Legends to supplementary figures

Supplementary Figure 1: Characterization of anti-TNfnIII 1-5 phage-derived scFv antibodies. Panel A: Histogram showing ELISA values recorded for antibody-containing periplasmic fractions of 96 clones (expressed in HB2151 cells) binding to coated TNfnIII 1-5. The y-axis shows the optical density at 450 nm. The selected clone TN64 is marked with an arrow. Panel B: Coomassie-stained SDS-PAGE gel (12%) showing purified TN64 (left) and its corresponding western blot (right) probed with mouse anti-His monoclonal antibody, followed by anti-mouse HRP conjugated secondary antibody. Panel C: Binding of variants of TNfnIII 1-5 with the scFv TN64: lane 1: molecular weight marker; lane 2: TNfnIII 1-5; lane 3: TNfnIII 1-3; lane 4: TNfnIII 3-5; lane 5: TNfnIII 3.

Supplementary Figure 2: Checking the cross-reactivity of TN64 with other ECM-related proteins. Panel A: Histogram showing ELISA values recorded for binding of TN64 antibody to fibronectin, fibrinogen, osteopontin and fibronectin-like type III repeats of Tenascin (1-5) (TNfnIII 1-5). The y-axis shows the optical density at 450 nm. Panel B: Osteopontin and TNfnIII1-5 were electrophoresed, transferred on nitrocellulose membrane and probed with either scFv Tn64 (lanes 1 and 3) or scFv O52 (lanes 2 and 4). Panel C: Fibrinogen and fibronectin were also checked for binding to TN64. Lanes 1 and 4 were probed with scFv TN64 whereas lanes 2 and 5 were probed with O52 (irrelevant control). Lane 3 was probed with anti-fibrinogen antibody and lane 6 with anti-fibronectin antibody (positive controls). Lane M indicates the molecular weight markers.

Supplementary Figure 3: Immunostaining of human lens epithelial cells to visualize the possible co-localization of the scFv antibodies with anti-tenascin-C antibody (Sigma Chemicals). Lens epithelial cells were probed with TN64, O52 (negative control) and anti-Tenascin-C antibody. scFvTN64 and O52 were visualized with c-myc-FITC conjugated antibody, whereas anti-Tenascin-C antibody was visualized by anti-mouse-PE conjugated antibody. Images were acquired using Olympus confocal microscopy.

Supplementary Figure 4: Evaluation of the effect of TN64 on the viability (Panel A) and proliferation (Panel B) of the HLE-B3 cells. HLE-B3 cells were seeded at the density of 5,000 cells per well of 96-well plate in the presence of complete media (MEM+20% FBS) and grown
overnight. Cells were serum starved and treated with scFv antibodies at different doses along with TGF-β2 (2ng/ml) for 24h in serum free condition. For cell viability, MTT assay was performed, showing no significant decrease in the viability till a concentration of 50ug/ml of TN64. O52 showed no effect on viability at any concentration. For proliferation, BrdU incorporation assay was done. BrdU was added after 10h of addition of scFv antibodies and cells were further incubated for 12-14h. TN64 showed significant decrease in proliferation starting from a concentration of 25ug/ml. scFv O52 had no effect on proliferation. p< 0.05 was considered statistically significant.

Supplementary Figure 5: Evaluation of expression levels collagen type-I (Panel A) and β-1 integrin (Panel B) by flow cytometry. Patient derived lens epithelial cells were cultured in complete media followed by serum starvation for 8 to 10h. Cells were treated with and without scFv antibodies along with TGF-β2 (2ng/ml). For collagen type-I, cells were fixed, permeabilized, blocked and incubated with mouse anti-collagen type-I antibody (Sigma Chemicals). For β1 integrin, cells were harvested, and incubated in blocking buffer, followed by incubation with primary mouse anti-β1 integrin antibody (Sigma Chemicals). FITC conjugated anti-mouse antibody was used as secondary antibody.