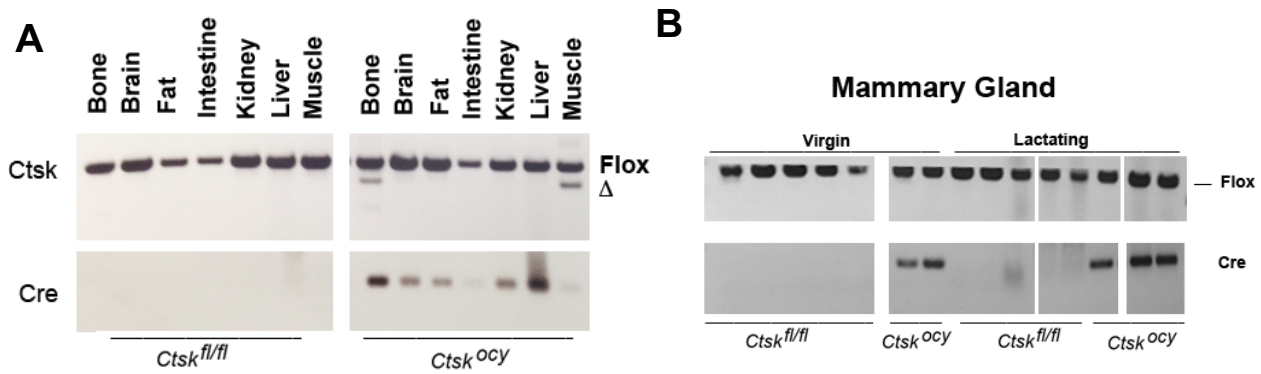

Figure S1. Conditional Inactivation of *Ctsk* using Dmp1Cre. A) Photographs of PCR amplicons derived from mouse genomic DNA isolated from a variety of tissues of *Ctsk*$^{fl/fl}$ and *Ctsk*^{ocy} mice. **B)** Photographs of PCR amplicons derived from mouse genomic DNA isolated from the mammary gland of *Ctsk*$^{fl/fl}$ and *Ctsk*^{ocy} mice.

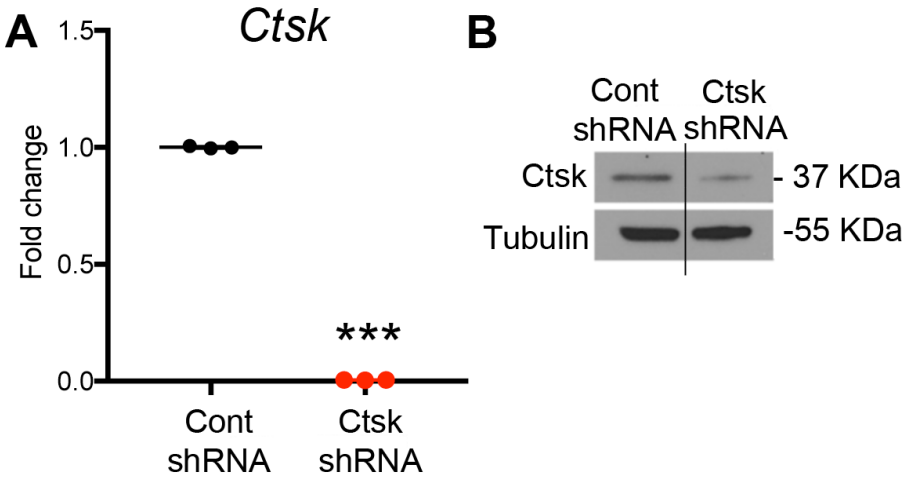

Figure S2. *Ctsk* silencing in Ocy454 cells. A) Efficiency of Ctsk deletion in Ocy454 cells. Control shRNA (black circles) and *Ctsk* shRNA (open circles) (TRCN0000030591). Data shown are means of triplicates ± SEM normalized to expression of 18S mRNA *=p< 0.05, (n=3). **B)** Densitometric analysis of Western blot analysis showing efficiency of Ctsk silencing. Beta-tubulin is shown as a loading control, (n=3). ***=p< 0.001comapred to control shRNA Ocy454 cells.

Table S1. μCT analysis of virgin and lactating *Ctsk*^{Ocy} mice and their control littermates

Parameters	Virgin		Lactating		Two-way ANOVA		
	***Ctsk*$^{fl/fl}$** (n=9)	***Ctsk*^{Ocy}** (n=9)	***Ctsk*$^{fl/fl}$** (n=7)	***Ctsk*^{Ocy}** (n=7)	**Deletion**	**Lactation**	**Interaction**
Cortical Bone							
Cross-sectional volume(mm^3)	1.15±0.02	1.14±0.02	1.13±0.03	1.15±0.02	NS	NS	NS
Cortical volume (mm^3)	0.478±0.008	0.477±0.010	0.418±0.013†‡	0.468±0.017$^{\#}$	NS	0.0064	0.0382
Marrow volume (mm^3)	0.672±0.011	0.666±0.010	0.709±0.018	0.684±0.026	NS	NS	NS
Cortical thickness (mm)	0.180±0.002	0.181±0.003	0.155±0.003†‡	0.172±0.007$^{\#}$	NS	0.0001	0.0417
Cancellous Bone							
BV/TV (%)	6.6±0.37	6.0±0.45	4.98±0.25†	6.5±0.31$^{\#}$	NS	NS	0.0085
Tb.Th (mm)	0.041±0.001	0.040±0.001	0.033±0.001†‡	0.035±0.001†‡	NS	0	NS
Tb.N (/mm)	3.38±0.12	3.29±0.10	3.30±0.09	3.40±0.06	NS	NS	NS
Tb.Sp (mm)	0.297±0.011	0.303±0.010	0.304±0.009	0.294±0.005	NS	NS	NS
Conn.D (/mm^3)	107±9	90±9	114±7	146±6$^{\dagger\ddagger\#}$	NS	0.0008	0.008
SMI (-)	2.72±0.06	2.80±0.08	2.64±0.05	2.36±0.07$^{\dagger\ddagger\#}$	NS	0.0007	0.0137

$^{\dagger}p<0.05$ compared to virgin *Ctsk*$^{fl/fl}$, Two-way ANOVA followed by Fisher's PLSD
$^{\ddagger}p<0.05$ compared to virgin *Ctsk*^{Ocy}
$^{\#}p<0.05$ compared to lactating *Ctsk*$^{fl/fl}$

Table S2. Histomorphometric analysis of virgin and lactating *Ctsk*^{Ocy} mice and their control littermates

Parameters	Virgin		Lactating		Two-way ANOVA		
	***Ctsk*$^{fl/fl}$** (n=9)	***Ctsk*^{Ocy}** (n=9)	***Ctsk*$^{fl/fl}$** (n=6-7)	***Ctsk*^{Ocy}** (n=6)	**Deletion**	**Lactation**	**Interaction**
BV/TV (%)	5.97±0.54	5.16±0.44	4.02±0.23†	5.52±0.40$^{\#}$	NS	NS	0.0161
Tb.Th (μm)	30.82±0.92	29.63±0.86	24.56±0.67†‡	27.94±0.81$^{\dagger\#}$	NS	0.0001	0.0163
Tb.N (/mm)	1.92±0.15	1.73±0.13	1.64±0.08	1.98±0.13	NS	NS	NS
Tb.Sp (μm)	518±49	574±47	597±31	489±32	NS	NS	NS
MS/BS (%)	36.02±1.30	33.66±1.09	29.72±1.47†‡	35.03±1.11$^{\#}$	NS	NS	0.0061
MAR (μm/day)	1.80±0.11	1.99±0.10	1.82±0.12	1.87±0.10	NS	NS	NS
BFR/BS ($\mu m^3/\mu m^2$/year)	234±13	243±10	197±15	239±13	NS	NS	NS
BFR/BV (%/year)	1570±70	1774±115	1629±117	1731±92	NS	NS	NS
Ob.S/BS (%)	21.75±1.85	18.51±0.96	16.07±1.11	20.74±2.39	NS	NS	0.0219
N.Ob/B.Pm (/mm)	17.82±1.61	14.85±1.05	14.06±1.10	18.15±2.47	NS	NS	0.0327
OS/BS (%)	11.14±2.81	6.29±1.35	9.96±1.61	10.00±3.06	NS	NS	NS
O.Th (μm)	2.37±0.21	2.24±0.14	2.24±0.16	2.81±0.20$^{\ddagger\#}$	NS	NS	NS
Oc.S/BS (%)	7.60±1.35	7.49±1.25	10.77±1.18†‡	6.88±0.31$^{\#}$	0.037	NS	NS
N.Oc/B.Pm (/mm)	3.76±0.56	3.56±0.53	4.62±0.55	2.88±0.23$^{\#}$	0.0451	NS	NS

$^{\dagger}p<0.05$ compared to virgin *Ctsk*$^{fl/fl}$, Two-way ANOVA followed by Fisher's PLSD
$^{\ddagger}p<0.05$ compared to virgin *Ctsk*^{Ocy}
$^{\#}p<0.05$ compared to lactating *Ctsk*$^{fl/fl}$

Table S3. μCT analysis of 12-wk-old *Ctsk*^{Ocy} male mice and their control littermates

Parameters	***Ctsk*$^{fl/fl}$** (n=9)	***Ctsk*^{Ocy}** (n=8)
Cortical Bone (midshaft femur)		
Cross-sectional volume (mm^3)	1.21±0.05	1.24±0.03
Cortical volume (mm^3)	0.522±0.017	0.537±0.009
Marrow volume (mm^3)	0.688±0.032	0.706±0.027
Cortical thickness (mm)	0.186±0.003	0.192±0.002
Cancellous Bone (distal femur)		
BV/TV (-)	0.099±0.005	0.103±0.003
Tb.Th (mm)	0.036±0.000	0.039±0.001*
Tb.N (/mm)	4.60±0.09	4.51±0.10
Tb.Sp (mm)	0.210±0.004	0.214±0.005
Conn.D (/mm^3)	199±13	193±14
SMI (-)	2.21±0.08	2.24±0.06

*p<0.05 compared to *Ctsk*$^{fl/fl}$, unpaired t-test

Table S4. Osteocyte lacunar analysis of virgin and lactating *Ctsk*^{Ocy} mice and their control littermates

Parameters	Virgin		Lactating		Two-way ANOVA		
	***Ctsk*$^{fl/fl}$** (n=6)	***Ctsk*^{Ocy}** (n=5)	***Ctsk*$^{fl/fl}$** (n=7)	***Ctsk*^{Ocy}** (n=6)	**Deletion**	**Lactation**	**Interaction**
Bone volume (x10^4 μm^3)	12.86±0.64	13.40±0.43	11.19±0.36†‡	12.54±0.29$^{\#}$	0.0437	0.0093	NS
Single Ocy lacunar area (μm^2)	23.28±1.32	24.76±1.66	31.23±1.31†‡	23.69±2.37$^{\#}$	NS	0.0376	0.0155
Total Ocy lacunar area (μm^2)	1449±155	1543±148	2242±106†‡	1484±177$^{\#}$	0.0221	0.0131	0.0088
Ocy lacunar perimeter (μm)	19.91±0.71	20.12±0.76	23.15±0.40†‡	20.34±0.98$^{\#}$	NS	0.0187	0.0476
Ocy number	63.67±4.05	64.00±4.69	72.86±3.20	62.17±2.06	NS	NS	NS
Ocy density (x10^{-4}/μm^3)	4.99±0.38	4.78±0.35	6.54±0.31†‡	4.97±0.18$^{\#}$	0.0073	0.0092	0.0423

$^{†}p$<0.05 compared to virgin *Ctsk*$^{fl/fl}$, Two-way ANOVA followed by Fisher's PLSD
$^{‡}p$<0.05 compared to virgin *Ctsk*^{Ocy}
$^{\#}p$<0.05 compared to lactating *Ctsk*$^{fl/fl}$
Ocy density: Ocy number/bone volume

Table S5. Serum, plasma, and urinary markers

Parameters	Virgin		Lactating		Two-way ANOVA		
	Ctsk$^{fl/fl}$ (n=5-9)	***Ctsk^{Ocy}*** (n=5-9)	***Ctsk$^{fl/fl}$*** (n=5-9)	***Ctsk^{Ocy}*** (n=5-9)	**Deletion**	**Lactation**	**Interaction**
PTH (pg/ml)	108.37±31.1	94.2±35	70.14±12†	106.14±40.5	NS	NS	0.05
PTHrP (pM)	1.3±0.13	1.48±0.11	1.95±0.23†	1.6±0.18	NS	<0.05	NS
1,25(OH)$_2$D (pmol/L)	104.1±52.2	113.54±51.7	776.7±199†‡	1029±227$^{\dagger\ddagger\#}$	NS	<0.001	NS
CTX-1 (ng/ml)	21.12±3.24	23.16±3	28.1±6†	25.2±3.3	NS	<0.05	NS
P1NP (ng/ml)	6.7±1.9	5.3±2.1	5.1±2	7.15±3.4	NS	NS	NS
Serum Ca(mg/dl)	7.86±0.088	7.65±0.18	7.95±0.16	8.09±0.18	NS	NS	NS
Milk Ca(mg/mg protein)	--------	--------	2.19±0.15	2.28±0.32	-	-	-

$^{\dagger}p<0.05$ compared to virgin *Ctsk$^{fl/fl}$*, Two-way ANOVA followed by Fisher's PLSD
$^{\ddagger}p<0.05$ compared to virgin *Ctsk^{Ocy}*
$^{\#}p<0.05$ compared to lactating *Ctsk$^{fl/fl}$*

Table S6. Q-RT-PCR.

Gene	Virgin		Lactating		Two-way ANOVA		
	Ctsk$^{fl/fl}$	***Ctsk^{Ocy}***	***Ctsk$^{fl/fl}$***	***Ctsk^{Ocy}***	**Deletion**	**Lactation**	**Interaction**
	(n=4-6)	(n=3-8)	(n=4-6)	(n=3-6)			
Rankl	1.09±0.17	4.6±0.49†	6.2±0.51†	4.0±0.9$^{\dagger\#}$	NS	0.0011	0.0001
Opg	1.05±0.19	2.23±0.63†	0.81±0.1‡	0.82±0.2‡	NS	0.030	NS
Rankl/Opg	1.1±0.23	2.85±0.86	8.06±0.81†‡	5.27±0.79$^{\dagger\ddagger\#}$	NS	<0.0001	0.0031
PthrP	1.7±0.61	6.52±2.5†	11.47±1.8†	9.89±1.55†	NS	0.001	0.0415
Sost	1.06±0.2	0.42±0.35†	0.07±0.02†	0.06±0.03	0.04	0.0002	NS
Mmp13	1±0.06	1.29±0.25	3.1±1.03	6.27±2.9†‡	NS	0.038	NS
Fgf23	1.15±0.28	0.47±0.07†	0.25±0.13†	0.18±0.05†	NS	0.007	NS
Ctsk	1.1±0.19	0.61±0.06	4.3±1.53†‡	1.8±0.64$^{\#}$	0.022	0.0024	NS

$^{\dagger}p<0.05$ compared to virgin *Ctsk$^{fl/fl}$*, Two-way ANOVA followed by Fisher's PLSD
$^{\ddagger}p<0.05$ compared to virgin *Ctsk^{Ocy}*
$^{\#}p<0.05$ compared to lactating *Ctsk$^{fl/fl}$*

Table S7. Three-point bending analysis of virgin and lactating *Ctsk*^{Ocy} mice and their control littermates

Parameters	Virgin		Lactating		Two-way ANOVA		
	***Ctsk*$^{fl/fl}$**	***Ctsk*^{Ocy}**	***Ctsk*$^{fl/fl}$**	***Ctsk*^{Ocy}**	**Deletion**	**Lactation**	**Interaction**
	(n=7)	(n=6-7)	(n=7)	(n=7)			
pMOI (mm^4)	0.42±0.01	0.42±0.01	0.36±0.02†‡	0.41±0.02	NS	0.0333	NS
I_{min} (mm^4)	0.149±0.004	0.146±0.004	0.128±0.007†‡	0.139±0.005	NS	0.0127	NS
Max bending moment (N)	14.57±0.68	13.78±0.30	9.67±0.39†‡	11.61±0.46$^{†‡\#}$	NS	0	0.0089
Failure moment (N-mm)	36.42±1.71	34.45±0.75	24.18±0.97†‡	29.04±1.56$^{†‡\#}$	NS	0	0.0089
Yield load (N)	12.61±1.00	10.31±0.45†	8.00±0.41†	9.01±0.93	NS	0.0006	0.0359
Post yield deformation (mm)	0.49±0.11	0.68±0.15	0.67±0.13	0.77±0.12	NS	NS	NS
Bending stiffness (N-mm^2)	1351±91	1331±57	945±52†‡	1100±49†	NS	0.0001	NS
Estimated modulus (GPa)	9.05±0.43	9.15±0.47	7.45±0.42†	7.97±0.45‡	NS	0.0044	NS
Fracture energy (mJ)	7.05±0.90	8.62±1.38	6.13±0.73	8.00±0.62	NS	NS	NS
Estimated strength (MPa)	168±5	161±1	131±4†‡	141±5†‡	NS	0	0.0450

$^{†}p<0.05$ compared to virgin *Ctsk*$^{fl/fl}$, Two-way ANOVA followed by Fisher's PLSD
$^{‡}p<0.05$ compared to virgin *Ctsk*^{Ocy}
$^{\#}p<0.05$ compared to lactating *Ctsk*$^{fl/fl}$