

SUPPLEMENTARY MATERIAL

**Phosphotungstic acid-enhanced microCT for
quantitative visualization of mouse mammary gland
morphology**

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Supplementary Table 1. Selected trabecular bone parameters from ASBMR nomenclature which were implemented to equivalently correspond to mammary gland parameters.

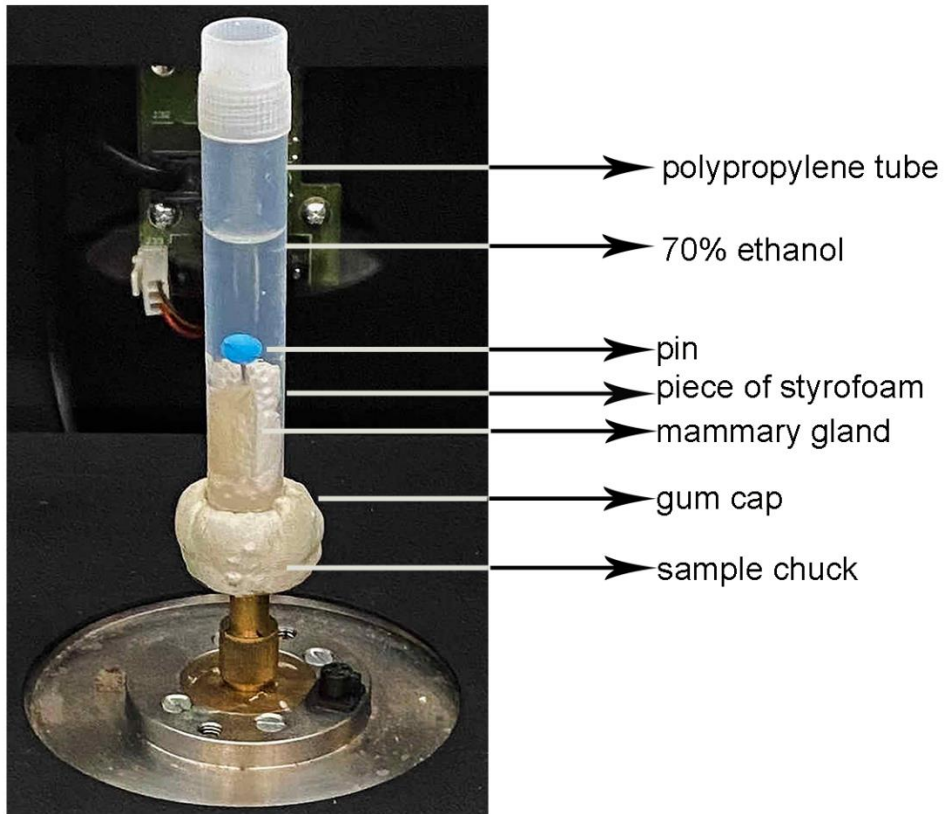
Unit	Bone parameters (ASBMR nomenclature)	Mammary gland parameters (revised nomenclature)
mm ³	Tissue volume (TV)	Tissue volume (TV)
mm ³	Bone volume (BV)	Ductal Volume (DV)
%	Percent bone volume (BV/TV%)	Percent ductal volume (DV/TV%)
mm	Trabecular thickness (Tb.Th)	Ductal thickness (D.Th)
mm ⁻¹	Trabecular number (Tb.N)	Ductal number (D.N)
mm	Trabecular separation (Tb.Sp)	Ductal separation (D.Sp)
mm ²	Bone surface (BS)	Ductal surface (DS)
mm ⁻¹	Bone surface density (BS/TV)	Ductal surface density (DS/TV)
–	Fractal dimension (FD)	Fractal dimension (FD)

Supplementary Table 2. Volumetric and morphometric parameters measured by PTA enhanced microCT in abdominal mammary glands from 4 distinct developmental stages (n=7 mice/group). Data are shown as mean values \pm SEM.

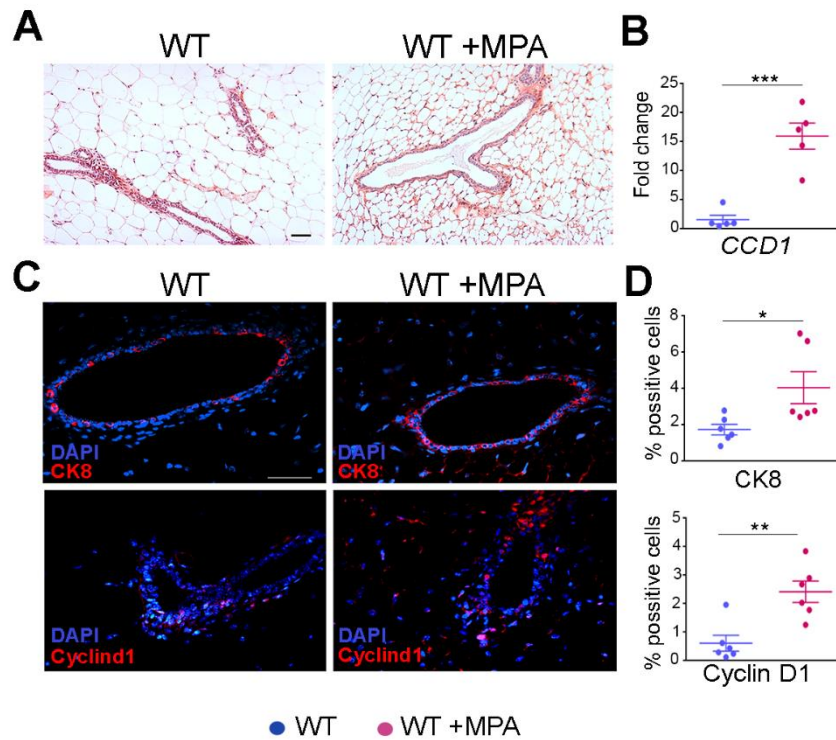
Mammary Parameters	Puberty (4-week)	Virgin adult (8-week)	Virgin adult (8-week)	Pregnancy (15.5 dpc)
Tissue volume (TV) (mm ³)	23.50 \pm 1.07	37.48 \pm 1.76	41.44 \pm 2.53	61.54 \pm 1.60
Ductal volume (DV) (mm ³)	0.44 \pm 0.05	2.24 \pm 0.17	1.73 \pm 0.22	13.59 \pm 0.68
Percent ductal volume (DV/TV%)	1.63 \pm 0.19	5.72 \pm 0.45	4.37 \pm 0.37	21.52 \pm 1.72
Ductal thickness (D.Th) (mm)	0.06 \pm 0.004	0.06 \pm 0.002	0.07 \pm 0.002	0.08 \pm 0.0009
Ductal number (D.N) (mm ⁻¹)	0.21 \pm 0.03	0.85 \pm 0.06	0.64 \pm 0.06	2.36 \pm 0.19
Ductal separation (D.Sp) (mm)	0.77 \pm 0.04	0.40 \pm 0.01	0.43 \pm 0.02	0.23 \pm 0.05
Ductal surface (DS) (mm ²)	27.21 \pm 2.52	121 \pm 10	99.06 \pm 7.81	584 \pm 33
Ductal surface density (DS/TV) (mm ⁻¹)	1.19 \pm 0.14	3.58 \pm 0.27	2.53 \pm 0.22	9.04 \pm 0.91
Fractal dimension (FD)	1.66 \pm 0.03	2.13 \pm 0.02	2.04 \pm 0.03	2.51 \pm 0.02

Supplementary Table 3. Volumetric and morphometric parameters measured by PTA enhanced microCT in abdominal mammary glands from MPA-treated and control mice (n=6 mice/group). Data are shown as mean values \pm SEM.

Mammary Parameter	WT control	WT +MPA	p-value
Tissue volume (TV) (mm ³)	42.26 \pm 1.94	43.28 \pm 2.23	0.735
Ductal volume (DV)(mm ³)	1.41 \pm 0.21	2.34 \pm 0.34	0.0430
Percent ductal volume (DV/TV%)	3.36 \pm 0.54	5.43 \pm 0.76	0.0506
Ductal thickness (D.Th) (mm)	0.065 \pm 0.002	0.078 \pm 0.004	0.0157
Ductal number (D.N) (mm ⁻¹)	0.52 \pm 0.08	0.68 \pm 0.07	0.1505
Ductal separation (D.Sp) (mm)	0.54 \pm 0.07	0.44 \pm 0.01	0.1918
Ductal surface (DS) (mm ²)	108 \pm 5	128 \pm 13	0.1633
Ductal surface density (DS/TV) (mm ⁻¹)	2.22 \pm 0.35	2.98 \pm 0.27	0.1146
Fractal dimension (FD)	2.11 \pm 0.020	2.19 \pm 0.026	0.0374



Supplementary Figure 1. Sample mounting preparation of mammary gland for microCT scanning. Mammary gland was stabilized in a piece of styrofoam with a pin and was placed into a microCT tube containing 70% alcohol. The tube was stuck with a gum cap in the microCT scanner chuck supplier.



Supplementary Figure 2. MPA induced expansion and proliferation of mammary gland epithelium. (A) Representative images from H/E staining sections of mammary glands from MPA-treated and control mice (WT), Scale bar=15 μ m, magnification=200x. (B) qPCR expression analysis showed increased mRNA levels of Cyclin D1 (*CCD1*) in MPA-treated mice compared to control mice (WT) (n=5 per group). (C) Immunofluorescent staining of mammary epithelial cells with cytokeratin 8 (CK8) and Cyclin D1. (D) Quantification of the percentage of positive cells for CK8 and Cyclin D1 in immunofluorescent images through ImageJ. Scale bar= 15 μ m, magnification=400x. Data are shown as mean values \pm SEM. T-test was performed for statistical analysis (* p<0.05, ** p<0.01).