Supplemental Materials

Molecular Biology of the Cell

Liu et al.

Table S1. Drp1 mutants used in yeast two-hybrid screen

Mutant	Point mutations	Mutant	Point mutations
1	Q314A, Y315A, Q316A, S317A, L318A	22	E437A, Q440A, R441A
2	L319A, N320A, S321A, Y322A	23	Q444A, N448A, Y449A
3	G323A, P325A, V326A, D327A	24	H445A, C446A, S447A
4	S330A, T332A	25	Q452A, E453A, L455A, R456A
5	Q335A, L336A, T338A	26	K459A, H461A, D462A
6	K339A, T342A, N346A	27	D462A, V465A, E466A
7	T351S, A 352D, K353G, Y354Q	28	K474A, L476A, P477A, V478A
8	S358A, E359A, L360A	29	E481A, M482A, H484A, N485A
9	R365A, C367A, H371A	30	K497A, P499A
10	Y368A, E372A	31	D503A, A504R
11	G375A, T377A, S380A	32	E611A, R612A, K615A
12	R376A, E379A	33	L613A, S616A, Y617A
13	V381A, D382A, P383A, L384A	34	R622A, K623A, N624A, Q626A
14	G385D, G386E	35	D627A, S628A, K631A
15	I390A, T394A, R397A	36	H635A, F636A, N639A, H640A
16	N398A, T400A	37	K642A, D643A, T644A, Q646A
17	L406A, F407A, V408A, P409A	38	S647A, E648A, V650A, G651A
18	F413A, E414A, L415A, L416A	39	K655A, S656A, S657A, L658A, L659A
19	R419A, R423A, E425A	40	D660A, D661A, T664A
20	E426A, P427A, L429A, R430A	41	E667A, D668A, M669A
21	E433A, L434A, H436A	42	K674A, E675A, D678A

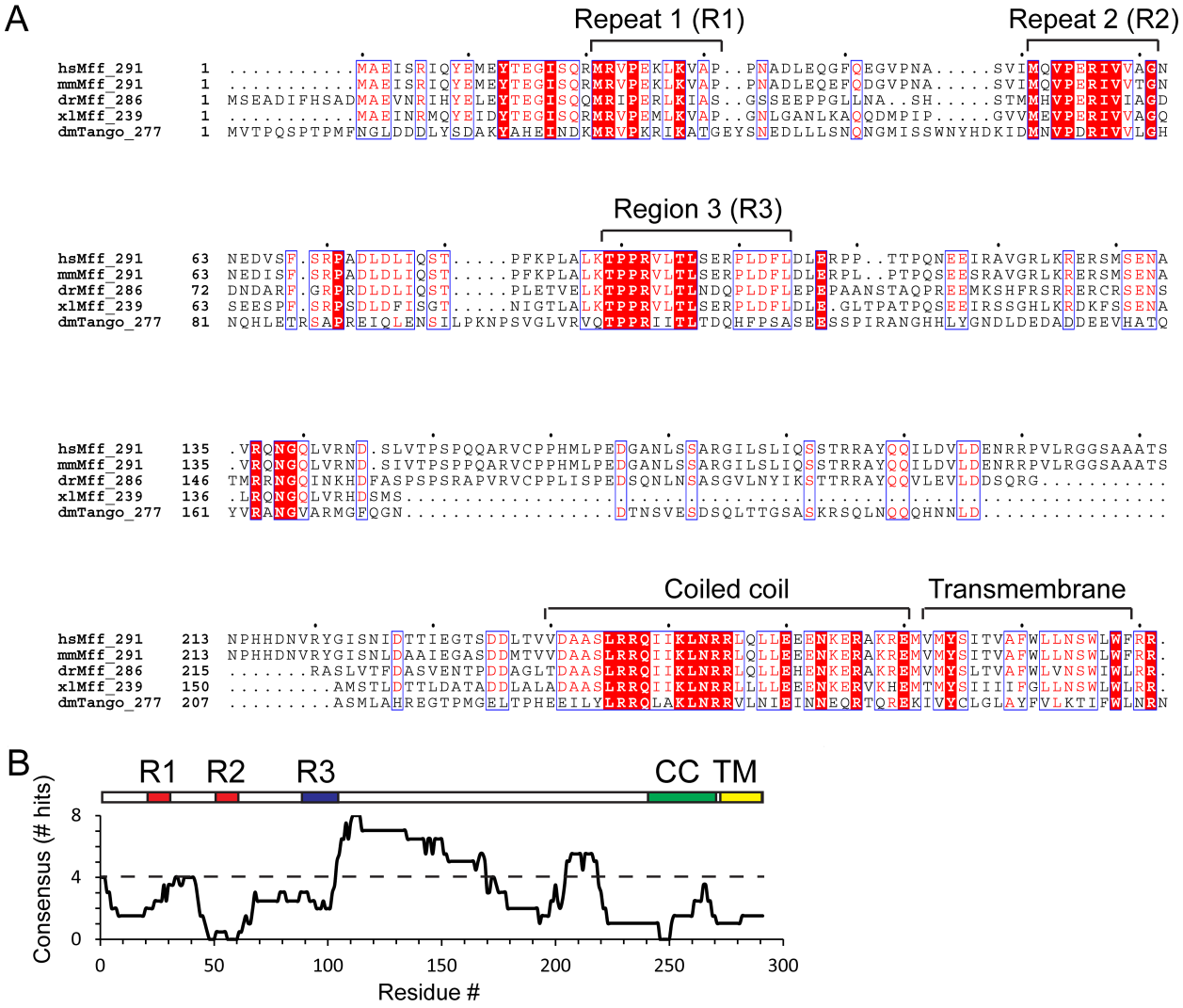

Figure S1. Mff sequence analysis.

(A) Multiple sequence alignment of Mff orthologs from human, mouse, fly, frog, and fish. Conserved residues are boxed in blue. Residues with 100% identity are highlighted in red with white text, and residues conserved in 70% of sequences are in red text. Notable regions included: Repeat 1 (R1) and Repeat 2 (R2) as previously identified (Gandre-Babbe and van der Bliek, 2008), an additional conserved region (R3), the coiled-coil domain (CC) and the transmembrane segment (TM). The Mff sequences used in this alignment are (RefSeq): *Homo sapiens* mitochondrial fission factor isoform b (NP_001263991.1), *Mus musculus* mitochondrial fission factor isoform 1, NP_083685.2, *Danio rerio* mitochondrial fission factor homolog A, (NP_001018402.2), *Xenopus laevis* mitochondrial fission factor homolog B, (NP_001085443.1), and *Drosophila melanogaster* transport and golgi organization 11, isoform A (NP_726111.1). Alignments were performed with MultAlin (Corpet, 1988) and formatted with ESPript (Robert and Gouet, 2014). (B) Disordered regions in Mff. DisMeta, the Disorder Prediction MetaServer (Huang *et al.*, 2014), was used to predict disordered regions in mouse Mff isoform 1. The consensus among 8 different predictors is plotted against each residue. A consensus above 50% of these predictors (dotted line) is indicative of disorder. The diagram of mouse Mff isoform 1 is aligned with the plot.

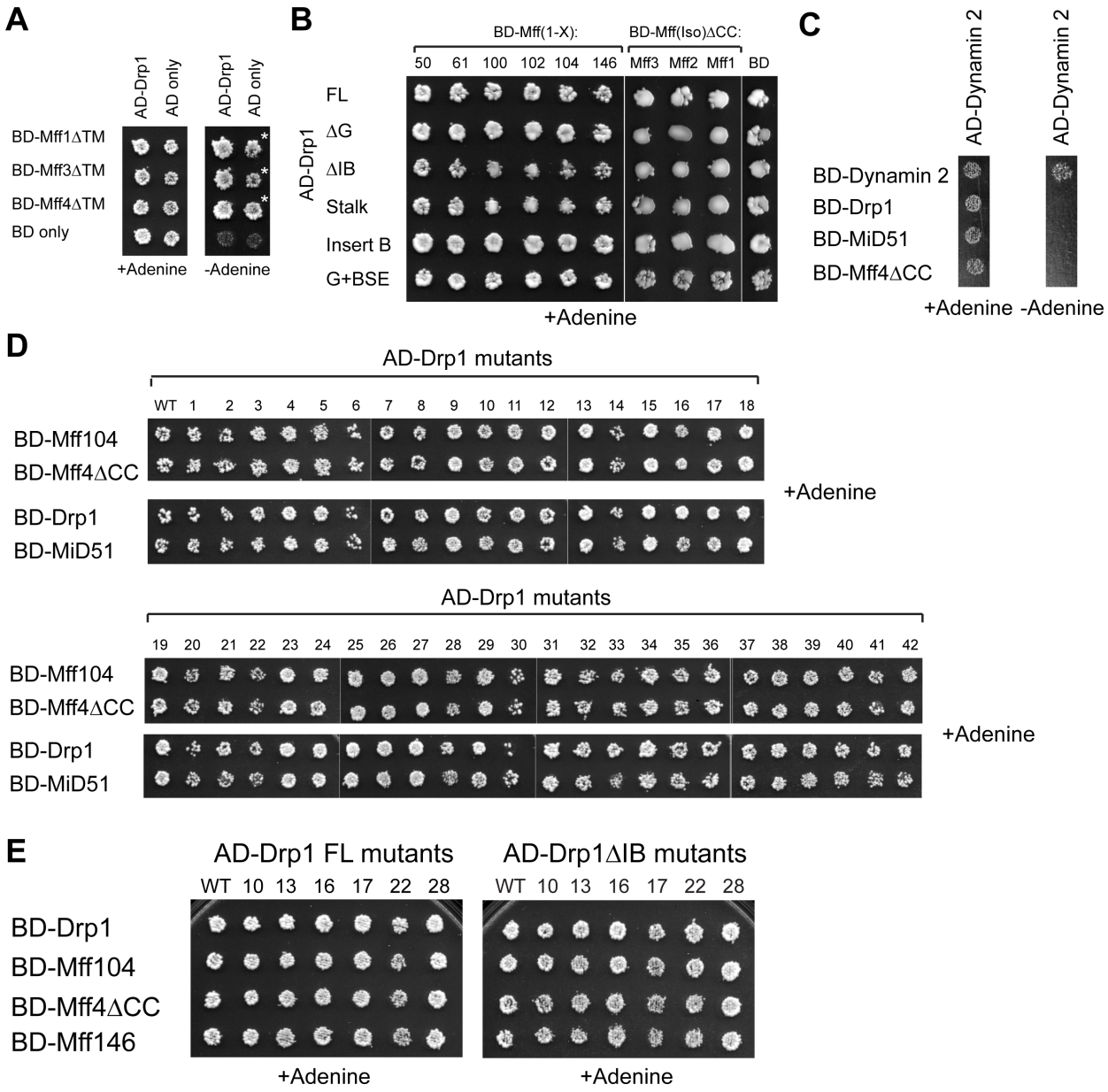

Figure S2. Yeast two-hybrid assay controls.

(A) Auto-activation of Mff lacking the transmembrane region. Mouse Mff isoforms 1, 3 and 4 with the transmembrane domain deleted (ΔTM) were expressed from the pGBDU vector as BD fusion proteins and tested against Drp1 expressed from the pGAD vector as AD fusion proteins. Growth on adenine-deficient plates, but not against AD or BD only, indicates an interaction. The growth of the Mff constructs against AD only on adenine-deficient plates (white asterisks) indicates auto-activation, making results with these constructs uninformative. (B) Diploid selection growths for the adenine-deficient plates in Figure 1E. (C) Lack of interaction between dynamin 2 and Mff. Mouse Dynamin 2, Drp1, MiD51, and Mff isoform 4 with the transmembrane domain deleted (Mff4ΔCC) were expressed as BD fusion proteins and tested against Dynamin 2 expressed as an AD fusion protein. Dynamin 2 interacted with itself, but did not interact with Drp1, MiD51, or Mff4ΔCC. (D) Diploid selection plates for the adenine-

deficient plates in Figure 3A. (E) Diploid selection plates for the adenine-deficient plates in Figure 3B.

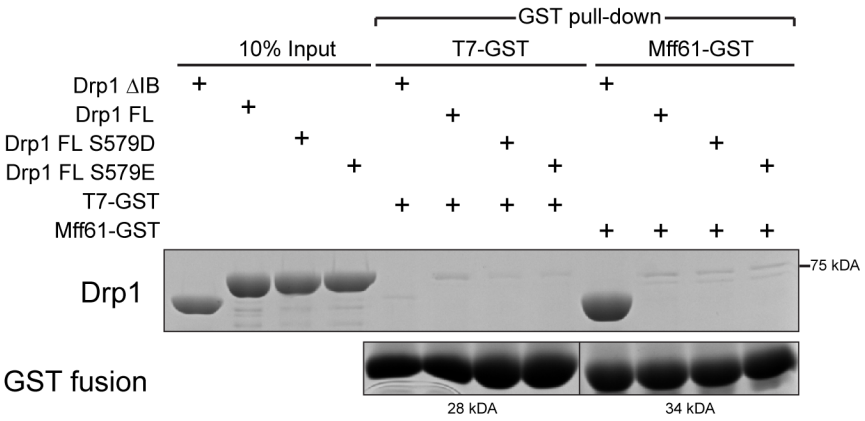

Figure S3. Phosphomimetic Drp1 does not interact with Mff61.
(A) GST pull-down assays for purified recombinant Drp1ΔIB, Drp1 (full-length), Drp1 S579D (full-length), and Drp1 S579E (full-length) versus purified recombinant Mff61-GST or T7-GST. The Coomassie-stained SDS-PAGE gel shows the input protein (lanes 1-4) and eluates from the pull-downs (lanes 5-12). In lanes 5-12, the bottom row shows the isolated GST fusion proteins. The top row depicts the co-immunoprecipitated proteins.