Supporting Information

Selective Fluorogenic Chemosensors for Distinct Classes of Nucleases

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Materials and reagents

The nucleoside phosphoramidites (A, T) and 3′-dT-CPG, 3′-phosphate CPG, Universal Support III columns were purchased from Glen Research. Solvents and reagents were purchased from Fisher Scientific, Aldrich or ACROS unless otherwise noted. Exonuclease T and $RecJ_f$ enzymes were purchased with 10X NEBuffer 4 (200 mM Tris-acetate, 500 mM potassium acetate, 100 mM magnesium acetate, 10 mM dithiothreitol, pH 7.9 at 25 °C) and 10X NEBuffer 2 (100 mM Tris-HCl, 500 mM NaCl, 100 mM $MgCl_2$, 10 mM dithiothreitol, pH 7.9 at 25 °C) from New England Biolabs, and S1 endonuclease was purchased with S1 Nuclease 10X Buffer (500mM sodium acetate, 2.8M NaCl, 45mM $ZnSO_4$, pH 4.5 at 25 °C) from Promega.

Oligodeoxynucleotide synthesis

5′-DMT 3′-cyanoethyl α-pyrene phosphoramidite (Y) was prepared according to published methods.[1] Probes were synthesized on a Applied Biosystems 394 DNA/RNA synthesizer, using 3′-dT-CPF, 3′-phosphate CPG or Universal Support III column on 1 μmol scale with DMT-off method. Coupling of each monomer used standard 3′ to 5′ cyanoethyl phosphoramidite chemistry with extended coupling time (999 s). Oligomers were deprotected by saturated NH_4OH for 17 hour at 55 °C except for YTTY. YTTY was cleaved from Universal Support III column using 2M NH_3 in MeOH solution first, then deprotected by saturated NH_4OH for 17 hours at 55 °C. Purification was carried out utilizing a Shimadzu Series HPLC with an Alltech C5 column with acetonitrile and TEAA buffer (50 mM, pH 8.5) as eluents. The identities were confirmed by MALDI-TOF-MS. The concentrations of oligonucleotides were determined by pyrene UV absorbance at 342 nm ($\varepsilon = 47000\ cm^{-1}\ M^{-1}$).[2] Uncertainty in concentration is ± 20%.

Quantum yield measurements

Fluorescence studies were performed on a Jobin Yvon-Spex Fluorolog 3 spectrophotometer equipped with a thermostat accurate to 0.1 °C. The emission spectra were obtained by exciting the samples at 340 nm and scanning the emission from 360 to 600 nm with a step of 1 nm. Quinine sulfate was used as a fluorescence quantum yield standard.[3]

In vitro enzymatic fluorescence assay

The probes (1 μM, 50 μL) were dissolved in 1X reaction buffer (450 μL) to prepare a stock solution of 100 nM. The samples were incubated at 37.0 °C with nuclease enzymes after mixing rapidly. The fluorescence spectra or the fluorescence intensity of solutions were recorded over varying timecourses. Data points with the enzymes were taken at 1, 5, 10, 20, 30, 60, 120, 180, 240 min; plots show straight lines connecting the points. Error in fluorescence intensity is estimated at ±5%.

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[1] (1) R. X.-F. Ren, N. C. Chaudhuri, P. L. Paris, S. R. IV and E. T. Kool, *J. Am. Chem. Soc.,* **1996,** 118, 7671-7678.
(2) J. Gao, S. Watanabe and E. T. Kool, *J. Am. Chem. Soc.,* **2004**, *126*, 12748-12749.

[2] J. Gao, C. Strassler, D. Tahmassebi and E. T. Kool, *J. Am. Chem. Soc.,* **2002**, *124,* 11590-11591.

[3] J. R. Lakowicz, Principles of Fluorescence Spectroscopy, 3rd ed., Springer, New York, **2006**.

[4] E. G. Folco, H. Lei, J. L. Hsu, R. Reed, *J. Vis. Exp.*, **2012**, 64, e4140.

Biological fluids fluorescence assay

The probes were added to PBS to a final volume of 100 nM and incubated at 37 °C for 5 minutes. The fluorescence emission spectrum following excitation at 342 nm was recorded. Serum, saliva, or urine (to a final concentration of 10% diluted with PBS) or sweat (to a final concentration of 8.3% diluted with PBS) was added and mixed, and the fluorescence emission spectrum following excitation at 342 nm was recorded at timepoints following addition of the biological fluid. Samples were incubated at 37 °C between readings. Due to high background, the readings for urine and serum were background corrected by subtracting the intensity of an identical sample without the sensor. The fluorescence emission of the background control sample did not vary significantly over the timecourse.

Cell lysate fluorescence assay

HeLa cells were grown to 90% confluence in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin in a humidified incubator at 37 °C with 5% CO_2. The protocol by Folco *et al*.[4] was followed with a few changes: The cells were collected by trypsinization at room temperature instead of scraping. Roche complete mini EDTA-free tablets were used instead of PMSF. The cells were lysed by passing 10-15 times through a 21 gauge needle and 5-10 times through a 16 gauge needle instead of through a dounce homogenizer. Following salt extraction of the nuclei, the cytoplasmic and nuclear fractions were adjusted to 20 mM HEPES pH 7.9, 100 mM KCl, 0.02 mM EDTA, 20% glycerol, 1x protease inhibitor, 0.5 mM DTT, 0.5 mM $CaCl_2$, and 2.5 mM $MgCl_2$. The fractions were concentrated using Amicon Ultra 3K centrifugal filters to a concentration of $1.7x10^{11}$ lysed cells/mL. 150 μL of either cytoplasmic of nuclear lysate was incubated with 5 μM nuclease sensor. Over a timecourse at 37 °C, aliquots were taken and diluted 50-fold before recording the fluorescence emission spectrum following excitation at 342 nm.

Table S1. Full List of Nuclease Sensor candidates and MALDI-TOF-MASS data

Sequence	Formula	MW	MALDI-TOF-MASS
5'-A-T-Y	C41H43N7O15P2	935.2	935.2
5'-A-Y-T	C41H43N7O15P2	935.2	935.6
5'-A-Y-T-T	C51H56N9O22P3	1239.3	1240.7
5'-T-T-Y	C41H44N4O17P2	926.2	926.4
5'-T-T-Y-A	C51H56N9O22P3	1239.3	1240.5
5'-Y-A-T-T	C51H56N9O22P3	1239.3	1239.7
5'-Y-S-S-T	C41H49N2O20P3	982.2	981.2
5'-Y-S-T	C36H40N2O15P2	802.2	802.9
5'-Y-S-T-T	C46H53N4O22P3	1106.2	1106.8
5'-T-S-Y-A_7	C106H124N37O50P9	2993.6	2995.0
5'-T-Y-A_8	C111H127N42O50P9	3126.6	3125.3
5'-Y-T-A_8	C111H127N42O50P9	3126.6	3126.5
5'-Y-S-T-T-S-Yp	C72H80N4O35P6	1746.3	1746.4
5'-Y-T-T-Yp	C62H62N4O25P4	1387.1	1387.1
5'-Y-T-T-Y	C62H61N4O22P3	1306.3	1306.6

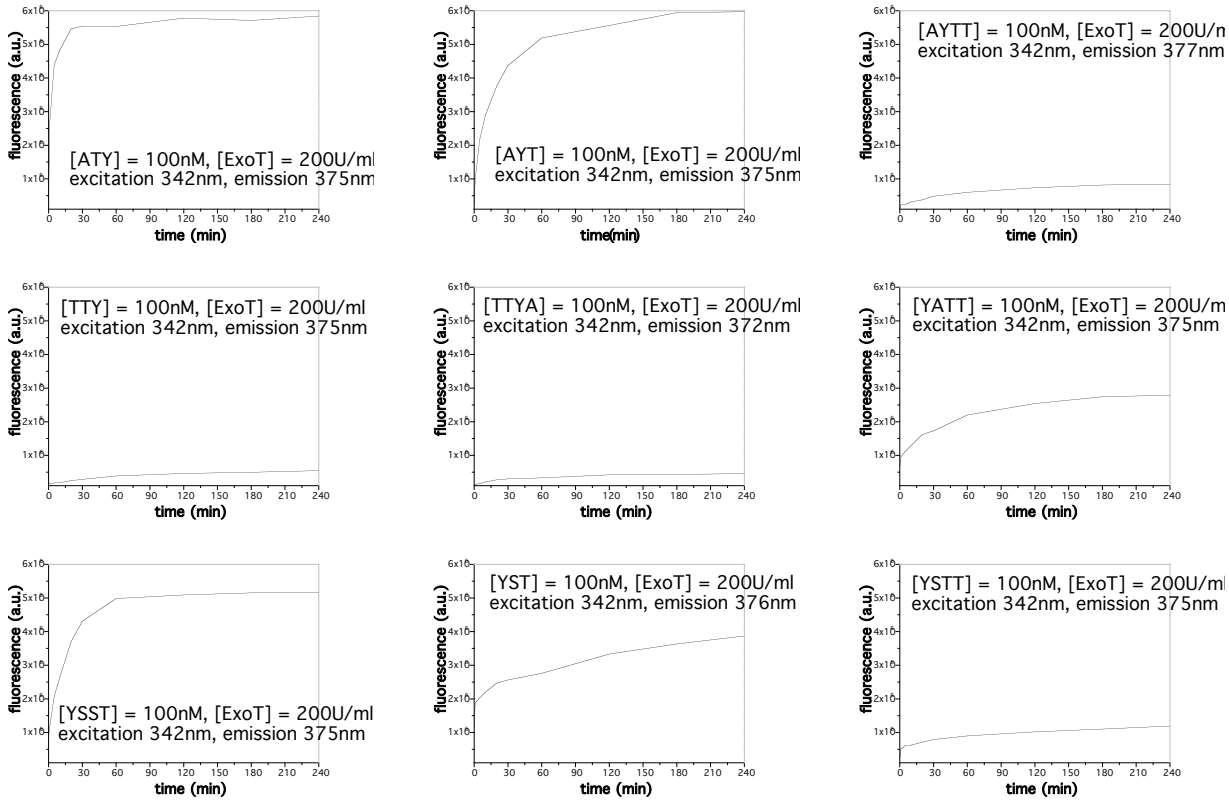

Figure S1. Enzyme responses of early candidate nuclease sensors with 3'-exonuclease ExoT. Conditions: [probe] = 100 nM, [Exo T] = 200U/mL, excitation 342 nm, 25 °C. T=thymidine, A=deoxyadenosine, Y=α-pyrene deoxyriboside, S=tetrahydrofuran abasic nucleoside.

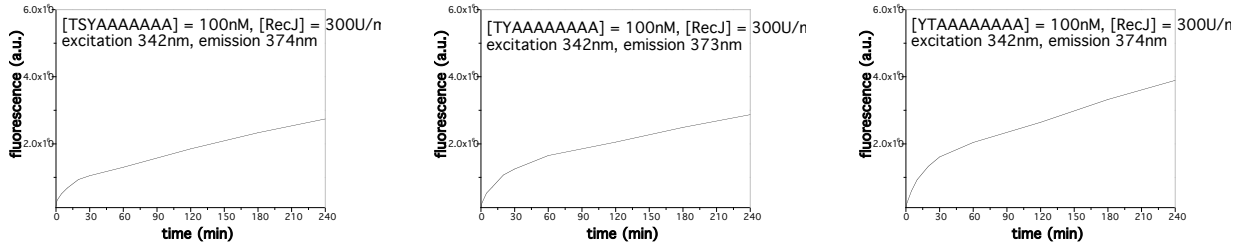

Figure S2. Enzyme responses of early candidate nuclease sensors with 5'-Exonuclease $RecJ_f$. Conditions: [probe] = 100 nM, [$RecJ_f$] = 300 U/mL, excitation 342 nm, 25 °C. T=thymidine, A=deoxyadenosine, Y=α-pyrene deoxyriboside, S=tetrahydrofuran abasic nucleoside.

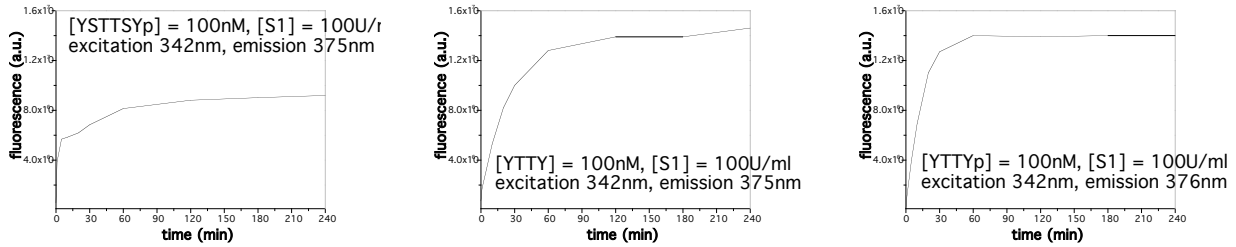

Figure S3. Enzyme responses of nuclease sensors with Endonuclease S1. Conditions: [probe] = 100 nM, [S1] = 100 U/mL, excitation 342 nm, 25 °C. T=thymidine, A=deoxyadenosine, Y=α-pyrene deoxyriboside, S=tetrahydrofuran abasic nucleoside.

Table S2. MALDI-TOF-MASS data and Quantum Yields of probes 1-4

Sequence	Formula	MW	MALDI-TOF-MASS	QY
5'-A-Y-T	C41H43N7O15P2	935.2	935.6	0.00878
5'-Y-T-A_8	C111H127N42O50P9	3126.6	3126.5	0.00554
5'-Y-T-T-Y	C62H61N4O22P3	1306.3	1306.6	0.0301
5'-Y-T-T-Yp	C62H62N4O25P4	1387.1	1387.1	0.0204

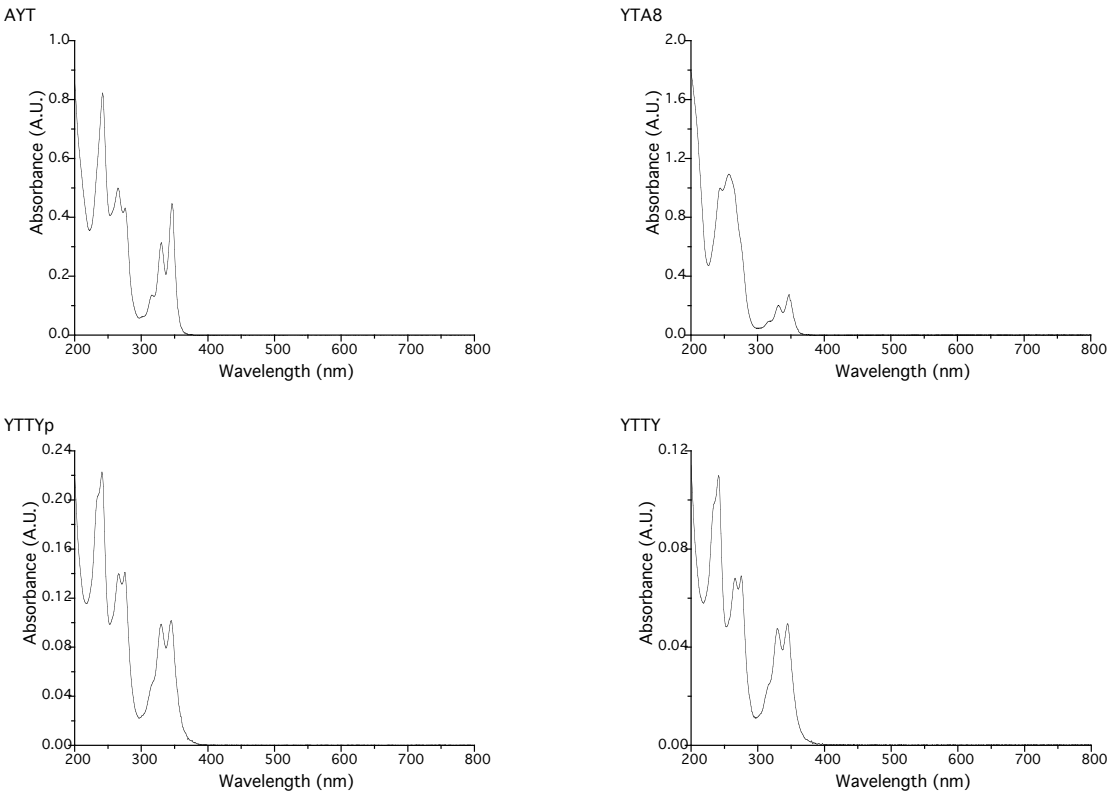

Figure S4. UV-vis absorption spectra of probes **1**-**4**. Conditions: [AYT] = 6.7 μM, [YTA_8] = 4.0 μM, [YTTYp] = 1.0 μM, [YTTY] = 0.48 μM in water.

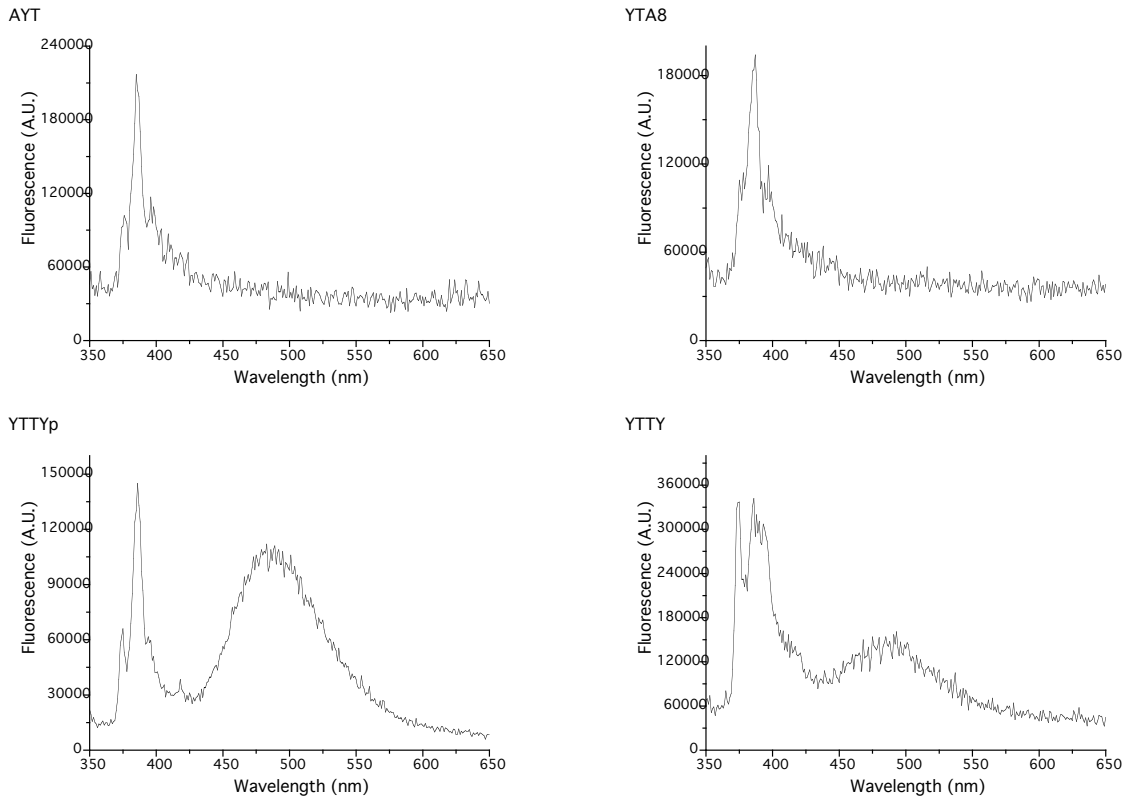

Fig. S5. Residual fluorescence spectra of quenched probes **1**-**4** (excitation 342 nm). Conditions: 0.1 μM probe in a buffer containing 50 mM KOAc, 20 mM Tris-acetate, 10 mM $Mg(OAc)_2$, 1 mM dithiothreitol, pH 7.9 at 25 °C for AYT; 10 mM Tris-HCl, 50 mM NaCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, pH 7.9 at 25 °C for YTA_8; 2.8 M NaCl, 45 mM $ZnSO_4$, 500 mM NaOAc, pH 4.5 at 25 °C for YTTYp and YTTY.

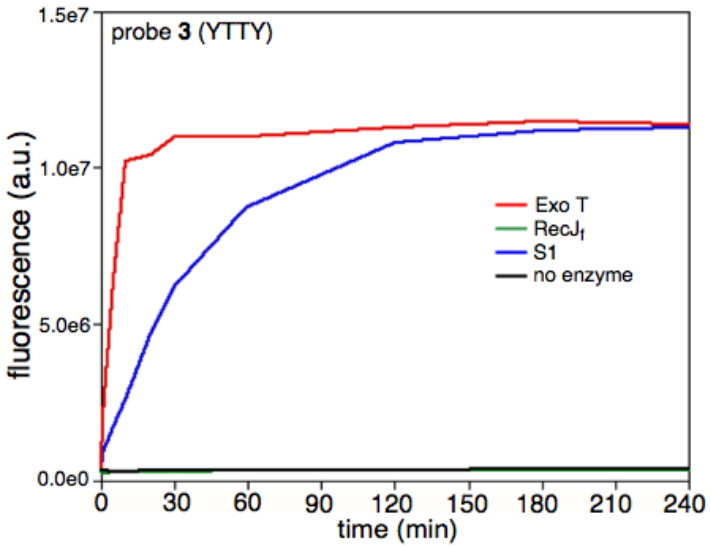

Figure S6. Enzyme selectivity profile of probe **3**. Conditions: 25 °C, excitation 342 nm, emission 375 nm for Exo T and S1, 374 nm for $RecJ_f$. [nuclease sensor] = 100 nM. [Exo T] = 25 U/mL, [$RecJ_f$] = 900 U/mL, [S1] = 100 U/mL.

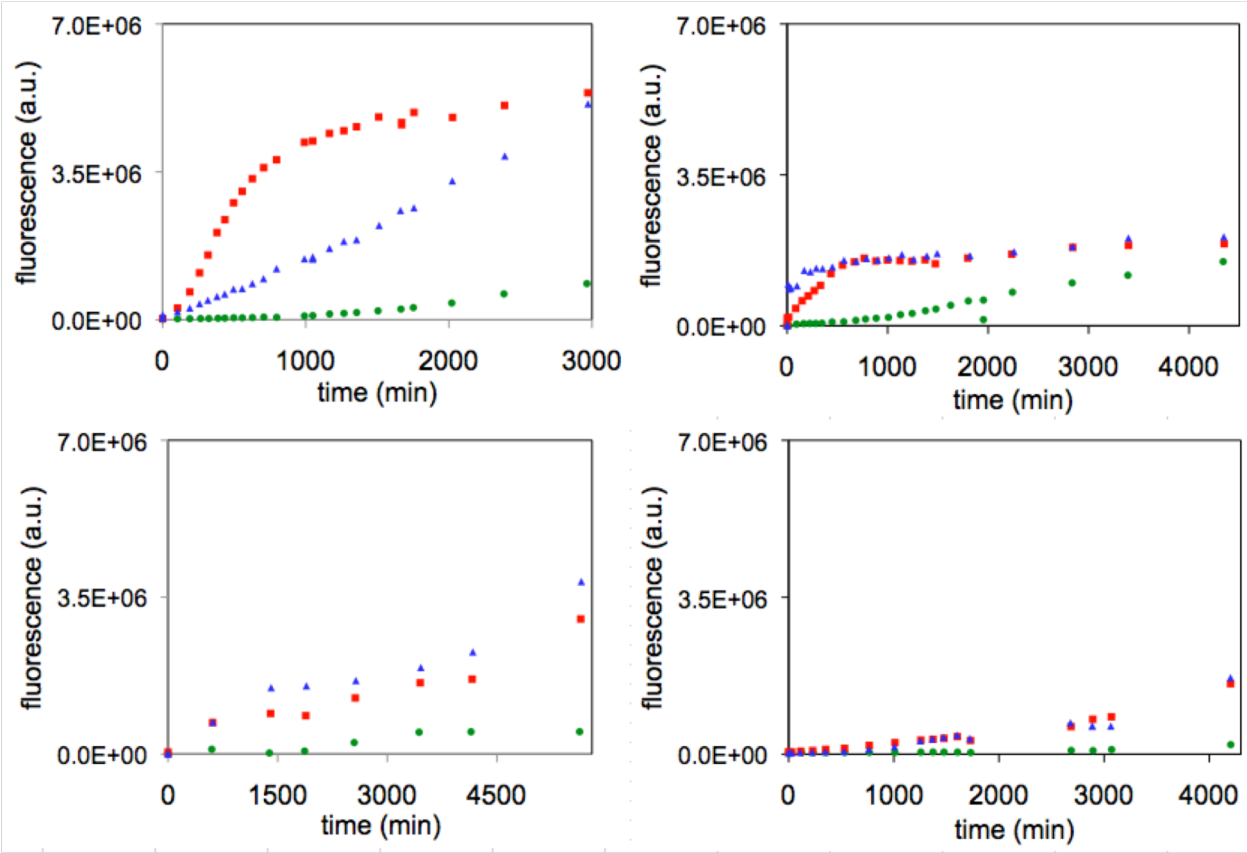

Figure S7. Full timecourses of fluorescence responses of nuclease sensors **1**, **2**, and **4** in biological fluids. (A) 10% saliva; (B) 10% serum; (C) 10% urine; (D) 8.3% sweat. Conditions: 37 °C, excitation 342 nm, emission 375 nm. [nuclease sensor] = 100 nM.