Supplementary Materials

HLA-B27-mediated activation of TNAP phosphatase promotes pathogenic syndesmophyte formation in ankylosing spondylitis

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Supplementary Methods

Characterization of MSCs (cont.). For adipogenic induction, α-MEM was supplemented with 10% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, 50 μg/mL ascorbate-2 phosphate, 100 nmol/L dexamethasone, 50 μg/mL indomethacin (Sigma-Aldrich) and 10 μg/mL insulin (Gibco). For chondrogenic induction, serum-free α-MEM was supplemented with 50 μg/mL ascorbate-2 phosphate, 100 nmol/L dexamethasone, 50 mg/mL insulin-transferrin-selenium-Premix (Gibco), 10 ng/mL transforming growth factor-β1 (PeproTech), 100 U/ml penicillin, and 100 μg/ml streptomycin. The medium was changed every 3 days. After the appearance of the morphologic features of differentiation, cells were fixed with 4% paraformaldehyde. Cells treated under adipogenic or chondrogenic culture conditions were stained with Oil red-O (Sigma-Aldrich) or Alcian Blue (ScyTek) to assess adipogenic or chondrogenic differentiation.

BMP-2 and DKK-1 measurements. Supernatants of AS MSCs and control MSCs cultured in osteogenic induction medium were collected at days 3 and 7. BMP-2 and DKK-1 levels were measured by ELISA kits (Abcam) according to the manufacturer's instructions using a microplate reader (Spectramax Gemini EM; Molecular Devices).

Measurement of intracellular ALP enzyme activity. A fluorometric ALP Activity Detection kit (Abcam) was used to measure intracellular ALP activity. Cells (5×10^4) were homogenized in

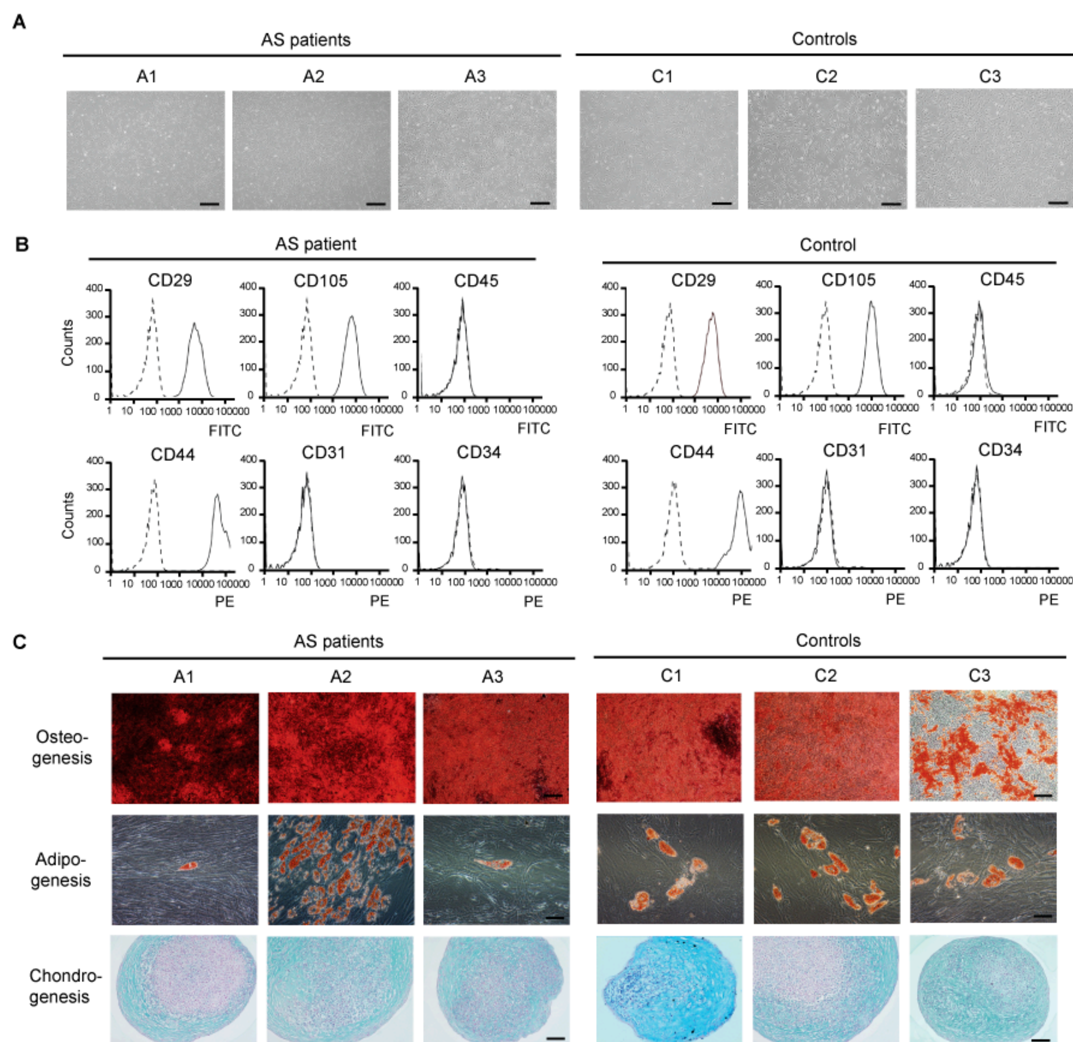
100 µL assay buffer and 4-methylumbelliferyl phosphate disodium salt substrate was added. ALP cleaves the phosphate group of the non-fluorescent 4-methylumbelliferyl phosphate substrate, yielding fluorescent 4-methylumbelliferone. A stop solution was added after 30 min and the fluorescence intensity at excitation/emission wavelengths of 360/440 nm was measured using a Fluorescence Microtiter Plate Reader (Spectramax Gemini; Molecular Devices). ALP activity= A/V/T (mU/mL); A is the amount of 4-methylumbelliferone generated by samples (nmol), V is the volume of sample added in the assay well (mL) and T is reaction time (min).

Human peripheral blood samples and populations (cont.). For disease activity scoring, a validated version of the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI; range, 0-10; scores $\geq$ 4 suggest suboptimal control of disease) is used. For radiographic severity assessment, the mSASSS is applied to assess the anterior parts of the cervical and lumbar vertebrae at 24 spine levels (range 0-3 at each spine level, range: 0-72). The Bath Ankylosing Spondylitis Functional Index (BASFI), a validated index to determine the degree of functional limitation, with the mean visual analogue scale (0 being "easy" and 10 "impossible") is applied to answer the questions in the test. The mean of the ten scales gives the BASFI score a value between 0 and 10. The Bath Ankylosing Spondylitis Global Score (BAS-G) consists of two questions that ask patients to indicate, on a 10cm visual analog scale, the effect of the disease on their well-being over the last week and the last 6 months. The mean of these two scores gives

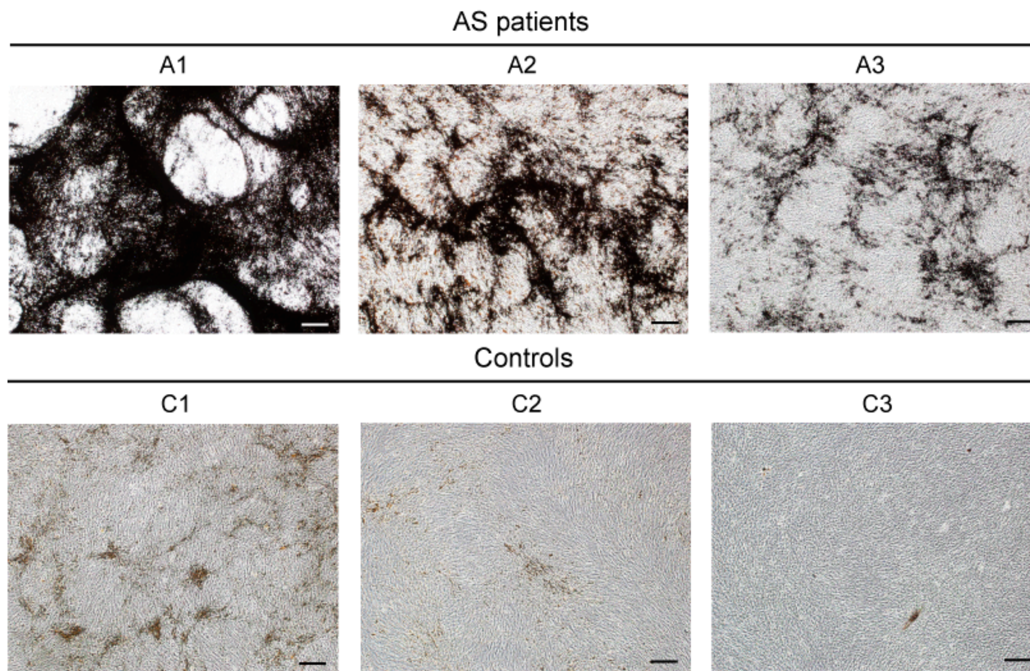
a BAS-G score of 0–10. The higher the score, the greater the perceived effect of the disease on the patient's well-being.

Cytokine release. MSCs were cultured under osteogenic induction to measure cytokine levels. Supernatants were harvested at days 3, 7, and 10. Cytokines were detected using a Human Milliplex kit (Merck Millipore) according to the manufacturer's instructions. Data were acquired by a Luminex 200 instrument (Luminex) and analyzed by Milliplex analyst v5.1 (Merck Millipore).

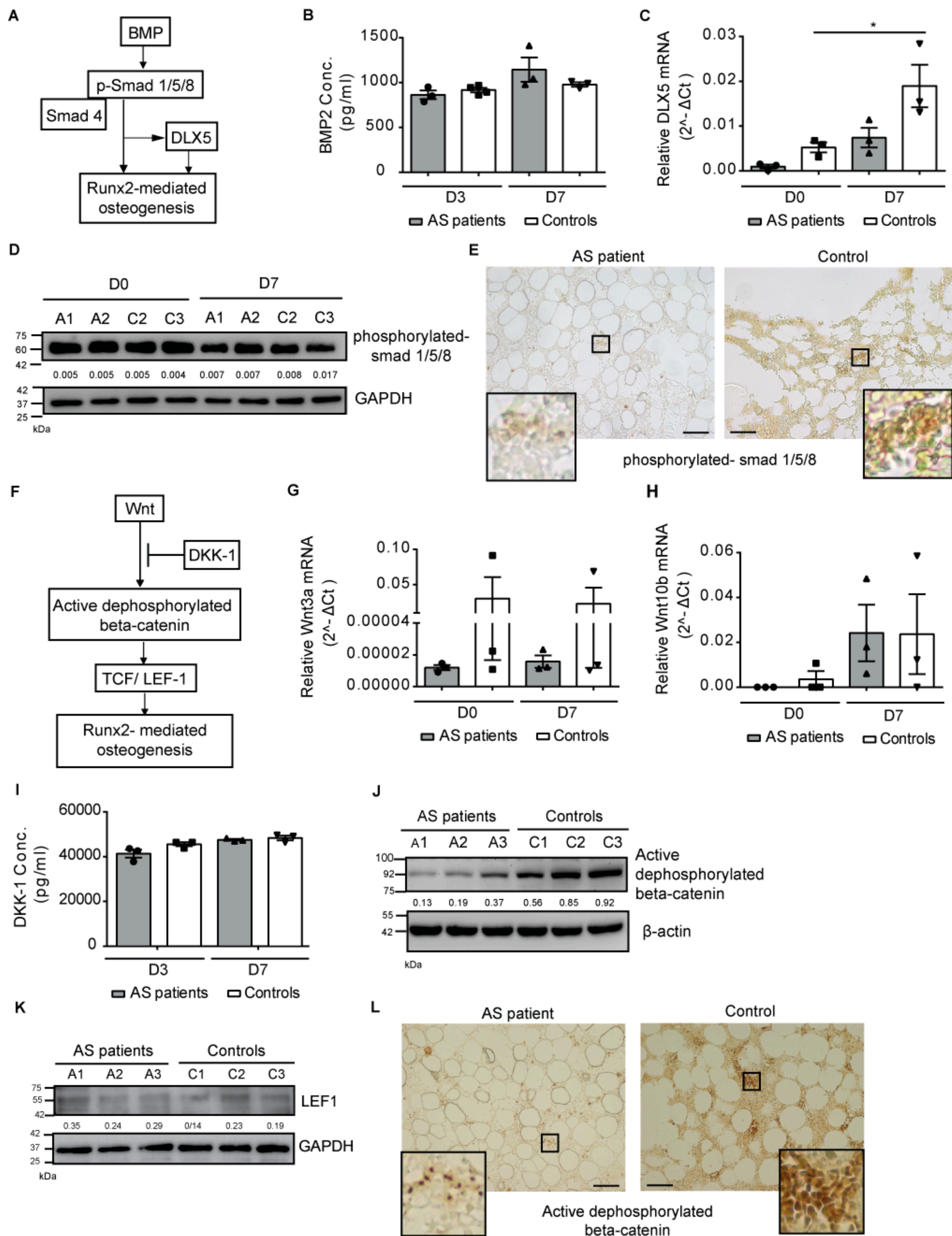
Supplementary Figures



Supplemental Figure 1. Characteristics of MSCs derived from entheseal tissues involved in spinal ankylosis from AS patients (AS MSCs) and non-AS controls (control MSCs). (**A**) Morphology of AS (A1, A2, and A3) MSCs and control (C1, C2, and C3) MSCs upon reaching confluence. Scale bar: 200 μm. (**B**) Flow cytometric analysis of the profiles of surface markers on AS MSCs and control MSCs. Dashed histograms represent staining with an isotype control antibody, mouse IgG1 antibody. The figure is representative of experiments from AS (A1, A2, and A3) MSCs and control (C1, C2, and C3) MSCs with similar results. (**C**) Differentiation potentials of AS (A1, A2, and A3) MSCs and control (C1, C2, and C3) MSCs under specific culture conditions for differentiation to three lineages (osteogenesis: Alizarin red S staining, scale bar: 200 μm; adipogenesis: Oil red-O staining, scale bar: 50 μm; chondrogenesis: Alcian blue staining, scale bar: 50 μm).

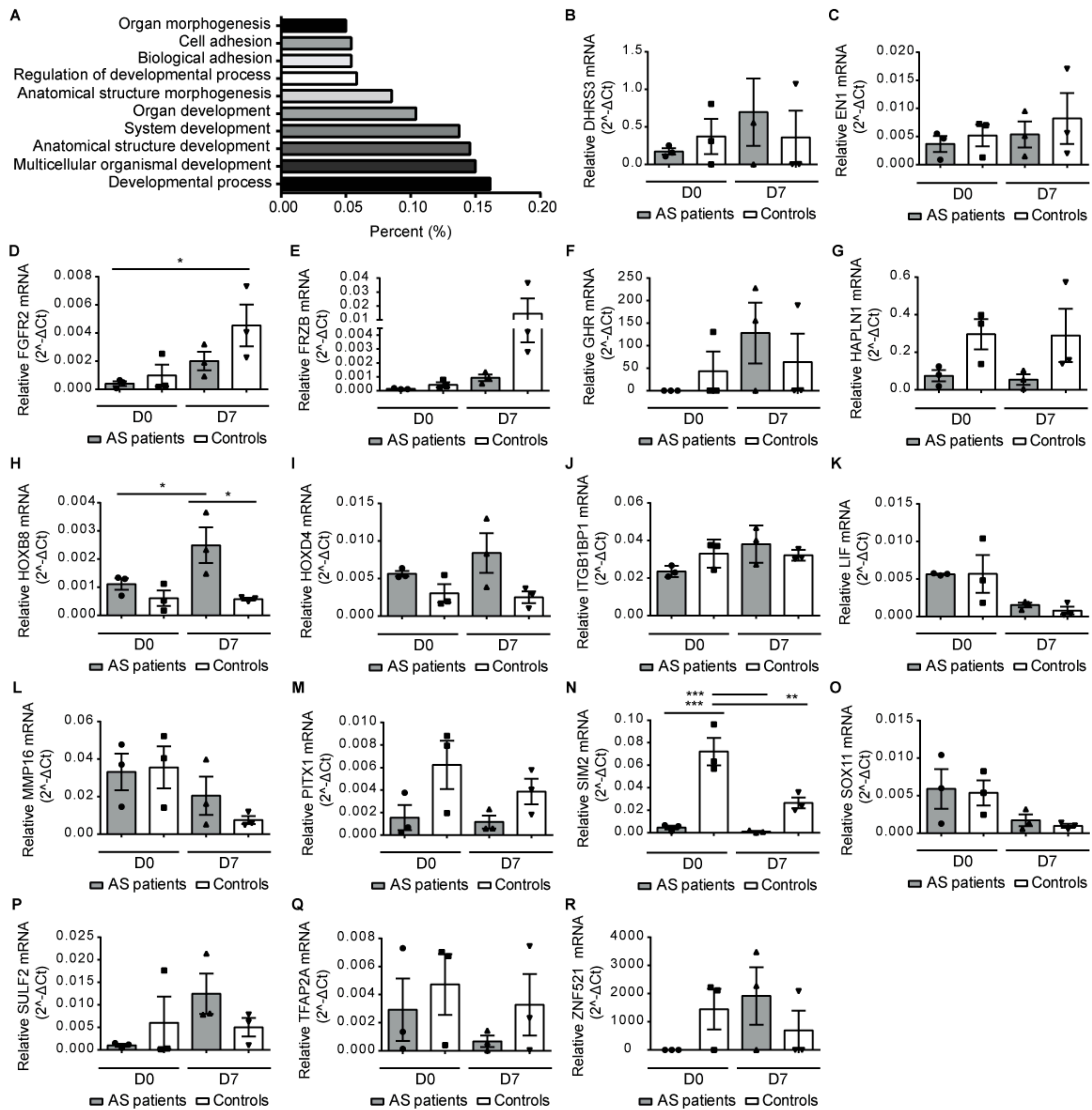


Supplemental Figure 2. von Kossa staining of MSCs under osteogenic induction. von Kossa staining showing enhanced mineralization in AS (A1, A2 and A3) MSCs at day 28 under osteogenic conditions compared with control MSCs (C1, C2 and C3). Scale bar: 200 μm.

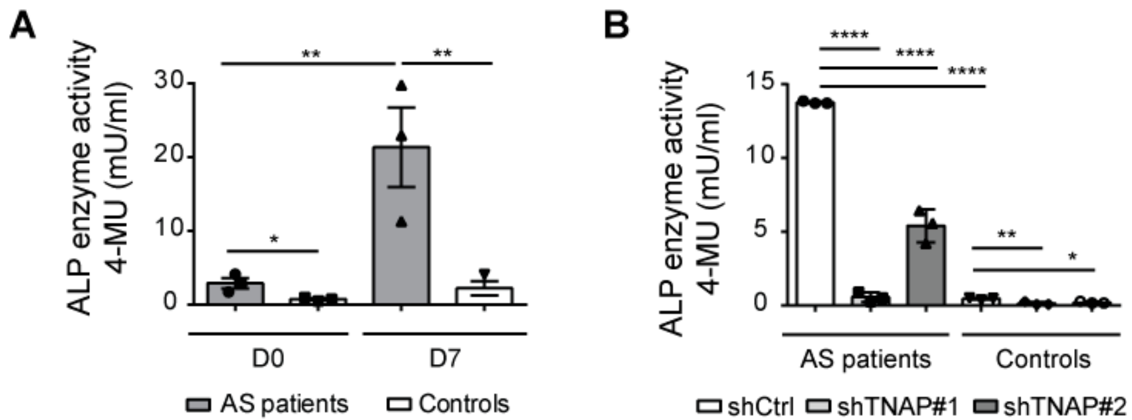


Supplemental Figure 3. BMP and Wnt pathways are not enhanced in *ex vivo* cultures of AS MSCs under osteogenic induction and BM specimens of AS patients. (**A**) Diagram of the BMP pathway in osteogenesis. (**B**) The concentration of BMP-2 from supernatants of AS MSCs and control MSCs at days 3 and 7 under osteogenic induction. (**C**) RT-QPCR of DLX5

expression in AS MSCs and control MSCs at days 0 and 7 under osteogenic induction. (**D**) Immunoblot analysis of phosphorylated-smad1/5/8 expression in AS (A1 and A2) MSCs and control (C2 and C3) MSCs at days 0 and 7 under osteogenic induction. (**E**) Immunohistochemical staining of the BM from AS patients and controls with a phosphorylated-smad1/5/8 antibody. Scale bar: 50 μm. Inset represents a high magnification of the boxed area. (**F**) Diagram of the Wnt pathway in osteogenesis. (**G**) RT-QPCR of Wnt3a mRNA expression in AS MSCs and control MSCs at days 0 and 7 under osteogenic induction. (**H**) RT-QPCR of Wnt10b mRNA expression in AS MSCs and control MSCs at days 0 and 7 under osteogenic induction. (**I**) The concentration of DKK-1 from supernatants of AS MSCs and control MSCs at days 3 and 7 under osteogenic induction. (**J**) Immunoblot analysis of active-dephosphorylated beta-catenin expression in AS (A1, A2, and A3) MSCs and control (C1, C2 and C3) MSCs at day 7 under osteogenic induction. (**K**) Immunoblot analysis of leukocyte enhancing factor (LEF) 1 expression in AS (A1, A2 and A3) MSCs and control (C1, C2 and C3) MSCs at days 7 under osteogenic induction. (**L**) IHC staining of the BM from AS patients and controls with an active-dephosphorylated beta-catenin antibody. Scale bar: 50μm. Inset represents a high magnification of the boxed area. All experiments were done in AS MSCs (A1, A2, and A3) and control MSCs (C1, C2 and C3) with experimental triplicates. Data are the mean ± SEM. *$P < 0.05$ denotes a significant difference by one-way ANOVA, followed by Tukey's HSD test.

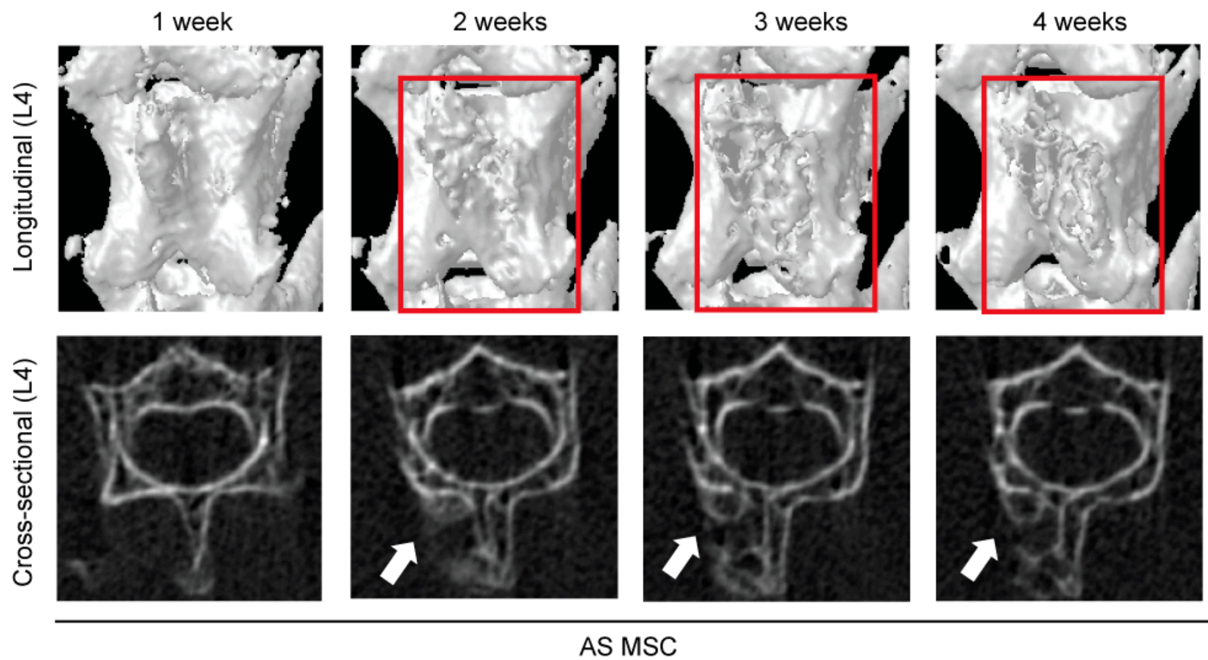


Supplemental Figure 4. Distribution of enriched gene ontology (GO) terms and validation of genes involved in osteogenesis by IPA based on microarray in AS MSCs and control MSCs under osteogenic induction. (**A**) Distribution of the ten most significant enriched GO terms in the biological process ontology showing the functional characteristics of differentially expressed gene sets in AS MSCs and control MSCs. (**B** to **R**) RT-QPCR showing the mRNA levels of *DHRS3* (**B**), *EN1* (**C**), *FGFR2* (**D**), *FRZB* (**E**), *GHR* (**F**), *HAPLN1* (**G**), *HOXB8* (**H**), *HOXD4* (**I**), *ITGB1BP1* (**J**), *LIF* (**K**), *MMP-16* (**L**), *PITX1* (**M**), *SIM2* (**N**), *SOX11* (**O**), *SULF2* (**P**), *TFAP2A* (**Q**), and *ZNF521* (**R**) in AS MSCs (A1, A2 and A3) and control MSCs (C1, C2 and C3) at days 0 and 7 under osteogenic induction. All experiments were done in AS MSCs (A1, A2 and A3) and control MSCs (C1, C2 and C3) with experimental triplicates. Data are the mean ± SEM. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ denote a significant difference by one-way ANOVA, followed by Tukey's HSD test.

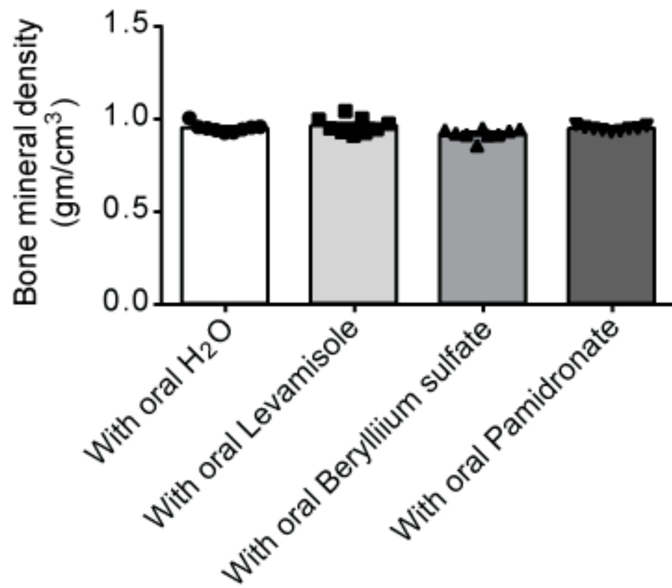


Supplemental Figure 5. Measurements of intracellular ALP enzyme activity. (A) Intracellular ALP enzyme activity in AS MSCs and control MSCs at the indicated days after osteogenic induction. **(B)** Intracellular ALP enzyme activity was suppressed by two independent shRNAs against TNAP in AS MSCs at day 7 under osteogenic induction. All statistical data in the "AS patient group" and "Control group" are from AS MSCs (A1, A2, and A3) and control MSCs (C1, C2, and C3), respectively, with three experimental repeats. Data are the mean ± SEM. *$P < 0.05$, **$P < 0.01$, and ****$P < 0.0001$ denote a significant difference by two-tailed Student's *t*-test (2 groups) or one-way ANOVA, followed by Tukey's HSD test.

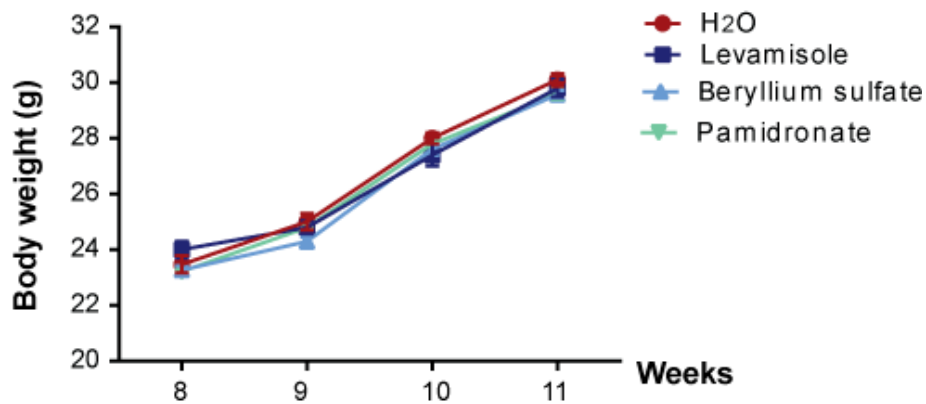
Supplemental Figure 6. Enhanced spontaneous mineralization in AS MSCs (A4, A5 and A6) cultured in growth medium with BGP. **(A)** Additional AS MSCs (A4, A5, and A6) and control MSCs (C3) were cultured in growth medium with BGP. Mineralization at day 20 was determined by ARS staining with quantification (**B**). (**C**) RT-QPCR showing the mRNA level of TNAP in the indicated MSCs at day 7. All experiments were done in AS MSCs (A4, A5 and A6) and control MSCs (C3) with experimental triplicates. Data are the mean ± SEM. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, and $****P < 0.0001$ denote a significant difference by one-way ANOVA, followed by Tukey's HSD test. Scale bar: 200 µm.



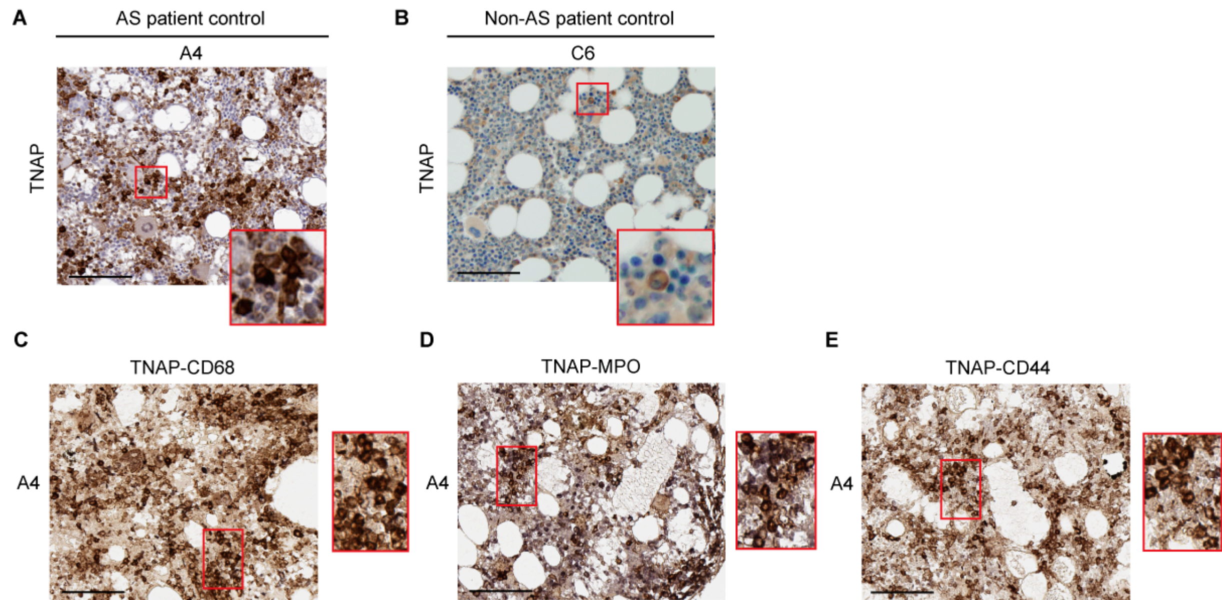
Supplemental Figure 7. Time sequence of the development of new bony appositions in NOD-SCID mice implanted with AS MSCs. Representative images of lumbar spine micro-computed tomography of NOD-SCID mice implanted with AS MSCs (derived from A1 with triplicates) in the artificial cortical defect of the right lamina of lumbar spine segment L4 at the indicated time points (1, 2, 3, and 4 weeks) after implantation. Longitudinal view over L4 (upper) and cross-sectional view over L4 (lower) are shown. New bony appositions are highlighted by a red rectangle (middle) and white arrow (right).



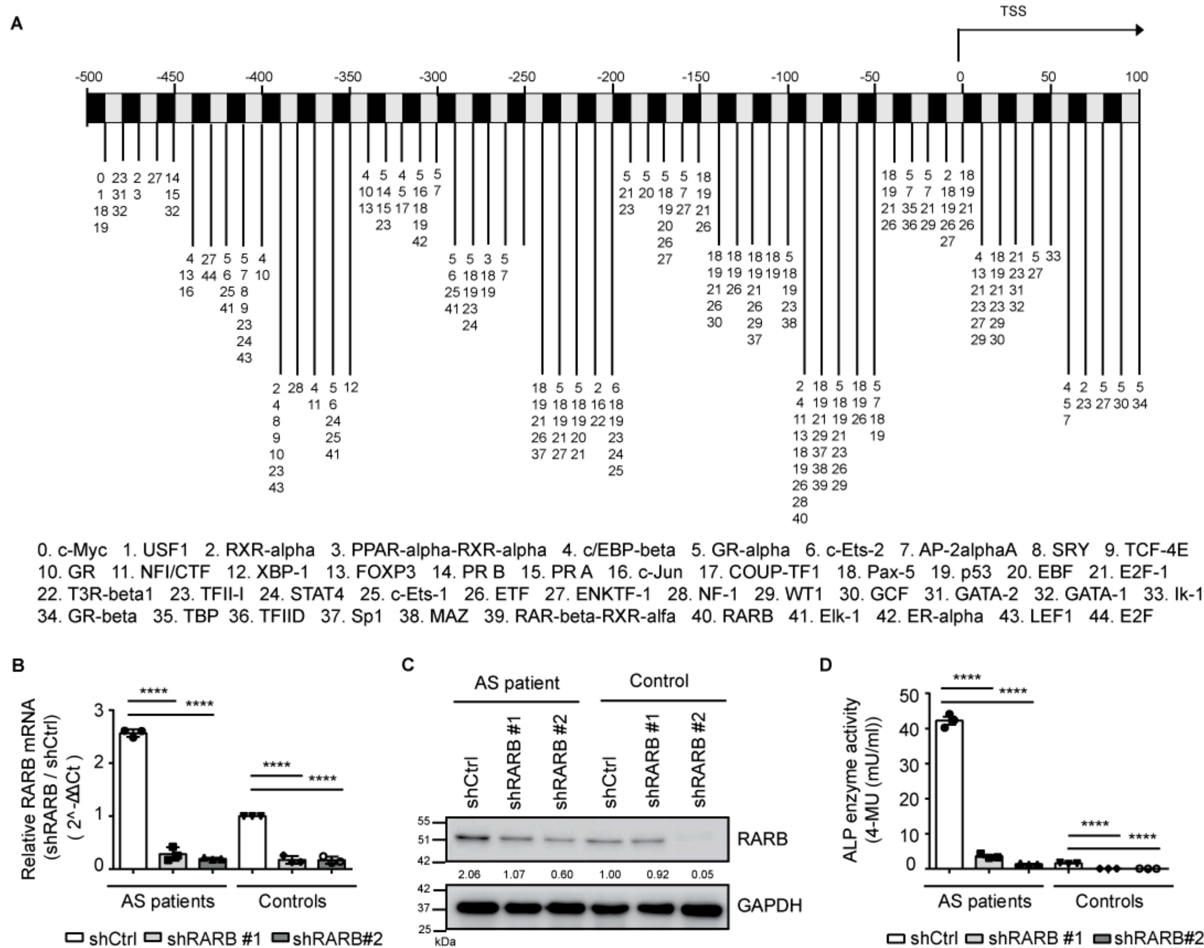
Supplemental Figure 8. Bone mineral density 12 weeks after the oral administration of TNAP inhibitors. Femoral bone mineral density was measured by micro-CT in NOD-SCID mice following implantation with AS MSCs (derived from triplicate experiments in A1, A2 and A3; n=3×3=9) and daily oral administration of H_2O (n=9), levamisole (10 mg/kg) (n=9), beryllium sulfate (7.5 mg/kg) (n=9), or pamidronate (0.3 mg/kg) (n=9).



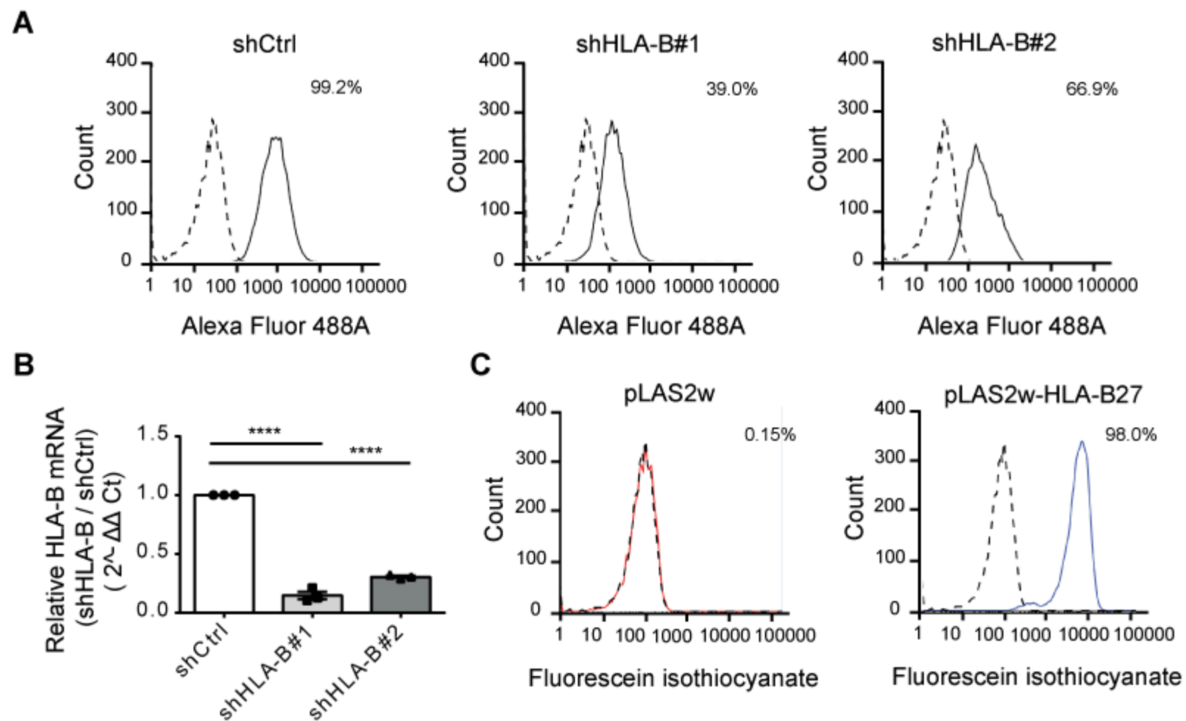
Supplemental Figure 9. Body weight change of NOD-SCID mice over the whole study period (8–11 weeks). NOD-SCID mice were implanted with AS MSCs (derived from triplicate experiments in A1, A2 and A3; n=3×3=9) in the artificial cortical defect of right lamina of lumbar spine segments L4 to L5 plus daily oral administration with H_2O (n=9), levamisole (10 mg/kg) (n=9), beryllium sulfate (7.5 mg/kg) (n=9), or pamidronate (0.3 mg/kg) (n=9)**.** Body weight changes at weeks 8 to 11 are shown. Data are the mean ± SEM.



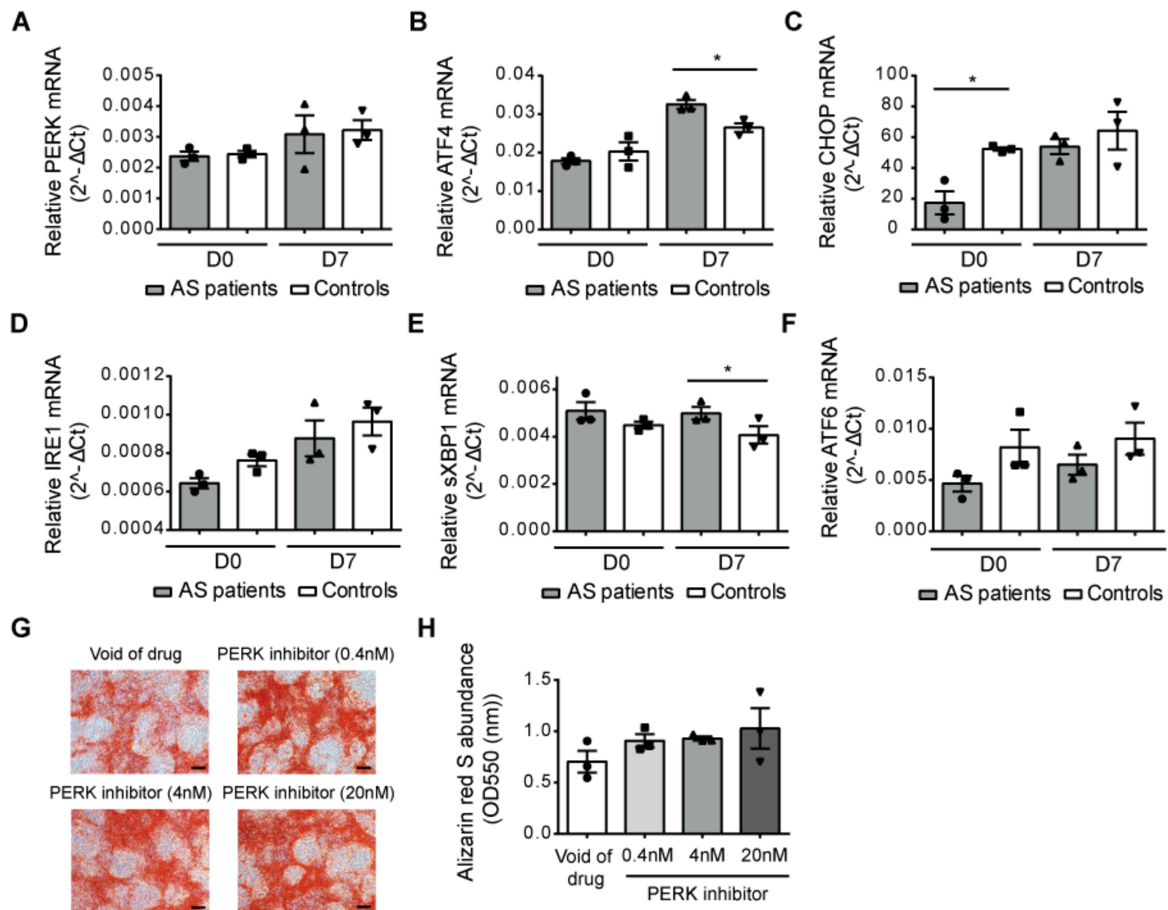
Supplemental Figure 10. TNAP expression in the BM of an AS patient and non-AS patient control. (**A** and **B**) IHC staining of the BM from an AS patient (A4) (**A**) and non-AS patient (C6) (**B**) with a TNAP-specific antibody. (**C** to **E**) Double IHC staining of BM sections from an AS patient (A4) with TNAP antibody (in brown) and indicated second primary antibodies (in blue). The co-localizations of TNAP/CD68 (monocyte lineage) (**C**), TNAP/myeloperoxidase (MPO) (myeloid lineage) (**D**), and TNAP/CD44 (MSC) (**E**) were visualized as dark-purple. Scale bar: 100 μm. Inset represents a high magnification of the boxed area.



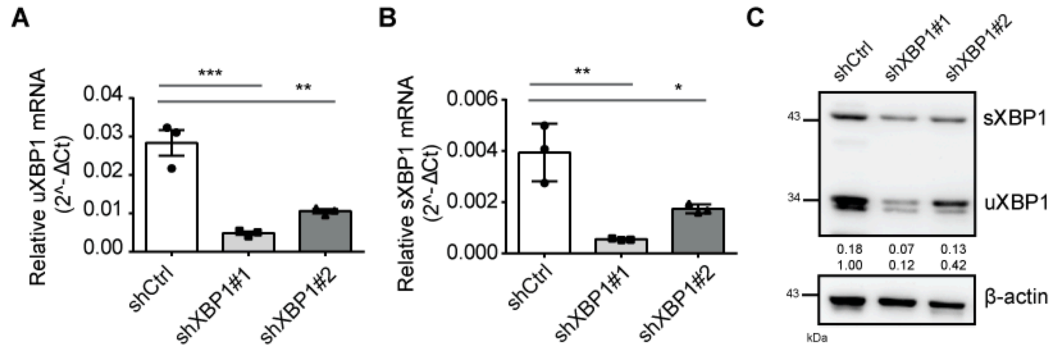
Supplemental Figure 11. Identification of the regulation of *ALPL* by RARB. (**A**) Putative transcription factors binding to the *ALPL* promoter were predicted by online software (PROMO and MALGEN on ALGGEN server). Overall, 44 transcription factors were predicted to bind to the promoter. (**B** and **C**) The knockdown efficiency of RARB in AS MSCs and control MSCs transduced with lentiviral vectors carrying two shRARB or shCtrl at day 7 under osteogenic induction. (**B**) RT-QPCR showing the mRNA levels of RARB in MSCs expressing shRARB or shCtrl normalized to the value of control MSCs expressing shCtrl. (**C**) Immunoblot analysis showing RARB knockdown. (**D**) Intracellular ALP enzyme activity in AS MSCs and control MSCs transduced with lentiviral vectors carrying two shRNAs against RARB or shCtrl at day 7 under osteogenic induction. All experiments done in the “AS patient group” and “Control group” were from AS MSCs (A1, A2, and A3) and control MSCs (C1, C2, and C3) with experimental triplicates. A representative immunoblot of AS (A1) MSCs and control (C3) MSCs is shown in (**C**). Data in (**B**) and (**D**) are the mean ± SEM. ****P < 0.0001 denotes a significant difference by one-way ANOVA, followed by Tukey’s HSD test.



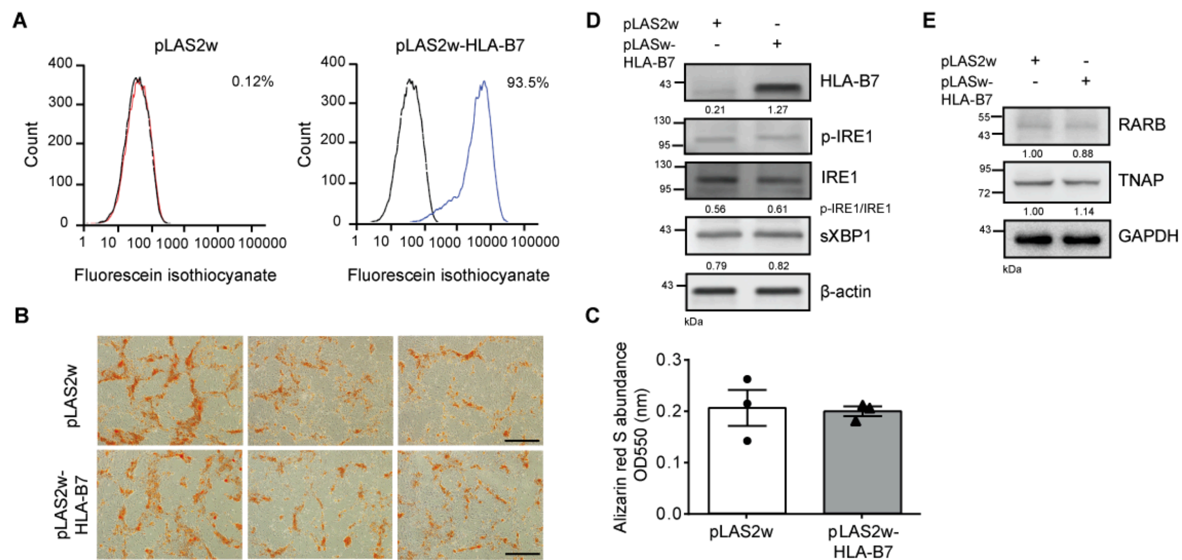
Supplemental Figure 12. Altered expression of HLA-B and HLA-B27 in AS or control MSCs. (**A**) Flow cytometric analysis of surface HLA-B expression on AS MSCs expressing shCtrl or shHLA-B. (**B**) RT-QPCR showing the knockdown efficiency of shHLA-B in AS MSCs normalized to the value of AS MSCs expressing shCtrl. (**C**) Flow cytometric analysis showing the surface HLA-B27 expression of control MSCs transduced with pLAS2w or pLAS2w-HLA-B27. Dashed histograms in (A) and (C) represent staining with an isotype control, mouse IgG1 antibody. The percentage of HLA-B(+) or HLA-B27(+) cells is shown in each histogram. All experiments were done with three AS MSCs (A1, A2, and A3) and three control MSCs (C1, C2, and C3). Representative histograms from three experiments are shown in **(A)** and **(C)**. Data in (**B**) are the mean ± SEM**.** ****P < 0.0001 denotes a significant difference by one-way ANOVA, followed by Tukey's HSD test.



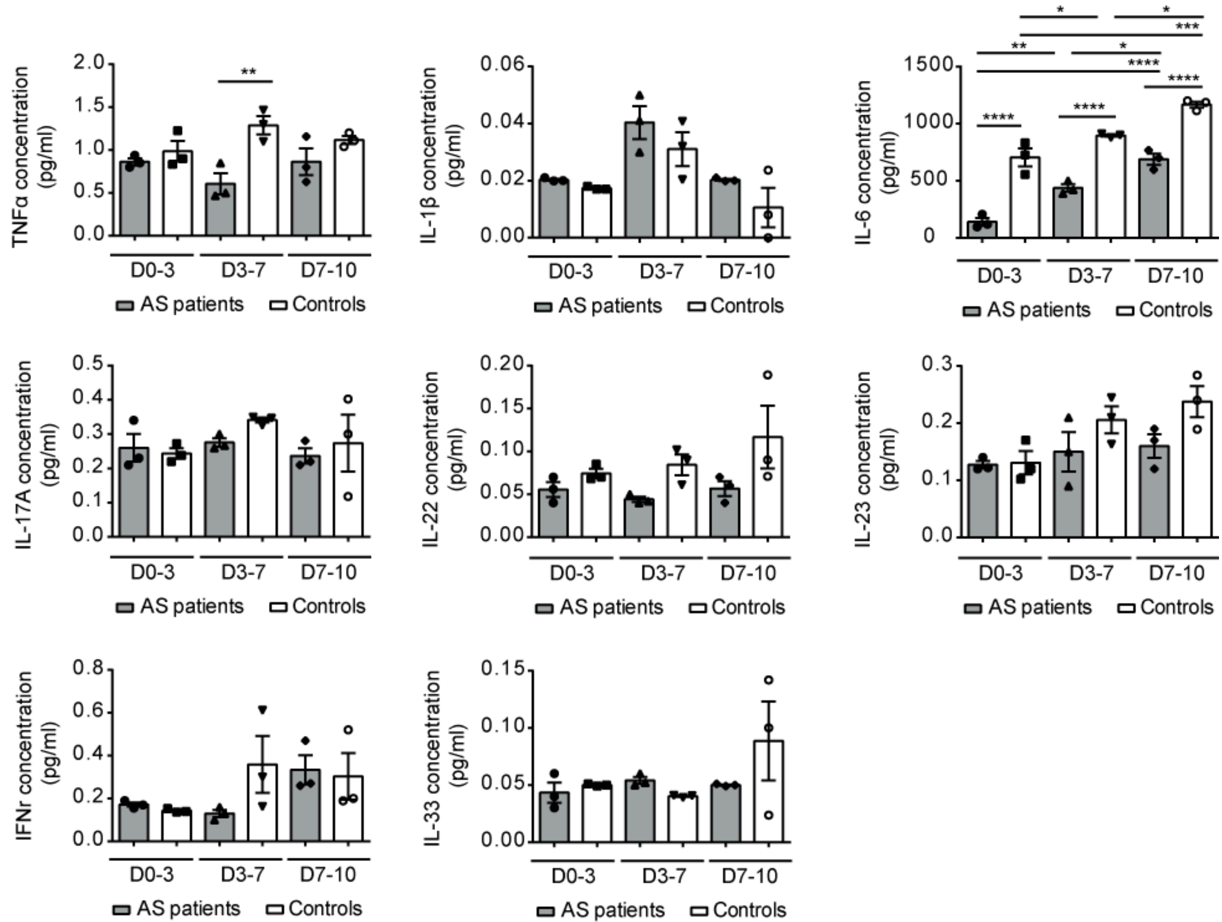
Supplemental Figure 13. Evaluation of the significance of UPR pathways in AS MSCs under osteogenic induction (**A** to **F**) RT-QPCR showing the mRNA levels of molecules involved in the three main UPR pathways, including PERK (**A**), ATF4 (**B**), CHOP(**C**), IRE1 (**D**), sXBP1 (**E**), and ATF6 (**F**) in AS MSCs (A1, A2 and A3) and control MSCs (C1, C2 and C3) at days 0 and 7 under osteogenic induction. (**G**) The effect of various concentrations of PERK inhibitors (0.4, 4, and 20 nM) on the mineralization of AS MSCs under osteogenic induction as determined by ARS staining with quantification (**H**). All experiments were done using three AS MSCs (A1, A2 and A3) and/or three control MSCs (C1, C2, and C3) with experimental triplicates. Representative results done in AS (A1) MSCs are shown in (**G**). Data in (**A**−**G** and **H**) are the mean ± SEM. $^*P < 0.05$ denotes a significant difference by one-way ANOVA, followed by Tukey's HSD test. Scale bar: 200 μm (**G**).



Supplemental Figure 14. The knockdown efficiency of XBP1 in AS MSCs transduced with shXBP1 or shCtrl. (**A** and **B**) RT-QPCR showing the mRNA levels of unspliced XBP1 (uXBP1) (**A**) and spliced XBP1 (sXBP1) (**B**) in AS MSCs transduced with lentiviral vectors expressing shXBP1 or shCtrl. (**C**) Immunoblot showing reduced uXBP1 and sXBP1 protein expressions after XBP1 knockdown. All experiments were done in AS MSCs (A1, A2 and A3) with three biological repeats. Data in (**A** and **B**) are the mean ± SEM. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ denote a significant difference by one-way ANOVA, followed by Tukey's HSD test. Representative results from AS (A1) MSCs are shown in (**C**).



Supplemental Figure 15. Overexpression of HLA-B7 does not lead to the upregulation of pIRE1/sXBP1/RARB/TNAP axis and enhanced mineralization in MSCs. (**A**) Flow cytometric analysis showing the surface HLA-B7 expression of control MSCs transduced with a control lentiviral vector (pLAS2w) or a vector expressing HLA-B7 (pLAS2w-HLA-B7). Histograms with black line represent staining with an isotype control, mouse IgG1 antibody. The percentage of HLA-B7(+) cells is shown in each histogram. (**B**) ARS staining of control MSCs expressing pLAS2w or pLAS2w-HLA-B7 at day 23 of osteogenic induction with quantification **(C)**. (**D**) Immunoblot showing the expressions of HLA-B7/pIRE1/sXBP1 in control MSCs transduced with pLAS2w or pLAS2w-HLA-B7. (**E**) Immunoblot showing RARB and TNAP expressions in control MSCs transduced with pLAS2w or pLAS2w-HLA-B7. All experiments were done in control MSCs (C1, C2 and C3) with two experimental repeats. Data are the mean ± SEM. Two-tailed Student t test was used. Representative images and results from control (C1) MSCs are shown in (**A, B, D** and **E**). Scale bar: 200 μm (**B**).



Supplemental Figure 16. Expression of cytokines secreted by AS MSCs (A1, A2 and A3) and control MSCs (C1, C2 and C3) under osteogenic induction at the indicated days. Data are the mean ± SEM. All experiments were done in AS MSCs (A1, A2 and A3) and control MSCs (C1, C2, and C3) with experimental duplicates. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, and $****P < 0.0001$ denote a significant difference by one-way ANOVA, followed by Tukey's HSD test.

Supplementary Tables

Supplemental Table 1. Characteristics of MSCs donors

Cases	AS patients [A]			Non-AS patients [B]		
	A1	**A2**	**A3**	**C1**	**C2**	**C3**
Age/sex	23/male	46/male	34/male	22/male	27/male	34/male
Status	AS patient	AS patient	AS patient	Non-AS control	Non-AS control	Non-AS control
HLA-B27	+	+	+	-	-	-

[A] MSCs were derived from entheseal tissues involved in spinal ankylosis from AS patients (A1, A2, and A3) that had undergone spinal wedge osteotomy.
[B] MSCs of non-AS individuals (C1, C2, and C3) who underwent traumatic surgery at similar sites were used as controls.
All donors were Taiwanese.

Supplemental Table 2. Genes up-regulated (> 2-fold) consistently at days 0, 3, and 7 after osteogenic induction in AS MSCs as compared with those in control MSCs.

Please see the excel file of "Supplemental Table 2".

Supplemental Table 3. Gene down-regulated (> 2-fold) consistently at days 0, 3, and 7 after osteogenic induction in AS MSCs as compared with those in control MCS.

Please see the excel file of "Supplemental Table 3".

Supplemental Table 4. Characteristics of additional AS MSCs donors

Cases	AS patients [A]		
	A4	**A5**	**A6**
Age/sex	40/male	41/male	32/male
Status	AS patient	AS patient	AS patient
HLA-B27	+	+	+

[A] MSCs were derived from entheseal tissues involved in spinal ankylosis from AS patients (A4, A5, and A6) who had undergone spinal wedge osteotomy. All donors were Taiwanese.

Supplemental Table 5. Characteristics of bone marrow slide donors

Cases	Ages	Sex
AS patients [A]		
A1	23	Male
A2	46	Male
A3	34	Male
A4	40	Male
Normal controls [B]		
C4	35	Male
C5	33	Male
Non-AS patient control [C]		
C6	29	Male

[A] AS patients were individuals who suffered from spinal ankylosis and underwent spinal wedge osteotomy of lumbar spines. Their bone marrow specimens of lumbar spines were from the surgical sites near entheseal tissues involved in spinal ankylosis

[B] Normal controls were individuals who underwent autopsies and their bone marrow slides of lumbar spines were purchased from US Biomax Company.

[C] Non-AS patient control was the individual who underwent the bone marrow biopsy of hip for lymphoma workup and was free of lymphoma in the bone marrow.

AS patients and non-AS patients were all Taiwanese. Normal controls were Caucasians.

Supplemental Table 6. Characteristics of AS patients with normal and increased serum BAP levels in "Taiwanese cohort"

Characteristics **(Patient number with normal/increased BAP)**	**Total AS patients** **(n = 104)**	**Patients with normal BAP** **(n=94)**	**Patient with increased BAP** **(n=10)**	***P* value**
Male/Female (94/10)	90/14	81/13	9/1	1.000
Age (years old) (94/10)	46 (12.1)	46 (12.0)	48 (14.0)	0.440
Onset age (years old) (92/10)	27 (10.4)	27 (10.3)	27 (11.6)	0.791
Disease duration (years) (92/10)	19 (11.2)	19 (11.1)	22 (12.4)	0.558
HLA-B27 positive/negative (92/9)	95/6	86/6	9/0	1.000
Serum BAP (μg/L) (94/10)	12.954 (5.538)	11.640 (3.846)	25.300 (3.287)	<0.001[A]
ESR (mm/hour) (91/10)	13.386 (12.751)	13.528 (13.305)	12.100 (5.859)	0.677
CRP (mg/dL) (92/10)	0.715 (1.030)	0.734 (1.070)	0.538 (0.551)	0.897
BASDAI (93/10)	2.680 (1.826)	2.698 (1.855)	2.520 (1.603)	0.902
BASFI (92/10)	1.108 (1.411)	1.055 (1.416)	1.600 (1.323)	0.119
BAS-G (94/10)	3.421 (2.782)	3.383 (2.870)	3.775 (1.805)	0.340
mSASSS (89/10)	31.051 (18.135)	29.876 (17.886)	41.500(17.834)	0.047 [A]

Values are shown as mean (standard deviation: SD).

P is determined by Mann-Whitney U or Fisher exact test between AS patients with normal and increased BAP.

[A] Statistically significant.

Supplemental Table 7. Characteristics of AS patients with normal and increased serum BAP levels in "British cohort"

Characteristics (Patient number with normal/increased BAP)	Total AS patients (n = 184)	Patients with normal BAP (n=137)	Patient with increased BAP (n=47)	*P* value
Male/Female (137/47)	136/48	98/39	38/9	0.209
Age (years old) (137/47)	53 (13.2)	53 (13.4)	54 (13.0)	0.612
Disease duration (years) (137/47)	22(13.3)	22 (13.2)	21 (13.8)	0.738
HLA-B27 positive/negative (137/47)	162/22	123/14	39/8	0.215
Serum BAP (μg/L) (137/47)	17.607 (8.055)	14.300 (3.555)	27.245 (9.656)	<0.001 [A]
CRP (mg/dL) (137/47)	1.104 (1.784)	0.989 (1.617)	1.442 (2.184)	0.027 [A]
BASDAI (137/47)	3.666 (2.063)	3.515 (2.036)	4.109 (2.100)	0.110
BASFI (137/47)	3.889 (2.382)	3.518 (2.169)	4.972 (2.657)	<0.001 [A]
BAS-G (137/47)	3.783 (2.438)	3.591 (2.381)	4.340 (2.539)	0.068
mSASSS (137/47)	19.630(22.191)	17.964 (22.083)	24.489(22.019)	0.036 [A]

Values are shown as mean (SD).

P is determined by Mann-Whitney U or Fisher exact test between AS patients with normal and increased BAP.

[A] Statistically significant.

Supplemental Table 8. Demography of AS patients and healthy controls in "Taiwanese cohort"

Characteristic	Total AS patients (n = 104)	Healthy controls (n = 50)	P values
Male/Female	90/14	32/18	0.003 [A]
Age (years old)	46 (12.1)	43 (14.6)	0.203
Serum BAP (μg/L)	12.954 (5.538)	6.296 (2.110)	<0.001 [A]

Values are shown as mean (SD).

P value is determined by Mann-Whitney U test or Fisher exact test.

[A] Statistically significant.

Supplemental Table 9. Demography of a longitudinal AS subgroup with retrospective follow-up of radiographic progression in "Taiwanese cohort"

Characteristic	**AS patients** (n = 37)
Male/Female	34/3
Age (years old)	52 (10.5)
Disease duration (years)	24 (11.9)
Interval of longitudinal radiographic follow-up (years)	5.7 (2.8)
ESR (mm/hour)	14.784 (15.013)
CRP (mg/dL)	0.953 (1.466)
BASDAI	2.730 (1.859)
BASFI	1.761 (1.682)
BAS-G	4.000 (2.967)
mSASSS	40.892 (18.403)
Yearly change in mSASSS	1.758 (1.715)
BASRI-total	11.216 (2.335)
Serum BAP (μg/L)	13.949 (5.608)

Values are shown as mean (SD).

Supplemental Table 10. The primers used in this study

Genes	Sequence of forward primer	Sequence of reverse primer
Primers used in RT-QPCR		
Runx2	5’-ACGCCATAGTCCCTCCTTTT-3’	5’-TCACTACCAGCCACCGAGAC-3’
TNAP	5’-AGACTGCGCCTGGTAGTTGT-3’	5’-CCTCCTCGGAAGACACTCTG-3’
Wnt3a	5’-ACCCCCTGTAAGGTTCCATC-3’	5’-TGTAGCTGGATGGAGTGCAG-3’
Wnt10b	5’-GAAGTTCTCTCGGGATTTCT-3’	5’-ATTTCCGCTTCAGGTTTTCA-3’
DLX5	5’-GCCATAAGAAGCAGAGGTAGG-3’	5’-AGAAGAGTCCCAAGCATCCGA-3’
Osteocalcin	5’-GTGCAGAGTCCAGCAAAGGT-3’	5’-CGATAGGCCTCCTGAAAGC-3’
Collagen1A1	5’-CCTGCGTGTACCCCACTCA-3’	5’-ACCAGACATGCCTCTTGTCCTT-3’
DHRS3	5’-GGGCACTGAGTGCCATTACTTC-3’	5’-CGGCATTGTTCACCAGGATGGT-3’
EN1	5’-GTGGTCAAAACTGACTCGCAGC-3’	5’-CCGCTTGTCCTCCTTCTCGTTC-3’
FGFR2	5’-GTGCCGAATGAAGAACACGACC-3’	5’-GGCGTGTTGTTATCCTCACCAG-3’
FRZB	5’-GCTACACAGAAGACCTATTTCCG-3’	5’-CCGTGGAATGTTTACCAGAGAGG-3’
GHR	5’-GCAGCTATCCTTAGCAGAGCAC-3’	5’-AAGTCTCTCGCTCAGGTGAACG-3’
HAPLN1	5’-CTGTTGTGGTAGCACTGGACTTA-3’	5’-TCACAGCATCCTGGTCCAGACA-3’
HOXB8	5’-GTCGCCCACACAGCTCTTCCC-3’	5’-AATAGGAACTCCTTCTCCAGCTC-3’
HOXD4	5’-GTGGTCTACCCCTGGATGAAGA-3’	5’-TCCAGTTCTAGGACTTGCTGCC-3’
ITGB1BP1	5’-GGTGTGTTACGATGACGGTCTG-3’	5’-GAGTCAAAAGCGGTGGATAAAACC-3’
LIF	5’-AGATCAGGAGCCAACTGGCACA-3’	5’-GCCACATAGCTTGTCCAGGTTG-3’
MMP16	5’-GATTCAGCCATTTGGTGGGAGG-3’	5’-CCCTTTCCAGACTGTGATTGGC-3’
PITX1	5’-GTACGCACTTCACAAGCCAGCA-3’	5’-GCTCGGTGAGGTTGGTCCACA-3’
SIM2	5’-TGTCTTGGCGAAAAGGAACGCG-3’	5’-CCACAATCTGGTAGCAGGAGTC-3’
SOX11	5’-GCTGAAGGACAGCGAGAAGATC-3’	5’-GGGTCCATTTTGGGCTTTTTCCG-3’
SULF2	5’-GGACTCCTTCTTGGTGGAGAGA-3’	5’-TACTCAGCACGCTGACACAGGT-3’
TFAP2A	5’-GACCTCTCGATCCACTCCTTAC-3’	5’-GAGACGGCATTGCTGTTGGACT-3’
ZNF521	5’-TGCCTCCAGGTGTTTGAATCGC-3’	5’-GCCAAGAGCAAGTCGGATCATC-3’
HLA-B	5’-TCATCCAGTGGGCTACGTG-3’	5’-TGTGTGTTCCGGTCCCAATA-3’
LEF1	5’-AGGGCTCCTGAGAGGTTTGT-3’	5’-GACGAGATGATCCCCTTCAA-3’
RARB	5’-TGAGAGGCATTGATCC-3’	5’-TCAGAAAAAGACGACCCAG-3’
BIP	5’-TGTTCAACCAATTATCAGCAAACTC-3’	5’-TTCTGCTGTATCCTCTTCACCAGT-3’
PERK	5’-GTCCGGAACCAGACGATGAG -3’	5’-GGCTGGATGACACCAAGGAA-3’
ATF4	5’-GTTCTCCAGCGACAAGGCTA-3’	5’-ATCCTGCTTGCTGTTGTTGG-3’
CHOP	5’-AGAACCAGGAAACGGAAACAGA-3’	5’-TCTCCTTCATGCGCTGCTTT-3’
ATF6	5’-GCTTTACATTCCTCCACCTCCTTG-3’	5’-GCTTTACATTCCTCCACCTCCTTG-3’
IRE1	5’-AGAGAAGCAGCAGACTTTGTC-3’	5’-GTTTTGGTGTCGTACATGGTGA-3’

sXBP1	5’-TGCTGAGTCCGCAGCAGGTG-3’	5’-GCTGGCAGGCTCTGGGGAAG-3’
uXBP1	5’-AGCACTCAGACTACGTGCACCTCT-3’	5’-CCAGAATGCCCAACAGGATATCAG-3’
GAPDH	5’-GTTGCTGTAGCCAAATTCGTTGT-3’	5’-GGTGGTCTCCTCTGACTTCAACA-3’
Primers used in qPCR of ChIP assay		
Frag 1	5'-CACTGGCTCTAGAATCACGCTC-3'	5'-CCCAAACTCTGCCCACTGAATG-3'
Frag 2	5'-AGTTTACAGCCAGCCCACTC-3'	5'-GTCTAAACTGCCCCATGCTTC-3'
Frag 3	5'-GGTGTTGAGCATCTCTGGATTG-3'	5'-GACCCATTTGTCTGTCTGCC-3'
Frag 4	5'-AAATGGATGGATGGATGCAC-3'	5'-TCCTAGAAGAGTACCTGACAA-3'
Frag 5	5'-AACAAAAACACTCTGCCCTC-3'	5'-AAATAGCAAAACCTCCACTCC-3'
Frag 6	5'-GACAGACAAAAACAAACACTCC-3'	5'-TCTTCCTACAGTTACTGCTTCC-3'
Frag 7	5'-TCTTAAACTGCGAAGCTGAAC-3'	5'-CTGATGACCTTGCTTCTTCC-3'
3'UTR	5'-GGAGGAAACAAGACTCCTGCAC-3'	5'- ATGAATGACATCAGCTCCCCCG -3'