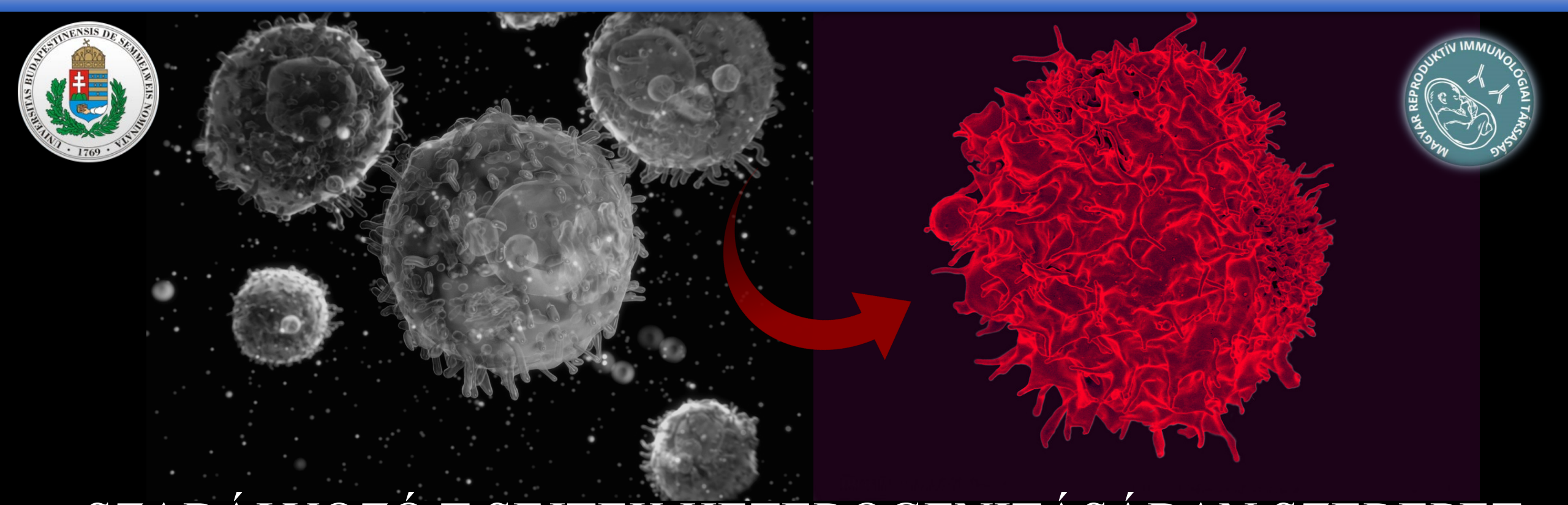


SZABÁLYOZÓ T-SEJTEK HETEROGENITÁSÁBAN SZEREPET JÁTSZÓ HSPE1 VIZSGÁLATA EGY-SEJT TRANSZKRIPTOMIKAI MÓDSZERREL

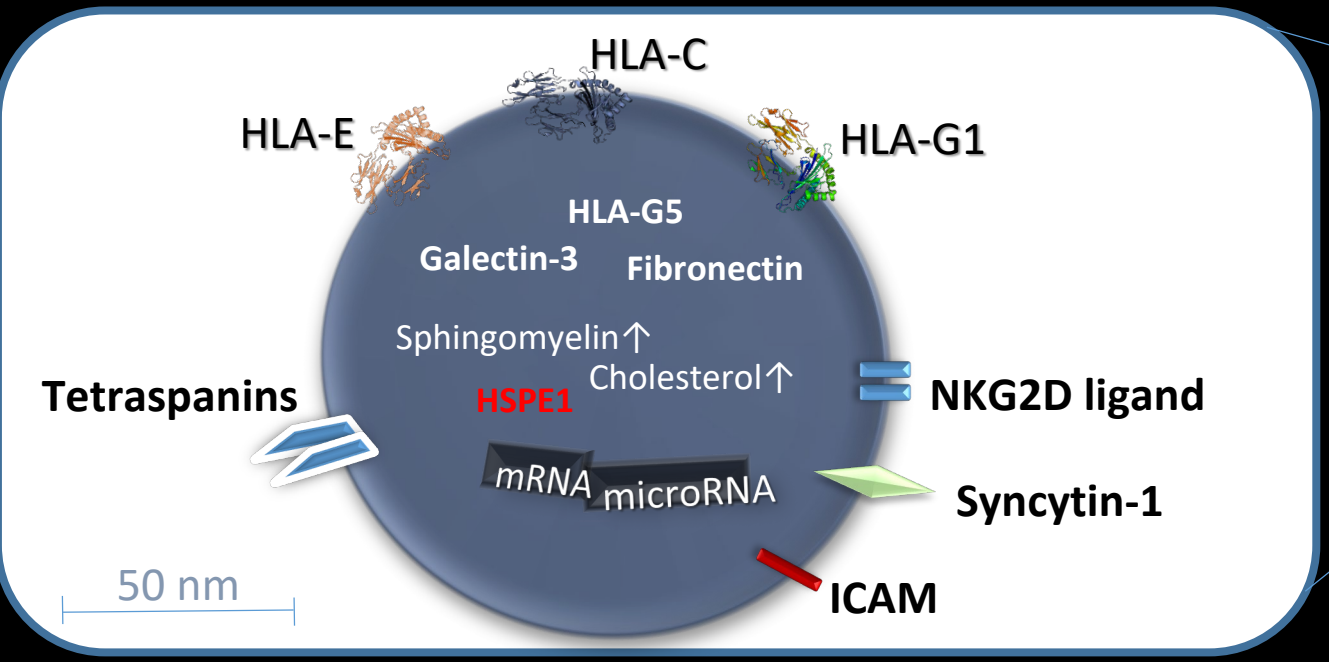
Kovács Árpád Ferenc[1], Fekete Nóra[1],Lumbach Réka[1], Kőhidai László[1], Buzás Edit[1], Pállinger Éva[1]

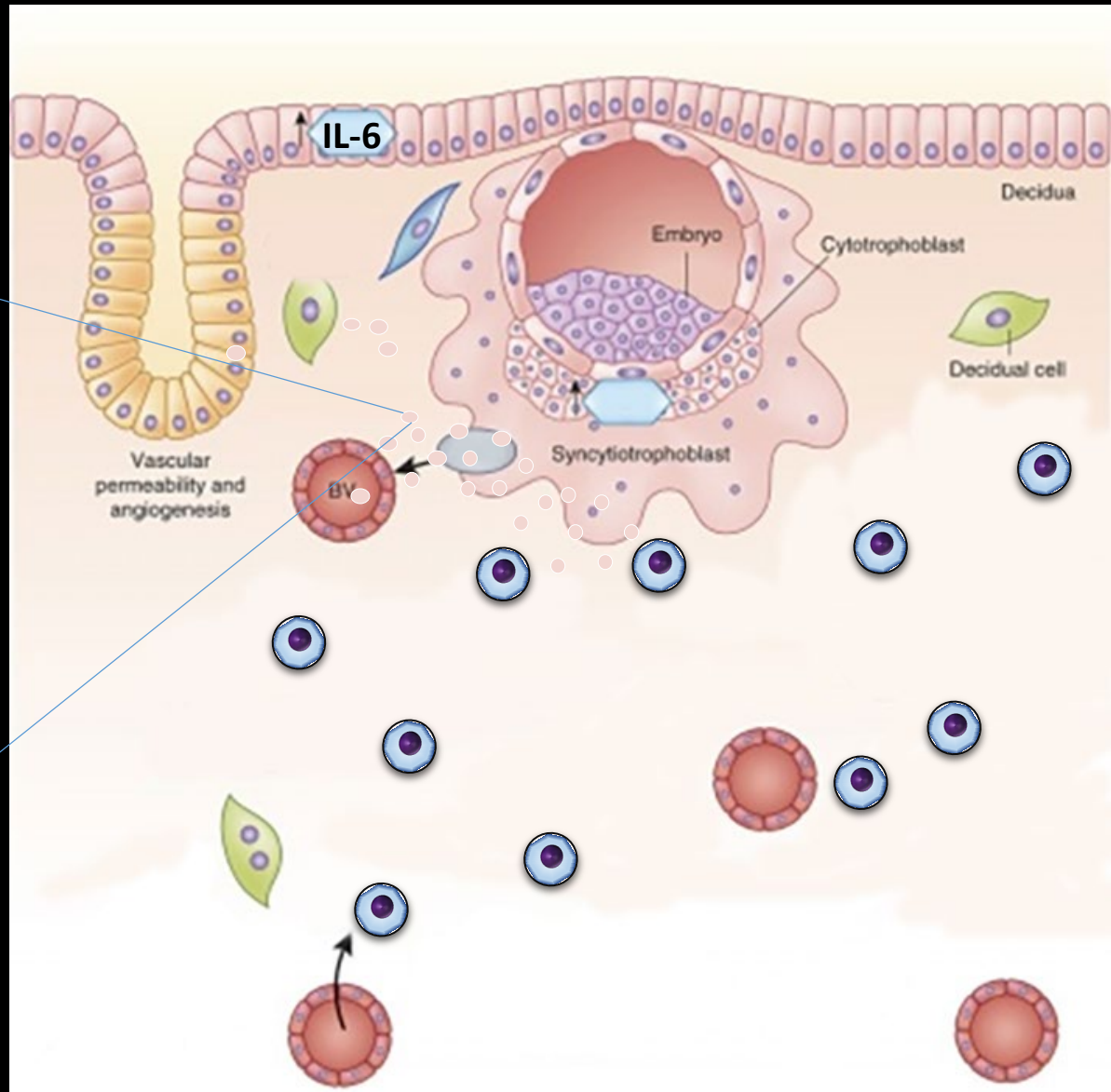
Semmelweis Egyetem, Genetikai, Sejt- és Immunbiológiai Intézet

2019/03/30

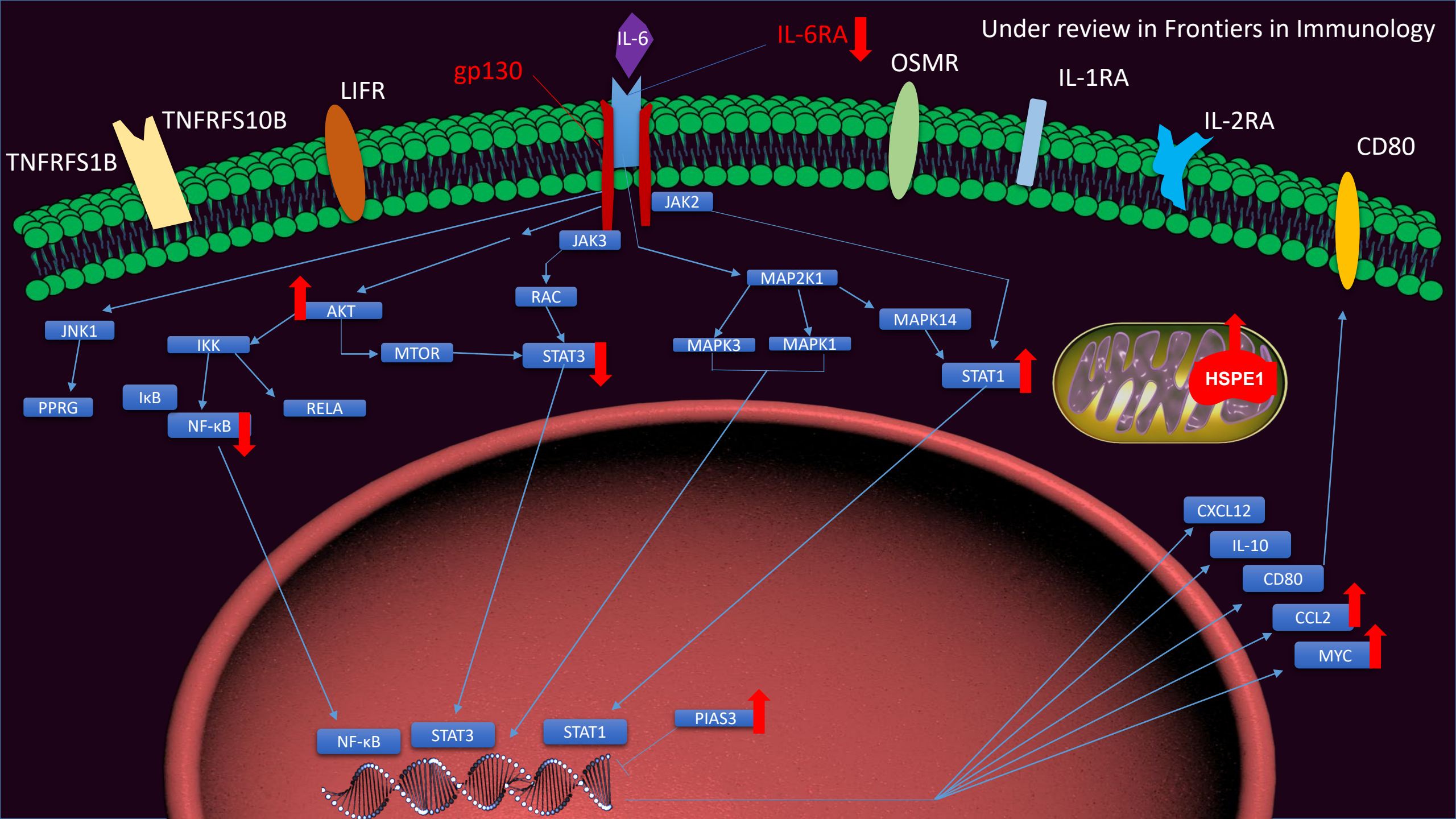
A T_{reg}-sejtek szerepe a humán reprodukcióban

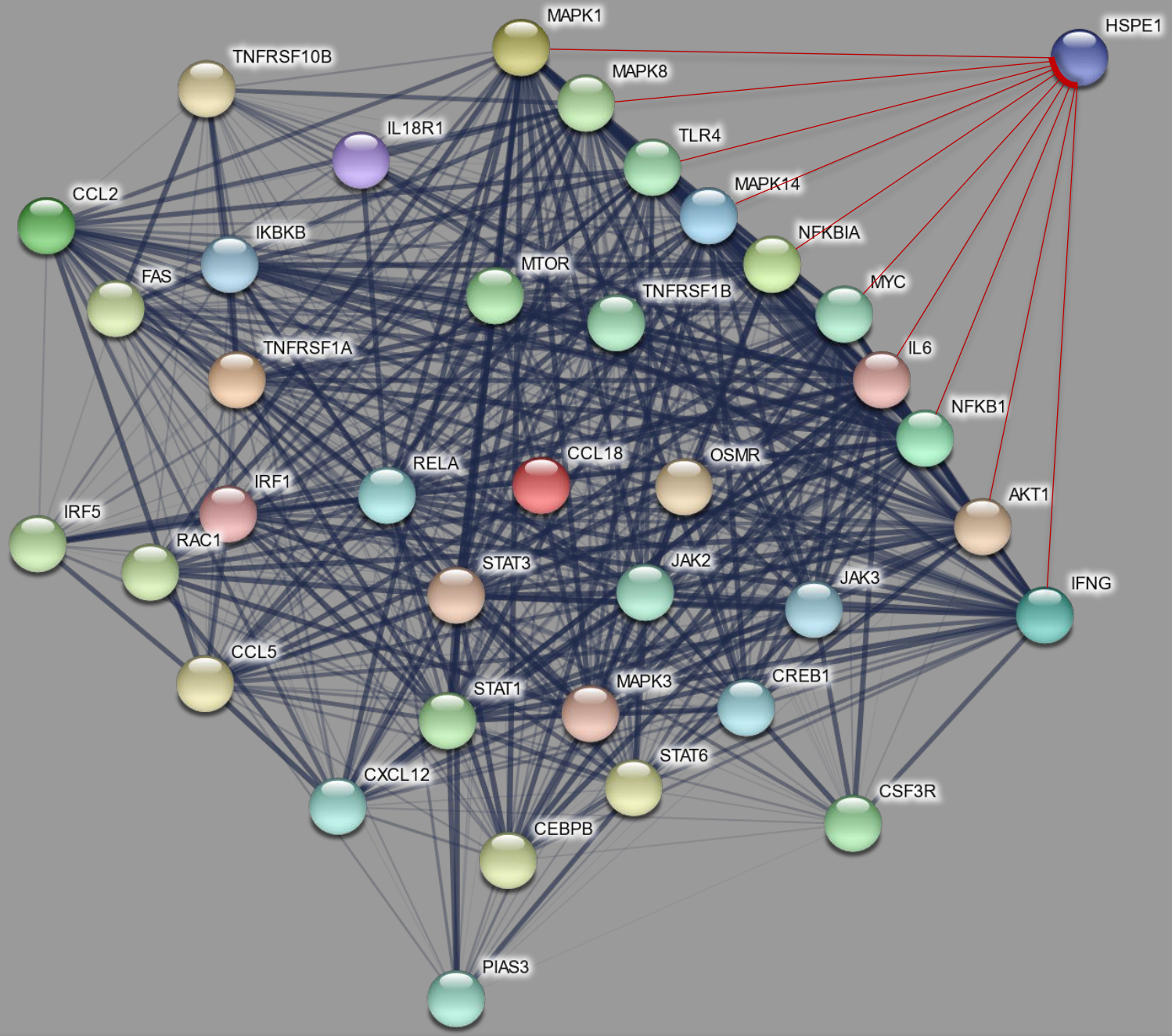
PERI-IMPLANTÁCIÓS IDŐSZAK

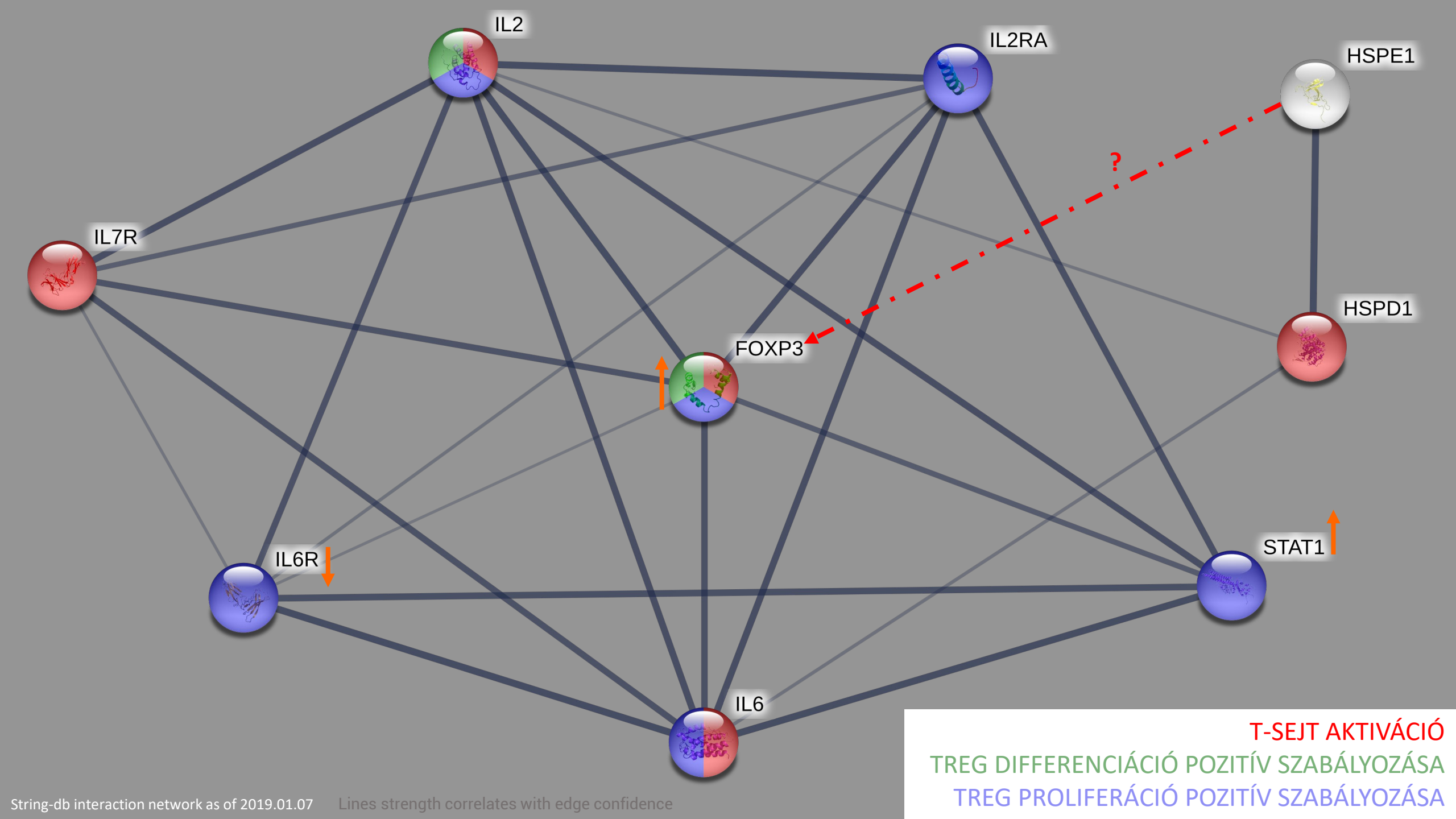




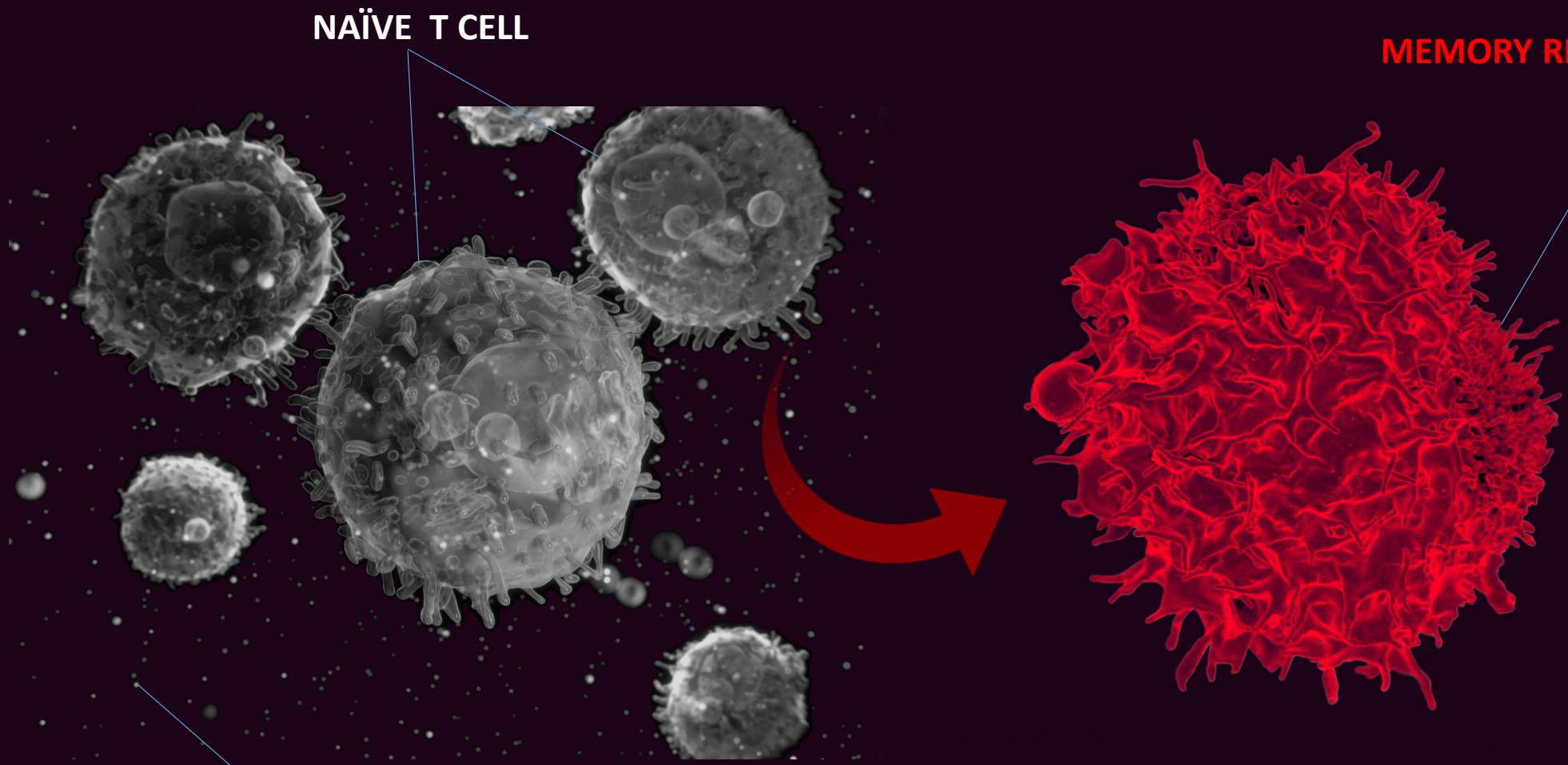
Schumacher et al. J Immun 2018; Kim et al. Dev Reprod 2017; Dekel et al. AJRI 2014


Under review in Frontiers in Immunology
IL-6
IL-6RA
gp130
OSMR
LIFR
IL-1RA
TNFRFS10B
IL-2RA
TNFRFS1B
CD80
JAK2
JAK3
MAP2K1
RAC
AKT
MAPK14
JNK1
IKK
MTOR
STAT3
MAPK3
MAPK1
STAT1
HSPE1
PPRG
IκB
RELA
NF-κB
CXCL12
IL-10
CD80
CCL2
MYC
NF-κB
STAT3
STAT1
PIAS3

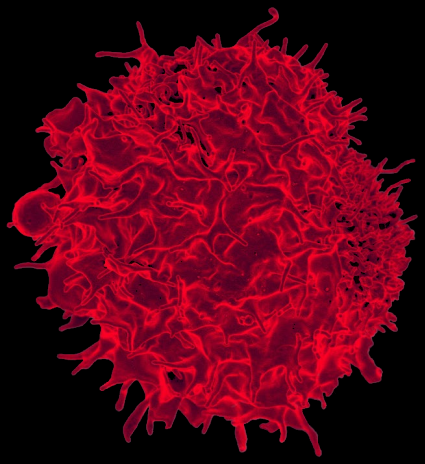

MAPK1
HSPE1
TNFRSF10B
MAPK8
IL18R1
TLR4
MAPK14
CCL2
IKBKB
NFKBIA
MTOR
FAS
MYC
TNFRSF1B
TNFRSF1A
IL6
NFKB1
CCL18
OSMR
RELA
IRF1
AKT1
IRF5
RAC1
STAT3
JAK2
JAK3
IFNG
CCL5
MAPK3
CREB1
STAT1
STAT6
CXCL12
CSF3R
CEBPB
PIAS3


IL2
IL2RA
HSPE1
IL7R
?
HSPD1
FOXP3
STAT1
IL6R
IL6
T-SEJT AKTIVÁCIÓ
TREG DIFFERENCIÁCIÓ POZITÍV SZABÁLYOZÁSA
TREG PROLIFERÁCIÓ POZITÍV SZABÁLYOZÁSA
String-db interaction network as of 2019.01.07
Lines strength correlates with edge confidence

HIPOTÉZIS


NAÏVE T CELL
MEMORY REGULATORY T CELL
TROPHOBLAST-DERIVED EXTRACELLULAR VESICLES

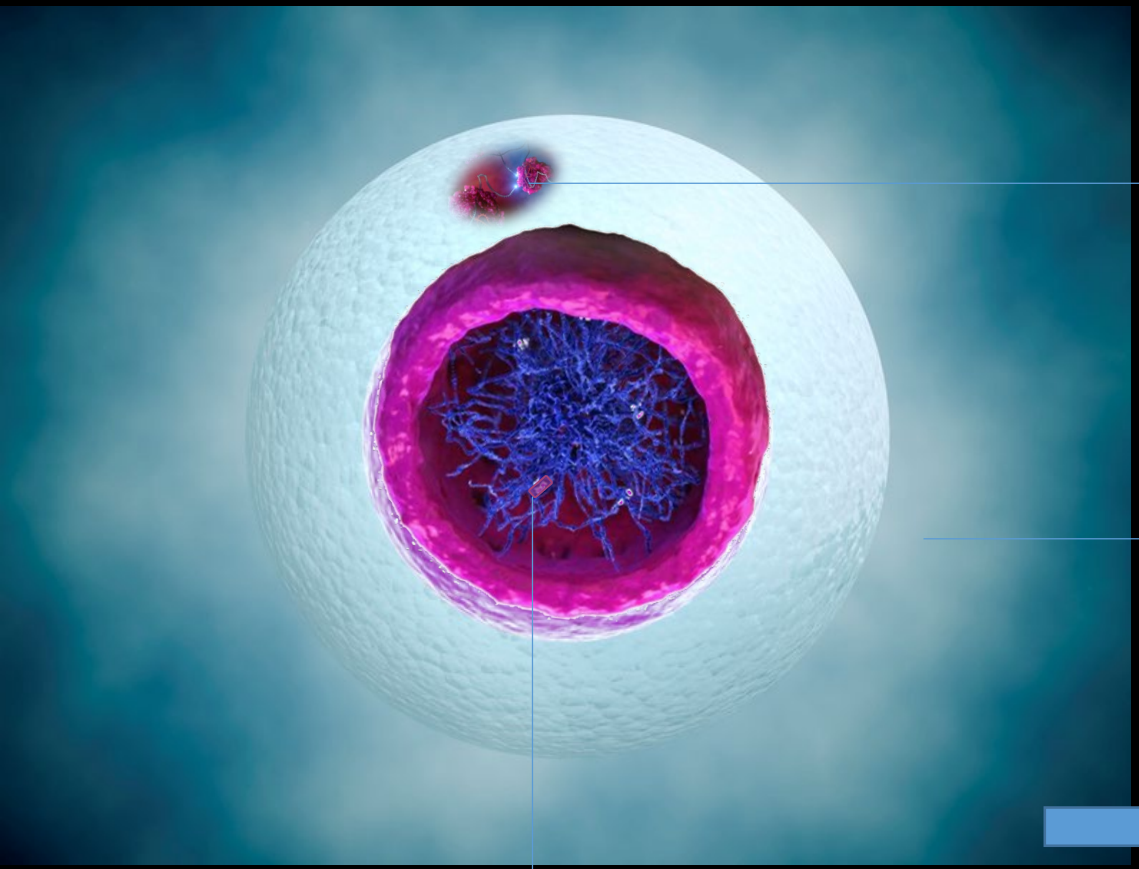
CÉLKÍTŰZÉS



Analysing the role of HSPE1:

1. **T_{reg} cell differentiation**
2. **T_{reg} cell heterogeneity**

MÓDSZEREK (1)



Intracellular HSPE1

- PROTEIN: FLOW CYTOMETRY
- mRNA: qPCR and **SINGLE CELL TRANSCRIPTOMICS**

Extracellular vesicles

- PROTEIN: ÁRAMLÁSI CITOMETRIA és TÖMEGSPEKTROMETRIA
- miRNS: NGS

FUNKCIONÁLIS VIZSGÁLAT

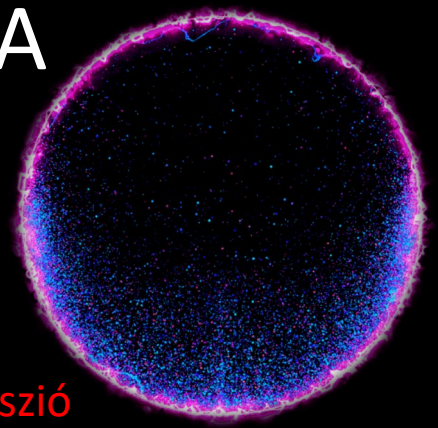
- SEJTMOZGÁS: HOLOMIKROSZKÓP
- CITOKINMINTÁZAT: ÁRAMLÁSI CITOMETRIA

NUCLEUS

Chromosome 2: *HSPE1* gene — DNA: SEQUENCING

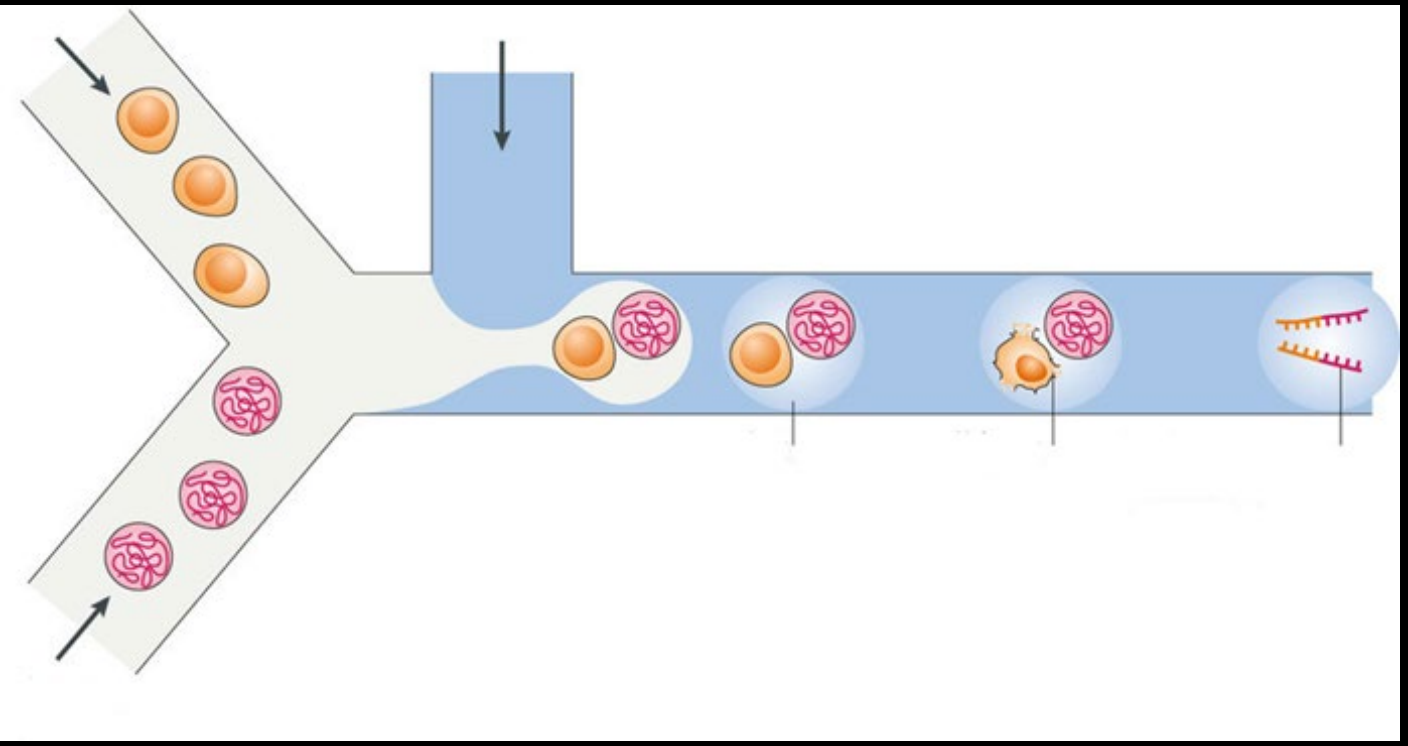
KNOCKOUT: RNP based CRISPR-Cas9 NLS

MÓDSZEREK (2) EGY-SEJT TRANSZKRIPTOMIKA



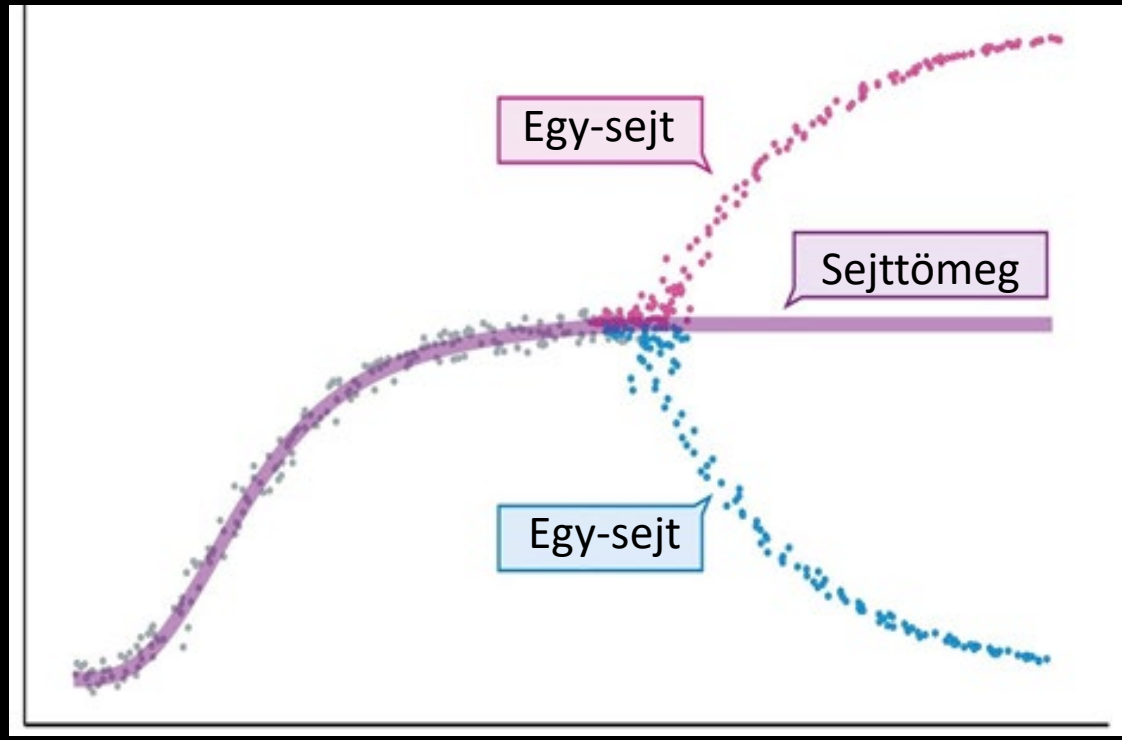
Sejt

Olaj

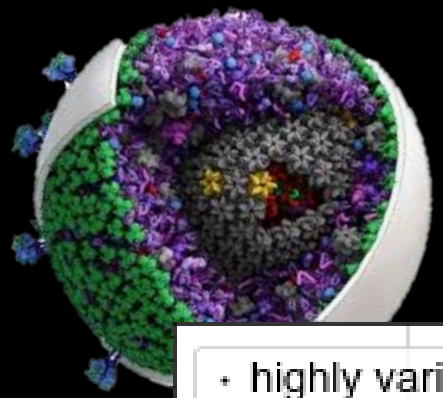


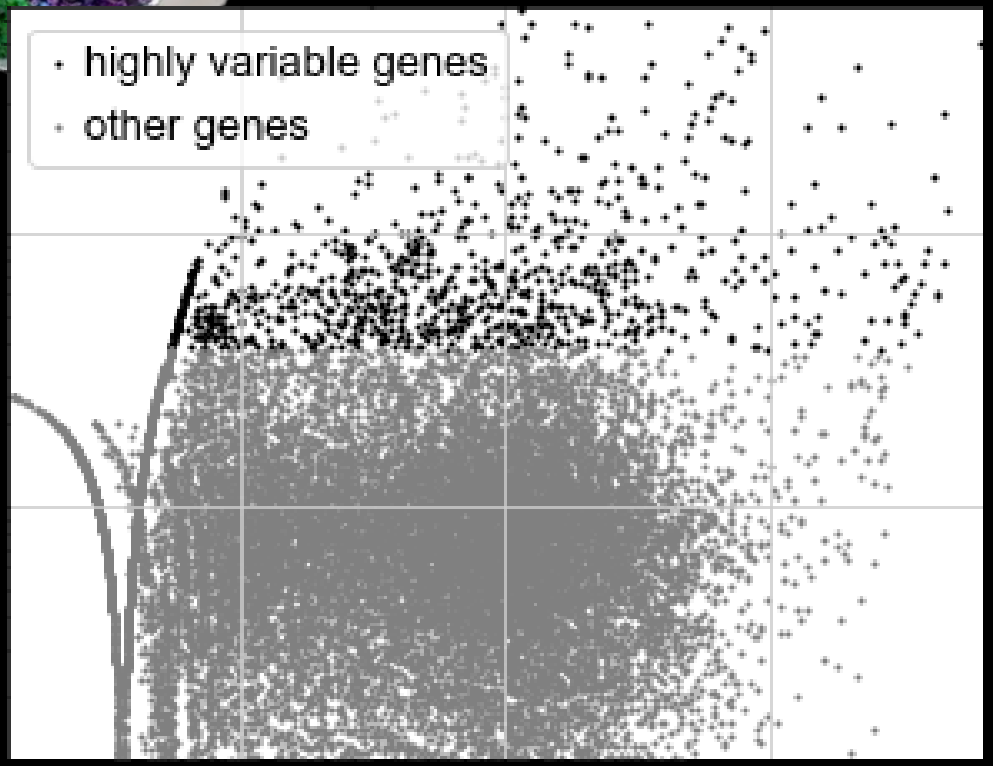
Azonosító

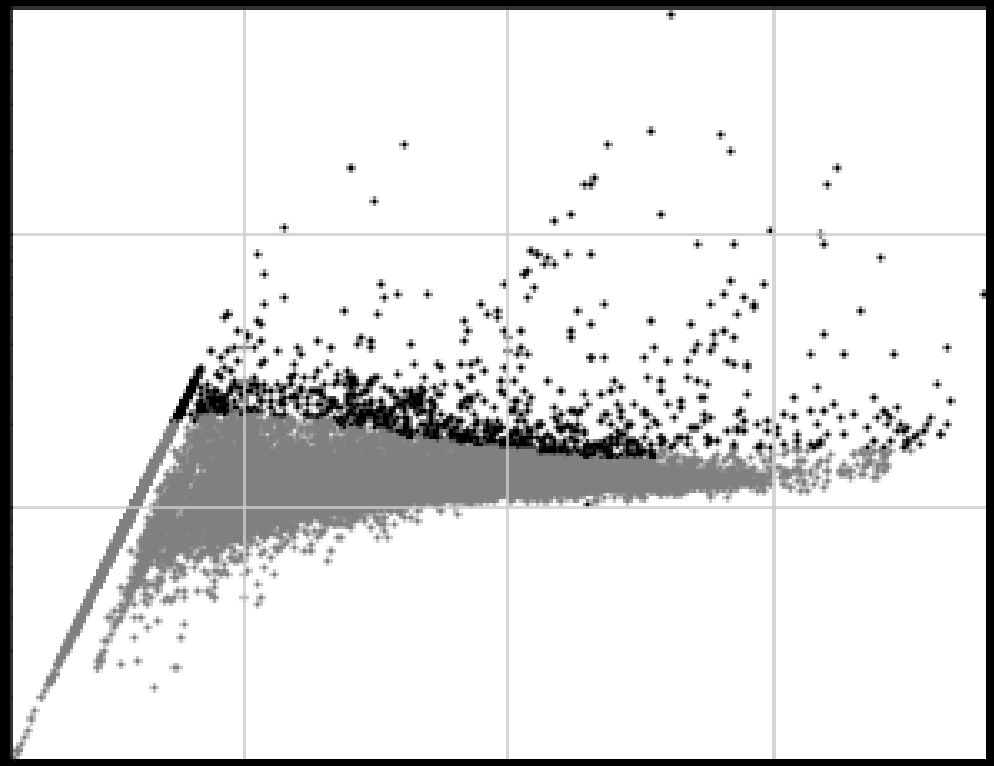
mRNS expresszió



4. PBMC SEJTEK EGY-SEJT TRANSZKRIPTOMIKAI VIZSGÁLATA

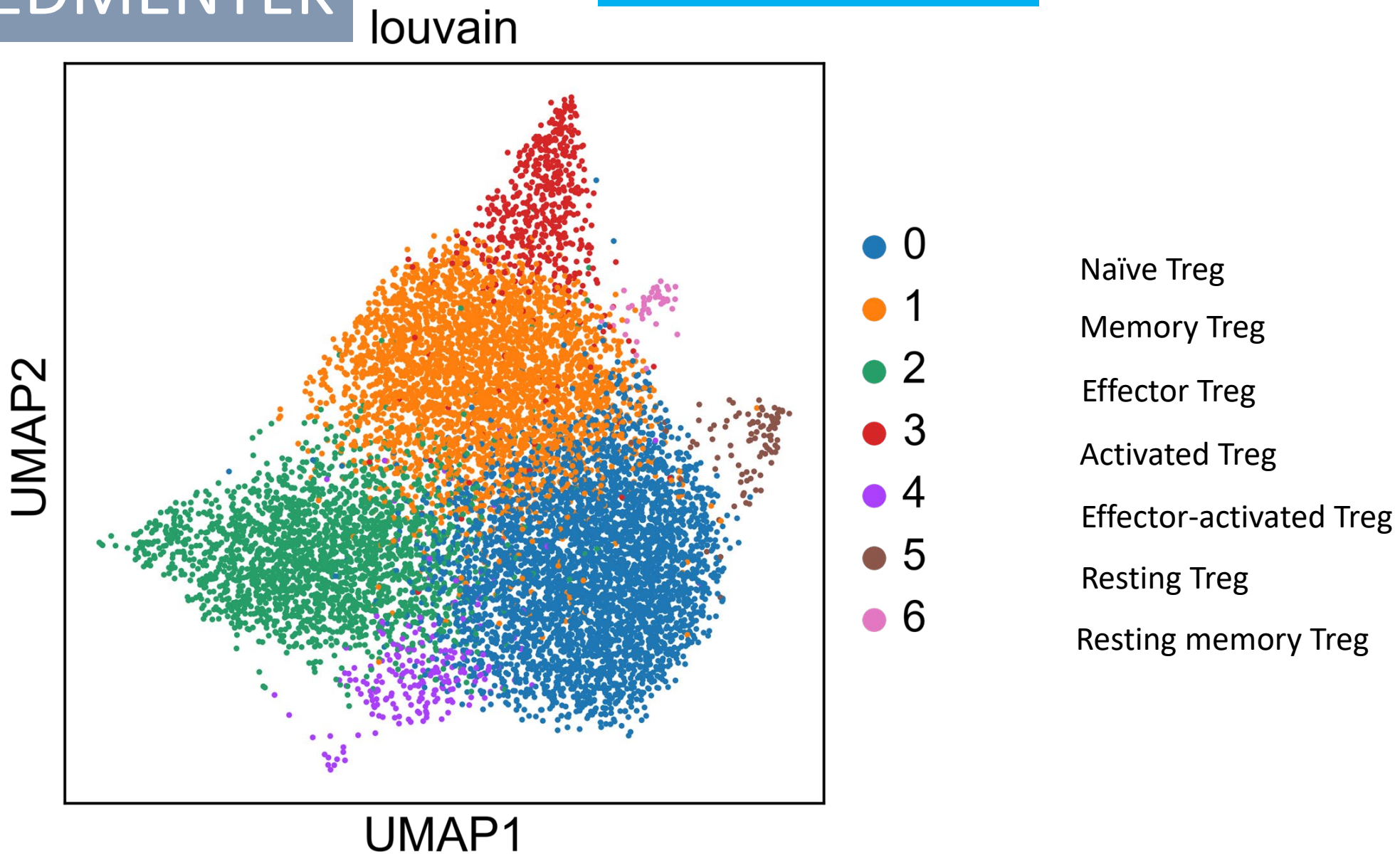






EREDMÉNYEK

CD4+ CD25+ Treg cells



Naïve Treg

Memory Treg

Effector Treg

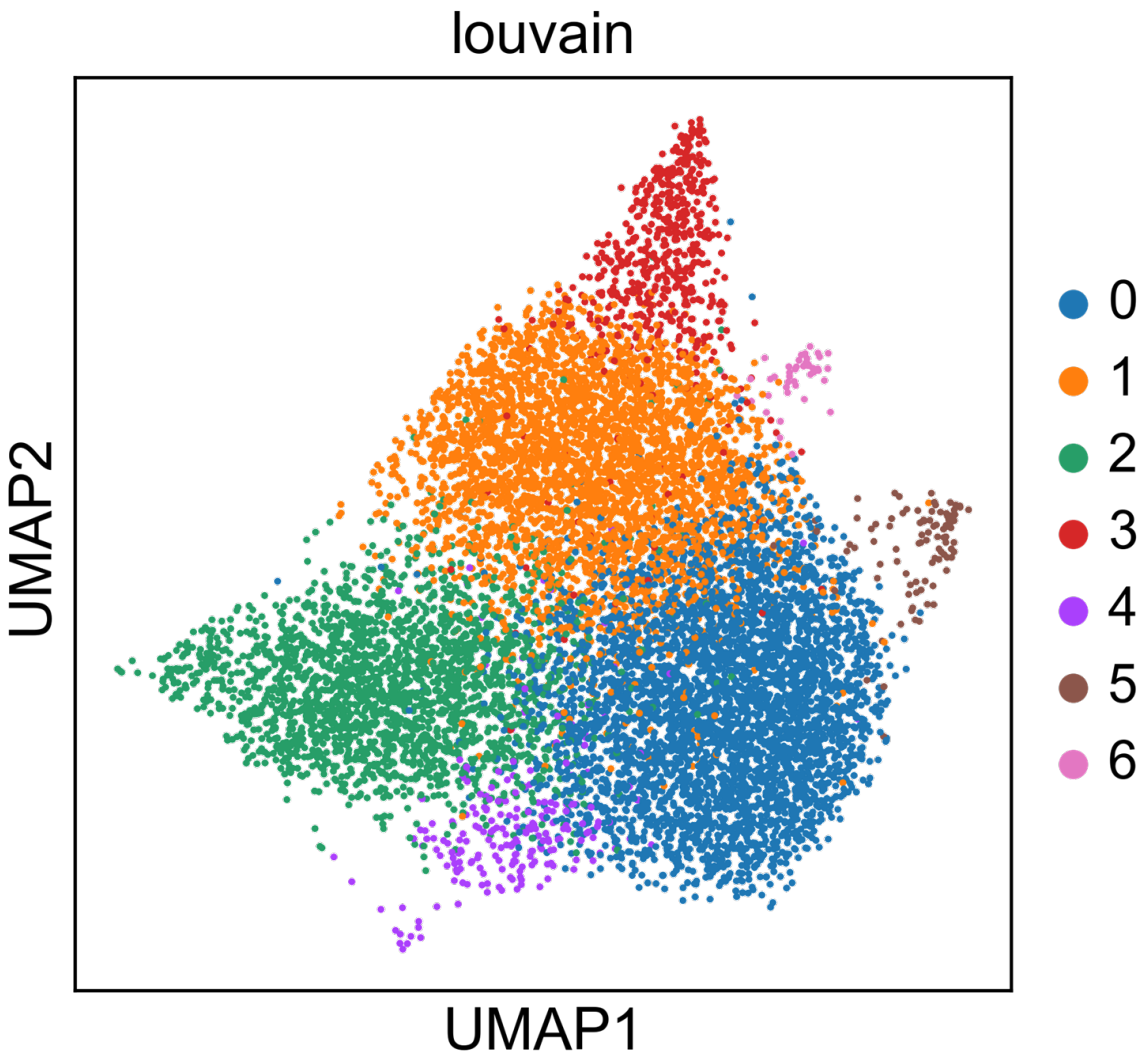
Activated Treg

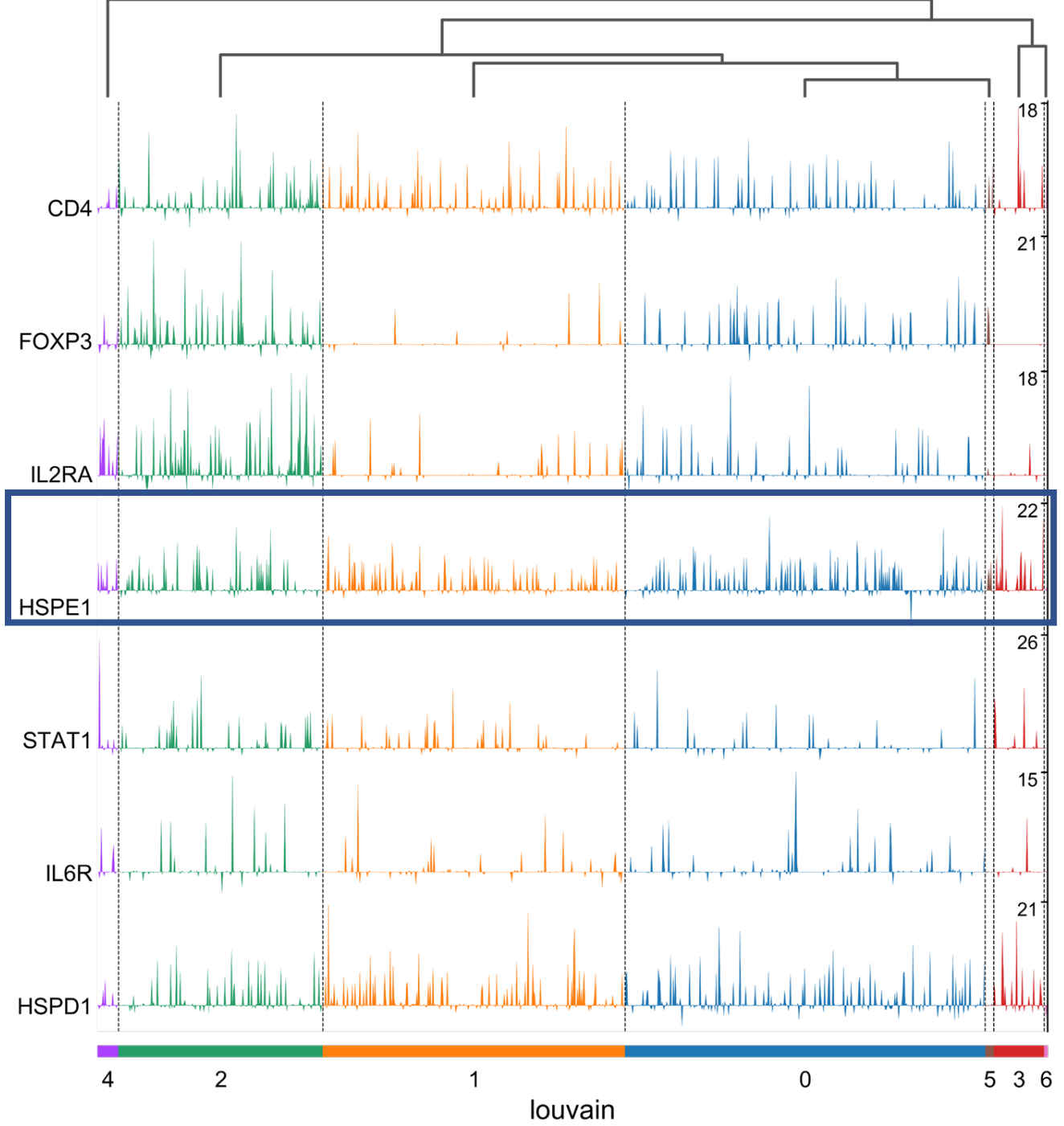
Effector-activated Treg

Resting Treg

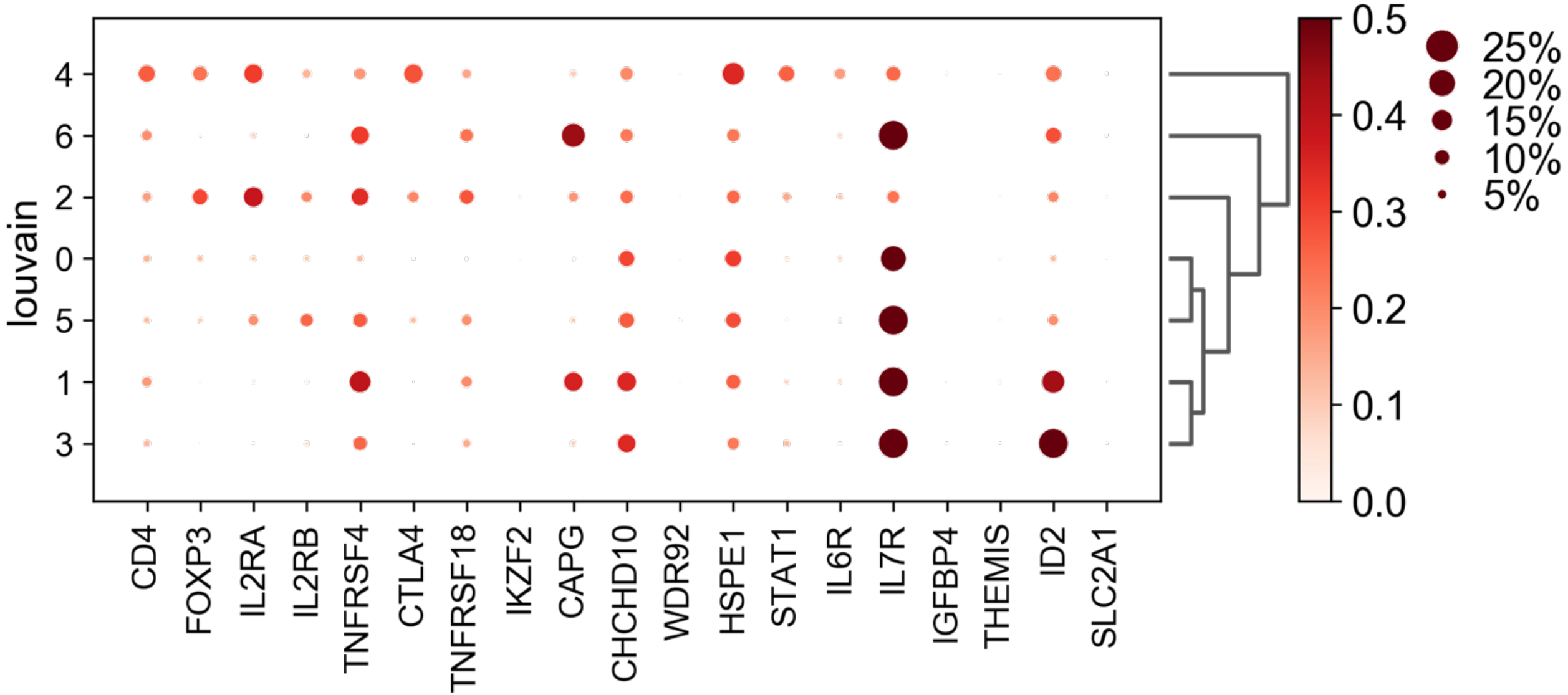
Resting memory Treg

CD4+ CD25+ Treg cells

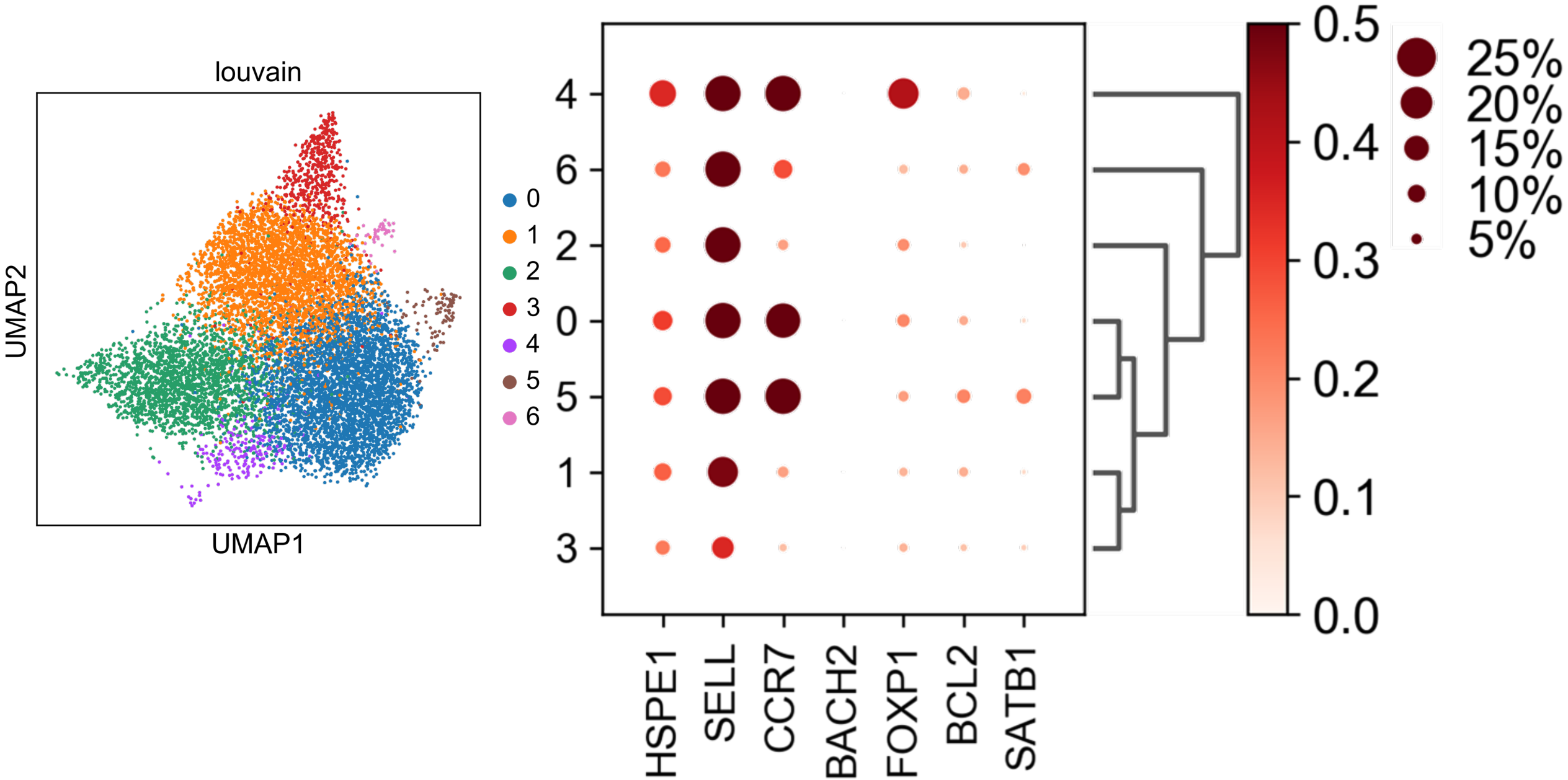




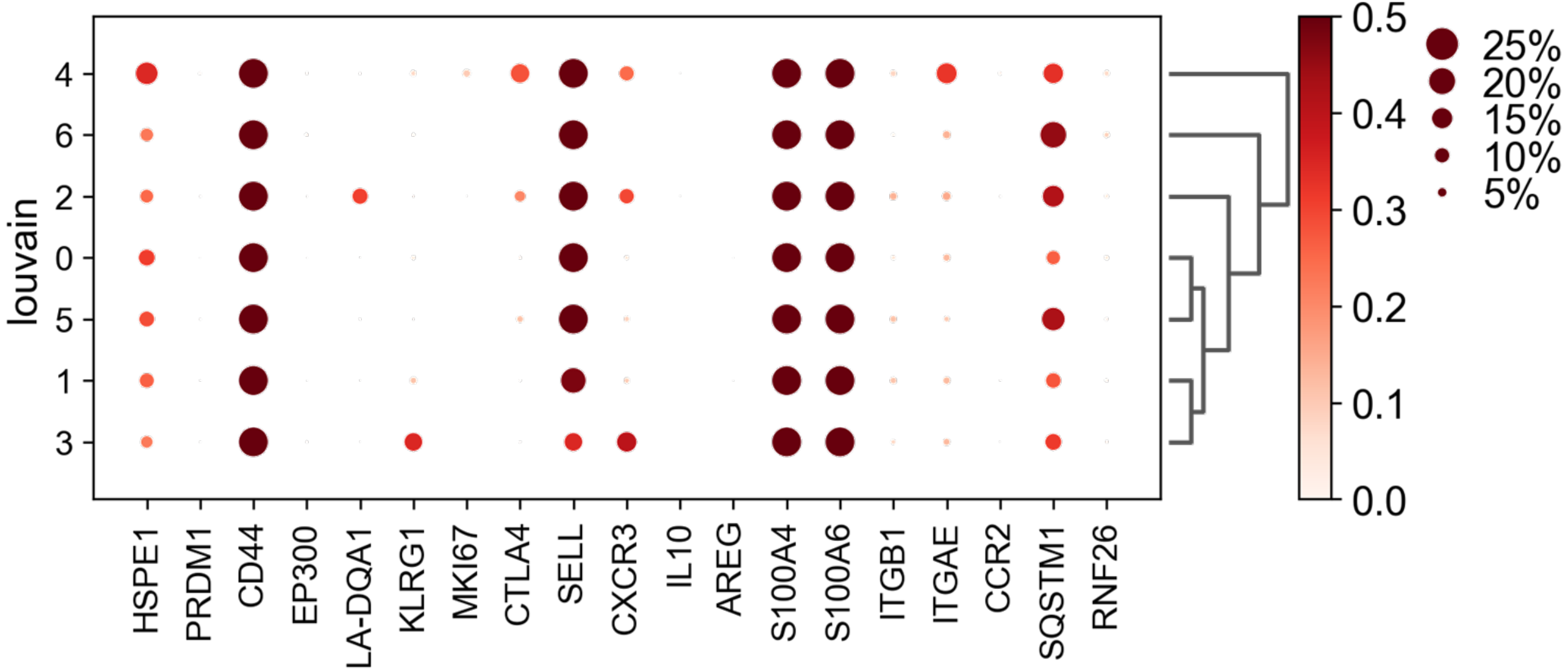
CD4+ CD25+ Treg cells – MARKER GENES



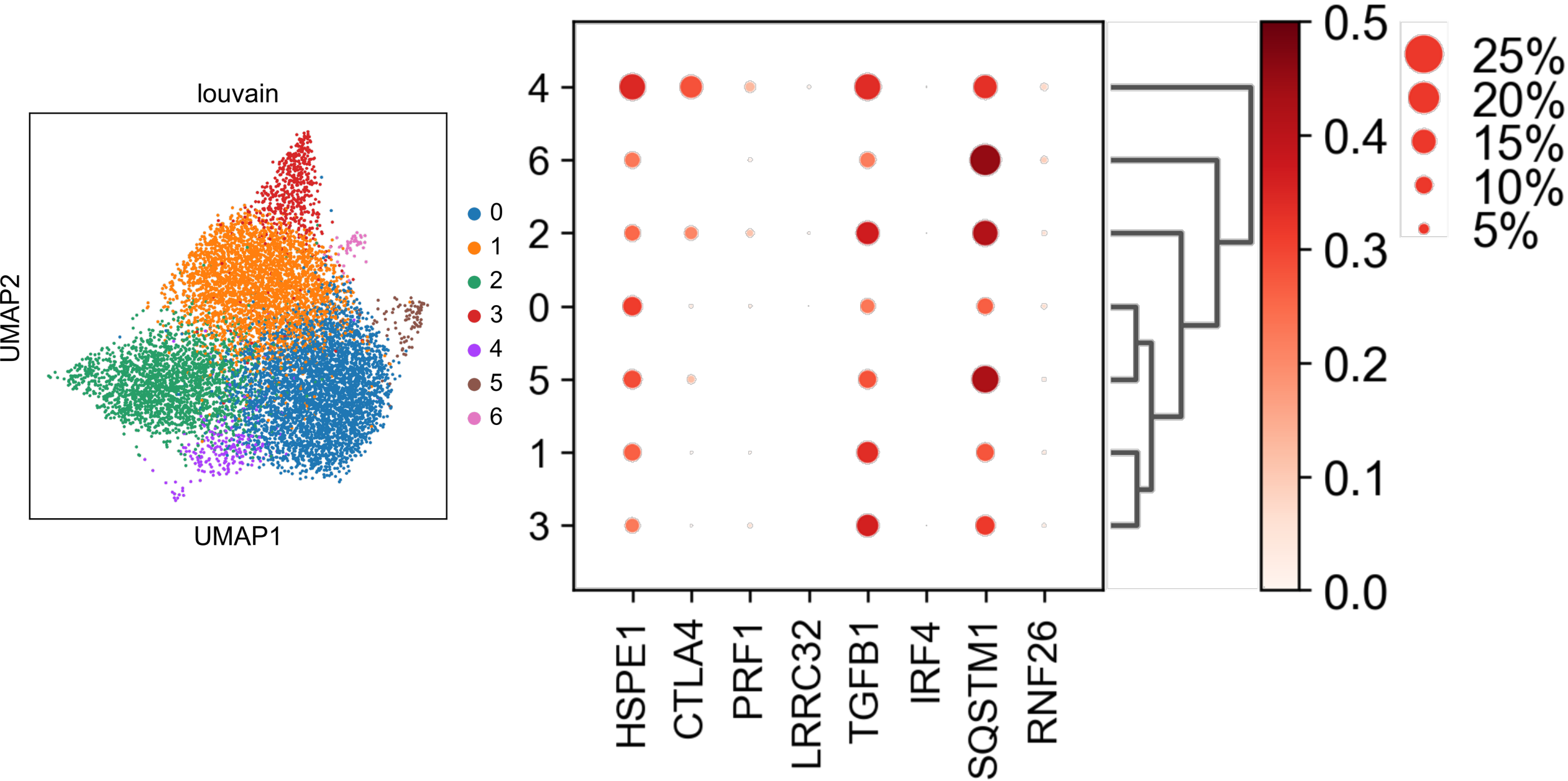
CD4+ CD25+ Treg cells – RESTING TREGS



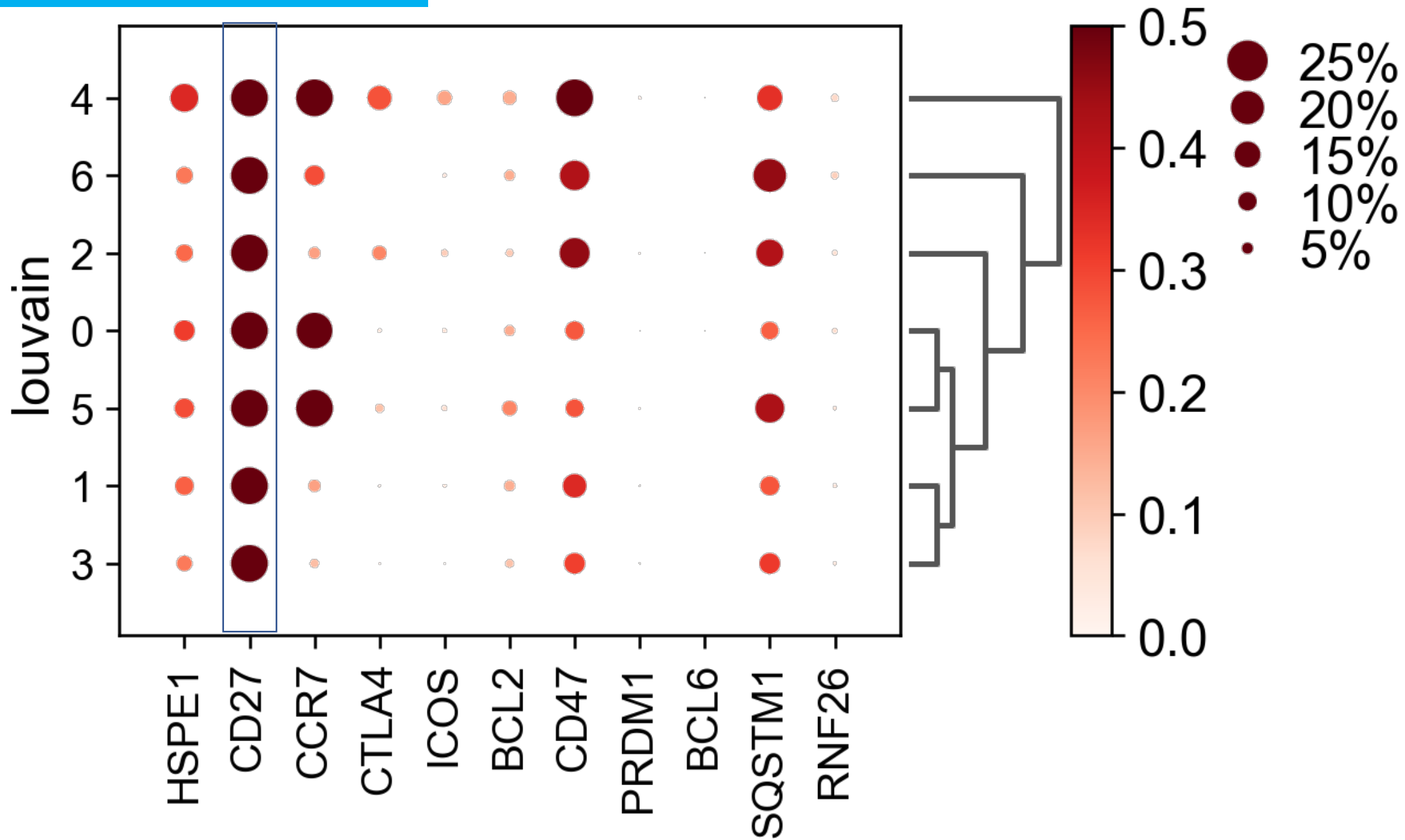
CD4+ CD25+ Treg cells – ACTIVATED TREGS



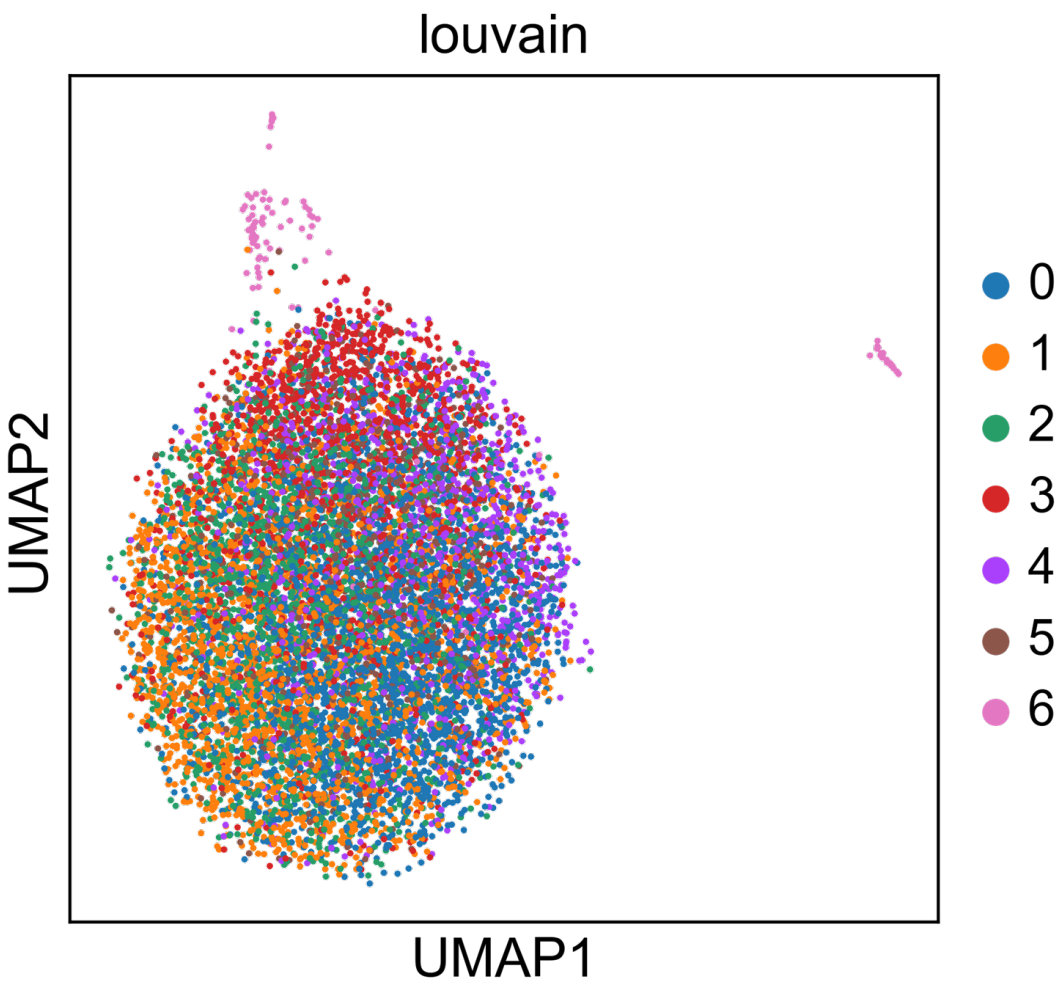
CD4+ CD25+ Treg cells – EFFECTOR TREGS

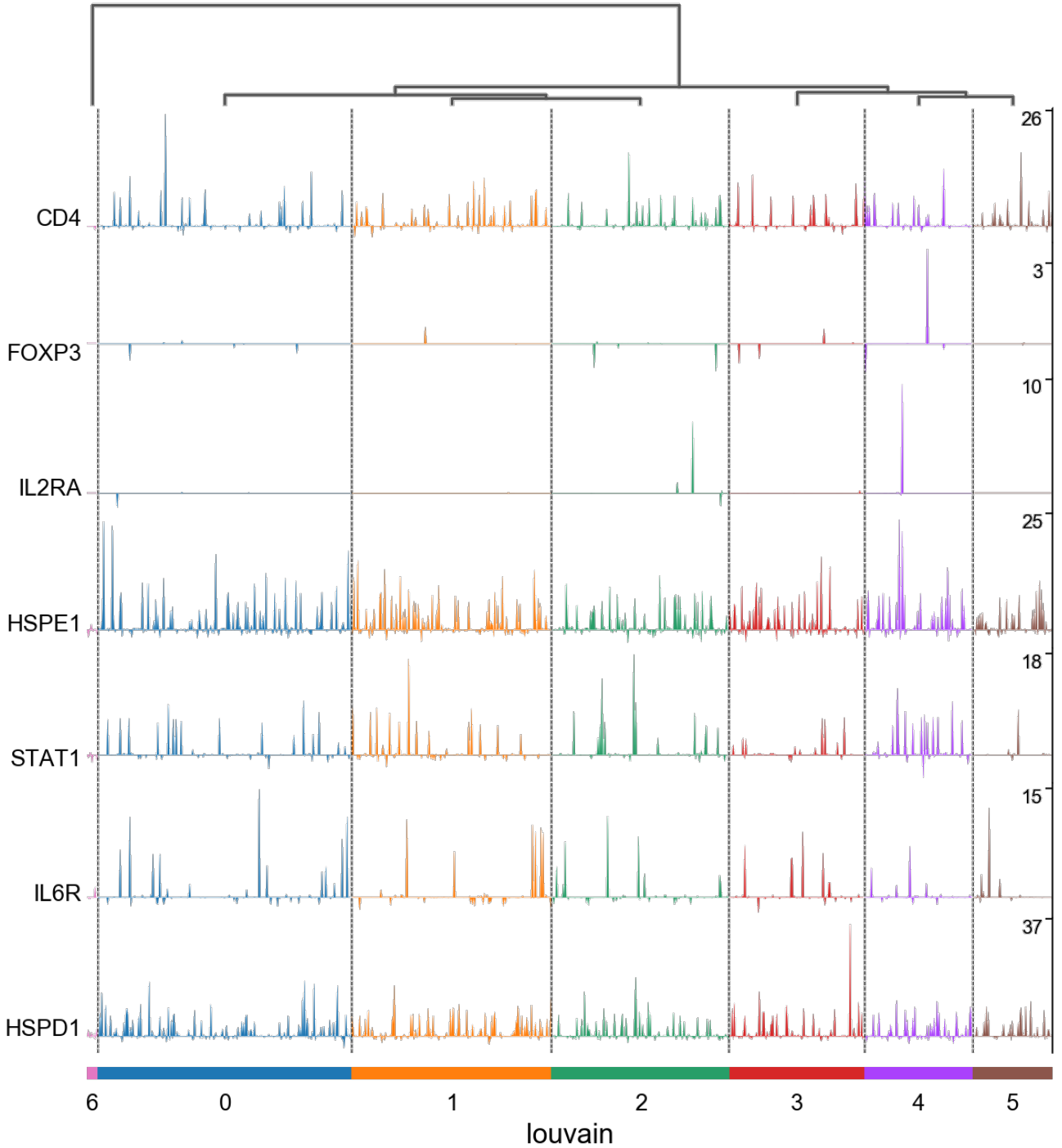


CD4+ CD25+ Treg cells – MEMORY TREGS

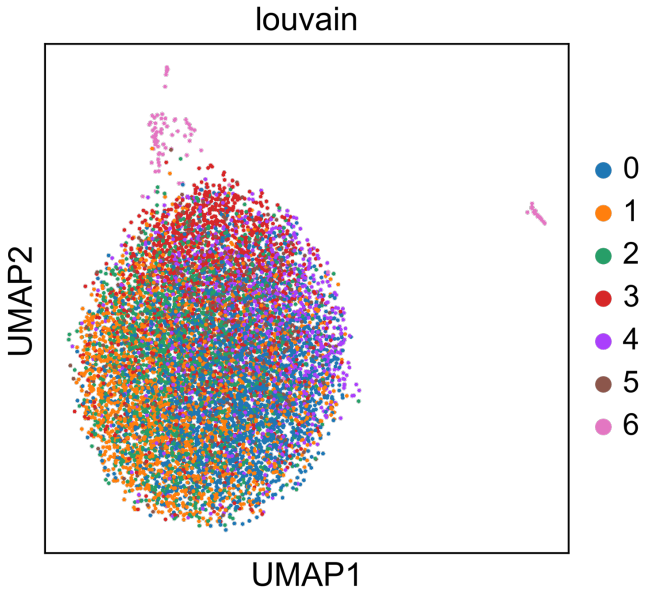


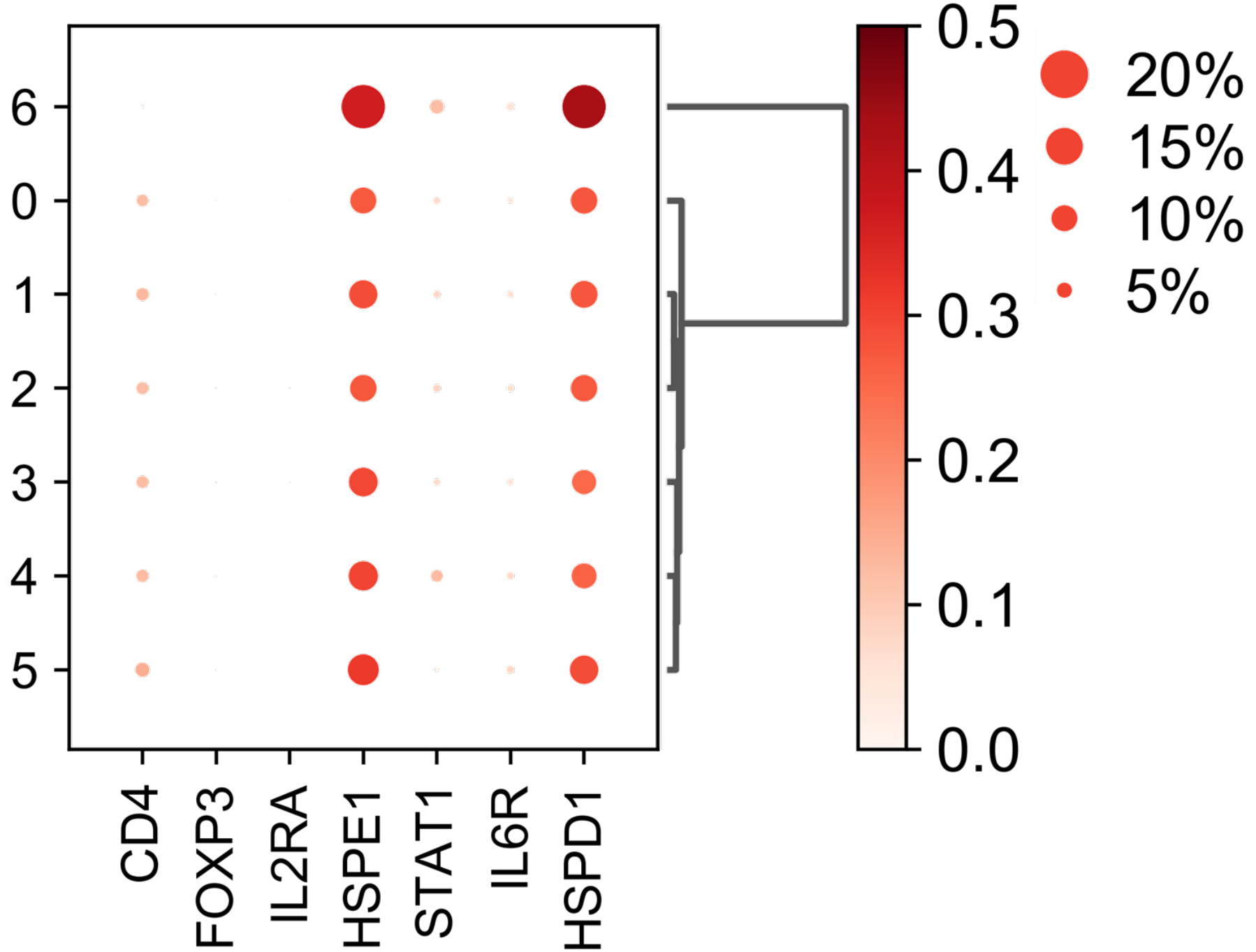
CD4+ CD45RA+ Naïve T cells





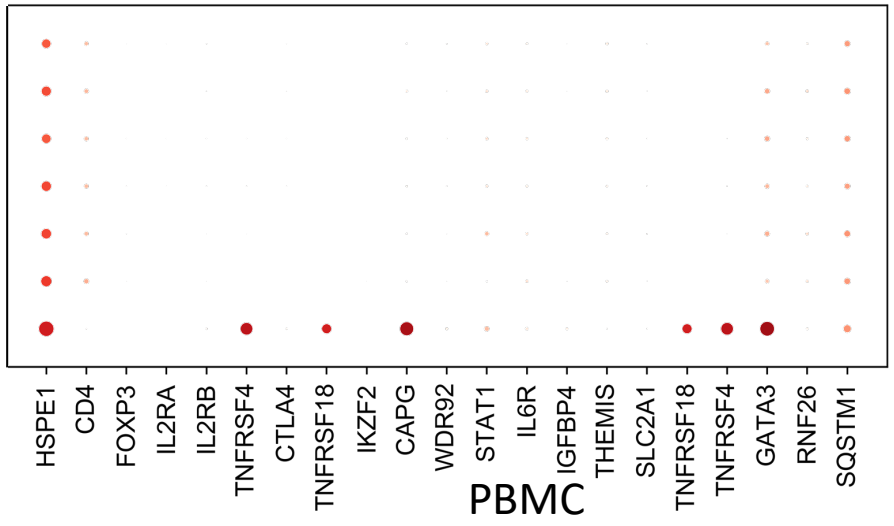
CD4+ CD45RA+ Naïve T cells



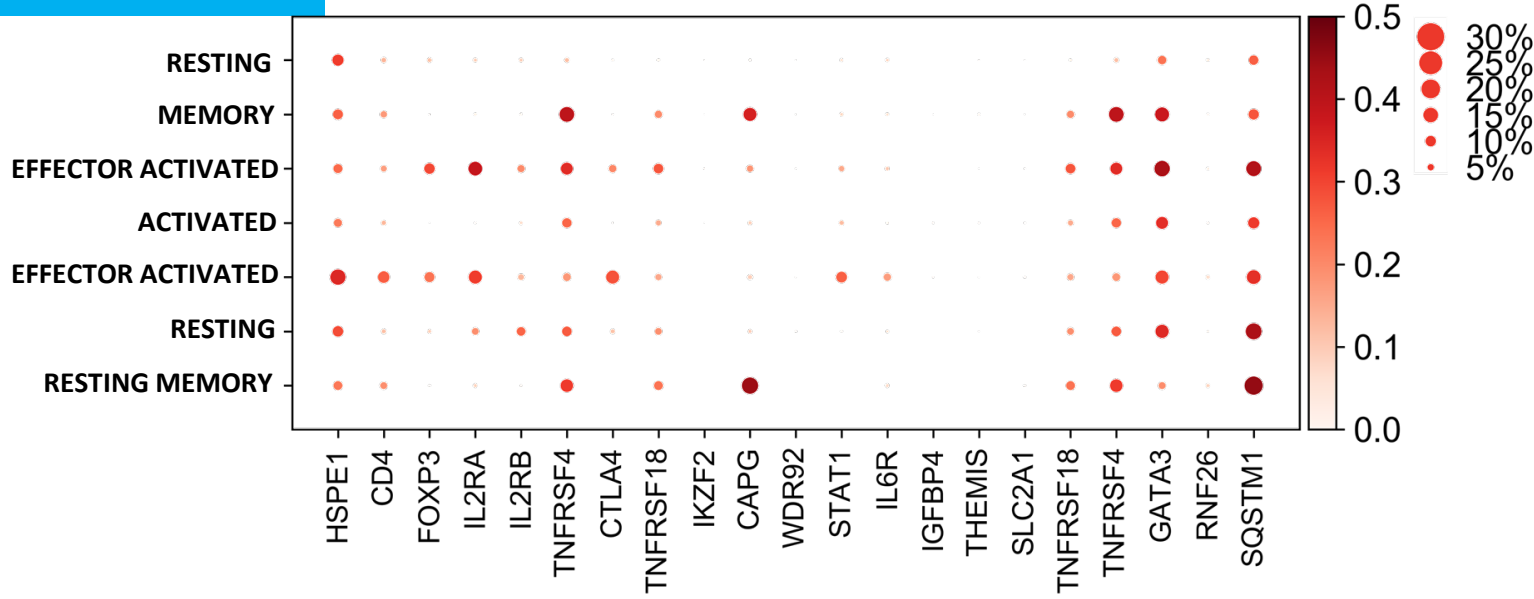


MARKER GENES

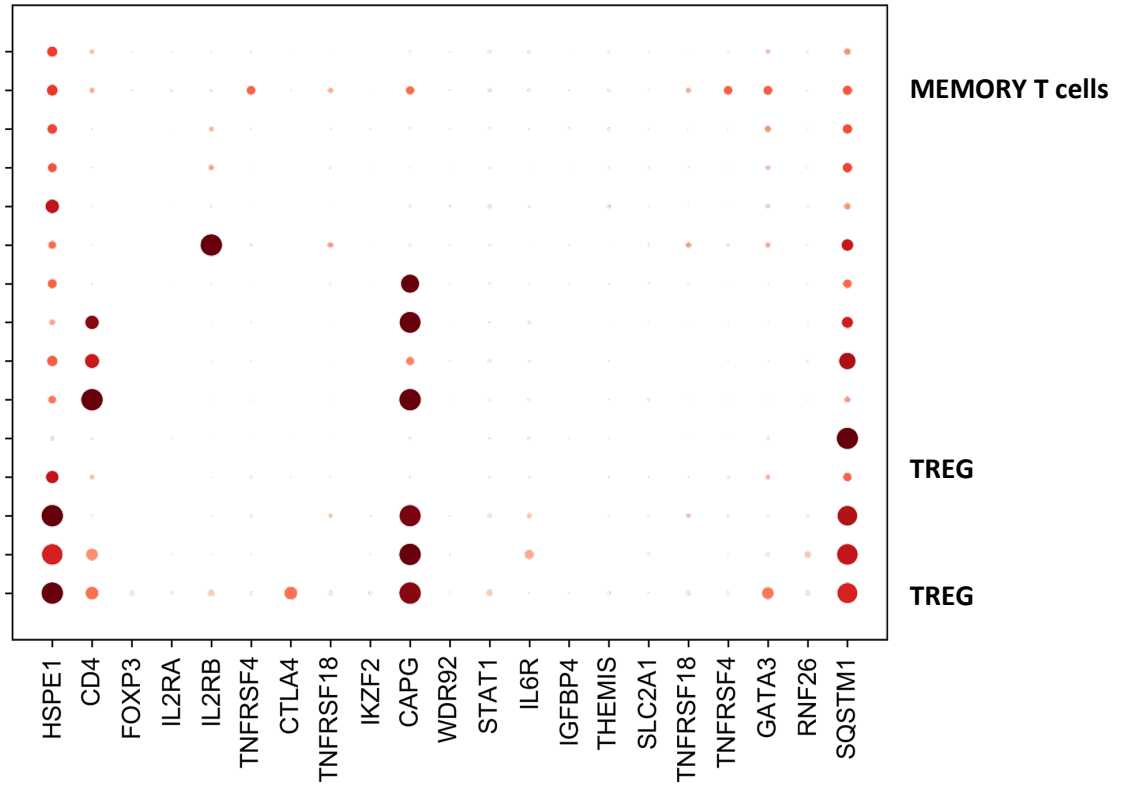
Tnaive



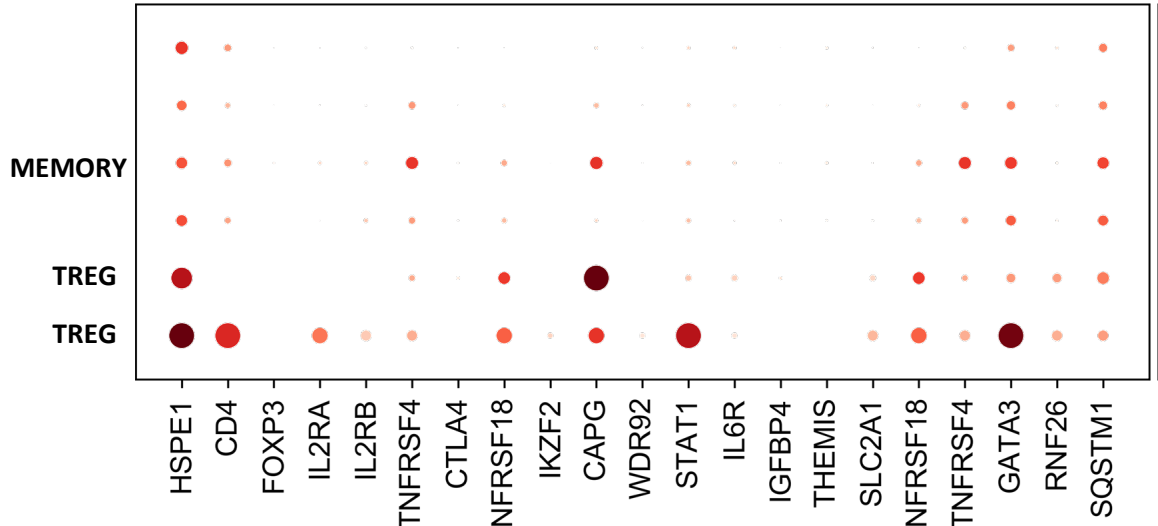
Tregs



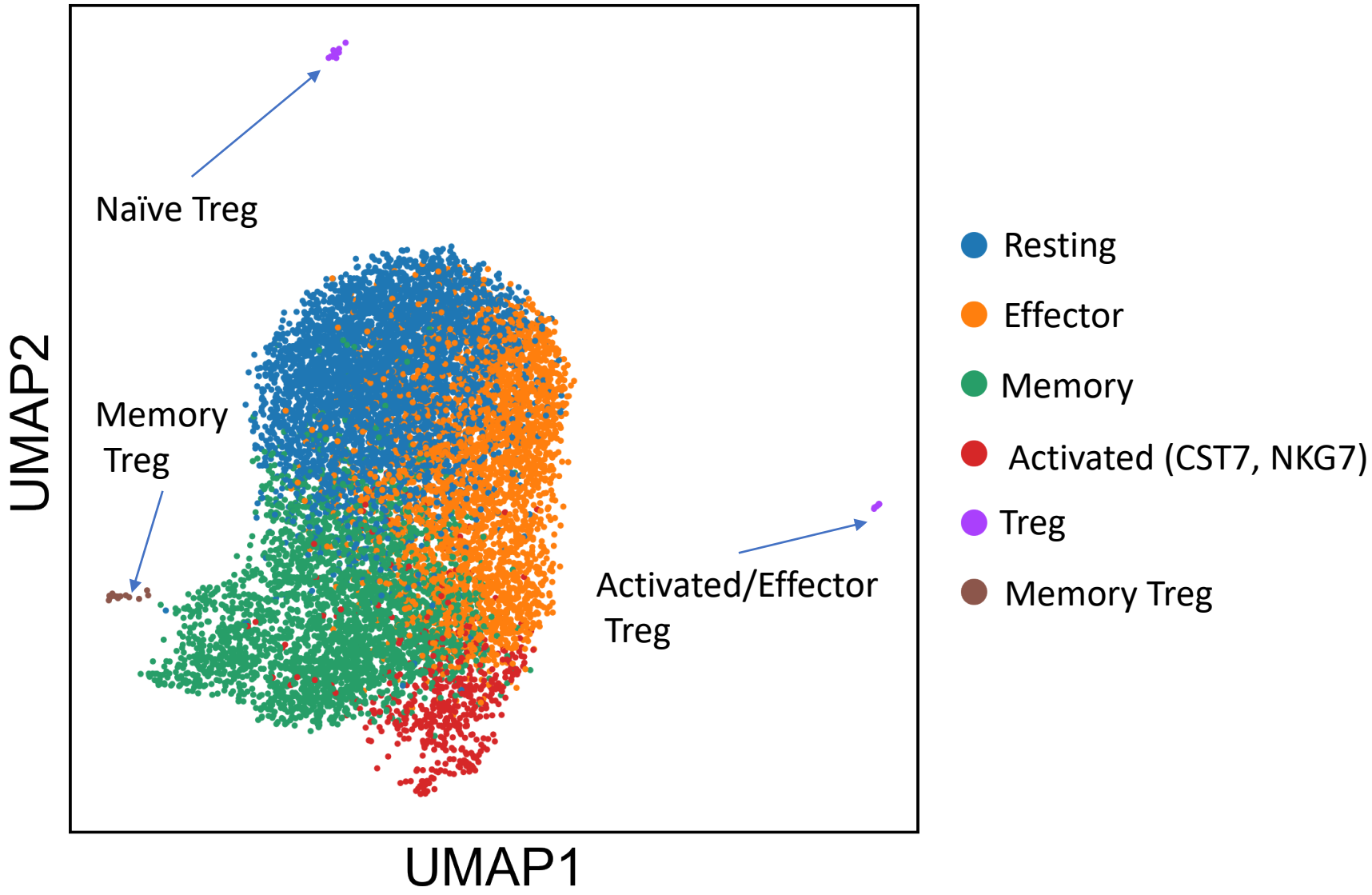
PBMC



CD4+ T cells

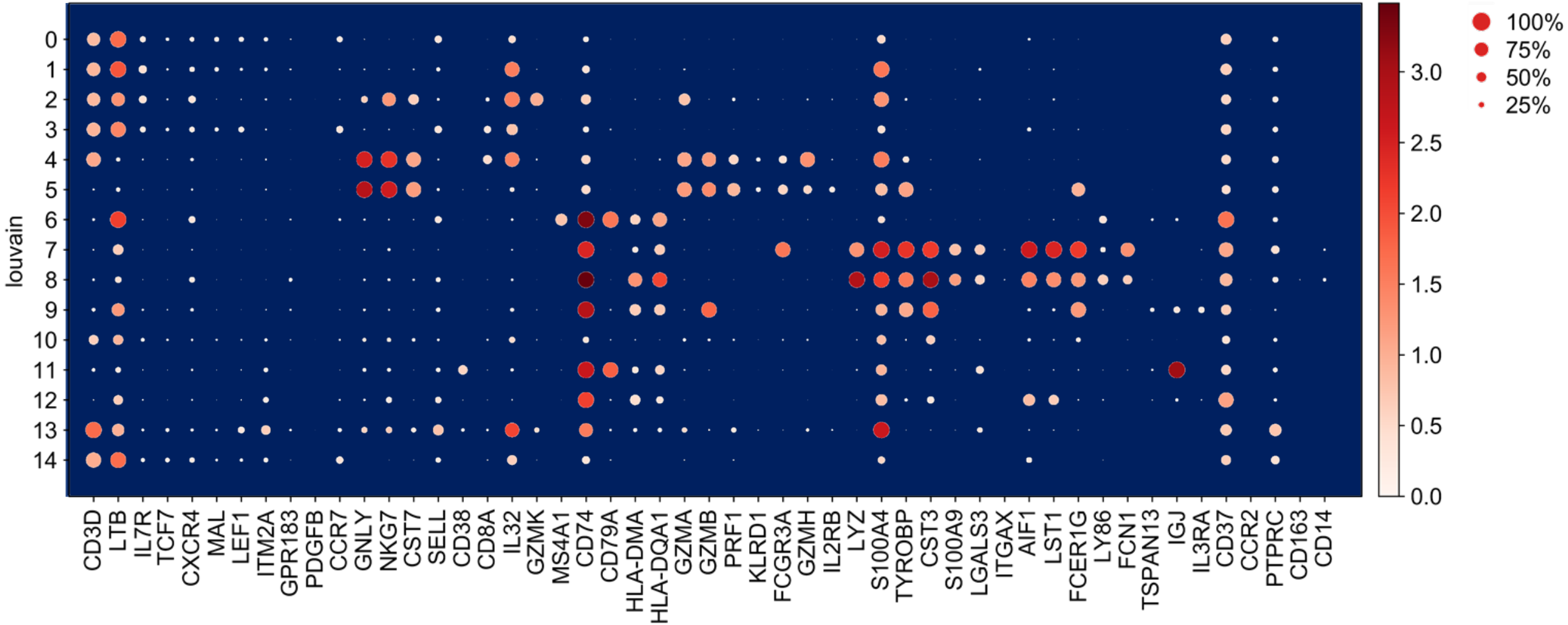


EREDMÉNYEK


CD4+ T CELLS
Naïve Treg
Memory
Treg
Activated/Effector
Treg
UMAP2
UMAP1
Resting
Effector
Memory
Activated (CST7, NKG7)
Treg
Memory Treg

EREDMÉNYEK

PBMC SEJTEK EGY-SEJT TRANSZKRIPTOMIKAI VIZSGÁLATA

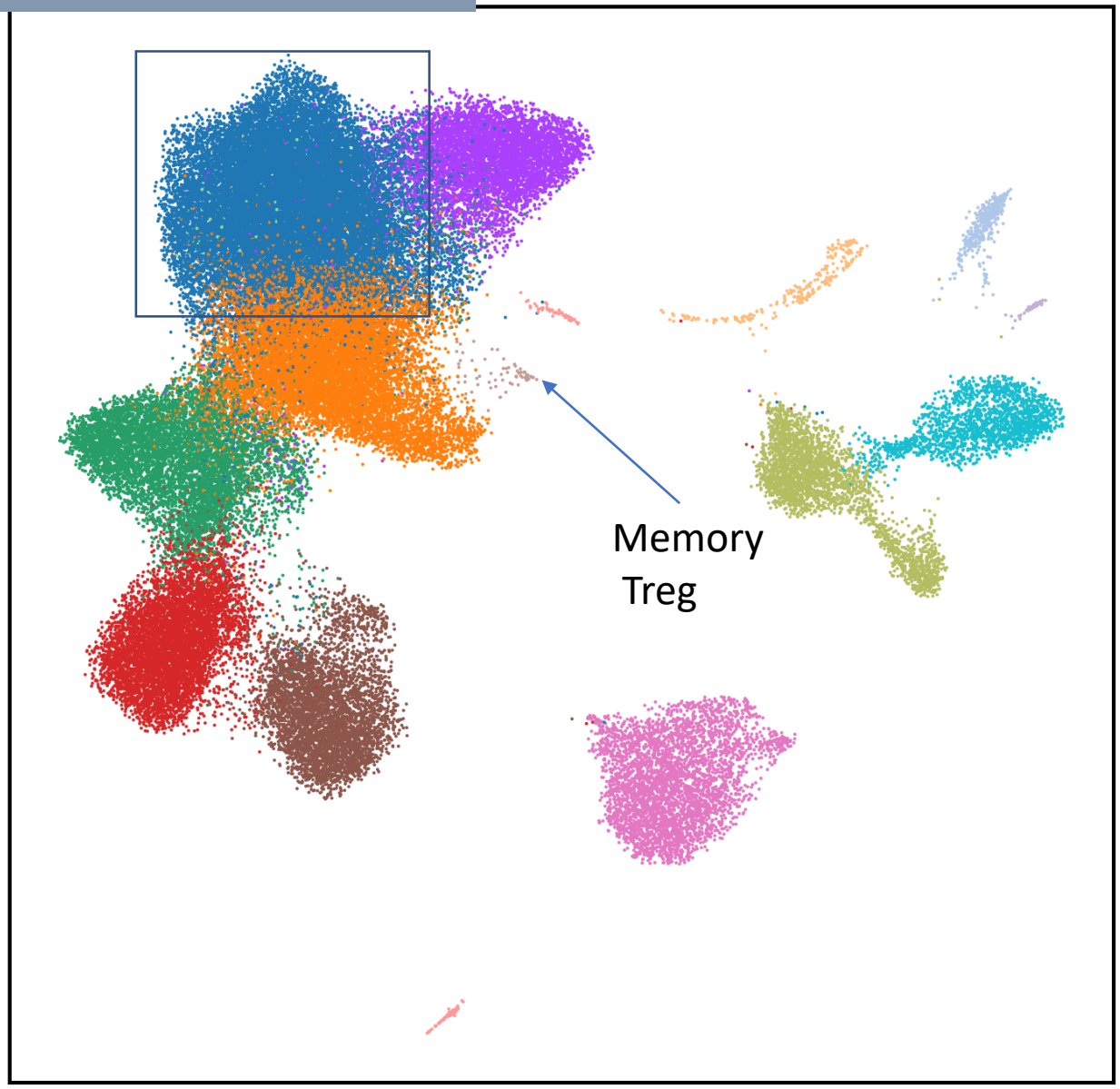


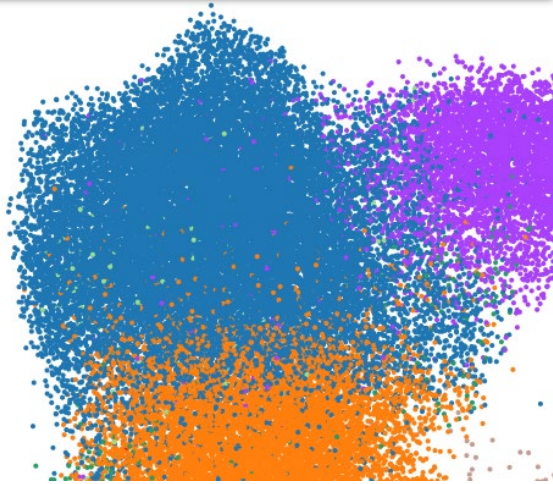
SIZE OF THE BULLETS IS PROPORTIONAL TO THE NUMBER OF CELLS EXPRESSING THE GENE, THE COLOR IS PROPORTIONAL WITH THE LEVEL OF GENE EXPRESSION

EREDMÉNYEK

Naïve Treg

PBMC

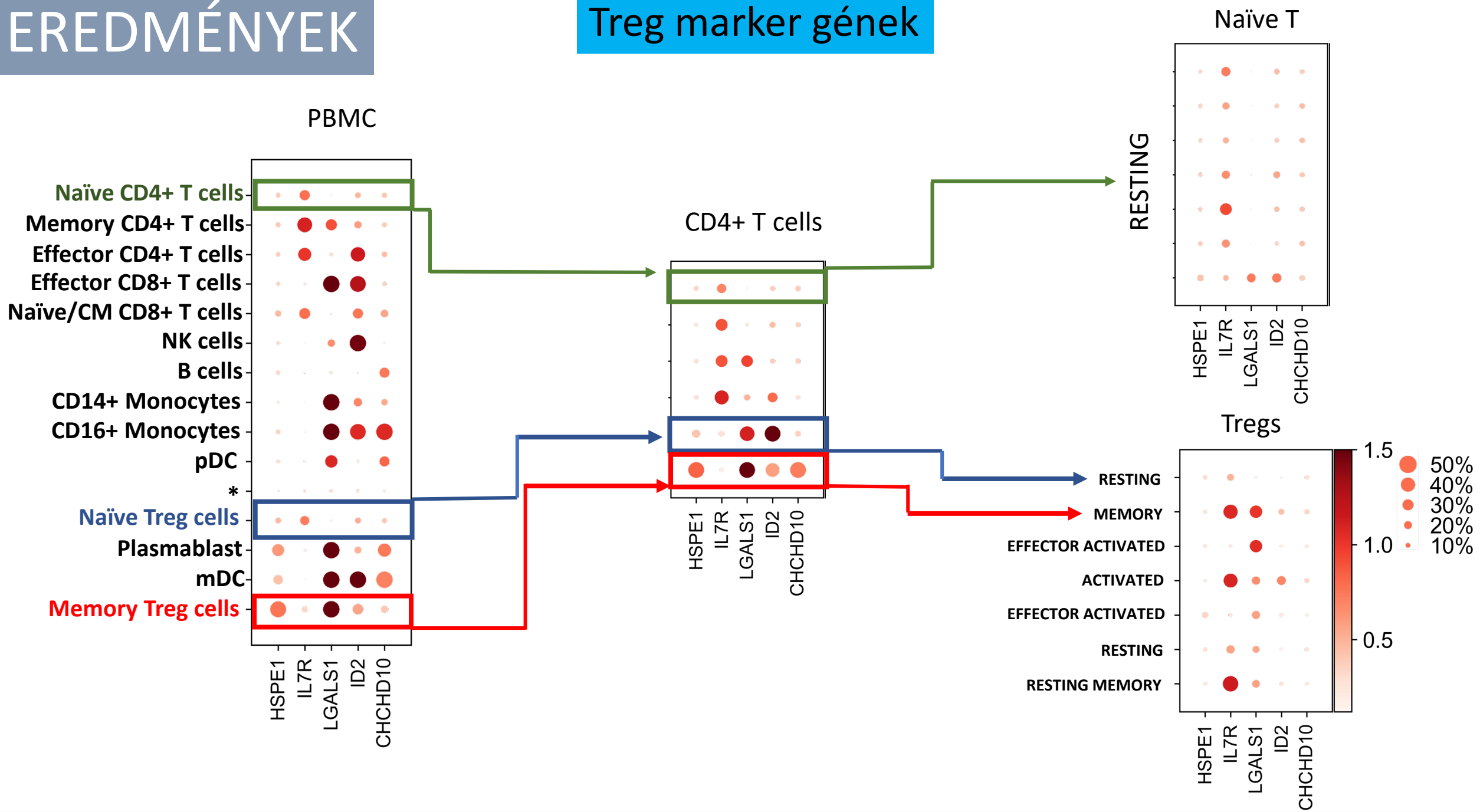




- 0 Naïve CD4+ T cells
- 1 Memory CD4+ T cells
- 2 Effector CD4+ T cells
- 3 Effector CD8+ T cells
- 4 Naïve/CM CD8+ T cells
- 5 NK cells
- 6 B cells
- 7 CD14+ Monocytes
- 8 CD16+ Monocytes
- 9 pDC
- 10 *
- 11 Naïve Treg cells
- 12 Plasmablast
- 13 mDC
- 14 Memory Treg cells

EREDMÉNYEK

Treg marker gének

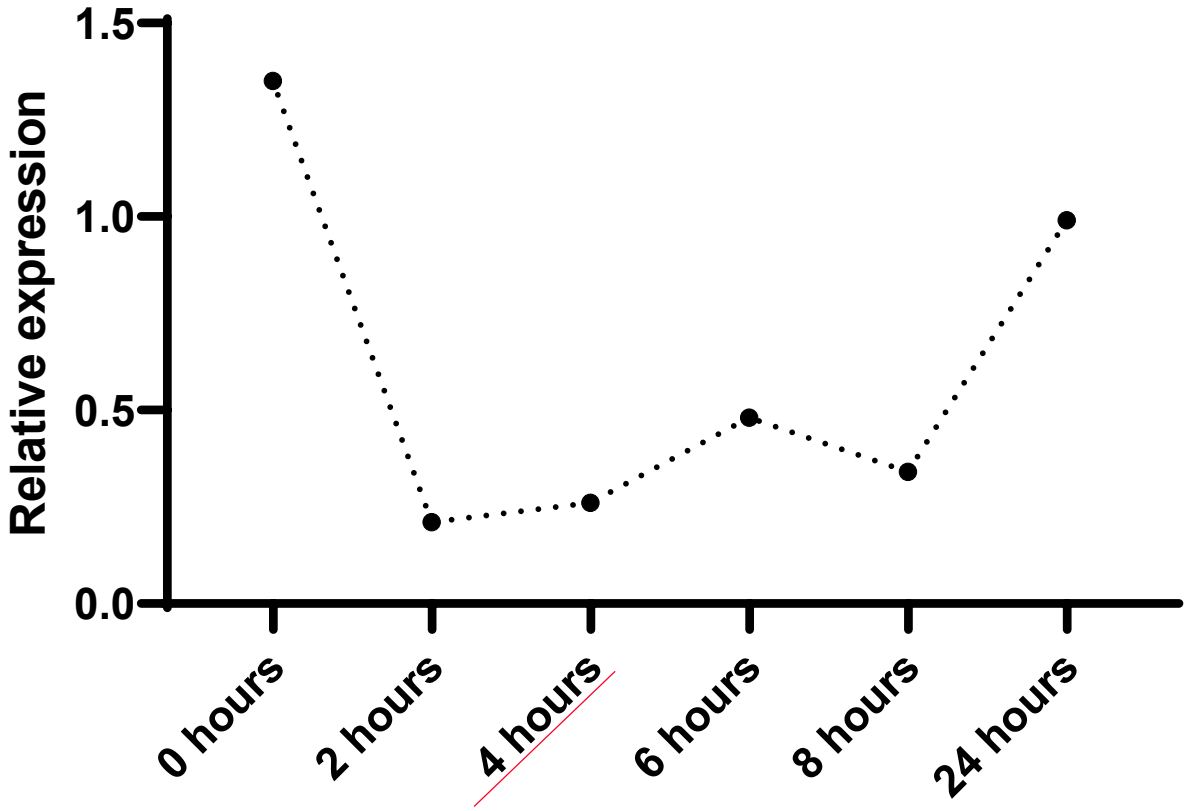


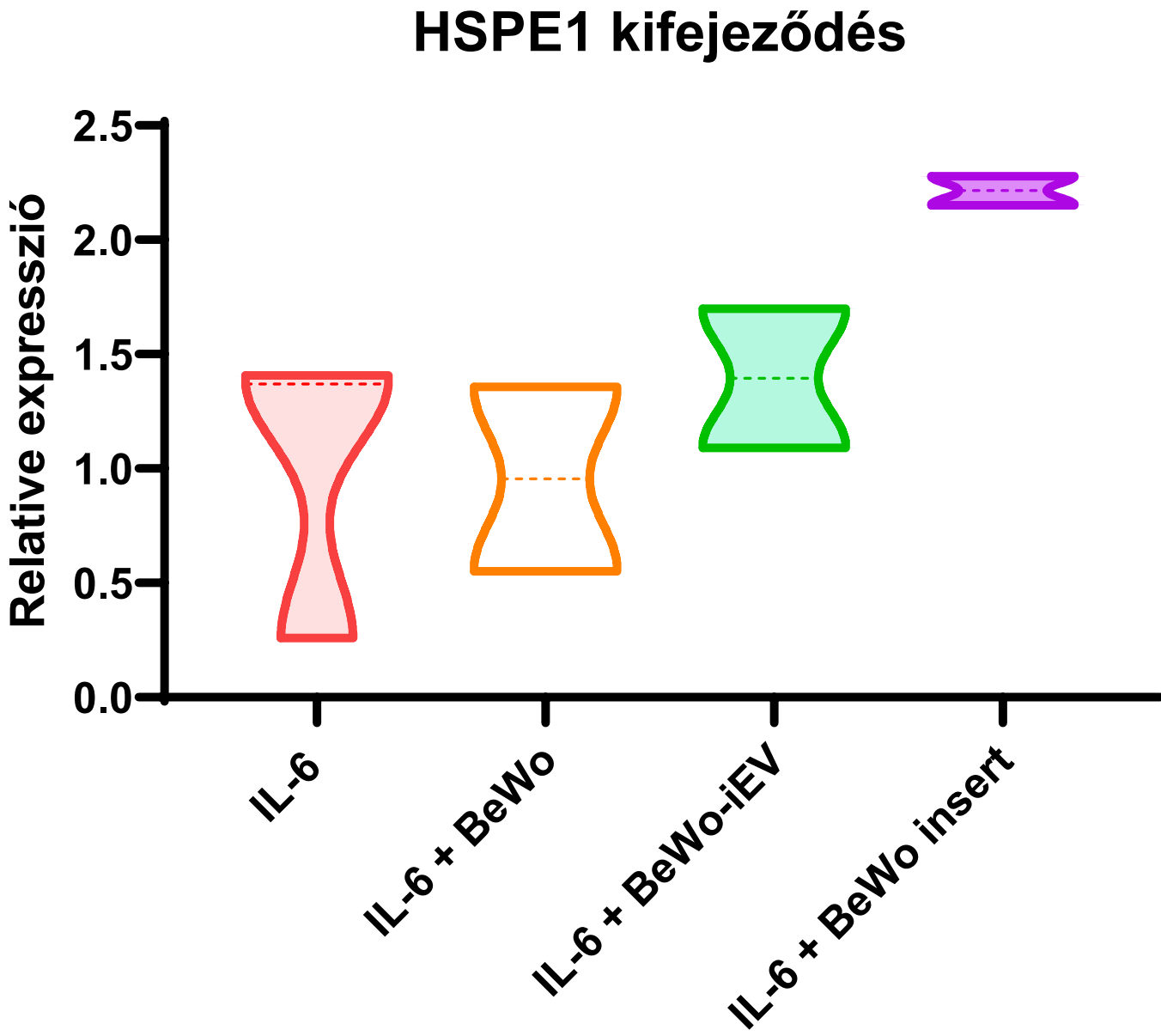
TREG CHARACTERIZATION

TREG LINAGE DEFINING MARKER	
FOXP3	
TREG MARKERS	
CELL SURFACE	INTRACELLULAR
IL2RA (CD25)	IKZF2
ENTPD1 (CD39)	CAPG
IL2RB (CD122)	CHCHD10
TNFRSF4 (CD134)	WDR92
CTLA4 (CD152)	
TNFRSF18 (CD357)	
GPR83	
IZUMO1R (JUNO)	
NEGATIVE MARKERS (OVEREXPRESSED IN CONVENTIONAL T CELLS)	
IL7R (CD127)	IGFBP4
	DAPL1
	THEMIS
	ID2
	SLC2A1 (effector T)

TREG SUBTYPES	
RESTING TREGS (limited immunosuppressive capacity)	SELL (CD62L); CCR7 (CD197), FOXP1, BCL2, SATB1, HSPE1
EFFECTOR TREGS	CTLA4 (CD152); TGFB1; PRDM1; PRF1, HSPE1, SQSTM1, LGALS1, ITGA4
TCR EARLY ACTIVATED TREGS	NR4A1 (↓); EGR1; EGR2; MYC; DUSP2
ACTIVATED TREGS	CD44; MKI67, **CTLA4**, **IL2RA** (VARIABLE EXPRESSION), CXCR3, S100A4, ITGB1, ITGAE, MKI67, SELL, SQSTM1, RNF26, HSPE1
TCR ACTIVATED TREGS	GATA3, ICOS, CTLA4, IL2RA, TNFRSF18, CD5
MEMORY TREGS	CD27 (prevents apoptosis of Treg), CCR7 (↓), CTLA4, ICOS, BCL2, CD47; PRDM1, BCL6, CCR4, SQSTM1, RNF26, HSPE1
POORLY SUPPRESSIVE RESTING TREGS	FOXP3 (↓), SATB1, BACH2 (BOTH REPRESSORS OF THE EFFECTOR FUNCTIONS)

EREDMÉNYEK





Normalized to HPRT

KÖVETKEZTETÉS

1. Tömegspektrometriás és áramlási citometriás módszerrel kimutattuk a HSPE1 jelenlétét a BeWo trophoblaszterű sejtvonal-eredetű extracelluláris vezikulákban

2. A BeWo-eredetű EVk kötődtek a CD4+ T-sejtekhez és IL6RA downregulációt, valamint növekedett IL-10 szecernálást indukáltak

3. Hét különböző sejtklasztert azonosítottunk a Treg sejtpopulációban

4. Meghatároztunk 4 panelt a Treg sejt altípusok azonosítására

5. A Treg sejt altípusokban a HSPE1 klaszterfüggő expressziót mutat

A HSPE1+ BeWo-eredetű EVk indukáló faktorként szerepelhetnek a Treg sejtek differenciációjában és a memória Treg sejtek expanziójában

KÖSZÖNETNYILVÁNÍTÁS

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EMBERI ERŐFORRÁSOK MINISZTÉRIUMA

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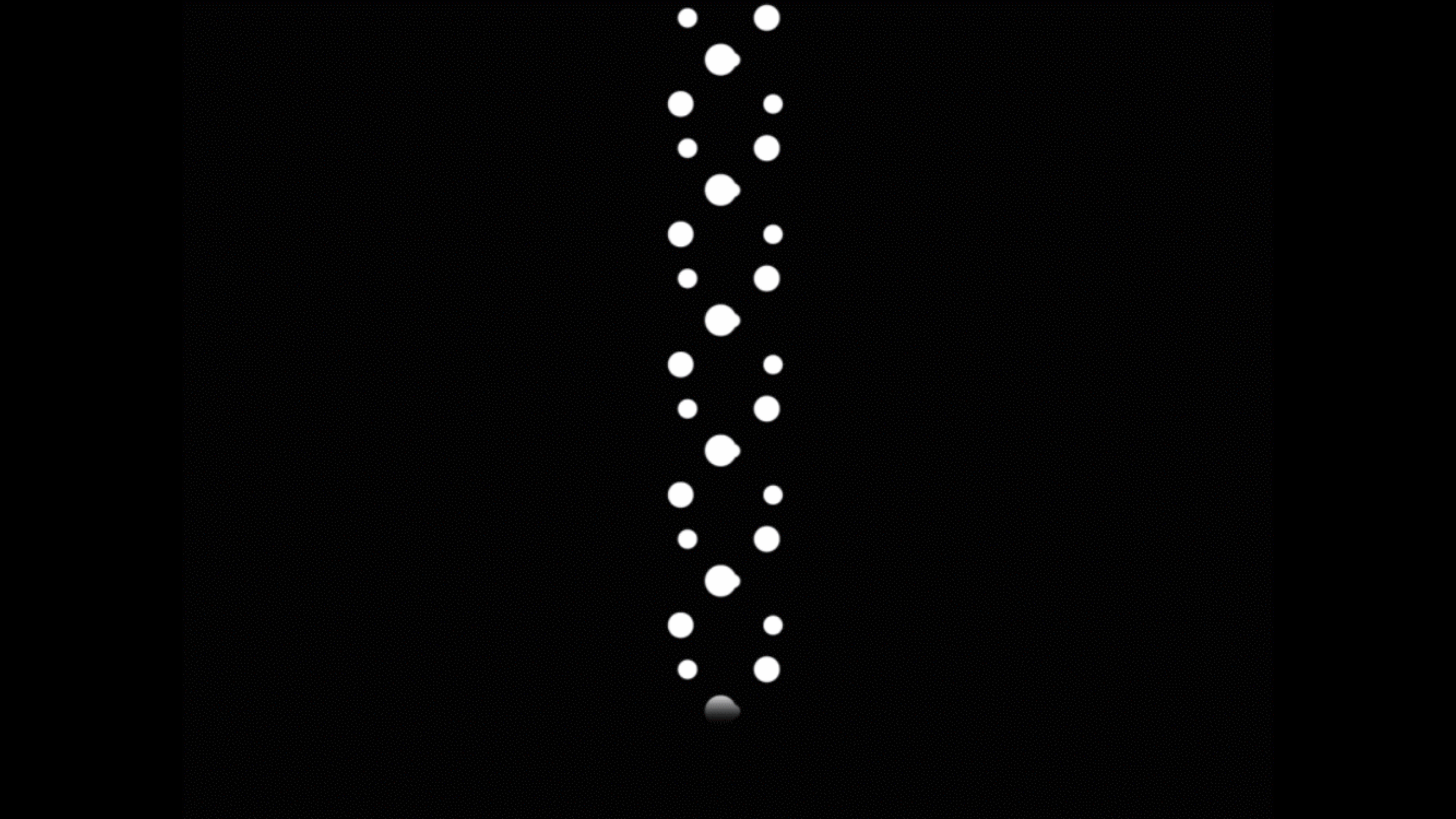
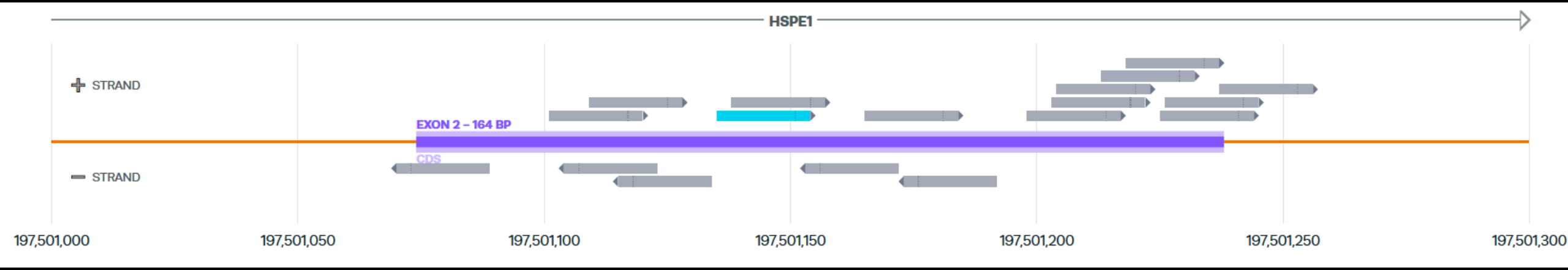
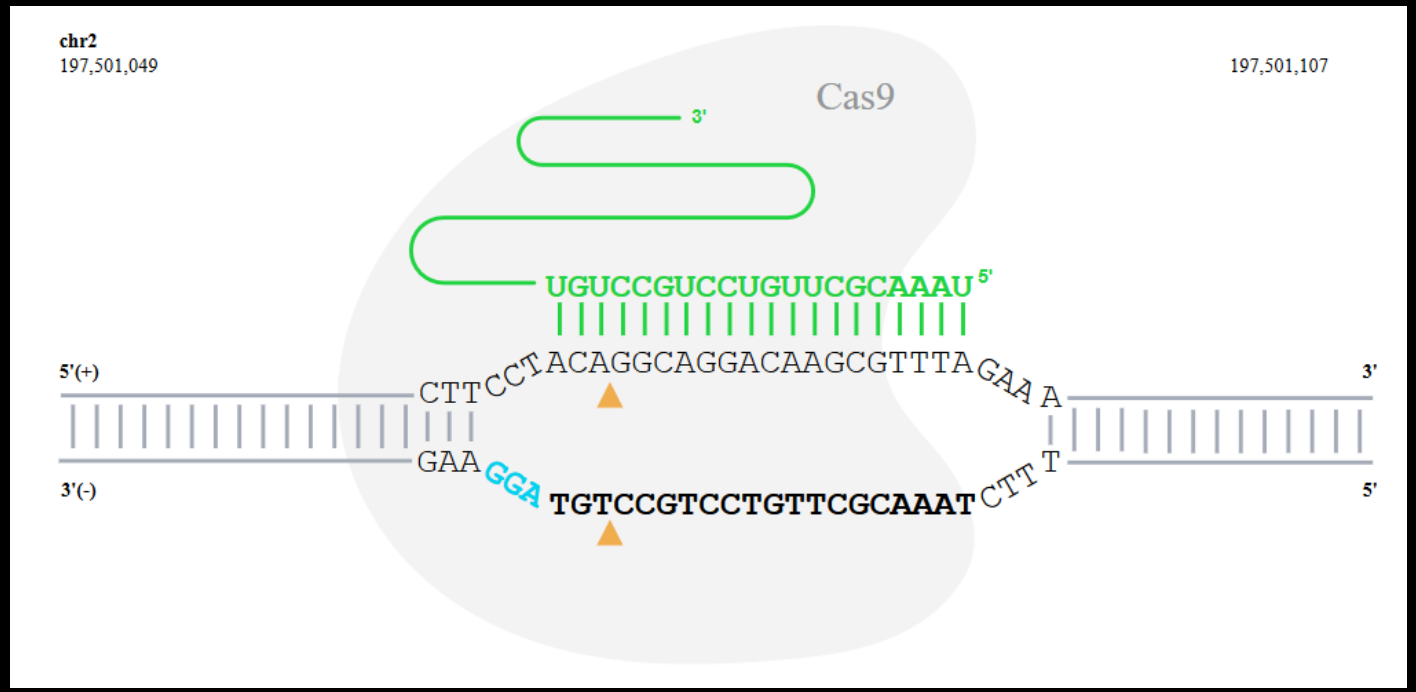


1. HSPE1 IMMUNMODULÁLÓ HATÁSÁNAK A VIZSGÁLATA A REGULATORIKUS T-SEJT EXPANZIÓRA (ÚNKP-18-3-IV-SE-14)





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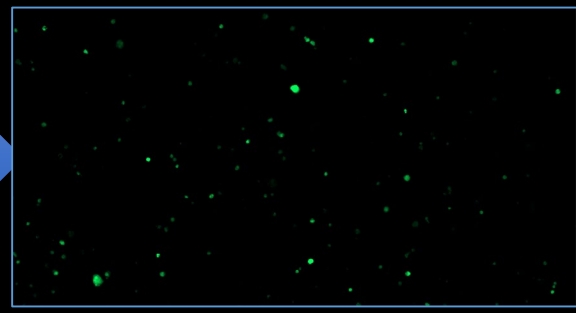
TRANSZFEKTÁLÁSI PROGRAM BEÁLLÍTÁSA ÉS OPTIMALIZÁLÁSA

Mintakód	Nucleofector program	Transzfekciós hatékonyság (FACS – GFP)	Viabilitás (FACS – PI)	FACS mérés	Fluoreszcens mikroszkóp
A1	CA-137	29 %	84.7 %		
A2	DS-150	41.4 %	71.1 %		
A3	CM-138	20.1 %	81.4 %		
A4	DS-120	16.5 %	89.7 %		
A5	CM-137	33.9 %	81.6 %		
A6	EH-100	54.8 %	50 %		
A7	CM-150	23.4 %	80.3 %		
A8	EO-100	23.7 %	33.1 %		
A9	DN-100	**42.1 %**	**72.9 %**		
A10	EN-138	**62.5 %**	**35.5 %**		
A11	DS-138	42.4 %	73 %		
A12	EN-150	**69.9 %**	**51.3 %**		
A13	DS-137	**62.7 %**	**67.5 %**		
A14	EW-113	49.4 %	25.9 %		
A15	DS-130	40.7 %	71.9 %		

TRANSZFEKCIÓS HATÉKONYSÁG
(FACS ÉS FLUORESZCENS MIKROSZKÓP)

PI VIABILITÁS
(FACS)

EN-150 ÉS DS137



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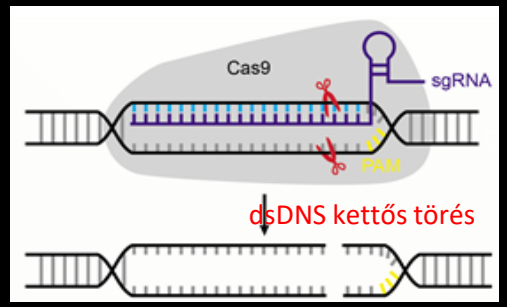
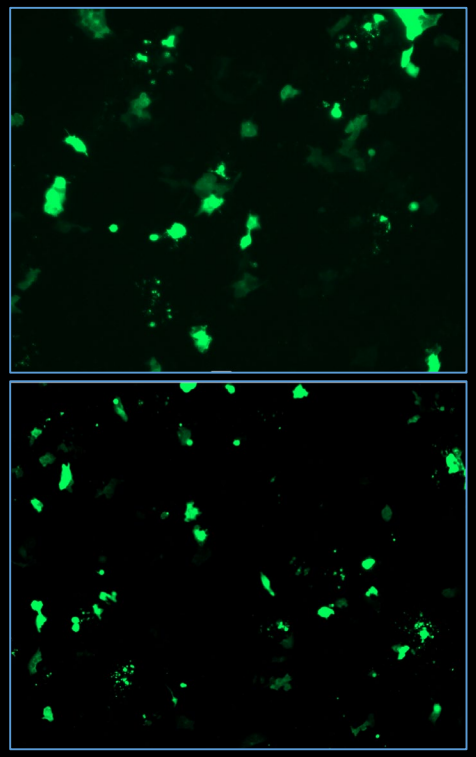


Indel → KO

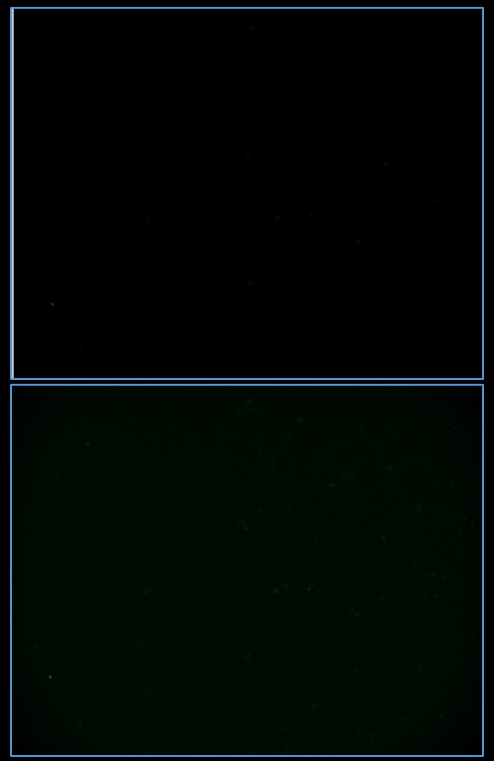
TRANSZFEKTÁLÁSI PROGRAM BEÁLLÍTÁSA ÉS OPTIMALIZÁLÁSA

3 TRANSZFEKCIÓS MÉDIUM: **SF, SE** ÉS **SG** → IRODALMI ADATOK → BEÁLLÍTÁSI KÍSÉRLET → **SF**

72 ÓRÁVAL A TRANSZFEKCIÓT KÖVETŐEN:



EN-150 + pMAX GFP



EN-150

pMAX GFP

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KLÓNSZELEKCIÓ