

**Supplementary Table S1 PCR primers used in this study**

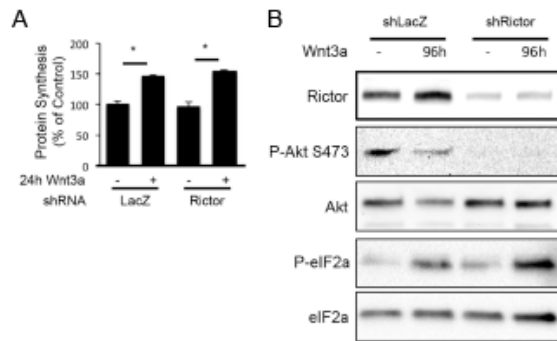
Gene Symbol	Forward	Reverse
18s	CGGCTACCATCAAGGAA	GCTGGAATTACCGCGGCT
Akp2	CCAACCTCTTTTGTGCCAGAGA	GGCTACATTGGTGTGAGCTTTT
Ibsp	CAGAGGAGGCAAGCGTCACT	GCTGTCTGGGTGCCAACACT
ATF4	GCATGCTCTGTTTCGAATGGA	CCAACGTGGTCAAGAGCTCAT
Ddit3	AAGCCTGGTATGAGGATCTGC	GGGGATGAGATATAGGTGCC
Gen2	CCACGAGATTCAGAGAAGGAAAG	TGGGTCTCTCTTAGAGGCATAG
Asns	CAAGGAGCCCAAGTTCAGTAT	GGCTGTCCTCCAGCCAAT
Glyt1	TCATGGCTTTGTCTGTCTGTCAT	GCGGCAGAGCTGGAACA
Lars	GAGCAGCAAGGGCAAATACTT	GAAAACGTGTGTCCCAAATGAAG
Tars	CCCTGGCCTGAATACATTAACAC	CGGCTTGCTATCTTTTGCTGC
Hspa5	GAGACTGCTGAGGCGTATTT	CAGCATCTTTGGTTGCTTGTC
Ero11	AGTCTGCGAGCTACAAGTATTC	TTCCTCACTCAGAGACTCATC
Lef1	CCTACAGCGACGAGCACTTTT	CCTTGCTTGGAGTTGACATCTG
Wnt7b	CAATGGTGGTCTGGTACCCAA	AGTCTCATGGTCCCTTTGTGGTT
Wnt10b	ATGAGAGGTTTTCGGTTGGAAA	CCTCCAAGAGCCTGACAAG

**Supplementary Table S2 shRNA sequences used in this study**

Gene Symbol	Insert Sequence
LacZ	GCGATCGTAATCACCCGAGTG
RFP	ACAACAGCCACAACGTCTATA
ATF4 - 1	GCGAGTGTAAGGAGCTAGAAA
ATF4 - 2	CCAGAGCATTCCTTTAGTTTA
GCN2 - 1	CGGTACTTCATTGAGTTTGAA
GCN2 - 2	GCCTGTCGAATGAAAGTGTTA
Rictor -1	CGAGACTTTGTCTGTCTAATT
Rictor -2	CGGTTCATACAAGAGTTATTT

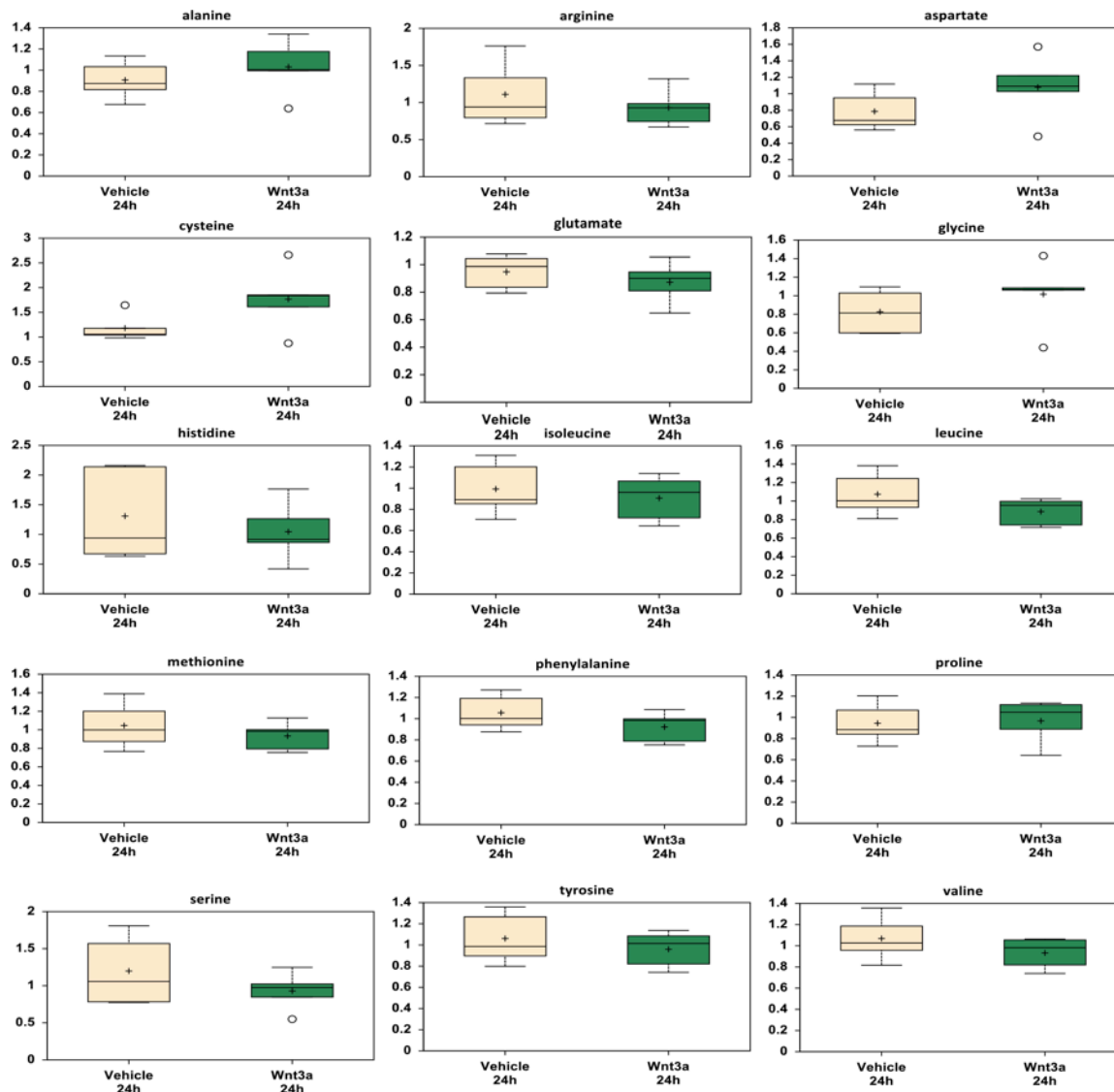
Results from shRNA-1 for each gene shown in paper.

## Supplemental Figures

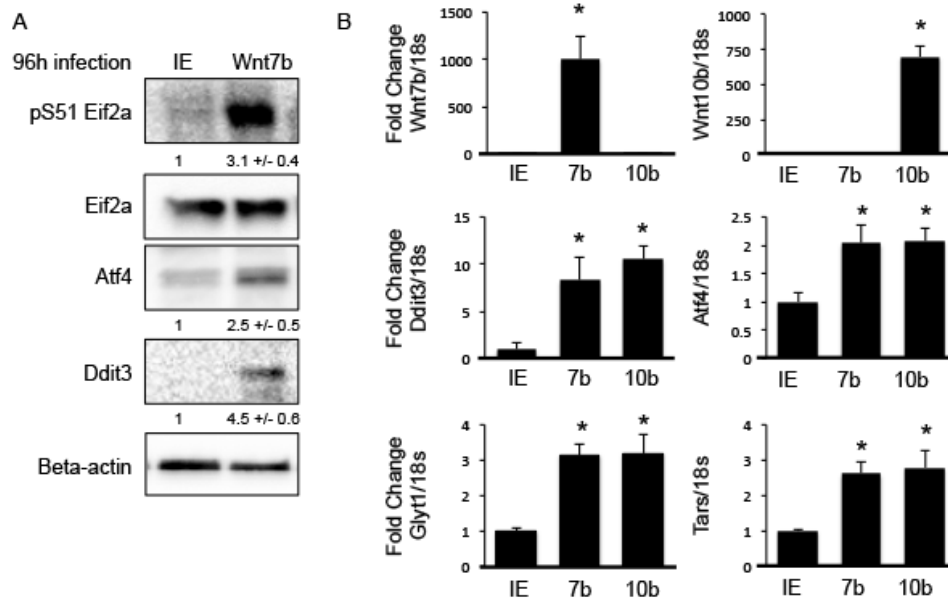


**Figure S1 mTORC2 signaling is dispensable for protein synthesis and ISR induction.**

(A) Metabolic labeling of protein synthesis in response to Wnt3a and Rictor knockdown. Error bar: STDEV. \* $p=0.05$ , Student's  $t$ -test.  $n=3$ . (B) Western blot analyses of ISR in response to Wnt3a and Rictor knockdown.

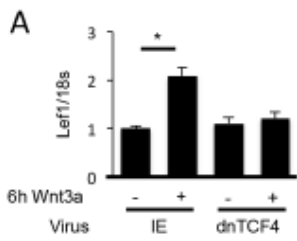


**Figure S2** Metabolomic studies of intracellular amino acid levels in response to Wnt3a or vehicle treatment for 24 hours. N=5. Box denotes upper and lower quartiles while line denotes the median and plus sign denotes the Mean. The whiskers denote the maximum and minimum values of the distribution. Circles denote outliers.



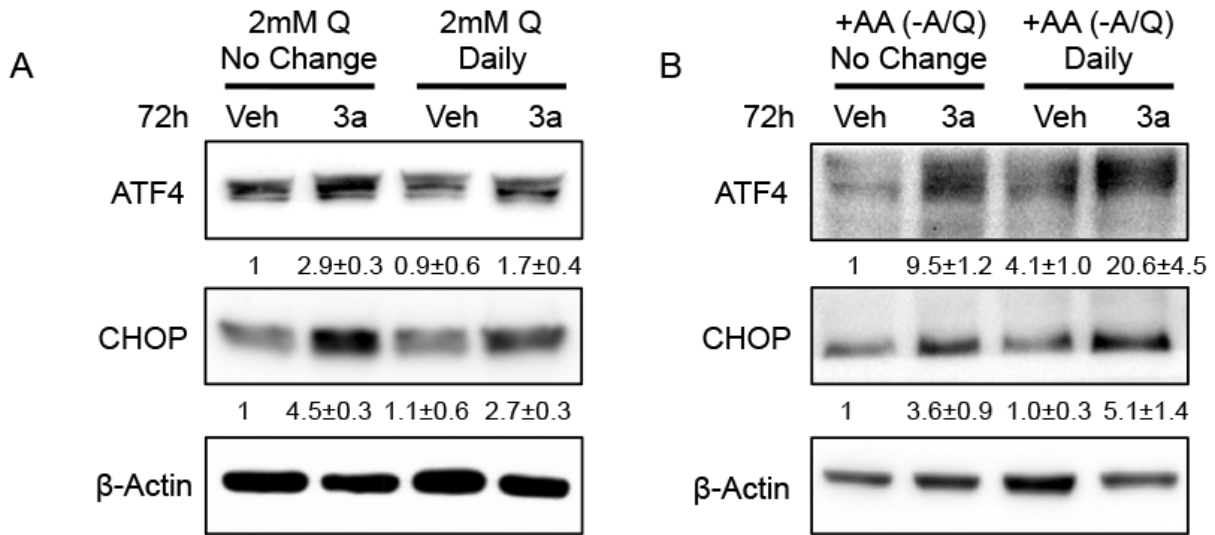
**Figure S3 Induction of anabolic genes and ISR in response to other osteogenic Wnts.**

(A) Western blot analyses of ISR in response to viral expression of Wnt7b. Phospho-proteins normalized to respective total protein; others normalized to  $\beta$ -actin. Images in (A) are derived from samples run on parallel gels. (B) qPCR analyses of anabolic genes in response to Wnt7b or Wnt10b. IE: control virus expressing GFP. Error bar: STDEV. \* $p=0.05$ , Student's *t*-test.  $n=3$ .



**Figure S4 dnTCF blocks induction of the  $\beta$ -catenin target gene Lef1.**

(A) qPCR analyses of the canonical target gene Lef1 in response to Wnt3a treatment for 6 hours in ST2 cells expressing dominant negative TCF4 (dnTCF4). Error bar: STDEV. \* $p=0.05$ , Student's *t*-test.  $n=3$ .



**Figure S5 Daily glutamine but not amino acid supplementation reduces ISR induction by Wnt3a.**

(A) Effect of daily supplementation of glutamine (A) or other amino acids (all except alanine and glutamine) (B) on ATF4 and CHOP induction by Wnt3a. Proteins normalized to β-actin. Fold change ± STDEV for Wnt3a over vehicle in three independent experiments.