SI Appendix

Rho-associated kinase is a therapeutic target in neuroblastoma

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Table S1: Rare variants in neuroblastoma patients (n=15). Rare protein changing variants not present in dpSNP, 1000 genome, ESP or in house controls. Somatic status not confirmed.

	Gene	Sample	Chr	Position	Reference	Observed	Cytoband	Zygosity	SNP	Exonic Function	Amino acid change
GEFs	AKAP13	NBL46R2	chr15	86163336	A	G	15q25.3	heterozygous	-	nonsynonymous SNV	AKAP13:uc010bnf.1:exon1:c.A14G:p.H5R
	ALS2	NBL3E2	chr2	202569898	C	G	2q33.1	heterozygous	-	nonsynonymous SNV	ALS2:uc002uyo.3:exon31:c.G4652C:p.C1551S
	ALS2	NBL4E1	chr2	202598080	CAGTATTTC	-	2q33.1	heterozygous	-	nonframeshift deletion	ALS2:uc002uyo.3:exon13:c.2491_2499del:p.831_833del,ALS2:uc002uyp.4:exon13:c.2491_2499del:p.831_833del
	ALS2	NBL3E2	chr2	202617935	A	T	2q33.1	heterozygous	-	nonsynonymous SNV	ALS2:uc002uyo.3:exon7:c.T1671A:p.D557E,ALS2:uc002uyp.4:exon7:c.T1671A:p.D557E,ALS2:uc002uyq.3:exon7:c.T1671A:p.D557E
	ARHGEF11	NBL52R2	chr1	156918243	C	T	1q23.1	heterozygous	-	nonsynonymous SNV	ARHGEF11:uc010phu.2:exon2:c.G101A:p.R34Q,ARHGEF11:uc001fqp.1:exon4:c.G410A:p.R137Q,ARHGEF11:uc001fqo.3:exon22:c.G1853A:p.R618Q,ARHGEF11:uc001fqn.3:exon23:c.G1973A:p.R658Q
	ARHGEF16	NBL14R2	chr1	3379907	C	G	1p36.32	heterozygous	-	nonsynonymous SNV	ARHGEF16:uc001akg.4:exon2:c.C259G:p.Q87E
	ARHGEF17	NBL14R2	chr11	73076579	G	A	11q13.4	heterozygous	-	nonsynonymous SNV	ARHGEF17:uc001otu.3:exon19:c.G5695A:p.V1899I
	ARHGEF2	NBL50R1	chr1	155934917	C	A	1q22	heterozygous	-	nonsynonymous SNV	ARHGEF2:uc001fmr,2:exon7:c,G502T:p,V168L,ARHGEF2:uc001fms,2:exon7:c,G583T:p,V195L,ARHGEF2:uc001fmt,2:exon7:c,G586T:p,V196L,ARHGEF2:uc010pgt,1:exon7:c,G505T:p,V169L,ARHGEF2:uc010pgu,1:exon10:c,G721T:p,V241L,ARHGEF2:uc001fmu,2:exon11:c,G718T:p,V240L
	ARHGEF25	NBL28R8	chr12	58010176	-	CTTGGAGA	12q13.3	heterozygous	-	nonframeshift insertion	ARHGEF25:uc001spb.3:exon14:c.1530_1531insCTT:p.Q510delinsQL,ARHGEF25:uc001spa.3:exon15:c.1212_1213insCTT:p.Q404delinsQL,ARHGEF25:uc009zpy.3:exon15:c.1647_1648insCTT:p.Q549delinsQL
	ARHGEF33	NBL50R1	chr2	39156066	G	A	2p22.1	heterozygous	-	nonsynonymous SNV	ARHGEF33:uc021vgd,1:exon3:c,G94A:p,E32K
	ARHGEF38	NBL17E2	chr4	106588301	C	T	4q24	heterozygous	-	stopgain SNV	ARHGEF38:uc003hxv.2:exon12:c.C1705T:p.Q569X
	ARHGEF40	NBL48R8cT	chr14	21548849	G	A	14q11.2	heterozygous	-	nonsynonymous SNV	ARHGEF40:uc001vzp.3:exon12:c.G2404A:p.E802K,ARHGEF40:uc010tln.2:exon12:c.G262A:p.E88K
	ARHGEF40	NBL19R6	chr14	21552099	C	T	14q11.2	heterozygous	-	nonsynonymous SNV	ARHGEF40:uc001vzo.1:exon12:c.C916T:p.P306S,ARHGEF40:uc001vzp.3:exon17:c.C3679T:p.P1227S,ARHGEF40:uc010tln.2:exon17:c.C1537T:p.P513S
	ECT2L	NBL19R6	chr6	139165651	G	A	6q24.1	heterozygous	-	nonsynonymous SNV	ECT2L:uc011edq.1:exon4:c.G491A:p.R164Q,ECT2L:uc021zfx.1:exon6:c.G698A:p.R233Q,ECT2L:uc003qif.2:exon7:c.G698A:p.R233Q
	FGD2	NBL47R4	chr6	36979531	C	G	6p21.2	heterozygous	-	nonsynonymous SNV	FGD2:uc010jwp.1:exon4:c.C428G:p.P143R

	FGD2	NBL13E5	chr6	36981404	G	A	6p21.2	heterozygous	-	nonsynonymous SNV	FGD2:uc010jwp.1:exon5:c.G547A:p.G183S
	ITSN2	NBL3E2	chr2	24522948	C	T	2p23.3	heterozygous	-	nonsynonymous SNV	ITSN2:uc002rfe.2:exon12:c.G1174A:p.E392K,ITSN2:uc002rff.2:exon12:c.G1174A:p.E392K,ITSN2:uc002rfg.3:exon12:c.G1174A:p.E392K,ITSN2:uc010eyd.2:exon13:c.G1249A:p.E417K
	MCF2	NBL47R4	chrX	138729056	C	T	Xq27.1	heterozygous	-	nonsynonymous SNV	MCF2:uc004faw.2:exon3:c.G32A:p.R11Q,MCF2:uc011mwn.1:exon3:c.G287A:p.R96Q,MCF2:uc011mwo.1:exon3:c.G32A:p.R11Q
	MCF2L	NBL47R4	chr13	113730406	A	C	13q34	heterozygous	-	nonsynonymous SNV	MCF2L:uc001vst.1:exon10:c.A1321C:p.N441H,MCF2L:uc001vsq.3:exon12:c.A1606C:p.N536H,MCF2L:uc001vsr.3:exon12:c.A1447C:p.N483H,MCF2L:uc001vss.4:exon12:c.A1429C:p.N477H,MCF2L:uc001vsu.3:exon12:c.A1606C:p.N536H,MCF2L:uc010tjr.2:exon12:c.A1435C:p.N479H,MCF2L:uc010tjs.2:exon12:c.A1429C:p.N477H
	OBSCN	NBL59R9	chr1	228403327	C	T	1q42.13	heterozygous	-	nonsynonymous SNV	OBSCN:uc001hsn.3:exon6:c.C1892T:p.T631I,OBSCN:uc009xez.1:exon6:c.C1892T:p.T631I
	OBSCN	NBL19R6	chr1	228444409	C	T	1q42.13	heterozygous	-	nonsynonymous SNV	OBSCN:uc001hsn.3:exon15:c.C4367T:p.A1456V,OBSCN:uc009xez.1:exon15:c.C4367T:p.A1456V
	OBSCN	NBL50R1	chr1	228475602	G	C	1q42.13	heterozygous	-	nonsynonymous SNV	OBSCN:uc001hsq,1:exon7:c,G1520C:p,S507T,OBSCN:uc001hsn,3:exon36:c,G9752C:p,S3251T,OBSCN:uc009xez,1:exon36:c,G9752C:p,S3251T
	OBSCN	NBL47R4	chr1	228509528	G	A	1q42.13	heterozygous	-	nonsynonymous SNV	OBSCN:uc001hsn.3:exon55:c.G14986A:p.E4996K,OBSCN:uc009xez.1:exon55:c.G14986A:p.E4996K
	OBSCN	NBL59R7	chr1	228559841	C	T	1q42.13	heterozygous	-	nonsynonymous SNV	OBSCN:uc001hsr.1:exon34:c.C5249T:p.S1750F,OBSCN:uc009xez.1:exon94:c.C21362T:p.S7121F
	OBSCN	NBL19R6	chr1	228560239	G	A	1q42.13	heterozygous	-	nonsynonymous SNV	OBSCN:uc001hsr.1:exon34:c.G5647A:p.V1883M,OBSCN:uc009xez.1:exon94:c.G21760A:p.V7254M
	PLEKHG1	NBL47R4	chr6	151054823	G	T	6q25.1	heterozygous	-	nonsynonymous SNV	PLEKHG1:uc003qnz.2:exon2:c.G6T:p.E2D,PLEKHG1:uc011eel.1:exon2:c.G126T:p.E42D,PLEKHG1:uc011eem.1:exon2:c.G183T:p.E61D,PLEKHG1:uc003qny.1:exon3:c.G6T:p.E2D
	PLEKHG1	NBL6E9	chr6	151055142	C	T	6q25.1	heterozygous	-	nonsynonymous SNV	PLEKHG1:uc003qnz.2:exon2:c.C325T:p.P109S,PLEKHG1:uc011eel.1:exon2:c.C445T:p.P149S,PLEKHG1:uc011eem.1:exon2:c.C502T:p.P168S,PLEKHG1:uc003qny.1:exon3:c.C325T:p.P109S
	PLEKHG3	NBL11E4	chr14	65209810	T	C	14q23.3	heterozygous	-	nonsynonymous SNV	PLEKHG3:uc001xhq.1:exon4:c.T1564C:p.F522L,PLEKHG3:uc010aqh.1:exon11:c.T1675C:p.F559L,PLEKHG3:uc001xhn.1:exon15:c.T2881C:p.F961L,PLEKHG3:uc001xho.1:exon17:c.T3049C:p.F1017L,PLEKHG3:uc001xhp.2:exon17:c.T3412C:p.F1138L
	PLEKHG3	NBL49R1	chr14	65198841	G	A	14q23.3	heterozygous	.	stopgain SNV	PLEKHG3:uc001xho.1:exon10:c.G1154A:p.W385X,PLEKHG3:uc001xhp.2:exon9:c.G1154A:p.W385X,PLEKHG3:uc001xhn.1:exon8:c.G986A:p.W329X,
	PREX2	NBL13E5	chr8	69002846	G	T	8q13.2	heterozygous	-	stopgain SNV	PREX2:uc011lez.1:exon18:c.G1951T:p.G651X,PREX2:uc003xxu.1:exon20:c.G2146T:p.G716X,PREX2:uc003xxv.1:exon20:c.G2146T:p.G716X
	PREX2	NBL4E1T	chr8	69009436	G	C	8q13.2	heterozygous	-	nonsynonymous SNV	PREX2:uc011lez.1:exon20:c.G2358C:p.K786N,PREX2:uc003xxu.1:exon22:c.G2553C:p.K851N,PREX2:uc003xxv.1:exon22:c.G2553C:p.K851N

	TIAM1	NBL50R1	chr21	32638517	G	A	21q22.11	heterozygous	-	nonsynonymous SNV	TIAM1:uc011adk.1:exon2:c.C772T:p.R258W,TIAM1:uc011adl.1:exon2:c.C772T:p.R258W,TIAM1:uc002yow.1:exon5:c.C772T:p.R258W
	TIAM2	NBL52R2	chr6	155575630	G	A	6q25.3	heterozygous	-	nonsynonymous SNV	TIAM2:uc003qqh.3:exon12:c.G1166A:p.C389Y,TIAM2:uc003qqg.3:exon20:c.G2327A:p.C776Y,TIAM2:uc003qqf.3:exon21:c.G2519A:p.C840Y,TIAM2:uc011efl.1:exon21:c.G2399A:p.C800Y,TIAM2:uc010kjj.3:exon23:c.G3077A:p.C1026Y,TIAM2:uc003qqe.3:exon25:c.G4391A:p.C1464Y,TIAM2:uc003qqb.3:exon28:c.G4391A:p.C1464Y
	TRIO	NBL49R1	chr5	14465735	G	A	5q15.2	heterozygous	.	nonsynonymous SNV	TRIO:uc003jfi.1:exon4:c.G658A:p.V220M,TRIO:uc003jff.2:exon37:c.G5749A:p.V1917M,TRIO:uc003jfh.1:exon32:c.G4696A:p.V1566M,
	VAV2	NBL59R9	chr9	136635539	G	A	9q34.2	heterozygous	-	nonsynonymous SNV	VAV2:uc004cet.1:exon13:c.C925T:p.R309W,VAV2:uc004cer.3:exon25:c.C2278T:p.R760W,VAV2:uc004ces.3:exon27:c.C2308T:p.R770W
GAPs	ARHGAP29	NBL18E2	chr1	94640251	T	C	1p22.1	heterozygous	-	nonsynonymous SNV	ARHGAP29:uc001dqj.4:exon23:c.A2960G:p.E987G
	ARHGAP12	NBL14R2	chr10	32197395	C	T	10p11.22	heterozygous	-	nonsynonymous SNV	ARHGAP12:uc001ivy.1:exon1:c.G383A:p.R128H,ARHGAP12:uc009xlr.1:exon2:c.G383A:p.R128H,ARHGAP12:uc001ivz.1:exon3:c.G389A:p.R130H,ARHGAP12:uc001iwb.1:exon3:c.G383A:p.R128H,ARHGAP12:uc001iwc.1:exon3:c.G383A:p.R128H,ARHGAP12:uc009xlq.1:exon3:c.G383A:p.R128H,ARHGAP12:uc009xls.2:exon3:c.G383A:p.R128H
	ARHGAP20	NBL19R6	chr11	110451877	G	T	11q23.1	heterozygous	-	nonsynonymous SNV	ARHGAP20:uc001plb.2:exon5:c.C422A:p.A141E,ARHGAP20:uc001pky.1:exon15:c.C1724A:p.A575E,ARHGAP20:uc009yyb.1:exon15:c.C1685A:p.A562E,ARHGAP20:uc001pkz.1:exon16:c.C1793A:p.A598E,ARHGAP20:uc001pla.1:exon16:c.C1685A:p.A562E
	ARHGAP20	NBL48R8cT	chr11	110454388	G	T	11q23.1	heterozygous	-	nonsynonymous SNV	ARHGAP20:uc001plb.2:exon3:c.C118A:p.Q40K,ARHGAP20:uc001pky.1:exon13:c.C1420A:p.Q474K,ARHGAP20:uc009yyb.1:exon13:c.C1381A:p.Q461K,ARHGAP20:uc001pkz.1:exon14:c.C1489A:p.Q497K,ARHGAP20:uc001pla.1:exon14:c.C1381A:p.Q461K
	ARHGAP5	NBL50R1	chr14	32560117	A	G	14q12	heterozygous	-	nonsynonymous SNV	ARHGAP5:uc001wrl.3:exon2:c.A242G:p.N81S,ARHGAP5:uc001wrm.3:exon2:c.A242G:p.N81S,ARHGAP5:uc001wrn.3:exon2:c.A242G:p.N81S
	ARHGAP5	NBL14R2	chr14	32624042	C	G	14q12	heterozygous	-	nonsynonymous SNV	ARHGAP5:uc001wrp.3:exon5:c.C602G:p.P201R,ARHGAP5:uc001wro.3:exon6:c.C614G:p.P205R,ARHGAP5:uc001wrl.3:exon7:c.C4397G:p.P1466R,ARHGAP5:uc001wrm.3:exon7:c.C4394G:p.P1465R,ARHGAP5:uc001wrn.3:exon7:c.C4397G:p.P1466R
	ARHGAP11A	NBL39R1	chr15	32929478	G	A	15q13.3	heterozygous	-	nonsynonymous SNV	ARHGAP11A:uc001zgy.1:exon12:c.G2504A:p.R835H,ARHGAP11A:uc010ubw.1:exon12:c.G1937A:p.R646H,ARHGAP11A:uc010ubx.1:exon13:c.G1937A:p.R646H
	ARHGAP23	NBL59R7	chr17	36666694	G	A	17q12	heterozygous	-	nonsynonymous SNV	ARHGAP23:uc021twd.1:exon24:c.G3962A:p.R1321H
	ARHGAP8, PRR5-ARHGAP8	NBL17E2	chr22	45258362	CAGGGGAG	-	22q13.31	heterozygous	-	frameshift deletion	ARHGAP8:uc003bfk.3:exon12:c.1189_1196del:p.397_399del,ARHGAP8:uc003bfj.3:exon13:c.1282_1289del:p.428_430del,ARHGAP8:uc011aqj.2:exon13:c.1348_1355del:p.450_452del,PRR5-

											ARHGAP8:uc011aqi.2:exon15:c.1555_1562del:p.519_521del,ARHGAP8:uc003bfd.3:exon17:c.1819_1826del:p.607_609del
	ARHGAP24	NBL14R2	chr4	86915914	C	G	4q21.3	heterozygous	-	nonsynonymous SNV	ARHGAP24:uc003hpm.3:exon6:c.C828G:p.S276R,ARHGAP24:uc003hpl.3:exon7:c.C822G:p.S274R,ARHGAP24:uc010ikf.3:exon7:c.C852G:p.S284R,ARHGAP24:uc003hpk.3:exon9:c.C1107G:p.S369R
	ARHGAP24	NBL18E2	chr4	86916141	G	A	4q21.3	heterozygous	-	nonsynonymous SNV	ARHGAP24:uc003hpm.3:exon6:c.G1055A:p.G352D,ARHGAP24:uc003hpl.3:exon7:c.G1049A:p.G350D,ARHGAP24:uc010ikf.3:exon7:c.G1079A:p.G360D,ARHGAP24:uc003hpk.3:exon9:c.G1334A:p.G445D
	ARHGAP24	NBL50R1	chr4	86916714	A	G	4q21.3	heterozygous	-	nonsynonymous SNV	ARHGAP24:uc003hpm,3:exon6:c,A1628G:p,N543S,ARHGAP24:uc003hpl,3:exon7:c,A1622G:p,N541S,ARHGAP24:uc010ikf,3:exon7:c,A1652G:p,N551S,ARHGAP24:uc003hpk,3:exon9:c,A1907G:p,N636S
	ARHGAP39	NBL50R1	chr8	145773193	G	A	8q24.3	heterozygous	-	nonsynonymous SNV	ARHGAP39:uc011llk.1:exon5:c.C1277T:p.S426L,ARHGAP39:uc003zds.1:exon6:c.C1277T:p.S426L,ARHGAP39:uc003zdt.1:exon6:c.C1277T:p.S426L
	ARHGAP4	NBL4E1T	chrX	153174571	G	A	Xq28	heterozygous	-	nonsynonymous SNV	ARHGAP4:uc004fjk.2:exon21:c.C2560T:p.R854C,ARHGAP4:uc011mzf.2:exon21:c.C2491T:p.R831C,ARHGAP4:uc004fjl.2:exon22:c.C2680T:p.R894C
	ARHGAP4	NBL19R6	chrX	153174912	G	A	Xq28	heterozygous	-	nonsynonymous SNV	ARHGAP4:uc004fjk.2:exon20:c.C2492T:p.S831L,ARHGAP4:uc011mzf.2:exon20:c.C2423T:p.S808L,ARHGAP4:uc004fjl.2:exon21:c.C2612T:p.S871L
	OPHN1	NBL59R9	chrX	67412761	-	A	Xq12	homozygous	-	stopgain SNV	OPHN1:uc004dww.4:exon15:c.1275_1276insT:p.D426_P427delinsX,OPHN1:uc011mpg.2:exon15:c.1275_1276insT:p.D426_P427delinsX
OTHER	ATRX	NBL19R6	chrX	76937199	C	G	Xq21.1	heterozygous	-	nonsynonymous SNV	ATRX:uc004ecq.4:exon8:c.G3435C:p.E1145D,ATRX:uc010nly.1:exon8:c.G3384C:p.E1128D,ATRX:uc004ecp.4:exon9:c.G3549C:p.E1183D,ATRX:uc004ecr.2:exon9:c.G3345C:p.E1115D,ATRX:uc004eco.4:exon10:c.G2904C:p.E968D,ATRX:uc010nlx.1:exon10:c.G3462C:p.E1154D
	ATRX	NBL47R4	chrX	76939744	C	A	Xq21.1	heterozygous	-	nonsynonymous SNV	ATRX:uc004ecq.4:exon8:c.G890T:p.C297F,ATRX:uc004ecr.2:exon8:c.G887T:p.C296F,ATRX:uc010nly.1:exon8:c.G839T:p.C280F,ATRX:uc004ecp.4:exon9:c.G1004T:p.C335F,ATRX:uc010nlx.1:exon9:c.G1004T:p.C335F,ATRX:uc004eco.4:exon10:c.G359T:p.C120F
	ATRX	NBL28R8	chrX				Xq21.1		-	Focal deletion exon 3-8	
	CCDC42	NBL39R1	chr17	8646993	T	C	17p13.1	heterozygous	-	nonsynonymous SNV	CCDC42:uc002gln.3:exon3:c.A245G:p.K82R,CCDC42:uc002glo.3:exon3:c.A245G:p.K82R
	CELSR1	NBL59R9	chr22	46777929	G	A	22q13.31	heterozygous	-	nonsynonymous SNV	CELSR1:uc011arc.1:exon13:c.C1865T:p.P622L,CELSR1:uc003bhw.1:exon21:c.C6902T:p.P2301L
	CELSR1	NBL14R2	chr22	46932653	A	G	22q13.31	heterozygous	-	nonsynonymous SNV	CELSR1:uc003bhw.1:exon1:c.T415C:p.C139R
	CELSR1	NBL59R9	chr22	46932653	A	G	22q13.31	heterozygous	-	nonsynonymous SNV	CELSR1:uc003bhw.1:exon1:c.T415C:p.C139R

	CSMD1	NBL47R4	chr8	2808772	A	T	8p23.2	heterozygous	-	nonsynonymous SNV	CSMD1:uc010lrg.3:exon28:c.T3741A:p.N1247K, CSMD1:uc011kwj.2:exon52:c.T8010A:p.N2670K, CSMD1:uc022aqr.1:exon66:c.T10065A:p.N3355K
	DAAM2	NBL3E2	chr6	39869211	A	G	6p21.2	heterozygous	-	nonsynonymous SNV	DAAM2:uc003oow.3:exon24:c.A2945G:p.K982R, DAAM2:uc003oox.3:exon24:c.A2942G:p.K981R
	FZD8	NBL48R8cT	chr10	35929895	C	A	10p11.21	heterozygous	-	nonsynonymous SNV	FZD8:uc001iyz.1:exon1:c.G463T:p.D155Y
	TENM4/ ODZ4	NBL39R1	chr11	78369680	C	T	11q14.1	heterozygous	-	nonsynonymous SNV	ODZ4:uc001ozk.4:exon5:c.G2408A:p.R803Q,ODZ4:uc001ozl.4:exon34:c.G7733A:p.R2578Q
	TENM4/ ODZ4	NBL3E2	chr11	78412590	C	T	11q14.1	heterozygous	-	nonsynonymous SNV	ODZ4:uc001ozl.4:exon28:c.G5068A:p.G1690R
	TENM4/ ODZ4	NBL48R8cT	chr11	78516464	C	T	11q14.1	heterozygous	-	stopgain SNV	ODZ4:uc001ozl.4:exon15:c.G2052A:p.W684X
	TENM2/ ODZ2	NBL48R8cT	chr5	167645875	C	T	5q34	heterozygous	-	nonsynonymous SNV	ODZ2:uc010jje.3:exon12:c.C2744T:p.T915I,ODZ2:uc003lzt.4:exon14:c.C3071T:p.T1024I,ODZ2:uc003lzr.4:exon19:c.C4262T:p.T1421I,ODZ2:uc010jjd.3:exon23:c.C4952T:p.T1651I
	TENM1/ ODZ1	NBL12E3	chrX	123556151	T	C	Xq25	heterozygous	-	nonsynonymous SNV	ODZ1:uc004euj.3:exon23:c.A4421G:p.K1474R,ODZ1:uc010nqy.3:exon24:c.A4442G:p.K1481R,ODZ1:uc011muj.2:exon24:c.A4439G:p.K1480R
	PRICKLE1	NBL18E2	chr12	42858223	T	G	12q12	heterozygous	-	nonsynonymous SNV	PRICKLE1:uc001rml.3:exon7:c.A1613C:p.D538A, PRICKLE1:uc001rnm.3:exon7:c.A1613C:p.D538A,PRICKLE1:uc010skv.2:exon7:c.A1613C:p.D538A,PRICKLE1:uc010skw.2:exon7:c.A1613C:p.D538A
	PRICKLE1	NBL19R6	chr12	42862582	G	A	12q12	heterozygous	-	nonsynonymous SNV	PRICKLE1:uc001rml.3:exon5:c.C434T:p.A145V,PRICKLE1:uc001rnm.3:exon5:c.C434T:p.A145V,PRICKLE1:uc010skv.2:exon5:c.C434T:p.A145V,PRICKLE1:uc010skw.2:exon5:c.C434T:p.A145V
	PTK7	NBL11E4	chr6	43112282	G	T	6p21.1	heterozygous	-	nonsynonymous SNV	PTK7:uc003oue.1:exon12:c.G1955T:p.R652L,PTK7:uc003ouc.1:exon14:c.G2177T:p.R726L,PTK7:uc003oud.1:exon14:c.G2225T:p.R742L,PTK7:uc003oub.1:exon15:c.G2345T:p.R782L,PTK7:uc011dve.1:exon15:c.G2369T:p.R790L
	RHO	NBL3E2	chr3	129251238	G	C	3q22.1	heterozygous	-	nonsynonymous SNV	RHO:uc003emt.3:exon3:c.G675C:p.Q225H
	ROCK2	NBL52R2	chr2	11357243	G	A	2p25.1	heterozygous	-	nonsynonymous SNV	ROCK2:uc002rbd.1:exon12:c.C1385T:p.A462V
	TRIOBP	NBL3E2	chr22	38119518	A	C	22q13.1	heterozygous	-	nonsynonymous SNV	TRIOBP:uc003ats.1:exon5:c.A439C:p.T147P,TRIOBP:uc003atu.3:exon5:c.A439C:p.T147P,TRIOBP:uc003atr.3:exon7:c.A955C:p.T319P,TRIOBP:uc003atq.1:exon13:c.A955C:p.T319P

Table S2. Summary of non silent mutation count in neuroblastoma in our won sequenced material and in published cohorts (n=383).

	Gene	Non silent mutations	Overall mutation frequency	Type of alteration	Ref
GEFS	AKAP13	2	0.52%	mis (2)	2
	ALS2	2	0.52%	mis (1), struct (1)	1, 2
	ARHGEF4	1	0.26%	mis (1)	2
	ARHGEF7	1	0.26%	struct (1)	4
	ARHGEF9	1	0.26%	mis (1)	2
	ARHGEF12	2	0.52%	mis (1), struct (1)	1, 2
	ARHGEF17	2	0.52%	mis (2)	2
	ARHGEF37	1	0.26%	mis (1)	4
	ARHGEF40	1	0.26%	mis (1)	3
	DNMBP	2	0.52%	mis (2)	2
	ECT2L	1	0.26%	mis (1)	2
	FGD4	1	0.26%	mis (1)	2
	FGD6	2	0.52%	mis (2)	2
	KALRN	1	0.26%	mis (1)	2
	MCF2L	1	0.26%	mis (1)	2
	MCF2L2	1	0.26%	fs (1)	2
	NGEF	1	0.26%	mis (1)	2
	OBSCN	5	1.31%	fs (2), mis (3)	2
	PLEKHG2	1	0.26%	mis (1)	2
	PLEKHG3	1	0.26%	fs (1)	2
	PREX1	1	0.26%	mis (1)	2
	PREX2	1	0.26%	mis (1)	2
	SOS1	1	0.26%	fs (1)	1
	SPATA13	2	0.52%	mis (2)	1,2
	TIAM1	4	1.04%	mis (3), stop (1)	1, 4
	TIAM2	1	0.26%	mis (1)	2
	TRIO	6	1.57%	struct (2), fs (1), mis (3)	1, 2
	VAV1	1	0.26%	mis (1)	3
	VAV2	1	0.26%	mis (1)	2
	VAV3	2	0.52%	struct (1), mis (1)	1,4
GAPs	ARHGAP10	2	0.52%	mis (2)	1
	ARHGAP15	1	0.26%	struct (1)	2
	ARHGAP20	2	0.52%	mis (2)	2
	ARHGAP23	1	0.26%	struct (1)	1
	ARHGAP25	1	0.26%	mis (1)	2
	ARHGAP26	1	0.26%	mis (1)	1
	ARHGAP31	1	0.26%	mis (1)	2
	ARHGAP32	1	0.26%	mis (1)	2
	ARHGAP4	1	0.26%	splice (1)	2
	ARHGAP5	1	0.26%	fs (1)	2

	ARHGAP6	2	0.52%	fs (1), mis (1)	2
	ARHGAP8	1	0.26%	mis (1)	2
	DLC1	3	0.78%	struct (2), mis (1)	1, 2
	GMIP	1	0.26%	fs (1)	2
	HMHA1	1	0.26%	fs (1)	2
	OPHN1	1	0.26%	splice (1)	2
	SH3BP1	1	0.26%	mis (1)	2
	SRGAP1	1	0.26%	mis (1)	2
	STARD8	1	0.26%	mis (1)	1
	TAGAP	3	0.78%	mis (3)	1, 2
OTHER	ATRX	35	9.14%	stop (2), mis (3), struct (28)	1, 2, 4
	CELSR1	1	0.26%	mis (1)	2
	CELSR2	3	0.78%	mis (2), fs (1)	1, 2
	CELSR3	2	0.52%	mis (1), fs (1)	2
	CSMD1	5	1.31%	mis (2), struct (3)	1, 2, 4
	CSMD2	2	0.52%	struct (1), mis (1)	1, 2
	CSMD3	3	0.78%	struct (2), mis (1)	1, 2, 4
	DAAM1	1	0.26%	mis (1)	2
	DVL2	1	0.26%	fs (1)	2
	FZD10	1	0.26%	mis (1)	2
	FZD2	1	0.26%	mis (1)	2
	FZD5	1	0.26%	mis (1)	4
	FZD6	1	0.26%	mis (1)	2
	FZD7	1	0.26%	mis (1)	2
	ODZ1/TENM1	3	0.78%	mis (3)	2, 4
	ODZ2/TENM2	4	1.04%	mis (1), struct (3)	1, 2, 4
	ODZ3/TENM3	6	1.57%	struct (6)	1, 2
	ODZ4/TENM4	4	1.04%	mis (4)	1,2
	PRICKLE3	1	0.26%	mis (1)	2
	PTPRD	6	1.57%	struct (6)	1, 4
	RND1	1	0.26%	struct (1)	1
	ROCK1	1	0.26%	mis (1)	2
	ROCK2	1	0.26%	mis (1)	2
	ROR2	1	0.26%	mis (1)	1
	SCRIB	1	0.26%	mis (1)	1
	TRIOBP	2	0.52%	mis (2)	2,4
	VANGL1	3	0.78%	mis (3)	2,3
	VANGL2	1	0.26%	mis (1)	2
	WNT5B	1	0.26%	struct (1)	1

Abbreviations; GEFs, guanine nucleotide exchange factors; GAPs, GTPaser Activating proteins; fs, frameshift; mis, missense; stop, stop gain; splice, splice site; struct, structural aberration.

Table S3. Genomic profile of the investigated neuroblastoma patients cohorts, in tumors with at least one somatic mutation in with Rho/Rac/ATRX associated gene, Rho/Rac associated gene and in the whole cohorts.

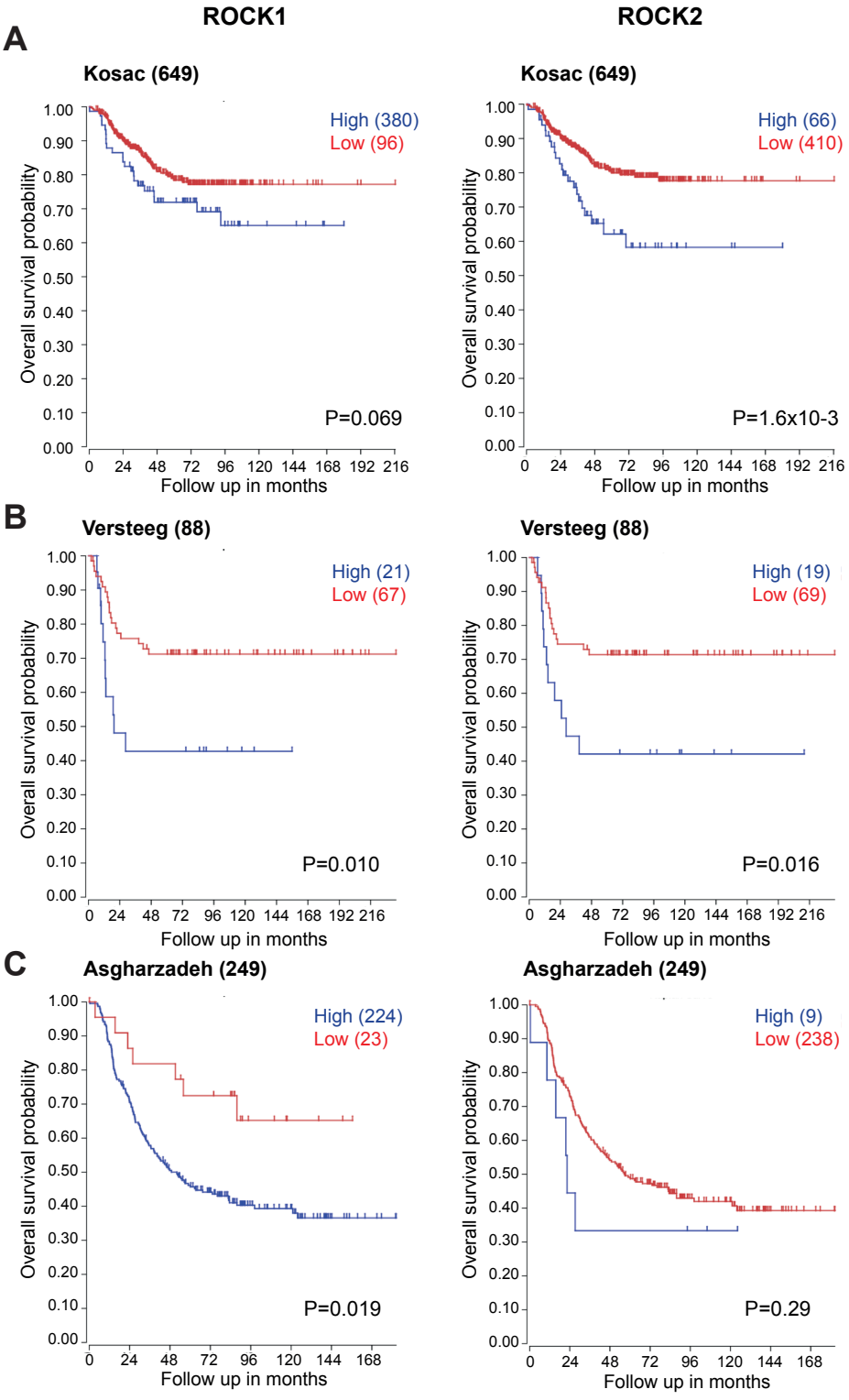
Set	(n)	Tumors with non silent mutations in genes in the Rho/Rac/ATRX pathway	Genomic profile of tumors with mutations in genes in the Rho/Rac/ATRX pathway			Tumors with non silent mutations in genes in the Rho/Rac* pathway	Genomic profile of tumors with mutations in genes in the Rho/Rac* pathway			Genomic profile of all tumors in the cohorts		
			11q-del	MYCN-amp	Other[#]		11q-del	MYCN-amp	Other[#]	11q-del	MYCN-amp	Other[#]
Pugh et al. 2013	240	71 (30%)	36 (51%)	17 (24%)	18 (25%)	55 (23%)	21 (38%)	17 (31%)	17 (31%)	103 (43%)	77 (32%)	60 (25%)
Molenaar et al. 2012	87	29 (33%)	NA	9 (31%)	NA	27 (31%)	NA	9 (33%)	NA	NA	23 (26%)	NA
Sausen et al. 2013	16	4 (25%)	1 (25%)	3 (75%)	0	4 (25%)	1 (25%)	3 (75%)	0	NA	8 (50%)	NA
Our material	40	13 (32.5%)	8 (62%)	2 (15%)	3 (23%)	11 (27.5%)	6 (55%)	2 (18%)	3 (27%)	16 (40%)	13 (32%)	11 (28%)
All	383	117 (30.5%)	45 (51%)	31 (26%)	21 (24%)	97 (25%)	27 (45%)	30 (31%)	20 (33%)	119 (42%)	121 (32%)	71 (25%)

**Alterations in Rho/Rac associated genes excluding ATRX;*
 # This group includes tumors with genomic signatures corresponding to 17q gain, other structural or numerical only profiles

Table S4. All Rho/Rac associated genes investigated in the analyses.

GEFS	ABR	ARHGEF15	ECT2	MCF2L2	TIAM2
	AKAP13	ARHGEF16	ECT2L	NET1	TRIO
	ALS2	ARHGEF17	FARP1	NGEF	VAV1
	ARHGEF1	ARHGEF18	FARP2	OBSCN	VAV2
	ARHGEF2	ARHGEF19	FGD1	PLEKHG1	VAV3
	ARHGEF3	ARHGEF25	FGD2	PLEKHG2	
	ARHGEF4	ARHGEF26	FGD3	PLEKHG3	
	ARHGEF5	ARHGEF33	FGD4	PREX1	
	ARHGEF6	ARHGEF34P	FGD5	PREX2	
	ARHGEF7	ARHGEF35	FGD6	RASGRF1	
	ARHGEF9	ARHGEF37	ITSN1	RASGRF2	
	ARHGEF10	ARHGEF38	ITSN2	SOS1	
	ARHGEF10L	ARHGEF40	KALRN	SOS2	
	ARHGEF11	BCR	MCF2	SPATA13	
	ARHGEF12	DNMBP	MCF2L	TIAM1	
GAPs	ARHGAP1	ARHGAP20	ARHGAP30	ARHGAP5	HMHA1
	ARHGAP10	ARHGAP21	ARHGAP31	ARHGAP6	OPHN1
	ARHGAP11A	ARHGAP22	ARHGAP32	ARHGAP8	SH3BP1
	ARHGAP11B	ARHGAP23	ARHGAP35	ARHGAP9	SRGAP1
	ARHGAP12	ARHGAP24	ARHGAP36	CHN1	SRGAP2
	ARHGAP15	ARHGAP25	ARHGAP39	CHN2	SRGAP3
	ARHGAP16P	ARHGAP26	ARHGAP4	DLC1	STARD13
	ARHGAP17	ARHGAP27	ARHGAP40	FAM13A	STARD8
	ARHGAP18	ARHGAP28	ARHGAP42	FAM13B	TAGAP
	ARHGAP19	ARHGAP29	ARHGAP44	GMIP	
OTHER	ATRX	FZD1	TENM4/ODZ4	RHOBTB3	ROCK1
	CDC42	FZD10	PRICKLE1	RHOC	ROCK2
	CELSR1	FZD2	PRICKLE2	RHOD	ROR1
	CELSR2	FZD3	PRICKLE3	RHOF	ROR2
	CELSR3	FZD4	PTK7	RHOG	SCRIB
	CSMD1	FZD5	PTPRD	RHOH	TRIOBP
	CSMD2	FZD6	RAC1	RHOJ	VANGL1
	CSMD3	FZD7	RAC2	RHOQ	VANGL2
	DAAM1	FZD8	RAC3	RHOU	WNT11
	DAAM2	FZD9	RHOA	RHOV	WNT5A
	DVL1	TENM1/ODZ1	RHOB	RND1	WNT5B
	DVL2	TENM2/ODZ2	RHOBTB1	RND2	
	DVL3	TENM3/ODZ3	RHOBTB2	RND3	

Abbreviations; GEFs, guanine nucleotide exchange factors; GAPs, GTPaser Activating proteins


ROCK1
ROCK2
A
Kosac (649)
High (380)
Low (96)
P=0.069
Kosac (649)
High (66)
Low (410)
P=1.6x10-3
Overall survival probability
Follow up in months
B
Versteeg (88)
High (21)
Low (67)
P=0.010
Versteeg (88)
High (19)
Low (69)
P=0.016
C
Asgharzadeh (249)
High (224)
Low (23)
P=0.019
Asgharzadeh (249)
High (9)
Low (238)
P=0.29

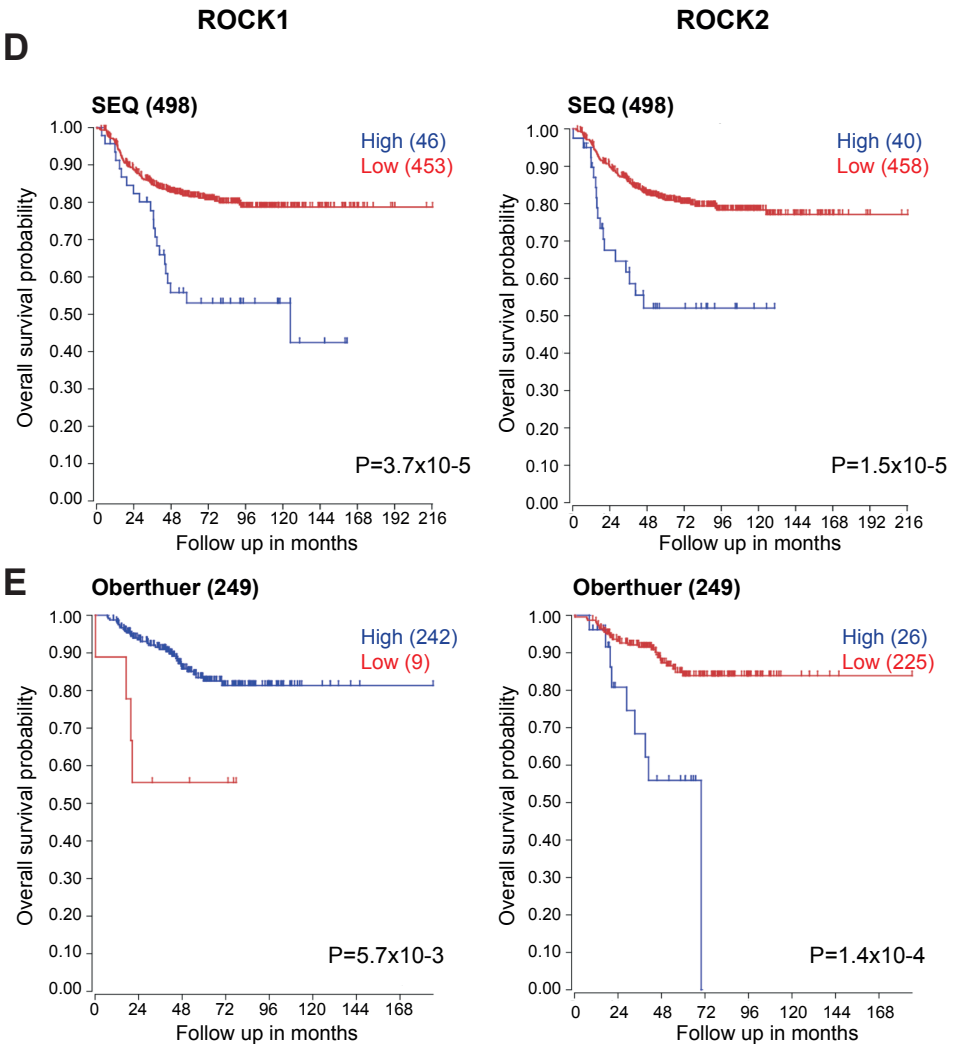


Fig. S1. Kaplan-Meier survival estimates of high/low *ROCK1* and *ROCK2* mRNA expression from five different neuroblastoma expression cohorts. *P* values from one-way ANOVA with Bonferroni post test. Data from R2: microarray analysis and visualization platform (http://r2.amc.nl)

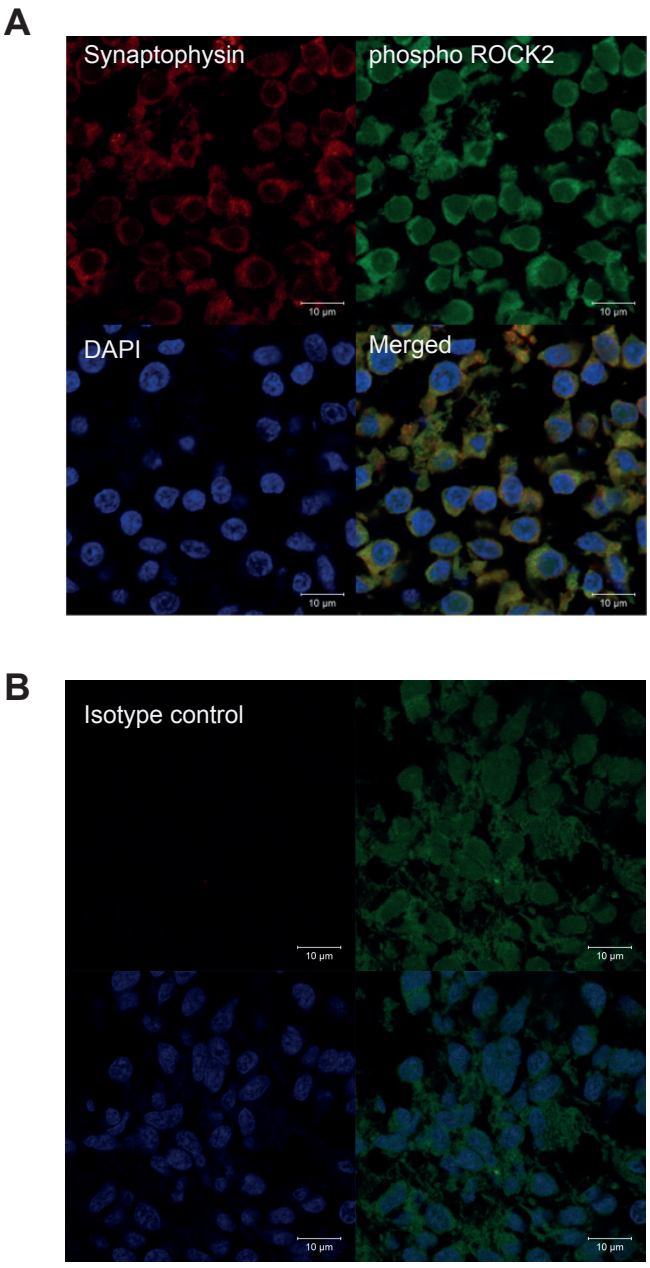


Fig. S2. Neuroblastoma tissue samples express activated ROCK2 in tumor cells. (*A*) Immunofluorescence staining of neuroblastoma tissue showing synaptophysin (red), phosphorylated ROCK2 (green), DAPI (blue) and overlay (yellow). (*B*) Isotype control.

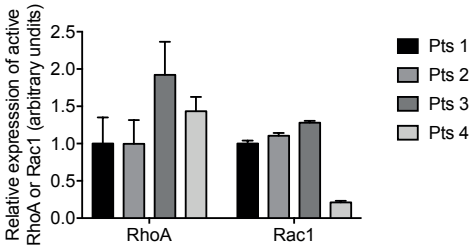


Fig. S3. Relative expression of active RhoA and Rac1 protein in human neuroblastoma samples. Showed as ratio of positive control and normalized to patient sample 1. Investigated by ELISA, in tumor material from the same four neuroblastoma samples as shown in Fig. 1. Mean of at least five determinations with S.E.M. are displayed.

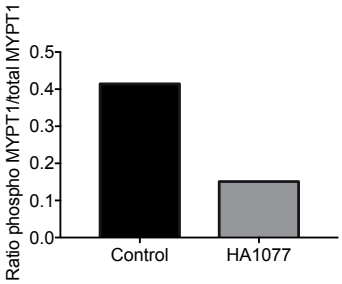


Fig. S4. The expression of phosphorylated MYPT1 was decreased in SK-N-BE(2) cells after treatment with HA1077. Here shown as ratio between phosphorylated MYPT1 and total MYPT1. Protein expression was determined with western blot (shown in Fig. 2*D*) and assessed with densitometry.

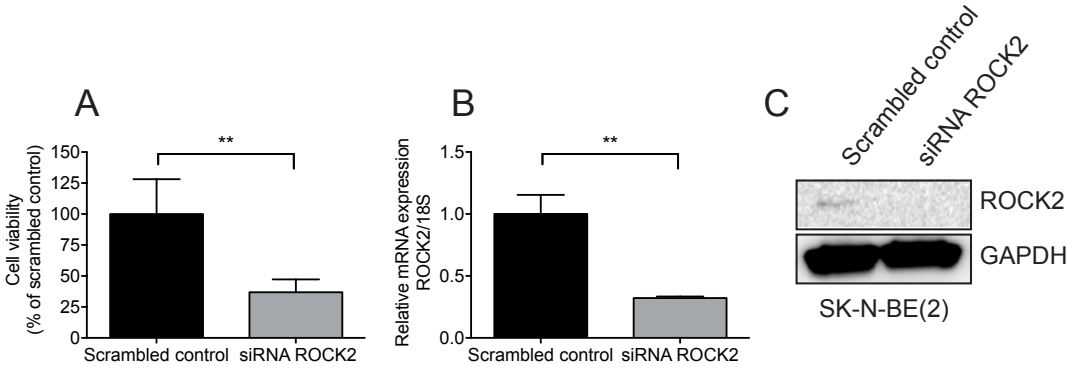


Fig. S5. Downregulating of *ROCK2* in neuroblastoma cells using a second siRNA sequence. (*A*) siRNA mediated inhibition of *ROCK2* suppressed the growth of SK-N-BE(2) neuroblastoma cells. Mean with S.D. is shown. (*B*) Measurement of *ROCK2* mRNA and protein in neuroblastoma cells transfected with *ROCK2* siRNA.

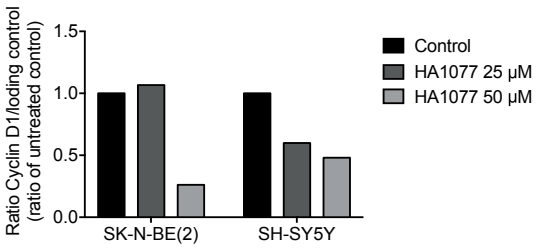


Fig. S6. The protein expression of cyclin D1 in neuroblastoma cells treated with HA1077 Here shown as ratio between cyclin D1 and loading control. Protein expression was determined with western blot (shown in Fig. 3*A*), SK-N-BE(2) at 96 hours and SH-SH5Y at 72 hours, and assessed with densitometry.

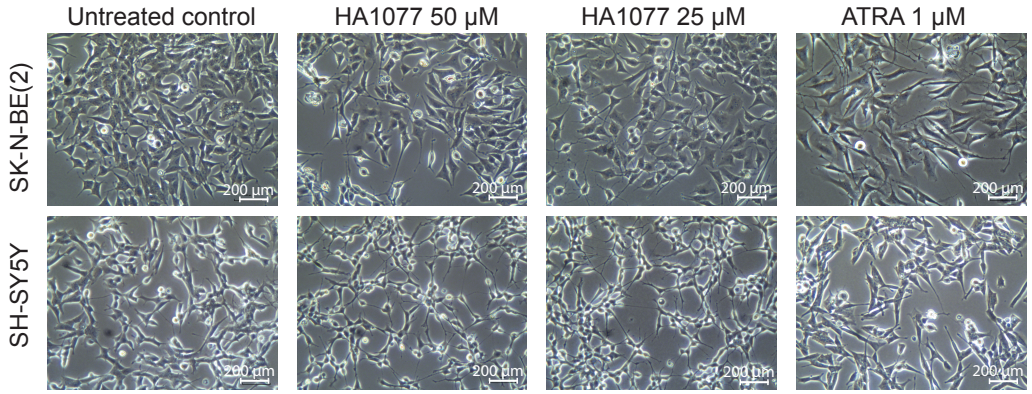


Fig. S7. Morphological differentiation of SK-N-BE(2) in response to HA1077 (50 µM, 72 hours) and retinoic acid (ATRA, 1 µM, 72 hours). Images were acquired using a phase contrast microscope (Nikon eclipse TS100, 20X objective). The experiment was repeated three times.

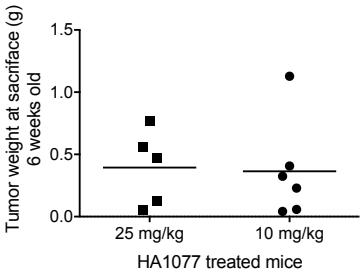


Fig. S8. Mice homozygous for the *MYCN* transgene (TH-*MYCN*$^{+/+}$) treated with two different doses of HA1077, 25 mg/kg (n=5) and 10 mg/kg (n=6) for ten days, respectively. The two HA1077 doses investigated did not differ in effect on tumor growth inhibition (mean 0.39 g vs. 0.36 g, t-test *P*=0.90). Individual tumor weights are shown, with a line representing the mean.

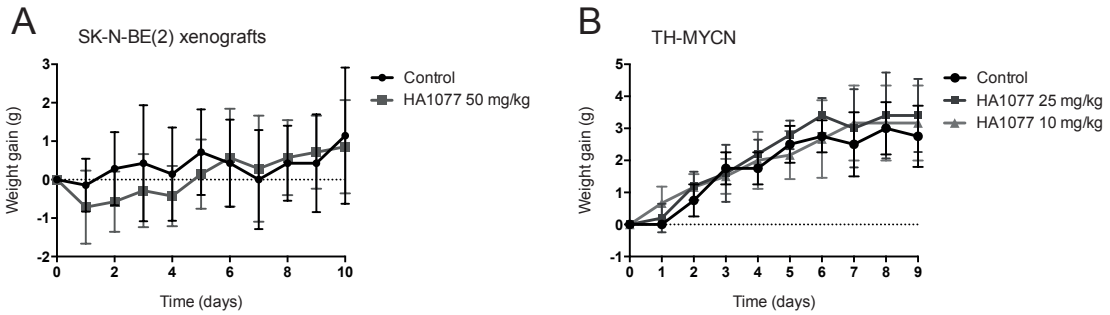


Fig. S9. Effect of HA1077 treatment on mouse body weight. No differences in weight gain for mice treated with HA1077 compared to untreated control mice were detected during the in vivo experiments. (*A*) Weight gain in nude mice engrafted with human SK-N-BE(2) cells treated with 50 mg/kg HA1077 (n=7) or vehicle (n=7) (day 10, end of treatment, t-test P=0.73, two-way ANOVA *P*=0.59 in regard to treatment). (*B*) Weight gain in TH-*MYCN*$^{+/+}$ mice treated with either HA1077 (25 or 10 mg/kg) or no treatment (day 9, end of treatment, one-way ANOVA *P*=0.75; two-way ANOVA *P*=0.75 for treatments). Mean with S.D. are displayed (A, B).

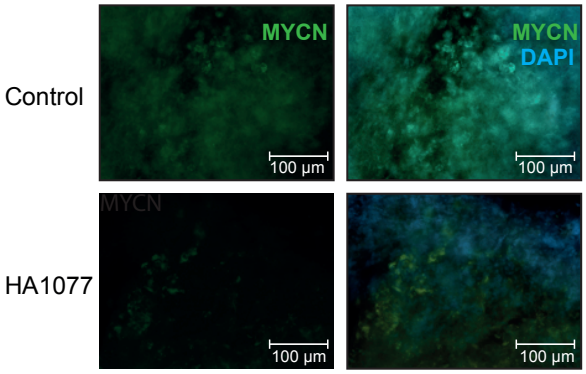


Fig. S10. MYCN expression in organotypic cultures of mice neuroblastomas. Tumors were sliced, put on insert membranes and grown *ex vivo* as organotypic cultures for 7 days before immunostaining for MYCN. TH-*MYCN*$^{(+/+)}$ tumors exposed to HA1077 (50 µM, 7 days) and non-treated tumors showed a marked inhibition of MYCN protein (images to the left: green staining for MYCN; to the right: with green MYCN staining and blue DAPI staining for nucleus, 20X).

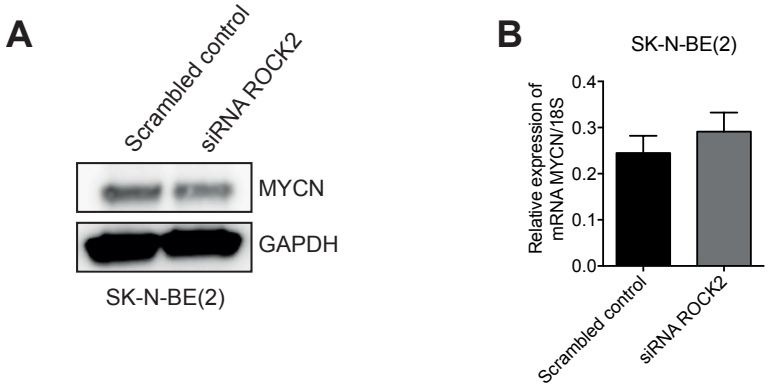


Fig. S11. *ROCK2* regulate MYCN protein levels in neuroblastoma cells. (*A, B*) SK-N-BE(2) neuroblastoma cells were transiently transfected with an alternative siRNA sequence targeting ROCK2. siRNA-mediated inhibition of *ROCK2* expression resulted in decreased MYCN protein levels (determined with western blotting) (A) but without affecting the mRNA levels of MYCN (studied with quantitative PCR) (B). Mean with S.D. of three determinations is presented.

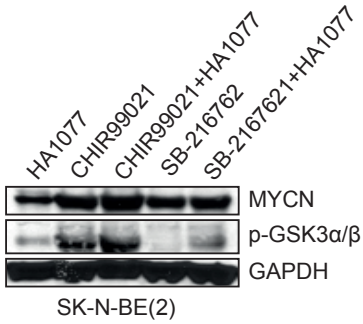


Fig. S12. GSK3β activity regulates MYCN protein levels in neuroblastoma. Western blot analysis demonstrating that the small-molecule GSK3β inhibitors, CHIR99021 and SB-216763 for 48 hours increase GSK3β phosphorylation (ser9) and elevated MYCN protein levels in combination with HA1077, SK-N-BE(2) neuroblastoma cells.

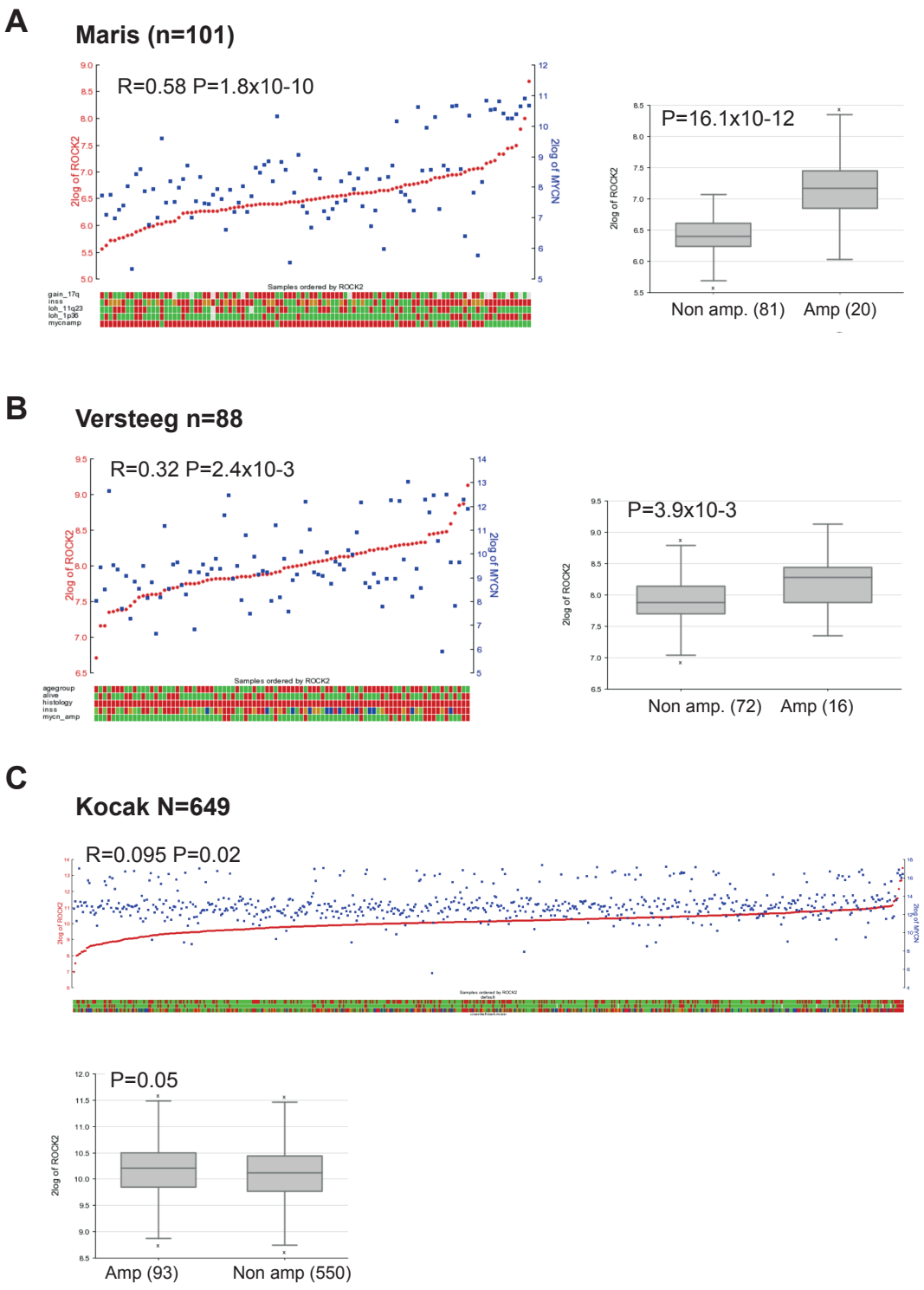


Fig. S13. *ROCK2* and *MYCN* expression is correlated in neuroblastoma tissue samples. A significant correlation between *ROCK2* and *MYCN* expression was observed throughout different public available and validated microarray datasets of neuroblastoma samples; (*A*) Maris dataset R=0.58, *P*=1.8x10^{-10}, n=101; (*B*) Versteeg dataset R=0.32, *P*=0.0024, n=88 and (*C*) Kocak dataset R=0.095, *P*=0.02, n=649. Also, *ROCK2* expression was significant higher in *MYCN*

amplified samples; (*A*) Maris dataset $P=16.1x10^{-12}$, n=101; (*B*) Versteeg dataset *P*=0.0039, n=88 and (*C*) Kocak dataset *P*=0.01, n=471. Analyses were done in R2: microarray analysis and visualization platform (http://r2.amc.nl).