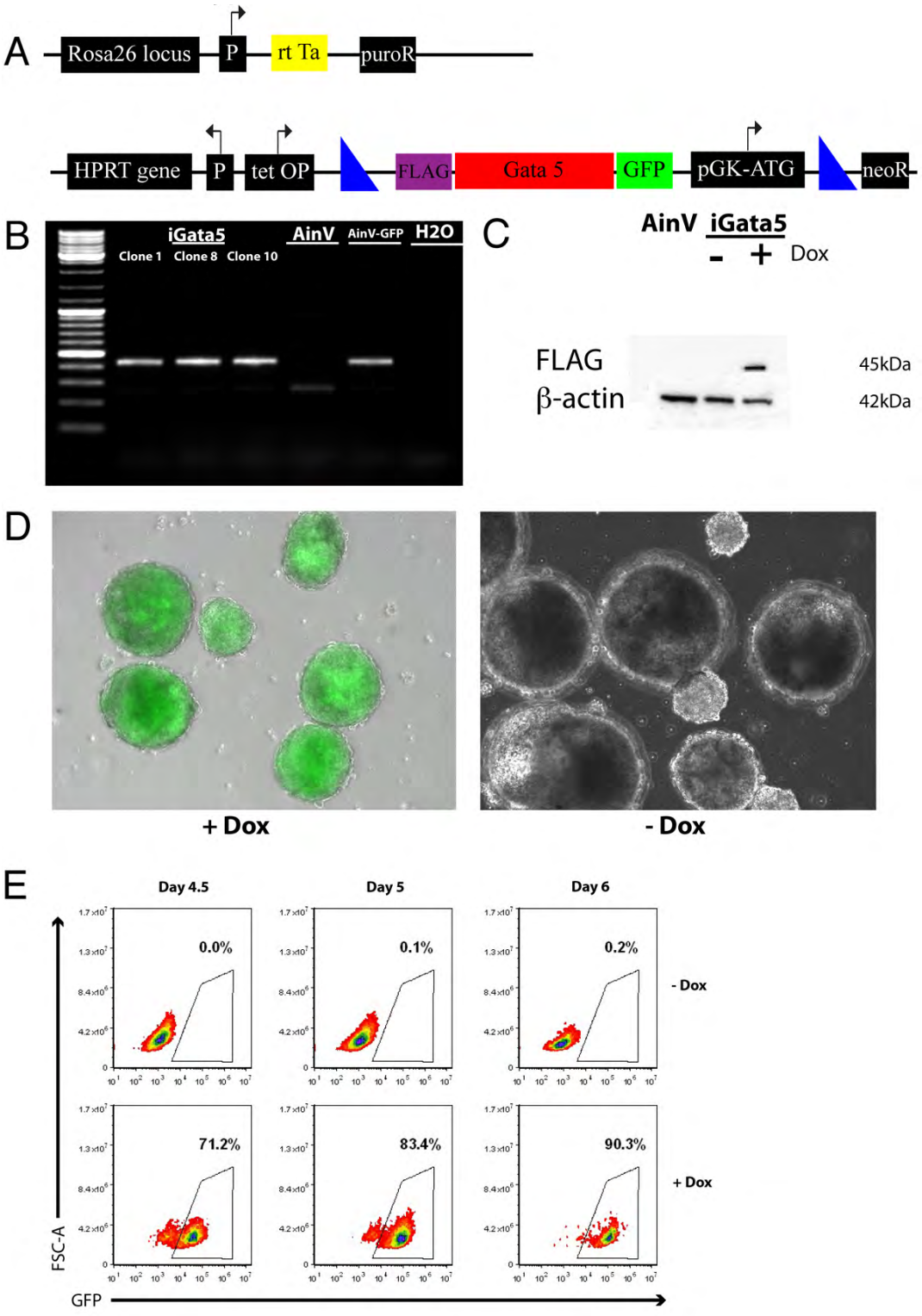

Fig. S1. Design and validation of iGata5ES cell lines. (**A**) In the parental Ainv ESC line, the reverse tet-transactivator protein (rtTa) is expressed from the Rosa26 locus. A loxP site (blue triangle) is engineered upstream of the HPRT promoter (P) and flanked by a tet operator (tet OP) site to generate a tetracycline-regulated promoter. It also includes a neomycin resistance gene that lacks a start codon (neoR). Cre-mediated recombination was used to target a flag-tagged *Gata5* cDNA, IRES:GFP cassette and phosphoglycerkinase promoter (pGK-ATG) into the loxP site, placing *Gata5* under conditional control. Both GATA5 and GFP are expressed, but only with addition of doxycycline. (**B**) Genomic PCR analysis (400 bp product) confirms single-copy site-specific integration of the cDNA-IRES:GFP cassette. Three separate clonal transgenic ESC lines are shown (clones 1, 8 and 10). The parental AinV18 line and H20 alone serve as negative controls, and a transgenic AinV clone with only a GFP cassette provides a positive control. (**C**) Western blot analysis of total cell extracts from parental AinV and uninduced (–) or doxycycline induced (+) iGata5ES-derived embryoid bodies. Protein detection was performed using a monoclonal anti-FLAG and anti-β-actin antibodies. (**D**) Fluorescence image (10×) of EBs derived from iGata5ES cells demonstrating strong GFP expression with doxycycline induction. (**E**) Flow cytometry analysis demonstrating GFP expression in EBs derived from iGata5ES at 12-48 hours after doxycycline induction.

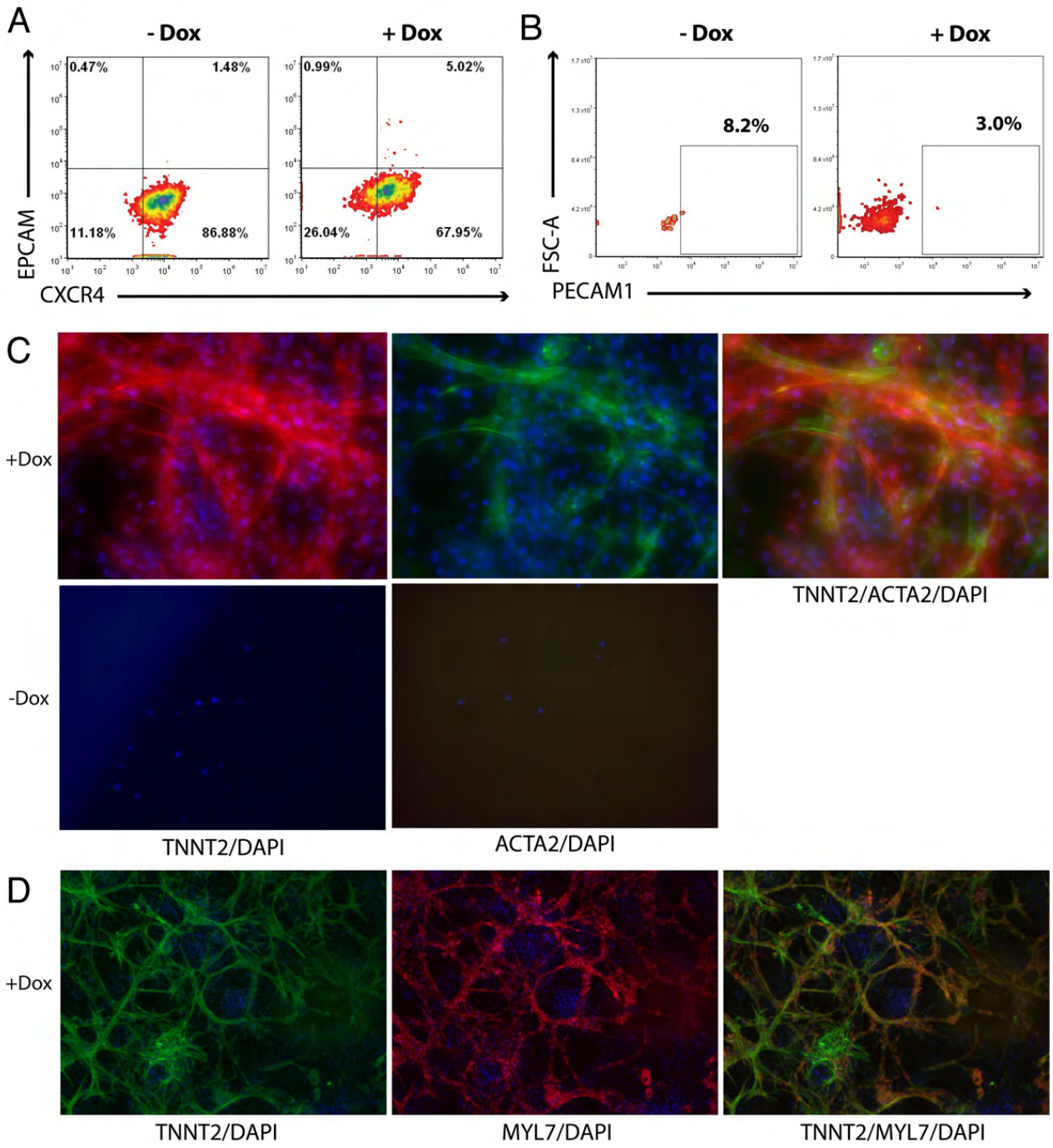

Fig. S2. *Gata5* expression directs the development of differentiated cardiac cells. (**A**) Flow cytometry analysis of day 6 EBs for EPCAM and CXCR4 with or without doxycycline induction of *Gata5*. (**B**) Flow cytometry analysis of day 12 EBs for PECAM1 with or without doxycycline induction of *Gata5*. (**C**) Induced (+Dox) and uninduced (–Dox) EBs were analyzed by immunohistochemistry for TNNT2 (left, red) and ACTA2 (middle, green) on day 12. Right panel is the merge. (**D**) Induced EBs were analyzed as in C for TNNT2 (left, green) and MYL7 (middle, red) on day 16. Right panel is the merge.

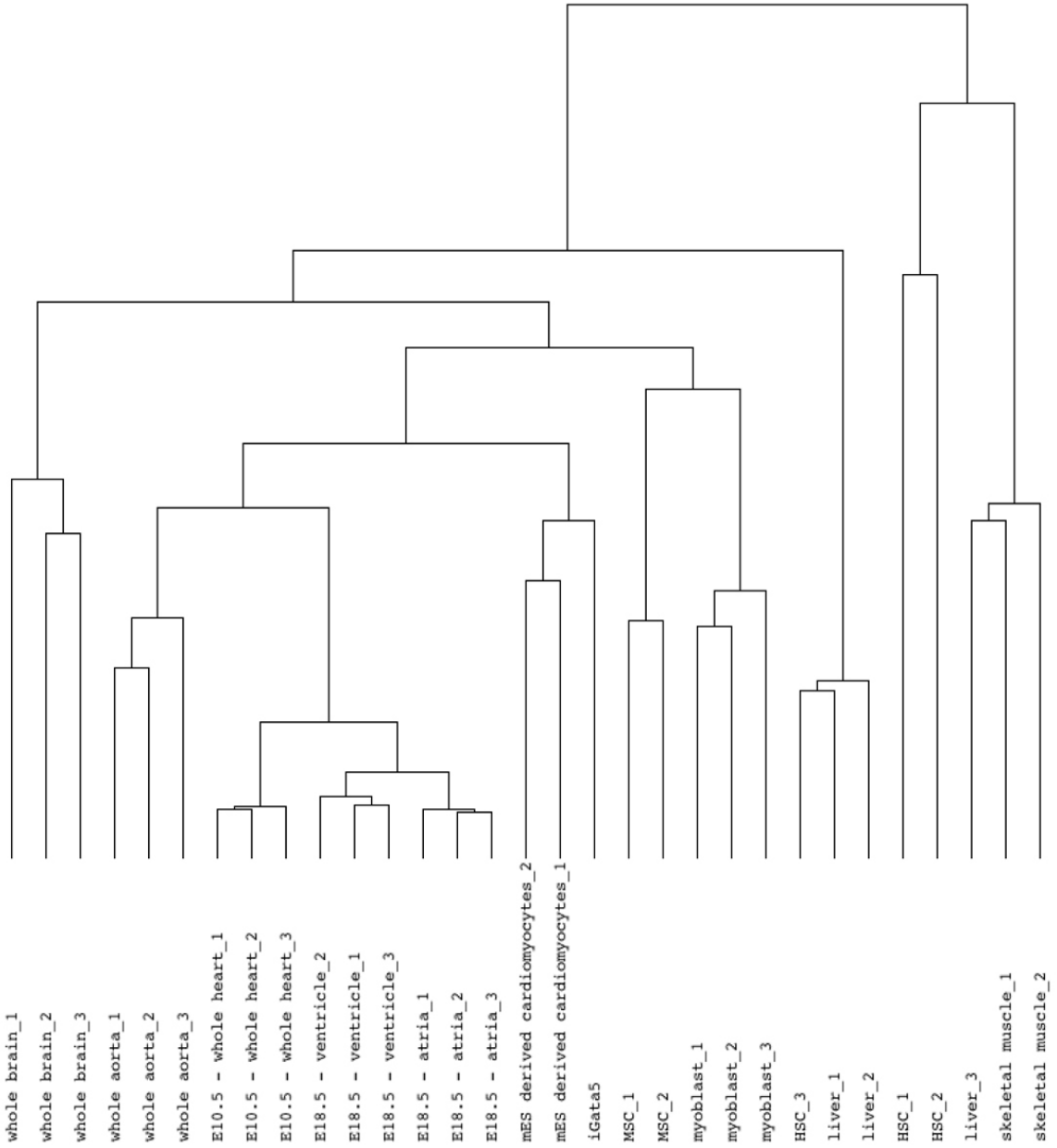

Fig. S3. RNA profiles of *Gata5*-induced EB derivatives cluster closely with mESC-derived cardiac precursors. ESC lines were induced with doxycycline at day 4, EB derivatives plated as a monolayer on fibronectin and RNA was isolated at day 16. After processing using a one-round in vitro transcription (IVT) system (MessageAmp Premier RNA Amplification Kit, Ambion/Applied Biosystems, Austin, TX), the cRNA was labeled with biotin, fragmented and hybridized to the Affymetrix Mouse Genome 430 Plus 2.0 GeneChip arrays (Santa Clara, CA). The array was scanned using GeneChip Scanner 3000 7G, and Affymetrix GeneChip Operating Software was used for image acquisition. The data normalization and statistical analysis were performed with Array Star 2 (DNASTAR, Madison, WI, USA). Shown is the dendrogram from an unsupervised hierarchical clustering of Gata5-induced EB derivatives in comparison with a variety of published microarray datasets (supplementary material Table S1).

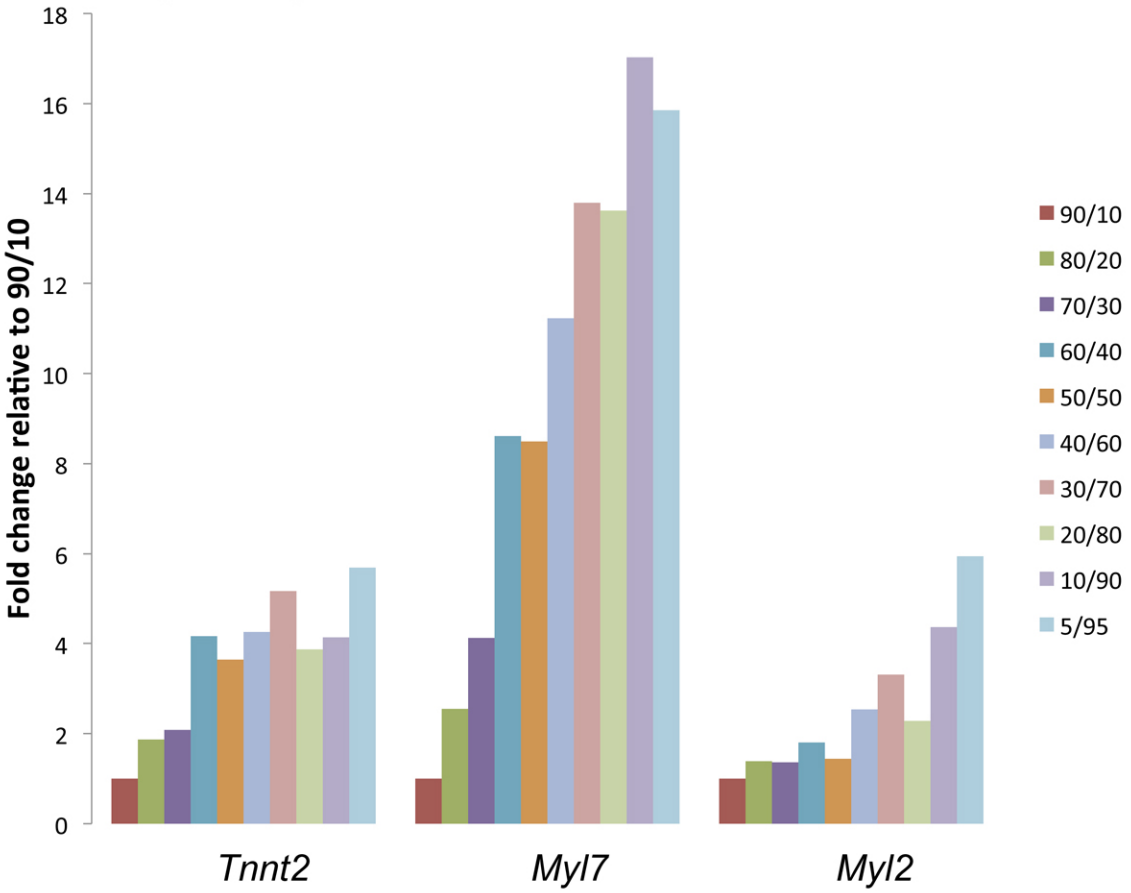

Fig. S4. Cardiac output is directly proportional to input of Gata5-expressing ESC derivatives. EB cultures were generated using mixed ratios of either parental AinV cells or the iGata5 doxycycline-inducible cells. EBs were induced at day 4 and harvested at day 6, and evaluated for expression of cardiac differentiation markers by qPCR, as in Fig. 1C. Shown are results from a representative experiment that was reproduced three times. For each gene product, transcript levels are normalized to the sample containing only 10% iGata5ES cells (far left red bar, 90/10). Moving to the right, each sample contained increasing percentage of iGata5ES cells, in the ratio of AinV to iGata5ES (e.g. 80/20). Although there is some sample variability, the increase in cardiac transcript levels is overall proportional to the input of iGata5ES cells.

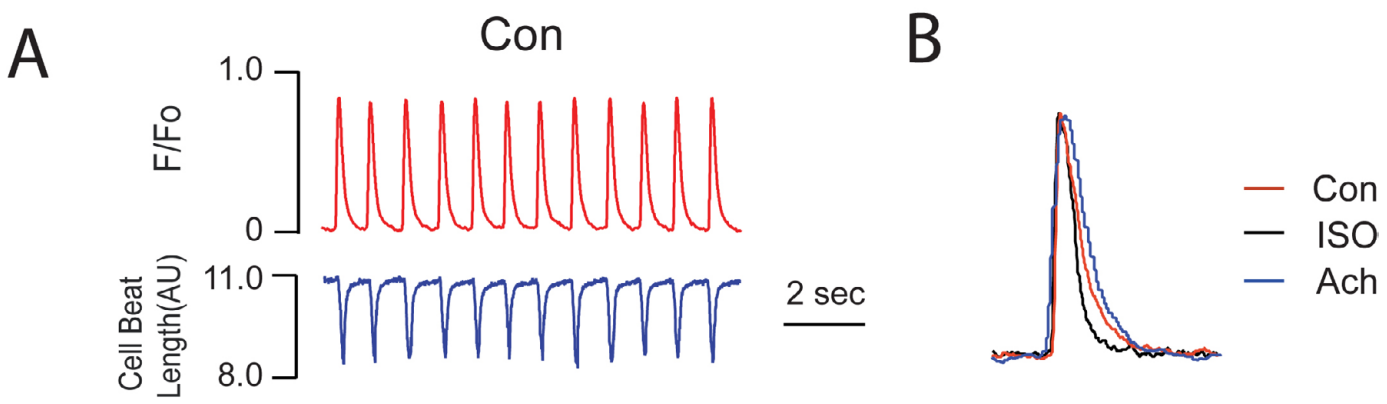

Fig. S5. *Gata5*-induced cardiac precursors demonstrate changes in Ca^{2+} oscillation kinetics. iGata5ES-derived cardiac precursors were dissociated with 0.25% trypsin-EDTA and plated onto laminin-coated coverslips at low density. Ca^{2+} fluorimetry was carried out using Fluo-4/AM. Cells were exposed to a dye-loading solution consisting of a standard Tyrodes solution. (**A**) A representative example of spontaneous Ca^{2+} oscillations and cell beat length changes in control iGata5ES-derived cardiac precursors. (**B**) Addition of 10 μM acetylcholine (Ach) or 1 μM isoproterenol (Iso) results in expected changes in Ca^{2+} oscillation kinetics as seen in superimposed tracings.

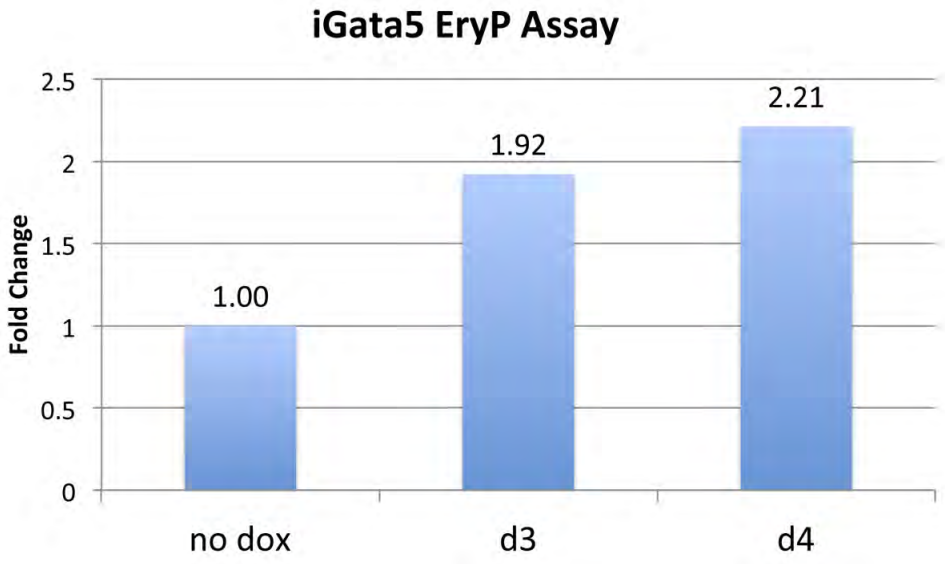

Fig. S6. Expression of Gata5 only modestly enhances hematopoiesis. In order to score hematopoietic progenitors, the iGata5ES cells were first re-conditioned to grow in the presence of serum on feeders, induced with doxycycline at day 3 or day 4. At day 6, an equivalent number of cells were plated with methycellulose in the presence of erythropoietin, and hematopoietic (primitive erythroid cell) colonies were counted 5 days later. Shown are representative results from a single experiment that was reproduced with similar results in three independent experiments.

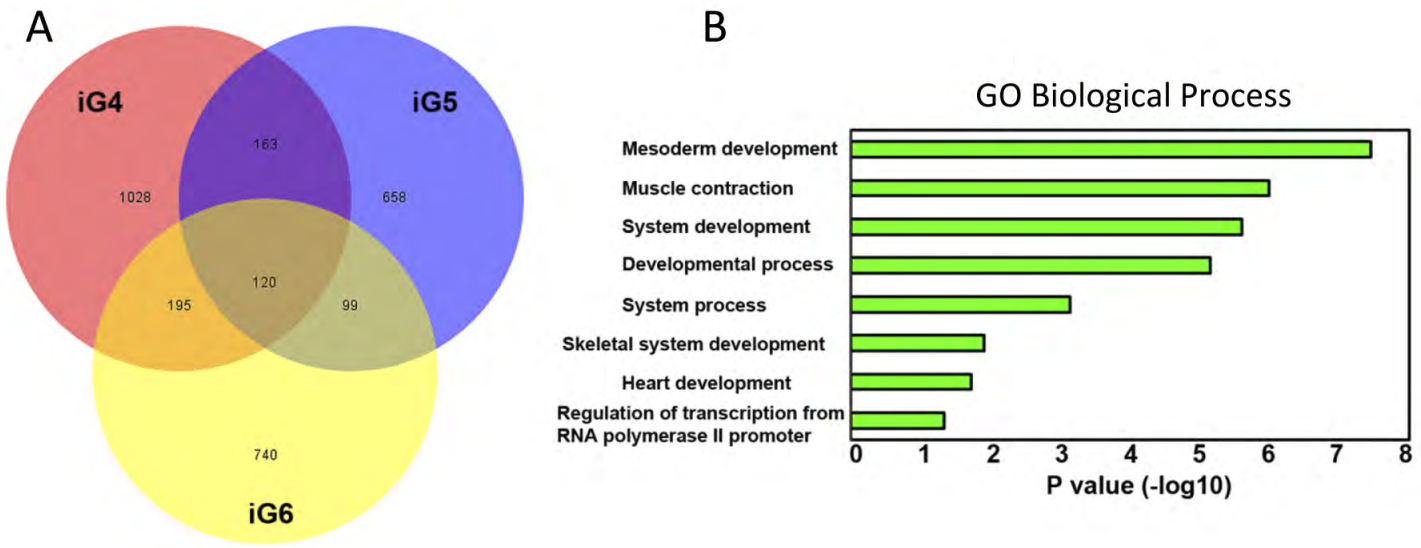

Fig. S7. Each of the GATA factors can robustly initiate cardiogenesis from ESCs. (**A**) In three independent experiments, EBs were induced with doxycycline at day 4 for 1 day and harvested for RNA profiling using Affymetrix microarrays, compared with uninduced controls. For each line, iGata4ES (iG4), iGata5ES (iG5) or iGata6ES (iG6), the gene sets activated or repressed at least fivefold compared with uninduced controls were compared (GenePattern). Venn diagrams indicate the overlap in these gene sets. (**B**) Gene ontology was analyzed for the triple-overlap set identified in A, comprising 120 genes that are highly associated with cardiac muscle developmental programs.

Movie 1. Monolayer of *Gata-5* induced EB derivatives displays extensive contractile activity. Real-time video of representative day 9 monolayer of EB derivatives on a fibronectin-coated well show extensive beating sheets of cardiomyocytes.

Table S1. List of microarray datasets used for hierarchical clustering

Label	GEO ID	Description
Whole brain 1,2,3	GSM 200695, 200696, 200697	C57BL/6 E9.5 whole brain
Whole aorta 1,2,3	GSM 238478, 238479, 238480	C57BL/6 2.5 to 4.5 month old whole aorta
E10.5 whole heart 1,2,3	GSM 25150, 25151, 25152	C57BL/6 E10.5 whole heart
E18.5 ventricle 1,2,3	GSM 25180, 25181, 25182	C57BL/6 E18.5 atrial chamber
E18.5 atria 1,2,3	GSM 25183, 25184, 25185	C57BL/6 E18.5 both ventricles
mES-derived cardiomyocytes 1,2	GSM 132680, 132681	mESC D3 transgenic line with neomycin resistance gene under the control of the alpha myosin heavy chain promoter (U71441)
MSC 1,2	GSM 476598, 476599	C57BL/6 bone marrow mesenchymal stem cells
HSC 1,2,3	GSM 291121, 291123	C57BL/6 8 week old hematopoietic stem cells
Myoblast 1,2,3	GSM 747404, 747405, 747406	C2C12 myoblast
Liver 1,2,3	GSM 300676, 300677, 300678	Mixed strain 28 day old liver
Skeletal muscle 1,2	GSM 385406, 385407	Wild type 7.5 month old gastrocnemius muscle

Table S2. List of qPCR primers

Gene	Forward (5′-3′)	Reverse (5′-3′)
Gapdh	CTAACATCAAATGGGGTGAGG	CGGAGATGATGACCCTTTTG
Nkx2.5	CATTTTACCCGGGAGCCTAC	CTTTGTCCAGCTCCACTGC
Tbx5	CAGGAGCACAGCCAAATTTAC	CCATGTACGGCTTCTTATAGGG
Gata4	TCTCACTATGGGCACAGCAG	GGGACAGCTTCAGAGCAGAC
FoxA2	GAGCCGTGAAGATGGAAGG	TCATGTTGCTCACGGAAGAG
Sox17	AGCAGAACCCAGATCTGCAC	GCTTCTCTGCCAAGGTCAAC
Nestin	AGGCGCTGGAACAGAGATT	GACATCTTGAGGTGTGCCAGT
Sox1	AACCCCAAGATGCACAACTC	TGTAATCCGGGTGTTCCTTC
Lmo2	ATCGAAAGGAAGAGCCTGGAC	GTCGATGGCTTTCAGGAAGTAG
Scl	AACAACAACCGGGTGAAGAG	CGCACTACTTTGGTGTGAGG
Cdh5 (*VE cad*)	ACCATCGCCAAAAGAGAGAC	TCTTGCCAGCAAACTCTCCT
Pecam1 (*Cd31*)	TGCACAGTGATGCTGAACAA	GCCTTCTGTCACCTCCTTTTT
Tnnt2 (*cTnt*)	CCTGCTGAGGCTGAACAGAT	CAGACATGCTCTCGGCTCTC
Myl7 (*Mlc2a*)	TTCTCATGACCCAGGCAGAC	CGTGGGTGATGATGTAGCAG
Myl2 (*Mlc2v*)	TGACCACACAAGCAGAGAGG	CCGTGGGTAATGATGTGGAC
Hcn4	GAGCCAGTACGCTCCAAACT	ACCTGAAGGAAGAAAGGAGCA
Gjc1 (*Conn45*)	TGGACTGCTGTAGTTACACTTTT	ACGAGAGGCACTTTTATTAAGTG

Table S3. List of antibodies

Primary	Secondary (flow cytometry)	Secondary (immunohistochemistry)
TNNT2 (CTNT) (Thermo, clone 13-11)	Anti-mouse IgG1 Alexa Fluor 647 (Invitrogen) Anti-mouse IgG Alexa Fluor 647 (Invitrogen) Anti-mouse IgG1 PE (eBioscience) Anti-mouse IgG1 FITC (Southern Biotech)	Anti-mouse IgG1 Alexa Fluor 568 (Invitrogen) Anti-mouse IgG1 FITC (Southern Biotech) Anti-mouse IgG Cy3 (Biomeda)
ACTA2 (SMA) (Abcam)	Anti-mouse IgG2a Alexa Fluor 488 (Invitrogen) Anti-mouse IgG Alexa Fluor 647 (Invitrogen)	Anti-mouse IgG2a Alexa Fluor 488 (Invitrogen)
PDGFRA (eBioscience)	*APC conjugated	n/a
KDR (FLK1) (eBioscience)	*PE conjugated	n/a
MYL7 (MLC2A) (Santa Cruz)	n/a	Anti-mouse IgG2b Alexa Fluor 568 (Invitrogen)