SUPPLEMENTAL FIGURES AND LEGENDS

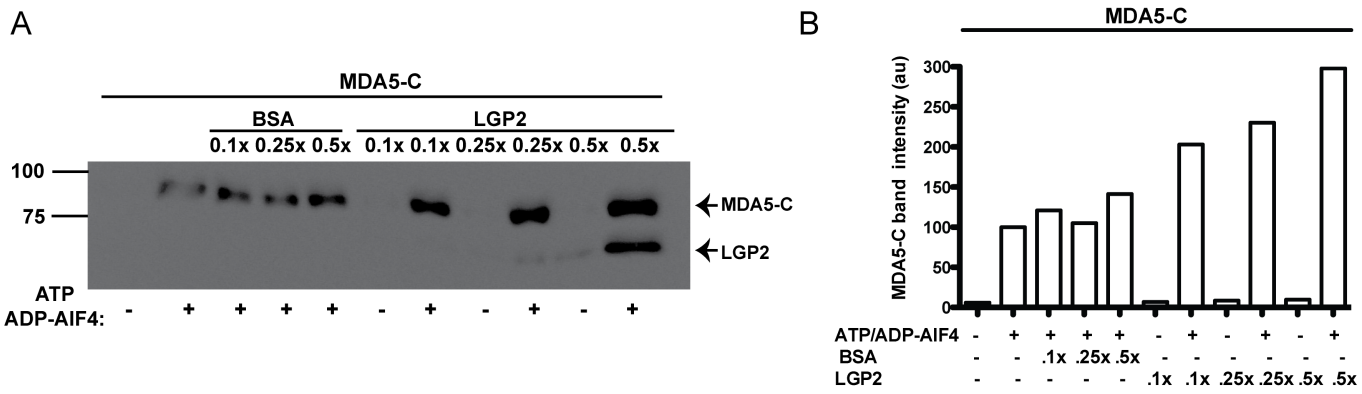

Figure S1. LGP2 enhancement of MDA5-RNA interaction requires ATP/ ADP-AlF$_4$ conditions, related to Figure 2.

A. Biotinylated poly(I:C) binding experiment conducted as in Figure 2A, comparing MDA5 pulldown and effects of LGP2 in buffer containing 500μM ATP prior to quenching with ADP-AlF$_4$ immediately prior to denaturation (+) to parallel samples in buffer lacking both ATP and ADP-AlF$_4$ (-).

B. Quantification of MDA5 band intensity from panel A. Data are normalized to the signal of MDA5 alone in the ATP/ ADP-AlF4 conditions.

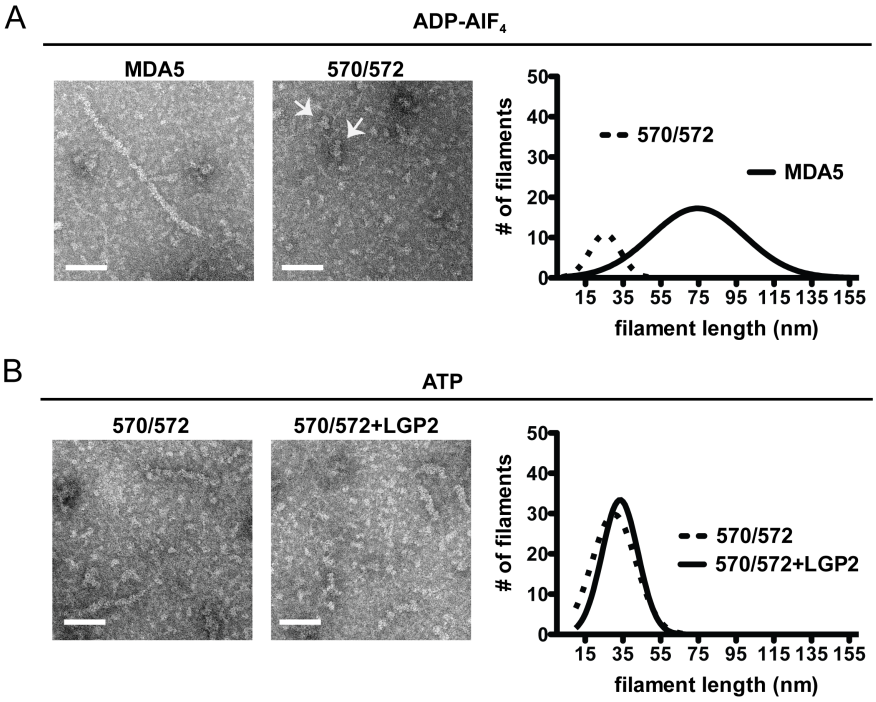

Figure S2. Additional analysis of the monomer-interface mutant, MDA5-570/572, related to Figure 3.

Electron micrographs (left) and quantification (right) of filaments formed by MDA5-570/572. As MDA5-570/572 filaments were scarce, images were taken specifically of fields that contained quasi-filaments, and the length distribution from 14 such images was quantified.

A. Direct comparison of WT and mutant MDA5. Electron micrographs illustrating MDA5 and MDA5-570/572 complexes formed under filament-favoring conditions and concentration (0.1μM). From 14 random selected images of MDA5, 137 filaments were measured with an average length of 75nm, while images selected to contain structures of MDA5-570/572 contained only 55 filaments with an average length of 25nm. Graph of filament lengths demonstrates obvious defect in generating structures longer than 18nm that could be quantified. MDA5-570/572 is impaired in filament formation and is not affected by LGP2.

B. Electron micrographs illustrating MDA5-570/572-dsRNA interactions and quasi-filaments at 3 fold higher concentration (0.3μM) than MDA5-WT used in panel A. For MDA5-570/572 alone, 80 filaments were measured with an average length of 30nm. For the mixed MDA5-570/572 and LGP2 sample, 77 filaments were measured with an average length of 34nm. Addition of LGP2 does not alter the quasi-filament length distribution.

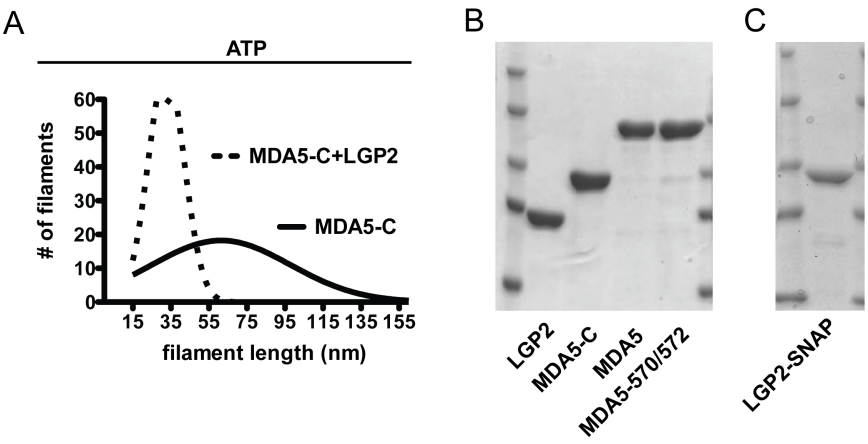

Figure S3. LGP2 purified from mammalian cells attenuates MDA5 filament formation (related to Figure 4).

Experiment conducted as in Figure 4 using an LGP2-SNAP fusion protein purified from transfected HEK293T cells.

A. Effect of LGP2 on MDA5 filament formation in ATP hydrolysis conditions. Similar to panel C of Figure 4, comparing MDA5-C alone to MDA5-C+LGP2 in the presence of ATP. Graph of filament size distributions is presented at right for 13 random images. For MDA5-C alone, 160 filaments were measured with an average length of 61nm. For the mixed MDA5-C+LGP2 sample, 185 filaments were measured with an average length of 33nm.

B. SDS-PAGE and Coomassie blue staining showing purity of proteins LGP2, MDA5-C, MDA5, and MDA5-570/572 isolated from baculovirus infected Sf9 cells.

C. SDS-PAGE and Coomassie staining of LGP2-SNAP purified from HEK293T cells.

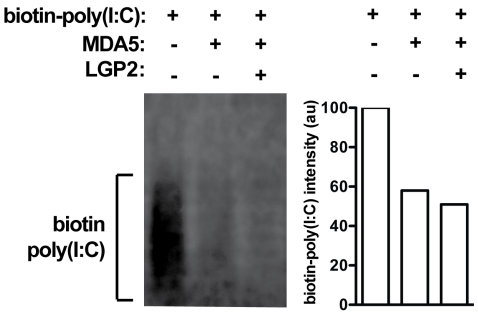

Figure S4. Quantification of biotin-poly(I:C)-RNP transfection efficiency, related to Figure 5.

293T cells were transfected with RNPs as in Figure 5. RNPs were assembled with 3μg total poly(I:C), with 2μg unlabeled poly(I:C) and 1μg biotinylated poly(I:C). Total RNA was harvested and analyzed by Northern blot followed by probing with HRP-conjugated streptavidin to detect transfected biotin-poly(I:C) (panel A). Naked poly(I:C) has a much higher transfection efficiency than RNP complexes, but RNPs containing MDA5 or MDA5+LGP2 display similar transfection efficiency. Panel B. Quantification of biotin-poly(I:C) signal..