Fig. A1

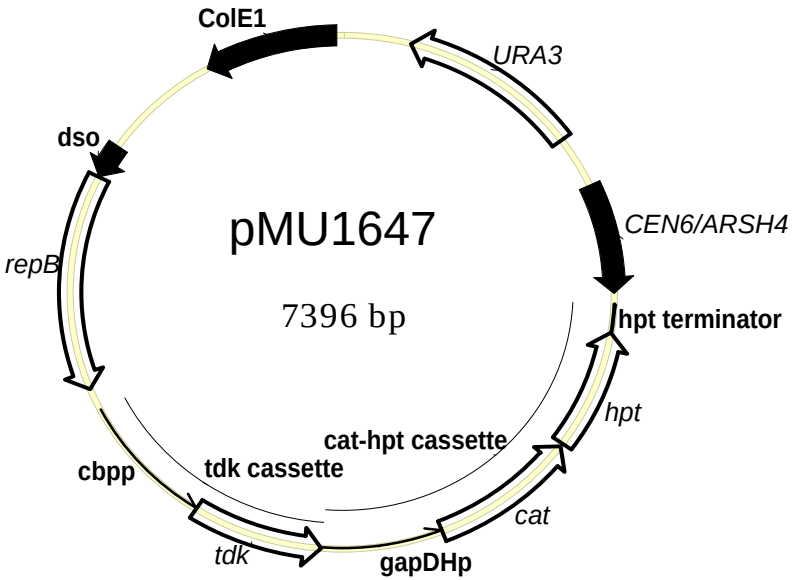
ColE1
URA3
dso
CEN6/ARSH4
pMU1647
repB
7396 bp
hpt terminator
hpt
cat-hpt cassette
cbpp
tdk cassette
cat
tdk
gapDHp

Table A1. Phenotype of counter selections contained on pMU1647 expressed as *C. thermocellum* colony forming units.

	colony forming units		
Strain	No Drug	+AZH	+FUDR
wt	6.0×10^8	4.4×10^2	2.8×10^8
wt + pMU1647	1.3×10^8	n.d.	5.0×10^6
Δ*hpt*	6.0×10^8	9.0×10^8	n.d.
Δ*hpt* + pMU1647	4.0×10^8	2.5×10^2	n.d.

Cultures were grown to an OD_{600} of 0.9 and dilution plated on the indicated medium. The cfu's reported resulted after 48 hours of incubation.

Table A2. Avicel fermentation products of *C. thermocellum* strains generated in this study[a].

Strains	Products formed g/l						[b] Final D.W. g	[c]Theoretical ethanol yield g/l	% Carbon recovery
	Cellobiose	Glucose	Acetate	Lactate	Ethanol	Pyruvate			
wt	2.1 +/- 0.01	2.81 +/- 0.16	2.74 +/- 0.03	2.49 +/- 0.02	1.32 +/- 0.04	n.d.	1.9 +/- 0.14	9.07	72
Δ*hpt*	1.94 +/- 0.05	2.49 +/- 0.02	2.77 +/- 0.01	2.42 +/- 0.03	1.64 +/- 0.00	n.d.	1.9 +/- 0.00	8.98	73
Δ*hpt*, Δ*ldh*	0.43 +/- 0.07	2.57 +/- 0.01	2.89 +/- 0.00	0.25 +/- 0.01	2.02 +/- 0.01	n.d.	1.4 +/- 0.07	9.56	60
Δ*hpt*, Δ*pta*	0.97 +/- 0.01	0.018 +/- 0.00	0.22 +/- 0.06	3.93 +/- 0.01	2.02 +/- 0.02	n.d.	0.6 +/- 0.00	10.39	45
Δ*hpt*, Δ*ldh*, Δ*pta*	0.50 +/- 0.01	0.16 +/- 0.00	0.02 +/- 0.08	0.35 +/- 0.03	2.56 +/- 0.01	0.31 +/- 0.02	1.15 +/- 0.21	9.33	33
Evolved Δ*hpt*, Δ*ldh*, Δ*pta*	0.53 +/- 0.00	0.06 +/- 0.01	0.16 +/- 0.04	0.11 +/- 0.01	5.61 +/- 0.14	n.d.	1.1 +/- 0.14	9.39	61

[a] Duplicate batch fermentations with initial pH 7.0 and 19.5 g/l Avicel, maintained at 55°C, sampled at 72 h.
[b] D.W. stands for dry weight, and is used to determine residual Avicel and calculate Avicel conversion
[c] Based on Avicel conversion
n.d., not detected

Table A3. Avicel fermentation products of *C. thermocellum-T. saccharolyticum* co-cultures

Co-culture	Products formed g/l						[c] Final D.W. g	[d]Theoretical ethanol yield g/l	% Carbon recovery
	Cellobiose	Glucose	Acetate	Lactate	Ethanol	Pyruvate			
wt strains[a]	0.78 +/- 0.08	0.63 +/- 0.28	4.04 +/- 0.02	2.58 +/- 0.01	3.21 +/- 0.15	n.d.	0.80 +/- 0.21	9.26	79
Engineered strains[a]	0.64 +/- 0.01	0.18 +/- 0.00	0.25 +/- 0.01	0.16 +/- 0.01	6.7 +/- 0.04	n.d.	0.63 +/- 0.11	9.35	76
Engineered strains[b]	0.20	n.d.	0.50	0.38	38.1	n.d.	8.4	47.5	82

[a] Duplicate batch fermentations with initial pH 6.75 and 17.2 g/l Avicel, maintained at 55°C, sampled at 120 h.

[b]Continuous stirred reactor fermentation (300 rpm) with initial pH 6.3 and 92.2 g/l Avicel, maintained at 55°C, sampled at 146 h.

[c] D.W., dry weight, and is used to determine residual Avicel and calculate Avicel conversion

[d] Based on Avicel conversion

n.d., not detected

Appendix figure legends:

Fig A1. pMU1647. A *C. thermocellum-E.coli-S.cerevisiae* shuttle vector with positive and negative selections for genetic manipulation in *C. thermocellum*. Replication factors: ColE1, *E. coli* origin; dso and *repB*, gram+ double stranded origin of replication and replication initiation protein for *C. thermocellum; CEN6/ARSH4, S. cerevisiae* replication elements. *URA3* is used as a positive selection marker in *S. cerevisiae*. The tdk cassette is a negative selectable marker in *C. thermocellum* and is comprised of the *C. thermocellum* cellobiose phosphorylase promoter (cbpp) and the *T. saccharolyticum* thymidine kinase gene (*tdk*). The cat-hpt cassette is a dual selection marker, allowing positive and negative selection in *C. thermocellum* and positive selection in *E. coli* and is comprised of the *C. thermocellum* glyceraldehyde 3-phosphate dehydrogenase promoter (gapDHp), the *cat* gene from pNW33n, and the *hpt* gene from *C. thermocellum*.