Mosaic Analysis with Double Markers Reveals Cell-Type-Specific Paternal Growth Dominance

Simon Hippenmeyer,1,2,* Randy L. Johnson,3 and Liqun Luo1,*
1Howard Hughes Medical Institute and Department of Biology, Stanford University, Stanford, CA 94305, USA
2IST Austria (Institute of Science and Technology Austria), 3400 Klosterneuburg, Austria
3Department of Biochemistry and Molecular Biology, University of Texas, MD Anderson Cancer Center, Houston, TX 77030, USA
*Correspondence: simon.hippenmeyer@ist.ac.at (S.H.), lluo@stanford.edu (L.L.)
http://dx.doi.org/10.1016/j.celrep.2013.02.002

SUMMARY

Genomic imprinting leads to preferred expression of either the maternal or paternal alleles of a subset of genes. Imprinting is essential for mammalian development, and its deregulation causes many diseases. However, the functional relevance of imprinting at the cellular level is poorly understood for most imprinted genes. We used mosaic analysis with double markers (MADM) in mice to create uniparental disomies (UPDs) and to visualize imprinting effects with single-cell resolution. Although chromosome 12 UPD did not produce detectable phenotypes, chromosome 7 UPD caused highly significant paternal growth dominance in the liver and lung, but not in the brain or heart. A single gene on chromosome 7, encoding the secreted insulin-like growth factor 2 (IGF2), accounts for most of the paternal dominance effect. Mosaic analyses implied additional imprinted loci on chromosome 7 acting cell autonomously to transmit the IGF2 signal. Our study reveals chromosome- and cell-type specificity of genomic imprinting effects.

INTRODUCTION

In diploid organisms, most genes are expressed from both parental chromosomes. However, a subset of genes in mammals and plants is subject to a unique mode of regulation called genomic imprinting (Barlow, 2011; Bartolomei and Ferguson-Smith, 2011), whereby either the maternal or paternal allele is preferentially silenced. Genomic imprinting is essential for embryonic development in mammals (Barton et al., 1984; McGrath and Solter, 1984; Surani et al., 1984). Deregulation of imprinting has been implicated in many diseases, including cancer and brain disorders such as Angelman and Prader-Willi syndromes (Feinberg, 2007; Mabb et al., 2011; Nicholls and Knepper, 2001). Despite the importance of imprinting in controlling prenatal growth, behavior, and metabolism of the whole organism (Ferguson-Smith, 2011; Wilkinson et al., 2007), the functional relevance of imprinting at the cellular level is poorly understood for most genes.

The analysis of mice carrying uniparental disomy (UPD) of whole chromosomes (somatic cells with two copies of either the maternal or paternal chromosome), as well as duplication and deficiency of defined chromosomal regions, has been fundamental for the identification and mapping of imprinted chromosomal regions and loci in the mouse genome (Cattanach and Kirk, 1985; Williamson et al., 2013). However, phenotypic analysis of mice with UPD, deletions, and duplications is limited due to the lack of assays with cellular resolution. Only very recently, the analysis of paternally or maternally inherited mutations with concurrent cell marker labeling has begun to reveal tissue-specific physiological functions for certain imprinted genes (Ferrón et al., 2011; Garfield et al., 2011). Here, we use the mosaic analysis with double markers (MADM) system (Zong et al., 2005) to probe the effects of genomic imprinting at the whole-chromosome level and with single-cell resolution across many tissues and cell types in the mouse.

RESULTS AND DISCUSSION

MADM Can Assess Genomic Imprinting Phenotypes with Single-Cell Resolution

MADM can generate Cre/LoxP-dependent mitotic recombination between homologous chromosomes and at the same time label genetically defined progeny with distinct fluorescent markers. Specifically, mitotic recombination at G2 phase followed by X segregation of recombinated chromosomes can produce fluorescently labeled progeny with chromosomal compositions distinct from parental cells (Zong et al., 2005). Even for chromosomes that do not harbor any mutations, G2-X events produce near-complete UPD for a particular chromosome carrying the MADM cassettes (Figures 1A and S1A). Consequently, imprinted genes located on such a chromosome will be homozygosed and either overexpressed by a factor of two or not expressed depending on their imprinting status. Furthermore, cells with unipaternal disomy are fluorescently labeled with GFP and sister cells with unimaterial disomy with tandem dimer Tomato (tdTomato), or vice versa (Figures 1A and S1A). Thus, MADM provides in principle a unique experimental platform to systematically assay the consequences of genomic imprinting at the whole-chromosome level by visualizing the single-cell phenotypes of defined UPDs in genetic mosaic animals.

Chromosome 7, but Not Chromosome 12, UPD Leads to Drastic Paternal Growth Dominance in the Liver

To test the potential effect of imprinted genes on specific chromosomes, we produced MADM cassettes for mouse chromosome 7
because mouse chromosome 7 is most enriched for imprinted genes and harbors several well-studied clusters of imprinted genes (Williamson et al., 2013). We inserted MADM cassettes near the centromere of chromosome 7 (Figures 1B and S1B; see Experimental Procedures for details) using a similar knockin strategy as previously described (Hippenmeyer et al., 2010). We then used a ubiquitous Cre driver (Hprt-Cre) to induce chromosome 7 UPD across the whole animal in a mosaic fashion.

When using GFP to label unipaternal disomy cells (PP), we found a massive expansion of green hepatocytes in the liver (Figures 1C and 1G) when compared with unimaternal disomy cells (MM) labeled by tdTomato. In a separate experiment in which we switched colors of UPD cells (tdTomato for PP, and GFP for MM), we found that red hepatocytes expanded as a consequence of the chromosome 7 UPD (Figure 1D). Thus, unipaternal disomy of chromosome 7 leads to a massive expansion of hepatocytes when compared with unimaternal chromosome 7 disomy.

We also created MADM cassettes near the centromere of mouse chromosome 12 for mosaic analysis of candidate genes on that chromosome (Figures 1B and S1C). Hprt-Cre together with the MADM transgenes on chromosome 12 also produced chromosome 12 UPD cells in a mosaic fashion across the whole animal and including the liver. In contrast to chromosome 7 UPD, hepatocytes carrying either paternal or maternal chromosome 12 UPD appeared similar in number (Figures 1E–1G).

### Imprinting Effects in Chromosome 7 UPD Display Cell-Type Specificity

We extended our analysis to additional tissues and cell types with mosaic chromosome 7 or chromosome 12 UPD. Qualitative (Figures 2A–2C and 2E–2G) and quantitative (Figure 2I) evaluation of hippocampal CA1 pyramidal neurons and cerebellar Purkinje cells in the brain, as well as cardiomyocytes in the heart, revealed no significant differences in cell number regardless of whether these cells carried unipaternal or unimaternal disomies for chromosome 7 or chromosome 12. By contrast, lung epithelia displayed a marked expansion of cells with unipaternal chromosome 7 but not chromosome 12 UPD (Figures 2D and 2H), similar...
to liver hepatocytes. We conclude that MADM-induced chromosome 7 but not chromosome 12 UPD results in cell-type-specific expansion with unipaternal disomy.

To exclude the possibility that the MADM markers on chromosome 7 themselves were selectively silenced due to imprinting, we generated mice containing constitutively expressed, reconstituted GFP (Hipp7gG/) and tdTomato (Hipp7TT/) markers, respectively, and intercrossed them to generate Hipp7gG/TT animals. All cells in every organ analyzed at postnatal day (P) 21 in Hipp7gG/TT expressed both markers uniformly (Figure S2). Therefore, the unequal ratio of hepatocytes and lung epithelia with unipaternal over unimaternal chromosome 7 UPD did not result from selective silencing of one marker in putative GFP+/tdTomato+ cells but reflects an imprinting phenotype whereby cells with unipaternal disomy have a growth advantage over unimaternal disomy.

**Paternal Growth Advantage in Chromosome 7 UPD Commences during Embryogenesis**

What is the developmental origin of cell number expansion in unipaternal chromosome 7 disomy? To address this question, we carried out a developmental time course analysis focusing on liver hepatocytes with MADM-induced chromosome 7 UPD. At embryonic day (E) 12, no expansion of the hepatocyte population with unipaternal disomy was apparent (Figure 3A). However, 4 days later at E16, unipaternal chromosome 7 disomy cells already outnumbered unimaternal chromosome 7 disomy cells (Figure 3B). We validated this result by clonal analysis using tamoxifen (TM)-induced CreER (Figures 3J and 3K). In liver 7 hepatocytes became more apparent at postnatal stages (Figures 3C–3F). Nevertheless, mosaic MADM-7 livers were neither larger than wild-type nor displayed any signs of tumors in mice of up to 6 months of age, suggesting that cell expansion due to unipaternal disomy still follows organ size control mechanism as in wild-type hepatocytes (Stanger, 2008). In contrast to hepatocytes with unipaternal chromosome 7 disomy, hepatocytes with either UPD for chromosome 12 displayed equal numbers postnatally up to 6 months (Figures 3G–3I).

**Ablation of a Single Gene on Chromosome 7, Igf2, Largely Mitigates Paternal Growth Advantage of Chromosome 7 UPD**

The paternal dominance of chromosome 7 disomy cells could, in principle, reflect the consequence of homozygosing many or just a few imprinted genes on chromosome 7. Because the “imprimeome” of chromosome 7 in the liver is currently unknown, we pursued a candidate gene approach to identify the causal gene. Chromosome 7 harbors several clusters of imprinted genes that either promote or antagonize growth, including paternally expressed Igf2 (insulin-like growth factor 2) (DeChiara et al., 1991), as well as maternally expressed H19 (Bartolomei et al., 1991) and Cdkn1c (cyclin-dependent kinase inhibitor 1C, aka p57kip2) (Hatada and Mukai, 1995; Matsuoka et al., 1995). The growth advantage of unipaternal disomy can be caused by overexpression of paternally expressed growth-promoting gene(s), absence of maternally expressed growth-antagonizing gene(s), or a combination of both. We began by assessing the involvement of IGF2 and introduced a null allele from the father into our
MADM analysis. Because IGF2 is paternally expressed in most cells (DeChiara et al., 1991), Igf2\(^{m/-}\) (m stands for the wild-type allele inherited from the mother) should in principle be equivalent to homozygous IGF2 \(^{--}\). Remarkably, we found a striking reduction of the large clusters of unipaternal chromosome 7 disomy cells at P21 in Igf2\(^{m/-}\)-MADM (Figures 4A and 4D compared with Figures 4B and 4E; Figures S3A and S3B).

To compare hepatocyte expansion with unipaternal chromosome 7 disomy produced by MADM in control mice and mice with paternal loss of Igf2, we quantified all green and red cells in entire liver sections to avoid selection bias and calculated their ratios (Figures 4G, S4A, and S4B). We found an 8.6±0.9-fold paternal expansion in control-MADM. The PP/MM ratio was reduced to 2.2±0.4-fold in animals with Igf2\(^{m/-}\)-MADM. To test whether the residual paternal dominance was due to IGF2 from a possible reactivation of the silenced maternal Igf2 allele, or in serum from distinct sources not subjected to imprinting (DeChiara et al., 1991), we also examined the liver from homozygous IGF2 \(^{--}\) mutants with MADM labeling (Figures 4C and S3C). We found a reduction of the PP/MM ratio (Figure 4F) to the same level (2.1±0.5) as when the Igf2 mutation was introduced only from the father. By contrast, maternal transmission of the Igf2 mutation had no effect on paternal dominance (Figures S3D, S4C, and S4D). Thus, the loss of the paternally inherited Igf2 fully accounts for the imprinting effect.

To extend our findings to other tissues, we also examined the effect of IGF2 on the paternal growth dominance in the lung epithelia. We found that paternal transmission of the Igf2 mutation also resulted in marked reduction of paternal growth dominance in the lung (Figures S4E–S4H), highlighting a general role of IGF2 imprinting in distinct cell types. Taken together, these findings indicate that Igf2 is a major factor driving unipaternal chromosome 7 growth dominance in the liver and lung.

Evidence that Additional Imprinted Factors on Chromosome 7 Act Cell Autonomously to Receive the IGF2 Signal

Although chromosome 7 harbors many imprinted genes controlling growth, we found that mutation of just one paternally expressed gene, Igf2, mitigates the major role of the paternal dominance effect. Our findings are in line with previous chimera studies implicating that duplication of distal chromosome 7 (where Igf2 is located) and Igf2 itself are responsible for causing overgrowth (Ferguson-Smith et al., 1991; McLaughlin et al., 1997). We further extended these studies with single-cell resolution by producing and visualizing UPD through rare mitotic recombination events enabled by MADM.

However, it is surprising that a secreted factor should be a major contributor in our experimental setting. Under the control-MADM-7 condition, all unipaternal disomy cells that overexpress Igf2 originate from mitotic recombination events in a single cell and are surrounded by a vast majority of cells with regular paternal Igf2 expression. Even with paternal growth dominance, unipaternal cells represent only a small fraction of the whole liver (see Figure S4A). We envision three possibilities to explain the paternal growth dominance that we observed in MADM-7 mice (Figure 4H). First, despite being a secreted factor, IGF2 acts predominantly cell autonomously. Furthermore, a 2-fold increase in IGF2 levels should greatly facilitate the growth of the unipaternal cells. We consider the first possibility unlikely because IGF2 is well known to bind IGF-binding proteins, enabling IGF2 distribution in the bloodstream and thus to signal growth of neighboring cells in paracrine and endocrine manners (Chao and D’Amore, 2008). Second, in addition to IGF2, another paternally expressed P factor acts cell autonomously to positively regulate IGF2 signal transmission. Again, this P factor should act in a dose-dependent fashion to account for the growth of unipaternal chromosome 7 disomy cells in a mostly wild-type background because it would also be present in 2-fold higher dosage levels, similar to IGF2. Third, a maternally
Figure 4. Igf2 Accounts for Most of the Paternal Dominance in Chromosome 7 UPD

(A–C) Schematic of Igf2 expression in MADM-7^G7T/G7T:Hprt-Cre^+/+. (A) Control-MADM-7. (B) Igf2 mutation on paternal chromosome. (C) Igf2 mutation on both paternal and maternal chromosomes. Note that due to Igf2 imprinting, most cells in (B) do not express Igf2.

(D–F) Labeling of uniparental chromosome 7 disomy (PP cells are green, and MM cells are red) in P21 control, paternal Igf2 mutant, or homozygous Igf2/C0/C0 mutant MADM animals corresponding to the schemes in (A–C). Scale bar, 110 μm.

(G) Quantification of PP/MM ratio in whole-liver sections from P21 MADM animals with genotypes indicated. Values represent mean ± SEM. *p < 0.05 and **p < 0.01.

(H) Three possible models to account for expansion of cells with unipaternal disomy in sparsely generated MADM-7 clones in wild-type animal. Assuming the crossing scheme in Figure 1A, cells with unipaternal chromosome 7 disomy (pUPD7) are labeled in green and cells with unimaternal chromosome 7 disomy (mUPD7) in red upon a G2-X MADM event. Green pUPD7 cells express a double dose of Igf2 and P factor(s) but lack M factor(s), where P or M factors represent additional imprinted genes on chromosome 7 that could act cell autonomously to promote or inhibit IGF2 signaling, respectively. In Model 1, IGF2 (double dose) signals strictly autocrine to only pUPD7, but not mUPD7. In Model 2, the double dose of additional imprinted P factor(s) acts in concert with secreted IGF2 to convey the growth advantage in pUPD7. In Model 3, the lack of M factor(s), which normally would tune down the IGF2 signal to a certain extent, promotes increased Igf2 growth signaling in pUPD7 cells. The cell-type specificity of UPD7 effects we observed may be a consequence of cell-type-specific expression of the M or P factors, or cell-type-specific interactions of these factors with Igf2.

See also Figures S3 and S4.
expressed M factor acts cell autonomously to negatively regulate IGF2 signal transmission. In unipaternal chromosome 7 disomy cells, the lack of the negative M factor results in greatly amplified IGF2 growth signaling.

In both the second and third possibilities above, the additional chromosome 7 P or M imprinted factors contribute, at most, a 2-fold difference independent of IGF2, and their major actions are manifested in the context of cell-autonomous IGF2 signal transmission. We favor the third model because it requires the least number of assumptions; indeed, Cdkn1c could represent an attractive candidate for the M factor because genetic interactions have been demonstrated between Igf2 and Cdkn1c (Caspary et al., 1999).

Although Igf2 displays a highly specific expression pattern in the brain, it is broadly expressed in peripheral embryonic tissues such as the liver, lung, and heart (Davies et al., 2002; DeChiara et al., 1991; Lehtinen et al., 2011). Because we have only detected a significant proliferation advantage in the liver and lung, but not in the heart or the brain, it is highly unlikely that the cell-type-specific effect of imprinting is accounted for by tissue-specific Igf2 expression patterns. Rather, the tissue specificity of the imprinting effect is likely caused by tissue-specific expression of the M or P factors, or their tissue-specific interactions with IGF2.

**Conclusions**

We have used the MADM strategy to create, and concomitantly visualize with distinct fluorescent colors, cells with unipaternal and unimatal near whole-chromosomal disomy. Consequently, it is possible to assay the phenotype of genomic imprinting at the single-cell resolution in virtually any tissue or organ and potentially for every chromosome in the mouse. The results from MADM of chromosome 7 and chromosome 12 revealed chromosomal as well as cell-type-specific imprinting phenotypes. The cell-type specificity of genomic imprinting indicates that certain genes exploit parentally controlled expression regulation in order to fulfill their appropriate physiological functions.

A salient advantage of the MADM method is the controlled generation of defined unipaternal and unimatal disomic cells that are labeled by two distinct fluorescent colors at the single-cell resolution. This provides a sensitive means to detect phenotypes for homozgyosing imprinted genes controlling cell proliferation (such as Igf2 presented in our study), size, and morphogenesis. An important feature is that MADM-induced UPD cells always display the combined phenotype of the entire cohort of imprinted genes located on a particular chromosome. This could be a limitation when it comes to identifying specific genes on the chromosome that contribute to the imprinting phenotypes. Therefore, the MADM approach complements rather than replaces conventional and/or conditional knockout experiments to study the function of imprinted genes. We presented an example of combining MADM to analyze whole-chromosome UPD with mutation in a selected candidate gene, Igf2, and deduced the quantitative contribution of Igf2 to the imprinting effect of the entire chromosome.

Finally, MADM analysis has been critical to study cell-autonomous functions of candidate genes (besides Igf2 described in this study) in cell proliferation, neuronal migration, dendrite morphogenesis, and tumor growth (Esponosa et al., 2009; Hippenmeyer et al., 2010; Liu et al., 2011; Muzumdar et al., 2007). The establishment of MADM cassettes on chromosome 7 and chromosome 12 now allows mosaic analysis of a vast majority of genes (~2,500 on chromosome 7, and ~300 on chromosome 12) on two new chromosomes in addition to our previous efforts (Hippenmeyer et al., 2010; Tasic et al., 2012; Zong et al., 2005). Together, MADM-7 and MADM-12 almost double the total number of mouse genes that can be subjected to MADM analysis.

**EXPERIMENTAL PROCEDURES**

**Generation of MADM-7 and MADM-12 Mice and Mouse Genetic Techniques**

MADM-7 and MADM-12 mice were generated following a previously described strategy (Hippenmeyer et al., 2010). In brief, for MADM-7 and MADM-12-targeting constructs, we identified suitable genomic loci on chromosome 7 (Hipp7) located at 2.13cm; ~0.7 kb downstream of exon 5 of theRpc9 gene and chromosome 12 (Hipp12) located at 1.71cm; ~16 kb downstream of exon 1 of theRab10 gene. The Hipp7 and Hipp12 genomic loci were cloned, GT and TG MADM cassettes (Hippenmeyer et al., 2010; Liu et al., 2011) inserted, and targeting vectors constructed using standard molecular biology procedures (details are available upon request). Linearized targeting vectors were then electroporated into R1 ES cells, and two correctly targeted GT and TG clones for each MADM-7 and MADM-12, respectively, were injected into blastocysts to generate chimeric mice. Homozygous MADM-7TGT, MADM-7TG/TG, MADM-12TGT/GT, and transheterozygous MADM-7GTGT, and MADM-12GTGT were born at Mendelian frequencies, had a normal lifespan, were fertile, showed no obvious averse phenotype, and did not exhibit GFP/tdTomato marker expression in the absence of Cre recombinase.

Heterozygote Igf2+/- (DeChiara et al., 1990), Jactin-CreER R26T (Guo et al., 2002), and hemizygote Hipp Tcr (Tang et al., 2002) mice have been described. Timed pregnancies were set up to generate embryos at defined developmental stages. For the generation of recombinant MADM-7GTGT, MADM-7GT/GT or MADM-7GTGT,igf we followed a standard breeding strategy as previously described (Hippenmeyer et al., 2010). The MADM experiments described in this study were carried out in mixed 129/C57Bl/6/Cd1 genetic background, and all experimental procedures were carried out in accordance with the Administrative Panel on Laboratory Animal Care protocol and the institutional guidelines by the Veterinary Service Center at Stanford University, and the institutional guidelines by the Veterinary Service Center at Stanford University.

For the induction of G2-X MADM clones in liver, we set up timed pregnancies, injected Tm intraperitoneally at E12, and isolated embryos at E16. Embryos were fixed in 4% PFA/PB overnight, cryoprotected in 30% sucrose/PBS,
embedded in OCT, and sectioned in the sagittal plane at 30 μm. The frequency of MADM clone generation was extremely low and varied between cohorts with slightly different genetic background.

**Computation of PP/MM Ratios**

Pyramidal cells in hippocampal area CA1 were counted in confocal images from 16 (MADM-+/GT;Hprt-Cre−/−) and 11 (MADM-12/+/GT;Hprt-Cre−/−) cyrosesions (derived from three animals for each genotype) encompassing the whole CA1 area. Purkinje cells in cerebella were counted in all consecutive sections from four (MADM-3/3/GT;Hprt-Cre−/−) and three (MADM-12/+/GT;Hprt-Cre−/−) entire half-brains derived from more than two animals each. For quantification of heart cardiomyocyte and liver hepatocyte populations, the green (GFP) and red (tdTomato) MADM signals were first extracted manually from confocal images derived from cyrosesions to separate them from the yellow GFP/“AdTomato” signal. The total areas of green and red signals, respectively, were then computed from binary images using a custom MATLAB script. The geometric means ± SEM of the paternal/maternal ratios were calculated in Excel, and significance was determined using Student’s t test: *p < 0.05 and **p < 0.01.

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes four figures and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2013.02.002.

**LICENSING INFORMATION**

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**ACKNOWLEDGMENTS**

We thank H. Zong for FLLFL MADM cassettes and discussions about MADM; Y. Cher-Tsai and the Stanford Transgenic Facility for generating knockin mice; A. Efstratiadis and C. Walsh for providing Igf2 mutant mice; C. Manalac, J. Zhong, K. Jaecle, C. Nguyen, and M. Shu for technical support; and H. A. Efstratiadis and C. Walsh for providing the original author and source are credited.

**REFERENCES**


Figure S1. MADM Principle for Imprinted Genes and Targeting Strategy to Generate MADM-7 and MADM-12, Related to Figure 1

(A) MADM is based upon Cre/LoxP-dependent interchromosomal recombination whereby two reciprocal chimeric marker genes (GT and TG) are reconstituted. Recombination in postmitotic cells or during the G1 phase of the cell cycle reconstitutes both green and red markers in the same cell, and produces yellow cells without altering the genotype (not shown). Recombination in G2 with both reconstituted marker genes (and corresponding chromosomes) segregating to the same daughter cell upon mitosis (Z-Segregation) also does not alter the genotype, and produces one yellow cell (left branch). In contrast, recombination in G2 followed by X-Segregation where the two recombinant chromosomes segregate into distinct daughter cells, results in each cell expressing either GFP (green) or tdTomato (tdT, red), and displays near complete uniparental chromosomal disomy (UPD, right branch). If the GT MADM cassette is inherited from the mother (M) and the TG MADM cassette from the father (P), as shown in this schematic, red cells harbor unimaternal chromosomal disomy (MM) and green cells unipaternal chromosomal disomy (PP). Consequently, maternally expressed genes (pink) are expressed at twice the normal dose and paternally expressed genes (blue) are not expressed in red cells with unimaternal disomy. By contrast, paternally expressed genes are expressed at twice the normal dose and maternally expressed genes are not expressed in green, unipaternal disomy cells. Thus, genes subject to imprinting are differentially expressed depending on the UPD. Non-imprinted genes (black) are not affected. Symbols used in this schematic and in Figure 1A are detailed in the key.

(B) Targeting of the Hipp7 genomic locus with GT and TG MADM cassettes, respectively. Top panel: the genomic organization of the Hipp7 locus. The insertion site (MADM-7 targeting locus) is located ~0.7kb telomeric to exon 5 of the Rps9 gene and ~4.8kb centromeric to exon 15 of the Lihb3 (PirB) gene. Middle panel: the Hipp7 locus with inserted GT cassette (MADM-7GT). Bottom panel: the Hipp7 locus with inserted TG cassette (MADM-7TG).

(C) Targeting of John12 genomic locus with GT and TG MADM cassettes, respectively. Top panel: the genomic organization of the John12 locus. The insertion site (MADM-12 targeting locus) is located ~16kb downstream of exon 1 of the Rab10 gene and ~39kb upstream of exon 1 of the Kif3c gene. Middle panel: the John12 locus with inserted GT cassette (MADM-12GT). Bottom panel: the John12 locus with inserted TG cassette (MADM-12TG).

GT and TG MADM cassettes have been described: for cassettes in MADM-12 see (Hippenmeyer et al., 2010); cassettes for MADM-7 include LoxP - FRT-Lox5171-Lox2272-FRT - (Liu et al., 2011; Tasic et al., 2012).
Figure S2. Biallelic and Global Expression of MADM from Genomic Hipp7 Locus, Related to Figure 2

(A) Breeding strategy to generate GG (green, Hipp7<sup>GG/+</sup>), TT (red, Hipp7<sup>TT/+</sup>) and GG/TT (yellow, Hipp7<sup>GG/TT</sup>) animals with reconstituted MADM marker genes. First, MADM<sup>7GT/7TG</sup>; HprtCre<sup>+/+</sup> animals were mated to wild-type. ‘Green GG’ (Hipp7<sup>GG/+</sup>) and ‘red TT’ (Hipp7<sup>TT/+</sup>) offspring animals (derived from germ cells where interchromosomal recombination has led to reconstituted GG and TT markers) were selected. Hipp7<sup>GG/TT</sup> were then crossed to Hipp7<sup>TT/+</sup> to generate Hipp7<sup>GG/TT</sup> animals.

(B–M) Marker expression from the Hipp7 locus in Hipp7<sup>GG/+</sup> (B, E, H, and K), Hipp7<sup>TT/+</sup> (C, F, I, and L) and Hipp7<sup>GG/TT</sup> (D, G, J, and M) in liver (B–D), heart (E–G), lung (H–J) and brain (hippocampus; K–M) at P21. In all images, both green and red channels from confocal microscopy were acquired and shown. Thus, Hipp7<sup>GG/+</sup> only express GFP; Hipp7<sup>TT/+</sup> only express tdT; Hipp7<sup>GG/TT</sup> express both with similar intensity, hence all cells are yellow. DAPI delineates CA1, CA3 and dentate gyrus regions in (K–M). Scale bar: 500 μm.
Figure S3. MADM Scheme for Analyzing the Imprinted Gene Igf2, Related to Figure 4

(A) Control MADM scheme (see Figure S1 for symbols). Imprinting (silencing) of the maternal Igf2 allele (white) and expression from the paternal allele (blue) are indicated in a cell before MADM is induced. The GT or TG cassette is located on the maternal (M) or paternal (P) chromosome, respectively. Different MADM events would produce labeled cells with the Igf2 genotypes shown at the bottom: 2x Igf2 for green cells, 1x Igf2 for yellow cells and no Igf2 for red cells.

(B) MADM scheme for paternal transmission of the mutant Igf2 allele. Because the maternal allele should be silenced, no Igf2 expression is predicted in green, red, and yellow cells.

(C) MADM scheme for transmission of the mutant Igf2 allele from both parents. No Igf2 expression occurs in green, red, and yellow cells.

(D) MADM scheme for maternal transmission of the mutant Igf2 allele. Because the maternal allele should be silenced in any case, the Igf2 expression pattern is predicted to resemble that of control MADM (A).
Figure S4. Detailed Analysis and Quantification of PP/MM Ratio in Igf2-MADM-7 Tissues, Related to Figure 4

(A) Representative images of whole liver sections assembled from individual confocal scans from P21 MADM-7 GT/+/HprtCre/+ (left: Igf2+/+), MADM-7 GT/TG/Igf2/HprtCre/+ (middle: Igf2m/-, Igf2 mutation from father), and MADM-7 GT/TG/Igf2/HprtCre/+ (right: Igf2m/-, homozygous Igf2 mutant). In all cases, green (GFP) cells harbor unipaternal Chr.7 disomy (PP), red (tdT) cells unimaternal Chr.7 disomy (MM) and yellow (GFP/tdT) cells are wild-type (PM). Note that the liver sections are significantly smaller in Igf2m/- and Igf2m/-/C0/C0 when compared to Igf2+/+, consistent with IGF2 being required for normal growth (DeChiara et al., 1990).

(B) Quantification of PP/MM ratios in P21 Igf2+/+, Igf2m/-, and Igf2m/-/C0/C0 MADM samples. Every data point represents the ratio of one confocal image (25-56 per compiled liver section from individual animals/genotypes). The large numeric variance reflects the variable sizes of clones with MADM labeling. Two extreme data points are highlighted with a star (indicates the highest) and square (lowest) PP/MM ratio in Igf2+/+. One representative whole liver section was quantified from n = 3 individual animals and per genotype.

(C and D) No mitigation of paternal dominance in MADM-7 liver with maternal transmission of Igf2 mutation. (C) Representative section of P21 liver with GT inherited from father and TG inherited from mother (MADM-7 GT/TG/Igf2/HprtCre/+). Thus, cells with unipaternal Chr.7 disomy (PP) appear in red (labeled by tdT), and are present at increased numbers when compared to cells with unimaternal Chr.7 disomy (MM) in green (labeled by GFP). (D) Representative section of P21 liver in MADM-7 GT/TG/Igf2/HprtCre/+ with GT inherited from father, and TG and the Igf2 mutation inherited from the mother. See Figure S3D for detailed MADM scheme. Note that red MM cells outnumber green PP cells similar as in (C), indicating that maternal inheritance of the Igf2 mutation has no functional relevance (due to imprinting status) since the paternal Igf2 allele is still overexpressed at double dose in red PP cells.

(E–H) Diminished paternal dominance in MADM-7 lung with paternal but not maternal transmission of Igf2 mutation. (E) Representative section of P21 lung with TG inherited from the father and GT inherited from the mother. Cells with unipaternal Chr.7 disomy (PP) are labeled in green by GFP expression, and are overabundant when compared to cells with unimaternal Chr.7 disomy (MM) labeled in red by tdT expression. (F) Paternal transmission of Igf2 mutation results in reduced paternal dominance as seen by the reduced number of green PP cells compared with (E). (G) Representative section of P21 lung with GT inherited from father and TG inherited from mother. Cells with unipaternal Chr.7 disomy (PP) are labeled in red by tdT expression, and are overabundant when compared to cells with unimaternal Chr.7 disomy (MM) labeled in green by GFP expression. (H) Maternal transmission of Igf2 mutation does not affect paternal dominance as seen by similar overabundance of red cells as in (G).

Scale bars: 1mm (A); 100 μm (C–H).