Supplemental Figures.

Supplemental Figure 1. Characterization of the MMTV-Spot14 overexpressing mammary gland. A) Immunohistochemical staining for Spot14 using the anti-hemagglutinin (HA) antibody to detect the C-terminal HA-tag engineered into the Spot14 transgene in 10-week virgin females. HA-tagged Spot14 expression was confined to the mammary epithelium component of the mammary gland. B) Spot14 transgene expression was quantified using qPCR primers that detect the HA sequence. C, D, and E) Representative whole mounts and histological sections of control and MMTV-Spot14 mammary glands from nulliparous, mid-pregnant, and mid-lactation mice, indicated no overt impairment of epithelial development exists in transgenic females.

Supplemental Figure 2. Glycolytic and lipogenic gene expression is maintained in Spot14 null MECs. Total RNA was isolated from lactation day 4 and day 10 MECs, converted to cDNA, and transcript copy number was quantified as previously described. Glycolytic and lipogenic gene expression mammary epithelial cells (MECs) at lactation day 4 and 10 was equivalent in Spot14 nulls, indicating that Spot14 does not influence gene expression in the lactating mammary gland.

Supplemental Figure 3. Quantitative regression curves and immunoblotting for FASN and Spot14. A) Standard regression curves using known amounts of recombinant FASN and Spot14 were used to quantify the amount of endogenous FASN and Spot14 from MEC lysates (panel B). C) Recombinant Spot14 folded in a manner consistent with its crystal structure, demonstrating alpha helical folding (blue) in contrast to boiled Spot14 that did not (red). D) Hydrolysis of myristoyl-CoA by Thioesterase II. Spot14 had no catalytic activity for the hydrolysis of myristoyl-CoA to liberate free myristic acid, nor did addition of Spot14 to the TE2 reaction enhance hydrolysis of myristoyl-CoA. Reaction buffer contained 25 mM potassium phosphate pH 8.0, 1 mM DTT, 0.5 mM EDTA, 0.1 mg/mL fatty acid free BSA, 10 μM myristoyl-CoA, 2 μg Thioesterase II, and/or 4 μg Spot14. Reaction was conducted at room temperature for 5 minutes.

Supplemental Figure 4. Non denatured native and anti-phosphothronine immunoblots for de novo fatty acid synthesis pathway enzymes. A) Immunoblots of non-denaturing native gels indicated the assembly of ACLY (homotetramer), ACC (homomultimer), and FASN (homodimer) known to be required for enzyme activity. No defect in the assembly of ACLY and ACC was observed in the Spot14 null MECs that could account for the lactation phenotype resulting in reduced *de novo* fatty acid synthesis. Loss of Spot14 did not alter the dimer assembly of FASN. B) Anti-phosphothreonine immunoblot and densitometry for immunoprecipitated FASN from lactation day 10 control and Spot14 null mammary glands. No significant difference was observed ($p = 0.4$).