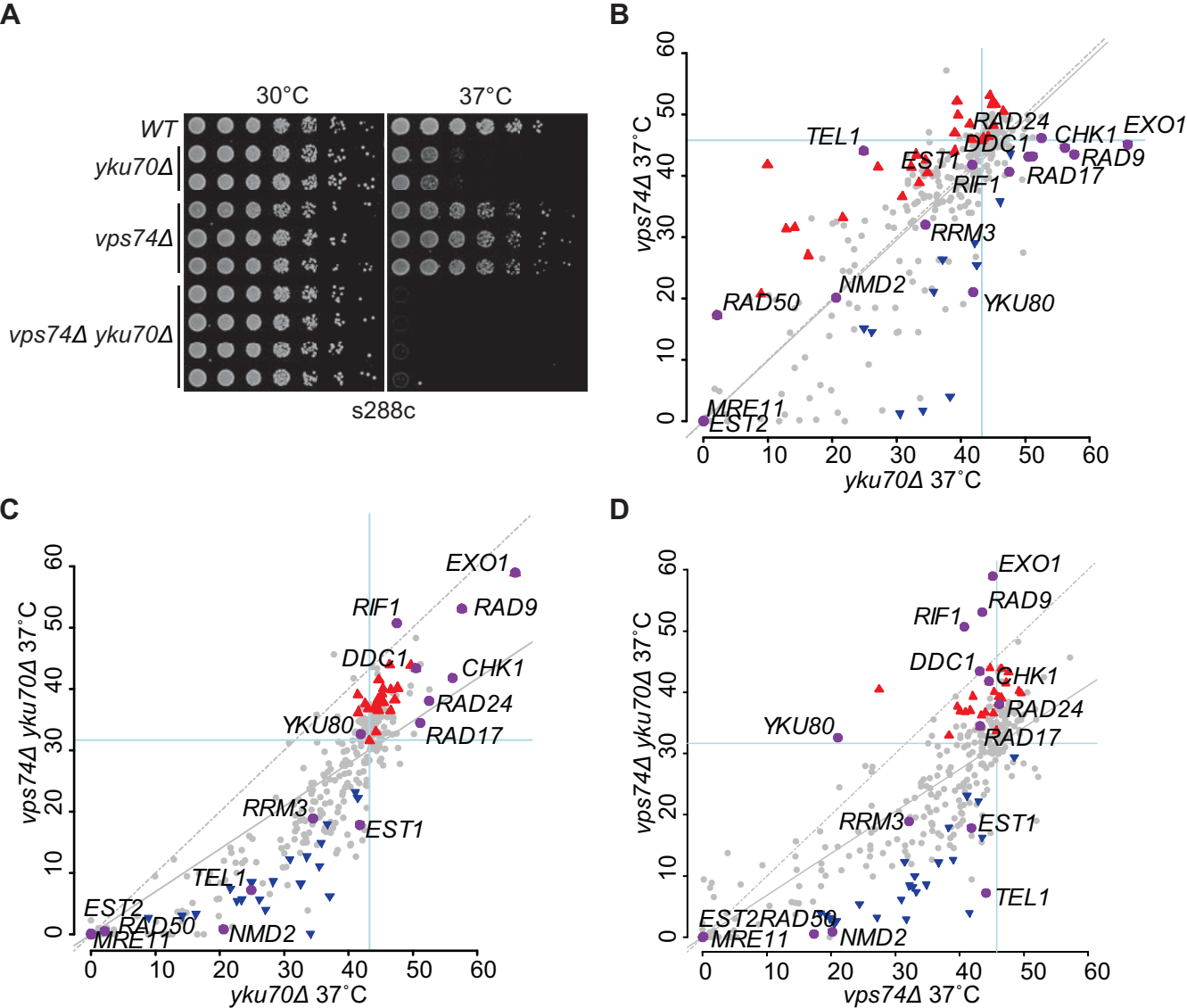


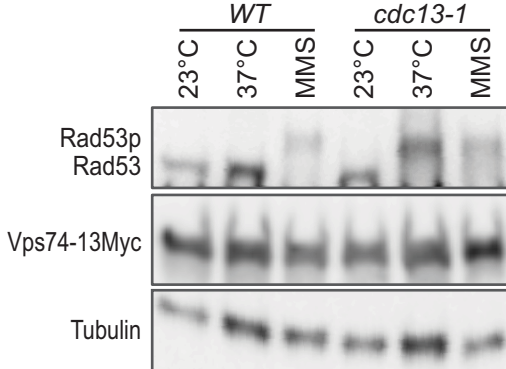
**Figure S1. *vps74Δ* decreases telomeric ssDNA of *cdc13-1* cells and slightly increases *yku70Δ* ssDNA.**

**(A)** Spot test assays as described in Figure 1. **(B)** Schematic model of chromosome V, right arm. **(C)** Cell cultures of the indicated genotypes were grown overnight at 23°C until OD600~0.5-0.8 and a further 4h at 36°C. DNA was extracted using the phenol method and the DNA was used to either measure ssDNA by in-gel assay (C) or QAOS (E). In-gel assay was performed as previously described (Dewar and Lydall 2012) using a IR-Dye800-labelled AC-rich oligonucleotide (m3157). **(D)** The gel in C was horizontally divided in two parts (Intermediate and Low). For each lane, the signal in each part was quantified and divided by the loading control signal. Quantifications were made using the ImageJ software. The mean of the two strains of each genotype is indicated and the "error" bars indicate the two independent measurements, or average deviation. Statistical analyses used the two-tailed unpaired T test (\* $P < 0.05$  and \*\* $P < 0.01$ ) performed with SigmaPlot (version 11). **(E)** QAOS was used to measure the ssDNA on the TG strand 600 bp from the right arm telomere in chromosome V (Booth et al. 2001). Data presentation and statistical analysis is as in D.



**Figure S2. *vps74Δ* exacerbates the checkpoint-dependent *yku70Δ* fitness defects.**

**(A)** Spot test assay as described in Figure 1 showing the fitness of the strains used in the mini-QFA (Figure 4). **(B)** More data as described in Figure 4.



**Figure S3. No evidence that Vps74 is phosphorylated in response to DNA damage.**

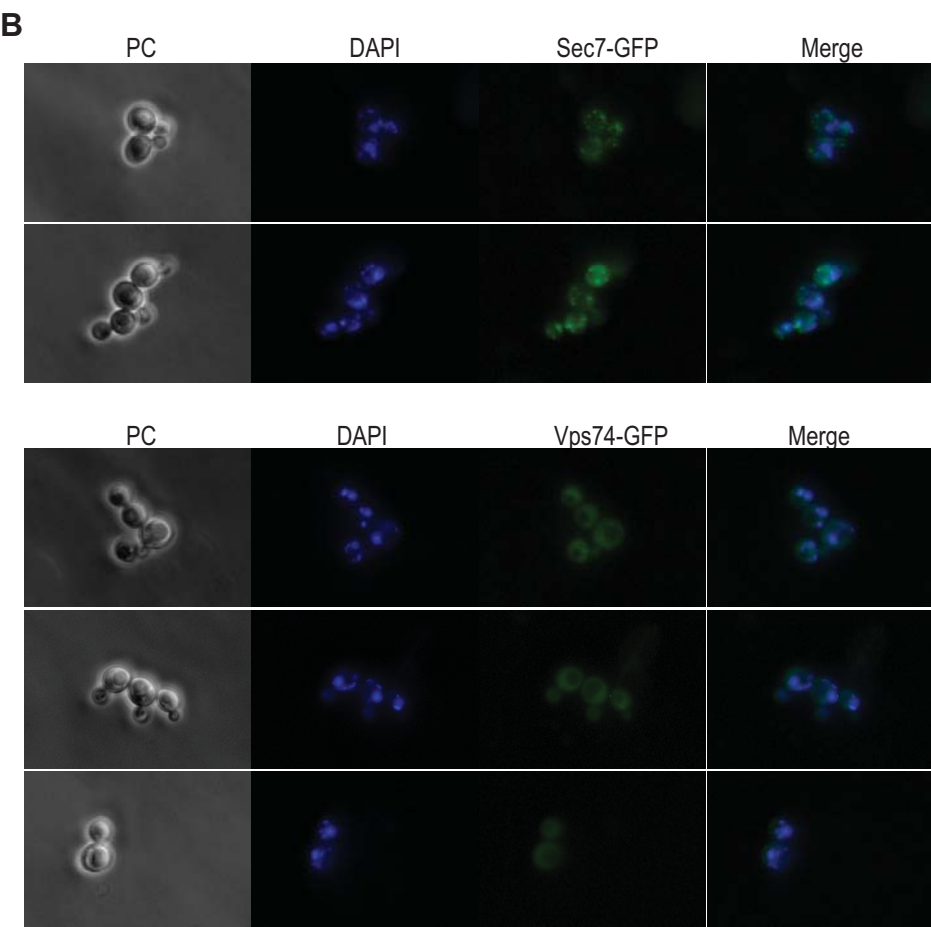
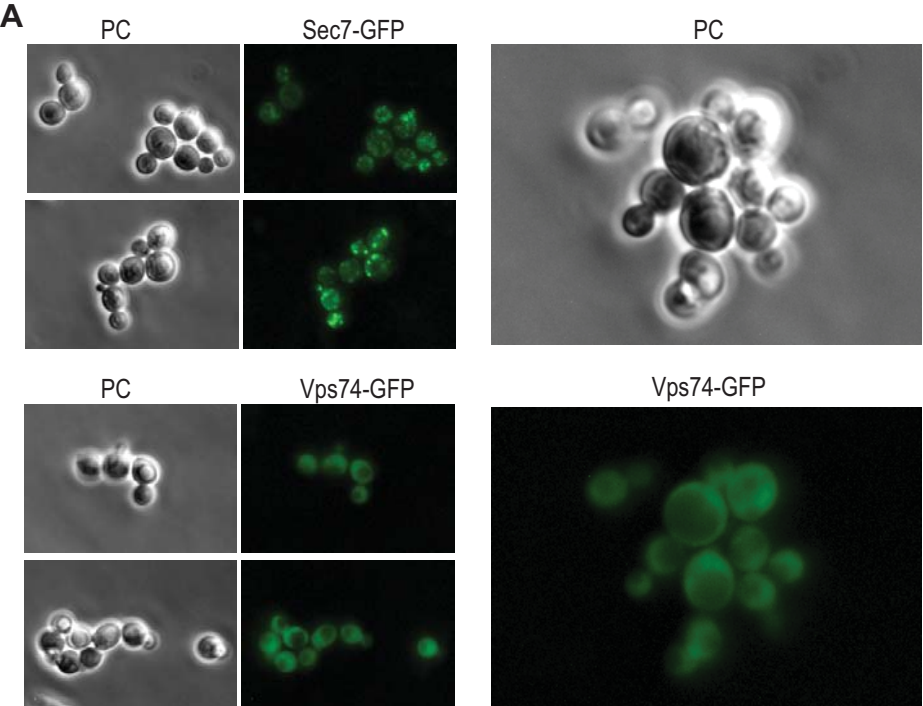
Western blot analysis of *WT* and *cdc13-1* carrying a Vps74-13-Myc tagged construct. Cell cultures were grown overnight at 23°C until early exponential phase (OD600~0.2). Each culture was divided in three cultures that were further incubated at 23°C, 37°C or in the presence of 0.1% MMS (23°C) for 180 min. Two independent strains of each genotype were analysed and one is shown.

A	Gene	Dist.	B	Gene	Dist.	C	Gene	Dist.
1	<b>vps74</b>	0	1	<b>pmt1</b>	0	1	<b>pmt2</b>	0
2	rad34	0.601	2	pmt2	0.579	2	hek2	0.568
3	pmt1	0.647	3	<b>vps74</b>	0.612	3	pmt1	0.579
4	pmp1	0.968	4	pmp1	0.65	4	pmp1	0.712
5	per1	1.033	5	rad34	0.824	5	sut1	0.76
6	pmt2	1.106	6	sut1	0.97	6	ydr090c	0.803
7	ted1	1.177	7	hek2	1.032	7	ssf2	0.814
8	sut1	1.193	8	<b>mnn2</b>	1.056	8	rad34	0.973
9	<b>mnn2</b>	1.291	9	ssh1	1.058	9	bph1	0.981
10	ngl2	1.322	10	ubp3	1.058	10	tmn3	1.002
11	ssh1	1.327	11	ydr090c	1.06	11	ydr431w	1.021
12	ydr090c	1.332	12	ngl2	1.064	12	<b>vps74</b>	1.036
13	yos9	1.348	13	per1	1.072	13	uga3	1.059
14	ysa1	1.367	14	ssf2	1.084	14	tae1	1.083
15	yhr033w	1.381	15	sol3	1.09	15	chd1	1.182
16	yel014c	1.419	16	ted1	1.097	16	rdh54	1.184
17	yfl054c	1.434	17	ecm32	1.118	17	sol3	1.194
18	ylr428c	1.440	18	uga3	1.156	18	opy1	1.203
19	ydr133c	1.484	19	mrpl50	1.163	19	ssa1	1.233
20	sol3	1.5	20	tae1	1.194	20	ubc13	1.256
21	ydr431w	1.573	21	yhr033w	1.217	21	yal004w	1.268
22	gga1	1.585	22	bph1	1.236	22	ssh1	1.292
23	rp119b	1.586	23	yal058c-a	1.239	23	sif2	1.298
24	ecm32	1.599	24	sif2	1.263	24	yhr033w	1.310
25	ura4	1.618	25	yal004w	1.263	25	<b>mnn2</b>	1.313

D

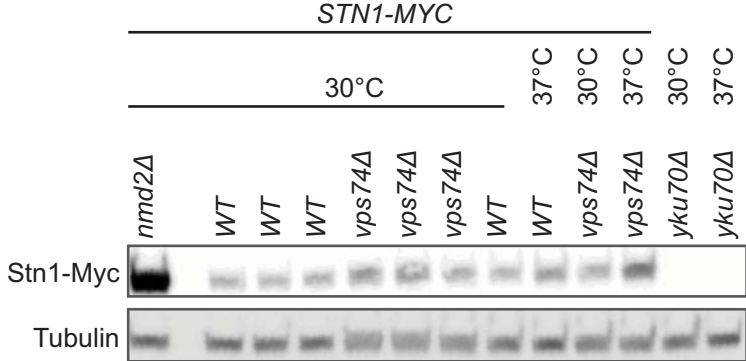
Protein	Function (from SGD)
Vps74	Golgi phosphatidylinositol-4-kinase effector and PtdIns4P sensor; interacts with the cytosolic domains of cis and medial glycosyltransferases, and in the PtdIns4P-bound state mediates the targeting of these enzymes to the Golgi; interacts with the catalytic domain of Sac1p, the major cellular PtdIns4P phosphatase, to direct dephosphorylation of the Golgi pool of PtdIns4P.
Pmt1	Protein O-mannosyltransferase of the ER membrane; transfers mannose from dolichyl phosphate-D-mannose to protein serine and threonine residues; 1 of 7 related proteins involved in O-glycosylation which is essential for cell wall rigidity; involved in ER quality control; amino terminus faces cytoplasm, carboxyl terminus faces ER lumen.
Pmt2	Protein O-mannosyltransferase of the ER membrane (like Pmt1); involved in ER quality control; acts in a complex with Pmt1p, can instead interact with Pmt5p; antifungal drug target.
Rad34	Protein involved in nucleotide excision repair (NER).
Pmp1	Regulatory subunit for the plasma membrane H(+)-ATPase Pma1p; forms unique helix and positively charged cytoplasmic domain that is able to specifically segregate phosphatidylserines.
Ted1	GPI-glycan remodelase; acts together with Emp24p/Erv25p in cargo exit from the ER.
Sut1	Zn(II)2Cys6 family transcription factor; positively regulates sterol uptake genes under anaerobic conditions; involved in hypoxic gene expression; relocalizes from the nucleus to the cytoplasm upon DNA replication stress.
Mnn2	Alpha-1,2-mannosyltransferase; responsible for addition of the first alpha-1,2-linked mannose to form the branches on the mannan backbone of oligosaccharides
Ng12	Protein involved in 5.8S rRNA processing.
Ssh1	Subunit of the Ssh1 translocon complex.
Sol3	6-phosphogluconolactonase; catalyzes the second step of the pentose phosphate pathway.
Ecm32	DNA dependent ATPase/DNA helicase; helicase belonging to the Dna2p-and Nam7p-like family of helicases that is involved in modulating translation termination.
Ubp3	Ubiquitin-specific protease involved in transport and osmotic response; inhibitor of gene silencing; protein abundance increases in response to DNA replication stress.
Hek2	RNA binding protein involved in asymmetric localization of <i>ASH1</i> mRNA; regulates telomere position effect and length.
Uga3	Transcriptional activator of GABA (gamma-aminobutyrate) genes.
Mrpl50	Mitochondrial ribosomal protein of the large subunit.
Ssf2	Protein required for ribosomal large subunit maturation.

**Figure S4. Vps74 and mannosyltransferases affect the fitness of telomere defective cells through similar pathways.**  
**(A-C)** List of gene deletions whose patterns are similar to *vps74Δ* (A), *pmt1Δ* (B) and *pmt2Δ* (C) across *cdc13-1 exo1Δ*, *stn1-13*, *yku70Δ*, *cdc13-1* and *cdc13-1 rad9Δ* genetic screens (Figure 5B) (Dubarry et al. 2015; Holstein et al. 2017). **(D)** Description of the genes that are simultaneously found to be similar to *vps74Δ*, *pmt1Δ* and *pmt2Δ* (from A-C).



**Figure S5. Vps74 localizes to the nucleus and cytoplasm.**

Cell cultures in late exponential phase carrying integrated *SEC7-GFP* (localizes to Golgi) or *VPS74-GFP* constructs were imaged under a fluorescence microscope. **(A-B)** Phase contrast, GFP and DAPI (B) pictures were taken with 600X amplification.



**Figure S6. Increased temperature and loss of Vps74 leads to increased Stn1-Myc levels.**

More data as in Figure 6A.