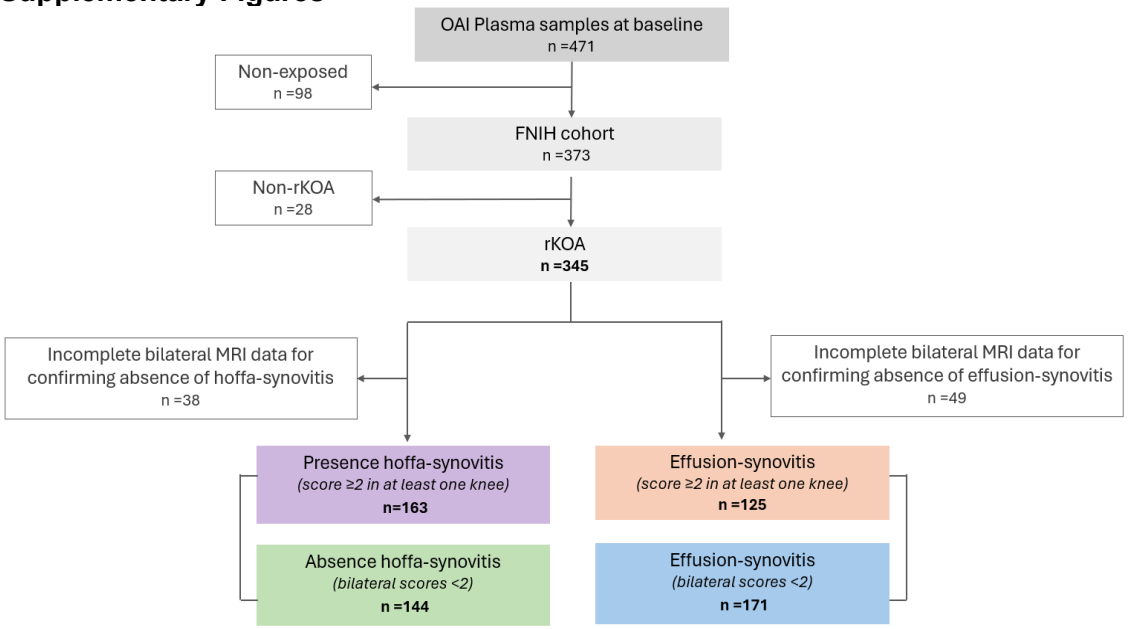
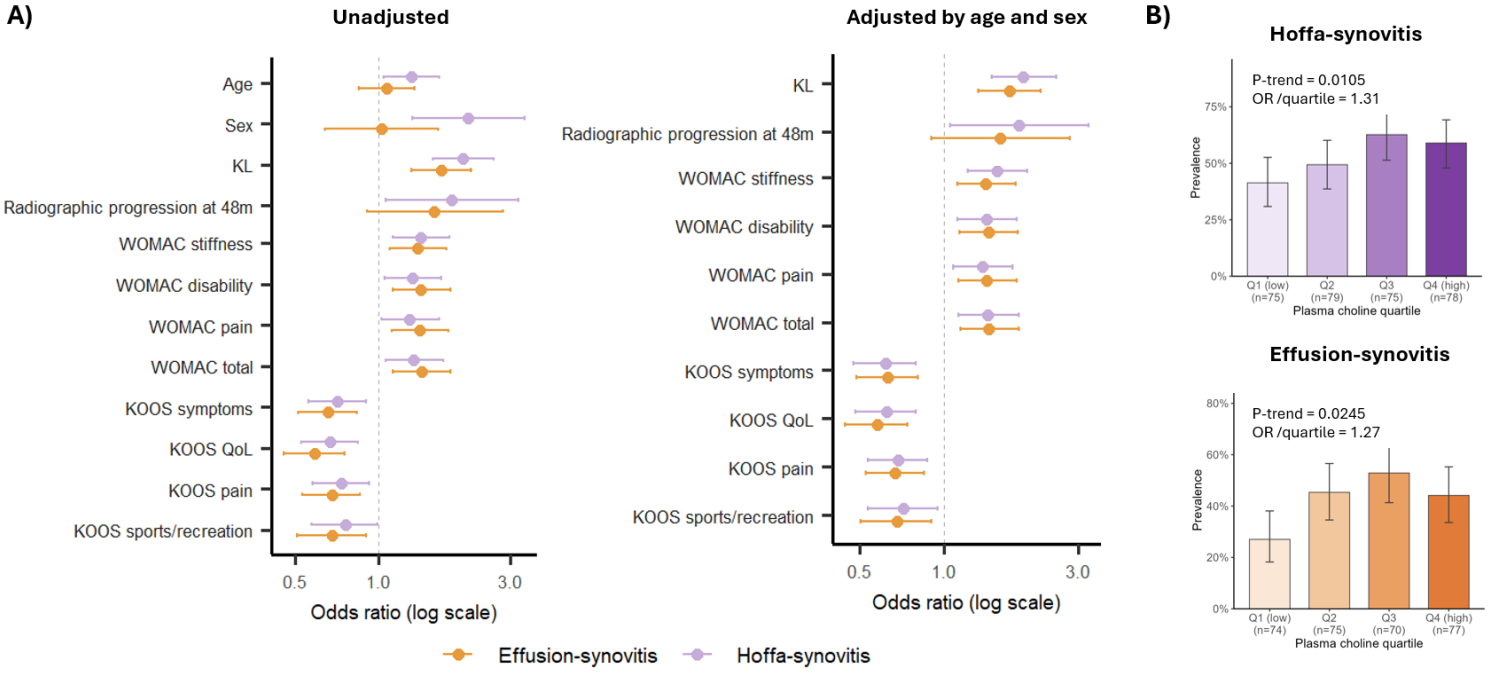
Supplementary material

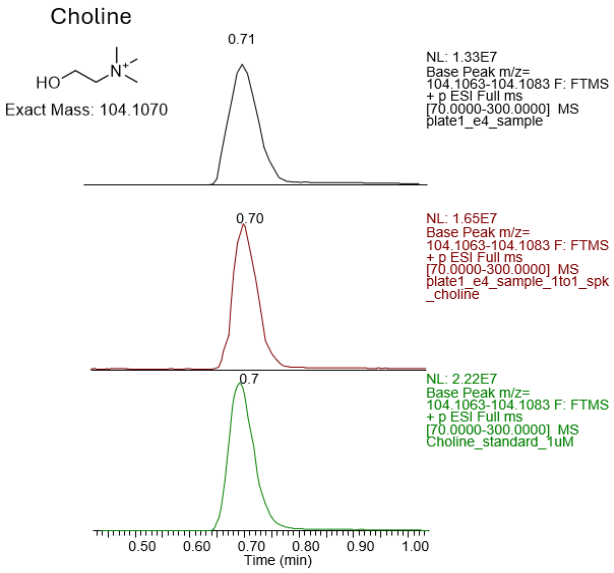
Supplementary Figures



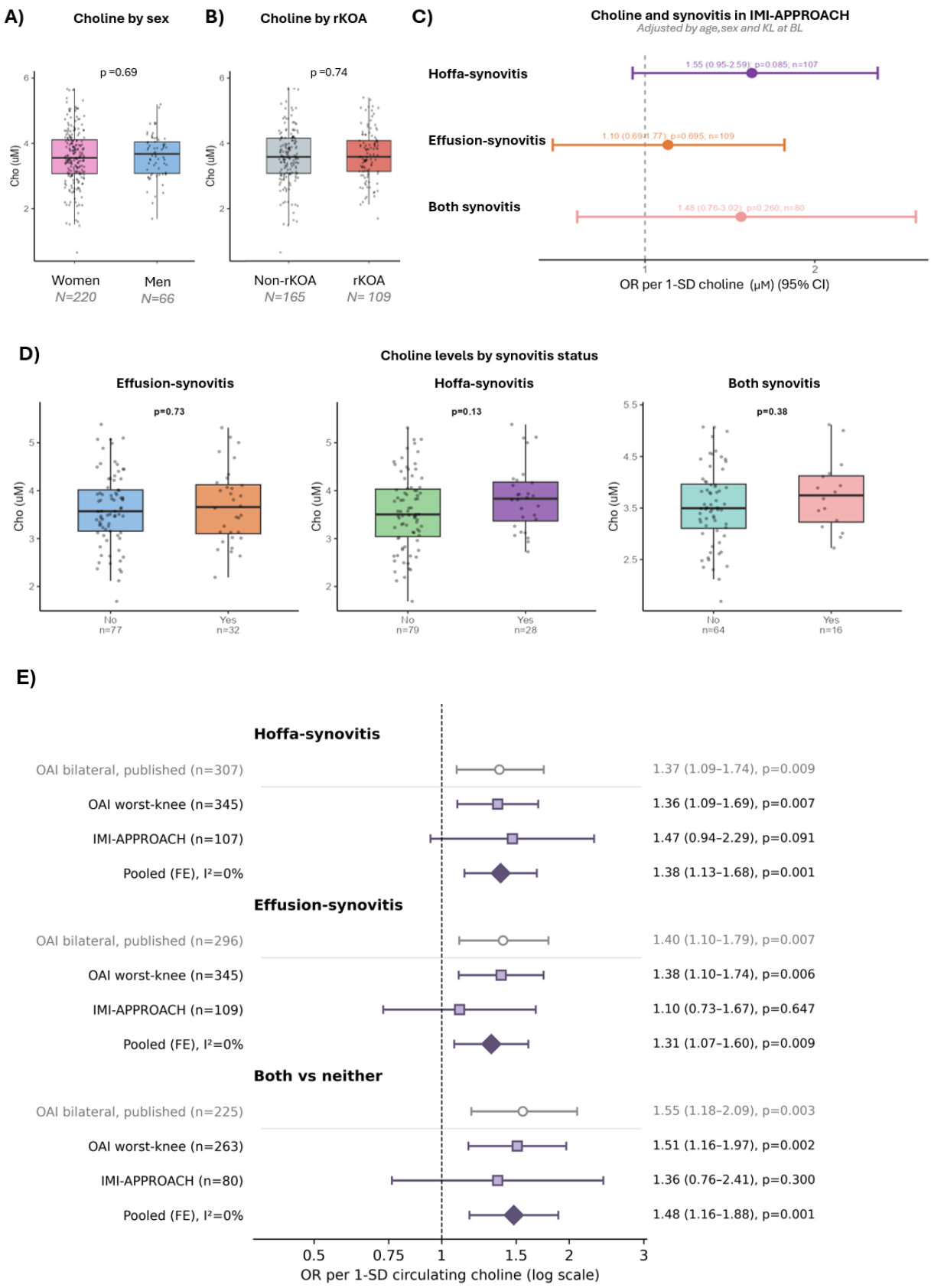
Supplementary Figure 1. Participant flowchart for the baseline cross-sectional analysis of non-contrast MRI-defined synovitis in participants with rKOA from the OAI cohort.



Supplementary Figure 2. Clinical, demographic, and plasma choline associations with MRI-defined synovitis in radiographic knee OA at baseline. (A) Odds ratios for the presence of each MRI-defined synovitis phenotype at baseline in participants with radiographic knee OA from the OAI cohort. Unadjusted estimates and adjusted for age and sex as covariates. Odds ratios (OR) are expressed per 1-SD increase for continuous variables and relative to the reference category for categorical variables. Points denote the OR and horizontal bars the 95% confidence interval. **(B)** Prevalence of MRI-defined synovitis phenotype across quartiles of baseline plasma choline. Trends and odds ratios per quartile increment were estimated by logistic regression.

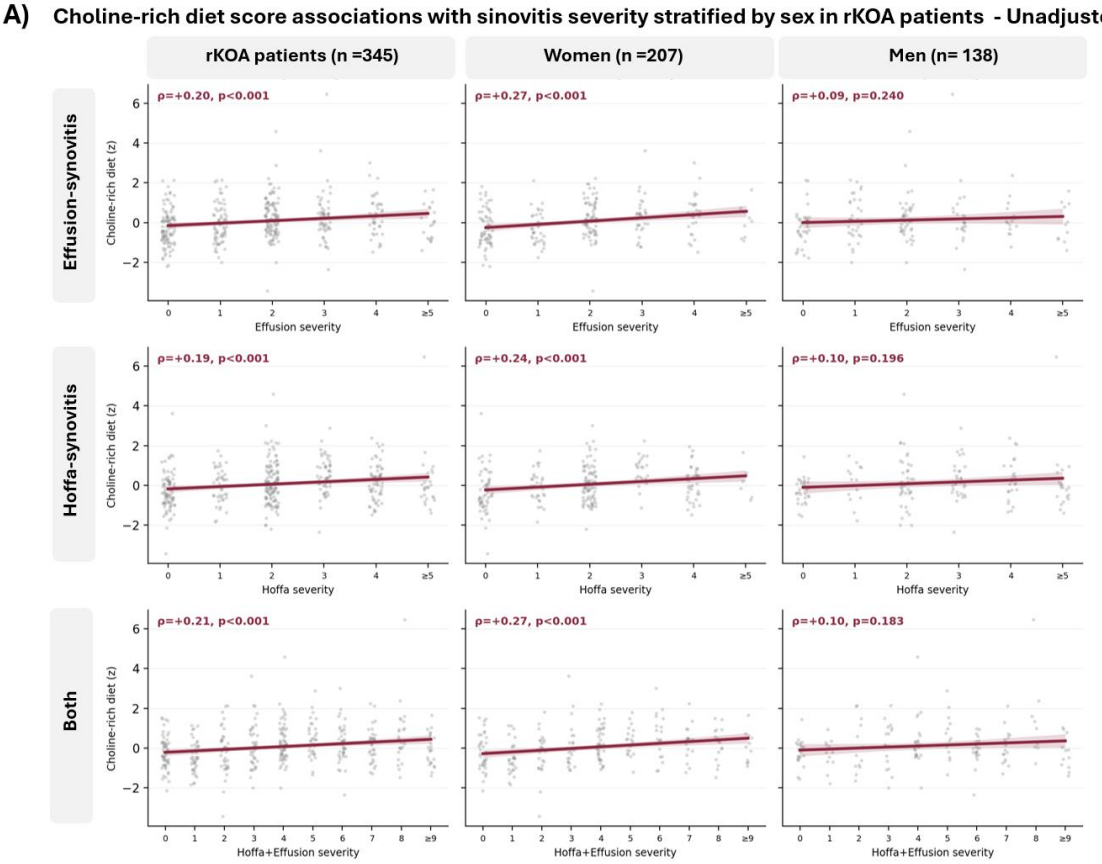


Supplementary Figure 3. Choline retention time and MS/MS spectral matching with an analytical standard.



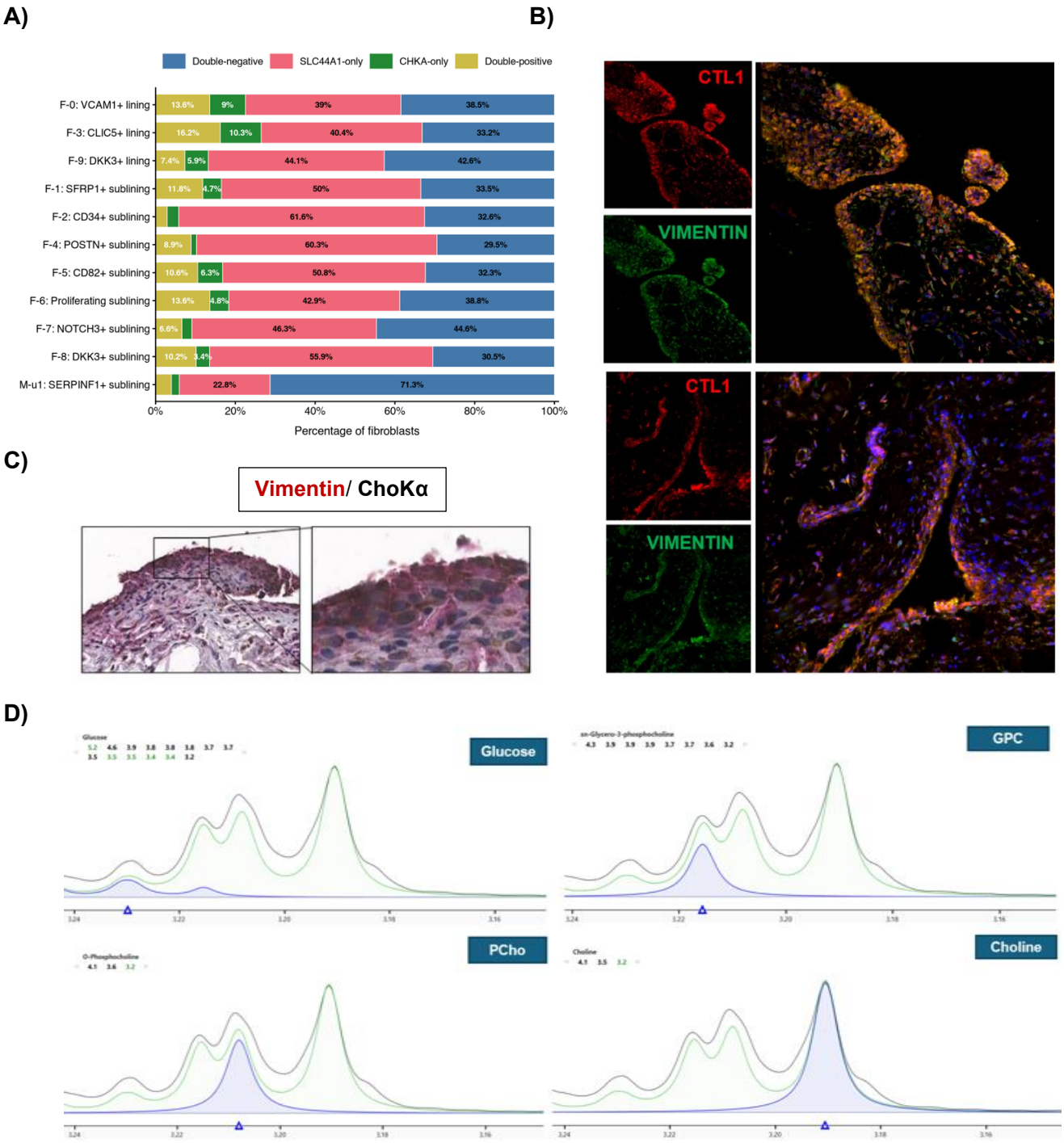
Supplementary Figure 4 | Serum choline across MRI-defined synovitis phenotypes in an external cohort (IMI-APPROACH). Absolute total choline concentrations were measured in baseline serum. (A) Serum choline according to sex in the full cohort (n = 286). (B) Serum choline according to radiographic knee OA status in the index knee (C) Odds ratios and 95% confidence intervals for Hoffa-synovitis, effusion-synovitis and the co-occurrence of both features per 1-SD

increase in serum choline in participants with rKOA. Models were adjusted for age, sex and index-knee KL grade. (D) Serum choline levels according to the presence or absence of effusion-synovitis, Hoffa-synovitis and both features in participants with rKOA. Because MRI assessment was restricted to the index knee, synovitis in the contralateral knee could not be excluded. Points represent individual participants; boxes show the median and interquartile range. (E) Odds ratios per 1-SD circulating choline for the presence of Hoffa-synovitis, effusion-synovitis and both phenotypes versus neither. Open circles show the primary bilateral OAI analysis for reference and were not included in the meta-analysis; filled symbols show harmonized single-knee models in OAI and IMI-APPROACH and their fixed-effect pooled estimate. Error bars are 95% confidence intervals.



Supplementary Figure 5. Associations between the choline-rich food score and knee synovitis severity. Associations are shown for all participants with rKOA (left), women (centre) and men (right) for effusion-synovitis, Hoffa-synovitis and their combined severity score. (A)

Unadjusted analyses. (B) Analyses adjusted for age, sex, BMI and baseline radiographic KL sum. Spearman correlations (ρ) and corresponding *P* values are displayed. Bilateral effusion- and Hoffa-synovitis scores ranged from 0–6 (scores ≥5 collapsed), and the combined score ranged from 0–12 (scores ≥9 collapsed).



Supplementary Figure 6. Molecular and metabolic characterization of the choline pathway in OA synovial tissue. (A) Distribution of *SLC44A1*- and *CHKA*-expressing synovial fibroblasts across fibroblast populations identified by single-cell RNA sequencing of OA synovial tissue. (B) Dual immunofluorescence for CTL1, the protein encoded by *SLC44A1*, and vimentin in synovial tissue from additional patients with end-stage OA. (C) Representative dual-color immunohistochemical staining for ChoKα and vimentin in end-stage OA synovium (n = 4). (D) Representative ^{1}H-NMR spectra showing signals used to identify and quantify glucose, free choline, phosphocholine (PCho), and glycerophosphocholine (GPC) in OA synovial tissue.

Supplementary Tables

Supplementary Table 1. Osteophyte volume in the DMM mouse model. Osteophyte volume was quantified by µCT in non-operated (Non-Op) and DMM-operated knees at the medial femoral condyle (MFC), medial tibial plateau (MTP), and combined medial compartment (MFC + MTP). Data are presented as mean ± SD. *P* values correspond to the effects of surgery, treatment, and their interaction. *n* = 9 PBS-treated mice and *n* = 10 MN58b-treated mice.

Osteophyte Volume (mm^2)											
	PBS		PBS		MN		MN		P		
	Non-Op		DMM		Non-Op		DMM				
	mean	SD	mean	SD	mean	SD	mean	SD	Surgery	Treatment	Surgery x treatment
MFC	0	0	0.116	0.042	0	0	0.075	0.042	0	0.051	0.051
MTP	0	0	0.223	0.111	0.009	0.029	0.169	0.06	0	0.301	0.137
MFC+MTP	0	0	0.34	0.141	0.009	0.029	0.244	0.055	0	0.109	0.041