Table 1. Ligand-induced split RLUC complementation in 293T cells expressing N-RLUC-hER$_{281-595}$-C-RLUC with 20 different single amino acid mutations generated at position 521 of hER

Con	E2	DES	OHT	Ral	Gen	ICI	521
1.537182	100	92.14092	96.78062	80.95113	1.396585	7.002063	G
3.83679	**6.002425**	**70.86455**	**84.1556**	**61.90189**	**2.682569**	**7.653152**	**T**
1.846348	1.748077	1.765008	5.852995	2.310649	1.541783	1.958053	Y
2.531851	2.288014	2.332733	5.140625	1.866223	1.603432	1.640789	F
0.120722	0.146854	0.130476	0.101767	0.143357	0.133236	0.140045	M
1.315613	1.333647	1.967622	2.583194	2.174469	1.227648	1.250835	N
1.115023	1.523748	1.111526	8.180391	3.245509	1.130481	1.567362	H
2.733177	16.49531	28.69449	57.56675	61.54101	3.000569	0.161024	C
1.900636	3.042711	1.858678	2.790409	2.006636	1.988969	2.082271	D
1.881313	2.038105	2.255625	14.37881	2.548413	1.846532	2.670055	Q
1.391432	**1.840091**	**1.647598**	**2.429531**	**1.843404**	**1.284512**	**1.862727**	**R**
1.394376	2.412049	1.706671	4.342681	1.500008	1.342665	2.049698	W
10.53263	**16.4747**	**20.83504**	**103.1782**	**271.3808**	**11.42756**	**22.88953**	**V**
3.404509	68.59239	44.08747	62.80657	99.23573	3.14135	14.18852	S
5.731169	8.996369	33.62661	30.82792	48.41414	6.269817	11.28751	I
5.814717	129.0186	86.91932	121.8301	118.1085	7.758232	18.28387	A
1.279543	2.064421	3.309735	7.539055	5.168413	1.813407	1.867879	L
1.354995	1.51197	1.679435	1.955292	1.906709	1.575828	2.024487	E
1.111526	1.176856	1.347449	2.804211	2.330893	1.075457	1.586133	P
1.442224	2.069757	1.830338	7.907662	6.010891	1.416828	2.49026	K

The intramolecular folding sensor with G521T mutation showed a 95% reduction in RLUC activity in response to the endogenous ER ligand 17β-estradiol (E2) ($P < 0.05$) without significantly affecting the other ligand-induced split RLUC complementation ($P < 0.05$). The complementation induced by the agonist 17β-estradiol (100%) was used for comparison.