Supplemental Table 1. Human ESC-derived cardiomyocyte action potential properties

		Amplitude (mV)	Upstroke velocity (mV/ms)	APD (ms)		RMP (mV)	N
				APD_{50}	APD_{90}		
H1 hESCCMs	Pacemaker-like	31.0	0.75	62.3	100.9	-27.7	1
	*Atrial-like	79±4.1	18.5±2.3	39.6±4.1	73.8±11.0	-30.2±3.5	12
	*Ventricular-like	92.7±7.5	17.3±2.7	111.1±17.3	150.0±18.8	-38.5±1.7	7
HES2 hESCCMs	Pacemaker-like	24.1	0.256	89.7	157.0	-23.2	1
	*Atrial-like	86.4±6.4	16.6±1.5	32.4±3.0	113.2±7.2	-24.1±2.0	11
	*Ventricular-like	90.9±4.8	17.5±1.5	259.6±39.8	370.8±49.9	-35.5±2.0	21

$APD_{50/90}$, action potential duration at 50% / 90% maximal amplitude, RMP, resting membrane potential

* The parameters are from trigged action potential in atrial-like and ventricular-like ESCCMs

Supplemental Table 2

Proteins with at least a two fold differential expression in H1 vs hES2

	Spot number	ave ratio (log)	SEM	up-regulated in hES2	up-regulated in H1	Protein Name	Accession No.	Protein MW	Protein PI	Pep.Count	Protein Score C. I. %	Total Ion Score	Total Ion C. I. %
	1	2.22	0.46		x	heat shock 70kDa protein 4 isoform a [Homo sapiens]	gi|38327039	94271.2	5.11	29	100	507	100
	2	1.09	0.46		x	valosin-containing protein [Homo sapiens]	gi|55662798	89265.7	5.14	36	100	367	100
	3	2.12	0.38		x	Alanyl-tRNA synthetase [Homo sapiens]	gi|15079238	106743.2	5.34	26	100	380	100
	4	-1.28	0.24	x		valosin-containing protein [Homo sapiens]	gi|55662798	89265.7	5.14	34	100	321	100
	5	1.50	0.53		x	ATP-dependent DNA helicase II [Homo sapiens]	gi|10863945	82652.3	5.55	34	100	289	100
	6	2.36	0.63		x	vinculin [Homo sapiens]	gi|57162633	116649.3	5.83	39	100	490	100
	7	-0.22	0.08	x		FLNA protein [Homo sapiens]	gi|15779184	88534.4	5.93	31	100	656	100
	8	1.46	0.83		x	eukaryotic translation elongation factor 2 [Homo	gi|4503483	95277	6.41	22	100	118	100
	9	3.26	0.71		x	eukaryotic translation elongation factor 2 [Homo	gi|4503483	95277	6.41	22	100	118	100
	10	1.93	0.67		x	Methylenetetrahydrofolate dehydrogenase 1 [Homo	gi|14602585	101467.3	6.75	19	100	104	100
	11	2.56	0.03		x	KHSRP protein [Homo sapiens]	gi|54648253	72851.3	8	23	100	430	100
	12	1.11	0.41		x	GARS protein [Homo sapiens]	gi|12652637	84594.4	7.85	24	100	590	100
	13	2.13	0.41		x	Villin 2 [Homo sapiens]	gi|46249758	69198.6	5.94	30	100	486	100
	14	-3.36	0.34	x		moesin [Homo sapiens]	gi|5419633	67777.8	6.08	25	100	368	100
	15	-2.10	0.80	x		Phosphoenolpyruvate carboxykinase 2 (mitochondrial)	gi|12655193	70653.6	7.57	26	100	140	100
	16	1.24	0.49		x	Phosphoenolpyruvate carboxykinase 2 (mitochondrial)	gi|12655193	70653.6	7.57	30	100	366	100
	18	0.99	0.39		x	transketolase [Homo sapiens]	gi|37267	67750.7	7.9	19	100	680	100
	19	2.11	0.44		x	transketolase [Homo sapiens]	gi|37267	67750.7	7.9	20	100	690	100
	21	1.57	0.38		x	dihydropyrimidinase-like 3 [Homo sapiens]	gi|4503379	61924.1	6.04	24	100	414	100
	22	2.18	0.40		x	dihydropyrimidinase-like 3 [Homo sapiens]	gi|4503379	61924.1	6.04	23	100	653	100
	23	1.19	0.40		x	CBS protein [Homo sapiens]	gi|13938263	60548.2	6.2	19	100	561	100
	24	-1.39	0.39	x		heterogeneous ribonuclear particle protein L - human	gi|106246	60149.2	6.65	18	100	457	100
	25	1.33	0.47		x	HNRPL protein [Homo sapiens]	gi|46812638	60195.2	6.65	14	100	166	100
	26	-3.34	0.22	x		Prolyl 4-hydroxylase, beta subunit [Homo sapiens]	gi|48735337	57080.7	4.76	22	100	604	100
	27	1.70	0.45		x	FK506 binding protein 4 [synthetic construct]	gi|60656373	51772.1	5.35	21	100	837	100
	28	1.83	0.41		x	FK506 binding protein 4 [synthetic construct]	gi|60656373	51772.1	5.35	17	100	464	100
	29	0.94	0.89		x	ATPase, H+ transporting, lysosomal 56/58kD, V1	gi|19913428	56464.9	5.57	15	100	199	100
	30	2.72	0.74		x	Keratin, type II cytoskeletal 8 (Cytokeratin 8) (K8) (CK	gi|2506774	53641.1	5.52	22	100	460	100
	31	0.78	0.42		x	dihydropyrimidinase-like 3 [Homo sapiens]	gi|4503379	61924.1	6.04	22	100	404	100
	33	3.16	0.06		x	phosphoglycerate dehydrogenase [Homo sapiens]	gi|56205096	56614.4	6.29	19	100	535	100
	34	-1.65	0.43	x		FSCN1 protein [Homo sapiens]	gi|14044046	54496.1	6.84	21	100	462	100
	35	-3.49	0.14	x		Guanine nucleotide binding protein (G protein), beta	gi|21619296	35054.6	7.6	17	100	600	100
	36	2.26	0.45		x	caspase 3, apoptosis-related cysteine protease [Homo	gi|27979119	31587.5	6.09	15	100	357	100
	37	1.41	0.42		x	ATP synthase, H+ transporting, mitochondrial F1	gi|50345982	54459.7	8.24	8	100	111	100
	38	0.99	0.52		x	UTP-glucose-1-phosphate uridylyltransferase (EC	gi|2136353	56929.8	8.43	20	100	628	100
	41	2.35	0.50		x	Sjogren syndrome antigen B (autoantigen La) [Homo	gi|32880067	46808.2	6.68	19	100	290	100
	44	1.83	0.36		x	S-adenosylhomocysteine hydrolase [Homo sapiens]	gi|30582233	47685.2	5.92	21	100	668	100
	46	-0.79	0.43	x		HNRPH1 protein [Homo sapiens]	gi|12655001	49198.4	5.89	14	100	615	100
	47	2.03	0.60		x	PAICS protein [Homo sapiens]	gi|17939425	47049.1	6.95	19	100	823	100
	48	2.19	0.78		x	RNA-binding protein LIN-28 [Homo sapiens]	gi|21842304	22728.1	8.35	14	100	324	100
	51	3.17	0.09		x	Nucleophosmin 1 [Homo sapiens]	gi|16307090	29446.2	4.47	5	100	173	100
	52	-0.76	0.31	x		tropomyosin alpha chain, cardiac and skeletal muscle -	gi|88929	32688.7	4.69	11	100	37	87.561
	53	-4.83	0.61	x		Unknown (protein for IMAGE:3538275) [Homo sapiens]	gi|16924319	40477.2	5.78	10	100	317	100
	55	3.60	0.16		x	growth-inhibiting gene 5 protein [Homo sapiens]	gi|41350397	26193.6	8.28	12	100	96	100
	56	1.06	0.46		x	TALDO1 protein [Homo sapiens]	gi|48257056	37385.4	6.35	10	100	97	100
	57	2.01	0.51		x	TALDO1 protein [Homo sapiens]	gi|48257056	37385.4	6.35	13	100	289	100
	58	2.08	0.55		x	TALDO1 protein [Homo sapiens]	gi|48257056	37385.4	6.35	15	100	387	100
	59	-2.93	0.50	x		PREDICTED: ribosomal protein P0 [Pan troglodytes]	gi|55639049	34251.8	5.71	13	100	540	100
	60	1.53	0.26		x	aspartate transaminase (EC 2.6.1.1) - human	gi|105387	46106.5	6.81	17	100	424	100
	61	2.03	0.53		x	Chain B, Binary Complex Of Human	gi|24159119	39567.3	7.58	5	100	450	100
	64	3.49	0.32		x	GD:MAPRE1 [Homo sapiens]	gi|5748523	29980.2	5.02	11	100	405	100
	65	-2.10	0.72	x		thioredoxin peroxidase [Homo sapiens]	gi|5453549	30520.8	5.86	10	100	420	100
	66	0.89	0.17		x	CArG binding factor [Homo sapiens]	gi|38327502	33193	8.16	7	100	160	100
	67	-1.26	0.39	x		cytosolic malate dehydrogenase [Homo sapiens]	gi|1255604	36403	6.91	8	100	374	100
	68	-2.45	0.75	x		Annexin A2, isoform 2 [Homo sapiens]	gi|18645167	38551.8	7.57	19	100	654	100

HES2 Culture

The HES2 line was cultured as described previously [5, 20]. Briefly, cells were grown on mouse embryonic fibroblasts (mEFs) pre-treated with 10 μg/ml mitomycin C (Sigma; St Louis, MO, USA) for 3h. Culture medium consisted of DMEM (Invitrogen, Carlsbad, CA, USA) containing 2mM l-glutamine(Invitrogen, Carlsbad, CA, USA), insulin-transferrin-selenium (Invitrogen, Carlsbad, CA, USA), non-essential amino acids (Invitrogen, Carlsbad, CA, USA), 90 μM β-mercaptoethanol (Gibco, Carlsbad, CA, USA), 20 U/ml penicillin (Invitrogen, Carlsbad, CA, USA), 20 μg/mL streptomycin (Invitrogen, Carlsbad, CA, USA), and 20% fetal calf serum (FCS) (Hyclone, Logan, UT). The HES2 line was passaged manually ("cut-and-paste"), by cutting colony pieces and removing them from the mEFs using dispase (10 mg/mL) for 2 min. The resulting HES2 colony pieces were washed twice in PBS then placed on fresh MEFs. .

HES2 Differentiation

Co-culture of the HES2 and END2 cells was carried out as described previously [5, 9]. In short, END2 cells were grown to 100% confluence and treated with 10 μg/mL mitomycin C (Sigma; St Louis, MO.) for 3 h. Undifferentiated HES2 cells were removed from the mEFs using 10mg/mL dispase (Invitrogen, Carlsbad, CA). Colonies were then washed twice with PBS, resuspended in hESC media and broken into pieces by repeated pipeting. These pieces were then transferred to the END2 cell layer and incubated at 37°C for 2–3 weeks in hESC medium lacking serum. The co-cultures were refreshed with medium lacking serum every 4–5 days. Areas of beating cardiomyocytes were scored by visual examination from day 7 onwards.

H1 Culture

The hESC line H1 (WiCell; Madison, WI) was maintained on irradiated mouse embryonic fibroblasts (mEFs) and propagated as previously described [1, 21, 22]. Briefly, the culture media consisted of Dulbecco's Modified Eagle's medium (DMEM)/F12 (Invitrogen Corp.; Carlsbad, CA) supplemented with 15% KO serum replacer (Invitrogen, Carlsbad, CA), 1 mM L-glutamine, 0.1 mM ß-mercaptoethanol (Sigma, St. Louis, MO 1% nonessential amino acids and 4 ng/ml fibroblast growth factor-β (β-FGF) (Invitrogen). Cells were grown in 6 well plates and passaged by adding 1 mL of 1mg/mL Collagenase IV (Invitrogen, Carlsbad, CA) and incubating for 5 minutes at 37°C. The entire well was then scraped and colonies were dissociated by pipeting up and down 20-30 times. Once dissociated, the colony pieces were pelleted at 1000 x g for 5 minutes and resuspended in culture media. The resulting suspension was plated on fresh mEFs at a density of 1:5. The culture media was then changed everyday.

H1 Differentiation

For cardiac differentiation, human EBs were formed from enzymatically dispersed hESCs suspended in petri dishes in the absence of LIF and β-FGF [8, 23, 24]. On day 7, suspended hEBs were plated onto gelatin-coated 6-well plates. The differentiation media, which contained 15% FBS and 0.1mM non-essential amino acids in DMEM, was replenished daily during differentiation. Spontaneously beating outgrowths, which typically appeared 2-3 days after plating, were identified by visual inspection.

Cardiomyocyte Isolation

Spontaneously beating HES2- and H1-derived cardiomyocytes were dissected from

hESC aggregates 14-21 days after the start of differentiation by a custom made glass knife and immediately washed with PBS. The cells were then incubated with 1mg/ml Collagenase IV (Invitrogen, Carlsbad, CA) at 37^{o}C for 30 min. After centrifugation, the cell pellet was incubated with KB solution containing 85 mM KCl, 30 mM K_2HPO_4, 5 mM $MgSO_4$, 1 mM EGTA, 2 mM Na_2-ATP, 5 mM pyruvic acid, 5 mM creatine, 20 mM taurine, and 20 mM d-glucose, at room temperature for 1hr with gentle mixing. The cell/KB solution mixture was added directly to a laminin treated glass coverslip and the cells were allowed to settle for 30 minutes at 37^{o}C. One ml of culture media (containing 15% FBS) was then added and the cells were incubated at 37^{o}C overnight. The media was refreshed the next day.

Electrophysiology

After dissociation, action potential (AP) recordings from single cells were done using the whole-cell patch-clamp technique. Patch pipettes were prepared from 1.5 mm thin-walled borosilicate glass tubes using a Sutter Micropipette Puller (P-97) and typically had resistances of 4-6 MΩ when filled with an internal solution containing (mM): 110 K^+ aspartate, 20 KCl, 1 $MgCl_2$, 0.1 Na-GTP, 5 Mg-ATP, 5 Na_2-phospocreatine, 1 EGTA, 10 HEPES, pH adjusted to 7.3 with KOH. The external Tyrode's bath solution consisted of (mM): 140 NaCl, 5 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 glucose, 10 HEPES, pH adjusted to 7.4 with NaOH. Upon seal formation and following patch break, APs were recorded using the current-clamp mode. Voltage recordings were filtered at 10 KHz. Axopatch 200B, Digitize 1322 and pClamp8 (Axon Burlingame, CA, USA) were used for data amplification and acquisition.

H1- or HES2-derived CMs were categorized into pacemaker-, atrial- or ventricular-like phenotypes, based on such common electrophysiological characteristics as the AP amplitude

(mV), upstroke velocity (mV/ms), APD50 and APD90 (ms), as well as the resting membrane potential (RMP, mV). We primarily used the AP profiles as signatures of different CM types. Nodal-like AP phenotype was defined as those that exhibited: **a)** prominent phase-4 depolarization, **b)** slow upstroke (dV/dt), **c)** small action potential amplitude (APA), **d)** relatively depolarized MDP, and **e)** spontaneous firing. By contrast, like others, we defined the ventricular-like phenotype as those that displayed: **i)** a significant plateau phase, **ii)** longer APD (vs. those of atrial and nodal), **iii)** rapid upstroke, and **iv)** a flat phase 4. Atrial APs were those that displayed a triangular shape. Of note, in comparison to neonatal and adult human ventricular and atrial CMs, the AP parameters of hESC-CMs exhibit MDP and upstroke velocities that are positive (~-40 vs. ~-80mV) and slow (~10V/s vs. 100-300V/s), respectively. These differences are in line with those reported previously for mouse ESC-, neonatal and adult CMs [25, 26].

2-D DIGE

Sample preparation: The cell samples were obtained by removing undifferentiated colonies of H1 and HES2 cells from their respective feeder layers by incubation with 1mg/ml collagenase IV for 5 minutes at 37°C followed by 10 minutes of 10mg/mL dispase digestion. Each sample contained cells collected from at least three independent passages. The cells were lysed in 2-D lysis buffer containing 30 mM Tris-HCl, pH8.8, 7 M Urea, 2 M thio-urea, and 4% CHAPS, and vigorously sonicated for 5 seconds using VirSonic 100 (VirTis). After vigorous shaking at room temperature for 30 minutes, the protein lysates were cleared by high speed centrifugation for 30 min. Supernatants were transferred to fresh eppendorf tubes, and protein concentrations are adjusted to 5 mg/ml. To minimize biological variations, triplicates of HES2 and H1 cell samples were resolved on 3 independent gels. In addition to the individual samples,

all 6 (3 of each hESC line) were combined to form an internal standard that was also resolved on each gel to minimize gel-to-gel differences as described previously [27, 28].

CyDye labeling: Each sample was labeled with a different CyDye. H1 cells were labeled with Cy5, while HES2 cells were labeled with Cy3 (GE Healthcare/Amersham). The internal standard was labeled with Cy2. Briefly, CyDyes were diluted 1:25 with dimethyl formamide immediately before reaction and 25 μg of protein lysate was mixed with 0.7 μl of diluted CyDye. The samples were then incubated on ice for 30 minutes, followed by the addition of 0.7 μl of 10 mM lysine to each of the samples to stop the reaction.

First dimension IEF: CyDye-labeled protein samples were mixed in a fresh vial and 100 μl of rehydration buffer (7 M urea, 2 M thiourea, 4% CHAPS, 2 mg/ml DTT, 1% bromophenol blue, 1% Pharmalyte for IEF, pH 3-10) was added, followed by 140 μl destreak solution (7 M urea, 2 M thiourea, 4% CHAPS, 1% bromophenol blue, 100 mM destreak, 2% Pharmalyte for IEF, pH 3-10) to a final volume of approximately 260 μl. The samples were mixed well and 250 μl was loaded per IEF strip (GE Healthcare/Amersham). IEF was then done for a total of 25000 volt-hours with standard conditions using Ettan IPGPhore II (as recommended by GE Healthcare/Amersham).

Second dimension SDS-PAGE: After the IEF, each IEF strip was incubated with 10 ml of 10 mg/ml DTT in equilibration solution (6 M urea, 30% Glycerol, 50 mM Tris-HCl, pH 8.8, 2% SDS) for 15 min with gentle shaking. Each IEF strip was then incubated with 10 ml of 45 mg/ml Iodoacetamide in equilibration solution (6 M urea, 30% Glycerol, 50 mM Tris-HCl, pH 8.8, 2% SDS) for 10 min with gentle shaking to block SH groups. The strips were then rinsed in SDS gel

running buffer once, inserted into a 9-12% gradient SDS gel and covered with 0.5% agarose sealing solution. Electrophoresis was performed at 16 ^{o}C.

*Gel scanning and data analysis***:** The resulting 2-D gel was scanned using a Typhoon Trio scanner (GE Healthcare/Amersham). A total of 3 images were generated for each gel at a resolution of 100μm using the spectrally resolvable dyes. These images were imported into the Decyder v6.5 software package. Using the Differential In Gel Analysis (DIA) module the normalized volume ratio of each Cy3 or Cy5 labeled, individually identified protein spot on a given gel was compared to the Cy2 labeled internal standard (corresponding to the same spot) from the same gel to quantitatively determine the fold change in the compared samples. The relative ratios of a given spot were determined from each of the 3 replicate gels.

Mass spectrometry.

Selected protein spots were excised from the gel using Ettan spot picker (GE Healthcare/Amersham). Following in-gel digestion and sample cleaning, tryptic digests were analyzed using matrix-assisted laser desorption/ionization (MALDI) mass spectrometry. The matrix used was a-cyano, 4-hydroxy cinnamic acid. A positive ion mass spectrum was obtained for the tryptic digest using MALDI-TOF/TOF technology (4700 Proteomics Analyzer, Applied Biosystems). Mass accuracy was better than 50 ppm (usually 10 ppm). Peaks were acquired in a reflector positive mode with mass range 900 to 3500. The top 10 monoisotopic peaks were further subjected to MS/MS analysis using TOF/TOF. Both MS and MS/MS data were submitted to the GPS Explorer workstation for database search using the MASCOT search engine (MATRIX science). The NCBInr human database was used for searching and the mass accuracy was calculated using trypsin autolytic peptide as a reference. Search parameters

allowed for a mass accuracy of ±50 ppm, one missed cleavage of trypsin, oxidation of methionine, and carbamido-methylation of cysteine.

Pathway Analysis

Ingenuity Pathway Analysis (IPA) software (www.ingenuity.com) was used for the analysis of the gene list generated by 2D-DIGE experiments. For Ingenuity pathway analysis, biological functions were assigned to the overall analysis based on findings in the scientific literature and those stored in the Ingenuity Pathways Knowledge Base. The calculations of significant score for networks in IPA are based on Fischer's Exact test. The p-values for Functions and Pathways are calculated using the right-tailed Fisher's Exact Test.