SUPPLEMENTAL MATERIAL

Cardiac copper deficiency activates a systemic signaling mechanism that communicates with the copper acquisition and storage organs

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Figure S1, related to Figure 2

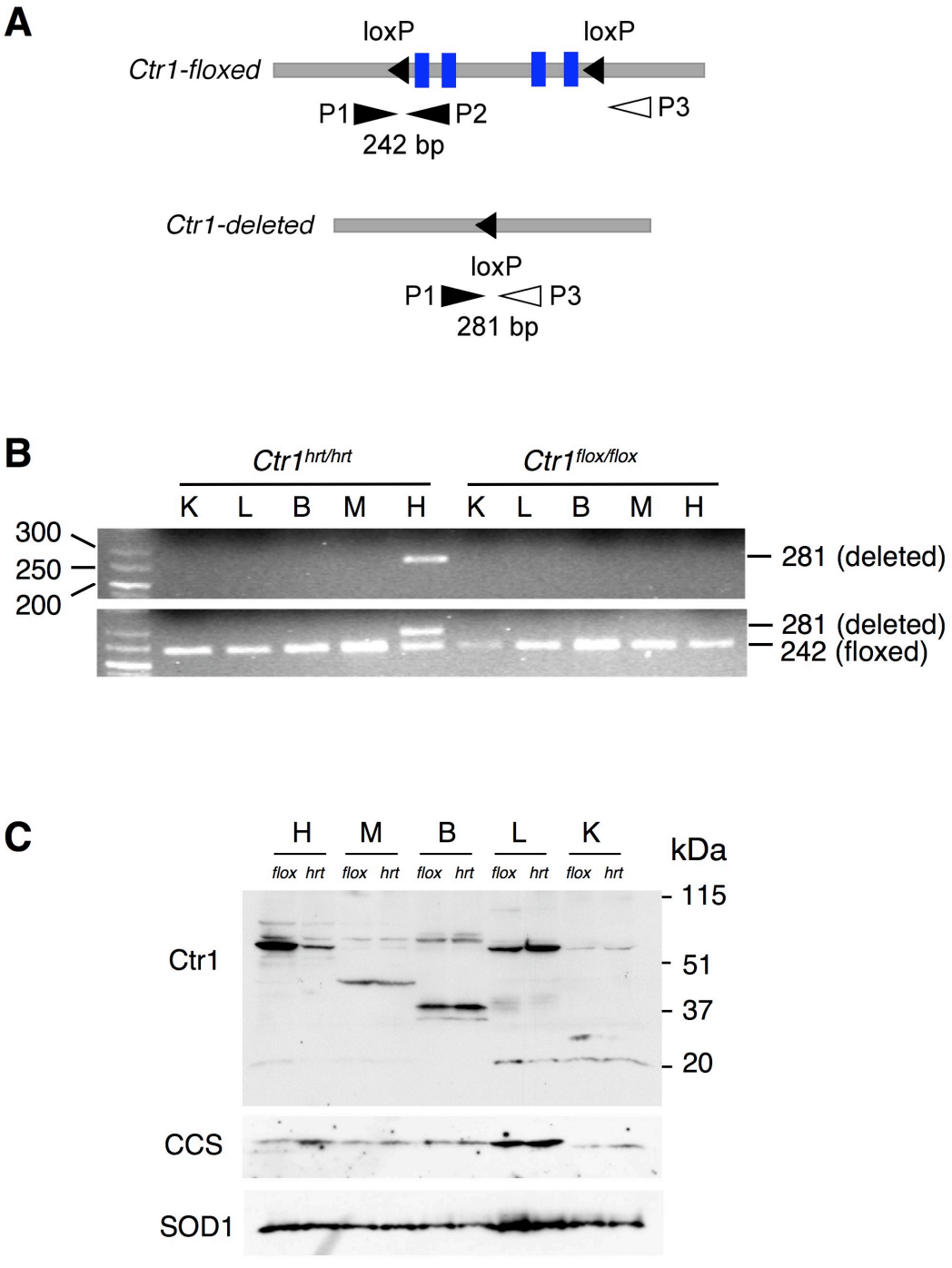


Figure S2, related to Figure 4

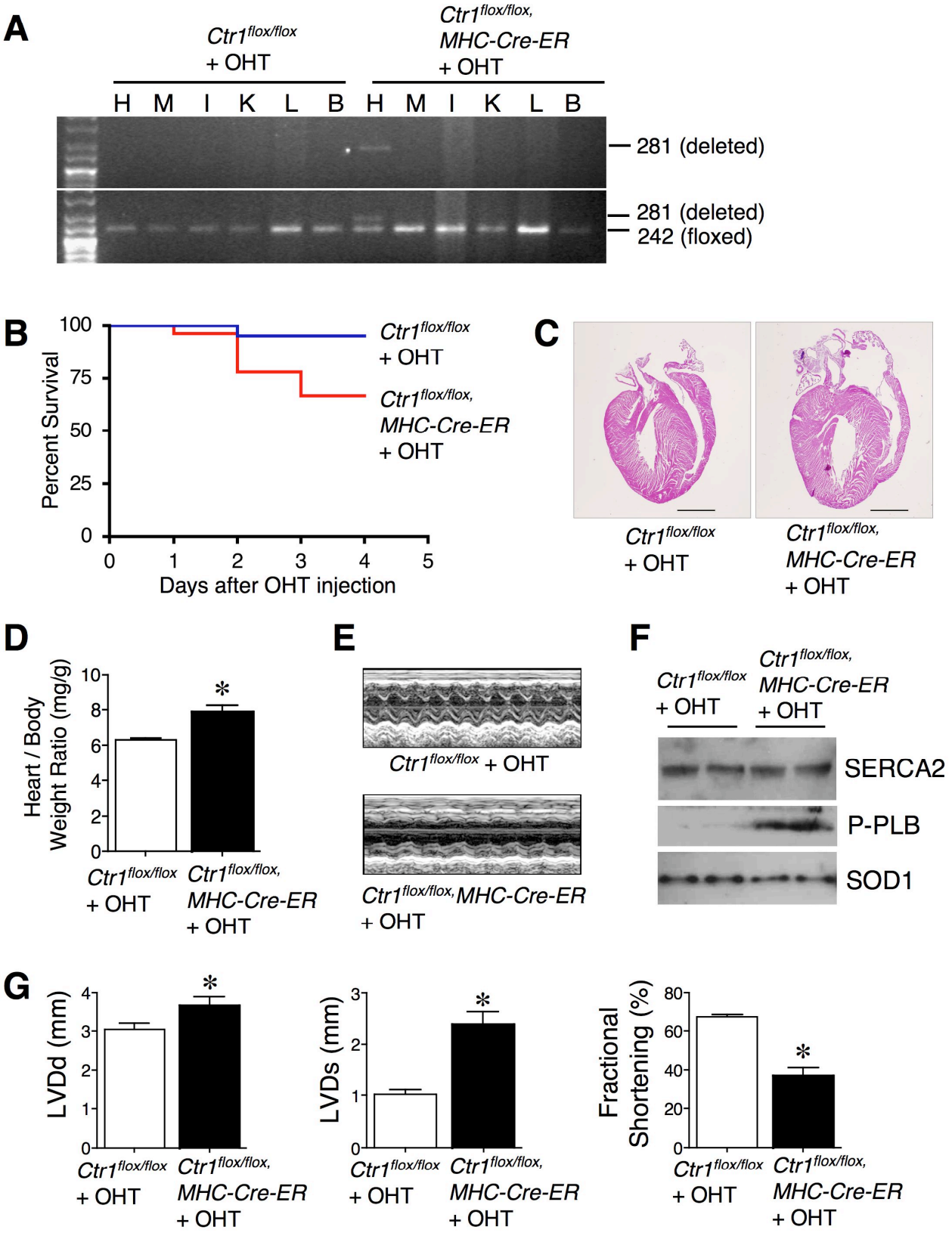
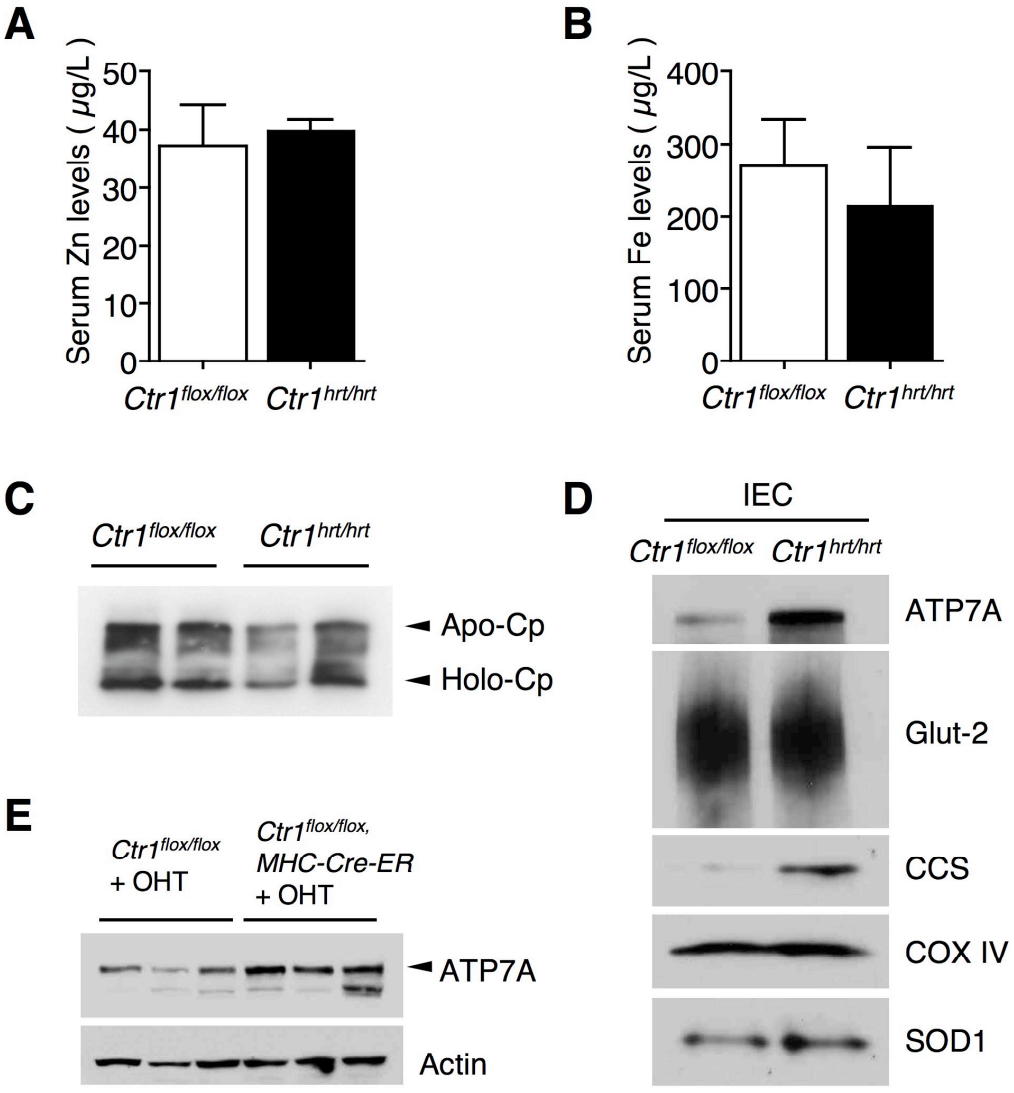


Figure S3, related to Figure 5



SUPPLEMENTAL FIGURE LEGENDS

Figure S1. (**A**) Targeting strategy used for cardiac-specific deletion of Ctr1. Mice harboring the *Ctr1*$^{flox/flox}$ allele (Ctr1-floxed) were bred with *MHC-Cre* transgenic mice. The locations of the Ctr1 exons (blue boxes), loxP recombination sites (small black arrowheads) and primers and their hybridization sites used for diagnostic PCR (P1, P2, P3) are shown as large arrowheads. Primers P1 and P2 give rise to a 242 bp PCR product indicative of the floxed allele whereas primers P1 and P3 give rise to a 281 bp PCR product indicative of a deleted allele. (**B**) Representative diagnostic PCR analysis of Ctr$^{flox/flox}$ and *Ctr1*$^{hrt/hrt}$ mouse tissues from postnatal day 10 mice. K, kidney; L, liver; B, brain; M, muscle; H, heart (**C**) Immunoblot of representative *Ctr1*$^{flox/flox}$ (flox) and *Ctr1*$^{hrt/hrt}$ (hrt) mouse tissues for Ctr1, CCS (copper chaperone for Cu, Zn superoxide dismutase) and SOD1 (Cu, Zn superoxide dismutase) (as loading control). Ctr1 exists predominantly as a monomer, dimer or trimer in different tissue extracts. H, heart; M, muscle; B, brain; L, liver; K, kidney.

Figure S2. (**A**) Diagnostic PCR analysis of *Ctr1*$^{flox/flox}$ and *Ctr1*$^{flox/flox}$*, MHC-Cre-ER* mice injected with OHT. The PCR product from *Ctr1*$^{flox/flox}$ mice is 242 bp, whereas the excision product from *Ctr1*$^{flox/flox}$*; MHC-Cre-ER* mice is 281 bp. H, heart; M,

muscle; I, intestine, K, kidney; L, liver; B, brain. (**B**) Kaplan-Meier plot showing survival of *Ctr1*$^{flox/flox}$ (blue, n=21) and *Ctr1*$^{flox/flox}$*; MHC-Cre-ER* mice (red, n=29) after the final OHT-injection. (**C**) Hematoxylin and eosin staining of cardiac sections from *Ctr1*$^{flox/flox}$ (left) and *Ctr1*$^{flox/flox}$*; MHC-Cre-ER* (right) mice injected with OHT (Scale Bar=2mm). (**D**) Ratio of heart weight to body weight (Hw/Bw) of OHT-treated *Ctr1*$^{flox/flox}$ (n=8) and *Ctr1*$^{flox/flox}$*; MHC-Cre-ER* mice (n=8) *P = 0.0005 versus *Ctr1*$^{flox/flox}$. Error bars, s.d. (**E**) Representative echocardiography of *Ctr1*$^{flox/flox}$ and *Ctr1*$^{flox/flox}$; *MHC-Cre-ER* mice injected with OHT. (**F**) SDS-PAGE analysis of total heart extracts of two representative 7 month-old *Ctr1*$^{flox/flox}$ and *Ctr1*$^{flox/flox}$*; MHC-Cre-ER* mice after OHT treatments. Tissues were probed for SERCA-2, P-PLB (phospho-phospholamban, Ser-16), and SOD1 as a loading control. (**G**) LVDd (*P = 0.0232 versus *Ctr1*$^{flox/flox}$), LVDs (*P < 0.0001 versus *Ctr1*$^{flox/flox}$) and % FS (*P < 0.0001 versus *Ctr1*$^{flox/flox}$) in OHT-injected *Ctr1*$^{flox/flox}$ (n=10) and *Ctr1*$^{flox/flox}$; *MHC-Cre-ER* mice (n=8) indicate deletion of cardiac Ctr1 in adult mice impairs cardiac contractility. Error bars, s.d.

Figure S3. (**A**) Serum Zn levels in *Ctr1*$^{hrt/hrt}$ mice (n=5) and *Ctr1*$^{hrt/hrt}$ (n=5) mice. Error bars, s.d., P = 0.5156 versus *Ctr1*$^{flox/flox}$. (**B**) Serum Fe levels in *Ctr1*$^{hrt/hrt}$ mice (n=5) and *Ctr1*$^{hrt/hrt}$ (n=5) mice. Error bars, s.d., P = 0.5994 versus *Ctr1*$^{flox/flox}$. (**C**) Ceruloplasmin (Cp) Cu metallation assays. One microliter serum from two *Ctr1*$^{flox/flox}$ and two *Ctr1*$^{hrt/hrt}$ mice was subjected to nonreducing SDS-PAGE and immunoblotting with anti-Cp antibody. Arrows indicate the positions of the apo- and

holo- forms of Ceruloplasmin. (**D**) Immunoblot analysis of ATP7A, Glut-2, CCS and COX IV in the intestinal epithelial cells (IEC) of *Ctr1*$^{hrt/hrt}$ mice and control mice. SOD1 levels were assayed as a loading control. (**E**) Immunoblot analysis of total Triton X-100 solubilized liver extracts from three representative 7 month-old *Ctr1*$^{flox/flox}$ and *Ctr1*$^{flox/flox}$*; MHC-Cre-ER* mice after OHT treatments for ATP7A and Actin (loading control).

SUPPLEMENTAL TABLE

Primer sequences for RT-PCR

ATP7A	Forward: 5'-atggagccaagtgtggatg-3' Reverse: 5'-ccaaggcagagtcagtggag-3'
ATP7B	Forward: 5'-atggatcccaggaagaactt-3' Reverse: 5'-gctggggacgagctggtgct-3'
GAPDH	Forward: 5'-atggtgaaggtcggtgtgaa-3', Reverse: 5'-agtggagtcatactggaaca-3'

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Drosophila Stocks and Breeding

W^{1118} stocks were obtained from the Bloomington *Drosophila* stock center. The TinCGal4 transgenic was a generous gift from Manfred Frasch (Lo and Frasch, 2001). The UAS-Ctr1A^{RNAi} construct was generated as described (Lee and Carthew, 2003); establishment of transgenic lines carrying the construct was carried out according to standard methods (Cooley et al., 1990). All crosses were performed and control lines maintained at 29 °C in a humidity-controlled incubator until OCT measurements were performed.

OCT Measurements

Fourteen wild type (W^{1118}) female flies, 12 TinCGal4 females, and 24 TinCGal4/UAS-Ctr1A^{RNAi} females, all of which were at least one week old, were used in optical coherence tomography (OCT) analysis. For the dorsal vessel specific knock-down of Ctr1A, two independent insertion lines of the UAS-Ctr1A^{RNAi} cassette, one on the 2nd chromosome and one on the 3rd chromosome, were crossed to TinCGal4 flies and subjected to OCT analysis. Data using the 3rd

chromosome insert are reported here, though similar results were obtained with the transgene inserted on the 2nd chromosome. OCT measurements were carried out as described (Wolf et al., 2006).

SUPPLEMENTAL REFERENCES

Cooley, L., Thompson, D., and Spradling, A.C. (1990). Constructing deletions with defined endpoints in Drosophila. Proc Natl Acad Sci U S A *87*, 3170-3173.

Lee, Y.S., and Carthew, R.W. (2003). Making a better RNAi vector for Drosophila: use of intron spacers. Methods *30*, 322-329.

Lo, P.C., and Frasch, M. (2001). A role for the COUP-TF-related gene seven-up in the diversification of cardioblast identities in the dorsal vessel of Drosophila. Mech Dev *104*, 49-60.

Wolf, M.J., Amrein, H., Izatt, J.A., Choma, M.A., Reedy, M.C., and Rockman, H.A. (2006). Drosophila as a model for the identification of genes causing adult human heart disease. Proc Natl Acad Sci U S A *103*, 1394-1399.