SUPPLEMENTAL MATERIAL

Extended Methods

Animal Protocol

All procedures were performed using standard non-survival surgical techniques in accordance with The Ohio State University Institutional Animal Care and Use Committee (OSU IACUC) guidelines. All animals were treated in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals-NIH Publication No. 85-23 (Revised). The Ohio State University complies with the Animal Welfare Act of 1966 (P.L. 89-544), as amended by the Animal Welfare Act of 1970 (P.L. 91-579), the Animal Welfare Act of 1976 (P.L. 94-279), and the Animal Welfare Act amendment of 1985(P.L.99-198). The Ohio State University is accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC). The Ohio State University conducts research in a manner consistent with the guidelines of NIH, USDA, and AAALAC. This study utilized a 38 kg female purpose-bred hound dog (Marshall Bioresources).

Ex-vivo Near-Infrared Optical Mapping

Following explantation, the intact canine heart was immediately preserved with cold cardioplegic solution (1-3°C). The whole intact atria were dissected from the ventricles and coronary artery leaks were ligated with silk sutures. The atrial preparation was coronary-perfused with oxygenated Tyrode solution at constant pressure (45-55 mmHg) and temperature (37°C).

The atrial preparation was immobilized with 10 μM blebbistatin (Abcam) and stained with near-infrared dye di-4-ANBDQBS (10-40 μM, University of Connecticut Health Center). Ex-vivo near-infrared optical mapping (NIOM) employed four CMOS cameras, as previously described[1] (1 high-resolution and 3 panoramic) and the same high-resolution MEA. Sub-epicardial optical

mapping of the whole coronary-perfused atria was conducted during SR, 300ms CL RA pacing, and pacing-induced sustained AF. Acetylcholine (ACh, 0.2μM, Sigma) was used ex-vivo to reproduce the effects of in-vivo VNS. AF was induced by burst atrial pacing during ACh perfusion.

Optical Mapping Data Analysis

Both in-vivo and ex-vivo NIOM data were analyzed through a custom-made Matlab program as recently described.[2, 3] Optical action potentials (OAPs) were processed by Gaussian binning, which takes a weighted average of neighboring pixels. Gaussian binning attenuated the distortion caused by the overlaying MEA, but did not affect the overall activation pattern. The effect of Gaussian binning on in-vivo NIOM OAPs and activation maps can be seen in **Figure S4**. NIOM activation maps were created by plotting the time of maximal optical action potential (OAP) derivative. In-vivo OAPs and their derivatives were compared to the nearest unipolar electrogram recorded by the MEA to confirm which OAP deflection represented local atrial activation and which deflections may be motion (**Figure [B]**).

Multi-Electrode Array (MEA) Mapping Analysis

MEA was used during in-vivo mapping as the current standard for measuring local epicardial surface activation, which validated the in-vivo optical signals. The electrograms from the endocardial quadripolar catheter (Biosense Webster) were recorded simultaneously by the MEA and NIOM systems and were used to synchronize the two. Local atrial activation was marked on the MEA unipolar electrograms at the point of maximum negative slope ($-dV/dt_{max}$). MEA activation maps were created from activation times using a custom Matlab program as recently described.[4]

Reference List

1. Li N, Csepe TA, Hansen BJ, Sul LV, Kalyanasundaram A, Zakharkin SO, Zhao J, Guha A, Van Wagoner DR, Kilic A, Mohler PJ, Janssen PM, Biesiadecki BJ, Hummel JD, Weiss R, Fedorov VV. Adenosine-Induced Atrial Fibrillation: Localized Reentrant Drivers in Lateral Right Atria due to Heterogeneous Expression of Adenosine A1 Receptors and GIRK4 Subunits in the Human Heart. *Circulation.* 2016;134:486-498.
2. Lou Q, Hansen BJ, Fedorenko O, Csepe TA, Kalyanasundaram A, Li N, Hage LT, Glukhov AV, Billman GE, Weiss R, Mohler PJ, Gyorke S, Biesiadecki BJ, Carnes CA, Fedorov VV. Upregulation of adenosine A1 receptors facilitates sinoatrial node dysfunction in chronic canine heart failure by exacerbating nodal conduction abnormalities revealed by novel dual-sided intramural optical mapping. *Circulation.* 2014;130:315-324.
3. Li N, Hansen BJ, Csepe TA, Zhao J, Ignozzi AJ, Sul LV, Zakharkin SO, Kalyanasundaram A, Davis JP, Biesiadecki BJ, Kilic A, Janssen PML, Mohler PJ, Weiss R, Hummel JD, Fedorov VV. Redundant and diverse intranodal pacemakers and conduction pathways protect the human sinoatrial node from failure. *Sci Transl Med.* 2017;9;eaam5607.
4. Hansen BJ, Zhao J, Li N, Zolotarev A, Zakharkin SO, Wang Y, Atwal J, Kalyanasundaram A, Abudulwahed SH, Helfrich KM, Bratasz A, Powell KA, Whitson B, Mohler PJ, Janssen PML, Simonetti OP, Hummel JD, Fedorov VV. Human atrial fibrillation drivers correlated with integrated functional and structural imaging to benefit clinical mapping. *J Am Coll Cardiol EP.* 2018;DOI: 10.1016/j.jacep.2018.08.024.

Online Figures

Figure S1.

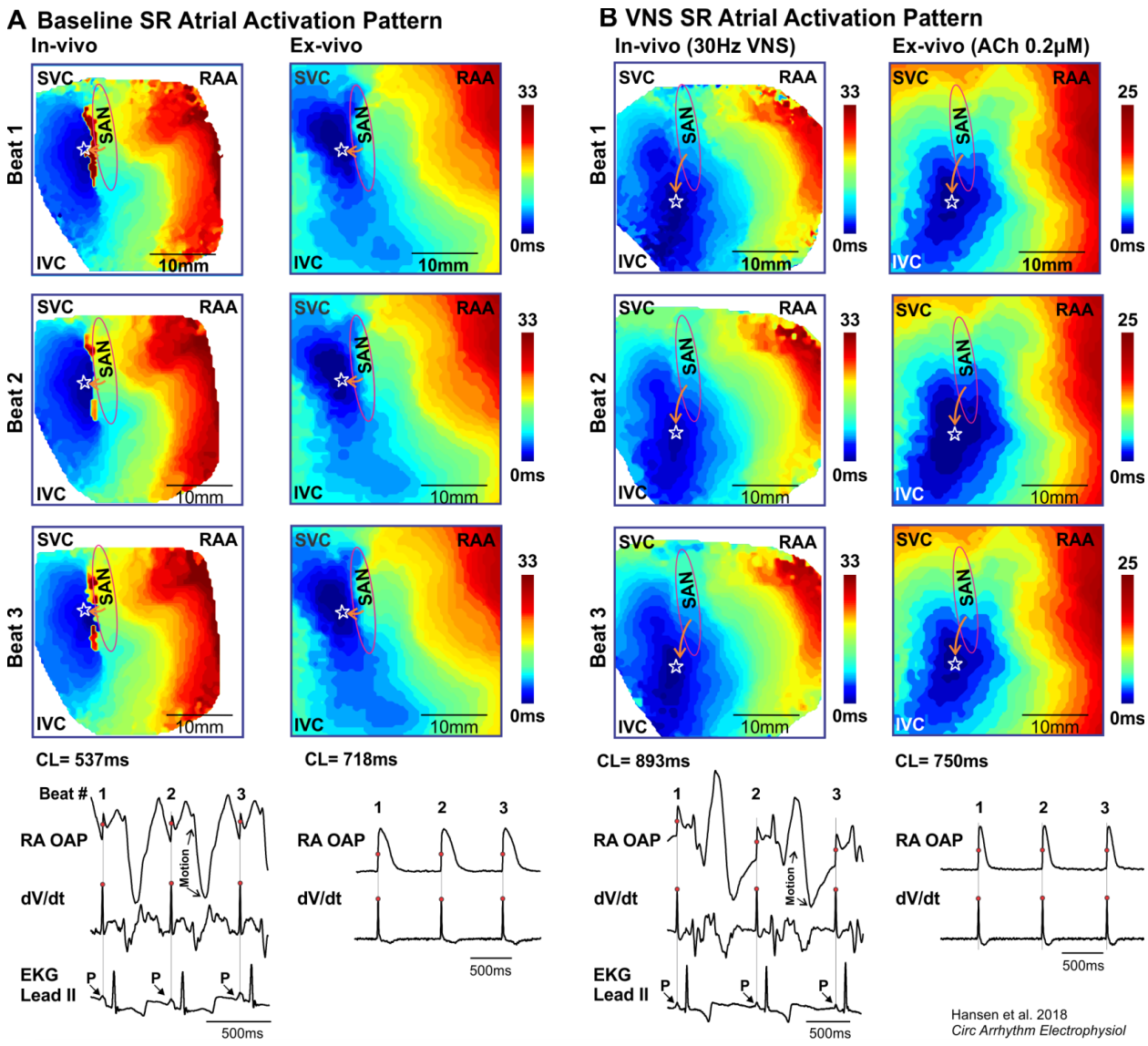

Figure S1. In-vivo vs ex-vivo NIOM atrial activation during sinus rhythm. A. Three sequential in-vivo (Left) and ex-vivo (Right) NIOM activation maps (Beats 1-3) of sinus rhythm (SR) at baseline conditions are shown from a recording without the multi-electrode array in-vivo. Pink oval indicates the sinoatrial node (SAN) region. Stars indicate the earliest atrial activation sites. Atrial optical action potentials (RA OAPs), their derivatives (dV/dt), and an in-vivo EKG trace are shown below. **B.** Three sequential in-vivo (Left) and ex-vivo (Right) NIOM activation

maps (Beats 1-3) of SR during vagal nerve stimulation (VNS) and 0.2 μM acetylcholine (ACh), respectively, are shown from a recording without the multi-electrode array in-vivo. Pink oval indicates the sinoatrial node (SAN) region. Stars indicate the earliest atrial activation sites. Atrial optical action potentials (RA OAPs), their derivatives (dV/dt), and an in-vivo EKG trace are shown below. Abbreviations: CL- cycle length; IVC/SVC-inferior/superior vena cava; RAA-right atrial appendage; SAN-sinoatrial node.

Figure S2.

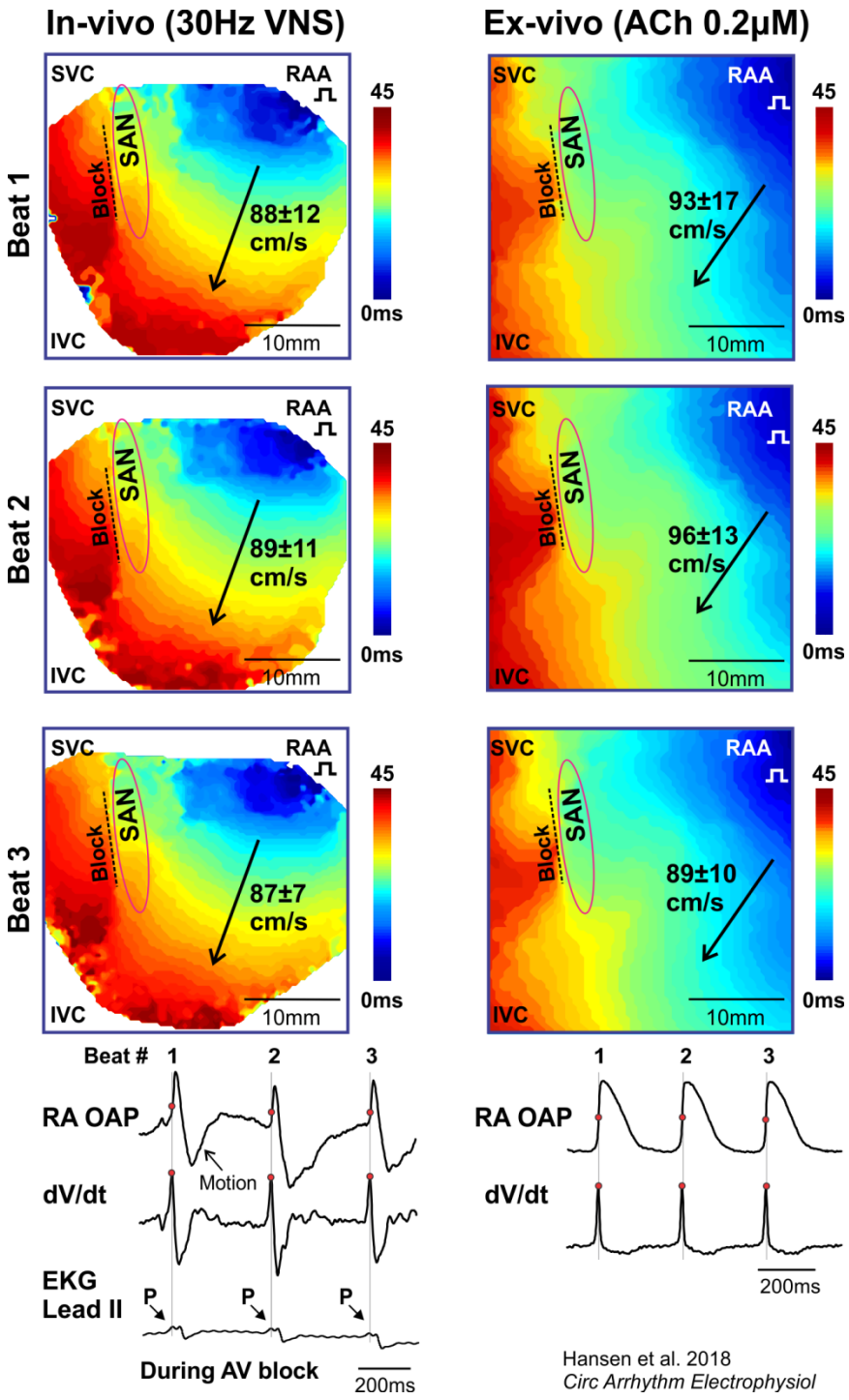

Figure S2. In-vivo vs ex-vivo NIOM atrial activation during right atrial pacing. Three sequential in-vivo (Left) and ex-vivo (Right) NIOM activation maps (Beats 1-3) of the right atrial appendage (RAA) pacing at 300ms cycle length (CL) during vagal nerve stimulation (VNS) and

0.2 μM acetylcholine (ACh), respectively, are shown from a recording without the multi-electrode array. Black arrows are labeled with local conduction velocity. Right Atrial Optical action potentials (RA OAPs), their derivatives (dV/dt), and an in-vivo EKG trace are shown below. Abbreviations as in Figure S1.

Figure S3.

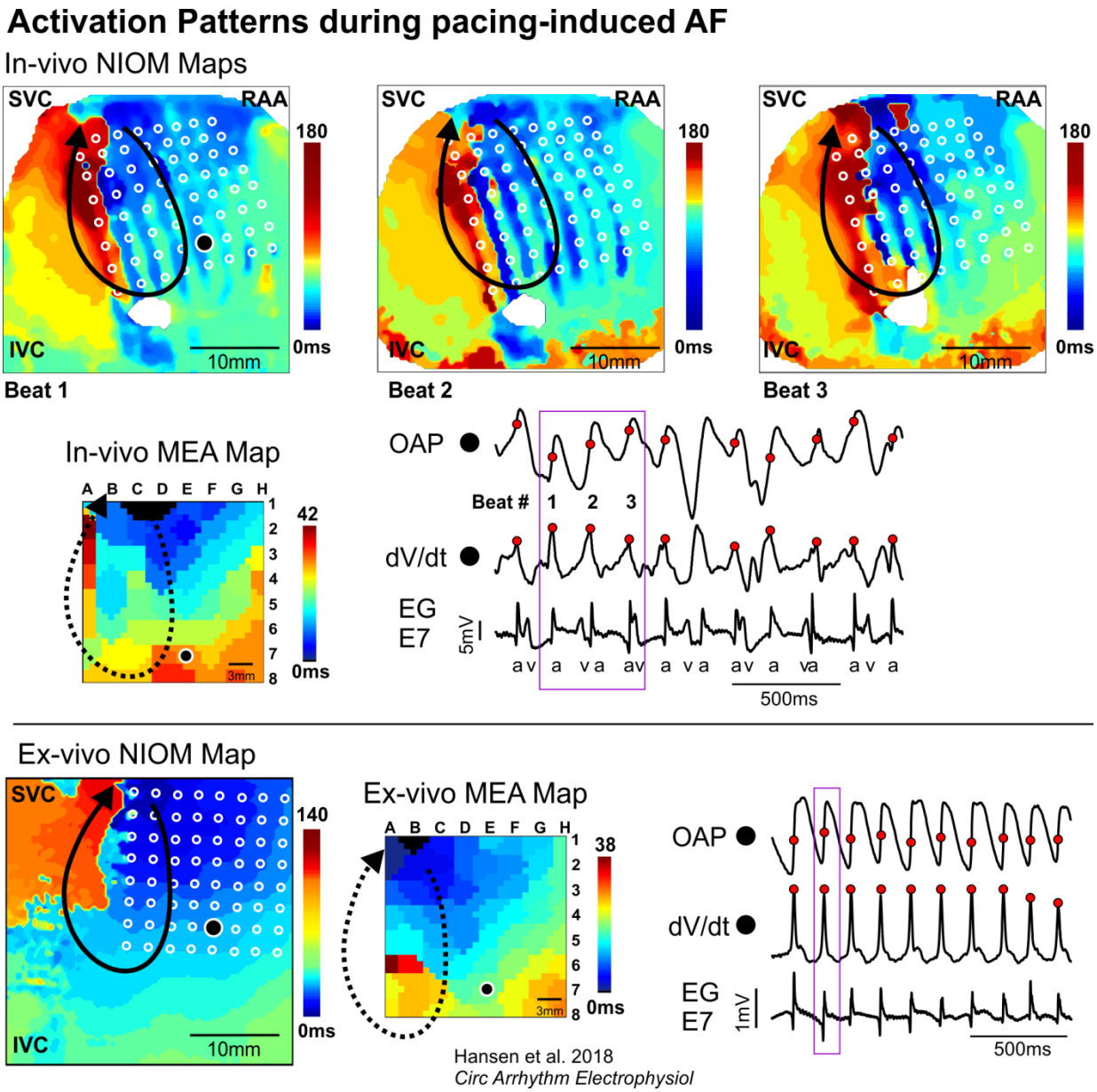

Figure S3. NIOM and multi-electrode mapping (MEA) of in-vivo and ex-vivo atrial fibrillation (AF) episodes. Top: Three sequential in-vivo activation maps (Beats 1-3) show a reentrant pattern around the sinoatrial node. Purple box indicates mapped time interval. Bottom: ex-vivo maps display a similar reentrant pattern. Example optical action potentials (OAPs), their derivatives (dV/dt) and unipolar electrograms (EG) from the nearest electrode are shown to the right. Black arrow represents reentry circuit. Abbreviations as in Figure S1.

Figure S4.

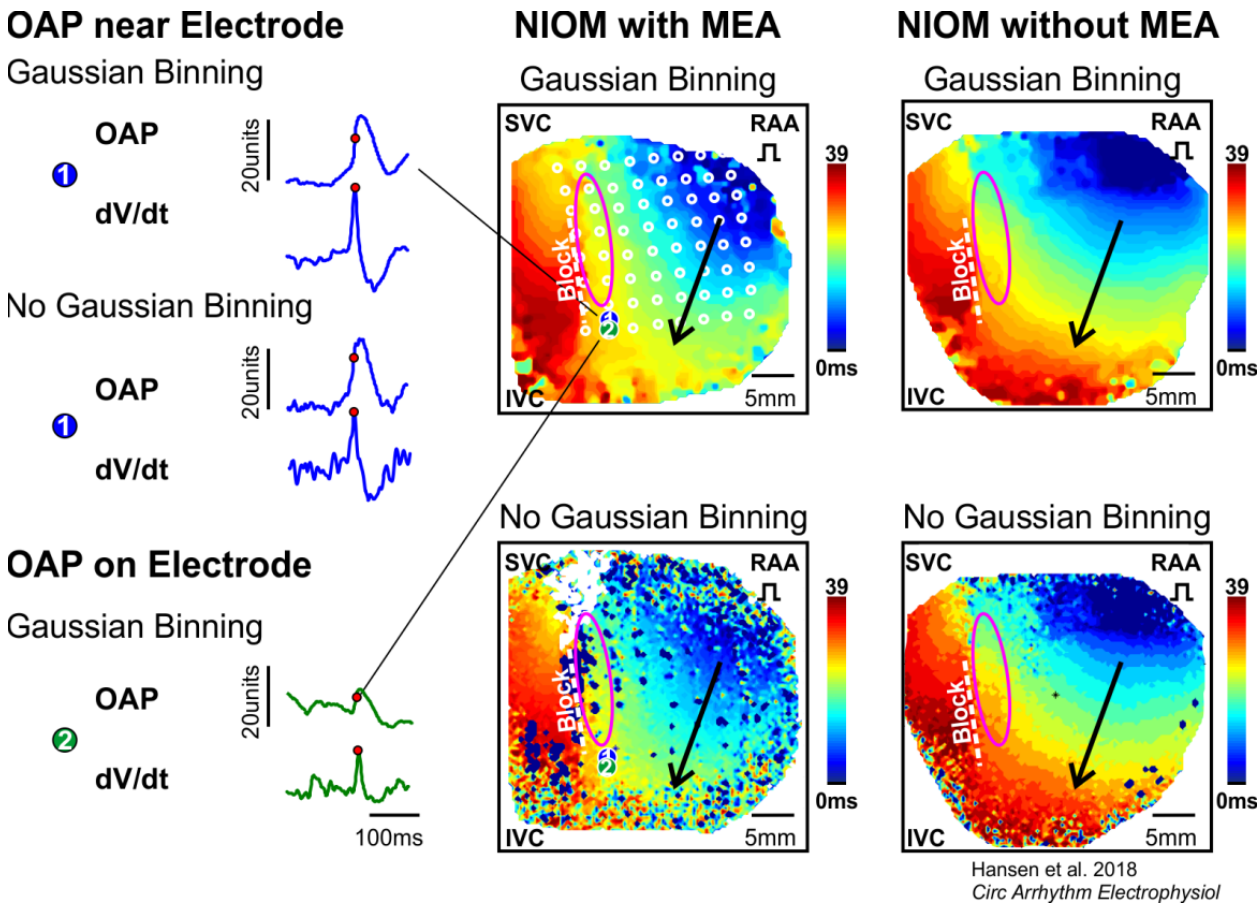

Figure S4. Effect of MEA on in-vivo atrial activation patterns seen by NIOM. Left. In-vivo Optical action potentials (OAPs) and their derivatives (dV/dt) from a pixel neighboring a mapping electrode pole as well as from directly overlaying the electrode with and without processing with Gaussian binning. Middle. Near-infrared optical mapping (NIOM) activation map during atrial pacing with the overlaying multi-electrode array (MEA) with (top) and without (bottom) processing with Gaussian binning. Pink oval denotes location of the sinoatrial node. Right. Near-infrared optical mapping (NIOM) activation map during atrial pacing without the overlaying multi-electrode array (MEA) with (top) and without (bottom) processing with Gaussian binning. Abbreviations as in Figure S1.