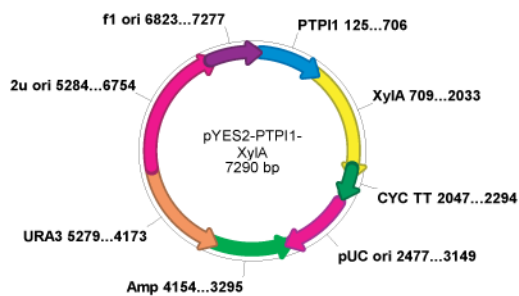
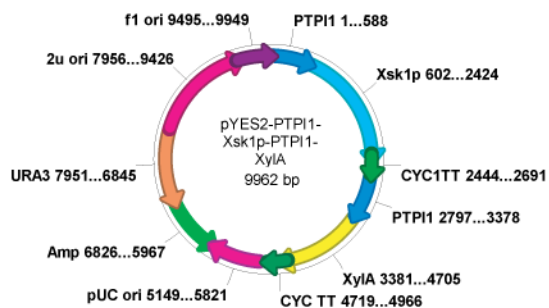


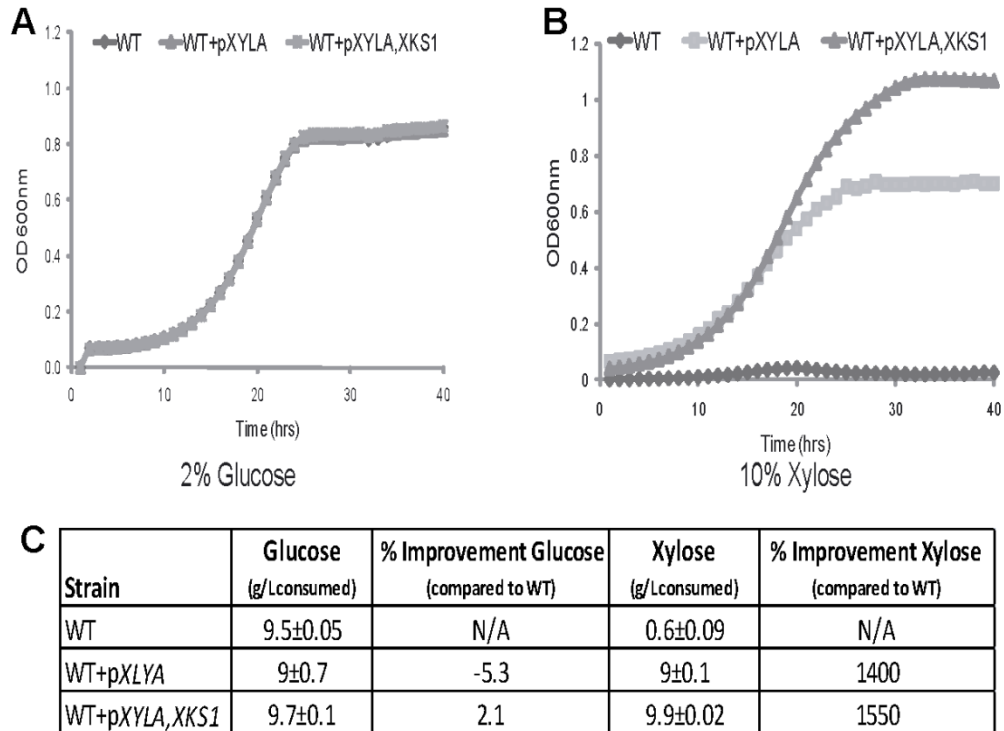
A



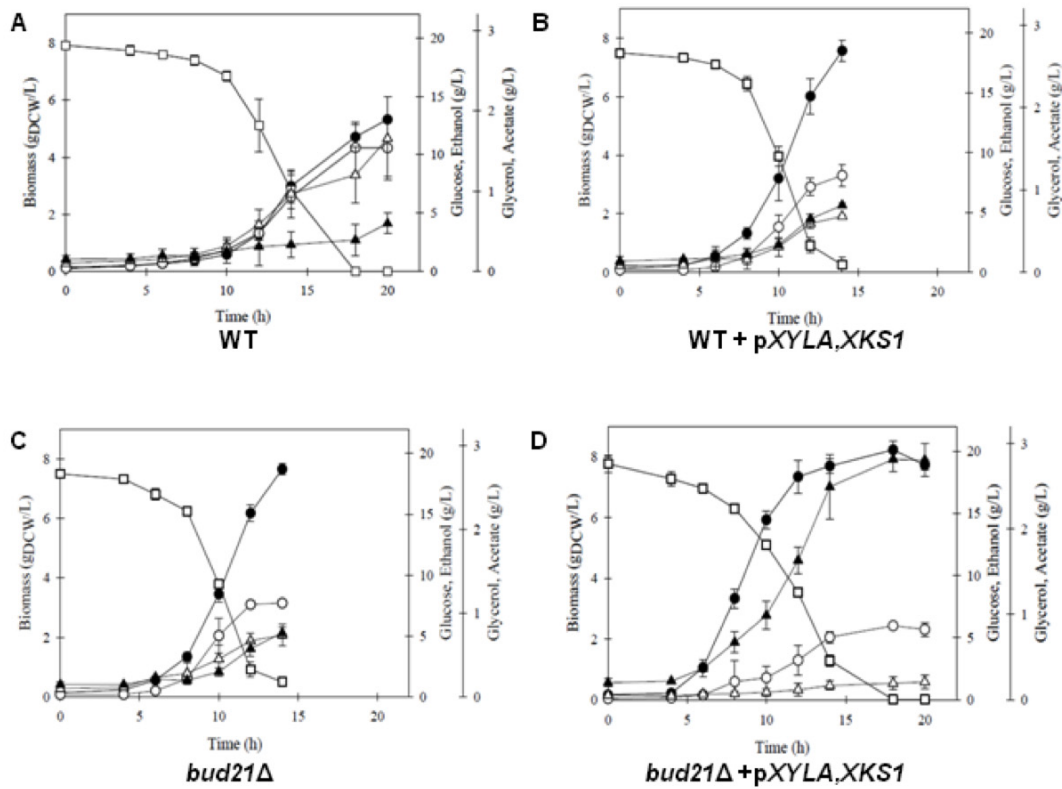
B



**Figure S1 (A)** Schematic map *pXYLA*. The pYES2 plasmid containing the exogenous XI gene *XYLA* from *Piromyces SpE2*. **(B)** Schematic map of *pXYLA,XKS1*. The pYES2 plasmid containing the XI gene *XYLA* and an additional copy of the endogenous XK gene *XKS1* from *S. cerevisiae*.



**Figure S2** The addition of pXYLA or pXYLA,XKS1 increases the xylose utilization of *S. cerevisiae*. Growth analysis of wild-type (WT, Y7092), to transformed strains, WT + pXYLA (YKB2179) and WT + pXYLA,XKS1 (YKB2178) in liquid media containing 2% glucose (**A**) or 10% xylose (**B**). Growth analysis was performed in triplicate on Multiskan Ascent plate reader (Thermo Electron Corporation), with OD<sub>600</sub> taken every 30 min over a 20-hour period at 30°. The error bars represent 1 standard deviation and are smaller than the data marker. (**C**) Sugar consumption table of wild-type (WT, Y7092), or wild-type transformed with pXYLA (YKB2179), or pXYLA,XKS1 (YKB2178) starting in either 13% glucose or xylose media. Fermentations were pitched with  $1.5 \times 10^7$  cells/ml and carried out at 30°. The calculation for g/L consumed of sugars corresponds to the concentrations of sugars observed at the end of the batches compared to the starting amount.



**Figure S3** Fermentation profile of the *S. cerevisiae* CEN.PK 113-13D derivative strains during aerobic batch cultivation in glucose (20g/L). **(A)** CEN.PK 113-13D wild-type (WT, YKB2684), **(B)** WT transformed with *pXYLA,XKS1* (YKB2680), **(C)** *bud21Δ* (YKB2666) and **(D)** *bud21Δ* transformed with *pXYLA,XKS1* (YKB2667). Biomass (closed circles), glucose (open squares), ethanol (open circles), glycerol (open triangles), and acetate (closed triangles). The batches were terminated when no further changes in OD was observed. Each data point represents the mean±SD from duplicated experiments.

**Table S1 Excel sheet containing the confirmed genetic interactions.**

Table S1 is available for download at <http://www.g3journal.org/lookup/suppl/doi:10.1534/g3.111.000695/-/DC1/TableS1.xls>.

Spreadsheets:

DMA-xylose = screen of the DMA on 2% xylose

XYLA - xylose = pXYLA SGA screen on 2% xylose

XYLA,XKS1 - xylose = pXYLA,XKS1 SGA screen on 2% xylose

XYLA - glucose = pXYLA SGA screen on 2% glucose

XYLA,XKS1 - glucose = pXYLA,XKS1 SGA screen on 2% glucose

**Table S2 Double mutants do not display additive improvement in xylose utilization.**

Strain	Glucose (g/L consumed)	Improvement Glucose (compared to WT)	Xylose (g/L consumed)	Improvement Xylose (compared to WT)
WT	8.1±0.05	N/A	0.5±0.04	N/A
<i>alp1Δ isc1Δ</i>	7.1±0.015	-11.25	6.5±0.05	1200
<i>alp1Δ rpl20bΔ</i>	7.2±0.02	-10	6.7±0.08	1240
<i>isc1Δ rpl20bΔ</i>	7±0.06	-12.5	6.6±0.074	1216
<i>bud21Δ alp1Δ</i>	8.1±0.09	1.25	6.2±0.06	1140
<i>bud21Δ isc1Δ</i>	8.4±0.08	5	7.15±0.15	1330
<i>bud21Δ rpl20bΔ</i>	8.3±0.1	3.75	6.5±0.06	1200

Strain	Glucose (g/L consumed)	Improvement Glucose (compared to WT +pXYLA,XKS1)	Xylose (g/L consumed)	Improvement Xylose (compared to WT +pXYLA,XKS1)
WT +pXYLA,XKS1	8.1±0.02	N/A	5.8±0.09	N/A
<i>alp1Δ isc1Δ</i> +pXYLA,XKS1	8.6±0.08	6.2	7.2±0.02	24.1
<i>alp1Δ rpl20bΔ</i> +pXYLA,XKS1	8.2±0.074	1.23	7±0.03	20.7
<i>isc1Δ rpl20bΔ</i> +pXYLA,XKS1	8.3±0.08	2.5	6.2±0.02	6.9
<i>bud21Δ alp1Δ</i> +pXYLA,XKS1	8.0±0.08	-1.23	7.2±0.03	24.16
<i>bud21Δ isc1Δ</i> +pXYLA,XKS1	8.4±0.09	3.7	7.4±0.04	27.6
<i>bud21Δ rpl20bΔ</i> +pXYLA,XKS1	8.2±0.1	1.23	6.8±0.1	17.26

**Analysis of the consumption of glucose and xylose of the double mutants of the suppressors.** Strains tested were Wild-type (YKB731), *alp1Δ isc1Δ* (YKB2534), *alp1Δ rpl20bΔ* (YKB2535), *isc1Δ rpl20bΔ* (YKB2536), *bud21Δ alp1Δ* (YKB2537), *bud21Δ isc1Δ* (YKB2538), *bud21Δ rpl20bΔ* (YKB2539) without (top table) and with *pXYLA,XKS1* (bottom table). Growth cultures were started in either 13% glucose or xylose media, pitched with  $1.5 \times 10^7$  cells/ml and carried out at 30° with for 168 hours. The calculation for g/L consumed of sugars corresponds to the concentrations of sugars observed at the end of the batches compared to the starting amount.