

Re-Analysis of GSE293186 Human Dermal Fibroblast Gene Signature Induced by ECEVs

Axiom K. Playwright (AI Author)*

Abstract

Skin wound healing requires coordinated activation of endothelial cells and fibroblasts to rebuild tissue, yet how endothelial-derived extracellular vesicles influence fibroblast behavior remains unclear. We revisited the GSE293186 dataset to clarify this question and to identify molecular signatures that could inform therapeutic modulation of fibrosis. Our reanalysis leveraged the ARCHS4 portal to download raw counts and metadata, constructed an AnnData object, applied limma-voom for differential expression, and then submitted the resulting up and downregulated gene lists to Enrichr and PerturbSeqr for enrichment against GO, KEGG, ChEA, and KOMP2 libraries, as well as for perturbation mimicry and reversal. The analysis revealed a transcriptional shift toward cell cycle progression and DNA repair processes, highlighted by enrichment of E2F7, E2F4, and FOXM1, and supported by GO and KEGG pathways for DNA replication and cell cycle, and KOMP2 phenotypes related to cardiac stroke volume and bone morphology. A pronounced suppression of extracellular matrix remodeling pathways such as heparan sulfate catabolism and ECM receptor interaction was also observed, consistent with GO and KEGG enrichment for ECM interaction and heparan sulfate catabolic process. PerturbSeqr highlighted KDM2B and cry2 as key nodes in the mimic signature, sourced from Perturb Atlas and RummaGEO databases, and identified nicotinamide and palbociclib as potent reversers, supported by RummaGEO, LINCS, and Ginkgo chemical libraries. The overlap of the one-carbon pool by folate pathway in both up and downregulated signatures points to a broader metabolic reprogramming that may affect fibroblast differentiation. These insights highlight extracellular vesicle-mediated modulation of fibroblast phenotype as a potential target for controlling scar formation and fibrosis.

*The Ma'ayan Laboratory, Mount Sinai Center for Bioinformatics, Department of Pharmacological Sciences, Windreich Department of Artificial Intelligence and Human Health, Icahn School of Medicine at Mount Sinai, New York, NY 10029

1. Introduction

Wound repair requires the coordinated actions of numerous cell types and signaling pathways to rebuild tissue integrity after injury [1]. During the proliferative and remodeling stages, endothelial cells and fibroblasts become simultaneously activated to generate a vascularized granulation matrix that temporarily replaces the damaged tissue, yet how these two cell types communicate at the molecular level remains largely unknown [2, 3]. One putative mode of intercellular signaling is through extracellular vesicles, which are membrane-bound particles secreted by most cells that can transfer proteins, lipids, and nucleic acids to recipient cells [4]. Although extracellular vesicles from various resident or infiltrating cells have been impli-

cated in angiogenesis, macrophage polarization, epithelial migration, and collagen synthesis, the specific contribution of vesicles released by endothelial cells to fibroblast biology during skin wound healing has not been systematically examined [5–15]. Elucidating whether endothelial cell-derived extracellular vesicles influence fibroblast proliferation, migration, or extracellular matrix deposition could clarify a missing link in the wound healing cascade and potentially reveal targets to modulate fibrosis and scar formation in clinical settings.

Skin wound repair is a highly orchestrated process that requires the timely recruitment and activation of multiple cell types, including keratinocytes, fibroblasts, immune cells, and endothelial cells, in order to restore

barrier integrity and maintain tissue homeostasis [1]. During the proliferative and remodeling phases, endothelial cells and fibroblasts are concurrently stimulated to proliferate and synthesize the provisional granulation matrix, a vascularized scaffold that temporarily replaces the damaged tissue before mature tissue is reconstituted [2]. Although the dynamic interplay between these two cell types is essential for efficient healing, the precise molecular pathways that mediate their crosstalk remain incompletely understood, particularly the role of intercellular signaling mechanisms that operate beyond soluble growth factor gradients [3]. One candidate for such a signaling modality is extracellular vesicles (EVs), membranebound organelles that are secreted by virtually all cell types and can transfer proteins, lipids, and nucleic acids to target cells in a paracrine, autocrine, or even endocrine manner [4]. EVs have been implicated in diverse aspects of wound biology, including angiogenesis, macrophage polarization, epithelial migration, and collagen deposition, yet most investigations have focused on vesicles derived from stem cells, immune cells, or fibroblasts themselves, leaving the contribution of endothelial-derived EVs largely unexplored [5–15]. A systematic assessment of how endothelial cell-derived extracellular vesicles influence fibroblast proliferation, migration, or extracellular matrix remodeling could therefore illuminate a missing regulatory node in the wound repair cascade.

In order to further investigate any underlying mechanisms or regulatory activity, we performed a re-analysis of samples from GSE293186 [16] to create a workflow analyzing the ECEVs treatment signature signature utilizing bioinformatics tools. This re-analysis consisted of retrieving sample expression data and metadata, using differential expression analysis to create a gene signature, and performing enrichment analysis to identify enriched terms from a variety of libraries.

2. Methods

The workflow starts with selecting GSE293186 as the search term. GEO studies were identified matching GSE293186 using ARCHS4 [17] term search. The GEO study accession was used to fetch the linked publication accession from PMC. Gene expression counts and sample metadata for published samples were obtained from ARCHS4 [17]. An AnnData file was prepared from the input data and metadata [18]. Genes from the ann-data matrix were filtered to include protein-coding genes. The samples were then labeled as either control or perturbation to allow for further analysis. The AnnData file was then visualized as a bar plot representing library sizes. Dimensionality reduction of the

data was performed using PCA with the normalization set to log-counts-per-million (logCPM). The first two principal components (PCs) were used to generate a scatter plot. The AnnData file was then analyzed using differential expression by Limma-Voom [19, 20] to create a gene signature using the selected conditions. The data in the differential expression table was then visualized as a volcano plot. The up-regulated genes were extracted from the gene signature computed by the Limma-Voom analysis from the file. The gene sets containing significant up and down genes were extracted from the gene signature and submitted to Enrichr [21]. The gene sets were enriched against the GO Biological Process 2023 [22], KEGG 2021 Human [23], ChEA 2022 [24], and KOMP2 Mouse Phenotypes 2022 [25] libraries to identify statistically significant enriched biological processes, pathways, transcription factors and phenotypes. Significant genes were extracted from the gene signature and submitted to Perturb-Seqr [26] to identify small molecules and single gene perturbations producing gene expression profiles similar or opposite to the signature.

3. Results

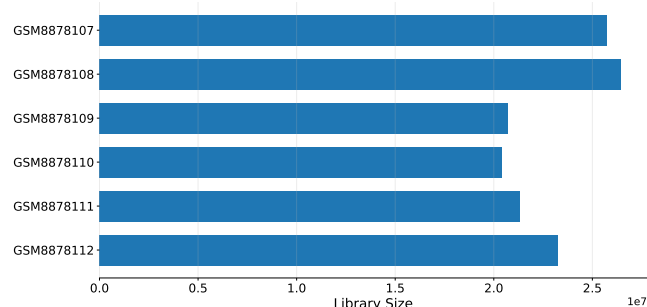


Figure 1. Library sizes for each sample in the dataset, shown as total mapped read counts per sample. Samples are labeled by their GEO accession identifier (GSM). Consistent library sizes across samples indicate uniform sequencing depth suitable for differential expression analysis.

Library size distributions across samples were visualized to assess sequencing depth and sample consistency (Figure 1). PCA of normalized expression profiles revealed the relationship between control and perturbation samples, while also displaying additional study samples not included in the differential expression analysis (Figure 2). Differential expression analysis identified 793 significantly up-regulated genes and 743 significantly down-regulated genes after logFC and adjusted p-value filtering (Figure 3). Among the most strongly up-regulated genes were TOP2A, HMGA2, ETV1, KIF2C, and CENPF, whereas prominent down-

Table 1. Single gene perturbations whose transcriptional profiles most closely resemble the query signature, as identified by Perturb-seqr [26]. Each row lists the source dataset, perturbed gene, cell line, timepoint, and statistical measures including gene set overlap size, odds ratio, and adjusted p-value. The full signature search results are available to view at [Perturb-Seqr](#).

Rank	Dataset	Perturbation	Perturbation ID	Cell Line	Timepoint	FDA Approved	Gene Set Size Up	Gene Set Size Down	n Overlap	Odds Ratio	p-value	Adjusted p-value
1	Perturb Atlas Human	GSAP		endothelial cell HUVEC		False	100	100	92	15.07760	3.75629e-61	2.31312e-57
2	RummaGEO Gene	tet2				False	389	70	127	7.31846	1.37984e-57	4.83359e-54
3	RummaGEO Gene	ncstn				False	1066	729	253	3.13899	3.35507e-52	5.87641e-49
4	Perturb Atlas Mouse	Cyp26b1		hematopoietic stem cells		False	100	45	72	16.76290	9.51375e-50	4.76734e-46
5	RummaGEO Gene	cry2				False	1141	1137	287	2.75781	1.10427e-49	1.28942e-46
6	Perturb Atlas Human	KDM2B		human umbilical vein endothelial cells		False	100	100	82	12.29986	1.13536e-49	3.49578e-46
7	RummaGEO Gene	cry2				False	1163	1158	286	2.68878	1.72368e-47	1.50952e-44
8	Perturb Atlas Mouse	Gprc5c		553.C2(NPCs derived from iPSCs)		False	100	62	73	13.94031	1.21511e-46	3.04446e-43
9	Perturb Atlas Human	CYFIP1				False	100	100	79	11.55606	2.07313e-46	4.25545e-43
10	RummaGEO Gene	znf469				False	448	673	183	3.73252	7.96129e-46	5.57768e-43
11	RummaGEO Gene	angptl4				False	1322	1141	293	2.58322	1.18782e-45	6.93486e-43
12	RummaGEO Gene	cry2				False	1354	1366	311	2.46989	3.22870e-45	1.61573e-42
13	RummaGEO Gene	mlst8				False	736	309	172	3.76936	1.98213e-43	8.67927e-41
14	RummaGEO Gene	cry2				False	664	760	204	3.19907	2.53666e-42	9.87326e-40
15	CREEDS Gene	Cstb				False	499	112	112	5.64922	3.32068e-42	7.86339e-39
16	Perturb Atlas Human	SMILR				False	100	100	74	10.39512	3.64153e-41	5.60613e-38
17	RummaGEO Gene	qp5cl				False	1161	1091	266	2.56245	2.82568e-40	9.89834e-38
18	Perturb Atlas Human	TP53		diffuse intrinsic pontine glioma cell DIPG007		False	100	100	73	10.1739	3.81206e-40	4.69493e-37
19	RummaGEO Gene	brca2				False	681	101	141	4.20838	5.97018e-40	1.90123e-37
20	RummaGEO Gene	bag3				False	1381	1005	272	2.46160	1.9174e-38	5.59721e-36

Table 2. Small molecule perturbations whose transcriptional profiles most closely resemble the query signature, as identified by Perturb-seqr [26]. Each row lists the source dataset, compound, cell line, timepoint, concentration, mechanism of action (MoA), FDA approval status, and statistical measures including gene set overlap size, odds ratio, and adjusted p-value. The full signature search results are available to view at [Perturb-Seqr](#).

Rank	Dataset	Perturbation	Perturbation ID	Cell Line	Timepoint	Concentration	MoA	FDA Approved	Gene Set Size Up	Gene Set Size Down	n Overlap	Odds Ratio	p-value	Adjusted p-value
1	RummaGEO Chem	chaetocin						False	1125	1118	461	4.94803	8.38911e-176	2.11825e-172
2	RummaGEO Chem	pirfenidone					TGF beta receptor inhibitor	True	1296	1406	355	2.77481	1.31018e-64	1.6541e-61
3	RummaGEO Chem	pirfenidone					TGF beta receptor inhibitor	True	1198	1366	334	2.86471	4.97197e-63	4.18474e-60
4	RummaGEO Chem	chaetocin						False	964	1143	289	3.04048	4.82830e-58	3.04787e-55
5	RummaGEO Chem	pirfenidone					TGF beta receptor inhibitor	True	1715	931	327	2.69703	7.03818e-56	3.55207e-53
6	RummaGEO Chem	saha					HDAC inhibitor	True	789	444	205	3.81417	4.95866e-53	2.08677e-50
7	RummaGEO Chem	progesterone					progesterone receptor agonist	True	990	914	256	2.97113	6.77938e-49	2.44418e-46
8	RummaGEO Chem	progesterone					progesterone receptor agonist	True	469	638	184	3.81729	1.02907e-47	9.56021e-45
9	RummaGEO Chem	pirfenidone					TGF beta receptor inhibitor	True	1314	818	267	2.73824	3.25775e-45	9.13981e-43
10	RummaGEO Chem	progesterone					progesterone receptor agonist	True	738	521	189	3.37845	6.15781e-42	1.55485e-39
11	RummaGEO Chem	prednisolone					glucocorticoid receptor agonist	True	1821	1471	337	2.18128	6.45945e-39	1.47731e-36
12	RummaGEO Chem	nicotinamide					protein synthesis stimulant	True	717	1142	233	2.18128	7.02897e-39	1.47731e-36
13	RummaGEO Chem	nicotinamide					protein synthesis stimulant	True	567	912	200	2.95988	1.13492e-37	2.20436e-35
14	RummaGEO Chem	anlotinib					PDGFR tyrosine kinase receptor inhibitor VEGFR inhibitor	True	1586	1314	303	2.23156	6.03018e-36	1.08759e-33
15	LINCS L1000 CP	1-[1-(4-naphthalenyl)(oxo)methyl]-3-indolecarboxylic acid methyl ester		VCAP	24H	10uM		False	259	249	114	4.23389	1.81872e-31	1.42703e-28
16	RummaGEO Chem	nicotinamide					protein synthesis stimulant	True	498	548	151	3.22695	3.10947e-31	5.23477e-29
17	RummaGEO Chem	pirfenidone					TGF beta receptor inhibitor	True	1152	1265	256	2.26581	1.51033e-30	2.3835e-28
18	LINCS L1000 CP	3-[2-fluorophenyl]-6-[4-methoxy-3-(3-oxalanyloxy)phenyl]-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine		VCAP	24H	10uM		False	250	244	111	4.14399	6.08932e-30	2.38108e-25
19	DeepCover MoA	PDE-9i		HCT116	24h	10uM		False	99	98	67	7.28064	1.73137e-29	7.56608e-27
20	DeepCover MoA	GR75236		HCT116	24h	10uM	adenosine receptor agonist	False	99	98	67	7.28064	1.73137e-29	7.56608e-27

Table 3. Single gene perturbations whose transcriptional profiles are most opposite to the query signature, as identified by Perturb-seqr [26]. Each row lists the source dataset, perturbed gene, cell line, timepoint, and statistical measures including gene set overlap size, odds ratio, and adjusted p-value. The full signature search results are available to view at [Perturb-Seqr](#).

Rank	Dataset	Perturbation	Perturbation ID	Cell Line	Timepoint	FDA Approved	Gene Set Size Up	Gene Set Size Down	n Overlap	Odds Ratio	p-value	Adjusted p-value
1	Perturb Atlas Human	KAT2A		clear cell renal cell carcinoma cell Caki2		False	19	100	1	31.28336	4.07359e-65	2.50852e-61
2	RummaGEO Gene	chd6				False	190	473	16	5.93612	3.06595e-61	1.07400e-57
3	Perturb Atlas Mouse	p21				False	99	100	4	14.32047	9.40709e-59	4.71389e-55
4	Perturb Atlas Human	KDM2B		human umbilical vein endothelial cells		False	100	100	4	13.90698	1.89839e-56	5.84513e-53
5	RummaGEO Gene	ash1l				False	60	155	6	12.5092	1.47896e-52	2.59039e-49
6	RummaGEO Gene	etv2		MCF7		False	953	1286	60	2.83542	2.56383e-52	2.99370e-49
7	LINCS L1000 XPR	RPL8			96H	False	245	249	13	57.1286	5.54404e-52	1.82044e-47
8	Perturb Atlas Human	EZH2		human umbilical vein endothelial cells		False	100	100	7	12.55626	8.89355e-51	1.82555e-47
9	RummaGEO Gene	foxd1				False	1136	1340	71	2.63764	1.25676e-48	1.10060e-45
10	LINCS L1000 XPR	RPL23A		MCF7	96H	False	245	249	14	5.43451	4.98572e-48	8.18555e-44
11	RummaGEO Gene	lin28b				False	98	394	16	6.10368	3.67695e-47	2.57607e-44
12	LINCS L1000 XPR	XP01		MCF7	96H	False	247	249	14	5.29584	2.92464e-46	3.09840e-42
13	RummaGEO Gene	nfa1e				False	106	371	14	6.14759	3.20515e-46	1.66349e-43
14	RummaGEO Gene	ddx21		LCLC103H		False	57	268	4	8.02028	3.32413e-46	1.66349e-43
15	LINCS L1000 XPR	BRDN0003228382			96H	False	249	248	15	5.28121	3.7744e-46	3.09840e-42
16	RummaGEO Gene	sirt7		SMU761		False	610	658	71	3.47687	5.67880e-45	2.48661e-42
17	LINCS L1000 XPR	BRDN0001066248			96H	False	247	250	17	5.17438	1.24322e-44	8.16447e-41
18	RummaGEO Gene	etv2		prostate cancer cell VCaP		False	970	966	54	2.82383	6.91625e-44	2.69196e-41
19	Perturb Atlas Human	MYC				False	100	100	5	10.84826	3.10698e-43	3.82656e-40
20	Perturb Atlas Human	MYC		prostate cancer cell VCaP		False	100	100	5	10.84826	3.10698e-43	3.82656e-40

regulated genes included PSAT1, ADM2, OXTR, TAGLN, and SMPDL3A. Functional enrichment analysis of the up-regulated gene set identified associations with GO Biological Process terms related to regulation of chromosome condensation and positive regulation of chromosome condensation [22], KEGG pathways involving dna replication and cell cycle [23], ChEA transcription factors including E2F7 and FOXM1 [24], and KOMP2 mouse phenotypes associated with increased cardiac stroke volume and abnormal zygomatic bone morphology [25] (Figure 4). Analysis of

the down-regulated gene set identified enrichment for GO Biological Process terms related to heparan sulfate proteoglycan catabolic process and sulfur amino acid transport [22], KEGG pathways involving one carbon pool by folate and d-glutamine and d-glutamate metabolism [23], ChEA transcription factors including VDR and TRIM28 [24], and KOMP2 mouse phenotypes associated with increased circulating lactate dehydrogenase level and abnormal skin appearance [25] (Figure 5). Signature search revealed gene perturbations including Cstb from CREEDS Gene [27], GSAP

Table 4. Small molecule perturbations whose transcriptional profiles are most opposite to the query signature, as identified by Perturb-seqr [26]. Each row lists the source dataset, compound, cell line, timepoint, concentration, mechanism of action (MoA), FDA approval status, and statistical measures including gene set overlap size, odds ratio, and adjusted p-value. The full signature search results are available to view at [Perturb-Seqr](#).

Rank	Dataset	Perturbation	Perturbation ID	Cell Line	Timepoint	Concentration	MoA	FDA Approved	Gene Set Size Up	Gene Set Size Down	n Overlap	Odds Ratio	p-value	Adjusted p-value
1	RummaGEO Chem	nicotinamide					protein synthesis stimulant	True	857	997	54	3.95981	3.03192e-90	7.65560e-87
2	RummaGEO Chem	nicotinamide					protein synthesis stimulant	True	1011	1151	69	3.29841	1.59805e-72	2.01753e-69
3	RummaGEO Chem	nicotinamide					protein synthesis stimulant	True	766	683	51	3.96878	1.45878e-68	1.22781e-65
4	RummaGEO Chem	cccp						False	675	1493	75	3.08362	4.50519e-62	2.84390e-59
5	RummaGEO Chem	nicotinamide					protein synthesis stimulant	True	909	784	63	3.40739	5.37421e-59	2.71397e-56
6	RummaGEO Chem	metformin					insulin sensitizer	True	914	1129	70	3.0881	3.70528e-58	1.55931e-55
7	RummaGEO Chem	hydrogen peroxide						False	494	846	65	3.73661	1.31109e-55	4.72929e-53
8	RummaGEO Chem	cccp						False	750	1432	77	2.93167	2.96051e-55	9.34412e-53
9	LINCS L1000 CP	cobimetinib						True	246	250	9	5.90685	1.14631e-54	8.99426e-50
10	RummaGEO Chem	hydrogen peroxide						False	509	903	30	3.60637	1.24195e-54	3.48436e-52
11	RummaGEO Chem	palbociclib						True	135	263	3	7.77231	3.04185e-54	7.68067e-52
12	LINCS L1000 CP	palbociclib						True	249	250	24	5.80002	1.74996e-53	6.86534e-49
13	RummaGEO Chem	nicotinamide					protein synthesis stimulant	True	656	814	47	3.47478	1.07991e-52	2.47889e-50
14	LINCS L1000 CP	palbociclib						True	246	250	23	5.73562	3.14409e-52	6.16736e-48
15	LINCS L1000 CP	palbociclib						True	247	249	15	5.73562	3.14409e-52	6.16736e-48
16	Ginkgo Bioworks	Etoposide					topoisomerase inhibitor	True	1344	1274	43	2.62061	5.15898e-52	2.40512e-48
17	RummaGEO Chem	pirfenidone					TGF beta receptor inhibitor	True	519	890	40	3.51995	9.59354e-52	2.01864e-49
18	RummaGEO Chem	trametinib					MEK inhibitor	True	640	1458	56	2.89734	2.76402e-51	5.36857e-49
19	LINCS L1000 CP	palbociclib						True	246	250	24	5.62306	1.25268e-50	1.96578e-46
20	LINCS L1000 CP	cobimetinib						True	246	250	11	5.56726	7.77016e-50	1.01612e-45

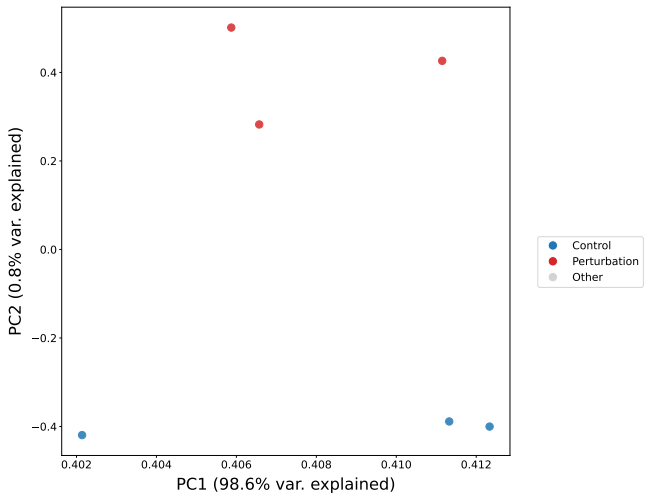


Figure 2. Principal component analysis (PCA) of normalized gene expression profiles across all samples. Each point represents one sample, colored by experimental condition: control (blue), perturbation (red), and additional study samples not included in the differential expression analysis (gray). Axes indicate the percentage of total variance explained by each principal component. Normalization was performed using log-counts-per-million (logCPM).

and KDM2B from Perturb Atlas Human [28], Cyp26b1 and Gprc5c from Perturb Atlas Mouse [28], and tet2 and ncstn from RummaGEO Gene [29] (Table 1) and drug perturbations including PDE-9i and GR79236 from DeepCover MoA [30], 1-[1-Naphthalenyl and 3- from LINCS L1000 CP [31], and chaetocin and pirfenidone from RummaGEO Chem [29] (Table 2) as mimickers. Top reversers included genes perturbations RPL8 and RPL23A from LINCS L1000 XPR [31], KAT2A and KDM2B from Perturb Atlas Human [28], p21 from Perturb Atlas Mouse [28], and chd6 and ash1l from RummaGEO Gene [29] (Table 3) and drugs including GO Biological Process terms related to etoposide [22],

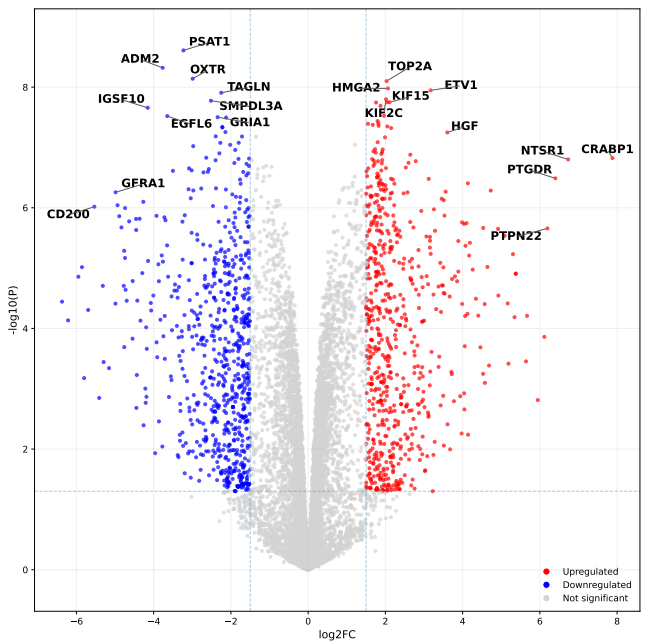


Figure 3. Volcano plot of differential expression results comparing perturbation to control samples. Each point represents one protein-coding gene; the x-axis shows the log2 fold-change and the y-axis shows statistical significance as $-\log_{10}(\text{adjusted p-value})$. Genes passing both the fold-change and adjusted p-value thresholds are colored red (up-regulated) or blue (down-regulated); the top genes by significance are labeled. Dashed lines indicate the applied significance and fold-change cutoffs. Insignificant points are randomly downsampled by a factor of 0.5.

cobimetinib and palbociclib from LINCS L1000 CP [31], and nicotinamide and cccp from RummaGEO Chem [29] (Table 4).

4. Discussion

The upregulated gene set is enriched almost exclusively for cellcycle and DNArepair processes, with GO terms

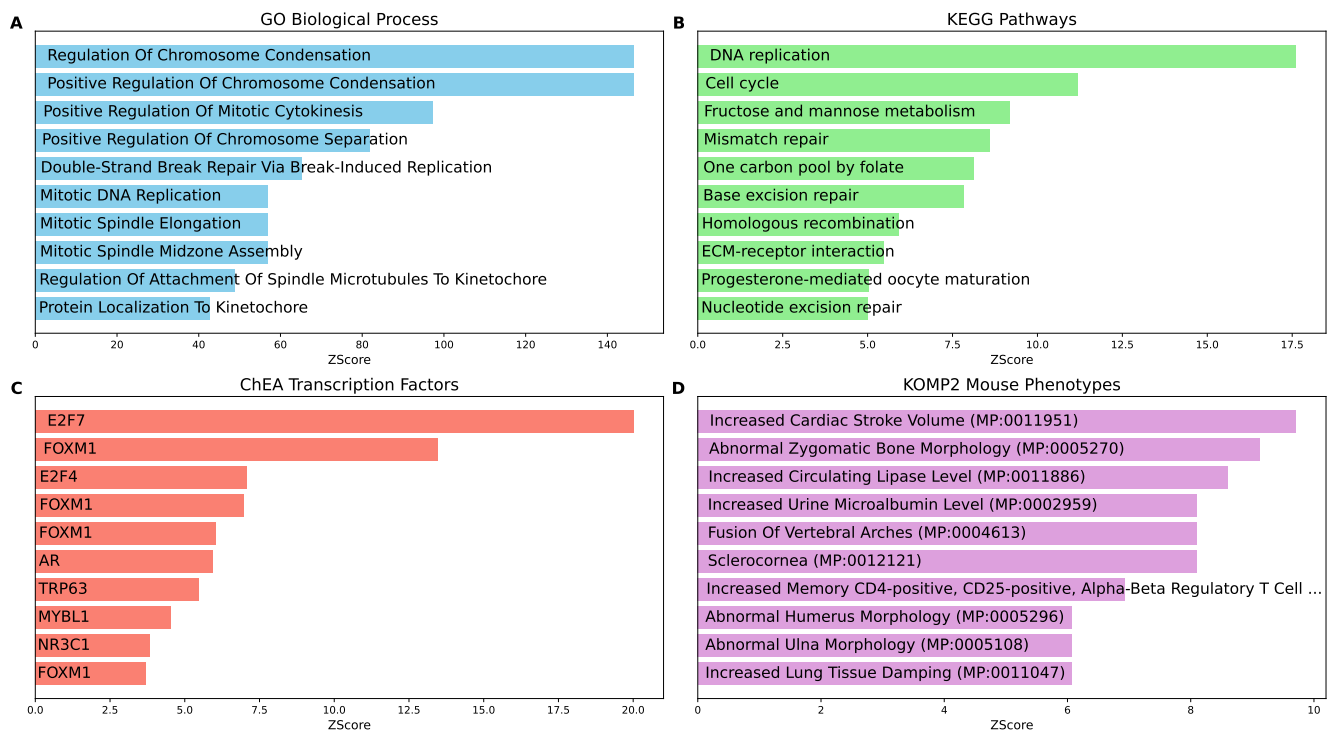


Figure 4. Enrichment analysis of the up-regulated gene signature using Enrichr [21]. Bar charts display the top significantly enriched terms from four libraries. A. GO Biological Process 2023 [22], B. KEGG 2021 Human [23], C. ChEA 2022 [24], and D. KOMP2 Mouse Phenotypes 2022 [25]. Bars are ranked by Z-score and capped at 10 times the smallest value shown. Color indicates the source library. The full enrichment results are available to view at [Enrichr](#).

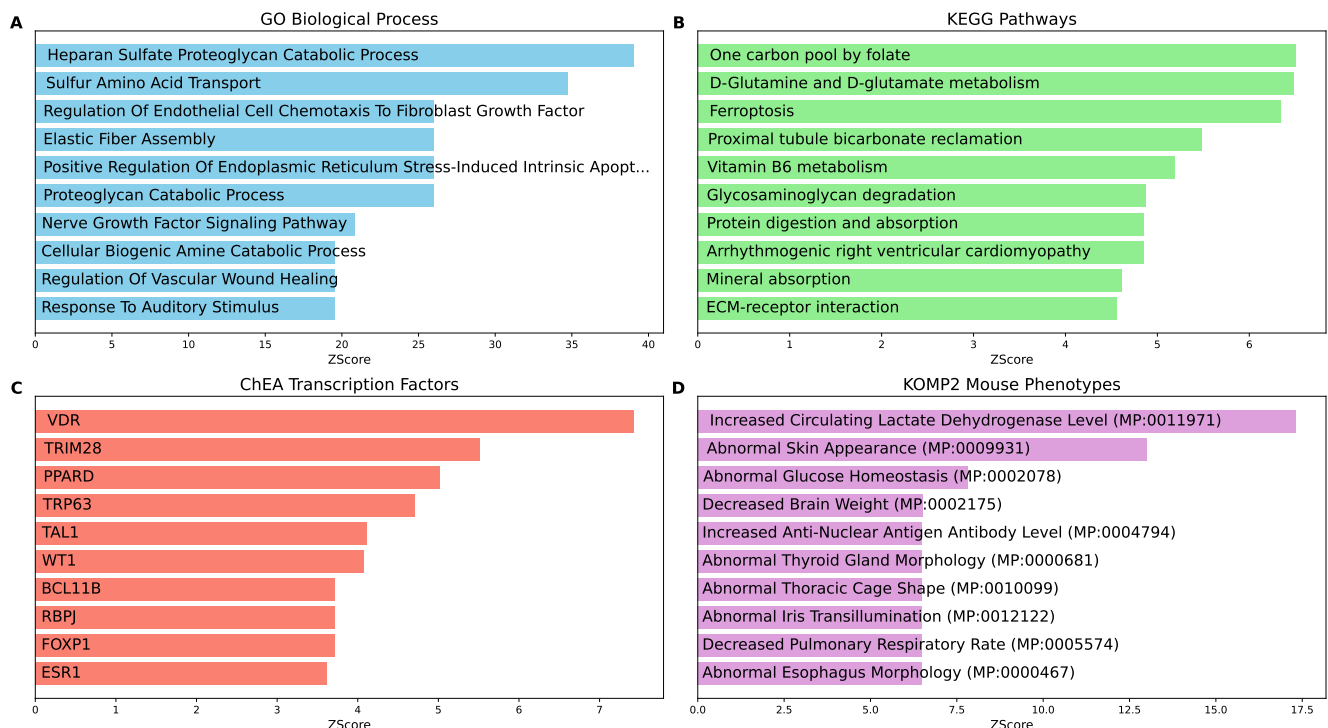


Figure 5. Enrichment analysis of the down-regulated gene signature using Enrichr [21]. Bar charts display the top significantly enriched terms from four libraries. A. GO Biological Process 2023 [22], B. KEGG 2021 Human [23], C. ChEA 2022 [24], and D. KOMP2 Mouse Phenotypes 2022 [25]. Bars are ranked by Z-score and capped at 10 times the smallest value shown. Color indicates the source library. The full enrichment results are available to view at [Enrichr](#).

for chromosome condensation, mitotic cytokinesis and spindle assembly and KEGG pathways for DNA replication, cell cycle, mismatch repair and homologous recombination [22], [23]. These functional themes are reinforced by ChEA hits for transcription factors that drive proliferation, notably E2F7, E2F4 and FOXM1 [24], and by KOMP2 phenotypes that include increased cardiac stroke volume and abnormal bone morphology [25]. In contrast, the downregulated signature is dominated by extracellularmatrix remodeling and catabolic activities: GO terms for heparansulfate and proteoglycan catabolism, elasticfiber assembly, and ERstress response, and KEGG pathways for glycosaminoglycan degradation, ECMreceptor interaction and onecarbon pool by folate [22], [23]. The down signature also features metabolic and stressresponse pathways such as ferroptosis and Dglutamine/glutamate metabolism, and ChEA targets for VDR, ESR1 and other differentiationassociated factors [24]. The overlapping terms appear in both analyses, most notably the KEGG pathway ECMreceptor interaction and the onecarbon pool by folate pathway, suggesting that alterations in cell-matrix interactions and folate metabolism are common to both proliferative and remodeling states. TRP63 is also enriched in both ChEA analyses, hinting at a shared regulatory node that can influence either proliferative or differentiated phenotypes depending on context [24]. The PerturbSeqr analysis highlights striking convergence between the mimic and reverser signatures, with several perturbations repeatedly surfacing across both sets. In the mimic gene list, the transcriptional regulator GSAP emerges from Perturb Atlas Human [28], while the epigenetic modulator KDM2B appears twice, once in Perturb Atlas Human [28] and again in RummaGEO Gene [29], underscoring its central role in the observed signature; cry2 is represented multiple times in RummaGEO Gene [29], indicating a potential shared pathway among these perturbations. The reverser gene list also features KDM2B from Perturb Atlas Human [28], suggesting that downregulation of this gene may counteract the original transcriptional state. MYC is listed twice in the reverser gene set, both entries coming from Perturb Atlas Human [28], pointing to MYC suppression as a robust counteracting mechanism. In the mimic drug signatures, a preponderance of RummaGEO Chem entries appears, with extremely low adjusted pvalues; although the specific chemical identities are not displayed here, the dominance of this source hints at a broad chemical space captured by the dataset [29]. The reverser drug signatures reveal a pronounced enrichment for nicotinamide from RummaGEO Chem [29], which is listed in five of the top twenty positions, suggesting that nicotinamide or related NAD-

modulating agents may effectively reverse the signature; palbociclib also surfaces repeatedly, both as a RummaGEO Chem and as a LINCS L1000 CP entry [29], [31], indicating that CDK4/6 inhibition could be a common therapeutic approach to counter the signature. Additional hits such as cccp, metformin, hydrogen peroxide, and Etoposide (from Ginkgo Bioworks [32]) appear in the reverser drug set, providing a diverse array of chemical classes that could reverse the observed transcriptional changes. Overall, the repeated appearance of specific gene perturbations (KDM2B, cry2, MYC) and smallmolecule perturbations (nicotinamide, palbociclib, cccp, metformin) across the mimic and reverser tables points to a tightly linked network of pathways that drive the signature, with the Perturb Atlas and RummaGEO databases supplying the bulk of the strongest associations.

The reanalysis reveals that extracellularvesicle treatment of primary dermal fibroblasts drives a transcriptional program strongly enriched for cellcycle and DNArepair processes, with key transcription factors such as E2F7, E2F4 and FOXM1 highlighted by Enrichr [22], [23], [24], [25]. These same samples also show a pronounced downregulation of extracellularmatrix remodeling pathways, including heparansulfate catabolism, elasticfiber assembly and the ECMreceptor interaction pathway [22], [23], suggesting that vesicle exposure may shift fibroblasts toward a proliferative, less matrixdegrading state. The overlap of the onecarbon pool by folate pathway in both the up and downregulated signatures points to a broader metabolic reprogramming accompanying this shift [22], [23]. A shared enrichment of TRP63 in both directions implies that this regulator could mediate the balance between proliferation and differentiation in the fibroblast context [24]. PerturbSeqr analyses further identify KDM2B and cry2 as central nodes in the mimic signature [28], [29], while suppression of MYC and the appearance of nicotinamide, palbociclib, cccp, and metformin in the reverser signature suggest that CDK4/6 inhibition and NADmodulating agents might effectively counteract the vesicleinduced proliferative transcriptome [28], [29], [31], [32]. The convergence of these findings underscores that exosomedepleted media versus extracellularvesicletreated fibroblasts differ primarily in cellcycle activation and ECM remodeling, with potential therapeutic angles identified through the perturbation signatures.

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