Supplementary Data File for: MHC genetic diversity confers transgenerational fitness advantage in dolphins

SUPPLEMENTARY TABLES:

Supplementary Table 1 | Correlation Matrix

	MHC Het Sites	Het PBR Sites	Msat Het	Soci-ability	Age at death	Age at last calf	Age last surv. calf	Calving Success	Repro Output 1	Repro Output 2	Offspring Viability 1	Offspring Viability 2
MHC Het Sites		0.9660	-0.0951	-0.2325	0.1118	0.0599	0.1331	0.3262	0.0155	-0.1214	0.3654	0.3282
MHC Het PBR Sites	<0.0001		-0.0167	-0.2371	0.1170	0.0189	0.0862	0.3008	0.0081	-0.1001	0.3323	0.2967
Msat Het	0.4509	0.8951		0.2678	-0.0897	-0.1271	-0.0134	-0.1164	0.1626	0.0599	-0.1669	-0.1334
Sociability	0.0477	0.0434	0.0310		-0.3690	-0.2189	-0.2174	-0.1854	0.1988	0.1387	-0.3869	-0.2213
Age at death	0.4255	0.4043	0.5488	0.0065		0.8689	0.8464	0.2690	-0.3987	-0.2242	0.4298	0.2909
Age at last calf	0.6198	0.8759	0.3130	0.0666	<0.0001		0.7963	0.3809	-0.0658	-0.2740	0.4528	0.4057
Age last surv. calf	0.3149	0.5165	0.9244	0.0981	<0.0001	<0.0001		0.3909	-0.1695	-0.3246	0.5177	0.4550
Calving Success	0.0049	0.0097	0.3556	0.1164	0.0515	0.0010	0.0022		0.4531	-0.4795	0.8539	0.9444
Repro Output 1	0.8965	0.9460	0.1956	0.0917	0.0031	0.5858	0.1992	<0.0001		-0.1072	0.0044	0.3248
Repro Output 2	0.3064	0.3992	0.6354	0.2418	0.1065	0.0208	0.0121	<0.0001	0.3667		-0.5861	-0.6277
Offspring Viability 1	0.0017	0.0046	0.1839	0.0009	0.0015	<0.0001	<0.0001	<0.0001	0.9711	<0.0001		0.8826
Offspring Viability 2	0.0046	0.0108	0.2894	0.0599	0.0346	0.0050	<0.0001	<0.0001	0.0051	<0.0001	<0.0001	

Pearson correlation *r* values are shown above the diagonal, and corresponding *P*-values shown below the diagonal. MHC Het Sites: The number of heterozygous sites for the entire MHC sequence for each female. MHC Het PBR Sites = The number of heterozygous sites for the putative peptide-binding region of the MHC sequences, as defined by previous studies on this region[14,15] for each female. Msat Het = the number of heterozygous sites per locus across 23 microsatellite loci. Repro Output 1 = observed reproductive output, based on confirmed sightings of calves. Repro Output 2 = potential maximum reproductive output. Offspring viability 1: observed offspring viability, based on confirmed sightings of calves. Offspring viability 2: potential minimum estimate. Sociability = the proportion of sightings of adult female with one or more other adults. Age at death = the estimated age at death of adult female, i.e. longevity; Age last calf = the estimated age of female when she produced her last offspring; Age at last surv. calf = the estimated age of female when she produced her last calf that survived to weaning. This is depicted as a correlation matrix heat map in Supplementary Fig. 1.

Supplementary Table 2 | Principal component regression (PCR). Table shows relationships between the predictor variables MHC heterozygosity and age variables versus dependent fitness variables described below. Predictor variables are defined as: (1) ‘Het Sites’ = the number of heterozygous sites of entire MHC II DQB sequence; (2) ‘Het PBR Sites’ = the number of heterozygous sites of parts of the MHC II DQB that code for putative peptide-binding regions (PBR), as defined by previous studies on this region[14,15]; (3) Age at death = the estimated age at death of adult female, i.e. longevity; (4) Age last calf = the estimated age of female when she produced her last offspring. (5) Age surv. calf = the estimated age of female when she produced her last calf that survived to weaning. Dependent variables are: Repro output 1 = observed reproductive output, based on confirmed sightings of calves; Repro output 2 = potential maximum reproductive output; Offspring viability 1 = observed offspring viability, based on confirmed sightings of calves; Offspring viability 2 = potential minimum estimate. Rows that are shaded represent significant positive relationships. The least square estimates of the regression coefficients (‘Estimate’), corresponding 95% confidence interval (asymptotic; 95% CI) and regression *t*-values are tabulated. Analysis of variance summary statistics of the analyses, including sum of squares (SS) for the regression (SS Reg) and total, i.e. combined SS of regression and residuals (SS total), *F* ratios (*F*) and *P*-values are shown below ‘ANOVA’. Goodness of fit results are presented as R-squared (R^2), i.e. coefficient of determination, and the standard deviation of the residuals (Sy.x).

									ANOVA				Goodness of Fit	
Dep. Var	Par. Est.	Variable	Estimate	SE	95% CI	|*t*|	*P* value	Sig.	SS Reg.	SS Total	*F*	*P* value	R^2	Sy.x
Calving Success	β0	Intercept	0.05473	0.03180	-0.009544 to 0.1190	1.721	0.0930	ns	0.0210	0.1075	4.847	0.0130	0.1951	0.0465
	β1	MHC Het	0.001181	0.000433	0.0003059 to 0.002056	2.728	0.0094	**						
	β2	MHC PBR	0.001669	0.000614	0.0004273 to 0.002910	2.717	0.0097	**						
	β3	Age at death	0.000393	0.000300	-0.0002129 to 0.0009993	1.311	0.1973	ns						
	β4	Age last calf	0.000324	0.000356	-0.0003949 to 0.001043	0.9114	0.3676	ns						
	B5	Age sur. calf	0.000579	0.000338	-0.0001037 to 0.001261	1.714	0.0943	ns						
Repro Output 1	β0	Intercept	0.2764	0.03956	0.1965 to 0.3564	6.988	<0.0001	***	0.0114	0.1453	1.704	0.1950	0.0785	0.0579
	β1	MHC Het	-0.00011	0.000539	-0.001198 to 0.0009785	0.2039	0.8394	ns						
	β2	MHC PBR	-0.00015	0.000764	-0.001690 to 0.001399	0.1905	0.8499	ns						
	β3	Age at death	-0.00068	0.000373	-0.001432 to 7.635e-005	1.816	0.0768	ns						
	β4	Age last calf	-0.00078	0.000443	-0.001671 to 0.0001181	1.754	0.0871	ns						
	B5	Age sur. calf	-0.00078	0.000420	-0.001624 to 7.396e-005	1.845	0.0724	ns						
Repro Output 2	β0	Intercept	0.3597	0.04341	0.2719 to 0.4474	8.285	<0.0001	***	0.0151	0.1764	1.871	0.1672	0.0856	0.0635
	β1	MHC Het	-0.00023	0.000591	-0.001428 to 0.0009606	0.3956	0.6945	ns						
	β2	MHC PBR	-0.00032	0.000838	-0.002015 to 0.001374	0.3817	0.7047	ns						
	β3	Age at death	-0.00076	0.000409	-0.001590 to 6.501e-005	1.862	0.0699	ns						
	β4	Age last calf	-0.00086	0.000486	-0.001843 to 0.0001206	1.773	0.0839	ns						
	B5	Age sur. calf	-0.00088	0.000461	-0.001817 to 4.694e-005	1.919	0.0621	ns						
Offspring Viability 1	β0	Intercept	0.1510	0.1293	-0.1104 to 0.4124	1.167	0.2500	ns	0.6837	2.116	9.549	0.0004	0.3232	0.1892
	β1	MHC Het	0.005854	0.001761	0.002295 to 0.009412	3.324	0.0019	**						
	β2	MHC PBR	0.008253	0.002498	0.003204 to 0.01330	3.304	0.0020	**						
	β3	Age at death	0.003175	0.001220	0.0007100 to 0.005641	2.603	0.0129	*						
	β4	Age last calf	0.003035	0.001447	0.0001100 to 0.005960	2.097	0.0424	*						
	B5	Age sur. calf	0.004249	0.001374	0.001473 to 0.007026	3.093	0.0036	**						
Offspring Viability 2	β0	Intercept	0.1130	0.1465	-0.1832 to 0.4091	0.7710	0.4452	ns	0.4358	2.273	4.744	0.0142	0.1917	0.2143
	β1	MHC Het	0.004860	0.001995	0.0008290 to 0.008891	2.437	0.0194	*						
	β2	MHC PBR	0.006856	0.002830	0.001137 to 0.01258	2.423	0.0200	*						
	β3	Age at death	0.002368	0.001382	-0.0004248 to 0.005161	1.714	0.0943	ns						
	β4	Age last calf	0.002207	0.001640	-0.001106 to 0.005521	1.346	0.1858	ns						
	B5	Age sur. calf	0.003226	0.001556	8.103e-005 to 0.006371	2.073	0.0446	*						

Supplementary Table 3 | Linear regression analysis for regression plots (Fig. 1a-c, main text). Association between the number of MHC heterozygous sites of entire sequence (predictor) and fitness measures (calving success, reproductive output and offspring viability).

Predictor: MHC Heterozygous Sites

Fitness	***n***	**Slope (SE)**	R^2	***F***	***p***	**sig.**[c]
Calving success	73	0.2869 (0.09868)	0.1064	8.46	0.0049	**
Repro Output 1[a]	73	0.01565 (0.1199)	0.00024	0.017	0.8965	ns
Repro Output 2[a]	73	-0.1261 (0.1224)	0.01473	1.064	0.3064	ns
Offspring viability 1[b]	71	1.458 (0.4471)	0.1335	10.63	0.0017	**
Offspring viability 2[b]	73	1.156 (0.3950)	0.1077	8.57	0.0046	**

[a] Repro Output 1 = observed reproductive output, based on confirmed sightings of calves; Repro Output 2 = potential maximum reproductive output. [b] Offspring viability 1 = observed offspring viability, based on confirmed sightings of calves. [b] Offspring viability 2 = potential minimum estimate of offspring viability. [c] sig. = statistical significance testing whether slope is significantly non-zero, based on linear regression: ns = not significant ($P \geq 0.05$), *$P < 0.05$; **$P < 0.01$.

Supplementary Table 4 | Two-tailed Mann-Whitney *U* tests: Comparing fitness measures for MHC heterozygous vs homozygous adult females

Mann-Whitney *U*: All Sites

FITNESS	**Het.**[c]	**Hom.**[c]	***n* Het.**[d]	***n* Hom.**[d]	***Mann-Whitney U***	***P***	**sig.**[e]
Calving success	0.1109 (0.008450)	0.06335 (0.01938)	66	7	130.0	0.0571	ns
Repro output 1[a]	0.1942 (0.009687)	0.2174 (0.03815)	66	7	177.5	0.3254	ns
Repro output 2[a]	0.3017 (0.02986)	0.3179 (0.02986)	66	7	191.5	0.4699	ns
Offspring viability 1[b]	0.5694 (0.03814)	0.2738 (0.07867)	64	7	90.5	0.0076	**
Offspring viability 2[b]	0.4153 (0.03371)	0.2190 (0.06730)	66	7	125	0.0451	*

[a] Repro Output 1 = observed reproductive output, based on confirmed sightings of calves; Repro Output 2 = potential maximum reproductive output. [b] Offspring viability 1 = observed offspring viability, based on confirmed sightings of calves. [b] Offspring viability 2 = potential minimum estimate of offspring viability. [c] mean fitness estimates for adult females that were heterozygous (‘Het.’) and homozygous (‘Hom’) for the MHC region. Standard errors are shown in brackets. [d] sample sizes for adult females that were heterozygous (‘Het.’) and homozygous (‘Hom’) for the MHC region. Standard errors are shown in brackets. [e] sig. = statistical significance testing for Mann-Whitney *U* test: ns = not significant ($P \geq 0.05$), $*P < 0.05$; $**P < 0.01$. Results for Mann-Whitney U tests, based on heterozygotes versus homozygotes for the peptide-binding region (PBR) of the MHC sequences are tabulated in Supplementary Table 5.

Supplementary Table 5 | Two-tailed Mann-Whitney *U* tests: Comparing fitness measures for MHC heterozygous vs homozygous adult females, based on peptide-binding region (PBR)

Mann-Whitney *U*: PBR Sites

FITNESS	Het.[c]	Hom.[c]	*n* Het.[d]	*n* Hom.[d]	*Mann-Whitney U*	*P*	sig.[e]
Calving success	0.1128 (0.008535)	0.06003 (0.01700)	64	9	153	0.0214	*
Repro output 1[a]	0.1941 (0.009954)	0.2127 (0.02988)	64	9	237	0.4003	ns
Repro output 2[a]	0.3028 (0.01011)	0.3065 (0.02496)	64	9	262	0.6705	ns
Offspring viability 1[b]	0.5780 (0.03828)	0.2796 (0.07826)	62	9	118.5	0.0040	**
Offspring viability 2[b]	0.4214 (0.03414)	0.2180 (0.06260)	64	9	155.5	0.0239	*

[a] Repro Output 1 = observed reproductive output, based on confirmed sightings of calves; Repro Output 2 = potential maximum reproductive output. [b] Offspring viability 1 = observed offspring viability, based on confirmed sightings of calves. [b] Offspring viability 2 = potential minimum estimate of offspring viability. [c] mean fitness estimates for adult females that were heterozygous (‘Het.’) and homozygous (‘Hom’) for the MHC region. Standard errors are shown in brackets. [d] sample sizes for adult females that were heterozygous (‘Het.’) and homozygous (‘Hom’) for the peptide-binding region (PBR)[14,15] of the MHC sequence. Standard errors are shown in brackets. [e] sig. = statistical significance testing for Mann-Whitney *U* test: ns = not significant ($P \geq 0.05$), $*P < 0.05$; $**P < 0.01$. Results of Mann-Whitney *U* tests, based on heterozygotes versus homozygotes for the entire MHC sequences are tabulated in Supplementary Table 4.

Supplementary Table 6 | Two-tailed Mann-Whitney *U* tests: Comparing MHC F_{IS} for high vs low fitness measures

Fitness Measure[a]		n^b	$F_{IS}{}^c$	*U*	*P*	*sig*
Calving Success	High	36	-0.120 (0.034)	1437	0.0438	*
	Low	36	0.057 (0.037)			
Repro Output 1	High	36	-0.030 (0.038)	1972	0.5208	ns
	Low	36	-0.071 (0.030)			
Repro Output 2	High	36	-0.024 (0.033)	1988	0.4632	ns
	Low	36	- 0.088 (0.031)			
Offspring Viability 1	High	35	-0.155 (0.039)	1421	0.0074	**
	Low	35	0.029 (0.031)			
Offspring Viability 2	High	36	-0.103 (0.037)	1448	0.0402	*
	Low	36	0.042 (0.035)			

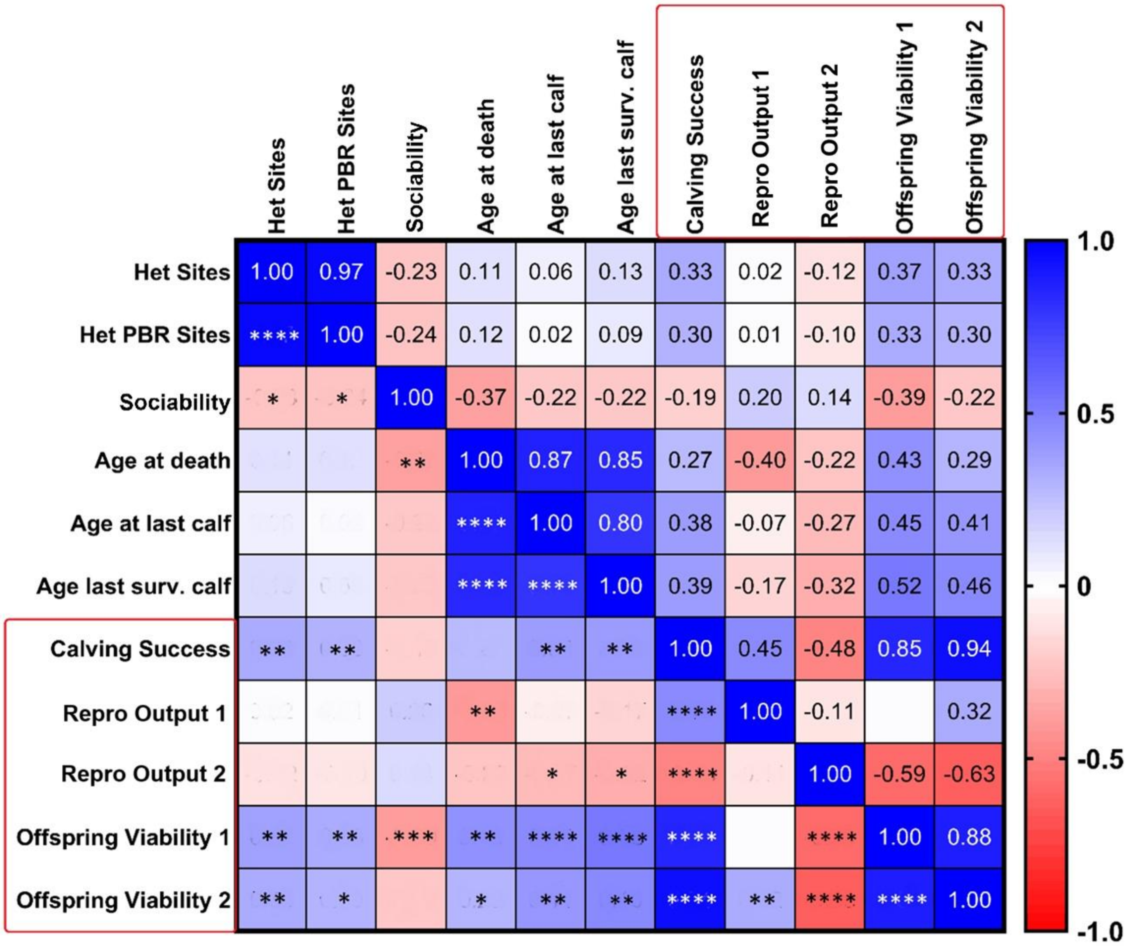
[a] Repro Output 1 = observed reproductive output, based on confirmed sightings of calves; Repro Output 2 = potential maximum reproductive output. [b] Offspring viability 1 = observed offspring viability, based on confirmed sightings of calves. [b] Offspring viability 2 = potential minimum estimate of offspring viability. ‘High’ = subset of sample with fitness values above the median; ‘Low’ = subset of sample with fitness values below the median. [b] *n* = the number of adult females/sequences in the sample. [c] F_{IS} = inbreeding coefficient (standard error in brackets) for the MHC locus. sig. = statistical significance comparing diversity measures for ‘high’ vs ‘low’, based on Mann-Whitney *U* tests: ns = not significant ($P \geq 0.05$), *$P< 0.05$; **$P < 0.01$.

Supplementary Table 7 | Two-tailed Mann-Whitney *U* tests: Comparing microsatellite F_{IS} for high vs low fitness measures

Fitness Measure[a]		n^b	F_{IS}^c	*U*	*P*	*sig*
Calving Success	High	32	0.0076 (0.032)	263.5	0.9871	ns
	Low	32	0.0193 (0.035)			
Repro Output 1	High	32	0.0010 (0.024)	227	0.4167	ns
	Low	32	0.0391 (0.032)			
Repro Output 2	High	32	0.030 (0.023)	240	0.5975	ns
	Low	32	0.011 (0.034)			
Offspring Viability 1	High	32	0.034 (0.033)	233.5	0.5031	ns
	Low	32	0.014 (0.032)			
Offspring Viability 2	High	32	0.038 (0.035)	235	0.5241	ns
	Low	32	0.010 (0.036)			

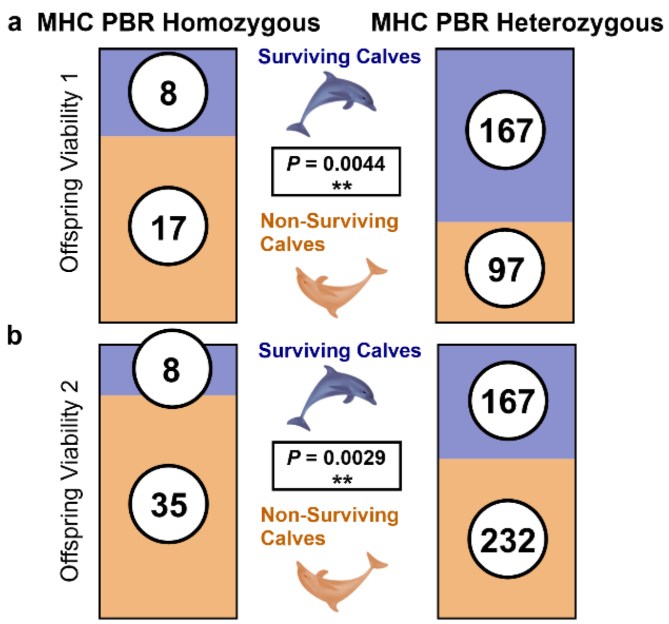
[a] Repro output 1 = observed reproductive output, based on confirmed sightings of calves; Repro output 2 = potential maximum reproductive output. [b] Offspring viability 1 = observed offspring viability, based on confirmed sightings of calves. [b] Offspring viability 2 = potential minimum estimate of offspring viability. 'High' = subset of sample with fitness values above the median; 'Low' = subset of sample with fitness values below the median. [b] *n* = the number of adult females/sequences in the sample. [c] F_{IS} = inbreeding coefficient (standard error in brackets) across 23 polymorphic microsatellite loci. sig. = statistical significance comparing diversity measures for 'high' vs 'low', based on Mann-Whitney *U* tests: ns = not significant ($P \geq 0.05$).

SUPPLEMENTARY FIGURES:



	Het Sites	Het PBR Sites	Sociability	Age at death	Age at last calf	Age last surv. calf	Calving Success	Repro Output 1	Repro Output 2	Offspring Viability 1	Offspring Viability 2
Het Sites	1.00	0.97	-0.23	0.11	0.06	0.13	0.33	0.02	-0.12	0.37	0.33
Het PBR Sites	****	1.00	-0.24	0.12	0.02	0.09	0.30	0.01	-0.10	0.33	0.30
Sociability	*	*	1.00	-0.37	-0.22	-0.22	-0.19	0.20	0.14	-0.39	-0.22
Age at death			**	1.00	0.87	0.85	0.27	-0.40	-0.22	0.43	0.29
Age at last calf				****	1.00	0.80	0.38	-0.07	-0.27	0.45	0.41
Age last surv. calf				****	****	1.00	0.39	-0.17	-0.32	0.52	0.46
Calving Success	**	**			**	**	1.00	0.45	-0.48	0.85	0.94
Repro Output 1				**			****	1.00	-0.11		0.32
Repro Output 2					*	*	****		1.00	-0.59	-0.63
Offspring Viability 1	**	**	***	**	****	****	****		****	1.00	0.88
Offspring Viability 2	**	*		*	**	**	****	**	****	****	1.00

Supplementary Fig. 1 | Correlation matrix heat map. Pearson correlation *r* values for significant correlations are shown above the diagonal, and corresponding significance, based on *P*-values shown as asterisks below the diagonal: $*P < 0.05$; $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$. Corresponding *P*-values are shown in Supplementary Table 1. Het Sites = The number of heterozygous sites for the entire MHC sequence for each female. Het PBR Sites = The number of heterozygous sites for the putative peptide-binding region of the MHC sequences, as defined by previous studies on this region[14, 15] for each female. Sociability = the proportion of sightings of adult female with one or more other adults. Age at death = the estimated age at death of adult female, i.e. longevity; Age last calf = the estimated age of female when she produced her last offspring; Age at last surv. calf = the estimated age of female when she produced her last calf that survived to weaning. Fitness measures are highlighted in red box: Repro Output 1 = observed reproductive output, based on confirmed sightings of calves. Repro Output 2 = potential maximum reproductive output. Offspring viability 1 = observed offspring viability, based on confirmed sightings of calves. Offspring viability 2= potential minimum estimate of offspring viability.



Supplementary Fig. 2 | Bar graphs & two-tailed Fisher's exact test results (MHC-PBR). Comparison between the number of surviving and non-surviving calves collectively produced by adult females that were homozygous or heterozygous for the MHC peptide-binding region (PBR). Stacked bar graphs show the relative percentages of surviving (blue) and non-surviving calves (orange) cumulatively produced by adult females who were homozygous or heterozygous for the entire MHC region. The numbers of surviving and non-surviving calves are shown in white font and black shading; percentages of surviving and non-surviving calves are shown in parentheses (black font). Fisher's exact test *P*-values are shown for each comparison in boxes between stacked bar graphs: $^{**}P < 0.01$. **a**, estimates for non-surviving calves based on confirmed sightings of calves (offspring viability 1); **b**, estimates for non-surviving calves, based on potential minimum estimate for offspring viability (offspring viability 2). Results for the entire MHC II DQB region are shown in Fig. 2 of main text.

References:

14. Heimeier, D., Baker, C.S., Russell, K., Duignan, P. J., Hutt, A., & Stone, G.S. Confirmed expression of MHC class I and class II genes in the New Zealand endemic Hector's dolphin (*Cephalorhynchus hectori*). *Mar. Mammal Sci.* **25**, 68–90 (2009).

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