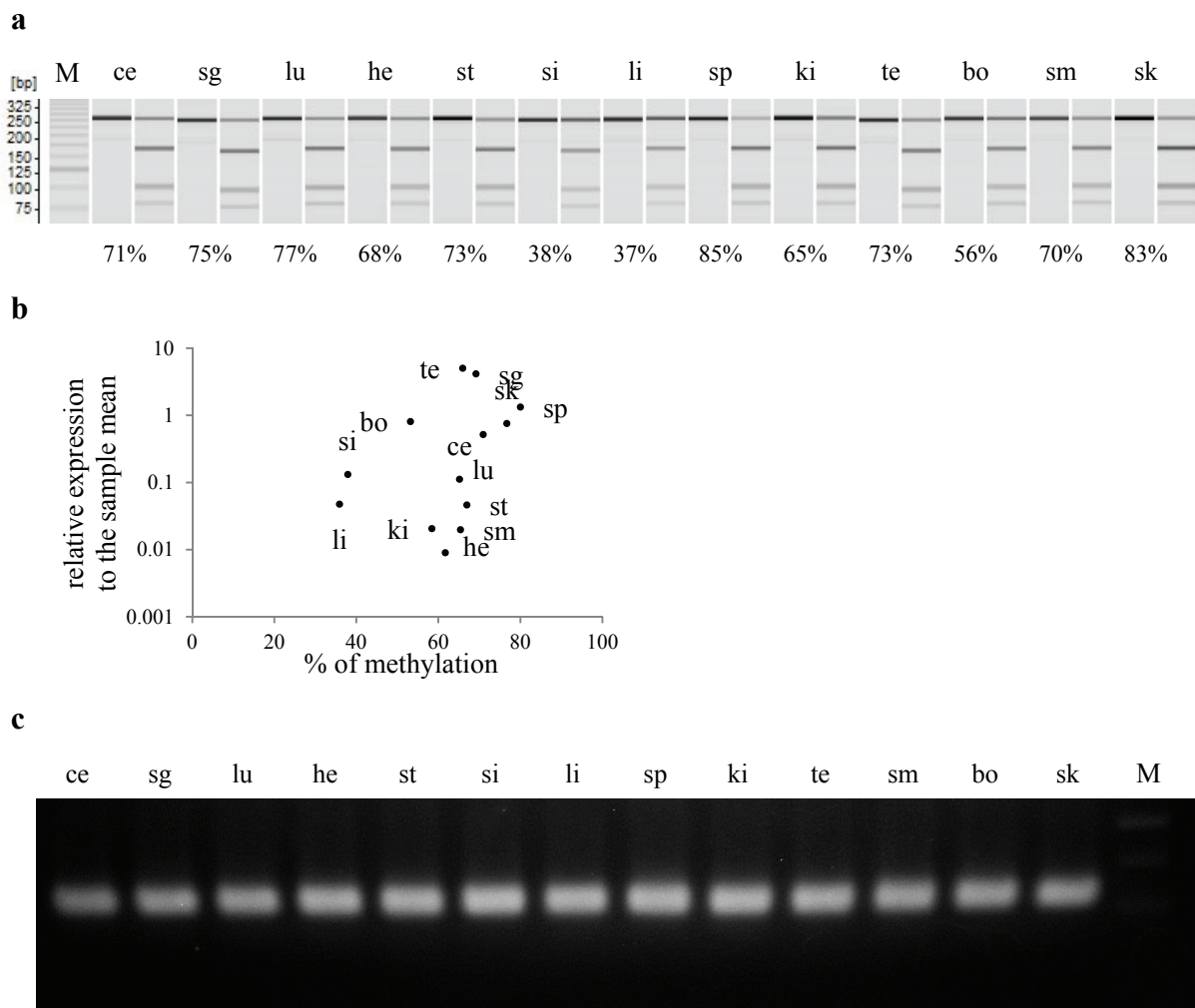


Wakitani_Supplementary Figure



Supplementary Figure. DNA methylation of the external region of Runx2-DMR.

a) A gel image as output from the results of combined bisulfite restriction analysis (CoBRA) targeting CpG-3,210 in mouse organs. Fragments digested with HpyCH4IV were loaded onto odd lanes except for the leftmost lane (showing a 25-bp DNA ladder; M). In the lanes immediately on the left, the fragment without HpyCH4IV digestion is loaded as an experimental control. b) Each organ is plotted on the scatter diagram. Vertical and horizontal scales indicate expression levels of Runx2 mRNA and the methylation rate of CpG-3,210, respectively. There is no significant correlation among mouse organs. c) Bisulfite PCR fragment digested with HpyCH4IV targeting CpG-1,297 is loaded. Methylation of CpG-1,297 was not detected in each organ. ce: cerebellum, sg: salivary glands, lu: lungs, he: heart, st: stomach, si: small intestine, li: liver, sp: spleen, ki: kidneys, te: testes, bo: bone, sm: skeletal muscle, and sk: skin.

Supplementary Table S1. The list of dogs used in this study

	Breed	Sex	Age	Statement
#1	Poodle (Toy)	male	3y	fracture
#2	Maltese	female	8y	disc injury
#3	Dachshund (Miniature)	male	6y10m	disc hernia
#4	Labrador Retriever	female	8y5m	disc hernia
#5	Poodle (Toy)	female	1y11m	portosystemic shunt patent ductus
#6	Dachshund (Miniature)	male	5y	disc hernia
#7	Dachshund (Kaninchen)	male	3y8m	disc hernia
#8	Dachshund (Miniature)	male	9y7m	disc hernia
#9	Poodle (Toy)	male	4y8m	disc hernia
#10	Dachshund (Miniature)	female	3y11m	disc hernia
#11	Beagle	female	3y	healthy
#12	Beagle	female	2y8m	healthy

Supplementary Table S2. The list of primers used in this study

Purpose Target	Primer sequence Forward	Reverse
CoBRA (CpG at Runx2 promoter in mouse)		
-7,885	AGGGGATAGAGGAATGATTTAATTG	TCCTCCCCTTTCTCTATACCATTAT
-6491, -6456	TGGGATTTGATTTTAGTTAGATAGG	AATACAAAACCCACCATTTTCAC
-4656, -4646, -4619	TTTAAATTGGTTAGTGGGTTTGAGA	AAAAATCAATACTATCACCCAACC
-3378, -3326, -3306	TTTATTTTGATATTTGGGTGAGTGG	CCCTCAAACCTCCCCTAACAATAA
-3210	TGTTTTTATTGTTAGGGGAAGTTG	AATTCCCAATCTTAAATCCCTTAAA
-2505	TTTGTGGGTTGTGTTATTAAGAAA	AAACCAACTTCCTAACCCATCTAA
-2060	GTGGTTTAGGGAGGTGATTTAAGTT	AATCACTACTTCTACCTCCCCTTTC
-1297	GATATTGTTTTGTTTGGTTTTTGG	CAACCTCAAATTCTAAACCTTCAA
-535, -478	TAATTAATGTGGGGTGGGGTTG	CCCACCTCACCCTCAAAACC
+1200, +1334	GTTGTGAGATTTTGGGTTTTTAG	ATCCCCAAATTTACTAAACACTCTC
+2369, +2557	GGAGAGAGAGGAAAGAAAGAAAGA	ATCTAACCTTTTCACACCACAAAA
Bisulfite sequencing (Runx2 promoter in mouse)		
-3371 to -3148	TTTATTTTGATATTTGGGTGAGTGG	AATTCCCAATCTTAAATCCCTTAAA
-2985 to -2649	ATGTTTAATGGAGGGAGGATGTT	CATACCTACCCCTCCTACCCTTA
-2505 to -2039	TTTGTGGGTTGTGTTATTAAGAAA	AATCACTACTTCTACCTCCCCTTTC
CoBRA (CpG at Runx2 promoter in dog)		
-2829	GTGGATTTTTTGGGAGAAGGATAGT	CTCCATAACCACATACTAACACTC
Real-time RT-PCR		
mouse Runx2	GACGAGGCAAGAGTTTCACC	GTCTGTGCCTTCTTGGTTCC
mouse Dlx5	TTATGCCGACTACGGCTACG	TGGTTTACCATTACCCATCCTC
mouse Gapdh	CCACCCAGAAGACTGTGGAT	GGATGCAGGGATGATGTTCT
dog Runx2	CCTTCCACTCTCAGTAAGAAGAGC	ATTCGTGGGTTGGAGAAGC
dog Dlx5	CTTACGCCGACTACAGCTACG	TGGTTTGCCATTACCCATC
dog Spp1	TGTGGCTTATGGACTGAGGTC	AGGACGGCATTGAAGTCATC
dog CD29	TGAATGCCAAATGGGACAC	GCTCATTTTCCCTCATACTTCG
dog CD44	TGCATCGCTGTCAATAGTCG	TGTTTACCAAGTGCACCATCTC
dog CD90	ACATGTGAACTCCGGCTCTC	GGCTTATGCCCTCACACTTG
dog CD105	ACTGTGGTGCTCAAGAAAGACC	TGCCACAGCTGGAGTAAGTG
dog Rpl13a	GAAGGAAAAGGCCAAGATCC	GAAGGAAAAGGCCAAGATCC