

FIG. 1A

Name	Amino Acid Mutation(s) in SEQ ID NO: 33	Acetylcholine									
		10 uM ach	30 uM ach	50 uM ach	100 uM ach	300 uM ach	500 uM ach	1 mM ach	3 mM ach	5 mM ach	10 mM ach
CODA71	-	15.61	63.31	73.16	74.94	65.94	62.78	74.24	76.27	77.15	77.96
CODA75	SEQ ID NO: 29	-12.88	-18.67	-0.29	-14.46	-16.31	-17.41	-16.46	-12.38	-20.88	-8.69
CODA217	W77F	-5.86			70.06			68.71	73.45		
CODA109	S170T	82.91	82.53	82.11	83.68			69.89	78.12		
CODA236	R101F	-18.24			60			66.07	71.38		
CODA237	R101M	-16.79			-18.63			46.28	69.31		
CODA238	R101W	-16.58			75.18			78.99	75.74		
CODA282	Y115D	6.81			1.19			-14.05	2.37		
CODA283	Y115E_N379K	-6.16			-11.12			-2.74	53.61		
CODA325	L131G	5.66			74.07			62.65	69.49		
CODA326	L131A	7.43			73.57			71.8	74.49		
CODA330	L131F	11.07			43.34			77.52	74.89		
CODA333	L131S	-10.11			-10.05			32.11	57.37		
CODA334	L131Q	-2.63	30.06		71.27			79.66	74.9		
CODA335	L131T	-9.13			-9.29			0.57	35.37		
CODA339	L131D	4.56			10.19			77.34	77.79		
CODA340	L131E	15.29	66.56		81.31			80.77	77.31		
CODA342	L131R	-2.57			-3.18			78.98	75.75		
CODA337	L131N	28.32			81.8			71.22	73.7		
CODA945	Q139E	-1.25			71.21			71.79	76.04		
CODA377	S172D	-3.19			0.07			71.7	76.95		
CODA405	Y210V	-6.31			-14.48			-2.82	1.06		
CODA407	Y210F	-3.75			-1.6			71.65	79.09		
CODA409	Y210W	-5.43			17.14			78.84	78.55		

FIG. 1A (continued)

Name	Amino Acid Mutation(s) in SEQ ID NO: 33	Acetylcholine									
		10 uM ach	30 uM ach	50 uM ach	100 uM ach	300 uM ach	500 uM ach	1 mM ach	3 mM ach	5 mM ach	10 mM ach
CODA534	L131S_S172D	-11.3			-27.53			2.84	14.65		
CODA535	L131T_S172D	-12.24			-26.23			-1.16	2.97		
CODA536	L131D_S172D	-12.91			-20.88			39.85	79.98		
CODA805	Y115D_S170T	-0.46			-4.93			-11.18	-3.53		
CODA806	Y115D_L131Q	9.47			6.12			-1.56	17.57		
CODA807	Y115D_L131E	15.45			13.77			27.42	53.62		
CODA952	Y140I	-1.97			2.79			58.81	66.9		
CODA965	Y140C	-21.71			3.49			63.34	51.92		
CODA1025	R101F_L131G	12.57		-3.46	12.65	-6.96	-6.77	19.42	65.06		
CODA1027	R101F_L131D	-1.62		-1.6	-2.62	-6.12	-7.71	-5.73	33.69		
CODA1039	Y115E_Y210W	-2.97		-3.94	0.35	-4.13	-5.18	1.21	26.69		
CODA1045	R101W_Y210V	-2.52			-11.98	-16.71	-12.87	-14.41	-7.4		-1.5
CODA1047	R101F_Y210V	-4.26			-13.05	-11.73	-10.75	-8.81	-6.77		-1.03
CODA1048	R101F_Y210F				-17.31	-13.7	-10.1	5.53	68.61		78.22
CODA1053	R101M_L131A	-3.98			-11	-7.92	7.36	57.28	60.54		79.46
CODA1054	R101M_L131F	-3.98			-10.34	-10.25	-11.46	1.77	36.81		74.02
CODA1055	R101W_Y115E_Y210W				-15.63	-12.05	-12.87	-11.55	12.68		37.58
CODA1056	R101F_Y115E_Y210W				-20.55	-19.32	-19.75	-14.08	14.07		25.7
CODA1138	W77F_R101F_L131D	-12.92	-15.36		-19.35	-14.79		37.5	61.73		
CODA1140	R101F_L131N_S172D	-20.07	-16.92		-25.8	-11.21		9.02	45		
CODA1157	Q139E_S172D	-36.89	-37.78		-25.67	-18.94		16.38	45.71		
CODA1173	S172D_Y210W							-6.12	11.31		

FIG. 1B

Name	Amino Acid Mutation(s) in SEQ ID NO: 33	CNL001											
		1nM CNL00	1nM CNL00	10nM CNL00	10nM CNL00	50nM CNL00	50nM CNL00	100nM CNL00	100nM CNL00	100nM CNL00	100nM CNL00	1uM CNL00	1uM CNL00
CODA71	-	3.727	20.231	81.096	82.368	83.62	84.482	84.297	85.368	85.647	86.358	86.358	86.358
CODA75	SEQ ID NO: 29	-8.496	-2.266	1.816	-4.649	-5.922	-5.597	-8.159	-5.058	-4.272	-1.281	-1.281	-1.281
CODA217	W77F	-22.273	-19.415	-9.563	6.398	61.938	66.837	79.791	80.908	83.444	83.905	83.905	83.905
CODA109	S170T	19.755	65.484	79.024	81.563	81.926	83.092	83	83.811	81.834	83.858	83.858	83.858
CODA236	R101F	9.048	26.601	71.533	72.948	75.895	74.238	76	75.861	76.367	76.162	76.162	76.162
CODA237	R101M	-2.114	-5.079	15.096	50.554	66.585	74.462	78	77.411	79.06	79.162	79.162	79.162
CODA238	R101W	32.42	54.768	75.149	75.339	75.881	77.844	78	78.575	81.126	79.482	79.482	79.482
CODA282	Y115D	-15.837	-6.519	-4.862	-8.244	-3.807	-2.871	-7.924	2.11	11.047	8.04	8.04	8.04
CODA283	Y115E_N379K	-0.713	-0.621	7.739	15.277	19.489	33.703	56.886	60.171	69.836	73.855	73.855	73.855
CODA325	L131G	74.429	78.715	79.033	79.322	79.966	80.091	81	79.701	79.289	80.581	80.581	80.581
CODA326	L131A	76.424	80.567	80.284	81.232	81.575	82.544	81	81.718	82.334	81.539	81.539	81.539
CODA330	L131F	76.289	78.896	79.339	80.245	80.831	80.605	81	80.663	81.293	81.428	81.428	81.428
CODA333	L131S	76.165	78.463	79.349	78.238	80.221	81.789	81.855	80.766	81.707	82.665	82.665	82.665
CODA334	L131Q	76.165	78.463	79.349	78.238	80.221	81.789	82	80.766	81.707	82.665	82.665	82.665
CODA335	L131T	-9.942	-0.475	17.889	38.46	61.741	69.159	73.635	76.092	80.633	79.637	79.637	79.637
CODA339	L131D	73.711	76.44	77.049	77.76	77.882	78.708	79.567	79.273	79.992	79.927	79.927	79.927
CODA340	L131E	76.413	77.144	78.258	78.763	79.757	79.327	79.011	79.948	79.855	80.618	80.618	80.618
CODA342	L131R	23.532	26.054	43.69	54.016	59.955	56.091	64.536	58.987	65.987	64.312	64.312	64.312
CODA337	L131N	76.917	79.554	79.479	79.506	81.465	81.297	82.619	81.722	81.96	81.773	81.773	81.773
CODA945	Q139E	6.65	13.512	78.678	82.723	84.91	84.849	85.046	84.642	85.493	86.071	86.071	86.071
CODA377	S172D	67.544	74.209	74.346	75.816	77.068	76.99	79.352	77.865	80.272	79.432	79.432	79.432
CODA405	Y210V	-16.246	-4.217	-6.142	-10.003	-11.054	-10.06	-3.832	-3.784	1.129	7.1	7.1	7.1
CODA407	Y210F	-10.845	-2.629	4.66	12.246	29.307	41.78	50.654	62.054	81.742	81.304	81.304	81.304
CODA409	Y210W	-8.914	-14.319	-1.444	4.393	11.897	11.434	12.719	16.053	14.077	15.495	15.495	15.495

FIG. 1B (continued)		CNL001										
Name	Amino Acid Mutation(s) in SEQ ID NO: 33	1nM CNL00	1nM CNL00	10nM CNL00	10nM CNL00	50nM CNL00	50nM CNL00	100nM CNL00	100nM CNL00	100nM CNL00	1uM CNL00	1uM CNL00
CODA534	L131S_S172D	23.438	28.59	56.882	56.777	65.044	67.373	68.666	70.357	73.019	73.527	73.527
CODA535	L131T_S172D	8.736	24.572	60.552	63.09	73.242	73.889	76.147	76.21	78.988	78.818	78.818
CODA805	Y115D_S170T	-10.729	-15.278	-20.403	-14.905	-14.303	-15.741	-12.637	-10.606	-13.535	-11.335	-11.335
CODA806	Y115D_L131Q	6.587	-5.179	-2.366	-1.272	2.763	1.995	3.091	1.45	7.331	9.103	9.103
CODA807	Y115D_L131E	0.885	-1.707	-0.567	0.043	5.141	9.409	9.796	11.009	27.914	29.109	29.109
CODA952	Y140I	0.368	-1.976	-3.923	4.348	74.107	75.866	80.323	81.996	83.489	82.208	82.208
CODA965	Y140C	-12.765	-12.608	68.134	71.394	80.298	82.159	82.404	82.14	82.34	80.649	80.649
CODA1025	R101F_L131G	-18.527	-21.577	14.063	49.818	77.765	79.23	80.544	80.695	81.191	82.385	82.385
CODA1027	R101F_L131D	24.049	36.735	52.927	61.449	67.308	68.77	69.049	71.613	71.811	69.907	69.907
CODA1039	Y115E_Y210W	-1.12	-3.941	6.275	11.943	12.949	18.635	17.576	18.319	19.329	20.672	20.672
CODA1045	R101W_Y210V	0.804	11.275	15.843	16.619	15.205	18.771	9.332	18.218	35.294	36.542	36.542
CODA1047	R101F_Y210V	-7.82	-8.382	-5.381	-1.431	-0.999	3.909	-0.823	-5.885	1.638	3.483	3.483
CODA1048	R101F_Y210F	9.114	0.845	28.944	53.629	68.581	74.379	80.541	80.774	83.143	81.751	81.751
CODA1053	R101M_L131A	-8.554	-13.711	39.631	66.809	82.122	82.031	83.312	83.986	84.363	83.877	83.877
CODA1054	R101M_L131F	21.932	39.689	54.192	64.111	71.409	70.989	74.148	71.722	71.378	71.411	71.411
CODA1055	R101W_Y115E_Y210W	-12.889	-13.295	-10.401	-7.981	-1.972	-1.661	0.277	4.325	2.88	3.259	3.259
CODA1056	R101F_Y115E_Y210W	-8.104	-12.153	-10.462	-4.735	-8.617	-1.606	-1.243	1.838	-0.567	4.663	4.663
CODA1138	W77F_R101F_L131D	-6.175	5.89	-2.032	7.601	9.263	4.423	24	30.448	73.823	73.271	73.271
CODA1140	R101F_L131N_S172D	52.592	61.248	71.678	71.881	73.807	75.649	76	76.329	76.168	74.076	74.076
CODA1157	Q139E_S172D	68.362	69.495	75.078	74.193	77.481	77.205	77	77.042	78.2	76.244	76.244
CODA1173	S172D_Y210W	16.321	30.264	61.978	65.343	73.006	74.903	76	76.759	76.928	76.619	76.619

FIG. 1C

Name	Amino Acid Mutation(s) in SEQ ID NO: 33	TC-6683						
		30nM TC-6683	300nM TC-6683	3uM TC-6683	30uM TC-6683	100uM TC-6683		
CODA71	-	-14.43	-13.08	-13.71	-14.02	-8.77		
CODA282	Y115D	0.365	-6.865	-4.927	-11.259	-11.894		
CODA325	L131G	-21.27	-18.33	-7.03	56.84	64.51		
CODA326	L131A	-16.28	-11.73	-2.38	-9.77	0.97		
CODA330	L131F	-22.26	-18.89	-9.45	-9.23	-10.75		
CODA333	L131S	-23.88	-21.46	-21.07	-16.15	-18.96		
CODA334	L131Q	-17.99	-23.26	-18.84	-16.92	-15.59		
CODA335	L131T	-13.84	-20.66	-17.86	-18.91	-16.88		
CODA339	L131D	-18.32	-17.25	-10	-8.51	-9.77		
CODA340	L131E	30.14	33.02	41.68	35.75	24.32		
CODA342	L131R	-16.26	-22.45	-13.48	-12.68	-10.33		
CODA337	L131N	-11.51	-13.17	-5.46	8.53	14.41		
CODA945	Q139E	-26.47	-18.62	-13.39	-16.6	-12.89		
CODA377	S172D	-20.23	-13.94	-14.17	1.86	43.61		
CODA534	L131S_S172D	-22.464	-23.976	-24.897	-20.474	-22.253		
CODA535	L131T_S172D	-29.362	-28.954	-23.708	-23.431	-18.324		
CODA536	L131D_S172D	-22.285	-23.926	-20.514	-3.078	7.37		
CODA807	Y115D_L131E	-26.801	-25.363	-23.651	-26.235	-24.182		
CODA952	Y140I	-27.7	-26.87	-31.2	-33.12	-24.67		
CODA965	Y140C	-8.21	-16.37	-19.48	-24.32	-24.99		
CODA1025	R101F_L131G	-1.645	3.043	0.082	2.449	10.437		
CODA1027	R101F_L131D	1.189	0.939	1.327	-0.845	-3.136		
CODA1039	Y115E_Y210W	-1.608	-3.491	-6.153	-9.263	-9.19		
CODA1045	R101W_Y210V	1.735	0.642	2.403	6.006	4.277		
CODA1047	R101F_Y210V	-24.195	-24.234	-21.291	-16.363	-24.9		
CODA1048	R101F_Y210F	-29.055	-33.935	-24.613	-21.752	-22.815		
CODA1054	R101M_L131F	-22.996	-27.055	-18.611	-13.879	-21.521		
CODA1055	R101W_Y115E_Y210W	-30.056	-30.801	-25.558	-18.295	-17.554		
CODA1056	R101F_Y115E_Y210W	-19.889	-22.443	-12.623	-10.728	-10.959		

FIG. 1D

FIG. 1D

		TC-5619/Bradanicline							
Name	Amino Acid Mutation(s) in SEQ ID NO: 33	1 nM brd	3 nM brd	10 nM brd	30 nM brd	100 nM brd	300 nM brd	1 uM brd	
CODA71	-	9.41	44.38	76.61	70.23	78.62	75.51		
CODA75	SEQ ID NO: 29	-13.39	-15.78	-13.47	-16.41		-17.56		
CODA217	W77F		-12.1		-14.72		-6.3		
CODA109	S170T		70.58		80.71		80.66		
CODA236	R101F		49.33		77.38		77.51		
CODA237	R101M		-14.87		69.93		76.57		
CODA238	R101W		74.7		76.78		77.01		
CODA282	Y115D	-0.54	-7.22	-2.82	-16.67	-0.02	-12.37	31.23	
CODA283	Y115E_N379K	0.59	-6.41	-1.58	-14.24	33.36	43.81	75.39	
CODA325	L131G		68.45		76.95		77.1		
CODA326	L131A		72.24		77.4		78.02		
CODA330	L131F	-12.4	25.12	72.19	76.35	78.94	77.06	78.61	
CODA333	L131S	-0.85	-2.05	-2.8	-2.07	30.34	50.56	72.42	
CODA334	L131Q		41.92		63.65		64.85		
CODA335	L131T	-2.39	-4.08	-3.3	-9.69	-1.05	10.53	34.72	
CODA339	L131D	4.12	19.15	68.98	77.29	81.41	75.79	82.26	
CODA340	L131E		68.57		76.85		78.49		
CODA342	L131R	-7.83	-12.89	-10.47	59.48	70.19	75.11	77.42	
CODA337	L131N		71.02		78.43		77.12		
CODA945	Q139E		14.15		78.18		76.8		
CODA377	S172D	0.77	-4.59	0.06	-10.48	0.25	-10.61	3.82	
CODA405	Y210V		9.92		4.37		59		
CODA407	Y210F		-0.53		8.71		76.26		
CODA409	Y210W	-13.45	-0.64	37.3	76.36	72.72	75.47	78.73	

FIG. 1D (continued)

		TC-5619/Bradanicline									
Name	Amino Acid Mutation(s) in SEQ ID NO: 33	1 nM brd	3 nM brd	10 nM brd	30 nM brd	100 nM brd	300 nM brd	1 uM brd			
CODA534	L131S_S172D	-1.13	-3.83	-3.78		-2.03		1.68			
CODA536	L131D_S172D	-1.24	0.25	-2.57	0.78	4.51	30.97	44.41			
CODA805	Y115D_S170T	-9.78	-12.06	-9.02	-16.3	-7.44	-10.93	-4.55			
CODA806	Y115D_L131Q	0.92	-4.65	0.38	-8.96	12.18	-0.3	45.47			
CODA807	Y115D_L131E	-1.52	-5.35	-3.49	-2.16	4.18	70.2	43.11			
CODA952	Y140I		-2.45		66.97		78.48				
CODA965	Y140C		-18.49		22.66		38.02				
CODA1025	R101F_L131G	-10.61	2.63	17.71	68.48		75.12				
CODA1027	R101F_L131D	-10.07	-0.8	1.74	53.95		76.74				
CODA1039	Y115E_Y210W	-5.55	6.78	-2.7	34.43		70.05				
CODA1045	R101W_Y210V	-1.431	-14.993	-5.52	15.49	72.105	71.411				
CODA1047	R101F_Y210V	-5.548	-9.432	-3.068	2.368	47.529	74.413				
CODA1048	R101F_Y210F	-2.084	-9.098	-2.587	55.818	76.957	74.268				
CODA1053	R101M_L131A	0.008	43.21	77.672	76.277	76.856	75.32				
CODA1054	R101M_L131F	6.609	-7.635	4.459	69.782	77.633	72.933				
CODA1055	R101W_Y115E_Y210W	-8.113	-11.136	38.755	39.111	61.378	52.398				
CODA1056	R101F_Y115E_Y210W	-7.894	-11.812	36.211	40.624	65.869	59.661				

FIG. 1E

Name	Amino Acid Mutation(s) in SEQ ID NO: 33	CNL002				100 nM CNL002	1 uM CNL002	30 uM CNL002
		1nM CNL002	10nM CNL002	50nM CNL002				
CODA71	-					49.61	74.15	75.21
CODA75	SEQ ID NO: 29					-7.94	-9.81	-15.27
CODA217	W77F					67.81	69.42	73.64
CODA109	S170T					73.48	70.03	
CODA236	R101F					-9.56	56.9	70.28
CODA237	R101M					-8.68	-2.24	66.99
CODA238	R101W					2.95	67.93	73.02
CODA282	Y115D					-9.7	-8.37	3.4
CODA283	Y115E_N379K					-5.81	9.69	-12.85
CODA325	L131G					45.62	74.72	77.4
CODA326	L131A					27.88	73.79	75.89
CODA330	L131F					-3.76	68.51	77.77
CODA333	L131S					-15.44	15.21	
CODA334	L131Q					49.9	80.72	80.97
CODA335	L131T					-14.53	6.45	57.43
CODA339	L131D					12.83	77.73	80.58
CODA340	L131E					49.97	67.8	64.58
CODA342	L131R					-17.2	8.12	57.24
CODA337	L131N					76.37	80.18	80.93
CODA945	Q139E					60.5	75.41	77.46
CODA377	S172D					59.35	79.26	79.96
CODA405	Y210V					-14.3	-7.56	-2.02
CODA407	Y210F					-12.47	-3.93	80.97
CODA409	Y210W					-14.92	-0.85	29.62

FIG. 1E (continued)

FIG. 1E (continued)

Name	Amino Acid Mutation(s) in SEQ ID NO: 33	CNL002				100 nM CNL002	1 uM CNL002	30 uM CNL002
		1nM CNL002	10nM CNL002	50nM CNL002				
CODA534	L131S_S172D				14.26	45.55	64.93	
CODA535	L131T_S172D				1.23	35.77	68.29	
CODA536	L131D_S172D				71.72	77.26	77.5	
CODA805	Y115D_S170T				-18.17	-15.2	-14	
CODA806	Y115D_L131Q				-10.03	-3.33	6.96	
CODA807	Y115D_L131E				-5.71	13.03	25.2	
CODA952	Y140I				32.58	69.08	73.28	
CODA965	Y140C				43.24	42.72	51.43	
CODA1025	R101F_L131G				12.48	46.04	39.82	
CODA1027	R101F_L131D				7.75	61.97	65.77	
CODA1039	Y115E_Y210W				21.85	47.37	59.97	
CODA1138	W77F_R101F_L131D	-2.209	43.044	73.418	81.66	81.69		
CODA1140	R101F_L131N_S172D	0.337	59.894	76.501	79.03	82.85		
CODA1157	Q139E_S172D				80.62	81.32		
CODA1173	S172D_Y210W				70.15	77.33		

FIG. 1F

FIG. 1F

		ABT-126										
Name	Amino Acid Mutation(s) in SEQ ID NO: 33	50 nM abt	100 nM abt	300 nM abt	500 nM abt	1 uM abt	3 uM abt	10 uM abt	30 uM abt	50 uM abt	100 uM abt	
CODA71	-	-1.98	24.27	56.8	76.28	73.16	70.98	72.62	77.49	77.96	77.81	
CODA75	SEQ ID NO: 29	-17.07	-16.76	-16.76	-14.68	-16	-14.11	-13.98	-21.05	-21.35	-24.12	
CODA217	W77F	37.81	57.31	12.61		66.05						
CODA109	S170T	55.83	68.89	72.52		70.96						
CODA236	R101F	-18.69	-23.01	-20.92		4.99						
CODA237	R101M	-24.34	-20.22	-20.57		-19.55						
CODA238	R101W	-14.97	-14.66	-16.35		50.02						
CODA282	Y115D	-21.22	-19.7	-15.55		-14.31						
CODA283	Y115E_N379K	-22.65	-20.55	-12.93		1.71						
CODA325	L131G	-17.39	6.89	38.65		45.82						
CODA326	L131A	-21.91	-0.5	34.86		56.66						
CODA330	L131F	-16.22	-11.89	39.23		66.5						
CODA333	L131S	-20.99	-16.21	-13.34		4.96						
CODA334	L131Q	23.65	61.83	76.44		77.68						
CODA335	L131T	-18	-16.39	-15.05		-5.56						
CODA339	L131D	-16.65	-12.3	18.5		59.09						
CODA340	L131E	2.2	25.39	36.48		50.76						
CODA342	L131R	-18.14	-18.14	-0.81	23.29	45.76	53.03	64.08	71.98	73.98	73.51	
CODA337	L131N	-11.64	34.79	60.08		65.99						
CODA945	Q139E	-8.27	4.76	30.88		60.66						
CODA377	S172D	-1.53	18.18	39.15		72.28						
CODA405	Y210V	-2.9	-2.06	-0.19		-3.64						
CODA407	Y210F	-13.07	0.1	5.97		50.23						
CODA409	Y210W	8.23	55.53	74.82	77.39	77.77	69.33	74.79	75.61	75.72	78.52	

FIG. 1F (continued)

		ABT-126									
Name	Amino Acid Mutation(s) in SEQ ID NO: 33	50 nM abt	100 nM abt	300 nM abt	500 nM abt	1 uM abt	3 uM abt	10 uM abt	30 uM abt	50 uM abt	100 uM abt
CODA534	L131S_S172D	-11.96	-10.72	11.65		-3.06					
CODA535	L131T_S172D	-11.09	-10.37	-4		4.05					
CODA536	L131D_S172D	-3.19	7.58	-1.65		31.29					
CODA805	Y115D_S170T	-11.83	-11.5	-8.01		-9.34					
CODA806	Y115D_L131Q	-1.81	-2.61	-3.78		-2.6					
CODA807	Y115D_L131E	-1.68	-2.25	-2.37		3.68					
CODA952	Y140I	-12.34	-11.57	-5.21		3.93					
CODA965	Y140C	-10.75	-4.11	18.93		52.64					
CODA1025	R101F_L131G	-11.53	-7.61	6.55		25.08					
CODA1027	R101F_L131D	-11.89	-16.07	-11.21		-9.38					
CODA1039	Y115E_Y210W	-17.32	-13.93	9.9	36.97	48.37	50.5	58.13	64.1	66.67	68.63
CODA1045	R101W_Y210V	-24.16	-24.7	-16.09		-11.61					
CODA1047	R101F_Y210V	-14.1	-11.72	-12.98		-15.09					
CODA1048	R101F_Y210F	-23.28	-21.78	-19.35		-16.33					
CODA1053	R101M_L131A	-27.01	-18.76	-17.8		-6.55					
CODA1054	R101M_L131F	-19.54	-18.28	-16.87		-11.77					
CODA1055	R101W_Y115E_Y210W	-21.36	-22.28	-14.63		-7.34					
CODA1056	R101F_Y115E_Y210W	-20.96	-21.95	-10.96		10.79					
CODA1138	W77F_R101F_L131D	-11.71	-5.13	15.09		56.63		75.93			
CODA1140	R101F_L131N_S172D	-7.87	-5.35	-3.67		3.64		5.91			
CODA1157	Q139E_S172D	-26.77	-24.64	-10.64		19.57		55.64			
CODA1173	S172D_Y210W	5.15	25.08	66.86		66.94		78.67			

FIG. 1G

Name	Amino Acid Mutation(s) in SEQ ID NO: 33	AZD-0328				
		100 nM azd0328	300 nM azd0328	1 uM azd0328	10 uM azd0328	30 uM azd0328
CODA71	-	73.59	78.09			76.05
CODA75	SEQ ID NO: 29	-8.83	-11.15			-11.92
CODA217	W77F	-7.57	7.08			81.59
CODA109	S170T	84.01	87.08			87.27
CODA236	R101F	-9.78	49.64			81.14
CODA237	R101M	-17.84	-11.98			81.2
CODA238	R101W	22.94	75.38			81.8
CODA282	Y115D	3.19	4.79			76.78
CODA283	Y115E_N379K	-2.1	38.2			79.44
CODA325	L131G	79.75	80.62			80.19
CODA326	L131A	80.39	82.07			82.23
CODA330	L131F	27.56	73.35			77.14
CODA333	L131S	38.49	67.13			80.61
CODA334	L131Q	80.55	83.15			82.53
CODA335	L131T	11.33	44.15			80.96
CODA339	L131D	62.87	77.03			78.63
CODA340	L131E	82.48	82.04			81.8
CODA342	L131R	1.79	66.58			80.55
CODA337	L131N	82.1	82.52			82.32
CODA945	Q139E	-5.79	58.47			66.57
CODA377	S172D	65.04	81.73			82.3
CODA405	Y210V	-8.13	-3.35			-2.86
CODA407	Y210F	0.85	1.25			82.08
CODA409	Y210W	-6.57	-4.84			81.64

FIG. 1G (continued)

Name	Amino Acid Mutation(s) in SEQ ID NO: 33	AZD-0328				
		100 nM azd0328	300 nM azd0328	1 uM azd0328	10 uM azd0328	30 uM azd0328
CODA534	L131S_S172D	-9.32	4.94			60.61
CODA535	L131T_S172D	-7.02	-0.48			70.16
CODA536	L131D_S172D	51.44	77.12			79.26
CODA805	Y115D_S170T	-0.71	-1.73			47.39
CODA806	Y115D_L131Q	12.15	30.92			68.65
CODA807	Y115D_L131E	24.49	45.34			71.21
CODA952	Y140I	-12.96	-13.21			68.5
CODA965	Y140C	-16.69	1.3			21.78
CODA1025	R101F_L131G	6.26	37.98			79.31
CODA1027	R101F_L131D	-4.76	-7.52			74.9
CODA1039	Y115E_Y210W	-10.45	-13			30.76

FIG. 1H

FIG. 1H

		Facitricline							
Name	Amino Acid Mutation(s) in SEQ ID NO: 33	30 nM fac	50 nM fac	100 nM fac	300 nM fac	1 uM fac	10 uM fac	30 uM fac	
CODA71	-	53.42		83.6	77.44			79.62	
CODA75	SEQ ID NO: 29	-11.68		4.35	-10.55			-5.25	
CODA217	W77F	-10.14			68.29			87.35	
CODA109	S170T	76.45		82.45	71.09			69.41	
CODA236	R101F	-28.54			72.98			87.6	
CODA237	R101M	-24.14			-14.38			88.78	
CODA238	R101W	-19.61			76.54			87.94	
CODA282	Y115D	10.17		24.77	51.04		79.76	81.7	
CODA283	Y115E_N379K	6.39			78.36		81.93	78.63	
CODA325	L131G	75.75			78.01			76.61	
CODA326	L131A	69.94			80.07			78.32	
CODA330	L131F	2.9			74.96		81.97	78.31	
CODA333	L131S	6.27			69.8		80.07	81.35	
CODA334	L131Q	83.07		85.36	84.19			82.75	
CODA335	L131T	1.24			46.92		79.06	81.06	
CODA339	L131D	12.67			80.4		82.18	82.14	
CODA340	L131E	82.51		83.6	82.92			82.68	
CODA342	L131R	-6.2			76.75		82.16	81.71	
CODA337	L131N	82.25			83.59			82.82	
CODA945	Q139E	-7.16			71.17			72.14	
CODA377	S172D	4.93			84.13		66.96	85.29	
CODA405	Y210V	-26.25			-23.52			0.83	
CODA407	Y210F	-21.94			-22.46			82.63	
CODA409	Y210W	-10.93			-5.82		60.97	57.77	

FIG. 1H (continued)

FIG. 1H (continued)		Faciniline							
Name	Amino Acid Mutation(s) in SEQ ID NO: 33	30 nM fac	50 nM fac	100 nM fac	300 nM fac	1 uM fac	10 uM fac	30 uM fac	
COD A534	L131S_S172D	-23.44			2.75		69.71	61.44	
COD A535	L131T_S172D	-21.36			4.17			65.94	
COD A536	L131D_S172D	24.15			80.17		81.34	79.9	
COD A805	Y115D_S170T	38.08		-14.37	19.41		67.33	79.67	
COD A806	Y115D_L131Q	41.32		59.51	74.86		78.53	84.45	
COD A807	Y115D_L131E	45.76		63.92	79.43		80.1	84.91	
COD A952	Y140I	-8.83			51.6			71.01	
COD A965	Y140C	-12.29			20.11			37.62	
COD A1025	R101F_L131G	-8.99			10.21			78.24	
COD A1027	R101F_L131D	-10.05			-12.54			70.96	
COD A1039	Y115E_Y210W	-13.46			5.43			49.06	

FIG. 1I

FIG. 1I

Name	Amino Acid Mutation(s) in SEQ ID NO: 33	TC-6987									
		1 uM tc6987	3 uM tc6987	5 uM tc6987	10 uM tc6987	30 uM tc6987	50 uM tc6987	100 uM tc6987	300 uM tc6987	500 uM tc6987	
CODA71	-	31.56	31.82	30.75	56.68	51.46	61.26	43.21	7.79	51.58	
CODA75	SEQ ID NO: 29	-11.79			-14.31					-19.65	
CODA217	W77F	-10.35			-13.93					-7.54	
CODA109	S170T	71.45			71.04					66.2	
CODA236	R101F	45.34			70.04					75.86	
CODA237	R101M	-21.62			21.52					60.33	
CODA238	R101W	72.47			72.04					76.56	
CODA282	Y115D	13.01			14.73					14.29	
CODA283	Y115E_N379K	-9.54			-7.06					-15.15	
CODA325	L131G	64.59			76.28					70.95	
CODA326	L131A	73.37			78.18					75.42	
CODA330	L131F	53.37			74.23					72.25	
CODA333	L131S	-3.87	-20.2	-15.25	4.92	14.75	21.33	29.7	20.79	44.37	
CODA334	L131Q	74.1			81.7					80.18	
CODA335	L131T	-0.14	-8.32	-2.13	0.13	-6.86	-9.5	-10.98	-12.16	-2.41	
CODA339	L131D	50	80.39	79.93	80.99	83.11	83.18	82.16	64.58	76.77	
CODA340	L131E	80.13			81.29					79.6	
CODA342	L131R	2.51			46.22					68.33	
CODA337	L131N	79.06			82.36					80.19	
CODA945	Q139E	-0.97			21.53					7.39	
CODA377	S172D	-9.74	-14.04	-0.95	-7.41	-16.12	-12.79	-11.77	-19.68	-17.02	
CODA405	Y210V	-22.63			-23.03					-40.88	
CODA407	Y210F	-15.22			-19.89					-33.32	
CODA409	Y210W	62.82			78.66					70.35	

FIG. 1I (continued)

Name	Amino Acid Mutation(s) in SEQ ID NO: 33	TC-6987								
		1 uM tc6987	3 uM tc6987	5 uM tc6987	10 uM tc6987	30 uM tc6987	50 uM tc6987	100 uM tc6987	300 uM tc6987	500 uM tc6987
CODA534	L131S_S172D	-20.99			-27.62					-24.22
CODA535	L131T_S172D	-21.8			-27.08					-21.56
CODA536	L131D_S172D	-18.24			-23.06					-19.3
CODA805	Y115D_S170T	31.9			25.54					21.13
CODA806	Y115D_L131Q	38.83			31.59					29.83
CODA807	Y115D_L131E	36.07			31.35					28.18
CODA952	Y140I	6.03			2.25					-0.24
CODA965	Y140C	2.73			30.37					33.07
CODA1025	R101F_L131G	-13.32			43.71					53.98
CODA1027	R101F_L131D	0.88			56.27					71.3
CODA1039	Y115E_Y210W	7.26			11					7.97

FIG. 1J

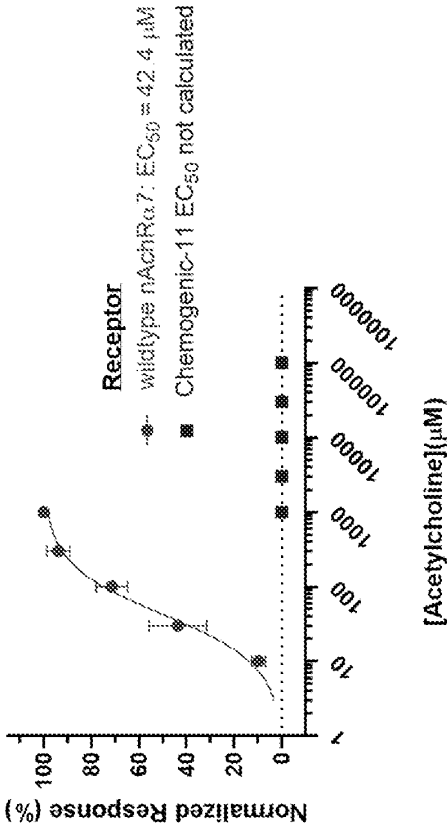
Name	Amino Acid Mutation(s) in SEQ ID NO: 33	Varenicline									
		3nM var	3nM var	30nM var	30nM var	300nM var	300nM var	500nM var	500nM var	1uM var	1uM var
CODA71	-	-2.36	-3.522	-4.255	-3.636	46.195	45.522	69.253	71.705	79.254	79.244
CODA75	SEQ ID NO: 29	4.161	-6.216	-5.173	-6.593	-5.733	-7.312	-10.35	-7.586	-5.637	-3.757
CODA217	W77F	5.662	7.57	13.968	19.079	16.183	18.025	23.366	19.206	34.69	30.504
CODA109	S170T	-20.96	-19.953	-16.094	-21.502	64.373	71.198	71.892	73.53	75.461	78.578
CODA236	R101F	19.949	22.334	15.764	16.213	23.135	22.987	35.048	34.188	71.031	71.005
CODA237	R101M	18.429	16.83	23.07	15.868	18.819	19.155	19.117	22.593	24.525	23.383
CODA238	R101W	17.896	12.564	16.263	22.117	77.492	79.056	80.186	80.065	81.263	80.702
CODA282	Y115D	2.111	-12.265	-4.433	-6.153	-0.861	-1.308	0.733	-4.633	-4.512	0.02
CODA325	L131G	-12.241	-9.415	75.334	72.278	78.305	77.83	75.936	76.788	77.805	77.352
CODA326	L131A	-5.939	-2.294	68.663	70.718	79.88	79.742	79.311	80.029	80.952	79.329
CODA330	L131F	-3.016	-1.452	-0.193	-1.853	6.915	6.513	27.593	29.863	67.499	65.57
CODA333	L131S	-4.782	1.048	2.084	5.022	12.162	12.979	29.238	25.614	43.863	39.346
CODA334	L131Q	-5.192	-4.327	-0.688	0.672	75.189	74.923	79.048	80.337	79.766	76.836
CODA335	L131T	0.398	3.947	5.31	7.666	4.327	3.275	1.835	4.863	9.471	7.505
CODA339	L131D	-0.902	-7.62	-6.009	-3.447	49.328	47.685	66.7	64.798	70.85	69.038
CODA340	L131E	-4.061	-0.568	62.964	63.631	74.912	75.084	76.346	75.876	75.805	74.856
CODA342	L131R	0.271	-3.613	-4.935	-4.363	0.282	2.32	23.777	18.376	66.17	66.088
CODA337	L131N	3.093	12.327	77.097	77.653	79.446	79.998	80.691	80.309	80.258	79.509
CODA945	Q139E	8.207	7.835	9.509	10.204	9.818	12.539	16.762	15.21	48.292	54.096
CODA377	S172D	-3.433	-5.737	-5.319	-6.175	-4.135	-8.614	-6.062	-6.125	-14.134	-15.373
CODA405	Y210V	7.317	7.643	9.457	9.134	15.503	11.954	13.427	10.784	9.032	11.591
CODA407	Y210F	2.441	7.326	1.275	12.96	13.119	2.355	11.539	6.571	8.397	7.309
CODA409	Y210W	7.776	-2.964	-5.568	3.024	2.815	3.06	1.766	2.819	1.844	-4.059

FIG. 1J (continued)

Name	Amino Acid Mutation(s) in SEQ ID NO: 33	Varenicline									
		3nM var	3nM var	30nM	30nM var	300nM var	300nM var	500nM var	500nM var	1uM var	1uM var
CODA952	Y140I	-22.522	-18.173	-15.306	-12.832	-14.671	-17.016	-16.713	-18.893	-15.847	-16.236
CODA965	Y140C	-17.716	-23.019	-14.993	-12.182	-11.731	-10.13	-12.518	-15.043	-13.385	-17.022
CODA1025	R101F_L131G	48.074	60.978	70.023	69.433	78.989	79.21	79.212	79.771	79.538	80.577
CODA1027	R101F_L131D	-3.367	-0.431	-0.761	2.989	2.966	10.211	15.278	20.84	37.104	42.439
CODA1039	Y115E_Y210W	-10.926	-7.395	-5.661	-1.244	-4.555	1.081	9.172	12.561	7.139	12.664
CODA1045	R101W_Y210V	6.745	8.397	14.1	19.095	16.83	21.507	21.586	23.131	31.591	26.479
CODA1047	R101F_Y210V	-3.035	-0.786	-5.181	0.669	-2.748	-6.708	-9.123	-9.869	-6.702	-7.26
CODA1048	R101F_Y210F	-17.119	-13.234	-7.747	4.193	4.149	1.512	-1.572	3.71	8.414	4.091
CODA1053	R101M_L131A	74.835	76.119	77.608	79.015	80.71	79.002	78.709	78.884	79.688	80
CODA1054	R101M_L131F	34.923	28.944	42.214	43.015	47.059	52.768	59.542	62.641	71.449	69.145
CODA1055	R101W_Y115E_Y210W	0.468	-10.015	-15.532	-14.462	-17.027	-15.59	-13.343	-10.702	-6.604	-7.612
CODA1056	R101F_Y115E_Y210W										
CODA1138	W77F_R101F_L131D	3.893	1.107	0.972	0.872	2.282	-0.288	3.637	-1.804	-1.84	6.36
CODA1140	R101F_L131N_S172D	-6.169	-10.445	-9.679	-10.878	-9.572	-8.873	-14.656	-11.304	-13.996	-16.128
CODA1157	Q139E_S172D	-0.225	-1.954	1.269	5.972	6.84	5.943	8.717	6.41	5.635	7.068
CODA1173	S172D_Y210W	9.211	7.193	7.821	9.078	6.933	8.36	5.501	7.04	7.174	5.185

FIG. 2A

Acetylcholine dose response



Chemogenic-11 (CR-11):
SEQ ID NO:33 with amino acid substitutions Y115D+L131Q

FIG. 2B

RG-3487 dose response

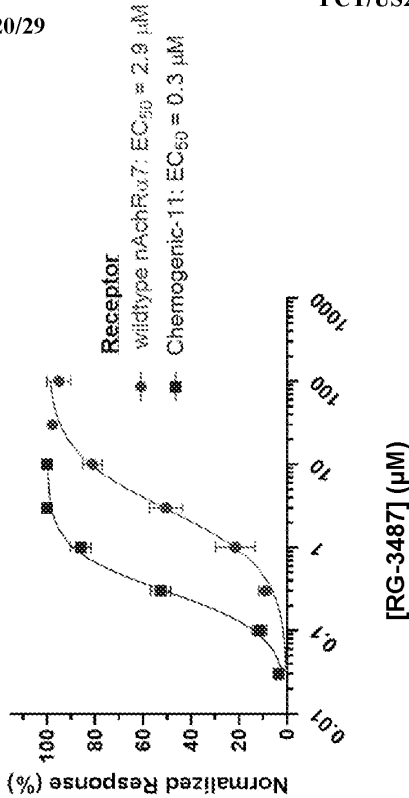


FIG. 3

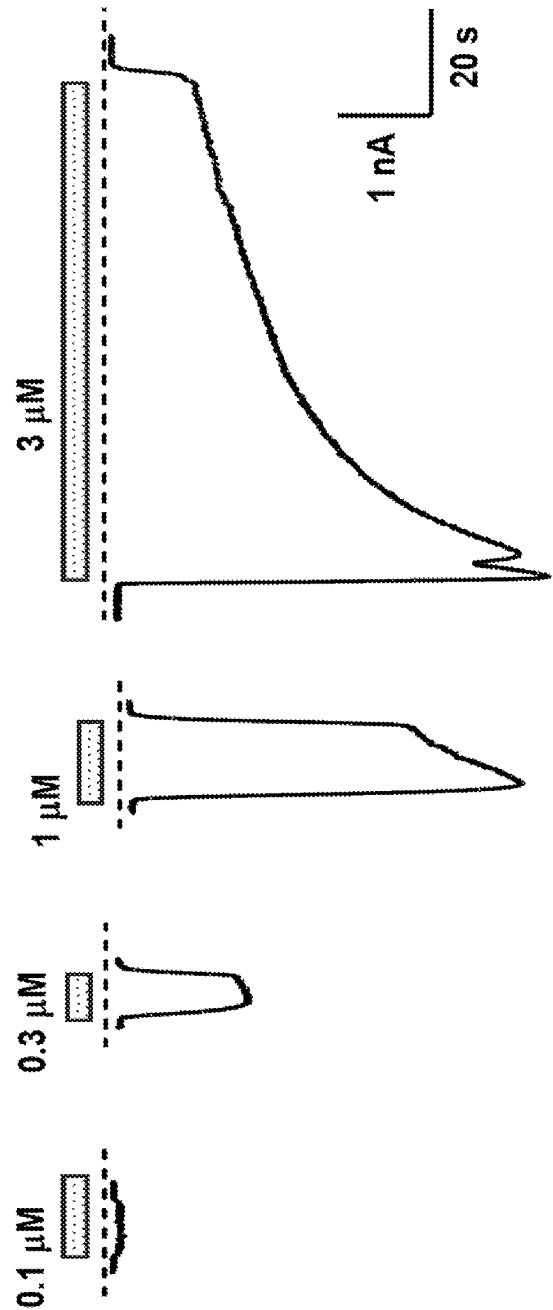


Fig. 4A

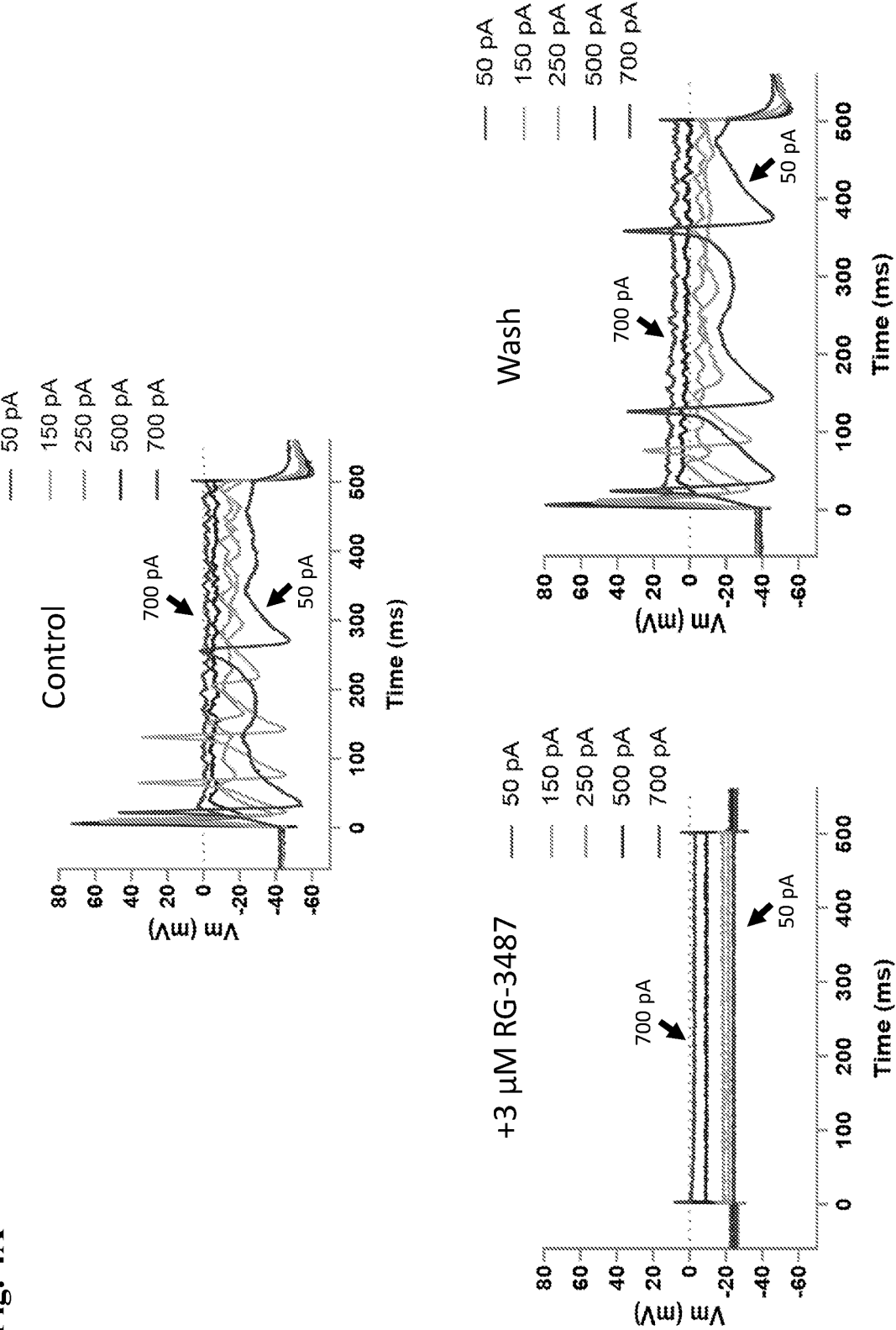


FIG. 4B

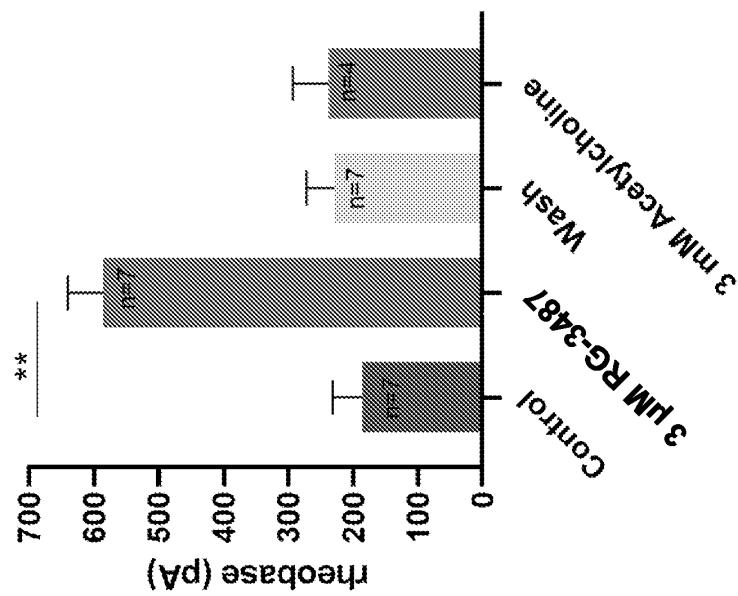


FIG. 5

Sequence of the Engineered Receptor	Normalized HA %	Normalized HA MFI	N	Normalized AB %	Normalized AB MFI	N
Y115D in SEQ ID NO: 33	0.91	0.74	2	1.23	1.04	2
Y115E in SEQ ID NO: 33	1.16	1.00	2	1.24	1.31	2
L131S in SEQ ID NO: 33	1.42	0.76	2	1.28	1.20	2
L131T in SEQ ID NO: 33	2.00	0.88	2	1.65	1.32	3
L131D in SEQ ID NO: 33	1.59	0.79	2	0.84	1.41	3
L131R in SEQ ID NO: 33	1.83	0.74	2	0.61	1.24	3
S172D in SEQ ID NO: 33	0.36	0.61	2	0.50	0.90	4
Y210W in SEQ ID NO: 33	0.96	1.09	2	0.88	0.62	4
L131S_S172D in SEQ ID NO: 33	0.47	1.10	8	0.44	0.91	3
L131T_S172D in SEQ ID NO: 33	0.33	0.86	9	0.43	0.72	2
L131D_S172D in SEQ ID NO: 33	0.40	0.87	9	0.48	0.87	2
Y115D_S170T in SEQ ID NO: 33	0.70	0.94	4	0.56	0.93	4
Y115D_L131Q in SEQ ID NO: 33	0.36	0.83	4	0.33	2.04	5
Y115D_L131E in SEQ ID NO: 33	0.91	1.00	4	0.78	1.14	4

FIG. 5 (continued)

Sequence of the Engineered Receptor	Normalized HA %	Normalized HA MFI	N	Normalized AB %	Normalized AB MFI	N
Y140I in SEQ ID NO: 33	1.41	1.53	2	0.54	2.64	2
R101F_L131G in SEQ ID NO: 33	0.98	0.63	2	0.46	0.90	2
R101F_L131D in SEQ ID NO: 33	0.61	0.49	2	0.50	0.68	2
Y115E_Y210W in SEQ ID NO: 33	1.44	0.68	2	0.34	1.08	2
R101W_Y210V in SEQ ID NO: 33	1.09	0.87	11	0.03	0.35	12
R101F_Y210V in SEQ ID NO: 33	1.13	0.65	4	0.03	0.38	4
R101F_Y210F in SEQ ID NO: 33	1.05	0.61	4	0.55	0.57	4
R101M_L131A in SEQ ID NO: 33	1.31	0.97	10	0.91	0.90	10
R101M_L131F in SEQ ID NO: 33	0.96	0.88	11	0.82	0.82	12
R101W_Y115E_Y210W in SEQ ID NO: 33	1.07	0.78	9	0.51	0.69	9
R101F_Y115E_Y210W in SEQ ID NO: 33	1.22	0.56	4	0.44	0.70	4

FIG. 6A

FIG. 6A

Engineered Receptor	Relative Total Expression in Cultured DRG neurons*		Relative Surface Expression in Cultured DRG neurons*		N coverslips	Expression ratio in Cultured DRG neurons*		Relative Surface Expression in HEK Cells	
	Soma	Processes	Soma	Processes		Total HA, axon : soma	Soma surface : total	% HA+	HA MFI
lentiviral vectors									
Wildtype Glycine Receptor α1	1	1	1	1	7				
L131R in SEQ ID NO: 33	0.33 ± .02	0.18 ± .03	0.55 ± .18	0.89 ± .50	5			2.28 ± .1.65	0.80 ± 0.20
Y210W in SEQ ID NO: 33	0.28 ± .03	0.12 ± .01	0.35 ± .06	0.30 ± .06	7			0.80 ± 0.23	0.76 ± 0.11
Y115E in SEQ ID NO: 33	0.45 ± .07	(0)	0.29 ± .06	0.25 ± .02	4			1.03 ± .16	1.07 ± 0.05
L131D in SEQ ID NO: 33	0.37 ± .07	0.19 ± .06	0.22 ± .05	0.35 ± .11	6			2.06 ± 1.55	1.04 ± .12
R101F, L131G in SEQ ID NO: 33	0.51 ± .09	(0)	0.25 ± .05	0.08 ± .05	4			0.78 ± 0.05	0.89 ± 0.01
R101F, L131D in SEQ ID NO: 33	0.19 ± .04	(0)	0.20 ± .05	0.08 ± .07	6			0.48 ± 0.01	0.69 ± 0.04
Y115E, Y210W in SEQ ID NO: 33	0.33 ± .03	0.09 ± .02	0.36 ± .05	0.51 ± .04	4				
empty vector	0.09 ± .03	0.01 ± .03	0.30 ± .10	0.15 ± .07	7				
AAV6 vectors									
Wildtype Glycine Receptor α1	1	1	1	1	12	0.07 ± .01	1.01 ± .06		
SEQ ID NO: 33	0.44 ± .10	0.18 ± .08	0.26 ± .07	0.26 ± .21	5	0.01 ± .00	0.55 ± .12		
Y210W in SEQ ID NO: 33	0.35 ± .04	0.16 ± .02	0.14 ± .06	0.10 ± .08	4	0.03 ± .00	0.43 ± .18	0.80 ± 0.23	0.76 ± 0.11
R101W, Y210V in SEQ ID NO: 33	0.62 ± .06	0.33 ± .08	0.40 ± .12	0.52 ± .14	5	0.02 ± .00	0.73 ± .24	0.99 ± 0.36	0.99 ± 0.14
R101F, Y210F in SEQ ID NO: 33	0.46 ± .17	0.05 ± .02	0.06 ± .06	0.01 ± .1	3	0.01 ± .00	0.24 ± .25	0.90 ± 0.32	0.95 ± 0.15
R101M, L131F in SEQ ID NO: 33	0.31 ± .05	0.17 ± .04	0.24 ± .03	0.39 ± .14	6	0.02 ± .00	0.78 ± .06	0.92 ± 0.38	1.06 ± 0.15
R101W, Y115E, Y210W in SEQ ID NO: 33	0.39 ± .04	0.22 ± .03	0.30 ± .04	0.39 ± .14	6	0.03 ± .00	0.77 ± .06	0.78 ± 0.10	0.84 ± 0.10
R101F, Y115E, Y210W in SEQ ID NO: 33	0.49 ± .02	0.11 ± .04	0.31 ± .06	0.08 ± .08	6	0.02 ± .01	0.63 ± .11		
L131G, Q139L, Y217F in SEQ ID NO: 16	0.28 ± .06	0.01 ± .01	0.04 ± .03	0.02 ± .03	4	0.00 ± .00	0.10 ± .08	1.02 ± .23	1.01 ± .11

FIG. 6B

FIG. 6B

Engineered Receptor	Relative Total Expression in Cultured hippocampal neurons*		Relative Surface Expression in Cultured hippocampal neurons*		N coverslips
	Soma	Processes	Soma	Processes	
	(normalized to wildtype glycine receptor)				
	lentiviral vectors				
Wildtype Glycine Receptor a1	1	1	1	1	12
SEQ ID NO: 33	0.44 ± .12	0.30 ± .08	0.61 ± .22	0.36 ± .11	4
L131R in SEQ ID NO: 33	0.65 ± .13	0.34 ± .07	0.29 ± .06	0.17 ± .03	5
Y210W in SEQ ID NO: 33	0.60 ± .05	0.27 ± .02	0.29 ± .05	0.18 ± .04	6
Y115E in SEQ ID NO: 33	0.58 ± .11	0.30 ± .04	0.42 ± .02	0.32 ± .03	4
L131D in SEQ ID NO: 33	0.54 ± .13	0.31 ± .06	0.29 ± .03	0.21 ± .03	6
R101F, L131G in SEQ ID NO: 33	0.38 ± .06	0.18 ± .02	0.38 ± .05	0.23 ± .02	3
R101F, L131D in SEQ ID NO: 33	0.24 ± .08	0.05 ± .03	0.10 ± .02	0.06 ± .02	2
Y115E, Y210W in SEQ ID NO: 33	0.29	0.19	0.48	0.35	1
	AAV6 vectors				
Wildtype Glycine Receptor a1	1	1	1	1	8
SEQ ID NO: 33	0.67 ± .26	0.35 ± .11	0.34 ± .11	0.23 ± .06	4
Y210W in SEQ ID NO: 33	0.65 ± .12	0.27 ± .03	0.24 ± .04	0.11 ± .02	4
R101M, L131F in SEQ ID NO: 33	0.57 ± .07	0.26 ± .02	0.12 ± .03	0.15 ± .11	4
R101W, Y115E, Y210W in SEQ ID NO: 33	0.39 ± .08	0.14 ± .02	0.13 ± .05	0.06 ± .03	4
R101F, Y115E, Y210W in SEQ ID NO: 33	0.38 ± .04	0.38 ± .12	0.13 ± .00	0.13 ± .13	2
L131G, Q139L, Y217F in SEQ ID NO: 16	0.38 ± .12	(-0.04 ± .04)	(-0.11 ± .07)	(-0.11 ± .04)	4

FIG. 6A

FIG. 6A

Engineered Receptor	Relative Total Expression in Cultured DRG neurons*		Relative Surface Expression in Cultured DRG neurons*		N coverslips	Expression ratio in Cultured DRG neurons*		Relative Surface Expression in HEK Cells	
	Soma	Processes	Soma	Processes		Total HA, axon : soma	Soma surface : total	% HA+	HA MFI
lentiviral vectors									
Wildtype Glycine Receptor al	1	1	1	1	7				
L131R in SEQ ID NO: 33	0.33 ± .02	0.18 ± .03	0.55 ± .18	0.89 ± .50	5			2.28 ± 1.65	0.80 ± 0.20
Y210W in SEQ ID NO: 33	0.28 ± .03	0.12 ± .01	0.35 ± .06	0.30 ± .06	7			0.80 ± 0.23	0.76 ± 0.11
Y115E in SEQ ID NO: 33	0.45 ± .07	(0)	0.29 ± .06	0.25 ± .02	4			1.03 ± .16	1.07 ± 0.05
L131D in SEQ ID NO: 33	0.37 ± .07	0.19 ± .06	0.22 ± .05	0.35 ± .11	6			2.06 ± 1.55	1.04 ± .12
R101F, L131G in SEQ ID NO: 33	0.51 ± .09	(0)	0.25 ± .05	0.08 ± .05	4			0.78 ± 0.05	0.89 ± 0.01
R101F, L131D in SEQ ID NO: 33	0.19 ± .04	(0)	0.20 ± .05	0.08 ± .07	6			0.48 ± 0.01	0.69 ± 0.04
Y115E, Y210W in SEQ ID NO: 33	0.33 ± .03	0.09 ± .02	0.36 ± .05	0.51 ± .04	4				
empty vector	0.09 ± .03	0.01 ± .03	0.30 ± .10	0.15 ± .07	7				
AAV6 vectors									
Wildtype Glycine Receptor al	1	1	1	1	12	0.07 ± .01	1.01 ± .06		
SEQ ID NO: 33	0.44 ± .10	0.18 ± .08	0.26 ± .07	0.26 ± .21	5	0.01 ± .00	0.55 ± .12		
Y210W in SEQ ID NO: 33	0.35 ± .04	0.16 ± .02	0.14 ± .06	0.10 ± .08	4	0.03 ± .00	0.43 ± .18	0.80 ± 0.23	0.76 ± 0.11
R101W, Y210V in SEQ ID NO: 33	0.62 ± .06	0.33 ± .08	0.40 ± .12	0.52 ± .14	5	0.02 ± .00	0.73 ± .24	0.99 ± 0.36	0.99 ± 0.14
R101F, Y210F in SEQ ID NO: 33	0.46 ± .17	0.05 ± .02	0.06 ± .06	0.01 ± .1	3	0.01 ± .00	0.24 ± .25	0.90 ± 0.32	0.95 ± 0.15
R101M, L131F in SEQ ID NO: 33	0.31 ± .05	0.17 ± .04	0.24 ± .03	0.39 ± .14	6	0.02 ± .00	0.78 ± .06	0.92 ± 0.38	1.06 ± 0.15
R101W, Y115E, Y210W in SEQ ID NO: 33	0.39 ± .04	0.22 ± .03	0.30 ± .04	0.39 ± .14	6	0.03 ± .00	0.77 ± .06	0.78 ± 0.10	0.84 ± 0.10
R101F, Y115E, Y210W in SEQ ID NO: 33	0.49 ± .02	0.11 ± .04	0.31 ± .06	0.08 ± .08	6	0.02 ± .01	0.63 ± .11		
L131G, Q139L, Y217F in SEQ ID NO: 16	0.28 ± .06	0.01 ± .01	0.04 ± .03	0.02 ± .03	4	0.00 ± .00	0.10 ± .08	1.02 ± .23	1.01 ± .11

FIG. 6B

FIG. 6B

	Relative Total Expression in Cultured hippocampal neurons*		Relative Surface Expression in Cultured hippocampal neurons*		N coverslips
	(normalized to wildtype glycine receptor)		(normalized to wildtype glycine receptor)		
	Soma	Processes	Soma	Processes	
Engineered Receptor	lentiviral vectors				
Wildtype Glycine Receptor α1	1	1	1	1	12
SEQ ID NO: 33	0.44 ± .12	0.30 ± .08	0.61 ± .22	0.36 ± .11	4
L131R in SEQ ID NO: 33	0.65 ± .13	0.34 ± .07	0.29 ± .06	0.17 ± .03	5
Y210W in SEQ ID NO: 33	0.60 ± .05	0.27 ± .02	0.29 ± .05	0.18 ± .04	6
Y115E in SEQ ID NO: 33	0.58 ± .11	0.30 ± .04	0.42 ± .02	0.32 ± .03	4
L131D in SEQ ID NO: 33	0.54 ± .13	0.31 ± .06	0.29 ± .03	0.21 ± .03	6
R101F, L131G in SEQ ID NO: 33	0.38 ± .06	0.18 ± .02	0.38 ± .05	0.23 ± .02	3
R101F, L131D in SEQ ID NO: 33	0.24 ± .08	0.05 ± .03	0.10 ± .02	0.06 ± .02	2
Y115E, Y210W in SEQ ID NO: 33	0.29	0.19	0.48	0.35	1
AAV6 vectors					
Wildtype Glycine Receptor α1	1	1	1	1	8
SEQ ID NO: 33	0.67 ± .26	0.35 ± .11	0.34 ± .11	0.23 ± .06	4
Y210W in SEQ ID NO: 33	0.65 ± .12	0.27 ± .03	0.24 ± .04	0.11 ± .02	4
R101M, L131F in SEQ ID NO: 33	0.57 ± .07	0.26 ± .02	0.12 ± .03	0.15 ± .11	4
R101W, Y115E, Y210W in SEQ ID NO: 33	0.39 ± .08	0.14 ± .02	0.13 ± .05	0.06 ± .03	4
R101F, Y115E, Y210W in SEQ ID NO: 33	0.38 ± .04	0.38 ± .12	0.13 ± .00	0.13 ± .13	2
L131G, Q139L, Y217F in SEQ ID NO: 16	0.38 ± .12	(-0.04 ± .04)	(-0.11 ± .07)	(-0.11 ± .04)	4