

## Supplementary Online Content

Kandaswamy R, McQuillin A, Sharp S, et al. Genetic association, mutation screening, and functional analysis of a Kozak sequence variant in the metabotropic glutamate receptor 3 gene in bipolar disorder. *JAMA Psychiatry*. Published online April 10, 2013.  
doi:10.1001/jamapsychiatry.2013.38.

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This supplementary material has been provided by the authors to give readers additional information about their work.

**eTable 1. Primer sequences and HRM mastermixes used for screening GRM3**

Primer name	Primer sequence	HRM Mastermix
GRM3_ExPa_F	TGGCGTAACGTGGGAGAGGA	
GRM3_ExPa_R	CCTTCAACGTATTCTTTCTGGAATGC	
GRM3_ExPb_F1	GCATTCCAGAAAGAATACGTTGAAAGG	
GRM3_ExPb_R1	GGAACCTTAAATCGGTGACG	
GRM3_Ex1a_F	CGTCACCGATTAAAGGTCC	
GRM3_Ex1a_R	CTAGAGCGCTTGGCTTTCAG	
GRM3_Ex1b_F	GGCTCGCAGTGTGCAGTTGA	
GRM3_Ex1b_R	AGCTCTTCCCAGCCGAGTCC	
GRM3_Ex1c_F	ATTAGATGCGACGGCTTCAG	
GRM3_Ex1c_R	TCCTCCTCTGGGACCCTTAC	
GRM3_Ex2a_F	GAGTGCCTGGTGTGTGGTG	
GRM3_Ex2a_R	CCAATGCATAGGTATCCCTTGAACA	
GRM3_Ex2c_F	TCAACGCCTGGAAGCCATGT	
GRM3_Ex2c_R	CCCAGTGAAACCCAGCCTCC	
GRM3_Ex3a_F	CCCTTTCCTGAAGCACACAC	
GRM3_Ex3a_R	GACTTGC GGATGTTGGAG	
GRM3_Ex3b_F	GCCATGGCTGAGATCTTGCG	
GRM3_Ex3b_R	CGCCTGTGGTTGCGTTTGT	
GRM3_Ex3c_F	GTTCGACCGCTACTTCCAG	
GRM3_Ex3c_R	GCCATGTCTGTGTACCAGC	
GRM3_Ex4a_F	TGGCACCCCTTTGATACTCAGTAATGG	
GRM3_Ex4a_R	CCTCAGGAAGGTCATAGCATCCA	
GRM3_Ex4b_F	TCACTGGGCAGAAACCTTATCGC	
GRM3_Ex4b_R	CATCGAAGATGCGGGCAATG	
GRM3_Ex4c_F	CCCTTGGTCAAAGCATCGGG	
GRM3_Ex4c_R	GGCCAACCAGATGATGCACG	
GRM3_Ex4d_F	ACCCTTGCAGAGAAGCGGGA	
GRM3_Ex4d_R	CAGGCTCATCATGGCATTGAA	
GRM3_Ex5c_F	GGCAAGCATTGAGAAGCACCC	
GRM3_Ex5c_R	CCCAGCACTTAGCACAAATACCTGAC	
GRM3_Ex6a_F	TCCCTCACCTCCTTCCCACC	
GRM3_Ex6a_R	CCCTGTCACCAATGCTCAGCTC	
GRM3_Ex6d_F	GAGCTGAGCATTGGTGACAGGG	
GRM3_Ex6d_R	CTGGGCAATTCTCGGCTTCC	
GRM3_HRM_ExPa_F	GCTTCATATAATTCAGTCCTTGG	Lightscanner (Idaho Technology, Salt Lake City, UT, US)
GRM3_HRM_ExPa_R	CTGCCAGCTACAGATAAAATCC	
GRM3_HRM_ExPb_F	TCTAGACATAGAAAGATGCCTG	AccuMelt (Quanta Biosciences, Gaithersburg,

		MD, US)
GRM3_HRM_Expb_R	TGCTATGGTTGTCGGATACAG	
GRM3_HRM_ExpC_F	AAGAAAGAAGAGGTTCTGTAGG	AccuMelt (Quanta Biosciences, Gaithersburg, MD, US)
GRM3_HRM_ExpC_R	TATAGGGGACTGCTGAATTATC	

<b>Primer name</b>	<b>Primer sequence</b>	<b>HRM Mastermix</b>
GRM3_HRM_Expd_F	TTTAAGAGGCGAGGTGGTAG	Sensimix (Bioline, London, UK)
GRM3_HRM_Expd_R	ATTCAATTGGACAGCCCCTG	
GRM3_HRM_ExpE_F	AAAGCAGCAGCCCAGCATTC	Sensimix (Bioline, London, UK)
GRM3_HRM_ExpE_R	GAACAATCTCTGGGTCAGGC	
GRM3_HRM_Ex1a_F	AGGGTTGCTAGGAAACAGGA	AccuMelt (Quanta Biosciences, Gaithersburg, MD, US)
GRM3_HRM_Ex1a_R	TCAGGTAAGCAGGGACTAAC	
GRM3_HRM_Ex1b_F	GGACCACAGGAGAGTTCTTG	AccuMelt (Quanta Biosciences, Gaithersburg, MD, US)
GRM3_HRM_Ex1b_R	GGGTATATCTGCAGAGTCTTC	
GRM3_HRM_Ex1c_F	CAAATCTACCCTGGCTTTTCG	AccuMelt (Quanta Biosciences, Gaithersburg, MD, US)
GRM3_HRM_Ex1c_R	CCTCCTCCTTCTTATCAG	
GRM3_HRM_Ex1d_F	ATTAGATGCGACGGCTTCAG	AccuMelt (Quanta Biosciences, Gaithersburg, MD, US)
GRM3_HRM_Ex1d_R	TTCCAAATCCAAGGGAGAAC	
GRM3_HRM_Ex1e_F	CACAAGTTGGCCATTTTCGAG	AccuMelt (Quanta Biosciences, Gaithersburg, MD, US)
GRM3_HRM_Ex1e_R	TGCTCTACCTCAACTTTCCC	

**eTable 2. All GRM3 GWAS SNPs analysed in UCL1 samples**

CHR	SNP	BP	A1	F_A	F_U	A2	CHISQ	P	OR	SE	L95	U95
7	rs1405881	85897433	C	0.10	0.11	T	0.13	0.72	0.95	0.15	0.71	1.27
7	rs1405880	85897732	C	0.10	0.10	G	0.00	0.96	0.99	0.15	0.74	1.33
7	rs274637	85898289	C	0.39	0.34	T	5.95	<b>0.01</b>	1.25	0.09	1.05	1.50
7	rs274621	85917450	C	0.38	0.33	T	7.63	<b>0.01</b>	1.29	0.09	1.08	1.55
7	rs802437	85930852	A	0.41	0.36	G	5.13	<b>0.02</b>	1.23	0.09	1.03	1.48
7	rs802439	85931065	A	0.29	0.31	G	1.01	0.32	0.91	0.10	0.75	1.10
7	rs802441	85931754	T	0.29	0.31	C	1.17	0.28	0.90	0.10	0.74	1.09
7	rs802459	85952689	T	0.40	0.35	C	5.47	<b>0.02</b>	1.24	0.09	1.04	1.49
7	rs802467	85973261	G	0.40	0.42	A	0.83	0.36	0.92	0.09	0.77	1.10
7	rs2189812	85993650	C	0.32	0.28	G	5.22	<b>0.02</b>	1.25	0.10	1.03	1.52
7	rs10238436	86014795	C	0.22	0.24	G	2.05	0.15	0.86	0.11	0.70	1.06
7	rs16888204	86014885	G	0.03	0.04	A	1.59	0.21	0.74	0.24	0.47	1.18
7	rs2237553	86019184	T	0.25	0.29	C	3.60	0.06	0.83	0.10	0.68	1.01
7	rs12704286	86019592	A	0.25	0.29	G	3.93	<b>0.05</b>	0.82	0.10	0.67	1.00
7	rs2237554	86023547	G	0.25	0.29	T	3.81	0.05	0.82	0.10	0.68	1.00
7	SNP_A-2126810	86031330	C	0.07	0.08	A	1.49	0.22	0.81	0.17	0.58	1.14
7	rs2888551	86032662	G	0.03	0.03	A	0.00	1.00	1.00	0.25	0.62	1.62
7	rs723631	86046243	C	0.24	0.29	G	6.18	<b>0.01</b>	0.78	0.10	0.64	0.95
7	rs17160978	86049959	T	0.04	0.04	C	0.00	0.97	1.01	0.24	0.63	1.61
7	rs17697445	86050097	A	0.03	0.03	G	0.00	0.97	1.01	0.24	0.63	1.62
7	rs2158786	86050669	A	0.24	0.29	G	6.29	<b>0.01</b>	0.78	0.10	0.64	0.95
7	rs16888210	86050734	T	0.04	0.04	C	0.00	0.97	1.01	0.24	0.63	1.61
7	rs2237559	86051267	A	0.04	0.04	G	0.00	0.97	1.01	0.24	0.63	1.61
7	rs2299221	86063065	T	0.04	0.04	A	0.56	0.45	0.85	0.22	0.54	1.31
7	rs12704289	86066792	C	0.07	0.08	T	0.98	0.32	0.85	0.17	0.61	1.18
7	rs7804907	86072859	T	0.24	0.28	C	5.61	<b>0.02</b>	0.79	0.10	0.65	0.96
7	rs2237563	86087819	G	0.26	0.19	A	16.94	<b>3.85 × 10<sup>-5</sup></b>	1.56	0.11	1.26	1.92
7	rs7806785	86090967	G	0.24	0.28	C	5.41	<b>0.02</b>	0.79	0.10	0.65	0.96
7	rs2299224	86092054	G	0.04	0.04	A	0.28	0.60	0.89	0.23	0.57	1.38
7	rs17697609	86097034	G	0.04	0.04	A	0.42	0.52	0.86	0.23	0.55	1.35
7	rs17161024	86105481	A	0.12	0.11	G	0.17	0.68	1.06	0.14	0.81	1.39
7	rs10952891	86111902	T	0.12	0.11	C	0.07	0.79	1.04	0.14	0.79	1.36
7	rs7788115	86116982	A	0.15	0.17	T	1.34	0.25	0.87	0.13	0.68	1.11
7	rs6955452	86117235	G	0.06	0.05	C	2.15	0.14	1.33	0.20	0.91	1.96
7	rs6955917	86117414	A	0.07	0.05	G	2.48	0.12	1.35	0.19	0.93	1.98
7	rs2299231	86134276	G	0.13	0.10	A	4.40	<b>0.04</b>	1.35	0.14	1.02	1.78
7	rs10236047	86157288	G	0.12	0.10	A	3.54	0.06	1.31	0.14	0.99	1.73
7	rs10258008	86180097	A	0.12	0.09	C	4.19	<b>0.04</b>	1.34	0.14	1.01	1.78
7	rs7781178	86180995	G	0.28	0.27	A	0.09	0.76	1.03	0.10	0.85	1.25

7	rs11974622	86181028	A	0.12	0.10	G	3.95	<b>0.05</b>	1.33	0.14	1.00	1.76
7	rs2519713	86189318	C	0.41	0.47	A	5.93	<b>0.01</b>	0.80	0.09	0.67	0.96

CHR, Chromosome; SNP, SNP ID; BP, Position in base pairs of SNP based on NCBI build 36 (hg18); A1, Allele 1; F\_A, Frequency of this allele in cases; F\_U, Frequency of this allele in controls; A2, Allele 2; CHISQ, Basic allelic test chi-square (1df); P, Asymptotic p-value for this test; OR, Estimated odds ratio (for A1, i.e. A2 is reference); SE, Standard error; L95, Lower bound of 95% confidence interval for odds ratio; U95, Upper bound of 95% confidence interval for odds ratio

**eTable 3. SNPs found by sequencing and high resolution melting curve analysis in *GRM3***

<i>GRM3</i> Gene region	SNP	Predicted effect on <i>GRM3</i> structure/function for novel SNPs	Chr 7 position (NCBI37/hg19)	BP MAF	CON MAF	HapMap MAF	1000 genome MAF (Mar 2012)
Promoter	rs274617 <sup>1</sup>		86271830	0.42	0.57	0.32	0.26
Promoter	rs274618 <sup>1</sup>		86272016	0.42	0.60	0.32	0.24
Promoter	rs166677 <sup>1</sup>	Alters binding of two transcription factors (TESS)	86272294	0.29	0.47	0.40	0.35
Promoter	rs274619 <sup>1</sup>		86272487	0.46	0.34	0.25	0.26
Promoter	rs274622 <sup>1</sup>		86272940	0.42	0.60	0.32	0.27
Promoter	ss518151300 <sup>2</sup>	Alters binding of one transcription factor (TESS)	86273209	0.0004	na	na	na
Exon 1 5' UTR	rs184681725 <sup>1</sup>		86273267	0.02	0.03	na	0.003
Exon 1 5' UTR	ss518151301 <sup>2</sup>	Alters binding of four transcription factors (TESS)	86273453	0.0005	na	na	na
Exon 1 5' UTR	rs2073549 <sup>1</sup>		86273584	0.07	0.05	0.01	0.02
Exon 1 5' UTR	ss518151302 <sup>2</sup>	Alters binding of eight transcription factors (TESS)	86273908	0.001	na	na	na
Exon 1 5' UTR	rs148754219 <sup>1</sup>		86274087	0.03	0	na	0.01
Intron 2	rs139639092 <sup>1</sup>		86395015	0.03	0	na	0.01
Exon 3	rs2228595 <sup>1</sup>	Coding synonymous (A293A)	86415987	0.05	0.02	0.06	0.06
Exon 4	rs17161026 <sup>1</sup>	Coding non-synonymous (G475D)	86468254	0.02	0	0.02	0.01

Chr, Chromosome; MAF, minor allele frequency; BP, bipolar cases; CON, Controls; UTR, untranslated region; na, not available; HRM, High resolution melt curve; <sup>1</sup> SNP detected by sequencing of 32 cases and 32 controls; <sup>2</sup> SNP detected by HRM screening of 1099 cases.

**eTable 4. Tests of association with potentially aetiological *GRM3* SNPs in the UCL sample**

SNP ID	Position (NCBI37/hg19)	N	Allele counts (MAF)	Genotype counts	P Value	OR (95% CI)
rs148754219	86274087					
Case		1062	A 19 (0.01); G 2105	AA 0; AG 19; GG 1043	0.005	4.20 (1.43-12.37)
Control		932	A 4 (0.002); G 1860	AA 0; AG 4; GG 928		
rs166677	86272294					
Case		1048	G 960 (0.46); A 1136	GG 221; AG 518; AA 310	0.19	1.09 (0.96-1.23)
Control		908	G 794 (0.44); A 1022	GG 176; AG 442; AA 290		
rs17161026	86415987					
Case		1056	T 30 (0.01); C 2082	TT 0; CT 30; CC 1026	0.51	1.21 (0.69-2.10)
Control		931	T 22 (0.01); C 1840	TT 0; CT 22; CC 909		
rs139639092	86395015					
Case		1057	A 21 (0.01); G 2093	AA 0; AG 21; GG 1036	0.53	1.24(0.64-2.41)
Control		934	A 15 (0.01); G 1853	AA 0; AG 15; GG 919		
rs2228595	86468254					
Case		1053	T 118 (0.06); C1988	TT 1; CT 116; CC 936	0.68	0.94 (0.72-1.23)
Control		931	T 110 (0.06); C 1752	TT 6; CT 98; CC 826		
rs2073549	86273584					
Case		1061	T 76 (0.04); A 2046	TT 1; AT 74; AA 986	0.85	0.97(0.69-1.35)
Control		932	T 69 (0.04); A 1795	TT 1; AT 67; AA 865		
rs184681725	86273267					
Case		1056	A 25 (0.01); C 2087	AA 0; AC 25; CC 1031	0.87	0.96 (0.54-1.69)
Control		927	A 23 (0.01); C 1831	AA 0; AC 23; CC 904		
ss518151300	86273209					
Case		1097	A 1 (0.0004); C 2139	AA 0; AC 1; CC 1069	0.35	NA
Control		1094	A 0 (0.00); C 1876	AA 0; AC 0; CC 938		
ss518151301	86273453					
Case		1098	T 1 (0.0005); C 2123	TT 0; TC 1; CC 1061	0.35	NA
Control		1094	T 0 (0.000); C 1870	TT 0; TC 0; CC 935		
ss518151302	86273908					
Case		1098	C 3 (0.001); T 2113	CC 0; TC 3; TT 1055	0.39	2.63(0.27-25.34)
Control		1094	C 0 (0.001); T 1855	CC 0; TC 1; TT 927		

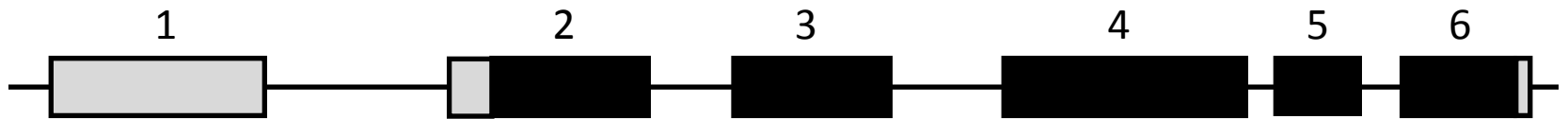
SNP, single nucleotide polymorphism; N, number of samples; MAF, minor allele frequency; OR, odds ratio; CI, confidence interval; NA, not applicable.



**eFigure 1. Alternatively spliced *GRM3* isoforms.**

**eFigure 1:** Four alternatively spliced *GRM3* isoforms are shown with their Ensembl database transcript numbers. Numbered exons are shown as boxes. Translated regions are shown in black and untranslated regions are shown in gray (adapted from Sartorius et al<sup>1</sup>).

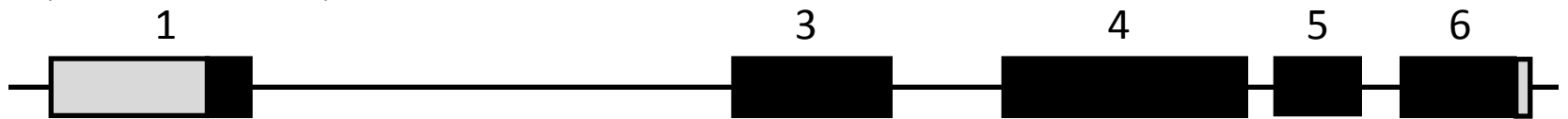
**GRM3** (ENST00000361669)



**GRM3 $\Delta$ 4** (ENST00000394720)



**GRM3 $\Delta$ 2** (ENST00000536043)



**GRM3 $\Delta$ 2 $\Delta$ 3** (ENST00000546348)



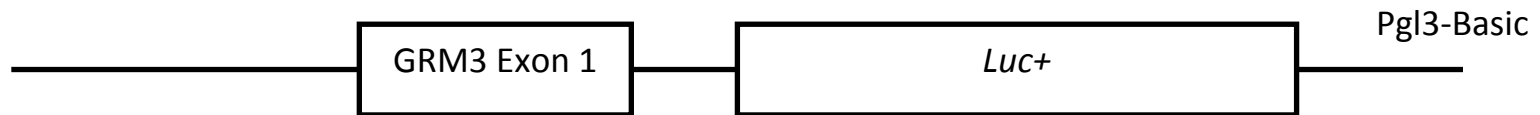


**eFigure 2. Functional analyses of the rs148754219 G/A variant**

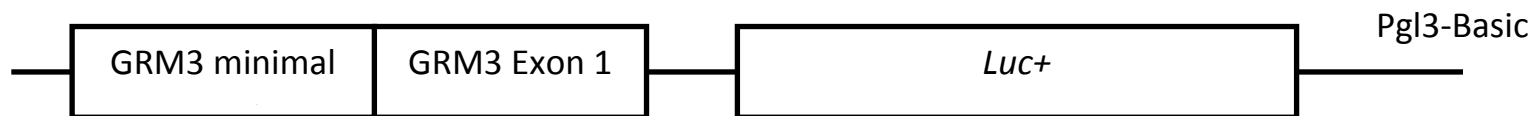
**eFigure 2A:** Luciferase (*Luc+*) reporter gene constructs designed to assess the impact of rs148754219 on transcription and/or translation. In clone E the native luciferase start codon is mutated so that translation can only begin at the start codon in *GRM3* exon 1.

**A**

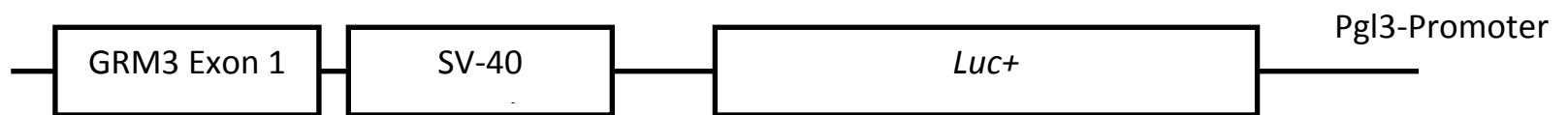
Clone A



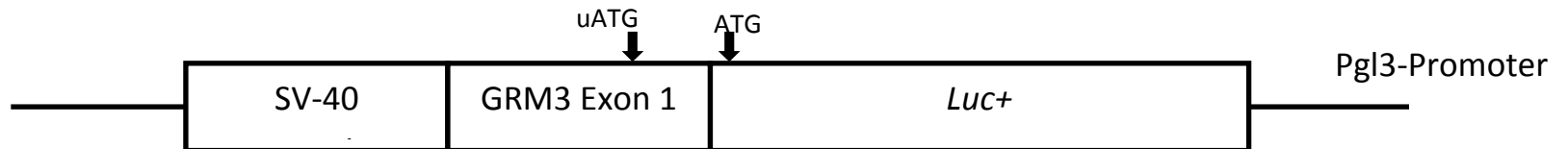
Clone B



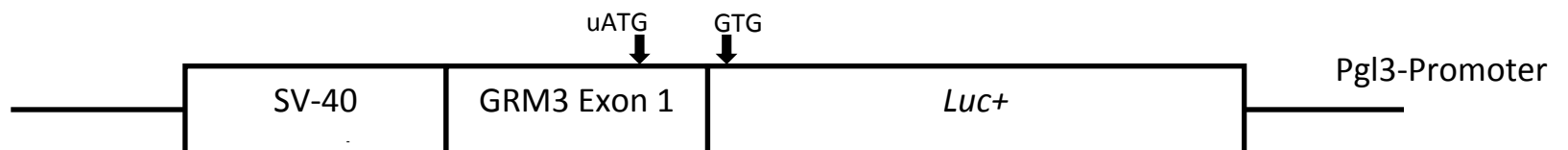
Clone C



Clone D

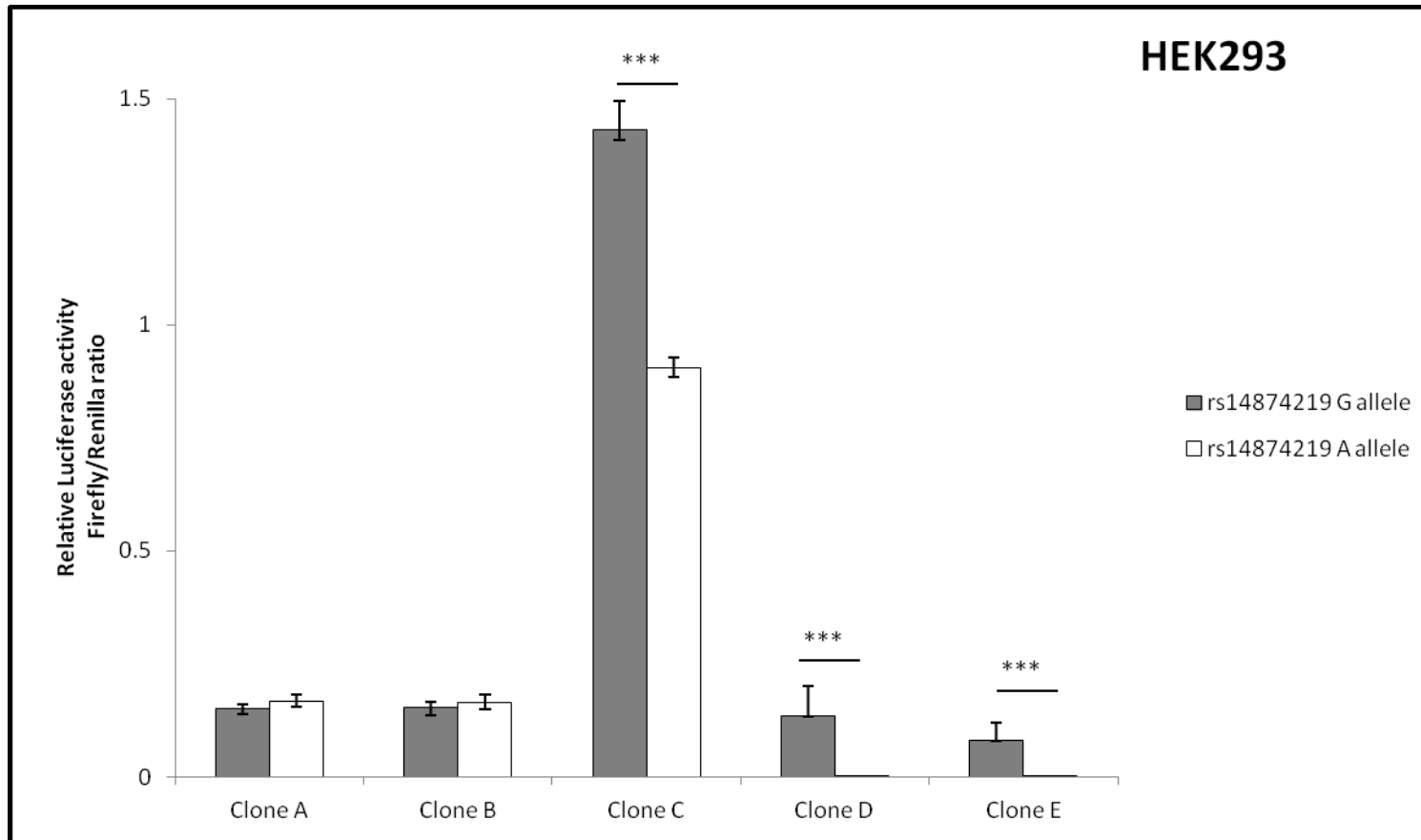


Clone E

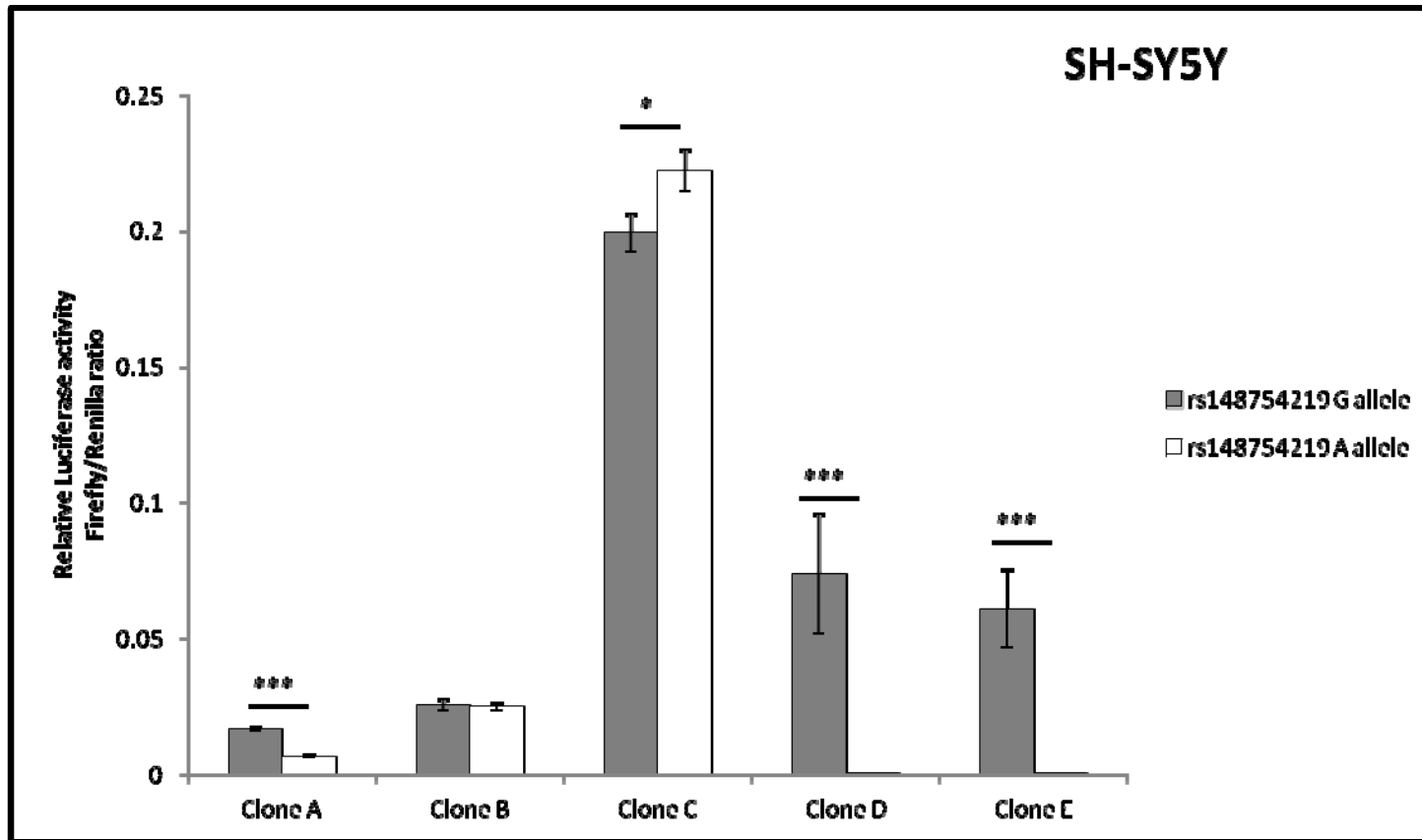


**eFigure 2B and C:** Relative luciferase activity of reporter gene assays for the experimental constructs in HEK293 (**B**) and SH-SY5Y (**C**) cells. Data expressed as mean  $\pm$  SEM of three independent experiments performed in triplicate ( $n = 9$ ). \* $P < 0.05$ ; \*\*\* $P < 0.001$ .

**B**



C



## **eReference**

1. Sartorius LJ, Nagappan G, Lipska BK, et al. Alternative splicing of human metabotropic glutamate receptor 3. *J Neurochem*. 2006;96(4):1139-1148.