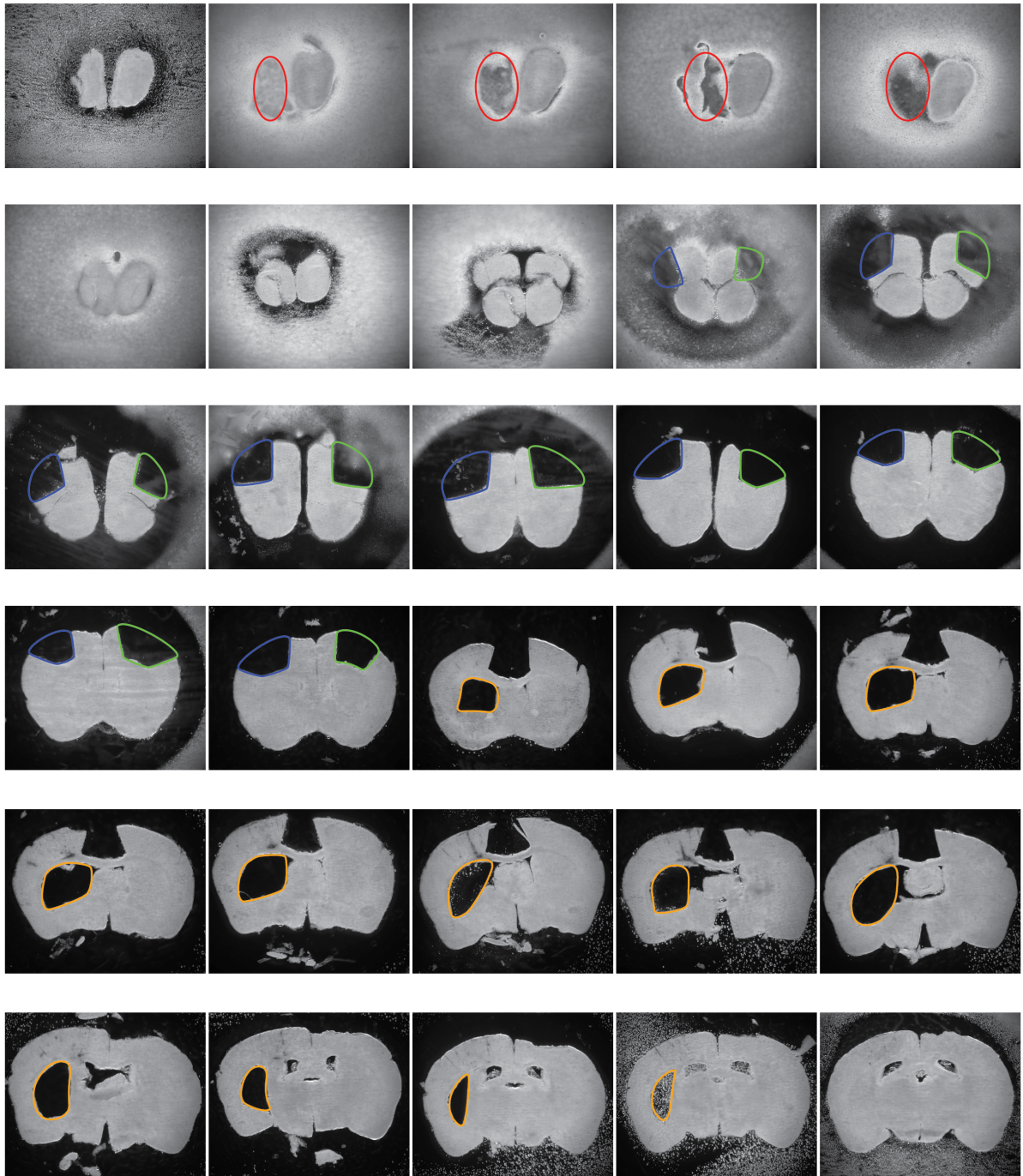
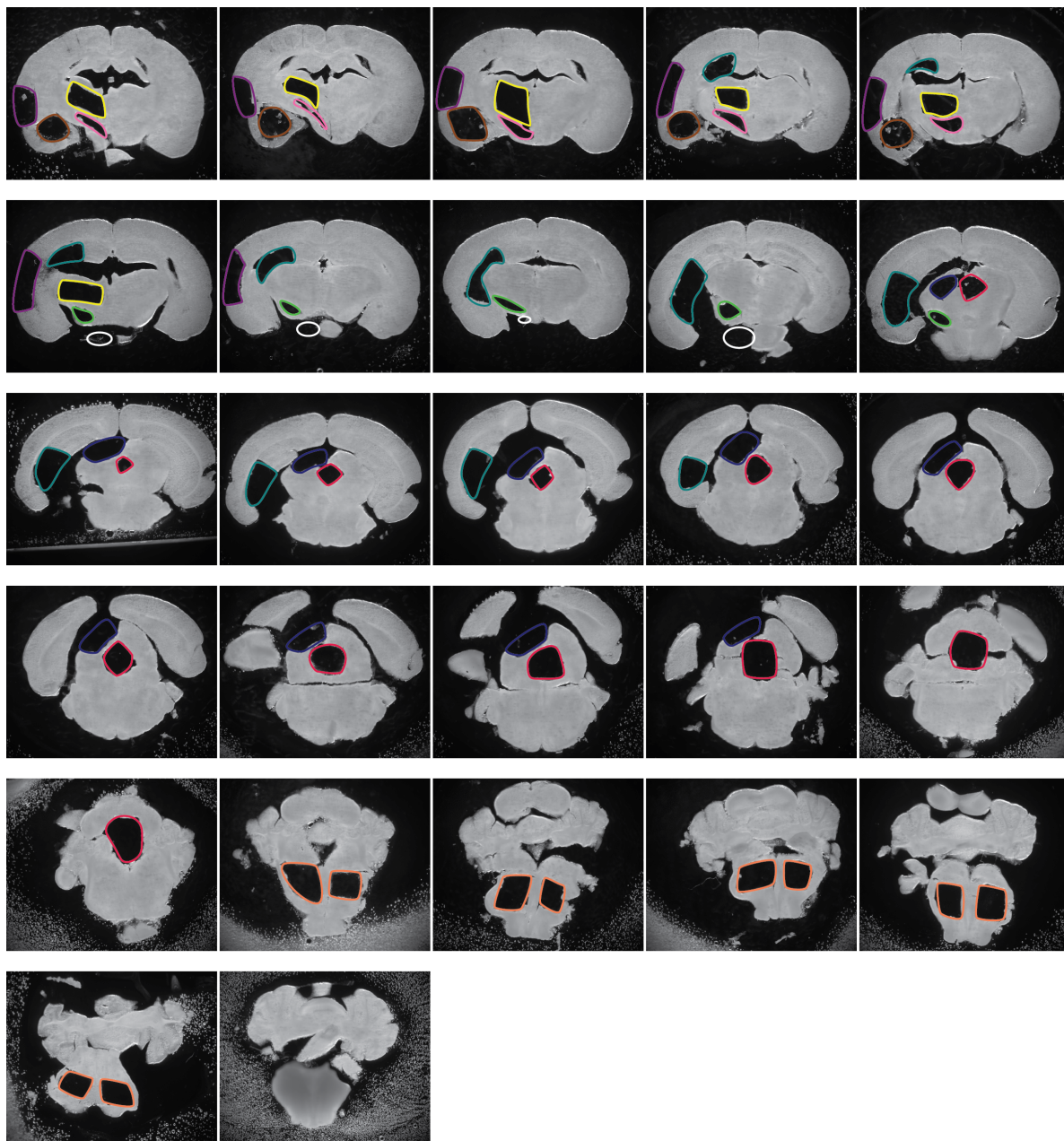

Supplementary information

**Specific expansion of motor cortical
projections in a singing mouse**

In the format provided by the
authors and unedited

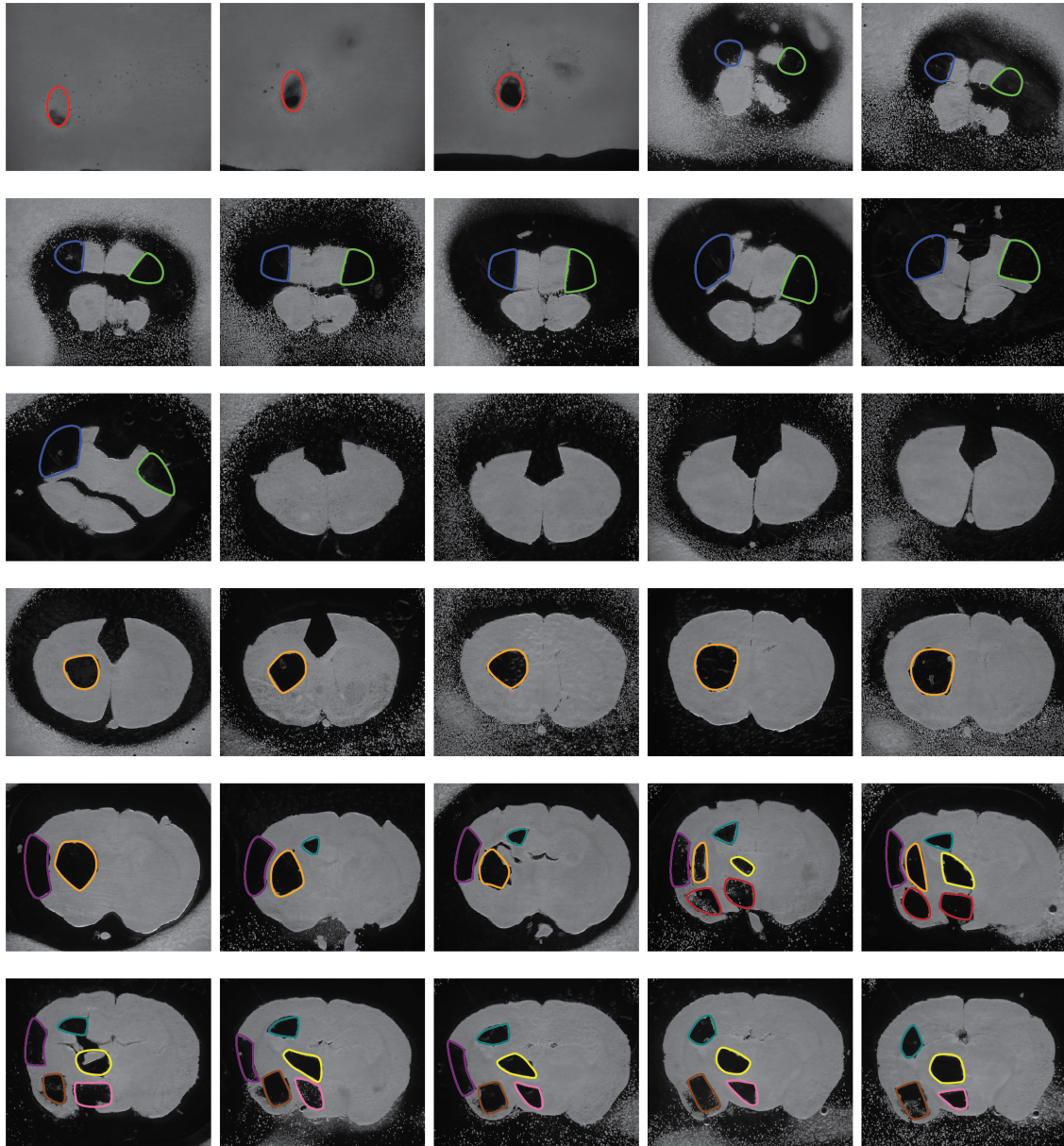


OB OMCi OMCc STR

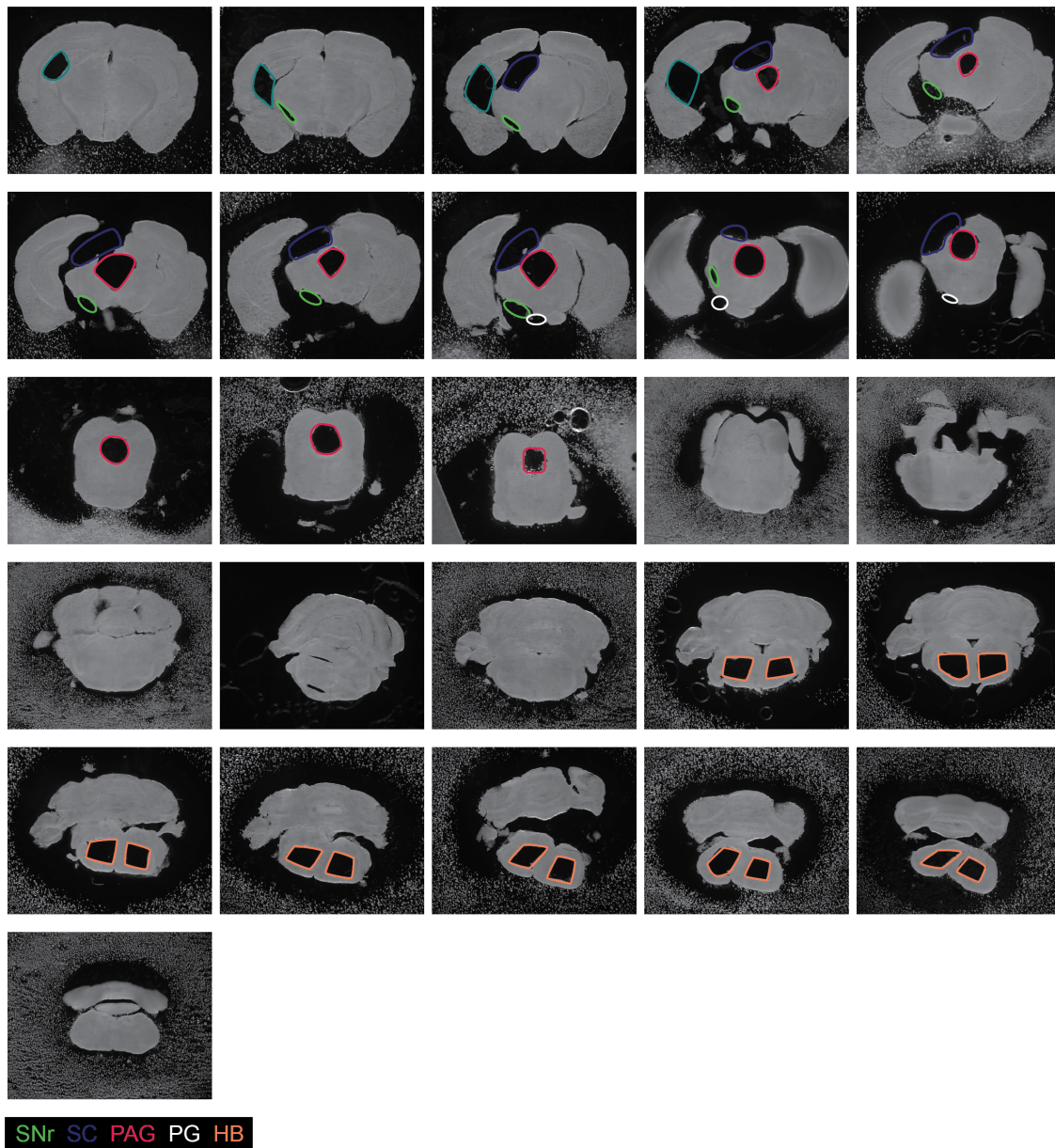


AudR TH HY HIP AMY SNr SC PAG PG HB

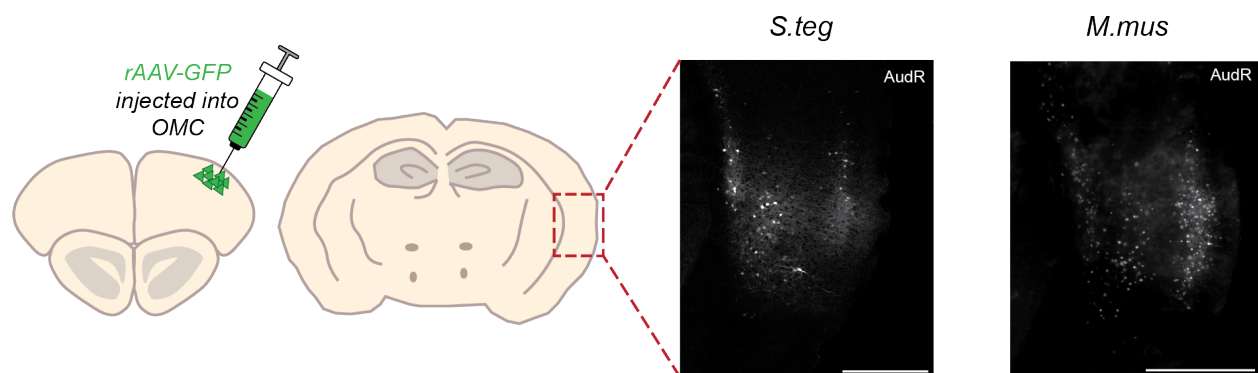
Supplementary Figure 1: **MAPseq dissections of a lab mouse.** Brightfield images of 200µm brain slices from a representative lab mouse. Outlined regions correspond to regions dissected, processed, and analyzed in MAPseq experiments. Each region is marked by a unique color. Region distortions are due to tissue displacement during dissection. Abbreviations: amygdala (AMY), auditory region (AudR), hindbrain (HB), hippocampus (HIP), hypothalamus (HY), olfactory bulb (OB), contralateral orofacial motor cortex (OMC_c), ipsilateral orofacial motor cortex (OMC_i), periaqueductal gray (PAG), pontine gray (PG), superior colliculus motor (SC_m), substantia nigra (SNr), striatum (STR), thalamus (TH).



OB OMCi OMCc STR AudR TH HY HIP AMY



Supplementary Figure 2: **MAPseq dissections of a singing mouse.** Brightfield images of 200µm brain slices from a representative singing mouse. Outlined regions correspond to regions dissected, processed, and analyzed in MAPseq experiments. Each region is marked by a unique color. Region distortions are due to tissue displacement during dissection. Abbreviations: amygdala (AMY), auditory region (AudR), hindbrain (HB), hippocampus (HIP), hypothalamus (HY), olfactory bulb (OB), contralateral orofacial motor cortex (OMC_c), ipsilateral orofacial motor cortex (OMC_i), periaqueductal gray (PAG), pontine gray (PG), superior colliculus motor (SC_m), substantia nigra (SNr), striatum (STR), thalamus (TH).



Supplementary Figure 3: **Retrograde viral tracing of OMC reveals synaptic inputs from AudR.** Schematic of retro-AAV carrying GFP injected in the OMC (*left*) and schematic of AudR (*middle left*). Two photon imaging of the AudR showing neurons projecting to OMC in singing mouse (*middle right*). Fluorescence microscopy imaging of the AudR showing neurons projecting to OMC in lab mouse (*right*). Scale bars represent 500 microns. Results shown here were replicated across 2 singing and 2 lab mice.

Table 1: Viruses used for bulk fluorescent tracing. Addgene and serotype information of AAVs used for bulk viral tracing experiments.

Virus Name	Addgene	Serotype	Experiment(s)
CamKII 0.4.Cre.SV40	105558	AAV9	OMC projection, Synaptic boutons
FLEX-tdTomato	28306	AAV9	OMC projection, trans-synaptic PAG
rAAV-hsyn-GFP	50465	AAV-rg	Singing mouse OMC retrograde
CAG-NLS-GFP	104061	AAV-rg	Lab mouse OMC retrograde
hSyn-Cre-WPRE	105553	AAV1	Trans-synaptic AudR and PAG
hSyn-Flex-mGFP-2A-synaptophysin-mRuby	71760	AAV1	Synaptic boutons
hSyn-DIO-mCherry	50459	AAV9	Trans-synaptic AudR
SynP-DIO-EGFP-WPRE-hGH	100043	AAV9	Trans-synaptic AudR
hSyn-FLEX-TeLC-P2A-EYFP-WPRE	135391	AAVDJ	Trans-synaptic PAG