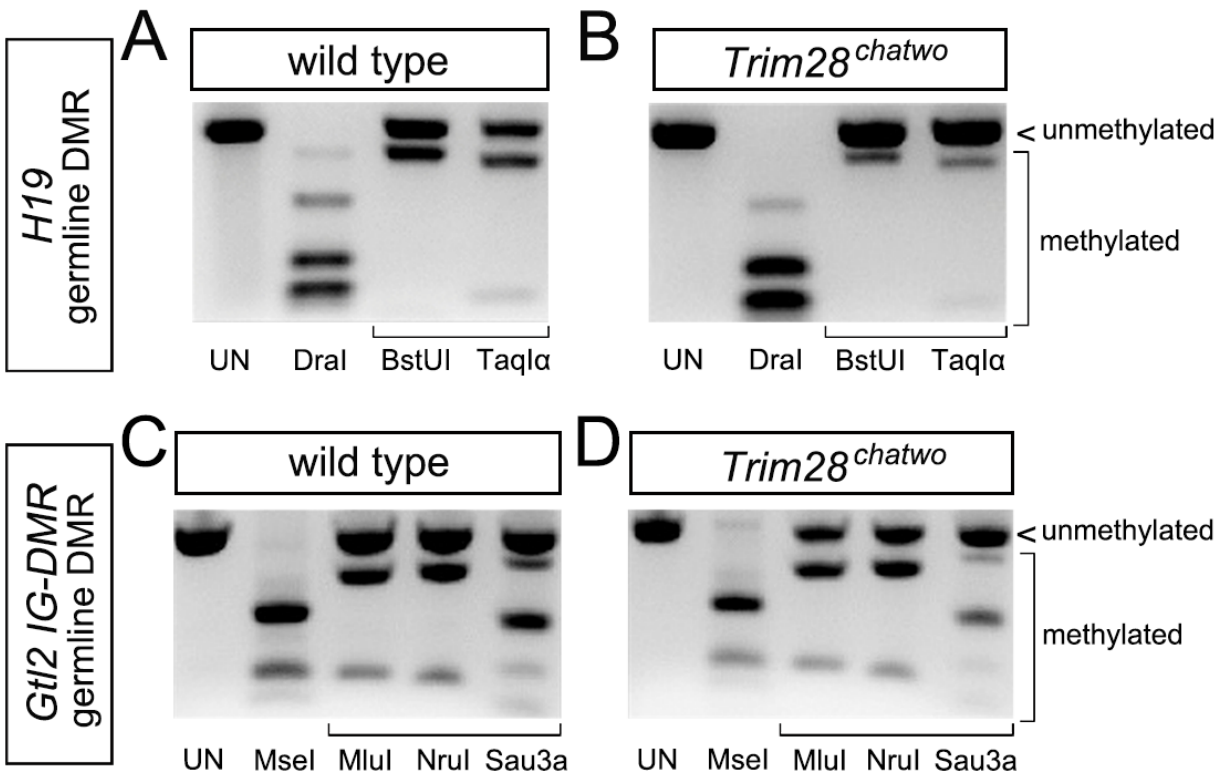

Figure S1. DNA methylation at the *H19* and *Gtl2* germline DMRs in *Trim28*^{chatwo} embryos. Related to Figure 3. DNA methylation at the *H19* (**A-B**) and *Gtl2* (**C-D**) germline DMRs was detected by combined restriction-bisulfite analysis (COBRA) in wild type (A, C) and *Trim28*^{chatwo} (B, D) embryos. Analysis was done on pools of 2-3 embryos collected at E8.5. Restriction with DraI (A-C) and MseI (D-F) measured the efficiency of bisulfite conversion. All other restriction enzymes (lanes with brackets) recognize the PCR product only if the original template contained methylated CpG dinucleotides. UN-undigested.

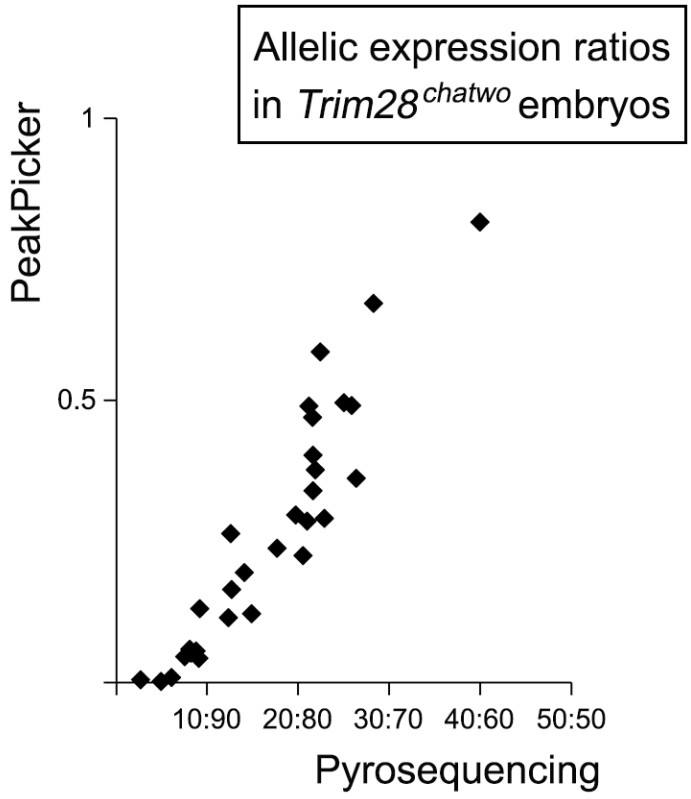

Figure S2. Relationship between PeakPicker and pyrosequencing quantification of allelic expression. Related to Figure 4. Graph showing pyrosequencing versus PeakPicker quantification of allelic expression ratios from analysis of the *H19*, *Gtl2,* and *Snrpn* imprinted genes in *Trim28*^{chatwo} embryos. Note that PeakPicker uses Sanger sequencing chromatograms to calculate the relative SNP allele ratio normalized to control peaks and produces a value between 0 (monoallelic expression) and 1 (equal expression from both alleles). In contrast, pyrosequencing provides a ratio that represents the relative expression from each allele, with values that range from 0:100 (monoallelic expression) to 50:50 (equal expression from each allele). Data shows a strong correlation between the two methods ($p<0.0001$).

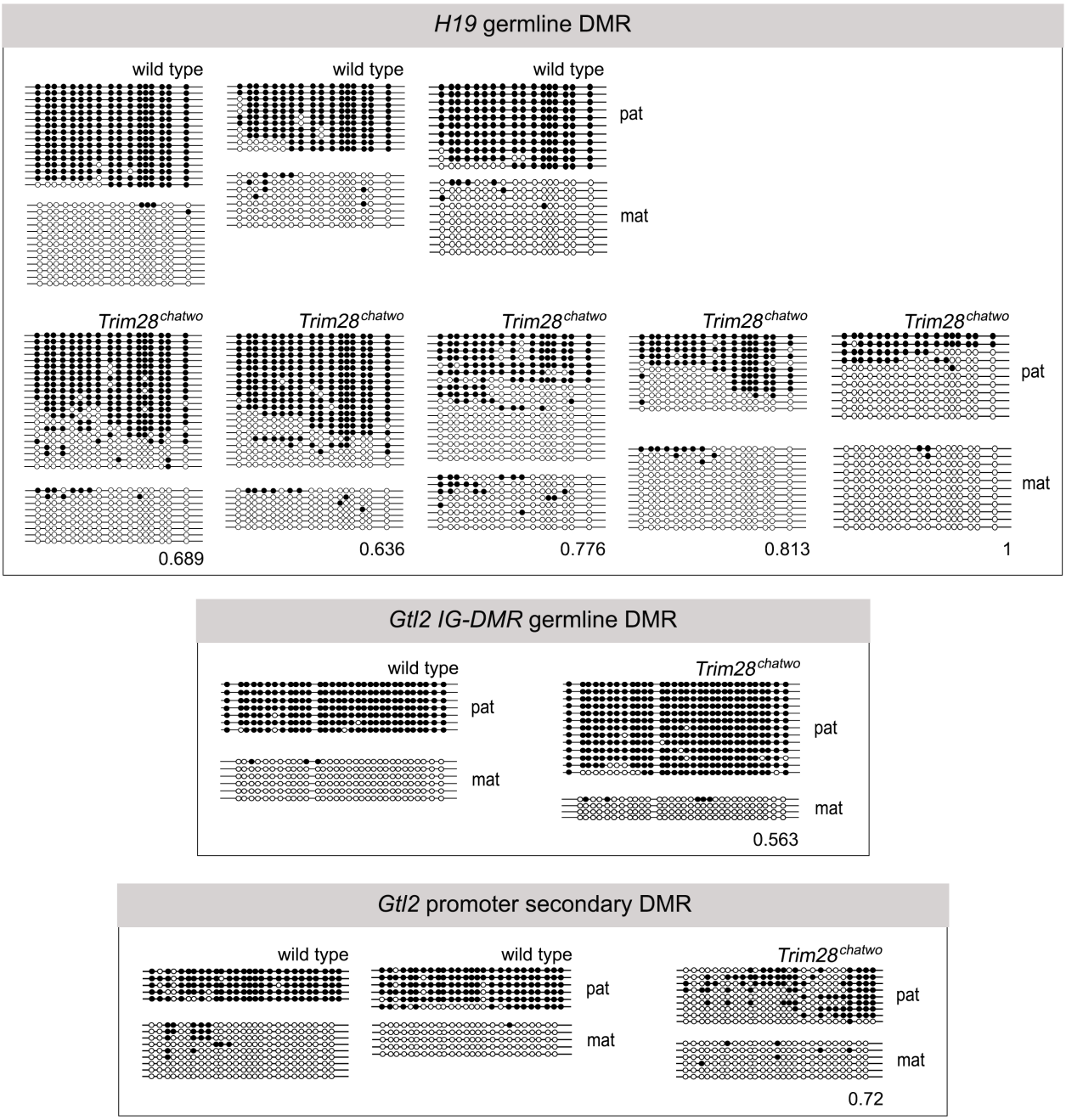

Figure S3. Bisulfite sequencing analysis of DNA methylation in *Trim28*^{chatwo} embryos. Related to Figures 4 & 5. Results from the analysis of DNA methylation at the *H19* germline DMR, the *Gtl2* germline DMR, and the *Gtl2* secondary DMR in additional wild type and *Trim28*^{chatwo} embryos to those shown in Figures 4 and 5. Methylated DNA is represented by filled circles. Unmethylated DNA is represented by empty circles. Maternal (mat) and paternal (pat) alelles were identified by allele-specific polymorphisms. The numbers at the bottom of each methylation plot indicate the PeakPicker allelic expression ratio for that particular embryo.

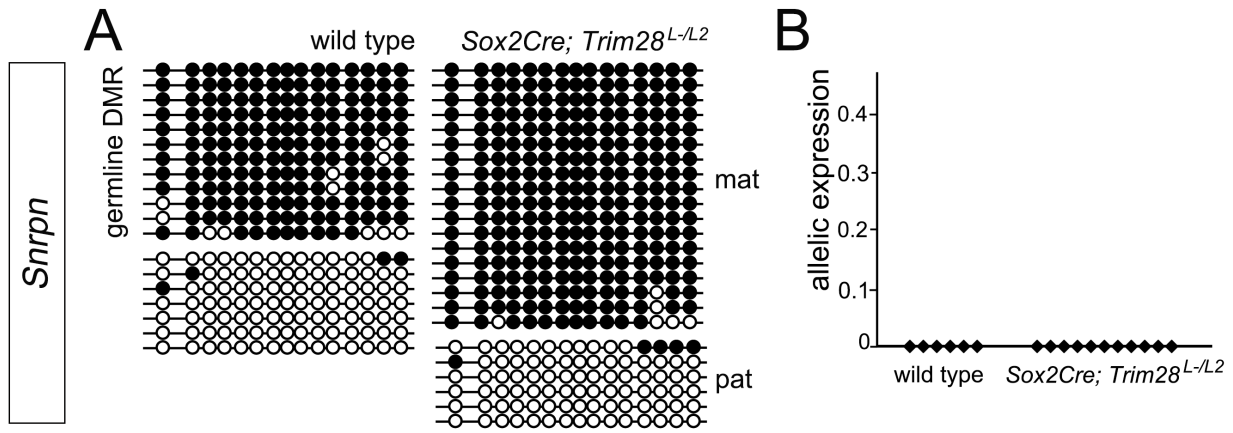

Figure S4. *Snrpn* DNA methylation and allelic expression in *Sox2Cre;Trim28*$^{L-/L2}$ embryos. Related to Figure 7. (**A**) Allele-specific DNA methylation of the *Snrpn* germline DMR as measured by bisulfite sequencing in wild type and *Sox2Cre;Trim28*$^{L-/L2}$ embryos. (**B**) *Snrpn* allelic expression ratios as quantified using the PeakPicker program. n = number of embryos analyzed.

Allelic Expression (Sanger Sequencing)

	Primer1	Primer2	SNP position (mm10)	SNP	Background	Source
H19	actgaaggcgaggatgacag	gttcaaggtagggggaggag	7: 142576519	G/A	FVB/CAST	
Gtl2	gccaaagccatcatctggaatc	cacagatgtagactcaacagtgaag	12: 109571394	T/C	FVB/CAST	Schmidt et al., 2000
Snrpn	cccaccaggaattagaggc	gcagtaagaggggtcaaaagc	7: 59983201	C/T	FVB/CAST	Szabo and Mann, 1995; Doherty et al., 2000
Gnas (Paternal)	ctggcagtgagatcagtgga	aaaatcgctgggttgaagg	2: 174298128, 174298129	TT/GA	FVB/CAST	
Airn	cacaggcaaacaaccctaca	ctcggcagagaaagttttgg	17: 12816566	T/C	FVB/CAST	
Kcnq1ot1	ccaccagcctcagcatattt	gaaacgatccacacggaagt	7: 143296059	T/C	FVB/CAST	
Peg3	gggggagaatcctccattta	tgggaactgaaaacgtgaca	7: 6709700	G/C	FVB/BL6	
Peg10	atgattttgcacccagatcc	tcaggcagaatggtgacaaa	6: 4757990	C/T	FVB/BL6	
Rasgrf1	ggctcatgatgaatgccttt	tacagaagcttggcgttgtg	9: 89991554	T/C	FVB/BL6	

Allelic Expression (Quantitative pyrosequencing)

	Primer1	Primer2	Pyro Primer	SNP position (mm10	SNP	Background
H19	TTTGGAGTCCCGGAGATAGCTTT	[Biotin-5]GCAAAGGATGAAGTAGGGCATGT	CCCAGTACCCACCTGT	7: 142575460	C/T	FVB/CAST
Gtl2	CCAGGACCCTCCAACTGTAAA	[Biotin-5]TCAGTCAGTAGGTGGGTCTCTCT	GAATAGTAGTGGAATTGTGT	12: 109571145	T/C	FVB/CAST
Snrpn	CTTGCTACGTGGGGAGAACTT	[Biotin-5]TAGGTACACCTGCTGGCACTC	GGCCCTCCACAGTCA	7: 59986552	T/G	FVB/CAST

Quantitative Reverse Transcriptase PCR

	Primer1	Primer2	Source
H19	ttgcactaagtcgattgcact	ggaactgcttccagactaggc	
Igf2	ttgtgctgcatcgctgcttac	tagacacgtccctctcggact	
Gtl2	gggcgcccacagaagaa	ggtgtgagccgatgatgtca	
Dlk1	ttaccggggttccttagagc	tgcattaatagggaggaaggg	
Rian	tcgagacacaagaggactcc	attggaagtctgagccatgg	Sekita et al., 2005
Mirg	ccttcctggatctctcgctt	gtgggagttgaaacatgggt	Wu et al., 2006
Peg3	cacgaagacgacaccaacag	gtctcgaggctccacatctc	Shule et al., 2007
Mest	atcccggtgcttcttctca	aggcagcaagcagcaact	
Impact	aagaacgcgcagacttatcg	ttattttctccacccactggt	
Snprn	ttggttctgaggagtgatttgc	ccttgaattccaccaccttg	Tsai et al., 1999
Peg10	gtctctactgtggcaatgg	gggaccttattcgttctgg	Zaiton et al., 2010
Airn	gtggattcaggtttcatg	ggcccagatatagaatgt	
Igf2r	tagttgcagctctttgcacg	acagctcaaacctgaagcg	
IAP 5' UTR	cgggtcgcggtaataaaggt	actctcgttccccagctgaa	Rowe et al., 2010
Actin	aagtgacgttgacatccg	gatccacatctgctggaagg	

Bisulfite Sequencing and COBRA

	Primer1	Primer2	Background
H19 primary outer	gagtatttaggaggtataagaatt	atcaaaaactaacataaacccct	FVB/CAST
H19 primary inner	gtaaggagattatgtttatttttgg	cctcattaatcccataactat	FVB/CAST
H19 secondary outer	gactggtcagcccttgagtc	cctcaccacaactcccactt	FVB/CAST
H19 secondary inner	ggtgaggagtgcccaaatta	gagctagcccctcagtcctt	FVB/CAST
Gtl2 primary outer	agatgtgttgtggatttaggttgtag	ctaaactacaatctatataatcacaacac	FVB/CAST
Gtl2 primary inner	aagtgttgtggtttgttatgggtaag	ccattcccaatctataaaaatattttaacc	FVB/CAST
Gtl2 secondary outer	gaagaatttttttatttggtgagtg	caacactcaaatcacccccc	FVB/CAST
Gtl2 secondary inner	gtttgaaaggatgtgtaaaaatg	ccccccacatctattctacc	FVB/CAST

PyroMark

	Primer1	Primer2	Pyro Primer
H19 primary pyro	gattatgggattatagatggtgat	[Biotin-5]aatcaacaaaatccacttactctctacaa	aggggagaaaatttaattag
H19 secondary pyro	agttagggttttttttaattggagtg	[Biotin-5]aaaacaataccaaaccctatcta	tttgttaattaattagtatatggt
Gtl2 primary pyro	ggtttatagtggatagttagtgt	[Biotin-5]cttccctcactccaaaaat	gttatggattggtgttaag
Gtl2 secondary pyro	ttgtagtttgggtgaggttatagtaatttg	[Biotin-5]aaccactcaccaaataaaaaattcttcta	tttatttagttagtttttagtatag

Genotyping

	Primer1	Primer2	Source	Notes
Trim28 (chatwo)	atgtgttgtgtggcccagta	acctcttggcacctgcaac	Shibata et al., 2011	Restrict with BslI
Trim28 (L -)	ggaatggttgttcattggtg	gcgagcacgaatcaaggtcag	Cammas et al., 2000	
Trim28 (L2)	ggaatggttgttcattggtg	accttggcccatttattgataaag	Cammas et al., 2000	
Cre	ctaggccacagaattgaaagatct	gtaggtggaaattctagcatcatcc		
D7Mit361	tactaccaatgctaagcgctacc	accagagtttgtgcccattc	MIT marker, MGI	CAST versus FVB SSLP near *H19* and *Kcnq1ot1*
D7Mit259	cccctcctcctgacctctt	gtctccatgggaaccacact	MIT marker, MGI	CAST versus FVB SSLP near *H19* and *Kcnq1ot1*
D7Mit316	ccacacaaatgccatacacg	tgtgcattcctgtatatttgtatgc	MIT marker, MGI	CAST versus FVB SSLP near *Snrpn*
D12Mit80	caacccagatgtcccttaaca	ctggaaggtttcacctagttgg	MIT marker, MGI	CAST versus FVB SSLP near *Glt2*
D2Mit265	aataataatcaaggttgtcattgaacc	tagtcaaaattcttttgtgtgttgc	MIT marker, MGI	CAST versus FVB SSLP near *Gnas*
D17Mit114	ggatccttagggctcacaca	gcctattttccaatttggca	MIT marker, MGI	CAST versus FVB SSLP near *Airn*

Supplemental Table I. Related to Experimental Procedures. Primers used for genotyping, quantification of allele-specific expression and DMR methylation.