Step 1 Isolation of notochordal cells from porcine tails (NC) and seeding into alginate beads at $4 \times 10^6$/mL and culture for 7 days

Culture with 10% serum containing medium

NC can be activated with serum?

Culture in serum-free medium

expressing unidentified activating cytokines

Medium was collected and called notochord conditioned medium (NCCM, 30 beads in 4mL media, 2 medium changes for 7 days)

Step 2 Start of co-culture with nucleus pulposus or annulus fibrosus cells from a single bovine tail and culture for 14 days

control: pure NPC or AFC

Co-culture with insert: NPC vs NC

Co-culture with insert: AFC vs NC

Culture with CM (of step 1) of NPC or AFC

All cultures set-up in normoxia (~20%) and hypoxia (2%), no cell-cell contact, cells are separated by polyethylene terephthalate (PET) high density membrane, pore size 4μm.