

Supplementary data

TmCactin plays an important role in Gram-negative and -positive bacterial infection by regulating expression of 7 AMP genes in *Tenebrio molitor*

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Figure S1. Genomic organization and sequence of *TmCactin*. (A) *TmCactin* gene structure has been shown containing the promoter, exon (E1-E7), and intron (I1-I6) regions. Translation start (ATG) and stop codons (TGA) are shown. (B) The gene sequence of *TmCactin* is depicted with the same colors as shown in (A). The *TmCactin* ORF sequences were identified by blast analysis and were aligned with the genomic sequence. *TmCactin* gene contains 7 exons interspersed with 6 introns.

Figure S2. Nucleotide and deduced amino acid sequence of *TmCactin*. *TmCactin* contains an ORF size of 2,833 bp that encodes a protein of 952 amino acids. Domain analysis by InterProScan 5 and blastp shows a conserved Cactin mid-region (open box), a C-terminal Cactus-binding region (gray box), and a zinc-finger domain (black box) in the amino acid sequence of *TmCactin*. Two regions for dsRNA synthesis are marked by blue (ds*TmCactin*#2) and green (ds*TmCactin*) arrows, and red arrows indicate the region for qPCR primers.

Figure S3. Validation experiments using a second *TmCactin* dsRNA. (A) Quantitative RT-PCR shows significant reduction in *TmCactin* mRNA in larvae injected with dsRNA against *TmCactin* (ds*TmCactin* #2) compared to *EGFP* dsRNA control-injected larvae. (B) Quantitative RT-PCR gel showing knockdown of *TmCactin* by both dsRNAs (ds*TmCactin* and ds*TmCactin* #2) compared to the

control *EGFP* dsRNA. *TmL27α* was used as a loading control. (C and D) Similar to the first dsRNA, injection with *dsTmCactin#2* also decreases larval survival rates after *E. coli* and *S. aureus* challenge compared to *EGFP* dsRNA control-injected larvae.

Figure S4. Quantitative RT-PCR confirms that *E. coli*- and *S. aureus*-induced expression of *Tene-1*, *Tene-4*, *Def-1*, *Def-2*, *Cole-1*, *Cole-2*, and *Att-1b* is reduced in larvae injected with *dsTmCactin#2*.

Supplementary Table 1. Top 100 blastp hits (all with E-values of 0.0) from TmCactin alignment to the NCBI nr database (last accessed Dec. 12, 2016).

Figure S1

[illegible]

Figure S2

ATG	CCA	AGA	AGT	GAC	CAT	TCT	CGA	CGA	CAG	AGG	TGG	AAA	AAG	AAA	GAG	AAA	CAC	AGA	60	GAA	GAC	GAC	TCA	AAA	CGT	CTT	GAA	TTT	GCG	CGA	ATG	CAA	TGC	GGC	AGC	GGC	AAG	AAG	1440																																																																																																																																																																																																																																																																																																																																																																																																																																																												
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Figure S3

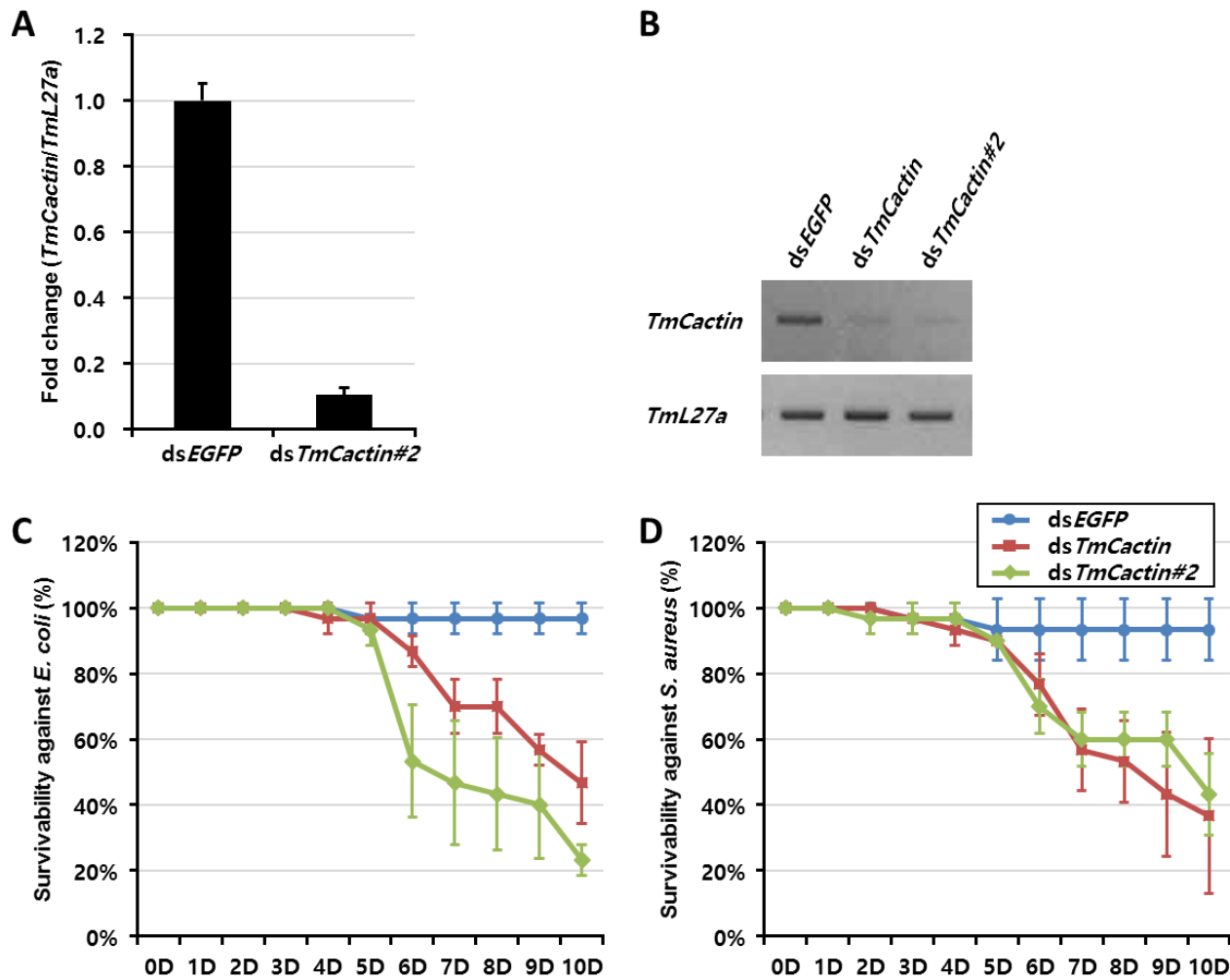
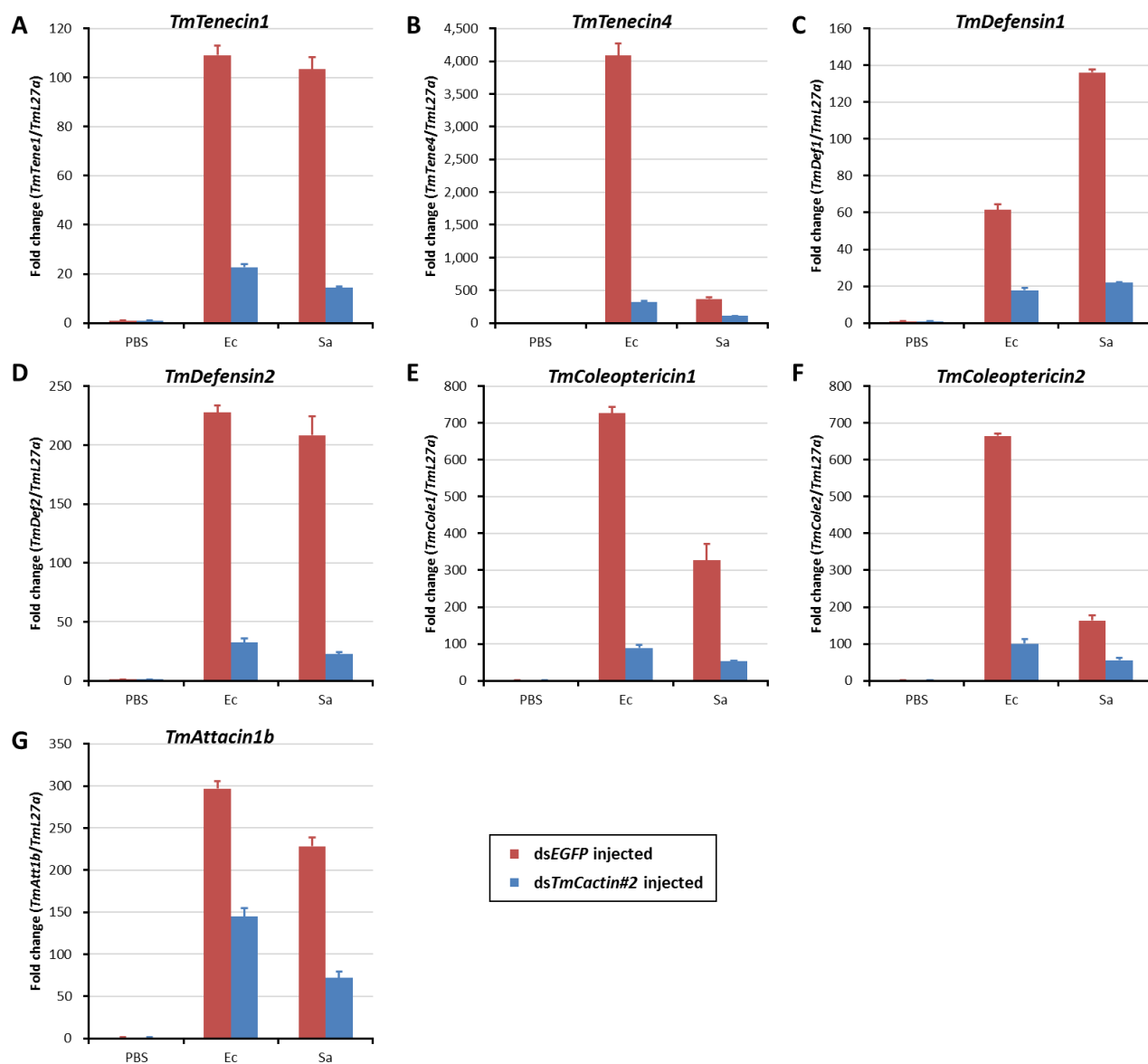


Figure S4



Supplementary Table 1. Top 100 blastp hits (all with E-values of 0.0) from TmCactin alignment to the NCBI nr database (last accessed Dec. 12, 2016).

Species	Common Name/Organism	GenBank Accession #	Sequence Description	Query Coverage	Alignment Length	Percent Similarity	Percent Identity
<i>Tribolium castaneum</i>	Red flour beetle	XP_008196004.1	Predicted cactin	60%	600	91%	87%
<i>Anoplophora glabripennis</i>	Long-horned beetle	XP_018564952.1	Predicted cactin	57%	545	92%	86%
<i>Agrilus planipennis</i>	Emerald ash borer	XP_018333554.1	Predicted cactin	56%	540	88%	79%
<i>Cyphomyrmex costatus</i>	Fungus-growing ant	KYM93587.1	Uncharacterized protein C19orf29	97%	1070	63%	50%
<i>Dendroctonus ponderosae</i>	Bark beetle	ENN71807.1	hypothetical protein YQE_11541	59%	574	88%	80%
<i>Pogonomyrmex barbatus</i>	Red ant	XP_011629906.1	Predicted cactin	57%	547	86%	73%
<i>Nicrophorus vespilloides</i>	Burying beetle	XP_017776307.1	Predicted cactin	57%	549	88%	77%
<i>Atta colombica</i>	Leafcutter ant	KYM76580.1	Uncharacterized protein C19orf29	90%	985	62%	49%
<i>Vollenhovia emeryi</i>	Myrmicine Japanese ant	XP_011861876.1	Predicted cactin	57%	547	85%	74%
<i>Atta cephalotes</i>	Leafcutter ant	XP_012057115.1	Predicted cactin	57%	547	85%	73%
<i>Atta colombica</i>	Leafcutter ant	XP_018056418.1	Predicted cactin isoform X1	57%	547	85%	73%
<i>Acromyrmex echinator</i>	Panamanian leafcutter ant	XP_011064726.1	Predicted cactin	57%	547	85%	73%
<i>Trachymyrmex cornetzi</i>	Fungus-growing ant	XP_018357566.1	Predicted cactin isoform X1	57%	547	85%	73%
<i>Pediculus humanus corporis</i>	Body louse	XP_002428222.1	Paramyosin, putative	57%	549	85%	73%
<i>Trachymyrmex cornetzi</i>	Fungus-growing ant	XP_018357567.1	Predicted cactin isoform X2	57%	547	85%	73%
<i>Linepithema humile</i>	Argentine ant	XP_012233515.1	Predicted cactin	57%	546	86%	73%
<i>Dinoponera quadriceps</i>	ant	XP_014477760.1	Predicted cactin	57%	550	86%	74%
<i>Harpegnathos saltator</i>	Indian jumping ant	XP_011152612.1	Predicted cactin	57%	551	86%	72%
<i>Harpegnathos saltator</i>	Indian jumping ant	EFN75758.1	Uncharacterized protein C19orf29	57%	551	86%	72%
<i>Cerapachys biroi</i>	Clonal raider ant	XP_011349145.1	Predicted cactin	57%	549	85%	73%
<i>Camponotus floridanus</i>	Florida carpenter ant	EFN62654.1	Uncharacterized protein C19orf29	80%	545	86%	72%
<i>Eufriesea mexicana</i>	Orchid bee	XP_017752636.1	Predicted cactin	57%	546	87%	75%

<i>Habropoda laboriosa</i>	Blueberry bee	XP_017790710.1	Predicted cactin	57%	549	87%	75%
<i>Camponotus floridanus</i>	Florida carpenter ant	XP_011264558.1	Predicted cactin	57%	547	84%	72%
<i>Ceratina calcarata</i>	Carpenter bee	XP_017883562.1	Predicted cactin isoform X2	57%	545	86%	75%
<i>Bombus impatiens</i>	Bumble bee	XP_003491995.1	Predicted Cactin	57%	546	86%	75%
<i>Dufourea novaeangliae</i>	Pickerel bee	XP_015439954.1	Predicted cactin	57%	550	86%	74%
<i>Apis cerana</i>	Indian honey bee	XP_016910227.1	Predicted cactin	57%	546	86%	75%
<i>Dufourea novaeangliae</i>	Pickerel bee	KZC06580.1	Uncharacterized protein C19orf29	57%	550	86%	74%
<i>Apis florea</i>	Red dwarf honey bee	XP_012345914.1	Predicted cactin isoform X2	57%	546	86%	75%
<i>Bombus terrestris</i>	Buff-tailed bumble bee	XP_003395486.1	Predicted cactin	57%	546	86%	75%
<i>Lasius niger</i>	Black garden ant	KMQ94182.1	Predicted cactin isoform 2	57%	545	86%	74%
<i>Apis florea</i>	Red dwarf honey bee	XP_003696278.1	Predicted cactin isoform X1	57%	546	86%	75%
<i>Trachymyrmex zeteki</i>	Fungus-growing ant	XP_018310944.1	Predicted cactin isoform X1	57%	545	86%	73%
<i>Apis florea</i>	Red dwarf honey bee	XP_012345915.1	Predicted cactin isoform X3	57%	546	86%	75%
<i>Trachymyrmex zeteki</i>		KYQ50290.1	Uncharacterized protein C19orf29	57%	545	86%	73%
<i>Megachile rotundata</i>	Alfalfa leafcutter bee	XP_003700357.1	Predicted cactin	57%	549	86%	74%
<i>Wasmannia auropunctata</i>	Little fire ant	XP_011696940.1	Predicted cactin	57%	547	85%	73%
<i>Polistes dominula</i>	European paper wasp	XP_015180263.1	Predicted cactin	57%	547	86%	74%
<i>Trachymyrmex septentrionalis</i>	Fungus-growing ant	XP_018342983.1	Predicted cactin	57%	547	85%	73%
<i>Orussus abietinus</i>	Wood wasp	XP_012275920.1	Predicted cactin	57%	545	86%	74%
<i>Atta colombica</i>	Leafcutter ant	XP_018056419.1	Predicted cactin isoform X2	54%	524	85%	74%
<i>Apis mellifera</i>	European honey bee	XP_016767776.1	Predicted low quality protein cactin	57%	546	86%	74%
<i>Monomorium pharaonis</i>	Pharaoh ant	XP_012529842.1	Predicted cactin	57%	548	85%	73%
<i>Athalia rosae</i>	Turnip sawfly	XP_012258533.1	Predicted cactin	57%	550	85%	74%
<i>Acromyrmex echinator</i>	Leaf-cutter ant	EGI59105.1	Uncharacterized protein C19orf29	54%	524	85%	74%

<i>Polistes canadensis</i>	Red paper wasp	XP_014599721.1	Predicted cactin-like	57%	548	86%	74%
<i>Ceratina calcarata</i>	Small carpenter bee	XP_017883559.1	Predicted cactin isoform X1	57%	573	82%	71%
<i>Solenopsis invicta</i>	Fire ant	XP_011172320.1	Predicted cactin	57%	547	85%	72%
<i>Neodiprion lecontei</i>	Pine sawfly	XP_015509948.1	Predicted cactin	57%	550	85%	74%
<i>Polistes canadensis</i>	Red paper wasp	XP_014609071.1	Predicted cactin-like	57%	548	86%	74%
<i>Trachymyrmex septentrionalis</i>	Fungus-growing ant	KYN38820.1	Uncharacterized protein C19orf29	57%	566	84%	71%
<i>Cephus cinctus</i>	Wheat stem sawfly	XP_015599202.1	Predicted cactin	57%	550	85%	73%
<i>Clastoptera arizonana</i>	Arizona spittle but	JAS19691.1	Hypothetical protein g.5547	57%	544	84%	73%
<i>Acyrtosiphon pisum</i>	Pea aphid	XP_001952287.2	Predicted cactin	56%	557	80%	68%
<i>Diuraphis noxia</i>	Russian wheat aphid	XP_015379807.1	Predicted cactin	56%	557	81%	68%
<i>Fopius arisanus</i>	Tephritid fruit fly parasitoid	XP_011307236.1	Predicted cactin	57%	562	83%	72%
<i>Bemisia tabaci</i>	Tobacco whitefly	XP_018916407.1	Predicted cactin	57%	550	82%	71%
<i>Cyphomyrmex costatus</i>	Fungus-growing ant	XP_018405760.1	Predicted cactin	54%	524	85%	74%
<i>Trachymyrmex zeteki</i>	Fungus-growing ant	XP_018310945.1	Predicted cactin isoform X2	54%	522	86%	74%
<i>Homalodisca liturata</i>	Smoketree sharpshooter	JAT00701.1	Hypothetical protein g.11009	57%	544	85%	75%
<i>Diachasma alloeum</i>	Wasp	XP_015123757.1	Predicted cactin	57%	548	84%	72%
<i>Microplitis demolitor</i>	Wasp	XP_008556943.1	Predicted cactin	57%	574	80%	70%
<i>Apis dorsata</i>	Giant honey bee	XP_006614047.1	Predicted cactin-like	57%	546	84%	73%
<i>Nasonia vitripennis</i>	Jewel wasp	XP_003425945.3	Predicted cactin	54%	523	81%	70%
<i>Ceratosolen solmsi marchali</i>	Fig wasp	XP_011502302.1	Predicted cactin	54%	525	81%	70%
<i>Copidosoma floridanum</i>	Wasp	XP_014214609.1	Predicted cactin	57%	548	80%	67%
<i>Cimex lectularius</i>	Bed bug	XP_014248225.1	Predicted low quality protein cactin	57%	544	80%	69%
<i>Trichogramma pretiosum</i>	Wasp	XP_014223848.1	Predicted low quality protein cactin	57%	544	78%	67%
<i>Halyomorpha halys</i>	Brown marmorated stink bug	XP_014290251.1	Predicted cactin	57%	545	82%	68%
<i>Cuerna arida</i>	Sharpshooter	JAS56516.1	Hypothetical protein g.16355	52%	499	84%	73%
<i>Daphnia magna</i>	Water flea	JAN43572.1	Cactin	57%	569	73%	59%
<i>Daphnia magna</i>	Water flea	JAN86127.1	Cactin	57%	569	73%	59%

<i>Daphnia magna</i>	Water flea	JAN52132.1	Cactin	57%	569	73%	59%
<i>Daphnia magna</i>	Water flea	JAN44925.1	Cactin	57%	569	73%	59%
<i>Daphnia magna</i>	Water flea	JAN46435.1	Cactin	57%	569	73%	59%
<i>Daphnia magna</i>	Water flea	KZS15704.1	Cactin	57%	562	74%	59%
<i>Rhipicephalus microplus</i>	Cattle tick	AIT40194.1	Cactin	58%	578	75%	60%
<i>Amblyomma aureolatum</i>	Tick	JAT94195.1	Putative cactin	57%	568	74%	60%
<i>Amblyomma sculptum</i>	Tick	JAU02627.1	Putative cactin	57%	567	74%	61%
<i>Daphnia magna</i>	Water flea	JAN58168.1	Cactin	57%	571	72%	58%
<i>Lucilia cuprina</i>	Sheep blowfly	KNC34812.1	Cactin	57%	549	72%	59%
<i>Lipotes vexillifer</i>	White-flag dolphin	XP_007460408.1	Predicted cactin	56%	555	72%	56%
<i>Tursiops truncatus</i>	Bottlenose dolphin	XP_004317108.1	Predicted low quality protein cactin	56%	555	72%	56%
<i>Ixodes scapularis</i>	Black-legged tick	XP_002409176.1	Conserved hypothetical protein	57%	580	73%	59%
<i>Orcinus orca</i>	Killer whale	XP_004286374.1	Predicted cactin	56%	553	71%	56%
<i>Lottia gigantea</i>	Sea snail	XP_009048707.1	Hypothetical protein LOTGIDRAFT_157867	55%	566	70%	58%
<i>Litopenaeus vannamei</i>	Whiteleg shrimp	AIZ03630.1	cactin	57%	575	74%	57%
<i>Drosophila simulans</i>	Fruitfly	XP_016040165.1	Uncharacterized protein Dsimw501_GD28616	58%	581	70%	56%
<i>Sorex araneus</i>	Common shrew	XP_004614888.1	Predicted cactin	55%	548	71%	56%
<i>Drosophila melanogaster</i>	Fruitfly	AAF66981.1	cactin	57%	571	70%	56%
<i>Drosophila melanogaster</i>	Fruitfly	NP_523422.4	cactin	57%	571	70%	56%
<i>Drosophila melanogaster</i>	Fruitfly	AER42874.1	LP09118p1	57%	571	70%	56%
<i>Alligator mississippiensis</i>	Alligator	KYO34526.1	cactin	59%	574	71%	57%
<i>Geospiza fortis</i>	Medium ground finch	XP_014165073.1	Predicted low quality protein cactin	56%	546	73%	58%
<i>Microtus ochrogaster</i>	Prarie vole	XP_005359049.1	Predicted cactin	58%	570	71%	56%
<i>Lepidothrix coronata</i>	Blue-crowned manakin/bird	XP_017692466.1	Predicted cactin	56%	549	73%	58%
<i>Camelus bactrianus</i>	Camel	XP_010965004.1	Predicted cactin	58%	579	72%	56%
<i>Alligator sinensis</i>	Alligator	XP_006027263.1	Predicted cactin	56%	548	72%	58%
<i>Charadrius vociferus</i>	Killdeer/bird	XP_009878904.1	Predicted cactin	56%	549	73%	57%