

Supplemental material, tables S1 and S2a-c

Adaptive evolution of virus-sensing toll-like receptor 8 in bats

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Table S1. Investigated bat species, sample accession numbers due to the IZW biobank and geographic origin.

Accession Nr	Species	Origin
IZW-CCV-E250/13	<i>Nyctalus noctula</i>	Germany
IZW-CCV-2011/13	<i>Nyctalus leisleri</i>	Germany
IZW-CCV-E263/13	<i>Pipistrellus nathusii</i>	Germany
XM_008156576	<i>Eptesicus serotinus</i>	unknown
IZW-CCV-E251/13	<i>Vespertillius murinus</i>	Germany
IZW-CCV-E260/13	<i>Myotis myotis</i>	Bulgaria
XM_006763795	<i>Myotis davidii</i>	unknown
XM_005880947	<i>Myotis brandtii</i>	unknown
XM_006088606	<i>Myotis lucifugus</i>	Canada
IZW-CCV-VP#1	<i>Vampyressa pusilla</i>	Costa Rica
IZW-CCV-VB#1	<i>Vampyressa bidens</i>	Costa Rica
IZW-CCV-CP-SP1	<i>Carollia perspicillata</i>	Costa Rica
IZW-CCV-PP#46	<i>Pteronotus parnelli</i>	Costa Rica
IZW-CCV-NL#2	<i>Noctilio leporinus</i>	Panama
IZW-CCV-NA-SP1	<i>Noctilio albiventris</i>	Panama
IZW-CCV-E31/14	<i>Rhinolophus ferrumequinuum</i>	Bulgaria
IZW-CCV-Reu17/13	<i>Rhinolophus euryale</i>	Bulgaria
IZW-CCV-E200/13	<i>Rhinolophus hipposiderus</i>	Bulgaria
XM_006906028	<i>Pteropus alecto</i>	unknown
IZW-CCV-66-08	<i>Eidolon helvum</i>	unknown

Table S2. Designed primers to amplify the chiropteran TLR8 gene.

Position ^a	Primer ^b	Direction	Sequence 3'-5'	Fragment ^c
1	F1long	forward	ACATGACCCCTTCMGTCTTTRCTGCTGACCTGC	1
1	PtF1a	forward	ACATGACCCCTTCAGTCTTTACTTCTG	1
8	Myof1a	forward	CCTTCCGTCTTTGCTGCTGACCTGC	1
21	F1Rhi	forward	TTGCTGACCTGCCTTGTCTCTG	1
22	F1	forward	TKCTGACCTGCCTTKTCCTGCT	1
35	PtF1b	forward	TTTCCTGCTAATCTCTGATTCCTGTG	1
35	MyoF1b	forward	TGTCCTGCTAACCTCRGATTCCTGTG	1
516	R1a	revers	AGCAGTTCCAGCCCARATTGAG	1
514	F2aRhi	forward	CTCTCTATTTGGGCTGGAAGT	2
515	F2a	forward	TCTCAATTTGGGCTGGAAGTGC	2
517	F2	forward	TCWATTTGRGCTGGAAGTGC	2
666	R1aRhi	revers	GTGTTGCTGAGATACAAGTTTGTGAG	1
756	R1	revers	AARCACCTYGGACAGTTCCCGCT	1
761	R1b	revers	GTTGAARCAACKTGGACAGTTC	1
776	R1bRhi	revers	CCTCCTGGACAAGGTGTACAG	1
872	R1c	revers	TGGAGGGAAGTGCTRGAGAGGTTG	1
1110	F3	forward	TTGCAYTTAARRGGTTATGTGTTCCAG	3
1110	F3Rhi	forward	TTGCACTTAGAAGGTTATGTGTTCCAG	3
1110	F3Vesp	forward	TTGCATTTAAGAGGTTATGTGTTCCAG	3
1076	F3Vamp	forward	CCAAAACCTTCTCTAATCTTACATCTC	3
1187	R2Rhi	revers	AAGTTAACACCCAAGTTGATAGTC	2
1187	R2Nyc	revers	AATAAAGTTAACGCCCAAGTTGATAGT	2
1187	R2Car	revers	AATGAAGTTCACGCCCAAGTCGATAGT	2
1192	R2	revers	AATAAARTTAAYVCCCAAGTTGA	2
1196	R2Noc	revers	TTTGCTTAATGAAGTTCACGCCCAAG	2
1551	F4Rhi	forward	CAAGCATTAAATGGAAGTGAATTTTCAG	4
1662	F4Noc	forward	TTTGCTTAATGAAGTTCACGCCCAAG	4
1692	F4	forward	CACTATTTTCMRAATMGCAGGGGT	4
1818	R3	revers	CCACTRAAACTAATTSTTYCAGGGA	3
1838	R3Rhi	revers	AATTGATCAAGGCAGTTGCCACT	3
2307	F5	forward	AACCCYTTWGAMTGYACCTGTGAC	5
2307	F5Rhi	forward	AACCCTTTTGACTGTACTTGTGAC	5
2426	R4	revers	AGCTCYAGAGTCAYAATRCTCTTC	4
2448	R4aRhi	revers	GGTGTCTGAAACACAGGTGGTTAG	4
2458	R4a	revers	GGCYGCAATGGTGTCTGAAACAC	4
2781	F5en	forward	GATAACCTCATGCAGAGCATCAAC	5
2992	R5vo	revers	TGGGRTTGTGAGGCCAYTGGAGGATGG	5
3092	R5long	revers	CRTCAGTTAGTATTGCTTAATGGAGTCAACAT AC	5
3102	R5	revers	RTCAGTTAGTATTGYYTAATGGA	5
3103	R5short	revers	CRTCAGTTAGTATTGCTTAATG	5

^a nucleotide position according to the nucleotide sequence of *Eidolon helvum*, starting point is the 3' end of forward primers and the 5' end of revers primers

^b forward and revers primers were used in different combinations, overspanning different fragment-lengths in different bat species

^c according to sequencing analyses

Table S3a. Nucleotide/amino acid sequence percent identities of the whole TLR8 sequences between investigated bat taxa[§] and between the equine and human orthologous.

	Vespertilioninae	Myotinae	Phyllostomidae	Mormoopidae	Noctilionidae	Emballonuridae	Rhinolophidae	Pteropidae	<i>Equus caballus</i>
Vespertilioninae	96/94								
Myotinae	89/87	97/95							
Phyllostomidae	83/79	82/79	96/96						
Mormoopidae	83/79	82/79	91/89						
Noctilionidae	81/77	81/77	88/87	88/86	99/99				
Emballonuridae	82/77	81/78	86/81	86/81	85/79				
Rhinolophidae	79/73	70/73	81/73	82/75	80/72	82/75	98/98		
Pteropidae	80/75	79/76	84/76	85/76	81/74	87/76	84/75	99/99	
<i>Equus caballus</i>	80/74	79/74	83/75	83/75	81/75	83/76	85/75	84/76	
<i>Homo sapiens</i>	77/70	76/70	80/72	80/72	78/71	79/71	80/70	81/72	84/77

[§] Vespertilioninae *Noctula noctula*, *N.leisleri*, *Pipistrellus. nathusi*, *Vespertilio murinus*, *Eptesicus fuscus*; Myotinae: *Myotis myotis*, *M. davidii*, *M. brandtii*, *M. lucifugus*; Phyllostomidae: *Vampyressa bidens*, *V. pusilla*, *Carollia perspicillata*; Mormopidae: *Pteronotus parnelli*; Noctilionidae: *Noctilio albiventris*, *N. leporinus*; Emballonuridae: *Saccopteryx bilineata*; Rhinolophidae: *Rhinolophus euryale*, *R. ferrumequinnum*, *R. hipposideros*; Pteropidae: *Eidolon helvum*, *Pteropus alecto*; *Equus caballus* (XM_014728737); *Homo sapiens* (XM_005274543).

Table S3b. Nucleotide/amino acid sequence percent identities of the TLR8 LRR ectodomain between investigated bat taxa[§] and between the equine and human orthologous.

	Vespertilioninae	Myotinae	Phyllostomidae	Mormoopidae	Noctilionidae	Emballourinae	Rhinolophidae	Pteropidae	<i>Equus caballus</i>
Vespertilioninae	95/93								
Myotinae	88/84	97/95							
Phyllostomidae	82/75	80/75	95/95						
Mormoopidae	82/76	80/76	90/88						
Noctilionidae	79/74	79/73	88/86	88/84	99/99				
Emballouridae	82/77	79/74	85/78	85/78	83/75				
Rhinolophidae	76/68	75/68	79/68	80/70	77/68	80/70	99/98		
Pteropidae	77/70	76/71	81/71	81/72	79/70	81/72	84/75	99/99	
<i>Equus caballus</i>	78/69	76/69	81/71	81/71	79/70	81/72	85/75	82/72	
<i>Homo sapiens</i>	75/65	73/65	78/67	78/67	75/67	77/67	77/66	78/67	82/74

[§] Vespertilioninae *Noctula noctula*, *N.leisleri*, *Pipistrellus. nathusi*, *Vespertilio murinus*, *Eptesicus fuscus*; Myotinae: *Myotis myotis*, *M. davidii*, *M. brandtii*, *M. lucifugus*; Phyllostomidae: *Vampyressa bidens*, *V. pusilla*, *Carollia perspicillata*; Mormopidae: *Pteronotus parnelli*; Noctilionidae: *Noctilio albiventris*, *N. leporinus*; Emballourinae: *Saccopteryx bilineata*; Rhinolophidae: *Rhinolophus euryale*, *R. ferrumequinnum*, *R. hipposideros*; Pteropidae: *Eidolon helvum*, *Pteropus alecto*; *Equus caballus* (XM_014728737); *Homo sapiens* (XM_005274543).

Table S3c. Nucleotide/amino acid sequence percent identities of the TLR8 TM and TIR domain between investigated bat taxa[§] and between the equine and human orthologous.

	Vespertilioninae	Myotinae	Phyllostomidae	Mormoopidae	Noctilionidae	Emballourinae	Rhinolophidae	Pteropidae	<i>Equus caballus</i>
Vespertilioninae	97/98								
Myotinae	94/97	98/99							
Phyllostomidae	89/93	89/94	96/99						
Mormoopidae	91/93	90/95	94/96						
Noctilionidae	90/92	89/93	91/94	92/93	99/100				
Emballouridae	92/94	91/95	91/95	92/95	91/93				
Rhinolophidae	90/94	90/94	91/94	92/93	90/92	92/94	99/99		
Pteropidae	90/95	89/94	90/95	91/93	89/92	91/95	92/94	100/100	
<i>Equus caballus</i>	90/94	89/95	90/94	91/93	91/93	91/93	93/92	92/92	
<i>Homo sapiens</i>	88/90	86/89	89/91	89/90	88/88	89/90	90/89	90/90	92/92

[§] Vespertilioninae *Noctula noctula*, *N.leisleri*, *Pipistrellus. nathusi*, *Vespertilio murinus*, *Eptesicus fuscus*; Myotinae: *Myotis myotis*, *M. davidii*, *M. brandtii*, *M. lucifugus*; Phyllostomidae: *Vampyressa bidens*, *V. pusilla*, *Carollia perspicillata*; Mormopidae: *Pteronotus parnelli*; Noctilionidae: *Noctilio albiventris*, *N. leporinus*; Emballourinae: *Saccopteryx bilineata*; Rhinolophidae: *Rhinolophus euryale*, *R. ferrumequinnum*, *R. hipposideros*; Pteropidae: *Eidolon helvum*, *Pteropus alecto*; *Equus caballus* (XM_014728737); *Homo sapiens* (XM_005274543).

Table S4. Positive selected sites identified by different model analyses for the Chiropteran TLR8 gene in relation to PSS of other taxa and to relevant binding and dimerization sites of the human TLR8 (hTLR8) molecule according to Tanji et al. (2013, 2015).

hTLR8 Position ^a	Persistent positive selected sites						Episodic positive selected sites		Ligand binding sites and dimerization sites in hTLR8				Amino acid alterations in bats	PSS of other taxa ^f
	M8	SLAC	FEL	REL	FUBAR	Prime	MEME	Branch ^b	Dim ^c	1. ^d	2. ^e	aa		
15			0.078			0.007								
32							0.007	aY						
39						0.018	0.010	Pt, Sb						Mammals
41				75										
43	0.93			120		0.082								
61						0.046	0.020	Pt						
62			0.082											
77			0.041	50	0.88		0.055	R, wMy						
88							0.040	Sb, Vm						
91						0.053								
104	0.97			395	0.90	0.041								
108	0.94			115										
109			0.031	100	0.94		0.051	R, Ef						
138			0.080			0.072								
146														Mammals
150	1.00			2720										Bats
157							0.006	Mb						
160														Mammals
167							0.045	Vm						
174				86	0.85									Mammals
178														Suidae
186														Mammals
191														Mammals
192						0.069								
195				74	0.91									
205						0.090								
214														Mammals
236			0.013	150	0.96		0.023	R, Sb						Mammals

238	0.96		0.086	592	0.90												
239				72	0.85	0.065	0.071	<i>M.lucifugus</i>									
240	0.92																
243						0.030											
249																	Mammals
261									u	x			F	F			
277	0.94	.072	0.017	443	0.97		0.016	aY, Sb									
279							0.028	aN, Pt									
280						0.072											
285	0.94			615	0.83												
290			0.081														
291											x		Y	Y-I			
311			0.043														
312	0.92			325		0.082											
314											x		K	K			
315											x		V	E			
319											x		E	E-Q			
338	0.96	.070	0.004	2101	0.98	0.019	0.005	aY, Pt, R, aN									Mammals
340											x		E	E-K-Q			
341,343,345											x		IDS	IDS			
348									u				Y	Y			
349										x			I	I-R-V			Mammals
350										x			K	K-Q			
351			0.046				0.043	aY, R, wMy		x			G	G-T-A-K			
352			0.049		0.91	0.008				x			S	Q-A-E-D-T			
353									u	x			Y	Y			
354			0.023	88	0.93		0.009	Sb, Vm, Ef									
355	0.90																
356			0.053	68	0.92		0.007	R									
365						0.041											
368	0.92		0.10	202	0.90												Bats
370											x		R	R-Q			
373											x		H	H-P			
375											x		R	R-K-E			
378									u	x			V	V			

385			0.079	57	0.89												
386							0.006	Rhip									
388																	Suidae Mammals
395						0.025											
397							0.002	Pt, Vm, Mb									
398											x		T		T		
400											x		N		N-D		
405									u	x			F		F		
407							0.029	Pt									
412			0.023	62	0.92	0.078	0.035	Sb									
415	0.93			105													Bats
416							0.041	R, wM									Mammals
417				161													
418							0.000	Pt, R, wMy									Mammals
419						0.050	0.039	wMy, Ef									
420			0.025	57	0.88	0.079	0.017	aN									
421	0.93			186	0.92		0.023	Pt, Sb, Vm									
422											x		I		I-V		
427									a				E		E-V-A-L		
429										x			R		R		
432									a				P		P		
436			0.066														
439							0.002	aN									
441																	Mammals
443			0.061														
447			0.030	51	0.91		0.043	Pt, R, Vm									
459																	Mammals
466											x		N		N		
468; 469											x		YH		YH		
470											x		F		S-N		
471											x		T		T		
472											x		R		K-T-N-H-E		Mammals
473											x		P		P-H		
474											x		L		L		

468																	
478						0.087											
479									u				C	C			
481	0.94		0.056	444	0.92												Mammals
491										x			N	N			Primates
494									u	x			F	F			
495									U				F	F			
498																	Mammals
506							0.002	R									Bats
508						0.080	0.018	aY, wMy									
509									u				C	C			
514									a				A	S			
516									a				S	G-D-A			
518									a				A	G-N			
520										x			V	V-A			
532			0.10														
539			0.10			0.081	0.038	Pt, aN, Ef									
541									a				R	R-K			
543,545										x			DD	DD			
566									a				H	H			
567,568									u				YF	YF			
573,574										x			VT	VT			
685							0.039	Pt									
693			0.07														
695				143													
606																	Mammals
608	0.99			921	0.91	0.09											
620							0.001	<i>N.albiventris</i>									
629																	Mammals
633			0.059														Mammals
636				76		0.043											
639																	Mammals
643			0.066														
656			0.053	50	0.89												
677	0.99	.070	0.005	13313	0.99		0.006	aY, Pt, R, Sb, Vm									Bats

685			0.046	70	0.92		0.053	Pt, Sb, wMy						
686			0.067											
689	0.91													Bats
691			0.044	168	0.93		0.019	R, Sb						
699	0.97			985										
712			0.069	145	0.90	0.087	0.002	aY, Pt						Bats Mammals
719							0.050	<i>R.euryale</i>						
721			0.086											
723	0.96		0.087	216	0.93	0.001	0.002	wMy, Vm						
730							0.044	Pt						
745			0.041											
761														Mammals
666														Mammals
790			0.087			0.001								
805			0.052			0.023								
811						0.021								
911						0.095								

^a putatively positive selected sites of bats are coloured according to figure 3.

^b aY (ancestral lineage of Yinpterochiroptera), R (ancestral lineage of Rhinolophidae), Pt (ancestral lineage of Pteropodidae), aN (ancestral lineage of Noctilionidae), wMy (ancestral lineage within Myotis), Sb (*S. bilineata*), Rhip (*R. hipposideros*), Vm (*V. murinus*), Mb (*M. brandtii*), Ef (*E. fuscus*).

^c sites involved in Dimerization, u: dimerization of the unliganded dimer, a: dimerization of the activated dimer.

^d 1. binding site: binding the uridine mononucleosid and small chemical ligands, induced the activated form.

^e 2. binding site: binding uridine rich ssRNA oligomers, induces binding of uridine at 1. site.

^f conserved amino acids are in bolt.

^g positively selected sites in bats (Escalera-Zamudio et al 2015), mammals (Areal et al. 2011), suidae (Darfour-Oduro et al. 2015) and primates (Wasliuk and Nachmann 2010), coincidence with sites of our bat PSS are marked bolt.

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