Supplemental Material

Itch-induced tick removal (IITR) is a common immune defense response against ixodid ticks in guinea pigs

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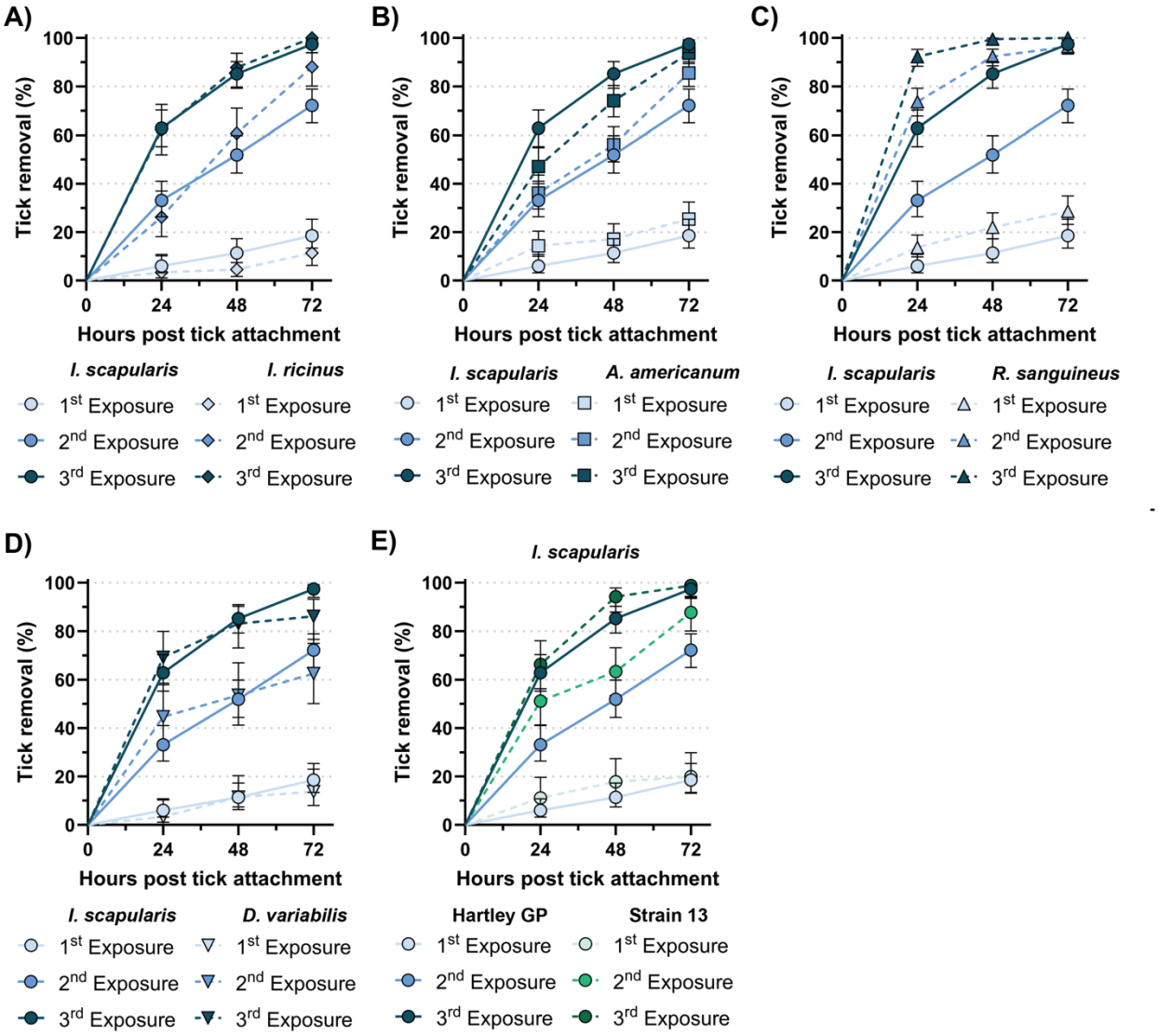
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1. Supplementary Figures

1.1 Supplementary Figure 1



Survival plots of other tick species overlayed with *I. scapularis*. (A-E) Smoothed, inverted Kaplan–Meyer plots showing probability of tick removal (%) for three weekly spaced consecutive tick exposures. Animals are tick-naïve for the 1st exposure and tick-sensitized for subsequent exposures. These are the same graph from Figure 1. *I. scapularis* removal success is overlayed with (A) *I. ricinus*, (B) *A. americanum*, (C) *R. sanguineus*, (D) *D. variabilis*, and (E) Strain 13 guinea pigs exposed 3-times to *I. scapularis* nymphs. Analyzed by Cox proportional hazards model (see **Supp. Table 2**).

2. Supplementary Tables

2.1 Supplementary Table 1. Statistical output of pairwise comparison of tick removal by Cox proportional hazards model

A)

Pairwise Comparison

I_scapularis

contrast	estimate	SE	df	z.ratio	p.value	sig.
1st - 2nd	-1.8678	0.2042	Inf	-9.1468	<0.0001	****
1st - 3rd	-2.8002	0.2045	Inf	-13.6942	<0.0001	****
2nd - 3rd	-0.9323	0.1284	Inf	-7.2595	<0.0001	****

Adjusted Hazards Ratios

I_scapularis

Factor	HR	2.5 %	97.5 %	p-value	sig.
Infestation2nd	6.4743	4.3388	9.6608	<0.0001	****
Infestation3rd	16.4475	11.0166	24.5557	<0.0001	****

B)

Pairwise Comparison

I_ricinus

contrast	estimate	SE	df	z.ratio	p.value	sig.
1st - 2nd	-2.9223	0.3449	Inf	-8.4728	<0.0001	****
1st - 3rd	-3.7636	0.3518	Inf	-10.6990	<0.0001	****
2nd - 3rd	-0.8413	0.1725	Inf	-4.8784	<0.0001	****

Adjusted Hazards Ratios

I_ricinus

Factor	HR	2.5 %	97.5 %	p-value	sig.
Infestation2nd	18.5832	9.4524	36.5340	<0.0001	****
Infestation3rd	43.1023	21.6309	85.8867	<0.0001	****

C)

Pairwise Comparison

A_americanum

Hours_pi	contrast	log_HR	SE	z	p.value	HR	lower.CL	upper.CL	p.adj	sig.
24	1st vs 2nd	-1.1116	0.2324	-4.7830	<0.0001	0.3290	0.2086	0.5189	<0.0001	****
24	1st vs 3rd	-1.4976	0.2251	-6.6539	<0.0001	0.2237	0.1439	0.3477	<0.0001	****
24	2nd vs 3rd	-0.3860	0.1618	-2.3853	0.0171	0.6798	0.4950	0.9335	0.0171	*
48	1st vs 2nd	-2.0746	0.1894	-10.9544	<0.0001	0.1256	0.0867	0.1821	<0.0001	****
48	1st vs 3rd	-2.4572	0.1919	-12.8047	<0.0001	0.0857	0.0588	0.1248	<0.0001	****
48	2nd vs 3rd	-0.3826	0.1250	-3.0601	0.0022	0.6821	0.5338	0.8715	0.0022	**
72	1st vs 2nd	-2.6380	0.2822	-9.3467	<0.0001	0.0715	0.0411	0.1243	<0.0001	****
72	1st vs 3rd	-3.0186	0.2883	-10.4700	<0.0001	0.0489	0.0278	0.0860	<0.0001	****
72	2nd vs 3rd	-0.3806	0.1867	-2.0382	0.0415	0.6834	0.4740	0.9855	0.0415	*

D)

Pairwise Comparison

D_variabilis

contrast	estimate	SE	df	z.ratio	p.value	sig.
1st - 2nd	-1.8992	0.3372	Inf	-5.6319	<0.0001	****
1st - 3rd	-2.6087	0.3249	Inf	-8.0292	<0.0001	****
2nd - 3rd	-0.7096	0.2247	Inf	-3.1575	0.0016	**

Adjusted Hazards Ratios

D_variabilis

Factor	HR	2.5 %	97.5 %	p-value	sig.
Infestation2nd	6.6802	3.4495	12.9368	<0.0001	****
Infestation3rd	13.5820	7.1846	25.6759	<0.0001	****

E)

Pairwise Comparison

R_sanguineus

contrast	estimate	SE	df	z.ratio	p.value	sig.
1st - 2nd	-2.3338	0.1507	Inf	-15.4830	<0.0001	****
1st - 3rd	-2.8038	0.1583	Inf	-17.7087	<0.0001	****
2nd - 3rd	-0.4701	0.0988	Inf	-4.7564	<0.0001	****

Adjusted Hazards Ratios

R_sanguineus

Factor	HR	2.5 %	97.5 %	p-value	sig.
Infestation2nd	10.3166	7.6778	13.8624	<0.0001	****
Infestation3rd	16.5075	12.1035	22.5140	<0.0001	****

F)

Pairwise Comparison

Strain_13

Hours_pi	contrast	log_HR	SE	z	p.value	HR	lower.CL	upper.CL	p.adj	sig.
24	1st vs 2nd	-1.6556	0.3334	-4.9653	<0.0001	0.1910	0.0993	0.3671	<0.0001	****
24	1st vs 3rd	-2.1310	0.3255	-6.5467	<0.0001	0.1187	0.0627	0.2247	<0.0001	****
24	2nd vs 3rd	-0.4754	0.1979	-2.4027	0.0163	0.6216	0.4218	0.9161	0.0163	*
48	1st vs 2nd	-2.4096	0.3238	-7.4412	<0.0001	0.0899	0.0476	0.1695	<0.0001	****
48	1st vs 3rd	-3.3080	0.3440	-9.6152	<0.0001	0.0366	0.0186	0.0718	<0.0001	****
48	2nd vs 3rd	-0.8984	0.2190	-4.1013	<0.0001	0.4072	0.2651	0.6256	<0.0001	****
72	1st vs 2nd	-2.8506	0.4966	-5.7407	<0.0001	0.0578	0.0218	0.1530	<0.0001	****
72	1st vs 3rd	-3.9964	0.5330	-7.4974	<0.0001	0.0184	0.0065	0.0523	<0.0001	****
72	2nd vs 3rd	-1.1458	0.3436	-3.3347	9e-04	0.3180	0.1622	0.6235	9e-04	***

The tables show the statistical output for the pairwise comparison and the Adjusted Hazard Ratios (HR) for the repeated exposures of Hartley or Strain 13 guinea pigs to various tick species (refer to Fig. 1). Tables refer to experiments done with (A) *I. scapularis*, (B) *I. ricinus*, (C) *A. americanum*, (D) *R. sanguineus*, (E), *D. variabilis* and (F) Strain 13 [guinea pigs]. P-value adjustments were done by False-Discovery Rate test (FDR). "" > 0.05, * ≤ 0.05, ** < 0.01, *** < 0.001, **** < 0.0001

2.2 Supplementary Table 2. Statistical output of pairwise comparison by tick exposure

A)

Pairwise Comparison

I_ricinus

contrast	estimate	SE	df	z.ratio	p.value	sig.
I_scapularis_1st - I_ricinus_1st	0.5456	0.4041	Inf	1.3502	0.2042	
I_scapularis_2nd - I_ricinus_2nd	-0.3104	0.2802	Inf	-1.1077	0.2872	
I_scapularis_3rd - I_ricinus_3rd	-0.0804	0.1658	Inf	-0.4850	0.6277	

B)

Pairwise Comparison

A_americanum

contrast	estimate	SE	df	z.ratio	p.value	sig.
I_scapularis_1st - A_americanum_1st	-0.3838	0.3431	Inf	-1.1186	0.2633	
I_scapularis_2nd - A_americanum_2nd	-0.2850	0.2077	Inf	-1.3721	0.1822	
I_scapularis_3rd - A_americanum_3rd	0.3154	0.1413	Inf	2.2313	0.0296	*

C)

Pairwise Comparison

R_sanguineus

contrast	estimate	SE	df	z.ratio	p.value	sig.
I_scapularis_1st - R_sanguineus_1st	-0.5268	0.2903	Inf	-1.8149	0.0745	+
I_scapularis_2nd - R_sanguineus_2nd	-1.0980	0.1726	Inf	-6.3626	<0.0001	****
I_scapularis_3rd - R_sanguineus_3rd	-0.6886	0.1068	Inf	-6.4457	<0.0001	****

D)

Pairwise Comparison

D_variabilis

contrast	estimate	SE	df	z.ratio	p.value	sig.
I_scapularis_1st - D_variabilis_1st	0.3158	0.7304	Inf	0.4324	0.7130	
I_scapularis_2nd - D_variabilis_2nd	0.0990	0.2913	Inf	0.3400	0.7339	
I_scapularis_3rd - D_variabilis_3rd	0.1448	0.1693	Inf	0.8551	0.4529	

E)

Pairwise Comparison

Strain_13

contrast	estimate	SE	df	z.ratio	p.value	sig.
I_scapularis_1st - Strain_13_1st	-0.1198	0.3004	Inf	-0.3989	0.6900	
I_scapularis_2nd - Strain_13_2nd	-0.4573	0.2230	Inf	-2.0508	0.0465	*
I_scapularis_3rd - Strain_13_3rd	-0.1704	0.1178	Inf	-1.4468	0.1585	

The tables show the statistical output for the pairwise comparison by tick exposure between the removal data of *I. scapularis* exposure and exposure to other tick of Hartley or Strain 13 guinea pigs (refer to **Supp. Fig. 1**). Tables refer to comparison of the *I. scapularis* removal data to

experiments done with (A) *I. ricinus*, (B) *A. americanum*, (C) *R. sanguineus*, (D) *D. variabilis*, and (E) Strain 13 [guinea pigs]. P-value adjustments were done by False-Discovery Rate test (FDR). “” > 0.05, * ≤ 0.05, ** < 0.01, *** < 0.001, **** < 0.0001

2.3 Supp. Table 3. Statistical output of the Bayesian Count-scale follow-up model

Countscale pairwise outcomes														
Dataset	model	outcome	level_1	level_2	contrast	scale	direction	evidence	mean	median	lower_95	upper_95	prob_greater_0	prob_less_0	prob_practically_small
Tick remaining at 24 h pta															
I_scapularis	Count-scale companion model	TicksRemaining	1st	2nd	2nd - 1st	ticks remaining out of 15	decrease	very strong directional evidence	-5.3884	-5.4116	-7.9090	-2.6936	0.0004	0.9996	0.0013
I_scapularis	Count-scale companion model	TicksRemaining	1st	3rd	3rd - 1st	ticks remaining out of 15	decrease	very strong directional evidence	-6.1988	-6.2399	-8.5584	-3.5762	0.0000	1.0000	0.0005
I_scapularis	Count-scale companion model	TicksRemaining	2nd	3rd	3rd - 2nd	ticks remaining out of 15	decrease	uncertain	-0.8104	-0.8240	-3.3459	1.7820	0.2672	0.7328	0.4710
A_americanum	Count-scale companion model	TicksRemaining	1st	2nd	2nd - 1st	ticks remaining out of 15	decrease	very strong directional evidence	-3.4917	-3.4721	-5.5751	-1.5484	0.0003	0.9997	0.0057
A_americanum	Count-scale companion model	TicksRemaining	1st	3rd	3rd - 1st	ticks remaining out of 15	decrease	very strong directional evidence	-4.7304	-4.7283	-6.8885	-2.6471	0.0000	1.0000	0.0002
A_americanum	Count-scale companion model	TicksRemaining	2nd	3rd	3rd - 2nd	ticks remaining out of 15	decrease	uncertain	-1.2387	-1.2365	-3.3590	0.8654	0.1268	0.8732	0.3968
R_sanguineus	Count-scale companion model	TicksRemaining	1st	2nd	2nd - 1st	ticks remaining out of 15	decrease	very strong directional evidence	-8.5194	-8.5591	-10.0926	-6.6835	0.0000	1.0000	0.0000
R_sanguineus	Count-scale companion model	TicksRemaining	1st	3rd	3rd - 1st	ticks remaining out of 15	decrease	very strong directional evidence	-11.0627	-11.1102	-12.3740	-9.4641	0.0000	1.0000	0.0000
R_sanguineus	Count-scale companion model	TicksRemaining	2nd	3rd	3rd - 2nd	ticks remaining out of 15	decrease	very strong directional evidence	-2.5432	-2.5033	-4.2358	-1.0894	0.0004	0.9996	0.0182
Total Scratch bouts															
I_scapularis	Count-scale companion model	ScratchBouts	1st	2nd	2nd - 1st	original outcome scale	increase	uncertain	5.8861	5.7243	-14.4799	27.4964	0.7281	0.2719	0.0706
I_scapularis	Count-scale companion model	ScratchBouts	1st	3rd	3rd - 1st	original outcome scale	increase	uncertain	13.9971	13.7228	-8.3912	38.0173	0.8976	0.1024	0.0318
I_scapularis	Count-scale companion model	ScratchBouts	2nd	3rd	3rd - 2nd	original outcome scale	increase	uncertain	8.1110	7.9104	-15.7594	33.2219	0.7606	0.2394	0.0548
A_americanum	Count-scale companion model	ScratchBouts	1st	2nd	2nd - 1st	original outcome scale	increase	very strong directional evidence	34.2132	33.2719	9.8490	63.4240	0.9972	0.0028	0.0014
A_americanum	Count-scale companion model	ScratchBouts	1st	3rd	3rd - 1st	original outcome scale	increase	very strong directional evidence	58.8774	57.4747	29.6608	96.3800	1.0000	0.0000	0.0002
A_americanum	Count-scale companion model	ScratchBouts	2nd	3rd	3rd - 2nd	original outcome scale	increase	suggestive directional evidence	24.6642	23.8514	-8.6958	62.6960	0.9356	0.0644	0.0132
R_sanguineus	Count-scale companion model	ScratchBouts	1st	2nd	2nd - 1st	original outcome scale	increase	uncertain	19.2643	18.2072	-15.9345	60.6334	0.8658	0.1342	0.0275
R_sanguineus	Count-scale companion model	ScratchBouts	1st	3rd	3rd - 1st	original outcome scale	increase	uncertain	21.6035	20.7089	-15.2581	64.4486	0.8878	0.1122	0.0213
R_sanguineus	Count-scale companion model	ScratchBouts	2nd	3rd	3rd - 2nd	original outcome scale	increase	uncertain	2.3392	2.3543	-42.3365	47.6619	0.5481	0.4519	0.0426

The table shows the pairwise comparison split by the two dependent variables. Bayesian statistic does not produce p-values by posterior probability values to determine strength of effect. The mean column refer to how many more scratch bouts / reduction in attached ticks are expected between one exposure round and another. If 0 is not included in the 95% confidence interval, than the difference is significant.

2.4. Supplementary Table 4. Statistical output of Cox proportional hazards model of tick exposed guinea pigs restricted by Elizabethan collar

A)

Pairwise Comparison

I_scapularis

contrast	estimate	SE	df	z.ratio	p.value	sig.
Unrestricted - Restricted	3.0453	0.3249	Inf	9.3716	<0.0001	****

Adjusted Hazards Ratios

I_scapularis

Factor	HR	2.5 %	97.5 %	p-value	sig.
InfestationRestricted	0.0476	0.0252	0.09	<0.0001	****

B)

Pairwise Comparison

A_americanum

contrast	estimate	SE	df	z.ratio	p.value	sig.
Unrestricted - Restricted	2.2983	0.3198	Inf	7.1861	<0.0001	****

Adjusted Hazards Ratios

A_americanum

Factor	HR	2.5 %	97.5 %	p-value	sig.
InfestationRestricted	0.1004	0.0537	0.188	<0.0001	****

C)

Pairwise Comparison

R_sanguineus

contrast	estimate	SE	df	z.ratio	p.value	sig.
Unrestricted - Restricted	2.6243	0.2898	Inf	9.0555	<0.0001	****

Adjusted Hazards Ratios

R_sanguineus

Factor	HR	2.5 %	97.5 %	p-value	sig.
InfestationRestricted	0.0725	0.0411	0.1279	<0.0001	****

The tables show the statistical output for the pairwise comparison and the Adjusted Hazard Ratios (HR) for sensitized guinea pigs once exposed to ticks with Elizabethan collar and once without (refer to **Fig. 2A-C**). The tables refer to exposures with (A) *I. scapularis*, (B) *A. americanum*, and (C) *R. sanguineus*. "" > 0.05, * ≤ 0.05, ** < 0.01, *** < 0.001, **** < 0.0001

2.5. **Supplementary Table 5.** Statistical output of the analysis of circulating lymphocyte counts in whole blood

A)

Two way Mixed ANOVA

I_scapularis

Factor	value	df1	df2	p.value	sig.
State	13.4990	1	5.4839	0.0122	*
Treatment	24.6455	2	7.0148	0.0007	***
State:Treatment	15.5081	2	7.0148	0.0027	**

One-way ANOVA

I_scapularis

Treatment	Effect	test	df1	df2	p.value	effsize	effsize_ci_lower	effsize_ci_upper	p.value.adj	sig.
Pre-Treatment	State	0.0208	1	7.6640	0.8891	0.0841	0.0166	0.8152	0.8891	
Pre-Infestation	State	15.3547	1	5.3351	0.0099	0.9408	0.7002	1.5033	0.0148	**
Post-Infestation	State	38.5027	1	6.0830	0.0008	1.0685	0.9462	1.2117	0.0023	***

One-way Repeated Measures ANOVA

I_scapularis

State	Effect	test	df1	df2	p.value	p.value.adj	sig.
FTY720	Treatment	58.1150	1.1103	5.5514	0.0003	0.0007	***
PBS	Treatment	0.6953	1.7190	8.5949	0.5040	0.5040	

Pairwise Comparison by within factor

I_scapularis

Level	Group	Group.1	psihat	ci.lower	ci.upper	p.value	p.crit	sig.
FTY720	Pre-Treatment	Pre-Infestation	2.3906	0.8600	3.9212	0.0027	0.0250	**
FTY720	Pre-Treatment	Post-Infestation	2.4807	1.2741	3.6874	0.0008	0.0169	***
FTY720	Pre-Infestation	Post-Infestation	0.0901	-0.3455	0.5258	0.4976	0.0500	
PBS	Pre-Treatment	Pre-Infestation	-0.1476	-2.3320	2.0368	0.8207	0.0500	
PBS	Pre-Treatment	Post-Infestation	0.3898	-0.8756	1.6553	0.3259	0.0169	
PBS	Pre-Infestation	Post-Infestation	0.5374	-2.0337	3.1086	0.4933	0.0250	

B)

Two way Mixed ANOVA

A_americanum

Factor	value	df1	df2	p.value	sig.
State	26.6579	1	6.7755	0.0014	**
Treatment	28.2159	2	6.3795	0.0007	***
State:Treatment	3.5418	2	6.3795	0.0923	+

Pairwise Comparison by within factor

A_americanum

Group	Group.1	psihat	ci.lower	ci.upper	p.value	p.crit	p.value.adj	sig.
Pre-Treatment	Pre-Infestation	2.2865	1.1336	3.4394	3e-04	0.0250	0.0004	***
Pre-Treatment	Post-Infestation	2.0805	1.3247	2.8363	<0.0001	0.0169	0.0001	****
Pre-Infestation	Post-Infestation	-0.2348	-1.1346	0.6649	0.4635	0.0500	0.4635	

Pairwise Comparison by between factor

A_americanum

test	df1	df2	p.value	effsize	effsize_ci_lower	effsize_ci_upper	sig.
11.3336	1	28.9095	0.0022	0.6577	0.2669	0.9494	**

C)

Two way Mixed ANOVA

R_sanguineus

Factor	value	df1	df2	p.value	sig.
State	87.0871	1	5.8153	0.0001	***
Treatment	21.8238	2	6.0394	0.0017	**
State:Treatment	12.9581	2	6.0394	0.0065	**

One-way ANOVA

R_sanguineus

Treatment	Effect	test	df1	df2	p.value	effsize	effsize_ci_lower	effsize_ci_upper	p.value.adj	sig.
Pre-Treatment	State	4.0528	1	7.0522	0.0837	0.7501	0.3534	1.0222	0.0837	+
Pre-Infestation	State	260.0203	1	5.7135	<0.0001	1.1667	1.0849	1.1872	<0.0001	****
Post-Infestation	State	148.3630	1	5.5220	<0.0001	1.1116	1.0538	1.1447	1e-04	****

One-way Repeated Measures ANOVA

R_sanguineus

State	Effect	test	df1	df2	p.value	p.value.adj	sig.
FTY720	Treatment	119.2776	1.2098	6.049	<0.0001	0.0001	****
PBS	Treatment	2.0118	2.0000	10.000	0.1844	0.1844	

Pairwise Comparison by within factor

R_sanguineus

Level	Group	Group.1	psihat	ci.lower	ci.upper	p.value	p.crit	sig.
FTY720	Pre-Treatment	Pre-Infestation	1.9361	1.1782	2.6940	0.0003	0.0169	***
FTY720	Pre-Treatment	Post-Infestation	1.9688	1.1775	2.7601	0.0003	0.0250	***
FTY720	Pre-Infestation	Post-Infestation	0.0327	-0.1844	0.2497	0.6177	0.0500	
PBS	Pre-Treatment	Pre-Infestation	0.5242	-0.8444	1.8928	0.2338	0.0250	
PBS	Pre-Treatment	Post-Infestation	0.1149	-1.2060	1.4358	0.7709	0.0500	
PBS	Pre-Infestation	Post-Infestation	-0.4093	-1.3044	0.4858	0.1670	0.0169	

The tables show the statistical output for the two-way mixed ANOVA and pairwise comparisons of circulating lymphocyte counts in whole blood (refer to **Fig. 6A-C**). This data assessed the effectiveness of FTY720 to remove naïve lymphocytes from circulations prior to treatment with FTY720 (Pre-Treatment), prior to the first tick exposure (Pre-Exposure) and after the first tick exposure (Post-Exposure). “” > 0.05,* ≤ 0.05,** < 0.01,*** < 0.001,**** < 0.0001

2.6. Supplementary Table 6. Statistical output of pairwise comparison of tick removal by Cox proportional hazards model

A)

Pairwise Comparison

I_scapualris

contrast	estimate	SE	df	z.ratio	p.value	sig.
1st_PBS - 1st_FTY720	0.0645	0.3840	Inf	0.1680	0.8666	
1st_PBS - 2nd_PBS	-1.4450	0.2862	Inf	-5.0493	<0.0001	****
1st_PBS - 2nd_FTY720	-0.3701	0.3554	Inf	-1.0414	0.3572	
1st_FTY720 - 2nd_PBS	-1.5095	0.3228	Inf	-4.6757	<0.0001	****
1st_FTY720 - 2nd_FTY720	-0.4346	0.3350	Inf	-1.2972	0.2919	
2nd_PBS - 2nd_FTY720	1.0750	0.2879	Inf	3.7333	4e-04	***

Adjusted Hazards Ratios

I_scapualris

Factor	HR	2.5 %	97.5 %	p-value	sig.
Infestation1st_FTY720	0.9375	0.4417	1.9901	0.8666	
Infestation2nd_PBS	4.2420	2.4208	7.4330	<0.0001	****
Infestation2nd_FTY720	1.4478	0.7215	2.9054	0.2977	

B)

Pairwise Comparison

A_americanum

contrast	estimate	SE	df	z.ratio	p.value	sig.
1st_PBS - 1st_FTY720	-0.6271	0.5341	Inf	-1.1740	0.2404	
1st_PBS - 2nd_PBS	-2.3024	0.4054	Inf	-5.6792	<0.0001	****
1st_PBS - 2nd_FTY720	-1.2208	0.4997	Inf	-2.4428	0.0219	*
1st_FTY720 - 2nd_PBS	-1.6753	0.4032	Inf	-4.1547	1e-04	****
1st_FTY720 - 2nd_FTY720	-0.5937	0.3621	Inf	-1.6398	0.1213	
2nd_PBS - 2nd_FTY720	1.0816	0.3562	Inf	3.0369	0.0048	**

Adjusted Hazards Ratios

A_americanum

Factor	HR	2.5 %	97.5 %	p-value	sig.
Infestation1st_FTY720	1.8722	0.6572	5.3334	0.2404	
Infestation2nd_PBS	9.9984	4.5169	22.1318	<0.0001	****
Infestation2nd_FTY720	3.3898	1.2729	9.0274	0.0146	*

C)

Pairwise Comparison

R_sanguineus

contrast	estimate	SE	df	z.ratio	p.value	sig.
1st_PBS - 1st_FTY720	0.9144	0.4817	Inf	1.8984	0.0576	+
1st_PBS - 2nd_PBS	-1.9040	0.2106	Inf	-9.0416	<0.0001	****
1st_PBS - 2nd_FTY720	-0.8243	0.4140	Inf	-1.9913	0.0557	+
1st_FTY720 - 2nd_PBS	-2.8184	0.5261	Inf	-5.3568	<0.0001	****
1st_FTY720 - 2nd_FTY720	-1.7387	0.4706	Inf	-3.6942	4e-04	***
2nd_PBS - 2nd_FTY720	1.0797	0.4724	Inf	2.2853	0.0334	*

Adjusted Hazards Ratios

R_sanguineus

Factor	HR	2.5 %	97.5 %	p-value	sig.
Infestation1st_FTY720	0.4008	0.1559	1.0301	0.0576	+
Infestation2nd_PBS	6.7125	4.4427	10.1422	<0.0001	****
Infestation2nd_FTY720	2.2803	1.0131	5.1328	0.0464	*

The tables show the statistical output for the pairwise comparison and the Adjusted Hazard Ratios (HR) for FTY720- and PBS-treated guinea pigs exposed twice to (A) *I. scapularis*, (B) *A. americanum*, or (C) *R. sanguineus* (refer to **Fig. 6D-F**). "" > 0.05, * ≤ 0.05, ** < 0.01, *** < 0.001, **** < 0.0001

3. Supplementary methods

3.1 Software packages

Statistical analysis of time-to-event (survival) data, two-way mixed ANOVA, and Bayesian MANOVA were executed in RStudio v.2026.04.1-526 using R v.4.6.0[1,2]. We employed the following R packages: ARTool v. 0.11.2[3], bayesplot v. 1.15.0[4], bookdown v. 0.46[5], brms v. 2.23.0[6], car v. 3.1.5[7], conflicted v. 1.2.0[8], DescTools v. 0.99.60[9], effectsize v. 1.0.2[10], emmeans v. 2.0.3 [11], easypackages v. 0.1.0[12], ggpubr v. 0.6.3[13], glmmTMB v. 1.1.14[14], gt v. 1.3.0[15], here v. 1.0.2[16], Hmisc v. 5.2.5[17], janitor v. 2.2.1[18], knitr v. 1.51[19], lme4 v. 2.0.1[20], MANOVA.RM v. 0.5.4[21], magrittr v. 2.0.5[22], MASS v. 7.3.65[23], metan v. 1.19.0[24], moments v. 0.14.1[25], multcomp v. 1.4.30[26], nlme v. 3.1.169[27], openxlsx v. 4.2.8.1[28], PMCMRplus v. 1.9.12[29], posterior v. 1.7.0[30], pwrss v. 1.0.0[31], rcompanion v. 2.5.2[32], readxl v. 1.4.5[33], rmarkdown v. 2.31[34], rstatix v. 0.7.3[35], RVAideMemoire v. 0.9.83.12[36], sjmisc v. 2.8.11[37], survival v. 3.8.6[38], survminer v. 0.5.2[39], tidyverse v. 2.0.0[40], tools v. 4.6.0[2]. Parametric and non-parametric tests were executed in GraphPad Prism v.11.0.0.

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