Supplementary Methods and Data

Methods

Genotyping

Genomic tail snip DNA was isolated and analyzed by PCR using the primers sets: i1Ex2F1: 5'-GAATC TGGAA AGAGT ACCAT TGTGA-3', i1Ex3R1: 5'-GTCTC CGAAG TCGAT TTTCA ACCTC-3' (WT) and NeoR3 : 5'-GATTG TCTGT TGTGC CCAGT CATAG-3' (KO) for $G\alpha_{i1}$(-/-) genotype; i2F8 5'-GATCA TCC GAGA TGGCT ACTCA GAAG-3, i2F14 5'-CAGGA TCATC CATGA AGATG GCTAC-3', i2R7 5'-CCCCT CTCAC TCTTG ATTTC CTACT GACAC-3' (WT), NeoR2 5'-GCACT CAAAC CGAGG ACTTA CAGAA C-3' (KO) for the $G\alpha_{i2}$ (-/-) genotype; i3Ex7R1: 5'-TTCAT GCTTT CATGC ATTCG GTTC-3', i3Ex6F1: 5'-GTGGC CAAAG ATCCG AACGA A-3' (WT) and NeoF1 : 5'-TGCCG AGAAA GTATC CATCA TG-3' (KO) for the $G\alpha_{i3}$ (-/-) genotype.

Gene Expression

The gene expression arrays and statistical analysis were carried out by a central service at University College, London (http://www.ich.ucl.ac.uk/services_and_facilities/lab_services/microarray_centre/). 100ng of total RNA was converted to single stranded (sense strand) DNA using the whole transcript sense target labeling kit (cat 901178, Affymetrix). The DNA was fragmented and end labeled with biotinylated nucleotides before hybridising to the Affymetrix Mouse Gene 1.0 ST arrays. Arrays were then washed and stained using the Hybridisation wash and stain kit (900720, Affymetrix) and scanned on the Affymetrix Scanner 3000. Raw data was summarised and normalised using the RMA algorithm [1] implemented in the Affymetrix

Expression Console software. LIMMA (Linear Models for Microarray Analysis) [2] was used to identify differentially expressed genes. LIMMA applies a modified t-test to each probe set which uses an empirical Bayes approach for estimating sample variances. The moderated t-statistic calculated by LIMMA is more robust than the ordinary t-statistic with small sample sizes. The p-values were corrected for multiple-testing using the Benjamini-Hochberg correction and a corrected p-value threshold of 0.05 was used to select differentially expressed genes.

Quantitative real-time PCR was performed using Taqman gene expression assays (Applied Biosystems) using either inventoried or made to order assays. We used the protocol which is given on their website (http://www3.appliedbiosystems.com/cms/groups/mcb_support/documents/generaldocuments/cms_041280.pdf). Briefly hearts were removed from each group of mice, washed with cold PBS and immediately placed in liquid nitrogen. They were ground in liquid nitrogen with a pestle and mortar and then RNA was extracted using RNeasy kit (cat no. 74104 Qiagen). cDNA was synthesized using High capacity cDNA reverse transcription Kit (4368814 Applied Biosystems). cDNA was quantified and 50ng/µl of DNA/20ul was used for the subsequent real time expression assay. All genes were assayed in triplicates and GAPDH was used as the house keeping gene. The assays from Applied Biosystems were are follows KCND2 (inventoried, Mm00498065 m1, 85 bp), KCND3 (made to order, Mm01302127 m1, 85 bp), KCNH2 (made to order, Mm01134905 m1, 90 bp), KCNQ1 (inventoried, Mm00434641 m1, 85 bp), KCNA4 inventoried, Mm01336166 m1, 132 bp), KCNJ2 (inventoried, Mm00434616 m1, 81 bp), KCNJ11 (inventoried, Mm00440050 s1, 85 bp), KCNB1 (inventoried, Mm00492791 m1, 73 bp), SCN5A (made to order, Mm01342505, 85 bp), SCN1B (inventoried, Mm00441210 m1, 86 bp), CACNA1C (made to order,

Mm01188838 m1, 88 bp) and CACNB2 (made to order, Mm01333544 m1, 82 bp). The samples were run on an Eppendorf Mastercycler Real Plex qPCR system.

Histology

Hearts were removed, rinsed in PBS, fixed overnight in 4% paraformaldehyde in PBS, and dehydrated in a methanol series. Dehydrated hearts were cleared with histoclear, wax embedded and sectioned at 7μm thickness. Sections were then stained with H+E or Masson's trichrome (both Sigma), and photographed. Five hearts of each genotype were examined. To grossly compare if there was cardiac hypertrophy we measured the ratio of the heart weight (mg) to the total body weight (g) and the ratio of the heart weight (mg) to tibial length (mm).

Isolation of cardiomyocytes

Mice were injected with heparin sodium (250 IU) and anaesthetised with a combination of ketamine/xylazine/atropine. The hearts were rapidly excised, cannulated and perfused with buffer containing (in mmol\L) 113 NaCl, 4.7 KCl, 0.6 KH2PO4, 0.6 Na2HPO4, 1.2 MgSO4.7H2O, 12 NaHCO3, 10 KHCO3, 30 taurine, 10 HEPES, 11 glucose and 10 2,3-butanedione monoxime, saturated with 95% O_2-5% CO_2 at 37°C. The hearts were perfused at 3 ml/min with perfusion buffer for 4 min, then with digestion buffer (perfusion buffer containing 0.9 mg/ml collagenase (Worthington type II), 0.125 mg/ml hyaluronidase and 12.5 μmol/L $CaCl_2$) for 10 min. The ventricles were then cut into several pieces and agitated in digestion buffer at 37^{o}C with oxygenation for 10 min twice. The supernatant was collected and 5% foetal calf serum was added. After centrifugation at 600 rpm for 3 min, the cell pellet was suspended in 10 ml of perfusion buffer containing 12.5 μmol\L $CaCl_2$ and the calcium concentration was gradually restored to 1 mmol\L over 20 min. The myocytes were

re-centrifuged at 600 rpm for 3 min, the cell pellet was suspended in culture medium (M-199 medium containing 2 mg/ml bovine serum albumin, 0.66 mg/ml creatine, 0.62 mg/ml taurine, 0.32 mg/ml carnitin hydrochloride, 10 units/ml penicillin, 10 μg/ml streptomycin and 25 μmol\L blebbistatin) and seeded onto sterilised laminin-coated coverslips for 60 min in humidified 5% CO_2-95% air at 37^0C. Myocytes were then gently washed once with blebbistatin-free culture medium to removed unattached cells use on the same day of isolation.

Electrophysiology

Action potential recordings: Action potentials were recorded in the current clamp mode. Ventricular myocytes were stimulated using a 5 ms current pulse. The resting membrane potential (Em), the magnitude of the initial depolarisation (Δ) and the action potential duration at which 50 and 90% repolarisation occurred (APD_{50} and APD_{90} respectively) were measured. The cells were clamped at -80 mV in an extracellular solution containing (mM): NaCl 135, KCl 5.4, $CaCl_2$ 2, $MgCl_2$ 1, NaH_2PO_4 0.33, H-HEPES 5, Glucose 10 (buffered to pH 7.4 with NaOH). The intracellular solution was (mM): K gluconate 110, KCl 20, NaCl 10, $MgCl_2$ 1, MgATP 2, EGTA 2, Na_2GTP 0.3 (buffered to pH 7.2 with KOH).

Ventricular cell restitution protocol: Cells were stimulated using a 5 ms current pulse. A pacing train of 4 stimulations separated by 200 ms (S1) was followed by a single stimulus (S2). The interval between S1-S2 ranged from 1000 ms to 20 ms. Our methods were adapted from published protocols [3, 4]. The same extracellular and intracellular solutions were used as described above for action potential recordings.

Calcium currents. Calcium currents were measured using calcium ions as the charge carrier [5]. Prior to whole cell configuration, ventricular myocytes were bathed in a solution

containing (mM): NaCl 140, KCl 6, MgCl2 1, CaCl2 2, glucose 10, H-HEPES 5 (buffered to pH 7.4 with NaOH). When the whole-cell configuration was obtained the extracellular solution was switched to a solution containing (mM): CaCl2 2, TEA-Cl 140, Cs-Cl 6, MgCl2 1, glucose 5, H-HEPES 5 (buffered to pH 7.4 with TEA-OH). The junction potential was measured to be around 3 mV, and was not corrected. The intracellular solution was (mM): CsCl2 125, MgCl2 1, EGTA 10, Na2GTP 0.3, H-HEPES 20 (buffered to pH 7.2 with CsOH). Currents were evoked during 400 ms depolarizing voltage steps to potential between -100 mV and +40 mV from a holding potential of -80 mV. Maximum currents were measured.

Calcium currents. Calcium channel currents were also measured with Ba^{2+} as the charge carrier. For recording the $I_{Ba,}$ external Na^{+} was substituted by isomolar tetraethylammonium (TEA), and extracellular Ca^{2+} by Ba^{2+}. The composition of the extracellular solution was (mM): TEA 135, KCl 5.2, $BaCl_2$ 2, $MgCl_2$ 1, NaH_2PO_4 0.33, H-HEPES 5, Glucose 10 (pH 7.4 buffered with TEA-OH). The intracellular solution was the same as the one used for AP recordings. Membrane currents were elicited with a series of 400 ms depolarising pulses to + 60 mV from a holding potential of – 80 mV in 10 mV increments. Maximum currents were measured.

Potassium currents. Voltage-gated K^{+} currents were evoked during a 4.5 s depolarizing voltage step to a test potential between -40 mV and +65 mV from a holding potential of -70 mV [6]. Voltage steps were given in 10 mV increments. The extracellular solution contained (mM): NaCl 135, KCl 5.4, CaCl2 1, MgCl2 1, CoCl2 2, NaH2PO4 0.33, H-HEPES 10, Glucose 5 (pH 7.4 buffered with NaOH). The intracellular solution was the same as the one used for AP recordings.

Figure Legend

Figure S1. *Cardiac histology in wild type and $G\alpha_{i2}$ (-/-) mice.* No detectable histological difference between hearts from $G\alpha_{i2}$ null (ko in figure) and control (wt in figure) mice. (A, B) Representative low power image of H+E stained short-axis sections through wild-type and Gαi2 null hearts respectively. Section at mid-papillary level. Five hearts of each genotype were examined. (C,D) High power images of left ventricular free wall of wild-type and $G\alpha_{i2}$ null hearts, H+E stained. (E, F) Masson's trichrome staining of wild-type and $G\alpha_{i2}$ null hearts, showing blue collagen stain in blood vessels (arrowed). Note low levels of collagen staining in both hearts. Scale bar; 100μm.

Reference List

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Table S1. Myocardial function as assessed by echocardiography. (LV; left ventricle).

	Control	$G\alpha_{i2}$ (-/-)	p value
Stroke Volume (µl)	43.0 (±2.0)	42.7 (±2.0)	0.92
Heart rate (1/min)	477 (±16)	479 (±8)	0.94
Cardiac Output (ml/min)	20.5 (±1.3)	20.4 (±0.8)	0.93
Peak Velocity (m/s)	0.79 (±0.02)	0.78 (±0.04)	0.73
LV End Diastolic Dimension (cm)	0.38 (±0.01)	0.38 (±0.01)	0.88
LV End Systolic Dimension (cm)	0.27 (±0.01)	0.27 (±0.02)	0.84
Ejection Fraction (%)	58 (±2.0)	55 (±4.0)	0.51
Diastolic Anterior Wall Dimension (cm)	0.065 (±0.003)	0.065 (±0.003)	1
Systolic Anterior Wall Dimension (cm)	0.08 (±0.00)	0.08 (±0.00)	0.62
Diastolic Posterior Wall Dimension (cm)	0.07 (±0.00)	0.07 (±0.00)	0.62
Systolic Posterior Wall Dimension (cm)	0.09 (±0.01)	0.09 (±0.00)	0.47

Figure S1