SUPPLEMENTAL MATERIALS

Membrane Insertion of the Pleckstrin Homology Domain Variable Loop 1 is Critical for Dynamin-catalyzed Vesicle Scission.

Supplementary Figure 1. Transferrin uptake was followed in tTA-HeLa cells infected for 16-18 hours with recombinant adenovirus encoding Dyn1 WT (●), RCLDyn1 G532C (▼), RCLDyn1 M534C (▽) or Dyn1 I533C (♦). The kinetics of internalization is plotted as a percentage of maximum uptake by Dyn1 WT.

Supplementary Figure 2. Basal GTPase activities (open symbols) of 0.5 μM Dyn1 WT (○) and RCLDyn1 I533C (□) at physiological salt (20 mM HEPES, pH 7.5. 150 mM KCl) and cooperative, assembly-stimulated GTP activities of the same (closed symbols ● for Dyn 1 WT and ■ for RCL Dyn1 I533C) at low ionic strength (20 mM HEPES, pH 7.5, No KCl) were measured as described under Methods. The concentration of P_i released is plotted as a function of time.

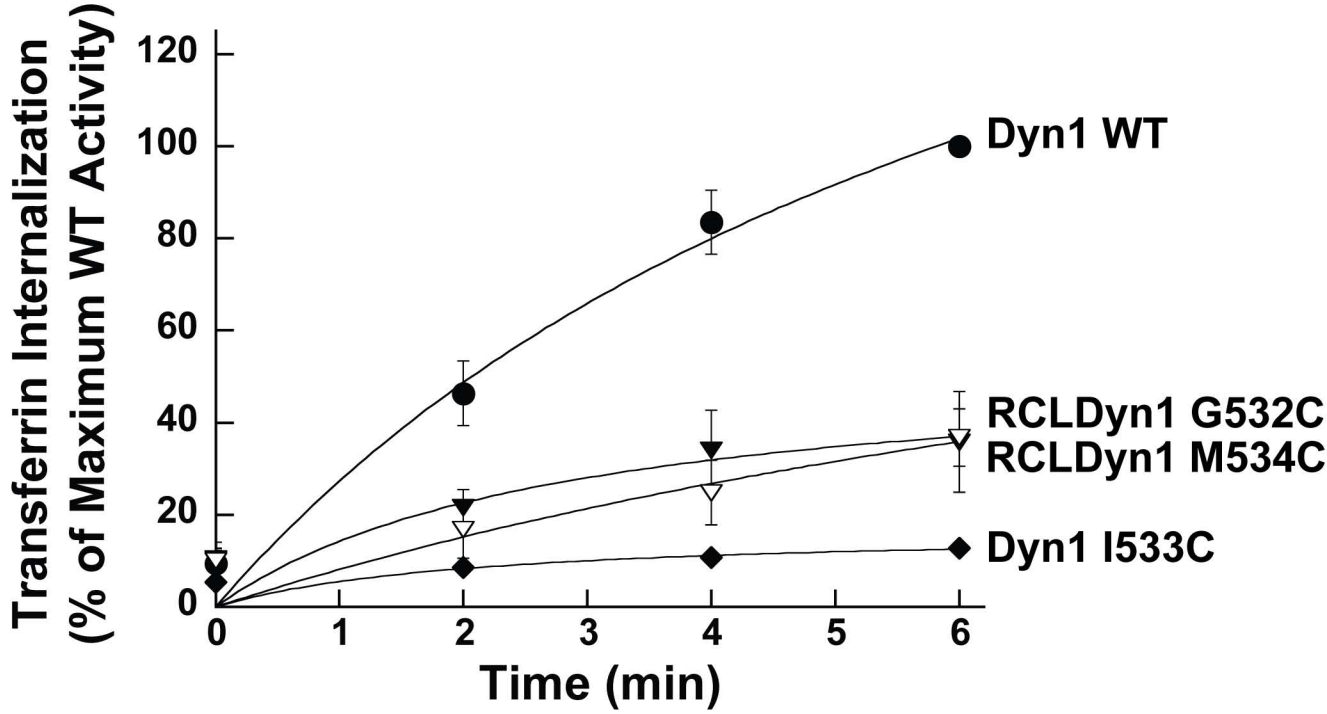
Supplementary Figure 3. (A) Liposome-stimulated GTPase activities at increasing concentrations of Dyn1 WT (●) and Dyn1 I533A (■). The rate of GTP hydrolysis in μM Pi released/min is plotted as a function of Dyn1 concentration. (B) Membrane fission activities of increasing concentrations of Dyn1 WT (●) and Dyn1 I533A (■) was determined on SUPER templates (6 μM total lipid) in the constant presence of GTP (1 mM) by a sedimentation assay as in Figure 1D.

Supplementary Figure 4. (A) Fluorescence images of RhPE-labeled SUPER templates showing the effect of addition of either 0.5 μM (final) Dyn1 I533C in the absence of GTP. Contrast inverted image of the same is shown for clarity. Images were acquired at a focal plane close to the coverslip. Note the absence of optically visible membrane tubules for Dyn1 I533C in comparison to Dyn 1 WT (Figure 3A *top panel*). (B) Fluorescence images of RhPE-labeled SUPER templates containing glycerol flow-generated membrane tubules showing the effect of addition of 0.5 μM (final) Dyn1 I533C in the constant presence of GTP. No fission of tubules into independent vesicles is observed when compared to Dyn1 WT (Figure 3B *bottom panel*). *Inset* Magnified fluorescence image of flow-generated membrane tubules showing no visible membrane constrictions for Dyn1 I533C. (C) Time-lapse fluorescence images showing the effect of addition of either 0.5 μM Dyn1 I533C to membrane tethers pulled out of RhPE-labeled SUPER templates in the presence of GTP.

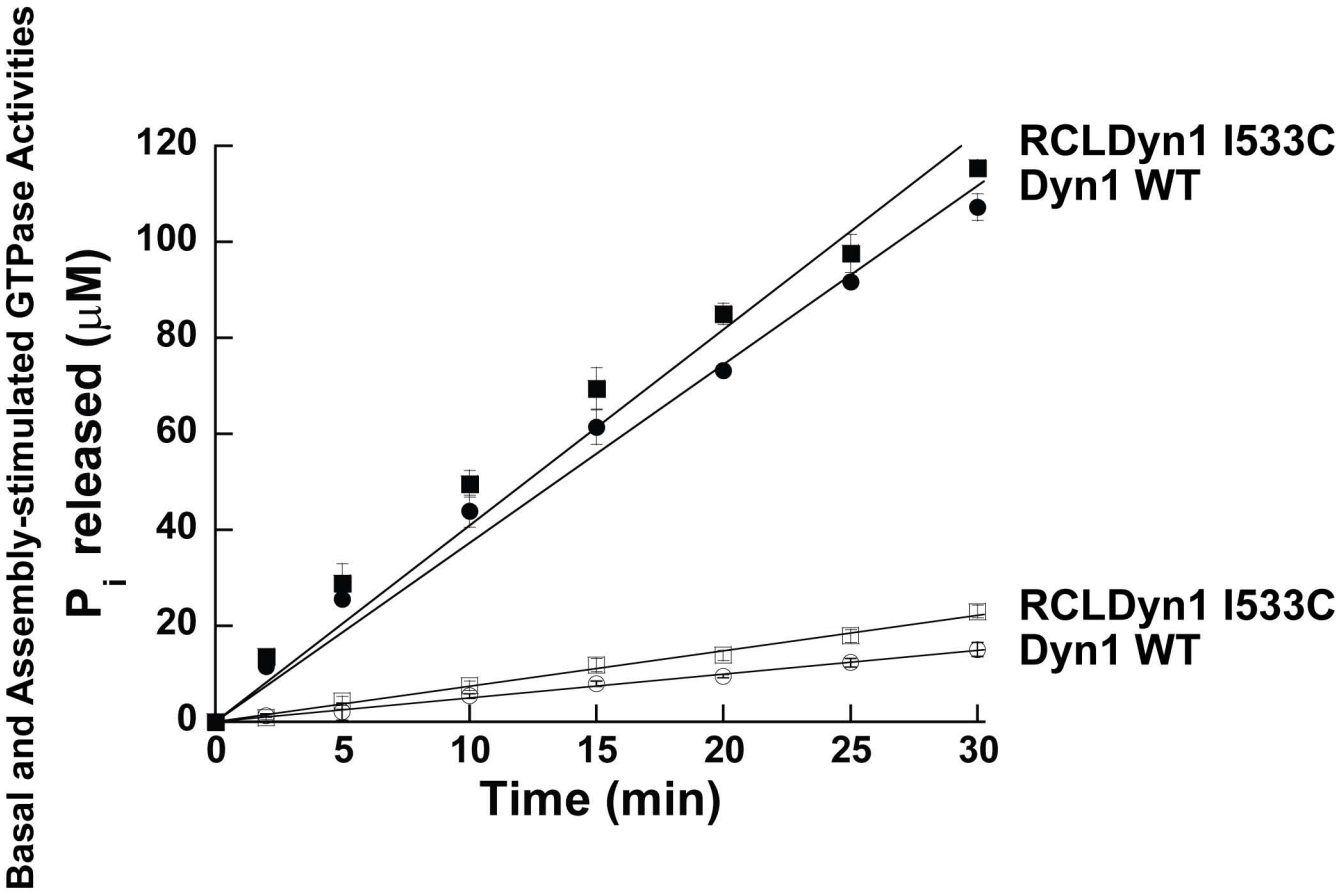
Supplementary Movie 1. Effect of 0.5 μM (final) Dyn1 WT and Dyn 1 I533A addition to SUPER templates in the presence of GTP (1 mM) is shown. Frames were captured at 1 fps and played at 7 fps.

Supplementary Movie 2. Effect of 0.5 μM (final) Dyn1 WT and Dyn 1 I533A addition to membrane tethers drawn out of SUPER templates in the presence of GTP (1 mM) is shown. Frames were captured at 1 fps and played at 7 fps.

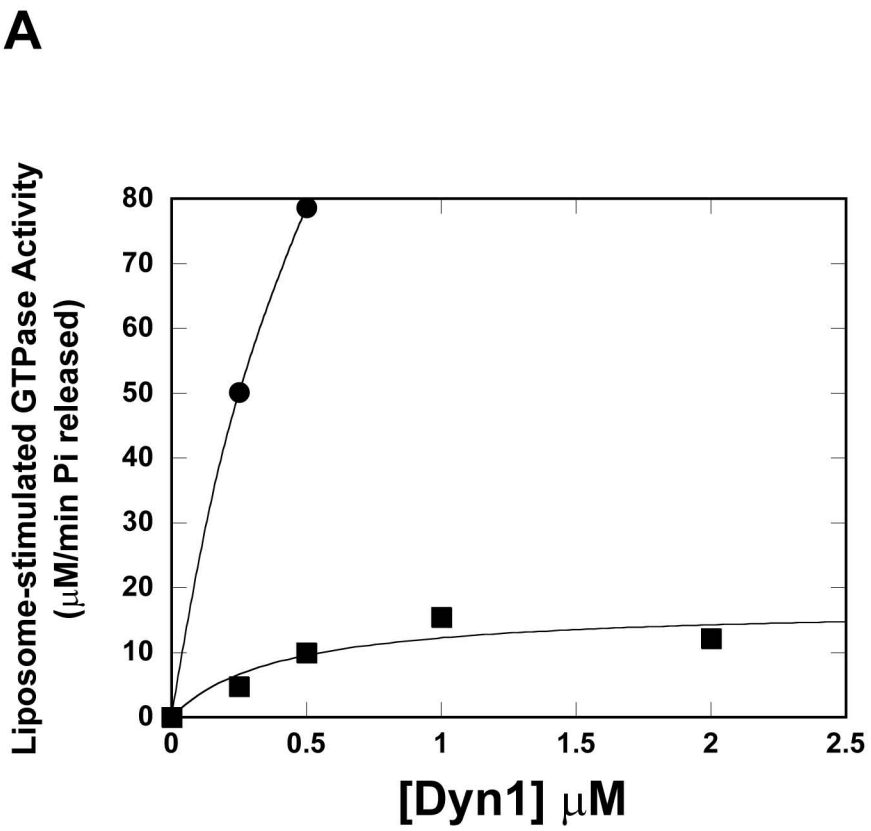
Supplementary Figure 1

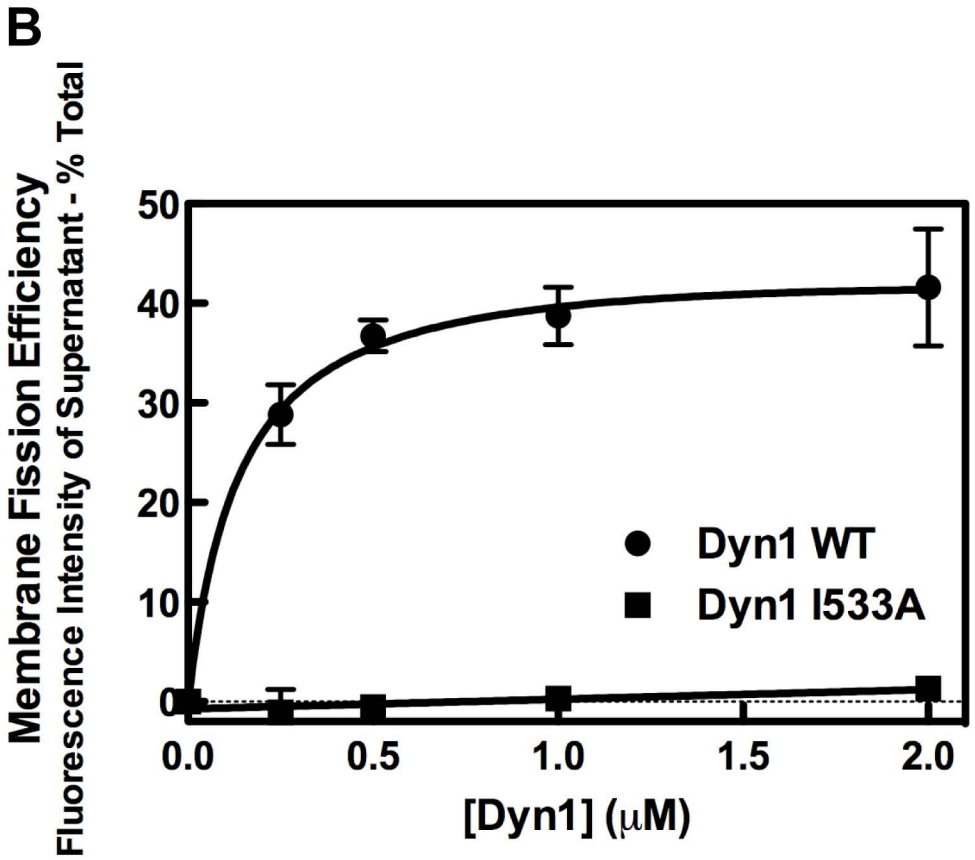


Supplementary Figure 2



Supplementary Figure 3





Supplementary Figure 4