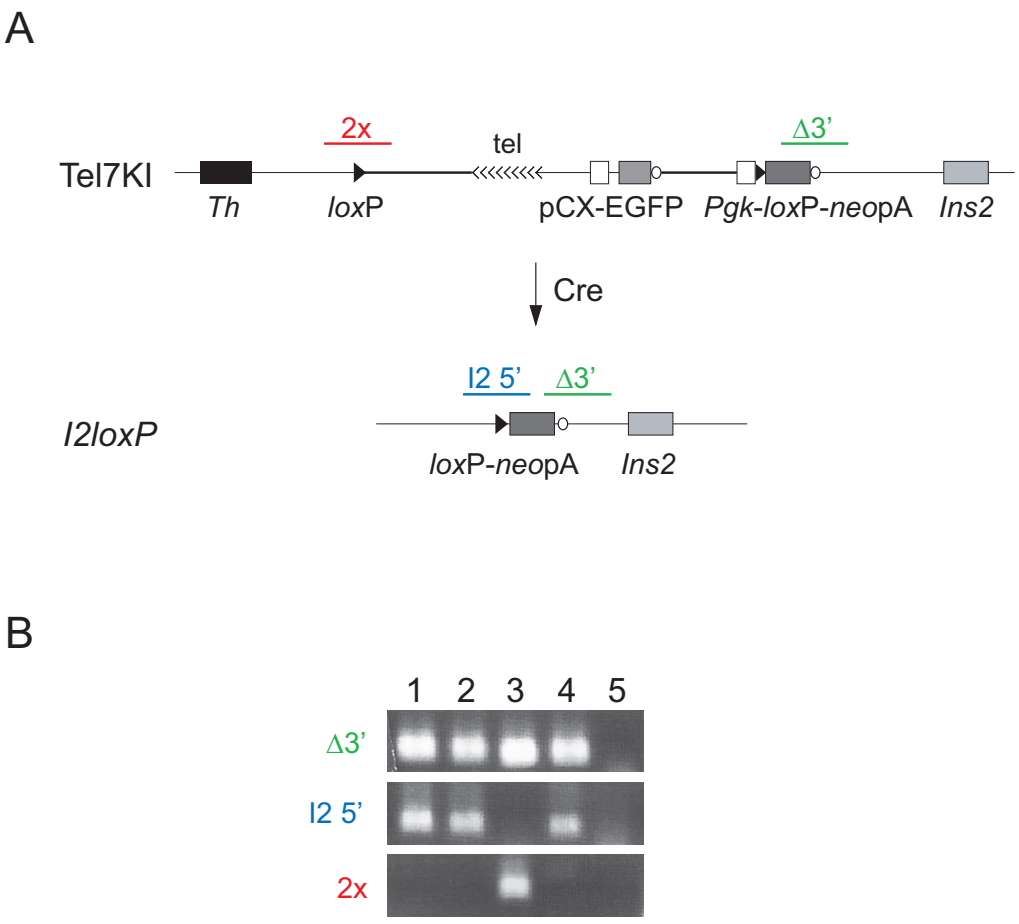

Fig. S1. Demonstration of the conditional excision of Tel7KI with Cre in ES cells.
(A) Schematic representation of the Tel7KI allele showing the conditional deletion of its *lox*P site-flanked elements, to reform the parental allele *I2loxP* by Cre excision. Diagnostic genomic PCR reactions amplifying a common element (Δ 3') as well as unique junctions (2x, I2 5') are positioned above the schemata. **(B)** Analysis of independent ES cell clones obtained after the transient transfection of a Tel7KI/+ cell line with a Cre expression vector. The samples analyzed are genomic DNA purified from Tel7KI/+ ES cells with (1 and 2) and without Cre transfection (3), the parental *I2loxP*/+ cells (4) and a water control (5).

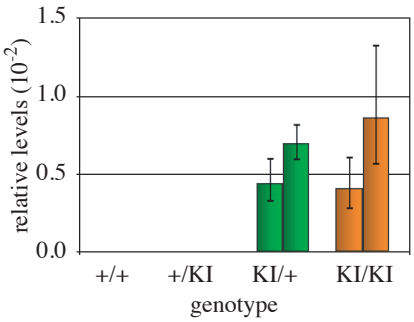

Fig. S2. Q-RT-PCR analysis of GFP expression from Tel7KI

GFP expression from Tel7KI was analyzed by quantitative RT-PCR on RNA purified from whole E12.5 embryos. Each bar represents a single embryo, and shows expression of EGFP transcript relative to Gapdh (10-2 scale). Error bars indicate standard deviations for technical triplicates.

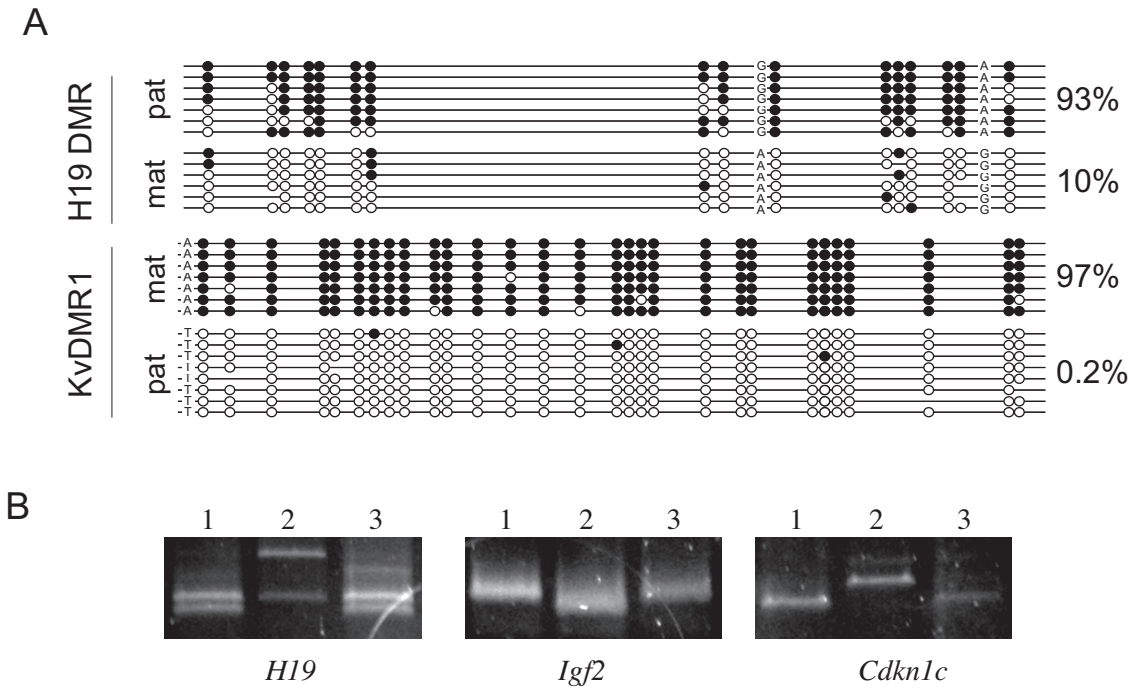

Fig. S3. Genomic imprints are maintained in cultured trophoblast giant cells.
(**A**) DNA methylation analysis of paternal transmission Tel7KI E8.5 EPCs differentiated into TGCs in vitro for 5 days. Sodium bisulfite-modified genomic DNA was analyzed for DNA methylation patterns at the *H19* DMR (IC1) as well as at KvDMR1 (IC2). The parental origin of the DNA strands analyzed was determined from sequence polymorphism within the regions studied (SNPs shown), such that each strand can be identified as being paternal (pat) or maternal (mat) in origin. H19 DMR (16 CpGs in 473 bp) and KvDMR1 (31 CpGs in 335 bp) analysis has been previously described (Davis et al., 1998; Umlauf et al., 2004; Oh et al., 2008). (**B**) Allele-specific expression analysis of the distal Chr7 imprinted genes *H19*, *Igf2*, and *Cdkn1c*. Analysis was performed on F1 hybrid conceptuses from a cross between a Tel7KI hemizygous animal (+/KI, *mus* background) and an animal homozygous for *M.m.castaneus* (*cast*) variants on distal Chr 7 (E12.5 *cast*/*mus* embryo, lane 1, E12.5 *mus*/*cast* embryo, lane 2) and compared to *cast*/*mus* F1 EPCs cultured for 5 days (lane 3). Each cDNA sample was amplified to detect a SNP in the genes of interest. PCR products were digested with a polymorphic restriction enzyme to determine the parental origin of the expressed allele.

Table S1. Oligonucleotides used in this study for genotyping, sodium bisulfite sequencing, quantitative RT-PCR.

Purpose	Sequence	Name	Reference
Genotyping (I2wt)	AGCACAGTCCCCTGTGTTCT	I2wt F	This study
	GTCTTCAACCCCATGTGACC	I2wt R	This study
Genotyping (Δ5')	CCAAAGAACGGAGCCGGTTG	PGK4	(1)
	TGAATGGGAAATGTGGTCCTTGG	M2G	(1)
Bisufite (β-a)	GGAGAGGTGYGGYGGTAGTTAATTAGAG	BABF6	This study
	TCATTAAACCAAACRCTAATTACAACCC	BABR4c	This study
	AAACCCCTCAAAACTTTCACRCAACCACAA	BABR5d	This study
Bisulfite (GFP ORF)	ATTATTTTCTAGATTGTTATGGTGAGTAAGGG	1870Fbis	(2)
	GAGGAGTTGTTTATYGGGGTGGTGTTT	1903Fbis	(2)
	TAACTATTATAATTATACTCCAACTTATACC	2303Rbis	(2)
Bisulfite (H19 DMR)	GAGTATTTAGGAGGTATAAGAATT	BMsp2t1	(3)
	ATCAAAAACTAACATAAACCCCT	BHha1t3	(3)
	TGTAAGGAGATTATGTTTTATTTTTGGA	BMsp2t2.2	(1)
	AACCTCATAAAACCCATAACTATA	BHha1t4.2	(1)
Bisulfite (KvDMR1)	GGTTATAAAGTTTAGGGGTTTTTAGATTTG	Kcnq1ot1 OF	(4)
	AAAACTTTTCTATTCAACTTAATTCCCAAC	Kcnq1ot1 OR	(4)
	GGTTTTAAGATTATTTTTGTTTTTGTAAGT	Kcnq1ot1 IF	(4)
	AATTCTCCTAAATATAATTTTTTTCTCAAC	Kcnq1ot1 IR	(4)
Q-PCR (GFP)	GCTCTGACTGACCGCGTTACT	BAE1F	This study
	GGACACGCTGAACTTGTGG	BAE1R	This study
Q-PCR (G3PDH)	ACCACAGTCCATGCCATCAC	G3PF	(1)
	TCCACCACCCTGTTGCTGTA	G3PR	(1)

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Table S2. Primer sequences for allele-specific RT-PCR

Name	Sequence	Size	Enzyme	Cast	129	Ref
H19rt1	CCTCAAGATGAAAGAAATGGT	641	*Sma*I/	244, 44,	244, 44,	(1)
H19rt2	AACACTTTATGATGGAACTGC		*Cac*81	222, 130	352	
Igf2F	CCATCAATCTGTGACCTCCTCTTG	200	*Tsp*5091			(2)
Igf2R	GGGTGTCAATTGGGTTGTTT					
P57S	GCCAATGCGAACGACTTC	364	*Taq*α1	257, 58, 49	306, 58	(3); Mann, unpublished
P574	TACACCTTGGGACCAGCGTACTCC					

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