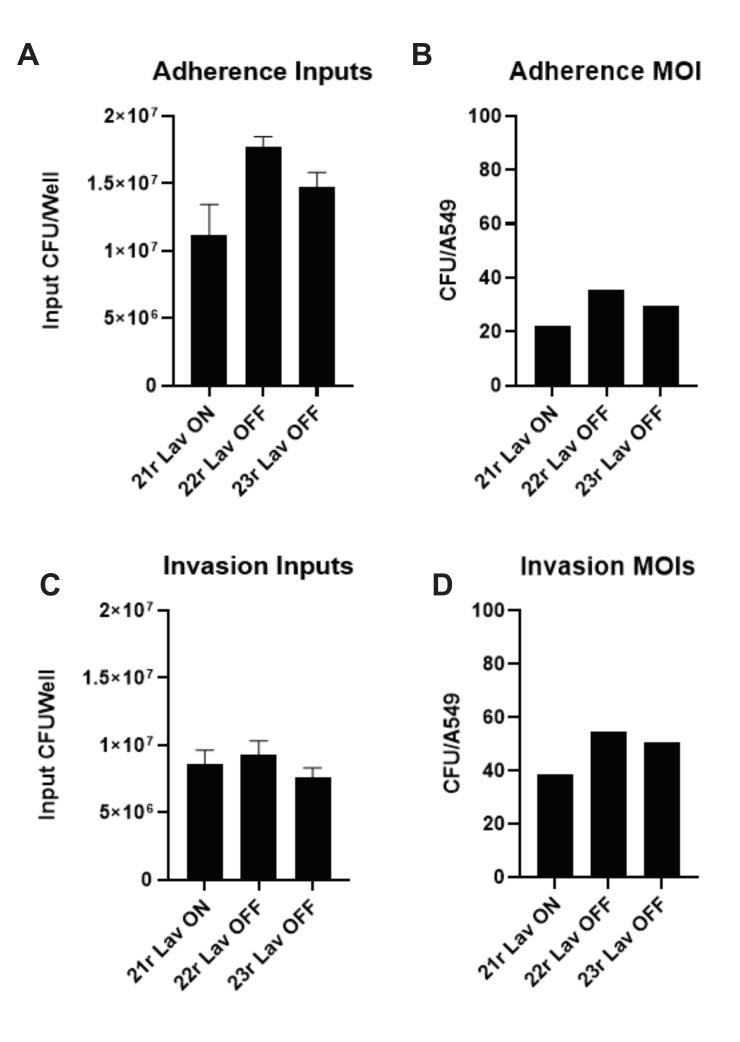


Supplementary Figure 1 - Full anti-Lav Western Blot and corresponding Coomassie gel. Whole-cell lysates from NTHi strain 86-028 NP with 21 SSR repeats (21r), 22 SSR re-peats (22r) or 23 SSR repeats (23r) present in the lav gene were run on a 4-12% Bis-Tris SDS PAGE gel and Coomassie stained to show standardised loading (A). Western Blotting (B) with mouse anti-Lav sera shows an SSR tract number of 21 (21r) puts the gene in-frame, and ON, with Lav protein detected at ~72kDa. The 22r and 23r populations have the lav gene out-of-frame and OFF, with no corresponding band detected in these samples.



A) Enriched Lav populations in vitro

	lav % ON;		lav % ON;			
	in sBHI Broth		on sBHI Agar		nr	
Day	21r ON	22r OFF	23r OFF	21r ON	22r OFF	23r OFF
input	80.6	17.4	17.3	80.6	17.4	17.3
1	87.5	12.3	18.1	91.3	9.1	9.3
2	89.8	13.4	11.9	91.3	9.2	9.2
3	87	14.4	14.1	91.4	8.8	9.3
4	89	13.7	12.9	91.1	8.8	12.7
5	80.1	16.9	14	91	9.4	10.1

B) A549 adherence and invasion assays

lav % ON; 21r ON			
Input	Adherence Out	Invasion Out	SSR No
	90.10%	89.50%	21
90.40%	90.10%	89.60%	21
	90.50%	89.80%	21

lav % ON; 22r OFF			
Input	Adherence Out	Invasion Out	SSR No
	9.60%	10.80%	22
8.50%	10.20%	10.70%	22
	10.50%	10.40%	22

lav % ON; 23r OFF			
Input			SSR No
	11.50%	12.00%	23
9.00%	11.00%	11.00%	23
	NA	11.10%	23

<i>lic1</i> A % ON; 21r ON			
Input	Adherence Out	Invasion Out	SSR No
	93.70%	94.00%	12
92.40%	93.20%	93.70%	12
	93.40%	94.60%	12

lic1A % ON; 22r OFF			
Input			SSR No
	93.90%	94.20%	12
92.90%	94.00%	93.90%	12
	95.90%	94.60%	12

lic1A % ON; 23r OFF			
Input	Adherence Out	Invasion Out	SSR No
	95.20%	93.60%	12
93.00%	94.30%	94.00%	12
	NA	93.60%	12

C) Nasal airway epithelial cell adherence assays

lav % ON; 21r ON			
Input	Adherence Out	SSR No	
89.90%	90.20%	21	
	90.00%	21	
	89.60%	21	

lav % ON; 22r OFF			
Input	Adherence Out	SSR No	
9.70%	9.40%	22	
	9.40%	22	
	10.70%	22	

lav % ON; 23r OFF			
Input	Adherence Out	SSR No	
10.30%	10.20%	23	
	10.60%	23	
	10.70%	23	

lic1A % ON; 21r ON			
Input	Adherence Out	SSR No	
96.70%	97.30%	12	
	97.00%	12	
	96.90%	12	

lic1A % ON; 22r OFF			
Innut		SSR No	
97.00%	96.80%	12	
	97.00%	12	
	97.10%	12	

lic1A % ON; 23r OFF		
Input	Adherence Out	SSR No
96.60%	96.70%	12
	95.70%	12
	97.00%	12

Supplementary Figure 3. Fragment analysis results to demonstrate stable expression of *lav* and *lic1A*. A) Results from multiple sub cultures of enriched Lav populations 21r ON, 22r OFF and 23r OFF on sBHI agar and in sBHI broth over a period of 5 days. Fresh sBHI plates or sBHI broth were reinoculated with a sample of the previous days' growth over five days; B) Inputs and outputs of the A549 adherence and invasion assays were used as a template in fragment analysis PCR reactions using either Lav_F+R primers, or Lic1A_F+R primers and checked for changes in the simple sequence repeat number present in each gene in order to determine if phase-variation of *lav* (top) or *lic1A* (bottom) was occurring in our enriched Lav populations (21r ON, 22r OFF and 23r OFF) during these assays; and C) Inputs and outputs of the nasal airway epithelial cell adherence assays were used as a template in fragment analysis PCR reactions using either Lav_F+R primers, or Lic1A_F+R primers and checked for changes in the simple sequence repeat number present in each gene in order to determine if phase-variation of *lav* (top) or *lic1A* (bottom) was occurring in our enriched Lav populations (21r ON, 22r OFF and 23r OFF) during these assays.

NA = we were unable to generate a PCR product for either lav or lic1A from this archived sample despite multiple

NA = we were unable to generate a PCR product for either lav or lic1A from this archived sample despite multiple attempts. All assays were carried out in triplicate with each output result equating to one replicate. SSR No = majority number of simple sequence repeats present in the respective tract based on PCR amplicon size

```
NTHi 86028NP B1.2
                   KFKKSFIISLFFSILYTSPLLAVVDYTYDNSKLTNEQIERLKKLRDRNSEYWKKETYLLK
NTHi R2866 B1.1
                   KFKKSFILSLLFSALYSSPLLA-VDYVYDKTKLTDDEITRLKKLRDRDSEYWKEETYFIK
                   NTHi 86028NP B1.2
                   SPPSNFPDIPVLFPKDSSVPFENIDNSKAISFYDOKYTEDYLVGFAOGFGVAKRNGDTEE
NTHi R2866 B1.1
                   STPQSPPNIPRLFPKNSFDSFENINNSKEISFYDKEFTEDYLVGFAQGFGVAKRNGETEE
                   NTHi 86028NP B1.2
                   PVRKYFKECLNTGNYNNDNCKANPLASSVSIKSDIFTPRPTIKNSHINSEILAVGNYTEL
NTHi_R2866_B1.1
                   TVRQYFKECLNTGKHSNDPTCKSYSTDAYNIKSDIFALNTIVKNSHINSEILAVGNYTKL
                    ** ******** **
                                    **********
NTHi 86028NP B1.2
                   MLAAQPSASSWAEHLYSDPGLSLTVQDNSHVIGQTIDLGFLQLTNSLWEPRWNSNIDYLV
NTHi R2866 B1.1
                   MLSAQH-SSTWAEHLYSNAELSLTVQDNSHVIGQTIDLGALILTNSLWEPRWNSNIDYLA
                   ** ** ** ****** ******
                                             Percent Identity Matrix
NTHi 86028NP B1.2
                   TENAEIR 247
                                                73.06% Identity
NTHi R2866 B1.1
                   TENADIR 245
                   ****:**
```

CLUSTAL O(1.2.4) multiple sequence alignment

```
KFSLISILASTFLFISNTSFAAAGDVPAYITQYLTHEKKEQ-TGDYWHYYYTYSLKSMQN
aeg HE07 B3.2
aeg HE40 B3.1
               KFSLIYSLLIAFLFIPYSV----SGEPKYVOEYFVKERGIOYFGEGSGASVFYSLKDMLD
               ***** * ****
                                  aeg HE07 B3.2
               PDSIVWKPVPQKIIDGLIKGWKACQN---SNDSDDCFLIGAPIPVLPAGIGLVGEDDFSD
aeg HE40 B3.1
               RNSVAWSPVPKQIIDYITNT-EACYFSHPDSPKDDCQLFGSNTYEKKWFL----TDFSE
               aeg HE07 B3.2
               GDVIOPEKENGTWDAPNAKHFILPFOEKRNVTSNGKDV--PRTLFOSYLYSPI---HKKR
               -QKYGAEEENGIFGNEYAKHFVYPFREDIPFYSESEKLYKLPVLYQSYLYDNYETEKTSR
aeg HE40 B3.1
                   * * * * * * *
aeg HE07 B3.2
               PKNALIDGKVYDVDVLAIDNYRFKFPNEPLRTLTLTVONRSEVRGATLOLWKMKLODSLW
aeg HE40 B3.1
               PLNALINNRVYDFNVLAIDNYRSKFPDET-RGLTLTVKNQSEVRGATLQLLKMVLQDSLW
               Percent Identity Matrix
               EPRFNSDVHHLETQNANIR
aeg_HE07_B3.2
                                250
                                             51.26% Identity
aeg_HE40_B3.1
               EPRFNSDVHHLETQNANIR
                                247
               *******
```

Supplementary Figure 4 – alignment Lav passenger domain alleles 1.1 vs 1.2, and alleles 3.1 vs 3.2