hydroxyapatite), chondrogenesis (toluidine blue, glycosaminoglycans), and adipogenesis (oil red o, lipid droplets) (Bar = 100 μM) (B). Subsequent gene analysis for markers of osteogenesis (Cbfa1) (Ci), chondrogenesis (Sox9) (Cii), and adipogeneis (PPARγ) (Ciii) normalized to both the housekeeping gene β-actin and undifferentiated 0 μM controls (**p<0.01, ****p<0.0001 vs. 0 μM) (mean +/- SD).

Figure 7. BIO-treated MSCs Retain Multipotent Potential in 3D

Differentiation of BIO-treated encapsulated MSCs (A). BIO-treated MSCs underwent proliferation in 2D for 3 weeks before encapsulation and subsequent induction of osteogenesis (von Kossa, hydroxyapatite), chondrogenesis (toluidine blue, glycosaminoglycans), and adipogenesis (oil red o, lipid droplets) (Bar = 50 μM) (B). Subsequent gene analysis for markers of osteogenesis (Cbfa1) (Ci), chondrogenesis (Sox9) (Cii), and adipogeneis (PPARγ) (Ciii) normalized to both the housekeeping gene β-actin and undifferentiated 0 μM controls (*p<0.05, **p<0.01 vs. 0 μM) (mean +/- SD).

12. Supplemental Data

Supplemental Figure S1. BIO Acts as an Agonist of Wnt/β-catenin Signaling

In the absence of BIO and Wnt ligand receptor frizzled interaction, active GSK3β, disheveled (DSH), adenomatosis polyposis coli (APC), and AXIN complex and act to ubiquitinate β-catenin, leading to its subsequent proteosomal degradation.

Addition of BIO specifically inhibits GSK3β by occupying its ATP binding pocket, preventing its activation and complexation with DSH, APC, and AXIN. As a result, increased cytosolic concentrations of active β-catenin are able to translocate into the nucleus and bind to the TCF/LEF family of transcription factors, resulting in elevated transcription of down stream Wnt target genes such as Sox2, NANOG, Oct4, and Cyclin D1.

Supplemental Figure S2. Chemical Structures of Synthesized Products

6-bromoindirubin-3'-oxime (BIO) (A), poly(lactide)-b-poly(ethylene glycol)-b-poly(lactide) dimethacrylate (PEGPLADM, n=227, m~2) (B), acrylate-PEG-RGDS (p=79) (C).

Supplemental Figure S3. Forward (FWD) and Reverse (REV) RT-PCR Primer Sequences

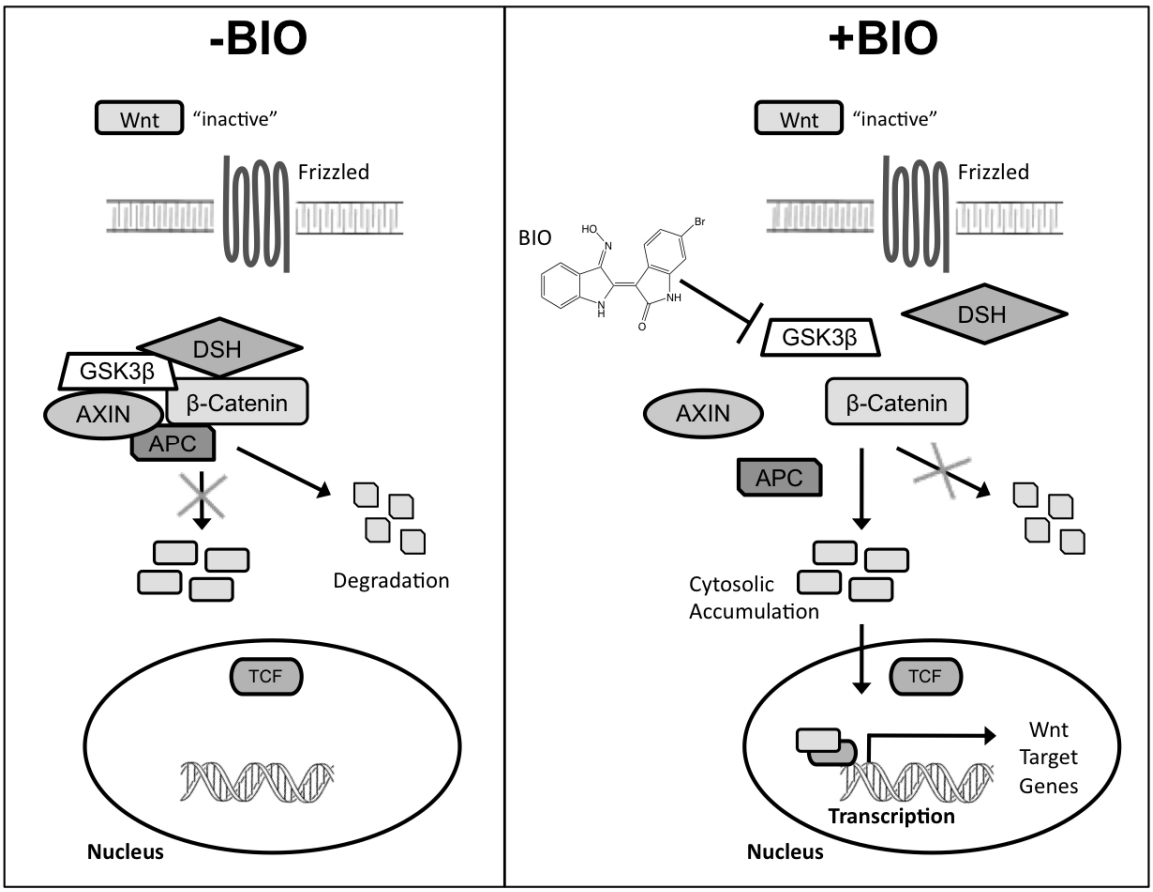
Gene	*Primer Sequence (5'-to-3')*	*Anneal.*	*Ref.*
β-actin	FWD: TGTGATGGTGGGAATGGGTCAG REV: TTTGATGTCACGCACGATTTCC	60 °C	Kotenko, S.V., et al. *Proc Natl Acad USA*. 97:1696. 2000
Sox2	FWD: AACCAGAAAAACAGCCCG REV: TTGCTGATCTCCGAGTTGTG	60 °C	Designed in house (Dr. Yufeng Dong)
NANOG	FWD: GATTTGTGGGCCTGAAGAAA REV: CAGATCCATGGAGGAAGGAA	57 °C	Designed in house (Dr. Yufeng Dong)
Oct4	FWD: CTGAAGCAGAAGAGGATCAC REV: GACCACATCCTTCTCGAGCC	57 °C	Szabo, E., et al. *Nature*. 468:521. 2010
Cyclin D1	FWD: GCCGAATTCTGGATGCTGGAGGTCTG REV: GCCGAATTCGGCTTCGATCTGCTCCT	60 °C	Sewing, A., et al. *J Cell Sci*. 104:545. 1993
Cbfa1	FWD: ATGCTTCATTCGCCTCACAAAC REV: CCAAAAGAAGTTTTGCTGACATGG	57 °C	Perinpanayagam, H., et al. *Arch Oral Biol*. 51:406. 2006
Sox9	FWD: TTTCCAAGACACAAACATGA REV: AAAGTCCAGTTTCTCGTTGA	60 °C	Lee, Y.J., et al. *J Korean Neurosurg S*. 43:149. 2008
PPARγ	FWD: TCTCTCCGTAATGGAAGACC REV: GCATTATGAGACATCCCCAC	55 °C	Takahashi, N., et al. *FEBS Lett*. 455:135. 1999

Supplemental Figure S4. Relative Gene Expression Profiles for 2D BIO-treated MSCs

Continuous gene profiles of the markers of undifferentiated stem cells, Sox2 (A), NANOG (B), and Oct4 (C), as well as the positive cell cycle regulator Cyclin D1 (D) following 24 hr BIO treatment of two-dimensional MSC populations. Gene profiles are normalized to both the housekeeping gene β-actin and 0 μM controls, and are expressed as a ratio of the initial gene expression obtained on day 0 prior to BIO treatment (*p<0.05, **p<0.01, ****p<0.0001 vs. 0 μM) (mean +/- SD).

Supplemental Figure S5. Relative Gene Expression Profiles for PEGPLADM encapsulated BIO-treated MSCs

Continuous gene profiles of the markers of undifferentiated stem cells, Sox2 (A), NANOG (B), and Oct4 (C), as well as the positive cell cycle regulator Cyclin D1 (D) following 24 hr BIO treatment of PEGPLADM encapsulated MSC populations. Gene profiles are normalized to both the housekeeping gene β-actin and 0 μM controls, and are expressed as a ratio of the initial gene expression obtained on day 0 prior to BIO treatment (*p<0.05, ****p<0.0001 vs. 0 μM) (mean +/- SD).



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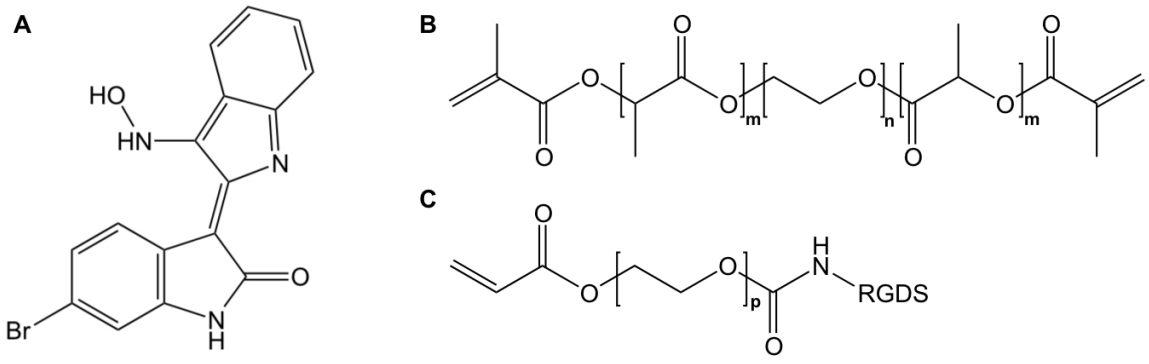

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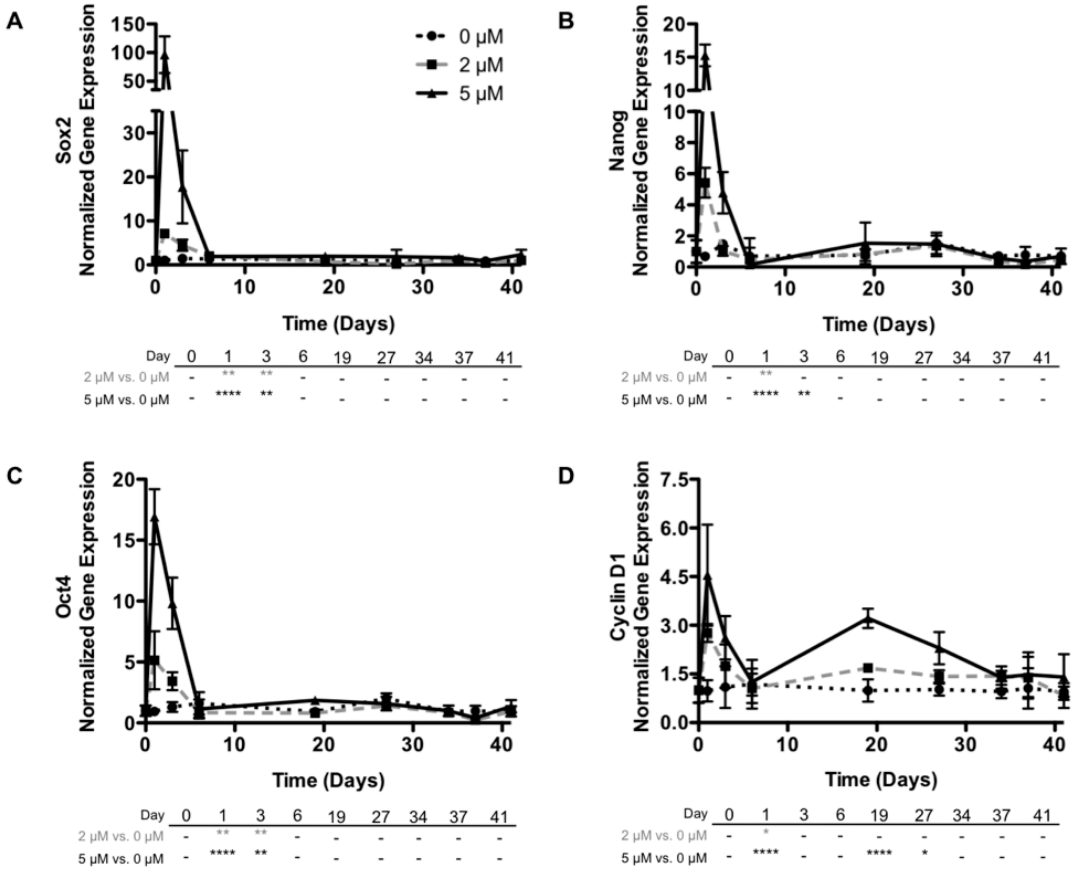

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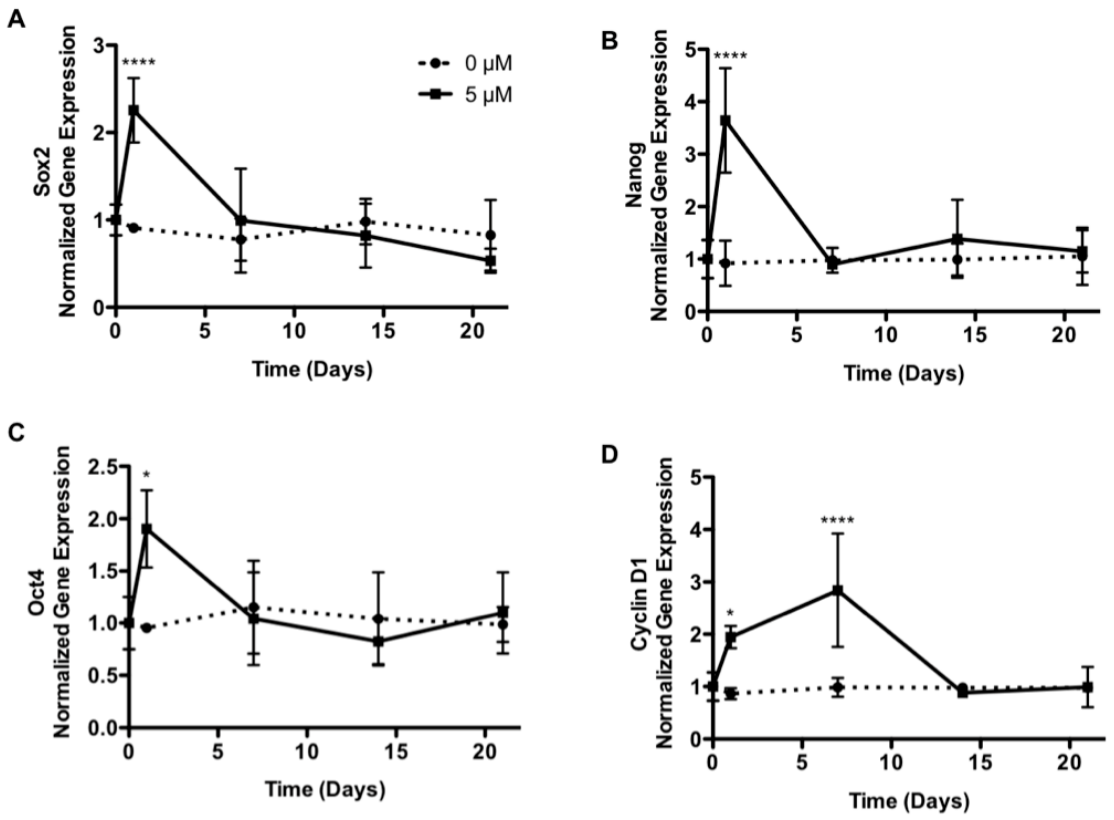

Supplemental Figure S5. Relative Gene Expression Profiles for PEGPLADM encapsulated BIO-treated MSCs

Continuous gene profiles of the markers of undifferentiated stem cells, Sox2 (A), NANOG (B), and Oct4 (C), as well as the positive cell cycle regulator Cyclin D1 (D) following 24 hr BIO treatment of PEGPLADM encapsulated MSC populations. Gene profiles are normalized to both the housekeeping gene β-actin and 0 μM controls, and are expressed as a ratio of the initial gene expression obtained on day 0 prior to BIO treatment (*$p<0.05$, ****$p<0.0001$ vs. 0 μM) (mean +/- SD).