Supplemental Information

Table S1. Strains and plasmids used in this study

***C. difficile* strains**	**Phenotype**	**Reference**
UK1	Ribotype 027	(1-3)
5108111	Ribotype 027	(4)
DH1834	Ribotype 027	(4)
DH1858	Ribotype 027	(4)
CD2315	Ribotype 078	(4)
M120	Ribotype 078	(5, 6)
M68	Ribotype 017	(7, 8)
JSC11	*cspC::ermB*	(1, 3)
CAA5	*sleC::ermB*	(3)
E. coli strains		
DH5α	F$^-$ endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG Φ80d*lacZ*ΔM15 Δ(*lacZYA-argF*)U169, hsdR17(r_K^- m_K^+), λ–	(9)
BL21(DE3)	F$^-$ ompT gal dcm lon hsdS$_B$(r_B^- m_B^-) λ(DE3 [lacI lacUV5-T7 gene 1 ind1 sam7 nin5])	Novagen
Plasmids		This study
pET22b	Expression vector	(Novagen)
pKS02	*cspB*-expressing plasmid	This study
pKS08	*sleC*-expressing plasmid	This study
pEK-K-CspC	Codon optimized *cspC*	This study
p*cspC-CO-Δ50CPD*	Codon optimized *cspC*-expressing plasmid	A. Shen (gift)

Figure S1. Complete germination data for *C. difficile* strains. Purified *C. difficile* spores of the indicated strains were suspended in BHIS medium alone (●) or medium supplemented with 2 mM (■), 5 mM (▲), 10 mM (▼), 20 mM (♦) or 50 mM (○) TA. Germination was monitored at

OD_{600} as described previously. Experiments were performed in triplicate and each experiment is shown. Data could not be displayed as the average due to inconsistent time points generated by the spectrophotometer between experiments.

Figure S2. Germination by certain *C. difficile* strains at lower TA concentrations. Purified *C. difficile* spores were suspended in BHIS medium alone or medium supplemented with the indicated TA concentrations. *C. difficile* 5108111, *C. difficile* CD2315, *C. difficile* DH1834, or *C. difficile* DH1858 spores suspended in BHIS medium alone (●) or medium supplemented with 1 mM (■), 2 mM (▲), 5 mM (▼), 10 mM (♦) or 20 mM (○) TA. Experiments were performed in triplicate and each experiment is shown. Data could not be displayed as the average due to inconsistent time points between experiments which is generated by the spectrophotometer.

Figure S3. Complete germination data for *C. difficile* spores suspended in medium supplemented with TA and CDCA. Purified *C. difficile* spores of the indicated strains were suspended in BHIS medium supplemented with 1 mM CDCA (●) or medium supplemented with 2 mM (■), 5 mM (▲), 10 mM (▼), 20 mM (♦) or 50 mM (○) TA and 1 mM CDCA. Germination was monitored at OD_{600} as described previously. Experiments were performed in triplicate and each experiment is shown. Data could not be displayed as the average due to inconsistent time points generated by the spectrophotometer between experiments.

Figure S4. Alignment of CspC protein sequences. The *cspC* gene was amplified from each of the *C. difficile* strains used in this study and sequenced. The translated sequence was aligned using the Clustal W method and only the sites where substitutions occurred are listed.

Figure S5. Alignment of CspB protein sequences. The *cspB* gene was amplified from each of the *C. difficile* strains used in this study and sequenced. The translated sequence was aligned using the Clustal W method and only the sites where substitutions occurred are listed.

Figure S6. Alignment of CspA protein sequences. The *cspA* gene was amplified from each of the *C. difficile* strains used in this study and sequenced. The translated sequence was aligned using the Clustal W method and only the sites where substitutions occurred are listed.

Figure S7. *C. difficile* spores require TA to initiate germination. Purified *C. difficile* spores were suspended in buffer supplemented with 100 mM glycine (●) or glycine and 10 mM taurocholic acid (○) and DPA release during spore germination was monitored using Tb^{3+} fluorescence, described previously, for 4 hours. (A) *C. difficile* UK1, (B) *C. difficile* 5108111, (C) *C. difficile* CD2315, (D) *C. difficile* DH1834, (E) *C. difficile* DH1858, (F) *C. difficile* M68, (G) *C. difficile* M120. Data points represent the average of 3 independent experiments and error bars represent the standard error of the mean.

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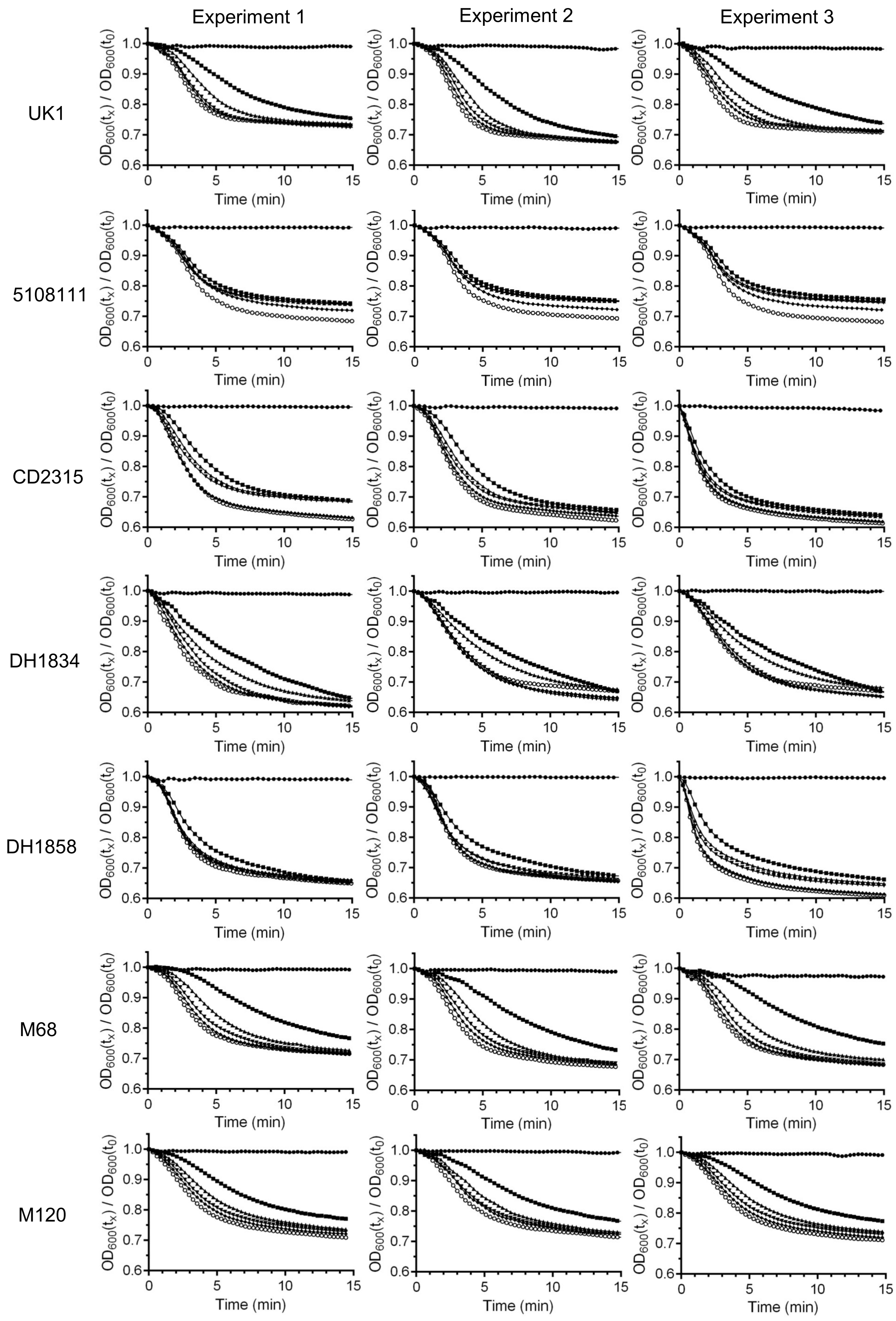
Experiment 1
Experiment 2
Experiment 3
UK1
5108111
CD2315
DH1834
DH1858
M68
M120
OD600(tx) / OD600(t0)
1.0
0.9
0.8
0.7
0.6
Time (min)
0
5
10
15

Figure S1

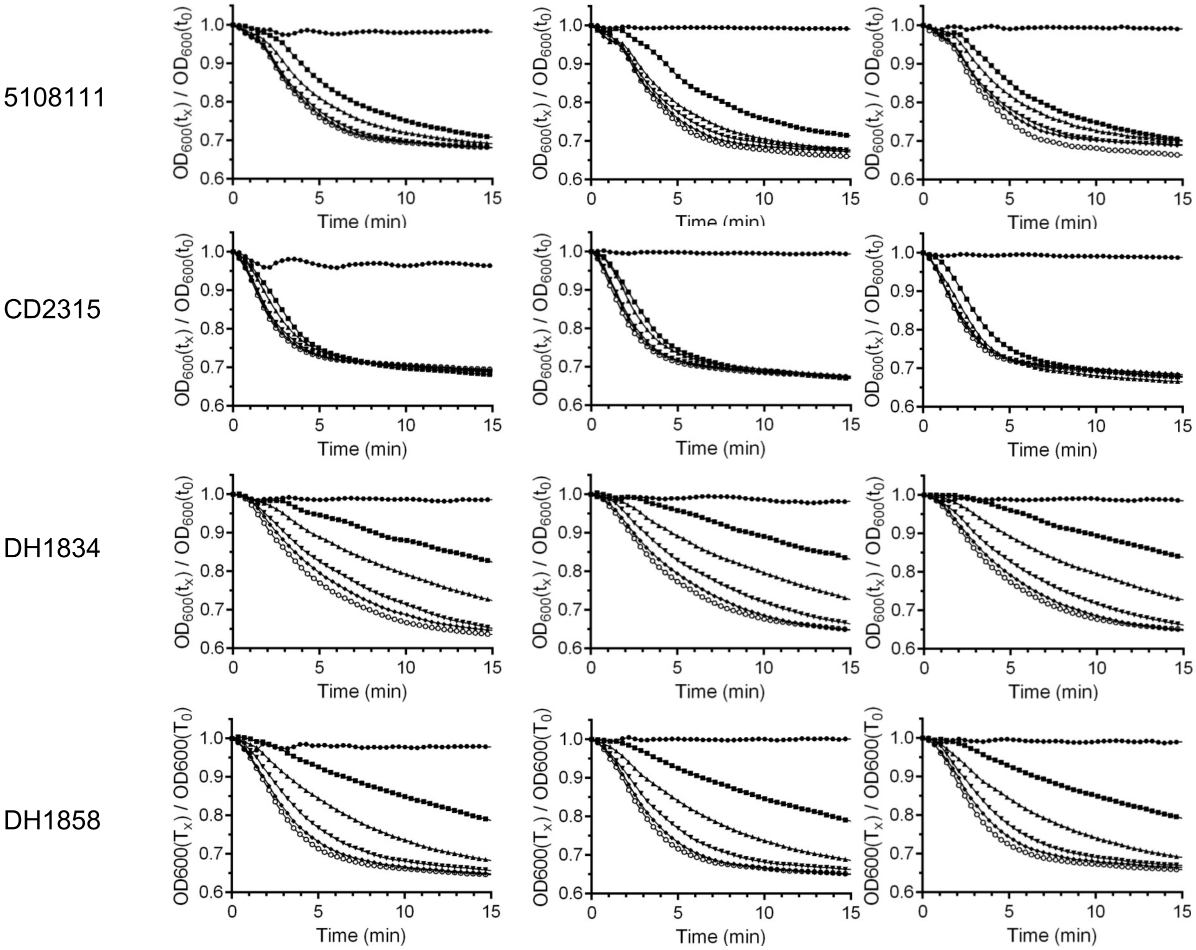
5108111
CD2315
DH1834
DH1858
$OD_{600}(t_x)$ / $OD_{600}(t_0)$
OD600(T_x) / OD600(T_0)
1.0
0.9
0.8
0.7
0.6
0
5
10
15
Time (min)

Figure S2

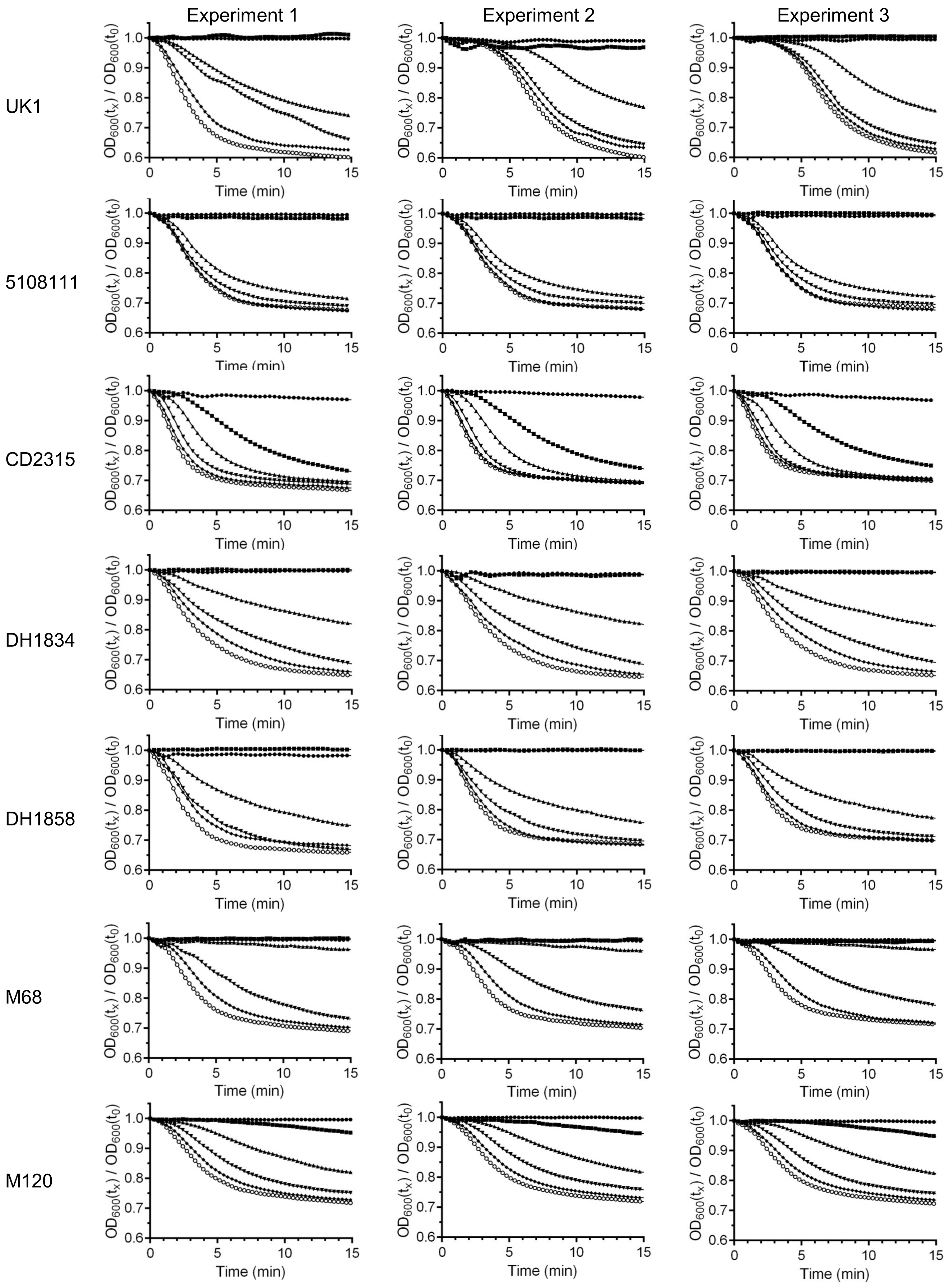
Experiment 1
Experiment 2
Experiment 3
UK1
5108111
CD2315
DH1834
DH1858
M68
M120
$OD_{600}(t_x) / OD_{600}(t_0)$
1.0
0.9
0.8
0.7
0.6
0
5
10
15
Time (min)

Figure S3

	1	12	24	39	59	72	82	84	121	151	184	187	206
UK1	M	D	R	V	E	N	E	D	T	Q	I	Q	T
5108111	M	D	R	V	E	N	E	D	T	Q	I	Q	T
DH1834	M	D	R	V	E	N	E	D	T	Q	I	Q	T
DH1858	M	D	R	V	E	N	E	D	T	Q	I	Q	T
CD2315	M	D	R	I	A	D	G	E	T	E	V	K	I
M120	M	D	R	I	A	D	G	E	T	E	V	K	I
M68	M	N	K	I	E	N	E	D	S	E	V	Q	T

	207	237	250	287	290	317	320	338	384	394	465	477	506	508
UK1	D	K	S	G	S	P	V	S	I	S	S	T	D	V
5108111	D	K	S	A	S	P	V	S	I	S	S	T	D	V
DH1834	D	K	S	G	S	P	V	S	I	S	S	T	D	V
DH1858	D	K	S	G	S	P	V	S	I	S	S	T	D	V
CD2315	G	R	G	G	S	L	V	A	T	G	P	T	N	I
M120	G	R	G	G	S	P	V	A	T	G	P	T	N	I
M68	D	K	G	G	N	P	I	S	I	G	S	A	D	V

Figure S4

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        1   62  110 211 267 287 311 360 362 367
UK1     M...I...D...R...Q...I...A...K...V...D
5108111 M...I...D...R...Q...I...A...K...V...D
DH1834  M...I...D...R...Q...I...A...K...V...D
DH1858  M...I...D...R...Q...I...A...K...V...D
CD2315  M...V...N...K...R...V...T...R...I...N
M120    M...V...N...K...R...V...T...R...I...N
M68     M...I...D...R...Q...I...A...K...V...D

       408 446 499 523
UK1     V...I...G...I
5108111 V...I...G...I
DH1834  V...I...G...I
DH1858  V...I...G...I
CD2315  I...V...V...V
M120    I...V...V...V
M68     I...V...G...I
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Figure S5

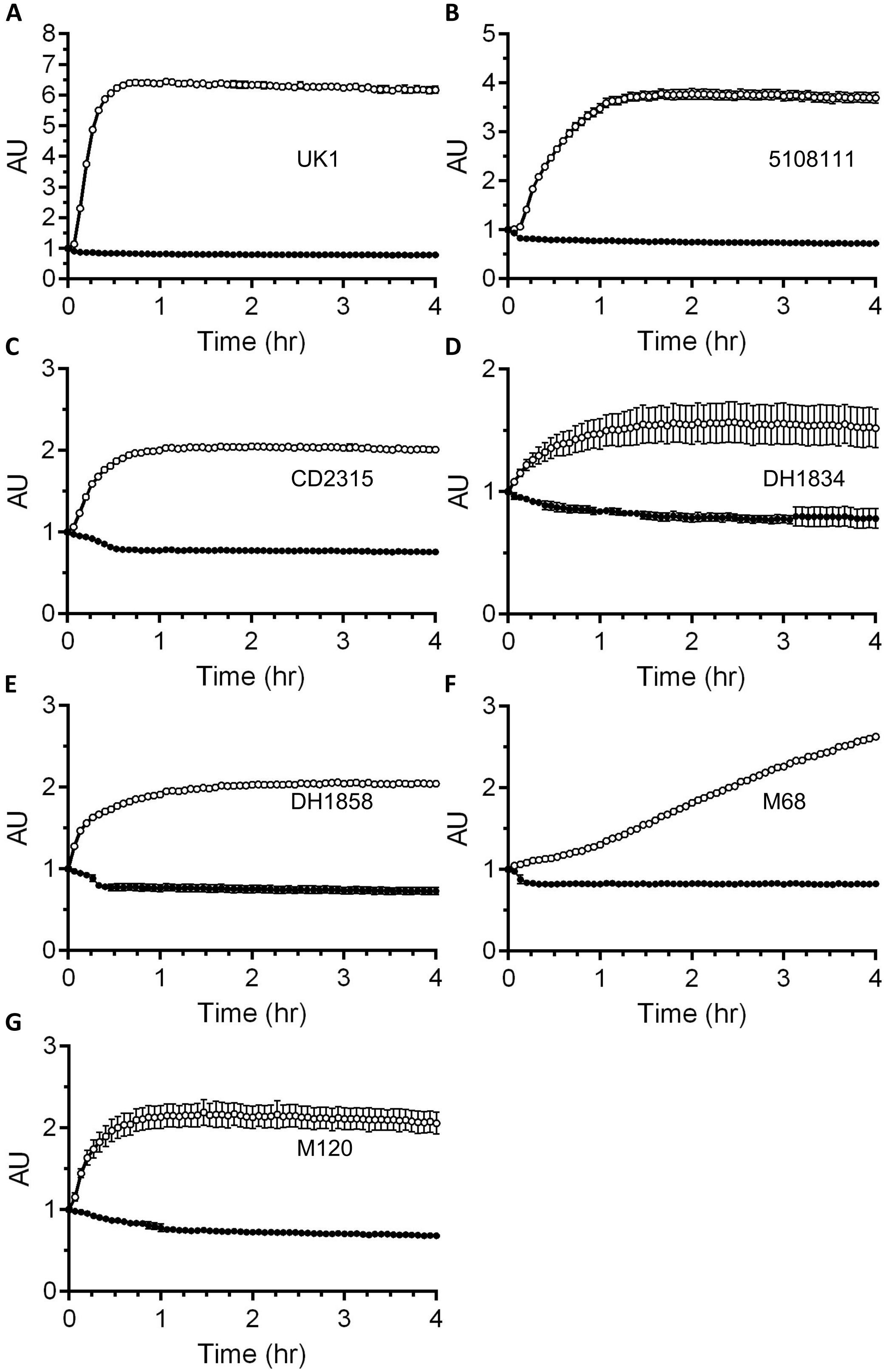
	550	553	555	557	558	564	569	572	574	580
UK1	K	E	R	K	F	I	D	N	I	A
5108111	K	E	R	K	F	I	D	N	I	A
DH1834	K	E	R	K	F	I	D	N	I	A
DH1858	K	E	R	K	F	I	D	N	I	A
CD2315	M	A	N	I	P	V	G	K	K	I
M120	M	A	N	I	P	V	G	K	K	I
M68	K	E	S	K	F	M	D	N	I	A

	607	608	612	622	624	629	633	644	645	669
UK1	M	S	F	S	N	S	R	V	S	M
5108111	M	S	F	S	N	S	R	V	S	M
DH1834	M	S	F	S	N	S	R	V	S	M
DH1858	M	S	F	S	N	S	R	V	S	M
CD2315	Q	S	L	S	D	N	Q	I	P	I
M120	Q	S	L	S	D	N	Q	I	P	I
M68	M	G	F	G	N	N	R	V	S	M

	701	723	745	766	778	786	793	810	816	823
UK1	P	T	E	S	I	I	D	K	R	M
5108111	P	T	E	S	I	I	D	K	R	M
DH1834	P	T	E	S	I	I	D	K	R	M
DH1858	P	T	E	S	I	I	D	K	R	M
CD2315	R	N	A	A	V	V	E	N	G	V
M120	R	N	A	A	V	V	E	N	G	V
M68	P	T	E	S	I	I	D	K	G	M

	837	850	869	870	884	895	914	917	984	1013	1021	1035
UK1	S	I	P	H	D	N	S	I	E	V	G	D
5108111	S	I	P	H	D	N	S	I	E	V	G	D
DH1834	S	I	P	H	D	N	S	I	E	V	G	D
DH1858	S	I	P	H	D	N	S	I	E	V	G	D
CD2315	V	V	S	H	E	S	T	V	K	I	S	N
M120	V	V	S	H	E	S	T	V	K	I	S	N
M68	S	I	P	Y	E	N	S	V	E	I	G	D

Figure S6


A
AU
8
7
6
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4
3
2
1
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UK1
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1
2
3
4
Time (hr)
B
AU
5
4
3
2
1
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5108111
0
1
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Time (hr)
C
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1
0
CD2315
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Time (hr)
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