

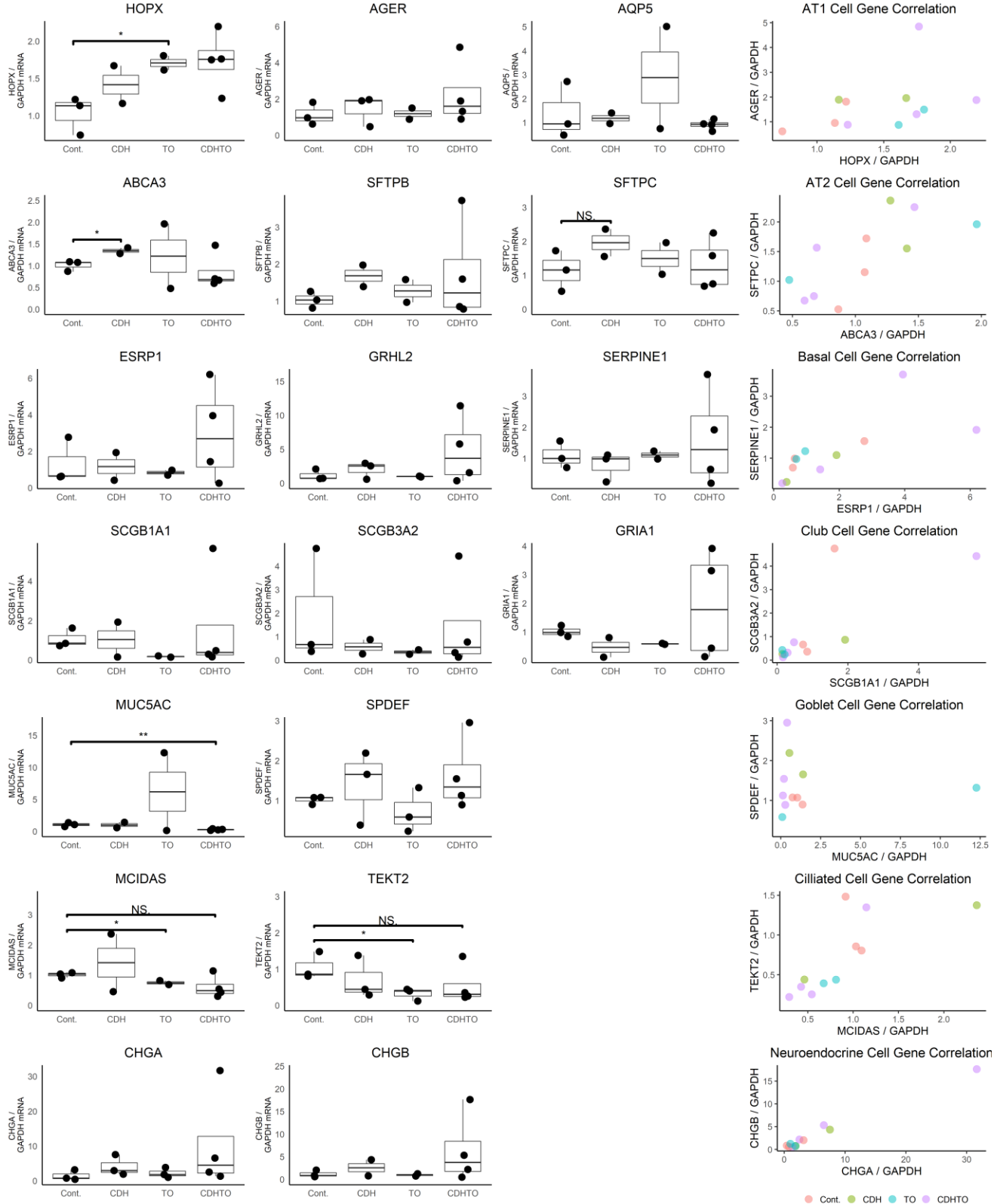
Fetal Tracheal Occlusion Increases Lung Basal Cells via Increased Yap Signaling

SUPPLEMENTAL MATERIAL

PCR Validation of Fetal Rabbit Lung mRNA-Seq Cell-Specific mRNAs

We quantified the mRNA levels of several cell-specific mRNAs in Control, CDH, TO, and CDHTO fetal rabbit lung. In general, the data showed good correlation of the cell-specific mRNAs with one another, a reduction in ciliated cell mRNAs, and increased basal cell and alveolar type 1 cell mRNAs (Supplemental Figure 1).

Lung Epithelial Cell-Specific Genes



Supplemental Figure 1: PCR quantification of cell-specific mRNAs in Fetal Rabbit lung. PCR for the AT1 cell markers Homeodomain-only protein homeobox (HOPX), Advanced Glycosylation End-products Receptor (AGER), and Aquaporin 5 (AQP5), the AT2 cell markers ATP Binding Cassette Subfamily A Member 3 (ABCA3), Surfactant Protein B (SFTPB), and SFTPC, the basal cell markers Epithelial Splicing Regulatory Protein 1 (ESRP1), Grainyhead Like Transcription Factor 2 (GHRL2), and Serpin Family E Member 1 (SERPINE1), the club cell markers Secretoglobin Family 1A Member 1 (SCGB1A1), SCGB3A2, and Glutamate Ionotropic Receptor AMPA Type Subunit 1 (GRIA1), the ciliated cell markers Multiciliate Differentiation And DNA Synthesis Associated Cell Cycle Protein (MCIDAS), and Tektin 2 (TEKT2), and the neuroendocrine cell markers Chromogranin A (CHGA) and CHGB were generally consistent with mRNA-seq data showing increased AT1 and basal cell mRNAs and decreased ciliated cell mRNAs.

Cell-specific Genes

Genes in Supplemental Table 1 were used for deconvolution of bulk mRNA-seq data.

Supplemental Table 1: Cell-Specific Genes

AT2	AT1	Basal	Ciliated	Club	Neuroendocrine	Endothelial	Myeloid	Matrix Fibroblast	Myofibroblast	Lipofibroblast
Egfl6	Tspan8	Igf1	3300002A11Rik	Gm15883	Crp	Elt1	Mx4A6D	Col3A1	Kcnj8	Adh1
Pla2G1b	Ctgf	Itga6	Lrrc71	Lrrc26	Ngfrap1	Aplnr	Clec4A3	Mxra8	Enpp2	Col12A1
Lgi3	Tinag1	Sna12	1700016K19Rik	Cckar	Ngfr	Plvap	Aie607873	Col1A1	Gpr64	Slc36A2
Lamp3	Mal2	Nrg1	Bc051019	Gpx2	Chga	Emcn	Fpr2	Cped1	Pdgfra	Slc46A2
Scd1	Vfndc1	Il13Ra2	Tekt2	Lypd2	Chgb	Ripply3	Sirpb1B	Fibin	Dnm3Os	Fhl1
Atg6w2c2	Lamc2	Ezh2	1700003M02Rik	Krt15	Pyv	Sox17	Ccr1	Vldlr	Agt	Shisa2
Hc	Ndst1	Tp63	1700012B09Rik	Cyp2F2	Sypl1	Clec1A	Clec4N	Gpc3	Hlf	Aars2
Slc26a1C9a9	Phactr1	Grlh2	D430036J16Rik	Gsta3	Edn1	Ushbp1	Sirpb1C	Col6A2	Tbx5	Ppp1R3F
Rbpjl	Gja1	Stat3	Stk33	Ehf	Calcr1	Clec14A	C5Ar1	Serpine2	H19	Col25A1
Ch13chi111	Cryab	Ngfr	4833427G06Rik	Homer2	Robo1	Kit	Sp1	Cp	Pdzr3	Fancx
Spink5	Ano1	Tubb6	1700026D08Rik	Kcnk2	Robo2	Tek	Fcgr3	Fblim1	Rhou	Thg1L
Bex2	Lmo7	Krt14	Nek5	Gsta4	Robo3	Myzap	Clec4A2	Palld	Fibin	Hebp1
Elf5	Scnn1A	Sce1	Nme5	Ace2	Robo4	Icam2	Ncf2	Snai2	Hhip	Lipe
Ppp1R14c	Sphk1	Sna12	Dnaa1	Aox3	Slt1	Rasip1	Mx4A6C	Col13A1	Prdm6	Thap2
Tfcp2l1cp111	Krt8	Serpine2	Ttli6	Aldh1A7	Slt2	Arhgef15	Mpeg1	Rbp1	Tgfb1	Mvk
Cxd15	Il18R1	Thbs1	Ccdc147	B430010I23Rik	Slt3	Tie1	Cd300lf	Cdo1	Igf1	Enpep
Kcnk3	Fam189a2	Dlk2	1700001L19Rik	S330417C22Rik	Jam2	Fcgr2B	Dpep1	Dkk3	Rras2	
Dram1	Flrt3	Bcam	Elmod1	Aldh1A1	Cdh5	Wfndc17	Ppp1R14A	Crispld2	Chst15	
Muc1	Mthfd1	Abi3Bp	Tekt4	Cxd17	Myct1	Cd84	Pbxdc2	Tbx4	Hoxb3	
Etv5	Tspan15	Lamb3	Efcab1	Acs1	Stmn2	Ifi204	Gyg	Ism1	Kctd5	
S100g	Slco3a1	Trp63	Ccdc37	Ptgr1	Prx	Igslf6	Angpt1	Tgfb3	Sprtn	
Tspan11	Fads3	Cald1	Ccdc96	Dapk2	Arap3	Pirb	Echdc2	Gm13889	Thy1	
Cebpa	Hopx	Fscn1	Kcnmb2	Tacstd2	Cd93	Tyrobp	Maf	Mdk	Plin2	
Retnla	Vegfa	Adamts1	RspH10B	Cldn10	Adcy4	Il18	Adarb1	Loxl2		
Bex1	Ppm1l	Plau	Ak7	Acs1	Flt1	Pld4	Lox1	Enpp2		
Myh7	Ager	Serpine1	Meig1	Rassf9	Ecsr	Ab124611	Nexn	Tnc		
Kcnj15	F3	Stc2	Ccdc135	Synpo	Gata2	Mx4A6B	Fxyd1	Agt		
Fabp5	Krt18	Cav1	4930451C15Rik	Au021092	Ppp1R16B	Lapmt5	Fmo2	Loxl2		
Irx3	Vwv1	Upp1	Cdhr3	Fam46C	Rasgpr3	Fcer1G	Gpx3	Rhou		
Irx1	Pdgfra	Ulp3	Tcte1	Scgb3A2	Ctla2B	Ctss	Angptl4	Crispld2		
Abca3	Cystm1	Aldh1L2	Map3K19	Acs2	Ptprb	Arhgap30	Plac9A	Tgfb1		
Matn4	Icam1	Psat1	Zbbx	Gss	Fgd5	Ccr2	Plac9B	Pdzr3		
Fasn	Rtkn2	Phlda1	Fam47E	Vfndc1	Ace	Alox5Ap	Adamts2	H19		
Rmdn2	2210011c24Rik	Il1Rn	Akap14	Stx19	Cldn5	Appb1ip	Ednra	Col27A1		
Wfndc2	Msln	Slc16A1	Lrrc48	Trf	Gplhbp1	Csf2Rb	Nebi	Col5A1		
Bex4	Gipc2	Slc7A5	Zfp474	Retnla	S1Pr1	Cybb	Ptprd	Net1		
Ank3	S100a14		1700028P14Rik	Scnn1B	Pecam1	Ulr1b4	Pcolce2	Hhip		
Slc34s1C2a2	Scnn1g		Ccdc121	Gsto1	Calcr1	Hds1	Meox2	Gm13889		
Dlk1	Spock2		Efcab10	Cgn	Tspan18	Plek	Hsd11B1	Ism1		
Myo5myO5Cc	Pdpn		2410004P03Rik	Rab25	Pcdh17	Lst1	6030408B16Rik	Tagln		
Lpcat1	Slc6a14		Sntn	Cd55	Ly6A	Pla2G7	Clec3B	Tpm2		
Irx2	Aqp5		Fank1	Fam13A	Ctla2A	Ptprc	Adh1	Dkk3		
PI4K2b	Col4a3		Agr3	Gstt3	Egfl7	Sifn2	Tcf21	Mdk		
Tc2t2c2Nn	Akap5		1600029I14Rik		Erg	Ly86	Spon1	Prdm6		
Dcxr	Sema3a		Fam1838		Palmd	Ccl6	Itga8	Tgfb3		
Ces1ceS1Dd	Sema3E		Gm867		Tspan7	Gp49A	Cacna1D	Fibin		
Tmem243	Sec14l3		Pifo		Acer2	Snx10	Lbh	Samd4		
Lcn2	Col4a4		Lrrc6		Cyrr1	Tnfaip2	Enpep	Tnfrsf12A		
Fgfr2	Tmem37		Ttcl8		Acvrl1	Mnda	Olfrml3	Pamr1		
Sftpb	Scnn1B		1700001C02Rik		Kdr	Ai662270	Cdh11	Thbs1		
Prr15prR15U	2310007B03Rik		1110017D15Rik		Eng	Ncf1	Ogn	Sdc2		
Sfta2	Cdkn2B		lqca		Podxl	Gda	Atp1A2	Aspn		
Sftpd	Krt19				Sema6A	Samsn1	GOS2	Tbx5		
Rbm47	Krt7				Nsg1	Cfp	Dpt	Pdlim3		
Sdc1	Pard6B				Nostrin	Sirpa	Mfap4	Spon2		
Cbr2	Gramd2				Esam	2010005H15Rik	Serping1	Fndc1		
Cldn3	Slc39a8				Ahr	Bcl2A1D	Dcn	Gpr64		
Nkx2-1	2200002d01Rik				Hyal2	Bcl2A1A	Mfap5	Olfrml2B		
Napsa	Rab11fip1				Ldb2	Bcl2A1B	C1S	Igf1		
Balapp21	Tacstd2				Edn1	Csf2Ra	Thbs2	Des		
Srx25	Eps8L2				2810025M15Rik	1810033B17Rik	Ptn	Kcnj8		
Exosc7	Fam174B				Rgs12	Cd300A	Prrx1	Wnt5A		
Acs14	Ugl2				Tmem2		Fbln1	Dnm3Os		
Lrk2	Mmp11				Efnb2		C1Rb	Rp23-103i12.13		
Itga9	Gprc5a				Ramp2		Cygb	Ltbp2		
Slc15s1C2a2	Myh14				Scn7A		Col14A1			
Sftpa1	Clic3				Impdh1		Pcolce			
Rab27rab27Bb	Pkp2				Mcam		Selm			
Acot1							C1Ra			
Gdc							Igf1bp5			
							Col6A3			
							Rcn3			
							Nkain4			
							Col5A1			
							Kdelr3			
							Aebp1			
							Fbn2			
							Postn			
							Aspn			
							Gas1			
							Adamts2			
							Loxl1			
							Ccdc80			
							Bicc1			
							Leprel2			
							Plagl1			
							Sfrp1			
							Lpar1			
							Gpc3			
							Igf1			
							Fbn1			
							Ltbp4			
							Lrrc17			
							Col5A2			
							Rarres2			
							Igf1bp6			
							Pamr1			
							Col1A1			
							Loxl2			
							Rian			
							Ogn			
							Nbl1			
							Serping1			
							C1Qtnf7			
							Col3A1			
							Col6A2			
							Smim1			
							Dnm3Os			
							Bgn			
							Clec3B			
							Pbxdc2			
							Cdh11			
							Ednra			

Mouse TO mRNA-Seq Gene Set Enrichment Analysis

Significantly different, 2-fold upregulated, and 2-fold downregulated DEGs were analyzed in ToppGene to identify key pathways and processes as described in Supplemental Figure 2.

Supplemental Figure 2: Gene Set Enrichment Analysis of DEGs in Mouse TO

GO Biological Process	p-value	Pathway	DEGs Down		GO Biological Process	p-value	Pathway	p-value
			GO Cellular Component	q-value				
neutrophil degranulation	0.0006978	Metal sequestration by antimicrobial proteins	neutrophil cell body membrane	0.00553	myofibril assembly	5.943E-28	Muscle contraction	5.483E-12
neutrophil activation involved in immune response	0.0007316	Neutrophil degranulation	cell body membrane	0.07114	striated muscle cell differentiation	6.894E-26	Striated Muscle Contraction	4.611E-11
neutrophil activation	0.0009076	IL-17 signaling pathway	fibrinogen complex	0.08099	striated muscle development	5.423E-25	Dilataseum Pathway	1.222E-06
neutrophil mediated immunity	0.0009223	Interleukin-4 and 13 signaling			muscle structure development	2.806E-24	Verapamil Pathway	1.222E-06
granulocyte activation	0.0009507	Innate Immune System		0.02789	sarcomere organization	2.997E-24	Dilated cardiomyopathy	3.202E-06
leukocyte degranulation	0.0009564				muscle cell development	8.16E-24	Dilated cardiomyopathy	1.202E-06
myeloid cell activation involved in immune response	0.0009327				cellular component assembly involved in morphogenesis	2.548E-22	Hypertrophic cardiomyopathy (HCM)	0.0000111
myeloid leukocyte mediated immunity	0.0002237				striated muscle tissue development	7.775E-22	Hypertrophic cardiomyopathy (HCM)	0.0000111
myeloid leukocyte activation	0.0009075				muscle cell differentiation	1.167E-21	Cardiac muscle contraction	0.0004888
leukocyte activation involved in immune response	0.001329				muscle tissue development	6.718E-21	Cardiac muscle contraction	0.0004888
cell activation involved in immune response	0.001593				muscle organ development	6.369E-19	Metal sequestration by antimicrobial proteins	0.002956
regulated exocytosis	0.00416				muscle system process	2.891E-17	Cardiac conduction	0.006476
leukocyte mediated immunity	0.008255				cardiac muscle tissue development	4.298E-17	Oxprenolol Pathway	0.02019
exocytosis	0.01084				heart contraction	1.08E-16	Nadolol Pathway	0.02019
exocytic process	0.01084				striated muscle contraction	1.202E-16	Pindolol Pathway	0.02019
vesicle fusion to plasma membrane	0.01084				heart process	2.695E-16	Penbutolol Pathway	0.02019
cellular transition metal ion homeostasis	0.01294				muscle contraction	7.248E-16	Propranolol Pathway	0.02019
immune effector process	0.01477				cardiac muscle tissue morphogenesis	4.390E-15	Nebivolol Pathway	0.02019
vesicle fusion	0.01576				actomyosin structure organization	4.706E-15	Bioprolol Pathway	0.02019
organelle membrane fusion	0.01646				blood circulation	4.935E-15	Metoprolol Pathway	0.02019
organelle fusion	0.01999				circulatory system process	9.03E-15	Eumolol Pathway	0.02019
leukocyte activation	0.02023				actin-mediated cell contraction	1.215E-14	Azobutolol Pathway	0.02019
vesicle organization	0.02117				muscle filament sliding	2.848E-14	Alprenolol Pathway	0.02019
secretion	0.027				muscle organ morphogenesis	1.122E-14	Betaxolol Pathway	0.02019
transition metal ion homeostasis	0.02762				actin-myosin filament sliding	4.595E-14	Potassium Channels	0.01818
defense response	0.029				heart development	2.082E-13	Adrenergic signaling in cardiomyocytes	0.03901
membrane fusion	0.03223				cardiac muscle contraction	2.948E-13	Labetalol Pathway	0.06223
cell activation	0.03176				circulatory system development	3.378E-13	Carvedilol Pathway	0.06223
cellular ion ion homeostasis	0.03409				regulation of heart contraction	6.474E-13	Interferon alpha/beta signaling	0.06458
response to inorganic substance	0.07909				actin filament-based movement	8.988E-13		
					cardiac muscle cell development	9.388E-13		
					actin filament-based process	3.151E-12		
					cardiac cell development	4.183E-12		
					anatomical structure formation involved in morphogenesis	7.072E-11		
					cardiac myofibril assembly	9.154E-11		
					supramolecular fiber organization	9.901E-11		
					cardiac muscle cell differentiation	2.834E-10		
					regulation of blood circulation	3.158E-10		
					cardiocyte differentiation	8.63E-10		
					heart morphogenesis	1.02E-09		
					regulation of system process	1.521E-09		
					skeletal muscle thin filament assembly	2.142E-09		
					cellular component morphogenesis	2.16E-09		
					muscle fiber development	4.246E-09		
					skeletal myofibril assembly	2.476E-08		
					ventricular cardiac muscle tissue morphogenesis	3.995E-08		
					regulation of striated muscle contraction	5.251E-08		
					cardiac ventricle morphogenesis	7.054E-08		
					ventricular cardiac muscle tissue development	3.556E-07		
					regulation of muscle system process	4.677E-07		
					metal ion transport	5.098E-07		
					skeletal muscle tissue development	6.452E-07		
					actin cytoskeleton organization	7.931E-07		
					animal organ morphogenesis	8.331E-07		
					potassium ion transmembrane transport	9.902E-07		
					secretion	0.0000136		
					export from cell	1.458E-06		
					striated muscle adaptation	1.512E-06		
					skeletal muscle organ development	1.772E-06		
					potassium ion transport	1.794E-06		
					skeletal muscle adaptation	1.848E-06		
					cardiac muscle fiber development	2.391E-06		
					secretion by cell	2.874E-06		
					muscle adaptation	4.798E-06		
					cardiac chamber morphogenesis	5.919E-06		
					cardiac ventricle development	7.365E-06		
					cardiac conduction	0.0000303		
					cardiac chamber development	0.00001606		
					regulation of muscle contraction	0.00001751		
					myotube differentiation	0.00002915		
					response to other organism	0.00004261		
					response to external biotic stimulus	0.00004467		
					peptide secretion	0.00004758		
					positive regulation of developmental process	0.00006194		
					establishment of protein localization to extracellular region	0.00008379		
					response to biotic stimulus	0.00008385		
					protein localization to extracellular region	0.00010388		
					regulation of heart rate	0.0001128		
					regulation of ion transmembrane transport	0.0001317		
					tissue morphogenesis	0.0001481		
					striated muscle myosin thick filament assembly	0.0001641		
					skeletal muscle myosin thick filament assembly	0.0001641		
					response to bacterium	0.0001766		
					regulation of transmembrane transport	0.0001977		
					positive regulation of transport	0.0002038		
					protein secretion	0.0002059		
					regulation of ion transport	0.0002721		
					myosin filament assembly	0.0002999		
					defense response	0.0003085		
					response to muscle stretch	0.0003286		
					myeloid leukocyte migration	0.0004314		
					regulation of actin filament-based movement	0.000496		
					regulation of the force of heart contraction	0.0005954		
					detection of muscle stretch	0.0005972		
					myosin filament organization	0.0006478		
					inorganic cation transmembrane transport	0.0006949		
					skeletal muscle contraction	0.001031		
					multicellular organismal signaling	0.001038		
					cation transport	0.001799		
					inorganic ion transmembrane transport	0.001839		
					actin filament organization	0.002312		
					biological adhesion	0.002464		
					regulation of peptide secretion	0.002617		
					negative regulation of cation transmembrane transport	0.00312		
					regulation of secretion	0.003126		
					leukocyte chemotaxis	0.003469		
					regulation of cardiac muscle contraction	0.003787		
					regulation of myotube differentiation	0.004051		
					adult heart development	0.004117		
					regulation of cardiac conduction	0.00462		
					ion transport	0.005222		
					regulation of protein secretion	0.006171		
					cation transmembrane transport	0.006283		
					positive regulation of myotube differentiation	0.006366		
					regulation of cation channel activity	0.006403		
					regulation of membrane potential	0.007054		
					regulation of immune system process	0.008005		
					regulation of secretion by cell	0.009363		
					cell adhesion	0.01065		
					regulation of transporter activity	0.01199		
					regulation of potassium ion transmembrane transport	0.01226		
					cell activation	0.01276		
					negative regulation of ion transmembrane transport	0.01295		
					inflammatory response	0.01346		
					regulation of potassium ion transport	0.01412		
					regulation of striated muscle cell differentiation	0.01434		
					musculoskeletal movement	0.0149		
					multicellular organismal movement	0.0149		
					apoptotic process involved in heart morphogenesis	0.01963		
					positive regulation of cation channel activity	0.02129		
					regulation of developmental growth	0.02325		
					regulation of cation transmembrane transport	0.0237		
					regulation of response to external stimulus	0.02596		
					positive regulation of striated muscle cell differentiation	0.0291		
					negative regulation of transmembrane transport	0.03019		
					regulation of growth	0.03151		
					striated muscle hypertrophy	0.03219		
					neurofilament bundle assembly	0.0326		
					sarcomerogenesis	0.0326		
					regulation of metal ion transport	0.03354		
					chemical homeostasis	0.03638		
					positive regulation of ion transport	0.03711		
					muscle hypertrophy	0.03761		
					neutrophil migration	0.0406		
					transmembrane transport	0.04338		
					neutrophil chemotaxis	0.04424		
					regulation of potassium ion transmembrane transporter activity	0.04452		
					leukocyte activation	0.04556		
					positive regulation of immune system process	0.04671		
					granulocyte chemotaxis	0.05087		
					monovalent inorganic cation transport	0.05285		
					regulation of ion transmembrane transporter activity	0.05584		
					granulocyte migration	0.05605		
					cation homeostasis	0.05865		
					myeloid leukocyte activation	0.05865		
					response to mechanical stimulus	0.07529		
					immune effector process	0.07578		
					ion homeostasis	0.07613		
					regulation of transmembrane transporter activity	0.08006		
					inorganic ion homeostasis	0.08108		
					calcium ion transport	0.08317		
					cellular divalent inorganic cation homeostasis	0.08602		

Primers Used for Cell-Specific mRNAs in Rabbit Lung

The primers in Supplemental Table 3 were used with SybrGreen RT-PCR to quantify cell-specific mRNAs.

Supplemental Table 3: Primers for Quantification of Rabbit Lung-Cell Specific mRNAs

<u>Primer</u>	<u>Target</u>	<u>Orientation</u>	<u>Sequence</u>
rGAPDH(812-1283)mRNA-F	GAPDH	Forward	CCTGGAGAAAGCTGCTAAGT
rGAPDH(812-1283)mRNA-R	GAPDH	Reverse	CGTTGCTGTCTGAGACTTTATTG
rABCA3(1416-1755)mRNA-F	ABCA3	Forward	CAGAGCCATCATGCACATATCA
rABCA3(1416-1755)mRNA-R	ABCA3	Reverse	TTCACCTTGACGCAGAAGAG
rSFTPC(112-414)mRNA-F	SFTPC	Forward	TGCACCTCAAACGTCTTCTC
rSFTPC(112-414)mRNA-R	SFTPC	Reverse	GCTGTCTGGAGCCATCTTC
rSFTPB(1097-14651)mRNA-F	SFTPB	Forward	CATCCAAAGCCCTCACTTCT
rSFTPB(1097-1461)mRNA-R	SFTPB	Reverse	CCAGCCTCTCTCTTCTGTATTT
rHOPX(213-481)mRNA-F	HOPX	Forward	TGGAGATCCTGGAGTACAAC
rHOPX(213-481)mRNA-R	HOPX	Reverse	CACAGCATTACACTGCCAAAC
rAGER(1285-1533)mRNA-F	AGER	Forward	CCCTAGTACCTGAAGGACTCTT□
rAGER(1285-1533)mRNA-R	AGER	Reverse	GTGATGTTCTGACCACCTACTG
rAQP5(1208-1472)mRNA-F	AQP5	Forward	CACTGCTGGTACCACCTTATT
rAQP5(1208-1472)mRNA-R	AQP5	Reverse	CCTGCCTCATCTTCCTTTCTT
rMCIDAS(1713-1976)mRNA-F	MCIDAS	Forward	TGTGTGCTCTCAGCTACTTATG
rMCIDAS(1713-1976)mRNA-R	MCIDAS	Reverse	CAACTTCCTTAGGTTCCCTCTC
rTEKT2(378-578)mRNA-F	TEKT2	Forward	GGACAAGTGTCTGACGGATTTA
rTEKT2(378-578)mRNA-R	TEKT2	Reverse	CGATGACCTCCACCTCTTTATG
rSPDEF(1068-1416)mRNA-F	SPDEF	Forward	CACCTGGACATCTGGAAATCA
rSPDEF(1068-1416)mRNA-R	SPDEF	Reverse	CTTTCGGATGATGCCCTTCT
rMUC5AC(903-1216)mRNA-F	MUC5AC	Forward	CTCCAACACCATTCCTCCAA
rMUC5AC(903-1216)mRNA-R	MUC5AC	Reverse	GCCAAACACAGGCACAATC
rMUC5B(929-1179)mRNA-F	MUC5B	Forward	CCTAAGCTGTGCACCTACAA
rMUC5B(929-1179)mRNA-R	MUC5B	Reverse	TTTCGTTGGGCTGGATGAG
rSCGB1A1(121-324)mRNA-F	SCGB1A1	Forward	CGAGATTTGCACACGTCATTG
rSCGB1A1(121-324)mRNA-R	SCGB1A1	Reverse	CTACATACACAGTGGGCTCTTC
rSCGB3A2(160-414)mRNA-F	SCGB3A2	Forward	ACTCTGCTATTGCCTTCCTTATT
rSCGB3A2(160-414)mRNA-R	SCGB3A2	Reverse	CTGTCTTTCTCTCCCTGATGTT
rGRIA1(3811-4084)mRNA-F	GRIA1	Forward	CACAGGTGGTACGGTGTATTT
rGRIA1(3811-4084)mRNA-R	GRIA1	Reverse	CACTGAGCTTGCATAGCATTTC
rGRHL2(2567-2871)mRNA-F	GRHL2	Forward	CGTCCCAAAGAGCCTGATAAA
rGRHL2(2567-2871)mRNA-R	GRHL2	Reverse	AGTAAACACGCCACATACC
rSERPINE1(477-741)mRNA-F	SERPINE1	Forward	CCTGGAACAAGGATGAGATCAG
rSERPINE1(477-741)mRNA-R	SERPINE1	Reverse	CGTTGAAGTAGAGGGCATTCA
rESRP1(1540-1767)mRNA-F	ESRP1	Forward	GGCTGCACAGAAGTGTCATA
rESRP1(1540-1767)mRNA-R	ESRP1	Reverse	AAAGCACAGAGGGCTGATAAA
rCHGA(1686-1909)mRNA-F	CHGA	Forward	TCTCGACCCTGGAATATCCTT□
rCHGA(1686-1909)mRNA-R	CHGA	Reverse	CGAAGAGCCCAGAACAGATTTA
rCHGB(1769-2254)mRNA-F	CHGB	Forward	GGAAAGAGCAGGACAGAGATTAC
rCHGA(1769-2254)mRNA-R	CHGA	Reverse	GGAATCGTAGAAGTCGGGAAAC

REFERENCES

- Al-Maary J, Eastwood MP, Russo FM, Deprest JA, Keijzer R. 2016. Fetal Tracheal Occlusion for Severe Pulmonary Hypoplasia in Isolated Congenital Diaphragmatic Hernia: A Systematic Review and Meta-analysis of Survival. *Annals of Surgery* **264**:929–933. doi:10.1097/SLA.0000000000001675
- Aydin E, Joshi R, Oria M, Varisco BM, Lim FY, Peiro JL. 2018. Fetal tracheal occlusion in mice: a novel transuterine method. *The Journal of surgical research* **229**:311–315. doi:10.1016/j.jss.2018.04.028
- Aydin E, Joshi R, Oria M, Lim F-Y, Varisco BM, Peiro JL. 2021a. Transuterine Fetal Tracheal Occlusion Model in Mice. *JoVE (Journal of Visualized Experiments)* e61772. doi:10.3791/61772
- Aydin E, Torlak N, Yildirim A, Bozkurt EG. 2021b. Reversible Fetal Tracheal Occlusion in Mice: A Novel Minimal Invasive Technique. *Journal of Surgical Research* **260**:278–283. doi:10.1016/j.jss.2020.11.080
- Boucherat O, Benachi A, Chailley-Heu B, Franco-Montoya ML, Elie C, Martinovic J, Bourbon JR. 2007. Surfactant maturation is not delayed in human fetuses with diaphragmatic hernia. *PLoS medicine* **4**:e237. doi:10.1371/journal.pmed.0040237
- Chapin CJ, Ertsey R, Yoshizawa J, Hara A, Sbragia L, Greer JJ, Kitterman JA. 2005. Congenital diaphragmatic hernia, tracheal occlusion, thyroid transcription factor-1, and fetal pulmonary epithelial maturation. *American journal of physiology Lung cellular and molecular physiology* **289**:L44–52. doi:10.1152/ajplung.00342.2004
- Chen J, Bardes EE, Aronow BJ, Jegga AG. 2009. ToppGene Suite for gene list enrichment analysis and candidate gene prioritization. *Nucleic Acids Research* **37**:W305–11. doi:10.1093/nar/gkp427
- Coughlin MA, Werner NL, Gajarski R, Gadepalli S, Hirschl R, Barks J, Treadwell MC, Ladino-Torres M, Kreutzman J, Mychaliska GB. 2015. Prenatally diagnosed severe CDH: mortality and morbidity remain high. *Journal of pediatric surgery*. doi:10.1016/j.jpedsurg.2015.10.082
- Cruz-Martinez R, Moreno-Alvarez O, Prat J, Krauel L, Tarrado X, Castanon M, Hernandez-Andrade E, Albert A, Gratacos E. 2009. Lung tissue blood perfusion changes induced by in utero tracheal occlusion in a rabbit model of congenital diaphragmatic hernia. *Fetal diagnosis and therapy* **26**:137–42. doi:10.1159/000254485
- Davey MG, Biard JM, Robinson L, Tsai J, Schwarz U, Danzer E, Adzick NS, Flake AW, Hedrick HL. 2005. Surfactant protein expression is increased in the ipsilateral but not contralateral lungs of fetal sheep with left-sided diaphragmatic hernia. *Pediatric pulmonology* **39**:359–67. doi:10.1002/ppul.20175
- Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, Batut P, Chaisson M, Gingeras TR. 2013. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* **29**:15–21. doi:10.1093/bioinformatics/bts635
- Dobrinskikh E, Al-Juboori SI, Oria M, Reisz JA, Zheng C, Peiro JL, Marwan AI. 2020. Heterogeneous Response in Rabbit Fetal Diaphragmatic Hernia Lungs After Tracheal Occlusion. *J Surg Res* **250**:23–38. doi:10.1016/j.jss.2019.12.025
- Du Y, Guo M, Whitsett JA, Xu Y. 2015. “LungGENS”: a web-based tool for mapping single-cell gene expression in the developing lung. *Thorax* ePub ahead of print. doi:10.1136/thoraxjnl-2015-207035
- Endale M, Ahlfeld S, Bao E, Chen X, Green J, Bess Z, Weirauch MT, Xu Y, Perl AK. 2017. Temporal, spatial, and phenotypical changes of PDGFR α expressing fibroblasts during late lung development. *Developmental biology*. doi:10.1016/j.ydbio.2017.03.020
- Engels AC, Brady PD, Kammoun M, Finalet Ferreira J, DeKoninck P, Endo M, Toelen J, Vermeesch JR, Deprest J. 2016. Pulmonary transcriptome analysis in the surgically induced rabbit model of diaphragmatic hernia treated with fetal tracheal occlusion. *Disease models & mechanisms* **9**:221–8. doi:10.1242/dmm.021626
- Harrison MR, Jester JA, Ross NA. 1980. Correction of congenital diaphragmatic hernia in utero. I. The model: intrathoracic balloon produces fatal pulmonary hypoplasia. *Surgery* **88**:174–82.
- Joshi R, Liu S, Brown MD, Young SM, Batie M, Kofron JM, Xu Y, Weaver TE, Apsley K, Varisco BM. 2016. Stretch regulates expression and binding of chymotrypsin-like elastase 1 in the postnatal lung. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **30**:590–600. doi:10.1096/fj.15-277350
- Kassambara A. 2020. ggpubr: Publication Ready Plots - Articles - STHDA. <http://www.sthda.com/english/articles/24-ggpubr-publication-ready-plots/>
- Kassambara A. 2020. rstatix: Pipe-Friendly Framework for Basic Statistical Tests.
- Krämer A, Green J, Pollard J, Tugendreich S. 2014. Causal analysis approaches in Ingenuity Pathway Analysis. *Bioinformatics* **30**:523–530. doi:10.1093/bioinformatics/btt703
- Lange AW, Sridharan A, Xu Y, Stripp BR, Perl AK, Whitsett JA. 2015. Hippo/Yap signaling controls epithelial progenitor cell proliferation and differentiation in the embryonic and adult lung. *J Mol Cell Biol* **7**:35–47. doi:10.1093/jmcb/mju046
- Love MI, Huber W, Anders S. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology* **15**:550. doi:10.1186/s13059-014-0550-8
- Mahoney JE, Mori M, Szymaniak AD, Varelas X, Cardoso WV. 2014. The hippo pathway effector Yap controls patterning and differentiation of airway epithelial progenitors. *Developmental cell* **30**:137–50. doi:10.1016/j.devcel.2014.06.003
- Meng Z, Moroishi T, Guan KL. 2016. Mechanisms of Hippo pathway regulation. *Genes & development* **30**:1–17. doi:10.1101/gad.274027.115
- Nantie LB, Young RE, Paltzer WG, Zhang Y, Johnson RL, Verheyden JM, Sun X. 2018. Lats inactivation reveals hippo function in alveolar type I cell differentiation during lung transition to air breathing. *Development (Cambridge, England)*. doi:10.1242/dev.163105
- Nguyen TM, van der Merwe J, Elowsson Rendin L, Larsson-Callertfelt A-K, Deprest J, Westergren-Thorsson G, Toelen J. 2021. Stretch increases alveolar type 1 cell number in fetal lungs through ROCK-Yap/Taz pathway. *Am J Physiol Lung Cell Mol Physiol* **321**:L814–L826. doi:10.1152/ajplung.00484.2020
- Peiro JL, Oria M, Aydin E, Joshi R, Cabanas N, Schmidt R, Schroeder C, Marotta M, Varisco BM. 2018. Proteomic profiling of tracheal fluid in an ovine model of congenital diaphragmatic hernia and fetal tracheal occlusion. *Am J Physiol Lung Cell Mol Physiol*

315:L1028–L1041. doi:10.1152/ajplung.00148.2018

- Penkala IJ, Liberti DC, Pankin J, Sivakumar A, Kremp MM, Jayachandran S, Katzen J, Leach JP, Windmueller R, Stolz K, Morley MP, Babu A, Zhou S, Frank DB, Morrissey EE. 2021. Age-dependent alveolar epithelial plasticity orchestrates lung homeostasis and regeneration. *Cell Stem Cell*. doi:10.1016/j.stem.2021.04.026
- Prat Ortells J, Albert A, Tarrado X, Krauel L, Cruz R, Moreno-Alvarez O, Fuste V, Castanon M. 2014. Airway and vascular maturation stimulated by tracheal occlusion do not correlate in the rabbit model of diaphragmatic hernia. *Pediatric research* **75**:487–92. doi:10.1038/pr.2013.244
- R Core Team. 2019. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing.
- Riccelli M, Gokey JJ, Aronow B, Perl A-KT. 2020. The elephant in the lung: Integrating lineage-tracing, molecular markers, and single cell sequencing data to identify distinct fibroblast populations during lung development and regeneration. *Matrix Biol*. doi:10.1016/j.matbio.2020.05.002
- Soldt BJ van, Qian J, Li J, Tang N, Lu J, Cardoso WV. 2019. Yap and its subcellular localization have distinct compartment-specific roles in the developing lung. *Development* **146**. doi:10.1242/dev.175810
- Tan SY, Krasnow MA. 2016. Developmental origin of lung macrophage diversity. *Development (Cambridge, England)* **143**:1318–27. doi:10.1242/dev.129122
- Varisco BM, Sbragia L, Chen J, Scorletti F, Joshi R, Wong HR, Lopes-Figueira R, Oria M, Peiro J. 2016. Excessive Reversal of Epidermal Growth Factor Receptor and Ephrin Signaling Following Tracheal Occlusion in Rabbit Model of Congenital Diaphragmatic Hernia. *Mol Med* **22**. doi:10.2119/molmed.2016.00121
- Vaughan AE, Brumwell AN, Xi Y, Gotts JE, Brownfield DG, Treutlein B, Tan K, Tan V, Liu FC, Looney MR, Matthay MA, Rock JR, Chapman HA. 2015. Lineage-negative progenitors mobilize to regenerate lung epithelium after major injury. *Nature* **517**:621–5. doi:10.1038/nature14112
- Vuckovic A, Herber-Jonat S, Flemmer AW, Ruehl IM, Votino C, Segers V, Benachi A, Martinovic J, Nowakowska D, Dzieniecka M, Jani JC. 2016. Increased TGF-beta: a drawback of tracheal occlusion in human and experimental congenital diaphragmatic hernia? *American journal of physiology Lung cellular and molecular physiology* **310**:L311-27. doi:10.1152/ajplung.00122.2015
- Yang Y, Riccio P, Schotsaert M, Mori M, Lu J, Lee DK, Garcia-Sastre A, Xu J, Cardoso WV. 2018. Spatial-Temporal Lineage Restrictions of Embryonic p63(+) Progenitors Establish Distinct Stem Cell Pools in Adult Airways. *Developmental cell* **44**:752-761.e4. doi:10.1016/j.devcel.2018.03.001
- Zuo W, Rostami MR, Shenoy SA, LeBlanc MG, Salit J, Strulovici-Barel Y, O'Beirne SL, Kaner RJ, Leopold PL, Mezey JG, Schymeinsky J, Quast K, Visvanathan S, Fine JS, Thomas MJ, Crystal RG. 2020. Cell-specific expression of lung disease risk-related genes in the human small airway epithelium. *Respiratory Research* **21**:200. doi:10.1186/s12931-020-01442-9