Supplemental Material

Data S1.

Supplemental Methods

Statistics - Pathway Analysis

iPathwayGuide scores pathways using the Impact Analysis method[1-3]. Impact analysis uses two types of evidence: i) the over-representation of differentially expressed (DE) genes in a given pathway and ii) the perturbation of that pathway computed by propagating the measured expression changes across the pathway topology. These aspects are captured by two independent probability values, pORA and pAcc, that are then combined in a unique pathway-specific p-value. The underlying pathway topologies, comprised of genes and their directional interactions, are obtained from the KEGG database.

The first probability, pORA, expresses the probability of observing the number of DE genes in a given pathway that is greater than or equal to the number observed, by random chance. Let us consider there are N genes measured in the experiment, with M of these on the given pathway. Based on the a priori selection of DE genes (genes with a log fold change differential expression of 0.6 and p=0.05), K out of M genes were found to be differentially expressed. The probability of observing exactly x differentially expressed genes on the given pathway is computed based on the hypergeometric distribution:

$$P(X=x|N,M,K) = \frac{\binom{M}{x}\binom{N-M}{K-x}}{\binom{N}{K}}$$

Table S1. Primer sequences used to assess gene expression.

Gene name	Primer Sequence (5’ -> 3’)
Aldehyde Dehydrogenase 2 (ADLH2)	TGAAGACGGTTACTGTCAAAGTGC AGTGTGTGTGGCGGTTTTTCTC
Superoxide Dismutase 2 (SOD2)	GGCGCCTCTCAGATAAACAG GGCTCATTGGGTCCTTGTTA
Heme Oxygenase 1 (HO-1)	CACAGCACTATGTAAAGCGTCT GTAGCGGGTATATGCGTGGG
Voltage-dependent anion-selective channel protein 1 (VDAC1)	CCCACATACGCCGATCTTGG GCTGCCGTTCACTTTGGTG
Citrate Synthase (CS)	GTTAGCTGGAGACGCTTTGG AGAGGCCTGGAAGGAAACAT
Histidine-rich calcium binding protein (HRC)	GAGACTCGGCAGAGAACCAC CATCACATCCACCCTCTCCT

Table S2. Antibodies used to assess protein expression.

Antibody	Catalog Number/Company	Dilution
4-hydroxynonenal **(**4HNE)	HNE11-S; Alpha Diagnostic International	1:1000
Voltage-dependent anion-selective channel protein 1 (VDAC1)	Ab14734; Abcam	1:2000
Dynamin 1-like **(**DRP1/DLP1)	611113; BD	1:2000
Mitochondrial fission factor **(**MFF)	17090-I-AP; Proteintech	1:1000
Mitochondrial dynamin like GTPase (OPA1)	612607; BD	1:2000
Enolase	sc-15343; Santa Cruz Biotechnology	1:2000

Table S3. Buffers and reagents used to measure oxygen consumption.

	Reagents	Final concentration in assay buffer
Oxygraph buffer	20 mM HEPES, 5 mM K_3PO_4, 0.2 mM EDTA, 2.5 mM $MgCl_2$, 10 mM KCl, 0.25 M sucrose, 1 mg/mL fatty acid free bovine serum albumin; pH 7.4	-
Malate	1 M malate	5 mM
Glutamate	2 M glutamate	5 mM
Succinate	1 M succinate	20 mM
ADP	0.5 M ADP	200 µM
Oligomycin	4 mg/ml oligomycin	0.5 µM
FCCP	1 M carbonyl cyanide-p-trifluoromethoxyphenylhydrazone	1 µM
Antimycin A	5 mM antimycin A	1 µM

Because the hypergeometric distribution is discrete, the probability of observing fewer than x genes on the given pathway just by chance can be calculated by summing the probabilities of randomly observing 0, 1, 2, ..., up to x-1 DE genes on the pathway:

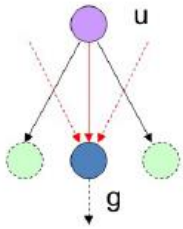
$$p_u(x-1) = P(X=1)+P(X=2)+\ldots+P(X=x-1) = \sum_{i=0}^{x-1} \frac{\binom{M}{i}\binom{N-M}{K-i}}{\binom{N}{K}}$$

iPathwayGuide calculates the probability of randomly observing a number of DE genes on the given pathway that is greater than or equal to the number of DE genes obtained from data, by computing the over-representation p-value: pORA = p (x) = 1 - p (x-1):

$$p_o(x) = 1 - \sum_{i=0}^{x-1} \frac{\binom{M}{i}\binom{N-M}{K-i}}{\binom{N}{K}}$$

The second probability, pAcc, is calculated based on the amount of total accumulation measured in each pathway. A perturbation factor is computed for each gene on the pathway using:

$$PF(g) = \alpha(g) \cdot \triangle E(g) + \sum_{u \in US_g} \beta_{ug} \frac{PF(u)}{N_{ds}(u)}$$



PF(g) is the perturbation factor for gene g, the term ΔE(g) represents the signed normalized measured expression change of gene g, and α(g) is a priori weight based on the type of the gene. The last term is the sum of the perturbation factors of all genes u, directly upstream of the target gene g, normalized by the number of downstream genes

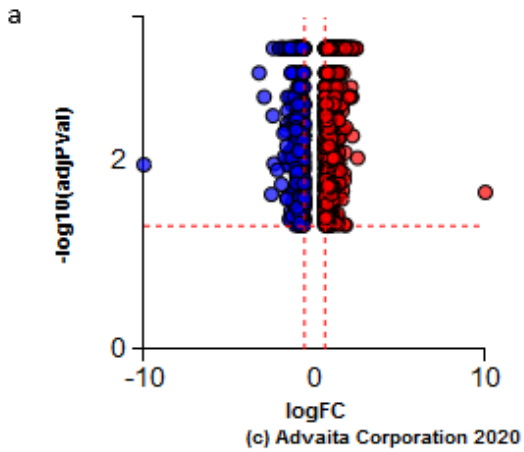
of each such gene N (u). The value of β quantifies the strength of the interaction between genes g and u. The sign of β represents the type of interaction: positive for activation-like signals, and negative for inhibition-like signals. Subsequently, iPathwayGuide calculates the accumulation at the level of each gene, Acc(g), as the difference between the perturbation factor PF(g) and the observed log fold-change:

$$Acc(g_i) = PF(g_i) - \triangle E(g_i)$$

Once all gene perturbation accumulations are computed, iPathwayGuide computes the total accumulation of the pathway as the sum of all absolute accumulations of the genes in a given pathway. The significance of obtaining a total accumulation (pAcc) at least as large as observed, just by chance, is assessed through bootstrap analysis. The two types of evidence, pORA and pAcc, are combined into an overall pathway score by calculating a p-value using Fisher's method. This p-value is then corrected for multiple comparisons using false discovery rate (FDR). Methods provided by https://ipathwayguide.advaitabio.com/report.

Figure S1. Volcano plots for each pair comparison for RNA-sequencing data.

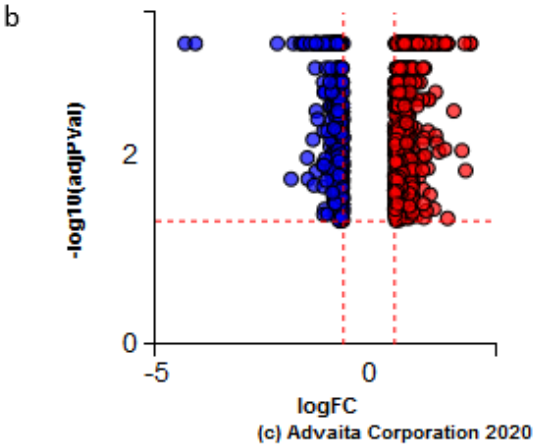
RV Sham vs. LV Sham



Organism: Mus musculus
Differentially Expressed (DE) genes: 1695
All genes with measured expression: 8049
DE thresholds:

- fold change: 0.6
- p-value: 0.05

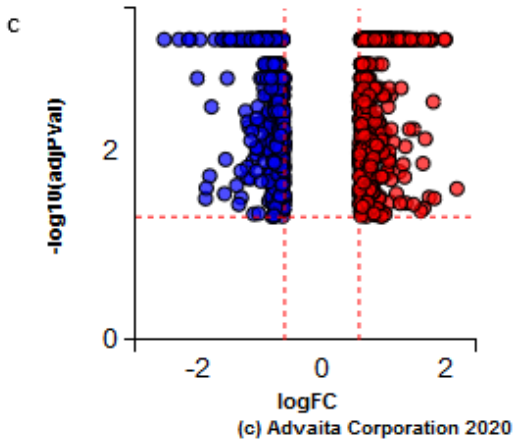
LV Sham vs. LV RVF



Organism: Mus musculus
Differentially Expressed (DE) genes: 887
All genes with measured expression: 7992
DE thresholds:

- fold change: 0.6
- p-value: 0.05

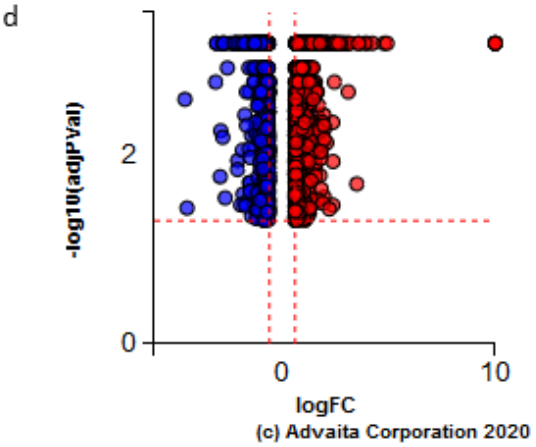
RV RVF vs. LV RVF



Organism: Mus musculus
Differentially Expressed (DE) genes: 1004
All genes with measured expression: 8075
DE thresholds:

- fold change: 0.6
- p-value: 0.05

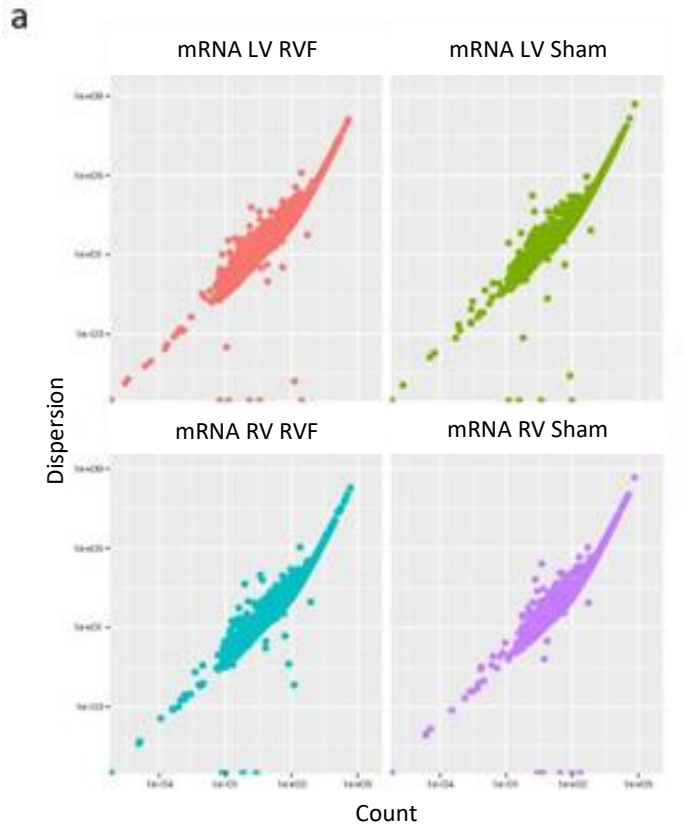
RV Sham vs. RV RVF



Organism: Mus musculus
Differentially Expressed (DE) genes: 1946
All genes with measured expression: 8190
DE thresholds:

- fold change: 0.6
- p-value: 0.05

(a) RV Sham vs. LV Sham; (b) LV Sham vs. LV RVF; (c) RV RVF vs. LV RVF; (d) RV Sham vs. RV RVF. For each pair, red and blue indicate the second dataset has increased or decreased expression compared to the first term, respectively. Genes with a log fold change differential expression (DE) of 0.6 (x-axis) and p=0.05 are shown. Significance is represented as negative log (base 10) of the p-value (y-axis). The figures were obtained with iPathwayGuide (www.advaitabio.com). RV – right ventricle, LV – left ventricle, RVF – RV failure.


a
mRNA LV RVF
mRNA LV Sham
mRNA RV RVF
mRNA RV Sham
Dispersion
Count

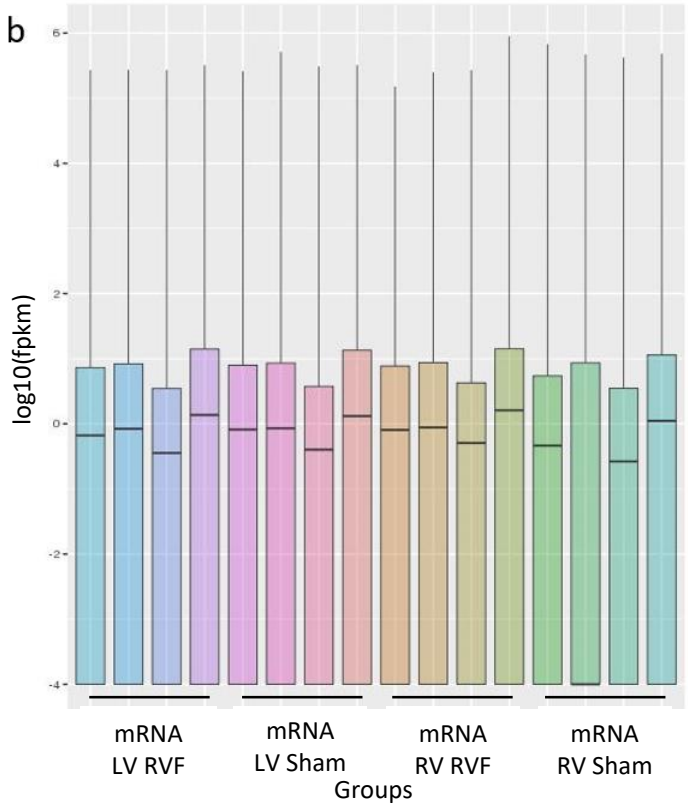
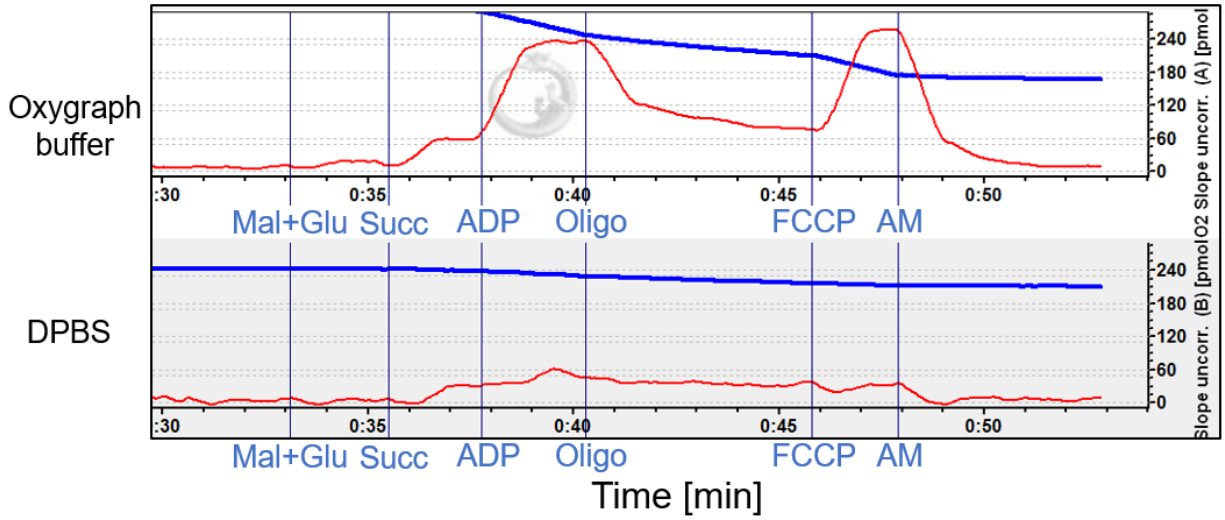

b
log10(fpkm)
6
4
2
0
-2
-4
mRNA LV RVF
mRNA LV Sham
mRNA RV RVF
mRNA RV Sham
Groups

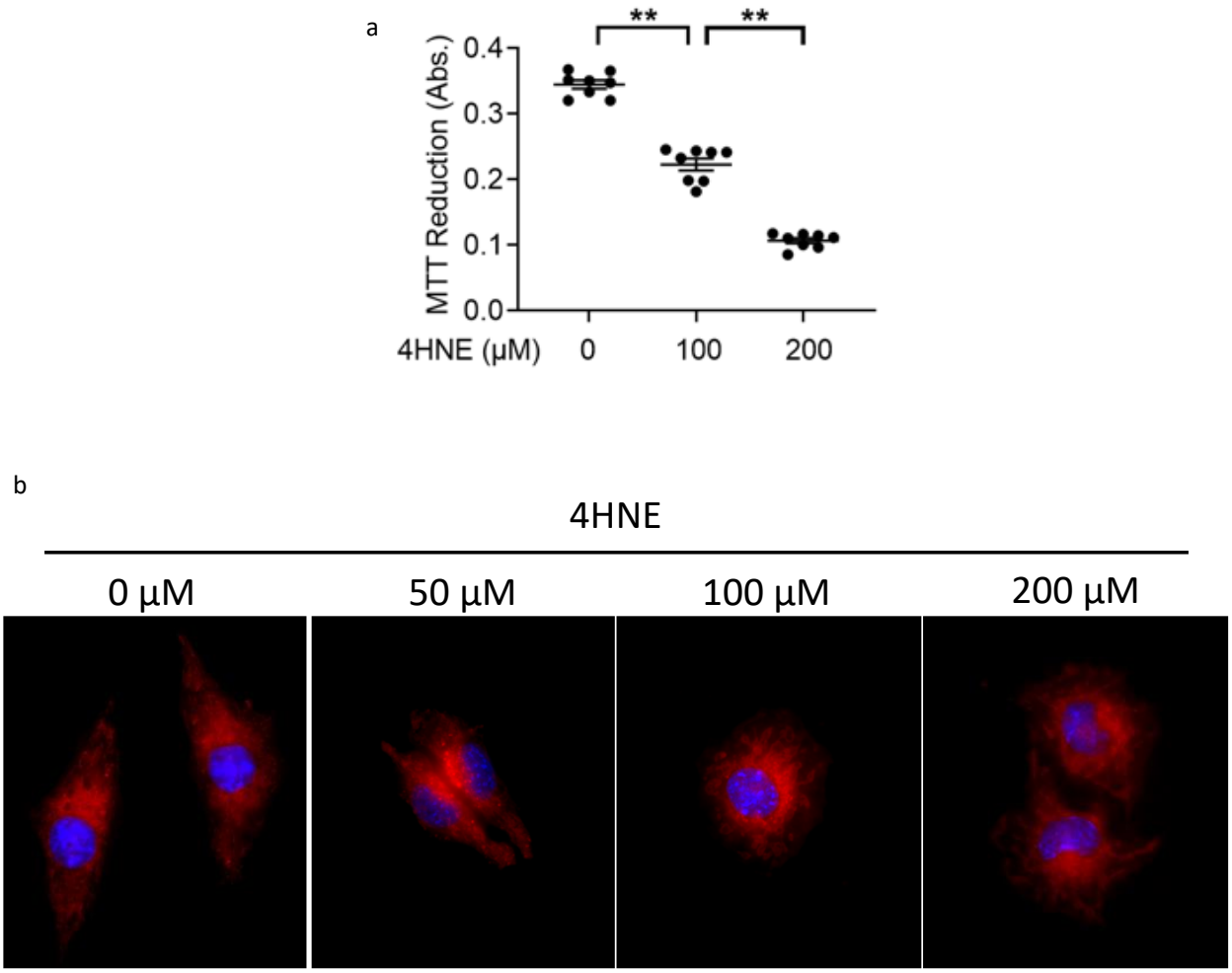
Figure S2. (a) CummeRbund Dispersion and (b) Box plots are shown to show the spread of the FPKM values in the individual samples (N=4/group). FPKM - Fragments Per Kilobase of transcript per Million mapped reads, RV – right ventricle, LV – left ventricle, RVF – RV failure.

Figure S3. Oxygraph buffer allows for mitochondrial substrates to permeate into tissues.



We used a high-resolution Oxygraph2K respirometer to measure oxygen consumption in RV myocardium. (a) A representative oxygen consumption [pmol/s/mL] tracing is shown for myocardial tissue (red curve). The blue curve represents oxygen concentration in the assay chamber. We evaluated oxygen consumption rates due to leak respiration during substrate utilization by complex I-NADH dehydrogenase and complex II-succinate dehydrogenase (green shades), and due to oxidative phosphorylation by complex V-ATP synthase (yellow shade). Minced heart tissue exhibits robust oxygen consumption in the (top) Oxygraph buffer, but not in (bottom) DPBS. Tissues suspended in DPBS failed to show any response even when the cell-permeable agent FCCP was administered, since the exogenous substrates (malate, glutamate, succinate, and ADP) could not readily enter the tissues. Mal+Glu – malate and glutamate; FCCP - carbonyl cyanide-p-trifluoromethoxyphenylhydrazone; AM – antimycin A; DPBS – Dulbecco's phosphate buffered saline.

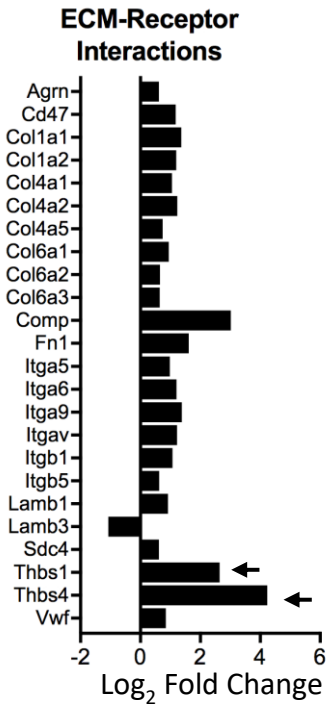
Figure S4. 4HNE dose testing in HL1 cardiomyocytes.



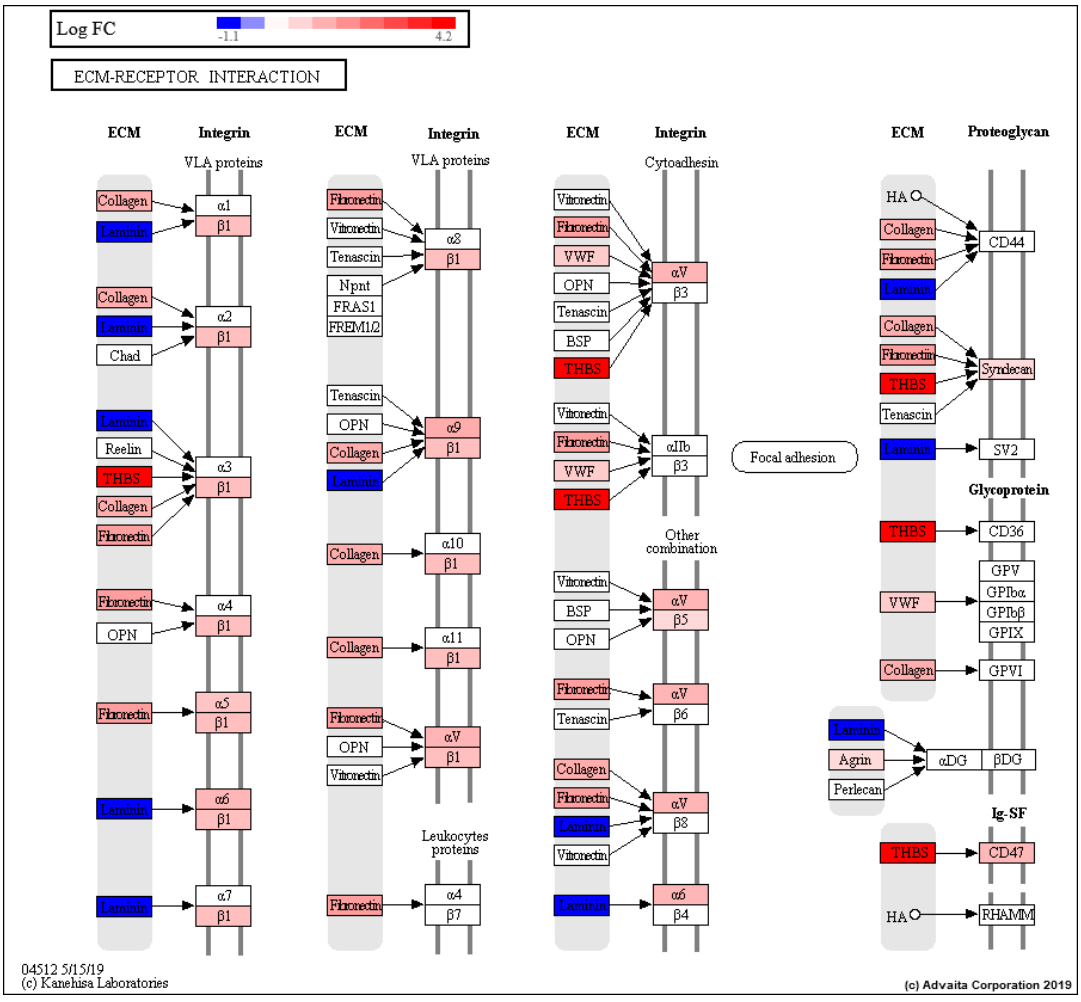
(a) MTT assay showed a decline in cell viability at 100 and 200 μM 4HNE-treatment. N=8/group, **p<0.001. (b) Mitochondrial morphology of cardiomyocytes following 1 hr 4HNE treatment. Cells were stained with Mitotracker™ Red CMX ROS and Hoechst 33342 for mitochondria and nuclear staining, respectively. 100 and 200 μM 4HNE-treated cells showed swollen mitochondria, a characteristic of imminent cell death while 50 μM 4HNE treated cells did not.

Figure S5. Extracellular matrix-receptor interactions are upregulated in the failing RV.

a



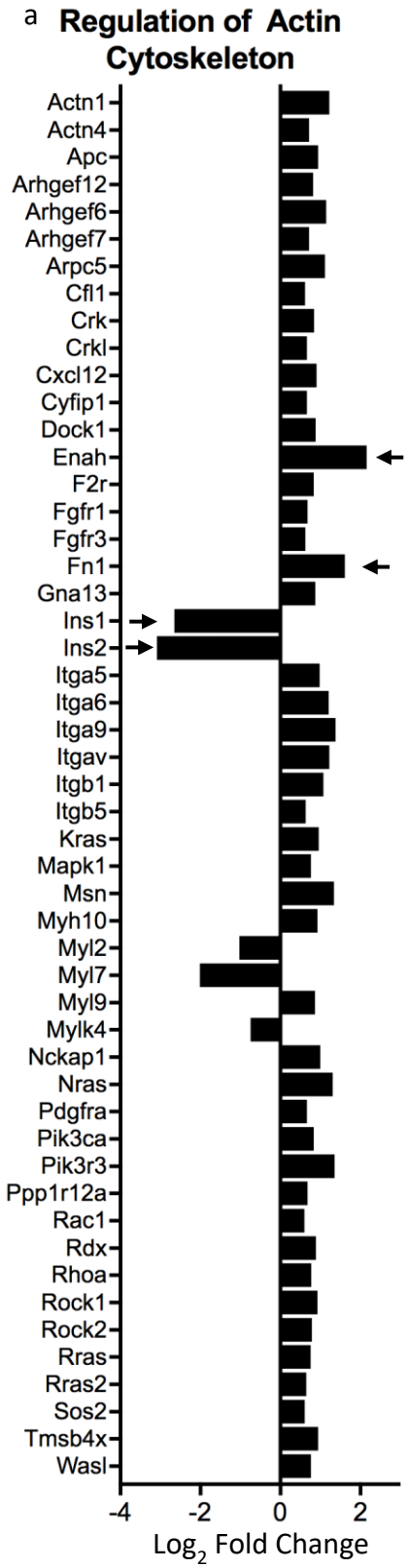
b



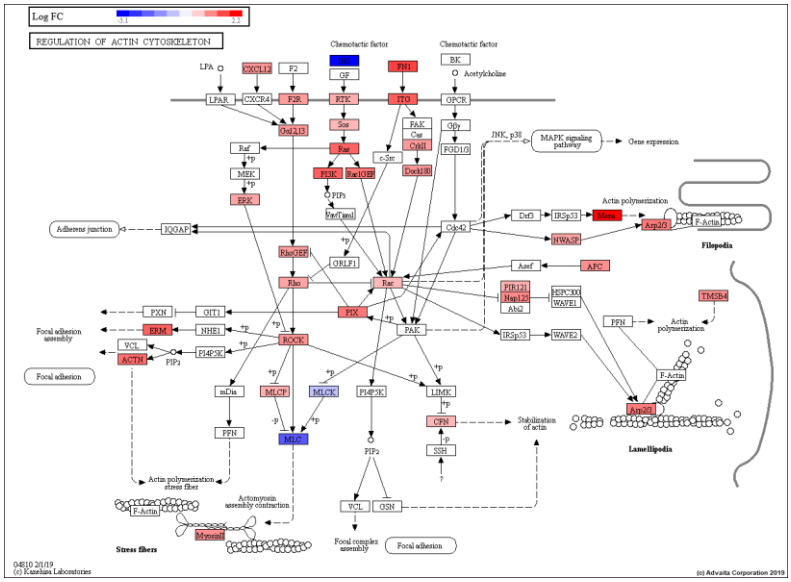
(a) Genes involved in ECM-receptor interactions are upregulated in RV failure vs. Sham with thrombospondins (arrows) being the most highly expressed genes. (b) Graphical representation of the dysregulated genes (N=4/group), Blue – downregulated, Red – upregulated, q-value = 2E-5. The figure was obtained with iPathwayGuide (www.advaitabio.com), using pathway data obtained from the KEGG resource (pathway #04512)[33-35]. ECM – Extracellular matrix; RV – Right ventricle.

Figure S6. Actin cytoskeleton regulation is upregulated in the failing RV.

a

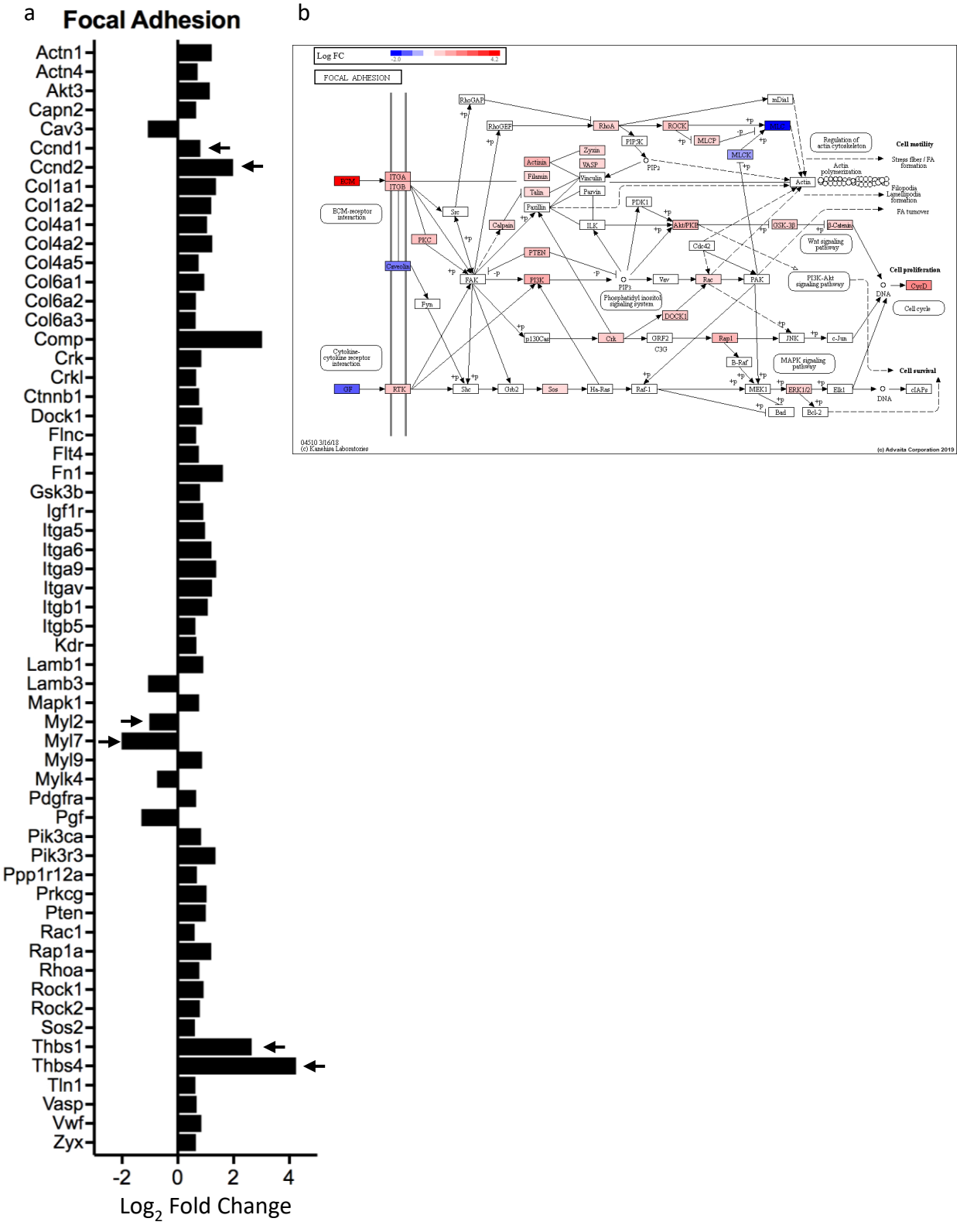


b



(a) Genes involved in actin polymerization are upregulated in RV failure vs. Sham with Enah and fibronectins (arrows) being the most highly expressed genes and insulin 1 and 2 (arrows) the most downregulated. (b) Graphical representation of the dysregulated genes (N=4/group), Blue – downregulated, Red – upregulated, q-value = 0.0002. The figure was obtained with iPathwayGuide (www.advaitabio.com), using pathway data obtained from the KEGG resource (pathway #04810)[33-35]. RV – Right ventricle; Enah – Enabled Homolog.

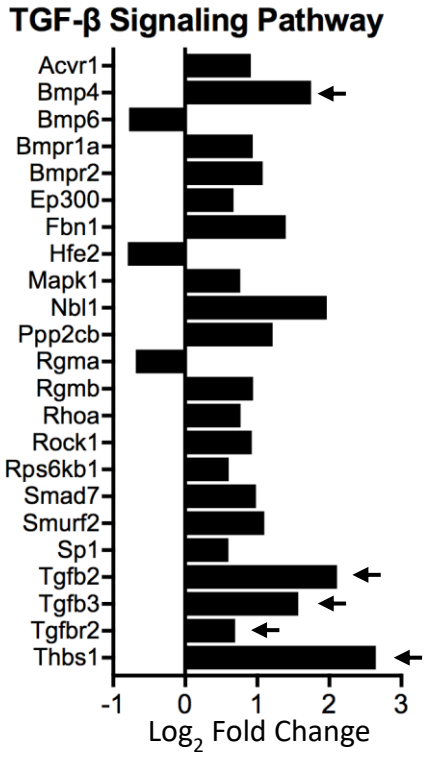
Figure S7. Focal adhesion pathways are upregulated in the failing RV.



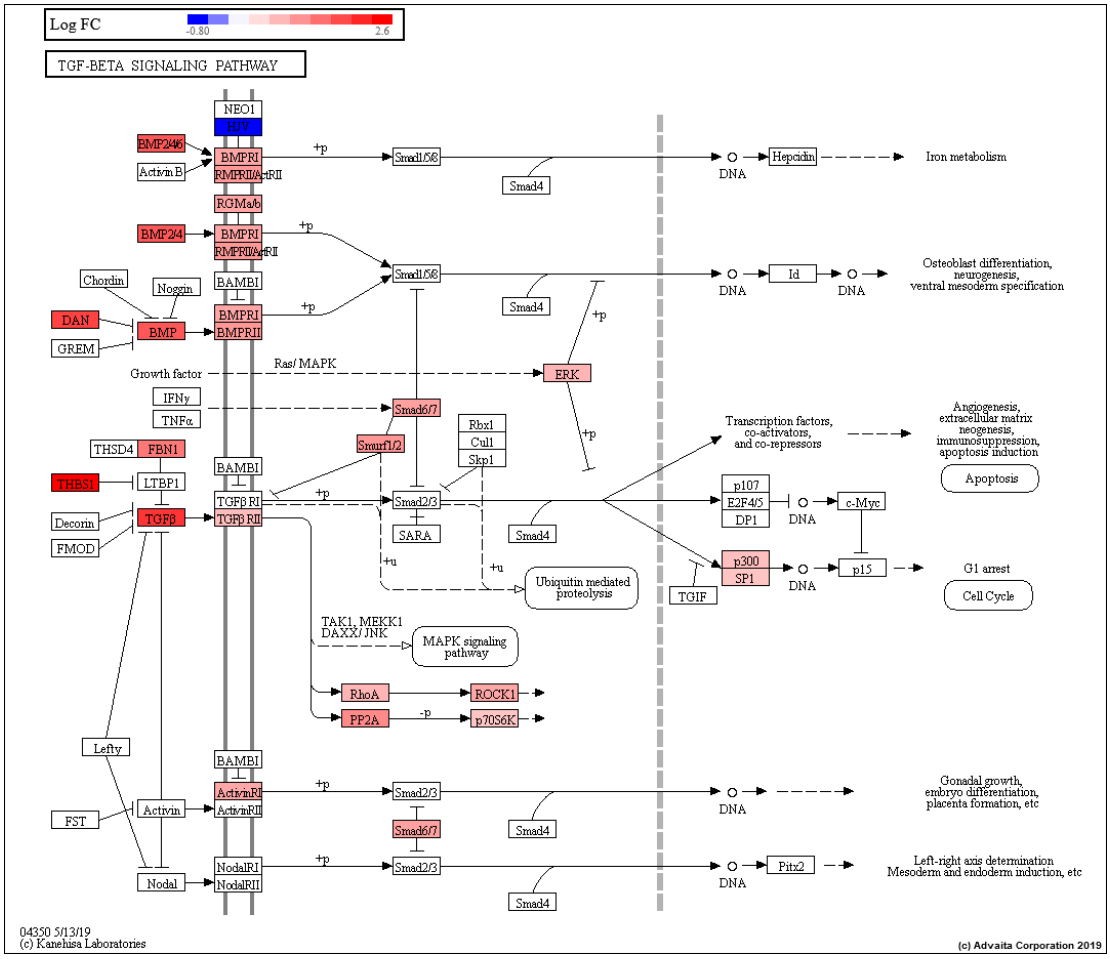
(a) Genes involved in focal adhesion are upregulated in RV failure vs. Sham with thrombospondins and cyclins (arrows) being the most highly expressed genes and myosin light chain 2 and 7 (arrows) the most downregulated. (b) Graphical representation of the dysregulated genes (N=4/group), Blue – downregulated, Red – upregulated, q-value = 0.0003. The figure was obtained with iPathwayGuide (www.advaitabio.com), using pathway data obtained from the KEGG resource (pathway #04510)[33-35]. RV – Right ventricle.

Figure S8. TGF-β signaling pathway is upregulated in the failing RV.

a



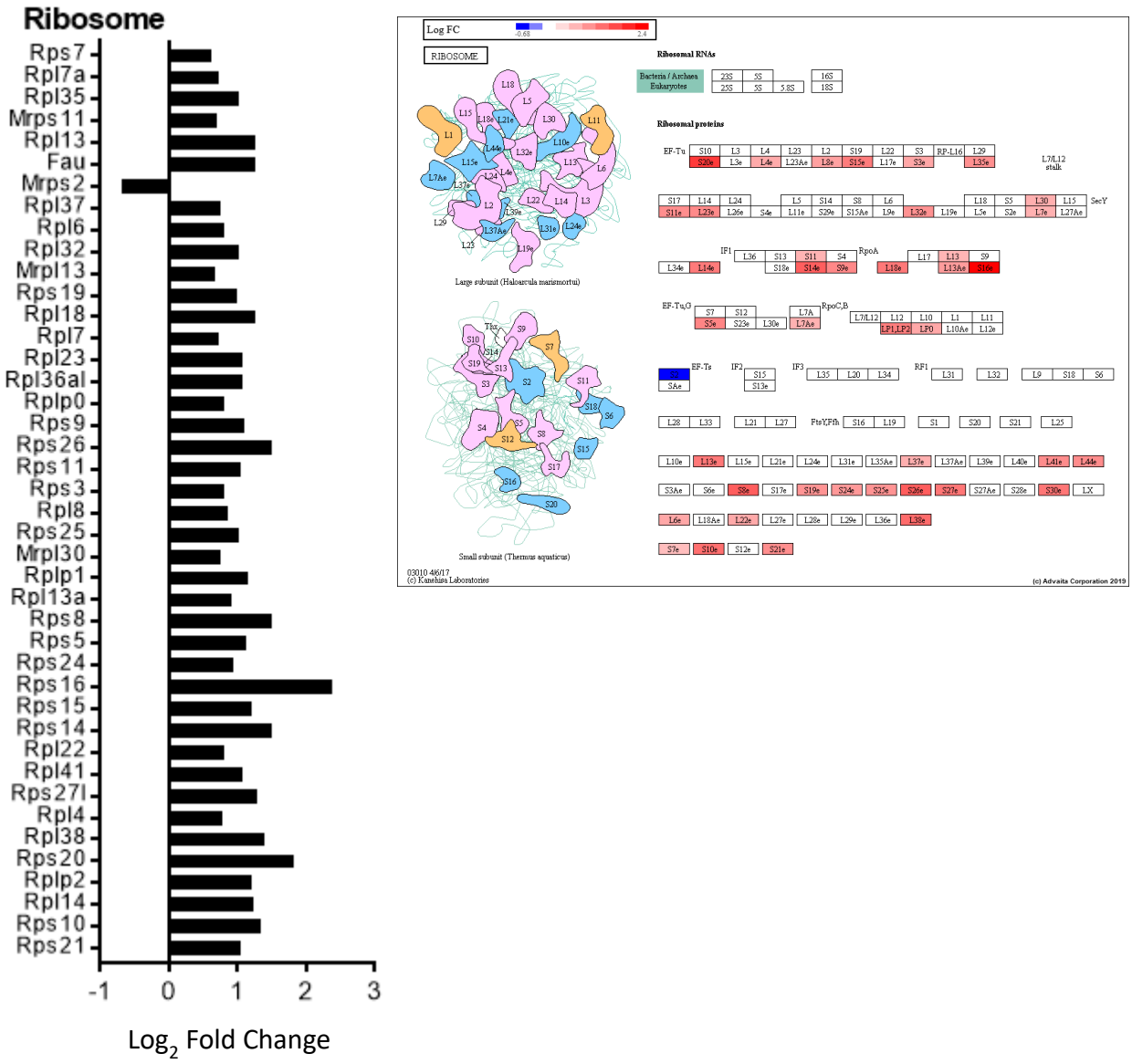
b



(a) Genes involved in TGF-β signaling are upregulated in RV failure vs. Sham with thrombospondin 1, TGF-β and its receptors and Bmp4 (arrows) being the most highly expressed genes. (b) Graphical representation of the dysregulated genes (N=4/group), Blue – downregulated, Red – upregulated, q-value = 0.006. The figure was obtained with iPathwayGuide (www.advaitabio.com), using pathway data obtained from the KEGG resource (pathway #04350)[33-35]. RV – Right ventricle.

Figure S9. Ribosome pathway is upregulated in the left ventricle in RV failure.

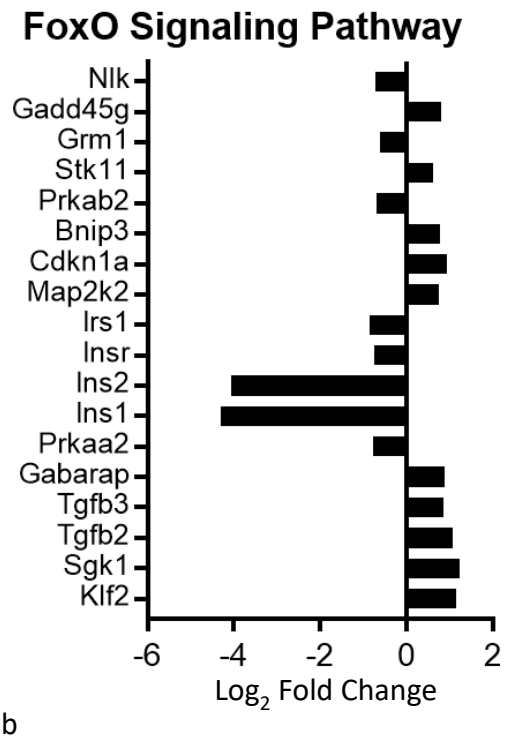
a b



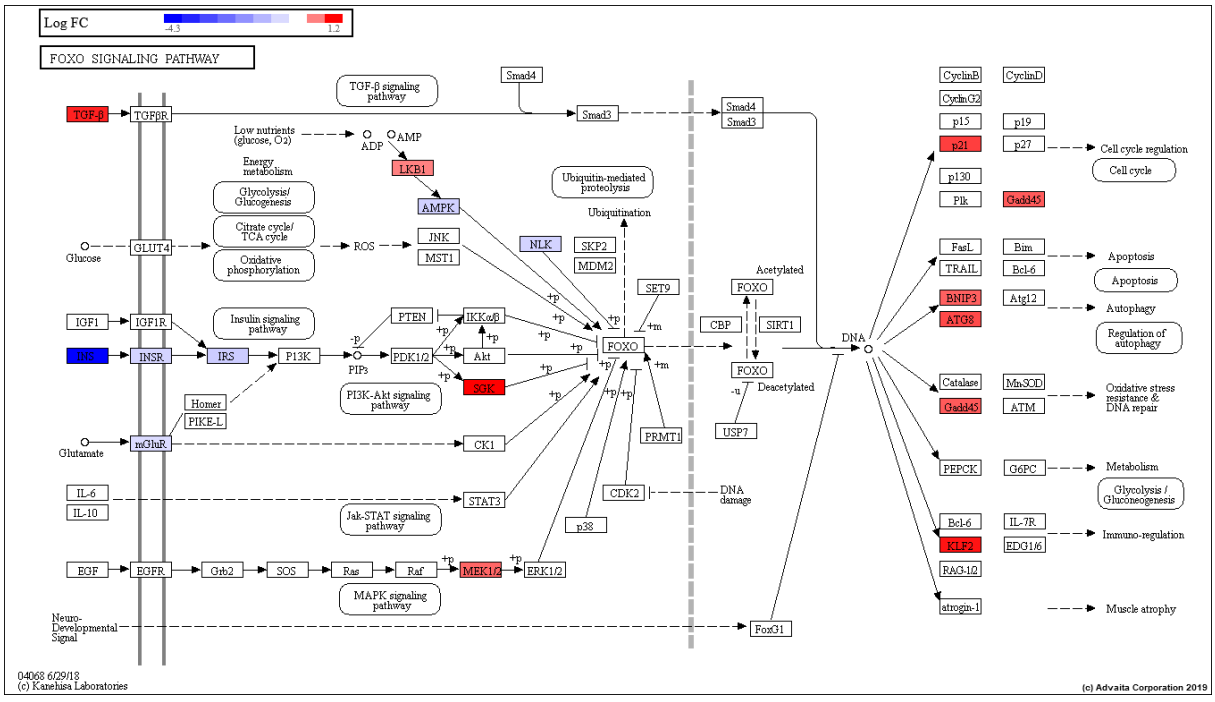
(a) Genes associated with ribosomes are upregulated in the left ventricle with RV failure vs. left ventricle from Sham. (b) Graphical representation of the differentially expressed genes (N=4/group), Blue – downregulated, Red – upregulated, q-value = 1.20E-13. The figure was obtained with iPathwayGuide (www.advaitabio.com), using pathway data obtained from the KEGG resource (pathway #03010)[33-35] .

Figure S10. FoxO signaling pathway is upregulated in the left ventricle in RV failure.

a



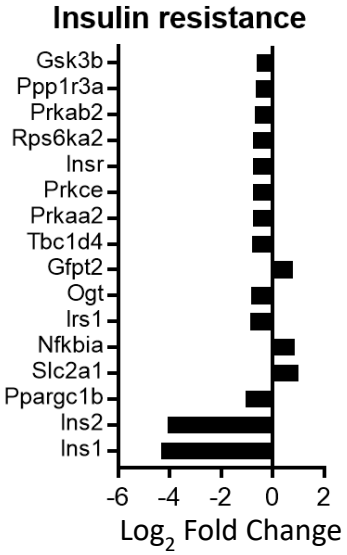
b



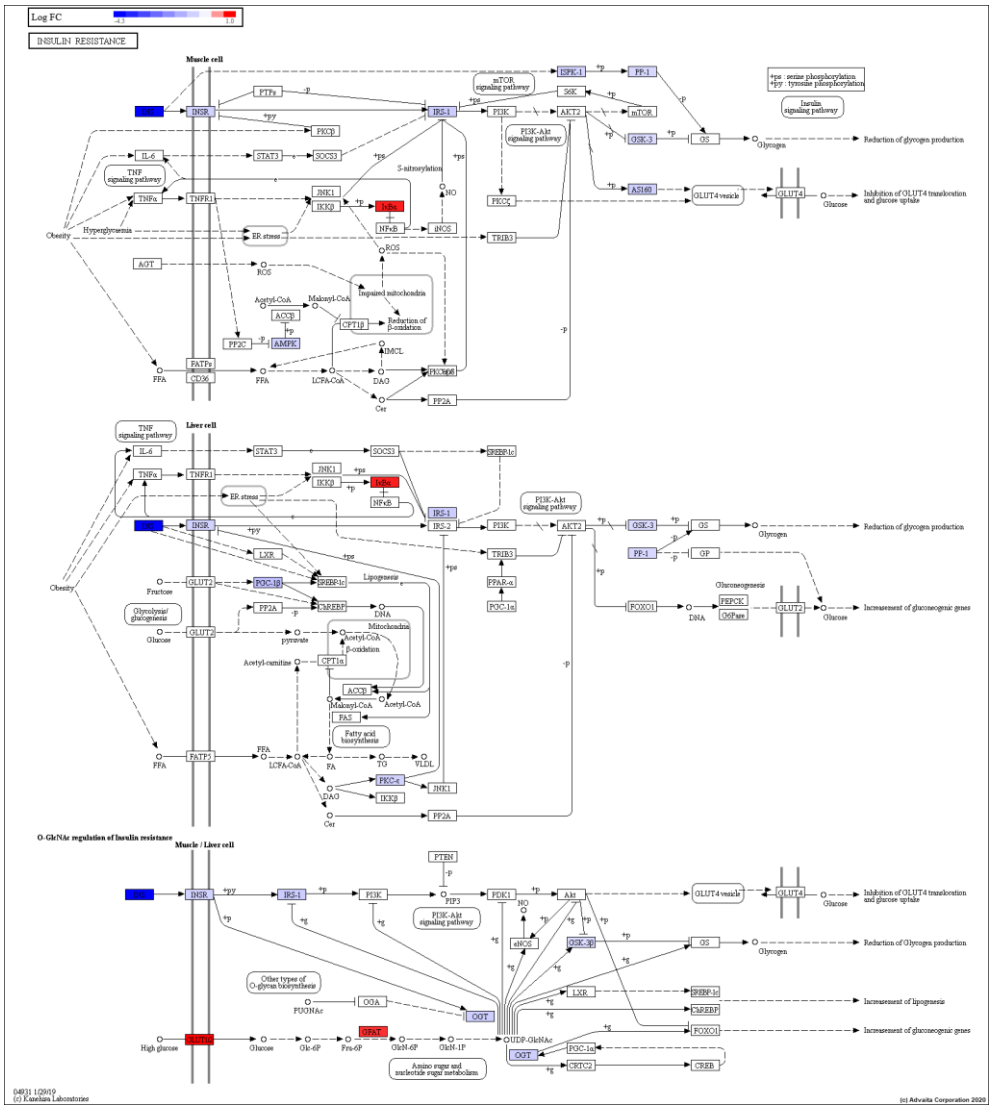
(a) Genes involved in FoxO signaling are perturbed in the left ventricle with RV failure vs. left ventricle from Sham. (b) Graphical representation of the differentially expressed genes (N=4/group), Blue – downregulated, Red – upregulated, q-value = 0.0011. The figure was obtained with iPathwayGuide (www.advaitabio.com), using pathway data obtained from the KEGG resource (pathway #04068)[33-35] .

Figure S11. **Insulin resistance pathway is downregulated in the left ventricle in RV failure.**

a



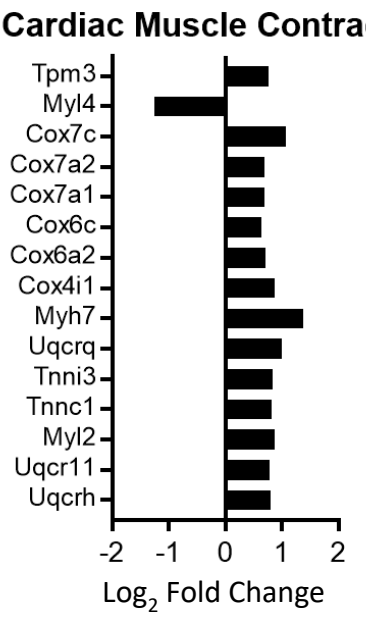
b



(a) Genes involved in insulin signaling are perturbed in the left ventricle with RV failure vs. left ventricle from Sham. (b) Graphical representation of the differentially expressed genes (N=4/group), Blue – downregulated, Red – upregulated, q-value = 0.003. The figure was obtained with iPathwayGuide (www.advaitabio.com), using pathway data obtained from the KEGG resource (pathway #04931)[33-35].

Figure S12. Cardiac muscle contraction pathway is upregulated in the left ventricle in RV failure.

a



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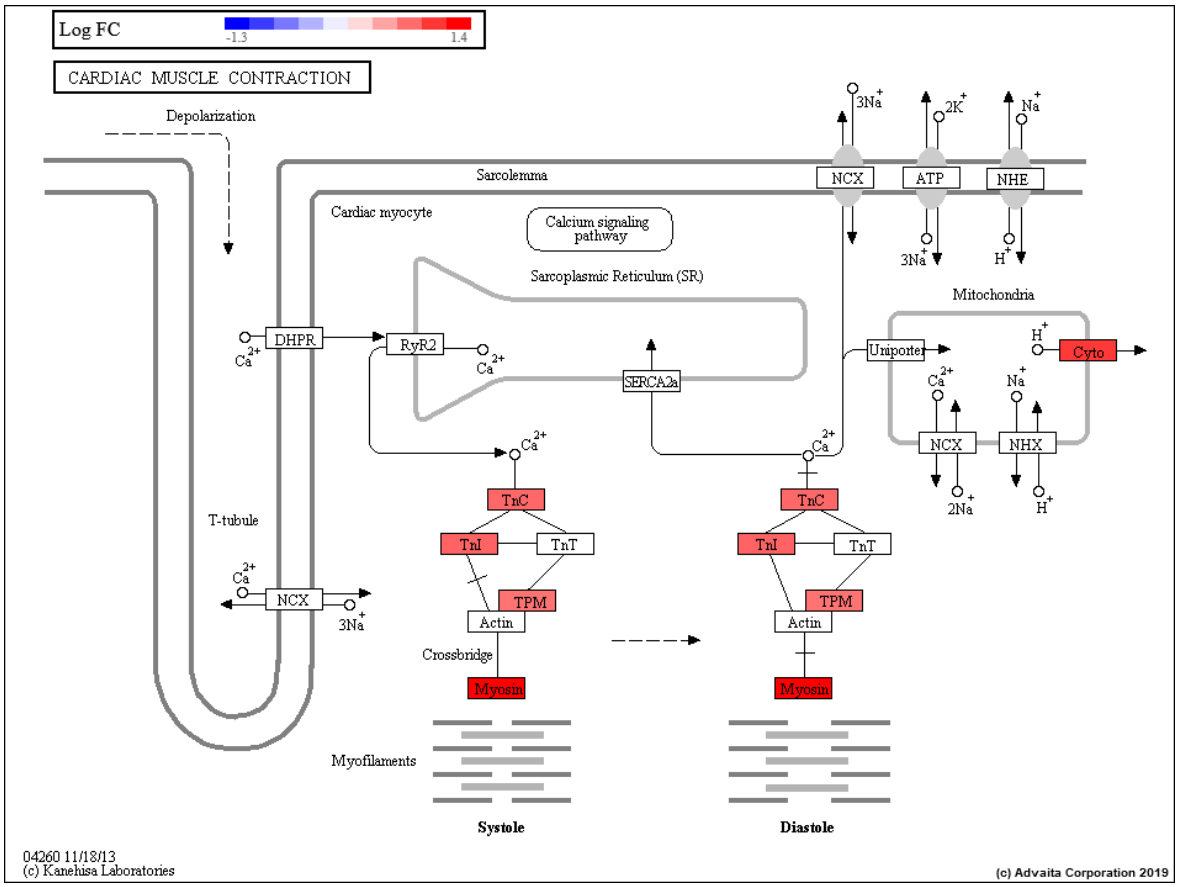

(a) Genes associated with cardiac muscle contraction are upregulated in the left ventricle with RV failure vs. left ventricle from Sham. (b) Graphical representation of the differentially expressed genes (N=4/group), Blue – downregulated, Red – upregulated, q-value = 0.0053. The figure was obtained with iPathwayGuide (www.advaitabio.com), using pathway data obtained from the KEGG resource (pathway #04260)[33-35].