

Small molecule-mediated tribbles homolog 3 promotes bone formation induced by bone morphogenetic protein-2

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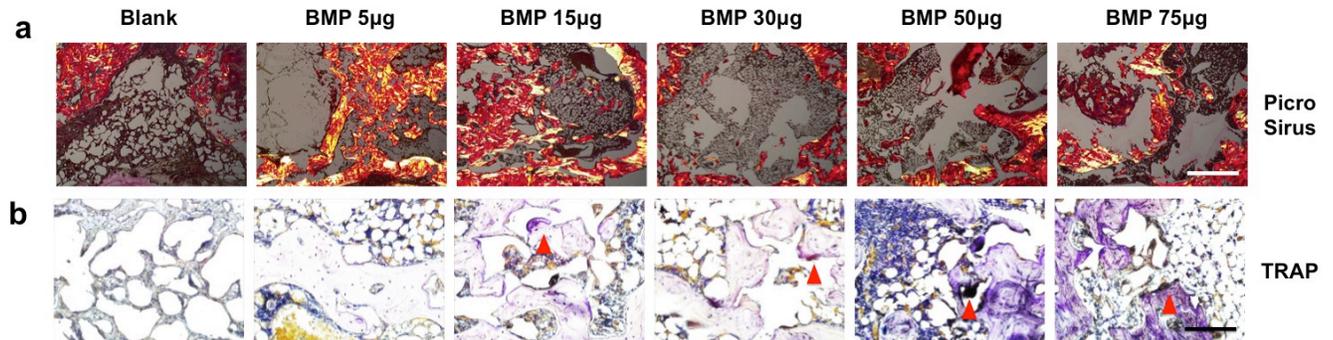
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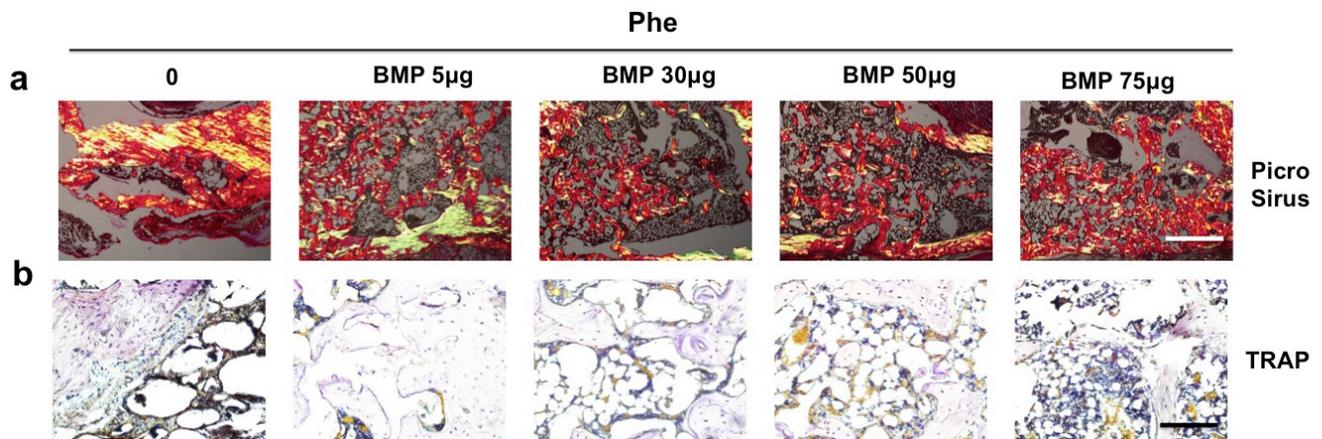
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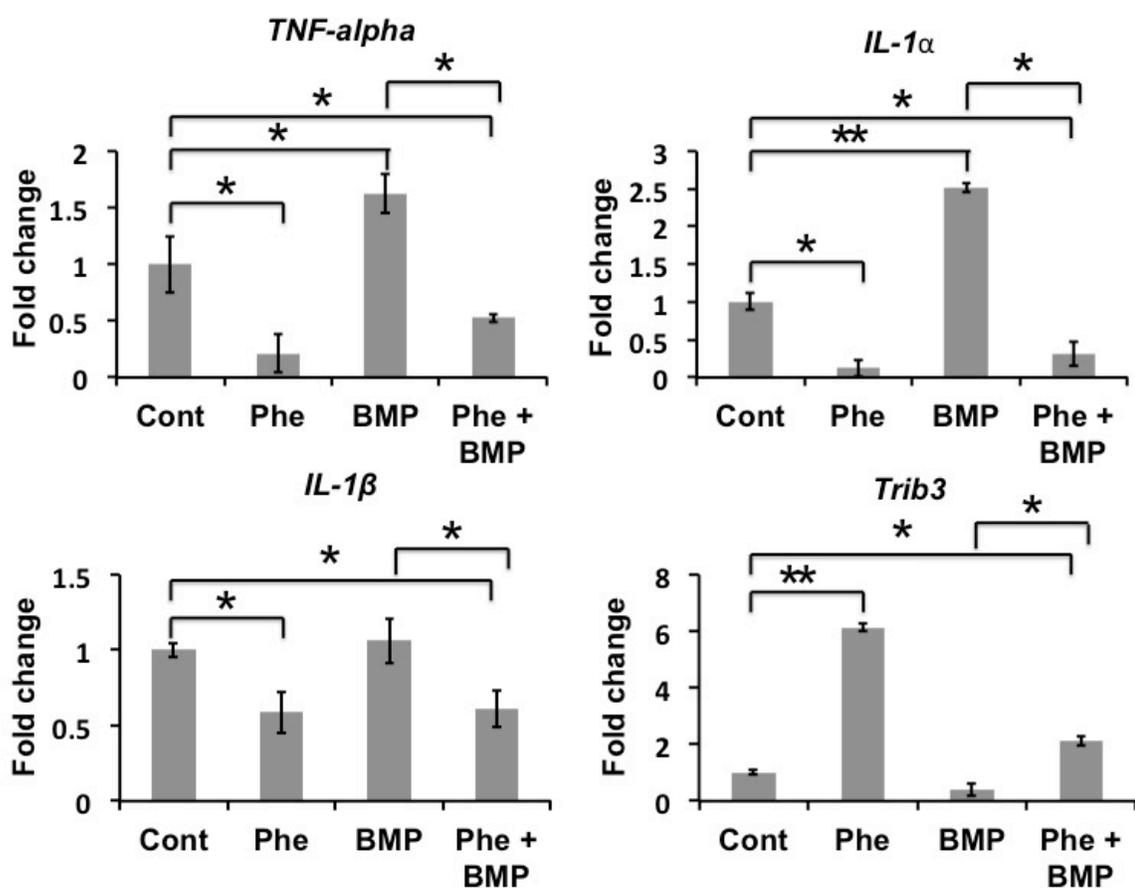
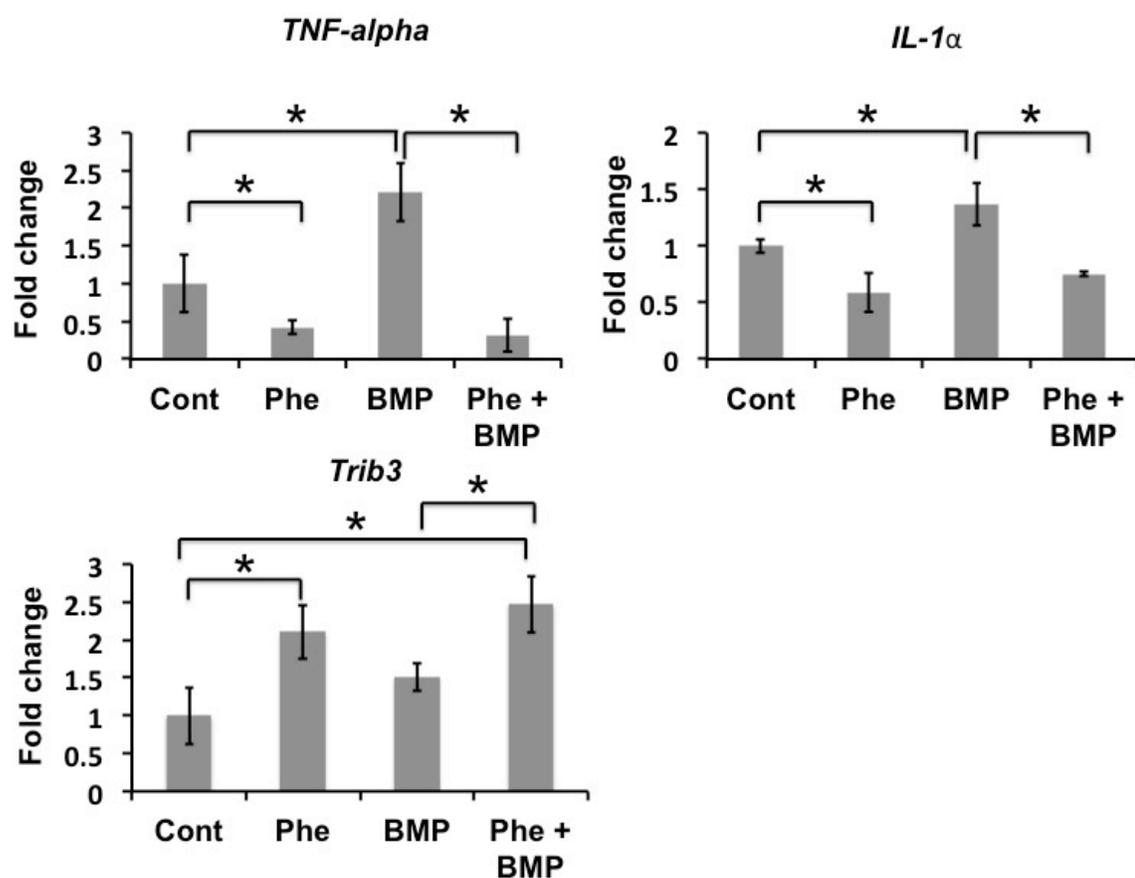
Supplementary Figures:



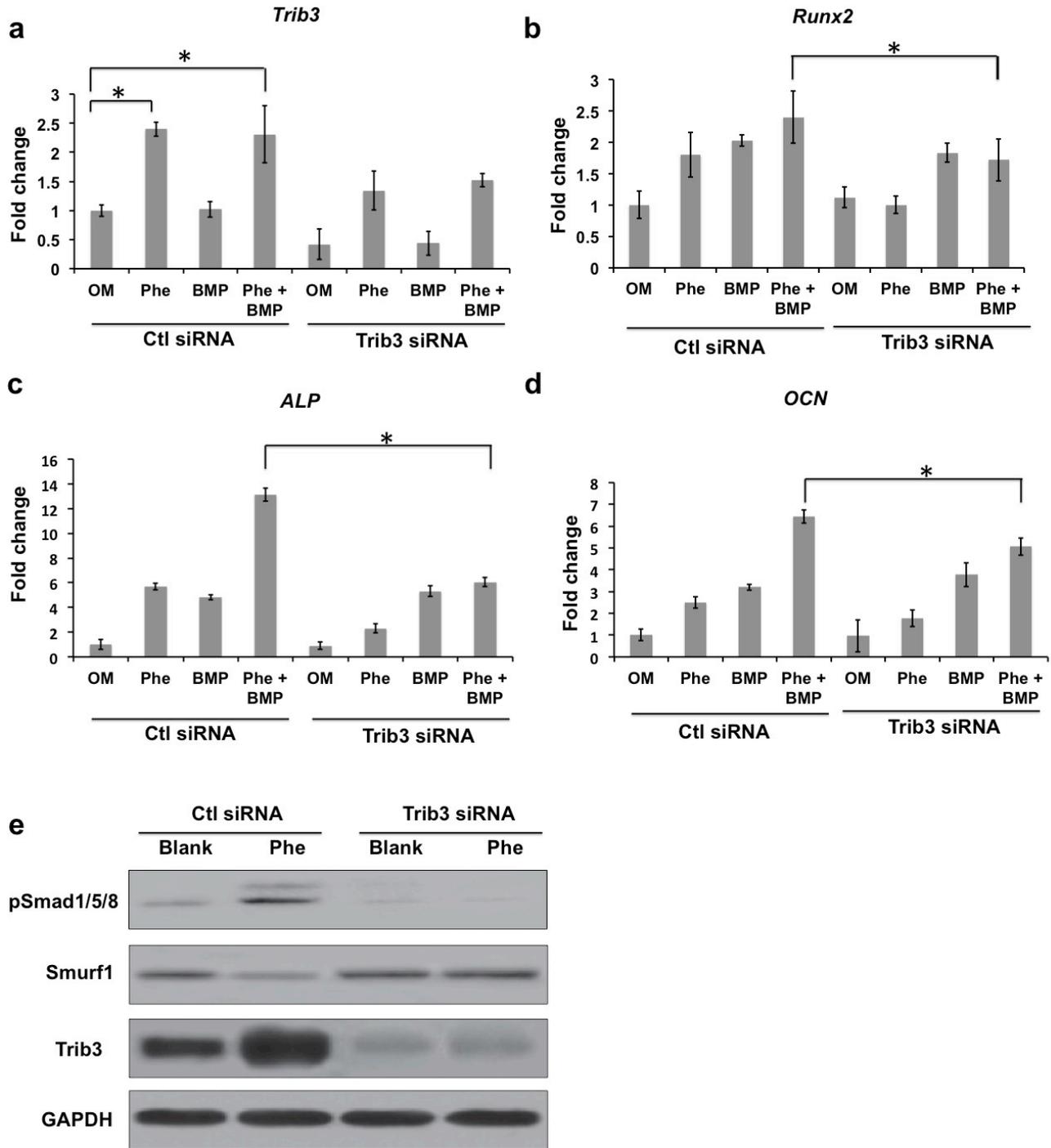
Supplementary Figure 1: High doses BMP2-induced cyst-like hollow bone formation detected in the rat mandible defect after 8 weeks postoperatively. (a) Picrosirius red staining with polarized images (scale bar = 500 µm); (b) TRAP staining for identifying osteoclasts (scale bar = 100 µm). Red arrowhead indicates osteoclasts along the edge of bone. Blank, blank scaffold; BMP, BMP2; TRAP, tartrate-resistant acid phosphatase.



Supplementary Figure 2: Extra use of phenamil (600 μ M, total 12 μ g per defect) suppressed high doses BMP2-induced cyst-like hollow bone formation in the rat mandible defect after 8 weeks postoperatively. (a) Picrosirius red staining with polarized images (scale bar = 500 μ m); (b) TRAP staining for identifying osteoclasts (scale bar = 100 μ m). Blank, blank scaffold; BMP, BMP2; Phe, phenamil; TRAP, tartrate-resistant acid phosphatase.

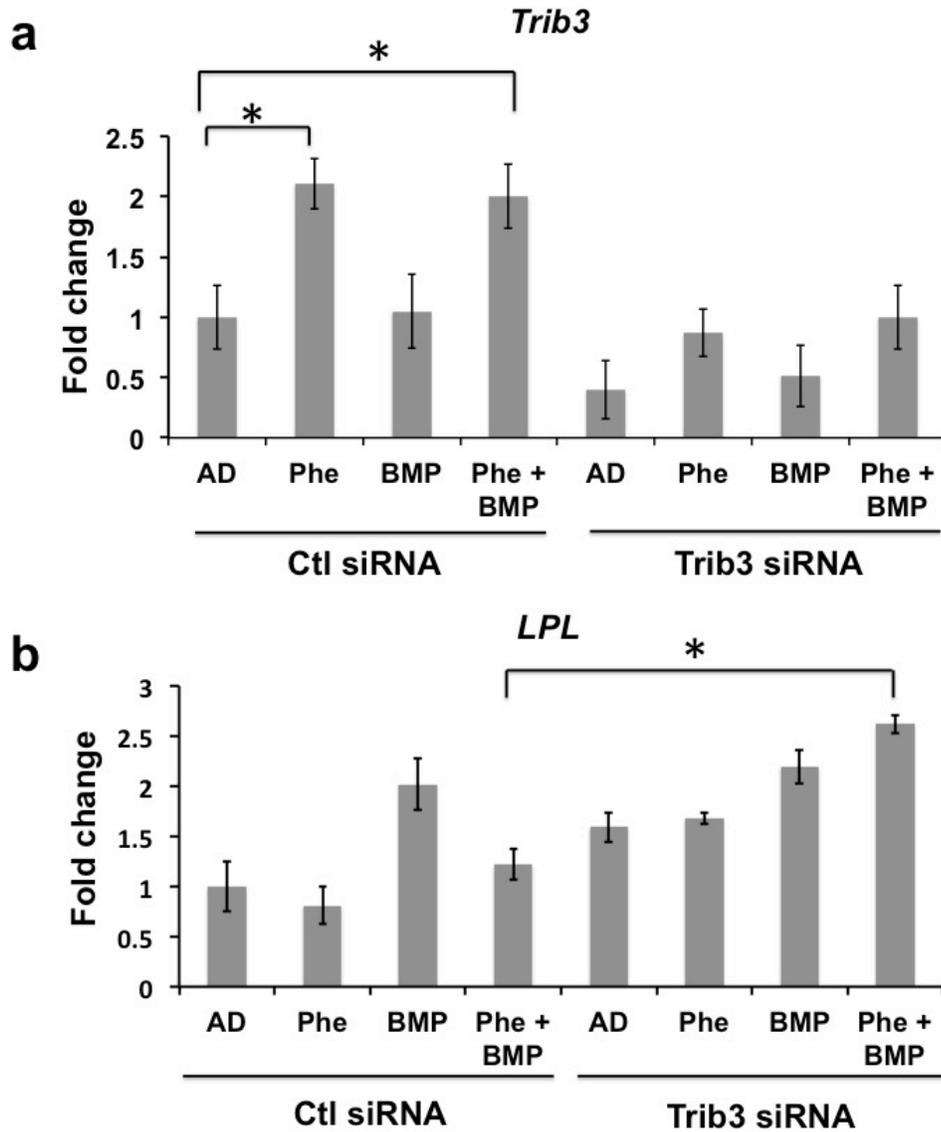
a**b**

Supplementary Figure 3: Phenamil (20 μ M) inhibited high doses BMP2 (500 ng/mL)-induced inflammatory gene expression in NIH3T3 (a) and C2C12 cells (b). Phenamil treatment with or without BMP2 also enhanced Trib3 expression in both NIH3T3 (a) and C2C12 cells (b). Data presented as means \pm SD (n=3/group); *p < 0.05, **p < 0.01.

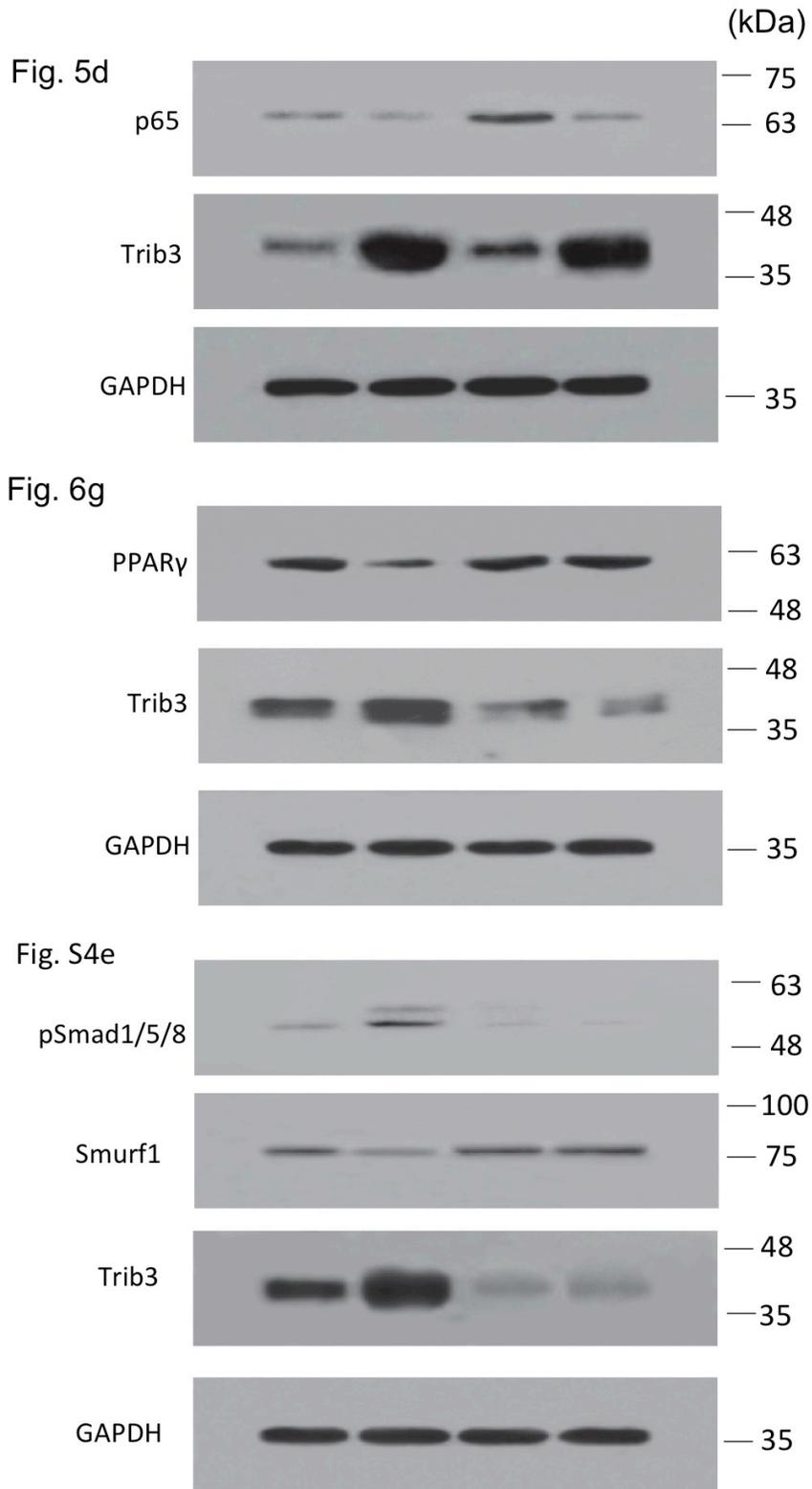


Supplementary Figure 4: (a-d) Knockdown Trib3 (a) blocked the synergistic effects of phenamil (20 μ M) on BMP2 (100 ng/mL)-induced osteogenic genes: *Runx2* (b), *ALP* (c), *OCN* (d) in hBMSCs. (e) Western-blot assay indicated that Trib3 knockdown in hBMSCs reversed

phenamil-mediated BMP signaling that was stimulated by the enhanced Trib3 to stabilize pSmad1/5/8 *via* inhibiting Smurf-1. Data presented as means \pm SD (n=3/group); *p < 0.05. Uncropped western blot gels are displayed in Supplementary Figure 6.



Supplementary Figure 5: (a,b) Trib3 knockdown (a) attenuated phenamil (10 μ M)-mediated inhibitory BMP2 (300 ng/mL)-induced adipogenic genes: *LPL* (b) in hBMSCs. Data presented as means \pm SD (n=3/group); *p < 0.05.



Supplementary Figure 6: Uncropped western-blot images related to Figure 5d, Figure 6g and Supplementary Figure 4e.

Supplementary Table 1: Primer sequences listed for real-time PCR assay.

Gene	Primer (Forward)	Primer (Reverse)
<i>Runx2 (h)</i>	TGGTACTGTCATGGCGGGTA	TCTCAGATCGTTGAACCTTGCTA
<i>ALP (h)</i>	ACTGGTACTCAGACAACGAGAT	ACGTCAATGTCCCTGATGTTATG
<i>Osteocalcin (h)</i>	CACTCCTCGCCCTATTGGC	CCCTCCTGCTTGGACACAAAG
<i>PPARγ (h)</i>	GGGATCAGCTCCGTGGATCT	TGCACTTTGGTACTCTTGAAGTT
<i>LPL (h)</i>	TCATTCCCGGAGTAGCAGAGT	GGCCACAAGTTTTGGCACC
<i>Trib3 (h)</i>	AAGCGGTTGGAGTTGGATGAC	CACGATCTGGAGCAGTAGGTG
<i>GAPDH (h)</i>	TGTGGGCATCAATGGATTTGG	ACACCATGTATTCCGGGTCAAT
<i>IL-1 α (m)</i>	CGAAGACTACAGTTCTGCCATT	GACGTTTCAGAGGTTCTCAGAG
<i>IL-1β (m)</i>	GAAATGCCACCTTTTGACAGTG	TGGATGCTCTCATCAGGACAG
<i>IL-6 (m)</i>	CCAAGAGGTGAGTGCTTCCC	CTGTTGTTCAGACTCTCTCCCT
<i>ALP (m)</i>	AATGAGGTCACATCCATCCTG	CACCCGAGTGGTAGTCACAA
<i>TNF-α (m)</i>	CCCTCACACTCAGATCATCTTCT	GCTACGACGTGGGCTACAG
<i>Trib3 (m)</i>	GCTAGAGATCTGAGCCGGGTA	AAGAGAGCCTGGCAAGAGG
<i>GAPDH (m)</i>	TGTGTCCGTCGTGGATCTGA	CCTGCTCACCACCTTCTGA