Cartibeads: three-step vs two-step methods

| Negative log FC | 3-step method | 2-step method |
|----------------|---------------|---------------|
| Platelet degranulation | *** | *** |
| TP53 regulates transcription of cell cycle genes | *** | *** |
| IL-4 and IL-13 signaling | *** | *** |
| RHO GTPases activate PAKs | ** | ** |
| Ephrin signaling | *** | *** |
| TP53 regulation of transcription of genes involved in... | *** | *** |
| Elastic fibre formation | *** | *** |
| ECM organization | *** | *** |
| Collagen biosynthesis | *** | *** |
| Collagen chain trimerization | *** | *** |
| Collagen degradation, formation | *** | *** |

| Positive Log FC | 3-step method | 2-step method |
|----------------|---------------|---------------|
| IL-4/-13 signaling | *** | *** |

**Cartibeads**: three-step vs two-step methods.

Changes in GAG content, measured in µg/barrel, are shown for 3-step and 2-step methods under 21% and 5% O2 conditions. Differences are statistically significant at p < 0.056.

**Figure S1**

(a) 3-step method vs 2-step method for different donors.

(b) Comparison of 2-step method vs 3-step method for Collagen biosynthesis and Collagen chain trimerization.

(c) Graph showing the comparison of 3-step vs 2-step methods for GAG content under 21% and 5% O2 conditions.

(d) Heatmap illustrating the differences in gene expression between 3-step and 2-step methods for various biological processes.
Figure S2

a

CD73

CD90

CD105

FL1 100.0

FL5 positive cells

FL4 subset

100.0

CD45

CD14

CD34

FL2 +

FL1 +

FL6 +

2.48

2.96

2.22

b

ASC  MSC  Donors 17  Donors 13  Donors 3

Osteocyte

Adipocyte

Chondrocyte
| ID    | Sex | Age | Pathology                      |
|-------|-----|-----|--------------------------------|
| Donor 1 | M   | 21  | Knee trauma                    |
| Donor 2 | M   | 47  | Knee trauma                    |
| Donor 3 | M   | 29  | Knee trauma                    |
| Donor 4 | F   | 65  | Knee osteoarthritis            |
| Donor 5 | M   | 80  | Knee osteoarthritis            |
| Donor 6 | F   | 64  | Knee osteoarthritis            |
| Donor 7 | M   | 69  | Knee osteoarthritis            |
| Donor 8 | M   | 87  | Femoral condyle necrosis       |
| Donor 9 | F   | 28  | Patellar dislocation           |
| Donor 10 | M   | 58  | Degenerative focal lesion      |
| Donor 11 | F   | 60  | Knee osteoarthritis            |
| Donor 12 | M   | 38  | Osteochondrosis Disseccance    |
| Donor 13 | M   | 68  | Knee osteoarthritis            |
| Donor 14 | M   | 63  | Knee osteoarthritis            |
| Donor 15 | F   | 74  | Knee osteoarthritis            |
| Donor 16 | M   | 82  | Knee osteoarthritis            |
| Donor 17 | M   | 76  | Ankle arthrosis                |
| Donor 18 | F   | 70  | Ankle arthrosis                |
| Donor 19 | M   | 74  | Ankle arthrosis                |
| Donor 20 | F   | 70  | Knee osteoarthritis            |
| Donor 21 | M   | 73  | Knee osteoarthritis            |
| Donor 22 | M   | 76  | Knee osteoarthritis            |
|       | Donor 16 | Donor 7 | Donor 10 |
|-------|----------|---------|----------|
| CD73  | 99.7     | 99.8    | 99.6     |
| CD90  | 93.2     | 99.7    | 99.7     |
| CD105 | 89.2     | 92.9    | 90.7     |
| Donor (CGH array) | Cell passage | Deletions | Duplications |
|------------------|--------------|-----------|--------------|
| Donor 2 (M, 47)  | P3 & P11     | nd        | nd           |
| Donor 3 (M, 29)  | P4 & P8      | nd        | nd           |
| Donor 4 (F, 65)  | P4           | nd        | nd           |
| Donor 5 (M, 80)  | P4           | Y loss    | nd           |
| Donor 6 (F, 64)  | P5           | nd        | nd           |
| Donor 7 (M, 69)  | P5           | nd        | nd           |
| Donor 10 (M, 58) | P4           | nd        | nd           |
| Donor 12 (M, 38) | P4           | nd        | nd           |
| Donor 13 (M, 68) | P5           | Y loss    | nd           |
| Donor 14 (M, 63) | P3           | Y loss    | nd           |

Nd = not detected
| Implant source         | Cell passage | Mice implanted | Tumor (6 months) |
|------------------------|--------------|----------------|------------------|
| Donor 2 (M, 47)        | P3           | 10             | 0                |
| Donor 3 (M, 29)        | P6           | 10             | 0                |
| Donor 11 (F, 60)       | P3           | 10             | 0                |
| Donor 12 (M, 38)       | P6           | 14             | 0                |
| Donor 15 (F, 74)       | P4           | 10             | 0                |
| A549 (adenocarcinoma)  |              | 8              | 100% (6 weeks)   |

Study mice at 6 months, normal skin and internal organs

Control mice at 6 weeks with internal organs damage (arrows show haemorrhaging)
| Gene     | Gene name                  | Nucleotide sequence                  | Size amplicon |
|----------|----------------------------|--------------------------------------|---------------|
| ACAN     | Aggrecan                   | Fwd AAGTCGTGGTGAAAGGCATC             | 109 pb        |
|          |                            | Rev GCAATGATGGCACTGTCTTG             |               |
| COL2A1   | Collagen type II alpha 1 chain | Fwd CTGGAAAAGCTGGTGAAAGG | Size 105 pb   |
|          |                            | Rev GGCCTGGATAACCTCTGTGA             |               |