

Supplementary information for:

A metabolic intermediate of the fructose-asparagine utilization pathway inhibits growth of a *Salmonella fraB* mutant

Anice Sabag-Daigle^{1,2}, Henry M. Blunk³, Anindita Sengupta⁴, Jikang Wu⁴, Alexander J. Bogard⁴, Mohamed M. Ali^{2,3,5}, Christopher Stahl³, Vicki H. Wysocki⁴, Venkat Gopalan⁴, Edward J. Behrman⁴, and Brian M. M. Ahmer^{1,2,3*}

¹ Department of Microbial Infection and Immunity, ² Center for Microbial Interface Biology, ³ Department of Microbiology, ⁴ Department of Chemistry and Biochemistry, The Ohio State University, Columbus, OH 43210.

⁵ Department of Medical Microbiology and Immunology, Faculty of Medicine, Mansoura University, Mansoura, Egypt.

* Corresponding Author:

The Ohio State University

Department of Microbial Infection and Immunity.

460 West 12th Ave

710 Biomedical Research Tower

Columbus, OH 43210

614-292-1919

ahmer.1@osu.edu

Supplementary Table S1. The intensity of transitions at 22 different run times and the average of the intensity from 22 scans (in bold) from *Salmonella* wild-type or $\Delta fraB::kan$ or Δfra island. S1A: Transition m/z 376→242 of 6-P-F-Asp with collision energy 15 eV. S1B: Transition m/z 376→125 of 6-P-F-Asp with collision energy 15 eV. S1C: Transition m/z 301→216 of spiked internal standard [^{13}C]-F-Asn with collision energy 13 eV.

Supplementary Table S1A		Transition 376→125				
	Run Time (min)	Intensity	Run Time (min)	Intensity	Run Time (min)	Intensity
wild-type	Replicate 1		Replicate 2		Replicate 3	
	0.00	0.00	0.00	229.00	0.00	0.00
	0.09	0.00	0.09	114.48	0.09	0.00
	0.17	0.00	0.17	0.00	0.17	0.00
	0.26	0.00	0.26	0.00	0.26	0.00
	0.34	0.00	0.34	0.00	0.34	0.00
	0.43	0.00	0.43	0.00	0.43	0.07
	0.51	0.00	0.51	0.00	0.51	89.00
	0.60	0.96	0.60	0.00	0.60	84.90
	0.69	326.63	0.69	0.00	0.69	0.00
	0.77	207.72	0.77	0.00	0.77	0.00
	0.86	89.77	0.86	0.00	0.86	0.00
	0.94	0.00	0.94	0.00	0.94	0.00
	1.03	0.00	1.03	0.00	1.03	0.00
	1.11	0.00	1.11	0.00	1.11	0.00
	1.20	0.00	1.20	0.00	1.20	0.00
	1.28	0.00	1.28	0.00	1.28	0.00
	1.37	0.00	1.37	0.00	1.37	0.00
	1.45	0.00	1.45	0.00	1.45	0.00
	1.54	0.33	1.54	0.00	1.54	0.00
	1.62	89.98	1.62	0.00	1.62	0.00
	1.71	85.66	1.71	0.00	1.71	0.00
	1.80	0.00	1.80	0.00	1.80	0.00
Average from 22 scans		36.41		15.61		7.91
$\Delta fraB::kan$	Replicate 1		Replicate 2		Replicate 3	
	0.00	105296.00	0.00	65520.00	0.00	89232.00
	0.09	83696.00	0.09	84603.73	0.09	82414.67
	0.17	65230.20	0.17	103671.09	0.17	75604.77
	0.26	46777.56	0.26	80855.27	0.26	87835.94
	0.34	95770.02	0.34	118093.44	0.34	108228.19
	0.43	65152.00	0.43	70618.93	0.43	113559.35
	0.51	95792.00	0.51	105109.55	0.51	88382.99
	0.60	12168.00	0.60	132641.92	0.60	122746.23
	0.69	57156.78	0.69	101877.70	0.69	140669.59
	0.77	102122.12	0.77	120417.38	0.77	112332.52
	0.86	87095.04	0.86	135178.14	0.86	92192.20

	0.94	99109.91	0.94	95788.18	0.94	127183.06
	1.03	109205.92	1.03	92546.17	1.03	88713.41
	1.11	119287.68	1.11	105802.41	1.11	86768.27
	1.20	108630.23	1.20	92026.16	1.20	134746.94
	1.28	98615.30	1.28	111126.88	1.28	85240.96
	1.37	91205.70	1.37	105286.88	1.37	100656.62
	1.45	94853.72	1.45	99465.04	1.45	129253.20
	1.54	98500.62	1.54	105218.18	1.54	95524.79
	1.62	90189.70	1.62	89972.77	1.62	93066.41
	1.71	98889.53	1.71	92224.35	1.71	97792.73
	1.80	67980.44	1.80	106004.67	1.80	84411.25
Average from 22 scans		86032.93		100638.58		101661.64

Δfra island	Replicate 1		Replicate 2		Replicate 3	
	0.00	88.00	0.00	330.00	0.00	298.00
	0.09	43.99	0.09	164.97	0.09	148.99
	0.17	0.00	0.17	0.04	0.17	0.00
	0.26	0.00	0.26	89.95	0.26	0.00
	0.34	0.00	0.34	0.00	0.34	0.00
	0.43	0.00	0.43	0.00	0.43	0.00
	0.51	0.00	0.51	0.00	0.51	0.04
	0.60	0.00	0.60	0.00	0.60	92.00
	0.69	0.00	0.69	0.00	0.69	89.00
	0.77	0.00	0.77	0.00	0.77	91.82
	0.86	0.00	0.86	0.00	0.86	0.00
	0.94	0.00	0.94	0.00	0.94	0.00
	1.03	0.00	1.03	0.00	1.03	0.00
	1.11	0.00	1.11	0.00	1.11	0.00
	1.20	0.00	1.20	0.00	1.20	0.00
	1.28	0.00	1.28	0.00	1.28	0.00
	1.37	0.00	1.37	0.00	1.37	0.00
	1.45	0.00	1.45	0.18	1.45	0.00
	1.54	0.00	1.54	90.00	1.54	0.00
	1.62	0.00	1.62	89.77	1.62	0.00
	1.71	0.00	1.71	0.00	1.71	0.00
	1.80	0.00	1.80	0.00	1.80	0.00
Average from 22 scans		6.00		34.77		32.72

Supplementary Table S1B		Transition 376→242				
	Run Time (min)	Intensity	Run Time (min)	Intensity	Run Time (min)	Intensity
wild-type	Replicate 1		Replicate 2		Replicate 3	
	0.00	0.00	0.00	109.00	0.00	0.00
	0.09	0.00	0.09	54.49	0.09	49.50
	0.17	0.00	0.17	0.00	0.17	99.00
	0.26	0.00	0.26	0.00	0.26	49.49
	0.34	0.00	0.34	0.00	0.34	0.00
	0.43	0.00	0.43	0.00	0.43	0.00
	0.51	0.00	0.51	0.00	0.51	0.00
	0.60	0.00	0.60	0.00	0.60	0.00
	0.69	0.00	0.69	0.00	0.69	0.00
	0.77	0.00	0.77	0.00	0.77	0.38
	0.86	0.00	0.86	0.00	0.86	278.78
	0.94	0.00	0.94	0.00	0.94	138.76
	1.03	0.00	1.03	0.00	1.03	0.00
	1.11	0.00	1.11	0.00	1.11	0.00
	1.20	0.00	1.20	0.00	1.20	0.00
	1.28	0.00	1.28	0.00	1.28	0.00
	1.37	0.00	1.37	0.00	1.37	0.00
	1.45	0.00	1.45	0.00	1.45	0.00
	1.54	0.00	1.54	0.00	1.54	0.00
	1.62	0.00	1.62	0.00	1.62	0.00
	1.71	0.00	1.71	0.00	1.71	0.00
	1.80	0.00	1.80	0.00	1.80	0.00
Average from 22 scans		0.00		7.43		28.00
Δ <i>fraB</i> :kan	Replicate 1		Replicate 2		Replicate 3	
	0.00	378432.00	0.00	428864.00	0.00	412224.00
	0.09	434240.00	0.09	374805.47	0.09	347475.38
	0.17	408061.44	0.17	320806.84	0.17	282805.72
	0.26	381898.41	0.26	420285.44	0.26	420268.75
	0.34	435263.19	0.34	407189.94	0.34	278215.00
	0.43	431104.00	0.43	463278.56	0.43	369078.25
	0.51	478208.00	0.51	440894.00	0.51	359033.22
	0.60	274688.00	0.60	356460.31	0.60	307317.38
	0.69	297284.41	0.69	336371.69	0.69	302773.34
	0.77	319870.31	0.77	380480.91	0.77	298595.00
	0.86	315561.25	0.86	381248.97	0.86	438069.50
	0.94	386010.47	0.94	381960.84	0.94	410904.91
	1.03	431163.59	1.03	389514.41	1.03	419113.97
	1.11	423654.38	1.11	397131.19	1.11	330717.13
	1.20	390840.69	1.20	404285.72	1.20	435400.47
	1.28	383351.31	1.28	402621.41	1.28	380231.28
	1.37	375837.22	1.37	400938.03	1.37	365068.44
	1.45	312673.84	1.45	386895.50	1.45	384534.28
	1.54	334365.72	1.54	396819.50	1.54	374184.94

	1.62	356220.00	1.62	406959.50	1.62	326823.66
	1.71	435390.53	1.71	488512.44	1.71	380461.09
	1.80	398142.69	1.80	488759.53	1.80	355754.28
Average from 22 scans		381011.88		402503.83		362684.09
Δ fra island	Replicate 1		Replicate 2		Replicate 3	
	0.00	92.00	0.00	0.00	0.00	0.00
	0.09	1564.29	0.09	0.00	0.09	0.00
	0.17	3034.81	0.17	0.00	0.17	0.00
	0.26	0.00	0.26	0.00	0.26	0.00
	0.34	0.00	0.34	0.00	0.34	0.00
	0.43	0.00	0.43	0.00	0.43	0.00
	0.51	0.00	0.51	0.00	0.51	0.00
	0.60	0.00	0.60	0.00	0.60	0.00
	0.69	0.00	0.69	1.69	0.69	0.00
	0.77	0.00	0.77	959.06	0.77	0.08
	0.86	0.00	0.86	479.06	0.86	42.96
	0.94	0.00	0.94	0.19	0.94	20.95
	1.03	0.00	1.03	89.84	1.03	0.00
	1.11	0.00	1.11	0.00	1.11	0.00
	1.20	0.00	1.20	0.00	1.20	0.00
	1.28	0.00	1.28	0.00	1.28	0.00
	1.37	0.00	1.37	0.00	1.37	0.00
	1.45	0.00	1.45	0.00	1.45	0.00
	1.54	0.00	1.54	0.00	1.54	0.00
	1.62	0.00	1.62	0.00	1.62	0.00
	1.71	0.00	1.71	0.00	1.71	0.00
	1.80	0.00	1.80	0.00	1.80	0.00

Supplementary Table S1C		Transition 301→216				
	Run Time (min)	Intensity	Run Time (min)	Intensity	Run Time (min)	Intensity
wild-type	Replicate 1		Replicate 2		Replicate 3	
	0.00	4855808.00	0.00	5217280.00	0.00	6034432.00
	0.09	4628947.50	0.09	5341720.50	0.09	6182912.00
	0.17	4402241.00	0.17	5466081.00	0.17	6331392.00
	0.26	4569217.50	0.26	5386110.00	0.26	5697567.50
	0.34	4734894.50	0.34	5290941.00	0.34	5859561.50
	0.43	4665233.50	0.43	5248424.50	0.43	6456441.50
	0.51	4594024.00	0.51	5610678.00	0.51	5301400.00
	0.60	4256597.00	0.60	5743850.00	0.60	5456564.00
	0.68	4893616.00	0.68	5876838.00	0.68	5173426.50
	0.77	4216108.00	0.77	5928141.00	0.77	5325438.00
	0.86	5215172.50	0.86	5508641.50	0.86	5043803.00
	0.94	4789414.50	0.94	5159774.00	0.94	5429924.50
	1.03	4852814.50	1.03	5788823.50	1.03	5816013.00
	1.11	4136416.50	1.11	5231310.00	1.11	6805656.00
	1.20	4996915.50	1.20	5639260.00	1.20	6880494.00
	1.28	4931519.50	1.28	5685614.50	1.28	5431173.00
	1.37	4909915.00	1.37	5380187.00	1.37	5376496.50
	1.45	4283769.00	1.45	5416060.50	1.45	6373634.00
	1.54	4658549.50	1.54	4259582.50	1.54	5346304.00
	1.62	4206973.50	1.62	4915073.50	1.62	5345182.00
	1.71	3782887.25	1.71	5569552.00	1.71	4937542.00
	1.79	4105774.75	1.79	5574673.00	1.79	5573198.50
Average from 22 scans		4576673.14		5419937.09		5735388.89
Δ <i>fraB::kan</i>	Replicate 1		Replicate 2		Replicate 3	
	0.00	6390784.00	0.00	6366208.00	0.00	4790272.00
	0.09	4556800.00	0.09	6036927.50	0.09	4767739.50
	0.17	6023823.00	0.17	5707516.00	0.17	4745121.50
	0.26	7490504.50	0.26	5041227.50	0.26	4502374.50
	0.34	7205162.50	0.34	5428375.00	0.34	4240588.00
	0.43	3493888.00	0.43	5814381.50	0.43	4502261.50
	0.51	6437888.00	0.51	5954668.50	0.51	5277353.00
	0.60	5686272.00	0.60	6093648.00	0.60	5024914.00
	0.68	5890600.00	0.68	5912835.00	0.68	4216811.00
	0.77	6094809.00	0.77	5260065.00	0.77	5080370.50
	0.86	5995117.00	0.86	5069989.00	0.86	5201254.00
	0.94	5308190.00	0.94	5190807.00	0.94	5331353.50
	1.03	6670302.50	1.03	5345509.50	1.03	5120014.00
	1.11	6613612.50	1.11	5513480.00	1.11	4473590.50
	1.20	7405299.50	1.20	5682643.50	1.20	5316013.00
	1.28	6083772.00	1.28	6023724.50	1.28	5134734.00

	1.37	6967314.00	1.37	5681643.50	1.37	4420241.50
	1.45	5669392.50	1.45	5341507.50	1.45	5369922.50
	1.54	5353365.00	1.54	5527297.00	1.54	4889648.00
	1.62	6022914.00	1.62	6444041.00	1.62	5127464.50
	1.71	5861864.50	1.71	6446893.50	1.71	5181757.50
	1.79	6721140.50	1.79	5921137.00	1.79	5233421.50
Average from 22 scans		6088309.77		5718387.50		4906691.82
Δ fra island	Replicate 1		Replicate 2		Replicate 3	
	0.00	7135232.00	0.00	6286336.00	0.00	5451776.00
	0.09	6648225.00	0.09	5635457.00	0.09	5424637.50
	0.17	6161864.00	0.17	4984942.50	0.17	5397431.50
	0.26	7329131.00	0.26	5268512.00	0.26	5026779.00
	0.34	6201920.50	0.34	5322773.00	0.34	4931768.50
	0.43	6940060.50	0.43	5376997.00	0.43	5404398.50
	0.51	7361177.00	0.51	5308376.00	0.51	4703230.50
	0.60	5945233.50	0.60	5242819.00	0.60	4699320.50
	0.68	5850912.00	0.68	7167728.50	0.68	5014424.50
	0.77	5687600.00	0.77	7011890.50	0.77	4882174.00
	0.86	5882010.50	0.86	6251434.50	0.86	4750582.00
	0.94	5972681.50	0.94	5684729.50	0.94	4876558.50
	1.03	7255659.00	1.03	5919862.50	1.03	5002110.50
	1.11	7082748.00	1.11	5986065.00	1.11	4945917.00
	1.20	6978949.50	1.20	5342195.50	1.20	4542485.00
	1.28	6329080.00	1.28	6381928.00	1.28	4551252.00
	1.37	6934855.50	1.37	5305456.00	1.37	5037180.50
	1.45	6384692.50	1.45	6529216.00	1.45	4731332.00
	1.54	6090958.00	1.54	5057578.50	1.54	4867471.00
	1.62	5506279.00	1.62	5554080.50	1.62	4979484.50
	1.71	6496944.50	1.71	5950441.50	1.71	4622257.50
	1.79	6112906.50	1.79	6316059.00	1.79	4022645.75
Average from 22 scans		6467687.27		5812949.00		4902964.40

Supplementary Table S2. The intensity of transitions at 22 different run times and the average of the intensity (shown in bold) for *Salmonella* wild-type spiked with 0, 20, 40, 160, 320 nmol of 6-P-F-Asp. S2A: Transition m/z 376→242 of 6-P-F-Asp with collision energy 15 eV. S2B: Transition m/z 376→125 of 6-P-F-Asp with collision energy 15 eV. S2C: Transition m/z 301→216 of spiked internal standard [¹³C]-F-Asn with collision energy 13 eV.

Supplementary Table S2A		Transition m/z 376→125								
Spiked 6-P-F-Asp (nmol)	0.00		20.00		40.00		160.00		320.00	
	Run Time (min)	Intensity	Run Time (min)	Intensity	Run Time (min)	Intensity	Run Time (min)	Intensity	Run Time (min)	Intensity
	0.00	0.00	0.00	11192.00	0.00	25376.00	0.00	123008.00	0.00	322560.00
	0.09	0.00	0.09	21988.00	0.09	36128.00	0.09	122575.91	0.09	304922.84
	0.17	0.00	0.17	19032.00	0.17	46880.00	0.17	122168.07	0.17	287305.00
	0.26	0.00	0.26	12254.02	0.26	30448.00	0.26	183805.91	0.26	302627.09
	0.34	0.00	0.34	5484.87	0.34	35191.54	0.34	180206.75	0.34	253275.33
	0.43	0.00	0.43	13808.87	0.43	39935.07	0.43	158146.70	0.43	288241.75
	0.51	0.00	0.51	22123.31	0.51	43280.00	0.51	160397.63	0.51	263957.44
	0.60	0.00	0.60	14118.35	0.60	34904.37	0.60	128195.40	0.60	291384.88
	0.69	0.00	0.69	18136.65	0.69	26538.07	0.69	130131.46	0.69	284086.13
	0.77	0.00	0.77	26074.75	0.77	42084.52	0.77	131985.92	0.77	276862.19
	0.86	0.00	0.86	20694.36	0.86	22509.33	0.86	80984.76	0.86	275865.16
	0.94	0.00	0.94	15878.93	0.94	39552.00	0.94	146022.98	0.94	362865.13
	1.03	0.00	1.03	14783.55	1.03	19094.38	1.03	135327.30	1.03	355287.69
	1.11	0.00	1.11	14326.89	1.11	42003.66	1.11	120180.77	1.11	365177.50
	1.20	0.00	1.20	13378.89	1.20	45120.95	1.20	144740.89	1.20	287131.28
	1.28	0.00	1.28	12464.86	1.28	45692.94	1.28	160453.84	1.28	273737.00
	1.37	0.00	1.37	25889.24	1.37	23269.32	1.37	162622.45	1.37	276840.97
	1.45	0.00	1.45	30609.20	1.45	30125.29	1.45	118524.63	1.45	289878.44
	1.54	0.00	1.54	29273.71	1.54	49431.23	1.54	122331.15	1.54	276631.06
	1.62	0.00	1.62	14662.38	1.62	52248.12	1.62	126078.33	1.62	284511.25
	1.71	0.00	1.71	17610.62	1.71	43485.88	1.71	110225.33	1.71	292463.50
	1.80	0.00	1.80	10192.34	1.80	26444.24	1.80	110794.37	1.80	288238.44
Average from 22 scans		0.00		17453.54		36351.95		135404.93		295629.55

Supplementary Table S2B		Transition m/z 376→242								
Spiked 6-P-F-Asp (nmol)	0.00		20.00		40.00		160.00		320.00	
	Run Time (min)	Intensity	Run Time (min)	Intensity	Run Time (min)	Intensity	Run Time (min)	Intensity	Run Time (min)	Intensity
	0.00	0.00	0.00	72848.00	0.00	141376.00	0.00	587264.00	0.00	1636352.00
	0.09	0.00	0.09	59200.00	0.09	128200.00	0.09	584831.50	0.09	1625852.88
	0.17	0.00	0.17	82416.00	0.17	115024.00	0.17	582441.88	0.17	1615139.75
	0.26	0.00	0.26	85040.77	0.26	204608.00	0.26	689622.31	0.26	1239408.25
	0.34	0.00	0.34	87669.45	0.34	164315.94	0.34	618482.19	0.34	1431080.13
	0.43	1.19	0.43	96962.67	0.43	124023.88	0.43	600779.81	0.43	1482377.88
	0.51	1525.10	0.51	101496.26	0.51	119136.00	0.51	556385.13	0.51	1280690.38
	0.60	2929.57	0.60	61011.16	0.60	114751.15	0.60	618484.31	0.60	1181678.88
	0.69	1463.28	0.69	120998.69	0.69	110373.60	0.69	611828.75	0.69	1426159.88
	0.77	0.00	0.77	77760.50	0.77	124718.26	0.77	605420.75	0.77	1414211.75
	0.86	0.00	0.86	78286.02	0.86	121741.67	0.86	596523.75	0.86	1294028.25
	0.94	0.00	0.94	90270.34	0.94	118769.88	0.94	615161.44	0.94	1409431.25
	1.03	1.41	1.03	121325.16	1.03	121170.33	1.03	612332.75	1.03	1095200.75
	1.11	656.53	1.11	118351.71	1.11	123588.26	1.11	603494.25	1.11	1544074.38
	1.20	1308.51	1.20	63585.57	1.20	154470.91	1.20	650994.81	1.20	1095740.88
	1.28	1112.59	1.28	119378.38	1.28	138406.86	1.28	645361.69	1.28	1210983.50
	1.37	555.37	1.37	83329.70	1.37	122330.67	1.37	639776.25	1.37	1072302.88
	1.45	3.22	1.45	100493.86	1.45	130143.40	1.45	650522.00	1.45	1210287.63
	1.54	1029.58	1.54	69729.33	1.54	136655.30	1.54	581179.81	1.54	1348132.00
	1.62	0.00	1.62	56662.05	1.62	117541.27	1.62	514583.69	1.62	1360403.00
	1.71	0.00	1.71	59478.60	1.71	131160.92	1.71	583833.50	1.71	1365267.00
	1.80	0.00	1.80	62385.79	1.80	139640.80	1.80	706737.75	1.80	1368735.25
Average from 22 scans		481.20		84940.00		131915.78		611638.29		1350342.66

Supplementary Table S2C		Transition m/z 301 → 216								
Spiked 6-P-F-Asp (nmol)	0.00		20.00		40.00		160.00		320.00	
	Run Time (min)	Intensity	Run Time (min)	Intensity	Run Time (min)	Intensity	Run Time (min)	Intensity	Run Time (min)	Intensity
	0.00	6759424.00	0.00	7116800.00	0.00	6689792.00	0.00	5249024.00	0.00	4375552.00
	0.09	6878743.50	0.09	6453248.00	0.09	6114872.00	0.09	4684177.50	0.09	4509210.00
	0.17	6997549.00	0.17	6225920.00	0.17	5539952.50	0.17	4119744.25	0.17	4642601.50
	0.26	5800731.00	0.26	6402099.50	0.26	5904384.00	0.26	4612397.00	0.26	4093093.50
	0.34	5214783.00	0.34	6577965.00	0.34	6269012.50	0.34	5125557.00	0.34	4375585.50
	0.43	6196063.50	0.43	6217821.00	0.43	6700971.50	0.43	4525772.00	0.43	4418749.00
	0.51	5990374.50	0.51	6376541.50	0.51	5957632.00	0.51	5136369.50	0.51	4741149.00
	0.60	5958235.00	0.60	6535695.50	0.60	6465635.50	0.60	5126078.00	0.60	4778021.00
	0.68	6575077.50	0.68	5688533.00	0.68	6973179.50	0.68	4506478.00	0.68	4815739.00
	0.77	6554359.00	0.77	5961367.50	0.77	6305606.50	0.77	4424951.00	0.77	4718582.50
	0.86	5731961.00	0.86	6640290.00	0.86	5990072.00	0.86	5015566.50	0.86	4712324.00
	0.94	6674223.50	0.94	6341399.00	0.94	6880552.00	0.94	5021451.50	0.94	4641670.00
	1.03	7052905.00	1.03	6043974.00	1.03	5948986.50	1.03	4916245.50	1.03	4579136.00
	1.11	6861609.50	1.11	6377719.00	1.11	5485464.00	1.11	4451603.50	1.11	4497119.50
	1.20	5900576.50	1.20	6588405.00	1.20	6270440.00	1.20	5055725.50	1.20	4383145.00
	1.28	6817500.50	1.28	6578582.50	1.28	6582760.00	1.28	5155470.50	1.28	4165192.50
	1.37	6336648.50	1.37	6291170.00	1.37	6894370.50	1.37	4608300.00	1.37	4364911.50
	1.45	6035473.50	1.45	6144709.50	1.45	6732421.00	1.45	4711188.00	1.45	4563929.50
	1.54	6368546.00	1.54	5999031.00	1.54	6570569.00	1.54	4947311.50	1.54	4551956.00
	1.62	6490814.50	1.62	6186388.50	1.62	6398089.00	1.62	4723824.00	1.62	4646255.50
	1.71	5918336.50	1.71	6375157.50	1.71	6448500.00	1.71	4759134.00	1.71	4738001.50
	1.79	6152878.50	1.79	6697550.00	1.79	6575024.00	1.79	4634067.50	1.79	4207721.50
Average from 22 scans		6330309.70		6355471.23		6349922.09		4795928.92		4523620.25

Supplementary Table S3. Normalization of transitions from 6-P-F-Asp with respect to the [¹³C]-F-Asn spiked internal standard. The ratios reported below are the average peak intensity of either transition m/z 376→125 or m/z 376→242 from 6-P-F-Asp relative to transition m/z 301→216 from the [¹³C]-F-Asn internal standard. Table S3A: Analyses of intensity ratios from data in Table S1 for *Salmonella* wild-type, $\Delta fraB::kan$ and Δfra island. Table S3B: Ratios determined using different amounts of spiked 6-P-F-Asp (based on primary data in Table S2); these data in turn were used to generate the standard curves in Fig. S2 (6-P-F-Asp spiked *Salmonella* wild-type).

Supplementary Table S3A						
	Transition ratios					
	Replicate 1		Replicate 2		Replicate 3	
Transition Strain used	$\frac{376 \rightarrow 125}{301 \rightarrow 216}$	$\frac{376 \rightarrow 242}{301 \rightarrow 216}$	$\frac{376 \rightarrow 125}{301 \rightarrow 216}$	$\frac{376 \rightarrow 242}{301 \rightarrow 216}$	$\frac{376 \rightarrow 125}{301 \rightarrow 216}$	$\frac{376 \rightarrow 242}{301 \rightarrow 216}$
wild-type	7.96E-06	0.00E+00	2.88E-06	1.37E-06	1.38E-06	4.88E-06
$\Delta fraB::kan$	1.41E-02	6.26E-02	1.76E-02	7.04E-02	2.07E-02	7.39E-02
Δfra island	9.28E-07	3.30E-05	5.98E-06	1.20E-05	6.67E-06	5.93E-07

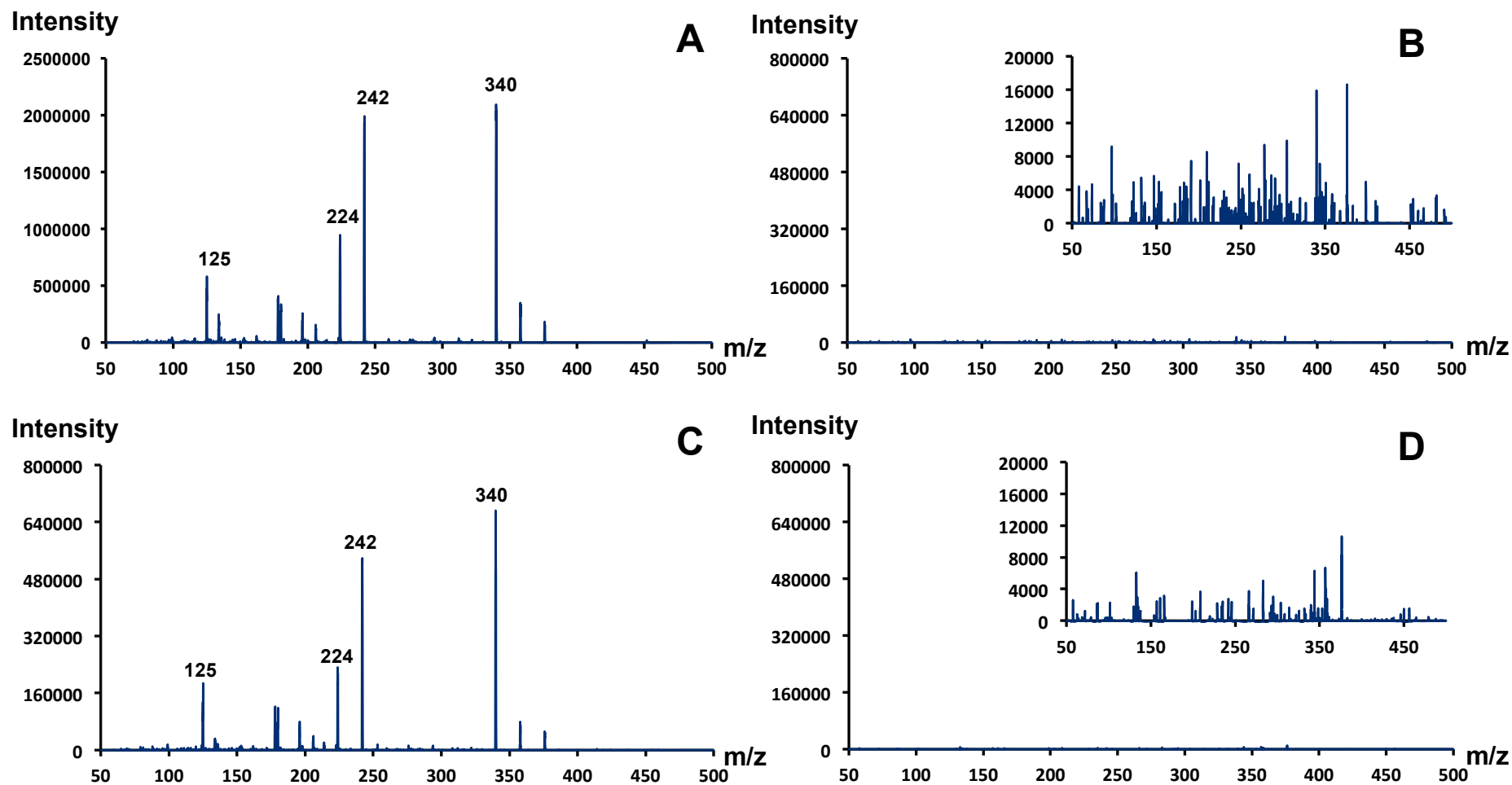
Supplementary Table S3B					
	Transition ratios				
Spiked 6-F-Asp (nmol)	0	20	40	160	320
Transition					
$\frac{376 \rightarrow 125}{301 \rightarrow 216}$	0.00E+00	2.75E-03	5.72E-03	2.82E-02	6.54E-02
$\frac{376 \rightarrow 242}{301 \rightarrow 216}$	7.60E-05	1.34E-02	2.08E-02	1.28E-01	2.99E-01

Supplementary Table S4. The amount of 6-P-F-Asp present in *Salmonella* wild-type, *fraB::kan* and delta *fra* island. While the qualitative trends are similar, there are some differences in the absolute amounts depending on which transition was used for the calculation. Table S4A represents the data computed from transition m/z 376→125 and the standard curve in Fig. S2A, while Table S4B is from m/z 376→242 and the standard curve in Fig. S2B. Two transitions were measured to confirm the overall trends; data from one transition (Table S4B) are shown as Fig. 3, while data from Table S4A are plotted in Fig. S3.

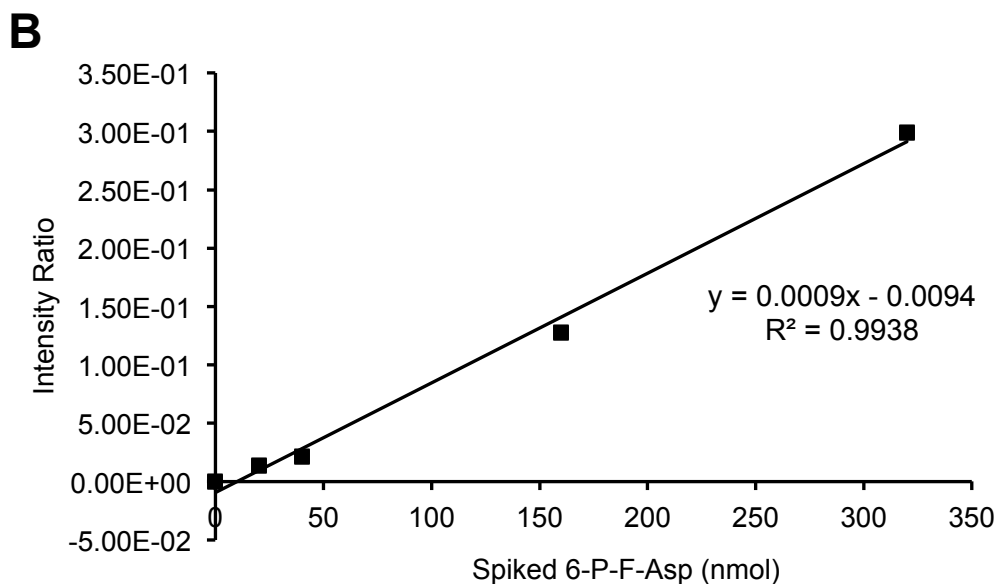
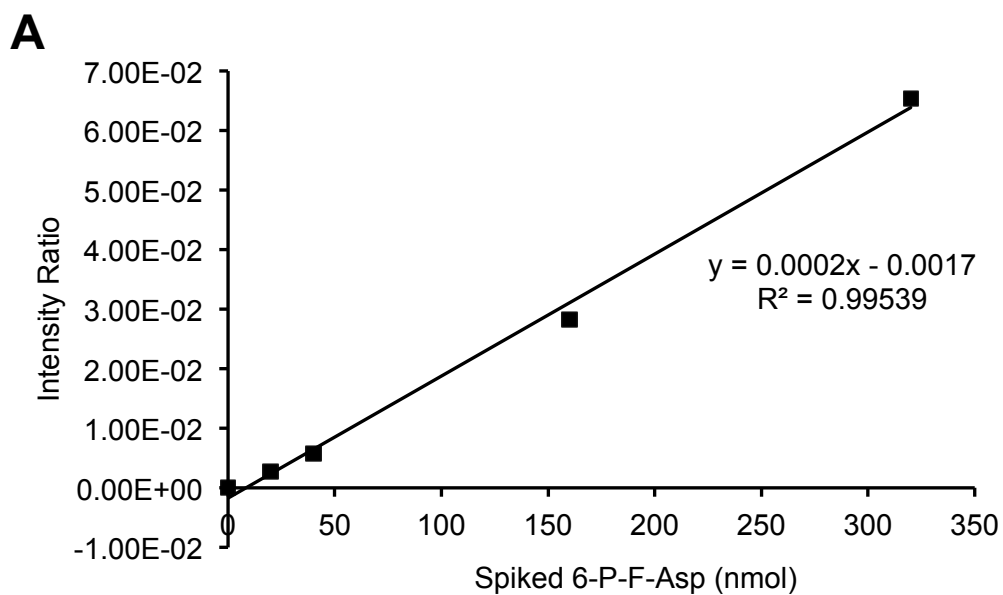
Supplementary Table S4A					
	6-P-F-Asp (nmol)				
Strain used	Replicate 1	Replicate 2	Replicate 3	Average	Standard deviation
wild-type	8.54*	8.51*	8.51*	8.52*	0.01
$\Delta fraB::kan$	80	110	100	100	10
Δfra island	8.50*	8.53*	8.53*	8.52*	0.01

Supplementary Table S4B					
	6-P-F-Asp (nmol)				
Strain used	Replicate 1	Replicate 2	Replicate 3	Average	Standard deviation
wild-type	10.444*	10.446*	10.450*	10.447*	0.002
$\Delta fraB::kan$	80	93	89	87	5
Δfra island	10.48*	10.46*	10.45*	10.46*	0.01

*Note: These measurements are below 20 nmol, the lowest concentration used for establishing the standard curve.



Supplementary Figure S1 Product-ion MS/MS spectra of m/z 376. Collision-induced dissociation (15 eV) of (A) *Salmonella* wild-type (14028) metabolome spiked with 320 nmol of 6-P-F-Asp, (B) *Salmonella* wild-type (14028), (C) $\Delta fraB::kan$ (HMB206) and (D) Δfra island (HMB215). Inset in panels B and D represents magnification to depict the signal/noise at this level of detection.



Supplementary Figure S2. Standard curves were constructed by separately plotting the change in peak intensity ratios for two different transitions of 6-P-F-Asp (m/z 376 \rightarrow 125, panel A; m/z 376 \rightarrow 242, panel B) as a function of the spiked 6-P-F-Asp. These transitions were normalized using the m/z 301 \rightarrow 216 from the [^{13}C]-F-Asn internal standard.

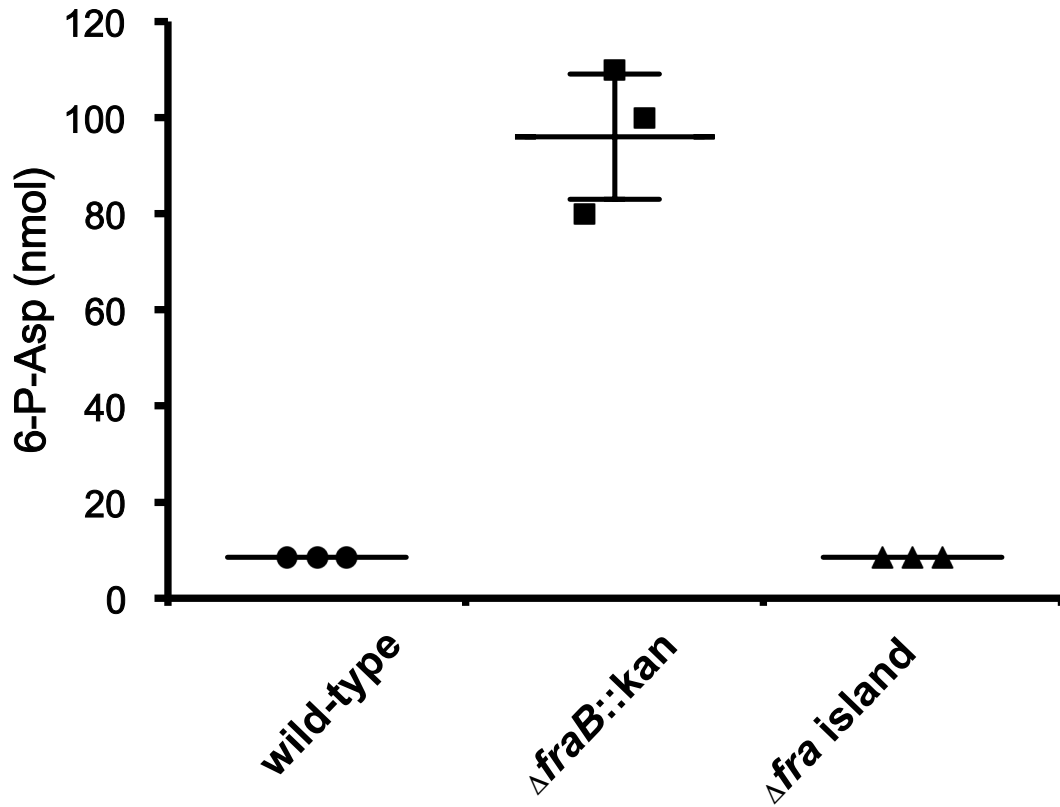


Figure S3. Each point represents mass spectrometry (MS)-based measurement of the levels of 6-P-F-Asp in one biological replicate. Samples were prepared by taking a one-fifteenth aliquot from the cell pellet of a 20-ml culture of *Salmonella* wild-type (14028) or $\Delta fraB::kan$ (HMB206) or Δfra island deletion (HMB215) mutant. Two transitions were measured to confirm quantitation; data from one transition (Table S4B) are shown as Figure 3, while data from a second transition (Table S4A) are plotted here. The values provided are the mean \pm standard deviation from three biological replicates. The lowest concentration on the standard curve was 20 nmol, so the values for wild-type and Δfra island deletion are very low; however, they indicate the reproducible absence of 6-P-F-Asp in these two strains.