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Supplemental Information

Contribution of Implanted, Genetically Modified Muscle Progenitor Cells Expressing BMP-2 to New Bone Formation in a Rat Osseous Defect

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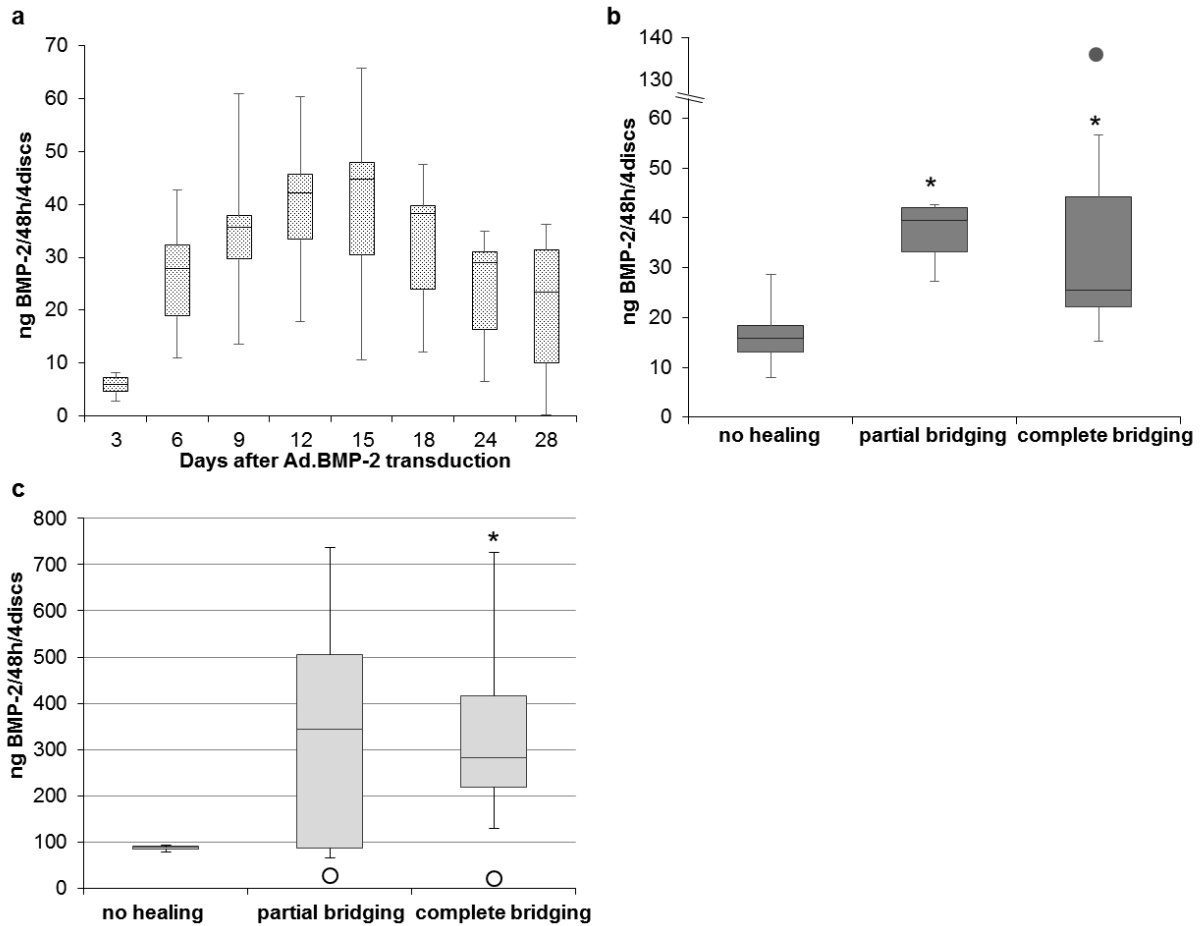


Figure S1: BMP-2 production by genetically modified rat muscle discs. BMP-2 was quantified in muscle supernatants using an ELISA kit specific for the human protein. (a) Time course of BMP-2 production *in vitro* by cultures of transduced muscle discs (n=8). (b) BMP-2 secretion by muscle discs at day 3 after Ad.BMP-2 transduction (n>8 per group) correlated to radiographic evaluation of repair 8 weeks after implantation. (c) BMP-2 secretion by muscle discs at the time of implantation (i.e., day 5 after transduction), correlated to radiographic evaluation of repair 8 weeks after implantation. Representative results are shown as box and whisker plots, with median as the horizontal bar, interquartile range calculated using Tukey hinges as the box, and the lowest and highest values represented as whiskers. Circle dots represent outliers. * denotes a significant difference ($p < 0.05$) compared to the “no healing” group.

Rat Number	Recipient	FK506 /SEW 2871	BMP-2 secretion (ng/48h/4discs)	Radiologic Bridging
1	Wild-type	+	220.5 ± 14.0	Complete
2	Wild-type	+	726.2 ± 35.1	Complete
3	Wild-type	+	219.8 ± 11.4	Complete
4	Wild-type	+	344.3 ± 16.4	Complete
5	Wild-type	+	129.3 ± 2.1	Complete
6	Wild-type	+	415.8 ± 2.4	Complete
7	Wild-type	+	415.1 ± 16.02	Complete
8	GFP	-	283.2 ± 33.5	Partial
9	GFP	-	405.4 ± 51.5	Partial
10	GFP	-	284.6 ± 21.4	None (pin loosening)
11	GFP	-	737.7 ± 20.1	Partial

Table S1. Bridging of femoral defects in wild-type and GFP⁺ rats by the implantation of muscle discs from GFP⁺ rats expressing BMP-2 in the absence or presence of immunosuppression

Gene	Accession number	Primer sequence 5'-3'	Product size (bp)	Primer efficiency
Rat GAPDH	NM 017008	F : GGTCGGTGTGAACGGATTTGG R : GCCGTGGGTAGAGTCATACTGGAC	148	95%
Rat ALP	NM 013059	F : ATGTCTGGAACCGCACTGAAC R : TTCTTTGTCAGGATCCGGAGG	166	99%
Rat Runx2	NM 001278483	F : GCCAGGTTCAACGATCTGAG R : GAGGCGGTCAGAGAACAAC	201	95%
Rat Col1A1	NM 053304	F : CTGACTGGAAGAGCGGAGAG R : GAGTGGGGAACACACAGGTC	111	96%
Rat osteopontin	M99252	F : AAGCCTGACCCATCTCAGAA R : ATGGCTTTCATTGGAGTTGC	114	98%
Rat osteocalcin	NM 013414	F : GCATTCTGCCTCTCTGACCT R : GGCTCCAAGTCCATTGTTGA	132	95%
Rat BMP-2	NM 017178	F : TAAAGCCTGCCACAGCCAGC R : TGTCCATCGCATCACAGCCG	122	98%
Human BMP-2	NM 001200	F : AACACTGTGCGCAGCTTCC R : CTCCGGGTTGTTTTCCAC	74	96%

Table S2. List and sequences of primers used for analysis of mRNA expression. Forward (F). Reverse (R).