- Electronic Supplementary File -

Characterization of the Z lineage
Major histocompatibility complex class I genes in zebrafish

Immunogenetics

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>Mhc1zla^{3781} (GenBank KC607872)

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ILLGAIGVTVWILKKKNIIGNGDEKHFVVSTVSGNKDENGFVVSTVSGNKDEKRSMPDGSADSGKGSSA-
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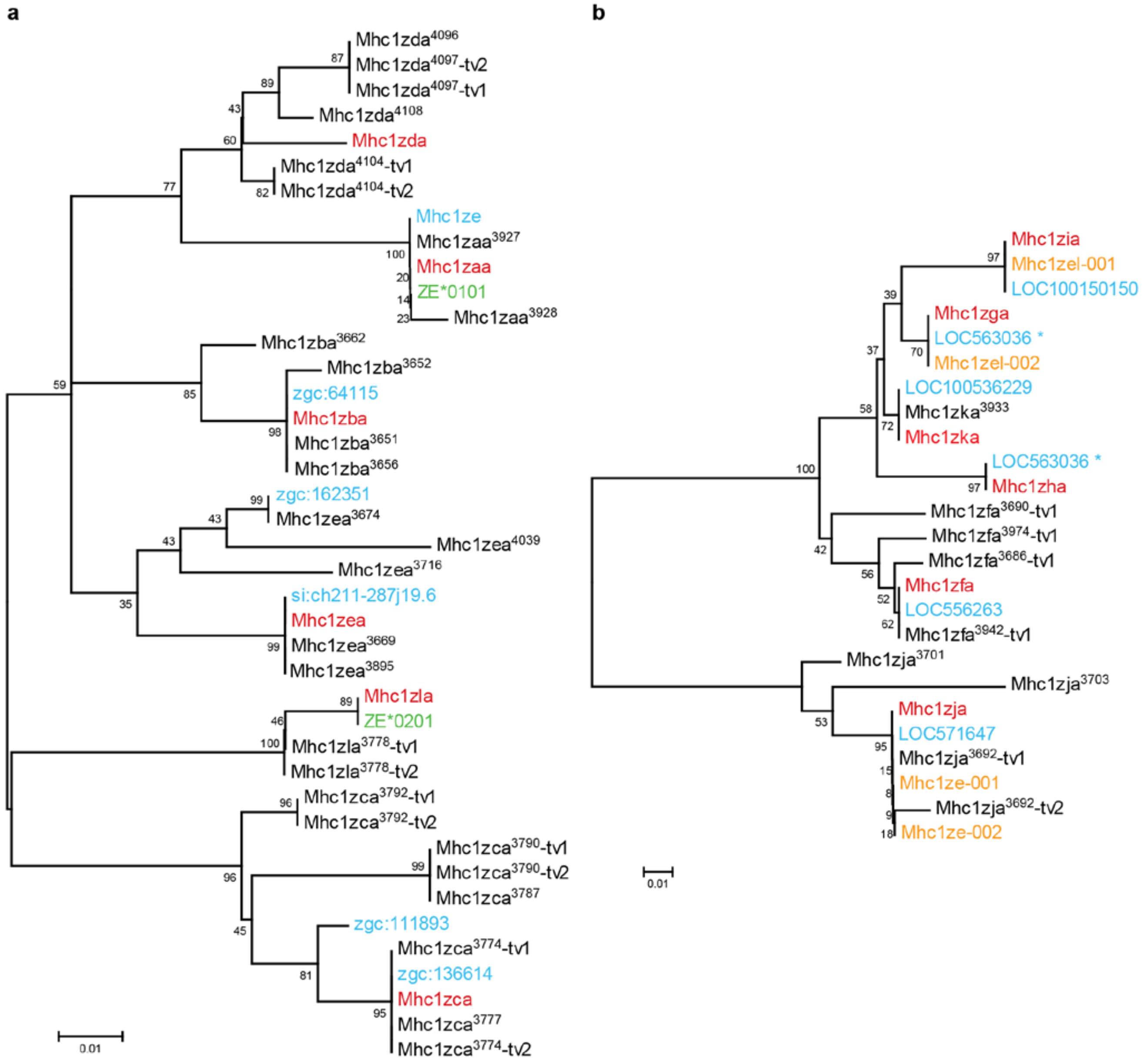
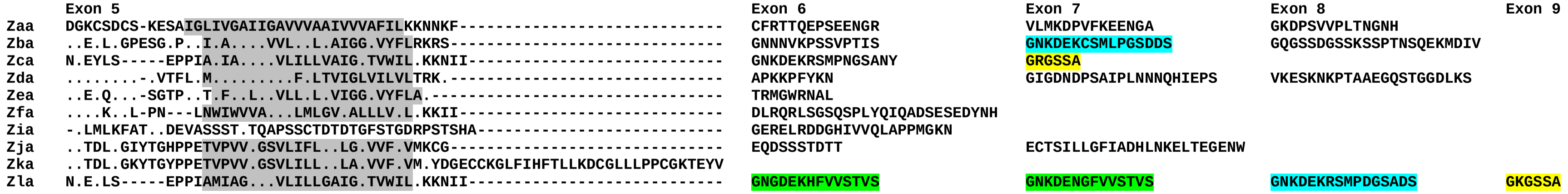
Figure S1. Full-length zebrafish MHC class I Z proteins. Proteins encoded by transcripts described in manuscript are shown. Transcript identity numbers are included as superscripts. Proteins encoded by alternatively spliced transcript variants (tv) are included. GenBank accession numbers are listed in parentheses. Non-functional transcripts are excluded from this list but are available in GenBank (KC607849, KC607856, KC607857, KC607858, KC607860, KC607861, KC607863, KC607865).



Chromosome 1 Group

Chromosome 3 Group

Figure S2. Organization and classification of zebrafish Z sequences. The α1-α3 peptide domains encoded by Z sequences identified in various databases were aligned and neighbor joining trees generated. Initial analyses indicated that two distinct sequence subgroups exist that were then analyzed separately. **a** One group includes all Z sequences from chromosome 1 (Zv9). **b** The other group includes all Z sequences from chromosome 3 (Zv9). Color-coding was employed to indicate the source of the sequence as follows: red = transcripts predicted from the reference genome (Zv9), green = full-length transcripts previously described (Kruiswijk, et al. 2002. J Immunol. 169(4):1936-47), blue = sequences identified from the zebrafish reference protein database (NCBI), orange = sequences identified through ZFIN and black = full-length transcripts described in this study (transcript identity numbers are included as superscripts). * Note that LOC563036 is a predicted transcript encoding both *Mhc1zga* and *Mhc1zha.*



	Exon 5	Exon 6	Exon 7	Exon 8	Exon 9
Zaa	DGKCSDCS-KESAIGLIVGAIIGAVVVAAIVVVAFILKKNNKF-----------------------------	CFRTTQEPSEENGR	VLMKDPVFKEENGA	GKDPSVVPLTNGNH	
Zba	..E.L.GPESG.P..I.A....VVL..L.AIGG.VYFLRKRS------------------------------	GNNNVKPSSVPTIS	GNKDEKCSMLPGSDDS	GQGSSDGSSKSSPTNSQEKMDIV	
Zca	N.EYLS-----EPPIA.IA....VLILLVAIG.TVWIL.KKNII----------------------------	GNKDEKRSMPNGSANY	GRGSSA		
Zda	........-.VTFL.M.........F.LTVIGLVILVLTRK.------------------------------	APKKPFYKN	GIGDNDPSAIPLNNNQHIEPS	VKESKNKPTAAEGQSTGGDLKS	
Zea	..E.Q...-SGTP..T.F..L..VLL.L.VIGG.VYFLA.--------------------------------	TRMGWRNAL			
Zfa	....K..L-PN---LNWIWVVA...LMLGV.ALLLV.L.KKII-----------------------------	DLRQRLSGSQSPLYQIQADSESEDYNH			
Zia	-.LMLKFAT..DEVASSST.TQAPSSCTDTDTGFSTGDRPSTSHA---------------------------	GERELRDDGHIVVQLAPPMGKN			
Zja	..TDL.GIYTGHPPETVPVV.GSVLIFL..LG.VVF.VMKCG------------------------------	EQDSSSTDTT	ECTSILLGFIADHLNKELTEGENW		
Zka	..TDL.GKYTGYPPETVPVV.GSVLILL..LA.VVF.VM.YDGECCKGLFIHFTLLKDCGLLLPPCGKTEYV				
Zla	N.E.LS-----EPPIAMIAG...VLILLGAIG.TVWIL.KKNII----------------------------	GNGDEKHFVVSTVS	GNKDENGFVVSTVS	GNKDEKRSMPDGSADS	GKGSSA

Figure S3. Alignment of transmembrane and cytoplasmic domains. The protein sequences from the C termini of the Z genes were aligned exon by exon. Identical residues are shown as dots while gaps are shown as dashes. The transmembrane domains are highlighted in gray and highly similar exons are highlighted in blue, green, or yellow.

Table S1. Zebrafish Z genes identified in the *de novo* genomic assemblies from double haploid homozygous AB (DHAB) and Tübingen (DHTu2) individuals.

Genomic Scaffold/BAC	Gene	DHAB	DHTu2
DKEY-28D10	*mhc1zaa*		
	mhc1zla		
Chr1, Scaffold 68	*mhc1zba*	✓	✓
	mhc1zca	✓	✓
	mhc1zda	✓[a]	✓
	mhc1zea	✓	✓
Chr3, Scaffold 307	*mhc1zfa*	✓	✓
Chr3, Scaffold 312	*mhc1zga*	✓	
	mhc1zha	✓	
	mhc1zia	✓	
	mhc1zja	✓	
Scaffold NA257	*mhc1zka*		✓

[a] Only able to identify exons encoding α3 to C terminus.

Table S2. Predicted sizes of Southern blot fragments containing Z gene α1 sequences based on *Hin*dIII restriction sites.

Gene	Genomic Location	Predicted Size (kb)
mhc1zca	Chr1, Scaffold 68	12.1
mhc1zaa	DKEY-28D10	11.1
mhc1zga + mhc1zha	Chr3, Scaffold 312	9.2
mhc1zba	Chr1, Scaffold 68	9.0
mhc1zia	Chr3, Scaffold 312	7.9
mhc1zja	Chr3, Scaffold 312	7.2
mhc1zea	Chr1, Scaffold 68	6.6
mhc1zka	Scaffold NA257	5.2
mhc1zda	Chr1, Scaffold 68	4.4
mhc1zfa	Chr3, Scaffold 307	4.3
mhc1zla	DKEY-28D10	4.0

Mhc1zaa

Mhc1zaa^{3927} 1 MAVFAVLFSAVMLLAIVPAWTEKHSLYYIYTALSRPVNLPGIHEFTAMGLLDDRQIDYYNSQEQKKIPKQPWMKEKMQED
Mhc1zaa^{3928} 1 MAVFAVLFSAVMLLAIVPAWTEKHSLYYIYTALSRPVNLPGIHEFTAMGLLDDRQIDYYNSQEQKKIPKQPWMKEKMQED

Mhc1zaa^{3927} 81 YWEKGTQSRKSKEQWFNVNVNILMERMRHNKSDVHVLQWRHGCEIESQDNNVRFSKGIDEYSYDGENFLSFDDADSQWVA
Mhc1zaa^{3928} 81 YWEKGTQSRKSKEQWFNVNVNILMERMRHNKSDVHVLQWRHGCEIESQDNNVWFSKGIDEYSYDGENFLSFDDADSQWVA

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Mhc1zaa^{3928} 161 PVDAALPTKRKWDNVPILNQYTKGYLEKECVDWLNKFREYGDEELKQGSAPKVHVFAKRYVNGKAKLKLTCLATGFYPKD

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Mhc1zaa^{3928} 241 VYLTIRKYRTALSDSEVESSGVRPNHDGTFQLRKSTYILEEEKAEYDCYVAHRTLNAPVVTTWDGKCSDCSKESAIGLIV

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Mhc1zda^{4097}	241	VYLTIRKYRMALSDKDLESSGVRPNHDGTFQLRKSTYIYEDEKAEYDCYVKHRTLGAPIIKKWDGKCSDCSKVTFLGMIV
Mhc1zda^{4108}	241	VYLTIRKYRMALPDKEFESSGVRPNHDGTFQLRKSTYIYEDEKAEYDCYVNHTTLGAPIIKKWDGKCSDCSKVTFLGMIV
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Mhc1zda^{4097}	321	GAIIGAVLVLTVIGLVILVLTIKKAPKKPFYKNGIGDNDPSAIPLNNNQHIEPSVKESKNKPTAAEGQSTSGDLKS
Mhc1zda^{4108}	321	GAIIGAVLVLTVIGLVILVLTRKKAPKKPFYKNGIGDNDPSAIPLNNNQHIEPSVKESKNKPTAAEGQSTGGDLKS
Mhc1zda^{4113}	321	GAIIGAVLVLTVIGLVILVLTRKKG---------IGDNDPSAIPLNNNQHIEPSVKESKNKPTAAEGQSTSGDLKS
Mhc1zda^{4098}	321	GAIIGAVLVLTVIGLVILVLTIKKG---------IGDNDPSAIPLNNNQHIEPSVKESKNKPTAAEGQSTSGDLKS
Mhc1zda^{4096}	321	GAIIGVVLVLTVIGLVILVLTIKKG---------IGDNDPSAIPLNNNQHIEPSVKESKNKPTAAEGQSTSGDLKS

Mhc1zea

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Mhc1zea^{3895} 1 MAVFAALFSVVMLFTVVPAWTEKHSLYYIYTALSRPVHLPGIYEFTAMGLLDDRQIDYYNSQEQKKIPKQHWMKEKMQED
Mhc1zea^{3716} 1 MAVFAALFSVVMLFTVVPAWTEKHSLYYIYTALSRPVDLPGIYQFTAMGLLDDRQIDYYNSQEQKKIPKQHWMKEKMQED
Mhc1zea^{3674} 1 MAVFAALFSVVMLFTVVPAWTEKHSLYYIYTALSRPVNLPGIYEFTAMGLLDDRQIDYYNSQEQKKIPKQHWMKEKMQED
Mhc1zea^{4039} 1 MAVFAALFSVVMLFTVVPAWTEKHSLYYIYTALSRPVHLPGIYEFIAMGLLDDRQIDYYNSQEQKKIPKQHWMKEKMQED

Mhc1zea^{3669} 81 YWEKGTQSRKSKEQWFNVNLKILMERMRHNNNSDVHVLQWRHGCEIDSQGNDVRFSKGIDEYSYDGRNFLAFDDADSQWV
Mhc1zea^{3895} 81 YWEKGTQSRKSKEQWFNVNLKILMERMRHNNNSDVHVLQWRHGCEIDSQGNDVRFSKGIDEYSYDGRNFLAFDDADSQWV
Mhc1zea^{3716} 81 YWEKGTQSRKSKEQWFNVNLKILMDRMRHNNNSDFHVLQWRHGCEIDSQDDDVRFSKGIDEYSYDGRNFLAFDDAESQWV
Mhc1zea^{3674} 81 YWEKGTQSRKSKEQWFNVNVGILMDRMRHNK-SDLHVLQWRHGCEIDSQDDDVRFSKGIDEYSYDGENFLAFDDAESRWV
Mhc1zea^{4039} 81 YWEKGTQSRKSKEQWFNVNLKILMDRMRHNNNSDLHVLQWRHGCEIDSQDDDVRFSKGIDEYSYDGKNFLAFDDAESRWV

Mhc1zea^{3669} 161 APVEEALPTKRKWDNVPILNQYTKGYLEKECVDWLNKFREYGDQELREGSPPDVHVFAKKIISGKAKLKLTCMVTGFYPK
Mhc1zea^{3895} 161 APVEEALPTKRKWDNVPILNQYTKGYLEKECVDWLNKFREYGDQELREGSPPDVHVFAKKIISGKAKLKLTCMVTGFYPK
Mhc1zea^{3716} 161 APVEEALPTKRKWDKVPILNQYTKGYLEKECVDWLNKFREYGDQELREGSPPDVHVFAKKIISGKAKLKLTCLVTGFYPK
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Mhc1zea^{4039} 161 APVEEALPTKRKWDNVDILNQYTKGYLEKECVDWLNKFREYGDQELGEGSPPDVHVFAKKIISGKAKLKLTCMVTGFYPK

Mhc1zea^{3669} 241 DVILTIRKYRTALSDNEVESSGVRPNPDGTFQLRKSTNIYEKAEYDCYVAHRTLKEPIIKKWDGECQDCSSGTPIGTIFG
Mhc1zea^{3895} 241 DVILTIRKYRTALSDNEVESSGVRPNPDGTFQLRKSTNIYEKAEYDCYVAHRTLNEPIIKKWDGECQDCSSGTPIGTIFG
Mhc1zea^{3716} 241 DVYLTIRKYRTALSDNEVESSGVRPNPDGTFQLRKSTNIYEKAEYDCYVAHRTLKEPIIIKWDGECQDCSSGTPIGTIFG
Mhc1zea^{3674} 240 DVILTIRKYRTALSDNEVESSGVRPNPDGTFQLRKSTNIYEKAEYDCYVSHRTLKEPIIKKWDGECQDCSSGTPIGTIVG
Mhc1zea^{4039} 241 DVYLTIRKYRTALSDNDLESSGVRPNHDGTFQLRKSTYINEKAEYDCYVSHRTLKEPIIKKWDGECQDCSSGTPIGTIFG

Mhc1zea^{3669} 321 ALIGVLLVLAVIGGAVYFLANTRMGWRNAL
Mhc1zea^{3895} 321 ALIGVLLVLAVIGGAVYFLANTRMGWRNAL
Mhc1zea^{3716} 321 ALIGVLLVLAVIGGAVYFLANTRMGWRNAL
Mhc1zea^{3674} 320 ALIGVLLVLAVIGGLVYFLANTRMGWRNAL
Mhc1zea^{4039} 321 ALIGVLLVLAAIGGAVYFLANTRMGWRNAL

Mhc1zfa

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Mhc1zfa^{3686} 1 MIMDVKVLTLLCVFLLYGALPLCRAEKHSLYYIYTGLSRPVDLPGIYEFSAMGLLDDRQIDSYNSREQRKIPKQQWMKEK
Mhc1zfa^{3974} 1 MIMDVKVFTSLCVLLFYGALPSCRAEKHSLYYIYTGLSRPVDLPGIYEFSAMGLLDDRQIDSYNSREQRKIPKQQWMKEK
Mhc1zfa^{3690} 1 MIMDVKVFTLLCAFLLNGALPSCRAEKHSLYYIYTGLSKPVDLPGIYEFSAMGLLDDRQIDSYNSREQINIPKQQWMKEK

Mhc1zfa^{3942} 81 MQEDYWEKGTQSRKSKEQWFNVNVHILMDRMRHNRLDLHVLQWRHGCEVEIKGSEVKFSKGIDEYGYDGENFLAFDEAES
Mhc1zfa^{3686} 81 MQEDYWEKGTQSRKSKEQWFNVNVHILMERMRHNRLDLHVLQWRTGCEVEIKGSEVKFSKGIDEYGYDGENFLAFDDAES
Mhc1zfa^{3974} 81 MQEDYWEKGTQSRKSKELWFNVNVNILMDRMRHNRLDLHVLQWRTGCEVEIKGSEVKFSKGIDEYGYDGENFLAFDDAES
Mhc1zfa^{3690} 81 MQEDYWEKGTQSRKSKEQWFNVNVHILMERMRHNRSDLHVLQWRTGCEVEIKGSEVKFSKGIDEYGYDGENFLAFDDAES

Mhc1zfa^{3942} 161 QWVAPVEEALPTKRKWDSVPILNQYTKGYLEKECVDWLNKFREYANEELRNGSPPEVHVFAVKSISDKTKLKLTCLATGF
Mhc1zfa^{3686} 161 QWVAPVEEALPTKRKWDSVPILNQYTKGYLEKECVDWLNKFREYANEELRNGSPPEVHVFAVKSISDKTKLKLTCLATGF
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Mhc1zfa^{3690} 161 QWVAPVEEALPTKRKWDSVPILNQYTKGYLEKECVDWLNKFREYANEELRNGSPPEVHVFAVKSISDKTKLKLTCLATGF

Mhc1zfa^{3942} 241 YSKDTMLVIRRNRLPEKKTESTGVRPNHDQTFQLRKSVEIEQDETDEYDCYMTHRTLKGPVIARWDGKCKDCLPNLNWIW
Mhc1zfa^{3686} 241 YSKDTMLVIRRNRLPEKKTESTGVRPNHDQTFQLRKSVEIEQDETDEYDCYMTHRTLKGPVIARWDGKCKDCLPNLNWIC
Mhc1zfa^{3974} 241 YSKDTMLVIRRNRLPEKKTESTGVRPNHDQTFQLRKSVEIEQDETDEYDCYMTHRTLKGPVIARWDGKCKDCLPNLNWIC
Mhc1zfa^{3690} 241 YSKDTMLVIRRNRLPEKKTESTGVRPNHDQTFQLRKSVEIEQDETDEYDCYMTHRTLKGPVIARWDGKCKDCLPNLNWIC

Mhc1zfa^{3942} 321 VVAGAVLMLGVVALLLVLLKKKIIDLRQRLSGSQSPLYQIQADSESEDYNH
Mhc1zfa^{3686} 321 VVAGAVLMLGVVALLLVLLKKKIIDLRQRLSGSQSPLYQIQADSESEDYNH
Mhc1zfa^{3974} 321 VVAGAVLMLGVVALLLVLLKKKIIDLRQRLSGSQSPLYQIQADSESEDYNH
Mhc1zfa^{3690} 321 VVAGAVLMLGVVALLPVLLKKKIIGSRQRLSGSQSPLYQIQADSESEDYNH

Mhc1zja

Mhc1zja^{3692} 1 MTSFDRSFTLLYLCLFHVIMSSFRAEKHSLYFIYTGLSRPVDLPGIYEFSAMGLLDDRQIDSYNSEEQRNIPKQQWMKEK
Mhc1zja^{4117} 1 MTSFDRSFTLLYLCLFHVIMSSFRAEKHSLYFIYTGLSRPVDLPGIYEFSAMGLLDDRQIDSYNSEEQRNIPKQQWMKEK
Mhc1zja^{3701} 1 MTSFDRSFTLLYLCLFHVILSSFRAEKHSLYFIYTGLSRPLDLPGIYEFSAMGLLDDRQIDSYNSEEQRNIPKQQWMKEN
Mhc1zja^{3703} 1 MTSFDKSFTLLYLCLFHVILSSFRAEKHSLYFIYTGLSRPLDLPGIYVFSAMGLLDDRQIDSYNSREQRNIPKQQWMKEN

Mhc1zja^{3692} 81 MQEDYWENRTQSRKEKQLWFYDNVHLLIDRNRQSTSVLHVLQWRHGCEVEKQGNEHSFNKSIDEYGYDGEDFLYYDDAES
Mhc1zja^{4117} 81 MQEDYRENRTQSRKEKQLWFYDNVHLLIDRNRQST---
Mhc1zja^{3701} 81 MQEDYWENRTQSRKSKELWFYDNVHLLIDRNRQSTSDLHVLQWRHGCEVEKQGNEHSFNKSINEYGYEGKDFLSFDEAES
Mhc1zja^{3703} 81 MQEDYWENRTQSRKEQQRWFYKNVHLLIDRNRQSTSDLHVLQWRHGCEVEKQGNEHSFNKSINEYGYEGKDFLSFDEAES

Mhc1zja^{3692} 161 RWVAPVEEALPTKRKWDNLPILNQYTKDYLEKECVDWLNKFREYADEELRKASPPDVFKLTRKSTKDETKLKLTCLATGF
Mhc1zja^{4117} 116 --
Mhc1zja^{3701} 161 RWVTAVEEALPTKRKWDSVPILNQYTKDYLEKECVDWLNKFREYVDEEFRKASPPDVYKFTKKSTKDETKLKLTCLATGF
Mhc1zja^{3703} 161 RWVTAVEEALPTKRKWDSVPILNQYTKDYLEKECVDWLNKFREYADEEFRKASPPDVYKFTKKSTKDETKLKLTCLATGF

Mhc1zja^{3692} 241 YPNDVMLNIRRYRVYLPEQETISTGVRPNHDQTFQLSKSVEIKEDQIDNYDCSVSHRTFKQPIIIKWDGTDLDGIYTGHP
Mhc1zja^{4117} 116 --SDGTDLDGIYTGHP
Mhc1zja^{3701} 241 YPNDVMLNIRRYRVYLPEQETISTGVRPNHDQTFQLSKSVEIKEDQIDNYDCSVSHRTFKQSIIIKWDGTDLDGKYTGYP
Mhc1zja^{3703} 241 YPNDVMLNIRRYRVYLPEQETISTGVRPNHDQTFQLSKSVEIKEDQIDNYDCSVSHRTFKQSIIIKWDGTDLDGKYTGYP

Mhc1zja^{3692} 321 PETVPVVIGSVLIFLAILGVVVFLVMKCG------------------------EQDSSSTDTTECTSILLGFIADHLNKE
Mhc1zja^{4117} 130 PETVPVVIGSVLIFLAILGVVVFLVMKCG------------------------EQDSSSTDTTECTSILLGFIADHLNKE
Mhc1zja^{3701} 321 PETVPVVIGSVLIFLAILAVVVFLVMNYDAVCLPRELQKTESQKTLQNQSSTTEQDSSSTDTTECTSILPGFIADHLNKE
Mhc1zja^{3703} 321 PETVPVVIGSVLIFLAILGVVVFLVMNYDAVCLPRELQKTESQKTLQNQSSTTEQDSSSTDTTDCTSILPRFNANHQYKV

Mhc1zja^{3692} 377 LTEGENW
Mhc1zja^{4117} 186 LTEGENW
Mhc1zja^{3701} 401 LTEGENW
Mhc1zja^{3703} 401 VTKEECI

Mhc1zla

Mhc1zla^{3778} 1 MAVLAVLFSAVMLLSVVPAWT---------EKHSLFYIYTALSRPVDLPGIYEFTAMGLLDDRQIDYYNSIDQKKIPKQP
Mhc1zla^{3781} 1 MAVLAVLFSAVMLLSVVPAWTDHVLLSFNTEKHSLFYIYTALSRPVDLPGIYEFTAMGLLDDRQIDYYNSIDQKKIPKQP

Mhc1zla^{3778} 72 WMKEKMQEDYWEKGTQSRKSKEQWFNVNVNILMDRMRHNKSDVHVLQWRHGCEIDSQGNDVRFSKGIDEYSYDGENFLAF
Mhc1zla^{3781} 81 WMKEKMQEDYWEKGTQSRKSKEQWFNVNVNILMDRMRHNKSDVHVLQWRHGCEIDSQGNDVRFSKGIDEYSYDGENFLAF

Mhc1zla^{3778} 152 DDAESRWVAPVEEALPTKRKWDNVPILNQYTKGYLEKECVDWLKKFREYGDQELRKVSPRDVHVFAKRYINGKDKLKLTC
Mhc1zla^{3781} 161 DDAESRWVAPVEEALPTKRKWDNVPILNQYTKGYLEKECVDWLKKFREYGDQELRKVSPRDVHVFAKRYINGKDKLKLTC

Mhc1zla^{3778} 232 LATGFYPKDVYLTIRKYRTALSDNDLESSGVRPNHDGTFQLRKSTYINEDEKAEYDCYVNHRTLKEPVIIRWNGECLSEP
Mhc1zla^{3781} 241 LATGFYPKDVYLTIRKYRTALSDNDLESSGVRPNHDGTFQLRKSTYINEDEKAEYDCYVNHRTLKEPVIIRWNGECLSEP

Mhc1zla^{3778} 312 PIAMIAGIIGVLILLGAIGVTVWILKKKNIIGNGDEKHFVVSTVSGNKDENGFVVSTVSGNKDEKRSMPDGSADSGKGSS
Mhc1zla^{3781} 321 PIAMIAGIIGVLILLGAIGVTVWILKKKNIIGNGDEKHFVVSTVSGNKDENGFVVSTVSGNKDEKRSMPDGSADSGKGSS

Mhc1zla^{3778} 392 A
Mhc1zla^{3781} 401 A

Figure S4. Sequence variation of MHC class I Z proteins. Proteins encoded by transcripts described in manuscript are shown aligned. Transcript identity numbers are included as superscripts. Proteins encoded by alternatively spliced transcript variants (tv) are included.