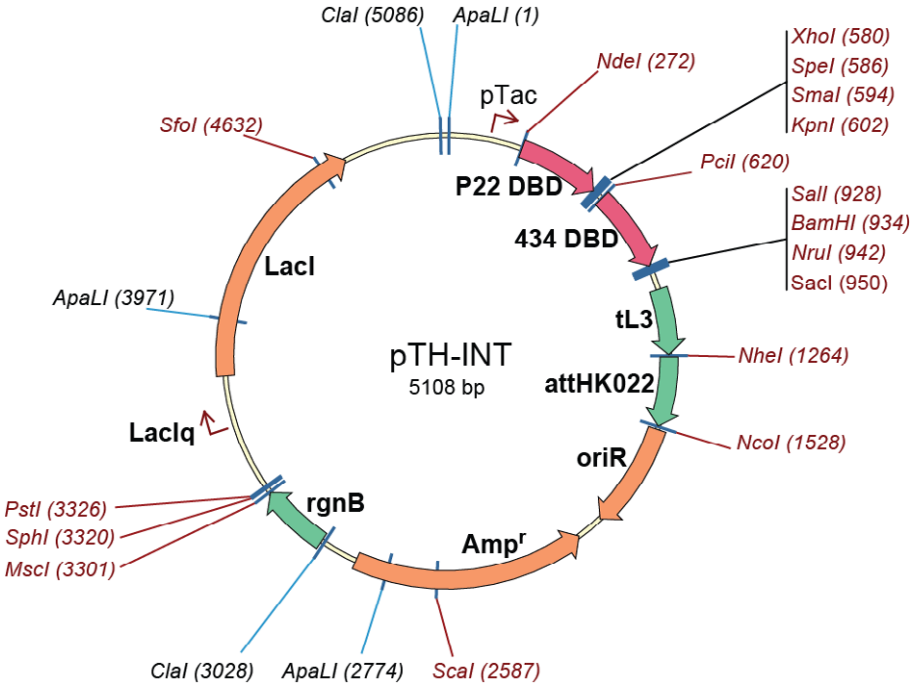
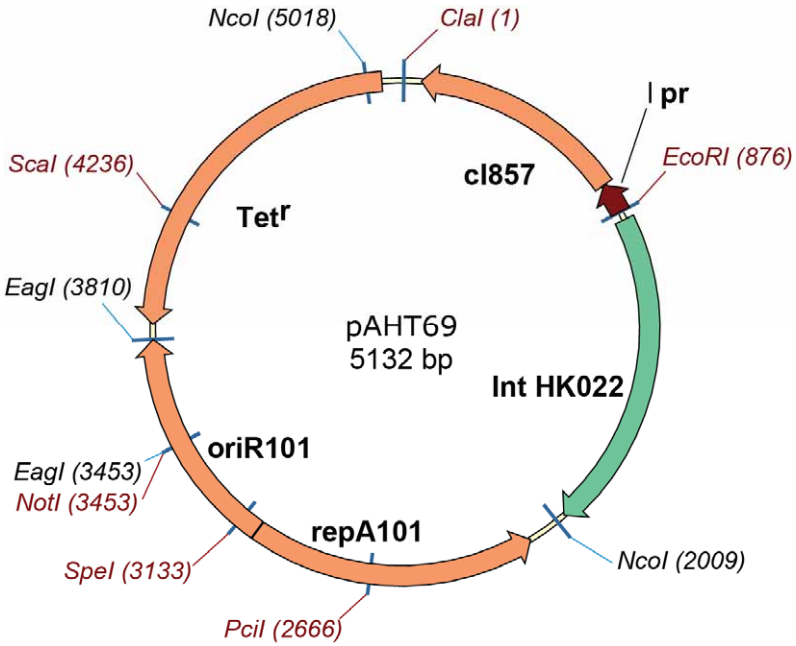
Supplementary information

pTH-INT structure and construction



pTH-INT was constructed on the pAH68[1] backbone (SphI – SacI), with LacIq and pTac promoter region from pAH55[1] (SphI – NdeI) and P22-polylinker (XhoI, SpeI, Sma, KpnI) and 434 polylinker (SalI, BamHI, NruI, SacI) from pTHCP14[2] (NdeI-SacI).

PAH69T helper plasmid structure and construction



pAHT69 was made on the pAH691 background with AmpR swapped to TetR (from pAH152) flanked by ClaI-EagI ites

Primers

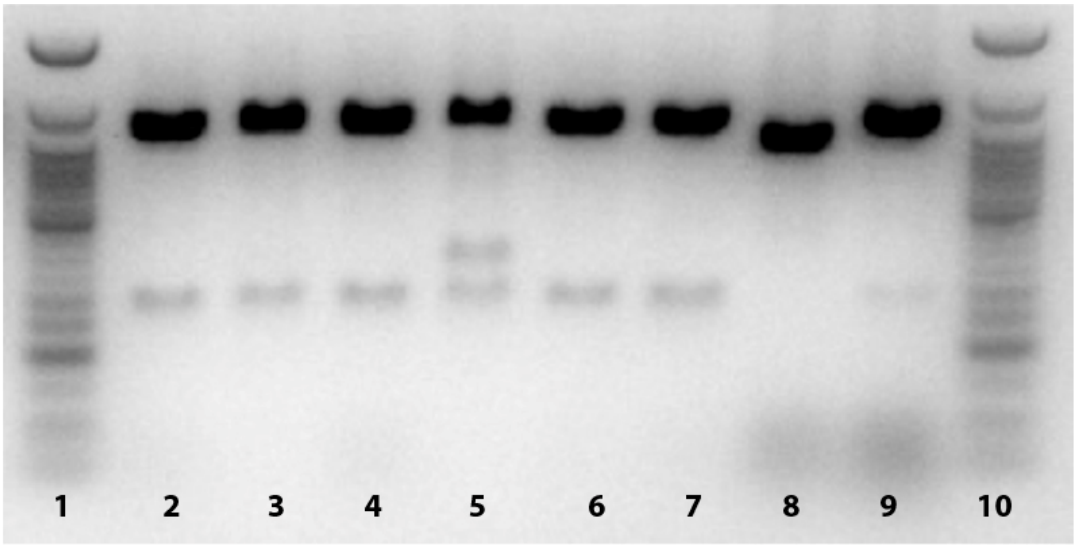
The following primers were used to clone the NS1, RIG-I (full length, CARD, helicase, RG) and TRIM25 into one of the multiple cloning sites of pTH-INT using the restriction endonucleases listed in the primer name. Primers P1-P4[1] were used to confirm chromosomal integration.

Primer Name	Primer Sequence
RIG-I card R NruI	GTTGTTTCGCGACTAGCTGTACAAGTTTGTATCAGACAC
RIG-I card F SalI	ACGCGTCGACATGACCACCGAGCAGCGACG
RD RIG R SacI	CGAGCTCTCATTTGGACATTTCTGCTGG
RD RIG F SalI	ACGCGTCGACATGGGTAGCAAGTGCTTCCTTCTG
Helicase R SacI	CGAGCTCTTACTTGCTCTTCCTCTGCCTCTGG
Helicase F SalI	ACGCGTCGACATGAGCCCATTTAAACCAAGAAA
NS1 R SacI	CGAGCTCTCAAACTTCTGACCTAATTGTTCC
NS1 F SalI	ACGCGTCGACATGGATCCAAACACTG
RIG-I R NruI	GTTGTTTCGCGATCATTTGGACATTTCTGCTGG
RIG-I F SalI	ACGCGTCGACATGACCACCGAGCAGCGACG
NS1 R KpnI	CGGGGTACCTCAAACTTCTGACCTAATTGTTCC
NS1 F XhoI	CCGCTCGAGATGGATCCAAACACTG
TRIM25 R KpnI	CGGGGTACCCTACTTGGGGGAGCAGATGG
TRIM 25 F XhoI	CCGCTCGAGATGGCAGAGCTGTGCCCCCTGG
P1	GGAATCAATGCCTGAGTG
P2	ACTTAACGGCTGACATGG
P3	ACGAGTATCGAGATGGCA
P4	GGCATCAACAGCACATTC

Chromosomal integration protocol

SNS126 cells[2] carrying the pAH69T helper plasmid were grown in 300 ml of SOB culture with 12.5 µg/ml tetracycline at 30°C to an optical density of 600 nm (OD600) of ca. 0.2, followed by incubation at 42°C to OD600 of 0.6 and then made electrocompetent using the standard protocol. These cells were electroporated with 5 µl of ~100 µg/ml of a pTH-INT plasmid, and suspended in SOC without antibiotics and incubated at 37°C for 45 minutes, followed by 45 minutes at 42°C. The resulting

solution was then spread onto selective agar (50 µg/ml ampicillin and 30 µg/ml spectinomycin) and incubated at 37°C overnight. Colonies were then tested for stable integration and loss of the helper plasmid and by colony PCR.[1] Isolated colonies were picked up with a plastic tip and suspended in 20 µl of water. Two microliters of the cell suspension, 5 pmol of each primer (P1-P4), and 0.5 U of Phire DNA polymerase (Finnzymes) were combined in Phire PCR buffer supplemented with MgCl2 to final concentration 5 mM, 4% DMSO and 12% sucrose with deoxynucleoside triphosphates in a final volume of 10 µl. PCR was carried out for 25 cycles with denaturing for 10 sec at 98°C, annealing for 10 sec at 55°, and extension for 15 sec at 72°C. Typical results for this PCR reaction are shown below.



Lane 1 & 10 are 50 bp DNA Ladder (New England Biolabs). Lane 2-7 are several verified integrants as evident by the bands at 289 bp and 824 bp. Lane 5 shows a multiple integrant as evident by the bands at 289 bp, 373 bp and 824 bp. Lane 8 is the negative control (host strain) and lane 9 is the positive control (previously obtained chromosomal integrant).[3]

ONPG assays

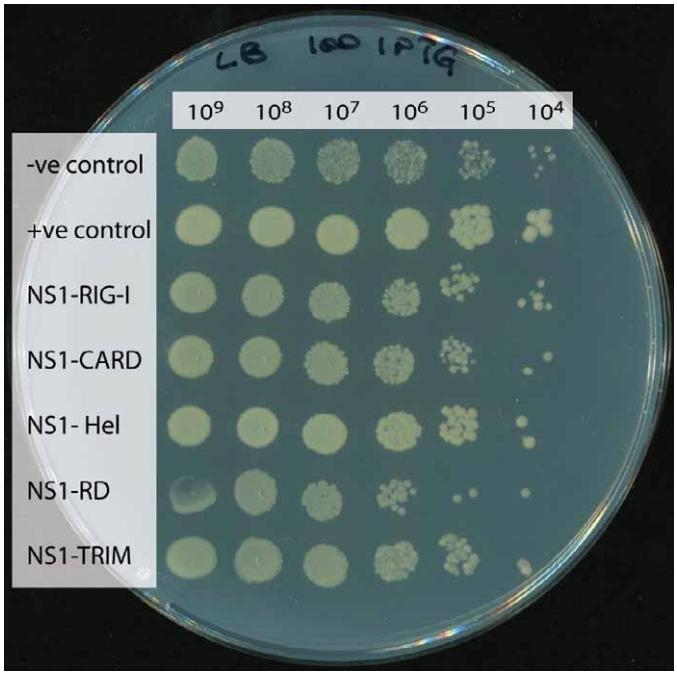
We tested NS1 interaction with RIG-I, its domains and TRIM25 by measuring the activity of the LacZ gene product (β-galactosidase) via ortho-nitrophenol galactoside (ONPG) assays. The standard protocol for ONPG assays was followed, with each point repeated in triplicate. For each strain (NS1-RIG-I, NS1-CARD, NS1-Hel, NS1-RD and NS1-TRIM25), cultures were grown overnight with shaking at 37□C in LB with Ampicillin (50 □g/ml) and Spectomycin (25 □g/ml). The following morning strains were sub-cultured (30 µL of culture in 3 mL LB with antibiotics) with varying levels of IPTG (0, 10, 25, 50, 100, 250 □□). The cultures were grown for 2.5 hours with shaking at 37 □C. The OD_{600} was recorded for each sample, and 0.3 ml of culture (plus a control containing 0.3 ml of LB) was added to 0.4 ml of Z Buffer (60mM $Na_2HPO_4.7H_2O$, 4 0mM NaH_2PO_4 H2O, 10mM KCl, 10mM $MgSO_4.7H_2O$ and 40mM ß-mercaptoethanol, pH 7.0) in a transparent microfuge tube. 1 drop of chloroform and 1 drop

of 0.1% SDS was added and each tube was vortexed for 10 second to lyse the cells. The tubes were incubated in a thermo-block at 37□C for 1 min after which 0.2mL of ONPG (4 mg/ml) was added to each tube, and the time taken for each solution to become yellow recorded in seconds. The reaction was stopped with 0.5 ml of 1M Na_2CO_3. The OD_{420} of each sample was recorded. The sample activity was calculated (for each sample) by diving the OD_{420} value by the amount of time (in seconds) it took the sample to turn yellow. The standardized activity is calculated by dividing the sample activity by the volume of culture used (0.3), and the specific activity is obtained by dividing the standardized activity by the OD_{600} value.

Drop spotting

Each RTHS strain (NS1-RIG-I, NS1-CARD, NS1-Hel, NS1-RD and NS1-TRIM25) was grown overnight with shaking, at 37□C in LB with Ampicillin (50□g/mL) and Spectomycin (25□g/mL). The following day 20□l of culture was diluted in 10% glycerol solution and ten-fold serial dilutions prepared in a sterile 96-well plate for each strain. 2.5 µl of each dilution was drop-spotted onto selective plates (200 ml Agar, 25 ml minimal media, 10 ml of 50% glycerol, 250 □l 1M $MgSO_4$, 50 □g/mL Ampicillin and 25 □g/mL Spectomycin), containing 25 µg/mL Kanamycin, 2.5 mM 3-amino-1,2,4-triazole and increasing levels of IPTG (10 µM, 25 µM, 50 µM and 100 µM), which induces the expression of the target proteins. We observed growth after 2 days.

All strains were also spotted on LB-agar, supplemented with 100 µM IPTG (figure below), eliminating the possibility that the observed lack of growth on minimal media supplemented with IPTG is due to toxicity of the proteins being expressed.



References

1. Haldimann, A.; Wanner, B. L., Conditional-replication, integration, excision, and retrieval plasmid-host systems for gene structure-function studies of bacteria. *J Bacteriol* **2001,** *183* (21), 6384-93.

2. Horswill, A. R.; Savinov, S. N.; Benkovic, S. J., A systematic method for identifying small-molecule modulators of protein-protein interactions. *Proc Natl Acad Sci U S A* **2004,** *101* (44), 15591-6.

3. Tavassoli, A.; Lu, Q.; Gam, J.; Pan, H.; Benkovic, S.; Cohen, S., Inhibition of HIV budding by a genetically selected cyclic peptide targeting the Gag-TSG101 interaction. *ACS Chem Biol* **2008,** *3* (12), 757-64.