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A Primers for quantitative Real-time polymerase chain reaction

Gene targeted	Sense	Antisense
β-actin	5′-TGTTACCAACTGGGACGACA-3′	5′-CTGGGTCATCTTTTCACGGT-3′
GAPDH	5′-ACCCAGAAGACTGTGGATGG-3′	5′-GGATGCAGGGATGATGTTCT-3′
Wnt1	5′-CAGCACCACTAGAGGAAACGA-3′	5′-CTGGGCACATATCTTACAGCATT-3′
Wnt2	5′-CCTCCGAAGTAGTCGGGAATC-3′	5′-GCAGGACTTTAATTCTCCTTGGC-3′
Wnt2b	5′-ACATGCAAGGCCCCTAAG-3′	5′-AGGATTGAGGGTAGAGGAAGG-3′
Wnt3a	5′-GTGAGGACATTGAATTTGGAGG-3′	5′-ACTTGAGGTGCATGTGACTG-3′
Wnt4	5′-TGCGAGGTAAAGACGTGCTG-3′	5′-CTTGAACTGTGCATTCCGAGG-3
Wnt5a	5′-CAACTGGCAGGACTTTCTCAA-3′	5′-CATCTCCGATGCCGGAACT-3′
Wnt5b	5′-GACTGACGCCAACTCCTG-3′	5′-TGCTCCTGATACAACTGACAC-3′
Wnt6	5′-TCAAGACTCTTTATGGATGCGC-3′	5′-ATGGCACTTACACTCGGTG-3′
Wnt7a	5′-ACGAGTGTCAGTTTCAGTTCC-3′	5′-AATCGCATAGGTGAAGGCAG-3′
Wnt7b	5′-AATGAGGCGGGCAGAAAG-3′	5′-TGCGTTGTACTTCTCCTTGAG-3′
Wnt8a	5′-TCATGTACGCAGTCACCAAG-3′	5′-TTTTCCCCGAACTCCACG-3′
Wnt8b	5′-GTACACCCTGACTAGAAACTGC-3′	5′-AAACTGCTTGGAAATTGCCTC-3′
Wnt9a	5′-GGTTCCTGTAGCCCACTAATG-3′	5′-GAAAGAGATGAAGCTCCCGTC-3′
Wnt9b	5′-CCAAGAGAGGAAGCAAGGAC-3′	5′-AACAGGTACGAACAGCACAG-3′
Wnt10a	5′-CGCTTCTCTAAGGACTTTCTGG-3′	5′-GTGGCATTTGCACTTACGC-3′
Wnt10b	5′-ATCGCCGTTCACGAGTGTC-3′	5′-GGAAACCGCGCTTGAGGAT-3′
Wnt11	5′-CCAAGCCAATAAACTGATGCG-3′	5′-GGCATTTACACTTCGTTTCCAG-3′
Wnt16	5′-AGTGCAGGCAACATGACCG-3′	5′-CCACATGCCGTACTGGACATC-3′
Lef1	5′-ACACCCTGATGAAGGAAAGC-3′	5′-GACCCATTTGACATGTACGG-3′
Tcf4	5′-ACGTTTGAGCTATCCATCCC-3′	5′-GTGCAGGAAGAGGTGCTGTA-3′
Axin2	5′-ACACATGCAGAAATGGGTCA-3′	5′-GGACGTCTGTGACAAGCAGA-3′
ALP	5′-ACCTTGACTGTGGTTACTGC-3′	5′-CATATAGGATGGCCGTGAAGG-3′
Oc	5′-TGTGACGAGCTATCAAACCAG-3′	5′-GAGGATCAAGTTCTGGAGAGC-3′
Osx	5′-GGAGACCTTGCTCGTAGATTTC-3′	5′-GGGATCTTAGTGACTGCCTAAC-3′
FABP4	5′-AAGAAGTGGGAGTGGGCTTT-3′	5′-AATCCCCATTTACGCTGATG-3′
PPAR-γ	5′-ATAGGTGTGATCTTAACTGCCG-3′	5′-CCAACAGCTTCTCCTTCTCG-3′

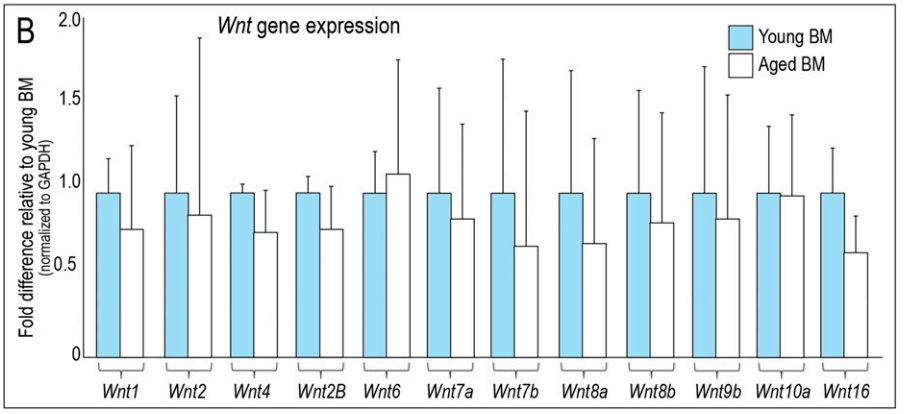

Fig. E-1

Fig. E-1A Primers used for quantitative real-time polymerase chain reaction. **Fig. E-1B** Quantitative real-time polymerase chain reaction to evaluate relative expression levels of Wnt ligands in bone marrow harvested from young (blue bars; n = 3) and aged (white bars; n = 3) bone marrow (BM) donors. Gene expression levels normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

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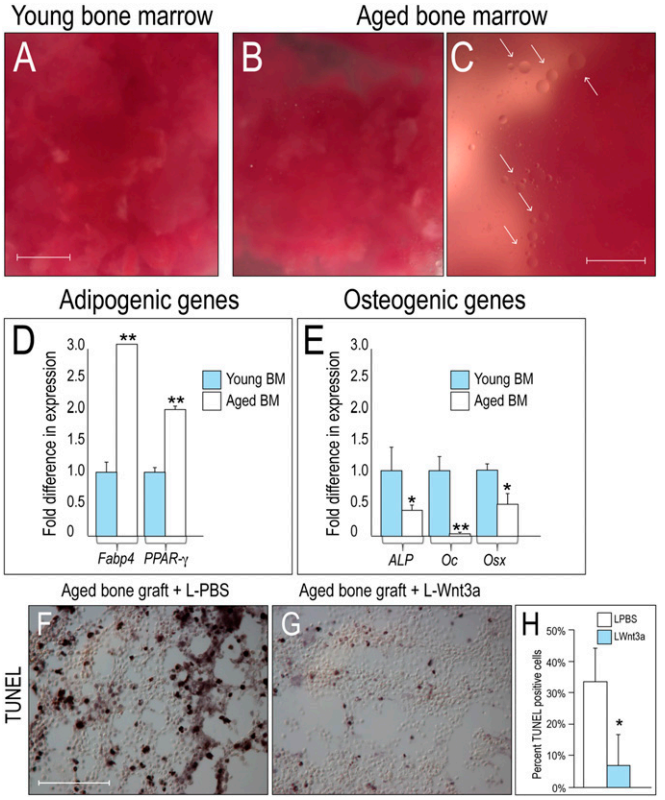

Fig. E-2

Aged bone marrow undergoes fatty degeneration. Compared with the gross appearance of bone marrow harvested from young donors **(Fig. E-2A)**, bone marrow from aged donors is fatty (**Fig. E-2B**); arrows indicate fat droplets (**Fig. E-2C**). **Fig. E-2D** Quantitative reverse transcription-polymerase chain reaction(qRT-PCR) analyses for the adipogenic genes fatty acid-binding protein 4 (Fabp4) and peroxisome proliferator-activated receptor gamma (PPAR-γ) in bone marrow (BM) from young animals (blue bars, n = 3) compared with bone marrow from aged animals (white bars; n = 3). **Fig. E-2E** qRT-PCR analyses for the osteogenic genes alkaline phosphatase (ALP), osteocalcin (Oc), and osterix (Osx) in bone marrow (BM) from young animals (blue bars; n = 3) compared with bone marrow from aged animals (white bars; n = 3). Gene expression levels normalized to beta-actin. **Figs. E-2F and E-2G** Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining in aged bone marrow following a twelve-hour incubation in liposomal phosphate-buffered saline solution (L-PBS) (**Fig. E-2F**) (n = 3) or L-Wnt3a (**Fig. E-2G**) (n = 3; effective concentration = 150 ng/mL), quantified in **Fig. E-2H**. Single asterisk denotes $p < 0.05$; double asterisk denotes $p < 0.01$. Scale bars: 1 mm (Fig. E-2A [scale bar in Fig. E-2A also applies to Fig. E-2B]); 400 μm (Fig. E-2C); and 50 μm (Fig. E-2F [scale bar in Fig. E-2F also applies to Fig. E-2G]).

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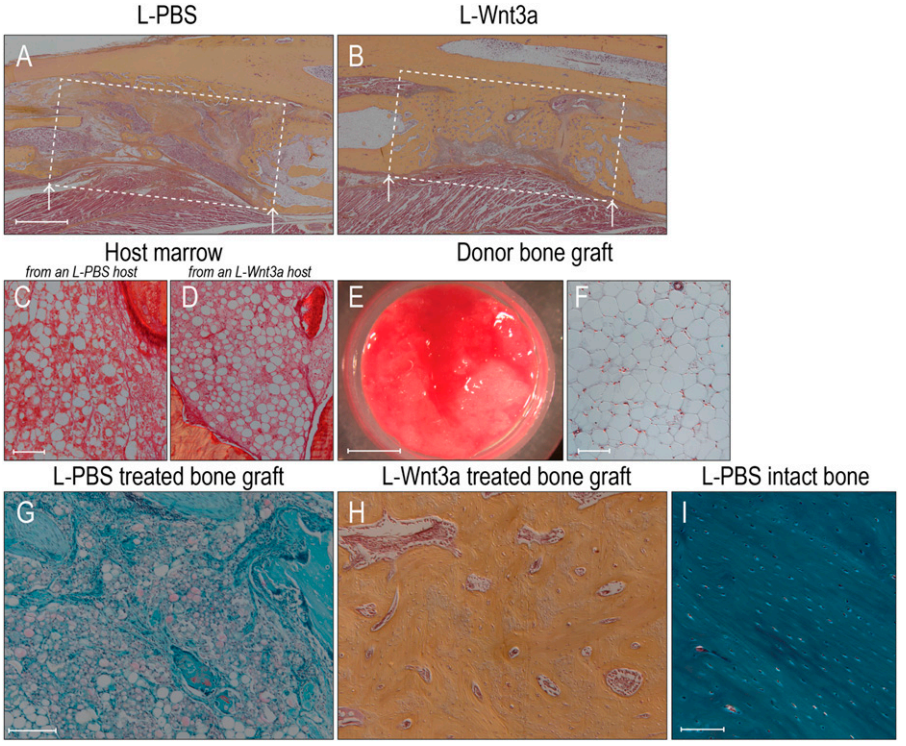

Fig. E-3

Movat pentachrome staining of injury site (boxed area) treated with aged bone marrow incubated in liposomal phosphate-buffered saline solution (L-PBS) (**Fig. E-3A**) or L-Wnt3a (**Fig. E-3B**). **Fig. E-3C and E-3D** Under polarized light, picrosirius red staining confirms that aged host rabbits treated with L-PBS (**Fig. E-3C**) or liposomal Wnt3a protein (L-Wnt3a) (**Fig. E-3D**) both showed evidence of fatty degeneration in their bone marrow. **Fig. E-3E** Morphology of fatty bone marrow harvested from an aged donor rabbit, prior to transplantation. **Fig. E-3F** Gömöri trichrome staining confirms that the graft consists of fatty bone marrow. **Fig. E-3G** Safranin O/fast green staining identifies cartilage, fibrocartilage, immature osteoid, and adipose in bone grafts treated with L-PBS. **Fig. E-3H** Pentachrome staining identifies new osteoid matrix with large blood vessel spaces formed in bone grafts treated with L-Wnt3a. Compare this new bone matrix with Gömöri trichrome (**Fig. E-3I**) staining of the host's intact bone cortex, which has a lamellar organization, typical of mature bone. Arrows mark the edge of intact bone. Scale bars: 500 μm (Fig. E-3A [scale bar in Fig. E-3A also applies to Fig. E-3B]); 200 μm (Fig. E-3C [scale bar in Fig. E-3C also applies to Fig. E-3D]); 250 μm (Fig. E-3E); 100 μm (Fig. E-3F); 200 mm (Fig. E-3G [scale bar in Fig. E-3G also applies to Fig. E-3H]); and 100 μm (Fig. E-3I).