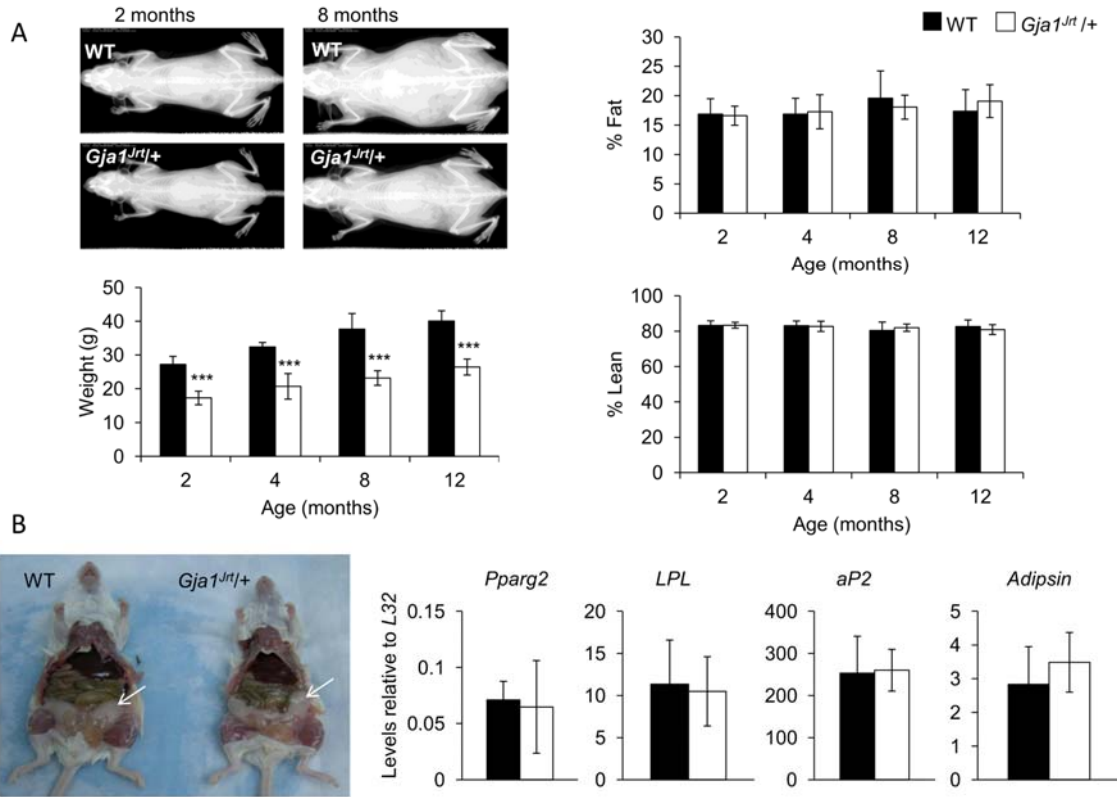


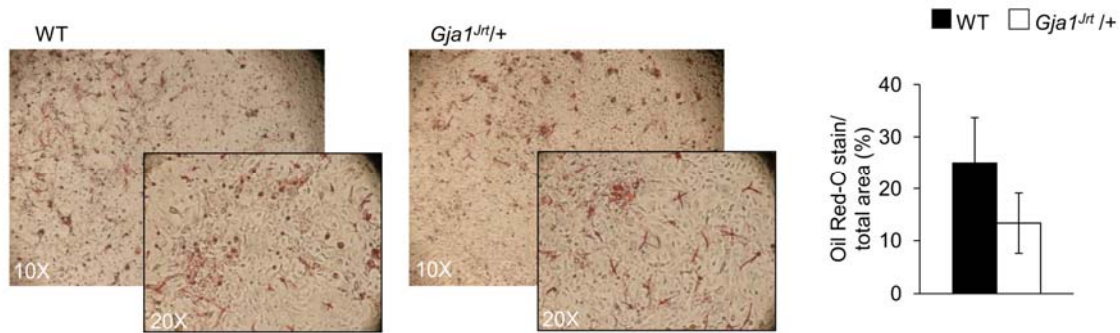
Supplemental Materials

Molecular Biology of the Cell

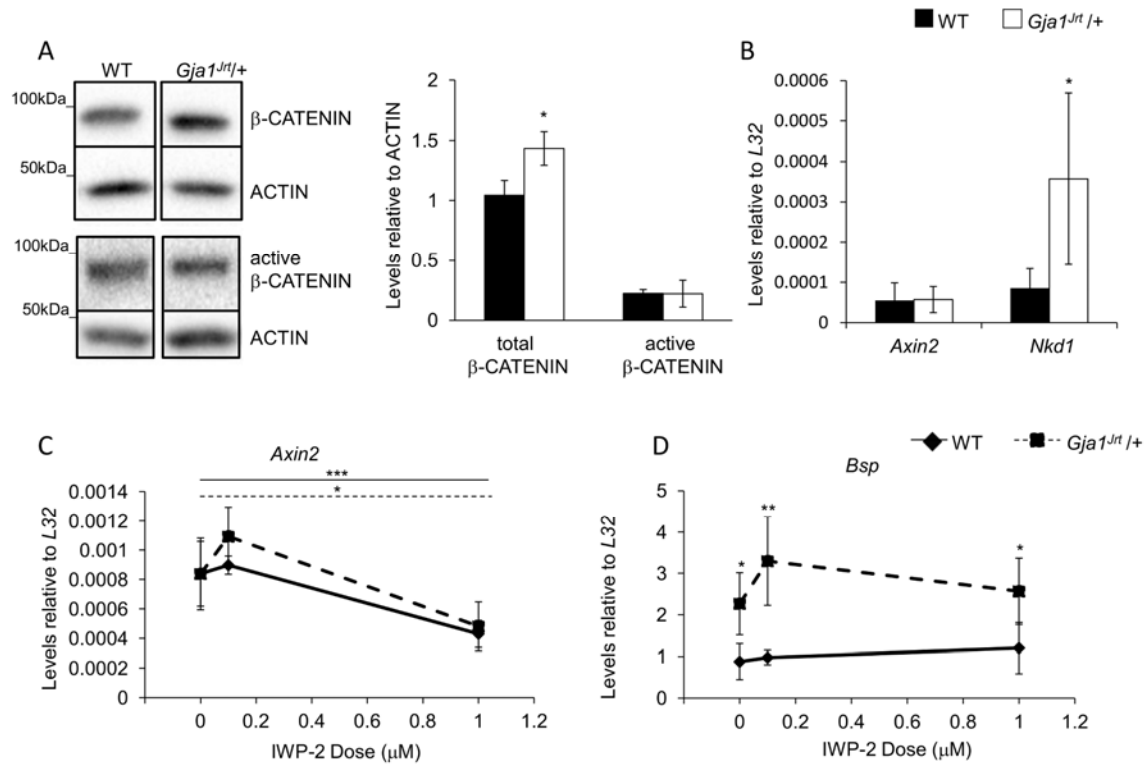
Zappitelli et al.



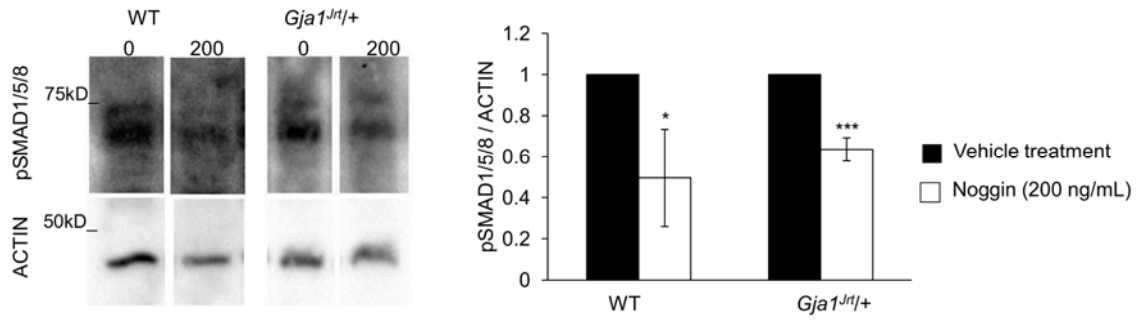
Supplemental Figure 1. The *Gja1^{Jrt}* mutation does not cause a systemic increase in adipogenesis or adipocyte activity. (A) Representative DEXA images of WT and *Gja1^{Jrt/+}* mice at 2 and 8 months of age. Measurements showed that *Gja1^{Jrt/+}* mice had significantly lower body weight than WT littermates at all ages. No differences in percentage fat or percentage lean mass were noted at any age; n = 9. (B) Representative images of WT and *Gja1^{Jrt/+}* epididymal fat pads. Expression of adipocyte-associated markers were unchanged in RNA isolated from epididymal fat pads of WT versus *Gja1^{Jrt/+}* mice; n ≥ 3. *p<0.05, **p<0.01 and ***p<0.001.



Supplemental Figure 2. Effect of the *Gja1^{Jrt}* mutation on formation of bone marrow-derived adipocytes *in vitro*. (A) Representative images of end-point adipogenic stromal cultures stained with Oil Red-O. The stromal cells were cultured under adipogenic conditions for approximately 6 days or until adipocytes could be identified by the presence of visible lipid droplets. (B) The amount of Oil Red-O staining was unaffected between genotypes; n = 3. *p<0.05, **p<0.01 and ***p<0.001.



Supplemental Figure 3. Changes in Wnt/ β -catenin signaling cannot account for the upregulation of *Bsp* expression in hyperactive *Gja1^{Jrt/+}* osteoblasts. (A) The level of total β -CATENIN protein was significantly increased in *Gja1^{Jrt/+}* versus WT confluent osteogenic stromal cultures derived from 4 month old mice. Levels of active β -CATENIN (antibody recognizes the active form of β -CATENIN, dephosphorylated on Ser37 and Thr41) were unchanged. Shown is one representative blot; $n \geq 4$. (B) Expression of direct β -catenin target genes, *Axin2* and *Nkd1*, were unaffected and increased, respectively, in *Gja1^{Jrt/+}* versus WT cells; $n \geq 8$. When confluent osteogenic stromal cells were treated with IWP-2, a Wnt signaling inhibitor, (C) the expression of *Axin2* decreased significantly, but (D) the expression of *Bsp* remained significantly increased in *Gja1^{Jrt/+}* versus WT cells. Expression of *Bsp* was unchanged by IWP-2 treatment in cells of both genotypes; $n \geq 3$. Solid and dashed lines indicate significant differences over dosage concentration in WT and *Gja1^{Jrt/+}* mice, respectively. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.



Supplemental Figure 4. Levels of pSMAD1/5/8 were significantly reduced in both WT and *Gja1^{Jrt/+}* stromal cells treated with 200ng/mL of Noggin versus vehicle treated cells. One representative blot is shown; n = 3. *p<0.05, **p<0.01 and ***p<0.001.

Supplemental Table 1. Quantitative RT-PCR primer sequences used in this study.

Gene	Direction	Sequence
<i>Adipsin</i>	Forward Reverse	TTGCAGGGGAGACTCCGGCAG CTCGGGTATAGACGCCCGGCT
<i>aP2</i>	Forward Reverse	TAACCCTAGATGGCGGGGCC AACACATTCCACCACCAGCTTGT
<i>Axin2</i>	Forward Reverse	GCATCGCAGTGTGAAGGCCAA AGCAGGTTCCACAGGCGTCA
<i>Bsp*</i>	Forward Reverse	CAGGGAGGCAGTGA CTCTTC AGTGTGGAAAGTGTGGCGTT
<i>Bmp2</i>	Forward Reverse	GAGGCGAAGAAAAGCAACAG GGGGAAGCAGCAACACTAGA
<i>Bmp4</i>	Forward Reverse	TTCCTGGTAACCGAATGCTGA CCTGAATCTCGGCGACTTTTT
<i>L32</i> [#]	Forward Reverse	CACAATGTCAAGGAGCTGGAAGT TCTACAATGGCTTTTCGGTTCT
<i>LPL</i>	Forward Reverse	GACTTGCCCTACGGCGCTCC AATCTCTTCCC GCGTCTGCTGC
<i>Nkd1</i>	Forward Reverse	GGAGGACAGCCGGCAAGAGTG ACCCGCAGTGTCTTGCTTGATG
<i>Pparg2</i>	Forward Reverse	TCGCTGATGCACTGCCTATG GAGAGGTCCACAGAGCTGATT
<i>Tcf7</i>	Forward Reverse	AGCCAGAAGCAAGGAGTTCACAGG GCAGGAAGGGGACAGGGGGTAG

Supplemental Table 2. List of antibodies used in this study.

Antigen	Host	Company	Catalogue ID
ACTIN	Rabbit	Sigma-Aldrich	A2066
β -CATENIN (active)	Mouse	Millipore	05-665
β -CATENIN (total)	Rabbit	Abcam	ab6302
CREB	Rabbit	Cell Signaling Technology	4820
Connexin 43	Rabbit	Invitrogen	71-0700
pCREB	Rabbit	Cell Signaling Technology	4276
SMAD 1	Rabbit	Invitrogen	38-5400
pSMAD 1/5/8	Rabbit	Cell Signaling Technology	9511
Anti-mouse IgG-HRP	Goat	Thermo Scientific	LE146795
Anti-rabbit IgG-HRP	Goat	Santa Cruz Biotech., Inc.	sc-2004