Optogenetic control of the BMP signalling pathway

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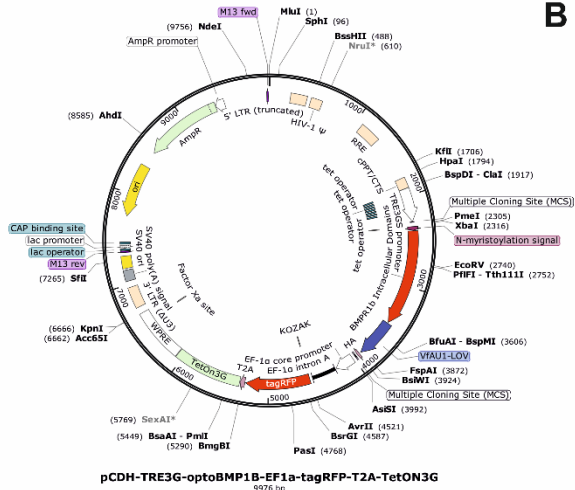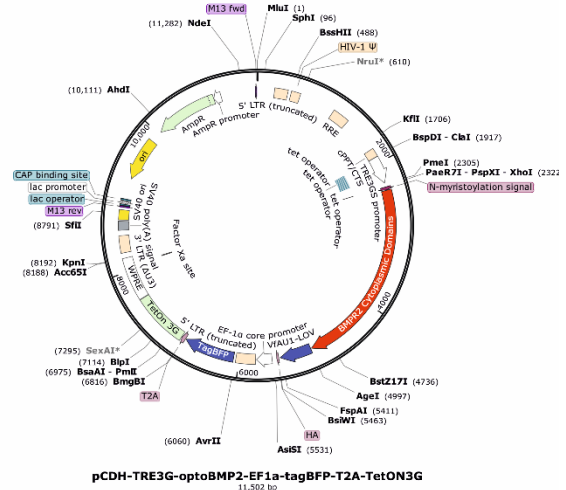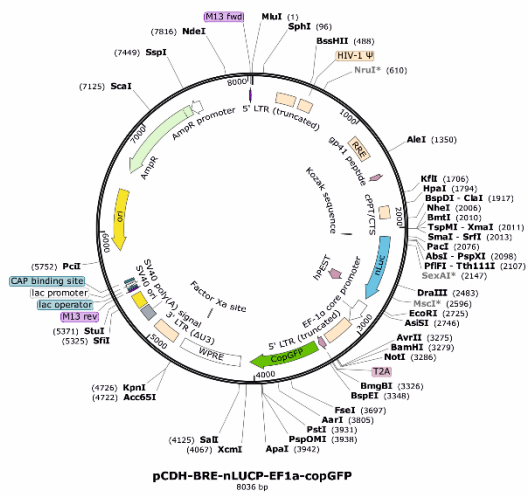
Figure S1 – Vector maps.

(A) Full vector map of TetOn-optoBMPR1B

(B) Full vector map of TetOn-optoBMPR2

**(C)** Full vector map of pCDH-BRE-nLUCP-EF1a-copGFP
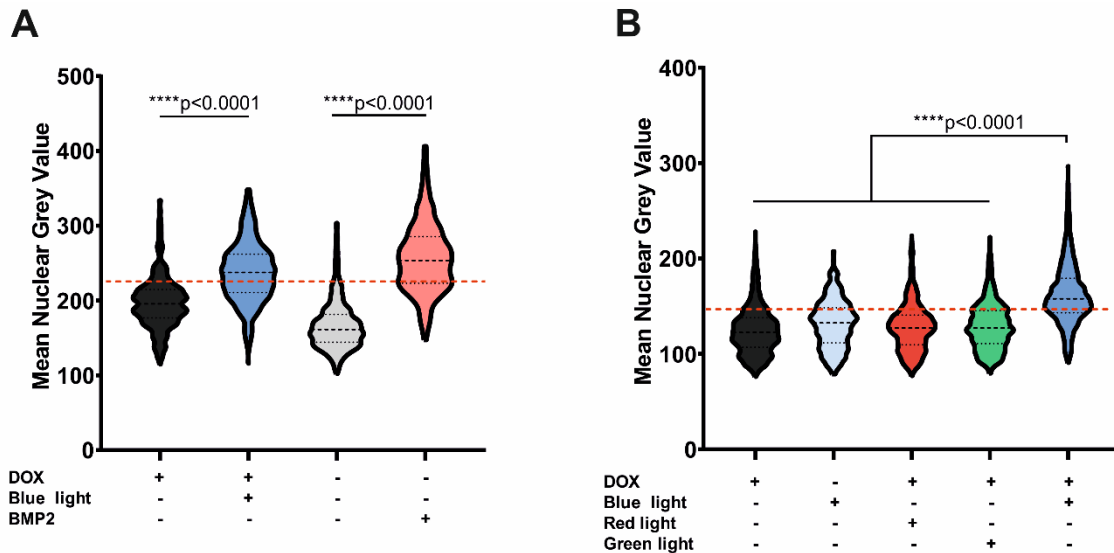
Figure S2. Single cell quantification of nuclear P-SMAD1/5 fluorescence. (A-B) Quantification of nuclear P-SMAD1/5 mean grey value when (**A**) cells were stimulated with blue light or 50ng/ml BMP2 and (**B**) stimulated with a range of light wavelengths. N = three different fields of view per condition across three independent experiments.

Data Information: Nuclei were identified with DAPI channel and relative nuclear fluoresence intensity of P-SMAD1/5 was measured with ImageJ. Quantification performed using three different fields of view per condition across three independent experiments. Violin plots represent all quantified data. Dotted red line indicates mean grey value threshold for positivity determined through analysis of non-stimulated controls. *P* values were generated using an ordinary one-way ANOVA (****p<0.0001).
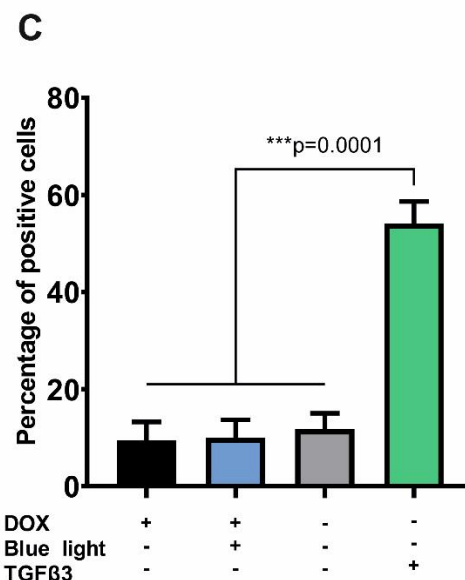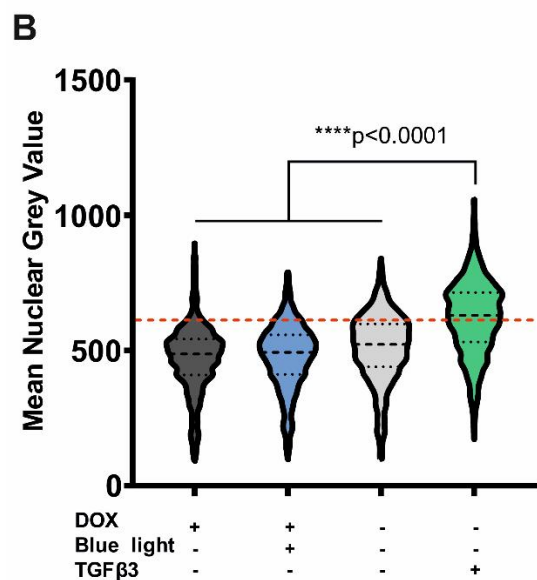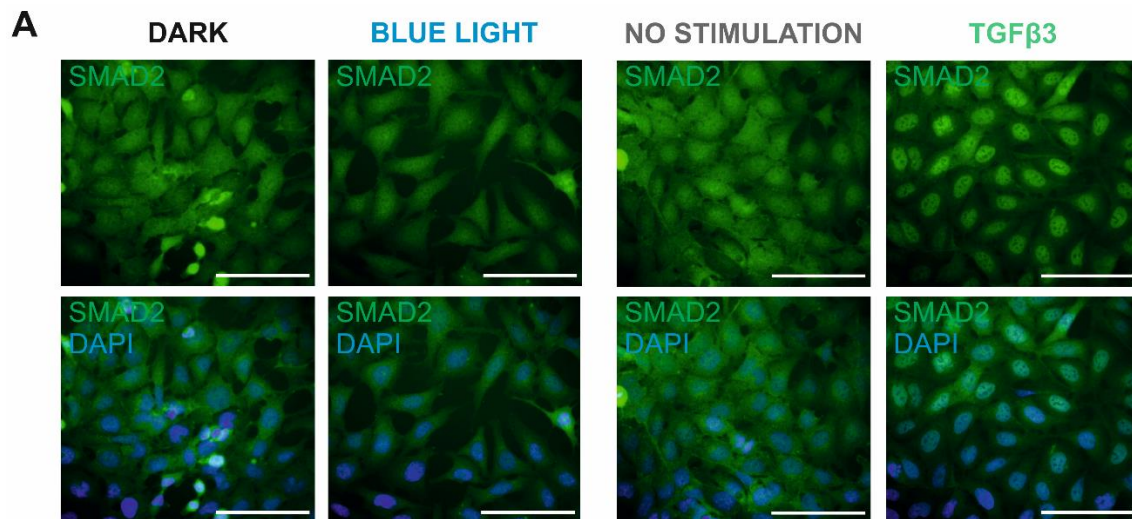
Figure S3. Analysis of SMAD2 nuclear translocation in optoBMP-TC28a2 cells. (**A**) Representative immunofluorescence images of cells stained for SMAD2 1 hour after initial stimulation. Cells were either stimulated with 15 minutes blue light illumination or kept in the dark after 24 hours doxycycline treatment and serum starvation (**LEFT**). To act as controls, cells were left untreated with doxycycline during serum starvation and either remain unstimulated or stimulated with 10ng/ml TGFβ3 (**RIGHT**). (**B**) Single cell quantification of nuclear SMAD2 mean grey value when cells were stimulated with 10ng/ml TGFβ3 or blue light illumination. (**C**) Percentage of nuclear-SMAD2 positive cells when cells were stimulated with 10ng/ml TGFβ3 or blue light illumination calculated from (**B**). Threshold for positivity calculated through analysis of unstimulated controls.

Data Information: Nuclei were identified with DAPI channel and relative nuclear fluorescence intensity of SMAD2 was measured with ImageJ. Scale bars represent 100μm. Quantification performed using three different fields of view per condition across three independent experiments. Violin plots (**B**) represent all quantified data. Dotted red line indicates mean grey value threshold for positivity determined through analysis of non-stimulated controls. *P* values were generated using an ordinary one-way ANOVA (***p<0.0005, ****p<0.0001).
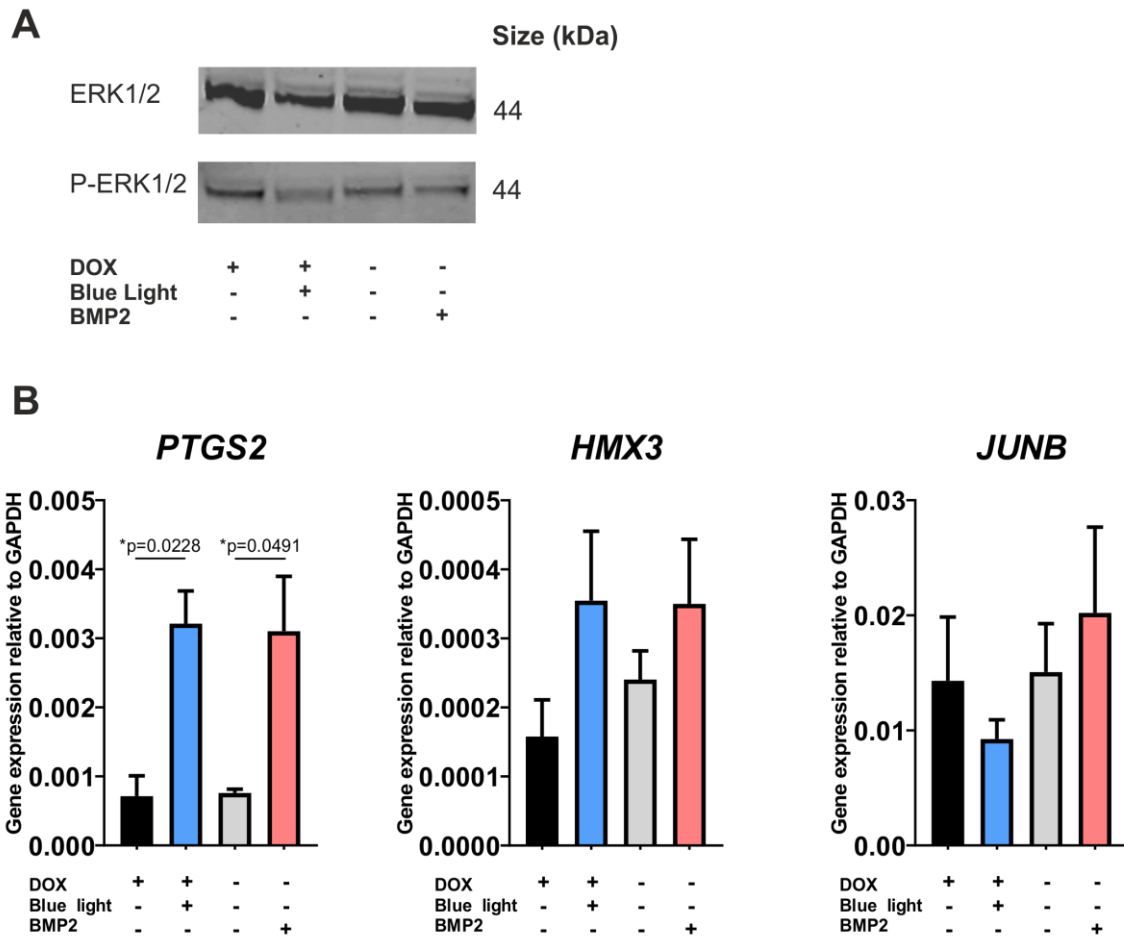
Figure S4. Activation of non-canonical signalling after blue light stimulation in optoBMP-TC28a2 cells. (**A**) Representative western blot for phosphorylated and total ERK1/2 MAPK 2 hours after stimulation with 50ng/ml BMP2 or blue light illumination. N = 3 runs. (**B**) Gene expression analyses of known non-canonical BMP signalling pathway targets. Cells were stimulated with blue light illumination or 50ng/ml BMP2 and analysed after 4 hours. Gene expression was normalised to GAPDH. N = four independent experiments.

Data information: Data presented in **B** represents mean values + SEM. *P* values were generated using an ordinary one-way ANOVA (*p<0.05).
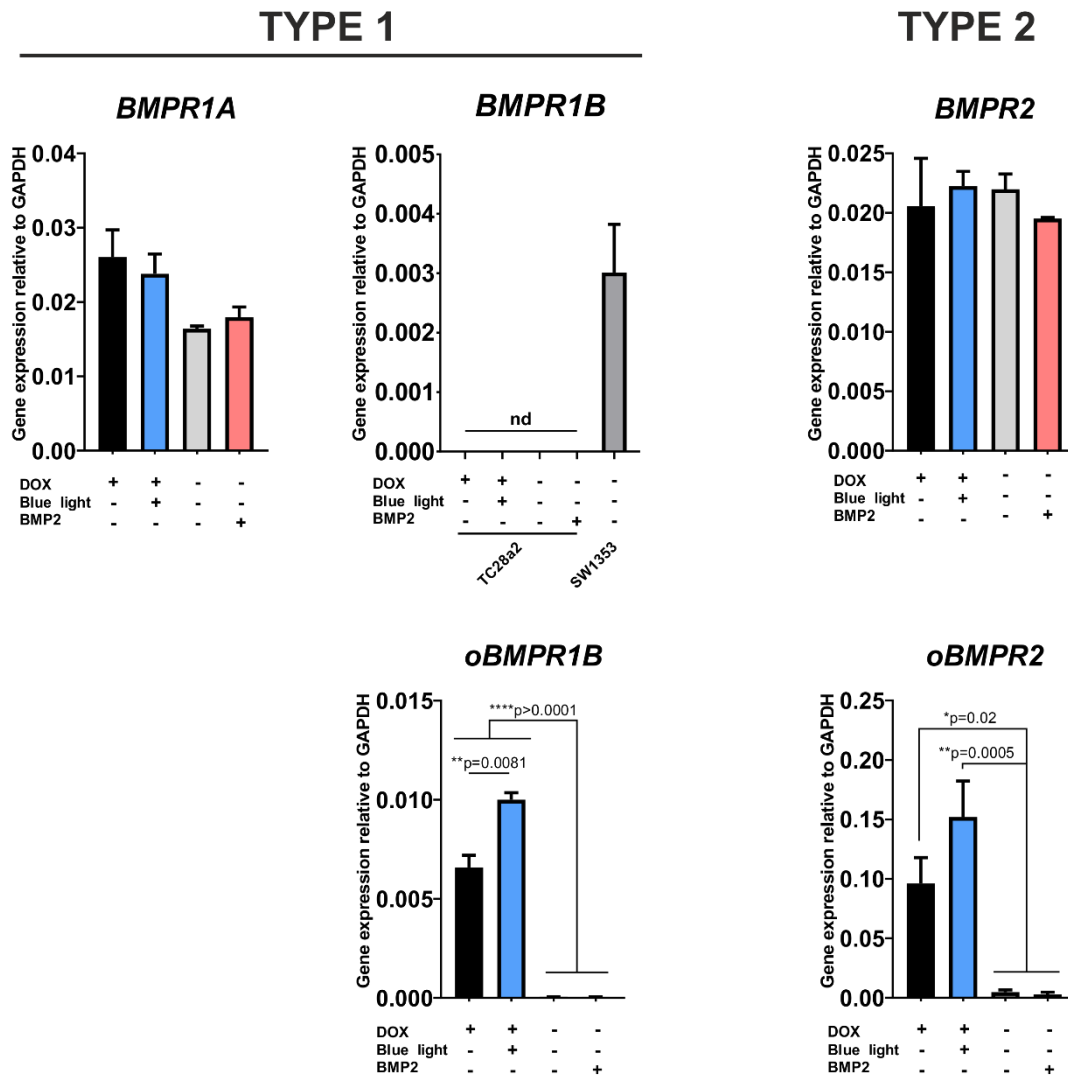
Figure S5 – Gene expression analyses of BMP-like receptors in optoBMP-TC28a2 cells.

Data Information: Native *BMPR1B* and *BMPR2* transcript was amplified through primer design targeting exons within the extra-cellular coding regions. *BMPR1B* transcript was not detected (nd) and primers were validated in an alternative chondrosarcoma SW1353 cell line. Optogenetic receptor transcript was detected through reverse direction primer design targeting the LOV domain coding region. Gene expression was normalised to GAPDH. N = four independent experiments. Bars represent mean values + SEM. *P* values were generated using an ordinary one-way ANOVA (*p<0.05, **p<0.01, ****p<0.0001).