

List of 10 most significant publications (in decreasing order of importance):

*Equal contribution

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1. Pradhan SJ, Reddy PC, Smutny M, Sharma A, Sako K, Oak MS, Shah R, Pal M, Deshpande O, Dsilva G, Tang Y, Mishra R, Deshpande G, Giraldez AJ, Sonawane M, Heisenberg CP#, **Galande S**#. (2021) Satb2 acts as a gatekeeper for major developmental transitions during early vertebrate embryogenesis. *Nat Commun.* 12:6094. doi: 10.1038/s41467-021-26234-7. PMID: 34667153

Zygotic genome activation (ZGA) initiates regionalized transcription underlying distinct cellular identities. A direct mechanistic link between the onset of ZGA and the tissue-specific transcription was unclear. In vertebrate embryos, pluripotency factors have been shown to provide transcriptional competence to activate the zygotic genome. However, how the precise timing of the onset of ZGA is controlled is unknown. More importantly, how changes mediated in the chromatin landscape during ZGA are channelized into patterns of gene expression during specification of cell types is less explored. In this study, we have addressed the molecular mechanisms underlying these intertwined processes. We have described a biphasic and bimodal requirement for the Satb2 during early embryonic development in zebrafish. Interestingly our study highlights the switch between functions of SATB2 from a repressor of transcription during ZGA to an activator of a special subset of neural crest progenitor cells during organogenesis. Analysis of the epigenetic landscape of zebrafish embryos revealed contrasting molecular activities of maternally deposited and zygotically synthesized Satb2. Maternal Satb2 prevents premature transcription of zygotic genes by influencing the interplay between pluripotency factors, while zygotic Satb2 activates transcription of the same group of genes during neural crest development and organogenesis. *satb2* mutant zebrafish recapitulate the corresponding mutant phenotypes in humans and mice.

2. Tripathi S., Gupta E., Naik N., Khare K., Mir R., Desai S., Humane S., Yadav S., Bal M., Saklani A., Patil P., Kamat S., and **Galande S**.# 2024. Statins attenuate Wnt/ β -catenin signaling by targeting SATB family proteins in colorectal cancer. *bioRxiv*. <https://www.biorxiv.org/content/10.1101/2024.08.23.609189v1>
(Under advanced stage of review in a reputed cancer journal)

Colorectal cancer is the second leading cause of cancer-related deaths worldwide, highlighting the need for improved treatments and advanced molecular research. A recent therapeutic approach focuses on repurposing drugs to target dysregulated pathways involved in tumorigenesis. Among these, statins, commonly known for lowering cholesterol, have attracted attention for their potential anti-cancer properties. Here, we provide direct

evidence for the same by assessing the impact of statin treatment on lipid, transcript, and protein levels. Our findings reveal that statins specifically target key components of the Wnt/ β -catenin pathway, a major factor in adenoma formation, including the SATB (Special AT-rich Binding protein) family proteins. While SATB1 is recognized as a regulator of tumorigenesis, particularly under Wnt signaling, SATB2 appears to exert an opposing role. We demonstrate that statin treatment reciprocally alters the expression pattern of these proteins. Furthermore, a human clinical trial evaluating statins as an anti-cancer therapy supports the hypothesis that differential expression of SATB proteins is crucial in tumorigenic outcomes. In conclusion, this modulation by statin treatment suggests promising new therapeutic avenues through drug repurposing.

3. Sharma A., Dsilva G.J., Deshpande G.# and **Galande S.#** 2024. Exploring the Versatility of Zygotic Genome Regulators: A Comparative and Functional Analysis. *Cell Reports* 43: 1-15. <https://doi.org/10.1016/j.celrep.2024.114680>.

Zygotic genome activation (ZGA), a vital conserved process, is modulated by a multitude of regulators that are not conserved and yet achieve a similar outcome. Recent observations have revealed how diverse mechanisms are employed by different proteins to achieve this shared goal in a variety of model systems. Miraculous journey leading up to ZGA is strewn with diverse twists and turns, with different facilitators as well as stumbling blocks, in the form of activators and suppressors. These are either maternally deposited or zygotically expressed and display a multitude of activities. Recent findings underscore the complexity of the regulatory networks involved in orchestrating ZGA, illustrating the adaptive nature of biological systems in employing multiple safeguard mechanisms to ensure accurate and reproducible developmental progression. In this systematic review we explored various molecular strategies that ZGA regulators have adopted, as documented in recent research discoveries. We show that across the evolutionary scale, such unique checks and balances ensure the homeostasis underlying the conserved outcome consisting of exquisite form and robust function.

4. Patta I, Madhok A, Khare SP, Gottimukkala KP, Verma A, Giri S, Dandewad V, Seshadri V, Lal G, Misra-Sen J and **Galande S#** (2020), Dynamic regulation of chromatin organizer SATB1 via TCR induced alternative promoter switch during T-cell development. *Nucleic Acids Res* 48:5873-5890. PMID: 32392347.

SATB1 is expressed in a lineage-specific manner in CD4⁺ T-cells. Analysis of RNA-seq data revealed multiple transcription start sites at the upstream regulatory region of SATB1. We further demonstrated that SATB1 gene is expressed via alternative promoters during T-helper (Th) cell differentiation. Thus, the promoter switch might play a crucial role in fine-tuning of SATB1 protein expression in a cell type-specific manner. While exploring the

mechanism(s) regulating the expression of SATB1 during T-cell development, four alternative promoters of *Satb1* in mouse thymocytes were predicted by ChIP-seq for H3K4me1/3 and their transcript variants were characterized. The discrepancy between the expression levels of SATB1 mRNA and protein in developing thymocytes was explainable by the differential translatability of *Satb1* transcript variants. Thus, we have discovered a new layer of regulation of gene expression that is post-transcriptional. The selective expression of a combination of *Satb1* transcript variants during T-cell development plays a crucial role toward the regulation of SATB1 protein levels in vivo. A novel mechanism for SATB1 regulation by a TCR signal-mediated alternative promoter switch was thus uncovered. This is a seminal finding in T cell biology.

5. Suresh V. *, Muralidharan, B. *, Pradhan, S. J. *, Bose, M., D'Souza, L., Parichha, A., Reddy, P. C., **Galande, S.**[#] and Tole, S.[#] 2023. Regulation of chromatin accessibility and gene expression in the developing hippocampal primordium by LIM-HD transcription factor LHX2. *PLoS Genetics* 19(8): e1010874. <https://doi.org/10.1371/journal.pgen.1010874>.

In the developing mouse brain, co-workers from Galande and Tole lab examined the hippocampal primordium (Hcp) and the adjacent neocortical primordium (Ncp) at embryonic day (E)12.5, when both structures predominantly contain progenitors and also contain newborn postmitotic neurons. We found that the Hcp displays strikingly distinct gene expression and chromatin accessibility from the Ncp. The Hcp chromatin is more accessible at over 14,000 loci compared with the Ncp, while the Ncp chromatin is more accessible at only 70 loci. We examined the transcription factor (TF) binding motifs on the DARs and compared these with TFs expressed in both tissues. TF LHX2 emerged as the top candidate that met these conditions. Loss of *Lhx2* selectively affected the accessibility of chromatin in the Hcp but not the Ncp. The majority of the DARs that displayed increased accessibility upon loss of *Lhx2* were also occupied by *Lhx2*. Transcriptomically, the Hcp displayed dysregulation of pathways related to DNA conformation, recombination, repair, replication, and organization. Comparing LHX2 occupancy with differentially expressed genes (DEGs) upon loss of *Lhx2*, we identified 4 key pathways dysregulated in the Ncp and Hcp: Wnt/ Hippo signaling, axon guidance, and pluripotency. Sequential filtering of the ChIP-seq, RNA-seq, and ATAC-seq data identified 14 genes that are controlled by *Lhx2* in the Hcp in terms of chromatin accessibility and mRNA expression. Some are known players in Ncp development, but none have thus far been examined in the Hcp. Our Hcp versus Ncp comparisons of chromatin accessibility, gene expression, and dysregulation upon loss of *Lhx2* offer a means to arrive at a mechanistic understanding of the range of distinct phenotypes in these structures when *Lhx2* is lost at different stages of development.

6. Mir R, Pradhan SJ, Patil P, Mulherkar R and **Galande S**[#]. (2015) Wnt/ β -catenin signaling regulated SATB1 promotes colorectal cancer tumorigenesis and progression. *Oncogene*. 35:1679-1691.

The regulation and role of chromatin organizer SATB1 implicated in the development and progression of colorectal cancer is poorly understood. The expression of SATB1 was found to be induced upon hyperactivation of Wnt/ β -catenin signaling and repressed upon depletion of TCF7L2 (TCF4) and β -catenin using colorectal cancer cell lines and the APC-min mutant zebrafish *in vivo* model. SATB1 was found to be a novel target of Wnt signaling, with its increased expression regulating TCF7L2. Both SATB1 and TCF7L2 are essential for coordinated regulation of Wnt responsive genes. This provides new therapeutic possibilities for cancers driven by hyperexpression of SATB1/Wnt signaling

7. Khare, S.P. *, Madhok, A. *, Patta, I., Sukla, K.K., Wagh, V.V., Kunte, P.S., Raut, D., Bhat, D., Kumaran, K., Fall, C. and Tatu, U., Chandak G.R., Yajnik C. S. #, **Galande, S.** # 2023. Differential expression of genes influencing mitotic processes in cord blood mononuclear cells after a pre-conceptional micronutrient-based randomised controlled trial: Pune Rural Intervention in Young Adolescents (PRIYA). *Journal of Developmental Origins of Health and Disease*, 14: 437-448. doi: 10.1017/S204017442200068X.

In the Pune Maternal Nutrition Study, vitamin B12 deficiency was seen in 65% of pregnant women, folate deficiency was rare. Maternal total homocysteine concentrations were inversely associated with offspring birthweight, and low vitamin B12 and high folate concentrations predicted higher offspring adiposity and insulin resistance. These findings guided a nested preconceptional randomised controlled trial 'Pune Rural Intervention in Young Adolescents' (PRIYA). Intervention improved maternal pre-conceptional and in-pregnancy micronutrient nutrition. Gene expression analysis in cord blood mononuclear cells in 88 pregnancies revealed 75 differentially expressed genes between the B12 + MMN and placebo groups. Based on the role of B-complex vitamins in the synthesis of nucleotides and S-adenosyl methionine, and the roles of vitamins A and D on gene expression, we propose that the multi-micronutrient intervention epigenetically affected cell cycle dynamics. Neonates in the B12 + MMN group had the highest ponderal index. Follow-up studies will reveal if the intervention and the altered biological processes influence offspring diabetes.

8. Mahajan S., Sen S., Sunil A., Srikanth P., Marathe S.D, Shaw K., Sahare M., **Galande S**[#], and Abraham N.A.[#] 2023. Knockout of ACE2 receptors lead to morphological aberrations in rodent olfactory centers and dysfunctions associated

with sense of smell. *Frontiers in Neuroscience*, 17:1180868. doi: 10.3389/fnins.2023.1180868.

ACE2 KO mice were generated using CRISPR-Cas9 based genome editing tools. This was my contribution during the pandemic, we worked hard to generate an animal model system that was not available in India to study COVID Pathogenesis. Reduced thickness of olfactory sensory neuron layer, and a decrease in the cross-sectional area of glomeruli were observed in these mice. ACE2 KO mice exhibited slower learning of odor discriminations at the threshold levels and novel odor identification impairments ACE2 KO mice failed to memorize the pheromonal locations while trained on a multimodal task, implying the aberrations of neural circuits involved in higher cognitive functions. ACE2 KO mice exhibited slower learning of odor discriminations at the threshold levels and novel odor identification impairments. Further, ACE2 KO mice failed to memorize the pheromonal locations while trained on a multimodal task implying the aberrations of neural circuits involved in higher cognitive functions. Our results thus provide the morphological basis for the sensory and cognitive disabilities caused by the deletion of ACE2 receptors and offer a potential experimental approach to study the neural circuit mechanisms of cognitive impairments observed in long COVID.

9. Shah R, Sharma A*, Kelkar A*, Sengupta K, and **Galante S**°. 2021. A novel cis regulatory element regulates human XIST in CTCF-dependent manner. *Mol Cell Biol*, 41(8):e0038220. doi: 10.1128/MCB.00382-20DOI: <https://doi.org/10.1128/MCB.00382-20>

X chromosome inactivation is initiated in diverse ways in different species. A cis regulatory element (cRE) within exon 1 of human *XIST* was identified and functionally characterized. In the initiation phase, pluripotency factors bind to this cRE and keep *XIST* repressed, while the same is enriched for CTCF in the maintenance phase, activating *XIST* transcription. A CRISPR-dCas9-KRAB-based interference approach targeting the cRE corroborated the significance of its manifesting transcriptional regulation of *XIST* in a CTCF- and YY1-dependent manner. A multi-component regulatory network involving pluripotency factors, YY1, and CTCF that shapes the transcriptional outcome from the *XIST* promoter was thus uncovered.

10. Reddy PC, Gungi A, Ubhe S, Pradhan SJ, Kolte A, **Galante S**°. (2019) Molecular signature of an ancient organizer regulated by Wnt/ β -catenin signalling during primary body axis patterning in Hydra. *Commun Biol*. 2:434. doi: 10.1038/s42003-019-0680-3. eCollection 2019.

As I am interested in understanding the evolution of epigenetic regulation and integration of the same with cellular signaling pathways, we chose to

study these processes using *Hydra*, one of the earliest multicellular eukaryotes, as a model system. Wnt/ β -catenin signaling is crucial for head organizer formation in *Hydra*. In this study, we explored the regulatory network involved and found that Wnt signaling controls genes important for tissue development. Most of the transcription factors (TFs) regulated by Wnt/ β -catenin are from the homeodomain and forkhead families. Silencing *Margin*, a Wnt-regulated homeodomain TF, prevents the ectopic tentacle growth usually caused by Wnt activation. We also discovered that β -catenin directly binds to and controls the *Margin* promoter. When *Margin* is overexpressed in zebrafish embryos, it causes body axis defects, indicating its role in axis patterning in higher eukaryotes. These findings suggest that homeobox TFs became regulated by Wnt/ β -catenin signaling, which likely contributed to the evolution of primary body axes in animals.