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Supplementary Materials for

Increased activity of TNAP compensates for reduced adenosine production and promotes ectopic calcification in the genetic disease ACDC

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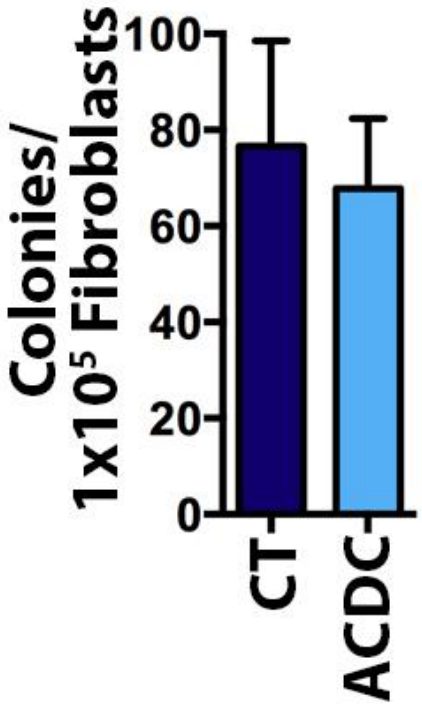


Fig. S1. Reprogramming efficiency in ACDC fibroblasts.

The dermal skin fibroblasts isolated from healthy control volunteers and patients with ACDC were reprogramed by activation of Yamanaka factors (Oct4, Sox2, Klf4, c-Myc) under feeder-free and chemically-defined conditions. Quantification of Tra1-60-positive colonies was performed at day 21 in both healthy control volunteers (n=6) and patients with ACDC (n=5). Data are mean ± SEM and analyzed by unpaired two-tailed student's t-test, no significant differences.

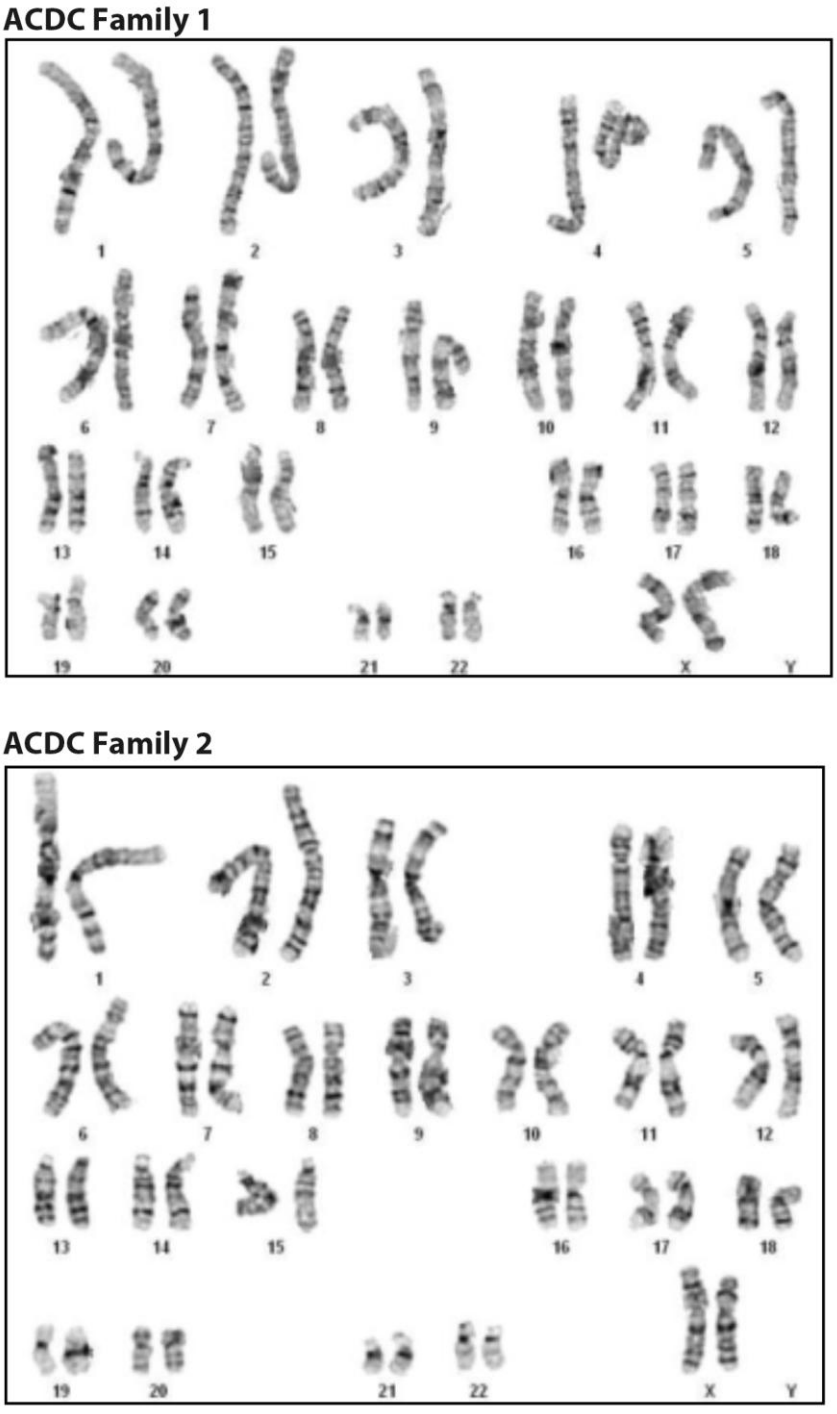


Fig. S2. Representative karyotypes of ACDC iPSCs.

The karyotype in all iPSCs lines was analyzed by using G-Band, and 20 total metaphases in each cell line were examined.

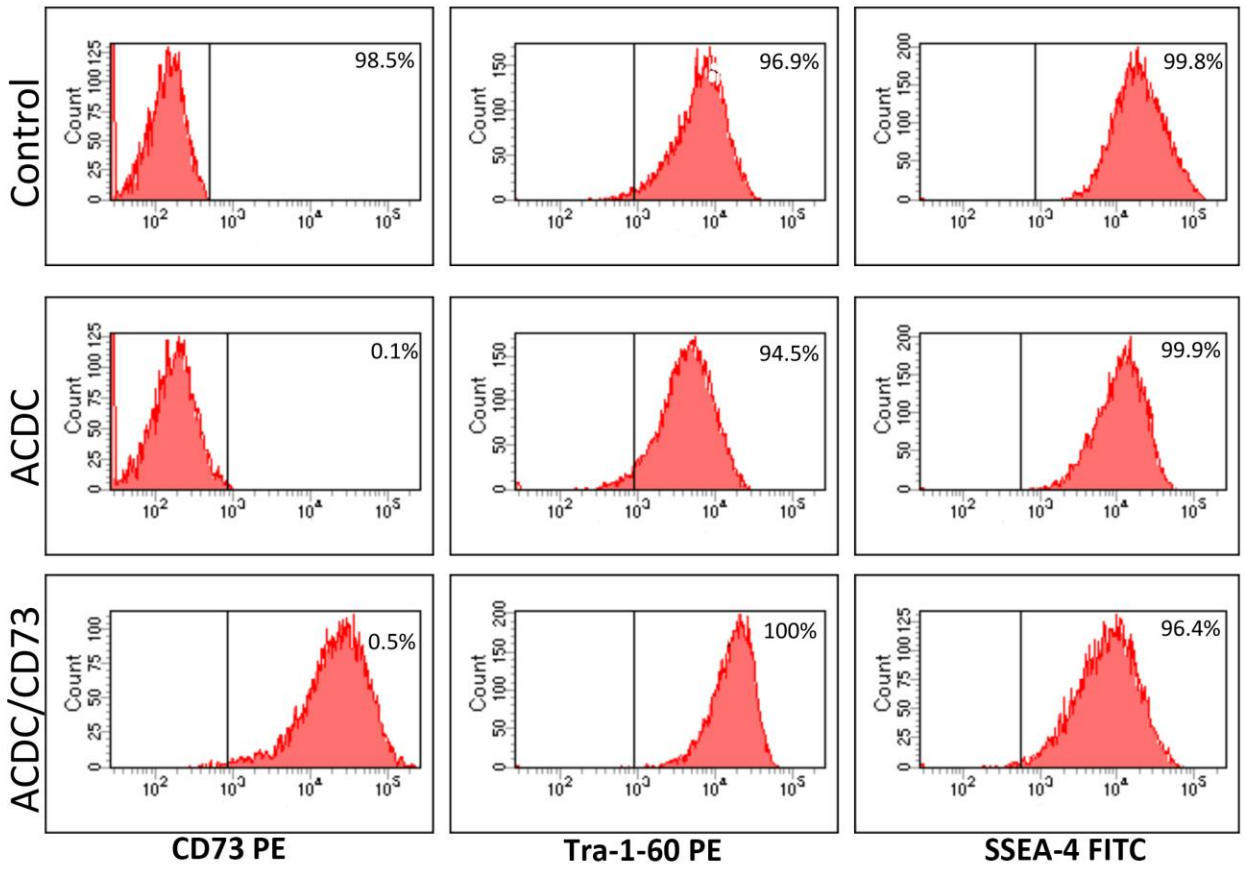


Fig. S3. Generation and characterization of ACDC iPSC lines overexpressing CD73.

The human CD73 cDNA was inserted into the AAVS1 safe harbor in two ACDC patient iPSCs using TALEN technology. All lines maintained the pluripotency potential and expression of Tra-1-60 and SSEA-4, and exhibited high expression of CD73 as measured by FACS (images shown are representative of 3 independent experiments).

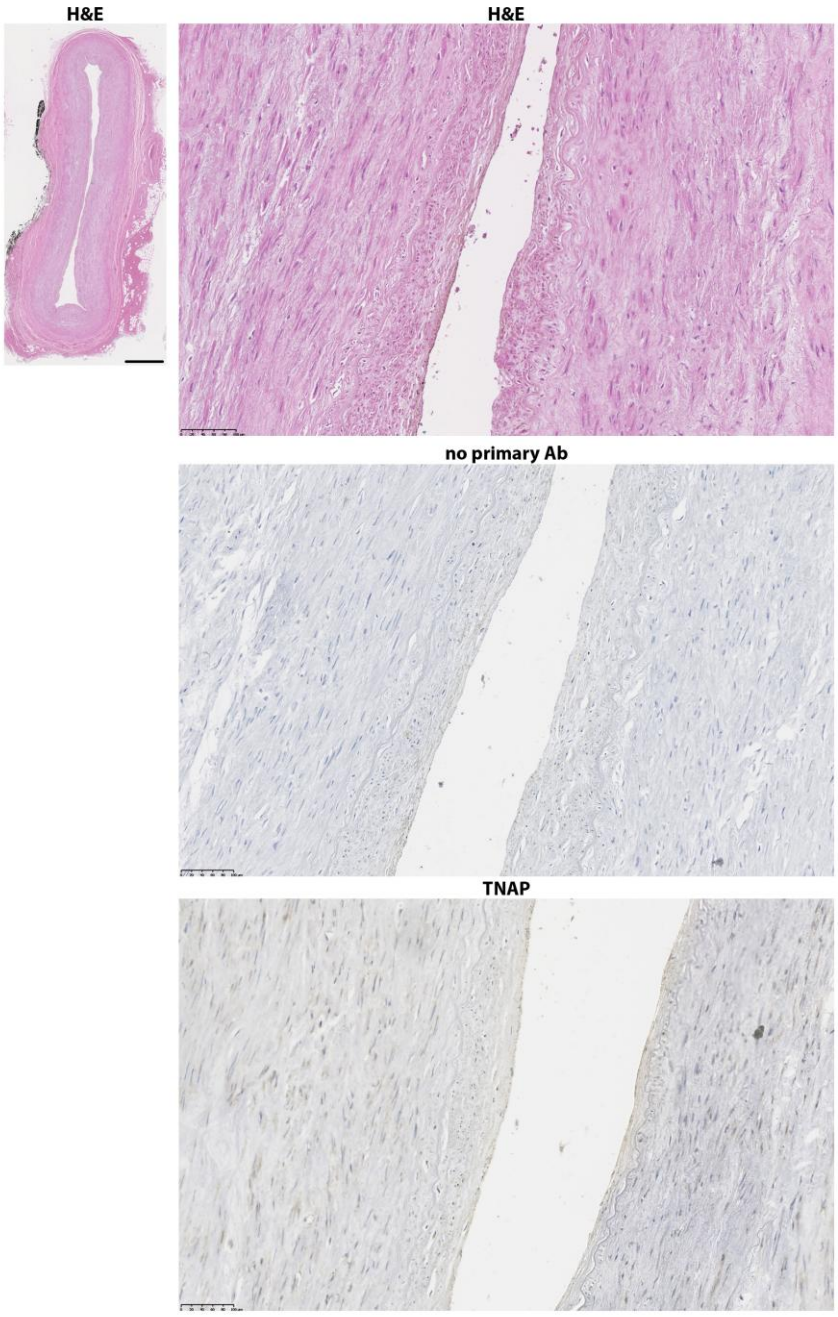


Fig. S4. TNAP is not present in control human artery that is free from calcification.

Histological hematoxylin and eosin (H&E) staining and immunohistochemical staining for TNAP performed on serial sections prepared from paraffin-embedded healthy femoral human artery. Scale bar for image of whole artery = 1mm; Scale bar for magnified images = 100μm

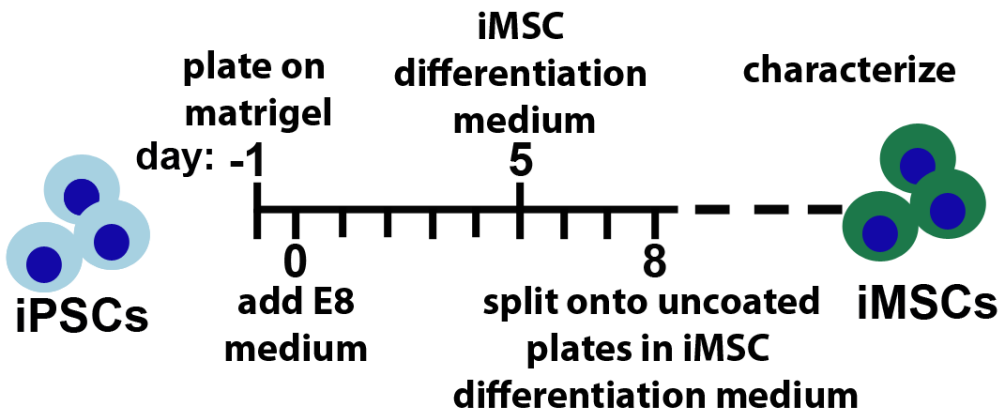


Fig. S5. Schematic of differentiation of iPSCs into iMSCs.

$1x10^5$ iPSCs were plated onto Matrigel-coated 6-well plates and cultured with E8 medium. The next day, the medium was replaced with mesoderm differentiation medium (MDM) for 5 days. At day 5, the medium was changed to MSC medium for additional 3 days. Subsequently, the cells were passaged and replated onto new culture plates with MSC medium.

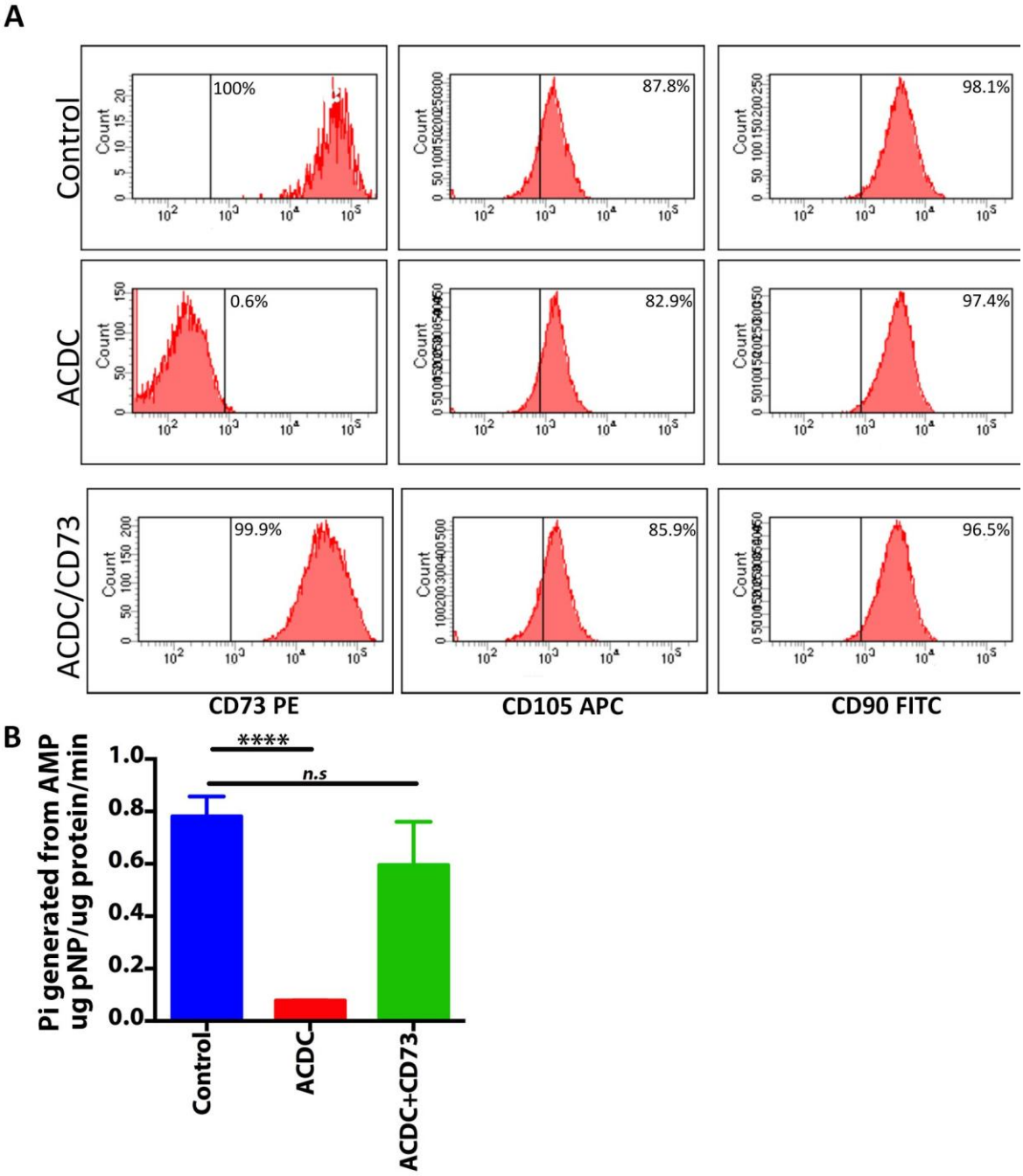


Fig. S6. CD73 overexpression rescues osteogenic defects in ACDC iMSCs.

A. Differentiation of iPSCs to iMSCs illustrates that ACDC iPSCs overexpressing CD73 show the typical iMSC expression pattern of CD73, CD105, and CD90 as analyzed by FACS. A representative from 3 independent experiments is shown. **B**. CD73 activity was detected in control (CT) iMSCs, ACDC iMSCs, and ACDC iMSCs overexpressing CD73. Data represent mean ± SD, n=3 each group ****$p<0.0001$ using unpaired two-tailed student's t test compared to control cell line.

Fig. S7. Overexpression of CD73 decreases TNAP in ACDC iPSCs.

Representative of imaging of TNAP staining showing that TNAP activity is reduced in ACDC iMSCs overexpressing CD73 at day 5 of osteogenic differentiation. Representative result of 3 independent stainings is shown.

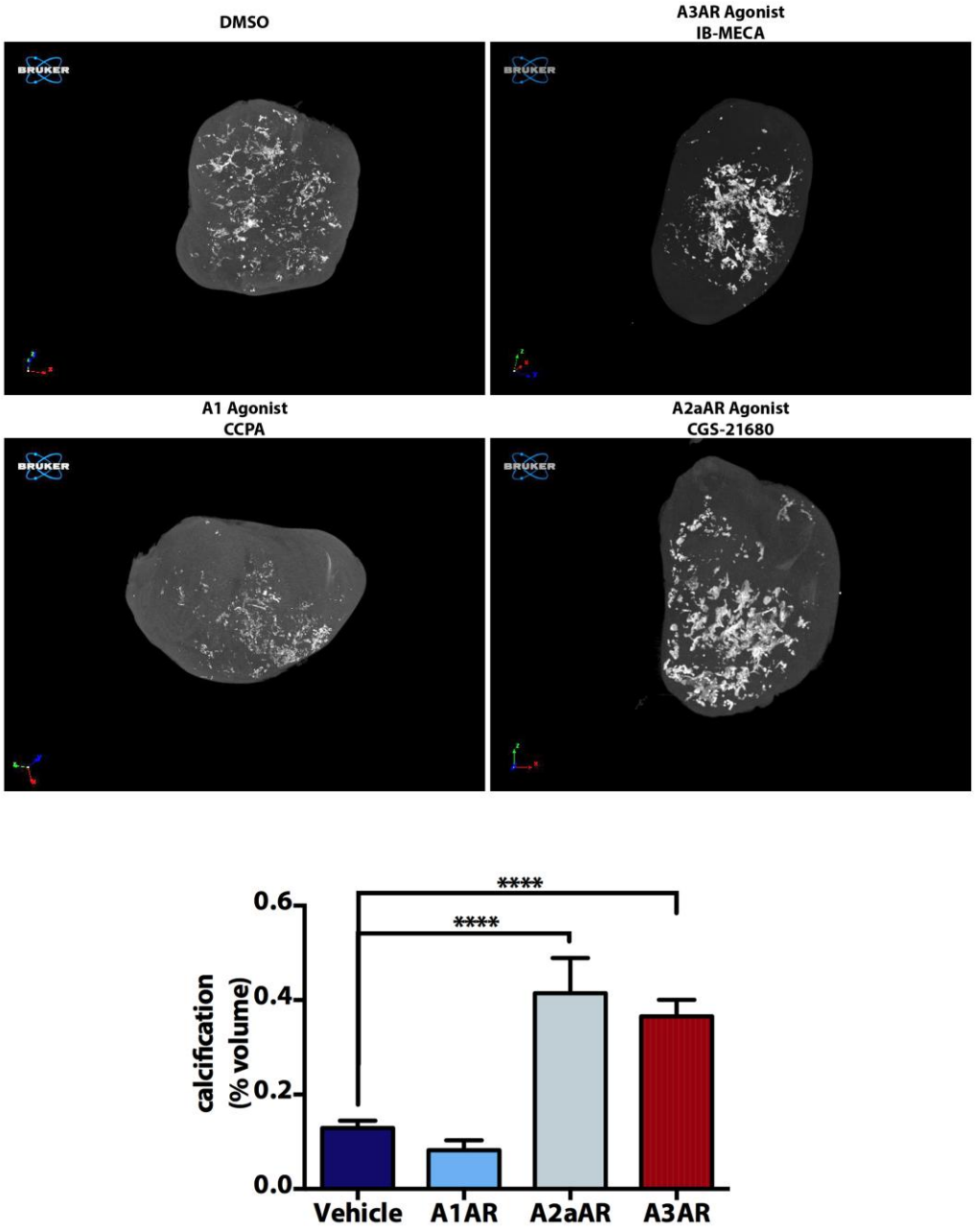


Fig. S8. Effect of AR agonists on calcification in teratomas.

Cells from one ACDC iPSC line were injected intramuscularly into NSG mice, then adenosine receptor agonists were injected introperitoneally daily. In the control group, an equal volume of DMSO in normal saline was injected. After 6 weeks, teratomas were harvested and scanned by tissue in vitro CT scanner, and calcification persentage were computed in CT Analyzer software. Data are mean ± SEM; DMSO (vehicle) n=12 teratomas; A1 (CCPA) n=3; A2a (CGS21680) n=5 ****p<0.0001; A3 (IB-MECA) n=3 ****p<0.0001) using two-tailed unpaired student's t test relative to control teratomas.

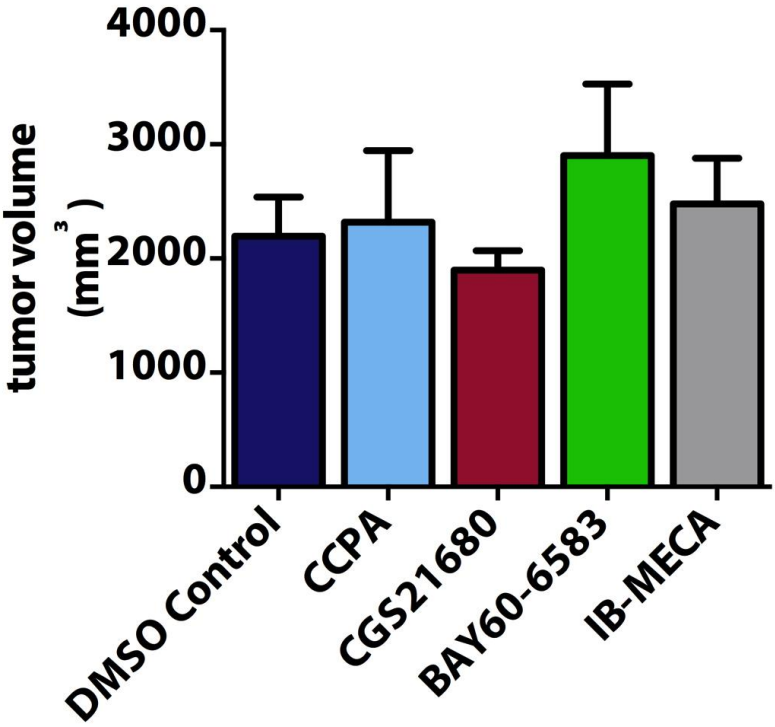


Fig. S9. AR agonists do not affect teratoma size.

Tumor volumes were measured in CT Analyzer software (mean ± SEM; DMSO n=12 teratomas; CCPA n=3; CGS21680 n=5; BAY60-6583 n=6; IB-MECA n=3. Data was analyzed by one-way ANOVA, multiple comparisons, differences were not significant compared to DMSO controls.

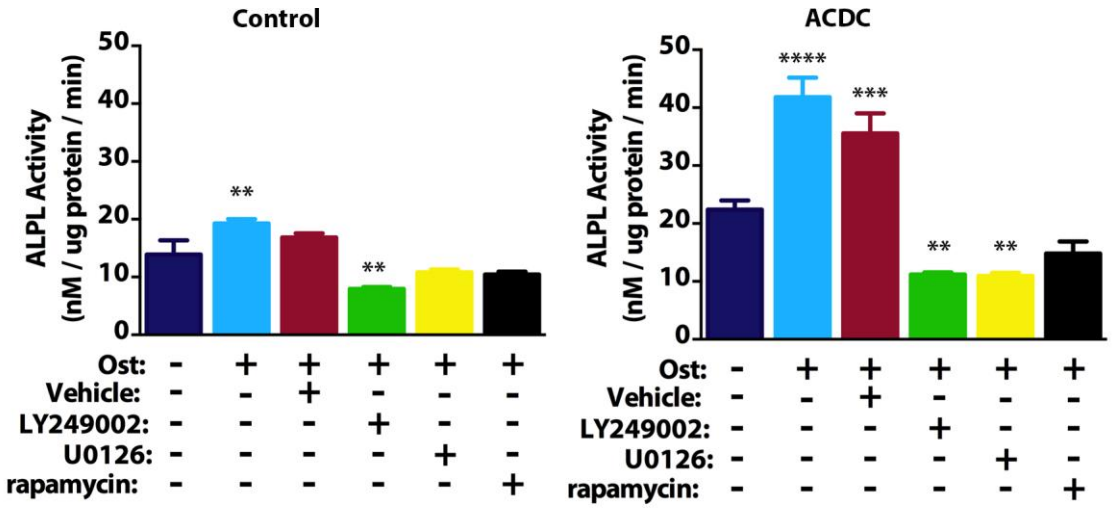
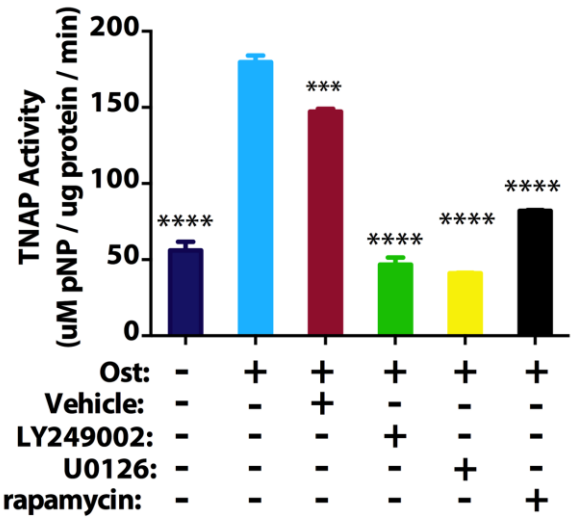


Fig. S10. TNAP activity in fibroblasts from control and ACDC patients.

Control (left) and ACDC (right) fibroblasts were cultured under osteogenic conditions (Ost) for five days and supplemented every other day with DMSO (vehicle), PI3K inhibitor (LY249002, 10uM), MEK1/2 inhibitor (U0126, 10uM), or mTOR inhibitor (rapamycin 200nM). Data shown are means ± SEM, n=3 patients cell lines run in triplicate in each group. For $^{**}p \leq 0.01$, $^{***}p \leq 0.001$, $^{****}P < 0.0001$ determined using one-way ANOVA with Dunnett's multiple comparison to no treatment conditions for each cell type.

A.



B.

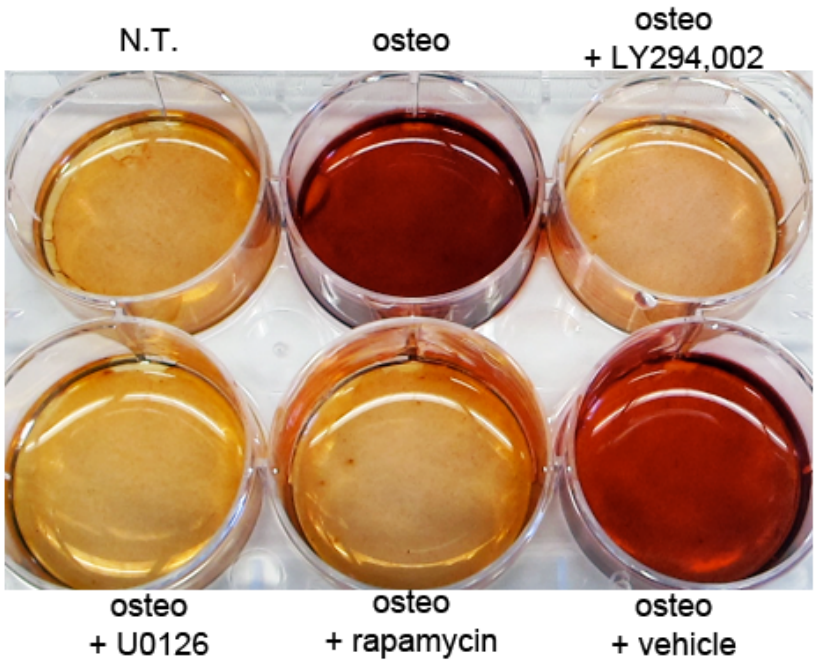


Fig. S11. TNAP activity and calcification in hMSCs.

Human MSCs were cultured under osteogenic conditions (Ost) for five days (**A**) or 21 days (**B**) and supplemented every other day with DMSO (veh), PI3K inhibitor (LY249002, 10uM), MEK1/2 inhibitor (U0126, 10uM), or mTOR inhibitor (rapamycin 200nM). Data shown are means ± SEM, n=3 independent experiments. $p<0.001$ as compared to Ost treatment, assessed by one-way ANOVA with Dunnett's multiple comparisons test. Image shown is representative of three separate experiments.

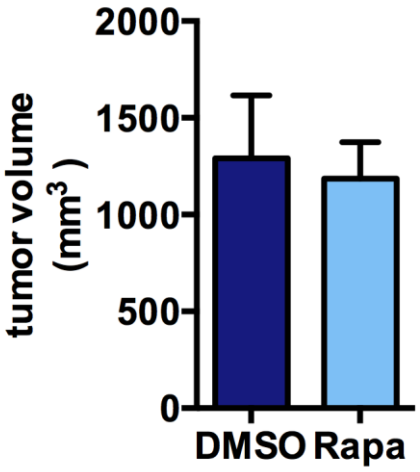


Fig. S12. Rapamycin treatment does not affect teratoma size.

Tumor volume were valued in CTAn (mean±SEM; DMSO n=6 teratomas; rapamycin n=3 teratomas. Data are mean ± SEM. No significant difference was detected using student t test, two-tailed, unpaired.