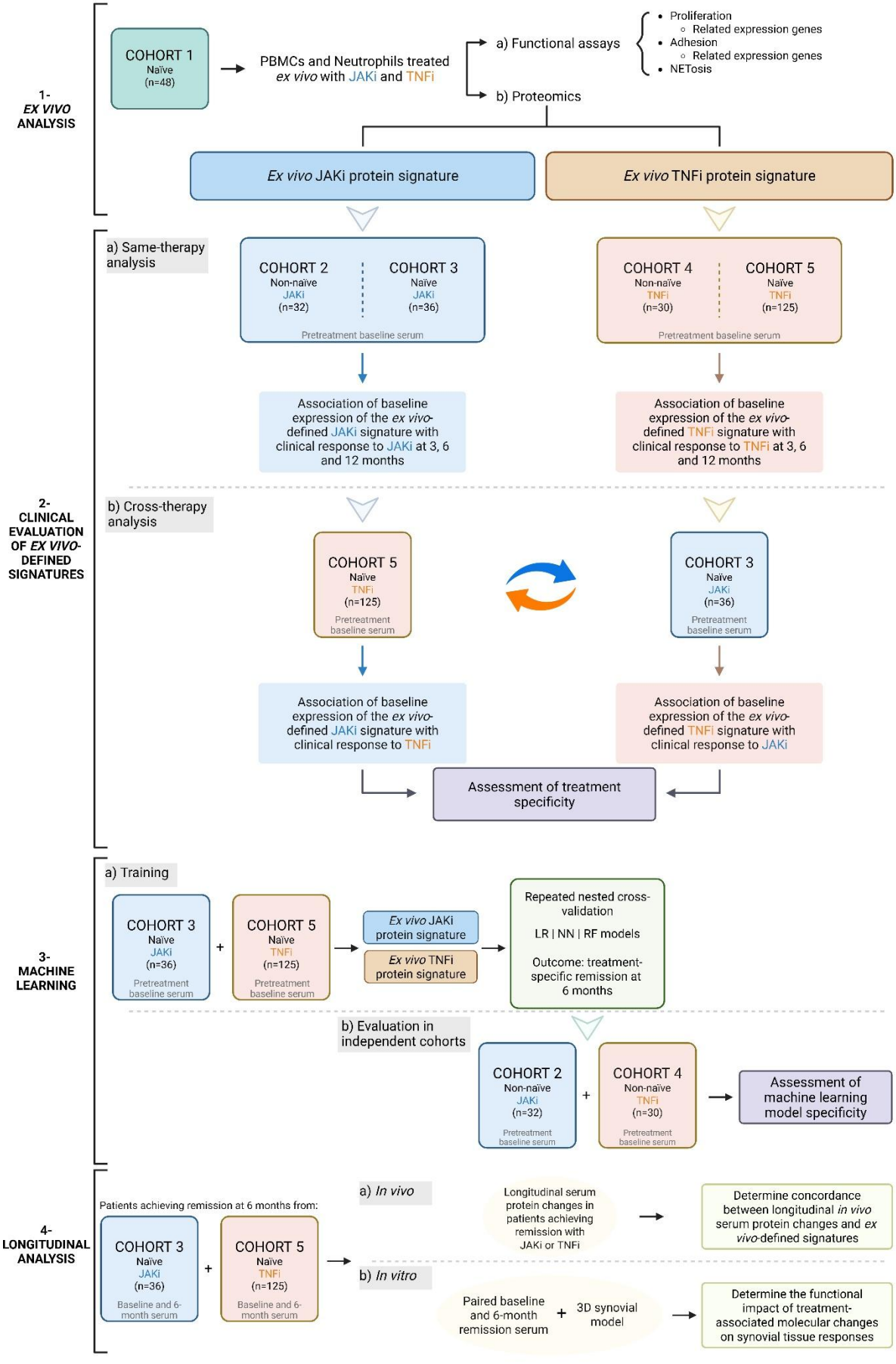
1- EX VIVO ANALYSIS
COHORT 1
Naïve
(n=48)
PBMCs and Neutrophils treated ex vivo with JAKi and TNFi
a) Functional assays
Proliferation
Related expression genes
Adhesion
Related expression genes
NETosis
b) Proteomics
Ex vivo JAKi protein signature
Ex vivo TNFi protein signature
2- CLINICAL EVALUATION OF EX VIVO-DEFINED SIGNATURES
a) Same-therapy analysis
COHORT 2
Non-naïve
JAKi
(n=32)
COHORT 3
Naïve
JAKi
(n=36)
Pretreatment baseline serum
COHORT 4
Non-naïve
TNFi
(n=30)
COHORT 5
Naïve
TNFi
(n=125)
Pretreatment baseline serum
Association of baseline expression of the ex vivo-defined JAKi signature with clinical response to JAKi at 3, 6 and 12 months
Association of baseline expression of the ex vivo-defined TNFi signature with clinical response to TNFi at 3, 6 and 12 months
b) Cross-therapy analysis
COHORT 5
Naïve
TNFi
(n=125)
Pretreatment baseline serum
COHORT 3
Naïve
JAKi
(n=36)
Pretreatment baseline serum
Association of baseline expression of the ex vivo-defined JAKi signature with clinical response to TNFi
Association of baseline expression of the ex vivo-defined TNFi signature with clinical response to JAKi
Assessment of treatment specificity
3- MACHINE LEARNING
a) Training
COHORT 3
Naïve
JAKi
(n=36)
Pretreatment baseline serum
COHORT 5
Naïve
TNFi
(n=125)
Pretreatment baseline serum
Ex vivo JAKi protein signature
Ex vivo TNFi protein signature
Repeated nested cross-validation
LR | NN | RF models
Outcome: treatment-specific remission at 6 months
b) Evaluation in independent cohorts
COHORT 2
Non-naïve
JAKi
(n=32)
Pretreatment baseline serum
COHORT 4
Non-naïve
TNFi
(n=30)
Pretreatment baseline serum
Assessment of machine learning model specificity
4- LONGITUDINAL ANALYSIS
Patients achieving remission at 6 months from:
COHORT 3
Naïve
JAKi
(n=36)
Baseline and 6-month serum
COHORT 5
Naïve
TNFi
(n=125)
Baseline and 6-month serum
a) In vivo
Longitudinal serum protein changes in patients achieving remission with JAKi or TNFi
Determine concordance between longitudinal in vivo serum protein changes and ex vivo-defined signatures
b) In vitro
Paired baseline and 6-month remission serum
3D synovial model
Determine the functional impact of treatment-associated molecular changes on synovial tissue responses

Supplementary Figure S1. Overview of the study design and analytical workflow.- (1) *Ex vivo* analysis: definition of JAKi- and TNFi-modulated protein signatures in Cohort 1. (2) Clinical evaluation: assessment of these signatures in pretreatment serum from Cohorts 2-5 and their association with subsequent clinical response. (3) Machine learning: evaluation of treatment-specific remission patterns in naïve patients using repeated nested cross-validation and comparison of LR, NN, and RF models, followed by evaluation of the same analytical framework in non-naïve patients. (4) Longitudinal analysis: comparison of baseline and 6-month serum profiles in remission achievers and functional assessment in a 3D synovial model.