Electronic supplementary information (ESI)

Cell-free and cytokine-free self-assembling peptide hydrogel-polycaprolactone composite scaffolds for segmental bone defects

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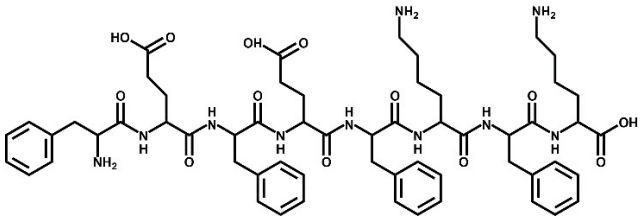
FEK8



FEK18

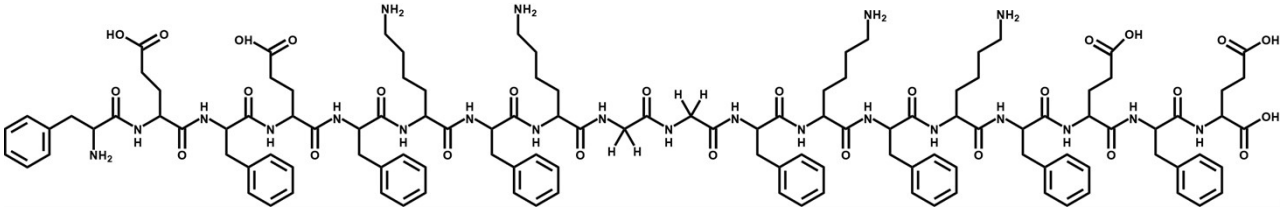


Figure S1. The chemical structures of FEK8 and FEK18.

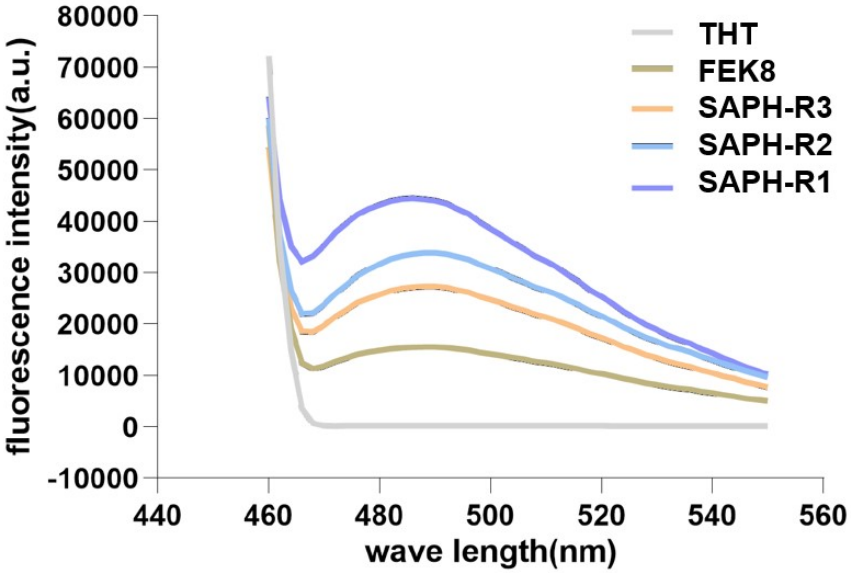


Figure S2. The UV-vis absorbance of ThT-treated SAPHs.

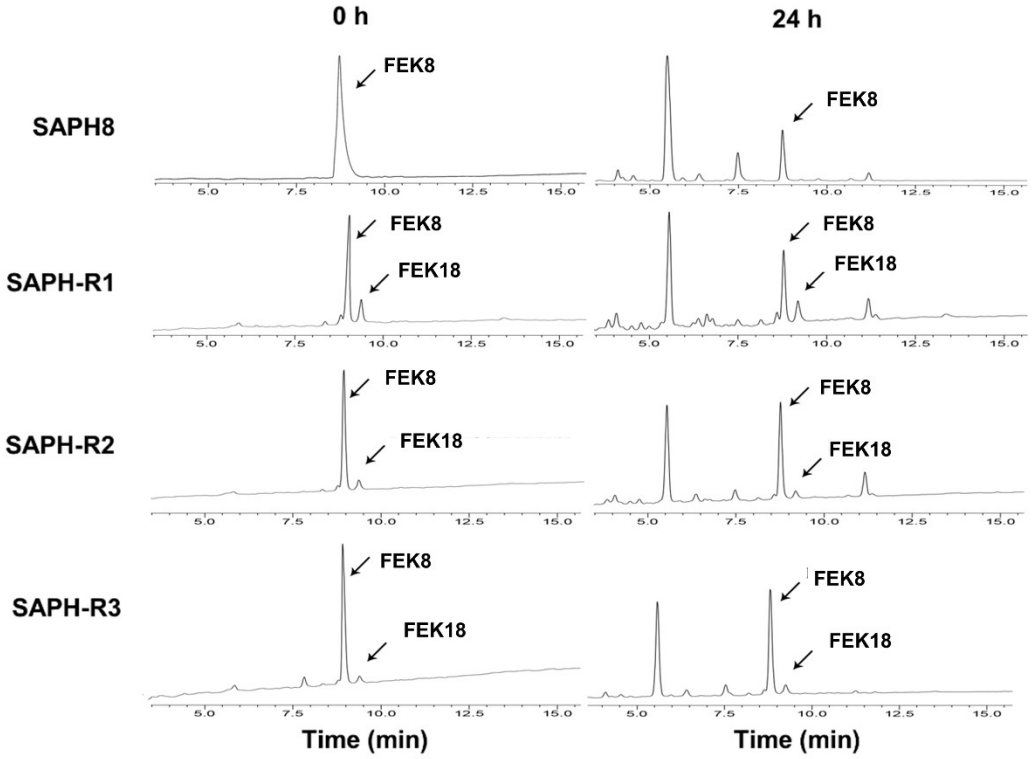


Figure S3. The HPLC retention peaks of SAPH treated with proteinase K for 0 h and 24 h. The SAPH8 peak areas were integrated to calculate the degradation rate.

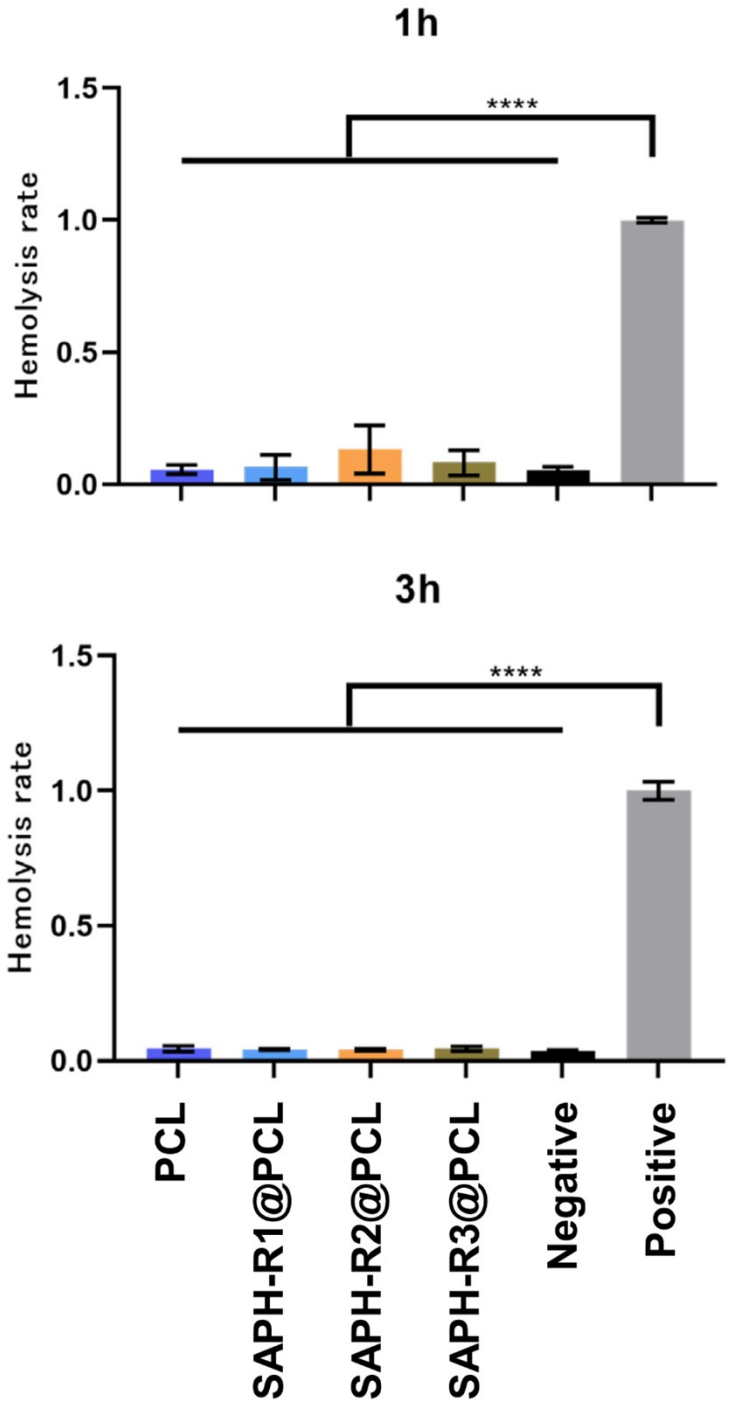


Figure S4. Haemolysis after incubating with PCL or SAPH-PCL composite scaffolds for 1h and 3 h (n =3). **** p <0.0001.

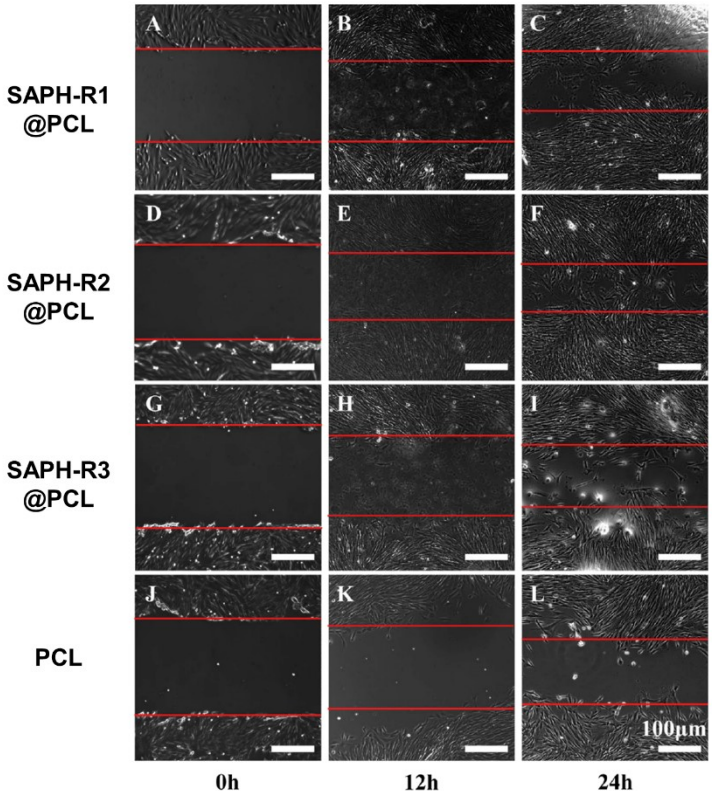


Figure S5. The microscopy images of BMSC migration in the scratch stimulation.

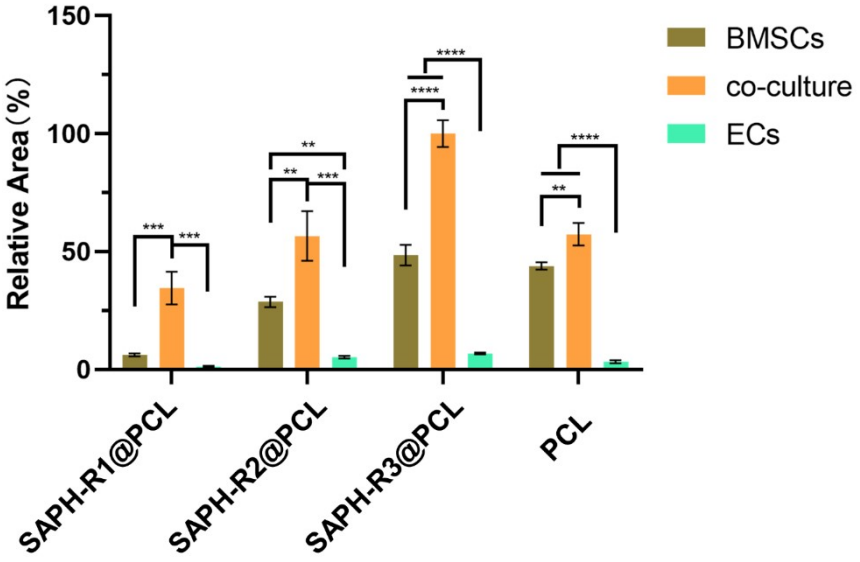


Figure S6. Statistical analysis of the alizarin red positive area in Figure 2E.

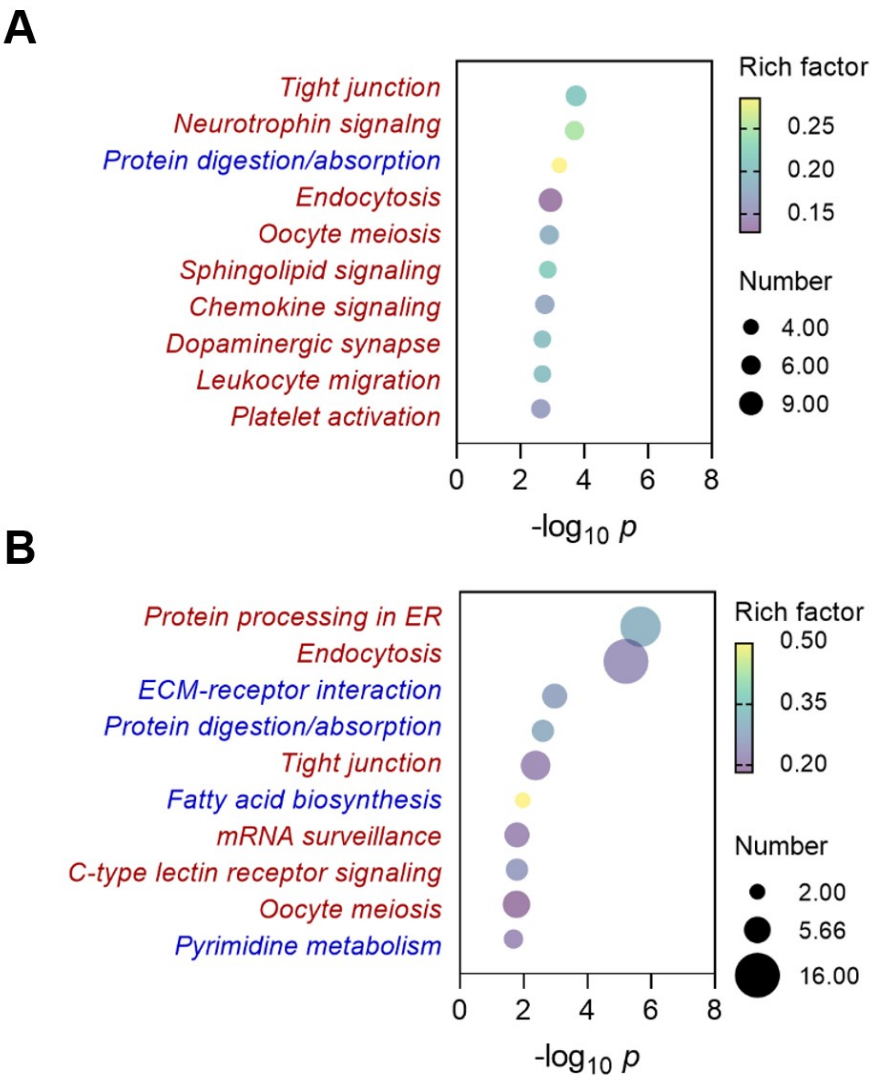


Figure S7. The top 10 KEGG pathways of the most significant expression differentiation between enrichment (A) PCL-4m vs CTR-4m and (B) SAPH-R3@PCL-4m vs CTR-4m. The differentiation was calculated using Fisher's exact test to determine the *p* value. Up and down regulation were shown as red and blue, respectively.

Table S1. The expression of differential proteins/differential in Wnt pathway.

KEGG Name	Accession	CTR-4m	PCL-4m	SAPH-R3@ PCL-4m	CTR-6m	PCL-6m	SAPH-R3@ PCL-6m
Protein							
CaMKⅡ	O77708	0.720±0.016	0.809±0.229	1.164±0.289	0.869±0.094	1.084±0.060	0.974±0.038
CaN	A0A5F9CZB0	0.754±0.028	0.860±0.097	1.155±0.136	0.930±0.022	0.999±0.021	1.001±0.034
CK2	G1SN56	0.825±0.021	1.075±0.045	0.937±0.054	0.969±0.126	0.979±0.024	1.006±0.016
CK2	A0A5F9D4P0	0.606±0.025	1.003±0.093	1.023±0.153	1.066±0.063	1.030±0.061	0.962±0.048
CK2	P67873	0.773±0.029	1.109±0.403	1.096±0.022	0.857±0.065	0.800±0.049	1.005±0.132
Duplin	G1TK69	1.817±0.078	0.538±0.080	0.629±0.364	0.948±0.180	0.707±0.017	0.717±0.239
PEDF	G1SCK5	2.119±0.360	0.652±0.159	1.148±0.352	0.779±0.222	1.067±0.112	1.080±0.282
Rac	G1TAX7	0.632±0.038	1.038±0.128	0.850±0.110	1.112±0.289	0.883±0.113	0.941±0.081
RhoA	G1T567	0.611±0.065	1.122±0.093	0.997±0.062	0.951±0.077	0.953±0.013	1.031±0.110
Skp1	G1TTU6	0.600±0.142	1.044±0.099	1.033±0.067	0.914±0.153	0.915±0.108	1.168±0.300
TBL1	G1TCY8	1.362±0.111	0.972±0.081	0.893±0.047	1.020±0.026	0.913±0.030	0.971±0.055
Phosphorylated protein							
pCaMKⅡ	O77708	0.148±0.005	0.400±0.208	0.763±0.441	0.613±0.197	0.857±0.069	0.649±0.095
pGSK-3β	A0A5F9DUX8	0.105±0.008	0.582±0.267	0.793±0.355	0.694±0.175	0.925±0.099	1.061±0.342
pPKA	Q95J97	0.380±0.041	0.675±0.235	0.805±0.187	0.852±0.048	1.013±0.067	0.963±0.025
(p) β-catenin	Catenin beta 1	0.280±0.003	0.648±0.334	0.702±0.124	0.766±0.186	0.955±0.146	0.821±0.047

Table S2. The expression of differential proteins/differential in TGFβ pathway.

KEGG Name	Accession	CTR-4m	PCL-4m	SAPH-R3@ PCL-4m	CTR-6m	PCL-6m	SAPH-R3@ PCL-6m
Protein							
FBN1	G1SKM2	3.598±0.217	1.201±0.625	0.679±0.071	0.832±0.080	0.742±0.143	0.660±0.081
LTBP1	A0A5F9DQH9	1.688±0.009	0.933±0.168	1.068±0.128	0.904±0.104	0.856±0.052	0.856±0.071
PP2A	P67777	0.562±0.001	1.193±0.078	1.009±0.103	0.848±0.109	0.975±0.005	1.043±0.073
PP2A	A0A5F9DM35	0.747±0.073	1.067±0.122	0.987±0.031	1.018±0.063	1.006±0.070	0.922±0.043
RhoA	G1T567	0.611±0.065	1.122±0.093	0.997±0.062	0.951±0.077	0.953±0.013	1.031±0.110
Skp1	G1TTU6	0.600±0.142	1.044±0.099	1.033±0.067	0.914±0.153	0.915±0.108	1.168±0.300
TGFβ	A0A5F9CDR7	2.147±0.169	1.054±0.133	1.379±0.107	1.027±0.070	1.650±0.096	1.536±0.101

Table S3. The expression of differential proteins/differential in NF-κB pathway.

KEGG Name	Accession	CTR-4m	PCL-4m	SAPH-R3@ PCL-4m	CTR-6m	PCL-6m	SAPH-R3@ PCL-6m
Protein							
BCL-10	G1TB43	0.794±0.013	0.924±0.145	0.959±0.003	0.995±0.184	0.960±0.050	0.946±0.050
BCR	P01879	1.910±0.283	1.449±0.428	0.802±0.082	0.812±0.069	1.002±0.074	0.740±0.062
BCR	G1TIU0	1.520±0.096	1.378±0.336	0.825±0.243	0.895±0.286	0.813±0.067	0.951±0.168
BTK	G1SZN9	1.406±0.001	0.940±0.055	0.841±0.100	0.955±0.037	0.898±0.107	0.878±0.125
CD14	G1SLB5	0.826±0.019	1.006±0.140	1.204±0.255	0.894±0.058	1.086±0.088	0.970±0.093
CK2	G1SN56	0.825±0.021	1.075±0.045	0.937±0.054	0.969±0.126	0.979±0.024	1.006±0.016
CK2	A0A5F9D4P0	0.606±0.025	1.003±0.093	1.023±0.153	1.066±0.063	1.030±0.061	0.962±0.048
CK2	P67873	0.773±0.029	1.109±0.403	1.096±0.022	0.857±0.065	0.800±0.049	1.005±0.132
CYLD	G1T481	1.016±0.001	1.356±0.067	1.339±0.190	1.571±0.251	1.487±0.005	1.219±0.082
ELKS	A0A5F9CI98	0.749±0.110	0.968±0.085	1.011±0.117	0.992±0.048	1.004±0.022	1.068±0.027
Lyn	A0A5F9CT28	0.820±0.005	1.047±0.035	0.879±0.026	1.076±0.173	0.931±0.077	0.890±0.033
VACAM1	A0A5F9DFM7	1.119±0.022	1.069±0.060	0.813±0.068	1.171±0.095	0.992±0.083	0.779±0.207
Phosphorylated protein							
pBCL-10	G1TB43	0.104±0.018	0.416±0.200	0.757±0.211	0.799±0.256	0.795±0.042	0.844±0.240
pPARP1	G1TEI0	0.141±0.050	0.694±0.120	0.568±0.073	1.023±0.024	0.727±0.117	0.581±0.006

Table S4. The expression of differential proteins/differential in HIF-1α pathway.

KEGG Name	Accession	CTR-4m	PCL-4m	SAPH-R3@ PCL-4m	CTR-6m	PCL-6m	SAPH-R3@ PCL-6m
Protein							
4EBP1	G1TEF8	1.432±0.011	0.909±0.207	0.937±0.089	0.825±0.070	1.006±0.220	1.082±0.173
CamK	O77708	0.720±0.016	0.809±0.229	1.164±0.289	0.869±0.094	1.084±0.060	0.974±0.038
eIF4E	A0A5F9DDW9	0.781±0.010	1.111±0.079	0.973±0.041	0.952±0.047	0.922±0.031	0.962±0.095
ENO1	A0A5F9D287	0.563±0.034	1.039±0.059	1.088±0.168	0.883±0.166	1.077±0.032	1.006±0.006
ENO1	P25704	0.611±0.176	0.656±0.178	1.076±0.365	0.752±0.226	1.351±0.090	1.035±0.310
HK	A0A5F9CVK2	1.163±0.089	1.047±0.189	1.124±0.159	0.808±0.076	1.117±0.078	0.913±0.063
HK	G1SRI8	0.817±0.065	1.044±0.069	1.081±0.037	0.941±0.012	0.946±0.025	1.011±0.062
HMOX1	A0A5F9DD47	0.724±0.048	0.970±0.071	1.098±0.059	0.937±0.053	1.045±0.090	1.114±0.040
LDHA	A0A5F9DPL1	0.607±0.002	0.959±0.007	0.950±0.176	1.026±0.167	1.06±0.0890	0.944±0.025
LDHA	G1TAJ3	0.551±0.065	0.762±0.143	1.066±0.207	0.778±0.156	1.268±0.055	1.054±0.179
PDK1	A0A5F9DMI0	1.111±0.041	1.003±0.032	0.882±0.050	1.101±0.167	0.927±0.136	0.903±0.216
PFKL	A0A5F9DGX0	0.821±0.110	0.606±0.121	0.984±0.367	0.785±0.255	1.222±0.051	1.084±0.225
PI3K	G1TVC1	1.573±0.273	1.003±0.089	0.902±0.030	1.076±0.050	0.899±0.099	0.884±0.182
rpS6	A0A5K1UJS7	0.736±0.041	1.007±0.256	0.981±0.039	0.947±0.116	0.931±0.072	1.081±0.039
TFRC	G1TCW1	0.810±0.032	1.033±0.392	0.792±0.047	1.040±0.133	0.927±0.097	1.041±0.023
TIMP1	A0A5F9CLN4	2.501±0.031	1.135±0.225	1.547±0.303	1.197±0.111	1.217±0.035	1.177±0.119
Phosphorylated protein							
pALDOA	G1T652	0.264±0.137	0.586±0.092	0.691±0.063	0.930±0.057	0.945±0.048	1.028±0.238
pALDOA	P00883	0.079±0.029	0.288±0.062	0.596±0.225	0.328±0.116	0.725±0.113	0.727±0.311
pALDOA	P00883	0.117±0.078	0.308±0.107	0.723±0.249	0.432±0.161	1.004±0.108	0.636±0.196
pALDOA	P00883	0.117±0.079	0.328±0.104	0.737±0.251	0.438±0.173	1.034±0.047	0.683±0.192
pALDOA	P00883	0.125±0.074	0.337±0.099	0.722±0.247	0.421±0.157	1.033±0.049	0.659±0.183
pALDOA	P00883	0.151±0.027	0.383±0.120	0.685±0.165	0.456±0.197	0.775±0.091	0.738±0.213
pALDOA	P00883	0.152±0.014	0.500±0.218	0.774±0.124	0.605±0.112	0.879±0.104	0.768±0.304
pALDOA	P00883	0.152±0.113	0.331±0.117	0.678±0.205	0.433±0.194	1.073±0.075	0.672±0.206
pALDOA	P00883	0.211±0.111	0.538±0.184	0.822±0.143	0.715±0.031	0.949±0.094	0.756±0.144
pALDOA	P00883	0.284±0.144	0.501±0.121	0.793±0.188	0.737±0.105	0.929±0.060	0.806±0.174
pCamK	O77708	0.148±0.005	0.400±0.208	0.763±0.441	0.613±0.197	0.857±0.069	0.649±0.095
pHMOX1	G1SZP6	0.204±0.074	0.530±0.022	0.438±0.168	0.872±0.049	0.673±0.232	0.828±0.155
pHMOX1	G1SZP6	0.238±0.031	0.414±0.168	0.480±0.019	0.843±0.207	0.695±0.156	0.897±0.335
pPHD	U3KPI6	0.222±0.051	0.442±0.183	0.936±0.381	0.767±0.136	0.901±0.055	0.947±0.400

Table S5. The expression of differential proteins in chemokine signalling, leukocyte migration, or platelet activation

KEGG Name	Accession	CTR-4m	PCL-4m	SAPH-R3@ PCL-4m	CTR-6m	PCL-6m	SAPH-R3@ PCL-6m
Protein							
ACTN1	A0A5F9CV24	0.516±0.114	1.224±0.078	1.170±0.462	0.795±0.141	1.003±0.174	0.976±0.157
ACTN2	G1TEM1	0.719±0.024	0.765±0.351	1.058±0.369	0.813±0.275	1.193±0.060	1.137±0.529
ACTN3	G1U4H8	0.554±0.018	0.619±0.232	0.942±0.235	0.742±0.213	1.275±0.150	1.350±0.482
ACTN4	A0A5F9D4S1	0.674±0.062	0.888±0.143	1.050±0.075	0.960±0.034	0.995±0.047	1.093±0.253
ARRB1	A0A5F9CCH4	0.788±0.128	0.996±0.114	0.759±0.107	1.115±0.241	0.957±0.057	1.109±0.035
BTK	G1SZN9	1.406±0.001	0.940±0.055	0.841±0.100	0.955±0.037	0.898±0.107	0.878±0.125
CDC42	G1U978	0.738±0.068	1.143±0.216	0.898±0.191	0.906±0.186	1.005±0.047	1.089±0.173
COL1A1	A0A5F9CXS8	6.445±0.815	0.489±0.264	0.518±0.127	0.897±0.64	1.029±0.397	0.449±0.019
COL1A1	G1T4A5	5.928±1.316	0.536±0.255	0.545±0.085	0.964±0.691	1.001±0.105	0.433±0.100
COL1A2	G1T2Z5	5.324±1.185	0.610±0.261	0.620±0.078	1.044±0.776	0.956±0.105	0.487±0.081
COL3A1	A0A5F9C9Q2	6.050±1.541	0.519±0.254	0.551±0.117	1.045±0.828	0.943±0.107	0.414±0.094
(C-X-C motif chemokines)	Q5VI85	0.857±0.092	1.133±0.26	0.790±0.261	1.086±0.062	0.866±0.014	1.187±0.038
FCER1G	A0A5F9CIF9	1.181±0.001	1.155±0.253	0.781±0.057	1.004±0.055	0.991±0.020	0.995±0.063
FERM	G1SCP8	0.580±0.050	0.908±0.032	1.122±0.172	0.926±0.124	1.048±0.043	1.072±0.153
FOXO3	G1SZC6	5.139±0.087	1.130±0.581	1.174±0.202	0.703±0.313	1.178±0.162	0.927±0.026
GNAI2	G1TRG8	0.713±0.040	1.02±0.029	0.975±0.128	0.997±0.110	1.001±0.04	1.044±0.098
GNAI3	G1SP68	0.806±0.017	1.068±0.093	1.100±0.249	0.973±0.025	0.976±0.086	0.949±0.111
GNAQ	G1TBW7	0.761±0.096	0.878±0.181	1.014±0.057	0.983±0.068	1.108±0.071	1.100±0.072
GNG5	A0A5F9CPL9	1.248±0.045	0.927±0.168	0.881±0.096	1.022±0.255	1.025±0.08	1.107±0.19
GPIIb	Q9TUN4	1.341±0.091	1.059±0.151	0.852±0.08	1.071±0.109	0.896±0.06	0.999±0.093
GRB2	G1SR27	0.735±0.100	1.046±0.033	1.002±0.092	0.926±0.076	0.978±0.018	0.972±0.023
ITGAL	A0A5F9C4C9	0.848±0.025	1.024±0.089	1.053±0.161	0.86±0.041	1.156±0.045	1.184±0.098
ITGAM	A0A5F9C476	1.099±0.066	0.891±0.241	0.792±0.086	1.225±0.183	0.929±0.075	0.969±0.011
ITGB	A0A5F9CHW0	1.471±0.043	0.992±0.023	0.905±0.050	1.023±0.059	0.955±0.052	0.945±0.083
LYN	A0A5F9CT28	0.820±0.005	1.047±0.035	0.879±0.026	1.076±0.173	0.931±0.077	0.890±0.033
MYL12B	G1T7S0	0.402±0.001	0.851±0.286	1.078±0.113	0.841±0.278	0.765±0.058	1.213±0.412
MYLPF	P02608	0.679±0.085	0.543±0.292	1.003±0.390	0.655±0.335	1.306±0.188	1.489±0.638
PECAM1	A0A5F9DIF8	1.367±0.146	1.065±0.163	1.022±0.197	0.859±0.083	0.981±0.117	0.928±0.069
PIK3CD	G1TVC1	1.573±0.273	1.003±0.089	0.902±0.03	1.076±0.050	0.899±0.099	0.884±0.182
PPP1CC	G1SWW8	0.754±0.019	1.203±0.019	0.920±0.151	1.082±0.081	0.891±0.090	0.955±0.070
PPP1R12A	G1TC70	0.800±0.034	0.952±0.091	1.014±0.009	0.938±0.083	0.918±0.041	1.038±0.110
Rac2	G1TAX7	0.632±0.038	1.038±0.128	0.850±0.110	1.112±0.289	0.883±0.113	0.941±0.081
RAP1A	A0A5F9D613	0.726±0.038	1.079±0.049	0.960±0.134	0.95±0.092	0.913±0.029	1.066±0.104
RAP1B	A0A5F9D525	0.680±0.065	1.042±0.046	0.986±0.011	0.902±0.098	0.955±0.138	1.216±0.137
(RGS domain-containing protein)	A0A5F9D1C3	0.746±0.057	1.016±0.131	0.898±0.014	1.089±0.125	0.896±0.069	0.968±0.116
RhoA	G1T567	0.611±0.065	1.122±0.093	0.997±0.062	0.951±0.077	0.953±0.013	1.031±0.110

STAT1	A0A5F9D806	0.731±0.092	1.167±0.210	0.953±0.175	0.976±0.176	1.103±0.061	0.950±0.074
(Tau kinase)	G1U0J7	0.851±0.019	1.128±0.022	1.044±0.165	0.858±0.008	0.935±0.101	1.054±0.164
Uncharacterized	G1SHY0	1.184±0.048	0.841±0.054	0.939±0.041	1.070±0.059	0.964±0.007	1.036±0.120
Uncharacterized	A0A5F9DKI8	0.807±0.077	0.996±0.073	0.943±0.082	1.047±0.041	0.989±0.011	0.957±0.050
VAMP8	G1SHE6	1.239±0.051	1.031±0.214	0.873±0.057	1.110±0.042	1.030±0.259	0.955±0.075
VCAM1	A0A5F9DFM7	1.119±0.022	1.069±0.060	0.813±0.068	1.171±0.095	0.992±0.083	0.779±0.207

Table S6. The expression of differential proteins involved in other pathways.

KEGG Name	Accession	CTR-4m	PCL-4m	SAPH-R3@ PCL-4m	CTR-6m	PCL-6m	SAPH-R3@ PCL-6m
Protein							
14-3-3	A0A5F9DHA9	0.574±0.059	0.888±0.044	1.026±0.055	0.965±0.039	1.058±0.052	1.124±0.122
14-3-3	G1SDY5	0.585±0.030	0.979±0.082	1.033±0.067	0.923±0.136	1.039±0.057	1.118±0.142
14-3-3	G1SIT9	0.652±0.090	0.895±0.085	0.993±0.086	1.044±0.136	1.047±0.140	1.129±0.161
14-3-3	G1SZD6	0.788±0.054	0.909±0.006	0.975±0.051	1.022±0.062	1.011±0.043	1.038±0.117
14-3-3	G1T7R2	0.500±0.022	0.876±0.009	1.024±0.130	1.044±0.039	1.023±0.054	1.046±0.037
4EBPs	G1TEF8	1.432±0.011	0.909±0.207	0.937±0.089	0.825±0.070	1.006±0.220	1.082±0.173
COL1A1	A0A5F9CXS8	6.445±0.815	0.489±0.264	0.518±0.127	0.897±0.640	1.029±0.397	0.449±0.019
COL1A1	G1T4A5	5.928±1.316	0.536±0.255	0.545±0.085	0.964±0.691	1.001±0.105	0.433±0.100
COL1A2	G1T2Z5	5.324±1.185	0.610±0.261	0.620±0.078	1.044±0.776	0.956±0.105	0.487±0.081
COL2A1	G1T5V9	3.964±1.462	0.664±0.189	1.442±1.069	0.859±0.499	0.793±0.152	0.419±0.049
eIF4E	A0A5F9DDW9	0.781±0.010	1.111±0.079	0.973±0.041	0.952±0.047	0.922±0.031	0.962±0.095
IBSP	G1SEM1	3.317±0.288	0.928±0.354	0.756±0.109	1.157±0.650	0.837±0.073	0.637±0.041
ITGA6	G1SD83	1.160±0.148	0.848±0.076	0.903±0.082	0.996±0.054	1.136±0.132	1.054±0.076
ITGAL	A0A5F9C4C9	0.848±0.035	1.024±0.109	1.053±0.197	0.860±0.050	1.156±0.055	1.184±0.120
ITGAM	A0A5F9C476	1.098±0.093	0.891±0.295	0.792±0.106	1.225±0.224	0.929±0.092	0.969±0.014
ITGB3	A0A5F9CHW0	1.471±0.060	0.992±0.028	0.905±0.062	1.023±0.072	0.955±0.064	0.945±0.102
ITGB4	A0A5F9DC01	0.829±0.059	0.873±0.200	1.065±0.110	0.845±0.104	1.164±0.158	1.157±0.300
LAMA2	G1SX80	1.589±0.223	0.893±0.251	0.900±0.134	0.917±0.374	1.011±0.061	1.034±0.194
LAMA3	A0A5F9DGZ2	1.269±0.103	0.844±0.128	0.908±0.066	1.134±0.145	1.142±0.083	0.870±0.101
S6	A0A5K1UJS7	0.736±0.041	1.007±0.256	0.981±0.039	0.947±0.116	0.931±0.072	1.081±0.039
SPP1	P31097	3.392±0.584	0.986±0.323	0.622±0.062	1.115±0.605	0.813±0.031	0.716±0.088
TGFβ	A0A5F9CDR7	2.147±0.169	1.054±0.133	1.379±0.107	1.027±0.070	1.650±0.096	1.536±0.101
TIMP1	A0A5F9CLN4	2.501±0.044	1.135±0.318	1.547±0.429	1.197±0.157	1.217±0.050	1.177±0.168
PDGFRL	G1SNF7	1.420±0.226	0.627±0.087	1.330±0.725	0.899±0.142	0.930±0.064	1.006±0.142
Phosphorylated protein							
(p) 14-3-3	G1TZP0	0.182±0.011	0.471±0.112	0.573±0.152	0.531±0.055	1.060±0.169	0.885±0.157
pCOL1A2	G1T2Z5	3.336±0.271	0.831±0.151	0.647±0.122	1.921±1.094	1.595±0.287	1.002±0.146
pFGF2	G1SDD5	0.145±0.069	0.563±0.103	0.773±0.151	0.733±0.200	0.938±0.237	1.171±0.344
pIBSP	G1SEM1	0.426±0.026	0.507±0.135	0.756±0.249	0.718±0.210	0.987±0.082	1.011±0.208
pIBSP	G1SEM1	1.124±0.168	0.566±0.129	0.653±0.158	0.899±0.198	0.985±0.090	0.840±0.080
pIBSP	G1SEM1	1.755±0.174	0.825±0.265	0.557±0.174	1.279±0.729	1.021±0.176	0.805±0.134
pMRC1	A0A5F9DHB6	0.185±0.035	0.724±0.561	1.100±0.410	0.724±0.287	0.724±0.062	0.882±0.103
pMSR1	A0A5F9C937	0.311±0.028	n.d.	0.847±0.306	0.765±0.066	0.881±0.094	0.819±0.160
pSPP1	P31097	1.191±0.113	0.735±0.273	0.654±0.199	0.725±0.088	0.933±0.316	0.564±0.195
pSPP1	P31097	1.290±0.123	0.783±0.474	0.605±0.142	0.702±0.068	0.850±0.191	0.526±0.083
pSPP1	P31097	1.529±0.206	0.827±0.474	0.732±0.183	0.647±0.103	0.857±0.150	0.634±0.216
pSPP1	P31097	1.704±0.202	0.682±0.226	0.607±0.158	0.923±0.283	1.042±0.251	0.576±0.184
pSPP1	P31097	1.844±0.001	0.871±0.401	0.715±0.211	0.747±0.081	0.871±0.161	0.597±0.211
pYAP/TAZ	G1TYX6	0.312±0.023	0.628±0.037	0.919±0.337	0.617±0.174	0.840±0.048	0.790±0.178

pYAP/TAZ	YAP1	0.355±0.061	0.412±0.209	0.840±0.105	0.924±0.127	0.889±0.051	0.911±0.176

** n.d.: not detected*

Experimental Section

1. Self-assembling peptide hydrogel synthesis

The FEK8 and FEK18 peptides, with structure shown in Fig. S1, were synthesized according to previously reported methods.[1] To prepare peptide solutions, 20.00 mg of FEK8 (18 μmol) or 20.86mg FEK18 (equivalent to 18 μmol FEK8) powders were dissolved into 1mL of Milli Q water under magnetic stirring, respectively. A series of synthetic peptide hydrogels were prepared based on peptide self-assembly, denoted as SAPH18, SAPH-R1, SAPH-R2, SAPH-R3, and SAPH8 according to their FEK18:FEK8 molar ratios (1:0, 1:2, 1:4, 1:6, and 0:1, respectively). To form the SAPH8 and SAPH18, the FEK8 and FEK18 solutions were heated at 80°C for 3h, followed by cooling down to ambient temperature and adjusting pH to 7.2, respectively. Similarly, the SAPH-R1, SAPH-R2, and SAPH-R3 hydrogels were prepared using this method by adjusting the FEK18/FEK8 solutions to 4.5mM/9mM, 3mM/12mM, and 2.25mM/13.5mM, respectively.

2. Preparation of SAPH-PCL composite scaffolds

The PCL scaffold model was designed by Auto CAD (version 2018) and printed by a fused deposition modeling (FDM) 3D printer (Creality CR-2020, Shenzhen, China) using the following parameters: nozzle diameter 0.4 mm, angle 0° and 90°, layer height 0.2 mm, filling rate 55%. To prepare SAPH-PCL composite scaffolds, the PCL scaffold was soaked in corresponding hydrogels for 12h, with occasional vacuum to degas. Accordingly, the SAPHs were infilled into 3D printed PCL scaffolds, denoted as SAPH-R1@PCL, SAPH-R2@PCL, and SAPH-R3@PCL, respectively.

3. Circular dichroism

The SAPH8, SAPH18, SAPH-R1, SAPH-R2, and SAPH-R3 samples were diluted at 1:150, then applied to a Chirascan circular dichroism spectrometer (Applied Photophysics, Leatherhead, UK). The spectrum between 185 to 245 nm was recorded, respectively.

4. Water contact angle

The water contact angles of the PCL, SAPH-R1@PCL, SAPH-R2@PCL, and SAPH-R3@PCL were detected (n=3) by a static water contact angle meter (Maishi DropMeter A100P, Ningbo, China), respectively.

5. Scanning electron microscopy observation

The PCL, SAPH-R1@PCL, SAPH-R2@PCL and SAPH-R3@PCL were snap-frozen at -20 °C and freeze-dried, respectively. Each sample was gold-plated using a JFC-1600 photoresister (JEOL Akishima, Japan) and the morphology was observed using an SEM (JSM-6510, JEOL) with an accelerating voltage of 5.0 kV.

PCL scaffold with infill density ranging from 30% to 70% were prepared and their pore sizes in SEM images were quantified by measuring the diameters of randomly picked 10 positions.

6. Mechanical characterization

Oscillating rheology analysis was carried out in SAPH-R1, SAPH-R2, and SAPH-R3 samples at 1% strain. The elastic moduli (G`) and viscous moduli (G``) were documented, respectively. An electronic universal material testing machine (Z050, Zwick/Roell) was used to test PCL scaffolds with infill density from 30% to 70%, or the SAPH-PCL composite scaffolds with fixed 55% infill density. The measurement was carried out by compression or tension under a speed of 1mm/min.

7. Thioflavin T (ThT) fluorescence assay

ThT solutions were added to the SAPH8, SAPH-R1, SAPH-R2, and SAPH-R3 solutions to make a final ThT concentration of 50 μM, respectively. After 5 min incubation, 100 μL of supernatant from each sample was transferred to a 96-well plate to record the emission spectrum from 460 to 550 nm under 442 nm excitation wavelength using a fluorescence microplate reader (BioTek Synergy 2, Winooski, VT).

8. Proteinase K degradation

For each sample, 1 mL SAPH was digested using proteinase K (Aladdin, Shanghai, China) at a final concentration of 4 U mL-1 at 37 °C with 200 rpm shaking. The degradation of SAPHs was analyzed using a high performance liquid chromatography (DIONEX 3000 system, Germany) and quantified by corresponding FEK8 peak area integrated at predetermined time points (0, 2, 4, 8, and 12 h), respectively.

9. Hemolytic assay

Fresh blood was collected from rabbit ears. After adding heparin as an anticoagulant, the red blood cells were collected by centrifugation at 1500 rpm for 15 min, then rinsed by 0.9% NaCl. The 50 μL of 5% red blood cell suspension was incubated with the SAPH-R1@PCL, SAPH-R2@PCL, SAPH-R3@PCL and the PCL scaffold (5mm×1mm, n=3) in a 96-well plate, respectively. Simultaneously, 50 μL of 5% red blood cells incubated with 200 μL of distilled water and 0.9% NaCl were used as positive and negative controls. After incubation at 37 °C for 1-5h, the supernatant was collected to measure the absorbance at 576 nm (n=3) using a UV spectrophotometer (Thermo EVOLUTION201, Waltham, MA). The hemolysis rate (%) was calculated by the following equation:

Hemolysis rate (%) = (A sample - A negative control) / (A positive control - A negative control) * 100%.

10. Cell culture

Rabbit BMSCs were obtained as described in our previous study.[2] In brief, bone marrow from rabbit legs was flushed out using cold PBS and cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. The medium was replenished every 3 days. After 10 days, the BMSCs were collected by 0.25% trypsin digestion for further works. Endothelial cells (ECs) were purchased from Procell CO., LTD (Wuhan, China) and cultured in an endothelial-specific culture medium (Procell). The BMSC/EC co-culture was achieved by 1:1 mixing of BMSCs and ECs in a mixture of DMEM and the endothelial-specific culture medium (equal volume). All the cells were cultured in a humidified incubator maintained at 37 °C with 5% CO2. All experiments were done in triplicate if no specific mention.

11. In vitro migration simulation

BMSCs were seeded in a 6-well plate at a density of 2×105 cells/well. When cells reached 90% confluency, 1mm in-width scratches were made using 200 μL pipette tips. After rinse, the PCL, SAPH-R1@PCL, SAPH-R2@PCL and SAPH-R3@PCL were put into the wells for another 24 h incubation in serum free medium, respectively. The migration was determined by measuring the scratch width before and after incubation using a CX23 microscope (Olympus, Japan) The migration distance was measured by Image J (version 1.53) and the migration rate was calculated sequentially.

12. Cell proliferation and live/dead staining

The PCL, SAPH-R1@PCL, SAPH-R2@PCL and SAPH-R3@PCL (Φ5mm×1mm) were soaked into 96-well plates containing 200 μL medium, respectively. To determine the cell proliferation, BMSCs, ECs, and BMSC/EC co-culture were seeded onto the scaffolds in corresponding wells at a density of 5×103 cells/well, respectively. After 1, 4, and 7 days of incubation, each sample was incubated with 10 μL of CCK-8 working solution (KeyGEN BioTech, China) for 1 h at 37 °C. Then the supernatant was transferred into a new plate to measure the absorbance at 450 nm using a microplate reader (BioTek Synergy 2).

To determine the live/dead cells, the cells were seeded using the same method as described at a density of 2×104 cells/well. After 3 days of incubation, the cells were stained with a Live/Dead staining kit (KeyGEN BioTech, China). The samples were then observed using a fluorescence microscope (XDS30, Shunyu, China) to detect the calcein AM-related live cell signals and PI-related dead cell signals.

13. Alkaline phosphatase (ALP) activity detection

The ALP activities were tested using an ALP activity kit (Beyotime, China). In brief, BMSCs, ECs, and BMSC/EC co-culture cells were seeded in a 96-well plate at 5×103 cells/well, respectively. After culturing with pre-soaked scaffolds (Φ5mm×1mm) for 7 and 14 days, the cells were collected and lysed on ice for 5 min using a lysis buffer. Then 50 μL supernatant from the lysate was mixed with 50 μL of substrate solution and incubated 30 min at 37 °C. A series of p-nitrophenol standards were prepared using the same method. After adding 100 μL of stopping solution, the UV absorbance at 405nm was recorded using a microplate reader (BioTek Synergy 2) to calculate the ALP concentration. Simultaneously, the total protein concentration of each lysate supernatant was quantified using a BCA protein assay kit (Beyotime, China).

14. Alizarin red S staining

BMSCs, ECs, and BMSC/EC co-culture cells were seeded in 6-well plates at 2×104/well and incubated with PCL, SAPH-R1@PCL, SAPH-R2@PCL, or SAPH-R3@PCL scaffold (Φ33mm×1mm) for 14 days, respectively. Then the cells and corresponding scaffolds were rinsed with PBS and fixed with 4% paraformaldehyde for 20 min. To determine the calcium mineralization using an alizarin red S staining method, the fixed cells were washed 3 times with PBS, followed by staining with alizarin red S solution (Beyotime, China) for 30 min at room temperature. After staining, the samples were rinsed and pictured under an invert phase contrast microscope.

15. Rabbit handling

Female New Zealand white rabbits (2.5-3.0 kg, 6 month-old) were purchased from Hengtai Experimental Animal Breeding CO., LTD (Wuxi, China). All animal handling procedures were approved by the ethics committee of Nanjing First Hospital (DWSY-22081207).

To establish a segmental ulna defect model, about 30% in length of the anterior ulna (1.2 cm of 4.0 cm) was removed by surgery under xylazine anaesthetizing. The PCL, SAPH-R1@PCL, SAPH-R2@PCL, and SAPH-R3@PCL scaffolds (Φ6mm×12mm) were implanted into the defected bone before incision stapling, respectively. For the CTR, the incision was stapled directly without any implantation to the defected ulna. To prevent infections, 20U of penicillin were injected intramuscularly to each rabbit after stapling and 3 days post surgery. After 4 and 6 months, the rabbits were sacrificed and the anterior legs (including radius and ulna) were peeled out for further experiments.

16. Micro-CT scanning and bone mass analysis

The defected ulna was cut off and scanned using a SkyScan 1176 Micro-CT machine (Bruker, Germany) at 70KV voltage and 9 μm pixel. The results were reconstructed to obtain the 3 dimensional models of bone.

Based on the 3D reconstruction, bone mass analysis was performed using Bruker's CTan software (Version 1.6.10.1) to determine the bone volume/tissue volume (BV/TV), the trabecular thickness (Tb.Th), and the bone mineralization density (BMD).

17. Histological staining

After Micro-CT scanning, the samples were fixed in 4% paraformaldehyde solution for 24 h, rinsed with running water overnight, and soaked in 10% EDTA to remove the mineralized tissue. Then the treated samples were rinsed and treated with ethanol gradient and xylene, followed by paraffin embedding. After cutting into 5 μm-in-thick sections using a microtome, the specimens were stained according to the standard Masson and H&E staining protocol. The specimens were imaged and assessed by histologists in a label-free manner, then typical images were chosen to present the whole specimen status.

18. Protein labeling, enrichment, and LC-MS/MS analysis

TMT labeled proteomic samples were prepared using filter aided proteome preparation (FASP) method as described previously.[3] In brief, tissue samples after 10% EDTA softening were lysed by SDT buffer (4% SDS, 100 mM Tris-HCl, and 1 nM DTT, pH 7.6), followed by BCA quantification. Then 200 μg proteins from each sample were rinsed with 8 M urea and 150 mM Tris-HCl (pH 8.0), followed by incubating with iodine acetamide for 30 min in dark. Each sample was digested with 4 μg of trypsin in 25 mM NH4HCO3 buffer at 37 °C overnight. The obtained peptide solutions were desalted using a C18 Cartidge column. For each sample, 100 μg of peptides were labeled with TMT (Thermo Fisher) according to the manufactory's instructions. The TMT-labelled peptides were fractionated using a Pierce high-pH reversed phase peptide fractionation kit (Thermo Fisher), then each flow-through was re-dispersed in 12 μL of 0.1% formic acid for sample injection. For phosphopeptide enrichment, the TMT-labelled samples were enriched using a high-select Fe-NTA phosphopeptides enrichment kit (Thermo Fisher), then redissolved in 20 μL of 0.1% formic acid for analysis.

After TMT labeling and Fe enrichment, the peptide samples/ phosphopeptide samples were separated using an Easy-nLCTM system (Thermo Fisher) equipped with Acclaim PepMap 100 C18 and Easy-column C18-A2 columns. The chromatographically separated samples were analyzed using a Q-Exactive mass LC-MS/MS spectrometer.

19. Quantitative proteomics

Raw data from LC-MS/MS were analyzed using Proteome Discoverer 2.4 database for peptide searching and quantification. Peptide mass tolerance was set as ±20 ppm, with 0.1 Da fragment mass tolerance. Carbamidomethylation on cysteine, TMT 6/10/16 on lysine, and TMT 6/10/16 on peptide N-termini were set

as fixed modifications, while oxidation on methionine, phosphorylation on tyrosine/serine/threonine (only for phosphopeptide samples) were set as variable modifications. The detected results were further quantified by peak integration and normalized by the area normalization method.

20. Proteomic data analysis

Differential proteins and differential phosphorylated proteins were sorted from the quantified protein with a threshold as p-value (from t-test) <0.05 and fold change > 1.2 or < 0.8. Principal component analysis (PCA) was performed using all quantified proteins/phosphorylated proteins and heat maps were generated using those statistically demonstrating differentiation. All identified proteins were employed for gene ontology (GO) annotation using Blast2GO software and plotted by R scripts. Subsequently, the annotated proteins were blasted against the online KEGG database to retrieve their KEGG orthology identifications. Afterward, the enrichment analysis was applied based on Fisher's exact test considering the whole quantified proteins as a background dataset. Benjamini-Hochberg correction for multiple testing was further applied to adjust derived p-values. And only functional categories and pathways with p-values < 0.05 were considered as significant.

21. Statistical analysis

Data analysis was performed using GraphPad Prism 8 software. Unpaired two-tailed t-test was used to compare between two groups. One-way ANOVA was used to compare between multiple groups with Turkeys' post-test. Results were presented as Mean ± SEM, with statistical significant signs (*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$).

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