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Supplementary Materials for

Programmable probiotics for detection of cancer in urine

Tal Danino, Arthur Prindle, Gabriel A. Kwong, Matthew Skalak, Howard Li, Kaitlin Allen, Jeff Hasty, Sangeeta N. Bhatia*

*Corresponding author. E-mail: sbhatia@mit.edu

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This PDF file includes:

Other Supplementary Material for this manuscript includes the following:
(available at www.sciencetranslationalmedicine.org/cgi/content/full/7/289/289ra84/DC1)

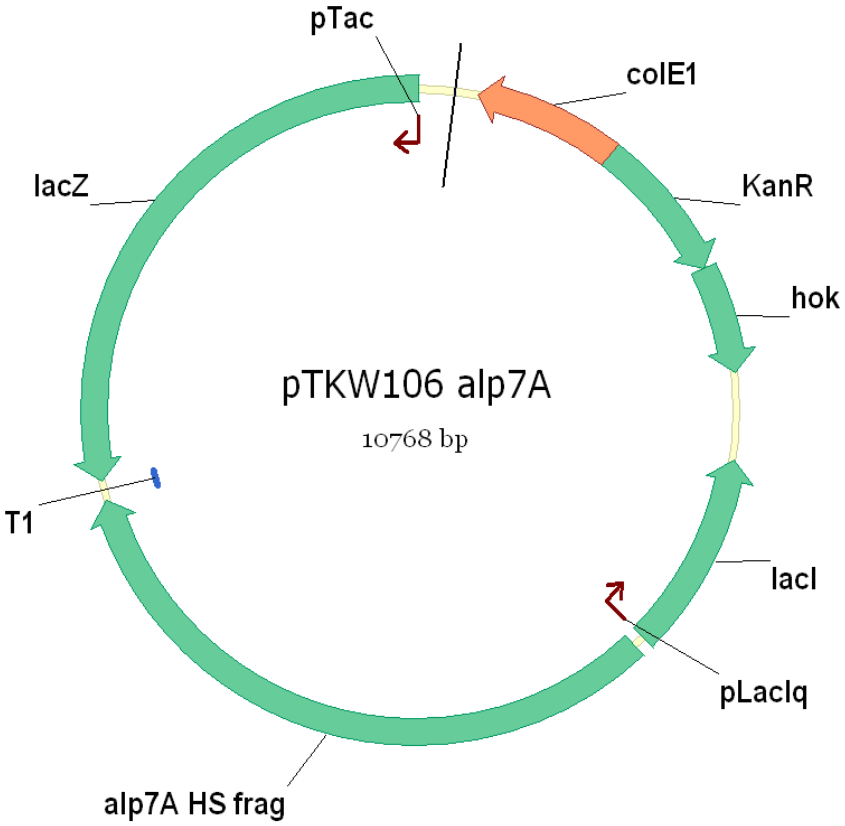

Fig. S1: Map of plasmid pTKW106 alp7A.

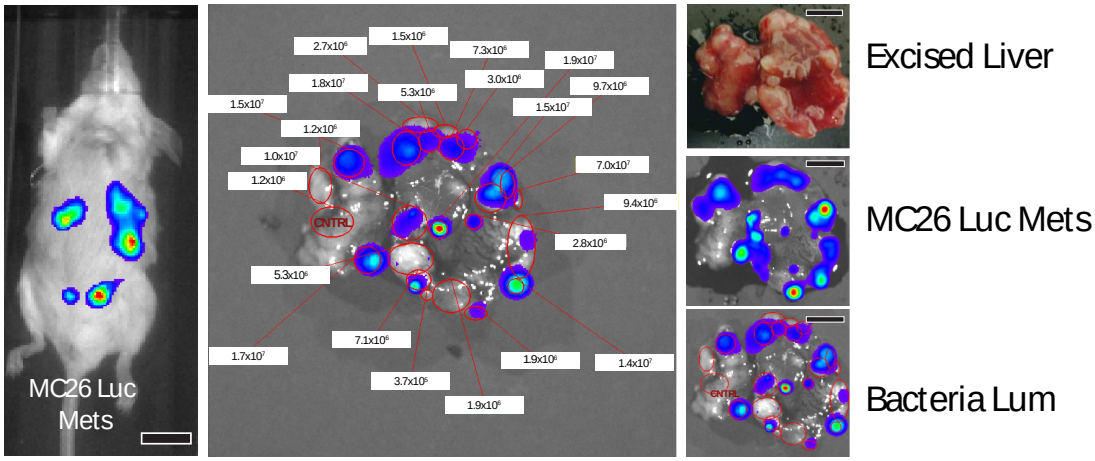

Fig. S2: Size and colonization of liver metastases. Mice with liver metastases (21 days) were injected intraperitonealy with luciferin and imaged using IVIS (left). Even in an intact host mouse, some liver metastases are visible. These signals derive from large tumors near the surface of the organ. 48 hours later, we orally administered PROP-Luc at $5x10^9$ CFU and waited an additional 24 hours for colonization to occur. To assay for correlation between tumor- and bacterial-derived luminescence, the liver was explanted and imaged using IVIS to first capture bacterial luminescence (Bacteria Lum image, bottom). The bacteria luxCDABE cassette produces luminescence without the addition of a substrate. We then soaked the liver in a D-luciferin solution after which luminescence derived from the firefly luciferase gene expressed by MC26-LucF cells was observed (MC26 Luc Mets, middle). Quantification of colonized metastases (middle, radiance values in p/sec/cm2/sr shown) is described in the Materials and Methods section and data is included in Table S1.

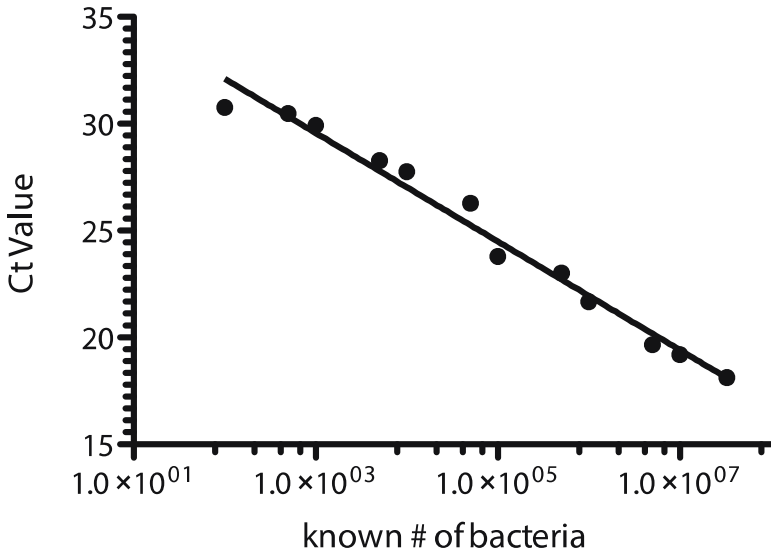

Fig. S3: Calibration curve for the qPCR calculation of the number bacteria in a tumor sample. Mean ± SEM, N=5 q-PCR replicates, error bars are smaller than the size of points.

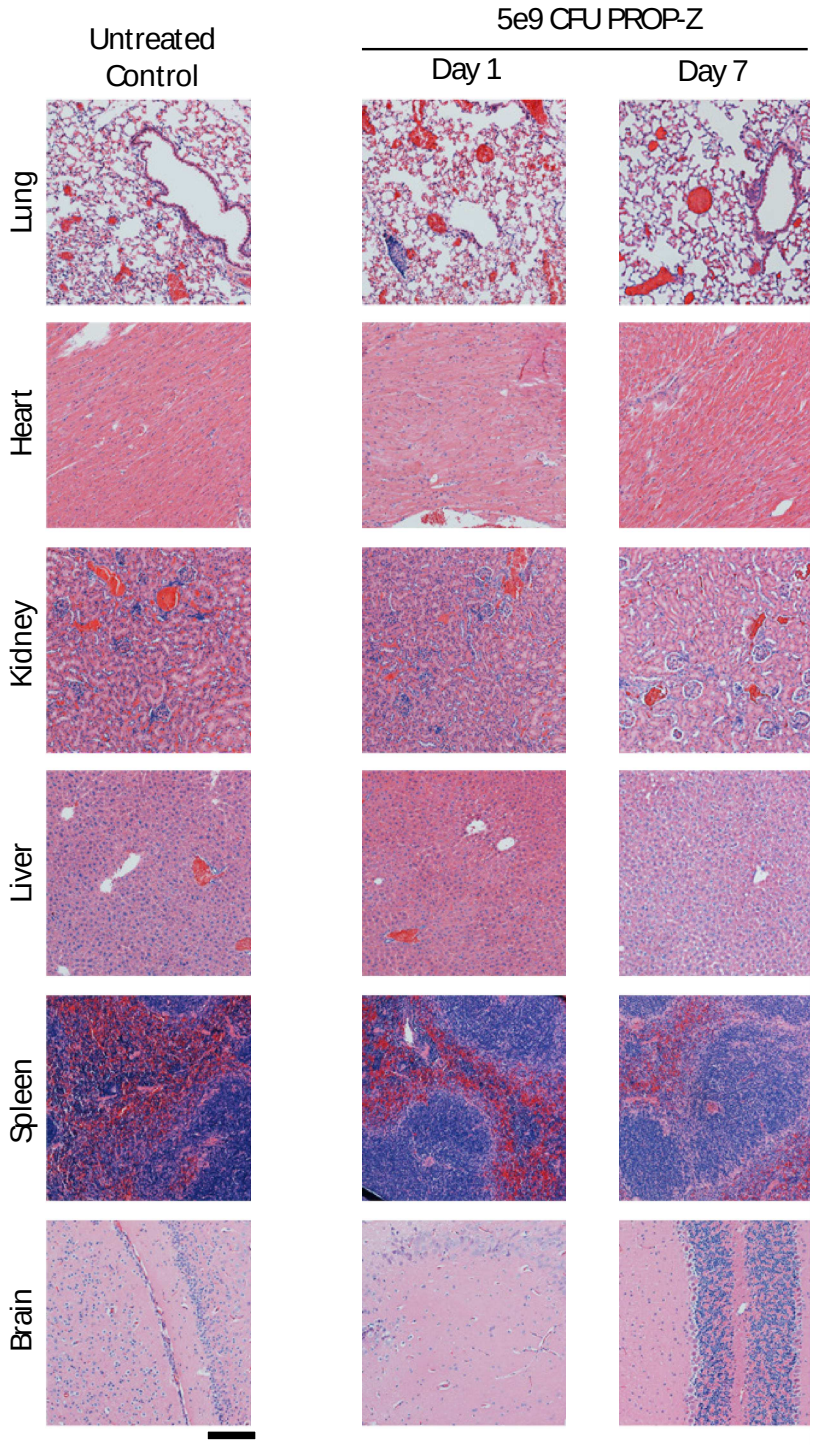

Fig. S4: Histopathological analysis of organs following oral delivery of PROP-Z. H&E staining of lungs, hearts, kidneys, livers, spleens, and brains of mice 1 and 7 days after oral gavage with $5x10^9$ PROP-Z, relative to untreated controls. Scale bar: 100 µm.

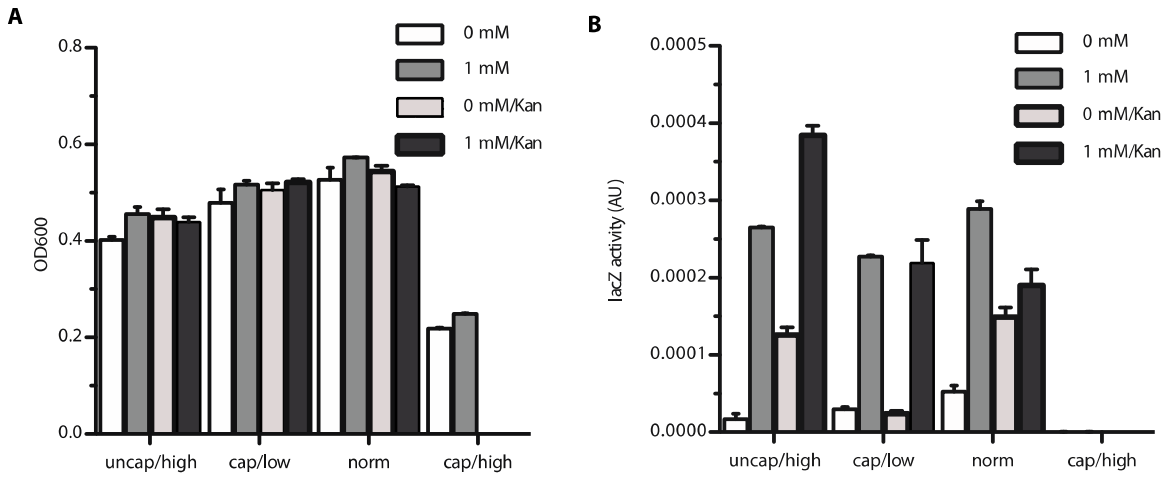

Fig. S5: Growth and activity of stabilized lacZ plasmid in different oxygen, inducer (IPTG), and antibiotic conditions. Oxygen conditions were varied for 48 hours by growing Mach1 (lacZ deficient) strain bacteria transformed with pTKW106alp7A in 14 mL Falcon tubes. These cultures were subcultured every 24 hours, and grown in tubes that were either capped or uncapped, and with either a high volume of media (14 mL) or low media volume (3 mL). Inducer (IPTG, 1.0 mM) and antibiotic (kanamycin, 50 ug/mL) conditions were also varied as indicated. Normal (norm) conditions are 3 mL media in an uncapped tube. Cultures were assayed for density (OD_{600}) in **(A)** and lacZ activity **(B)**. Mean ± SEM, N=3 for each column shown above.

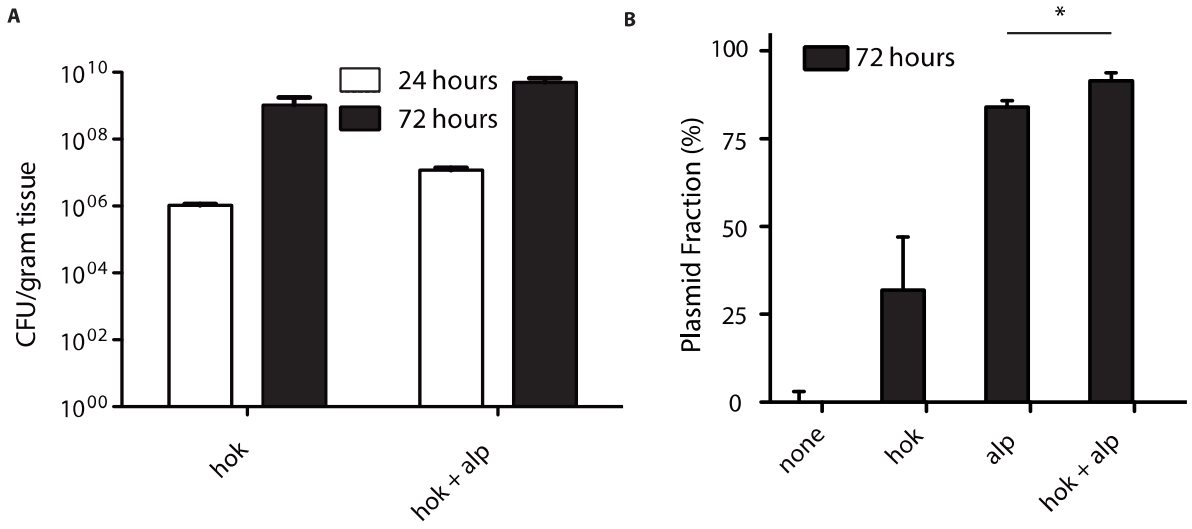

Fig. S6: CFU and stability of constructs in subcutaneous tumor models. Nude mice bearing subcutaneous tumors derived from the LS174T_LucF cell line were injected intravenously with $1x10^6$ PROP (EcN-luxCDABE) bacteria with either the pTKW106 (hok only), pTKW106alp7A (hok+alp), pTKW106_delhok (none), or pTK106alp7A (alone) plasmids. **(A)** Absolute levels of bacteria present in explanted, dissociated tumors were determined at two time points by colony counts in the presence of kanamycin and erythromycin. Growth rates of the bacteria bearing both stability plasmids are not slower than the parent strain. **B)** Plasmid stability was determined at 72 hours by performing differential colony counts in the presence of erythromycin (resistance gene present on integrated luxCDABE) or erythromycin and kanamycin (resistant only with the retention of pTKW106 plasmids). N=4-6 tumors in each column shown above, * indicates $P<0.05$, Student's t-test.

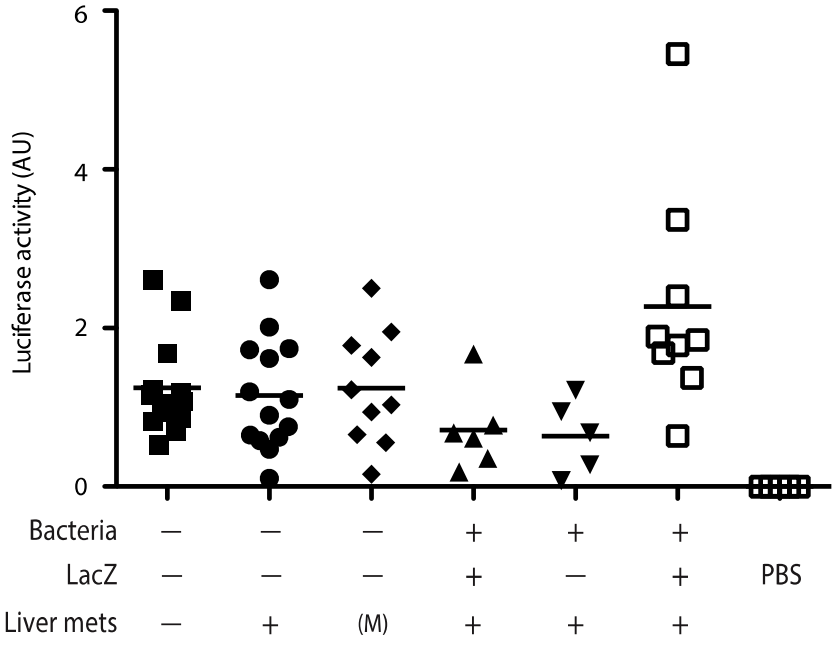

Fig. S7: Background cleavage of Lugal from urine assay. We performed assays on a variety of test cases to determine the dominant source of background cleavage of Lugal. The case without administration of PROP-Z to Liver Mets (-Bacteria, -lacZ, -LM), representing healthy mice, has the narrowest spread, and the introduction of the surgery procedure itself, either with cells (-Bacteria, -lacZ, +LM) or PBS (-Bacteria, -lacZ, Mock), generates variability into the urine cleavage assay. The PBS control shows an *in vitro* incubation of LuGal for 2 hours at room temperature with no significant LuGal cleavage observed. In vitro, we observed reporter cleavage by homogenized liver tissue from healthy mice, but no significant signals were obtained after incubation with whole blood. 3 way ANOVA was performed as in Table S2 where +PROPZ (+Bacteria, +LacZ) causes the most significant difference between +Liver Mets and – Liver Mets groups, $P < 0.005$.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
PROP-Z	1	2.975e+12	2.975e+12	4.324	0.04373
LM	1	1.304e+12	1.304e+12	1.896	0.17587
nonlacZ	1	2.394e+12	2.394e+12	3.480	0.06912
PROP-Z:LM	1	6.414e+12	6.414e+12	9.322	0.00392
Residuals	42	2.890e+13	6.881e+11		

Table S2. Results of three-way factorial ANOVA. We performed a 3 way ANOVA on PROP-Z, Liver Metastasis (LM), and nonlacZ variables from the data in Fig. S7. The P value (P=0.17587) indicates that the difference in urine assay value between liver metastasis and healthy mice before administration of PROP-Z is not statistically significant. Thus, the background cleavage of our substrate is mostly from a healthy mouse (-Bacteria, -lacZ, -Liver Metastasis). The introduction of PROP-Z causes the most significant difference between +LM and –LM experiment design (P=0.00392). Df=Degrees of Freedom, Sum Sq=Sum of the squares, Mean Sq=Mean of Sq, F-value=F-statistic, Pr (>F)=P-value.