

1 **Supplementary Video 1** The MTD of Src can compensate for the deletion of MA to  
2 promote polarized assembly. HEK293 cells were transfected with constructs  
3 expressing MLV Env and Src $\Delta$ MA Gag-GFP (green). At 4 hour post-transfection,  
4 cells were co-cultured with uninfected target XC cell expressing mCAT-1-mCherry  
5 (red). A virus producing cell in contact with a target cell was monitored by spinning  
6 disc confocal microscopy. Z-stacks of red and green images of each time point were  
7 merged into single images to generate a quick time movie. The movie is played at 5  
8 frames/s for a period of 3 hours.

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10 **Supplementary Video 2** Wild-type MLV Gag is targeted to the MLV synapse. A time  
11 lapse imaging experiment as in video 1 was performed for HEK293 cells transfected  
12 with constructs expressing wild-type Gag-YFP (green) and MLV Env. The time point  
13 when virus assembly initiated was set to zero. Video S2 represents the data source for  
14 the analysis of wt Gag and wt Env presented in Figure 7.