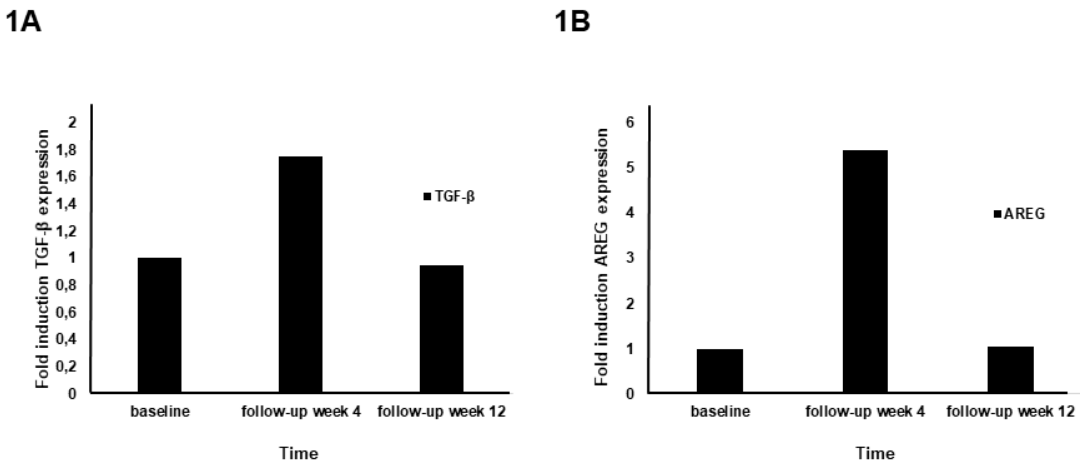
Supplemental Material

Supplemental figure 1



Supplemental figure 1. Adoptive Treg transfer is associated with enhanced TGF-β expression and tissue repair. mRNA expression levels of TGF-β **(A)** and amphiregulin **(B)** were measured by qRT-PCR and normalized to the housekeeping gene HPRT in gut biopsies derived before adoptive Treg transfer (baseline) and 4 weeks and 12 weeks after adoptive Treg transfer. TGF-β, transforming growth factor beta; AREG, amphiregulin.

Voskens C, *et al. Gut* 2022;0:1–5. doi: 10.1136/gutjnl-2022-327075

Supplemental table 1 Lot-release of the Treg product intended for clinical use

Parameter				**Specification**
Cell count	Cell count alive/ml	8.44	X 10^6/ml	≥ 5.0
Vitality	Cell count alive/dead	89.7	%	≥ 75
Phenotype	$CD4^+$	98.9	%	≥ 90
	$CD25^+$	85.1	%	≥ 80
	CD127+	1.4	%	≤ 10
	$CD4^+CD25^+CD127^{low/-}$	81.6	%	≥ 70
	$CD8^+$	0.0	%	≤ 0.5
	$CD19^+$	0.0	%	≤ 1.0
	$CD56^+$	0.0	%	≤ 1.0
Suppression	Tresp+Treg (1+1)	99.6	%	≥ 80
	Tresp+Treg (5+1)	96.7	%	≥ 60
	Tresp+Treg (10+1)	87.3	%	≥ 50
Expander beads	Remaining bead count	< 400	n/100 x 10^6	< 1000
Sterility	Microbiological growth	negative	-	negative
	Endotoxin	< 0.75	IU/ml	< 5
	Mycoplasma DNA	negative	-	negative

Supplemental table 2 Previous ulcerative colitis related medications

Previous therapies	Duration of treatment	Reason for discontinuation
Azathioprine	2007-2016	Loss of response
Golimumab	2017-2018	Severe skin allergic reactions
Vedolizumab	2018-2019	Non-response
Ustekinumab	2019-2021	Non-response

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Supplemental table 3 Concomitant medication during the study

Concomitant therapies	Dose
5-aminosalicylic acid	4 g / day
Ursodeoxycholic acid	1 g / day

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Supplemental table 4 Clinical markers during the study

	Baseline	Treg transfer	Follow-up week 2	Follow-up week 4	Follow-up week 8	Follow-up week 12
Mayo Score SF	2	3	3	2	2	1
Mayo Score RB	1	1	0	0	0	0
PGA	2	3	1	2	0	1
Partial Mayo Score	5	7	4	4	2	2

SF= Stool frequency; RB= Rectal bleeding; PGA= Physician Global Assessment

Supplemental table 5 Laboratory parameters

Parameter	normal range	baseline	Follow-up week 2	Follow-up week 4	Follow-up week 12
Calprotectin	0 - 50 µg/g	5637.0	2325.6	2925.0	3029.9
Leucocytes	(4 - 11) x 10e3/µl	6.69	6.17	7.64	5.21
Haemoglobin	(11.8 - 15.5) g/dl	14.2	13.3	13.3	13.6
Thrombocytes	(160 - 400) x 10e3/µl	411	392	392	347
neutrophils	%	69.0	52.2	57.4	53.9
eosinophils		2.1	1.6	1.7	1.7
basophils		0.9	1.1	0.8	1.0
lymphocytes		18.4	32.1	29.2	29.4
monocytes		9.6	13.0	10.9	14.1
potassium	(3.6 - 4.8) mmol/l	4.3	4.0	4.1	4.3
sodium	(135 - 145) mmol/l	138	136	139	140
creatin-kinase	(< 170) U/l	39	103	26	35
AST	(< 35) U/l	176	91	63	158
ALT	(< 35) U/l	165	76	57	152
LDH	(< 250) U/l	177	102	110	167
Gamma-GT	(< 40) U/l	938	679	289	859
alk. Phosphatase	(35 - 105) U/l	1089	811	490	1065
Glucose	(70 - 110) mg/dl	118	80	75	86
Calcium	(2.20 - 2.65) mmol/l	2.28	2.31	2.18	2.28
urea	(17 - 43) mg/dl	23	17	17	20
Kreatinin	(0.51 - 0.95) mg/dl	0.64	0.68	0.63	0.71
Bilirubin total	(< 1.1) mg/dl	1.8	1.5	1.1	1.5
Magnesium	(0.7 - 1.1) mmol/l	0.8	0.8	0.8	0.8
Albumin	(35 - 55) g/l	35.7	37.2	34.7	38.3
CRP	(< 5) mg/l	16.4	8.0	9.8	14.4

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