Supplementary Information for

A microbial-electronic optical communication language for intestinal monitoring and regulation

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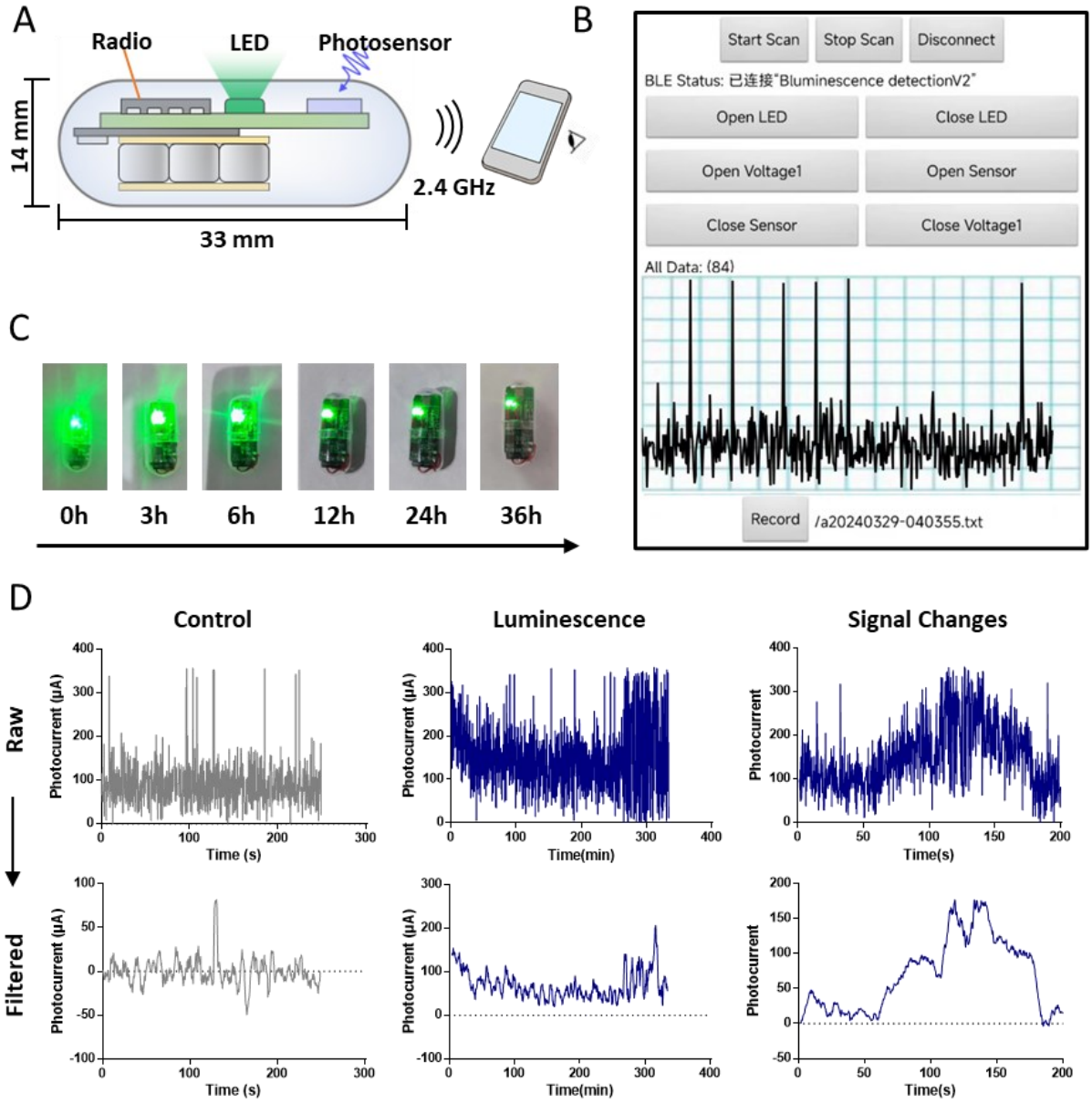

Fig. S1. Basic properties of the electronic capsule used in this work.

(**A**) The capsule was consisted of a photosensor to detect EcN bioluminescence, a LED to activate optogenetic circuit in EcN, and a wireless Bluetooth module sending 2.4 GHz signals to smartphones. The section diameter of the electronic capsule was about 14 mm and the long-end length was about 33 mm (**B**) The user interface of the APP of smartphones when detecting and recording microbial bioluminescence signal. (**C**) The LED in the electronic capsule could emit green light for at least 36 h. (**D**) The processing of raw photoelectric sensing data through a sliding average filtering algorithm for smooth output when the capsule was placed under different conditions of luminescence.

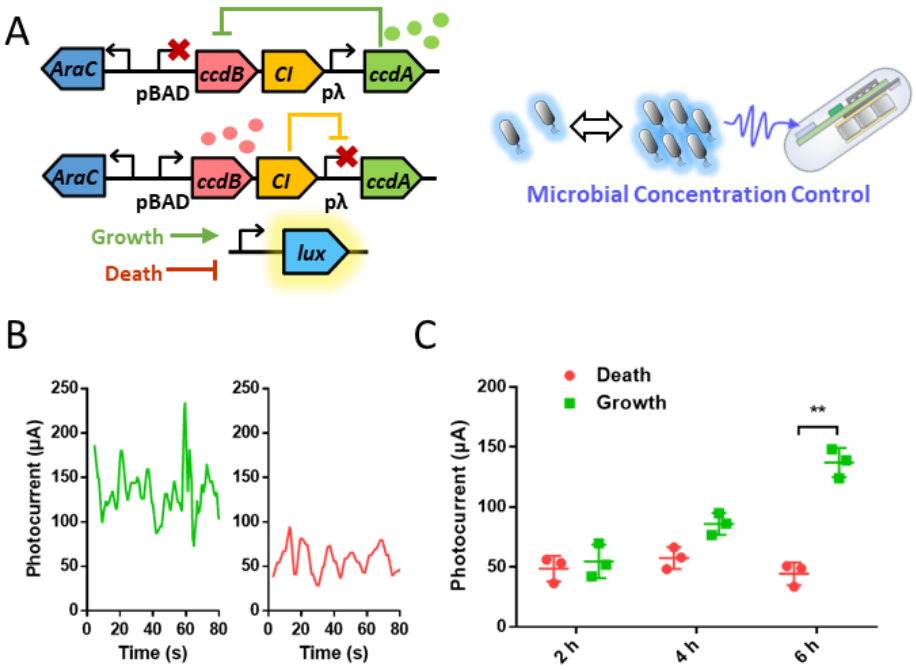

Fig. S2. Monitoring of microbial cell growth-death change using electronic capsule.

(**A**) A scenario was devised to monitor arabinose-induced surviving bacterial density changes via electronic capsule. Wherein the engineered EcN was equipped with a survival-death switch genetic circuit controlled by L-arabinose as an inducer (**B**) The monitoring of the bioluminescence signals reflecting the engineered bacterial density changes via the electronic capsule in the form of photocurrent. (**C**) Detection of the different surviving strain groups with (Red) or without (Green) arabinose-induction via our electronic capsule. Data are presented as the mean ± SD. n=3. * $0.01<p<0.05$, ** $0.001<p<0.01$, *** $p<0.001$.

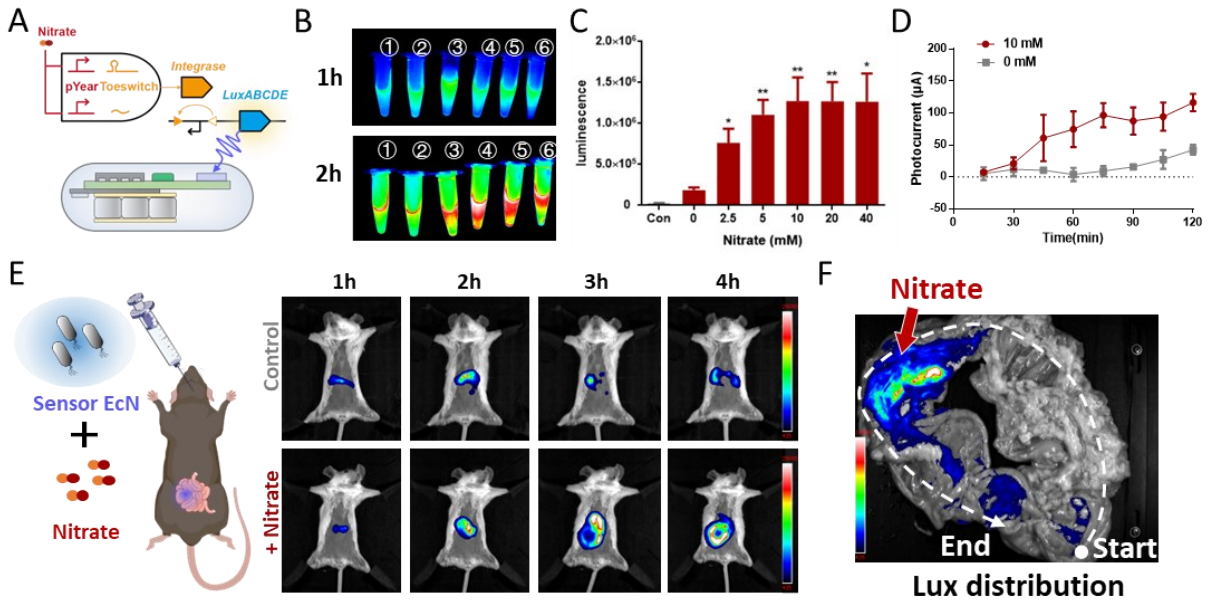

Fig. S3. Monitoring of microbial information in response to nitrate via electronic capsule.

(**A**) A designer genetic circuit for sensing nitrate to activate expression of an integrase to promote the expression of *luxABCDE* module to produce luciferase. (**B**) Bioluminescence changes of the luciferase-expressing EcN within 2 h after sensing different concentrations of KNO_3. From 1 to 6 respectively represented the strains in Eppendorf tubes sensing 0, 2.5, 5, 10, 20, 40 mM of KNO_3. (**C**) Measurement of the bioluminescence of the luciferase-expressing EcN sensing different conditions of nitrate. (**D**) Detection of the bioluminescence of the nitrate-sensing EcN within 2 hours via the electronic capsule. (**E**) The response of nitrate-sensing EcN to different concentrations of nitrate within 1 to 4 hours illustrated by bioluminescence changes. (**F**) The photo of the real swine intestinal section containing nitrate-sensing EcN used in the related experiments in Fig. 2F, G. The capsule was pulled from the "Start" point to the "End" point slowly to simulate the peristalsis in the gut. Data are presented as the mean ± SD. n=3. * $0.01<p<0.05$, ** $0.001<p<0.01$, *** $p<0.001$.

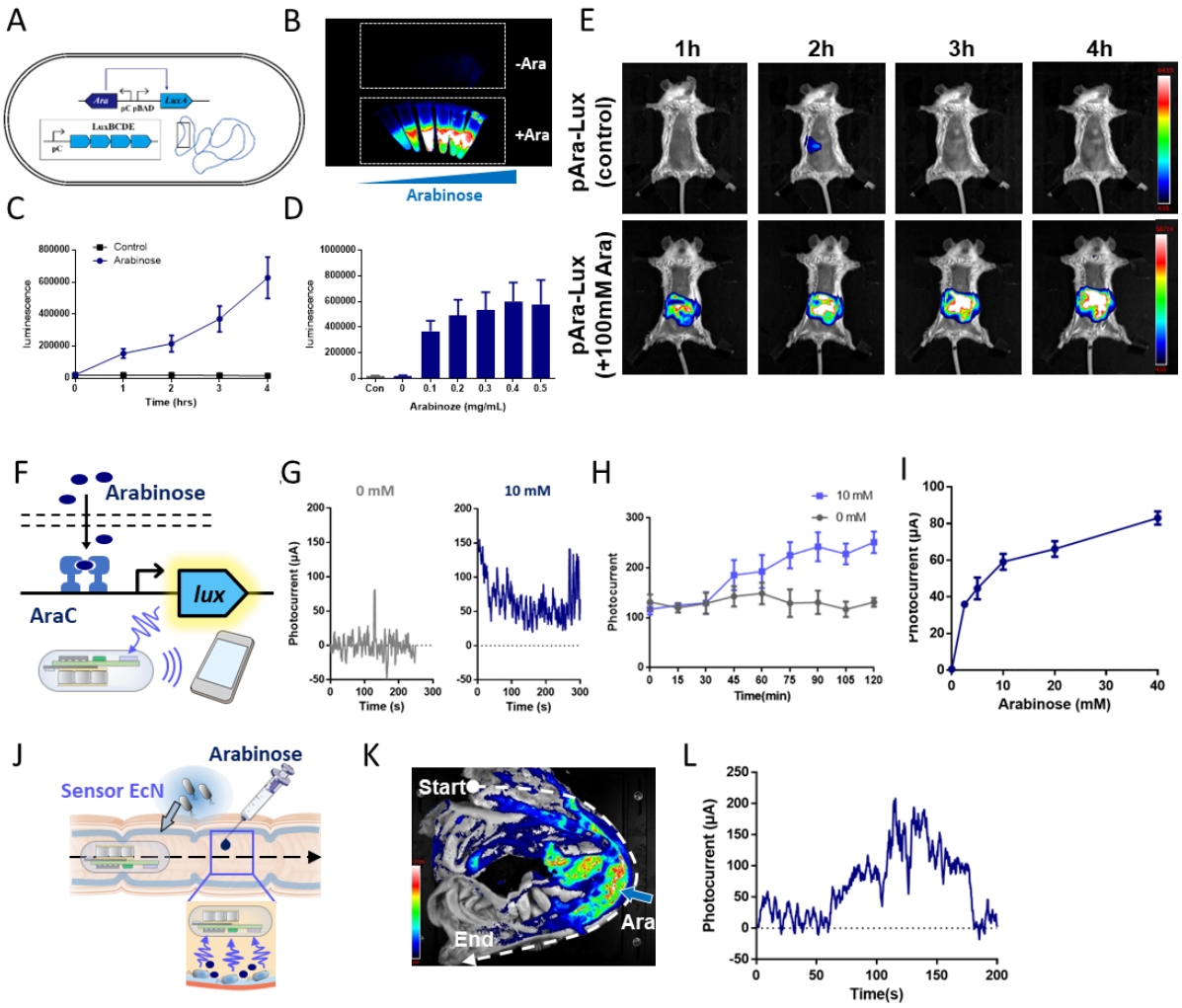

Fig. S4. Monitoring of microbial information in response to arabinose via electronic capsule. (**A**) A designer genetic circuit in EcN for sensing L-arabinose to activate expression of *luxA* combined with constitutively expressed *luxBCDE* in genome to produce luciferase. (**B**) Bioluminescence changes of the luciferase-expressing EcN within 2 h after sensing different concentrations of arabinose. From left to right respectively represented the strains in Eppendorf tubes sensing 0, 0,1, 0.2, 0.3, 0.4, 0.5 mg/mL of arabinose. (**C**) Measurement of the bioluminescence changes of the luciferase-expressing EcN after sensing 10 mM arabinose within 4 h. (**D**) Measurement of the bioluminescence of the luciferase-expressing EcN sensing different conditions of arabinose. (**E**) Detection of the bioluminescence changes of the arabinose-sensing EcN in mice model within 4 hours. (**F**) The cooperation of arabinose-sensing EcN with photo sensing electronic capsule *in vitro*. (**G**) Direct photocurrent changes in the electronic capsule when detecting the bioluminescence changes of the EcN sensing 10 mM arabinose. (**H**) Detection of the bioluminescence of the arabinose-sensing EcN within 2 hours via the electronic capsule. (**I**) Detection of the bioluminescence of the EcN sensing different concentrations of arabinose within 2 hours via the electronic capsule. (**J**) The monitoring of the nitrate distribution via the electronic capsule while passing through an intestinal tissue segment containing evenly distributed arabinose-sensing EcN. (**K**) The photo of the real swine intestinal section containing nitrate-sensing EcN

used in the related experiments in Fig. 2F, G. The capsule was pulled from the “Start” point to the “End” point slowly to simulate the peristalsis in the gut. (**L**) The changes of photocurrent presented by electronic capsule when it passed slowly through the intestinal section. Data are presented as the mean ± SD. n=3. * $0.01<p<0.05$, ** $0.001<p<0.01$, *** $p<0.001$.

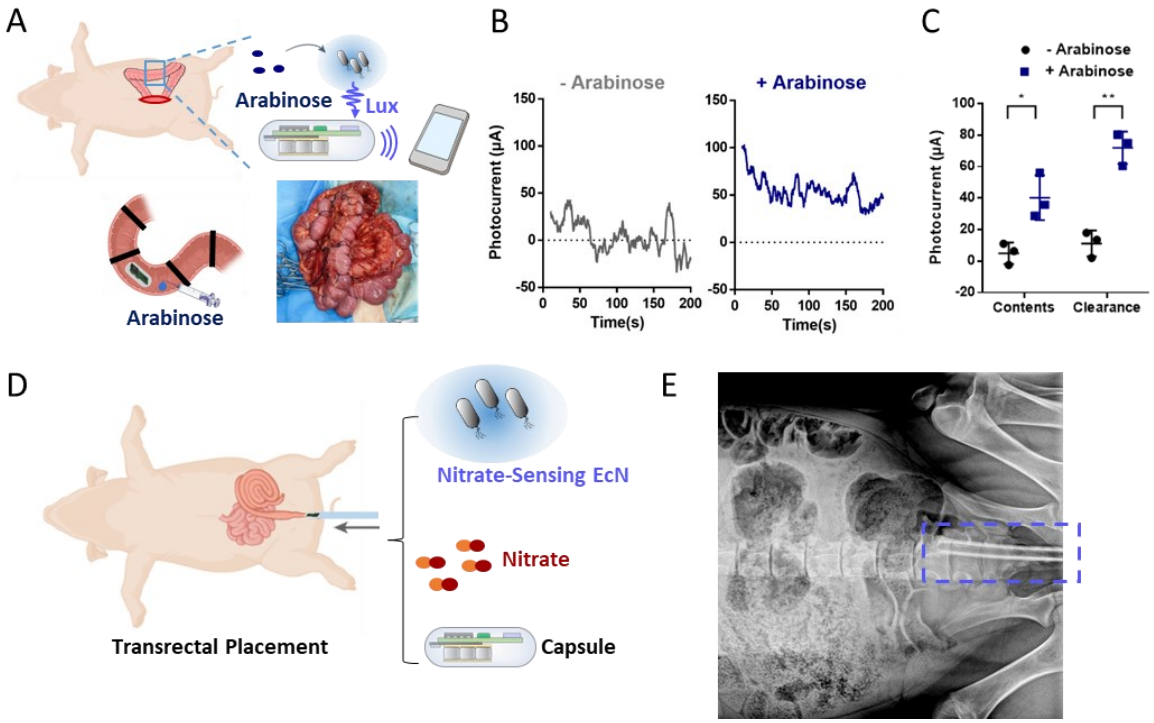

Fig. S5. Monitoring of microbial information in the gut of live pigs.

(**A**) To realistically simulate the process of monitoring the bioluminescent signals of the engineered EcN by using the capsule in the colon in *vivo*, the colon of anesthetized pigs was exposed through surgery for simulation experiments. The colon was tied into several segments by wrapping it with sutures to provide different operations. Capsule was surgically placed to monitoring the statuses in different segments. (**B**) Comparison of the photocurrent values when adding arabinose or not. (**C**) Regardless of whether intestinal contents were cleared or not, the capsule was loaded to monitor the response of arabinose-sensing EcN to the inducer arabinose in the form of increased photocurrent. (**D**) Simulation of diagnosis of nitrate in liv pigs by introducing the nitrate-sensing EcN, the nitrate, and the capsule in the rectum. (**E**) X-ray photo of the pig where a moderately soft pipeline was used to assist placing of the electronic capsule inside the rectum. Data are presented as the mean ± SD. n=3. * $0.01<p<0.05$, ** $0.001<p<0.01$, *** $p<0.001$.

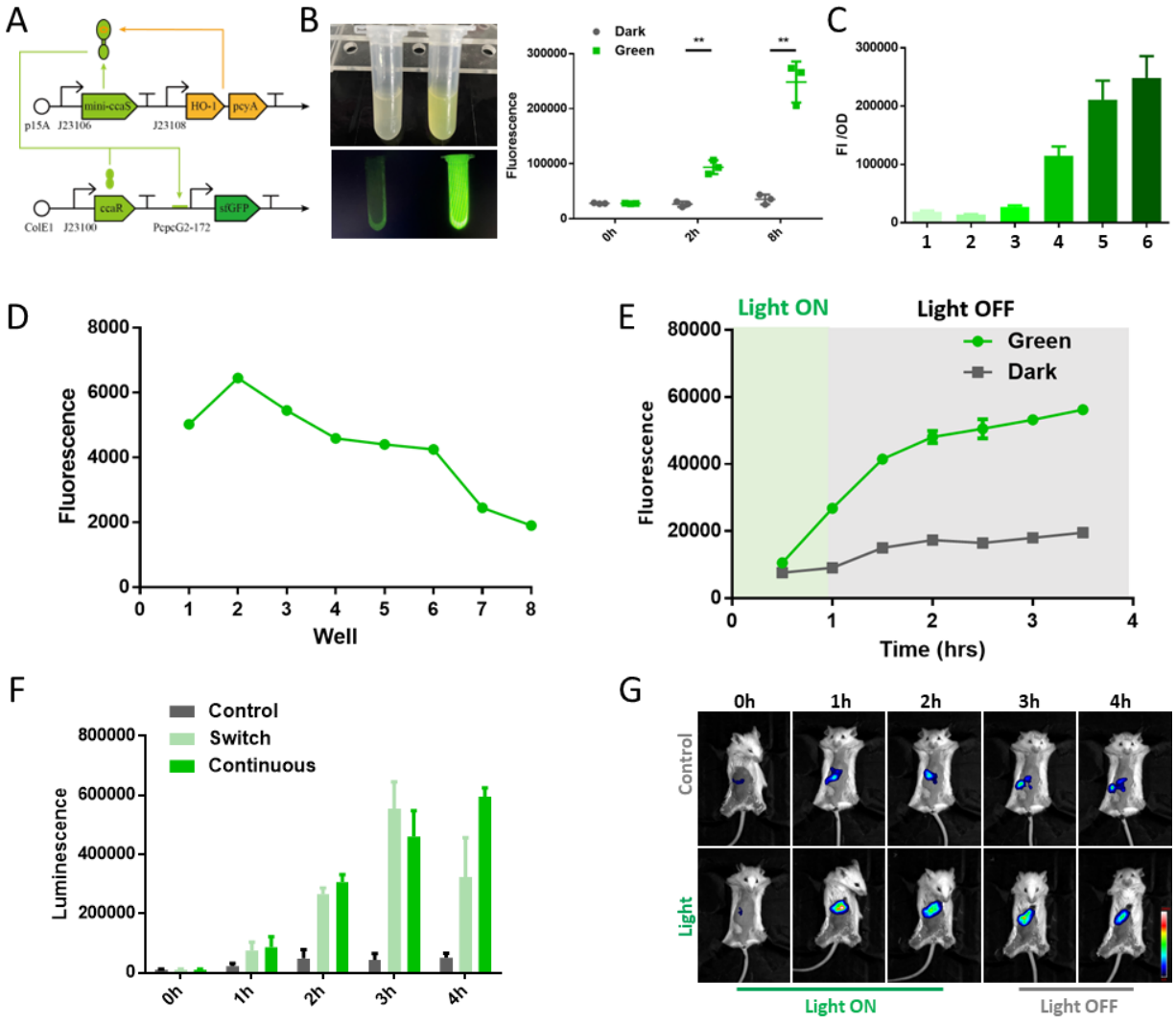

Fig. S6. The characterization of the EcN equipped with CcaS-CcaR two-component optogenetic circuit under the control of remote green light.

(**A**) Construction of the CcaS-CcaR two-component optogenetic circuit in EcN. (**B**) The activation of GFP expression after 2 h irradiation on the EcN tubes with green light. (**C**) Comparison of activated expression strength among CcaS-CcaR two-component optogenetic circuit and other inducible or constitutive expression circuits harbored in EcN. 1: EcN-MUT1-GFP; 2: EcN-MUT2-GFP; 3: EcN-SC101-GFP; 4: EcN-RSF-GFP; 5: EcN-Ara-GFP (after 8 hours of 0.2% arabinose induction); 6: EcN-Opto-GFP (after 8 hours of green light induction). (**D**) The changes of the GFP fluorescence density after 2 or 8 h green light irradiation on the EcN. Measurement of the GFP fluorescence densities in the EcN tubes in Fig. 3B experiment. (**E**) The green light was shut off after 1 h irradiation on the EcN, compared with strain under dark conditions during the whole period. (**F**) Comparison of the luciferase luminescence changes in the EcN strains under conditions of totally dark, continuous green-light irradiation, and On-to-Off green-light irradiation. (**G**) Detection of the bioluminescence changes of the optogenetic-activated EcN in mice model within 4 hours. Data are presented as the mean ± SD. n=3. * $0.01<p<0.05$, ** $0.001<p<0.01$, *** $p<0.001$.

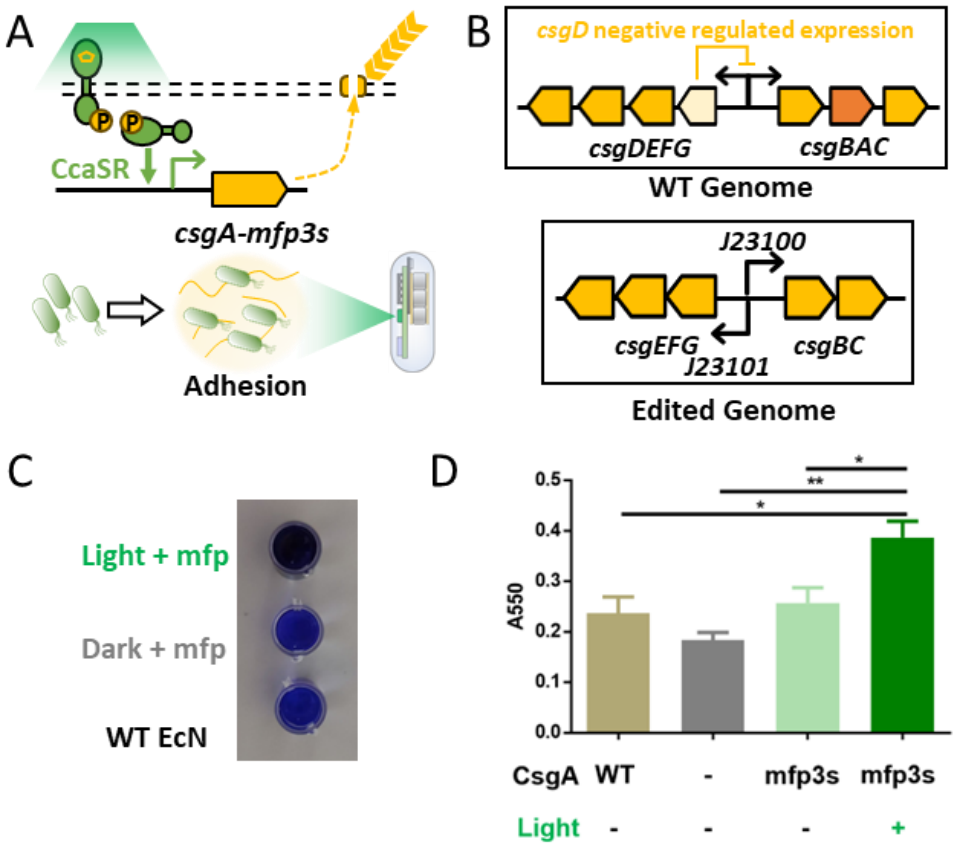

Fig. S7. Regulation of microbial adhesion via electronic capsule.

(**A**) Scheme diagram of the regulation of EcN adhesion under the control of the green-light activated optogenetic circuit for CsgA-mfp3s expression. (**B**) The gene *csgD* an *csgA* were deleted from EcN genome in the EcN for CsgA-mfp3s expression. (**C**) Crystal violet staining experiments showed that the strain's adhesion to plastic microporous substrates was enhanced under capsule-to-EcN light control (**D**) Comparison of different EcN groups expressing CsgA-mfp3s or not under the green light control or not. Data are presented as the mean ± SD. n=3. * $0.01<p<0.05$, ** $0.001<p<0.01$, *** $p<0.001$.

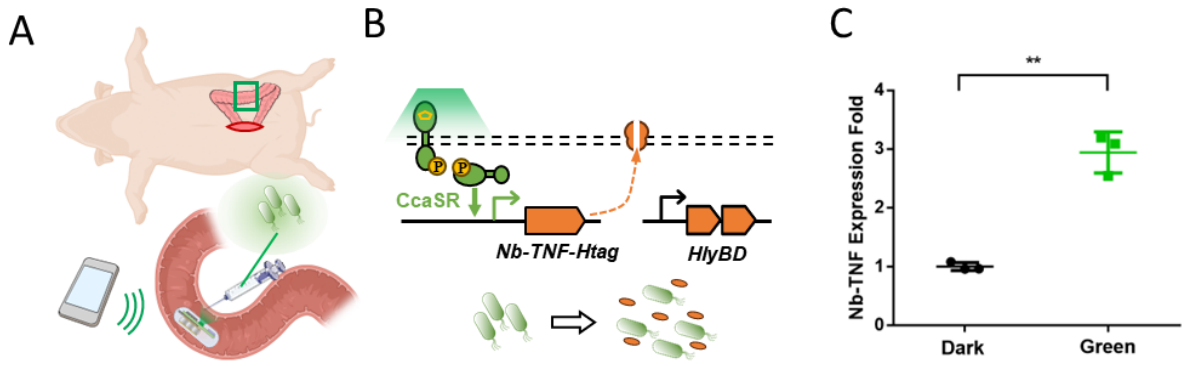

Fig. S8. Regulation of Nb-TNF expression and secretion in live pigs under the control of electronic capsule.

(**A**) To realistically simulate the process of the capsule regulating the optogenetic-controlled EcN in the colon *in vivo* while facilitating the sampling analysis for the secretion of the target protein, the capsule was surgically placed into the colon of anesthetized pigs. At the same time, the EcN with a CFU of 10^9 was injected near the capsule. (**B**) Scheme diagram of the regulation of Nb-TNF expression and secretion under the control of the green light emitted by the capsule. (**C**) Comparison of the Nb-TNF mRNA amounts under the green light irradiation of the capsule for 2 h. Data are presented as the mean ± SD. n=3. * $0.01<p<0.05$, ** $0.001<p<0.01$, *** $p<0.001$.

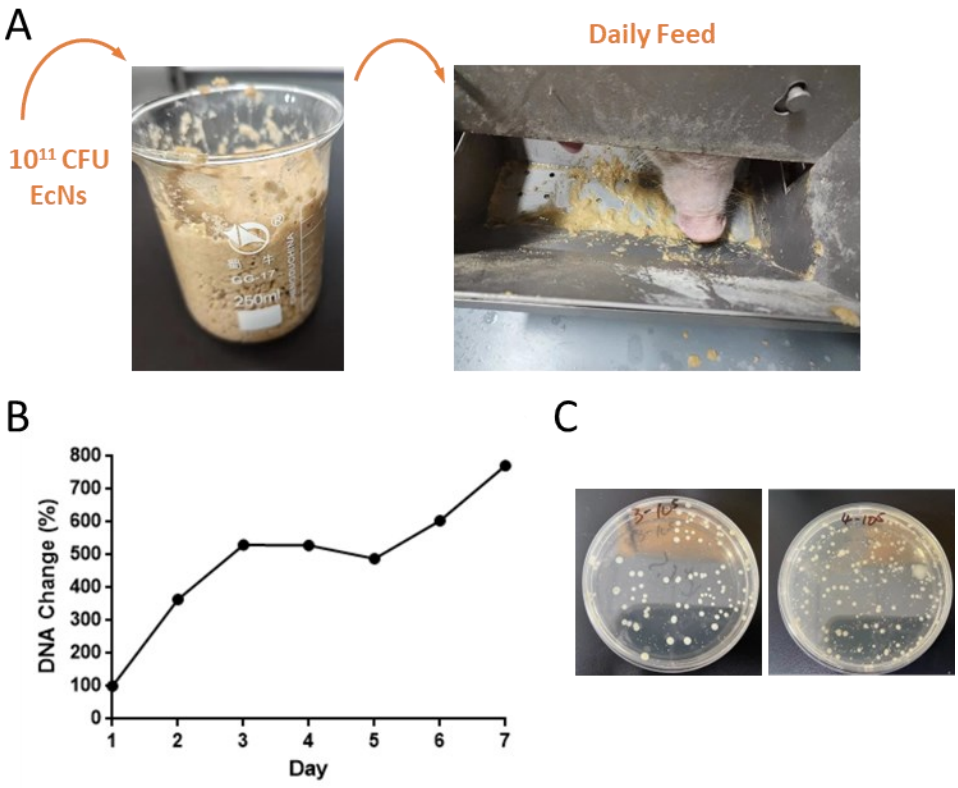

Fig. S9. Characterization of the engineered EcN strains orally administrated in live pigs.
(**A**) Mixing of the engineered EcN into daily feed for pig administration. (**B**) qRT-PCR detection of the *luxE* gene amouts through DNA extraction from pig feces. (**C**) Plate cultivation of the fresh fecal samples on plates containing 50 mg/L of kanamycin to screen the EcN containing the *kanR* plasmid.

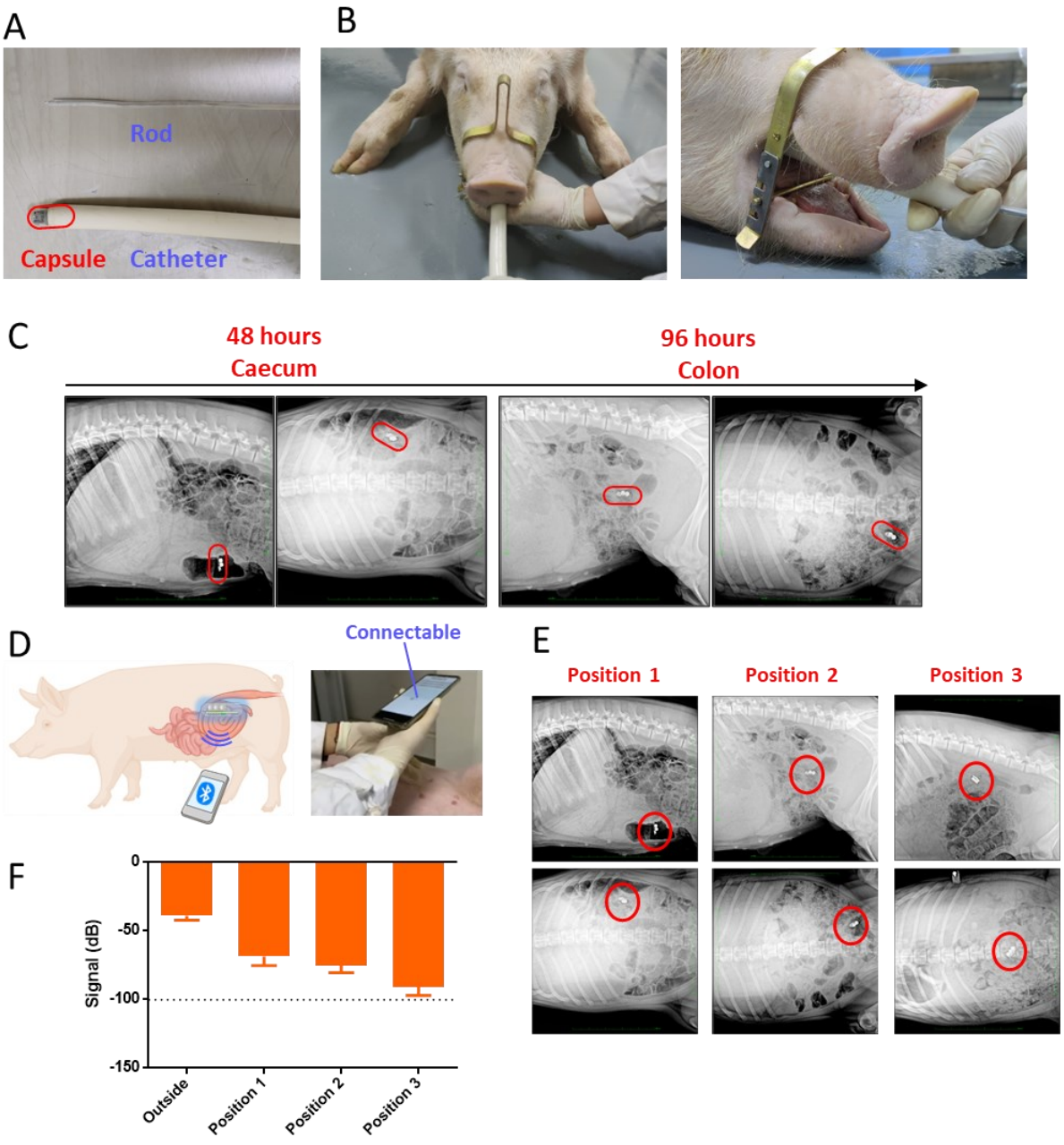

Fig. S10. Characterization of electronic capsule orally administrated in live pig intestine.

(**A**) Photo of the rod and the catheter used in Fig. 3J experiment for delivering the electronic capsule orally to the stomach. (**B**) Actual photo of the pigs while the catheter was pushed in pig mouth for avoiding the damage of capsule by pig chewing force. (**C**)The electronic capsule would pass through caecum after about 48 h and through colon after about 96 h. (**D**) The electronic capsule maintained connectable in the whole period in swine gastrointestinal tract. (**E**) X-ray photos of the electronic capsule at three different positions in live pig. (**F**) The Bluetooth signal intensity for the capsule *in vivo* fluctuated with its location due to tissue depth, yet remained above the connectivity threshold (>100 dB) even at its deepest within the body.

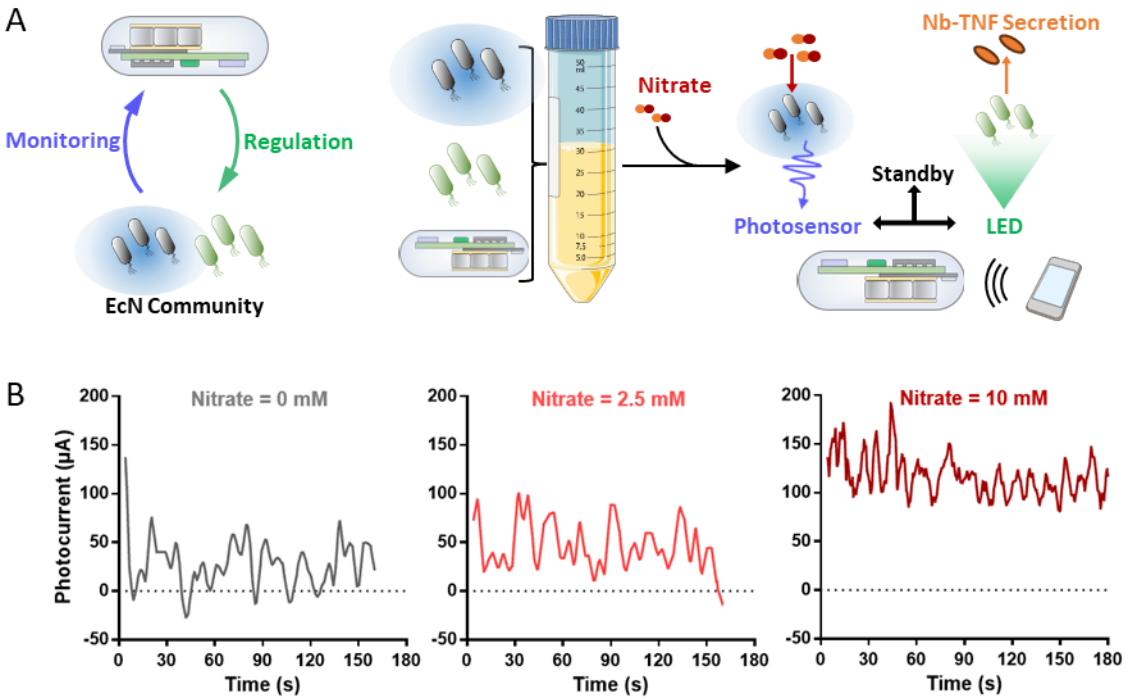

Fig. S11. Monitoring-regulating cycle based on the bidirectional optical communication between the electronic capsule and EcN community *in vitro*.

(**A**) In a complete monitoring-regulating cycle *in vitro*, a first sensor EcN strain transmitted bioluminescent signals to the capsule to detect environmental molecules of the bacterial community, and a second effector EcN strain received optogenetic signals from the capsule to regulate the function of the bacterial community. (**B**) The different nitrate concentrations were detected by electronic capsule and be transferred into distinguished photocurrent values.

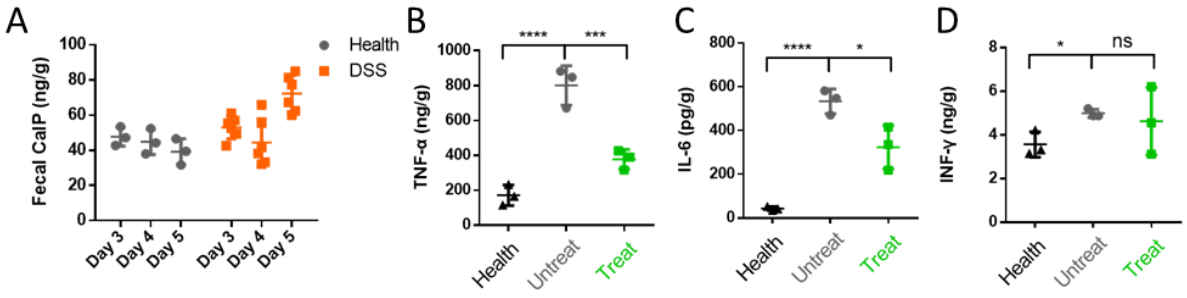

Fig. S12. Characterization of the pig colitis alleviation via EcN-capsule communication.

(A) The concentrations of fecal CalP were measured at different days from the beginning of pig colitis modeling in Fig. 4B. (B) Comparison of the concentrations of TNF-α in the colon samples of different pig groups, namely health pig, untreated pig, and treated pig via our EcN-capsule communication. (C) Comparison of the concentrations of IL-6 in the colon samples of different pig groups, namely health pig, untreated pig, and treated pig via our EcN-capsule communication. (D) Comparison of the concentrations of INF-γ in the colon samples of different pig groups, namely health pig, untreated pig, and treated pig via our EcN-capsule communication. Data are presented as the mean ± SD. n=3~6. * $0.01<p<0.05$, ** $0.001<p<0.01$, *** $p<0.001$.

Table S1. The engineered EcN strains used in this study.

No.	Name	Key characteristics	Reference
1	EcN	*E. coli* Nissle 1917 ΔpMUT1 ΔpMUT2 (cryptic plasmids)	Lab stock (*31*)
2	EcN-CSG	EcN Δ*csgA, ΔcsgD*::pJ23101-*csgEFG*,pJ23100-*csgBC*	
3	EcN-CSG-LuxBCDE	EcN-CSG Δ*lacZ*::pJ23100-*LuxBCDE*	
4	EcN-Lux	EcN harboring pLuxABCDE	
5	EcN-Opto-GFP	EcN harboring p15A-ccaS-PCB and pGreen-sfGFP	Designed referring to (*22*)
6	EcN-Ara-GFP	EcN harboring pAra-sfGFP	
7	EcN-RSF-GFP	EcN harboring pRSF-sfGFP	
8	EcN-SC101-GFP	EcN harboring pSC101-sfGFP	
9	EcN-MUT1-GFP	EcN harboring pMUT1-sfGFP	
10	EcN-MUT2-GFP	EcN harboring pMUT2-sfGFP	
11	EcN-Opto-NbTNF	EcN harboring pHlyBD and p15A-ccaS-PCB and pGreen-Nb-TNF-H60	
12	EcN-Opto-CsgA-mfp	EcN-CSG harboring p15A-ccaS-PCB and pGreen-csgA-mfp	Designed referring to (*24*)
13	EcN-Ara-ccdBA-Lux	EcN harboring pAra-ccdB-CI-ccdA and pLux	
14	EcN-Opto-Lux	EcN-CSG-LuxBCDE harboring p15A-ccaS-PCB and pGreen-LuxA	
15	EcN-Ara-Lux	EcN-CSG-LuxBCDE harboring pAra-LuxA	
16	EcN-NO-Lux	EcN-CSG-LuxBCDE harboring pNO-LuxA	

Table S2. The constructed plasmids used in this study.

No.	Name	Key characteristics	Reference
1	pLuxABCDE	Kan^R, T5-*LuxABCDE*	
2	pGreen-sfGFP	pUC ori, Kan^R, pJ23100-*ccaR*-B0015, pcpcG2-*sfGFP(LAA)*-rrnB T1	Designed referring to (*22*)
3	p15A-ccaS-PCB	p15A ori, Chl^R, pJ23106-*mini ccaS*, pJ23108-*HO1-pcyA*-L3S1P22	Designed referring to (*22*)
4	pRSF-sfGFP	pRSF ori, Amp^R, pJ23100-*sfGFP*	
5	pSC101-sfGFP	pSC101 ori, Amp^R, pJ23100-*sfGFP*	
6	pMUT1-sfGFP	pMUT1 ori, Amp^R, pJ23100-*sfGFP*	
7	pMUT2-sfGFP	pMUT2 ori, Kan^R, pJ23100-*sfGFP*	
8	pAra-sfGFP	p15A ori, Chl^R, pBAD-*sfGFP*, pC-*AraC*	
9	RSF-NbTNF-H60	pRSF ori, Amp^R, pJ23100-*Nb-TNF-H60*	Designed referring to (*25*)
10	pGreen-Nb-TNF-H60	pUC ori, Kan^R, pJ23100-*ccaR*-B0015, pcpcG2-*Nb-TNF-H60*-rrnB T1	
11	pGreen-LuxA	pUC ori, Kan^R, pJ23100-*ccaR*-B0015, pcpcG2-*LuxA*-rrnB T1	
12	pHlyBD	pSC101 ori, Amp^R, pJ23100-*HlyBD*	
13	pGreen-csgA-mfp	pUC ori, Kan^R, pJ23100-*ccaR*-B0015, pcpcG2-*CsgA-mfp3s*-rrnB T1	Designed referring to (*24*)
14	pAra-ccdB-CI-ccdA	p15A ori, Chl^R, pBAD-*ccdB-CI*, pλ-*ccdA*, pC-*AraC*	
15	pNO-LuxA	pRSF ori, Amp^R, pYeaR-Toehold switch-*LuxA*, pYeaR-Trigger	Designed referring to (*20, 21*)
16	pAra-LuxA	p15A ori, Chl^R, pBAD-*LuxA*, pC-*AraC*	

Table S3. Disease activity index (DAI) score of pigs in the DSS-induced colitis trail.

Group	**Score**												
Untreated	0	0	0	4	8	10	11	11	11	12	12	12	12
Treated	0	0	0	4	8	10	11	11	8	8	8	8	8
Date	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**

Definition of DAI (*4*):

0-2: no obviable colitis symptoms.

3-5: mild inflammation, diarrhea, and other systematic symptoms. For example, the feces became moist and soft; rectal temperature measured usually exceeding 38 ℃.

6-10: moderate inflammation, diarrhea, and other systematic symptoms. For example, the feces became more moist and softer, and difficult to shape; rectal temperature measured exceeding 38 ℃, sometimes exceeding 39 ℃; food consumed decreased.

11-12: severe inflammation, diarrhea, and other systematic symptoms. For example, the feces became watery or even bloody; food consumed decreased; high body surface temperature that was easily perceivable by human hands; weakened behavior and significantly reduced mobility.

Movie S1. Remote control of electronic capsule-loaded LED in the intestine.

To visualize the remote control of the electronic capsule *in vivo*, and examine its functionality, we placed the capsule into pig's rectum transanally with ~ 30 cm depth, repeatedly turned on and off the capsule-loaded LED under remote Bluetooth control on the smartphone APP, and recorded the LED status via colonoscopy.

Movie S2. The simulated process of administrating the electronic capsules into the pig's esophagus.

The process of placing electronic capsules into the esophagus of pigs was demonstrated through the simulation video. Under the lubrication of glycerol, we inserted a soft catheter with an electronic capsule at one end into the pig's esophagus to a depth of ~ 40cm, and then use a guide rod to push the capsule deep into the esophagus.

Movie S3. The wireless connectable time range when the electronic capsule was in the intestine of pigs.

When the electronic capsule was at its position as shown in fig. S10C right (96 hours after orally administration), the available range of the motivation of user's smartphone, maintaining Bluetooth connectivity, was recorded.