PBX Transcription Factor Drives Pulmonary Vascular Adaptation to Birth - Supplemental Data

Figure S1.
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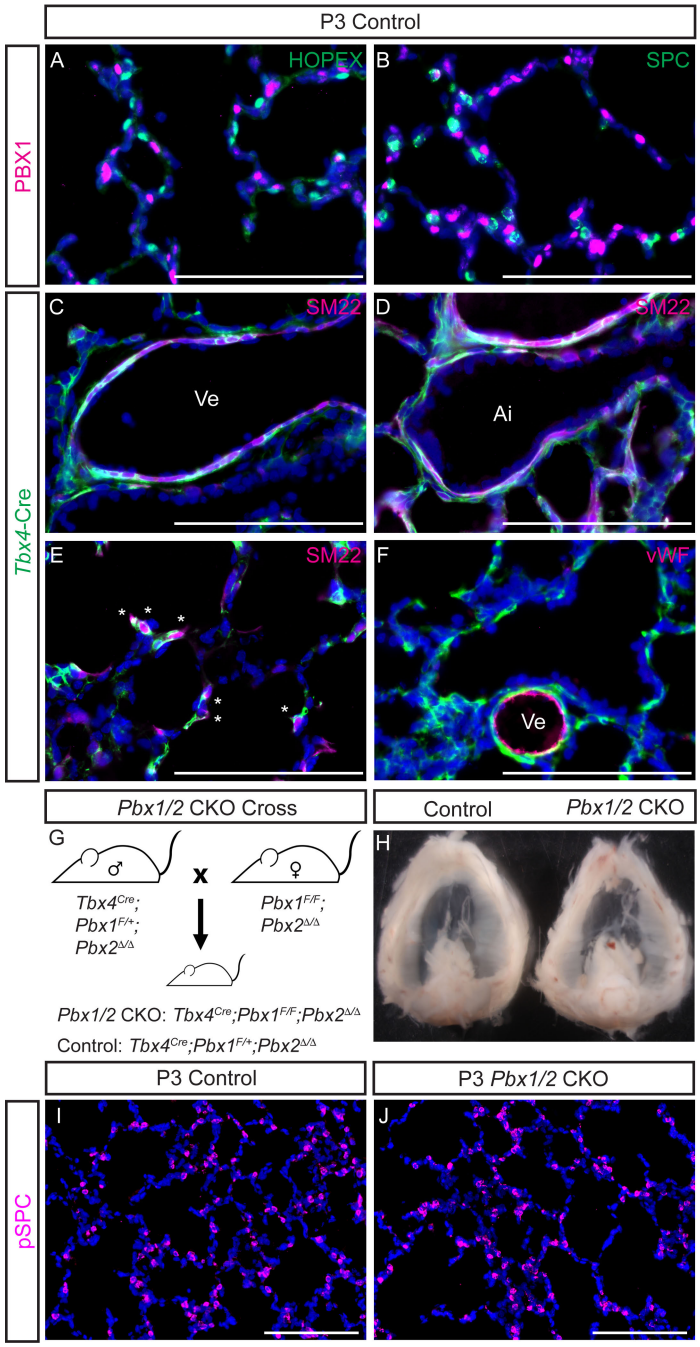

Figure S1. *Tbx4*$^{-Cre}$ mediated deletion of PBX1/2. (A, B) At P3, PBX1 is not expressed in alveolar type I cells stained by HOPEX or type II cells stained by pSPC. (C – F) *Tbx4*$^{-Cre}$ activity overlaps with SM22 stained vascular (Ve) smooth muscle cells, airway (Ai) smooth muscle cells, and alveolar myofibroblasts (*), but not vWF stained vascular endothelium. (G) Genetic cross to create *Pbx1/2* CKO and control mice. (H) *Pbx1/2* CKO mice have normal diaphragm development. (I, J) *Pbx1/2* CKO mice have normal pSPC staining in the alveolar region compared to controls. Scale bar = 100 μm. Abbreviations (Ai – airway, Ve – vessel).

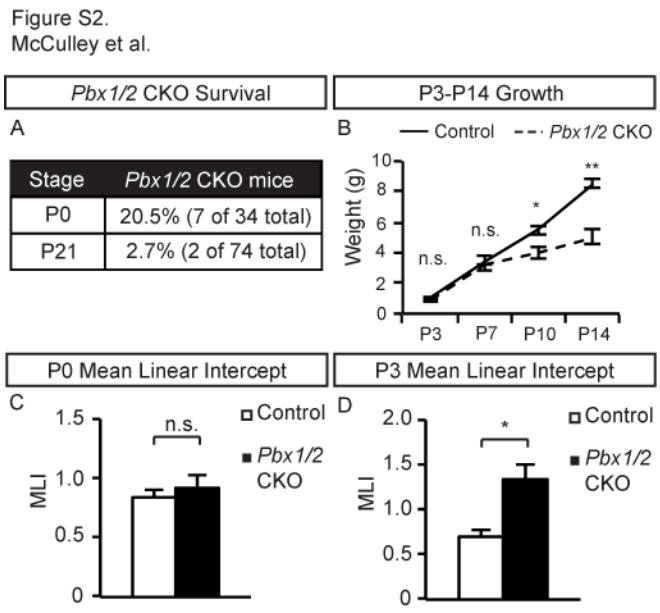

Figure S2. *Pbx1/2* CKO mice survive until birth but fail to thrive and die between 2 – 4 weeks of age. (A) *Pbx1/2* CKO mice are present at the expected Mendelian ratio at birth, but with only rare survivors beyond 3 weeks of age. (B) *Pbx1/2* CKO mice are of normal weight at P3 and P7 but are smaller than controls at P10 (*p = 0.02), and P14 (***p = 0.0006). (C, D) MLI measurement of lung alveolar region complexity showed that *Pbx1/2* CKO mice have normal complexity at P0 (p = 0.55) but reduced complexity at P3 (*p = 0.02). For MLI statistical analyses, 3 samples were included from each group and comparisons were made using a Student's t-test.

Figure S3.
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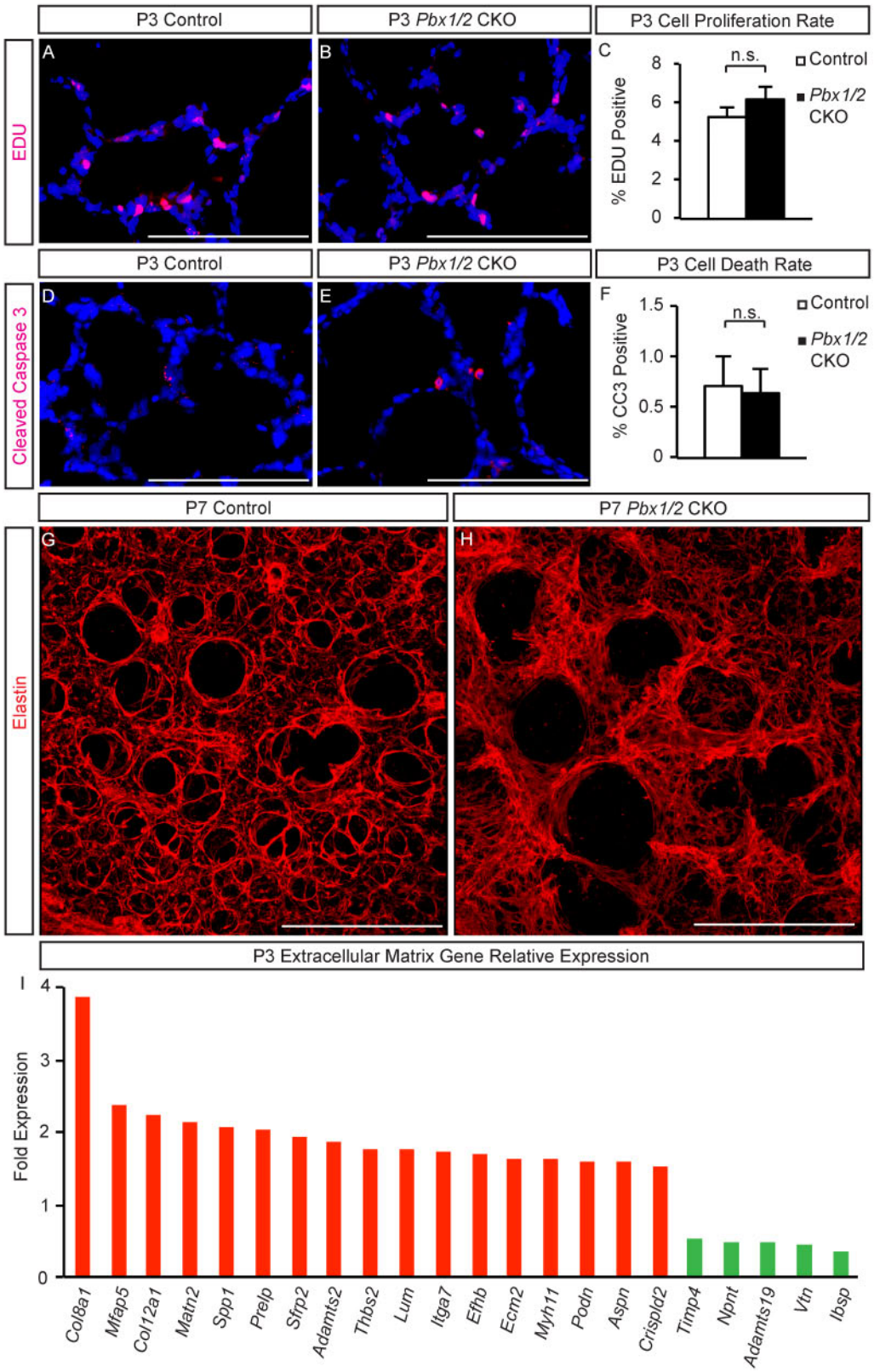
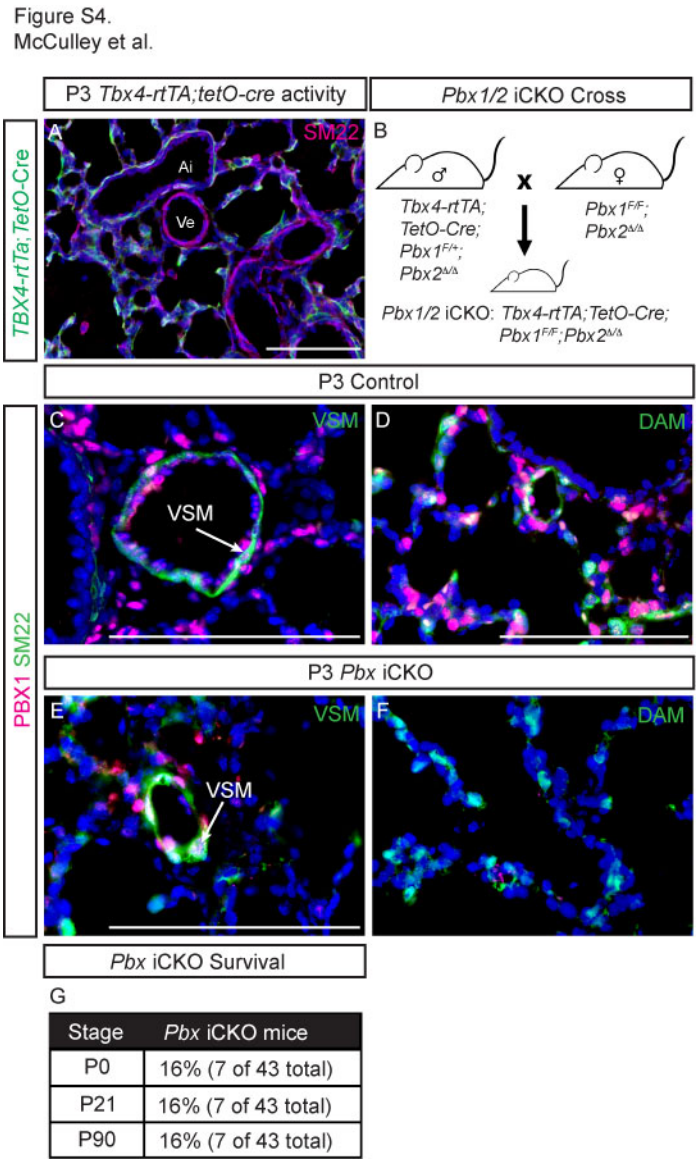


Figure S3. *Pbx1/2* CKO mice have defective alveologenesis. (A – C) EdU staining for proliferating cells demonstrates no difference in the rate of cell proliferation in control and *Pbx1/2* CKO mice at P3. (D – F) Cleaved Caspase-3 staining for cells undergoing apoptosis demonstrates no difference in the rate of cell death in control and *Pbx1/2* CKO mice at P3. (G, H) Elastin staining of the 100-μm vibratome sections from the alveolar region of the lung with three-dimensional reconstruction shows that, unlike control mice that have a well-defined extracellular Elastin network, *Pbx1/2* CKO mice have a diffuse and disorganized network of Elastin. (I) Gene expression microarray analysis demonstrates changes in the expression of genes required for extracellular matrix production, organization, and turnover in P3 *Pbx1/2* CKO mice relative to controls. Chart includes genes with relative expression changes greater than 1.5 fold compared to expression in controls. For all statistical analyses, 4 samples were included from each group and comparisons were made using a Student's t-test.



Stage	*Pbx* iCKO mice
P0	16% (7 of 43 total)
P21	16% (7 of 43 total)
P90	16% (7 of 43 total)

Figure S4. *Tbx4-rtTA; TetO*-Cre mediated deletion of PBX1/2. (A) *Tbx4-rtTA; TetO*-Cre is active in mesenchyme cells of the alveolar region, but not in SM22 stained vascular smooth muscle cells. (B) Genetic cross to generate *Pbx* iCKO mice. (C, D) At P3, control mice have PBX1 expression in both SM22 stained vascular smooth muscle (VSM, C) and distal alveolar myofibroblasts (DAM, D). (E, F) *Pbx* iCKO mice have PBX1 expression in SM22 stained vascular smooth muscle cells (VSM, E) but not in distal alveolar myofibroblast cells (DAM, F). (G)

Pbx iCKO mice survive until birth (P0) and are well appearing at weaning (P21) and early adulthood (P90) with 100% survival. Scale bar = 100 μm. Abbreviations (Ai – airway, DAM – distal alveolar myofibroblast, VSM – vascular smooth muscle, Ve – vessel).

Figure S5.
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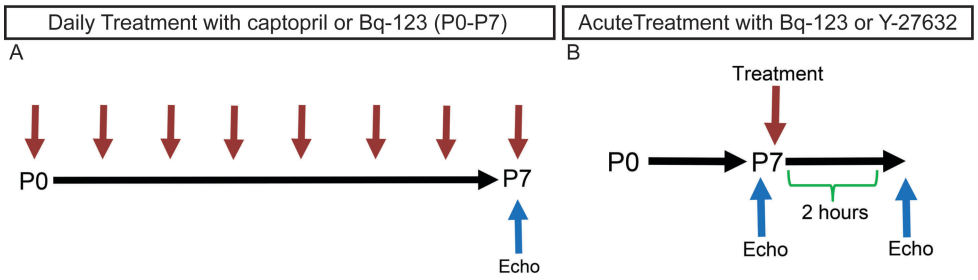

Figure S5. Postnatal and acute treatment schedules. (A) Daily treatment of control and *Pbx1/2* CKO mice with angiotensin converting enzyme (captopril) or endothelin receptor A antagonist (Bq-123) with echocardiography at P7. (B) Echocardiography at P7 in control and *Pbx1/2* CKO mice followed by acute treatment with Bq-123 or Rho-kinase inhibitor (Y-27632) with repeat echocardiogram measurement 2 hours later.

Figure S6.
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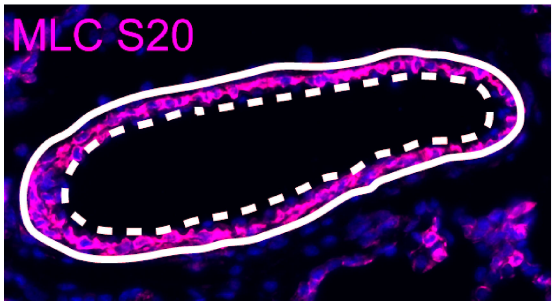

Figure S6. Phosphorylated myosin light chain (MLC-S20) staining intensity quantification. The staining intensity was recorded as intensity per unit area. Both the MLC S20 staining intensity and the area were quantified by measuring the difference between the outer (solid line) and the inner (dashed line) using Image J.

Table S1. Embryonic day 14 (E14) microarray gene expression analysis. Gene ID, gene name, ratio of gene expression in E14 *Pbx1/2* CKO lungs compared to E14 controls, and direction of change. Results limited to fold change ≥ 1.5.

Gene ID	Gene Name	Gene Identifier	Ratio	Direction
Prokr2	Prokineticin receptor 2 (Prokr2), mRNA	NM_144944	2.76	Up
Adamdec1	ADAM-like, decysin 1 (Adamdec1), mRNA	NM_021475	2.22	Up
Neto1	Neuropilin (NRP) and tolloid (TLL)-like 1 (Neto1), mRNA	NM_144946	1.98	Up
Lgi1	Leucine-rich repeat LGI family, member 1 (Lgi1), mRNA	NM_020278	1.93	Up
Fat3	FAT tumor suppressor homolog 3 (Drosophila) (Fat3), mRNA	NM_001080814	1.88	Up
Ptprz1	Protein tyrosine phosphatase, receptor type Z, polypeptide 1, mRNA (cDNA clone IMAGE:3590815)	NM_001081306	1.88	Up
Ctse	Cathepsin E, mRNA (cDNA clone MGC:5924 IMAGE:3601519)	NM_007799	1.86	Down
Tnc	Tenascin C (Tnc), mRNA	NM_011607	1.82	Up
Fat3	FAT tumor suppressor homolog 3 (Drosophila) (Fat3), mRNA	NM_001080814	1.77	Up
Ube2v1	Ubiquitin-conjugating enzyme E2 variant 1, mRNA (cDNA clone MGC:25512 IMAGE:2646198)	NM_023230	1.75	Up
Lgr5	Leucine rich repeat containing G protein coupled receptor 5 (Lgr5), mRNA	NM_010195	1.7	Up
Olfr59	Olfactory receptor 59, mRNA (cDNA clone MGC:170786 IMAGE:8862181)	NM_011002	1.64	Down
Rgs5	Regulator of G-protein signaling 5, mRNA (cDNA clone MGC:46953 IMAGE:3962083)	Rgs5	1.63	Up
Ccl11	Chemokine (C-C motif) ligand 11, mRNA (cDNA clone MGC:41147 IMAGE:1527856)	NM_011330	1.62	Down
Vmn2r54	Vomeronasal 2, receptor 54 (Vmn2r54), mRNA	NM_001081449	1.62	Down
Dvl3	Dishevelled 3, dsh homolog (Drosophila) (Dvl3), mRNA	NM_007889	1.61	Up
B3gnt3	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 3, mRNA (cDNA clone MGC:31283 IMAGE:4218953)	BC026418	1.61	Down
Slitrk4	SLIT and NTRK-like family, member 4, mRNA (cDNA clone MGC:144017 IMAGE:40096585)	NM_178740	1.6	Up
Esco1	Establishment of cohesion 1 homolog 1 (S. cerevisiae), mRNA (cDNA clone IMAGE:3598095)	NM_001081222	1.6	Down
Lrp1	Lipoprotein receptor-related protein (Lrp1)	NM_008512	1.6	Up
Fat3	FAT tumor suppressor homolog 3 (Drosophila) (Fat3), mRNA	NM_001080814	1.6	Up
Prokr1	Prokineticin receptor 1 (Pkr1)	NM_021381	1.59	Up
P2ry14	Purinergic receptor P2Y, G-protein coupled, 14, mRNA (cDNA clone MGC:38677 IMAGE:5356997)	NM_133200	1.59	Up
Fat3	FAT tumor suppressor homolog 3 (Drosophila) (Fat3), mRNA	NM_001080814	1.58	Up
Hspg2	Perlecan (heparan sulfate proteoglycan 2), mRNA (cDNA clone IMAGE:3497830)	NM_008305	1.58	Up
Pam	Peptidylglycine alpha-amidating monooxygenase (Pam), mRNA	NM_013626	1.58	Up
Smpx	SMPX protein (Smpx)	NM_025357	1.57	Down
Pom121	Nuclear pore membrane protein 121 (Pom121), mRNA	NM_148932	1.57	Up
Kcnip1	A-type potassium channel modulatory protein 1.2 (Kchip1.2)	NM_027398	1.56	Up
Fat3	FAT tumor suppressor homolog 3 (Drosophila) (Fat3), mRNA	NM_001080814	1.56	Up
Dock6	Dedicator of cytokinesis 6, mRNA (cDNA clone IMAGE:5005183)	NM_177030	1.56	Up
Polr2a	Polymerase (RNA) II (DNA directed) polypeptide A (Polr2a), mRNA	NM_009089	1.56	Up
Cldn1	Claudin 1, mRNA (cDNA clone MGC:5767 IMAGE:3491319)	NM_016674	1.54	Down
St6gal2	Beta galactoside alpha 2,6 sialyltransferase 2, mRNA (cDNA clone MGC:169766 IMAGE:8861161)	NM_172829	1.54	Up
V1rd22	Vomeronasal 1 receptor, D22 (V1rd22), mRNA	NM_207548	1.54	Down

Zmiz1	Zinc finger, MIZ-type containing 1, mRNA (cDNA clone MGC:86031 IMAGE:6856060)	NM_183208	1.53	Up
Ppbp	Chemokine subfamily B Cys-X-Cys	NM_023785	1.53	Up
Dock6	Dedicator of cytokinesis 6, mRNA (cDNA clone IMAGE:5005183)	NM_177030	1.53	Up
Dock6	Dedicator of cytokinesis 6, mRNA (cDNA clone IMAGE:5005183)	NM_177030	1.53	Up
Fat3	FAT tumor suppressor homolog 3 (Drosophila) (Fat3), mRNA	NM_001080814	1.53	Up
Prelp	Proline arginine-rich end leucine-rich repeat, mRNA (cDNA clone MGC:25825 IMAGE:4165772)	NM_054077	1.52	Up
Fat3	FAT tumor suppressor homolog 3 (Drosophila) (Fat3), mRNA	NM_001080814	1.52	Up
Lama5	Laminin, alpha 5, mRNA (cDNA clone IMAGE:5037040)	NM_001081171	1.52	Up
Taf1d	TATA box binding protein (Tbp)-associated factor, RNA polymerase I, D (Taf1d), transcript variant 2, mRNA	BC110660	1.52	Up
Fam65a	Family with sequence similarity 65, member A, mRNA (cDNA clone MGC:40645 IMAGE:5400266)	NM_001081241	1.52	Up
Abi3bp	ABI gene family, member 3 (NESH) binding protein, mRNA (cDNA clone IMAGE:4982180)	NM_001014423	1.52	Up
Nmnat2	Nicotinamide nucleotide adenylyltransferase 2, mRNA (cDNA clone MGC:113818 IMAGE:30532596)	NM_175460	1.52	Up
Lzts1	Leucine zipper, putative tumor suppressor 1, mRNA (cDNA clone MGC:170979 IMAGE:8862374)	BC127607	1.52	Up
Csdc2	Cold shock domain containing C2, RNA binding (Csdc2), mRNA	NM_145473	1.51	Up
Trp53i11	Trp53 inducible protein 11, mRNA (cDNA clone IMAGE:4984921)	NM_001025246	1.51	Up
Far2	Fatty acyl CoA reductase 2, mRNA (cDNA clone MGC:67167 IMAGE:6809131)	NM_178797	1.51	Down
Jup	Plakoglobin	NM_010593	1.51	Up
Gm129	Gene model 129, (NCBI), mRNA (cDNA clone MGC:170135 IMAGE:8861530)	NM_001033302	1.51	Down
Olfr1079	Olfactory receptor 1079 (Olfr1079), mRNA	NM_146407	1.51	Up
Fat3	FAT tumor suppressor homolog 3 (Drosophila) (Fat3), mRNA	NM_001080814	1.5	Up
Kdm5d	Smcy (Smcy)	NM_011419	1.5	Down
Ccr2	Chemokine (C-C motif) receptor 2 (Ccr2), mRNA	NM_009915	1.5	Down

Table S2. Postnatal day 0 (P0) microarray gene expression analysis. Gene ID, gene name, ratio of gene expression in P0 *Pbx1/2* CKO lungs compared to P0 controls, and direction of change. Results limited to fold change ≥ 1.8.

Gene ID	Gene Name	Gene Identifier	Ratio	Direction
Inmt	Indolethylamine N-methyltransferase, mRNA (cDNA clone MGC:19191 IMAGE:4236077)	NM_009349	22.1	Down
Rgs5	Regulator of G-protein signaling 5, mRNA (cDNA clone MGC:46953 IMAGE:3962083)	Rgs5	4.01	Up
C7	CDNA clone IMAGE:9007301	C7	3.91	Down
Col8a1	Collagen, type VIII, alpha 1, mRNA (cDNA clone MGC:18687 IMAGE:3674027)	NM_007739	3.91	Up
Rgs5	Regulator of G-protein signaling 5, mRNA (cDNA clone MGC:46953 IMAGE:3962083)	NM_009063	3.76	Up
Fmo2	Flavin containing monooxygenase 2, mRNA (cDNA clone MGC:28212 IMAGE:3990305)	NM_018881	3.43	Down
Vsnl1	Visinin-like protein 1	NM_012038	3.34	Down
Zbtb16	PLZF gene	NM_001033324	3.31	Down
Slc36a2	Solute carrier family 36 (proton/amino acid symporter), member 2 (Slc36a2), mRNA	NM_153170	3.3	Down
Serpina3k	Serine (or cysteine) peptidase inhibitor, clade A, member 3K, mRNA (cDNA clone MGC:30243 IMAGE:5050045)	NM_009252	3.22	Down
Prkg2	Protein kinase, cGMP-dependent, type II (Prkg2), mRNA	NM_008926	3.17	Down
Slc27a6	Solute carrier family 27 (fatty acid transporter), member 6 (Slc27a6), mRNA	NM_001081072	3.17	Down
Cpa1	Carboxypeptidase A1 (Cpa1), mRNA	NM_025350	3.05	Down
Ppp4r4	Protein phosphatase 4, regulatory subunit 4, mRNA (cDNA clone MGC:132871 IMAGE:40060480)	NM_028980	3.04	Down
Lrrc15	Leucine rich repeat containing 15 (Lrrc15), mRNA	NM_028973	3.02	Up
Adcy8	Adenylate cyclase 8 (Adcy8), mRNA	NM_009623	3.01	Down
St6gal2	Beta galactoside alpha 2,6 sialyltransferase 2, mRNA (cDNA clone MGC:169766 IMAGE:8861161)	NM_172829	2.97	Up
Fhl5	Four and a half LIM domains 5 (Fhl5), mRNA	NM_021318	2.88	Up
Cdo1	Cysteine dioxygenase 1, cytosolic, mRNA (cDNA clone MGC:18800 IMAGE:4194939)	NM_033037	2.81	Down
Kcnmb1	Potassium large conductance calcium-activated channel, subfamily M, beta member 1, mRNA (cDNA clone MGC:13890 IMAGE:3988005)	NM_031169	2.75	Up
Mfap5	Microfibrillar associated protein 5, mRNA (cDNA clone MGC:35969 IMAGE:3982519)	NM_015776	2.71	Up
Kcna2	Potassium voltage-gated channel, shaker-related subfamily, member 2 (Kcna2), mRNA	NM_008417	2.68	Down
Adh1	Alcohol dehydrogenase 1 (class I), mRNA (cDNA clone MGC:18885 IMAGE:4238555)	NM_007409	2.67	Down
Enpep	Glutamyl aminopeptidase (Enpep), mRNA	NM_007934	2.66	Down
Actg2	Actin, gamma 2, smooth muscle, enteric, mRNA (cDNA clone MGC:6022 IMAGE:3591323)	NM_009610	2.6	Up
Prelp	Proline arginine-rich end leucine-rich repeat, mRNA (cDNA clone MGC:25825 IMAGE:4165772)	NM_054077	2.52	Up
Cacna1d	L-type voltage-gated calcium channel Cav1.3(1a) subunit (cacna1d gene)	NM_028981	2.5	Down
Myh11	Myosin, heavy polypeptide 11, smooth muscle, mRNA (cDNA clone MGC:13920 IMAGE:3989495)	NM_013607	2.5	Up
Srd5a2l2	Steroid 5 alpha-reductase 2-like 2 (Srd5a2l2), mRNA	NM_153801	2.47	Down
Cyp2e1	Cytochrome P450, family 2, subfamily e, polypeptide 1, mRNA (cDNA clone MGC:18528 IMAGE:4223797)	NM_021282	2.47	Down
Kcnq5	Voltage-gated potassium channel KCNQ5 (Kcnq5)	NM_023872	2.46	Down
Figf	C-fos induced growth factor (Figf), mRNA	NM_010216	2.43	Down

Podn	Podocan, mRNA (cDNA clone MGC:106502 IMAGE:30609151)	NM_172874	2.36	Up
Phex	Phosphate regulating gene with homologies to endopeptidases on the X chromosome (hypophosphatemia, vitamin D resistant ricket	NM_011077	2.35	Down
Cnn1	Calponin 1 (Cnn1), mRNA	NM_009922	2.33	Up
Dclk1	Doublecortin-like kinase 1, mRNA (cDNA clone IMAGE:5006471)	NM_019978	2.25	Up
Gprc6a	G protein-coupled receptor, family C, group 6, member A (Gprc6a), mRNA	NM_153071	2.22	Down
Tpm2	Tropomyosin 2, beta, mRNA (cDNA clone MGC:35840 IMAGE:4990394)	NM_009416	2.21	Up
Scube2	Signal peptide, CUB domain, EGF-like 2 (Scube2), mRNA	NM_020052	2.21	Down
Dpep1	Dipeptidase 1 (renal), mRNA (cDNA clone MGC:6318 IMAGE:2812088)	NM_007876	2.2	Down
Amph	Amphiphysin, mRNA (cDNA clone IMAGE:5357091)	NM_175007	2.15	Down
Apoc1	Apolipoprotein C-I, mRNA (cDNA clone MGC:30241 IMAGE:5124986)	NM_007469	2.15	Down
Rcan2	Calcineurin inhibitory protein ZAKI-4	NM_030598	2.14	Up
Il16	Interleukin 16, mRNA (cDNA clone IMAGE:4167410)	NM_010551	2.13	Down
Chi3l3	Chitinase 3-like 3 (Chi3l3), mRNA	NM_009892	2.1	Down
Tagln	Transgelin, mRNA (cDNA clone MGC:6045 IMAGE:3600413)	NM_011526	2.1	Up
Wisp1	WNT1 inducible signaling pathway protein 1 (Wisp1), mRNA	NM_018865	2.1	Up
Lgr5	Leucine rich repeat containing G protein coupled receptor 5 (Lgr5), mRNA	NM_010195	2.07	Up
Slit2	SLIT2 (Slit2)	NM_178804	2.06	Down
Col12a1	Collagen, type XII, alpha 1 (Col12a1), mRNA	NM_007730	2.05	Up
Stfa2	Stefin A2, mRNA (cDNA clone MGC:169628 IMAGE:8861023)	NM_001082545	2.04	Down
Bex4	Brain expressed gene 4, mRNA (cDNA clone MGC:116687 IMAGE:30677421)	NM_212457	2.04	Down
Nebl	Nebulette-like mRNA, partial sequence	BC119802	2.04	Down
Sync	Syncoilin (Sync), mRNA	NM_023485	2.03	Up
Scel	Sciellin (Scel), mRNA	NM_022886	2.01	Down
Cfh	Complement component factor h (Cfh), mRNA	NM_009888	2.01	Down
Nrxn1	Neurexin I, mRNA (cDNA clone IMAGE:30544573)	NM_020252	2.01	Up
C1qtnf2	C1q and tumor necrosis factor related protein 2, mRNA (cDNA clone MGC:40933 IMAGE:5375090)	NM_026979	2.01	Up
Hist1h2ab	Histone cluster 1, H2ab, mRNA (cDNA clone MGC:151487 IMAGE:40126429)	NM_175660	2	Up
Slitrk6	SLIT and NTRK-like family, member 6, mRNA (cDNA clone MGC:170290 IMAGE:8861685)	NM_175499	2	Up
Aoc3	Amine oxidase, copper containing 3 (Aoc3), mRNA	NM_009675	2	Up
Pole	Polymerase (DNA directed), epsilon, mRNA (cDNA clone IMAGE:3496540)	NM_011132	1.99	Up
Nrcam	Neuron-glia-CAM-related cell adhesion molecule (Nrcam), transcript variant 2, mRNA	NM_176930	1.99	Down
Tk1	Thymidine kinase 1, mRNA (cDNA clone MGC:11539 IMAGE:3969287)	NM_009387	1.99	Up
Mctp2	Multiple C2 domains, transmembrane 2 (Mctp2), mRNA	NM_001024703	1.98	Down
Itga8	Integrin alpha8	NM_001001309	1.97	Down
Sfrp2	Secreted frizzled-related protein 2, mRNA (cDNA clone MGC:25299 IMAGE:4487469)	NM_009144	1.96	Up
Stfa1	Stefin A1 (Stfa1), mRNA	NM_001082543	1.96	Down
Atp6v0a4	Vacuolar proton translocating ATPase a4 isoform	NM_080467	1.96	Down
Slc7a10	Solute carrier family 7 (cationic amino acid transporter, y+ system), member 10 (Slc7a10), mRNA	NM_017394	1.95	Down
Ifi205	(strain C57Bl/6) mRNA sequence	NM_172648	1.95	Down
Eif2s3y	Eukaryotic translation initiation factor 2, subunit 3, structural gene Y-linked (Eif2s3y), mRNA	NM_012011	1.95	Up

Dlk1	Delta-like 1 homolog (Drosophila) (Dlk1), mRNA	NM_010052	1.94	Down
Tnc	Tenascin C (Tnc), mRNA	NM_011607	1.94	Up
Adamts15	A disintegrin-like and metallopeptidase (reprolysin type) with thrombospondin type 1 motif, 15, mRNA (cDNA clone IMAGE:349199	NM_001024139	1.93	Down
Ltbp2	Latent transforming growth factor beta binding protein 2 (Ltbp2), mRNA	NM_013589	1.92	Up
Rsad2	Viperin (Vig1)	NM_021384	1.92	Up
Myl9	Myosin, light polypeptide 9, regulatory (Myl9), mRNA	NM_172118	1.91	Up
1190002H 23Rik	Response gene to complement 32	NM_025427	1.91	Down
Adamts2	A disintegrin-like and metallopeptidase (reprolysin type) with thrombospondin type 1 motif, 2, mRNA (cDNA clone IMAGE:4004191	NM_175643	1.91	Up
Bex4	Brain expressed gene 4, mRNA (cDNA clone MGC:116687 IMAGE:30677421)	NM_212457	1.91	Down
Slc6a17	Solute carrier family 6 (neurotransmitter transporter), member 17, mRNA (cDNA clone MGC:198667 IMAGE:9054248)	NM_172271	1.9	Up
Enpp2	Ectonucleotide pyrophosphatase/phosphodiesterase 2, mRNA (cDNA clone MGC:6665 IMAGE:3499038)	NM_015744	1.88	Down
Kdm5d	Smcy (Smcy)	NM_011419	1.88	Up
Serpina1b	Serine (or cysteine) preptidase inhibitor, clade A, member 1B, mRNA (cDNA clone MGC:18593 IMAGE:4194027)	NM_009244	1.88	Up
Uty	Ubiquitously transcribed tetratricopeptide repeat gene, Y chromosome (Uty), mRNA	NM_009484	1.87	Up
Ddx3y	DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, Y-linked, mRNA (cDNA clone MGC:29067 IMAGE:5043470)	NM_012008	1.86	Up
Mmp8	Matrix metallopeptidase 8 (Mmp8), mRNA	NM_008611	1.85	Down
Gria1	Glutamate receptor, ionotropic, AMPA1 (alpha 1) (Gria1), transcript variant 2, mRNA	NM_001113325	1.85	Down
Bmp7	Bone morphogenetic protein 7, mRNA (cDNA clone MGC:18610 IMAGE:4218495)	NM_007557	1.84	Up
Upk1b	Uroplakin Ib	NM_178924	1.84	Down
Acss3	Acyl-CoA synthetase short-chain family member 3 (Acss3), transcript variant 1, mRNA	NM_001142804	1.83	Down
Xdh	Similar to hypothetical protein MGC37588, mRNA (cDNA clone MGC:28125 IMAGE:3980327)	NM_011723	1.83	Down
Slc38a5	Solute carrier family 38, member 5, mRNA (cDNA clone MGC:173142 IMAGE:40057282)	NM_172479	1.83	Down
Pcdh7	Protocadherin 7 (Pcdh7), transcript variant 2, mRNA	NM_018764	1.83	Up
Prim1	DNA primase, p49 subunit (Prim1), mRNA	NM_008921	1.82	Up
Thbs2	Thrombospondin 2, mRNA (cDNA clone IMAGE:3583417)	NM_011581	1.82	Up
Sgcd	Sarcoglycan, delta (dystrophin-associated glycoprotein) (Sgcd), mRNA	NM_011891	1.82	Up
Cox4i2	Cytochrome c oxidase subunit IV isoform 2 (Cox4i2), nuclear gene encoding mitochondrial protein, mRNA	NM_053091	1.82	Down
Tcf19	Transcription factor 19 (Tcf19), mRNA	NM_025674	1.82	Up
Cldn1	Claudin 1, mRNA (cDNA clone MGC:5767 IMAGE:3491319)	NM_016674	1.81	Down
P4ha3	Procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), alpha polypeptide III, mRNA (cDNA clone MGC:99449	NM_177161	1.81	Up
Fam162b	Family with sequence similarity 162, member B (Fam162b), mRNA	BC147085	1.81	Down
Loxl2	Lysyl oxidase-like protein 2	NM_033325	1.81	Up
Nebl	Nebulette (Nebl), mRNA	Nebl	1.81	Down
Serpina3c	Serine (or cysteine) peptidase inhibitor, clade A, member 3C (Serpina3c), mRNA	NM_008458	1.8	Down
Olfr172	Olfactory receptor 172 (Olfr172), mRNA	NM_147001	1.8	Up

Table S3. Postnatal day 3 (P3) microarray gene expression analysis. Gene ID, gene name, ratio of gene expression in P3 *Pbx1/2* CKO lungs compared to P3 controls, and direction of change. Results limited to fold change ≥ 1.8.

Gene ID	Gene Name	Gene Identifier	Ratio	Direction
Inmt	Indolethylamine N-methyltransferase, mRNA (cDNA clone MGC:19191 IMAGE:4236077)	NM_009349	13.85	Down
Ucp1	Uncoupling protein 1 (mitochondrial, proton carrier), mRNA (cDNA clone MGC:13965 IMAGE:4191276)	NM_009463	8.21	Up
Reg3g	Regenerating islet-derived 3 gamma (Reg3g), mRNA	NM_011260	6.73	Up
Scgb3a1	UGRP2 type B	NM_170727	5.88	Up
Rgs5	Regulator of G-protein signaling 5, mRNA (cDNA clone MGC:46953 IMAGE:3962083)	Rgs5	4.72	Up
Fabp4	Fatty acid binding protein 4, adipocyte (Fabp4), mRNA	NM_024406	4.69	Up
Slc27a6	Solute carrier family 27 (fatty acid transporter), member 6 (Slc27a6), mRNA	NM_001081072	4.36	Down
Cpa1	Carboxypeptidase A1 (Cpa1), mRNA	NM_025350	4.3	Down
Vsnl1	Visinin-like protein 1	NM_012038	4.25	Down
Adcy8	Adenylate cyclase 8 (Adcy8), mRNA	NM_009623	3.92	Down
Col8a1	Collagen, type VIII, alpha 1, mRNA (cDNA clone MGC:18687 IMAGE:3674027)	NM_007739	3.85	Up
Rgs5	Regulator of G-protein signaling 5, mRNA (cDNA clone MGC:46953 IMAGE:3962083)	NM_009063	3.73	Up
Ctse	Cathepsin E, mRNA (cDNA clone MGC:5924 IMAGE:3601519)	NM_007799	3.48	Down
Phex	Phosphate regulating gene with homologies to endopeptidases on the X chromosome (hypophosphatemia, vitamin D resistant ricket	NM_011077	3.41	Down
Slc36a2	Solute carrier family 36 (proton/amino acid symporter), member 2 (Slc36a2), mRNA	NM_153170	3.36	Down
Serpina1b	Serine (or cysteine) preptidase inhibitor, clade A, member 1B, mRNA (cDNA clone MGC:18593 IMAGE:4194027)	NM_009244	3.21	Up
Nrxn1	Neurexin I, mRNA (cDNA clone IMAGE:30544573)	NM_020252	2.91	Up
Fhl5	Four and a half LIM domains 5 (Fhl5), mRNA	NM_021318	2.9	Up
Enpep	Glutamyl aminopeptidase (Enpep), mRNA	NM_007934	2.85	Down
Slc7a10	Solute carrier family 7 (cationic amino acid transporter, y+ system), member 10 (Slc7a10), mRNA	NM_017394	2.79	Down
Cox8b	Cytochrome c oxidase, subunit VIIIb, mRNA (cDNA clone MGC:41187 IMAGE:1515187)	NM_007751	2.74	Up
Pcdh9	Protocadherin 9, mRNA (cDNA clone MGC:190003 IMAGE:9088190)	NM_001081377	2.67	Up
Pcdh9	Protocadherin 9, mRNA (cDNA clone MGC:190003 IMAGE:9088190)	NM_001081377	2.61	Up
Unc5c	Unc-5 homolog C (C. elegans) (Unc5c), mRNA	NM_009472	2.57	Down
Fam171b	Family with sequence similarity 171, member B, mRNA (cDNA clone IMAGE:4501762)	NM_175514	2.55	Up
Cidea	Cell death-inducing DNA fragmentation factor, alpha subunit-like effector A (Cidea), mRNA	NM_007702	2.54	Up
Hsd3b2	Hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 2 (Hsd3b2), mRNA	NM_153193	2.47	Down
Ppp4r4	Protein phosphatase 4, regulatory subunit 4, mRNA (cDNA clone MGC:132871 IMAGE:40060480)	NM_028980	2.4	Down
Adipoq	Adiponectin, C1Q and collagen domain containing, mRNA (cDNA clone MGC:41360 IMAGE:1329799)	NM_009605	2.38	Up
Mfap5	Microfibrillar associated protein 5, mRNA (cDNA clone MGC:35969 IMAGE:3982519)	NM_015776	2.38	Up
Slit2	SLIT2 (Slit2)	NM_178804	2.37	Down
Csmd3	CUB and Sushi multiple domains 3 (Csmd3), mRNA	NM_001081391	2.36	Up

Kcnq5	Voltage-gated potassium channel KCNQ5 (Kcnq5)	NM_023872	2.35	Down
St6gal2	Beta galactoside alpha 2,6 sialyltransferase 2, mRNA (cDNA clone MGC:169766 IMAGE:8861161)	NM_172829	2.34	Up
Csmd3	CUB and Sushi multiple domains 3 (Csmd3), mRNA	NM_001081391	2.34	Up
Cnn1	Calponin 1 (Cnn1), mRNA	NM_009922	2.31	Up
Car3	Carbonic anhydrase 3, mRNA (cDNA clone MGC:18583 IMAGE:4195712)	NM_007606	2.3	Up
Slc38a11	Solute carrier family 38, member 11 (Slc38a11), mRNA	NM_177074	2.29	Up
Amph	Amphiphysin, mRNA (cDNA clone IMAGE:5357091)	NM_175007	2.29	Down
Fam162b	Family with sequence similarity 162, member B (Fam162b), mRNA	BC147085	2.26	Down
Col12a1	Collagen, type XII, alpha 1 (Col12a1), mRNA	NM_007730	2.25	Up
Rsad2	Viperin (Vig1)	NM_021384	2.25	Down
Serpina1e	Serine (or cysteine) peptidase inhibitor, clade A, member 1E (Serpina1e), mRNA	NM_009247	2.24	Up
Kcnmb1	Potassium large conductance calcium-activated channel, subfamily M, beta member 1, mRNA (cDNA clone MGC:13890 IMAGE:3988005)	NM_031169	2.24	Up
A2m	Alpha-2-macroglobulin, mRNA (cDNA clone IMAGE:5346024)	NM_175628	2.24	Down
Cacna1d	L-type voltage-gated calcium channel Cav1.3(1a) subunit (cacna1d gene)	NM_028981	2.23	Down
Kcnj5	Potassium inwardly-rectifying channel, subfamily J, member 5 (Kcnj5), mRNA	NM_010605	2.22	Up
Figf	C-fos induced growth factor (Figf), mRNA	NM_010216	2.21	Down
Sstr2	Somatostatin receptor 2 (Sstr2), transcript variant 2, mRNA	NM_009217	2.18	Down
Nkg7	Natural killer cell group 7 sequence (Nkg7), mRNA	NM_024253	2.17	Down
Cox4i2	Cytochrome c oxidase subunit IV isoform 2 (Cox4i2), nuclear gene encoding mitochondrial protein, mRNA	NM_053091	2.16	Down
Matn2	Matrilin 2, mRNA (cDNA clone MGC:5875 IMAGE:3492881)	NM_016762	2.15	Up
Adm	Adrenomedullin (Adm), mRNA	NM_009627	2.15	Up
Lrrc15	Leucine rich repeat containing 15 (Lrrc15), mRNA	NM_028973	2.12	Up
Retnla	Resistin like alpha, mRNA (cDNA clone MGC:35890 IMAGE:4189285)	NM_020509	2.11	Up
Nebl	Nebulette (Nebl), mRNA	Nebl	2.11	Down
Adamdec1	ADAM-like, decysin 1 (Adamdec1), mRNA	NM_021475	2.1	Up
Spp1	Osteopontin (OPN)	NM_009263	2.08	Up
Wisp1	WNT1 inducible signaling pathway protein 1 (Wisp1), mRNA	NM_018865	2.08	Up
Cox7a1	Cytochrome c oxidase, subunit VIIa 1 (Cox7a1), mRNA	NM_009944	2.07	Up
Pcdh20	Protocadherin 20, mRNA (cDNA clone IMAGE:3986792)	NM_178685	2.05	Up
Prelp	Proline arginine-rich end leucine-rich repeat, mRNA (cDNA clone MGC:25825 IMAGE:4165772)	NM_054077	2.02	Up
Hs6st3	Heparan sulfate 6-O-sulfotransferase 3 (Hs6st3), mRNA	NM_015820	1.99	Down
Ncam1	Neural cell adhesion molecule 1 (Ncam1), transcript variant 2, mRNA	NM_001081445	1.99	Up
Ctsk	Cathepsin K (Ctsk), mRNA	NM_007802	1.98	Up
C1qtnf3	C1q and tumor necrosis factor related protein 3, mRNA (cDNA clone IMAGE:3989958)	NM_030888	1.97	Up
Opcml	Opioid binding protein/cell adhesion molecule-like, mRNA (cDNA clone MGC:99974 IMAGE:30542711)	NM_177906	1.96	Down
Emr4	EGF-like module containing, mucin-like, hormone receptor-like sequence 4 (Emr4), mRNA	NM_139138	1.93	Down
Sfrp2	Secreted frizzled-related protein 2, mRNA (cDNA clone MGC:25299 IMAGE:4487469)	NM_009144	1.92	Up
Ppp1r3c	Protein phosphatase 1 binding protein PTG	NM_016854	1.91	Down
Pde1a	Phosphodiesterase 1A, calmodulin-dependent (Pde1a), mRNA	NM_016744	1.91	Up
Cldn1	Claudin 1, mRNA (cDNA clone MGC:5767 IMAGE:3491319)	NM_016674	1.9	Down
Cass4	Cas scaffolding protein family member 4 (Cass4), transcript	NM_001033538	1.9	Down

	variant 2, mRNA			
Scube2	Signal peptide, CUB domain, EGF-like 2 (Scube2), mRNA	NM_020052	1.89	Down
Lgr5	Leucine rich repeat containing G protein coupled receptor 5 (Lgr5), mRNA	NM_010195	1.89	Up
Rspo2	R-spondin 2 homolog (Xenopus laevis), mRNA (cDNA clone IMAGE:30054159)	NM_172815	1.89	Down
Itih5	Inter-alpha (globulin) inhibitor H5, mRNA (cDNA clone MGC:70205 IMAGE:3490706)	NM_172471	1.89	Up
Cdo1	Cysteine dioxygenase 1, cytosolic, mRNA (cDNA clone MGC:18800 IMAGE:4194939)	NM_033037	1.88	Down
Olfr50	Olfactory receptor 50, mRNA (cDNA clone MGC:157535 IMAGE:40135208)	NM_146946	1.87	Down
Crabp1	Cellular retinoic acid binding protein I (Crabp1), mRNA	NM_013496	1.87	Down
Slc38a5	Solute carrier family 38, member 5, mRNA (cDNA clone MGC:173142 IMAGE:40057282)	NM_172479	1.87	Down
Slc4a1	Solute carrier family 4 (anion exchanger), member 1 (Slc4a1), mRNA	NM_011403	1.86	Down
Lass3	Longevity assurance 3-like protein variant 2 (Lass3)	DQ646881	1.86	Up
Adamts2	A disintegrin-like and metallopeptidase (reprolysin type) with thrombospondin type 1 motif, 2, mRNA (cDNA clone IMAGE:4004191	NM_175643	1.85	Up
Pgf	Placental growth factor, mRNA (cDNA clone MGC:11485 IMAGE:2649577)	NM_008827	1.85	Up
Spef2	Sperm flagellar 2, mRNA (cDNA clone MGC:163919 IMAGE:40130565)	NM_177123	1.85	Up
Saa3	Serum amyloid A 3 (Saa3), mRNA	NM_011315	1.84	Up
Ablim3	Actin binding LIM protein family, member 3, mRNA (cDNA clone MGC:106450 IMAGE:30534236)	NM_198649	1.84	Down
Nrcam	Neuron-glia-CAM-related cell adhesion molecule (Nrcam), transcript variant 2, mRNA	NM_176930	1.84	Down
Adh1	Alcohol dehydrogenase 1 (class I), mRNA (cDNA clone MGC:18885 IMAGE:4238555)	NM_007409	1.84	Down
Neto1	Neuropilin (NRP) and tolloid (TLL)-like 1 (Neto1), mRNA	NM_144946	1.83	Up
Camk1g	Calcium/calmodulin-dependent protein kinase I gamma (Camk1g), mRNA	NM_144817	1.82	Down
Csmd3	CUB and Sushi multiple domains 3 (Csmd3), mRNA	NM_001081391	1.82	Up
Nebl	Nebulette (Nebl), mRNA	NM_028757	1.81	Down
Fst	Follistatin (Fst), mRNA	NM_008046	1.8	Up

Table S4. Quantitative real-time PCR primers.

β-actin	5’-CGGCCAGGTCATCACTATTGGCAAC-3’ 5’-GCCACAGGATTCCATACCCAAGAAG-3'
Adcy8	5’-AGGATTTACCAACCTCTCTACGAC-3’ 5’-GGTGCGAGTAACGACACAACTTTA-3'
Kcnmb1	5’-TGCCTTTGGGTCAATGTATCAG-3’ 5’-GACGTGGAGTTCAGTTGCTCTG-3’
Actg2	5’-ATGGGCCAGAAAGACAGCTA-3’ 5’-GAGTGAGAGCACAGCCTGAA-3’
Rgs5	5’-TTGGGAATTCTCCTCCAGAA-3’ 5’-AGAAGCTTGTCCAGGGACTG-3’
Cacna1d	5’-TCGTGGAGATTTCCAGAAGC-3’ 5’-TCGAAAGCAGCAAAACTTCC-3’
Nppc	5’-ATGCACCTCTCCCAGCTGAT-3’ 5’-ATCGGTCTCCCTTGAGATTG-3’
Acta2	5’-AGACAGCTATGTGGGGGATG-3’ 5’-GTTGGCCTTAGGGTTCAGTG-3’
Pde5a	5’-GCAAGATGCAAACAAAATCAA-3’ 5’-TGCAAAGCAAACTTCCAATG-3’
Ptgis	5’-GGCTGGACTTCCATCCCTAT-3’ 5’-ACTGCCTGCTTCTGTGGAGT-3’
eNOS	5’-GTGTGAAGGCAACCATTCTG-3’ 5’-ATTGCCAAATGTGCTGGTC-3’
Edn1	5’-AGTGTGTCTACTTCTGCCACCTG-3’ 5’-TGCCTGGTCTGTGGCCTTATTG-3’
Agt	5’-TGCGGAGGCAAATCTGAACAAC-3’ 5’-AAAGTGCAGCGTGCCTGAG-3’