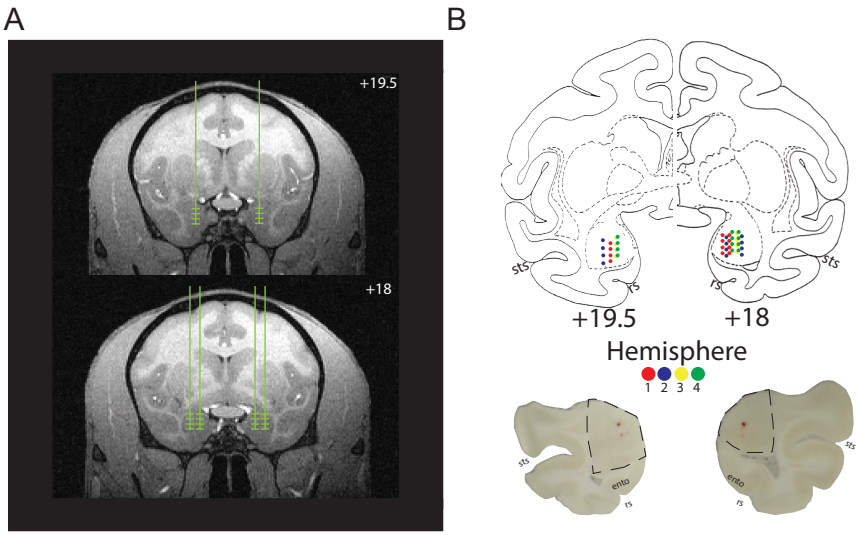
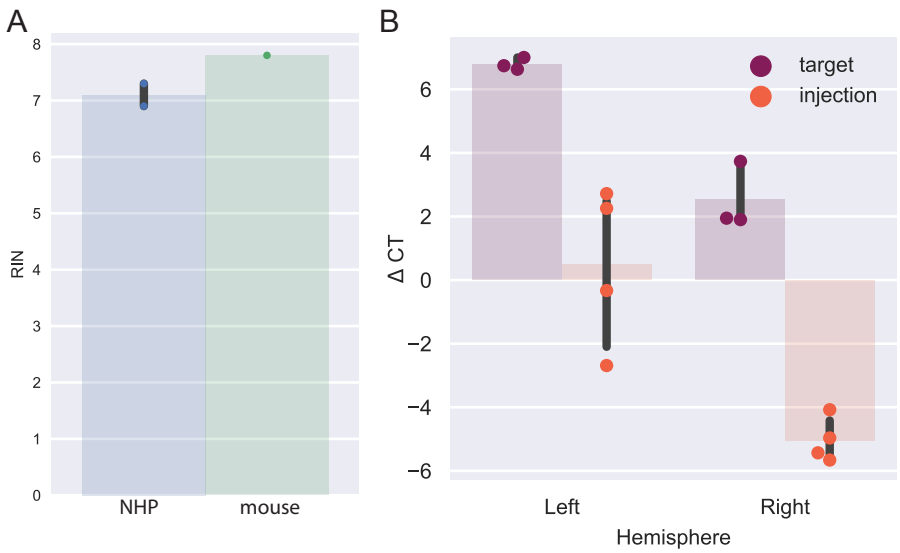
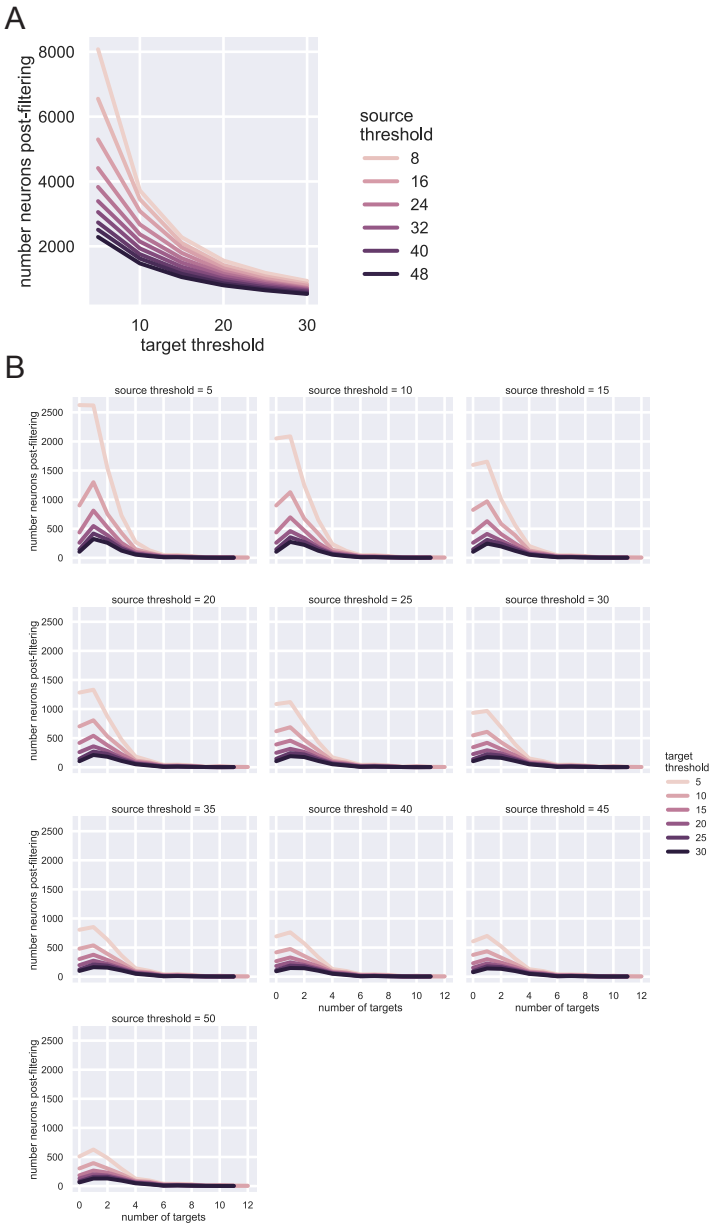


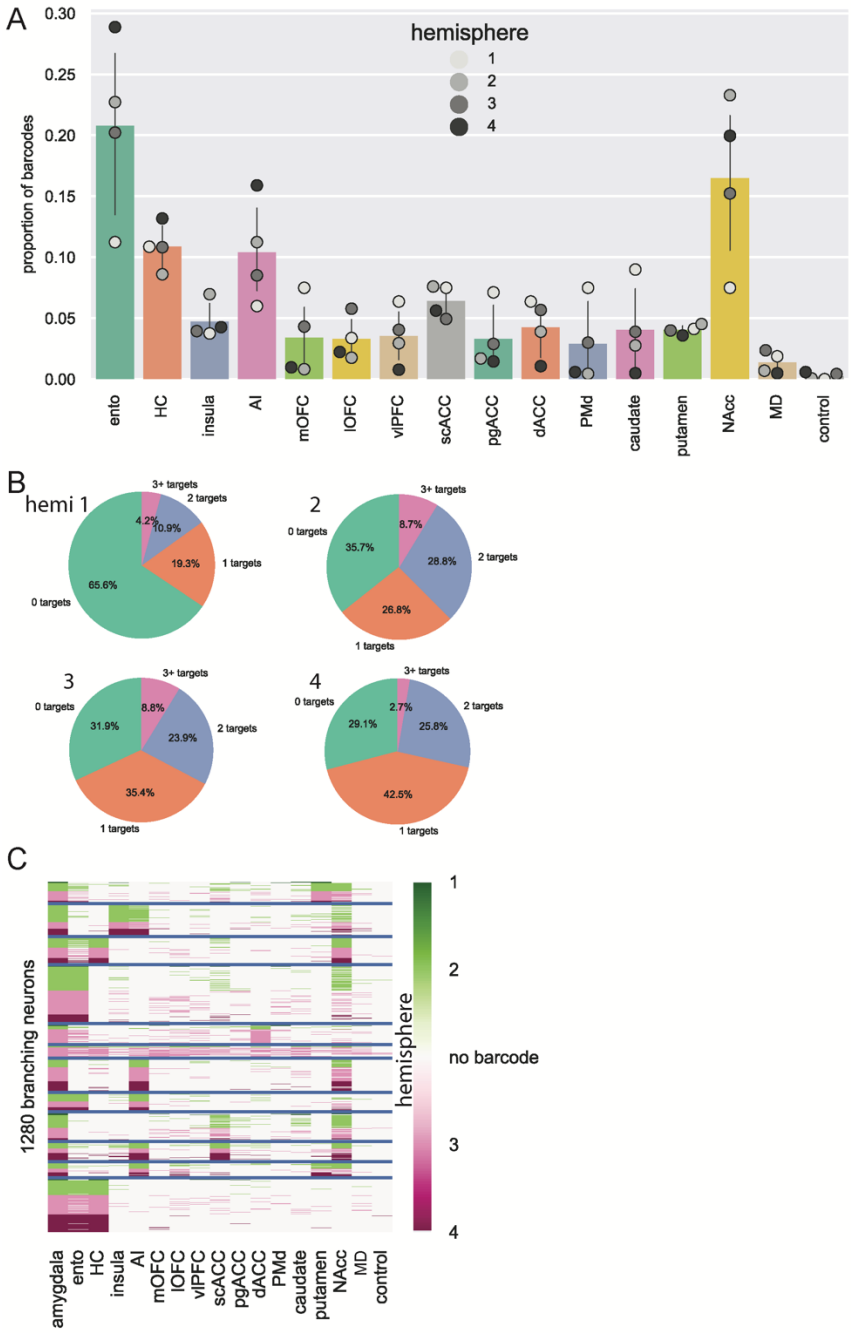
Supplemental Figure 1: Anatomical verification. Related to Figure 1. **A**) Representative MRI images showing anterior (top) and middle (bottom) injection targets within amygdala. Vertical lines indicate intended injection tracks, while horizontal lines indicate injection depths along those tracks. Intraaural distance is indicated in mm. **B**) Locations of injections for individual animals (colors); anterior injection on the left (one track in M/L plane per animal), middle on the right (two tracks in M/L plane per animal). Example tissue sections shown below with the extent of the amygdala surrounded by the dotted line; injection tracks are marked by light blood spots on the sections (ento refers to entorhinal cortex, rs rhinal sulcus, sts superior temporal sulcus).



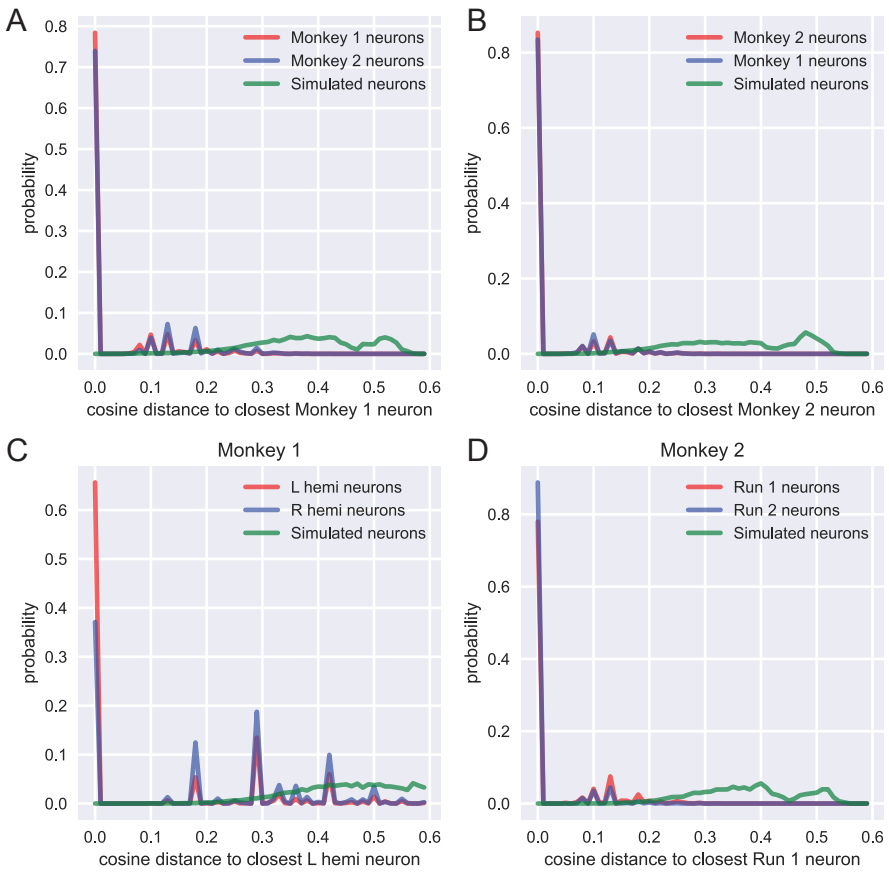
Supplemental Figure 2: Optimization of MAPseq for use in macaques. Related to Figure 1. **A)** RNA Integrity Number (RIN) values from fixed non-human primate (NHP) tissue (blue, left) are comparable to fixed samples from mouse brains also injected with sindbis virus for MAPseq (green, right). **B)** ΔCT (difference in cycle threshold between barcode and housekeeping gene actin; negative values indicate more barcode than actin) values from qPCR indicate that sindbis virus for MAPseq is indeed producing robust barcode expression in macaque neurons. A difference in ΔCT of about 3 between target and injection indicates approximately 10x more barcode in injection sites; both hemispheres here are well above that threshold. Injection samples for the ΔCT analysis were the three or four amygdala samples in which we could see visible needle tracks. Target samples were three or four mid-NAcc samples, as we wanted to assess the amount of barcode transported to one of the strongest projection targets.



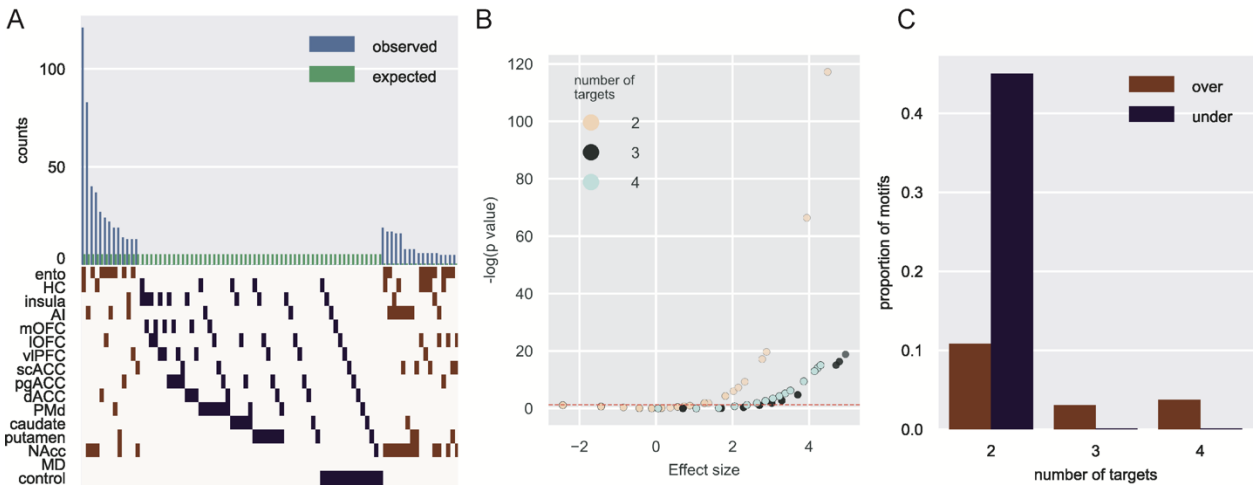
Supplemental Figure 3. Filtering parameters do not dramatically change recovered barcodes. Related to Figure 1. **A**) Number of barcodes surviving filtering for different source thresholds (color of line) and target thresholds (x-axis). Sufficient source threshold eliminates majority of noise. **B**) Effect of thresholding on number of projection targets per neuron. Each plot is one source threshold, while colored lines reflect different target thresholds. The shape of the distributions is lightly flattened by increasing projection threshold, while again, source threshold is responsible for most of the noise.



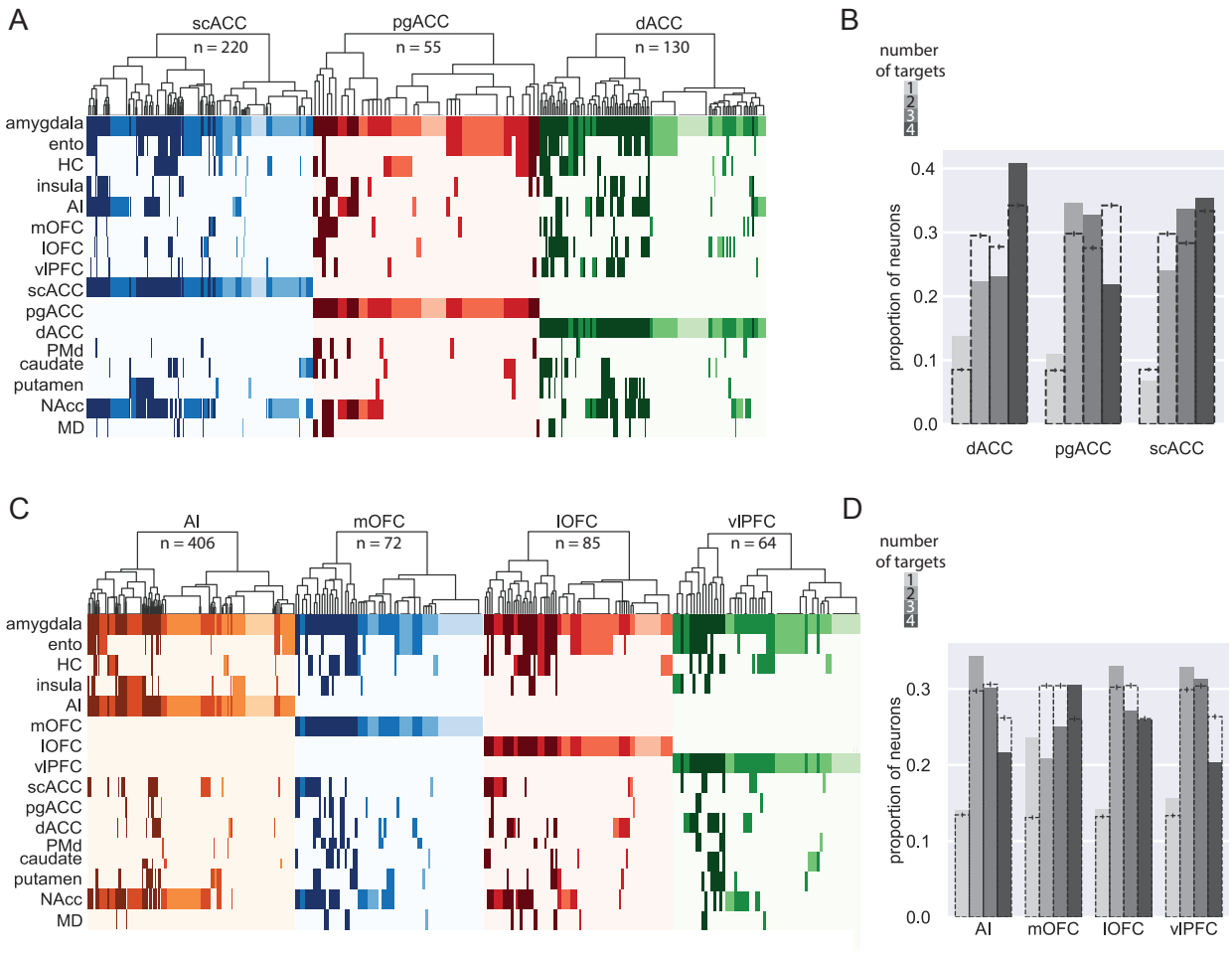
Supplemental Figure 4: MAPseq is consistent across animals. Related to Figure 1. Hemispheres 1 and 2 are from one animal, and hemispheres 3 and 4 from the other. **A**) Overall barcode distribution across areas. Colored bars represent the mean across all 4 hemispheres sequenced, error bars are standard deviation, and individual points reflect counts within each hemisphere's data separately. **B**) Number of targets for each neuron across hemispheres – roughly equal proportions of 1-target and 2+-target neurons, with most variance observed in proportion of 0-target neurons. **C**) K-means clustered projection patterns of neurons which branch to multiple targets, labelled by hemisphere. Note that most clusters are comprised of neurons from multiple hemispheres.



Supplemental Figure 5: Comparison across hemispheres. Related to Figure 1. Density plot of cosine distance between two actual samples (red/blue) and simulated neurons from a uniform distribution (green). **A**) Monkey 1 neurons (both hemispheres) as a basis are more similar to monkey 2's neurons (Kolmogorov-Smirnov test, D = 0.12, p = 0.81) than the simulated neurons (D = 0.52, p < 0.0001. **B**) Same for monkey 2 as a basis. **C**) Within monkey 1, the two hemispheres are more similar to each other (D = 0.18, p = 0.27) than the random neurons (D = 0.43, p < 0.0001). **D**) Within monkey 2, the two sequencing runs were more similar to each other (D = 0.22, p = 0.12) than the simulated neurons (D = 0.48, p < 0.0001).



Supplemental Figure 6: Over- and under-represented branching motifs compared to a uniform null distribution. Related to Figure 2. **A)** Observed (blue) and expected (green) counts of neurons with projections to multiple areas (top). Specific over (red) or under-represented (blue) branching motifs by area (bottom). **B)** Volcano plot of probability of all possible branching motifs with 2 (cream), 3 (grey), 1and 4 (turquoise) target areas. Positive effect size indicates over-representation compared to the null distribution and negative effect size indicates under-representation. The red dashed line marks the level of statistical significance such that any points above it are significantly over- or under-represented after FDR correction. **C)** Proportion of significantly over- (red) and under- (blue) represented 2-, 3- and 4- target area branching motifs.



Supplemental Figure 7: Single-neurons and branching degrees for ventral- and medial frontal cortex-projecting neurons. Related to Figures 3 and 4. **A)** Single-neuron projection patterns for non-overlapping populations of neurons targeting either scACC (blue), pgACC (red), or dACC (green). **B)** Degrees of branching for each medial frontal cortex-projecting population; dashed bars indicate the mean of 1000 shuffles of the data, downsampled for equal numbers of neurons from each population; error bars indicate 95% confidence intervals around the mean of the shuffles. **C)** Single-neuron projection patterns for non-overlapping populations of neurons targeting either AI (yellow), mOFC (blue), lOFC (red), or vlPFC (green). **D)** Same as B, for orbital-projecting neurons.