Supplemental Figures:

Supplemental Figure 1 related to Figure 1:
Confirmation of chimerism. Blood chimerism was confirmed in parabiotic pairs by measuring the frequency of donor-derived blood cells from one partner ($CD45.1^+$) in the spleen of the other partner ($CD45.2^+$). Partner-derived cells typically represented 40-50% of splenocytes, consistent with establishment of parabiotic cross-circulation. Because old CD45.1+ mice are not commercially available we could not verify the establishment of chimerism in old parabiotic pairs using this system; however, our extensive experience with this model (Pietramaggiori et al., 2009; Wagers et al., 2002; Wright et al., 2001) as well as published (Ruckh et al., 2012) and unpublished data from GFP^{young}/WT^{old} pairs strongly support the conclusion that cross-circulation is established effectively in these fully isogenic pairs.

Supplemental Figure 2 related to Figure 3:
Design of the experiment and assessment of cardiac mass
(A) Young isochronic, heterochronic and old isochronic parabiotic mice were generated. 10 weeks after surgery mice were sacrificed and tissues harvested for analysis.
(B) Graph representing the heart weight / tibia length ratio after 10 weeks of parabiosis,
Data shown as mean ± s.e.m.

Supplemental Figure 3 related to Figure 7:
Spleen has a significantly higher level of GDF11 expression among the analyzed tissues and shows a significant age dependent reduction in GDF11 expression and protein synthesis.
(A) Expression of GDF11 in tissues harvested from young (3 months old) mice. Real-time PCR transcript measurements are normalized to levels in the liver. The gene expression in the spleen was significantly higher (* $P<0.05$) when compared with all the other tissues.
(B) Expression of GDF11 in the spleen harvested from young (3 months old) and old (24 months old) mice. Real-time PCR transcript measurements are normalized to levels in young mice.
(C) Western blot analysis of GDF11 in the spleen from young and old mice. Densitometry (arbitrary units, mean ± s.e.m) of GDF11 normalized to α-tubulin. Data shown as mean ± s.e.m.

Supplemental Figure 4 related to Figure 7
GDF11 specifically binds cardiomyocytes *in vivo* at the intercalated discs.
Immunohistochemistry was performed using a rabbit monoclonal GDF11 antibody on left ventricle mid sections in 24 months old C57Bl/6 female mice. Note that these data suggest that GDF11 acts on cardiomyocytes in vivo, but these data do not exclude the possibility of an effect on non-myocyte cells in the heart, such as fibroblasts and endothelial cells. Scale bar = 20μm

Supplemental Figure 5 related to Figure 7: **GDF11 levels can be persistently increased for 24 hours in plasma after a single intraperitoneal bolus.**
GDF11 levels in plasma were evaluated by Western analysis at the indicated times after a single intraperitoneal injection of 0.1 mg/kg of recombinant GDF11 (n=3).

Supplemental Figure 6 related to Figure 7:
Supplementation of rGDF11 did not prevent development of cardiac hypertrophy after pressure overload by transverse aortic constriction in young mice.
(A) Graph representing the heart weight / tibia length ratio after 30 days of treatment with rGDF11 or vehicle. The ratio in mice that were injected with rGDF11 (n=10) was not significantly different than the ratio measured in mice that were injected with vehicle (n=9) *(9.08+/- 0.71vs. 9.89+/-0.69 mm/mg, P=ns)*
(B) Left ventricular myocyte cross-sectional area measured after PAS staining after 30 days of treatment with rGDF11 or vehicle. Cardiomyocyte cross sectional area was not significantly different in the two groups (*(286.4±12.89 μm^2 in rGDF11 treated, 304.2±17.3 μm^2 in vehicle treated, P=ns)*
(C) Table with echocardiographic data after 30 days of treatment with rGDF11 or vehicle. No significant differences were noted in echocardiographic parameters of ventricular remodeling or function. AWT=anterior wall thickness; PWT=posterior wall thickness; EDD=end diastolic dimension; ESD=end systolic dimension; FS=fractional shortening.
Data shown as mean ± s.e.m.

Supplemental Tables:

Serum analytes **(SOMAscan)**
Collectin kidney 1
Cathepsin D
Dickkopf-related protein 4
*Erythrocyte membrane protein 4.1	Protein 4.1R*
Esterase D
***Growth-differentiation factor 11	BMP-11***
Hemoglobin
*Interleukin-1 receptor accessory protein	IL-1 RAcP	IL1 R3*
*Natural killer group 2 member D	NKG2D*
Ras-related C3 botulinum toxin substrate 1
*GTP-binding nuclear protein Ran	ARA24*
*TIMP3	Tissue inhibitor of metalloproteinases 3*
Thymidylate synthase

Supplemental Table 1 related to Figure 7:
List of serum analytes identified by proteomics analysis. The table summarizes the 13 serum analytes that reliably distinguish young mice from old mice.

	Vehicle (n=7)	GDF11 (n=6)
AWT (mm)	1.39±0.02	1.39±0.01
PWT (mm)	1.10±0.02	1.09±0.04
ESD (mm)	1.25±0.04	1.25±0.03
EDD (mm)	2.99±0.09	3.20±0.05
FS (%)	57.9±1.6	60.9±1.1

Supplemental Table 2 related to Figure 7:
Echocardiographic data after 30 days of treatment with rGDF11 or vehicle in 23 months old C57Bl/6 male mice. No significant differences were noted in echocardiographic parameters. AWT=anterior wall thickness; PWT=posterior wall thickness; EDD=end diastolic dimension; ESD=end systolic dimension; FS=fractional shortening.
Data shown as mean ± s.e.m.

Supplemental experimental procedures:

Flow cytometry

All flow cytometry was performed on freshly isolated, unfixed splenocytes kept one ice during all incubation steps. Cells were blocked with HBSS/2%FBS for 10min prior to resuspension at a concentration of $1x10^6$ cells per 250uL. Cells were incubated for 30min in directly conjugated primary antibodies specific for CD45.1 (eBioscience) and CD45.2 (eBioscience) and washed twice in HBSS, prior to flow analysis. Conjugated isotype control antibodies were used in all experiments.

Gene expression analysis

To quantify expression genes commonly induced by hypertrophic stimuli, hearts from different experimental groups were excised and snap frozen in liquid nitrogen 4 weeks after surgery. RNA was extracted with Trizol reagent (Sigma), transcribed into cDNA with the High Capacity cDNA Reverse Transcription kit (Applied Biosystems) using random primers, and subsequently analyzed by real-time PCR on an Applied Biosystems 7300 Real Time PCR System using SYBR Green (Applied Biosystems) or TaqMan (Applied Biosystems) and primers for *ANP* (left: 5′- TCGTCTTGGCCTTTTGGCT-3′; right: 5′-TCCAGGTGGTCTAGCAGGTTCT-3′), *BNP* (left: 5′-AGGGAGAACACGGCATCATT-3′; right: 5′- GACAGCACCTTCAGGAGAT-3′), *SERCA-2* (left: 5'- TGGAACAACCCGGTAAAGAGT-3'; right: 5'-CACCAGGGGCATAATGAGCAG-3'), *GDF11* (Mm01159973m1 TaqMan Gene Expression Assays, Life technologies) . Results were normalized to expression of

TATA binding protein and presented as fold increase relative to young isochronic animals based on the $\Delta\Delta C_t$ method.

Metabolomic and lipidomic profiling analysis

LC-MS/MS analysis. Plasma metabolomic profiling was performed on a 4000 QTRAP triple quadrupole mass spectrometer (Applied Biosystems/Sciex, Foster City, CA) with a Turbo V electrospray source coupled to an HPLC system including an HTS PAL autosampler (Leap Technologies, Carrboro, NC) and a 1200 series binary pump (Agilent Technologies, Santa Clara, CA). This LC-MS/MS system was used for polar metabolites analysis employing hydrophilic-interaction liquid chromatography (HILIC) and also for lipid analysis, each requiring distinct methods of plasma extraction, LS/MS acquisition methods and instrument configurations. The MultiQuant software v. 2.0.2 (AB SCIEX, Foster City, CA) was used for automated peak integration and metabolite peaks also were manually reviewed for quality of integration (Roberts et al., 2012).

HILIC: Hydrophilic-interaction liquid chromatography is suitable for analyzing hydrophilic metabolites; including amino acids, nucleotides and neurotransmitters. Ten microliters of plasma were extracted with 90 µL of 74.9:24.9:0.2 vol/vol/vol acetonitrile/methanol/formic acid containing 0.2 µg/mL (final concentration) of isotopically labeled valine-d8 and phenylalanine-d8 (Sigma-Aldrich; St Louis, MO). The samples were vortexed for 30 seconds, centrifuged (10 minutes, 10,000 rpm, 4°C) and the supernatants were injected directly into the LC/MS system. Samples underwent hydrophilic interaction chromatography using a 150 x 2.1 mm Atlantis HILIC Silica column (Waters,

Milford, MA): mobile-phase A, 10 mM ammonium formate and 0.1% formic acid; and mobile-phase B, acetonitrile with 0.1% formic acid. The column was eluted isocratically with 5% mobile-phase A for 0.5 minutes followed by a linear gradient to 60% mobile-phase over 10 minutes and then back to 5% mobile-phase A for 17 minutes. Electrospray ionization (ESI) was used in positive multiple reaction monitoring (MRM) ion mode. Declustering potentials and collision energies were optimized for each metabolite by infusion of reference standards before sample analyses. The ion spray voltage was 5 kV, the source temperature was 425°C and the MRM window was set to 70 msec. Formic acid, ammonium acetate, LC/MS grade solvents, and valine-d8 were obtained from Sigma-Aldrich (St. Louis, MO), with the remainder of isotopically-labeled analytical standards obtained from Cambridge Isotope Laboratories, Inc. (Andover, MA). The samples were run in a randomized order to minimize internal variation and were interspaced by mouse pooled plasma samples to account for temporal drift across all analyzed metabolites. The internal standard peak areas were monitored for quality control and individual samples with peak areas differing from the group mean by more than 2 standard deviations were reanalyzed. Metabolites analyzed were selected based on the following criteria: 1) known structural identity; 2) distribution across multiple biochemical pathways; 3) reliable measurement using LC/MS in a high throughput fashion; and, 4) low rate of missingness on our platform (<1%).

Lipid analysis: Ten microliters of plasma were extracted with 190 µl of isopropanol containing 0.25 µg/ml (final concentration) 1-dodecanoyl-2-tridecanoyl-sn-glycero-3-phosphocholine (Avanti Polar Lipids, Alabaster, AL).

After centrifugation, supernatants were injected directly, followed by reverse-phase chromatography using a 150 x 3.0 mm Prosphere HP C4 column (Grace, Columbia, MD): mobile-phase A, 95:5:0.1 vol/vol/vol 10mM ammonium acetate/methanol/acetic acid; and mobile-phase B, 99.9:0.1 vol/vol methanol/acetic acid. The column was eluted isocratically with 80% mobile-phase A for 2 minutes followed by a linear gradient to 20% mobile-phase A over 1 minute, a linear gradient to 0% mobile phase A over 12 minutes, then 10 minutes at 0% mobile-phase A and a linear gradient to 80% mobile phase A over 9 minutes. MS analyses were carried out using electrospray ionization and Q1 scans in the positive ion mode. Ion spray voltage was 5.0 kV, the source temperature was 400°C and the declustering potential was 70 V. For each lipid analyte, the first number denotes the total number of carbons in the lipid acyl chain(s) and the second number (after the colon) denotes the total number of double bonds in the lipid acyl chain(s).

Immunohistochemistry

Mouse hearts were fixed with 4% paraformaldehyde, paraffin embedded, sectioned, and stained with standard immunohistochemistry microscopy methods as previously described. An antigen retrieval step was used in all experiments, by heating samples in a citrate-based buffer (Dako) to 95°C for 20 min. Primary antibodies were used as follows: rabbit GDF11 antibody 1:500 (Abcam)
A biotinylated anti-rabbit secondary followed by ABC reagent and DAB (Vector Laboratories) were used for immunohistochemistry.

Induced pluripotent stem cell-derived human cardiomyocytes

Induced pluripotent stem cell-derived human cardiomyocytes (iPSC-CM) were obtained from Cellular Dynamics International (CDI) and cultured according to the manufacturer's instructions. Briefly, cells were plated at ~580,000 viable cells per well in 5ug/ml fibronectin-coated 6 well plates in CDI Plating Medium. Medium was changed after 2 days to CDI Maintenance Medium, and 2 additional changes with this medium were performed at days 4 and 6 post-plating. At the latter point, cells were observed to be beating homogenously. At 7 days post-plating, medium was changed to serum-free DMEM (low glucose) and cells were incubated for an additional 24h. At this time, cells were exposed to either control serum free media, or the same media with 50nM myostatin (Peprotech) or 50nM rGDF11 (Peprotech) for 15 mins. Lysates were collected and western analyses were performed using standard methods. Antibodies used were from Cell Signaling Technology: phospho-Fox01/Fox03a (9464), phospho-SMAD2 (3108), phospho-SMAD3 (9520), GAPDH (2118).

Western Blot Analysis

Western blot analyses were performed as described previously (Seki et al., 2009). Membranes (polyvinylidene fluoride, PerkinElmer Life Sciences) were incubated with primary antibodies (anti-GDF11 diluted 1:1000, from Abcam) and detected with horseradish peroxidase-conjugated antibodies (1:2000, from Bio-Rad) and enhanced chemiluminescence (PerkinElmer Life Sciences). Spleen western blot analyses were performed with membranes (immune-Blot PVDF membrane, Bio-Rad) incubated with primary antibodies (anti-GDF11, Abcam,

1:500 dilution and alpha-tubuline, Sigma, 1:1000 dilution) and detected with IRDye conjugated antibodies (1:10000 dilution, Li-Cor). Membranes were scanned with Odyssey CLx Infrared Imaging System (Li-Cor) and quantified by densitometry with the Image Studio Software (Li-Cor).

Transverse Aortic Constriction and Echocardiography

Transverse aortic constriction (TAC) surgery and Echocardiography were performed in in vivo studies using blinded protocols.

Supplemental References:

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Seki, K., Sanada, S., Kudinova, A.Y., Steinhauser, M.L., Handa, V., Gannon, J., and Lee, R.T. (2009). Interleukin-33 prevents apoptosis and improves survival after experimental myocardial infarction through ST2 signaling. Circ Heart Fail *2*, 684-691.