Supplementary table 1

Position	Native Amino Acid	Frequency	Random Forest Ranking	Mutation	Frequency	Estimate (log(D-luc/4'Br))	Standard Error	t value	Pr(>|t|)	Significance
211	P	196	18.67	A	7	0.30839	0.18982	1.625	0.10762	
				S	6	-0.0713	0.23635	-0.302	0.76367	
				T	2	0.08635	0.27811	0.31	0.75689	
214	T	208	10.42	A	2	0.22054	0.25565	0.863	0.39053	
				S	1	0.31082	0.31859	0.976	0.33179	
218	R	181	97.4	A	2	-0.6817	0.23314	-2.924	0.00434	**
				H	5	-0.1292	0.31859	-0.405	0.68605	
				K	23	0.66895	0.09273	7.214	1.43E-10	***
220	S	198	19.33	N	13	0.16193	0.33322	0.486	0.62813	
226	I	209	-3.28	V	2	-0.4745	0.73781	-0.643	0.52173	
227	F	198	0.35	C	1	-0.2112	0.31843	-0.663	0.5089	
				W	4	-0.0487	0.28951	-0.168	0.86683	
				Y	8	0.07492	0.13087	0.572	0.5684	
229	N	190	15.7	T	21	-0.2349	0.18888	-1.244	0.21673	
232	I	210	0	T	1	0.17555	0.32366	0.542	0.58883	
237	I	200	-4.78	V	11	0.05426	0.15272	0.355	0.72317	
239	S	196	-2.99	I	1	0.55528	0.46132	1.204	0.23177	
				T	13	0.30491	0.20534	1.485	0.14096	
				V	1	-0.3416	0.39311	-0.869	0.38714	
240	V	194	22.89	A	9	0.27524	0.17373	1.584	0.11652	
				G	1	0.23078	0.3638	0.634	0.52741	
				I	3	-0.0028	0.25232	-0.011	0.99113	
				L	2	0.62078	0.3638	1.706	0.09128	.
				M	2	0.11961	0.29372	0.407	0.68477	
241	V	198	8.13	A	9	0.00528	0.18827	0.028	0.97767	
				F	1	-0.9705	0.48142	-2.016	0.0467	*
				L	1	NA	NA	NA	NA	
				M	2	-0.4318	0.29795	-1.449	0.15067	
243	F	201	15.33	L	1	-0.0997	0.39904	-0.25	0.80329	
				M	9	-0.0694	0.13886	-0.5	0.61837	
246	G	192	9.84	A	19	-0.4631	0.24468	-1.893	0.06151	.
247	F	195	63.21	L	4	-0.2604	0.24334	-1.07	0.28725	
				S	1	NA	NA	NA	NA	
				Y	11	-0.5138	0.18159	-2.83	0.00571	**
249	M	150	35.41	F	9	0.19395	0.2297	0.844	0.40065	
				I	6	0.44579	0.27541	1.619	0.10892	
				L	46	0.03409	0.08591	0.397	0.69239	
250	F	178	23.77	A	4	-0.2625	0.2519	-1.042	0.30012	
				C	6	0.25221	0.19822	1.272	0.20641	
				G	1	0.57044	0.46814	1.219	0.22611	
				H	2	-0.5907	0.23678	-2.495	0.01437	*
				M	6	-0.2362	0.1506	-1.568	0.12021	
				S	2	0.72441	0.30415	2.382	0.01927	*
				T	7	-0.08	0.19599	-0.408	0.68395	
				V	1	0.15515	0.4816	0.322	0.74807	
				Y	4	-0.1121	0.16827	-0.666	0.5068	
251	T	210	0	I	1	NA	NA	NA	NA	
252	T	208	-4.28	S	3	-0.0501	0.54804	-0.091	0.92729	
255	Y	175	39.24	F	21	-0.4338	0.19608	-2.212	0.02938	*
				H	12	-0.4664	0.17548	-2.658	0.00926	**
				I	1	-0.7957	0.67763	-1.174	0.24332	
				N	2	-0.8542	0.31773	-2.688	0.00851	**
256	L	196	5.34	I	7	-0.2893	0.24769	-1.168	0.24579	
				M	6	-0.0489	0.21685	-0.225	0.82224	
				V	2	-0.1631	0.47519	-0.343	0.73221	
257	I	159	30.32	A	1	-0.5082	0.31734	-1.602	0.11266	
				C	3	-0.0694	0.38171	-0.182	0.85618	
				F	5	0.38618	0.28158	1.371	0.17353	
				L	14	0.06181	0.20057	0.308	0.75864	
				M	13	0.12343	0.17659	0.699	0.48633	
				R	3	0.27062	0.22695	1.192	0.23612	
				S	3	-0.0553	0.23705	-0.233	0.81617	
				V	8	0.42431	0.23297	1.821	0.07178	.
				Y	2	0.75755	0.40014	1.893	0.06144	.
260	F	207	9.51	G	1	-0.1392	0.31859	-0.437	0.66321	
				I	1	-0.2192	0.31859	-0.688	0.49317	
				N	1	-0.2792	0.31859	-0.876	0.38311	
				Y	1	0.10082	0.31859	0.316	0.75238	
264	L	189	31.8	F	1	-0.2897	0.37298	-0.777	0.43932	
				M	13	-0.3601	0.21138	-1.704	0.09179	.
				V	8	0.00896	0.2792	0.032	0.97448	
265	M	210	0	T	1	-0.8745	0.73781	-1.185	0.23893	
266	Y	209	2.65	H	2	-0.0988	0.87985	-0.112	0.91087	
267	L	210	0	F	1	0.05555	0.32366	0.172	0.86409	
269	E	209	2.47	G	2	-0.0795	0.25565	-0.311	0.75664	
279	D	210	0	N	1	0.7202	0.7657	0.941	0.34936	
281	K	210	0	E	1	-0.064	0.7331	-0.087	0.93062	
282	I	209	-5.28	T	1	NA	NA	NA	NA	
				V	1	0.08489	0.49072	0.173	0.86304	
283	Q	210	0	R	1	0.17555	0.32366	0.542	0.58883	
284	S	208	-2.78	V	3	0.08257	0.21939	0.376	0.70749	
286	L	196	24.55	I	2	-0.9751	0.48336	-2.017	0.04654	*
				M	4	-0.281	0.20038	-1.402	0.16411	
				V	9	0.55493	0.31594	1.756	0.08231	.
287	L	196	17.08	A	1	0.43402	0.46005	0.943	0.34791	
				I	1	-0.6259	0.42133	-1.486	0.14079	
				M	4	-0.2324	0.24791	-0.938	0.35089	
				V	9	0.36644	0.20177	1.816	0.07257	.
288	V	188	20.36	A	4	0.10941	0.42788	0.256	0.79875	
				F	2	0.78822	0.285	2.766	0.00685	**
				G	1	-0.2254	0.65275	-0.345	0.73062	
				I	1	0.65384	0.7185	0.91	0.36517	
				L	8	0.05214	0.23996	0.217	0.82845	
				M	6	-0.2228	0.32173	-0.693	0.49029	
				N	1	0.86248	0.63283	1.363	0.17621	
290	T	210	0	A	1	0.00446	0.50387	0.009	0.99295	
293	S	210	0	N	1	0.19511	0.85279	0.229	0.81953	
294	F	208	-2.12	L	1	-0.0444	0.32366	-0.137	0.89107	
				S	2	0.06635	0.38763	0.171	0.86446	
295	F	203	-11.5	L	8	0.23481	0.66098	0.355	0.72321	
301	I	210	0	T	1	NA	NA	NA	NA	
305	D	210	0	E	1	NA	NA	NA	NA	
310	H	209	6.41	R	2	0.10082	0.31859	0.316	0.75238	
311	E	197	22.44	C	14	0.16082	0.31859	0.505	0.61491	
312	I	209	0.37	V	2	-0.0492	0.31859	-0.154	0.87764	
313	A	197	22.27	G	14	NA	NA	NA	NA	
314	S	88	43.43	A	2	0.24328	0.23923	1.017	0.31181	
				C	51	-0.021	0.10384	-0.202	0.84049	
				T	58	-0.0921	0.10553	-0.873	0.38493	
				V	12	0.12736	0.14241	0.894	0.37344	
315	G	209	3.81	H	1	-0.3713	0.39338	-0.944	0.3477	
				T	1	-0.2377	0.48899	-0.486	0.62805	
316	G	127	68.74	A	13	-0.3285	0.12704	-2.586	0.01126	*
				R	1	NA	NA	NA	NA	
				S	28	-0.5065	0.08789	-5.763	1.07E-07	***
				T	42	-0.1567	0.09474	-1.654	0.10148	
321	L	209	-0.74	R	2	NA	NA	NA	NA	
326	A	207	-7.86	V	4	NA	NA	NA	NA	
334	P	208	3.67	S	3	-0.2754	0.48692	-0.566	0.57299	
337	R	208	-14.1	K	2	-0.6126	0.25525	-2.4	0.01838	*
				P	1	-0.0598	0.33262	-0.18	0.85769	
338	Q	207	25	C	2	-0.4257	0.42175	-1.009	0.31547	
				L	1	-0.69	0.46969	-1.469	0.14521	
				V	1	-0.4423	0.47466	-0.932	0.35384	
342	L	210	0	A	1	-1.8681	0.32673	-5.718	1.30E-07	***
347	S	199	86.35	A	8	-1.2661	0.1773	-7.141	2.02E-10	***
				G	4	-1.8726	0.22135	-8.46	3.68E-13	***
348	A	210	0	G	1	NA	NA	NA	NA	

Supplementary table 1. Additional information related to figure 1 · p < 0.1, * p < 0.05, ** p < 0.01 *** p < 0.001. Positive values in the "Estimate" column indicate a preference for D-luc, while negative values indicate a preferece for 4'Br-luc.

Supplementary Table 2

Position	PDB#	Region	Rosetta	RF	1	2	3	AA's in sequenced variants	iAChSnFR0.6	iSeroSnFR0.0	iSeroSnFR0.1	iSeroSnFR0.2	iSeroSnFR	Notes
3	41	Lower lobe					Rand.	F,V	V					
4	42	Lower lobe					Rand.	F,V	V					
7	45	Lower lobe	X	-2.01				A,S	S					
8	46	Binding pocket	X	0.28		SSM	SSM	E,H,I,L,P,Q,S,T,V,Y	I					
9	47	Binding pocket	X	2.30		SSM	SSM	A,D,I,K,L,N,P,Q,V,Y	I	N				
10	48	Binding pocket	X	-1.06		SSM	SSM	C,D,F,H,L,P	F	H				
11	49	Binding pocket	X	1.60			SSM	A,T	T					
12	50	Binding pocket	X	0.23			SSM	A,E,D	E					
13	51	Binding pocket	X	-1.55			SSM	A,D,E,Q,P,T	Q					
14	52	Lower lobe	X	-1.00				I,Q	I					
19	57	Lower lobe					Rand.	L,M	M					
20	58	Lower lobe					Rand.	L,V	V			L		
29	67	Lower lobe					SSM	D,P,Y	D					Random mutation in round 3 in improved variant
34	72	Lower lobe					SSM	A,C,R,T	R					Random mutation in round 3 in improved variant
38	76	Lower lobe					SSM	C,F,K,L	L					Random mutation in round 3 in improved variant
40	78	Binding pocket/lower lobe	X	-0.25				A,D,G,L	G					
41	79	Binding pocket/lower lobe	X	-0.02		SSM		A,E,F,G,I,L,P,Q,S,T,V	T	V				
42	80	Binding pocket/lower lobe	X	1.11				D,N	N					
55	93	Lower lobe				Rand.		N,Y	N					
59	97	Lower lobe					Rand.	F,I	I					Mutation occured after iSeroSnFR was discovered
61	99	Lower lobe				Rand.	SSM	A,G,L,M,V	V		L			
62	100	Binding pocket/lower lobe	X	-0.55				A,E	E					
63	101	Binding pocket/lower lobe	X	0.72				H,Y	Y					
64	102	Binding pocket/lower lobe	X	0.11			SSM	A,H,L,T,V	T	V				
65	103	Binding pocket/lower lobe					SSM	G	G					
66	104	Binding pocket/lower lobe	X	7.74	SSM			A,D,H,I,K,N,P,S,T,V,Y	T		Y			
70	108	Interface	X	-1.00				D,I	D					
73	111	Linker adjacent loop					SSM	A,S,T	T					
L1.1	112	Linker 1					SSM		Y					
L1.2	113	Linker 1					SSM	F,P	P					
L1.3	114	Linker 1					SSM	E,L,R	E					
L1.4	115	Linker 1					SSM		P					
GFP153	120	GFP/strand 7				Rand.	SSM	I,L,T	T		I			Effect of I was negligable by GLM, but was never confirmed
GFP157	124	GFP/7-8 loop					Rand.	H,Q	Q					Mutation occured after iSeroSnFR was discovered
GFP169	136	GFP/strand 8					SSM	H,L,Y	H		Y			
GFP192	159	GFP/9-10 loop					SSM		P			S	P	
GFP196	163	GFP/9-10 loop					SSM	A,L,P,S	P			L	P	L eliminates all mammalian expression
GFP231	198	GFP/11-1 linker					Rand.	F,L	L					
L2.1	357	Linker 2					SSM	P,S	P					
L2.2	358	Linker 2					SSM	A,K,Q,P	P					
L2.3	359	Linker 2					SSM	A,P	P					
L2.4	360	Linker 2					SSM	C,G,P,S,T	G					
77	361	Linker adjacent loop				Rand.	SSM	I,T	T			A		
102	386	Lower lobe					Rand.	L,P	P					
106	390	Binding pocket/hinge	X	0.71				D,E,M,N,R	N					
107	391	Binding pocket/hinge	X	-0.42		SSM		A,D,N,S	N	A				
108	392	Binding pocket/hinge	X	-2.21		SSM		A,D,E,P,R,S,T	T	S				
109	393	Binding pocket/upper lobe	X	0.39		SSM		F,H,W,Y	Y	F	Y			
110	394	Binding pocket/upper lobe	X	1.60				R,T,V	T	V				
112	396	Upper lobe				SSM		A,V	A					
130	414	Upper lobe					Rand.	A,S	A					Mutation occured after iSeroSnFR was discovered
140	424	Upper lobe					Rand.	A,V	A					
141	425	Binding pocket/upper lobe	X	0.72		SSM	SSM	D,N,Q,S,T	T	N				
142	426	Binding pocket/upper lobe	X	1.42			SSM	A,I,L,M,P,R,T	M					
143	427	Binding pocket/upper lobe	X	3.42	SSM			F,H,L,M,P,S,T,V	F		M			
144	428	Binding pocket/upper lobe	X	1.53				F,H	F					
147	431	Binding pocket/upper lobe	X	-0.46			SSM	D,K,N,Q,R,S,T	K			N		
148	432	Binding pocket/upper lobe					SSM	H,P	P					
149	433	Binding pocket/upper lobe					SSM	D,Y	D					
169	453	Binding pocket/upper lobe					SSM	A,D,V	D					
170	454	Binding pocket/upper lobe	X	4.57	SSM			A,D,G,H,L,M,N,P,R,S,T	M	H	A			
173	457	Binding pocket/upper lobe	X	-0.59			SSM	A,L,M,P,Q,R,T	R	M		P		
174	458	Binding pocket/upper lobe	X	0			SSM	A,F,H,P,S,Y	Y					
176	460	Upper lobe				Rand.		A,V	A					
186	470	Binding pocket/upper lobe	X	-1.16				A,D	D					
188	472	Binding pocket/upper lobe	X	2.51		SSM	SSM	C,D,F,G,H,I,K,L,M,P,R,T,Y	F		T			
189	473	Binding pocket/upper lobe	X	0.06			SSM	A,E,L,M,R	A					
190	474	Binding pocket/upper lobe	X	0.52			SSM	A,H,S,T	T					
191	475	Binding pocket/upper lobe					SSM	C,D,S	D					
211	495	Binding pocket/hinge	X	0.06				G,P,R	P					
212	496	Binding pocket/hinge					SSM	H,Y	Y					
221	505	Lower lobe					Rand.	D,E	D					
252	536	Binding pocket/lower lobe					SSM	R	R					

Supplementary table 2. Addiitonal information related to Figure 1. Summary of positions tested during sensor engineering, and mutations introduced.

Supplementary Table 3

Summary of the Gaussian Regression model (built using glm):

Note Singularities were found in the modeling and are indicated by an NA in the following table. This is often the case when variables are linear combinations of other variables, or the variable has a constant value. These variables will be ignored when using the model to score new data and will not be included as parameters in the exported scoring routine.

Call:

glm(formula = Norm5HT ~ ., family = gaussian(identity), data = crs$dataset[, c(crs$input, crs$target)])

Deviance Residuals:

Min	1Q	Median	3Q	Max
-0.23834	-0.013295	0.004073	0.017042	0.23219

Coefficients: (2 not defined because of singularities)

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	0.264147	0.007505	35.167	<2E-16	***
Pos66D	-0.074688	0.06343	-1.177	0.24107	
Pos66H	-0.04404	0.067121	-0.656	0.51285	
Pos66I	0.06477	0.037844	1.711	0.08929	.
Pos66K	0.036014	0.064759	0.556	0.57905	
Pos66N	-0.265513	0.064759	-4.1	7.10E-05	***
Pos66P	0.111056	0.048996	2.267	0.025	*
Pos66V	-0.009171	0.067121	-0.137	0.89153	
Pos66Y	0.230738	0.030511	7.563	5.46E-12	***
Pos143L	0.017094	0.040089	0.426	0.6705	
Pos143M	0.505723	0.106518	4.748	5.18E-06	***
Pos143S	0.040451	0.028731	1.408	0.16145	
Pos143T	-0.240873	0.072141	-3.339	0.00109	**
Pos143V	0.040291	0.064759	0.622	0.53488	
Pos170A	0.133016	0.031509	4.222	4.43E-05	***
Pos170G	-0.267192	0.064759	-4.126	6.42E-05	***
Pos170L	0.02359	0.019403	1.216	0.2262	
Pos170P	0.055877	0.020024	2.79	0.00603	**
Pos170R	0.077359	0.049535	1.562	0.1207	
Pos170S	0.027526	0.064759	0.425	0.67147	
Pos188C	0.055963	0.064759	0.864	0.38903	
Pos188D	-0.016659	0.027657	-0.602	0.54796	
Pos188G	0.084843	0.064759	1.31	0.19237	
Pos188I	0.064468	0.064759	0.996	0.32127	
Pos188K	0.01733	0.070855	0.245	0.80715	
Pos188L	0.044601	0.027743	1.608	0.11025	
Pos188P	0.089914	0.05036	1.785	0.07644	.
Pos188R	-0.042585	0.03299	-1.291	0.19897	
Pos188T	0.078718	0.046043	1.71	0.08963	.
GFP153I	NA	NA	NA	NA	

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for gaussian family taken to be 0.004414231)

Null deviance: 4.11291 on 168 degrees of freedom

Residual deviance: 0.55921 on 135 degrees of freedom

AIC: -415.58

Number of Fisher Scoring iterations: 2

==== ANOVA ====

Analysis of Deviance Table

Model: gaussian, link: identity

Response: Norm5HT

Terms added sequentially (first to last)

	Df	Deviance	Resid. Df	Resid. Dev
NULL	168	4.1129		
Pos41	1	0.0972	167	4.0157
Pos66	8	2.7726	159	1.2431
Pos109	1	0.0126	158	1.2305
Pos143	5	0.1450	153	1.0855
Pos170	6	0.2231	147	0.8624
Pos188	9	0.0634	138	0.7990
Pos55	1	0.0074	137	0.7917
Pos78	1	0.1359	136	0.6558
Pos176	1	0.0965	135	0.5992
GFP153	0	0	135	0.5592

[1] "\n"

Time taken: 0.02 secs

Rattle timestamp: 2016-06-21 12:24:20 EKUnger

==

Supplementary table 3. Additional information related to Figure 1. Summary of the gaussian regression model (GLM) performed after the first round of site-saturated mutagenesis. Note that these are raw values. In figure 1, values have been normalized such that the parent (iSeroSnFR0.0) would be equal to 1.