SUPPLEMENTARY FIGURE LEGENDS

Figure S1: TLR3 has a positive prognostic value in several types of sarcomas

(A) Expression level of TLR3 in normal tissues. Publicly available transcriptomic data obtained from GTEX portal. **(B)** Event-free survival probability of FP-RMS samples stratified according to TLR3 expression (*p-value = 0.049, log-rank test). Microarray transcriptomic dataset was provided by Javed Khan's laboratory. **(C)** Overall survival probability of FN-RMS samples stratified according to TLR3 expression (p-value = 0.078, log-rank test). Publicly available E-TABM-1202 transcriptomic data were downloaded from ArrayExpress. **(D)** Overall survival probability of Ewing sarcoma samples stratified according to TLR3 expression (***p-value < 0.0001, log-rank test). Publicly available transcriptomic data from Kuijjer's cohort were downloaded from R^2 cancer. **(E)** Overall survival probability of leiomyosarcomas (TCGA 2022-v32 Sarcoma cohort), stratified according to TLR3 expression (***p-value < 0.0001, log-rank test).

Figure S2: Poly(I:C) treatment drives TLR3-induced cell death in pediatric sarcoma cells.

(A) Immunoblot of TLR3 from MNNG/HOS osteosarcoma cells, treated or not with IFN-1 for 24 h. GAPDH is used as a loading control. One representative image out of 3 independent experiments is shown. Asterisks denote unspecific bands. **(B)** Immunoblot of TLR3 from the patient-derived cell line RMS-CLB1, treated or not with IFN-1 for 24 h. GAPDH is used as a loading control. One representative image out of 3 independent experiments is shown. Asterisks denote unspecific bands. **(C)** Viability of MNNG/HOS osteosarcoma cells treated or not with IFN-1 and/or Poly(I:C) for 72 h was analyzed by cytometry (DAPI/Acridine Orange). Mean valu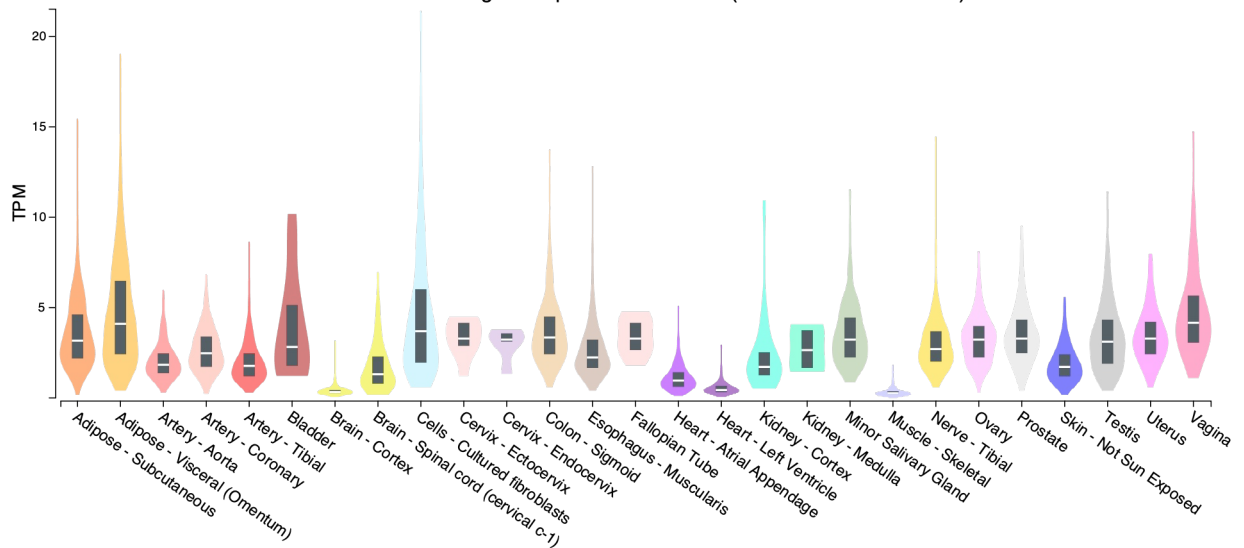es ± s.t.d are represented (n=3, *p-value = 0.05). **(D)** Viability of the patient-derived RMS-CLB1 cell line treated or not with IFN-1 and/or Poly(I:C) for 72 h was analyzed by cytometry (DAPI/Acridine Orange). Mean values ± s.t.d are represented (n=3, *p-value = 0.05).
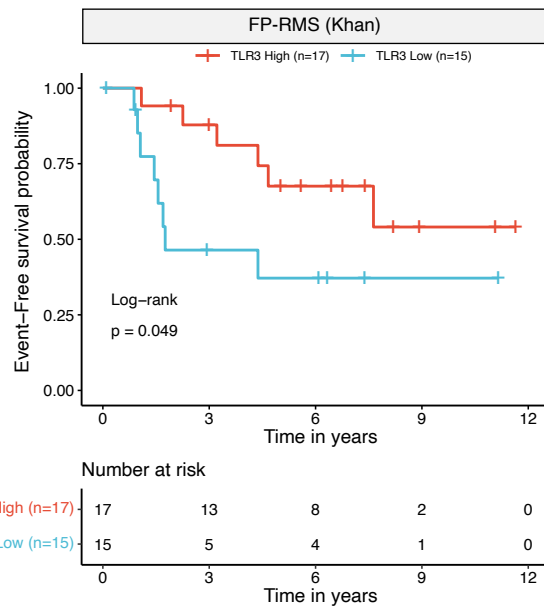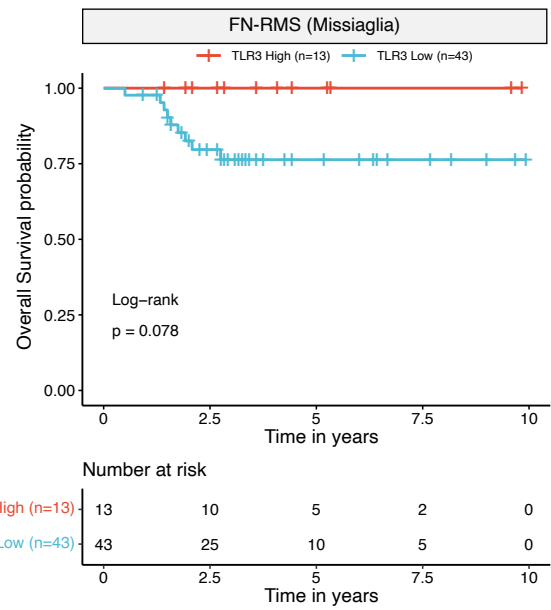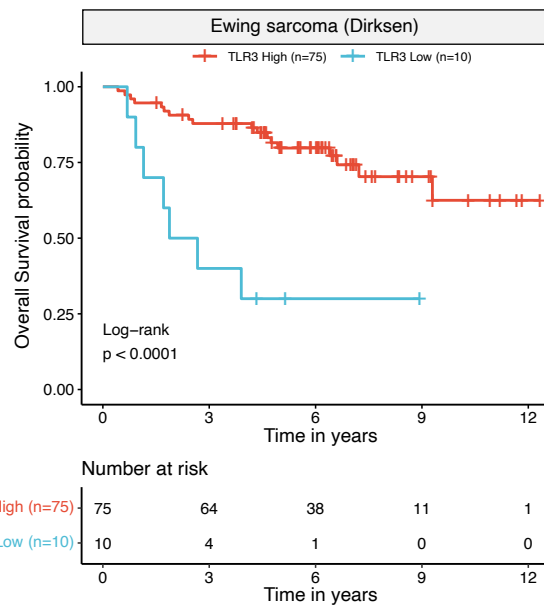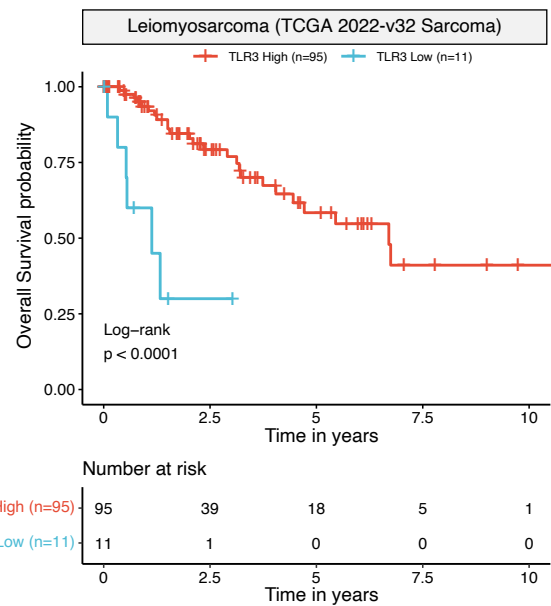

Figure S3: **RD and RH30 rhabdomyosarcoma cell lines homozygous for the L412F variant of TLR3 are resistant to Poly(I:C).**

(A) Immunoblot of TLR3 from RD rhabdomyosarcoma cells, treated or not with IFN-1 for 24 h. GAPDH is used as a loading control. One representative image out of 3 independent experiments is shown. Asterisks denote unspecific bands. **(B)** Poly(I:C) does not lead to an

increase in Caspase-3 activity in RD cells *in vitro*. Mean values ± s.t.d of caspase-3 activity quantified according to the fluorescence emitted by cleavage of a DEVD-AFC 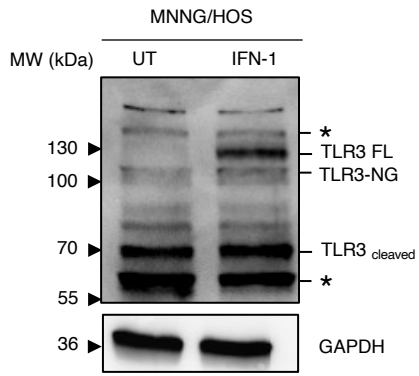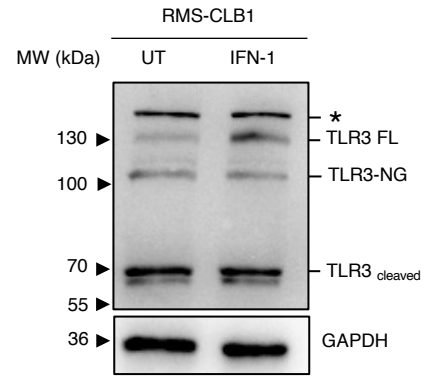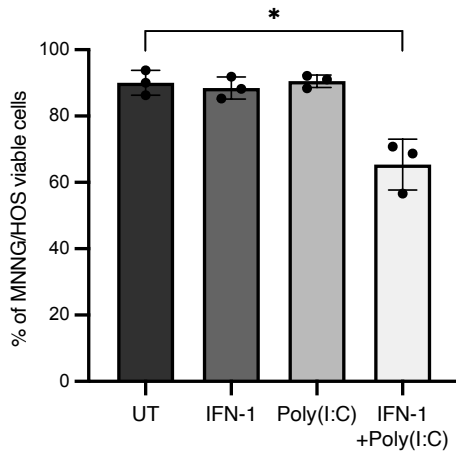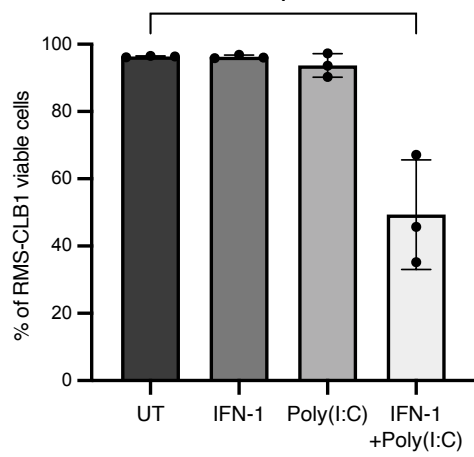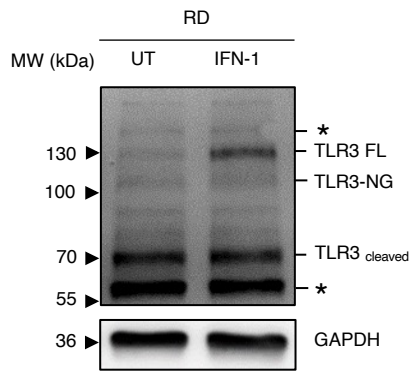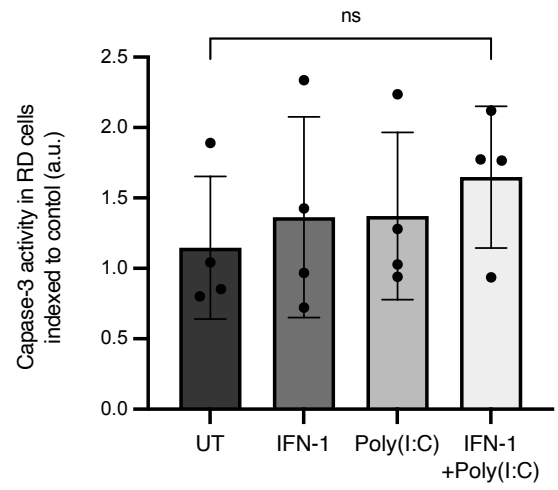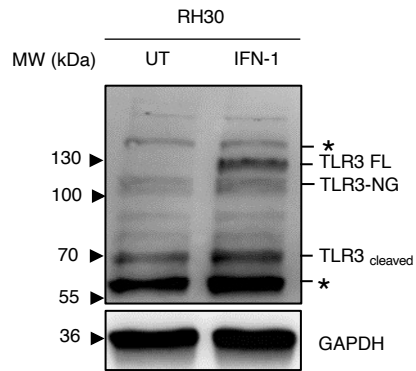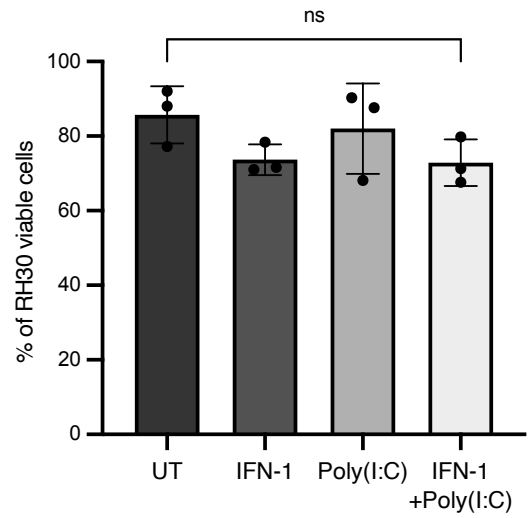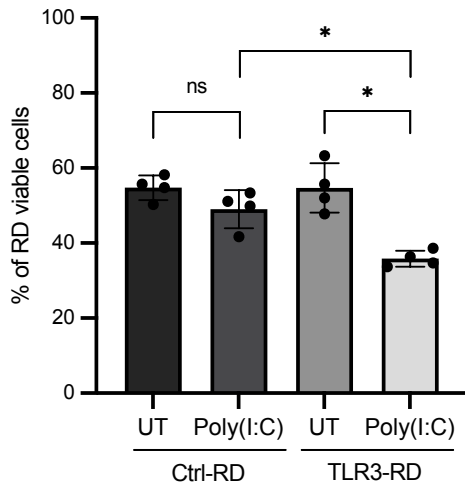peptide are represented (n = 4, ns p-value = 0.1714). **(C)** Immunoblot of TLR3 from RH30 rhabdomyosarcoma cells, treated or not with IFN-1 for 24 h. GAPDH is used as a loading control. One representative image out of 3 independent experiments is shown. Asterisks denote unspecific bands. **(D)** Viability of RH30 RMS cells treated or not with IFN-1 and/or Poly(I:C) for 72 h was analyzed by cytometry (DAPI/Acridine Orange). Mean values ± s.t.d are represented (n = 3, ns p-value = 0.1). **(E-F)** Transient transfection of the WT allele of TLR3 is sufficient to restore cell death upon Poly(I:C) treatment in RD cells. **(E)** Viability of RD rhabdomyosarcoma cells transfected or not with TLR3 and treated with Poly(I:C) for 48 h was analyzed by cytometry (DAPI/Acridine Orange). Mean values ± s.t.d are represented (n=3, *p-value< 0.05). **(F)** Immunoblot of transfected CFP tagged-TLR3 in RD rhabdomyosarcoma cells. One representative image out of 3 independent experiments is shown. Transfected and endogenous forms of TLR3 are indicated. Asterisks denote unspecific bands. Ctl: RD transfected with an empty vector. Transf. : RD transfected with a CFP-TLR3 L412 encoding vector.

A Bulk tissue gene expression for TLR3 (ENSG00000164342.12)

B FP-RMS (Khan)

C FN-RMS (Missiaglia)

D Ewing sarcoma (Dirksen)

E Leiomyosarcoma (TCGA 2022-v32 Sarcoma)

A

MNNG/HOS

MW (kDa) UT IFN-1

130

100

70

55

36 GAPDH

*
TLR3 FL
TLR3-NG
TLR3 cleaved
*

B

RMS-CLB1

MW (kDa) UT IFN-1

130

100

70

55

36 GAPDH

*
TLR3 FL
TLR3-NG
TLR3 cleaved

C

% of MNNG/HOS viable cells

*

100

80

60

40

20

0

UT   IFN-1   Poly(I:C)   IFN-1
                          +Poly(I:C)

D

% of RMS-CLB1 viable cells

*

100

80

60

40

20

0

UT   IFN-1   Poly(I:C)   IFN-1
                          +Poly(I:C)

A

RD

MW (kDa) | UT | IFN-1

130
100
70
55

* TLR3 FL
TLR3-NG
TLR3 cleaved
*

36 GAPDH

B

ns

Capase-3 activity in RD cells indexed to contol (a.u.)

UT | IFN-1 | Poly(I:C) | IFN-1 +Poly(I:C)

C

RH30

MW (kDa) | UT | IFN-1

130
100
70
55

* TLR3 FL
TLR3-NG
TLR3 cleaved
*

36 GAPDH

D

ns

% of RH30 viable cells

UT | IFN-1 | Poly(I:C) | IFN-1 +Poly(I:C)

E

% of RD viable cells

ns | * | *

UT | Poly(I:C) | UT | Poly(I:C)
Ctrl-RD | TLR3-RD

F

RD

MW (kDa) | Ctl | Transf.

160
130
100
70
55

TLR3-CFP
TLR3 FL
TLR3-NG
TLR3 cleaved
*

36 GAPDH

Supplementary Table 1 – Cutoffs used for the survival analyses

Figure	Dataset	Tumor	TLR3 Cutoff	Cutoff method	Log2 normalised expression
1B	Missiaglia	FP-RMS	2,659058	Maxstat	Yes
1C	Kuijjer	Osteosarcoma	176,7	KaplanScan	No
S1A	Khan	FP-RMS	7,262	Maxstat	Yes
S1B	Missiaglia	FN-RMS	3,427901	Maxstat	Yes
S1C	Dirksen	Ewing Sarcoma	31,8	KaplanScan	No
S1C	TCGA 2022-v32 Sarcoma	Leiomyosarcoma	1,95	Maxstat	Yes