SUPPLEMENTARY MATERIAL

Common variation at 6q16 within *HACE1* and *LIN28B* influences susceptibility to neuroblastoma

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I. SUPPLEMENTARY TABLES

Supplementary Table 1. Neuroblastoma patient characteristics.

Characteristic	Discovery[§] (European Ancestry) Blood DNA n=2,101	Replication[§] (African American) Blood DNA n=365	Primary Neuroblastomas[§] Tumor RNA n=87
Age			
< 1 yr	736 (36)	99 (27)	37 (43)
≥ 1 yr	1,324 (64)	263 (73)	50 (57)
Not Available	41	3	0
INSS Stage			
Stage 1	388 (20)	49 (14)	25 (29)
Stage 2	272 (14)	47 (13)	1 (1)
Stage 3	327 (16)	61 (18)	19 (22)
Stage 4	875 (44)	172 (49)	42 (48)
Stage 4S	123 (6)	22 (6)	0 (0)
Not Available	116	14	0
MYCN			
Not Amplified	1,566 (82)	278 (84)	71 (82)
Amplified	342 (18)	54 (16)	16 (18)
Not available	193	33	0
Histology			
Favorable	932 (56)	139 (49)	43 (51)
Unfavorable	735 (44)	144 (51)	41 (49)
Not available	434	82	4
DNA index			
Hyperdiploid	616 (66)	63 (61)	60 (75)
Diploid	316 (34)	46 (39)	20 (25)
Not available	1169	246	7
Risk			
Low	705 (36)	98 (29)	26 (30)
Intermediate	386 (20)	63 (19)	18 (21)
High	853 (44)	176 (52)	43 (49)
Not available	157	28	0

[§] Frequency within each clinical/biological subset is listed in parentheses.

Supplementary Table 2. Association results at previously reported loci.

SNP	Chr	Position[a]	A1/A2[b]	A1 Freq in cases	A1 Freq in controls	Allelic P-value	OR[c]
BARD1							
rs3768716	2	215635794	G/A	0.287	0.227	8.61E-14	1.377
rs17487792	2	215643500	T/C	0.286	0.225	1.12E-13	1.376
rs7587476	2	215653887	T/C	0.309	0.246	4.14E-14	1.372
rs6435862	2	215672546	G/T	0.342	0.283	8.36E-12	1.319
rs6744811	2	215677308	C/A	0.317	0.371	1.27E-09	0.784
rs2121283	2	215710338	A/G	0.314	0.365	1.18E-08	0.795
rs11677798	2	215724550	T/C	0.366	0.416	3.70E-08	0.807
rs2592232	2	215725751	C/T	0.370	0.309	1.26E-11	1.309
rs10498025	2	215749256	G/A	0.311	0.254	9.40E-12	1.328
rs10498026	2	215749350	A/G	0.413	0.476	2.40E-11	0.775
LINC00340/FLJ44180							
rs4712653	6	22125964	C/T	0.534	0.459	1.69E-15	1.354
rs9295536	6	22131929	A/C	0.506	0.430	7.82E-16	1.357
rs6939340	6	22140004	A/G	0.451	0.524	1.74E-14	0.747
LMO1							
rs4758051	11	8238639	G/A	0.511	0.452	3.83E-10	1.267
rs110419	11	8252853	G/A	0.441	0.511	1.26E-13	0.755
HSD17B12							
rs11037575	11	43671734	C/T	0.458	0.407	4.89E-08	1.231

[a] Human genome build hg19.
[b] A1: Allele 1; A2: Allele 2.
[c] OR: odds ratio (cases versus controls for A1).

Supplementary Table 3. Genotype counts and statistical significance for 6q16 SNPs.

SNP (*Gene*)	Genotype / Allele	Discovery*			Italian Replication*			African American Replication*			Combined	
		Cases (N = 2101)	Controls (N = 4202)	P€	Cases (N = 351)	Controls (N = 780)	P€	Cases (N = 365)	Controls (N = 2491)	P¥	Meta P£	OR§ (95% CI±)
rs4336470 (*HACE1*)	CC	1034 (49.5)	1772 (42.4)		176 (50.3)	348 (44.6)		58 (16.0)	237 (9.5)			
	CT	870 (41.6)	1917 (45.8)		136 (38.9)	329 (42.2)		157 (43.1)	1035(41.6)			
	TT	186 (8.9)	494 (11.8)		38 (10.8)	103 (13.2)		149 (40.9)	1215 (48.9)			
	C	2938 (70.3)	5461 (65.3)	1.8×10^{-8}	488 (69.7)	1025 (65.7)	0.06	273 (37.5)	1509 (30.3)	1.4×10^{-3}	2.7×10^{-11}	1.26 (1.18-1.35)
	T	1242 (29.7)	2905 (34.7)		212 (30.3)	535 (34.3)		455 (62.5)	3465 (69.7)			
rs9404576 (*HACE1*)	TT	1032 (49.3)	1772 (42.3)					58 (15.9)	236 (9.5)			
	TG	875 (41.8)	1924 (46.0)					158 (43.3)	1038(41.6)			
	GG	186 (8.9)	491 (11.7)					149 (40.8)	1216 (48.9)			
	T	2939 (70.2)	5468 (65.3)	3.4×10^{-8}				274 (37.5)	1510 (30.3)	1.3×10^{-3}	1.8×10^{-10}	1.27 (1.18-1.36)
	G	1247 (29.8)	2906 (34.7)					456 (62.5)	3470 (69.7)			
rs4079063 (*HACE1*)	AA	695 (33.3)	1190 (28.4)					37 (10.2)	143 (5.8)			
	AG	1003 (48.1)	2098 (50.2)					143 (39.3)	806 (32.7)			
	GG	387 (18.6)	896 (21.4)					184 (50.5)	1517 (61.5)			
	A	2393 (57.4)	4478 (53.5)	4.0×10^{-5}				217 (29.8)	1092 (22.1)	1.3×10^{-4}	1.3×10^{-7}	1.20 (1.12-1.29)
	G	1777 (42.6)	3890 (46.5)					511 (70.2)	3840 (77.9)			
rs2499663 (*HACE1*)	CC	696 (33.1)	1190 (28.3)					38 (10.4)	144 (5.8)			
	CT	1015 (48.3)	2112 (50.3)					143 (39.2)	829 (33.3)			
	TT	389 (18.6)	898 (21.4)					184 (50.4)	1518 (60.9)			
	C	2407 (57.3)	4492 (53.5)	4.5×10^{-5}				219 (30.0)	1117 (22.4)	1.5×10^{-4}	1.6×10^{-7}	1.21 (1.13-1.29)
	T	1793 (42.7)	3908 (46.5)					511 (70.0)	3865 (77.6)			
rs2499667 (*HACE1*)	AA	702 (33.4)	1192 (28.4)					35 (9.6)	132 (5.3)			
	AG	1011 (48.2)	2116 (50.3)					142 (38.9)	821 (33.0)			
	GG	387 (18.4)	894 (21.3)					188 (51.5)	1538 (61.7)			
	A	2415 (57.5)	4500 (53.5)	2.6×10^{-5}				212 (29.0)	1085 (21.8)	2.6×10^{-4}	1.2×10^{-7}	1.21 (1.13-1.29)
	G	1785 (42.5)	3904 (46.5)					511 (71.0)	3897 (78.2)			
rs17065417 (*LIN28B*)	AA	1785 (85.0)	3337 (79.4)		298 (84.9)	600 (78.6)		300 (82.2)	1952 (78.4)			
	AC	299 (14.2)	815 (19.4)		48 (13.7)	155 (20.3)		61 (16.7)	507 (20.3)			
	CC	17 (0.8)	49 (1.2)		5 (1.4)	8 (1.1)		4 (1.1)	32 (1.3)			
	A	3869 (92.1)	7489 (89.1)	1.8×10^{-7}	644 (92.0)	1355 (88.8)	0.03	661 (90.6)	4411 (88.5)	0.129	1.2×10^{-8}	1.38 (1.23-1.54)
	C	333 (7.9)	913 (10.9)		58 (8.0)	171 (11.2)		69 (9.4)	571 (11.5)			

* No deviations from Hardy-Weinberg equilibrium were observed (P>0.001) in all cohorts
€ P-values were calculated by allelic test
¥ P-value calculated by logistic regression with percent African admixture as covariate [9]
£ Meta-analysis P-value using METAL [10]
§ OR: odds ratio of risk allele based on meta-analysis
± CI: confidence interval

Supplementary Table 4. Association results for 6q16 SNPs with first 20 PCs as covariates.

		Discovery cohort European Ancestry			
SNP	**A1/A2 (protective/ risk allele)**	**Freq A1 Cases (n=2101)**	**Freq A1 Controls (n=4202)**	**Allelic P[a]**	**Logistic Regression P[b]**
rs4336470	T/C	0.30	0.35	1.8×10^{-8}	2.8×10^{-8}
rs9404576	G/T	0.30	0.35	3.4×10^{-8}	3.4×10^{-8}
rs4079063	G/A	0.43	0.47	4.0×10^{-5}	3.3×10^{-5}
rs2499663	C/T	0.43	0.47	4.5×10^{-5}	3.5×10^{-5}
rs2499667	G/A	0.43	0.47	2.6×10^{-5}	1.7×10^{-5}
rs17065417	C/A	0.08	0.11	1.8×10^{-7}	4.5×10^{-6}

[a] Standard Chi-square test for allelic association.

[b] Logistic regression with first twenty principal components (PCs) as covariates.

Supplementary Table 5. Association results for 6q16 SNPs conditioned on rs4336470.

SNP	BP[a]	Discovery P-value[b]	Italian Replication P-value [b]	African American Replication P-value[c]
rs4336470	105180785	N/A[d]	N/A[d]	N/A[d]
rs9404576	105184640	N/A[d]	-	0.86
rs4079063	105266118	0.69	-	0.06
rs2499663	105300570	0.65	-	0.05
rs2499667	105319125	0.83	-	0.07
rs17065417	105406274	2.5×10^{-4}	0.07	0.19

[a] Human genome build hg19.
[b] Logistic regression conditioned on rs4336470.
[c] Logistic regression with percent African admixture as covariate, conditioned on rs4336470.
[d] N/A: not available; SNP is conditioning SNP or is in complete linkage with conditioning SNP.

Supplementary Table 6. Correlation of rs4336470 genotypes (CC, TC, TT) with clinical variables.

				P-value [2]	
	CC[1]	CT[1]	TT[1]	CC vs TT	CT vs TT
Stage 4	438 (50%)	357 (41%)	77 (9%)	1.000	0.4464
Not Stage 4	536 (49%)	471 (43%)	95 (9%)		
***MYCN* Amp**	176 (51%)	130 (38%)	36 (11%)	0.3932	0.1851
***MYCN* Not Amp**	760 (49%)	665 (43%)	130 (8%)		
High risk	420 (50%)	350 (41%)	78 (9%)	0.5557	0.9616
Not High risk	540 (50%)	454 (42%)	90 (9%)		
DNA Index Hyperdiploid	238 (42%)	264 (46%)	62 (11%)	0.0246	0.0324
DNA Index Diploid	161 (51%)	129 (41%)	23 (7%)		
Unfavorable Histology	370 (51%)	298 (41%)	62 (8%)	0.9269	0.9172
Favorable Histology	467 (51%)	382 (41%)	77 (8%)		
Age >= 1 year	648 (49%)	546 (42%)	120 (9%)	0.5543	0.8849
Age < 1 year	364 (50%)	311 (42%)	60 (8%)		

1: The CC, CT and TT genotypes represent homozygous risk genotype, heterozygous risk genotype and homozygous non-risk (protective) genotype, respectively.
2: Two-sided Fisher's exact test.

Supplementary Table 7. Correlation of rs17065417 genotypes (AA, AC, CC) with clinical variables.

				P-value [2]	
	AA[1]	AC[1]	CC[1]	AA vs CC	AC vs CC
Stage 4	741 (85%)	129 (14%)	5 (1%)	0.5986	0.5881
Not Stage 4	941 (85%)	157 (14%)	9 (1%)		
***MYCN* Amp**	281 (83%)	56 (17%)	2 (<1%)	0.2315	0.2846
***MYCN* Not Amp**	1311 (86%)	214 (14%)	3 (<1%)		
High risk	722 (85%)	127 (15%)	4 (<1%)	0.4108	0.3963
Not High risk	928 (85%)	152 (14%)	9 (1%)		
DNA Index Hyperdiploid	509 (84%)	96 (16%)	0 (0%)	n.a.	n.a.
DNA Index Diploid	276 (89%)	32 (10%)	1 (<1%)		
Unfavorable Histology	629 (86%)	101 (14%)	3 (<1%)	0.6641	0.3845
Favorable Histology	757 (85%)	133 (15%)	2 (<1%)		
Age >= 1 year	1121 (85%)	189 (14%)	11 (1%)	0.6014	0.7939
Age < 1 year	624 (85%)	107 (15%)	5 (<1%)		

1: The CC, CT and TT genotypes represent homozygous risk genotype, heterozygous risk genotype and homozygous non-risk (protective) genotype, respectively.
2: Two-sided Fisher's exact test

Supplementary Table 8. Pairwise interaction between most significant SNPs at five loci (case/control).

CHR1	SNP1	GENE1	CHR2	SNP2	GENE2	OR	P[a]
2	rs7587476	BARD1	6	rs9295536	LINC00340	1.101	0.109
2	rs7587476	BARD1	6	rs4336470	HACE1	0.905	0.127
2	rs7587476	BARD1	6	rs17065417	LIN28B	0.942	0.577
2	rs7587476	BARD1	11	rs110419	LMO1	1.009	0.879
2	rs7587476	BARD1	11	rs11037575	HSD17B12	1.063	0.309
6	rs9295536	LINC00340	6	rs4336470	HACE1	0.963	0.520
6	rs9295536	LINC00340	6	rs17065417	LIN28B	0.936	0.495
6	rs9295536	LINC00340	11	rs110419	LMO1	0.914	0.099
6	rs9295536	LINC00340	11	rs11037575	HSD17B12	1.001	0.988
6	rs4336470	HACE1	6	rs17065417	LIN28B	0.998	0.984
6	rs4336470	HACE1	11	rs110419	LMO1	1.082	0.176
6	rs4336470	HACE1	11	rs11037575	HSD17B12	0.999	0.990
6	rs17065417	LIN28B	11	rs110419	LMO1	1.231	0.028[b]
6	rs17065417	LIN28B	11	rs11037575	HSD17B12	0.909	0.311
11	rs110419	LMO1	11	rs11037575	HSD17B12	0.940	0.252

[a] P-value from --epistasis option in plink.
[b] P-value not significant after Bonferroni adjustment for multiple testing (P = 0.420)

Supplementary Table 9. Pairwise interaction between most significant SNPs at five loci (case-only).

CHR1	SNP1	GENE1	CHR2	SNP2	GENE2	P[a]
2	rs7587476	BARD1	6	rs9295536	LINC00340	**0.002**[c]
2	rs7587476	BARD1	6	rs4336470	HACE1	0.089
2	rs7587476	BARD1	6	rs17065417	LIN28B	0.343
2	rs7587476	BARD1	11	rs110419	LMO1	0.618
2	rs7587476	BARD1	11	rs11037575	HSD17B12	0.195
6	rs9295536	LINC00340	6	rs4336470	HACE1	0.975
6	rs9295536	LINC00340	6	rs17065417	LIN28B	0.528
6	rs9295536	LINC00340	11	rs110419	LMO1	0.349
6	rs9295536	LINC00340	11	rs11037575	HSD17B12	0.733
6	rs4336470	HACE1	6	rs17065417	LIN28B	n/a[b]
6	rs4336470	HACE1	11	rs110419	LMO1	0.433
6	rs4336470	HACE1	11	rs11037575	HSD17B12	0.325
6	rs17065417	LIN28B	11	rs110419	LMO1	0.098
6	rs17065417	LIN28B	11	rs11037575	HSD17B12	0.259
11	rs110419	LMO1	11	rs11037575	HSD17B12	0.715

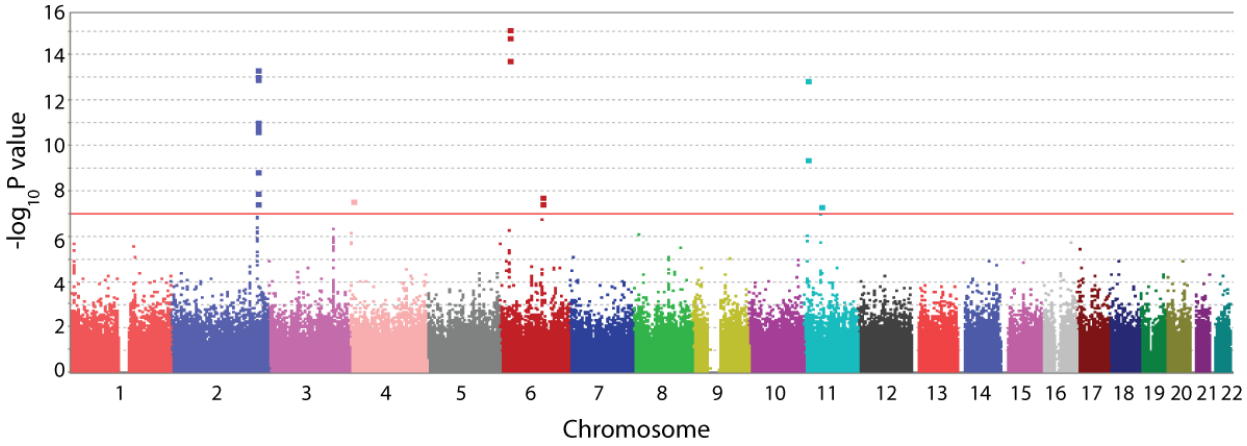
[a] P-value from --epistasis option in plink.
[b] Interaction test for rs4336470 and rs17065417 not reported due to insufficient numbers.
[c] Interaction remains significant after Bonferroni adjustment (P = 0.028).

Supplementary Table 10. Genotyped and imputed SNP associations at 6q16 ($P < 1 \times 10^{-6}$).

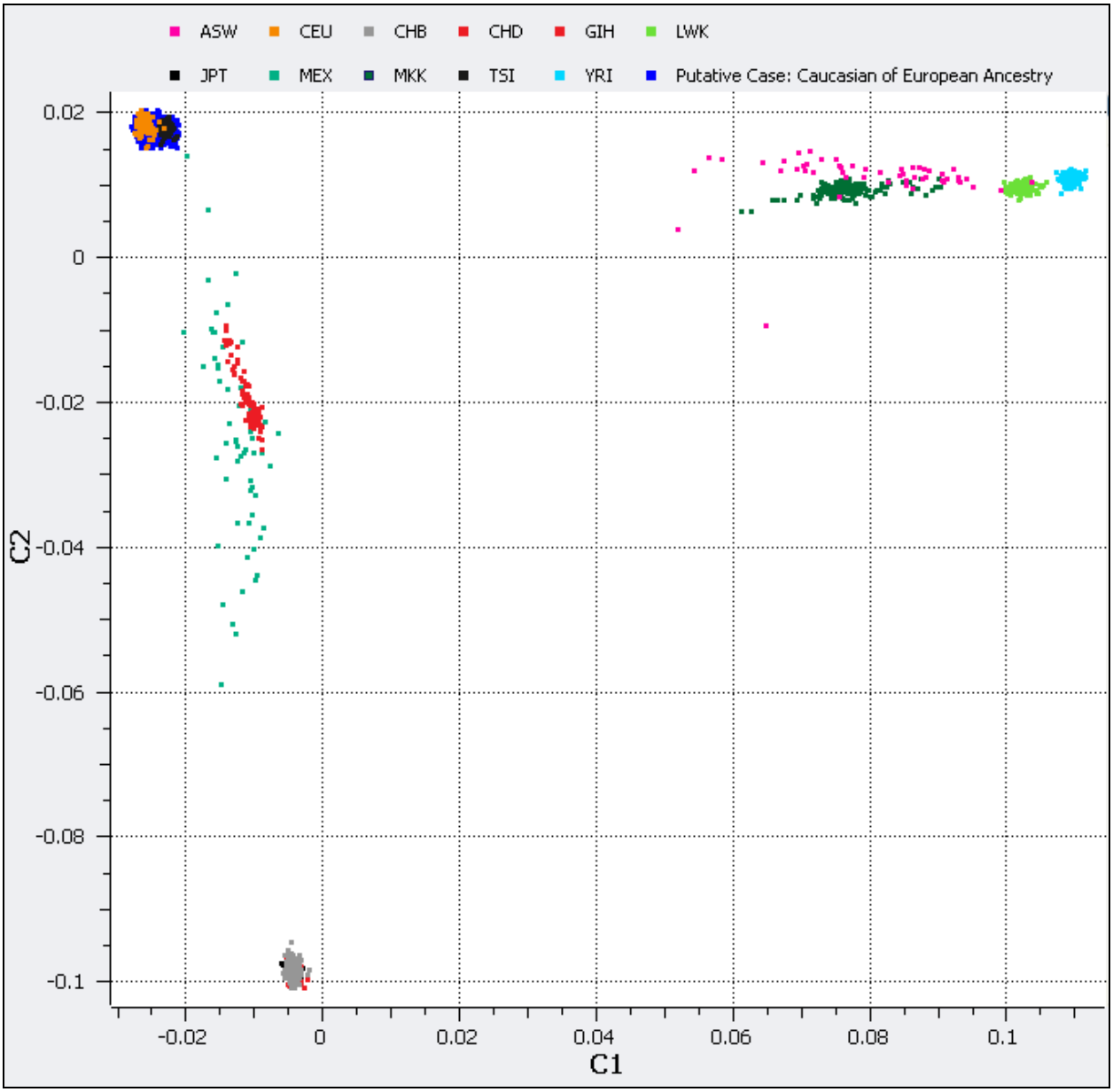
Identifier	Position[1]	Major Allele[2]	Minor Allele[2]	Cases MAF[3]	Controls MAF[3]	OR[4]	OR Lower	OR Upper	P-value[5]
6-105142638	105142638	C	G	0.40	0.45	0.810	0.747	0.878	3.75E-07
6-105146360	105146360	G	T	0.40	0.45	0.812	0.750	0.880	3.99E-07
6-105146656	105146656	T	C	0.40	0.46	0.810	0.748	0.877	2.90E-07
6-105147784	105147784	G	A	0.03	0.05	0.585	0.475	0.721	2.84E-07
6-105149439	105149439	T	C	0.40	0.45	0.811	0.749	0.878	3.14E-07
6-105149713	105149713	C	G	0.40	0.45	0.811	0.749	0.878	3.17E-07
6-105154880	105154880	G	A	0.40	0.45	0.813	0.751	0.880	3.89E-07
6-105155782	105155782	C	A	0.40	0.45	0.817	0.754	0.885	7.27E-07
6-105158405	105158405	T	C	0.40	0.45	0.815	0.752	0.882	5.19E-07
6-105158634	105158634	T	A	0.40	0.45	0.815	0.753	0.883	5.52E-07
6-105173726	105173726	G	A	0.30	0.35	0.795	0.734	0.861	1.40E-08
6-105174273	105174273	T	C	0.30	0.35	0.795	0.734	0.861	1.40E-08
6-105175533	105175533	C	T	0.33	0.38	0.796	0.731	0.866	1.23E-07
rs4336470	105180785	C	T	0.30	0.35	0.795	0.733	0.861	1.47E-08
rs9404576	105184640	T	G	0.30	0.35	0.798	0.737	0.865	2.62E-08
6-105185421	105185421	T	C	0.40	0.44	0.820	0.758	0.888	8.85E-07
6-105185807	105185807	C	G	0.31	0.36	0.812	0.750	0.880	2.55E-07
6-105190335	105190335	T	G	0.29	0.34	0.804	0.741	0.872	1.27E-07
6-105191814	105191814	C	T	0.32	0.36	0.815	0.753	0.882	2.94E-07
6-105194651	105194651	A	G	0.30	0.35	0.795	0.734	0.861	1.41E-08
6-105197091	105197091	G	T	0.30	0.35	0.795	0.734	0.861	1.42E-08
6-105197874	105197874	A	G	0.30	0.35	0.796	0.735	0.862	1.57E-08
6-105232233	105232233	A	C	0.31	0.36	0.815	0.753	0.883	3.36E-07
6-105235757	105235757	A	G	0.31	0.36	0.815	0.753	0.883	3.36E-07
6-105358164	105358164	G	A	0.12	0.15	0.751	0.671	0.841	7.79E-07
6-105360252	105360252	C	A	0.12	0.15	0.751	0.671	0.840	6.70E-07
6-105362716	105362716	T	C	0.12	0.15	0.755	0.674	0.844	9.96E-07
6-105404562	105404562	A	C	0.08	0.10	0.697	0.609	0.798	1.11E-07
rs17065417	105406274	A	C	0.08	0.11	0.706	0.619	0.805	1.24E-07
6-105406324	105406324	C	T	0.07	0.10	0.700	0.611	0.801	1.69E-07
6-105415911	105415911	G	A	0.08	0.11	0.704	0.616	0.805	1.88E-07
6-105419286	105419286	T	A	0.08	0.11	0.698	0.610	0.799	1.06E-07
6-105425731	105425731	G	A	0.04	0.06	0.592	0.487	0.720	5.72E-08
6-105430503	105430503	A	T	0.08	0.11	0.698	0.610	0.798	1.04E-07
6-105432994	105432994	G	C	0.08	0.11	0.705	0.617	0.806	2.06E-07
6-105434907	105434907	A	G	0.08	0.11	0.698	0.610	0.798	1.04E-07
6-105436993	105436993	G	A	0.08	0.11	0.698	0.610	0.798	1.04E-07
6-105439354	105439354	A	G	0.08	0.11	0.703	0.616	0.802	1.09E-07
6-105441404	105441404	T	G	0.08	0.11	0.698	0.610	0.798	1.04E-07
6-105444115	105444115	T	C	0.08	0.11	0.698	0.610	0.798	1.04E-07
6-105454799	105454799	T	A	0.08	0.11	0.702	0.614	0.803	1.59E-07
6-105462201	105462201	T	A	0.08	0.10	0.703	0.615	0.804	1.89E-07
6-105469367	105469367	A	G	0.07	0.10	0.697	0.608	0.799	1.70E-07
6-105469476	105469476	C	T	0.07	0.10	0.697	0.608	0.800	1.77E-07
6-105475109	105475109	C	T	0.07	0.10	0.693	0.604	0.796	1.22E-07
6-105480449	105480449	A	T	0.06	0.09	0.647	0.557	0.751	5.87E-09
6-105480607	105480607	A	T	0.06	0.09	0.644	0.554	0.748	4.41E-09
6-105480725	105480725	T	C	0.06	0.09	0.646	0.557	0.751	6.37E-09
6-105480816	105480816	G	A	0.06	0.09	0.649	0.559	0.754	9.89E-09

1. Human genome build hg19
2. Alleles with respect to forward strand
3. MAF: minor allele frequency
4. Odds ratio with respect to minor allele
5. SNPTEST p-value

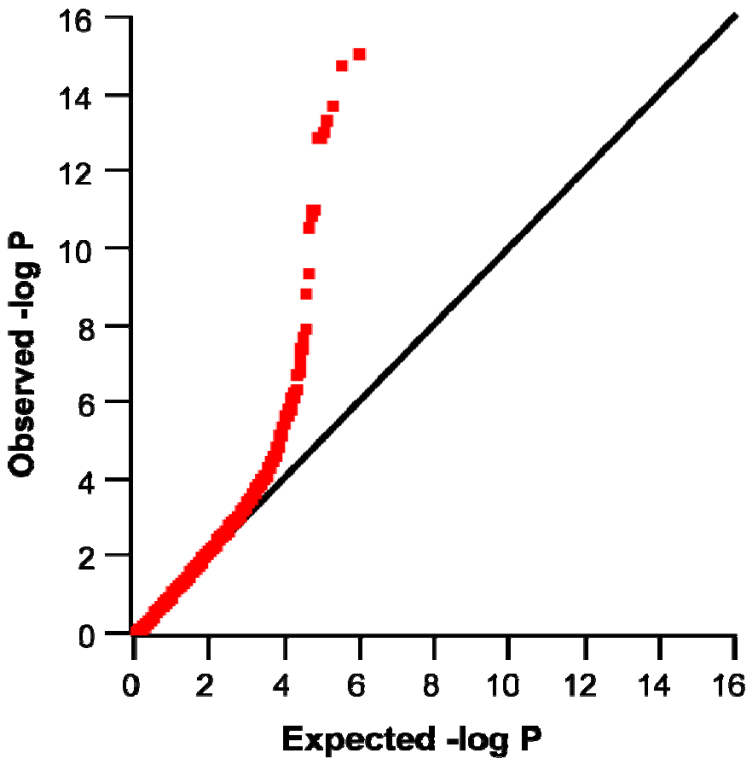
II. SUPPLEMENTARY FIGURES



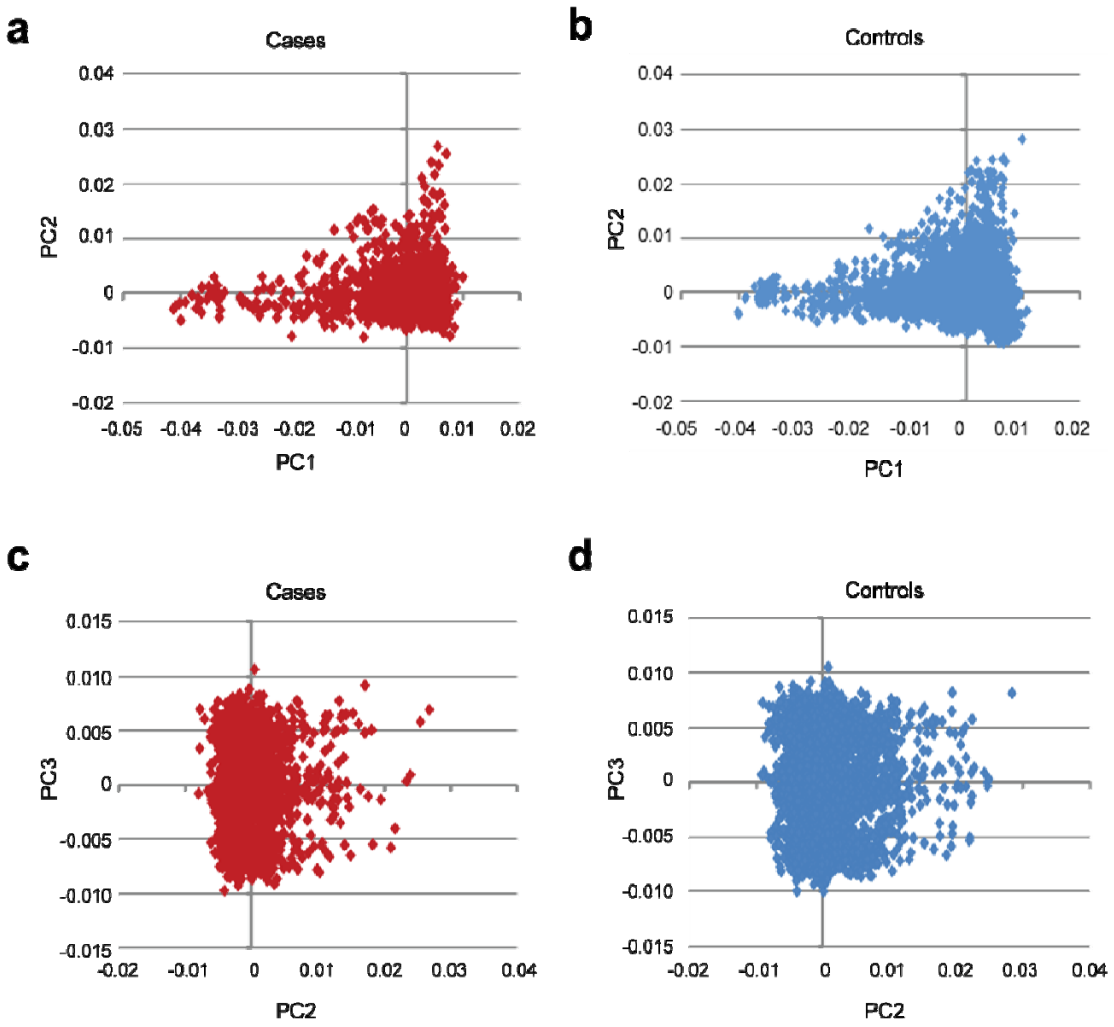
Supplementary Figure 1. Manhattan plot of discovery results. Level of significance ($-\log_{10}$ transformed P values) for each SNP along the genome in chromosomal order is plotted. Red line: genome-wide significance threshold based on Bonferroni adjustment. New association signal identified is at chromosome 6q16.



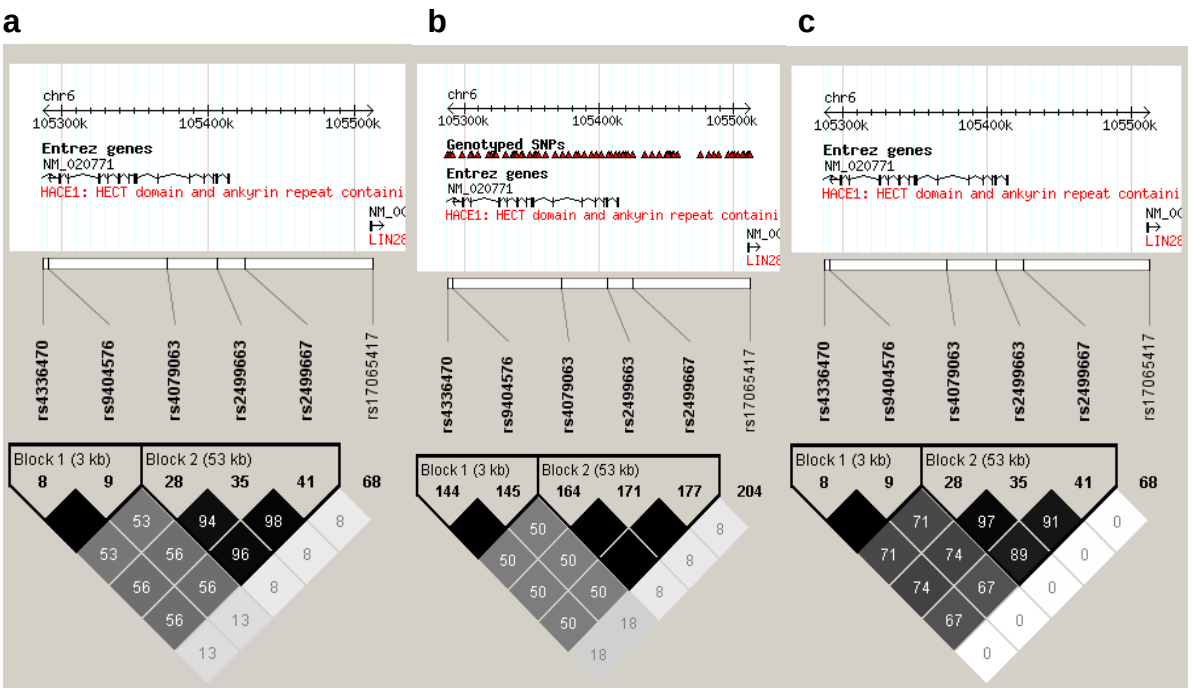
Supplementary Figure 2. MDS plot of HapMap3 populations and neuroblastoma cases of European Ancestry. Plot generated from SVS 7.2 Software (Golden Helix; Bozeman, MT).



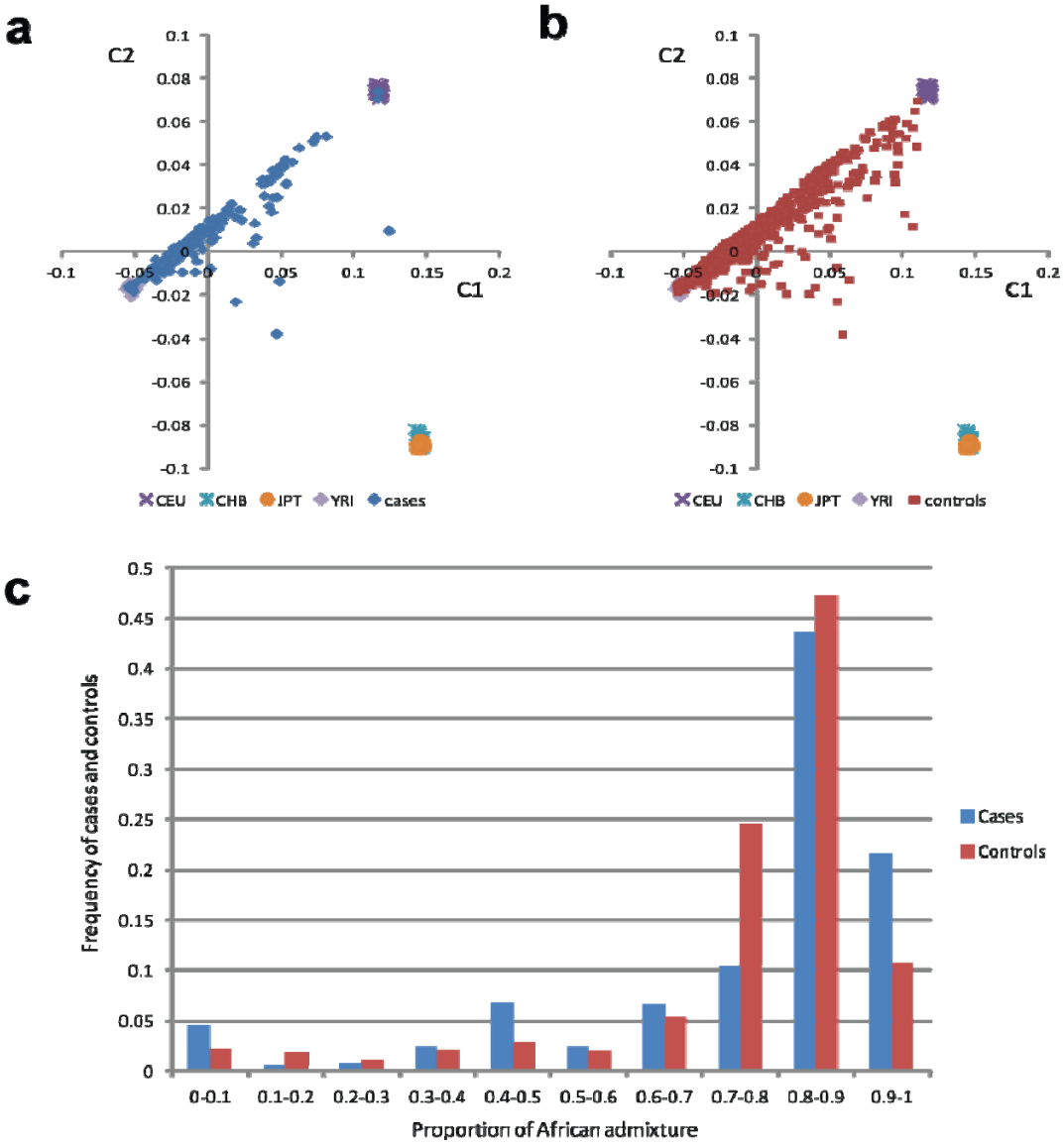
Supplementary Figure 3. Quantile-Quantile plot of the expected and observed P-values. SNPs passing quality control in the discovery phase are plotted; known neuroblastoma-associated SNPs have not been removed. The genomic inflation factor is 1.14.



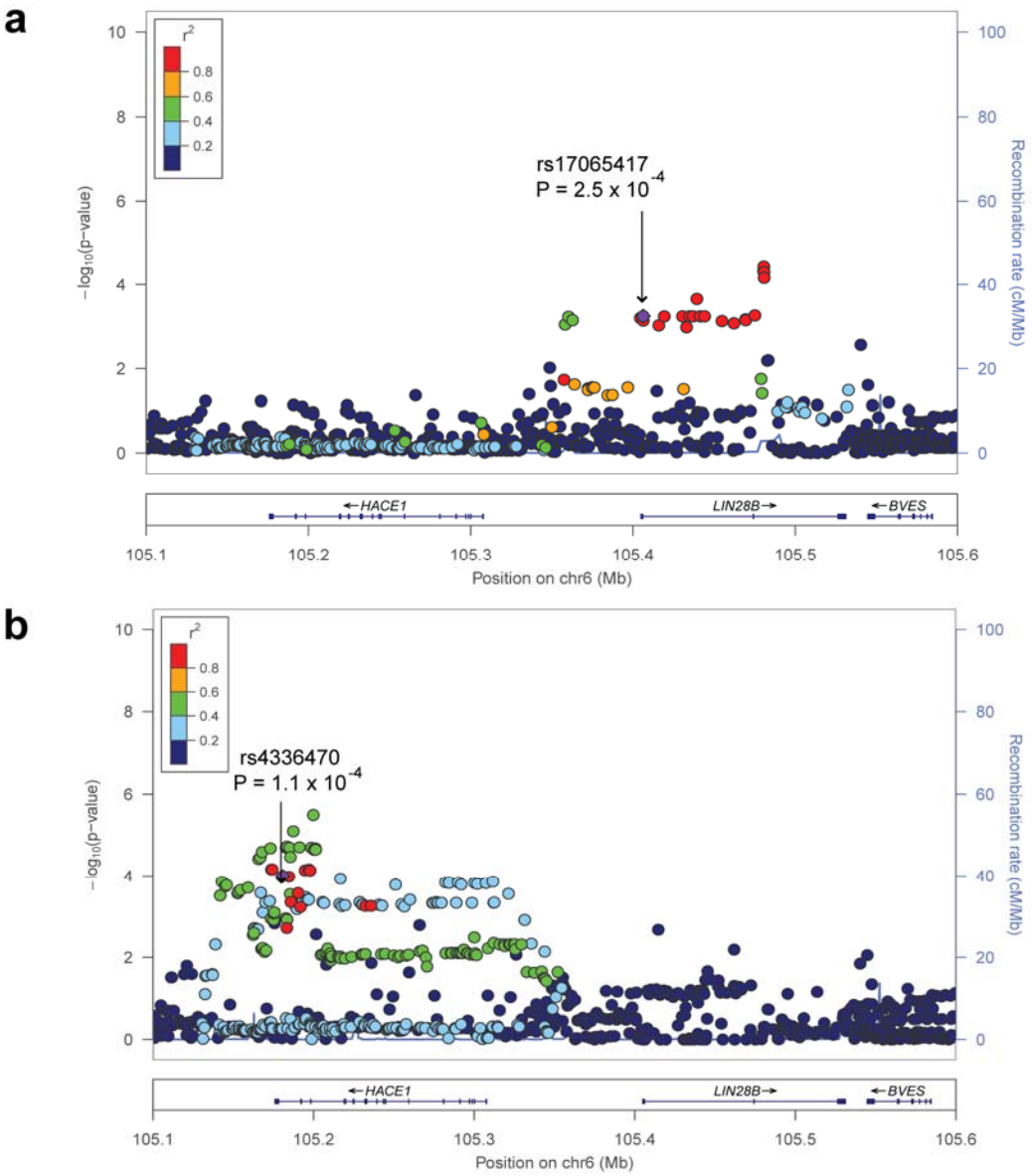
Supplementary Figure 4. Plots of first three principal components for Discovery phase. No clear evidence of stratification was observed. Including the first three principal components in a logistic regression analysis did not alter the inflation factor (lambda = 1.136), suggesting the elevated lambda was not the result of gross population stratification issues.



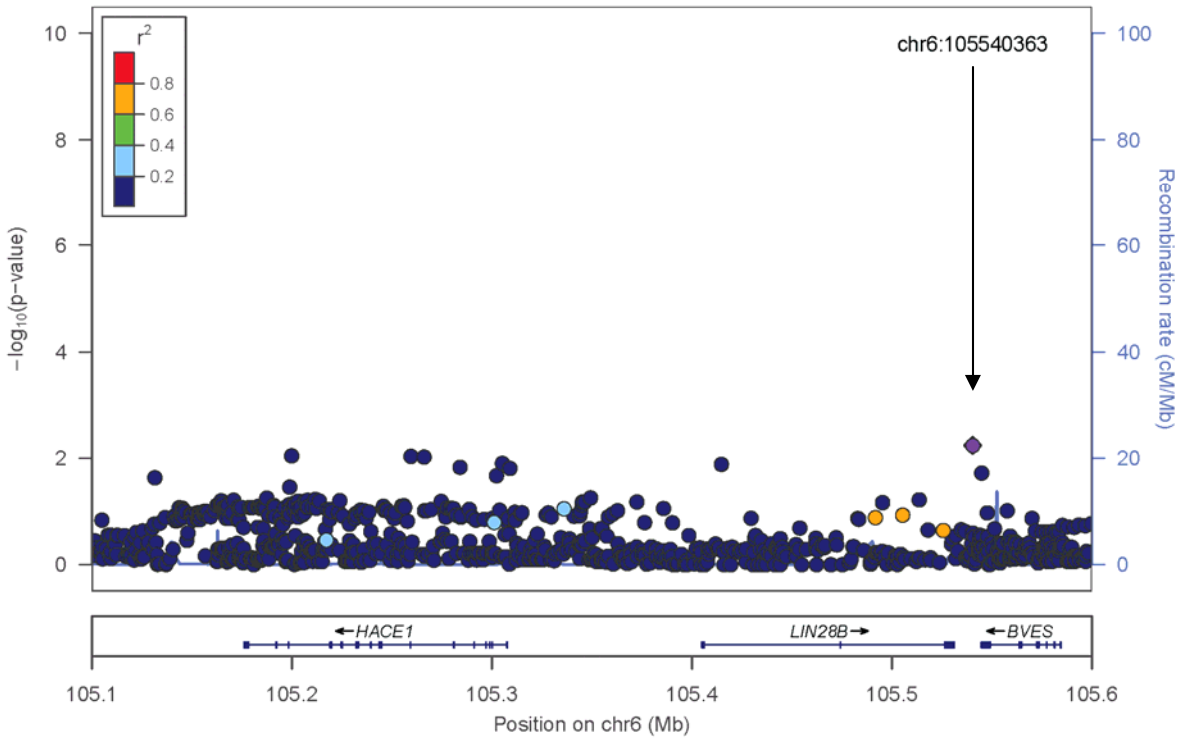
Supplementary Figure 5. Linkage Disequilibrium (LD) plot of significant SNPs from Discovery phase a) HapMap3 CEU population. b) HapMap3 TSI population. c) HapMap3 YRI population.



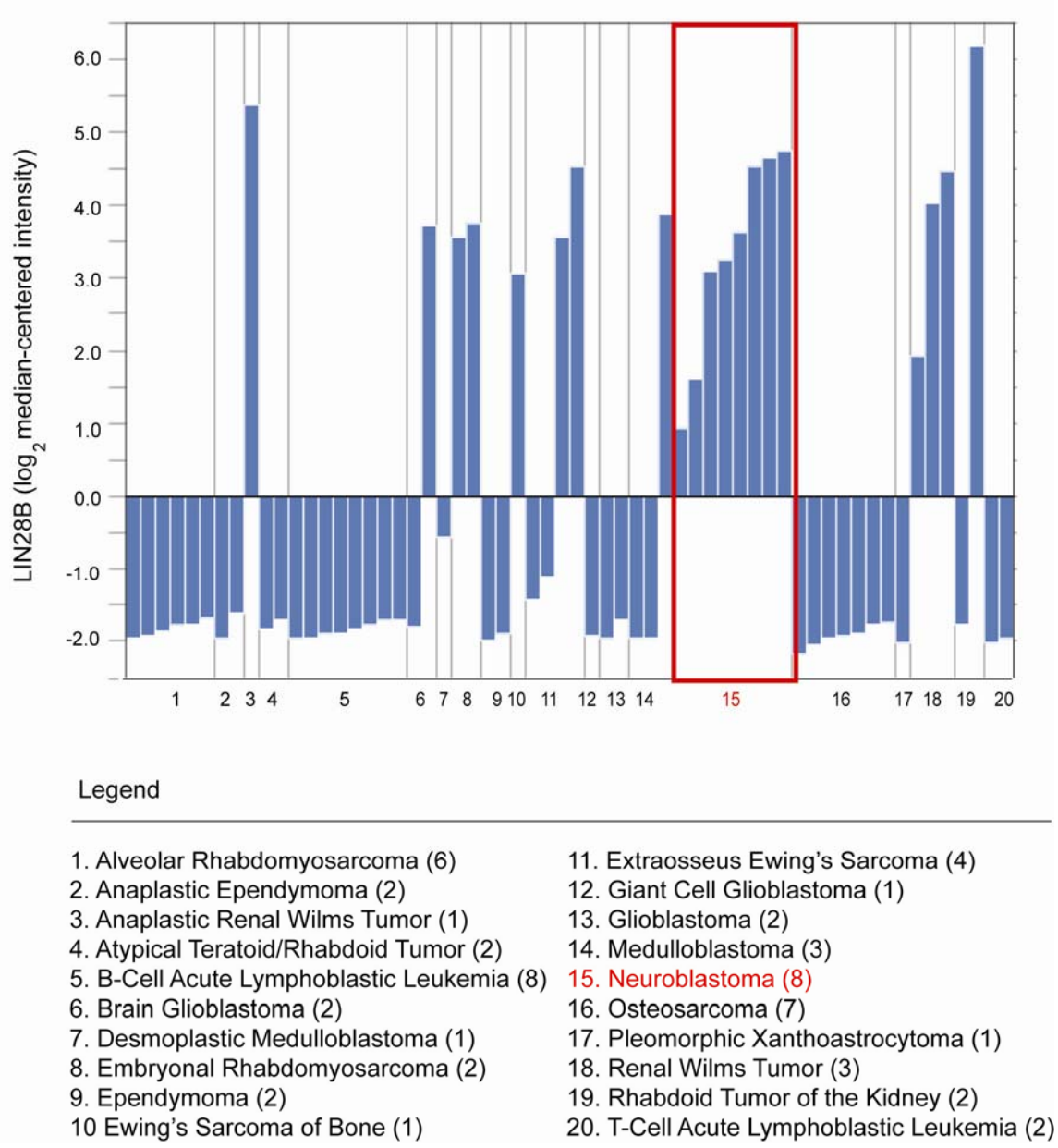
Supplementary Figure 6. MDS and admixture plots for African American replication. Plotted are first two components of MDS analysis for cases **(a)** and controls **(b)** along with the CEU, CHB, JPT and YRI HapMap populations. **(c)** Histogram of the proportion of African admixture in cases and controls.



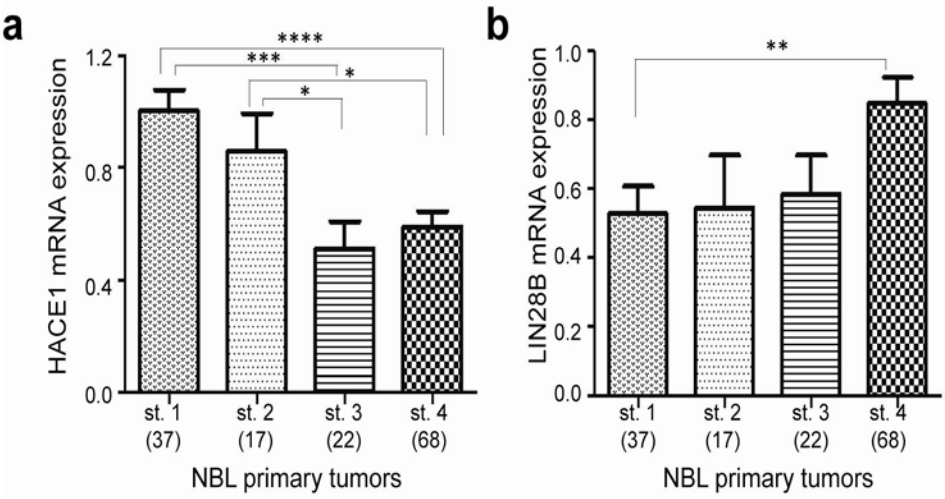
Supplementary Figure 7. Conditional association results for genotyped and imputed SNPs at 6q16. Genomic position based on hg19. **a.** conditioned on rs4336470. SNPs mapping within or downstream of *HACE1* are no longer statistically significant indicating they represent a single association signal. P-values for SNPs mapping to *LIN28B* are slightly attenuated but remain highly significant, suggesting they contribute independently to the observed association signal at 6q16. **b.** conditioned on rs17065417.



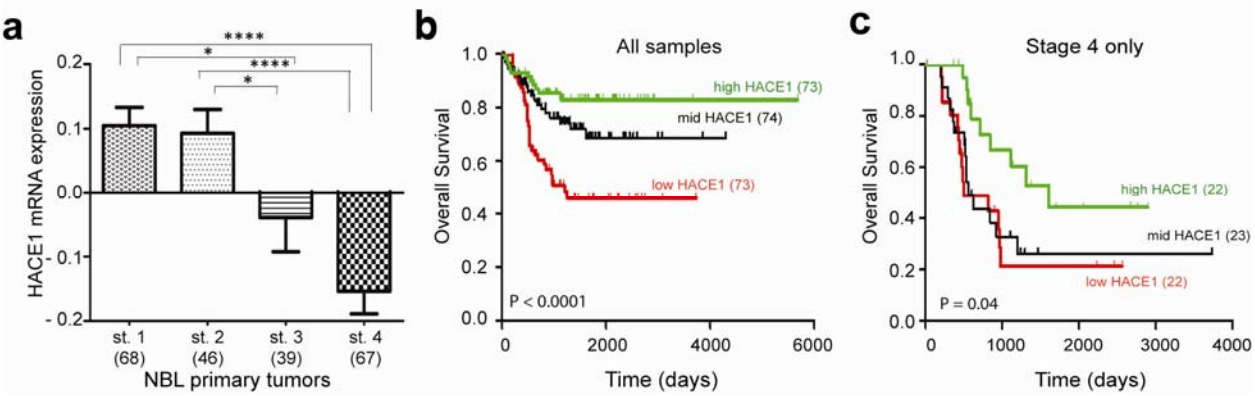
Supplementary Figure 8. Association results for genotyped and imputed SNPs at 6q16 conditioned on both rs4336470 and rs17065417. Marker colored purple is the most significant SNP after conditioning, but is no longer significant after adjustment for multiple testing. Association signal across entire region is abolished after conditioning on both rs4336470 and rs17065417. Genomic positions based on hg19.



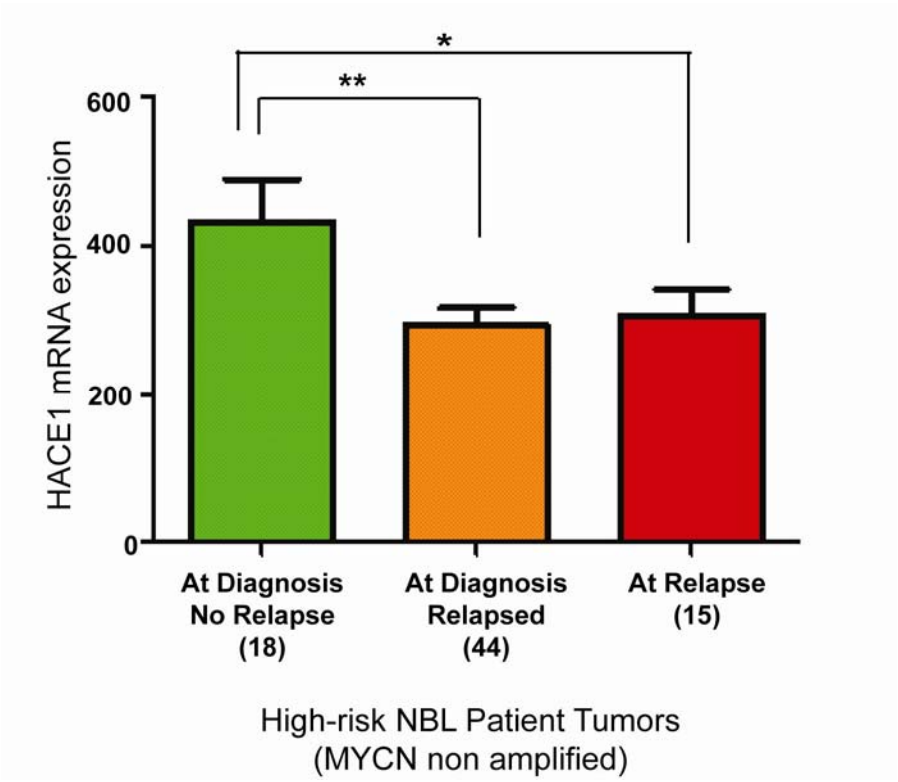
Supplementary Figure 9. *LIN28B* expressed higher in neuroblastoma compared to most other pediatric cancers. Data from Neale et al.(1) are plotted using Oncomine (http://www.oncomine.org).



Supplementary Figure 10. *HACE1* and *LIN28B* expression are associated with disease stage in neuroblastoma. Expression of *HACE1* (**a**) and *LIN28B* (**b**) in 144 primary neuroblastomas is shown according to INSS stage in bar graph format (error bars: SEM). Data are from published cDNA array available through the National Cancer Institute (NCI; http://home.ccr.cancer.gov/oncology/oncogenomics). **** P < 0.0001, *** P < 0.001; ** P < 0.01, * P < 0.05.



Supplementary Figure 11. Decreased *HACE1* expression is associated with advanced stage disease and poor overall survival. a. Expression of *HACE1* in 220 primary neuroblastomas is shown according to INSS stage in bar graph format (error bars: SEM). Data are from a custom Agilent mRNA expression array published by Oberthuer and colleagues(2) and were downloaded from the Oncogenomics website at the National Cancer Institute (NCI; http://home.ccr.cancer.gov/oncology/oncogenomics). **** P < 0.0001, * P < 0.05. **b.** Decreased *HACE1* expression in diagnostic tumors predicts for worse overall survival; Kaplan Meier analysis shown. Neuroblastoma patients are grouped based on tertiles of *HACE1* expression in tumors. Log rank p-value shown. **c.** Decreased *HACE1* in predicts for worse overall survival within the aggressive stage 4 tumors; Kaplan Meier based on tertiles of *HACE1* expression. Log rank p-value shown.



Supplementary Figure 12. Decreased *HACE1* expression associated with disease progression in high-risk MYCN non-amplified tumors. Expression of *HACE1* in seventy-seven high-risk neuroblastomas without MYCN amplification is shown in bar graph format (error bars: SEM). Data are from the Affymetrix U133 mRNA expression array published by Asgharzadeh and colleagues(3). *HACE1* expression is significantly higher in high-risk tumors at diagnosis that were relapse-free for 5-years. ** $P < 0.01$; * $P < 0.05$.

III. SUPPLEMENTARY REFERENCES

1. Neale, G., Su, X., Morton, C.L., Phelps, D., Gorlick, R., Lock, R.B., Reynolds, C.P., Maris, J.M., Friedman, H.S., Dome, J., Khoury, J., Triche, T.J., Seeger, R.C., Gilbertson, R., Khan, J., Smith, M.A., and Houghton, P.J. 2008. Molecular characterization of the pediatric preclinical testing panel. *Clin Cancer Res* 14:4572-4583.

2. Oberthuer, A., Berthold, F., Warnat, P., Hero, B., Kahlert, Y., Spitz, R., Ernestus, K., Konig, R., Haas, S., Eils, R., Schwab, M., Brors, B., Westermann, F., and Fischer, M. 2006. Customized oligonucleotide microarray gene expression-based classification of neuroblastoma patients outperforms current clinical risk stratification. *J Clin Oncol* 24:5070-5078.

3. Asgharzadeh, S., Pique-Regi, R., Sposto, R., Wang, H., Yang, Y., Shimada, H., Matthay, K., Buckley, J., Ortega, A., and Seeger, R.C. 2006. Prognostic significance of gene expression profiles of metastatic neuroblastomas lacking MYCN gene amplification. *J Natl Cancer Inst* 98:1193-1203.