Supporting Information

Armelin-Correa et al. 10.1073/pnas.1317036111

SI Materials and Methods

DNA FISH. Mice were transcardially perfused with 4% (wt/vol) paraformaldehyde and the noses were dissected, embedded in Optimal Cutting Temperature formulation (OCT) (Tissue-Tek), and sectioned on a cryostat. Briefly, sections (14 μm) were permeabilized with 0.5% saponin/0.5% Triton X-100 for 30 min, treated with 0.1 N HCl for 10 min, and kept in 50% (vol/vol) formamide with 2× SSC for at least 4 h. Sections were then kept in 70% (vol/vol) formamide with 2× SSC for 1.5 h, denatured for 13 min at 75 °C, immersed in ice-cold 70% (vol/vol) ethanol, and passaged through another ethanol series before being air dried. The appropriate probe was applied to each slide and incubated in a humidifed chamber at 37 °C for 60 h. The slides were washed in 50% (vol/vol) formamide, 2× SSC, pH 7.0, at 45 °C. Slides were then washed with 0.1× SSC prewarmed to 60 °C in a 45 °C water bath before being transferred to 4× SSC, 0.05% Tween 20 for 5 min at room temperature. A blocking solution with 0.05% Tween 20 and 3% (wt/vol) BSA in 4× SSC was applied to the slide and incubated for 30 min at room temperature. A mixture containing Alexa Fluor 488 conjugate avidin (Molecular Probes) or antidigoxigenin conjugated with rhodamine (Roche), 4× SSC, 3% (wt/vol) BSA and 0.05% Tween 20 was applied to each slide, which were incubated at 37 °C for 1 h in darkness and then washed in 4× SSC, 0.05% Tween 20 in darkness at 42 °C before incubation with DAPI. Samples were mounted with Vectashield antifade mountant (Vectorlabs).

Immunostaining of the Olfactory Epithelium. Immunodetection of OMP, GAP43, and CK14 was performed as described for the immuno-DNA FISH experiments. Briefly, sections (14 μm) were permeabilized with 0.5% saponin/0.5% Triton X-100 for 30 min and treated with 0.1 N HCl for 10 min. The samples were blocked for 30 min with 1% BSA, 3% (vol/vol) horse serum, and 0.1% Triton X-100 in 1× PBS. Primary antibodies were: anti-OMP (Wako, no. 544–10001), anti-GAP43 (Abcam, no. Ab134019), and anti-CK14 (Abcam, no. Ab7800).

Statistical Analysis. Statistical analysis was performed by using GraphPad Prism. Group distribution of gene loci was analyzed with the χ^2 test and allele distances to the gravity center of the heterochromatin blocks were analyzed with Tukey's multiple comparison test.

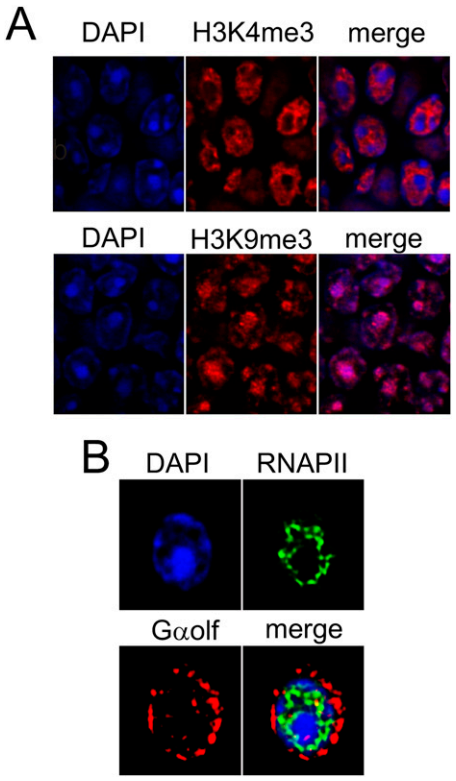


Fig. S1. Transcription compartments in the nuclei of olfactory neurons. (*A*) Immunofluorescence detection of H3K4me3 and H3K9me3 in nuclei from olfactory neurons, with DAPI staining in blue, as indicated. (*B*) Double immunofluorescence detection of active RNA polymerase II (RNAPII) (green) and Gαolf (red) in dissociated olfactory neurons. A representative Gαolf positive olfactory neuron is shown. RNAPII was detected using 1:100 mouse monoclonal IgM anti-RNA pol II phosphoS2 clone H5 (Abcam) and Gαolf was detected using 1:100 rabbit polyclonal Gαolf K-19 (Santa Cruz Biotechnology). Slides were analyzed on an Olympus BX61 microscope equipped with a Hamamatsu C10600 camera. Deconvolved images were generated by using Autoquant X2.1.

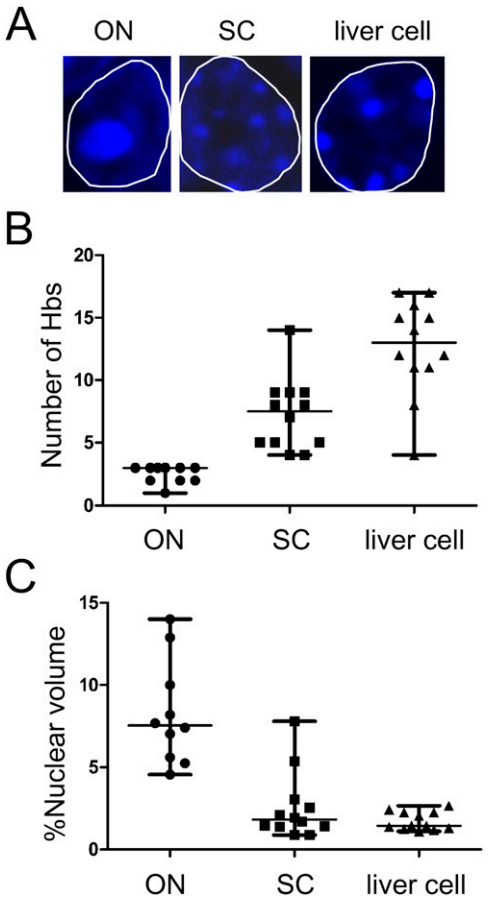
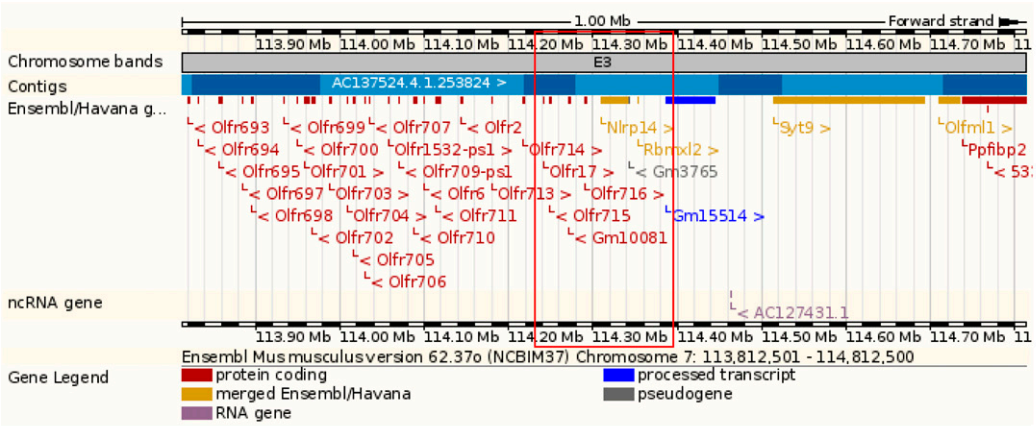


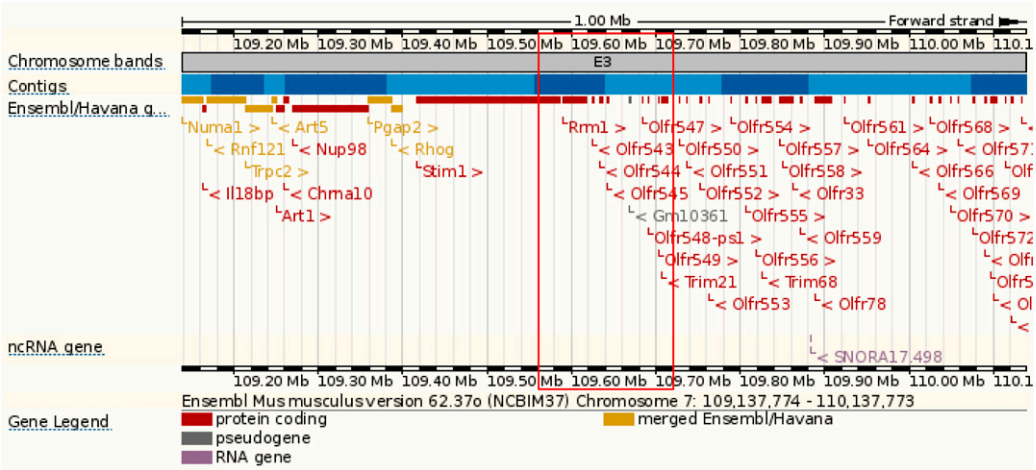
Fig. S2. Organization of constitutive heterochromatin in olfactory neurons, supporting cells and liver cells. (*A*) Representative images of DAPI-stained nuclei from an olfactory neuron (ON), or supporting cell (SC) in the olfactory epithelium, or from a liver cell. Heterochromatin can be clearly distinguished as intensely DAPI-stained regions (1). Outline shows the boundary of a single nucleus. Nuclei are not shown to scale. (*B*) The number of constitutive heterochromatin blocks (HBs) visualized in nuclei from ONs, SCs, and liver cells is shown. Ten cells were counted for each cell type. (*C*) The percentage of the total nuclear volume occupied by the largest heterochromatin block in nuclei from ONs, SCs, and liver cells is shown. Ten cells were counted for each cell type.

1. Takizawa T, Gudla PR, Guo L, Lockett S, Misteli T (2008) Allele-specific nuclear positioning of the monoallelically expressed astrocyte marker GFAP. *Genes Dev* 22(4):489–498.

P2 BAC: RP23-240P9 chr7:114229826-114389914



S6 BAC: RP23-311N6 chr7:109558898-109716649



OMP BAC: RP23-330N5 chr7:105142677-105347065

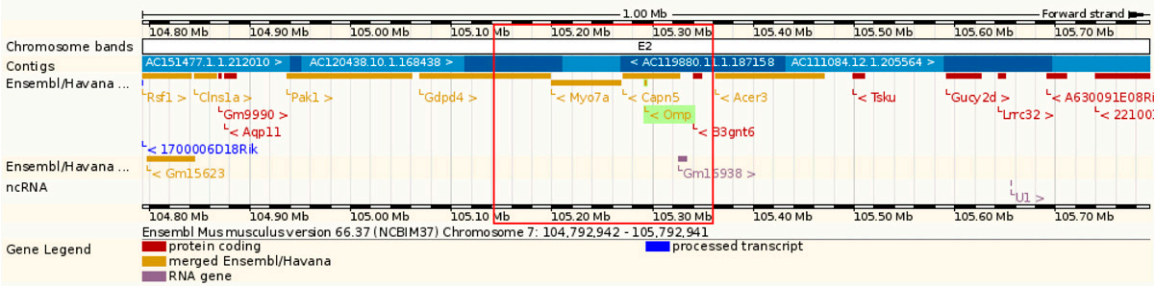
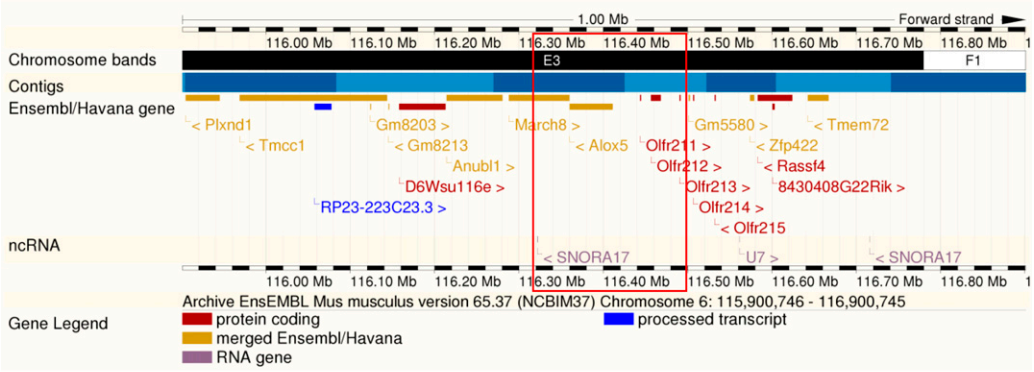


Fig. S3. (Continued)

olfr211 BAC: RP23- 238L17 chr6:116313652-116487839



mOR28 BAC: RP23-204N18: chr14:52948005-53146863

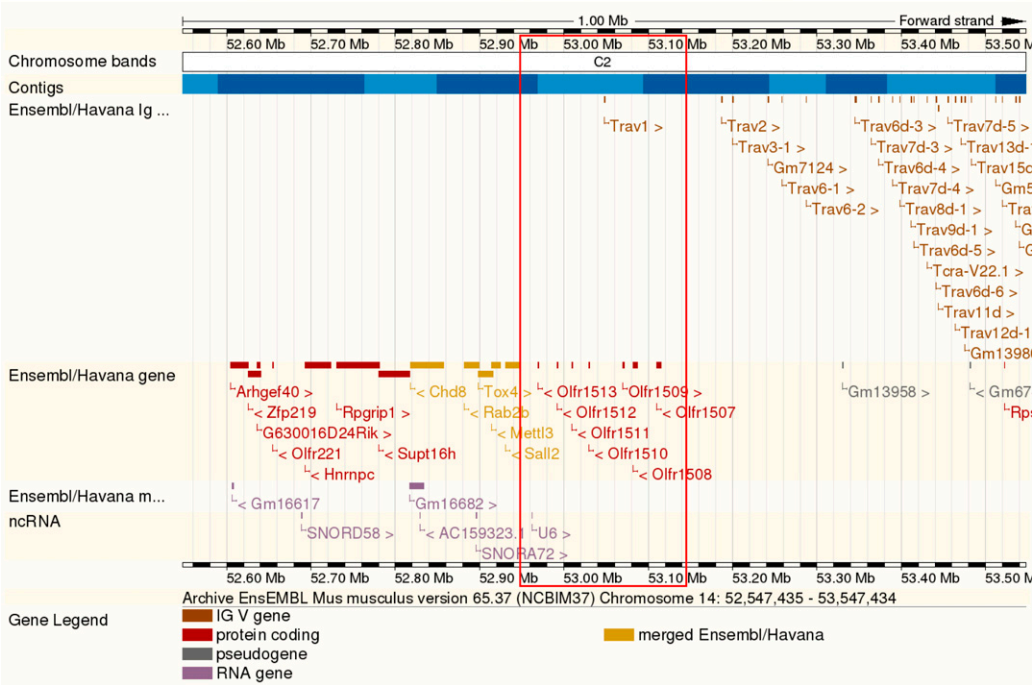


Fig. S3. Genes located in the regions covered by the BAC clones. Ensembl diagrams showing the detailed regions of the BAC clones. The regions corresponding to each BAC clone are boxed in red. The genomic positions of the BAC clones used in this study are RP23-330N5 (chr7: 105,142,677–105,347,065); RP23-311N6 (chr7: 109,558,898–109,716,649); RP23-240P9 (chr7: 114,229,826–114,389,914); RP23-238L17 (chr6: 116,313,652–116,487,839); and RP23-204N18 (chr14: 52,948,005–53,146,863). Gene and BAC locations were taken from University of California Santa Cruz Genome Bioinformatics (http://genome.ucsc.edu/index.html).

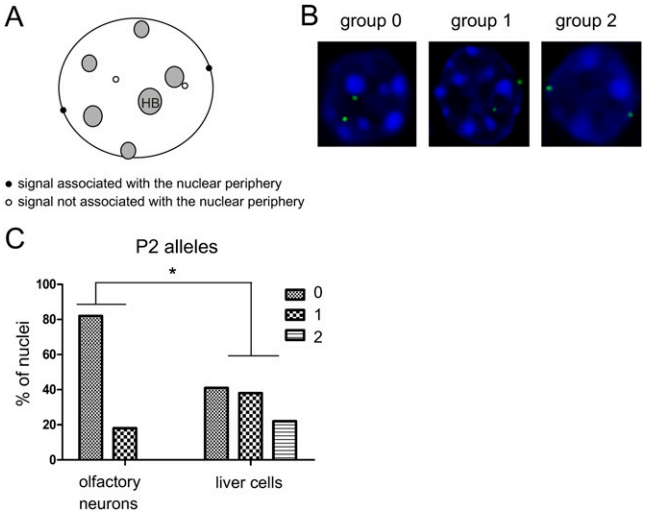


Fig. S4. Positioning of P2 OR gene loci in liver cells. The positioning of the P2 alleles relative to the nuclear periphery in both nuclei of liver cells and nuclei of olfactory neurons was analyzed. (*A*) Schematic representation of a nucleus from a liver cell with heterochromatin blocks shown in gray. Examples of signals that were considered to be associated with the nuclear periphery (●) or not (○) are shown. (*B*) Scoring system used to analyze the nuclei. Representative DNA FISH images are shown of nuclei where no allele (group 0), one allele (group 1), or two alleles (group 2) are associated with the nuclear periphery. DNA FISH signals are shown in green; heterochromatin is visualized by DAPI (blue). Images are deconvolved single z-sections. In these images, both alleles are seen in the same optical plane. (*C*) Three-dimensional DNA FISH was used to determine the positions of the P2 loci relative to the nuclear periphery in olfactory neurons and liver cells. Sixty olfactory neuron nuclei and 32 liver cell nuclei were scored and classified into one of the three groups. As previously described (1), we found that many of the liver cells are polyploid. Here we have analyzed only the nuclei that are diploid. Group distribution of OR gene loci in nuclei of olfactory neurons and liver cells were significantly different (*$P < 0.05$, χ^2 test).

1. Duncan AW, et al. (2010) The ploidy conveyor of mature hepatocytes as a source of genetic variation. *Nature* 467(7316):707–710.

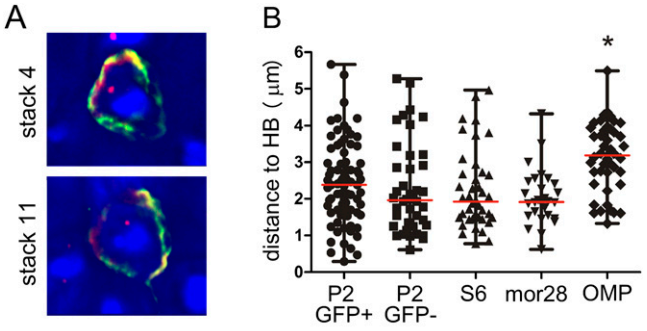
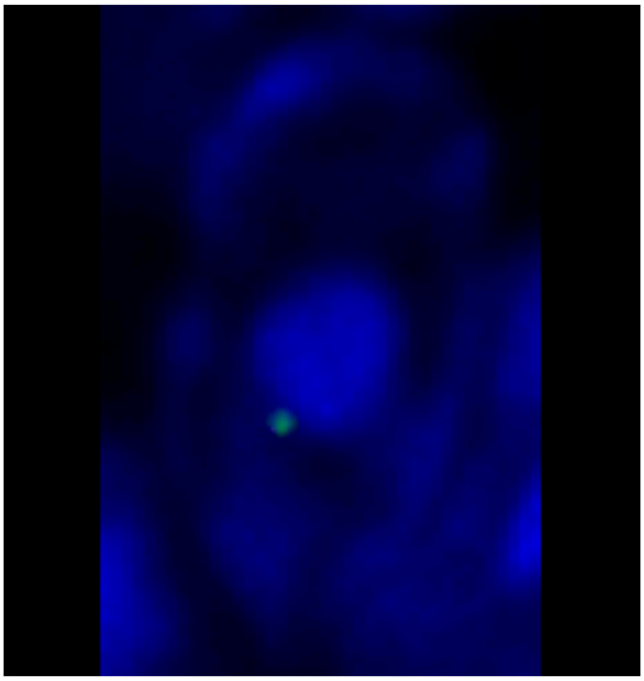


Fig. S5. Positioning of OR gene loci in olfactory nuclei. (*A*) Representative immuno-DNA FISH image of a GFP positive olfactory neuron (green) showing the P2 OR gene loci (red). The allele visualized in stack 11 is associated with the constitutive heterochromatin block, and the allele visualized in stack 4 is located farther away from it, in the euchromatic environment. (*B*) Distances of the OR and OMP gene loci to the center of the heterochromatin blocks. Three-dimensional DNA FISH was used to measure the distances between the P2 loci and the center of the heterochromatin blocks in olfactory neurons that express the P2 OR gene (GFP^+) or do not express the P2 OR gene (GFP^-). Each dot represents the distance measured between an individual allele and the gravity center of the heterochromatin block. Eighty alleles from GFP^+ olfactory neurons and 42 alleles from GFP^- olfactory neurons were analyzed. The distances of the OMP alleles (44 alleles), S6 alleles (42 alleles), and mOR28 alleles (28 alleles) to the center of the heterochromatin blocks are also shown. Medians for P2, S6, and mOR28 are, respectively, 2.0 μm, 1.9 μm, and 1.9 μm, and the median distance between the OMP alleles and the center of the heterochromatin blocks is 3.2 μm. The distances obtained for the P2 alleles in the GFP^+ and GFP^- neurons (2.4 μm and 2.0 μm, respectively) are not significantly different ($P > 0.5$). The distances obtained for the OMP alleles are significantly different from the distances obtained for the OR alleles ($P < 0.05$) (one-way ANOVA and Tukey's multiple comparison posttest). (*C*) Colocalization with H3K27me3 was analyzed in group 1 neurons. The percentages of group 1 neurons showing one allele that is colocalized with H3K27me3 in GFP^- or GFP^+ neurons are shown.

Table S1. Locations of gene loci in relation to heterochromatin blocks

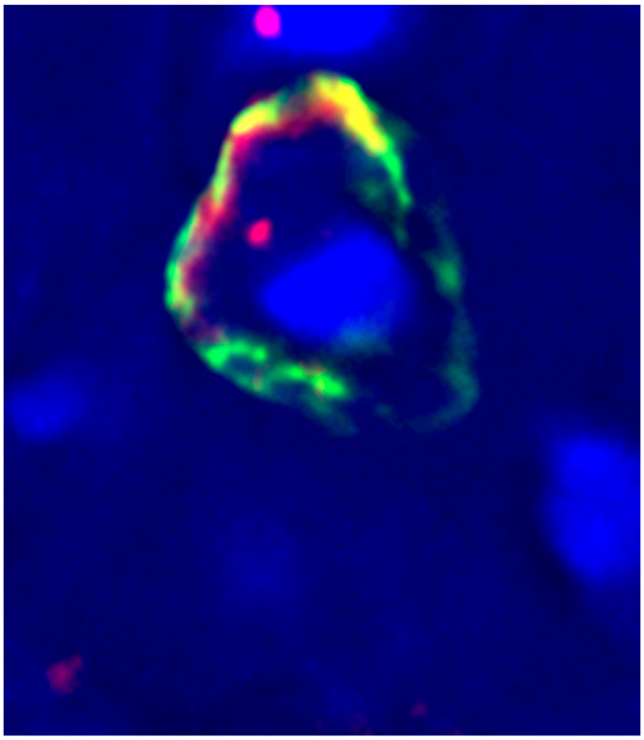
Loci	Group 0 (%)	Group 1 (%)	Group 2 (%)	Total
OMP	48 (79)	10 (16)	3 (5)	61
P2	19 (30)	29 (45)	16 (25)	64
S6	27 (39)	28 (40)	15 (21)	70
mOR28	11 (37)	13 (43)	6 (20)	30
Olfr211	11 (37)	16 (53)	3 (10)	30

The numbers of nuclei from olfactory neurons that have no allele (group 0), one allele (group 1), or two alleles (group 2) associated with heterochromatin blocks are shown.



Movie S1. Localization of the two P2 gene alleles within the nucleus of an olfactory neuron by using 3D DNA FISH. The allele visualized first is associated with a large heterochromatin block, whereas the second allele is not.

Movie S1



Movie S2. Localization of the two P2 gene alleles within the nucleus of a GFP^+ olfactory neuron by using immuno-3D DNA FISH. Two dimensional images of this neuron are shown in Fig. S5*A*.

Movie S2