

Supplementary Table 1: Diet Formulation

Ingredients	Control Diet (AIN-93M)	TC Diet (10%)	FOS Diet (10%)
	(g/kg diet)	(g/kg diet)	(g/kg diet)
TC¹ or FOS	0.0	100.0	100.0
Carbohydrates			
Cornstarch (g)	465.7	383.6	365.7
Maltodextrin (g)	155.0	155.0	155.0
Sucrose (g)	100.0	100.0	100.0
TC (g)	0.0	82.1	
Protein			
Casein (g)	140.0	133.5	140.0
TC (g)	0.0	6.5	
Fat			
Soybean oil (g)	40.0	39.4	40.0
TC (g)	0.0	0.59	
Fiber			
Cellulose(g)	50.0	48.1	50.0
TC (g)	0.0	1.92	
Vitamin mix	10.0	10.0	10.0
Mineral			
Mineral mix ²	13.4	13.4	13.4
Calcium Carbonate (40.04% Ca)	12.5	12.3	12.5
Sodium Phosphate, monobasic (25.81 % P)	5.6	5.5	5.6
Potassium Phosphate, monobasic (22.76% P)	2.4	2.4	2.4
Sucrose	1.1	1.3	1.1
Choline Bitartrate	2.5	2.5	2.5
L-cysteine	1.8	1.8	1.8

¹TC (tart cherry) analysis performed by NP Analytical Laboratories (St Louis, MO).² Calcium and phosphorus deficient mineral mix (TD 98057 Envigo Laboratories) was used so that calcium and phosphorus could be adjusted for the TC diet.

Supplementary Table 2: Primer Sequence List for qRT-PCRs

Symbol	Name	Sequence
<i>Bmp2</i>	Bone morphogenetic protein 2	QF 5'- GGACATCCGCTCCACAAA -3' QR 5'- GGCGCTTCCGCTGTTT-3'
<i>Bsp</i>	Bone sialoprotein	QF 5'- ACACCCCAAGCACAGACTTTTG -3' QR 5'- TCCTCGTCGCTTTCCTTCACT -3'
<i>Ccr7</i>	C-C Motif Chemokine Receptor 7	QF 5'- GGTGGCTCTCCTTGTCATTTTC -3' QR 5'- GTGGTATTCTCGCCGATGTAGTC -3'
<i>Colla1</i>	Type I collagen called the pro- α 1	QF 5'- CGTCTGGTTTGGAGAGAGCAT -3' QR 5'- GGTCAGCTGGATAGCGACATC -3'
<i>Cx43</i>	Connexin 43	QF 5'- ACAGCGGTTGAGTCAGCTTG-3' QR 5'- GAGAGATGGGAAGGACTTGT-3'
<i>Cxc12</i>	C-X-C Motif Chemokine Ligand 12	QF 5'- CAAGCATCTGAAAATCCTCAACAC -3' QR 5'- TCTTCAGCCGTGCAACAATC -3'
<i>Cxcl10</i>	C-X-C Motif Chemokine Ligand 10	QF 5'- AGTGCTGCCGTCATTTTCTG -3' QR 5'- ATTCTCACTGGCCCGTCAT-3'
<i>Cxcr4</i>	C-X-C Motif Chemokine Receptor 4	QF 5'- TCAGTGGCTGACCTCCTCTT -3' QR 5'- CTTGGCCTTTGACTGTTGGT-3'
<i>Dmp-1</i>	Dentin matrix acidic phospho protein 1	QF 5'- CTGTCATTCTCCTTGTGTTTCCT -3' QR 5'- CAAATCACCCGTCCTCTCTTC -3'
<i>Gapdh</i>	Glucose 6 phosphate dehydrogenase	QF 5'- GAACGCAAAGCTGAAGTGAGACT -3' QR 5'- TCATTACGCTTGCCTGTTGGT -3'
<i>Gpr41</i>	G protein-coupled receptor 41	QF 5'-TCCTGGCATCGGCTCACT -3' QR 5'- TGTAGGTTGCATTTCCCCAGTA -3'
<i>Gpr43</i>	G protein-coupled receptor 43	QF 5'- TTCCCATGGCAGTCACCAT -3' QR 5'- GGGCTGCGTGAGCATGAT -3'
<i>Gpr109a</i>	G protein-coupled receptor 109a	QF 5'- TCGAAAGAAAACATTGGGTGAA -3' QR 5'- CCCCCTGAGCTCCACACT-3'
<i>Hprt1</i>	Hypoxanthine-guanine phosphoribosyl transferase	QF 5'- GCCTAAGATGAGCGCAAGTTG -3' QR 5'- TACTAGGCAGATGGCCACAGG -3'
<i>Il6</i>	Interleukin 6	QF 5'- GAGGATACCACTCCCAACAGACC -3' QR 5'- AAGTGCATCATCGTTGTTTCATACA -3'
<i>Il10</i>	Interleukin 10	QF 5'- GGTTGCCAAGCCTTATCGGA -3' QR 5'- ACCTGCTCCACTGCCTTGCT -3'
<i>Il17</i>	Interleukin 17	QF 5'- ATCCCTCAAAGCTCAGCGTGTC -3' QR 5'- GGGTCTTCATTGCGGTGGAGAG-3'
<i>Il22</i>	Interleukin 22	QF 5'- TGCCTTTCCTGACCAAACCTCA -3' QR 5'- CTGGTCGTCACCGCTGATG -3'
<i>Il23</i>	Interleukin 23	QF 5'- CGCCAAGGTCTGGCTTTTTTAT -3' QR 5'- CAGGCTCCCCTTGAAGATG -3'
<i>Mepe</i>	Matrix extracellular phospho glycoprotein	QF 5'- CCCCAGAGCAGCAAAGGTA -3' QR 5'- CTCCGCTGTGACATCCCTTTA -3'
<i>Ocn (Bglap2)</i>	Osteocalcin (Bone gamma-carboxyglutamate protein 2)	QF 5'- TGAGCTTAACCCTGCTTGTGACGA -3' QR 5'- AGGGCAGCACAGGTCCTAAATAGT -3'
<i>Opg</i>	Osteoprotegerin	QF 5'- TCCCGAGGACCACAATGAAC -3' QR 5'- TGGGTTGTCCATTCAATGATGT -3'
<i>Opn</i>	Osteopontin	QF 5'- ACTCCAATCGTCCCTACAGTCG -3' QR 5'- TGAGGTCCTCATCTGTGGCAT -3'
<i>Osx</i>	Osterix	QF 5'- GAAGTTCACCTGCCTGCTCTGT -3' QR 5'- CGTGGGTGCGCTGATGT -3'
<i>Phex</i>	Phosphate regulating endopeptidase X linked	QF 5'- GGCATGACTGCTGTAAGATCAGAT -3' QR 5'- AGCTCCATTGACATAAGGCACT -3'

<i>Rankl</i>	Receptor activator of nuclear factor kappa-B ligand	QF 5'- TCTGCAGCATCGCTCTGTTC -3' QR 5'- AGCAGTGAGTGCTGTCTTCTGATATT- 3'
<i>Runx2</i>	Runt-related transcription factor 2	QF 5'- CGACAGTCCCAACTTCCTGT-3' QR 5'- CGGTAACCACAGTCCCATCT -3'
<i>Sost</i>	Sclerostin	QF 5'- ACCGGGCGGAGAATGG -3' QR 5'- GCTGTACTCGGACACATCTTTGG -3'
<i>Tgfb</i>	Transforming growth factor beta	QF 5'- CCCTATATTTGGAGCCTGGA -3' QR 5'- CTTGCGACCCACGTAGTAGA -3'
<i>Tnfa</i>	Tumor necrosis factor alpha	QF 5'- CTGAGGTCAATCTGCCCAAGTAC -3' QR 5'- CTTACAGAGCAATGACTCCAAAG-3'
<i>Vcam1</i>	Vascular Cell Adhesion Molecule 1	QF 5'- TGAACCCAAACAGAGGCAGAGT -3' QR 5'- GGTATCCCATCACTTGAGCAGG -3'
<i>Wnt10b</i>	Wingless-type MMTV integration site family, member 10b	QF 5'- ATGCGGATCCACAACAACAG-3' QR 5'- TTCCATGGCATTGCACTTC- 3'

Supplementary Table 3: Additional Trabecular and Cortical Bone Microarchitectural Parameters of the Lumbar Vertebra and Tibia

	Con		TC		FOS		<i>P-values</i>		
	- CD25	+ CD25	- CD25	+ CD25	- CD25	+ CD25	CD25	Diet	CD25*Diet
Vertebra Trabecular Bone									
TbN (1/mm ²)	4.18 ± 0.07	4.29 ± 0.10	4.20 ± 0.08	4.46 ± 0.01	5.00 ± 0.07 [#]	4.97 ± 0.11 [#]	0.1238	< 0.0001	0.2547
TbTh (μm)	51.58 ± 0.79	51.55 ± 1.04	49.36 ± 0.57 [#]	49.95 ± 0.89 [#]	55.08 ± 0.37 [#]	54.88 ± 0.91 [#]	0.8579	< 0.0001	0.8764
TbSp (μm)	238.00 ± 4.35	230.38 ± 6.45	232.86 ± 4.47	222.88 ± 5.30	196.13 ± 3.44 [#]	198.63 ± 5.68 [#]	0.2317	< 0.0001	0.2317
ConnDens (1/mm ³)	160.48 ± 7.66	164.89 ± 7.40	158.07 ± 5.37	187.15 ± 7.30	240.54 ± 9.97 [#]	241.51 ± 14.92 [#]	0.1026	< 0.0001	0.1414
SMI	1.28 ± 0.07	1.36 ± 0.06	1.46 ± 0.02 [#]	1.46 ± 0.07 [#]	0.73 ± 0.04 [#]	0.70 ± 0.09 [#]	0.8116	< 0.0001	0.5710
Tibia Trabecular Bone									
TbN (1/mm ²)	3.84 ± 0.08	3.79 ± 0.13	3.76 ± 0.10	3.64 ± 0.09	4.39 ± 0.10 [#]	4.51 ± 0.08 [#]	0.8119	< 0.0001	0.4473
TbTh (μm)	47.22 ± 0.68	45.19 ± 0.89	45.74 ± 1.31	45.40 ± 1.10	47.69 ± 0.64 ^{\$}	48.18 ± 0.65 ^{\$}	0.3937	0.0330	0.3581
TbSp (μm)	255.43 ± 5.00	259.94 ± 9.18	261.88 ± 7.07	270.14 ± 6.88	219.78 ± 5.54 [#]	212.53 ± 3.98 [#]	0.7301	< 0.0001	0.4665
ConnDens (1/mm ³)	81.73 ± 4.69	78.67 ± 7.40	93.10 ± 9.34	81.66 ± 6.02	145.96 ± 12.34 [#]	154.87 ± 7.22 [#]	0.5925	< 0.0001	0.4719
SMI	2.14 ± 0.10	2.17 ± 0.06	1.99 ± 0.09 [#]	1.97 ± 0.04 [#]	1.83 ± 0.08 [#]	1.80 ± 0.04 [#]	0.8420	0.0001	0.9173
Tibia Cortical Bone									
MedullaryArea (μm ²)	28.1 ± 0.60	27.90 ± 0.80	27.7 ± 0.60	27.60 ± 1.10	30.9 ± 0.70 [#]	29.10 ± 0.70 [#]	0.3417	0.0076	0.4884
Porosity (%)	3.88 ± 0.06	3.93 ± 0.11	3.93 ± 0.04	3.77 ± 0.12	4.1 ± 0.10	4.07 ± 0.13	0.2124	0.0809	0.7615

Control diet = Con; Tart cherry diet = TC; Fructooligosaccharide diet = FOS; Isotype control antibody = - CD25; CD25 antibody = +CD25.

Data presented as mean ± SE. n=8 mice/group. When only main effects of diet were detected, # indicates differences between TC or FOS diets vs. Con diet ($P<0.05$).\$ indicated differences between TC vs. FOS diet groups ($P<0.05$).

Supplementary Table 4: T- Lymphocyte Absolute Counts in Ileum and Bone Marrow

	Con		TC		FOS		<i>P-values</i>		
	- CD25	+ CD25	- CD25	+ CD25	- CD25	+ CD25	CD25	Diet	CD25*Diet
Ileum T-Lymphocytes									
CD3 ⁺ CD4 ⁺ (1 x10 ³)	15.43 ± 3.42	27.00 ± 6.12	18.90 ± 3.32	18.17 ± 2.56	10.77 ± 1.27	14.84 ± 2.82	0.0743	0.1048	0.4038
Treg (CD4 ⁺ CD25 ⁺ Foxp3 ⁺)	431 ±102.31 ^b	55 ±12 ^c	821 ± 129 ^a	23 ± 7.01 ^d	457 ± 53 ^{ab}	26 ± 4.01 ^{cd}	< 0.0001	0.8014	0.0030
Th-17 (CD4 ⁺ IL-17 ⁺)	163 ± 40 ^b	223 ± 59 ^{ab}	168 ± 48 ^b	87 ± 19.34 ^b	126 ± 28 ^b	358 ± 44.98 ^a	0.0998	0.0848	0.0131
Bone Marrow T-Lymphocytes									
CD3 ⁺ CD4 ⁺ (1 x 10 ³)	4.47 ± 0.85	3.40 ± 0.94	5.52 ± 1.16	3.79 ± 0.41	14.15 ± 1.10 [#]	10.64 ± 0.92 [#]	0.0391	< 0.0001	0.7621
Treg (CD4 ⁺ CD25 ⁺ Foxp3 ⁺)	886 ± 165	6.75 ± 3.74	788 ± 202	3.5 ± 1.43	2566 ± 121 [#]	11.83 ± 2.18 [#]	< 0.0001	< 0.0001	0.2407
Th-17 (CD4 ⁺ IL-17 ⁺)	60 ± 14	61 ± 20	54 ±11.61	57 ± 15	147 ± 26 [#]	163 ± 19.66 [#]	0.9469	< 0.0001	0.6876

Control diet = Con; Tart cherry diet = TC; Fructooligosaccharide diet = FOS; Isotype control antibody = - CD25; CD25 antibody = +CD25.

Data presented as mean ± SE. n=8/group. Superscript letters note significant interactions (CD25*Diet) and groups that share the same superscript letter are not significantly different from each other. When only main effects of diet were detected, # indicates differences between TC or FOS diets vs. Con diet ($P<0.05$). \$ indicates differences between the TC vs. FOS diet groups ($P<0.05$).

Supplementary Table 5: Bone Gene Expression Data

	Con		FOS		<i>P-values</i>		
	- CD25	+ CD25	- CD25	+ CD25	CD25	Diet	CD25*Diet
Osteoblastogenesis							
<i>Runx 2</i>	1.00 ± 0.14	1.38 ± 0.15	1.39 ± 0.17	1.12 ± 0.21	0.8852	0.5806	0.1806
Osteoblast Markers							
<i>Bsp</i>	1.00 ± 0.23	1.10 ± 0.15	1.78 ± 0.31	0.93 ± 0.16	0.2121	0.4246	0.0923
<i>Bglap2</i>	1.00 ± 0.18	1.59 ± 0.22	2.15 ± 0.38	1.87 ± 0.48	0.8661	0.0848	0.3625
<i>Opn</i>	1.00 ± 0.19	0.85 ± 0.15	1.52 ± 0.23	0.93 ± 0.18	0.1563	0.2700	0.3641
Rank: Rankl:Opg Axis							
<i>RankL</i>	1.00 ± 0.16	0.91 ± 0.1	0.90 ± 0.22	0.81 ± 0.12	0.7798	0.1754	0.8669
<i>Opg</i>	1.00 ± 0.11	0.74 ± 0.17	1.01 ± 0.27	0.73 ± 0.12	0.3621	0.8459	0.3917
<i>RankL:Opg</i>	1.00 ± 0.27	1.48 ± 0.37	0.72 ± 0.06	1.63 ± 0.15	0.0820	0.2180	0.4943

Control diet = Con; Fructooligosaccharide diet = FOS; Isotype control antibody = - CD25; CD25 antibody = +CD25.

Data presented as mean ± SE. n=6/group

Supplementary Figure 1.

Representative 3-D images generated from microCT scans of the (A) lumbar vertebral body and (B) midshaft of the tibia. Control diet = Con; Tart cherry diet = TC; Fructo-oligosaccharide diet = FOS; Isotype control antibody = -CD25; CD25 antibody = +CD25.

