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Figures and figure supplements

The activity-dependent histone variant H2BE modulates the life span of olfactory neurons

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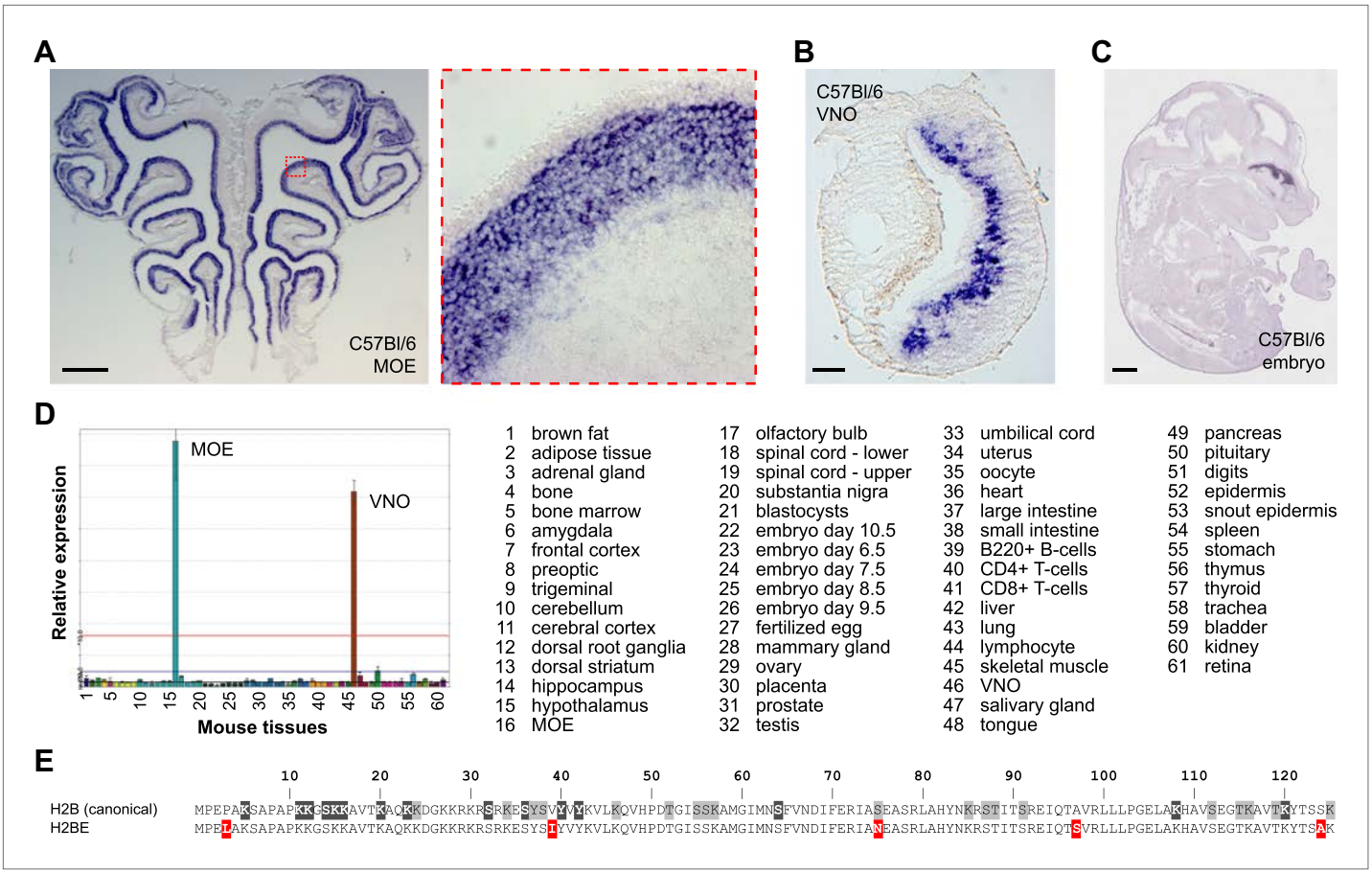

Figure 1. Mouse H2BE is detected exclusively in chemosensory neurons. (**A**) Analysis of *H2be* mRNA in the MOE of an 8-week old mouse, showing expression limited to sensory neurons. Boxed region is magnified (right). (**B**) Analysis of *H2be* mRNA in the VNO of an 8-week old mouse, showing expression limited to sensory neurons, especially in the apical zone. (**C**) Analysis of *H2be* mRNA in a sagittal section of an E14.5 mouse embryo (image from genepaint.org; ID: ES2590). (**D**) Profile of *H2be* mRNA levels in 61 mouse tissues (listed, right), showing exclusive expression in MOE and VNO. Data from GNF, now maintained by BioGPS (http://biogps.org/). (**E**) Alignment of H2BE and canonical H2B sequences. H2BE variant positions are highlighted in red; H2B PTM sites supported by >5 or ≤5 reports are highlighted in dark and light gray, respectively (http://www.phosphosite.org). Scale bars for (A), 500 μm; (B), 100 μm; (C), 1000 μm.

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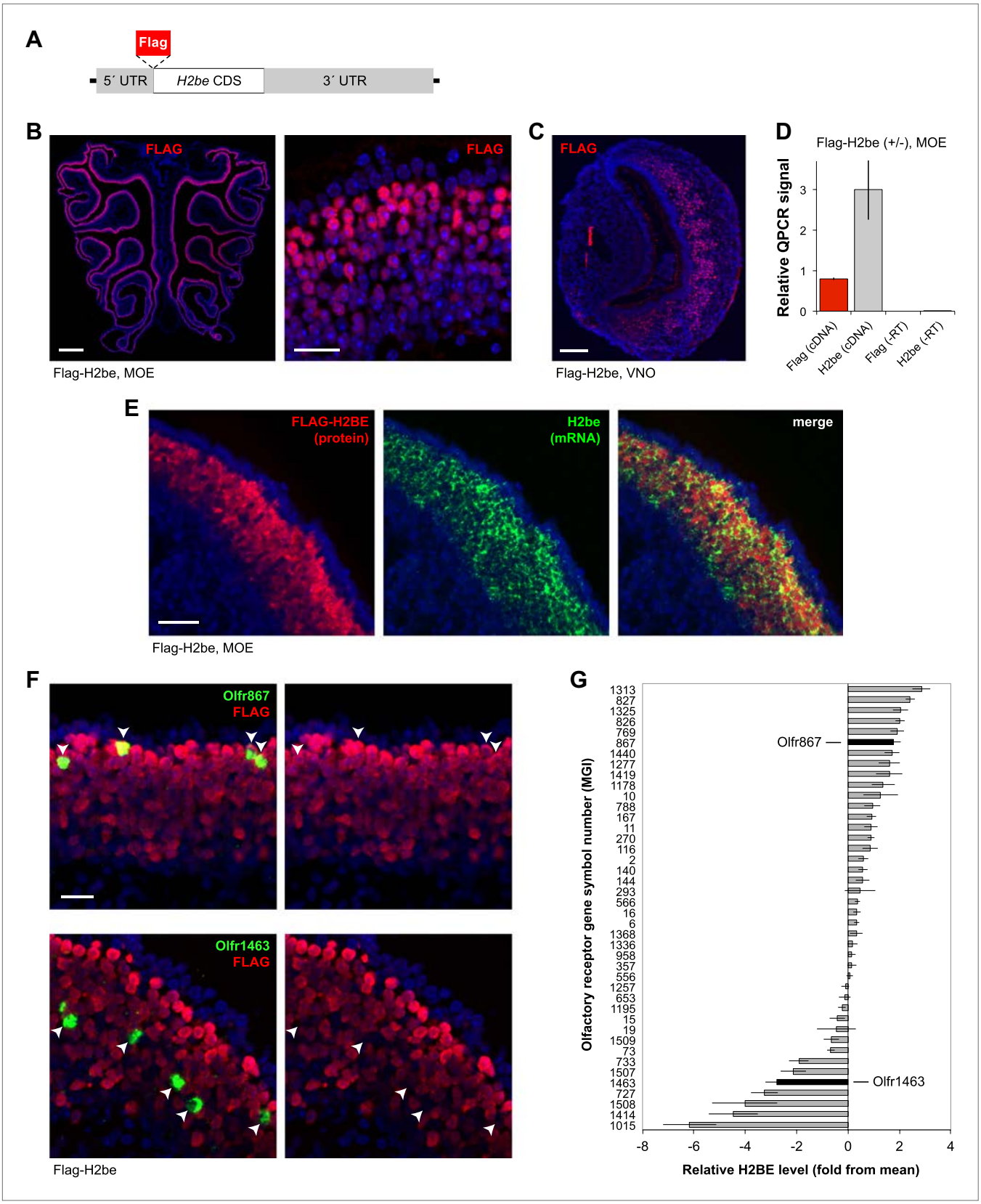

Figure 2. Generation of an *H2be:Flag-H2be* transgenic mouse (referred to as Flag-H2be) reveals that H2BE levels are stereotyped according to OR identity. (**A**) Flag-H2be transgenic construct, generated through modification of a BAC containing the *H2be* genomic region by insertion of a

Figure 2. Continued on next page

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FLAG-encoding sequence immediately upstream of the *H2be* CDS. (**B**,**C**) Representative images of FLAG-H2BE in MOE (B) and VNO (C) from 10-week old Flag-H2be transgenic mice. (**D**) Quantitative PCR (qPCR) analysis of Flag-H2be transgene mRNA levels in whole MOE tissue from three-week old Flag-H2be$^{(+/-)}$ transgenic mice. Signals were normalized to a value of three, corresponding to a primer pair recognizing all three *H2be* mRNAs (two endogenous and one transgenic). A primer pair specific for the Flag-tagged transgenic allele produces a normalized signal of approximately 1, indicating similar per-allele expression levels for the transgenic and endogenous *H2be* alleles. Negative control samples (−RT) were prepared by omitting reverse transcriptase during cDNA synthesis. (**E**) Colocalization analysis of FLAG-H2BE protein and *H2be* mRNA in the MOE of a 3-week old Flag-H2be$^{(+/-)}$ transgenic mouse. Observation of occasional basally-located neurons that are *H2be*-mRNA-positive and FLAG-H2BE-negative is likely due to an expected lag in protein production and accumulation following *H2be* transcription onset during neuronal development. (**F**) Colocalization analysis of *Olfr867* or *Olfr1463* (arrowheads) and FLAG-H2BE, showing representative ORs associated with high or low levels of H2BE, respectively. Mouse age: 10 weeks. (**G**) Quantification of average H2BE levels in neurons expressing specific ORs (*n* = from 4 to 36 neurons per OR examined; mean, 18). Gene symbols are from the Mouse Genome Informatics database (MGI; http://www.informatics.jax.org/). Scale bars for (B, left), 500 μm; (B, right) and (F), 20 μm; (C) and (E), 100 μm.

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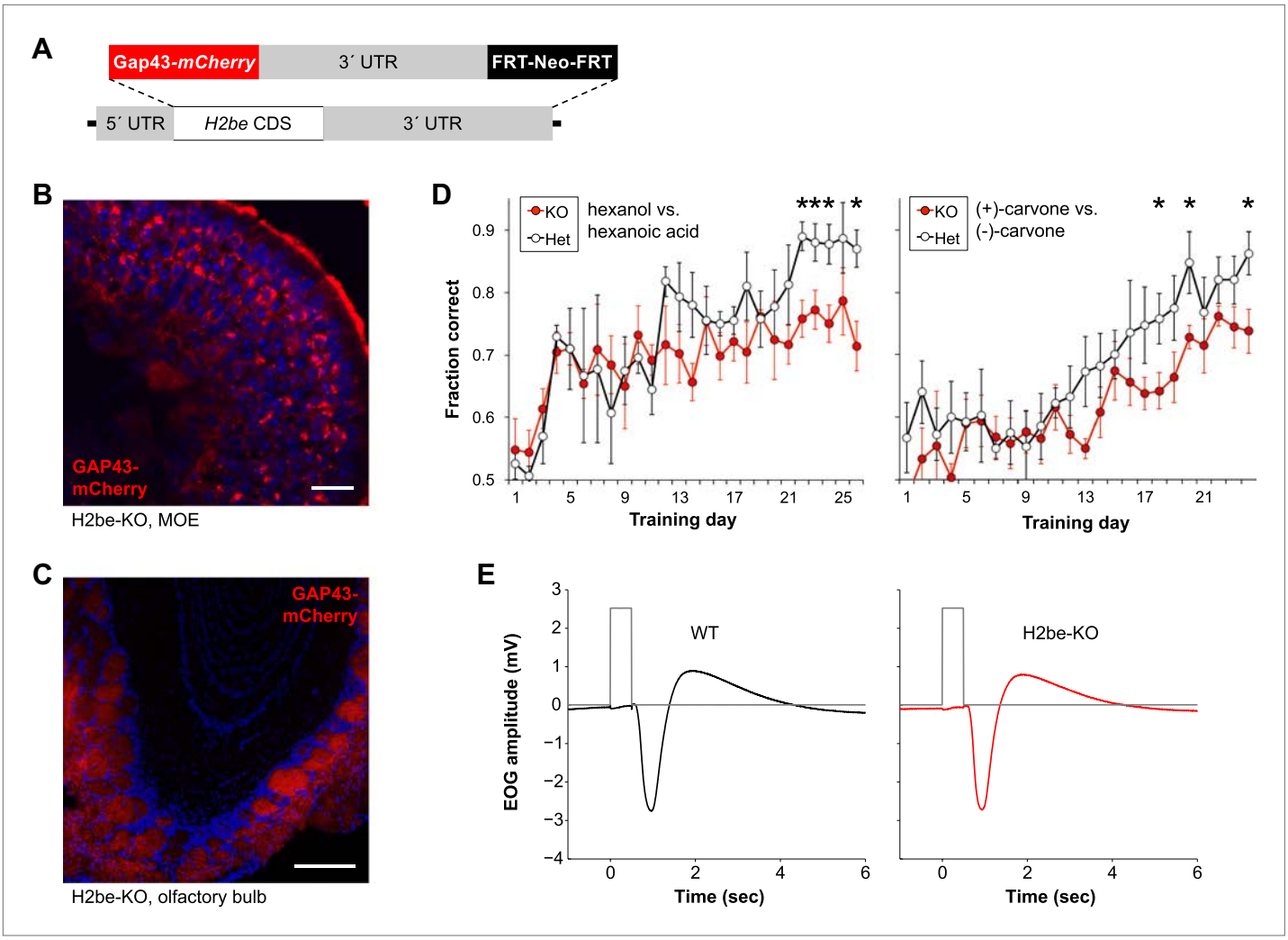

Figure 3. Generation of an H2be-KO/GAP43-mCherry-KI mouse line (referred to as H2be-KO) reveals that loss of *H2be* causes defects in olfaction. (**A**) H2be-KO allele, constructed through replacement of the endogenous *H2be* CDS with a membrane-targeted mCherry-encoding sequence (*Gap43-mCherry*). (**B**,**C**) Intrinsic GAP43-mCherry fluorescence in the MOE (B) and OB (C) of H2be-KO mice, showing GAP43-mCherry localization to the cell membranes and processes of olfactory neurons. Mouse ages: (B), 6 months; (C), 2 months. Scale bar for (B), 20 μm; (C), 200 μm. (**D**) Performance of approximately 3-month old water-restricted H2be-KO and control littermates in discriminating between hexanol/hexanoic acid (left) or (+)/(−)-carvone (right) odor pairs to obtain water (n = 5 per genotype). *p<0.05. (**E**) Effects of *H2be* loss-of-function on odor-evoked electrical responses in the MOE. Electro-olfactogram traces (black and red) represent average responses to a 0.5-s stream of air from the head space of a 1% solution of isoamyl-acetate in mineral oil. Gray traces show timing of switching between the delivery of clean, de-odorized air (low), and odor-containing air (high). Results shown are representative of multiple trials, odorants, and concentrations; experimental procedures were adapted from those described previously (***Waggener and Coppola, 2007***).

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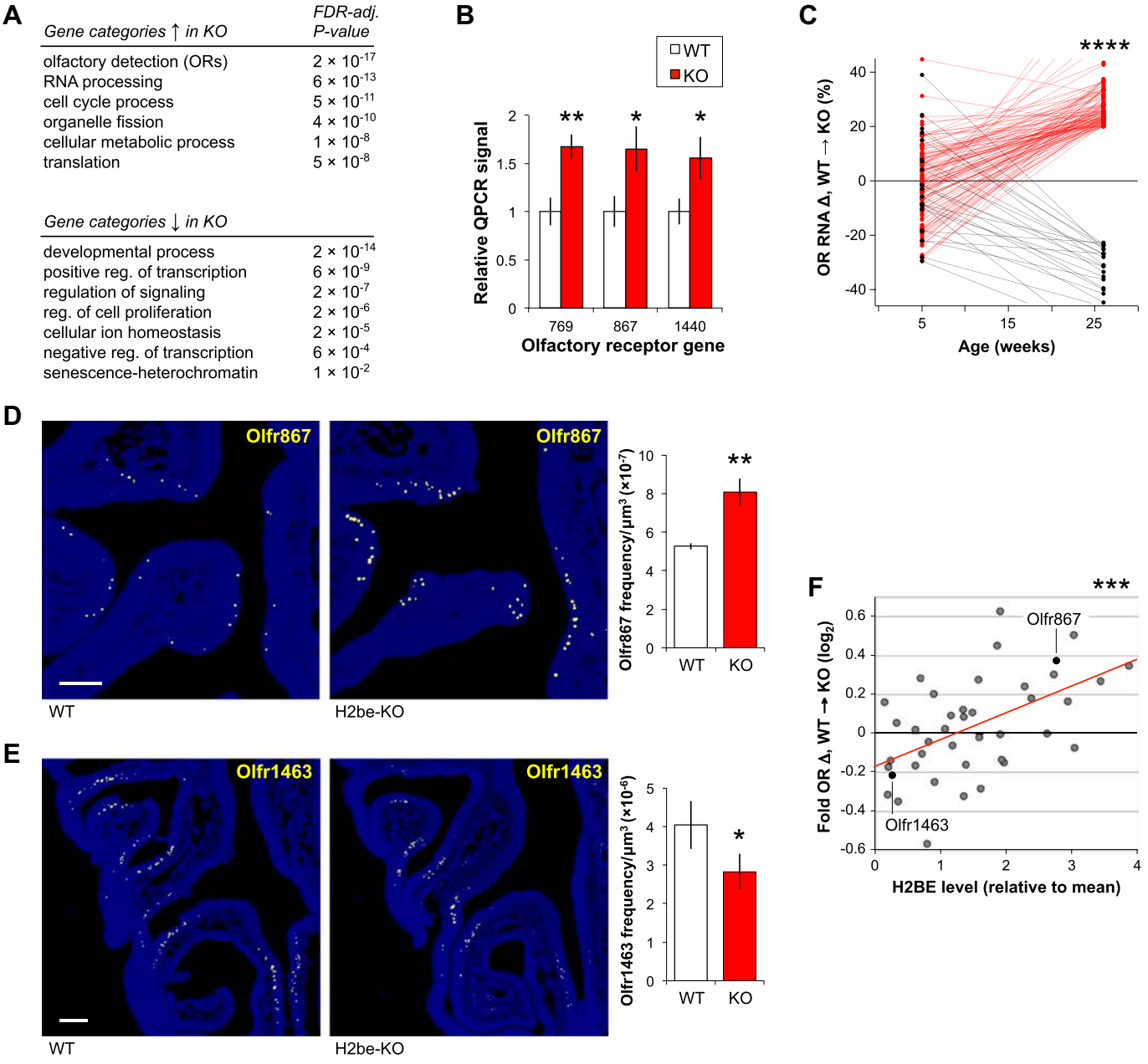

Figure 4. Loss of *H2be* causes defects in gene expression and OR expression frequencies. (**A**) Enriched gene ontology categories among genes up- (top) or down-regulated (bottom) in 6-month old H2be-KO vs WT MOEs, based on microarray analyses of whole MOE tissue (*n* = 6 samples/genotype, 2 animals/sample). (**B**) Multiplex qPCR analysis of OR mRNA levels in MOE tissue of six-month old H2be-KO and WT mice. Signals represent normalized ratios of specific OR mRNAs to *Cnga2*, which is unaltered in H2be-KO mice and used as an internal control (*n* = 6). (**C**) Expression differences in H2be-KO and WT MOEs (based on microarray analysis), plotted as a function of age. For simplicity, only ORs with differences >20% at age 6 months are shown, with up- and down-regulated ORs shown in red and black, respectively; statistical analysis included all ORs interrogated. (**D**,**E**) Representative images (left) and quantification (right) of *Olfr867* (D) and *Olfr1463* (E) expression frequencies in 6-month old H2be-KO and WT littermates (*n* = 3 mice, 10 sections per mouse). Scale bars, 200 µm. (**F**) Relationship between OR gene expression defects in 6-month old male H2be-KO mice (based on microarray analysis) and stereotypical H2BE levels as measured in male Flag-H2be mice. Red line, best fit. *p<0.05, **p<0.01, ***p<0.001, ****p<10^{-30}.

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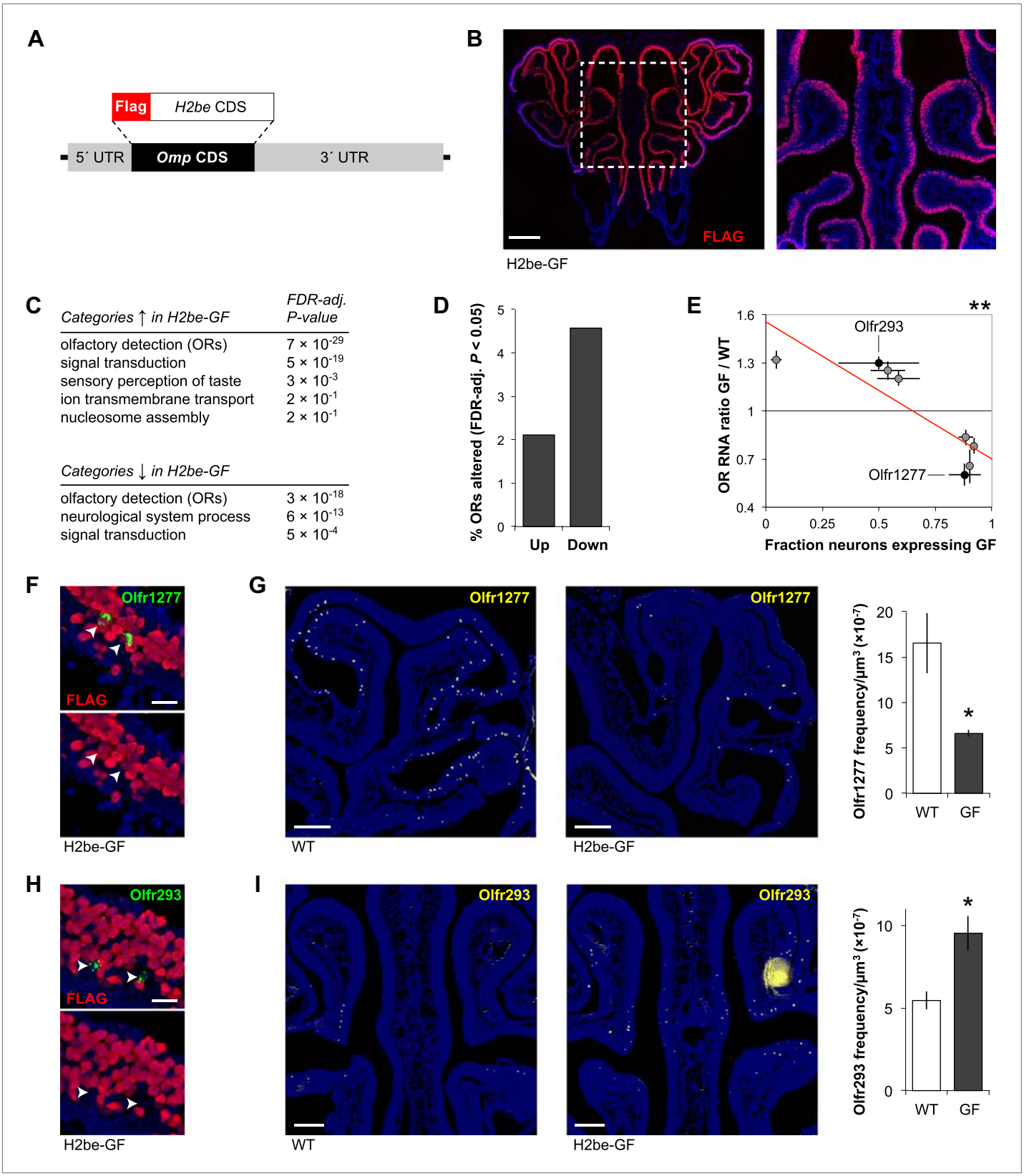

Figure 5. Generation of an *Omp:Flag-H2be* transgenic mouse (referred to as H2be-GF) reveals that ectopic overexpression of *H2be* in olfactory neurons alters gene expression and OR expression frequencies. (**A**) H2be-GF transgenic construct, generated through replacement of the *Omp* CDS with a

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Figure 5. Continued

FLAG-H2BE-encoding sequence in a vector containing the *Omp* genomic region. (**B**) Analysis of FLAG-H2BE in the MOE of a 5-week old H2be-GF mouse, showing high transgene expression in all mature neurons, except for a band near zone 2 (***Sullivan et al., 1996***). Boxed region is magnified (right). (**C**) Gene ontology categories enriched among genes up- (top) or down-regulated (bottom) in 5-week old H2be-GF vs WT MOEs, based on microarray analyses of whole MOE tissue (n = 4 samples, 3 animals per sample) and WT (n = 6 samples, 2 animals per sample) mice. (**D**) Percentage of OR genes with significantly differential expression (FDR-adjusted $p<0.05$) in 5-week old H2be-GF and WT mice. (**E**) Relationship between OR gene expression defects in H2be-GF mice and transgene co-expression penetrance. Red line, best fit. (**F**,**H**) Colocalization of *Olfr1277* or *Olfr293* (arrowheads) and FLAG-H2BE in 5-week old H2be-GF mice, showing representative ORs associated with high- or low transgene penetrance, respectively. (**G**,**I**) Representative images (left) and quantification (right) of *Olfr1277* (G) and *Olfr293* (I) expression frequencies in 5-week old H2be-GF and WT littermates (n = 3 mice; 10 sections per mouse). *$p<0.05$, **$p<0.01$. Scale bars for (B), 500 μm; (F) and (H), 20 μm; (G) and (I), 200 μm.

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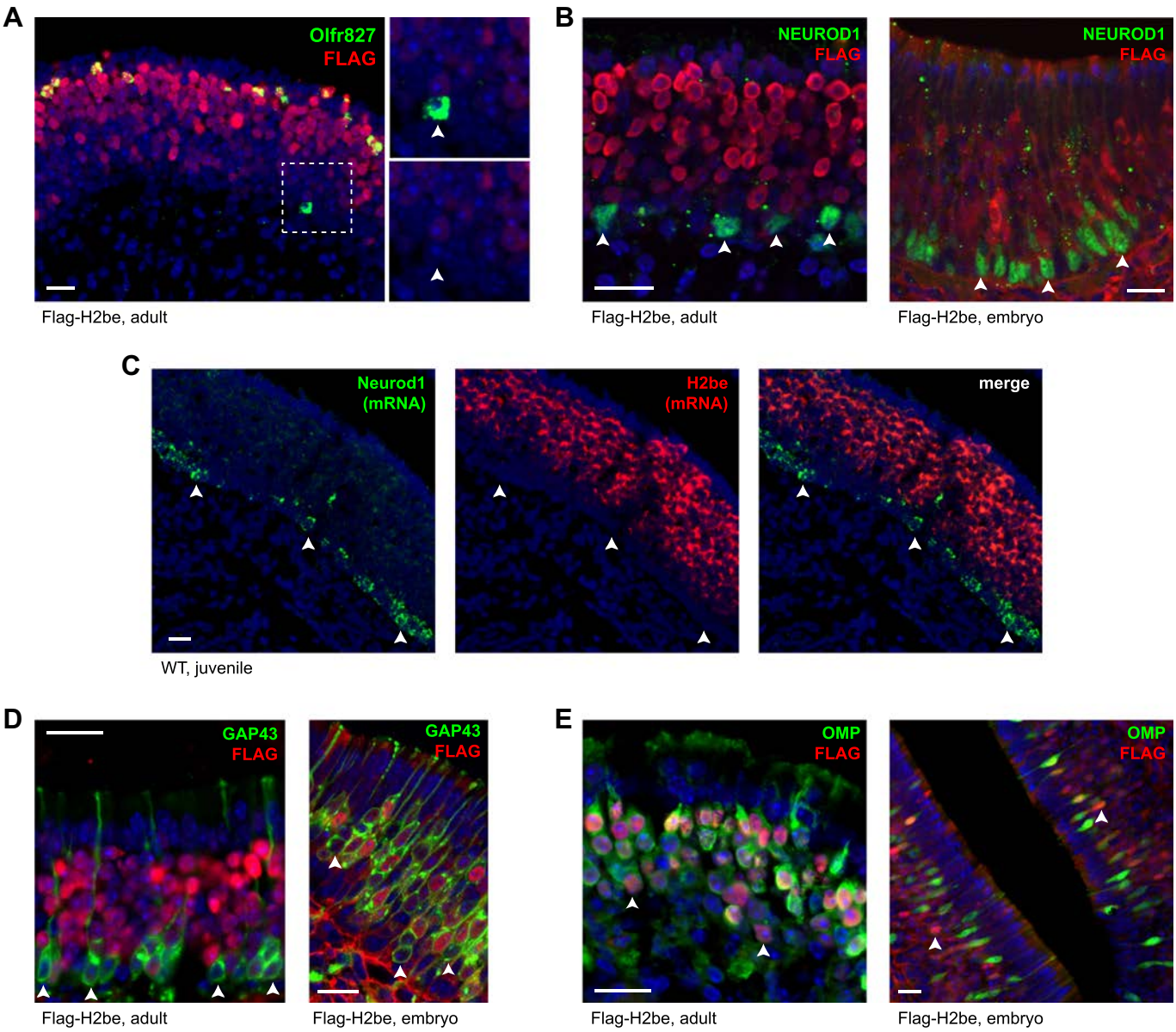

Figure 6. The expression onset of *H2be* follows OR choice during neuronal development. (**A**) H2BE is undetectable in an *Olfr827*$^+$ neuron that is newly-differentiated (note its basal position in the epithelium; boxed and magnified, right), but expressed at uniformly high levels in mature *Olfr827*$^+$ neurons. (**B**,**D**,**E**) MOE expression patterns in adult (left) or embryo (right) of *H2be* relative to *Neurod1*, *Gap43*, and *Omp*, which have expression onsets prior to, concurrently with, and following OR choice, respectively. All NEUROD1$^+$ cells (B) and a fraction of basal GAP43$^+$ (newly-differentiated) neurons (C) are H2BE$^-$ (arrowheads), whereas a fraction of H2BE$^+$ neurons are OMP$^-$ (E; arrowheads). (**C**) Colocalization of endogenous *H2be* and *Neurod1* mRNAs in the MOE of a 3-week old WT mouse. Mouse ages: (A), 3 months; (B, left), 4 months; (C), 3 weeks; (D, left) and (E, left), 10 months. Scale bars for (A) to (E), 20 μm.

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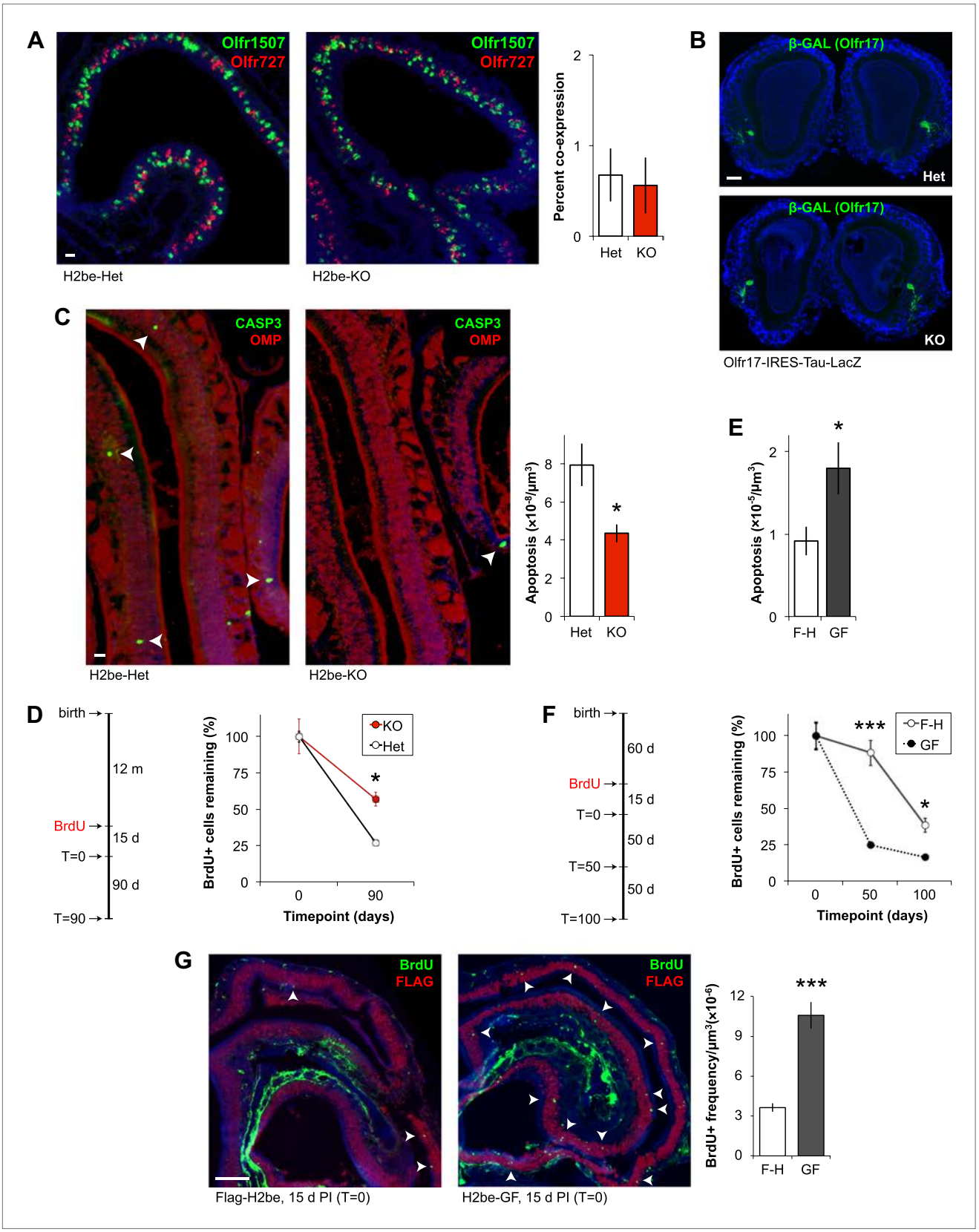

Figure 7. H2BE affects olfactory neuronal longevity, not OR choice. (**A**) Representative images (left) and quantification (right) of *Olfr1507* and *Olfr727* co-expression frequencies in H2be-KO and control littermates (*n* = 10 sections). (**B**) Labeled axons of *Olfr17*-expressing neurons form indistinguishable

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glomeruli in the OBs of H2be-KO and control littermates. (**C**,**E**) Representative images (C, left; arrowheads) and quantification (C, right; E) of apoptosis in mature neurons of H2be-KO (C) or H2be-GF (E) mice compared to controls (C: *n* = 3 mice, 12 sections per mouse; E: *n* = 10 sections). (**D**,**F**) Schematic of experimental analysis timeline (left) and quantification (right) of relative $BrdU^+$ neuron frequencies in H2be-KO (D) and H2be-GF (F) mice compared to controls, respectively (D: *n* = 3 mice per timepoint, 12 sections per mouse; F: *n* = 10 sections per timepoint). F-H: Flag-H2be. (**G**) Representative images (left) and quantification (right) of neurogenesis in H2be-GF (GF) mice and Flag-H2be (F-H) controls by analysis of frequencies of $BrdU^+$ neurons (arrowheads) 15 days post-injection of BrdU (*T* = 0 timepoint; *n* = 10 sections). *p<0.05, ***p<0.001. Mouse ages: (A) and (E), 4 months; (B) and (G), 2 months; (C), 15 months. Scale bars for (A) and (C), 20 μm; (B), 200 μm; (G), 100 μm.

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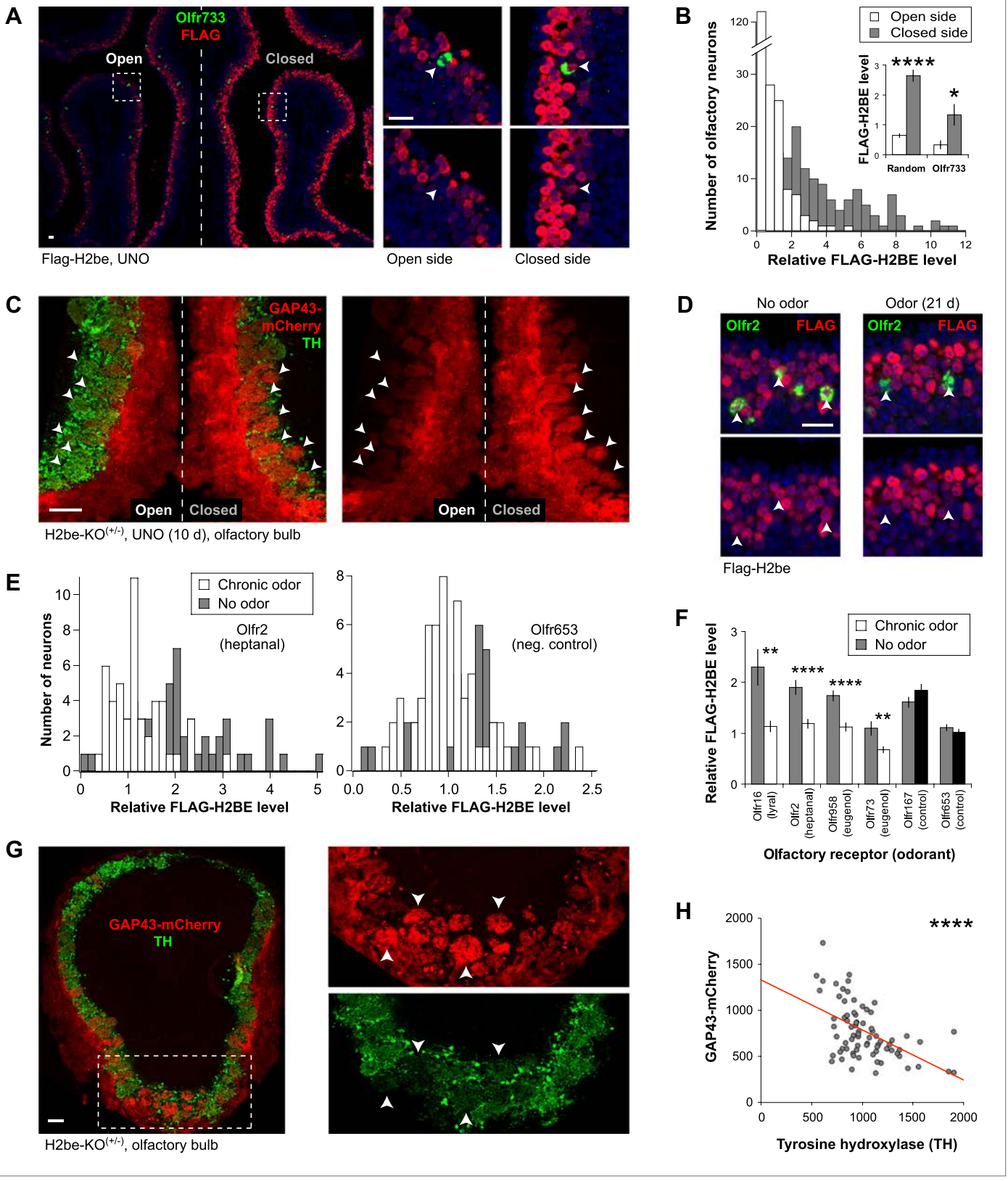

Figure 8. *H2be* is regulated by activity. (**A**,**B**) Effects of unilateral naris occlusion (UNO) on H2BE level in the MOE. (**A**) Representative images of FLAG-H2BE and *Olfr733* colocalization (boxed regions magnified, right; *Olfr733*$^{+}$ neurons, arrowheads). (**B**) Distributions (main) and averages (inset) of

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H2BE level within nuclei of randomly-sampled (main; inset, left; *n* = 200) or *Olfr733*$^{+}$ (inset, right; *n* = 10) neurons on the two sides of the MOE. **(C)** Effects of UNO on intrinsic GAP43-mCherry fluorescence in glomeruli (arrowheads) within the OB of an H2be-KO heterozygous mouse. Reduced tyrosine hydroxylase (TH; a marker of olfactory activity) staining on the closed side indicates completeness of naris closure. (**D**–**F**) Effects of odor exposure on H2BE level in olfactory neurons. Representative images (D) and quantification (E, left) of FLAG-H2BE in Olfr2^{+} neurons (D, arrowheads) exposed to odors or mineral oil (no odor). (E, right) Quantification of FLAG-H2BE in Olfr653^{+} neurons (chosen randomly as a negative control) exposed to odors or mineral oil (no odor). (**F**) Average H2BE level within neurons expressing odor-stimulated or control ORs (*n* = 20–60). (**G**,**H**) Representative image (G; boxed region magnified, right) and quantification (H) of the relationship between GAP43-mCherry and tyrosine hydroxylase intensities within glomeruli of an H2be-KO heterozygous mouse. Red line, best fit. *p<0.05; **p<0.01; ****p<0.0001. Mouse ages: (A) and (C), 4 weeks; (D), 8 weeks; (G), 10 weeks. Scale bars for (A) and (D), 20 μm; (C) and (G), 100 μm.

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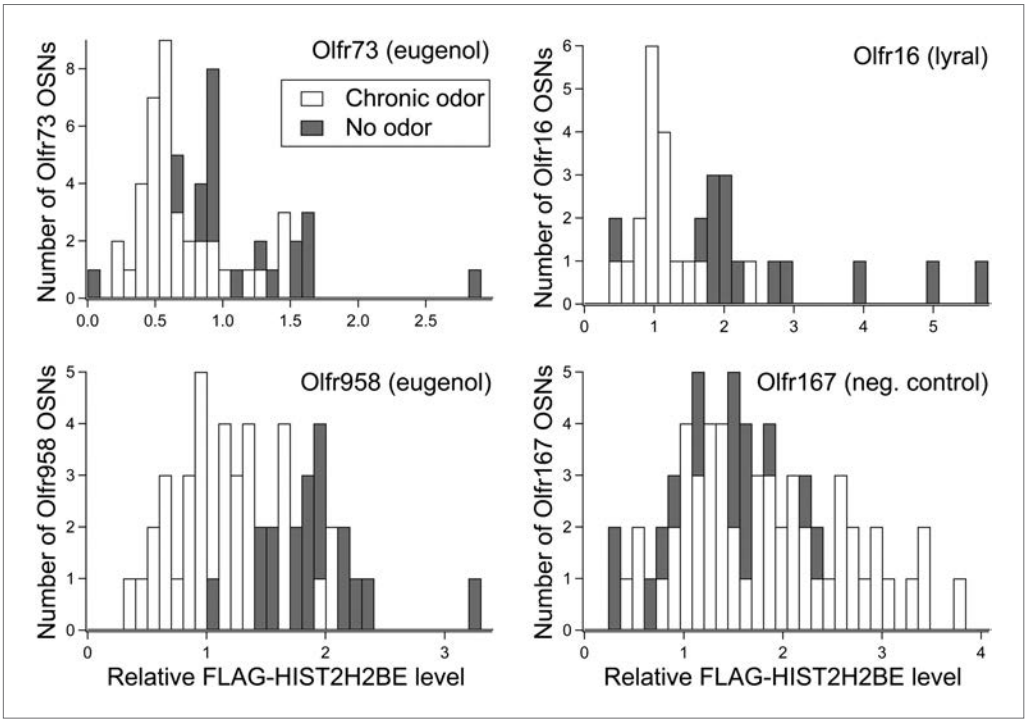

Figure 8—figure supplement 1. Distribution of relative H2BE levels within neurons expressing Olfr73, Olfr958, Olfr16, and Olfr167 for odor-exposed and control littermates. Stimulating odors are indicated, except for Olfr167, which serves as a negative control.

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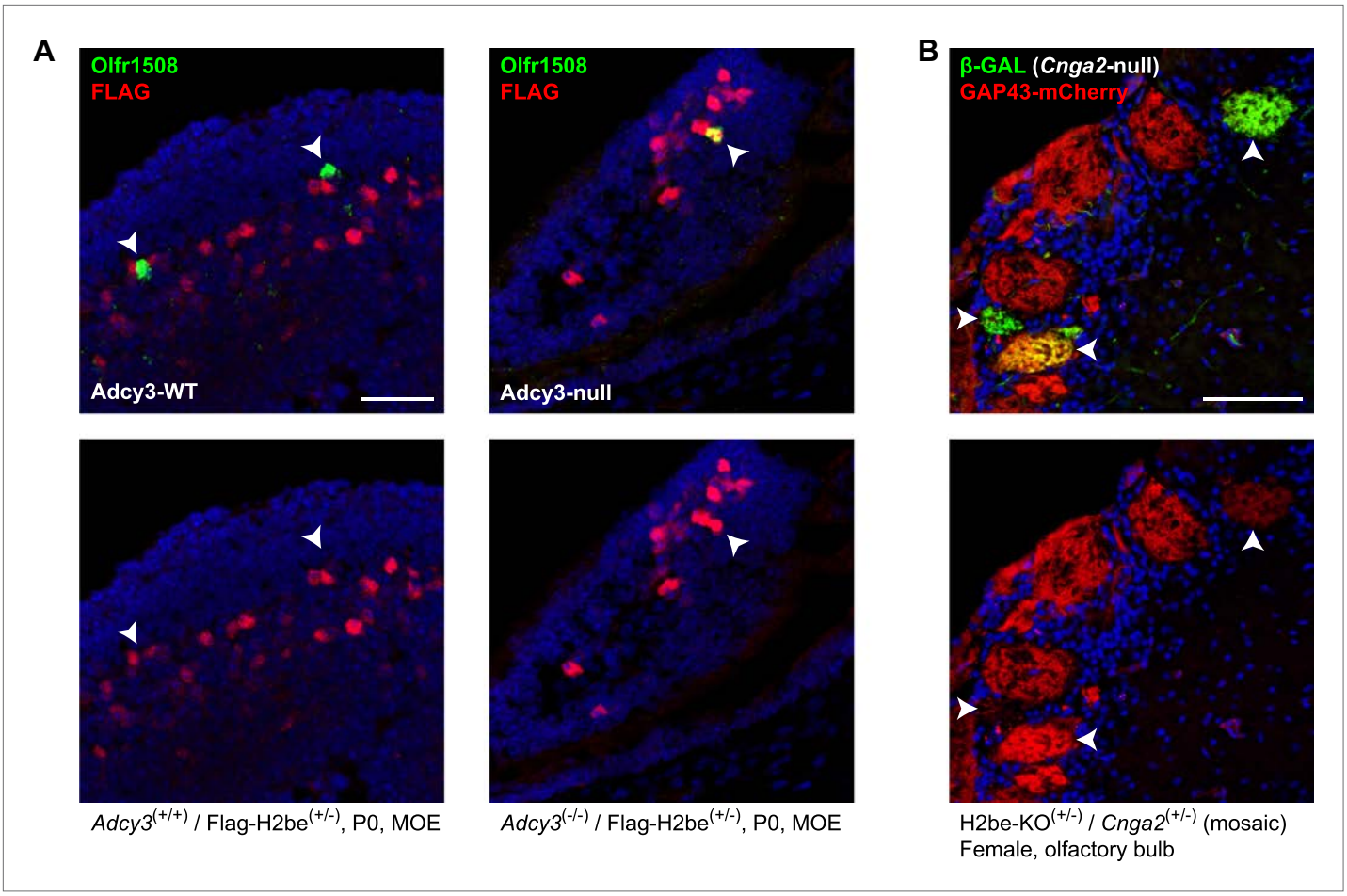

Figure 9. H2BE levels are cAMP-, but not Ca^{2+}-dependent. (**A**) Representative example of the effects of *Adcy3* loss-of-function on H2BE levels in newborn (P0) mice. In *Adcy3*$^{(+/+)}$ mice, *Olfr1508*$^{+}$ neurons contain extremely low H2BE levels (left, arrowheads), while in *Adcy3*$^{(-/-)}$ mice, *Olfr1508*$^{+}$ neurons frequently contain extremely high levels (right, arrowhead), indicating that cAMP participates in the negative regulation of H2BE. Note: age P0 was chosen due to the low postnatal survival rate of *Adcy3*$^{(-/-)}$ mice. (**B**) Effects of *Cnga2* loss-of-function on *H2be* expression. Mice in which *Cnga2* (an X-chromosomal gene necessary for odor-evoked Ca^{2+} signaling) was replaced with the *Tau-LacZ* gene (***Zhao and Reed, 2001***) were crossed with H2be-KO mice to generate three-week old Cnga2-KO-Tau-LacZ$^{(+/-)}$/H2be-KO-Gap43-mCherry$^{(+/-)}$ compound heterozygous females. In these mice, one half of new olfactory neurons express TAU-LACZ instead of CNGA2 and project to glomeruli distinct from neurons expressing *Cnga2* (***Zheng et al., 2000***). Analysis of β-GAL^{+} (TAU-LACZ) and GAP43-mCherry intensities within glomeruli revealed that *Cnga2*$^{-}$ neurons (β-GAL^{+}; arrowheads) do not have higher levels of GAP43-mCherry than Cnga2^{+} neurons (β-GAL^{-}), indicating that *H2be* expression is not negatively regulated by Ca^{2+} signaling. Scale bars for (A), 40 μm; (B), 100 μm.

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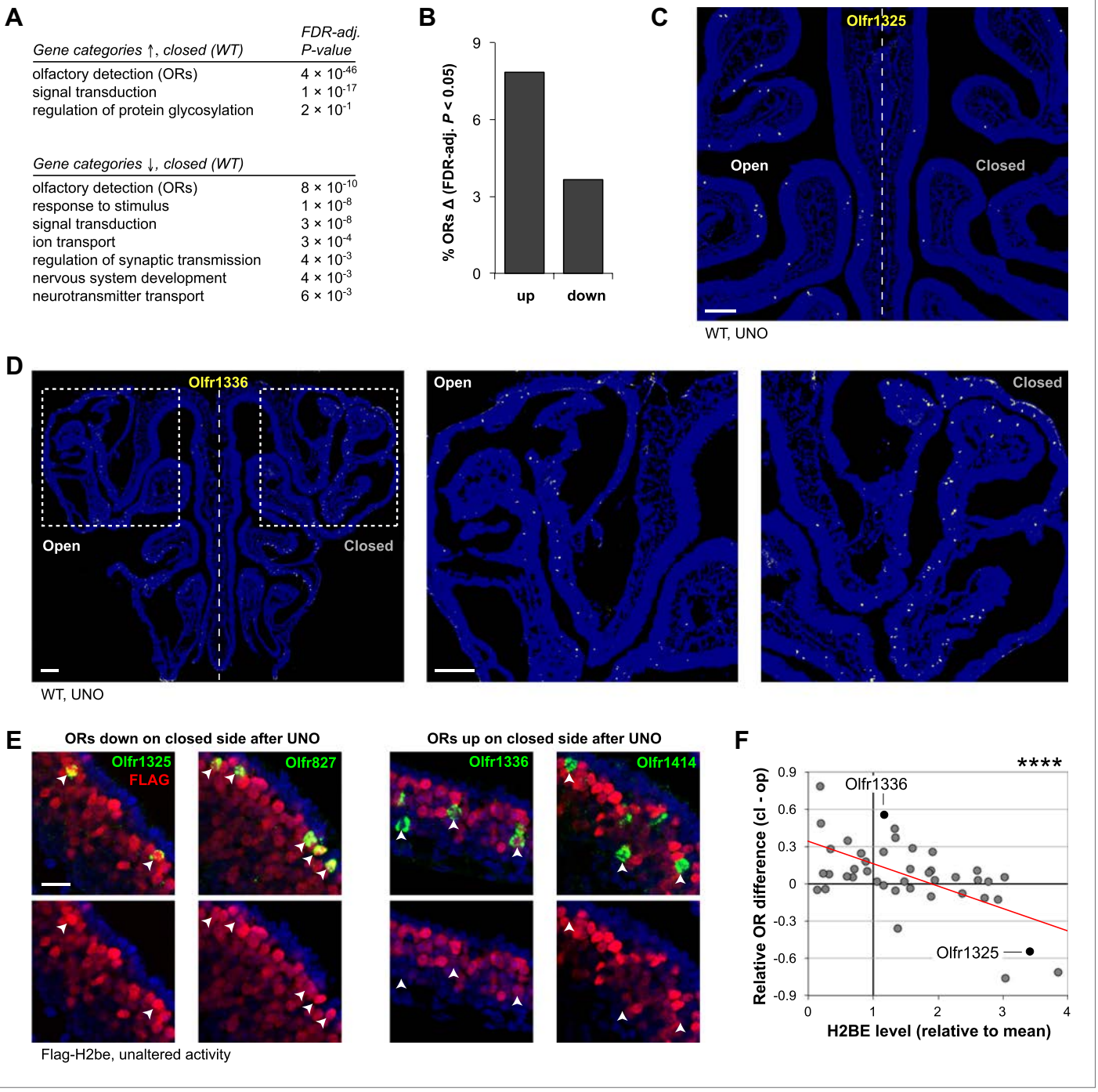

Figure 10. Unilateral naris occlusion (UNO) alters gene expression and OR expression frequencies. (**A**) Gene ontology (biological process) terms enriched at the top of a gene list ranked descendingly according to differential expression on the two MOE halves from 5-week old WT mice subjected to UNO (21 days), based on microarray analysis (n = 3 samples per MOE side, four animals per sample). (**B**) Percentage of OR genes with significantly differential (FDR-adjusted p<0.05) expression on the two sides of the MOE of WT mice after UNO (21 days; values from microarray data). (**C**,**D**) Representative images of *Olfr1325* (C) and *Olfr1336* (D) expression in the MOE after UNO. (**E**) Representative images of normal FLAG-H2BE levels in neurons associated with ORs that are down- (left) or up-regulated (right) in frequency after olfactory deprivation. (**F**) Relationship between UNO-mediated OR gene expression differences on the two sides of the MOE (values from microarray data for WT mice subjected to UNO for 21 days) and associated FLAG-H2BE levels measured in intact mice. Red line, best fit; ****p<0.0001. Mouse ages: (C) and (D), 5 weeks; (E), 12 weeks. Scale bars for (C) and (D), 200 μm; (E), 20 μm.

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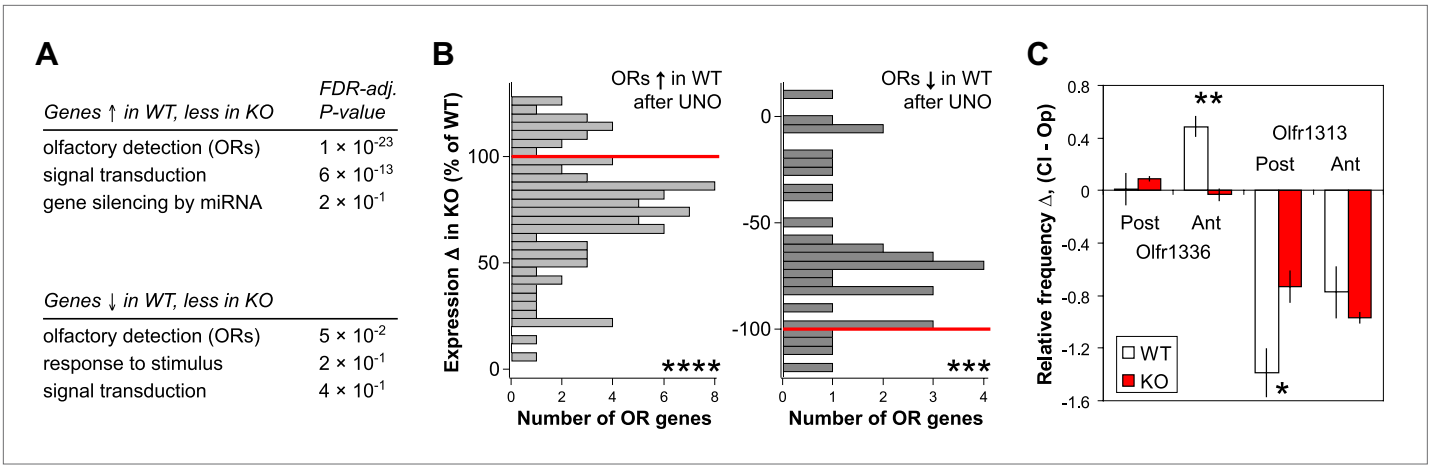

Figure 11. *H2be* affects activity-dependent gene expression. (**A**) Gene ontology (biological process) terms enriched among genes with UNO-mediated expression differences in WT mice (log_2 fold-change > 0.3; unadjusted p<0.02), but at least 20% less altered expression in H2be-KO compared to WT mice after UNO, based on microarray analysis of MOE halves from 5-week old WT and H2be-KO mice subjected to UNO (21 days; *n* = 3 samples per MOE side, four animals per sample). (**B**) Histograms of UNO-mediated OR expression differences on the closed and open sides of the MOE of H2be-KO mice as a percentage of the corresponding WT differences (normalized to 100% or −100%; red lines) for ORs significantly up- (left) or down-regulated (right) in WT mice after olfactory deprivation (FDR-adjusted p<0.05; values from microarray data). (**C**) Comparison of UNO-altered *Olfr1336* and *Olfr1313* frequencies in 5-week old WT and H2be-KO mice subjected to UNO (21 days). Values correspond to relative OR expression frequency differences on the two sides of the MOE according to the anterior (Ant)/ posterior (Post) position (*n* = 3 mice, five sections per region per mouse). Note: UNO appears to affect OR frequencies differently in the anterior and posterior regions of the MOE. *p<0.05; **p<0.01***p<0.001; ****p<0.0001.

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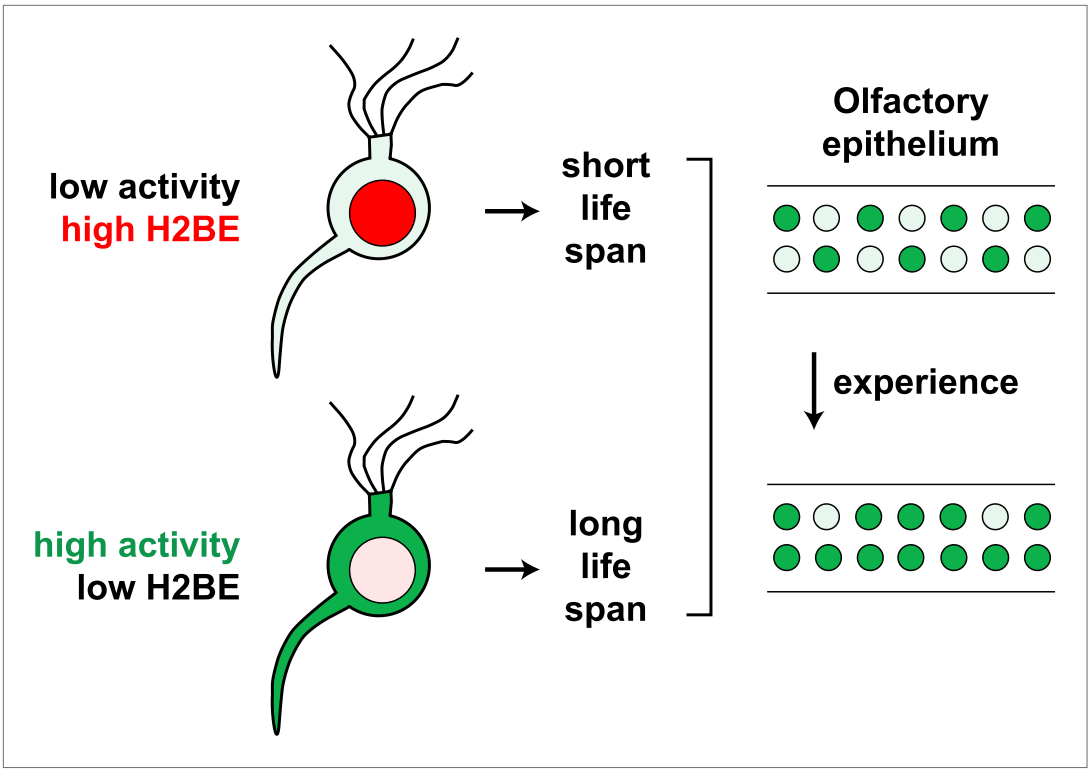

Figure 12. Model for the effects of neuronal activity on H2BE expression level, life span and resulting neuronal representation.

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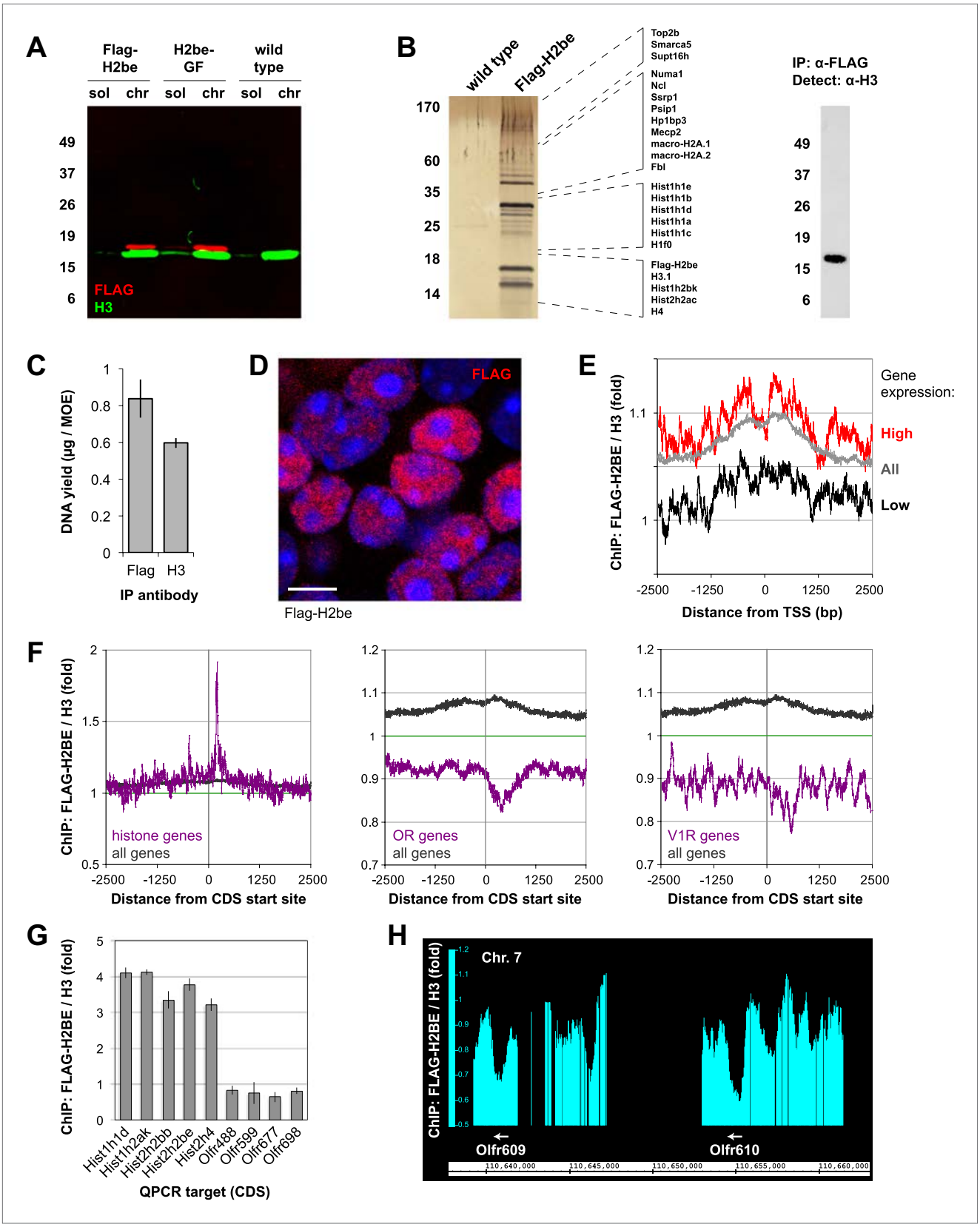

Figure 13. Chromatin incorporation and localization of FLAG-H2BE. (**A**) Two-color western analysis of FLAG-H2BE and H3 in soluble nucleoplasm (sol) and chromatin (chr) fractions of unfixed MOE cell nuclei from 16-week old Flag-H2be, H2be-GF, and WT mice. (**B**) SDS-PAGE analysis (left), mass

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spectrometric identification (listed, middle), and western analysis (right) of proteins associated with immunoprecipitated FLAG-H2BE-containing native mononucleosomes from MOE tissue of Flag-H2be transgenic mice. Proteins identified by mass spectrometry are listed according to their approximate electrophoretic mobility. (**C**) Quantification of DNA immunoprecipitated from crosslinked and fragmented chromatin derived from MOE tissue of Flag-H2be transgenic mice. (**D**) Representative image of FLAG-H2BE localization within olfactory neurons of a 10-week old Flag-H2be mouse. Scale bar, 5 μm. (**E**) Genome-wide ChIP analysis of relative FLAG-H2BE levels with respect to distance from the transcript start sites (TSS) for all mouse genes (grey), and genes expressed at high (red) and low (black) levels in olfactory neurons. (**F**) Genome-wide ChIP analysis of relative FLAG-H2BE levels with respect to distance from the CDS start sites for mouse histone (left), OR (middle), and vomeronasal type 1 receptor (V1R; right) genes in comparison to all genes. (**G**) Quantitative PCR analysis of relative FLAG-H2BE levels in the protein-coding regions of representative histone and OR genes. Analyses were performed on ligation-mediated-PCR-amplified Flag-H2BE and H3 ChIP DNA samples. (**H**) Genome-wide ChIP analysis shows depleted FLAG-H2BE levels within representative OR CDS regions.

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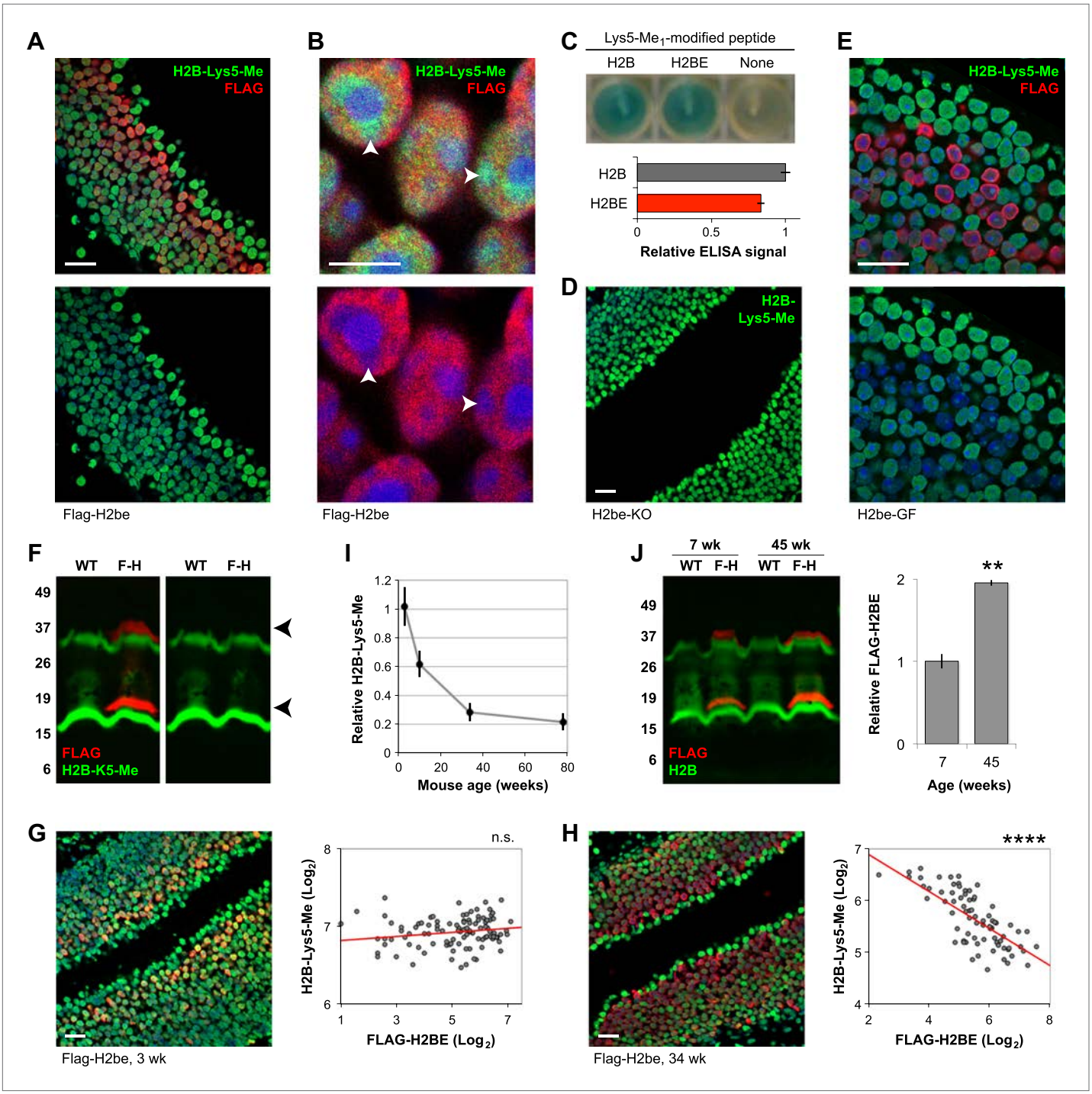

Figure 14. H2BE's post-translational modifications (PTMs) differ from those of canonical H2B. (**A**,**B**,**D**,**E**) Representative images of H2B-Lys5-Me (A, B, D, and E) and FLAG-H2BE (A, B, and E) staining in the MOE of Flag-H2be (A and B), H2be-KO (D) or H2be-GF (E) mice. (B) High-magnification image of FLAG-H2BE and H2B-Lys5-Me colocalization shows that H2BE is depleted in nuclear regions enriched for H2B-Lys5-Me (arrowheads). Mouse ages: (A), (B), and (E), 10 weeks; (D), 34 weeks. (**C**) Confirmation of reactivity of the anti-H2B-Lys5-Me$_1$ polyclonal antibody with the Lys5-Me PTM in the context of the H2BE protein sequence. Image (top) and quantification (bottom) of an ELISA assay for peptides corresponding to canonical H2B or H2BE and containing the Lys5-Me PTM. (**F**) Two-color fluorescent western analysis of Lys5-Me modification of FLAG-H2BE in MOE lysates from WT and Flag-H2be (F-H) mice. No detectable H2B-Lys5-Me staining of the FLAG-H2BE bands (red; arrowheads) is observed. Approximate molecular weights (kDa) are indicated (left). The bands observed at approximately 30–35 kDa likely correspond to histone dimers. (**G–I**) Age-dependence of H2BE accumulation. (G and H) Images (left) and quantification (right) of FLAG-H2BE and H2B-Lys5-Me co-localization in 3- (G) and 34-week old (H) mice. Red lines, best fits. (I)

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Quantification of H2B-Lys5-Me levels in high-H2BE neurons (relative to apical sustentacular cells; *n* = 20 nuclei from two images per timepoint). (**J**) Two-color fluorescent western analysis (left) and quantification (right) of FLAG-H2BE relative to total H2B as a function of age in MOE lysates from WT and Flag-H2be (F-H) mice. Approximate molecular weights (kDa) are indicated (left). The bands observed at approximately 30–35 kDa likely correspond to histone dimers. **p<0.01; ****p<0.0001; n.s., not significant. Scale bar for (A), (D), (E), (G), and (H), 20 μm; (B), 5 μm.

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