Supplemental Information

Programming controlled adhesion of *E. coli* to target surfaces, cells and tumors with synthetic adhesins.

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***Running title*:** Programmable *E. coli* adhesion

***Keywords*:** adhesins/ cell surface antigens / *E. coli* / synthetic biology / tumor targeting

Note: # These authors contribute equally to this work

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Supplemental Methods

Plasmid constructions.

pNVfib: A DNA fragment encoding a VHH binding human fibrinogen [1] was cloned between *Sfi*I and *Not*I sites of pNeae2.

pDisplay-TirM-tm: the DNA sequence corresponding to amino acid residues 252 to 360 of the translocated intimin receptor of EHEC was amplified by PCR from pET28a-$TirM_{EHEC}$ [2] template with oligonucleotide primers Sfi-TirM-For and TirM-Xma-Rev. The amplified fragment was digested with *Sfi*I and *Xma*I and cloned into the same sites of pDisplay vector. Subsequently, a DNA fragment encoding mWasabi (GeneBank Accession Number EU024648) was synthesized (GeneArt, Life Technologies) with flanking *Sma*I and *Sac*II restriction sites and cloned into pDisplay.

pGE: This 1436 bp Km^R-suicide plasmid contains two I-SceI sites flanking a multiple cloning site (MCS) with the following restriction sites: *Xma*I, *Xho*I, *Bsa*I, *Sac*I, *Nde*I, *Xba*I, *Hind*III, *Spe*I, *Avr*II, *Sph*I and *Xma*I. This plasmid was constructed ligating three DNA fragments, one encompassing the R6K origin of replication, a Km resistance cassette and the MCS. A DNA fragment of 424 bp corresponding to R6K origin of replication was PCR amplified from plasmid pEMG [3] with R6K1 and R6K2 primers. This PCR product has *Xma*I and I-*Sce*I sites at its 5'-end and an *Asc*I site at its 3'-end. The Km resistance cassette was amplified by PCR from pEMG with Km1 and Km2 primers. This PCR product of 958 bp has an *Asc*I site at its 5'-end and I-*Sce*I and *Xma*I sites at its 3'-end. The MCS was obtained annealing oligonucleotides MCS1 and MCS2 generating a DNA fragment of 74 bp with XmaI cohesive ends. PCR fragments of R6K and Km were digested with *Xma*I and *Asc*I, and ligated to the MCS.

pGE*flu*: This suicide plasmid is a pGE-derivative with two homology regions (HR) of ca. 500 bp flanking the *flu* gene that were amplified by PCR from the chromosomal DNA of *E. coli* K-12 MG1655. The 5'-HR was amplified with oligos XhoI-yeeP and SacI-yeeP and cloned between *Xho*I-*Sac*I sites of pGE. The 3'-HR was amplified with oligonucleotides Ang43-Spe and and Ang43-Sph and cloned into *Spe*I and *Sph*I restriction sites of pGE vector backbone.

pGE*flu*-SAgfp: The sequence encoding NVgfp fusion was obtained by digesting pNVgfp with *Xba*I and *Hind*III and cloned into same sites of pGE*flu*. Promoter P_{N25} sequence, obtained by hybridizing oligonucleotides PN25-Sac-Xba1 and PN25-Sac-Xba2, was cloned upstream NVgfp between *Sac*I and *Xba*I sites.

pGE*flu*-SAtir: The sequence encoding the VHH anti-TirM and C-terminal myc-tag was obtained by digestion of pNVtir [2] with *Nco*I and *Hind*III and cloned into the same sites of pGE*flu*-SAgfp replacing the VHH anti-GFP and myc-tag of SAgfp.

pGE*mat-lux*: This suicide plasmid is a pGE-derivative contains the *luxCDABE* operon with P_2 constitutive promoter and two homology regions (HR) of ca. 500 bp flanking the *matB* gene of *E. coli* K-12 MG1655. The 5'-HR was amplified by PCR using primers matA-XhoI and matA-SacI and cloned between *Xho*I and *Sac*I sites of pGE. The 3'-HR was amplified by PCR using primers matBC-SpeI and matBC-SphI and cloned into *Spe*I and *Sph*I sites of pGE. The *luxCDABE* operon was obtained by *Hind*III and *Spe*I digestion of pSEVA226 [4] and cloned into the same sites of pGE vector backbone. The P_2 promoter sequence was cloned between *Sac*I and *Hind*III sites of pGE. The constitutive P_2 promoter is a synthetic tandem promoter based on P_{A1} and Ptac [5] with the following sequence (5'-

TTATCAAAAAGAGTATTGGCTTAAAGTCTAACCTATAGGATACTTAC

AGCCATCGAGAGGGACACGGCGAATCTAGAGTCGACCTGCAGGCATGCAAGC

TCTTCTGAAATGAGCTGTTGACAATTAATCATGGGCTCGTATAATAGATTCAT-3')

***E. coli* strain constructions**.

EcM1SAgfp: The EcM1 strain carrying pACBSR (Cm^R) was transformed with pGE*flu*-SAgfp (Km^R) and cointegrants were selected on LB-Km-Cm plates. Cointegrants were resolved by I-SceI expression and individual Km sensitive colonies were checked by PCR with primer pairs: VHH-Sfi2 and VHH-Not for VHH detection; Yoe1 and NeaeR1 for checking upstream integration site; Neae4-YeeR2 for checking downstream integration site.

EcM1SAtir: The EcM1 strain carrying pACBSR (Cm^R) was transformed with pGE*flu*-SAtir (Km^R) and cointegrants were selected on LB-Km-Cm plates. Cointegrants were resolved by I-SceI expression and individual Km sensitive colonies were checked by PCR with primer pairs: VHH-Sfi2 and VHH-Not for VHH detection; Yoe1 and NeaeR1 for checking upstream integration site; Neae4-YeeR2 for checking downstream integration site.

EcM1*lux*Δ*flu*: The EcM1 strain carrying pACBSR (Cm^R) was transformed with pGE*flu* (Km^R), and cointegrants were selected on LB-Km-Cm plates. Cointegrants were resolved by I-SceI expression and individual Km sensitive colonies were checked by PCR with primers Yoe1 and YeeR2 to confirm the *flu* gene deletion. Subsequently, EcM1Δ*flu* strain bearing pACBSR was transformed with pGE*mat-lux* (Km^R). Cointegrants were resolved by I-SceI expression and individual Km sensitive colonies were tested for light emission. To ensure site specific integration of the *lux* operon, the bioluminescent colonies were checked by PCR primer pairs: ykgL-For and LuxC-Rev for upstream integration site and LuxE-For and yagX-Rev for downstream integration site.

EcM1luxSAgfp: The EcM1SAgfp strain carrying pACBSR (Cm^R) was transformed with pGE*mat-lux* suicide plasmid (Km^R). Cointegrants and individual bioluminescent colonies were selected and screened as described above for strain EcM1*lux*Δ*flu*.

EcM1luxSAtir: The EcM1SAtir strain carrying pACBSR (Cm^R) was transformed with pGE*mat-lux* suicide plasmid (Km^R). Cointegrants and individual bioluminescent colonies were selected and screened as described above for strain EcM1*lux*Δ*flu*.

Supplementary Table 1. *E. coli* strains and plasmids used in this study

Name	Genotype and relevant properties	Reference
***E. coli* strains**		
DH10B-T1^{R}	(F- λ-) *mcrA* Δ*mrr-hsdRMS-mcrBC* φ80*lacZDM15* Δ*lacX74 recA1 endA1 araD139* Δ*(ara, leu)7697 galU galK rpsL (Str^{R}) nupG tonA*	Novagen
BW25141	(F- λ-) Δ*(araD-araB)567*, Δ*lacZ4787*(::rrnB-3), Δ*(phoB-phoR)580*, *galU95*, Δ*uidA3*::*pir*, *recA1*, *endA9*(del-ins)*::FRT*, *rph-1*, Δ*(rhaD-rhaB)568*, *hsdR51.*	*6*
CC118 λ*pir*	Δ(*ara-leu*) *araD* Δ*lacX74 galE galK phoA20 thi- rpsE rpoB argE(Am) recA1,* λ*pir*	*7*
MG1655	K-12 (F- λ-)	*8*
EcM1	MG1655Δ*fim*A-H	*2*
EcM1SAgfp	EcM1Δ*flu::P$_{N25}$-SAgfp*	This work
EcM1SAtir	EcM1Δ*flu::P$_{N25}$-SAtir*	This work
EcM1*lux*Δ*flu*	EcM1Δ*flu* Δ*matB::P$_{2}$-luxCDABE*	This work
EcM1*lux*SAgfp	EcM1Δ*flu::P$_{N25}$-SAgfp* Δ*matB::P$_{2}$-luxCDABE*	This work
EcM1*lux*SAtir	EcM1Δ*flu::P$_{N25}$-SAtir* Δ*matB::P$_{2}$-luxCDABE*	This work
Plasmids		
pAK-Not	(Cm^{R}), lacI^{q}-P*lac* promoter, pBR322 ori	*2*
pNeae	pAK-Not-derivative; Neae polypeptide [Intimin$_{EHEC}$ (1-659)-E-His-tag]	*9*
pNeae2	pAK-Not-derivative; Neae-myc polypeptide [Intimin$_{EHEC}$ (1-659)-E-His-myc-tag]	*2*
pNVgfp	pNeae2-derivative; VHH anti-GFP fused to Neae2 [Intimin$_{EHEC}$ (1-659)-E-Vgfp-myc-tag]	*2*
pNV$_{fib}$	pNeae2-derivative; VHH anti-Fibrinogen fused to Neae2 [Intimin$_{EHEC}$ (1-659)-E-Vfib-myc-tag]	*1*
pDisplay	(Amp^{R}, G418^{R}) for display of peptides on the plasma membrane of mammalian cells (P$_{CMV}$-IgK signal peptide-HA-polylinker-myc tag-PDGFR transmembrane domain)	Life technologies
pDisplay-GFP-tm	pDisplay derivative; GFP-tm fusion (display of GFP)	*10*
pDisplay-TirM-tm	pDisplay derivative, TirM-tm fusion (display of TirM$_{EHEC}$ and mWasabi)	This work
pGE	(Km^{R}), R6K-ori, polylinker flanked by two I-SceI restriction sites.	This work
pGE*flu*	pGE derivative; with homology regions flanking the *flu* gene of *E. coli* K-12	This work
pGE*flu*-SAgfp	pGE*flu* derivative; P$_{N25}$-SAgfp (constitutive expression of SA binding GFP)	This work
pGE*flu*-SAtir	pGE*flu* derivative; P$_{N25}$-SAtir (constitutive expression of SA binding TirM).	This work
pGE*mat-lux*	pGE derivative; P$_{2}$-*luxCDABE* with homology regions flanking the *matB* gene of *E. coli* K-12	This work
pACBSR	(Cm^{R}), p15A-ori, P$_{BAD}$ promoter, I-SceI endonuclease, λ Red	*11*

Supplementary Table 2. Oligonucleotides used in this study

Name	Sequence (5'- 3')	Use
Sfi TirM For	GTCCTCGCAACTGCGGCCCAGCCGGCCCAGGCGCTTGCATTGACGCCGG	pDisplay-TirM-tm construction
TirM Xma Rev	TCCCCCCGGGCGAACCACCACCCGATGAAACTTTCAGCTCCTCCTG	pDisplay-TirM-tm construction
PN25-Sac-Xba 1	CTCATAAAAAATTTATTTGCTTTCAGGAAAATTTTTCTGTATAATAGATTCATAAATTTGAGAGAGGAGTTT	PN25 promoter generation
PN25-Sac-Xba 2	CTAGAAACTCCTCTCTCAAATTTATGAATCTATTATACAGAAAAATTTTCCTGAAAGCAAATAAATTTTTTATGAGAGCT	PN25 promoter generation
Xho yeeP	CCGCTCGAGATGACCGTGCCCTGTCTGTGGATG	*flu* 5' Homology Region
Sac yeeP	CCGGAGCTCTCAGAAGAAAATCCAGTTCCATACCGC	*flu* 5' Homology Region
Ang43 Spe	GACTAGTTTCCACTGCAGGCAGCGGGATGACGTTCTC	*flu* 3' Homology Region
Ang43 Sph	ACATGCATGCCCAGCCAGCGAATATGGAACAACCGGGTTATG	*flu* 3' Homology Region
Yoe1	CGGTTCACAGGCAATTGGCGGTATTGTTAAC	5' check SAs integration in *flu*
NeaeR1	TGTTGTGCCGCATAATTTAATGCCTTGTCATC	5' check SAs integration in *flu*
Neae4	CGTAATGGCAATAGCTCTAACAATGTA	3' check SAs integration in *flu*
YeeR2	ACATCAGTGACGGTGAAATATCGTACTGTAACG	3' check SAs integration in *flu*
MatA XhoI	CCGCTCGAGCTGAACTGATTGTGGATATCGACAG	*mat* 5' Homology Region
MatA SacI	CCGGAGCTCTGCATTTCTTCCCGAGTTGAATTGAGG	*mat* 5' Homology Region
MatBC SpeI	GACTAGTGCATCTGGAGCGGCGACGTTAGCGTAC	*mat* 3' Homology Region
MatBC SphI	ACATGCATGCCACAGCGCTGCGGTTGGCATTATCG	*mat* 3' Homology Region
YkgL For	ACTCAGTCTCCTCCCTTTGCG	5' check *lux* integration in *mat*
LuxC Rev	TGCCAACAGATGTACAGATTTACC	5' check *lux* integration in *mat*
LuxE For	TATATCATAACCGGAGGCGGCTGG	3' check *lux* integration in *mat*
YagX Rev	ACTTATGTCAGCAGCGCTGGC	3' check *lux* integration in *mat*
VHH Sfi2	GTCCTCGCAACTGCGGCCCAGCCGGCCATGGCTCAGGTGCAGCTGGTGGA	Check integration SAs
VHH Not	GGACTAGTGCGGCCGCTGAGGAGACGGTGACCTGGGT	Check integration SAs
R6K1	TCCCCCCGGGTAGGGATAACAGGGTAATCCATGTCAGCCGTTAAGTGTTCCTGTGTC	pGE vector construction
R6K2	TTGGCGCGCCGATCTGAAGATCAGCAGTTCAACC	pGE vector construction
Km1	TTGGCGCGCCGACGTCTTGTGTCTCAAAATCTCTG	pGE vector construction
Km2	TCCCCCCGGGATTACCCTGTTATCCCTATTATTAGAAAAATTCATCCAGCATCAG	pGE vector construction
MCS1	CCGGTCTCGAGACGCGTGAGCTCCATATGTCTAGAGCTAGCAAGCTTACTAGTCCTAGGGCATGCA	pGE vector construction
MCS2	CCGGTGCATGCCCTAGGACTAGTAAGCTTGCTAGCTCTAGACATATGGAGCTCACGCGTCTCGAGA	pGE vector construction

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Supplementary Figures

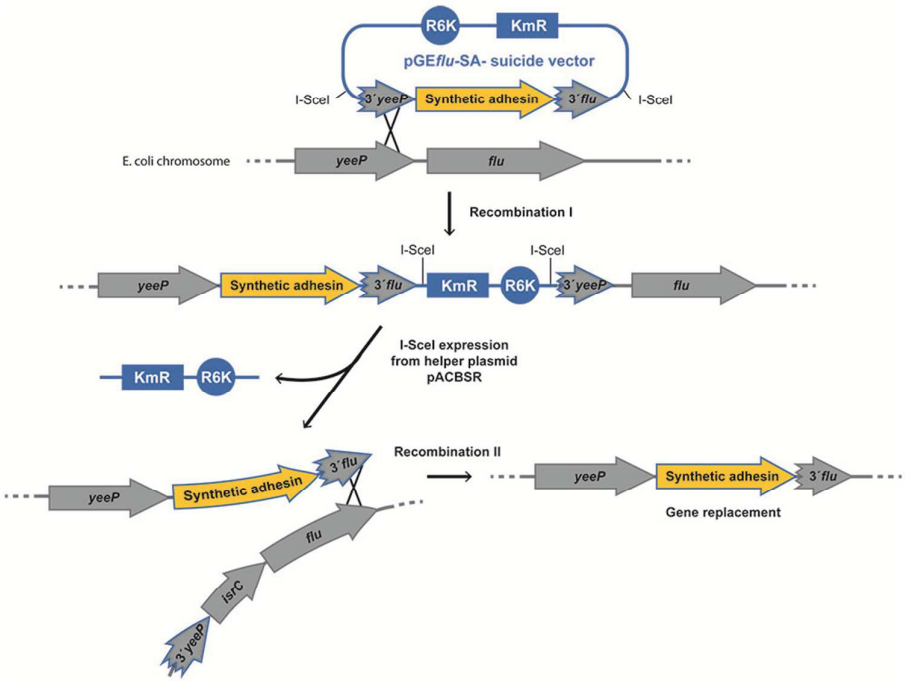

Figure S1. Site-specific integration of synthetic adhesin gene in the chromosome of *E. coli*. Scheme showing the integration of a synthetic adhesin gene in the *flu* locus of *E. coli* K-12 chormosome. Integration is done using a pGE*flu*-SA suicide plasmid containing the pi-dependent R6K origin of replication, a kanamycin resistance gene (Km^R) and a synthetic adhesin gene cassette of the desired specificity under the control of the P_{N25} constitutive promoter. The SA gene cassette is flanked by two homology regions (HRs), corresponding to the 3'-ends of the *yeeP* and *flu* genes, and two I-SceI restriction sites. Homologous recombination of the suicide plasmid with the chromosome (Recombination I) leads to a Km^R-cointegrant that is later resolved by the expression of I-SceI endonuclease from the helper plasmid pACBSR. The double strand breaks generated by I-SceI cleavage are repaired by a second homologous recombination (Recombination II) that could revert the cointegrant to the wild type situation or lead to the chormosomal integration of the SA-gene cassette replacing the *flu* gene (as depicted).

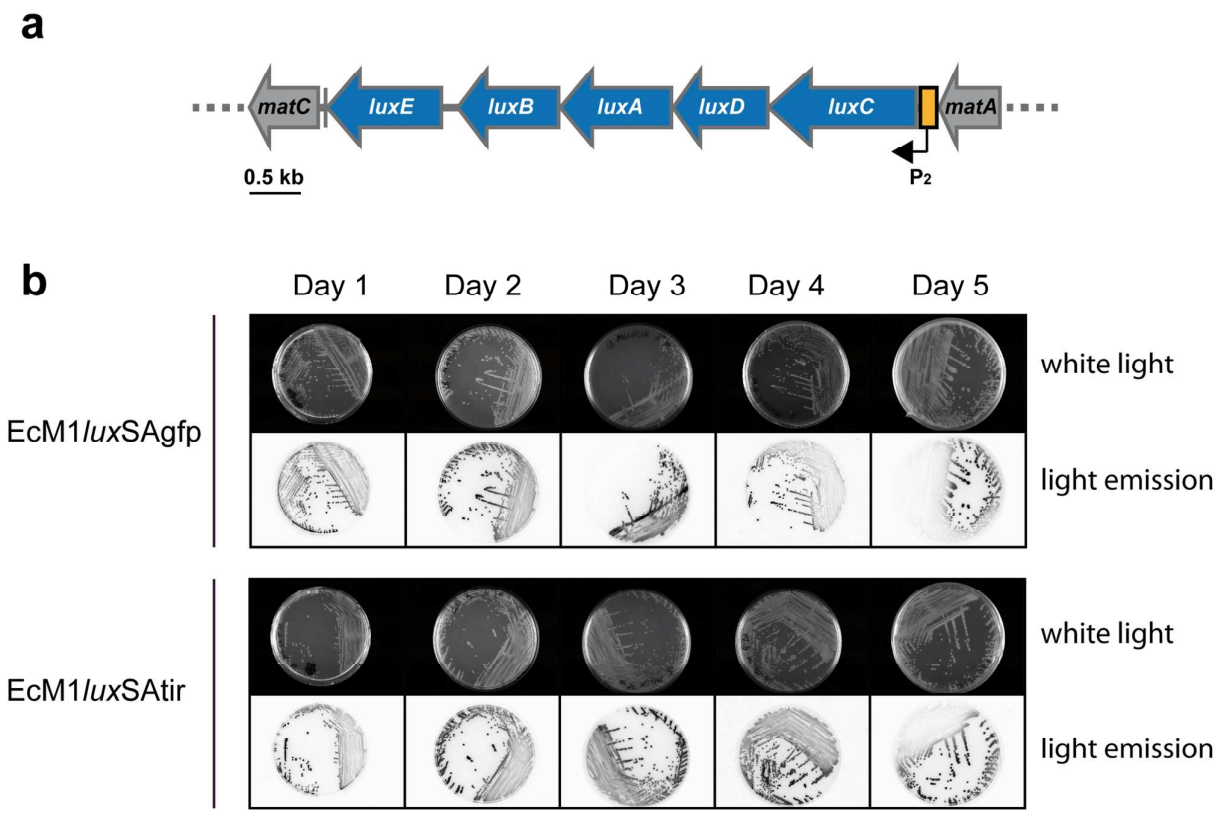

Figure S2. Bioluminescence of engineered *E. coli* strains expressing synthetic adhesins. (**a**) Scheme showing the *luxCDABE* operon under the control of the P2 constitutive promoter inserted in the chromosome of engineered *E. coli* strains replacing the *matB* gene. (**b**) Stability of the bioluminescence from EcM1*lux*SAgfp and EcM1*lux*SAtir strains grown in liquid LB at 37 ^{o}C for the indicated days, with a daily dilution (1:2000) with fresh LB medium. A sample of these cultures from each day was streaked on LB agar plates and white light and light emission images from the plates were acquired. All individual colonies in the plates show strong bioluminescence.

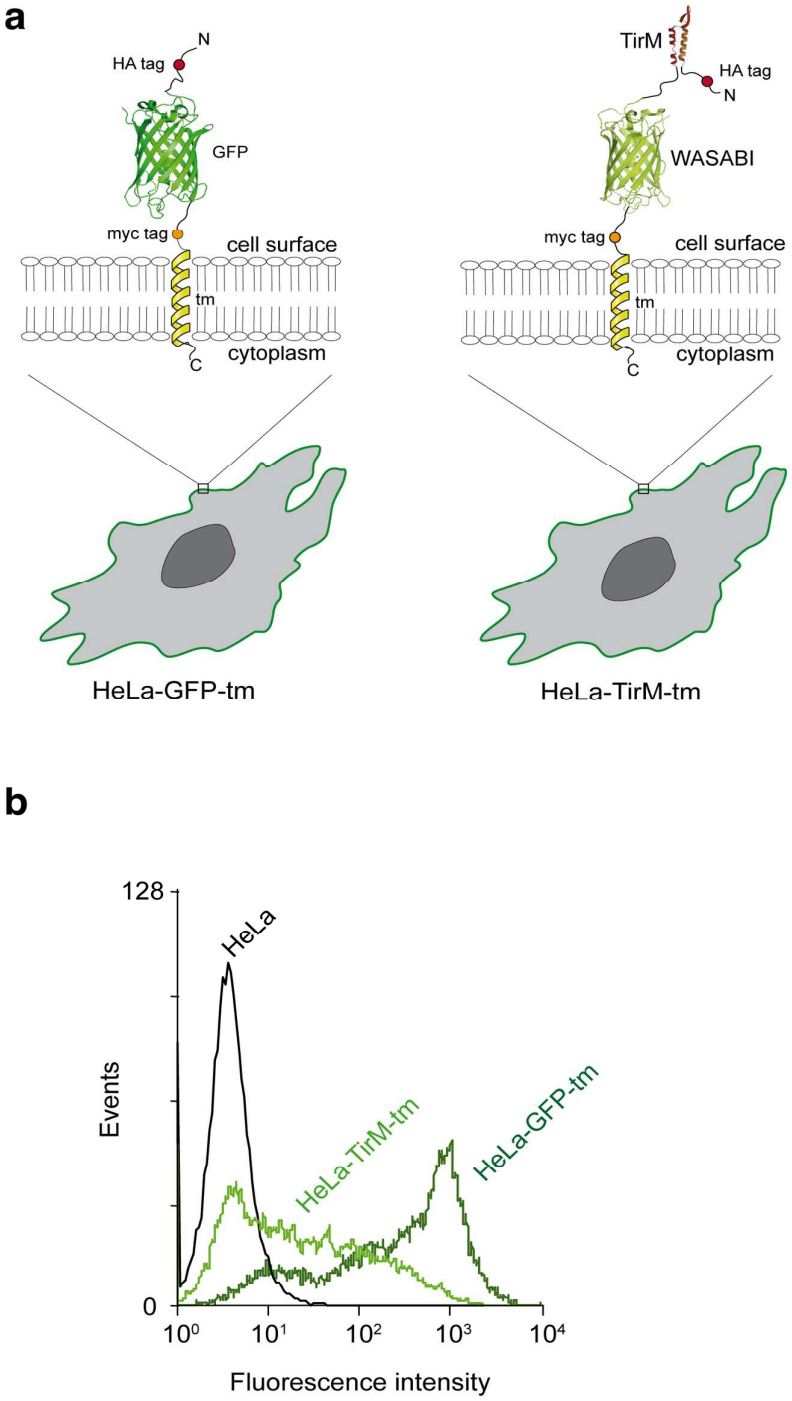

Figure S3. HeLa-GFP-tm and HeLa-TirM-tm cells. (**a**) Model showing GFP-tm and TirM-tm protein fusions in the plasma membrane of stably transfected HeLa-GFP-tm and HeLa-TirM-tm cells, respectively. The transmembrane domain (tm) from the platelet derived growth factor receptor (PDGFR) anchors the fusion proteins in the plasma membrane, displaying on the cell surface GFP or mWasabi-TirM protein domains. (**b**) Flow cytometry analysis of HeLa-GFP-tm and HeLa-TirM-tm cells showing fluorescence levels corresponding to the expression of protein fusions GFP-tm and TirM-tm by these cell populations. Untransfected HeLa cells were used as a negative control.

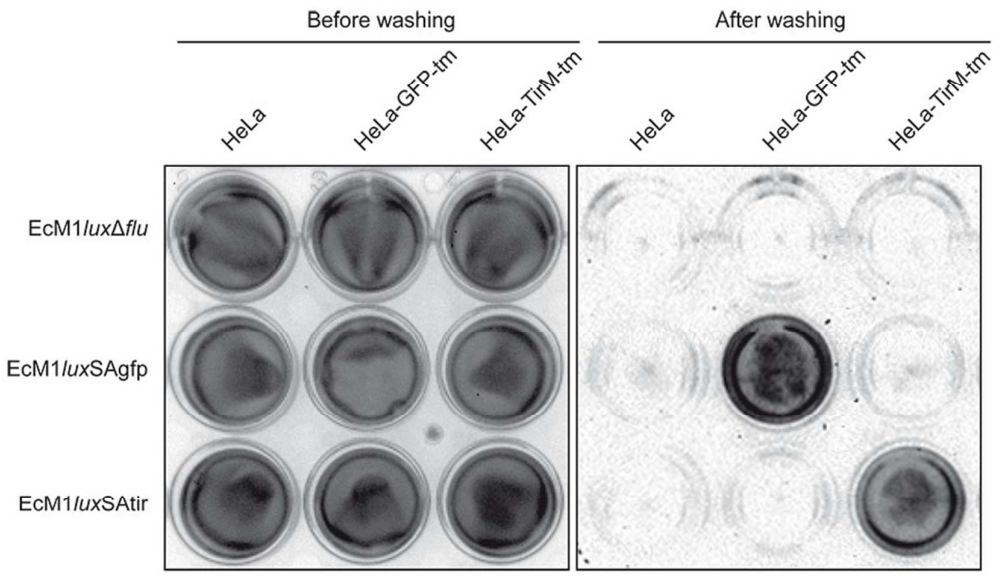

Figure S4. Bioluminescence assay testing the adhesion of engineered *E. coli* to target mammalian cells. HeLa, HeLa-GFP-tm and HeLa-TirM-tm cells were grown in a tissue culture plate and infected with EcM1*lux*Δ*flu,* EcM1*lux*SAgfp and EcM1*lux*SAtir as indicated. After 1 h infection, the plate was monitored for light emission before (left) and after (right) washing of unbound bacteria with PBS, revealing positive bioluminiscence signals when the engineered bacterial strains with a SAs infects its target cell.

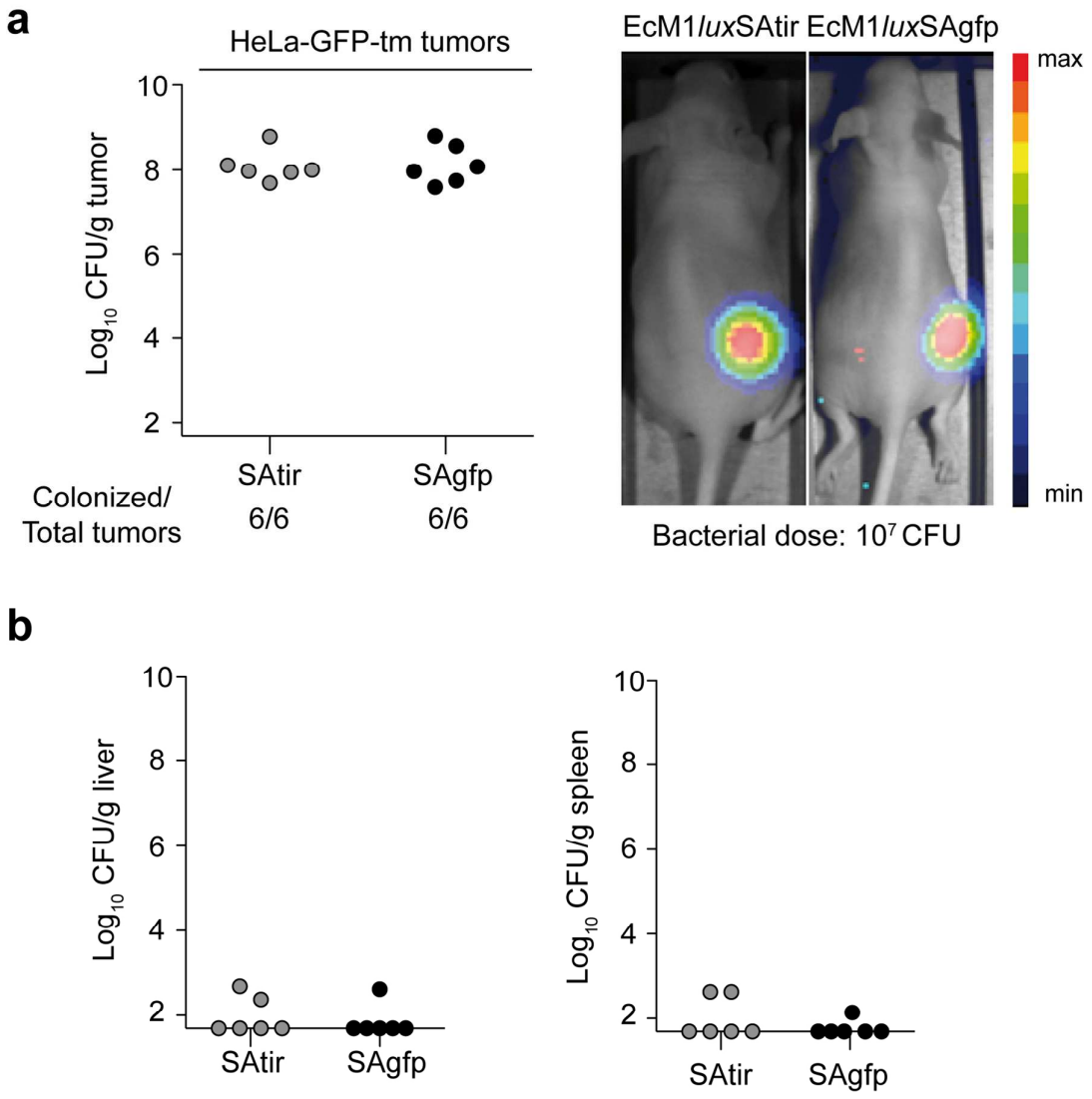

Figure S5. ***In vivo*** **colonization of tumors with high doses of** ***E. coli*** **expressing synthetic adhesins.** (**a**) Bacterial colonization of HeLa-GFP-tm solid tumors by EcM1*lux*SAtir (SAtir) and EcM1*lux*SAgfp (SAgfp), as indicated, after their intravenous administration with a dose of $1x10^7$ CFU/mouse. Infected tumor-bearing mice (experimental groups n=6) were euthanized 4-days post-administration and the number of CFU in each tumor was determined. Each circle represents the CFU determined per gram of tumor (Log_{10} CFU/g) for each animal in the different experimental groups. The ratio of colonized tumors in each group is shown at the bottom. On the right, bioluminescence live imaging of HeLa-GFP-tm tumor-bearing mouse infected with $1x10^7$ CFU of EcM1*lux*SAtir (left image) or EcM1*lux*SAgfp (right image). Images are overlays of photographic white-light and bioluminescence signals from a representative tumor-bearing mouse infected with each strain, as indicated on top. The intensities of the bioluminescence signals are represented in pseudocolor according to the scale bar. (**b**) Graphs showing bacterial titers in livers (left) and spleens (right) from those animals with a HeLa-GFP-tm tumor colonized in A by EcM1*lux*SAtir (SAtir) or EcM1*lux*SAgfp (SAgfp) strains. Each circle in the graph represents the CFU determined per gram of tissue (Log_{10} CFU/g).

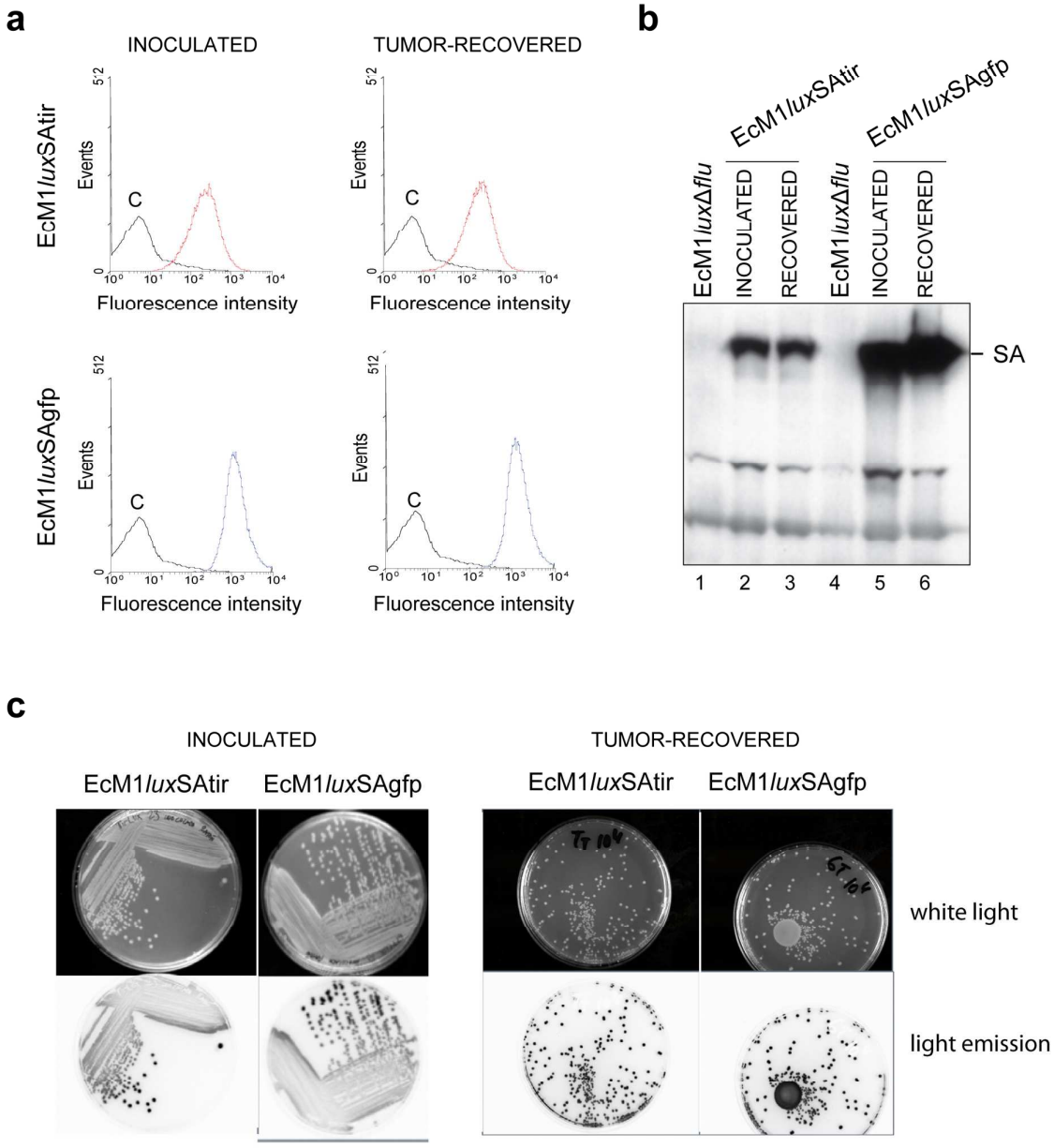

Figure S6. Expression of synthetic adhesins and bioluminescence in engineered *E. coli* recovered from colonized tumors. (a) The expression of SAs in the inoculated and tumor-recovered bacteria from strains EcM1luxSAtir and EcM1luxSAgfp, as indicated, was analyzed by flow cytometry. Bacteria were recovered 4-days post-infection from colonized tumors as in Figure S5. Bacteria in flow cytometry were stained with anti-myc mAb and secondary anti-mouse IgG-Alexa 488. Control strain (C, black line) was EcM1*luxΔflu*. (**b**) The expression of SAs in the inoculated and tumor-recovered bacteria from strains EcM1luxSAtir and EcM1luxSAgfp, as indicated, was analyzed by Western blot. The SA protein fusions were detected with anti-myc mAb and anti-mouse IgG-POD conjugate. (**c**) Analysis of bioluminescence of inoculated and tumor-recovered EcM1*lux*SAgfp or EcM1*lux*SAtir bacteria grown in LB agar plates at 37^0C. Images of white light and light emission from the plates were acquired. All individual colonies show strong bioluminescence.

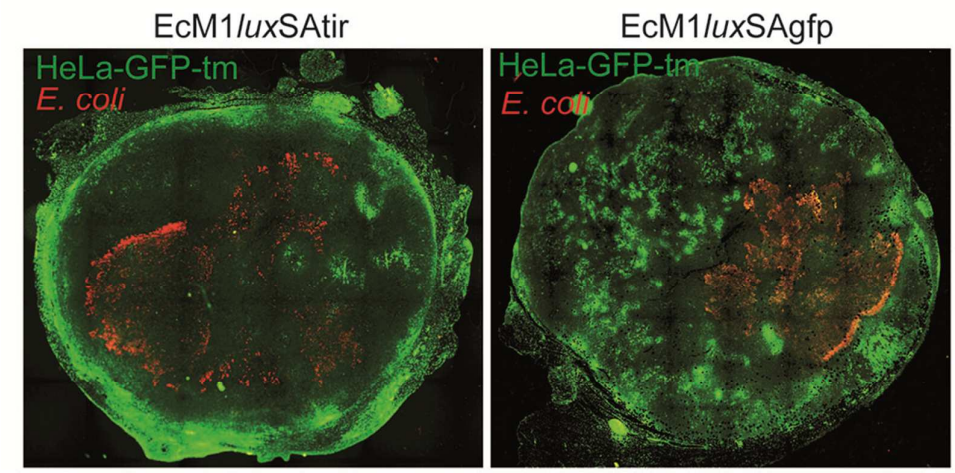

Figure S7. Bacterial distribution in solid HeLa-GFP-tm tumors. Histological cross-sections of colonized HeLa-GFP-tm solid tumors four days post-infection with ~1x10^7 CFU of EcM1*lux*SAtir or EcM1*lux*SAgfp strains per mouse, as indicated. Bacteria were stained with anti-*E. coli* polyclonal antibodies and anti-rabbit-Alexa-594 antibodies (red). The green color corresponds to the fluorescence of GFP in HeLa-GFP-tm cells.