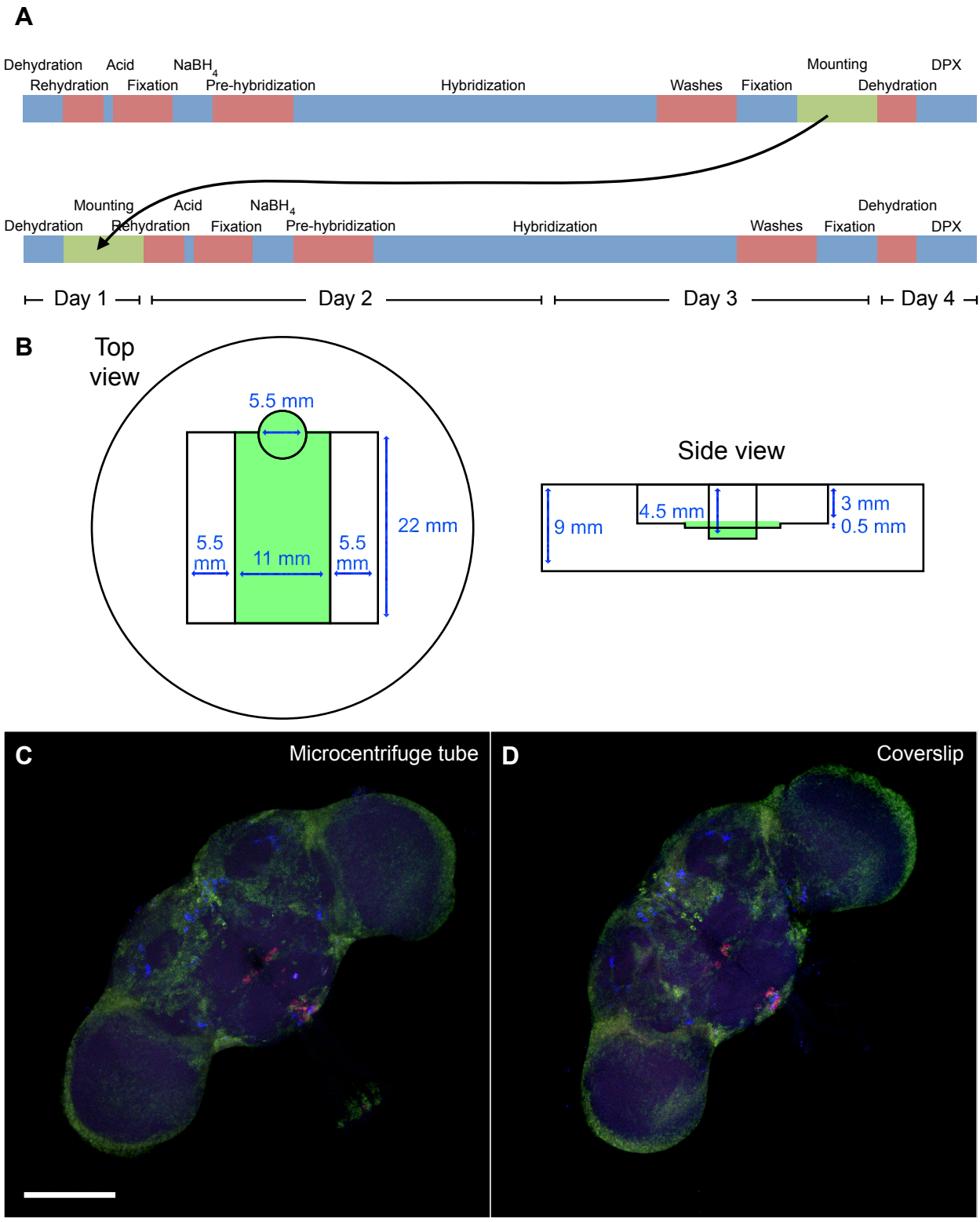

Figure S5. Comparison of the "microcentrifuge tube" and "coverslip" FISH protocols.
(A) Timeline of FISH processing steps. Both protocols involve similar steps, but sample mounting is moved towards the beginning of the procedure in the coverslip protocol. This simpliies subsequent sample handling while maintaining the identity of many individual brains throughout the protocol. (B) Top and side view schematics of the hybridization chamber for the coverslip protocol. Area to be illed with hybridization solution is colored green. (C-D) The two protocols produce FISH labeling of similar quality. Drosophila brains were labeled with FISH probes for *ChAT* (Cy3; blue), *pale* (Cy5; green), and *Tβh* (AF594; red) mRNAs using either microcentrifuge tube (C), or coverslip (D) protocols.