Supplemental Inventory:

Figure S1. This figure supports Figure 1 and shows marker localization in E14.5 skin and sorted cell populations by immunofluorescence and qRT-PCR.

Figure S2. This figure supports Figure 2 and shows consistent gene expression patterns are maintained after cDNA amplification, prior to library manufacture. It also shows quality metrics of the RNA-sequencing.

Figure S3. This figure supports Figure 5 and shows additional new signature genes identified for all isolated cell types, verified by qRT-PCR analysis and immunofluorescence staining.

Figure S4. This figure supports Figure 6 and shows additional heat maps of select signaling pathways for which factors are expressed in heterogeneous cell types.

Supplemental table legends.

Supplemental experimental procedures. These procedures include information on RNA-sequencing and computational analyses. Primers are listed for qRT-PCR.

Figure S1

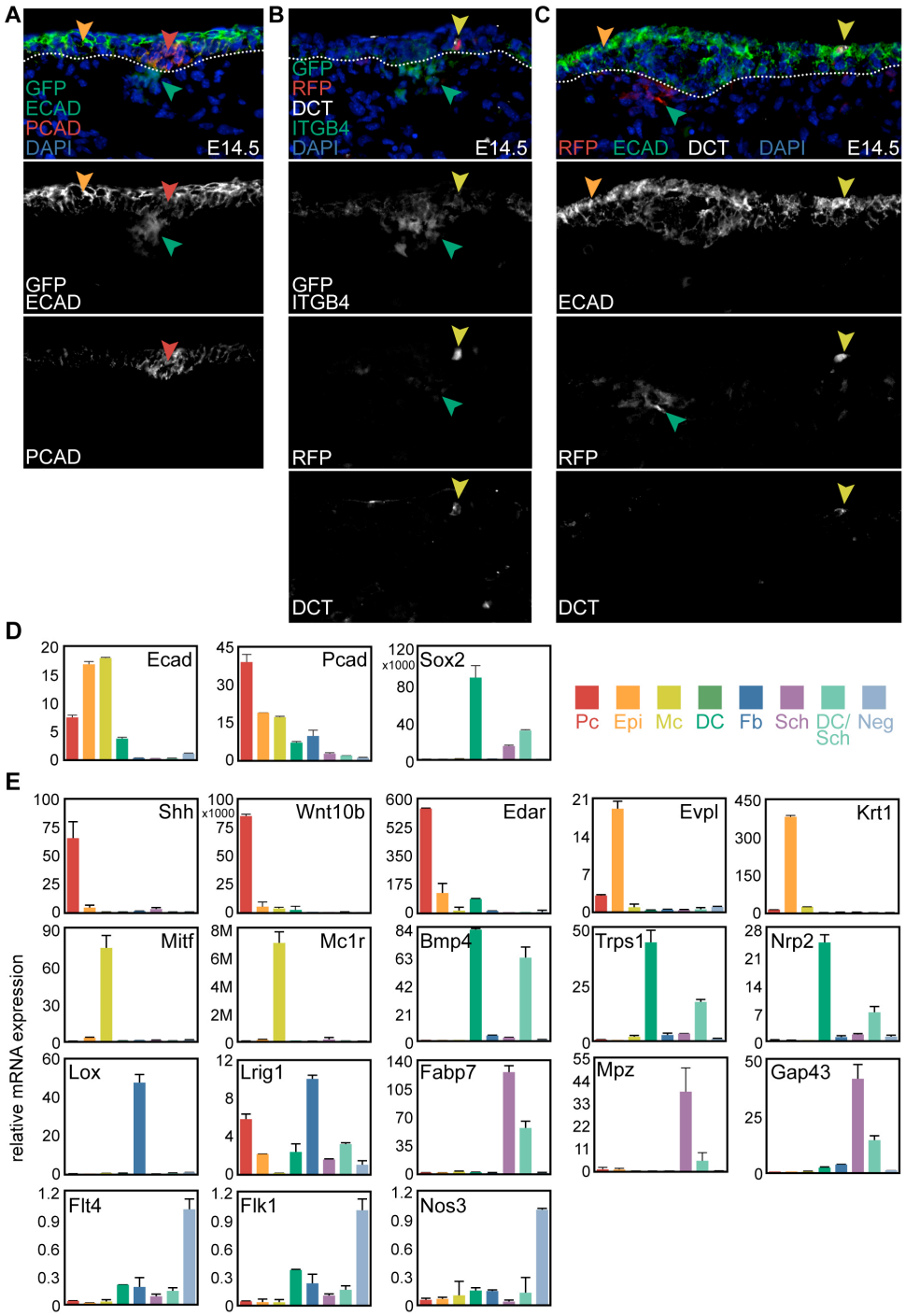

Figure S1. Supports Figure 1 data. Reporter/antibody labeling of embryonic skin and marker gene verification in target cell types. Color key indicates samples associated with each arrowhead/bar.
(A) Co-staining of ECAD and PCAD on Sox2^{GFP} skin. Note ECAD and GFP are both depicted in the green channel.
(B) Co-staining of ITGB4, which marks the basement membrane and DCT, which marks melanocytes, on Sox2^{GFP}/Lef1-RFP skin. Note ITGB4 and GFP are both depicted in the green channel. One melanocyte is seen sitting in the epidermal compartment.
(C) Co-staining of ECAD and DCT on Lef1-RFP skin. Note ECAD^{+}RFP^{+}DCT^{+} melanocyte.
(D) Markers used to isolate cells were confirmed enriched in the appropriate sorted cell types by qRT-PCR analysis. Data are mean ± SD from 2 measurements.
(E) Confirmed enrichment of additional known markers for each sorted cell type by qRT-PCR analysis.

Figure S2

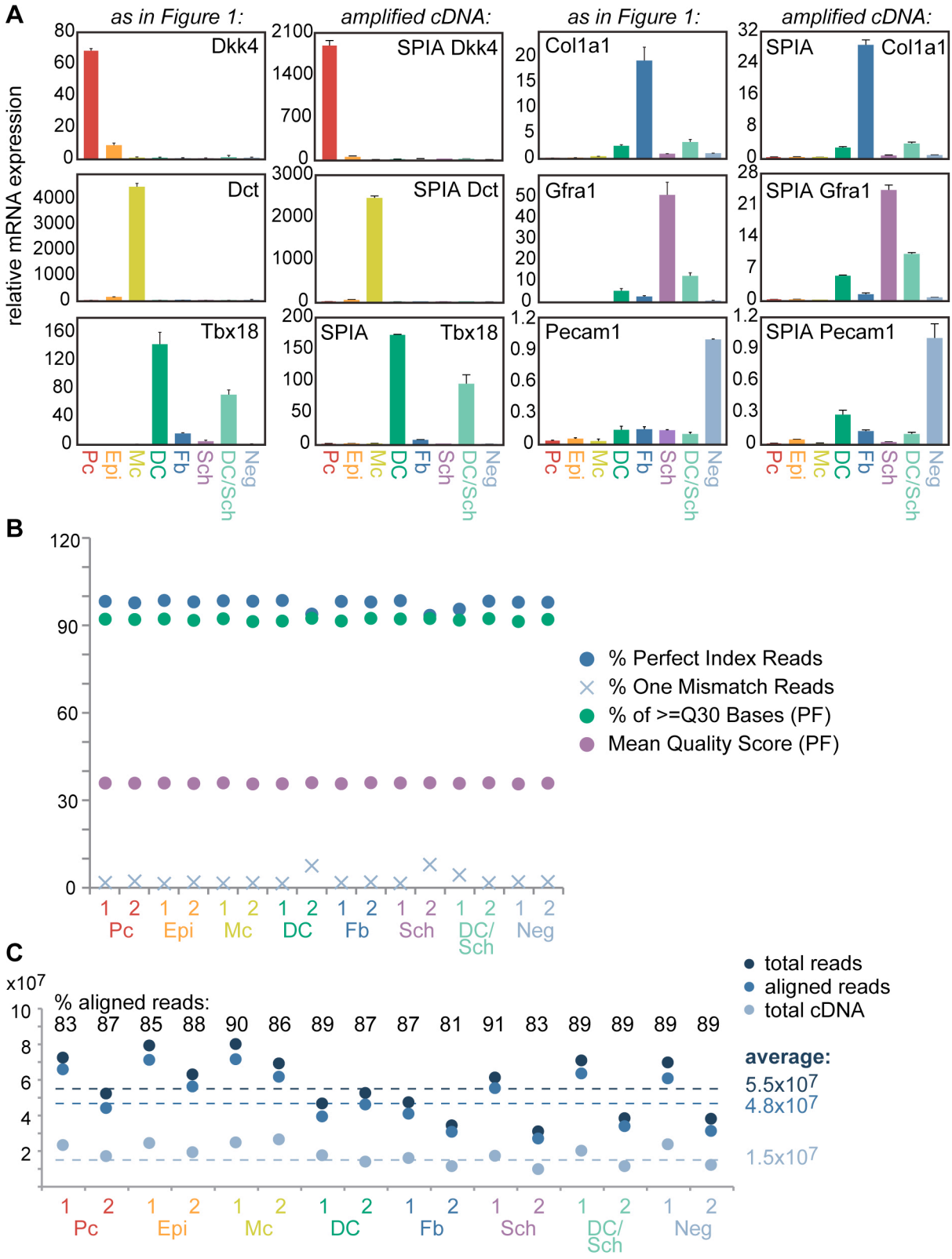

Figure S2. Supports Figure 2 data. Validation of RNA-sequencing methods and results.
(A) qRT-PCR on NuGEN-amplified cDNA (SPIA samples) reveals gene expression patterns mirror those found with qRT-PCR on conventional cDNA manufactured with Invitrogen SuperScript III.
(B) % Perfect Index Reads and Mean Quality Scores for each of the 16 samples after RNA-sequencing.
(C) Total reads, aligned reads, and total cDNA for each of the 16 samples after RNA-sequencing.

Figure S3

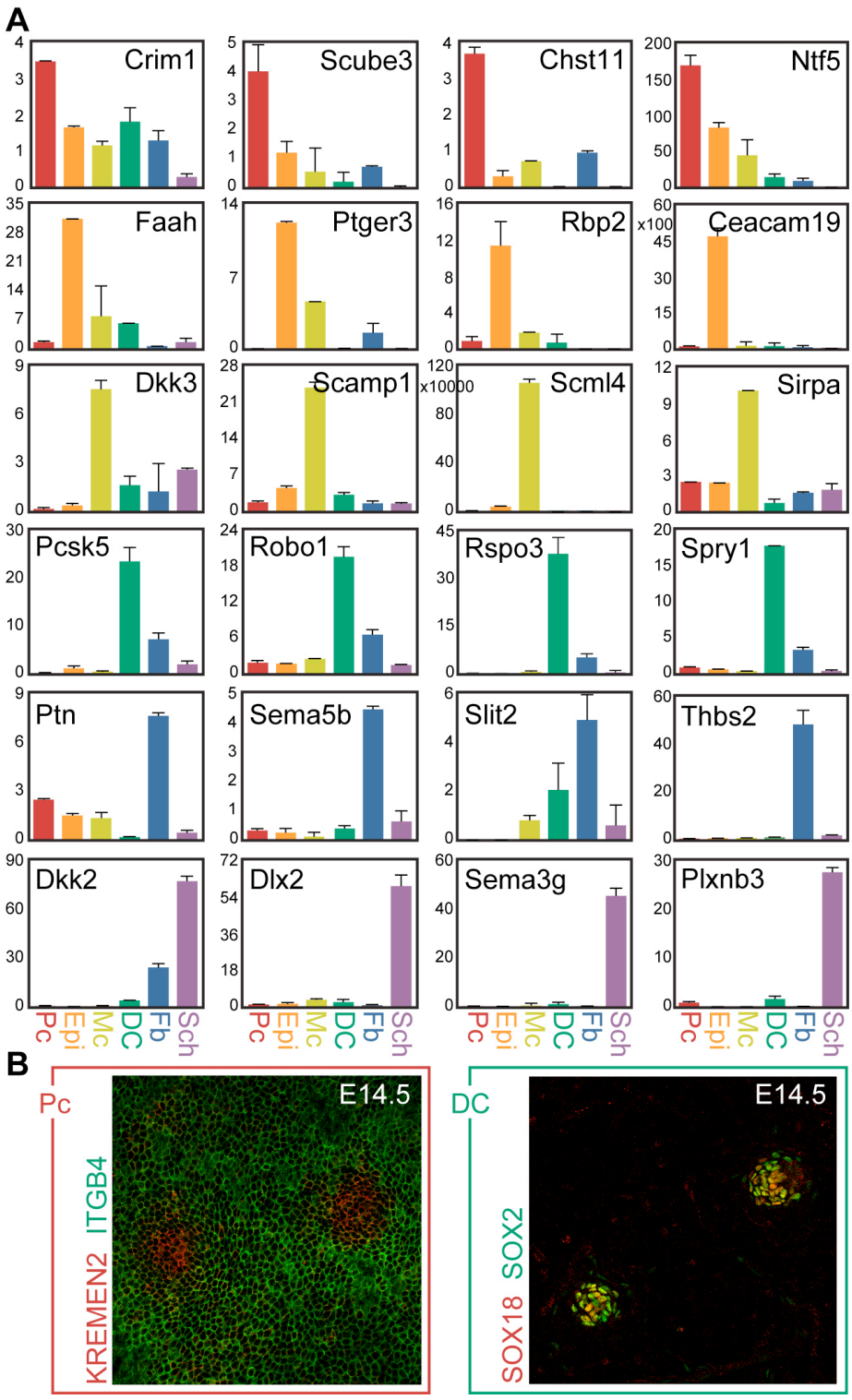

Figure S3. Supports Figure 5 data. qRT-PCR and immunofluorescence verification of signature genes.
(A) qRT-PCR verification of select signature genes for each cell type. Data are mean ± SD from 2 measurements.
(B) Immunofluorescence staining verification of selected signature genes for the Pc and DC. Whole-mount view of E14.5 skin.

Figure S4

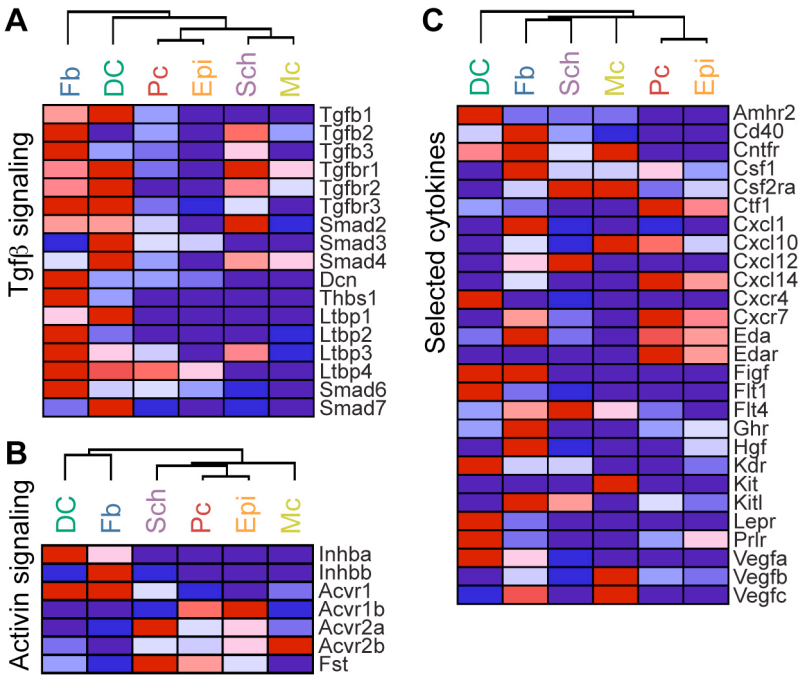

Figure S4. Supports Figure 6. Multiple signaling factors are specifically expressed by distinct cell types in embryonic skin.
Genes involved in Tgfβ (A), Activin (B) and cytokine (C) signaling were mined from the KEGG database and represented in a heat map if expressed (FPKM >1) in embryonic skin.

SUPPLEMENTAL TABLE LEGENDS

Supplemental tables available as .xls:

Table S1. Supports Figure 4. Signature gene lists.
Table S2. Supports Figure 4. Enriched genes in epithelial (Pc + Epi), dermal (DC + Fb), and neural crest (Sch + Mc) cells.
Table S3. Supports Figure 4. Results of Enrichr GO analysis.
Table S4. Supports Figure 4. Overlap between embryonic cell signatures and signatures of their adult cell counterparts.
Table S5. Supports Figure 6. Results of Enrichr KEGG analysis.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

RNA-sequencing analysis

All raw RNA sequencing reads were mapped to the mouse genome (mm10) with TopHat v2.0.3 (Trapnell et al., 2009) coupled with the Bowtie2 (Langmead and Salzberg, 2012) aligner with default parameters. Transcriptomes were assembled and fragments per kilo-base per million reads (FPKM) for each gene were computed with Cufflinks v2.1.1 (Trapnell et al., 2010) with default parameters. Differentially expressed genes (DEGs) were identified using Cuffdiff (with default parameters except for the library normalization method was upper quartile normalization, where FPKMs were scaled via the ratio of the 75 quartile fragment counts to the average 75 quartile value across all libraries) and ANOVA, and the Fisher exact test was used for enrichment analysis with the Benjamini-Hochberg correction for multiple hypotheses testing with FDR significance cut off $q<0.05$. Hierarchical clustering analyses for samples were performed with an FPKM matrix of either all detected genes or DEGs. The FPKM matrix was $\log_{10}$ transformed and standardized across each gene using z-scores so that the relative gene expression values across samples were 0 centered. Hierarchical clustering was performed for both genes and samples with Euclidean distance and average linkage functions. Principle component analyses (PCA) were performed for samples with the scikit-learn Python package and visualized in 3D plot using the Matplotlib Python package. Gene ontology enrichment analysis was carried out using Enrichr (Chen et al., 2013). Heatmaps were generated by integrating data from the KEGG pathways database (Kanehisa and Goto, 2000; Kanehisa et al., 2014) and with Genepattern (Reich et al., 2006). KEGG pathway enrichment analysis was performed with Enrichr (Chen et al., 2013).

Accession numbers

RNA-seq data have been deposited at the NCBI-GEO under the accession number GSE00000.

Primer list for qRT-PCR:

Gene	Forward primer (5' to 3')	Reverse primer (5' to 3')
Ascl4	CGTCGCTGTGCTAAAAAGGACA	AGACAAAAGGGCAAGAGAAACAGTG
Bmp4	TCCACTGGCTGATCACCTCAAC	AGTCCAGCTATAGGGAAGCAGTTTG
Bmp7	CCCAGCTCAACGCCATCTCT	TTCCGGTTGGGGAGGTGAG
Cd274	TAGGTTTTCTCCCCCATCCTTCT	CAAGTGAGGCGTCTGTGTTTGAG
Ceacam19	ATCACTTTCCCTCCAGACTCCAG	AGAAGCCCCATTTTACTCACAGC
Chst11	CAACCCCAAAGTCCCTTGTAGTAAA	GCCTTCGCTAGCACTGGAAAGT
Col1a1	GTGGGAGGGAACCAGATTG	GCAACAGTCGCTTCACCTAC
Crim1	AAGCCAGGGAGATGGAAAGC	AGTGTAGCCCCTGGAAATGC
Ctgf	TGGGGACAATGACATCTTTGAGTC	TTCCTCCCACGGTAGTTAAAAACAC
Dab1	GGTCCCAAGAACTAGACAGCAAGAA	GACCCCATAGCCACTGAAGTTGA
Dcc	GATGGCCTGTCTGGGTTTCA	CATGGGATGGAAGGTTGCCT
Dct	AGCAGCCAACGACCCTGTGT	CCTTTGCGAAGCCTTCTGTATTG
Dkk2	CTAAAACATGGGGAACGCTGAAG	CAACTTGGCCAGGACTACTGATTG
Dkk3	GGGGCTGTGCTAGCATTGATACT	CTGGGCATTCTCACAAGACTGC
Dkk4	CTAACGTGCCGAAGTCAGGTG	GGAGATTGGGCTTTATTTATGTGC
Dkkl1	CCCATGGACTTCCGAGACCTT	CGGGGGCTCTTTTGCTTCTAC
Dlk1	TGGCTGTGTCAATGGAGTCT	TTCTCCAGGTCCACGCAAGT
Dlx2	GGCCACTTTTAGGCCATCCT	TCACGGGGGTAGGTGATAGG
Dpt	GAATATGAGGGCCAGGAAAACCT	GTGCCATGGGAAAGGGAGAAT
Dsc1	CAGGAGGAAGAAGGGCTAGAGTTTT	GAGAATTTGGGGAGCTATGATTGG
Ecad	GCTGCCCCGAAA ATGAAAAG	AAGACCGGCTGGGTAAACTCTG
Edar	AGGCTGCCCTAAGTAGTTCATTGAG	ATAGGGCATGCCAGCAAACC
Enpp2	CGCCCTGATGTCCGTGTATCT	ACGGCTAGTCTTCCGGTAGAAATC
Ephb3	TACGGCTCAATGACGGACAGTT	TAGGTGGGGTCTGATGGGTCAT
Erbb3	GTTAGGGGGCGTTTACATTGAGA	ACTGAGGGGCACAGATGGTCTT
Evpl	GCTGCCGCAAAGATCCTCTAA	GAGCCCAACACCACAAACGAT
Faah	CTATAAAGCAGAAGTCCCTGGGTATG	TTGGTAAAGTGTCTGGCAAACAGTAG
Fabp7	TCATAACAGCGAACAGCAACGATATC	GGGAAACGTGACCAAACCAACT
Fgf10	ATTTCCCCCTGTATGCATCCTAAC	TTCCCACGGAGGCAGAACTC
Fgf20	ACGCCGCATGTCTCTGGATAA	AAAGTCCCATCTCAGTGTGGTGTG
Flk1	GTGGCGTTTCCTACTCCTAATGAGA	CACCCAGCAGAAACCCTGAGTT
Flt4	GAAGGGCGGACATGACACAAC	GCCCCTGAAGCTTTCCTTGAC
Fmn1	CTATTGGACCCAGATACCCTTTACCT	TTCCCCTCTTTGCCTGAGTGAT
Foxi3	AACTCCATCCGCCACAACCTAT	CTGCCCTTCTGATTTTGATGTCC
Gap43	ATGTGCCTGCTGCTGTCACTG	CTCGCCATAACAACACCAAGAAAC
Gapdh	CGTAGACAAAATGGTGAAGGTCGG	AAGCAGTTGGTGGTGCAGGATG
Gas7	TCCTGCCTGTATGCTGGTTCAC	GGTGGGGTGGAAAACACATTG
Gfra1	ACAGCGCTTCTGGCAGTTGATA	TGCTGGCCCTCTAGATCCATAAC
Grem1	CAGGGCTGTAGTTGGCTTTGTCATT	CTTACACCCGCGGTCAAGTGAAT
Grhl3	GATCGGTTCTGACGTTCACTGTTT	GTGCCGAGGAAGTCAATAAGAAAGT
Hhip	TCAGTAACGGCCCTTTGGTTG	TGGGCAGGTTGAACTGTGACTC
Irx1	TCTCGCAGATGGGCTCTCAGTAT	TTTGGTGGGGTACGGGTTCTT
Krt1	TTACTCCCGAGGGACCAAATAAAG	TGTTACCATGGGACTCAGACTGCT

Krtdap	CAACAGAGGGCCTTAACAATGAGTT	TCCATGCTTGCCTCCTCTTCTAC
L1cam	AGGCCACAGTTTGAGGGAAAAG	CAGGCTAGCCAGGGAGAAAGAA
Lox	TGAGGAAGGGCCAAACATCTAAC	CGTGGGATCGAATAGCAACAAG
Lrig1	ACGTGAGGCCTTCAATCAGC	AAGGGAACTAACTTGGCGAG
Mc1r	AGGGGAGGCTGTTGGCTTATC	CGGGACCGTGGTTTCCTAAAT
Mitf	CACTGGGGAGAAGTTGATGTTGATA	AGTGCTGCGGACCATACAGAAA
Mpz	AGATGGAGCTTCGCAAAGATGAG	ACCTAGACCCGGGAAAAAGAGG
Myo7a	ACTGGCCCCTGTCATAAGCACTA	TAGCAGGGACCCACCACATAACT
Ndnf	TGGTACCCAATGCCATGTGTAGA	CTTAGTGGGCCTGGGTCTCATT
Nos3	CCGGAAAGAGGGATTGTGTCA	GCCGGAGGAACCTTCAAGATT
Nrp2	GGAGGCATGACCGATTGTGTC	TGGCCTGTCTGTCCTGTCCAT
Ntf5	TCTCCCGGAGGAACTTGACATTA	GTGCCAGGCAACCAGAAACAG
Ovol1	ATGTGAACAGCCGCGTGTATGT	AACCAGTGGGGGTGGAGAAAA
Pcad	AGTGGGGAAGTCGATTCAAGAAAC	AGGCGGGAGACCTTTAGACATTC
Pcsk5	GGCCGAAAGTGGAAAGAAACC	GCACGGGAAGTCCTGACAATAAC
Pecam1	TGTTGCTGGGTCATTGGAGGTCA	TTGTCAAGCGAAGGATAGATAAGA
Plp1	GGACGGCGAAGTTGTAAGTGG	TGTTGTATGGCTCCTGGTGTTTG
Plxnb3	TAAGGTCCCAGATGGAGCAACA	GGGTGCCCTTCATTGAAAGTAAG
Ptger3	GACCATCAAAGCCCTGGTGT	CTTCAGGTTGTTCATCATCTGGCA
Pthlh	CCCAGCTTAAGGACGCATTGA	CAGTTTCCTGGGGAGACAGTTTG
Ptn	CTTGGGGAGAATGTGACCTCAATAC	GTGGCGTCTTTTAATCCAGCATC
Rbp2	TTCCGCAACTACGACCTGGATTTCA	TTTCCCGTGCCCATCACTTCTTTTT
Robo1	CTCGGAATTGCCAAGTGATGC	TTGTGGGGGTCAAGCCTGATA
Scamp1	TGTTACTGCAGCCATGAGACGAG	CACGCACCTACACAATACACAAACAT
Scml4	ATCAGGCAGTTCCCACATACAGAA	TTATTAGAGGCCTCCCCTGACTTG
Scube3	GACATGCATTGAAACCTGTGCT	TGTTTAAGCGGCACTCGTCT
Sema3g	TGGGTGTATGGGTGGCTCTA	GCCTCCACCTTCTCTCACAC
Sema5b	ACCCCTATGGAATTCAAGACACTGA	CTTACTGGCTAGGCAGCAAGTTCTT
Shh	ACGAGGATGGAGCCTGTAGTTTGT	GGGTGTGTGTGGCACGCTTTATTT
Sirpa	CCATGTTCTTGGGCTGTCTTCTAA	AGGGCGCTTCTGTTCTCTTCTTT
Slit2	CTATCTGCCACATGTCCTCACAAG	GACCCTGGGGTTTTACTGAAAGA
Sox2	TACTGGCAAGACCGTTTTCGTG	TATTGGAATCAGGCTGCCGAG
Spib	AGTCTTTTCCGTTCAAGTGCTACAG	GTTTTAAGGGCCTCTTCATCGTC
Spry1	TTCCCAGCCTTCTCCTTACAGC	CAGGGGCAAATCAGACAGGAAT
Tbx18	ATGGCCTCCAGAATGCGTATG	TGTCCCCCATCAAGCCTGTT
Thbs2	GCAGTGCGCCTTTACTTTATGGT	ATAGTCCCACGCTTCCTGTTTCA
Trps1	AGCCCAGGGTTCATTGACTAAAAG	AAGCCAGGCACATGACTCAAGTAG
Tyr	AGCGGGTAAGAGCACTGACTGTT	GCCCAAGAGCCAAAGGAATG
Tyrp1	CAAGGCCACCACAAAGTCACAG	CGCAGGCCTCTAAGATACGAGAA
Wnt10b	GAACAGCTCTGGGGGTGTAG	GTTCTGGGCTGTAGTGGAGG

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