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Supplemental Material to:

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Large offspring syndrome: a bovine model for the human loss-of-imprinting overgrowth syndrome Beckwith-Wiedemann

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Supplemental Figure 1. Biallelic expression of *KCNQ1OT1* in LOS fetuses. Shown is Sanger sequencing data of *KCNQ1OT1* RT-PCR product in tissues analyzed in AI-B799 (control), ART-J835LOS and ART-J489ALOS fetuses. The columns show the chromatograph for each tissue of each fetus. . Values below the chromatograph are the percentage of *KCNQ1OT1* expressed from the maternal allele. Arrows show the double peaks of SNP2 site (refer to Figure 2) in ART-J835LOS and ART-J489ALOS fetuses. LOS: large offspring syndrome; RT-PCR: reverse transcription-polymerase chain reaction; SNP: single nucleotide polymorphism.

Supplemental Figure 2. Analysis of *PHLDA2* expression. **(A)** The *PHLDA2* RNA was determined by quantitative RT- PCR in several tissues from eight AI fetuses (diamonds; AI), two LOS fetuses with biallelic expression of *KCNQ1OT1* (triangle = ART-J835LOS; square = ART-J489ALOS; bi-LOS), and five LOS fetuses with correct imprinting of *KCNQ1OT1* (circles; mono-LOS). The short line among the diamonds and circles represents the average level of the individuals. *PHLDA2* level was normalized to the expression of *GAPDH*. **(B)** The threshold cycle (Ct) of *PHLDA2* was normalized to the reference gene *GAPDH* in each tissue from the eight AI fetuses. The data are expressed as mean ± SEM. Tissues that do not share the common characters have significant difference ($*P<0.05$). LOS: large offspring syndrome;

Supplemental Figure 3. Loss of methylation of KvDMR1 on the maternal allele is associated with biallelic expression of *KCNQ1OT1* in LOS fetuses. DNA was treated with sodium bisulfite prior to PCR, and PCR product was cloned before sequencing. Sequencing data was used to determine the DNA methylation status at the KvDMR1. Shown on top is a depiction of the 10th intron of the maternally-expressed gene *KCNQ1* and its direction of transcription is shown with an arrow. The region harbors the promoter of the antisense long ncRNA *KCNQ1OT1* (shown as dashed arrow), which is also an imprinting control region known as KvDMR1. A 385 bp region of the KvDMR1 was used to determine the DNA methylation status of 37 CpG sites (ovals). A SNP (vertical arrow) between *B. t. indicus* and *B. t. taurus* was used to determine the parental origin of the alleles and only maternal alleles are shown here. Five tissues from two fetuses are shown. Filled and open circles represent methylated and unmethylated CpG dinucleotides, respectively. Missing circles are sequencing data of low quality. Each line denotes an individual DNA strand. The level of maternal *KCNQ1OT1* expression is shown in the center and next to the strands. Tail tissues were collected for the purpose of DNA analysis, precluding its use for gene expression determinations. **NA**: Not available.

Supplemental Figure 4. **KvDMR1 is unmethylated on the paternal alleles.** DNA was treated with sodium bisulfite prior to PCR, and PCR product was cloned before sequencing. Sequencing data was used to determine the DNA methylation status. Only paternal alleles are shown here – maternal allele results can be found in Figure 5 and Supplemental Figure 3. Five tissues from 4 fetuses are shown. Filled and open circles represent methylated and unmethylated CpG dinucleotides, respectively. Missing circles are sequencing data of low quality. Each line denotes an individual DNA strand and the number to the left of the strands represents the number of strands with that phenotype when greater than one.

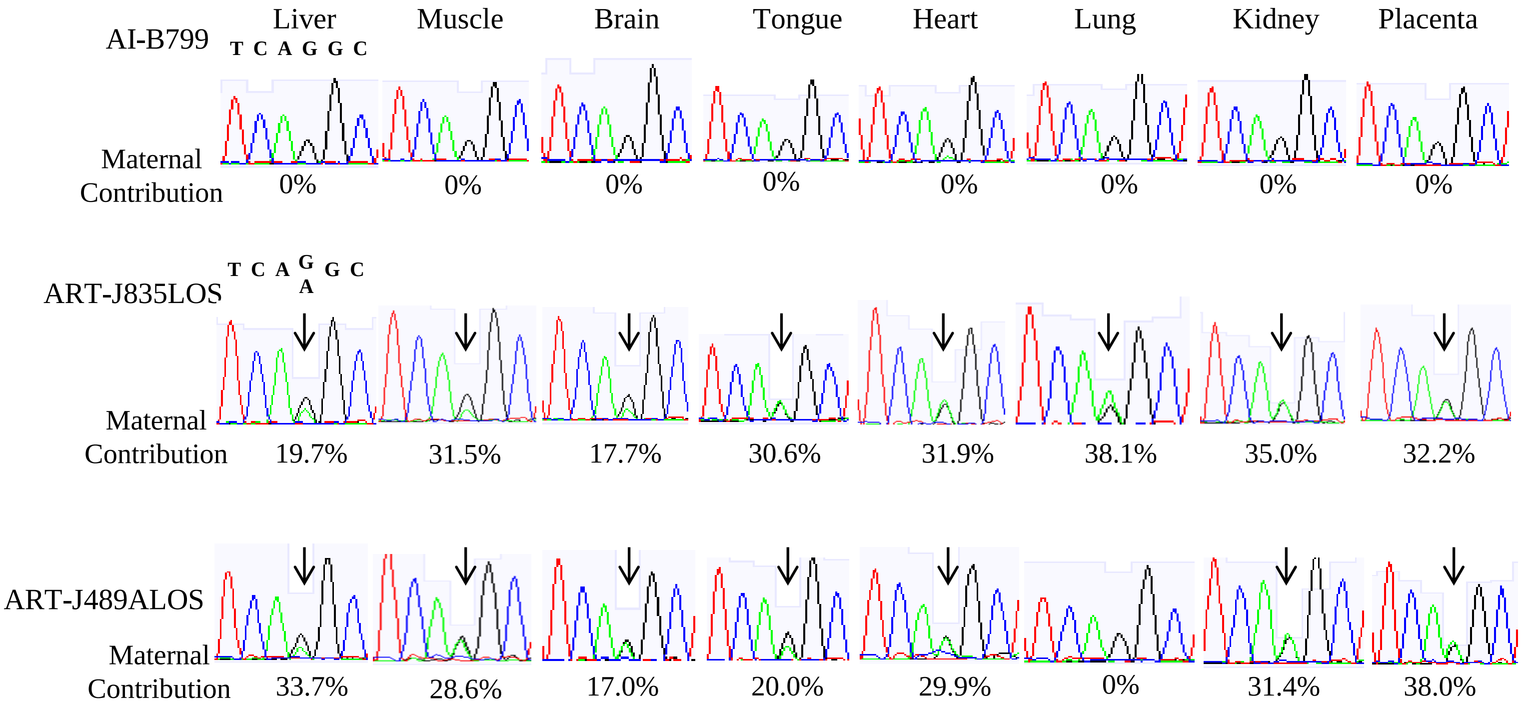
Supplemental Figure 5. **Differential methylation of the *H19/IGF2* ICR in tail and brain of AI control fetuses.** DNA was treated with sodium bisulfite prior to PCR, and PCR product was cloned before sequencing. Sequencing data was used to determine the DNA methylation status. Shown on top is a depiction of the maternally-expressed gene *H19* (shown as an arrow) and the ICR is represented as a black box in which the vertical lines represent 8 predicted CTCF binding sites (31). A 318 bp region which covers the 5th CTCF binding site was used to determine the DNA methylation status of 21/20 CpG sites (ovals). An extra CpG site on paternal alleles is a result of the SNP that "CG" in *B. t. indicus* (paternal) and "CA" in *B. t. taurus* (maternal). A SNP (vertical arrow) between *B. t. indicus* and *B. t. taurus* was used to determine the origin of the parental allele. Two tissues from 2 fetuses are shown. Filled and open circles represent methylated and unmethylated CpG dinucleotides, respectively. Missing circles are sequencing data of low quality. Each line denotes an individual DNA strand.

Supplemental Table 1

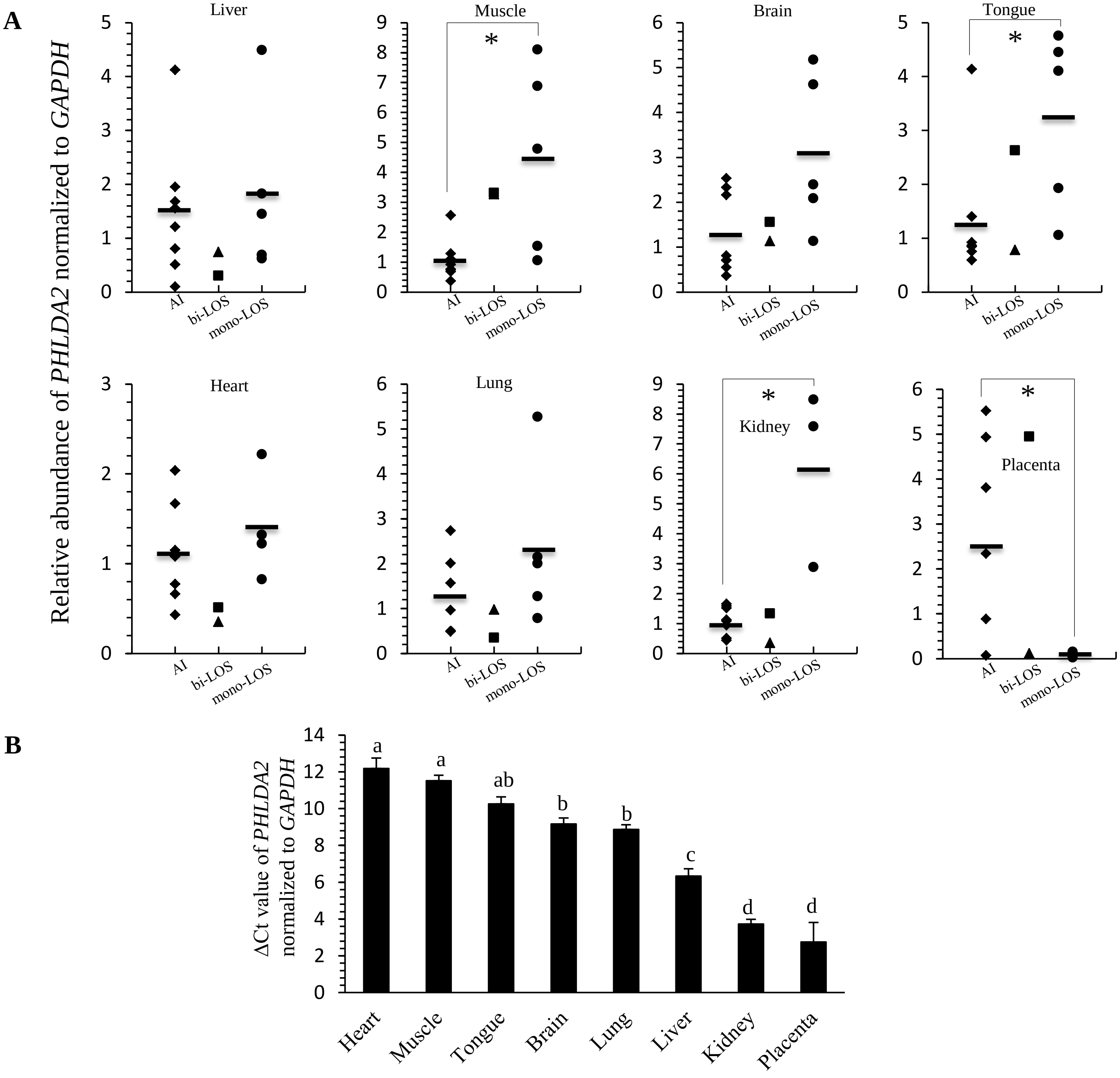
The five tables show the calculated allelic expression of *KCNQ1OT1, CDKN1C, KCNQ1, PHLDA2, H19* and *IGF2* imprinting status in each tissue for each fetus. *KCNQ1OT1* and *IGF2* are paternally-expressed; and *CDKN1C*, *PHLDA2*, *KCNQ1* and *H19* are maternally-expressed. The six genes are located on chromosome 29. Each table shows the data of one gene. Gene symbol and tissues are listed on the left side of the table (i.e. Liver, Muscle, Brain, Tongue, Heart, Lung, Kidney and Placenta). J835, R868B *et al.*, are fetus numbers, and they are arranged based on fetal body weight from large to small in ART group. First seven ART fetuses from left to right present with macrosomia (> 97th percentile body weight of controls). Sex of each fetus is listed above the fetus numbers. The numbers on the top are bodyweight in grams of each fetus. Two columns show the way (ART or AI) the fetuses were generated. The numbers in the tables are the percentage of mRNA expressed from the normally repressed allele. Only samples that had at least 10% expression from the repressed allele were considered biallelic. For example, the liver of the fetus J835 had biallelic expression of *KCNQ1OT1* (19.7% expression from the paternal allele (normally repressed)) and the liver of the fetus R868B had monoallelic expression of *KCNQ1OT1* (0% expression from the repressed allele). “NE” means expression not detectable based on current methods. A “--”means sample not analyzed. ART= assisted reproductive technologies; AI= artificial insemination; F=female; M=male.

Supplemental Table 2

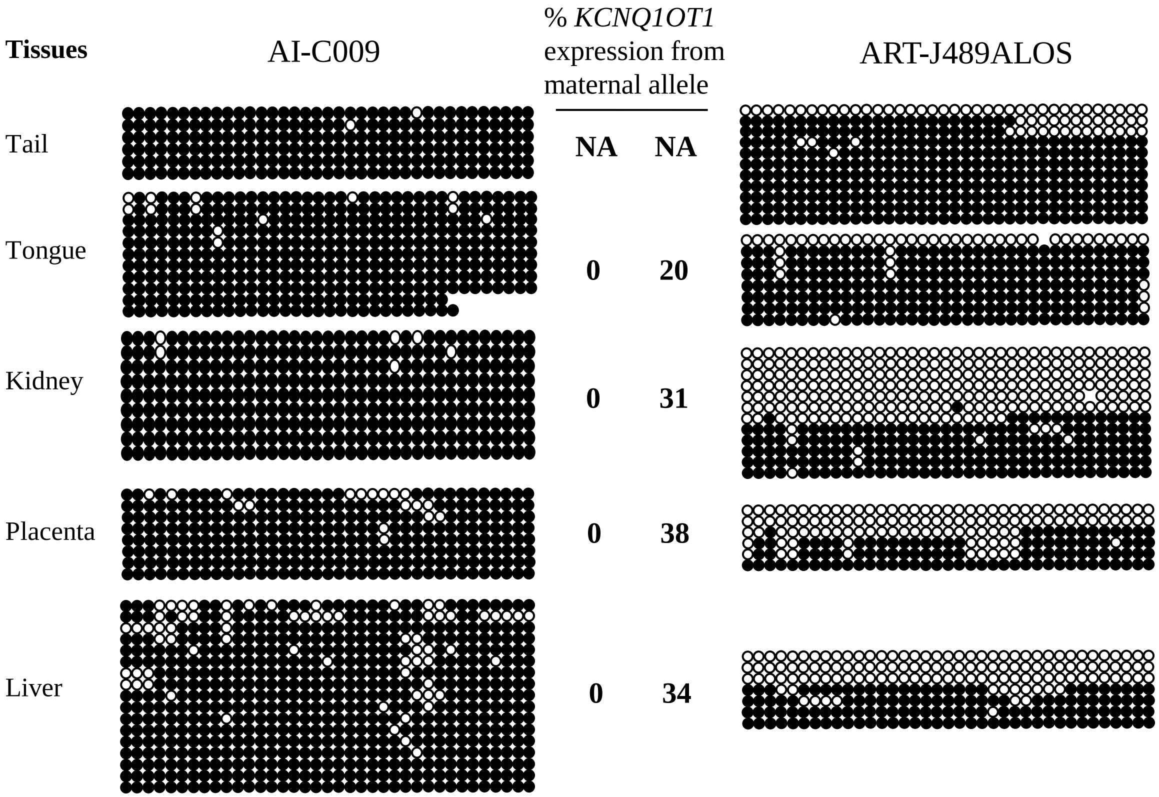
1. RT-PCR primers, conditions, allelic expression assay restriction enzymes, digested product size and PAGE concentrations and Real-time PCR probes. NA: not applicable.
2. Primers and conditions for bisulfite converted KvDMR1, *CDKN1*C exon 2, and *H19/IGF2* ICR. NA: not applicable.



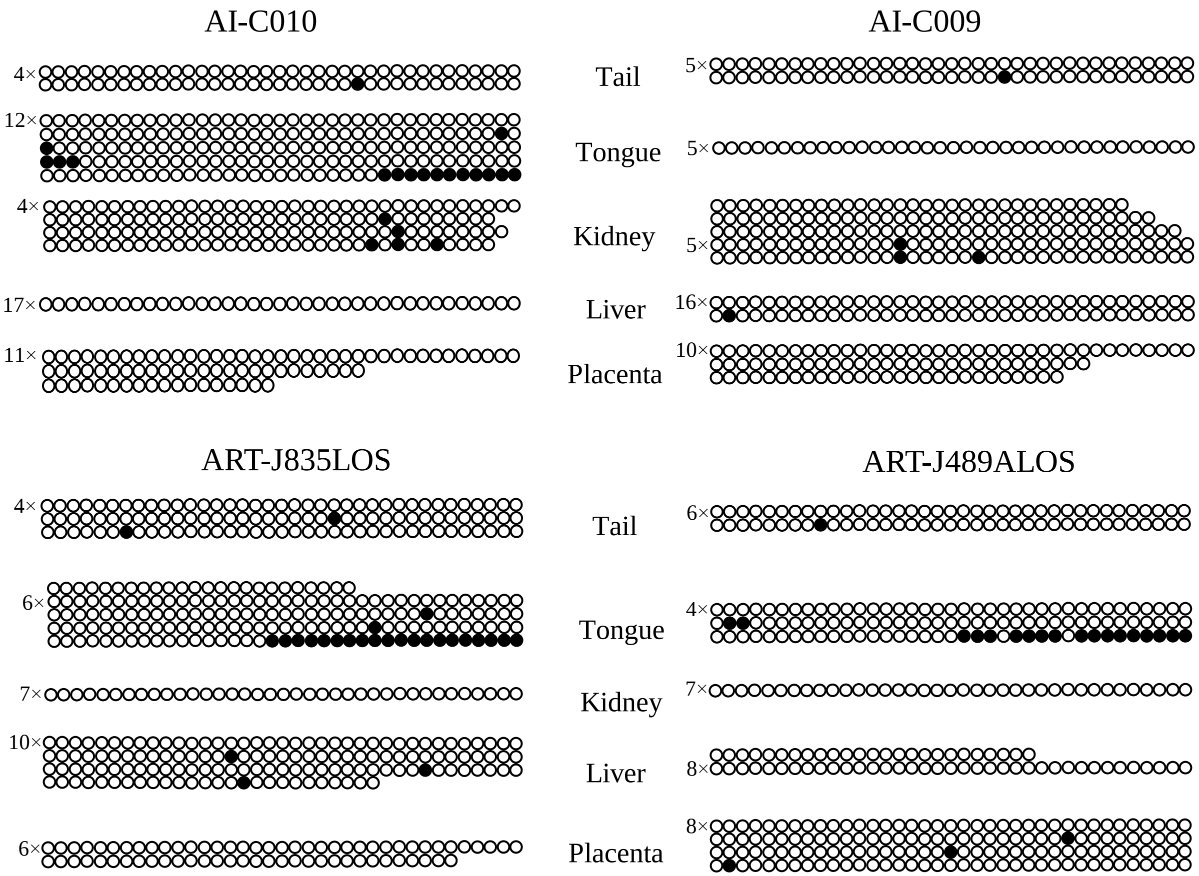
Supplemental Figure 1



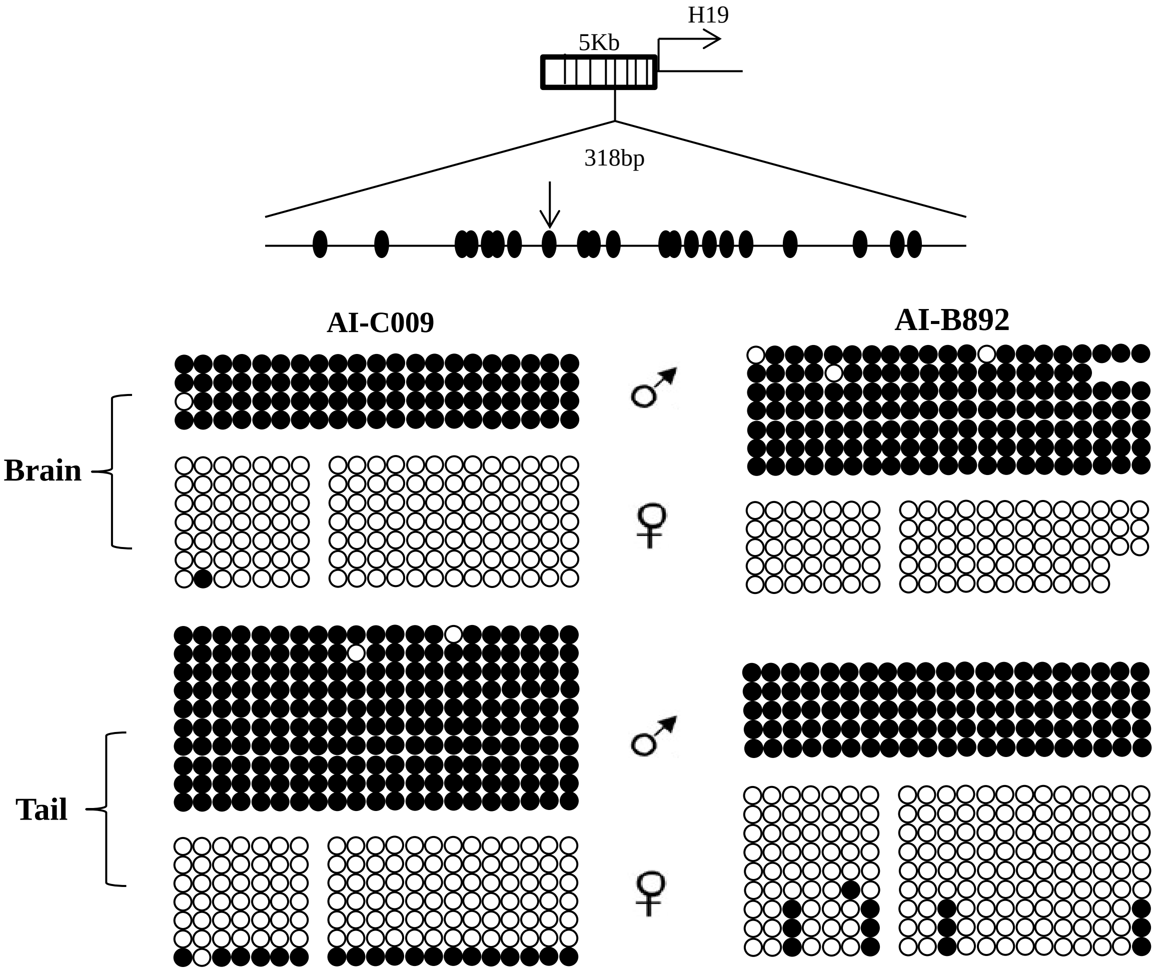
Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5

KCNQ1OT1	**ART**																										
	>97% bodyweight							<97% bodyweight																			
Bodyweight	714g	620g	584g	518g	514g	482g	478g	474g	472g	466g	466g	464g	462g	460g	432g	430g	428g	422g	420g	410g	400g	390g	376g	372g	360g	352g	352g
Sex	F	F	M	F	F	M	M	M	M	M	F	M	M	M	M	M	M	M	M	M	M	F	F	M	F	F	F
Fetus No#	J835	R868B	J183b	J355	J489A	K120	K290b	K169b	J183A	J498A	R893	K282	K075	J817A	R868A	K525	K169A	J566a	J566b	K290A	J498b	J817b	J619	K648	J649	6312	J489b
Liver	19.7	0	0	0	33.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Muscle	31.5	0	0	0	28.6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Brain	17.7	0	0	0	17.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tongue	30.6	0	0	0	20.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Heart	31.9	0	0	0	29.9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lung	38.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Kidney	35.0	0	0	0	31.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Placenta	32.2	0	0	0	38.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	--

KCNQ1OT1	**AI**					
	>97%	<97%				
Bodyweight	496g	416g	410g	408g	404g	380g
Sex	M	F	M	F	F	M
Fetus No#	C010	C009	C002	B799	B892	B773
Liver	0	0	0	0	0	0
Muscle	0	0	0	0	0	0
Brain	0	0	0	0	0	0
Tongue	0	0	0	0	0	0
Heart	0	0	0	0	0	0
Lung	0	0	0	0	0	0
Kidney	0	0	0	0	0	0
Placenta	0	0	0	0	0	0

Supplemental Table S1.1

CDKN1C	**ART**																						
	>97% bodyweight							<97% bodyweight															
Bodyweight	714g	620g	584g	518g	514g	482g	478g	474g	466g	466g	464g	462g	460g	430g	422g	420g	410g	390g	376g	372g	360g	352g	352g
Sex	F	F	M	F	F	M	M	M	M	F	M	M	M	M	M	M	M	F	F	M	F	F	F
Fetus No#	J835	R868B	J183b	J355	J489A	K120	K290b	K169b	J498A	R893	K282	K075	J817A	K525	J566a	J566b	K290A	J817b	J619	K648	J649	6312	J489b
Liver	0	0	0	0	19.9	0	0	0	0	0	0	0	0	0	0	0	11.6	0	0	0	0	0	0
Muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Brain	12.4	35.2	16.9	38.0	12.7	19.1	20.3	21.6	27.0	20.2	25.5	11.8	14.6	18.5	27.3	28.3	27.8	31.5	19.9	23.8	25.0	17.4	21.5
Tongue	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Heart	0	0	0	20.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lung	0	0	11.7	0	0	0	0	0	0	0	0	0	0	0	12.9	0	0	10.6	0	0	0	0	0
Kidney	16.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Placenta	0	0	0	NE	0	0	0	0	0	0	0	0	0	0	0	0	0	NE	0	0	0	0	--

CDKN1C	**AI**					
	>97%	<97%				
Bodyweight	496g	416g	410g	408g	410g	380g
Sex	M	F	M	F	F	M
Fetus No#	C010	C009	C002	B799	B892	B773
Liver	0	0	0	0	0	0
Muscle	0	0	0	0	0	0
Brain	22.0	16.1	15.7	23.2	23.2	27.7
Tongue	0	0	0	0	0	0
Heart	0	0	0	0	0	0
Lung	15.5	0	0	15.7	0	0
Kidney	0	0	0	0	0	0
Placenta	0	0	0	0	0	0

Supplemental Table S1.2

	ART																						
KCNQ1	>97% bodyweight							<97% bodyweight															
Bodyweight	714g	620g	584g	518g	514g	482g	478g	474g	466g	466g	464g	462g	460g	430g	422g	420g	410g	390g	376g	372g	360g	352g	352g
Sex	F	F	M	F	F	M	M	M	M	F	M	M	M	M	M	M	M	F	F	M	F	F	F
Fetus No#	J835	R868B	J183b	J355	J489A	K120	K290b	K169b	J498A	R893	K282	K075	J817A	K525	J566a	J566b	K290A	J817b	J619	K648	J649	6312	J489b
Liver	0	0	13.0	14.9	0	0	15.3	22.0	14.1	12.5	0	20.1	16.2	20.5	0	0	12.5	15.2	18.1	15.6	11.0	0	16.4
Muscle	20.6	10.0	23.2	20.0	29.4	18.6	13.6	11.2	22.8	12.1	0	17.9	20.6	17.3	22.0	22.0	10.8	12.9	14.8	11.6	18.5	0	18.5
Brain	29.9	19.7	41.1	36.2	42.9	41.3	37.6	38.8	35.2	28.5	20.9	15.2	38.0	44.9	31.4	16.7	44.4	30.4	34.8	43.8	35.8	23.8	33.0
Tongue	13.7	21.6	17.6	25.2	27.0	17.0	26.7	19.9	22.2	14.4	10.6	23.8	10.0	24.8	0	17.3	20.9	17.7	9.8	18.3	14.6	16.8	28.0
Heart	23.8	27.2	26.3	13.6	31.3	27.0	31.6	34.2	29.7	23.2	23.0	27.7	29.5	30.4	32.4	26.9	31.4	27.9	30.6	37.7	31.9	35.1	28.8
Lung	13.1	0	28.0	0	17.8	16.3	16.2	20.0	0	10.9	14.1	20.8	18.4	19.0	16.7	15.6	15.2	0	19.7	0	21.3	0	17.5
Kidney	20.7	0	0	17.3	35.4	20.0	18.1	16.0	13.9	22.7	13.3	0	23.0	12.5	18.5	20.3	20.7	0	11.9	0	28.2	17.8	11.3
Placenta	22.3	0	0	NE	46.9	14.3	0	35.4	0	23.8	0	38.0	23.3	26.2	38.0	14.3	30.6	0	34.0	21.1	0	38.5	--

	AI					
KCNQ1	>97%	<97%				
Bodyweight	496g	416g	410g	408g	410g	380g
Sex	M	F	M	F	F	M
Fetus No#	C010	C009	C002	B799	B892	B773
Liver	0	10.5	0	0	0	0
Muscle	0	16.7	16.4	14.3	0	13.5
Brain	23.5	15.9	14.7	26.7	28.1	37.8
Tongue	26.5	15.0	0	0	26.4	16.9
Heart	27.7	14.8	23.6	19.3	25.9	25.1
Lung	11.2	19.4	0	0	20.6	15.0
Kidney	0	16.2	0	0	13.5	15.0
Placenta	47.2	0	12.3	39.3	28.5	14.4

Supplemental Table S1.3

	ART									
PHLDA2	>97% bodyweight							<97%		
Bodyweight	714g	620g	584g	518g	514g	482g	478g	390g	372g	360g
Sex	F	F	M	F	F	M	M	F	M	F
Fetus No#	J835	R868B	J183b	J355	J489A	K120	K290b	J817b	K648	J649
Liver	0	0	0	0	0	0	0	0	0	0
Muscle	0	35.3	50.0	35.1	37.6	0	0	19.9	0	100
Brain	80.0	36.3	17.0	41.3	100	70.5	45.0	55.7	0	47.3
Tongue	100	0	0	0	0	0	39.3	29.5	100	0
Heart	0	0	0	20.1	0	0	35.1	34.7	0	13.8
Lung	51.9	51.3	0	55.3	0	0	100	0	0	12.3
Kidney	18.7	0	9.8	12.7	21.0	0	15.1	20.0	0	13.8
Placenta	0	0	0	0	0	0	0	NE	0	0

	AI					
PHLDA2	>97%	<97%				
Bodyweight	496g	416g	410g	408g	404g	380g
Sex	M	F	M	F	F	M
Fetus No#	C010	C009	C002	B799	B892	B773
Liver	0	0	0	0	0	0
Muscle	0	0	0	35.3	22.4	0
Brain	67.9	0	40.4	29.4	53.0	0
Tongue	33.4	100	0	41.4	0	32.2
Heart	58.1	83.1	0	100	48.9	100
Lung	51.6	0	64.4	0	0	0
Kidney	30.5	19.4	26.2	21.8	12.5	41.4
Placenta	0	0	0	0	0	0

	ART													
IGF2	>97%							<97%						
Bodyweight	714g	620g	584g	518g	514g	482g	478g	474g	466g	466g	464g	462g	372g	352g
Sex	F	F	M	F	F	M	M	M	M	F	M	M	M	F
Fetus No#	J835	R868B	J183b	J355	J489A	K120	K290b	K169b	J498A	R893	K282	K075	K648	6312
Liver	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Brain	24.3	24.6	35.5	25.8	26.1	30.1	25.3	34.1	13.6	0	14.0	9.3	0	0
Tongue	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Heart	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lung	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Kidney	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Placenta	0	0	0	0	0	0	0	0	0	0	0	0	0	0

	AI				
IGF2	>97%	<97%			
Bodyweight	496g	416g	404g	392g	380g
Sex	M	F	F	F	M
Fetus No#	C010	C009	B892	B783	B773
Liver	0	0	0	0	0
Muscle	0	0	0	0	0
Brain	NE	38.9	32.2	0	39.2
Tongue	0	0	0	0	0
Heart	0	0	0	0	0
Lung	0	0	0	0	0
Kidney	0	0	0	0	0
Placenta	0	0	0	0	0

Supplemental Table S1.4

	ART																						
H19	>97% bodyweight							<97% bodyweight															
Bodyweight	714g	620g	584g	518g	514g	482g	478g	474g	466g	466g	464g	462g	460g	430g	422g	420g	410g	390g	376g	372g	360g	352g	352g
Sex	F	F	M	F	F	M	M	M	M	F	M	M	M	M	M	M	M	F	F	M	F	F	F
Fetus No#	J835	R868B	J183b	J355	J489A	K120	K290b	K169b	J498A	R893	K282	K075	J817A	K525	J566a	J566b	K290A	J817b	J619	K648	J649	6312	J489b
Liver	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Brain	0	0	0	0	0	NE	0	0	0	0	0	0	0	0	0	0	0	NE	0	0	0	NE	0
Tongue	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Heart	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lung	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Kidney	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Placenta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	--

	AI					
H19	>97%	<97%				
Bodyweight	496g	416g	410g	408g	404g	380g
Sex	M	F	M	F	F	M
Fetus No#	C010	C009	C002	B799	B892	B773
Liver	0	0	0	0	0	0
Muscle	0	0	0	0	0	0
Brain	0	0	0	0	0	0
Tongue	0	0	0	0	0	0
Heart	0	0	0	0	0	0
Lung	0	0	0	0	0	0
Kidney	0	0	0	0	0	0
Placenta	0	0	0	0	0	0

Supplemental Table S1.5

Gene Symbol	SNP information			Primers (5 → 3)	PCR annealing Temp (°C)	PCR amplicon size (bp)	Expressed allele	Restriction enzyme	Digested Indicus allele (bp)	Digested Taurus allele (bp)	PAGE(%)
	NCBI Accession No#	*Bos. Indicus*	*Bos. Taurus*								
KCNQ1OT1	NW_003104648.1 2971334, 2971399	G, G	C, A	Forward: TTCTTCCCAAGGCTGCCAGAAA	60.7	460	Paternal	NA Sanger Seq	NA	NA	NA
				Reverse: TCTGCTCCTTCCAGCTGTAGGTT							
CDKN1C	NW_003104648.1 2777626	T	C	Forward: ATGAGAGCCTCGTGCTCAAAGA	60.7	443	Maternal	AvaII	232, 133, 78	365, 78	7%
				Reverse: AGTGAGGTGCCTTTGGCATAACCA							
KCNQ1	NW_003104648.1 3083541	A	G	Forward: ATCTATGTCCGGAAGCCGTC	59.5	300	Maternal	DdeI	142, 89, 50, 19	231, 50, 19,	7%
				Reverse: ATCCAGGTCCAGGTCCTCA							
PHLDA2	NW_003104648.1 2737363	A	G	Forward: AATTGCTGACCAGGGCAAGGAT	61.6	207	Maternal	NA SSCP	NA	NA	8%
				Reverse: CCGAGTGCTTTATTGGAATGGTGG							
H19	NW_003104648.1 3559835	T	C	Forward: GATATGGTCCGGTGTGATGGAGAGAGCA	62.8	752	Maternal	BsiHKAI	609, 108, 35	609,143	18%
				Reverse: TTCGGAGCCTCCAGACTCGGTG							
IGF2	NW_003104648.1 3471637	T	C	Forward: TTTCTGACCGCTGGGTCGCTT	62.0	172	Paternal	NA SSCP	NA	NA	8%
				Reverse: AGCTCACTAGTCGGGCTGC							

Real-Time Taqman Probe	
CDKN1C	Bt03250987_m1
PHLDA2	Bt03249477_g1
IGF2	Bt03259226_m1
GAPDH	Bt03210913_g1

Supplemental Table S2.1

DMR Symbol	SNP information			Primers (5 → 3)	PCR annealing Temp (°C)	Betaine	$MgCl_2$	PCR amplicon size (bp)
	NCBI Accession No#	*Bos. Indicus*	*Bos. Taurus*					
KvDMR1	NW_003104648.1 2960379	C	G	Forward: GTGAGGAGTATGGTATTGAGG	62.1	NA	4mM	385
				Reverse: TCAACCCTCTCAACCAATAAC				
*CDKN1C*exon2	NA	NA	NA	Forward: GGGATTATAATTTTTAGTTGGATATG	58.8	NA	4mM	363
				Reverse: GCCCCGAAATCCCTAAATAC				
H19/IGF2 ICR	NW_003104648.1 3556134	G	A	Forward: AGTGTGTAGAGGATATTGG	53.6	1M	4mM	318
				Reverse: CTCCTCTCCTAACTTCAAC				

Supplemental Table S2.2