The following supplement accompanies the article

Characterization of genetic variation and basis of inflammatory bowel disease in the Toll-like receptor 5 gene of the red wolf and the maned wolf

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Fig. S1. Primers used

Primer	Manufacturer	Direction	Primer sequence
TLR5 Fragment 1	Eurofins MWG (Huntsville, USA)	Forward	5'-GTT TCT CAA GGA CCC AGC AC-3'
		Reverse	5'-TCC TGA AGG CTT CTC TGT CG-3'
TLR5 Fragment 2	Eurogins MWG (Huntsville, USA)	Forward	5'-GCT GCA CCT GAA CCA CAA C-3'
		Reverse	5'-TGA AGA GGG AGA ACG TGA GG-3'

Fig. S2. PCR cycling conditions

Frag1 cycling conditions		
Cycle Number	Settings:	
1	95°C	10 minutes
35	95°C	1 minute
	57°C	1 minute
	72°C	2 minutes
1	72°C	7 minutes
Frag2 cycling conditions		
Cycle Number	Settings:	
1	95°C	8 minutes
2	95°C	30 seconds
	64°C	30 seconds
	72°C	1 minute
2	95°C	30 seconds
	62°C	30 seconds
	72°C	1 minute
2	95°C	30 seconds
	60°C	30 seconds
	72°C	1 minute
2	95°C	30 seconds
	58°C	30 seconds
	72°C	1 minute
2	95°C	30 seconds
	56°C	30 seconds
	72°C	1 minute
1	72°C	15 minutes

Fig. S3. Polymorphic positions between domestic dog (DD), maned wolf (MW) and red wolf (RW) sequences

```
DD    ATGGGAGGAGAGCGTGCGTGCAGAGGAAGCAGCACGTGCCAAGTCCCAGC 50
MW
RW

DD    CCTGGAAGTGCGTGGAGAGGCGGCCGGAGCCCCCGTGTTGCACCCCCCGC 100
MW
RW

DD    CTCCCCGCTGGGCCTCCTTCCTTTGCATCCCTGGGGCCCCTGGATGTCAT 150
MW
RW

DD    CGAGTCATCGGGGGCCTTCCTGGCCACTCTGGCCGCTGCCGCCTGCCCCA 200
MW
RW

DD    GGACGTGCGCCCCAGCTGGCCCGTGTGTCCAGCCCCTGCTGCACCCTGTG 250
MW
RW

DD    CACAGAGCGGACGTGTGGCACTTGTCCAGATGACGGGCGCCCTGAGCCGC 300
MW
RW

DD    GCCGCGCTCGCCCCCACAGGCCTGGGCAGGGGGTGGGAGGGGGATGCACT 350
MW
RW

DD    GACCCGTCAGGCTGGGCGCTTCGCGGATGGTGGCCCGAAGGACCAGCGTG 400
MW
RW
```

```
DD    TGCGTGCTGACCCGGGCCGTGTGTGGAGCGCGCAGGGGGCGGAGGGGCGG 450
MW
RW

DD    GCCCCCGGCACTGGGCGGGGGCGGCACCAGTATCCTCCGCCTGCCATTTT 500
MW
RW

DD    CCCCGAAGCCCTGCACGCATCCCGATTGAGTGACGGCAAACAGACTCTCC 550
MW
RW

DD    TCAAGGTAAGTGTTTCTCAAGGACCCAGCACGGCGCTGAGTGCGCGTCCC 600
MW                                  ------------------
RW

DD    GCCGGGCGCACGTGTGGGGGGAGGCAGGTGCCCGTCCAGGGGCCCCCGTC 650
MW    ---A---C-------------------------------------A--
RW              ------------------------R----------------

DD    CTGGGCCCCGGGGTGGCGTTGGGCTTGCACGGCTGTGTTTCCGTCCCGCA 700
MW    -C---------------------------------------------
RW    -Y---------------------------------------R-------

DD    GGATCATGGGCCGCCAGCTGGGCCGCACGCTGGGGCTGCTGCTTGTGGCC 750
MW    ------------S----------T--G-----------------------
RW    --------------------------G-R--------------------

DD    GGCGCCGTGGCCGCAGCATCCTGCTGCGTGGCTGACGGCCGGAGGGCCCT 800
MW    --------------G-----------------C--T--------------
RW    --------------G-----------------------------------
```

```
DD    GTACCGCTCCTGCAACCTCAGCCAGGTGCCCCCGGTCCCCAGCACCACCG 850
MW    ----------------------------------------T-------
RW    ------------------------------------------------

DD    AGATCCTCCTGCTGAGCTTCAACTACATCCGGGCCGTCACCCGCGCCTCG 900
MW    --R-------------------------------------------------
RW    ------------------------------------------------

DD    TTCCCCCTCCTGGAGCGGCTGCAGCTGCTGGAGCTGGGGACGCAGCAGAC 950
MW    ---------------------
RW    ------------------------------------------------

DD    GCCCTTCAGCGTCGACAGAGAAGCCTTCAGGAACCTGCCCAACCTGCGCA 1000
MW
RW

DD    CCCTGGACCTGGGCAACAGCCGGGTGGATTTCCTGCATCCCGACGCCTTC 1050
MW
RW

DD    CAGGGGCTGCCCCACCTGCAGGAACTCCGGCTGTTCGCCTGTGGCCTCTC 1100
MW
RW

DD    CGACGTCGTGTTGACAGACGGTTATTTCAGAAACCTGGGGGCTTTGTTGC 1150
MW
RW

DD    GCCTGGACCTGTCCAAAAATCAGATTGGGAGCCTCGAGCTTCACGCCTCC 1200
MW
RW

DD    TTCCGGGAGCTGGGTTCCCTGAGGTCCGTGGACTTTTCCCTCAACCGGAT 1250
```

MW

RW

DD CCCGGCTGCGTGTGAGCAGGGGCTCAGGCCCCTGCAGGGCAAGGCGCTCT 1300

MW

RW

DD CCCTTCTGAACCTCGCGGCCAATGGCCTGTACAGCCGGGCCCCCGTGGAC 1350

MW

RW

DD TGGGGGCGGTGCGGGAACCCGTTCAGGAATGTGGTCCTGGAGACCCTGGA 1400

MW

RW

DD CGTGTCTAACAACGGCTGGACCGCAGACGTCACGGGCAACGTCACCAGGG 1450

MW

RW

DD CCATCGGTGGGAGCCAGATCTCCTCCTTGGTGCTCGCCCACCACATCATG 1500

MW

RW

DD GGGCAGGGGTTTGGCTTCCGGAACATCCGGGACCCTGACCGGAGCACGTT 1550

MW

RW

DD CGCGGGGCTGGCCGGGAGCTCGGTGCTGCGGCTGGATCTGTCGCACGGCT 1600

MW

RW

DD TCGTCTTCTCCCTGAACGCCCGACTGTTCGAGGTGCTCGGGGACCTGAAG 1650

MW

```
RW

DD    CTCCTGGACCTCGCCCACAACAAGATCAACAGGATCGCGGGAGAAGCGTT 1700
MW
RW

DD    TCACGGCCTCGGCAGCGTCCAGGTTCTCAACCTGTCGCACAATCTCCTGG 1750
MW
RW

DD    GCGAGCTCTATGACTCTGACTTCTCGGGGCTCGCGGAGGTCGCCTACATT 1800
MW
RW

DD    GACCTGCAGCACAATCACATCGGGATCATCCAGGACCAGACGTTCAGATT 1850
MW
RW

DD    CCTGGGGGCGCTTCGGACCCTGGATCTCCGCGACAACGCCCTCAAAACCG 1900
MW
RW

DD    TTTCCTTCGTGCCCAGCATAGACACCATCTTCCTGGGCAACAACAAGCTG 1950
MW
RW

DD    GAGACCGTGTCCCACATGGACCTCACAGCCAGCTTCCTGGAGCTGTCGGA 2000
MW
RW

DD    CAACAGGCTGGAGGACCTGGGCGACCTCTACTCGCTCCTCCGGGTCCCTG 2050
MW
RW
```

```
DD    CCCTGCAGGTCCTCATCCTCAACCGCAACCGCCTGTCCGCGTGCCGTGGC 2100
MW
RW

DD    GGACACGGCCCCACGGGCAGCGTCGGCCCAGAGAGGCTCTTCCTCGGGAG 2150
MW
RW

DD    CAACATGCTGCAGCTGGCCTGGGAGACCGGGCGGTGCTGGGACGTGTTCC 2200
MW
RW

DD    GGGGGCTGCCCCGGCTCCGGGTGCTGCACCTGAACCACAACTACCTGGCC 2250
MW
RW

DD    GCCCTCCCGCCGGGGCTGCTGCGGGACCTCACGGCGCTGAGGGGCCTCGA 2300
MW                      ------------------------------
RW                      ---R-----------R--------------

DD    CCTGAGCGCCAACAGGCTGAGCACGCTGTCCCGGGGCGACCTGCCTGCTG 2350
MW    ------T-----------------------------------------
RW    -------------------------------------------------

DD    CCTTGGAGGTGCTGGATGTGTCCAGGAACCAGCTCCTGTCCCTGGACCCC 2400
MW    -------------------------------------------------
RW    -------------------------------------------------

DD    GGGCTGCTCGCCCCGCTCAGAGCCGTGGACCTAACGCACAACAAGTTCAT 2450
MW    -------------------------------------------------
RW    -------------------------------------------------
```

```
DD    CTGCGGCTGCGAGCTCCGTCCCTTGGTGAGGTGGCTCAACCGGACCAACG 2500
MW    ------------------------------------------------
RW    ------------------------------------------------

DD    TCACTGTGTTCGGGTCCCGCGCAGACGTGCGCTGCGCCTACCCCAGCTTG 2550
MW    ----C---------------A--------------------------C-
RW    ----C-------------------------------------------C-

DD    CTTGCGGGGACGCCCCTGTCCTCTGTCTCCATGGAGGGCTGTGACGACGA 2600
MW    ----------------------
RW    ----------------------

DD    GGAGGCCCTGCGGACCCTCACGTTCTCCCTCTTCATCTTCTCCACCGTCG 2650
MW
RW

DD    GGGTCACGCTGTTCCTCCTGGCCGTCCTCGTGGCCGCCAAGCTCCGGGGC 2700
MW
RW

DD    CTTTGCTTCCTCTGTTACAAGGCGGCCCGGCGCCTCCTGCCTGCGGGGCC 2750
MW
RW

DD    CGCCGAGGACGGAGCGCCCGACGCGTACCAGTACGACGCCTACCTGTGCT 2800
MW
RW

DD    TCAGCGGCAGAGACTTCGAGTGGGTGCAGCGCGCGCTGCTCAGGCACCTG 2850
MW
RW

DD    GACGCTCAGTACAGCTCCCGAAACAGGCTGAACCTGTGCTTCGAGGAGAG 2900
```

MW

RW

DD GGACTTCGTCCCGGGGCGGGAGCACATCGCCAACATCCAGGACGCCGTGT 2950

MW

RW

DD GGAGCAGCCGCAAGGTGGTCTGTCTGGTGAGCAGGCACTTCCTCCGCGAC 3000

MW

RW

DD GGGTGGTGCCTGGAGGCCTTCGCGGCCGCGCGGAGCCGCTGCGCGTCCCA 3050

MW

RW

DD CCTGGACGGCGCCCTCGTCCTGGTGGTCGTGGGCTCCCTGTCGCAGTACC 3100

MW

RW

DD AGCTGAGGAGGCACCCGGCCATCGGGGGCTTCGTGCGGCAGCGCCGGTAC 3150

MW

RW

DD TTGAGGTGGCCCGAGGATCTGCAGGACGTGGGCTGGTTCCTGGACACGCT 3200

MW

RW

DD CTCCCGACACATCCTGCAGGAGCAGAGGGGCGCGCGCGGGGATGGCGGCA 3250

MW

RW

DD TCCCGCTGCGCACCGTGGCGGCCGGGCGCCGACCTCACTGCACCAGGGTC 3300

MW

```
RW

DD    GGGAGGCGCCGACCTCACTGCACCAGGGTCCGGGGGCGCCGACCTCACTG 3350
MW
RW

DD    CACCGGGGCCCCGGGGGGCGCCGACCTCACTGCACCGGGGTCCGGGGGGC 3400
MW
RW

DD    GCCGACCTCACTGCACCGGGGTCCGGGGGACGCCGACCTCACTGCACCGG 3450
MW
RW

DD    GGCCCCGGGGGGCGCCGACCTCACTGCACCGGGGCCCCGGGGGGCGCCGA 3500
MW
RW

DD    CCTCACTGCACCAGGGTCGGGAGGCGCCGACCTCAGTGCACCAGGGACCG 3550
MW
RW

DD    GGGGCGCCGACCTCACTGCACCGGGGCCCCGGGGGCGCTGCTCCTCCGCG 3600
MW
RW

DD    GCGGGCGCCCGGGTCCGACAAAGGGCGAGGGCGCGAGCGGTGCGGCGCAG 3650
MW
RW

DD    GAGCTCAGGGTCCGCGCGGAGGACCCGGGAGCACACACGGCCCGAGGAGC 3700
MW
RW
```

DD CGCCCCGCGCCCGCCCCGCCCTCGGTCGCGCCCCCGCCGCAGCCCCAGCC 3750
MW
RW

DD CCCCGCGCCCCCGCCCGGCCCCGCCGCCGCCCCCCGCGGCTGCCCCGACC 3800
MW
RW

DD CTGCCCGCCCTCCGCCCGCGCGGGCTGCCCGGCCCCGCGTCCTTACCCGG 3850
MW
RW

DD TCTCCCGGCCCCGCGGCGCGGGGGGCGGCGGCGGCGGCGGCGGCGGC 3900
MW
RW

DD GGGGCCGGGACGCGTCCACGCAGGAGACAGGCGCCCCCGACGCGCCGGCC 3950
MW
RW

DD CCGATGCGGACCCGGAGCCGGCTTCCGCCTCCCGCCGGAAGGCGTCCCGA 4000
MW
RW

DD GCAGGACCGGAAGTCCCGCCCCGGCGGCTGAGGGGGGCGGCCGGGGGCGG 4050
MW
RW

DD TCGTGTCAGCAGTTCGGCGGGGCGGGGGGTCACACCGACGTCCGTGGGCT 4100
MW
RW

DD GCCCCCGGCCTCGGGCGGGCCGCGTGTTTCTCCGTCCGCCCGCTCCTCGC 4150

MW

RW

DD CAGACCGCCGGGTCGCGGCGGGGGAGGGGGCGGGGCGGGGCGGGACGCAG 4200

MW

RW

DD GAGGGGCGGGGCGTGGGAGAGGCGGGGGCGGGGCGCGAGAGGGGCGGGGC 4250

MW

RW

DD GGGGCGGGGCGTGGGA 4266

MW

RW