Supplementary Appendix 1. Echocardiographic assessment.

Patients underwent a screening evaluation bubble study with TTE and/or TCD ultrasound. All patients underwent preprocedural TOE, according to the American Society of Echocardiography recommendations, to assess the presence of PFO, to evaluate the morphology and size of fossa ovalis, to identify atrial septal aneurysm (ASA, defined as excursion of the septal tissue of greater than 10 mm from the plane of the atrial septum into the right atrium or left atrium or a combined total excursion right and left of 15 mm), the presence of stretched PFO with left-to-right shunt and the evidence of Eustachian Valve and/or Chiari Network, to measure tunnel length and PFO width. We grouped in the terms "atrial septal hypermobility" both the presence of ASA and also every swinging atrial septum. Highly experienced operators guided the procedure by TOE using bicaval and short axis 2D view and 3D multiplanar imaging reconstruction to better characterise the PFO morphology, optimise the positioning of the stitch and check the final result. The grading of RLS was semi-quantitative according to the number of microbubbles detected in the left atrial within 5 cardiac cycles and performed both at rest and after Valsalva manoeuvre. Delayed appearance of microbubbles in the left atrium, indicates intrapulmonary shunting and could be a reason for false-positive bubble test. Hence, the importance of checking if the bubbles, which opacified the left atrium, entered from the atrial septum and not from the pulmonary veins. Grading of RLS was considered as follows: grade 0=no microbubbles, grade 1 (mild)=1 to 9 microbubbles, grade 2 (moderate)=10 to 19 microbubbles, grade 3 (severe) $\geq$ 20 microbubbles. Moreover, the microbubble test was performed in all patients to assess the early intraprocedural result by saline injection from the inferior vena cava. All the 116 patients that were successfully treated with the NobleStitch (Heartstitch) system were then evaluated with TTE and TCD with bubble test within 6 months after the index procedure. If a significant RLS was found (RLS $\geq$ 2), a transoesophageal

echocardiography study was organised to assess the mechanism and to confirm the severity of the residual shunt.

Supplementary Appendix 2. Statistical analysis.

Continuous variables were analysed for distribution using the Kolmogorov-Smirnov test. According to their distribution, continuous variables were expressed as mean±standard deviation or median and interquartile range (IQR), as appropriate. Categorical variables were expressed as absolute numbers and percentages and were compared by chi-square or Fisher's exact tests, as appropriate.

Unpaired Student's t-tests were used to compare continuous parameters following a normal distribution, whereas Mann-Whitney U tests were used to compare continuous variables with skewed distribution. A 2-sided p-value <0.05 was required for statistical significance.

The data were analysed with SPSS statistics software (version 25, IBM Corp.).
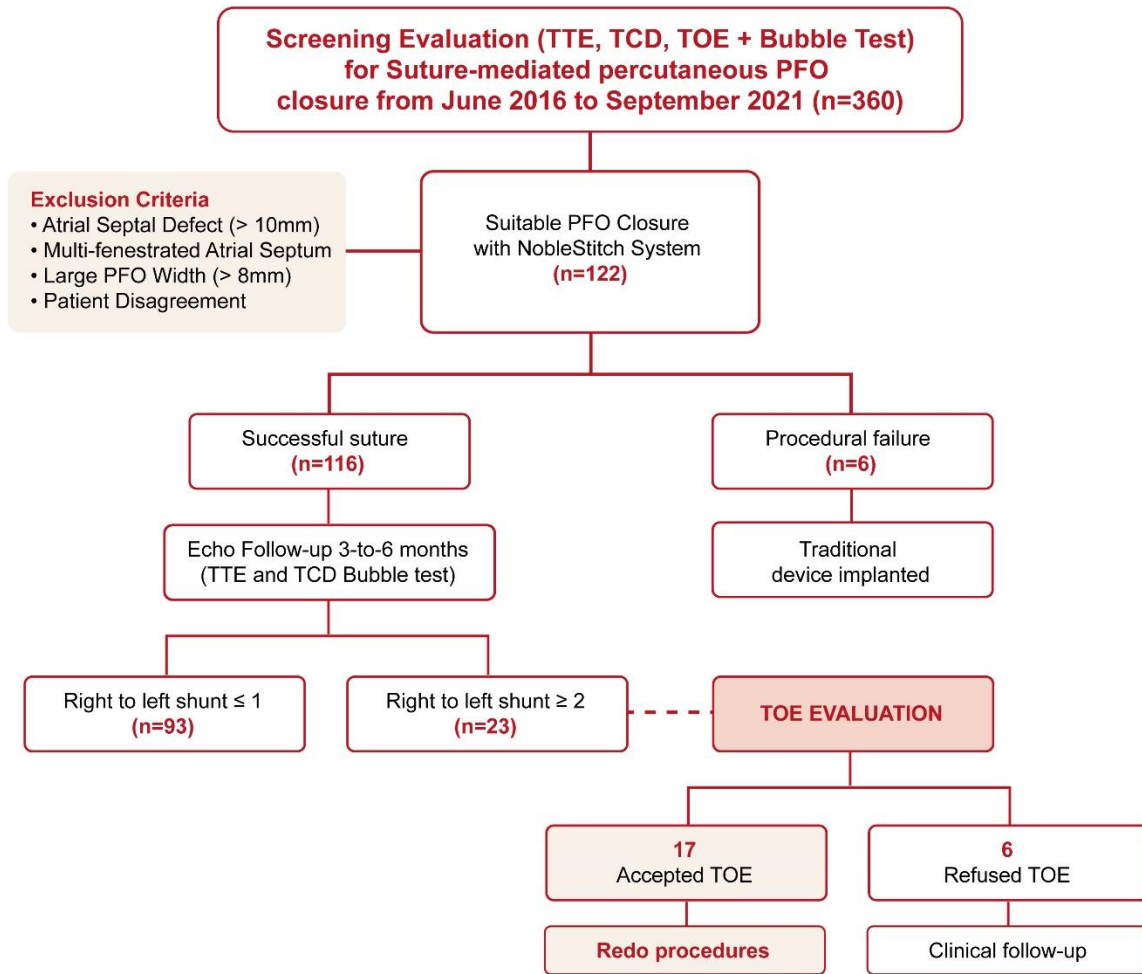
Supplementary Figure 1. Study design.
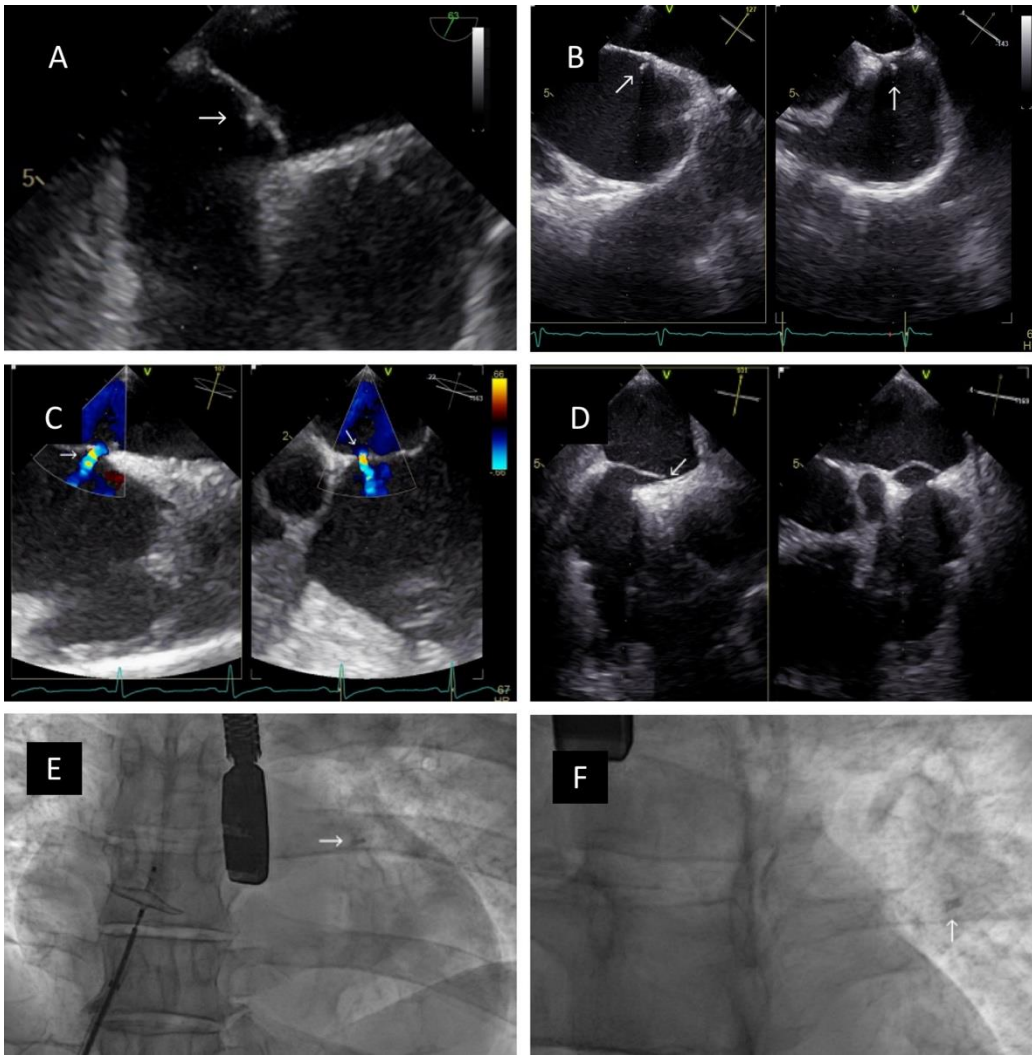
Supplementary Figure 2. Assessment of ineffective PFO closure mechanisms after the NobleStitch procedure

A. Short axis view showing incomplete detachment of stitch, positioned on the septum primum;

B. X-plane view showing stitch placed on the septum secundum;

C. X-plane view of atrial septal tear with left-to-right colour shunt;

D. X-plane view confirming persistent PFO without stitch;

E. Angiographic cranial LAO view showing KwiKnot embolisation into the lung;

F. Angiographic cranial LAO view focused on embolised knot.