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Supplementary Data

Exploration of neuroblastoma xenograft models for tumor extracellular RNA profiling in murine blood plasma

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1. Supplemental Figures

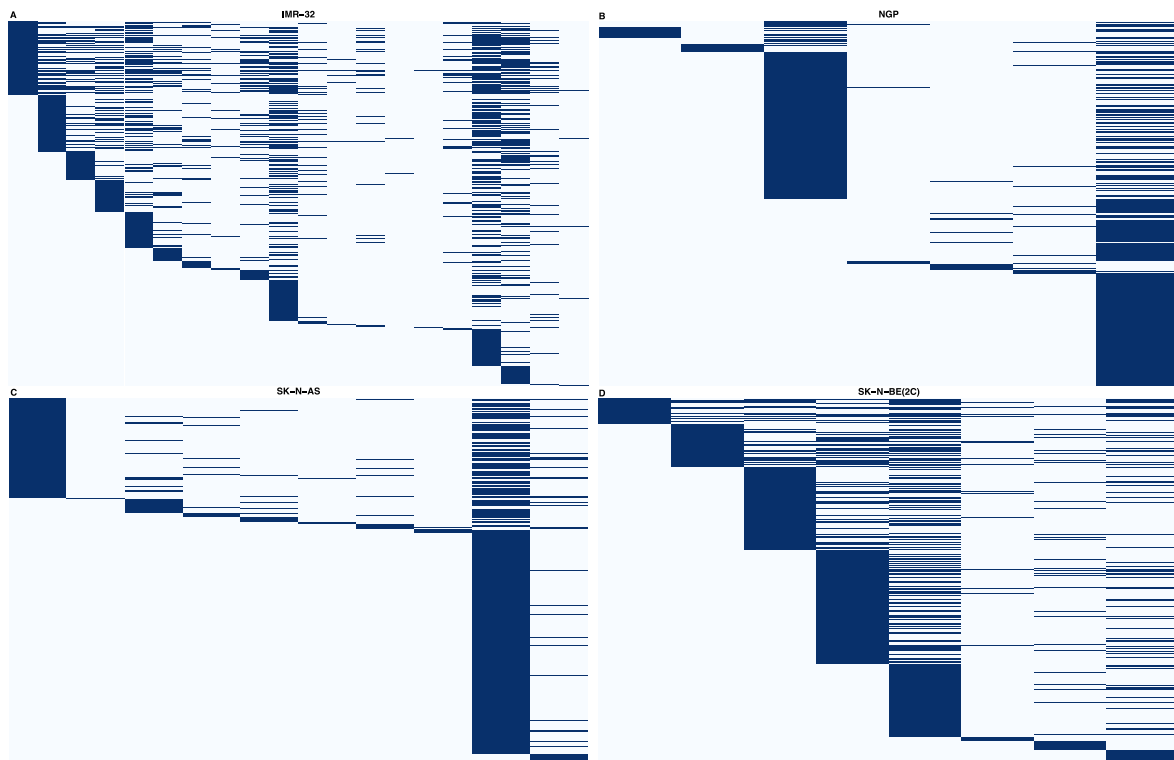


Figure S1. The overlap of genes between the mice engrafted with IMR-32 (A: $n = 16$), NGP (B: $n = 3$), SK-N-AS (C: $n = 8$) or SK-N-BE(2C) (D: $n = 8$). Based on a binary count table, heatmaps were created. In the heatmap, different columns represent different samples while the rows represent the genes detected in one of the samples. A blue line represents a gene that is detected in the plasma of a sample.

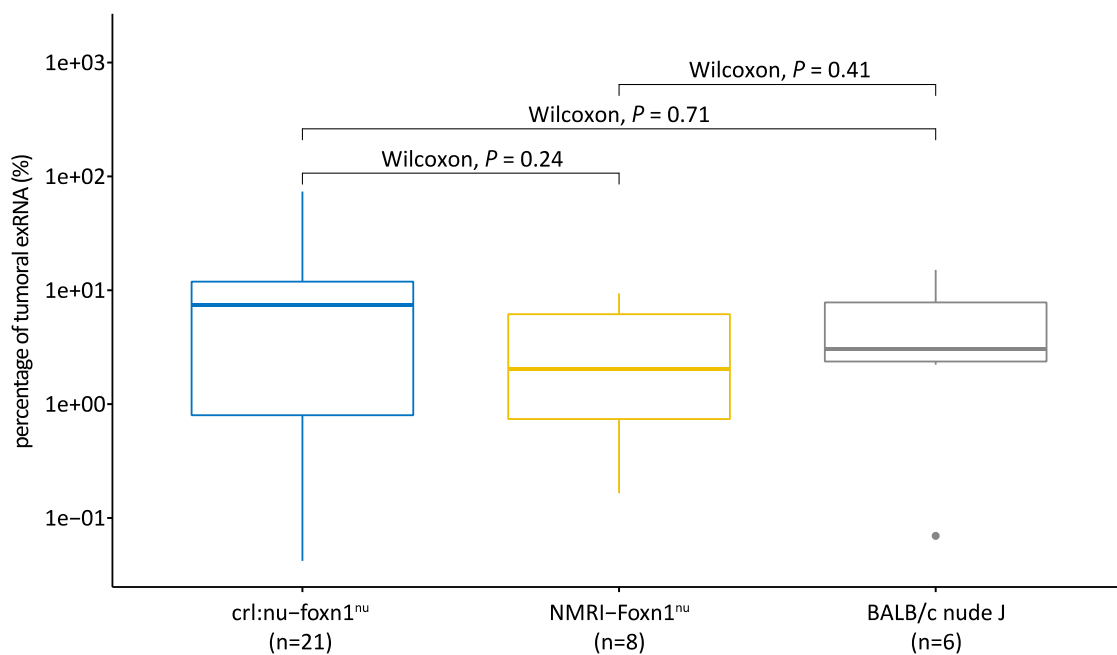


Figure S2. Percentage of tumoral exRNA in the different engrafted mouse strains.

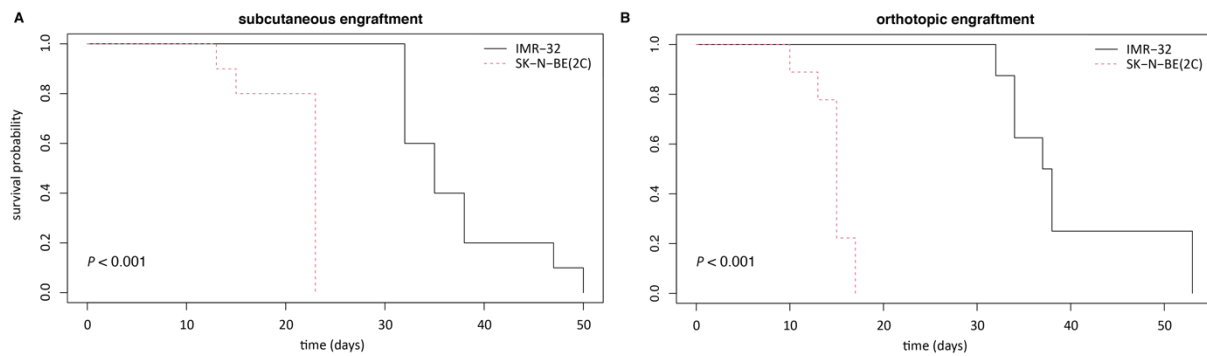


Figure S3. Kaplan-Meier curve depicting the probability of survival in function of the lifespan (days) for four different mouse cohorts: subcutaneously engrafted mice with either IMR-32 or SK-N-BE(2C) cells (**A**) and orthotopically engrafted mice with either IMR-32 or SK-N-BE(2C) cells (**B**). P values of log rank tests are indicated. The lifespan of the mice is defined by the tumor size, as animals were sacrificed at maximally allowed tumor sizes. All animals from the engraftment site cohort are included ($n = 37$).

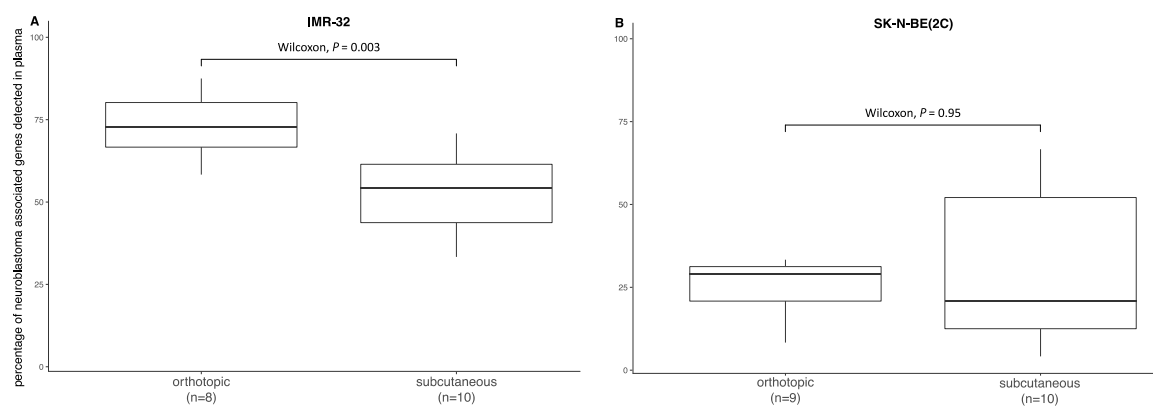


Figure S4. Percentage of neuroblastoma associated genes, detected in the blood plasma of orthotopically and subcutaneously engrafted mice with IMR-32 (**A**) or SK-N-BE(2C) (**B**) cells.

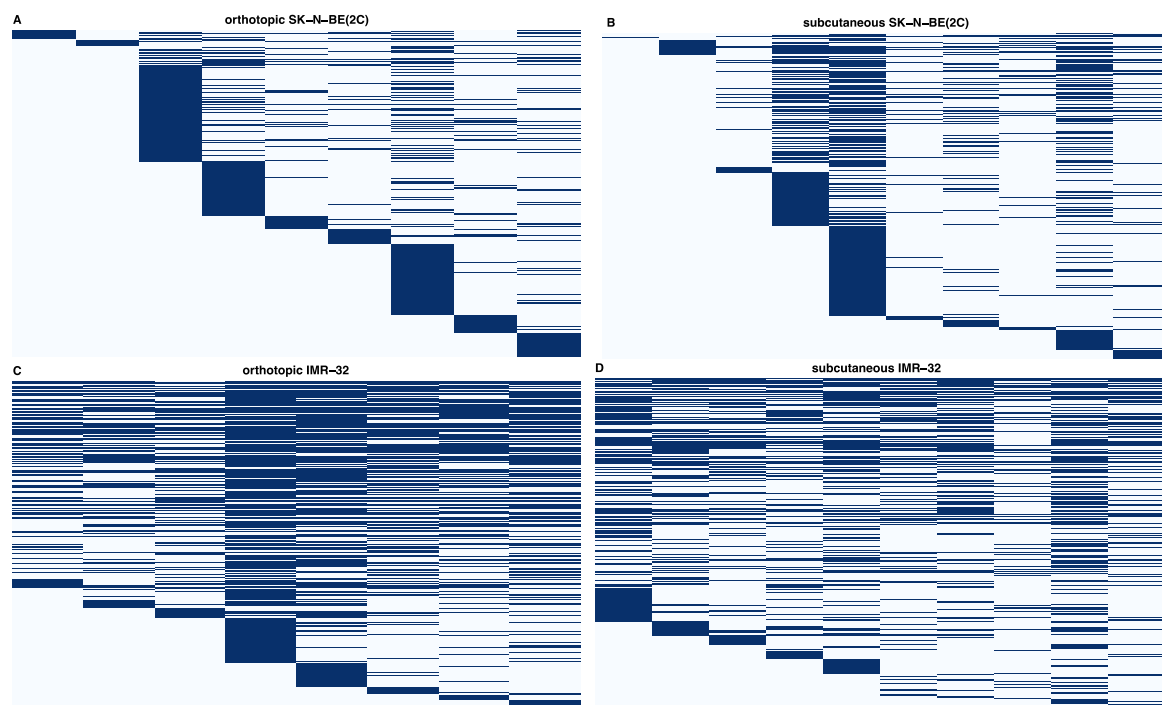


Figure S5. Gene overlap in orthotopically (**A**: $n = 9$) and subcutaneously (**B**: $n = 10$) engrafted SK-N-BE(2C) xenografts, and orthotopically (**C**: $n = 8$) and subcutaneously (**D**: $n = 10$) engrafted IMR-32 xenografts. Based on a binary count table, heatmaps were created. In the heatmap, different columns represent different samples while the rows represent the genes detected in one of the samples. A blue line represents a gene that is detected in the plasma of a sample.

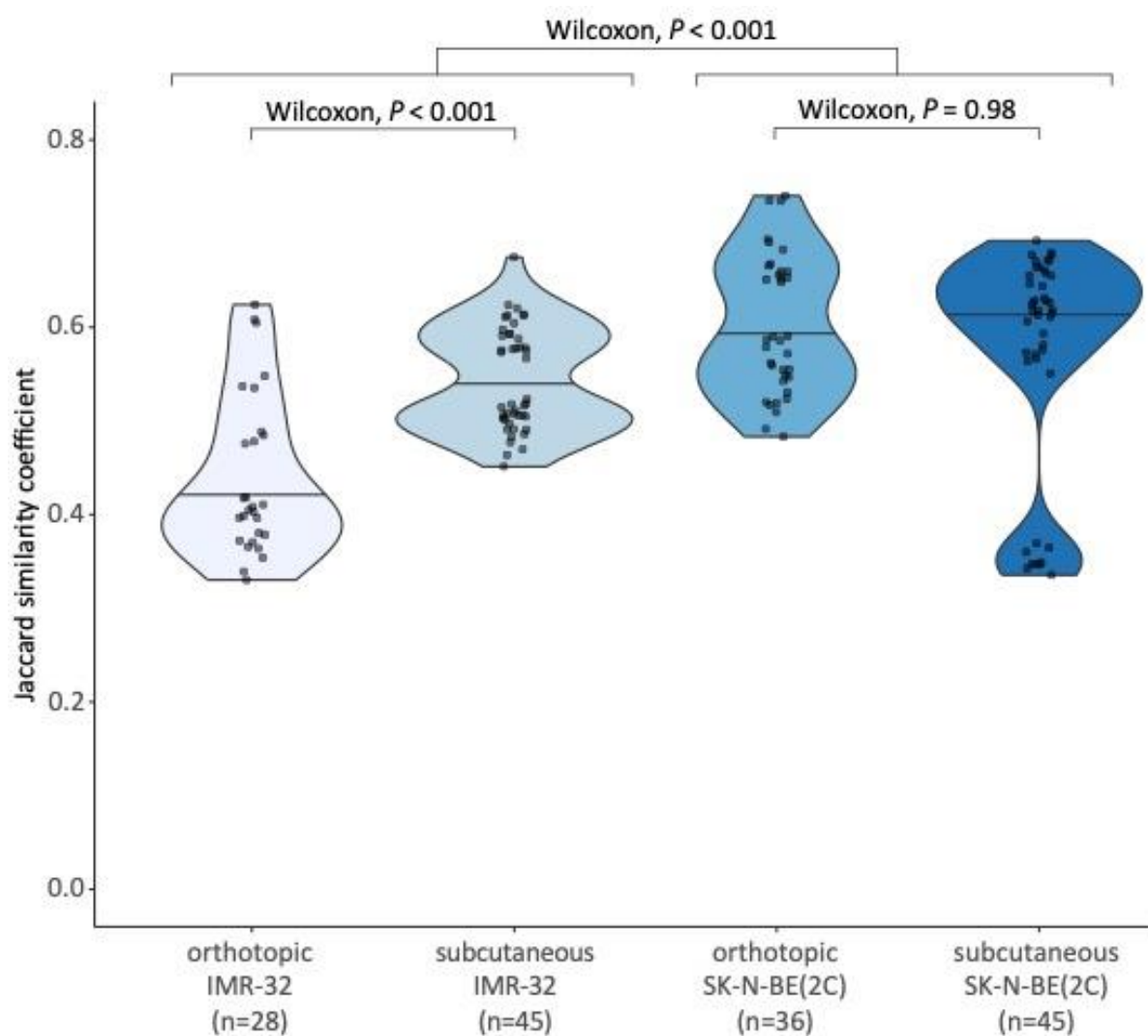


Figure S6. Jaccard similarity coefficient obtained by pairwise comparison of murine gene counts (transcripts per million) of the samples within each model (orthotopic IMR-32, subcutaneous IMR-32, orthotopic SK-N-BE(2C) and subcutaneous SK-N-BE(2C)). The horizontal black line in each model represents the median.

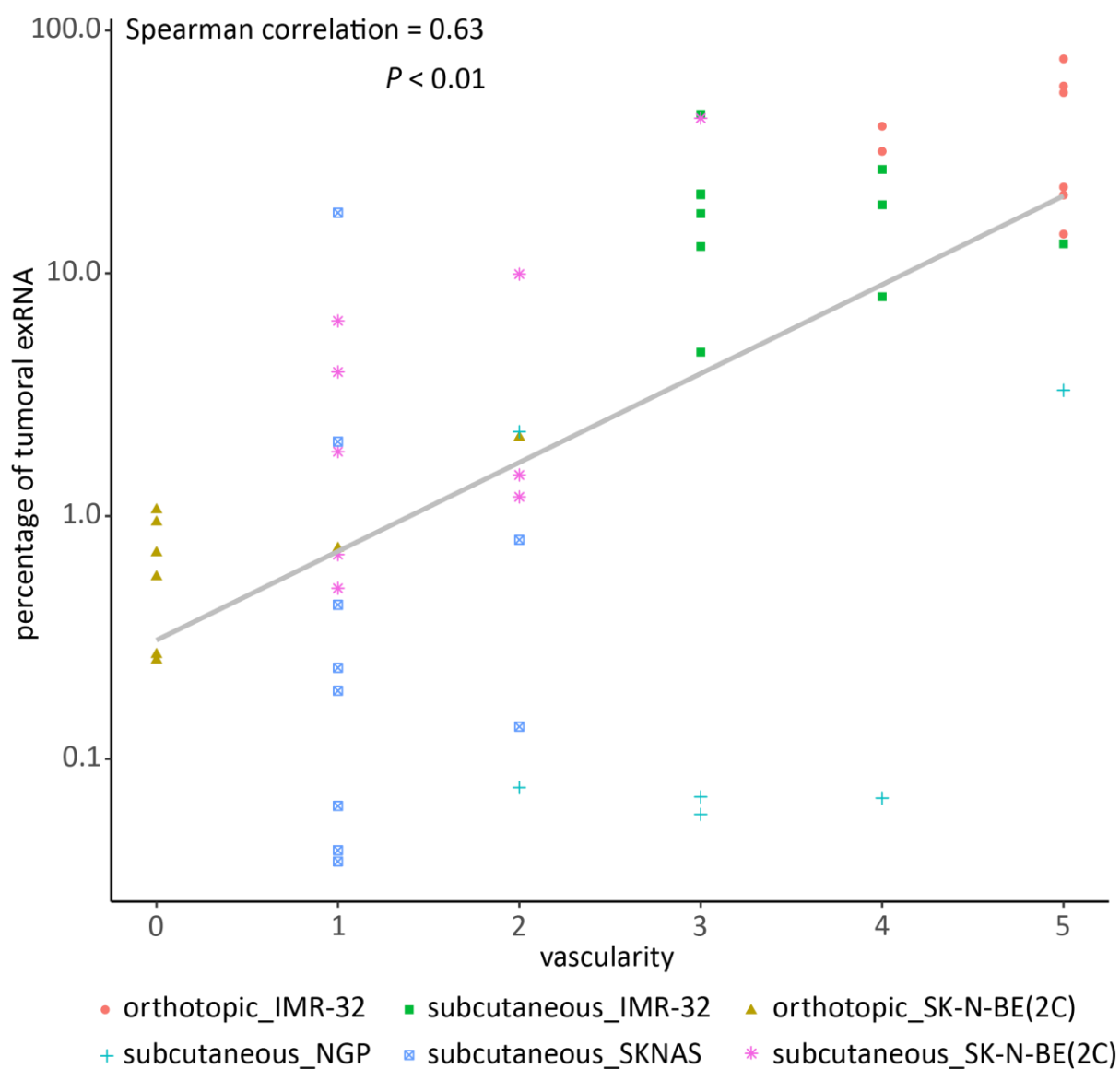


Figure S7. Correlation analysis of the percentage of tumoral exRNA in function of vascularity. The gray line represents the linear regression line. All animals from the engraftment site cohort are included, and NGP and SK-N-AS engrafted mice from the cell line cohort ($n = 53$).

2. Supplemental Tables (cf. supplemental excel file Table S2)

Table S1. Characteristics and genetic background of the cell lines that were engrafted in the mice included in our study [40–42]. Empty cells are either wild type or no reported aberration.

Cell line characteristics and genetic background	NGP	IMR-32	SK-N-AS	SK-N-BE(2C)
location of biops	lung	abdomen	bone marrow	bone marrow
cell type	neurite bearing	neurite bearing	substrate adherent	intermediate
MYCN	amplification	amplification		amplification
ALK				
TP53	mutation		mutation	mutation
MDM2	amplification			
CDK4	amplification			
TERT				
ATRX				mutation
NRAS			mutation	
PTPN11				
ATM		mutation		
CDKN2A				
KRAS				
PIK3CA	mutation			
11q loss	yes	yes	yes	
17q gain	yes	yes	yes	yes
1p loss		yes	yes	yes

Table S2. Overall, the sequencing data is of good quality. For each RNA sample (RNA ID), the matching mouse ID, biomaterial ID, sample type, mouse cohort, tumor size, plasma input volume and added spike concentrations are shown, as well as haemolysis levels measured by NanoDrop technology (absorbance of light at 414 nm) and RNA sequencing QC results. Sequencing depth: total read number; % trimmed: percentage total base pairs trimmed; % dups R1 or R2: percentage of R1 or R2 reads marked as duplicate; % GC R1 or R2: average GC content percentage of R1 or R2 reads; length R1 or R2: average sequence length (in bp) of R1 or R2 reads; % aligned to combined, human or mouse genome: percentage of reads aligned to the combined, human or mouse genome; exonic depth of combined, human or mouse genome: number of reads mapping to exons in the combined, human or mouse genome. The Sequin/ERCC ratio reflects RNA purification efficiency and should be relatively constant within a single experiment.