Supplemental Material

Detailed Methods

Mouse models

The Gαq-40 transgenic mice were generated on the FVB/N background in the Dorn laboratory, and have been described previously [1, 2]. Four pairs of 8 week old male nontransgenic FVB/N and Gαq-40 transgenic mouse hearts were used for RNA sequencing studies. Echocardiographic and cardiac catheterization studies were performed using standard methods [1, 3].

Sequencing and data processing

Four barcoded libraries were combined in equimolar (10 nmol/L) amounts and diluted to 4 pmol/L for cluster formation on a single Illumina Genome Analyzer II flowcell lane. Basecalling of DNA clusters was performed using Illumina's processing pipeline software (version 1.5) and 36-nt sequences, with quality scores, were obtained in Illumina's SCARF text format. The first 4 nucleotides of each 36-nt read corresponded to barcodes (3 nucleotides with an additional, invariant T). The UNIX functions grep, sed and awk were used to sort the sequences according to barcode, remove the barcoding nucleotides, and convert sequence data to FASTQ format. After barcode removal, the 32 base mRNA sequence reads were mapped to transcripts annotated in NCBI release 37 of the mouse genome using the publicly available packages Bowtie (release 0.12.0) (http://bowtie-bio.sourceforge.net/index.shtml) [4], (release 1.0.12) (http://tophat.cbcb.umd.edu/) [5], and Cufflinks (release 0.7.0) (http://cufflinks.cbcb.umd.edu/) [6]. Bowtie maps short reads to reference sequences such as whole genomes. TopHat and Cufflinks map known and novel splice junctions, use annotation files to compute which aligned sequences map to the known transcriptome, and take into account transcript isoform diversity (alternative splicing). Cufflinks may be used with gene annotation files to calculate overall gene expression in terms of Reads Per Kb of exon per Million mapped reads (RPKM), a parameter previously defined in [7]. (The current release of Cufflinks (0.8.1) uses the term Fragments Per Kb of exon per Million mapped reads (FPKM) [6], which is equivalent to RPKM for single-end sequencing, as we have performed here.) We used the default options supplied with these software packages in our analyses.
Annotation files in gtf format (http://mblab.wustl.edu/GTF22.html), including mRNAs, ESTs and noncoding elements such as ribosomal RNAs and miRNA precursors, were downloaded from Ensembl (ftp://ftp.ensembl.org/pub/current_gtf/mus_musculus/). We used the Cufflinks module (above) with annotation files from which ESTs, rRNAs and miRNA precursors had been manually removed, to focus on sequence reads mapping to coding genes. We analyzed only those RNA elements that had expression signals in at least 2 of 4 biological replicates.

The gtf annotation files from Ensembl contain all possible separate transcripts (identified with ENST labels) for each gene (identified with ENSG labels). In order to assess alternative splicing events, we used the Cufflinks module with these same gtf annotation files, but forced calculation of RPKM values for separate transcripts (ENSTs) rather than for entire genes (ENSGs). The presence of alternatively spliced products was evaluated by manually examining Cufflinks output files for ENST entries corresponding to an ENSG entry of interest.

Example shell script for extraction of two barcoded sequences from Illumina pipeline 1.5 SCARF text file (rawsequence.txt), removal of 4-nucleotide barcodes, and conversion to FASTQ format

Extract from SCARF text file (rawsequence.txt):

…
HWI-EAS158:7:1:18:1532#0/1:ATGTGCCGGGCGAGCCGCTCTGCGCTAGGCGCTCAG:ab`aaaabbabb`ba
ababaaa`aaaaaaaaa`aaa
HWI-EAS158:7:1:17:190#0/1:TGATGTTGACTTTGTCCACCTGGAACTTGGTCTCAA:
aaaaabaaaaaaaaa`a`a^__`aa`^`a]a]_`__
…

Shell script:

```
grep ':ATGT' rawsequence.txt > ATGT-barcoded.seq
sed 's/:ATGT/:/' ATGT-barcoded.seq | awk '{gsub(":","\t");print}' | awk '{print "@" $1 "_" $2 "_" $3 "_"
$4 "_" $5 "\n" $6 "\n" "+" "\n" substr($7,5,36) }' > ATGTsequences.fastq
grep ':TGAT' rawsequence.txt > TGAT-barcoded.seq
sed 's/:TGAT/:/' TGAT-barcoded.seq | awk '{gsub(":","\t");print}' | awk '{print "@" $1 "_" $2 "_" $3 "_"
$4 "_" $5 "\n" $6 "\n" "+" "\n" substr($7,5,36) }' > TGATsequences.fastq
```

Example shell script for mapping of FASTQ sequences to the mouse genome by Tophat, followed by annotation and RPKM calculations for whole genes, using Cufflinks with a gtf annotation file

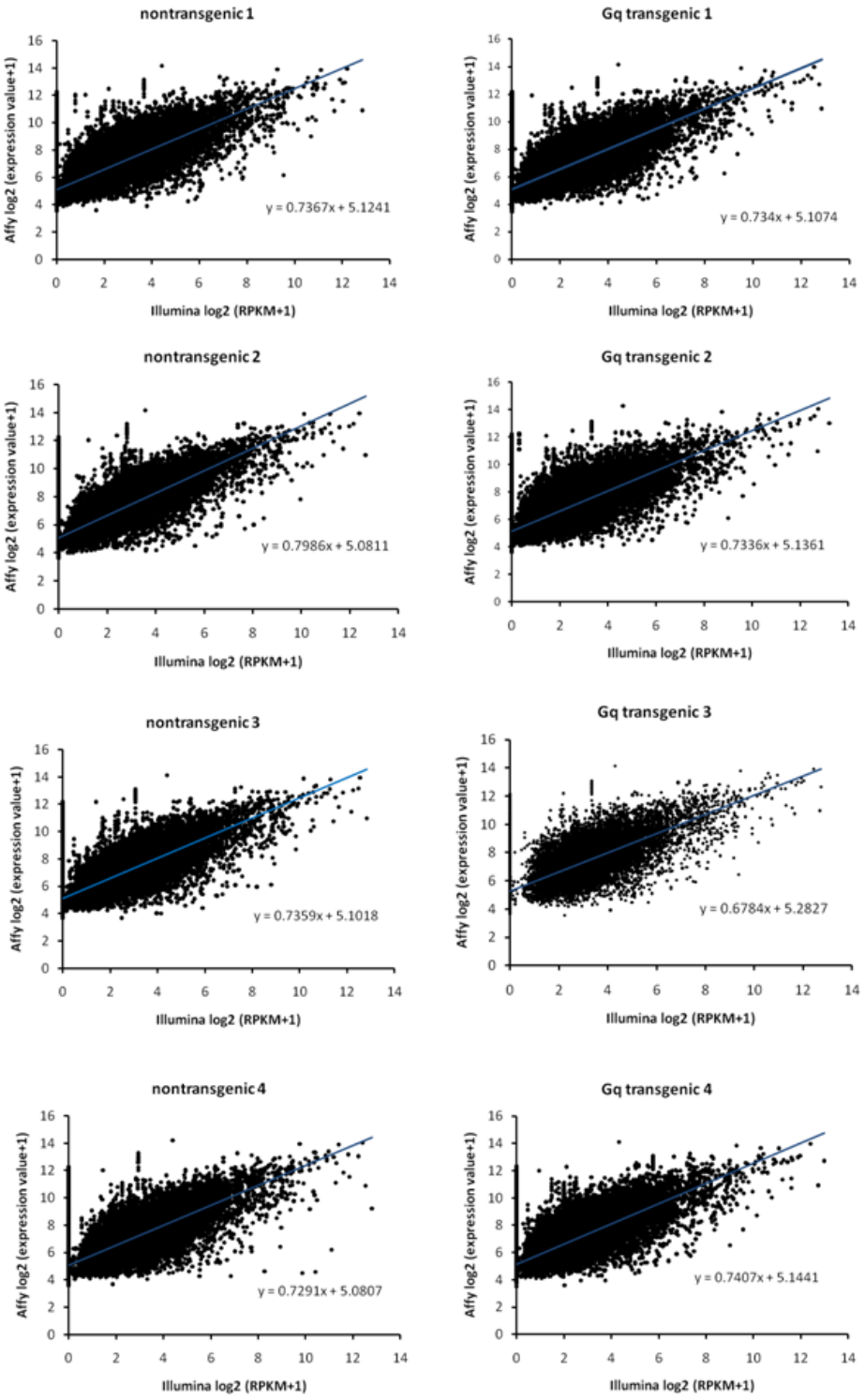
```
tophat --solexa1.3-quals -o ~/ATGTsequences-processed ~/bowtie_database/m_musculus/indexname
~/ATGTsequences.fastq
cd ~/ATGTsequences-processed
cufflinks -G ~/bowtie_database/m_musculus/index.gtf accepted_hits.sam
```

Example shell script for mapping of FASTQ sequences to the mouse genome by TopHat, followed by annotation and RPKM calculations for separate transcript components to permit alternative splicing analysis

```
tophat --solexa1.3-quals -o ~/ATGTsequences-processed ~/bowtie_database/m_musculus/indexname
~/ATGTsequences.fastq
cd ~/ATGTsequences-processed
cufflinks accepted_hits.sam
cuffcompare -o summstats.txt -r ~/bowtie_database/m_musculus/index.gtf transcripts.gtf
```

Reverse-transcription quantitative PCR

One microgram of total cardiac RNA was reverse-transcribed into cDNA using oligo-dT priming (qScript Flex cDNA kit, Quanta Biosciences). One-twentieth of each preparation was used for each individual qPCR, using one of the following TaqMan probes (Applied Biosystems): *Nppa,* Mm01255747_g1; *Atp2a2*, Mm00437634_m1; *Acta1*, Mm00808218_g1; *Myh7*, Mm00600555_m1; *Aqp4*, Mm00802131_m1; *Bcl2*, Mm00477631_m1; *Edn3*, Mm00432986_m1; *Fhl2*, Mm00515781_m1; *Nrg1*, Mm00626552_m1; *Tac1*, Mm00436880_m1; *Tuba1a*, Mm00846967_g1; *Gapdh,* 4352339E.



Supplemental Figure I. Correspondence of gene expression determined by RNA sequencing vs microarray, for 8 individual hearts.U Gene expression determined by RNA sequencing (Illumina) is plotted against gene expression determined by microarrays (Affymetrix). Values shown are $\log_2$(RPKM+1) for RNA sequencing (x-axis) and $\log_2$(Affymetrix signal units+1) for microarrays. A value of 1 was added to RPKM or Affymetrix signal units to avoid taking the log of 0.

Supplemental Table I: Sequencing read totals and alignment to reference genome for barcoded RNASeq samples.

	Lane 1					**Lane 2**					**Both lanes**
	ntgATGT	ntgTGAT	ntgTGCT	ntgTGTT	Total	GqTCAT	GqTCCT	GqTCTT	GqTGGT	Total	Total
Recognized barcoded reads	3426712	2349661	1735078	1694057	9205508	2882044	2020058	1343763	2765911	9011776	18217284
Total raw reads					11216862					9591887	20808749
% barcode detection					82.1					94.0	87.5
Reads aligned to reference genome	1885165	1546833	1304290	766577	5502865	1676644	1334172	411077	1607088	5028981	10531846
% alignment	55.0	65.8	75.2	45.3	59.8	58.2	66.0	30.6	58.1	55.8	57.8

Supplemental Table I. Alignment of sequencing reads to the mouse genome. The number of sequencing reads with recognizable barcodes was compared to the total number of sequencing reads obtained for the entire sequencing lane. In addition, the percentage of barcoded reads that were successfully aligned to the mouse genome was calculated.

Supplemental Table II is supplied as an Excel datasheet.

Supplemental Table II. Highly expressed genes in nontransgenic mouse heart, determined by RNA sequencing. The 234 highest-expressing genes (equal or greater than 60 copies per cell) in nontransgenic mouse hearts (mean ± SEM, n=4) are shown, ranked from highest to lowest expression. 1 copy per cell corresponds to an RPKM value of 3 according to Mortazavi et al. [7].

Supplemental Table III is supplied as an Excel datasheet.

Supplemental Table III. Highly expressed genes in Gq mouse hearts, determined by RNA sequencing. The 236 highest-expressing genes (equal or greater than 60 copies per cell) in Gq transgenic mouse hearts (mean ± SEM, n=4) are shown, ranked from highest to lowest expression.

Supplemental Table IV is supplied as an Excel datasheet.

Supplemental Table IV. Complete gene expression profile in individual nontransgenic and Gq mouse hearts, determined by RNA sequencing. All genes detected by RNA sequencing in individual hearts are shown, ranked from highest to lowest copy number per cell in nontransgenic hearts (mean ± SEM). Genes detected in only 1 of the 4 biological replicates (no value for standard error) were excluded from further analysis.

Supplemental Table V: Highly-expressed genes in mouse hearts by GO category

GO-ID	Description	Genes
6915	apoptosis	ACTC1,BAG3,ITM2B,NDUFA13,PSAP,SOD2,TNS1,VDAC1
7166	cell surface receptor linked signal transduction	AES,CRYAB,GNAS,GNB2L1
5856	cytoskeleton	ACTA1,ACTB,ACTC1,ACTN2,CSRP3,DES,GSN,HSPB7,IVNS1ABP,LDB3,LMOD2,MAP1LC3A,MYBPC3,MYH6,MYH7,MYL2,MYL3,MYL4,MYL7,MYOM1,MYOZ2,PAM,TNNT2,TPM1,TTN,TUBA4A
5783	endoplasmic reticulum	ATP2A2,HERPUD1,HRC,MGST3,PLN,PTGDS,S100A1,SLN,SRL
6006	glucose metabolic process	ALDOA,ENO3,LDHA,LDHB,MDH1,MDH2,OGDH,PDHA1,PDK2,PFKM,PGAM2,TPI1
6629	lipid metabolic process	ACAA2,ANKRD23,APOE,ACADL,ACADM,ATP5A1,ACADVL,ATP5B,CPT1B,ECH1,FABP3,HADHA,LPL,PHYH,PSAP,PTGDS,TPI1
5746	mitochondrial respiratory chain	COX6B1,COX7B,CYC1,COX6C,COX7A1,MT-CO1,COX7A2,MT-CO2,MT-CYTB,MT-ND3,MT-ND4,NDUFA1,NDUFA10,NDUFA11,NDUFA13,NDUFA2,NDUFA3,NDUFA4,NDUFA6,NDUFA8,NDUFB10,NDUFB5,NDUFB7,NDUFB8,NDUFB9,NDUFC1,NDUFS2,NDUFS6,NDUFS7,NDUFV1,NDUFV2,UQCR,UQCRC1,UQCRC2,UQCRFS1,UQCRH,UQCRQ
5739	mitochondrion	ACAA2,ACO2,AK1,ACADL,ACADM,ATP5A1,ACADVL,ATP5B,ATP5C1,ATP5D,ATP5E,ATP5F1,ATP5G1,ATP5G3,ATP5H,ATP5J,ATP5J2,ATP5K,ATP5O,BRP44,CHCHD10,CKMT2,COQ9,COX17,COX4I1,COX5A,COX5B,COX6A2,COX6B1,COX6C,COX7A1,COX7A2,COX7B,COX8B,CPT1B,CS,CTSD,CYC1,ECH1,ETFB,GBAS,HADHA,HSP90AB1,IDH2,IDH3B,IDH3G,LARS2,MDH2,MT-ATP6,MT-CO1,MT-CO2,MT-CYTB,MT-ND1,MT-ND3,MT-ND4,MT-ND6, NDUFA1,NDUFA10,NDUFA11,NDUFA13,NDUFA2,NDUFA3,NDUFA4,NDUFA6,NDUFA8,NDUFB10,NDUFB5,NDUFB7,NDUFB8,NDUFB9,NDUFC1,NDUFS2,NDUFS6,NDUFS7,NDUFV1,NDUFV2,NNT,OGDH,OXCT1,PDHA1,PDK2,PINK1,PSAP,SDHA,SDHB,SDHC,SLC25A3,SLC25A4,SOD2,UQCR,UQCRC1,UQCRC2,UQCRFS1,UQCRH,UQCRQ,VDAC1
30016	myofibril	ACTA1,ACTC1,ACTN2,ANKRD1,ANKRD23,CRYAB,CSRP3,DES,HSPB1,LDB3,MYBPC3,MYH6,MYH7,MYL2,MYOM1,MYOZ2,PDLIM5,PYGM,TCAP,TNNI3,TNNT2,TPM1,TTN
5634	nucleus	AES,ANKRD1,ANKRD23,CS,CSRP3,EEF1A2,FABP4,FHL2,HOPX,HSPB1,IVNS1ABP,LDB3,NDUFA13,PAM,PDE4DIP,PTGDS,TTN

GO-ID	Description	Genes
7242	intracellular signaling cascade	ATP2A2,GNAS,GNB2L1,MT1,PINK1,PSAP,SOD2,TNS1
45449	regulation of transcription	AES,ANKRD1,FABP4,FHL2,HOPX,PAM
6950	response to stress	ACADM,APOE,CRYAB,CTSD,GPX3,HERPUD1,HSP90AB1,HSPB1,HSPB6,HSPB7,HSPB8,IDH2,MB,SOD2,TNS1,VDAC1
6416	translation	EEF1A2,EEF2,LARS2,RPL8,RPLP1,RPS5,RPS9
6810	transport	APOE,ATP1A1,ATP2A2,ATP5A1,ATP5B,ATP5C1,ATP5D,ATP5E,ATP5F1,ATP5G1,ATP5G3,ATP5H,ATP5J,ATP5J2,ATP5K,ATP5O,COX17,CPT1B,CYC1,ETFB,FABP3,FABP4,FTH1,FXYD1,GSN,HBA-A1,HBA-A2,HBB-B1,HBB-B2,MB,MT-ATP6,MT-CO1,MT-CO2,MT-CYTB,MT-ND3,MT-ND4,MYH6,NDUFA1,NDUFA10,NDUFA11,NDUFA13,NDUFA2,NDUFA3,NDUFA4,NDUFA6,NDUFA8,NDUFB10,NDUFB5,NDUFB7,NDUFB8,NDUFB9,NDUFC1,NDUFS2,NDUFS6,NDUFS7,NDUFV1,NDUFV2,NNT,PLN,PSAP,PTGDS,SDHA,SDHB,SDHC,SLC25A3,SLC25A4,TUBA4A,UQCR,UQCRC1,UQCRC2,UQCRFS1,UQCRH,UQCRQ,VDAC1

Supplemental Table V. Genes expressed at greater than 60 copies per cell in nontransgenic hearts, determined by RNA sequencing, are presented by Gene Ontology categories (GO-IDs).

Supplemental Table VI is supplied as an Excel datasheet.

Supplemental Table VI. Differential gene expression in Gq-overexpressing hearts compared to nontransgenic hearts, as determined by microarrays. Genes were defined as differentially expressed at $P<0.001$ (FDR<0.03), magnitude of fold-change ≥ 1.3. Genes are ranked by fold-change in expression, from highest to lowest. Data from RNA sequencing for the same genes are shown, and significant regulation reported by RNA sequencing is indicated.

Supplemental Table VII: Differential gene expression in Gq-overexpressing compared to nontransgenic hearts

Gene symbol	ntg copies/cell	Gq copies/cell	Fold-change (RNASeq)	p-value (RNASeq)	Fold-change (array)	p-value (array)	regulated (array)
Upregulated							
Gnaq	2	574	365.9	1.4E-06	15.1	6.9E-15	Y
Nppa	43	2604	60.2	5.9E-04	13.4	2.6E-10	Y
Gdf15	2	62	26.5	4.2E-04	4.0	9.8E-07	Y
Fgf6	0.4	5	11.2	3.4E-05	4.1	7.5E-08	Y
Thbs4	0.7	6	8.6	6.3E-04	9.7	3.8E-09	Y
Acta1	127	897	7.0	2.5E-05	2.6	1.8E-06	Y
Synpo2l	20	117	5.9	1.4E-04	2.3	5.1E-07	Y
Bcl2	0.4	2	5.4	1.6E-04	2.8	9.3E-09	Y
Nppb	124	635	5.1	2.0E-06	3.0	1.0E-07	Y
Phlda1	7	30	4.5	8.0E-04	1.9	9.4E-05	Y
Scgb1c1	4	16	4.1	7.5E-04	2.7	1.2E-05	Y
Rcan1	19	75	4.0	6.5E-05	2.3	1.1E-06	Y
Etv5	1	4	3.7	4.4E-04	2.6	1.0E-05	Y
Arc	0.3	1	3.7	4.4E-04	1.1	2.3E-01	
Casq1	11	41	3.6	1.3E-05	2.5	8.4E-09	Y
Klhl34	2	6	3.6	7.1E-04	1.6	1.8E-03	
Dusp27	3	9	3.4	7.7E-04	2.4	7.6E-06	Y
Dbn1	1	4	3.3	5.6E-06	1.6	5.3E-07	Y
Nuak1	5	16	3.3	2.8E-04	2.4	1.4E-05	Y
Ankrd1	204	671	3.3	2.0E-06	1.5	9.3E-06	Y
Cyr61	15	47	3.2	5.3E-05	2.3	4.0E-05	Y
Ppm1e	0.5	2	3.1	2.0E-04	2.8	3.4E-07	Y
Fbxl7	0.2	0.6	3.1	3.4E-04	1.6	3.1E-05	Y
Tnfrsf12a	20	64	3.1	2.9E-04	2.3	3.9E-07	Y
Ckap4	3	10	3.0	9.3E-05	1.9	2.1E-05	Y
Mybpc2	4	13	3.0	1.6E-05	2.3	1.2E-06	Y
Otud1	7	22	3.0	3.1E-05	2.4	2.6E-04	Y
Masp1	2	6	2.9	6.8E-05	1.7	1.4E-04	Y
Spry4	1	3	2.8	8.2E-05	1.3	3.3E-02	
Irx5	0.7	2	2.8	1.8E-04	1.4	1.2E-03	
Uck2	5	13	2.8	6.4E-04	2.3	5.3E-04	Y
Col1a1	11	29	2.7	3.2E-04	2.3	2.0E-09	Y
Col15a1	9	23	2.6	6.2E-04	1.7	1.1E-05	Y
Abra	11	29	2.6	1.1E-04	2.1	8.8E-06	Y
Pfkp	4	9	2.6	8.6E-05	1.7	1.7E-04	Y

Gene symbol	ntg copies/cell	Gq copies/cell	Fold-change (RNASeq)	p-value (RNASeq)	Fold-change (array)	p-value (array)	regulated (array)
Arhgap24	2	4	2.4	5.0E-04	1.2	3.2E-03	
Rasa3	1	3	2.4	7.7E-05	1.5	5.6E-04	Y
Frmd6	2	4	2.4	2.4E-04	1.5	6.4E-04	Y
Napepld	0.3	0.6	2.3	9.6E-04	1.5	7.9E-04	Y
Thap1	0.9	2	2.3	1.1E-03	-1.1	2.5E-01	
Xirp2	19	44	2.3	4.8E-04	2.3	2.9E-07	Y
Serpinb6b	3	7	2.3	1.7E-04	1.9	1.9E-07	Y
Itgb5	10	22	2.3	7.8E-05	1.8	3.5E-08	Y
Surf6	0.6	1	2.2	2.4E-05	1.0	4.7E-01	
Tjap1	5	12	2.2	4.8E-04	1.7	2.2E-05	Y
Camta1	5	12	2.2	5.6E-05	1.7	3.4E-07	Y
Itga9	4	9	2.2	3.0E-04	1.8	8.4E-07	Y
Qsox1	4	9	2.2	1.2E-04	1.5	1.4E-06	Y
Btg2	4	8	2.1	6.8E-04	1.7	9.4E-04	Y
Hn1	12	26	2.1	1.6E-04	1.7	6.1E-06	Y
Nfatc2	2	3	2.0	2.4E-04	1.5	1.5E-05	Y
Myom2	66	135	2.0	1.8E-05	1.6	2.7E-03	
Enah	18	37	2.0	3.1E-04	1.7	2.2E-05	Y
Nrap	55	112	2.0	4.8E-05	1.6	7.4E-06	Y
Col1a2	11	21	1.9	7.6E-04	1.9	3.5E-08	Y
Lrrc10	55	105	1.9	2.3E-04	1.7	8.9E-05	Y
Prkab2	3	5	1.9	1.1E-03	1.5	1.5E-03	
Slc38a2	26	46	1.8	6.6E-04	1.5	3.4E-05	Y
Tsc22d1	32	57	1.8	8.3E-04	1.3	5.9E-04	
Heyl	2	4	1.8	1.0E-03	1.3	2.9E-05	
Ttpal	2	3	1.8	9.5E-04	1.3	1.5E-04	
Grb10	11	19	1.7	5.5E-05	1.4	4.3E-05	Y
Sorbs2	39	67	1.7	3.3E-04	1.6	5.9E-05	Y
Prmt2	2	3	1.7	9.1E-04	1.4	1.3E-04	Y
Mmp2	8	13	1.7	1.5E-04	1.5	9.0E-05	Y
Mybpc3	261	424	1.6	2.3E-05	1.2	7.1E-05	
Lamb2	41	65	1.6	5.6E-04	1.3	3.2E-05	
Bambi	1	2	1.6	7.5E-04	1.2	5.7E-03	
Myh10	2	3	1.6	7.4E-04	1.4	2.6E-05	Y
Habp4	5	8	1.5	5.5E-04	1.2	2.2E-03	
Plxnb2	9	14	1.5	1.0E-03	1.3	2.1E-04	
Msn	11	17	1.5	6.1E-04	1.5	8.8E-07	Y
Hspb6	182	267	1.5	5.6E-04	1.0	7.9E-01	
Dnaja4	13	20	1.5	4.1E-04	1.3	1.0E-03	Y
Plxna1	2	3	1.4	7.5E-04	1.1	2.0E-01	

Gene symbol	ntg copies/cell	Gq copies/cell	Fold-change (RNASeq)	p-value (RNASeq)	Fold-change (array)	p-value (array)	regulated (array)
Cxcr7	10	14	1.4	7.2E-04	1.1	4.0E-01	
Arl6ip5	20	26	1.3	2.0E-04	1.1	4.5E-01	
Downregulated							
Idh3b	85	63	-1.3	6.6E-04	-1.2	1.2E-04	
Dlat	27	20	-1.3	1.0E-03	-1.2	3.6E-04	
Fmo1	5	4	-1.4	5.0E-04	-1.2	1.6E-01	
Ank	21	15	-1.4	2.8E-04	-1.3	9.7E-06	Y
Pdha1	78	54	-1.4	9.3E-04	-1.2	2.5E-05	
Hadha	128	88	-1.4	7.3E-04	-1.3	5.3E-06	
Acadvl	99	68	-1.5	1.1E-04	-1.6	2.4E-07	Y
Strn3	6	4	-1.5	3.8E-04	1.1	1.2E-01	
Rxrg	8	5	-1.6	6.9E-04	-1.5	9.5E-05	Y
Fyco1	22	13	-1.6	4.8E-04	-1.5	8.9E-07	Y
Sh3kbp1	8	5	-1.7	9.1E-04	-1.2	1.0E-03	
Ndufv2	99	58	-1.7	3.6E-04	-1.2	2.6E-04	
Rhobtb1	28	16	-1.7	8.8E-04	-1.4	1.4E-04	Y
Mapkapk3	3	2	-1.7	8.1E-04	-1.6	9.3E-05	Y
Atp2a2	811	458	-1.8	1.8E-04	-1.2	2.1E-05	
Acaa2	96	51	-1.9	4.6E-05	-1.7	3.9E-07	Y
Npc1	8	4	-1.9	2.7E-05	-1.8	4.4E-07	Y
Bcs1l	4	2	-1.9	5.4E-04	-1.1	3.4E-02	
Qrsl1	3	2	-2.0	2.3E-04	-1.5	2.5E-04	Y
Hsdl2	15	7	-2.0	8.2E-04	-1.5	5.2E-06	Y
Nphp3	18	9	-2.0	7.6E-05	1.0	9.6E-01	
Aldh6a1	20	10	-2.0	2.4E-04	-1.5	3.7E-07	Y
Decr1	35	17	-2.0	5.3E-04	-1.6	1.6E-07	Y
Suclg2	11	5	-2.0	8.1E-04	-1.5	1.5E-06	Y
Epm2aip1	2	1	-2.1	6.2E-04	-1.1	9.5E-02	
Grb14	24	12	-2.1	2.5E-04	-1.7	4.1E-08	Y
Car14	20	9	-2.2	2.0E-04	-2.0	1.2E-07	Y
Mtr	23	10	-2.3	3.2E-04	-1.0	7.3E-01	
Fah	6	2	-2.5	9.3E-05	-2.3	3.9E-05	Y
Nqo1	8	3	-2.6	8.6E-04	-1.7	2.2E-05	Y
Scn4a	3	1	-2.6	6.9E-05	-2.5	7.0E-06	Y
A2bp1	7	2	-2.6	4.8E-04	-1.9	4.0E-07	Y
Slc22a3	0.8	0.3	-2.8	3.3E-04	-2.2	2.4E-06	Y
Lrtm1	33	12	-2.8	1.9E-04	-2.6	6.8E-07	Y
Pex11a	2	0.8	-2.8	3.8E-04	-1.8	6.6E-05	Y
Olfml2b	5	2	-2.9	6.6E-04	-1.9	2.3E-06	Y
Mylk4	14	5	-3.1	2.7E-04	-2.1	1.1E-05	Y

Gene symbol	ntg copies/cell	Gq copies/cell	Fold-change (RNASeq)	p-value (RNASeq)	Fold-change (array)	p-value (array)	regulated (array)
Fhl2	167	50	-3.4	8.7E-05	-2.6	5.1E-08	Y
Slc38a3	10	3	-3.5	1.6E-05	-2.5	8.3E-07	Y
Nr4a3	3	0.7	-3.5	8.3E-05	-2.5	1.7E-04	Y
Efnb3	11	3	-3.9	4.7E-05	-2.4	4.5E-07	Y
Dusp18	7	1	-5.2	6.7E-05	-4.2	1.6E-07	Y
Scn4b	5	1	-5.2	3.8E-04	-3.9	3.9E-06	Y
Whrn	4	0.7	-5.3	6.8E-04	-2.2	6.7E-07	Y
Dixdc1	0.5	0.1	-6.3	3.3E-04	-1.5	1.9E-04	Y
Ces3	19	2	-10.0	2.5E-04	-7.7	2.4E-11	Y
Cfd	9	0.7	-12.6	1.2E-04	1.2	1.7E-02	
Tmem163	5	0.2	-28.3	6.9E-04	1.2	5.7E-02	

Supplemental Table VII. Differential gene expression in Gq-overexpressing compared to nontransgenic hearts. 125 genes were defined as differentially expressed using RNA sequencing ($P<0.001$, FDR=0.1, magnitude of fold-change ≥ 1.3). Genes are listed with numbers of copies per cell, and ranked by fold-change in expression, from highest to lowest. Data from microarrays for the same genes are shown, and significant regulation reported by microarrays ($P<0.001$, fold-change ≥ 1.3) is indicated.

Supplemental Table VIII is supplied as an Excel datasheet.

Supplemental Table VIII. Ingenuity Pathways Analysis networks. The 77 upregulated and 48 downregulated genes in Gq hearts, defined by RNA sequencing, were used as input for automated signaling network analysis. Gene input lists were subdivided into a high-expressing (>20 copies/cell) set (first datasheet tab), a low-expressing (<20 copies/cell) set (second datasheet tab), or not divided (third worksheet tab). The 'Focus Molecules' column denotes the number of genes from the input lists that participate in a given signaling network.

Barcode sense oligo	5'-ACACTCTTTCCCTACACGACGCTCTTCCGATCTagtT-3'
Barcode antisense oligo	5Phos-actAGATCGGAAGAGCTCGTATGCCGTCTTCTGCTTG-3'
Forward PCR primer	5'-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT-3'
Reverse PCR primer	5'-CAAGCAGAAGACGGCATACGAGCTCTTCCGATCT-3'
Sequencing primer	5'-ACACTCTTTCCCTACACGACGCTCTTCCGATCT-3'

Supplemental Table IX. Nucleotide sequences for custom 'barcoded' Illumina sequencing adapters. Top: Example oligonucleotides for Illumina sequencing adapters, using the barcode 'agt'. Middle: Generic primers, independent of barcode, used for PCR amplification of gel-purified Illumina libraries. Bottom: Generic oligonucleotide for sequencing libraries on the Illumina Genome Analyzer.

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