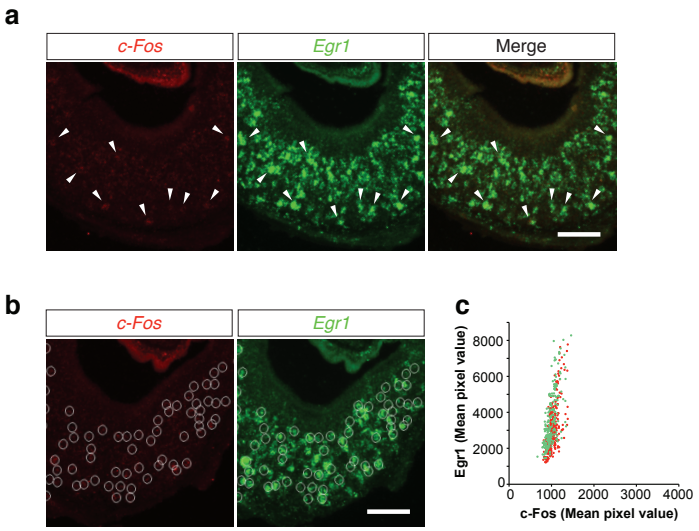
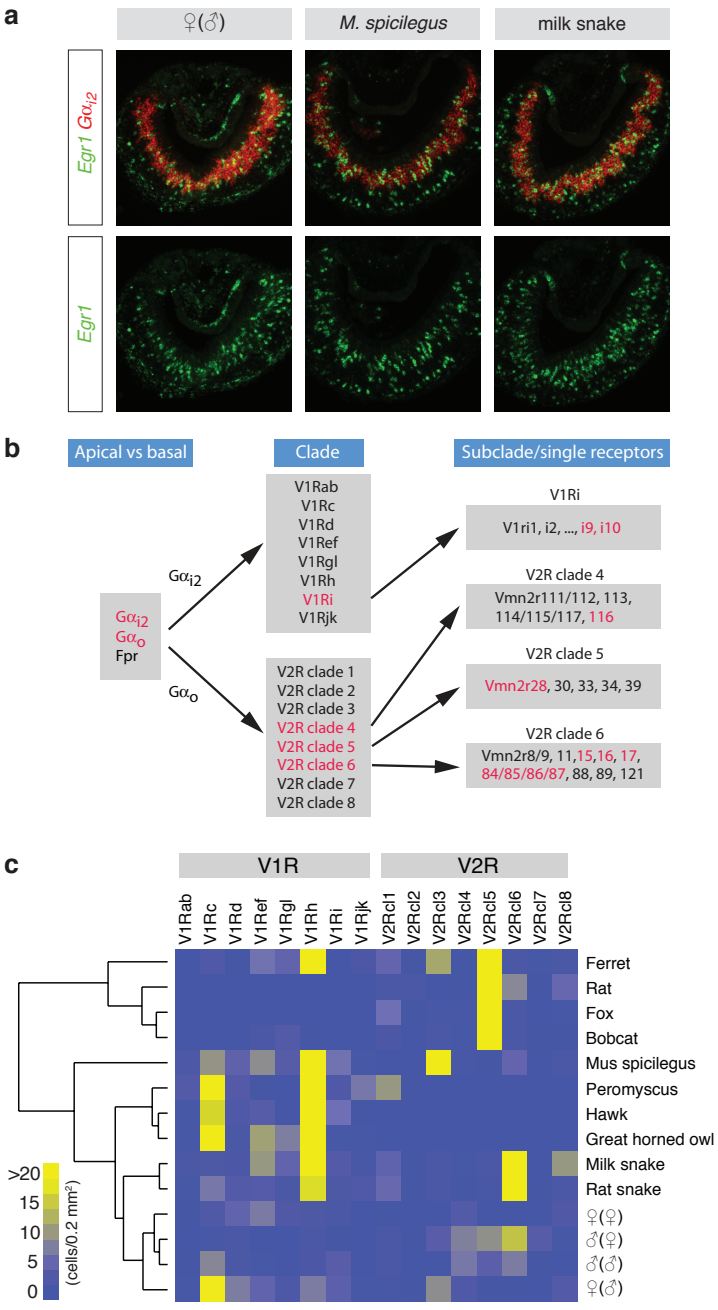
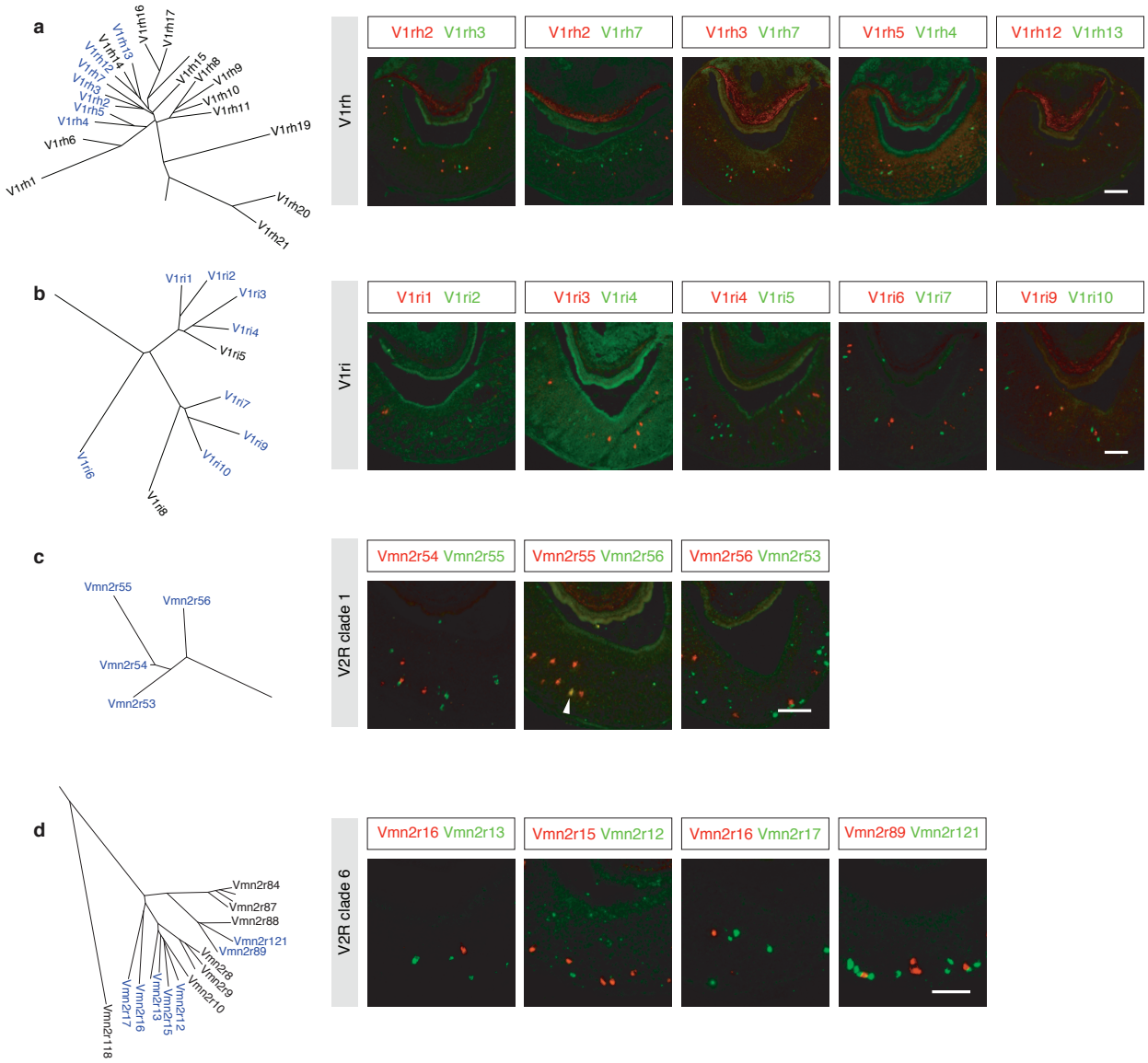


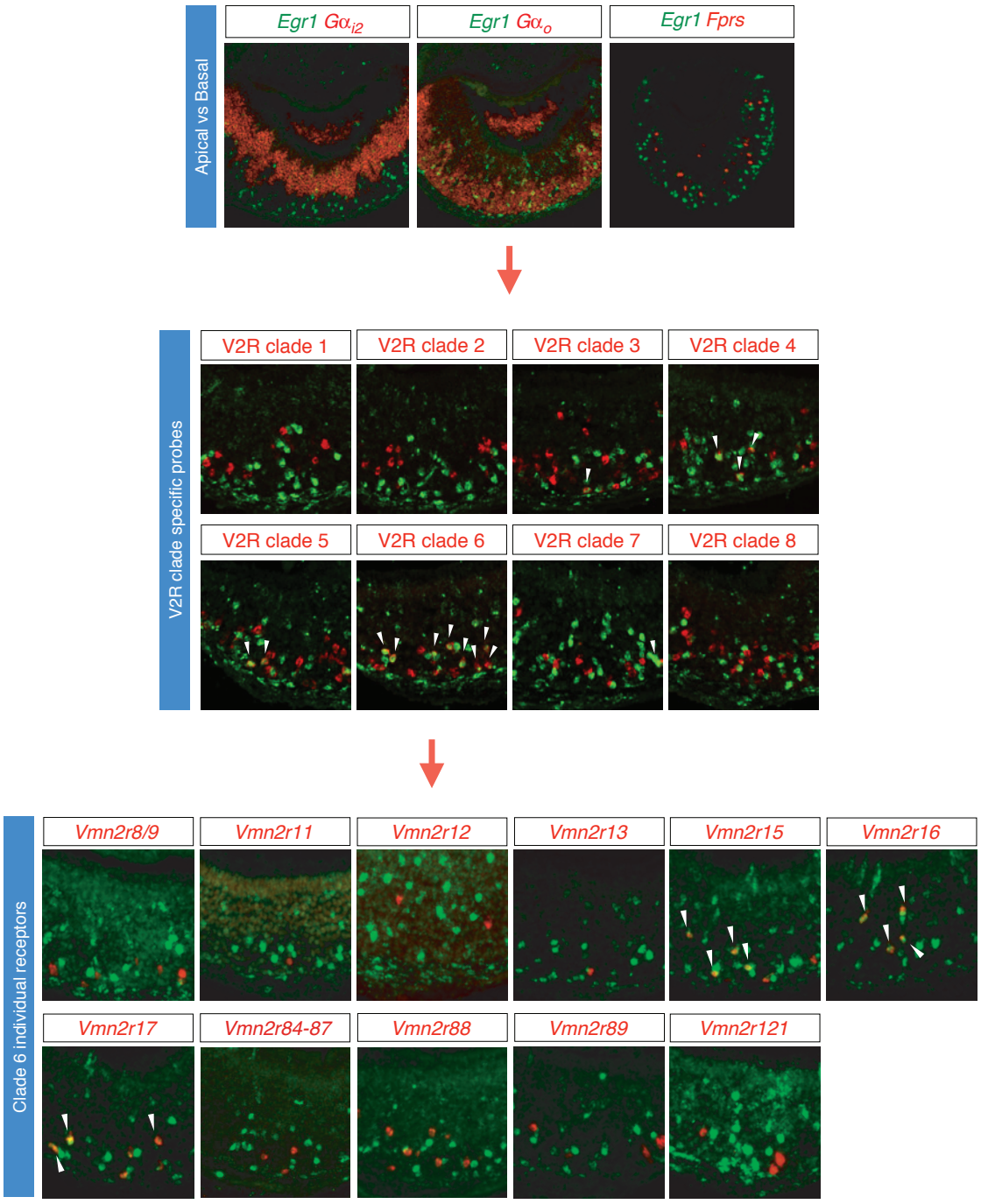
Supplementary figure 1: c-Fos labels a subset of Egr1-positive cells.
a, Double RNA in situ hybridization with probes against c-Fos (red) and Egr1 (green) in a VNO slice from an animal exposed to a mix of heterospecific and conspecific cues. Cells expressing c-Fos (arrowheads) appear all co-labeled with Egr1.
b-c, Linear correlation between c-Fos and Egr1 signal intensity. We measured the intensity of c-Fos and Egr1 hybridization signals in raw images of VNO sections, one VNO slice each for three animals (as marked by circles in **b**). Intensities of c-Fos and Egr1 signals are plotted in **c**. Red dots correspond to cells picked from the c-Fos channel and green dots mark cells selected from the Egr1 channel. Importantly, c-Fos and Egr1 signal intensity exhibited a linear relationship, with all c-Fos positive cells also displaying Egr1 expression. Note that the intensity of Egr1 signals is much higher, thus also offering greater sensitivity over c-Fos expression.



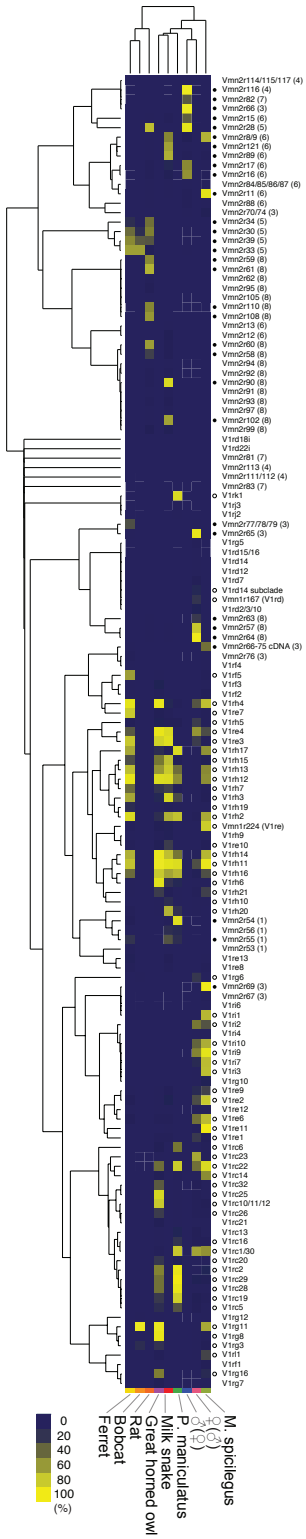
Supplementary figure 2: Mapping vomeronasal receptors expressed in activated VNO neurons.
a, Conspecific and heterospecific cues activate large subpopulations of V1R and V2R expressing neurons. Top panels show double in situ hybridization with probes against $G\alpha_{i2}$ (red), a marker of V1R-expressing neurons, and Egr1 (green). Bottom panels show Egr1 alone. Female CD-1 mice were used for all experiments except where indicated. ♀(♂) represents female cues detected by males. Bar, 100 µm.
b, Schematic of the hierarchical approach used to identify the receptor family, clade and then individual receptor gene expressed by activated VNO neurons, here with the specific example of the detection of male conspecific cues in male mice. **c**, Survey of VR clade activation identifies unique signatures among animal cues. Responses to mammalian predators form a distinct cluster, and involve the common activation of V2Rs from clade 5. Similarly, responses to avian predators, snakes, or mice of both sexes generate distinct clusters of receptor activation, indicating that mice detect unique chemical signatures in each animal group. The heat map represents the number of Egr1/VR double positive cells per 0.2 mm^2.



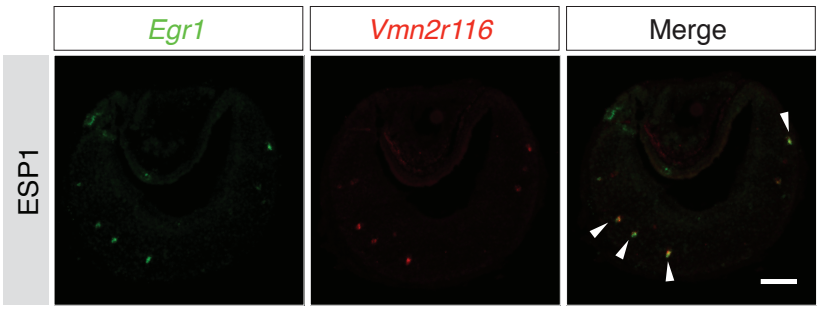
Supplementary figure 3: Specificity of VNO receptor probes.
Dual color in situ hybridization with probes directed at closely related receptors. **a**, discrimination among the V1Rh clade, **b**, V1Ri clade, **c**, V2R clade 1, **d**, V2R clade 6 demonstrate the strict receptor specificity of the designed probes. Receptor genes shown in the figure are indicated in blue. In the vast majority of the sections analyzed, a single vomeronasal receptor gene was found expressed in a given neuron, though rare exceptions were also seen for example in (**c**) (arrow). Bar, 100 µm.



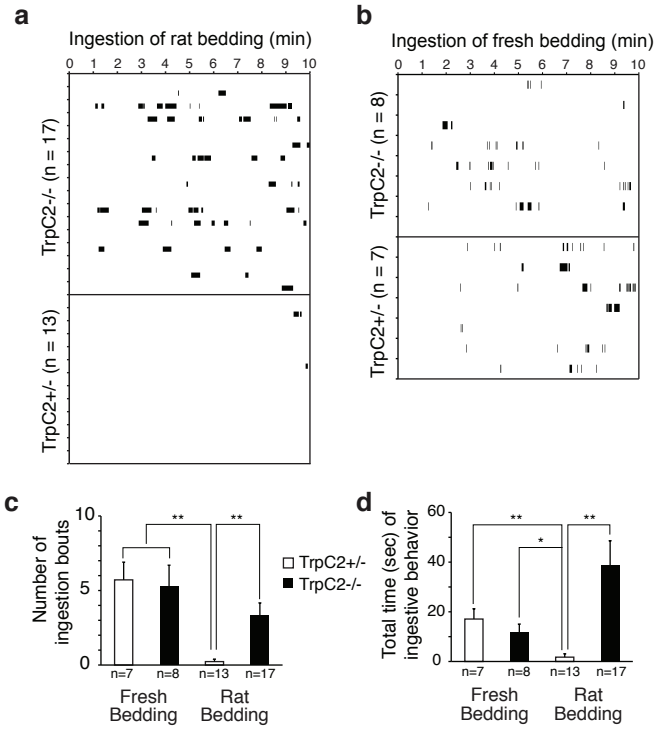
Supplementary figure 4: Hierarchical identification of vomeronasal receptors in females that are activated by male conspecific cues.
Egr1 (green) and cell type specific markers Gαi2, Gαo, and FPR (red) were used to first specify the families of activated receptors among V1Rs, V2Rs and FPRs, respectively. The majority of Egr1 positive cells induced upon male conspecific cue exposure reside in the Gαo-positive zone expressing V2Rs. Subsequently, we used clade- and then specific receptor probes to identify individual VRs expressed in activated neurons. In this example, data are shown that led to the identification of specific receptors to male cues among V2R clade 6. The arrows indicate co-localization between Egr1 and cell-type specific markers.



Supplementary figure 5: Hierarchical clustering of single receptor specificity.
Hierarchical clustering of single receptor specificity.
A co-localization matrix representing each stimulus was analyzed using hierarchical clustering. The clustering of receptors reveals clear segregation corresponding to V1Rs (marked by open circles) or V2Rs (marked by closed circles). The molecular clades for receptors with “Vmn” nomenclature are indicated in parentheses.

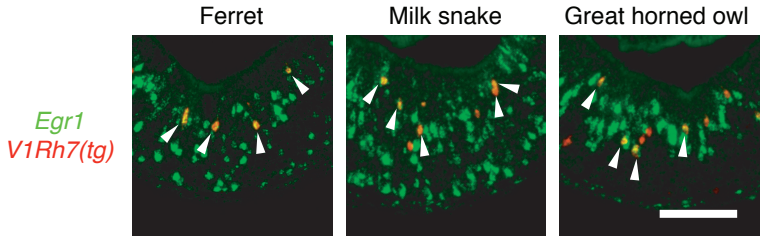


Supplementary figure 6: ESP1 induces Egr1 expression in Vmn2r116/V2Rp5 VSNs.
In situ hybridization detecting Egr1 in a VNO (female CD-1) exposed to recombinant ESP1 peptide. Egr1 is exclusively induced in cells expressing Vmn2r116 (V2Rp5), the specific receptor for ESP1. Bar, 100 µm.

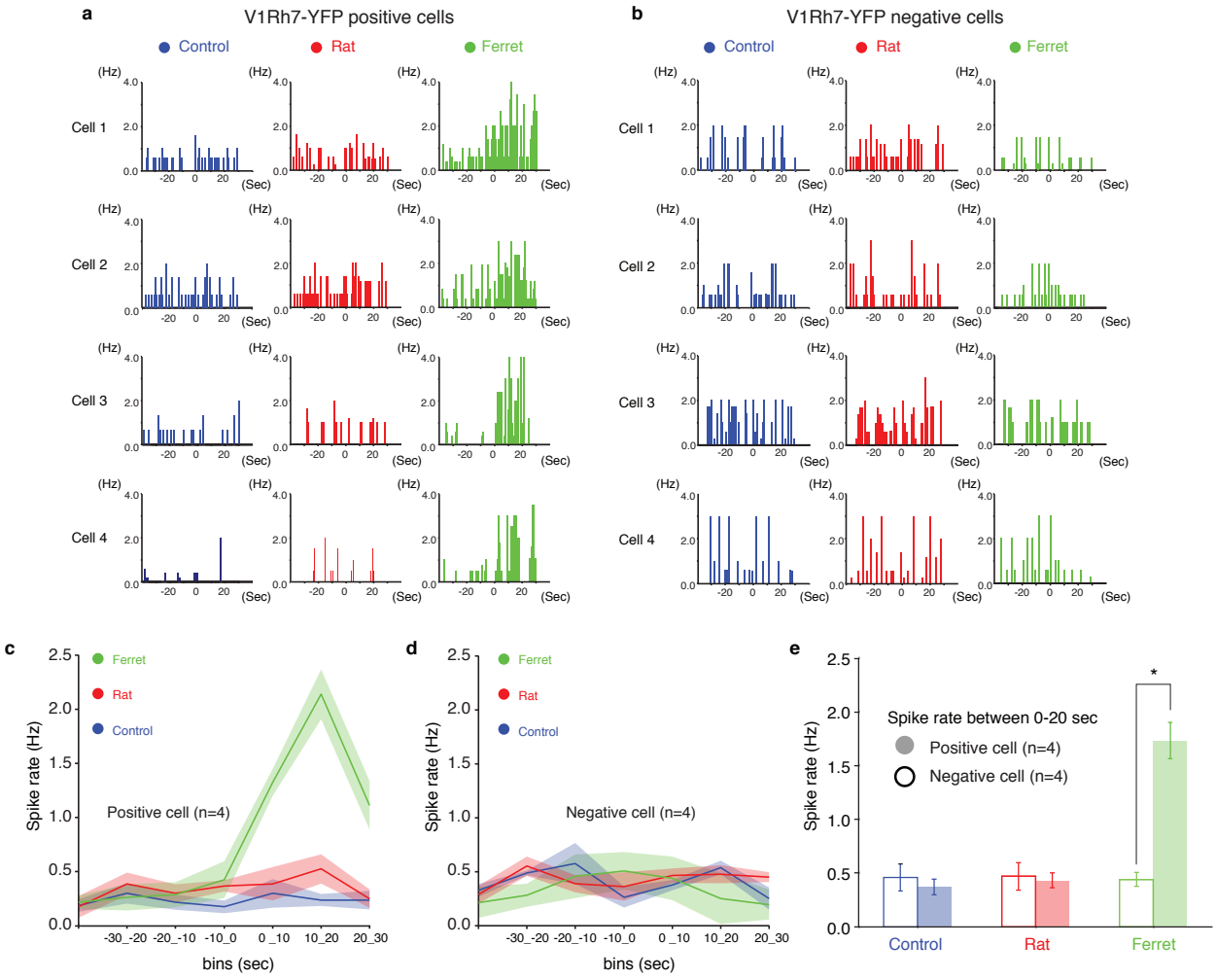


Supplementary figure 7: VNO detection of predator cues suppresses ingestive behavior.

a,b, Raster plots quantifying ingestive behavior in a 10 min experiment. a, ingestion of fresh bedding b, ingestion of rat bedding. Ingestive behavior is frequently observed when fresh bedding (shredded corn cob) is introduced to the home cage. In contrast, ingestive behavior is dramatically suppressed by rat stimuli in wild type, but not animals lacking vomeronasal function (TrpC2-/- mice), indicating that vomeronasal sensory cues are critical for suppression of feeding in the presence of predator cues. **c,d,** Quantitation of ingestive behavior. c, total number of feeding bouts, d, cumulative time of ingestive behavior.
*: $p<0.05$ and **: $p<0.01$ in two-tailed Student's t-test.

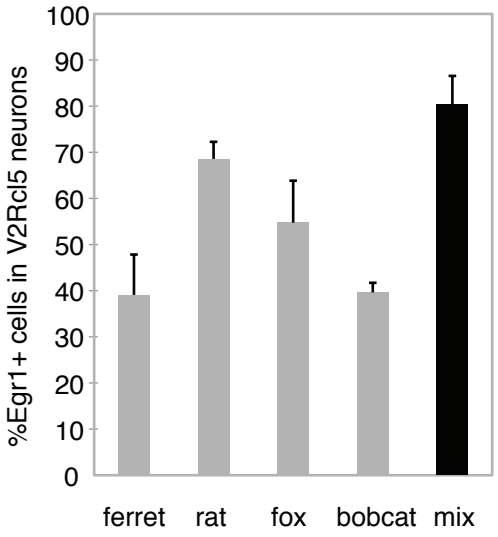


Supplementary figure 8: A transgenic strategy confirmed the specificity of the receptor V1rh7 for predator cues.
Transgenic females expressing the reporter tau-YFP under the control of the V1rh7 locus were exposed to predator cues from ferret, milk snake, or great horned owl. V1rh7 expressing VNO neurons displaying the YFP reporter (red) show Egr1 induction following predator exposure, thus confirming the receptor identification performed by RNA in situ hybridization with a probe directed at V1rh7. Bar, 100 µm.

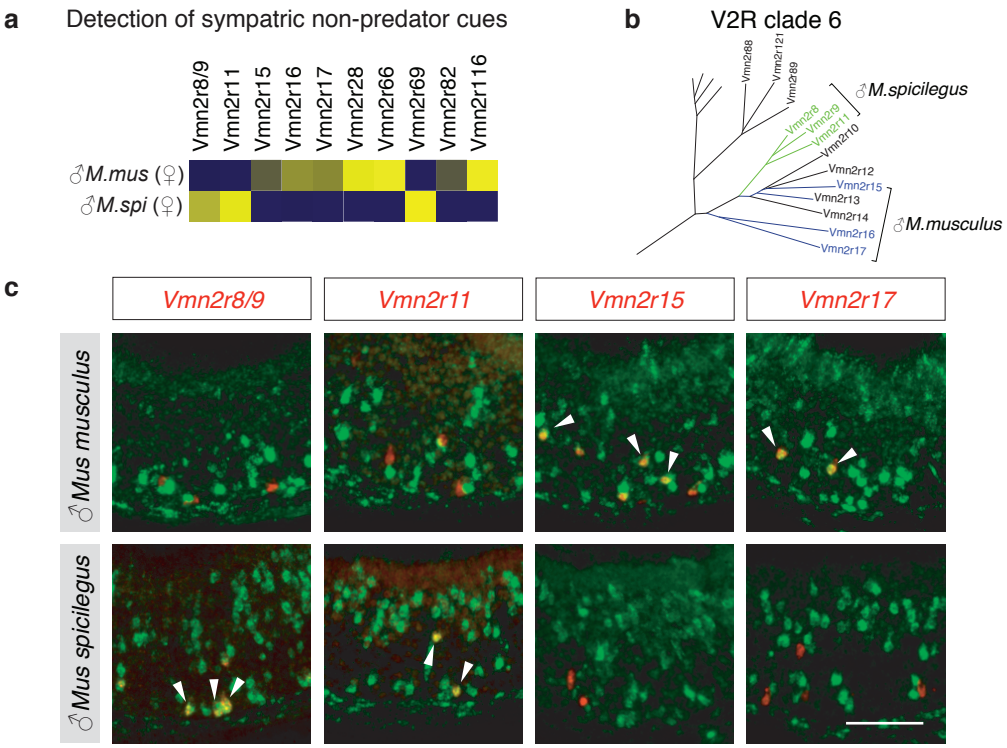


Supplementary figure 9: Response specificity of V1Rh7 neurons analyzed with VNO slices from a V1Rh7-ires-tau-YFP BAC transgenic mouse line.

a, Peristimulus time histograms displayed in 1 sec bins showing the responses of 4 V1Rh7-YFP labeled neurons (5 trials each) under three different stimulus conditions: i) Control buffer (blue), ii) 1:200 dilution of rat urine (red) and iii) ferret stimuli (green). The horizontal axis marks time in sec. The vertical axis marks the frequency in Hz. Data shows selective increase in the firing rate of V1Rh7-YFP positive neurons in response to ferret stimuli. **b**, Data showing the responses of 4 V1Rh7-YFP negative neurons in the same 3 conditions. **c** shows the average temporal response profile of 4 V1Rh7-YFP positive neurons in response to three different stimulus conditions mentioned, similarly **d** shows the same for 4 negative neurons (10 sec bins). The data demonstrates selective increase in the activity of V1R7h-YFP neurons to ferret stimuli. **e**, Bar plots showing the comparison of average spike rate (average for 0-20 sec) of V1Rh7 positive and negative neurons (four positive and four negative) under three different stimulus conditions (time t = 0 is the minimum time required for the 20mM KCl solution to evoke a response in the recorded neuron). Average firing rates for positive cells were 0.368±0.072, 0.420±0.061 and 1.732±0.170 Hz for control, rat, and ferret stimulus, respectively. * indicates $p < 0.05$ in Wilcoxon ranksum test.

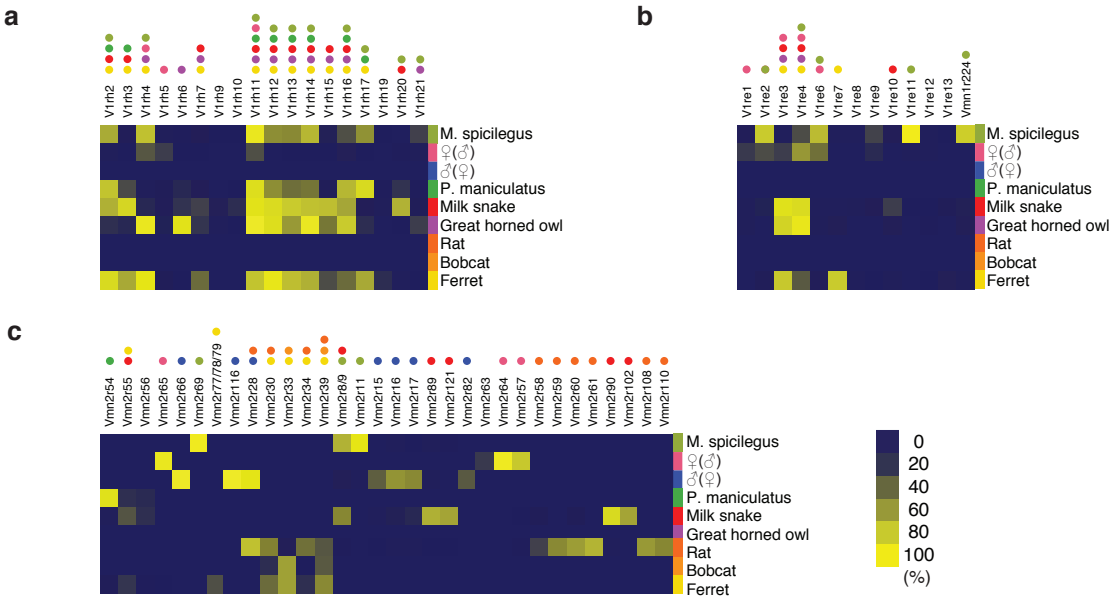


Supplementary figure 10: Vomeronasal neurons activated by distinct mammalian predators partially overlap.
Female mice exposed to four individual mammalian predator cues or to a mix of the four were analyzed for Egr1 induction in V2R clade 5 neurons. Four VNO slices per animal in three animals were analyzed. The exposure of mixed bedding does not dramatically increase the % co-localization from the each individual bedding exposure, indicating that the receptors activated by four mammalian predators largely overlap. Error bars represent s.e.m.



Supplementary figure 11: Receptor repertoires to sympatric non-predator cues.

a, Comparison between V2R receptors that detect *M. musculus* (M. mus) and *M. spicilegus* (M. spi) cues. A heat map represents the co-localization between Egr1 and expression of representative V2R receptor genes (yellow is 100%, blue 0% overlap). **b**, V2R clade 6 contains subsets of receptors detecting conspecific or highly related Mus spicilegus cues. Panels in **c** show in situ hybridization of Egr1 (green) and individual vomeronasal receptors (red), with arrows marking co-localization of Egr1 and receptor signals. Bar, 100 µm.



Supplementary figure 12: Differential specificity of information encoded by V1Rs and V2Rs.

a,b, V1Rs appear to detect chemical cues that are common to multiple animal groups. Heat map of receptor activation by distinct chemosignals (**a**) V1rh receptors (**b**) V1re and V1rf receptors. **c**, In contrast, most V2Rs appear activated by only a single class of animal cues, suggesting that V2Rs can more precisely encode the biological context of the scent than V1Rs.

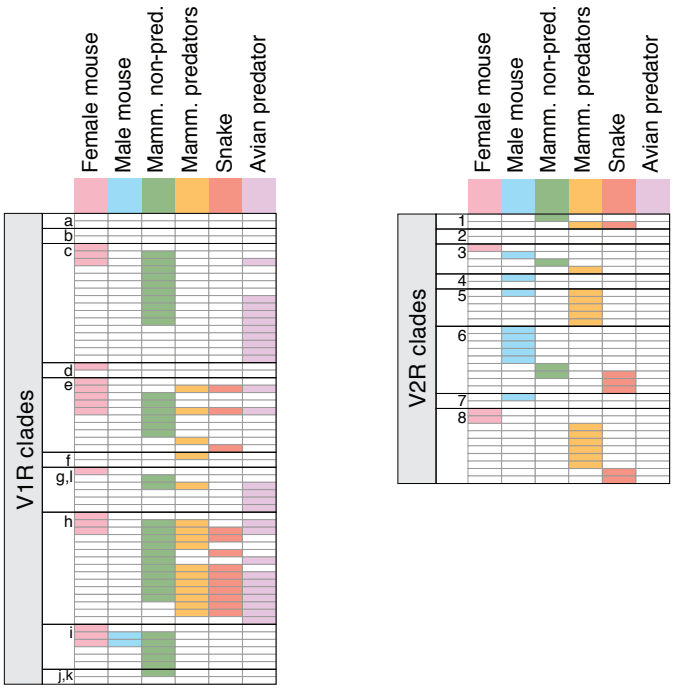
Supplementary table 1: Hierarchical mapping of vomeronasal receptors

The summary of hierarchical mapping conducted in this study is presented in table format.

	Female (in female)	Female (in male)	Male (in female)	Male (in male)	M. spicilegus	P. maniculatus	Ferret	Bobcat	Rat	Fox	Milk snake	Ratsnake	Great horned owl	Hawk
V1Rs, V2Rs, or Fprs														
Gai2	+	+	-	+	+	+	+	+	-	-	+	+	+	+
Gao	+	+	+	+	+	+	+	+	+	+	+	+	-	-
Fpr mix	-	-	-	-	-	-	-	-	-	-	-	-	-	-
V1Rs														
V1Rab Clade	-	-	-	-	-	-	-	-			-	-	-	-
V1Rc Clade	+	+	-	-	+	+	-	-			-	+	+	+
V1rc1/30	-	+			+	+							-	
V1rc2	-	-			-	+							+	
V1rc5	-	-			-	+							-	
V1rc6	-	-			-	+							-	
V1rc10/11/12	-	-			-	-							+	
V1rc13	-	-			-	-							-	
V1rc14	-	-			+	-							-	
V1rc16	-	-			-	+							-	
V1rc19	-	-			-	+							+	
V1rc20	-	-			-	-							+	
V1rc21	-	-			-	-							-	
V1rc22	-	+			+	+							+	
V1rc23	-	+			-	-							-	
V1rc25	-	-			-	-							+	
V1rc26	-	-			-	-							+	
V1rc28	-	-			-	+							+	
V1rc29	-	-			-	+							+	
V1rc32	-	-			-	-							+	
V1Rd Clade	+	+	-	-	-	-	-	-			-	-	-	-
V1rd2/3/10	-	-												
Vmn1r167 subclade*	-	+												
V1rd14 subclade**	-	-												
V1rd7	-	-												
V1rd12	-	-												
V1rd14	-	-												
V1rd15/16	-	-												
V1rd18	-	-												
V1rd22	-	-												
*Vmn1r167 subclade includes Vmn1r167, 169, 170, 171, 175, 176														
**V1rd14 subclade includes V1d13-21														
V1Ref Clade	+	+	-	-	+	-	+	-			+	-	+	-
V1re mix	+	+			+		+				+		+	
V1re1	-	+			-		-				-		-	
V1re2	+	+			+		-				-		-	
V1re3	+	+			-		+				+		+	
V1re4	-	+			+		+				+		+	
V1re6	+	+			+		-				-		-	
V1re7	-	-			-		+				-		-	
V1re8	-	-			-		-				-		-	
V1re9	-	-			+		-				-		-	
V1re10	-	-			-		-				+		-	
V1re11	-	-			+		-				-		-	
V1re12	-	-			-		-				-		-	
V1re13	-	-			-		-				-		-	
Vmn1r224	-	-			+		-				-		-	
V1rf mix	-	-			-		+				-		-	
V1rf1					-		-						-	
V1rf2					-		-						-	
V1rf3					-		-						-	
V1rf4					-		-						-	
V1rf5					-		+						-	
V1Rgl Clade	+	+	-	-	+	-	-	+			-	-	+	-
V1rg3	-	-			-			-					+	
V1rg5	-	-			-			-					-	
V1rg6	+	+			-			-					-	
V1rg7	-	-			-			-					-	
V1rg8	-	-			-			-					+	
V1rg10	-	-			-			-					-	
V1rg11	-	-			+			+					+	
V1rq12	-	-			-			-					-	
Vmn1r77	-	-			-			-					+	
V1rl	-	-			+			-					-	
V1Rh Clade	-	+	-	-	+	+	+	-			+	+	+	+
V1rh2	-	-			+	+	+				+		-	
V1rh3	-	-			-	+	+				+		-	
V1rh4	-	+			+	-	+				-		+	
V1rh5	-	+			-	-	-				-		-	
V1rh6	-	-			-	-	-				-		+	
V1rh7	-	-			-	-	+				+		+	
V1rh9	-	-			-	-	-				-		-	
V1rh10	-	-			-	-	-				-		-	
V1rh11	-	+			+	+	+				+		+	
V1rh12	-	-			+	+	+				+		+	
V1rh13	-	-			+	+	+				+		+	
V1rh14	-	-			+	+	+				+		+	
V1rh15	-	-			-	-	+				+		+	
V1rh16	-	-			+	+	+				+		+	
V1rh17	-	-			+	+	+				-		-	
V1rh19	-	-			-	-	-				-		-	
V1rh20	-	-			-	+	-				+		-	
V1rh21	-	-			+	-	-				-		+	
V1Ri Clade	+	+	-	+	+	-	-	-			-	-	-	+
V1ri1	-	-		-	+									
V1ri2	-	+		-	-									
V1ri3	-	-		-	+									
V1ri4	-	-		-	-									
V1ri6	-	-		-	-									
V1ri7	-	-		-	+									
V1ri9	+	+		+	+									
V1ri10	+	+		+	+									
V1Rjk Clade	-	-	-	-	-	+	-	-			-	-	-	-
V1rj2						-								
V1rj3						-								
V1rk1						+								

Supplementary table 1: Hierarchical mapping of vomeronasal receptors
The summary of hierarchical mapping conducted in this study is presented in table format.

	Female (in female)	Female (in male)	Male (in female)	Male (in male)	M. spicilegus	P. maniculatus	Ferret	Bobcat	Rat	Fox	Milk snake	Ratsnake	Great horned owl	Hawk
V2Rs														
V2R Clade 1	-	-	-	-	-	+	+	-	-	+	+	+		
Vmn2r53						-	-				-			
Vmn2r54						+	-				-			
Vmn2r55						-	+				+			
Vmn2r56						-	-				-			
V2R Clade 2	-	-	-	-	-	-	-	-	-	-	-	-		
V2R Clade 3	-	+	+	-	+	-	+	-	-	-	-	-		
Vmn2r65		+	-		-		-							
Vmn2r66			+		-									
Vmn2r67			-		-									
Vmn2r69			-		+									
Vmn2r70/74			-		-									
Vmn2r76		-	-		-		-							
Vmn2r77/78/79		-	-		-		+							
V2R Clade 4	-	-	+	+	-	-	-	-	-	-	-	-		
Vmn2r111/112			-	-										
Vmn2r113			-	-										
Vmn2r114/115/117			-	-										
Vmn2r116			+	+										
V2R Clade 5	-	-	+	+	-	-	+	+	+	+	-	-		
Vmn2r28			+	+			-	-	+					
Vmn2r30*			-	-			+	-	+					
Vmn2r33*			-	-			+	+	-					
Vmn2r34*			-	-			-	-	+					
Vmn2r39*			-	-			+	+	+					
* These probes are expected to recognize more than one receptor, but to label largely non-overlapping populations of the V2R Clade 5														
V2R Clade 6	-	-	+	+	+	-	-	-	+	-	+	+		
Vmn2r8/9			-	-	+				-		+			
Vmn2r11			-	-	+				-		-			
Vmn2r12			-	-	-				-		-			
Vmn2r13			-	-	-				-		-			
Vmn2r15			+	+	-				-		-			
Vmn2r16			+	+	-				-		-			
Vmn2r17			+	+	-				-		-			
Vmn2r84/85/86/87			-	+	-				-		-			
Vmn2r88			-	+	-				-		-			
Vmn2r89			-	-	-				-		+	+		
Vmn2r121			-	-	-				-		+	+		
V2R Clade 7	-	-	+	-	-	-	-	-	-	-	-	-		
Vmn2r80/81/82			-											
Vmn2r81			-											
Vmn2r82			+											
Vmn2r83			-											
V2R Clade 8	-	+	-	-	-	-	-	-	+	-	+	-		
V2Rcl8 subclade 1 (Vmn2r57-90)		+							-		+			
Vmn2r63		-							-		-			
Vmn2r64		+							-		-			
Vmn2r57		+							-		-			
Vmn2r58									+		-			
Vmn2r59									+		-			
Vmn2r60									+		-			
Vmn2r61									+		-			
Vmn2r62									-		-			
Vmn2r90									-		+			
V2Rcl8 subclade 2 (Vmn2r91-110)		-							+		+			
Vmn2r91									-		-			
Vmn2r92									-		-			
Vmn2r93									-		-			
Vmn2r94									-		-			
Vmn2r95									-		-			
Vmn2r97									-		-			
Vmn2r99									-		-			
Vmn2r102									-		+			
V2Rcl8 subclade 2-2 (Vmn2r105-110)									+		-			
Vmn2r105									-					
Vmn2r108									+					
Vmn2r110									+					



Supplementary table 2: Graphical representation of vomeronasal receptor function

A summary of results presented in the supplementary table 1 was rendered graphically. We classified observed responses according to six different types of stimuli: conspecific female, conspecific male, mammalian non-predator (Peromyscus and Mus spicilegus), mammalian predator (rat, fox, ferret, and bobcat), snake, and avian predator. Each horizontal bar corresponds to a single receptor (or a group of receptors detected by a single probe). To highlight the specificity of receptors identified in this study, we omitted uncharacterized receptors in this table.