

AQTUAL

TURNING CHRONIC DISEASE TREATMENT INTO DATA-DRIVEN CARE

Introduction: clinical need for functional, tissue-informed liquid biopsy

Chronic immune-mediated diseases and cancers are driven by dynamic tissue-specific programs that are difficult to access longitudinally in routine clinical practice. Synovium, gut, skin, and tumor tissue biopsies are invasive, not easily repeatable, and often impractical across the full spectrum of chronic conditions. As a result, most clinical decisions still rely on serum proteins, autoantibodies, cross-sectional imaging, and oncology-focused tools such as tumor genotyping panels and circulating tumor DNA (ctDNA) mutation assays, all of which incompletely capture underlying disease activity, cell-type contribution, and tissue involvement.

Circulating cell-free DNA (cfDNA) has emerged as an attractive liquid biopsy analyte, with most current clinical and research assays targeting short nucleosomal fragments (cfDNA_{nuc}) to measure copy number, sequence variation, methylation, or coarse fragmentation patterns. These fragmentomic and methylation approaches provide valuable information on genome integrity, global chromatin state, and tissue-of-origin inference, but they are limited in capacity to capture regulatory DNA elements and do not directly report gene-level transcriptional activity. There is a clear need for a cfDNA technology that resolves both epigenomic and transcriptomic information at the gene and pathway level, with sufficient tissue resolution to support mechanism-anchored diagnostics, prognosis, and therapy selection across heterogeneous chronic diseases and oncologic indications.

Aqtual's technology

During apoptosis and other forms of regulated cell death, chromatin is fragmented and cleared through nuclease and phagocytic pathways, releasing multiple classes of cfDNA into the circulation (**Figure 1**). These include conventional nucleosomal fragments and longer fragments derived from regulatory, transcriptionally active chromatin (cfDNA_{ac}) that are protected from degradation by transcription factors and associated regulatory protein complexes. The genomic locations of cfDNA_{ac} fragments are enriched at promoters, gene bodies, enhancers, and insulators, thus retaining both epigenetic and transcription-coupled signatures of the dying cell.

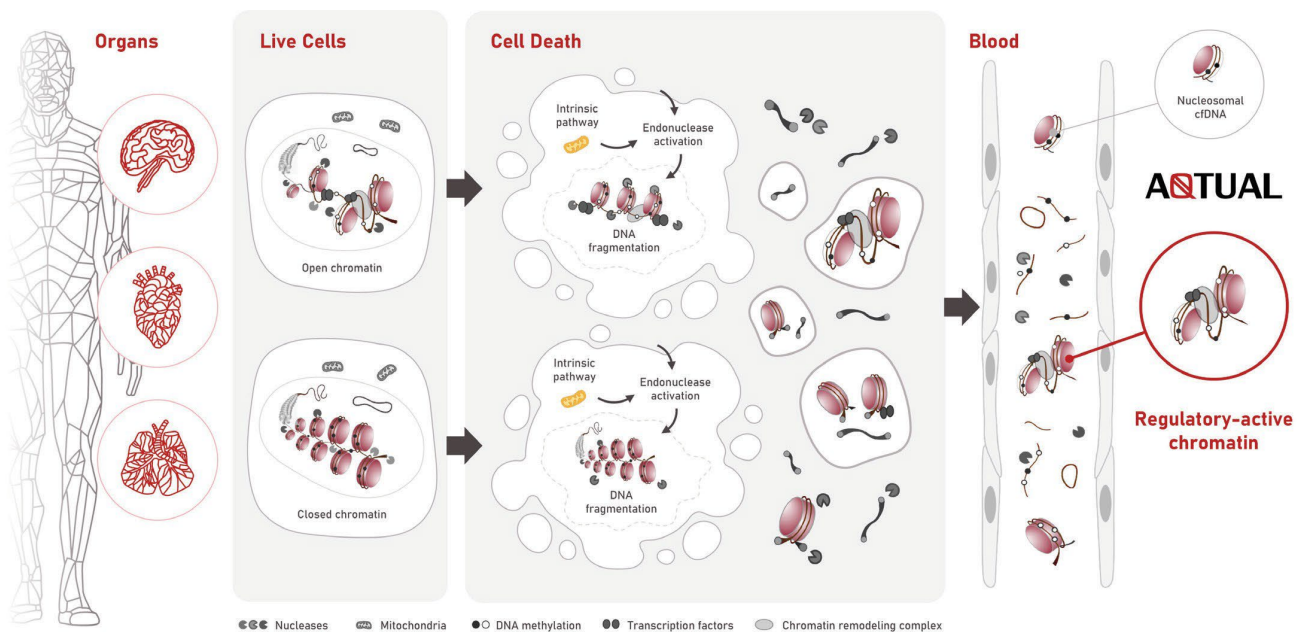


FIGURE 1. Cell death and subsequent release of cfDNA components into the blood. Living cells have different chromatin profiles (open or closed) which determine their gene expression profiles and, in turn, reflect their cell-type and the tissue from which they originated. Nucleic acid fragments enter the bloodstream upon apoptosis or necrosis. This circulating DNA is available for analysis and can provide valuable molecular information.

Aqtual's novel approach to cfDNA workflow

Traditional liquid biopsy workflows extract short cfDNA_{nuc} fragments with peak sizes to 167 bp and 334 bp, representing the mono- or di-nucleosome profile, respectively. In contrast, Aqtual's platform selectively enriches longer cfDNA fragments derived from active chromatin. (Figure 2). The workflow comprises plasma isolation, cfDNA_{ac} enrichment, library preparation and sequencing, followed by computational normalization and quantification of cfDNA_{ac} counts at predefined regulatory regions. This design explicitly targets regulatory protein-protected chromatin, yielding a cfDNA signal that integrates epigenomic state and transcriptional activity at each locus.

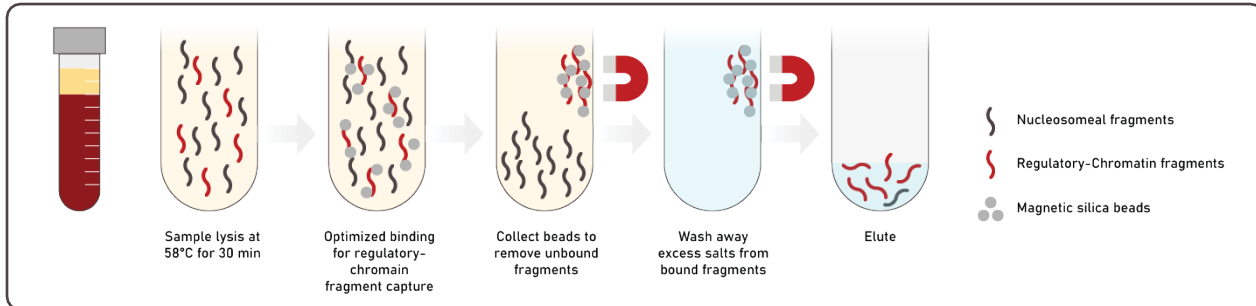


FIGURE 2. Schematic of chromatin capture workflow. Aqtual's proprietary sample processing workflow to enrich for longer cfDNA_{ac} fragments.

Reconstructing gene expression using robust cfDNA_{ac} signals

Aqtual's platform specifically enriches for regulatory-active chromatin (cfDNA_{ac}) that include both epigenetic and transcriptomic signals from promoters, gene bodies, enhancers, and insulators (Figure 3a). By capturing cfDNA_{ac} derived from promoters and gene bodies, Aqtual's platform generates an integrated signal of gene expression, offering a comprehensive molecular view of events both preceding gene expression (promoter) and during active gene expression (gene bodies).

Aqtual's normalized counts from a healthy cohort were mapped against the Genotype-Tissue Expression (GTEx) whole blood gene expression profiles to validate cfDNA_{ac} signals. The cfDNA_{ac} signals derived from gene bodies and promoters showed a strong correlation with whole-blood gene expression levels obtained from GTEx¹ (Figure 3b-c). Specifically, the results that the cfDNA_{ac} scores for the gene bodies and promoters closely matched the GTEx whole blood gene expression, as measured by transcript per million (TPM), with a 95% and 89% correlation, respectively (Figure 3b-c). The cfDNA_{ac} signals derived from the gene bodies also revealed a strong correlation of 85% with promoter cfDNA_{ac} signals (Figure 3d), consistent with the expectation that both promoters and gene bodies should be associated with gene expression.

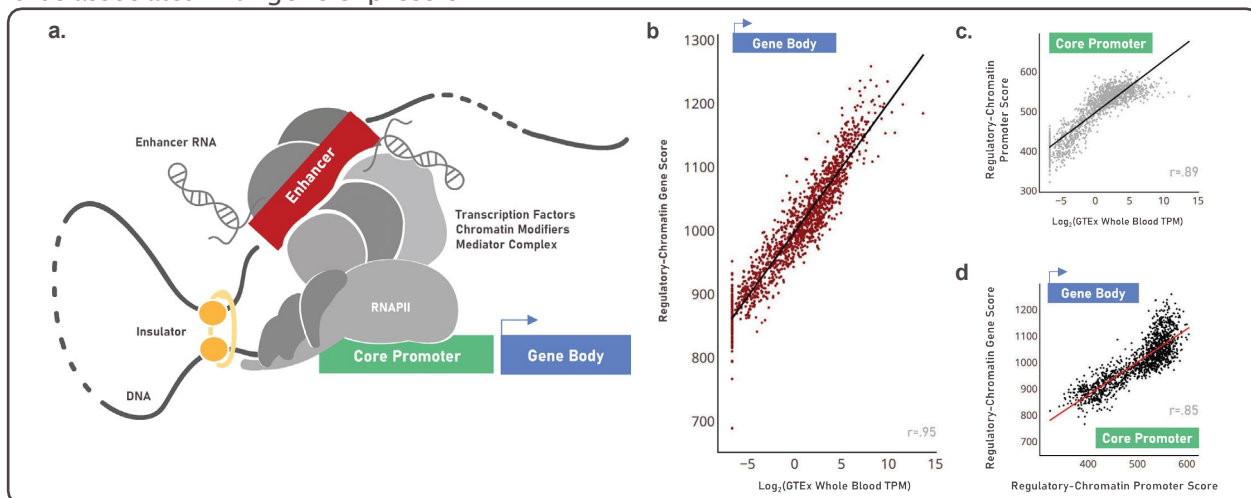


FIGURE 3. Regulatory-active chromatin fragments (cfDNA_{ac}) correlate with gene expression. **a.** Schematic of an active transcription complex. Highlighted are the regulatory regions, which are involved from the start through the end of transcription, captured by Aqtual's platform: insulator (yellow), enhancer (red), promoter (green) and gene body (blue). **b-c.** The cfDNA_{ac} signals from gene bodies and promoters compared to whole blood gene expression levels from GTEx. **d.** Correlation of cfDNA_{ac} signals from gene body and promoter regions.

Collectively, these results demonstrate the ability of Aqtual's platform to capture actively transcribed regions of the genome without the use of mRNA which can be used to provide insights into active gene expression.

Active chromatin fragments represent regulatory protein-protected regions of the chromatin

To confirm that Aqtual's assay is capturing bona fide regulatory chromatin rather than nucleosomal cell-free DNA, we compared cfDNA_{ac} fragment distributions with independent maps of active regulatory elements from the Encyclopedia of DNA Elements (ENCODE) and the Roadmap Epigenomics Consortium. The ENCODE/Roadmap efforts defined promoters, enhancers, and other regulatory regions using histone Chromatin Immunoprecipitation followed by Sequencing (ChIP-seq) and transcription factor binding profiles through an antibody-dependent process². The results shown in **Figure 4** indicate a clear overlap between cfDNA_{ac} signals and ENCODE mappings at the promoter regions (compare red regulatory-active chromatin track vs gray ENCODE track), while cfDNA_{nuc} fragments showed no enrichment (compare black nucleosomal track vs gray track).

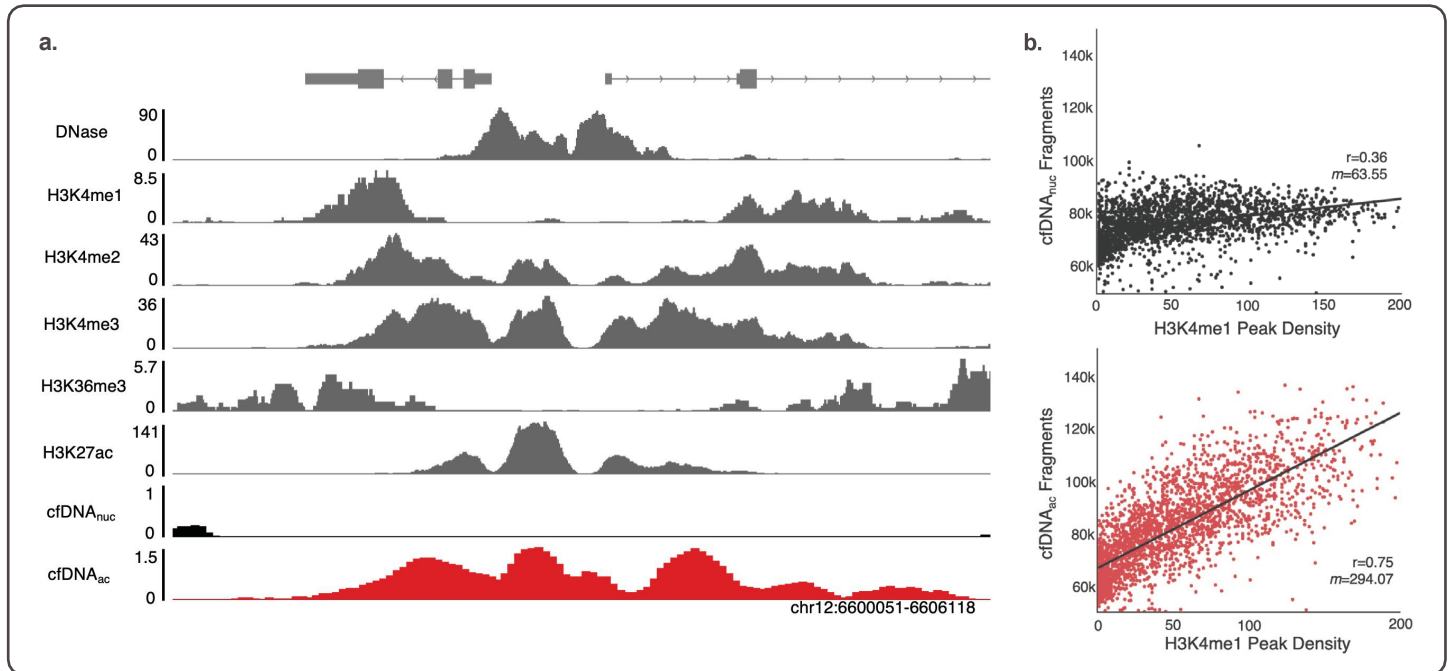


FIGURE 4. Genome browser view of regulatory-active chromatin signals at promoter regions of chromosome 12. **a.** Top tracks (gray) are signals for selected histone modifications obtained from the Roadmap Epigenomics Consortium, and the bottom tracks represent nucleosomal (black) and regulatory-active chromatin (red) signals. **b.** Pearson correlation comparing number of nucleosomal (black) or regulatory-active chromatin fragments (red) with the density signal of one histone modification (H3K4me1).

Genome browser views at representative loci on chromosome 12 (4a) illustrate this pattern: the cfDNA_{ac} tracks closely follow promoter-proximal histone marks such as H3K4me3 and H3K4me1 from Roadmap Epigenomics (gray tracks), while cfDNA_{nuc} remains relatively flat across the same regions (Figure 4a). At a genome-wide scale, the number of cfDNA_{ac} fragments within promoters correlates strongly with H3K4me1 peak density (Figure 4b), consistent with cfDNA_{ac} originating from protein-protected, open chromatin at active or poised regulatory elements, whereas nucleosomal fragments show minimal correlation. In addition to promoter overlap, the fragment characteristics of cfDNA_{ac} further support a regulatory, protein-protected origin. cfDNA_{ac} fragments are longer than typical mono-nucleosomal cfDNA_{nuc} fragments and exhibit positioning patterns centered on transcription factor and co-factor binding sites rather than on canonical nucleosome dyads. This is consistent with models in which regulatory complexes and histone modifications shield stretches of open chromatin from complete degradation during cell death, yielding a distinct footprint in circulation that preserves information about transcriptional activity and chromatin state.

Collectively, these orthogonal comparisons establish that Aqtual's assay is selectively sampling actively transcribed and regulatory chromatin in blood, recapitulating ChIP-defined promoter and enhancer landscapes without relying on RNA or antibody-based ChIP-seq.

Why Aqtual's platform differs from what is currently available

Most liquid biopsy platforms today are designed to isolate short DNA fragments collectively referred to as nucleosomal cfDNA (cfDNA_{nuc}), wrapped around the smallest unit of chromatin - nucleosome. Gene activity is then inferred indirectly from cfDNA_{nuc} using methylation signatures or coarse fragmentation features (**Figure 5**), which primarily report on nucleosome positioning and global chromatin state rather than directly quantifying transcription. These fragmentomics approaches can also support tissue-of-origin and cell-type inference, but their sensitivity and resolution are fundamentally limited compared with analyses that target regulatory, protein-protected active chromatin.

By contrast, platforms that aim to interrogate gene regulation at regulatory elements often rely on antibody-based chromatin immunoprecipitation followed by sequencing (ChIP-seq), an elaborate workflow that depends on a restricted set of known histone marks or transcription factors and again only infers gene expression from selected epigenetic features. In both cases—cfDNA_{nuc}-based methylation/fragmentation assays and ChIP-seq-based epigenomic profiling—the result is a partial view of gene regulation that lacks a direct, genome-wide, gene-resolved measurement of active transcriptional programs⁷.

Aqtual has the only liquid biopsy platform that provides a direct reconstruction of gene expression data without the use of RNA-seq. This platform has been validated against two orthogonal datasets — gene expression profiles from GTEx and epigenetic profiles from ENCODE.

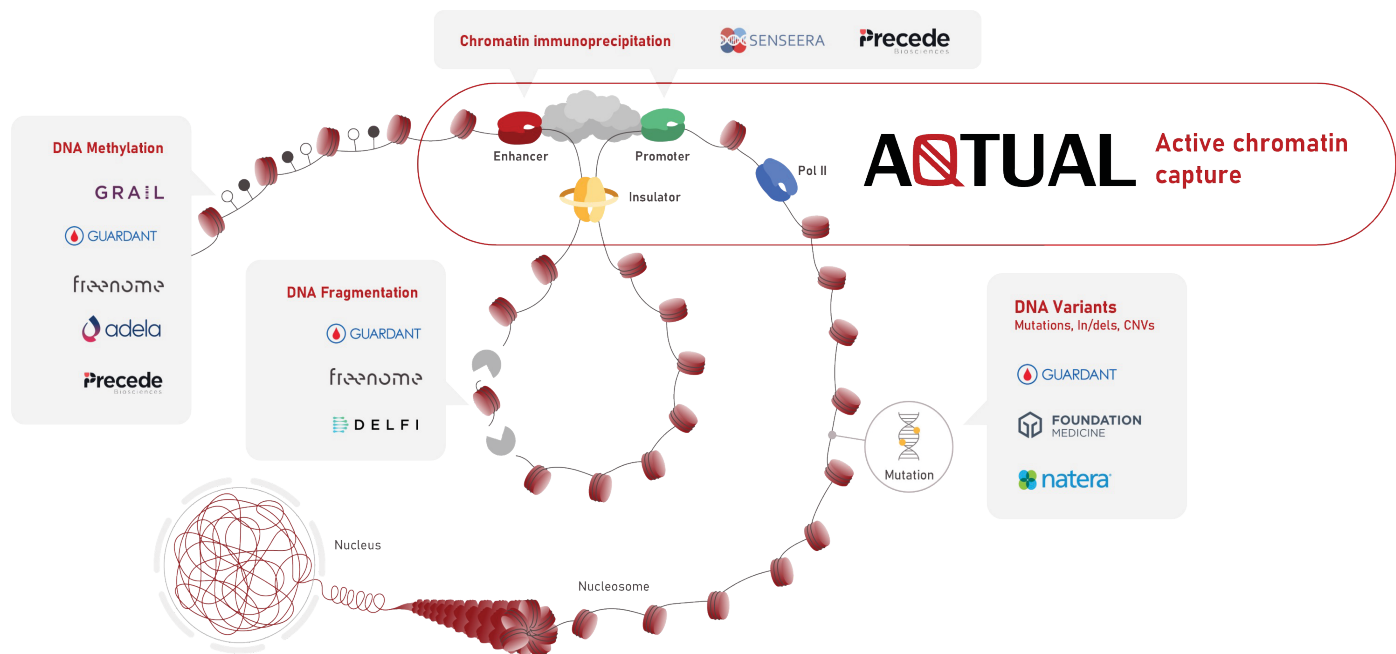


FIGURE 5. Different molecular signals are captured by various cfDNA platforms.

Demonstrating tissue-resolved disease signatures: initial application in rheumatoid arthritis (RA)

The initial application for Aqtual's platform has been in RA, where the integration of precision medicine into routine clinical practice remains limited^{3,6}. Early data show that Aqtual's non-invasive cfDNA_{ac} platform can identify synovium-specific gene expression signatures in the blood of patients with RA by detecting both organ-specific and systemic biological processes. Performance data were presented at an American College of Rheumatology (ACR) annual meeting^{8,9} demonstrating:

1. Aqtual's platform identified synovial gene expression signatures in patients with RA, marking the first blood-based detection of synovial signatures.
2. Aqtual's platform identified synovial signatures in patients with RA with an overall sensitivity of 90.8% and specificity of 96.1% (**Table 1**). Importantly, this performance was maintained in the seronegative population.
3. Aqtual's platform was able to differentiate RA patients from healthy controls and those with other conditions, including osteoarthritis and a range of inflammatory conditions, regardless of seropositivity

status (Table 1).

	OVERALL*	SERONEGATIVE	SPECIFICITY	
	Mean (SD)	Mean (SD)		Mean (SD)
AUC	0.991 (0.001)	0.971 (0.001)	Normal/Healthy	100% (0%)
Sensitivity, %	90.8 (0.94)	83.7 (2.03)	Ankylosing spondylitis	93.8% (3.44%)
Specificity, %	96.1 (0.64)	95.4 (0.69)	Crohn's Disease	100% (0%)
Positive Likelihood Ratio	24.13 (4.495)	21.46 (3.99)	Psoriasis	91.1% (3.04%)
Negative Likelihood Ratio	0.10 (0.001)	0.17(0.002)	Psoriatic arthritis	76.0% (5.48%)
			Ulcerative colitis	100% (0%)
			Osteoarthritis	86.7% (4.66%)

TABLE 1. Performance characteristics for RA and other autoimmune conditions. AUC: area under the curve; RA: rheumatoid arthritis.

*Seropositive and seronegative

Cohort used for cross-disease interrogation

To further evaluate Aqtual's cfDNA_{ac} technology across diverse immune-mediated and degenerative conditions, we analyzed plasma samples from a well-characterized biorepository cohort obtained from BioOptions (Brea, CA). This cohort comprised 191 individuals contributing 229 plasma samples, including 89 patients with rheumatoid arthritis (RA), 12 with osteoarthritis (OA), and 61 with other inflammatory or autoimmune conditions, as well as 29 healthy controls. The non-RA inflammatory subset included patients with ankylosing spondylitis (AS, 13 patients/13 samples), Crohn's disease (CD, 10/11), psoriasis (PsO, 18/18), psoriatic arthritis (PsA, 10/10), and ulcerative colitis (UC, 10/10), see **Table 2**.

	Number of Samples	Number of Patients
RA: Rheumatoid Arthritis	89	89
OA: Osteoarthritis	12	12
AS: Ankylosing spondylitis	13	13
CD: Crohn's disease	11	10
PsO: Psoriasis	18	18
PsA: Psoriatic arthritis	10	10
UC: Ulcerative colitis	10	10
Healthy controls	66	29

TABLE 2. Cohort description

Next, we outline how gene-level cfDNA_{ac} signatures are distributed across the disease cohort, illustrating how a single platform can resolve organ- and compartment-anchored programs from blood without requiring tissue biopsy.

Psoriatic arthritis: Blood-based detection of a joint and enthesis-anchored circuit

Psoriatic arthritis is characterized by synovitis, enthesitis, and dactylitis, with a dominant pathology centered on synovial hyperplasia, fibroblast activation, and neoangiogenesis coupled to infiltrating lymphocyte and myeloid cell populations. To assess whether Aqtual's blood-based cfDNA_{ac} platform can resolve this tissue-specific regulatory program, we compared plasma samples from 10 PsA patients to 102 non-PsA controls (including RA, AS, IBD, PsO, OA, and healthy donors) and identified genes with statistically significant differential regulatory activity.

The PsA-enriched cfDNA_{ac} signature resolves into two coordinated compartments: a stromal axis dominated by extra-cellular matrix (ECM) and fibroblast-activation genes, as well as an immune axis enriched for lymphocyte-trafficking and GPCR/G-protein signaling modules (**Figure 6**). On the stromal side, upregulation of COL8A2, COL9A2, MMP23B, NBL1, TINAGL1, ARID1A, KDM1A, ECE1, EPHA8, and EPHB2 indicates collagen remodeling, fibroblast activation, and angiogenesis at synovial and enthesal sites. On the immune side, elevated S1PR1, GPR153, GNG12, GNB1, PRKCZ, and TNFRSF14 highlights GPCR-guided lymphocyte and myeloid trafficking with T-cell co-stimulation. Together, these cfDNA_{ac} profiles recapitulate the core PsA tissue biology of ECM-active, angiogenic fibroblasts coupled to GPCR-directed immune infiltrates, showing that Aqtual's platform can resolve joint- and enthesis-anchored disease programs from blood alone.

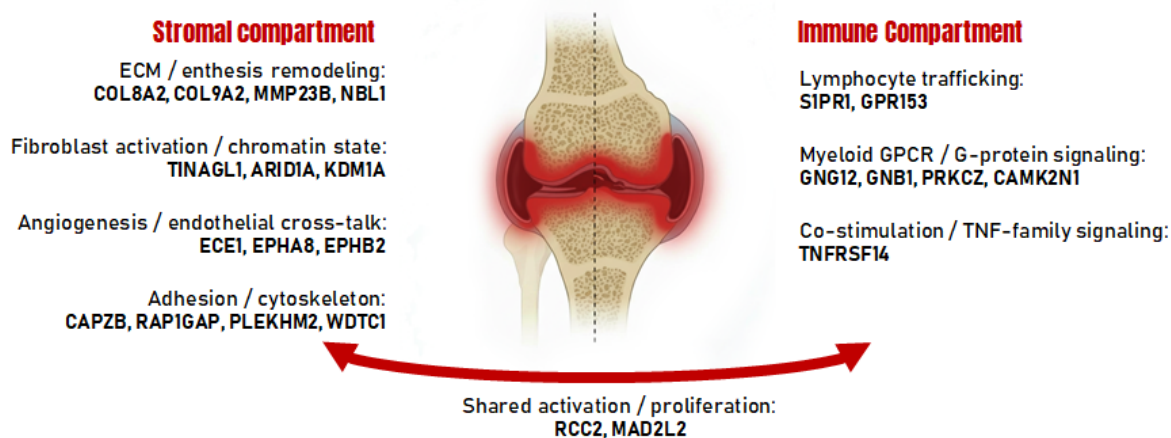


FIGURE 6. Psoriatic Arthritis stromal-immune circuit

Psoriasis: epidermal–dermal stromal and immune programs resolved from blood

In psoriatic skin disease, Aqtual's cfDNA_{ac} scores reveal a blood-detectable regulatory program that is clearly distinct from the joint/enthesis signature observed in PsA, see Figure 7. The PsO-enriched gene set is dominated by epidermal and dermal stromal signals, including basement-membrane and junctional components (AGRN, DST, KAZN, BVES), focal-adhesion/ECM-linkage genes (FBLIM1, MXRA8), and matrix-remodeling factors (MMP23A, RUNX2). In parallel, immune and epithelial programs are reflected by complement/innate interface (MASP2), MAPK-linked stress signaling (MAPK13, MAPK14, PIM1), and immune transcription factors (BACH2, FOXP4), together with shared chromatin and signaling regulators (CHD5, PRDM2, BRD2, BRPF3, PHF3, GNB1, PRKCZ). These modules align with prior psoriatic plaque tissue studies showing altered dermal–epidermal junctions, heightened metalloproteinase activity, complement deposition, and widespread epigenetic re-programming in keratinocytes and infiltrating T cells¹⁰⁻¹⁵.

These results indicate that Aqtual's blood-based active-chromatin footprint can resolve tissue-specific regulatory programs in related psoriatic diseases: a skin-dominant ECM/complement/keratinocyte program in PsO versus a joint-dominant fibroblast/vascular/immune-trafficking program in PsA, both consistent with and extending existing tissue and single-cell literature.

Stromal Compartment Epidermal/Dermal

Basement membrane / junction
AGRN, DST, KAZN, BVES

Focal adhesion / ECM
FBLIM1, MXRA8

Matrix remodeling
MMP23A, RUNX2

Immune Compartment

Complement / innate interface
MASP2

MAPK / stress signaling
MAPK13, MAPK14, PIM1

Immune transcription factors
BACH2, FOXP4

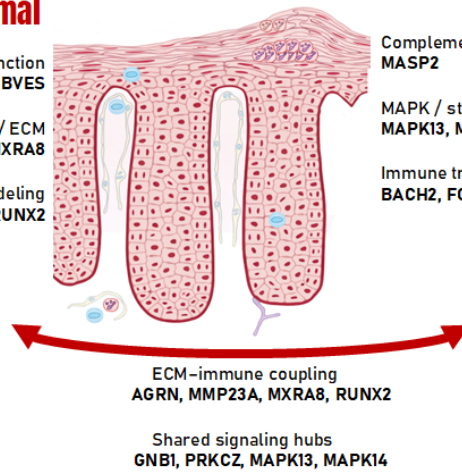


FIGURE 7. Stromal-immune regulatory circuits in psoriatic skin captured by cfDNA_{ec}.

Ankylosing spondylitis: enthesal/spinal stromal remodeling linked to Th17-complement immune programs

In ankylosing spondylitis (AS), which primarily targets the entheses and spinal ligaments, Aqtual's assay detects a circulating regulatory program consistent with fibroblast-driven matrix remodeling at these sites¹⁶. The stromal component features upregulation of MMP23B and AGRN together with chromatin regulator CHD5, indicating active extracellular-matrix turnover, basement-membrane organization, and epigenetically tuned mesenchymal activation that align with the progression from inflammatory erosions to new bone formation in AS.

On the immune side, AS samples show enrichment of canonical spondyloarthritis pathways, including IL-23/IL-17-linked T-cell programs and interferon-responsive myeloid activation, reflected by TNFRSF18 (GITR), PIK3CD, and ISG15. Complement-lectin pathway activation via MASP2 connects matrix damage to innate amplification at enthesal and spinal insertion sites, while shared signaling hubs such as GNB1 and PRKCZ couple GPCR/kinase signaling in both stromal and immune cells. Together, these cfDNA-derived signatures support the model shown in **Figure 8**, depicting immune-stromal coupling via matrix damage and complement (MMP23B, MASP2) and shared intracellular signaling (PIK3CD, PRKCZ) that links Th17-skewed immunity to pathological enthesal and spinal remodeling in AS.

Stromal Compartment

ECM / entheses and spinal remodeling:
MMP23B, AGRN, CHD5, PHF13

Fibroblast activation:
CHD5, PHF13

Complement / lectin-pathway stromal interface:
MASP2

Immune Compartment

Th17 / co-stimulated T-cell activation:
TNFRSF18 (GITR), PIK3CD

Interferon-responsive myeloid activation:
ISG15

GPCR / kinase signaling in lymphoid/myeloid cells:
GNB1, PRKCZ

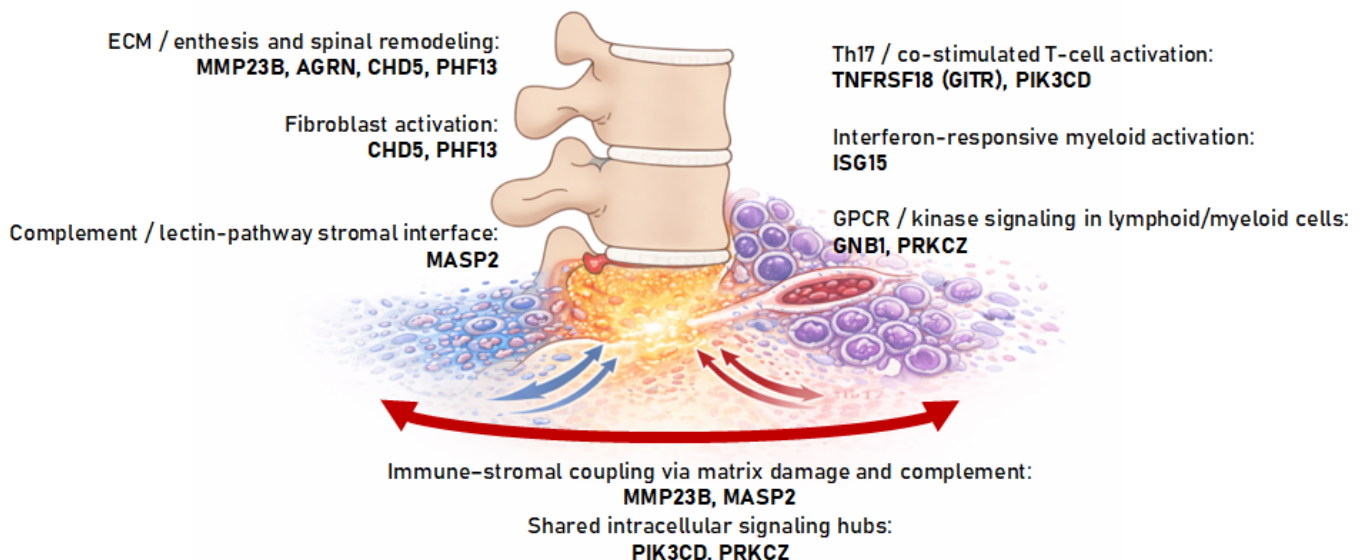


FIGURE 8. Immune-stromal coupling in ankylosing spondylitis: matrix damage, complement activation, and shared signaling hubs

Integrated immune–stromal regulatory programs in IBD

Comparing ulcerative colitis (UC) and Crohn’s disease (CD) to adjacent immune-mediated diseases (RA, PsA, AS, OA) with Aqtual’s chromatin scores shows that IBD is dominated by integrated immune–stromal activation rather than isolated epithelial injury. In both UC and CD, we observe coordinated up-regulation of checkpoint-associated, chronically activated T-cell programs (HAVCR2/TIM-3, TOX2, TRAV21, CMIP) together with immune-mediated apoptosis (FASLG), consistent with sustained adaptive immune pressure in the intestinal mucosa. Parallel enrichment of myeloid autophagy and phagolysosomal modules (MORN2, NPC2, CTSA, TOM1) and humoral components (C1S, IGHJ2P) indicates a metabolically active innate compartment and ongoing plasma-cell activity rather than transient inflammation, see **Figure 9**^{17,18}.

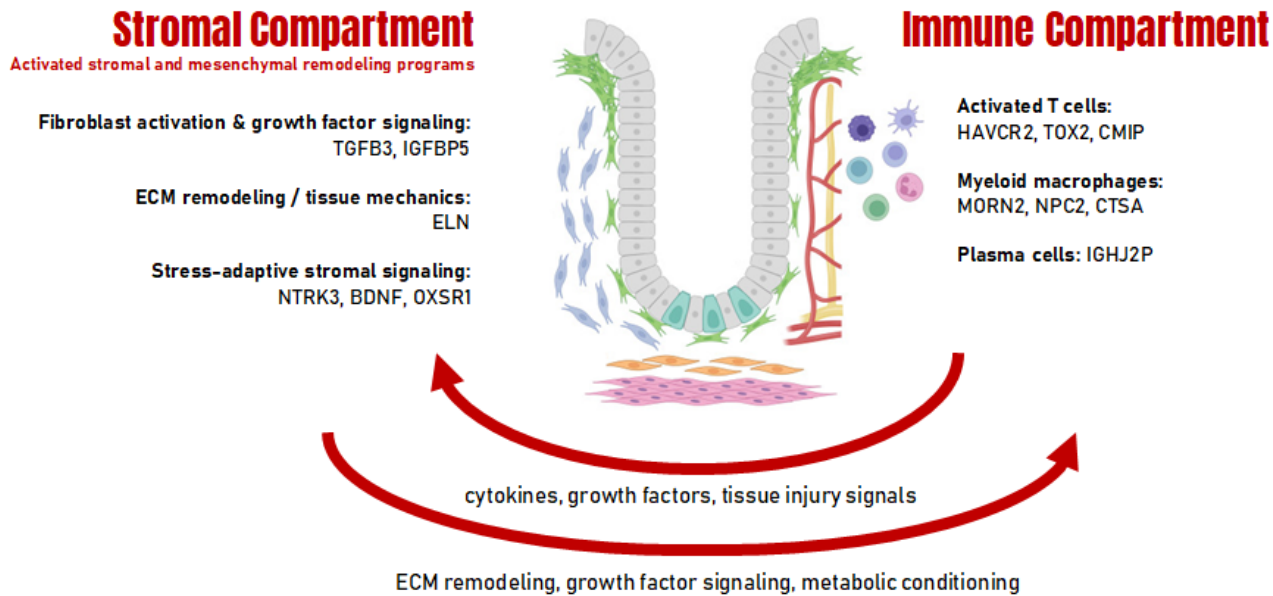


FIGURE 9. Integrated immune–stromal circuits in ulcerative colitis and Crohn’s disease

On the stromal side, both diseases show matrix and growth-factor remodeling (ELN, TGFB3), a mesenchymal-immune interface centered on fibroblast/vascular cross-talk (IGFBP5, AAMP), and stress-adaptive and metabolic programs (OXSR1, PLIN2), with additional neurovascular signatures (BDNF, NTRK3) suggesting neuroimmune conditioning of the gut wall. Thus, the chromatin signatures measured in blood recapitulate tissue-specific immune, stromal, metabolic, and neurovascular programs in IBD that are typically defined from intestinal biopsies^{19,20}.

Platform-level view: blood-based access to organ-specific regulatory programs across autoimmune and chronic disease

Together, these data from multiple indications provide evidence that blood-based profiling of regulatory chromatin can serve as an integrative readout of tissue and compartment-specific immune activity. By resolving synovial and enthesal fibroblast-immune programs in the arthritides, epidermal and dermal axes in psoriasis, and coordinated mucosal immune–stromal circuits in IBD, the Aqtual platform begins to bridge the long-standing gap between mechanistic tissue atlases and the constraints of real-world clinical sampling. Anchoring this capability in clinically annotated cohorts such as the 1,400-plus patient prospective PRIMA-102 study enables direct translation into products, including Aqtual’s RA Therapy Selection test and additional diagnostic and prognostic assays under development, that are designed for use in routine rheumatology practice. In doing so, Aqtual directly addresses the technical limitations that have historically prevented blood-based assays from truly replacing tissue biopsies—recovering stable, organ-resolved regulatory programs from plasma and thereby unlocking tissue biology without repeated invasive sampling. The same program-level framework provides a natural interface for pharma partnerships, supporting indication and target selection, tissue-aware response biomarkers, and adaptive, mechanistically informed trial designs that finally align liquid-biopsy readouts with the tissue states that matter for drug development.

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