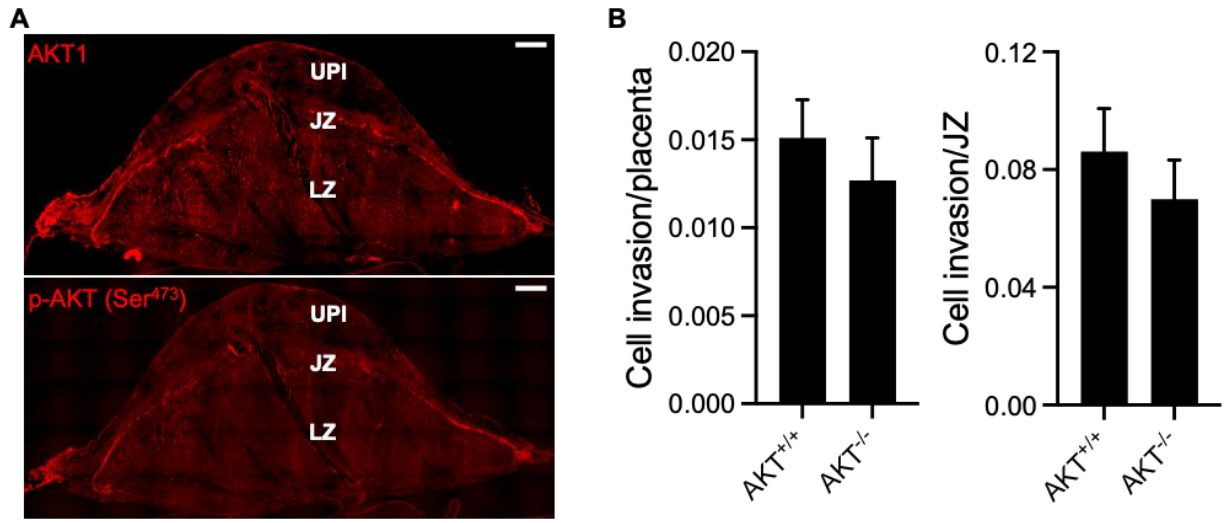

Fig. S1. Placental AKT protein distribution and intrauterine trophoblast cell invasion in wild type and *Akt1* null placentation sites. **A)** Distribution of AKT1 (top) and phospho (p)-AKT4 (Ser^{473}) proteins (bottom) in the gestation day (**gd**) 18.5 placentation site. **B**) Area occupied by invasive trophoblast cells (cytokeratin-positive cells) within the uterine-placental interface was quantified and is presented relative to areas associated with the entire placenta or the junctional zone (**JZ**). Graphs represent means ± SEM (n = 6/group).

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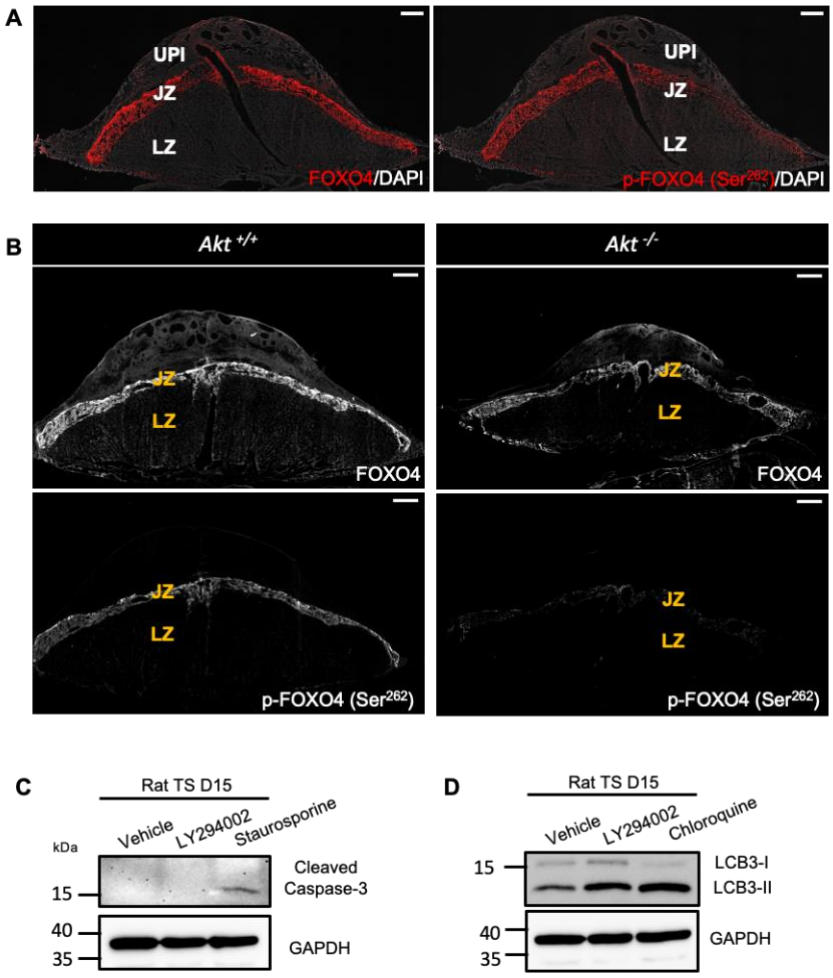

Fig. S2. Relationship of PI3K/AKT signaling to FOXO4 distribution and functions. A) Distributions of FOXO4 and phospho (p)-FOXO4 (Ser^{262}) proteins in gestation day (**gd**) placentation sites. **B)** Distribution of FOXO4 and phospho (p)-FOXO4 (Ser^{262}) proteins in *Akt1*$^{+/+}$ and *Akt1*$^{-/-}$ placentas at gd 18.5. UPI: uterine-placental interface; JZ: junctional zone; and LZ: labyrinth zone. **C**) Western blot analysis of cleaved caspase-3 in rat TS cells exposed to vehicle (DMSO), LY294002 (10 μM), or staurosporine (1 μM). Staurosporine was used as a positive control. **D**) Western blot analysis for serine palmitoyltransferase long chain base subunit 3 (**LCB3**) in rat TS cells exposed to vehicle (DMSO), LY294002 (10 μM), or chloroquine (50 μM). Chloroquine was used as a positive control. Please note that inhibition of PI3K/AKT signaling did not have a detectable effect on the formation of cleaved caspase-3 but did have a modest effect on LCB3 accumulation.

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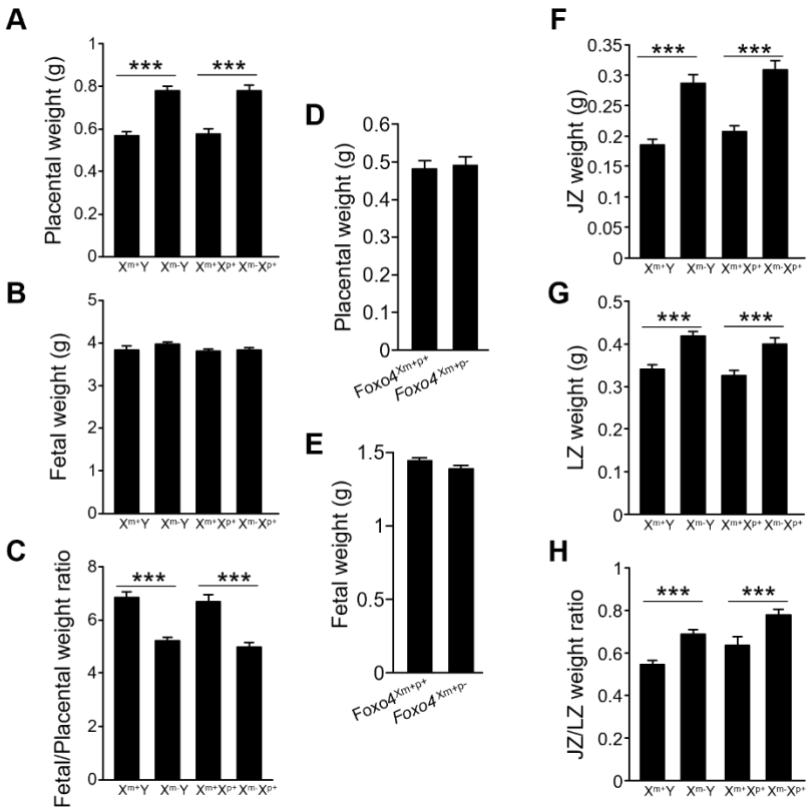

Fig. S3. Placentas **(A)** and fetuses **(B)** were dissected from *Foxo4* heterozygous females mated with wild type males at gd 20.5 and weighed; **C**, fetus/placenta ratio. Placentas **(D)** and fetuses **(E)** were dissected from wild type females mated with *Foxo4* hemizygous null males at gd 18.5 and weighed. **F-H**) Placentas from heterozygous females mated with wild type males were then separated into junctional zone (**JZ, F**) and labyrinth zone (**LZ, G**) compartments, and weighed; **H**, JZ/LZ weight ratio. $X^{m+}Y$, n = 19; $X^{m-}Y$, n = 22; $X^{m+}X^{p+}$, n = 15; $X^{m-}X^{p+}$, n = 15 from 6 dams. Graphs represent means ± SEM. Asterisks denote statistical differences (***P<0.001) as determined by Student's or Welch's t-test.

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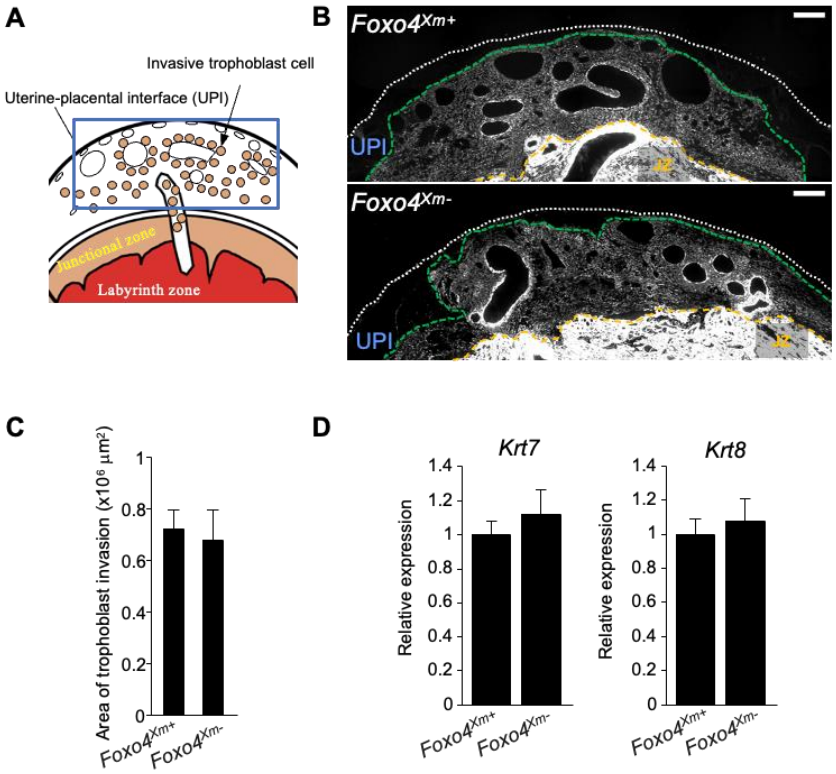

Fig. S4. FOXO4 deficiency does not affect either intrauterine trophoblast invasion or the intrauterine invasive trophoblast cell phenotype. A) Schematic representation of a late gestation placentation site. The uterine-placental interface, site for intrauterine trophoblast invasion, is highlighted in the boxed area. **B)** Trophoblast cells were immunostained for pan-cytokeratin (**KRT**). Representative images are shown. The extent of intrauterine trophoblast invasion is demarcated using a green dashed line. The white dotted line represents the outer border of the uterus, and the yellow dashed line represents the uterine border with the placenta. Scale bars = 500 µm. **C)** The area of intrauterine trophoblast invasion is graphically depicted (n = 6/group). Graphs represent means ± SEM. **D)** RT-qPCR measurements of *Krt7* and *Krt8* transcripts, signature markers for invasive trophoblast cells, within dissected uterine-placental interface tissue specimens at gd 18.5 (*Foxo4*$^{Xm+}$, n = 12; *Foxo4*$^{Xm-}$, n = 12). Graphs represent means ± SEM.

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Table S1. Genotype of offspring following *Akt1* heterozygous breeding.

Offspring from $Akt1^{+/-}$ x $Akt1^{+/-}$	Offspring genotype		
	$Akt1^{+/+}$	$Akt1^{+/-}$	$Akt1^{-/-}$
Total	40	92	42
Ratio (%)	23	52.9	24.1

Table S2. RNAseq AKT1 WT vs KO JZ

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Table S3. AKT KO JZ Pathway analysis

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Table S4. Genotype of offspring following *Foxo4* hemizygous male x wild type female breeding.

Offspring from $X^{-}Y$ x XX	Offspring genotype	
	$X^{m+}Y$	$X^{m+}X^{p-}$
Total	40	44
Ratio (%)	47.6	52.4

Table S5. RNAseq Foxo4 WT vs KO JZ

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Table S6. Foxo4 KO JZ Pathway analysis

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Table S7. RNAseq Foxo4 KD in rTSC

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Table S8. Foxo4 KD rTS Pathway analysis

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Table S9. Primers used for genotyping

Primer name	Sequence
Akt1 Fwd	TGAGTCCATTCTGGAGGACTAGAC
Akt1 Rev1	TTGCCCAGTAGCTTCAGGTACTC
Akt1 Rev2	GAGGGAAGGTTAGGGACTAGCC
Foxo4 Fwd	AGAAGGTACCCACGGAGGGA
Foxo4 Rev1	CCACACAGTTCCTGCTGTACATAG
Foxo4 Rev2	CTCCTTGGAGTGGCACCTTC

Fwd, forward
Rev, reverse

Table S10. List of primers used for RT-qPCR

Gene	Forward primer	Reverse primer	Accession no.	Amplicon size (bp)
Ccn3	CATGGTTCGGCCTTGTGAG	TGGATTTCAGGGACTTCTTGGT	NM_030868.2	89
Ccnd1	AATGCCAGAGGCGGATGAGA	CGTTGTGCGGTAGCAGGAGA	NM_171992.5	190
Ccne1	TGCAGGCGAGGATGAGA	GAAGTCCTGTGCCAAGTAGAATG	NM_001100821.1	98
Cdc6	CAGGCGAGCTATTGAAATTGTG	GACTTGGGATATGTGAGCGAGA	NM_001108298.1	130
Cdk1	GTTGACATCTGGAGCATAGG	CTCTACTTCTGGCCACACTT	NM_019296.2	144
Mcm5	TGTCCAGGATTTCACCAAACA	CACTTGAGGCGGTAAAGCAC	NM_001399204.1	123
Prl8a4	CTGAAACCCTCTGTAATCTTGCTG	GTCTCGTCCCTCTTAATCAGTTTTG	NM_021580.1	112
Krt7	CGGAATGGAACCTGTGAA	GTAGATGTAGTCTTGATGGAATAAG	NM_001047870.2	150
Krt8	TGGGCCAGGAGAAGCTGAA	CACATCCTTGATGAGGACAAA	NM_199370.1	140
Foxo4	CGGAATGCCTGGGGAAA	ATGTACCTTGATGAACTTGCTGTG	NM_001106943.1	213
Grb7	TACCACCTGGAGAGAAGAGAGAGAG	GGGCTCAGATCCAGTTCCA	NM_053403.2	141
Erbb3	TGCGTTGCCAGTTGTCC	CCGTGCTTATCTACTTCCATCTTGT	NM_017218.3	92
JamL	TCGGCCTTGATGGGATG	CACGCTGAGGCTGGAGTAGTAG	XM_032909807.1	129
Lpal2	AAGGAGATGCCAACCAACAAA	GCCATTCTTCCCTCTCCTGA	NM_001109578.1	125
Mmp12	GCTGGTTCGGTTGTTAGG	GTAGTTACACCCTGAGCATAC	NM_053963.2	100
Gstm1	CTGGACGCCTTCCCAAA	TAGCAAGGGCCTACTTGTTACTCC	NM_017014.2	145
Txnip	GTCTCAGCAGTGCAAACAGACC	AAGCTCAAAGCCGAACTTGTACTC	NM_001008767.2	139
Alox5ap	TGTGGGCAATGTTGTGCTC	GCTTTGCGCCTTGCTTTC	NM_017260.2	100
Nupr1	GCCCACACTTCCCAGCA	ACCTCCACCGACGACATAAGA	NM_053611.2	102
Gapdh	GACATGCCGCCTGGAGAAAC	AGCCCAGGATGCCCTTTAGT	NM_017008.4	92

Table S11. shRNA sequences

shRNA	Sequence
Foxo4 shRNA 1	CCGGTGCAGTCCTTGTCCTCGAAACTCGAGTTTCGAGGACAAGGACTGCTTTTTG
Foxo4 shRNA 2	CCGGTGCTTGCATCTCCTACTGAACTCGAGTTCAGTAGGAGATGCAAGCTTTTTG