



**HAL**  
open science

## Comparison of Atrial Remodeling Caused by Sustained Atrial Flutter Versus Atrial Fibrillation

Jean-Baptiste Guichard, Patrice Naud, Feng Xiong, Xiaoyan Qi, Nathalie L'heureux, Roddy Hiram, Jean-Claude Tardif, Raymond Cartier, Antoine da Costa, Stanley Nattel

► **To cite this version:**

Jean-Baptiste Guichard, Patrice Naud, Feng Xiong, Xiaoyan Qi, Nathalie L'heureux, et al.. Comparison of Atrial Remodeling Caused by Sustained Atrial Flutter Versus Atrial Fibrillation. *Journal of the American College of Cardiology*, 2020, 76, pp.374 - 388. 10.1016/j.jacc.2020.05.062 . hal-03492539

**HAL Id: hal-03492539**

**<https://hal.science/hal-03492539v1>**

Submitted on 22 Aug 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

## **Comparison of Atrial Remodeling Caused by Sustained Atrial Flutter vs. Atrial Fibrillation**

Jean-Baptiste Guichard, MDa,b, Patrice Naud, PhDa, Feng Xiong, PhDa, Xiaoyan Qi, PhDa, Nathalie L'Heureuxa, Roddy Hiram, PhDa, Jean-Claude Tardif, MDa, Raymond Cartiera, Antoine Da Costa, MD, PhDb, Stanley Nattel, MDa,c,d,e

From the aDepartment of Medicine and Research Center Montreal Heart Institute and Université de Montréal, Montreal, Quebec, Canada; bDepartment of Cardiology, University Hospital of Saint-Étienne, University Jean Monnet, Saint-Étienne, France; cDepartment of Pharmacology and Therapeutics, McGill University Montreal, Canada; dInstitute of Pharmacology, West German Heart and Vascular Center, Faculty of Medicine, University Duisburg-Essen, Essen, Germany; eIHU LIRYC and Fondation Bordeaux Université, Bordeaux, France.

**Short title:** Atrial Flutter-Induced Atrial Remodeling

**Funding sources:** The Canadian Institutes of Health Research (Foundation Grant 148401) and the Heart and Stroke Foundation of Canada (G-16-00012708).

**Potential Conflict of Interest:** Dr. Guichard received grant support from Abbott Labs. No other author has any potential conflicts to disclose.

**Acknowledgements:** The authors thank Lucie Lefebvre for secretarial help with the manuscript, and Chantal St-Cyr for technical assistance.

### **Corresponding author:**

Stanley Nattel, MD

Department of Medicine and Research Center Montreal Heart Institute and Université de Montréal

5000 Belanger St E, Montreal H1T 1C8, Quebec, Canada

E-mail: stanley.nattel@icm-mhi.org

Phone : 514-376-3330 ext 3587

Fax : 514-593-2493

Twitter handle: @StanleyNattel; @guichard\_jb

Tweet: Atrial fibrillation (AF) and #flutter (AFL), cause electric remodeling but only #AF causes #inflammation+#fibrosis. Keep patients with AFL out of AF!

## **ABSTRACT**

**Background:** Atrial flutter (AFL) and atrial fibrillation (AF) are associated with AF-promoting atrial remodeling, but no experimental studies have addressed remodeling with sustained AFL.

**Objective:** To define the atrial remodeling caused by sustained AFL and/or AF.

**Methods:** Intercaval radiofrequency-lesions created a substrate for sustained isthmus-dependent AFL, confirmed by endocavity mapping. Four groups (6 dogs/group) were followed for 3 weeks: 1) sustained AFL; 2) sustained AF (600-bpm atrial tachypacing); 3) AF superimposed on an AFL-substrate (AF+AFLs); 4) Sinus rhythm with an AFL-substrate (SR+AFLs; control-group). All dogs had atrioventricular-node ablation and ventricular pacemakers at 80-bpm to control ventricular rate.

**Results:** Monitoring confirmed spontaneous AFL-maintenance >99% of the time in AFL-dogs. At terminal open-chest study, left-atrial (LA) effective refractory period was reduced similarly with AFL, AF+AFLs and AF, while AF-vulnerability to extrastimuli increased in parallel. Induced-AF duration increased significantly in AF+AFLs and AF, but not AFL. AF+AFLs-dogs had shorter cycle-lengths and substantial irregularity versus AFL-dogs. LA-volume increased in AF+AFLs and AF, but not AFL-dogs, versus SR+AFLs. Optical mapping showed significant conduction-slowing in AF+AFLs and AF but not AFL, paralleling atrial fibrosis and collagen-gene upregulation. Left-ventricular function did not change in any group. Transcriptomic analysis revealed substantial dysregulation of inflammatory and extracellular-matrix signaling pathways with AF but not AFL.

**Conclusions:** Sustained AFL causes atrial repolarization-changes like AF, but unlike AF or AF+AFLs does not induce structural remodeling. These results provide novel insights into AFL-induced remodeling and suggest that early intervention may be important to prevent irreversible fibrosis when AF intervenes in an AFL-patient.

**Condensed Abstract:** Atrial flutter (AFL) and atrial fibrillation (AF) are closely related and both are associated with AF-promoting atrial remodeling, but no experimental studies have addressed the remodeling effects of sustained AFL vs AF. Here, we compared for the first time AFL-induced remodeling versus AF alone and AF superimposed on an AFL-substrate over a 3-week period in a dog model. AF and AFL produced similar refractory-period abbreviation, but only AF induced inflammatory signaling, fibrosis, conduction slowing and stabilization of the AF-substrate. These findings highlight the pro-inflammatory effects of AF and its importance in promoting fibrosis and arrhythmia-stabilization.

**Key words:** atrial fibrillation, atrial flutter, atrial remodeling, fibrosis

### **Abbreviations and Acronyms**

APD = action-potential duration

A-TP = atrial tachypacing

CTI = cavotricuspid isthmus

CV = conduction velocity

ECM = extracellular-matrix

EPS = electrophysiological study

ERP = effective refractory period

LVEF = LV ejection fraction

qPCR = quantitative reverse transcription polymerase chain reaction

RAA = right-atrial appendage

Atrial flutter (AFL) is a relatively common macroreentrant atrial arrhythmia. Patients with AFL often experience atrial fibrillation (AF) at some point in their natural history, and at times may manifest one or the other arrhythmia. AF and AFL are known to share many pathophysiological features, both in terms of underlying pathology and of dynamic electrophysiological interaction (1,2). AF induces complex atrial remodeling that promotes its own recurrence and maintenance, with both electrical and structural components (3,4). AFL also induces atrial remodeling, causing action-potential duration (APD) and effective refractory period (ERP) abbreviation in patients similar to characteristic changes caused by AF (5,6).

In addition to the short-term APD-ERP changes caused by AF, longer-term AF promotes the development of atrial fibrotic remodeling (7,8), likely via profibrotic consequences of molecules secreted by atrial cardiomyocytes activated at increased rates (9). Whether AFL is capable of promoting structural remodeling is unknown. Studies of AFL patients remote from arrhythmia episodes show extensive structural and conduction abnormalities (10), but whether these are due to the underlying atrial pathology causing AFL or to arrhythmia-induced structural remodeling that fails to reverse is unclear.

Frame et al. first developed a dog model of AFL, employing a Y-shaped lesion to permit anatomically-based re-entry around the tricuspid valve (11) and used it extensively to study the mechanisms and pharmacological responses of AFL. The model was subsequently employed to produce sustained AFL in sheep (12), demonstrating ERP-abbreviation and increased AF-susceptibility after 28 days of arrhythmia. However, the authors did not investigate whether sustained AFL leads to structural or molecular remodeling. Furthermore, patients with an AFL-substrate often manifest AF, and appear to have more extra-pulmonary vein AF-sources than AF-

patients without an associated AFL-substrate (13). Whether the remodeling associated with AF and subsequent arrhythmias differs depending on the presence of an AFL-substrate is unknown.

The present study was designed to develop and apply a chronic dog model of AFL to determine: 1) Whether sustained AFL can cause structural remodeling of the type caused by sustained AF; 2) Whether the remodeling caused by AF in the presence of an AFL-substrate shows different properties and/or arrhythmic predisposition from AFL maintained by an AFL-substrate or AF without an AFL-substrate; 3) The effects of AFL-substrate ablation on arrhythmia susceptibility in the presence and absence of tachycardia-remodeling induced by sustained AFL or AF.

## **Methods**

Because of space limitations, only a summary of essential methods is presented here. For full detailed methods, see Supplemental Appendix.

All animal-handling procedures were approved by the Montreal Heart Institute Animal Research Ethics Committee and conformed to the guidelines of the Canadian Council on Animal Care. Dogs were anesthetized for the initial procedure with ketamine (5.3 mg/kg, intravenous)/diazepam (0.25 mg/kg, intravenous)/isoflurane (1.5%), intubated, and ventilated. A pacing lead in the right ventricle (RV) attached to a VVI pacemaker was used to demand-pace the RV at 80 bpm. Radiofrequency (RF)-ablation was applied to create atrioventricular block. Open-chest surgery was then performed to establish a substrate for AFL-maintenance (Figure 1A). A transmural line of block was achieved by applying RF energy between the blades of a bipolar radiofrequency-ablation device (Atricure, Cincinnati, OH). A Y-shaped RA lesion was created involving the superior vena cava (SVC), the inferior vena cava (IVC) and the ostium of the right-atrial (RA) appendage (RAA), as shown schematically in Figures 1A and 1B. A pacing

lead was inserted into the RAA and attached to a subcutaneous pacemaker in the neck. Among dogs with an anatomic AFL substrate, an additional lead was inserted into the RAA and exteriorized in the neck to re-induce AFL if AFL spontaneously terminated. Analgesics and antibiotics were routinely provided postoperatively.

### *Experimental groups*

Twenty-four mongrel dogs (weight, 25.6-40.6 kg; 6/group) were studied in the following groups (**Figure 2**). (1) Sinus rhythm (SR)+AFL-substrate, with AFL-substrate and SR during 3-week follow-up (control group); (2) AFL+AFL-substrate, with AFL-substrate and AFL maintenance during 3-week follow-up; (3) AF+AFL-substrate, with AFL-substrate and AF maintained by 600 bpm atrial tachypacing (A-TP) during 3-week follow-up; (4) AF-only, without AFL-substrate; AF maintained (600 bpm A-TP) during 3-week follow-up.

### *Atrial flutter model*

AFL was induced by 10 Hz RA-pacing for 15 seconds in AFL+AFL-substrate dogs. The surface electrocardiogram (ECG) was monitored twice-daily to confirm AFL-maintenance. Tachycardia with typical ECG features of AFL (**Figure 1C**) was detected on  $99.1 \pm 1.4\%$  of the EKG-recordings in AFL+AFL-substrate dogs. At the end of the 3-week follow-up, in vivo endocavity mapping (EnSite 3D-mapping system; St. Jude Medical, St. Paul, MN) was performed on 4 dogs each from SR+AFL-substrate-, AFL+AFL-substrate, and AF+AFL-substrate groups. Double-loop macroreentry in a figure-of-8 fashion was observed in all AFL-substrate+AFL dogs (**Figure 1D**). One limb of the circuit was located around the tricuspid valve and the other around the surgically-induced RA lateral lesion. The flutter cycle-length averaged  $168 \pm 17$  ms (range 136-192 ms) and endocavity mapping always revealed activity over  $>90\%$  of the flutter-cycle (**Video 1**). The post-pacing interval following overdrive pacing adjacent to the

CTI was always <20 ms (mean  $11.1 \pm 5.3$  ms) greater than the tachycardia cycle length, suggesting that macroreentry was CTI-dependent.

#### *Open-chest electrophysiological study (EPS)*

Dogs were anesthetized with ketamine/diazepam/isoflurane, intubated, and mechanically ventilated. Any AF or AFL was direct-current cardioverted. Vulnerability in each dog was defined as the percentage of 6 pacing-sites (left-atrial, LA, appendage; LA inferior and superior wall; RAA, RA inferior and superior wall) at which AF was inducible with a single S2. To measure arrhythmia duration, tachyarrhythmia was induced 10 times with 2-s burst pacing (10-Hz, 4×threshold-current). EPSs were performed for each dog with an AFL-substrate before and after placing ligatures across the CTI and posterolateral RA-wall.

#### *Echocardiography*

Transthoracic echocardiography was performed at baseline and on the last study day prior to euthanasia, with an M3S probe (2.0-4.3 Megahertz) and a Vivid 7 Dimension system (GE Healthcare Ultrasound, Horten, Norway) under sedation with acepromazine (0.07 mg/kg i.m.). For details, see Supplemental Appendix.

#### *Optical mapping*

The LA was dissected free and perfused through its coronary artery with Krebs solution. After 30 minutes for stabilization and electrical/mechanical uncoupling with blebbistatin (15  $\mu$ M), the heart was loaded with di-4-ANEPPS (10 M, 0.1 mL, Biotium, Inc., Hayward, CA). A charge-coupled device (CardioCCD, RedShirtImaging, LLC, Decatur, GA) was used to record LA free wall fluorescence at 2 kHz focused on a 15x15-mm square region. Results were analyzed with custom-written software. For details, see Supplemental Appendix.

#### *Real-time quantitative reverse transcription polymerase chain reaction (qPCR)*

LA-samples were homogenized in lysis buffer and RNA was isolated with Nucleospin RNA II, including DNase treatment to prevent genomic contamination. Messenger RNAs (mRNAs) were reverse-transcribed and quantitative PCR was performed with TaqMan for HPRT and  $\beta$ 2-microglobulin, as well as KCNJ2, KCND3, collagen-1 (COL1A1), and collagen-3 (COL3A1). SyBr green primers were used to quantify SCN5A, L-type calcium-channels (CACNA1C and CACNA1D), Kv7.1 (KCNQ1 and KCNE1), KCNIP2, KCNH2, KCNJ3, KCNJ5, connexin-43 (GJA1) and connexin-40 (GJA5). For details, see Supplemental Appendix.

#### *Transcriptomic analysis*

Flash-frozen LA-samples were homogenized in Trizol to extract nucleic acids. Microarray (Canine Genome Array 2.0, Affymetrix) data were analyzed with the Transcriptome Analysis Console. Gene Ontology enrichment pathway analysis was performed with the Gene Ontology Consortium online tool powered by Panther (<http://geneontology.org>), and confirmed with the Reactome Pathway browser (<http://reactome.org>). For details, see Supplemental Appendix.

#### *Western blot*

Protein samples were separated by electrophoresis on 4-20% sodium dodecyl sulfate-polyacrylamide gels and transferred electrophoretically onto polyvinylidene difluoride membranes. Membranes were blocked in a Tris-buffered saline (TBS) solution and incubated overnight at 4°C with primary antibodies. After washing with TBS-Tween/1% serum-albumin, membranes were hybridized with horseradish peroxidase-conjugated secondary antibody. For details, see Supplemental Appendix.

#### *Histology*



Sections (6  $\mu\text{m}$ ) were cut at room temperature and stained with Masson's Trichrome. Stained images were digitized, and the fibrotic area was analyzed with Image Pro 9.3 (Media Cybernetics, Rockville, MD). LA-fibrosis (expressed as percent cross-sectional area) was quantified by an observer blinded to intervention-group.

#### *Data analysis*

Continuous variables are expressed as mean  $\pm$  SEM, unless otherwise stated. Multiple-group non-repeated measures comparisons were obtained with 1-way ANOVA and Tukey tests. Repeated-measures analyses were performed for normally distributed data with 2-way ANOVA and Tukey tests. Distribution normality was tested with a Shapiro-Wilk test. Two-tailed  $p < 0.05$  indicated statistical significance. The authors had full access to and take responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

## **Results**

#### *Electrophysiologic changes*

All dog-groups with 21-day tachyarrhythmia showed significantly reduced ERPs (**Figure 3A**). Vulnerability to induction of any atrial tachyarrhythmia (whether AF or AFL) was similarly increased in AFL-substrate+AFL, AFL-substrate+AF and AF groups compared to AFL-substrate+SR (**Figure 3B**). Vulnerability to tachyarrhythmia-induction remained elevated after AFL-substrate ablation in both AFL-substrate+AFL and AFL-substrate+AF groups (Figure 3B). This finding contrasted with the effect of ablation on the duration of induced tachyarrhythmia in AFL-substrate groups (**Figure 3C**). Whereas all AFL-substrate dogs showed inducible sustained AFL before substrate-ablation, interruption of the AFL-circuit dramatically reduced tachyarrhythmia duration in AFL-substrate+AFL and AFL-substrate+SR groups. For AFL-substrate dogs that had been kept in AF electrically for 3 weeks, tachyarrhythmia was maintained

spontaneously for over 20 minutes in all dogs prior to AFL-substrate interruption but in contrast to AFL-substrate+AFL dogs, tachyarrhythmia duration did not decrease significantly after AFL-substrate ablation (**Figure 3C**). No electrophysiological differences were noticed between AFL-substrate+SR and acute control dogs, except for the inducibility of sustained AFL in the presence of the AFL-substrate in AFL-substrate+SR dogs (Supplemental Figure 1).

Whereas **Figure 3C** shows atrial tachyarrhythmia vulnerability independently of whether the induced arrhythmia was AFL or AF, **Figure 3D** shows the inducibility of AF only. The presence of an AFL-substrate was not associated with enhanced inducibility of AF per se in the absence of tachyarrhythmic remodeling. AF-inducibility in AFL-substrate+AFL dogs was significantly greater than in AFL-substrate+SR dogs, reflecting the effect of tachycardia-induced remodeling, but quantitatively less ( $p=0.08$ ) than in AFL-substrate+AF dogs. Figure 4A shows representative examples of atrial electrograms recorded during atrial tachyarrhythmia in each of the groups studied. For atrial tachyarrhythmia induced in the presence of the AFL substrate, the stability of the arrhythmia was the same among all AFL-substrate groups, but the tachyarrhythmia cycle length in dogs subjected to 3 week-AF was 30% shorter than after 3 week-exposure to AFL or SR (Figure 4B), reflecting the mixed fibrillo-flutter pattern of arrhythmia in the AFL-substrate+AF dogs (Figure 4A). The intermediate nature of arrhythmia induced in AFL-substrate+AF dogs is further reflected by greater cycle-length variability in these animals versus AFL-substrate+AFL dogs and less variability versus AF dogs (**Figure 4C**).

#### *Hemodynamic changes*

AFL-substrate+AF and AF-dogs showed significant increases in LA volume compared to AFL-substrate+SR, whereas AFL-substrate+AFL dogs did not show a significant increase (**Figure 5A**). AF-dogs and AFL-substrate+AFL dogs showed a significantly reduced LA

emptying fraction (Figure 5B). None of the tachyarrhythmia dogs (whether maintained in AFL or in AF) showed significant LVEF changes compared to AFL-substrate+SR (**Figure 5C**).

Ex vivo electrophysiologic changes revealed by optical mapping. Ex-vivo study and optical mapping were performed in all groups shown in **Figure 2**, with the results illustrated in **Figure 6**. No conduction velocity differences were found between AFL-substrate+SR- and AFL-substrate+AFL groups, but there was a significant reduction (by ~25%) in conduction velocity for AFL-substrate+AF and AF groups (**Figures 6A and B**). AFL-substrate+AFL, AFL-substrate+AF and AF dogs all showed significant decreases in APD50, 80 and 90 compared to AFL-substrate+SR dogs (**Figure 6C**), consistent with the in vivo ERP data.

#### *Differences in factors determining conduction velocity*

Examples of Masson-trichrome stained atrial-tissue sections from each group are shown in **Figure 7A**. Fibrous-tissue content appears to be increased in the AFL-substrate+AF- and AF-samples. **Figure 7B** confirms this impression, with a significant, 4-fold increase in atrial fibrous-tissue content in AFL-substrate+AF and AF dogs compared to AFL-substrate+SR and AFL-substrate+AFL dogs. Collagen-gene expression was enhanced in AFL-substrate+AF and AF dogs for collagen 1 and 3 compared to AFL-substrate+AFL dogs (**Figure 7C**).

Changes in connexin expression are shown in Supplemental Figure 2. No significant changes in GJA1 mRNA expression, total connexin-43 and phosphorylated connexin-43 expression were found (Supplemental Figures 2A and B). Connexin-40 (GJA5) mRNA and protein were only downregulated in the AF-group, and not in groups with AFL-substrate, compared to AFL-substrate+SR group (Supplemental Figures 2A and C).

Supplemental Figure 3 shows the mRNA expression data for a wide range of cardiac ion-channel subunits. SCN5A mRNA expression was significantly decreased in AF dogs versus

AFL-substrate+SR dogs (Supplemental Figure 3A). AFL-substrate+AF dogs also showed quantitatively smaller SCN5A expression, without statistical significance. No significant changes relative to AFL-substrate+SR dogs were found for mRNA expression of calcium (Supplemental Figure 3B) or potassium-channel subunits (Supplemental Figure 3C).

### *Transcriptomal analysis*

The results of transcriptomal analysis are shown in **Figure 8**. Large, statistically-significant differences were seen between AF-dogs and AFL-substrate+SR dogs in extracellular-matrix (ECM) pathways and pathways involved in inflammation (Figure 8A). In contrast, AFL-substrate+AFL dogs (when compared to AFL-substrate+SR) showed no evidence of inflammatory pathway dysregulation and only relatively modest changes in ECM-organization pathways (11 pathways vs 29 for AF vs AFL-substrate+SR;  $-\log_{10}(\text{p value})$  of 4.2 vs 9.7; **Figure 8B**). When AF was compared to AF-substrate+AFL, enrichment in ECM and inflammatory pathways was noted (**Figure 8C**), and the superposition of AF on an AFL-substrate strongly activated ECM-pathways when compared to AFL alone (Figure 8D). An analysis of the pathways exclusive to AF-dogs vs AFL-substrate+SR (obtained by subtracting those occurring with AFL-substrate+AFL vs AFL-substrate+SR) revealed strong dysregulation of inflammation-related signaling (Supplemental Figure 4A), whereas no similar changes were seen for pathways exclusively altered in AFL-dogs (Supplemental Figure 4B). A closer look at the differential-pathway reactome in AF (Supplemental Figure 5) shows a wide range of inflammatory pathways and ECM-related signaling (the most significant changes are in bright yellow), whereas none of these pathways were seen with AFL (Supplemental Figure 6). Inflammation-related pathways uniquely associated with AF included cytokine and interleukin signaling, as well as tumor necrosis factor-alpha receptor pathways. ECM systems included

ECM-organization, elastin-fiber related molecules and the control of collagen-fiber trimer formation. Taken together, the transcriptomic data indicate that AF activates inflammatory and ECM signaling pathways that are unaffected with sustained AFL.

## **Discussion**

### *Main findings*

We have found that sustained AFL induces atrial refractory period and APD abbreviation similar to that caused by AF, along with significantly enhanced vulnerability to atrial tachyarrhythmia induction by atrial extrasystoles. However, unlike the atrial-remodeling effect of sustained AF, whether in the presence or absence of an AFL-substrate, AFL alone did not induce atrial fibrosis, slow atrial conduction, cause activation of inflammation or ECM pathways, or increase the sustainability of AF (**Central Illustration**).

### *Comparison to previous studies of atrial remodeling*

The literature contains extensive information about AF-associated remodeling (13). Sustained AF abbreviates atrial APD and ERP, by a combination of effects on atrial K<sup>+</sup>- and Ca<sup>2+</sup>-currents, and ERP-changes are closely linked to increased vulnerability to AF-induction by atrial premature beats (13). In addition, AF is closely linked to atrial fibrosis, although the evidence that AF itself causes fibrosis in the absence of LV-function changes is somewhat less clear (13). Much less is known about the atrial remodeling caused by AFL. Short episodes (average duration 8.6 minutes) of AFL reduce atrial ERP, with the changes reversing rapidly (within 10 minutes) after rhythm reversion (6). Chronic AFL (mean duration 17 months) also showed ERP-abbreviation, which was much slower to reverse, with reversal noted 30 days after conversion to sinus rhythm (6). The monophasic-APD changes after conversion to sinus rhythm of chronic AFL are quantitatively similar to those with AF (5). Atrial tachypacing at a rate

similar to that of AFL in man (300 bpm) produces atrial ERP-shortening similar to that caused by a rate that reproduces AF-induced ERP-remodeling, 400 bpm (14).

Only one experimental study has examined the atrial remodeling caused by sustained AFL (12). Morton et al. studied AFL in sheep, induced by a surgical Y-shaped lesion similar to the substrate that we studied and maintained for a period of 28 days. They noted a marked and rapid decrease in atrial ERP, which was statistically significant within 3 days, reaching an approximately 45%-decrease at 28 days (12). Conduction-velocity changed more slowly, approaching steady-state (a 28%-decrease) between 14 and 28 days (12). They did not compare the changes to those caused by AF, did not assess structural remodeling and did not examine the effects of AF superimposed on an AFL-substrate.

Our study is the first to compare the effects of sustained AFL on atrial electrophysiology and structure, to compare the changes to those produced by AF and to study the effects of AF occurring in the presence of a structural substrate for AFL. We found that AFL and AF produced binary remodeling effects- while AFL-induced remodeling fully resembled that caused by AF on atrial ERP and atrial vulnerability, only AF caused significant structural remodeling and enhanced AF-sustainability (whether in the absence or presence of an AFL-substrate). When sustained AF was superimposed on an underlying AFL-substrate, the induced tachyarrhythmia had features intermediate between those of AFL and AF (**Figure 4**), with greater regularity than AF but a significantly shorter cycle-length and greater irregularity than AFL induced with an AFL-substrate and AFL-induced remodeling. Similarly, only AF (whether in the absence or presence of an AFL-substrate) produced significant LA-enlargement. Of note, the significant LA fibrosis (**Figure 7**) and conduction-slowing (**Figure 6**) caused by AF occurred in the absence of any LV-dysfunction (**Figure 5C**), in agreement with experimental data suggesting that rapid

atrial-cardiomyocyte firing can activate atrial fibroblasts directly by secreting profibrotic molecules (9).

Prior studies of AF-associated remodeling have suggested a variety of mechanisms that may contribute to conduction-slowing. These include atrial fibrosis (15), connexin-changes (15–17) and Na<sup>+</sup>-channel downregulation (18). In the present study, significant Na<sup>+</sup>-channel expression-changes occurred exclusively in the AF-only dogs (Supplemental Figure 3A), as did connexin-40 downregulation (Supplemental Figure 2C); neither of these alterations were noted in dogs with AF+AFL-substrate despite their conduction-slowing which equalled that in AF-only dogs. Thus, it is unlikely that the connexin-40 or Na<sup>+</sup>-channel expression changes contributed significantly to conduction slowing. On the other hand, clear atrial fibrosis and collagen-gene upregulation occurred only in AFL-substrate+AFL and AF dogs (**Figure 7**), closely paralleling the changes in conduction-velocity (**Figure 6**) and suggesting a primary role for atrial fibrosis in the latter. Connexin-43 was not significantly altered in any group (**Figure 2B**), although a trend to decreased phospho-connexin-43 expression was seen in AF-only dogs.

#### *Study Limitations*

We created an anatomical substrate for AFL with an approach similar to that originally described by Frame et al. in acute experiments (11) and later applied by Morton et al. (12) to induce sustained AFL in sheep (12). We documented the responsible macroentry circuit with optical mapping and found that AFL was sustained in AFL-substrate+AFL dogs without the need for reinduction.

We used radiofrequency-induced atrioventricular block and ventricular demand pacing at 80 bpm to preclude any possibility of tachycardiomyopathy. While this approach produces atrioventricular dissociation, which could result in atrial remodeling as previously reported in a

goat model (19), this is unlikely in our study as no differences were seen in atrial vulnerability, LA-ERP, or the duration of atrial tachyarrhythmia (after ablation of the flutter substrate) between the AFL-substrate+SR group (followed for 3 weeks in sinus rhythm with atrioventricular-block and ventricular pacing) and acute control dogs (Supplemental Figure 1).

We cannot exclude the possibility that atrial remodeling might be different with longer-lasting AFL and/or AF, as our arrhythmia duration was limited to 3 weeks.

#### *Novel elements and potential significance*

The present study is the first to examine atrial electrical and structural remodeling in a chronic model of AFL in comparison to the results of electrically-maintained AF for the same period and to analyze the results of AF-induced remodeling superimposed on an AFL-substrate as would occur in AFL-patients that develop AF. AFL-induced remodeling differed from that caused by AF (with or without an AFL-substrate) by failing to cause atrial fibrosis or collagen-gene upregulation. This finding suggests that the extensive right-atrial conduction disturbances and evidence of scarring seen in AFL patients (10,20) are likely due to the atrial cardiomyopathy causing AFL rather than due to the AFL itself.

We also noted prominent activation of inflammatory pathways in AF that were absent in AFL. There is extensive evidence for a role of chronic inflammation in the AF-substrate (21). Recent work points to the participation of cardiomyocyte inflammatory signaling in the development of paroxysmal (22), persistent (22) and post-operative (23) AF, along with the effectiveness of targeting unresolved atrial inflammation in suppressing inflammatory signaling and AF-promoting fibrosis in an animal model of AF associated with right-heart disease (24). Atrial fibrosis is now a well-established mechanistic paradigm in AF (25). The present findings extend this prior work by linking AF-induced inflammatory and ECM pathway activation to



atrial fibrotic remodeling and associated AF-promotion and by showing that, in contrast, these changes are not induced by a similar duration of AFL. Further work is needed to determine more precisely why and how these changes are produced by AF and not AFL.

Our findings may also have therapeutic implications. Since AF-associated fibrosis appears to be largely irreversible (15,26,27) it may be important to detect AF occurring in AFL patients and intervene early to prevent the progression of fibrosis due to AF-induced structural remodeling, which might limit the effectiveness of therapy by catheter ablation.

### **Conclusions**

We have used a chronic dog model of sustained AFL to probe the atrial remodeling associated with an AFL-substrate, depending on whether the associated rhythm is sinus, AFL or AF, and to compare this to the remodeling caused by AF alone. Sustained AFL causes refractory-period and action-potential duration abbreviation like that of AF, along with enhanced atrial vulnerability to extrastimulation, but unlike AF does not induce structural remodeling. When AF is superimposed on an AFL-substrate, substantial structural remodeling occurs. These results provide new insights into AFL-induced remodeling and suggest that early intervention may be important to prevent further irreversible atrial fibrosis when AF occurs in an AFL-patient.

## **Perspectives**

Competency in Medical Knowledge: Atrial flutter and atrial fibrillation both remodel the atrium, but only atrial fibrillation causes inflammatory signaling and fibrosis.

Translational Outlook: Future studies should explore interventions that target inflammatory signaling to prevent fibrosis caused by atrial fibrillation.

## References

1. Manolis AS. Contemporary Diagnosis and Management of Atrial Flutter: A Continuum of Atrial Fibrillation and Vice Versa? *Cardiol Rev* 2017;25:289–97.
2. Waldo AL. Atrial fibrillation and atrial flutter: Two sides of the same coin! *Int J Cardiol* 2017;240:251–2.
3. Nattel S, Burstein B, Dobrev D. Atrial remodeling and atrial fibrillation: mechanisms and implications. *Circ Arrhythm Electrophysiol* 2008;1:62–73.
4. Schotten U, Verheule S, Kirchhof P, Goette A. Pathophysiological mechanisms of atrial fibrillation: a translational appraisal. *Physiol Rev* 2011;91:265–325.
5. Franz MR, Karasik PL, Li C, Moubarak J, Chavez M. Electrical remodeling of the human atrium: similar effects in patients with chronic atrial fibrillation and atrial flutter. *J Am Coll Cardiol* 1997;30:1785–92.
6. Sparks PB, Jayaprakash S, Vohra JK, Kalman JM. Electrical remodeling of the atria associated with paroxysmal and chronic atrial flutter. *Circulation* 2000;102:1807–13.
7. Avitall B, Bi J, Mykitysey A, Chicos A. Atrial and ventricular fibrosis induced by atrial fibrillation: evidence to support early rhythm control. *Heart Rhythm* 2008;5:839–45.
8. Martins RP, Kaur K, Hwang E, et al. Dominant frequency increase rate predicts transition from paroxysmal to long-term persistent atrial fibrillation. *Circulation* 2014;129:1472–82.
9. Burstein B, Qi X-Y, Yeh Y-H, Calderone A, Nattel S. Atrial cardiomyocyte tachycardia alters cardiac fibroblast function: a novel consideration in atrial remodeling. *Cardiovasc Res* 2007;76:442–52.
10. Stiles MK, Wong CX, John B, et al. Characterization of atrial remodeling studied remote from episodes of typical atrial flutter. *Am J Cardiol* 2010;106:528–34.

11. Frame LH, Page RL, Hoffman BF. Atrial reentry around an anatomic barrier with a partially refractory excitable gap. A canine model of atrial flutter. *Circ Res* 1986;58:495–511.
12. Morton JB, Byrne MJ, Power JM, Raman J, Kalman JM. Electrical remodeling of the atrium in an anatomic model of atrial flutter: relationship between substrate and triggers for conversion to atrial fibrillation. *Circulation* 2002;105:258–64.
13. Nattel S, Harada M. Atrial remodeling and atrial fibrillation: recent advances and translational perspectives. *J Am Coll Cardiol* 2014;63:2335–45.
14. Shiroshita-Takeshita A, Mitamura H, Ogawa S, Nattel S. Rate-dependence of atrial tachycardia effects on atrial refractoriness and atrial fibrillation maintenance. *Cardiovasc Res* 2009;81:90–7.
15. Burstein B, Comtois P, Michael G, et al. Changes in connexin expression and the atrial fibrillation substrate in congestive heart failure. *Circ Res* 2009;105:1213–22.
16. Igarashi T, Finet JE, Takeuchi A, et al. Connexin gene transfer preserves conduction velocity and prevents atrial fibrillation. *Circulation* 2012;125:216–25.
17. Bikou O, Thomas D, Trappe K, et al. Connexin 43 gene therapy prevents persistent atrial fibrillation in a porcine model. *Cardiovasc Res* 2011;92:218–25.
18. Gaspo R, Bosch RF, Bou-Abboud E, Nattel S. Tachycardia-induced changes in Na<sup>+</sup> current in a chronic dog model of atrial fibrillation. *Circ Res* 1997;81:1045–52.
19. Neuberger H-R, Schotten U, Blaauw Y, et al. Chronic atrial dilation, electrical remodeling, and atrial fibrillation in the goat. *J Am Coll Cardiol* 2006;47:644–53.
20. Medi C, Teh AW, Roberts-Thomson K, Morton JB, Kistler PM, Kalman JM. Right atrial remodeling is more advanced in patients with atrial flutter than with atrial fibrillation. *J Cardiovasc Electrophysiol* 2012;23:1067–72.

21. Harada M, Van Wagoner DR, Nattel S. Role of inflammation in atrial fibrillation pathophysiology and management. *Circulation* 2015;79:495-502.
22. Yao C, Veleva T, Scott L Jr, et al. Enhanced Cardiomyocyte NLRP3 Inflammasome Signaling Promotes Atrial Fibrillation. *Circulation* 2018;138:2227-42.
23. Heijman J, Muna P, Veleva T, et al. Pre-existing atrial inflammatory and calcium/calmodulin-dependent kinase II signaling create a vulnerable substrate for post-operative atrial fibrillation. *Circ Res* (in revision).
24. Hiram R, Xiong F, Naud P, et al. The inflammation-resolution promoting molecule resolving D1 prevents atrial proarrhythmic remodeling in experimental right heart disease. (*Cardiovasc Res*, in revision)
25. Nattel S. Molecular and cellular mechanisms of atrial fibrosis in atrial fibrillation. *JACC Clin Electrophysiol* 2017;3:425-35.
26. Cha T-J, Ehrlich JR, Zhang L, et al. Dissociation between ionic remodeling and ability to sustain atrial fibrillation during recovery from experimental congestive heart failure. *Circulation* 2004;109:412-8.
27. Shinagawa K, Shi YF, Tardif JC, Leung TK, Nattel S. Dynamic nature of atrial fibrillation substrate during development and reversal of heart failure in dogs. *Circulation* 2002;105:2672-78.

## Figure Legends

**Figure 1. Overview of atrial flutter chronic dog model.** A) Schematic of the Y-shaped lesion and anatomical substrate for AFL (1=Surgical Y-shaped lesion, 2=macroreentrant circuit across right atrium); B) Right lateral atrial photograph showing radiofrequency-induced Y-shaped lesion (blue arrows); C) EKGs from AFL-dogs at different time-points during follow-up, D), E) Right atrial endocavity mapping at 3 weeks in an atrial-flutter dog. Dual-loop macroreentry maintained the arrhythmia, with counterclockwise lateral right atrial (D, longer dashes) and clockwise para-tricuspid (E, shorter dashes) circuits. IVC=inferior vena cava; RAA=right atrial appendage; SVC=superior vena cava.

**Figure 2. Schematic of groups and interventions.** Parallel groups were studied (n=6/dog-group), to evaluate effects of spontaneously-maintained AFL in the presence of an AFL-substrate, electrically-maintained AF in the presence of an AFL-substrate and electrically-maintained AF without an AFL-substrate, in comparison to a control group with SR in the presence of an AFL-substrate. Abbreviations: AFL=atrial flutter; AF=atrial fibrillation; ATP=atrial tachypacing; SR=sinus rhythm.

**Figure 3. Electrophysiological effects of 3-week atrial flutter (AFL) and AF.** Changes in A) left-atrial (LA) effective refractory period (ERP); B) Inducibility of AF and/or AFL; C) AF/AFL duration; D) Inducibility of AF. For selected analyses, results are shown before and after ablation of AFL-substrate. \* P<0.05, \*\*P<0.01, \*\*\* P<0.001 versus AFL-substrate + SR, ### P<0.001 versus AFL-substrate+AFL, †††P<0.001 versus before AFL-ablation, by 1-way ANOVA with Tukey-test. Abbreviations: AFL=atrial flutter; AF=atrial fibrillation; SR = sinus rhythm.

**Figure 4. Tachyarrhythmia properties after 3-week AFL and/or AF.** A) Examples of atrial electrograms during tachycardia. B) Mean+SEM tachycardia cycle-lengths; C) Mean+SEM

standard deviation (SD) of cycle-lengths during tachycardia; # P<0.05, ## P<0.01 versus AFL-substrate+AFL by 1-way ANOVA with Tukey-test. †††P<0.001 vs. AFL Substrate+AF by 1-way ANOVA with Tukey-test. AF=atrial fibrillation; AFL=atrial flutter; SR=sinus rhythm.

**Figure 5. Echocardiographic changes with 3-week AFL and AF.** Echocardiographic indices are shown for: A) Left-atrial (LA) volume; B) LA Emptying Fraction; C) Left-ventricular ejection fraction (LVEF). \* P <0.05 versus AFL-substrate+SR by 1-way ANOVA with Tukey-test. # P<0.05 versus AFL-substrate+AFL by 1-way ANOVA with Tukey test. AF=atrial fibrillation; AFL=atrial flutter; SR=sinus rhythm.

**Figure 6. Effects of 3-week AFL and/or AF on conduction and repolarization properties on optical mapping.** A) Representative left-atrial activation maps from each group; B) Mean+SEM Conduction Velocities; C) Mean±SEM action-potential duration (APD). \*P<0.05, \*\*P<0.01 versus AFL-substrate+SR by 1-way ANOVA with Tukey-test. # P<0.05, ## P<0.01 versus AFL-substrate+AFL by 1-way ANOVA with Tukey-test. AFL=atrial flutter; AF=atrial fibrillation; AFLs=atrial flutter substrate; APD=action potential duration; BCL=basal cycle length; SR=sinus rhythm.

**Figure 7. Structural remodeling with 3-week AFL and/or AF. Effects on atrial fibrosis (A and B)) and the expression of collagen genes Col1A1 and Col3A1 (C)).** \* P<0.05, \*\* P<0.01, \*\*\* P<0.001 by 1-way ANOVA with Tukey test. # P<0.05, ## P<0.01, ### P<0.001 versus AFL substrate + AFL by 1-way ANOVA with Tukey-test. AF=atrial fibrillation; AFL=atrial flutter; SR=sinus rhythm.

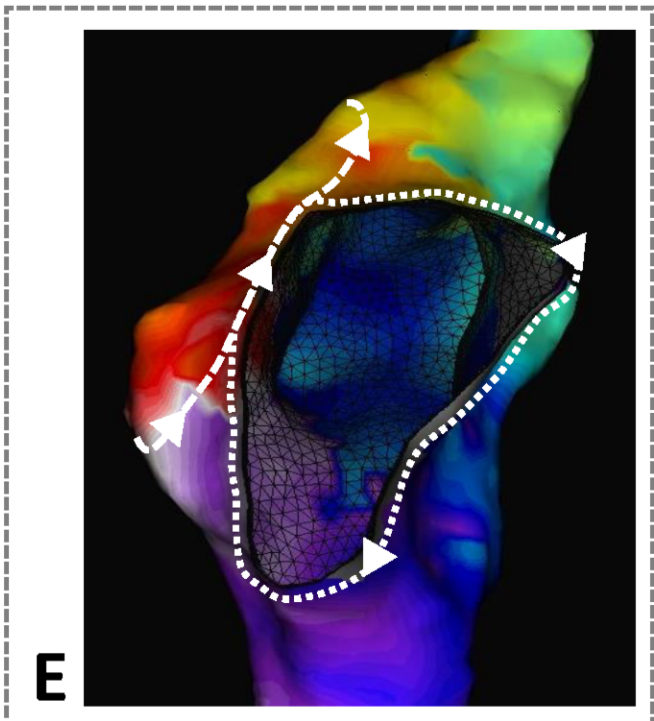
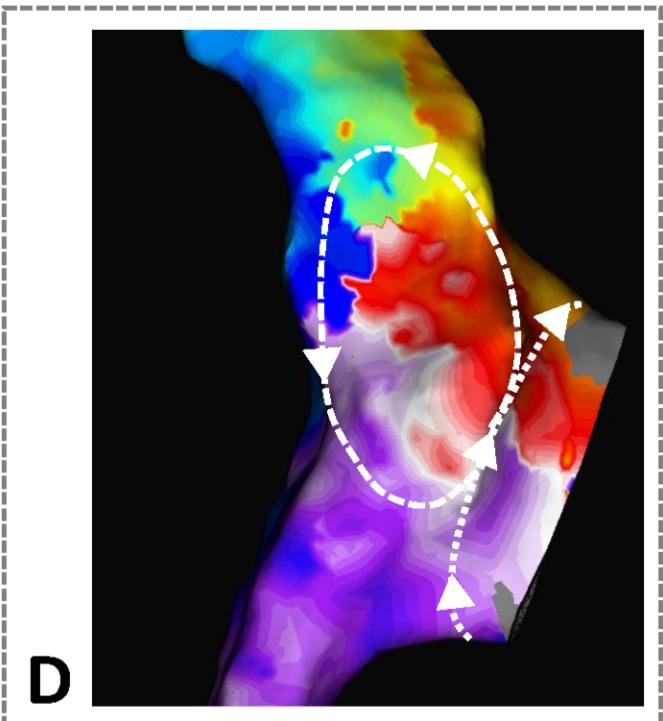
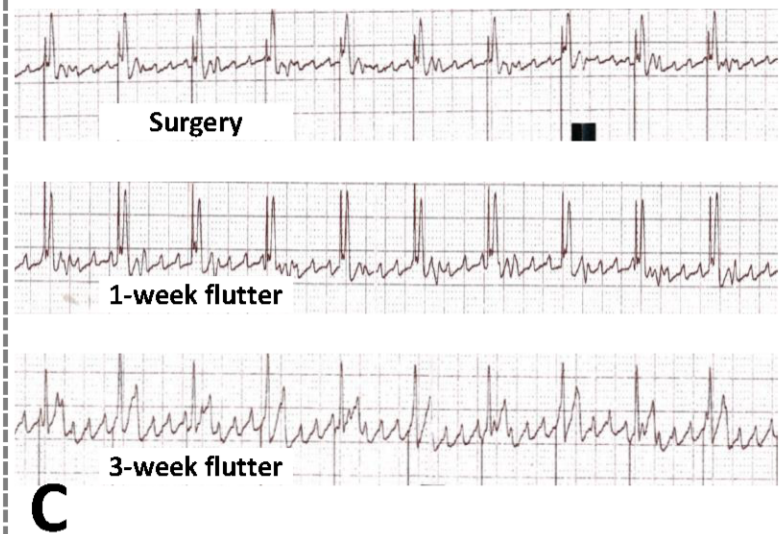
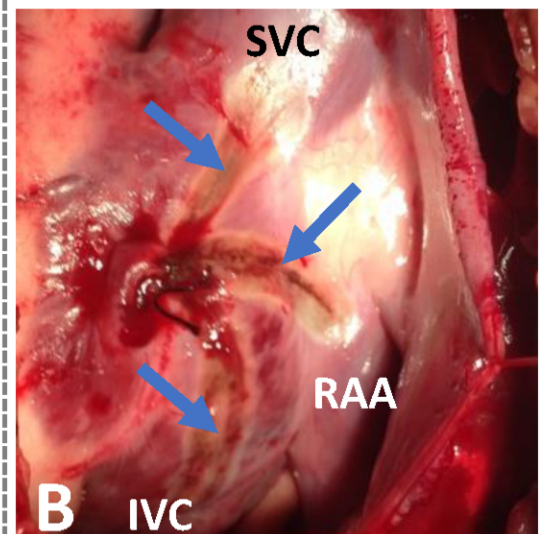
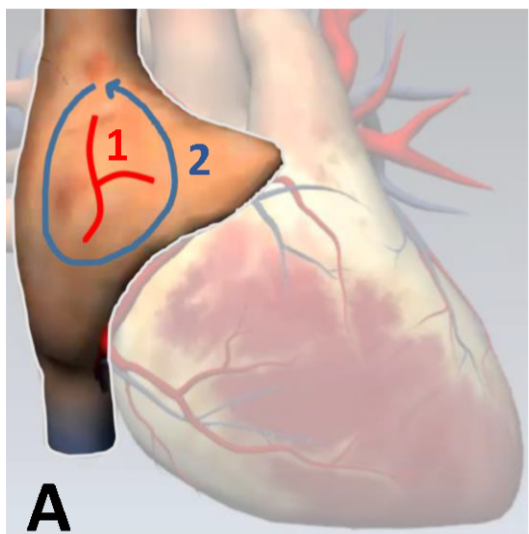
**Figure 8. Gene ontology analysis.** The x axes represent the  $-\log_{10}$  (p value) showing the over-represented pathways according to the identified differentially expressed genes for: A) AF vs AFL substrate+SR; B) AFL substrate+AFL vs AFL substrate+SR; C) AF vs AFL

substrate+AFL; D) AFL substrate+AFL vs AFL substrate+AFL). In brackets are the numbers of differentially expressed genes in the pathway/total number of genes in the pathway. (N=6 animals per intervention). AF=atrial fibrillation; AFL=atrial flutter; SR=sinus rhythm.

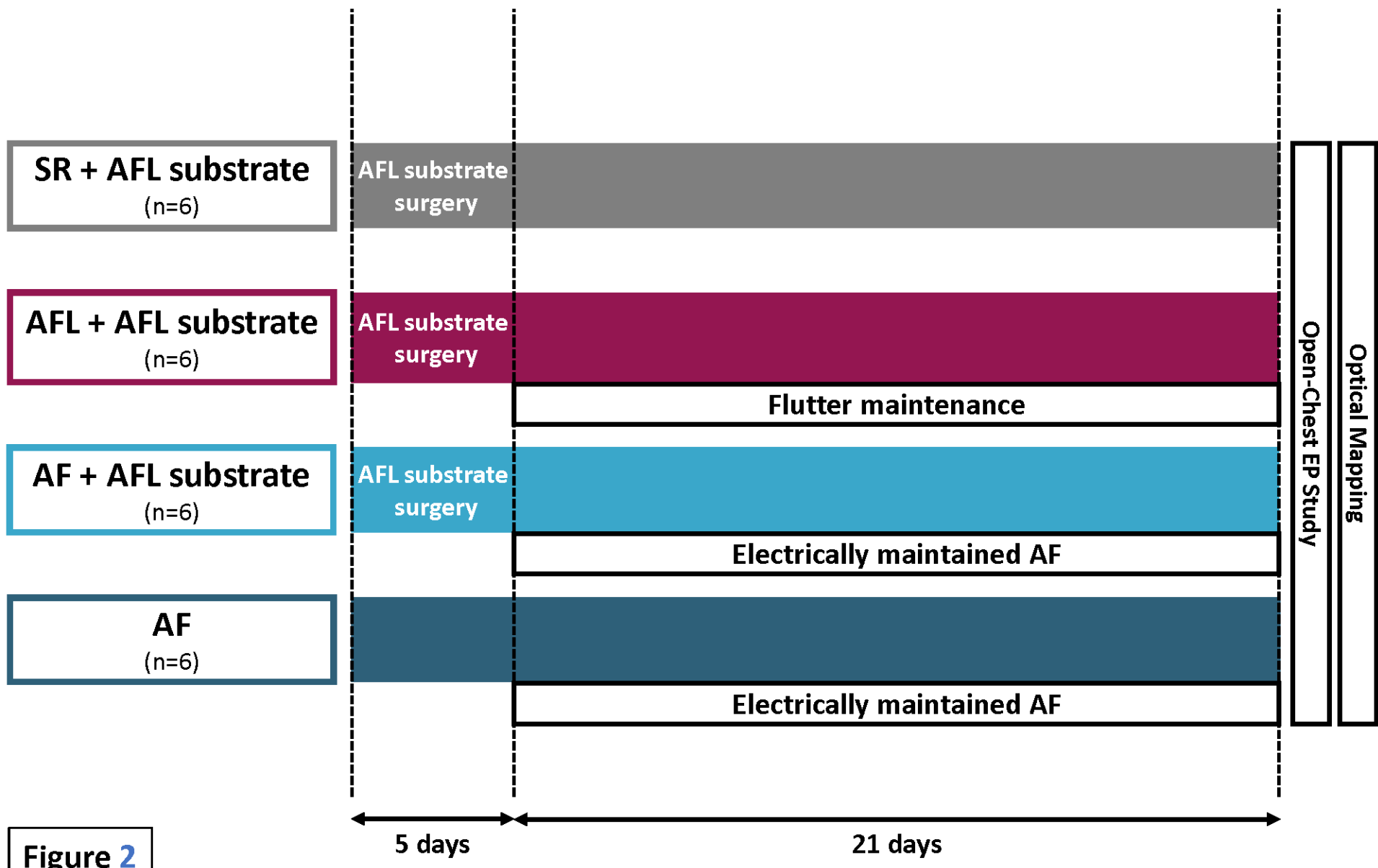
**Central Illustration: Differential arrhythmia-induced atrial remodeling between sustained AFL and sustained AF.** AFL and AF reduced APD and ERP, increasing vulnerability to AF-induction by premature extrasystoles, while only AF produced inflammatory signaling associated with LA-dilation, fibrosis and conduction slowing, and increased AF-persistence. Abbreviations: AFL=atrial flutter, AF=atrial fibrillation, APD=action potential duration, ERP=effective refractory period, LA=left atrium, ECM=extracellular matrix.

**Video 1: Anterolateral (left) and anteroinerior views (right) of right atrium.** In vivo endocavity mapping (EnSite 3D-mapping system; St. Jude Medical, St. Paul, MN) showed a stable macroreentry circuit as indicated by the movie, showing electrical activation in white. The inset shows an isochrone map during one cycle, with the direction of the reentrant wavefront shown by the white dashed lines. Dual-loop macroreentry maintained the arrhythmia, with counterclockwise lateral right atrial (longer dashes) and clockwise para-tricuspid (shorter dashes) circuits.

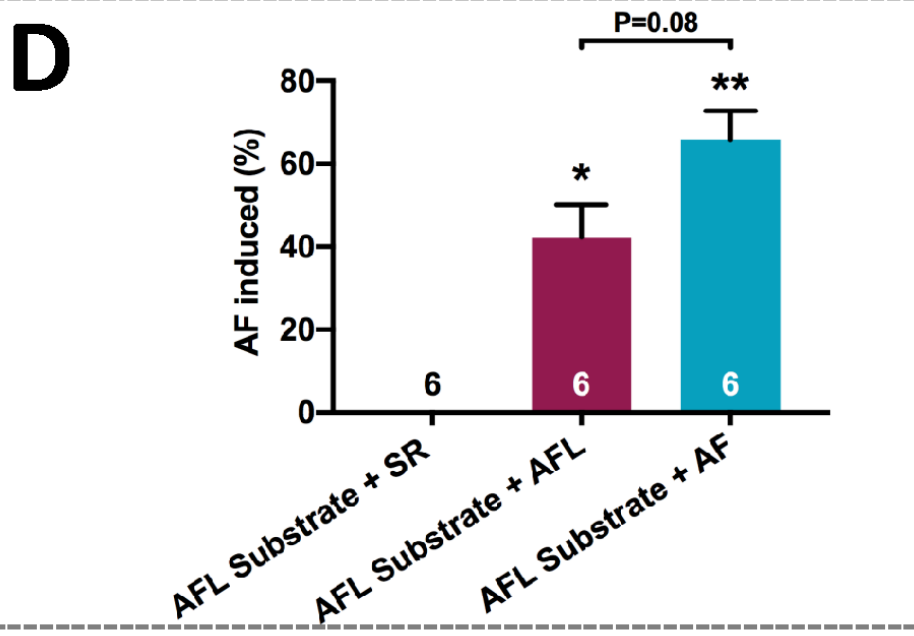
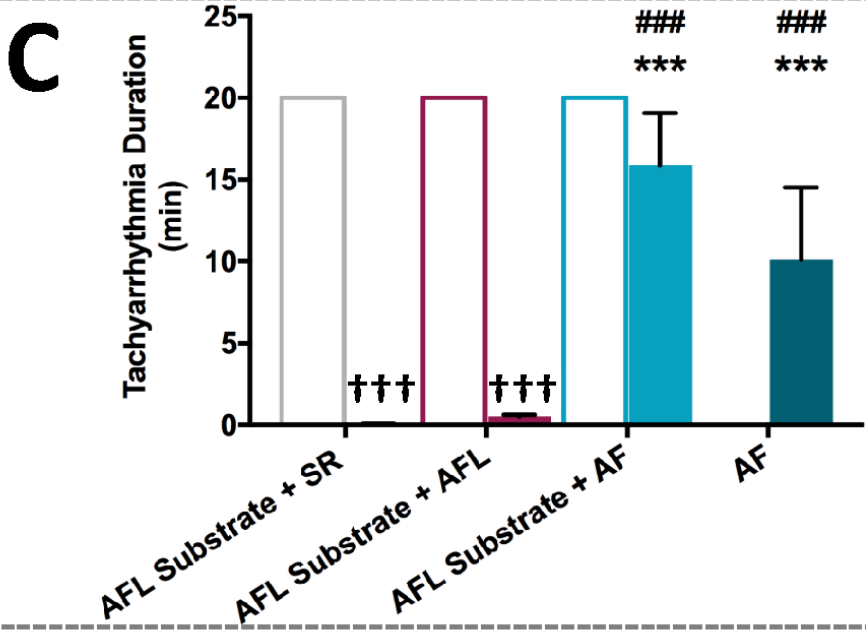
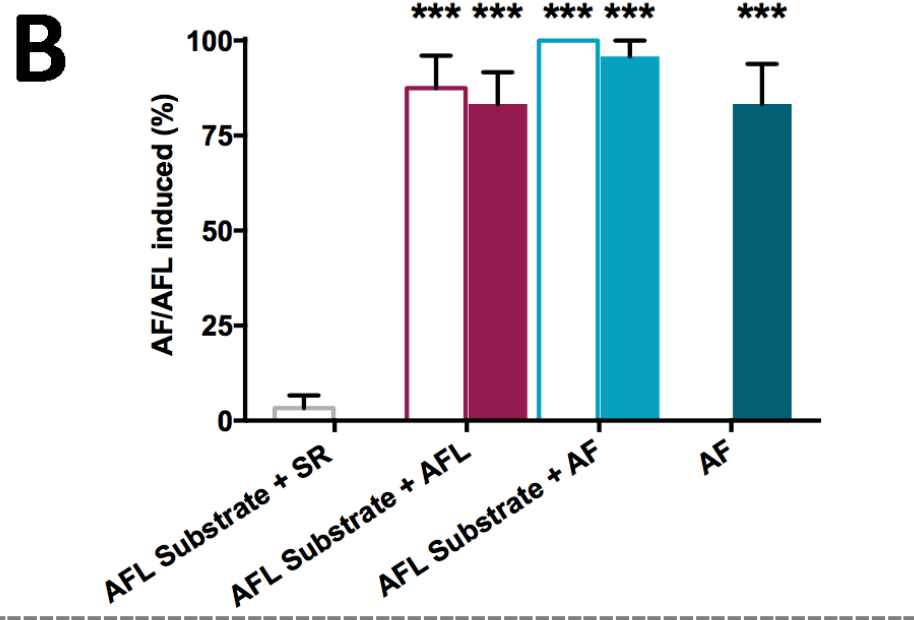
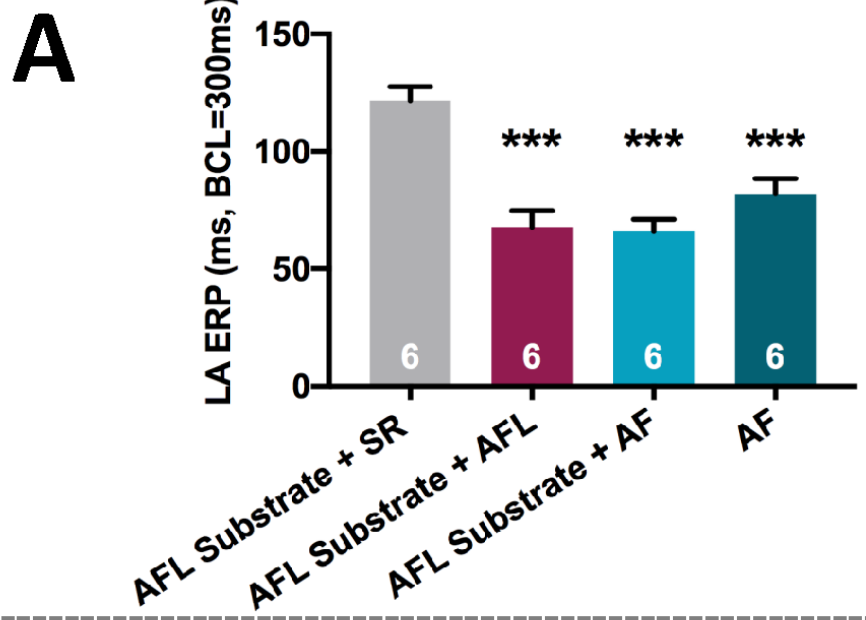




**Figure 1**



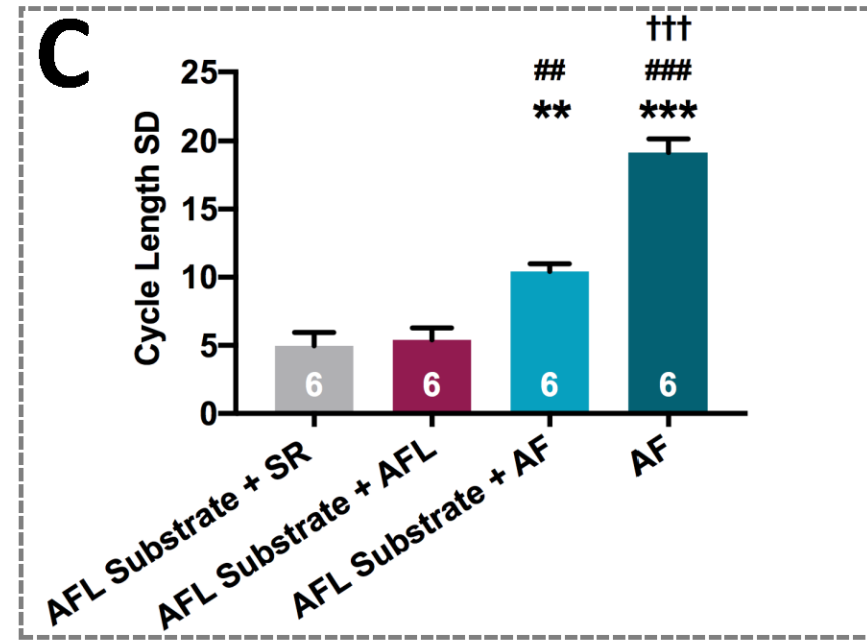
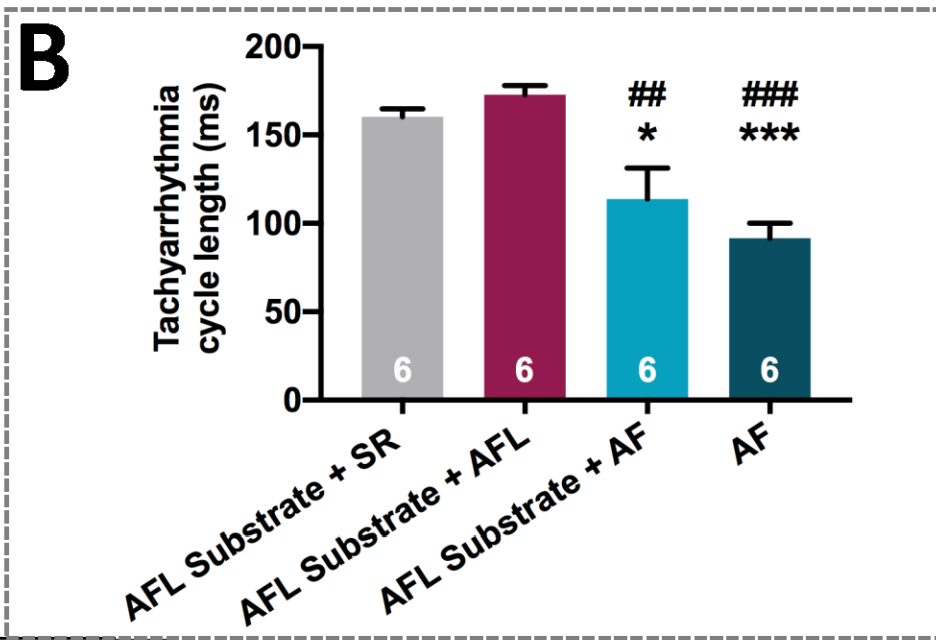
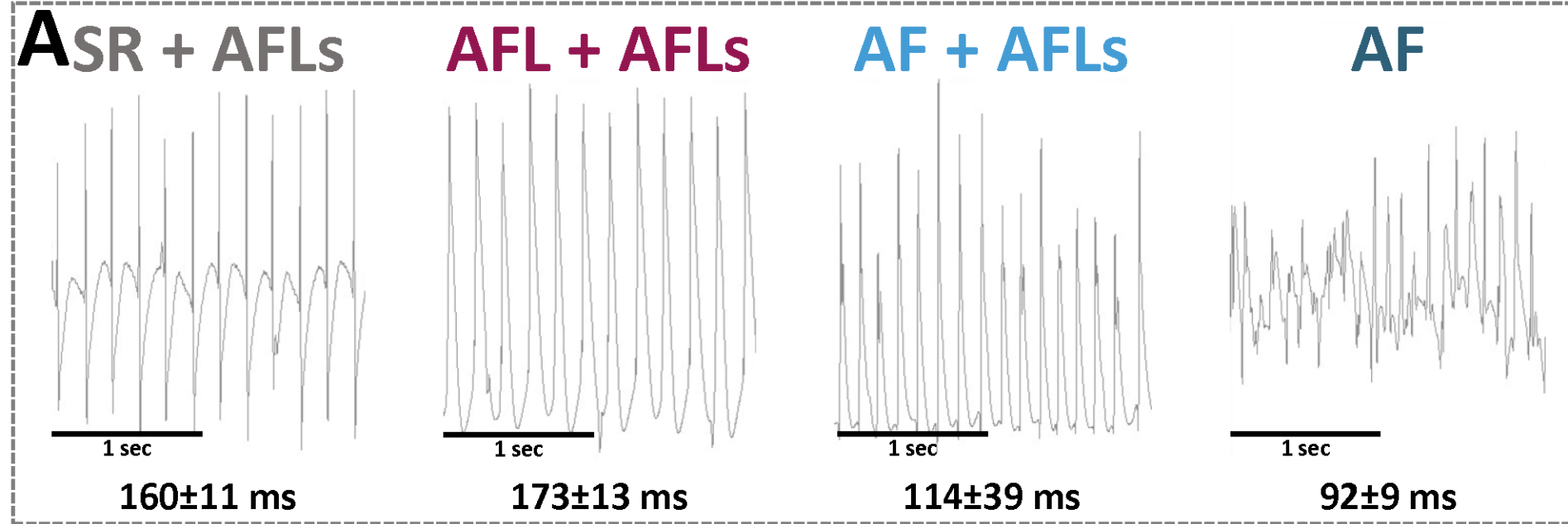
**Figure 2**



□ Before AFL Ablation    ■ After AFL Ablation

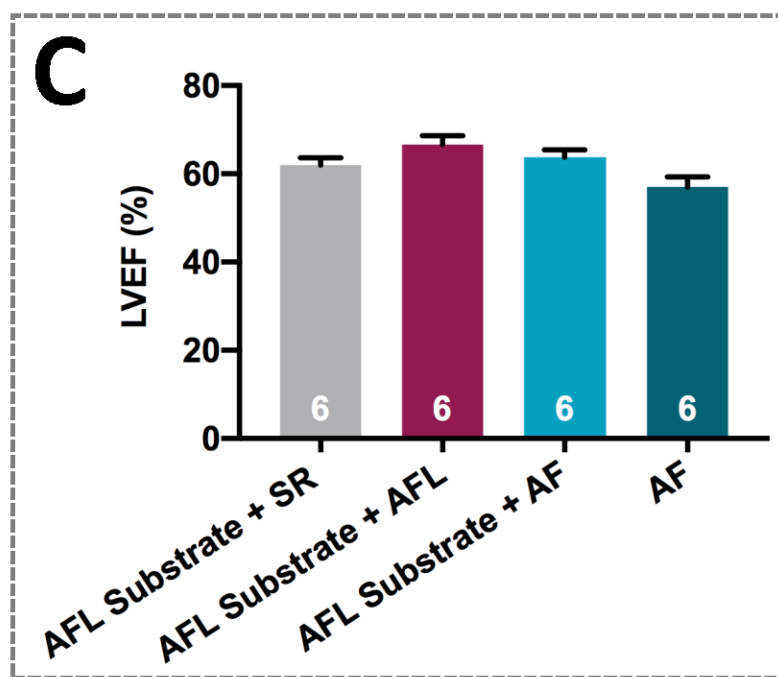
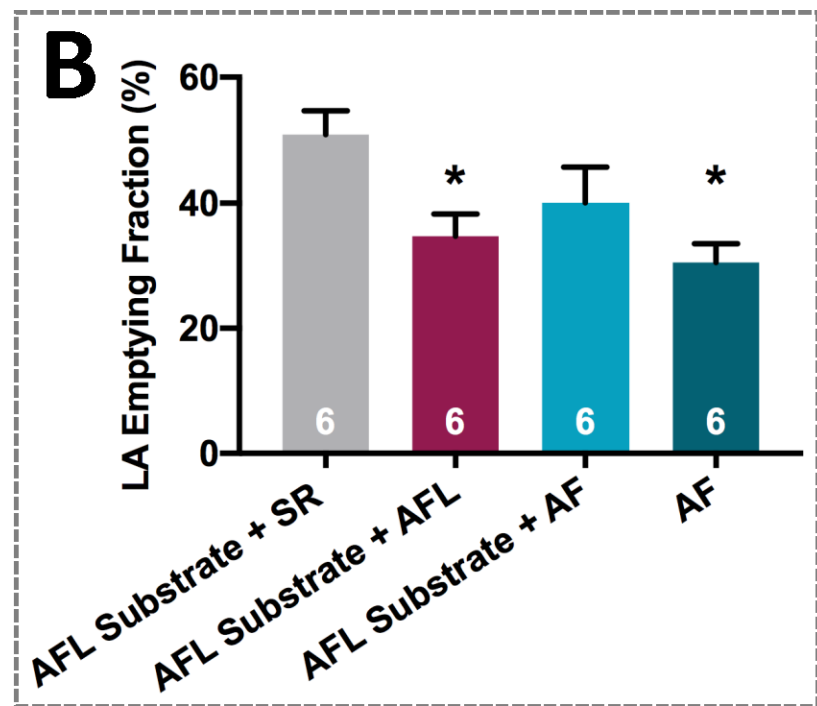
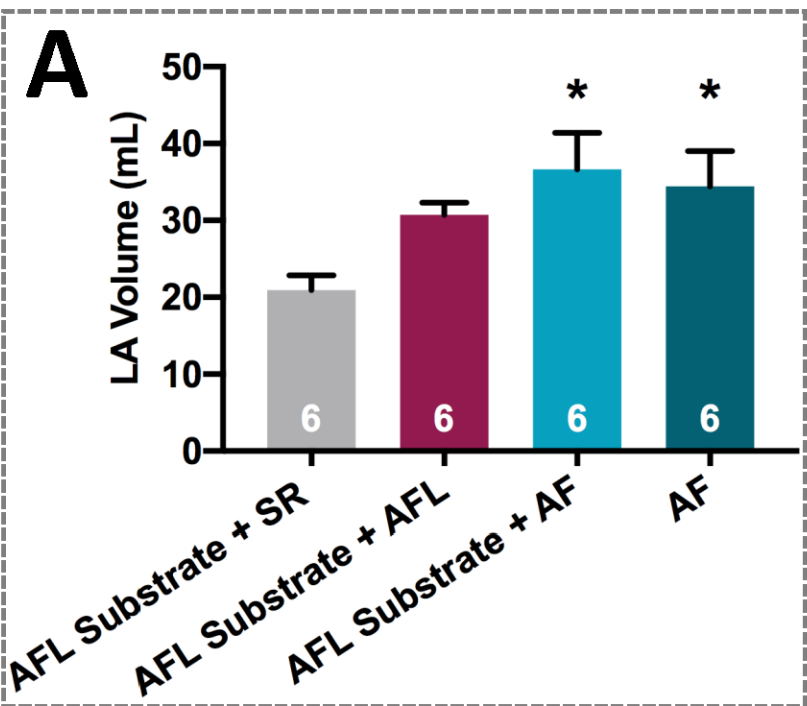
**Figure 3**

\* P<0.05, \*\* P<0.01, \*\*\* P<0.001 vs. AFL substrate + SR; ### P<0.001 vs. AFL substrate + AFL  
 ††† P<0.001 vs. Before AFL ablation



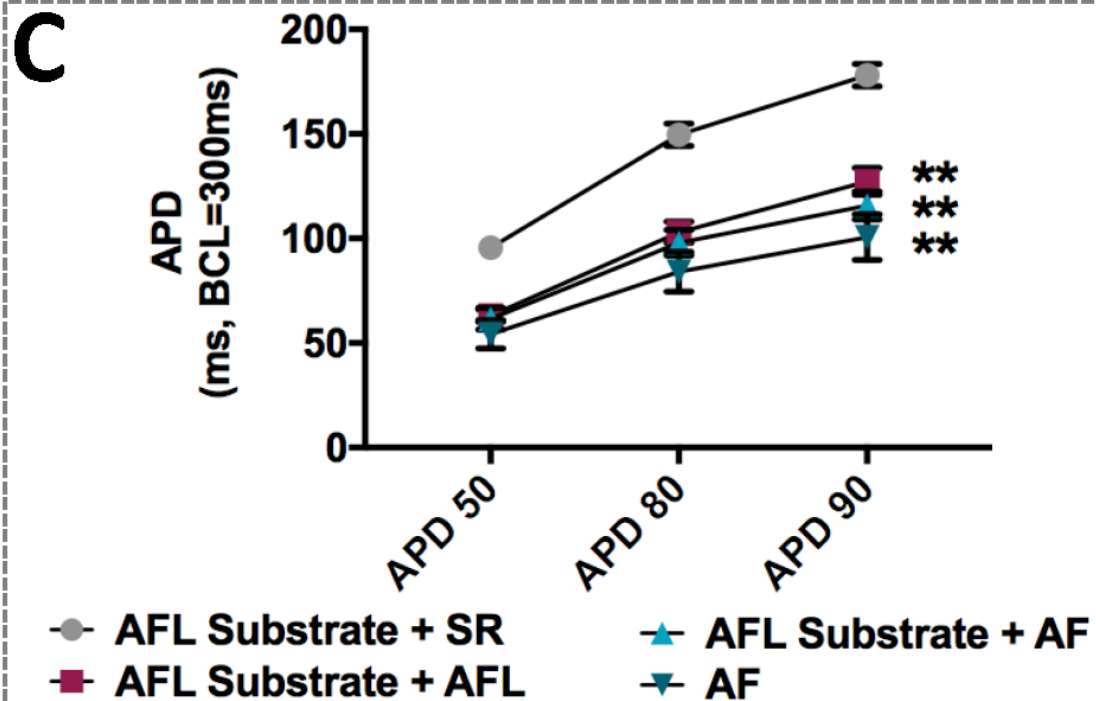
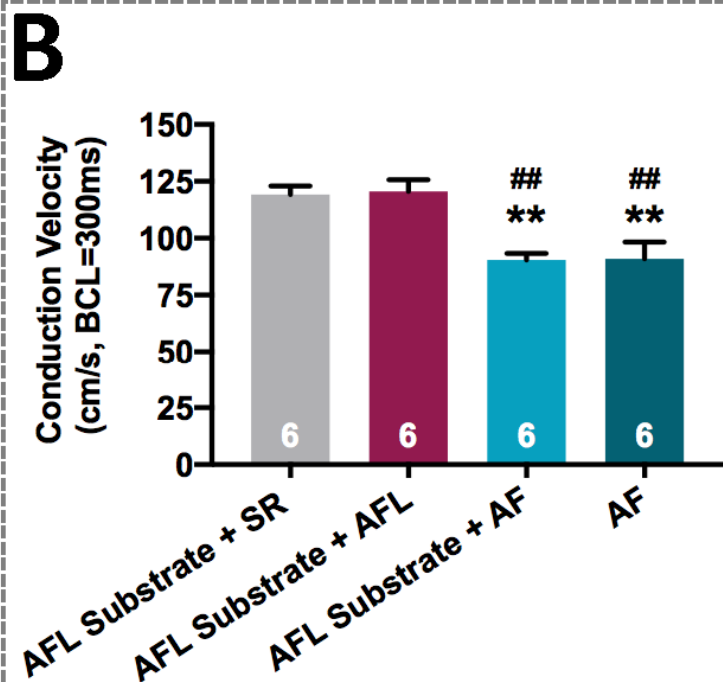
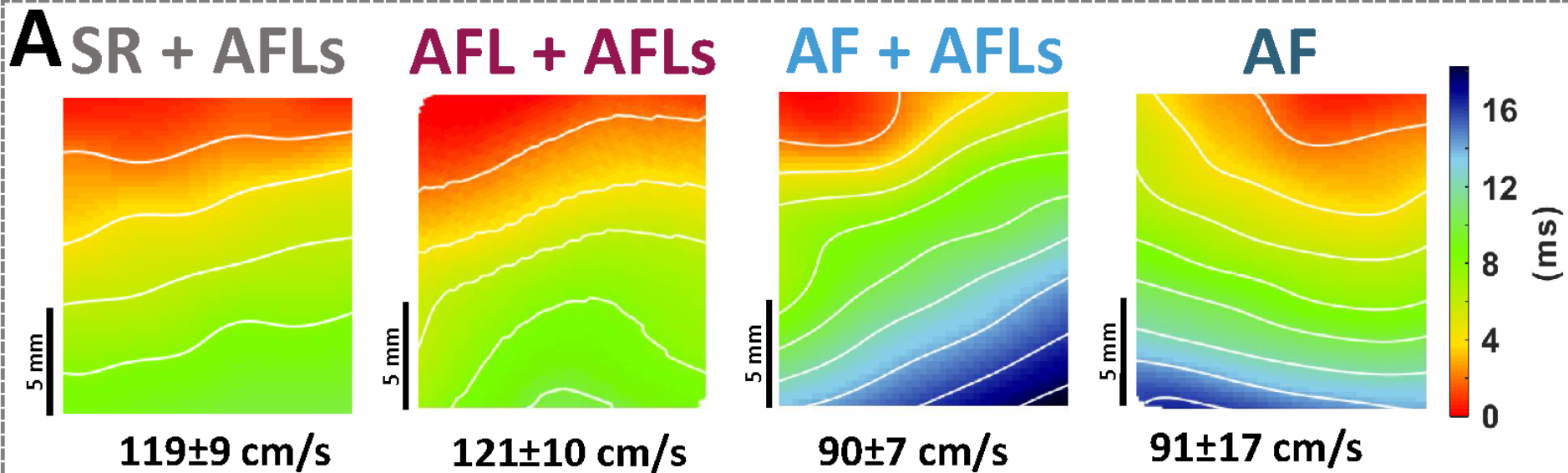
**Figure 4**

•  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$  vs. AFL Substrate+SR; ##  $P < 0.01$  ###  $P < 0.001$  vs. AFL Substrate+AFL; †††  $P < 0.001$  vs. AFL Substrate+AF



\* P<0.05 vs. AFL substrate + SR

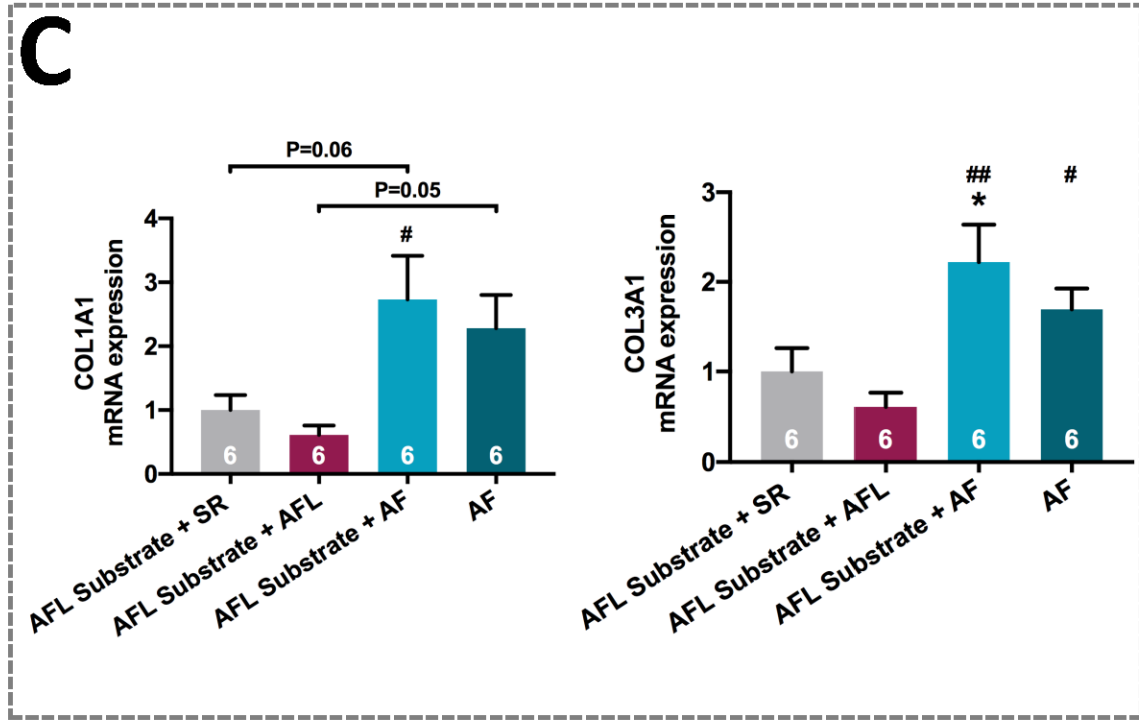
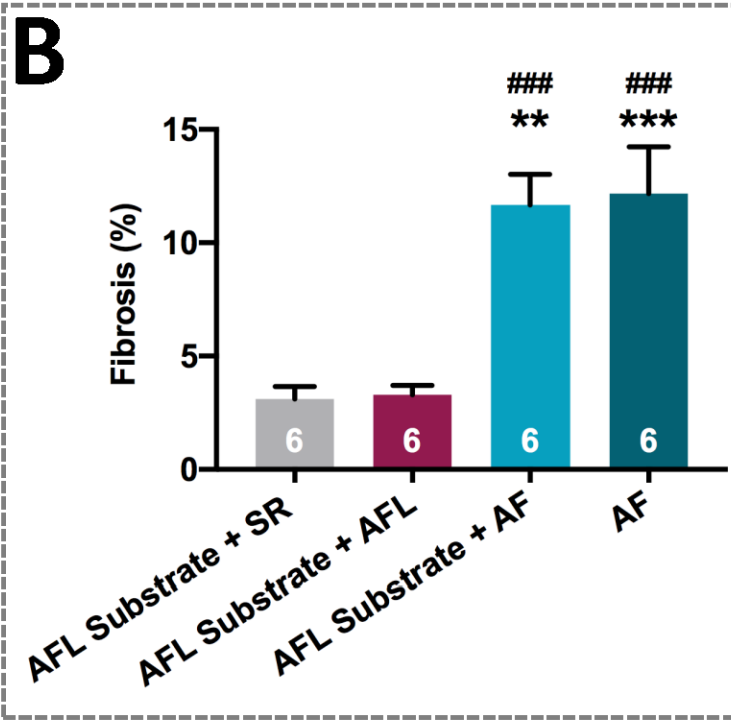
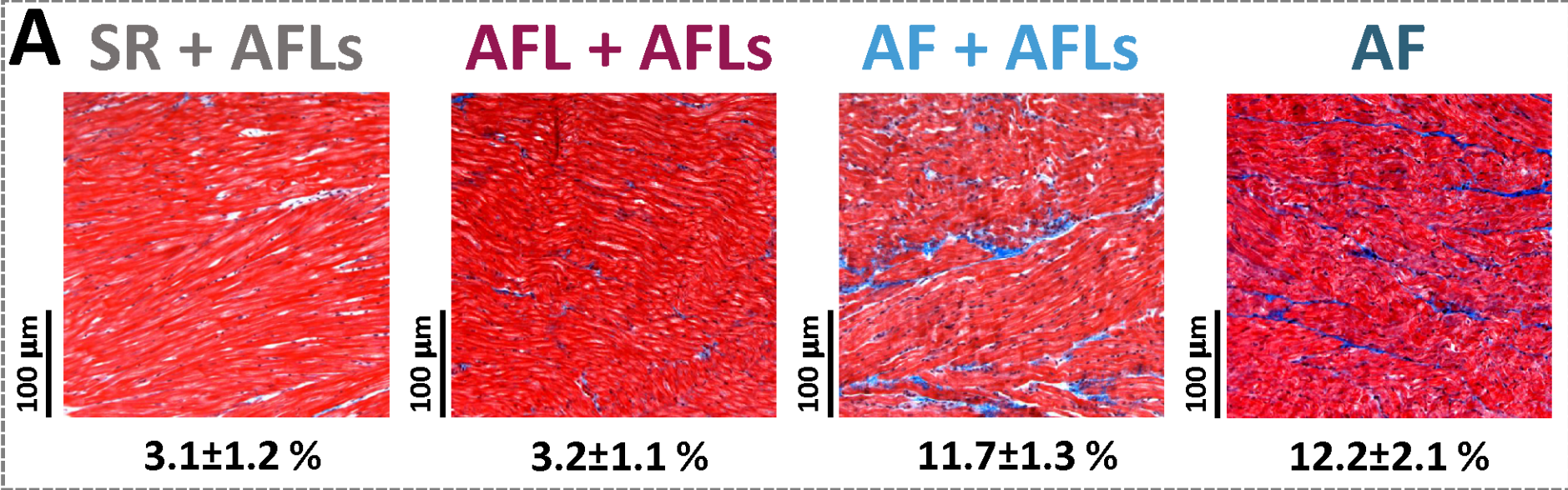
Figure 5



**Figure 6**

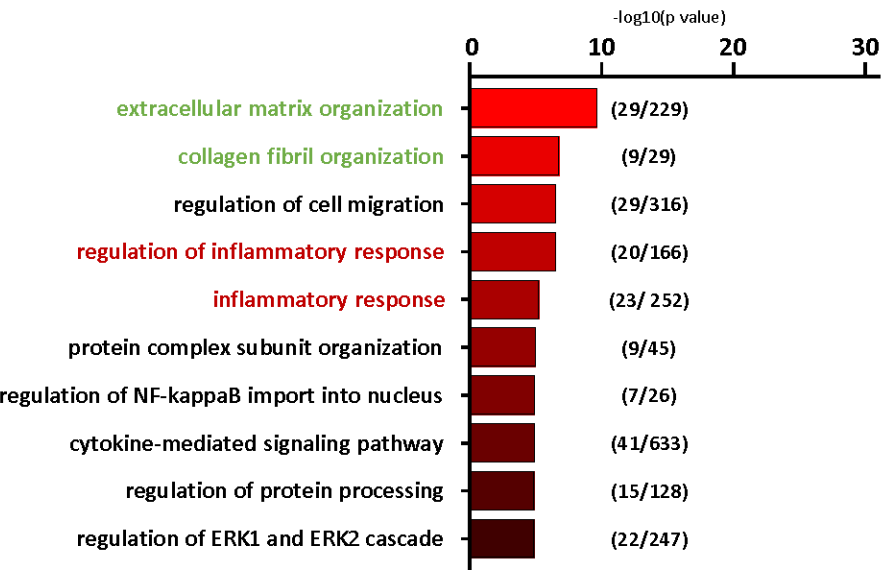
# P<0.05, ## P<0.01 vs. AFL substrate + AFL

\* P<0.05, \*\* P<0.01 vs. AFL substrate + SR

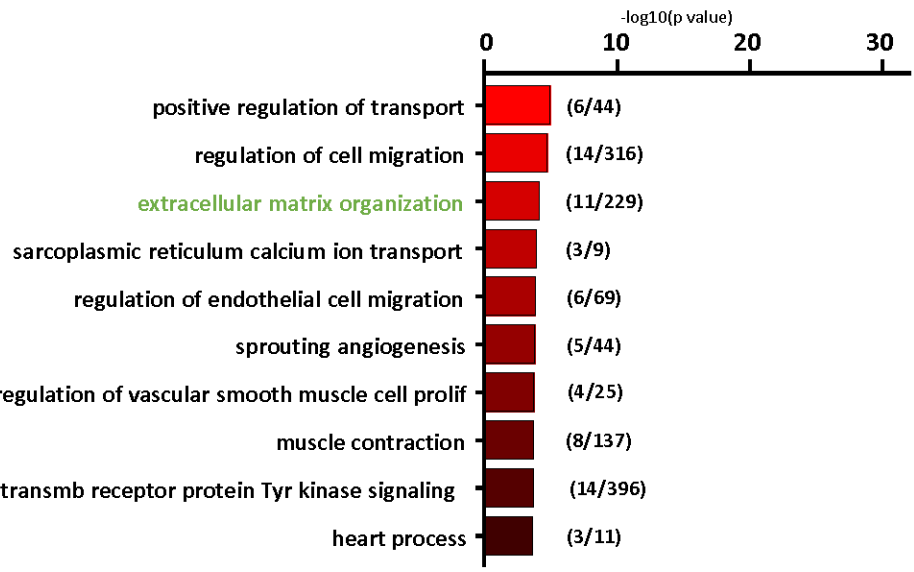


**Figure 7** \* P<0.05, \*\* P<0.01, \*\*\* P<0.001 vs. AFL substrate + SR # P<0.05, ## P<0.01, ### P<0.001 vs. AFL substrate + AFL

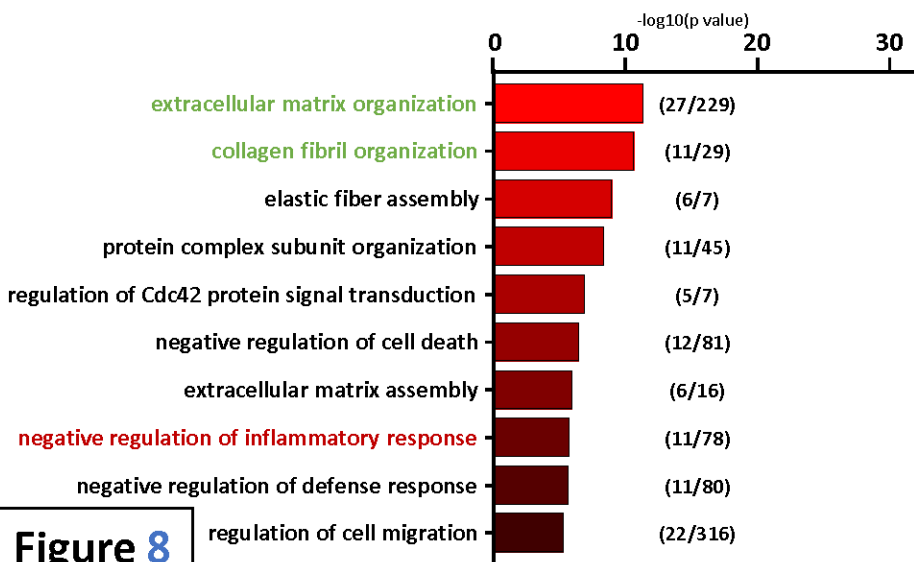
# A AF vs SR+AFLs



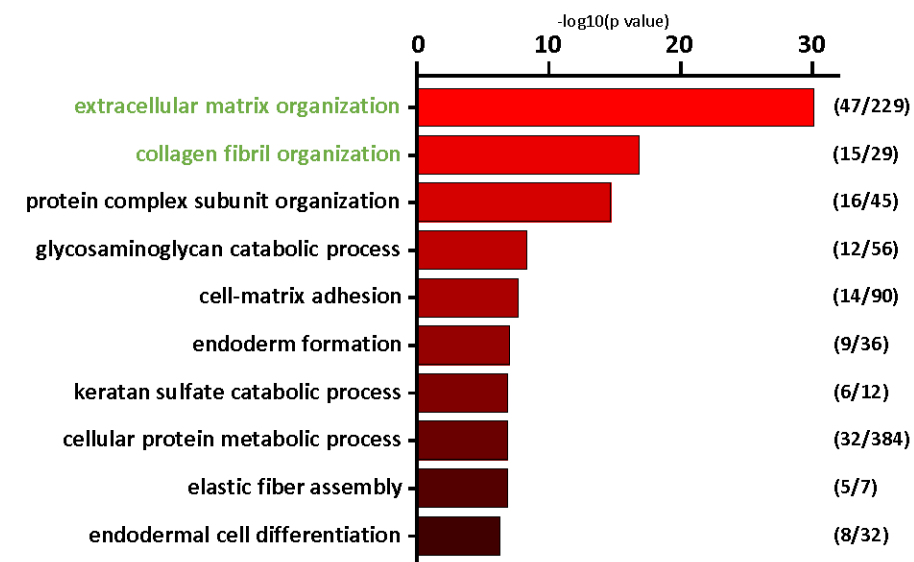
# B AFL+AFLs vs SR+AFLs



# C AF vs AFL+AFLs



# D AF+AFLs vs AFL+AFLs



**Figure 8**