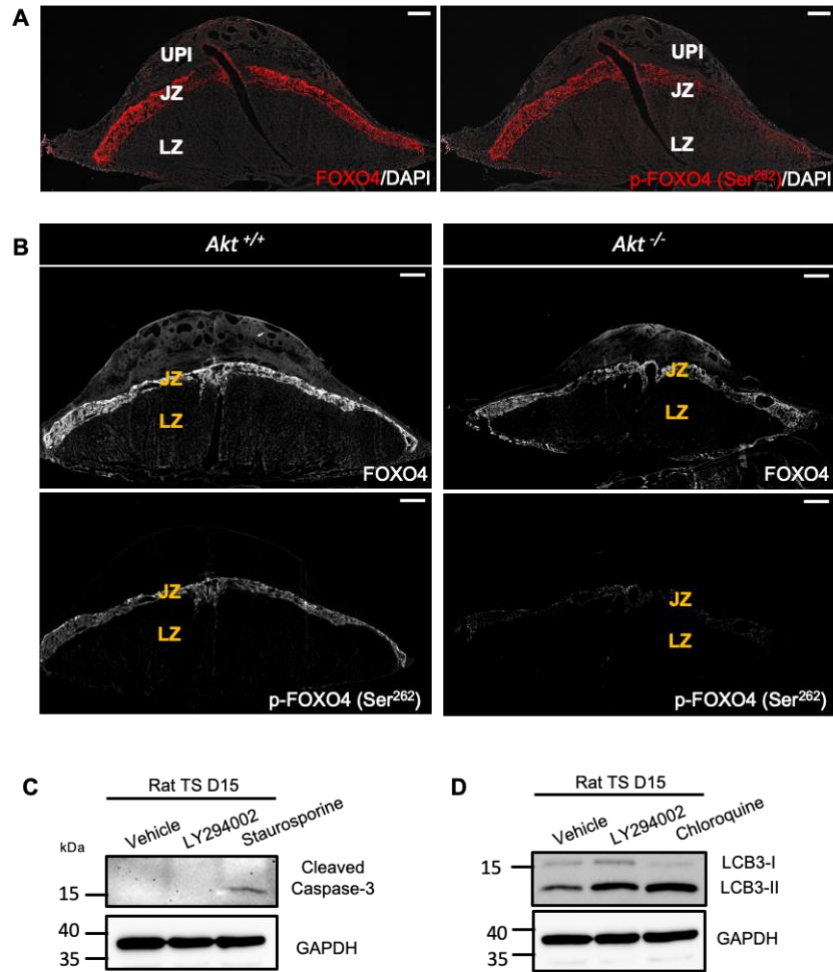
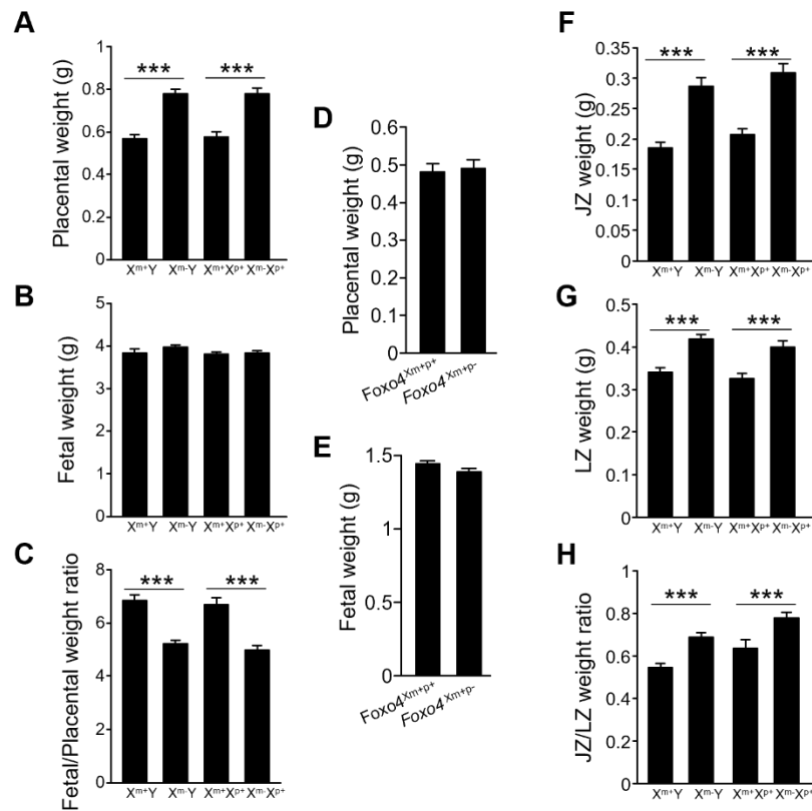


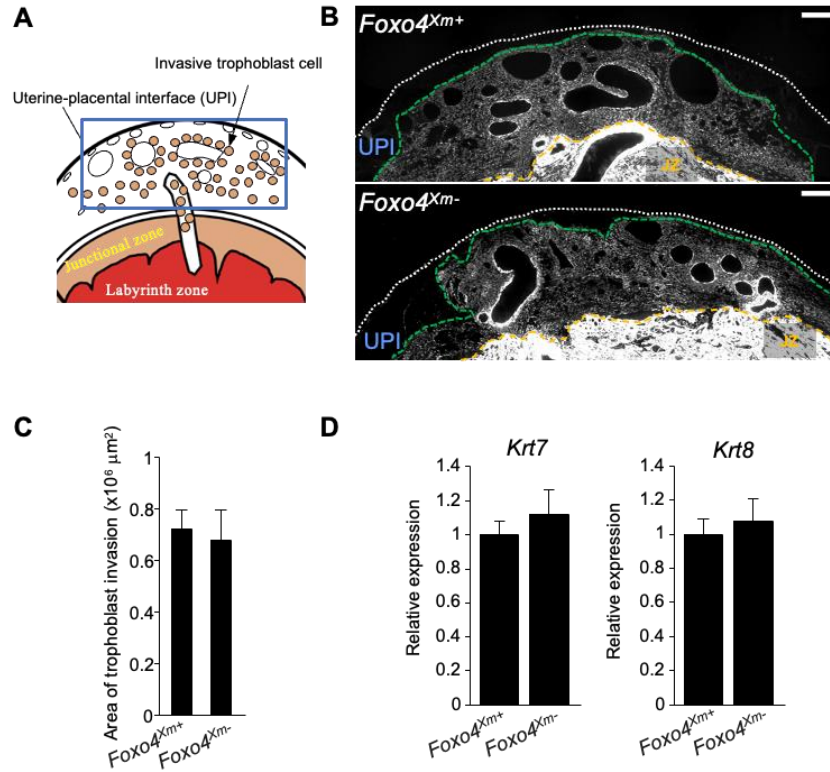
**Fig. S1. Placental AKT protein distribution and intrauterine trophoblast cell invasion in wild type and *Akt1* null placentation sites.** **A)** Distribution of AKT1 (top) and phospho (p)-AKT4 (Ser<sup>473</sup>) proteins (bottom) in the gestation day (gd) 18.5 placentation site. **B)** Area occupied 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).



**Fig. S2. Relationship of PI3K/AKT signaling to FOXO4 distribution and functions. A)** Distributions of FOXO4 and phospho (p)-FOXO4 (Ser<sup>262</sup>) proteins in gestation day (gd) placentation sites. **B)** Distribution of FOXO4 and phospho (p)-FOXO4 (Ser<sup>262</sup>) proteins in *Akt1*<sup>+/+</sup> and *Akt1*<sup>-/-</sup> 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  $\mu$ M), or staurosporine (1  $\mu$ M). 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  $\mu$ M), or chloroquine (50  $\mu$ M). 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.



**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  $\pm$  SEM. Asterisks denote statistical differences (\*\*\*)  $P < 0.001$  as determined by Student's or Welch's *t*-test.



**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  $\mu\text{m}$ . **C)** The area of intrauterine trophoblast invasion is graphically depicted ( $n = 6/\text{group}$ ). Graphs represent means  $\pm$  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<sup>Xm+</sup>*,  $n = 12$ ; *Foxo4<sup>Xm-</sup>*,  $n = 12$ ). Graphs represent means  $\pm$  SEM.

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

Offspring from <i>Akt1</i> <sup>+/-</sup> x <i>Akt1</i> <sup>+/-</sup>	Offspring genotype		
	<i>Akt1</i> <sup>+/+</sup>	<i>Akt1</i> <sup>+/-</sup>	<i>Akt1</i> <sup>-/-</sup>
Total	40	92	42
Ratio (%)	23	52.9	24.1

**Table S2. RNAseq AKT1 WT vs KO JZ**

[Click here to download Table S2](#)

**Table S3. AKT KO JZ Pathway analysis**

[Click here to download Table S3](#)

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

Offspring from <b>X<sup>-</sup>Y x XX</b>	Offspring genotype	
	<b>X<sup>m+</sup>Y</b>	<b>X<sup>m+</sup>X<sup>p-</sup></b>
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
<i>Akt1</i> Fwd	TGAGTCCATTCTGGAGGACTAGAC
<i>Akt1</i> Rev1	TTGCCAGTAGCTTCAGGTA
<i>Akt1</i> Rev2	GAGGGAAGGTTAGGGACTAGCC
<i>Foxo4</i> Fwd	AGAAGGTACCCACGGAGGGA
<i>Foxo4</i> Rev1	CCACACAGTTCCTGCTGTACATAG
<i>Foxo4</i> 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)
<i>Ccn3</i>	CATGGTTCGGCCTTGTGAG	TGGATTTACAGGACTTCTTGGT	NM_030868.2	89
<i>Cend1</i>	AATGCCAGAGGCGGATGAGA	CGTTGTGCGGTAGCAGGAGA	NM_171992.5	190
<i>Ccne1</i>	TGCAGGCGAGGATGAGA	GAAGTCCTGTGCCAAGTAGAATG	NM_001100821.1	98
<i>Cdc6</i>	CAGGCGAGCTATTGAAATTGTG	GACTTGGGATATGTGAGCGAGA	NM_001108298.1	130
<i>Cdk1</i>	GTTGACATCTGGAGCATAGG	CTCTACTTCTGGCCCACTT	NM_019296.2	144
<i>Mcm5</i>	TGTCCAGGATTTACCAAACA	CACTTGAGGCGGTAAGCAC	NM_001399204.1	123
<i>Prl8a4</i>	CTGAAACCCTCTGTAATCTTGCTG	GTCTCGTCCCTCTAATCAGTTTG	NM_021580.1	112
<i>Krt7</i>	CGGAATGGAACCTGTGAA	GTAGATGTAGTCTTGATGGAATAAG	NM_001047870.2	150
<i>Krt8</i>	TGGGCCAGGAGAAGCTGAA	CACATCCTTGATGAGGACAAA	NM_199370.1	140
<i>Foxo4</i>	CGGAATGCCTGGGGAAA	ATGTACCTTGATGAACCTGCTGTG	NM_001106943.1	213
<i>Grb7</i>	TACCACCTGGAGAGAAGAGAGAGAG	GGGCTCAGATCCAGTTCCA	NM_053403.2	141
<i>ErbB3</i>	TGCGTTGCCAGTTGTCC	CCGTGCTTATCTACTCCATCTTGT	NM_017218.3	92
<i>JamL</i>	TCGGCCTTGATGGGATG	CACGCTGAGGCTGGAGTAGTAG	XM_032909807.1	129
<i>Lpal2</i>	AAGGAGATGCCAACCAACAAA	GCCATTCTCCCTCCTGA	NM_001109578.1	125
<i>Mmp12</i>	GCTGGTTCGGTTGTTAGG	GTAGTTACACCCTGAGCATAC	NM_053963.2	100
<i>Gstm1</i>	CTGGACGCCTTCCAAA	TAGCAAGGGCCTACTTGTACTCC	NM_017014.2	145
<i>Txnip</i>	GTCTCAGCAGTGCAAACAGACC	AAGCTCAAAGCCGAACCTGTACTC	NM_001008767.2	139
<i>Alox5ap</i>	TGTGGGCAATGTTGTGCTC	GCTTTGCGCCTTGCTTTC	NM_017260.2	100
<i>Nupr1</i>	GCCACACTTCCAGCA	ACCTCCACCGACGACATAAGA	NM_053611.2	102
<i>Gapdh</i>	GACATGCCGCCTGGAGAAAC	AGCCCAGGATGCCCTTAGT	NM_017008.4	92

**Table S11. shRNA sequences**

shRNA	Sequence
<i>Foxo4</i> shRNA 1	CCGGTGCAGTCCTTGTCTCGAACTCGAGTTTCGAGGACAAGGACTGCTTTTTG
<i>Foxo4</i> shRNA 2	CCGGTGCCTTGCATCTCCTACTGAACTCGAGTTCAGTAGGAGATGCAAGCTTTTTG