

SUPPLEMENTAL FIGURES

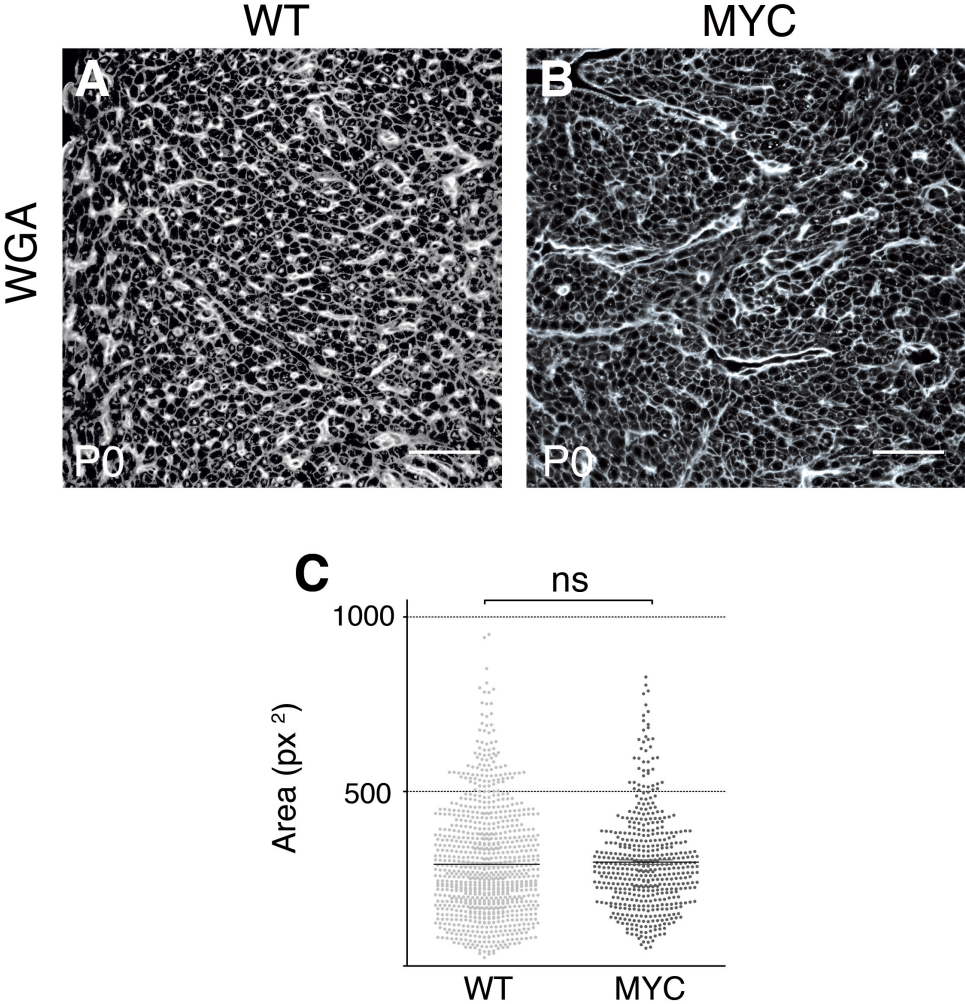


Figure S1 (related to Figure 2). Morphometric analysis in newborn mice of cardiomyocytes exposed to Myc overexpression during gestation

A and **B**, Confocal sections showing wheat germ agglutinin (WGA) staining of P0 hearts from *Nkx2.5Cre*-recombined *iMOS^{WT}* (WT) (**A**) and *iMOS^{T1-Myc}* (MYC) (**B**) mosaics. Bar, 50 μ m. **C**, Analysis of 2-D cardiomyocyte size in *iMOS^{WT}* (WT) and *iMOS^{T1-Myc}* (MYC) mosaics (N= 507 and 902 cells, respectively).

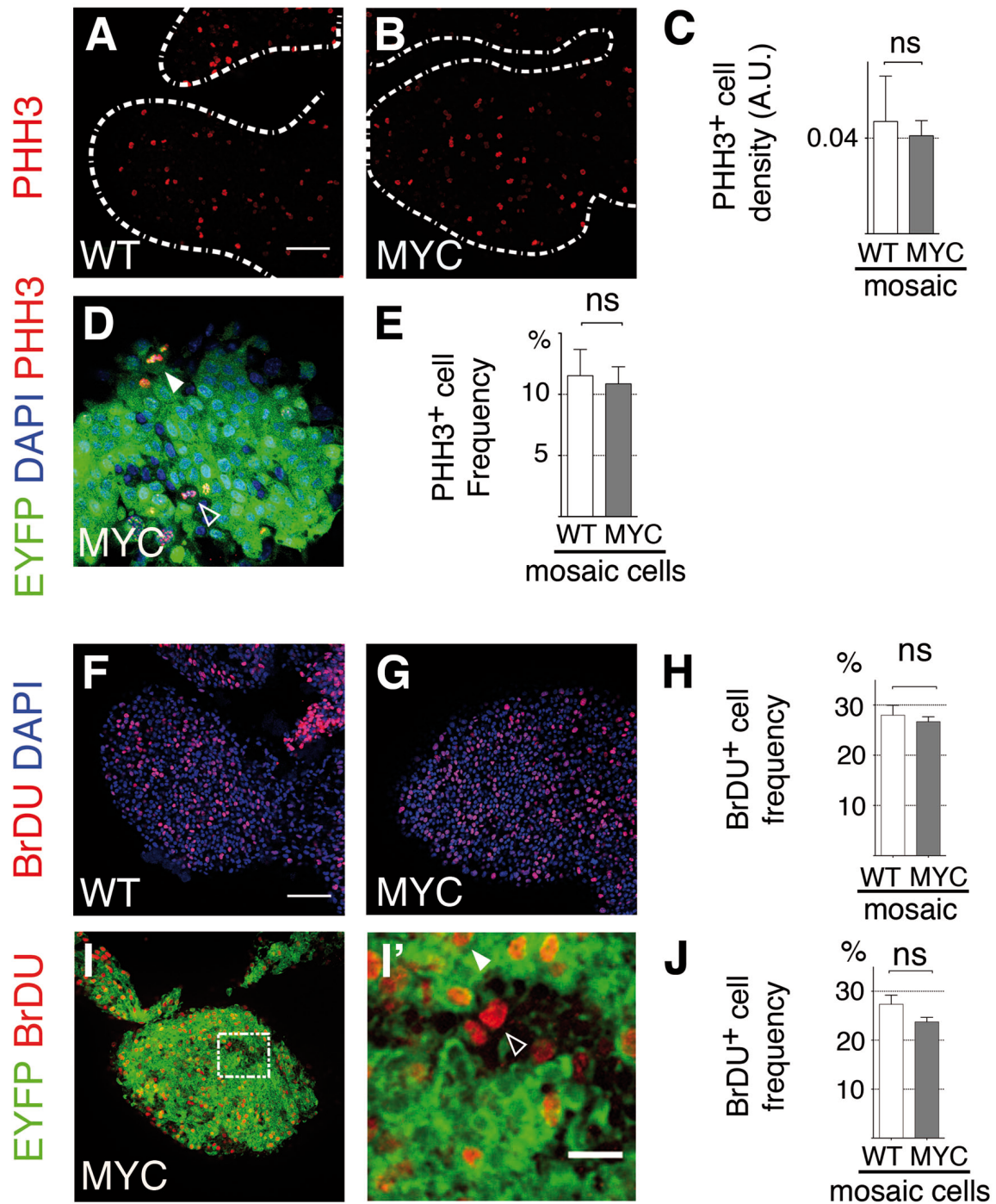


Figure S2 (related to Figure 4). Analysis of cell proliferation upon Myc mosaic overexpression in the developing heart

A, B, Z-projections from 30 μm confocal stacks obtained from E10.5 left ventricles of *iMOS*^{WT} (WT) and *iMOS*^{T1-Myc} (MYC) mosaics, showing anti PHH3 staining to mark cell division. Bar, 50 μm . **C**, Total PHH3 density measured as number of positive cells per arbitrary area units at E10.5 in *iMOS*^{WT} (WT) and *iMOS*^{T1-Myc} (MYC) mosaics (N=4 hearts, N \geq 20 positive

cells/heart). **D**, Confocal section of a MYC mosaic left ventricle at E10.5, showing the distribution of EYFP⁺, ECFP⁺ and PHH3⁺ cells. Bar, 50 μ m. **E**, PHH3⁺ nuclei frequency in the EYFP and ECFP populations of *iMOS*^{T1-Myc} mosaics (N=5 embryos and \geq 680 nuclei). The filled arrowhead marks an EYFP⁺, PHH3⁺ cell and the empty arrowhead, an ECFP⁺, PHH3⁺ cell. Data are means \pm SEM.*p<0.1; **p<0.05; ***p<0.001. **F,G**. Confocal sections from E10.5 left ventricles of *iMOS*^{WT} (WT) and *iMOS*^{T1-Myc} (MYC) mosaics showing anti BrDU staining. **H**. BrDU⁺ cardiomyocyte frequency analyzed at E10.5 in *iMOS*^{WT} (WT) and *iMOS*^{T1-Myc} (MYC) mosaics (N=4 hearts, N \geq 20 positive cells/heart). **I**, Confocal section of an *iMOS*^{T1-Myc} mosaic (MYC) E10.5 left ventricle showing the distribution of EYFP⁺ and BrDU⁺ cells. **I'**, detailed image of the boxed area in **I**. **J**, BrDU⁺ cardiomyocyte frequency in the EYFP-Myc (MYC) and ECFP-WT (WT) populations of the *iMOS*^{T1-Myc} mosaic (N=4). Bar, 20 μ m. Filled arrowhead shows an EYFP⁺, BrDU⁺ cell and empty arrowhead, an ECFP⁺, BrDU⁺ cell. Data are mean \pm SEM.*p<0.1; **p<0.05; ***p<0.001

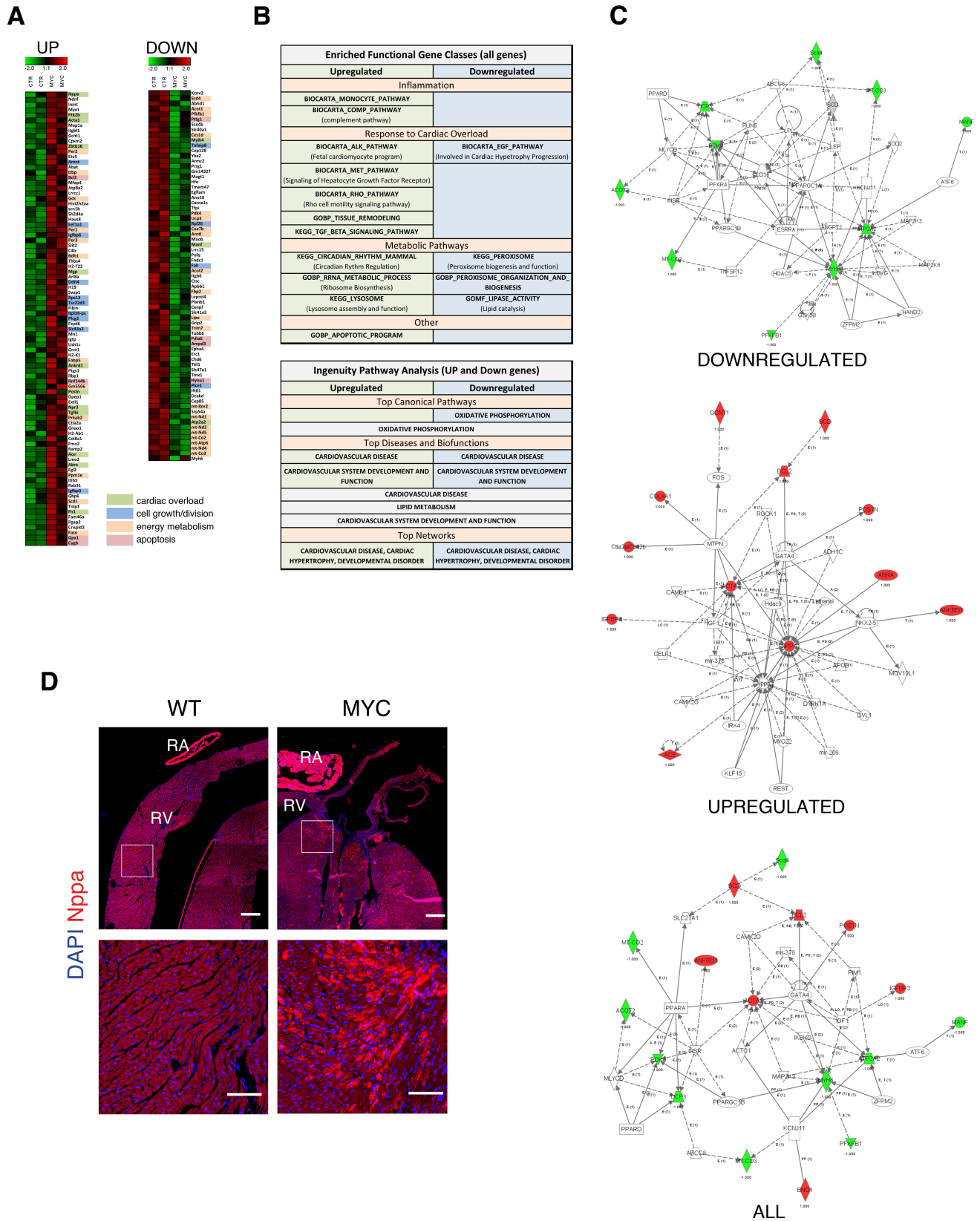


Figure S3 (related to Figure 7). Transcriptomic analysis of the changes induced by Myc mosaic overexpression in the adult heart

A, representation of the list of genes up- or down-regulated in *iMOS*^{T1-Myc} mosaics with respect to controls and their expression levels. Criteria for inclusion were adjusted p -value ≤ 0.05 and expression levels ≥ 10 counts/million reads in at least one condition. Genes are colour-coded for involvement in predominant known functional classes for this gene set (see legend). **B**, top table; significant ($p \leq 0.05$) gene sets enriched in up- or down-regulated genes, including all genes in the analysis. Lower table; Top Ingenuity pathways and networks detected in the gene set shown in **A** ($p \leq 0.01$). Pathways include those detected in the independent analysis of the upregulated and downregulated genes and in their joint analysis. **C**, Representation of the Top Networks found regulated in the Ingenuity analysis of the downregulated (top), upregulated (center) and joint list of genes (bottom). **D**, expression analysis of Nppa protein in adult *iMOS*^{WT} (WT) and *iMOS*^{T1-Myc} (MYC) mosaics. Top panels show low magnification images of immunofluorescence detection of Nppa and boxed areas are magnified in the bottom panels. RA, right atrium, RV, right ventricle. Bars, 100 μ m top; 50 μ m bottom.

SUPPLEMENTAL MOVIE

Movie S1 (related to Figure 4). 3D reconstruction animation showing the distribution of apoptotic cells and induced EYFP-Myc cells in the OFT region of *Islet1MerCreMer*-recombined *iMOS*^{T1-Myc} E10.5 hearts.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

BrDU incorporation assay

Pregnant females were administered BrDU intraperitoneally (3mg at 10mg/ml) 2 hours prior to embryo extraction; embryos were processed as mentioned previously. Adult mice were given BrDU in drinking water (0,5mg/ml) for one month, starting at 5 weeks from birth. To detect BrDU, embryos or isolated cardiomyocytes were treated with DNaseI for 30 minutes and staining was performed using anti BrDU antibody (BD biosciences).

3D-embryonic heart reconstruction and apoptosis quantification

Embryonic *iMOS* MYC E10.5 hearts were reconstructed using Imaris x64 software.

Quantification of Myc protein and EYFP expression

Myc protein expression was quantified on confocal sections using ImageJ. Medial LV sections were analyzed. The nuclear region was defined from the DAPI stain and the average intensity of Myc immunofluorescent detection with Cy3 was measured within the DAPI-defined nuclear areas. Values were classified into defined intensity intervals and represented as interpolated curves derived from the original frequencies.

EYFP intensity was analyzed in confocal images of isolated cardiomyocytes using ImageJ. Cells were detected by autofluorescence in the green channel and the average EYFP intensity was measured for each detected cardiomyocyte in arbitrary units. Values were classified into intervals and assigned to EYFP-negative, -medium or -high expression categories, which were defined identically for all experimental genotypes.

Echocardiography

Mice were anesthetized by isoflurane inhalation (1.25%) and examined with a 30-MHz transthoracic echocardiography probe. Images were obtained with Vevo 770 (VisualSonics, Toronto, Canada) from *iMOS*^{T1-Myc}; α MHCmerCremer and wild type littermates. Short-axis, long-axis, B-mode and two-dimensional M-mode views were obtained as previously described (Cruz-Adalia et al., 2010). Left ventricle function was estimated from the ejection fraction, obtained from M-mode echocardiographic images by a blinded echocardiography expert. For these measurements, a long- or short-axis view of the heart was selected to obtain an M-mode registration in a line perpendicular to the left ventricular septum and posterior wall at the level of the mitral chordae tendinea. For the exercise challenge mice were swimming for 90 min 5 days/week during two months.

RNASeq library production

In order to assess the gene expression changes in *iMOS*^{T1-Myc} hearts upon mosaic Myc overexpression, total RNA was extracted at from hearts induced to recombine with tamoxifen at t1, according to the scheme in Figure 5. 2 *iMOS*^{T1-Myc}; α *MHCmerCremer* hearts were used as experimental hearts and 2 *iMOS*^{T1-Myc} littermates without α *MHCmerCremer* were used as control. RNA was isolated using standard procedures and analyzed using RNA-Seq. Total RNA was quantified by absorbance at 260 nm in a NanoDrop spectrophotometer and its integrity was determined using an Agilent Bioanalyzer (Santa Clara, CA). Total RNA (1 μ g) was used with the TruSeq RNA Sample Preparation v2 Kit (Illumina, San Diego, CA) to construct index-tagged cDNA libraries. The quality, quantity and the size distribution of the Illumina libraries were determined using the DNA-1000 Kit (Agilent Bioanalyzer). Prepared cDNA libraries were applied to an Illumina flow cell for cluster generation (True Seq SR Cluster Kit V2 cBot) and sequence-by-synthesis single reads of 75 base length using the TruSeq SBS Kit v5 (Illumina) were generated on the Genome Analyzer Iix following the standard RNA sequencing protocol.

RNASeq data analysis

Sequencing adaptor contaminations were removed from reads using cutadapt software (<http://code.google.com/p/cutadapt/>) and the resulting reads were mapped and quantified on the transcriptome (Ensembl gene-build 70) using RSEM v1.2.3 (Li and Dewey, 2011). Only genes with at least 2 counts per million in at least 1 sample were considered for statistical analysis. Data were then normalized and differential expression tested using the bioconductor package EdgeR (Robinson et al., 2010). We considered as differentially expressed those genes with a Benjamini-Hochberg adjusted p-value ≤ 0.05 . Analysis of the data was performed using Gene set enrichment analysis and Ingenuity pathways analysis software (Biobase International). The data have been deposited in the NCBI GEO database with the accession number GSE58858.

SUPPLEMENTAL REFERENCES

Cruz-Adalia, A., Jimenez-Borreguero, L.J., Ramirez-Huesca, M., Chico-Calero, I., Barreiro, O., Lopez-Conesa, E., Fresno, M., Sanchez-Madrid, F., and Martin, P. (2010). CD69 limits the severity of cardiomyopathy after autoimmune myocarditis. *Circulation* 122, 1396-1404.

Li, B., and Dewey, C.N. (2011). RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC bioinformatics* 12, 323.

Robinson, M.D., McCarthy, D.J., and Smyth, G.K. (2010). edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics (Oxford, England)* 26, 139-140.