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Supplementary information

Global hyperactivation of enhancers stabilizes human and mouse naive pluripotency through inhibition of CDK8/19 Mediator kinases

In the format provided by the authors and unedited

Supplementary Information

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Supplementary Information: Page#1:
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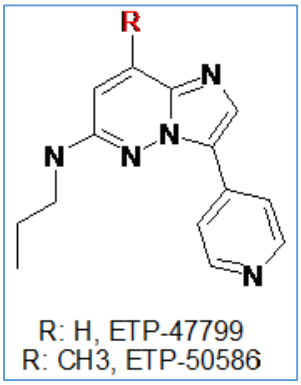
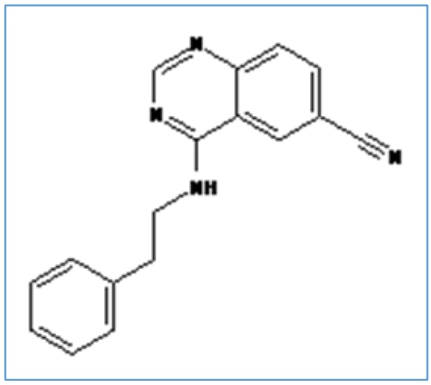


CDK8/19i = ETP-47799

This study;
CNIO Experimental Therapeutics Programme

CDK8/19-inhibitor ETP-47799 253.3 g/mol
-referred to throughout this study as “CDK8/19i”

CDK8/19-inhibitor ETP-50586 267.63 g/mol
-used for Kinome Analysis in Suppl Information page 2.

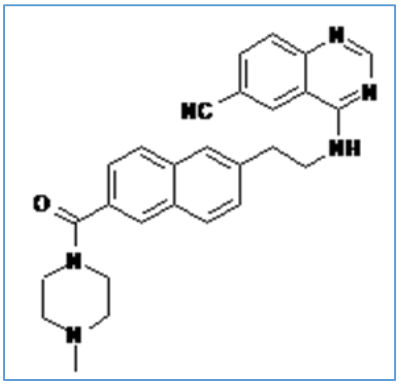


SnxA; Senexin A

Porter et al., 2012, PNAS;
Ref: 83

CDK8/19-inhibitor

320.0 g/mol

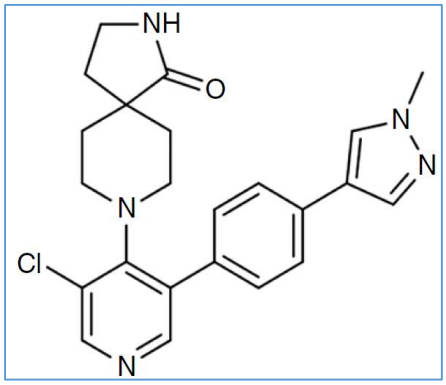


SnxB; Senexin B

Porter et al., 2012, PNAS;
Ref: 83

CDK8/19-inhibitor

370.0 g/mol



CCT251545

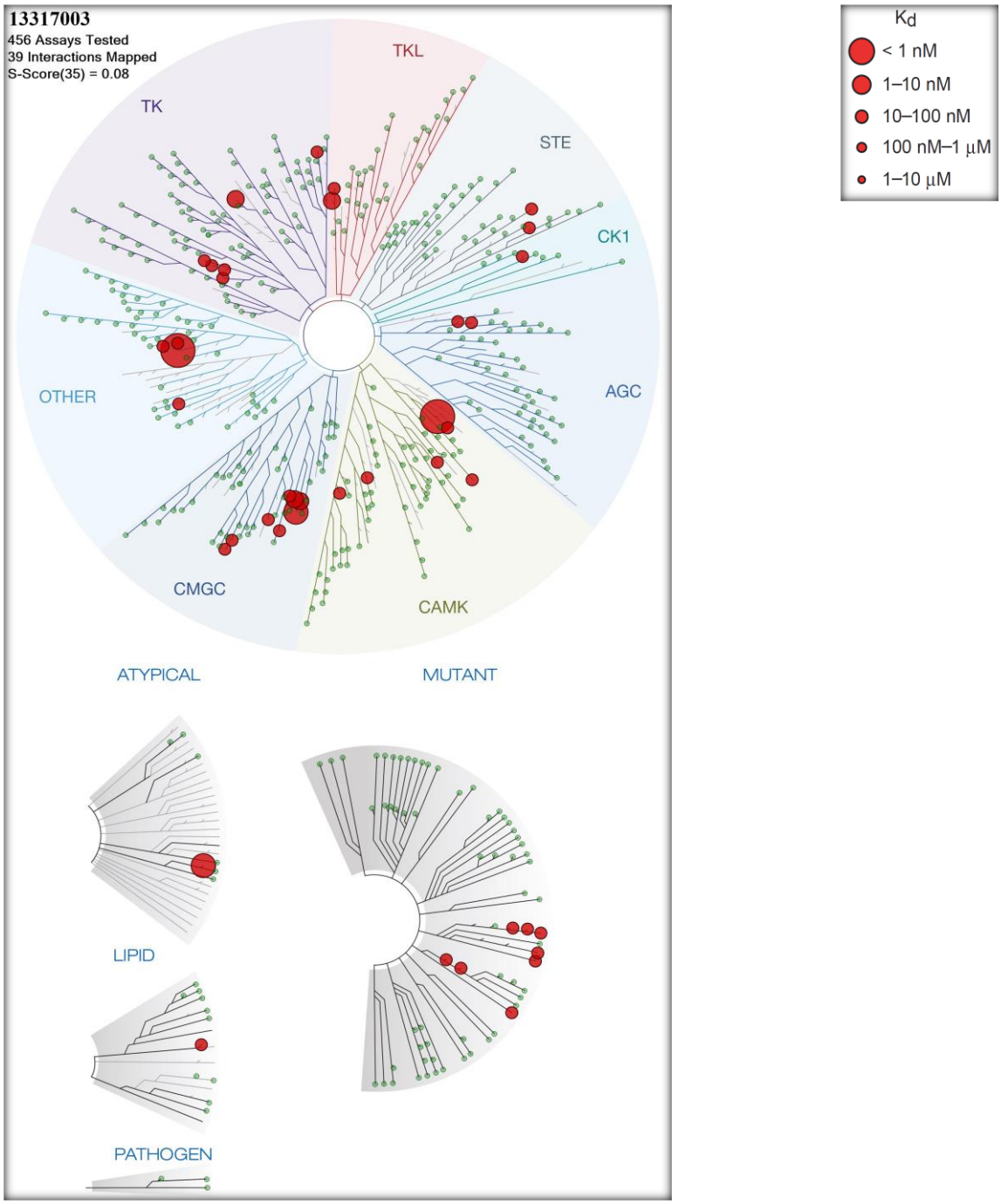
Dale et al., 2015, Nat Chem Biol;
Ref: 31

CDK8/19-inhibitor

421.91g/mol

Structural details of four CDK8/19 inhibitors used in this study. These four CDK8/19 inhibitor molecules differ in structure, molecular weight, and putative off-target effects (see Supplementary Table 1 for full characterization). However, they display high inhibitory action against CDK8/19, and they all improve PSC culture (see notes in Supplementary Table 1 for concentrations used, inhibitor assays, and observations in PSC culture).

Kinomescan: in vitro high-throughput screen of inhibitor specificity. Kinomescan results are shown in a screen of 456 known human kinases.



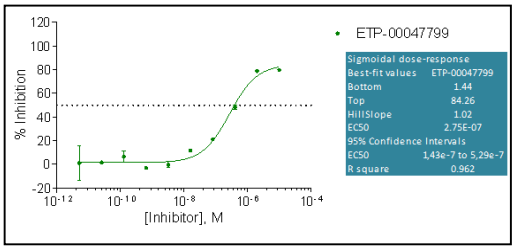
Kinomescan: in vitro high-throughput screen of inhibitor specificity. Kinomescan results are shown in a screen of 456 known human kinases. S = Selectivity Score = 0.08. Estimated Kd is shown for potential hits in a red dot, with scale shown on right. The 456 human kinases tested, their Kd, and their percent remaining activity in the presence of 1μM of CDK8/19 inhibitor CNIO-ETP -50586 (relative to the inhibitor-free control at 100%), are listed in **Supplementary Table 1**.

Note, the Kinomescan does not include the presence of partner proteins like Cyclins, in the assays which examine CDK function. The highest off-target hits for kinases most sensitive to the inhibitor were studied further and ruled-out by checking if these kinases were expressed in PSCs, and if expressed, selective inhibitors for the off-targets were tested in PSC culture (for example Imanitib and CLKi, see **Supplementary Table 1**), or ruled-out by directly testing with recombinant proteins in-house or by using alternate CDK8/19 inhibitors SenexinA, SenexinB, or CCT251545 (see **Supplementary Table 1**).

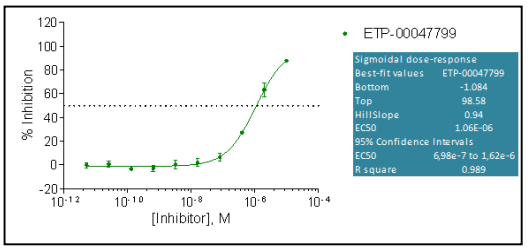
Kinomescan: https://www.discoverx.com/technologies-platforms/competitive-binding-technology/kinomescan-technology-platform.

Supplementary Information: Page#3:
Inhibitor kinetic data to assess specificity of CDK8/19i-CNIO-47799. Related to **Supplementary Table 1**.

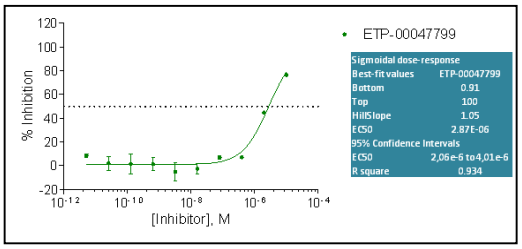
CDK1/CyclinB



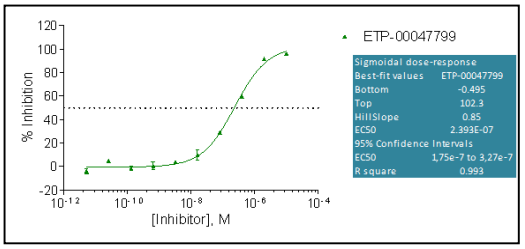
CDK2/CyclinA2



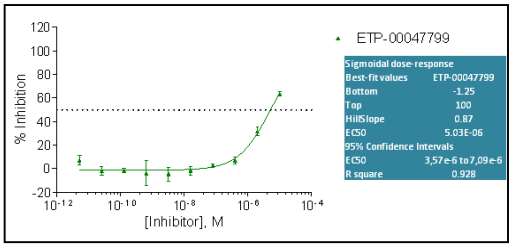
CDK4/CyclinD1



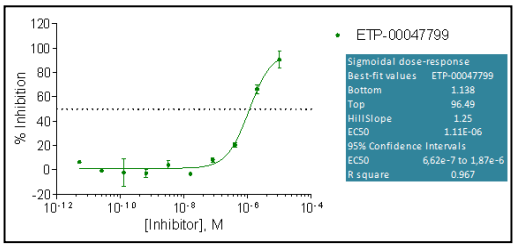
CDK5/p25



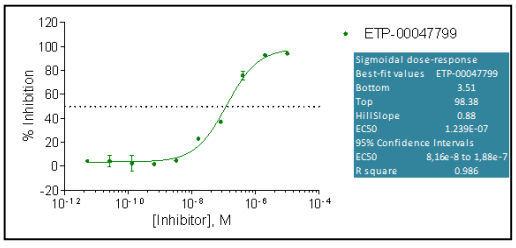
CDK6/CyclinD1



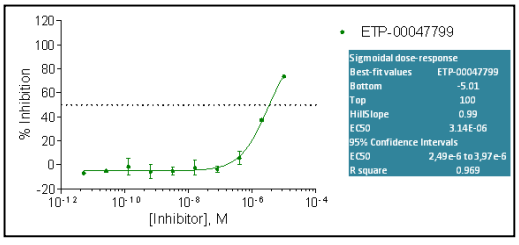
CDK7/CyclinH/MAT1



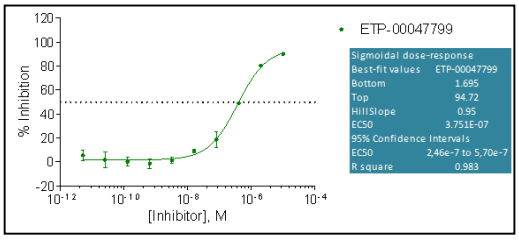
CDK9/Cyclin T1



GSK3Bβ



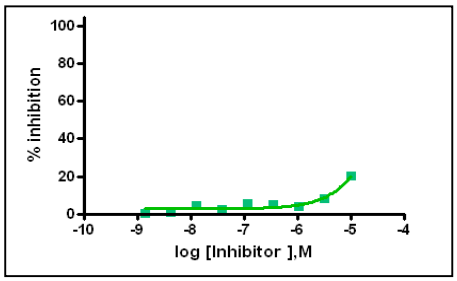
DRYK1A



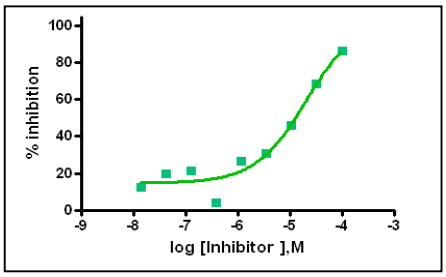
Inhibitor kinetic data to assess specificity of CDK8/19i-CNIO-47799 versus potential off-targets suggested by Kinomescan with related compound CDK8/19i-CNIO-50586 (see page 1,2) but which are ruled out here by weak inhibition or in the additional assays in **Supplementary Table 1**. Examples are shown of inhibitory kinetics of the CDK8/19i-CNIO-47799 compound versus the indicated recombinant protein kinases, as assessed by Lanthascreen Technology. Data are Mean+/-SD of n=3 technical replicates. See also: **Methods**.

Inhibitor kinetic data to assess specificity of CDK8/19i-CNIO-47799. Related to **Supplementary Table 1**.

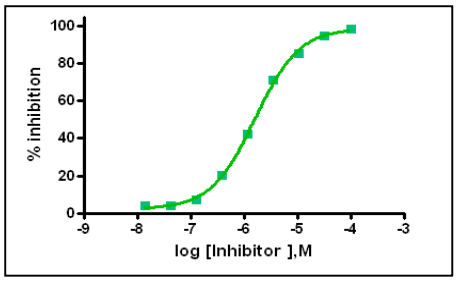
mTOR



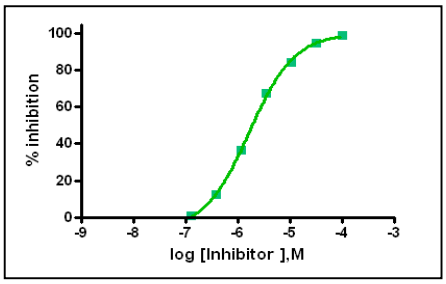
PI3Kα



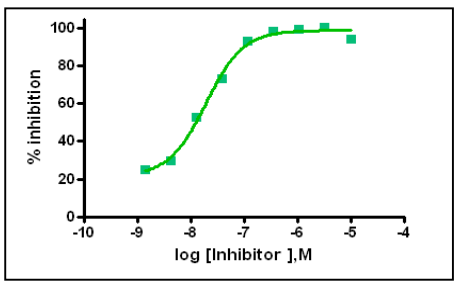
PIM1



PIM2

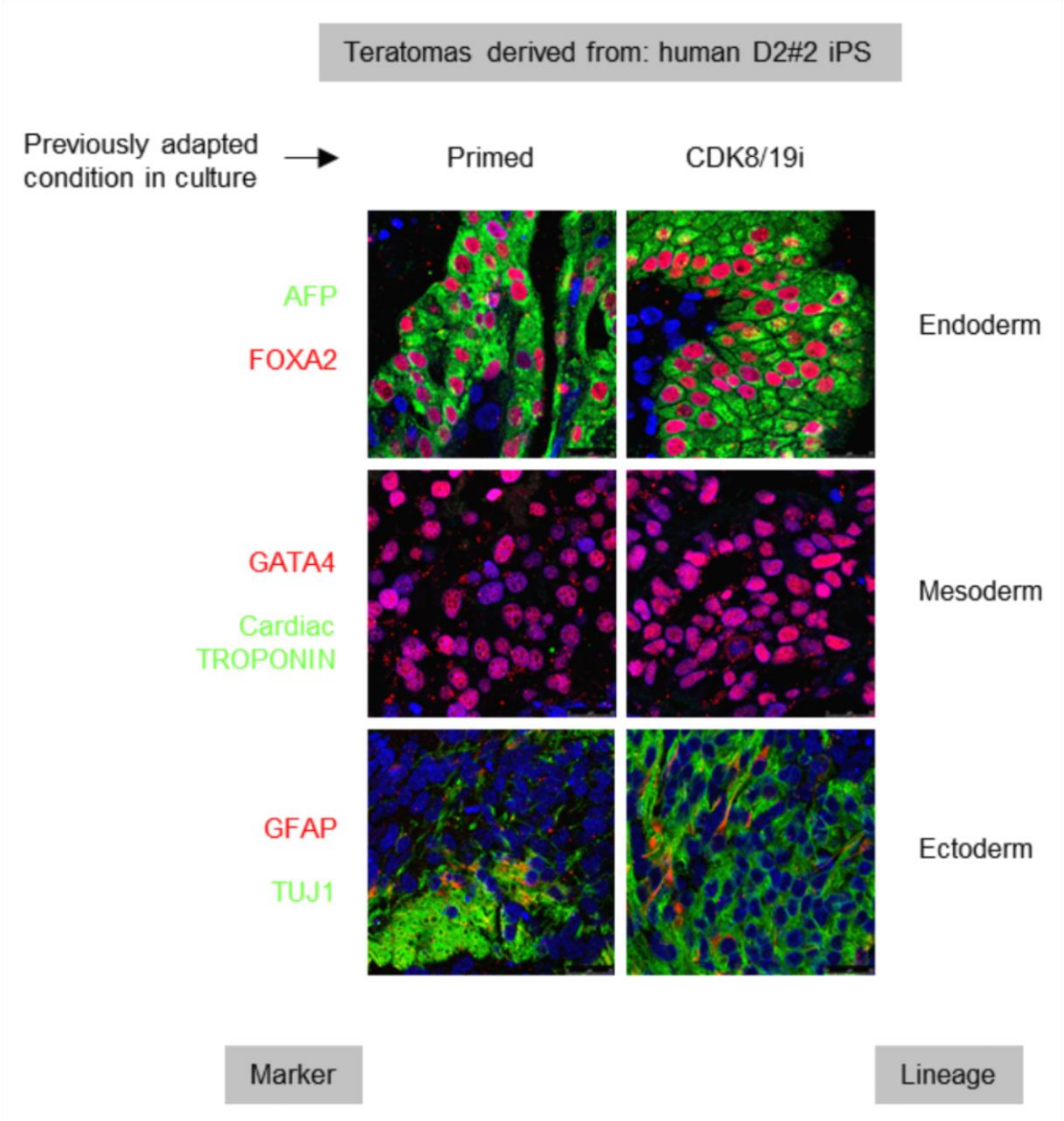


FLT3



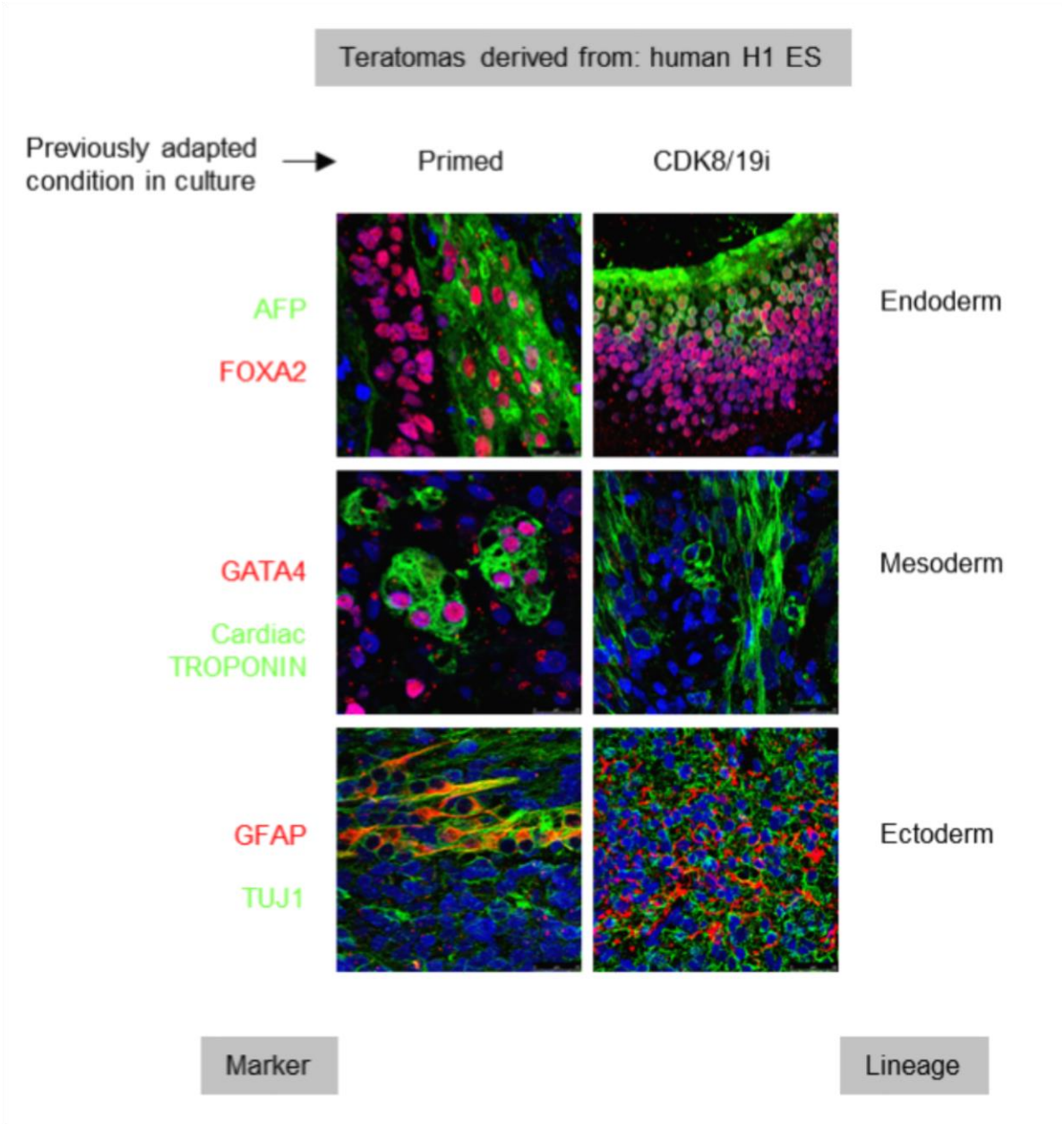
Inhibitor kinetic data to assess specificity of CDK8/19i-CNIO-47799 versus potential off-targets suggested by Kinomescan with related compound CDK8/19i-CNIO-50586 (see page 1,2) but which are ruled out here by weak inhibition or in the additional assays in **Supplementary Table 1**. Examples are shown of inhibitory kinetics of the CDK8/19i-CNIO-47799 compound versus the indicated recombinant protein kinases, as assessed by Lanthascreen Technology. Data are Mean+/-SD of n=3 technical replicates. See also: **Methods**.

Supplementary Information: Page#5:
Examples of immunofluorescence staining for embryonic germ layer lineage markers in teratomas generated from the human PSC line: D2#2 (human iPS).



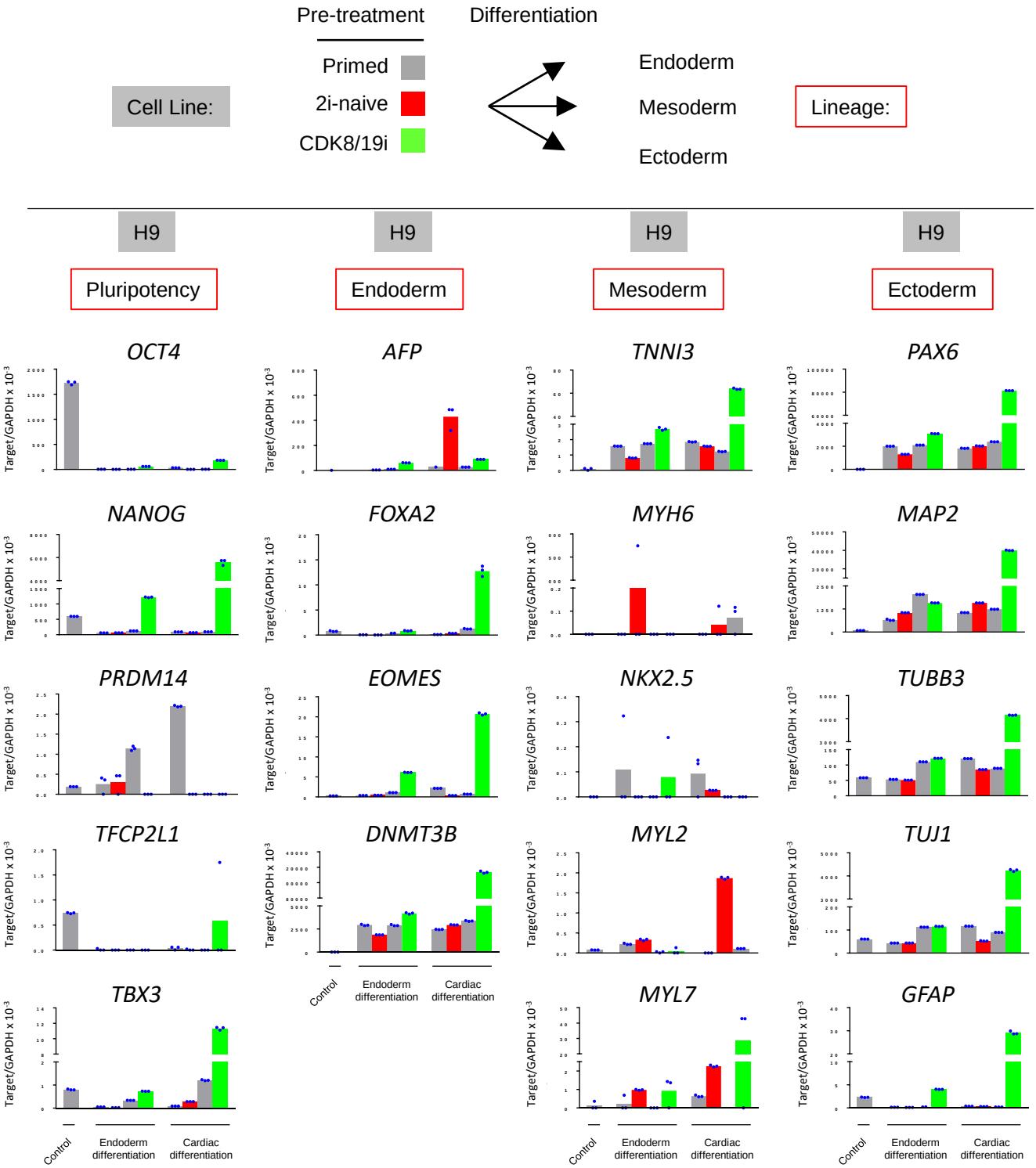
Examples of immunofluorescence staining for embryonic germ layer lineage markers in teratomas generated from the human PSC line: D2#2 (human iPS). PSCs were injected into nude mice testes and teratomas were collected after several weeks (see: Methods). 1 or 2 sections of each teratoma were stained for the indicated markers. See Supplementary Table 1 for summary of efficiency of teratoma generation and all lineage markers analyzed/detected in teratomas from H1 and D2#2 PSCs.

Supplementary Information: Page#6:
Examples of immunofluorescence staining for embryonic germ layer lineage markers in teratomas generated from the human PSC line: H1 (human ES).



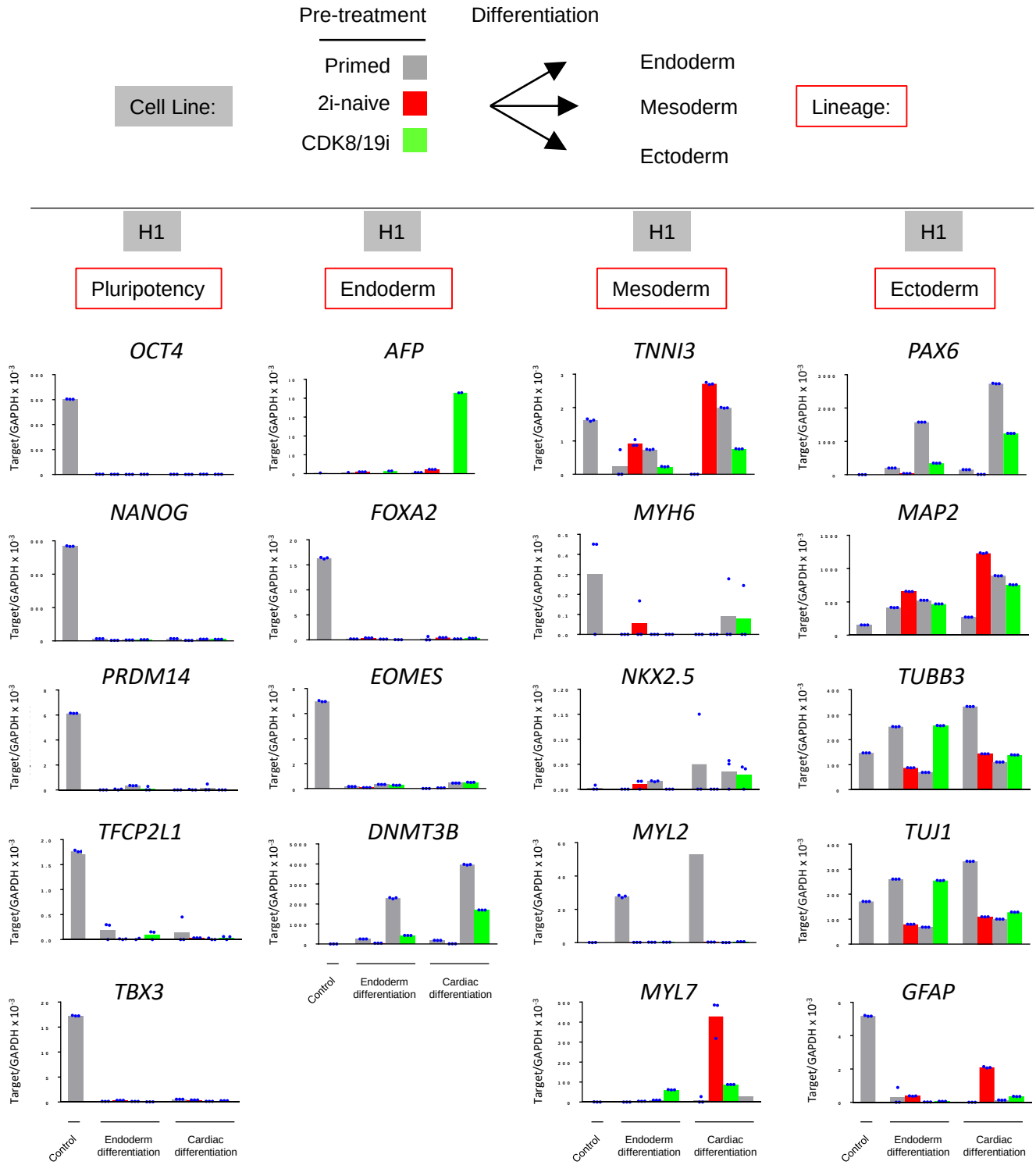
Examples of immunofluorescence staining for embryonic germ layer lineage markers in teratomas generated from the human PSC line: H1 (human ES). PSCs were injected into nude mice testes and teratomas were collected after several weeks (see: Methods). 1 or 2 sections of each teratoma were stained for the indicated markers. See Supplementary Table 1 for summary of efficiency of teratoma generation and all lineage markers analyzed/detected in teratomas from H1 and D2#2 PSCs.

Supplementary Information: Page#7:
Summary of human PSC-derived Embryoid Body analyses by qRT-PCR for embryo germ layer markers, shown per cell line and per culture condition. Related to **Fig.3B**



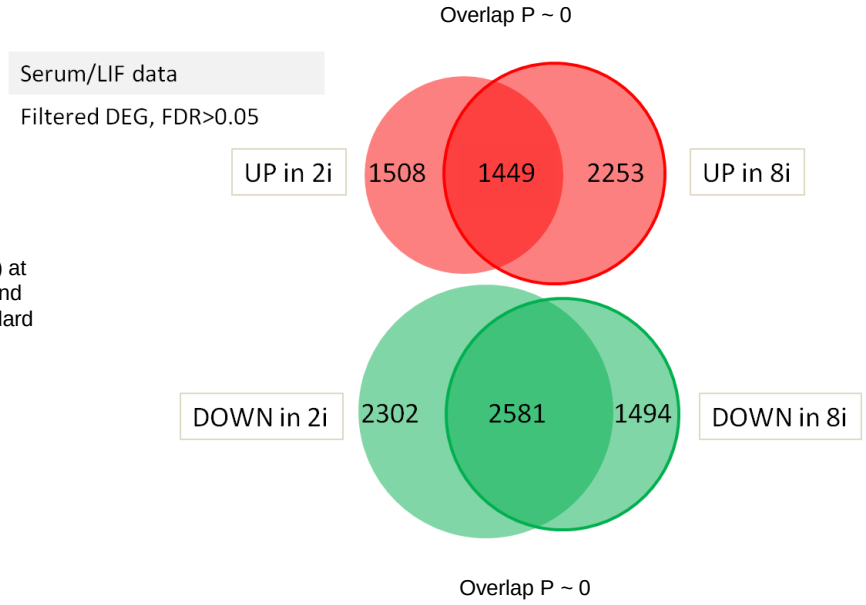
H9 Human PSCs were cultured to adapt to the indicated conditions, then the inhibitors were removed and differentiation initiated. Presence of markers of the 3 embryonic germ layers was detected by qRT-PCR. Control = undifferentiated primed state human PSCs. A specific neural-directed differentiation protocol was not performed, since EBs maintained in the same differentiation conditions used for endoderm or mesoderm also spontaneously gave rise to neural cell clusters. Note: DNMT3B is not a specific marker of any one lineage, but it is repressed in pluripotency and up-regulated upon PSC differentiation. Data: Mean of n=3 technical replicates indicated. See **Methods** for primers and differentiation protocols used. See also: **Source Data for Supplementary Information**.

Summary of human PSC-derived Embryoid Body analyses by qRT-PCR for embryo germ layer markers, shown per cell line and per culture condition. Related to **Fig.3B**

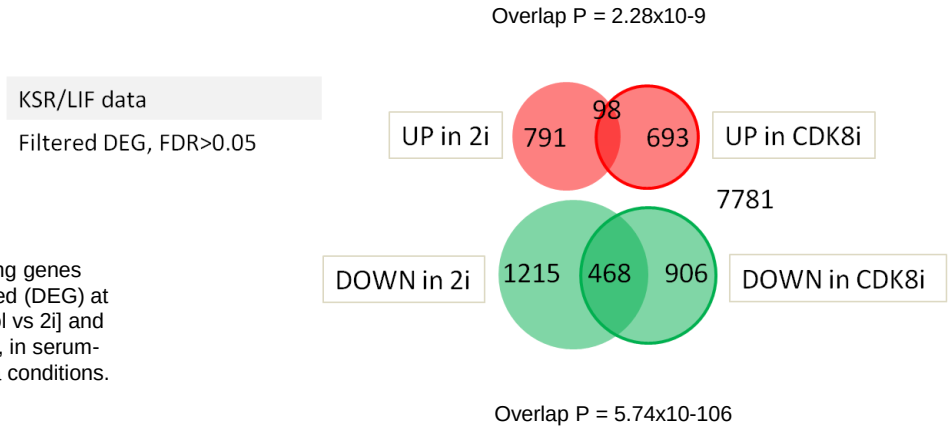


H1 Human PSCs were cultured to adapt to the indicated conditions, then the inhibitors were removed and differentiation initiated. Presence of markers of the 3 embryonic germ layers was detected by qRT-PCR. Control = undifferentiated primed state human PSCs. A specific neural-directed differentiation protocol was not performed, since EBs maintained in the same differentiation conditions used for endoderm or mesoderm also spontaneously gave rise to neural cell clusters. Note: DNMT3B is not a specific marker of any one lineage, but it is repressed in pluripotency and up-regulated upon PSC differentiation. Data: Mean of n=3 technical replicates indicated. See **Methods** for primers and differentiation protocols used. . See also: **Source Data for Supplementary Information**.

Supplementary Information: Page#9:
Venn diagram of overlapping genes in the comparisons indicated for inhibitors in standard control serum/LIF, or serum/free KSR/LIF based media. Related to **Supplementary Table 2**, and **Extended Data Fig.3F**.



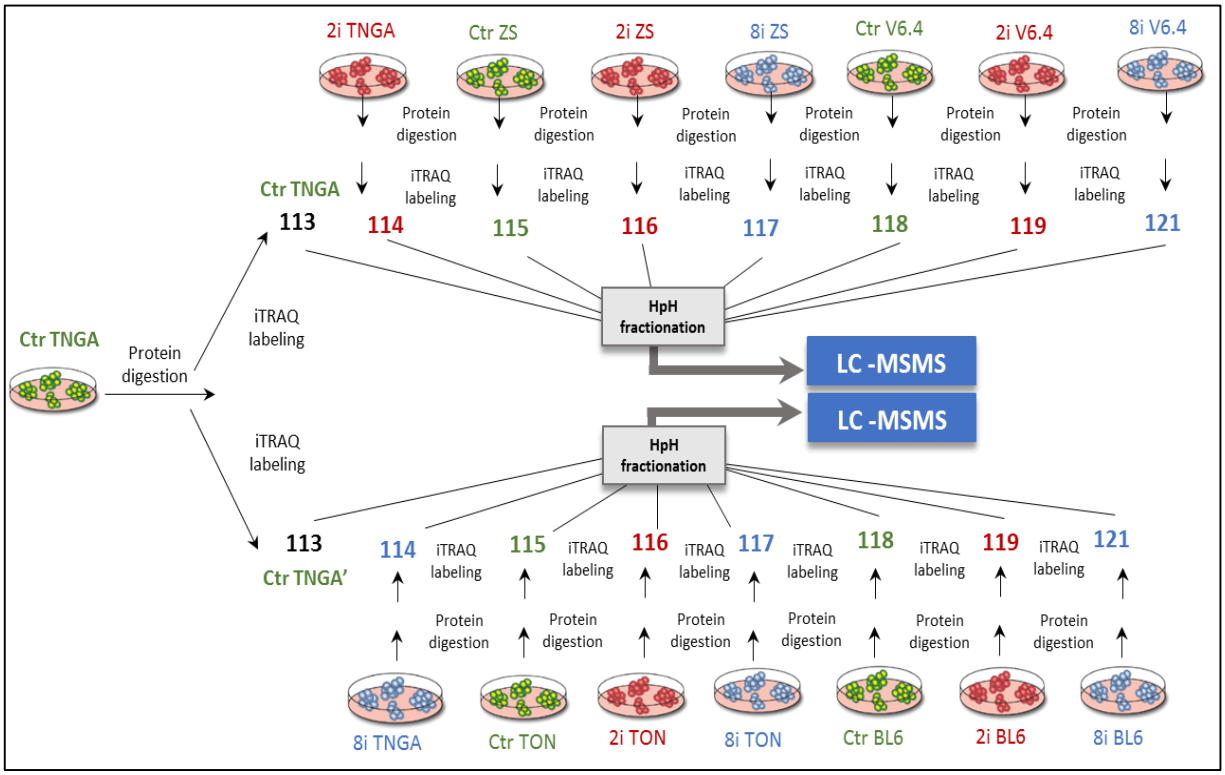
Venn diagram showing genes differentially expressed (DEG) at FDR<0.05, in [Control vs 2i] and [Control vs Cdk8/19i], in standard serum/LIF media conditions.



Venn diagram showing genes differentially expressed (DEG) at FDR<0.05, in [Control vs 2i] and [Control vs Cdk8/19i], in serum-free/LIF (KSR) media conditions.

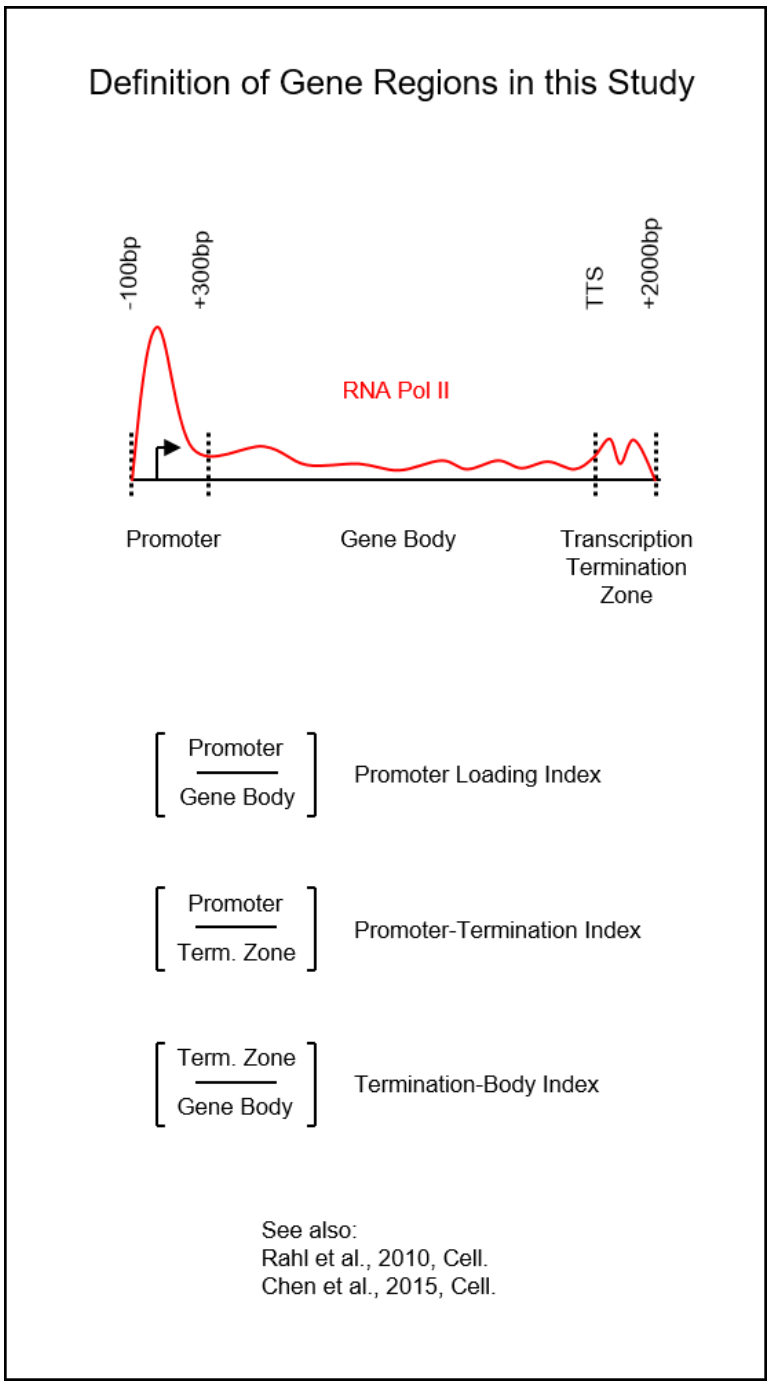
The differentially expressed genes were first selected in 2i or CDK8/19i versus the control condition: serum/LIF (lupper) or KSR/LIF (lower). Then the overlap of differentially expressed genes in 2i or CDK8/19i was compared. See **Supplementary Table 2, section #5**, for the list of genes in each region of the Venn diagrams. See **Supplementary Table 2** for the full list of genes detected and their differential expression in serum/LIF media. See **Source Data for Extended Data Fig.3F**, for the full list of genes detected and their differential expression in serum-free KSR/LIF media.

Supplementary Information: Page#10:
iTRAQ proteomic isobaric labelling scheme of samples in this study for iTRAQ8plex analysis.



To quantify multiple samples using iTRAQ 8-plex, two multi-plex experiments were performed in parallel. The organization of the samples is shown above to assist data re-analysis. Samples were organized in a labelling scheme such that each treatment (2i or CDK8/19i) was in the same analysis run as their control samples. TNGA control sample was added to both iTRAQ experiments. The complete labelling scheme is shown in Illustration above.

Schematic for the definition of genic regions in this study of RNA Pol II abundance.



Schematic for the definition of genic regions in this study of RNA Pol II abundance.. ChIP-seq reads were quantified for each gene region as shown in the schematic, and are listed for all RefSeq genes in columns in **Supplementary Table 6**. See also **Source Data** for calculations with ChIP-seq data in the **Figures 5,6**.