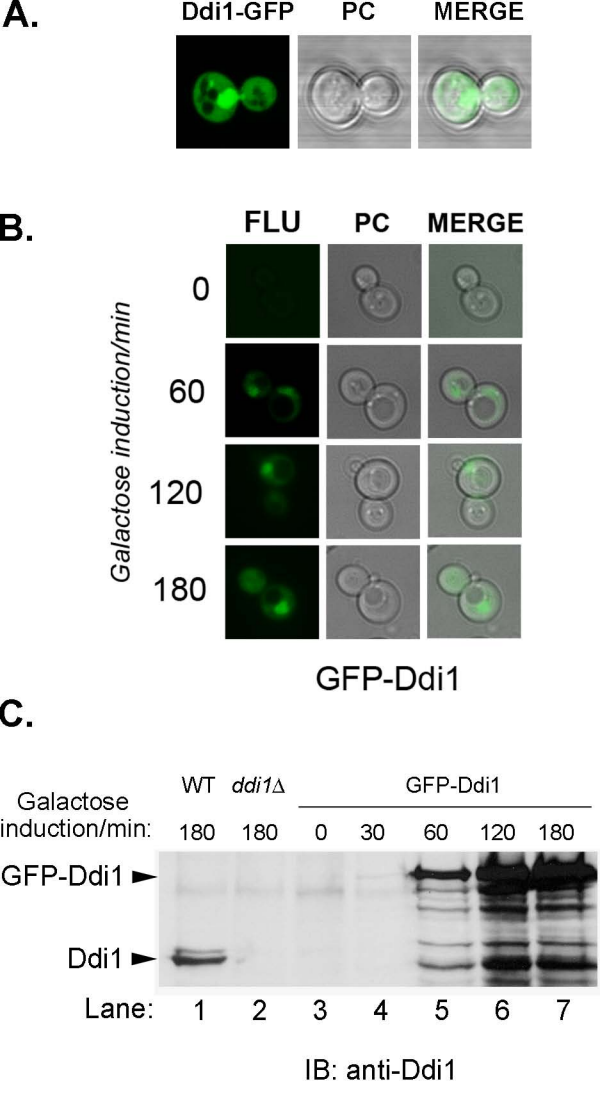
E07-05-0462 Gerst

Supplementary Figure 1. Different GFP-tagging strategies show that Ddi1 is enriched in the nucleus. (A) Wild-type cells (WT; W303) expressing *DDI1-GFP* from a multi-copy plasmid (pADH-DDI1-GFP) were grown to the mid-log phase and observed bu confocal microscopy. Both phase contrast (*PC*) and merged light and fluorescence panels (*MERGE*) are shown. (B and C) GFP-Ddi1 induced from a *GAL1* promoter show nuclear enrichment at all times after induction. Wild-type cells expressing *GFP-DDI1* from the genome (GGY12) were grown in medium containing raffinose as a carbon source to mid-log phase, washed with TE buffer, and then grown in galactose-containing media. At different time points (0, 30, 60, 120, and 180 min, as indicated) cells were harvested and examined either by fluorescent microscopy (B) or by Western analysis (C). *FLU* indicates fluorescence, *PC* indicates phase contrast, while *MERGE* indicates the merge of the windows. In (C) *GFP-DDI1* expressing cells (GGY12; lanes 3-7), wild-type (WT - W303 cells; lane 1) and *DDI1* deficient (*ddi1*Δ - VL2 cells; lane 2) cells were grown under the same conditions as described above, and shifted to galactose-containing media for up to 180 min (as indicated). Arrowheads indicate the positions of native Ddi1 and galactose-induced GFP-Ddi1 in the Western blot. Protein expression was performed using anti-Ddi1 (1:5000) antibodies. Blot was over-exposed to show the presence of native Ddi1 in lane 1, hence the appearance of breakdown products of GFP-Ddi1 in lanes 5-7.

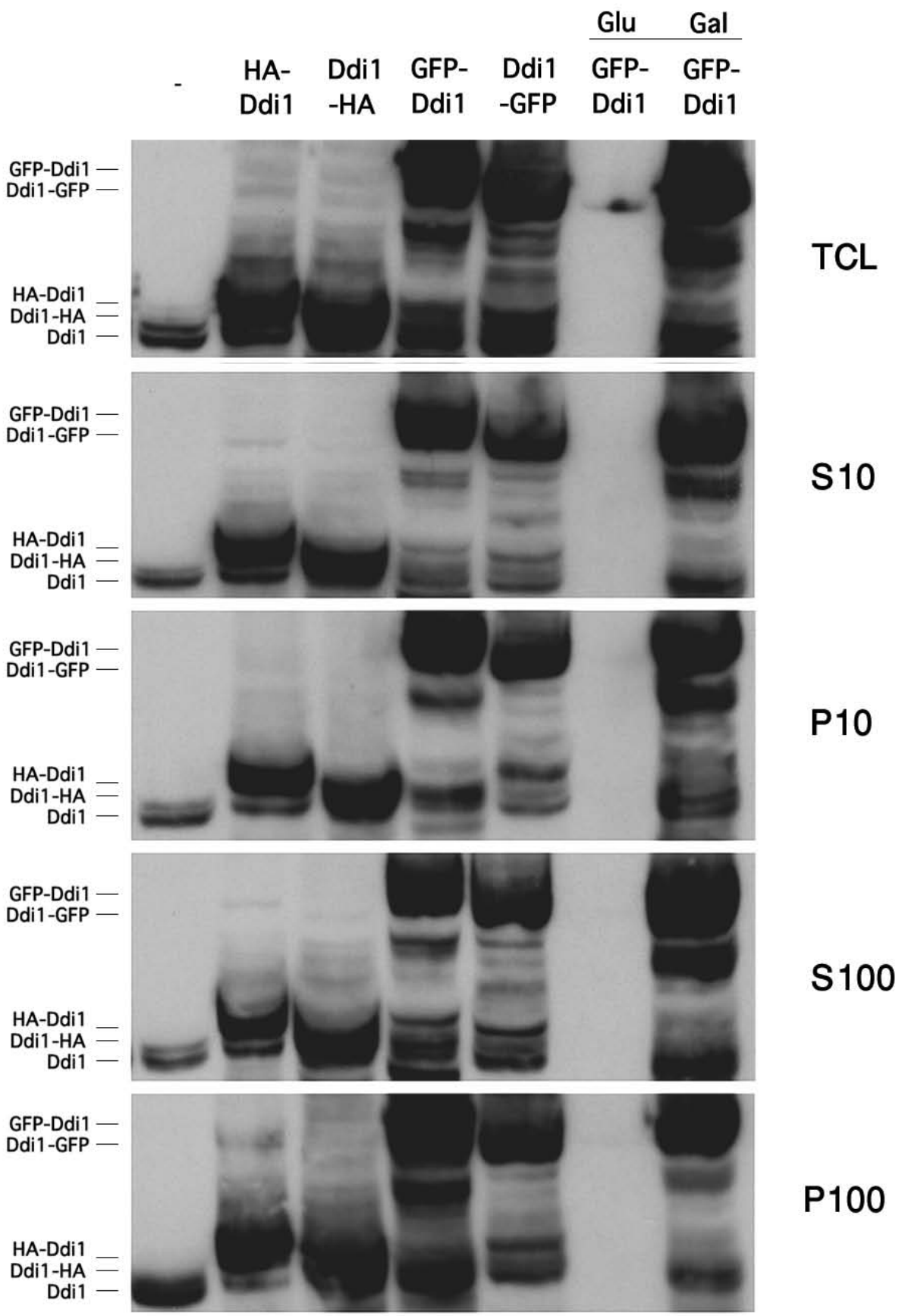
Supplementary Figure 2. The different tagged forms of Ddi1 show the same sub-cellular pattern of distribution. Wild-type yeast (W303) expressing a control vector (-), a multicopy plasmid expressing Ddi1 tagged either at the amino and carboxy terminal with HA (HA-Ddi1 and Ddi1-HA, respectively – note that Ddi1-HA appears smaller due to usage of a shortened HA tag), or a multicopy plasmid expressing Ddi1 tagged either at the amino and carboxy terminal with GFP and HA (GFP-Ddi1 and Ddi1-GFP, respectively – note that Ddi1-GFP appears smaller due to usage of a shortened HA tag) were grown to log phase and lysed using glass beads. In parallel, wild-type cells expressing *GFP-DDI1* from the genome (GGY12) were first grown in medium containing glucose as a carbon source to mid-log phase, washed with TE buffer, and then either grown in galactose-containing media for 6 hours or maintained in glucose-containing medium prior to lysis. After a low speed spin (600 x *g*) to remove unbroken cells, total cell lysates (TCLs) from the cells were subjected centrifugation at 10,000 x *g* to yield supernatant (S10) and pellet (P10) fractions. Next, the S10 was subjected to centrifugation at 100,000 x *g* to yield supernatant (S100) and pellet (P100) fractions. Both pellet fractions were resuspended and samples from all fractions (containing equal amounts of protein (40 μg)) were electrophoresed on a 7.5% SDS-PAGE gel prior to blotting and immunodetection with anti-Ddi1 antibody (1:5000). After detection, densitometry was employed to determine the relative amounts of native, HA-tagged, and GFP-tagged Ddi1 present in a given fraction vis á vis the different strains examined. In the TCLs the ratio of native Ddi1 to tagged Ddi1 (HA or GFP, including GFP-Ddi1 grown on galactose, but not glucose) was approximately 1:4 [*e.g.* 1:3.5 (native:HA-Ddi1); 1:3.6 (native:Ddi1-HA); 1:4 (native:GFP-Ddi); 1:4 (native:Ddi1-GFP); and 1:4.4

(native:GFP-Ddi1 on galactose]. This indicates that the expression levels were similar in the different strains expressing tagged Ddi1. In the S10, P10, S100, and P100 fractions, the native to tagged Ddi1 ratios were also very similar, approximately 1:3-4 in all fractions for all tagged forms examined (*i.e.* in the same order listed above: P10 - 1:2.6:2.7:3.4:2.9:3.3; S10 – 1:2.6:2.6:3.2:2.8:3.4; P100 – 1:3.3:3.7:4.1:3.7:4.2;3.6:3.7; S100 – 1:2.2:2.9:3.5:3.2:4.3). These results indicate that the distribution of the different tagged forms of Ddi1 is more or less equal within a given fraction and, therefore, the proteins can be said to behave similarly.

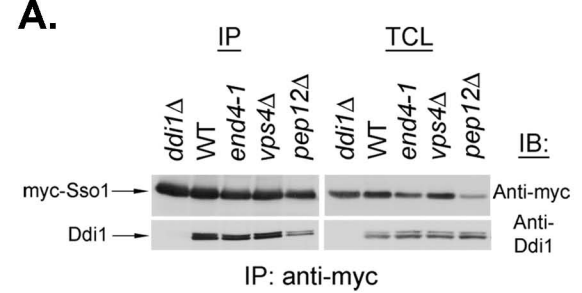
Supplementary Figure 3. (A) Ddi1 binds Sso1 in different mutants of endocytic transport. Wild-type cells (WT; BY4741) or mutants (*e.g*. *ddi1*Δ, *end4-1*, *vps4*Δ, and *pep12*Δ) over-producing myc-Sso1 (pADH-myc-SSO1) were lysed and subjected to co-IP by anti-myc antibodies. Detection of the immunoprecipitated proteins was performed using anti-myc (1:1000) and anti-Ddi1 (1:5000) antibodies. (B) GFP-Sso1 accumulates in the class E compartment in *vps4*Δ cells. Wild type (WT; BY4741), *DDI1* (*ddi1*Δ), and a *vps* class E mutant (*vps4*Δ) cells were transformed with a single-copy plasmid producing GFP-Sso1 (YCp50-GFP-Sso1) and observed using fluorescent microscopy. Arrows point out an enlarged compartment adjacent to the vacuole where GFP-Sso1 accumulates.

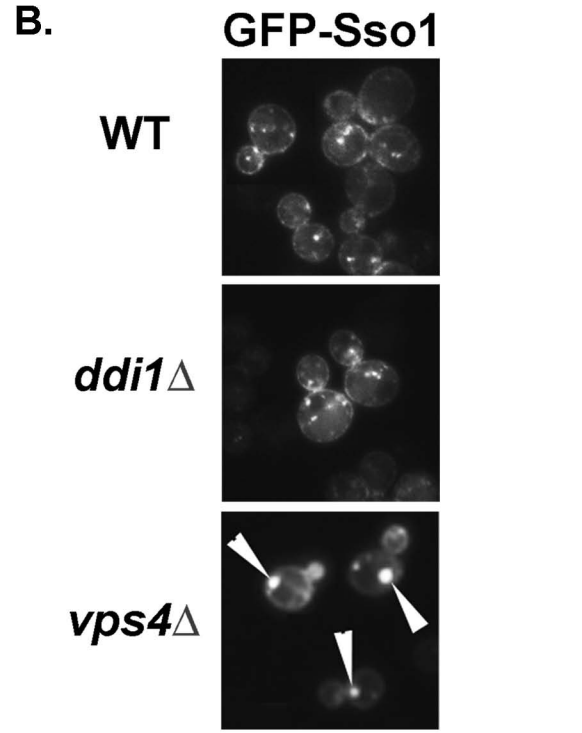


Supplementary Figure 1.



Supplementary Figure 2.





Supplementary Figure 3.