Lorecivivint (SM04690), a Potential Disease-Modifying Osteoarthritis Drug, Inhibits CLK2 and DYRK1A, Novel Molecular Regulators of Wnt Signaling, Chondrogenesis, and Inflammation

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Disclosures

- All authors are employees or shareholders of Samumed, LLC

- Lorecivivint is an investigational agent not approved by the FDA or any other regulatory agency

Osteoarthritis (OA) and the Wnt pathway

Degenerative tissue remodeling is due to mechanical forces and inflammation[1]

Overexpressed Wnt proteins and pathway mutations are associated with OA[2-5]

Increased Wnt signaling drives bone formation, cartilage breakdown, and inflammation[6-9]

Hypothesis: Inhibiting the Wnt pathway reduces inflammation while protecting and regenerating cartilage

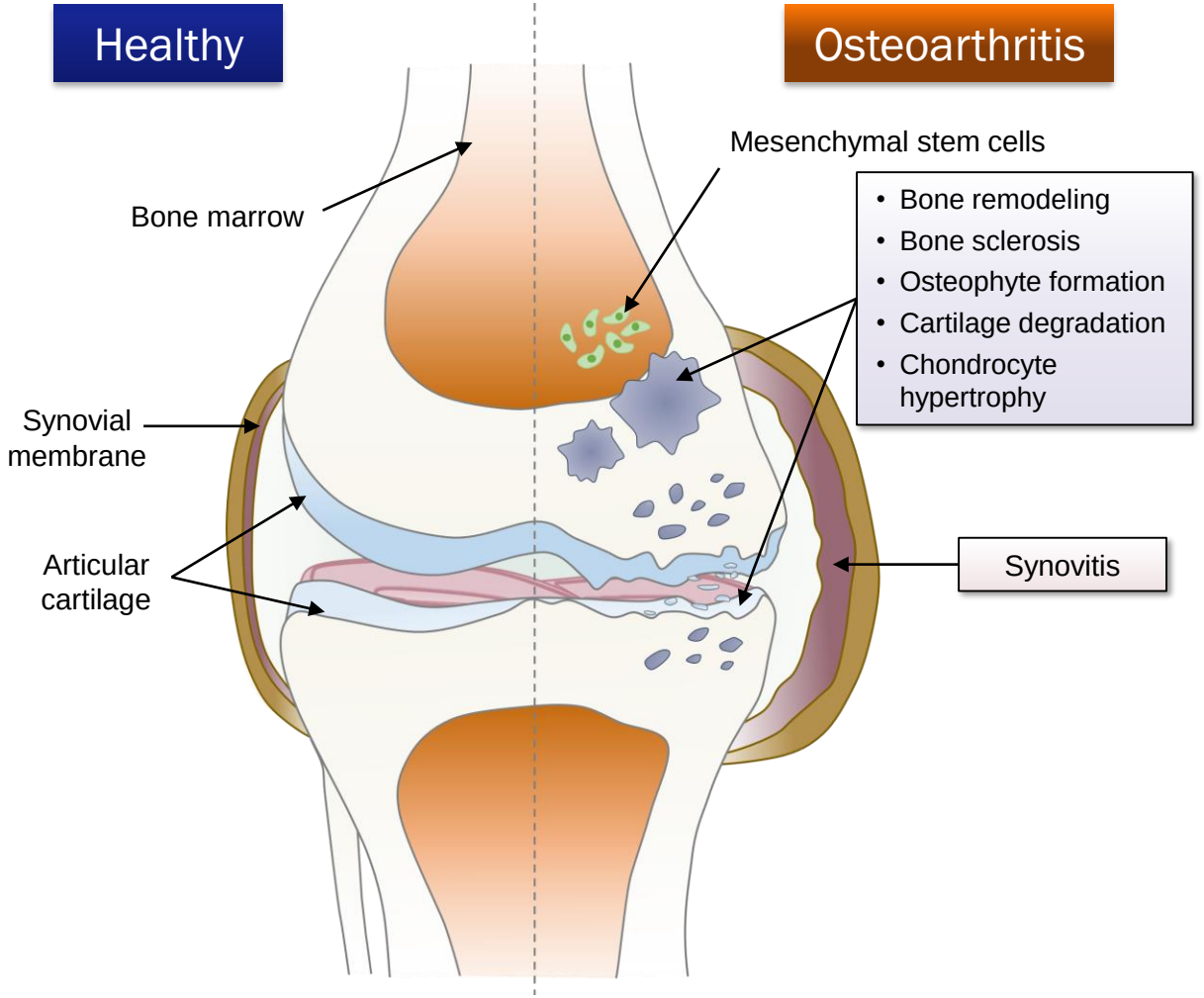


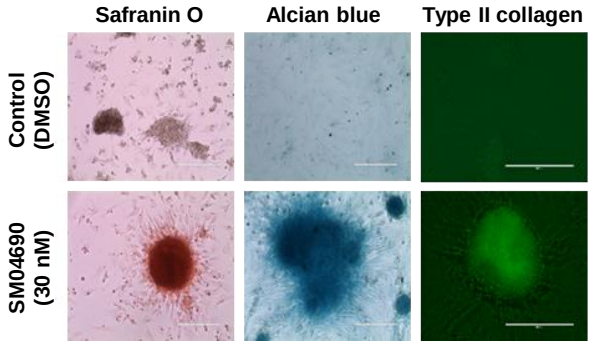
Figure adaptation: Bush and Beier. *Nature Medicine.* 2013

1. Loeser R. *Arthritis Rheum.* 2006
2. Hamerman D. *N Engl J Med.* 1993
3. Yuasa T, et al. *Lab Invest.* 2008
4. Ma B and Hottiger MO. *Frontiers Immun.* 2016
5. Sokolove J and Lepus CM. *Ther Adv Musculoskelet Dis.* 2013
6. Blom AB, et al. *Arthritis Rheum.* 2009
7. Monteagudo S, et al. *Nat Commun.* 2017
8. Rudnicki JA and Brown AM. *Dev Biol.* 1997
9. Thomas RS, et al. *Arthritis Res Ther.* 2011

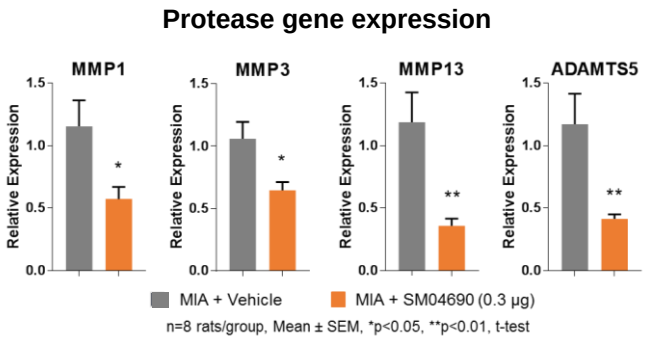
Lorecivivint (LOR; SM04690) preclinical development

***In vitro* assays and animal models of OA**

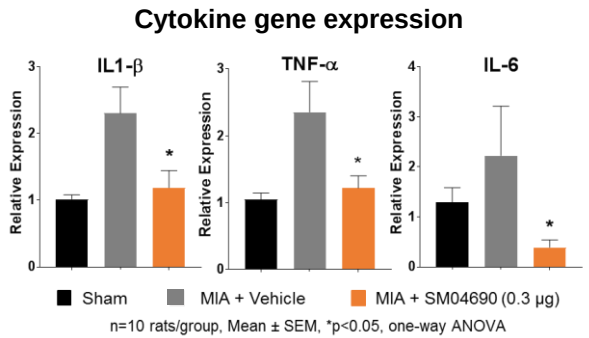
hMSC assays → **Chondrocyte Regeneration**



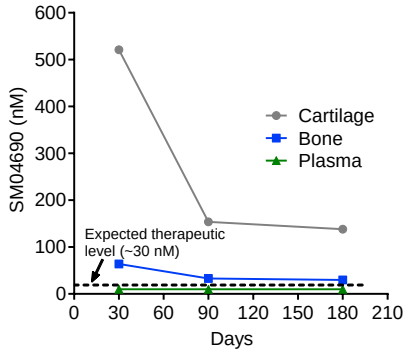
Protease assays → **Cartilage Protection**

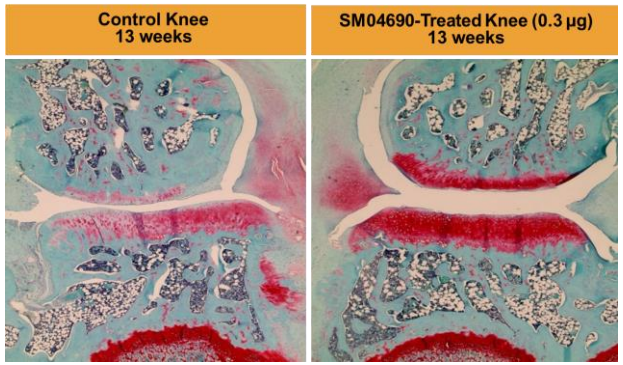


Cytokine assays → **Anti-inflammation**



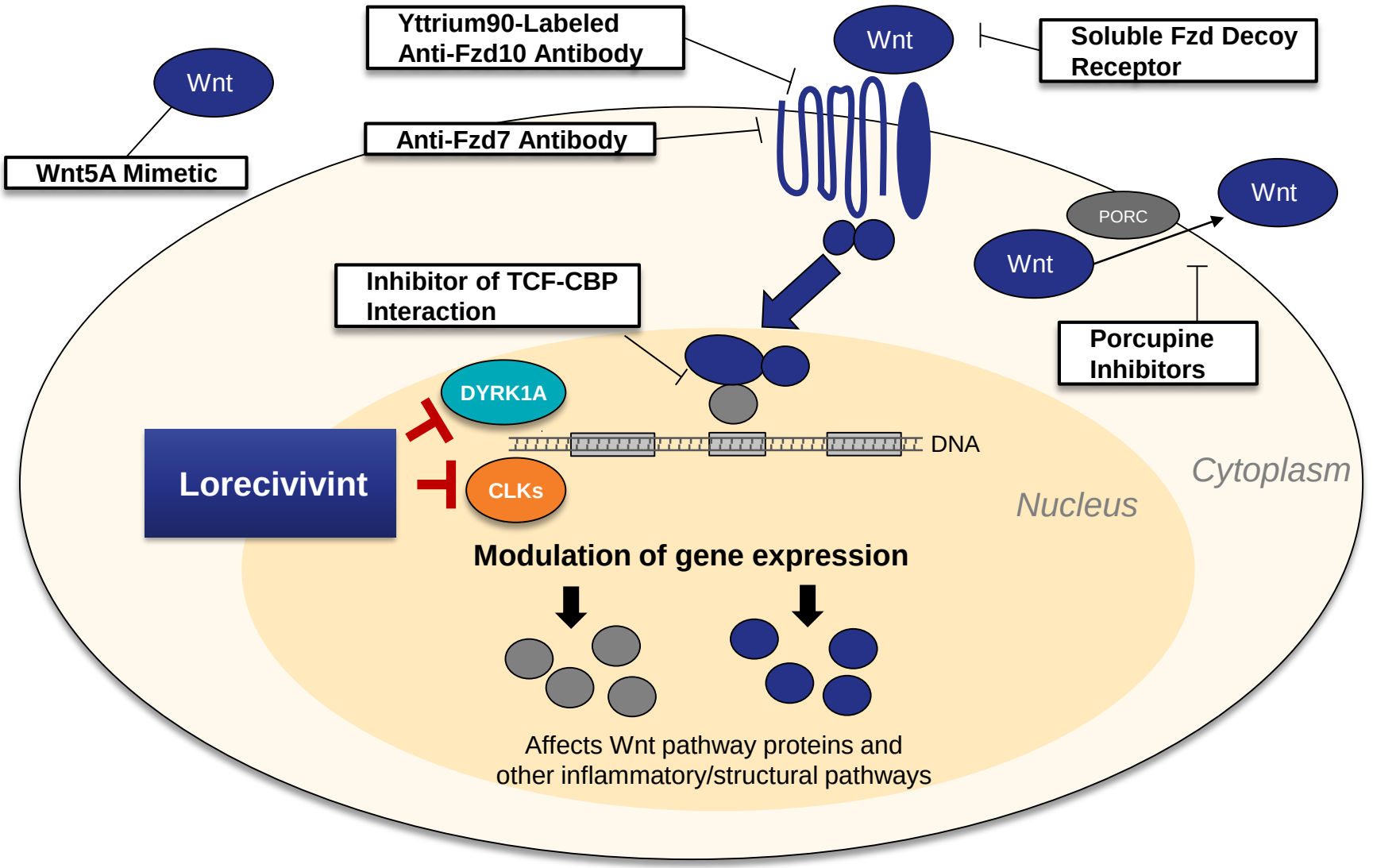
Animal models → **Sustained Local PK**



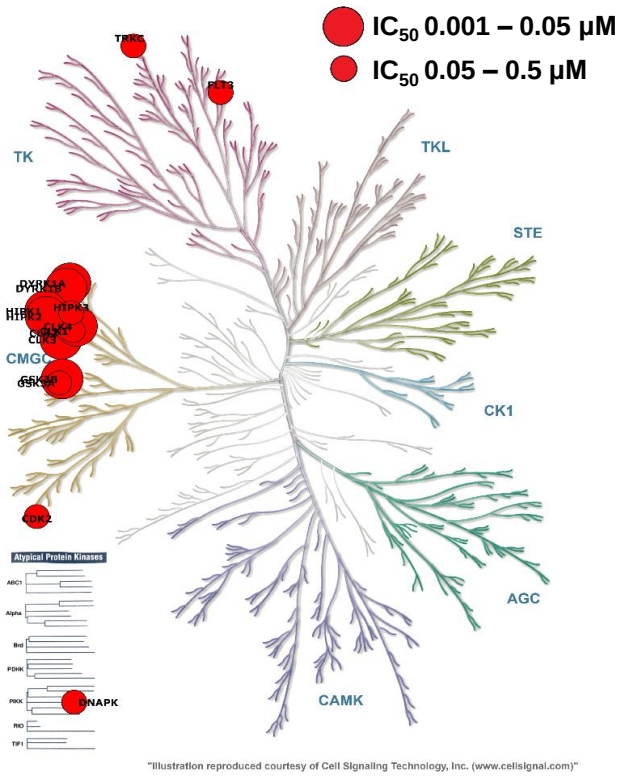


Improved Joint Health
(Animal models)

LOR inhibits the Wnt pathway through a unique MOA



LOR is a potent and selective kinase inhibitor



318 kinases tested *in vitro*

Kinase Tested	% Inhibition LOR (0.5 μM)	IC_{50} (nM)	Fold IC_{50} >CLK2
CLK2	98	5.8	1.0
CLK3	100	44.3	7.6
DYRK1A	99	26.9	4.6
DYRK1B	94	41.2	7.1
GSK3β	92	37.8	6.5
HIPK1	95	33.2	5.7
HIPK2	95	16.8	2.9

DNA

↓ **Transcription** ← **DYRK1A**

Pre-mRNA

↓ **mRNA processing e.g., splicing** ← **CLK2**

mRNA

↓ **Translation**

Protein

Alternative splicing regulation of gene expression

DNA

Transcription

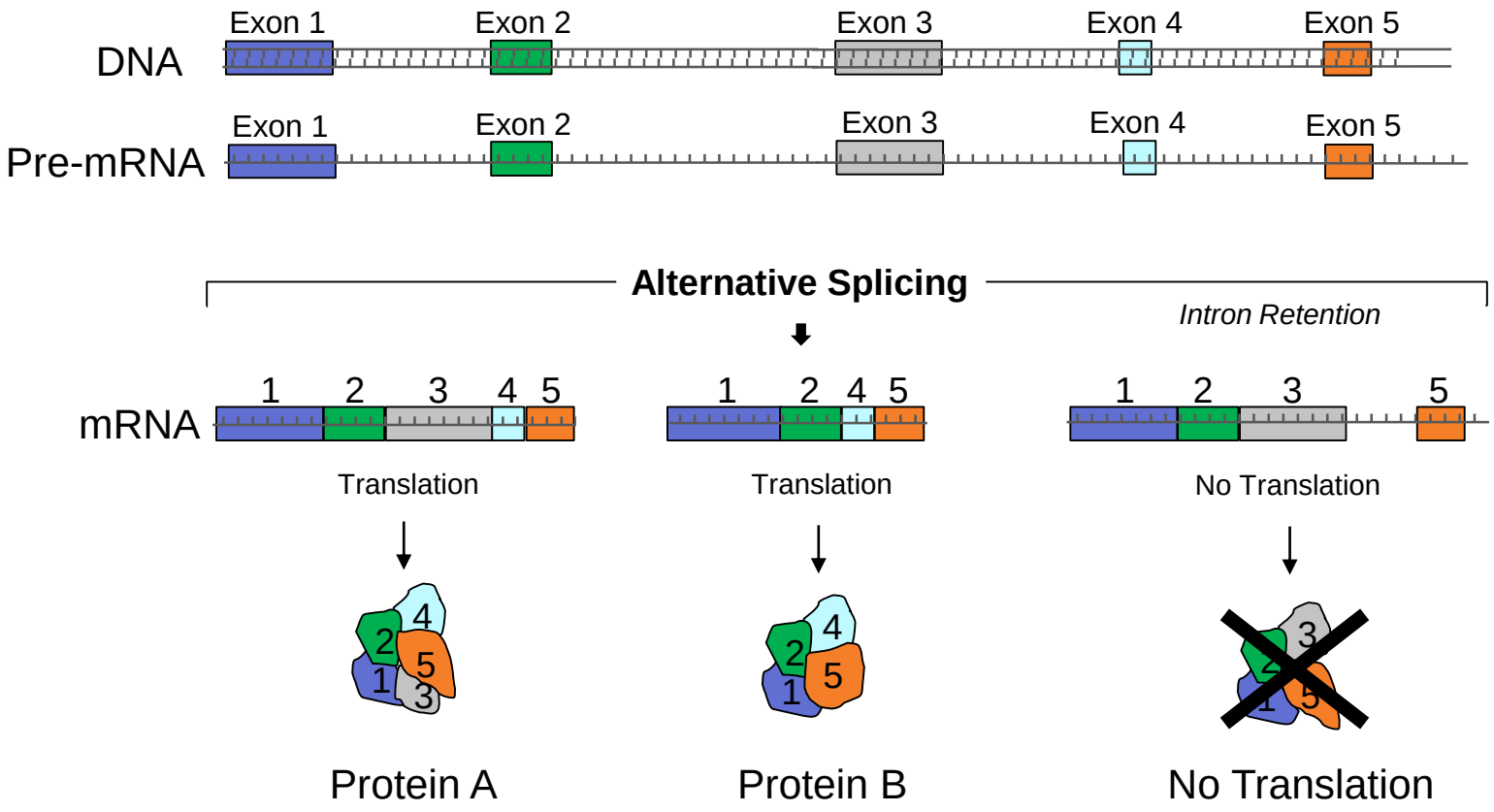
Pre-mRNA

mRNA processing
e.g., splicing

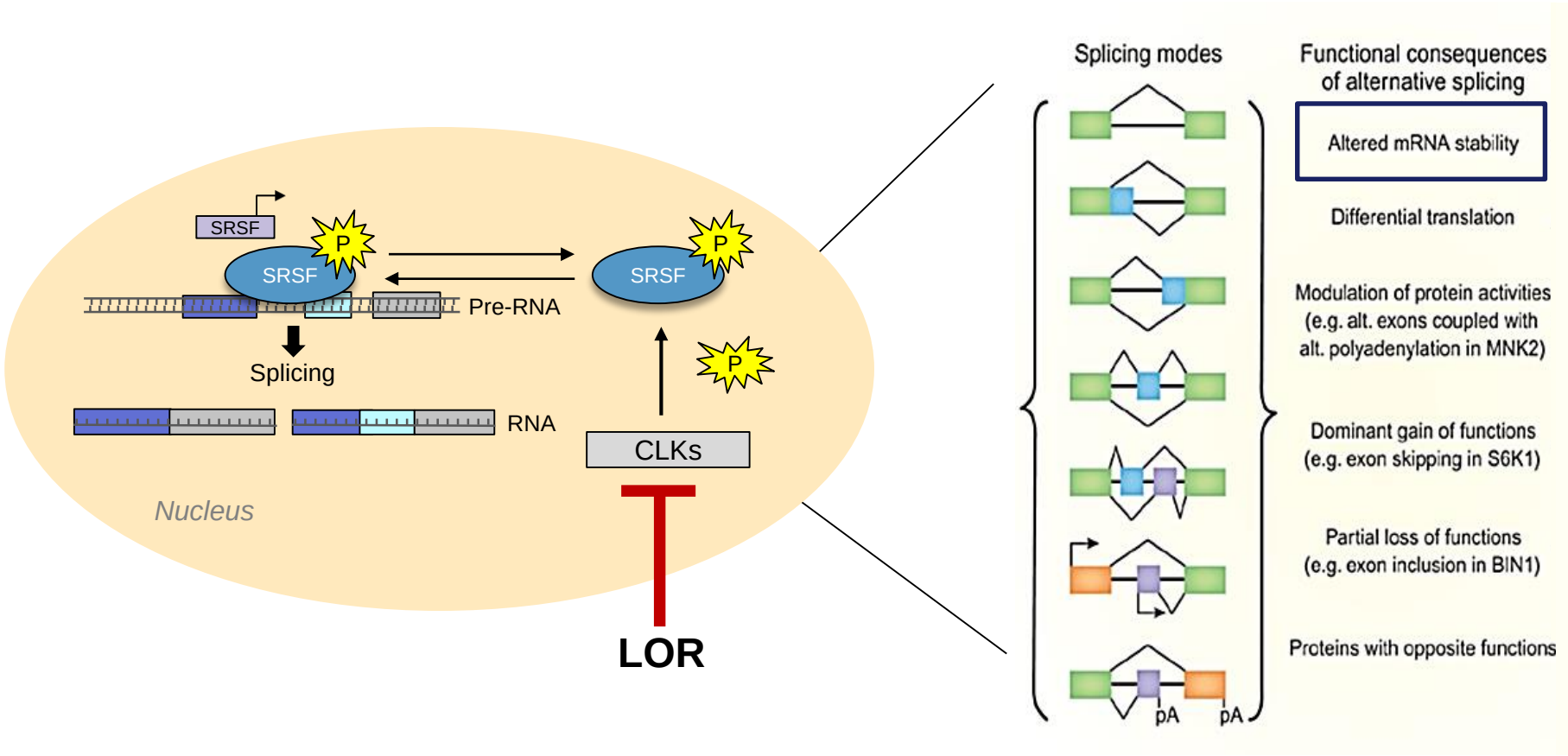
mRNA

Translation

Protein



CDC-like kinases (CLKs)



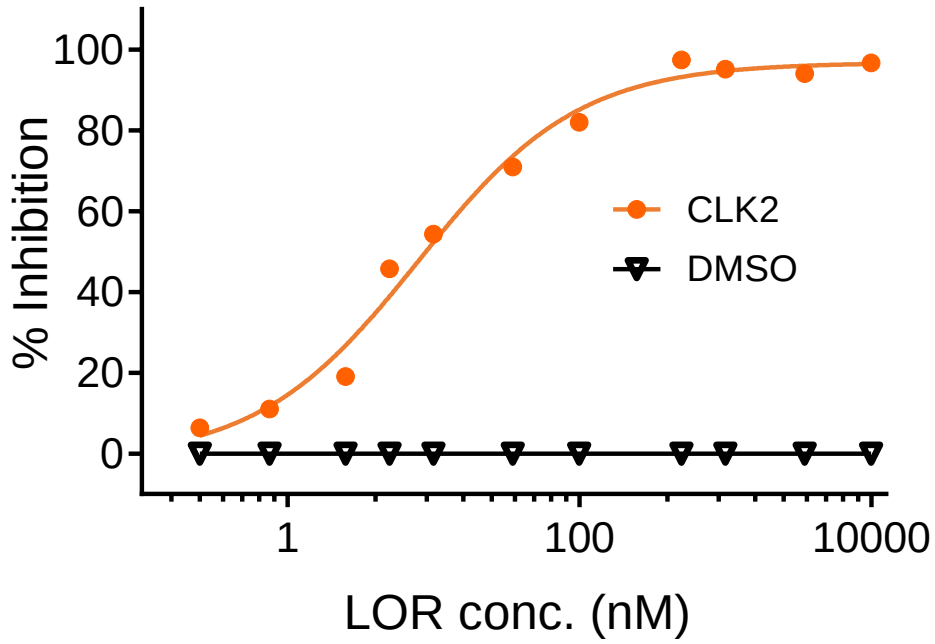
Directly affects transcription or alternative splicing of genes

Alteration of transcription factors can subsequently impact target genes of implicated pathway

1. Ben-David Y, et.al. *EMBO J.* 1991
2. Colwill K, et al. *EMBO J.* 1996
3. Mott B, et al. *Biorganic Med. Chem. Letter.* 2009
4. Riggs J, et al. *J. Med. Chem.* 2017

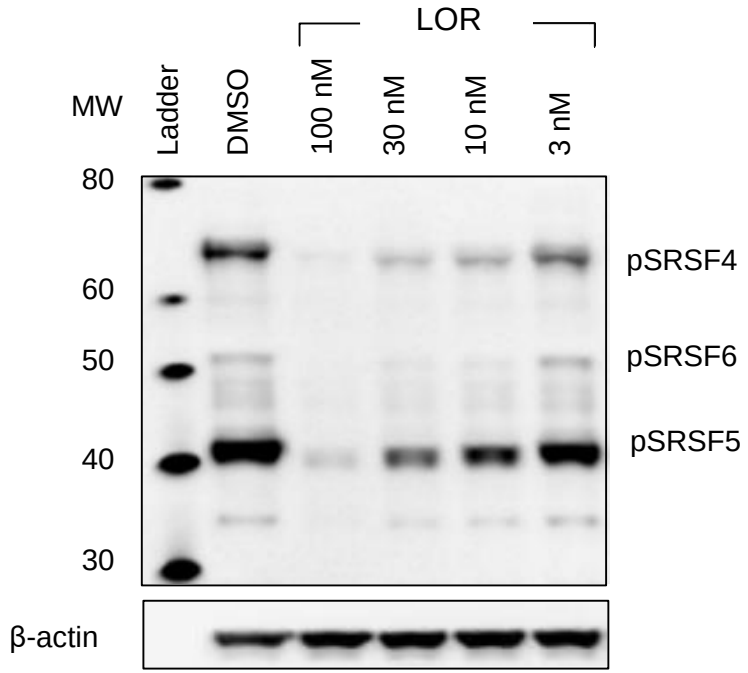
LOR inhibited CLK-mediated SRSF phosphorylation

LOR
(*In vitro* CLK2 biochemical kinase assay)



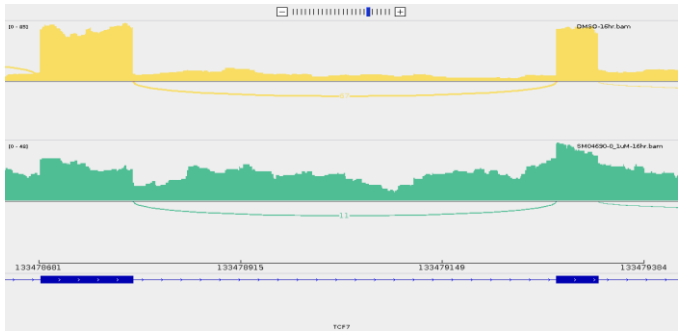
CLK2 IC_{50} = 7.8 nM

SRSF
(hMSCs *in vitro*)



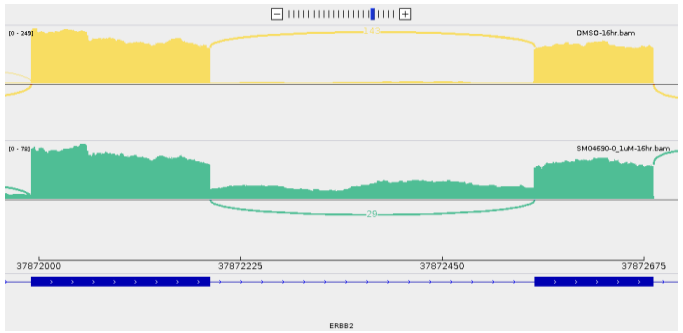
LOR induced intron retention and modulated alternative splicing

TCF7



DMSO / LOR

ERBB2

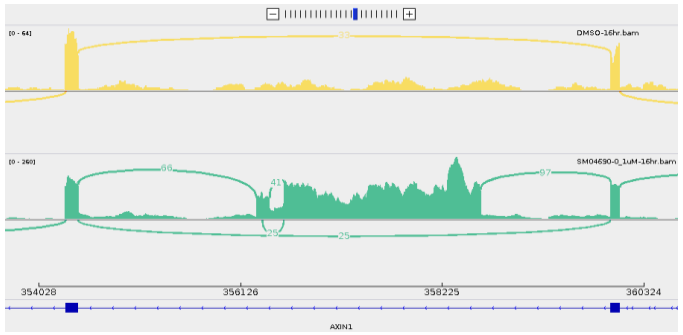


DMSO / LOR

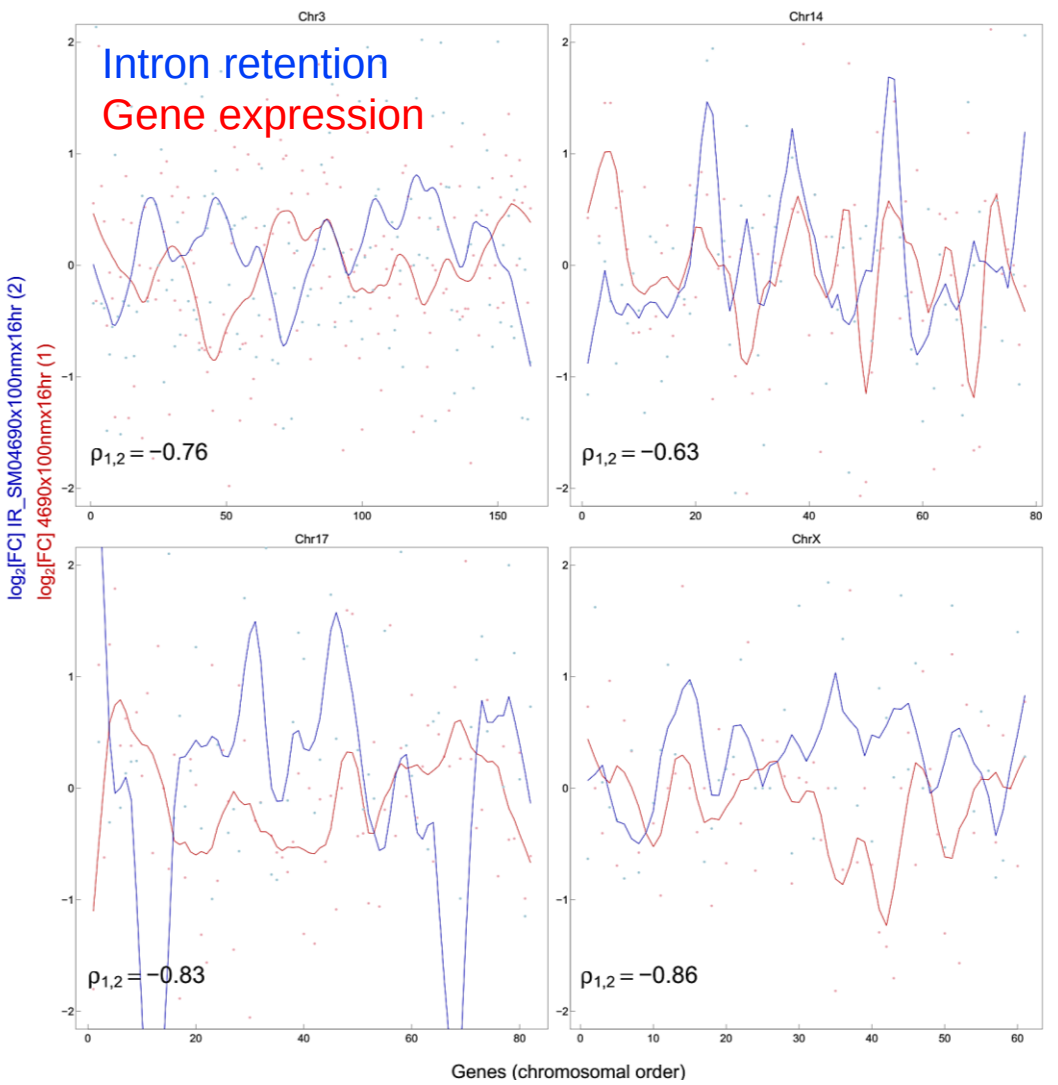
DVL2

DMSO / LOR

AXIN1



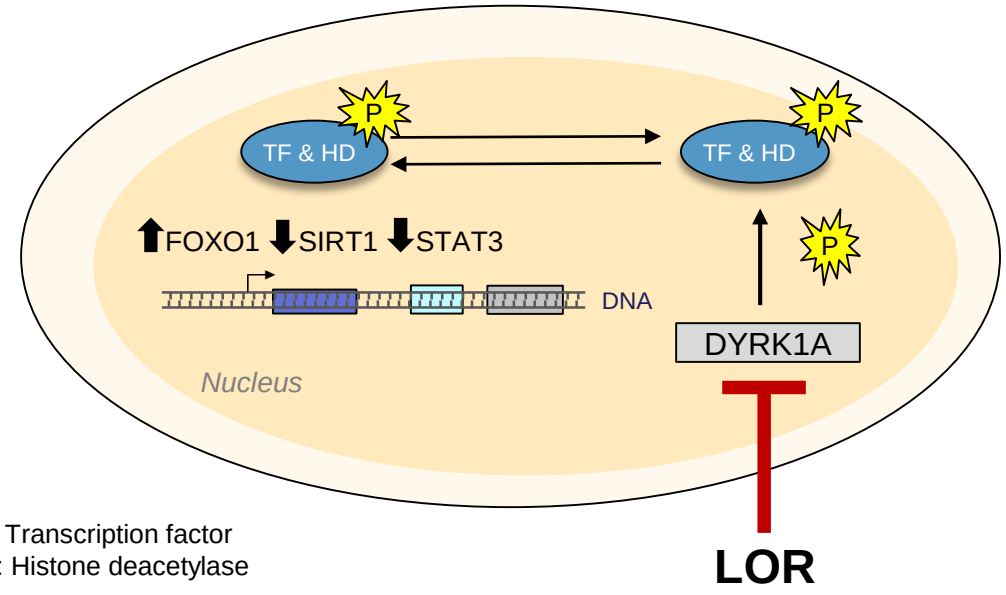
DMSO / LOR



RNA sequencing in hMSCs

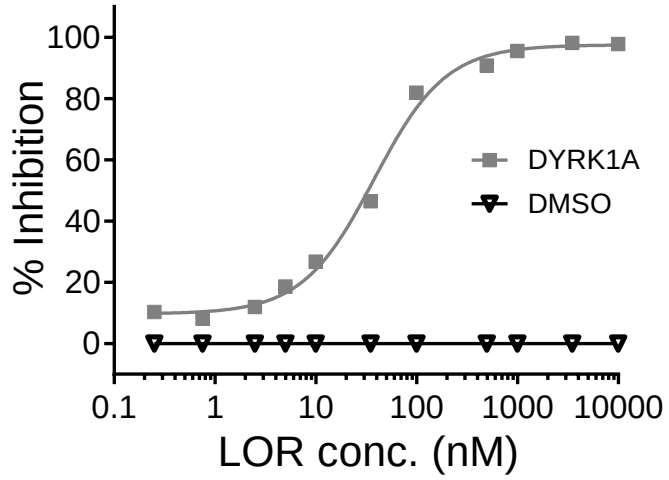
LOR inhibited DYRK1A

- DYRK1A inhibition
  - Reduced Wnt signaling[1] (benefited chondrocytes)
  - Reduced SIRT1[1,2] and increased FOXO1[3,4] (benefited chondrocytes)
  - Reduced STAT3[5] (inhibited inflammation)



TF: Transcription factor
HD: Histone deacetylase

LOR
(*In vitro* DYRK1A biochemical kinase assay)



DYRK1A IC_{50} = 26.9 nM

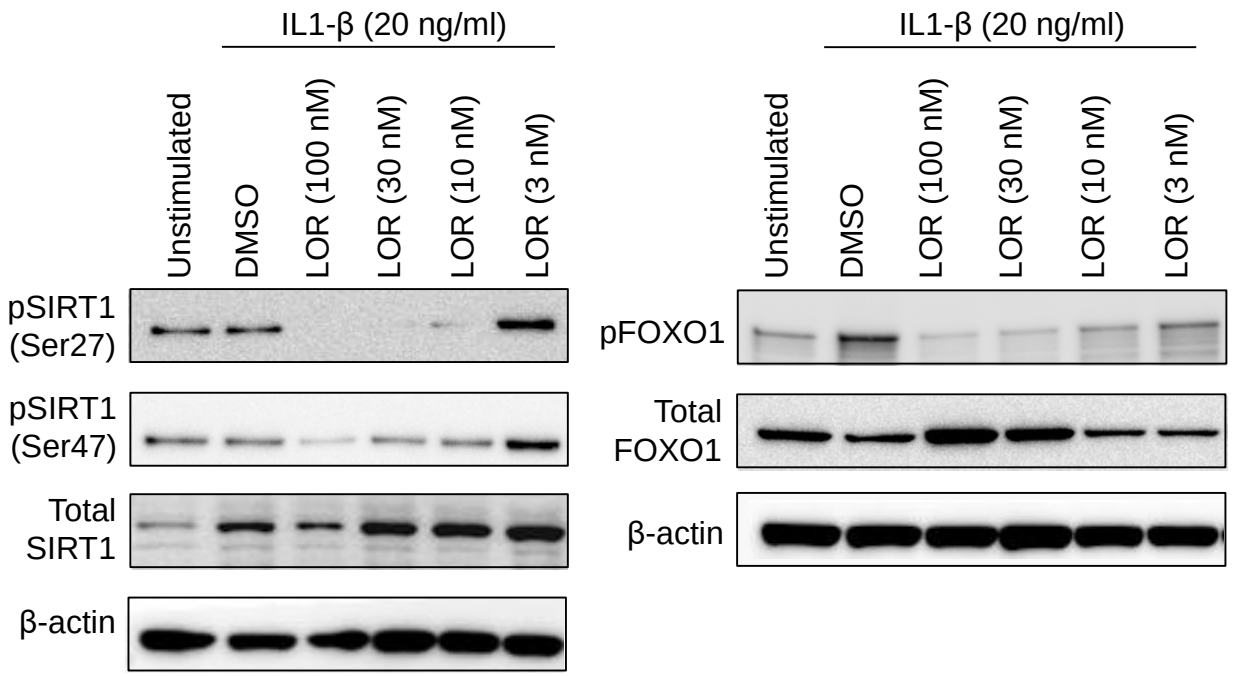
1. Monteagudo S, et.al. *Nat Commun.* 2017
2. Khor B, et al. *eLife*. 2015
3. Guo X, et.al. *J Biol Chem*. 2010
4. Matsuzaki T. *Sci Transl Med*. 2018
5. Akasaki Y, et.al. *Osteoarthritis Cartilage*. 2014

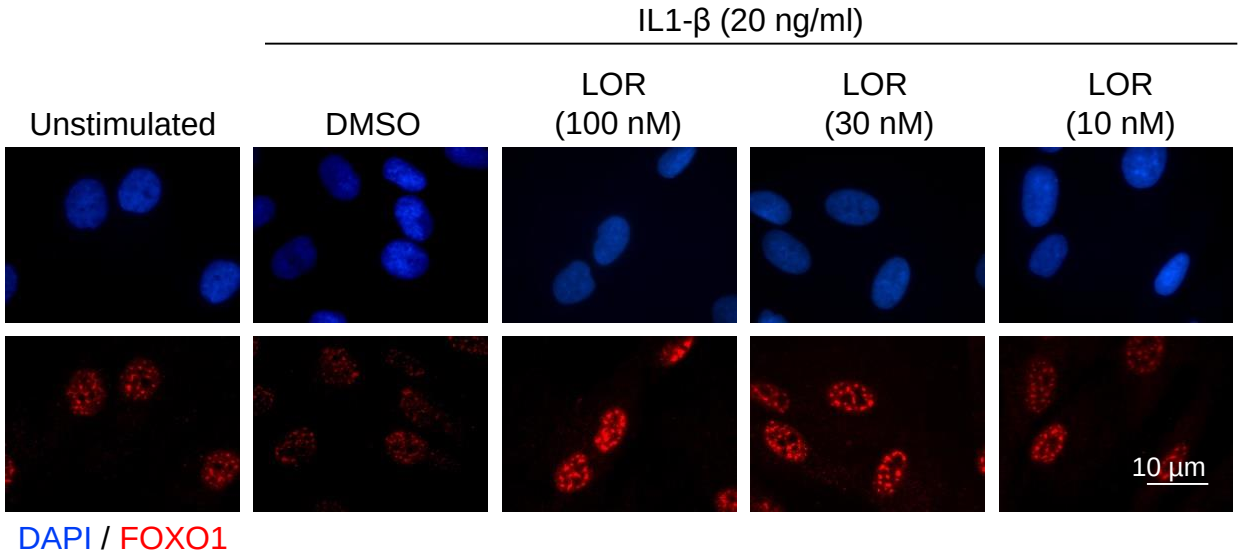
LOR inhibited SIRT1 and FOXO1 phosphorylation

Reduced FOXO1 phosphorylation led to increased nuclear FOXO1 levels

SIRT1
(hMSCs *in vitro*)

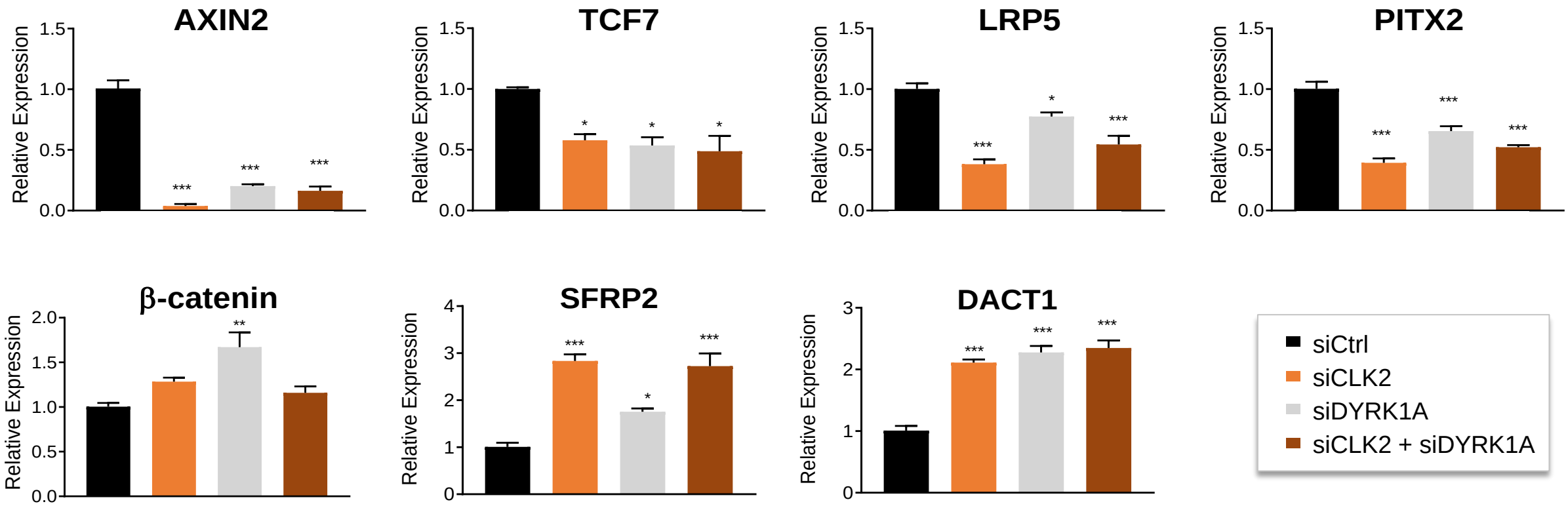
FOXO1
(Chondrocytes *in vitro*)





CLK2 and DYRK1A knockdowns inhibited the Wnt pathway

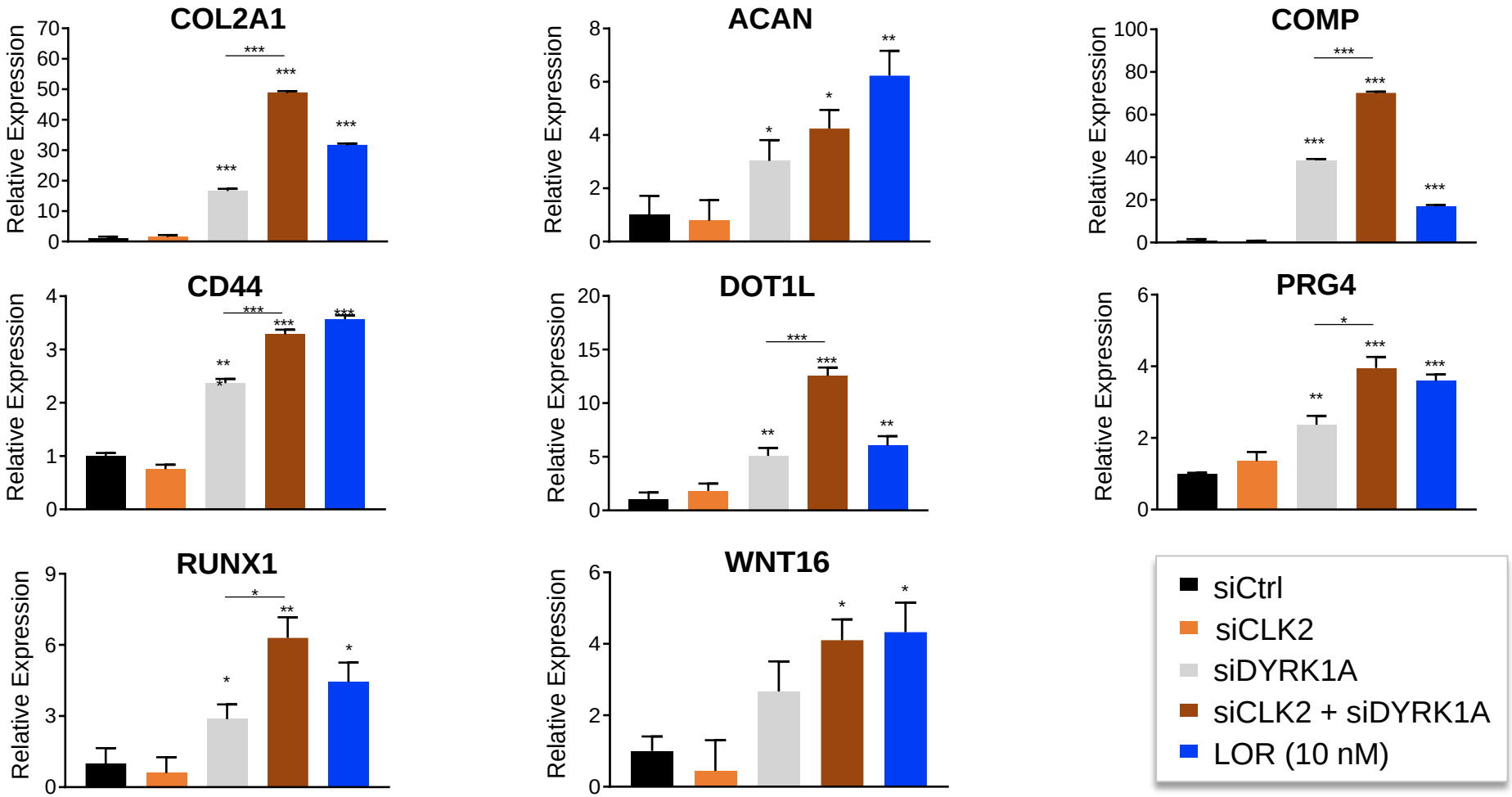
- Knockdowns inhibited Wnt pathway genes and upregulated secreted Wnt inhibitors SFRP2 and DACT1



In vitro siRNA knockdown effects in hMSCs identified by NanoString panel and validated by qPCR

*P<0.05, **P<0.01, ***P<0.001 vs. siCtrl

CLK2/DYRK1A knockdown induced chondrocyte differentiation

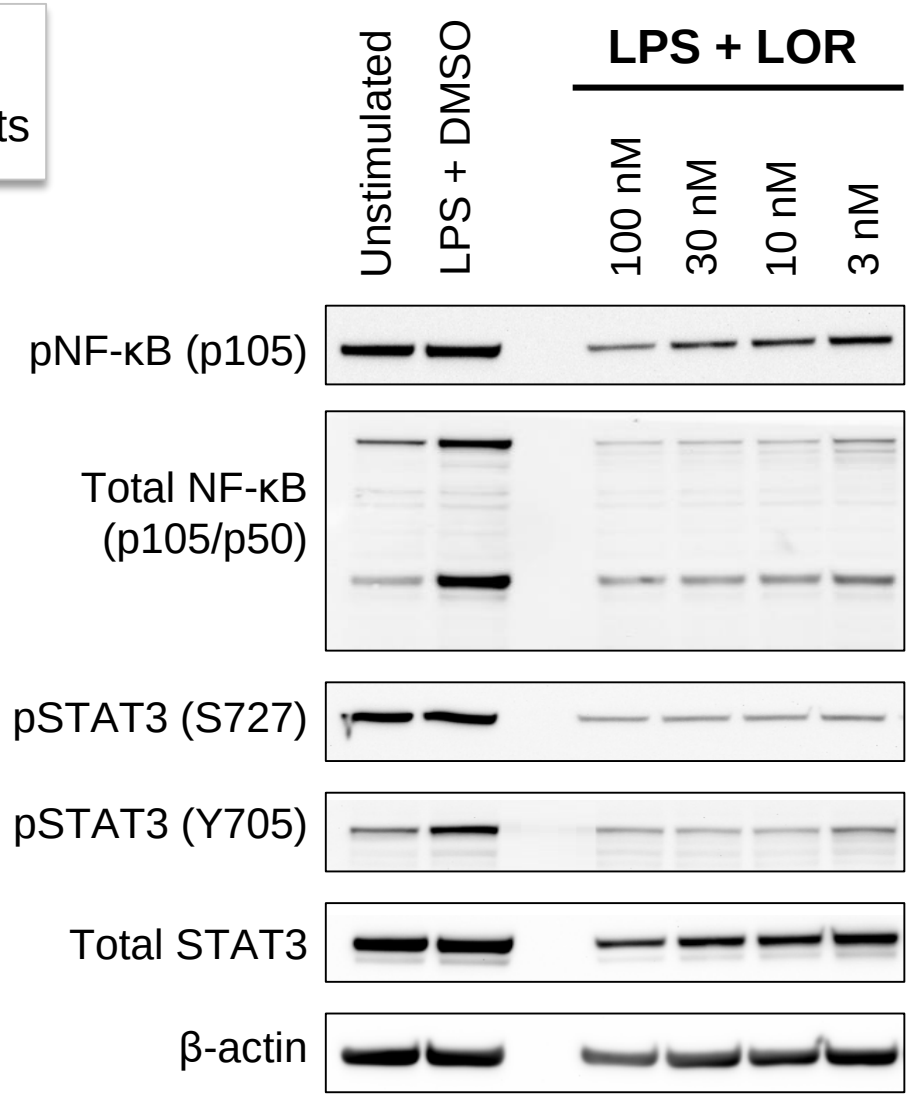


In vitro siRNA knockdown effects in hMSCs identified by NanoString panel and validated by qPCR

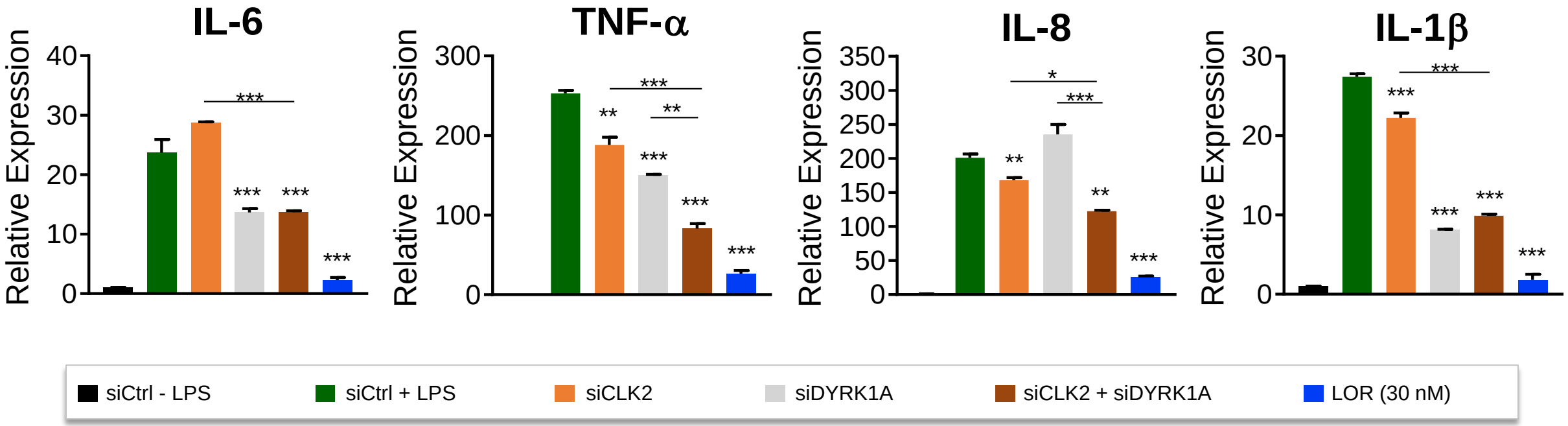
**P*<0.05, ***P*<0.01, ****P*<0.001 vs. siCtrl

LOR decreased phosphorylation of NF-κB and STAT3

NF-κB and STAT3
In vitro LPS-stimulated synovial fibroblasts

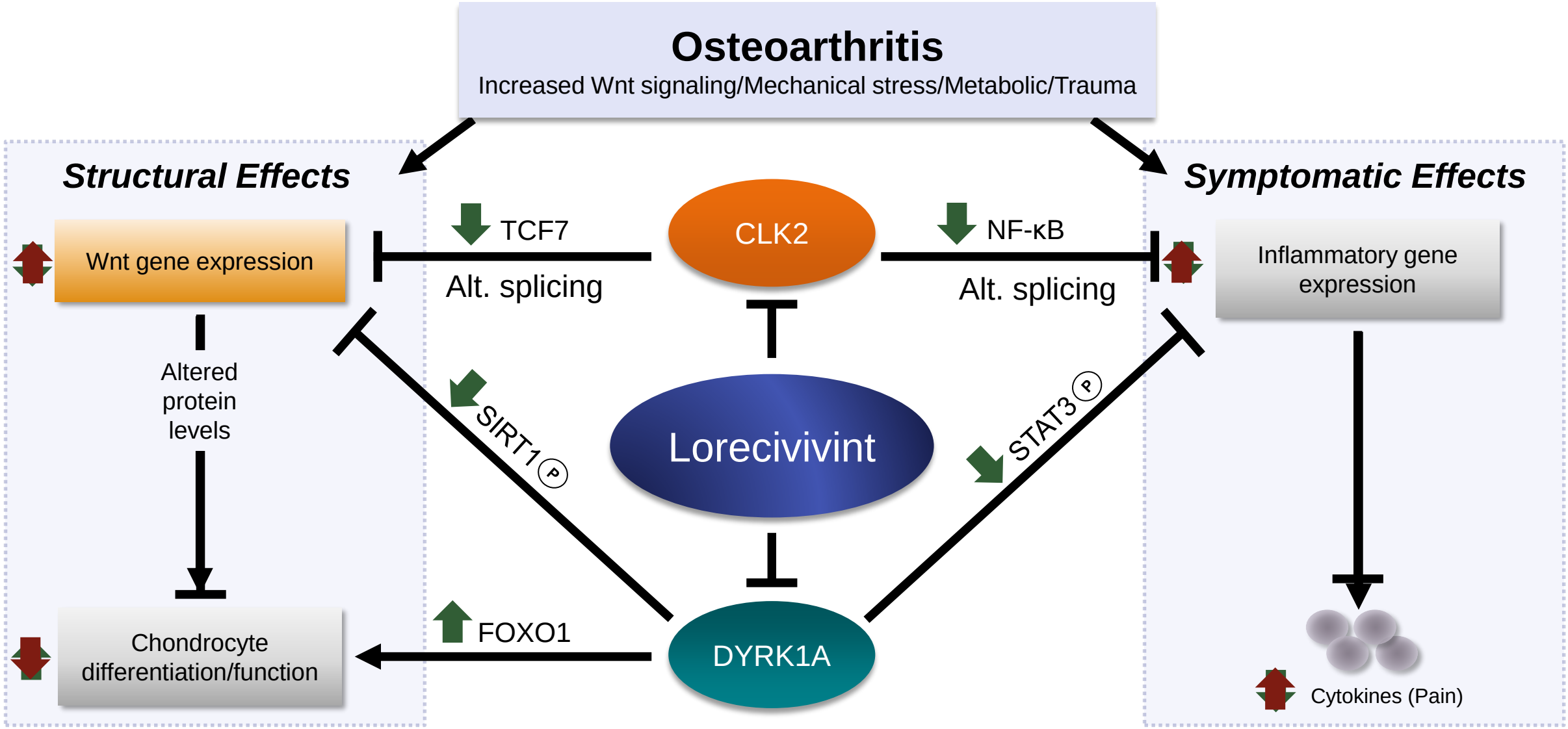


Inhibition of CLK2 and DYRK1A reduced inflammation



In vitro siRNA knockdown effects in BEAS-2B cells
Cytokines measured by qPCR
Mean ± SEM; **P*<0.05, ***P*<0.01, ****P*<0.001 vs. vehicle

LOR MOA



STAT3: Signal transducer and activator of transcription 3, SIRT1: Sirtuin 1, TCF7: Transcription factor 7, NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B cells, FOXO1: Forkhead box O1

LOR summary

- The intranuclear kinases CLK2 and DYRK1A, dual targets of LOR, are novel targets for modulation of Wnt signaling, chondrocyte biology, and inflammation
- LOR protected cartilage, induced chondrogenesis, and reduced inflammation *in vitro* and *in vivo*
- Phase 3 human clinical trials are ongoing

Thank you