Supplementary Figure legends

Supplementary Figure 1. *In vitro* activity screen of PLK1 siRNA sequences. Activity of native PLK1 siRNA sequences targeting human PLK1 mRNA were assessed in the HT29 cell viability assay. Cells were treated with SNALP formulated PLK1 or Luc siRNA at 1 nM (white bar), 5 nM (grey bar), and 25 nM (black bar). Cell viability was assessed at 72 h using CellTiter Blue resazurin dye. Two rounds of siRNA design (A & B, C) were conducted. Sequence numbers represent siRNA target site in hPLK1 mRNA open reading frame (refseq NM_005030)

Supplementary Figure 2. *In vivo* characterization of the interferon response induced by SNALP-formulated siRNA. **a**) Time course for the induction of serum IFNα and liver IFIT1 mRNA after i.v. administration of SNALP formulated native (unmodified) ApoB1 siRNA. Balb/c mice (n = 4 per group) were administered 2.5 mg/kg siRNA or lipid vehicle; serum IFNα (pg/mL) and IFIT1 mRNA (relative to GAPDH) from whole liver lysates were assessed after 4, 8, and 16 h by ELISA and bDNA assay respectively. **b,c**) Measurement of IFIT1 mRNA induction in target tissues can resolve residual immunostimulatory activity within siRNAs. Mice were treated with the native ApoB-1 siRNA or ApoB-1 siRNAs containing selective 2OMe nucleotides in either the sense (S) strand or both strands (S+AS). **b**) Serum IFNα and **c**) liver IFIT1 mRNA were assessed 4 h after administration (mean + SD, n = 4). Residual immunostimulatory activity, in the absence of systemic cytokine induction was evident by IFIT1 mRNA induction in

ApoB-1 2'OMe(S) treated mice. This response was fully abrogated by the incorporation of additional 2'OMe nucleotides into the AS strand of the siRNA duplex. All siRNAs retained full RNAi activity. ApoB-1 siRNA (5'-3') sequences: sense - GUCAUCACACUGAAUACCAAU; 2'OMe sense – GUCAUCACACUGAAUACCAAU AS – AUUGGUAUUCAGUGUGAUGACAC; 2'OMe AS – AUUGGUAUUCAGUGUGAUGACAC (2'OMe nucleotides underlined)

Supplementary Figure 3. Detection of the PLK1424-specific and KSP2263-specific mRNA cleavage products and by 5'RACE-PCR *in vitro*. A) HT29 cells were treated with 10 nM SNALP-formulated PLK1424, Luc siRNA or PBS. RNA was isolated 24 hours after transfection and assayed for the specific PLK1 mRNA cleavage product by 5'-RACE-PCR. B) Neuro2a cells were treated with SNALP-formulated KSP2263, PLK773 siRNA or PBS. RNA was isolated 24 hours after transfection and assayed for the specific mouse KSP mRNA cleavage product by 5'-RACE-PCR. The identity of the RNAi specific 476bp PLK1 mRNA and 102bp KSP mRNA cleavage products were confirmed by direct oligonucleotide sequencing (M. Robbins unpublished observation)

Supplementary Figure 4. PLK1424 SNALP confers significant survival advantages in the hepatic Hep3B – nu/nu mouse model. Mice bearing established hepatic tumors were treated with PLK1424-2/A or Luc-U/U SNALP (2 mg/kg twice weekly between d11 and d28 after tumor seeding) and monitored for tumor burden until euthanasia defined by humane endpoints as described in the

accompanying paper. Data represent 2 independent studies. Median survival of PLK1424 vs Luc in Study **A**) = d 45 and d 67 respectively; p = 0.02. and study **B**) = d 42 and undefined respectively, p = 0.008, Log-rank Mantel Cox test. All animals surviving beyond day 80 were found to be tumor free at termination of the study on day 100.

Supplementary Figure 5. PLK1424-2/A SNALP significantly reduces macroscopic tumor burden after completion of dosing. Results are from individual mice described in Figure 8C. Livers from **A**) PBS control and **B**) PLK1424-2/A SNALP treated mice showing macroscopic tumor burden in the left lateral hepatic lobe. **C**) Body weights of individual mice shown in A and B over the duration of the study from day 8 – day 21 after tumor seeding. Loss of body weight directly correlated with tumor burden in individual mice.

Supplementary Figure 6. **PLK1 SNALP is well tolerated in mice**. Groups of CD1 ICR mice were administered PBS, PLK773-1/B or Luc-U/U SNALP to assess potential cumulative toxicities associated with either PLK1 silencing or the lipid vehicle. Mice were treated twice weekly at 2 mg/kg siRNA, equivalent to the efficacious dosing regimen in tumor studies. Clinical chemistry and complete blood counts were evaluated 24 h after dose 5 (day 15) and dose 9 (day 29). siRNA treatment induced no significant changes in **A)** serum liver enzymes Alanine aminotransferase (ALT), aspartate aminotransferase (AST) or sorbitol dehydrogenase (SDH); **B)** Total wbc, lymphocyte or neutrophil counts and **C)**

platelet counts at either 15 or 29 days treatment duration. All values are mean +/- SD (n = 6). No changes in red blood cell parameters were observed (A.Judge unpublished observations).

Supplementary Figure 7. Induction of monoastral spindle formation by KSP2263 siRNA. HeLa cells were treated for 16 h with A) Luc or B) KSP2263 siRNA at 20 nM then immunostained for α-tubulin (FITC, green). DNA was stained with DAPI (blue) and flourescent images captured and overlayed. Control cells show normal bipolar spindles at metaphase compared to monoastral spindles in KSP2263 treated cells.

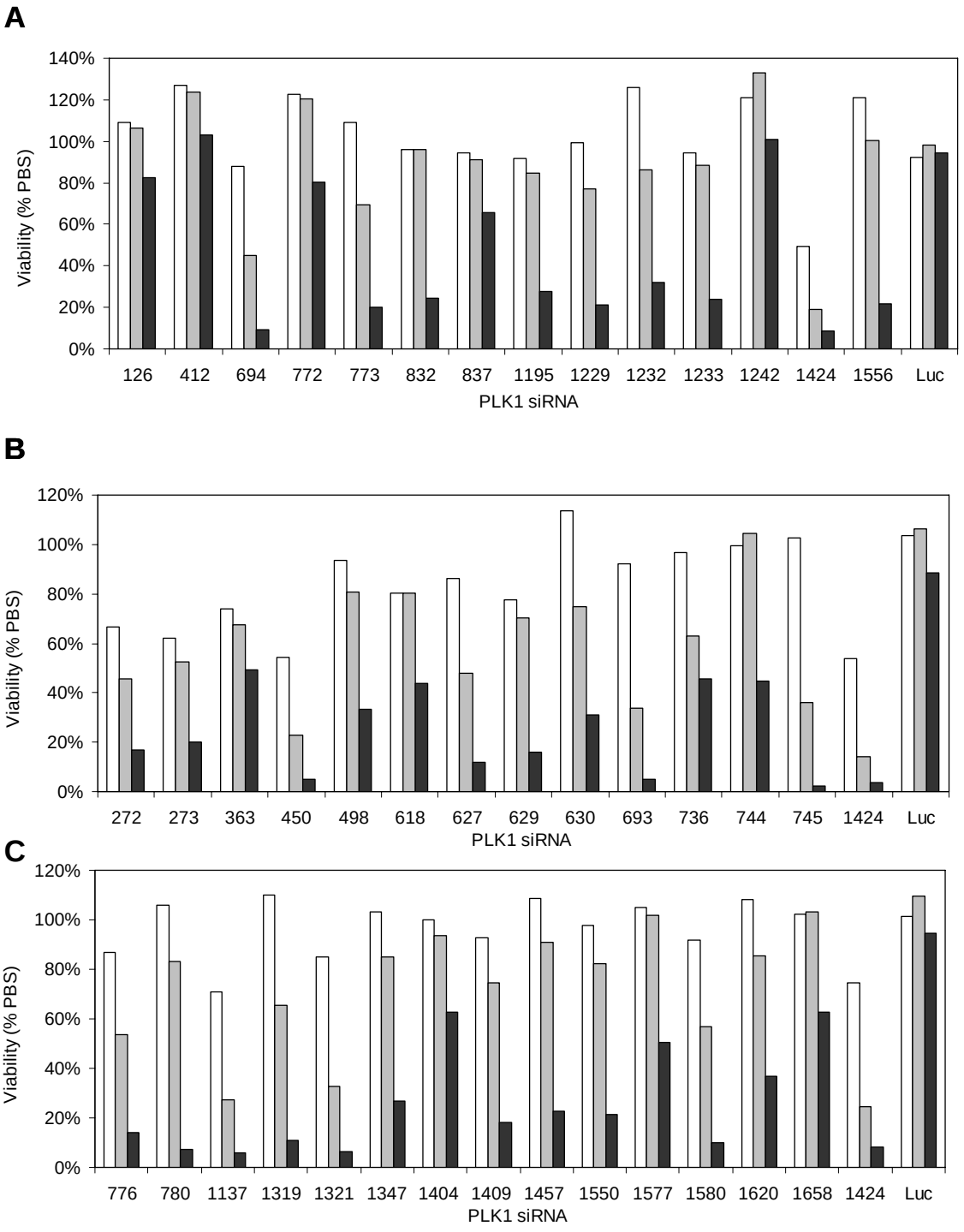
Supplementary Figure 8. Comparison of PLK1424 SNALP comprising either PEG-cDMA or PEG-cDSA in the hepatic tumor model. **A**) Blood clearance of ^{3}H-labelled SNALP(according to Judge *et al.* 2006 Mol Ther. 13: 328-337) comprising either PEG-cDMA or PEG-cDSA following IV administration in mice. Data are expressed as mean % injected dose (+/- SD, n = 4) remaining in whole blood at 0.25, 0.5, 1, 2, 4 and 8 h after injection. **B**) PLK1 mRNA silencing in hepatic Hep3B tumors 24 h after single 2 mg/kg administration of either PLK1424-2/A SNALP formulations (mean PLK1:GAPDH ration +/- SD, n = 4 mice). **D**) Treatment with PLK1424-2/A SNALP comprising either PEG-cDMA or PEG-cDSA confers significant survival advantages in scid/beige mice bearing intrahepatic Hep3B tumors. Mice were administered PLK1424-2/A SNALP comprising PEG cDMA or PEG-cDSA or Luc-U/U SNALP (PEG-cDMA) at 2

mg/kg twice weekly between d 10 and d 28 after seeding (6 doses). Time to euthanization due to tumor burden was assessed based on clinical scores as a humane surrogate to survival. Both PLK1424-2/A SNALP compositions provided significant survival advantage over control ($p < 0.05$, Log-rank Mantel Cox test).

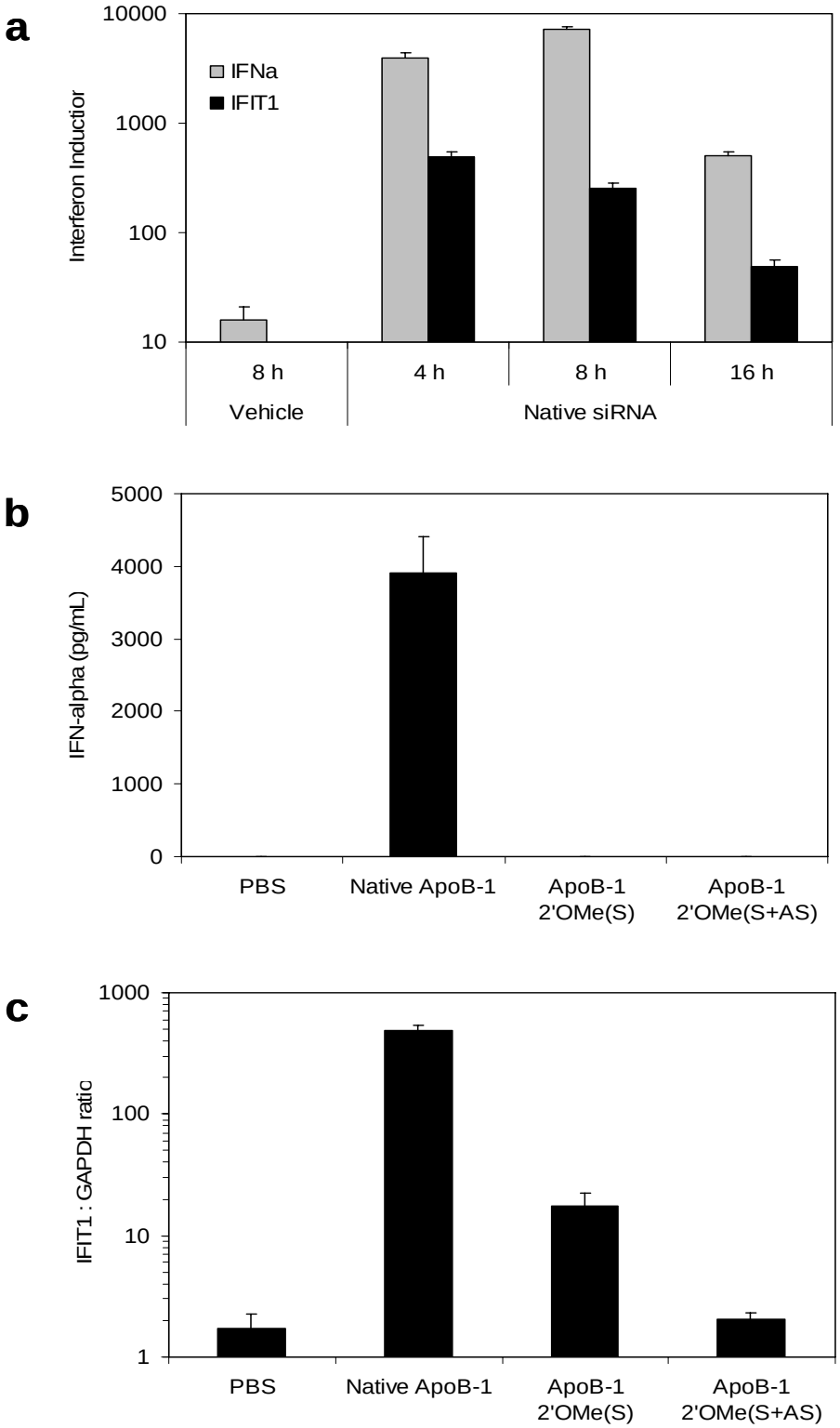
Supplementary Table 1. siRNA sequences targeting human PLK1

Name	Sequence 5'-3'
126	GGUCCUAGUGGACCCACGCUU
272	AGCCGCACCAGAGGGAGAAUU
273	GCCGCACCAGAGGGAGAAGUU
363	GGACAACGACUUCGUGUUCUU
412	CUCCUGGAGCUGCACAAGAUU
450	GCCUGAGGCCCGAUACUACUU
498	CCUGCACCGAAACCGAGUUUU
618	GAGGAAGAAGACCCUGUGUUU
627	GACCCUGUGUGGGACUCCUUU
629	CCCUGUGUGGGACUCCUAAUU
630	CCUGUGUGGGACUCCUAAUUU
693	GGUGGAUGUGUGGUCCAUUUU
694	GUGGAUGUGUGGUCCAUUGUU
736	GUGGGCAAACCACCUUUUGUU
744	ACCACCUUUUGAGACUUCUUU
745	CCACCUUUUGAGACUUCUUUU
772	GAGACCUACCUCCGGAUCAUU
773	AGACCUACCUCCGGAUCAAUU
776	CCUACCUCCGGAUCAAGAAUU
780	CCUCCGGAUCAAGAAGAAUUU
832	GCCGCCUCCCUCAUCCAGAUU
837	CUCCCUCAUCCAGAAGAUGUU
1137	GCAGCUGCACAGUGUCAAUUU
1195	GAGGCUGAGGAUCCUGCCUUU
1229	GGGUCAGCAAGUGGGUGGAUU
1232	UCAGCAAGUGGGUGGACUAUU
1233	CAGCAAGUGGGUGGACUAUUU
1242	GGUGGACUAUUCGGACAAGUU
1319	CACGCCUCAUCCUCUACAAUU
1321	CGCCUCAUCCUCUACAAUGUU
1347	CAGCCUGCAGUACAUAGAGUU
1404	UCCCAACUCCUUGAUGAAGUU
1409	ACUCCUUGAUGAAGAAGAUUU
1424	AGAUCACCCUCCUUAAAUAUU
1457	UGAGCGAGCACUUGCUGAAUU
1550	CCCGCAGCGCCAUCAUCCUUU
1556	GCGCCAUCAUCCUGCACCUUU
1577	GCAACGGCAGCGUGCAGAUUU
1580	ACGGCAGCGUGCAGAUCAAUU
1620	GCUCAUCUUGUGCCCACUGUU
1658	UCGACGAGAAGCGGGACUUUU

Supplementary Figure 1

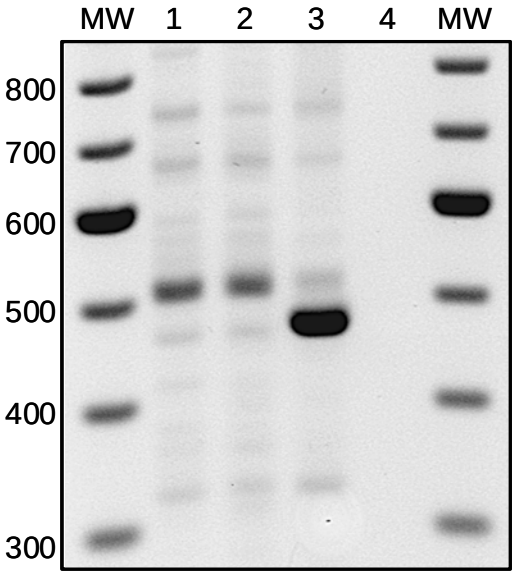


Supplementary Figure 2



Supplementary Figure 3

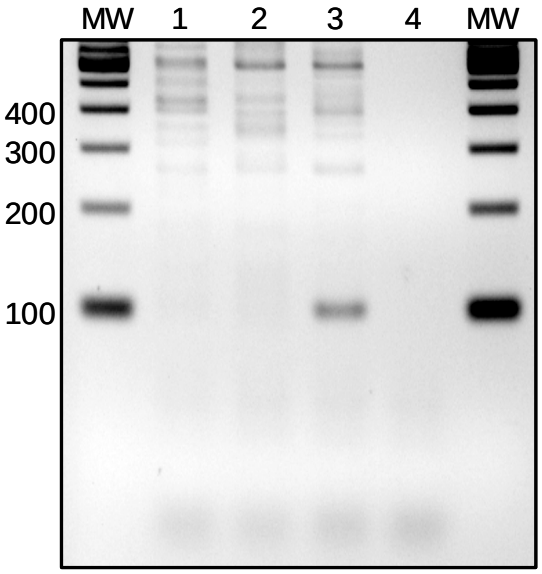
PLK1424-specific RACE-PCR



1. HT29 + PBS
2. HT29 + Luc SNALP
3. HT29 + PLK1424 SNALP
4. No template

MW. 100 bp MW ladder

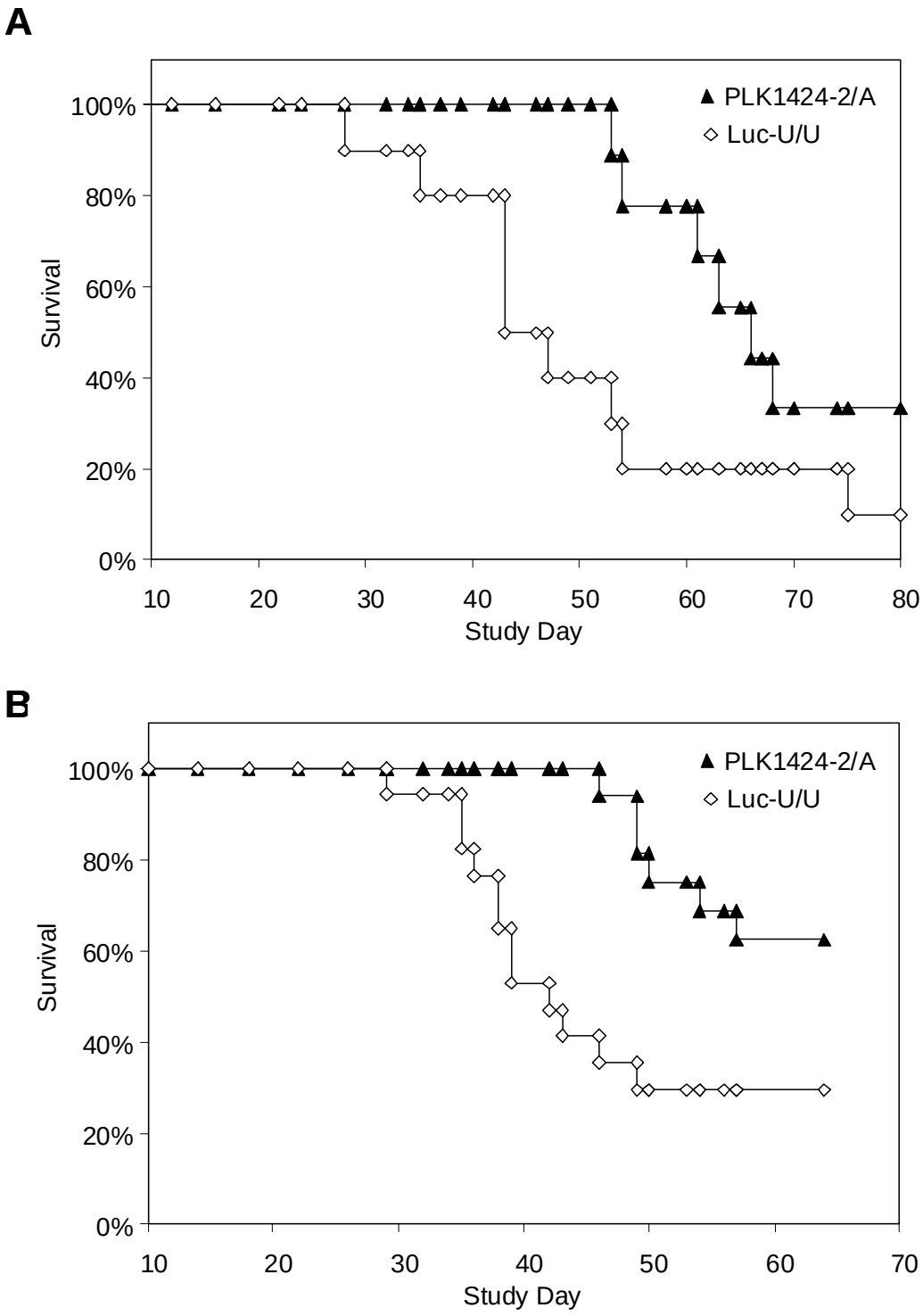
KSP2263-specific RACE-PCR



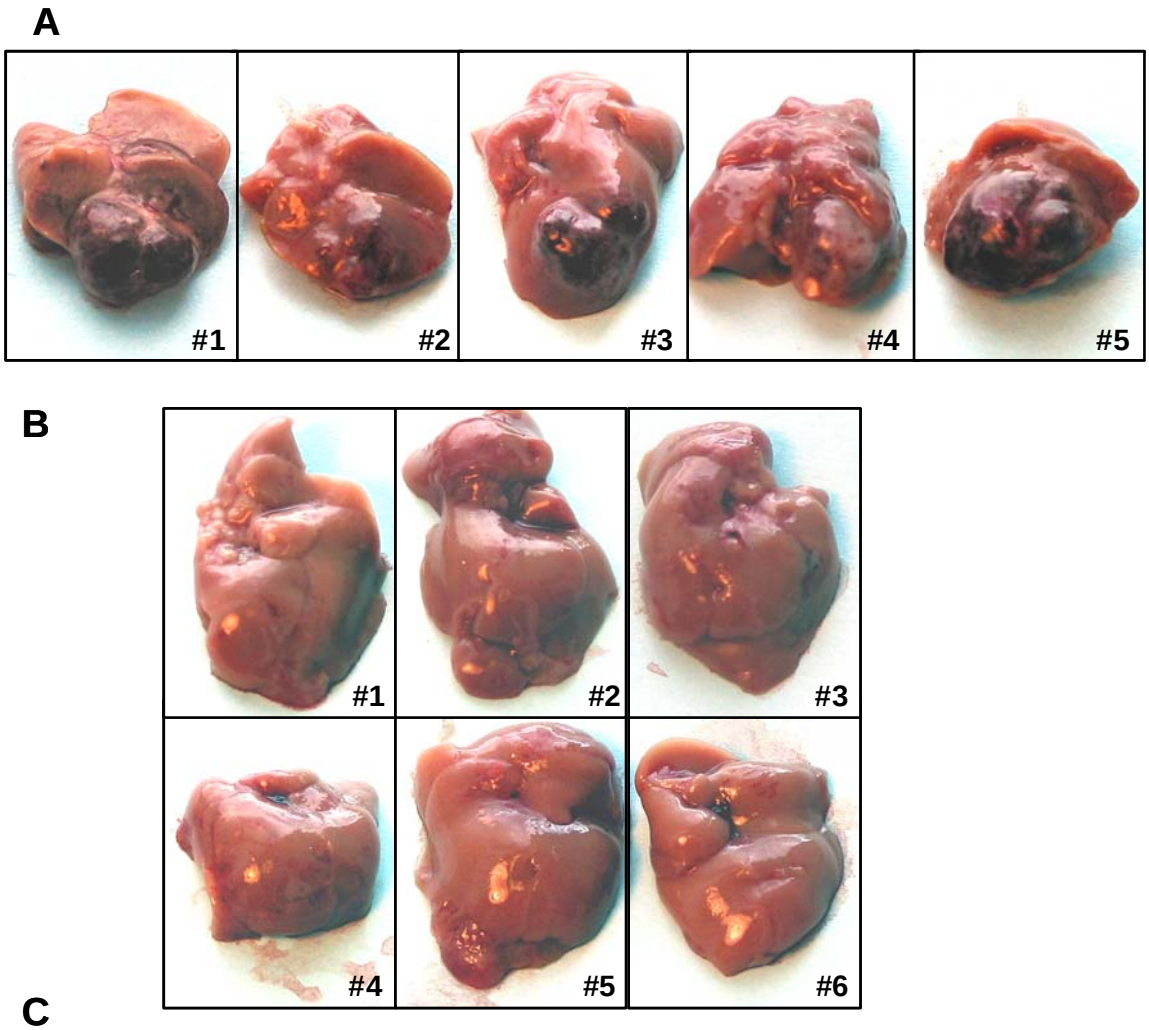
1. Neuro2a + PBS
2. Neuro2a + PLK773 SNALP
3. Neuro2a + KSP2263 SNALP
4. No template

MW. 100 bp MW ladder

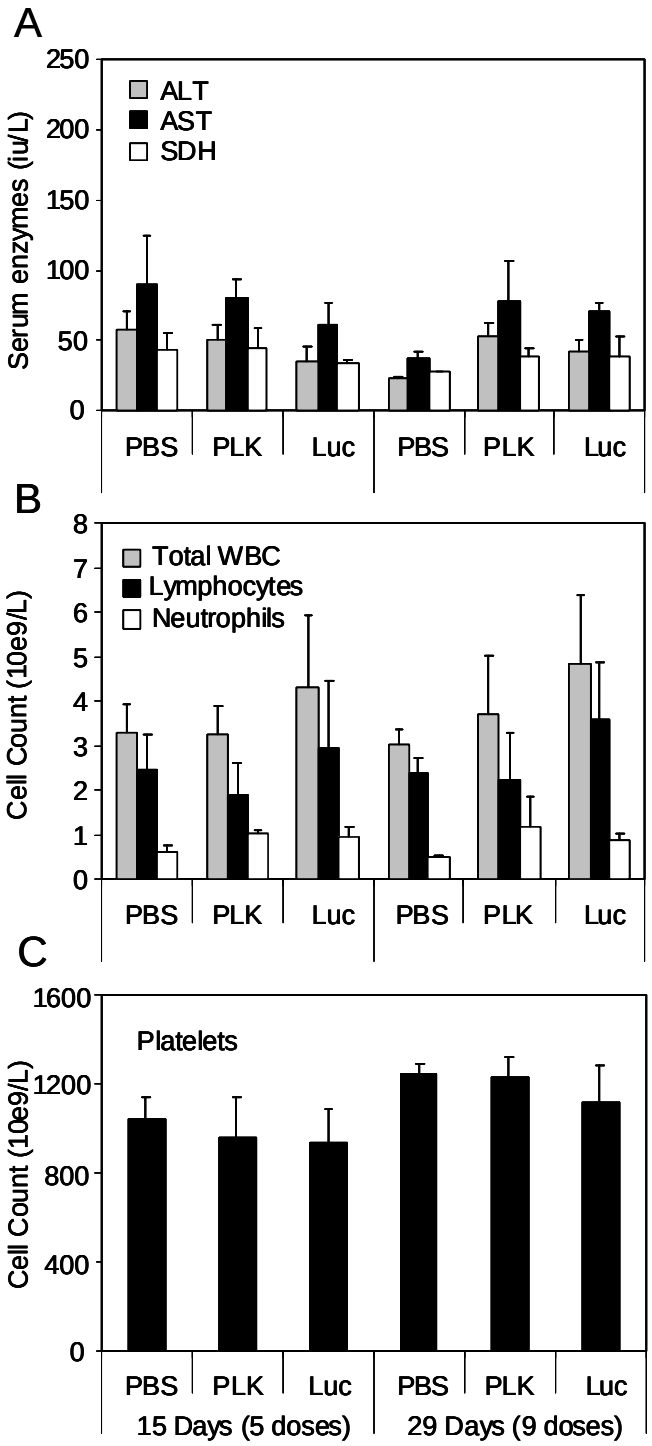
Supplementary Figure 4



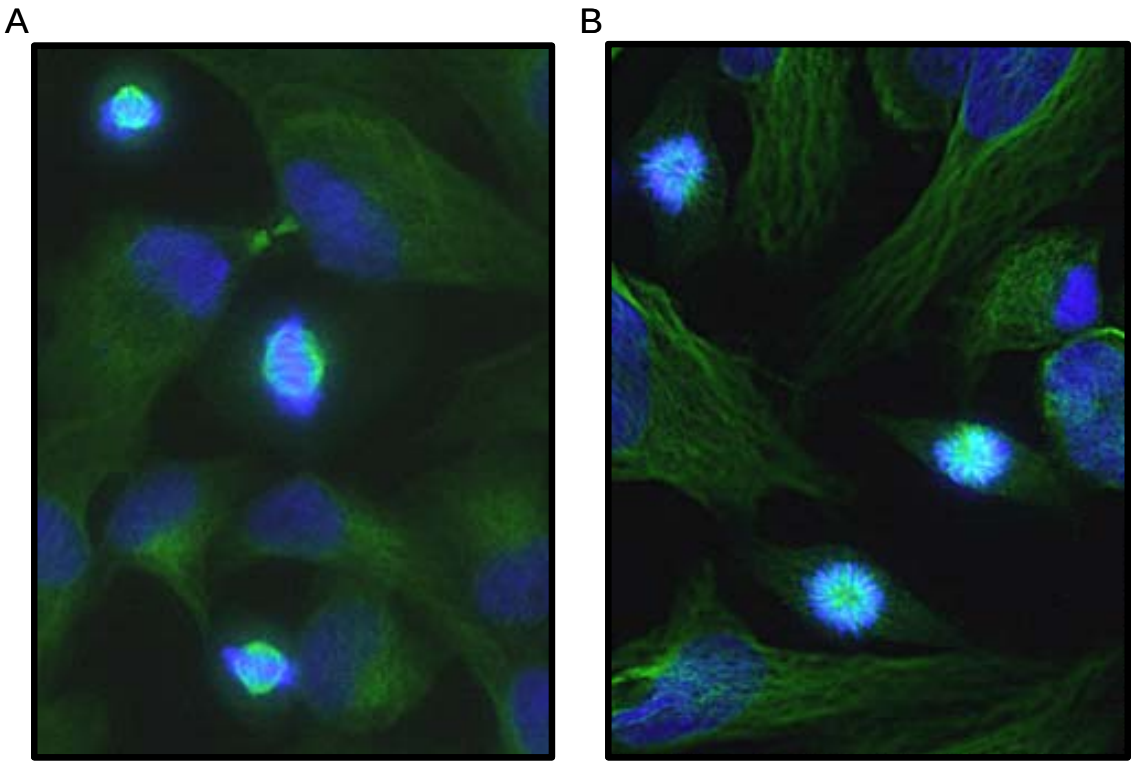
Supplementary Figure 5



Supplementary Figure 6



Supplementary Figure 7



Supplementary Figure 8