Supplementary Figure 1. Individual experimental mouse data for rabies virus-based monosynaptic retrograde tracing.

(A,**B)** Monosynaptic retrograde tracing data for five Vip-IRES-Cre mice (**A**, green) and four Ndnf-IRES-Cre (**B**, magenta). **Top.** Distribution of starter cells across cortical regions. **Middle**. Distribution of starter cells across cortical depth. Numbers indicate the total number of starter cells for each mouse. **Bottom**. Distribution of presynaptic RV-nEGFP$^+$ cells across auditory thalamic regions. Numbers indicate the total number of presynaptic cells within auditory thalamic regions for each mouse.

A1: primary auditory cortex; AuD: auditory dorsal area; AuV: auditory ventral area; S1: primary somatosensory cortex; S1BF: primary somatosensory cortex, barrel field; S1ULp: primary somatosensory cortex, upper lip region; S2: secondary somatosensory cortex; TeA: temporal association cortex; V2La: secondary visual cortex, lateral area, anterior region; V2Li: secondary visual cortex, lateral area, intermediate region; MGBv: ventral division of the medial geniculate body; MGBm: medial division of the medial geniculate body; MGBd: dorsal division of the medial geniculate body; MZMG: marginal zone of the medial geniculate body; PIN: posterior intralaminar nucleus; PP: peripeduncular nucleus.

Supplementary Figure 2. Rabies virus-based monosynaptic retrograde tracing validation.

(A) Schematic of the approach to test the specificity of our starter cell populations for the monosynaptic retrograde tracing experiments. Vip/Ndnf-WT mice were injected with the same Cre-dependent viruses (TVA-mCherry and RVG) and RV-nEGFP used in the experimental mice, following the same procedure. This control was carried out in two Vip- and two Ndnf-WT mice.

(B) Example confocal images of ACx regions of Vip-WT and Ndnf-WT mice, injected with Cre-dependent TVA-mCherry, Cre-dependent RVG, and RV-nEGFP. No starter cells were found in the ACx of Vip/Ndnf-WT, confirming the specificity of our Cre-dependent virus expressing TVA-mCherry. Scale bar: 50 µm.

(C) Example epifluorescence images of auditory thalamic sections showing no RV-nEGFP$^+$ neurons, consistent with the absence of starter cells in ACx. Calretinin immunostaining delineates the higher-order thalamic areas.

(D) Schematic of the experimental approach to test for viral leak expression of RV-nEGFP. Vip- and Ndnf-IRES-Cre mice were injected with the Cre-dependent AAV expressing TVA-mCherry, omitting the

injection of the Cre-dependent RVG required for monosynaptic retrograde infection. Subsequently, RV-nEGFP was injected following the same protocol employed in the experimental mice.

(E) Example confocal images of ACx regions of Vip-IRES-Cre and Ndnf-IRES-Cre mice, injected with TVA-mCherry and RV-nEGFP. White arrows indicate representative starter cells in ACx, identified by the co-localization of mCherry and EGFP. Scale bar: 50 µm.

(F) Example epifluorescence images of brain sections containing the auditory thalamic regions showing no RV-nEGFP^{+} neurons despite the presence of starter cells in ACx. Calretinin immunostaining delineates the higher-order auditory thalamus.

(G) Distribution of starter cells across cortical depth in Vip/Ndnf-IRES-Cre control mice. Inset indicates the total number of starter cells in the cortex. Data corresponds to two mice of each genotype.

(H) Distribution of starter cells in individual Vip-IRES-Cre (green) and Ndnf-IRES-Cre (magenta) mice used as controls to test for viral leak expression of RV-nEGFP. Inset indicates the total number of starter cells in each mouse.