Supplemental Figures

Figure Legends

Figure S1. *In vitro* differentiation of osteoclasts from BMM cells infected with pMX-puro (con), wild-type HA-GSK-3β (WT), constitutively active HA-GSK-3β S9A (S9A), or catalytically inactive HA-GSK-3β K85R (K85R), respectively. TRAP staining was performed 4 days after RANKL stimulation. $TRAP^+$ MNCs (> 3 nuclei) were counted. Data represent means ± SD. **P* < 0.05, ***P* < 0.01.

Figure S2. BMMs infected with retroviruses expressing either empty vector (EV), HA-GSK-3β S9A (S9A), or HA-GSK-3β K85R (K85R) were cocultured with osteoblasts for 7 days in the presence of $1,25(OH)_2D_3$ and PGE_2. TRAP staining was performed and $TRAP^+$ MNCs were counted. Data represent means ± SD. **P* < 0.05, ***P* < 0.01. Scale bar, 200 μm.

Figure S3. Generation of Tg mice expressing GSK-3β S9A mutant under the control of TRAP promoter. (A) Schematic diagram of the TRAP-GSK-3β-S9A construct. Constitutively active GSK-3β S9A cDNA was fused to 1.8 kb of the mouse TRAP gene promoter. GT or GP primer indicates specific primers for the transgene. (B) Genomic DNA isolated from the tail was analyzed by PCR using specific primers for the transgene. (C) BMMs isolated from GSK-3β S9A Tg (TG) mice or wild-type littermates (WT) were cultured in the presence of RANKL as indicated. The expression of transgene was confirmed by RT-PCR analysis using a specific primer (GT primer) for GSK-3β S9A or β-actin.

Figure S4. GSK-3α had no significant effect on RANKL-induced osteoclast differentiation. *In vitro* differentiation of osteoclasts from BMMs infected with pMX-puro (con), V5-GSK-3α (WT), or catalytically inactive V5-GSK-3αK 148A (K148A), respectively. TRAP staining

was performed 4 days after RANKL stimulation. $TRAP^+$ MNCs containing more than 3 nuclei were counted. GSK-3α expression following infection was confirmed by Western blot analysis using the V5 antibody and then reprobed with GAPDH as a loading control. Data represent means ± SD.

Figure S1

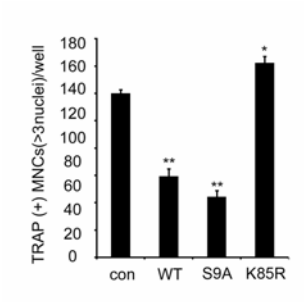

TRAP (+) MNCs(>3nuclei)/well
180
160
140
120
100
80
60
40
20
0
**
**
*
con
WT
S9A
K85R

Figure S2

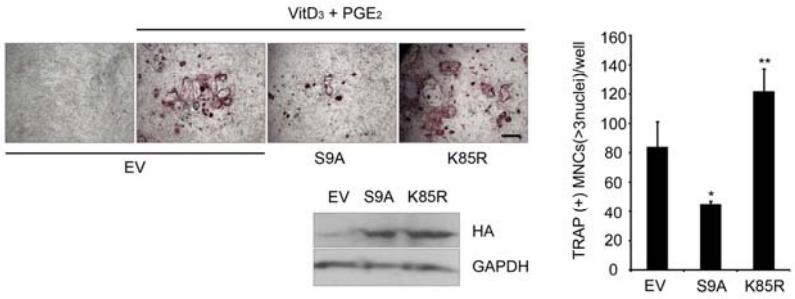

VitD3 + PGE2
EV
S9A
K85R
EV S9A K85R
HA
GAPDH
TRAP (+) MNCs(>3nuclei)/well
160
140
120
100
80
60
40
20
0
*
**
EV
S9A
K85R

Figure S3

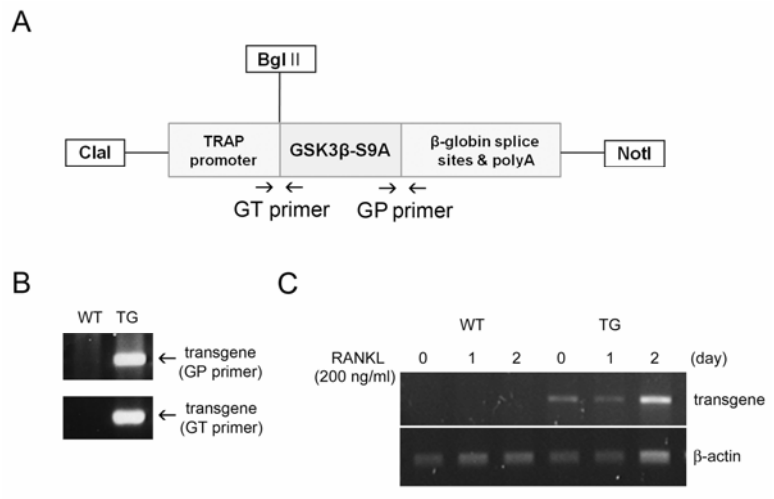
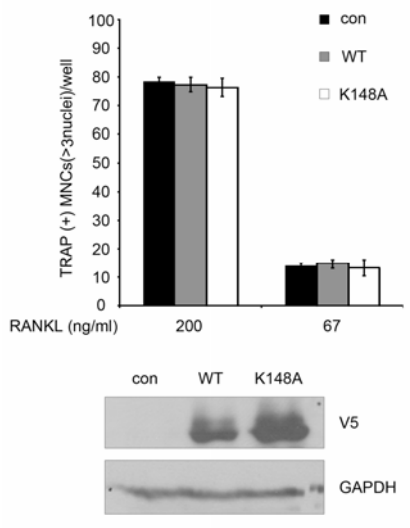

A
BglII
ClaI
TRAP promoter
GSK3β-S9A
β-globin splice sites & polyA
NotI
GT primer
GP primer
B
WT TG
transgene (GP primer)
transgene (GT primer)
C
WT
TG
RANKL (200 ng/ml)
0 1 2 0 1 2 (day)
transgene
β-actin

Figure S4


100
90
80
70
60
50
40
30
20
10
0
TRAP (+) MNCs(>3nuclei)/well
con
WT
K148A
RANKL (ng/ml)
200
67
con
WT
K148A
V5
GAPDH