Supporting Information

Younis et al. 10.1073/pnas.1719278115

SI Text

Transcriptome Analysis Using Heart. In cardiac muscle tissues, the expression of 123 and 182 genes was found to be significantly changed in *Zbed6*$^{-/-}$ and *Igf2*$^{pA/mG}$ females, respectively, with 70 DE genes in common (Fig. S3*A*). The GO analysis of DE genes in *Zbed6*$^{-/-}$ and *Igf2*$^{pA/mG}$ showed a significant enrichment in genes related to the extracellular space (Fig. S3*B*). The expression profile of the cardiac muscle of males revealed fewer DE genes than in females, with only five DE genes in common between *Zbed6*$^{-/-}$ and *Igf2*$^{pA/mG}$ males (Fig. S3*C*). Moreover, the change in expression of *Igf2, Enho*, and *Tmem8* genes among genotypes in cardiac muscle were strikingly similar to the pattern observed in skeletal muscle (Fig. S3*D*). Furthermore, the paired immunoglobin-like type 2 receptor-associating neural protein (*Pianp*) gene was among the most significantly upregulated genes in *Zbed6*$^{-/-}$ cardiac tissues (Fig. S3*E*) This gene is predicted as a direct target of ZBED6, with a ZBED6 binding site at its promoter, as observed for the *Enho* promoter (Fig. S4*B*).

Transcriptome Analysis of Kidney Tissue. This revealed significant changes in the expression of 165 genes in *Zbed6*$^{-/-}$, but only 36 genes in *Igf2*$^{pA/mG}$ in male mice (Fig. S3*F*). The GO analysis of the DE genes in kidney of male *Zbed6*$^{-/-}$ mice showed a significant enrichment of genes related to extracellular exosome, mitochondrial membrane, and oxidative phosphorylation (Fig. S3*G*). Moreover, transcriptome analysis of kidney of female mice revealed ~250 DE genes in each of the *Zbed6*$^{-/-}$ and *Igf2*$^{pA/mG}$ groups, with 159 DE genes (~60%) in common (Fig. S3*H*). The GO analysis of the DE genes in *Zbed6*$^{-/-}$ kidney revealed a significant enrichment of genes related to extracellular space and transporter activities (Fig. S3 *I* and J). *Igf2* was markedly upregulated in the kidney of both *Zbed6*$^{-/-}$ and *Igf2*$^{pA/mG}$ males and females (Fig. S3*K*). Furthermore, *Enho* and *Pianp* were upregulated only in kidney of *Zbed6*$^{-/-}$ mice; there were no changes in *Tmem8* expression in any genotype (Fig. S3*K*).

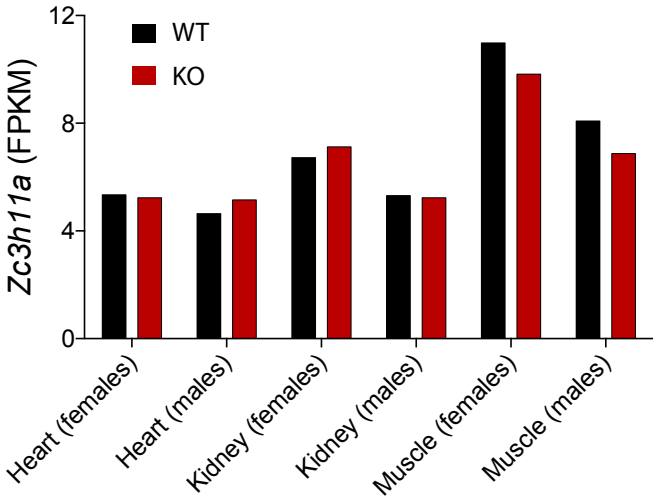

Fig. S1. No altered expression of the *Zc3h11a* gene in *Zbed6*$^{-/-}$ mice. The RNAseq data did not reveal any altered expression of the "host" gene *Zc3h11a* after *Zbed6* inactivation in any of the tissues studied.

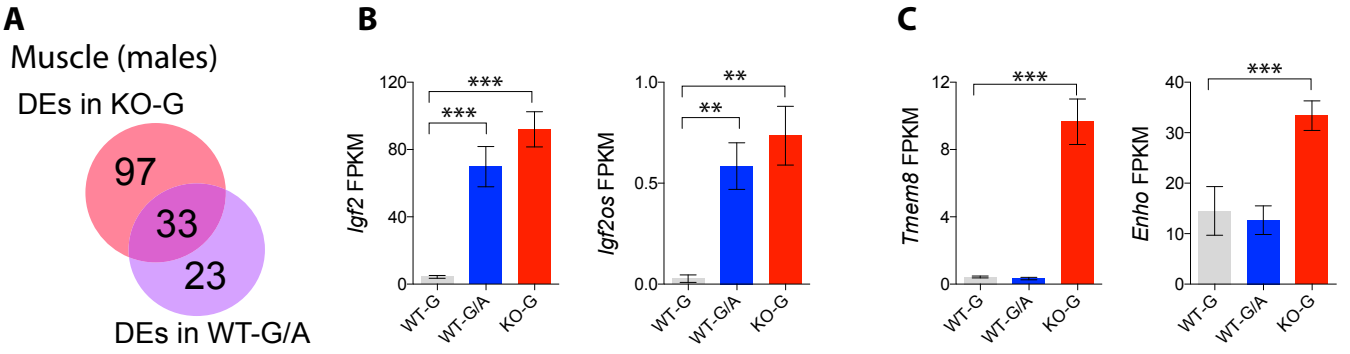

Fig. S2. Transcriptome analysis of the tibialis anterior muscle in *Igf2*$^{pA/mG}$ and *Zbed6*$^{-/-}$ male mice. (*A*) Overlap between DE genes in skeletal muscle of KO-G and WT-G/A male mice. (*B*) The expression of *Igf2* and *Igf2os*. (*C*) Expression analysis of *Tmem8* and *Enho* genes. Results are means ± SEM **P* < 0.05; ***P* < 0.01; ****P* < 0.001, after Benjamini–Hochberg correction for multiple testing.

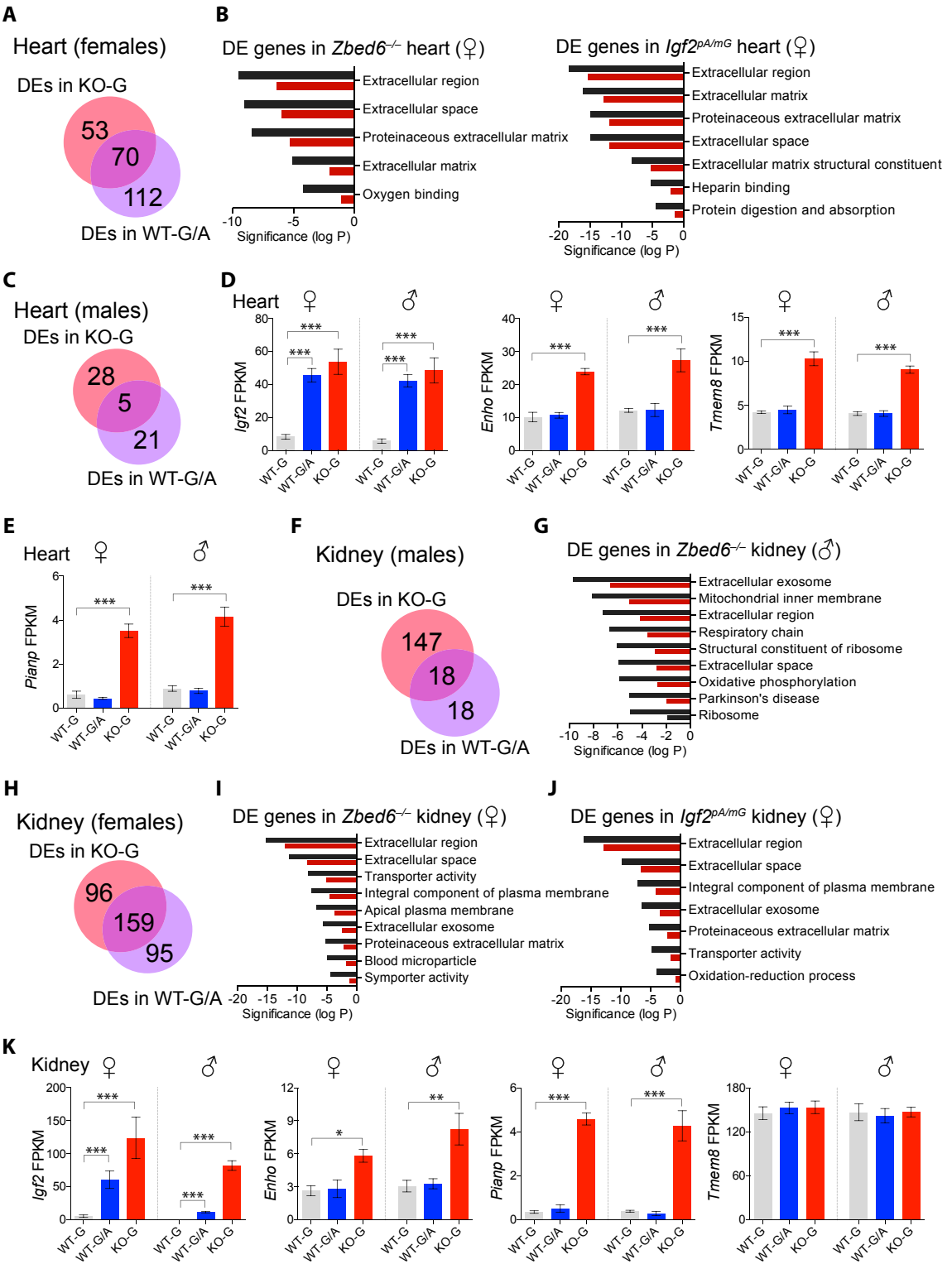

Fig. S3. Transcriptome analysis of heart and kidney tissue in *Igf2*$^{pA/mG}$ and *Zbed6*$^{-/-}$ mice. Overlap between DE genes in cardiac muscle of KO-G and WT-G/A female mice (*A*) and gene ontology (GO) analysis of DE genes in this comparison (*B*). (*C*) Overlap between DE genes in cardiac muscle of KO-G and WT-G/A male mice. (*D* and *E*) Expression of *Igf2*, *Enho*, *Tmem8*, and *Pianp* transcripts as measured by FPKM values in cardiac muscle of male and female mice. (*F*) Overlap between DE genes in kidney of KO-G and WT-G/A male mice and GO analysis of DE genes in kidney tissue of male KO-G mice. (*G*) GO analysis of DE genes in kidney of KO-G male mice. (*H*) Overlap between DE genes in kidney of KO-G and WT-G/A female mice. (*I* and *J*) GO analysis of DE genes in kidney of KO-G (*I*) and WT-G/A (*J*) female mice. (*K*) Expression of *Igf2*, *Enho*, *Tmem8*, and *Pianp* in kidney tissue of male and female mice. Results are means ± SEM **P* < 0.05; ***P* < 0.01; ****P* < 0.001, after Benjamini–Hochberg correction for multiple testing. (*B*, *G*, *I*, and *J*) Black bars represent *P* values, and red bars represent false discovery rate (FDR).

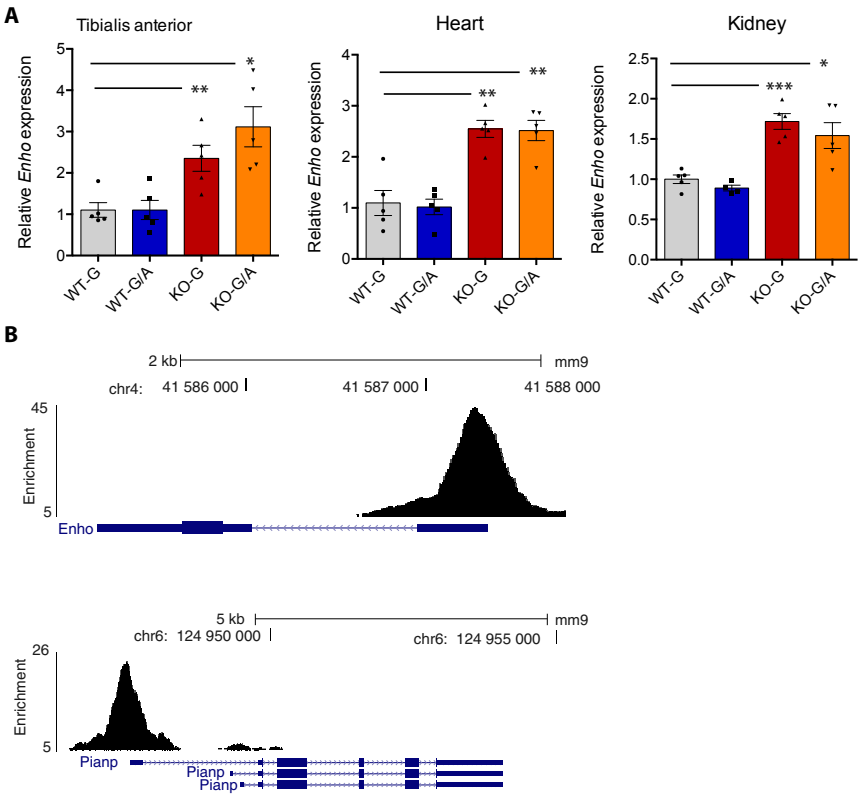

Fig. S4. qPCR analysis and previously published ZBED6 ChIP-seq data. (*A*) qPCR analysis of *Enho* expression in adult tissues (muscle, heart, and kidney) from the four *Zbed6/Igf2* genotypes; the relative expression compared with the WT-G group is indicated. Results are means ± SEM $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; Student's *t* test. (*B*) ChIP-seq data from Jiang et al. (1) showing the ZBED6 peak over the *Enho* and *Pianp* promoters in mouse myoblast C2C12 cells.

1. Jiang L, et al. (2014) ZBED6 modulates the transcription of myogenic genes in mouse myoblast cells. *PLoS One* 9:e94187.

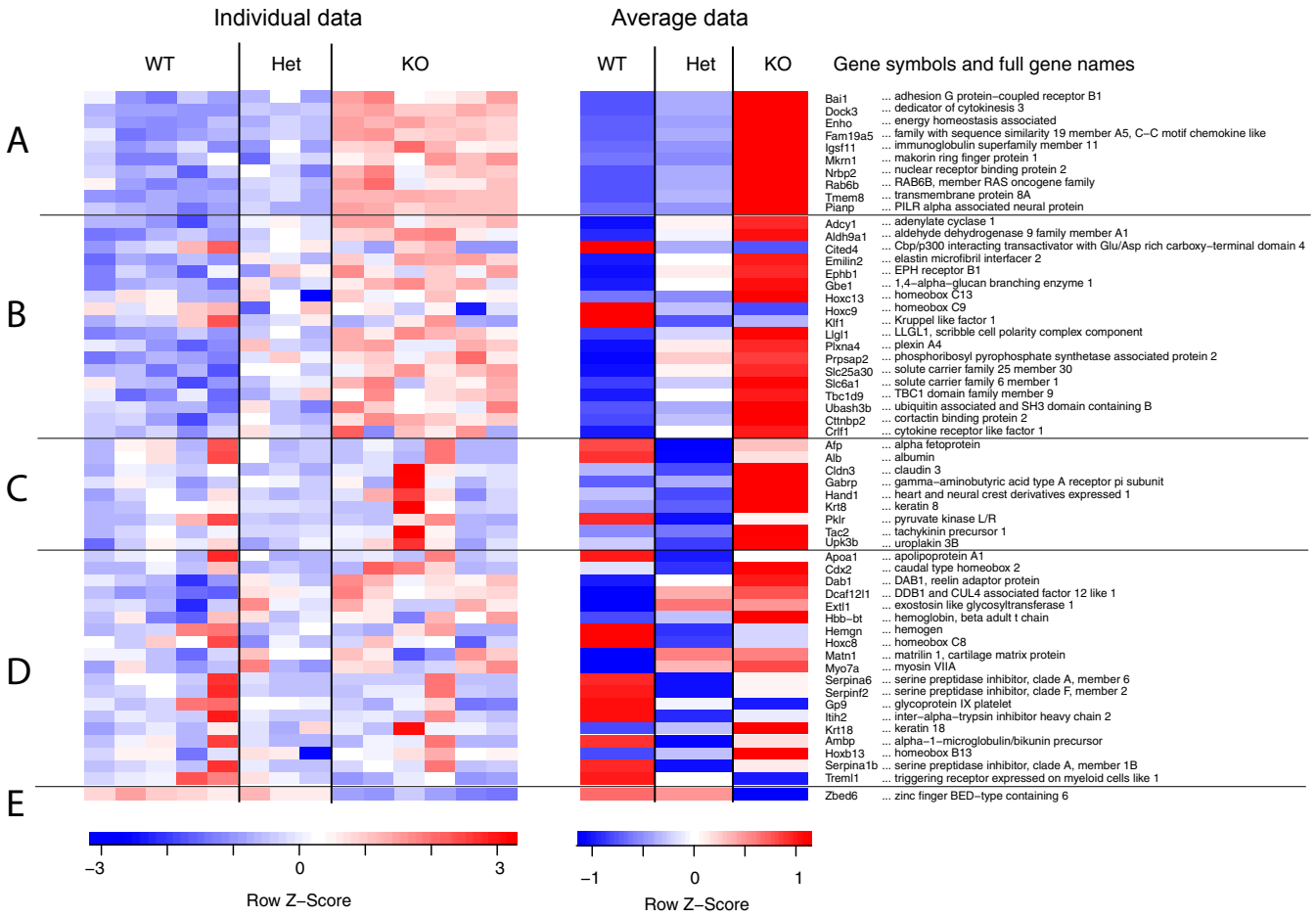

Fig. S5. DE between *Zbed6*$^{+/+}$ (WT) and *Zbed6*$^{-/-}$ (KO) mice in fetal muscle tissue based on RNA-seq data including data on *Zbed6*$^{+/-}$ heterozygotes (Hets). (*Left*) Individual data. (*Right*) Average data for each genotype class. (*A*) Genes associated with ZBED6 ChIP-seq peaks showing significant DE between Hets and KOs. (*B*) Genes associated with ZBED6 ChIP-seq peaks but with no significant DE between Hets and KOs. (*C*) Genes not associated with any ZBED6 ChIP-seq peaks but showing significant DE between Hets and KOs. (*D*) Genes not associated with ZBED6 ChIP-seq peaks and no significant DE between Hets and KOs. (*E*) Expression of *Zbed6* in the three different genotypes.

Dataset S1. Gene ontology analysis of differentially expressed genes in *Zbed6* knock-out (KO) or *Igf2* knock-in (KI) mice skeletal muscle (tibialis anterior), heart, and kidney tissues

Dataset S1

Dataset S2. Lists of all differentially expressed genes in *Zbed6* knock-out (KO) or *Igf2* knock-in (KI) mice in skeletal muscle (tibialis anterior), heart, and kidney tissues

Dataset S2