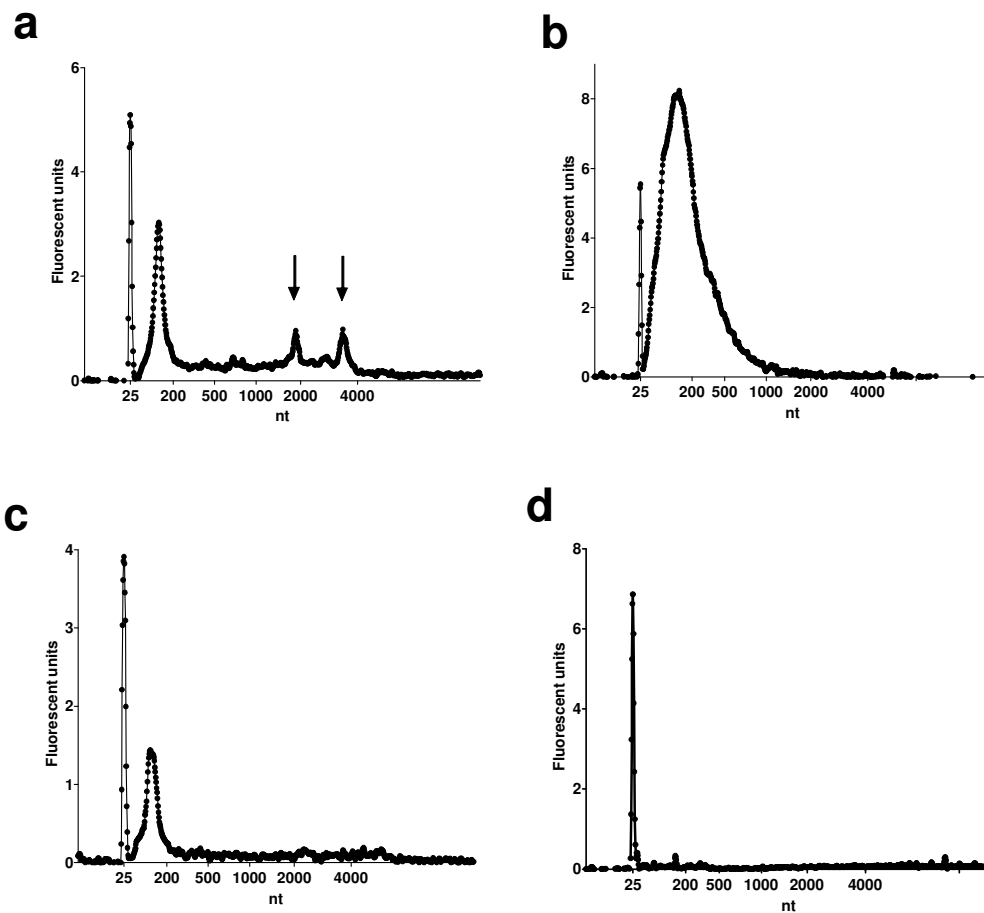# Supplementary Information


Supplementary Figure S1. Bioanalyzer profiles of tumor and normal cell exoRNA and exoDNA. (a) GBM 11/5 exoRNA was analyzed on the Bioanalyzer using an RNA chip, 18S and 28S rRNA peaks are detectable (arrowheads). **(b)** GBM11/5 exoDNA was analyzed on the Bioanalyzer using the RNA Pico Chip. Sizes ranged from 25 – >1000 nt with a peak at 200 nt. **(c)** ExoRNA from human fibroblasts HF19 was extracted and analyzed as in (a). The RNA yield was too low to yield distinct 18S and 28S rRNA. **(d)** ExoDNA from human fibroblasts HF19 was not detectable on the Bioanalyzer even after a 30x concentration.
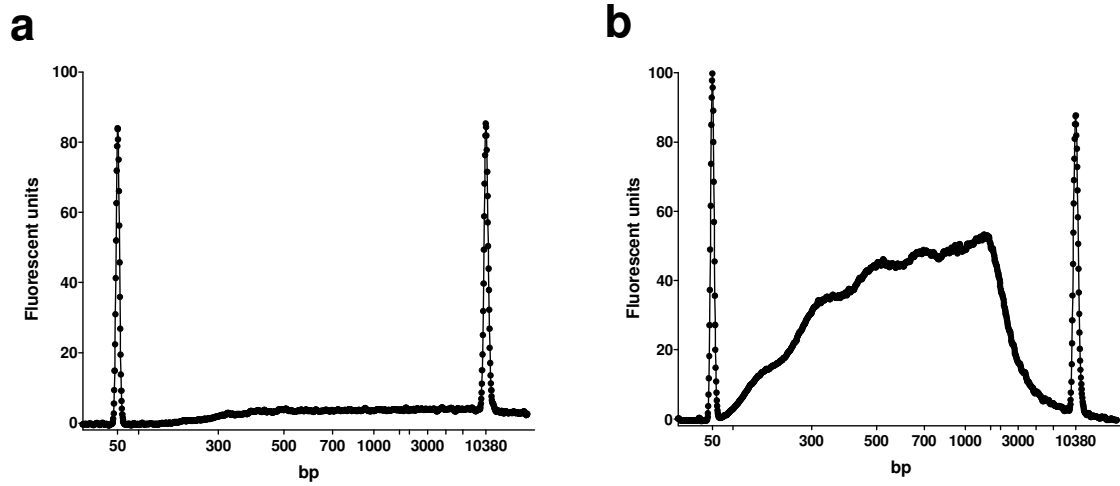
Supplementary Figure S2. **Microvesicles contain single stranded DNA.** DNA was purified from externally DNase-treated microvesicles from medulloblastoma D384 cells. **(a)** exoDNA was not detected using a chip that detects dsDNA. **(b)** The same sample as in (a) was subjected to second strand synthesis with Superscript Double-Stranded cDNA synthesis kit (Invitrogen) according to manufacturer's recommendation after which the DNA was readily detected.
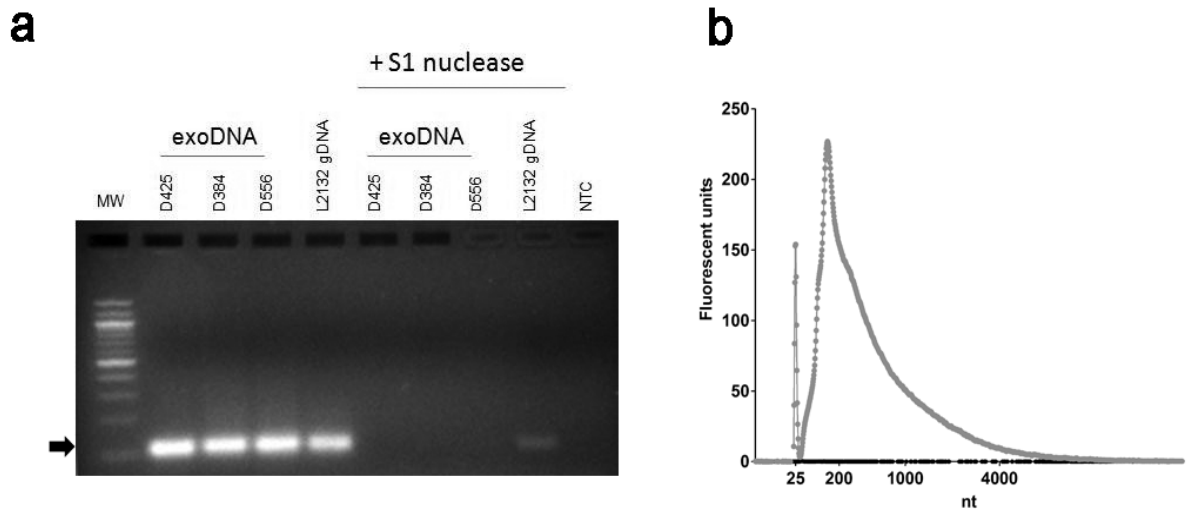
Supplementary Figure S3. exoDNA is sensitive to S1 nuclease. ExoDNA was extracted from three medulloblastoma cell lines (D425, D384 and D556) and gDNA was extracted from L2132 normal fibroblasts as a control double stranded DNA. The samples were mock treated or treated with S1 nuclease enzyme which degrades single-stranded nucleic acids. **(a)** Agarose gel electrophoresis of GAPDH PCR products (112 bp, shown by arrow) from the different samples; **(b)** representative bioanalyzer profile (RNA Pico Chip) of exoDNA before (gray line) and after S1 nuclease treatment (black line).
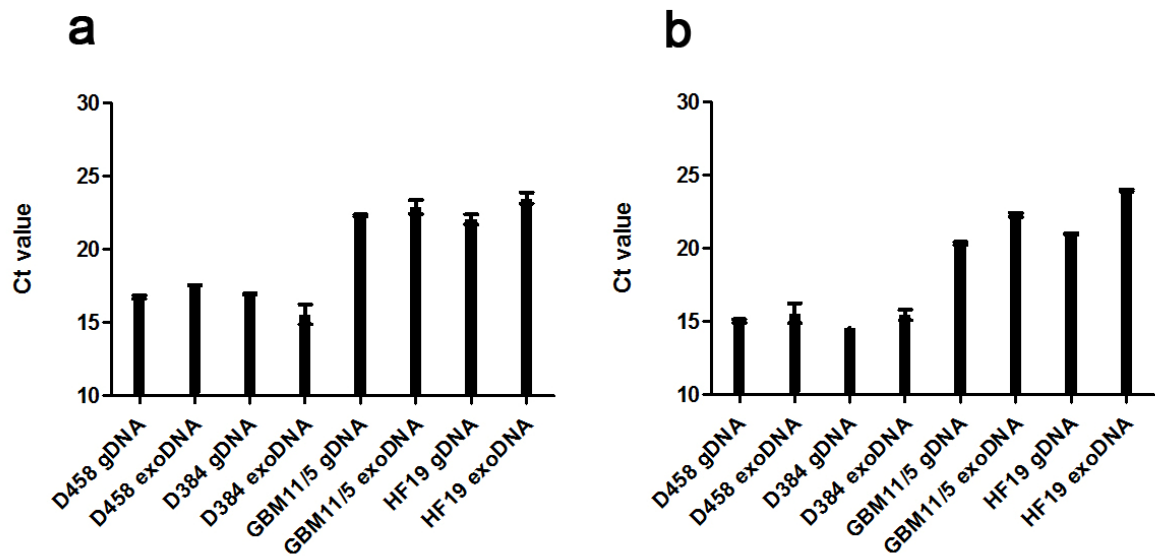
Supplementary Figure S4. **Detection of the c-Myc amplified genomic DNA in microvesicles**. (**a**) c-Myc amplification was detected for medulloblastoma cell lines D458 and D384 at the genomic and exoDNA level, while glioblastomas (11/5) and fibroblasts had lower levels of c-Myc. (**b**) The level of c-Myc amplification at the genomic and exoDNA level was comparable to that of POU5F1B gene sequence (*AF268618*) which is found 319 kb upstream of the c-Myc gene in the genome, but still within the commonly amplified region. Results are expressed as average ±SEM (n=3).
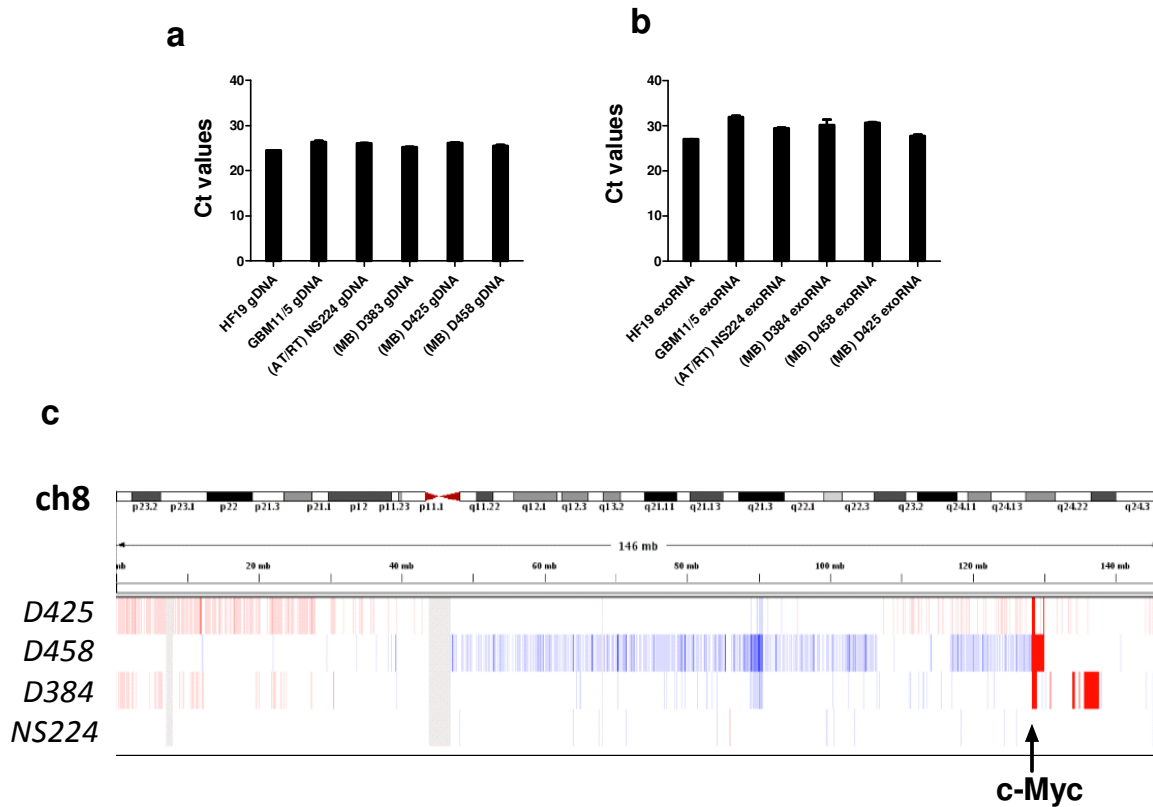
Supplementary Figure S5. Evaluation of c-Myc copy numbers in tumor cell lines and n-Myc levels in cells and microvesicles. (a) No amplification of the n-Myc gene was shown by qPCR in genomic DNA isolated from medulloblastoma, rhabdoid tumor, GBM, and normal fibroblasts; and similar levels **(b)** were found by qRT-PCR for exoRNA derived from their corresponding cells. Results are expressed as average ±SEM (n=3). **(c)** Genomic amplification in the c-Myc genomic region was analyzed in medulloblastoma lines, D425, D458 and D384, as well as rhabdoid tumor line, NS224, using an Affymetrix 250K SNP array.
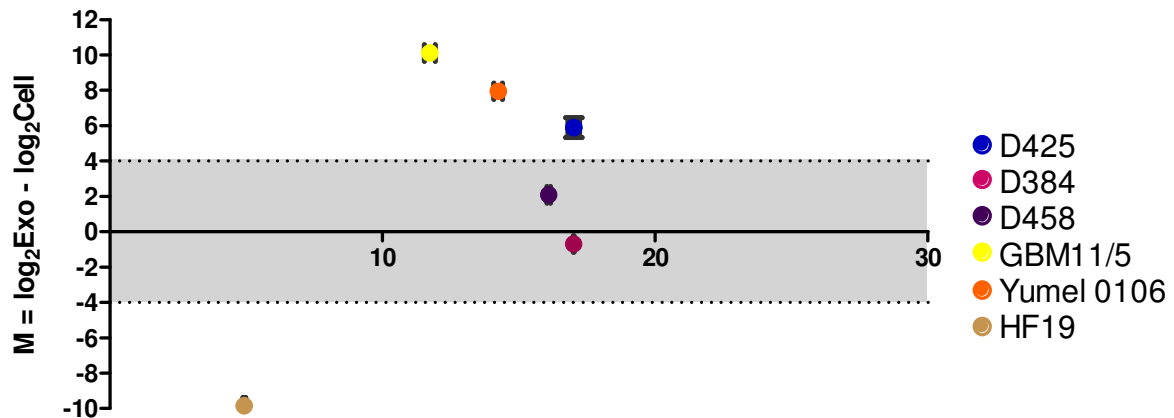
Supplementary Figure S6. HERV-K RNA is abundant and enriched in several tumor cell lines. qRT-PCR was carried out for HERV-K RNA in cell RNA and exoRNA from three medulloblastoma (D425, D384 and D458), one GBM (11/5), one melanoma (0106) and one human fibroblast (HF19) line and the Ct values were normalized to *GAPDH*. The relative levels are presented as MA plots (see Supplementary Methods) ± SEM (n = 3-6). The x-axis represents abundance of HERV-K RNA in the cell lines and the y-axis represents relative microvesicle (y>0) or cellular enrichement (y<0). *Note.* HERV-K RNA was not detectable in exoRNA from normal human fibroblasts (HF19), so it was given a Ct value of 36 (below detection limit). MA plots were generated from the qPCR Ct values that were normalized to the house keeping gene *GAPDH*. Amplification failures were given the default Ct value of 36, since no specific amplification occurred above that cycle. The log ratios of the levels were calculated (Ct values are log2) as M = (36 - CtMicrovesicle) - (36 - CtCell) and A = 0.5 × ((36 - CtMicrovesicle) + (36 - CtCell)).
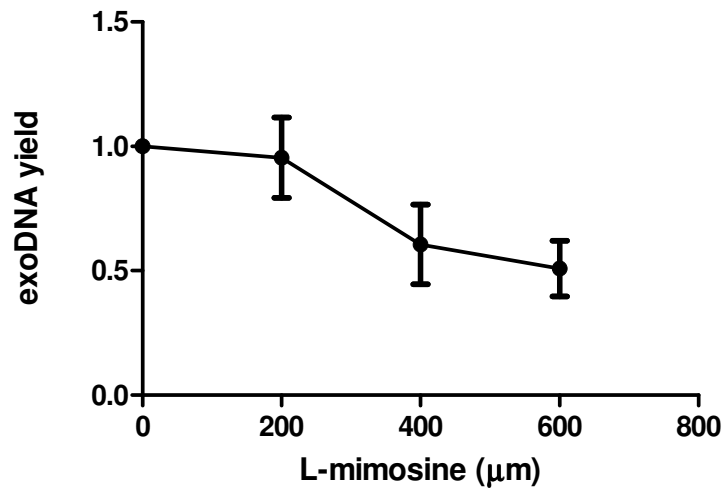
Supplementary Figure S7. Inhibition of DNA replication results in reduced exoDNA yield.
D384 cells (2×10^6 cells/well) were seeded in 6-well plates and treated with increasing dosage of
L-mimosine (200, 400 and 600 µM) (Sigma-Aldrich, St. Louis, MO), an inhibitor of DNA
replication or mock treated. The drug was added at time point one and microvesicles were
isolated from the medium after 48 hrs; ssDNA was extracted using the Qiagen PCR purification
kit. Cell viability was assessed by cell count using the Countess automated cell counter
(Invitrogen). ssDNA yields were quantified using the Bioanalyzer and the yields were compared
to mock treated cells (normalized to 1.0). Results are expressed as average ±SEM (n=3).

Supplementary Table S1. List of PCR primer sequences used.

	Forward	Reverse
n-Myc (exon 2)	TCTACCCGGACGAAGATGAC	AGCTCGTTCTCAAGCAGCAT
c-Myc (exon 3)	TCAAGAGGCGAACACACAAC	TAACTACCTTGGGGGCCTTT
c-Myc (spanning intron 2)	CCTACCCTCTCAACGACAGC	CTCTGACCTTTTGCCAGGAG
c-Myc (exon1; *in vivo*)	CAACCCTTGCCGCATCCAC	AGTCGCGTCCTTGCTCGG
GAPDH	CTCTGCTCCTCCTGTTCGAC	ACGACCAAATCCGTTGACTC
HERV-K6	GGAGAGAAGCTGTCCTGTGG	TGACTGGACTTGCACGTAGG
LINE1	TAAGGGCAGCCAGAGAGAAA	GCCTGGTGGTGACAAAATCT
ALU	CATGTGGGTTAGCCTGGTCT	TTCCCACATTGCGTCATTTA
POU5F1B	ATCCTGGGGGTTCTATTTGG	CTCCAGGTTGCCTCTCACTC