Supporting Information

Lonfat et al. 10.1073/pnas.1310704110

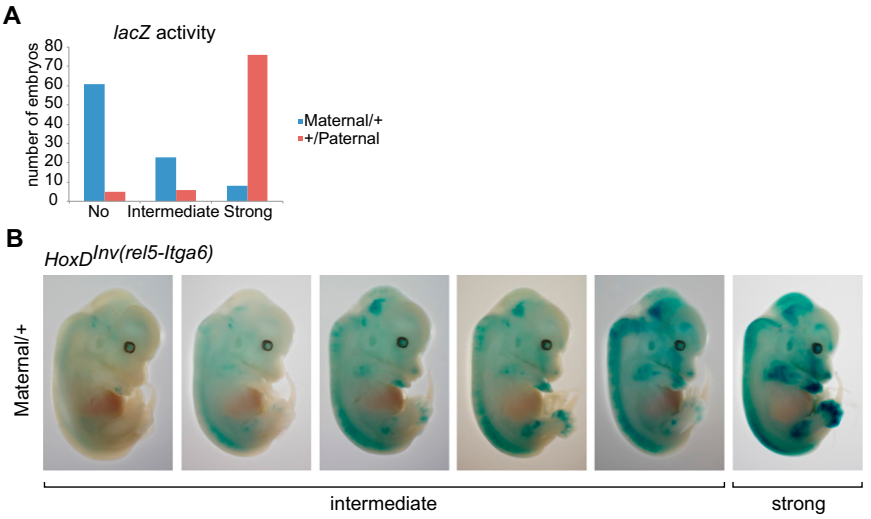

Fig. S1. Variegation of transgene silencing on the maternal chromosome. (*A*) Summary of the strength of *lacZ* staining in heterozygous embryos carrying the transgene after either maternal or paternal transmission. (*B*) A substantial percentage of Maternal/+ embryos carrying the $HoxD^{Inv(rel5\text{-}Itga6)}$ inversion escapes the repression of the reporter *lacZ*. These escapers show staining with variable extents (qualified as intermediate) ranging from almost no activity to no repression at all (strong), which is similar to +/Paternal animals expression. They are characterized by distinct, heterogenous, and mostly clonal patterns of expression, with patches and stripes of *lacZ* activity.

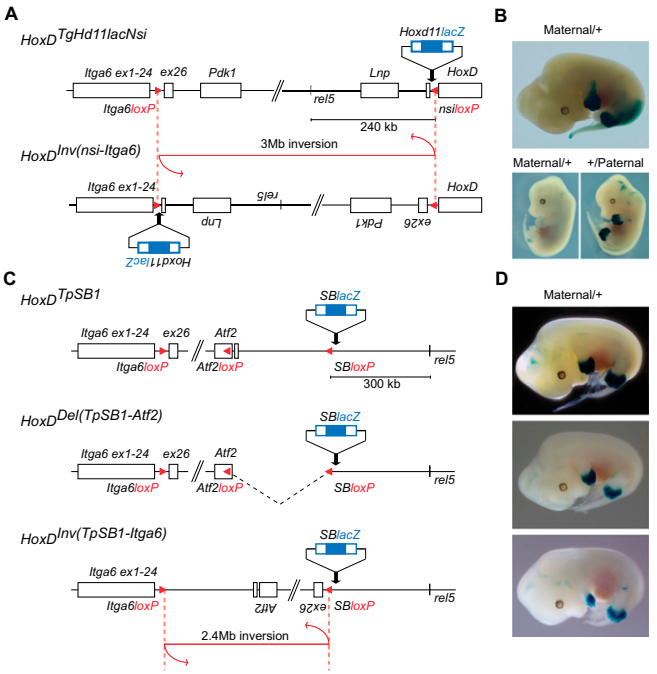

Fig. S2. The imprinting is *Itga6* locus-specific. (*A*) Scheme of the *HoxD*^{TgHd11lacNsi} allele carrying an *Hoxd11lacZ* transgene (blue) along with a *loxP* site (red). Using another *loxP* site on the same chromosome within the *Itga6* locus (1), we induced the *HoxD*$^{Inv(TgHd11lacNsi\text{-}Itga6)}$ inversion, which repositioned the *Hoxd11lacZ* transgene into *Itga6* (on the bottom) at the same position where the *Hoxd9lacZ* transgene is located in the *HoxD*$^{Inv(rel5\text{-}Itga6)}$ allele. (*B*) Whole-mount staining of β-*gal* reporter activity. Before inversion, the transgene inherited from either (*Upper*) the mother or the father (not shown for clarity) displays the exact same strong expression, similar to the endogenous *Hoxd11*. After inversion, a strong maternal imprinting of the *Hoxd11lacZ* transgene is scored, with no staining compared with the paternal transmission of the transgene. (*C*) Different insertion sites within the *HoxD* landscape show no imprinting effect on a reporter transgene. *HoxD*^{TpSB1} is the insertion of a Sleeping Beauty (SB) transposon 300 kb upstream of *rel5*. SB contains a reporter *lacZ* associated with a minimal promoter of the β-*Globin* gene (blue) and a *loxP* site cloned upstream (red) (2); *lacZ* staining of the transgene in embryos heterozygous for SB shows no allelic difference when transmitted through either (*D*) a female or a male. *HoxD*$^{Del(TpSB1\text{-}Atf2)}$ is a deletion between a *loxP* site in the *Atf2* gene (red) (3) and the SB *loxP* site. The relocation of the SB transgene 290 kb closer to the *Itga6* locus shows no parental influence on the expression of the *lacZ*. Maternal/+ and + /Paternal embryos staining are similar. The *HoxD*$^{Inv(TpSB1\text{-}Itga6)}$ inversion addresses the effect of *Itga6* exon 26 and its telomeric landscape on *lacZ* reporter expression. Using a *loxP* in the *Itga6* locus and the SB *loxP* (upstream the *lacZ*), a *cre* recombinase-mediated inversion relocated the last exon of *Itga6* near the reporter gene still located at the TpSB1 position. Neither the exon 26 nor the *Itga6* telomeric landscape induced imprinting on SB transgenes. Both parental inherited transgenes showed the same *lacZ* expression (Maternal/+ embryo for the inversion shown in *D*).

1. Gimond C, et al. (1998) Cre-loxP-mediated inactivation of the alpha6A integrin splice variant in vivo: Evidence for a specific functional role of alpha6A in lymphocyte migration but not in heart development. *J Cell Biol* 143(1):253–266.
2. Ruf S, et al. (2011) Large-scale analysis of the regulatory architecture of the mouse genome with a transposon-associated sensor. *Nat Genet* 43(4):379–386.
3. Shah M, et al. (2010) A role for ATF2 in regulating MITF and melanoma development. *PLoS Genet* 6(12):e1001258.

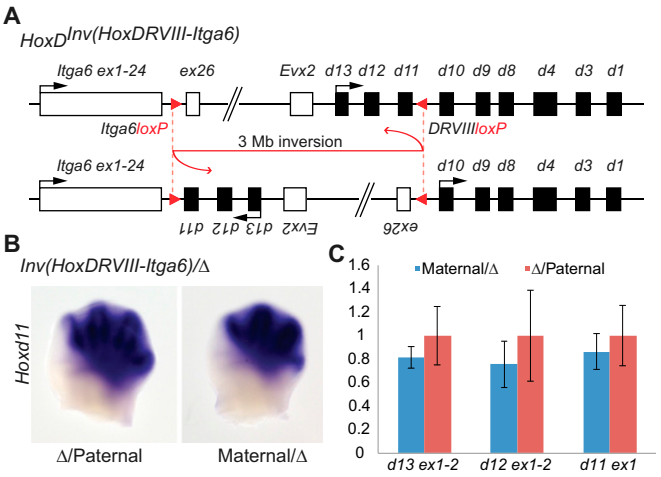

Fig. S3. *lacZ* sequence-dependent imprinting specific to the *Itga6* locus. (*A*) Scheme of the *HoxD*$^{Inv(HoxDRVIII\text{-}Itga6)}$ allele, which is an inversion between a *loxP* site (red) located between *Hoxd11* and *Hoxd10* and a *loxP* site within the *Itga6* gene [red; same as for the *HoxD*$^{Inv(rel5\text{-}Itga6)}$ allele]. *Hoxd11*, *Hoxd12*, and *Hoxd13* are now relocated in the *Itga6* locus, and the position of *Hoxd11* corresponds to the location of the imprinted *Hoxd9lacZ* transgene in the *HoxD*$^{Inv(rel5\text{-}Itga6)}$ allele. (*B* and *C*) Mice heterozygous for the *HoxD*$^{Inv(HoxDRVIII\text{-}Itga6)}$ inversion were crossed with animals carrying a deletion from *Hoxd8* to *Hoxd13* [*HoxD*$^{DelRXIIDel(13\text{-}8)}$, depicted by the Δ] to ensure a correct allelic comparison of *Hoxd11* expression. (*B*) *Hoxd11* expression in E12.5 digits after inversion with the allele inherited from either the father or the mother. Whole-mount in situ hybridizations of *Hoxd11* show no parent-specific transmission effect. (*C*) Quantitative RT-PCRs (RT-qPCRs) on digits mRNA confirms that all relocated genes on both parental alleles ($n = 4$) have similar expression levels.

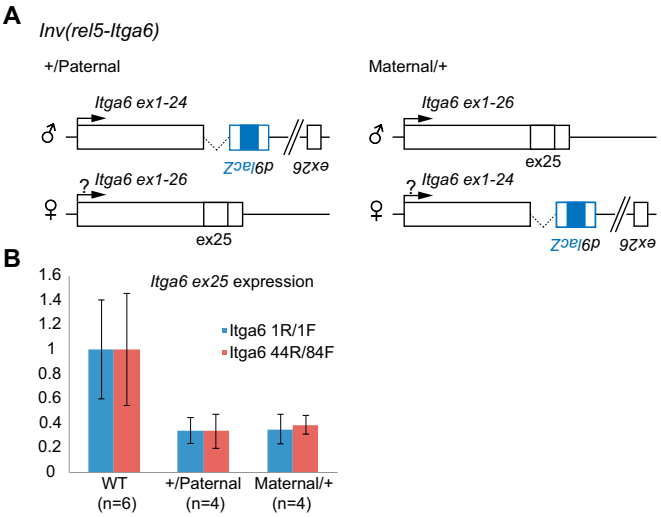

Fig. S4. The *Itga6* locus is not imprinted. (*A*) Scheme of the *Itga6* locus in heterozygous *HoxD*$^{Inv(rel5\text{-}Itga6)}$ animals. The *loxP* site within *Itga6* comes with a deletion of exon 25 (1). (*B*) Specific RT-qPCR primers designed for the *Itga6* exon 25 were used to compare expression levels of *Itga6* in either the Maternal/+ or the +/Paternal *HoxD*$^{Inv(rel5\text{-}Itga6)}$ configurations. No significant parental bias is scored, at least for the expression of *Itga6* exon 25.

1. Gimond C, et al. (1998) Cre-loxP-mediated inactivation of the alpha6A integrin splice variant in vivo: Evidence for a specific functional role of alpha6A in lymphocyte migration but not in heart development. *J Cell Biol* 143(1):253–266.

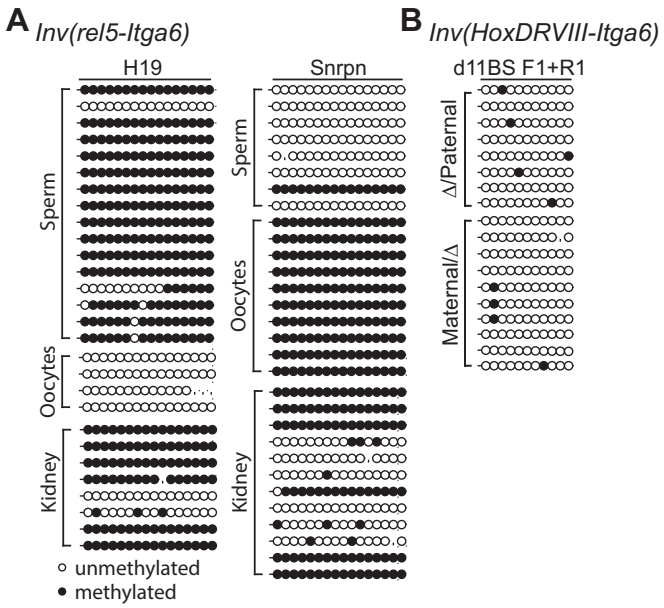

Fig. S5. Controls for DNA methylation. (*A*) The germ cells samples used in the study display the expected control patterns of methylation for *H19* (methylated in sperm) and *Snprn* (methylated in oocytes). (*B*) Bisulfite sequencing analysis of *Hoxd11* in the $HoxD^{Inv(HoxDRVIII\text{-}Itga6)}$ allele. No differential methylation was found when *Hoxd11* was positioned within *Itga6*. Primer sequences are in Table S1.

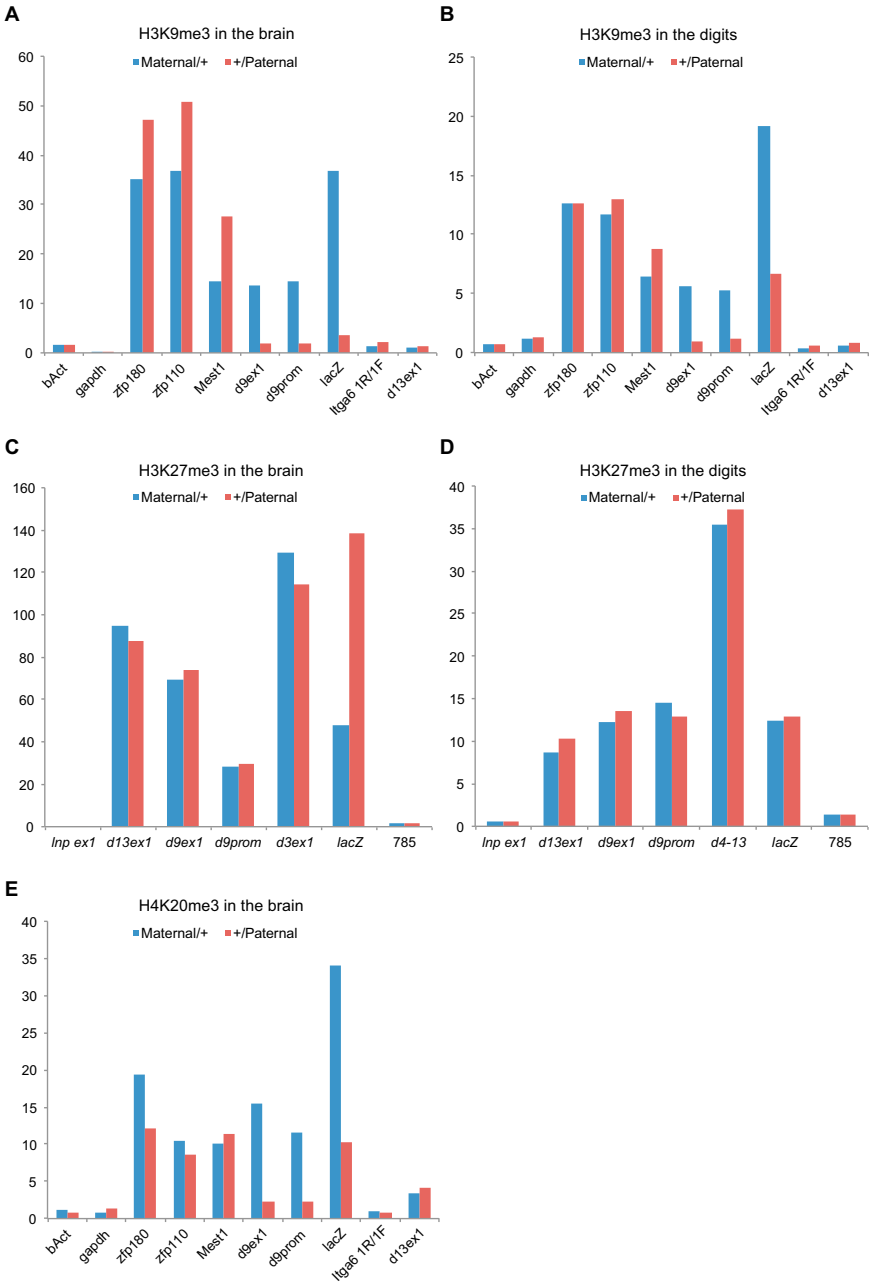

Fig. S6. Repressive histone methylation marks cover the silenced transgene. ChIP followed by qPCR on E12.5 forebrain or presumptive digits from *HoxD*$^{Inv(rel5\text{-}Itga6)}$ embryos using (*A* and *B*) H3K9me3, (*C* and *D*) H3K27me3, and (*E*) H4K20me3 antibodies. Primer sequences are given in Table S1.

Table S1. List of primers used in the study

Gene	Sequence
RT-qPCR	
Itga6 1F	AAG ATC ATT ACG ATG CCA CCT
Itga6 1R	TGC ATC GGA AGT AAG CCT CT
Itga6 44F	ATG CCA CCT ATC ACA AGG CTG
Itga6 84R	AGA CGG CTG AGT ATG GAT CTC AG
lacZ F	ATC AGG ATA TGT GGC GGA TGA
lacZ R	TGA TTT GTG TAG TCG GTT TAT GCA
Dlx1 ex1-2 F	GGC TAC CCC TAC GTC AAC TC
Dlx1 ex1-2 R	TTT TTC CCT TTG CCG TTA AAG C
Dlx2 ex1-2 F	AAC CAC GCA CCA TCT ACT C
Dlx2 ex1-2 R	TGA CCT GAG TTT GGG TGA G
Dlx2 ex2-3 F	ACA ATG TCT CCT ACT CCG C
Dlx2 ex2-3 R	TCA AGG TCT TCC TTG TCC G
ZFP180-3′-F	CCG TAC AGG TGC AAT CTG TG
ZFP180-3′-R	GTT TGT AGC TCT GGC GGA AC
ZFP110-3′-F	AAC GAC CGC TCA GCC ATC TC
ZFP110-3′-R	GCC TGG TAA GGT GGG AAC TC
β-Actin F	AGC CAA CTT TAC GCC TAG CGT
β-Actin R	TCT CAA GAT GGA CCT AAT ACG GC
Gapdh F	AGG TCG GTG TGA ACG GAT TTG
Gapdh R	TGT AGA CCA TGT AGT TGA GGT CA
Bisulfite sequencing	
hoxd9lacBS F	AAG GGG GAT GTG TTG TAA GG
hoxd9lacBS R	TCA CAA CAA CCC CCA ATT TA
d11BS F1	GGT TTG TAT TTT TGT TTT TGG TGT TA
d11BS R1	ATA TAA AAT TCC CAC CCC CAC
4C	
lacZD2 F	GTC GTT TGC CGT CTG AAT TT
lacZD2 R	GAG GGG ACG ACG ACA GTA TC

HoxD primers for RT-qPCR were described in ref. 1.

1. Montavon T, Le Garrec J-F, Kerszberg M, Duboule D (2008) Modeling Hox gene regulation in digits: Reverse collinearity and the molecular origin of thumbness. *Genes Dev* 22(3): 346–359.