

**Appendix for: O-GlcNAcylation mediates Wnt-stimulated bone formation by
rewiring aerobic glycolysis**

Table of Contents

Appendix Table S1. Related to Fig. 3.....	2
Appendix Table S2. Related to Fig. 3.....	2
Appendix Table S3	3
Appendix Table S4	4

Appendix Table S1. Related to Fig. 3.

μ CT analyses of distal metaphysis of the femurs

Group	BV/TV (%)	Tb.N (1/mm)	Tb.Th (mm)	Tb.Sp (mm)
Ctrl+PBS	22.50 $\pm$ 5.34	5.48 $\pm$ 0.21	0.06 $\pm$ 0.008	0.18 $\pm$ 0.009
OgtCKO+PBS	10.14 $\pm$ 2.11*	3.93 $\pm$ 0.71*	0.044 $\pm$ 0.004	0.26 $\pm$ 0.059*
Ctrl+Scl-Ab	43.35 $\pm$ 6.52**	6.67 $\pm$ 0.36**	0.08 $\pm$ 0.009**	0.13 $\pm$ 0.013
OgtCKO+Scl-Ab	26.62 $\pm$ 1.82***	5.54 $\pm$ 0.30***	0.066 $\pm$ 0.007***	0.17 $\pm$ 0.011

Data are shown as mean $\pm$ SD, n=5.

BV/TV = bone volume over tissue volume; Tb.N = trabecular number; Tb.h = trabecular thickness; Tb.Sp = trabecular space. Data acquired from 100 μ CT slices (1.31 mm) immediately below the distal femoral growth plate. *p<0.05, OgtCKO+PBS versus Ctrl+PBS; **p<0.05, Ctrl+Scl-Ab versus Ctrl+PBS; ***p<0.05, OgtCKO+Scl-Ab versus Ctrl+Scl-Ab, two-way ANOVA followed by Tukey's multiple comparisons test.

Appendix Table S2. Related to Fig. 3.

μ CT analyses of diaphyseal cortical bone of the femurs

Group	Ct.Th (mm)	Tt.Ar (mm ²)	Ct.Ar (mm ²)	Ct.Ar/Tt.Ar (%)
Ctrl+PBS	0.15 $\pm$ 0.014	1.60 $\pm$ 0.19	0.62 $\pm$ 0.087	38.39 $\pm$ 1.63
OgtCKO+PBS	0.11 $\pm$ 0.016*	1.31 $\pm$ 0.099*	0.41 $\pm$ 0.062*	31.12 $\pm$ 3.23*
Ctrl+Scl-Ab	0.21 $\pm$ 0.008**	1.92 $\pm$ 0.20**	0.90 $\pm$ 0.10**	46.93 $\pm$ 0.94**
OgtCKO+Scl-Ab	0.11 $\pm$ 0.011***	1.43 $\pm$ 0.13***	0.45 $\pm$ 0.055***	31.30 $\pm$ 2.01***

Data are shown as mean $\pm$ SD, n=5. Ct.Th = cortical thickness; Tt.Ar = total area; Ct.Ar = cortical area; Ct.Ar/Tt.Ar = cortical area over total area. Data acquired from 50 μ CT slices (0.655 mm) of the mid-diaphyseal of the femur. *p<0.05, OgtCKO+PBS versus Ctrl+PBS; **p<0.05, Ctrl+Scl-Ab versus Ctrl+PBS; ***p<0.05, OgtCKO+Scl-Ab versus Ctrl+Scl-Ab, two-way ANOVA followed by Tukey's multiple comparisons test.

Appendix Table S3

siRNA sequences

Gene name		sequence (5' to 3')
siNC	sense	UUCUCCGAACGUGUCACGUTT
siNC	anti-sense	ACGUGACACGUUCGGAGAATT
siGfat1	sense	GGAAUCUUGCCAAAUCUGUTT
siGfat1	anti-sense	ACAGAUUUGGCAAGAUUCCTT
siOgt	sense	GCUGAUGUCUGCCUGGAUAdTdT
siOgt	anti-sense	UAUCCAGGCAGACAUCAGCdTdT
siOga	sense	GCUGCACGAGAAUAUGAAAdTdT
siOga	anti-sense	UUUCAUAUUCUCGUGCAGCdTdT
si- β -catenin#1	sense	CAGUUGUCAAUUUGAUUAATT
si- β -catenin#1	anti-sense	UUAUAUCAAUUGACAACUGTT
si- β -catenin#3	sense	GGGCAGUAUGCAAUGACUATT
si- β -catenin#3	anti-sense	UAGUCAUUGCAUACUGCCCTT
siHK2	sense	GGACAGAACAUGGCGAGUUTT
siHK2	anti-sense	AACUCGCCAUGUUCUGUCCTT

Appendix Table S4

Primer sequences.

Gene name		sequence (5' to 3')
<i>β-actin</i>	Forward	AGATGTGGATCAGCAAGCAG
	Reverse	GCGCAAGTTAGGTTTTGTCA
<i>Alpl</i>	Forward	CCAACTCTTTTGTGCCAGAGA
	Reverse	GGCTACATTGGTGTTGAGCTTTT
<i>Bglap</i>	Forward	CCAACCGAGTCATTTAAGGCT
	Reverse	GCTCACGTCGCTCATCTTG
<i>Ogt</i>	Forward	TCCTGATTTGTACTGTGTTCGC
	Reverse	AAGCTACTGCAAAGTTCGGTT
<i>Gfat1</i>	Forward	TGGTGTGCGGAGTGAACATAA
	Reverse	GTGTGCTCTATCACGGCACTT
<i>Hk2</i>	Forward	CTAAGGGGTTCAAGTCCAGTGG
	Reverse	AGACCAATCTCGCAGTTCTGA
<i>Runx2</i>	Forward	CCAACCGAGTCATTTAAGGCT
	Reverse	GCTCACGTCGCTCATCTTG
<i>Sp7</i>	Forward	CCCTTCTCAAGCACCAATGG
	Reverse	AAGGGTGGGTAGTCATTTGCATA
<i>Ibsp</i>	Forward	CAGAGGAGGCAAGCGTCACT
	Reverse	GCTGTCTGGGTGCCAACACT