Heterogeneity in old fibroblasts is linked to variability in reprogramming and wound healing

Nature. 2019 Oct;574(7779):553-558. doi: 10.1038/s41586-019-1658-5. Epub 2019 Oct 23.

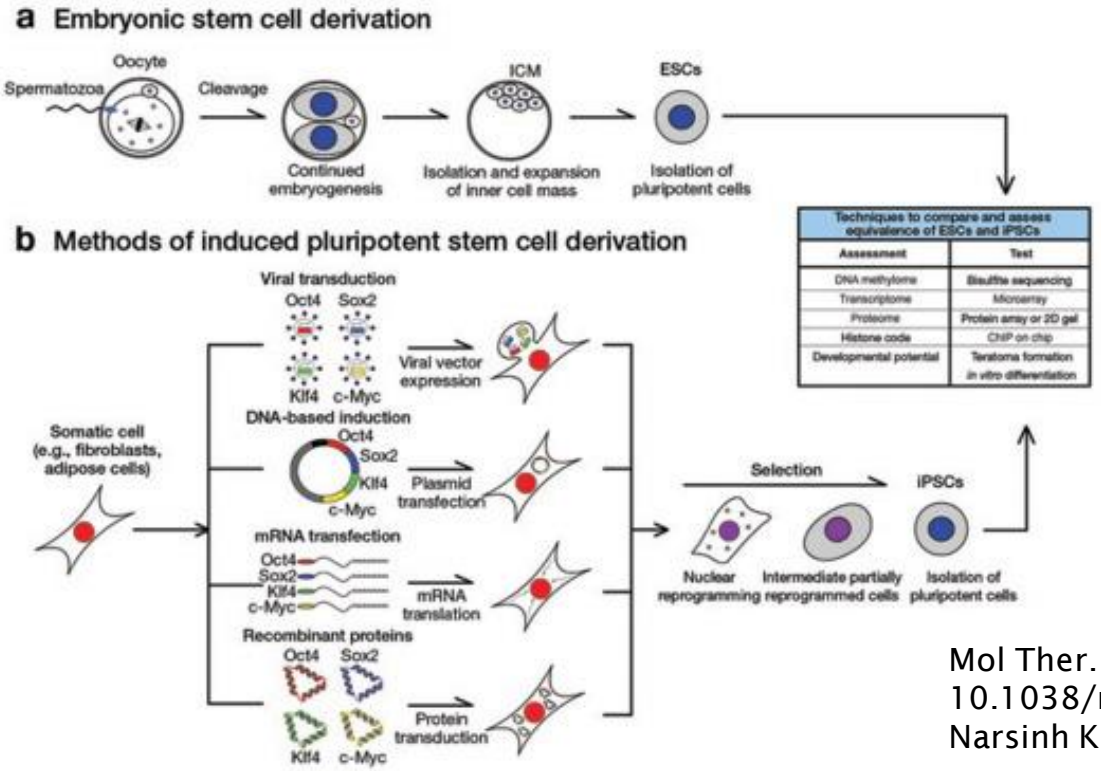
Mahmoudi S1, Mancini E1, Xu L1,2, Moore A3,4, Jahanbani F1, Hebestreit K1, Srinivasan R4,5, Li X1, Devarajan K1, Prélot L1, Ang CE4,6,7, Shibuya Y4,7, Benayoun BA1,8, Chang ALS9, Wernig M4,7, Wysocka J4,5, Longaker MT3,4, Snyder MP1, Brunet A10,11.

Methods

- C57/Bl6
- Young mice: 3 months
- Old mice: 28-29 months
- Mouse FBs: Primary FB isolation from young and old mice from skin, ears and lungs, isolated with Liberase
- Human FBs: Primary FBs from male volunteers of different ages with four biological grandparents of Ashkenazi Jewish descent; 4mm pre-auricular punch biopsy
- Cytokine profiling: 24h plating of cells in serum- and feeder-free medium; Luminex multi-analyte human and mouse assay
- Cellular reprogramming: lentiviral vector *OCT4, KLF4, SOX2, MYC;* Virus produced in human HEK293T-cells by PEI-transfection

Methods: Reprogramming/ iPS-generation

- Takahashi, Yamanaka: Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors. Cell 2006
- "Yamanaka-Cocktail" = 4 transcription factors (TF) to reprogram cells into induced pluripotent stem cells: *OCT4*, *KLF4*, *SOX2*, *MYC*

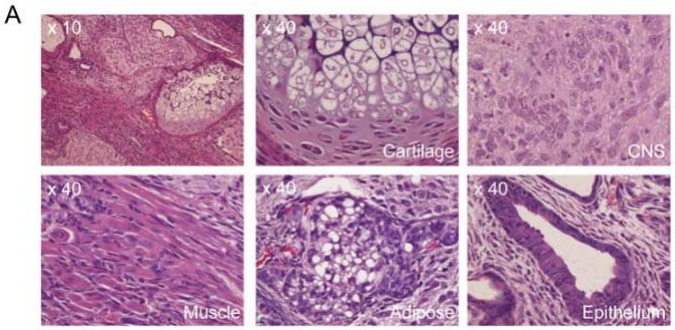


Mol Ther. 2011 Apr;19(4):635-8. doi: 10.1038/mt.2011.41.
Narsinh KH1, Plews J, Wu JC.

Methods: Reprogramming/ iPS-generation

How to reprogram:

- Generate (e.g. in HEK-cells) or buy virus containing desired transcription factors
- Plate e.g. FBs, add virus + Polybrene (to enhance infection);
- plate cells with irradiated feeder cells
- Switch to embryonic stem cell medium
- After 13-15d, assess cells for distinct mES morphology, plate each iPS clone individually
- Assess for reprogramming efficiency: staining for AP and SSEA1 (markers for pluripotency)



Takashi, Yamanka, Cell 2006

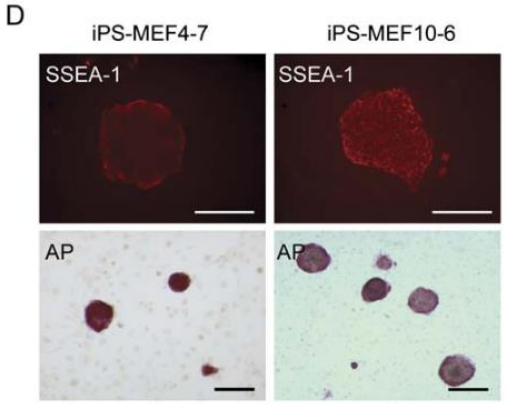


Fig. 1: Primary fibroblasts from old mice secrete inflammatory cytokines and show increased variability in reprogramming efficiency between mice.

Assessing reprogramming efficiency

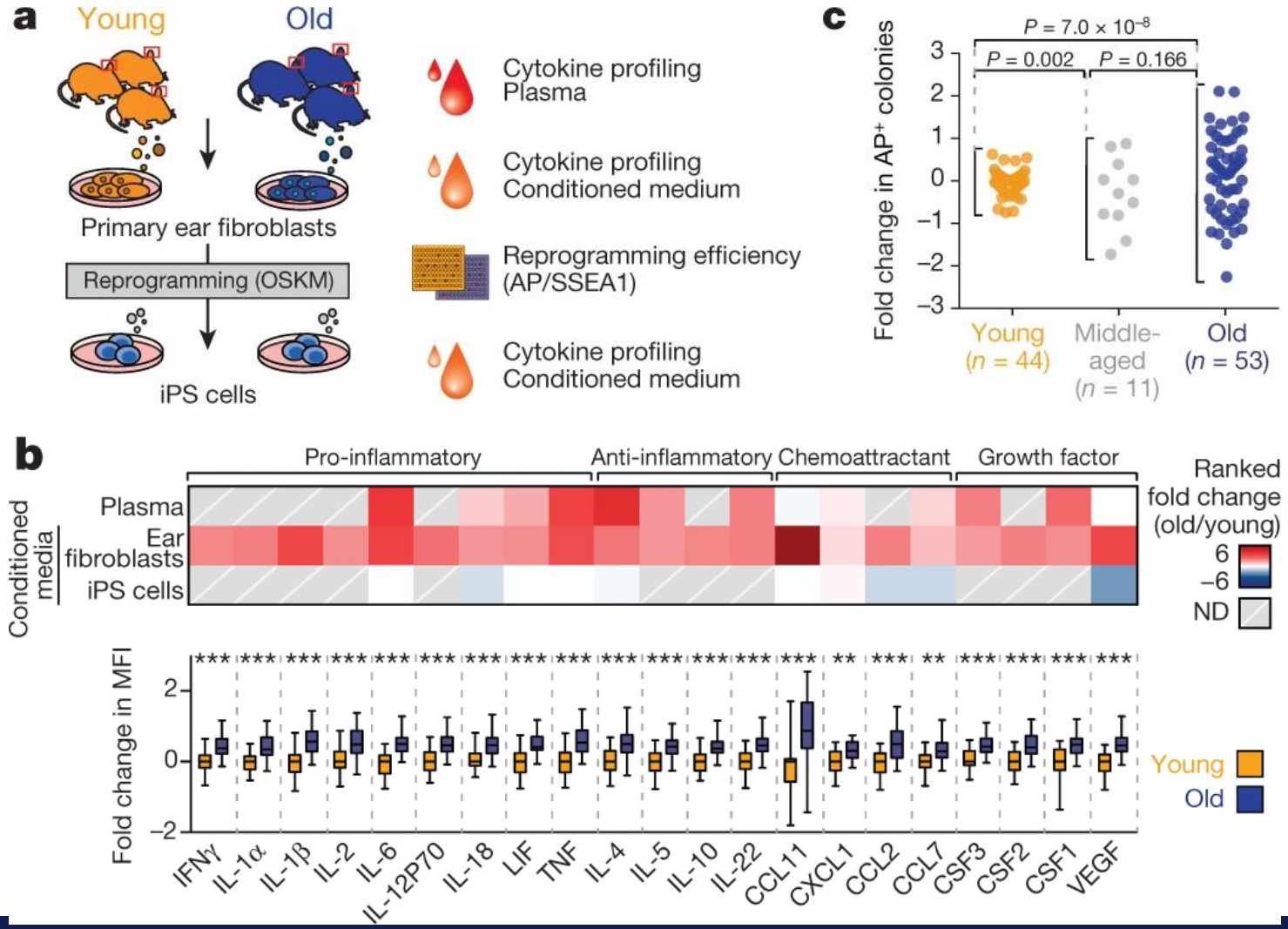
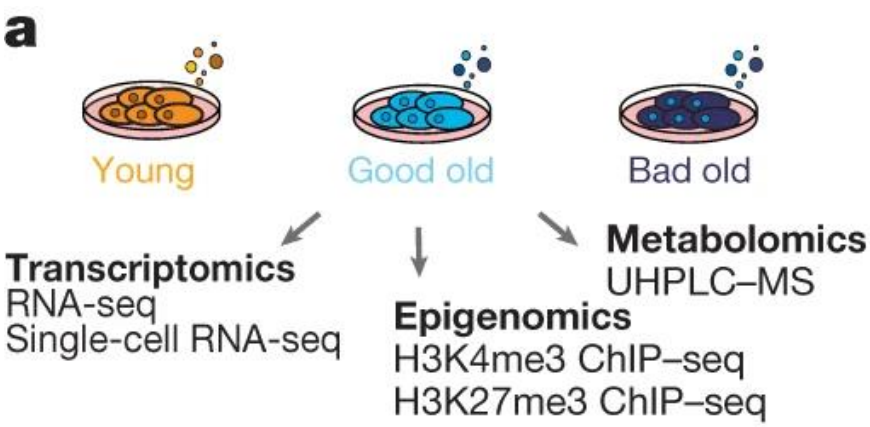
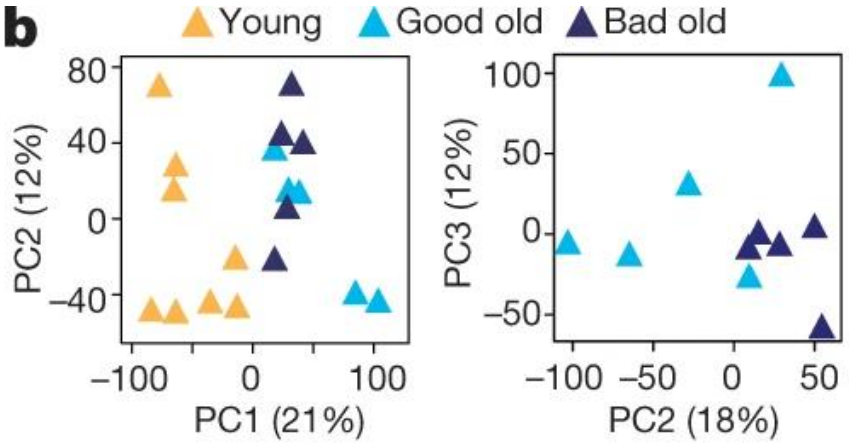


Fig. 2: Old fibroblast cultures exhibit a signature of an inflammatory activated state, which is associated with variability in reprogramming efficiency.





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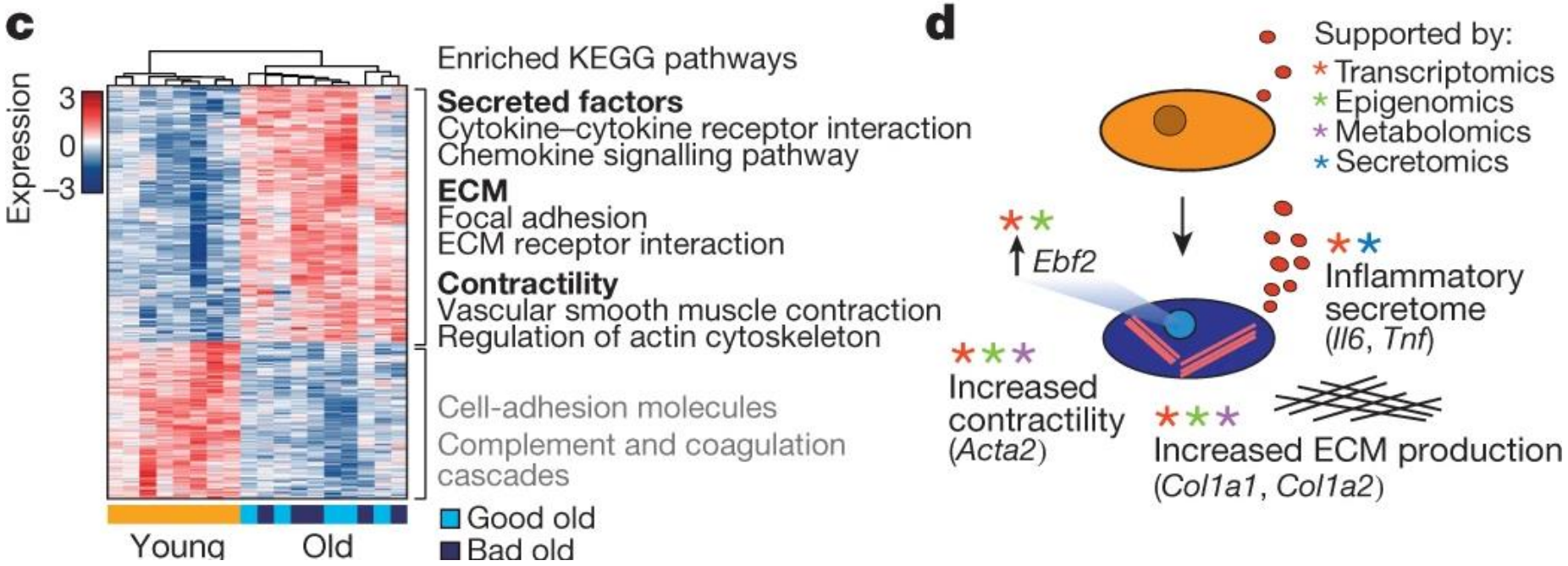
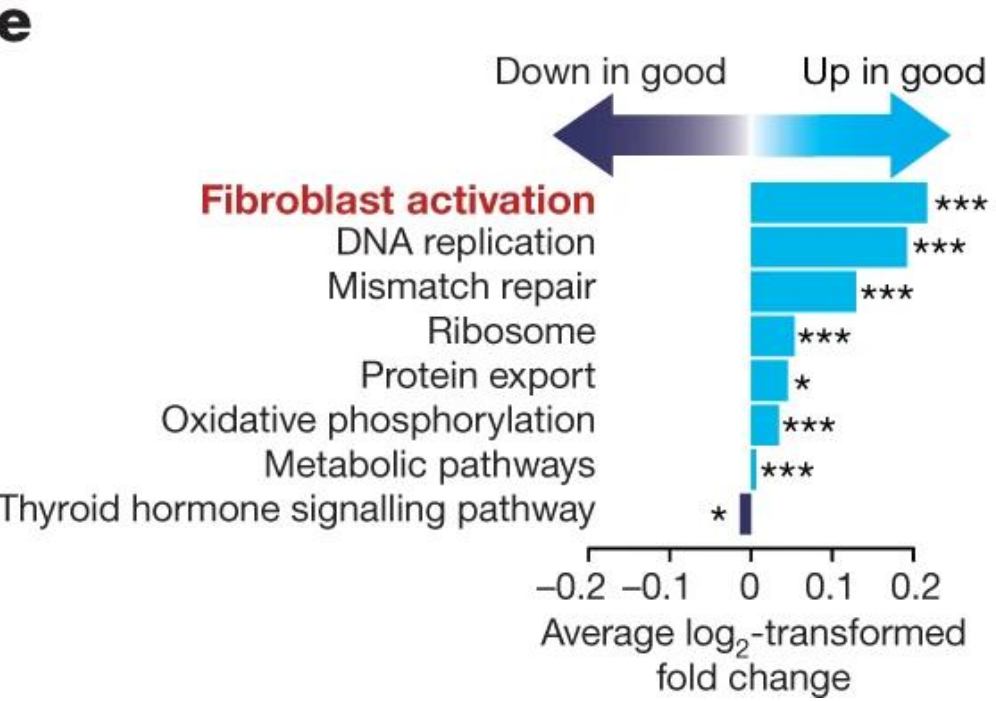


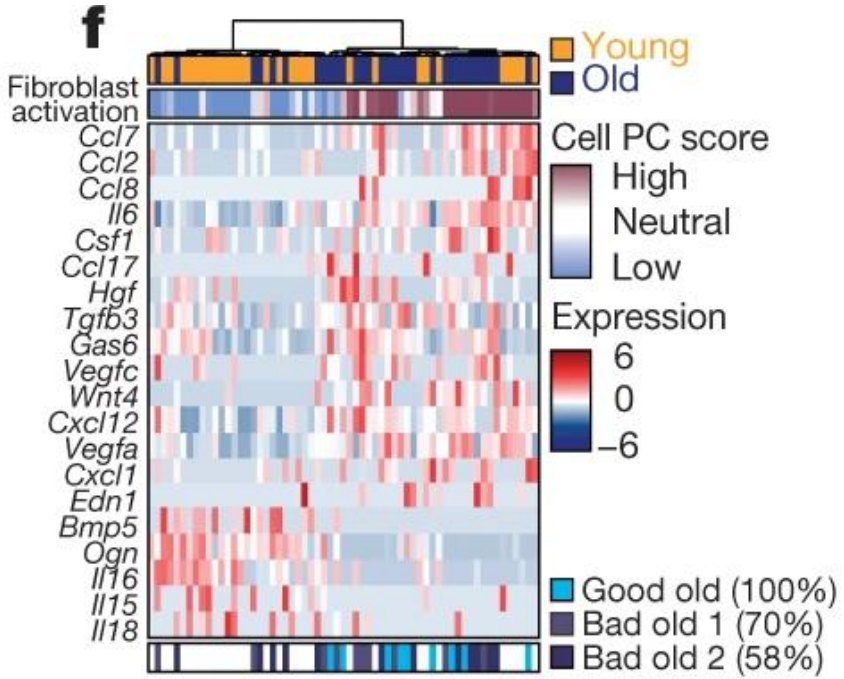
EBF2 = potential driver for FB activation

Fig. 2: Old fibroblast cultures exhibit a signature of an inflammatory activated state, which is associated with variability in reprogramming efficiency.

Pathway analysis of FBs
with good or bad reprogramming



PAGODA-analysis of scRNAseq from
young, good and bad old FBs



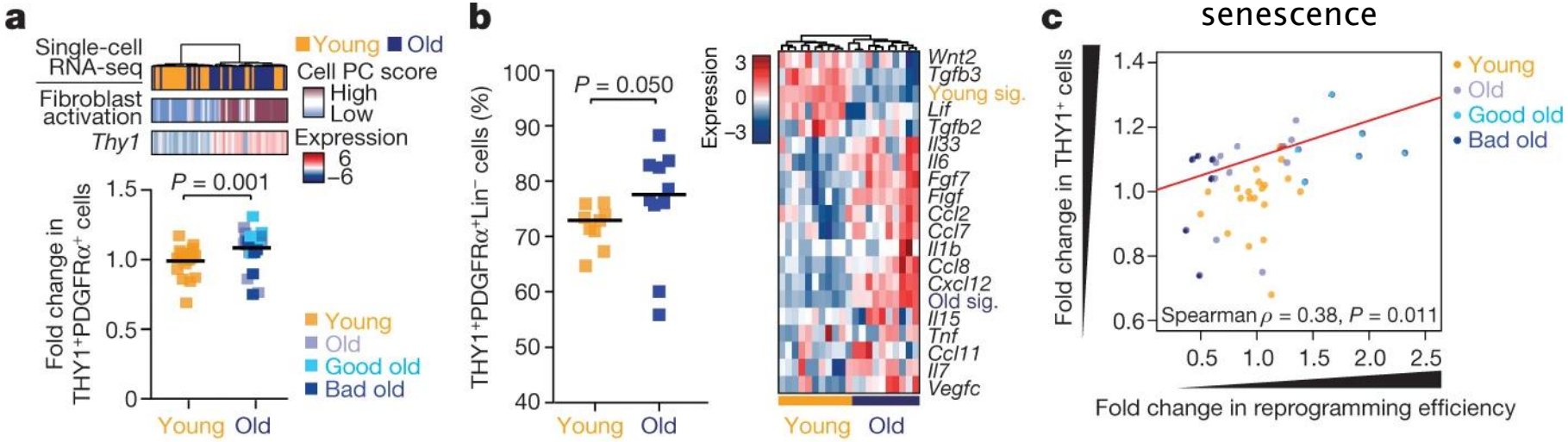
- Old FBs →more „activated FBs“
- Activated FBs = better reprogramming
- Activated FBs = myofibroblasts = profibrogenic
- Activated FBs = proliferate, no senescence markers (Figure E5b-e)

Figure 3: Age-associated increase in activated fibroblasts and the cytokines that they secrete drive part of the variability in reprogramming between mice.

Old FBs contain more *Thy1*+ FBs, and express more *Ebf2*

More Thy1+ in ears of old mice and higher FB activation signature

Better reprogramming= more THY1+, more proliferation, less senescence



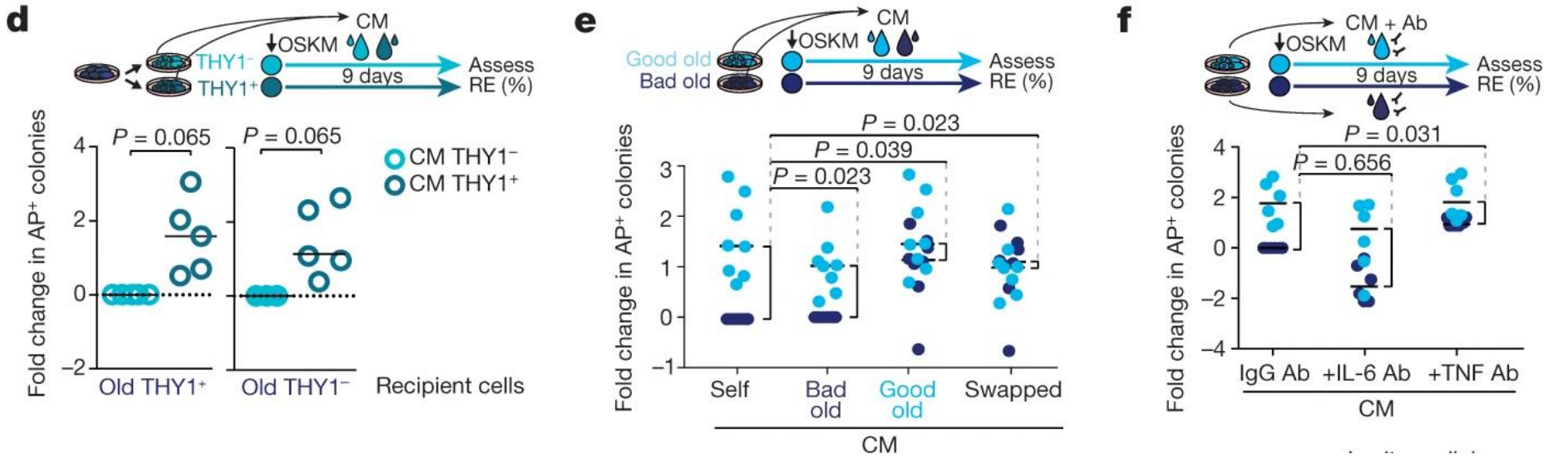
THY1 = activation marker, PDFGFRa= FB-marker

Figure 3: Age-associated increase in activated fibroblasts and the cytokines that they secrete drive part of the variability in reprogramming between mice.

Conditioned medium (CM) from activated (=THY1+) FB enhanced reprogramming

Swapping of CM reduces differences in reporgramming efficiency

IL6 enhances & TNF reduces reprogramming efficiency



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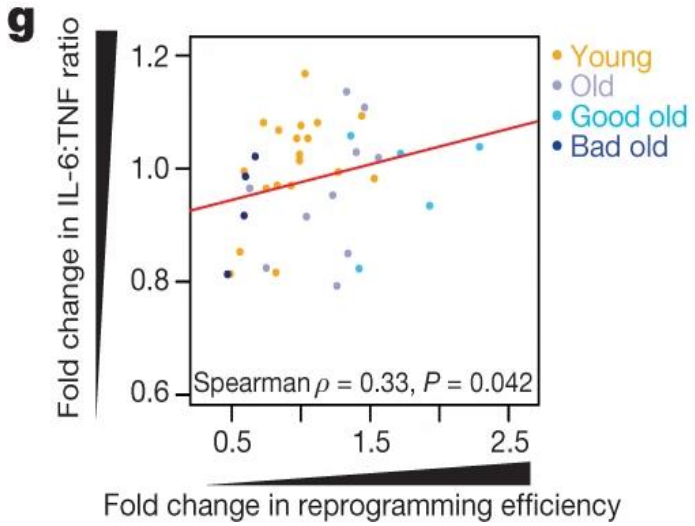
IL6:TNF correlates with RE

Activated:non-activated FB and their cytokines drive variability between FB cultures in old mice



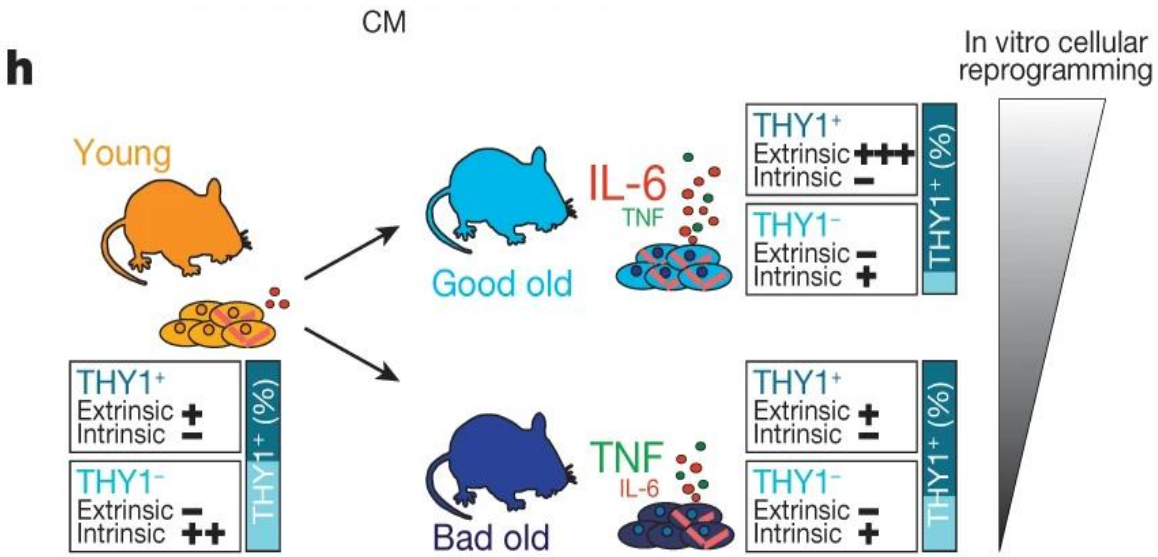
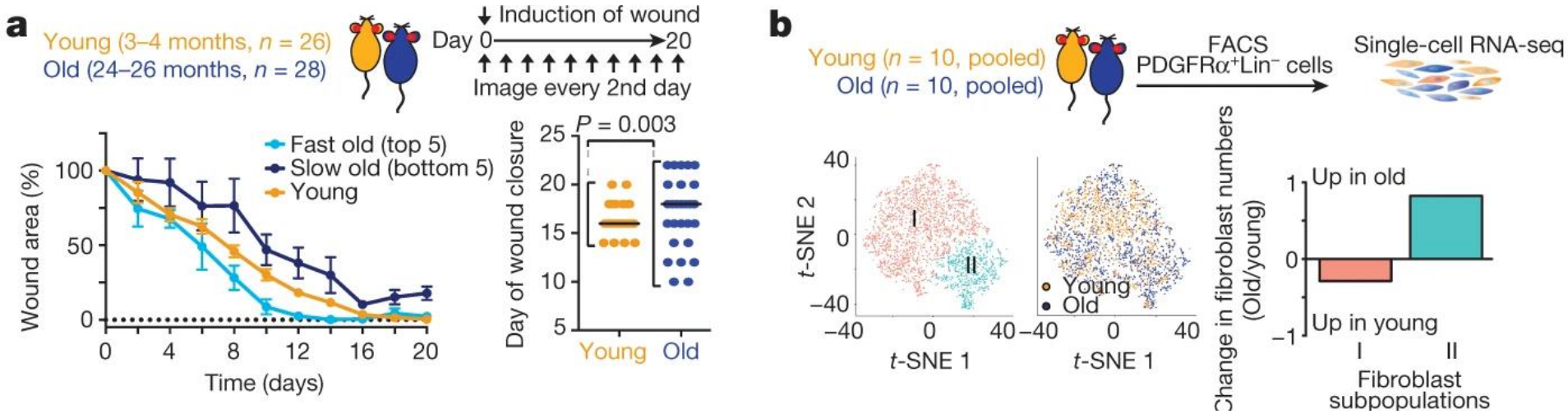


Figure 4: Wound healing rate is variable between old mice and correlates with fibroblast subpopulations with distinct cytokine signatures.



Increased variability in wound healing rate old vs young

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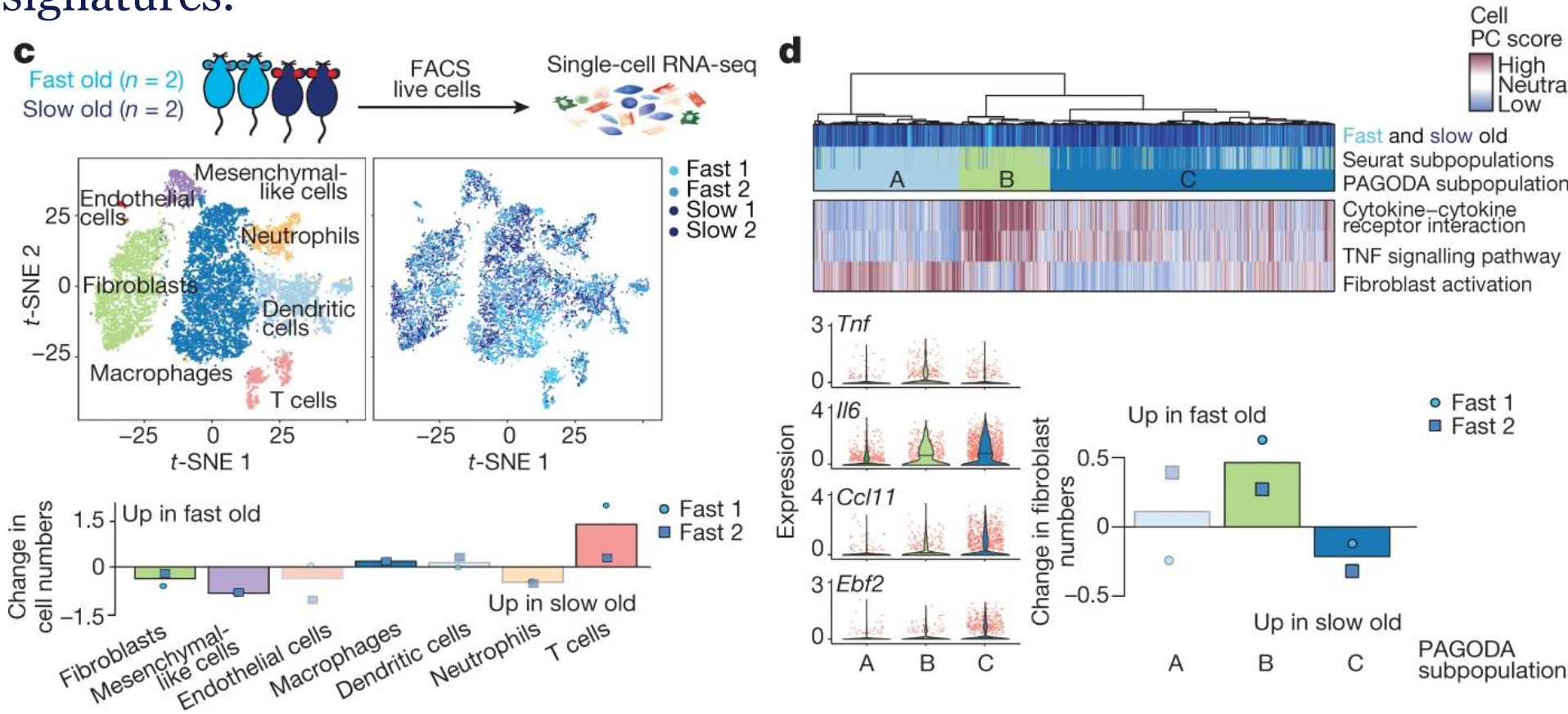


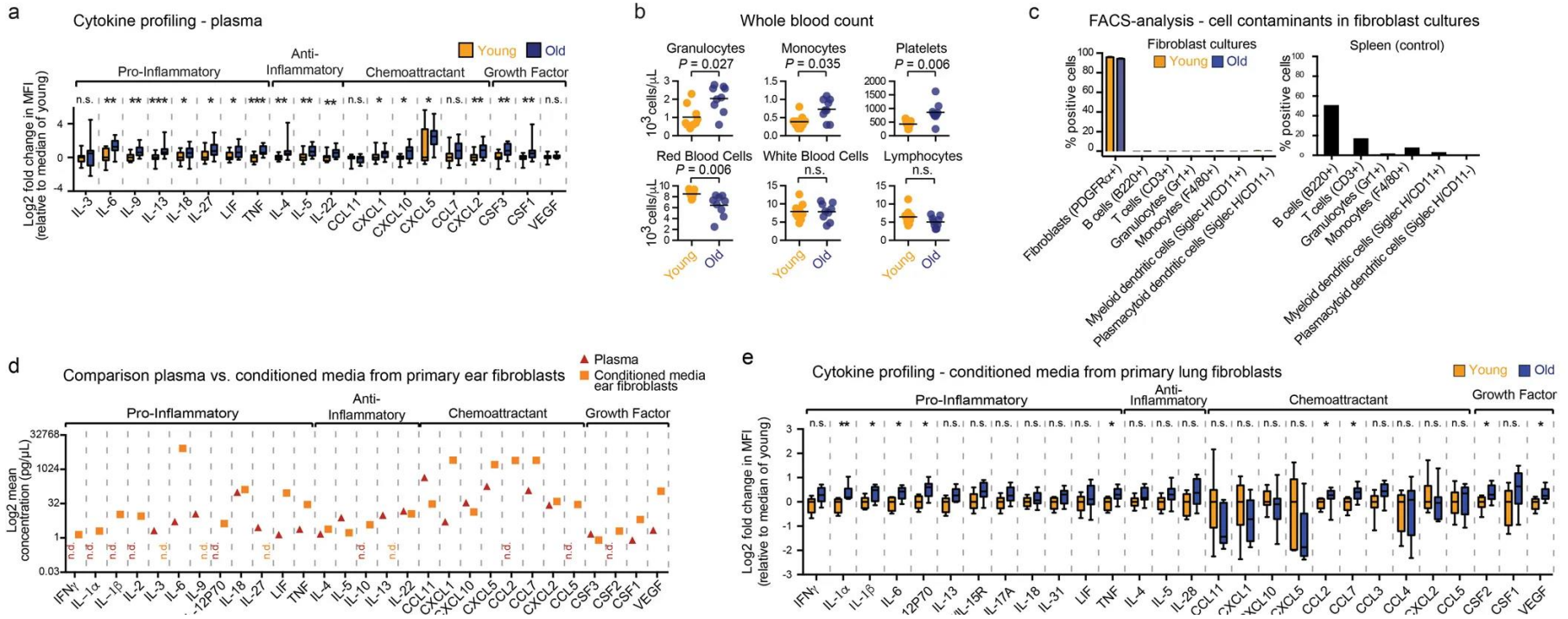
- B more abundant in fast healing old,
- Increased cytokine expression: TNF up →
- TNF associated with fast wound healing + bad reprogramming

- C more in slow healing old, higher in other cytokines, e.g. Ccl11
- Higher in TF *Ebf2*

Discussion

- Conclusive story?
- Relevance?
  - There is no iPS-induction (?)/reprogramming in vivo?!
  - Only technical relevance?
- Would you rather have fibrosis or senescence?

Extended Data 1: Extended Data Fig. 1 Primary old fibroblasts from mouse ear, mouse lungs and human skin secrete high levels of inflammatory cytokines, and the ability of individual cultures from ear fibroblasts to reprogram is stereotypical.



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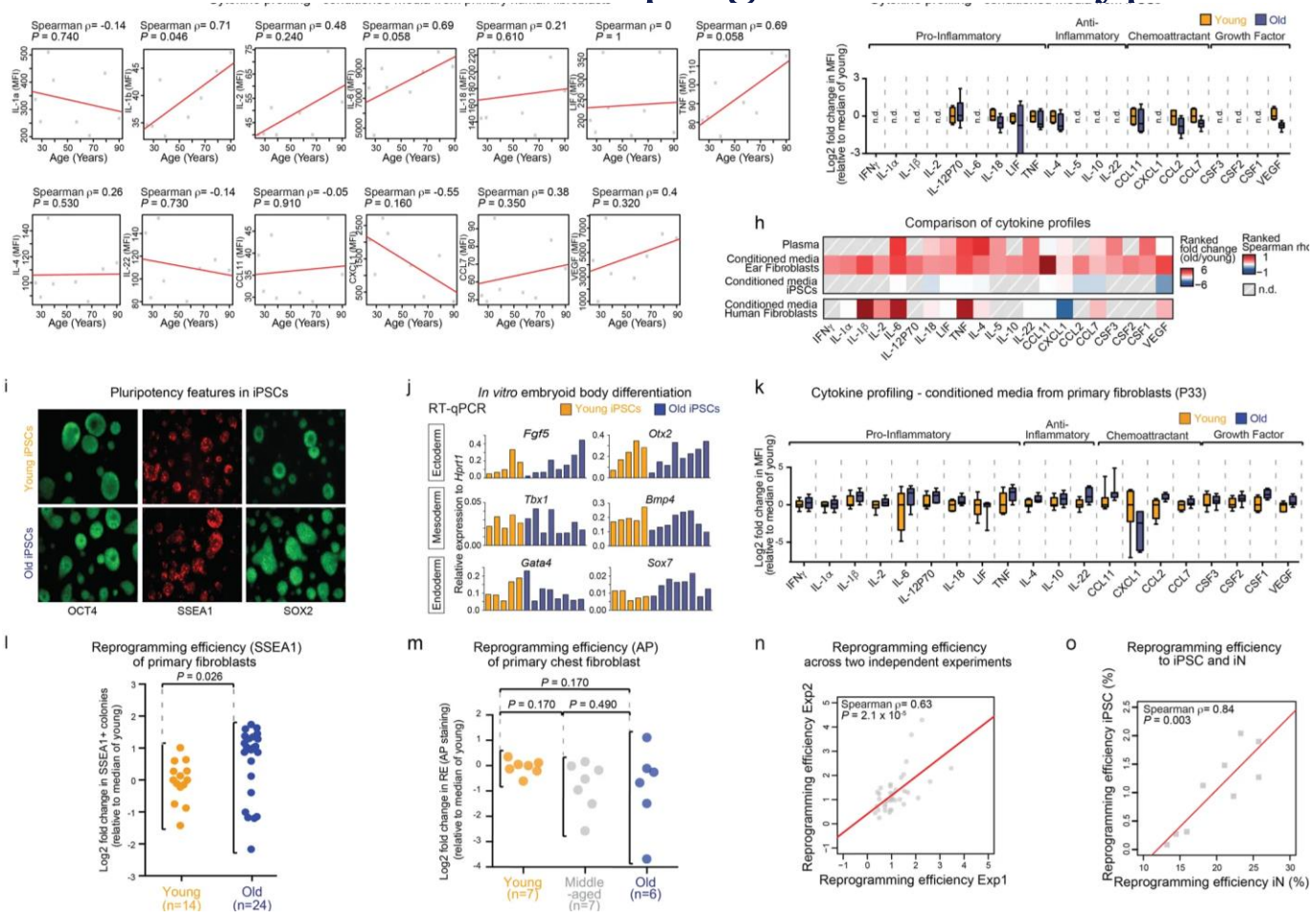
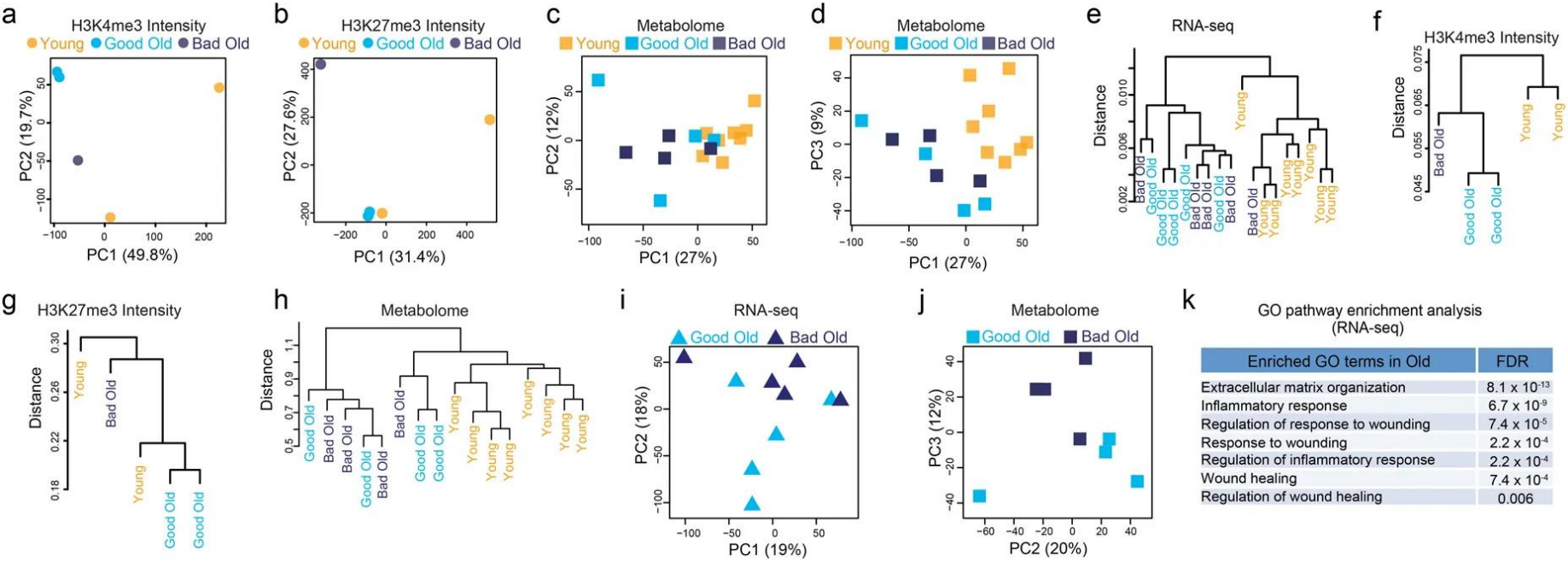


Extended Data Fig. 2 Old fibroblasts exhibit distinct transcriptomic, epigenomic and metabolomics profiles compared to young fibroblasts.



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GO pathway enrichment analysis (RNA-seq)

Enriched GO terms in Old	FDR
Extracellular matrix organization	8.1×10^{-13}
Inflammatory response	6.7×10^{-9}
Regulation of response to wounding	7.4×10^{-5}
Response to wounding	2.2×10^{-4}
Regulation of inflammatory response	2.2×10^{-4}
Wound healing	7.4×10^{-4}
Regulation of wound healing	0.006

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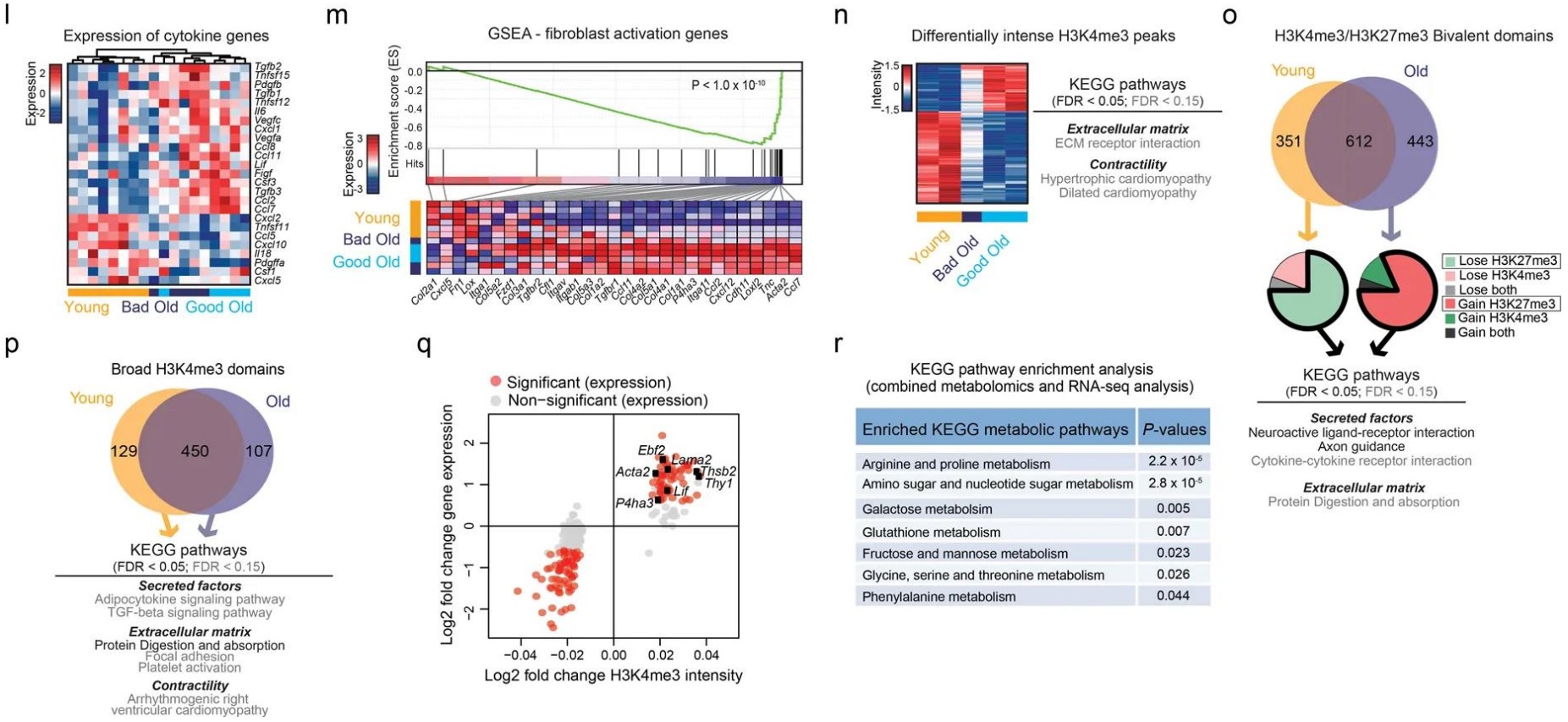

Enriched KEGG metabolic pathways	*P*-values
Arginine and proline metabolism	2.2×10^{-5}
Amino sugar and nucleotide sugar metabolism	2.8×10^{-5}
Galactose metabolsim	0.005
Glutathione metabolism	0.007
Fructose and mannose metabolism	0.023
Glycine, serine and threonine metabolism	0.026
Phenylalanine metabolism	0.044

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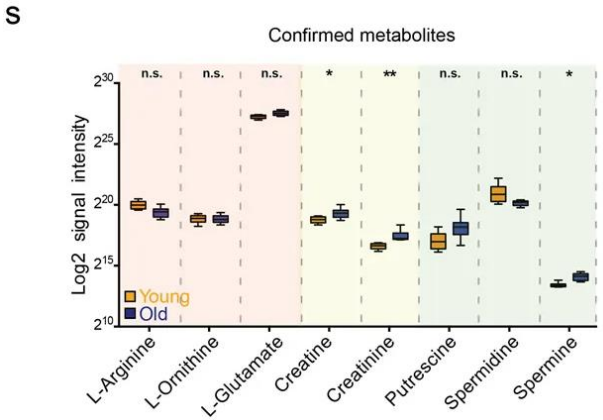

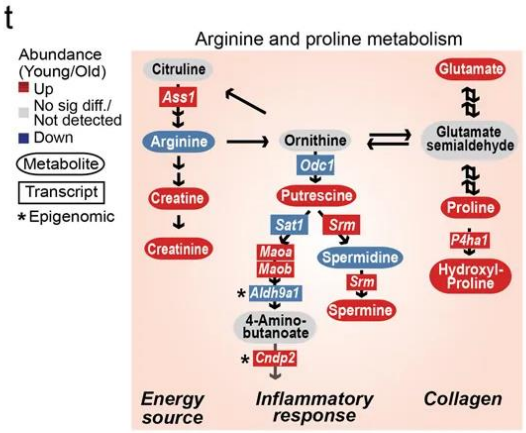

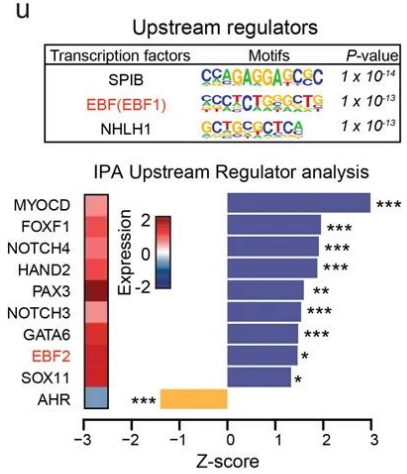

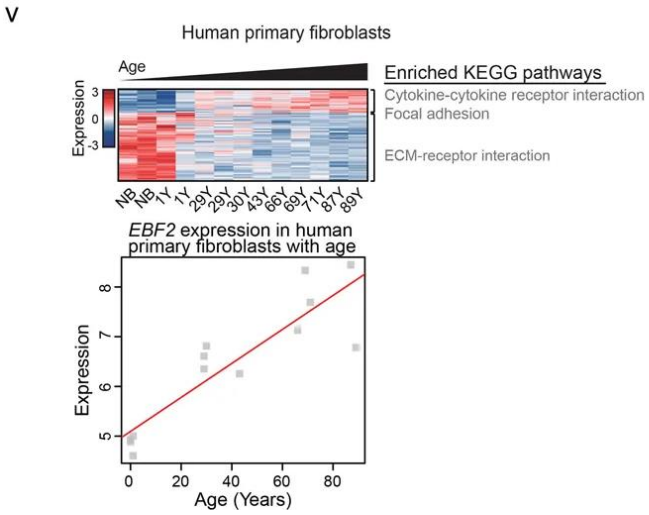

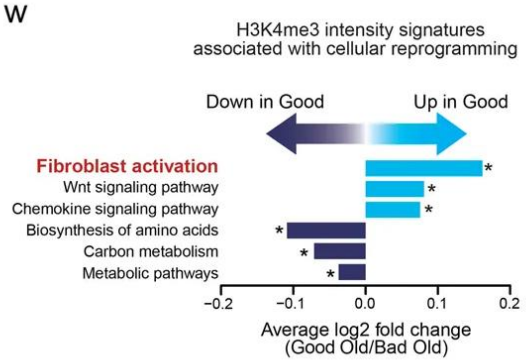

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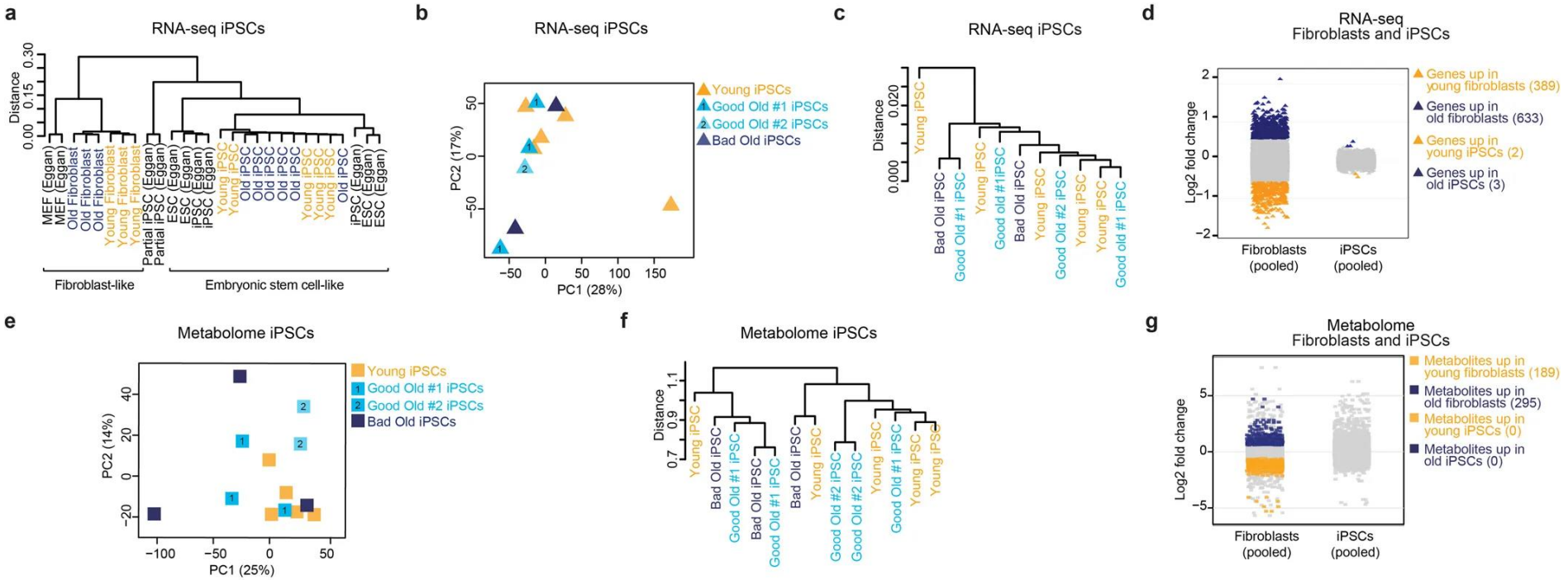

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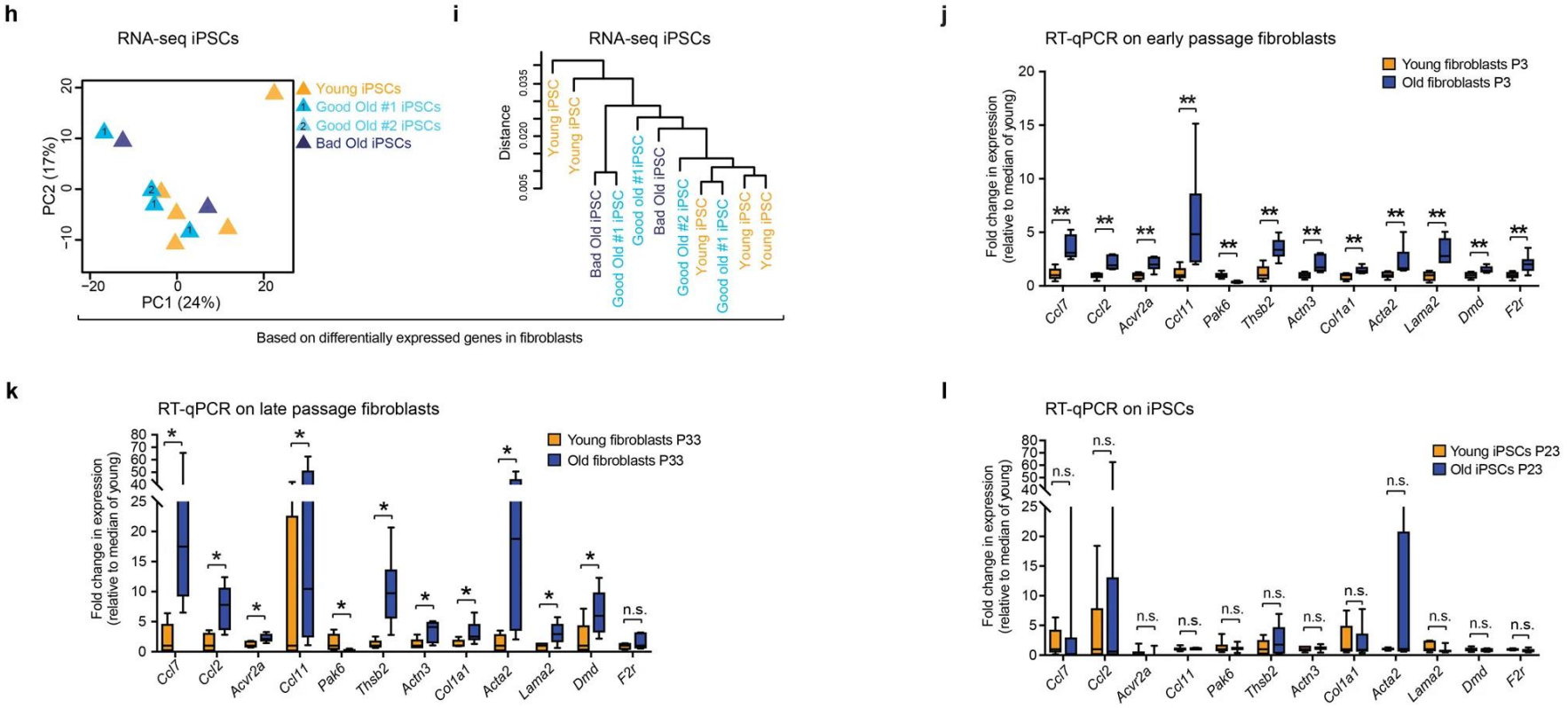

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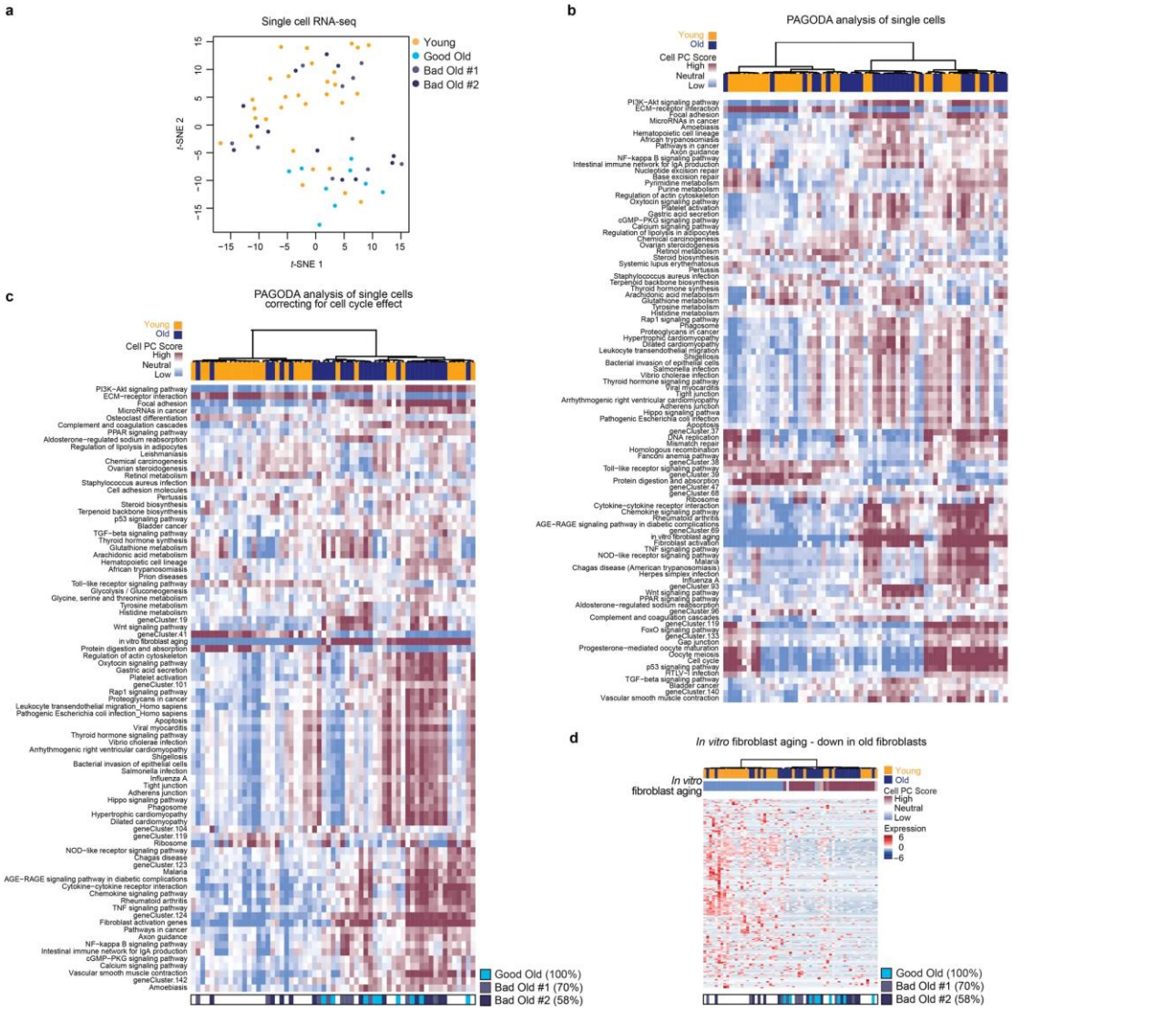

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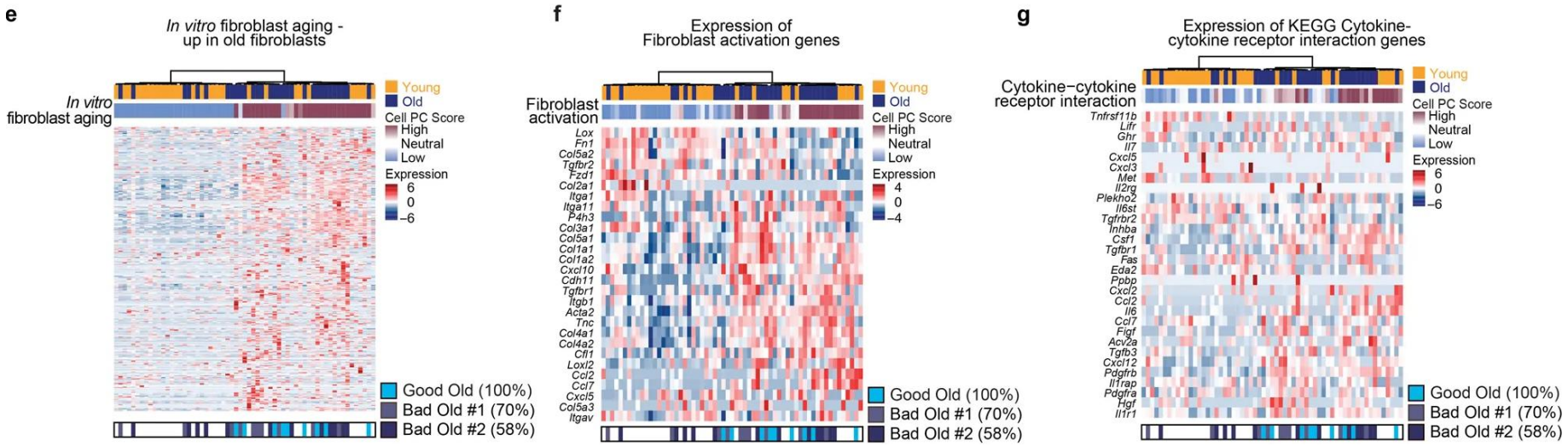

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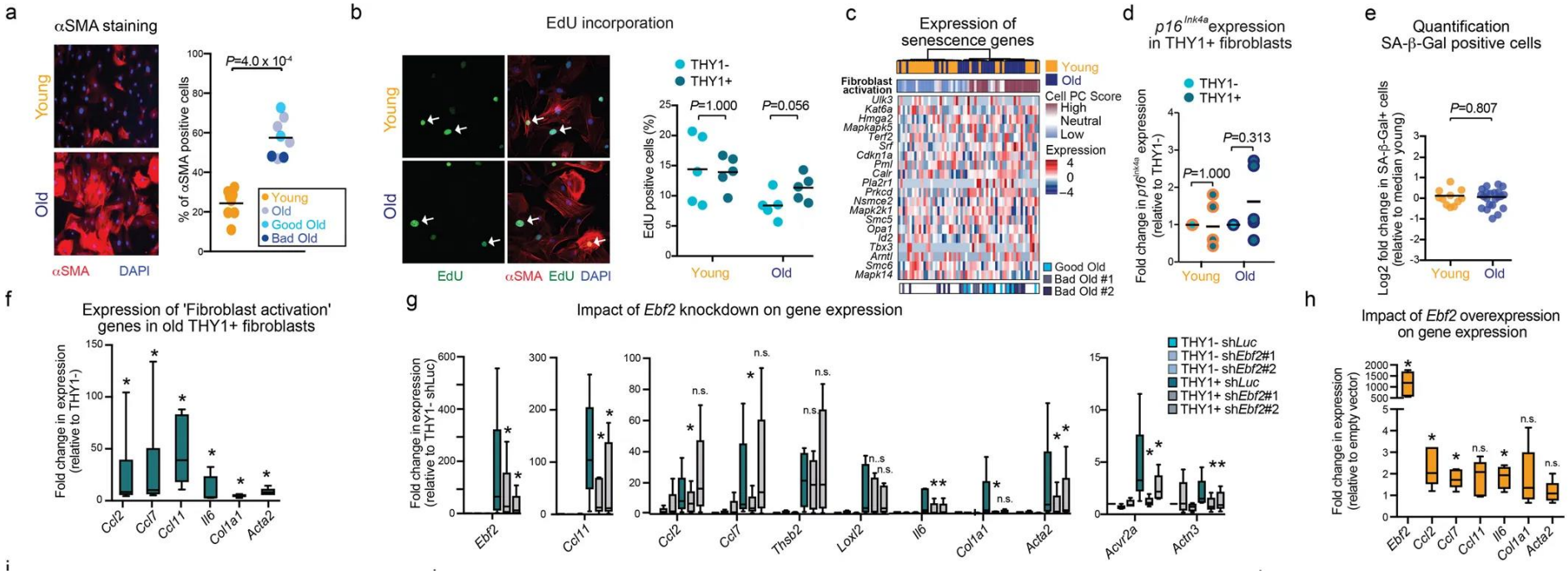

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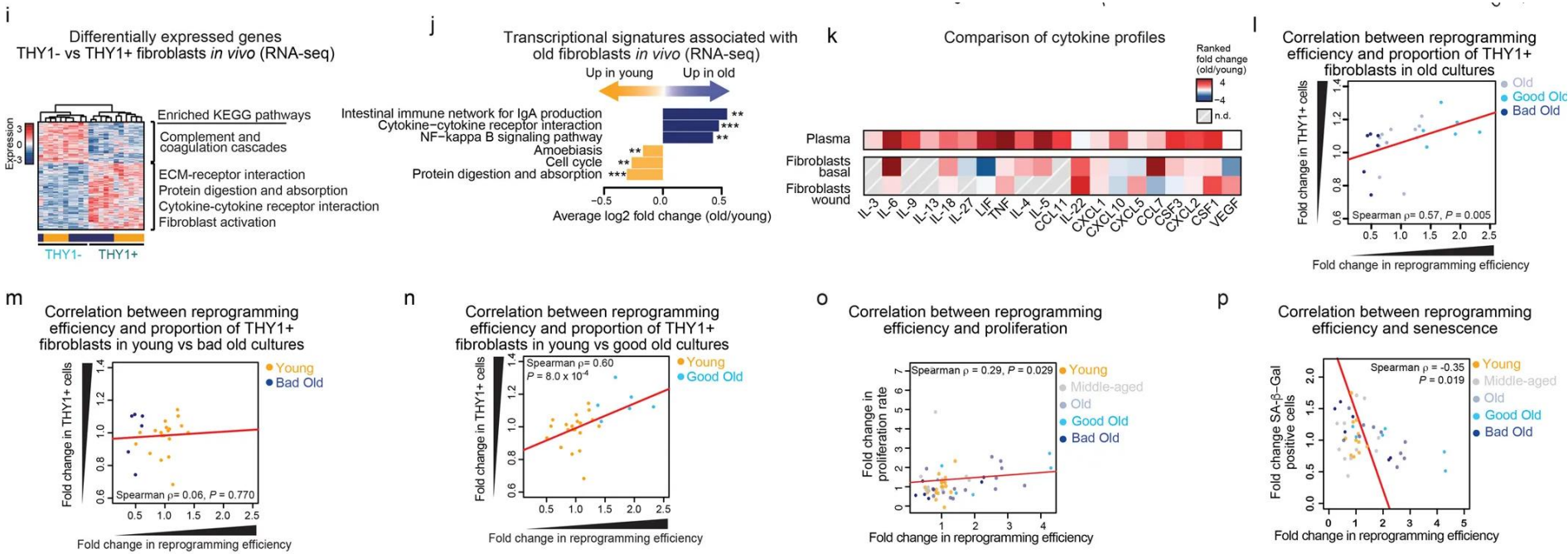

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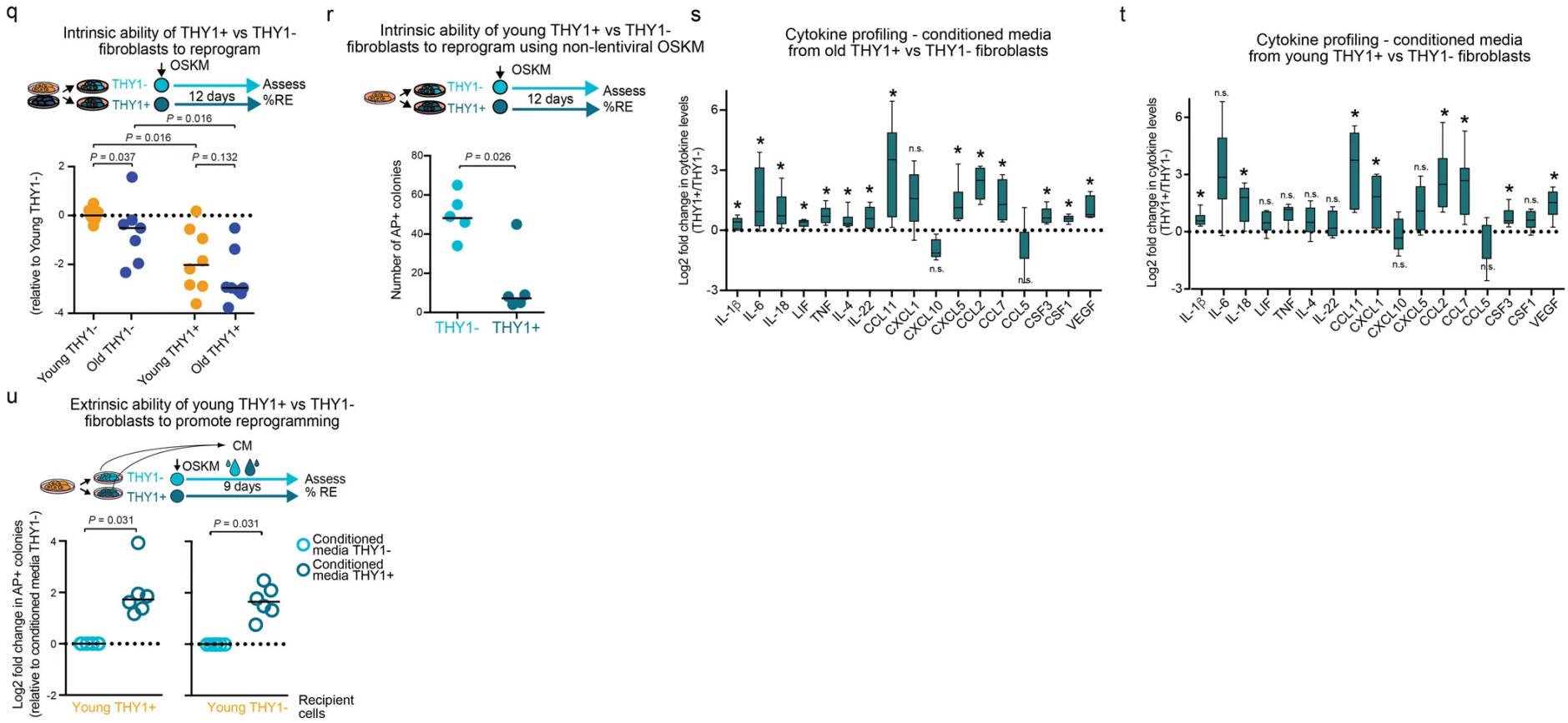

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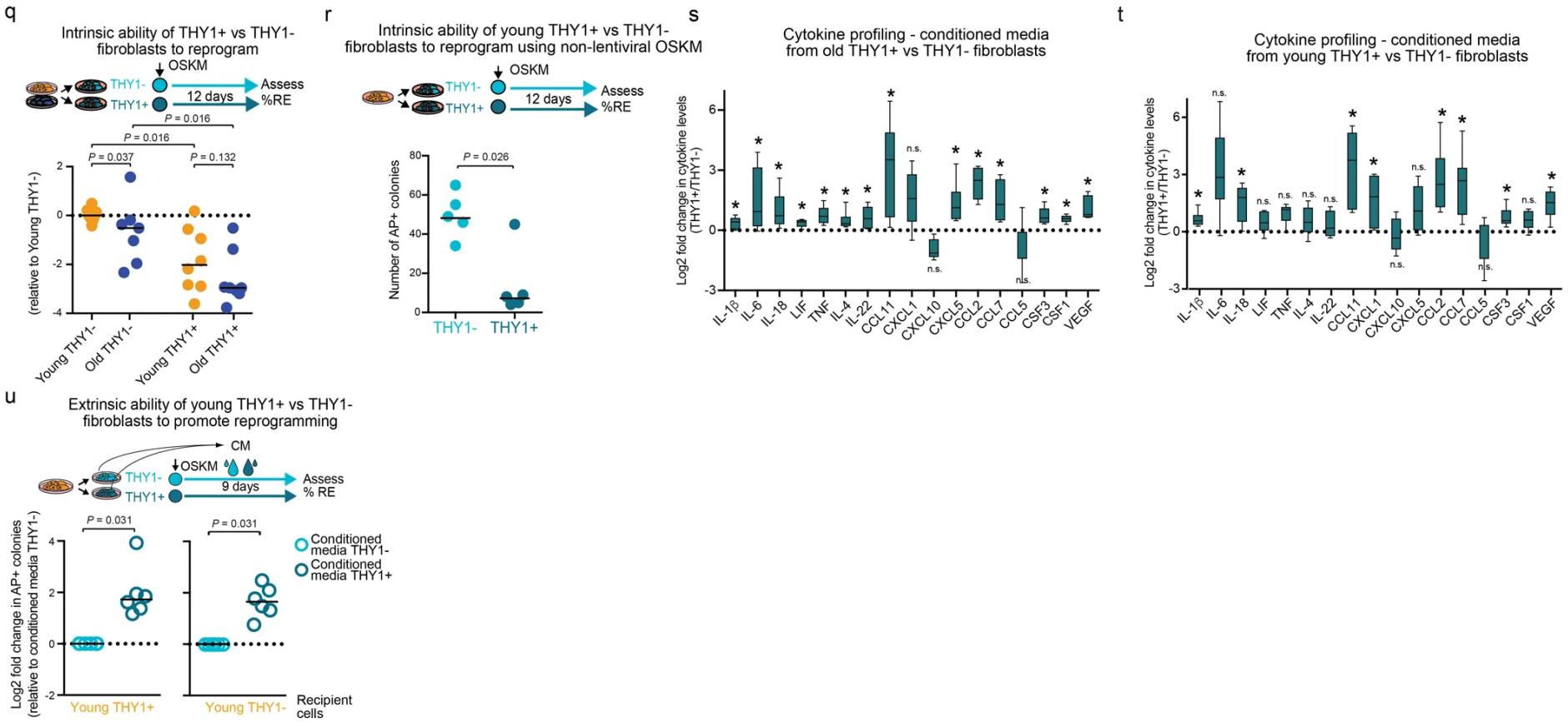

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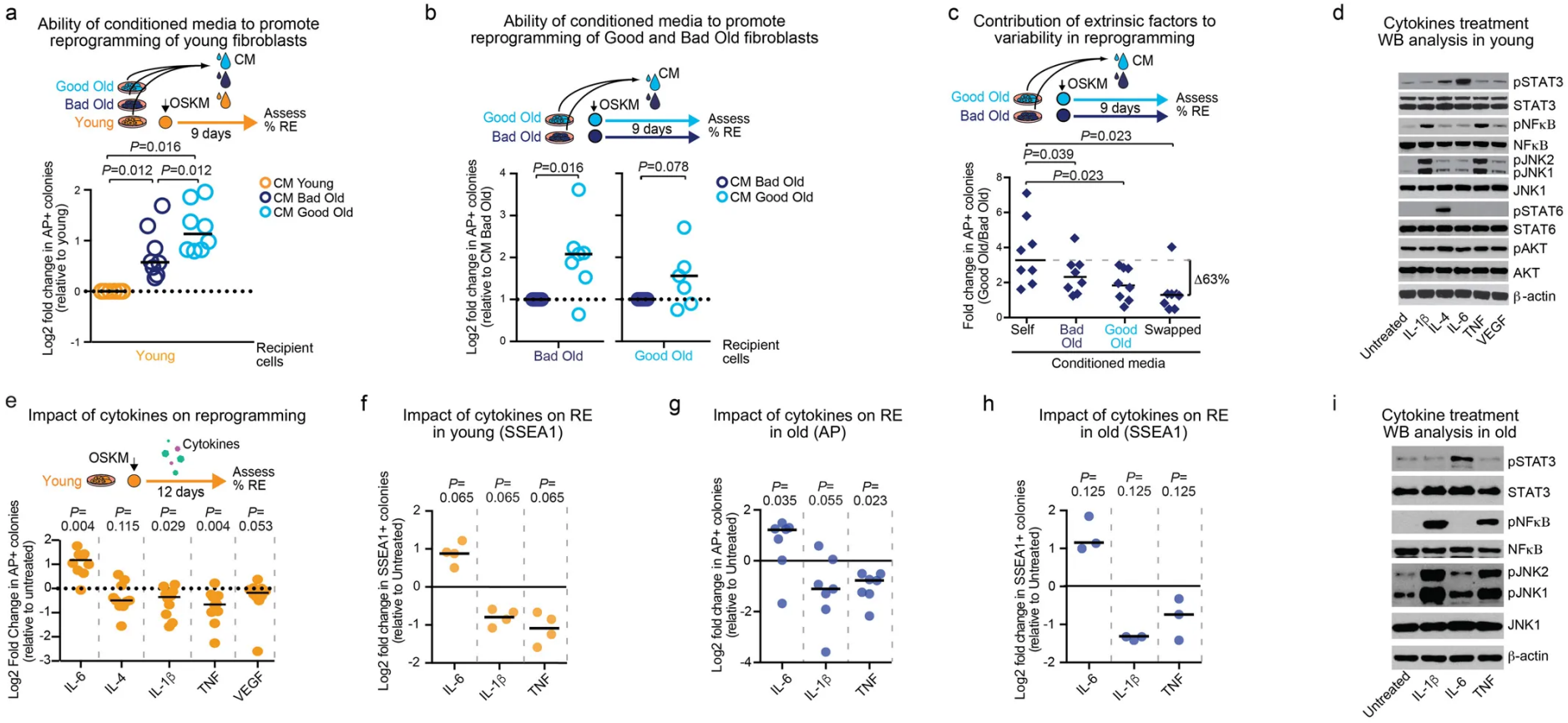

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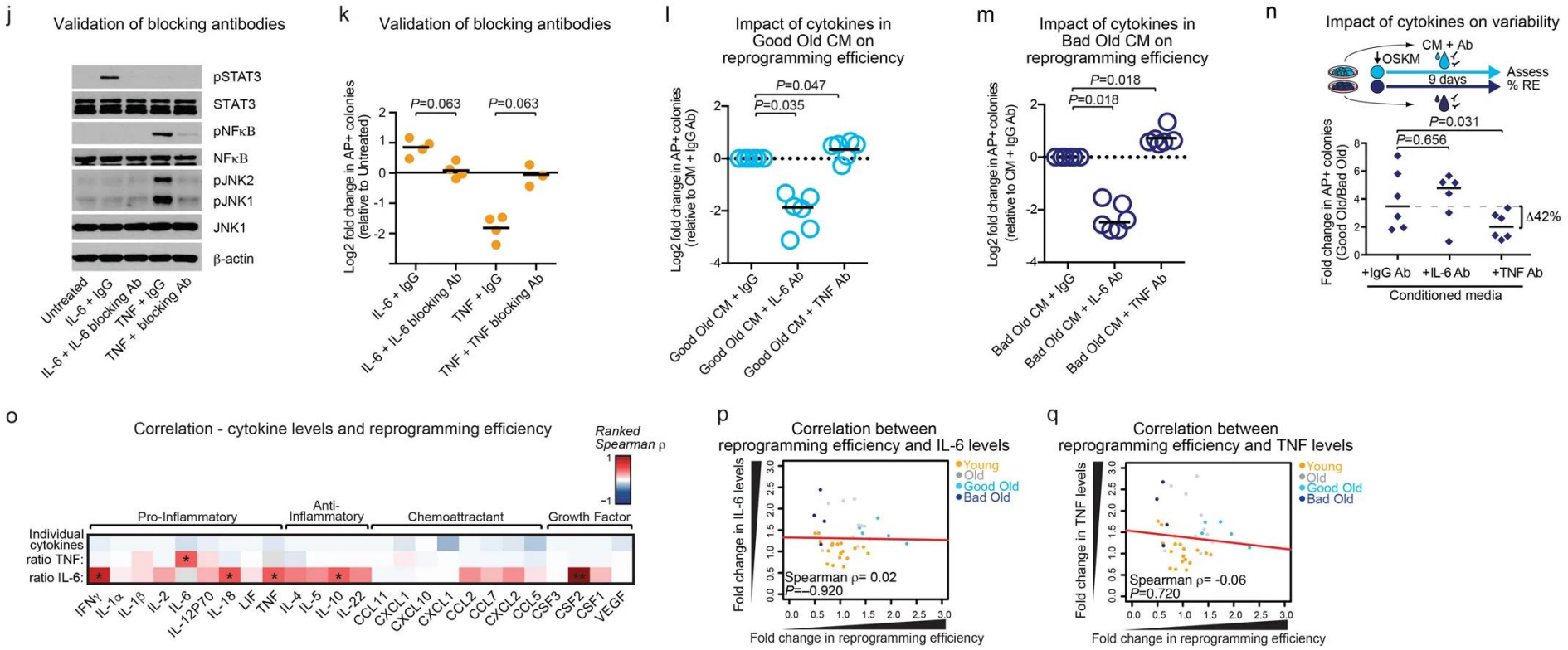

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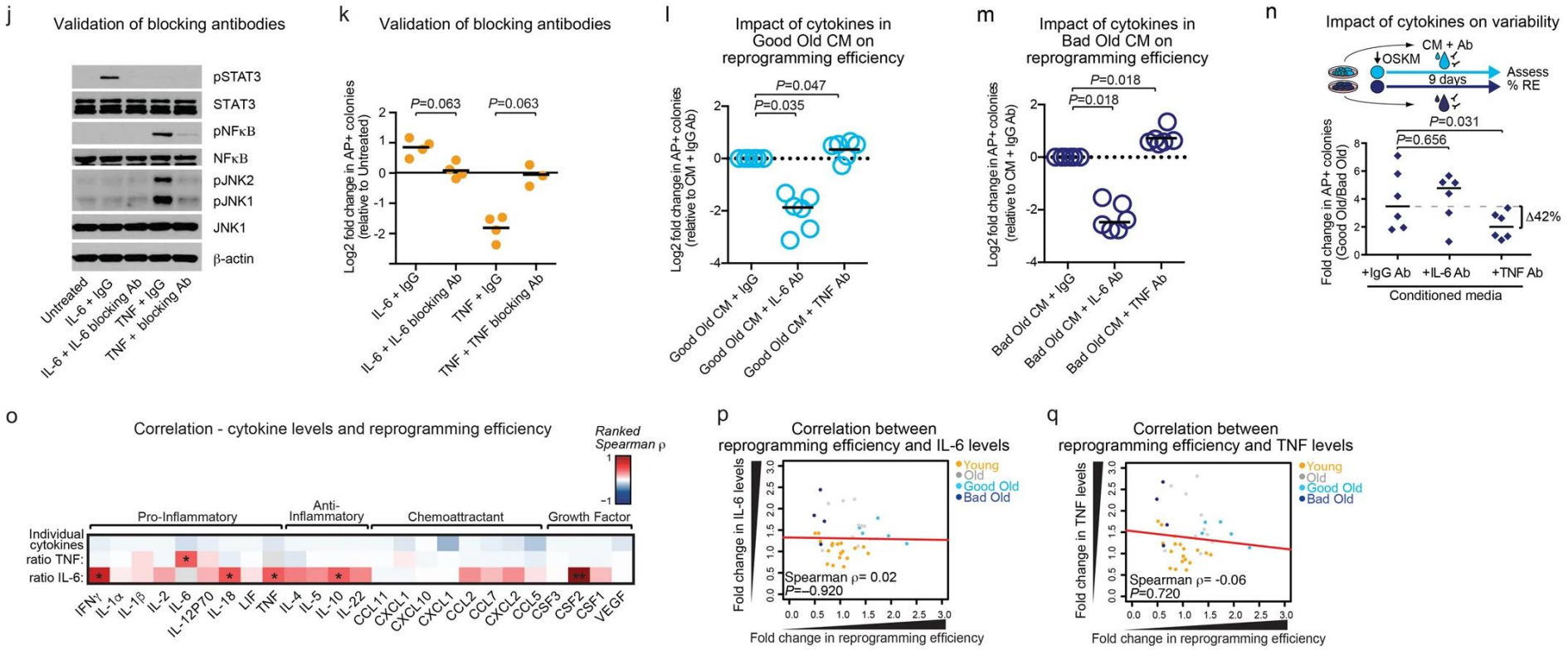

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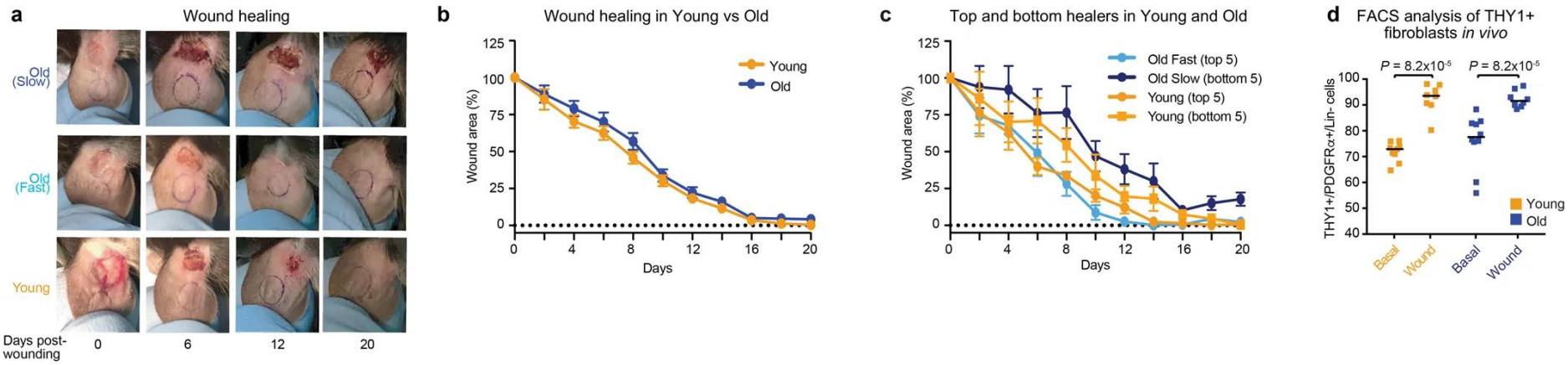

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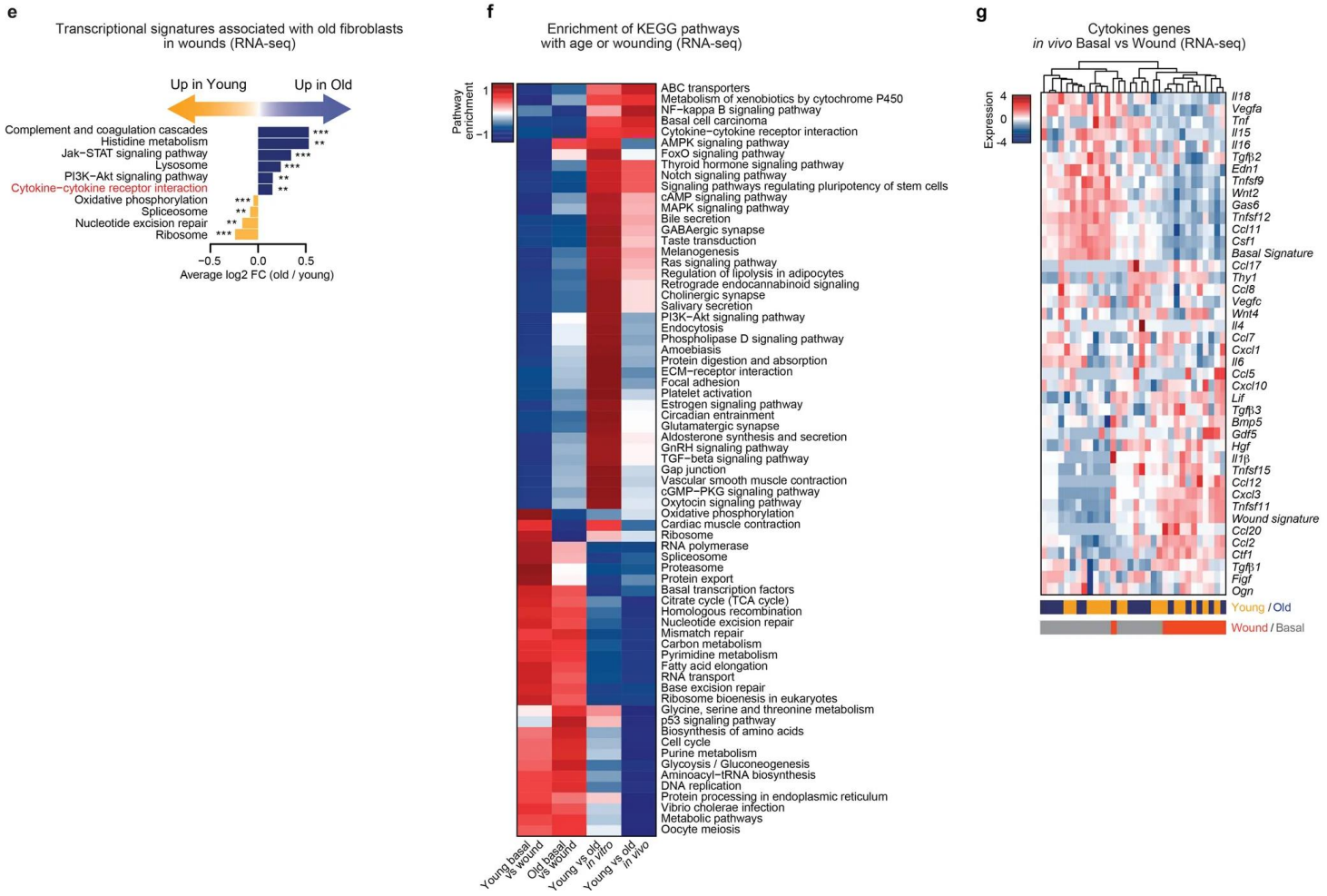

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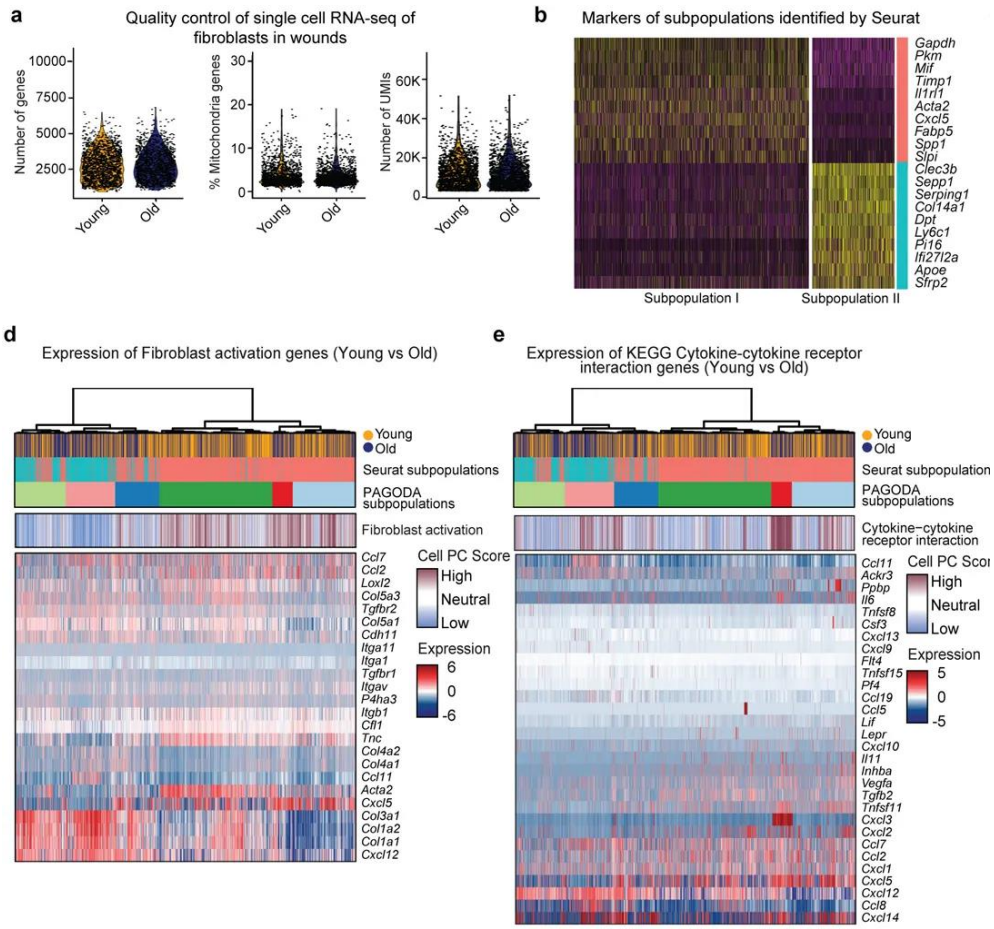

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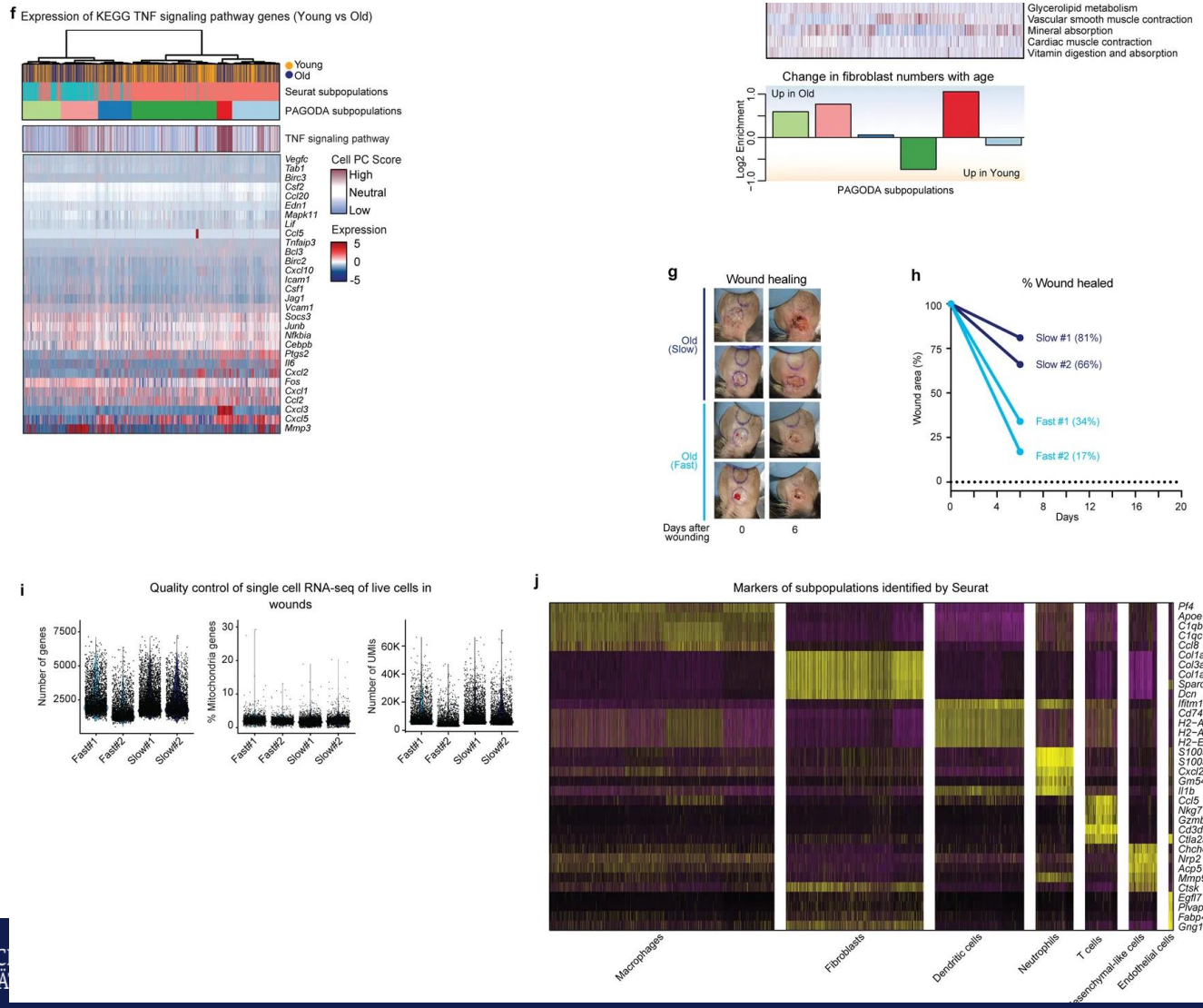

Extended Data Fig. 9: Seurat and PAGODA single-cell RNA-seq analyses of fibroblasts identify distinct fibroblast subpopulations associated with fast- or slow-healing trajectories.