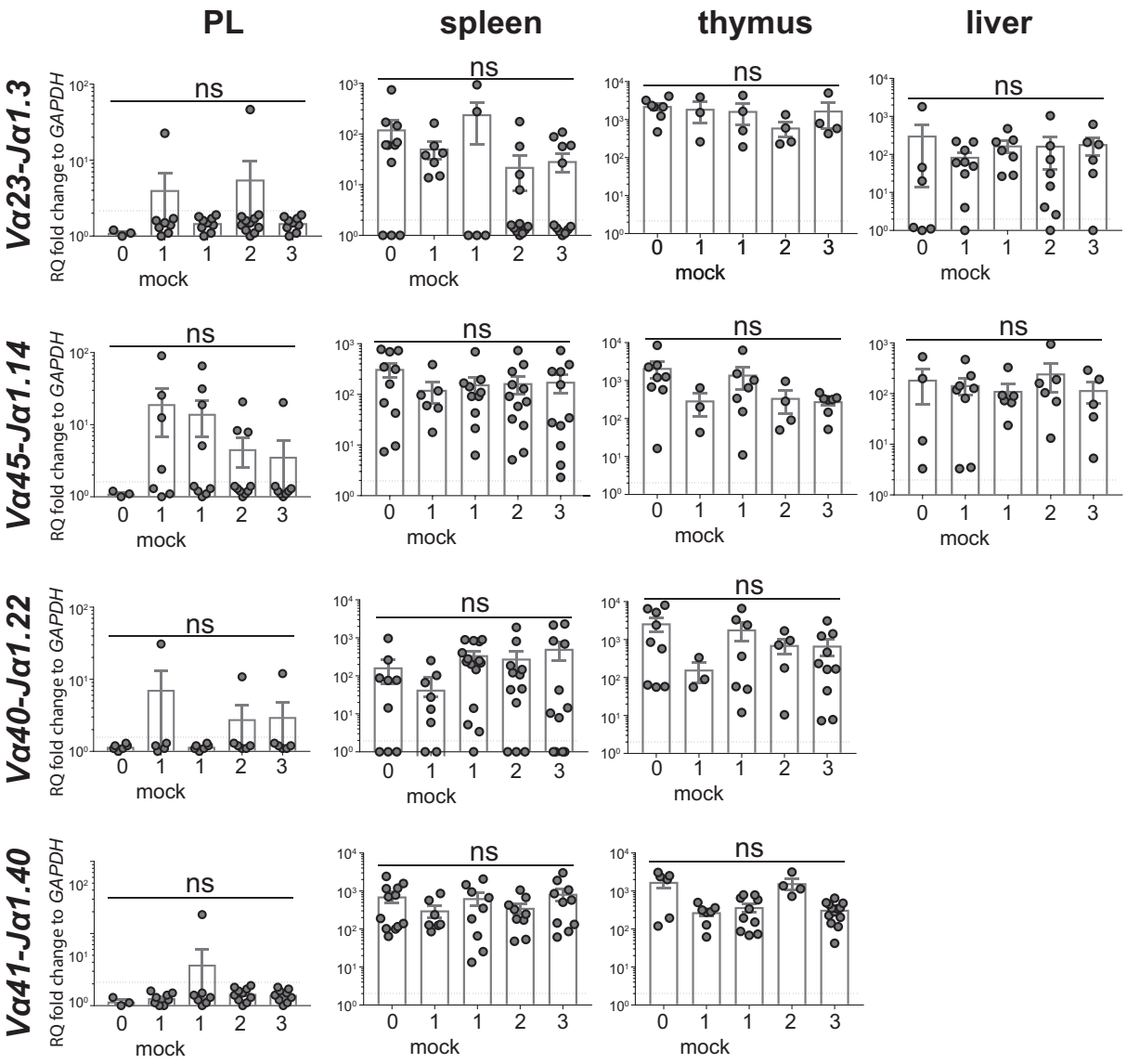
Supplementary Material

Supplementary Table 1A: List of qPCR primer sequences

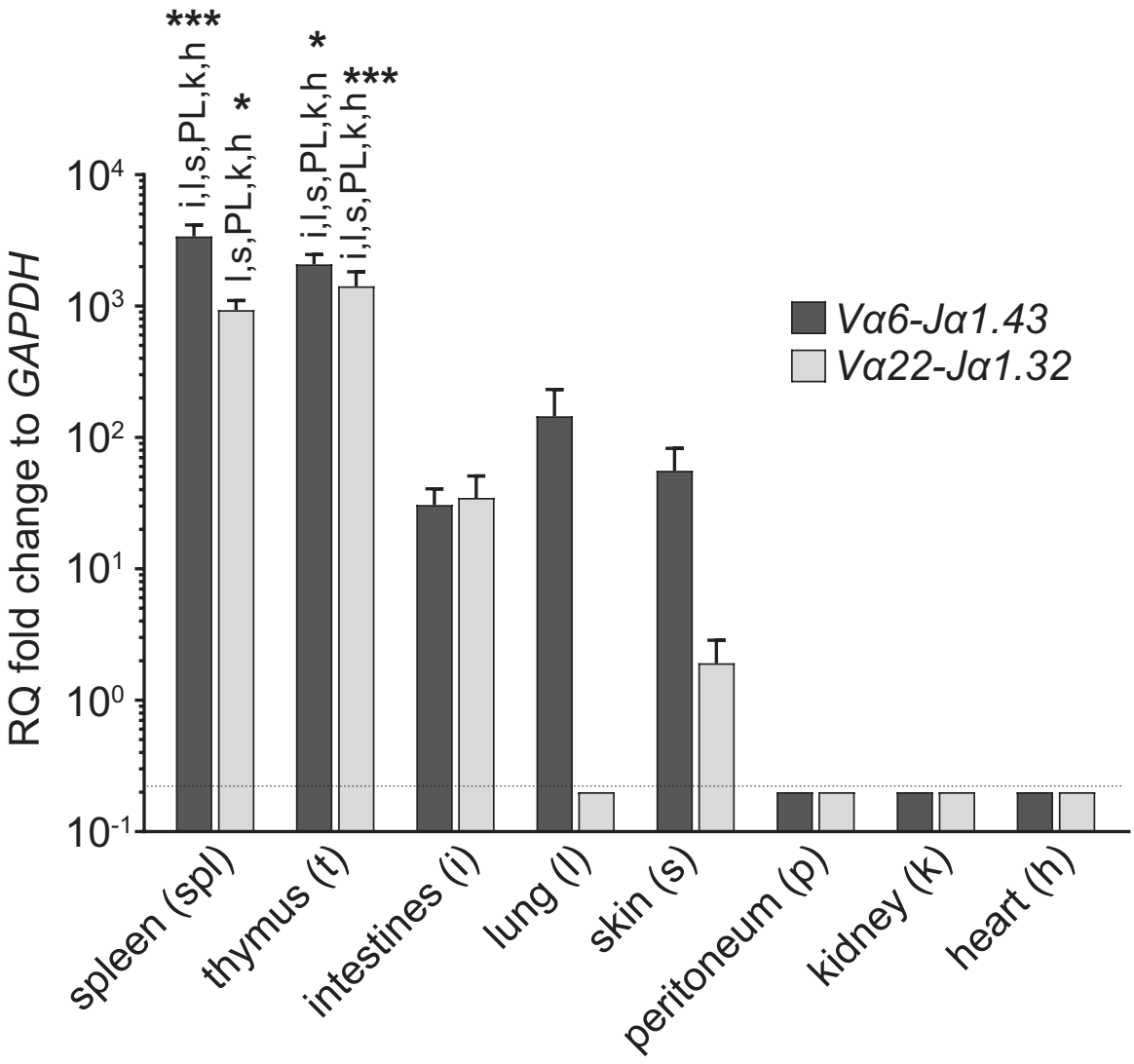
		Primer sequence
Genomic *XNC10 α2*	F	5'-cattagaccactacccagacttg-3'
	R	5'-aggaaggtgcagtttcagtt-3'
Genomic *XNC5*	F	5'-agggtgccattcgtgtttat-3'
	R	5'-ctctgtggtctcacctttcttc-3'
qPCR primers		
GAPDH	F	5'-gacatcaaggccgccattaagact-3'
	R	5'-agatggaggagtgagtgtcaccat-3'
XNC1	F	5'-catttgaggtgtggcaaagg-3'
	R	5'-gcagctcacatgcagatttc-3'
XNC4	F	5'-accaaagttgcccaggaata-3'
	R	5'-gcagctcacaggcattatttg-3'
XNC10	F	5'-ctccatcgcattcgtctttc-3'
	R	5'-tcttcaacaccagtcttgttt-3'
XNC11	F	5'-cacagatggcaggtttctca-3'
	R	5'-tgtagctcacaggcatttctac-3'
Vα6-Jα1.43 iTCR	F	5'-ggaattgctggttaataatttggga-3'
	R	5'-aaatgtgagtttgtggaaccccc -3'
Vα22-Jα1.32 iTCR	F	5'-atgtggtggacggacatacag-3'
	R	5'-tggaaccagctccagcattag-3'
Vα23-Jα1.3 iTCR	F	5'-cgcgataaactcaaaggaagaata-3'
	R	5'-ctttccatagcctattccagta-3'
Vα40-Jα1.22 iTCR	F	5'-tccagcctaccatggaagaag-3'
	R	5'-catatttccatagccaccagtact-3'
Vα41-Jα1.40 iTCR	F	5'-cccagcctcccaagaaggaggtc-3'
	R	5'-ggtaagtttattccagcctcctgt-3'
Vα45-Jα1.14 iTCR	F	5'-tccgttaaagagaaggattcccag-3'
	R	5'-ctcccagccactaccagaataag-3'
perforin	F	5'-cctgcttcacaccgttactt-3'
	R	5'-agaatgttcccagcactcttc-3'
FasL	F	5'-ggagaactcacgctgatgaa-3'
	R	5'-ggttaaacagagcacccagata-3'
iNOS	F	5'-aaccgtaagccaaagaagga-3'
	R	5'-tggttctggcagccacagt-3'
Arg1	F	5'-tccaagggacagccaagaag-3'
	R	5'-ctcgaacatcattgccaaattc-3'

Supplementary Table 1B: List of primer sequences

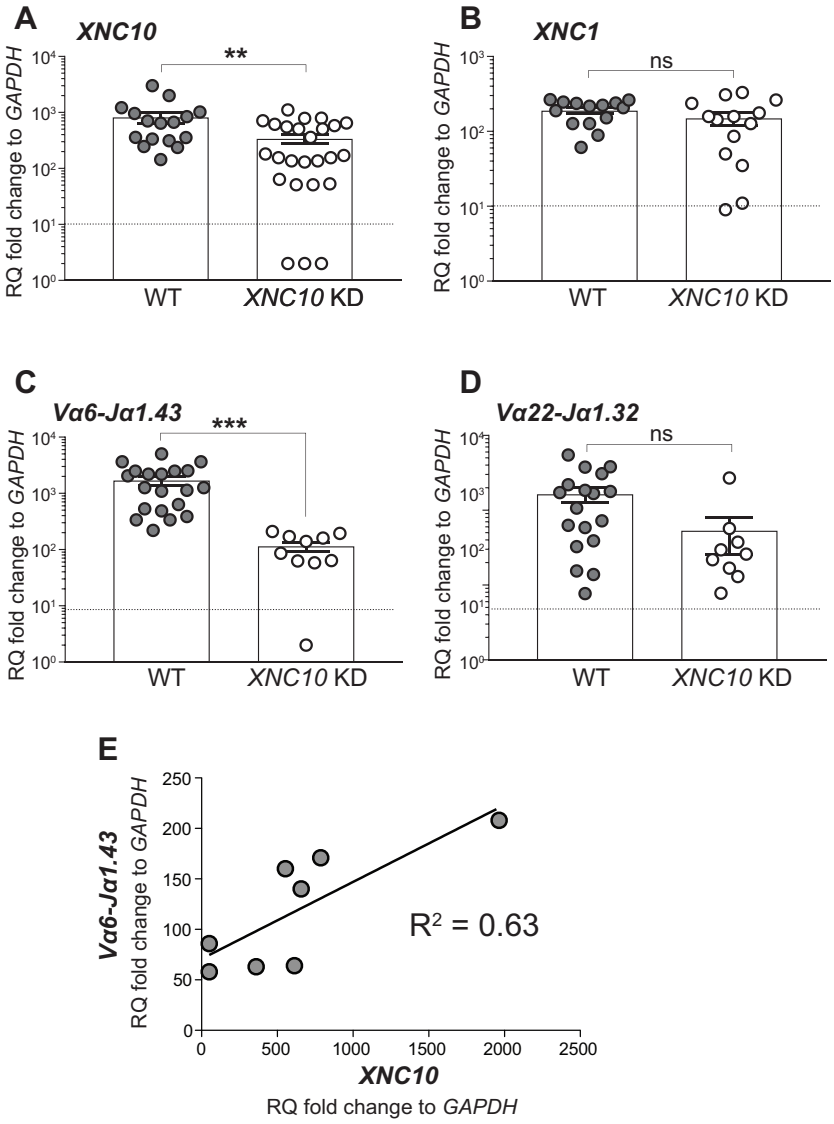
Construction of XNC10-tRFP-puro homology template		
XNC10-HAL	F1	5’-cgatgactggcttcttgtgc-3’
XNC10-HAL	F2	5’-tgcggattggttgggtgaag-3’
XNC10-HAL	R1	5’-ctcgggacctggggttttc-3’
XNC10-HAL	R2	5’-actgggctgtcgggacactg-3’
nested-XNC10	F	5’-gagtatgtggggatcacaatggg-3’
nested-XNC10	R	5’-ggtgcagtttcagttgactgatg-3’
1a-HAL-HindIII	F	5’-atgattacgccaagctttacatttcactggcgaatcagagcac-3’
1b-HAL-HindIII	F	5’-atgattacgccaagcttctggactccgtggctccctg-3’
2-HAL	R	5’-agctcacatccattctttattggcag-3’
3-HAR-Nhe	F	5’-agaatggatgtgagctagcgacggcagcattcgtggtaatgaag-3’
4a-HAR-XbaI	R	5’-gaattgggccctctagacctggggttttccagataacaaatctttcc-3’
4b-HAR-XbaI	R	5’-gaattgggccctctagatctttccataatttgcatcttcctacc-3’
SpeI-EF1a	F	5’-actagttgctccggtgcccgtcagtgg-3’
SpeI-SV40	R	5’-actagtcatcatttgagtcaattccagacatg-3’
sequencing pA		5’-gagtatgtggggatcacaatggg-3’
sequencing pB		5’-cgcactctagttatgccactgg-3’
sequencing pC		5’-ggtgcagtttcagttgactgatg-3’
sequencing pD		5’-cgaatttggactattccctagtcg-3’
XNC10α2-tRFP-puro	F	5’-cccattccacaaagacaggata-3’
XNC10α2-tRFP-puro	R	5’-aggggccataacccgtaaag-3’
RNAi-mediated XNC10 deficiency		
BbsI-sgRNA-XNC10α2	top	5’-caccgggatgtgagctgagtgagga-3’
BbsI-sgRNA-XNC10α2	bottom	5’-aaactcctcactcagctcacatccc-3’



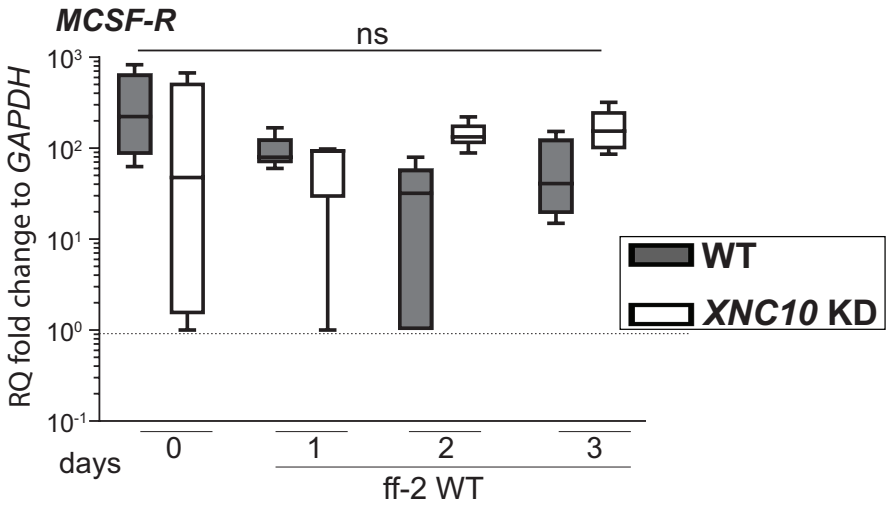
Supplementary Figure 1. iTCR rearrangements that are not altered after ff-2 tumor transplantation. Relative expression of four iTCR α chains: *Vα23-Jα1.3*, *Vα45-Jα1.14*, *Vα40-Jα1.22*, *Vα41-Jα1.40* was monitored in the peritoneal cavity, spleen, thymus, and liver after challenge with $1x10^5$ ff-2 WT tumor cells. Tadpoles at the developmental stage 54-55 were used. Peritoneal lavages, spleen, thymus, and liver were collected from un-, mock- and tumor-challenged tadpoles to assess gene expression by qPCR. Mock challenge was performed by injecting APBS only. Each dot represents one tadpole. Dotted black line indicates the limit of qPCR detection. Gene expression was normalized against endogenous *GAPDH* expression and represented as fold change compared to the lowest level of expression. Results are pooled from three independent experiments and presented as the mean ± SEM (n = 3 – 15). One-way ANOVA followed by post hoc Tukey's multiple comparisons tests were used to determine significance of differences among groups. ns, no statistical significance among any paired groups.



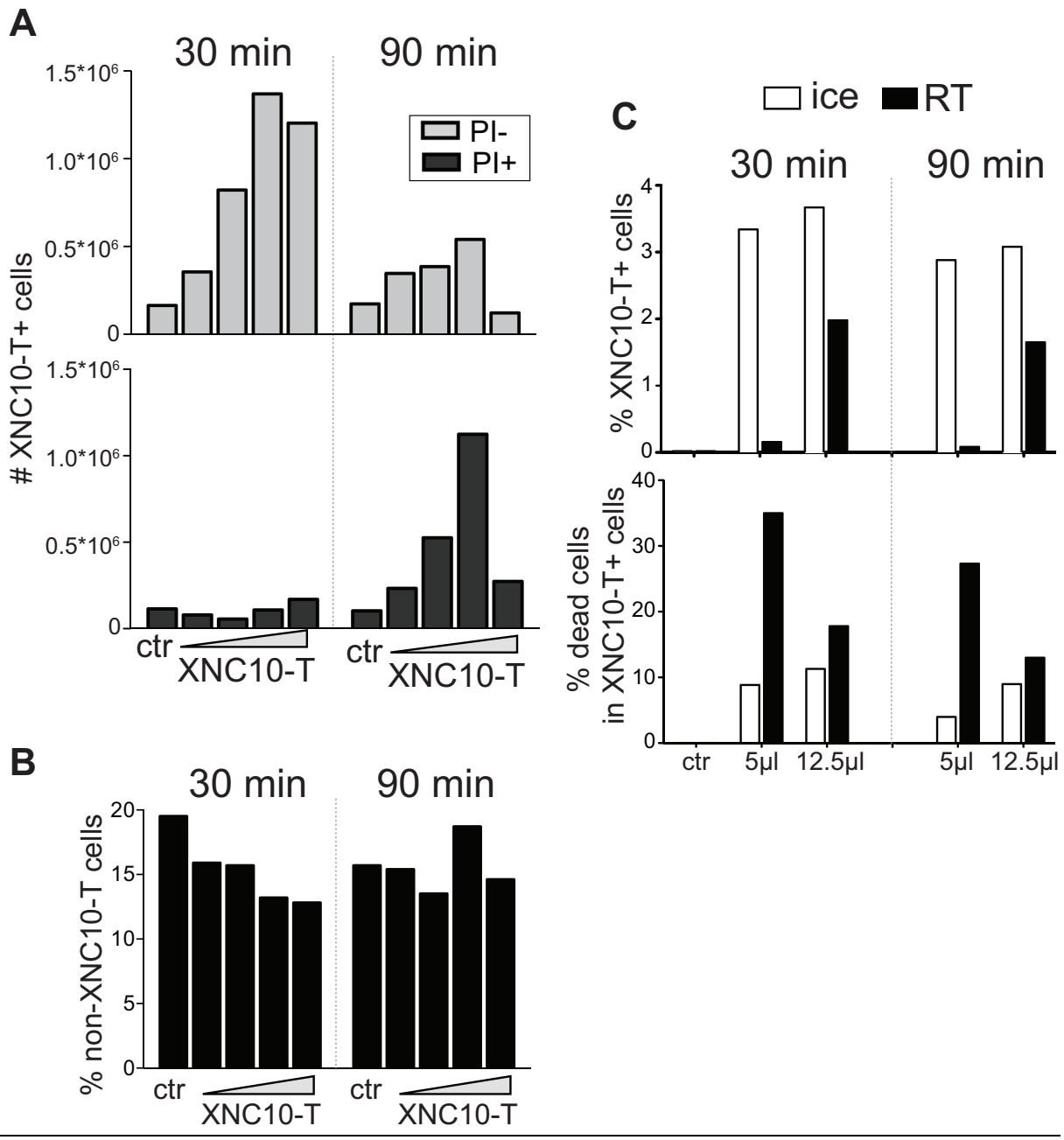
Supplementary Figure 2. Relative expression of *Vα6-Jα1.43* and *Vα22-Jα1.32* iTCR rearrangements in different tissues of unchallenged F tadpoles. Dotted line indicates the qPCR detection limit. Gene expression was normalized against *GAPDH* expression and represented as fold change compared to the lowest level of expression. Results are pooled from three independent experiments and presented as the mean ± SEM (n = 6 – 21). One-way ANOVA followed by post hoc Tukey's multiple comparisons tests were used to determine significance of differences among groups and defined as * $p < 0.05$, *** $p < 0.0005$.



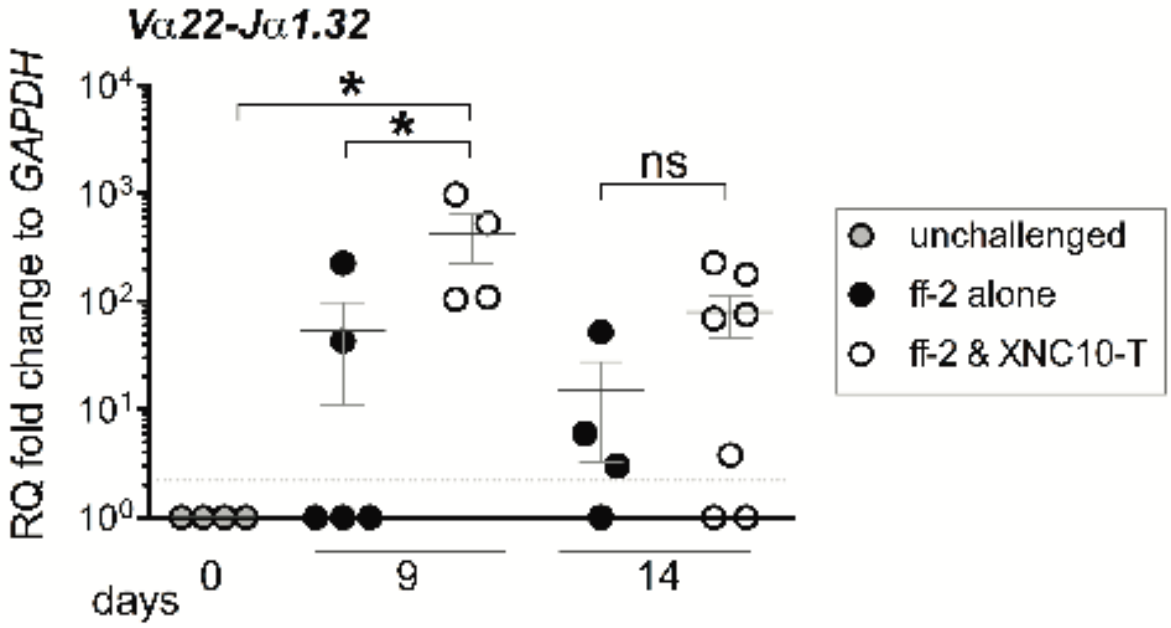
Supplementary Figure 3. Unchallenged *XNC10* KD F transgenic tadpoles have deficiency in *XNC10* and *Vα6-Jα1.43* iTCR transcript levels. Spleens of *XNC10* deficient (KD) Tg inbred F and WT inbred F control tadpoles at the developmental stage 54-55 were used to assess transcript levels of *XNC10* (A), *XNC1* (B), *Vα6-Jα1.43* (C), and *Vα22-Jα1.32* (D) by qPCR. (E) Correlation of XNC10 silencing with reduced *Vα6-Jα1.43* gene expression per individual tadpoles, assessed in spleens of unchallenged *XNC10* KD inbred F tadpoles. Gene expression was normalized against *GAPDH* expression and represented as fold change compared to the lowest level of expression. Results were pooled from two independent experiments and presented as the mean ± SEM (n = 5 – 15). Two-tailed unpaired t test was used to determine significance of differences between groups and defined as ** $p < 0.005$, *** $p < 0.0005$. ns, no statistical significance.



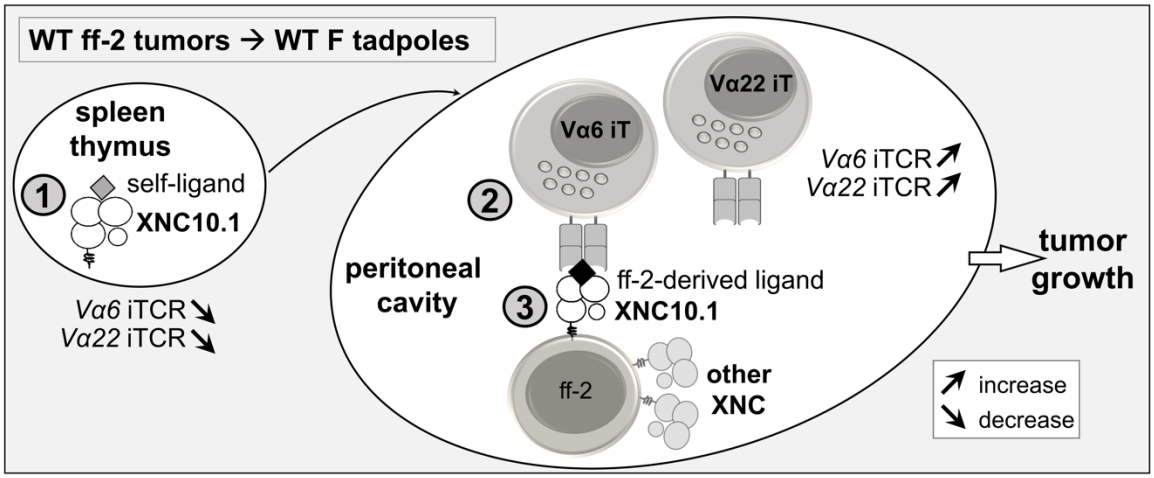
Supplementary Figure 4. No differences between WT or *XNC10* KD inbred F hosts in relative abundance of *MSCF-R* transcripts after ff-2 tumor transplantations. WT or *XNC10* KD Tg inbred F tadpoles at the developmental stage 54-55 were ip transplanted with $1x10^5$ ff-2 WT tumor cells. Following tumor transplantation, peritoneal lavages were collected to assess gene expression by qPCR. Gene expression was normalized against *GAPDH* expression and represented as fold change compared to the lowest level of expression. Results were pooled from three independent experiments and presented as the mean ± SEM (n = 9 – 20). Two-tailed unpaired t test was used to determine significance of differences between groups. ns, no statistical significance.



Supplementary Figure 5. Effects of XNC10-Tetramer treatment. (A) XNC10-T induced Vα6 iT cell death *ex vivo* represented as cell numbers. Freshly isolated splenocytes from an outbred *X. laevis* adult were stained with 4 different concentrations of APC-conjugated XNC10-T (5.0, 7.5, 10.0 and 12.5 µg/12.5 µl) for 30 min and 90 min. (B) XNC10-T did not affect the viability of cells other than Vα6 iT cells *ex vivo*. Freshly isolated splenocytes from an adult outbred *X. laevis* were stained with 4 different concentrations of APC-conjugated XNC10-T (5.0, 7.5, 10.0 and 12.5 µg/12.5 µl) for 30 min and 90 min. Live/dead cell viability dye – propidium iodide (PI) was used to stain dead cells. (C) Enhanced XNC10-T induced Vα6 iT cell death *ex vivo* at room temperature (RT).



Supplementary Figure 6. Changes in *Vα22-Jα1.32* iTCR transcript levels following ff-2 tumor transplantation and XNC10-T administration. Tadpoles at the developmental stage 54-55 were used and peritoneal cells were collected for transcriptional analysis. Each dot represents one tadpole. Dotted line indicates the qPCR detection limit. Gene expression was normalized against *GAPDH* expression and represented as fold change compared to the lowest level of expression. Results are presented as the mean ± SEM (n = 4 – 7). One-way ANOVA followed by post hoc Tukey's multiple comparisons tests were used to determine significance of differences among groups and defined as * $p < 0.05$. ns, no statistical significance.



	Target	Transplantation	Tumor outcome
1	KD of host *XNC10*	ff-2 WT tumors → XNC10.1 KD F tadpoles	no growth
2	Vα6-T cell depletion	ff-2 WT & XNC10.1-T → WT F tadpoles	enhanced growth
3	KO of tumor *XNC10*	ff-2 XNC10 KO → WT F tadpoles	tumor rejection

Supplementary Figure 7. Working model of interactions between *Xenopus* ff-2 lymphoid tumor and two different iT cell subsets in the peritoneal cavity of tumor-challenged tadpoles. Upon intraperitoneal tumor transplantation, both Vα6 iT and Vα22 iT cells infiltrate into the peritoneal cavity. Vα6 iT cells either directly or indirectly limit tumor growth. Expression of XNC10 on tumor cells suppresses Vα6 iT cell cytotoxicity, promoting tumor growth. The ligand for Vα22 iT cells is unknown. By impairing Vα6 iT cells or tumor expression of XNC10 genes, tumor growth can be manipulated. Accordingly, *XNC10* deficient ff-2 tumor cells are rejected by WT inbred F tadpoles. Alternatively, blocking Vα6 iT cell function with XNC10-tetramer enhances ff-2 WT tumor growth. Surprisingly, F tadpoles with *XNC10* deficiency reject transplanted ff-2 tumors.