

Mutant	pEC50	Peak (nA)	BzATP Efficacy	Suramin Inhibition (%)	PPADS Inhibition (%)	MTSES (% change)	MTSET (% change)
WT	6.10 ± 0.07	8503 ± 590	0.48 ± 0.02	52 ± 2	44 ± 7	5 ± 3	10 ± 8
E52C	6.02 ± 0.09	10372 ± 1030	0.44 ± 0.06	69 ± 2	65 ± 5	-5 ± 6	-2 ± 6
K53C	5.63 ± 0.07	6736 ± 1207	0.50 ± 0.04	72 ± 4	66 ± 1	-14 ± 6	20 ± 9
G54C	6.27 ± 0.17	10459 ± 1096	0.62 ± 0.01	47 ± 5	38 ± 4	-17 ± 7	6 ± 10
Y55C	5.88 ± 0.03	4447 ± 1115	0.26 ± 0.06	91 ± 4*	72 ± 4	-3 ± 1	-2 ± 2
Q56C	5.84 ± 0.21	7730 ± 950	0.54 ± 0.06	60 ± 2	68 ± 14	2 ± 2	25 ± 3
T57C	6.09 ± 0.09	7421 ± 1236	0.44 ± 0.13	57 ± 7	48 ± 6	24 ± 4	20 ± 5
S58C	5.79 ± 0.14	4938 ± 1354	0.29 ± 0.02	77 ± 3	79 ± 8	4 ± 5	56 ± 13
S59C	5.74 ± 0.25	6436 ± 716	0.35 ± 0.01	60 ± 6	59 ± 15	14 ± 3	51 ± 11
G60C	5.77 ± 0.16	6855 ± 1319	0.29 ± 0.02	58 ± 12	62 ± 8	3 ± 5	115 ± 20***
L61C	6.49 ± 0.08	7417 ± 1232	0.45 ± 0.03	56 ± 8	60 ± 8	-2 ± 3	34 ± 4
I62C	5.99 ± 0.10	8024 ± 1429	0.24 ± 0.03*	75 ± 8	92 ± 2**	-34 ± 5**	-6 ± 4
S63C	6.25 ± 0.07	7156 ± 1843	0.36 ± 0.03	68 ± 6	64 ± 2	-30 ± 6*	16 ± 11
S64C	6.15 ± 0.18	9421 ± 1085	0.58 ± 0.01	58 ± 5	63 ± 12	-5 ± 5	-60 ± 3***
V65C	5.92 ± 0.05	8260 ± 1233	0.53 ± 0.13	57 ± 3	69 ± 10	1 ± 2	7 ± 6
S66C	5.83 ± 0.10	8392 ± 1263	0.28 ± 0.05	73 ± 4	66 ± 2	4 ± 2	-8 ± 13
V67C	6.21 ± 0.14	6175 ± 894	0.40 ± 0.02	64 ± 5	59 ± 8	-1 ± 4	10 ± 7
K68C	2.83 ± 0.08***	993 ± 88***	0.03 ± 0.02***	92 ± 0*	58 ± 8	-91 ± 3***	-99 ± 0***
L69C	6.11 ± 0.13	8516 ± 2120	0.60 ± 0.08	81 ± 4	89 ± 4	5 ± 3	26 ± 3
K70C	4.99 ± 0.03***	4011 ± 1661	0.05 ± 0.01***	97 ± 0**	27 ± 5	-32 ± 6*	-64 ± 3***
G71C	5.78 ± 0.03	4943 ± 1156	0.30 ± 0.04	91 ± 3*	83 ± 6	1 ± 2	-22 ± 9
L72C	6.26 ± 0.19	7905 ± 1281	0.83 ± 0.02***	49 ± 8	35 ± 3	-34 ± 4**	-44 ± 4
A73C	6.18 ± 0.18	8688 ± 1315	0.83 ± 0.08***	66 ± 2	78 ± 5	-4 ± 5	8 ± 1
V74C	5.67 ± 0.10	7362 ± 1572	0.30 ± 0.07	71 ± 3	59 ± 5	-5 ± 11	-5 ± 1
T75C	6.07 ± 0.06	10562 ± 429	0.01 ± 0.01***	90 ± 4*	45 ± 14	-4 ± 4	-2 ± 2
Q76C	6.02 ± 0.05	5598 ± 636	0.31 ± 0.12	62 ± 4	73 ± 6	-8 ± 7	22 ± 7
L77C	6.14 ± 0.12	10263 ± 1277	0.27 ± 0.05	42 ± 9	36 ± 6	-18 ± 6	-7 ± 13
P78C	6.14 ± 0.03	9634 ± 1798	0.48 ± 0.07	69 ± 2	49 ± 5	17 ± 4	15 ± 10
G79C	6.09 ± 0.03	9888 ± 1057	0.24 ± 0.04	48 ± 5	33 ± 5	-4 ± 2	31 ± 6
L80C	6.17 ± 0.02	10328 ± 584	0.33 ± 0.02	66 ± 3	38 ± 8	-4 ± 13	17 ± 4
G81C	5.92 ± 0.09	7006 ± 831	0.17 ± 0.04***	61 ± 12	78 ± 3	-5 ± 8	-17 ± 8
P82C	6.03 ± 0.02	10055 ± 867	0.45 ± 0.09	54 ± 2	38 ± 8	-40 ± 7**	11 ± 6
Q83C	5.97 ± 0.13	6285 ± 1783	0.46 ± 0.03	60 ± 8	19 ± 4	-10 ± 7	-21 ± 6
V84C	6.11 ± 0.24	10279 ± 924	0.40 ± 0.09	72 ± 10	44 ± 4	-12 ± 7	39 ± 16
W85C	6.04 ± 0.17	8694 ± 917	0.12 ± 0.02***	82 ± 6	72 ± 5	-19 ± 9	-3 ± 6
D86C	6.04 ± 0.03	4572 ± 558	0.39 ± 0.03	88 ± 3	62 ± 7	-34 ± 4**	-31 ± 7
V87C	6.72 ± 0.09	7773 ± 1345	0.50 ± 0.08	53 ± 6	23 ± 9	-38 ± 8**	-24 ± 10
A88C	6.08 ± 0.30	9398 ± 886	0.63 ± 0.10	64 ± 6	65 ± 3	-7 ± 2	-2 ± 4
D89C	6.14 ± 0.14	6881 ± 1175	0.33 ± 0.04	46 ± 9	87 ± 1	-18 ± 3	12 ± 7
Y90C	5.83 ± 0.05	6741 ± 306	0.02 ± 0.01***	95 ± 1**	62 ± 10	-22 ± 5	-8 ± 1
V91C	6.05 ± 0.07	6440 ± 362	0.27 ± 0.05	84 ± 1	43 ± 6	-4 ± 14	74 ± 16*
F92C	4.01 ± 0.21***	6032 ± 970	0.03 ± 0.01***	55 ± 10	83 ± 5	10 ± 2	18 ± 1
P93C	5.64 ± 0.04	6929 ± 343	0.04 ± 0.01***	96 ± 0**	70 ± 6	-55 ± 3***	15 ± 9
A94C	6.23 ± 0.01	5930 ± 471	0.29 ± 0.04	67 ± 2	81 ± 3	-22 ± 8	40 ± 10
Q95C	5.85 ± 0.10	8213 ± 535	0.12 ± 0.02***	90 ± 3*	41 ± 8	-11 ± 4	23 ± 5
G96C	5.28 ± 0.10	7084 ± 563	0.13 ± 0.04***	68 ± 11	68 ± 10	-38 ± 4**	12 ± 4

Supplementary table i: Summary of effects of P2X1 receptor mutants E52C to G96C

pEC₅₀ values are shown as calculated from experimental data. pEC₅₀ is -log₁₀ of the EC₅₀. Peak current values were taken from the 1st application of a maximal concentration of ATP (100 μM to 10 mM). For the partial agonist data, values correspond to the efficacy of the partial agonist (100 μM) compared with ATP (100 μM). Suramin and PPADS inhibition data was taken from the inhibition of an EC₉₀ concentration of ATP in the presence of an IC₅₀ concentration of the antagonist. MTS data was calculated as a percentage change of the response to an EC₅₀ concentration of ATP in the presence and absence of the MTS reagent (1mM). Conserved residues are shown in a black box. *p<0.05; **p<0.01; ***p>0.001. n = 3–12.

Mutant	pEC ₅₀		% change of peak response
	Control	With MTSES	
I62C	5.99 ± 0.10	6.31 ± 0.07	-28 ± 3**
S63C	6.25 ± 0.07	6.13 ± 0.24	-19 ± 3*
K68C	2.83 ± 0.08	***	-99 ± 0***
K70C	4.98 ± 0.04	3.52 ± 0.11***	-38 ± 4**
L72C	6.26 ± 0.19	5.86 ± 0.17	-26 ± 4*
P82C	6.03 ± 0.02	6.16 ± 0.11	-38 ± 2**
D86C	6.04 ± 0.03	5.96 ± 0.19	-28 ± 2**
V87C	6.72 ± 0.09	6.20 ± 0.13*	-50 ± 2**
P93C	5.64 ± 0.04	5.14 ± 0.02***	-39 ± 5*
G96C	5.28 ± 0.10	4.72 ± 0.07***	-30 ± 3**

Supplementary table ii. Effect of MTSES reagents on P2X1 receptor mutants.

pEC₅₀ values were calculated from the experimental data in the absence and the presence of MTSES (1mM). pEC₅₀ is $-\log_{10}$ of the EC₅₀. The change of the peak response is shown as a percentage change of the maximal response of the mutant P2X receptor in the presence of MTSES. Conserved residues are shown in a black box.

Mutant	pEC ₅₀		% change of peak response
	Control	With MTSET	
G60C	5.77 ± 0.16	6.57 ± 0.04^{***}	58 ± 9^{**}
S64C	6.15 ± 0.18	5.84 ± 0.11	-54 ± 6[*]
K68C	2.83 ± 0.08	***	-98 ± 1^{***}
K70C	4.98 ± 0.04	4.65 ± 0.04	-55 ± 5^{**}
V91C	6.05 ± 0.07	6.29 ± 0.08	25 ± 4[*]

Supplementary table iii. Effect of MTSET reagents on P2X1 receptor mutants.

pEC₅₀ values were calculated from the experimental data in the absence and the presence of MTSET (1mM). pEC₅₀ is $-\log_{10}$ of the EC₅₀. The change of the peak response is shown as a percentage change of the maximal response of the mutant P2X receptor in the presence of MTSET. Conserved residues are shown in black boxes.