

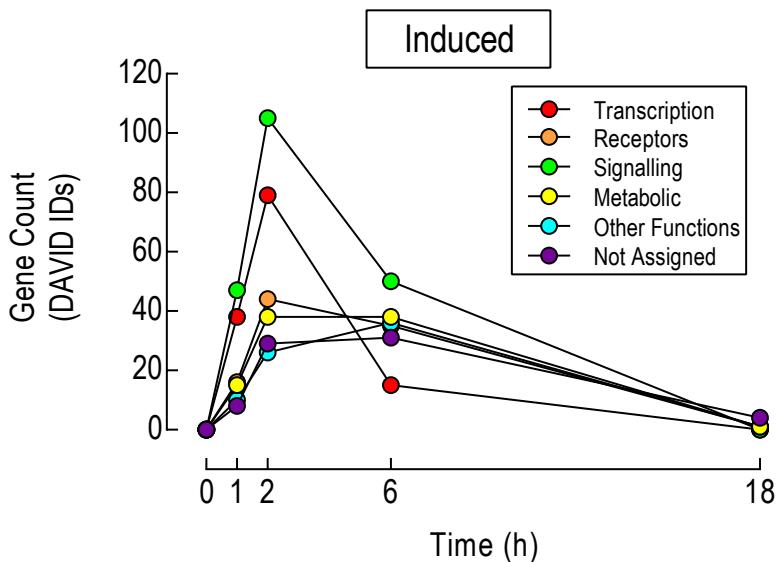
## Supplemental Material

# Analysis of the Indacaterol-Regulated Transcriptome in Human Airway Epithelial Cells Implicates Gene Expression Changes in the Adverse and Therapeutic Effects of $\beta_2$ -Adrenoceptor Agonists

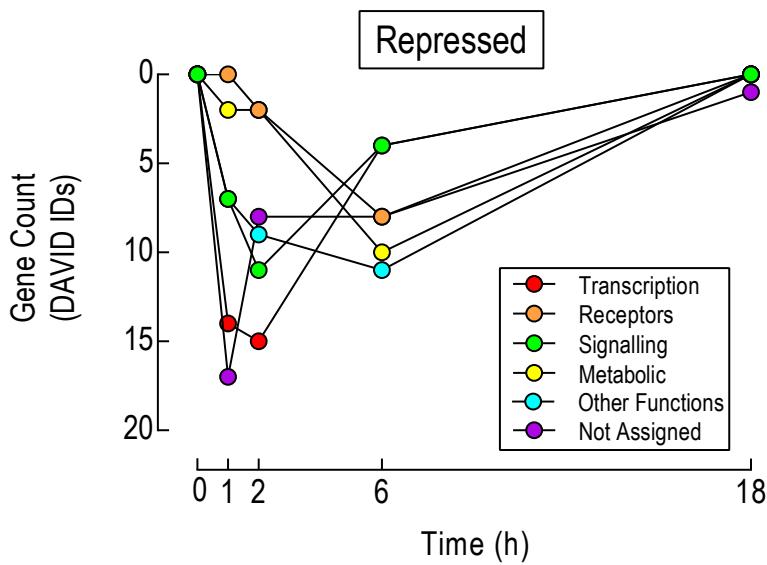
Dong Yan, Omar Hamed, Taruna Joshi, Mahmoud M. Mostafa, Kyla C. Jamieson, Radhika Joshi, Robert Newton & Mark A. Giembycz

*Departments of Physiology & Pharmacology (D.Y., O.H., K.C.J., T.J., R.J., M.A.G.) and Cell Biology & Anatomy (R.N., M.M.M.), Snyder Institute for Chronic Diseases, Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada.*

(A)

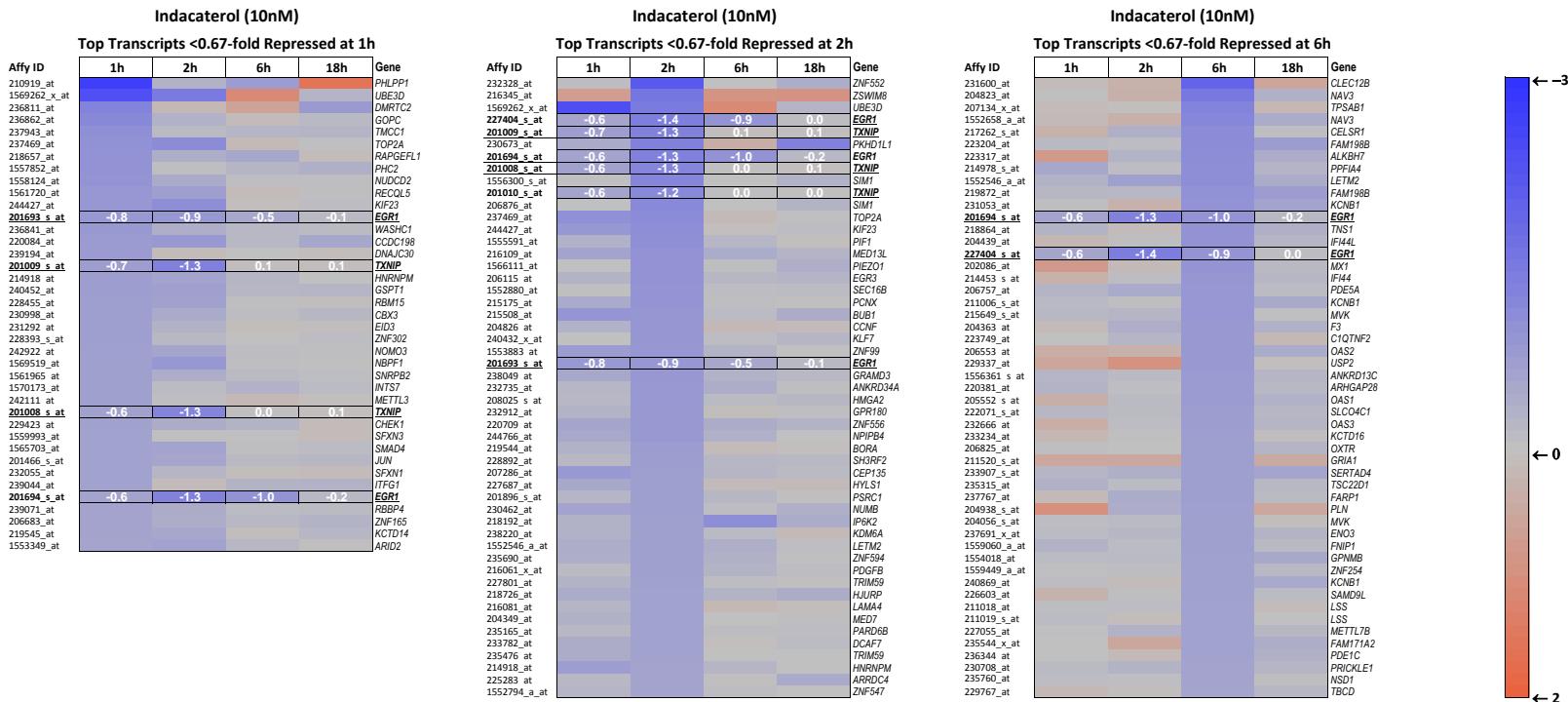


(B)



**Supplemental Fig. 1.** Effect of indacaterol on the number of significantly induced and repressed genes (panels A and B respectively) at 1h, 2h, 6h and 18h that were annotated with one of the six broad GO terms used in figure 4.





**Supplemental Fig. 3.** Heat maps showing the effect of indacaterol on gene repression in BEAS-2B cells treated with indacaterol (10nM) for 1h, 2h and 6h. The top repressed transcripts by probe set that were statistically different from time-matched, vehicle-treated cells at each time-point (FDR P value <0.1) were ranked highest to lowest and presented as heat maps relative to changes of the same transcripts at the other two time-points. The colour scale is logarithmic (base 2) with the most intense blue and red colours representing a 8-fold decrease ( $\log_2 = -3$ ) and an 8-fold increase ( $\log_2 = 2$ ) in gene expression respectively. The right hand-side and left hand-side of every row in each heat map shows the gene name and corresponding Affymetrix identification number (Affy ID). Genes validated by PCR are underlined and bolded in black, and their expression level changes are shown in each cell in log2 format.

**Supplemental TABLE 1.** Donor demographics.

Donor ID#	Age	Gender	Ethnicity	Smoker	Cause of Death	Source of Epithelial Cells
p42	18	Male	Caucasian	No	Head Trauma	Right Lung
p48	22	Male	Caucasian	Yes	Anoxia	Left Lung
p72	62	Male	Caucasian	No	Intracerebral Haemorrhage	Right Lung
p78	60	Male	Hispanic	Yes	Head Trauma	Left Lung
p82	42	Male	Caucasian	Yes	Intracerebral Haemorrhage	Right Lung

**Supplemental TABLE 2.** Primer pairs for real-time PCR. Official HGNC gene symbols are shown. When appropriate common gene symbols are provided in parentheses. Generic primers were used for genes that encode multiple isoforms.

Gene	Oligonucleotides	Accession Number(s)
<b>AVPII</b>		NM_021732.2
Forward	5' -AAGGCTGTGGAAGAGAGTTGG-3'	
Reverse	5' -TGTGGATGCTCACACTGAAGG-3'	
<b>BDNF</b>		NM_001352269.1, NM_001352270.1, NM_001352267.1, NM_001352271, NM_020734.4, NM_001352268.1, NM_001297776.1, XM_017019690.1, XM_017019683.1, XM_017019684.1, XM_017019685.1, XM_017019686.1 XM_017019687.1, XM_017019688.1, XM_017019689.1
Forward	5' -GGCGGCAGACAAAAAGACTG-3'	
Reverse	5' -TGGGATTGCACTTGGTCTCG-3'	
<b>C5ARI</b>		NM_001736.3
Forward	5' -TCCTCCGGAACGTGTTGACT-3'	
Reverse	5' -AGCGCGTGAATGACTTGCT-3'	
<b>CD200</b>		NM_005944.5, NM_001004196.2
Forward	5' -GGACTGTGACCGACTTTAACCAA-3'	
Reverse	5' -AGCAATAGCGGAACTGAAAACC-3'	
<b>CDKN1C (p57<sup>kip2</sup>)</b>		NM_000076.2, NM_001122630.1, NM_001122631.1
Forward	5' -CTGTCCGGGCCTCTGATCT-3'	
Reverse	5' -CATCGCCCGACGACTTCT-3'	
<b>CEBPD</b>		NM_005195
Forward	5' -GGAGATGCAGCAGAACAGTTGGT-3'	
Reverse	5' -CGCGCTGGTGCAGCTT-3'	
<b>DEPDC7</b>		NM_139160.2, NM_001077242.1
Forward	5' -GACTCTCAGGAAGATGAGTGG-3'	
Reverse	5' -GTCTGGTTGCTCAGGAAAGC-3'	
<b>DMBT1</b>		NM_004406.2, NM_007329.2, NM_017579.2, NM_001320644.1
Forward	5' -GGTGGCATCCAACAACACTACAA-3'	
Reverse	5' -GTAGGATGGCTGGAGAATGT-3'	
<b>DUSP1 (MKP1)</b>		NM_004417.3
Forward	5' -CGCGCAAGTCTTCTTCCTCA-3'	
Reverse	5' -GATGCTTCGCCTCTGCTTCA-3'	
<b>EDN1</b>		NM_001955.4, NM_001168319.1
Forward	5' -TGATTTCTCTCTGCTGTTGTGG-3'	
Reverse	5' -GCGCCTAAGACTGCTGTTCT-3'	

<b>Gene</b>	<b>Oligonucleotides</b>	<b>Accession Number(s)</b>
<b><i>EGR1</i></b>		NM_001964.2
Forward	5' -ACCTGACCGCAGAGTCTTT-3'	
Reverse	5' -GAGTGGTTGGCTGGGTAA-3'	
<b><i>EPHA4</i></b>		NM_001304537.1, NM_001304536.1, NM_004438.4, XM_005246374.2
Forward	5' -GGATCATTGGAGATGGGGCT-3'	
Reverse	5' -GTATTACTCCGTCTCCGGCT-3'	
<b><i>FGFR2</i></b>		NM_000141.4, NM_022970.3, NM_001144913.1, NM_023029.2
Forward	5' -CATCGCATTGGAGGCTACAAG-3'	NM_001144914.1, NM_001144915.1, NM_001144916.1,
Reverse	5' -GGGACCACACTTCATAATGAG-3'	NM_001144917.1, NM_001144918.1, NM_001144919.1.
<b><i>GAPDH</i></b>		NM_002046.5, NM_001256799.2
Forward	5' -ATGGAAATCCCATCACCATCTT-3'	
Reverse	5' -CAGCATGCCCACTTG-3'	
<b><i>GAS1</i></b>		NM_002048.2
Forward	5' -CAGATTGTCGCCAGTGAGGA-3'	
Reverse	5' -TCTGGGCCGCAGATACAAACA-3'	
<b><i>IL6</i></b>		NM_000600.4, NM_001318095.1
Forward	5' -GAGTAGTGAGGAACAAGCCAG-3'	
Reverse	5' -GTCAGGGGTGGTTATTGCATC-3'	
<b><i>IL11</i></b>		NM_000641.3, NM_001267718.1
Forward	5' -GGGACCACAAACCTGGATTTC-3'	
Reverse	5' -CGCAGGTAGGACAGTAGGT-3'	
<b><i>NR4A1</i></b>		NM_002135.4, NM_173157.2, NM_001202233.1, NM_001202234.1
Forward	5' -GCATGGTGAAGGAAGTTGTCCG-3'	
Reverse	5' -GGGGCTGCTGGGTTTGAA-3'	
<b><i>NR4A2</i></b>		NM_006186.3
Forward	5' -GGCCCATGTCGACTCCAA-3'	
Reverse	5' -GTCAGGGTTGCCCTGGAA-3'	
<b><i>NR4A3</i></b>		NM_006981.3, NM_173200.2, NM_173199.2
Forward	5' -GTAGACAAGAGACGTCGAAACC-3'	
Reverse	5' -CCTCTCCTCCCTTCAGACTAT-3'	
<b><i>PDE4D</i></b>		NM_006203.4, NM_001104631.1, NM_001165899.1, NM_001197218.1, NM_001197219.1, NM_001197220.1, NM_001197221.1, NM_001197222.1 NM_001197223.1
Forward	5' -CTCTCTGAAATGAGTCGGTCTG-3'	
Reverse	5' -CCTCTGAGTTGGAGAAGGAAT-3'	

<b>Gene</b>	<b>Oligonucleotides</b>	<b>Accession Number(s)</b>
<b>PDK4</b>		NM_002612.3
Forward	5' -GCTGCCATGAAGCAGCTACTG-3'	
Reverse	5' -CGCAAAATGCAAAAGAAGTTCT-3'	
<b>PPP1R3C</b>		NM_005398.6
Forward	5' -CCTAATGAGCTGCACCAGAACATG-3'	
Reverse	5' -TCACAGGTGGTGAATGTGCC-3'	
<b>RGS2</b>		NM_002923
Forward	5' -CCTCAAAAGCAAGGAAAATATATACTGA-3'	
Reverse	5' -AGTTGTAAAGCAGCCACTTGTAGCT-3'	
<b>RIMKLB</b>		NM_001352269.1, NM_001352270.1, NM_001352267.1, NM_001352271, NM_020734.4, NM_001352268.1, NM_001297776.1, XM_017019690.1, XM_017019683.1, XM_017019684.1, XM_017019685.1, XM_017019686.1, XM_017019687.1, XM_017019688.1
Forward	5' -GCGGGGTCACAGAGGTAAAG-3'	
Reverse	5' -GGAACAGGTATGGCGCTTCA-3'	
<b>SGK1</b>		NM_001291995.1, NM_001143676.1, NM_001143677.1, NM_001143678.1
Forward	5' -ATGGCCTGCCGCCCTTTTAT-3'	
Reverse	5' -CAGGAGGTGTCTTGCAGGAAT-3'	
<b>SLC7A2</b>		NM_001164771.1, NM_001008539.3, NM_003046.5, XM_017013747, XM_005273610.4, XM_005273611.4, XM_005273612.4
Forward	5' -GGATTCTTAGCTTCCTCGTG-3'	
Reverse	5' -GCCTGGTGTGGCATGAAC-3'	
<b>SMOC1</b>		NM_022137.5, NM_001034852.2, XM_005267995.1, XM_005267996.1
Forward	5' -GGCGTTCACCGACTACTGT-3'	
Reverse	5' -AGCGTCCTACTTCTTGCT-3'	
<b>SOCs3</b>		NM_003955.3
Forward	5' -GATTCTCCTTCAATTCTCAGCTT-3'	
Reverse	5' -ATTAGTTCAGCATTCCGAAGTGT-3'	
<b>STEAP4</b>		NM_001205316.1, NM_001205315.1, NM_024636.3
Forward	5' -GTTCCGATTTGTCCAGTCCA-3'	
Reverse	5' -GGCTGAGGAATCTCTCCCAC-3'	
<b>TXNIP</b>		NM_006472.5, XM_017000085.1
Forward	5' -AGACCAGCCAACAGGTGAGA-3'	
Reverse	5' -AGGAAGCTCAAAGCCGAAC-3'	













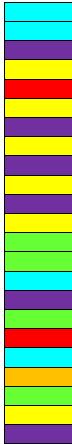
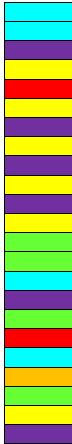
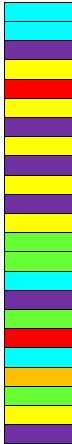
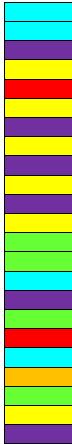
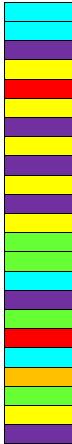
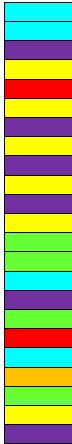
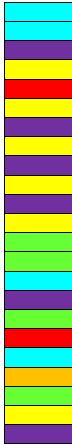
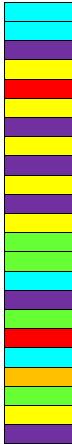
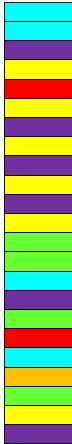
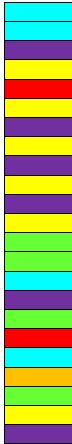
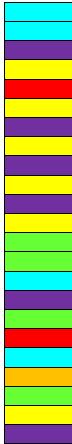
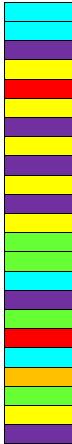
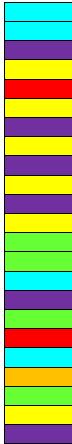
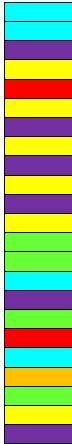
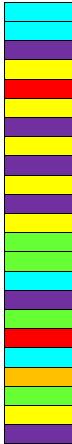
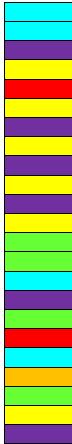
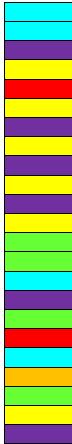
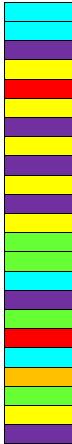
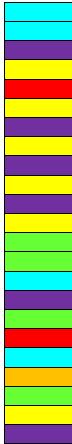
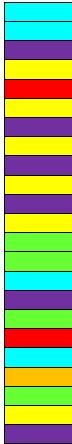
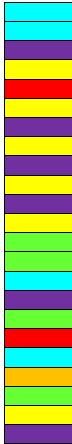
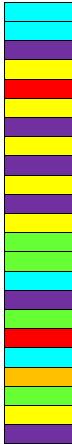
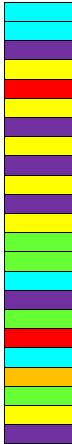




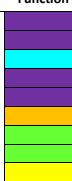
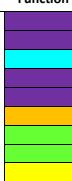
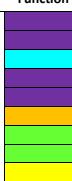
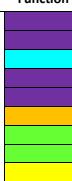
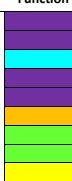
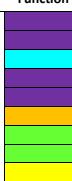
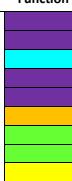
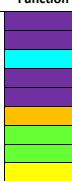






201858_s_at	5552	<b><i>SRGN</i></b>	6.80E-03	1.53	serglycin	
1555950_a_at	1604	<b><i>CD55</i></b>	1.74E-02	1.53	CD55 molecule, decay accelerating factor for complement (Cromer blood group)	
227657_at	57484	<b><i>RNF150</i></b>	3.90E-02	1.53	ring finger protein 150	
229812_at	84196	<b><i>USP48</i></b>	4.12E-02	1.53	ubiquitin specific peptidase 48	
231015_at	28999	<b><i>KLF15</i></b>	7.74E-02	1.53	Kruppel-like factor 15	
218113_at	23670	<b><i>TMEM2</i></b>	9.95E-02	1.53	transmembrane protein 2	
226322_at	83857	<b><i>TMTC1</i></b>	1.70E-03	1.52	transmembrane and tetratricopeptide repeat containing 1	
219501_at	55068	<b><i>ENOX1</i></b>	2.20E-03	1.52	ecto-NOX disulfide-thiol exchanger 1	
210015_s_at	4133	<b><i>MAP2</i></b>	2.60E-03	1.52	microtubule associated protein 2	
208055_s_at	26091	<b><i>HERC4</i></b>	2.69E-02	1.52	HECT and RLD domain containing E3 ubiquitin protein ligase 4	
226811_at	54855	<b><i>FAM46C</i></b>	8.29E-02	1.52	family with sequence similarity 46, member C	
203455_s_at	6303	<b><i>SAT1</i></b>	9.47E-02	1.52	spermidine/spermine N1-acetyltransferase 1	
220227_at	1002	<b><i>CDH4</i></b>	1.30E-03	1.51	cadherin 4, type 1, R-cadherin (retinal)	
229310_at	114818	<b><i>KLHL29</i></b>	1.00E-02	1.51	kelch-like family member 29	
210664_s_at	7035	<b><i>TFPI</i></b>	2.43E-02	1.51	tissue factor pathway inhibitor (lipoprotein-associated coagulation inhibitor)	
209683_at	81553	<b><i>FAM49A</i></b>	4.80E-02	1.51	family with sequence similarity 49, member A	
228796_at	131034	<b><i>CPNE4</i></b>	5.13E-02	1.51	copine IV	
205193_at	23764	<b><i>MAFF</i></b>	8.39E-02	1.51	v-maf avian musculoaponeurotic fibrosarcoma oncogene homolog F	
205462_s_at	3241	<b><i>HPCAL1</i></b>	1.30E-03	1.50	hippocalcin-like 1	
239352_at	55117	<b><i>SLC6A15</i></b>	4.30E-03	1.50	solute carrier family 6 (neutral amino acid transporter), member 15	
213280_at	23108	<b><i>RAP1GAP2</i></b>	2.18E-02	1.50	RAP1 GTPase activating protein 2	
220148_at	64577	<b><i>ALDH8A1</i></b>	4.05E-02	1.50	aldehyde dehydrogenase 8 family, member A1	
233506_at	9689	<b><i>BZW1</i></b>	9.02E-02	1.50	basic leucine zipper and W2 domains 1	

**Indacaterol (18h)**  
 (9 Probe Sets = 9 Gene IDs)

Affy Probe Set ID	Entrez Gene ID	Official Gene Symbol	FDR P-Value	Fold change	UniGene Name	Function
237328_at	55195	<b><i>C14orf105</i></b>	9.76E-02	3.27	chromosome 14 open reading frame 105	
1556420_s_at	388403	<b><i>YEL2</i></b>	4.21E-02	2.24	yippee like 2	
216639_at	27286	<b><i>SRPX2</i></b>	7.81E-02	2.02	sushi-repeat containing protein, X-linked 2	
220006_at	79825	<b><i>EFCC1</i></b>	4.21E-02	1.98	EF-hand and coiled-coil domain containing 1	
237222_at	123722	<b><i>FSD2</i></b>	9.76E-02	1.85	fibronectin type III and SPRY domain containing 2	
208335_s_at	2532	<b><i>ACKR1</i></b>	3.93E-02	1.59	atypical chemokine receptor 1 (Duffy blood group)	
220227_at	1002	<b><i>CDH4</i></b>	3.67E-02	1.55	cadherin 4, type 1, R-cadherin (retinal)	
1554202_x_at	57010	<b><i>CABP4</i></b>	9.76E-02	1.54	calcium binding protein 4	
220335_x_at	23491	<b><i>CES3</i></b>	3.53E-02	1.53	carboxylesterase 3	

Transcripts used for real-time PCR validation are bolded and underlined.

\*Some genes are recognised by more than one probe set and some probe sets recognise more than one gene.





**Indacaterol (18h)****(1 Probe Set ≡ 1 Gene ID)**

Affy Probe Set ID*	Entrez Gene ID	Official Gene Symbol	FDR P-Value	Fold change	UniGene Name	Function
240876_x_at	145645	<i>C15orf43</i>	9.51E-02	0.51	chromosome 15 open reading frame 43	

Transcripts used for real-time PCR validation are bolded and underlined.

\*Some genes are recognised by more than one probe set and some probe sets recognise more than one gene.



Indacaterol (2h)						
Annotation Cluster 1		Enrichment Score: 7.64	Genes	Count	P -Value	Benjamini
UP_SEQ_FEATURE		DNA-binding region:Basic motif	ATF3, BHLHE40, CEBPA, CEBPD, CREB3L2, EPAS1, FOS, FOSB, FOSL2, HES1, JUNB, MAFF, MAFK, MYC, NFI3, PHTF2, TCF21	17	4.90E-09	4.60E-06
Annotation Cluster 2		Enrichment Score: 6.81	Genes	Count	P -Value	Benjamini
GOTERM_BP_DIRECT		positive regulation of transcription from RNA polymerase II promoter	ATF3, ATXN7, BMP2, CEBPA, CEBPD, CITED4, CREB3L2, EDN1, EPAS1, ETS2, FGFR2, FOS, FOSB, FOSL2, FOXC1, FOXC2, FOXO1, FSTL3, GREM1, HES1, HOXA5, IL6, IL11, INHBA, JAG1, JUNB, KLF4, LPIN1, MAFF, MAFK, MEF2D, MYC, NFATC1, NOG, NR4A1, NR4A2, NR4A3, PPARGC1A, PROX2, REL, RGCC, SERPINE1, SIX2, SMAD2, SOX4, SOX9, TCF21, TFAP2A, TFAP2C, VGLL2, WNT5A, ZBTB38, ZNF462	53	3.20E-13	7.50E-10
GOTERM_MF_DIRECT		transcriptional activator activity, RNA polymerase II core promoter proximal region sequence-specific binding	CEBPA, CEBPD, CREB3L2, EPAS1, FOS, FOSB, FOXC2, HOXA5, JUNB, KLF4, MEF2D, MYC, NR4A1, NR4A2, NR4A3, SIX2, SMAD2, SOX4, SOX9, TCF21, TFAP2A, TFAP2C	22	8.60E-10	4.40E-07
GOTERM_BP_DIRECT		transcription from RNA polymerase II promoter	ATF3, CEBPA, CEBPD, CREB3L2, CTDP1, EPAS1, FOS, FOSB, FOXC1, FOXC2, HIVEP1, HIVEP2, HOXA5, JUNB, KLF4, MAFF, MAFK, MEF2D, MYC, NFATC1, NFI3, REL, RUNX3, SIX2, SMAD2, SOX4, SOX9, TCF21, TFAP2A, TFAP2C, TSC22D1	32	1.40E-09	1.10E-06
GOTERM_MF_DIRECT		transcription factor activity, sequence-specific DNA binding	ATF3, BCL6, BHLHE40, BNC1, CEBPA, CEBPD, CREB3L2, EPAS1, ETS2, FOS, FOSB, FOSL2, FOXC1, FOXC2, HES1, HIVEP2, HIVEP3, HOXA5, ID3, KLF3, KLF4, KLF9, MAFF, MAFK, MEF2D, MYC, NFATC1, NFI3, NR2F2, PRDM1, REL, RUNX3, SIX2, SMAD2, SOX4, SOX9, TBX3, TFAP2A, TFAP2C, TGIF1, TSC22D1, WNT5A, ZBTB38	43	2.50E-08	4.30E-06
GOTERM_CC_DIRECT		nucleus	ANKRD37, AREG, ARID5B, ATF3, ATXN7, BAG1, BCL6, BCOR, BHLHE40, BNC1, BTG1, C8orf4, C9orf72, C15orf48, CASP9, CDKN1C, CEBPA, CEBPD, CITED4, CPEB4, CREB3L2, CSNK1E, CTDP1, DGKD, DLC1, DUSP1, DUSP4, EPAS1, ERF1, ETS2, FERMT2, FGFR2, FILIP1L, FOS, FOSB, FOSL2, FOXC1, FOXC2, FOXO1, FSTL3, GDF15, GEM, HERC4, HES1, HIVEP1, HIVEP2, HIVEP3, HLX, HOXA5, ID3, IGFBP3, ING1, IRF2BP2, IRX1, IRX2, IRX4, ISG20, JMY, JUP, KLF3, KLF6, KLF9, LAT52, LBH, LMCD1, LPIN1, MAFF, MAFK, MBD, MEF2D, MYC, NEDD9, NFATC1, NFI3, NFKB1, NR2F2, NR4A1, NR4A2, NR4A3, PHTF2, PIP5K1A, PMAIP1, PPARGC1A, PPM1D, PRDM1, PRKCE, PROX2, PTP4A1, REL, RGCC, RGS2, RHOB, RUNX3, SGK1, SH3BP4, SIK1, SIK2, SIX2, SLC2A14, SMAD2, SNAI2, SNRK, SOX4, SOX9, SPRY2, STC1, TBX3, TCF21, TFAP2A, TFAP2C, TGIF1, TLE1, TLE3, TNFAIP3, TRIB1, TSC22D1, USP36, VGLL2, WWC1, ZBTB38, ZNF331, ZNF462, ZNF503	123	2.90E-06	7.10E-04
GOTERM_CC_DIRECT		nucleoplasm	A1CF, ARID5B, ATF3, ATXN7, BCL6, BNC1, C9orf72, CREB3L2, CSNK1E, CTDP1, DUSP4, DUSP5, ELL2, EPAS1, ETS2, FERMT2, FGFR2, FOS, FOSB, FOXC1, FOXC2, FOXO1, FSTL3, HES1, HLX, ID3, ING1, ISG20, JMY, JUNB, KLF3, KLF9, LBH, LMCD1, LPIN1, MAFF, MAFK, MCTP2, MEF2D, MYC, NFATC1, NR4A1, NR4A2, NR4A3, PFKFB3, PIP5K1A, PPARGC1A, PRDM1, REL, SAP30, SGK1, SIX2, SMAD2, SOX4, SOX9, SPIDR, TFAP2A, TFAP2C, TGIF1, TLE1, TLE3, USP2, USP48	62	4.30E-03	1.40E-01
GOTERM_BP_DIRECT		transcription, DNA-templated	ARID5B, ATXN7, BCL6, BCOR, BHLHE40, BNC1, CEBPA, CEBPD, CITED4, EPAS1, ERN1, ETS2, FOSL2, FOXO1, FSTL3, HES1, HLX, ID3, IRF2BP2, KLF3, KLF6, KLF9, LBH, LMCD1, LPIN1, MAFF, MEF2D, MN1, NFKB1, NR2F2, NR4A1, NR4A2, NR4A3, PHTF2, PRDM1, PROX2, SAP30, SMAD2, SNAI2, TBX3, TGIF1, TLE1, TLE3, VGLL2, WWC1, ZBTB38, ZNF331, ZNF462, ZNF503	49	7.50E-03	1.80E-01
Annotation Cluster 3		Enrichment Score: 3.73	Genes	Count	P -Value	Benjamini
GOTERM_BP_DIRECT		neural crest cell development	EDN1, FOXC1, FOXC2, SNAI2, SOX9	5	5.50E-05	7.40E-03
GOTERM_MF_DIRECT		transcription factor activity, RNA polymerase II distal enhancer sequence-specific binding	BHLHE40, CEBPA, FOXC1, FOXC2, NFATC1, SNAI2, SOX9, VGLL2	8	1.40E-04	7.00E-03
GOTERM_BP_DIRECT		Notch signaling pathway	BMP2, CEBPA, FOXC1, FOXC2, HES1, JAG1, MYC, SNAI2, SOX9	9	8.60E-04	3.80E-02
Annotation Cluster 4		Enrichment Score: 3.41	Genes	Count	P -Value	Benjamini
GOTERM_MF_DIRECT		growth factor activity	AREG, BDNF, BMP2, CTGF, DKK1, FGF9, DF15, HBEGF, IL6, IL11, INHBA, JAG1, NTF3, VEGFC	14	4.80E-06	4.90E-04
GOTERM_BP_DIRECT		cell-cell signaling	AREG, BDNF, BMP2, CCL20, CTGF, EDN1, EFNA1, FGF9, FGFR2, GDF15, GREM1, IL11, IL15, INHBA, NTF3, TFAP2C	16	3.70E-05	5.70E-03
GOTERM_MF_DIRECT		cytokine activity	AREG, BMP2, EDN1, GDF15, GREM1, IL6, IL11, IL15, INHBA, WNT5A	10	3.50E-03	1.10E-01
Annotation Cluster 5		Enrichment Score: 3.34	Genes	Count	P -Value	Benjamini
GOTERM_BP_DIRECT		cell fate commitment	BMP2, FGFR2, GAS1, PRDM1, SMAD2, SPRY2, WNT4, WNT5A	8	1.30E-05	2.90E-03
GOTERM_BP_DIRECT		epithelial to mesenchymal transition	BMP2, FGFR2, NOG, SNAI2, SOX9, WNT4, WNT5A	7	2.30E-05	4.00E-03
Annotation Cluster 6		Enrichment Score: 2.16	Genes	Count	P -Value	Benjamini
GOTERM_CC_DIRECT		integral component of plasma membrane	BDKRB2, CSAR1, CD200, CD55, CLDN1, DLCK1, EFNA1, FGFR2, GPRCSA, GPR37, HBEGF, HLA-DQA1, HSG51, IGF1R, IL5, IL1RAP, JAG1, KCNJ2, KCNK1, KCNK15, NPC1, PAG1, PLAUR, PLPP3, PLXNA2, RAPGEF2, SCARA5, SEMA6D, SLC2A3, SLC7A1, SLC7A2, SLC7A5, SLC7A11, SLC16A6, SLC19A2, SLC22A4, SLC22A5, SLC23A2, SLC38A2, SLC04A1, STEAP4, TFRC, THBD, TRABD2A, TM4SF1	45	1.20E-05	1.40E-03
GOTERM_CC_DIRECT		extracellular space	ADAMTS15, AREG, B4GALT1, BMP2, C9orf72, CCL2, CCL20, CES3, CTGF, CXCL2, DKK1, DMBT1, EDN1, EFNA1, FGF9, FGFR2, FSTL3, GDF15, HBEGF, IGFBP3, IGFBP4, IL6, IL6R, IL11, IL15, KIT, LMCD1, LPL, LYPD3, METRN1, NOG, PAPPA, SERPINE1, SMPDL3A, STC1, STC2, TFRC, THBD, ULBP2, VEGFC, WNT4, WNT5A	41	8.30E-05	6.70E-03
GOTERM_CC_DIRECT		extracellular region	BDNF, BMP2, CCL2, CCL20, CD55, CHSY1, CRISPLD2, CTGF, CXCL2, DKK1, DMBT1, EDN1, EFNA1, FGF9, FGFR2, FSTL3, GDF15, HBEGF, IGFBP3, IGFBP4, IL6, IL6R, IL11, IL15, KIT, LMCD1, LPL, MFAP5, NOG, NPC1, NTF3, PAPPA, SERPINE1, STC2, TFRC, VEGFC, WNT4, WNT5A	39	8.20E-03	2.00E-01



GOTERM_CC_DIRECT	integral component of plasma membrane	ADGRG2, AVPR2, BDKRB2, CSAR1, CD200, CD55, CDH4, CNR1, DCLK1, EPHAS5, FGFR2, GPC3, GPRC5A, IGF1R, KCNK15, LPAR6, NPC1, NRXN3, PLPP3, PTPRO, SLC6A15, SLC7A2, SLC7A11, SLC16A6, SLC22A3, SLC22A4, SLC22A5, SLC26A2, STEAP4, TM4SF1, ACKR1, ADAM12, ADAM22, ADGRD1, AKAP12, ANTXR1, ATP1B3, AVPR2, B4GALT1, BDKRB2, CSAR1, CACNA2D3, CD55, CD200, CDH4, CFTR, CLMP, CNR1, COBL, DNER, DOCK4, ENO1, EPHAS5, FGFR2, GAS1, GNAL, GPC3, GPRC5A, GUCY1A2, IGF1R, ITPR1, JUP, KCNG1, KCNK15, LPAR6, LPL, MCAM, MDM2, NFASC, NOS3, PCDH9, PHLDB2, PLPP3, PTPRO, RAP1GAP2, RGS2, SIRPA, SLC6A15, SLC7A2, SLC7A11, SLC16A14, SLC22A3, SLC22A4, SLC22A5, SLC26A2, SLC04A1, STEAP4, SYTL2, TFP1, WNT4, WNT5A	32	3.70E-05	7.30E-03
GOTERM_CC_DIRECT	plasma membrane	AVPR2, B4GALT1, BDKRB2, CSAR1, CACNA2D3, CD55, CD200, CDH4, CFTR, CLMP, CNR1, COBL, DNER, DOCK4, ENO1, EPHAS5, FGFR2, GAS1, GNAL, GPC3, GPRC5A, GUCY1A2, IGF1R, ITPR1, JUP, KCNG1, KCNK15, LPAR6, LPL, MCAM, MDM2, NFASC, NOS3, PCDH9, PHLDB2, PLPP3, PTPRO, RAP1GAP2, RGS2, SIRPA, SLC6A15, SLC7A2, SLC7A11, SLC16A14, SLC22A3, SLC22A4, SLC22A5, SLC26A2, SLC04A1, STEAP4, SYTL2, TFP1, WNT4, WNT5A	62	7.60E-04	7.20E-02
GOTERM_CC_DIRECT	integral component of membrane	ACKR1, ADAM12, ADAM22, ADGRD1, ADGRG2, ANTXR1, AVPR2, B4GALT1, BDKRB2, C15orf48, C10TNF, CD200, CDH4, CFTR, CLMP, CMTM6, CNR1, DNER, EPHAS5, FGFR2, GALNT15, GAS1, GPAT3, GPRC5A, GRAMD1B, HRK, IGF1R, ITPR1, LPAR6, MARC1, MCAM, NETO1, NFASC, NPC1, NRXN3, PAPPA, PCDH9, PDE3A, PHLDB2, PLD5, PLXDC2, PTPRO, RNF150, SHISA2, SIRPA, SLC6A15, SLC7A11, SLC16A6, SLC16A14, SLC22A3, SLC22A4, SLC22A5, SLC26A2, SLC04A1, SPTLC3, SPTSSA, STEAP4, TM4SF1, TMEM2, TMT1C, UGT1A8, UGT2B28, UST, WNT5A	64	5.10E-02	5.20E-01
<b>Annotation Cluster 3</b>		<b>Enrichment Score: 2.10</b>	<b>Genes</b>	<b>Count</b>	<b>P-Value</b>
GOTERM_BP_DIRECT	cell fate commitment	FGFR2, GAS1, HEY1, PRDM1, WNT4, WNT5A	6	1.10E-04	7.70E-02
GOTERM_BP_DIRECT	epithelial to mesenchymal transition	FGFR2, WNT4, WNT5A	3	4.90E-02	9.10E-01
<b>Annotation Cluster 4</b>		<b>Enrichment Score: 2.10</b>	<b>Genes</b>	<b>Count</b>	<b>P-Value</b>
GOTERM_BP_DIRECT	cAMP catabolic process	PDE3A, PDE4D, PDE7B, PDE10A	4	4.60E-04	2.00E-01
GOTERM_MF_DIRECT	3',5'-cyclic-AMP phosphodiesterase activity	PDE3A, PDE4D, PDE7B, PDE10A	4	4.90E-04	1.80E-01
GOTERM_MF_DIRECT	3',5'-cyclic-nucleotide phosphodiesterase activity	PDE3A, PDE4D, PDE10A	3	2.50E-02	7.60E-01
GOTERM_MF_DIRECT	cAMP binding	PDE3A, PDE4D, PDE10A	3	2.70E-02	7.40E-01
<b>Annotation Cluster 5</b>		<b>Enrichment Score: 2.08</b>	<b>Genes</b>	<b>Count</b>	<b>P-Value</b>
GOTERM_BP_DIRECT	quaternary ammonium group transport	SLC22A3, SLC22A4, SLC22A5	3	1.10E-03	3.10E-01
GOTERM_MF_DIRECT	quaternary ammonium group transmembrane transporter activity	SLC22A3, SLC22A4, SLC22A5	3	1.10E-03	2.00E-01
<b>Annotation Cluster 6</b>		<b>Enrichment Score: 2.02</b>	<b>Genes</b>	<b>Count</b>	<b>P-Value</b>
GOTERM_CC_DIRECT	proteinaceous extracellular matrix	ADAMTS1, COL4A3, CRISPLD2, GPC3, SFTP2, SMOC1, TFP1, VCAN, WNT4, WNT5A	10	1.70E-03	6.50E-02
GOTERM_CC_DIRECT	endoplasmic reticulum lumen	ADAMTS1, CES3, COL4A3, WNT4, WNT5A	5	1.30E-01	7.70E-01
<b>Annotation Cluster 7</b>		<b>Enrichment Score: 1.73</b>	<b>Genes</b>	<b>Count</b>	<b>P-Value</b>
GOTERM_MF_DIRECT	symporter activity	SLC16A6, SLC16A14, SLC22A4, SLC22A5	4	1.70E-02	6.80E-01
<b>Annotation Cluster 8</b>		<b>Enrichment Score: 1.56</b>	<b>Genes</b>	<b>Count</b>	<b>P-Value</b>
KEGG_PATHWAY	Pathways in cancer	BDKRB2, CASP9, CEBPA, COL4A3, FGFR2, FOXO1, IGF1R, JUP, LPAR6, MDM2, WNT4, WNT5A	12	7.60E-03	4.80E-01

Indacaterol-induced genes (defined by using Affymetrix probe sets) with significant expression level changes of >1.5-fold (based on a FDR P-value of <0.1) were subjected to functional annotation clustering using DAVID (default parameters). Clusters with enrichment scores >1.3 are shown.

**Supplemental TABLE 6.** Functional Annotation Clustering of Genes Significantly Repressed by Indacaterol in BEAS-2B Cells at 1h, 2h and 6h.

**Indacaterol (1h)**

Annotation Cluster 1	Enrichment Score: 1.35	Genes	Count	P-Value	Benjamini
GOTERM_BP_DIRECT	DNA repair	CHEK1, EID3, RECQL5	3	5.40E-02	8.90E-01

**Indacaterol (2h)**

Annotation Cluster 1	Enrichment Score: 2.72	Genes	Count	P-Value	Benjamini
GOTERM_BP_DIRECT	cell division	BUB1, BORA, CCNF, HMGA2, PARD6B, PSRC1	6	1.20E-03	3.20E-01
GOTERM_BP_DIRECT	mitotic nuclear division	BUB1, BORA, CCNF, HMGA2	4	2.00E-02	7.80E-01
GOTERM_CC_DIRECT	cytosol	BORA, BUB1, CEP135, KIF23, PARD6B, PKHD1L1, PSRC1, SEC16B, TXNIP	9	5.30E-01	1.00E+00
Annotation Cluster 2	Enrichment Score: 1.91	Genes	Count	P-Value	Benjamini
GOTERM_MF_DIRECT	DNA binding	EGR1, EGR3, HJURP, HMGA2, KLF7, SIM1, TOP2A, ZNF99, ZNF547, ZNF556, ZNF594, ZNF738	12	1.40E-03	1.30E-01
GOTERM_BP_DIRECT	transcription, DNA-templated	EGR3, KLF7, MED13L, SIM1, TXNIP, ZNF99, ZNF547, ZNF552, ZNF556, ZNF594, ZNF738	11	1.20E-02	7.10E-01
GOTERM_CC_DIRECT	nucleus	CCNF, EGR1, EGR3, HJURP, HMGA2, HYLS1, IP6K2, KDM6A, KIF23, KLF7, NUMB, PARD6B, SIM1, TOP2A, TXNIP, ZNF99, ZNF547, ZNF552, ZNF556, ZNF594, ZNF738	21	1.50E-02	7.20E-01
GOTERM_MF_DIRECT	nucleic acid binding	KLF7, ZNF99, ZNF552, ZNF547, ZNF556, ZNF594, ZNF738	7	2.70E-02	7.70E-01
GOTERM_BP_DIRECT	regulation of transcription, DNA-templated	EGR3, HMGA2, ZNF99, ZNF552, ZNF547, ZNF556, ZNF594, ZNF738	8	5.60E-02	8.90E-01
GOTERM_CC_DIRECT	intracellular	KIF23, TRIM59, ZNF99, ZNF547, ZNF552, ZNF556, ZNF738	7	9.10E-02	8.10E-01
GOTERM_MF_DIRECT	metal ion binding	EGR1, EGR3, KDM6A, KLF7, ZNF99, ZNF547, ZNF552, ZNF556, ZNF594	9	1.10E-01	9.80E-01
GOTERM_MF_DIRECT	transcription factor activity, sequence-specific DNA binding	EGR1, EGR3, KLF7, SIM1, ZNF552	5	1.90E-01	1.00E+00
GOTERM_MF_DIRECT	zinc ion binding	EGR1, KLF7, SH3RF2, TRIM59, ZSWIM8	5	3.00E-01	1.00E+00

**Indacaterol (6h)**

Annotation Cluster 1	Enrichment Score: 2.31	Genes	Count	P-Value	Benjamini
GOTERM_BP_DIRECT	type I interferon signaling pathway	EGR1, MX1, OAS1, OAS2, OAS3	5	1.30E-05	3.70E-03
GOTERM_MF_DIRECT	2'-5'-oligoadenylate synthetase activity	OAS1, OAS2, OAS3	3	2.80E-05	2.70E-03
GOTERM_BP_DIRECT	response to virus	IFI44, MX1, OAS1, OAS2, OAS3	5	1.10E-04	1.60E-02
GOTERM_BP_DIRECT	defense response to virus	IFI44L, MX1, OAS1, OAS2, OAS3	5	5.10E-04	4.90E-02
GOTERM_MF_DIRECT	nucleotidyltransferase activity	OAS1, OAS2, OAS3	3	1.60E-03	7.50E-02
GOTERM_BP_DIRECT	negative regulation of viral genome replication	MX1, OAS1, OAS3	3	3.70E-03	2.40E-01
GOTERM_MF_DIRECT	double-stranded RNA binding	OAS1, OAS2, OAS3	3	7.90E-03	2.30E-01
GOTERM_BP_DIRECT	interferon-gamma-mediated signaling pathway	OAS1, OAS2, OAS3	3	1.10E-02	4.20E-01
GOTERM_MF_DIRECT	transferase activity	OAS1, OAS2, OAS3	3	1.90E-02	3.70E-01
GOTERM_BP_DIRECT	immune response	OAS1, OAS2, OAS3	3	2.50E-01	1.00E+00
GOTERM_MF_DIRECT	ATP binding	MVK, NAV3, OAS1, OAS2, OAS3	5	4.20E-01	1.00E+00

Indacaterol-induced genes (defined by using Affymetrix probe sets) with significant expression level changes of <0.67-fold (based on a FDR P-value of <0.1) were subjected to functional annotation clustering using DAVID (default parameters). Clusters with enrichment scores >1.3 are shown.