Supplementary Materials:

Table S1. Peptides used in this study

Name	Name in original reference	N-terminal modification	Sequence	C-terminal modification	Mw	Purity	Source
Biotin-TAT (47-57)	TAT	biotin-	YGRKKRRQRRR	OH	1786.2		Eurogentec/Anaspec
CmR	CmR	biotin-{Ahx}{Ahx}{Ahx}	KRDRGGGCMGTINTATAAC-NH2	NH2	2445.93	97%	GenScript
cRADyK	cyclic RAD		Cyclo[RAD-D-Phe-K-biotin-PEG-PEG]		1134.33	95%	Peptides International
cRGDyK	cyclic RGD		Cyclo[RGD-D-Phe-K-biotin-PEG-PEG]		1120.3	97%	Peptides International
F3	F3	biotin-{Ahx}{Ahx}{Ahx}	EPQRRSARLSAKPAPPKPEPKPKKAPAKK	NH2	3754.61	97%	GenScript
GE11	GE11	biotin-{Ahx}{Ahx}{Ahx}	YHWYGYTPQNVI	NH2	2105.46	97%	GenScript
Hemopressin	Hemopressin (HP)	biotin-{Ahx}{Ahx}{Ahx}	PVNFKFLSH	NH2	1653.04	96%	GenScript
iNGR	iNGR	biotin-{Ahx}{Ahx}{Ahx}	CRNGRGPDC	NH2	1539.9	98%	GenScript
iRGD	iRGD	biotin-{Ahx}{Ahx}{Ahx}	CRGDKGPDC	NH2	1512.87	97%	GenScript / solubility
iso-d-T7	T7 (D-isomer)	biotin-{Ahx}{Ahx}{Ahx}	{d-HIS}{d-ARG}{d-PRO}{d-TYR}{d-ILE}{d-ALA}{d-HIS}	NH2	1457.79	98%	GenScript
Lyp1	Lyp-1	biotin-{Ahx}{Ahx}{Ahx}	CGNKRTRGC	NH2	1556.95	96%	GenScript / solubility
NGR18	NGR-2C-TNF1–11	NH2	CNGRCGVRSSSRTPSDKY	{Ahx}{Ahx}{Ahx}-K-biotin	2707.14	96%	GenScript
NGR5	NGR-5	NH2	YNGRT	{Ahx}{Ahx}{Ahx}-K-biotin	1345.66	97%	GenScript / solubility
NTP	C3, NTP (NCAM-1 targeting peptide)	biotin-{Ahx}{Ahx}{Ahx}	ASKKPKRNIKA	NH2	1805.27	98%	GenScript
pAGP	pAGP	biotin-{Ahx}{Ahx}{Ahx}	CAGPRTRRC	NH2	1582	95%	GenScript
RMS-I	RMS-I	biotin-{Ahx}{Ahx}{Ahx}	CQQSNRGDRKRC	NH2	2013.43	99%	GenScript
RMS-II	RMS-II	biotin-{Ahx}{Ahx}{Ahx}	CMGNKRSAKRPC	NH2	1913.43	96%	GenScript
RMS-P3	RMS-P3	biotin-{Ahx}{Ahx}{Ahx}	CMGTINTRTRRC	NH2	1974.48	95%	GenScript
RMS-P3-3G		biotin-{Ahx}{Ahx}{Ahx}	GGGCMGTINTRTRRC	NH2	2145.59	96%	GenScript
shTmR (aka TmRggg)		biotin-{Ahx}{Ahx}{Ahx}	KRDRCMGTINTRTRRC	NH2	2530.1	95%	GenScript
T7	T7	biotin-{Ahx}{Ahx}{Ahx}	HAIYPRH	NH2	1457.77	98%	GenScript
tLyp1	tLyp-1	biotin-{Ahx}{Ahx}{Ahx}	CGNKRTR	NH2	1398.8	95%	GenScript
TmR	TmR	biotin-{Ahx}{Ahx}{Ahx}	KRDRGGGCMGTINTRTRRC	NH2	2701.26	95%	GenScript
uPA	U11	biotin-{Ahx}{Ahx}{Ahx}	VSNKYFSNIHWGC	NH2	2119.5	96%	GenScript

Table S2. Published work supporting expression of selected targets in RMS cell lines or tumors.

Target	Uniprot	Gene	Evidence in the literature
Furin	P09958	FURIN	RMS patient samples and cell lines [1-3]
Neural Cell Adhesion Molecule 1 / CD56 (NCAM-1)	P13591	NCAM1	Preferentially expressed in RMS [4]. This publication
Cannabinoid Receptor 1 / CB1	P21554	CNR1	Gene expression regulated by PAX3/FOXO1 [5]
Epidermal Growth Factor Receptor c-ErbB-1	P00533	EGFR	Reviewed in [6]. E.g., expression in 31/66 (47%) cases; FN: 26/34, 76%, FP: 5/32, 16% [7]
Transferrin Receptor 1 / CD71 (TFR1)	P02786	TFRC	Overexpression in A204 cells [8]
Urokinase Plasminogen Activator Receptor (uPAR)	Q03405	PLAUR	uPAR targeted in RD RMS cells by bispecific uPAR/EGFR immunotoxin [9] and in Rh30 RMS cells [10].
Nucleolin (Protein C23)	P19338	NCL	RD cell line [11,12] RMS PDX (n=5) [11] FN-RMS / ERMS cells [13] This publication
Aminopeptidase N / CD13 (APN)	P15144	ANPEP	Expression in soft tissue sarcomas [14]
Complement component 1 Q subcomponent-binding protein, mitochondrial, p32	Q07021	C1QBP	No direct evidence in the literature
Neuropilin-1	Q9H2D9	NRP1	No direct evidence in the literature
Integrin $\alpha_v\beta_3$	P06756 P05106	ITAV ITGB3	Expression in Rh30 cells [15,16] and RD cells [17]

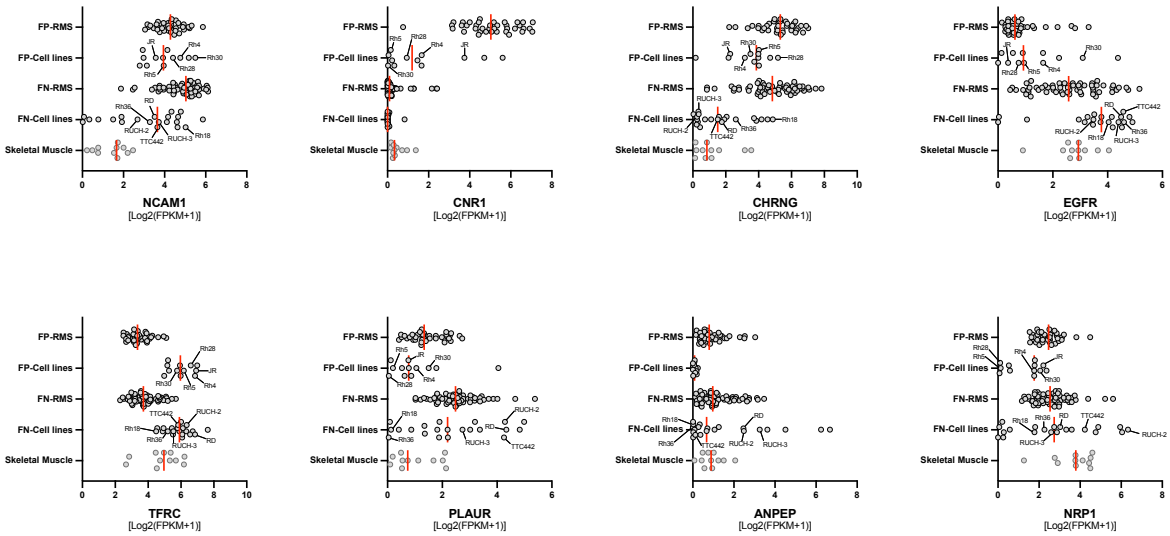

Figure S1. Targeted Receptors Expression in RMS cell lines, tumors, and skeletal muscles. Whole transcriptome sequencing data for cell surface proteins were extracted from Brohl et al. [18]. The data set from the online Supplementary Material Excel file mmc4.xlsx - S3A.2.Ex_CellSurfaceProteins contains data from 60 FN RMS tumors, 38 FP-RMS tumors, 21 FN-RMS cell lines, 12 FP-RMS cell lines, 11 skeletal muscles tissues Log2 values of Fragments Per Kilobase Million (FPKM) are indicated for each selected gene. Highlighted are RMS cell lines available in our laboratory. When compared to skeletal muscles, NCAM1, CNR1, CHRNG, PLAUR, present higher transcripts numbers in RMS samples. Transcripts expression of EGFR, TFRC, NRP1, ANPEP is not strikingly higher in RMS samples.

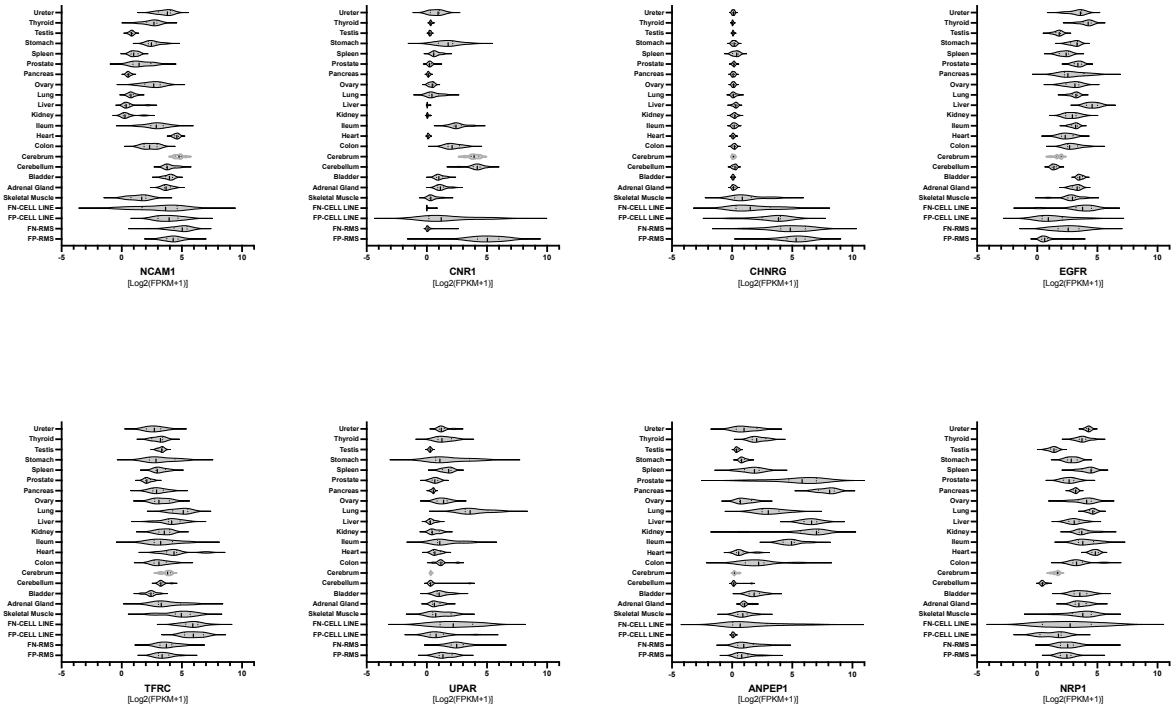

Figure S2. Targeted Receptors Expression in RMS cell lines, tumors, and normal tissues. Whole transcriptome sequencing data for cell surface proteins were extracted from Brohl et al. [18]. The data set from the online Supplementary Material Excel file mmc4.xlsx - S3A.2.Ex_CellSurfaceProteins contains data from 60 FN RMS tumors, 38 FP-RMS tumors, 21 FN-RMS cell lines, 12 FP-RMS cell lines, 11 skeletal muscles tissues), and additional control tissues. Log2 values of Fragments Per Kilobase Million (FPKM) are indicated for each selected gene. Transcripts in 19 control tissues are shown. Striking is the almost complete absence of expression of CHNRG in all tissues analyzed, except for skeletal muscles, where expression is however lower than in RMS tumors and cell lines.

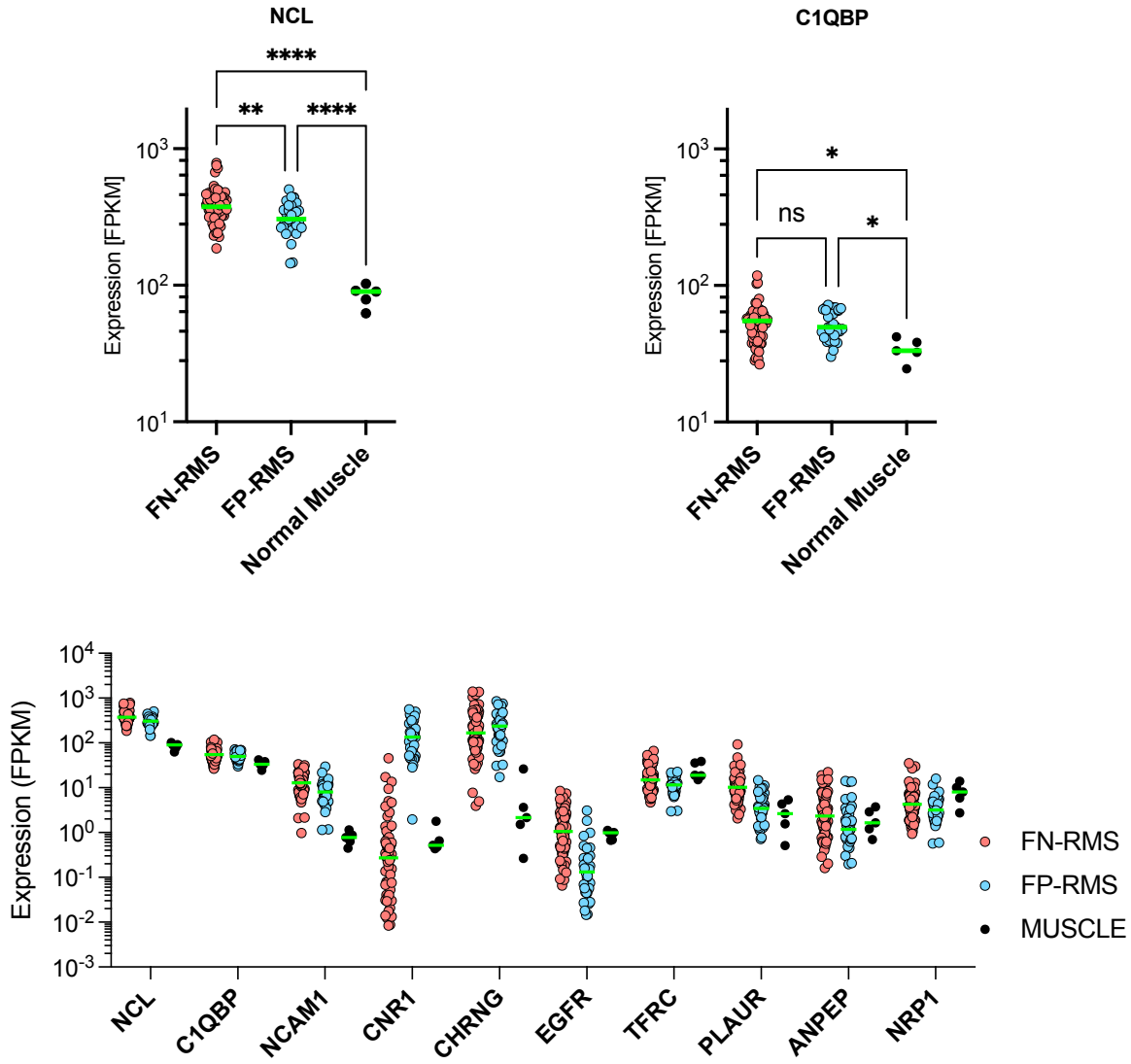

Figure S3. Expression of selected surface targets including nucleolin and p32 in RMS cell lines, tumors, and skeletal muscle. Since Nucleolin and p32 are not included in Fig.S1 and Fig.S2 as they are not 'classical' surface proteins, we have extracted expression data from a published RNA sequencing of 34 FP-RMS, 66 FN-RMS, and 5 normal skeletal muscles where transcripts were quantified by RNA sequencing [19] (GEO accession number GSE108022). Displayed are Fragments Per Kilobase Million (FPKM).

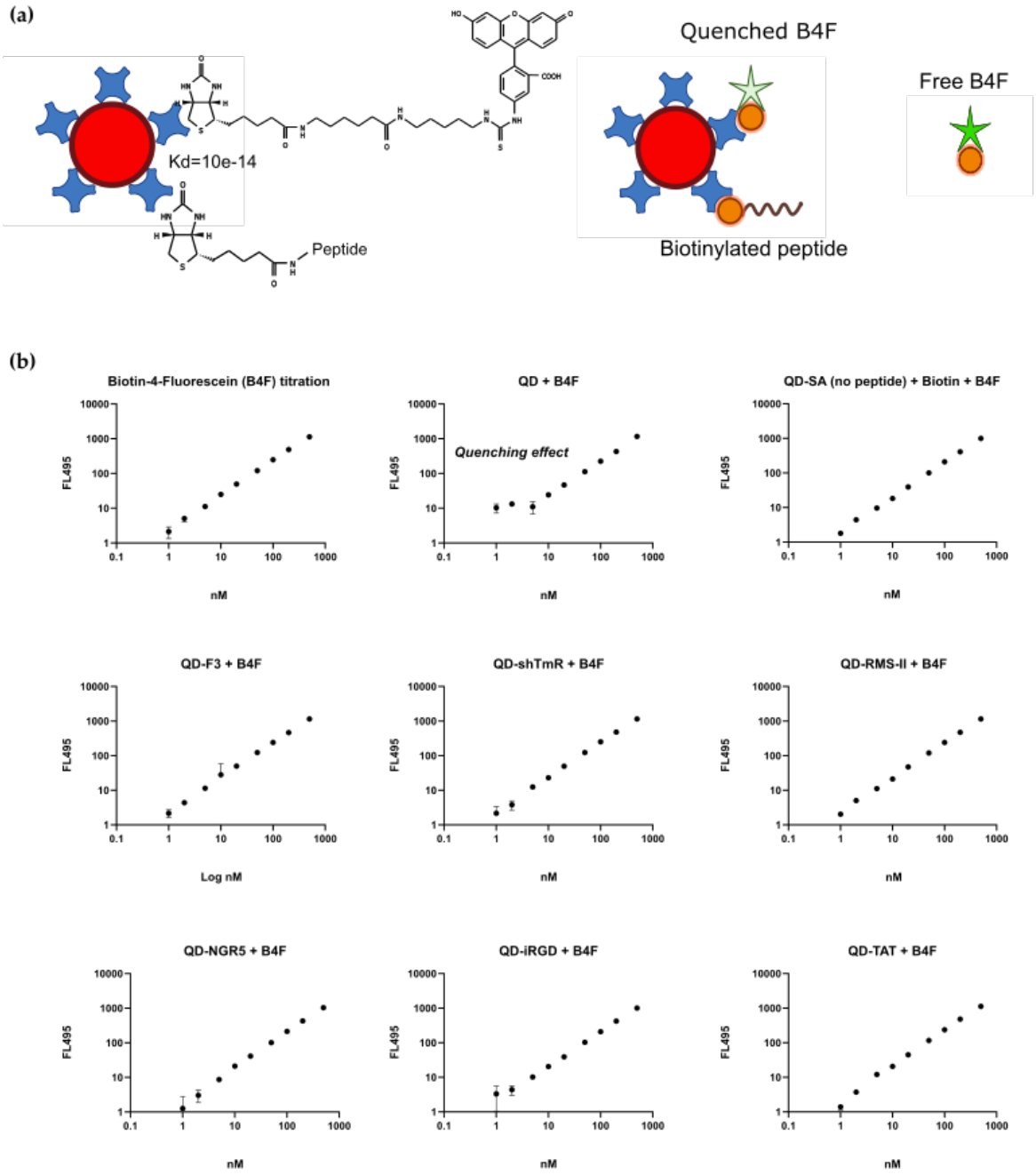

Figure S4. Quality control for conjugation of streptavidin-QD and biotin-Peptides by B4F quenching. **(a)** Schematic illustration of the quenching process taking place when Biotin-4-FITC binds to QDs. **(b)** Representative results obtained for the different biotinylated peptides. When Biotin-4-Fluorescein was incubated from 1 to 500nM with QD-SA alone (QD + B4F) the quenching was visible at concentrations lower than 10nM. Preincubated of QD-SA with free biotin abrogated this effect, as well as the conjugation with biotinylated peptides, indicating successful conjugation.

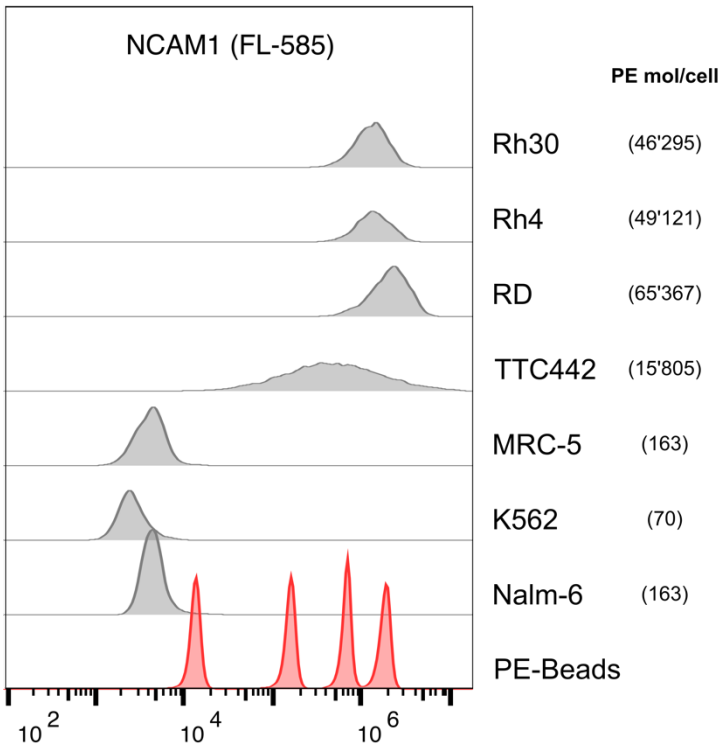

Figure S5. Surface expression of NCAM1 on RMS cell lines and control cells. Cells were detached by Accutase and after washing were incubated with PE-conjugated anti-NCAM1. Quantibrite PE-Beads (340495, BS Biosciences) were used according to the manufacturer instructions. The four peaks correspond to (from left to right) 1700, 14200, 39400, 133400 molecules PE per bead. Quantification was performed by assuming one molecule PE per antibody.

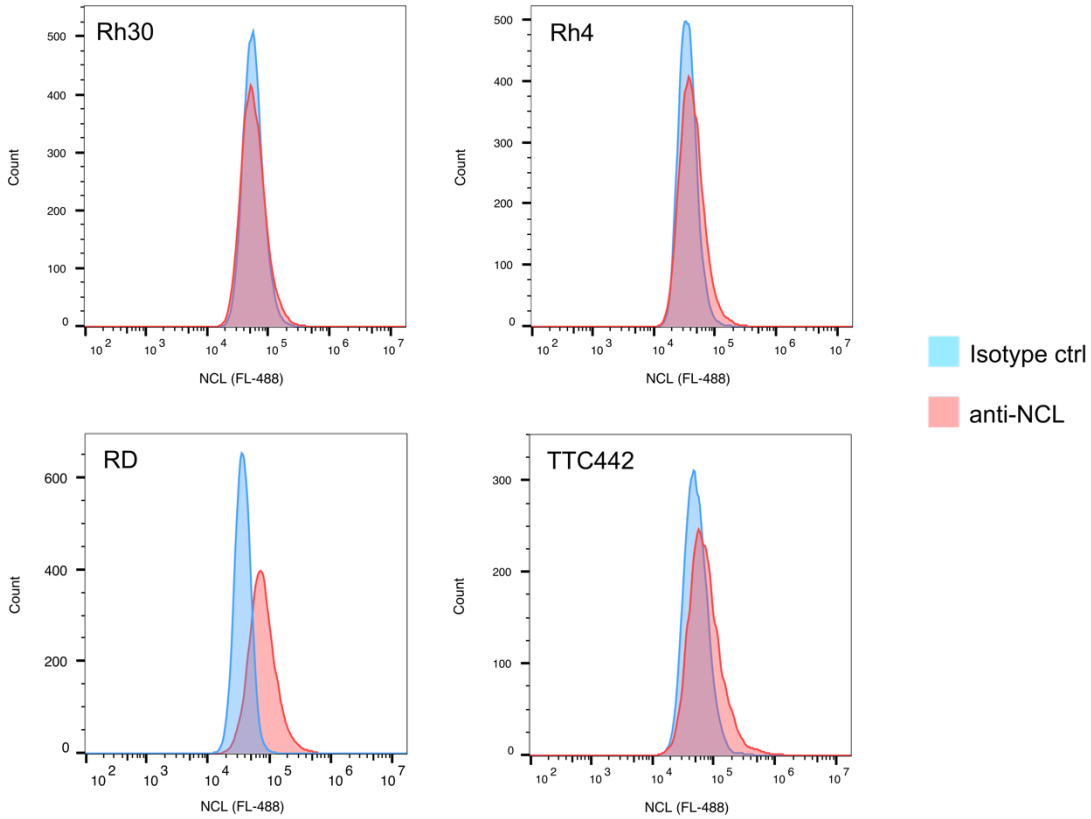

Figure S6. Surface detection of Nucleolin on RMS cells. Cells were detached with Accutase and after washing in PBS/2%BSA 100'000 were incubated with anti-Nucleolin polyclonal Rabbit Antibody (N2662, Sigma) diluted 1/50, for 1h at 4°C. After washing, secondary antibody Goat anti-Rabbit IgG (H+L) Cross-adsorbed, Alexa488 (A11008, Thermofisher) 1/500 was incubated for 30 min at 4°C. Rabbit IgG Isotype Control (02-6102, Thermofisher) was used as control. After washing, cells were fixed with 1%PFA/PBS and measured by flow cytometry. No consistent shift could be observed for all cell lines analyzed. Only RD cells did show a clear increased fluorescence upon incubation with anti-Nucleolin antibody, as compared to isotype control.

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