

WNT5a-ROR Signaling Is Essential for Alveologenesis

Supplementary Materials:

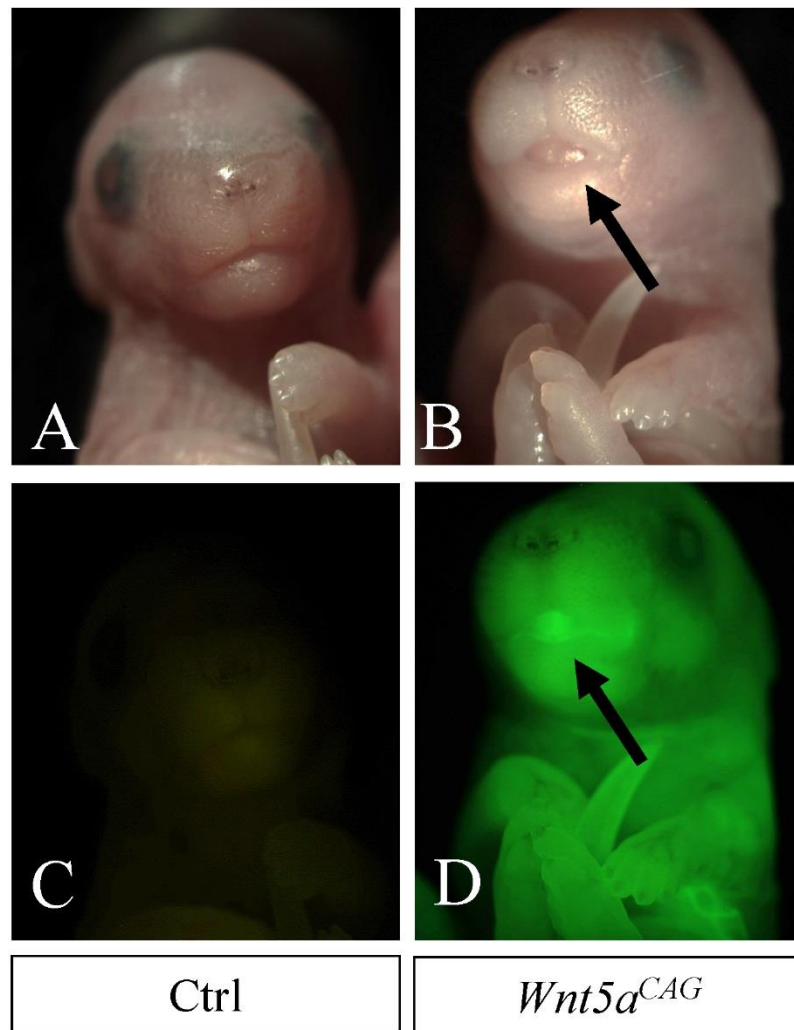


Figure S1: Craniofacial defects of E18 *Wnt5a*^{CAG} embryos.

A-D: Frontal view of E18 control (A, C) and *Wnt5a*^{CAG} (B, D) craniofacial phenotype. Panels A & B show the bright field images and Panels C & D show the green fluorescent images of control and *Wnt5a*^{CAG} embryos, respectively. Arrow indicates the “open” mouth phenotype in *Wnt5a*^{CAG}.

Table S1: Expression of cell type specific markers during saccular and alveolar phases of lung development.

Saccular phase cell specificity is based on mouse E18.5 scRNA-seq in LungGENS (LungGENS: <https://research.cchmc.org/pbge/lunggens>). Alveolar phase cell specificity is based on mouse P7 scRNA-seq in LungGENS for endothelial and myofibroblast markers, and publications [32,41,42] listed in the table for AT1 and AT2 markers.

Gene Symbol	Gene Name	Saccular Phase	Alveolar Phase (reference)
Aqp5	Aquaporin 5	AT1	AT1 (32)
Cav1	Caveolin 1	AT1, EC	AT1 (42)
Emp2	Epithelial Membrane Protein 2	AT1	AT1 (42)
Hopx	HOP Homeobox	AT1	AT1 (32)
Rage (Ager)	Advanced Glycosylation End-Product Specific Receptor	AT1	AT1 (42)
Rtkn2	Rhotekin 2	AT1, Ciliated Cells	AT1 (42)
Pdpn	Podoplanin	AT1	AT1 (32)
Sftpc	Surfactant Protein C	AT2	AT2 (32)
Sftpb	Surfactant Protein B	AT2	AT2 (41)
Abca3	ATP Binding Cassette Subfamily A Member 3	AT2, Club Cells	AT2 (32)
Cd31(Pecam1)	Platelet And Endothelial Cell Adhesion Molecule 1	EC	EC
Emcn	Endomucin	EC	EC
Flk1 (KDR)	Kinase Insert Domain Receptor	EC, Club Cells	none
Tek	TEK Receptor Tyrosine Kinase	EC	EC
Acta2	Actin Alpha 2, Smooth Muscle	MF/SMC	MF/SMC
Tagln	Transgelin	MF/SMC	MF/SMC
Des	Desmin	none	MF/SMC
Dbn1	Drebrin 1	MF/SMC	MF/SMC
Tgfb1	Transforming Growth Factor Beta Induced	MF/SMC	MF/SMC
Fbln1	Fibulin 1	MF/SMC, Ciliated Cells	MF/SMC
Eln	Elastin	MF/SMC, LipoFB/MatrixFB	MF/SMC
Colla1	Collagen Type I Alpha 1 Chain	LipoFB/MatrixFB	MatrixFB
Pdgfra	Platelet Derived Growth Factor Receptor Alpha	MF/SMC	MF/SMC
Pdgfrb	Platelet Derived Growth Factor Receptor Beta	MF/SMC	none

Abbreviations: **AT1:** Alveolar epithelial type 1, **AT2:** Alveolar epithelial type 2, **EC:** Endothelial Cells, **MF:** Myofibroblasts, **SMC:** Smooth Muscle Cells, **LipoFB:** Lipofibroblasts, **MatrixFB:** Matrix fibroblasts, **none:** $-\log(p\text{-value}) < 2.996$ for most differentially expressed cell type.

Table S2: Changes of cell migration genes in *Pdgfra* deficient SCMF identified by RNA-seq analysis (GSE126457, [25]).

Gene	Fold Changes	P-value	FDR
Actc1	-7.9	0.00E+00	0.00E+00
Bmp2	2.94	4.25E-39	2.38E-37
Meox2	-7.06	6.50E-24	2.37E-22
Lum1	-14.74	1.55E-40	9.06E-39
Cyr61	2.27	0.00E+00	0.00E+00
Cnn1	-9.68	3.57E-168	7.53E-166
Fgf18	-2.22	6.32E-04	3.96E-03

Table S3: Antibody information (*: discontinued).

Anitbody & Reagents	Company	Catelog #
Mouse anti-GFP	Santa Cruz, CA	SC-9996
Rabbit anti-ACTA2	Novus, CO	NB600531*
Rabbit anti-ACTA2	Millipore, MA	ABT1487
Goat anti-TAGLN	Abcam, MA	AB10135
Rabbit anti-HOPX	Santa Cruz, CA	SC-30216*
Rabbit anti-SFTPC	Millipore, MA	AB3786
Hamster anti-PDPN	DSHB	8.1.1

Table S4: Sequences of real-time RT-PCR primers.

Gene name (mouse)	realtime RT-PCR Primer Sequences
Emcn	5'-TGC TTC AAG CGA CTG TTC TTT-3' 5'-GCC GGT GTT GGA ATA GAG TC-3'
Flk1	5'-CAGTGGTACTGGCAGCTAGAA-3' 5'-ACAAGCATACGGGCTTGT-3'
Cd31	5'-GAGTACGAGGTGAAGGTGCAT-3' 5'-CGCCTTCTGTACCTCCTT-3'
Tek	5'-CAT AGG AGG AAA CCT GTT CAC-3' 5'-GCC CCC ACT TCT GAG CTT-3'
Acta2 (aSMA)	5'- CCCACCCAGAGTGGAGAA-3' 5'- ACATAGCTGGAGCAGCGTCT-3'
Tagln	5'-CCTTCCAGTCCACAAACGAC-3' 5'-GTAGGATGGACCTTGTGG-3'
Desmin (Des)	5'-GCG TGA CAA CCT GAT AGA CG-3' 5'-TGG AT TCC TCC TGT AGT TTG G-3'
Dbn1	5'-CCT GCA CCC TTC AAC CAC-3' 5'-GAA TAG GAG TGG GTG CCA TC-3'
Tgfb1	5'-GCT GCG AGT CTT TGT TTA TCG-3' 5'-CCT CTT ATC ATG GGC AGC A-3'
Eln	5'-GCTGCTGCTAAGGCTGCTAA-3' 5'-AGCACCTGGGAGCCTAACTC-3'
Coll1a1	5'-CAT GTT CAG CTT TGT GGA CCT-3' 5'-GCA GCT GAC TTC AGG GAT GT-3'
Fbln1	5'-GTGCAAGGCTGGCTTCTATP-3' 5'-GATAGCGCTGGCACTCGT-3'
Pdgfra	5'-GTCGTTGACCTGCAGTGGAA-3' 5'-CCAGCATGGIGATACCTTTGT-3'
Pdgfrb	5'-TCAAGCTGCAGGTCAATGTC-3' 5'-CCATTGGCAGGGTGTACTC-3'
Ng2	5'-CTTGGCCTTGTGGTTCAGAT-3' 5'-CACCTCCAGGTGGTTCCTCC-3'
Adrp	5'-CCTCAGCTCTCCTGTTAGGC-3' 5'-CACTACTGCTGCTGCCAATT-3'

Gene name (human)	Realtime RT-PCR Primer Sequences
WNT5a	5'-CGTCTGGAAGCAGACGTTTC-3' 5'-TCACGCCTCCTGATCTCC-3'
ROR1	5'-GCAAGGGGAAATAGAAAATCAG-3' 5'-GAAGGAATGGCGAACTGAGA-3'
ROR2	5'-CCCCTCATTAACCAGCACAA-3' 5'-TTCCAAACCGGTCCTCT-3'

Gene name (mouse)	realtime RT-PCR Primer Sequences
Slfbp (SpB)	5'-AACCCACACCTCTGAGAAC-3' 5'-GTGCAGGCTGAGGCITGT-3'
Slfpc (SpC)	5'-GGTCCGTGATGGAGAGTCCAC-3' 5'-GATGAGAAGCGTTTGAGGT-3'
Abca3	5'-TGT GGC GAC TGA GAA CCT T-3' 5'-CAC TGA TGT CCG GTT CTG C-3'
Aqp5	5'-CAGACCTCAGAGATTTGTGAAG-3' 5'- CAGAAATAAATAAGATGGCAC-3'
Caveolin1 (Cav1)	5'-CCA GGG AAA CCT CCT CAG A-3' 5'-CCG GAT GGG AAC AGT GTA GA-3'
Emp2	5'-AAG CAG GGA GAG AGG TTC GT-3' 5'-GCT CCG ATC ATG ACA CAC AG-3'
Hopx	5'-ACCACGCTGTGCCTCATC-3' 5'-GCGCTGCTTAAACCATTTC-3'
Rage	5'-GTGTCGGGCAACTAACAGG-3' 5'-CTGGCTTCCAGGAATCTG-3'
Rtkn2	5'-TGIATGGCAACGTGTGCTG-3' 5'-CCAGACCTTTCTCTGTTTC-3'
Pdpu(T1a)	5'-CAGTGTGTCTGGGTTTTGG-3' 5'-TGGGGTCAACAATATCATCTTCA-3'
Wnt5a	5'-TAA GCC CGG GAG TGG CTT TGG-3' 5'-GGG CGA AGG AGA AAA ACG TGG-3'
Actc1	5'-CCTCTCTGGAGAAGAGCTATGA-3' 5'-AATGAAAGAGGGCTGGAGAG-3'
Bmp2	5'-AGATCTGTACCGCAGGCACT-3' 5'-GTTCTCCACGGCTTCTTC-3'
Meox2	5'-AAT CTA GAC CTC ACT GAA AGA CAG G-3' 5'-CTT GCT GTC CCC CTT TGA-3'
Lum	5'-CAG CAA CAT TCC GGA TGA G-3' 5'-TCA TTG TGA GAT AAA CGC AGG T-3'
Cyr61	5'-GGATCTGTGAAGTGCCTCT-3' 5'-CTGCATTTCTTGCCCTTTT-3'
Cnn1	5'-CGGCTTGTCTGTGAAGTAA-3' 5'-ACCCCTCAATCCACTCTCT-3'
Fgf18	5'-AGGACGGGGACAAGTATGC-3' 5'-GGACTTGACTCCCGAAGGT-3'
Tbp	5'-CAGCCAAGATTACGGTAGAT-3' 5'-CCAATGACTCCAATGACCCT-3'