Supplemental Material

Supplemental Figure Legends:

Supplemental Figure 1: ISHAGE enumeration of canine HPCs (CD34+ CD45_{dim} VEGFR2- progenitor cells)

- A. After incubation of cells with fluorophore-conjugated antibodies to CD45 and CD34, data are displayed as a dotplot of side scatter (SSC) vs. CD45 fluorescence. All CD45 positive events are gated in a region R1.
- B. Data in R1 is displayed as a dotplot of side scatter vs. CD34 fluorescence. A CD34 positive cluster of low side scatter should be apparent which is gated R2.
- C. Data from R2 are displayed in a dotplot SSC vs CD45 fluorescence. The CD45-dim population is gated R3.
- D. Returning to the SSC vs CD45 dotplot and switching off all gates again, a new gate R4 is created around the homogenous, CD45 positive and low side scatter lymphocyte population.
- E. R4 is displayed as a dotplot of side scatter vs. forward scatter. This shows the phenotypic characteristics of the lymphocytes and blasts, which are similar to those of hematopoetic precursors. A region R5 is created to set the boundaries of these defining phenotypic features.
- F. Contents of R1x R2 x R3 are displayed as in E. All events outside R5 are excluded from the final count, which represents the number of CD34+ CD45_{dim} positive cells per 60,000 events. These cells are uniformly VEGFR2 negative.

Supplemental Figure 2: Enumeration of canine CD34+ CD45- VEGFR2+ progenitor cells (EPCs)

A. After incubation of cells with fluorophore-conjugated antibodies to CD45 and CD34, data are displayed as a dotplot of side scatter (SSC) vs. CD45 fluorescence. All CD45 negative events are gated in a region R1.

B. Data in R1 is displayed as a dotplot of side scatter vs. CD34 fluorescence. A CD34 positive cluster of low side scatter should be apparent which is gated R2.

C. Data from R2 are displayed in a dotplot SSC vs CD45 fluorescence. The CD34+ CD45-negative population is visible by gating on R1 * R2.

D. In a dotplot of SSC vs VEGFR2 a new gate R3 is created around all VEGFR2+ events.

E. In the same dotplot, CD34+ CD45- VEGFR2+ events are demonstrated by gating on R1 * R2 * R3. Contents of R1x R2 x R3 are displayed in a dotplot of SSC vs. FSC (forward scatter). The low side scatter, low forward scatter nature of these cells is apparent.

Supplemental Figure 3: A: Hematopoietic (HPC) and B: endothelial progenitor cell (EPC) counts in a canine pacing model of LV dysfunction, expressed as absolute counts. Cells were enumerated from paced dogs at baseline and at 10 days, compared using a Wilcoxon signed-rank test.

Supplemental Figure 4: Neurohumoral correlates of progenitor cell counts: Spearman Rank correlation (ρ) of change in progenitor cell counts from paced dogs over 10 days compared with plasma aldosterone level (A), canine B-type natriuretic peptide (cBNP)

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(B) levels, and canine atrial natriuretic peptide levels (ANP) (C) at 10 days. For clarity, correlations are shown in a linear fashion.

Supplemental Figure 5. Immunodetection of VEGFR2 expression in canine cells.

A. Canine aortic endothelial cells (Cell applications, Inc.) stained with control IgG.

Hoechst counterstain depicts nuclei. B. Canine aortic endothelial cells stained with anti-

VEGFR2 and Hoechst. C. FACS analysis of canine endothelial cells using IgG and the

anti-VEGFR2 antibodies











