Expanded View Figures

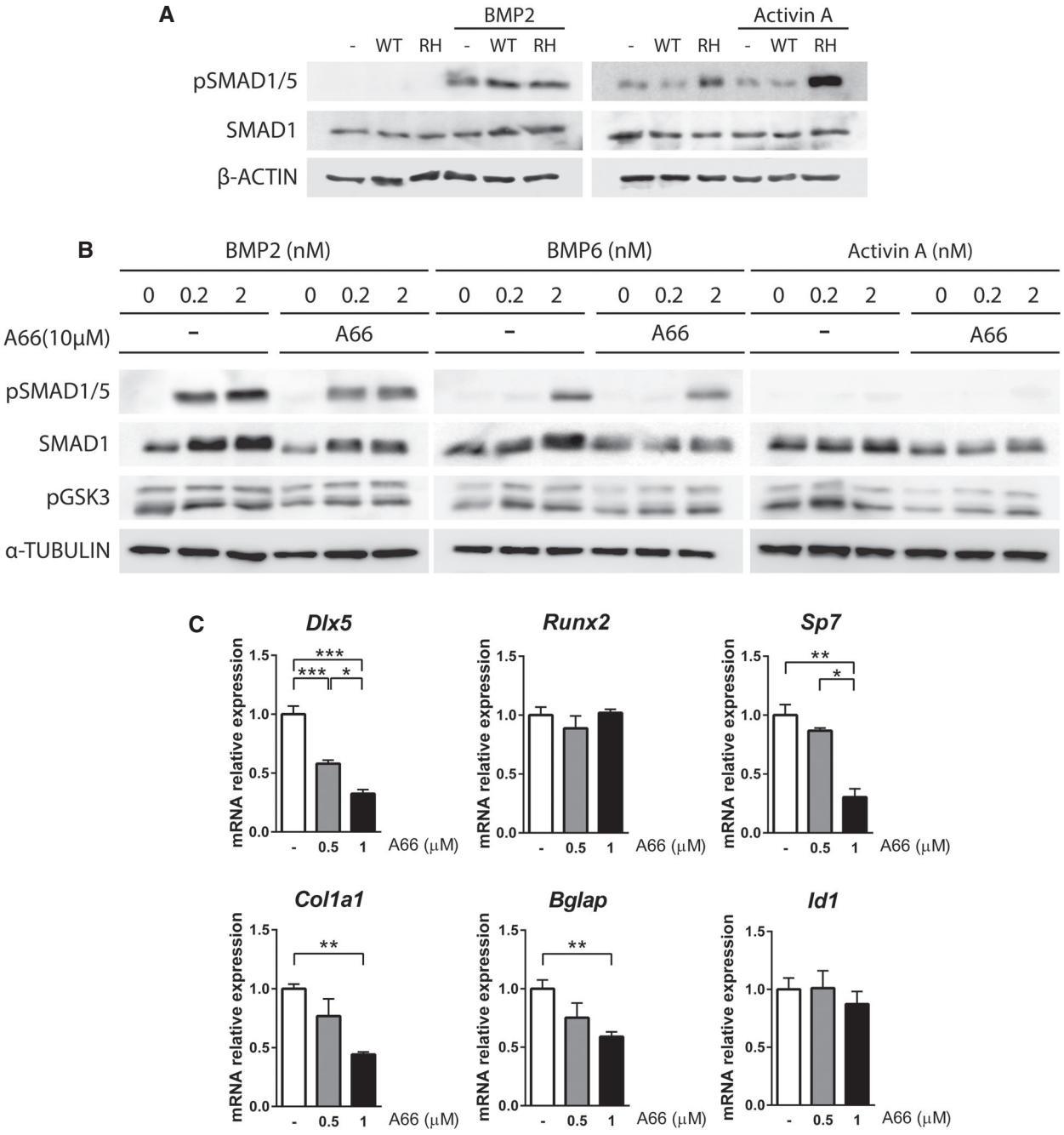

Figure EV1. Dose selection and MSC osteogenic differentiation.

A Immunoblots of MSCs expressing wild-type *Acvr1* (WT) or *Acvr1*^{R206H} (RH). MSCs were serum-starved for 16 h and then treated with 2 nM BMP2 or 2 nM activin A for 1 h.

B Immunoblots of MSCs. Cells were serum-starved and treated with 10 μM A66 for 16 h and then treated with the indicated concentrations of BMP2, BMP6, or activin A for 1 h.

C MSCs were cultured for 14 days in osteogenic media in the presence of the indicated concentrations of A66. Data shown as mean ± SEM (n = 5 per group). *P < 0.05, **P < 0.01, ***P < 0.001; one-way ANOVA.

Source data are available online for this figure.

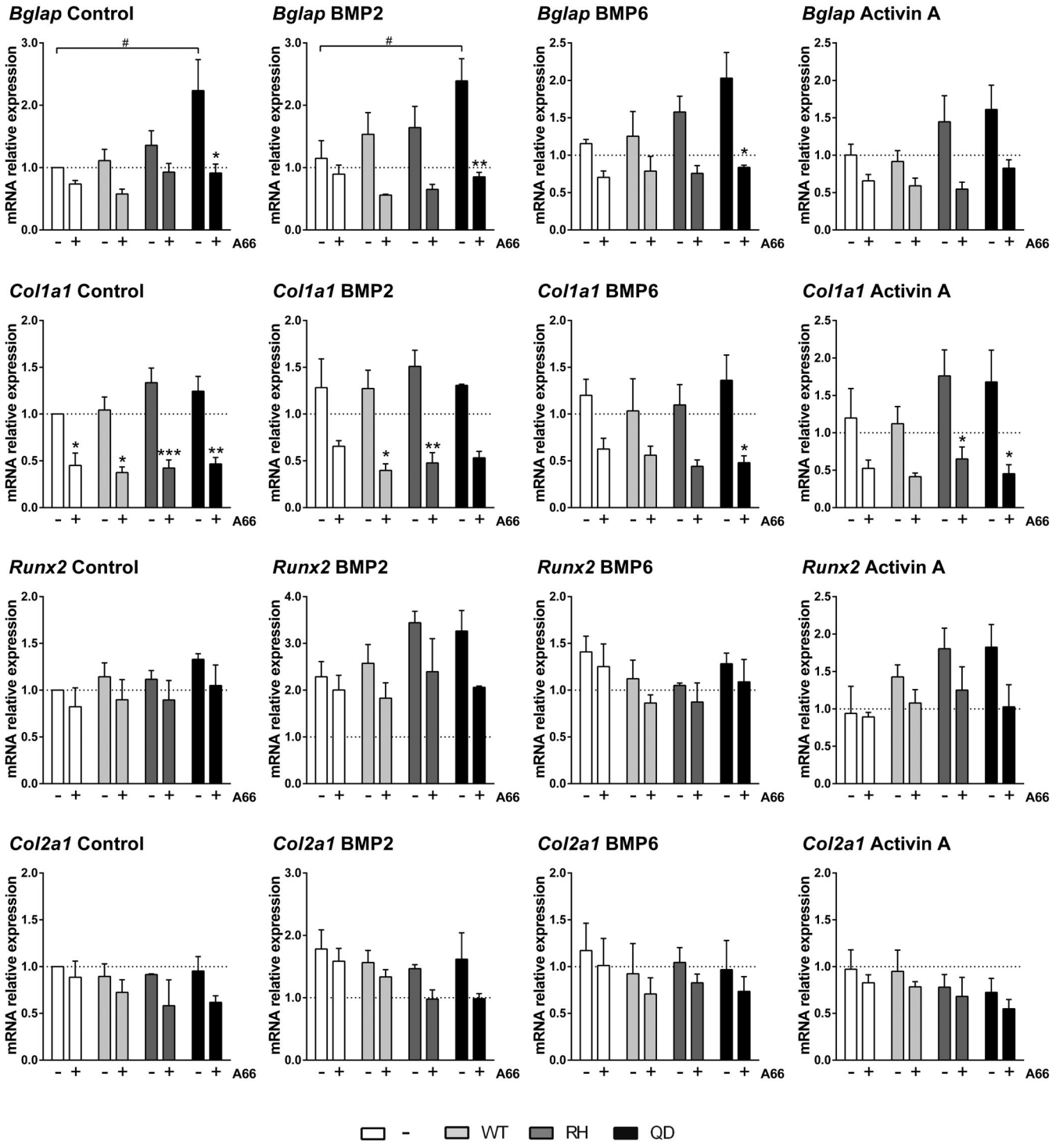

Figure EV2. PI3Kα inhibition reduces the specification of cell progenitors into chondrogenic and osteogenic lineages.

mRNA expression of osteoblast and chondroblast-specific genes of MSCs expressing wild-type *Acvr1*, *Acvr1*^{R206H}, or *Acvr1*^{Q207D}. MSCs were serum-starved and treated with 10 μM A66 for 16 h and then treated with 2 nM BMP2, 2 nM BMP6, or 2 nM activin A for 2 h. Data shown as mean ± SEM (n = 4 per group). * or $^{\#}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; two-way ANOVA. Asterisks refer to significance between MSCs treated with or without A66 in each case. Similarly, # refers to significance between *Acvr1*^{R206H} or *Acvr1*^{Q207D} MSCs of each group compared to mock-transfected cells untreated with A66.

Figure EV3. PI3Kα inhibition with BYL719 in MSCs.

A Immunoblots of MSCs. Cells were serum-starved and treated with a range of BYL719 concentrations for 16 h and then with 2 nM BMP2 for 1 h.

B Immunoblots of MSCs expressing wild-type *Acur1* (WT) or $Acur1^{R206H}$ (RH) variants. MSCs were serum-starved and treated with 10 μM BYL719 for 16 h and then with 2 nM BMP6 or 2 nM activin A for 1 h.

C mRNA expression of osteoblast and chondroblast-specific genes of MSCs expressing wild-type *Acur1*, $Acur1^{R206H}$, or $Acur1^{Q207D}$. MSCs were serum-starved and treated with 10 μM BYL719 for 16 h. Data shown as mean ± SEM (n = 4 per group). * or $^{\#}P < 0.05$, ** or $^{\#\#}P < 0.01$, *** or $^{\#\#\#}P < 0.001$; two-way ANOVA. Asterisks refer to significance between MSCs treated with or without BYL719 in each case. Similarly, # refers to significance between untreated groups transfected with different forms of *Acur1*.

Source data are available online for this figure.

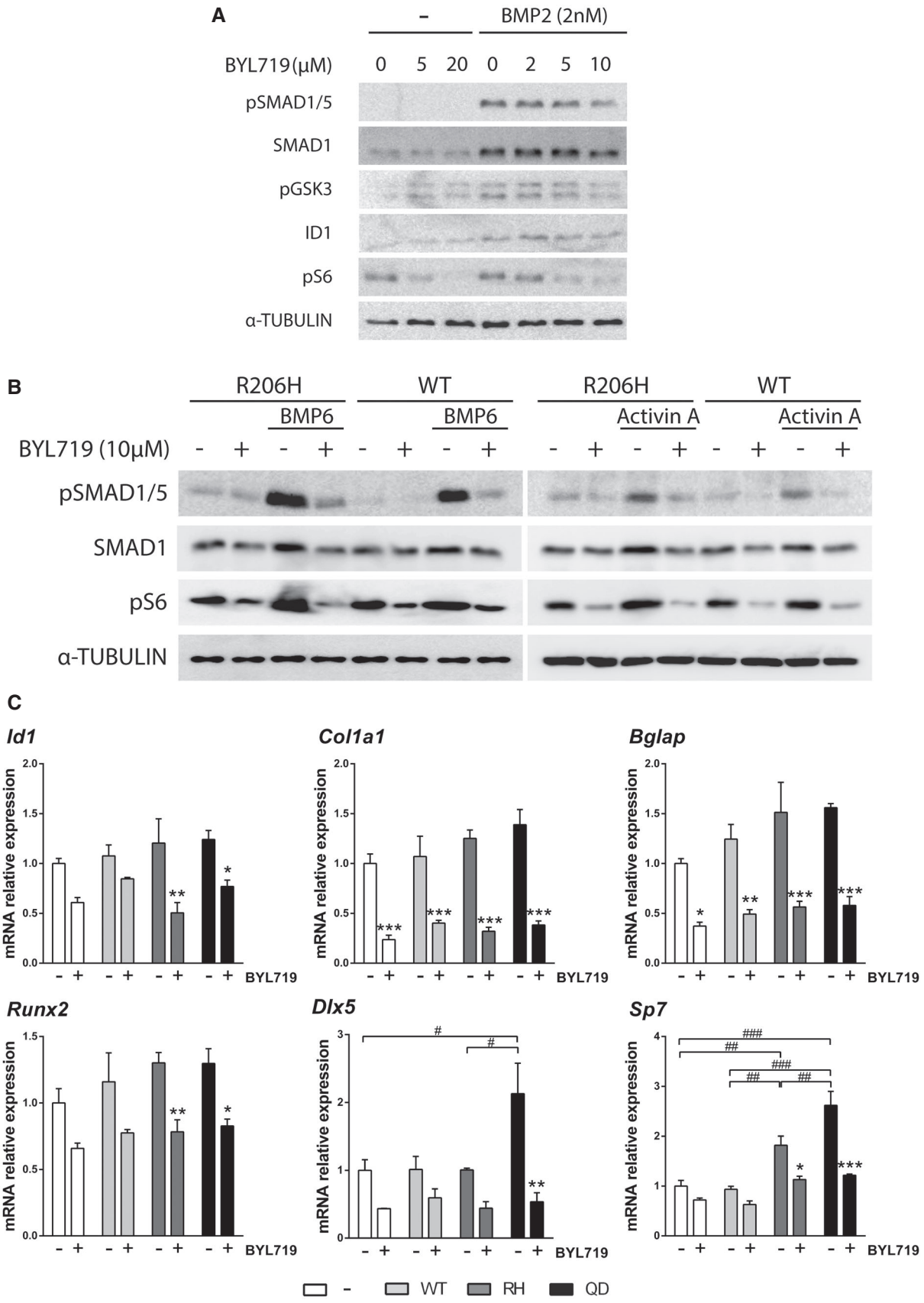
A
–
BMP2 (2nM)
BYL719(μM)
0 5 20 0 2 5 10
pSMAD1/5
SMAD1
pGSK3
ID1
pS6
α-TUBULIN
B
R206H
WT
R206H
WT
BMP6
BMP6
Activin A
Activin A
BYL719 (10μM)
pSMAD1/5
SMAD1
pS6
α-TUBULIN
C
Id1
Col1a1
Bglap
Runx2
Dlx5
Sp7
mRNA relative expression
BYL719
WT
RH
QD

Figure EV3.

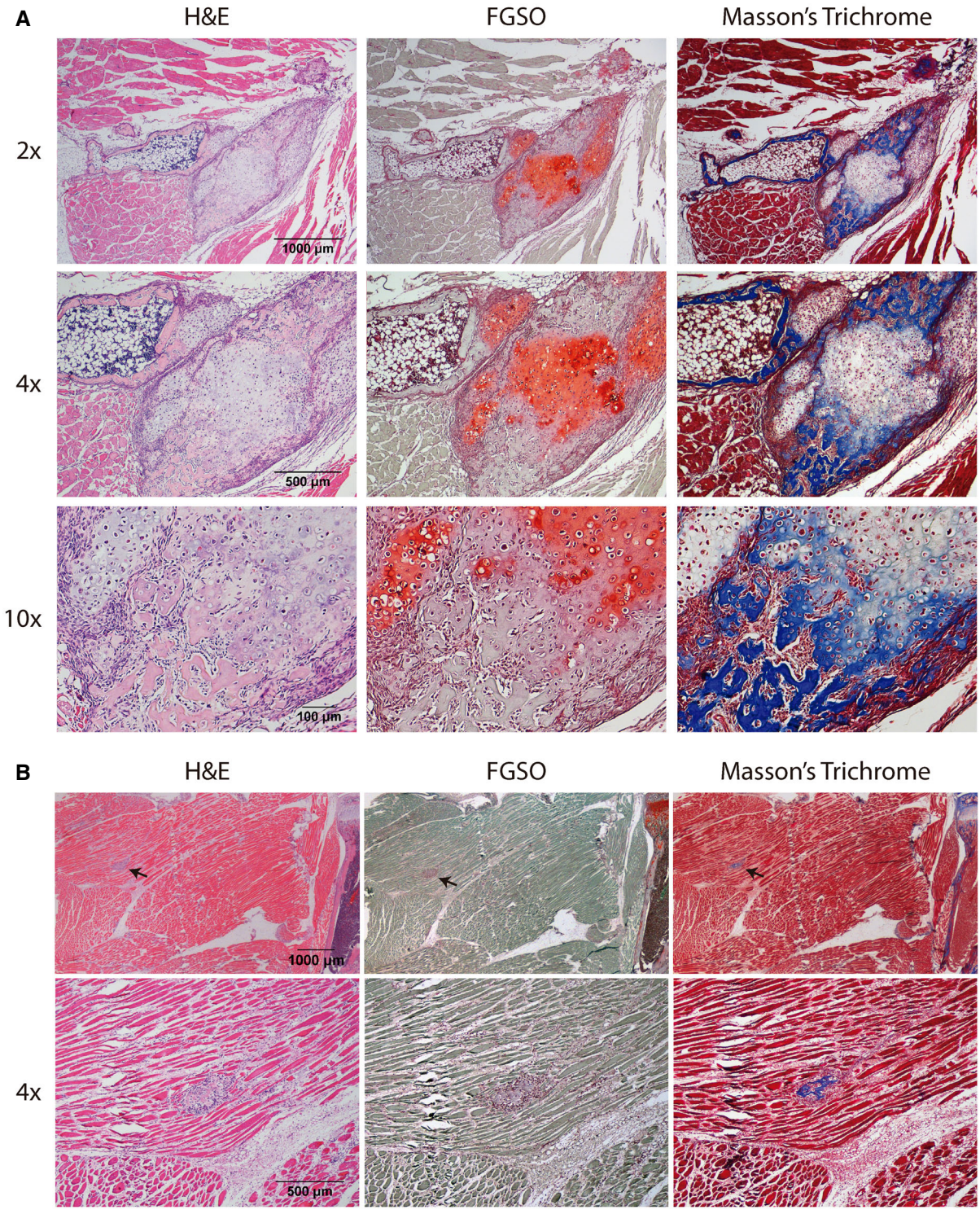

Figure EV4. Detailed histological images of endochondral heterotopic ossification.

A Representative images of HO of *Acvr1*^{Q207D} mice treated with vehicle: hematoxylin and eosin (H&E), fast green/safranin O (FGSO), and Masson's trichrome staining. Microscopy images shown with 2× (scale bar = 1,000 µm), 4× (scale bar = 500 µm), and 10× (scale bar = 100 µm).

B Representative images of the single case of heterotopic ossification in *Acvr1*^{Q207D} mice treated with intermittent BYL719. Images obtained with stereomicroscope (scale bar = 1,000 µm). Microscopy images shown with 4× (scale bar = 500 µm). Black arrows show the localization of heterotopic ossification.