Supplementary table S1. Transformation efficiencies of different *T. saccharolyticum ATP-pfk* expression plasmids each with a different predicted translation initiation rate for the *ATP-pfk*, and the corresponding PFK activities of the obtained colonies. The "no pfk control" is an empty vector (i.e. no *ATP-pfk* CDS in the plasmid); plasmid with "0" translation rate is a promoterless expression plasmid. Where there is no PFK activity measured (denoted by "-" sign), it is because no transformants were obtained for that level of *ATP-pfk* translation/expression. One unit (U) of activity is defined as formation of one umol of product per minute.

Predicted Translation Initiation Rate (arbitrary units)	Transformation efficiency (CFU/ug plasmid DNA)		PPi-PFK (U/mg CFE)		ATP-PFK (U/mg CFE)	
	Average	Standard deviation	Average	Standard deviation	Average	Standard deviation
No pfk control	36400	11597	2.44	0.46	<0.01	0.10
0	4050	1626	2.69	1.28	<0.01	0.03
400	6020	764	2.11	0.78	<0.01	0.02
4000	0	0	-	-	-	-
8000	76820	97835	1.73	0.94	<0.01	0.04
14000	0	0	-	-	-	-
27000	0	0	-	-	-	-
95000	0	0	-	-	-	-

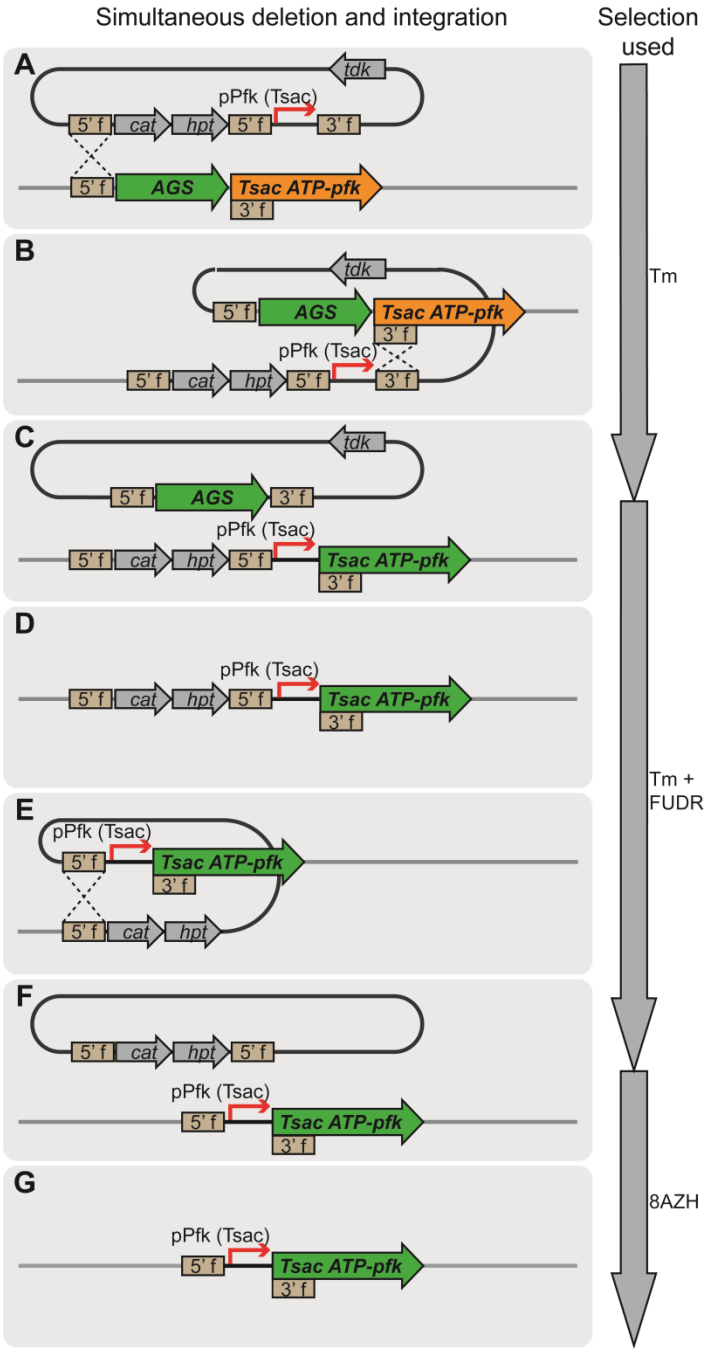

Figure S1. Scheme for expressing the *T. saccharolyticum ATP-pfk* in *C. thermocellum*. Gray arrows in the figure represent selection/counter-selection genes: "Tm" represents positive selection for presence of the thiamphenicol resistance marker gene, *cat*, with thiamphenicol; "Tm + FUDR" represents positive selection of the *cat* gene with thiamphenicol and negative selection against the presence of the thymidine kinase gene, *tdk,* with FUDR; "8AZH" represents negative selection with the compound 8-azahypoxanthine against the presence of the hypoxanthine phosphoribosyltransferase (*hpt*) gene, brown boxes represent homology regions, with "5'f" being an upstream homology region to the , and "3'f" being a downstream homology region. A green arrow represents an actively-expressed gene, and a

yellow arrow represents an inactive (not expressed/translated) gene. Red arrow represents a promoter. Figure was adapted from Olson and Lynd, 2012. A. After initial transformation of the deletion plasmid into *C. thermocellum*; the plasmid DNA undergoes a single recombination event at a homology region on the chromosome, in this case one of the upstream homology region indicated by "5'f". B. Having undergone one recombination event, a second recombination event occurs between the downstream homology region on the plasmid and the corresponding locus on the chromosome. C. The net effect of the double recombination events is the replacing of the ADP-glucose synthase (AGS) operon (which now resides on the plasmid vector) with the *T. saccharolyticum* ATP-*pfk* promoter, the *cat-hpt* selection cassette, and another upstream homology region; this chromosomal configuration will be referred as the "merodiploid". D. Selection with thiamphenicol and FUDR results in positive selection for the merodiploid cell, and negative selection against the AGS-operon containing plasmid vector. E-F. Similar to B-C, recombination occurs between the upstream homology regions (one that was originally present on the chromosome, and another that was introduced by the plasmid), resulting in removal of the *cat-hpt* selection cassette from the chromosome. G. Selection with 8AZH results in removal of the *cat-hpt* selection cassette to obtain a clean insertion of the *T. saccharolyticum* ATP-*pfk* promoter to drive expression of the *T. saccharolyticum* ATP-*pfk* gene.

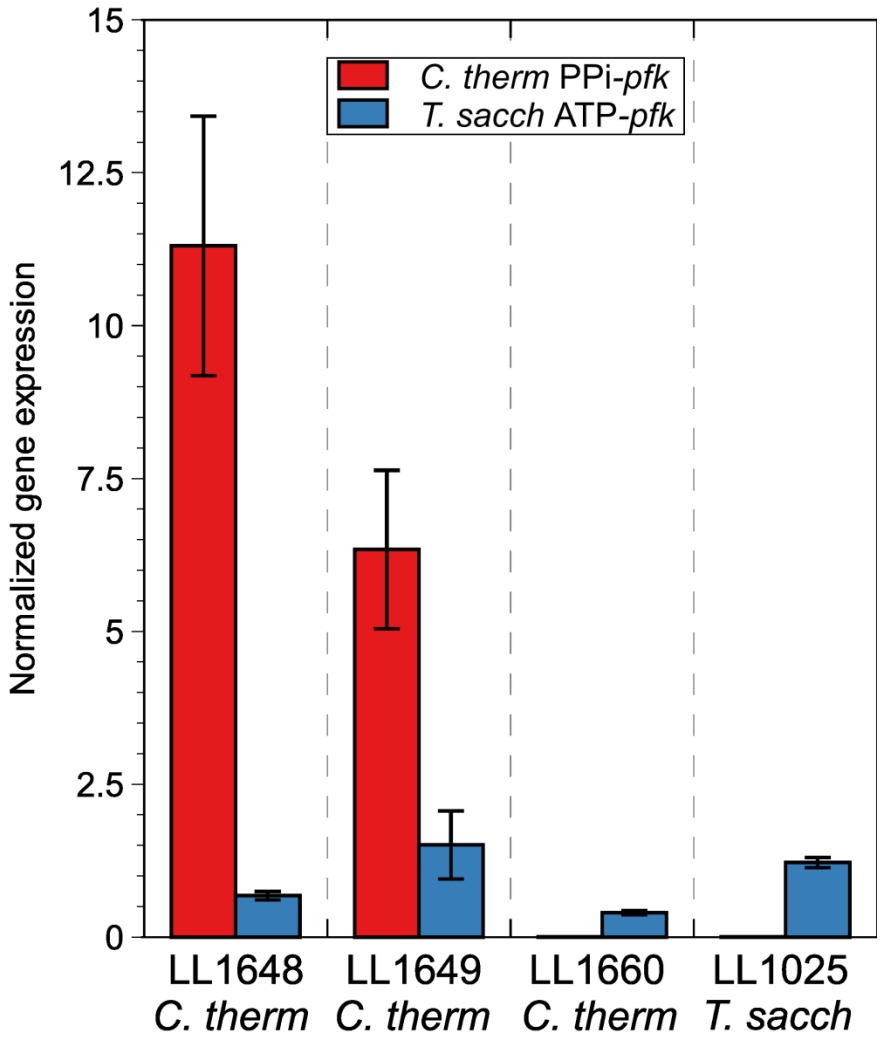

Figure S2. Relative gene expression (normalized against the species' *recA* expression), of *PPi-pfk* and *ATP-pfk* in select *C. thermocellum* strains, and in wild type *T. saccharolyticum*. *C. therm* – *C.* thermocellum, *T. sacch* – *T.* saccharolyticum. Data is the mean of two biological replicates, error bars represent the minimum and maximum values.

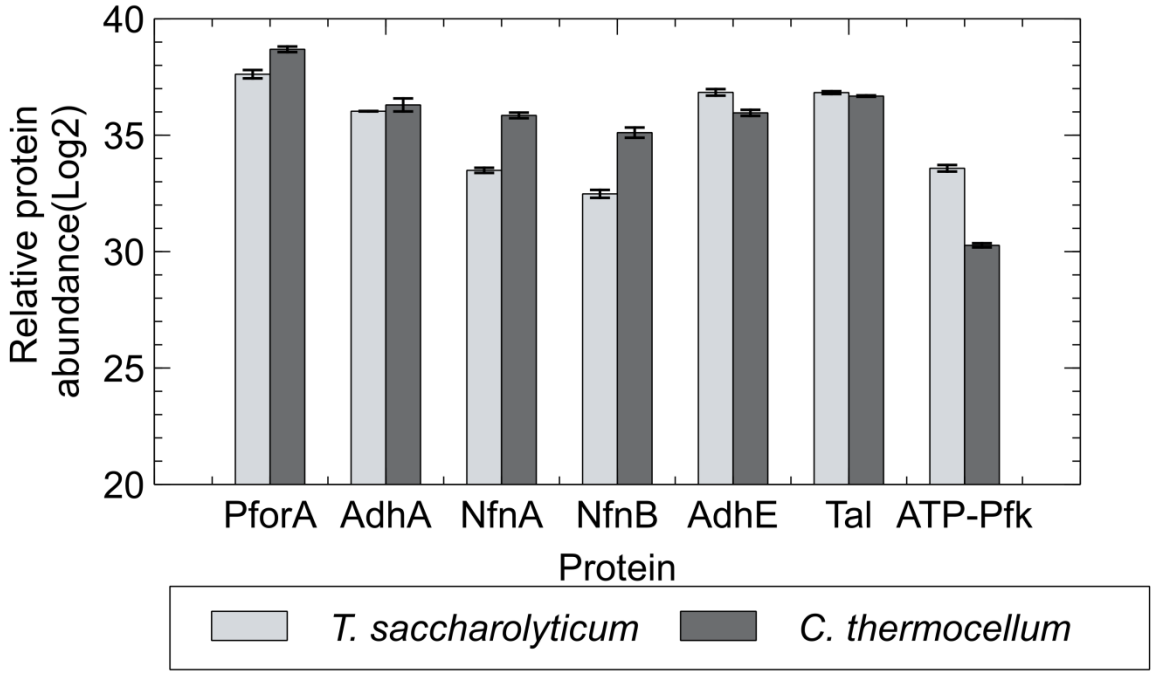

Figure S3. Relative protein abundances of select *T. saccharolyticum* proteins that are expressed in both *C. thermocellum* strain LL1649 and wild type *T. saccharolyticum*. Error bars represent one standard deviation (n=3).

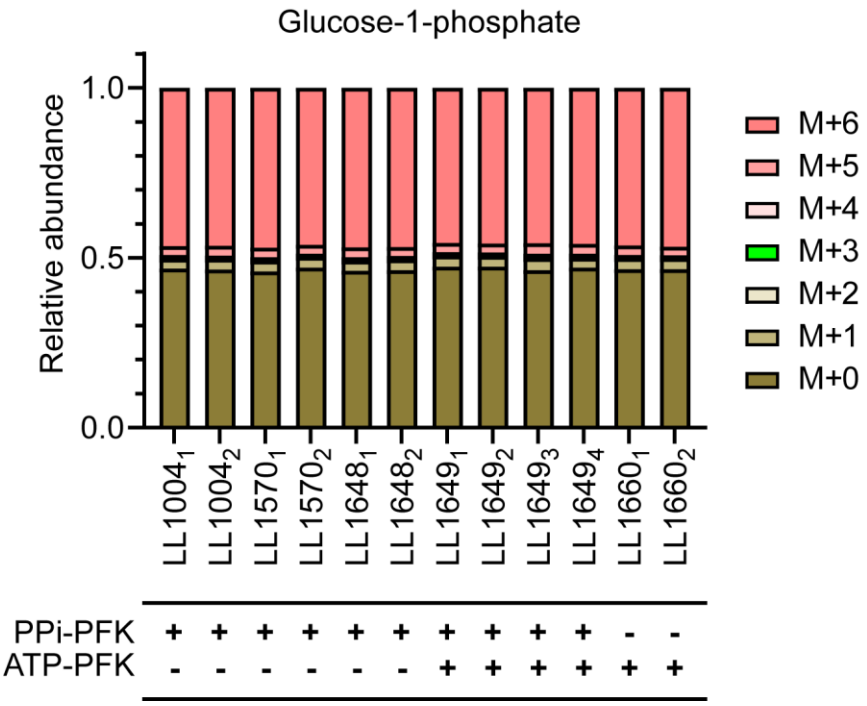

Figure S4. ^{13}C labeling for glucose-1-phosphate (G1P) for cells fed a 50:50 mixture of uniformly labeled and naturally labeled glucose. Subscript after strain ID number indicates biological replicate. The color represents the number of labeled carbon atoms, with m+6 representing six ^{13}C-labeled carbon atoms, m+5 representing five labeled carbon atoms, etc. The cofactor specificity of the PFK reaction is indicated below the strain name: “+” indicates presence of a reaction, “-“ indicates absence of a reaction.

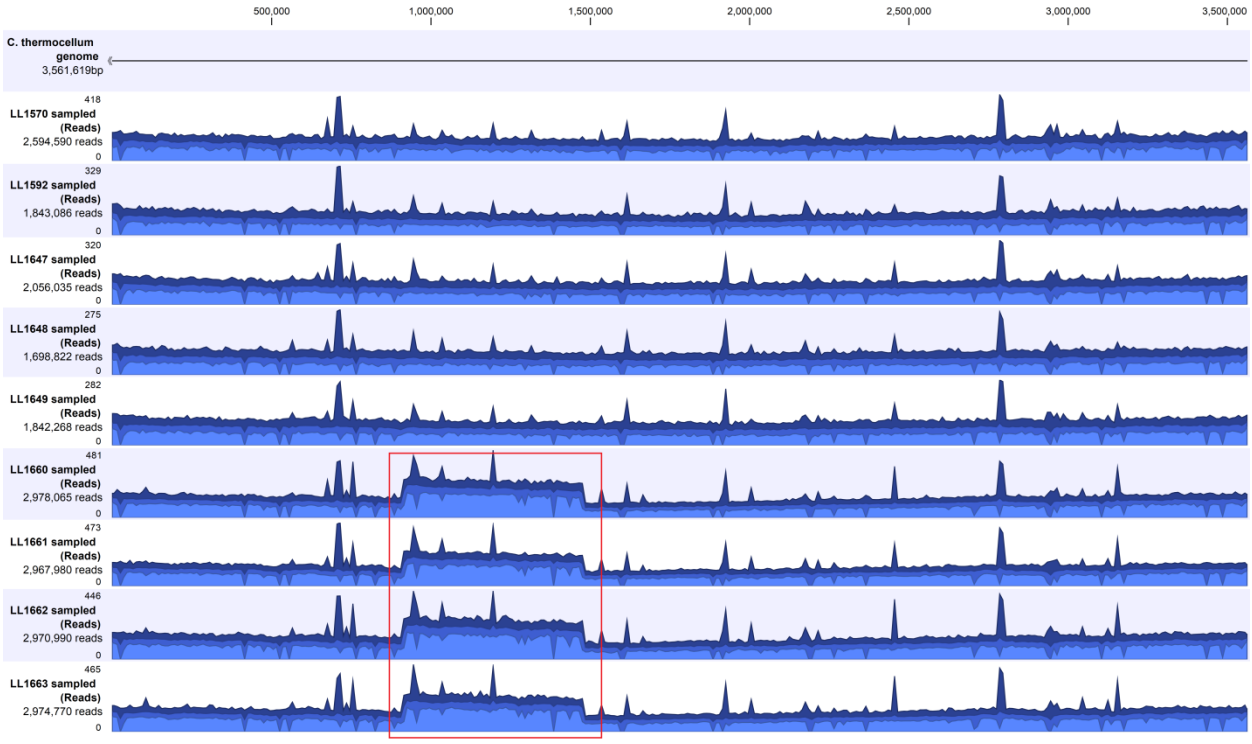

Figure S5. Partial genome duplication observed in strain LL1660 and three of its sister colonies (highlighted by the red box), from position ~913,400 bp to ~1,478,000 bp. Genome coordinates indicated at the top of the figure. For each genome, the read depth is indicated in blue. The scale of read depth is given on the left side of the figure. The maximum read depth varies between 275 and 481. For each strain, the total number of reads are also given. This varies from ~1.7 million to ~3 million, and depends on the amount of DNA used for WGS analysis.