Supplemental Materials

Transfection with luciferase (Luc) reporters: (-577) human Col2 vs. mouse Col2 plus Luc control Previous experiments tested marker gene type II collagen (Col2) promoter-driven reporters for tracking MSC chondrogenesis. Early passage human mesenchymal cells (hMSCs) (P0 or P1) were grown in high-FBS human mesenchymal cell media (DMEM Low-Glucose and 20% FBS from Gibco plus FGF-basic from Peprotech) until 80% confluence. hMSCs were then passaged with 0.05% trypsin-EDTA (Gibco) and partitioned into aliquots of 5 x 10^5 cells for each transfection reaction. Transfection was accomplished as described in the main text for either the human Col2 based (-577 region of the promoter) reporter or mouse Col2 based reporter plasmid. Freshly transfected hMSCs were seeded in hMSC media and grown until 80% confluence. Cells were then trypsonized as described above and seeded at 5 x 10^5 cells per well in a 96-well, conical bottom, plate and centrifuged as described to generate pellets. hMSC pellets were incubated at 37°C, 5% CO_2 in pellet chondrocyte differentiation media for up to three weeks. During the three-week incubation period, hMSC pellets were selected at Days 4, 9, 14, 16 and 19 and imaged using the PerkinElmer/Xenogen IVIS system. Final histology analysis was performed on Day 21 with IHC staining for Col2.

Figure S1A shows all three reporter constructs: the triple fusion reporter (TFR) consisted of firefly luciferase, monomeric red fluorescent protein, and truncated herpes viral thymidine kinase driven by the constitutively active myeloproliferative sarcoma virus LTR (MPSV). Only the Luc was used as the control. The two Col2 promoter-driven reporters (mouse and human) have the same backbone. The human Col2 promoter was truncated -577 proximal to the promoter for maximal luciferase activity. The second reporter (green fluorescent protein) was also driven by constitutively active MPSV for histology.

Figure S1B is a collection of sequential bioluminescent imaging (BLI) of pellets transfected with luciferase reporters taken at 10 min after the addition of imaging substrate D-luciferin. Figure S1C shows the time activity curves from the pellets. The raw data is in log scale showing exponential decay. Both (human and mouse) Col2 signals were very low in signal intensity comparing to the Luc control. When normalized to that of the Luc control, they peaked on different days although the general trend was similar. Figure S1D shows immunohistochemical (IHC) staining for Col2 at Day 21 for pellets transfected with human Col2 promoter driven Luc, wild type (wt, with out reporter transfection), and Luc control. By Day 21, Col2 staining was strong (redish) across all pellets.

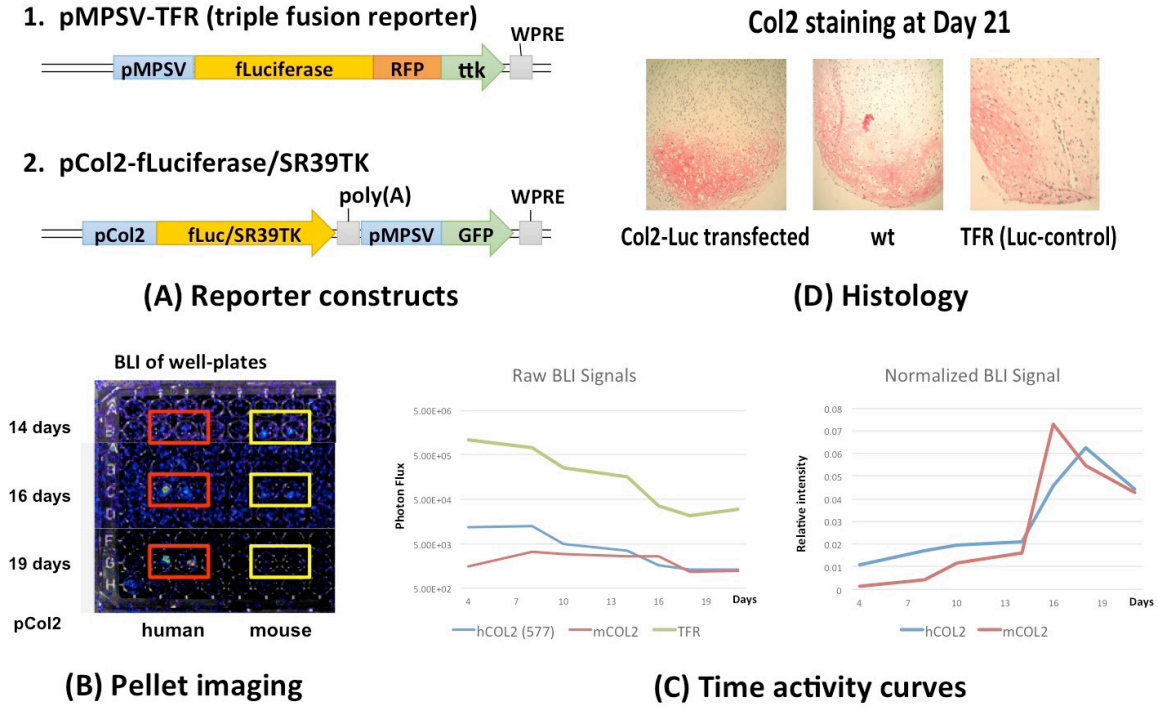

Figure S1: Marker gene (collagen type II or Col2) based imaging for tracking Col2 expression time course with BLI.
A) Reporter constructs: top, the triple fusion reporter (TFR, consisted of firefly luciferase, monomeric red fluorescent protein, and truncated herpes viral thymidine kinase. Only fLuc was used as the control) driven by the constitutively active myeloproliferative sarcoma virus LTR (MPSV); bottom, the Col2 promoter-driven reporter (two versions with mouse and human Col2 promoters, the human Col2 promoter was truncated -577 proximal to the promoter for maximal luciferase activity). The second reporter (green fluorescent protein) was also driven by constitutively active MPSV for histology.
B) Pellet imaging: sequential bioluminescent imaging (BLI) of pellets transfected with luciferase reporters. This is a collection of well-plate imaging taken at 10 min after the addition of imaging substrate D-luciferin.
C) Time activity curves from the pellets. Left: raw data in log scale; Right: both (human and mouse) Col2 signals were normalized to that of the Luc control.
D) Histology with Col2 staining of (Day 21) pellets transfected with human Col2 promoter driven Luc, wild type (wt, with out reporter transfection), and Luc control.

Figure S2 shows the full results from current bioluminescent imaging (BLI) of pellets transfected with the luciferase reporters. On Days 3, 6, 10, 12, 14, 16, 18, 20 and 26, cell pellets were selected for BLI using an IVIS spectrum imager (PerkinElmer, CA). During each imaging session, the wells containing pellets were added with the substrate D-luciferin potassium salt from Promega (Madison, WI) and imaged at 1, 5, 10 and 15 min after the addition of D-luciferin. During each BLI session, 1-min imaging exposure was performed at preset time points after incubation with substrate D-luciferin. The inserts are representatives of well-plate imaging taken at 10 min after the addition of imaging substrate D-luciferin. The exposure time after that time point increased to 5 min to get more photons for the image. ROIs were placed over the pellets for calculating the intensity of the signal in the unit of p/s/cm²/sr for averaged radiance and displayed in log scale.

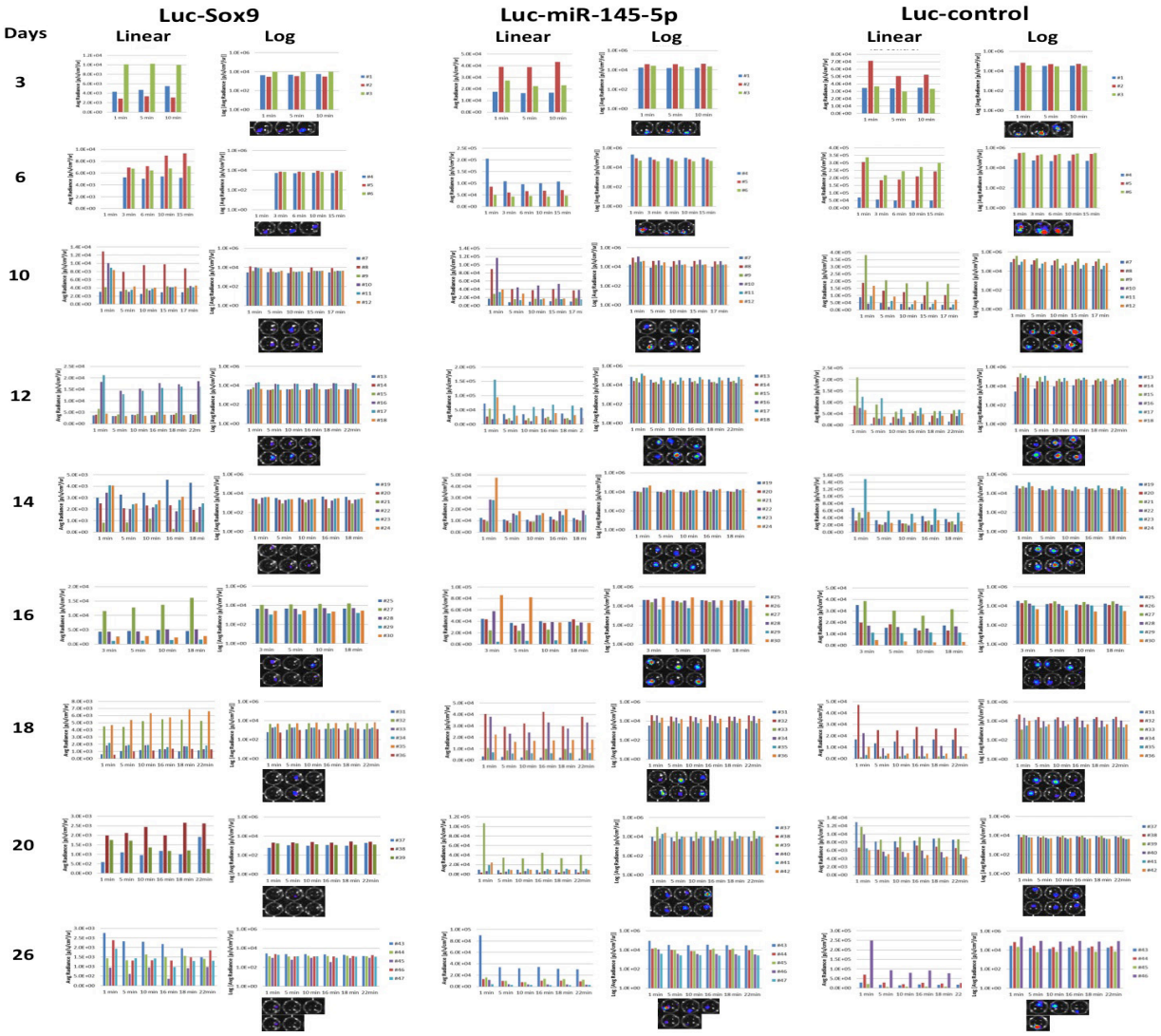

Figure S2: Bioluminescent imaging (BLI) of pellets transfected with luciferase reporters. The inserts are representatives of well-plate imaging taken at 10 min after the addition of imaging substrate D-luciferin. BLI was performed at preset time points after incubation with substrate D-luciferin. Each exposure was 1 min. ROIs were placed over the pellets for calculating the intensity of the signal in the unit of p/s/cm²/sr for averaged radiance and displayed in both linear and log scales.

Figure S3 Sox9 transcript (mRNA) binding sites for hsa-mir-145-5p. Top: the mirSVR score (for miRNA target prediction) is -0.3203 and the PhastCons score (for evolutionary conservation) is 0.4449; Bottom: the mirSVR score is -1.0271 (the cutoff is -0.1) and the PhastCons score is 0.5725 (the cutoff is 0.57).

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        3' ucCCUAAGGACCCUUUUGACCUg 5' hsa-miR-145
             | |  ||    | :||||||||
   267:5' gcGAAAUCAACGAGAAACUGGAc 3' SOX9


        3' ucccuAAGGACCCU--UUUGACCUg 5' hsa-miR-145
                 ||  :||   |  ||||||||
  1385:5' uguuuUUGUUGAAAACAAACUGGAa 3' SOX9
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Figure S3: Potential Sox9 transcript (mRNA) binding sites for hsa-mir-145-5p.