



Fig. S9. Difference map histograms. Differences arise from the presence of additional protein mass, conformational changes, or noise. Unlike positive differences due to new protein, positive differences due to conformational changes and noise should be accompanied by corresponding negative differences. Thus the histogram for the "control" difference map between the two ΔN -VPS4p half-data-set reconstructions exhibited a monotonic symmetric peak around zero extending to approximately $\pm 5\sigma$. In sharp contrast, the other two difference maps (full-length Vps4p minus ΔN -VPS4p and Vta1p-Vps4p minus full-length Vps4p) had large shoulders of positive differences beyond $+3.5\sigma$ which are not matched by corresponding negative differences and which reflect the presence of authentic additional densities.