Figure S1 (Wu *et al.*)

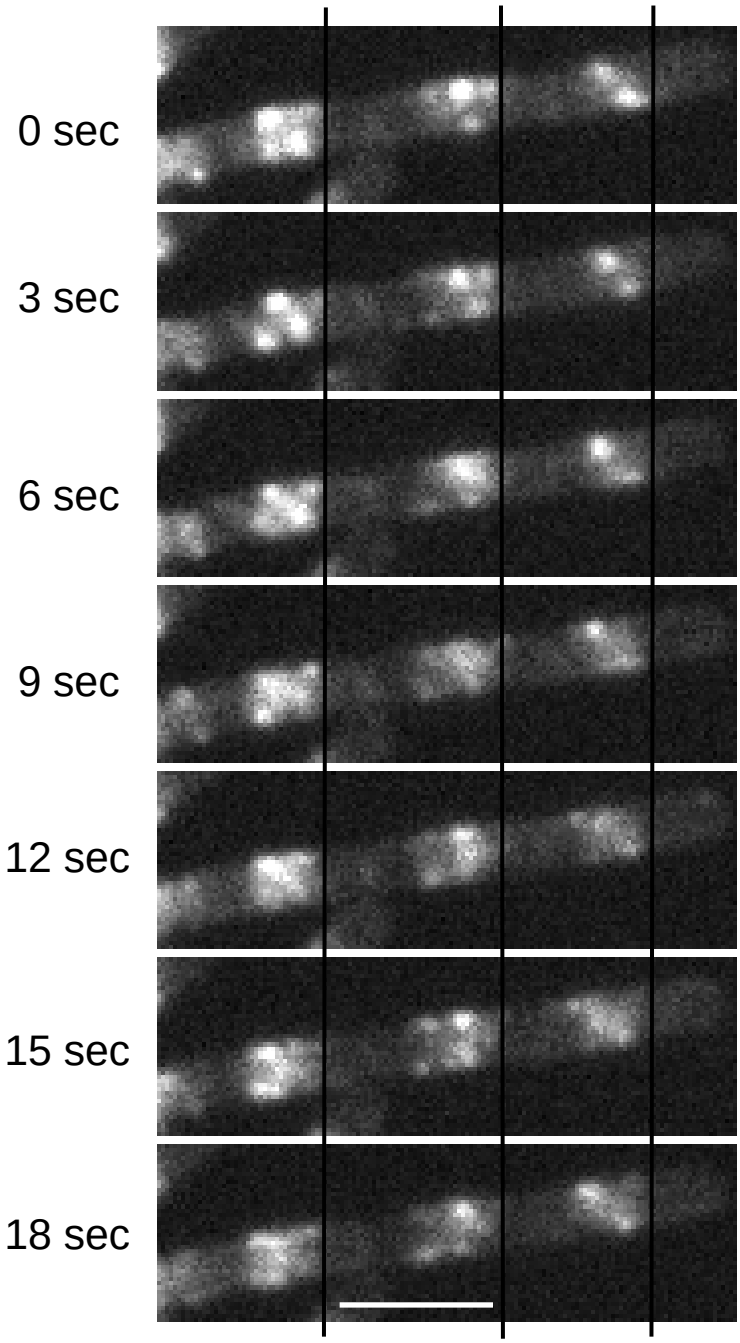
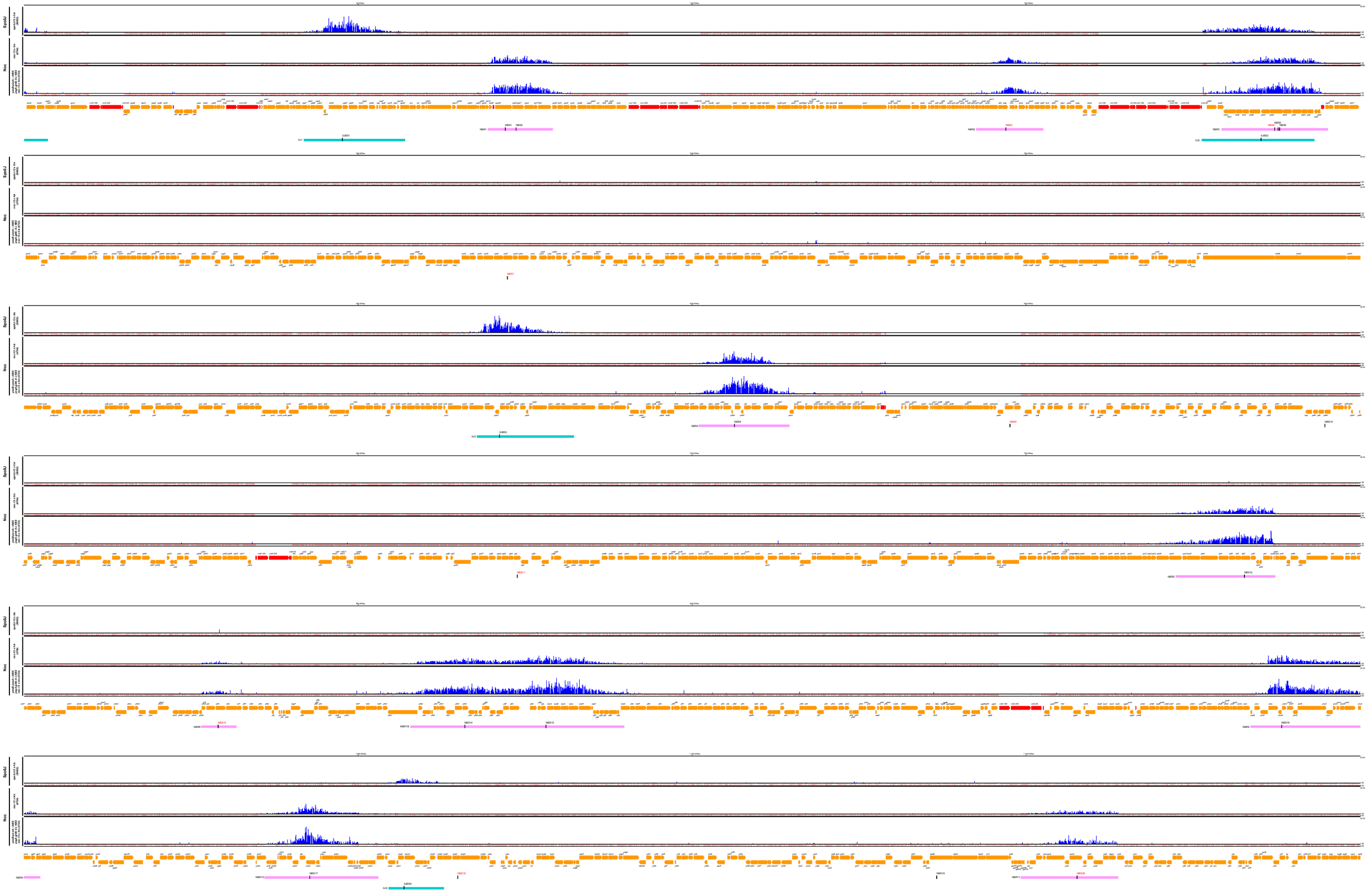
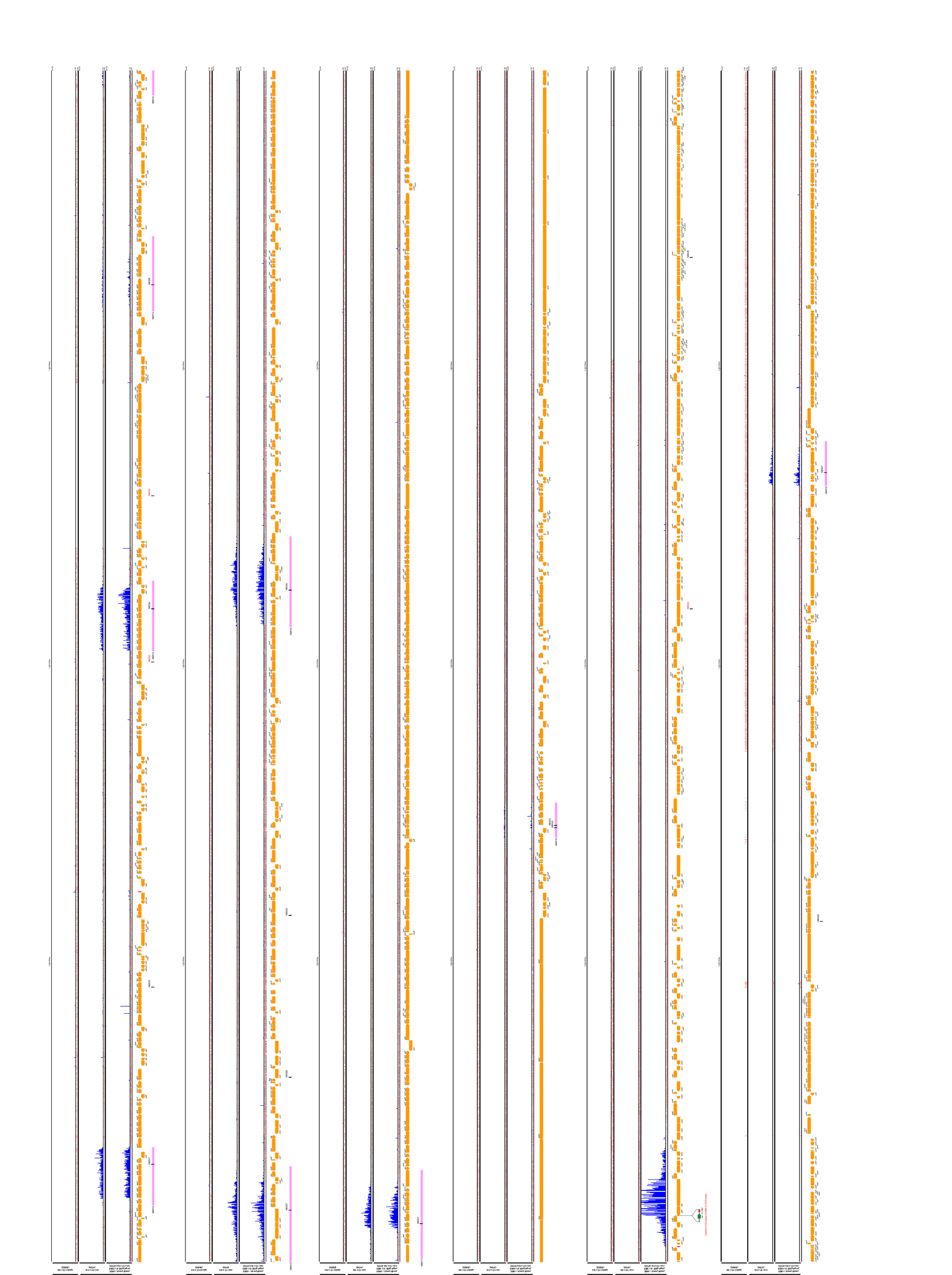
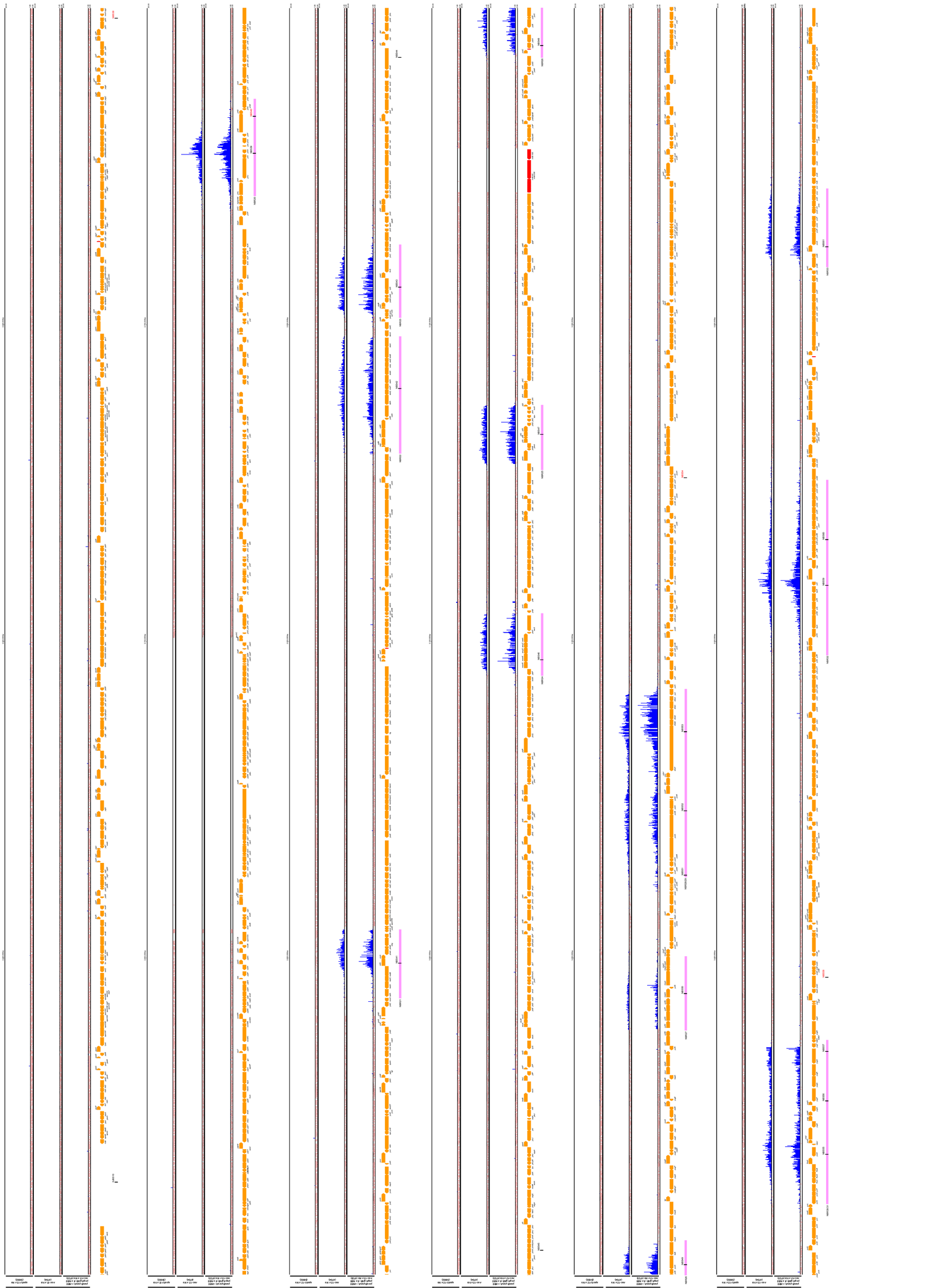


Fig S2 (Wu *et al.)*







SpoIJ
Noc
Deletion of NBS in 4723 strain

Fig S3 (Wu *et al.*)

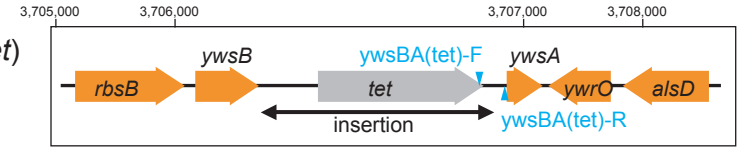
(A)

	Strain	4723	4724	4727	4728	4729	
Chromosome modifications	*ywsB-ywsA*	ΔNBS	ΔNBS	*tet*	*tet*	ΔNBS	
	yogA-gltB	8xNBS	–	*kan*	–	–	
	ymfC-ymfD	–	8xNBS	–	*kan*	–	
	spoVFA	–	–	–	–	2xNBS	
	dilution	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3	
ywsBA(tet)-F & -R	ChAP						◄(a)
	Sup						◄(a) (b)
		yogA(kan)gltB-R	ymfC(kan)D-R	yogA(kan)gltB-R	ymfC(kan)D-R	yogA(kan)gltB-R	
kan-F &	ChAP						◄(c) (e) ◄(d) (f)
	Sup						◄(c) (e) ◄(d) (f)
spoVFB-F & -R	ChAP						◄(g)
	Sup						◄(g)
ydbO-F & -R	ChAP						◄(h)
	Sup						◄(h)
Primers	template						

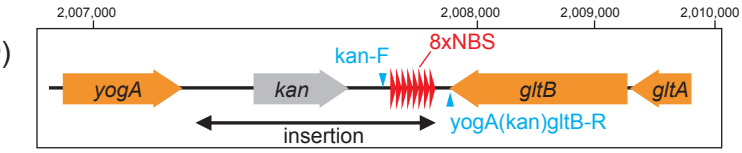
(B)

(a) *ywsB-ywsA::tet*

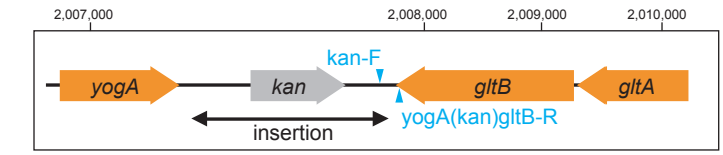
(b) *ywsB-ywsA::*ΔNBS(*tet*)



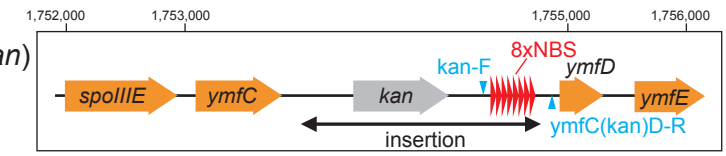
(c) *yogA-gltB::*8xNBS(*kan*)



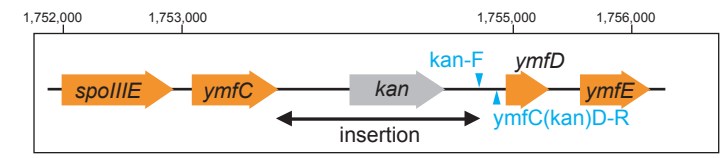
(d) *yogA-gltB::kan*



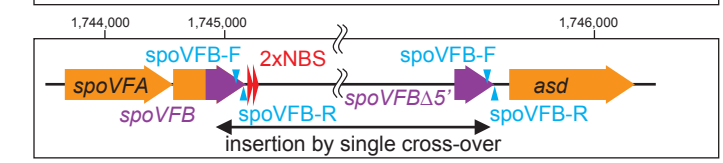
(e) *ymfC-ymfD::*8xNBS(*kan*)



(f) *ymfC-ymfD::kan*



(g) *spoVFA::*2xNBS



(h) *ydbO*

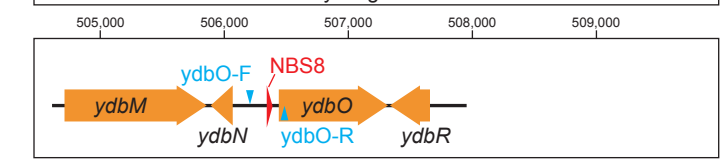


Fig S4 (Wu *et al.*)

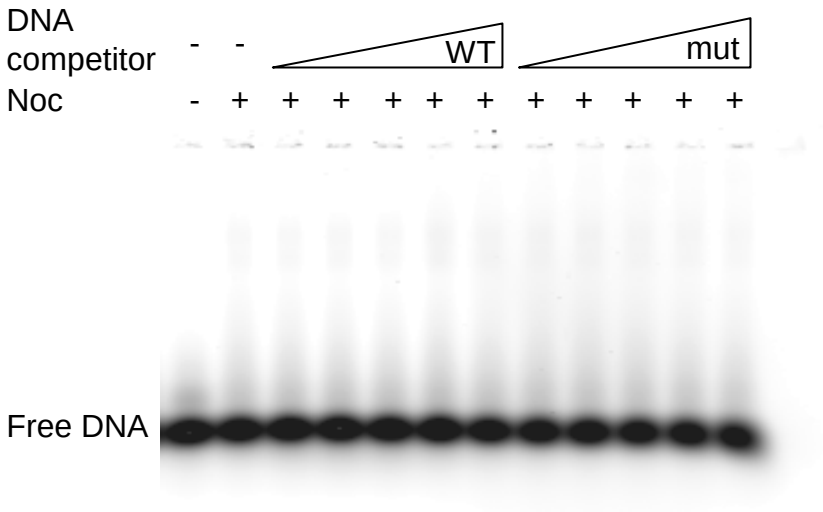


Figure S5 (Wu et al)

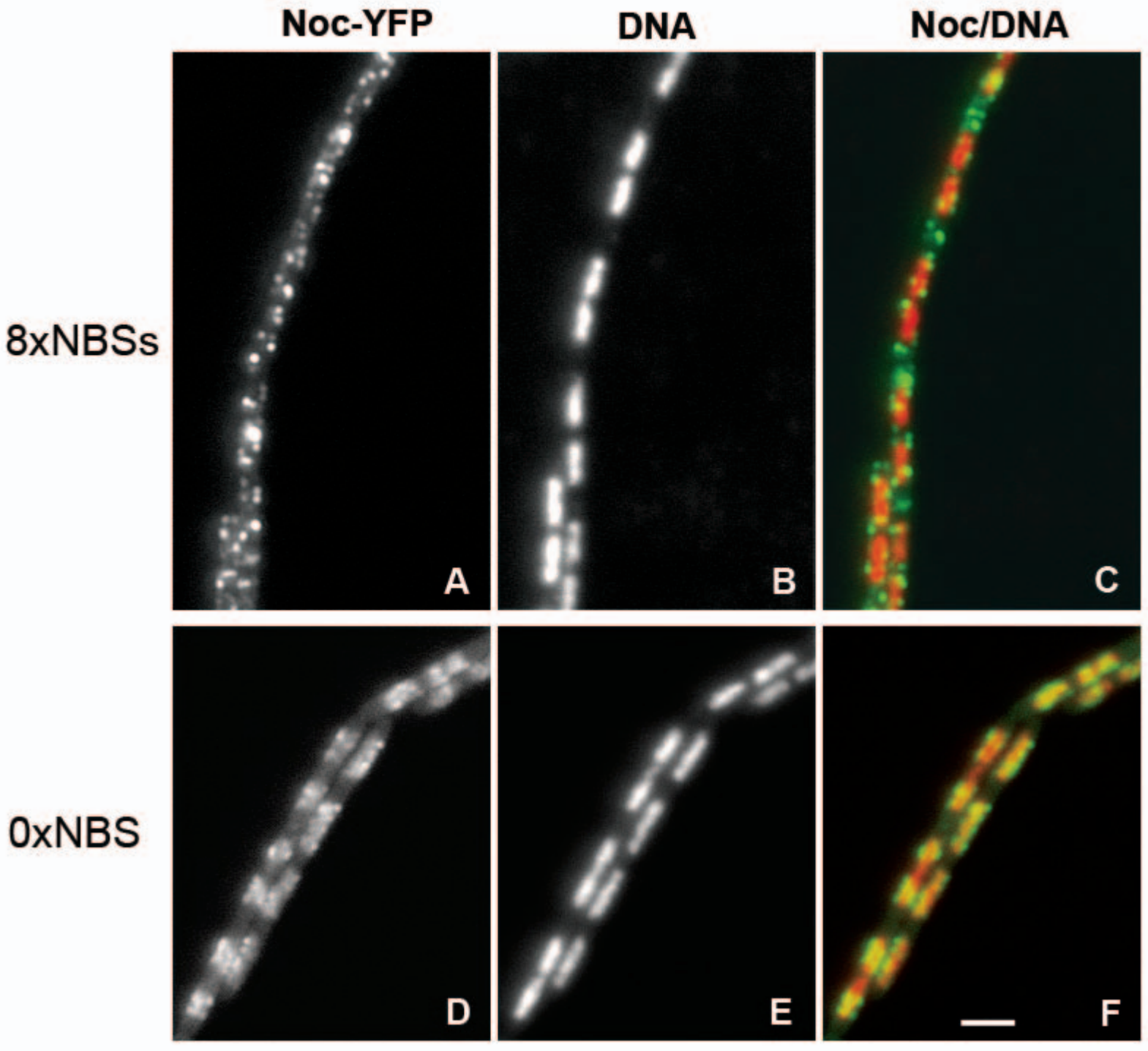


Table S1

	binding region [a]				binding sequence [b]					
	ID	start	end	length (kbp)	ID	PWM Score	sequence	other NBS [c]	start	end
Noc							ATTTCCCGGGAAAT (consensus)			
	NBR1	69,424	79,118	9.7	NBS1	15.20	ATTTCCCGaGAAAa		72,015	72,028
					NBS2	16.44	ATTTCCCGGGAAAa	NBS43	73,631	73,644
	NBR2	142,528	152,527	10.0	*NBS3*	*14.87*	ATTgCgtGGGAAAT		146,960	146,973
	NBR3	179,286	195,163	15.9	*NBS4*	*15.01*	ATTTtCaGGGAAAT		187,198	187,211
					NBS5	15.56	ATgTCCCGGGAAAT		187,701	187,714
					NBS6	16.18	ATTcCCtGGGAAAT	NBS24	187,956	187,969
	ND [d]				*NBS7*	*15.10*	ATTTCCttGGAAAg		272,185	272,198
	NBR4	501,045	514,543	13.5	NBS8	15.99	ATTgCCCGGGAAAc		506,319	506,332
	(phage) [e]				*NBS9*	*15.10*	ATTTCCaaGGAAAg		547,503	547,516
	ND				NBS10	15.49	ATTgCCCGcGAAAT		594,729	594,742
	ND				*NBS11*	*15.13*	ATTgCCaaGGgAAT		673,920	673,933
	NBR5	772,414	787,284	14.9	NBS12	16.47	ATTgCCCGGGcAAT		782,691	782,704
	NBR6	826,549	831,774	5.2	*NBS13*	*14.92*	AaTcCCCGGGAAAT		829,022	829,035
	NBR7	857,841	889,812	32.0	NBS14	15.97	ATTTCtCGGGAAAT	NBS 39, 68	865,963	865,976
	NBR8				NBS15	15.91	ATTTCCaaGGAAAT		878,110	878,123
	NBR9	983,632	1,002,353	18.7	NBS16	16.40	ATTTCCCGGGAAAg	NBS66	988,267	988,280
	NBR10	1,035,971	1,052,980	17.0	NBS17	15.54	ATTcCCgaGGAAAT		1,042,746	1,042,759
	ND				*NBS18*	*15.12*	tTTaCCCGGGAAAc		1,064,797	1,064,810
	ND				NBS19	15.60	ATTgCtCGGGAAAT		1,136,603	1,136,616
	NBR11	1,149,211	1,163,760	14.6	*NBS20*	*14.91*	ATTTCCCGGcAAAa		1,157,631	1,157,644
	NBR12	1,209,448	1,219,187	9.7	NBS21	15.16	ATagCCCGGGAAAT		1,216,340	1,216,353
	ND				NBS22	15.69	ATTgCCCGGGcAAa		1,246,089	1,246,102
	ND				*NBS23*	*14.92*	ATTaCCCcGGgAAa		1,300,864	1,300,877
	NBR13	1,302,538	1,314,295	11.8	NBS24	16.18	ATTcCCtGGGAAAT	NBS6	1,309,612	1,309,625
	(phage)				*NBS25*	*15.04*	ATTTCCacGGAAAc		1,327,478	1,327,491
	NBR14	1,359,589	1,372,178	12.6	NBS26	15.66	ATTTaCCGGGAAAT		1,364,056	1,364,069
	NBR15	1,395,873	1,416,015	20.1	NBS27	16.74	ATTaCCCGGGAAAT		1,408,639	1,408,652
	ND				NBS28	15.35	ATTTCCtGaGAAAT	NBS36,62,73	1,430,963	1,430,976
	ND				NBS29	16.10	ATTaCCgGGGAAAT		1,458,161	1,458,174
	NBR16	1,506,620	1,521,783	15.2	NBS30	16.57	ATTTCCgGGGAAAT	NBS70	1,512,772	1,512,785
	NBR17	1,600,540	1,615,333	14.8	NBS31	16.07	ATTgCCCGGGAAAa		1,606,379	1,606,392
	NBR18	1,871,232	1,877,034	5.8	NBS32	15.16	ATTTCtCGGGAAAg	NBS33	1,872,877	1,872,890
					NBS33	15.16	ATTTCtCGGGAAAg	NBS32	1,873,274	1,873,287
	ND				*NBS34*	*14.97*	ATTcCCtGGGgAAg		2,109,707	2,109,720
	(phage)				NBS35	16.03	ATTTCCCGGGcAAg		2,168,634	2,168,647
	(phage)				NBS36	15.35	ATTTCCtGaGAAAT	NBS28,62,73	2,257,123	2,257,136
	NBR19	2,330,277	2,337,738	7.5	NBS37	16.00	ATTcCCCGGGAAAg		2,332,505	2,332,518
	ND				*NBS38*	*14.96*	ATTgCCgGaGAAAT		2,598,414	2,598,427
	NBR20	2,770,362	2,785,662	15.3	NBS39	15.97	ATTTCtCGGGAAAT	NBS14, 68	2,777,087	2,777,100
	ND				*NBS40*	*14.92*	ATTTCCgGaGgAAT		2,782,892	2,782,905
	NBR21	2,843,648	2,854,479	10.8	NBS41	15.48	ATTaCCgaGGAAAT		2,849,169	2,849,182
	NBR22	2,929,677	2,948,154	18.5	NBS42	16.34	ATTaCCCGGGgAAT		2,939,921	2,939,934
	NBR23	2,951,132	2,962,647	11.5	NBS43	16.44	ATTTCCCGGGAAAa	NBS2	2,955,940	2,955,953
	ND				NBS44	15.31	ATTgCCCGGaAAAT	NBS45	2,992,211	2,992,224
	ND				NBS45	15.31	ATTgCCCGGaAAAT	NBS44	3,003,833	3,003,846
	NBR24	3,094,616	3,104,422	9.8	NBS46	16.22	ATTgCCtGGGAAAT		3,097,098	3,097,111
	NBR25	3,127,110	3,137,321	10.2	NBS47	16.53	ATTTCCaGGGAAAT		3,132,695	3,132,708
	NBR26	3,192,210	3,205,375	13.2	NBS48	15.83	ATTgCCgGGGcAAT		3,194,104	3,194,117
					NBS49	16.20	ATTgCCgGGGAAAT		3,201,557	3,201,570
	NBR27	3,238,587	3,250,257	11.7	NBS50	15.79	ATTgCCgGGGgAAT		3,244,378	3,244,391

	NBR28	3,262,776	3,292,455	29.7	NBS51	15.68	ATTTgCCGGGAAAT		3,263,064	3,263,077
					NBS52	15.81	ATTTCCCaGGAAAa		3,273,229	3,273,242
	NBR29				NBS53	17.21	ATTTCCCGGGAAAT		3,285,728	3,285,741
	ND				*NBS54*	*15.10*	ATTTCCgaGGAAAc		3,325,833	3,325,846
	NBR30	3,411,168	3,437,030	25.9	NBS55	15.31	ATTgtCCGGGAAAT		3,418,984	3,418,997
	NBR31				NBS56	15.56	ATgTCCCGGGAAAT		3,427,425	3,427,438
					NBS57	15.41	ATTcCCCaGGAAAa		3,435,226	3,435,239
	ND				*NBS58*	*15.13*	ATTTCCttGGAAAa		3,447,075	3,447,088
	NBR32	3,497,825	3,525,469	27.6	NBS59	16.84	ATTgCCCGGGAAAT		3,508,841	3,508,854
					NBS60	15.82	ATTTCCtGGGAAAa		3,516,074	3,516,087
	NBR33	3,559,126	3,571,479	12.4	NBS61	16.03	ATTTCCCGGGGAAa		3,562,307	3,562,320
	NBR34	3,701,174	3,718,878	17.7	NBS62	15.35	ATTTCCtGaGAAAT	NBS28,36, 7	3,706,775	3,706,788
	ND				*NBS63*	*15.02*	ATTcCCCcGGcAAa		3,753,679	3,753,692
	ND				*NBS64*	*14.94*	ATTcCttGGGAAAT		3,826,860	3,826,873
	NBR35	3,877,522	3,905,167	27.6	NBS65	15.28	ATTctCCGGGAAAT		3,885,840	3,885,853
	NBR36				NBS66	16.40	ATTTCCCGGGAAAg	NBS16	3,901,296	3,901,309
	NBR37	3,919,086	3,933,964	14.9	NBS67	15.80	ATTTCCgGGGAAAa		3,927,398	3,927,411
					NBS68	15.97	ATTTCtCGGGAAAT	NBS14, 39	3,930,376	3,930,389
	NBR38	3,955,818	3,978,799	23.0	NBS69	15.35	ATTTCttGGGAAAT		3,968,799	3,968,812
	NBR39	4,029,664	4,049,613	20.0	NBS70	16.57	ATTTCCgGGGAAAT	NBS30	4,038,650	4,038,663
					NBS71	15.31	ATTgCCCtGGAAAc		4,041,348	4,041,361
	NBR40	4,085,587	4,092,485	6.9	NBS72	15.29	ATTTCCaGaGAAAT		4,089,302	4,089,315
	NBR41	4,129,017	4,143,359	14.3	NBS73	15.35	ATTTCCtGaGAAAT	NBS28,36, 6	4,136,398	4,136,411
	ND				NBS74	15.54	ATTgCCaaGGAAAT		4,150,991	4,151,004
Spo0J [f]							TGTTTCACGTGAAACA (consensus)			
	0J1	41,890	57,001	15.1	0JBS1	23.21	TGTTaCACGTGAA ACA		47,640	47,655
	0J2	176,308	193,136	16.8	0JBS2	23.10	TGTTaCACGTGtA ACA		185,161	185,176
	0J3	467,785	482,280	14.5	0JBS3	21.88	cGTTcCAtGTGAA ACA		471,151	471,166
	0J9	1,054,553	1,062,814	8.3	0JBS9	21.13	aGTTcCACaTGAA ACg		1,056,855	1,056,870
	ND				0JBS10	20.20	TGTTcCAtaaGAA ACA		2,414,565	2,414,580
	0J4	3,903,912	3,916,703	12.8	0JBS4	23.21	TGTTaCACGTGAA ACA	0J5	3,910,454	3,910,469
	0J5	4,128,420	4,174,412	46.0	0JBS5	23.21	TGTTaCACGTGAA ACA	0J4	4,146,458	4,146,473
	0J6				0JBS6	22.71	cGTTTCACGTGAA ACA		4,151,806	4,151,821
	0J7				0JBS7	22.57	TGTTTCAtGTGAA ACA		4,165,780	4,165,795
	0J8	4,187,425	3,529	30.7	0JBS8	23.24	TGTTcCACGTGAA ACA		4,204,760	4,204,775

[a] Peaks of Noc- and Spo0J-binding regions were automatically detected by the methods as described in materials and methods. Note that some peaks NBR7 & 8, NBR28 & 29, NBR30 & 31, NBR35 & 36 and 0J05, 06 & 07 were merged.

[b] Bases different from the predicted consensus binding sequences are indicated in red; NBSs with PWM score values < 15.15 are

[c] ID of NBS at other genome position is shown if exist.

[d] Not detected.

[e] The GeneChip used in this study does not have probes in phage-related regions.

[f] Spo0J-binding peaks were detected by using date set previously published (Ishikawa et al., 2007).

Supplemental Data

Figure S1 Dynamic localization of Noc-YFP captured using timelapse microscopy. Strain 4702 with a *noc-yfp* fusion (and without the native *noc*) growing exponentially in CH medium containing 0.3 % xylose was imaged at 28°C using a Spinning Disc Confocal System. The black lines mark the left or the right edge of the YFP signals at the beginning of the timelapse experiment (t0). Scale bar, 2 μm.

Figure S2 DNA binding profiles of Spo0J and Noc proteins on the *B. subtilis* genome revealed using ChAP-chip analysis. Binding signals of Spo0J (SI002, top), and Noc in a wild-type strain (4704, middle) and in a mutant strain with NBS-insertion at *yogA* region and deletion at *ywsA* region (4723, bottom) are shown. Top and bottom lines indicate signal intensities of 20 and 0 respectively. Middle lines exhibit threshold values to detect binding regions. Noc and Spo0J binding regions detected by our algorithm are shown as pink and green boxes with IDs, respectively. Positions of Noc binding sequence (NBS) with high score of PWM value and Spo0J binding sequence (*parS*) are also indicated by vertical lines under gene map. The insertion of 8 copies of NBS between *yogA* and *gltB,* and the deletion of NBS by replacing with a *tet* cassette between *ywsB* and *ywsA* in strain 4723 are also indicated.

Figure S3 Enrichment of DNA at selected NBRs as detected by ChAP using specific primer pairs. (A) ChAP analysis of the various strains harbouring an NBS deletion and /or insertion. Modified chromosomal regions are indicated in the upper part of the table. DNA fragments co-purified with Noc (ChAP) and total DNA extracted before ChAP-purification (Sup) were diluted 16-fold and 64-fold, respectively, and used as templates for quantitative PCR after a further 4-fold serial dilutions (lane 1, 2 and 3). Lower part of the panel shows the PCR products obtained with either the 'total' DNA (Sup), or ChAP-

purified DNA (ChAP), using primer pairs shown to the left. Two different reverse primers were used with kan-F and are indicated above the DNA bands. Primer pairs ydbO-F and ydbO-R are used to amplify the NBS located in the *ydbO* gene, which has been used for gel-shift assay (Figure 5). (B) Schematic map of the chromosomal regions that have been modified, showing the locations of the NBSs (in red), and the locations of the primers (in blue). a to h represent the PCR products obtained (in A) from the corresponding regions (in B).

Figure S4 Mutant protein Noc(K164A) is not able to bind the NBS in vitro. In the gel-shift assay the Noc(K164A)-12xHis was incubated with 25 nM of a Cy5-labelled probe containing the NBS from the *ydbO* gene. Unlabelled competitor DNA (wild type NBS or a mutant NBS) was present at concentrations of 0, 125 nM, 250nM, 500nM, 1μM or 2 μM.

Figure S5 Localization of Noc-YFP in cells harbouring a multicopy plasmid carrying an NBS array (strain 4707; panels A-C) or without an NBS (strain 4706; panels D-F), grown in the presence of 0.3% xylose. A and D show the localization of Noc-YFP; B and E are images of nucleoids stained with DAPI; and C and F are overlayed images with Noc-YFP shown in green and DNA (DAPI) in red. Scale bar, 2 μm.