Supplementary Material

Supplementary Table 1. List of capture and detection antibodies used for the sandwich immunoassays development.

Target protein	Capture antibody (Prod number, company)	Detection antibody (Prod number, company)	Serum sample dilution
ITIH1	HPA041639, Atlas Antibodies	HPA042049, Atlas Antibodies	1:30
S100A6	HPA007575, Atlas Antibodies	CAB047354,NCI-CPTC	1:10
C3	BSI 0263, BioSystems International	BSI 0190, BioSystems International	1:2000

Supplementary Table 2. Univariate analysis for clinical covariates.

Covariates	OR (95% CI)	pvalue
Age	1.059 (1.023-1.096)	0.001
Gender	1.830 (1.020-3.293)	0.043
BMI	1.260 (1.158-1.371)	8.427E-10
Family history of knee replacement	0.944 (0.424-2.103)	0.889
Frequent knee bending activity	3.530 (1.865-6.679)	1.06E-4
Alignment		0.002
varus vs neither	0.523 (0.230-1.189)	0.122
valgus vs neither	2.07 (0.993-4.315)	0.052
Alignment valgus negative	0.894 (0.820-0.975)	0.012
PASE	0.998 (0.994-1.001)	0.161
History of knee injury	2.083 (1.032-4.206)	0.041
History of knee surgery	1.364 (0.517-3.596)	0.531
WOMAC pain score	1.196 (1.038-1.379)	0.014
Joint space width	0.984 (0.651-1.489)	0.940

OR, odd ratio; CI, confidence interval; BMI, Body mass index; PASE, Physical Activity Scale for the Elderly; WOMAC, Western Ontario and McMaster Universities Arthritis Index pain; JSW, joint space width

Supplementary Table 3. Stepwise-multivariable regression analysis and Receiver Operating Curve analysis for the clinical model.

Variables	OR (95%CI)	pvalue
Age	1.076 (1.033-1.121)	4.41E-4
Gender	2.863 (1.402-5.848)	0.004
BMI	1.301 (1.180-1.434)	1.309E-10
History of knee inJury	3.057 (1.309-7.138)	0.010
WOMAC pain	1.114 (0.964-1.288)	0.143
Constant	3.00E-6	5.725E-10
AUC (95% CI)	0.816 (0.756-0.875)	2.193E-14
% Specificity (95% CI)	67.5 (58.7-75.4)	
% Sensitivity (95% CI)	84.8 (76.7-91.8)	

OR, odd ratio; CI, confidence interval; AUC, area under the curve; BMI, body mass index; WOMAC, Western Ontario and McMaster Universities Arthritis Index pain

Supplementary Table 4. Characteristics of the Receiver operating characteristic (ROC) analysis for the different predictive models.

Predictive models	Exploratory cohort				
	AUC (95% CI)	NPV % (95% CI)	PPV% (95% CI)	Sensitivity% (95% CI)	Specificity % (95% CI)
CV	0.816 (0.756-0.875)	85.7 (79.5-91.8)	66.3 (60.3-72.8)	84.8 (76.7-91.8)	67.5 (58.7-75.4)
ITIH1	0.783 (0.718-0.849)	77.2 (71.8-83.0)	72.9 (65.4-80.0)	68.3 (59.3-77.0)	80.7 (73.6-87.0)
CV +ITIH1	0.871 (0.822-0.920)	85.2 (79.3-90.8)	76.3 (69.2-83.5)	81.4 (73.2-89.5)	80.7 (73.6-87.7)
C3	0·727 (0·656-0·799)	69.1 (64.9-73.6)	78.6 (67.9-88.6)	46.5 (36.0-56.9)	90.3 (84.2·-95.6)
CV +C3	0.842 (0.787-0.897)	81.6 (76.0-87.3)	75.6 (68.0-83.5)	75.5 (66.2-83.7)	81.5 (74.5-88.6)
S100A6	0.605 (0.526-0.684)	72.5 (61.8-82.6)	49.3 (45.0-53.8)	81.4 (73.2-89.5)	36.8 (28.0-45.6)
CV + S100A6	0.827 (0.770-0.883)	87.3 (80.9-93.5)	65.8 (60.1-72.4)	87.2 (80.2-94.1)	65.7 (57.0-74.5)
ITIH1+C3+S100A6	0.794 (0.731-0.857)	79.5 (73.0-85.2)	67.4 (60.2-74.7)	74.4 (65.1-82.5)	72.8 (64.0-80.7)
CV +ITIH1+C3+S100A6	0.874 (0.825-0.923)	82.8 (77.3-88.2)	79.5 (72.2-87.0)	76.7 (67.4-84.8)	85.0 (78.0-91.2)

AUC,area under the curve; CI, confidence interval; NPV,negative predictive value; PPV,positive predictive value; CV, clinical covariates

Supplementary Table 5. Predictive model combining clinical covariates and ITIH1 (full model).

Variables	B	pvalue	OR (95% CI)
Age	0.063	0.005	1.065 (1.020-1.113)
Gender	0.856	0.029	2.354 (1.090-5.082)
BMI	0.261	1.42E-6	1.298 (1.167-1.443)
WOMAC pain	0.076	0.330	1.079 (0.926-1.257)
History of knee injury	0.784	0.109	2.190 (0.840-5.704)
ITIH1	0.004	1.87E-6	1.004 (1.002-1.006)
Constant	-15,817	5.8E-11	

B, coefficient;OR, odd ratio; BMI, Body mass index; WOMAC, Western Ontario and McMaster Universities Arthritis Index pain

Supplementary Table 6. Receiver operating characteristic (ROC) analysis for the different predictive models in the replication cohort and Integrated Discrimination Analysis (IDI) to evaluate the additional value of ITIH, C3 and S100A6 to the clinical covariates.

Predictive models	AUC (95% CI)	IDI (95% CI); pvalue
ITIH1	0.713 (0.624-0.802)	
C3	0.708 (0.618-0.799)	
S100A6	0.654 (0.559-0.749)	
CV	0.753 (0.670-0.838)	
CV + ITIH1	0.786 (0.704-0.867)	0.090 (0.034, 0.145); p=0.002
CV + C3	0.770 (0.687-0.853)	0.029 (-0.004, 0.061); p=0.082
CV + S100A6	0.769 (0.686-0.852)	0.0377 (0.010, 0.065); p=0.007
CV +ITIH1+C3+S100A6	0.778 (0.665-0.860)	0.087 (0.031, 0.148); p=0.006

CV, clinical covariates; AUC,area under the curve; CI, confidence interval; IDI, integrated discrimination improvement

Supplementary Table 7. Probability of cumulative incidence (%) by each interval censored according to the different ITIH1 categories.

interval-censured	ITIH1 levels		
left-right	high	intermediate	low
0-12	30.7	18.6	4.9
12-24	40.6	27.1	10.4
24-36	55.4	34.9	13.2
36-48	64.5	35.8	14.9
48-72	64.5	35.8	15.9
72-96	65.4	37.6	18.7
96-999	100	100	100