Synchronized cycles of bacterial lysis for in vivo delivery

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背景

化疗不能精确作用于实体瘤，容易“误伤”周围组组织与细胞，表现为疗效不显著和副作用明显；

如何打入实体肿瘤的内部?

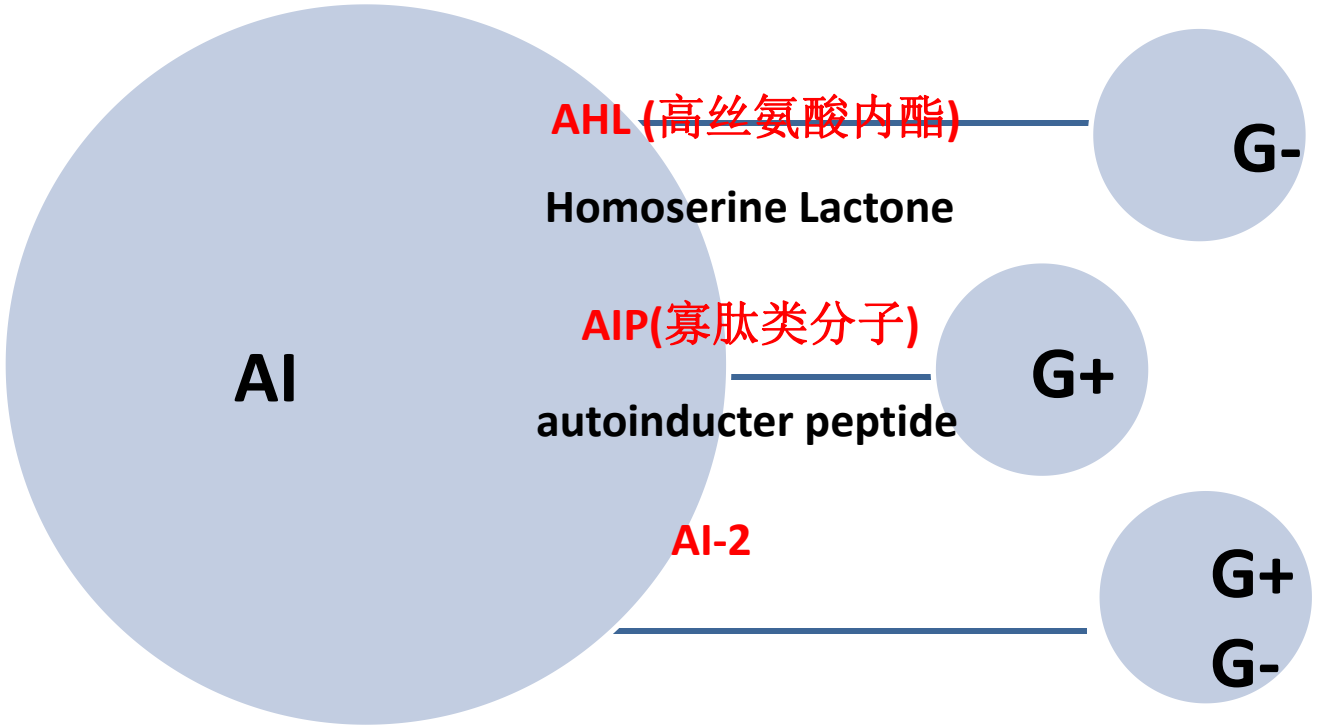
在不断的进化过程中，有些细菌获得了优先在实体瘤病灶环境中生长的特性；

研究者们尝试让细菌携带治疗实体瘤的药物到达病灶环境，进行精确、有效地杀伤肿瘤细胞，然而：细菌增殖较快，数量呈现指数增长，其后果是杀伤力太强，人体承受不住。

如何解决病灶环境中携带治疗药物的细菌数量过多的问题?

背景

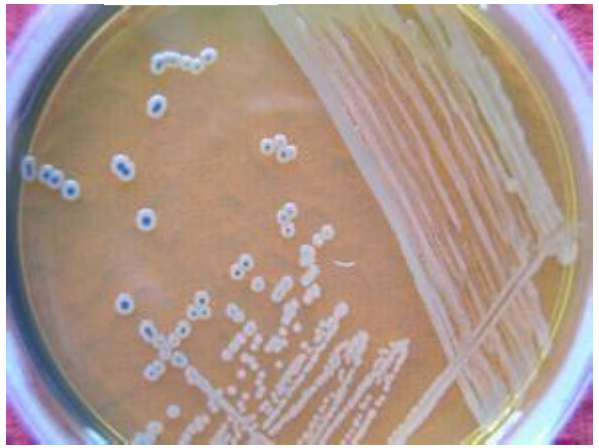
群体感应（Quorum Sensing）：一些细菌之间存在信息交流，合成并释放一种被称为自诱导物质(autoinducer, AI) 的信号分子，胞外的AI浓度能随细菌密度的增加而增加，达到一个临界浓度时，AI能启动菌体中相关基因的表达，调控细菌的生物行为。

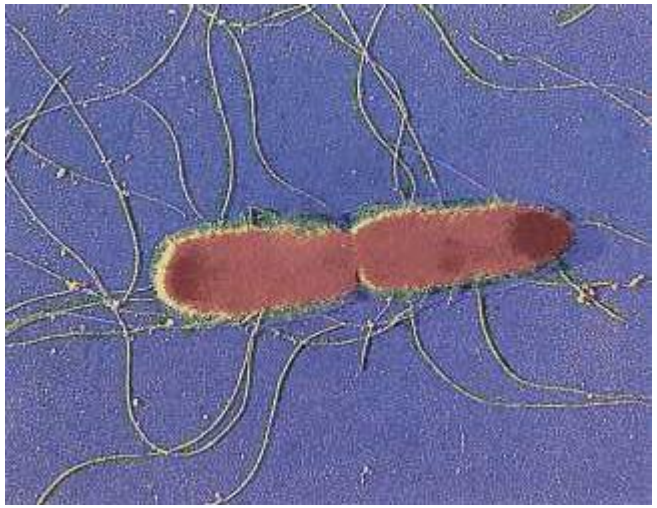


扩大地盘！

AI can diffuse to neighbouring cells and thus provides an intercellular synchronization mechanism

背景





沙门氏菌(*Salmonella* sp.)

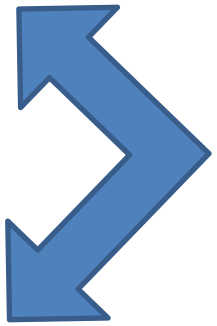
沙门氏菌具有复杂的抗原结构，约有1000多种。一般沙门氏菌具有菌体(O)抗原、鞭毛(H)抗原和表面抗原(荚膜或包膜抗原)三种抗原。

感染沙门氏菌的人或带菌者的粪便污染食品，可使人发生食物中毒。

当前研究

构建了三种*Salmonella* sp.工程菌，分别表达三种抗癌药物。

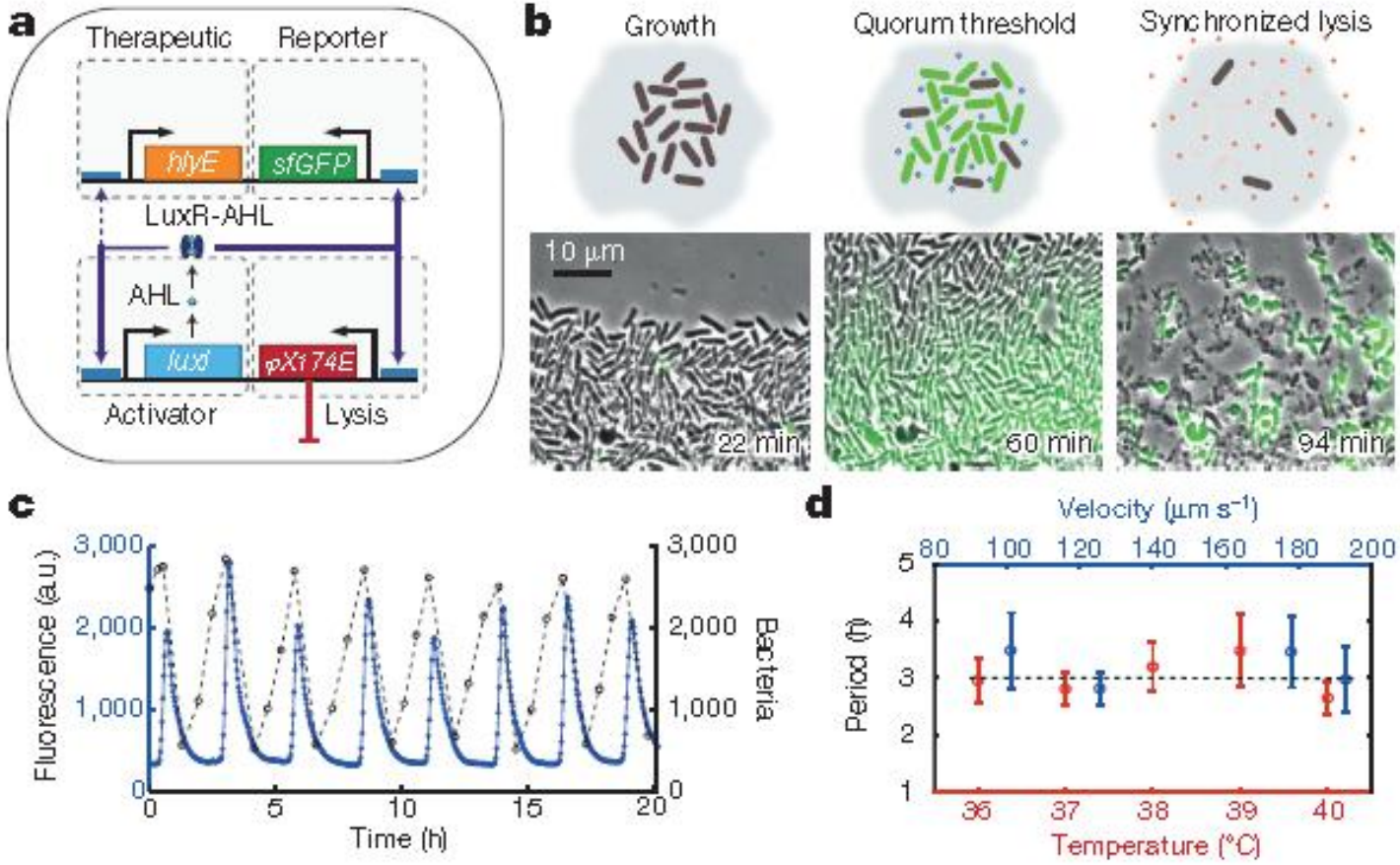
利用细菌的群体感应现象，在*Salmonella* sp.工程菌中装上可以产生AHL的表达系统，然后再安装一个受AHL浓度控制的自杀系统。

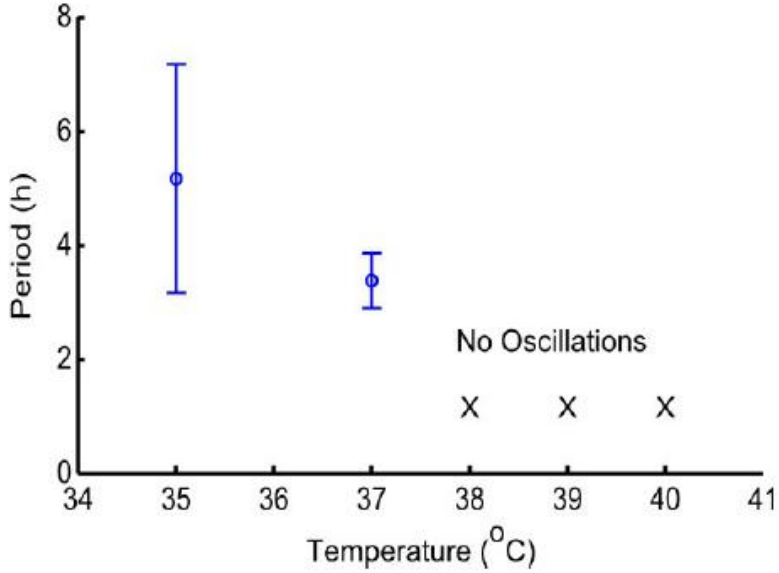


使工程菌数量达到一定域值后，发生大规模同时自杀同步裂解的行为，将药物释放。幸存少量的工程菌，再次进行繁殖与同步裂解的周期。

- ✓ **解决了药物精确定位于肿瘤细胞的问题**
- ✓ **解决了杀伤力太强的问题。**

结果：肿瘤组织明显缩小，延长50%的寿命。（肿瘤萎缩）





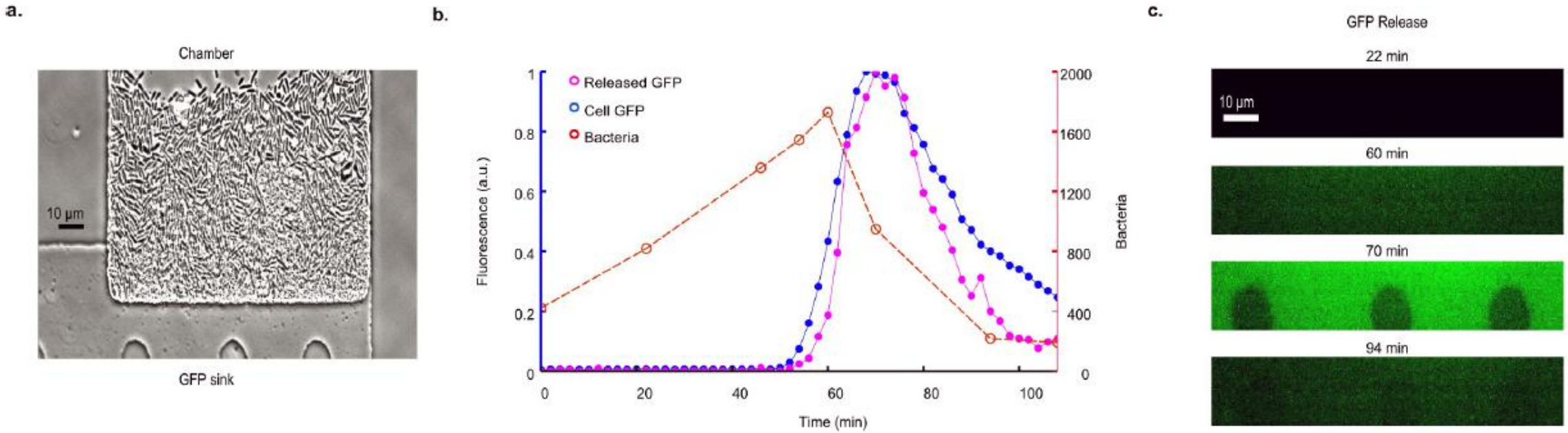
Salmonella enterica* subsp. *enterica typhimurium

鼠伤寒减毒肠道沙门氏菌亚种

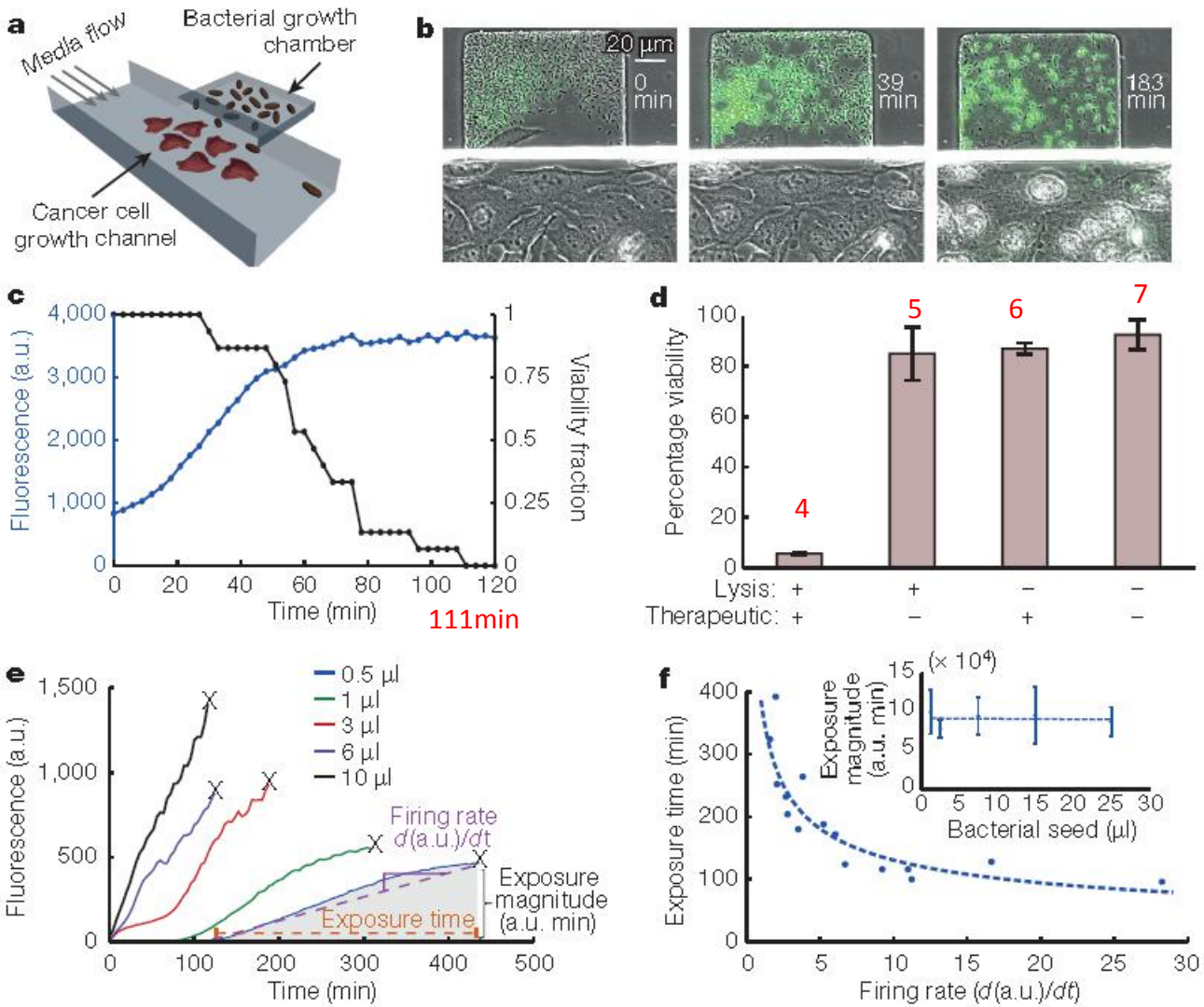
Construction and characterization of the SLC (synchronized lysis circuit) 同步裂解循环

Extended Data Table 1 | A list of strains and respective plasmids used in this study

Strain #	Strain Name	Host Bacterium	Plasmid(s)
1	MOD47	SL1344, M913	pTD103 luxI (-LAA) sfGFP + pZA35 X714E (+LuxR)
2	MOD46a	SL1344, M913	pTD103 luxI sfGFP + pZA35 X714E (+LuxR)
3	MOD67	SL1344, M913	pTD103 luxI (-LAA) sfGFP + pZA35 X714E (+LuxR) ptac::HlyE
4	MOD61	SL1344, ELH1301	pTD103 luxI sfGFP + pZA35 X714E (+LuxR) ptac::HlyE
5	MOD64	SL1344, ELH1301	pTD103 luxI sfGFP + pZA35 X714E (+LuxR)
6	MOD65	SL1344, ELH1301	pZA35 ptac::HlyE
7	ELH1301	SL1344, ELH1301	N/A
8	MOD105	SL1344, ELH430	pZE25 luxI luxCDABE hok/alp + pZA35 X714E (+LuxR) pLux::HlyE hok/alp
9	EcN-luxCDABE	Nissle 1917	N/A
10	MOD101	SL1344, ELH1301	pZE25 luxI luxCDABE hok/alp + pZA35 X714E (+LuxR) pLux::HlyE hok/alp
11	MOD102	SL1344, ELH1301	pZE25 luxI luxCDABE hok/alp + pZA35 X714E (+LuxR) ptac::HlyE hok/alp
12	MOD69	SL1344, ELH1301	pTD103 LuxCDABE hok/alp + pZA35 X714E (+LuxR) ptac::HlyE hok/alp
13	MOD29	JS006, BW25113	pTD103 luxI sfGFP + pZA35 X714E (+LuxR)
14	MOD110	SL1344, ELH1301	pZE25 luxI luxCDABE hok/alp + pZA35 X714E (+LuxR) pLux::CDD-iRGD hok/alp
15	MOD112	SL1344, ELH1301	pZE25 luxI luxCDABE hok/alp + pZA35 X714E (+LuxR) ptac::mCCL21 hok/alp



Investigating lysis-mediated intracellular Release



***In vitro* co-culture with HeLa cell**

a, Schematic of the microfluidic co-culture with cancer cells and bacteria. Fluidic resistance was modified in this chip to achieve stable near-stagnant flow reduction to allow for cancer cell adherence and for diffusion of released therapeutic from the trap to the channel.

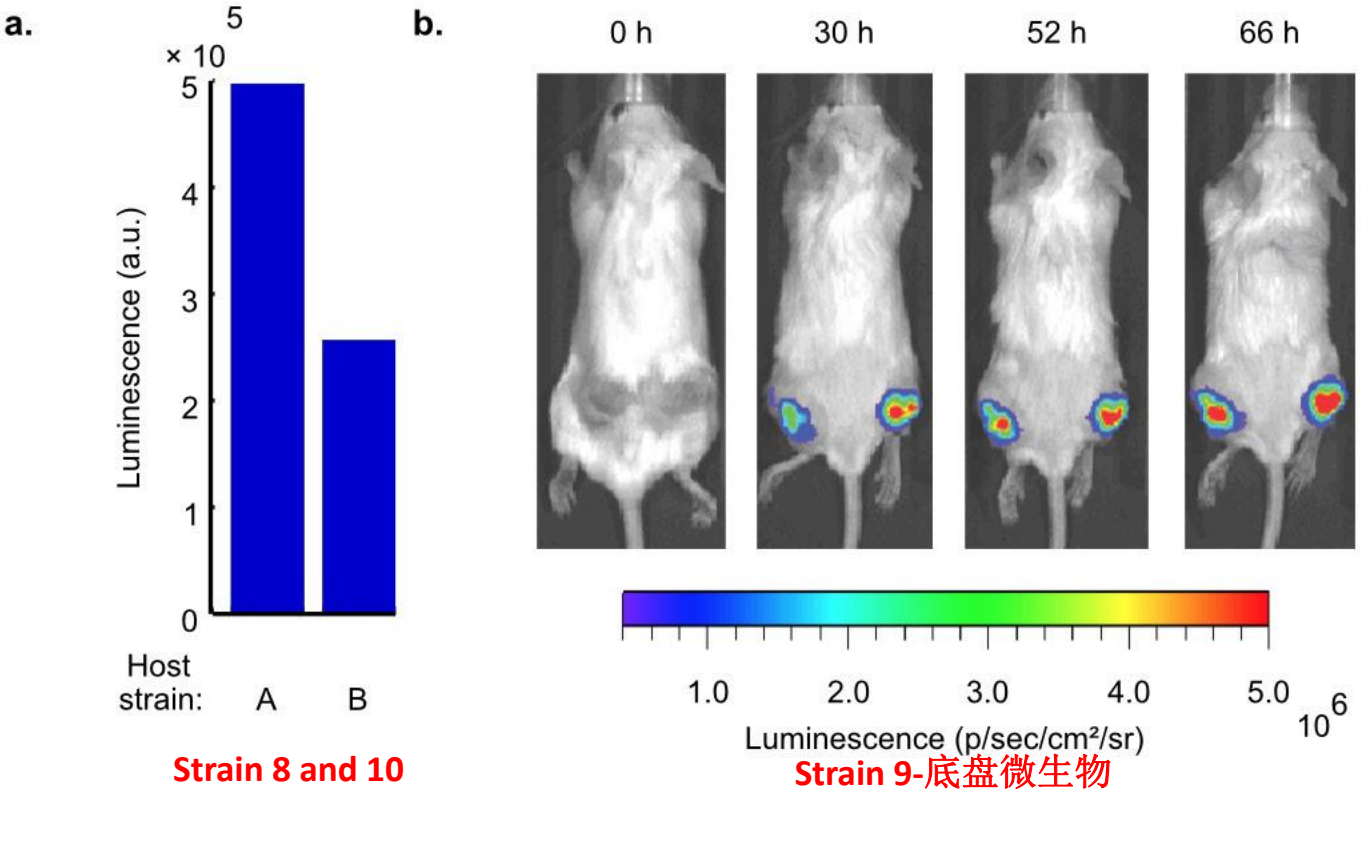
b, Frames from the co-culture time series sequentially visualizing *S. Typhimurium* (strain 3) firing, lysis and HeLa cell death.

c, Fluorescent profile of the bacteria and HeLa cell viability fraction (number of live cells/number of dead cells in image frames) from b with time.

d, Percentage viability of HeLa cells co-cultured with supernatant from *S. Typhimurium* culture harbouring the SLC + HlyE (strain 4), the SLC only (strain 5), constitutive hlyE only (strain 6), or no plasmid (strain 7). Error bars indicate ± 1 s.e. averaged over three measurements.

e, Fluorescence profile of the SLC + HlyE (strain 4) co-cultured with HeLa cells at various initial seeding densities. The x symbols on the graph mark the point of complete HeLa cell death.

f, The toxin exposure time, measured from the initial presence of fluorescence to HeLa cell death, as a function of the sfGFP production rate (see example in e). Although the time to death depends on seeding, the total magnitude of exposure remains conserved (inset). Error bars indicate ± 1 s.e. for three measurements.



Strain 8 and 10

Strain 9-底盘微生物

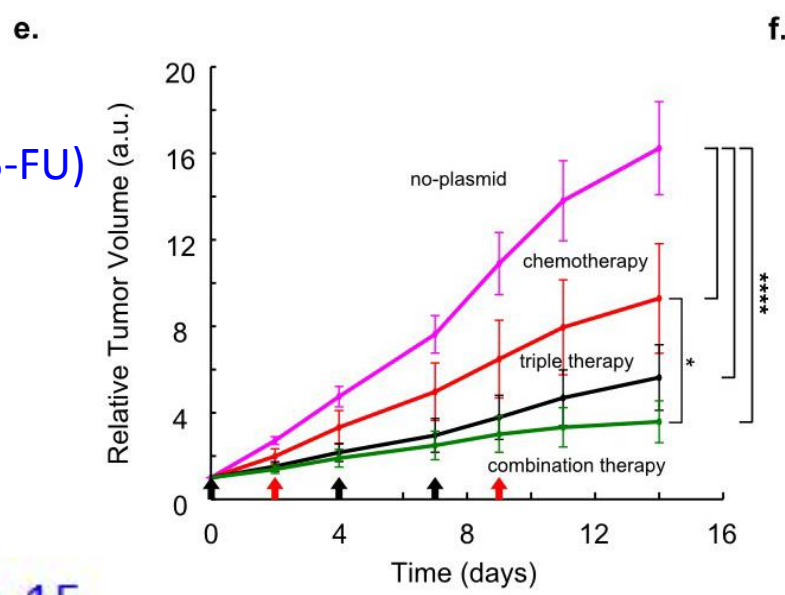
Strain 8 and 9

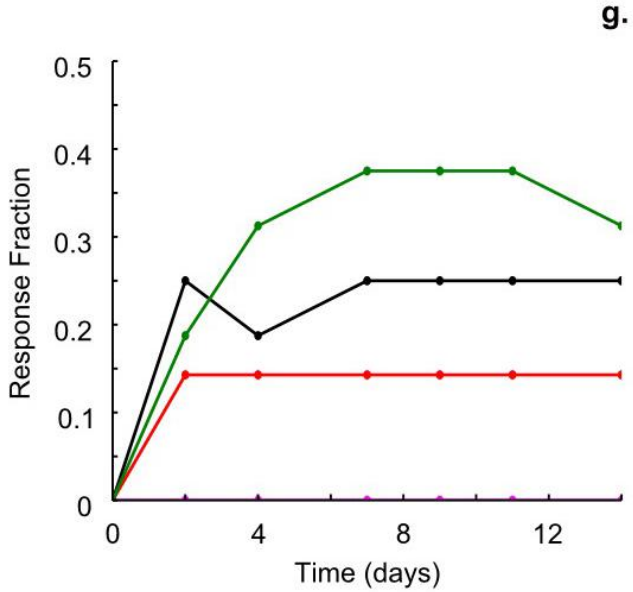
Strain 9-底盘微生物

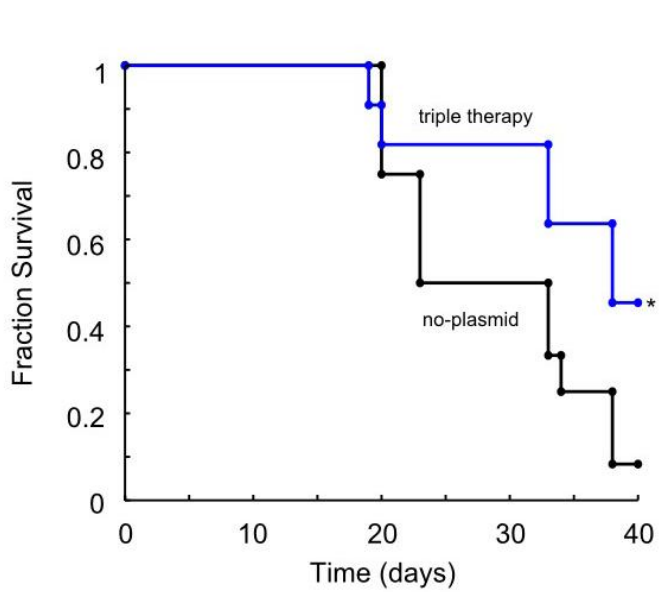
***In vivo* expression and therapy testing with MC26 cell-1**

结直肠癌

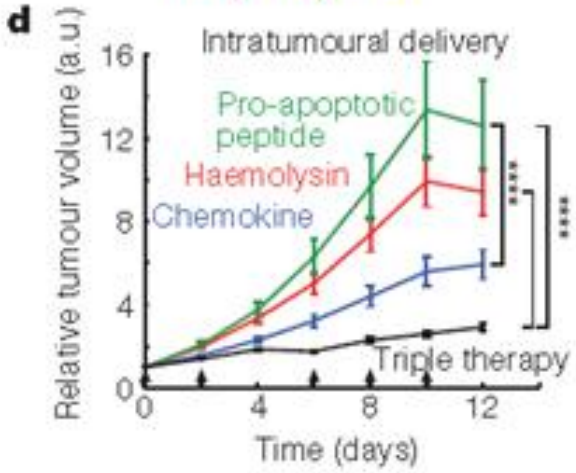
5-fluorouracil (5-FU)





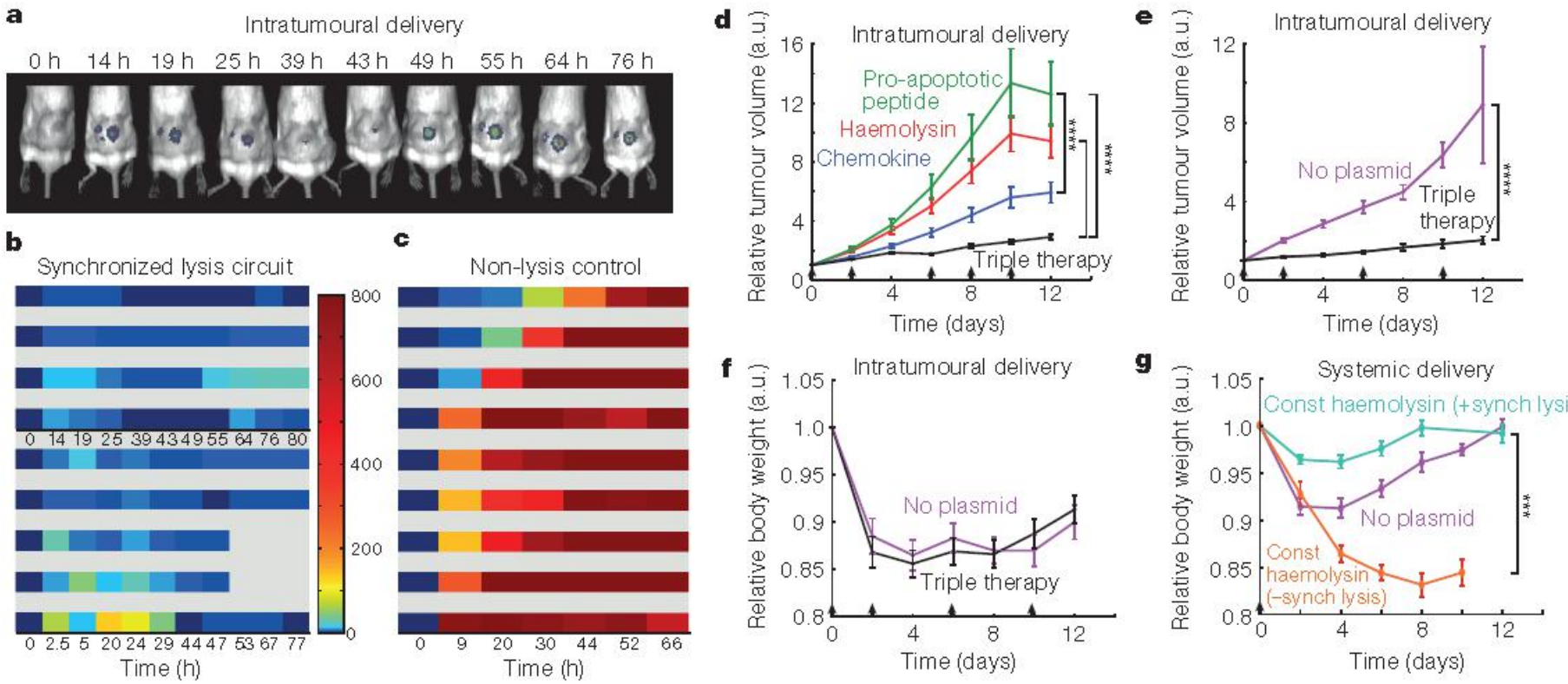


14, 10, 15



***In vivo* expression and therapy testing with MC26 cell-2**
结直肠癌

14, 10, 15



***In vivo* co-culture with MC26 cell**

Pro-apoptitic peptide（CDD-iRGD）：生产一种蛋白这种药物，从癌细胞内部着手，诱导肿瘤细胞自杀身亡。**strain14**

Haemolysin (HylE)：溶血素分子，通过破坏肿瘤的细胞膜，达到摧毁肿瘤细胞的目的。**strain10**

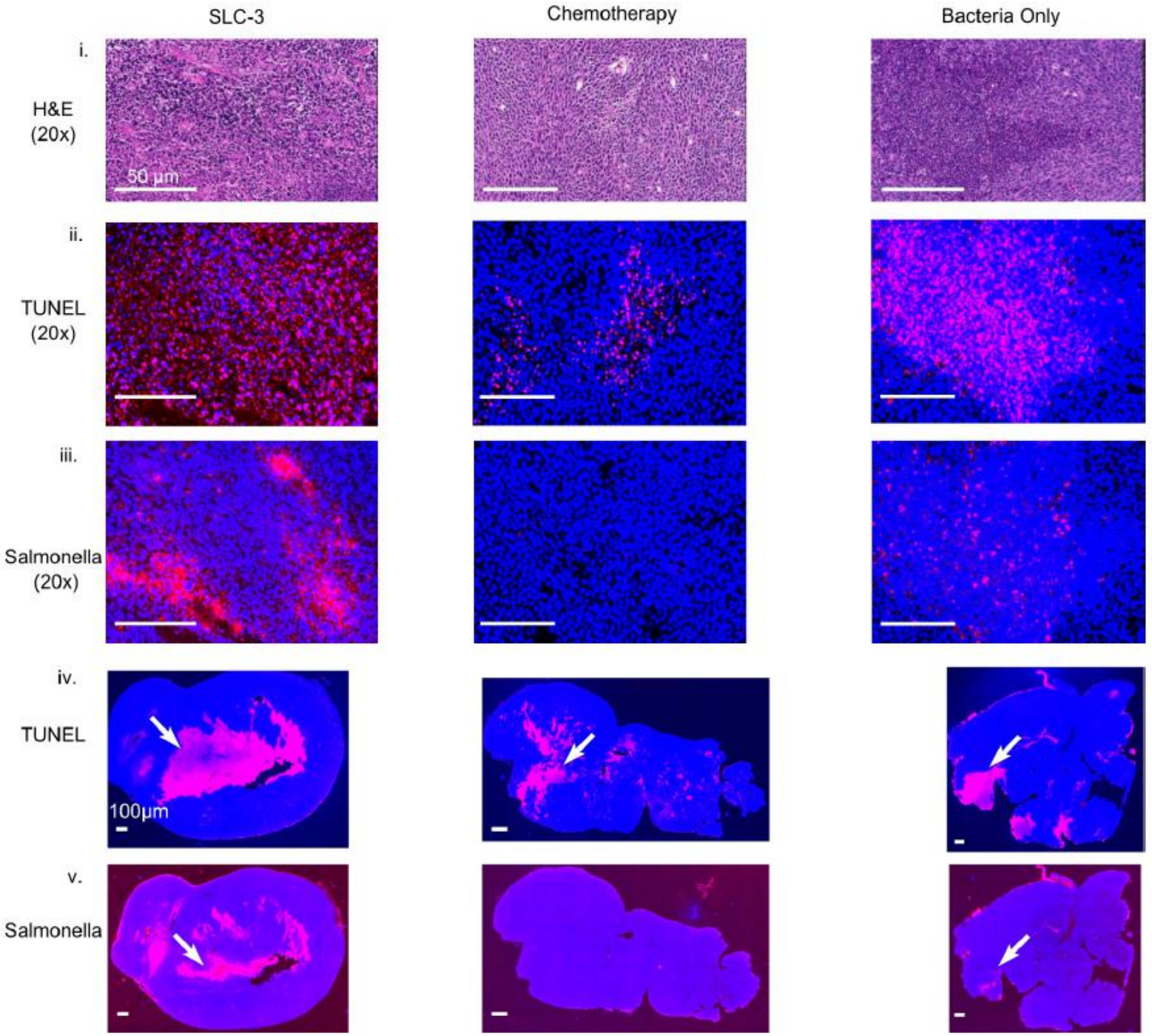
Chemokine（ CCL21）：产生另一种蛋白，这个蛋白会激活人体免疫系统，调动人体T细胞和DC细胞等围攻肿瘤。**Strain15**

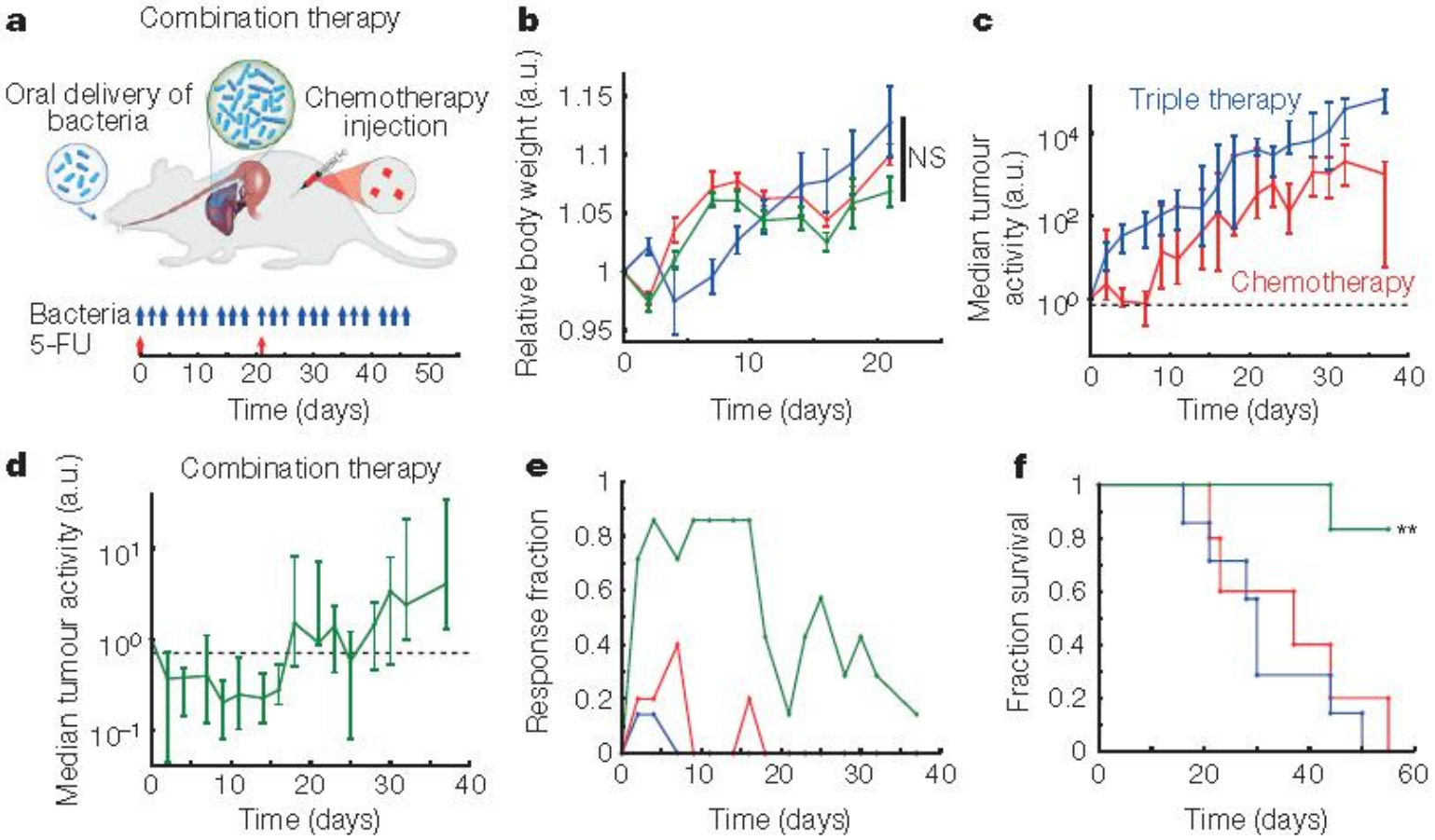
Histological analysis

Se

***Salmonella*: observed by anti-*Salmonella* antibodies, showing localization of *Salmonella* within tumours**

TUNEL staining: indicated higher levels of apoptosis and cell death in SLC-3 treated tumours





SLC bateria:口服
5-FU：腹腔内注射

肝内同系结直肠癌转移模型验证表明：将**SLC bacteria**与**5-FU**结合治疗结直肠癌效果最好，可大大缩小肿瘤块体积，延长癌症小鼠的寿命（约**50%**）

读后

SHARE REPORT

Commensal *Bifidobacterium* promotes antitumor immunity and facilitates anti–PD-L1 efficacy

Ayelet Sivan[1,*], Leticia Corrales[1,*], Nathaniel Hubert[2], Jason B. Williams[1], Keston Aquino-Michaels[3], Zachary M. Earley[2], Franco W. Benyamin[1], Yuk Man Lei[2], Bana Jabri[2], Maria-Luisa Alegre[2], Eugene B. Chang[2], Thomas F. G

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* These authors contributed equally to this wo

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DOI: 10.1126/science.aac4255

SHARE RESEARCH ARTICLE CANCER

Intratumoral injection of *Clostridium novyi*-NT spores induces antitumor responses

Nicholas J. Roberts[1,*], Linping Zhang[2,*], Filip Janku[3,*], Amanda Collins[2,*], Ren-Yuan Bai[4,*], Verena Staedtke[4,5,*], Anthony W. Rusk[6], David Tung[2], Maria Miller[2], Jeffrey Roix[2], Kristen V. Khanna[6], Ravi Murthy[7], Robert S. Benjamin[8], Thorunn Helgason[3], Ariel D. Szvalb[9], Justin E. Bird[10], Sinchita Roy-Chowdhuri[11], Halle H. Zhang[2], Yuan Qiao[1], Baktiar Karim[12], Jennifer McDaniel[13], Amanda Elpiner[14], Alexandra Sahora[15], Joshua Lachowicz[16], Brenda Phillips[17], Avenelle Turner[18], Mary K. Klein[19], Gerald Post[13], Luis A. Diaz Jr[1,20], Gregory J. Riggins[4], Nickolas Papadopoulos[1], Kenneth W. Kinzler[1], Bert Vogelstein[1], Chetan Bettegowda[1,4], David L. Huso[12], Mary Varterasian[2], Saurabh Saha[2,*,†] and Shibin Zhou[1,*]

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* These authors contributed equally to this work.

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DOI: 10.1126/scitranslmed.3008982

- 该遗传回路的稳定性？

裂解质粒的丢失

AHL域值的变化 → 细菌数量的变化 →

- 有没有更好的底盘微生物或者有助于机体免疫物？

Bifidobacterium （双歧杆菌）

Clostidrium novyi （诺维氏梭菌）

……

ARTICLE

Insights into t potential of m

Christian Rinke[1], Patrick Schwientek[1], Alex
Aaron Darling[3,4], Stephanie Malfatti[1], Brand
George Tsiamis[8], Stefan M. Sievert[9], Wen-T
Ramunas Stepanauskas[5], Edward M. Rubin

Genome sequencing enhances our unde and functional diversity that shapes th limited phylogenetic breadth, owing to apply single-cell genomics to target and belonging to 29 major mostly unchart additional genomic information, we a propose two new superphyla. We unco and challenge established boundaries be stop codon, an archaeal-type purine sy Bacteria. The single-cell genomes also habitats, facilitating organism-level in representation of the tree of life and pr on our planet.

Microorganisms are the most diverse and abundan Earth, occupying every possible metabolic niche. these organisms have not been obtained in pure cu recently become aware of their presence mainly independent molecular surveys based on conserved small subunit ribosomal RNA; SSU rRNA) or thr cing (metagenomics)[1,2]. As an increasing numbe

（肠道、高温、高盐、高压、洞穴...）

ARTICLE

An environm large and dist

Micheal C. Wilson[1,2*], Tetsushi Mori[3*], Ch
Christine Gernert[6], Ursula A. E. Steffens[2],
Alexander O. Brachmann[1], Cristian Gurgu
Ikuro Abe[9], Shigeki Matsunaga[5], Jörn Kal

Cultivated bacteria such as actinomyce such 'talented' producers represent or uncultured majority is generally perce whether taxa containing talented bac covery of such producers. Two phyl megabases and multiple, distinct bio sponge *Theonella swinhoei*. Almost all single phylotype. '*Entotheonella*' spp. here as candidate phylum 'Tectomicr compounds provide significant opport

More than half of the known natural products w tumour or antiviral activity are of bacterial orig pounds were isolated from cultivated representat groups: filamentous actinomycetes, Myxobacte members of the genera *Pseudomonas* and *Bac* teria, which are proposed to form 70% of all kn represent a particularly promising source for n taxa. However, except for individual biosynth from environmental sources[3,4], the true meta microbes remains unexplored. Two such pat

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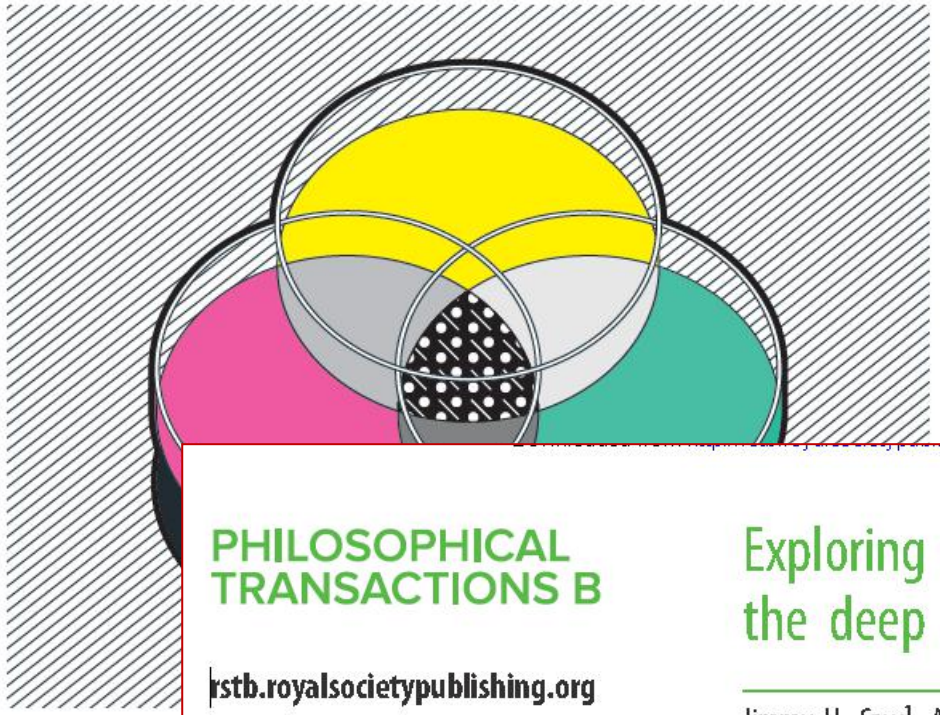

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Microbiologists a universe of unkno

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Research

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Accepted: 26 May 2015

One contribution of 17 to a theme issue 'Eukaryotic origins: progress and challenges'.

Exploring microbial dark matter to resolve the deep archaeal ancestry of eukaryotes

Jimmy H. Saw[1], Anja Spang[1], Katarzyna Zaremba-Niedzwiedzka[1], Lina Juzokaite[1], Jeremy A. Dodsworth[2,†], Senthil K. Murugapiran[2], Dan R. Colman[3], Cristina Takacs-Vesbach[3], Brian P. Hedlund[2], Lionel Guy[4] and Thijs J. G. Ettema[1]

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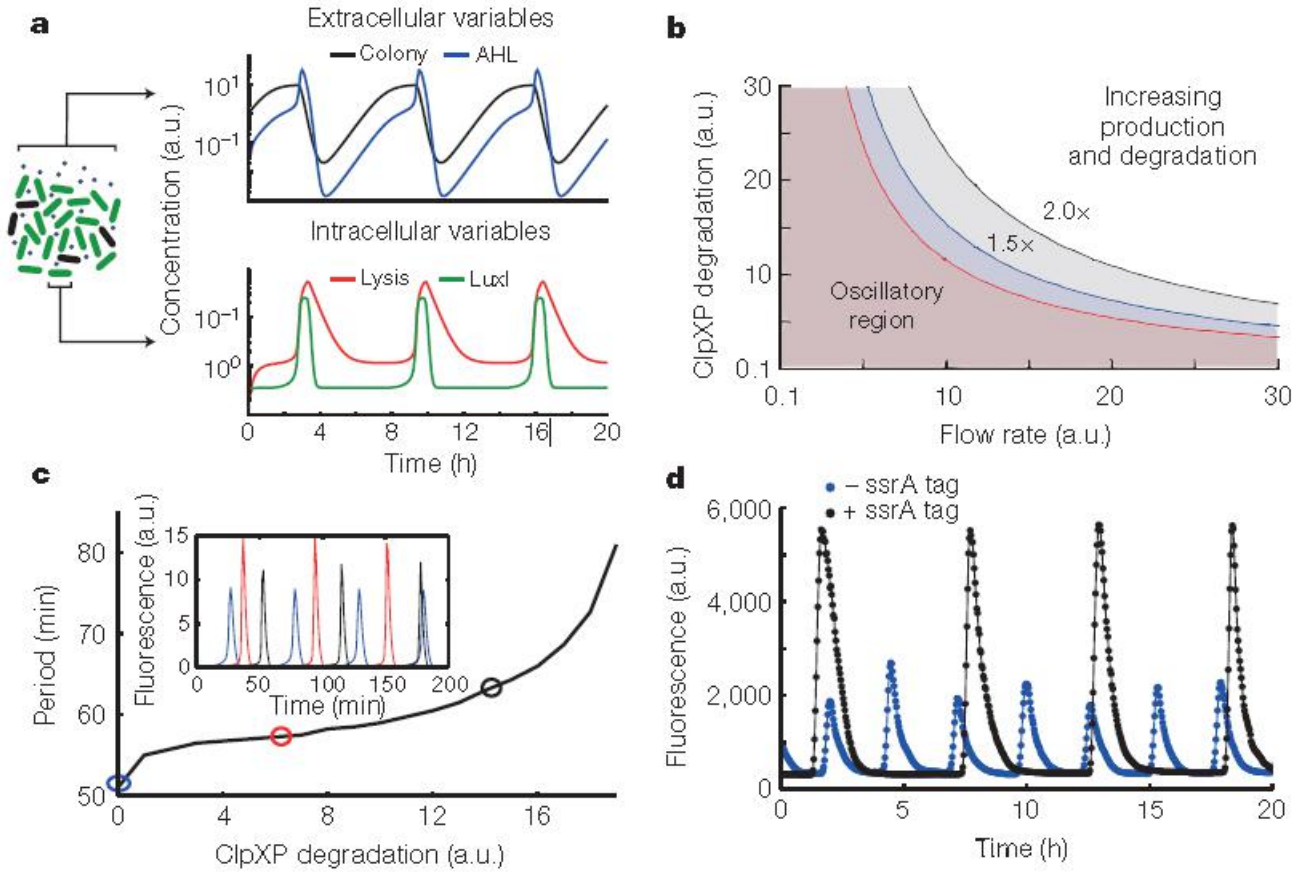
[2]School of Life Sciences, University of Nevada Las Vegas, Las Vegas, NV, USA
[3]Department of Biology, University of New Mexico, Albuquerque, NM, USA
[4]Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden

The origin of eukaryotes represents an enigmatic puzzle, which is still lacking a number of essential pieces. Whereas it is currently accepted that the process of eukaryogenesis involved an interplay between a host cell and an alphaproteobacterial endosymbiont, we currently lack detailed information regarding the identity and nature of these players. A number of studies have provided increasing support for the emergence of the eukaryotic host cell from within the archaeal domain of life, displaying a specific affiliation with the archaeal TACK superphylum. Recent studies have shown that genomic exploration of yet-uncultivated archaea, the so-called archaeal 'dark matter', is able to pro-

THANK YOU!



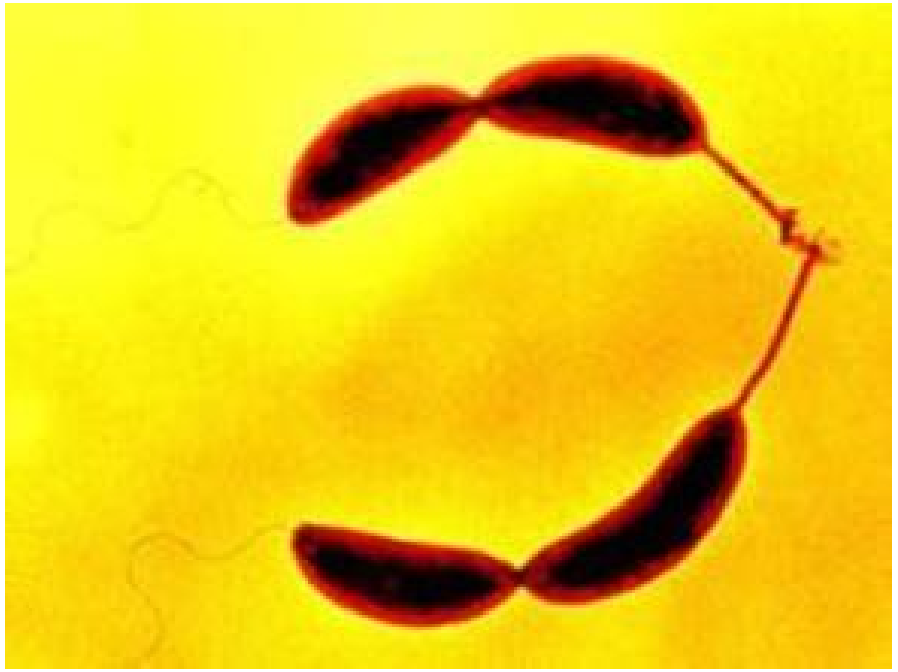
a, The model consists of intracellular variables (lysis protein E and LuxI concentrations) and extracellular variables (colony size and AHL concentrations). A time series of colony size (black), colony AHL (blue), intracellular LuxI (green) and lysis protein concentrations (red) Are shown on the right.

b, The region in the model parameter space for **ClpXP**-mediated degradation and flow where the model output is oscillatory increases with higher production and degradation terms.

c, Results from the computational model showing the ability to tune the oscillatory period by varying ClpXP-mediated degradation of LuxI.

d, Fluorescence profiles showing lysis oscillations for LuxI ssrA (black, strain 2) and LuxI non-ssrA (blue, strain 1) tagged versions of the circuit.

ssrA tag (mediated protein degradation)可以增加SLC的稳定性。



新月柄杆菌
Caulobacter crescentus

ClpXP蛋白酶控制细菌细胞的生长与裂解，存在于多个微生物物种中。

ClpXP蛋白酶存在于细菌各个不同的生长阶段，但是其仅仅会在特定的时刻来破坏其靶点。

Cell, 2014