TSG-6 is weakly chondroprotective in murine OA but does not account for FGF2-mediated joint protection

Linyi Zhu*[a], PhD, Shannah Donhou*[a], BSc, Annika Burleigh[a], PhD, Jadwiga Miotla Zarebska[a], PhD, Marcia Curtinha[a] BSc, Ida Parisi[a], PhD, Sumayya Nafisa Khan[a], PhD, Francesco Dell'Accio[b], MD PhD, Anastasios Chanalaris[a], PhD, Tonia L Vincent[a], MD PhD.

[a]Kennedy Institute of Rheumatology, Arthritis Research UK Centre for OA Pathogenesis, University of Oxford, UK

[b]William Harvey Research Institute, Queen Mary, University of London, UK

*Equal contribution

Corresponding author:

tonia.vincent@kennedy.ox.ac.uk

0044-1865 612645

Keywords: TSG-6, osteoarthritis, FGF2, chondroprotection

Funding: Arthritis Research UK, Kennedy Trust for Rheumatology Research

The authors have no conflicts to declare.

Abstract

Objective: TSG-6 is an anti-inflammatory protein highly expressed in osteoarthritis (OA), but its influence on the course of OA is unknown.

Methods: Cartilage injury was assessed by murine hip avulsion or re-cutting rested explants. 42 previously validated injury genes were quantified by real-time PCR in whole joints post DMM (6h and 7days). Joint pathology was assessed 8 and 12 weeks following destabilisation of the medial meniscus (DMM) in 10 week old male and female FGF2$^{-/-}$, TSG-6$^{-/-}$, TSG-6^{tg} (overexpressing), FGF2$^{-/-}$;TSG-6^{tg} (8 weeks only) mice and strain-matched, wild type controls. In vivo cartilage repair was assessed 8 weeks following focal cartilage injury in TSG-6^{tg} and control mice. FGF2 release following cartilage injury was measured by ELISA.

Results: TSG-6 mRNA upregulation was strongly FGF2-dependent upon injury *in vitro* and *in vivo*. 15 inflammatory genes were significantly increased in TSG-6$^{-/-}$ joints including *IL1α*, *Ccl2* and *Adamts5* compared with wild type. Six genes were significantly suppressed in TSG-6$^{-/-}$ joints including *Timp1, Inhibin βA* and *podoplanin* (known FGF2 target genes). FGF2 release upon cartilage injury was not influenced by levels of TSG-6. Cartilage degradation was significantly increased at 12 weeks post DMM in male TSG-6$^{-/-}$ mice, with a non-significant 30% reduction in disease, seen in TSG-6^{tg} mice. No differences were observed in cartilage repair between genotypes. TSG-6 overexpression was unable to prevent accelerated OA in FGF2$^{-/-}$ mice.

Conclusion: TSG-6 influences early gene regulation in the destabilised joint and exerts a modest late chondroprotective effect. Although strongly FGF2 dependent, TSG-6 does not explain the strong chondroprotective effect of FGF2.

INTRODUCTION

Tumour necrosis factor alpha (TNF-α) stimulated gene 6 (TSG-6) is a secreted product of TNF-α treated cells (1), which encodes for a 35kDa multifunctional protein, consisting of Link and CUB (complement protein subcomponents C1r/C1s, urchin embryonic growth factor and bone morphogenetic protein 1) modules (2-4). TSG-6 is expressed in response to a range of pro-inflammatory mediators (1, 2, 5) and is involved in a number of physiological processes including cervical ripening (6) and ovulation (7).

In murine models of inflammatory arthritis, TSG-6 has been shown to protect the joint against damage. Delivery of recombinant protein led to a reduction in proteoglycan-induced arthritis while deletion of TSG-6 in the same murine model led to increased severity of arthritis (8, 9). Chondroprotection was seen in mice overexpressing TSG-6 in a collagen-induced arthritis model (10) and in antigen-induced arthritis where mice expressing a cartilage-specific transgene of TSG-6 had reduced aggrecan and cartilage degradation (11).

TSG-6 has been detected at high levels in the synovial fluid of patients with rheumatoid arthritis and osteoarthritis (12, 13) and TSG-6 levels are also highly elevated in human synovial fluid following joint injury (14). Moreover, TSG-6 enzymatic activity, detected by the transfer of heavy chains from inter-alpha-inhibitor (IαI) to hyaluronan, has been identified as a biomarker for knee OA progression, such that increased TSG-6 activity is associated with a higher risk of total knee replacement (15).

We have previously shown that TSG-6 is strongly regulated in the mouse joint early following surgical destabilisation of the medial meniscus (DMM), a model of OA. This regulation was deemed to be highly mechanosensitive as regulation was abrogated if the mice were prevented from mobilising on their destabilised joint (16). We also showed that both *in vivo* and *in vitro* injury-induced regulation of TSG-6 was strongly dependent on fibroblast growth factor 2 (FGF2), a growth factor that is released from the cartilage matrix upon injury or loading (17,

18). Our previous work showed that mice deficient in FGF2 develop markedly accelerated OA upon surgical joint destabilisation and upon ageing, indicating a chondroprotective role for FGF2 *in vivo* (19). FGF2 may be chondroprotective through its ability to suppress ADAMTS5, one of the principal pathogenic aggrecan degrading enzyme in cartilage. Indeed, *Adamts5* was elevated in $FGF2^{-/-}$ mice and FGF2 was able to suppress IL-1 induced aggrecanase activity, in human articular cartilage explants *in vitro* (20). Whether this is a direct response or whether suppression of ADAMTS5 is through an intermediate protein such as TSG6, is unknown.

In view of the strong anti-inflammatory role of TSG6 in other arthritis models, and its strong FGF2-dependent gene regulation, in this paper we explore the hypothesis that the chondroprotective properties of FGF2 may in part be mediated through TSG-6. We examine the course of disease after deletion or overexpression of TSG-6 and ask whether TSG-6 overexpression is able to prevent accelerated disease that is seen in $FGF2^{-/-}$ mice.

MATERIALS AND METHODS

Animals. Animal experiments were carried out after gaining ethical approval in agreement with local policy. 4-6 mice per cage were housed at 21°C in standard individually ventilated cages, maintained under a 12 hour light/dark cycle. Mice were fed a certified mouse diet (RM3; Special Dietary Systems) and water *ad libitum*. TSG-6 constitutive knockout ($TSG\text{-}6^{-/-}$) and cartilage specific (Col2 driven, Balb/c background) constitutive over-expressing TSG-6 transgenic mice ($TSG\text{-}6^{tg}$) (11) were obtained from Katalin Mikecz, Rush University Medical Center, Illinois, USA. $TSG\text{-}6^{-/-}$ animals were backcrossed onto a C57BL/6 background (for 9 generations) and were bred as heterozygotes to generate wildtype and knockout littermate controls. $FGF2^{-/-}$ mice were originally purchased from Jackson Laboratory and were backcrossed onto a pure C57BL/6 background (9 generations). $TSG\text{-}6^{tg}$ (Balb/c) mice were crossed with $FGF2^{-/-}$ (C57BL/6) to generate mixed litters of $FGF2^{-/-}$;$TSG\text{-}6^{tg}$ and $FGF2^{-/-}$;TSG-

6^{WT} (mixed background). Balb/c mice were obtained from Charles Rivers, UK laboratory. Figure 1 shows the schematic of the experiment design which summarized the in vivo experiments performed including a total number of mice of each genotype in each experiment.

Surgical induction of OA. 10 week old mice were anesthetized by inhalation of Isoflurane (3% induction and 1.5-2% maintenance) in 1.5-2 L/min oxygen. Following surgery, mice received a subcutaneous injection of Vetergesic (Alstoe Animal Health Limited). The mice were fully mobile 5 minutes after withdrawal of isoflurane. OA was induced by cutting the medial menisco-tibial ligament as previously described (16). For sham surgeries, the joint was opened under anaesthesia but the menisco-tibial ligament was left intact. Murine joints were harvested, and the skin and muscle bulk removed (16).

Histological assessment. Dissected knees were fixed in 10% formalin, decalcified in dilute formic acid, and embedded in paraffin. Coronal sections through the whole joint (80 μm apart) were cut and stained with Safranin O. Severity of cartilage destruction was assessed by a modified Osteoarthritis Research Society International OA grading system as previously described (19) by 2 blinded, independent scorers. At least 8 sections were evaluated per joint. Results were expressed as the summed score, which is calculated by adding the 3 highest scores together from any given joint section (all four joint compartments). Osteophytes were scored by size (0-3) and maturity (0-3) (n = 5 per group) as previously described by Little *et al* (21).

Focal cartilage injury and histological assessment. Focal cartilage injury was carried out under a dissection microscope as previously described by Eltawil *et al* (22). Briefly, the joint was opened, the patella was dislocated and a longitudinal full thickness injury was made in the patellar groove using the tip of a 25 G needle. Patellar dislocation was reduced and the joint capsule and skin were sutured closed. The contralateral knee was left as an un-operated control. Three transverse sections at 100 μm intervals (22) were scored for cell morphology (0-4), matrix staining (0-4), filling of the defect (0-4), and osteochondral junction repair (0-2)

according to the modified Pineda scale; a high score indicating better cartilage repair (scores were inverted in Eltawil *et al* (22)). Sums of these 4 categories were given for each section and an average score was calculated (max score = 14).

Cartilage injury. Mice (5–6 weeks old) were culled by CO_2, and the acetabulofemoral (hip) joints were exposed by blunt dissection. The femoral cap (cartilage) was avulsed using forceps, as described previously (19). Murine hip cartilage was avulsed (avulsion injury) directly into serum-free Dulbecco's Modified Eagle's Medium (DMEM) and incubated for 48 h. Some explant were rested for 48 h after avulsion, then either re-cut (cut with a scalpel into 4 pieces) or treated with FGF2 (250ng/ml) in fresh DMEM and left for 4 h prior to RNA extraction. For FGF2 release cartilage explants (post avulsion) were immediately placed in 100 µl serum-free DMEM at 37^0C for 30 min. The medium collected (injury conditioned medium), was stored at -80^0C until FGF2 measurement.

FGF2 measurement in cartilage avulsion injury conditioned medium. FGF2 levels in the injury conditioned medium were assayed in duplicate on single spot ultra-sensitive V-PLEX bFGF kit (catalog no. K151MDD) from Meso Scale Discovery (MSD, Meso Scale Discovery, 1601 Research Blvd, Rockville, MD). The assay was carried out according to the manufacturer's instructions. Plates were read using MSD SPECTOR Imager 2400 measuring electrochemiluminescence. FGF2 concentrations were extrapolated from a standard curve calculated using a four-parameter logistic fit using MSD Discovery Workbench software version 3.

RNA extraction and Real-Time PCR. Four murine femoral heads were snap frozen and stored at -80°C. For whole joints, the joint was harvested at defined anatomical positions (patella insertion on tibia and quadriceps insertion on femur), and the skin and muscle removed as previously described (23). The femoral head cartilage or joints were pulverized using a PowerGen 125 Polytron instrument (Fisher Scientific), and RNA was extracted using Qiagen

RNeasy Mini Kit according to the manufacturers' instructions. RNA was reverse transcribed using a High Capacity cDNA kit (Applied Biosystems) and analysed on 384-well custom-made TaqMan microfluidic cards.

Statistical analysis. Differences in gene expression levels between WT (C57BL/6) and TSG-$6^{-/-}$ joints were analysed by unpaired multiple t-tests and the p-values corrected for multiplicity (q-values) using a family wise false discovery ratio of 5%. q-values are used in Table 1. Analysis of variance (ANOVA) with Sidak *post hoc* testing to adjust for multiplicity was used to compare TSG-6 gene expression differences between WT and $FGF2^{-/-}$ joints. Mann-Whitney U test was used to analyse TSG-$6^{-/-}$ and TSG-6^{tg} histological pathology scores. Where three genotypes were considered side by side (Figure 5), ANOVA was performed. p values less than 0.05 were considered statistically significant. Spearman r correlation test was used to assess a relationship between fold gene expression and histological score. Where data was not normally distributed (tested using the D'Agostino normality test), and in the case of non-parametric data like osteophyte and focal cartilage data, Mann Whitney tests were used. Statistical testing was performed using GraphPad Prism 7 and SPSS 26.0 (IBM) software.

RESULTS

TSG-6 is induced upon injury in an FGF2-dependent manner. We first confirmed TSG-6 regulation following cartilage injury *in vivo* and *in vitro* as previously shown by our group (23). TSG-6 was strongly induced upon re-cutting injury or FGF2 stimulation (Figure 2a). It was also strongly upregulated in whole joints after DMM in an FGF2-dependent manner (Figure 2b); upregulation of TSG-6 being suppressed in $FGF2^{-/-}$ mice.

Deletion of TSG-6 leads to increased inflammatory genes and decreased FGF2-dependent gene regulation following DMM. We next wanted to see how TSG-6 influenced the regulation of inflammatory response genes in the whole joint following DMM. TaqMan microfluidic cards were prepared for a number of known inflammatory and FGF2-dependent genes previously found to be upregulated in whole joints following DMM or after *in vitro* cartilage injury (16, 17). Twenty-eight genes were significantly regulated at any point post-surgery compared with the 0 hour unoperated controls. 15 genes (Table 1) were significantly upregulated in TSG-6$^{-/-}$ joints compared with WT (C57BL/6) joints 6 hours after DMM, including inflammatory response genes such as *Adamts5*, *Ccl2* and *IL-1α*. These findings appear consistent with published studies showing an anti-inflammatory effect of TSG-6. 6 genes were significantly suppressed in TSG-6$^{-/-}$ joints compared with WT (C57BL/6) joints 6 hours after DMM including *Inhibin βA*, *Tnfrsf12a*, *Podoplanin* and *Timp1*. Interestingly, 5 out of the 6 genes that were strongly suppressed in TSG-6$^{-/-}$ joints had previously been shown to be highly FGF2 dependent *in vivo* and *in vitro* (17).

Male TSG-6$^{-/-}$ show a modest late increase in disease following DMM. Gene expression profiles suggested an increase in inflammatory mediators in the joint associated with a reduction of FGF2-dependent genes. As FGF2 has been shown to be chondroprotective (19) we hypothesised that TSG-6$^{-/-}$ mice might develop accelerated OA following DMM. We examined the susceptibility of 10-week-old male and female WT (C57BL/6) and TSG-6$^{-/-}$ mice to DMM-induced OA and compared the summed scores 8 and 12 weeks after surgery. No differences were seen between TSG-6$^{-/-}$ and WT (C57BL/6) mice in either males or females at 8 weeks post DMM (Figure 3a-b). At 12 weeks post DMM, TSG-6$^{-/-}$ male mice, but not female mice, had a statistically significant 50% increase in mean disease score (21.9 ± 10.1) compared with WT (C57BL/6) DMM controls (14.2 ± 4.7) (Figure 3a). No disease was seen in the contralateral joints (of DMM operated mice) of either genotype at either time point.

Osteophytes are established early (from 1 week) post DMM (24). At 8 weeks post DMM, there were no significant changes in osteophyte size or maturity in male mice (Figure 3c-d). This was also the case at 12 weeks (data not shown).

Male transgenic mice show a non-significant 30% reduction in disease. We next examined the susceptibility of male and female TSG-6^{tg} mice following DMM. Cartilage degradation was assessed by histology 8 and 12 weeks after DMM. Male TSG-6^{tg} mice showed a 30% reduction in mean disease score at 12 weeks post DMM that did not reach statistical significance after correcting for multiple testing ($p = 0.066$, Mann-Whitney U test) (Figure 4a). No differences were observed in sham-operated joints between genotypes at either 8 or 12 weeks post surgery. There were no significant differences between any of the female experimental groups (Figure 4b). As male mice showed a non-significant reduction in disease mean score at 12 weeks post DMM, we looked at the amount of transgene expressed and whether this correlated with the cartilage degradation score. There was no correlation between the amount of transgene expressed (in the contralateral joint) and the severity of cartilage damage 12 weeks post DMM (Figure 4c). Nor was there a difference in osteophyte size or maturity in male mice 8 weeks post DMM (Figure 4d-e).

In order to see whether TSG6 might be mediating the protection afforded by FGF2, we tested whether overexpression of TSG-6 would compensate for loss of FGF2. TSG-6^{tg} mice were crossed with FGF2$^{-/-}$ mice to generate mixed litters of FGF2$^{-/-}$;TSG-6^{tg} and FGF2$^{-/-}$;TSG-6^{WT} mice (on a mixed background). Deletion of FGF2 (FGF2$^{-/-}$;TSG-6^{WT}) led to severe disease compared with wild type C57BL/6 or Balb/c mice consistent with our previous publication (19). Overexpression of TSG-6 (FGF2$^{-/-}$;TSG-6^{tg}) was unable to compensate for loss of FGF2 (Figure 4f-g) and disease scores did not correlate with transgene level (Figure 4h).

Overexpression of TSG-6 does not affect healing of focal cartilage defects. Chondroprotection may be mediated by enhanced cartilage repair within the joint. We

considered whether TSG-6 affected the bioavailability of FGF2, a repair cytokine, after articular cartilage injury, and whether overexpression of TSG-6 would influence the healing of cartilage *in vivo*. To address the former, conditioned medium from injured mouse hips from FGF2$^{-/-}$, WT(Balb/c), TSG-6$^{+/-}$(heterozygotes), TSG-6$^{-/-}$ (homozygotes), and TSG-6^{tg} mice were assayed for FGF2 (Figure 5a). Levels of FGF2 were equivalent between groups (apart from that generated by FGF2$^{-/-}$ cartilage). These results suggested that the bioavailability of FGF2 after injury was not determined by TSG-6 levels. Next, we generated full thickness defects in the patellar groove of TSG-6^{tg}, WT (Balb/c) and C57BL/6 mice using a model of cartilage regeneration that has been shown to be strain and age-dependent (22). 10-week-old male and female Balb/c mice produced superior repair compared with C57BL/6 controls 8 weeks after surgery (Figure 5c-d). No difference in repair was seen between TSG-6^{tg} and WT (Balb/c) in either male or female mice.

DISCUSSION

TSG-6 mRNA is strongly upregulated post DMM and has a major influence on suppressing inflammatory genes as well as influencing FGF2-dependent genes early following induction of OA. *In vivo* data demonstrated increased cartilage degradation 12 weeks post DMM in male TSG-6$^{-/-}$ mice and a non-significant 30% reduction of mean disease score in TSG-6^{tg} mice at the same time point (12 weeks). These data collectively suggest that TSG-6 has a real, albeit modest chondroprotective role in the joint. Despite its strong FGF2 dependence, TSG6 overexpression appeared to be unable to reverse accelerated disease seen in FGF2$^{-/-}$ mice indicating that it is not the principal driver of FGF2-dependent chondroprotection. It was possible that the increase in mRNA did not translate to an increase in protein but we were unable to validate this by immunohistochemistry (data not shown). When we considered gender, female mice developed very modest disease post DMM, as previously shown (25).

Little disease progression was seen over time in these mice and there was no detectable effect of genotype.

The function of TSG-6 remains poorly understood and it remains unclear whether modest joint protection is afforded by its anti-inflammatory or other actions. The anti-inflammatory actions of TSG-6 may be related to the ability of the Link module of TSG-6 to bind to chemokines from the CXC and CC families (26) inhibiting neutrophil migration (27-29) or interfering with their matrix binding partners, heparin and heparan sulfate (HS) (30, 31). Murine OA induced by DMM is characterised by transient synovitis apparent immediately post-surgery but little overt neutrophilic infiltration is seen beyond 2 weeks (Vincent unpublished data). We did not attempt to measure levels of inflammatory cells in the OA joints, although there were increases in leukocyte markers such as CD14 and CD68 in whole joint extracts of TSG-$6^{-/-}$ compared with WT (C57BL/6) animals early post DMM. Nor did we specifically assess synovitis by histological scoring as this is difficult to do using coronal joint sections. Other inflammatory genes that were up-regulated in the TSG-$6^{-/-}$ joints included cytokines such as *Il1α*, *Ccl2* and *Il6*. Although these molecules have proposed pro-catabolic actions in the joint, they are probably being made by non-leukocytic cells e.g. chondrocytes, and published, as well as unpublished data from our group, do not support a role for these molecules in driving disease (32, 33). If TSG-6 is not acting by inhibiting leukocyte migration to suppress OA, it may be controlling cartilage loss by down-regulating the protease network (34, 35). This is partly mediated by the formation of a complex between the Link module of TSG-6 with inter-alpha-inhibitor (IαI) (36), a serine protease inhibitor. The inhibitory effect of this complex is specific for plasmin, a key activator of several MMPs, that is induced in murine OA by direct mechanical injury (16).

Our data show that TSG-6 promotes several FGF2-dependent genes with putative anti-inflammatory/repair functions e.g. the tissue inhibitor of metalloproteinase, *Timp1* and *inhibin*

βA, the dimer of which forms *activin A* (a TGFβ family member). These genes are strongly induced *in vivo* and *in vitro* upon cartilage injury (17) and have purported chondroprotective actions by anti-catabolic and pro-repair roles. The fact that they are also TSG-6 dependent suggests either that TSG-6 can influence these genes directly (by an unknown mechanism), or that TSG-6 affects the regulation or bioavailability of FGF2. We speculated that TSG-6 could be influencing the binding of FGF2 in the pericellular matrix of cartilage, where it resides and is released upon tissue injury (37, 38). However, the latter did not appear to be the case as the release of FGF2 upon cartilage injury was not influenced by the level of TSG-6 expression. Other complex actions of TSG-6 have been described such as heavy chain transfer-mediated stabilisation of the ECM (39, 40) and interference of tissue derived morphogenetic proteins such as BMP2 (41), which could possibly account for the influence that we are describing.

In the past decade, interest has turned to the role of TSG-6 in mesenchymal stem cells (MSCs); secreted TSG-6 is thought to mediate their immunomodulatory and tissue-protective properties (42, 43). TSG-6, as well as FGF2, regulate morphology and crucial cellular processes for the maintenance of stemness and biological properties of MSCs (44, 45). However, if the principal role of TSG-6 is to act on MSCs to enhance their repair capacity then we should have expected to see a change in repair score after focal cartilage injury. Our results show over-expression of TSG-6 has no influence on this repair. The focal cartilage injury model has not previously been explored in Balb/c mice and shows that this strain repairs well, in a similar fashion to DBA/1 mice (22). To fully exclude a pro-repair action of TSG-6, it would be necessary to perform the focal cartilage defect in transgenic mice back-crossed onto a non-repairing strain such as C57BL/6. This is beyond the scope of the current project.

TSG-6 activity, measured by heavy chain transfer, has been described as a biomarker for disease progression and is associated with increases in other inflammatory mediators including TIMP1, MMP3 and IL-6 (15, 46). Our data do not support a pro-disease role for TSG-6 in OA

297 suggesting that correlation with disease progression may be epiphenomenal rather than causal.
298 This is probably also the case following an acute knee injury, where synovial fluid TSG-6
299 levels follow a similar pattern to several other inflammatory response proteins (14), which in
300 part reflects the severity of the injury. Despite considerable efforts by a number of groups, the
301 precise mechanism of action of TSG-6 remains elusive and its therapeutic potential in OA,
302 speculative.
303
304 Limitations:
305 We did not perform in depth analysis of the bone in TSG-$6^{-/-}$ mice. Deletion of TSG-6 has been
306 shown to influence bone microarchitecture by modulating both osteoblast and osteoclast
307 function, which could potentially affect the biomechanical response in the joint following
308 DMM (41, 47). This is unlikely to have influenced the TSG-6 overexpressing mice as
309 overexpression was driven by Type II collagen in these mice and the effects should be more
310 restricted to the articular cartilage. Neither did we perform synovitis scoring nor pain
311 assessments on these mice. This was due to limitations imposed by coronal sectioning of the
312 joints which we routinely perform for OA cartilage scoring. Due to poor breeding of $Tsg6^{+/-}$
313 (heterozygotes) we were limited by the number of animals available and did not perform sham
314 operations in this strain or examine the effect of genotype with age, which in our experience
315 requires very much larger numbers. We recognise, discussed above, the limitation of examining
316 focal cartilage repair in Balb/c mice when they already appear to have moderately good
317 intrinsic repair capability (not known at the start of our experiment). We also recognise the
318 difficulties of trying to make conclusions from data that either just succeed or just fail to reach
319 statistical significance after stringent correction for multiple testing. We powered this study to
320 detect a 40% change in disease score between genotypes at any time point. A retrospective
321 power calculation indicates that we needed four additional wild type mice (n=16 WT, n=20

322 TSG-6^{Tg}, at 12 weeks post DMM) for the 30% reduction in disease to reach statistical
323 significance.
324

REFERENCE

1. Lee TH, Lee GW, Ziff EB, Vilcek J. Isolation and characterization of eight tumor necrosis factor-induced gene sequences from human fibroblasts. Molecular and cellular biology. 1990;10(5):1982-8.
2. Milner CM, Day AJ. TSG-6: a multifunctional protein associated with inflammation. Journal of cell science. 2003;116(10):1863-73.
3. Lee TH, Wisniewski H-G, Vilcek J. A novel secretory tumor necrosis factor-inducible protein (TSG-6) is a member of the family of hyaluronate binding proteins, closely related to the adhesion receptor CD44. The Journal of cell biology. 1992;116(2):545-57.
4. Tammi MI, Day AJ, Turley EA. Hyaluronan and homeostasis: a balancing act. Journal of Biological Chemistry. 2002;277(7):4581-4.
5. Day AJ, Milner CM. TSG-6: A multifunctional protein with anti-inflammatory and tissue-protective properties. Matrix Biology. 2018.
6. Fujimoto T, Savani RC, Watari M, Day AJ, Strauss III JF. Induction of the hyaluronic acid-binding protein, tumor necrosis factor-stimulated gene-6, in cervical smooth muscle cells by tumor necrosis factor-α and prostaglandin E2. The American journal of pathology. 2002;160(4):1495-502.
7. Fülöp C, Kamath RV, Li Y, Otto JM, Salustri A, Olsen BR, et al. Coding sequence, exon–intron structure and chromosomal localization of murine TNF-stimulated gene 6 that is specifically expressed by expanding cumulus cell–oocyte complexes. Gene. 1997;202(1-2):95-102.
8. Szántó S, Bárdos T, Gál I, Glant TT, Mikecz K. Enhanced neutrophil extravasation and rapid progression of proteoglycan‐induced arthritis in TSG‐6–knockout mice. Arthritis & Rheumatism: Official Journal of the American College of Rheumatology. 2004;50(9):3012-22.
9. Bárdos T, Kamath RV, Mikecz K, Glant TT. Anti-inflammatory and chondroprotective effect of TSG-6 (tumor necrosis factor-α-stimulated gene-6) in murine models of experimental arthritis. The American journal of pathology. 2001;159(5):1711-21.
10. Mindrescu C, Thorbecke G, Klein M, Vilček J, Wisniewski HG. Amelioration of collagen‐induced arthritis in DBA/1J mice by recombinant TSG‐6, a tumor necrosis factor/interleukin‐1–inducible protein. Arthritis & Rheumatism. 2000;43(12):2668-77.
11. Glant TT, Kamath RV, Bárdos T, Gál I, Szántó S, Murad YM, et al. Cartilage‐specific constitutive expression of TSG‐6 protein (product of tumor necrosis factor α–stimulated gene 6) provides a chondroprotective, but not antiinflammatory, effect in antigen‐induced arthritis. Arthritis & Rheumatism. 2002;46(8):2207-18.
12. Bayliss M, Howat S, Dudhia J, Murphy J, Barry F, Edwards J, et al. Up-regulation and differential expression of the hyaluronan-binding protein TSG-6 in cartilage and synovium in rheumatoid arthritis and osteoarthritis. Osteoarthritis and Cartilage. 2001;9(1):42-8.
13. Wisniewski H-G, Maier R, Lotz M, Lee S, Klampfer L, Lee TH, et al. TSG-6: a TNF-, IL-1-, and LPS-inducible secreted glycoprotein associated with arthritis. The Journal of Immunology. 1993;151(11):6593-601.
14. Watt FE, Paterson E, Freidin A, Kenny M, Judge A, Saklatvala J, et al. Acute molecular changes in synovial fluid following human knee injury: association with early clinical outcomes. Arthritis & rheumatology. 2016;68(9):2129-40.
15. Wisniewski H-G, Colón E, Liublinska V, Karia RJ, Stabler TV, Attur M, et al. TSG-6 activity as a novel biomarker of progression in knee osteoarthritis. Osteoarthritis and cartilage. 2014;22(2):235-41.
16. Burleigh A, Chanalaris A, Gardiner MD, Driscoll C, Boruc O, Saklatvala J, et al. Joint immobilization prevents murine osteoarthritis and reveals the highly mechanosensitive nature of protease expression in vivo. Arthritis & Rheumatism. 2012;64(7):2278-88.

17. Chong KW, Chanalaris A, Burleigh A, Jin H, Watt FE, Saklatvala J, et al. Fibroblast growth factor 2 drives changes in gene expression following injury to murine cartilage in vitro and in vivo. Arthritis & Rheumatism. 2013;65(9):2346-55.
18. Vincent TL, Hermansson MA, Hansen UN, Amis AA, Saklatvala J. Basic fibroblast growth factor mediates transduction of mechanical signals when articular cartilage is loaded. Arthritis & Rheumatism: Official Journal of the American College of Rheumatology. 2004;50(2):526-33.
19. Chia SL, Sawaji Y, Burleigh A, McLean C, Inglis J, Saklatvala J, et al. Fibroblast growth factor 2 is an intrinsic chondroprotective agent that suppresses ADAMTS‐5 and delays cartilage degradation in murine osteoarthritis. Arthritis & Rheumatism: Official Journal of the American College of Rheumatology. 2009;60(7):2019-27.
20. Sawaji Y, Hynes J, Vincent T, Saklatvala J. Fibroblast growth factor 2 inhibits induction of aggrecanase activity in human articular cartilage. Arthritis & Rheumatism: Official Journal of the American College of Rheumatology. 2008;58(11):3498-509.
21. Little C, Barai A, Burkhardt D, Smith S, Fosang A, Werb Z, et al. Matrix metalloproteinase 13–deficient mice are resistant to osteoarthritic cartilage erosion but not chondrocyte hypertrophy or osteophyte development. Arthritis & Rheumatism: Official Journal of the American College of Rheumatology. 2009;60(12):3723-33.
22. Eltawil N, De Bari C, Achan P, Pitzalis C, Dell'Accio F. A novel in vivo murine model of cartilage regeneration. Age and strain-dependent outcome after joint surface injury. Osteoarthritis and cartilage. 2009;17(6):695-704.
23. Chong KW, Chanalaris A, Burleigh A, Jin H, Watt FE, Saklatvala J, et al. Fibroblast growth factor 2 drives changes in gene expression following injury to murine cartilage in vitro and in vivo. Arthritis Rheum. 2013;65(9):2346-55.
24. Borges PDN, Vincent TL, Marenzana M. Automated assessment of bone changes in cross-sectional micro-CT studies of murine experimental osteoarthritis. PloS one. 2017;12(3):e0174294.
25. Ma H-L, Blanchet T, Peluso D, Hopkins B, Morris E, Glasson S. Osteoarthritis severity is sex dependent in a surgical mouse model. Osteoarthritis and cartilage. 2007;15(6):695-700.
26. Dyer DP, Salanga CL, Johns SC, Valdambrini E, Fuster MM, Milner CM, et al. The anti-inflammatory protein TSG-6 regulates chemokine function by inhibiting chemokine/glycosaminoglycan interactions. Journal of Biological Chemistry. 2016;291(24):12627-40.
27. Dyer DP, Thomson JM, Hermant A, Jowitt TA, Handel TM, Proudfoot AE, et al. TSG-6 inhibits neutrophil migration via direct interaction with the chemokine CXCL8. The Journal of Immunology. 2014;192(5):2177-85.
28. Getting SJ, Mahoney DJ, Cao T, Rugg MS, Fries E, Milner CM, et al. The link module from human TSG-6 inhibits neutrophil migration in a hyaluronan-and inter-α-inhibitor-independent manner. Journal of Biological Chemistry. 2002;277(52):51068-76.
29. Milner C, Tongsoongnoen W, Rugg M, Day A. The molecular basis of inter-α-inhibitor heavy chain transfer on to hyaluronan. Biochemical Society Transactions. 2007;35(4):672-6.
30. Mahoney DJ, Mulloy B, Forster MJ, Blundell CD, Fries E, Milner CM, et al. Characterization of the Interaction between Tumor Necrosis Factor-stimulated Gene-6 and Heparin IMPLICATIONS FOR THE INHIBITION OF PLASMIN IN EXTRACELLULAR MATRIX MICROENVIRONMENTS. Journal of Biological Chemistry. 2005;280(29):27044-55.
31. Marson A, Robinson DE, Brookes PN, Mulloy B, Wiles M, Clark SJ, et al. Development of a microtiter plate-based glycosaminoglycan array for the investigation of glycosaminoglycan–protein interactions. Glycobiology. 2009;19(12):1537-46.
32. Fukai A, Kamekura S, Chikazu D, Nakagawa T, Hirata M, Saito T, et al. Lack of a chondroprotective effect of cyclooxygenase 2 inhibition in a surgically induced model of osteoarthritis in mice. Arthritis & Rheumatism. 2012;64(1):198-203.
33. Zarebska JM, Chanalaris A, Driscoll C, Burleigh A, Miller R, Malfait A, et al. CCL2 and CCR2 regulate pain-related behaviour and early gene expression in post-traumatic murine osteoarthritis but contribute little to chondropathy. Osteoarthritis and cartilage. 2017;25(3):406-12.

34. Nagyeri G, Radacs M, Ghassemi-Nejad S, Tryniszewska B, Olasz K, Hutas G, et al. TSG-6 protein, a negative regulator of inflammatory arthritis, forms a ternary complex with murine mast cell tryptases and heparin. Journal of Biological Chemistry. 2011;286(26):23559-69.
35. Wisniewski H-G, Hua J, Poppers DM, Naime D, Vilcek J, Cronstein BN. TNF/IL-1-inducible protein TSG-6 potentiates plasmin inhibition by inter-alpha-inhibitor and exerts a strong anti-inflammatory effect in vivo. The Journal of Immunology. 1996;156(4):1609-15.
36. Rugg MS, Willis AC, Mukhopadhyay D, Hascall VC, Fries E, Fülöp C, et al. Characterization of complexes formed between TSG-6 and inter-α-inhibitor that act as intermediates in the covalent transfer of heavy chains onto hyaluronan. Journal of Biological Chemistry. 2005;280(27):25674-86.
37. Vincent T, Hermansson M, Bolton M, Wait R, Saklatvala J. Basic FGF mediates an immediate response of articular cartilage to mechanical injury. Proceedings of the National Academy of Sciences. 2002;99(12):8259-64.
38. Vincent T, McLean C, Full L, Peston D, Saklatvala J. FGF-2 is bound to perlecan in the pericellular matrix of articular cartilage, where it acts as a chondrocyte mechanotransducer. Osteoarthritis and Cartilage. 2007;15(7):752-63.
39. Briggs DC, Birchenough HL, Ali T, Rugg MS, Waltho JP, Ievoli E, et al. Metal ion-dependent heavy chain transfer activity of TSG-6 mediates assembly of the cumulus-oocyte matrix. Journal of Biological Chemistry. 2015;290(48):28708-23.
40. Fülöp C, Szántó S, Mukhopadhyay D, Bárdos T, Kamath RV, Rugg MS, et al. Impaired cumulus mucification and female sterility in tumor necrosis factor-induced protein-6 deficient mice. Development. 2003;130(10):2253-61.
41. Mahoney DJ, Mikecz K, Ali T, Mabilleau G, Benayahu D, Plaas A, et al. TSG-6 regulates bone remodeling through inhibition of osteoblastogenesis and osteoclast activation. Journal of Biological Chemistry. 2008;283(38):25952-62.
42. Mosna F, Sensebe L, Krampera M. Human bone marrow and adipose tissue mesenchymal stem cells: a user's guide. Stem cells and development. 2010;19(10):1449-70.
43. Romano B, Elangovan S, Erreni M, Sala E, Petti L, Kunderfranco P, et al. TNF-Stimulated Gene-6 Is a Key Regulator in Switching Stemness and Biological Properties of Mesenchymal Stem Cells. STEM CELLS. 2019;37(7):973-87.
44. Bianchi G, Banfi A, Mastrogiacomo M, Notaro R, Luzzatto L, Cancedda R, et al. Ex vivo enrichment of mesenchymal cell progenitors by fibroblast growth factor 2. Experimental Cell Research. 2003;287(1):98-105.
45. Lai W-T, Krishnappa V, Phinney DG. Fibroblast Growth Factor 2 (Fgf2) Inhibits Differentiation of Mesenchymal Stem Cells by Inducing Twist2 and Spry4, Blocking Extracellular Regulated Kinase Activation, and Altering Fgf Receptor Expression Levels. STEM CELLS. 2011;29(7):1102-11.
46. Chou C-H, Attarian DE, Wisniewski H-G, Band PA, Kraus VB. TSG-6–a double-edged sword for osteoarthritis (OA). Osteoarthritis and cartilage. 2018;26(2):245-54.
47. Mahoney DJ, Swales C, Athanasou NA, Bombardieri M, Pitzalis C, Kliskey K, et al. TSG‐6 inhibits osteoclast activity via an autocrine mechanism and is functionally synergistic with osteoprotegerin. Arthritis & Rheumatism. 2011;63(4):1034-43.

Figure Legends

Figure 1. Summary of experiment design.

Figure 2. TSG-6 is regulated in an FGF2-dependent manner. (a) Regulation of TSG-6 gene expression by re-cutting injury or FGF2 stimulation of murine cartilage explants. Murine hip cartilage (from male animals) was avulsed (avulsion injury) directly into serum-free DMEM and incubated for 48 h. Rested cartilage was either re-cut (re-cut injury) or treated with FGF2 (250ng/ml) in fresh DMEM and left for 4 h prior to RNA extraction. Each point represents the mRNA from 4 hips pooled. *Tsg6* levels were expressed relative to *Gapdh* and normalized to the control. (b) RNA was extracted from male wild type or FGF2$^{-/-}$ mouse joints (n=3) at specified times post DMM. *Tsg6* mRNA levels were expressed relative to *18s* and normalized to the WT 0 h control. Statistical significance was determined by ANOVA with Sidak *post hoc* testing, comparing (a) treated compared with rested controls and (b) WT with FGF2$^{-/-}$ for each time point.

Figure 3. Increased cartilage degradation in male TSG-6$^{-/-}$ mice 12 weeks post DMM. Histological chondropathy scores and representative histologic sections of knee cartilage 8 or 12 weeks after DMM in (a) male and (b) female WT (C57BL/6) and TSG-6$^{-/-}$ mice. Black arrows in the images indicate cartilage defects. Scale bar = 200 μm. Statistical significance was determined by Mann Whitney U tests. Mean osteophyte scores: (c) size and (d) maturity in WT (C57BL/6) and TSG-6$^{-/-}$ male mice 8 weeks post DMM. Also shown are contralateral joint controls. CL-contralateral.

Figure 4. Joint pathology in TSG^{tg} mice at 8 and 12 weeks post DMM. Histological chondropathy scores (left) and representative histologic sections (right) of knee cartilage 8 and

12 weeks after Sham or DMM in (a) male and (b) female TSG-6^{tg} and WT (Balb/c) mice. Black arrows in the images indicate cartilage defects. Scale bar = 200 μm. Statistical significance was determined by Mann Whitney U tests. There were no significant differences seen between any of the female experimental groups by Two-way-ANOVA. (c) Fold increase of *Tsg6* over WT transgene (by RT-qPCR) was plotted against summed cartilage score for male mice 12 weeks post DMM. R squared correlation statistical analysis was performed. Mean osteophyte scores: (d) size and (e) maturity, were carried out in WT (Balb/c) and TSG-6^{tg} male mice 8 weeks after DMM. (f) Histological scores (left) and (g) representative histologic sections (right) of knee cartilage 8 weeks after DMM in male WT(C57BL6), FGF2$^{-/-}$;TSG-6^{tg} and FGF2$^{-/-}$;TSG-6^{WT} mice. Balb/c histology scores are derived from Figure 4a. Statistical significance was determined by One-way ANOVA with Sidak *post hoc* testing. (h) Level of *Tsg6* expression was plotted against summed cartilage score for male FGF2$^{-/-}$;TSG-6^{tg} mice 8 weeks post DMM. R squared correlation statistical analysis was performed.

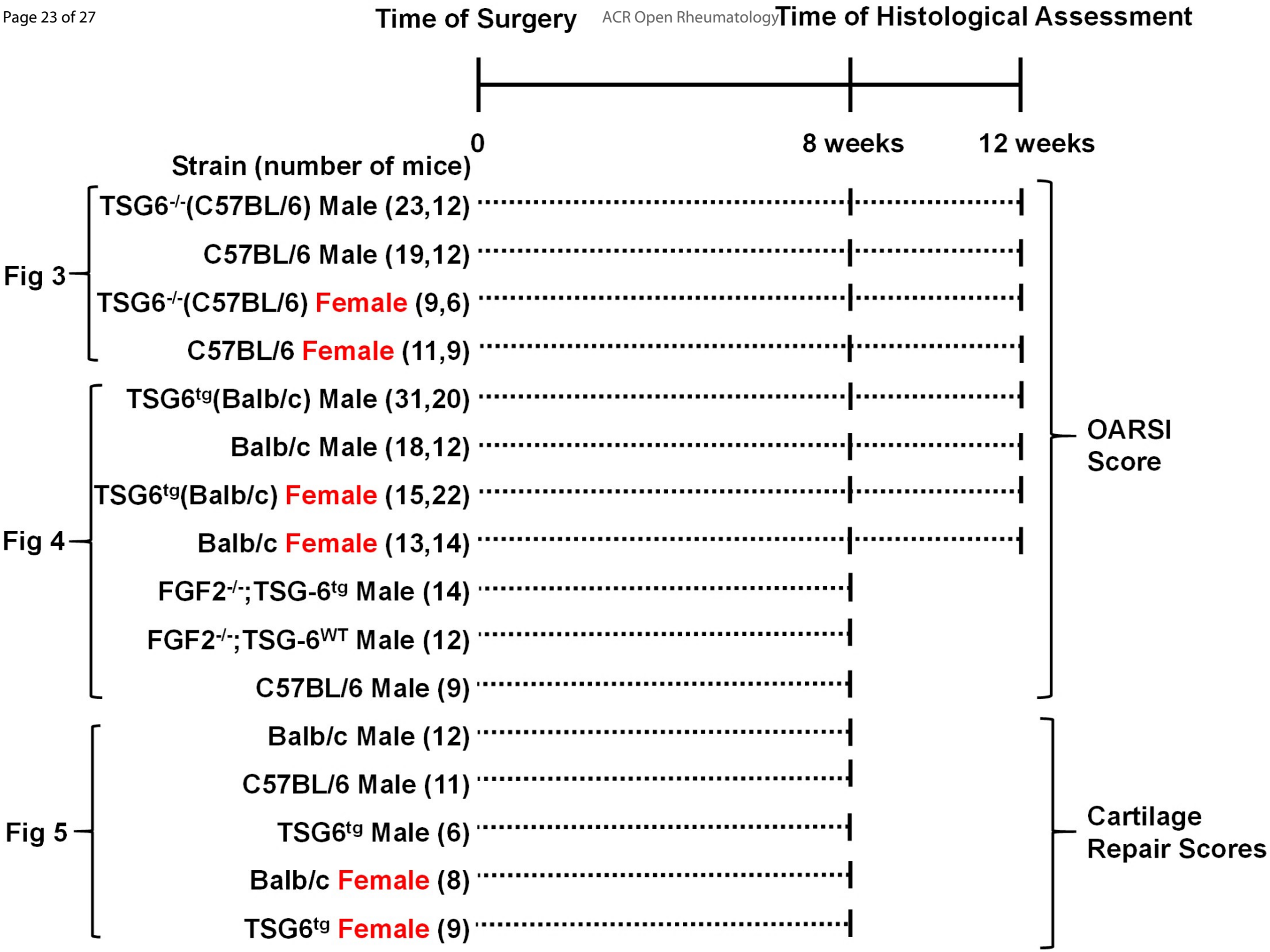
Figure 5. TSG-6^{tg} mice do not have enhanced cartilage repair capacity. (a) FGF2 levels were measured by ELISA (MSD PLEX bFGF assay) in conditioned medium collected from injured mouse hips (a mixture of male and female) from WT(Balb/c), TSG-6^{tg}, TSG-6$^{+/-}$ (heterozygotes), and TSG-6$^{-/-}$ (KO, homozygotes) mice. (b) Transverse section of the joint showing the position of patella groove. (c) Cartilage repair scores (left) and representative histological images of Safranin-O stained sections (right) 8 weeks after focal cartilage injury in male WT (Balb/c mice) TSG-6^{tg}, and C57BL/6 mice, n = 6-12 mice per group. (d) Cartilage repair scores after focal cartilage injury in female WT (Balb/c) and TSG-6^{tg} mice. Scale bar = 200 μm. Mann Whitney U tests were used to determine statistical significance.

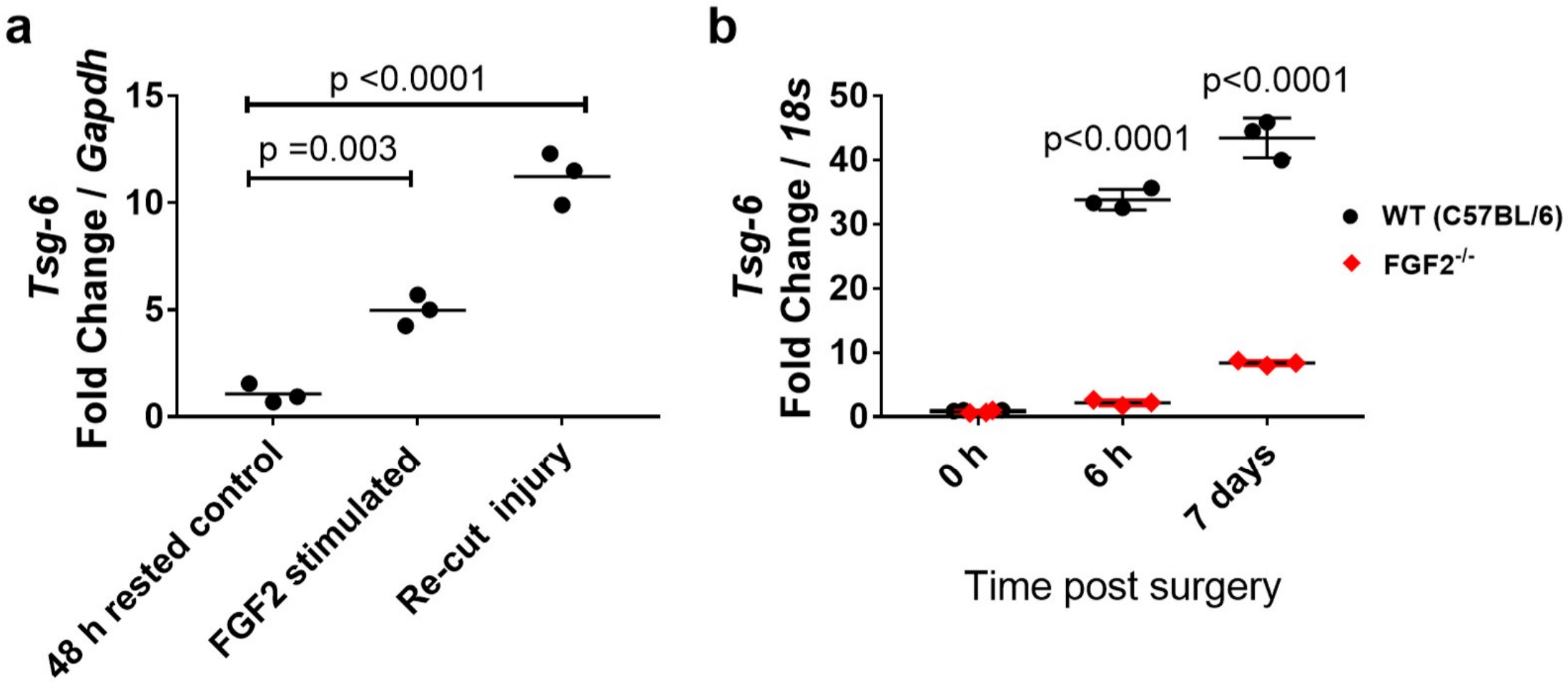
Table 1. Gene expression profiles of whole joint in TSG-6$^{-/-}$ and WT following DMM.

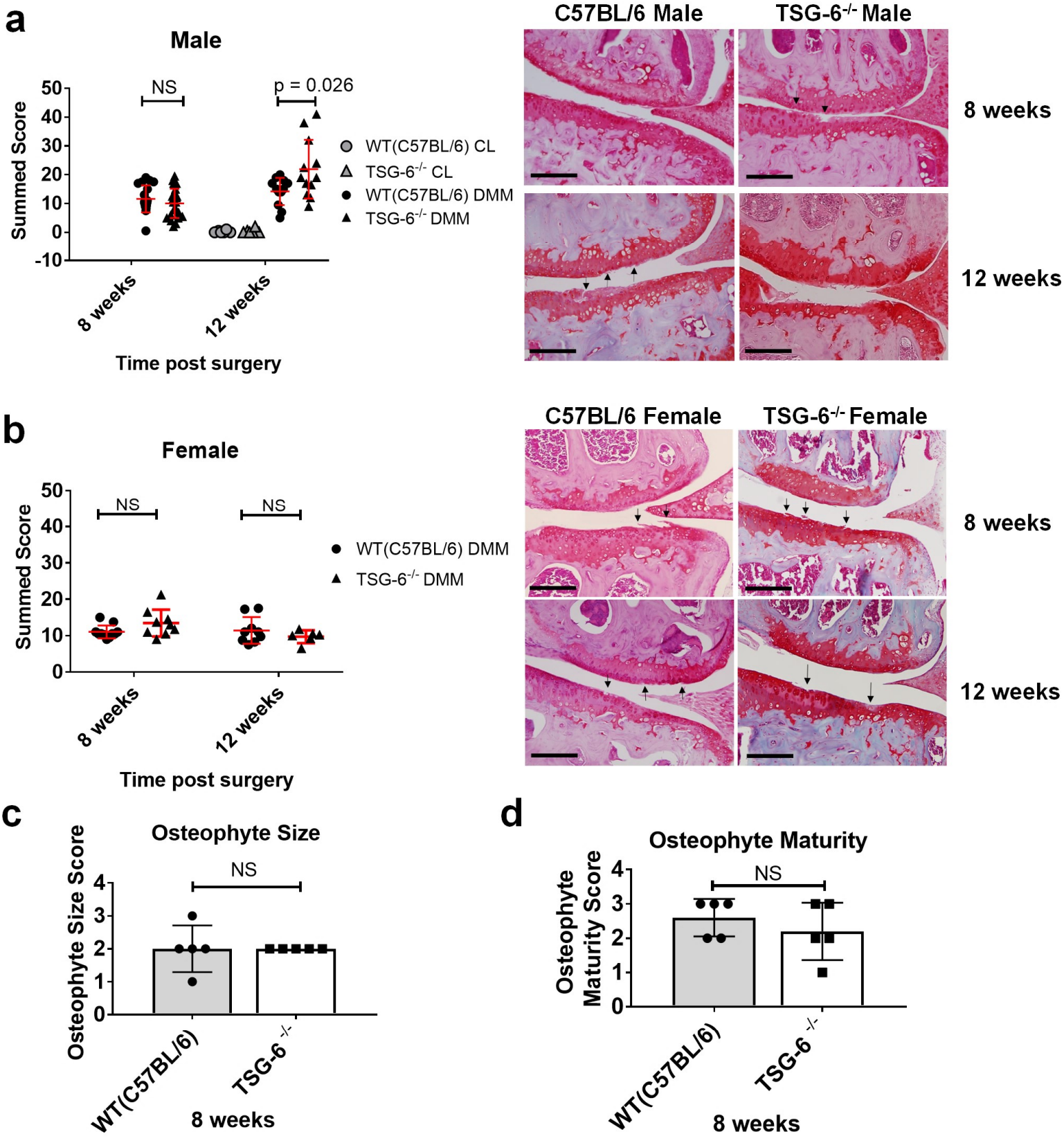
Subscript:The effect of TSG-6 deletion on relative mRNA levels for injury response genes in whole murine joints of WT (C57BL/6) and TSG-$6^{-/-}$ mice (n = 3 mice per group) 0 h, 6 h or 7 days post DMM. Values are the mean fold change ± SEM. Results are expressed relative to *18s*. Statistical significance was determined using unpaired multiple t-tests and the p-values corrected for multiplicity (q-values) using a discovery ratio of 5%. Genes highlighted were shown to be significant (light grey = increase, dark grey = decrease). *Adam* – a disintegrin and metalloproteinase; *Adamts* – a disintegrin and metalloproteinase with thrombospondin motifs; *Ctgf* – connective tissue growth factor; *F3* – coagulation factor III; *Has1* - hyaluronan synthase 1; Inhba - inhibin beta A; *Nos* – nitric oxide synthase; *Pdpn*- podoplanin; *Ptges* - prostaglandin E synthase; *Ptgs2* -Prostaglandin-endoperoxide synthase 2; *Sfrp2* - secreted frizzled-related protein 2; *Timp1* – tissue inhibitor of metalloproteinase 1; *Tnfrsf12a* -tumor necrosis factor receptor superfamily member 12A (TWEAK receptor); *Wisp2* - WNT1-inducible-signaling pathway protein 2.

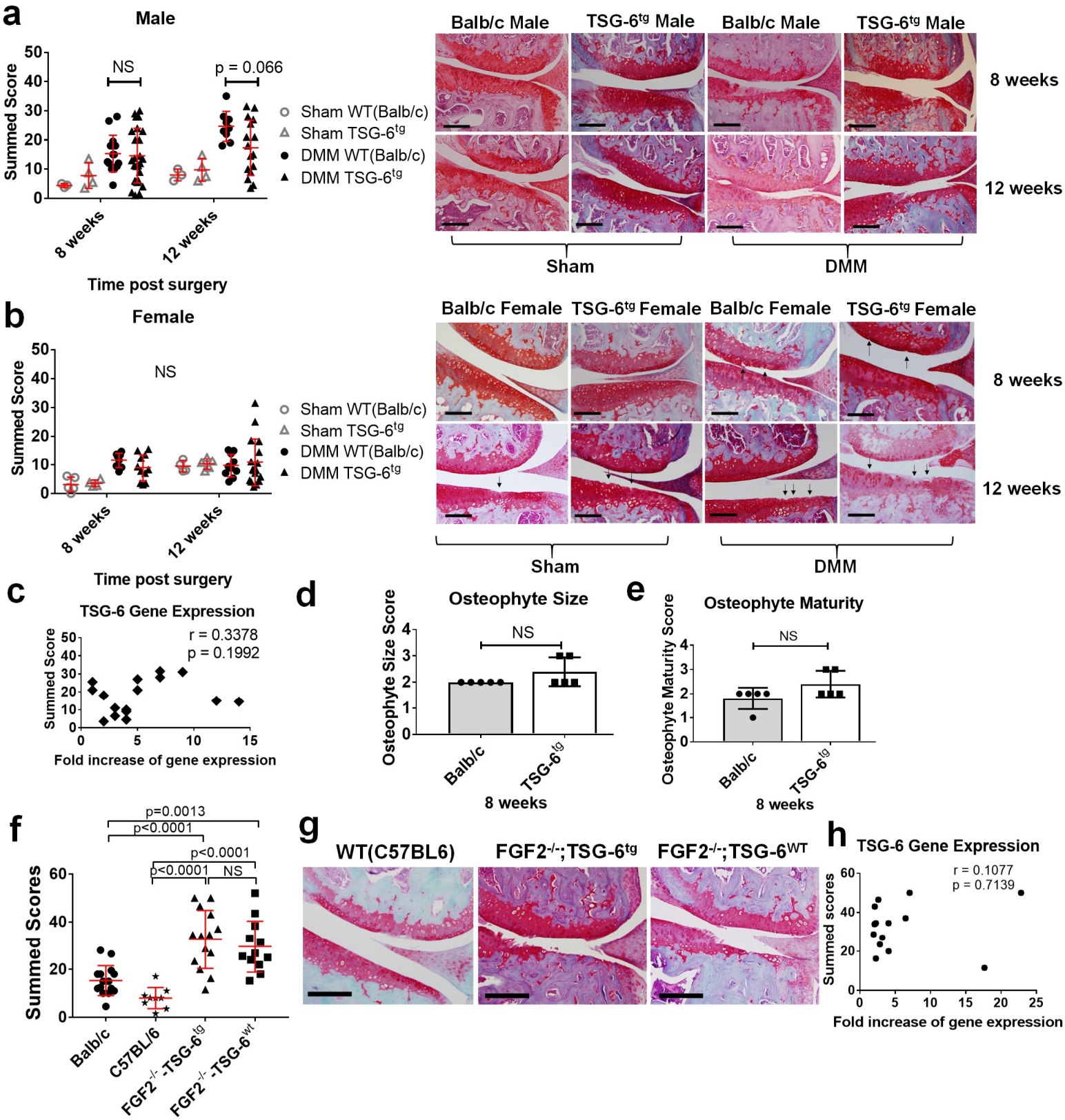
	0h (Mean fold of change ± SEM)			6h/0h (Mean fold of change ± SEM)			7d/0h (Mean fold of change ± SEM)		
Gene ID	**WT**	**TSG-6$^{-/-}$**	***q* value**	**WT**	**TSG-6$^{-/-}$**	***q* value**	**WT**	**TSG-6$^{-/-}$**	***q* value**
Aggrecan	1.00 ± 0.06	1.03 ± 0.03	0.6424	0.89 ± 0.08	1.02 ± 0.12	0.4529	0.71 ± 0.02	0.81 ± 0.11	0.3972
Adam8	1.07 ± 0.17	1.11 ± 0.09	0.8616	2.41 ± 0.06	3.70 ± 0.33	0.018	1.31 ± 0.02	3.01 ± 0.24	0.0022
Adam9	1.12 ± 0.08	1.10 ± 0.09	0.8712	2.22 ± 0.07	2.84 ± 0.24	0.065	2.20 ± 0.11	1.47 ± 0.17	0.0232
Adamts1	0.98 ± 0.12	1.04 ± 0.05	0.6512	7.25 ± 1.54	2.40 ± 0.20	0.0351	3.10 ± 0.58	0.27 ± 0.04	0.008
Adamts15	0.98 ± 0.02	1.17 ± 0.09	0.1026	12.70 ± 0.65	13.54 ± 0.94	0.5087	7.34 ± 0.67	13.66 ± 0.71	0.0029
Adamts4	1.21 ± 0.12	1.14 ± 0.07	0.6099	3.90 ± 0.26	4.42 ± 0.34	0.2941	1.87 ± 0.22	2.19 ± 0.09	0.2514
Adamts5	1.17 ± 0.09	1.09 ± 0.11	0.6417	1.50 ± 0.13	3.10 ± 0.10	0.0006	1.57 ± 0.21	2.35 ± 0.15	0.042
Arginase 1	0.96 ± 0.10	0.82 ± 0.11	0.3997	84.23 ± 4.06	309.06 ± 32.50	0.0024	3.14 ± 1.05	317.81 ± 13.19	< 0.0001
Arginase 2	1.15 ± 0.12	1.20 ± 0.08	0.7661	1.05 ± 0.01	13.72 ± 0.89	0.0001	1.15 ± 0.09	13.29 ± 0.71	< 0.0001
Ccl2	1.09 ± 0.08	1.03 ± 0.02	0.494	131.41 ± 10.93	239.79 ± 8.64	0.0015	20.90 ± 0.64	29.31 ± 2.56	0.0332
Ccl5	1.03 ± 0.02	1.19 ± 0.12	0.2392	1.52 ± 0.07	1.97 ± 0.06	0.0081	0.73 ± 0.30	2.21 ± 0.81	0.163
Ccl7	0.96 ± 0.03	1.04 ± 0.03	0.1759	5.57 ± 1.93	10.04 ± 2.48	0.228	2.33 ± 0.29	18.31 ± 2.68	0.0041
Ccr2	0.75 ± 0.14	0.98 ± 0.46	0.654	1.11 ± 0.04	1.21 ± 0.10	0.4285	1.41 ± 0.26	2.38 ± 0.64	0.2298
Ccr5	1.21 ± 0.11	1.04 ± 0.16	0.428	3.50 ± 0.15	11.44 ± 0.79	0.0006	1.32 ± 0.15	19.71 ± 3.24	0.0048
Cd14	0.94 ± 0.16	1.09 ± 0.19	0.5612	5.16 ± 0.72	8.34 ± 0.51	0.0228	1.89 ± 0.51	2.90 ± 0.12	0.1236
Cd68	1.12 ± 0.34	0.65 ± 0.10	0.252	2.14 ± 0.05	4.98 ± 0.90	0.0345	1.35 ± 0.17	3.18 ± 0.33	0.0083
Col2a1	1.11 ± 0.09	1.10 ± 0.03	0.9419	0.31 ± 0.04	0.43 ± 0.17	0.5567	0.19 ± 0.04	0.34 ± 0.12	0.3177
Ctgf	0.74 ± 0.21	0.27 ± 0.13	0.1327	0.20 ± 0.05	0.35 ± 0.12	0.3289	0.17 ± 0.04	0.44 ± 0.18	0.2205
F3	1.10 ± 0.09	1.00 ± 0.14	0.6016	1.13 ± 0.09	12.94 ± 0.25	< 0.0001	2.14 ± 0.14	2.82 ± 0.13	0.0224
Has1	1.21 ± 0.11	1.15 ± 0.18	0.7819	3.73 ± 0.26	8.70 ± 0.55	0.0013	0.57 ± 0.29	3.07 ± 0.87	0.052
Has2	1.08 ± 0.08	1.05 ± 0.04	0.7588	2.04 ± 0.04	3.24 ± 0.69	0.1542	2.35 ± 0.34	2.64 ± 0.10	0.4547
Il1a	1.07 ± 0.04	1.04 ± 0.03	0.5471	1.21 ± 0.06	2.71 ± 0.09	0.0002	1.02 ± 0.01	2.51 ± 0.22	0.0026
Il1b	1.00 ± 0.00	1.16 ± 0.08	0.1215	5.17 ± 0.16	5.31 ± 0.65	0.8441	1.92 ± 0.21	2.77 ± 0.56	0.2268
Il1r1	1.18 ± 0.12	0.86 ± 0.19	0.2308	3.65 ± 0.39	7.07 ± 0.42	0.0039	1.31 ± 0.04	2.24 ± 0.35	0.0559
Il1rl1	1.03 ± 0.02	1.21 ± 0.10	0.1517	2.02 ± 0.16	2.11 ± 0.15	0.6776	1.47 ± 0.22	1.45 ± 0.18	0.9486
Il33	1.09 ± 0.05	1.30 ± 0.01	0.0127	3.20 ± 0.26	5.48 ± 0.32	0.0052	1.70 ± 0.35	4.53 ± 0.09	0.0014
Il6	1.25 ± 0.13	1.16 ± 0.25	0.7802	16.84 ± 1.67	50.02 ± 11.37	0.0447	0.99 ± 0.33	123.72 ± 14.94	0.0012
Inhba	1.06 ± 0.17	0.82 ± 0.09	0.2862	2.73 ± 0.05	0.93 ± 0.27	0.0027	0.70 ± 0.26	0.82 ± 0.09	0.6733
Mmp13	0.96 ± 0.02	1.50 ± 0.33	0.1815	0.42 ± 0.04	4.48 ± 1.67	0.0721	0.61 ± 0.24	6.92 ± 2.29	0.0519
Mmp19	1.17 ± 0.11	0.93 ± 0.23	0.3946	3.36 ± 0.25	3.56 ± 0.11	0.5015	1.82 ± 0.20	5.86 ± 1.68	0.0756

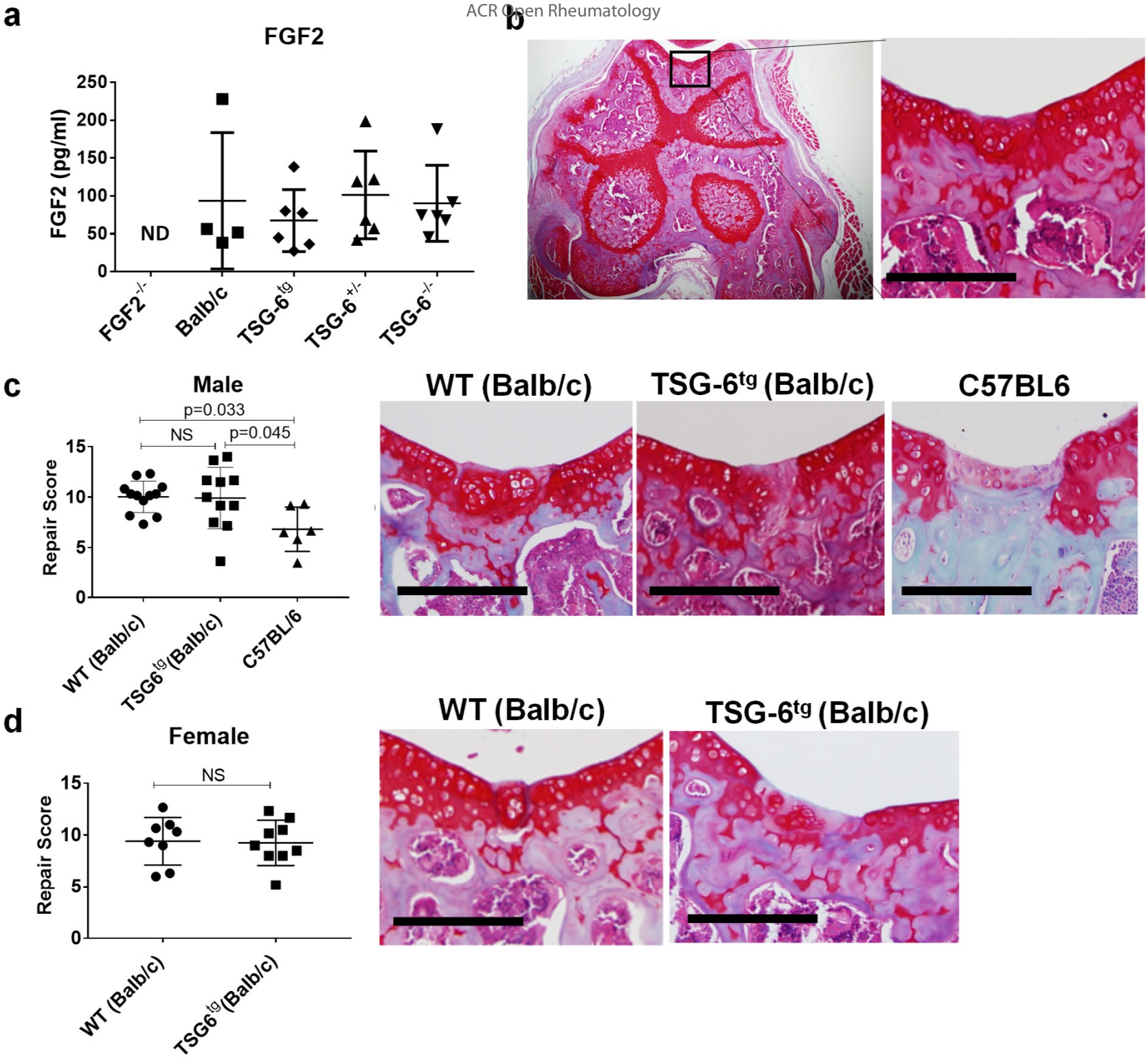
Mmp3	1.36 ± 0.18	1.26 ± 0.26	0.7757	6.01 ± 0.00	3.21 ± 0.30	0.0008	2.01 ± 0.00	9.84 ± 0.34	< 0.0001
Mmp8	1.19 ± 0.05	1.03 ± 0.01	0.0286	2.04 ± 0.02	3.69 ± 0.82	0.1137	1.01 ± 0.00	3.43 ± 1.25	0.125
Nos2	1.00 ± 0.00	1.16 ± 0.14	0.3365	14.73 ± 1.86	25.34 ± 3.53	0.0566	8.18 ± 0.74	21.75 ± 1.83	0.0023
Pdpn	0.97 ± 0.15	0.98 ± 0.19	0.9671	6.87 ± 0.22	2.09 ± 0.82	0.0048	2.30 ± 0.21	1.38 ± 0.16	0.0256
Ptges	0.85 ± 0.15	1.20 ± 0.08	0.1083	1.37 ± 0.09	1.99 ± 0.01	0.0021	1.25 ± 0.25	2.58 ± 0.40	0.0495
Ptgs2	1.09 ± 0.06	1.05 ± 0.01	0.5115	14.40 ± 1.41	21.76 ± 2.12	0.0443	9.04 ± 1.05	23.83 ± 2.28	0.0042
Sfrp2	0.92 ± 0.19	1.05 ± 0.02	0.5473	0.58 ± 0.22	2.93 ± 0.56	0.0171	3.11 ± 0.45	3.44 ± 0.37	0.5949
Timp1	0.93 ± 0.09	0.44 ± 0.01	0.0068	4.90 ± 0.26	1.57 ± 0.04	0.0002	1.24 ± 0.16	1.78 ± 0.28	0.1737
Tnfrsf12a	1.05 ± 0.07	0.83 ± 0.14	0.243	5.83 ± 0.30	1.08 ± 0.15	0.0001	2.58 ± 0.23	1.12 ± 0.12	0.0047
TSG-6	1.14 ± 0.14			31.19 ± 0.13			45.27 ± 1.17		
Wisp2	0.96 ± 0.05	1.09 ± 0.07	0.1775	7.03 ± 0.24	6.66 ± 0.47	0.5142	4.24 ± 0.23	3.08 ± 0.29	0.034
Wnt16	0.92 ± 0.11	0.88 ± 0.10	0.7704	0.95 ± 0.04	0.44 ± 0.03	0.0007	0.79 ± 0.15	0.73 ± 0.17	0.8061


Time of Surgery
Time of Histological Assessment
0
8 weeks
12 weeks
Strain (number of mice)
TSG6-/-(C57BL/6) Male (23,12)
C57BL/6 Male (19,12)
TSG6-/-(C57BL/6) Female (9,6)
C57BL/6 Female (11,9)
TSG6tg(Balb/c) Male (31,20)
Balb/c Male (18,12)
TSG6tg(Balb/c) Female (15,22)
Balb/c Female (13,14)
FGF2-/-;TSG-6tg Male (14)
FGF2-/-;TSG-6WT Male (12)
C57BL/6 Male (9)
Balb/c Male (12)
C57BL/6 Male (11)
TSG6tg Male (6)
Balb/c Female (8)
TSG6tg Female (9)
Fig 3
Fig 4
Fig 5
OARSI Score
Cartilage Repair Scores


a
Tsg-6
Fold Change / Gapdh
15
10
5
0
p <0.0001
p =0.003
48 h rested control
FGF2 stimulated
Re-cut injury
b
Tsg-6
Fold Change / 18s
50
40
30
20
10
0
p<0.0001
p<0.0001
WT (C57BL/6)
FGF2-/-
0 h
6 h
7 days
Time post surgery


a
Male
NS
p = 0.026
Summed Score
50
40
30
20
10
0
-10
WT(C57BL/6) CL
TSG-6-/- CL
WT(C57BL/6) DMM
TSG-6-/- DMM
8 weeks
12 weeks
Time post surgery
C57BL/6 Male
TSG-6-/- Male
8 weeks
12 weeks
b
Female
NS
NS
Summed Score
WT(C57BL/6) DMM
TSG-6-/- DMM
8 weeks
12 weeks
Time post surgery
C57BL/6 Female
TSG-6-/- Female
8 weeks
12 weeks
c
Osteophyte Size
NS
Osteophyte Size Score
WT(C57BL/6)
TSG-6 -/-
8 weeks
d
Osteophyte Maturity
NS
Osteophyte Maturity Score
WT(C57BL/6)
TSG-6 -/-
8 weeks


a
Male
Summed Score
NS
p = 0.066
Sham WT(Balb/c)
Sham TSG-6tg
DMM WT(Balb/c)
DMM TSG-6tg
8 weeks
12 weeks
Time post surgery
Balb/c Male
TSG-6tg Male
Balb/c Male
TSG-6tg Male
8 weeks
12 weeks
Sham
DMM
b
Female
Summed Score
NS
Sham WT(Balb/c)
Sham TSG-6tg
DMM WT(Balb/c)
DMM TSG-6tg
8 weeks
12 weeks
Time post surgery
Balb/c Female
TSG-6tg Female
Balb/c Female
TSG-6tg Female
8 weeks
12 weeks
Sham
DMM
c
TSG-6 Gene Expression
Summed Score
r = 0.3378
p = 0.1992
Fold increase of gene expression
d
Osteophyte Size
Osteophyte Size Score
NS
Balb/c
TSG-6tg
8 weeks
e
Osteophyte Maturity
Osteophyte Maturity Score
NS
Balb/c
TSG-6tg
8 weeks
f
Summed Scores
p=0.0013
p<0.0001
p<0.0001
p<0.0001
NS
Balb/c
C57BL/6
FGF2-/--TSG-6tg
FGF2-/--TSG-6wt
g
WT(C57BL6)
FGF2-/-;TSG-6tg
FGF2-/-;TSG-6WT
h
TSG-6 Gene Expression
Summed scores
r = 0.1077
p = 0.7139
Fold increase of gene expression


a
FGF2
FGF2 (pg/ml)
250
200
150
100
50
0
ND
FGF2-/-
Balb/c
TSG-6tg
TSG-6+/-
TSG-6-/-
b
c
Male
p=0.033
NS
p=0.045
Repair Score
15
10
5
0
WT (Balb/c)
TSG6tg(Balb/c)
C57BL/6
WT (Balb/c)
TSG-6tg (Balb/c)
C57BL6
d
Female
NS
Repair Score
15
10
5
0
WT (Balb/c)
TSG6tg(Balb/c)
WT (Balb/c)
TSG-6tg (Balb/c)