Enhancer selection dictates gene expression responses in remote organs during tissue regeneration

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Acute trauma stimulates local repair mechanisms but can also impact structures distant from the injury, for example through the activity of circulating factors. To study the responses of remote tissues during tissue regeneration, we profiled transcriptomes of zebrafish brains after experimental cardiac damage. We found that the transcription factor gene cebpd was upregulated remotely in brain ependymal cells as well as kidney tubular cells, in addition to its local induction in epicardial cells. cebpd mutations altered both local and distant cardiac injury responses, altering the cycling of epicardial cells as well as exchange between distant fluid compartments. Genome-wide profiling and transgenesis identified a hormone-responsive enhancer near cebpd that exists in a permissive state, enabling rapid gene expression in heart, brain and kidney after cardiac injury. Deletion of this sequence selectively abolished cebpd induction in remote tissues and disrupted fluid regulation after injury, without affecting its local cardiac expression response. Our findings suggest a model to broaden gene function during regeneration in which enhancer regulatory elements define short- and long-range expression responses to injury.

Results

Local and remote cebpd induction during heart regeneration. Zebrafish can regenerate cardiac tissue throughout life, based on injury-stimulated cardiomyocyte (CM) proliferation15. To assess long-range responses to cardiac regeneration in zebrafish, we employed a genetic ablation system to destroy ~60% of CMs upon tamoxifen exposure (Fig. 1a,b)16. We then collected whole brain transcriptomes at 7 days post tamoxifen incubation (d.p.i.), at which point cardiac tissues had activated regenerative programs and sequenced transcriptomes. In whole-animal management of tissue regeneration in which injury-responsive enhancer elements expand the range and functions of key regulatory genes.

Organisms are connected circuits of organ systems, and injuries to one organ can, in some cases, alter the whole body physiology and the functions of distant organs. For example, heart failure causes the elevation of renal venous pressure, fostering the development of tubular hypertrophy, renal fibrogenesis and renal venous congestion, further contributing to renal failure6. Conversely, electrolyte disruption, water retention and neurohormonal dysregulation caused by acute and chronic kidney injuries can cause cardiac dysfunction in patients35. Recent studies have identified mechanisms of crosstalk by which tissues distant from an injury can impact regeneration and functional recovery. For example, in response to leg muscle injury, satellite cells and fibro-adipogenic progenitors in the contralateral leg switch from quiescence to an alert state, improving the capacity to respond to a second injury6,19. Studies involving a range of species, tissues and injury contexts have begun to illuminate circulating factors that modulate regeneration or regeneration-associated physiology21-24. Thus, although the most obvious signature of regeneration is the growth and remodelling of local tissues, injury also influences a multidimensional communication network involving circulatory systems and distant tissues.

Here we explore long-range tissue interactions during regeneration by assessing the brain transcriptomes of zebrafish subjected to severe cardiac injury. Our findings suggest a concept in whole-animal management of tissue regeneration in which injury-responsive enhancer elements expand the range and functions of key regulatory genes.

Results

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potential heterogeneity in distant tissue responses and because sampling does not capture expression changes in rare cell populations. Among differentially expressed transcription factor genes, CCAAT enhancer binding protein delta (cebpd) showed the highest increase in RNA levels (Fig. 1e). Cebpd is a basic-leucine zipper transcription factor encoded by an intronless gene. The rodent homologue C/EBPδ is expressed in various central nervous system cells and has been implicated in biological events such as memory formation, neurite outgrowth, inflammation and energy metabolism. HOMER analysis revealed enrichment of C/EBP binding motifs at the promoter regions of genes showing higher brain RNA levels during heart regeneration, suggesting transcriptional
activation by Cebpδ (Extended Data Fig. 1b). By in situ hybridization (ISH), cebpδ showed weak, if any, detectable expression in brains of uninjured zebrafish, but was notably induced in the ependymal tissue lining the ventricles of the telencephalon, optic tectum, hypothalamus and rhombencephalon during heart regeneration. We noted little or no expression in brain parenchymal compartments (Fig. 1h). Additional genes displayed similar expression features (Fig. 1i,j), revealing a dynamic brain program instigated during heart regeneration, with ependymal layer cells as a prominent reactive population.

C/EBP transcription factors have been implicated in murine cardiac injury responses. Zebrafish cardiac ventricles typically displayed low cebpδ expression throughout muscle in the absence of injury, with variability among animals. We also observed occasional expression in epicardial cells that envelop the chamber. Upon induced CM ablation, cebpδ expression was clearly and consistently detected in the epicardium and associated cells on the cardiac periphery at 7 d.p.i., revealing a local cebpδ expression response (Fig. 1k,l). Together, these findings implicate cebpδ in both local and distant responses to cardiac regeneration.

**cebpδ expression is a selective response to injury.** Local and distal induction of cebpδ expression could conceivably be responsive to one or more components of heart regeneration. To decouple cardioinduction of distant responses to cardiac regeneration.

To determine whether distinct cardiac injuries other than genetic ablation induce cebpδ in remote tissues, we partially resected ventricular apices and assessed cebpδ expression by ISH (Fig. 2d). Apical resection, like induced CM ablation, activates local regenerative programs; however, it differs in that it does not disrupt both chambers or elicit signs of heart failure like oedema and reduced endurance. Following resection, cebpδ was induced in CMs at 1 day post resection/amputation (d.p.a.), diminished in CMs, but detectable in the epicardial layer by 3 d.p.a., and consistently expressed in wound epicardial cells at 7 d.p.a. (Fig. 2e). By contrast, we did not detect induction of cebpδ in brain after cardiac resection injuries at any timepoint (Fig. 2e), suggesting that brain cebpδ activation is a selective response to cardiac damage that is severe enough to elicit heart failure.

Next, we analysed additional organs for cebpδ expression after CM ablation. We noticed weak cebpδ expression by ISH in renal marrow, glomeruli and tubules of uninjured animals, and that expression in tubules increased prominently after cardiac injury (Fig. 2f,g). We did not observe a comparable extent of cebpδ induction in several other organs or tissues we examined (Extended Data Fig. 2). Brain ependymal and renal tubular cells are in contact with cerebrospinal fluid (CSF) or tubular compartments, and a defining symptom of heart failure is venous congestion and oedema, suggesting a possible relationship of cebpδ induction with fluid regulation. To disrupt fluid homeostasis, we first induced acute kidney injury by intraperitoneal injection of gentamicin (Fig. 2h). cebpδ was induced in the brain ventricular lining by 3 days post injection without detectable renal induction (Fig. 2i). Next, we performed full spinal cord transections, mechanically disrupting CSF dynamics (Fig. 2k). At one week post spinal cord injury (w.p.i.), cebpδ was prominently induced in ependymal cells surrounding the central canal. Notably, cebpδ

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**Fig. 1 | cebpδ is induced in local and remote tissues during heart regeneration.** a. Schematic of profiling whole brain transcriptomes after CM ablation.

b. Heatmap of genes with changes in the brain during heart regeneration. P < 0.05, FC > 1.2.

c. RNA-seq browser tracks of msrb2 and lpin1 showing increased brain expression (Fig. 2c), indicating that increases in brain cebpδ expression are predominantly a response to cardiac injury rather than CM proliferation.

d. Volcano plot showing differential gene expression in the brain during heart regeneration. Pink dots indicate highlighted genes and blue dots with decreased RNA levels (P < 0.05, FC < -1.2). Grey dots indicate genes with no significant changes. Orange dots indicate genes with increased RNA levels (P < 0.05, FC > 1.2).

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**Fig. 2 | cebpδ expression features with different stimuli and in various tissues.** a. Schematic of induced cardiogenesis and tissue collection for cebpδ ISH.

b. ISH on heart sections indicating cebpδ induction in epicardium during cardiogenesis. Arrows indicate violet ISH signals. n = 5 animals for both groups.

c. ISH on brain sections indicating little or no cebpδ induction in brain during induced cardiogenesis. n = 10 animals for both groups.

d. Schematic of cardiac injury and tissue collection for cebpδ ISH.

e. Top: ISH on resected hearts demonstrating cebpδ induction in CMs at 1 d.p.a. and in epicardium at 3 and 7 d.p.a. Bottom: ISH on brain sections indicating little or no cebpδ induction in brain after ventricular resection. For heart ISH, n = 5 animals for uninjured control, 1, 3 and 7 d.p.a. For brain ISH, n = 10 animals for uninjured, n = 15 animals for 1, 3 and 7 d.p.a.

f. Schematic of CM ablation and head kidney collection for cebpδ ISH.

g. ISH of cebpδ on kidney sections after CM ablation. cebpδ is induced in kidney tubules after CM ablation. n = 8 CreER− and 9 CreER+ animals.

h. Schematic of acute kidney injury and collection of brain for cebpδ ISH and whole kidney for qPCR. ISH on brain sections indicating cebpδ induction in optic tectum ependyma after kidney injury. n = 10 animals for uninjured and for 3 d.p.i. qPCR indicating no cebpδ induction in kidney after kidney injury. n = 8 animals for both uninjured and 3 d.p.i. Data are presented as mean ± s.e.m.; an unpaired two-tailed t-test was used to calculate P values.

i. Schematic of spinal cord transection and tissue collection for cebpδ ISH. Diagrams illustrate the relative position of brain and spinal cord cross-sections (in I) to the injury site.

j. ISH on tissue cross-sections, indicating cebpδ induction in ependymal cells (arrows) in the injured spinal cord and the brain. n = 15 animals for uninjured and n = 10 animals for 1 w.p.i. Dashed lines in I outline the central canal. Scale bars, 100 μm (b,c,e,g,l).
expression was not localized exclusively to the site of transection, but induced uniformly along the anteroposterior axis of the spinal cord and rostrally into the brain ependyma (Fig. 2l). These findings, in addition to results we describe later, suggest a mechanism in which remote injury-induced brain cebpδ expression is influenced at least in part by alterations in fluid control compartments.
**cebpd** mutations alter local and distant injury responses. To determine whether **cebpd** has required functions during heart regeneration, we deleted the majority of the **cebpd** coding sequence using clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 methods, removing all detectable **cebpd** expression (Fig. 3a–c). **cebpd** homozygous mutants survived normally to adulthood and were fertile.
To assess the effects of *cebpd* mutations on heart regeneration, we first performed ventricular resection and examined the cardiac anatomy 30 days later. We found no gross differences in muscle- 

To investigate the effects of *cebpd* mutations on distant responses, we first assessed the exercise capacity and stress sensitivity of *cebpd* mutants in the context of CM ablation-induced heart failure, finding no differences (Figs. 3k–m). We next adapted published techniques to test fluid regulation in *cebpd* mutants, labelling the interstitial fluid by fluorescent dye injection (Fig. 3n). *cebpd* mutant fish displayed a capacity similar to that of wild-type clutchesmate to transfer fluid solutes to vasculature in the absence of injury (Fig. 3o, p and Extended Data Fig. 3e). Notably, whereas severe cardiac injury did not change the dynamics of dye transfer in wild-type zebrafish, *cebpd* mutants displayed an ~46% reduction in transfer versus when uninjured (Fig. 3o, p and Extended Data Fig. 3e). Transscriptome sequencing of *cebpd* tissues revealed decreased RNA levels of the aquaporins *aqp7* and *aqp10a* in *cebpd* kidneys during heart regeneration, consistent with altered renal water regulation (Extended Data Fig. 4c, d). GO analysis of differentially expressed genes indicated enrichment of transmembrane transporter activities in brain and kidney, including the zebrafish orthologue of the described Cebpd-regulated renal lactate transporter (Extended Data Fig. 4e–i and Supplementary Tables 2 and 3). Altogether, our experiments indicate that Cebpd is involved in a local response to cardiac injury (epicardial proliferation) as well as a remote response (intercompartmental fluid exchange).

Regulatory sequences for remote gene expression responses. To understand how *cebpd* levels are controlled during heart regeneration, we searched for *cis*-regulatory elements. First, we established transgenic reporter fish using large bacterial artificial chromosome (BAC) sequences surrounding *cebpd* coding sequences, replacing the *cebpd* start codon with an EGFP-polyA cassette. One BAC line (108cebpd12:EGFP) included 108-kb upstream and 12-kb downstream sequences with respect to the *cebpd* start codon (Fig. 4a), whereas a second line (35cebpd84:EGFP) included 35-kb upstream and 84-kb downstream sequences (Fig. 4a). The two lines displayed differences in enhanced green fluorescent protein (EGFP) signals that persisted from larval to adult stages, most distinctly visualized in caudal fin and head structures (Extended Data Fig. 5a,b). Following induced CM ablation, 35cebpd84:EGFP fish sharply increased reporter gene expression locally in cardiac tissue and distally in the ventricular lining of the brain (Fig. 4b, c), mimicking endogenous *cebpd* patterns. By contrast, we did not detect EGFP reporter gene expression in hearts or brains of induced 108cebpd12:EGFP fish (Fig. 4b, c). We infer from these findings...
that an ~72-kb region downstream of the cebpδ start codon contains sequences key for directing cebpδ expression during cardiac regeneration.

We hypothesized that one or more DNA regulatory elements responsible for distant injury-activated expression of cebpδ in brain exist within the identified 72-kb sequences. To locate these,
we used the assay for transposase-accessible chromatin using sequencing (ATAC-seq) to assess samples of whole brain open chromatin sequences from uninjured fish or those subjected to CM ablation (Fig. 4d). From this assay, we identified 1,386 regions with increased chromatin accessibility and 1,071 with reduced accessibility genome-wide ($P < 0.05$; Supplementary Table 4).
Transcriptomes and chromatin accessibility of whole kidney marrow (WKM) revealed a largely distinct set of induced genes and accessible regions, indicating tissue specificity (Extended Data Fig. 6a–f, Supplementary Tables 5–8). Bias-free transcription factor footprint enrichment test (BIFET) sequencing analysis revealed over-representation of predicted binding sites for C/EBP family members (Cebp and Cebpd; \( P < 0.05 \)) in sequences within brain chromatin that increase accessibility during heart regeneration (Fig. 4f and Supplementary Table 9), suggesting that Cebpd has preferential access in this context. Other top enriched motifs were for factors involved in brain development and function (POU5F1, FOXC1, POU3F2, ZNF24), histone modifications, DNA methylation and DNA–DNA interactions, we analysed recently published genome-wide datasets for enhancers, including H3K27Ac enrichment (Fig. 5a), H3K4me3 is associated with marks indicative of active chromatin that increase accessibility during heart regeneration (Extended Data Fig. 4a and Supplementary Table 10). Of regions with significantly increased accessibility, a portion are linked to genes with increased RNA levels, indicative of potential enhancers. Within the 72-kb cebp downstream region covered only by 35cebpd84, we identified a 1.2-kb sequence located \( \pm 44 \) kb downstream of cebp showing particularly high accessibility in the absence of injury (referred to hereafter as cebp-linked enhancer (CEN)) (Fig. 4g). CEN increased accessibility further after CM ablation. A/B compartmentalization analysis indicated a conversion of cebp and CEN regions from the B compartment to A compartment after heart injuries (Extended Data Fig. 7a), suggesting changes in the chromatin architecture at CEN from a relatively closed (transcriptional inactive) to relatively open (active) environment. Analysis of regions upstream and downstream of cebp genes in multiple species revealed high conservation of CEN in genomes of the teleosts fugu and carp but not in coelacanth, Xenopus, murine and human genomes (Extended Data Fig. 7c). In summary, profiling revealed many candidate enhancers to influence gene expression in brain during heart regeneration, with one of these sequences, CEN, contained within the large, regeneration-responsive region downstream of cebp coding sequences.

Tissue regeneration enhancer elements (TREES) are a class of regulatory elements with the capacity to trigger expression of a gene specifically or preferentially during a regeneration context\(^3\). TREES have been inferred through epigenetic profiles or experimentally validated by transgenesis in many species and regeneration settings\(^7\). To test whether CEN has properties of a TRE, we fused CEN to the minimal promoter cfos and reporter gene EGFP and established stable transgenic lines (Fig. 4h). We then genetically induced CM ablation and collected heart and brain tissues. We found that cardiac injury activated reporter gene expression, both locally in epicardium of injured hearts and remotely in the ependymal lining of brains (Fig. 4i). These results indicate that CEN contains DNA sequences that are sufficient to activate gene expression in local and distant tissues during heart regeneration.

Corticosteroid receptors regulate distant cebp expression. To investigate mechanisms by which CEN controls cebp expression, we analysed recently published genome-wide datasets for histone modifications, DNA methylation and DNA–DNA interactions in many zebrafish tissues\(^4\). In adult brain, kidney, heart and skeletal muscle, CEN is associated with marks indicative of active enhancers, including H3K27Ac enrichment (Fig. 5a), H3K4me3 deficiency (Fig. 5a) and low CpG methylation (Fig. 5b). By contrast, CEN is highly methylated and associated with weak H3K27Ac
signals in testes samples (Fig. 5a,b). Hi-C analysis of genome-wide, three-dimensional chromatin organization indicated strong interactions between CEN and the cebp promoter region in uninjured brain tissue. Three additional chromatin regions adjacent to cebp also showed apparent contact with the cebp promoter and CEN (Fig. 5c). These data suggest that CEN physically associates

\[ \text{CEN}^{+/+} \text{DMSO} \]
\[ \text{CEN}^{-/-} \text{DMSO} \]
\[ \text{CEN}^{+/+} \text{Dex} \]
\[ \text{CEN}^{-/-} \text{Dex} \]
\[ \text{CEN}^{+/+} \text{Spir} \]
\[ \text{CEN}^{-/-} \text{Spir} \]

7 d.p.i., Collect brain, head kidney and heart for ISH

4 d.p.i., Collect brain for ISH

Treat with DMSO/dexamethasone/spironolactone

Hormone receptor agonist treatment, head kidney

Fluorescence intensity (a.u.)

| Fluorescence intensity (a.u.) |
|-----------------------------|
| 0.5                         |
| 0.4                         |
| 0.3                         |
| 0.2                         |
| 0.1                         |
| 0.0                         |

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NATURE CELL BIOLOGY

| VOL 24 | MAY 2022 | 685–696 | www.nature.com/naturecellbiology

694
with cebp mediated expression in tissues.

First we assessed cebp expression in CEN mutant tissues at 7 days after CM ablation. CEN mutations caused no detectable changes in cebp expression in regenerating hearts by ISH, with epicardial expression similar to wild-type clutches (Fig. 6c). This finding was consistent with quantitative polymerase chain reaction (qPCR) analysis, which revealed normal elevation of cebp transcript levels following injury (Fig. 6d). Notably, we detected little or no cebp expression in CEN−/− brain ependymal cells or renal tubular cells, consistent with selective absence of this injury response (Fig. 6e,f). Furthermore, deletion of CEN substantially reduced corticosteroid-induced cebp expression in the brain and kidney (Fig. 6g–i). This finding indicates that CEN contains sequences essential for steroid hormone-induced cebp expression in tissues that also display remote expression responses upon heart injury, and further implicates GRs and MRs in remote regulation of cebp after cardiac injury. Published datasets describing cardiac chromatin during heart regeneration indicate many sequences surrounding cebp enriched with active histone marks or deficient in repressive marks, suggesting additional regulatory features that provide redundancy in epicardial cells. By contrast, CEN was the only prominent region with high accessibility reads above background in the 72 kb downstream of cebp in brain chromatin of animals receiving cardiac injuries (Extended Data Fig. 7b).

Next we tested whether mutations in the CEN-regulated remote circuit caused similar alterations as cebp coding sequence mutations in fluid regulation. We found an ~49% reduction in the transfer of fluorescent dye from intraperitoneal to vasculature compartments in CEN mutant fish after cardiac injuries compared with their uninjured mutant siblings (Fig. 6j−l and Extended Data Fig. 9), indicating that CEN-mediated cebp induction is required to restore fluid dynamics perturbed by severe cardiac injury.

**Discussion**

Here we have provided a case example of a gene, cebp, that is involved in tissue repair proximate to an injury event, as well as in physiological sequelae that can impact remote tissues. We show by profiling and molecular genetics in zebrafish that the framework of enhancer regulatory elements surrounding a gene makes possible this division of labour (Extended Data Fig. 10). We postulate that the available binding proteins and local chromatin environment distinguish expression responses in various tissues near and far from an injury event, through selection from the menu of TREs. The TREAT we describe, CEN, can detect hormone signals and instruct gene expression away from the injury in brain and kidney cells. We refer to CEN and other such regulatory sequences as ‘remote-TREs’ (r-TREs), as they represent a subclass with sentinel abilities to detect distant injuries. The candidate r-TREs reported here were inferred from bulk tissue chromatin profiling, which probably masks the identification of r-TREs. Assessment of purified cell populations or use of single-cell-based profiling technology could resolve the heterogeneity of responses in complex tissues and increase the success of identifying r-TREs in poorly represented cell populations. Moreover, although dynamism of chromatin accessibility has identified enhancers responding to local injuries in multiple studies, DNA sequences in an already open state like CEN can conceivably function as r-TREs, thus increasing the importance of in vivo enhancer validation assays.

Broadly, our study highlights gene regulation and physiological responses at the level of whole-animal biology during tissue regeneration. We show evidence that distant cebp induction during heart regeneration is regulated by signalling through GR and/or MR. Functions of corticosteroids and related hormones have been examined in multiple species and regeneration contexts; these factors are stable in circulation and can impinge on a variety of physiological targets that can impact regeneration, including pain, behaviour, fluid regulation, temperature regulation and the recruitment of inflammatory and immune cells. These and other distant responses must integrate with morphogenetic responses of cells at the injury site in the cinematography of regeneration. Understanding control of the enhancer landscape in cells throughout a regenerating organism can reveal systems-level gene regulation that deepens our knowledge of how and why regeneration occurs.

**Online content**

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at [https://doi.org/10.1038/s41556-022-00906-y](https://doi.org/10.1038/s41556-022-00906-y).

Received: 7 September 2021; Accepted: 23 March 2022; Published online: 5 May 2022
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Methods

Zebrafish. Procedures involving the use of animals under ethical guidelines were approved by the Institutional Animal Care and Use Committee at Duke University (protocol A005-21-01). The wild-type and transgenic fish used in this study are of the outbred Blue zebrafish strain. Embryos were raised in 28°C egg water until 5 d.p.f. and then transferred to the aquarium system. Adult fish of both sexes (4–12 months old) were used in this study unless specified in the text. All animal models used here are summarized in Supplementary Table 12a.

For CM ablation, Z-CAT fish (Tg(cmlc2:CreER; bactin2-loxp-loxP-stop-loxP)−) were treated with 5 μM tamoxifen for 24 h (d.p.i.). CreER− fish were employed as controls. Brains and hearts were collected at 14 days post tamoxifen incubation (d.p.i.). For cardiac ventricle resection, adult fish were anaesthetized and placed ventral side up on sponge. A small incision was made with straight iridectomy scissors to open the pericardial sac and 20% of cardiac ventricle was removed using curved iridectomy scissors. Hearts and brain were collected at 17 h (ref. 17). Tamoxifen-treated CreER− fish were used as uninjured controls in all genetic ablation experiments. Hearts, brains and kidneys were collected for histological assays at 7 days post tamoxifen incubation (7 d.p.i.). For cardiac ventricle resection, adult fish were anaesthetized and placed ventral side up on sponge. A single dose of 40 μg of gentamicin (15730060, Invitrogen) was intraperitoneally injected into zebrafish. At 3 days post gentamicin injection, brains and kidneys were collected for histology assays and qPCR.

Generation of cebpδ mutant zebrafish. The cebpδ knockout allele was generated with a pair of guide RNAs (gRNAs) using CRISPR-Cas9 technology. The gRNAs were designed using the CHOPCHOP website15, and the target sequences were 5′-GCCAGCTCATGTCATCGTGCGGCCG-3′ and 5′-CGAAGGCTCTTTGGTGTCCCGGGGGG-3′ (PAM sequences underlined). gRNAs were generated through in vitro transcription using 17 T7 polymerase and co-injected with Cas9 protein (CP01-200, PNA Bio) into one-cell-stage embryos. Animals with cebpδ knockout alleles were screened by PCR with the primers cebpδF: 5′-ACACCTTTCTCTGGGACACGGCC-3′ and cebpδR: 5′-CTTCATGCCGCATCAGTATG-3′. Deletions were confirmed by Sanger sequencing (Eton Biosciences). The allele designation for this line is pds54.

Generation of Tg(BAC108cepdbd12:EGFP) and Tg(BAC35cepdbd8:EGFP) zebrafish. The BAC clone CH211-1772E3 contains 108 kb upstream and 12 kb downstream sequences of the cebpδ start codon (Supplementary Table 12d). The BAC clone CH211-180G16 contains 35 kb upstream and 84 kb downstream sequences of the cebpδ start codon (Supplementary Table 12d). The 108 kb upstream and 35 kb downstream transgenic line was identified by replacing the cebpδ translational start codon with an EGFP-polycistronic cassette using Red/ET recombineering technology (Gene Bridges). 5′-TCTGGCTTTGGGACACGGCC-3′ and cebpδR: 5′-CCATCATCTGTGCTTCAACCTGTTAC-3′. The wild-type allele was identified by PCR with the primers cebpδF: 5′-ACACCTTTCTCTGGGACACGGCC-3′ and cebpδR: 5′-CTTCATGCCGCATCAGTATG-3′. Deletions were confirmed by Sanger sequencing (Eton Biosciences). The allele designation for this line is pds54.

Generation of CEN mutant zebrafish. CEN was deleted using a pair of gRNAs using CRISPR-Cas9 technology, as described above. The target sequences were 5′-AGATTTTAGAGGTGCCTAGAGGCGGGGGG-3′ and 5′-TCTGCTGGGATGTGGAGGCGC-3′ (PAM sequences underlined). To generate knockout animals, gRNAs were co-injected with Cas9 protein (CP01-200, PNA Bio) into one-cell-stage embryos. Animals with CEN alleles were screened by PCR with the primers CENseqF: 5′-CAGGGAGAATAATATTCCAGAGACTGTC-3′ and CENseqR: 5′-CTTATTTAAGAAGGCTCTGCCCTCTG-3′. The wild-type allele was identified by PCR with the primers CENseqF: 5′-GTCATGCTCAGATCACCACCCGGG-3′ and CENseqR: 5′-GTATTTAGGAGGTGCCTAGAGGCGGGGGG-3′. The allele designation for this line is pds54.

Generation of Tg(CEN-cfos:EGFP) fish. The CEN sequence was amplified from zebrasin genomic DNA using the primers 5′-CAGGGGATTAATATTCCAGAGACTGTC-3′ and 5′-CTTATTTAAGAAGGCTCTGCCCTCTG-3′. CEN was subcloned upstream of cfos:EGFP-polycistronic cassette by Gateway Cloning, using Gateway LR Cloning Kit (11790120, Thermo Scientific). Two 1-2 kb flanks of CEN were amplified by the CENfos:EGFP-polycistronic cassette were used to generate transgenic animals. Nine stable transgenic lines were identified by PCR using the primers CENseqF: 5′-GGATCCTGCTCAGATCACCACCCGGG-3′ and CENseqR: 5′-GTGTCAGATGACTCTAGG-3′. The allele designations for the lines used in this study are pd56 and pd57.

Drug treatments. For GR and MR agonist and antagonist treatment, dexamethasone (D1756, Sigma-Aldrich), spironolactone (S3378, Sigma-Aldrich), RU486 (Mifepristone, M8046, Sigma-Aldrich) and eplerenone (E0905, TCI chemicals) were dissolved in dimethyl sulfoxide (DMSO) to make stock solutions. Concentrated stock adult fish were incubated in 5 μM RU486 or 50 μM eplerenone (or both), or DMSO as vehicle control, from 3 days post tamoxifen treatment to 6 days post tamoxifen treatment. Water was changed every 24 h for 4 days. At 4 days post treatment, heart, brain and kidney were collected for histology analysis. Z-CAT fish treated with tamoxifen were incubated in 5 μM RU486 or 50 μM eplerenone (or both), or DMSO as vehicle control, from 3 days post tamoxifen treatment to 6 days post tamoxifen treatment. Water was changed every 24 h. At 7 days post tamoxifen treatment (4 days post drug treatment), the brain was collected for qPCR.

RNA-seq and analysis. Z-CAT fish were treated with tamoxifen to induce CM ablation and the possible effects of CM ablation. We included only male zebrafish in our study and employed tamoxifen-treated (CreER−) siblings as uninjured controls. At 7 days post tamoxifen incubation (d.p.i.), whole brain, whole kidney marrow or whole kidney were collected for RNA sample preparation. Tissues from ten fish were pooled together and sequenced as one biological sample, and two to three biological replicates were sequenced in each experiment. RNA was extracted using Trizol reagent (T9424, Sigma-Aldrich) and treated with DNase1 to degrade genomic DNA. Samples were purified with a Quick-RNA Miniprep kit (R1054, Zymo Research) and submitted to the Duke Center for Genomic and Computational Biology or Beijing Genomics institute (BGI) for library preparation and sequencing using Illumina HiSeq4000 or NovaSeq6000 or DNBSEQ platforms.

RNA-seq reads were trimmed by Trimm Galore (v.0.4.1, with -q 15) and mapped with TopHat (v.2.1.1, with the parameters -b very-sensitive -no-coverage-search and supplying the UCSC danRer10 refSeq annotation) to the zebrafish genome (GRCz10). The mapped reads were filtered by MAQ (no smaller than 20) and counted using HTSeq-counts (v.0.6.0). Bioconductor package DESeq2 (v.1.30.1 or v.1.28.1) was employed to analyse differential expressions (DE), and a two-sided Wald significance test was performed. Gene Ontology enrichment tests were performed to analyze enriched biological processes and molecular functions by clusterProfiler (v.3.16.1 or 3.18.1). The coverage depth tracks were plotted by trackViewer (v.1.25.3), heatmaps were drawn using heatmap (v.2.10.0 and 2.12.0) and heatmaps and scatterplot were created by EnhancedVolcano (v.1.6.0 or 1.8.0). The coverage depths were normalized by depthtool (v.3.1.3) using RPM (reads per kilo base per million mapped reads) for RNA-seq and RPGC (number of reads per bin / scaling factor for 1x average coverage) for ATAC-seq. The enriched motifs in promoter regions of differentially expressed genes were analyzed by Homer (v.1.0.4). All software and algorithms used in this manuscript are summarized in Supplementary Table 12a, with citations to their published descriptions when available.

The RNA-seq results for whole kidney samples from cebpδ mutants and their wild-type siblings were analysed by the STAR-Salmon-Deseq2 pipeline. RNA-seq reads were trimmed by Trim Galore (v.0.6.7) and mapped with STAR (v.2.6.14) to zebrafish genome (GRCz10). The mapped reads were quantified by salmon with transcripts in UCSC danRer10 annotation downloaded from illumina Genome. The transcript counts were summarized to gene level by tximport (v.1.18.0). Bioconductor package DESeq2 (v.1.30.1) was used to analyse differential expressions.

ATAC-seq and analysis. Brains and whole kidney marrows were collected from male Z-CAT fish at 7 days post tamoxifen treatment. Samples from ten individual animals were pooled together for tissue dissociation. Brains were freshly homogenized with a Dounce homogenizer, and whole kidney marrow was isolated by aspiration as previously described16. A total of 100,000 cells/nuclei were used for library preparation. ATAC-seq libraries were prepared as previously described17 and sequenced at Duke Center for Genomic and Computational Biology with the HiSeq4000 platform. ATAC-seq reads were trimmed by Trim Galore (v.0.4.1, with -q 15) and mapped to the zebrafish genome (GRCz10 using bowtie2 (v.2.2.5, with the parameters -vvery-sensitive). The mapped reads were filtered by MAQ (no smaller than 30) by samtools (v.1.5) and duplicated reads were removed by picard (v.1.91). The peaks were called by MACS2 (v.2.1.0, with -shift -100 -extsize 200 -q 0.05 -g 1.5e9) and filtered by irreproducible discovery rate (IDR)7. The Bioconductor package DiffBind (v.2.14.0) was used for differential open region analysis, and a two-sided Wald significance test was performed with the
The Virtual 4C plot was performed as previously described67. For the enriched motif test, the values were adjusted by the Benjamin–Hochberg method. The heat sensitivity test was performed as previously described. Briefly, hearts were collected at 30 d.p.a. and fixed in 4% PFA at 4°C overnight before being frozen and sectioned at 10μm. Sections were stained with the primary anti-ropoxin-T antibody (MS-295-PAB, Thermo Scientific, 1:100) followed by secondary antibody Alexa Fluor 488 (A11032, Thermo Fisher Scientific, 1:250), then imaging using a Leica DM6000 compound microscope with Leica Application Suite X (v.3.4.2) and a ×20 objective lens. The same slides were then used for AFOG staining. All antibodies and chemicals used for histological analysis are summarized in Supplementary Table 12b.

Swim assays. A stepped velocity test was used to assess zebrafish swim capacity, as previously described with minor modifications6. Briefly, zebrafish (no more than 17 per group) were forced to swim against an increasing water current in a 5-litre swim tunnel before surgery to determine their baseline swim capacity. Fish after Cα2l1−/−| MEF2C antibody (MS-295-PAB, Thermo Scientific, 1:100) followed by secondary antibody Alexa Fluor 488 (A11032, Thermo Fisher Scientific, 1:250), then imaging using a Leica DM6000 compound microscope with Leica Application Suite X (v.3.4.2) and a ×20 objective lens. The same slides were then used for AFOG staining as described in ref. 4. All antibodies and chemicals used for histological analysis are summarized in Supplementary Table 12b.

Histology. ISH was performed on cryosections of paraformaldehyde-fixed adult heart, brain, spinal cord and kidney with the aid of an InSituPro robot (Intavis). Hearts and kidneys were dissected at 10μm, brains at 12μm and spinal cords at 16μm. All ISH probes were digoxigenin-labelled RNA probes cloned from adult zebrafish tissues. All primers used for cloning ISH probes are listed in Supplementary Table 12e. Hearts were stained with primary anti-troponin-T antibody (MS-295-PABX, Thermo Fisher Scientific, 1:250) and subsequent catalytic colour reaction with NBT (nitroblue tetrazolium; 11383213001, Sigma-Aldrich) and subsequent alkaline phosphatase antibody (11093274910, Sigma-Aldrich) and subsequent fluorescence intensity using Fiji (v.2.3.0).

Statistical reproducibility. Clutchesmates were randomized into different treatment groups for each experiment. No animal was excluded from the analysis unless the animal died during the procedure. All measurements were acquired from distinct samples and no sample was measured repeatedly. The numbers of animals used for each experiment are indicated in the figure legends. Other experimental or tissue assays were performed with 7–20 animals in each group and replicates are indicated in the figure or figure legends. Statistical values are displayed as mean ± s.d. or mean ± s.e.m., as indicated in the figure legend. Normality tests were performed for all applicable experiments. Statistical differences were calculated using unpaired two-tailed Student’s t-tests when a normality test was passed, or a two-sided Mann–Whitney test otherwise. Fisher’s exact test was employed for assessing muscle regrowth and cardiac scar. A log-rank (Mantel–Cox) test was employed for comparing survival curves. P values are included in the figures or figure legends. Other experimental or tissue assays were performed with 7–20 animals in each group and replicates are indicated in the figure or figure legends. Statistical values are displayed as mean ± s.d. or mean ± s.e.m., as indicated in the figure legend. Normality tests were performed for all applicable experiments. Statistical differences were calculated using unpaired two-tailed Student’s t-tests when a normality test was passed, or a two-sided Mann–Whitney test otherwise. Fisher’s exact test was employed for assessing muscle regrowth and cardiac scar. A log-rank (Mantel–Cox) test was employed for comparing survival curves. P values are included in the figures or figure legends. All statistical analyses were performed with GraphPad Prism9. RNA-seq and ATAC-seq data involve two or three biologically independent samples for each group. Tissues from ten fish were pooled together for one biological replicate. All chromatin immunoprecipitation sequencing (ChIP-seq) and Hi-C data from GSE1340554 involve two biologically independent samples for each group. WGBS data from GSE1340555 involve one biological sample for each group. Cardiac ChIP-seq data from GSE81862, GSE75894 and GSE569284 involve two to three biological replicates for each group.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this Article.

Data availability

RNA-seq and ATAC-seq data are deposited in the NCBI GEO database under accession nos. GSE158079 and GSE193638. The brain, kidney, heart, muscle and
kidney H3K27ac, H3K4me3, WGBS and the deep sequencing of brain Hi-C data are downloaded from GEO: GSE134055. Other heart-related ChIP-seq data were downloaded from GSE81862, GSE75894 and GSE96928. Unique reagents generated in this study, and all data supporting the findings of this study, are available from the corresponding author upon reasonable request. Source data are provided with this paper.

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Acknowledgements
We thank Duke Zebrafish Core for animal care, Duke Center for Genomic and Computational Biology for advice, A. Dickson and K. Oonk for assistance with ISH, J. Kang, Y. Diao, J. A. Goldman, M. Pronobis, V. Cigliola, R. Yan, K. Ando, L. Slota-Burtt and R. Karra for comments on the manuscript and Y. Diao, J. Rawls, B. Black and N. Bursac for discussions. We acknowledge research support from NIH (R01 HL155607 to J.C.; R35 GM 124820 to F.Y.; R35 HL150713 and R01 HL136182 to K.D.P.) and from AHA and Fondation Leducq to K.D.P.

Author contributions
Conceptualization was provided by F.S. and K.D.P., wet lab investigations by F.S., A.R.S., J.C. and A.S., bioinformatic analysis by J.O., L.S., Y.L., H.Y., F.Y. and G.E.C., writing of the manuscript by F.S. and K.D.P., funding acquisition by K.D.P., G.E.C. and F.Y., and supervision by K.D.P., G.E.C. and F.Y.

Competing interests
The authors declare no competing interests.

Additional information
Extended data is available for this paper at https://doi.org/10.1038/s41556-022-00906-y.
Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41556-022-00906-y.
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Peer review information Nature Cell Biology thanks Josep Saura and the other, anonymous, reviewers for their contribution to the peer review of this work. Peer reviewer reports are available.

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Extended Data Fig. 1 | Bioinformatic analysis of brain RNA-seq during cardiac regeneration. a, Gene Ontology analysis demonstrating biological pathways with gene enrichment, p < 0.05. Counts indicate the number of genes with significantly changed expression in each biological pathway. b, HOMER analysis demonstrating enriched transcription factor binding motifs at the promoter regions (upstream 2 kb to downstream 500 bp) of genes with increased brain RNA levels during heart regeneration.
Extended Data Fig. 2 | Expression of cebpδ is not induced in several tissues in adult zebrafish during heart regeneration. ISH on sections of nasal epithelium, spleen, liver, skeletal muscle, and intestine, indicating that cebpδ expression is not noticeably induced in these tissues during heart regeneration. Tissues were harvested 7 days after tamoxifen administration, which induced CM ablation in CreER+ animals. n = 10 animals for all groups. Scale bars: 100 µm.
Extended Data Fig. 3 | See next page for caption.
Extended Data Fig. 3 | Cardiac muscle regeneration and fluid regulation in cebpd mutant animals. a, Representative sections of cardiac ventricles 30 days after resection (dpa) in wild-type and cebpd−/− animals, stained with AFOG. Scale bar: 100 μm. n reported in (b). b, Semiquantitative assessment of cardiac injuries based on muscle and scar morphology, indicating no significant difference between cebpd−/− animals and their wild-type siblings. 1: Robust regeneration. 2: Partial regeneration 3: Blocked regeneration. A single trial was performed, with n = 11 animals for each group. Data were analysed using a Fisher’s exact test. c, Sections of 7 days post resection (dpa) ventricles staining with markers for CM nuclei (Mef2; green) and cell cycle entry (EdU; red). Dashed lines outline the approximate resection injury site. Scale bars: 100 μm. d, Quantification of the CM cycling index at 7 dpa indicating no significant differences in cebpd knockout animals compared to their wild-type siblings. A single trial was performed with n = 8 cebpd+/− animals, 9 cebpd−/− animals, and 12 cebpd−/− animals. Mean ± s.d. Data were analysed using an unpaired two-tailed Student’s t-test. e, Fluorescence images of tailfin vasculature indicating that significantly less fluorescent dextran was able to reach the circulatory system of cebpd−/− fish after cardiac injuries. Data are quantified in Fig. 3. n = 16 CreER− and 21 CreER+ animals for cebpd+/+, and n = 22 CreER− and 18 CreER+ animals for cebpd−/−. Scale bars: 500 μm.
Extended Data Fig. 4 | See next page for caption.
Extended Data Fig. 4 | Transcriptome profiling of cebpd mutant brain and kidney during heart regeneration. a,b, Volcano plot showing differential gene expression in the cebpd⁻/⁻ brain (a) and kidney (b) during heart regeneration. 521 genes (158 genes increased and 363 genes decreased) and 1662 genes (787 increased and 875 decreased) were differentially regulated in the brain and kidney of cebpd⁻/⁻ animals, respectively. Full lists of differentially expressed genes are shown in Supplementary Tables 2 and 3. Pink dots: highlighted genes. Blue dots: genes with decreased RNA levels (p < 0.05, FC < −1.2). Grey dots: genes with no significant changes. Orange dots: genes with increased RNA levels (p < 0.05, FC > 1.2). c, RNA-seq browser track of aqp7 showing decreased transcript levels of aqp7 (Log₂ FC = −1.062, p = 0.0012) in cebpd⁻/⁻ kidney during heart regeneration. d, RNA-seq browser track of aqp10a showing decreased transcript levels of aqp10a (Log₂ FC = −1.045, p = 0.0020) in cebpd⁻/⁻ kidney during heart regeneration. e, RNA-seq browser track of slc5a8l showing decreased transcript levels of slc5a8l (Log₂ FC = −0.515, p = 0.0034) in cebpd⁻/⁻ kidney during heart regeneration. f,g, Gene Ontology analysis of differentially expressed genes in cebpd⁻/⁻ brain during heart regeneration demonstrating top biological pathways (f) and molecular functions (g) with gene enrichment. Counts indicate the number of genes with significantly changed expression in Gene Ontology term. h,i, Gene Ontology analysis of differentially expressed genes in cebpd⁻/⁻ kidney during heart regeneration demonstrating top biological pathways (h) and molecular functions (i) with gene enrichment. Counts indicate the number of genes with significantly changed expression in Gene Ontology term.
Extended Data Fig. 5 | Two BAC sequences direct distinct gene expression patterns in zebrafish. 

**a**, Two BAC transgenic lines show distinct larval EGFP fluorescence patterns. Boxed area is magnified on bottom left, and a magnified dorsal view is shown on bottom right. 108cebpd12:EGFP is prominent in skeleton and strong in jaw, indicated by yellow arrows. 35cebpd84:EGFP is weak in skin with low expression in jaw, indicated by yellow arrows. Scale bars: 500 µm. This expression is consistent in all animals used in this study.

**b**, Two BAC transgenic lines show distinct adult EGFP fluorescence patterns. 108cebpd12:EGFP is strong in caudal fin rays and nasal cavity, indicated by yellow arrows. 35cebpd84:EGFP is weak in skin, indicated by yellow arrows. Scale bars: 500 µm. This expression is consistent in all animals used in this study.
Extended Data Fig. 6 | See next page for caption.
Extended Data Fig. 6 | Transcriptome and epigenetic analysis of whole kidney marrow. a, Schematic of CM ablation and whole kidney marrow (WKM) collection for RNA-seq and ATAC-seq. b, Volcano plot showing differential gene expression in WKM after cardiac injury. Blue dots: genes with decreased RNA levels (p < 0.05, FC < −1.2). Grey dots: genes without significant changes. Orange dots: genes with increased RNA levels (p < 0.05, FC > 1.2). c, Gene ontology analysis of top biological pathways with gene enrichment. p < 0.05. Counts indicate the number of genes with significantly changed expression in each biological pathway. d, Heatmap of chromatin regions with changes in accessibility in the brain and kidney after CM ablation. p < 0.05. e, Heatmaps of RNA-seq and ATAC-seq data representing putative enhancer elements linked to genes with significant transcriptional changes in WKM after a cardiac injury. f, BIFET analysis indicating enriched transcription factor binding to open regions in WKM chromatin regions cardiac injury. Red: p < 0.05. Blue: p ≥ 0.05.
Extended Data Fig. 7 | Epigenetic features of CEN and its sequence conservation across species. a, A/B compartment analysis indicates a chromatin B to A compartment switch at cebp and CEN loci. Dashed lines indicate cebp and CEN region. b, Browser tracks indicating assays from this study and others. Brain ATAC-seq and Brain RNA-seq, uninjured and 7 days after CM ablation (7 dpi; this study); CM H3.3 occupancy, uninjured and 7 dpi (GSE81862); H3K27Ac occupancy, uninjured and 7 dpi (GSE75894); H3K27me3 and H3K4me3 occupancy in ventricular Gata4+ CMs, uninjured and 5 days post resection (GSE96928). Dashed lines indicate CEN. c, mVista plot of genomic regions around cebp indicating high conservation of zebrafish CEN with cyprinid fish and low conservation with amphibians and mammals. Calculation window = 100 bp, conservation identity = 70%.
Extended Data Fig. 8 | Expression of corticosteroid receptors in the brain and kidney. a, b, RNA-seq browser track of nr3c1 (a) and nr3c2 (b) indicating that expression of nr3c1 in both brain and whole kidney marrow (WKM) are not significantly changed during heart regeneration, indicated as ‘injured’ in tracks. c, ISH on sections of brain and kidney from uninjured animals demonstrating nr3c1 and nr3c2 expression in multiple regions including ependymal cells and renal tubules. n=5 animals for each group. Scale bar: 100 μm.
Extended Data Fig. 9 | CEN is required for fluid homeostasis during heart regeneration. Fluorescence images of tailfin vasculature indicating that less fluorescent dextran transferred to the circulatory system of CEN−/− fish after cardiac injuries. A single trial with $n = 9$ animals for both groups of CEN+/+, and $n = 11$ CreER− and $n = 10$ CreER+ animals for CEN−/− animals, was performed. Data are quantified in Fig. 6. Scale bars: 500 µm.
Extended Data Fig. 10 | Model describing CEN element functions in local and remote tissues. (Top) Cardiac injury induces cebpδ expression locally, regulating epicardial activation during heart regeneration. CEN is sufficient but not required for directing local cebpδ induction, likely due to existence of redundant enhancers. (Bottom) Cardiac injury leads to transcriptional activation by bound corticosteroid receptors in remote tissues. CEN exists in an open, permissive structure, topologically close to its promoter and poised for corticosteroid receptor binding. Ligand-bound corticosteroid receptors trigger CEN-directed cebpδ expression in distant tissues, contributing to fluid homeostasis in animals undergoing injury-induced regeneration.
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Software and code

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Data collection

Confocal images were collected using Zeiss LSM 700 confocal microscope with Zen 2010 B SP1
Compound scope images were collected using Leica DM6000 compound microscope with Leica Application Suite X (v.3.4.2)
Dissecting scope images were collected using Zeiss AXIO Zoom.V16 with ZEN pro 2012.
Quantitative PCR was performed on a Roche LightCycler 480 with Software release 1.5.0 SP4.

Data analysis

Image processing and imaging data analysis were performed with Fiji (v.2.3.0).
All statistical analyses were performed with GraphPad Prism 9.
Software and algorithm used for bioinformatic analyses including:
Tophat2 [Trapnell et al., 2009; v 2.1.1]; Trim Galore [v 0.4.1 & v 0.6.7]; samtools [Li et al., 2009; V 1.5]; HiSeq Counts [Anders et al., 2010; v 0.6.0] DESeq2 [Love et al., 2014; v 1.28.1 & v 1.30.1]; clusterProfiler [Yu et al., 2012; v 3.16.1 & 3.18.1]; trackViewer [Ou and Zhu, 2019; v 1.25.3 & v 1.31.1]; phenom (Gu et al., 2016; v 1.0.12); complexHeatmap (Gu et al., 2016; v 2.4.3); Bowtie2 (Langmead & Salzberg, 2012; v 2.2.5); picard (v 1.91); MACS2 (Zhang et al., 2008; V 2.1.0); DiffBind (Ross-Innes et al., 2012; v 2.14.0); ChIPpeakAnno (Zhu et al., 2010; v 3.22.3); BIFET [You et al., 2013; v 1.8.0] motifDB (v 1.28.0); MotifIV::motifDistances function (v 1.42.0); motifStack (Ou et al., 2018; v 1.30.0); motifmatcher (v 1.10.0 & v 1.12.0); EnhancedVolcano (v 1.6.0 & 1.6.0); Homer (Heinz et al., 2010; v 4.4.10.4); deepTools (Ramirez et al., 2016; v 3.1.5); STAR (v 2.6.10); tximport (v 1.18.0); ATACseqQC::footprintsScanner function (v 1.16.0); compactmap package (v 1.9.2); samtools (v 1.5); mVISTA (Mayor et al., 2000)

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- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy.

RNA-seq and ATAC-seq data are deposited in NCBI GEO database under accession number GSE158079 and GSE193630. The brain, kidney, heart, muscle and kidney H3K27ac, H3K4me3, WGBS and the deep sequencing of brain Hi-C data are downloaded from GEO: GSE134055 35. Other heart-related ChIP-seq data were downloaded from GSE81862, GSE75894 and GSE96928. Unique reagents generated in this study, and all data supporting the findings of this study, are available from the corresponding author upon reasonable request.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- [x] Life sciences
- [ ] Behavioural & social sciences
- [ ] Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nrr-reporting-summary-list.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size

No statistical method was used to predetermine sample size. Samples sizes were chosen based on available literature with similar methodologies: Shoffner et al., 2020; Han et al., 2019; Cao et al., 2016. n number for each experiment and each group was indicated in figure legends.

Data exclusions

No data were excluded from analysis.

Replication

All experiments were performed on multiple animals and data were pooled. ISh experiments were repeated with total number of animals indicated in figure legends. Other behavioral experiments or tissue assays were performed with 7 - 20 animals in each group and replicates indicated in figures or figure legends.

Randomization

Siblings, mixed males and females, were randomly allocated in control and experimental groups unless specified in the method and figure legend.

Blinding

Researchers were blinded when performing animal surgeries. Samples were renamed with randomized numbers to perform data quantification. Experiments involved tamoxifen-induced CM ablations are impossible to blind as animals develop signs of heart failure including gasping and edema, which are not evident in control animals. Researchers was blinded to phenotypes of animals in analysis of wild-type and mutant fish.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| [x] | Antibodies            |
| [ ] | Eukaryotic cell lines |
| [x] | Palaeontology and archaeology |
| [ ] | Animals and other organisms |
| [x] | Human research participants |
| [x] | Clinical data         |
| [ ] | Dual use research of concern |

Methods

| n/a | Involved in the study |
|-----|-----------------------|
| [ ] | ChIP-seq              |
| [ ] | Flow cytometry        |
| [x] | MRI-based neuroimaging |

Antibodies

| Antibodies used |
|-----------------|
| Recombinant Anti-MEF2A + MEF2C antibody (Abcam, ab197070): 1:100 dilution |
| Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488 (Thermo Scientific, A11034): 1:250 dilution |
Animals and other organisms

Policy information about **studies involving animals; ARRIVE guidelines** recommended for reporting animal research

**Laboratory animals**

This study involved wild-type and transgenic fish of the outbred Ekkwill strain. Adult animals of both sex were used with an age range from 4-12 month old, unless specified in the Methods section or figure legends. A full list of transgenic lines used in the manuscript is summarized and reported in a supplementary table.

**Wild animals**

The study did not involve wild animals

**Field-collected samples**

The study did not involve field collected samples

**Ethics oversight**

Animal use was approved by the Institutional Animal Care and Use Committee at Duke University, Protocol #A005-21-01.

Note that full information on the approval of the study protocol must also be provided in the manuscript.