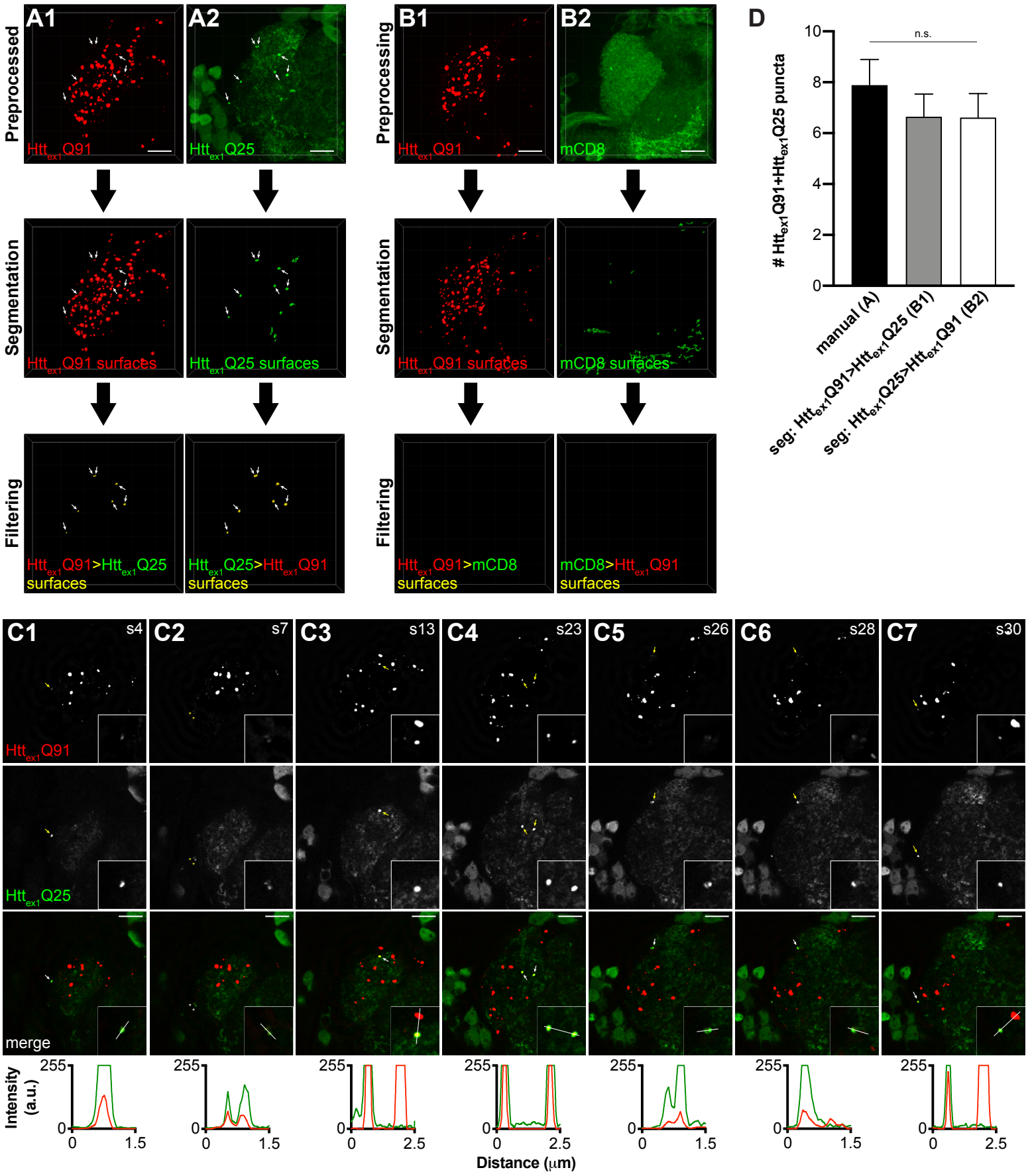
A1
A2
B1
B2
Preprocessed
Preprocessing
Segmentation
Filtering
Htt$_{ex1}$Q91
Htt$_{ex1}$Q25
mCD8
Htt$_{ex1}$Q91 surfaces
Htt$_{ex1}$Q25 surfaces
mCD8 surfaces
Htt$_{ex1}$Q91>Htt$_{ex1}$Q25 surfaces
Htt$_{ex1}$Q25>Htt$_{ex1}$Q91 surfaces
Htt$_{ex1}$Q91>mCD8 surfaces
mCD8>Htt$_{ex1}$Q91 surfaces
D
n.s.
# Htt$_{ex1}$Q91+Htt$_{ex1}$Q25 puncta
manual (A)
seg: Htt$_{ex1}$Q91>Htt$_{ex1}$Q25 (B1)
seg: Htt$_{ex1}$Q25>Htt$_{ex1}$Q91 (B2)
C1
C2
C3
C4
C5
C6
C7
s4
s7
s13
s23
s26
s28
s30
Htt$_{ex1}$Q91
Htt$_{ex1}$Q25
merge
Intensity (a.u.)
Distance (μm)

**Figure 1 – figure supplement 2. Semi-automatic quantification of seeded wtHtt$_{ex1}$ aggregates.** (A and B) Semi-automatic image processing workflow for high-magnification confocal z-stacks of DA1 glomeruli from 14 day-old adult males expressing Htt$_{ex1}$Q91-mCherry in DA1 ORNs and either (A1,2) Htt$_{ex1}$Q25-GFP or (B1,2) mCD8-GFP in GH146+ PNs. Raw data were preprocessed by deconvolution to reduce noise (*top panels*), segmented in the mCherry (A1 and B1) or GFP (A2 and B2) channels (*middle panels*), and filtered for co-localizing fluorescence signal in the other channel (*bottom panels*). Arrows in (A1 and A2) indicate seven Htt$_{ex1}$Q91+Htt$_{ex1}$Q25 puncta identified by this method. Scale bars = 10 μm. (C1-7) Selected single 0.35 μm confocal z-slices from the same confocal stack shown in (A1 and A2). Slice number is indicated at the top right of each image. Individual Htt$_{ex1}$Q91+Htt$_{ex1}$Q25 puncta identified by semi-automated image segmentation in (A) are indicated with arrows (yellow in individual channels, white on merged images) in each slice. Two additional co-localized Htt$_{ex1}$Q91+Htt$_{ex1}$Q25 puncta identified by manual counting are indicated with asterisks in (A2). Scale bars = 10 μm. Insets show Htt$_{ex1}$Q91+Htt$_{ex1}$Q25 puncta at higher zoom (inset dimensions = 9.12 μm x 9.12 μm). Htt$_{ex1}$Q91-mCherry (*red*) and Htt$_{ex1}$Q25-GFP (*green*) fluorescence intensity profiles for lines indicated in merged insets are shown below images. Lines were scanned from leftmost to rightmost point. (D) Comparison of manual quantification (C) vs semi-automated segmentation approaches (A1 and A2) for 14 day-old males with the same genotype in (A and C). Data are shown as mean ± SEM; n.s. = not significant by one-way ANOVA followed by Tukey's multiple comparisons test.