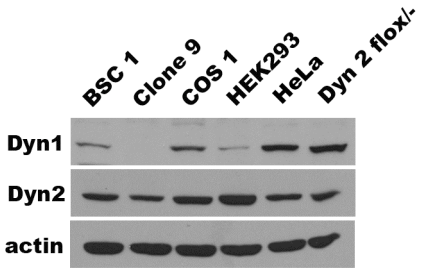
Liu et al.: Supplemental Material

Isoform and splice-variant specific functions of dynamin-2 revealed by analysis of conditional knock-out cells.



Supplemental Figure 1
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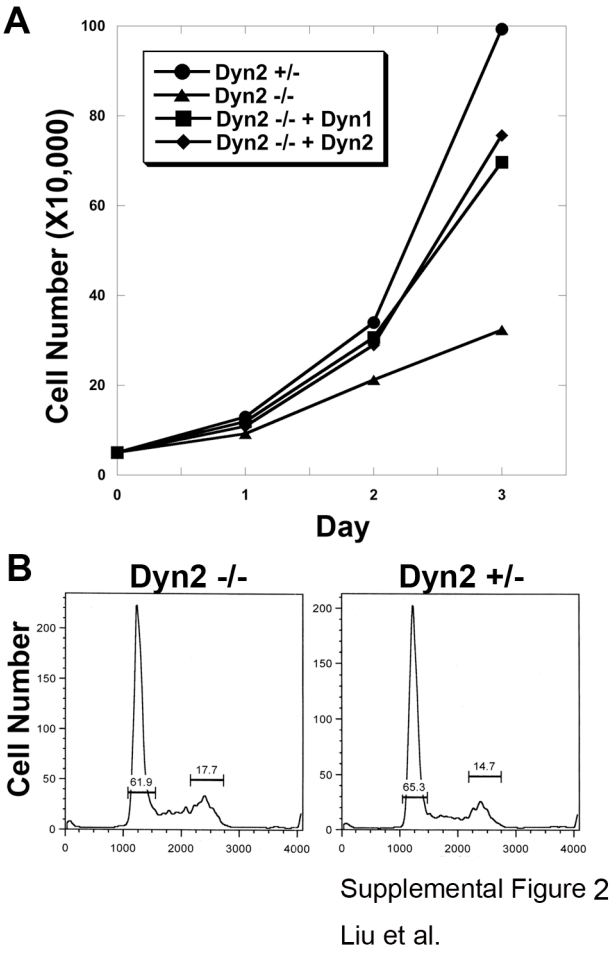
Supplemental Figure 1. Dynamin isoforms expression. Cell lysates from BSC-1, Clone-9, COS-1, HEK293, HeLa and our Dyn2 +/- cells were blotted with anti-Dyn1, Dyn2 and actin antibodies to revel their relative expression level in these cultured cells. Clone-9 is the only cell line that we have tested doesn't express Dyn1, and Dyn1 is relatively higher in HeLa and our Dyn2+/- cells.



Supplemental Figure 2

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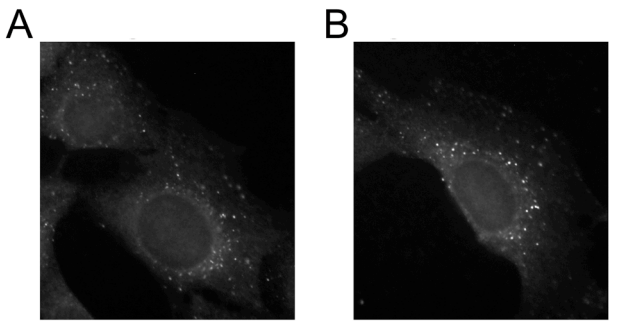
Supplemental Figure 2. Cell growth and cell cycle progression in Dyn2 KO cells. (A) After seeding 5,000 cells of control ($Dyn2^{flox}$/- cells express GFP from retrovirus infection), Dyn2 KO (expressing GFP, too), Dyn2 KO contain Dyn1-IRES-GFP or Dyn2-IRES-GFP cells on 35mm dishes, cell number were determined daily. (B) Cell cycle progression was analyzed by PI staining of control and Dyn2 KO cells and flow cytometry analysis as described in Supplemental Method.



Supplemental Figure 3
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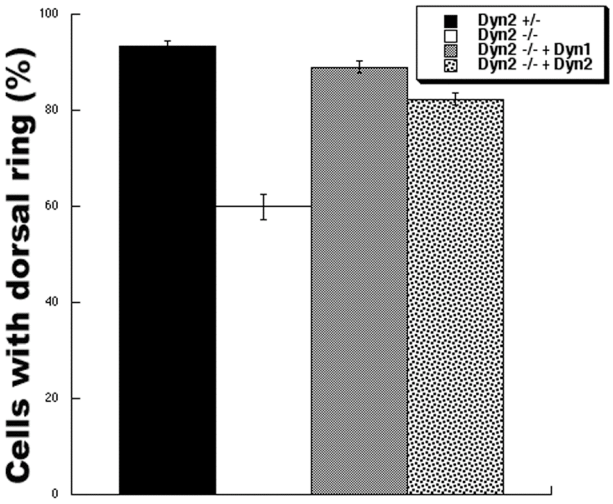
Supplemental Figure 3. Endocytosis of fluorescently-labeled folate in control (A) and Dyn2 KO cells (B). Cells were incubated with 2μg/ml rhodamine-folate (gift from M. G. Finn) for 30 min on ice and shifted to 37°C for 10 min. After return to ice, surface folate was removed by acid stripping, cells were fixed and imaged for internalized folate.



Supplemental Figure 4

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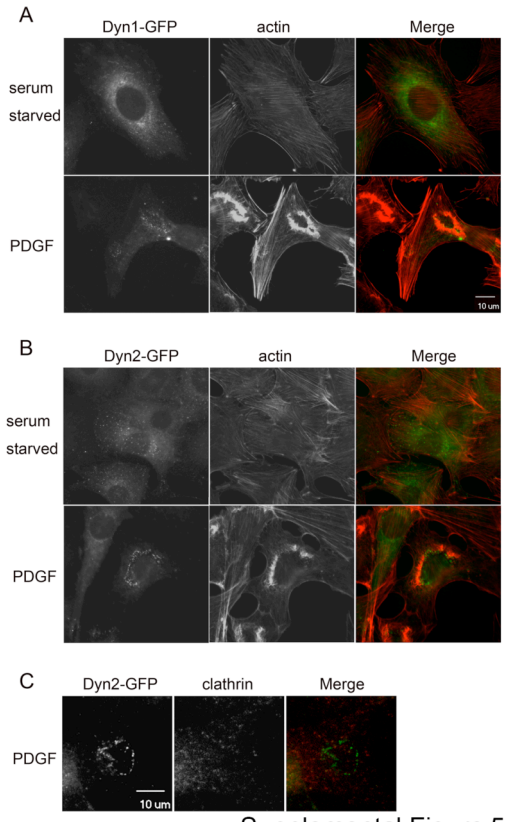
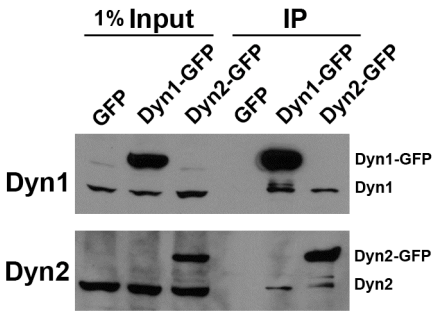
Supplemental Figure 4. Quantification of dorsal ring formation in Dyn2 KO cells. Control, Dyn2 KO or Dyn2 KO cells expressing either Dyn1 or Dyn2 were stimulated by PDGF for 5 min. and fixed and stained with Alexa Flour 568-phalloidin. Three independent experiments and 100 cells for each experiment were counted and scored for the cells having dorsal ruffles.



Supplemental Figure 5. Dynamin localizes to the dorsal ruffles. Both Dyn1-GFP (A) and Dyn2-GFP (B) localize to the dorsal ruffle after PDGF stimulation. Cells expressing either Dyn1-GFP or Dyn2-GFP were stimulated with PDGF for 5 min and stained with Alexa Flour 568-phalloidin. (C) Dyn2-GFP associated with dorsal ruffles does not co-localize with clathrin. Cells expressing Dyn2-GFP and clathrin-mCherry were stimulated with PDGF and stained with Alexa Flour 568-phalloidin.

Supplemental Figure 7. Hetero-oligomerization of Dyn1 and Dyn2. Cell lysate from $Dyn2^{flox}$/- cells expressing Dyn1-GFP, Dyn2-GFP or GFP alone were immuno-precipitated by anti-GFP antibody and detected by anti-Dyn1 or Dyn2 antibodies.



Supplemental Figure 6

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Supplemental Figure 6. Hetero-oligomerization of Dyn1 and Dyn2. Cell lysate from $Dyn2^{flox}$/- cells expressing Dyn1-GFP, Dyn2-GFP or GFP alone were immuno-precipitated by anti-GFP antibody and detected by anti-Dyn1 or Dyn2 antibodies.