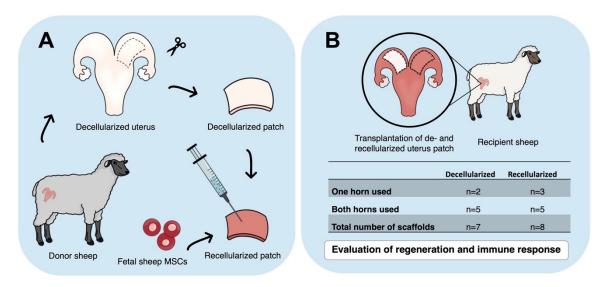
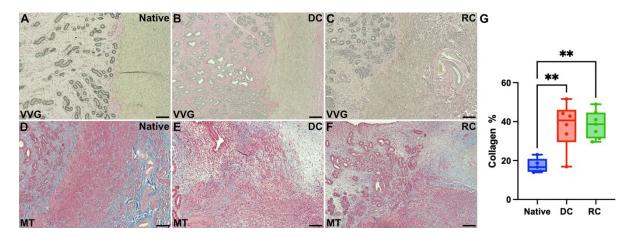
Electronic Supplementary Material (ESI) for Biomaterials Science. This journal is © The Royal Society of Chemistry 2024

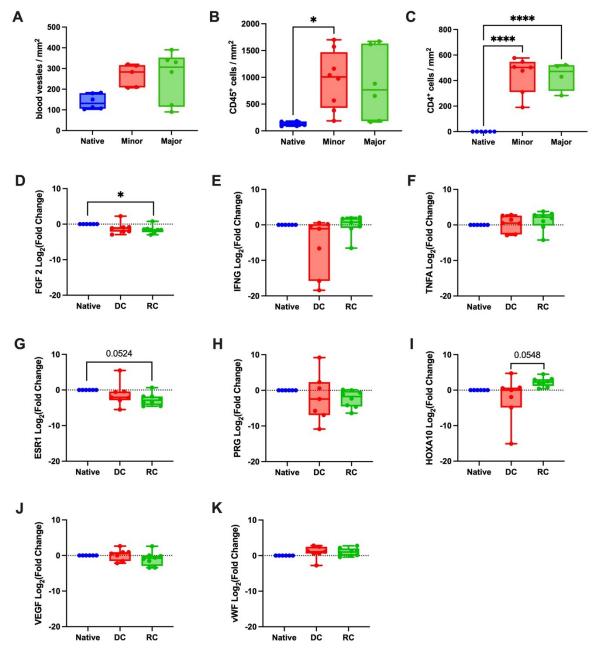
## **SUPPLEMENTARY FIGURES**



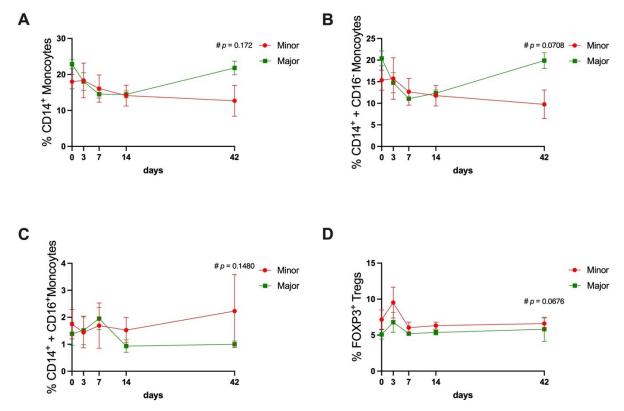
Supplementary Figure 1. Overview of the study design. (A) Whole uteri from donor sheep were decellularized to obtain  $2 \times 3$  cm tissue patches, which, for the recellularization group, were repopulated using fetal sheep mesenchymal stem cells. (B) A total of 10 recipient sheep got a uterus patch transplantation. Transplantations were done in two rounds. On the first one (n = 5), only one uterine horn received the graft (2 received a DC graft and 3 an RC graft). On the second one (n = 5), both uterine horns received a transplanted graft, one DC and the other one an RC graft. DC: decellularized; MSCs: mesenchymal stem cells; RC: receilularized.



Supplementary Figure 2. Elastin staining was detected in native (A) but also both experimental groups (B,C). Collagen was also visualized (D,E,F) and quantified (G) in native and both experimental groups. DC: decellularized; MT: Masson's trichrome staining; RC: recellularized; VVG: Verhof-van Gieson staining. Scale bars =  $125 \mu m$ , \*\*p < 0.01



Supplementary Figure 3. The number of blood vessels quantified was significantly higher in the recellularized group (A). Infiltration of CD45+(B) and CD4+ (C) showed a significant increase compared to the native control. Fold changes of both experimental groups (DC and RC) are calculated in relation to the native control group. Target genes were fibroblast growth factor 2 (FGF2; D), interferon-gamma (IFNG; E), tumor necrosis alpha (TNFA; F), estrogen receptor 1 (ESR1; G), progesterone receptor (PGR; H), homeobox A10 (HOXA10; I), vascular endothelial growth factor (VEGF; J), and von Willebrand factor (vWF; K). CD45+; Leukocytes, CD4; T-cells, DC: decellularized; ns: non-significant; RC: recellularized. \*\*p < 0.05 \*\*p < 0.01, \*\*\*\*p < 0.0001.



Supplementary Figure 4. The systemic immune response FACS analysis revealed no changes between groups (minor and major regeneration groups) or across time in the percentage of monocytes (A), either if considering their classical (CD14+CD16+; C) phenotypes. The percentage of regulatory T cells also remained the same between groups (D).

Supplementary Table 1. Primers and probes design. NCBI accession for each of the genes and nucleotide sequences for forward (FW) and reverse (RV) primers and probes are shown in the table. Intercalating dyes and quenchers included in the probes are also

Gene	NCBI accession	Primers sequences 5'→3'	5'→3' dye and quenchers in probes
PGR	XM_015100878.3	FW: TCTGCTGACAAGTCTGAA	•
		RV (as): GGTCATCAATATGTAAGTTCCG	
		P: CTCAGTGGTCAAGTGGTCTAAGTCA	6-FAM/ZEN/IBFQ
ESR1	XM_027972563.2	FW: GCACTTTACCCACATCATG	
		RV (as): GGCTGTTCCCAAAACAAG	
		P: TCGCTCGGTTCTATGTGGCA	HEX/ZEN/IBFQ
HOXA10	XM 004007939	FW: AGCTCTATTTACTTCTAATCTTAA	
	_	RV (as): CACAGCTTTTATTCTTCTATAAG	
		P: GTÁATGCTGCTGTGCGTGGAA	HEX/ZEN/IBFQ
VEGF	NM 001025110	FW: CTGCTGTAATGACGAAAG	
	_	RV (as): TGCTGTAGGAAACTCATC	
		P: CCACTGAGGAGTTCAACATCACCA	6-FAM/ZEN/IBFQ
vWF	XM 027967983	FW: CATCGTGACCTTTGATGG	
	_	RV (as): GCAGAATCCGTTATGGAG	
		P: GGAGCAGGACTTGGAGGTGAT	HEX/ZEN/IBFQ
IFNG	NM_001009803	FW: GCCATCAATGAACTCATC	
	_	RV (as): CAGGAGAACCATTACATTG	
		P: TGATGAATGACCTGTCGCCAA	6-FAM/ZEN/IBFQ
FGF2	NM 001009769.1	FW: GAACGATTGGAGTCTAATAAC	
	_	RV (as): TGGGTCCAAGTTTATACTG	
		ACTCCAGTTGGTATGTGGCACT	6-FAM/ZEN/IBFQ
TNFA	NM 001024860.1	FW: GATGCTGATTTGGTGACTG	
	_	RV (as): CTCAGGACACTTTATTTCTCG	
		P: TACATCACTGAACCTCCGCTCC	6-FAM/ZEN/IBFQ
PPIH	XM 027968560	FW: GATGGACTTCTGGTGATG	
	_	RV (as): CCTCTGGACTACATCTCC	
		P: AAGCTGCCTGTGGTGATCTCA	HEX/ZEN/IBFQ

Supplementary Table 2. Monoclonal antibodies used for the flow cytometry analysis. AF: alexa fluor; BV: brilliant violet; CD: cluster of differentiation; Cy: cyanine; FITC: fluorescein isothiocyanate; Ig: immunoglobulin; MHC: major histocompatibility complex; NCR1: natural cytotoxicity triggering receptor 1; NK: natural killer; PE: phycoerythrin; PerCP: peridinin chlorophyll protein.

## Characterization of monocytes, macrophages, and NK cells

Monoclonal antibody target protein	Fluorochrome	Clone	Catalogue No.
CD14	AF488	Tuk4	BioRad (MCA1568A488)
CD68	AF647	SK3	BioRad (MCA341A647)
CD16 (primary antibody)	-	VPM64	BioRad (MCA919GA)
NKp46 (CD335/NCR1)	PE	AKS1	BioRad (MCA2365PE)
IgG1(secondary antibody)	BV421	A85-1 (RUO)	BD Biosciences (562580)

## Characterization of $T_{\text{Regs}}$ , cytotoxic T cells, and helper T cells

Monoclonal antibody target protein	Fluorochrome	Clone	Catalogue No.
FOXP3	PerCP-Cy5.5	FJK-16s	eBioscience <sup>TM</sup> (45-5773-82)
CD25	FITC	9.14	BioRad (MCA2218F)
MHC2	PE	28.1	BioRad (MCA2225PE)
MHC2	FITC	28.1	BioRad (MCA2228F)
CD4	AF647	44.38	BioRad (MCA2213A647)
CD45R (primary antibody)	-	20.96	BioRad (MCA2221GA)
CD8	PE	38.65	BioRad (MCA2216PE)
IgG1(secondary antibody)	BV421	A85-1 (RUO)	BD Biosciences (562580)