Peak <sup>a</sup>	Gene <sup>b</sup>	WT αSwrA⁰	WT αDegU⁰	<i>swrA</i> αDegU <sup>c</sup>	Class <sup>d</sup>	Ref <sup>e</sup>
213927	skfA				1	
293411	yczC/yccF				1	
300782	ycdA/ycdB				3	(5,6,10,11)
302324	ycdC				1	(11)
376912	srfAA				1	(6,7)
478800	ydaJ				1	
889865	yfjA/malA				3	
1023281	ctrA				1	
1117009	comK/yhzC				3	
1264976	yjcM/yjcN				3	
1289306	yjhA				1	
1372068	ykhA/ykgA				ND	
1372668	hmp				ND	
1599132	bpr				1	(6,10,11,13)
1665617	rnc				ND	
1691171	flgB (P <sub>flache</sub> )				2	(1,5,6,8,10-12)
1924010	ynel				1	
1998239	ppsA				1	
2033801	exIX				1	
2204071	yopS/yopR				3	
2219547	yonU				1	
2272024	yolC/yolD				1	
2308418	degR/ypzA				1	
2397791	motl				3	
2551865	yqhG (intragenic)				3	
2819207	yrvJ (intragenic)				1	
3028129	iscS/braB				1	
3105979	ytvA/ytvB				1	(6,11)
3208278	тсрА				2	(6,11)
3210376	tlpA				2	
3244781	maeN				ND	
3257423	degQ				3	(9)
3276581	yukE				3	
3293560	besA				1	
3535733	sacB/pbpE				3	
3622315	swrA				1	(5)
3640693	flgM				1	(4,6,11)
3701166	rbsR/pgsB				ND	
3718673	ywrE				1	
3728282	ywqH				2	

## TABLE S1: SwrA/DegU ChIP-Seq WT vs swrA Enrichment Summary

3882923	yweA		1	
3902219	ywdA		1	
3923156	slrA/ywcC		1	
3941940	sacX		1	(2)
3979458	cydA/cimH		1	
3989186	yxkC		3	(6,11)
3996762	ухјЈ		1	
4030682	wapA		2	(3,6,11)
4039337	ухіВ		3	
4104395	yxaJ/yxal		1	
4204590	yaaD		3	

<sup>a</sup>Peak indicates the location in base pairs of the center of the peak of ChIP-seq signal.

<sup>b</sup>Gene indicates the closes proximal gene to peak center. Two genes are indicated when it wasn't clear which of the two genes might be regulated by the DNA binding activity. Two peaks are intragenic and are labeled with (intragenic).

<sup>o</sup>Data lanes are presented with the genotype of the parent and the primary antibody ( $\alpha$ ) used for ChIP-seq analysis. Data is presented as: dark gray, indicates a major peak with over 50 input units, light gray indicates a minor peak with between 5-50 input units, and white box, indicates the absence of a peak with less than 5 input units of peak intensity.

<sup>d</sup>Class indicates the way in which the DegU-dependent peak intensity changed in response to various genetic backgrounds. Class 1 represents the DegU-dependent peaks that were abolished in the absence of SwrA, Class 2 represents the DegU-dependent peaks that were reduced in the absence of SwrA, and Class 3 represents the DegU-dependent peaks that did not change in intensity in the absence of SwrA. ND, indicates that we were unable to class the target with certainty often due to very low peak intensity. <sup>e</sup>References indicate publications in which the target was either predicted or demonstrated.

Peak <sup>a</sup>	Gene <sup>b</sup>	degQ <sup>+++</sup> αDegUª	swrA degQ <sup>+++</sup> αDegUª	Class <sup>b</sup>
45389	abrB/metS			1
213927	skfA			1
293411	yczC/yccF			1
300782	ycdA/ycdB			1
302324	ycdC			1
376912	srfAA			1
478800	ydaJ			1
749613	yeeF			1
859668	yfkN/yfkM			1
889865	yfjA/malA			1
1023281	ctrA			1
1056366	yhaZ			1
1117009	comK/yhzC			3
1121049	yhjC			1

#### TABLE S3: SwrA/DegU ChIP-Seq degQ<sup>+++</sup> vs swrA degQ<sup>+++</sup> Enrichment Summary

1151193	yisl		1
1264976	yjcM/yjcN		2
1289306	yjhA		1
1291068	yjiA		1
1354187	ykcB/mhqA		1
1372068	ykhA/ykgA		3
1372668	hmp		3
1398916	ykoM(intragenic)		1
1507445	fruR		3
1599132	bpr		1
1665617	rnc		1
1691171	flgB		2
1783589	pksC		1
1924010	ynel		1
1940485	eglS		1
1998239	ppsA		1
2033801	exlX		1
2076944	yobO		1
2204071	yopS/yopR		1
2219547	yonU		1
2272024	yolC/yolD		1
2308418	degR/ypzA		3
2397791	dgrA		2
2551865	yqhG(intragenic)		2
2581676	pstS		1
2661024	yqcG		1
2773724	cypB/yrkH		3
2773754	yrkH/cypB		3
2780534	yrhG		1
2818395	yrzK/yrvJ		1
2819207	yrvJ(intragenic)		1
3028129	iscS/braB		1
3105979	ytvA/ytvB		1
3208278	тсрА		2
3210376	tlpA		1
3239698	yufN		1
3244781	maeN		1
3257423	degQ		2
3276581	yukE		2
3293560	besA		1
3535733	sacB/pbpE		3
3615618	yvkC/csbA		1
3622315	swrA		1

3640693	flgM		1
3701166	rbsR/pgsB		1
3718673	ywrE		1
3728282	ywqH		2
3775553	ywmD		1
3882923	yweA		1
3902219	ywdA		1
3907807	vpr		1
3923156	sIrA/ywcC		1
3941940	sacX		1
3979458	cydA/cimH		1
3989186	yxkC		3
3996762	ухјЈ		1
4014576	yxiP/yxiO		1
4030682	wapA		3
4035905	bgIP		1
4039337	ухіВ		3
4104395	yxaJ/yxal		1
4204590	yaaD		3

<sup>a</sup>Data lanes are presented with the genotype of the parent and the primary antibody ( $\alpha$ ) used for ChIP-seq analysis. Data is presented as: dark gray, indicates a major peak with over 50 input units, light gray, indicates a minor peak with between 5-50 input units, and open box indicates the absence of a peak with less than 5 input units of peak intensity.

<sup>b</sup>Class indicates the way in which the DegU-dependent peak intensity changed in response to various genetic backgrounds. Class 1 represents the DegU-dependent peaks that were abolished in the absence of SwrA, Class 2 represents the DegU-dependent peaks that were reduced in the absence of SwrA, and Class 3 represents the DegU-dependent peaks that did not change in intensity in the absence of SwrA. ND, indicates that we were unable to class the target with certainty often due to very low peak intensity.

Peak <sup>a</sup>	Gene <sup>b</sup>	degU <sup>hy32+++</sup>	swrA degU <sup>hy32+++</sup>	
45000		αDegUª	αDegUª	Class
45389	abrB/metS			1
1105819	apr <b>⊢</b>			3
3293560	besA			1
4035905	bgIP			1
1599132	bpr			1
3253560	comA			3
1117009	comK/yhzC			3
3256928	comQ			1
3716154	cotB			1
1023281	ctrA			1
3979458	cydA/cimH			1
2773724	cypB/yrkH			3
3257423	degQ			2
2308418	degR/ypzA			2
3645362	degU			3
2397791	dgrA			2
1940485	eglS			1
2033801	exIX			2
1691171	flgB			2
3640693	flgM			1
1507445	fruR			3
266397	glnJ(intragenic)			3
1372668	hmp			3
3028129	iscS/braB			1
1387091	ispA/rsbRB			3
2765963	levR			2
1018839	lytE/phoA			3
3244781	maeN			1
3208278	тсрА			2
449657	mtIA/ycnL			3
828536	pel			3
1783589	pksC			3
1998239	ppsA			2
2581676	psts			1
3701166	rbsR/capB			1
1665617	rnc			1
3535733	sacB/pbpE			3
3941940	sacX			2
3464400	sdpA/sdpB			1

## TABLE S4: SwrA/DegU ChIP-Seq *degU*<sup>hy32+++</sup> vs *swrA degU*<sup>hy32+++</sup> Enrichment Summary

3971006	sigY/yxIA		3
213927	skfA		1
3923156	slrA/ywcC		1
376912	srfAA		1
3622315	swrA		1
3210376	tlpA		2
3907807	vpr		1
4030682	wapA		3
4204590	yaaD		3
48076	yabD		1
185198	ybbB		2
190271	ybbF(intragenic)		3
193600	ybbJ		2
300782	ycdA/ycdB		3
302324	ycdC		2
293411	yczC/yccF		3
478800	ydaJ		1
548818	yddM		1
594245	ydfK		1
743573	yeeB(intragneic)		1
744752	yeeC		1
749613	yeeF		2
889865	yfjA/malA		3
859668	yfkN/yfkM		1
821653	yfmG		1
1056366	yhaZ		1
1121049	yhjC		1
1151193	yisl		1
1264976	yjcM/yjcN		2
1289306	yjhA		1
1291068	yjiA		1
1354187	ykcB/mhqA		1
1372068	ykhA/ykgA		2
1398916	ykoM(intragenic)		1
1410764	ykoY(intragenic)		1
1476554	ykuD		1
1442885	ykvO/ykvN		2
1473462	ykzT/cheV		2
1552212	ylaN		3
1781134	ymzD		1
1924010	ynel		1
2069142	yobl		1
2073577	yobL(intragenic)		1

2076944	yobO(intragenic)		1
2114594	yojN		1
2272024	yolC/yolD		1
2221441	yonR		2
2219547	yonU		1
2204071	yopS/yopR		2
2700775	yqaB/yqaC		2
2661024	yqcG		1
2698615	yqdA		1
2551865	yqhG(intragenic)		2
2841492	yrbD		1
2780534	yrhG		1
2773754	yrkH/cypB		3
2802030	yrrl(intragenic)		3
2819207	yrvJ(intragenic)		1
2818395	yrzK/yrvJ		1
3105979	ytvA/ytvB		1
3190799	yubF		1
3239698	yufN		2
3276581	yukE		2
3615618	yvkC/csbA		1
3902219	ywdA		1
3882923	yweA		2
3874914	ywfA		2
3775553	ywmD		1
3756632	ywoB/amtB		3
3728282	ywqH		2
3718673	ywrE		1
4104395	yxaJ/yxal		1
4039337	ухіВ		3
4014576	yxiP/yxiO		1
3996762	yxjJ		2
3989186	yxkC		3
4194290	yyaK/yyaJ		1
4176028	yybJ(intragenic)		1
4175313	yybK(intragenic)		1
4174633	yybK/yybL		1
4173156	yybN		1
4133714	yydB/yydC		1
4132875	yydC		1
4127781	vvdF		1

<sup>a</sup>Data lanes are presented with the genotype of the parent and the primary antibody ( $\alpha$ ) used for ChIP-seq analysis. Data is presented as: dark gray, indicates a major peak with over 50 input units, light gray,

indicates a minor peak with between 5-50 input units, and open box indicates the absence of a peak with less than 5 input units of peak intensity.

<sup>b</sup>Class indicates the way in which the DegU-dependent peak intensity changed in response to various genetic backgrounds. Class 1 represents the DegU-dependent peaks that were abolished in the absence of SwrA, Class 2 represents the DegU-dependent peaks that were reduced in the absence of SwrA, and Class 3 represents the DegU-dependent peaks that did not change in intensity in the absence of SwrA. ND, indicates that we were unable to class the target with certainty often due to very low peak intensity.

reporters	wt	∆swrA	swrA***	∆degU	∆degU swrA⁺**
flache	142 ± 7	46 ± 3	307 ± 19	48 ± 4	41 ± 5
flgM	17 ± 1	6 ± 1	29 ± 1	4 ± 1	4 ± 1
swrA	27 ± 8	15 ± 4	54 ± 10	12 ± 1	12 ± 5
ухјЈ	31 ± 0	10 ± 5	78 ± 8	10 ± 1	5 ± 3
тсрА	20 ± 5	2 ± 1	51 ± 8	8 ± 1	10 ± 2
ynel	16 ± 1	16 ± 1	13 ± 4	20 ± 1	18 ± 2
ytvA	12 ± 1	15 ± 3	13 ± 1	13 ± 1	18 ± 2
ydaJ	4 ± 1	6 ± 1	5 ± 2	3 ± 0	4 ± 1
ywdA	6 ± 1	8 ± 2	9 ± 2	5 ± 1	5 ± 0
yweA	53 ± 4	23 ± 5	41 ± 6	45 ± 1	47 ± 10

TABLE S5a: Expression of reporters in Figure 2A and 2B (Miller units)<sup>a</sup>

<sup>a</sup>± is the standard deviation of three replicates.

### TABLE S5b: Expression of reporters in Figure 2C (Miller units)<sup>a</sup>

reporters	wt	swrA	swrA degU
flache	230 <u>+</u> 8	100 <u>+</u> 10	91 <u>+</u> 8
flgM	7 <u>+</u> 2	4 <u>+</u> 1	2 <u>+</u> 0.5
swrA	19 <u>+</u> 0.5	10 <u>+</u> 0	6 <u>+</u> 1
ухјЈ	10 <u>+</u> 3	3 <u>+</u> 5	3 <u>+</u> 0.6
тсрА	21 <u>+</u> 7	3 <u>+</u> 1	3 <u>+</u> 0

<sup>a</sup>± is the standard deviation of three replicates.

## TABLE S5c: Expression of various *flache* reporters in Figure 2D (Miller units)<sup>a</sup>

reporters	wt	swrA	swrA***
wt	230 <u>+</u> 8	100 <u>+</u> 10	458 <u>+</u> 21
site1+2	90 <u>+</u> 3	80 <u>+</u> 21	109 <u>+</u> 11
+20	35 <u>+</u> 8	17 <u>+</u> 2	52 <u>+</u> 1
-35-+1	10 <u>+</u> 0.08	9 <u>+</u> 0.4	9 <u>+</u> 1
UAS <sup>r</sup>	34 <u>+</u> 2	23 <u>+</u> 1	48 <u>+</u> 2

<sup>a</sup>± is the standard deviation of three replicates.

## TABLE S5d: Expression of *P*<sub>flache</sub> in Figure S11 (Miller units)<sup>a</sup>

Strain	Miller units
wt	119 <u>+</u> 4
degUhy32 <sup>++</sup>	90 <u>+</u> 19
swrA	31 <u>+</u> 1
degUhy32 <sup>++</sup>	

 $a_{\pm}$  is the standard deviation of three replicates.

### **TABLE S6: Primers**

Number	Primer sequence
353	TTGCGGAATTCTTGTCAATTAAAGGAGATGGTATTTCA
354	TTGCGGGATCCCACGCACAATACTTGCCCTCTTC
1782	CTATATGCTTATTGTAAGAAATAACAGG
1921	AGGAGGAATTCAGGTACTTATATCAAGGTACTAAACAA
3042	CTGCTGAATTCAGCTCTTGTCGTATCCATATTTGCT
5116	AGGAGGAATTCTGCTTATTATTTGTTCGGTTTC
5117	CTCCTGGATCCCAATCGATTTCCACAGATAATAG
5118	AGGAGGAATTCGTGAGAAAGCTCGCGGTAC
5119	CTCCTGGATCCCTTTTTATGCTACCCTTCATATC
5120	AGGAGGAATTCCATAACGCCAAAAGCCAGTCC
5121	CTCCTGGATCCCCAAAAAAGTTTTGGTACTCTC
6064	AGGAGGGATCCGCTGAAGAAAAGTCGGCTTG
6069	AGGAGAAGCTTATCCGCTCAGTCTGCAGTG
6481	AGGAGGAATTCCGGCAAATTGAAATCGGCTGT
6482	AGGAGGGATCCGAAGCAAATCAGACGCTCAC
6483	AGGAGGAATTCGCCTGAGCTAAAAGCCATGC
6484	AGGAGGGATCCCAGAAAAAAAAAAGTAGGTTATCG
6487	AGGAGCAATTGGCTTCGTCATTCTGGGCTTC
6488	AGGAGGGATCCAGGCCGTCAGCTTGCTATG
6489	AGGAGCAATTGGATCTTCGAGAGGATTCAAAG
6490	AGGAGGGATCCAATTGATTGTTGAAATTTGGCCA
7227	AGGGAATTCCACGAACTTCATAGACTTTATG
7228	AGGGGATCCTCAGTTTTTTCACCCTCAATATC
7229	AAAGAATCCCCCCAATGCCT
7230	CGTCTGTTTTCTGACTCATATT
7231	CAAAAAGTTTCAAAAATGCCGAA
7459	GACATCGATTAGAGAAGGCAA
7460	CGTTGCAGTCTTTAAACAATCT
7461	TGATTTGCAGCTTCAAACAGC
7462	ATGCATATTAACTCGTTAGAAAG
7463	TGTGTTCAATTCCGCAAAATAG
7464	ACAGTATTACCTGATGACCTG
7465	GTCAGATCTTTTTGGAGGCTT
7466	GCCTGCGATTATGTAATTTGC
7548	/56-FAM/TTCCTGGGTTGAAAGTCTTTC
7549	/5HEX/ATAAGCTCAAATCCACTTACCT
7550	/56-FAM/TCAGATCACTCATCTTCCTAAT
7551	/5HEX/ACGGATTGTGTTCCAAATTGAT
7554	TGCACTGACGCTTGAAAGAAT
7555	GCTTCCGTGTAATTGATCTTTG
7562	/56-FAM/CTAAATACAATCCGTATTCACC
7563	/5HEX/TCTATTGTACGGATAAAATGCC

7564	/56-FAM/TGATTTGCAGCTTCAAACAGC
7565	/5HEX/ACAATACTTGCCCTCTTCAATT
7566	/56-FAM/TATCGCCGCTTCAGCTGAG
7567	/5HEX/CAATATGACAACCGGTATGATG
7568	/56-FAM/CATTATGAAAGCTGGTGGCG
7569	/5HEX/ATATCCATTGTCACAAGGTCC
7597	TAACCGTGCAGCAGCGTTAT
7598	CCTGAATATGTTGTTAAGGCAC
7817	AGGAGGAATTCAGGAGGATTATTTATCATGGCT
7818	AGGAGGTCGACGCATTAATCACATTCATTCACC
7819	TAGTTCTAACAATCTAGGACTTTTTTCCTAGTTGCAAAATAGATAA
7820	TTATCTATTTTGCAACTAGGAAAAAAGTCCTAGATTGTTAGAACTA
7821	TAGTTCTAACAATCTAGGACAAAAAACCTAGTTGCAAAATAGATAA
7822	TTATCTATTTTGCAACTAGGTTTTTTGTCCTAGATTGTTAGAACTA
7823	AATGTAGTTCTAACAATCTAATACTTTATACCTAGTTGCAAA
7824	TTTGCAACTAGGTATAAAGTATTAGATTGTTAGAACTACATT
7825	TTTGCAACTAGGTATAAAGTATTAGATTGTTAGAACTACATT
7826	AATTATCTATTTTGCAACTAATTATAAAGTCCTAGATTGTTA
7827	AATGTAGTTCTAACAATCTAATACTTTATAATTAGTTGCAAA
7828	TTTGCAACTAATTATAAAGTATTAGATTGTTAGAACTACATT
7869	TAGTTCTAACAATCTAGGACGGGGGGGCCTAGTTGCAAAATAGATAA
7870	TTATCTATTTTGCAACTAGGCCCCCCGTCCTAGATTGTTAGAACTA
7871	TAGTTCTAACAATCTAGGACCCCCCCCCAGTTGCAAAATAGATAA
7872	TTATCTATTTTGCAACTAGGGGGGGGGGGCCCTAGATTGTTAGAACTA
7885	AGGAGGTCGACTGCATTAATCACATTCATTCACC
7886	ATCTAGGACTTTATACCTAGTTTAATTGTGAGGACATTTTTTA
7887	TAAAAAAATGTCCTCACAATTAAACTAGGTATAAAGTCCTAGAT
7888	ATCTAGGACTTTATACCTAGTTTACACGAACTTCATAGACTTTATG
7889	CATAAAGTCTATGAAGTTCGTGTAAACTAGGTATAAAGTCCTAGAT
7979	ATCTAGGACTTTATACCTAGTTGACATTTTTTACACGAACTTC
7980	GAAGTTCGTGTAAAAAAATGTCAACTAGGTATAAAGTCCTAGAT
7981	TTATACCTAGTTTAAGCTCGTAGCAAAATAGATAATTGTGAGGA
7982	TATCTATTTTGCTACGAGCTTAAACTAGGTATAAAGTCCTAGAT
7983	TAAGCTCGTACTTGACTGAAGCAAAATAGATAATTGTGAGGA
7984	TTCAGTCAAGTACGAGCTTAAACTAGGTATAAAGTCCTAGAT
8005	ATCTAGGACTTTATACCTAGTTTACACGAACTTCATAGACTTTATG
8006	CATAAAGTCTATGAAGTTCGTGTAAACTAGGTATAAAGTCCTAGAT
8007	CTCCTGGATCCTCAGTTTTTTTCACCCTCAATATCCT
8008	AGGAGGAATTCCGTTCTGTTATTGTGAACGCA
8205	
8206	
8207	AGGAGGCTAGCACTCTGCTGCACTAAGAACTTCATAGACTTTATGCCTGTTATTTC
8208	CTCCTGGATCCGAAATTGCATTAATCACATTCATTCAC

## **TABLE S7: Plasmids**

Plasmid	Genotype	Reference
pAM01	amyE::P <sub>sac</sub> x-lacZ cat amp	
pAM08	amyE::P <sub>ydaJ</sub> -lacZ cat amp	
pAM09	amyE::P <sub>ywdA</sub> -lacZ cat amp	
pAM11	amyE::P <sub>ytvA</sub> -lacZ cat amp	
pAM12	amyE::P <sub>yweA</sub> -lacZ cat amp	
pAM19	amyE::P <sub>ycdC</sub> -lacZ cat amp	
pAM27	amyE::P <sub>flache(-35-+1)</sub> -lacZ cat amp	
pAM39	$\Omega P_{flache} DegUBS(6T)$ oriBsTs mls amp	
pAM40	$\Omega P_{flache} DegUBS(6A)$ oriBsTs mls amp	
pAM41	ΩP <sub>flache</sub> DegUBS(site1) oriBsTs mls amp	
pAM42	ΩP <sub>flache</sub> DegUBS(site2) oriBsTs mls amp	
pAM43	ΩP <sub>flache</sub> DegUBS(site1+2)oriBsTs mls amp	
pAM48	$\Omega P_{flache} DegUBS(6G)$ oriBsTs mls amp	
pAM49	$\Omega P_{flache} DegUBS(6C)$ oriBsTs mIs amp	
pAM56	$\Omega P_{flache} DegUBS(+10)$ oriBsTs mls amp	
pAM67	$\Omega P_{flache} DegUBS(+20)$ oriBsTs mls amp	
pAM68	$\Omega P_{flache} DegUBS(-10)$ oriBsTs mls amp	
pAM69	$\Omega P_{flache} DegUBS(-20)$ oriBsTs mls amp	
pAM72	amyE:: P <sub>flache</sub> DegUBS(site1+2)-lacZ cat amp	
pAM77	amyE:: P <sub>flache</sub> DegUBS(+20)-lacZ cat amp	
pDG268	amyE::lacZ cat amp	(18)
pDP139	amyE::Pflache-lacZ cat amp	(14)
pDP144	amyE::P <sub>swrA</sub> -lacZ cat amp	
pDP145	amyE::P <sub>flgM</sub> -lacZ cat amp	(14)
pDP155	amyE::P <sub>hag</sub> -GFP cat amp	(14)
pDP462	amyE::P <sub>ynel</sub> -lacZ cat amp	
pDP463	amyE::P <sub>mcpA</sub> -lacZ cat amp	
pDP464	amyE::P <sub>yxjJ</sub> -lacZ cat amp	
pDP521	amyE::P <sub>sinl</sub> -lacZ cat amp	
pDP522	amyE::P <sub>tlpA</sub> -lacZ cat amp	
pDP616	ΩP <sub>flache</sub> RANDO-UAS oriBsTs mls amp	
pDP617	amyE:: PflacheRANDO-UAS-lacZ cat amp	
pEC20	amyE::P <sub>hyspank</sub> -degQ spec amp	(15)
pminiMAD	oriBsTs mls amp	(19)
pNW43	pT7 DegU-His6 amp	(16)
pSM94	pT7-GST-SwrA amp	(17)
pYH8	pT7 DegS-His6 kan	(4)

#### SUPPLEMENTAL REFERENCES

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#### SUPPLEMENTAL FIGURE LEGENDS

**Figure legend S1: SwrA does not bind to DNA directly.** Electrophoretic mobility shift assays (EMSA) with the indicated radiolabeled promoter fragments (classes in parentheses) and  $\mu$ M of protein, either GST-SwrA (left), unphosphorylated DegU-His<sub>6</sub> (center) or DegU-P-His<sub>6</sub> phosphorylated by DegS-His<sub>6</sub> and ATP (right). Ø indicates that no protein was added.

**Figure legend S2:** SwrA and DegU are mutually required for swarming. Quantitative swarm expansion assays of the following strains: gray circles, WT (3610), open circles, *swrA* (DS2415), closed circles, *swrA*<sup>+++</sup> induced with 1 mM IPTG (DS8094), open squares, *degU* (DS3649), closed squares, *degU*<sup>+++</sup> induced with 1 mM IPTG (DS3534), open triangles, *swrA degU*<sup>+++</sup> induced with 1 mM IPTG (DS8111), closed triangles, *degU swrA*<sup>+++</sup> induced with 1 mM IPTG (DS8259) and closed diamonds, *degU swrA* (DS6262). Each data point is the average of three replicates. Wildtype was included as a control while gathering the data displayed.

**Figure legend S3: Enlarged peaks from ChIP-seq experiments.** Each panel indicates a 4 kb window corresponding to the promoter region of the likely gene indicated in the upper left. The class of binding from table 1 is indicated as I, II, or III in upper left parenthetical. Green trace, SwrA ChIP-seq from wild type cells; blue trace, DegU ChIP-seq from wild type cells; magenta trace, DegU ChIP-seq from *swrA* mutant cells. X-axis is genome position in kb.

**Figure legend S4: MEME analysis of DegU binding sites.** 200bp sequence surrounding ChIP-seq peaks were grouped as indicated in the upper left and run through the MEME suite to predict a putative inverted repeat to which SwrA and/or DegU might bind. Note that MEME analysis of the class II sites looks particularly strong but relatively few sites are in this class. Sequences used to generate this figure are presented in **Table S2**.

#### Figure legend S5: DegU ChIP sequencing in strains overexpressing DegQ and DegU<sup>hy32</sup>.

A) ChIP-Seq analysis using a primary antibody to DegU ( $\alpha$ DegU) in wild type (top) and swrA mutant (bottom) in which DegQ had been artificially overexpressed by growth in the presence of 1 mM IPTG. The pgsB gene necessary for poly-glutamate production was also mutated due to the extreme mucoidy resulting from DegQ overexpression. Data is presented as number of sequences reads per kilobase (kb) pair position in the chromosome such that SwrA-enriched sequences appear as peaks. The following strains were used to generate this panel:  $degQ^{+++}$ (DK9504) and  $\Delta swrA degQ^{+++}$  (DK9525). B) ChIP-Seg analysis using a primary antibody to DegU ( $\alpha$ DegU) in  $\Delta$ degU (top) and  $\Delta$ degU swrA mutant (bottom) in which the hyperactive DegU<sup>hy32</sup> allele had been artificially overexpressed by growth in the presence of 1 mM IPTG. The pgsB gene necessary for poly-glutamate production was also mutated due to the extreme mucoidy resulting from DegU<sup>hy32</sup> overexpression. Data is presented as number of sequence reads per kilobase (kb) pair position in the chromosome such that SwrA-enriched sequences appear as peaks. The following strains were used to generate this panel:  $\Delta degU$ degUhy32<sup>+++</sup> (DK9523), ΔswrA ΔdegU degUhy32<sup>+++</sup>(DK9524). C) Each panel indicates a 4 kb window corresponding to the promoter region of the likely gene indicated in the upper left. Blue trace, DegU ChIP-seq from wild type cells in which DegQ was overexpressed; red trace, DegU ChIP-seq from *swrA* mutant cells in which DegQ was overexpressed. D) Each panel indicates a 4 kb window corresponding to the promoter region of the likely gene indicated in the upper left. Purple trace, DegU ChIP-seg from *degU* mutant cells in which DegU<sup>hy32</sup> was artificially overexpressed; red trace, *deqU swrA* mutant cells in which DeqU<sup>hy32</sup> was artificially overexpressed.

Figure legend S6: DegU-P protects DNA in additional target promoter regions only the presence of SwrA. DNasel sequencing footprint analysis of the A)  $P_{figM}$  B)  $P_{mcpA}$  C)  $P_{swrA}$  and D)  $P_{vnel}$ . 300-400 bp fragments of dsDNA were fluorescently labeled on the forward strand, the

indicated protein was added, followed by partial digestion with DNase I and sequencing of the digested fragments. Top panel, 1 uM BSA added; middle panel 3  $\mu$ M of DegU-P-His<sub>6</sub> added; bottom panel 3  $\mu$ M of DegU-P-His<sub>6</sub> and 1 $\mu$  M of GST-SwrA added. Orange bars indicate the region of protection in the presence of SwrA/DegU-P. Peaks indicate the number of sequences reads terminating at that location on one strand.

Figure legend S7: Sequence alignment of sequence immediately upstream of that shown in Figure 5C. This alignment is included to show that sequence conservation upstream of the  $P_{flache}$  promoter breaks down farther upstream of the DegU binding site, UP element and promoter boxes.

Figure legend S8. Neither SwrA nor DegU act like repressors in a strain in which the DegU binding site was moved 20 base pairs closer to the  $P_{flache}$  promoter. Fluorescence micrographs of strains in which the DegU binding site was moved 20 base pairs closer to the  $P_{flache}$  promoter in strains mutated for *swrA* (top, DB613) and *degU* (bottom, DB795). Membrane, false colored red;  $P_{hag}$ -GFP expression, false colored green. Scale bar is 8 µm. Note, no  $P_{hag}$ -GFP expression was detected above background in either strain.

Figure legend S9. Suppressors of  $P_{flache}$  site1+2 and  $P_{flache}$  UAS' mutant restore swarming motility. Quantitative swarm expansion assays of the following strains: A) gray circles, suppressors of  $P_{flache}$  site1+2 mutant and B) black circles, suppressors of  $P_{flache}$  (UAS') mutant. Each data point is the average of three replicates. Wildtype,  $P_{flache}$  site1+2 and  $P_{flache}$  UAS' were included as controls while gathering the data displayed. Details of strains used to generate this figure are mentioned in Table1.

Figure legend S10. Predicted aligned error matrices of DegU-DegU and DegU-DegU-SwrA-SwrA complexes.

**Figure legend S11. DegUhy32 does represses motility downstream of** *P*<sub>flache</sub>. A) βgalactosidase activity of *P*<sub>flache</sub> fused to the *lacZ* gene. *degUhy32* was artificially overexpressed by growth in the presence of 1 mM IPTG. Error bars are the standard deviation of three replicates. The following strains were used to generate this panel: *wildtype* (DB643), *degUhy32*<sup>+++</sup>(DB1159) and *swrA degUhy32*<sup>+++</sup> (DB1160). Average miller units are presented in **Table S5d.** B) Fluorescence micrographs of wildtype (DB456), *degUhy32*<sup>+++</sup>(DB1161) and *swrA degUhy32*<sup>+++</sup> (DB1162). *degUhy32* was artificially overexpressed by growth in the presence of 1 mM IPTG. Membrane, false colored red; *P*<sub>hag</sub>-GFP expression, false colored green. Scale bar is 8μm. Note, no *P*<sub>hag</sub>-GFP expression was detected above background in *degUhy32*<sup>+++</sup> and *swrA degUhy32*<sup>+++</sup>.

**Figure legend S12. EMSAs for the densitometry analysis in Figure 4.** Electrophoretic mobility shift assays (EMSA) with the indicated radiolabeled promoter fragment and protein. Ø indicates that no protein was added. Left panels, an increasing about of SwrA was added to a constant 0.3 µM of DegU. Right panels, an increasing amount of SwrA was added to a constant 0.3µM of DegU-P phosphorylated by DegS and ATP. Gray carets indicate the position of DNA shifted by the presence of either DegU or DegU-P alone. Black carets indicated the position of DNA supershifted by the presence of either DegU or DegU-P and SwrA. \* Indicates aggregation.

	<u>SwrA (μM)</u>	DegU (μM)	<u>DegU-P (μM)</u>
	Ø0000 Ø0	0.000	Ø0.00 Ø
$P_{_{\it flache}}({\sf II})$	******	è <sup>ii</sup>	
$P_{_{yxjJ}}(I)$			
$P_{_{flgM}}(I)$			
$P_{_{mcpA}}(II)$			
$P_{swrA}(I)$			
$P_{_{ynel}}(I)$			
$P_{\tiny comK}(III)$	1	k	**
$P_{_{hag}}$			











		SigD		
-250	-35 box	<b>(</b>	-10 box	-121
subtilis GAAAGTCTTTCTATGTAATAATTTTAATAAATTTTGCATTTT	I CTTC <mark>A</mark> AA.	AAGTTTCAAAAAA	GCCGAAAA	GAAAGGAGAAAAAAACAGAAATTCTGCTATTTTCAGGCTTATATCAAGGC
altitudinis TTAAAATCTCACTAATCAAAAAACCCTTTTCGGCCGAAATAA	CGCTGAA	AAGGGTTTTTTTC	TATAAAAA	AAGAGGATTTGTCCGAAAAATGACACTCTTTTTTCCAAGAAACCCCCTTA
amyloliquefaciens TAGGTGAAAAGTTGCAGTTTAAAGTTGCTTATTGGAATCTGA	CTGAGAT,	AATCCATCTTGC1	GTGT <mark>G</mark> AAA	AAAGGAGGAAGAAAAAATTCTGCTATTTTCAGTCTTATATTAAAAAA
atropheus TAAAGTTGATTTTTCCTTT <u>TT</u> TATCTGAGA <u>T</u> AATCCATCTCG	GTTTGAAT	TTTGTGAAAAGC	CCGAAAAC	ATAAGGAGAGATCACTGAAAAAGCATCTATTTTCTGGCTTATATCAGAGG
<i>cabrialesii</i> GA <mark>AA</mark> GTCT <mark>TTC</mark> TTT <mark>GTAA</mark> TAA <mark>TTTTAATAA</mark> ACTTATGTTTT	CTTCAAA	AAGACTCAAAAAT	GACGAAAA	IAATAAAGAAAAAAAAAAAATTCTGCTATTTTCAGGCTTATATCAAAGC
glycenifermentans TTGTCAACCTTTATAAAATTATATTATTGTAATTTTTGAAAC	CATAAACT	CAATATTATCAG7	CTTGTGAC	CTCGCTAAAAAATATTCTGATATATTTTTTAGTGAGCTCCATTTCATTT
haloterans AAAAGTCTTTC <mark>TTT</mark> GTAATAA <mark>TTTTAATAAA</mark> GTTTATTATTT	CTTCATA	AAAACAGAAAAAA	GACGAAAT	AAATGGGGAAAATACAGAAATTCAGCTATTTTCAGCCTTATATCAATGT
haynesii TAAAATCTIGTCGAGAATIGTAAAAACCATAAGCCTATIGTA	A GGAAT AA'	TAGGCTTAAAGTC	TATGATCI	3GAAGCAAAAAAAAACTCGTAAGAAATATCTATTTTCCGAATAGGTCTTT
<i>inaquosorum</i> GAAAGTCTTTCTTTGTAATAATTTTAATAAAATTTACATTTT	'I CTTCA AA'	TAGACATAAAAAI	GACGAAAA	FAATAAAGAAAAAAAAAAAAATTCTGCTATTTTCAGGCTTATATCAAAGC
intestinalis GAAAGTCTITCTTTGTAATAATTTTAATAAATCTCATAAATG	J CTTTAAA.	AAGGCTCAAAAAI	GACGAAAA	SATTATAGAAAAAAAAAAAATTCTGCTATTTTCAGGCTTATATTAAGGA
licheniformis TTTAAACTATATTATCGTAATTTTTCATATCAAAAAACAAC	AAAACGC	GGTGCAAAATCAF	TGCGTTAC	ITGAATAAAAGATATACTAACATAATTTTTTGAAACTTTAATGAACGTA
<i>mojavensis</i> AAAAGTCTITCICIGTAATATGTTAATAAAATTGATGATTTT	CTTCATAA	AAACAGA <u>AA</u> AAAG	ACGAT <u>AA</u> Z	IGAATGGGGAAATACAGAAATTAAGCTATTTTCAGCCTTATATTAATGT
nakamurai ATGGGTGAAAAGA GCAG TTAAAGTTGATTAATGAC GGCG	;TCTGBGB'	TATCCATTTTGC	TGTGTGG	AGAGAGAGAAGTTACAGAAATTCCG <mark>CTA</mark> TTTTCAGGCTTATATCAAACA
paralicheniformis TTTTAAACTATATTATCGTAATTTTTTCATATCAAAAACAAC	AAAATGC	GGTGCTG <u>AA</u> TCAF	TGCGTTGC	ITGAATAAAAAATATACTAACATAATGTTTTGAAACTTTAACAAACGTA
pumilus ATTTAAAATCTCATTAATCAAAAACCCTTTTCAGCAGAAATA	AAGCTGA	AAAGGGTTTTTTT	TTATAAAA	AAAGAGGATTTGTCCGAAAAATGACACTCTTTTTTCCAAGAAAACCCTT
rugosus GARAGTCTTTCTTTGTAATAATTTTTAATAAMTCTCGCAAATT	TCTTGAAA.	AAGGCTTAAAAAT	GACGAAAA	GAATATAGAAAAAACAGAAATTCTGCTATTTTCAGGCTTATATTAAGGC
safensis TTAAAATCICAUUAATCAAAAACCCTTTTTGGCCGAAMTAAA	J GCTGAAA	AGGGTTTTTTTAT	GTGAAAAA	ATGAGGATTTGACCGAAAAAIIGTCACIICTTTIITTCCAAGAAACCCCTTA
siamensis TAGGTGAAAAGTIGGAGTITAAAGTTGATTATTGGAATCTGA	I CTGAG T	AATCCATCTTGCT	GTGTGAG	AAAAGGGGAAGATACAGAAATTCAGCTATTTTCAGTCTTATATTAACCA
sonorensis TTA ATTC TG CGAGAA TGTAAAAACCATAAGCCTATTGT	'AAGAAATA	ATAGGCTTAAAGT	CTATGATC	TGGGAGCAAAAAAAAATCGTAATATATATATCTATTTTTACGAATAGGTATT
spizizenii GAAAGTCTITCTIIGTAATAATTTTTAATAAATCTCATAAATG	JI CTTTAAA.	AAGGCTCAAAAA	GACGAAAA	JATTATAGAAAAAAAAAAAATTCTGCTATTTTCAGGCTTATATTAAGGA
stercoris GAAAGICTITCIG GTAA GATITTAATAAAICTTIGCATATT	T CTTCAAA.	AAGTTTCAAAAA	GACG <u>AAA</u> A	GAAAGGAGAAAAAACACAAATTCTGCTATTTTCAGGCTTATATCAAGGC
tequilensis CTA GCTT TATT CCTGGGTTAAAAGTCTTTCCTTGT ATAA	J TTTAATA	AAGTTTGAATTTC	ACTTCAAA	
vallismortis GANGTET TETT GTEATATTTTTAATAATETAACATTE	CATAAAA	ATAGCTTA AAAAA	GGTGAGAA	
	CTGAGAT	A SCOTTOCATCTTGCT	GIGIGAAA	
znangznouensis TTTTAATCICATTAATCAAAAACCCTTTTTTGGCCGAATTAAA	I GUTGAAA	AGGGTTTTTTTTA	GTGAAAAA AABCAAAAA	
xiamenesis TITEAAATUTUAMMAATUAAAAUUUTTITTAGUUGAMATAA	MIGUIG AVAV	MANAGGGTTTTTTTT	AATGAAAA	







Predicted aligned error matrices





