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Title: The cardiac conduction system: single-cell transcriptomic profiling and the generation of novel intraoperative optical imaging agents

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Introduction
Coordinated beating of the heart requires a specialized subset of heart cells known as the cardiac conduction system (CCS). Damage to any component of the CCS can result in severe cardiac arrythmias and even sudden death. Due to the current inability to visually distinguish conduction cells from the surrounding working myocardium, accidental damage to the CCS, via inadvertent incision and/or suture placement during cardiac surgeries, remains a significant source of morbidity and mortality in the perioperative setting.

Methods and Results
Wild-type, embryonic day 16.5 mouse hearts were harvested and all major components of the CCS were isolated through microdissection. Tissues were then digested into single-cell suspensions, mRNA was reverse transcribed and barcoded before high-throughput sequencing and bioinformatics analyses were performed to uncover both known and a host of previously unknown CCS-specific genes. A subset of the novel CCS-specific genes were then validated by immunohistochemistry (IHC), fluorescence *in situ* hybridization (FISH) and whole-mount immunolabeling with volume imaging (iDISCO+) within the tissues of mice and/or humans. Next, two CCS-specific cell surface markers were used as molecular targets for the generation of antibody-based optical imaging agents. These optical imaging tools demonstrated high sensitivity and specificity in labeling the entire CCS *in vivo* following a single intravenous injection in mice. Specificity was confirmed within intact, whole hearts using both closed-field NIR imaging and iDISCO+. In addition to timecourse and dosage analyses, biodistribution was evaluated as well as safety validation by surface ECG assessment.

Conclusions
This study provides a comprehensive transcriptional landscape of the entire CCS at single-cell resolution for the first time. Further, capitalizing on novel CCS-specific cell surface markers, novel optical imaging agents created in this study provide a proof-of-principle for the *in vivo* labeling of cardiac substructures for the first time and lay the foundation for translational opportunities in the real-time visualization of the CCS during cardiothoracic surgeries and other cardiac interventions.