

**Enrichment of CD146⁺ adipose-derived stem cells in combination
with articular cartilage extracellular matrix scaffold promotes
cartilage regeneration**

Supplementary materials

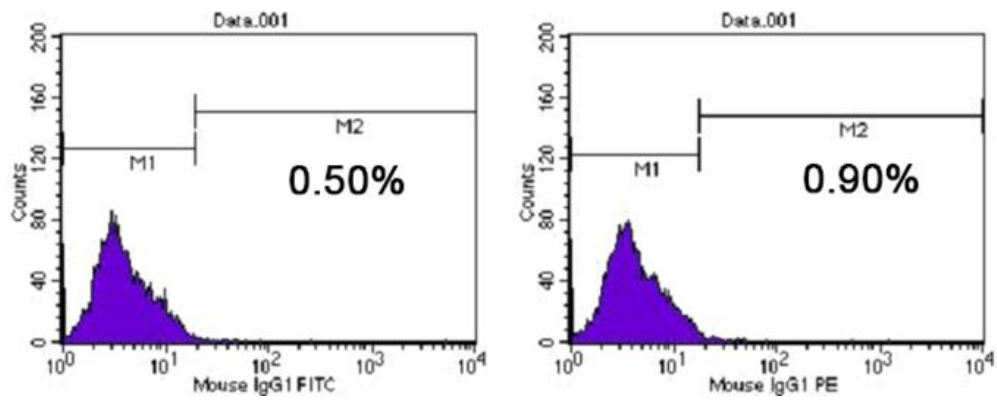


Figure S1. Isotype control of flow cytometry

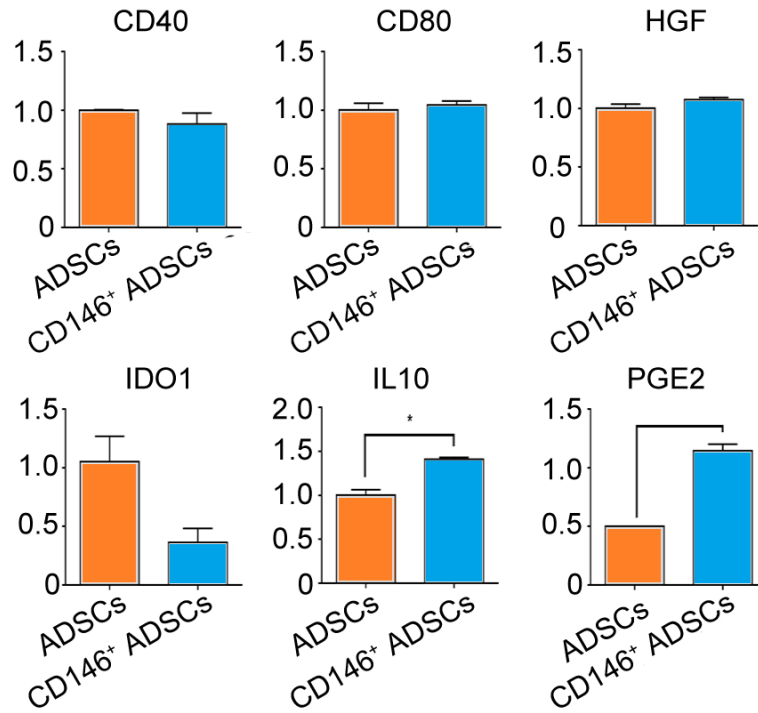


Figure S2. Quantification of mRNA levels of *CD40*, *CD80*, *HGF*, *IDO1*, *IL10*, and *PGE2* in CD146⁺ cells and hADSCs by qRT-PCR. Data represent mean \pm SD from three independent experiments.

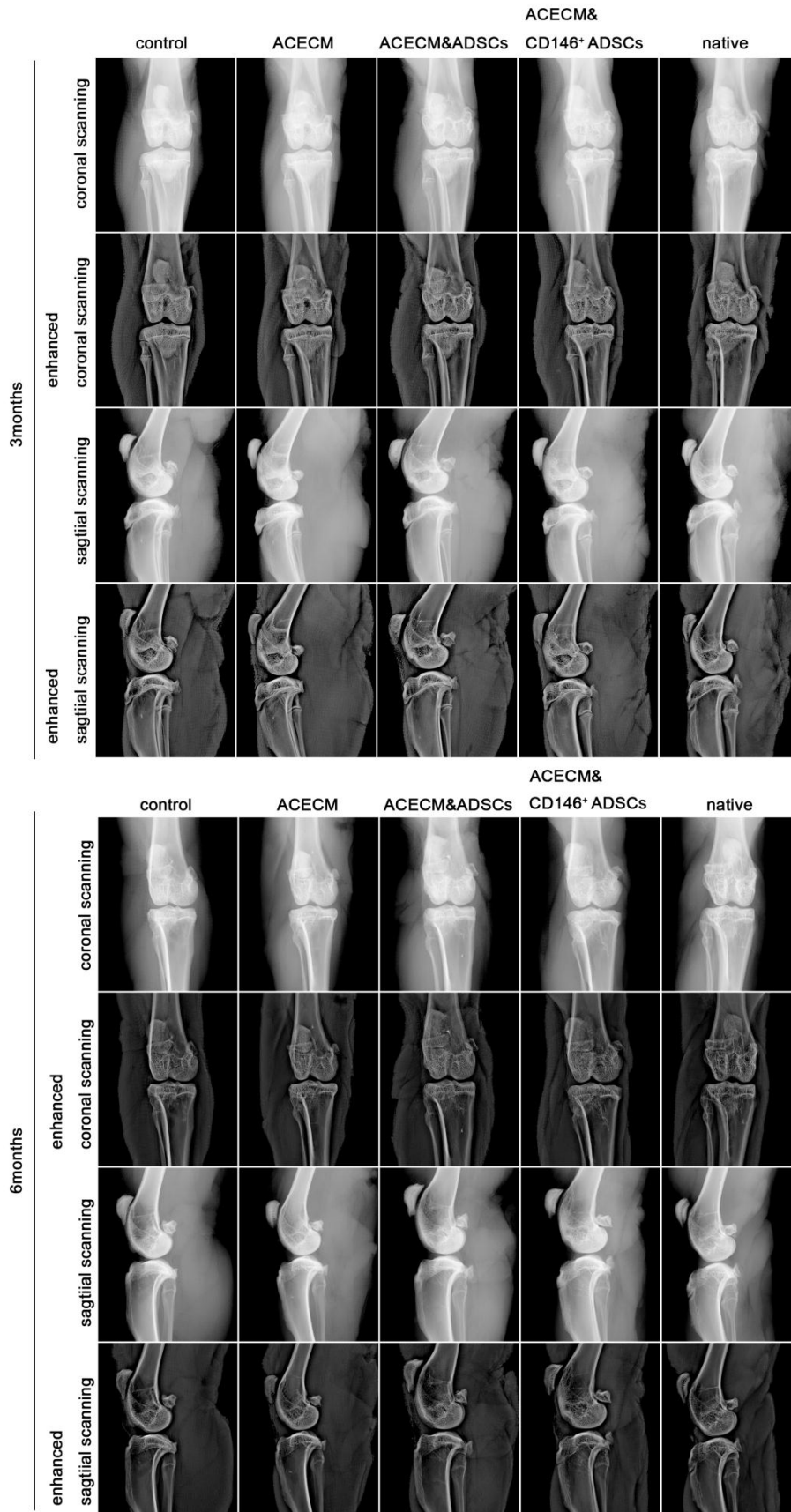


Figure S3. X-ray images of rabbit knees at 3 months and 6 months after surgery.

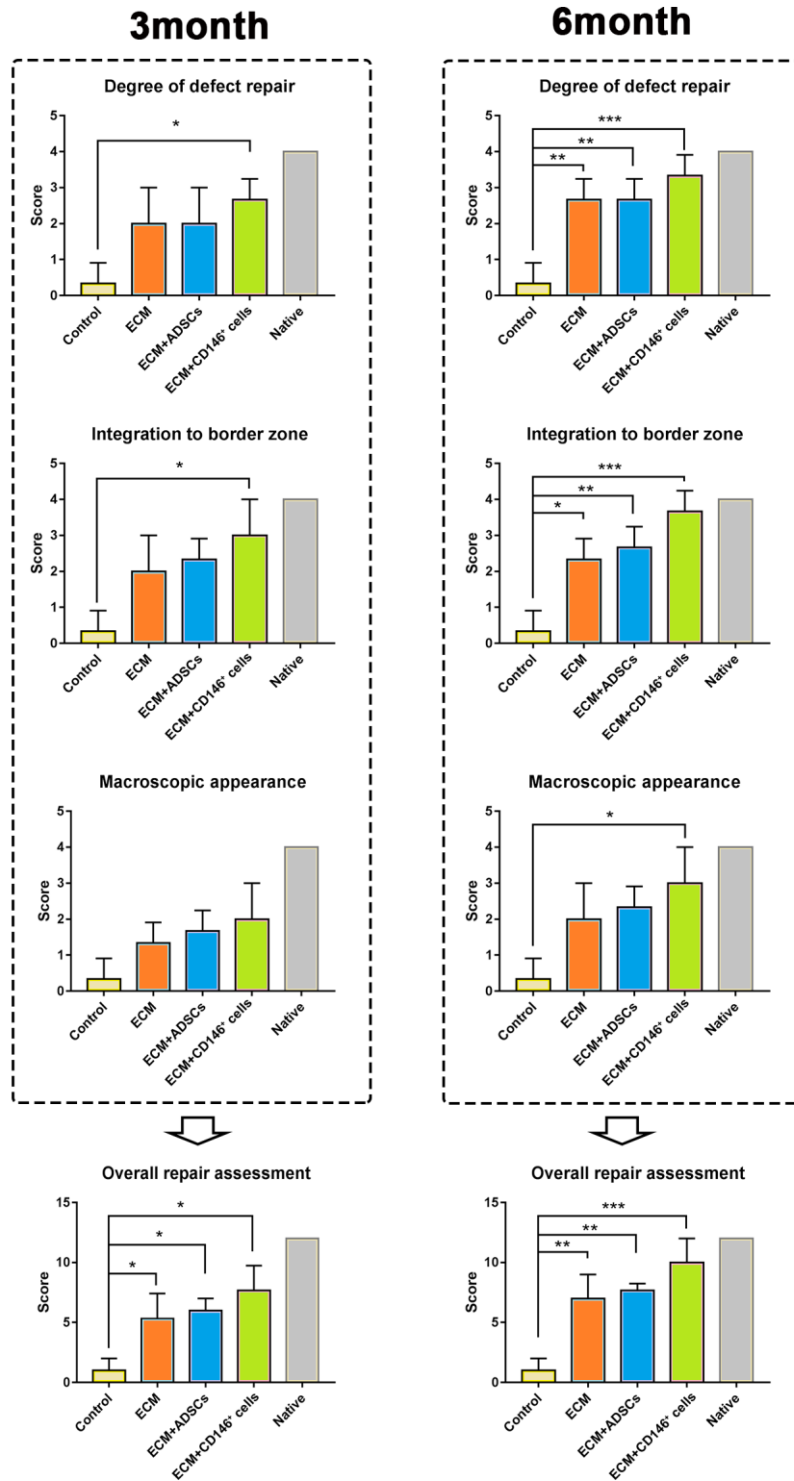


Figure S4. ICRS scoring system for macroscopic evaluation of cartilage repair after 3 and 6 months. The results represent the mean \pm standard error of the mean. * $P < 0.05$, ** $P < 0.01$.

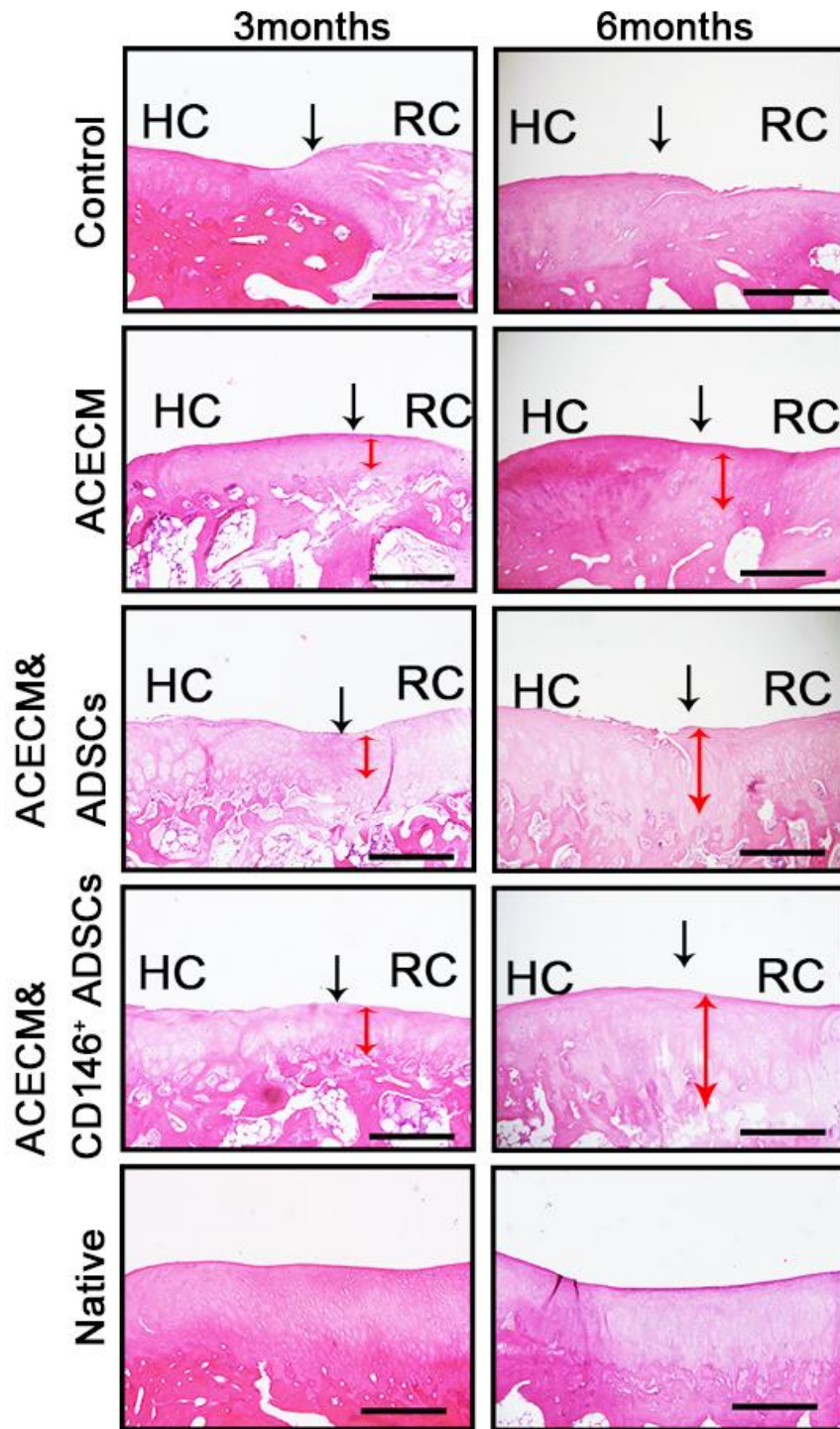


Figure S5. Histological analysis of cartilage defect after 3 and 6 months by H&E staining. The black solid arrows denote the repair interface. The red solid arrows denote the depth of the repaired cartilage. HC, host cartilage; RC, repaired cartilage. Scale bar: 200 μ m.

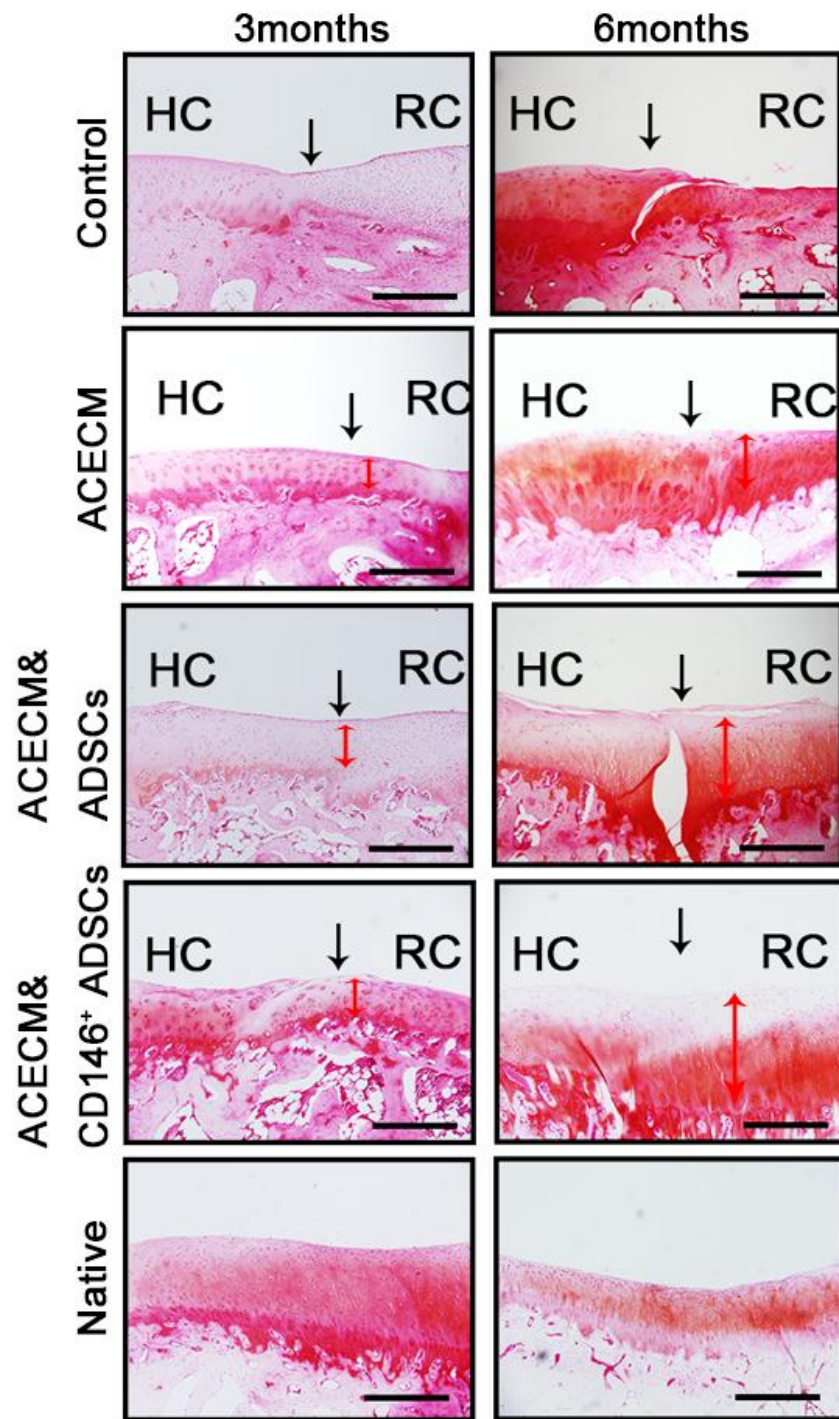


Figure S6. Histological analysis of cartilage defect after 3 and 6 months by Safranin O staining.

The black solid arrows denote the repair interface. The red solid arrows denote the depth of the repaired cartilage. HC, host cartilage; RC, repaired cartilage. Scale bar: 200 μ m

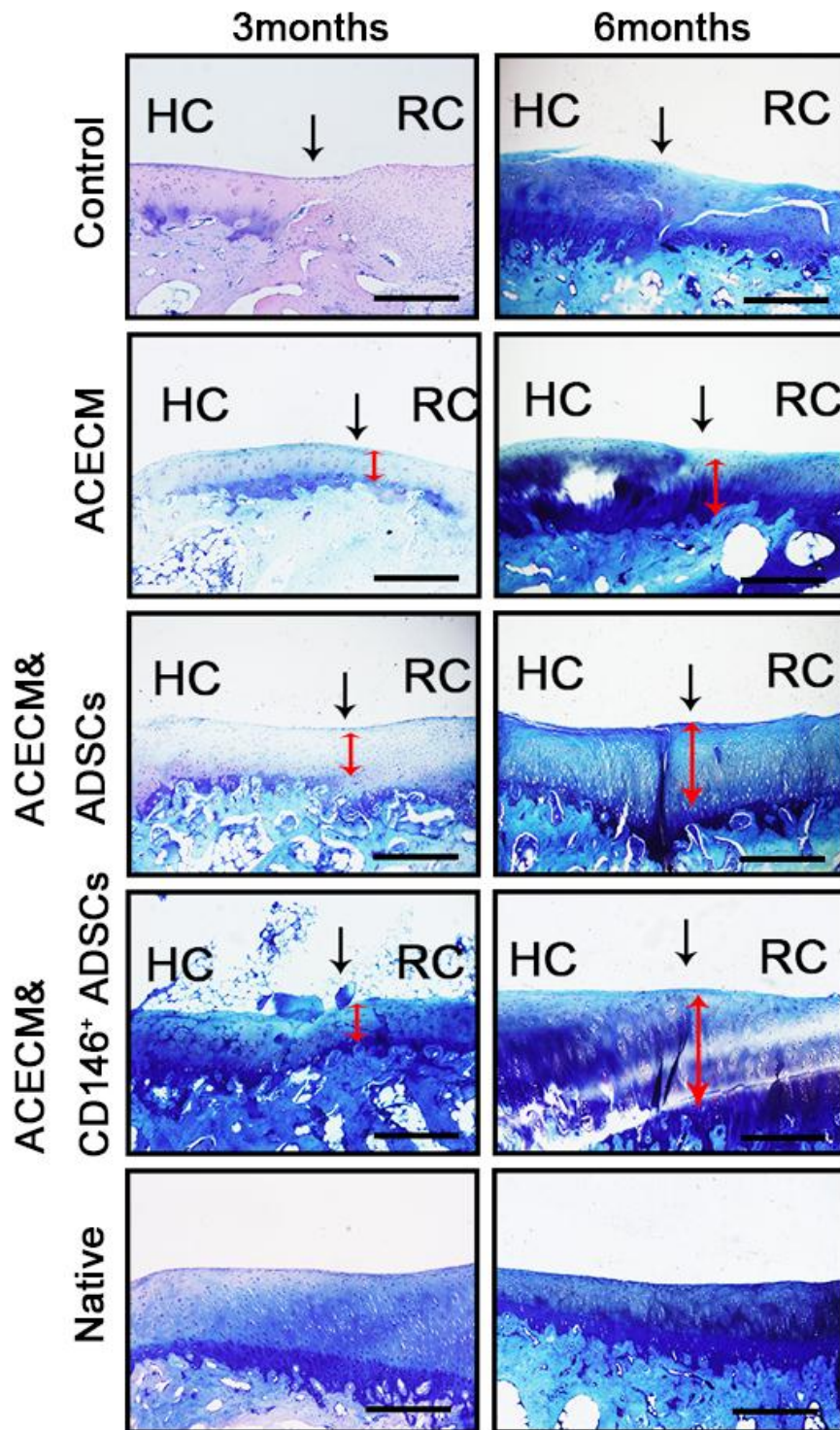


Figure S7. Histological analysis of cartilage defect after 3 and 6 months by Toluidine Blue staining. The black solid arrows denote the repair interface. The red solid arrows denote the depth of the repaired cartilage. HC, host cartilage; RC, repaired cartilage. Scale bar: 200 μ m.

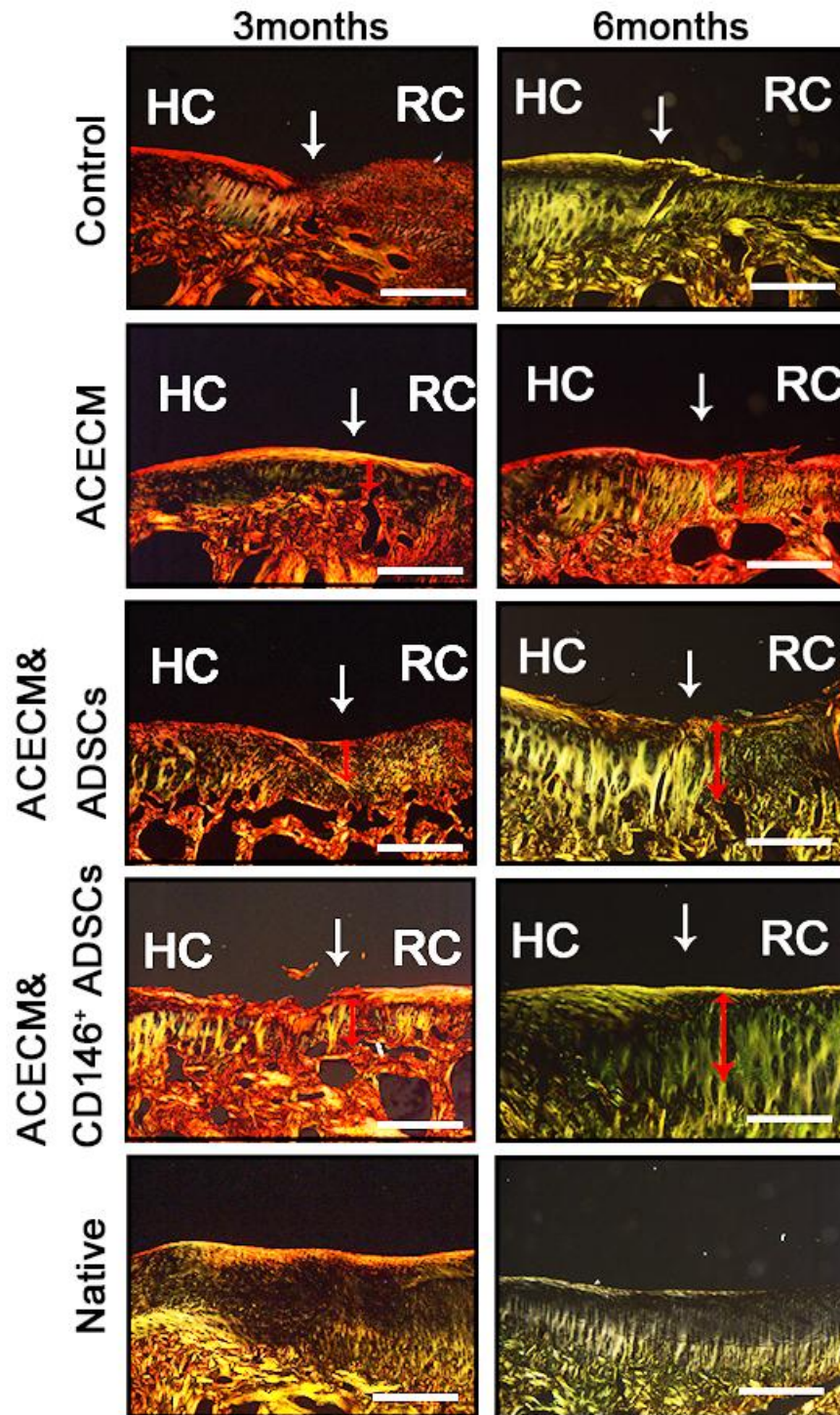


Figure S8. Histological analysis of cartilage defect after 3 and 6 months by Sirius Red staining.

The black solid arrows denote the repair interface. The red solid arrows denote the depth of the repaired cartilage. HC, host cartilage; RC, repaired cartilage. Scale bar: 200 μ m.

Table S1. List of Primers Used for RT-PCR Analysis

Gene	Forward primer	Reverse primer
CD80	GCTGGCTGGTCTTTCTCACT	GTGCCAGCTCTTCAACAGAA
CD40	CTCACCTCGCTATGGTTCGT	GGCACAAAGAACAGCACTGA
IdO1	GATGAAGAAGTGGGCTTTGC	CAGGCAGATGTTTAGCAATGA
PGE2	CCTTCTTCGAAAGTTTTGCC	ATGTGCAGTTGCCCTCTGTA
HGF	CGCTGGGAGTACTGTGCAAT	CCCTGTAGCCTTCTCCTTGA
IL-10	GTGGAGCAGGTGAAGAATGC	GCCACCCTGATGTCTCAGTT
ACTIN	ACCTTCTACAATGAGCTGCG	CCTGGATAGCAACGTACATGG
GAPDH	CAAGAAGGTGGTGAAGCAGG	GGTGTCGCTGTTGAAGTCAG

Table S2. Score System for MRI Evaluation

Criteria	Findings	Scores
Repaired cartilage signal	Isointense	2
	Hyperintense	1
	Hypointense	0
Repaired lesion morphologic features	Flush	2
	Proud	1
	Depressed	0
Repaired cartilage fill	Good (67%-100%)	2
	Moderate (34%-66%)	1
	Poor (0%-33%)	0
Peripheral repaired cartilage integration	No gap	2
	Small (gap of ≤ 2 mm)	1
	Large (gap of > 2 mm)	0
Subchondral edema	None	3
	Mild (< 1 cm ²)	2
	Moderate (1-3 cm ²)	1
	Severe (> 3 cm ²)	0
Osseous overgrowth	No	1
	Yes	0

Table S3. International Cartilage Repair Society Macroscopic
Evaluation of Cartilage Repair

Cartilage repair assessment ICRS	Points
Degree of defect repair	
In level with surrounding cartilage	4
75% repair of defect depth	3
50% repair of defect depth	2
25% repair of defect depth	1
0% repair of defect depth	0
Integration to border zone	
Complete integration with surrounding cartilage	4
Demarcating border <1 mm	3
3/4th of graft integrated, 1/4th with a notable border >1 mm width	2
1/2 of graft integrated with surrounding cartilage, 1/2 with a notable border >1 mm	1
From no contact to 1/4th of graft integrated with surrounding cartilage	0
Macroscopic appearance	
Intact smooth surface	4
Fibrillated surface	3
Small, scattered fissures or cracks	2
Several, small or few but large fissures	1
Total degeneration of grafted area	0
Overall repair assessment	
Grade I: normal	12
Grade II: nearly normal	11–8
Grade III: abnormal	7–4
Grade IV: severely abnormal	3–1

ICRS, International Cartilage Repair Society.

Table S4. International Cartilage Repair Society Visual Histological Assessment Scale for Cartilage Repair

Feature	Points
I. Surface	
Smooth/continuous	3
Discontinuities/irregularities	0
II. Matrix	
Hyaline	3
Mixture: hyaline/fibrocartilage	2
Fibrocartilage	1
Fibrous tissue	0
III. Cell distribution	
Columnar	3
Mixed/columnar clusters	2
Clusters	1
Individual cells/disorganized	0
IV. Cell population viability	
Predominantly viable	3
Partially viable	1
<10% viable	0
V. Subchondral bone	
Normal	3
Increased remodeling	2
Bone necrosis/granulation tissue	1
Detached/fracture/callus at base	0
VI. Cartilage mineralization (calcified cartilage)	
Normal	3
Abnormal/inappropriate location	0