

SUPPLEMENTAL MATERIAL

Data S1.

SUPPLEMENTAL METHODS

Estimation of P-wave Alternans

The customized algorithm for the estimation of PWA begins with QRS detection. Following initial QRS detection²⁷, fiducial points (R-waves) are identified using a template-matching based QRS detection algorithm²⁶. Using these fiducial points, an isoelectric PR segment is selected for every beat and is used as the zero amplitude reference throughout the remainder of the analysis. Then an 'average' QRS complex is estimated. For each QRS complex, a correlation coefficient with the 'average' beat is estimated and used for ventricular erroneous beat detection; a beat will be considered erroneous if its correlation coefficient is less than a threshold (CC_t) value of 0.90 or if the difference between current R-to-R (RR) waveform interval and the median RR interval from the preceding 7 beats is less/more than a threshold (RR_t) value of 20%. The process of template matching and RR interval detection is designed to eliminate erroneous beats such as premature ventricular complexes from the analysis. If a beat meets both threshold criteria, it is classified as 'good' and retained for analysis. In addition, a goodbeat percentage is calculated for all sequences as a moving average of the number of good sequences in a window of 128 beats. This is indicative of the number of consecutive good beats in the ECG recording and only sequences that meet a threshold of 80% are used for further processing.

After ventricular erroneous beat detection, erroneous atrial beats (such as premature atrial contractions) are detected in the same way that erroneous ventricular beats are detected: the correlation coefficient (CC_t) between the present beat P-wave and the average P-wave of the 128-beat sequence is set at 0.90. Once all erroneous *ventricular and atrial* beats are detected, then for each erroneous beat, the P-wave of that and the subsequent beat are removed from the sequence of beats and substituted with a median odd or even beat P-wave (estimated from all good odd or even beats), depending on whether the erroneous beat was an odd or an even one.

For purposes of PWA estimation to eliminate the effect of respiration (that may cause signal wandering), we subtract the baseline (defined as the mean value of the

electrocardiographic PQ interval) of each beat from the corresponding P-wave. The next step of the algorithm involves creating a matrix of all 128 beats in which a window that reflects the atrial depolarization for each beat is identified according to its fiducial point (Figure 2). Then, the power spectrum is estimated for each time-aligned sequence of sample points (Figure 2) within the selected atrial waveform (i.e. P-wave reflecting atrial depolarization)²⁶. Subsequently, the power spectra for each sample point within the waveform are averaged and the statistical estimates of alternans (i.e. alternans voltage, noise and K-score), are obtained as previously described²⁶. Briefly, P-wave alternans are estimated as follows:

$$\text{alternans voltage } (\mu\text{V}) = \sqrt{\text{alternans peak} - \mu_{\text{noise}}}$$

$$K_{\text{score}} = \frac{\text{alternans peak} - \mu_{\text{noise}}}{\sigma_{\text{noise}}}$$

where, the alternans peak is the peak of the power spectrum corresponding to 0.5 cycles/beat and the mean (μ_{noise}) and the standard deviation (σ_{noise}) of the spectral noise are estimated in a predefined noise window. The alternans voltage is a direct measure of the presence of alternans, while the K_{score} is a measure of the statistical significance of the alternans voltage.

Estimation of Heart Rate Variability Measures

To investigate the effect of chronic LLTS on heart rate variability (HRV) we evaluated several time domain and non-linear measures of HRV. First, for each sham and active patient, RR Interval values were calculated during control (no tragus stimulation) and LLTS at all three time points: baseline, 3 months and 6 months.

The Standard Deviation of RR Intervals (SDRR), a measure of long term HRV and the Root Mean Square of Successive Differences (RMSSD) of the RR intervals, a common indicator of short term HRV, was calculated. Additionally, NN₅₀ count, defined as the number of times the change in consecutive RR intervals exceeds 50 ms and pNN₅₀, the percentage of consecutive RR intervals that differ by more than 50 ms ((NN₅₀ Count/RR Interval Count)*100%), were also calculated.

We also estimated the SD2/SD1 ratio, where, SD1 and SD2 are non-linear measures of short (SD1) and long (SD2) term HRV calculated from Poincare maps and based on RR intervals.

SD1/SD2 ratio correlates with LF/HF ratio and is used as a measure of autonomic balance; parasympathetic nervous system (PNS) and sympathetic nervous system (SNS) activity contribute to LF power, and PNS activity primarily contributes to HF power. A low LF/HF, or high SD2/SD1 ratio reflects parasympathetic dominance. SD1 and SD2 are estimated as follows:

$SD1^2 = 0.5 * SDDS^2$ (where SDDS = standard deviation of successive differences of RR intervals)

$SD2^2 = 2 * SDRR^2 - 0.5 * SDDS^2$

Mean values of each HRV measure were generated for all patients during control and LLTS. Comparison of HRV measures were performed at baseline, 3 months and 6 months between sham and active groups.

Effect of LLTS on P-wave Duration and QT, QT_c, PR, T_{peak}-T_{end} Intervals

After identification of fiducial points (P_{on}, P_{off}, Q_{peak}, R_{peak}, T_{peak} and T_{end}) from the ECG waveform using wavelet transform²⁷, P-wave duration and QT, PR and T_{peak}-T_{end} intervals were calculated for each beat. Corrected QT interval, QT_c, was calculated based on Bazett's formula as the QT interval for each beat divided by the square root of the preceding RR interval. Mean values were generated for all patients during control and LLTS. Comparison of ECG Intervals were performed at baseline, 3 months and 6 months between sham and active groups.

SUPPLEMENTAL RESULTS

Effect of Chronic Low Level Tragus Stimulation on Heart Rate, P-wave Duration and QT, QT_c, PR, T_{peak}-T_{end} Intervals

Summary results of chronic LLTS on heart rate (Figure S1), RR-interval (Figure S2), P-wave duration (Figure S3), QT-interval (Figure S4), QT_c interval (Figure S5), PR-interval (Figure S6), and T_{peak}-T_{end} interval (Figure S7), respectively, across all active and sham patients, during control and LLTS are presented as median (horizontal solid line), 75-25% percentiles (box) and 90-10% percentiles (error bars). At 6 months after chronic LLTS, active patients had significantly larger QT-intervals compared to sham patients during both control and LLTS. However, this effect was

not observed in the corrected QT intervals after adjustment for heart rates. Statistical comparison was performed using 1-way ANOVA.

Effect of Chronic Low Level Tragus Stimulation on Heart Rate Variability

Summary results of chronic LLTS on SDRR (Figure S8), RMSSD (Figure S9), NN₅₀ count (Figure S10), pNN₅₀ and SD2/SD1 ratio (Figures S11 and S12), across all active and sham patients, during control (no tragus stimulation) and LLTS are presented as median (horizontal solid line), 75-25% percentiles (box) and 90-10% percentiles (error bars). Chronic LLTS did not lead to any significant changes in HRV in either sham or active patients.

Effect of Chronic Low Level Tragus Stimulation on P-wave Alternans

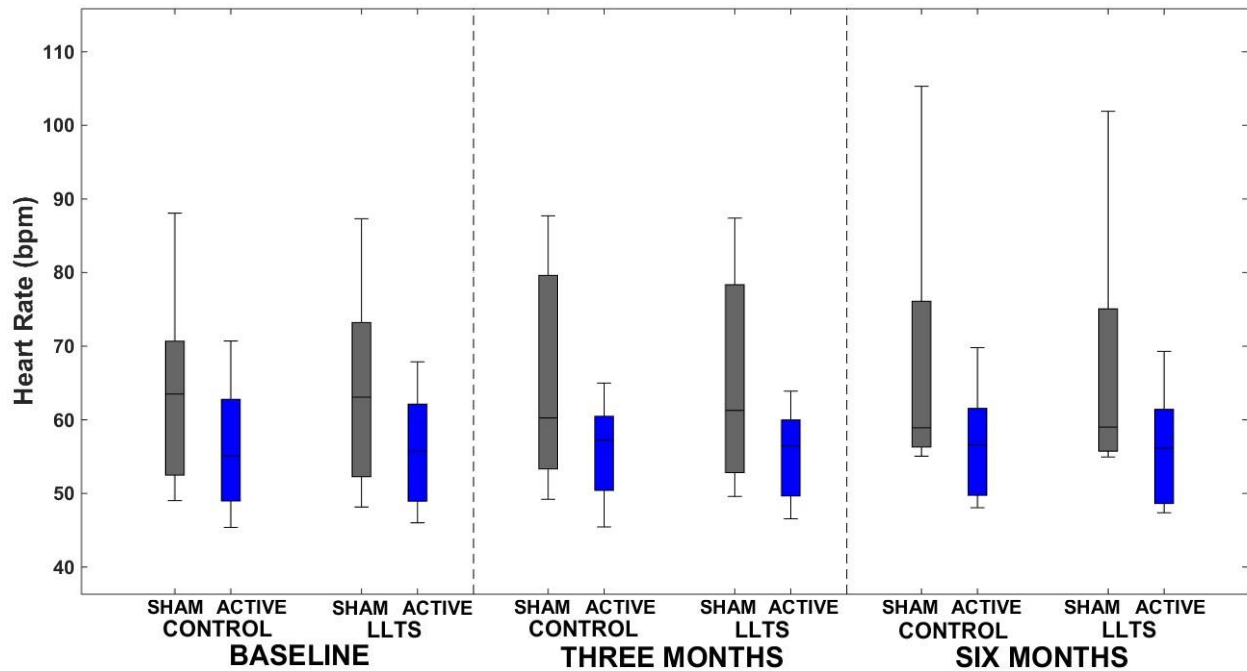
Figures OS13, OS14 and OS15 demonstrate summary results of chronic LLTS on Δ PWA voltage, Δ PWA K_{score} and Δ PWA burden across all active and sham patients, during control (no tragus stimulation) and LLTS respectively. While no significant effect of LLTS was observed in sham patients, the active group exhibited significantly lower Δ alternans, Δ K_{score} and Δ PWA burden values during LLTS as compared to control.

Identifying Early Markers of Effective LLTS Treatment

Active patients were categorized into two groups based on the effect of chronic LLTS: (A1) Patients demonstrating a drop in Δ PWA voltage and K_{score} with LLTS compared to control, and (A2) Patients with no drop in Δ PWA voltage and K_{score} with LLTS compared to control, after 3 or 6 months of chronic LLTS (Figures OS16A and OS16B, respectively).

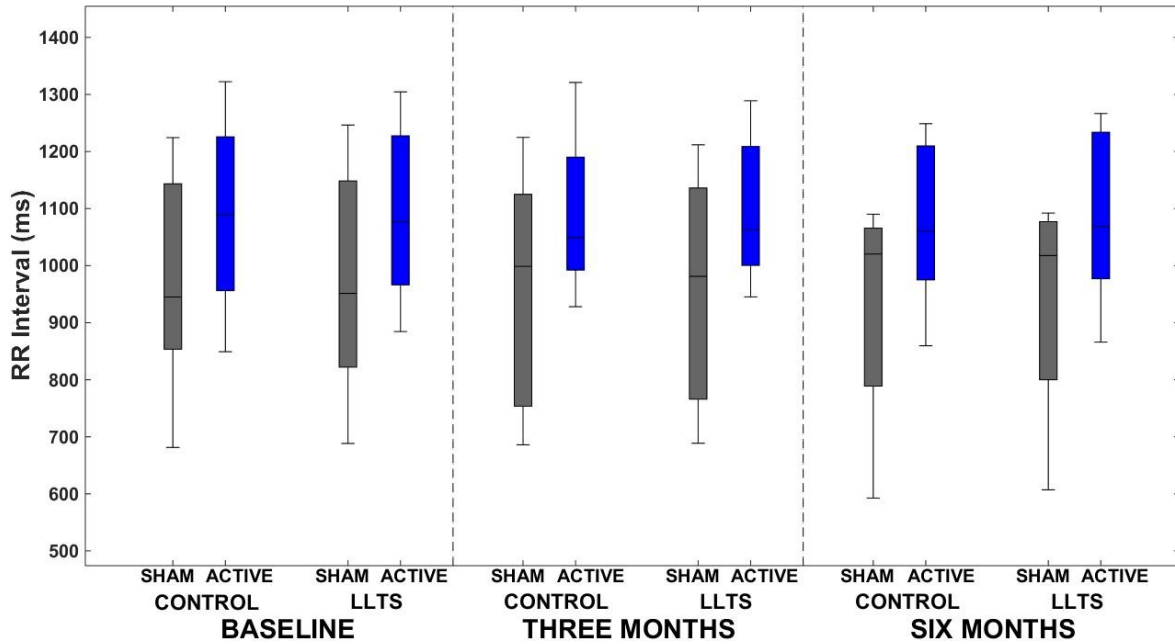
After categorizing the active patients into the two groups, for each group, the acute (at baseline) effect of LLTS on PWA voltage and K_{score} was investigated. In Figures OS16A and OS16B, we observe that active patients who demonstrate a drop in PWA voltage and K_{score} after either 3 or 6 months of chronic LLTS, show an early response to acute LLTS at baseline as well. Specifically, group A1 demonstrates an increase in PWA voltage and K_{score} with acute LLTS, while group A2 shows no change in these parameters with acute LLTS.

Figure S1. Summary results of low level tragus stimulation (LLTS) on Heart Rate across all active (with chronic LLTS) and sham (no chronic LLTS) patients.



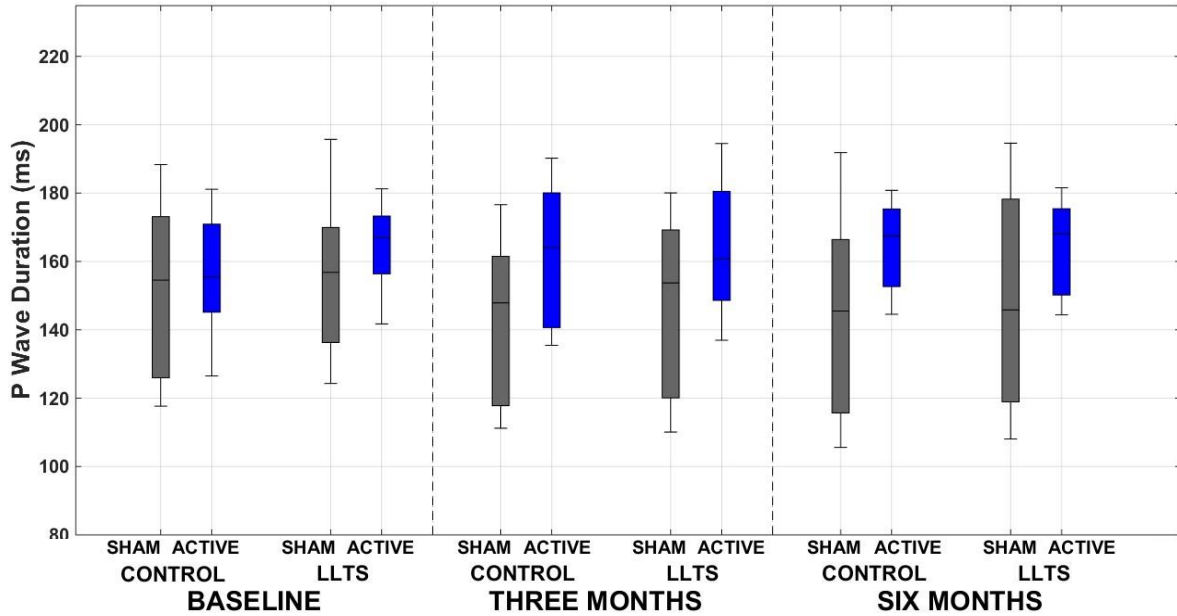
Data are presented as median (horizontal solid line), 75-25% percentiles (box) and 90-10% percentiles (error bars). Heart rate during control (no tragus stimulation) and LLTS is compared between sham and active patients at three time points: baseline, 3 months and 6 months. Sample sizes are sham (n = 12, 10, 8) and active (n= 15, 14, 14) for baseline, 3 months and 6 months, respectively. Patients that experienced episodes of AF during ECG recording or had atrial pacing, AV pacing or junctional rhythm were excluded from the analysis.

Figure S2. Summary results of low level tragus stimulation (LLTS) on RR Interval across all active (with chronic LLTS) and sham (no chronic LLTS) patients.



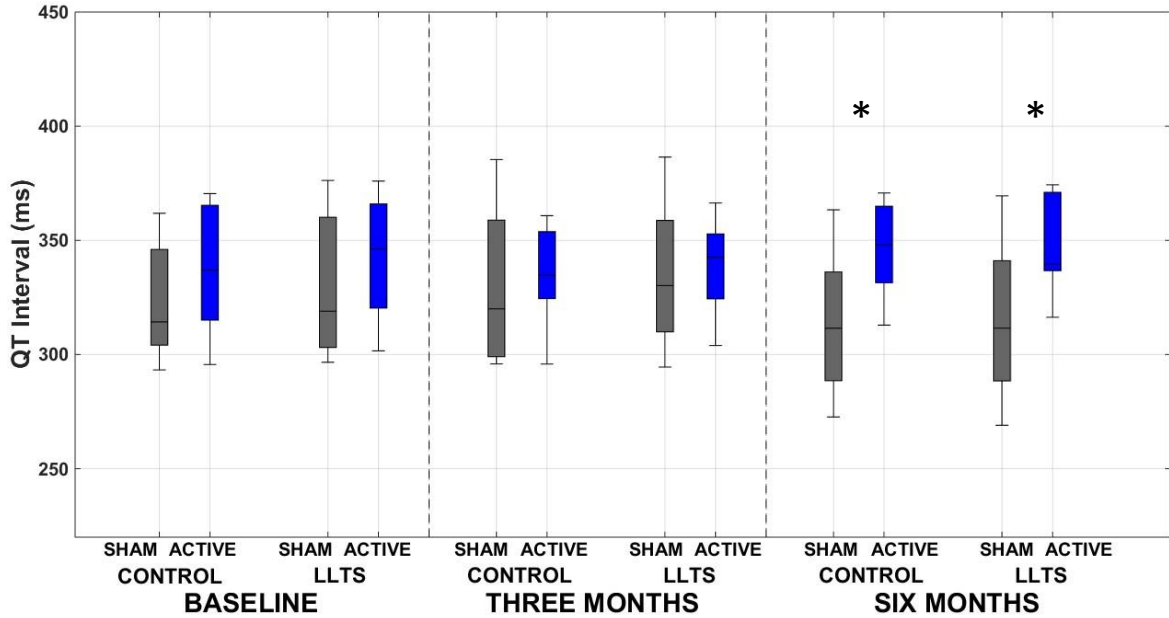
Data are presented as median (horizontal solid line), 75-25% percentiles (box) and 90-10% percentiles (error bars). RR interval during control (no tragus stimulation) and LLTS is compared between sham and active patients at three time points: baseline, 3 months and 6 months. Sample sizes are sham (n = 12, 10, 8) and active (n= 15, 14, 14) for baseline, 3 months and 6 months, respectively. Patients that experienced episodes of AF during ECG recording or had atrial pacing, AV pacing or junctional rhythm were excluded from the analysis.

Figure S3. Summary results of low level tragus stimulation (LLTS) on P-wave duration across all active (with chronic LLTS) and sham (no chronic LLTS) patients.



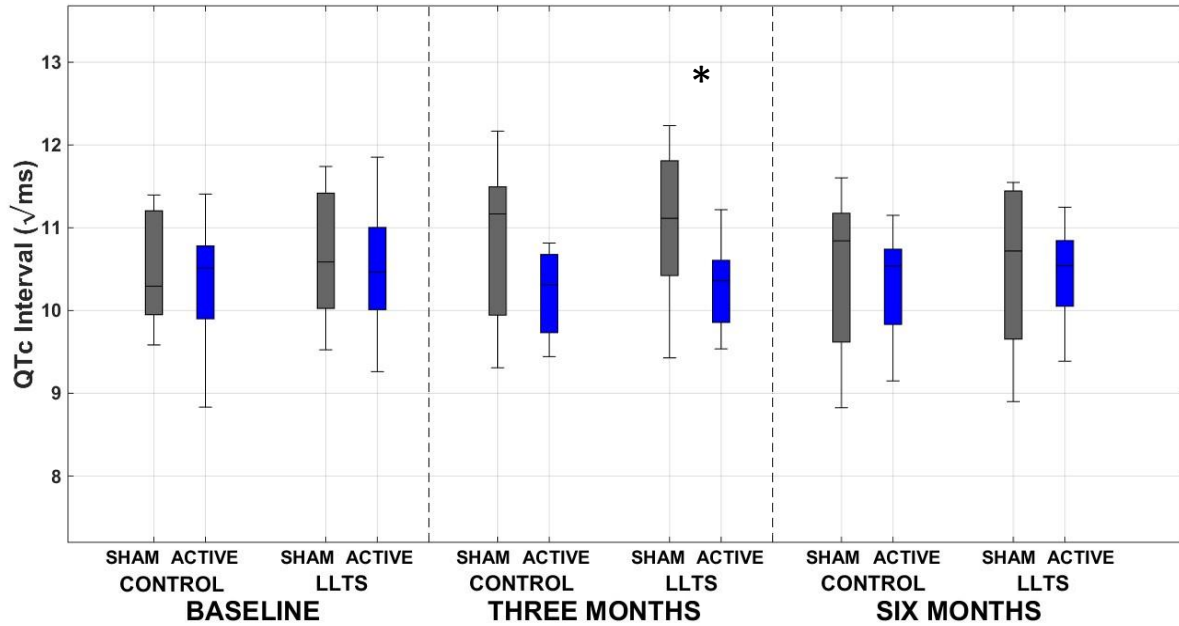
Data are presented as median (horizontal solid line), 75-25% percentiles (box) and 90-10% percentiles (error bars). P-wave duration during control (no tragus stimulation) and LLTS is compared between sham and active patients at three time points: baseline, 3 months and 6 months. Sample sizes are sham (n = 12, 10, 8) and active (n= 15, 14, 14) for baseline, 3 months and 6 months, respectively.

Figure S4. Summary results of low level tragus stimulation (LLTS) on QT-interval duration across all active (with chronic LLTS) and sham (no chronic LLTS) patients.



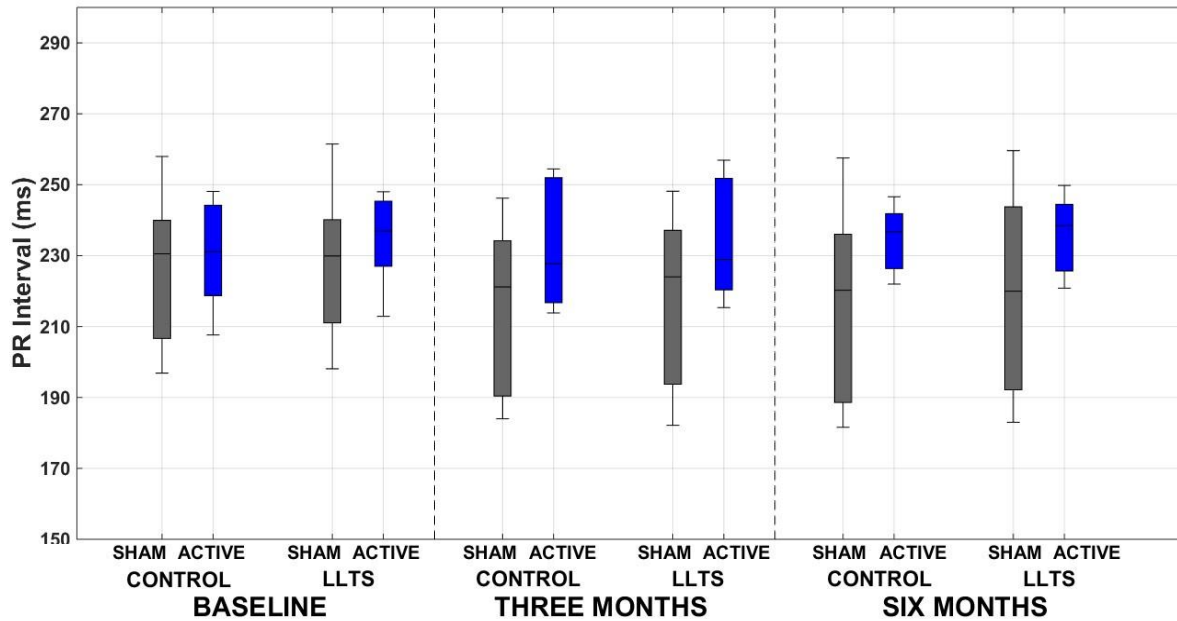
Data are presented as median (horizontal solid line), 75-25% percentiles (box) and 90-10% percentiles (error bars). QT interval during control (no tragus stimulation) and LLTS is compared between sham and active patients at three time points: baseline, 3 months and 6 months. Sample sizes are sham (n = 12, 10, 8) and active (n= 15, 14, 14) for baseline, 3 months and 6 months, respectively. '*' denotes statistical significance of p < 0.05, using 1-way ANOVA

Figure S5. Summary results of low level tragus stimulation (LLTS) on QTc-interval duration across all active (with chronic LLTS) and sham (no chronic LLTS) patients.



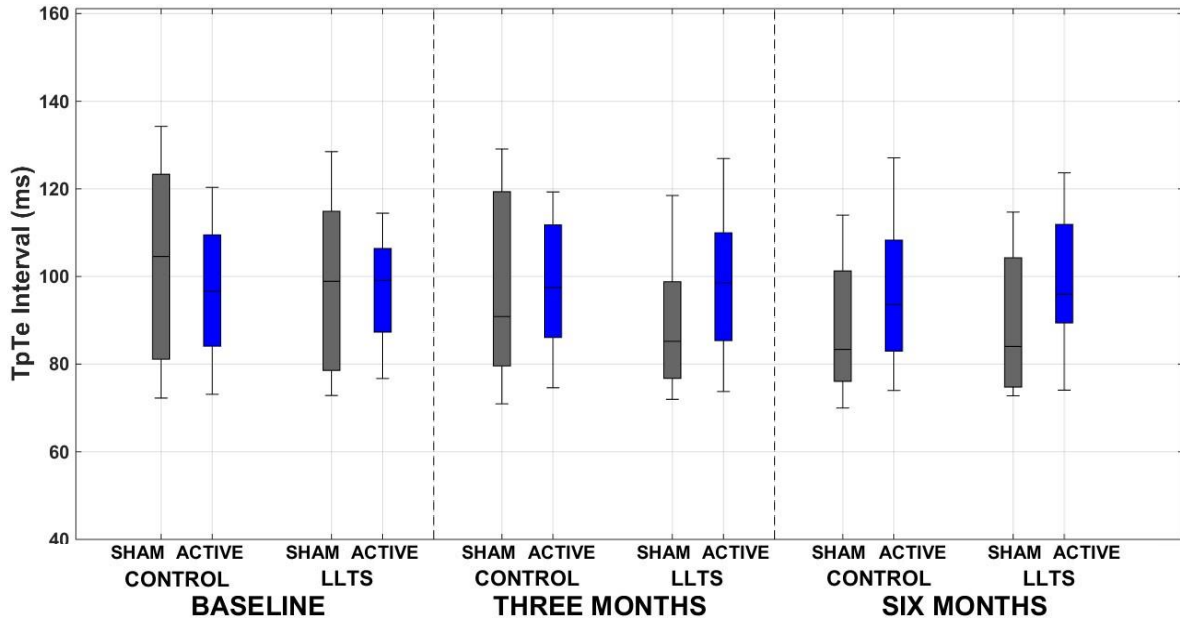
Data are presented as median (horizontal solid line), 75-25% percentiles (box) and 90-10% percentiles (error bars). QTc interval during control (no tragus stimulation) and LLTS is compared between sham and active patients at three time points: baseline, 3 months and 6 months. Sample sizes are sham (n = 12, 10, 8) and active (n= 15, 14, 14) for baseline, 3 months and 6 months, respectively. '*' denotes statistical significance of p < 0.05, using Kruskal-Wallis

Figure S6. Summary results of low level tragus stimulation (LLTS) on PR-interval duration across all active (with chronic LLTS) and sham (no chronic LLTS) patients.



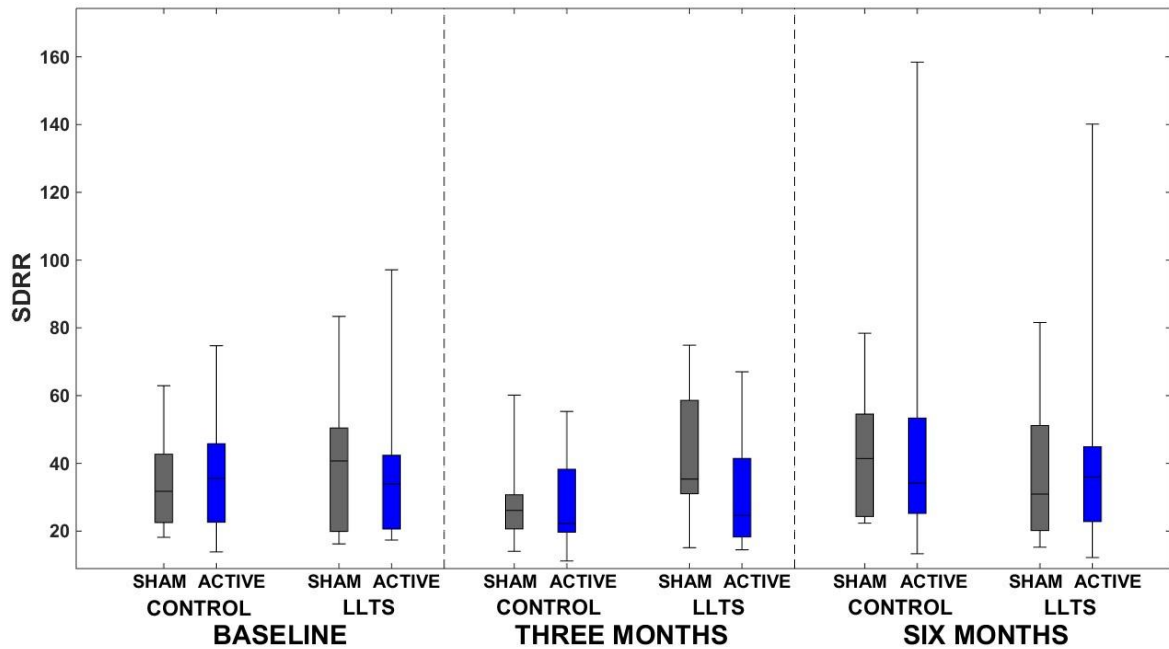
Data are presented as median (horizontal solid line), 75-25% percentiles (box) and 90-10% percentiles (error bars). PR interval during control (no tragus stimulation) and LLTS is compared between sham and active patients at three time points: baseline, 3 months and 6 months. Sample sizes are sham (n = 12, 10, 8) and active (n= 15, 14, 14) for baseline, 3 months and 6 months, respectively.

Figure S7. Summary results of low level tragus stimulation (LLTS) on $T_{peak}-T_{end}$ duration across all active (with chronic LLTS) and sham (no chronic LLTS) patients.



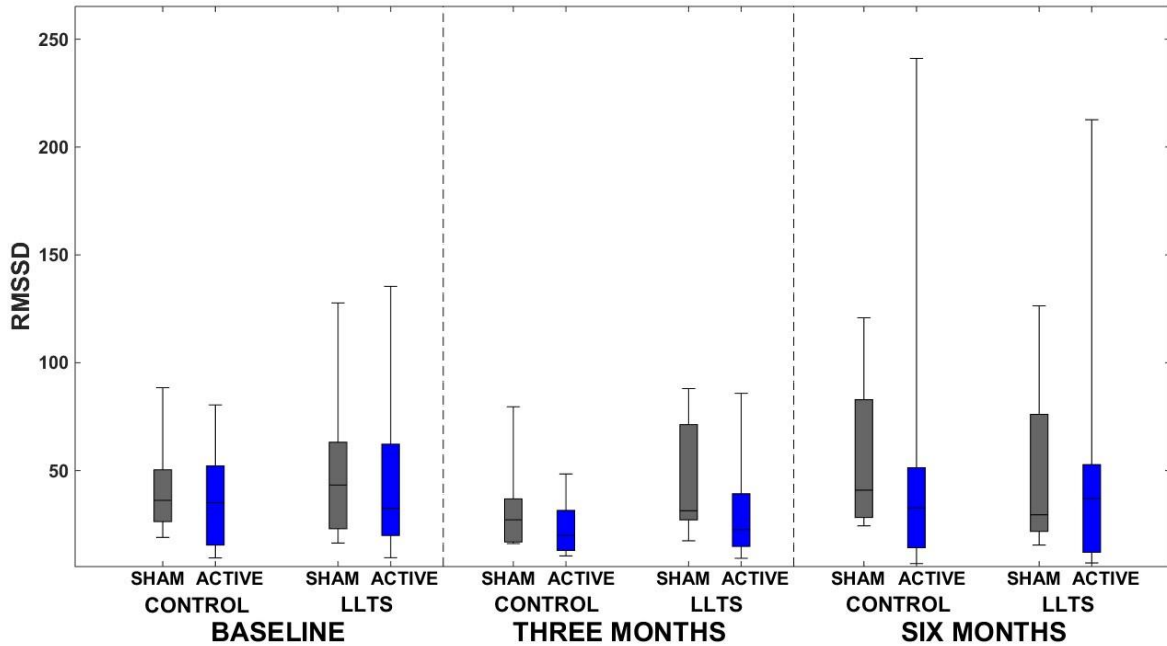
Data are presented as median (horizontal solid line), 75-25% percentiles (box) and 90-10% percentiles (error bars). $T_{peak}-T_{end}$ duration during control (no tragus stimulation) and LLTS is compared between sham and active patients at three time points: baseline, 3 months and 6 months. Sample sizes are sham ($n = 12, 10, 8$) and active ($n = 15, 14, 14$) for baseline, 3 months and 6 months, respectively.

Figure S8. Summary results of low level tragus stimulation (LLTS) on SDRR across all active (with chronic LLTS) and sham (no chronic LLTS) patients.



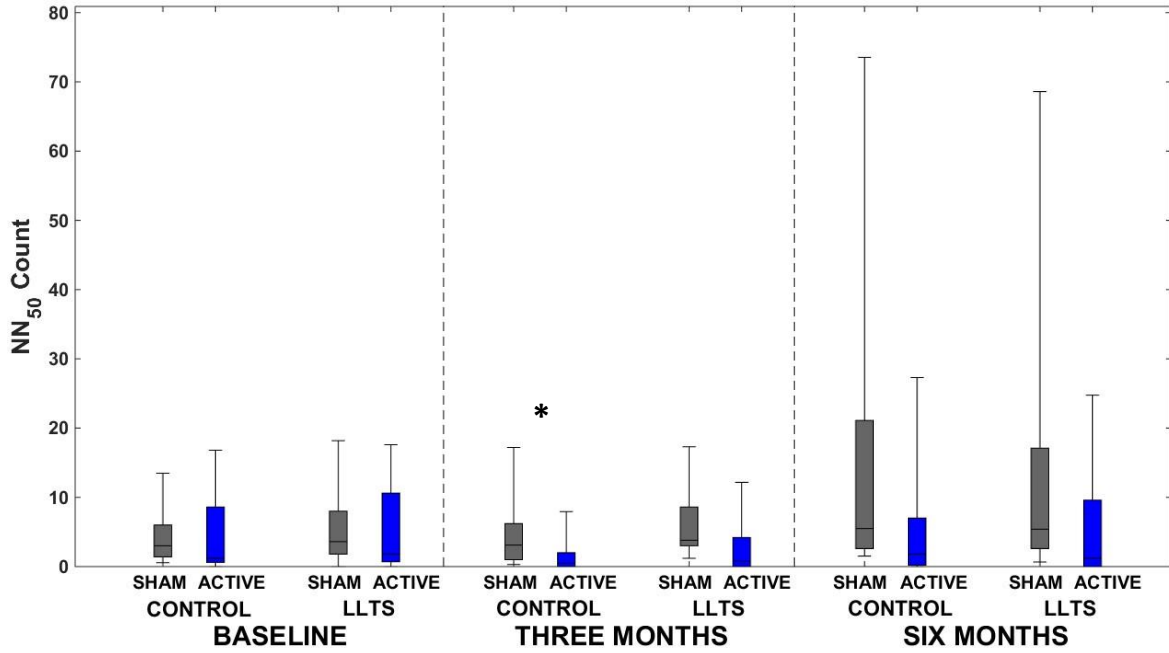
Data are presented as median (horizontal solid line), 75-25% percentiles (box) and 90-10% percentiles (error bars). Heart rate during control (no tragus stimulation) and LLTS is compared between sham and active patients at three time points: baseline, 3 months and 6 months. Sample sizes are sham (n = 12, 10, 8) and active (n= 15, 14, 14) for baseline, 3 months and 6 months, respectively. Patients that experienced episodes of AF during ECG recording or had atrial pacing, AV pacing or junctional rhythm were excluded from the analysis.

Figure S9. Summary results of low level tragus stimulation (LLTS) on RMSSD across all active (with chronic LLTS) and sham (no chronic LLTS) patients.



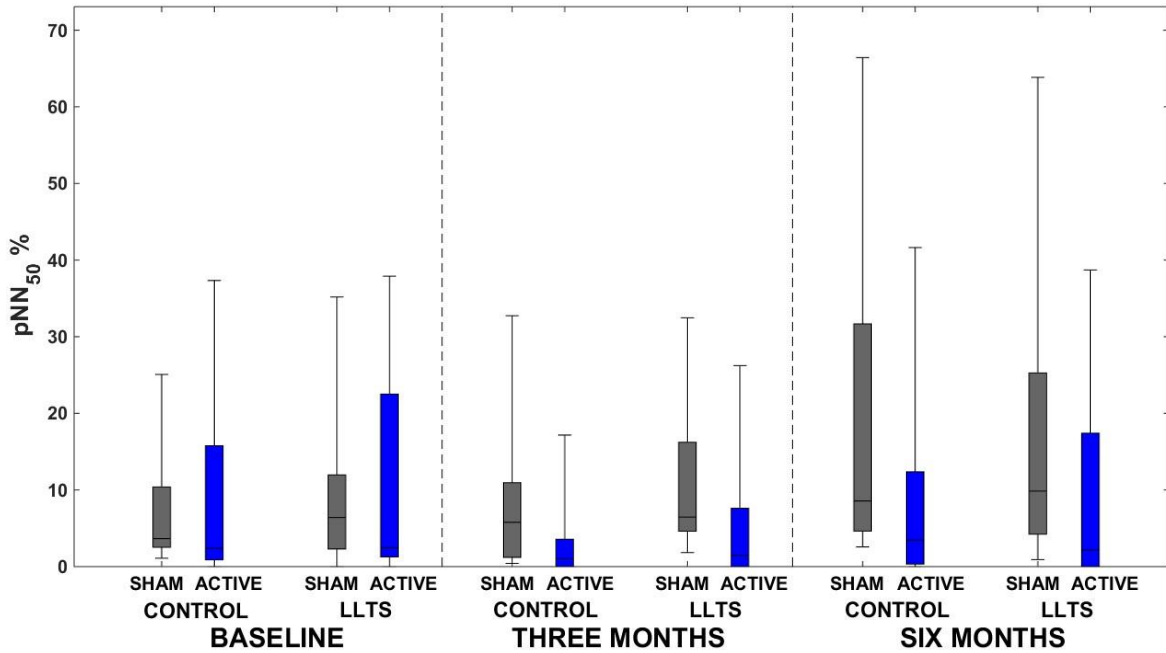
Data are presented as median (horizontal solid line), 75-25% percentiles (box) and 90-10% percentiles (error bars). Heart rate during control (no tragus stimulation) and LLTS is compared between sham and active patients at three time points: baseline, 3 months and 6 months. Sample sizes are sham (n = 12, 10, 8) and active (n= 15, 14, 14) for baseline, 3 months and 6 months, respectively. Patients that experienced episodes of AF during ECG recording or had atrial pacing, AV pacing or junctional rhythm were excluded from the analysis.

Figure S10. Summary results of low level tragus stimulation (LLTS) on NN₅₀ Count across all active (with chronic LLTS) and sham (no chronic LLTS) patients.



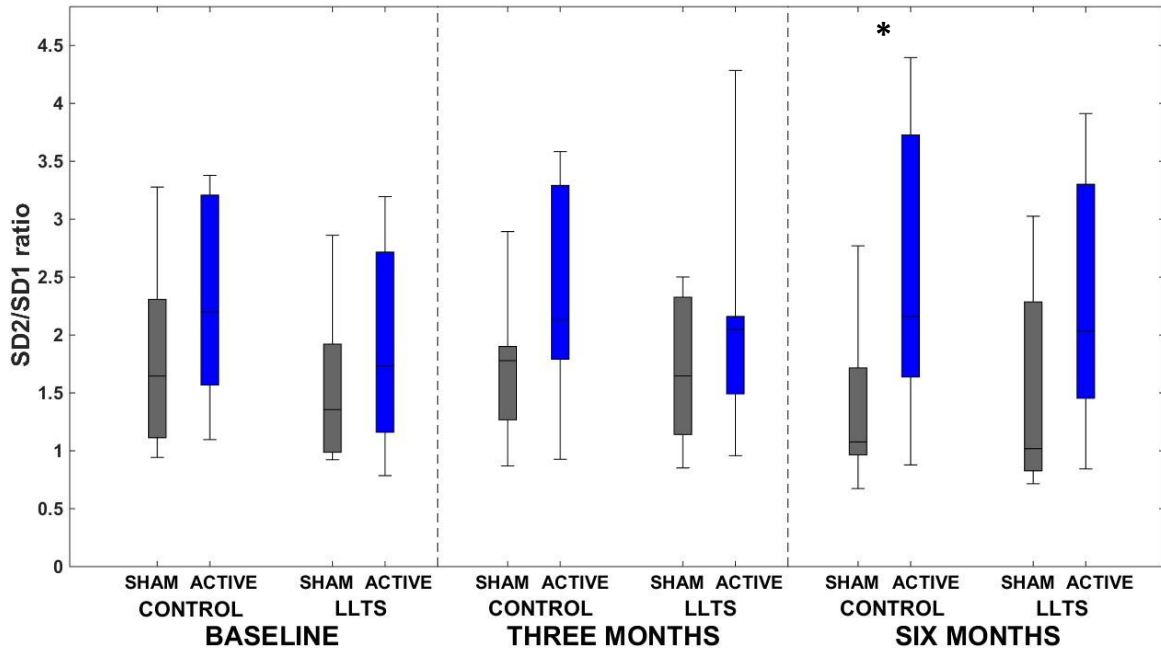
Data are presented as median (horizontal solid line), 75-25% percentiles (box) and 90-10% percentiles (error bars). Heart rate during control (no tragus stimulation) and LLTS is compared between sham and active patients at three time points: baseline, 3 months and 6 months. Sample sizes are sham (n = 12, 10, 8) and active (n= 15, 14, 14) for baseline, 3 months and 6 months, respectively. Patients that experienced episodes of AF during ECG recording or had atrial pacing, AV pacing or junctional rhythm were excluded from the analysis. “*” denotes statistical significance of $p < 0.05$, using Kruskal-Wallis

Figure S11. Summary results of low level tragus stimulation (LLTS) on pNN₅₀% across all active (with chronic LLTS) and sham (no chronic LLTS) patients.



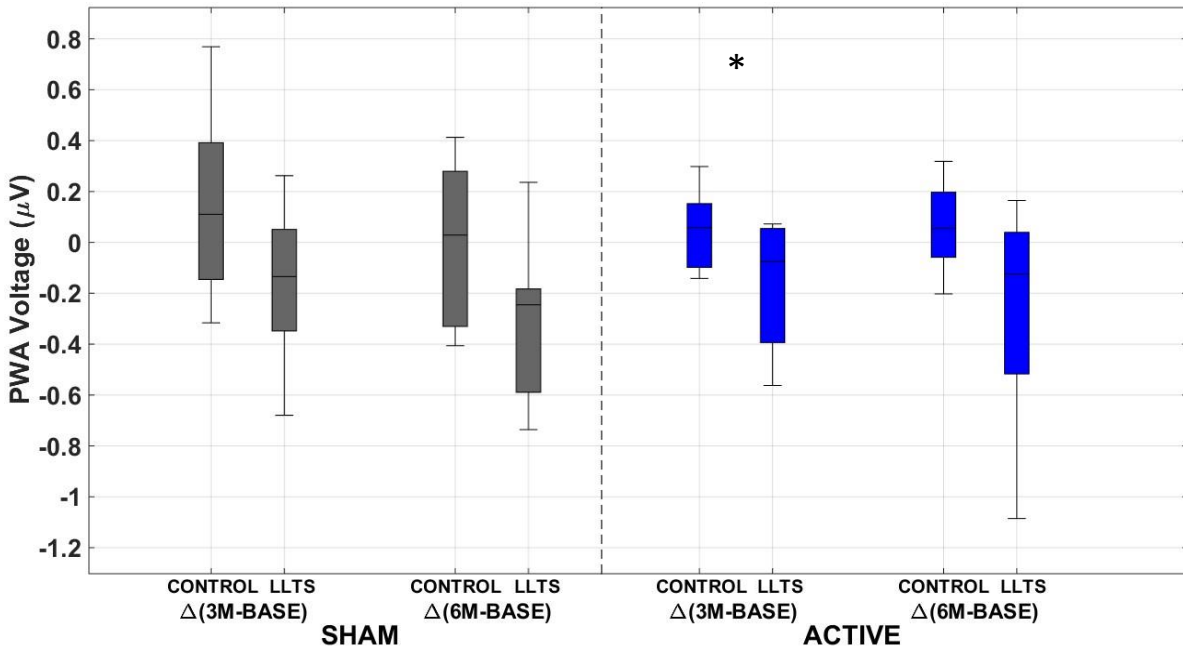
Data are presented as median (horizontal solid line), 75-25% percentiles (box) and 90-10% percentiles (error bars). Heart rate during control (no tragus stimulation) and LLTS is compared between sham and active patients at three time points: baseline, 3 months and 6 months. Sample sizes are sham (n = 12, 10, 8) and active (n= 15, 14, 14) for baseline, 3 months and 6 months, respectively. Patients that experienced episodes of AF during ECG recording or had atrial pacing, AV pacing or junctional rhythm were excluded from the analysis.

Figure S12. Summary results of low level tragus stimulation (LLTS) on SD2/SD1 ratio across all active (with chronic LLTS) and sham (no chronic LLTS) patients.



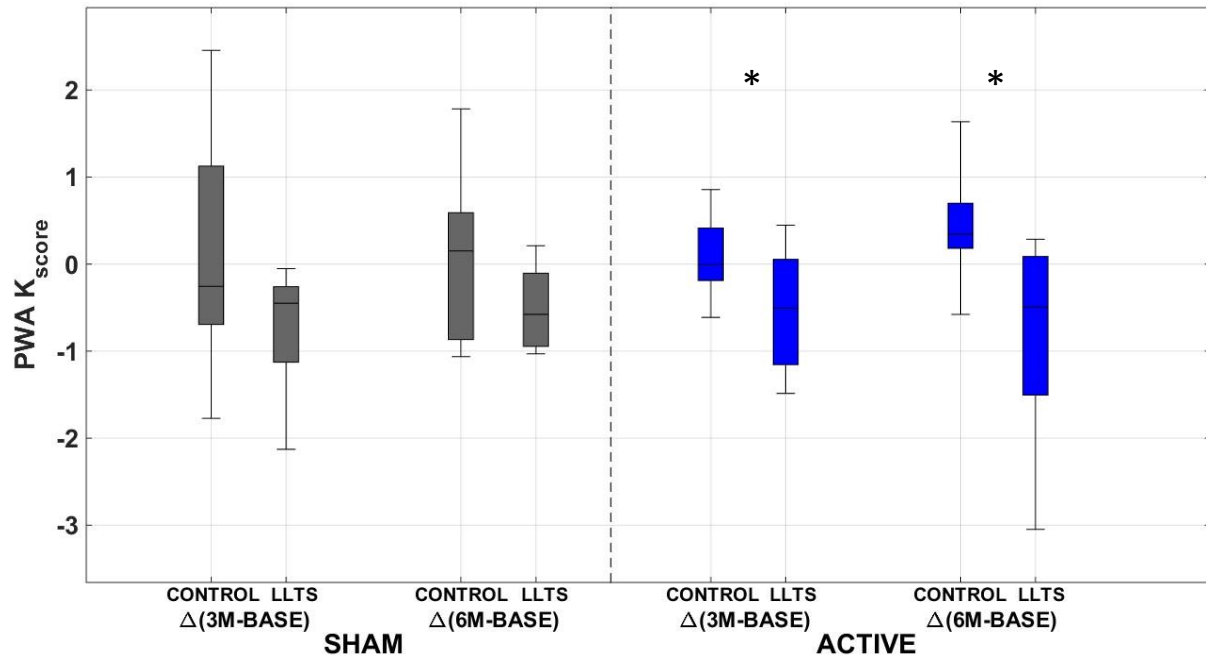
Data are presented as median (horizontal solid line), 75-25% percentiles (box) and 90-10% percentiles (error bars). Heart rate during control (no tragus stimulation) and LLTS is compared between sham and active patients at three time points: baseline, 3 months and 6 months. Sample sizes are sham (n = 12, 10, 8) and active (n= 15, 14, 14) for baseline, 3 months and 6 months, respectively. Patients that experienced episodes of AF during ECG recording or had atrial pacing, AV pacing or junctional rhythm were excluded from the analysis. ‘*’ denotes statistical significance of $p < 0.05$, using Kruskal-Wallis.

Figure S13. Summary results of chronic low level tragus stimulation (LLTS) on Δ P-wave alternans (Δ PWA) voltage across all active (with chronic LLTS) and sham (no chronic LLTS) patients, during control (no LLTS) and LLTS.



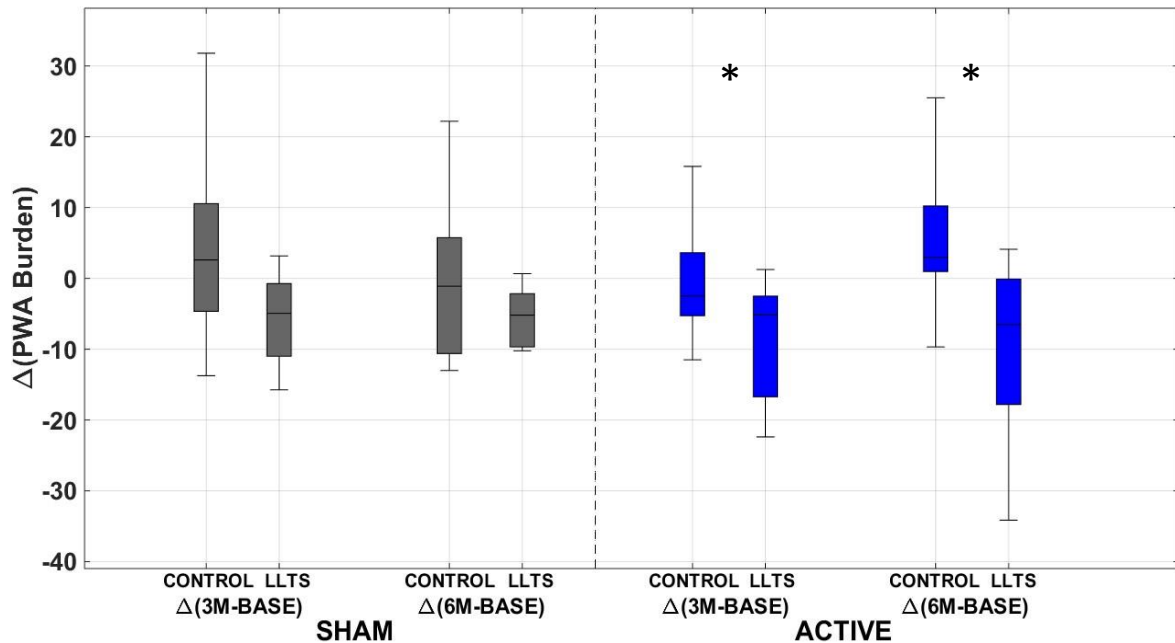
Δ PWA values are calculated between the three time points: 3 months-baseline Δ (3M-BASE) and 6 months-baseline Δ (6M-BASE). Δ alternans voltage during control and LLTS is compared between sham and active patients. Statistical comparison was performed using 1-way ANOVA. ‘*’ denotes statistical significance of $p < 0.05$. Sample sizes are sham: Δ (3M-BASE) control (n = 9), Δ (3M-BASE) LLTS (n = 9), Δ (6M-BASE) control (n = 7), Δ (6M-BASE) LLTS (n = 6); and active: Δ (3M-BASE) control (n = 11), Δ (3M-BASE) LLTS (n = 10), Δ (6M-BASE) control (n = 10), Δ (6M-BASE) LLTS (n = 8). Patients that experienced episodes of AF during ECG recording or had atrial pacing, AV pacing or junctional rhythm were excluded from the analysis.

Figure S14. Summary results of chronic low level tragus stimulation (LLTS) on Δ P-wave alternans (Δ PWA) K_{score} across all active (with chronic LLTS) and sham (no chronic LLTS) patients, during control (no LLTS) and LLTS.



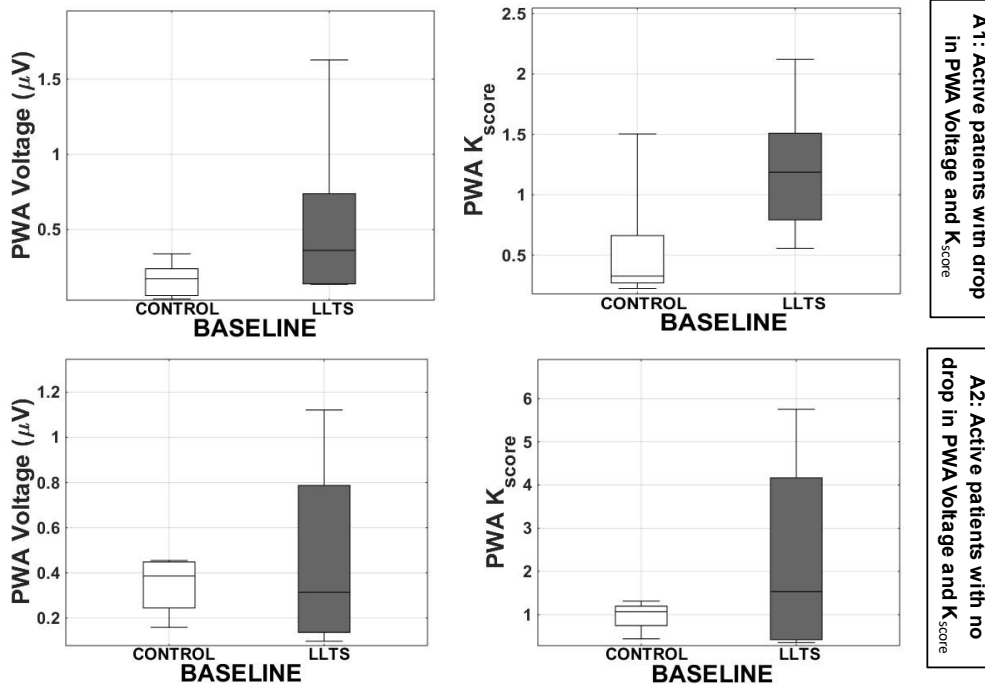
Δ PWA values are calculated between the three time points: 3 months-baseline $\Delta(3M-BASE)$ and 6 months-baseline $\Delta(6M-BASE)$. Δ PWA K_{score} during control and LLTS is compared between sham and active patients. Statistical comparison was performed using 1-way ANOVA. "*" denotes statistical significance of $p < 0.05$. Sample sizes are sham: $\Delta(3M-BASE)$ control ($n = 9$), $\Delta(3M-BASE)$ LLTS ($n = 9$), $\Delta(6M-BASE)$ control ($n = 7$), $\Delta(6M-BASE)$ LLTS ($n = 6$); and active: $\Delta(3M-BASE)$ control ($n = 11$), $\Delta(3M-BASE)$ LLTS ($n = 10$), $\Delta(6M-BASE)$ control ($n = 10$), $\Delta(6M-BASE)$ LLTS ($n = 8$). Patients that experienced episodes of AF during ECG recording or had atrial pacing, AV pacing or junctional rhythm were excluded from the analysis.

Figure S15. Summary results of chronic low level tragus stimulation (LLTS) on Δ P-Wave alternans (Δ PWA) burden across all active (with chronic LLTS) and sham (no chronic LLTS) patients, during control (no tragus stimulation) and LLTS.



Δ PWA burden values are calculated between the three time points: 3 months-baseline Δ (3M-BASE) and 6 months-baseline Δ (6M-BASE). Δ PWA burden during control and LLTS is compared between sham and active patients. 1-way ANOVA was used for statistical comparison. '*' denotes statistical significance of $p < 0.05$. Sample sizes are sham: Δ (3M-BASE) control (n = 9), Δ (3M-BASE) LLTS (n = 9), Δ (6M-BASE) control (n = 7), Δ (6M-BASE) LLTS (n = 6); and active: Δ (3M-BASE) control (n = 11), Δ (3M-BASE) LLTS (n = 10), Δ (6M-BASE) control (n = 10), Δ (6M-BASE) LLTS (n = 8). Patients that experienced episodes of AF during ECG recording or had atrial pacing, AV pacing or junctional rhythm were excluded from the analysis.

Figure S16 (A) active patients are categorized into two groups based on effect of chronic LLTS: (A1; n=5) Patients demonstrating a drop in ΔP -Wave alternans (ΔPWA) voltage and K_{score} with LLTS compared to control (no tragus stimulation), and (A2; n=4) patients with no drop in ΔPWA voltage and/or K_{score} with LLTS compared to control, after three months of chronic LLTS.



(B) active patients are categorized into two groups based on effect of chronic LLTS: (A1; n=6) Patients demonstrating a drop in Δ PWA voltage and K_{score} with LLTS compared to control, and (A2; n=2) Patients with no drop in Δ PWA voltage and/or K_{score} with LLTS compared to control, after six months of chronic LLTS. For both LLTS and control, delta values are calculated using mean PWA voltage and K_{score} at 3 months (or 6 months) and baseline, Δ (3M-BASE) (or Δ (6M-BASE)). For each group, acute (at baseline) effect of LLTS on PWA voltage and K_{score} is observed. Kruskal-Wallis test was used for comparison and '*' denotes statistical significance of $p < 0.05$.

