OBJECTIVES/SPECIFIC AIMS: The objective of this study is to assess the efficacy of combined AF and NP repairs to prevent degenerative changes and restore native disc morphology in an in vivo large animal model. We hypothesize that combined repairs will prevent disc degeneration following injury to a greater extent than the individual repairs after 6 weeks in vivo, and as demonstrated through disc height measurements and disc morphology. METHODS/STUDY POPULATION: A total of 8 skeletal mature female Finn sheep were used in this study. Following a previously described method, IVDs from L1 to L6 of the lumbar spine were exposed using a lateral access, extraperitoneal approach. IVDs were randomized into 5 treatment groups: 1) intact discs, 2) discs injured via a 3 cm x 1 cm box annulotomy and partial nucleotomy, 3) injury followed by a high density collagen (HDC) AF patch, 4) injury followed by injection of a modified hyaluronic acid (HA) into the NP, and 5) injury followed by both the HDC AF patch and HA NP injection. The HDC treatment was 15 mg/mL type-I collagen mixed with 0.06 mM riboflavin, injected at the defect site and crosslinked in situ with blue light. The NP injection was HA modified with C16 side chains to increase the viscosity of the hydrogel (HYADD 4®). At 6 weeks post-operatively, sheep were sacrificed and had 3T magnetic resonance images (MRI) taken of their lumbar spine. Disc height analysis and Pfirrmann grading were performed on each segment using MR images. Additionally, quantitative MRI analyses were performed using a custom MATLAB algorithm that segments NP from the surrounding tissue and directly measures the NP volume. ANOVA with Tukey’s HSD was used to determine statistical significance between groups for disc height and quantitative MRI analyses, and the Kruskal-Wallis test with Mann-Whitney tests was used to statistically analyze Pfirrmann Grades. All animal use followed approved IACUC protocol. RESULTS/ANTICIPATED RESULTS: As shown in axial MR images (Figure 1A), intact discs had a hypointense NP with a distinct border to the AF. The discs receiving injury with no treatment had hypointense NP with no distinct border between the AF and NP. Individual and combined treatment with the HA NP injection and HDC AF patch appeared to preserve the hypointense NP signal and AF/NP border. Intact control discs were not degenerated and had an average Pfirrmann grade of 1 (Figure 1B), while injured, untreated discs had significant degeneration with an average Pfirrmann grade of 3. Discs receiving the HA NP injection and collagen AF patch individually showed fewer signs of degeneration than the injured alone, and the combined treatment resulted in the least amount of degeneration with Pfirrmann grades not significantly different than the intact controls. Disc height index confirmed the trends seen in the Pfirrmann grades (Figure 1C), where injured discs lost 20% of the intact disc height, the individual NP and AF repairs restored 5-10% of intact disc height, and the combined repairs preserved 90% of the intact disc height. The NP voxel count of all treatment groups were similar to the intact controls (Figure 1D). DISCUSSION/SIGNIFICANCE OF IMPACT: The objective of this study was to determine how combined AF and NP can prevent degenerative changes to the disc in a large animal in vivo model. Pfirrmann grading and disc height index results show that the greatest preservation of disc morphology was seen with combined AF and NP repairs, while the individual strategies prevented degenerative changes better than injury with no treatment. It appears the HA NP injection restores lost water content to the disc following injury, and the AF collagen patch plays a role in maintaining the NP repair within the disc. This is the first study to our knowledge to attempt combined AF and NP repairs in an in vivo large animal model. Combining NP and AF repairs leads to significantly improved outcomes following disc injury, which warrants the translation of combined repairs into the clinic to improve patient outcomes with degenerative disc disease involving NP and AF.