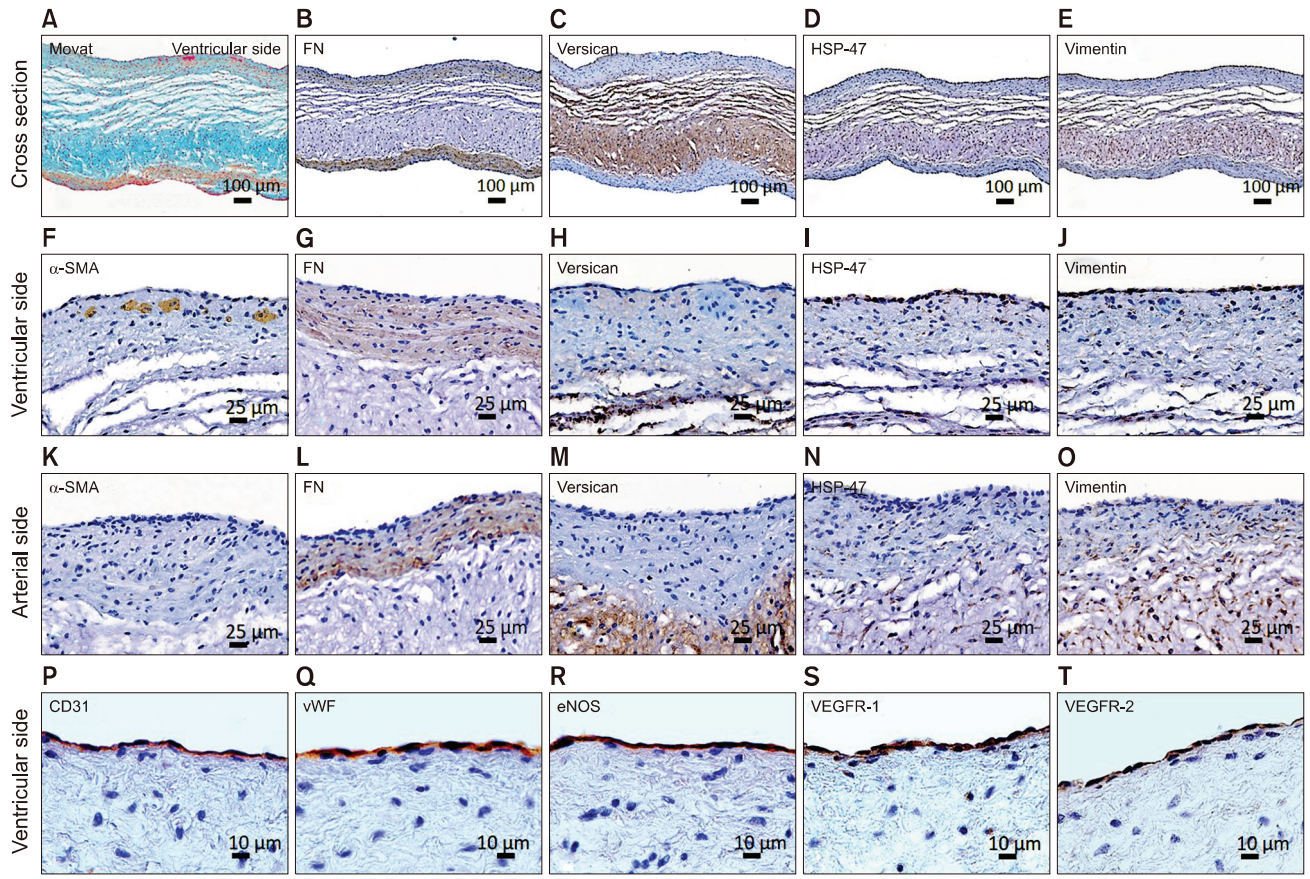

Fig. S1. Histological characterization of native porcine pulmonary valve leaflet (A∼T). Russell-Movat pentachrome staining showed the distribution of proteoglycan (blue color), collagen (yellow color), and fibrin (red color) within the leaflet (A). For panels (B∼T), presence of the protein of interest is indicated by brown staining; nuclei are counterstained with hematoxylin (blue). Expression of fibronectin (B) was mostly localized to the ventricular and arterial sides (G, L) whereas versican (C) was mostly detected within the spongiosa and fibrosa (H, M). Collagen-binding protein HSP 47 (D, I, N) and the intermediate filament protein vimentin (E, J, O) were expressed within all layers of the leaflet. A few cells showed positive staining for α-SMA on the ventricular side (F) but not on the arterial side (K). Expression of endothelial cell markers CD31 (P), vWF (Q), e-Nos (R), VEGFR-1 (S), and VEGFR-2 (T) was detected in the cells covering the surface of the leaflets (only the ventricular side is shown). No signal was detected in the control slides (data not shown).

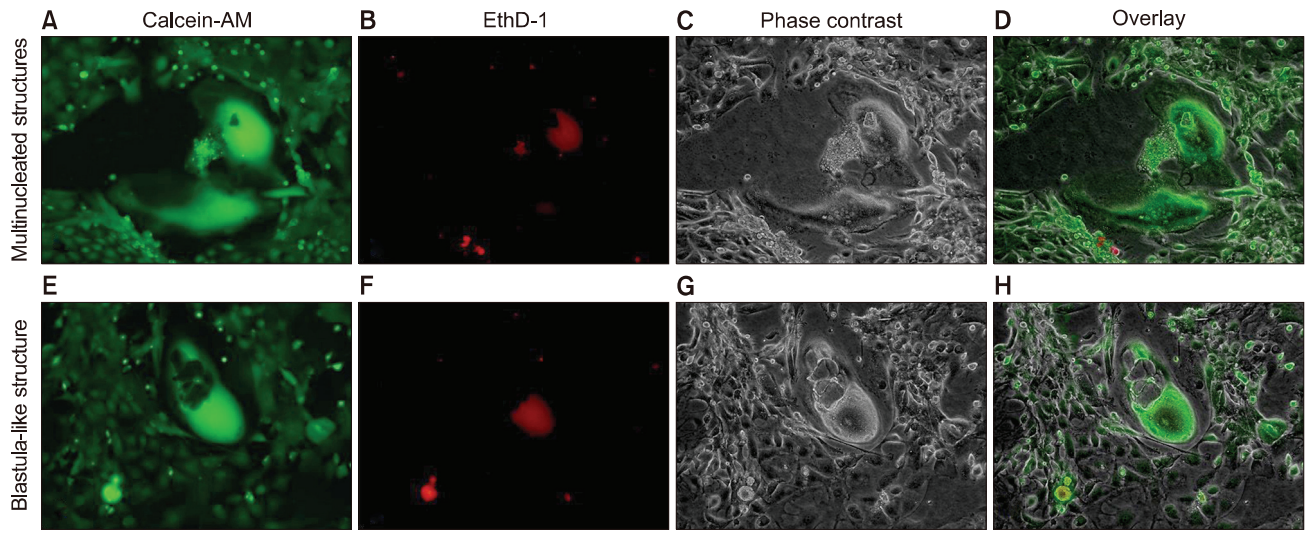

Fig. S2. Viability of cellular structures derived from in vitro culture of pulmonary valve leaflets. Live multinucleated cells and colonies were stained with Calcein-AM (green fluorescence) and dead cells with EthD-1 (red fluorescence).

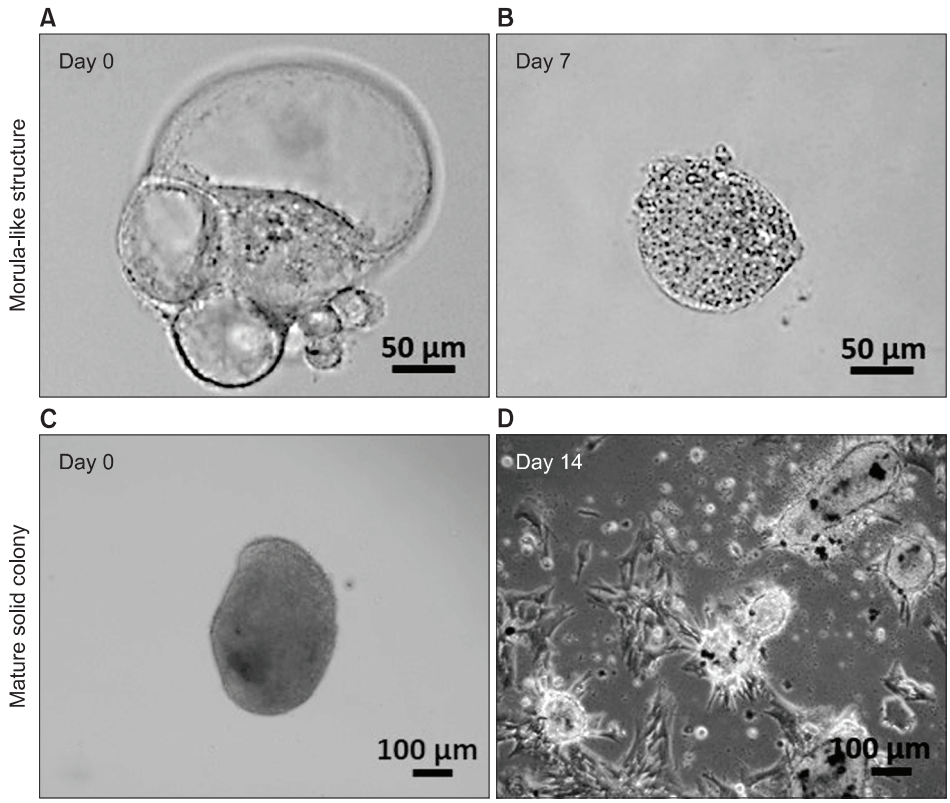

Fig. S3. Differentiation potential of morula-like structures and mature colonies. An isolated morula-like structure at Day 0 (A) degenerated after 7 days of culturing in DMEM (B), whereas an isolated mature colony at Day 0 (C) differentiated into fibroblast-like cells and formed new dense colonies after 14 days of culturing in DMEM (D).

Video S1. 3D imaging of blastula-like structure. Cytoskeleton staining of non-adherent colonies shows the position of cells on the surface of a hollow sphere. Pseudocolors have been used to visualize nuclei (green) and cytoskeleton (red).

Video S2. In vitro time-lapse imaging of fluorescently labeled cells for 10 days. Partial cell fusion was detected by delivering the cellular content of a green-labeled cell to a red-labeled cell (white arrow head) close to the niche area. Green fluorescent label was detected in the red cell at 3 days after the exchange, and green label accumulated (or was generated) in the recipient cell during the next 3 days. By 6 days, the red cell has become green in color and showed a reversible consolidation of cellular content. Accumulation of both green and red fluorescent label was detected in some non-adherent cells (yellow in overlay). Migration of some of the cells to the niche area was facilitated by highly motile non-adherent cells (yellow arrow head) followed by complete fusion of the cells with the niche multi-nucleated structure.