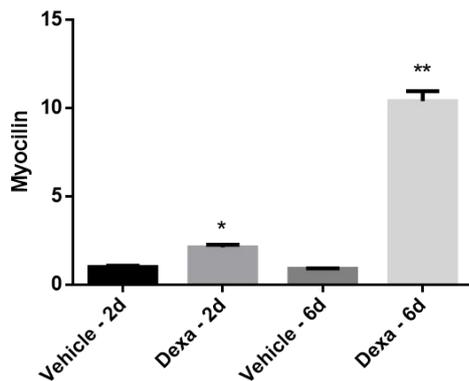
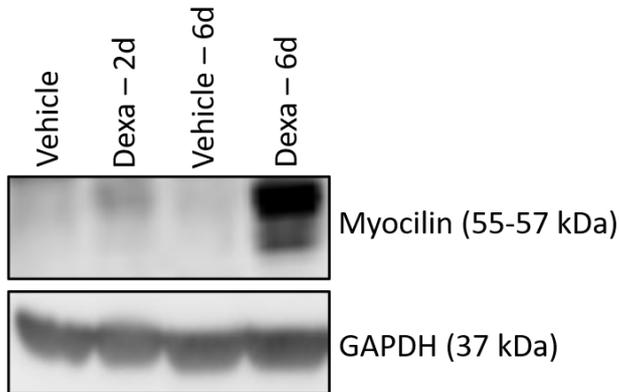
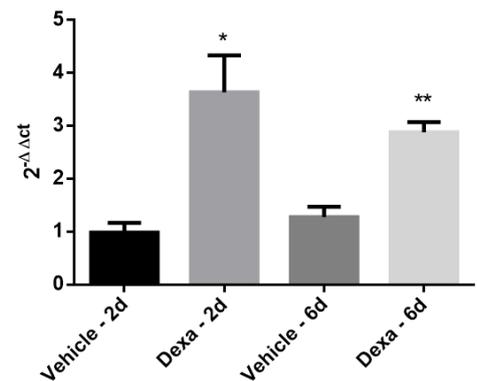


SUPPLEMENTARY FIGURES

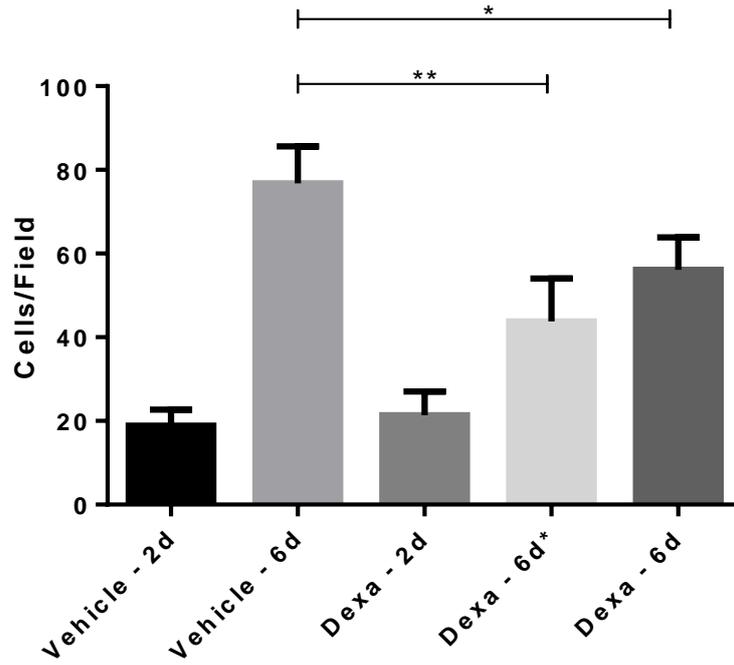
A



B

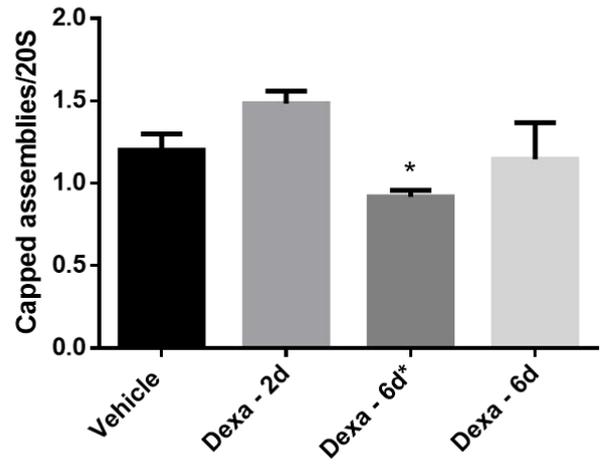
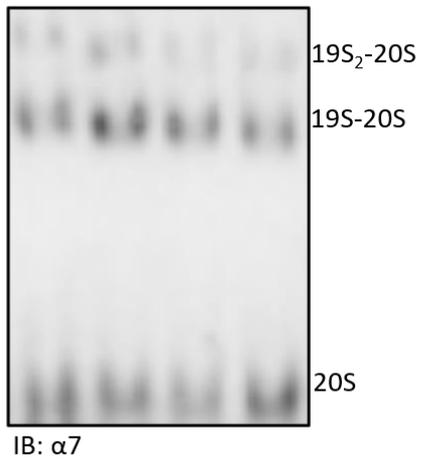
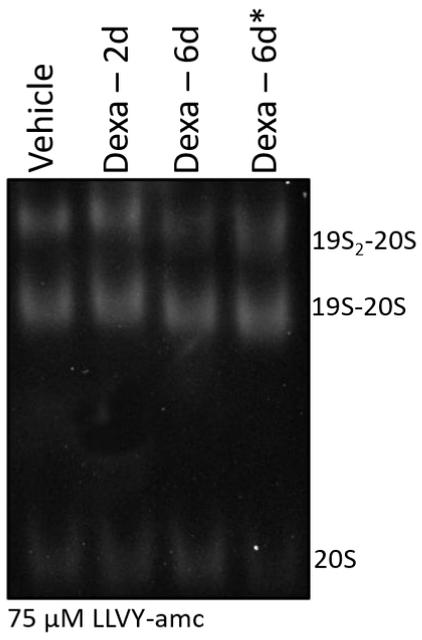


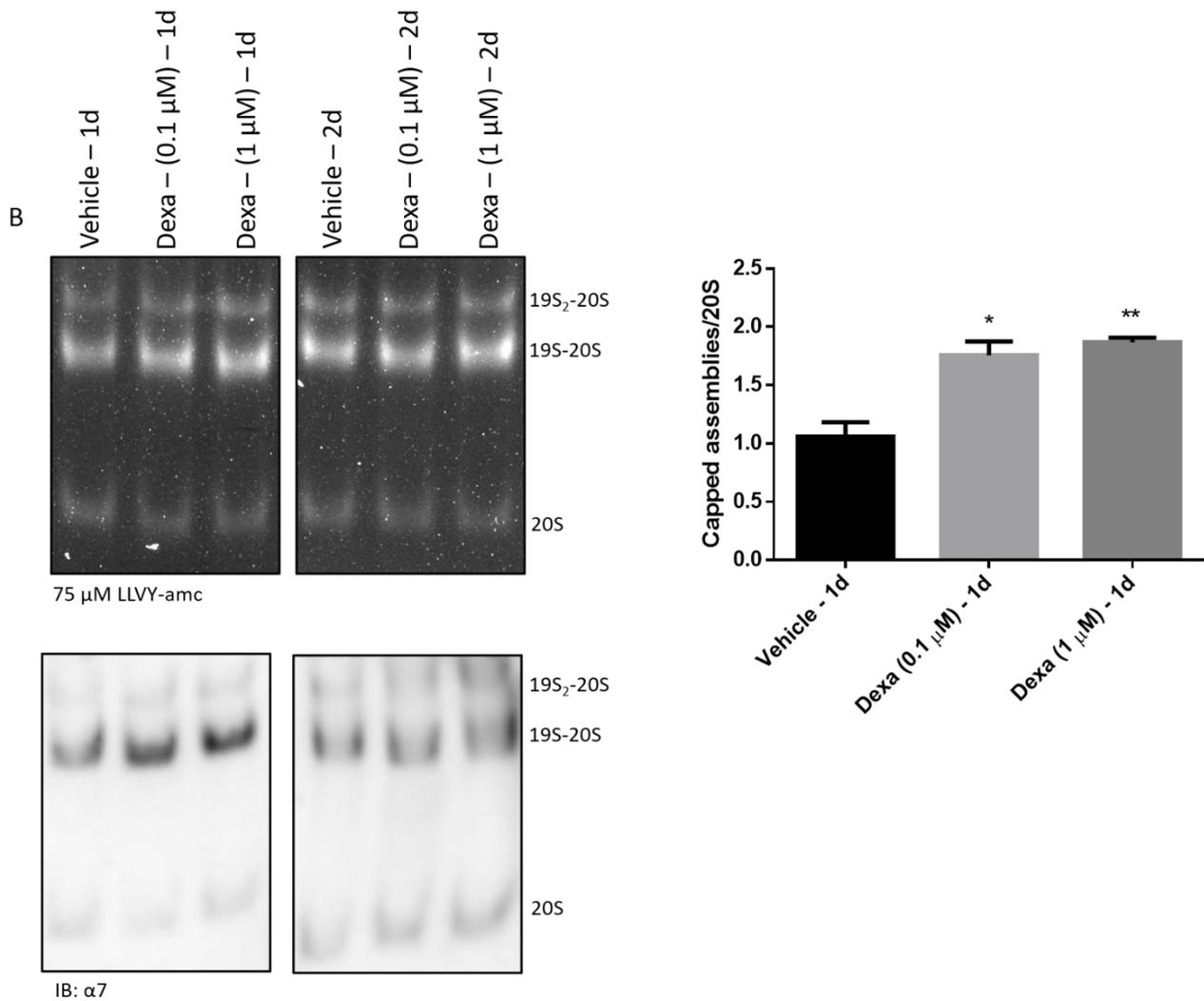
**Figure 1: Validation of TMC strain identity.** (A) Myocilin staining of Dexa-2d and Dexa-6d cells (and correspondent Vehicle cells) by denaturing and reducing Wb. GAPDH was used as internal control. A representative image of three independent experiments is here shown (n=3). One-way ANOVA followed by Tukey's post-hoc significance test. \* $p < 0.001$ ; \*\* $p < 0.00001$ ; (B) RT-PCR analysis of myocilin gene expression. GAPDH was used as internal control. Values were determined through the  $2^{-\Delta\Delta Ct}$  formula. \* $p < 0.0001$ , \*\* $p < 0.0015$ .



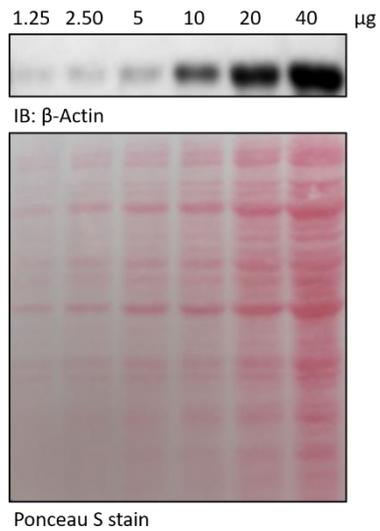
**Figure 2: TMCs cell count by Trypan blue.** Cells of each experimental condition were counted in 9 different fields upon Trypan blue staining. A representative image of two independent experiments is here shown (n=9). One-way ANOVA followed by Tukey's post-hoc significance test. \* $p < 0.0001$ , \*\* $p < 0.004$

A



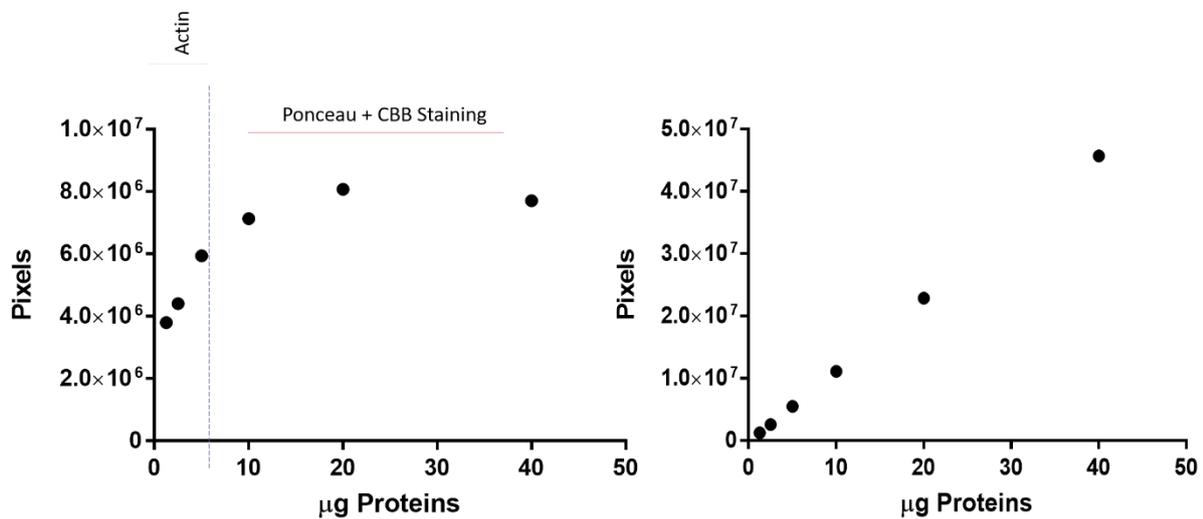


**Figure 3: Assessment of proteasome activity and assembly by native-gel electrophoresis.** (A) Proteasome particles were separated by mass/charge through native gel electrophoresis. The three main assemblies were probed with 75  $\mu$ M LLVY-amc (*upper panel*). The identity of the particles was verified by staining them with an anti- $\alpha$ 7 antibody (*lower panel*) \* $p < 0.003$ ; (B) proteasome assemblies of TMCs stimulated for 1 day and 2 days with 0.1  $\mu$ M and 1  $\mu$ M Dexa. \* $p < 0.0004$ , \*\* $p < 0.0001$ . In all cases quantification is reported as the sum of capped assemblies intensity vs that of 20S as they appear upon immunostaining. A representative experiment of three independent observations is reported ( $n=3$ ); One-way ANOVA followed by Tukey's post-hoc significance test.



Actin – Dynamic Linear Range

Ponceau S Staining – Dynamic Linear Range



**Figure 4: Determination of  $\beta$ -actin and Ponceau S staining dynamic linear range.** Serial dilution (from 40  $\mu\text{g}$  to 1.25  $\mu\text{g}$ ) of a TMCs lysate were analysed by Western blotting and probed with the anti- $\beta$ -actin antibody. Bands intensity was determined by ImageJ and data plotted to visualize the range within which the signal was below the saturation threshold. Thereafter, proteins demanding  $\mu\text{g}$  loading higher than the saturation threshold normalized to Ponceau S staining (and confirmed by CBB, not shown).