Insulin-like growth factor 2 (IGF2) is the major fetal growth hormone in mammals. We identify zinc finger protein 568 (ZFP568), a member of the rapidly evolving Kruppel-associated box–zinc finger protein (KRAB-ZFP) family linked primarily to silencing of endogenous retroelements, as a direct repressor of a placental-specific Igf2 transcript (designated Igf2-P0) in mice. Loss of Zfp568, which causes gastrulation failure, or mutation of the ZFP568-binding site at the Igf2-P0 promoter causes inappropriate Igf2-P0 activation. Deletion of Igf2 can completely rescue Zfp568 gastrulation phenotypes through late gestation. Our data highlight the exquisite selectivity with which members of the KRAB-ZFP family repress their targets and identify an additional layer of transcriptional control of a key growth factor regulating fetal and placental development.

Zfp568 is an essential factor that regulates convergent extension during gastrulation (9–11). To determine genes that may be regulated by Zfp568, we crossed mice harboring a floxed Zfp568 allele fused to a C-terminal green fluorescent protein (GFP) tag (Zfp568-GFPFL/Zfp568WT)) (fig. S1A) with Rosa26-CreERT2 mice and derived ESC and trophoblast stem cell (TSC) lines from blastocysts (fig. S1B) (12). Treatment of cells with 4-hydroxytamoxifen (4-OHT), which activates the CreERT recombinase, resulted in deletion of Zfp568 and loss of ZFP568-GFP protein (fig. S1, C and D). Only two genes were significantly affected by acute Zfp568 deletion in ESCs and TSCs: Zfp568 itself and Igf2-P0, which was activated an average of eightfold (Fig. 1A; figs. S1C and S2A). A Kruppel-associated box–zinc finger protein (KRAB-ZFP), as the direct repressor of Igf2-P0 in early development.

To determine whether ZFP568 directly represses Igf2-P0, we performed chromatin immunoprecipitation sequencing (ChIP-seq) with antibodies against GFP on Zfp568-GFPFL/Zfp568WT) ESCs and TSCs. We found 137 and 86 high-confidence ZFP568-binding peaks in ESCs and TSCs, respectively (table S2), which include a peak upstream of the Igf2-P0 promoter (Fig. 2A). Motif analysis revealed a highly significant binding motif of 21 to 24 base pairs (bp) in both ESCs and TSCs, with a large fraction of the most highly enriched peaks containing the target motif, including Igf2-P0 (fig. S4A). The most-enriched 29 peaks were also shared by both ESCs and TSCs (fig. S4B). ZFP568 binding was only weakly correlated with KRAB-associated protein-1 (KAPI) and SET domain bifurcated 1 (SETDB1) binding and was not substantially marked by trimethylated histone H3
lysine 9 (H3K9me3) (fig. S4B). In contrast, the Igf2-p0 promoter contained a strong H3K9me3 signal that was completely lost upon Zfp568 deletion (fig. 2A). Furthermore, deletion of the KRAB-ZFP corepressors SetDb1 and Trim28/KAPI resulted in derepression of Igf2-p0 and loss of H3K9me3 (fig. S4, C and D). Additionally, there was a corresponding loss of DNA methylation at an Igf2-p0 CpG island designated DMR0, but not at DMR1 or DMR2, upon loss of Zfp568 (fig. S5, A to C).

To verify the binding site of Zfp568, we performed luciferase reporter assays using minimal putative Zfp568-binding sites and the full Igf2-p0 promoter (fig. S6, A and B). Expression of Zfp568 significantly repressed reporters containing the minimal Igf2-p0 binding site or with the consensus binding site (fig. 2B). Likewise, Zfp568 repressed the full Igf2-p0 reporter (fig. S6B). Point mutation of the KRAB domain that mimics the previously described Chato mutation (11) prevented repression of the reporters (fig. S6C). Furthermore, triplet scrambling of the Igf2-p0 binding site or deletion of paired zinc fingers had a significant impact on transcriptional repression (fig. S6, D and E). Because the binding site contains a CpG dinucleotide, we speculated that Zfp568 binding to its target may be methylation sensitive (fig. S6B). However, bisulfite sequencing in ESCs demonstrated that these CpG sites are not methylated (fig. S6F). Furthermore, we expressed in Escherichia coli and purified the 11–zinc-finger array of Zfp568 and measured its binding affinity to a 26-nt double-stranded oligonucleotide encompassing the Igf2-p0 binding site using fluorescence polarization (33). We found that the Zfp568 zinc fingers bound specifically to the Igf2-p0 binding site with a Ka of ~8 nM (fig. 2C). Methylation of the CpG site caused a modest reduction (about threefold) in binding affinity, whereas methylation of the two CpA sites had a more significant impact on transcriptional repression (fig. S6, D and E). Because the binding site–mutant ESCs with 4-OHT (to delete Zfp568) did not further increase the expression of Igf2-p0 (fig. 3B), indicating that Zfp568 repression activity is binding site dependent. ChIP-seq and DNA methylation analysis revealed loss of H3K9me3 in Zfp568 binding site–mutant ESCs and loss of DNA methylation at DMR0 (fig. 3C and fig. S7D). These data demonstrate that Zfp568 maintains a heterochromatin state at the Igf2-p0 promoter by direct interaction with its binding site.

Consistent with our ESC and TSC data, we also found Igf2-p0 levels to be increased in Zfp568 KO embryos (fig. 4A and fig. S8, A to D), which fail to complete gastrulation and which display phenotypes similar to the previously described Chato mutants, including convergent-extension failure and a yolk sac membrane–ruffling phenotype (that could be easily visualized and scored using autofluorescence microscopy) and arrest by embryonic day 9 (9) (fig. S8, E and F) (9, 10). Zfp568 embryonic tissues also displayed reduced methylation specifically at Igf2 DMR0 (fig. S8, G and H). We thus reasoned that overexpression of Igf2 may contribute to the early embryonic lethality of Zfp568 mutants. To test this idea, we crossed Zfp568 knockout (KO) mice with Igf2 KO mice (14) (containing a deletion in the final exon, thus disrupting all Igf2 transcript variants) to determine if deletion of Igf2 could rescue the lethal phenotype. Loss of paternal Igf2 expression was sufficient to completely restore viability of Zfp568 KO/Igf2 KO embryos through mid-gestation, as Zfp568 KO/Igf2 KO, Igf2 p0/* embryos and fetuses were found at near Mendelian ratios and indistinguishable from Zfp568 WT littersmates at E12.5 to 18.5 (fig. 4B).
and Igf2-P0 KO mice. We thank K. Pfeifer and C. Gebert for Igf2 KO mice. We thank S. Coon, J. Iben, and T. Li for Next Generation Sequencing (NGS) support. This work was supported by NIH grants 1ZIAHD008933 (T.S.M.) and GM049245-23 (A.P. and X.C.), and Future Scientists Exchange Program of the China Scholarship Council (CSC) (Y.W.). NGS data have been deposited in the Gene Expression Omnibus (GEO) database (GSE84832). Zfp568-GFPFL mice are available from C.-K.J.S. under a material transfer agreement with the Academia Sinica, Taipei, Taiwan, Republic of China.

SUPPLEMENTARY MATERIALS
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Materials and Methods
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A placental growth factor is silenced in mouse embryos by the zinc finger protein ZFP568

Peng Yang, Yixuan Wang, Don Hoang, Matthew Tinkham, Anamika Patel, Ming-An Sun, Gernot Wolf, Mairead Baker, Huan-Chieh Chien, Kuan-Yu Nick Lai, Xiaodong Cheng, Che-Kun James Shen and Todd S. Macfarlan

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Embryo viability relies on placental repression
The insulin-like growth factor (IGF) signaling pathway controls maternal supply of and fetal demands for nutrients. Yang et al. report that the essential KRAB-zinc finger protein ZFP568 specifically and directly represses a placental-specific IGF2 transcript during early mouse development. Elimination of ZFP568 in vivo leads to the inappropriate early activation of transcription, which results in embryonic death owing to overexpression of IGF2 peptide. Thus, the specific, targeted preimplantation repression of a promoter is essential for viability. Science, this issue p. 757