Shin et al., http://www.jcb.org/cgi/content/full/jcb.201401137/DC1

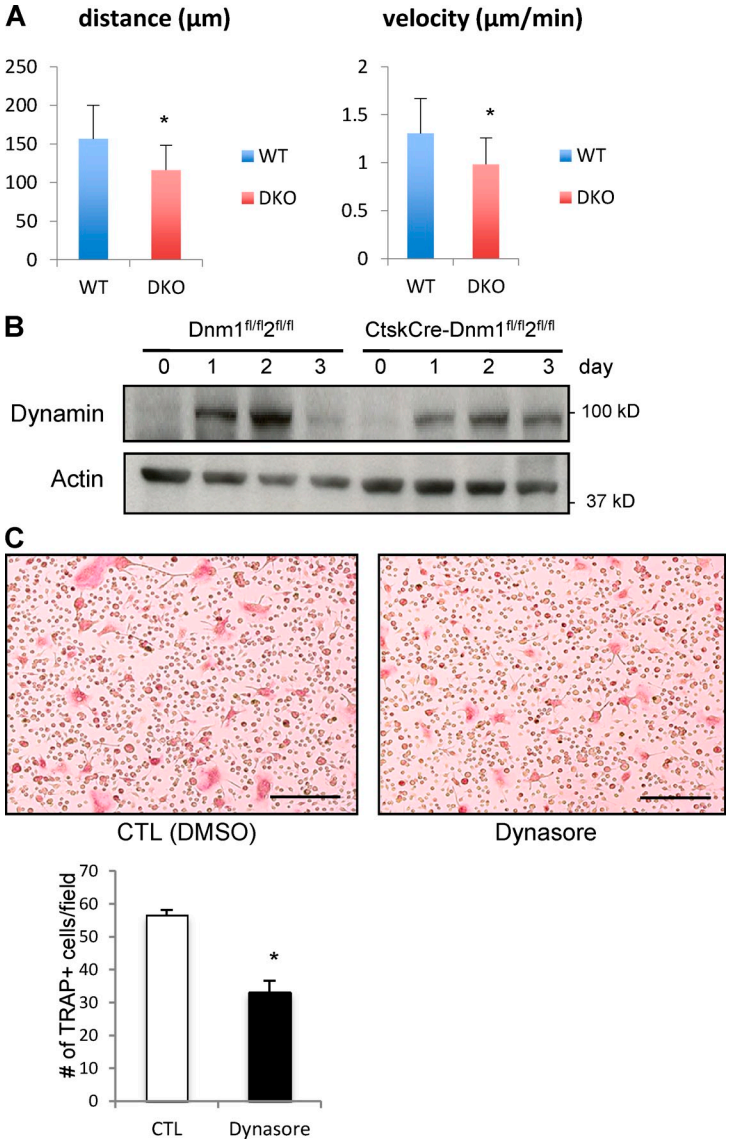


Figure S1. **Characterization of osteoclasts with dynamin depletion or inhibition.** (A) Analysis of CTL and DKO OCP motility using time-lapse microscopy. Migration tracks of 12 individual cells for each genotype were analyzed to determine velocity and migration distance in each of six independent experiments. Data are mean ± SE (error bars). $P < 0.001$, tracking 2 h, $n = 55$. (B) Western blot analysis of dynamin protein expression in osteoclasts derived from CTL and *Dnm*-DKO;Ctsk-Cre mice. Cells were differentiated with RANKL and M-CSF treatment. Actin was used as a loading control. Dynamin was decreased by 40–50% and 50–60% at days 1 and 2, respectively, in Ctsk;Dnm-DKO cells. Quantification of protein bands was performed using Pxi Gel Doc (Syngene). (C) The effect of the dynamin inhibitor dynasore on osteoclast fusion. Osteoclasts cultured with M-CSF and RANKL for 2 d were further differentiated with 100 µM dynasore for 6 h and fixed for TRAP staining. Data are mean ± SE (error bars); *, $P < 0.01$. Bars, 200 µm.

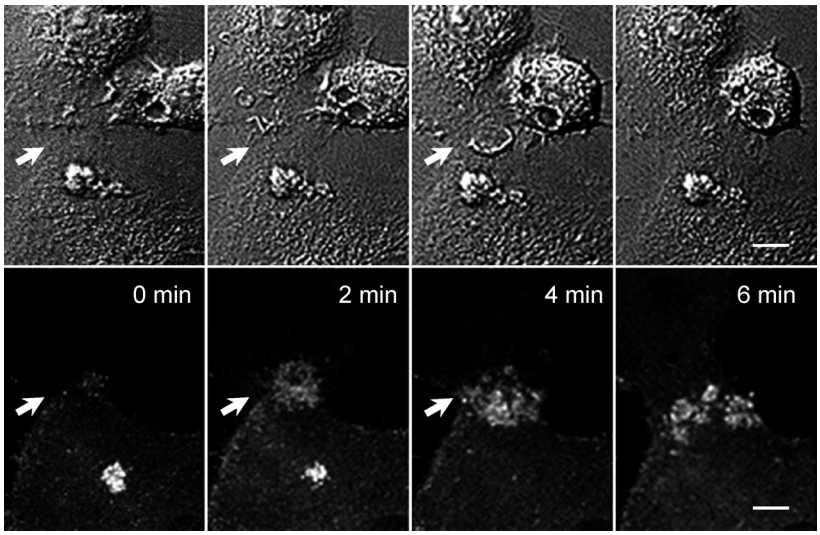


Figure S2. **Time-lapse confocal images of two osteoclasts in fusion.** Primary OCPs were transduced with GFP-actin and induced to differentiate with RANKL and M-CSF. Top: brightfield images; bottom: EGFP images. Images were captured every 2 min. At time 0 min, an osteoclasts (OCL) expressing GFP-actin (bottom) makes a contact with a GFP-negative OCL (top). Podosome clusters (arrows) are highly enriched at sites of fusion at 2 min, then reorganize and dissociate as fusion process continues (4 min and 6 min). Bars, 10 µm.

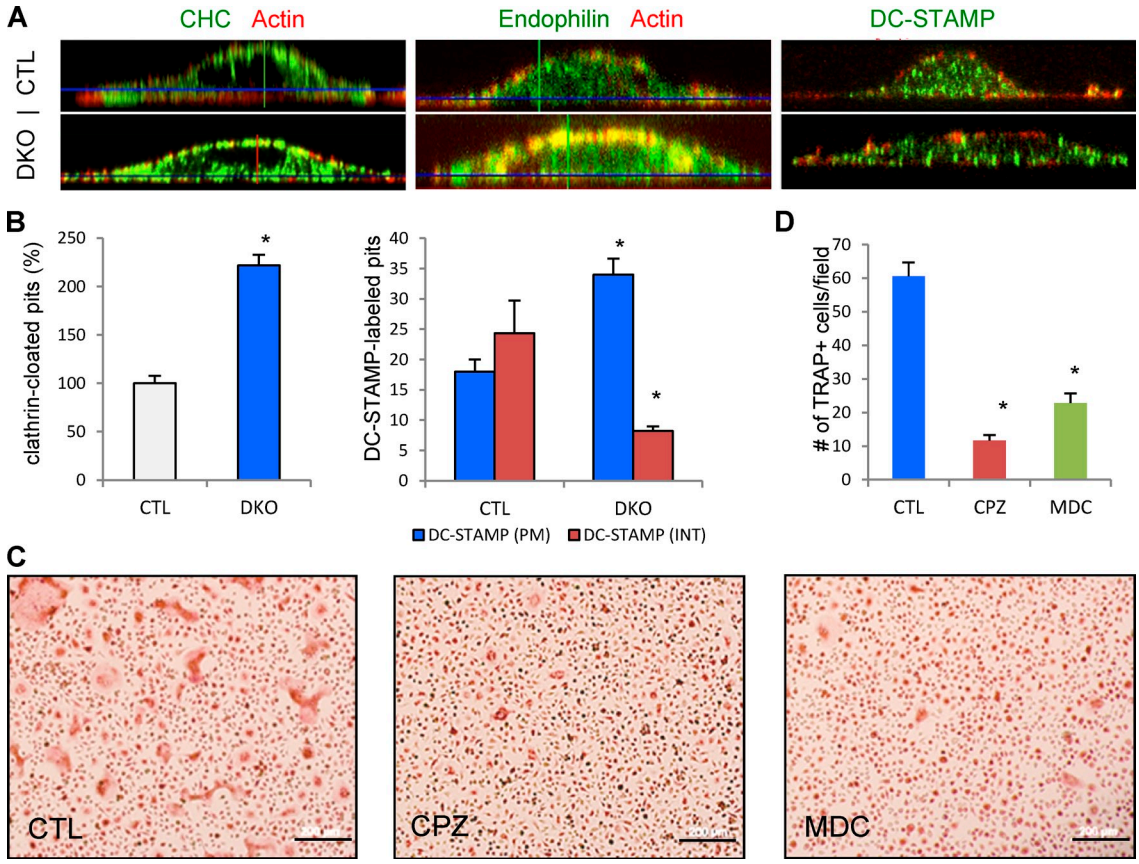


Figure S3. **Endocytosis is involved in osteoclast fusion.** (A) Confocal z-series images of OCPs immunostained with anti-CHC, anti-endophilin, or anti–DC-STAMP (shown in green) along with rhodamine-phalloidin for F-actin. (B) Quantification of clathrin-coated pits labeled with anti-CHC was obtained from 13 CTL cells and 12 DKO cells. Puncta labeled with anti–DC-STAMP were quantified separately for association with plasma membrane (PM) and intracellular (INT) localization. (C) Effects of treatment with endocytosis inhibitors on OCP fusion. Osteoclasts cultured with RANKL and M-CSF for 2 d were treated with CPZ (6 µg/ml) or monodansylcadaverine (MDC, 70 µM) for 6 h in the presence of RANKL and M-CSF, then fixed and stained for TRAP. Bars, 200 µm. (D) Quantification of the number of TRAP+ cells (more than three nuclei) in C. Data are from three independent experiments. Error bars indicate SEM. *, $P < 0.01$.

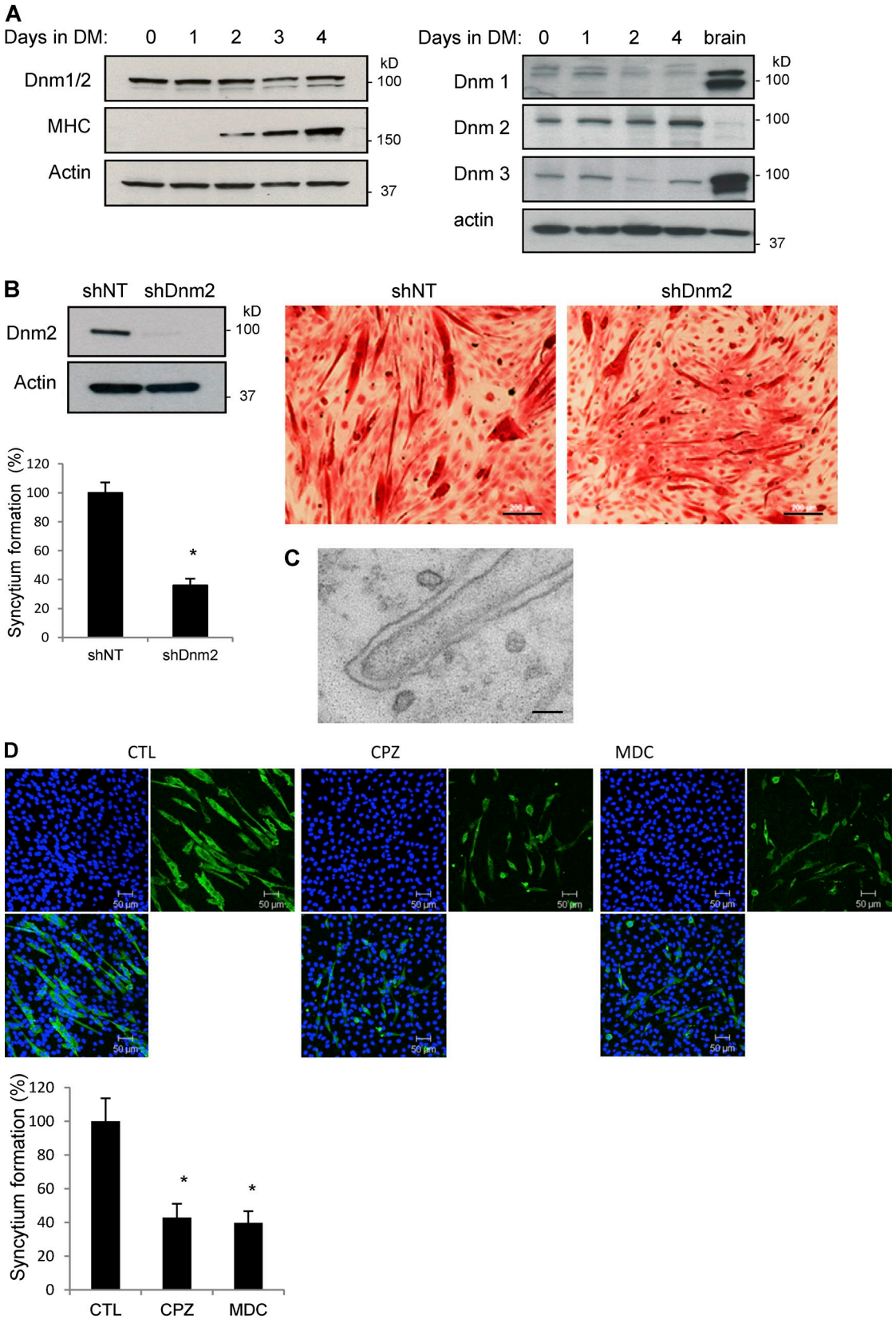


Figure S4. **Dynamin is essential for myoblast fusion.** (A) Expression of dynamin isoforms and MHC proteins during the differentiation of C2C12 in differentiation media (DM) for the indicated days. (B) Effect of dynamin knockdown on myoblast fusion. C2C12 cells were transduced with shRNAs of non-targeting (NT) or Dnm2, and cultured in differentiation medium. The expression level of dynamin 2 was analyzed by Western blotting. C2C12 cells were fixed and stained with H&E after being transduced with the indicated shRNAs and differentiated for 96 h. The extent of syncytium formation was quantified from at least seven randomly chosen microscopic fields. Data are means ± SEM (error bars) from three independent experiments. *, $P < 0.01$. Bars, 200 µm. (C) Electron microscopy image of two apposing C2C12 myoblasts, showing a finger-like membrane protrusion invading into the adjacent cell. Bar, 100 nm. (D) Representative images of C2C12 cells treated with CPZ or MDC for 6 h after incubating for 40 h in differentiation media. Myotubes were visualized with immunostaining with anti-MHC antibody (green) and TOPRO (blue). Bar, 50 µm. The extent of syncytium formation was normalized to CTL cells. All data are means ± SEM (error bars). *, $P < 0.01$.

Table S1. **Histomorphometric analysis of 6-wk-old female Ctsk-cre;Dnm1$^{fl/fl}$Dnm2$^{fl/fl}$ mice and their control littermates**

| Parameter | Dnm1$^{fl/fl}$Dnm2$^{fl/fl}$ ($n$ = 4) | Ctsk-cre;Dnm1$^{fl/fl}$Dnm2$^{fl/fl}$ ($n$ = 4) |
|---|---|---|
| BV/TV (%) | 5.6 ± 1.9 | 13.0 ± 1.7[a] |
| Tb.Th (µm) | 40.5 ± 11.5 | 44.5 ± 6.0 |
| Tb.N (/mm) | 1.37 ± 0.09 | 2.95 ± 0.36[a] |
| Tb.Sp (µm) | 693.5 ± 60.1 | 297.9 ± 39.4[a] |
| Ob.S/BS (%) | 27.9 ± 0.3 | 24.8 ± 4.1 |
| N.Ob/T.Ar (/mm$^2$) | 42.2 ± 1.4 | 86.2 ± 24.9[a] |
| N.Ob/BS (/mm) | 15.5 ± 1.4 | 14.4 ± 3.0 |
| OV/TV (%) | 0.19 ± 0.13 | 0.25 ± 0.07 |
| OS/BS (%) | 22.3 ± 5.7 | 17.2 ± 3.8 |
| O.Th (µm) | 3.00 ± 1.24 | 2.42 ± 0.46 |
| Oc.S/BS (%) | 1.43 ± 0.11 | 0.42 ± 0.11[a] |
| N.Oc/T.Ar (/mm$^2$) | 1.76 ± 0.05 | 1.40 ± 0.37[a] |
| N.Oc/BS (/mm) | 0.65 ± 0.04 | 0.24 ± 0.06[a] |
| ES/BS (%) | 1.61 ± 0.19 | 0.50 ± 0.10[a] |

Results are mean ± SD.
[a]$P < 0.05$ versus data from control mice, unpaired $t$ test.