Supplementary materials for:

Rewiring bile salt sensing enables rapid and portable monitoring of liver dysfunction via engineered bacteria.R

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These supplementary materials contain:

- Supplementary text.
- Supplementary figures S1 to S19.
- Supplementary tables 1 to 5.

Supplementary text.

NGS primers used in this study

I. 1st round PCR for adaptor and UMI barcode:

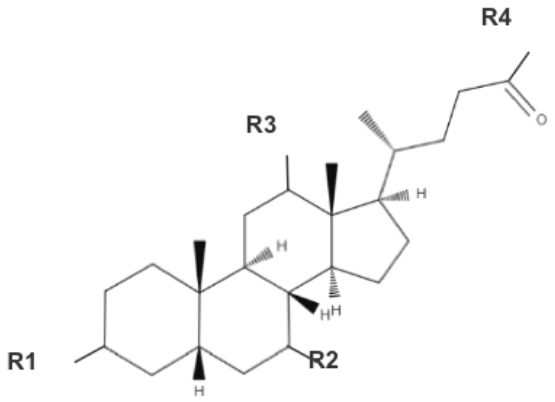
TcpP_Sensing_NGS_1st_Fw:
TCGTCGGCAGCGTCagatgtgtataagagacagNNNNNNNNCCAGAAACTTAGCGAGCAGAAG

TcpP_Sensing_NGS_1st_Rv:
GTCTCGTGGGCTCGGagatgtgtataagagacagNNNNNNNNgccacctgggatttccg

II. 2nd round PCR for index insertion:

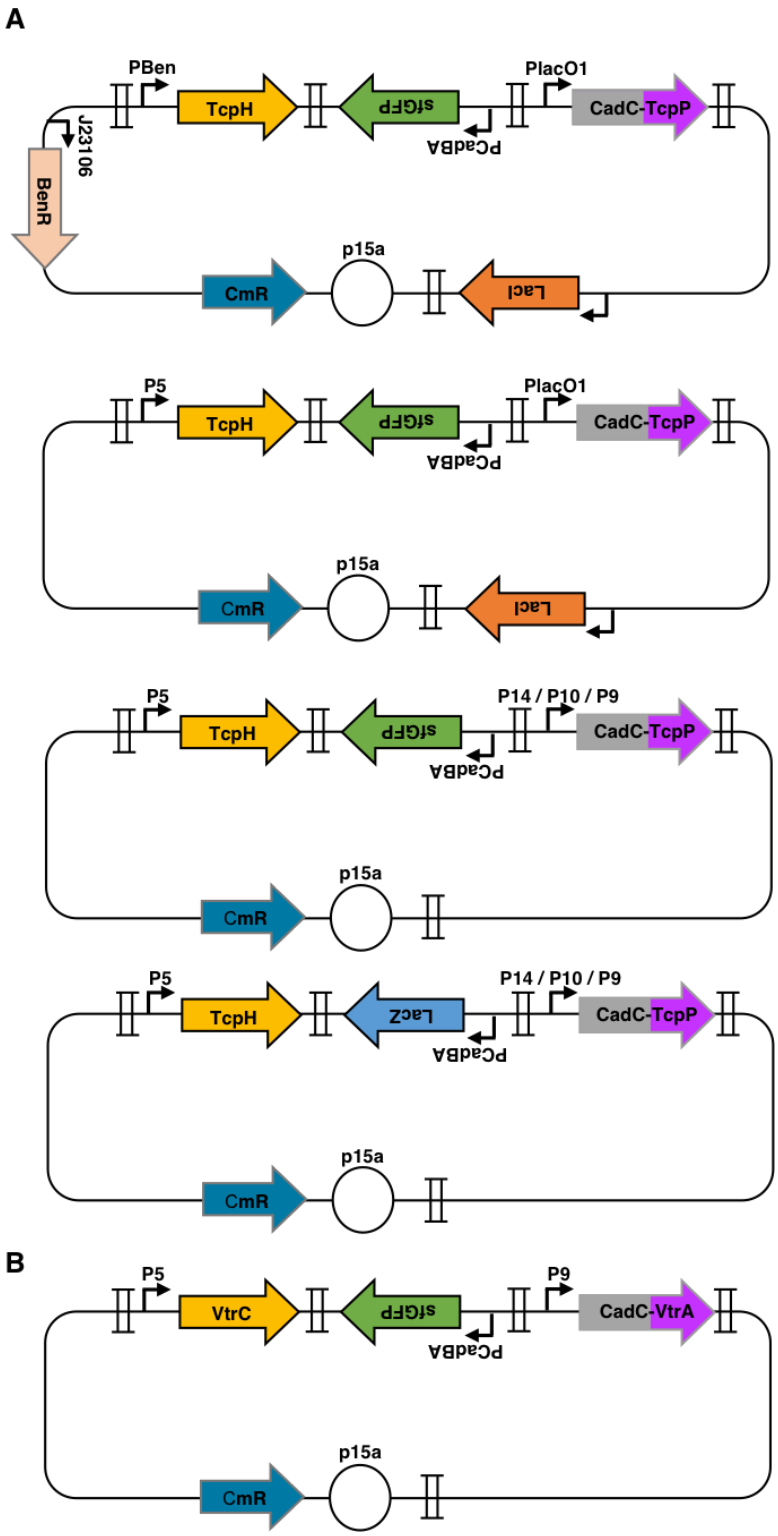
P5_bc1	aatgatacggcgaccaccgagatctacacAACAACACtcgtcggcagcgtc
P5_bc2	aatgatacggcgaccaccgagatctacacAACGGTGGtcgtcggcagcgtc
P5_bc3	aatgatacggcgaccaccgagatctacacACCTTCTTtcgtcggcagcgtc
P5_bc4	aatgatacggcgaccaccgagatctacacATTGAGCCtcgtcggcagcgtc
P5_bc5	aatgatacggcgaccaccgagatctacacCCAGGAAGtcgtcggcagcgtc
P5_bc6	aatgatacggcgaccaccgagatctacacCGTCCAATtcgtcggcagcgtc

P7_bc1	caagcagaagacggcatacgagatCGTCCAATgtctcgtgggctcgg
P7_bc2	caagcagaagacggcatacgagatGTGTCGTTgtctcgtgggctcgg
P7_bc3	caagcagaagacggcatacgagatAACAACACgtctcgtgggctcgg
P7_bc4	caagcagaagacggcatacgagatACCTTCTTgtctcgtgggctcgg
P7_bc5	caagcagaagacggcatacgagatCCAGGAAGgtctcgtgggctcgg
P7_bc6	caagcagaagacggcatacgagatAACGGTGGgtctcgtgggctcgg

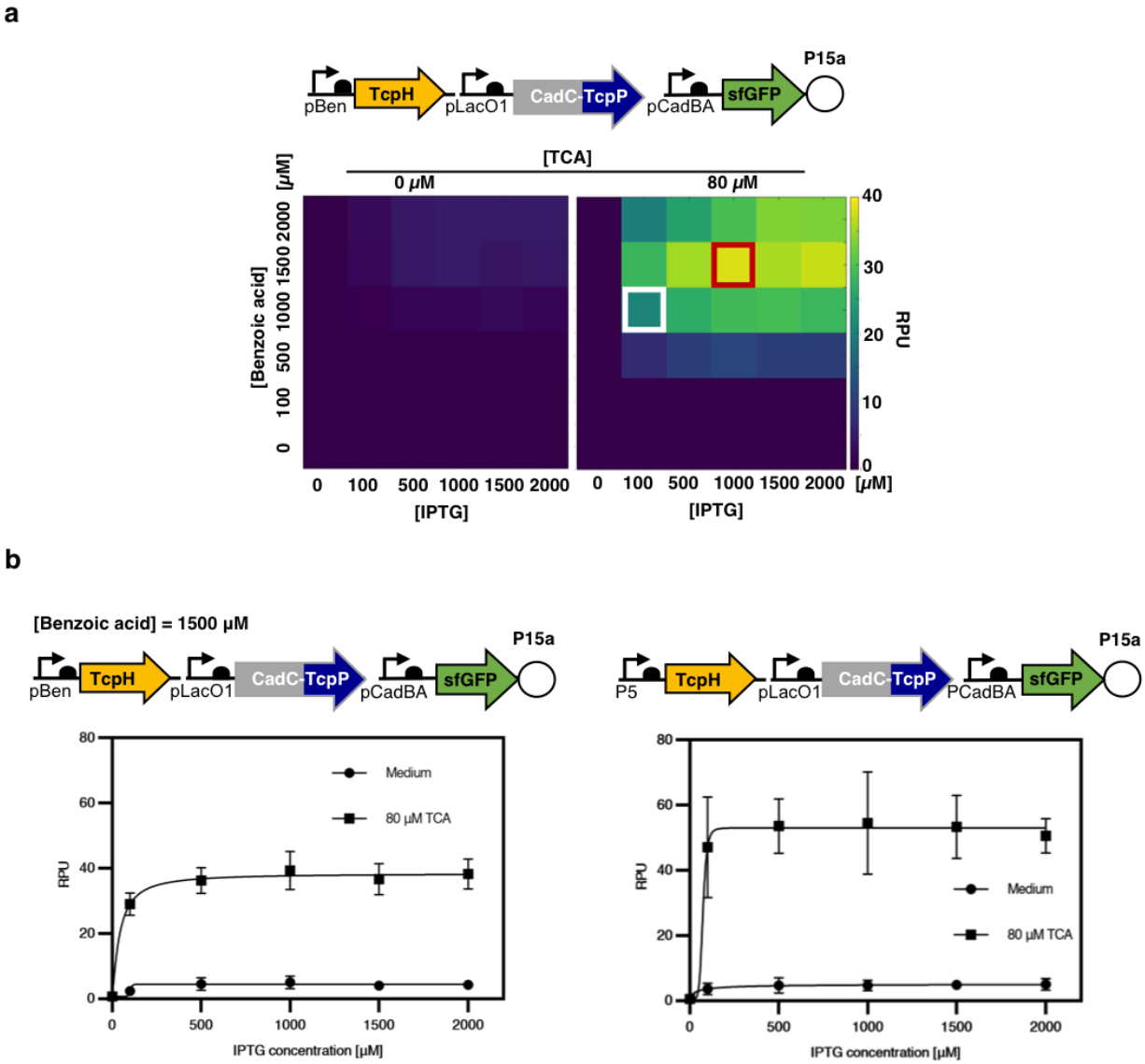


Name	Abbreviation	R1	R2	R3	R4
Cholic acid	CA	OH	OH	OH	OH
Glycocholic acid	GCA	OH	OH	OH	$NHCH_2COO^-$
Taurocholic acid	TCA	OH	OH	OH	$NHCH_2CH_2SO_3^-$
Chenodeoxycholic acid	CDCA	OH(α)	OH(α)	H	OH
Glycochenodeoxycholic acid	GCDCA	OH(α)	OH(α)	H	$NHCH_2COO^-$
Taurochenodeoxycholic acid	TCDCA	OH(α)	OH(α)	H	$NHCH_2CH_2SO_3^-$
Ursodeoxycholic acid	UDCA	OH(α)	OH(β)	H	OH
Glycoursodeoxycholic acid	GUDCA	OH(α)	OH(β)	H	$NHCH_2COO^-$
Deoxycholic acid	DCA	OH	H	OH	OH
Glycodeoxycholic acid	GDCA	OH	H	OH	$NHCH_2COO^-$
Taurodeoxycholic acid	TDCA	OH	H	OH	$NHCH_2CH_2SO_3^-$
Lithocholic acid	LCA	OH	H	H	OH

Supplementary Figure 1. Chemical structures of different bile salts and corresponding abbreviation used in this study.

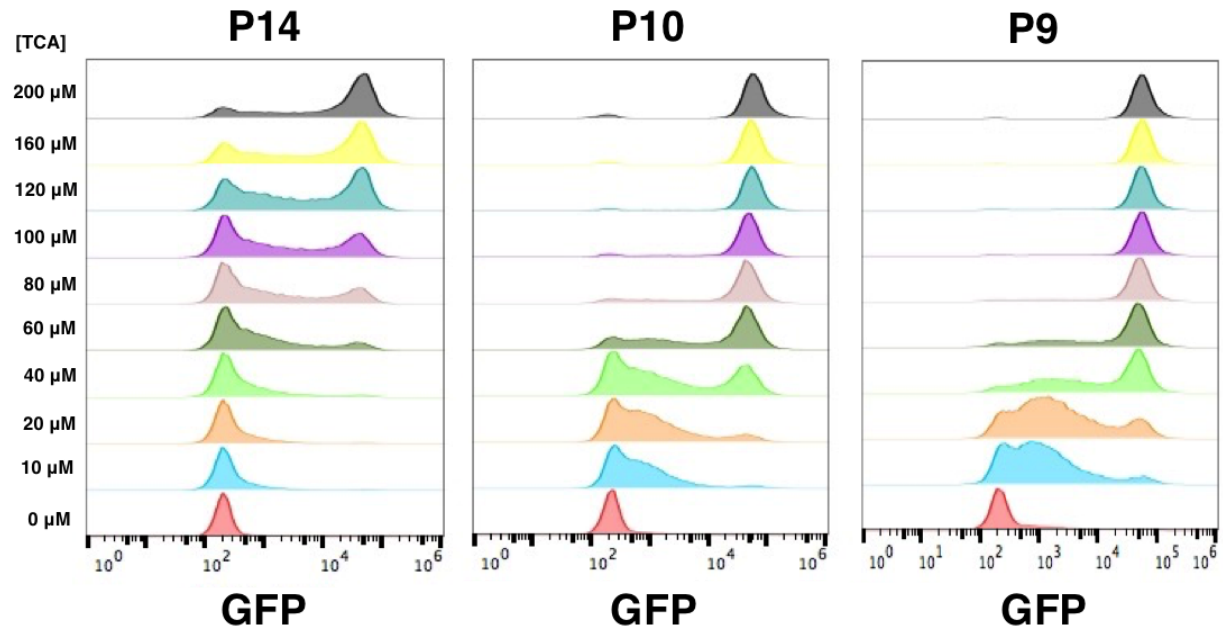


Supplementary Figure 2: Plasmid maps of (A) inducible, mixed type and constitutive CadC-TcpP/TcpH system; and (B) constitutive CadC-VtrA/VtrC system. *BenR*, the transcription factor BenR. *LacI*, the transcription factor LacI. CmR, the chloramphenicol resistance gene. Promoters are depicted by arrows; RBS are shown as solid half-circles; terminators are presented by II symbols.

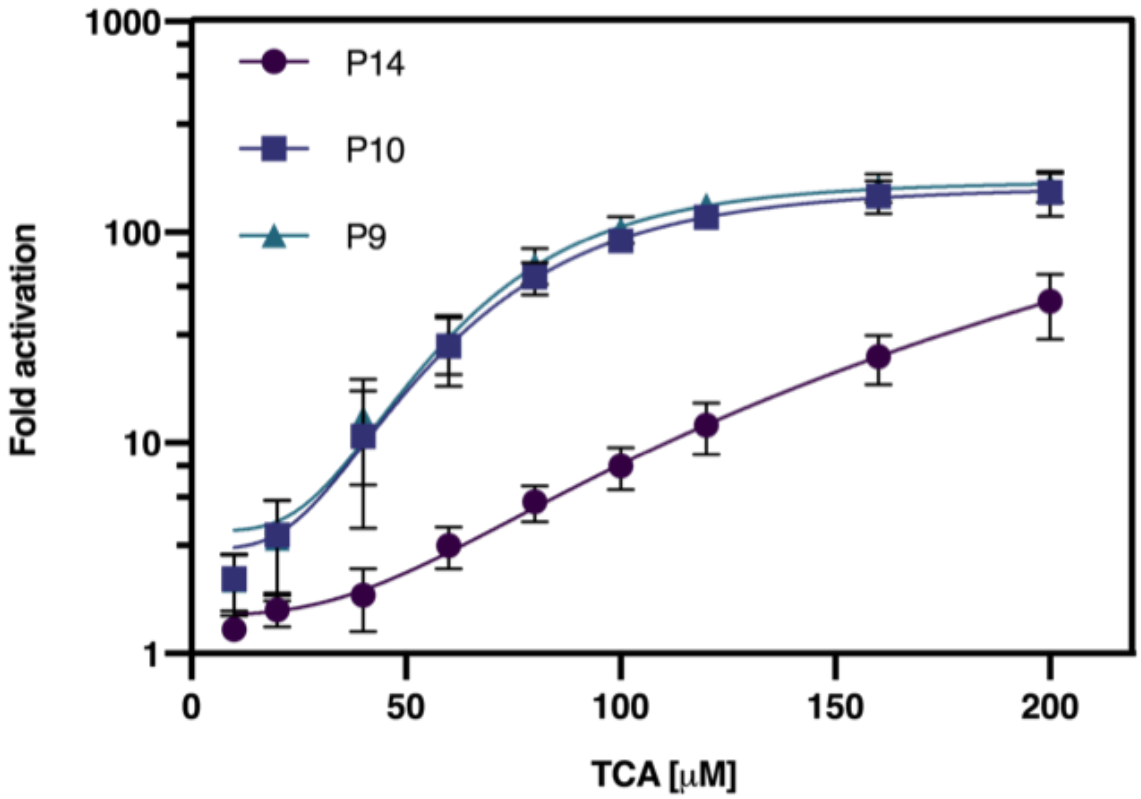


Supplementary Figure 3: System performance of inducible CadC-TcpP/TcpH system. (a) Response of inducible CadC-TcpP/TcpH system with or without the presence of ligand TCA at different expression levels induced by various concentrations of IPTG and benzoic acid. Schematic diagram of each genetic component and their corresponding control elements are listed above. RPU, reference promoter unit. The response with highest signal-to-noise ratio is highlighted with white square and the response with highest dynamic range is highlighted with red square. We found that there was an optimal expression ratio between the two proteins, i.e. maximizing protein expression did not lead to better output. (b) Comparing the different response between pLacO1-CadC-TcpP system with benzoic acid inducible (left panel) or constitutively expressed (right panel) TcpH, respectively. Inducible expression systems are useful to explore the parameter space of engineered systems. Yet the final bactosensor should operate using genes driven by optimal constitutive promoters. Constitutive expression reduces overall system complexity, DNA and metabolic footprint, and facilitates biosensor manipulation for real-world applications. Using constitutive promoters also helps avoiding toxicity or interference from the inducer molecule. Here we use the data from the inducible CadC-TcpP/TcpH system with highest

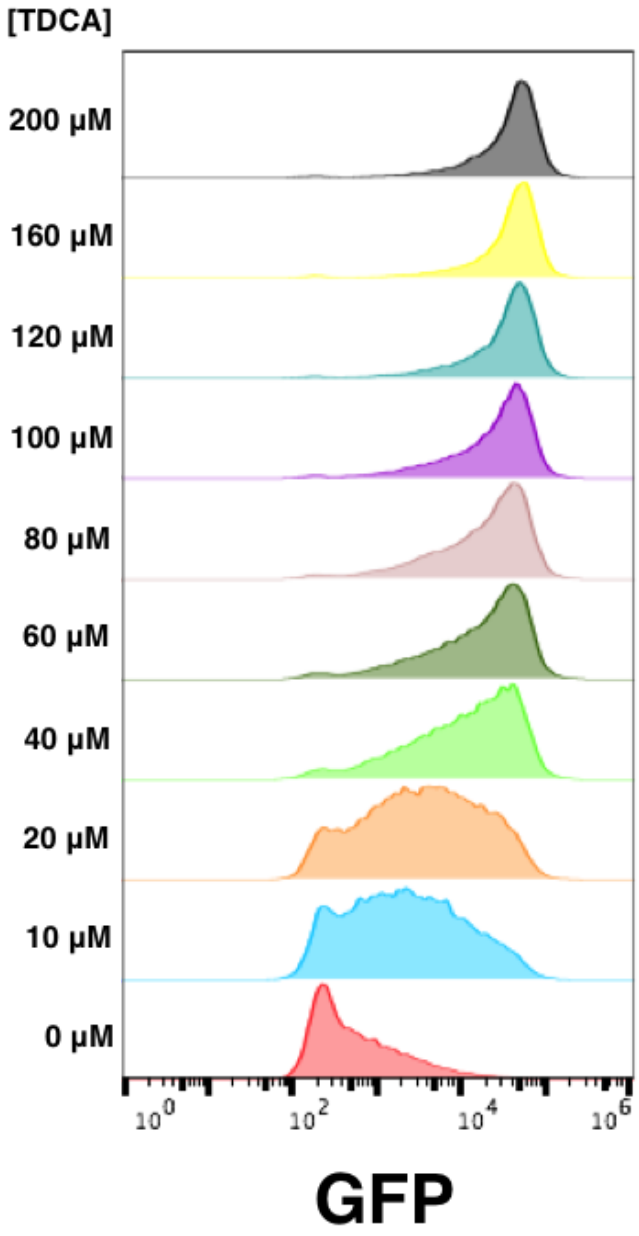
dynamic range (induced by 1500 μM benzoic acid, Fig. S2a) to compare with the data from PlacO1-CadC-TcpP with constitutive expressed TcpH. We first placed the TcpH gene under the control of the strong constitutive promoter P5 [43], while keeping CadC-TcpP expression under IPTG control (right panel). The resulting combination showed a significant improvement in signal output level (swing) and signal-to-noise ratio compared to the system in which TcpH expression was under the control of the inducible pBEN promoter (left panel). These results suggested that the signal drop observed in Fig.S3a at high benzoic acid concentration might be due to a deleterious effect of the inducer benzoic acid on the cellular physiology.



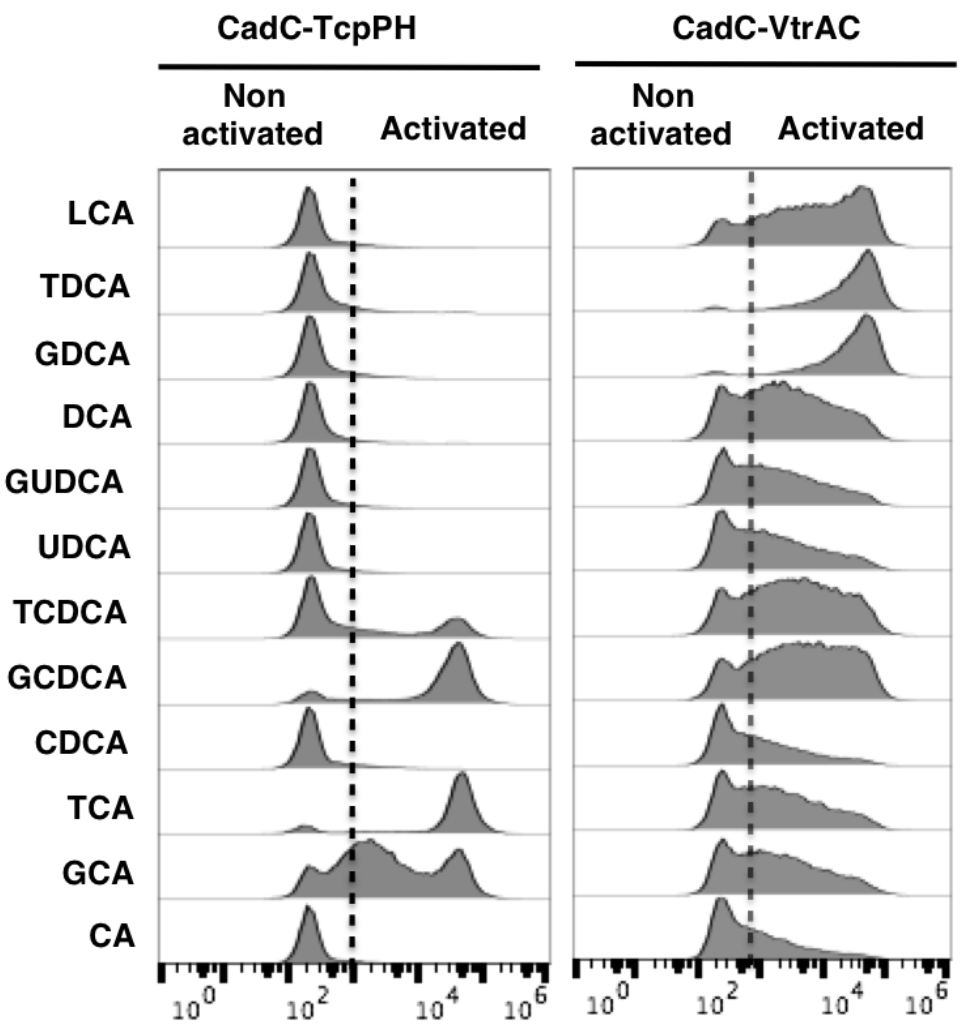
Supplementary Figure 4: Response of CadC-TcpP promoter variants (with P5_TcpH) to increasing concentration of ligand TCA. The TCA concentration is labeled at the left-y-axis.



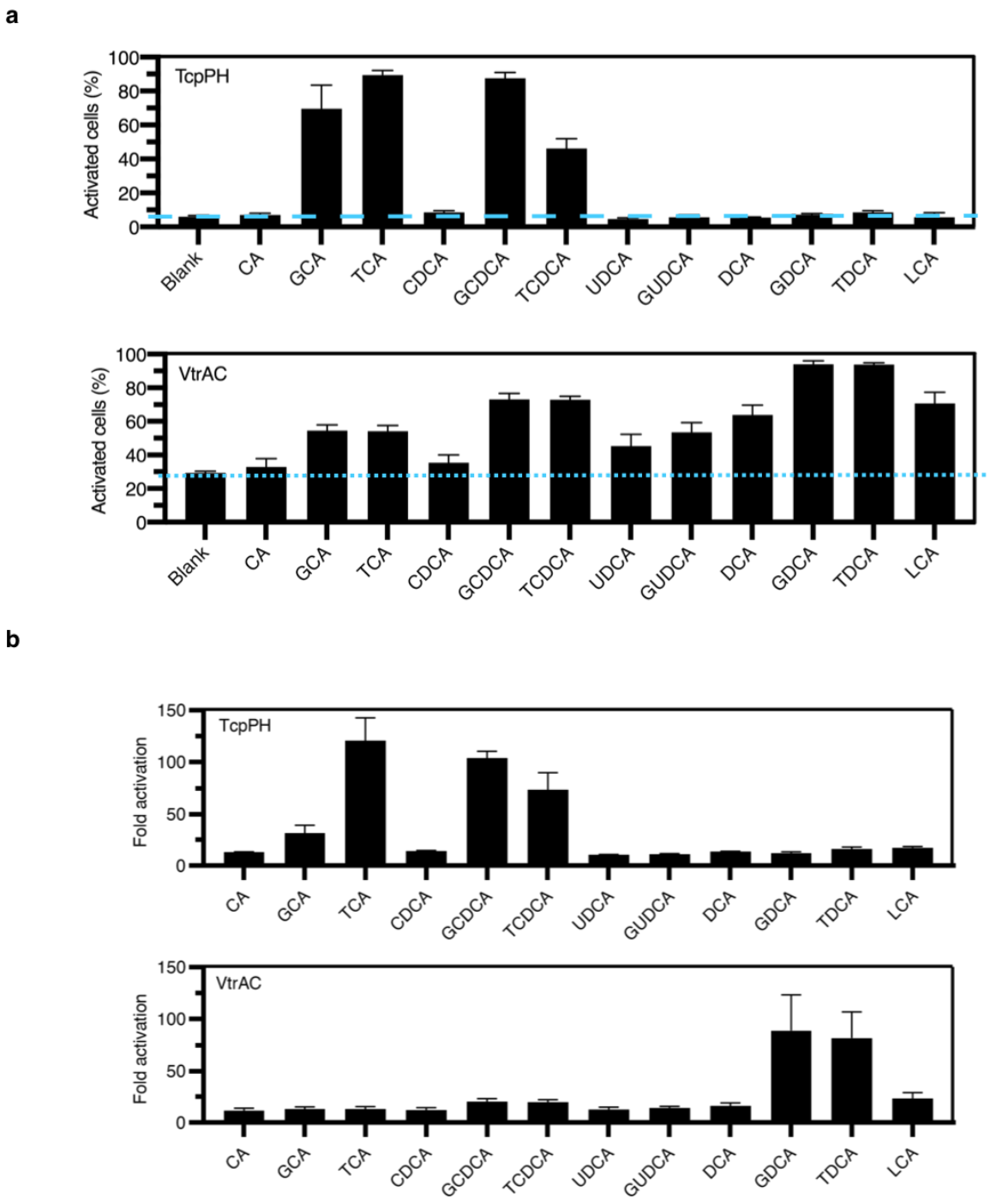
Supplementary Figure 5: Activation fold of CadC-TcpP promoter variants (with P5-TcpH) responded to different concentrations of ligand TCA.



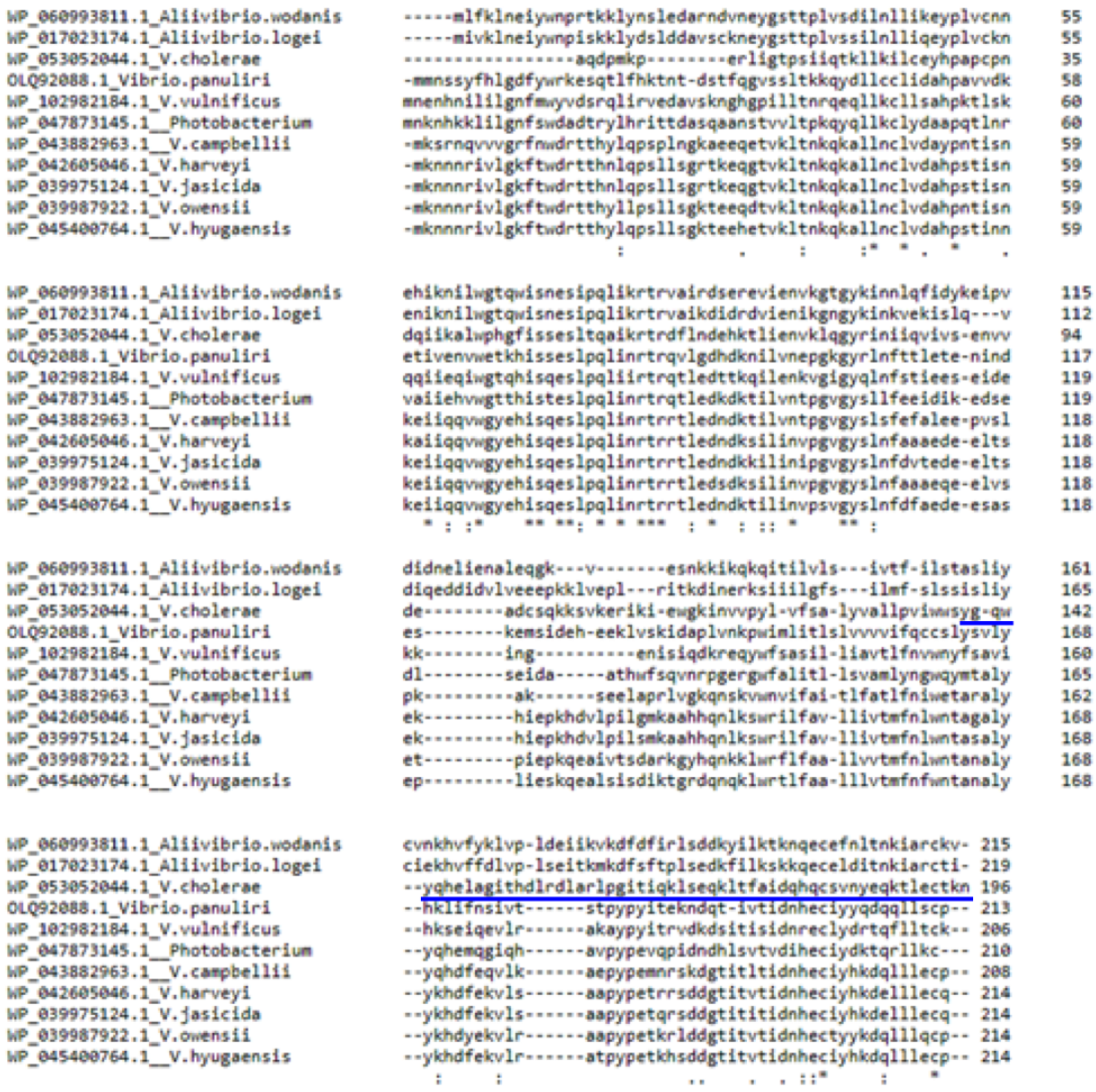
Supplementary Figure 6: Response of P9-CadC-VtrA_P5-VtrC to different concentrations of TDCA. Different concentration of TDCA used for titration are labeled at the left-y-axis.



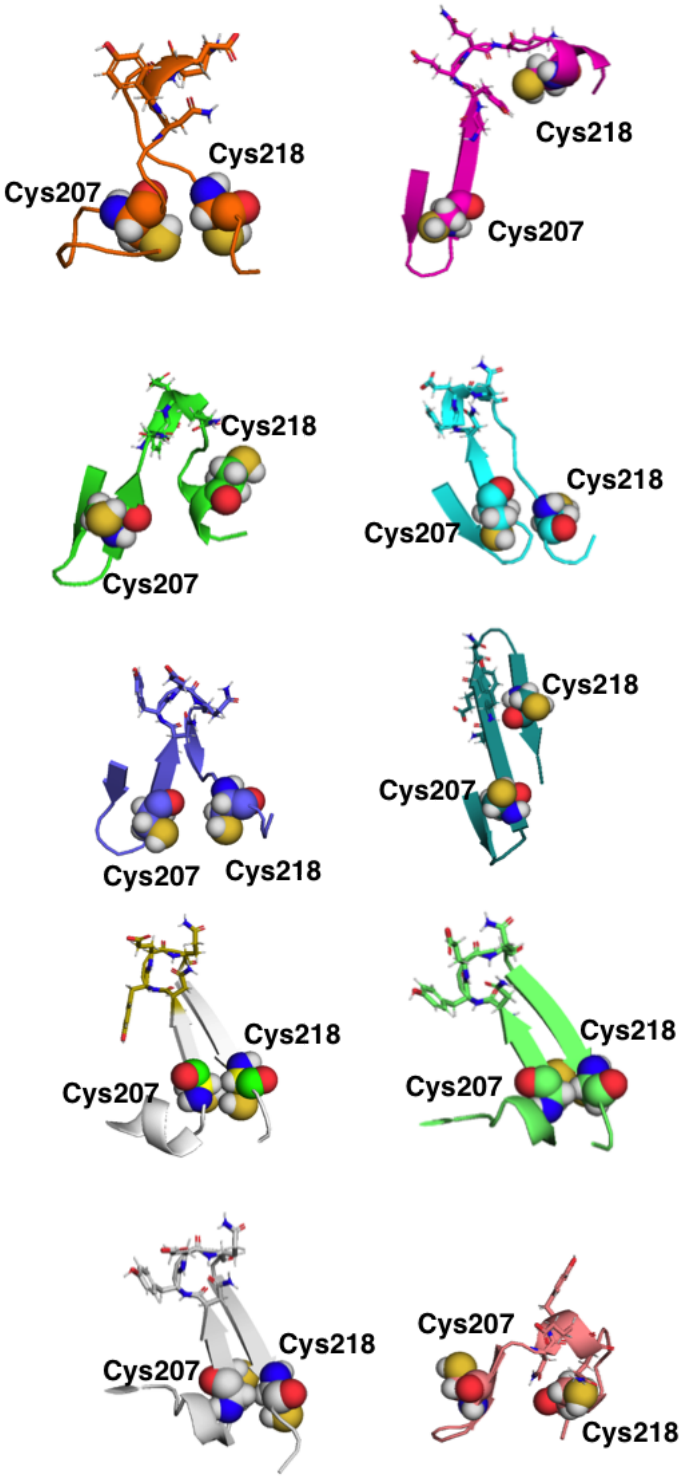
Supplementary Figure 7: Response of CadC-TcpPH and CadC-VtrAC system to different type of bile salts. The activated cells and non-activated cells are further distinguished by gating. The different bile salts used for profiling are labeled in the left-y-axis. Different from the TcpPH system which shows significant preference to primary conjugated bile salts, the VtrAC system shows different levels of preference to all bile salts. This result might reveal the potential of the hydrophobic inner chamber formed by VtrA/VtrC heterocomplex for the protein engineering of ligand specificity against different type of bile salts.



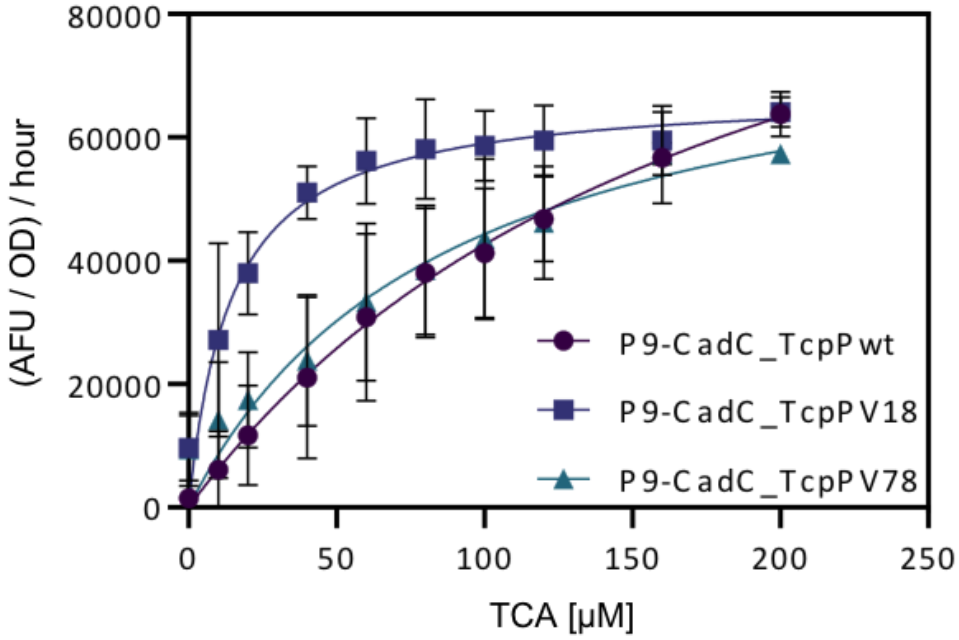
Supplementary Figure 8: Response of CadC-TcpPH and CadC-VtrAC system to different bile salts are presented as (a) percentage of activated cells and (b) activation folds. The population of activated cells are calculated through the gating shown in Fig. S8. The activation fold is calculated as the ratio of the geometric mean of activated cells divided by the geometric mean of non-activated cells.



Supplementary Figure 9: Multiple sequence alignment of the periplasmic domains from TcpP homologous proteins. The periplasmic bile salt sensing domain of TcpP is highlighted by blue line.



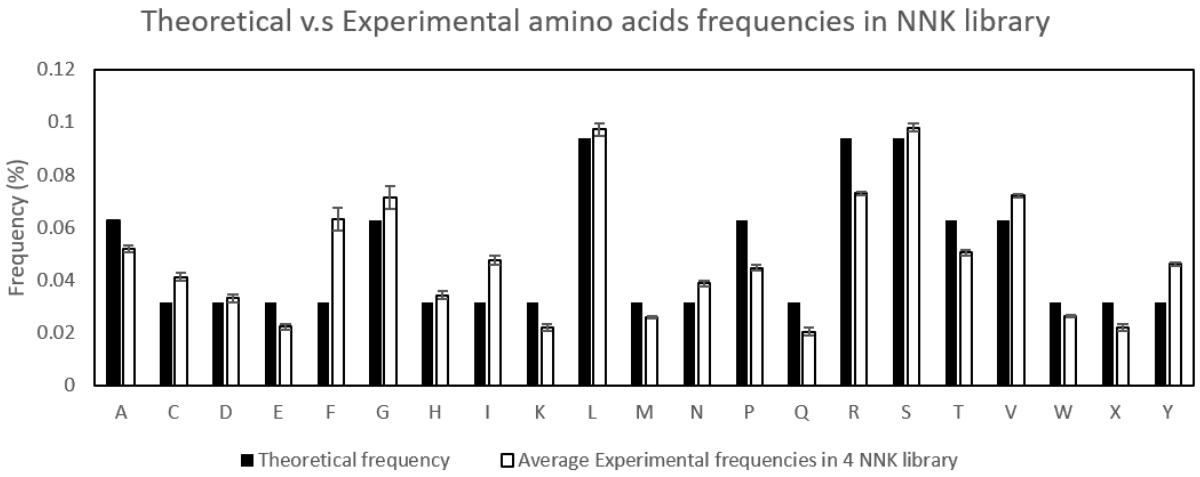
Supplementary Figure 10: Molecular modeling of TcpP periplasmic sensing domain



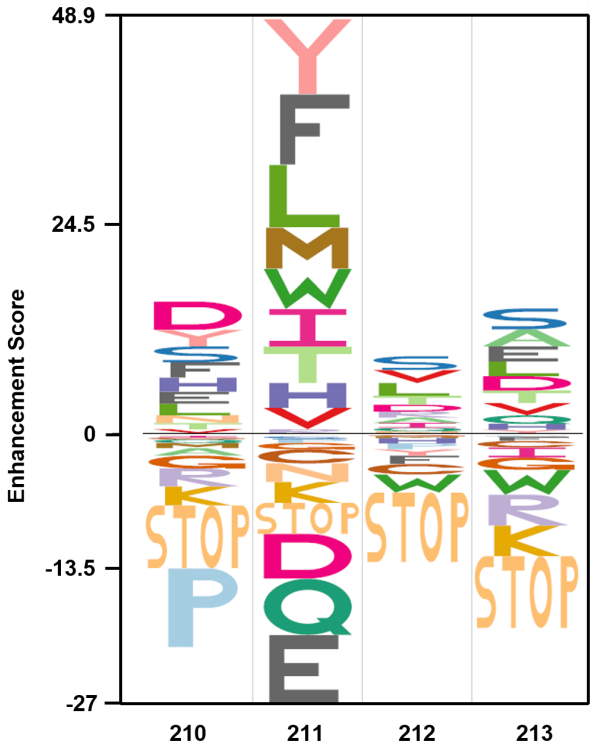
	TcpPwt	TcpPV18	TcpPV78
Vmax	123084	67529	83036
Km	188.8	14.44	87.4

Supplementary Figure 11: Kinetics analysis of CadC-TcpPwt and CadC-TcpPmut-18

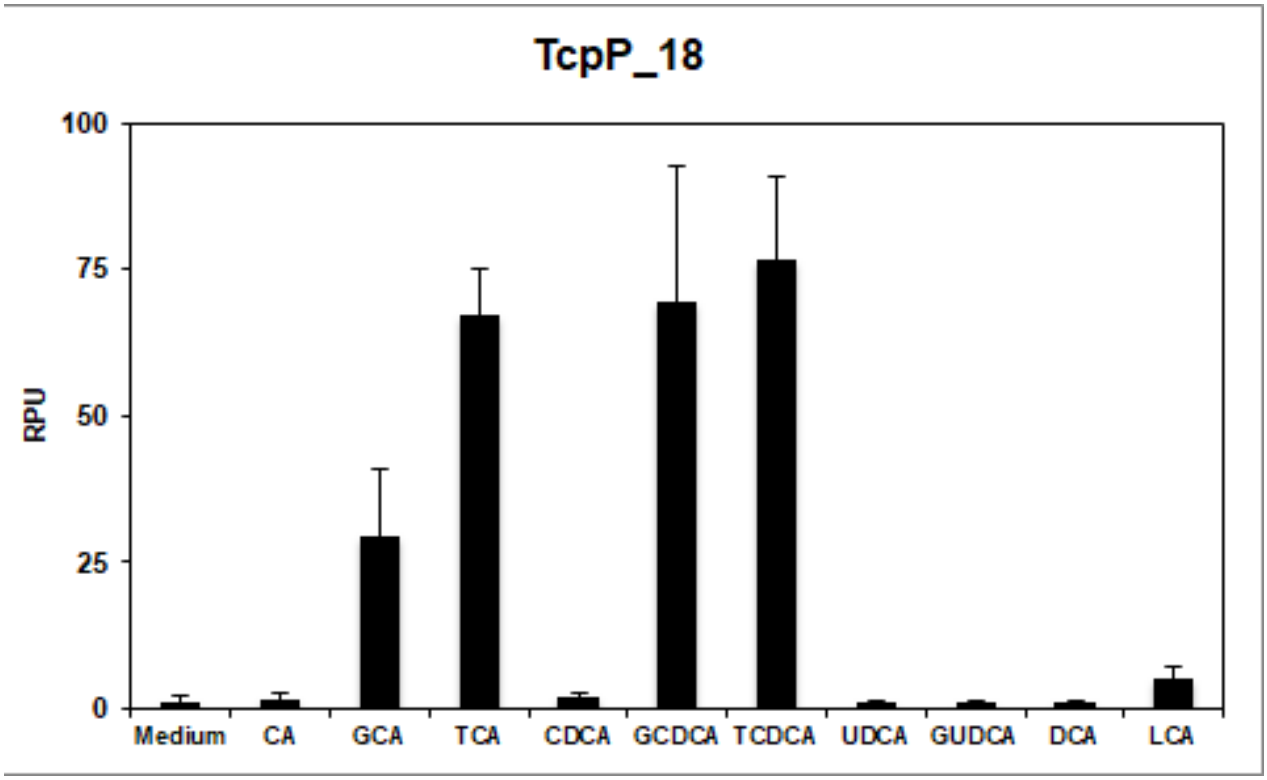
a



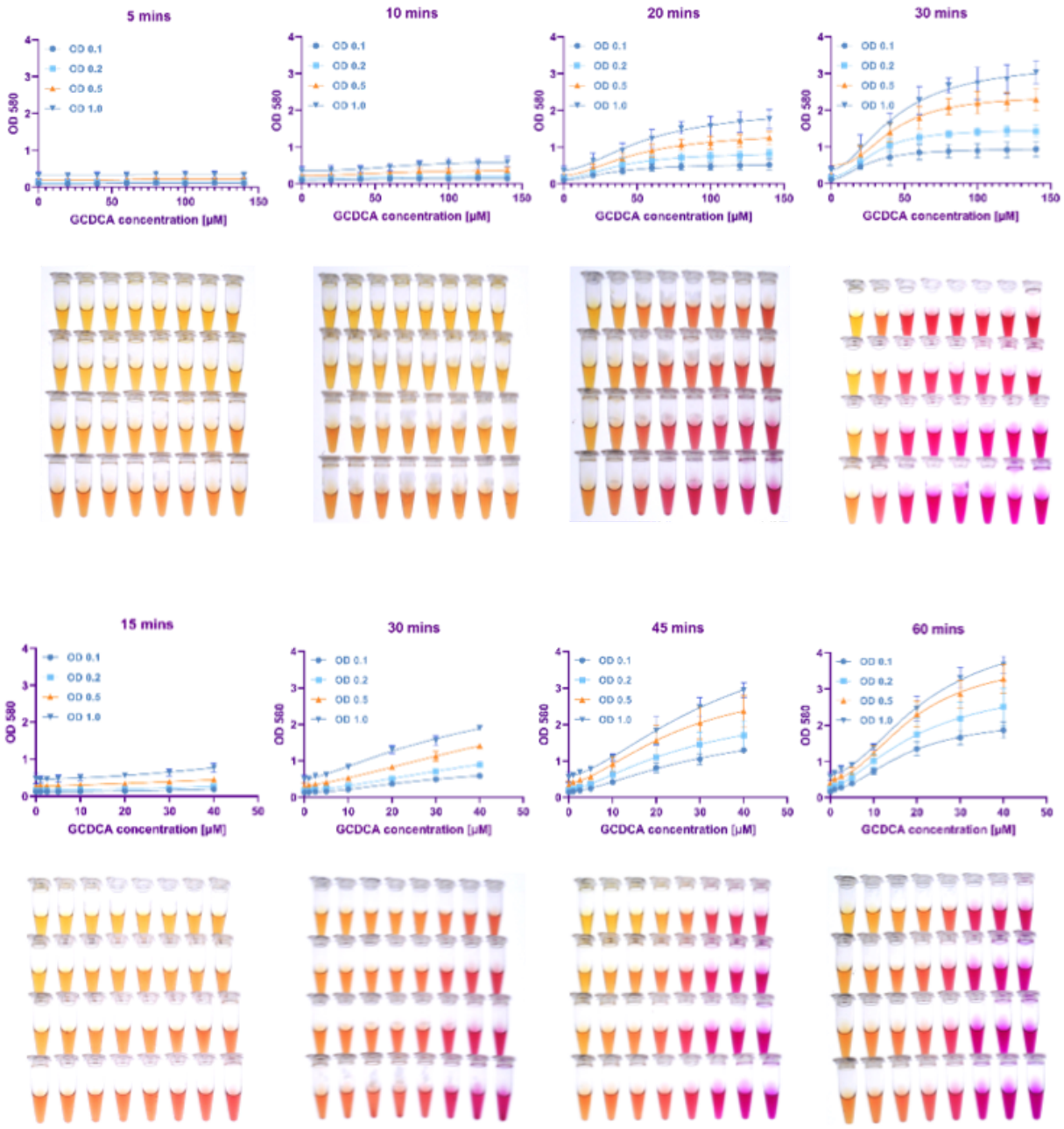
b



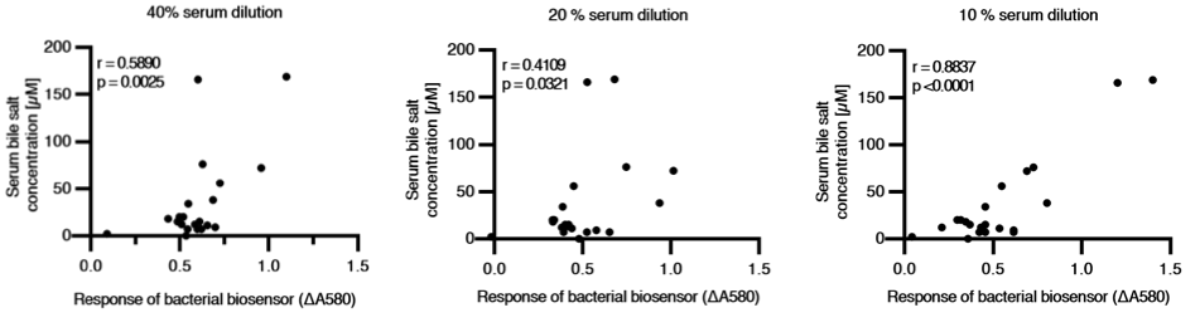
Supplementary Figure 12: (a) Comparison of theoretical and experimental amino acid frequencies in preselected NNK library. For verifying the quality of preselected TcpP synthesized 4 x NNK library, the summary counts of NGS results of each position were calculated as frequencies. The average values were further compared with theoretical frequencies of NNK library. **(b) The EDlogo plot of TcpP functional variants (from 3rd round of selection) compared with background frequencies (from preselected library) to highlight the enrichment and depletion score.** The y-axis of the logo is the enhancement score of each amino acid. STOP: the 'TAG' stop codon in NNK degenerate codons.



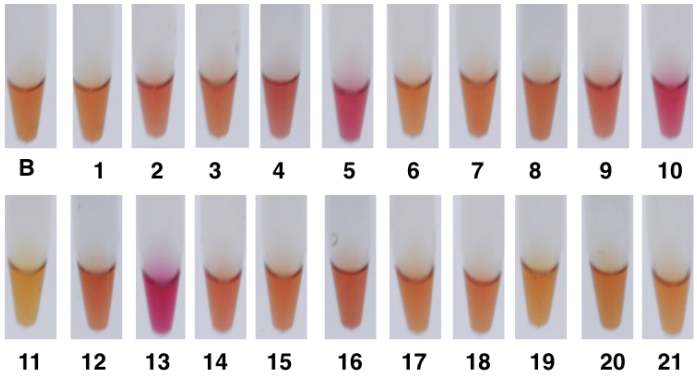
Supplementary Figure 13: Bile salt response profile of TcpP18



Supplementary Figure 14: Fine tuning system performance of TcpP18-LacZ through varying cell number per reaction and sample incubation time.



Supplementary Figure 15: Determination of serum dilution rate in fresh bacterial biosensor cells.

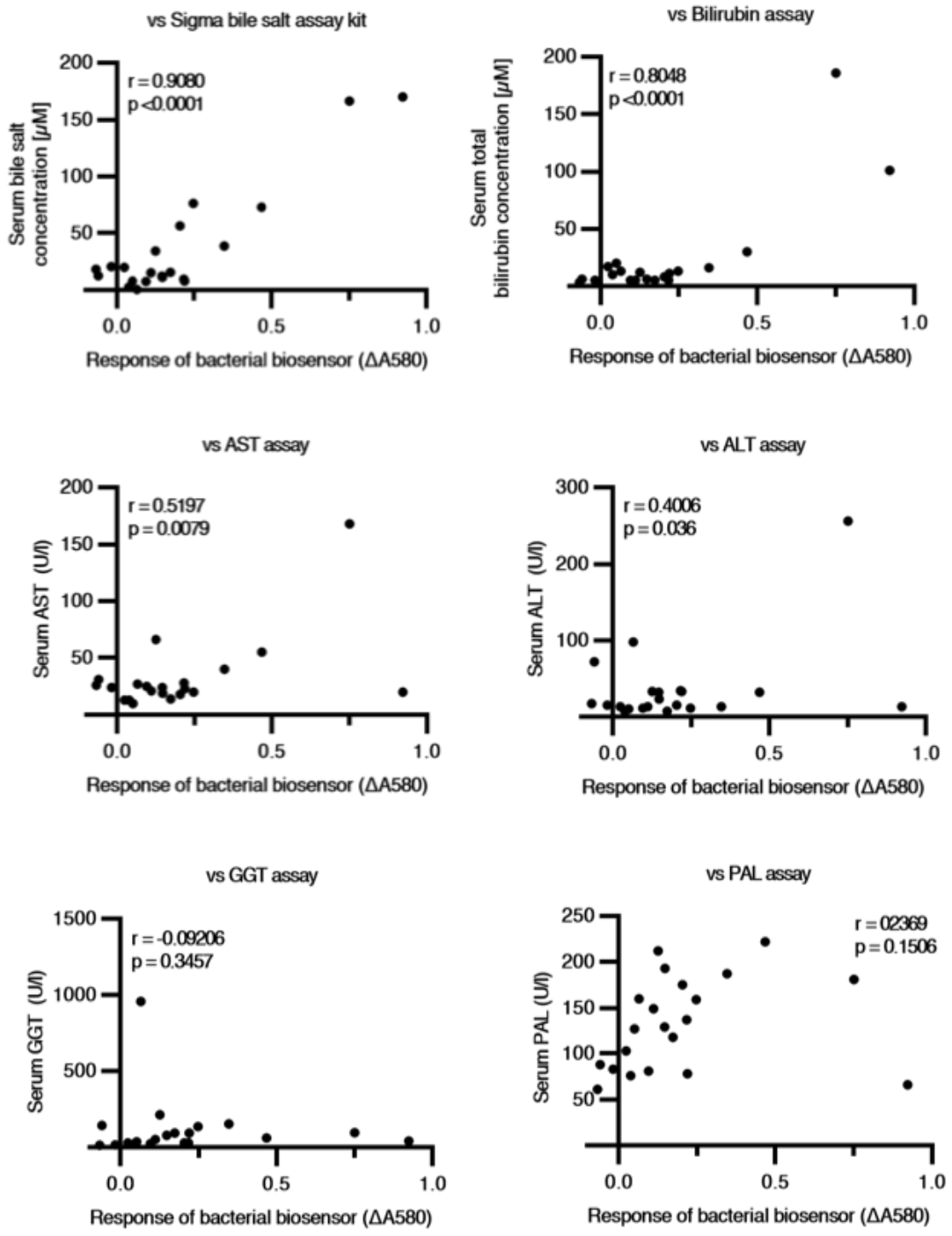


 # Blank = serum only

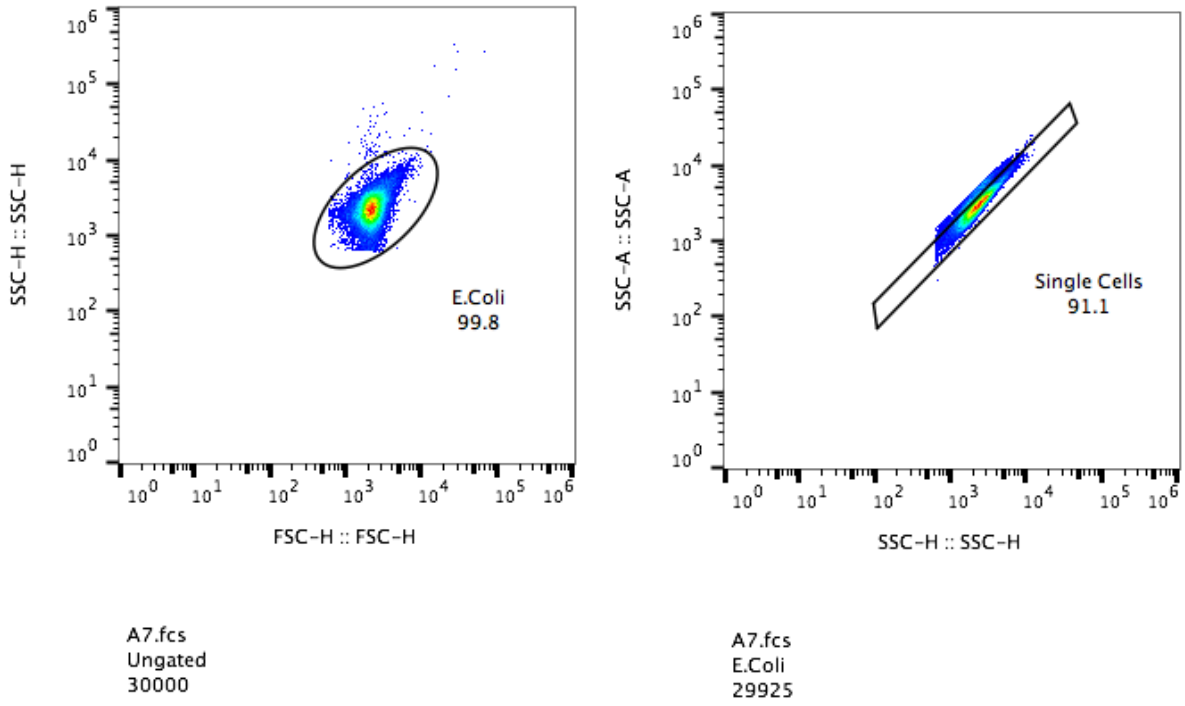
Sample	Blank	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10
A580	0,77	0,80	0,89	0,86	0,98	1,52	0,83	1,01	0,82	1,11	1,23
Stdeva	0,05	0,12	0,06	0,06	0,14	0,17	0,10	0,19	0,13	0,17	0,10
Sample	Patient 11	Patient 12	Patient 13	Patient 14	Patient 15	Patient 16	Patient 17	Patient 18	Patient 19	Patient 20	Patient 21
A580	0,70	0,94	1,69	0,98	0,91	0,97	0,91	0,88	0,75	0,79	0,71
Stdeva	0,15	0,09	0,20	0,13	0,11	0,08	0,06	0,20	0,07	0,15	0,06

Sample		Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10
ΔA580	(-Blank)	0,04	0,13	0,10	0,22	0,75	0,06	0,25	0,05	0,35	0,47
Sample	Patient 11	Patient 12	Patient 13	Patient 14	Patient 15	Patient 16	Patient 17	Patient 18	Patient 19	Patient 20	Patient 21
ΔA580	-0,07	0,17	0,92	0,22	0,15	0,20	0,15	0,11	-0,02	0,02	-0,06

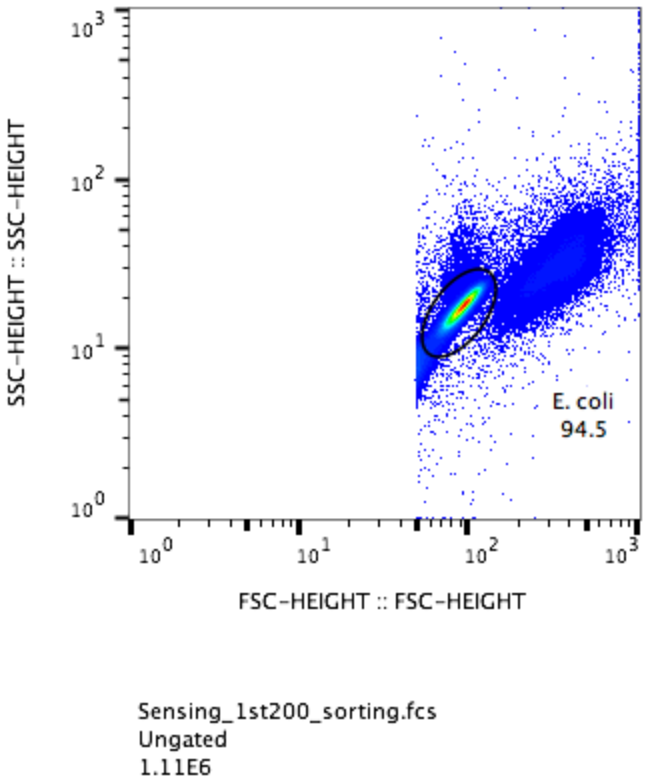
Supplementary Figure. 16 Visible color changes of bactosensor in clinical serum sample and measured absorbances values for each patient.



Supplementary Figure. 17 Comparison of the data of bactosensor in clinical serum samples from 21 liver transplantation patients to different liver biomarkers. These biomarkers includes bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), and alkaline phosphatase (ALP).



Supplementary Figure 18: Figure exemplifying the flow cytometry gate strategy. Gates were designed based on FSC-H vs SSC-H graphs to remove debris from the analysis (left pannel) and SSC-A vs SSC-H to doublet discrimination (right panel).



Supplementary Figure 19: Figure exemplifying the cell sorter gate strategy. Gates were designed based on FSC-H vs SSC-H graphs to remove debris from the analysis.

Supplementary File1: NGS summary counts of preselected synthetic NNK library and selected (Evo3) TcpP loop functional variants

Table S1 Genetic parts

Component	Nucleotide sequences
CadC DNA binding domain and juxtamembrane linker	ATGCAACAACCTGTAGTTCGCGTTGGCGAATGGCTTGTTACTCCGTCCATAAACCAAATTAGCCGCAATGGGCGTCAAC TTACCCTTGAGCCGAGATTAATCGATCTTCTGGTTTTCTTTGCTCAACACAGTGGCGAAGTACTTAGCAGGGATGAACTT ATCGATAATGTCTGGAAGAGAAGTATTGTCACCAATCACGTTGTGACGCAGAGTATCTCAGAACTACGTAAGTCATTAAAA GATAATGATGAAGATAGTCCTGTCTATATCGCTACTGTACCAAAGCGCGGCTATAAATTAATGGTGCCGGTTATCTGGTACA GCGAAGAAGAGGGAGAGGAAATAATGCTATCTTCGCCTCCCCCTATACCAGAGGCGGTTCCTGCCACAGATTCTCCCT CCCACAGTCTTAACATTCAAAACACCGCAACGCCACCTGAACAATCCCCAGTTAAAAGCAAACGA
TcpP transmembrane domain and periplasmic domain	GTGGTGCCGTATCTGGTGTTTTCAGCGTTATATGTTGCCTTATTACCTGTCATTTGGTGGTCATACGGGCAATGGTATCAA CACGAATTGGCCGGGATTACTCACGATTTACGTGACTTAGCCCGTCTTCCTGGAATCACAATCCAGAAACTTAGCGAGC AGAAGTTGACCTTTGCTATCGACCAGCATCAATGTTCGGTGAATTACGAACAAAAGACGTTGGAATGTACTAAGAACTAAT AA
TcpH	ATGCACAAGAAGTTAAAGGCTTGGGGAGGGGCCACTGGTCTGTTTGTGGTCGCCCTGGGGGTAACAATTATTGCTCTT CCTATGCGCCAGAAAAATAGCCACGGGACCATGATTATTGATGGAACCGTTACTCAAATCTTTAGCACCTACCAGGGCAA TCTGTCTAATGTGTGGCTTACTCAGACGGACCCTCAAGGTAATGTTGTTAAATCATGGACTACACGCTACCAAACATTGC CTGACCCTTCATCACAAAAGCTGAATCTGATCCCCGATTACTCCCAATCGAATGCTTCTCGTGATTACAATGTATTGTCGA TCTATCAACTTGGCAAAGGGTGCTTTCTGGCTTTTCCTTACAAATTGCTGACGGCGGAAAAGATGTGGTTCTCGTGTCA GAGCGATTTCTAA
VtrA transmembrane domain and periplasmic domain	ATTCTTCTTATCAGCATTATTTTGCAGTTGGCTTTCATCATCTATGTGGCTTATAGCTACACCAGTATTTTCGTGTCGTCCAC CGCAAAGGACGACTATCCCAGTTTATCGTTCCAACAAGACTACGTCTACATCTTTTCTTCAGACTTTCAATTAAGCGAGG AGCTGGGCGTGGCCCTTATCAACGCTTTAAGTGCAAAAGAAATTGTACCCGAGCGTCTTTACGTTATGTTGAATGACAAG ACCATCTCGTTCAGTTTTATCAGCAAGAATAAGAAGTCCAAGAATCGCGTATTAAGCACGGAAAAGAAACTCAATTATAAG CATATCTCCGAGTACATCGTCAATGAGATTGAGTACTAATAA
VtrC	ATGAAACTTAATATTAAGCGTCTGCATTTGAGCCTGACCCTGATGAGTGTCGTCATGCTGCTGGTAATCATCTACAATAATT TCTTTCAACCCGTGCACTTCTACGAAACGTCATACAAGTACCAAGCGGCGGACTCGACGTACATGCACGACGTCGCGA TCAATGTCTCGATTAAGGGCAACCACTTCACCTCAGATATCATCATCCGCGAGCTGGTGAAGTCAGAGAACAAGAATTAC TATAACGTTATTGGACATGGAGACATCATTCAAAAGAACACCCATCAATACTACTTGAACTTCGATAACATCGACGTTTACA CGGGTACTAATAAAGCGAACATGAAGCCGTATAAGGAACCGACTAGCATCTCCTCGCTCATCAACAAGTCAAATAACATT CGCGTTGTTTACTTATCGGAAGAGTATGTTGTGGTAGAGTTCTTCTTTTATGATGGACAGATTATCACATTGCATCGCTATTA ATAA
sfGFP	ATGTCAAAAGGAGAAGAACTTTTTACAGGTGTAGTACCTATCTTGGTTGAATTGGATGGTGATGTTAACGGTCACAAATTT TCTGTACGTGGTGAAGGTGAAGGTGATGCAACTAACGGTAAATTGACACTTAAATTCATTTGTACAACTGGAAAACTTCCT GTTCCTTGGCCTACTCTTGTTACAACATTGACATATGGAGTACAATGTTTTTCACGTTATCCTGATCATATGAAACGTCACG ATTTTTTTAAATCTGCTATGCCAGAAGGTTATGTACAAGAACGTACAATTTCATTTAAAGATGACGGAACATATAAAACACGT GCTGAAGTAAAATTCGAAGGTGACACTCTTGTTAATCGTATCGAATTGAAAGGAATCGATTTCAAAGAAGATGGTAACATT TTGGGACACAAACTTGAATACAACTTCAACTCTCATAATGTTTATATCACAGCTGACAAACAAAAAAACGGTATTAAAGCTA ATTTTAAAATTCGTCACAATGTTGAAGATGGATCTGTTCAATTGGCTGATCATTATCAACAAAATACACCAATCGGAGACGG ACCAGTATTGCTTCCAGATAACCACTACCTTTCTACTCAATCAGTTCTTTCAAAAGATCCTAACGAAAAACGTGACCATAT GGTACTTCTTGAATTTGTTACAGCAGCAGGTATCACTCACGGTATGGACGAACTTTATAAATAA
Promoters	**Nucleotide sequences**
P14	TTGACAATTAATCATCCGGCTCGTATAATGTGTGGA
P10	TTTCAATTTAATCATCCGGCTCGTATAATGTGTGGA
P9	TTGCCTCTTAATCATCGGCTCGTATAATGTGTGGA
pCadBA	ATCCATTGTAAACATTAAATGTTTATCTTTTCATGATATCAACTTGCGATCCTGATGTGTTAATAAAAAACCTCAAGTTCTCAC TTACAGAAACTTTTGTGTTATTTCACCTAATCTTTAGGATTAATCCTTTTTTCGTGAGTAATCTTATCGCCAGTTTGG

Table S2-5 Patient characteristics

Table S2. Patients characteristics (n=21)

Characteristics	**Value**
Age (years)	57.5±10.4
Male, n (%)	15 (71.4)
Underlying liver disease	
Alcoholic cirrhosis, n (%)	7(33.3)
Hepatocellular carcinoma, n (%)	4 (19)
Virus related cirrhosis (HBV, HCV), n (%)	2 (9.5)
Primary sclerosing cholangitis or primitive biliary cholangitis	5 (24)
Other (non-alcoholic steato-hepatitis, Budd-Chiari,auto immune hepatitis)	3 (14.2)
Time from liver transplantation to serum analysis in months, median (SD)	8± 38
Still alive at the end of the study , n (%)	18 (85.7)

Table S3. Patients characteristics (n=21)

Patient number	Age range	Sex	Cause of transplant	Year of transplant
1	50-69	m	Alcoholic	2020
2	60-69	f	NASH	2019
3	60-69	m	HCC	2019
4	60-69	m	Alcoholic	2019
5	30-39	m	vascular cirrhosis	2020
6	60-69	m	HCV	2006
7	40-49	f	auto-immune acute hepatitis	2020
8	60-69	m	PSC	2020
9	60-69	f	cirrhosis NASH	2020
10	40-49	f	PSC	2014
11	60-69	f	autoimmune cirrhosis	2019
12	>70	m	budd chiari disorder	2019
13	60-69	m	alcoholic cirrhosis	2013
14	30-39	m	PSC	2019
15	>70	m	NASH	2019
16	60-69	m	ischemic cholangitis	2019
17	50-59	m	HVC/HCC/alcoholic cirrhosis	2019
18	50-59	f	HVC/HCC/alcoholic cirrhosis	2019
19	50-59	m	HBV	2018
20	60-69	f	PSC	2020
21	40-49	f	HBV/HCV/HDV	2016

PSC: Primary Sclerosing Cholangitis
HBV: Hepatitis B Virus
HCV: Hepatitis C Virus
HCC: Hepato Cellular Carcinoma
NASH: Non-Alcoholic SteatoHepatitis

Table S4. Patients characteristics (n=21)-Hepatic tests and bactosensor measurements

Patient number	ASAT	ALAT	GGT	Pal	Total bilirubin	hospital bile salt	Bile salt (sigma kit)	BacSensor (OD 580)
1	13	7	18	76	10	4,7	2,56	0,07
2	66	33	214	212	12	24	34,44	0,16
3	25	11	24	81	5	7,5	7,59	0,13
4	23	33	94	78	11	5,4	7,86	0,32
5	168	256	96	181	186	193,1	166,21	0,91
6	27	98	959	160	13	2,7	0,62	0,06
7	20	11	136	159	13	19	76,22	0,43
8	10	10	36	127	20	7,9	7,86	0,16
9	40	13	154	187	16	25	38,74	0,51
10	55	32	60	222	30	55,8	72,91	0,40
11	26	17	14	61	3	10,4	18,24	0,05
12	14	7	94	118	5	13,2	15,65	0,16
13	20	13	41	66	101	77	169,91	1,11
14	28	34	28	137	5	10,6	9,63	0,32
15	19	23	78	193	6	11,3	11,10	0,24
16	18	15	29	175	8	40,9	56,58	0,25
17	24	32	81	129	5	8,4	12,07	0,14
18	21	13	50	149	5	11,2	15,37	0,07
19	24	15	17	83	5	3,6	20,71	0,00
20	13	13	28	103	17	12,6	20,08	0,02
21	31	72	143	88	6	3,2	12,60	-0,08

Table S5. Patients acute clinical conditions

Patient number	Acute clinical condition
1	follow up
2	bypass/switch duodéno pancréas
3	follow up
4	type 2 diabetes
5	transplant liver and kidney, graft rejection/ biliary stenosis
6	graft rejection and biliary duct obstruction/stenting 48 H before blood test
7	Kehr drain
8	follow up
9	sepsis (acute general infection) and hepatic artery stenosis
10	follow up and new cirrhosis
11	fiever
12	ischemic cholangitis
13	type 2 diabetes
14	follow up
15	follow up
16	follow up, phosphatase alcaline d'origine osseuse, portage BLSE urinaire
17	follow up and bile duct stenosis
18	follow up
19	acute colic infection
20	follow up
21	angiocholitis