ng/ml) or plasmin (1 μg/ml) as indicated. The number of multinucleated TRAP positive cells was determined from four different cultures. The data represent the mean ± SEM. *, $P<0.01$; **, $P<0.05$.

Figure 3. ***Plasmin induced the OPG expression in OBs.***

(A to D) OBs from the WT mice were cultured for 24 hours in either the absence or presence of plasmin (1 μg/ml). Plasmin-induced expression of OPG gene in OBs from the WT mice was evaluated by qRT-PCR (A) or a Western blot analysis (B). In C and D, some cultures were further treated with plasmin inhibitors: α2AP (200 nM), EACA (25 mM), and aprotinin (10 μg/ml). Then, the expression of OPG mRNA in OBs from the WT mice was measured by qRT-PCR (C) or a Western blot analysis (D). (E) OBs from the WT mice were stimulated with 1 μg/ml plasmin for the indicated periods. Phosphorylation of ERK1/2 and p38 MAPK were evaluated by a Western blot analysis using antibodies to ERK1/2 and p38 MAPK. (F) OBs from the WT mice were pretreated with 30 μM PD98059 or 30 μM SB203580 for 60 min and then stimulated with 1 μg/ml plasmin for 24 hours. The expression of OPG in OBs from the WT mice was evaluated by a Western blot analysis. In G and H, the OPG expression in OBs from the $Plg^{+/+}$ and $Plg^{-/-}$ mice was evaluated by qRT-PCR (G) or a Western blot analysis (H). In I and J, OBs from the $Plg^{-/-}$ mice were cultured for 24 hours in the absence or presence of plasmin (1 μg/ml). Then, the OPG expression in OBs from the $Plg^{-/-}$ mice was evaluated by qRT-PCR (I) or a Western blot analysis (J). The data represent the mean of 3 individual experiments ± SEM. *, $P<0.01$; **, $P<0.05$.

Figure 4. ***Effects of Plg-deficiency on the ability of OBs to induce osteoclastogenesis of Raw 264.7 cells***

(A) Raw264.7 cells and OBs from the $Plg^{+/+}$ and $Plg^{-/-}$ mice were co-cultured for 3 days in the absence or presence of IL-1β or PGE_2. (B) Raw264.7 cells and OBs from the $Plg^{-/-}$ mice were co-cultured for 3 days in the absence or presence of plasmin. Mature OCs were identified as multinucleated TRAP positive cells. The number of multinucleated TRAP positive cells was determined from six different cultures. The data represent the mean ± SEM. *, $P<0.01$.

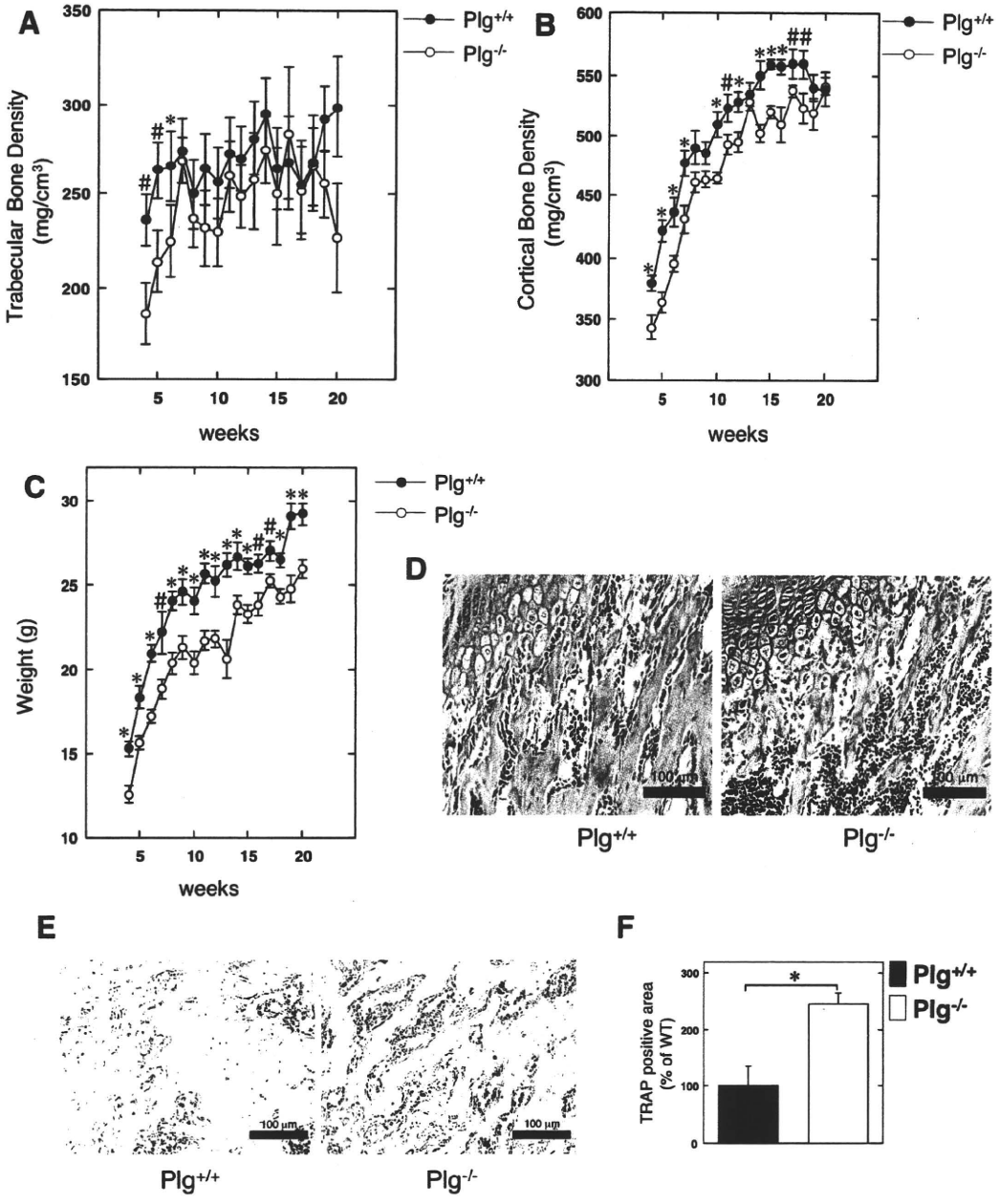
Figure 5. ***Effect of Plg-deficiency on the ALP activity in OBs***

ALP activity in OBs from the $Plg^{+/+}$ and $Plg^{-/-}$ mice was evaluated (n=4). The data represent the mean ± SEM. *, $P<0.01$.

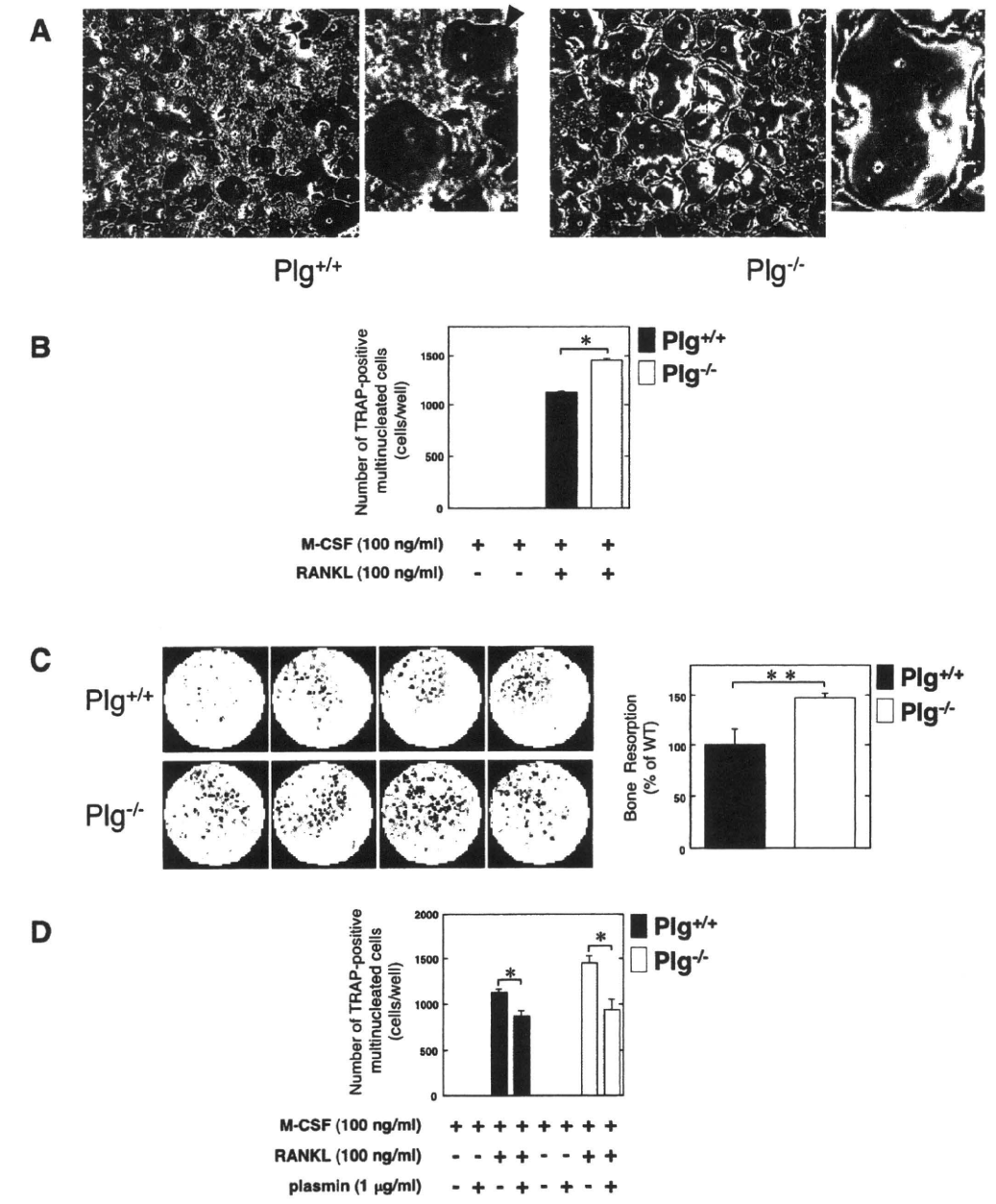
Figure 6. ***Rescue of the down-regulated BMD in Plg-deficient mice by the injection of plasmin***

Intraperitoneal injection with saline or plasmin (1 mg/kg) in the 5-week-old male $Plg^{-/-}$ mice was carried out weekly for up to 3 weeks. Then, the trabecular BMD (A), the cortical BMD (B), and the weight (C) in the male $Plg^{-/-}$ mice were measured by pQCT (n=3). The data represent the mean ± SEM. *, $P<0.01$.

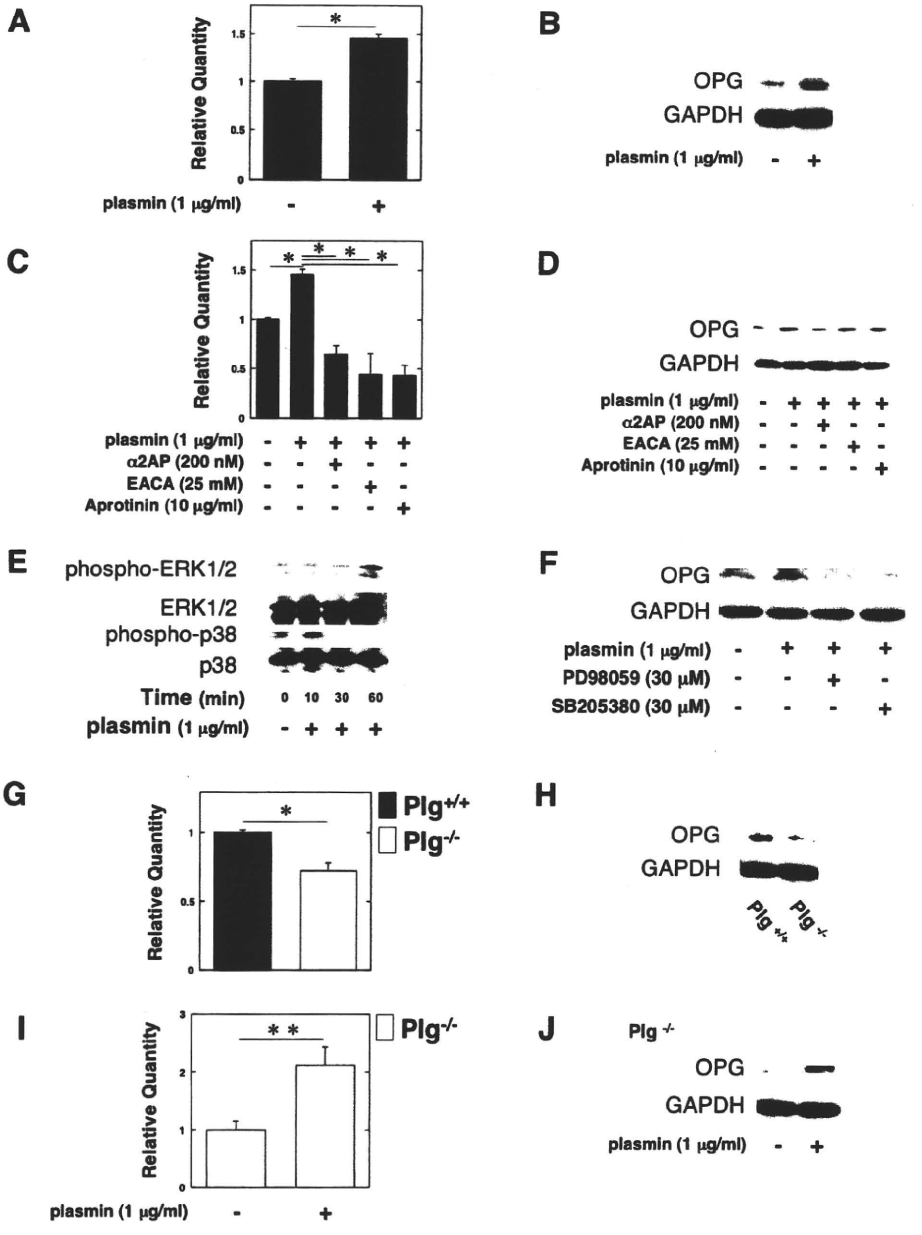
Kanno et al 2010, Figure 1



Kanno et al 2010, Figure 2



Kanno et al 2010, Figure 3



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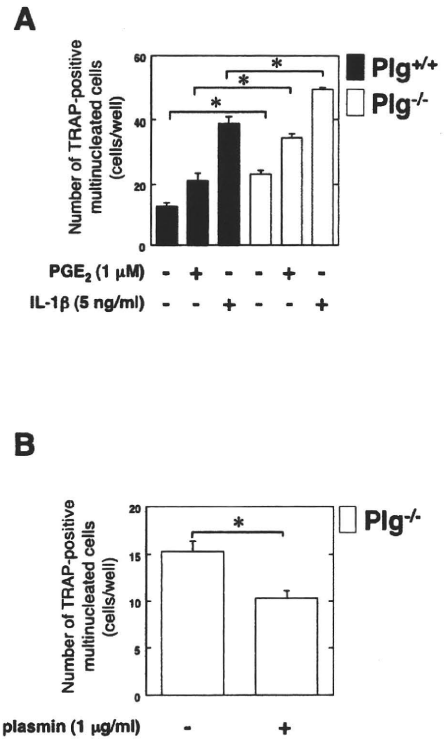
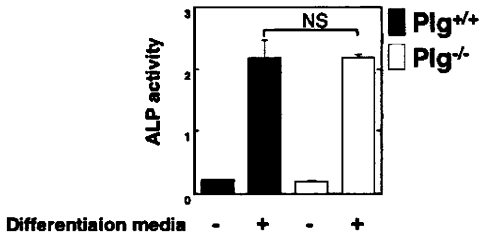


Kanno et al 2010, Figure 5



Kanno et al 2010, Figure 6