

Supporting Information

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Bone-Targeted Delivery of Cell-Penetrating-RUNX2 Fusion Protein in Osteoporosis Model

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Supporting Information

Bone Targeting Delivery of Cell-Penetrating Protein-RUNX2 Fusion Protein Using Hydroxyapatite Binding Tag in Osteoporosis Model

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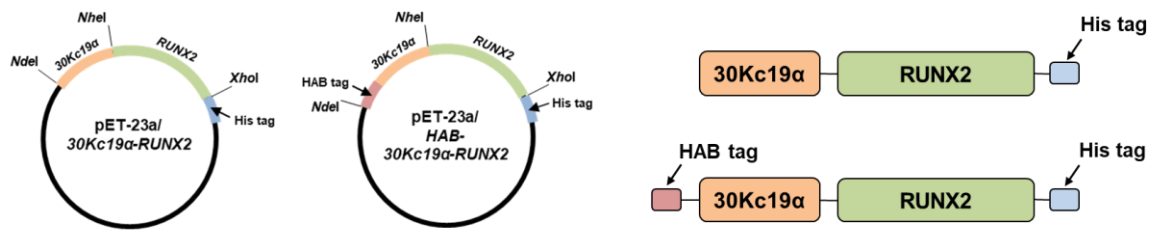
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MDDDDDDDC**ADSDVPNDILEEQLYNSVVADYDSAVEKSKHLYEKKSEVITNVVNKLIRNNKMNCMEYAY**
QLWLQGSKDIVRDCFPVEFRLIFAENASMASNSLFSAVTPCQQSFFWDPSTSRRFSPSSSLQPGKMSDVS
VAAQQQQQQQQQQQQQQQQQQQQQQQQQQQEEAAAAAAAAAAAAAAAAAAVPRLRPPHDNRT
MVEIADHPAELVRTDSPNFLCSVLPSHWRCNKTLPVAFKVVALGEPDGTVVTVMAGNDENYSaelRNAsA
VMKNQVARFNDLRFVGRSGRGKSFTLITVFTNPPQVATYHRAIKVTVDGPREPRRHQKLDDSKPSLFS
SDLGRIPHPSMRVGVPPQNPRPSLNSAPSPFNQGGQSQITDPRQAQSSPPWSYDQSYPSYLSQMTSP
SIHSTPLSSTRGTGLPAITDVPRRISDDDTATSDFCLWPSSLSKKSQAGASELGPFS DPRQF
PSISSLTERFSNPRMHYPATFTYTPPVTSGLMSLGSATTHYHTYLPYPYGGSSQSQSGPFTSSTPYLYGTSS
ASYQFPMVPGGDRSPSRMVPPCTTNSGSTALLNPNLNQNDGVDADGSHSSSPTVLNSSGRMDES
VWRPYPKKKRKRKVLHHHHHHH

Figure S1. Amino acid sequences of HAB-30Kc19α-RUNX2. The amino acid sequences of 30Kc19α and RUNX2 are indicated by orange and green letters, respectively. HAB tag (DDDDDDDC) and His tag are located at the N-terminus and C-terminus of recombinant proteins.

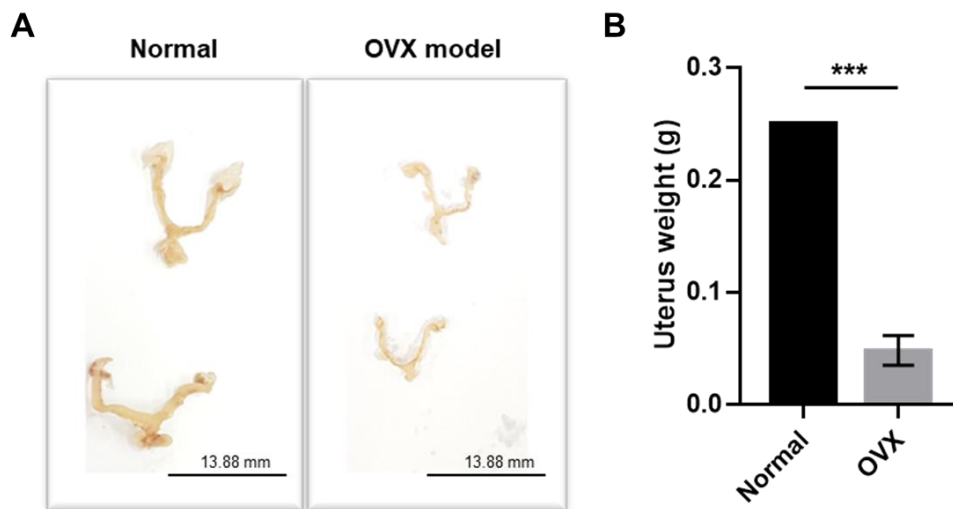


Figure S2. Establishment of a mouse model for osteoporosis. The osteoporosis model was generated by the removal of ovaries. (A) Morphological changes in the uterus after ovariectomy (OVX). The uterus size of the OVX became smaller than that of the normal group. Scale bar: 13.88 mm (B) Weight changes in the uterus after OVX. Reduction in the uterus weight confirming successful ovary removal. *** $p < 0.001$

Gene	Primer sequence (5'→3')	
<i>GAPDH</i>	Forward	TTCACCACCATGGAGAAGGC
	Reverse	TCTTCTGGGTGGCAGTGATG
<i>RUNX2</i>	Forward	GCGCATTCCTCATCCCAGTA
	Reverse	GGCTCAGGTAGGAGGGGTAA
<i>SPP1</i>	Forward	GCATCACCTGTGCCATAACCAG
	Reverse	TCCTGGCTGTCCACATGGTC
<i>BGLAP</i>	Forward	TATTGGCCCTGGCCGCACTT
	Reverse	CTCACACACCTCCCTCCTG

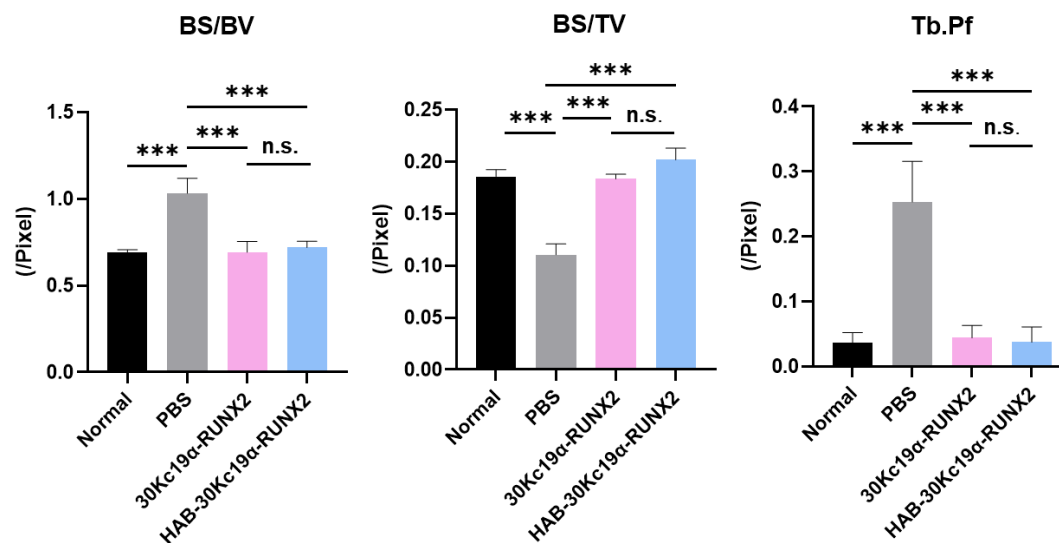


Figure S3. In vivo bone regeneration of recombinant protein treatment in postmenopausal osteoporosis model. Four weeks after the osteoporosis model was generated by ovariectomy (OVX) in Balb/c mice, PBS (50 μ L), 30Kc19 α -RUNX2 (50 μ g, 1 μ g μ L⁻¹) and HAB-30Kc19 α -RUNX2 (50 μ g, 1 μ g μ L⁻¹) were injected via tail vein injection four times every 1 week ($n=3$). Micro-CT parameters such as bone surface to bone ratio (BS/BV), bone surface density (BS/TV), trabecular pattern factor (Tb.Pf) was measured. *** $p < 0.001$

Table S1. Primer sequences used in qRT-PCR.