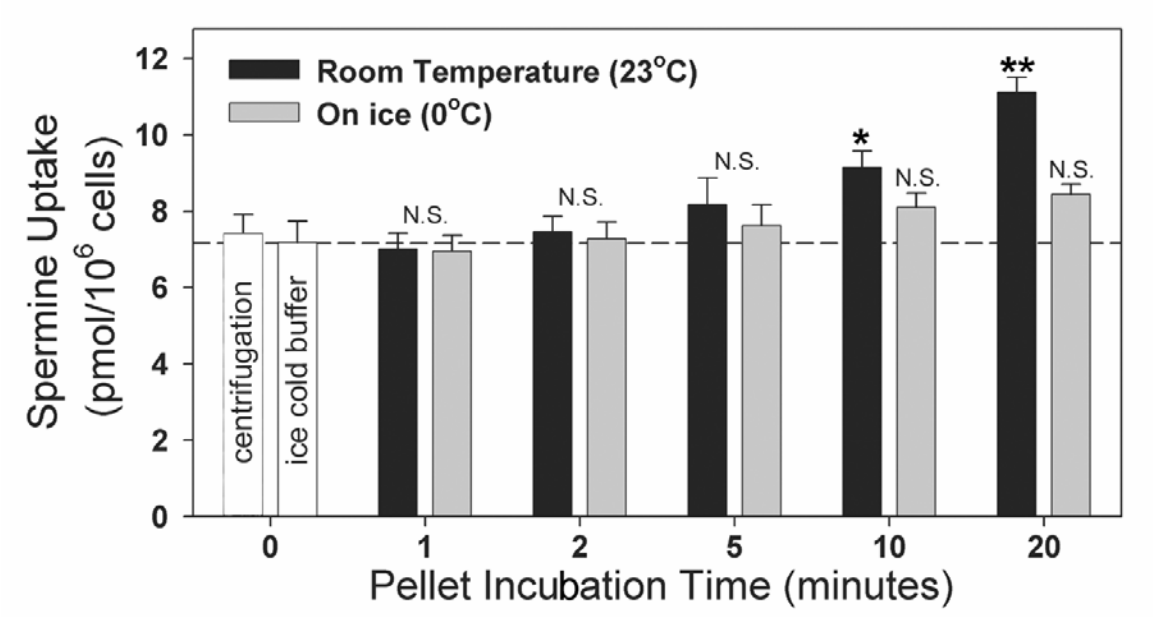

Figure S1. Centrifugation of cell suspensions effectively stops the uptake of radiolabeled substrates. *Drosophila* S2 cells were incubated with 500 nM [^{14}C]spermine for 5 minutes. The reaction was then stopped by either room temperature "centrifugation" as described in our manuscript, or by the addition of 3 volumes of "ice cold buffer" as indicated. The measured amount of spermine transport (open bars) was similar using either method, suggesting that centrifugation served to stop the reaction as well as rapidly lowering the temperature and diluting the substrate. The dotted line represents the average of these values and serves as a baseline for the remainder of the experiment testing the effects of centrifugation.

To determine whether residual transport occurred after stopping the reaction using centrifugation, the pelleted cells were incubated either at room temperature (black bars) or on ice (0°C, grey bars) for the specified amount of time before lysis. Bars represent means ± SEM of three independent experiments done in triplicate. The data indicate that the pelleted cells do not take up statistically significant amounts of radiolabeled substrate for at least 5 minutes at room temperature and for at least 20 minutes on ice (not significant, N.S.). After extended incubation at room temperature, the pelleted cells can take up additional substrate: +20% relative to the baseline after 10 minutes (*, $p<0.02$) and +50% after 20 minutes (**, $p<0.0001$).

All samples were processed by centrifugation at room temperature for 30 seconds followed by removal of the supernatant, which took three to five seconds per sample. (It is likely that the cells pelleted after several seconds, but 30s centrifugation was used for convenience.) The pelleted cells were washed twice sequentially by rapidly adding 1.5 ml of ice cold buffer followed by centrifugation at room temperature for 30 seconds. After the last wash, the buffer was quickly aspirated to prevent the pellet from diffusing and/or the radiolabeled substrate from effluxing. After removing the second wash from the last tube from a given experiment, the cells were lysed as described in the Materials and Methods section of our manuscript. For each experiment we used a maximum of six assay tubes to ensure that all of these steps were performed very rapidly. The entire

procedure was complete by about 3 to 4 minutes. For most of the procedure, the cells were in a pellet, and the data above indicates that transport does not occur for up to 5 minutes for pelleted cells at either room temperature or on ice. Furthermore, immediately after the first spin, the bulk of the substrate had been removed.

It remains possible that very small amounts of transport occurred while the cells were in a compact pellet, but these amounts are not likely to alter the conclusions of our manuscript. We also acknowledge that we do not yet know why simply pelleting the cells serves to slow if not stop the transport reaction. Indeed, we were surprised when this procedure appeared to work better than a filtration assay. Nonetheless, our data strongly suggest under the conditions that we have used here, pelleting the cells provides a convenient method to dramatically slow and essentially stop polyamine transport.