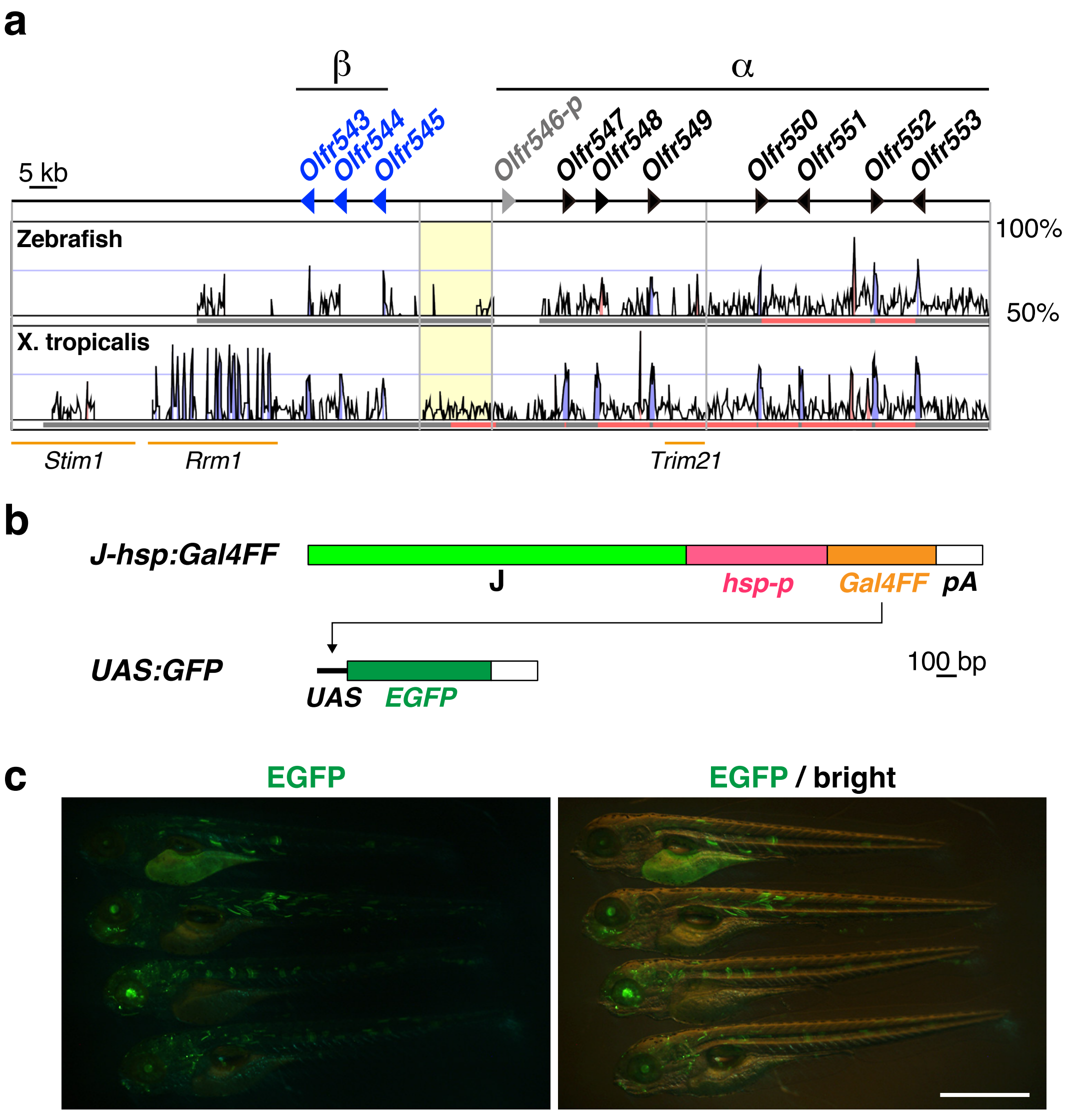
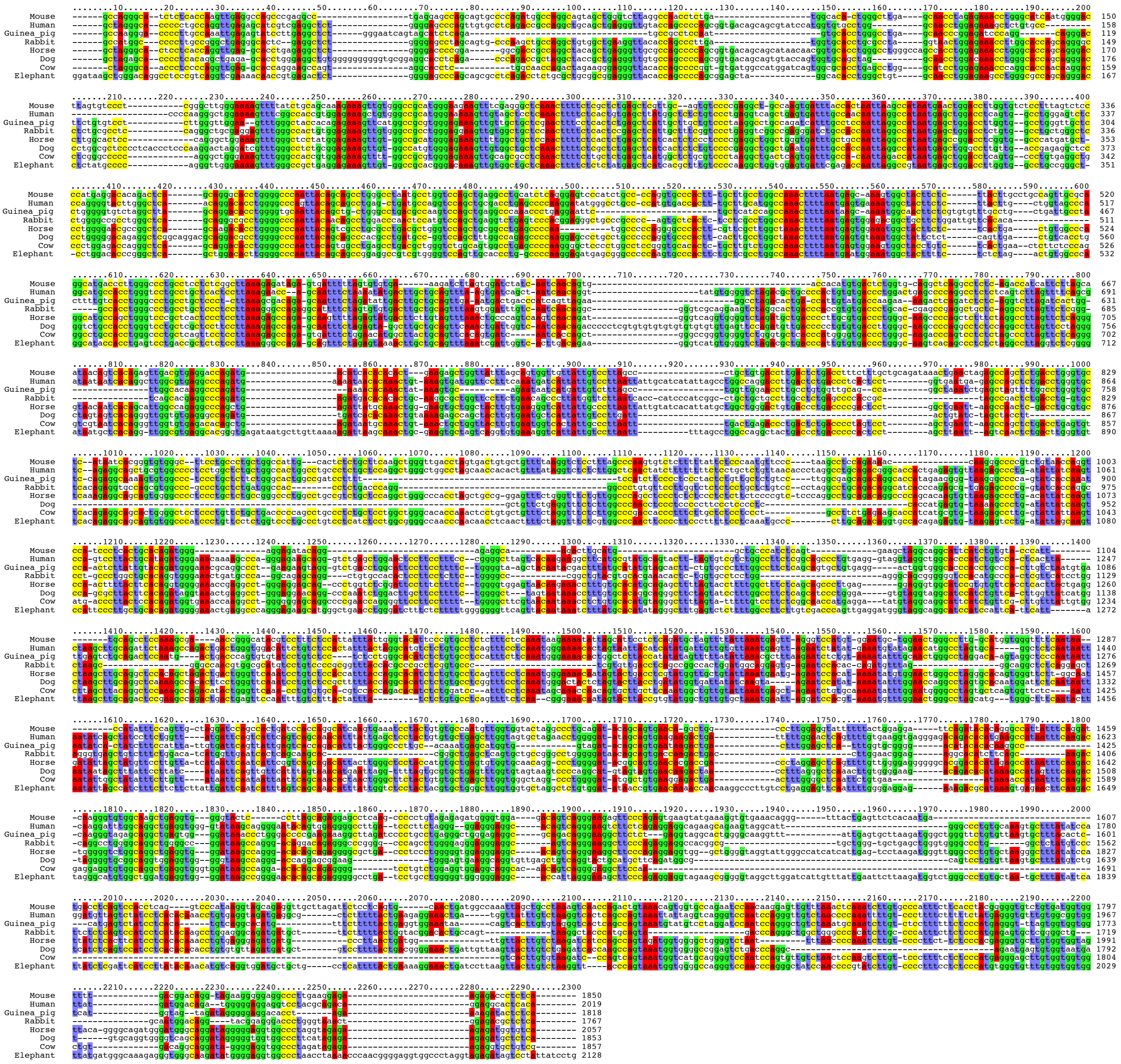


**Supplementary Figure 1 | Conservation and functional analyses of the J element in nonmammals.**

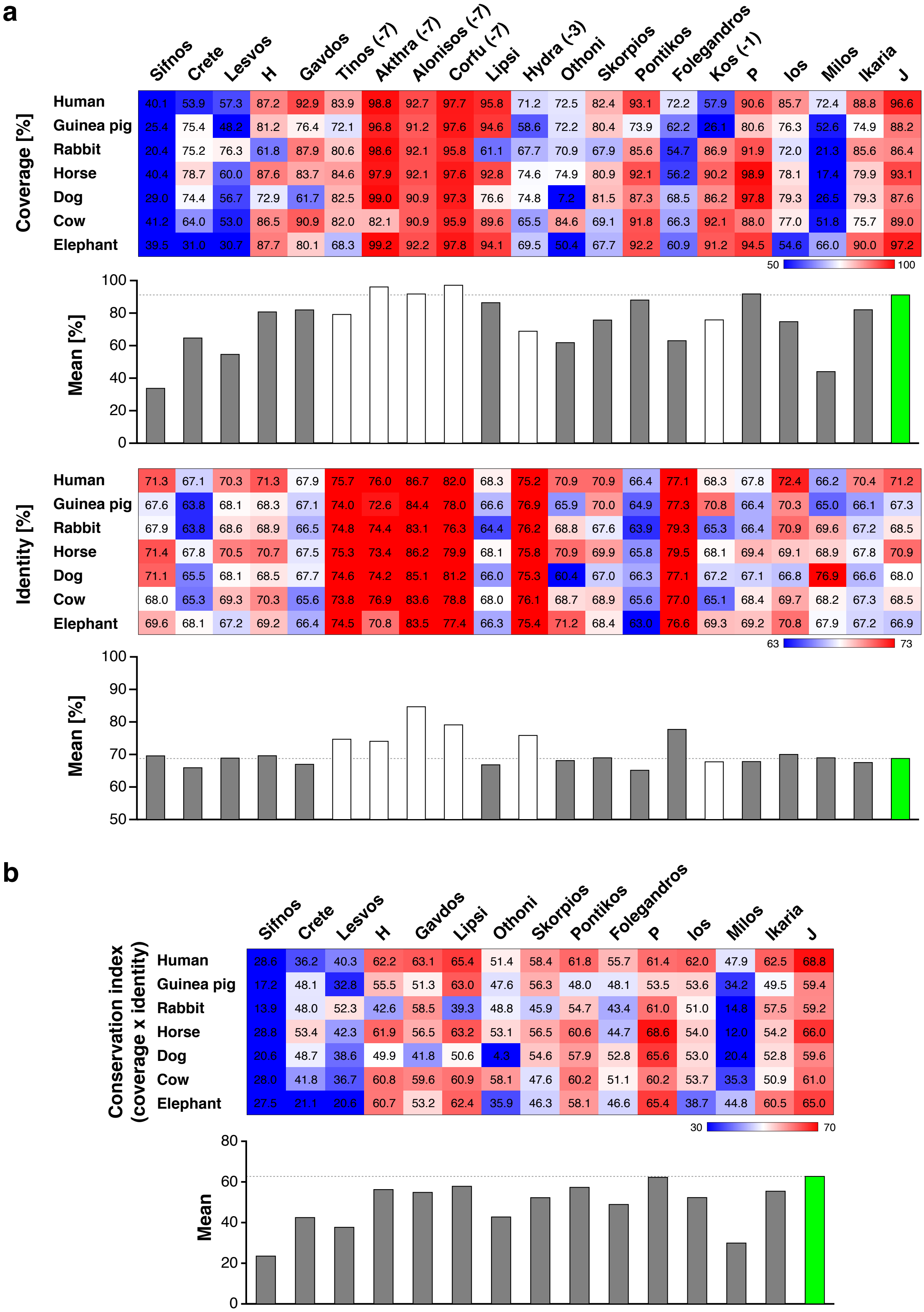
**(a)** Genomic region of the *Olfr544* locus (same as Fig. 1b) and nucleotide percent identity plots between mouse and zebrafish or *X. tropicalis* using VISTA. No significant conservation in the intergenic region was detected, even when a 50-bp calculation window was used for high-sensitivity detection.

**(b,c)** Transient reporter assay of enhancer activity of the J element in zebrafish. **(b)** Strategy of the transient reporter assay in zebrafish. The J element was fused to the *hsp* basal promoter (*hsp-p*), *Gal4FF* (a modified GAL4 transcription activator), and a polyadenylation sequence (*pA*). To test the enhancer function of the J element, the J-hspGal4FF driver construct was injected into *UAS:GFP* zebrafish embryos. **(c)** Whole-mount fluorescent images of injected larvae at 4 dpf (days post-fertilization). Specific GFP expression was not observed in the nose (n = 77), indicating the J element did not show any enhancer activity in the transient reporter assay in zebrafish. The scale bar is 500 µm.



**Supplementary Figure 2 | Multiple alignment of the J element.**

Multiple alignment of the J element for mouse and 7 representative placental mammalian species, human (Primates), guinea pig (Rodentia), rabbit (Lagomorpha), horse (Perissodactyla), dog (Carnivora), cow (Cetartiodactyla), and African elephant (Proboscidea).



| Coverage [%] | Sifnos | Crete | Lesvos | H | Gavdos | Tinos (-7) | Akthra (-7) | Alonisos (-7) | Corfu (-7) | Lipsi | Hydra (-3) | Othoni | Skorpios | Pontikos | Folegandros | Kos (-1) | P | Ios | Milos | Ikaria | J |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Human | 40.1 | 53.9 | 57.3 | 87.2 | 92.9 | 83.9 | 98.8 | 92.7 | 97.7 | 95.8 | 71.2 | 72.5 | 82.4 | 93.1 | 72.2 | 57.9 | 90.6 | 85.7 | 72.4 | 88.8 | 96.6 |
| Guinea pig | 25.4 | 75.4 | 48.2 | 81.2 | 76.4 | 72.1 | 96.8 | 91.2 | 97.6 | 94.6 | 58.6 | 72.2 | 80.4 | 73.9 | 62.2 | 26.1 | 80.6 | 76.3 | 52.6 | 74.9 | 88.2 |
| Rabbit | 20.4 | 75.2 | 76.3 | 61.8 | 87.9 | 80.6 | 98.6 | 92.1 | 95.8 | 61.1 | 67.7 | 70.9 | 67.9 | 85.6 | 54.7 | 86.9 | 91.9 | 72.0 | 21.3 | 85.6 | 86.4 |
| Horse | 40.4 | 78.7 | 60.0 | 87.6 | 83.7 | 84.6 | 97.9 | 92.1 | 97.6 | 92.8 | 74.6 | 74.9 | 80.9 | 92.1 | 56.2 | 90.2 | 98.9 | 78.1 | 17.4 | 79.9 | 93.1 |
| Dog | 29.0 | 74.4 | 56.7 | 72.9 | 61.7 | 82.5 | 99.0 | 90.9 | 97.3 | 76.6 | 74.8 | 7.2 | 81.5 | 87.3 | 68.5 | 86.2 | 97.8 | 79.3 | 26.5 | 79.3 | 87.6 |
| Cow | 41.2 | 64.0 | 53.0 | 86.5 | 90.9 | 82.0 | 82.1 | 90.9 | 95.9 | 89.6 | 65.5 | 84.6 | 69.1 | 91.8 | 66.3 | 92.1 | 88.0 | 77.0 | 51.8 | 75.7 | 89.0 |
| Elephant | 39.5 | 31.0 | 30.7 | 87.7 | 80.1 | 68.3 | 99.2 | 92.2 | 97.8 | 94.1 | 69.5 | 50.4 | 67.7 | 92.2 | 60.9 | 91.2 | 94.5 | 54.6 | 66.0 | 90.0 | 97.2 |

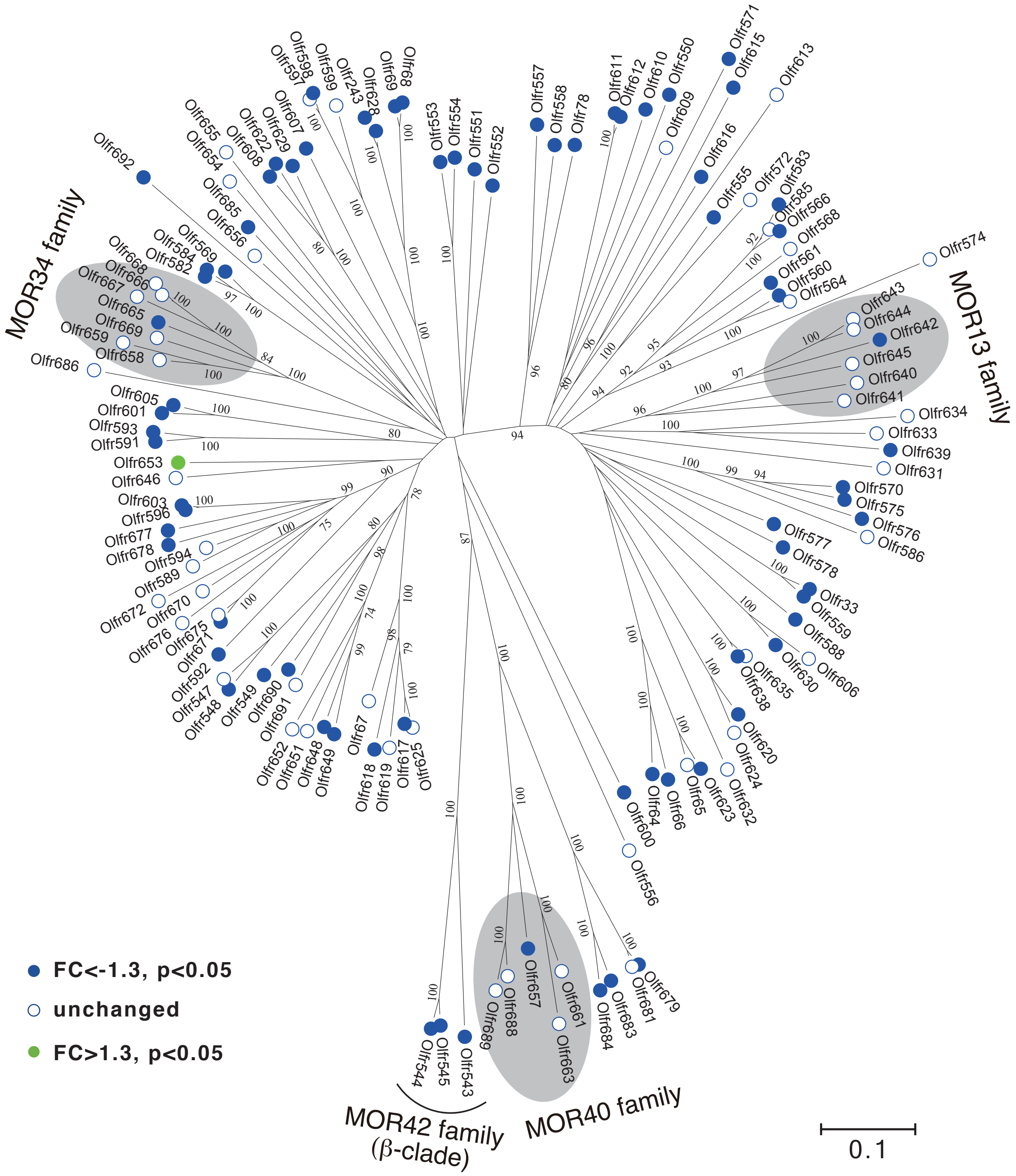
| Identity [%] | Sifnos | Crete | Lesvos | H | Gavdos | Tinos (-7) | Akthra (-7) | Alonisos (-7) | Corfu (-7) | Lipsi | Hydra (-3) | Othoni | Skorpios | Pontikos | Folegandros | Kos (-1) | P | Ios | Milos | Ikaria | J |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Human | 71.3 | 67.1 | 70.3 | 71.3 | 67.9 | 75.7 | 76.0 | 86.7 | 82.0 | 68.3 | 75.2 | 70.9 | 70.9 | 66.4 | 77.1 | 68.3 | 67.8 | 72.4 | 66.2 | 70.4 | 71.2 |
| Guinea pig | 67.6 | 63.8 | 68.1 | 68.3 | 67.1 | 74.0 | 72.6 | 84.4 | 78.0 | 66.6 | 76.9 | 65.9 | 70.0 | 64.9 | 77.3 | 70.8 | 66.4 | 70.3 | 65.0 | 66.1 | 67.3 |
| Rabbit | 67.9 | 63.8 | 68.6 | 68.9 | 66.5 | 74.8 | 74.4 | 83.1 | 76.3 | 64.4 | 76.2 | 68.8 | 67.6 | 63.9 | 79.3 | 65.3 | 66.4 | 70.9 | 69.6 | 67.2 | 68.5 |
| Horse | 71.4 | 67.8 | 70.5 | 70.7 | 67.5 | 75.3 | 73.4 | 86.2 | 79.9 | 68.1 | 75.8 | 70.9 | 69.9 | 65.8 | 79.5 | 68.1 | 69.4 | 69.1 | 68.9 | 67.8 | 70.9 |
| Dog | 71.1 | 65.5 | 68.1 | 68.5 | 67.7 | 74.6 | 74.2 | 85.1 | 81.2 | 66.0 | 75.3 | 60.4 | 67.0 | 66.3 | 77.1 | 67.2 | 67.1 | 66.8 | 76.9 | 66.6 | 68.0 |
| Cow | 68.0 | 65.3 | 69.3 | 70.3 | 65.6 | 73.8 | 76.9 | 83.6 | 78.8 | 68.0 | 76.1 | 68.7 | 68.9 | 65.6 | 77.0 | 65.1 | 68.4 | 69.7 | 68.2 | 67.3 | 68.5 |
| Elephant | 69.6 | 68.1 | 67.2 | 69.2 | 66.4 | 74.5 | 70.8 | 83.5 | 77.4 | 66.3 | 75.4 | 71.2 | 68.4 | 63.0 | 76.6 | 69.3 | 69.2 | 70.8 | 67.9 | 67.2 | 66.9 |

| Conservation index (coverage x identity) | Sifnos | Crete | Lesvos | H | Gavdos | Lipsi | Othoni | Skorpios | Pontikos | Folegandros | P | Ios | Milos | Ikaria | J |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Human | 28.6 | 36.2 | 40.3 | 62.2 | 63.1 | 65.4 | 51.4 | 58.4 | 61.8 | 55.7 | 61.4 | 62.0 | 47.9 | 62.5 | 68.8 |
| Guinea pig | 17.2 | 48.1 | 32.8 | 55.5 | 51.3 | 63.0 | 47.6 | 56.3 | 48.0 | 48.1 | 53.5 | 53.6 | 34.2 | 49.5 | 59.4 |
| Rabbit | 13.9 | 48.0 | 52.3 | 42.6 | 58.5 | 39.3 | 48.8 | 45.9 | 54.7 | 43.4 | 61.0 | 51.0 | 14.8 | 57.5 | 59.2 |
| Horse | 28.8 | 53.4 | 42.3 | 61.9 | 56.5 | 63.2 | 53.1 | 56.5 | 60.6 | 44.7 | 68.6 | 54.0 | 12.0 | 54.2 | 66.0 |
| Dog | 20.6 | 48.7 | 38.6 | 49.9 | 41.8 | 50.6 | 4.3 | 54.6 | 57.9 | 52.8 | 65.6 | 53.0 | 20.4 | 52.8 | 59.6 |
| Cow | 28.0 | 41.8 | 36.7 | 60.8 | 59.6 | 60.9 | 58.1 | 47.6 | 60.2 | 51.1 | 60.2 | 53.7 | 35.3 | 50.9 | 61.0 |
| Elephant | 27.5 | 21.1 | 20.6 | 60.7 | 53.2 | 62.4 | 35.9 | 46.3 | 58.1 | 46.6 | 65.4 | 38.7 | 44.8 | 60.5 | 65.0 |

**Supplementary Figure 3 | Conservation analysis of the mouse OR enhancers conserved in all tested mammals.**

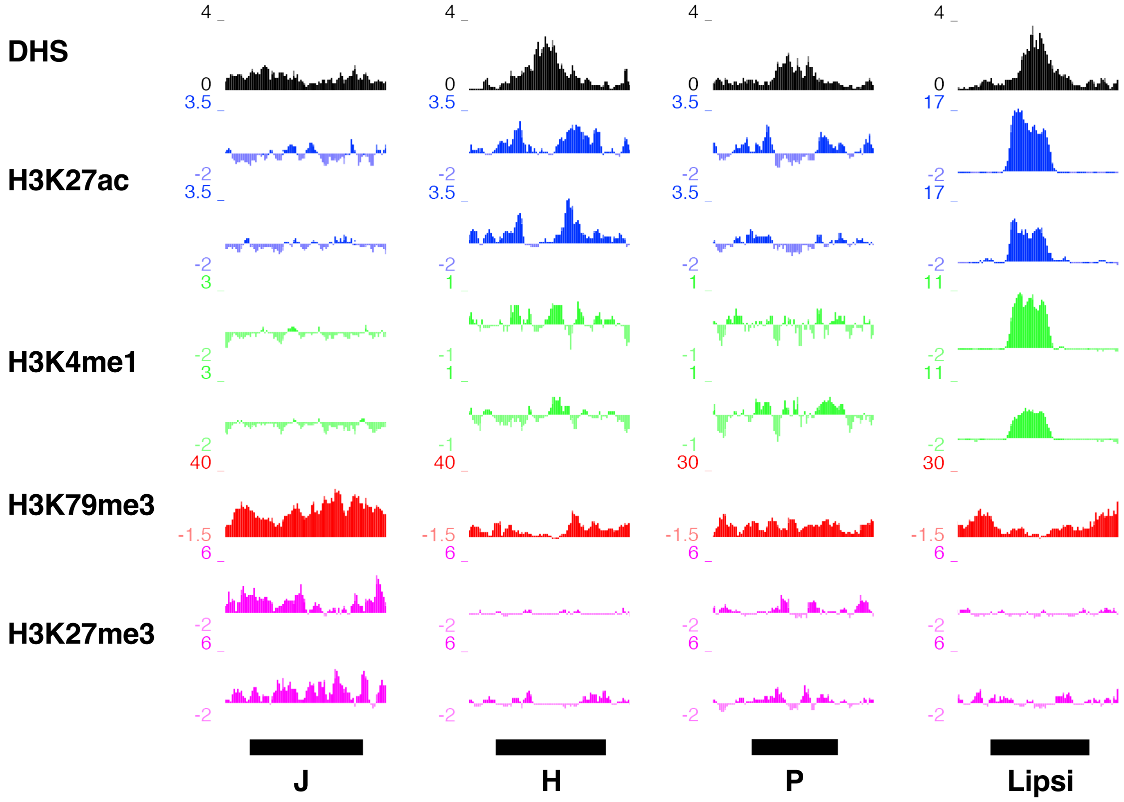
**(a)** The J element and 20 of 35 previously identified OR enhancers were conserved in all seven tested placental mammalian genomes. Coverage of alignment region (upper part) and nucleotide identity (lower part) of the mouse OR enhancers for other mammalian species are summarized. The graph shows the mean for the seven species. Sifnos, Crete, Lesvos, H, Gavdos, Lipsi, Othoni, Skorpios, Pontikos, Folegandros, P, Ios, Milos, Ikaria, and J elements are conserved and are located in proximity to OR genes in all tested placental mammalian genomes (J, green bar; the others, grey bars in graph). However, Tinos, Akthra, Alonisos, Corfu, Hydra, and Kos do not reside close to OR genes in at least one non-mouse genomes (white bars in graph). The number of genomes containing enhancers that do not reside close to the OR gene is shown in parentheses. The bottom graph shows the mean among the seven species.

**(b)** Summary of the conservation analysis for each enhancer-species combination of the selected 15 elements, which are all conserved in the seven tested mammals and linked to OR genes. The conservation index is defined by multiplying the percentage coverage by the nucleotide identity. Nine elements (H, Gavdos, Lipsi, Skorpios, Pontikos, P, Ios, Ikaria, and J) showed relatively high average conservation indexes (mean > 50). The J element was one of the most highly conserved of the 15 conserved OR enhancer elements.

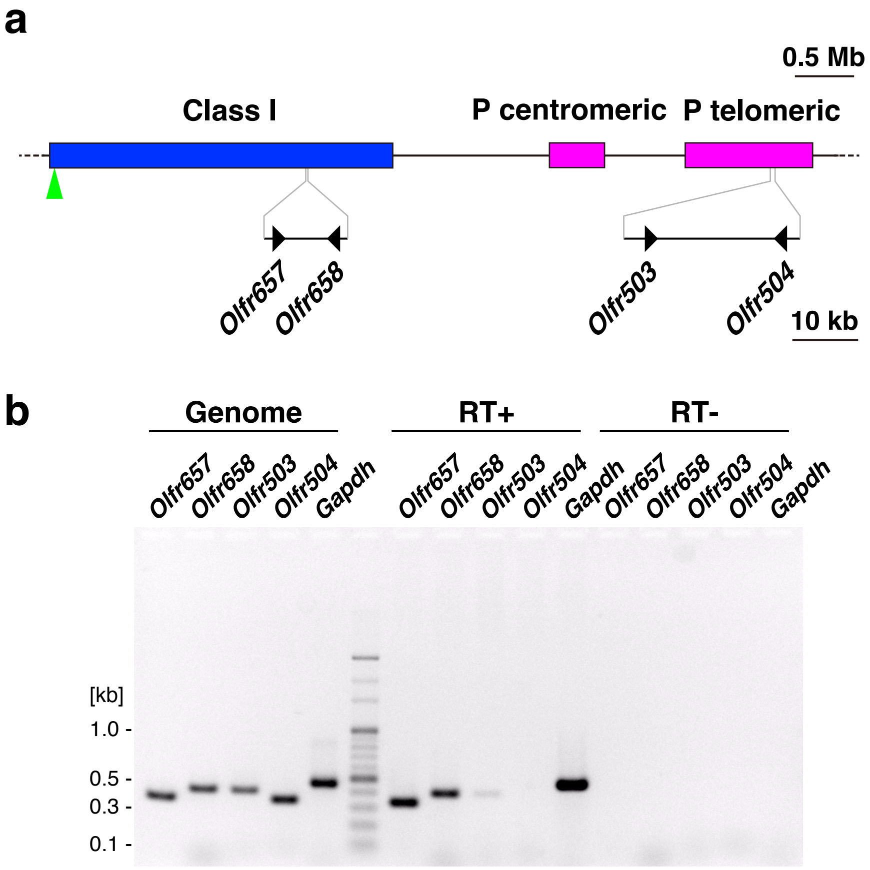


**Supplementary Figure 4 | Phylogenetic analysis of the effect of ΔJ on the expression of class I OR genes.**

An unrooted phylogenetic tree of the mouse class I OR family using 129 intact class I OR genes was constructed by the neighbor-joining method. Class I OR family is classified into 42 subfamilies. Differentially expressed genes (1.3-fold change, $p < 0.05$) are indicated by filled circles (downregulated, blue; upregulated, green), whereas unaffected genes are represented by open circles. The class I genes whose expression levels were affected by ΔJ were not biased against specific subfamilies; however, the MOR13, MOR34, and MOR40 subfamilies included many J-independent genes (grey shaded). Bootstrap values obtained from 500 resamplings are shown only for nodes with bootstrap values > 70%.



**Supplementary Figure 5 | An epigenetic signature for the J and class II enhancer elements.** Sequencing tracks for DNaseI hypersensitivity (DHS) sequencing and chromatin immunoprecipitation sequencing on the J and class II enhancer element regions (H, P, and Lipsi). Sequencing data were retrieved from GSE55174 and GSE52464 (single data for DHS and H3K79me3; two replicates for H3K27ac, H3K4me1, and H3K27me3) deposited in Gene Expression Omnibus. Note these sequence data were obtained from whole MOE preparations. Class II enhancer elements have a well-defined DHS peak, are enriched for H3K27ac and H3K4me1, and are missing H3K79me3 and H3K27me3 modifications. In contrast, the J element does not have significant DHS, H3K27ac and H3K4me1 peaks, and is enriched for H3K79me3 and H3K27me3, indicating that epigenetic landscape of the J element is different from previously characterized enhancer elements for class II genes.



**Supplementary Figure 6 | Expression analysis of *Olfr503* and *Olfr504*.**

**(a)** Genomic organization of *Olfr503* and *Olfr504* and the homologous genes *Olfr657* and *Olfr658*. *Olfr657* and *Olfr658* reside in the 3-Mb class I OR cluster; *Olfr503* and *Olfr504* are located in the P telomeric cluster. The green triangle represents the J element.

**(b)** RT-PCR analysis for the four class I OR genes and *Gapdh* on RT+ (with reverse transcriptase) or RT- (without reverse transcriptase) cDNA derived from wild-type MOE. Genomic DNA was used as a positive control. The expression of *Olfr657* and *Olfr658* is clearly detected, whereas *Olfr503* and *Olfr504* expression is not detected or is barely detected.

**Supplementary Table 1 | ISH analysis in ΔJ mice.**

| OR gene probe | n | Expression [% of WT] (mean ± SEM) | Microarray analysis fold change value |
|---|---|---|---|
| **Class I gene** | | | |
| Olfr543 | 3 | 16 ± 6.4 | -1.61 |
| Olfr544 | 5 | 2.1 ± 0.5 | -3.57 |
| Olfr547 | 3 | 50 ± 12 | -1.03 |
| Olfr552 | 4 | 4.6 ± 1.6 | -6.36 |
| Olfr33 | 5 | 35 ± 3.7 | -2.29 |
| Olfr584 | 4 | 72 ± 21 | -1.70 |
| Olfr607 | 4 | 26 ± 4.4 | -3.14 |
| Olfr639 | 3 | 19 ± 2.5 | -3.28 |
| Olfr653 | 5 | 159 ± 13 | 1.56 |
| Olfr657 | 4 | 3.9 ± 1.6 | -3.37 |
| Olfr690 | 4 | 56 ± 7.9 | -1.58 |
| Olfr692 | 5 | 29 ± 4.9 | -1.39 |
| **Atypical class I gene** | | | |
| Olfr556 | 3 | 76 ± 4.5 | -1.05 |
| Olfr686 | 3 | 78 ± 14 | -1.08 |
| **Class II gene (dorsal)** | | | |
| Olfr151 | 3 | 106 ± 21 | -1.05 |
| Olfr521 | 5 | 109 ± 17 | -1.06 |
| **Class II gene (ventral)** | | | |
| Olfr17 | 4 | 97 ± 9.1 | -1.10 |
| Olfr693 | 4 | 100 ± 20 | -1.04 |

Summary of ISH analysis for 18 tested OR genes (12 class I, 2 atypical class I and 4 class II genes). Percentage of expression was calculated by dividing the average number per section of ΔJ by that of wild-type in each experiment pair. The fold change value of the microarray data (Supplementary Data 2) was displayed on the right column (ratio of signals between ΔJ and wild-type mice; positive value means ΔJ per wild-type, negative value means wild-type per ΔJ).

## Supplementary Table 2 l ISH analysis in ΔJ heterozygous and homozygous mice.

| OR gene probe | Expression [% of WT] | | | | | | One-way ANOVA p-value |
|---|---|---|---|---|---|---|---|
| | J/J | | J/ΔJ | | ΔJ/ΔJ | | |
| | n | (mean ± SEM) | n | (mean ± SEM) | n | (mean ± SEM) | |
| Olfr544 | 5 | 100 ± 13 | 4 | 75 ± 7.6 | 5 | 1.9 ± 0.3 | < 0.0001 |
| Olfr552 | 4 | 100 ± 7.7 | 4 | 66 ± 16 | 4 | 4.9 ± 1.9 | 0.0004 |
| Olfr33 | 5 | 100 ± 14 | 4 | 75 ± 9.2 | 5 | 34 ± 3.3 | 0.0014 |
| Olfr657 | 4 | 100 ± 12 | 4 | 80 ± 11 | 4 | 3.5 ± 1.3 | 0.0001 |
| Olfr692 | 5 | 100 ± 13 | 4 | 90 ± 10 | 5 | 29 ± 6.4 | 0.0008 |
| Olfr653 | 5 | 100 ± 16 | 4 | 93 ± 5.1 | 4 | 179 ± 29 | 0.0178 |

Summary of ISH analysis for 6 tested class I OR genes. Percentage of expression was calculated by dividing the average number per section of ΔJ heterozygous (J/ΔJ) or homozygous (ΔJ/ΔJ) by that of wild-type (J/J). P values were calculated by the One-way ANOVA in each *Olfr* probes.

**Supplementary Table 3 | List of PCR primers.**

| Target | Forward primer sequence | Reverse primer sequence | Product size [bp] |
|---|---|---|---|
| **Homology arm for deletion of Tg-220** | | | |
| Tg-U79 | CCCAAGCTTATCACCAGGGACAACTTGG | CGGGATCCAGAGGTGCAAGAAACTGAC | 1201 |
| Tg-U29 | GGCCATTCAAGCTTCTACTCTCTAC | CGGGATCCACTTCCATGACCTGAGTC | 1260 |
| Tg-U16 | CTCAAGCTTGTCTGCCCTCTCTGAC | CGGGATCCTTGACGGTGGCTCTGATTG | 1223 |
| **Reporter transgene** | | | |
| Olfr544 promoter | GCTCTAGAGCACACCCCTTCAGCTCCTC | TCCCCGCGGCAGGACTCACACAGGCAGGG | 921 |
| 5´-end of 13 kb region | ACGCGTCGACTCAGTATCAGGCCATTC | CGGGATCCTAGTTCAGCTGTCGCTTTCC | 2414 |
| 3´-end of 13 kb region | CGGGATCCATAGATCCGCCTACTTCCAC | GCTCTAGAGAGGGCTCAACATTGCTTTG | 876 |
| **Zebrafish transgene** | | | |
| 1.9 kb J element | CCGCTCGAGTAGTTTTCTGTGAGAGGGTCTCTTC | CTAGTCTAGAGGGGGACAAAGCCAGGGCAT | 1894 |
| **Mouse genotyping** | | | |
| J (intact) allele | GCTTAGATTCTCCTCAGTGCAAC | CACTTGCCTGCTACTGTCATTC | 432 |
| ΔJ allele | ACTGGCATCATCTCTACATCAGC | CACTTGCCTGCTACTGTCATTC | 299 for ΔJ line#8<br>(2281 for intact allele) |
| Venus | GCAAGCTGACCCTGAAGCTG | TTGCTCAGGGCGGACTGGTA | 508 |
| GFP | GGCATCAAGGTGAACTTCAAGATCC | CTTTACTTGTACAGCTCGTCCATGC | 242 |
| SacB | GCTGAATACAACGGCTATCACG | TCTCTCAGCGTATGGTTGTCG | 449 |
| CmR | GAGGCATTTCAGTCAGTTGCTC | CGGCATGATGAACCTGAATCG | 517 |
| **RT-PCR** | | | |
| Olfr657 | CCTTCAATAGTTCCAAGTCCCA | AAGATACCGGACTCCATGCC | 343 |
| Olfr658 | GTCATGTCAGTGCAGAATAGCA | AGTAACGCAGAGGGTAGCAG | 406 |
| Olfr503 | TCATGTCAGCGAGGAATAACC | GAGTAATGCAGAGGGTAGCAA | 406 |
| Olfr504 | AGACTTCAATAGTTCCAAGTTCCT | GCAGATACCAGACTCCATGCT | 346 |
| Gapdh | ACCACAGTCCATGCCATCAC | ATGTAGGCCATGAGGTCCAC | 467 |

**Supplementary Table 4 l Off-target analysis for ΔJ mice.**

| Target | Chr | Seed sequence (20-bp + PAM) | Forward primer sequence | Reverse primer sequence | Mutation |
|---|---|---|---|---|---|
| gRNA1 target | 7 | `tctcattgccacccggatga`**`tgg`** | - | - | - |
| OT-1 | 10 | `cactgctgccacccggatga`**`agg`** | `tttgtacttgagctggtgaggc` | `tgtctcttgccaccacactg` | No |
| gRNA2 target | 7 | `gcccctccaccgtacttgca`**`ggg`** | - | - | - |
| No potential off-target sites | | | | | |

**Supplementary Table 5 I List of riboprobes.**

| Gene | Synonym | OR class | Accession # | Nucleotide position | Cross-hybridized gene | Reference |
|---|---|---|---|---|---|---|
| **OR coding probe** | | | | | | |
| Olfr543 | MOR42-2 | I | NM_001011782 | 38-987 | - | |
| Olfr544 | MOR42-3, S6 | I | NM_020289 | 14-915 | Olfr545 | |
| Olfr547 | MOR31-4 | I | NM_147079 | 31-910 | - | |
| Olfr552 | MOR28-1 | I | NM_147102 | 15-927 | - | |
| Olfr556 | MOR41-1 | atypical I | NM_146754 | 82-994 | - | |
| Olfr33 | MOR11-2 | I | NM_147073 | 1-957 | Olfr559 | |
| Olfr78 | MOR18-2, MOL2.3 | I | NM_130866 | 1140-2102 | - | |
| Olfr578 | MOR7-1 | I | NM_147115 | 4-790 | - | |
| Olfr584 | MOR30-2 | I | NM_147054 | 71-748 | Olfr569, Olfr582 | |
| Olfr607 | MOR33-3P | I | XM_006544227 | 1-843 | - | |
| Olfr639 | MOR12-1 | I | NM_147084 | 1-922 | - | |
| Olfr653 | MOR33-1 | I | NM_147074 | 32-960 | - | |
| Olfr657 | MOR40-13 | I | NM_146312 | 1-913 | Olfr504 | |
| Olfr672 | MOR32-4 | I | NM_146760 | 9-919 | - | |
| Olfr686 | MOR35-1 | atypical I | NM_147069 | 39-948 | - | |
| Olfr690 | MOR31-2, Ors18 | I | NM_020290 | 8-1002 | - | |
| Olfr692 | MOR36-1 | I | NM_146355 | 23-952 | - | |
| Olfr521 | MOR101-2 | II | NM_146356 | 19-963 | Olfr520 | |
| Olfr693 | MOR283-8 | II | NM_146453 | 13-928 | - | |
| Olfr54 | MOR126-1 | II | NM_010997 | 31-933 | Olfr1375 | |
| Olfr73 | MOR174-9, mOR-EG | II | NM_054090 | 3-904 | - | |
| Olfr878 | MOR163-1 | II | NM_146798 | 233-909 | - | |
| Olfr17 | MOR263-5, P2 | II | AF247657 | 54–948 | - | Fuss et al., (2007) |
| Olfr151 | MOR171-2, M71 | II | AF281061 | 64-930 | Olfr160 | Fuss et al., (2007) |
| Olfr19 | MOR140-1, M12 | II | AY073758 | 172–869 | - | Fuss et al., (2007) |
| **Olfr545 specific probe** | | | | | | |
| Olfr545 3'UTR | MOR42-1, S50 | I | NC_000073.6 | 102492367-102493333 | - | |
| **Others** | | | | | | |
| GFP | - | - | U55762 | 677-1416 | Venus | |