Supplemental Figure Legends, Clasquin et al.

Supplemental figure 1. Related to Figure 1. Representative analytical data supporting the identification of metabolites.

(A-C) Standards were spiked into metabolite extracts. Chromatographic retention times of the standards and endogenous compounds were compared. Note that S1P chromatographically separates from its isomer, S7P.

(D-F) MS^2 was obtained for synthetic standards and for the endogenous compounds. Fragmentation was obtained in (-) ionization mode with (D) 15 eV for S7P and O8P, (E) 10 eV for S1P and 15eV for O1P, and (F) 20 eV for SBP and OBP. In all graphs, the y-axis represents ion counts in arbitrary units. Note that common fragments are lost between S7P and O8P, S1P and O1P, and SBP and OBP with the octulose compound fragments having one additional CH_2O unit resulting in a fragment 30 amu greater. The compounds all fragment to 97 amu, corresponding to $[H_2PO_4]^-$.

Supplemental figure 2. Related to Figure 2. Kinetics of Shb17 on FBP. Data include those presented in Figure 2, but also include additional data across the full kinetic range.

Supplemental figure 3. Related to Figure 3. Effects of site-directed mutations on Shb17 catalytic activity. The indicated Shb17 protein variant was added to a mixture containing both SBP and FBP. After a 20 min incubation at 30°C SBP (A), FBP (B), and S7P (C) levels were measured by LC-MS. WT enzyme also completely depleted SBP, converting it to S7P, while more modestly depleting FBP. The mutant enzymes H189A and S65A show decreased but still significant SBPase activity, while displaying no FBPase activity. The other mutant enzymes show little to no SBPase or FBPase activity.

Supplemental figure 4. Related to Figure 4. *In vivo* synthesis of SBP and OBP.

(A) The labeling pattern of S7P is similar in wild type and *shb17*Δ cells fed a mixture of 70% unlabeled and 30% uniformly labeled [U-$^{13}C_6$]-glucose. S7P can be synthesized through pentose phosphate pathway reactions or by Shb17. The similarity in the labeling pattern between the mutant and wild type suggests that much of the S7P pool is produced by the pentose phosphate pathway in cells grown under minimal conditions.

(B) Effect of the DAmP allele of fructose bisphosphate aldolase (FBA) and *shb17*Δ on metabolite pool sizes. Metabolite is indicated for each graph. Strains are indicated by numbers. 1: wild type, 2: *shb17*Δ, 3: *shb17*Δ FBA1-DAmP, 4: FBA1-DAmP. Mass spectral intensities are mean ± SE of $N \geq 9$ independent experiments with each strain.

(C) Kinetics of labeling of SBP after switching *shb17*Δ cells with wild type fructose bisphosphate aldolase (*FBA1-wt*), or the Decreased Abundance by mRNA Perturbation allele (*FBA1-DAmP*) into [U-$^{13}C_6$]-glucose.

Supplemental table 1. Relates to Figure 3. Crystallographic data collection and model refinement statistics

	SHB17 H13A + SBP (PDB 3OI7)
Data collection	
Space group	P_1
Cell dimensions	
a,*b*,*c* (Å)	a=58.6 b=74.9 c=83.6, a=90.0, b=89.9, g=77.2
Wavelength	1.54178
Resolution (Å)	50-2.2 (2.24-2.20)
[1]R_{merge}	0.080(0.112)
I / σI	9.9(3.0)
Completeness (%)	82.6(36.9)
Redundancy	1.6(1.3)
Refinement	
Resolution (Å)	27.5-2.40
[2]No. reflections	46894/2567
[3]R_{work} / R_{free}	22.8/26.0
No. atoms	8833
Protein	8452
Major ligand	(SBP)88
Other ligands/ion	47
Solvent	246
Average Isotropic *B*-factors	23.9
R.m.s deviations	
Bond lengths (Å)	0.012
Bond angles (°)	1.36
Ramachandran plot	
Most favored (%)	89.7
Additionally allowed (%)	9.9
Generously allowed (%)	0.4
Disallowed (%)	0

Values in parentheses are for the highest resolution shell.

[1] $R_{merge} = \sum_{hkl} \left| I - \langle I \rangle \right| / \sum_{hkl} I$

[2]R_{free} calculated using 5% of total reflections randomly chosen and excluded from the refinement

[3] $R_{work} = \sum \left| F_{obs} - F_{calc} \right| / \sum \left| F_{obs} \right|$ where F_{obs} and F_{calc} are the observed and the calculated structure factors, respectively.

Supplemental Table 2. Relates to Figure 5. Yeast strains.

Strain name	**Relevant genotype**	**Source**
Wild type	FY4 (ACY308) and FY5 (ACY309) haploids	(Winston et al., 1995)
shb17Δ	*MATa* and *MATα shb17Δ::ClonNAT*	this study
FBA1-DAmP	*MATa ykl060c-DAmP*	this study
shb17Δ FBA1-DAmP	*MATa shb17Δ::ClonNAT fba1-DAmP*	this study
"Single Mutants" Figure 5	1. ACY1097:*MATa shb17Δ::ClonNAT* 2. ACY1101:*MATa tal1Δ::KanMX* 3. ACY1107:*MATa zwf1Δ::KanMX*	this study
"Double Mutants" Figure 5	1. ACY1102:*MATa shb17Δ::ClonNAT tal1Δ::KanMX* 2. ACY1109:*MATa shb17Δ::ClonNAT zwf1Δ::KanMX* 3. ACY1122: *MATa tal1Δ::KanMX zwf1Δ::KanMX*	this study
"Triple Mutant" Figure 5	ACY1108: *MATa shb17Δ::ClonNAT, tal1Δ::KanMX zwf1Δ::KanMX*	this study
Supplemental Table 3	1. ACY1100: *MATa nqm1Δ::HygMX tal1Δ::KanMX* 2. ACY1103: *MATa nqm1Δ::HygMX* 3. ACY1104: *MATa nqm1Δ::HygMX shb17Δ::ClonNAT, tal1Δ::KanMX* 4. ACY1105: *MATa nqm1Δ::HygMX shb17Δ::ClonNAT tal1Δ::KanMX zwf1Δ::KanMX* 5. ACY1106: *MATa nqm1Δ::HygMX shb17Δ::ClonNAT tal1Δ::KanMX* 6. ACY1121: *MATa nqm1Δ::HygMX tal1Δ::KanMX zwf1Δ::KanMX*	this study

All strains are in FY4 and/or FY5 strain background.

Supplemental Table 3. Relates to Figure 5. Optical density during growth of indicated mutant strains (strains are mutants for various combinations of the sedoheptulose bisphosphatase *SHB17*, the transaldolase *TAL1*, the whole genome duplicate of *TAL1*, *NQM1*, and the glucose-6-phosphate dehydrogenase *ZWF1*.

Supplemental Table 4. Relates to Figure 5. Media composition applied to measuring flux through Shb17 using [6-$^{13}C_6$]-glucose. Liquid media was of the identical nutrient composition but lacked agarose.

Media type	Description
YNB (minimal media)	• 6.7 mg/mL Bacto Yeast Nitrogen Base without amino acids • 2% dextrose • 30 mg/mL Ultrapure, tripe washed agarose
YNB + amino acids	• 6.7 mg/mL Bacto Yeast Nitrogen Base without amino acids • 2% dextrose • 50 mg/L tryptophan • 20 mg/L histidine-HCl • 20 mg/L arginine-HCl • 20 mg/L methionine • 30 mg/L tyrosine • 100 mg/L leucine • 30 mg/L isoleucine • 100 mg/L lysine-HCl • 50 mg/L phenylalanine • 100 mg/L glutamic acid • 100 mg/L aspartic acid • 150 mg/L valine • 200 mg/L threonine • 400 mg/L serine • 35 mg/L alanine • 35 mg/L cysteine • 35 mg/L proline • 30 mg/mL Ultrapure, triple washed agarose
YNB + amino acids + adenine + uracil	• 6.7 mg/mL Bacto Yeast Nitrogen Base without amino acids • 2% dextrose • 50 mg/L tryptophan • 20 mg/L histidine-HCl • 20 mg/L arginine • 20 mg/L methionine • 30 mg/L tyrosine • 100 mg/L leucine • 30 mg/L isoleucine • 100 mg/L lysine-HCl • 50 mg/L phenylalanine • 100 mg/L glutamic acid • 100 mg/L aspartic acid • 150 mg/L valine • 200 mg/L threonine • 400 mg/L serine • 50 mg/L adenine sulfate • 20 mg/L uracil • 30 mg/mL Ultrapure, triple washed agarose
YNB + amino acids + adenine + uracil + palmitate	• YNB + amino acids + adenine + uracil • 40 µM palmitate complexed to 6.8µM BSA

YNB + amino acids + adenine + uracil + polysorbate 80	• YNB + amino acids + adenine + uracil • 1% 50/50 (v/v) polysorbate 80 / ethanol
YNB + amino acids + adenine + uracil + polysorbate 80 + ergosterol	• YNB + amino acids + adenine + uracil • 1% of 2mg/mL ergosterol in 50/50 (v/v) polysorbate 80 / ethanol (final concentration of 20 μg/mL.)

Supplemental Methods:

Chemicals and enzymes

HPLC-grade water, methanol and acetonitrile (OmniSolv, EMD Chemical), fructose-6-phosphate disodium salt and erythrose (70% w/v syrup) were obtained from VWR International (West Chester, PA). Tributylamine, acetic acid, magnesium chloride (98%), ribose-5-phosphate barium salt hexahydrate (≥99%), thiamine-pyrophosphate, spinach aldolase (0.27 U/mg), rabbit muscle aldolase (11.5 U/mg), transketolase (0.16 U/mg), and triose phosphate isomerase (4120 U/mg) were purchased from Sigma-Aldrich (St. Louis, MO). Fructose-1,6-bisphosphate trisodium salt octahydrate (98%) was obtained from AK Scientific, Mountain View, CA. Zinc acetate dihydrate (99.7%) was from J.T. Baker, Phillipsburg, NJ, and tris(hydroxymethyl)methanamine (99+%) was from Thermo Fisher Scientific, Pittsburg, PA.

Yeast strains and media components

S. cerevisiae strains were derived from the synthetic genetic analysis (SGA) deletion set (BY4743 background) (Tong et al., 2001) with the genotype *MAT**a** ura3Δ0 leu2Δ0 his3Δ1 lys2Δ0 met15Δ0 can1Δ::LEU2*$^{+}$*-MFA1pr-HIS3/CAN1 ykr043cΔ::kanMX* and a *yar047cΔ::kanMX* control in the same background. Prototrophic deletions were also created by homologous recombination using the YKR043CΔ::ClonNAT allele amplified by PCR from the above deletion. Prototrophic controls were wild type FY4 (Mat a) and FY5 (Mat α)(Winston et al., 1995).

Ergosterol, palmitate, amino acids, and all other media components were obtained from Sigma-Aldrich (St. Louis, MO). Polysorbate 80, FCC was obtained from Spectrum Chemicals (New Brunswick, NJ). Ultra fatty acid free BSA was obtained from Roche (Indianapolis, IN). [^{15}N]-NH_4Cl, [U-$^{13}C_6$]-glucose and [6-$^{13}C_1$]-glucose were obtained from Cambridge Isotope Laboratories (Andover, MA). Minimal media was made with 6.7 g/L Difco Yeast Nitrogen Base without amino acids plus 2% (w/v) glucose. For the SGA-derived auxotrophic strains, minimal media were supplemented with 20 mg/L uracil, 20 mg/L L-methionine, and 30 mg/L L-lysine HCl. Plates were prepared with 15 g/L triple washed ultrapure agarose (Bennett et al., 2008).

For Shb17 flux experiments, amino acid, adenine sulfate and uracil supplements were added to concentrations recommended in Methods in Yeast Genetics (Amberg, 2005). For the amino acid supplementation, alanine, cysteine and proline, which are not included in the supplementation recipe in Methods in Yeast Genetics, were added at 35 mg/L, thereby providing supplementation for 17 amino acids (Katja Schwartz, personal communication). Ergosterol stock was 2 mg/mL in 50/50 (v/v) Tween 80 / ethanol, dissolved by overnight stirring, then added to yield a final supplementation of 20 µg/mL. Sodium palmitate was prepared at a concentration of 1 mM in 150 mM NaCl solution at 70° C. The sodium palmitate was complexed at 37°C to 0.2 mM fatty-acid free BSA in 150 mM NaCl as described by Seahorse Biosciences; final mixture was 40 µM palmitate with 6.8µM BSA. Fatty acid-BSA complexes were stored up to one week at -20°C. Supplemental Table 4 provides full recipes for all media.

For experiments involving short term ^{13}C-labeling (kinetic flux profiling of the DaMP aldolase mutants or the [6-$^{13}C_1$]-glucose experiments) and absolute quantitation of FBP and SBP (Bennett et al., 2008), yeast were grown on filters on an agarose-media support as described in (Yuan et al, 2008, Crutchfield et al. 2010). This allowed rapid, non-disruptive transfer of cells from unlabeled to labeled media, and rapid quenching of metabolism. All data was corrected for naturally occurring ^{13}C (Yuan et al., 2008).

LC/MS Analysis

Instrumentation was an Accela U-HPLC system, HTC PAL autosampler, and a stand-alone orbitrap mass spectrometer with an electrospray ionization source (Thermo Fischer Scientific). The reverse-phase ion pairing LC method consists of a Phenomenex Synergy Hydro-RP column (100 x 2 mm, 2.5 µm particle size, Phenomenex, Torrance, CA) and an aqueous / organic gradient run at at 200 µL/min. Mobile phase A was 97:3 water:methanol with 10 mM tributylamine and 15 mM acetic acid. Mobile phase B was methanol. The gradient was 0 min, 0% B; 2.5 min, 0% B; 5 min, 20% B; 7.5 min, 20% B; 13 min, 55% B; 15.5 min, 95% B; 18.5 min, 95% B; 19 min, 0% B; 25 min, 0% B. The column temperature was maintained at 25°C, and the autosampler at 5°C. The injection volume was 10µL. The stand-alone orbitrap mass spectrometer was run in negative ionization mode. Data was collected at 100,000 resolution at 1 Hz under automatic gain control (target $3x10^6$ ions) and a maximum injection time of 100 µS. Instrument run parameters were sheath gas flow rate at 25 (arbitrary units,) auxiliary gas flow rate at 8 (arbitrary units,) sweep gas flow rate at 3 (arbitrary units,) spray voltage at 3 kV, capillary temperature at 325°C, capillary voltage at -50 V, tube lens voltage at -100 V. The scan range was 85 to 1000 m/z, with a more restricted scan range at certain points in the run to avoid artifacts from phosphate and sulfate accumulation in the orbitrap (see Lu et al., 2010 for details). Peaks differing between wild-type and knock-out strains were determined using in-house developed, open-source software MAVEN (Melamud et al., 2010) , whose algorithms closely resembled those of XC-MS (Smith et al., 2006). MAVEN was also used to quantitate isotope-labeled forms.

To obtain MS/MS and MS/MS/MS fragmentation patterns, we used a similar chromatography method to the above (for details, see (Lu et al., 2008)) coupled by negative mode electrospray ionization to either a triple quadrupole mass spectrometer or an ion trap-orbitrap hybrid mass spectrometer.

The number of carbons and nitrogens in each molecule was determined by the method of (Hegeman et al., 2007). Yeast cultures were grown with uniformly isotopically labeled nutrients for > 20 generations to ensure complete labeling of metabolites prior to LC-MS analysis.

Synthesis of Sedoheptulose-1,7-bisphosphate (SBP)

An aqueous solution of cofactors, substrates, and buffers was made with the given final concentrations: thiamine-pyrophosphate (1.1 µM), fructose-6-phosphate (2.5 mM), fructose-1,6-bisphosphate (5.7 mM), magnesium chloride (110 µM), and tris(hydroxymethyl)methanamine (4.7 mM). The enzymes rabbit muscle aldolase (750 µL of 0.1 U/µL in 50mM Tris), transketolase, (50 µL of 0.0032 U/µL in 50mM Tris) and triose phosphate isomerase (78 µL of 9.58 U/µL in 50mM Tris) were added to 6.6 mL of the cofactor and substrate solution to produce 750 mL of final reaction mixture containing thiamine-pyrophosphate (1 µM), fructose-6-phosphate (2.2 mM), fructose-1,6-bisphosphate (5 mM), magnesium chloride (100 µM), tris(hydroxymethyl)methanamine (10 mM), rabbit muscle aldolase (10 U/mL), transketolase (0.021 U/mL) and triose phosphate isomerase (100 U/mL). The reaction was incubated overnight at 37^{o}C and then flash frozen and stored at -80^{o}C until needed. The reaction was characterized by direct infusion negative mode ESI-MS. Based on these data, and assuming equal ionization efficiencies between phosphosugars, the reaction conversion was 27%. Due to the formation of xylulose-5-phosphate as a side product of the transketolase reaction with fructose-6-phosphate and glyceraldehyde-3-phosphate, an octulose-bisphosphate was also formed. SBP purity was ~ 80% of the C7 and C8 sugar bisphosphates. To remove other byproducts, SBP was further purified by reversed phase ion pairing LC using a larger scale version of the method of (Lu et al., 2010). Specifically, the column was Phenomenex AXIA Synergi 4µm Hydro-RP 80Å, 250 x 21.2 mm. Mobile phase was 97:3 water:methanol with 10 mM tributylamine and 15 mM acetic acid. Mobile phase B was methanol. Flow rate was 15 mL/min. The gradient was 0 min, 0% B;

5 min, 0% B; 10 min, 20% B; 20 min, 20% B; 35 min, 65% B; 38 min, 95% B; 42 min, 95% B; 43 min, 0% B; 50 min, 0% B. Injection volume ranged from 1.5 to 4 mL. 15 to 30 mL fractions were collected, tested for presence of SBP and FBP by LC/MS, and concentrated on a SpeedVac. This purification resulted in a final mixture containing FBP:SBP:OBP in the approximate ratio 2:1:0.25.

Synthesis of Sedoheptulose-1-phosphate (S1P)

An aqueous solution of cofactors, substrates, and buffers was made with the given final concentrations: fructose-1,6-bisphosphate (25.4 mM), erythrose (63.4 mM), zinc acetate (6.3 mM) and tris(hydroxymethyl)methanamine (58.5 mM). The enzymes spinach aldolase (28 µL of 0.05 U/µL in 50mM Tris) and triose phosphate isomerase (1.5 µL of 9.58 U/µL in 50mM Tris) were added to 110 mL of the cofactor and substrate solution to produce 139 µL of final reaction mixture containing fructose-1,6-bisphosphate (20 mM), erythrose (50 mM), zinc acetate (5 mM) and tris(hydroxymethyl)methanamine (50 mM), aldolase (10 U/mL), and triose phosphate isomerase (100 U/mL). The reaction was incubated overnight at 37^{o}C and then flash frozen and stored at -80^{o}C until used. The reaction conversion was determined to be 54% by LC-MS/MS.

Synthesis of D-*glycero*-D-*altro*-Octulose-1,8-bisphosphate (OBP)

An aqueous solution of cofactors, substrates, and buffers was made with the given final concentrations: fructose-1,6-bisphosphate (25 mM), ribose-5-phosphate (62.5 mM), zinc acetate (5 mM) and tris(hydroxymethyl)methanamine (50.4 mM). The enzymes spinach aldolase (40 µL of 0.05 U/µL in 50mM Tris) and triose phosphate isomerase (2.1 µL of 9.58 U/µL in 50mM Tris) were added to 160 mL of the cofactor and substrate solution to produce 139 µL of final reaction mixture containing fructose-1,6-bisphosphate (20 mM), ribose-5-phosphate (50 mM), zinc acetate (5 mM) and tris(hydroxymethyl)methanamine (50 mM), aldolase (10 U/mL), and triose phosphate isomerase (100 U/mL). The reaction was incubated overnight at 37^{o}C and then flash frozen and stored at -80^{o}C until used. The reaction conversion was measured at 42% by LC-MS/MS.

Rapid LC-MS/MS method appropriate for analyzing SBP in low complexity samples

Samples were injected onto a Phenomonex Luna NH_2 5µm 100Å 50 x 2.0 mm column at 15°C, 10µL injection volume. Mobile phase was a 12 min gradient of 20mM NH_4HCO_3, 20mM NH_4OH 95/5 H_2O/CH_3CN (A) and CH_3CN (B) flowing at 0.15mL/min. From 0-6 min B was decreased from 30% to 0, holding at 0 until 10 min, then back to 30% at 10.5min. The LC was coupled to a Finnigan TSQ Quantum Ultra triple quadrupole MS (Thermo Electron Corporation, San Jose, CA) with an ESI source running in negative ionization mode with parameters listed under *Formula and Structural Assignment*. ESI spray voltage was 3000V. Nitrogen was used as the sheath gas and auxiliary gas at 30 psi and 10 psi, respectively. Argon was the collision gas at 1.5mTorr. Scan width was 0.1 m/z and scan time was 0.1 sec. Capillary temperature was 325°C. Monitored parent/product ions (collision energy) were 339/97 (25) FBP, and 369/97(25) for SBP.

Absolute Quantitation of FBP and SBP Wild-type liquid cultures were started in YNB + 2% [U-$^{13}C_6$]-glucose, grown overnight at 30˚C, then set back to ~4 x 10^6 cells/mL as measured by Coulter counter. When cells reached ~1 x 10^7 cells/mL, 1.7mL aliquots were filtered onto solid agarose plates with identical media supplementation. Once cultures reached mid-log phase (~2.5 x 10^7 cells/mL), filter cultures were transferred to pre-chilled plates containing 0.8 mL of -20˚C 40/40/20 acetonitrile/methanol/water containing either 2.1 x 10^{-5} M FBP or 2.0 x 10^{-7} M SBP. The remainder of the metabolite extraction protocol is described in the methods section under "*Screening for Metabolic Phenotypes"*. Identically grown cultures were used for determining cell count and median cell size. Detailed procedure is reported in Bennett et al., 2008.

Protein Purification and Enzymatic Assays

For expression in *E. coli*, the coding sequence of Shb17 was amplified from the genome by PCR and cloned within the NdeI and XhoI sites of the pET-15 vector (EMD Biosciences), which provides an N-terminal 6x histidine tag. The sequence verified plasmid was transferred to Rosetta 2 (DE3) pLysS cells (EMD Biosciences). Protein expression was induced with IPTG. Cells were lyzed by freeze-thaw in 50 mM sodium phosphate pH 7, 300 mM NaCl, 1 mM PMSF. Protein was bound to HisPur Cobalt resin (Thermo Scientific) and eluted in the same buffer with the addition of 150 mM imidazole. The purified enzyme was buffer exchanged into 10 mM Tris-HCl pH 7.0, 50 mM NaCl, 1 mM DTT using Econopac desalting columns (Biorad). Enzymatic activity was identified by incubating purified Shb17 protein with SBP, S1P and OBP at approximately 1000 ng/mL in a buffer of 10mM Bis-Tris-Propane-HCl, 10mM $MgCl_2$, 1mM DTT pH 7.0. After twenty minutes of incubation at 30°C, reactions were quenched by placing at 5°C, adding acetonitrile to 20% final volume, and vortexing. Analysis was by LC-MS or targeted LC-MS/MS.

For kinetic experiments, all substrates were dissolved in water. C-terminally His_6-tagged Shb17 protein was quantified by Bradford assay (Biorad). Reactions contained 10mM Bis-Tris-Propane-HCl pH 7.0, 10 mM $MgCl_2$, 1 mM DTT, 10 µg/mL BSA , and Shb17. Shb17 concentrations ranged from 2.5 to 8 µg/mL for reactions with FBP, and from 0.1 to 1.25 µg/mL for reactions with SBP. Note that the SBP solutions also contained residual FBP used during the SBP synthesis. Reactions in 30 µL volume were allowed to proceed for 15 min at 30°C before quenching by the addition of 30 µL 20% acetic acid. Samples were diluted prior to LC/MS analysis. Calibration curves were prepared in parallel in an identical matrix lacking Shb17. A rapid LC-MS/MS method was developed and validated for the quantitation of SBP and FBP to accommodate the large number of samples generated during kinetic experiments.

Crystallography

Crystals of SHB17(H13A) were grown at room temperature by hanging-drop vapor diffusion using 2 µL of protein (29 mg/ml) and 2 µL of reservoir solution. Crystals were found using a reservoir solution of 0.1M Hepes 7.5, 10% isopropanol, 22% PEG4K, 4% glycerol, with 0.03 mg/ml trypsin added to the protein solution (Dong et al., 2007). These crystals were then soaked for 10 minutes in well solution plus 10 mM sedoheptulose-1,7-bisphosphate, and then cryoprotected using Paratone-N oil (Hampton Research) and flash-frozen in liquid nitrogen.

The structure of SHB17(H13A) with bound sedoheptulose-1,7-bisphosphate was solved by molecular replacement from the protein coordinates of SHB17(H13A) solved with fructose-1,6-bisphosphate (PDB code 3LL4). Briefly, diffraction data were generated using a home-source Rigaku FRE Superbright generator equipped with osmic confocal mirrors, and the data was collected on a Rigaku Saturn A200 image plate (Rigaku Americas, TX). Diffraction data were integrated and scaled using HKL2000 (Otwinowski, 1997). Four molecules of Ykr043C were found using the molecular replacement program PHASER (McCoy et al., 2005). Additional density was found in the active site in the same position as the sugar moiety in the fructose-1,6-bisphosphate complex, however the resulting sugar had a clear ring conformation rather than a linear one. The density was best fit by a beta-furanose conformation of sedoheptulose-1,7-bisphosphate, which was modeled using REEL from the PHENIX package (Afonine et al.; Moriarty et al., 2009). Following structure solution and ligand modeling, the model was improved with alternate cycles of manual building and automated and manual water-picking using COOT (Emsley and Cowtan, 2004) and restrained refinement against a maximum likelihood target with 5% of the reflections randomly excluded as an R_{free} test set using REFMAC5(Winn et al., 2001), as implemented using the CCP4 package (Collaborative

Computational Project, number 4), as well as rebuilding and subsequent refinement using PHENIX (Afonine et al.). The final model contains two dimers of SHB17(H13A), each representing residues 3-263, and shows excellent stereochemistry overall with 99.6% of the residues in the most favored and additional allowed regions of the Ramachandran plot (Laskowski, 1993). All statistics for data collection and structure refinement are summarized in Supplemental Table 1.

References for Extended Supplemental Methods:

Afonine, P.V., Grosse-Kunstleve, R.W., Chen, V.B., Headd, J.J., Moriarty, N.W., Richardson, J.S., Richardson, D.C., Urzhumtsev, A., Zwart, P.H., and Adams, P.D. phenix.model_vs_data: a high-level tool for the calculation of crystallographic model and data statistics. J Appl Crystallogr *43*, 669-676.

Amberg, D.C., Burke, D.J., Strathern, J.N. (2005). Methods in Yeast Genetics (Cold Spring Harbor, NY, Cold Spring Harbor Laboratory Press).

Bennett, B.D., Yuan, J., Kimball, E.H., and Rabinowitz, J.D. (2008). Absolute quantitation of intracellular metabolite concentrations by an isotope ratio-based approach. Nat Protoc *3*, 1299-1311.

Crutchfield C.A., Lu W, Melamud E, Rabinowitz J.D. (2010). Mass spectrometry-based metabolomics of yeast. Methods Enzymol. 2010;470:393-426.

Dong, A., Xu, X., Edwards, A.M., Chang, C., Chruszcz, M., Cuff, M., Cymborowski, M., Di Leo, R., Egorova, O., Evdokimova, E., et al. (2007). In situ proteolysis for protein crystallization and structure determination. Nat Methods 4, 1019-1021.

Emsley, P., and Cowtan, K. (2004). Coot: model-building tools for molecular graphics. Acta Crystallogr D Biol Crystallogr *60*, 2126-2132.

Laskowski, R.A., MacArthur, M.W., Moss, D.S. & Thornton, J.M. (1993). PROCHECK: a program to check the stereochemical quality of protein structures. J Appl Crystallogr *26*, 283-291.

Lu, W., Clasquin, M.F., Melamud, E., Amador-Noguez, D., Caudy, A.A., and Rabinowitz, J.D. (2010). Metabolomic analysis via reversed-phase ion-pairing liquid chromatography coupled to a stand alone orbitrap mass spectrometer. Anal Chem *82*, 3212-3221.

McCoy, A.J., Grosse-Kunstleve, R.W., Storoni, L.C., and Read, R.J. (2005). Likelihood-enhanced fast translation functions. Acta Cryst *D61*, 458-464

Melamud, E., Vastag, L., and Rabinowitz, J.D. (2010). Metabolomic Analysis and Visualization Engine for LC-MS Data. Anal Chem.

Otwinowski, Z., Minor, W. (1997). Processing of X-ray Diffraction Data Collected in Oscillation Mode. Methods in Enzymology *276*, 307-326.

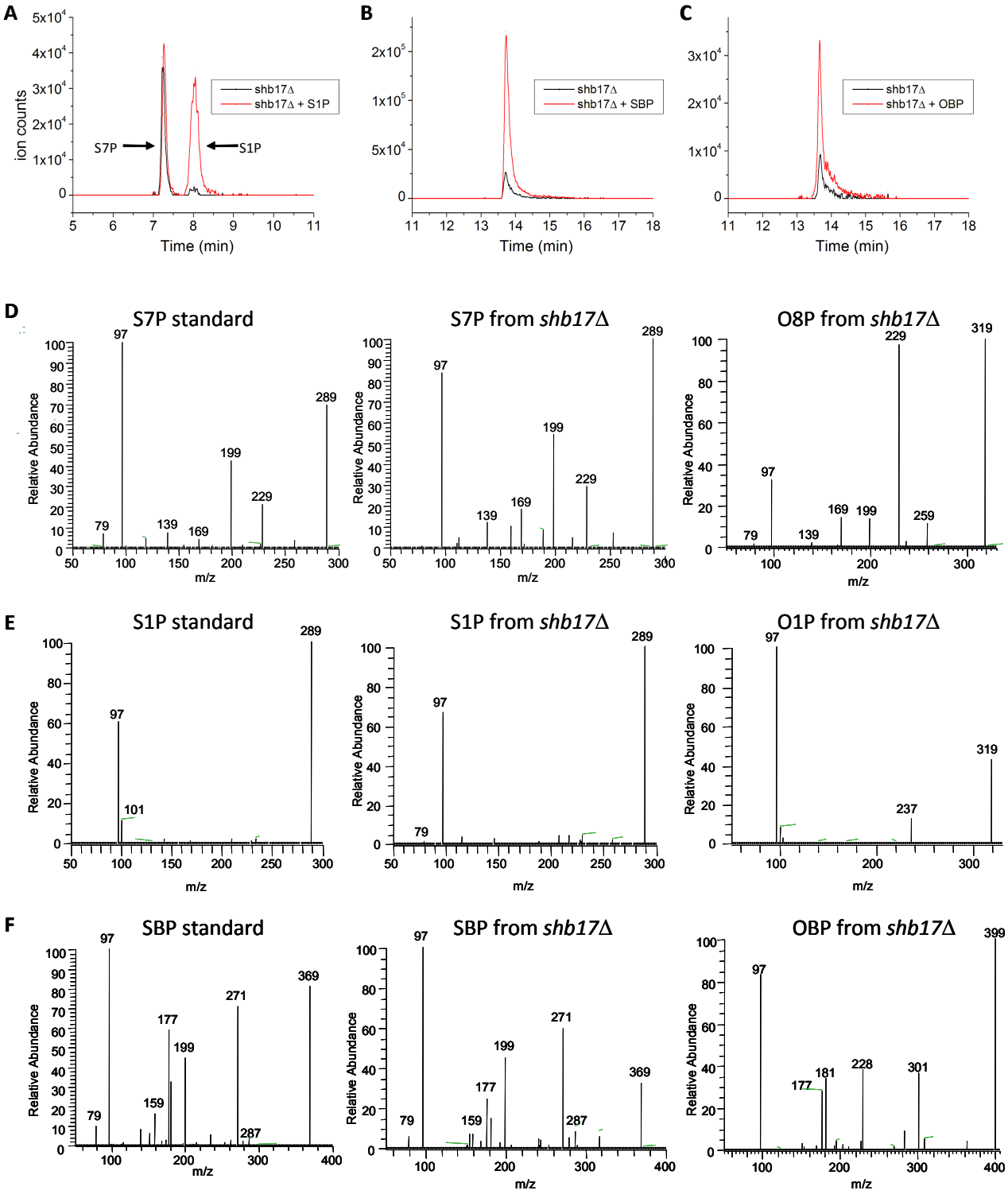
Moriarty, N.W., Grosse-Kunstleve, R.W., and Adams, P.D. (2009). electronic Ligand Builder and Optimization Workbench (eLBOW): a tool for ligand coordinate and restraint generation. Acta Crystallogr D Biol Crystallogr *65*, 1074-1080.

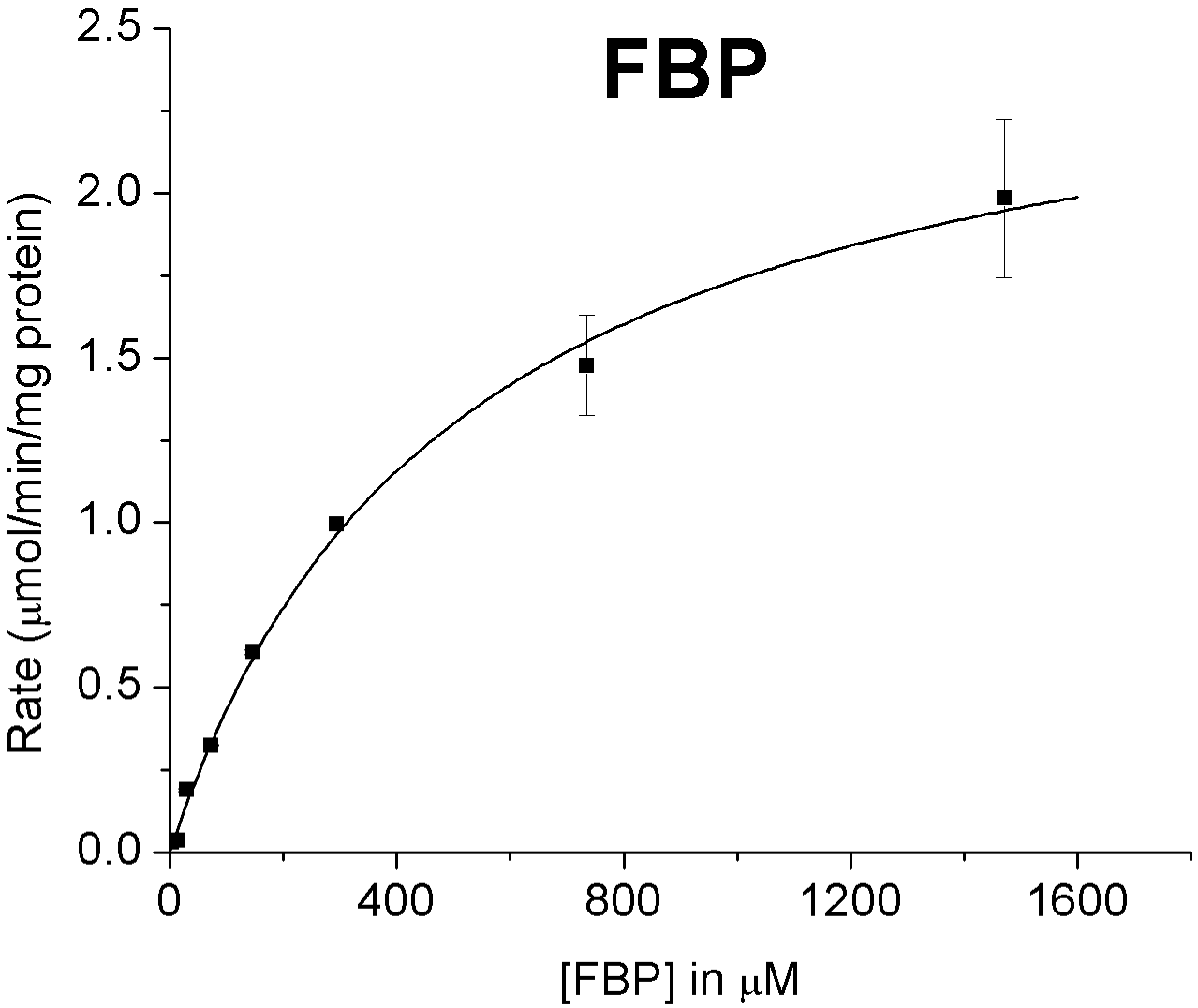
Smith, C.A., Want, E.J., O'Maille, G., Abagyan, R., and Siuzdak, G. (2006). XCMS: processing mass spectrometry data for metabolite profiling using nonlinear peak alignment, matching, and identification. Anal Chem *78*, 779-787.

Tong, A.H., Evangelista, M., Parsons, A.B., Xu, H., Bader, G.D., Page, N., Robinson, M., Raghibizadeh, S., Hogue, C.W., Bussey, H., et al. (2001). Systematic genetic analysis with ordered arrays of yeast deletion mutants. Science 294, 2364-2368.

Winston, F., Dollard, C., and Ricupero-Hovasse, S.L. (1995). Construction of a set of convenient Saccharomyces cerevisiae strains that are isogenic to S288C. Yeast *11*, 53-55.

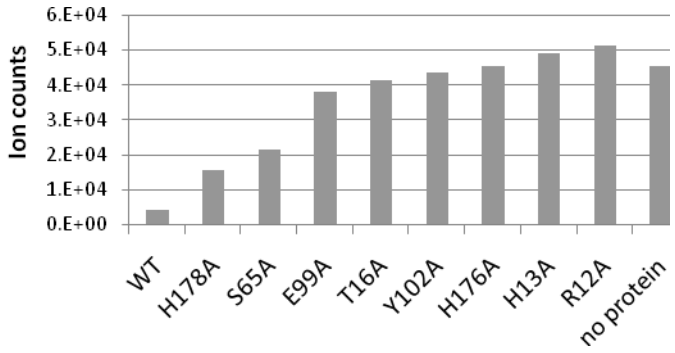
Winn, M.D., Isupov, M.N., and Murshudov, G.N. (2001). Use of TLS parameters to model anisotropic displacements in macromolecular refinement. Acta Crystallogr D Biol Crystallogr *57*, 122-133.
Yuan, J., Bennett, B.D., and Rabinowitz, J.D. (2008). Kinetic flux profiling for quantitation of cellular metabolic fluxes. Nat Protoc 3, 1328-1340.


A
ion counts
shb17Δ
shb17Δ + S1P
S7P
S1P
Time (min)
B
shb17Δ
shb17Δ + SBP
Time (min)
C
shb17Δ
shb17Δ + OBP
Time (min)
D
S7P standard
S7P from shb17Δ
O8P from shb17Δ
Relative Abundance
m/z
E
S1P standard
S1P from shb17Δ
O1P from shb17Δ
F
SBP standard
SBP from shb17Δ
OBP from shb17Δ

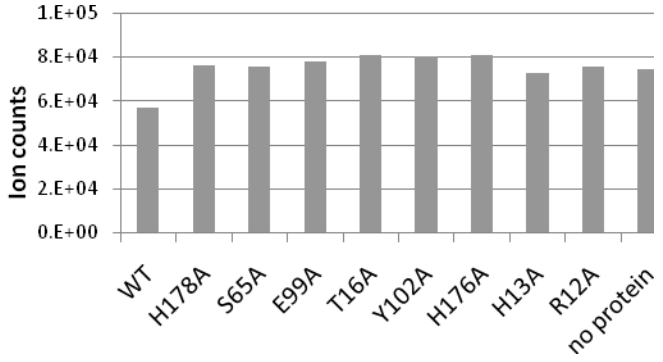

FBP
Rate (μmol/min/mg protein)
2.5
2.0
1.5
1.0
0.5
0.0
0
400
800
1200
1600
[FBP] in μM

Supplemental Figure 3

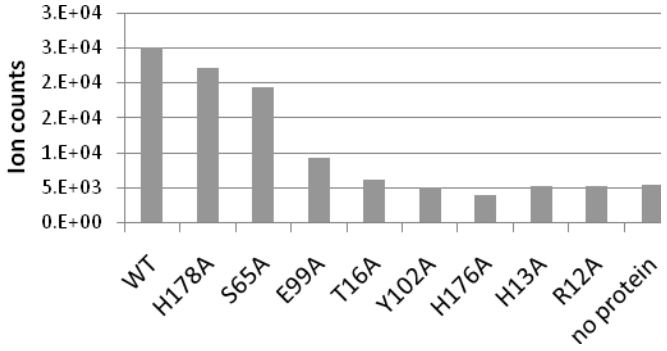
A. Sedoheptulose 1,7-bisphosphate

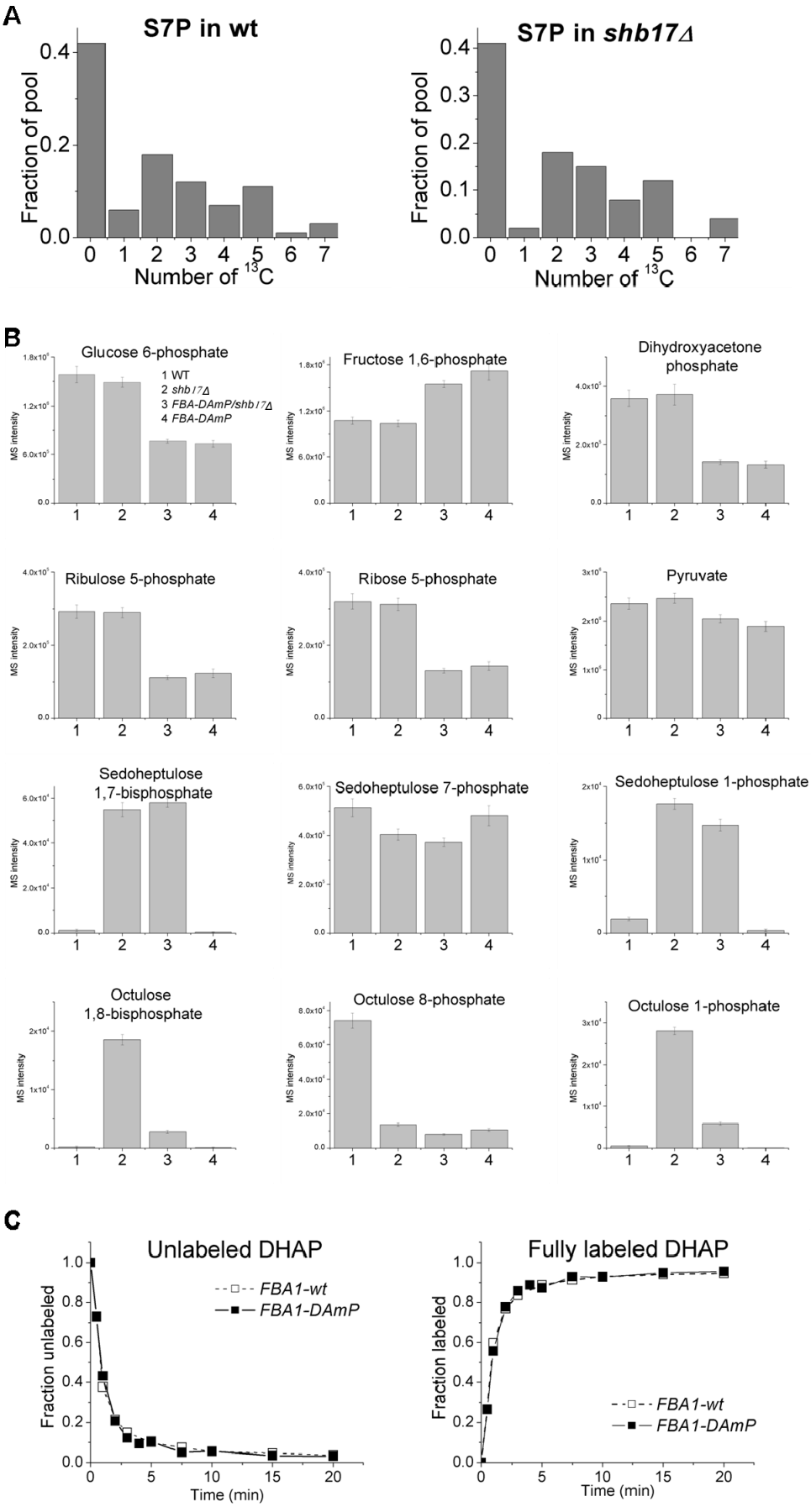


B. Fructose 1,6-bisphosphate



C. Sedoheptulose 7-phosphate




A
S7P in wt
Fraction of pool
Number of 13C
S7P in shb17Δ
Fraction of pool
Number of 13C
B
Glucose 6-phosphate
1 WT
2 shb17Δ
3 FBA-DAmP/shb17Δ
4 FBA-DAmP
MS intensity
Fructose 1,6-phosphate
Dihydroxyacetone phosphate
Ribulose 5-phosphate
Ribose 5-phosphate
Pyruvate
Sedoheptulose 1,7-bisphosphate
Sedoheptulose 7-phosphate
Sedoheptulose 1-phosphate
Octulose 1,8-bisphosphate
Octulose 8-phosphate
Octulose 1-phosphate
C
Unlabeled DHAP
FBA1-wt
FBA1-DAmP
Fraction unlabeled
Time (min)
Fully labeled DHAP
Fraction labeled
Time (min)