

Supporting Information - Figures

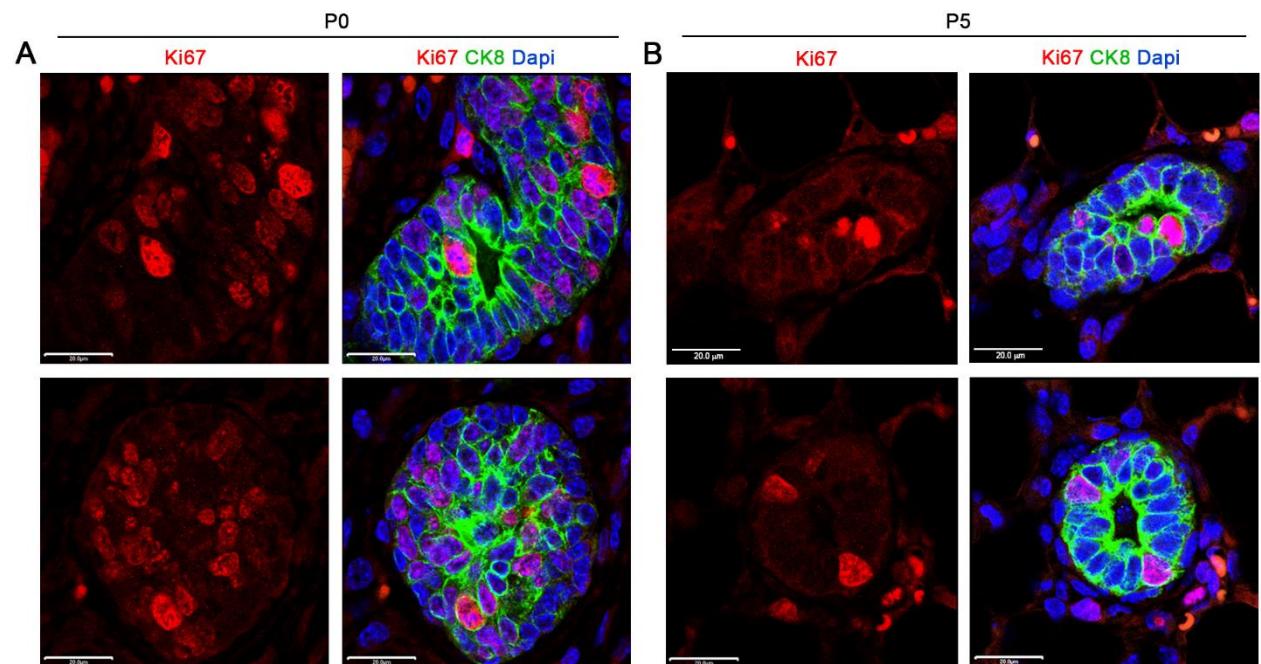


Figure S1. Early postnatal mammary gland morphogenesis coincides with widespread proliferation in the epithelial compartment.

(A,B) Immunofluorescence of Ki67 and CK8 in wild-type P0 (A) and P5 (B) mammary gland cross-sections with DAPI counter-staining (scale bars = 20 µm). Images are representative of 3 replicates.

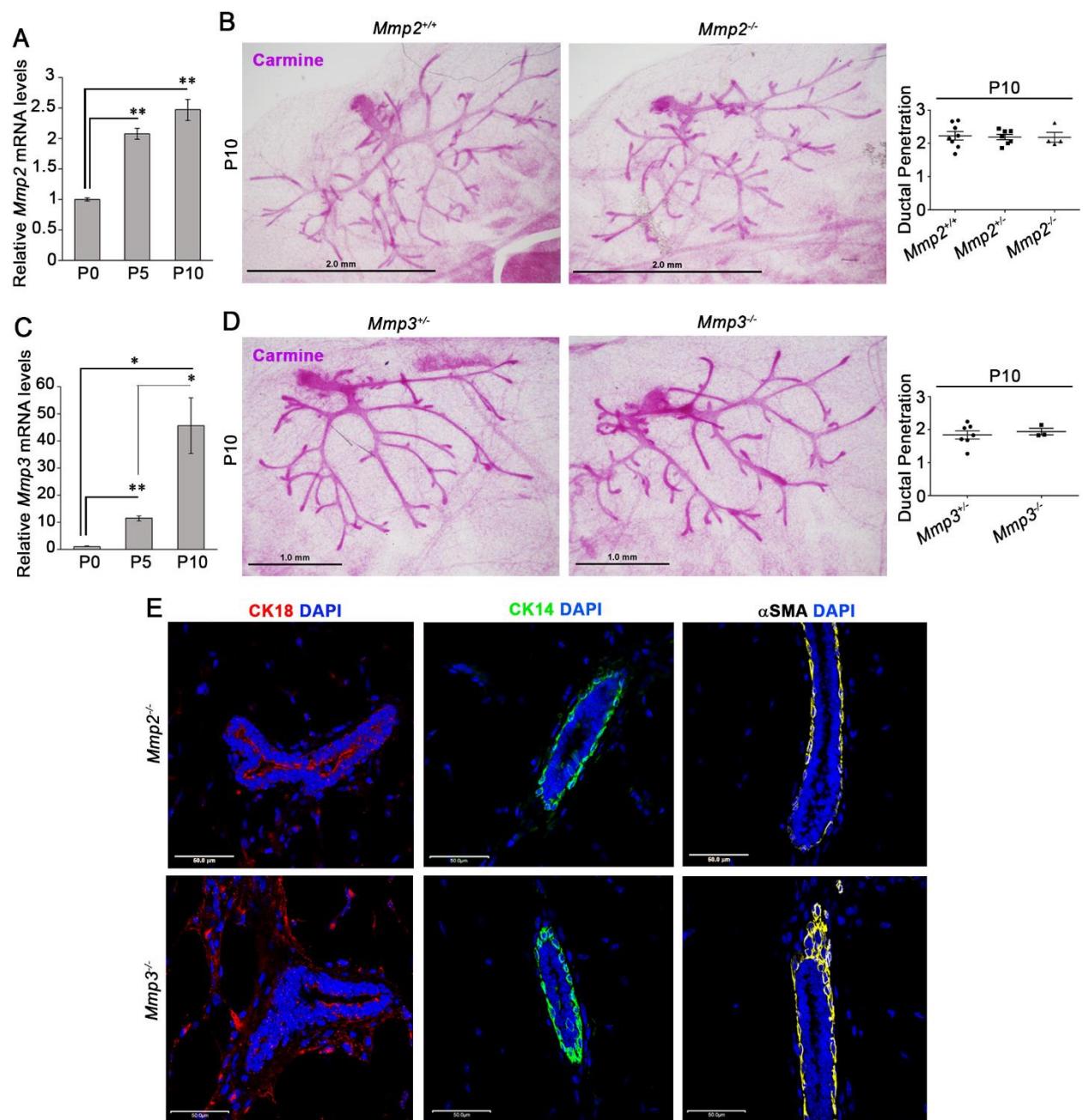


Figure S2. *Mmp2* and *Mmp3* global knockouts establish early postnatal mammary glands. (A) qPCR of *Mmp2* at P0, P5 and P10 in wild-type mammary glands. Results are expressed as mean expression levels \pm SEM at P0 (n=4), P5 (n=3) and P10 (n=4) relative to *Arbp*. **p<0.001, *p<0.05. (B) Carmine-stained whole mounts of mammary glands isolated from P10 *Mmp2*^{+/+} vs. *Mmp2*^{-/-} mice (scale bar = 2.0 mm) with quantifications of duct penetration (DP) (mm). Results are plotted with mean \pm SEM for *Mmp2*^{+/+} (n=8), *Mmp2*^{+/+} (n=7) and *Mmp2*^{-/-} (n=7). (C) qPCR of *Mmp3* at P0, P5 and P10 in wild-type mammary glands. Results are expressed as mean expression levels \pm SEM at P0 (n=4), P5 (n=3) and P10 (n=4) relative to *Arbp*. *p<0.05, **p<0.001. (D) Carmine-stained whole mounts of mammary glands isolated from P10 *Mmp3*^{+/+} vs. *Mmp3*^{-/-} mice (scale bar = 1.0 mm) with quantifications of duct penetration (DP) (mm). Results are plotted with mean \pm SEM for *Mmp3*^{+/+} (n=8), *Mmp3*^{+/+} (n=7) and *Mmp3*^{-/-} (n=7). (E) Immunofluorescence images of CK18 (red), CK14 (green), and αSMA (yellow) in *Mmp2*^{-/-} and *Mmp3*^{-/-} mammary glands. Nuclei are stained with DAPI (blue).

and *Mmp2*^{-/-} (n=4) mice. (C) qPCR of *Mmp3* at P0, P5 and P10 in wild-type mammary glands. Results are expressed as mean ± SEM for P0 (n=3), P5 (n=3) and P10 (n=4) relative to *Arbp*. **p<0.001, *p<0.05. (D) Carmine-stained whole mounts of P10 mammary glands (scale bars = 1.0 mm) from *Mmp3*^{+/+} mice (n=7) and *Mmp3*^{-/-} mice (n=3) with quantification of DP (mm). Results are plotted with mean ± SEM. (E) Immunofluorescence of CK18, CK14, and αSMA in P10 *Mmp2*^{-/-} and *Mmp3*^{-/-} mammary glands with DAPI counter-staining (scale bars = 50 μm). P values were calculated with unpaired t-tests. Images shown are representative of 2 replicates.

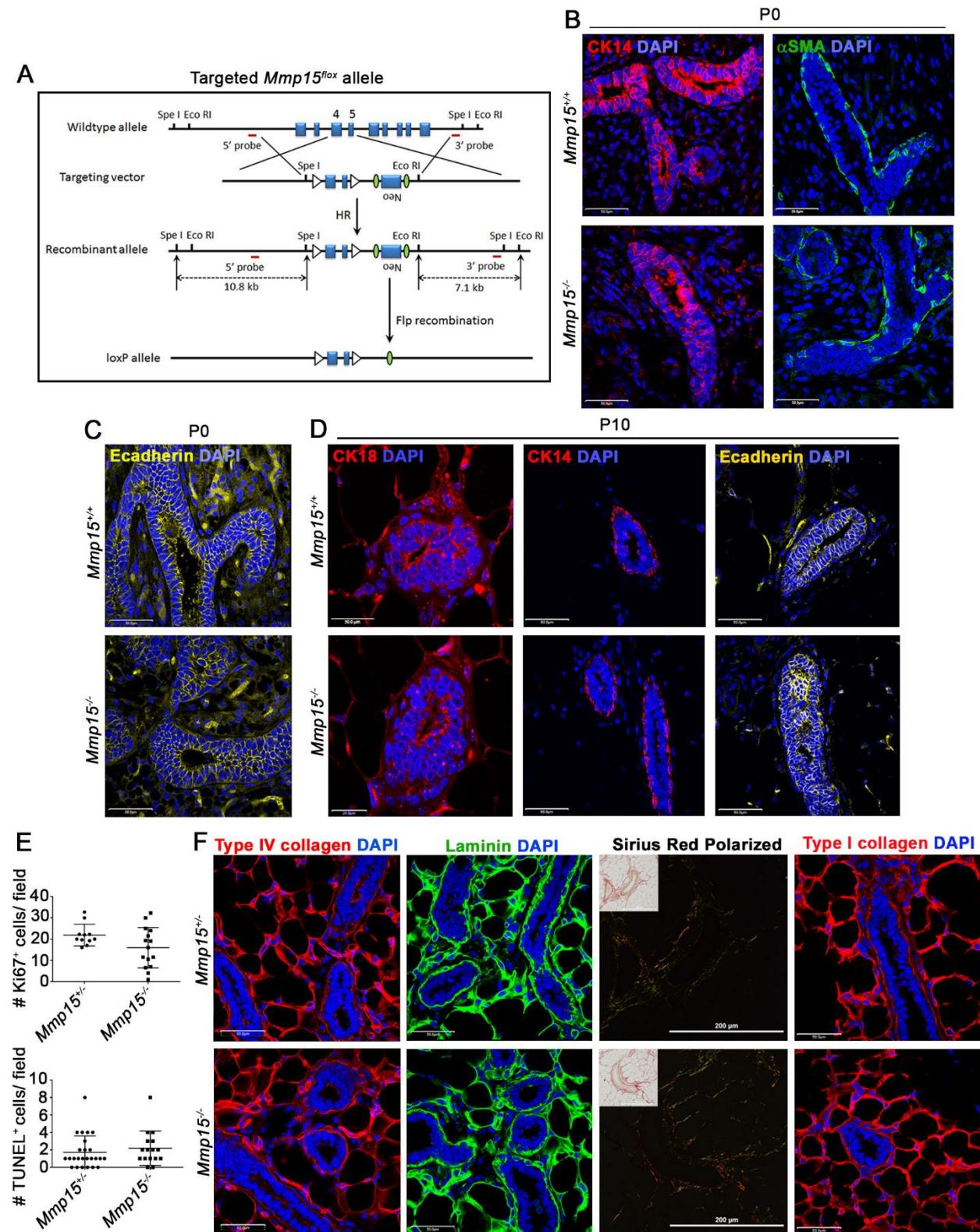


Figure S3. MMP15-independent orchestration of mammary epithelial cell organization, mammary cell proliferation and ECM. (A) Schematic of *Mmp15* global knockout targeting strategy and the *Mmp15*^{fl/fl} allele. (B-C) Immunofluorescence of CK14, αSMA and E-cadherin in P0 *Mmp15*^{+/+} and *Mmp15*^{-/-} mammary glands with DAPI nuclear counter-staining (scale bars = 50 μm). (D) Immunofluorescence of CK18, CK14, and E-cadherin in P10 *Mmp15*^{+/+} and *Mmp15*^{-/-} mammary glands with DAPI counter-staining. (E) Quantification of average number of Ki67-positive cells per field and TUNEL-positive cells per field from immunofluorescence of P5 mammary glands (60X magnification). Results are plotted with mean values of n>10 fields per genotype. (F) Immunofluorescence of type IV collagen, laminin, and type I collagen with DAPI counter-staining in P5 *Mmp15*^{+/+} and *Mmp15*^{-/-} mammary glands (scale bars = 50 μm). Polarized light images of Sirius Red staining of P5 mammary gland cross-sections are also shown. Insets show the corresponding bright-field images (scale bar = 200 μm). All images are representative of n≥2 replicates.

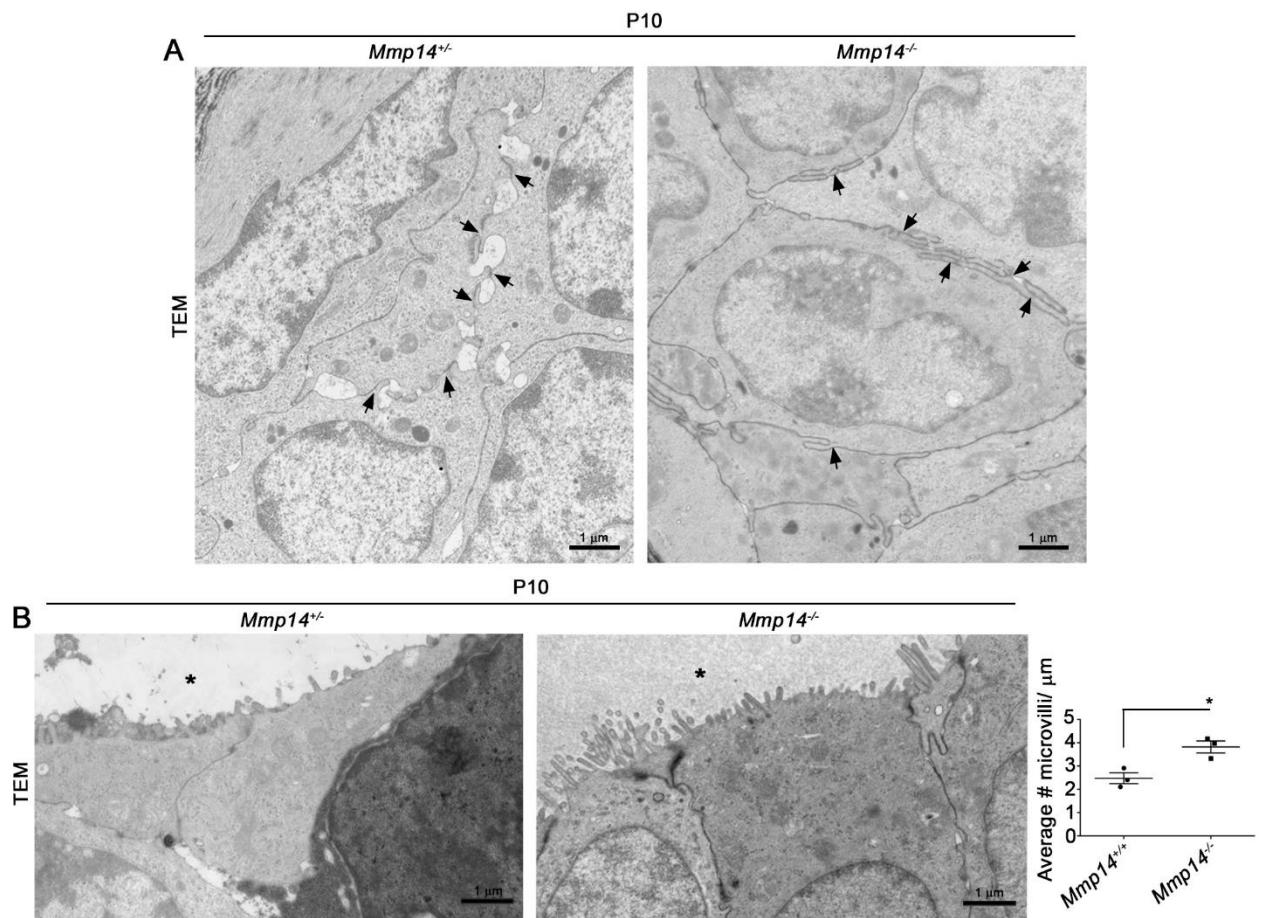


Figure S4. *Mmp14*-dependent regulation of epithelial cell junctions and microvilli *in vivo*. (A) Transmission electron micrographs (TEM) of representative P10 *Mmp14^{+/+}* and *Mmp14^{-/-}* mammary ducts (15000X) (n=3 per genotype). Arrows denote cell-cell junctions and adjacent spaces in *Mmp14^{+/+}* ducts with corresponding regions in *Mmp14^{-/-}* ducts (scale bars = 1 μm). (B) TEM of P10 mammary duct microvilli (15000X) with quantification of average number of microvilli per μm plotted with mean \pm SEM (n=3 per genotype). *p<0.02. Asterisks mark the mammary duct lumen (scale bar = 1 μm). P value was calculated with an unpaired t-test.

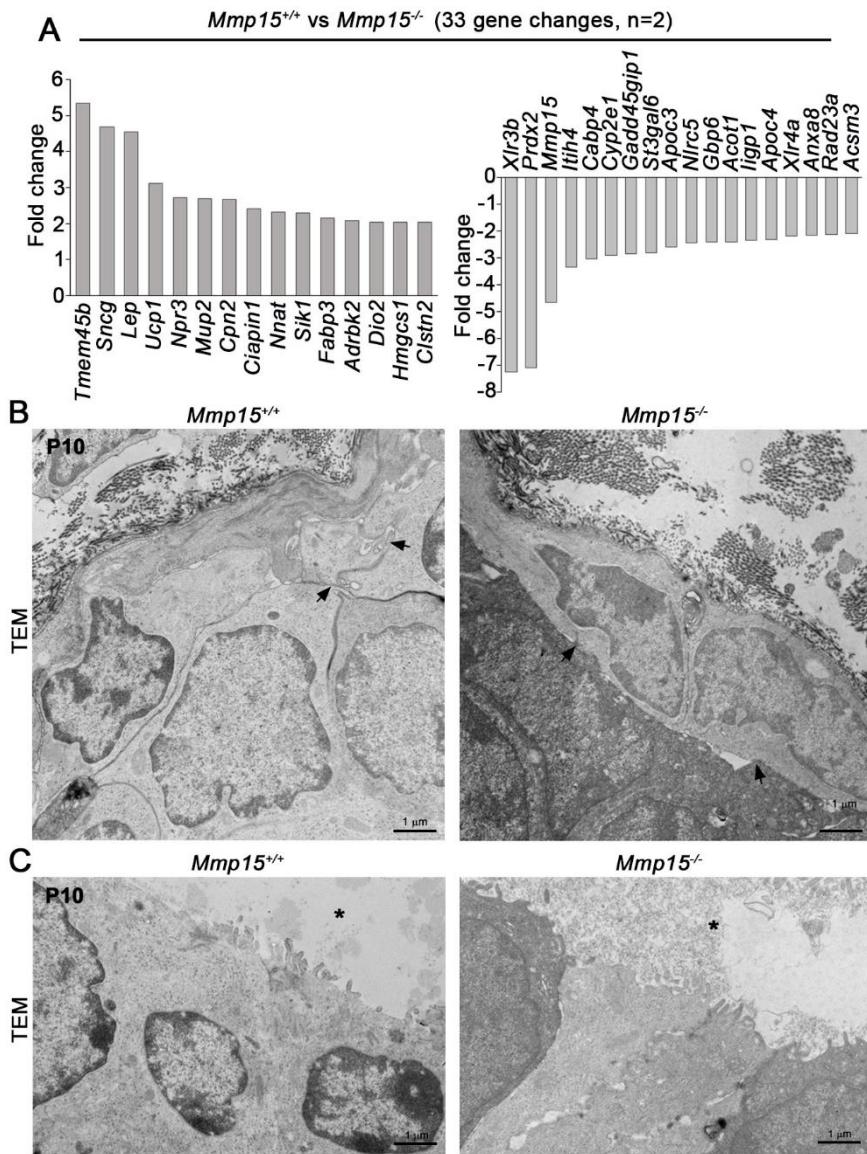


Figure S5. *Mmp15* knockout mammary glands maintain global gene expression profile and epithelial ultrastructure.

(A) Charts of the up-regulated and down-regulated genes in P10 *Mmp15^{-/-}* mammary glands relative to *Mmp15^{+/+}* mammary glands (n=2 per genotype). (B) TEM of representative *Mmp15^{+/+}* and *Mmp15^{-/-}* mammary ducts (15000X) (scale bar = 1 μm). Arrows mark corresponding cell junctions (n=2 per genotype). (C) TEM of representative P10 mammary duct microvilli (15000X) (n=2 per genotype; scale bars = 1 μm). Asterisks mark the mammary duct lumen.

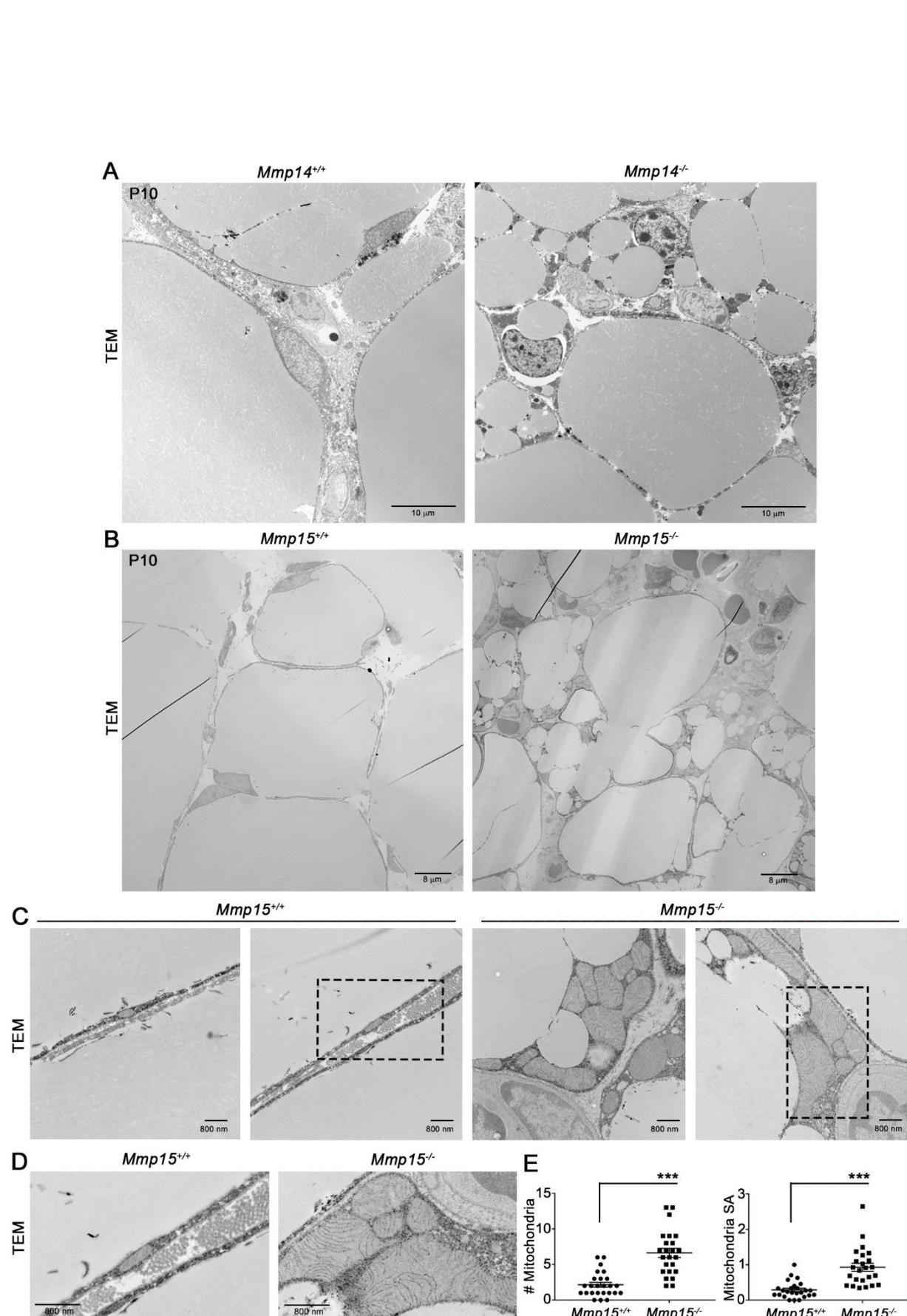


Figure S6. MMP14- and MMP15-dependent regulation of mammary adipocyte development *in vivo*.

(A) Transmission electron micrographs (TEM) of representative P10 *Mmp14^{+/−}* and *Mmp14^{−/−}* mammary adipocytes (1950X, n=3 per genotype, scale bars = 10 μm). (B) TEMs of representative P10 *Mmp15^{+/+}* and *Mmp15^{−/−}* mammary adipocytes (2000X, n=2 per genotype, scale bars = 8 μm). (C) TEMs of representative mitochondria in P10 *Mmp15^{+/+}* and *Mmp15^{−/−}* adipocytes (20,000X, n=2 per genotype, scale bars = 800 nm). Dotted lines circumscribe mitochondria for close-up imaging of cristae (D). (E) Quantifications of mitochondrial number and size (surface area, SA) in *Mmp15^{+/+}* and *Mmp15^{−/−}* TEMs (n=2 mice per genotype, n=25 fields per genotype). ***p<0.00001, as calculated with an unpaired t-test.

Table S1. qPCR Primers

Gene	Forward Primer	Reverse Primer
<i>Gapdh</i>	5'-TGAAGCAGGCATCTGAGGG-3'	5'-CGAAGGTGGAAGAGTGGGAG-3'
<i>Arbp</i>	5'-CACTGGTCTAGGACCCGAGAA-3'	5'-AGGGGGAGATGTTCAGCATGT-3'
<i>Mmp2</i>	5'-TCTGGAGCGAGGATAACCCAA-3'	5'-TTCCAGGAGTCTGCGATGAGC-3'
<i>Mmp3</i>	5'-GTT CCT GAT GTT GGT GGC TT-3'	5'-AGC CTC TCC TTC AGA GAT CC-3'
<i>Mmp15</i>	5'-ACATGTCCACCATGCGCTCT-3'	5'-TACCATGATGTCAGCCTCC-3'
<i>Mmp14</i>	5'-CTGCCATTGCCGCCATGCAAAA-3'	5'-TGGCGTGGCACTCTCCCATACT-3'
<i>Ucp1</i>	5'-AGGCTTCCAGTACCAATTAGGT-3'	5'- CTGAGTGAGGCAAAGCTGATTT-3'
<i>Dio2</i>	5'-AATTATGCCTCGGAGAACCG-3'	5'-GGCAGTTGCCTAGTGAAGGT-3'
<i>Fabp3</i>	5'-ACCTGGAAGCTAGTGGACAG-3'	5'-TGATGGTAGTAGGCTTGGTCAT-3'
<i>Prdm16</i>	5'-CCACCAAGACTTCGAGCTACG-3'	5'-ACACCTCTGTATCCGTAGCA-3'
<i>Pgc1a</i>	5'-CCACTTCAATCCACCCAGAAAG-3'	5'-TATGGAGTGACATAGAGTGTGCT-3'
<i>Leptin</i>	5'-GAGACCCCTGTGTCGGTTC-3'	5'-CTGCGTGTGTGAAATGTCATTG-3'

Table S2. Genotyping Primers

Gene	Forward Primer(s)	Reverse Primer	PCR Product Size
<i>Mmp14</i> ^{WT}	5'-TAGGCCTGGAACATTCTAACGATC-3'	5'-CTTTGTGGGTGACCCTGACTTGC-3'	900bp
<i>Mmp14</i> ^{KO}	5'-TGCAGGCCAGAGGCCACTTGTGT-3'	5'-CTTTGTGGGTGACCCTGACTTGC-3'	950bp
<i>Mmp14</i> ^{lacZ(+)}	5'-ACCTGCGTGCAATCCATCTT-3'	5'-ATGATGGCGGAGGGATCGTTAG-3'	350bp
<i>Mmp14</i> ^{lacZ(-)}	5'-TGAGGTGGAAAACACGACCAG-3'	5'-ATGATGGCGGAGGGATCGTTAG-3'	180bp
<i>Mmp15</i> ^{WT}	5'-CCGCCACCAAGCCTCACTGTCT-3'	5'-AAAGCCACCCACGCCATCAAAC-3'	400bp
<i>Mmp15</i> ^{KO}	5'-CGCCACCAAGCCTCACTGTCT-3'	5'-AATTGCTGGGATGGAGGAAGGTA-3'	470bp
<i>Mmp15</i> ^{lacZ}	5'-GAGATGGCGCAACGCAATTATG-3'	5'-TGCACGTCCCATTCTCATGC-3'	292bp
<i>Mmp2</i> ^{WT}	5'-GTGCTACTGCAGGATAAACTGATG-3'	5'-CCGGGACAGGAACGTACTGGTTC-3'	794bp
<i>Mmp2</i> ^{KO}	5'-GCGCCTACCGGTGGATGTGGAATGTGT GCG-3'	5'-CCGGGACAGGAACGTACTGGTTC-3'	310bp
<i>Mmp3</i> ^{WT}	5'-ACCGGATTTCCAAGACAGAGTG-3'	5'-GCATCTCCATTAATCCCTGGTCC-3'	325bp
<i>Mmp3</i> ^{KO}	5'-AGGATCTCCTGTCATCTCACCTGCTC CTG-3'	5'-AAGAACTCGTCAAGAAGGCGATAGAA GGCG-3'	492bp