Supplemental Materials and Methods

MEC isolation

Modifications to the MEC isolation procedure as described (Rudolph et al., 2009) were made for MEC isolation of transgenic mice. Specifically, after removal of lymph nodes, the MGs were removed, minced, and digested with 1 mg/mL collagenase type 1 (Worthington Biochemical Corporation, LS004196) in Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (HyClone, 11330-032), for 80 min in a 37°C rotor. Collagenase was then quenched with 0.5% fetal bovine serum, and the digested cell suspension was pelleted by centrifugation. Erythrocytes were then removed by successive washes of the cell pellet in Dulbecco's phosphate-buffered saline with calcium and magnesium (Hyclone SH30264.01) followed by 2-second centrifugations at 1500 rpm until pellet was no longer red. Cell pellets were then split into fractions for protein analysis, RNA isolation, and lipid extraction.

Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Quantification of miRNA was determined by calculating the relative copy number of each miRNA or the comparative threshold cycle (C_t) method. To estimate relative copy number, a standard curve was run using five four-fold dilutions of a P14 sample in duplicates. To calculate $2^{-\Delta\Delta Ct}$, the C_t values for RNAU6 were subtracted from C_t values of the miRNA to achieve the ΔC_t value. The ΔC_t of a control sample was then subtracted from each ΔC_t to achieve the relative miRNA levels ($\Delta\Delta C_t$). Fold change is calculated ($2^{-\Delta\Delta Ct}$). Statistical differences in miRNA expression between P14 and L2 samples were analysed by *t*-test.

Immunohistochemistry

Primary antibodies used were anti-FASN (Abcam ab22759, 1:8000, used for Fig. 2B and BD Biosciences 610962, Franklin Lakes, NJ, USA, 1:3,200, used for Fig. 6B), anti-MYB (Millipore 05-175, Billerica, MA, USA, 1:50), anti-adipophilin (Fitzgerald 20-AP002, Acton, MA, USA, 1:1600), anti-ACACA (Cell Signaling Technology 3676, Danvers, MA, USA, 1:1,600) and anti-CC3 (Cell Signaling Technology 9661, 1:1,600). Primary antibody detection was performed with either EnVision+ System-HRP labeled polymer secondary (Agilent Technologies K4001,K4003) or secondary antibody labeled with biotin (Dako/Agilent Technologies E0433, Carpinteria, CA,

1:500; Jackson Immuno Research, West Grove, PA, USA, 1:250) followed by incubation with streptavidin-HRP (Dako, 1:1000) and 3, 3′-diaminobenzidine (Dako) detection.

Immunoblot

The primary antibodies used were anti-pSTAT5 (Tyr694) (Cell Signaling Technology, 9351, 1:1000), anti-STAT5B (Millipore, 06-554, 1:1000), anti-STAT5A (Millipore, 06-968, 1:500), anti-whey acidic protein (WAP) (Santa Cruz, 398276, 1:300), anti-FASN (Cell Signaling Technology, 9363, 1:1000), anti-ACACA (Cell Signaling Technology, 3676, 1:1000), anti-OLAH (generous gift of Dr. Stuart Smith, Children's Hospital Oakland Research Institute, Oakland, CA, USA, 1:500), and anti-α-tubulin loading control (Sigma, T5168, 1:20,000). Secondary antibodies used were goat anti-mouse or rabbit Alexa Fluor 680® (Molecular probes, Grand Island, NY, USA)

Oligo primers for cloning of mouse 3′ UTR for Fasn

For *Fasn*-3′-UTR-WT: forward 5′-ATTGAGCTCACCTGCCGACCACCATGAAG-3′ and reverse 5′-CGGCTCGAGATTTCAGTCTTGTTCCACTCA-3′; For *Fasn*-3′-UTR-mut: forward 5′-CCCTAAAACTAGAGAAGCCATGTGGGGAAG-3′ and reverse 5′-CATGGCTTCTCTAGTTTTAGGGGGTTCTGG-3′.

Oligo primers for cloning of mouse 3′ UTR for Stat5b

For *Stat5b*-3′-UTR-WT site 1: forward 5′-GTAAATTATTTATTGGGAGATGAGTTTTTAAAAGCTGCTG-3′ and reverse 5′-CTCATCTCCCAATAAATAATTTACTACACAGGAGTTTG-3′; For *Stat5b*-3′-UTR-mut site 1: forward 5′-GTAAATTATTTACGAGGCGATGAGTTTTTAAAAGCTGCTG-3′ and reverse 5′-CTCATCGCCTCGTAAATAATTTACTACACAGGAGTTTG-3′; For *Stat5b*-3′-UTR-WT site 2: forward 5′-GTACCTGGACATGGGAGAGGTTTTTAACTGGAAAGTG-3′ and reverse 5′-GTTAAAAACCTCTCCCATGTCCAGGTACACCCTCAG-3′; For *Stat5b*-3′-UTR-mut site 2: forward 5′-GTACCTGGACACTAGACAGGTTTTTAACTGGAAAGTG-3′ and reverse 5′-GTTAAAAACCTGTCTAGTGTCCAGGTACACCCTCAG-3′.Detailed methods for generating *Fasn*-3′-UTR-mut and *Stat5b*-3′-UTR-mut plasmids are available upon request.

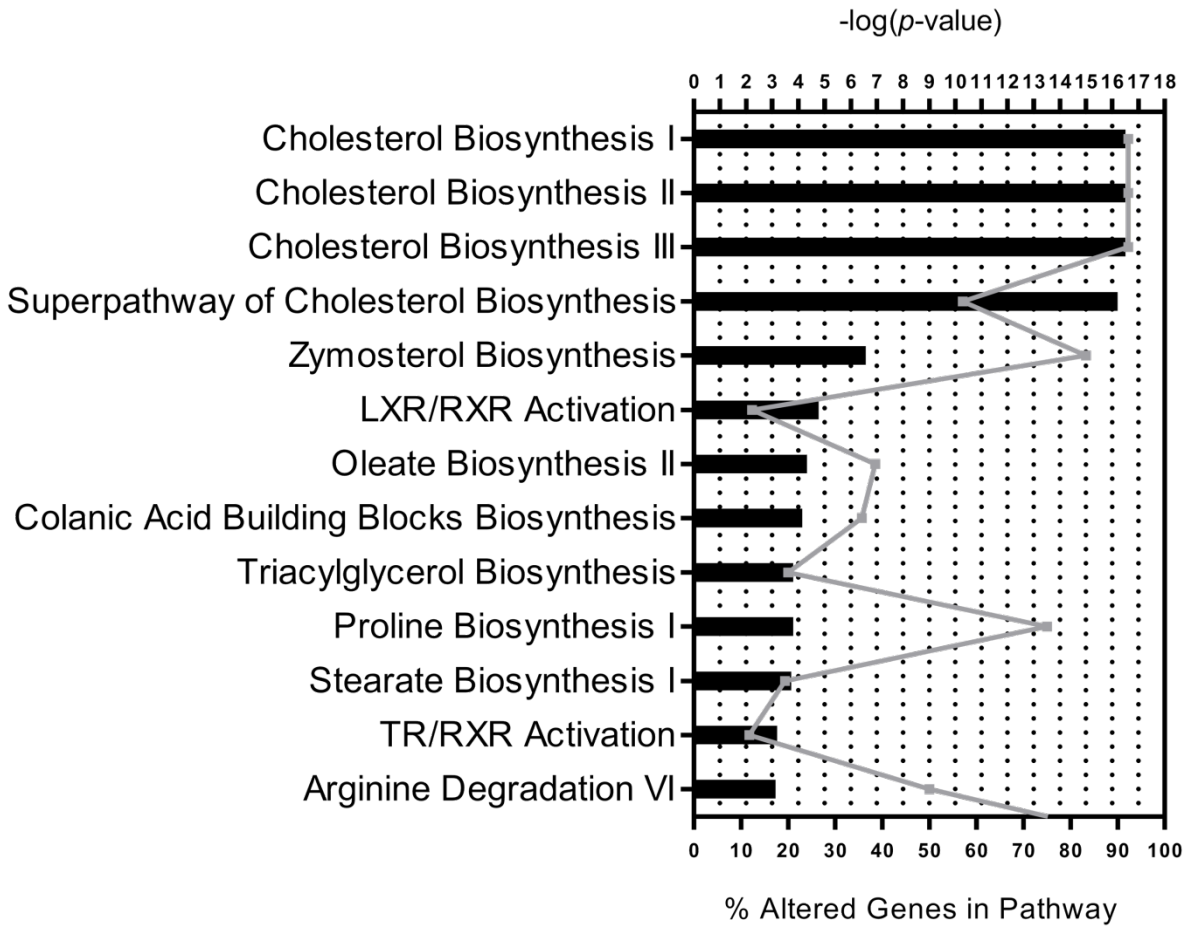
Total lipid extraction

Briefly, 7-20 mg of MEC pellet was suspended in 250 µL of 100 mM potassium phosphate buffer pH 7.2, mixed by inversion, acidified with 20 µL of 1 M HCl, 500 µL of 100% methanol was added, and samples were homogenized by rotor/stator. Lysates were cleared by centrifugation and supernatant transferred to glass tubes. Total lipids were extracted with 2 × 1 mL of isooctane/ethyl acetate 3:1 v/v and vortexed vigorously. The organic phase was collected and taken to dryness by evaporation under nitrogen gas at 40°C, and samples were resuspended in 250 µL of isooctane.

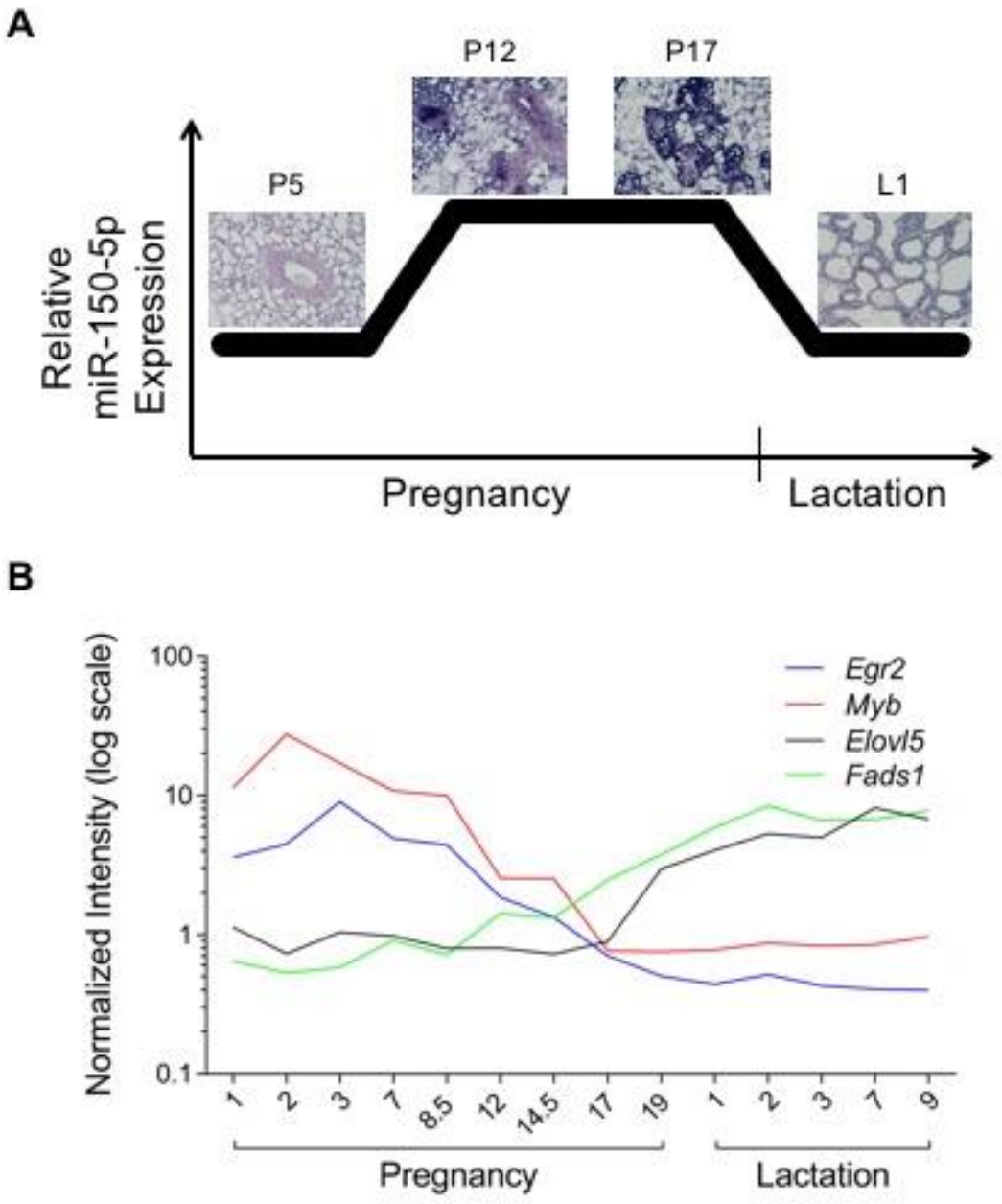
Quantification of MEC TAG and fatty acids

50 µL of isooctane suspended total lipid was taken to dryness under nitrogen gas, samples were resuspended in 200 µL dichloromethane that contained 15 µL of a 10% nonaethylene glycol monododecyl ether (Sigma Aldrich, St. Louis, MO) dissolved in dichloromethane (wt/vol). Samples were incubated for 5 min at 25°C and taken to dryness at 40°C for 25 minutes to ensure organic solvent was completely evaporated. Pellets contained triglyceride/nonionic surfactant complexes, to which 200 µL of reverse osmosis water was carefully added without mixing and incubated at 40°C for 10 min and followed by a gentle vortex. A standard regression curve was made using 80 nmol of tripalmitin (Sigma Aldrich, St. Louis MO) combined with 25 µL of 10% nonaethylene glycol monododecyl ether in dichloromethane (wt/vol), incubated and dried as above, suspended in 100 µL of reverse osmosis water, and dilutions of 20, 10, 5, 2.5, 1.25, 0.625, and 0.3125 nmol tripalmitin were used. Total TAG from the organic fraction was quantified relative to known tripalmitin standard using a modified colorimetric assay (Van Veldhoven et al., 1997) and are expressed as mM concentrations. Triglyceride Reagent and Free Glycerol Reagent were purchased from Sigma Aldrich (St. Louis, MO) and diluted according to the manufacturer's instructions.

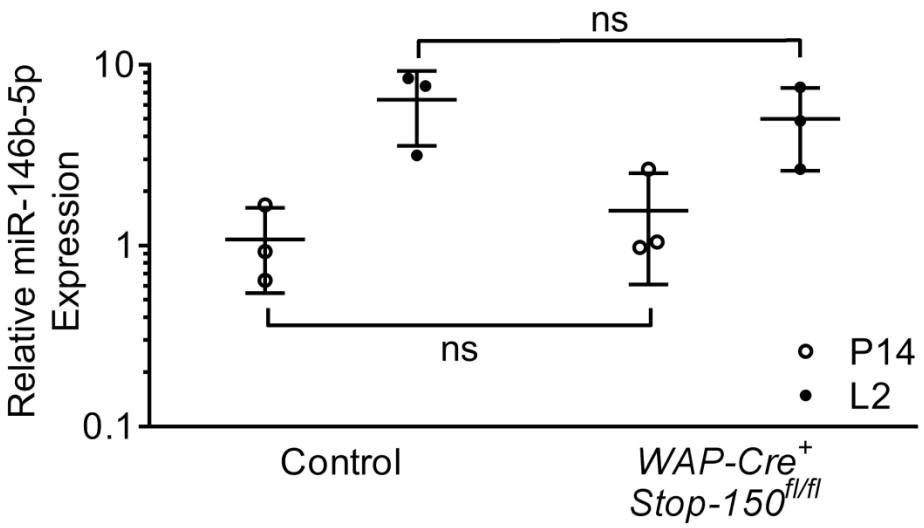
Supplemental Figures



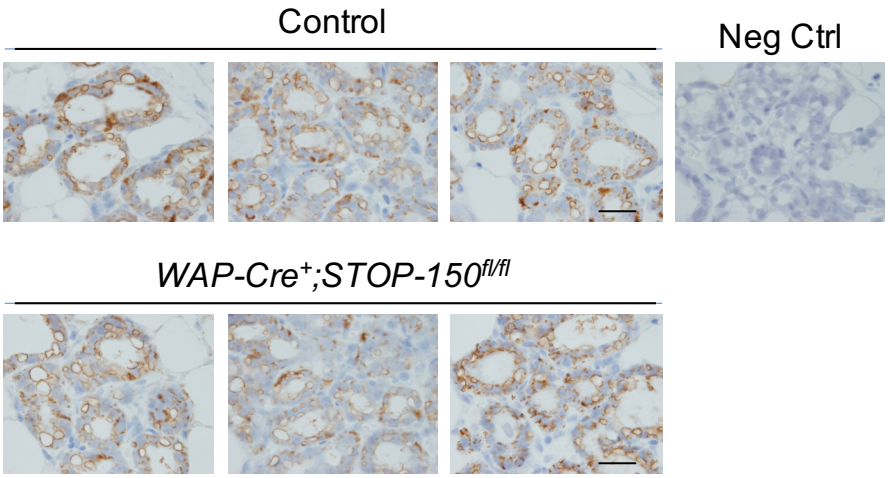
Supplemental Figure 1. Key pathways involved in secretory activation are altered in pregnancy versus lactation. Top 13 pathways represented by mRNAs changed in P14 versus L2 as measured by GeneChip Mouse Gene 1.0 ST array in CD1 mammary epithelial cells analyzed by IPA. Pathways are sorted in order of decreasing statistical significance (-log of *p*-value), represented by black bars with values indicated on the top axis. Grey line represents the percent of genes in each pathway that were altered with values indicated on the bottom axis.



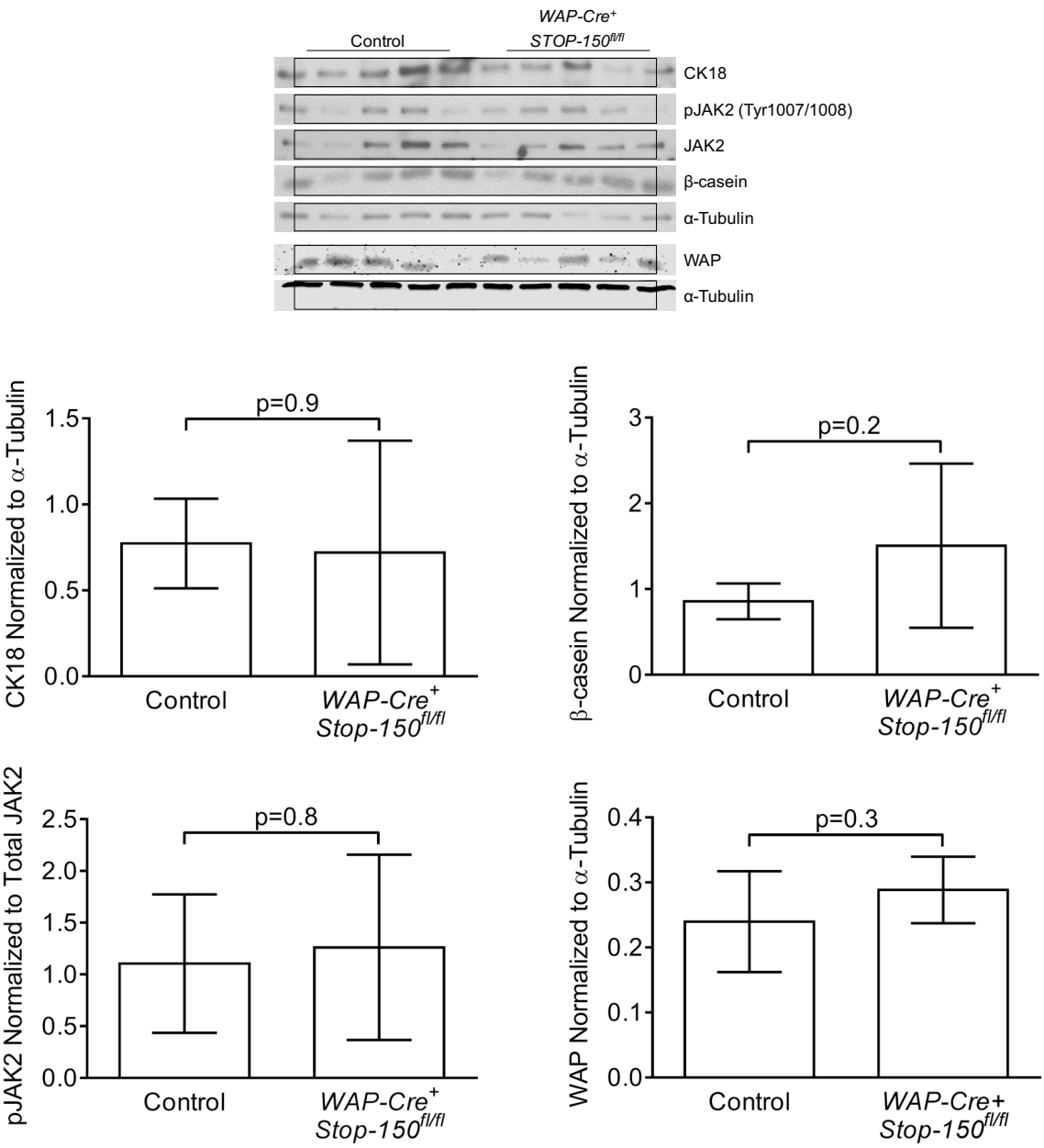
Supplemental Figure 2. miR-150-5p plays a potentially different role at mid-pregnancy. **(A)** A simplified expression pattern of mature miR-150-5p in the normal mammary gland based on ISH performed on whole mammary glands from C57BL/6 mice at P5, P12, P17 and L2, 400× magnification. **(B)** Time course of normalized expression values (log2 transformed) of a selection of validated or predicted miR-150-5p target transcripts from whole mammary gland (4 mice per time point), from a published data set (Rudolph et al., 2003)**.**



Supplemental Figure 3. Constitutive expression of miR-150 in mammary epithelium throughout lactation did not affect the normal expression pattern of miR-146b-5p, which increases at L2 compared to P14. TaqMan qRT-PCR was used to quantify mature miR-146b-5p expression in RNA isolated from L2 and P14 MECs from the indicated genotype normalized to RNAU6. Error bars represent standard deviation, n=3, unpaired *t*-test.



Supplemental Figure 4. Constitutive miR-150 did not suppress lipid droplet formation. IHC for adipophilin in mammary glands at P18 (3 mice per indicated genotype) and negative control (no primary antibody), scale bar = 20μm.



Supplemental Figure 5. Constitutive miR-150 does not affect JAK2 activation or milk protein expression downstream of STAT5. Immunoblot analysis of isolated L2 mammary epithelial cells. Blots were probed with primary antibodies, washed and incubated with appropriate peroxidase-conjugated secondary antibodies, and developed using enhanced chemiluminescence. The primary antibodies used were anti-CK18 (Santa Cruz 28264), anti-pJAK2 (Tyr1007/1008) (Cell Signaling, C80C3), anti-JAK2 (Cell Signaling, D2E12), anti-β-casein (Santa Cruz, 17969), and anti-α-tubulin loading control (Sigma, T5168). Film was scanned using a CanoScan 8600F (Canon, Tokyo, Japan) and densitometry of bands were quantified with ImageJ (National Institutes of Health). Immunoblot for WAP was done by Odyssey infrared imager described in methods.

Supplemental Table 1. Genespring analyzed GeneChip miRNA 1.0 ST data. Affymetrix GeneChip miRNA 1.0 ST microarray results with signal values, *p*-values ($p<0.05$) and fold change for CD1 mammary epithelial cells at pregnancy day 14 versus lactation day 2.

Click here to Download Table S1

Supplemental Table 2. Genespring analyzed GeneChip Mouse Gene 1.0 ST data. Affymetrix GeneChip Mouse Gene 1.0 ST microarray results with signal values, *p*-values ($p<0.05$) and fold change for CD1 mammary epithelial cells at pregnancy day 14 versus lactation day 2.

Click here to Download Table S2

Supplemental Table 3. The majority of significantly downregulated miRNAs at L2 compared to P14 are predicted to target lipid synthesis genes that show a concomitant increase between P14 and L2. Significant downregulated miRNAs are listed on the left, sorted from large to small fold change indicated in parenthesis. Genes involved in lipid synthesis that were TargetScan predicted targets of each miRNA are listed on the right. Numbers in parenthesis indicate fold increase of gene expression at L2 compared to P14 in the Affymetrix Mouse Gene 1.0 ST array. miRNAs that are part of the miR-17/92 cluster are in bold.

Significantly decreased miRNAs predicted to target lipid synthesis genes that increased between P14 and L2

miRNA (-FC)	Predicted Gene Targets (+FC)
miR-150-5p (7.6)	*Olah* (10.7), *Fasn* (6.6), *Fads1* (5.5), *Elovl5* (4.5), *Elovl6* (4.0), *Elovl1* (3.7),
miR-342-3p (4.4)	*Me1* (9.6), *Scd2* (7.2), *Fasn* (6.6), *Fads2* (4.2), *Elovl6* (4.0)
miR-20b-5p (3.8)	*Insig1* (12.1), *Pank3* (3.9)
miR-146a-5p (2.9)	*Fads2* (4.2)
miR-361-5p (2.8)	*Fasn* (6.6), *Scd1* (5.9), *Elovl6* (4.0), *Pank3* (3.9)
miR-342-5p (2.7)	*Scd2* (7.2), *Acly* (4.9)
miR-191-5p (2.6)	*Me1* (9.6), *Elovl6* (4.0)
miR-425-5p (2.5)	*Lpl* (7.8), *Fasn* (6.6), *Elovl5* (4.5), *Thrsp* (4.0), *Pank3* (3.9), *Tpi1* (1.5)
miR-155-5p (2.5)	*Lpl* (7.8), *Fads2* (4.2), *Pank3* (3.9)
miR-18a-5p (2.5)	*Scd2* (7.2), *Acaca* (6.6), *Fads1* (5.5), *Fads2* (4.2), *Elovl1* (3.7)
miR-106a-5p (2.5)	*Insig1* (12.1), *Pank3* (3.9)
miR-17-3p (2.4)	*Acacb* (8.8), *Fasn* (6.6), *Fads1* (5.5), *Acly* (4.9), *Pank3* (3.9)
miR-130b-3p (2.3)	*Slc25a1* (10.9), *Acacb* (8.8), *Elovl6* (4.0)
miR-92a-3p (2.3)	*Insig1* (12.1), *Pank3* (3.9)
miR-17-5p (2.3)	*Insig1* (12.1), *Elovl6* (4.0), *Pank3* (3.9)
miR-150-3p (2.1)	*Insig1* (12.1), *Acaca* (6.6), *Fads1* (5.5), *Elovl6* (4.0), *Pank3* (3.9)
miR-29a-3p (2.1)	*Insig1* (12.1), *Lpl* (7.8), *Scd1* (5.9), *Fads1* (5.5)
miR-20a-5p (2.1)	*Insig1* (12.1), *Pank3* (3.9)
miR-106b-5p (2.1)	*Elovl6* (4.0), *Pank3* (3.9)
miR-15b-5p (2.1)	*Me1* (9.6), *Fasn* (6.6), *Scd1* (5.9), *Fads1* (5.5), *Slc2a1* (3.3)
miR-185-5p (2.1)	*Fads1* (5.5), *Agpat1* (2.1), *Elovl6* (4.0)
miR-106b-3p (2.0)	*Insig1* (12.1), *Elovl6* (4.0), *Pank3* (3.9)
miR-532-5p (2.0)	*Fasn* (6.6), *Acly* (4.9), *Elovl5* (4.5)

Supplemental Table 4. RPPA data. RPPA results include mean net intensities. Individual *p*-values and fold changes comparing control versus WAP-Cre^{+}; Stop-150$^{fl/fl}$ mammary epithelial cells are given for P14 and L2.

Click here to Download Table S4

Supplemental Table 5. Genespring analyzed GeneChip Mouse Transcriptome Assay 1.0 data. Affymetrix GeneChip Mouse Transcriptome Assay 1.0 microarray results with signal values, *p*-values ($p<0.05$) and fold change for CD1 mammary epithelial cells from WAP-Cre^{+}; Stop-150$^{fl/fl}$ mice versus controls at lactation day 2.

Click here to Download Table S5

Supplemental Table 6. The majority of significantly downregulated miRNAs at L2 compared to P14 are predicted to target milk protein genes that show a concomitant increase between P14 and L2. Significant downregulated miRNAs are listed on the left, sorted from large to small fold change indicated in parenthesis. Milk protein genes that were TargetScan predicted targets of each miRNA are listed on the right. Numbers in parenthesis indicate fold increase of gene expression at L2 compared to P14 in the Affymetrix Mouse Gene 1.0 ST array. miRNAs that are part of the miR-17/92 cluster are in bold.

Significantly decreased miRNAs predicted to target milk protein genes that increased between P14 and L2

miRNA (-FC)	**Predicted Gene Targets (+FC)**
miR-150-5p (7.6)	*Egf* (6.7), *Btn1a1* (4.3), *Mfge8* (4.0)
miR-342-3p (4.4)	*Ltf (9.4), Lpo* (8.2), *Xdh* (6.3)
miR-20b-5p (3.8)	*Ltf* (9.4)
miR-146a-5p (2.9)	*Egf* (6.7)
miR-361-5p (2.8)	*Cel* (12.9)
miR-342-5p (2.7)	*Muc1* (8.5), *Egf* (6.7), *Mfge8* (4.0)
miR-140-3p (2.6)	*Egf* (6.7), *Xdh* (6.3)
miR-191-5p (2.6)	*Xdh* (6.3)
miR-425-5p (2.5)	*Ltf* (9.4), *Egf* (6.7), *Btn1a1* (4.3)
miR-155-5p (2.5)	*Egf* (6.7), *Xdh* (6.3), *Btn1a1* (4.3)
miR-18a-5p (2.5)	*Btn1a1* (4.3)
miR-106a-5p (2.5)	*Ltf* (9.4)
miR-17-3p (2.4)	*Ltf* (9.4), *Egf* (6.7)
miR-17-5p (2.3)	*Ltf* (9.4)
miR-150-3p (2.1)	*Btn1a1* (4.3), *Mfge8* (4.0), *Csn1s2b* (1.8)
miR-29a-3p (2.1)	*Wfdc3* (13.1)
miR-185-5p (2.1)	*Xdh* (6.3)
miR-532-5p (2.0)	*Csn2* (1.3)