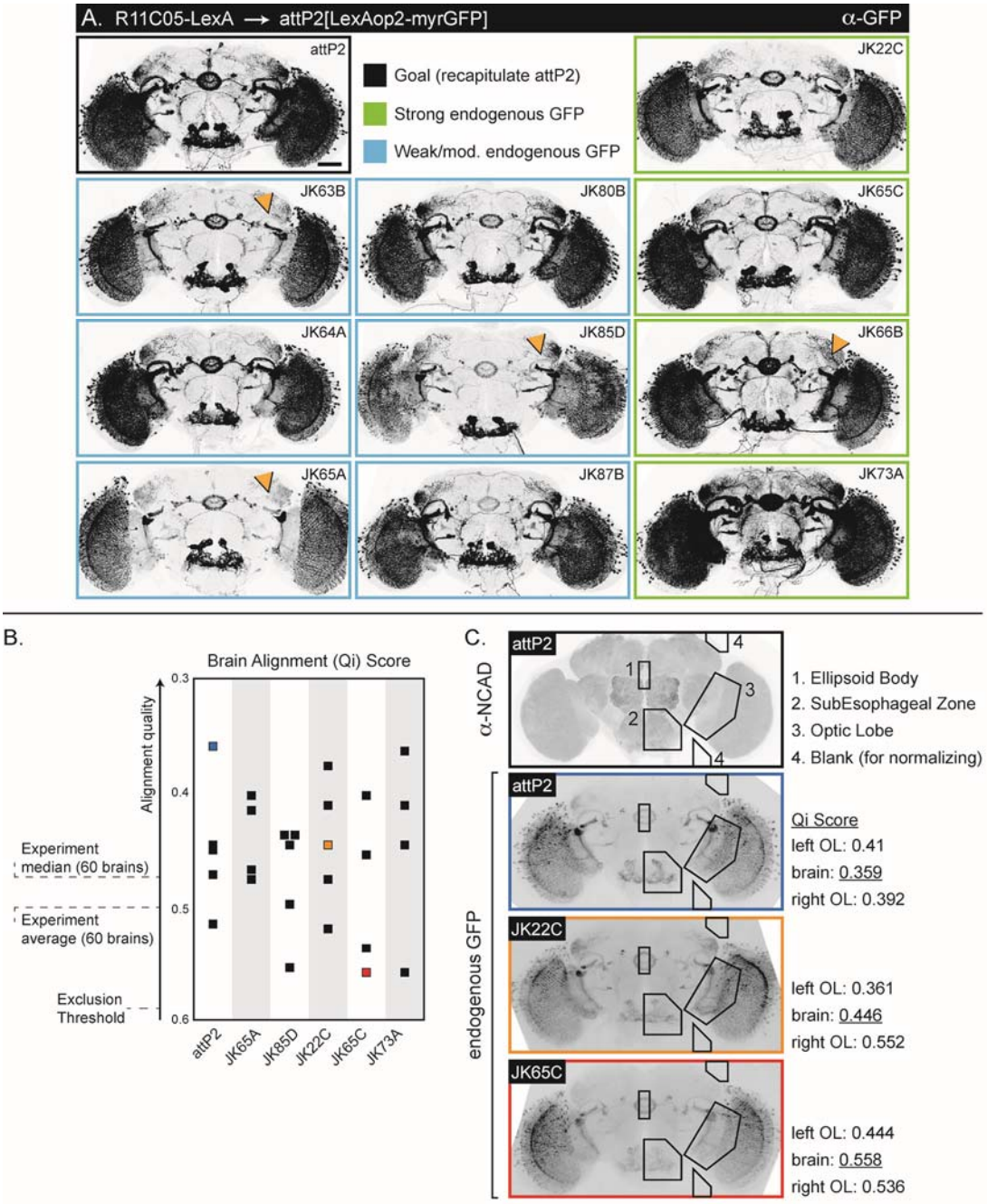

Figure S3. Characterization of R11C05-LexA expression in attP2 and several new candidate landing sites.

Figure S3 Characterization of R11C05-LexA expression in *attP2* and several new candidate landing sites. (A) To further characterize the expression of R11C05-LexA in the best new candidate landing sites, brains were stained with anti-GFP and imaged quantitatively by confocal microscopy. Images are representative samples from each line; the border around each image indicates the strength of native GFP fluorescence, as judged during the visual screen (see Figure 2B and 2C). Orange arrowheads indicate qualitative differences between R11C05-LexA expression in new candidate landing sites vs. *attP2*. (B) Alignment quality of brains presented in Figure 2D: Brains were imaged quantitatively for native GFP fluorescence by confocal microscopy, then stacks were aligned to a reference brain. Samples were assigned alignment (Qi) scores for each optic lobe and the central brain (smaller Qi indicates better alignment), and samples with brain-Qi > 0.59 were excluded from the analysis shown in Figure 2D. Samples represented by the blue, orange, and red squares are shown in panel C, with regions of interest (ROIs) drawn and complete Qi scores. (C) ROIs used for quantitative comparison of R11C05-LexA expression: Top - ROIs superimposed over a maximum projection of the neuropil, stained by anti-N-Cadherin. Bottom - ROIs drawn over three examples of aligned brains; the border color of each image indicates the sample in panel B with the matching color. To quantify signal in each ROI, the mean fluorescence intensity (mFI) of the ellipsoid body (1), subesophageal zone (2), and optic lobe (3) were computed, then the average mFI of the two blank regions (4) was subtracted.