#### Supplementary figures

**Fig. S1** Characterizations of decellularized heart. (a) Immunofluorescent staining of native and decellularized heart thin sections showing the presence/absence of DAPI-positive nuclei (blue), actinin (green) or Cx43 (red). Nuclei and contractile proteins were not detected in decellularized heart. Scale bars, 50  $\mu$ m. (b) DNA quantification of native hearts and decellularized heart. Values are means ± SD, n=3. (c) Masson's trichrome staining of the decellularized matrix showed good retention of collagen.

**Fig. S2** Characterization of NSCs specific marker. (a) Sphere NSCs was identified and immunostained to identify nestin-the NSCs specific marker. DAPI-nucleus (b) Photograph of recellularized constructs under the perfusion culture on day 14 post recellularization. (c) Immunofluorescent staining of recellularized constructs for vWF- vascularization specific marker. (d) Immunofluorescent staining of recellularized constructs for DAPI and nestin after different days (day7, day14) of culturing. The nestin+ cells were counted over days of perfusion (Right figure). Error bars represent SD (n=3), determined using unpaired t-test. \*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001.

**Fig. S3** Co-immunostaining of neuron specific marker MAP2 (red) and astrocytes specific marker GFAP (green) after 7days or 14 days of perfusion culturing.

**Fig. S4** Characterization of cell type specific markers. (a) GFAP (green) and α-actinin (red), (b)Cx43 (green) and α- actinin (red) after 14 days of culturing. (c) Flow cytometry analysis characterized the percentage of α-actinin positive cells.

**Fig. S5** Histological analysis of recellularized constructs after different days (day7, day14) of perfusion. The sections of the recellularized constructs were immunostained with anti-GATA4, anti-c-kit and anti-Nkx2-5 antibodies.

**Fig. S6** Characterizations of decellularized brain. (a) SEM images of native brain and decellularized brain. (b) H&E staining of sections from the native brain and decellularized brain. (c) Immunostaining of native brain and decellularized brain with anti-laminin, anti-Collagen I, anti-Collagen III, anti-Collagen IV and anti-Fibronectin antibodies. No nuclear staining (DAPI) was observed in decellularized brain.

**Fig. S7** Quantitative proteomic profiling of decellularized heart. (a) The schematic image of the TMT labeling comparatively proteomic analysis of decellularized heart matrix and decellularized brain matrix. (b) Summary of the differently expressed proteins between decellularized heart and decellularized brain.

**Fig. S8** Analysis of the differently expressed proteins between decellularized heart and decellularized brain. (a) Functional enrichment analysis. (b) KEGG Pathway enrichment analysis.

**Fig. S9** GO-based enrichment and clustering analysis of up-regulated proteins (heart-vs-brain) based on cellular component. Q1 (0< Ratio T/C < 1/2), Q2 (1/2<= Ratio T/C < 1/1.5), Q3 (1.5 < Ratio T/C <=2) and Q4 (Ratio T/C >2).

**Fig. S10** GO-based enrichment and clustering analysis of up-regulated proteins (heart-vs-brain) based on molecular function. Q1 (0< Ratio T/C < 1/2), Q2 (1/2<= Ratio T/C < 1/1.5), Q3 (1.5 < Ratio T/C <=2) and Q4 (Ratio T/C >2).

**Fig. S11** GO-based enrichment and clustering analysis of up-regulated proteins (heart-vs-brain) based on biological process. Q1 (0< Ratio T/C < 1/2), Q2 ( $1/2 \le$  Ratio T/C < 1/1.5), Q3 ( $1.5 \le$  Ratio T/C <=2) and Q4 (Ratio T/C >2).

**Fig. S12** NSCs were seeded in 2D heart decellularized coating wells and cultured for 14 days. Immunofluorescent analysis of NSCs with neural lineage specific markers GFAP, MAP2 and cardiac specific markers α-actinin, GATA4.

















10<sup>2</sup> 10<sup>3</sup>





Laminin

Col IV



b

Name	Identified	Quantified	
Proteins	3477	2275	

Table 1 Summary of identified and quantified proteins

Name	Up-regulated	Down-regulated
Proteins	364	573

Table 2 Summary of differentially quantified proteins

а		-log10(Fisher' exact test p value)						
u		0	20	40	60	80	100	120
	extracellular matrix						10	0
en	extracellular region		11.43					
noc	proteinaceous extracellular matrix		11.09					
Ĕ	contractile fiber		10.01					
ŭ	contractile fiber part		9.98					
ulai	myofibril		9.73					
Gelle	extracellular region part		8.71					
0	extracellular matrix component		6.66					
_	extracellular matrix structural constituent		5.51					
ion	calcium ion binding		4.05					
nct	structural molecule activity	. 3	3.31					
F	glycosaminoglycan binding	2	.85					
ular	structural constituent of muscle	2	.85					
leci	copper ion binding	2	.59					
Mo	extracellular matrix binding	2	.31					
_	enzyme inhibitor activity	2	.29					
	muscle contraction		7.13					
	muscle system process		7.12					
	striated muscle tissue development		5.88					
	cardiac chamber development		5.61					
ess	cardiac ventricle development		5.52					
00	organ morphogenesis		5.5					
I Pr	muscle tissue development		5.43					
gica	muscle organ development		5.31					
golo	cardiac chamber morphogenesis		5.15					
Bio	circulatory system development	-	5.1					
	cardiovascular system development		5.1					
	ventricular cardiac muscle tissue morphogenesis		5.08					
	extracellular structure organization		4.7					
	extracellular matrix organization		4.7					



-ig. 35				Zscore(-log10(Fisher's test P value))
Cellu	lar C	omp	oner	nt 📃
01	02	03	04	-1.5 -1 -0.5 0 0.5 1 1 Value
GI	QZ	00	QT	cell body
				proteasome complex
	_			neuronal cell body
				synaptic vesicle membrane
				membrane region
				vacuolar membrane
				synapse
				bounding membrane of organelle
				synapse part
				endocytic vesicle
				Golgi apparatus part
				microtubule cytoskeleton
				organelle envelope lumen
				recycling endosome membrane
				cytoplasmic membrane-bounded vesicle
				cytoplasmic vesicle
				endosomal part
				midbody
				endosome membrane
				cytoplasmic vesicle part
				microtubule
				membrane raft
				recycling endosome
				brush border
				lysosomal membrane
				organelle membrane adherens junction
				extracellular region
				contractile fiber
				fibrillar collagen trimer
				myosin complex
				intracellular non-membrane-bounded organ
				non-membrane-bounded organelle
				myosin filament
				muscle myosin complex
				actin cytoskeleton
				sarcomere
				striated muscle thin filament
				cytoskeleton
				troponin complex extracellular matrix component
				Z disc
				myofibril
				extracellular space
				intermediate filament
				A band
				costamere
				collagen type V trimer
				extracellular matrix
				anchoring junction
				myosin II complex collagen trimer
				I band



#### **Biological Process**

Zscore(-log10(Fisher's test P value))





