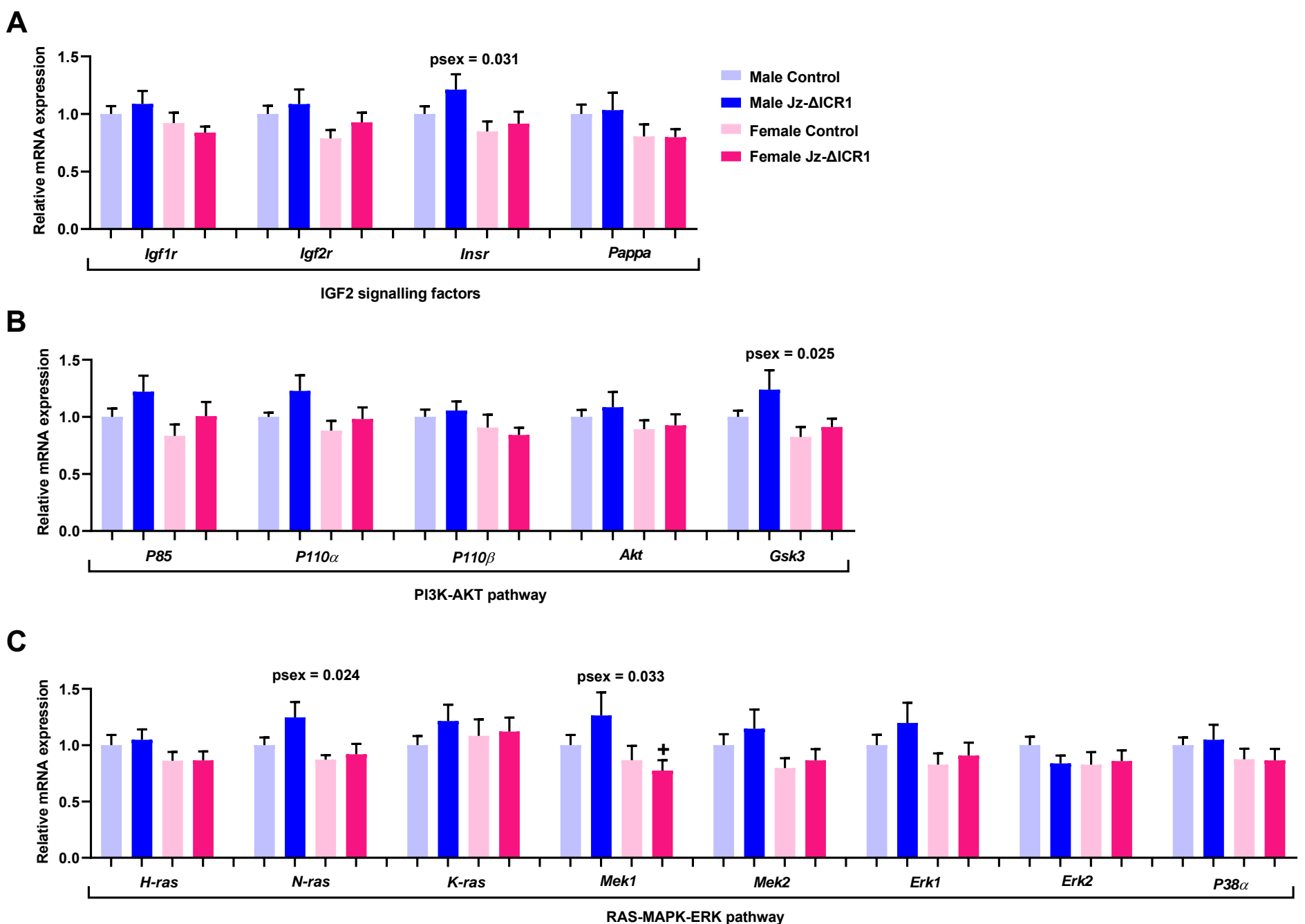
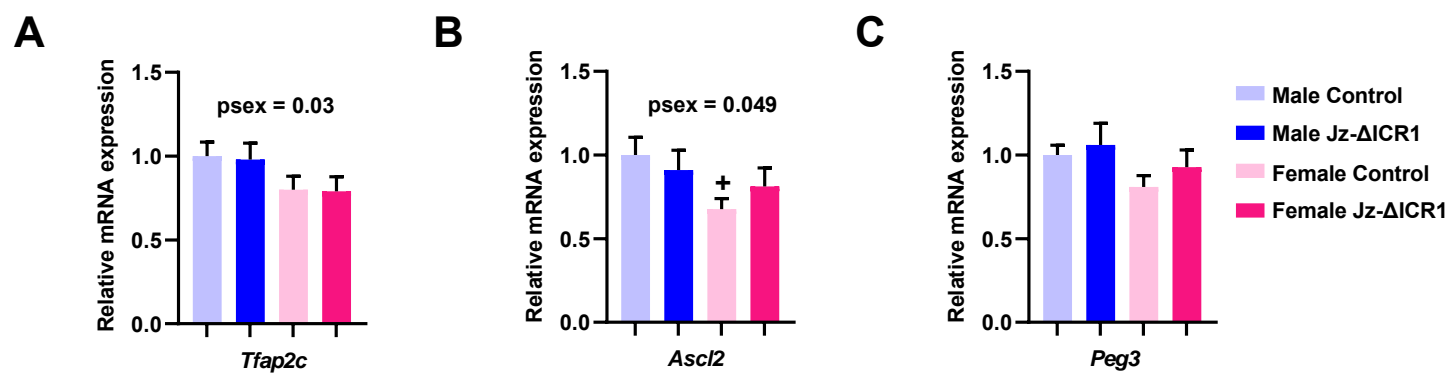


**Fig. S1. Negative controls for *in situ* hybridization and immunohistochemistry experiments in D16 mouse placentas.** Sense *in situ* hybridization probes for (A) *H19* and (B) *Psg23*. Secondary antibody only control used in immunohistochemistry experiments for (C) cleaved caspase-3 and (D) Ki67. Black boxes represent the area magnified in the image below. Black bar represents 1 mm. Red bar represents 100  $\mu$ m.



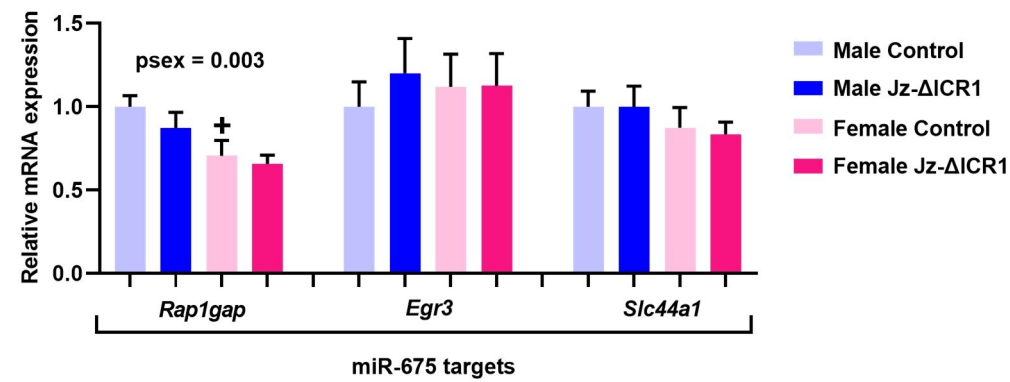
**Fig. S2. Jz- $\Delta$ ICR1 does not alter the gene expression of IGF2 signalling factors or downstream members of the PI3K-AKT and MAPK pathways.** Expression of (A) IGF2 signalling factors, (B) PI3K-AKT pathway genes and (C) RAS-MAPK-ERK pathway genes in Jz samples relative to the geometric mean of housekeeping genes (*Hprt* and *Ywhaz*) using qPCR (n = 8-10 per genotype/sex, across 11 litters). Data were obtained on D16. Values presented as mean + SEM with significance assessed by two-way ANOVA and pairwise T-test (psex < 0.05 = +).



**Fig. S3. Jz-ΔICR1 does not alter the gene expression of *Tfap2c*, *Ascl2* or *Peg3*.** Expression of (A) *Tfap2c*, (B) *Ascl2* and (C) *Peg3* in Jz samples relative to the geometric mean of housekeeping genes (*Hprt* and *Ywhaz*) using qPCR (n = 9-10 per genotype/sex, across 11 litters). Data were obtained on D16. Values presented as mean + SEM with significance assessed by two-way ANOVA and pairwise T-test (psex < 0.05 = +).

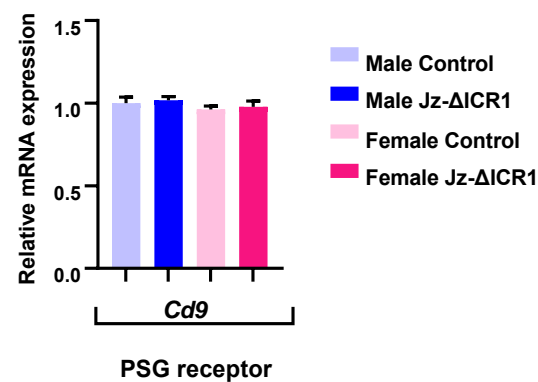
Fig. S4.

A



**Fig. S4. Jz-ΔICR1 does not alter the gene expression of miR-675 targets.** (A) Expression of miR-675 target genes in Jz samples relative to the geometric mean of housekeeping genes (*Hprt* and *Ywhaz*) using qPCR (n = 8-10 per genotype/sex, across 11 litters). Data were obtained on D16. Values presented as mean + SEM with significance assessed by two-way ANOVA and pairwise T-test (psex < 0.05 = +).

A



**Fig. S5. Jz-ΔICR1 did not alter the expression of the PSG receptor Cd9 in the Lz.** (A) Expression *Cd9* in in Lz samples relative to the geometric mean of housekeeping genes (*Hprt* and *Polr2a*) with Jz-ICR1Δ using qPCR (n = 9-10 per genotype/sex, across 11 litters). Data were obtained on D16. Values presented as mean + SEM with significance assessed by two-way ANOVA.

**Table S1. The effect of Jz-ΔICR1 on conceptus weight, placental volume and the proportion of cell types within the Jz and Db of D16 mouse placentas in males and females.**

	Male		Female		Overall P Values		
	Control	Jz-ΔICR1	Control	Jz-ΔICR1	Genotype	Sex	Interaction
Fetal weight (mg)	420.4 ± 8.4	429.1 ± 11.8	407.5 ± 12.7	412.3 ± 14.2	0.6646	0.1667	0.7708
Placental weight (mg)	123.1 ± 4.0	120.7 ± 3.2	108.2 ± 4.6 †	110.8 ± 4.3	0.9560	0.0008	0.7987
Fetal:placental weight ratio	3.5 ± 0.1	3.5 ± 0.1	3.8 ± 0.2	3.8 ± 0.1	0.8413	0.0198	0.7029
Db volume (mm <sup>3</sup> )	18.9 ± 1.1	14.3 ± 1.0 *	15.7 ± 0.5	15.5 ± 0.7	0.0104	0.2645	0.0679
Jz volume (mm <sup>3</sup> )	45.0 ± 2.3	54.1 ± 2.9	33.9 ± 2.9 †	48.1 ± 1.7 *	<0.0001	0.0018	0.3148
Lz volume (mm <sup>3</sup> )	52.1 ± 2.4	46.3 ± 1.9	47.4 ± 1.1	44.4 ± 1.3	0.0179	0.0680	0.4417
Jz SpT volume (mm <sup>3</sup> )	27.5 ± 0.9	33.4 ± 1.2 *	22.3 ± 1.7 †	29.7 ± 1.2 *	<0.0001	0.0016	0.5768
Jz GC volume (mm <sup>3</sup> )	15.8 ± 1.4	18.5 ± 1.8	10.6 ± 1.2	16 ± 1.0 *	0.0065	0.0091	0.3494
Jz TGC volume (mm <sup>3</sup> )	1.8 ± 0.2	2.2 ± 0.3	1.0 ± 0.2	2.0 ± 0.2 *	0.0077	0.0649	0.2109
Db S volume (mm <sup>3</sup> )	11.6 ± 0.6	8.5 ± 0.5 *	10.2 ± 0.6	11.1 ± 0.8 †	0.3655	0.0800	0.0034
Db GC volume (mm <sup>3</sup> )	6.2 ± 0.5	5.0 ± 0.5	4.7 ± 0.2	4.8 ± 0.5	0.2235	0.0718	0.1724
Db V volume (mm <sup>3</sup> )	1.0 ± 0.2	0.9 ± 0.2	0.8 ± 0.3	0.8 ± 0.2	0.5724	0.4757	0.7436
Jz GC / Jz SpT	0.57 ± 0.04	0.55 ± 0.04	0.48 ± 0.04	0.55 ± 0.04	0.5264	0.2328	0.2640
Db GC / Jz GC	0.42 ± 0.05	0.29 ± 0.02	0.49 ± 0.06	0.31 ± 0.04 *	0.0037	0.3990	0.6375
Db GC / (Jz GC + Db GC)	0.29 ± 0.03	0.22 ± 0.02	0.32 ± 0.03	0.23 ± 0.02 *	0.0044	0.4158	0.7247
Average Jz SpT cell size (μm <sup>2</sup> )	308.3 ± 18.4	288.7 ± 17.8	305.5 ± 15.5	294.4 ± 20.5	0.4151	0.9379	0.8192
Average Jz GC cell size (μm <sup>2</sup> )	211.6 ± 20.9	211.4 ± 14.8	174.2 ± 15.7	186.6 ± 18.7	0.7357	0.1041	0.7292
Average number of Jz SpT	91917.9 ± 7033.4	123267.1 ± 3010.1 *	70356.7 ± 9146.4	101629.7 ± 6729.3 *†	0.0006	0.0083	0.9957
Average number of Jz GC	76471.8 ± 7866.7	100092.8 ± 13213.5	60704.5 ± 7437.7	99236.4 ± 11529.4 *	0.0107	0.4356	0.4832

Weight data displayed for males (Control n = 29 and Jz-ΔICR1 n = 27, from individual pups across 13 litters) and females (Control n = 17 and Jz-ΔICR1 n = 27, from individual pups across 13 litters). Control:Jz-ΔICR1 male = 52:48 and female = Control:Jz-ΔICR1 = 39:61. Volume data: n = 8 per sex/genotype, across 7 litters. Cell size data: n = 4 per sex/genotype, across 4 litters. Values presented as mean ± SEM with significance assessed by two-way ANOVA and pairwise T-test (p<sub>genotype</sub> < 0.05 = \*, p<sub>sex</sub> < 0.05 = †). Decidua (Db), Junctional zone (Jz), Labyrinth zone (Lz), Jz spongiotrophoblasts (Jz SpT), Jz glycogen cells (Jz GC), Jz trophoblast giant cell (Jz TGC), Db stroma (Db S), Db glycogen cell (Db GC) and Db vessel (Db V).

**Table S2. QPCR primers with references for those previously published.**

Gene	Forward	Reverse	Product size (bp)	Reference
<i>Ascl2</i>	GAGCAGGAGCTGCTTGACTT	CAGTCAGCACTTGGCATTG	176	Tunster et al., 2010
<i>Cd9</i>	GCTGGGATTGTCTTCGGGT	GGGTTTCATCCTTGCTCCGTA	151	
<i>Flt1</i>	AAAGGCTGAGCATCACTCCC	GGACTCCCTGCATCACTAACAA	190	
<i>Gbe1</i>	GGACGCTGAGATGTACACGA	AGTCCAACCATTGAGGATGC	167	Tunster et al., 2010
<i>Gys1</i>	GGAGAAATTTGGGAGGAAGC	GTCAAGATGGGGTCTGAGGA	183	Tunster et al., 2010
<i>H19</i>	CATTCTAGGCTGGGGTCAAA	GCCCTTCTTTTCCATTCTCC	172	Keniry et al., 2012
<i>Hsd11b1</i>	GAGGAAGGTCTCCAGAAGTA	ATGTCCAGTCCGCCCAT	143	Chin et al., 2017
<i>Hsd11b2</i>	GGCTGGATCGCGTTGTC	CGTGAAGCCCATGGCAT	132	Chin et al., 2017
<i>Pappa</i>	TGCAATAGGTCAGAGTGTGC	GGGTATGTCACGCACTGTCA	91	
<i>Pcdh12</i>	GCCTGGTTAGGCTCTCTGTG	TTATTTCCCTCGGTGGTTTGG	151	Tunster et al., 2010
<i>Peg3</i>	AGCACATCCCCTGTACGAA	TCTGCATTTGACCCCTGGAT	190	
<i>Polr2a</i>	CACTGTCATCACCCCTGACC	ATACTGGCTGTTTCCCTGTC	148	
<i>Prl6a1</i>	CTTACTGCCTGCCACACATC	CTCCAAGCTGTCAACATACTGA	114	
<i>Prl7b1</i>	CAGCACATCAATAGCCTTGC	TTGGTGATTTGAGTGGCAA	160	Tunster et al., 2016
<i>Rapgap1</i>	CTGCAACTTCCGCAACAAGG	ATGTGTTCCCGATATGCCG	108	
<i>Slc2a1</i>	GCTTATGGGCTTCTCCAACT	GGTGACACCTCTCCACATAC	123	Xu et al., 2015
<i>Slc2a3</i>	GATCGGCTCTTCCAGTTTG	CAATCATGCCACCAACAGAG	176	Tunster et al., 2010
<i>Slc38a1</i>	CCTTCACAAGTACCAGAGCAC	GGCCAGCTCAAATAACGATGAT	127	Chen et al., 2015
<i>Slc38a2</i>	TAATCTGAGCAATGCGATTGTG	AGATGGACGGAGTATAGCGAAA	129	Chen et al., 2015
	G	A		
<i>Slc38a4</i>	GCGGGGACAGTATTCAGGAC	GGAACTTCTGACTTTCGGCAT	102	Chen et al., 2015
<i>Slc44a1</i>	TCCGCCGCGCAGAGC	TCTTGTCTGTCACCTGTTG	147	
<i>Tfap2c</i>	CCTGCTCAGCTCCACGTC	CCTCCATTTTGGACTTTGC	129	
<i>Ugp2</i>	CTGGTGAAATCGCTCAAGT	TTACATTCAGGCCTCCATCC	187	Tunster et al., 2010
<i>Vegfa</i>	GAAGCTACTGCCGTCGATT	CTTCATCGTTACAGCAGCC	126	

**Table S3. Primary antibodies used for protein expression analysis**

Primary antibody	Species	Manufacturer	Catalogue number	Dilution	Incubation time
Igf1r	Rabbit	Santa Cruz	sc-713	1:1000	1 O/N at 4°C, 2h at RT
Igf2r	Rabbit	Cell Signalling	15128	1:2000	1 O/N at 4°C, 2h at RT
Insr	Rabbit	Santa Cruz	sc-711	1:400	1 O/N at 4°C, 2h at RT
P85	Rabbit	Milipore	06-195	1:1000 in 5% BSA	2h at RT
P110α	Rabbit	Cell Signalling	4249	1:1000	1 O/N at 4°C, 2h at RT
P110β	Rabbit	Cell Signalling	3011	1:1000	1 O/N at 4°C, 3h at RT
pAkt Ser473	Rabbit	Cell Signalling	9271	1:500	1 O/N at 4°C, 3h at RT
T-Akt	Rabbit	Cell Signalling	9272	1:1000	1 O/N at 4°C, 2h at RT
pGsk3 Ser21/9	Rabbit	Cell Signalling	9331	1:1000	1 O/N at 4°C, 1h at RT
T-Gsk3	Rabbit	Cell Signalling	9315	1:1000	1 O/N at 4°C, 1h at RT
pP38	Rabbit	Cell Signalling	4511	1:1000	1 O/N at 4°C, 1h at RT
T-P38	Rabbit	Cell Signalling	8690	1:1000	1 O/N at 4°C, 1h at RT
pMapk Thr202/Tyr204 (Erk1/2)	Rabbit	Cell Signalling	4370	1:1000	1 O/N at 4°C, 2h at RT
T-Mapk (Erk1/2)	Rabbit	Cell Signalling	4695	1:1000	1 O/N at 4°C, 2h at RT

Abbreviations: O/N = overnight, RT = room temperature.

## Supplementary reference list

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