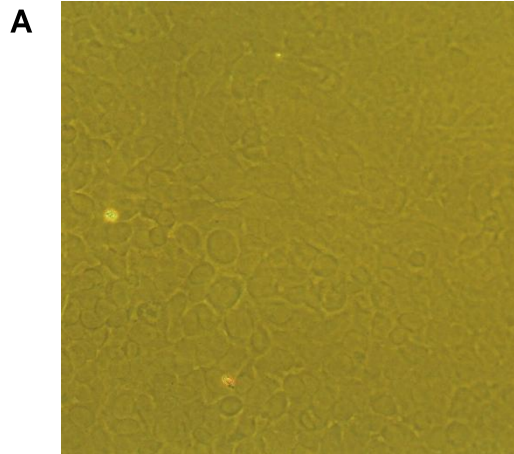
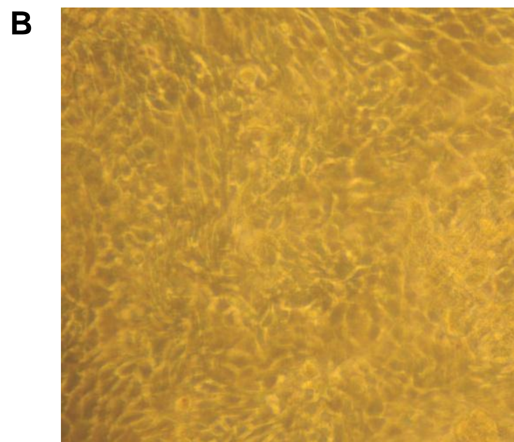
Table S1. Oligonucleotide primers.

Targeted gene	Primer type	Primer sequence	Application
NMB0342	Forward	ACAGCCGCTTCATTATGTGGAA	qRT-PCR
	Reverse	GCTGCCCCACAGGAACA	qRT-PCR
	Reporter	CCAGCCAAAACAAAAC	qRT-PCR
	Forward	TAGCAGGGCTTTGGCTTCTTCG	PCR
	Reverse	CGATCCCTATGTTCATGCAG	PCR
	Forward	GGATTGGTGCTGATTGTGGTATTC	RT-PCR
	Reverse	TCGCCTTCAAGCCGTTTTTAC	RT-PCR
NMB0343	Forward	AACACCTCAAACGGCTGGAGAC	RT-PCR
	Reverse	TGCATGAACATAGGGATCGTCTTC	RT-PCR
NMB0344	Forward	GCCGTTTTGAAGGCGTAAGC	qRT-PCR
	Reverse	GCCGCCTGAAAACAAATCTTTGAG	qRT-PCR
	Reporter	AACCGCCAGAAAAC	qRT-PCR
	Forward	TCGCCACCTATACCGTTAC	PCR
	Reverse	CTGGACTTCCTGCGTACC	PCR
	Forward	GGACATTATGCCGTTTTGGTCG	RT-PCR
	Reverse	TGGAAATACTCGTCAGGGGTAGCC	RT-PCR
NMB0345	Forward	GACAAGAAACCGTCCTTCAAAACC	qRT-PCR
	Reverse	GGCGATATGCAATGCGTATGC	qRT-PCR
	Reporter	CCGTTCAAGCCATATTT	qRT-PCR
	Forward	ATGTTGCTGGCAACAGACG	PCR
	Reverse	GCGGTTTTTTTCTGTTTCATGG	PCR
	Forward	AAGTGGTCAATACCGTGGTCGCAC	RT-PCR
	Reverse	AGGATTTCGCCCAACTGGAC	RT-PCR
NMB0346	Forward	GGATAAGGATGTCCAAAACCGC	PCR
	Reverse	AAACCGTCAAAAGCCTGCTCGTCG	PCR
NMB0347	Forward	GCAGATCATGCAGCAGGC	PCR
	Reverse	TGCTCGCCTTCGTTGAACA	PCR
	Forward	ATAGCGACACAGAAAAAGATGC	RT-PCR
	Reverse	AGCGTAAACTGCGACACCAG	RT-PCR
NMB0348	Forward	CCTTTTCAGACGGCATTGC	PCR
	Reverse	GAGTGGAAACGAGCAGGTCT	PCR
	Forward	GAGGCATACGGTTACAACGAGG	RT-PCR
	Reverse	GAAATCGGCAACGGTTTGG	RT-PCR
NMB0152	Forward	TGATTCAAATGCAGACCATCTT	PCR
	Reverse	TTTCATAAATCGCTCAGTACGC	PCR

A



B



C

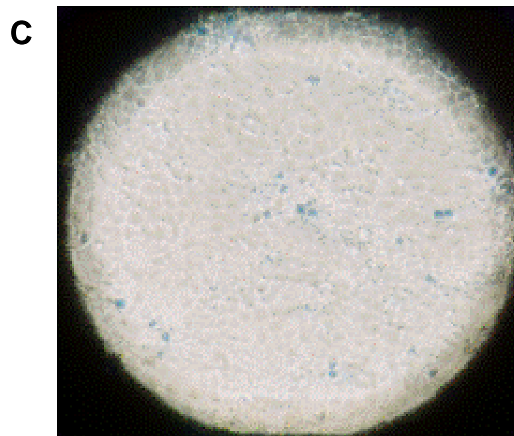


FIG S1. Integrity of 16HBE14 epithelial monolayers by inverted microscopy. View of intact uninfected epithelial monolayers at 4h (**A**) and three weeks (**B**). (**C**) Trypan blue-stained monolayer after 72-hour co-incubation with N. meningitidis MC58.

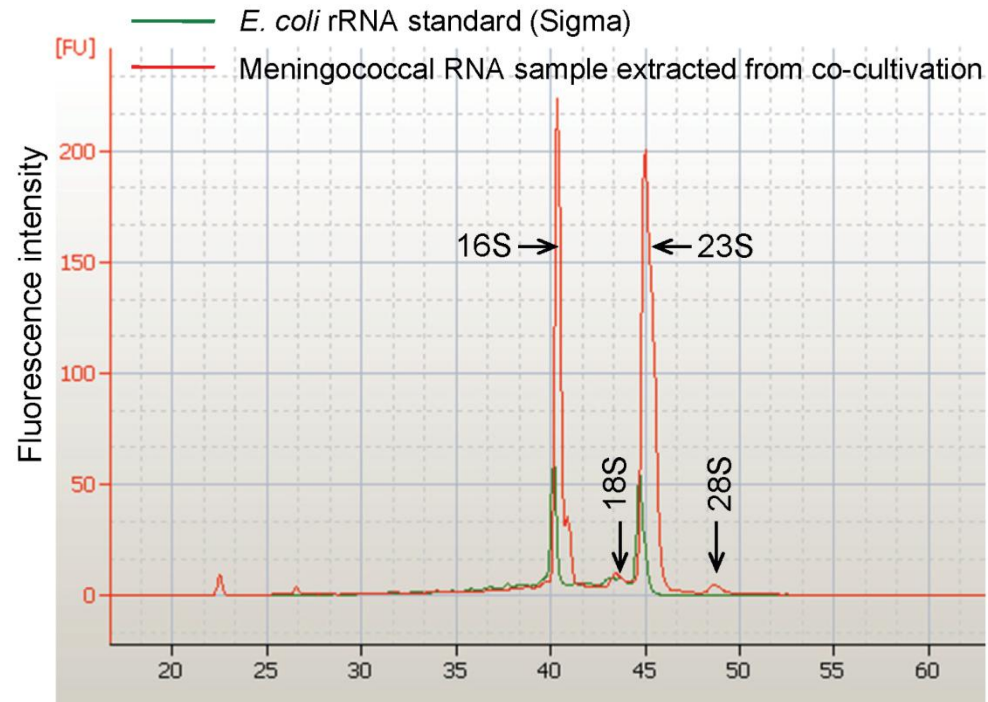


FIG S2. Analysis of meningococcal RNA obtained from epithelial co-cultivation experiment using Bioanalyzer 2100. An E. coli rRNA standard (green) is superimposed onto meningococcal RNA sample (red). 16S and 23S represent meningococcal RNA. 18S and 28S represent human RNA.

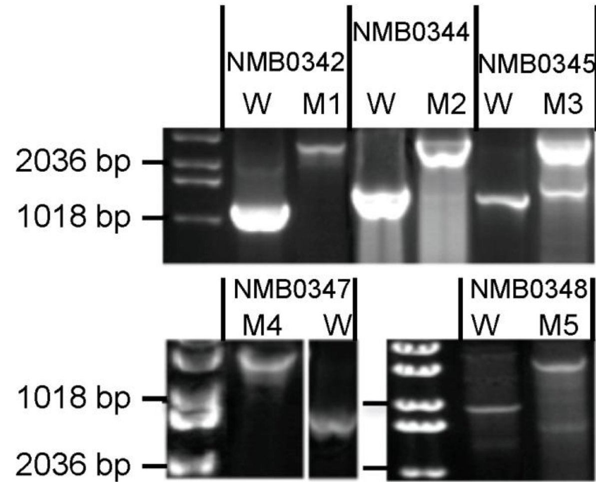


FIG S3. PCR products separated on agarose gel by electrophoresis to confirm insertion of the kanr cassette into the targeted genes. PCR reactions were performed using primers flanking each of the targeted genes (NMBxxxx) and DNA extracted from each of mutants (M#) in comparison with the DNA from wild type MC58 (W). Molecular weight markers were run alongside and indicated. In each mutant-wild type group (separated by vertical lines) mutant DNA produced a band (or a major band, in cases of multiple bandings) of higher molecular weight.

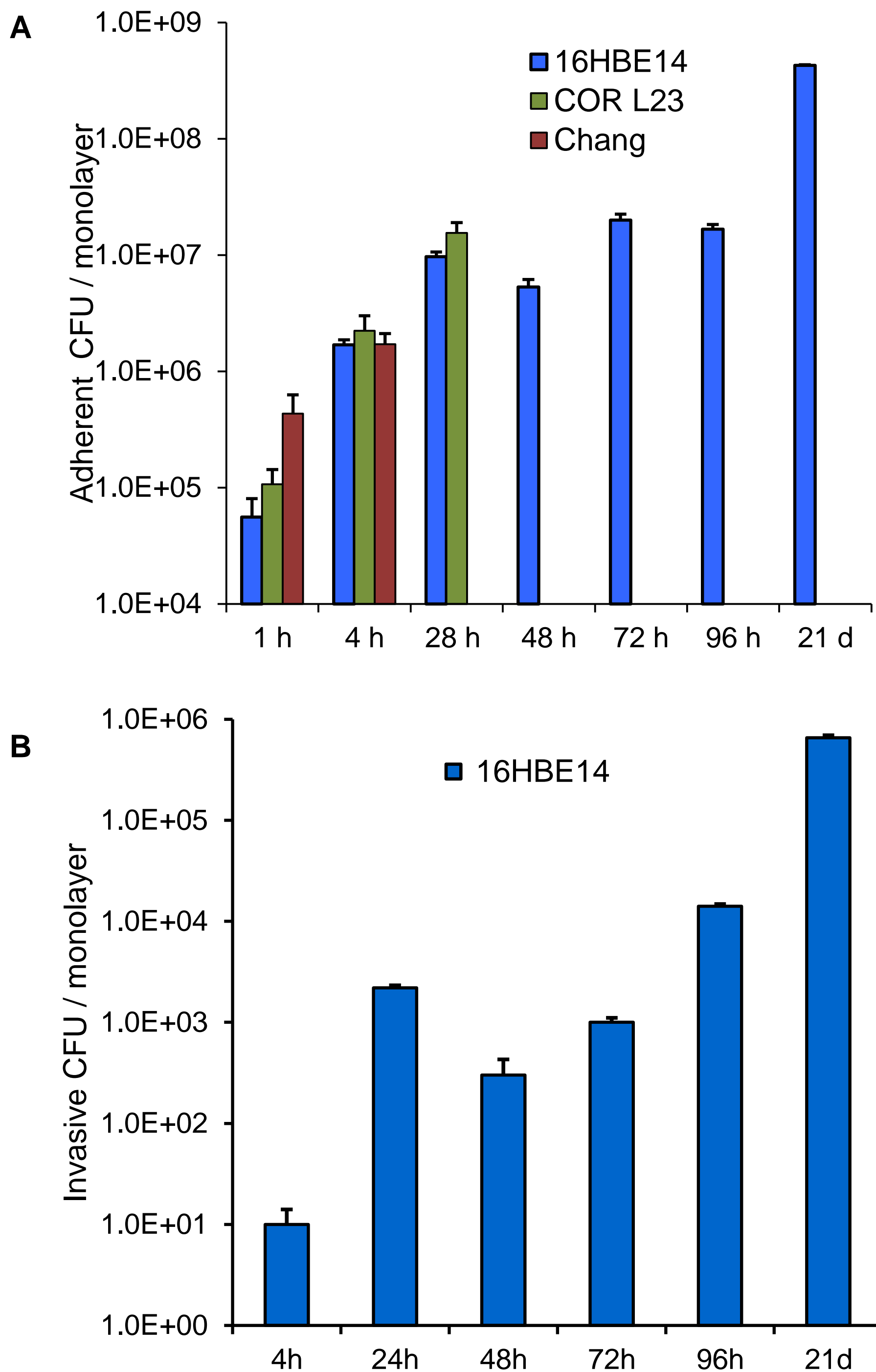


FIG S4. Epithelial adherence and invasion. Monolayers of 16HBE14, COR-L23, and Chang cells were incubated with *N. meningitidis* MC58 for the time periods indicated and (**A**) epithelial adherent, or (**B**) internalised (protected from externally-applied gentamicin – the gentamicin protection assay) CFU were obtained from three biological replicates. Error bars indicate standard error of the mean (SEM).

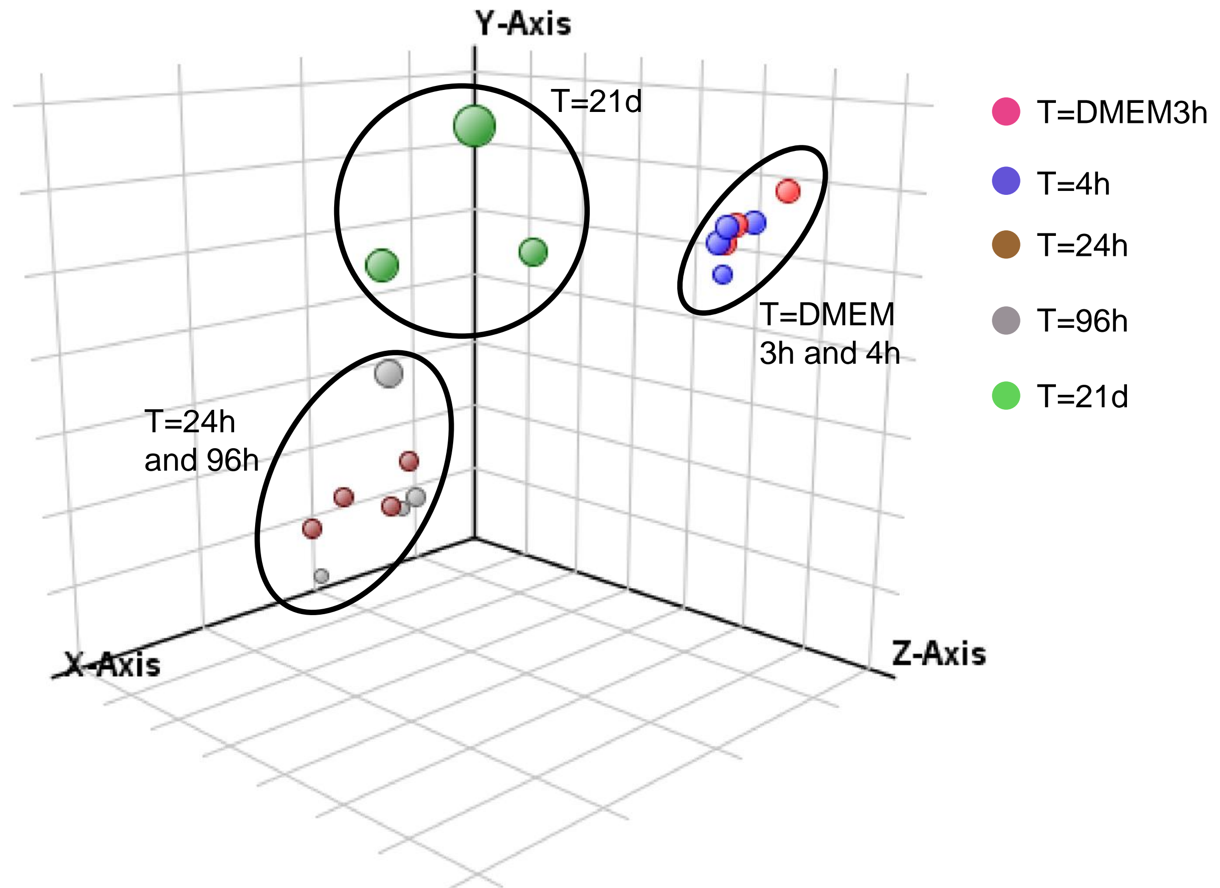


FIG S5. Principal component analysis (PCA) of trasnscriptomes from individual biological replicates of each time point groups (coloured dots). X-axis: 1st component (37.7% variance); Y-axis: 2nd component (34.9% variance); Z-axis: 3rd component (27.3% variance). Circles indicate closely clustered transcriptomes.

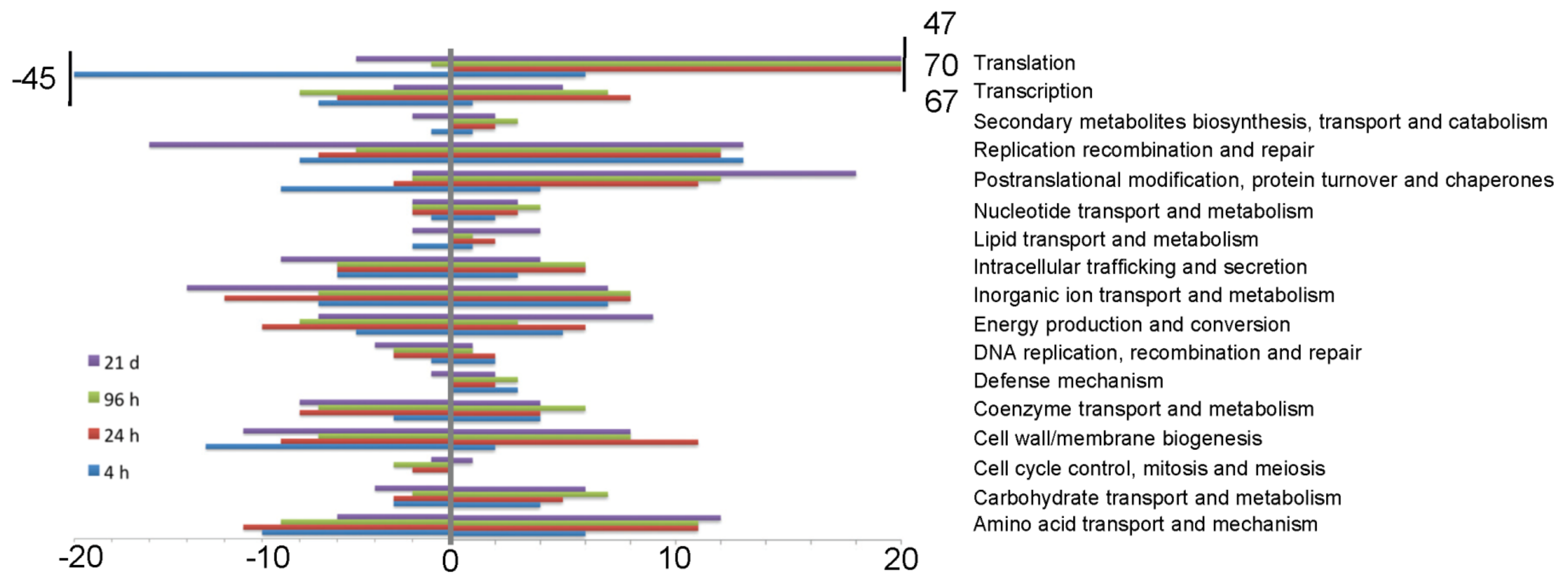


FIG S6. Schematic representation of the number of differentially expressed meningococcal genes. Genes were identified in epithelial co-cultivation experiments at different time points (colour coded) and classified into functional groups according to NCBI Cluster of Orthologous Groups.

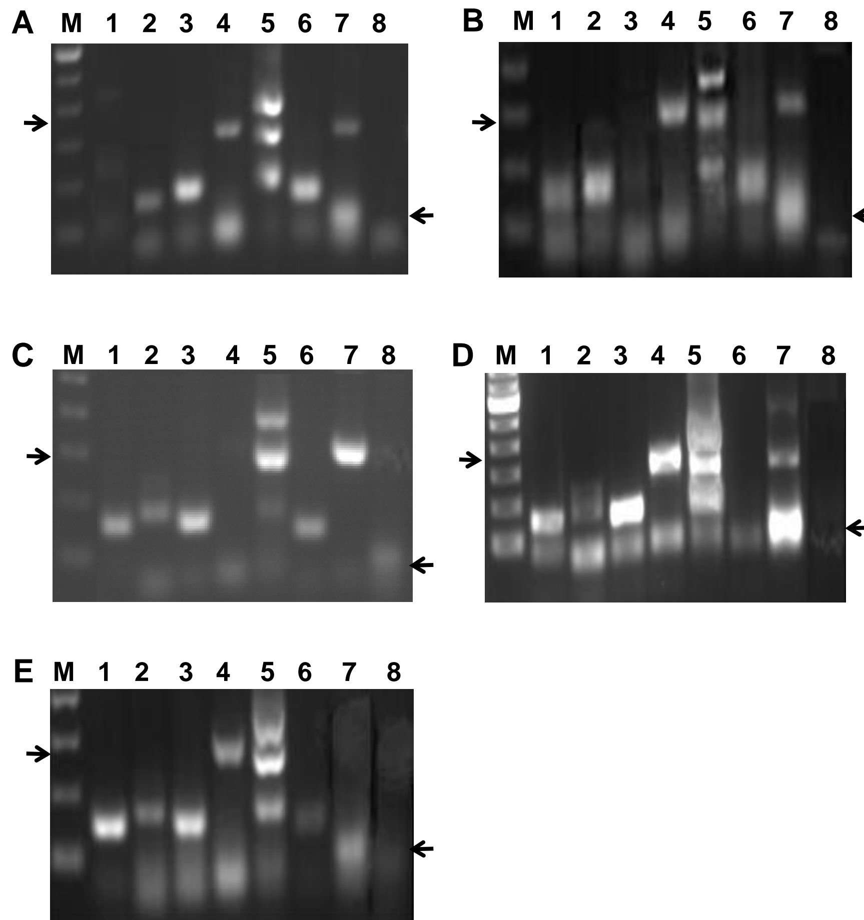


FIG S7. Detection of expression of genes belonging to *NMB0342* – *NMB0348* locus by RT-PCR. RNA samples were prepared from meningococcal mutants with targeted disruption in gene *NMB0342* (**A**), *NMB0344* (**B**), *NMB0345* (**C**), *NMB0347* (**D**) and *NMB0348* (**E**). RT-PCR products were generated using gene-specific primers for *NMB0342* (**lane 1**), *NMB0343* (**lane 2**), *NMB0344* (**lane 3**), *NMB0345* (**lane 4**) *NMB0346* (**lane 5**), *NMB0347* (**lane 6**) and *NMB0348* (**lane 7**) with expecting size of 148, 170, 155, 292, 267, 139 and 290 bp, respectively. **Lane M**: 100 bp marker. **Lane 8** (negative controls): RNA samples being treated with RNaseA before RT-PCR reactions using primers for *NMB0343*. Due to the presence of non-specific products, on the left and right of each panel an arrow is placed to indicate expecting size of RT-PCR products generated by *NMB0346*-specific (**lane 5**) and *NMB0348*-specific (**lane 7**) primers, respectively. Note: Due to the small size of RT-PCR products (preferred by RT-PCR primer design) the product bands appeared to be defused, especially when high voltage was not able to be applied to electrophoresis (e.g. panel **B**).

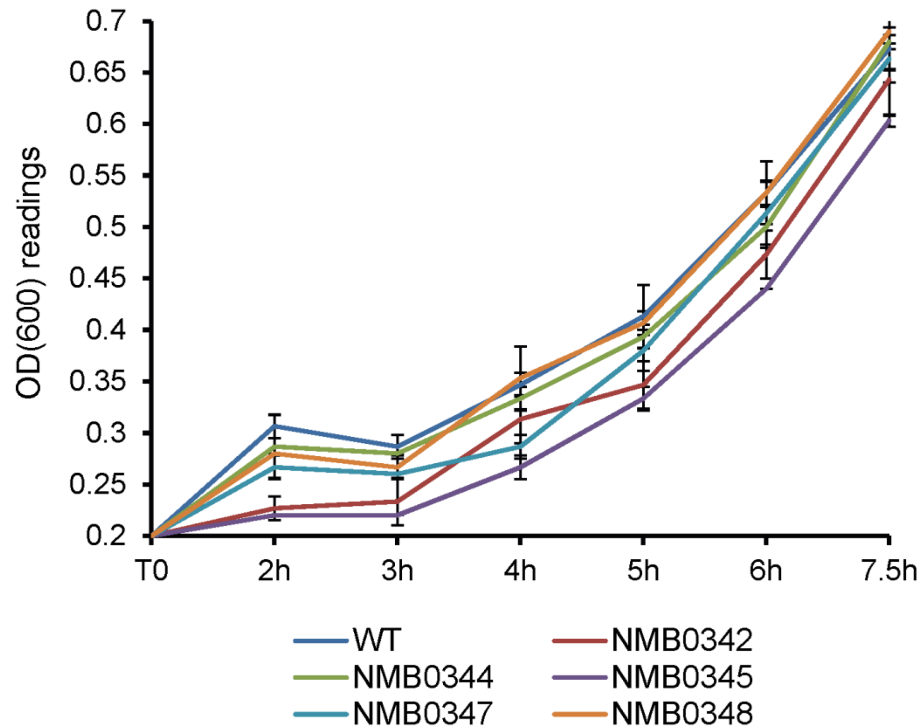


FIG S8. Meningococcal growth in co-cultivation medium. The growth was monitored by measuring OD(600nm) (y-axis) at different incubation time points (x-axis). WT denotes wild type MC58 and a gene number (NMBxxxx) denotes the MC58 mutant strain with the corresponding gene being disrupted.