Supplementary information

Materials and methods:

Expression and purification of dynamin mutants

Wild type dynamin and mutants were expressed by transient transfection in Sf9 cells and purified by affinity chromatography using GST-tagged amphiphysin-II SH3 domain as described previously[4]. For *E. coli* expression, Dynamin^{RCL} R15C/R730C cDNA was subcloned from pIEX6 insect cell expression plasmid into pTriEx-3 multisystem expression vector using *NcoI* and *NotI* restriction enzymes. E.coli BL21 DE3 cells carrying TriEx-3 plasmid expressed high yields of dynamin upon overnight IPTG induction at 25 °C. For dynamin purification purposes, both *E. coli* and insect cell lysates were treated similarly.

Chemical Crosslinking

Chemical crosslinking was performed as previously described[4]. Briefly, 20 µg of dynamin mutants in 100 µl of HEPES buffer (20 mM HEPES (pH 7.5), 150 mM KCl, 1 mM EGTA and 2 mM $MgCl_2$) was incubated with 20 µM 1,1-Methanediyl Bismethanethiosulfonate (MTS-1-MTS, Toronto Research Chemicals) for 10 min at room temperature, and then quenched with 40 µM N-Ethylmaleimide (NEM, Thermofisher Pierce). Samples were run on 7.5% SDS-PAGE precast gels (Bio-RAD) after denaturing with 6X SDS sample buffer containing 10% SDS, followed by heat denaturation for 10 min at 95-100 °C.

Intact Mass spectrometry

For MALDI MEGA-TOF measurements, protein samples were first desalted in 70% Acetonitrile, 0.01% TFA with Ziptip (Millipore) pipette tips and spotted on a MALDI plate pre-crystallized with sinapinic acid (SA, Thermofisher Pierce). An additional layer of matrix (SA) ensured proper mixing of sample and matrix. Calibration was performed with IgG (160 kDa) and BSA (65 kDa) and data accumulation was performed using 100 accumulations of 100 laser pulses each.

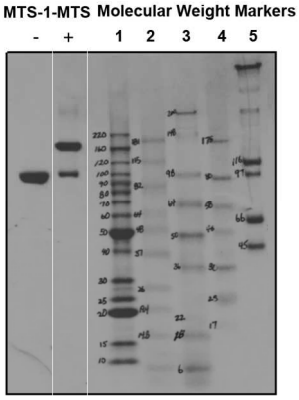

Figure S1. Coomassie-blue stained gel showing migration of non-crosslinked and crosslinked Dyn1^{RCL} R15C/R730C relative to several commercially available molecular weight markers. (1) BenchMark Protein ladder, unstained (Invitrogen Cat. No. 10747-012, MWs 200, 160, 120, 100, 90, 80, 70, 60, 50, 40, 30, 25,, 20, 5, 10 kD); (2) BenchMark pre-stained protein ladder (Invitrogen Cat. No. 10748-010, MWs 181, 115, 82, 64, 48, 51, 26, 18.4, 14.8 kD); (3) SeeBlue Plus2 pre-stained protein standard (Invitrogen Cat No. LC5925. MWs 200, 148, 98, 64,50, 36, 22, 18, 6 kD); (4) Pre-stained protein marker (New England Biolabs Cat. No. P77085, MWs 175, 80, 58, 46, 30, 25, 17); (5) Unstained high range molecular weight standards (Bio-Rad, Cat. No. 161-0303, MWs 4200, 116, 97, 66, 45 kD). The unlabeled higher MW species likely correspond to rabbit muscle myosin monomers (200kD) and dimers (400 kD) that result from incomplete denaturation of standards.