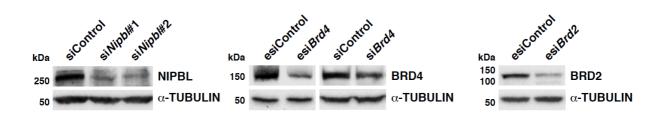
## The Cornelia de Lange Syndrome-associated factor NIPBL interacts with BRD4 ET domain for transcription control of a common set of genes

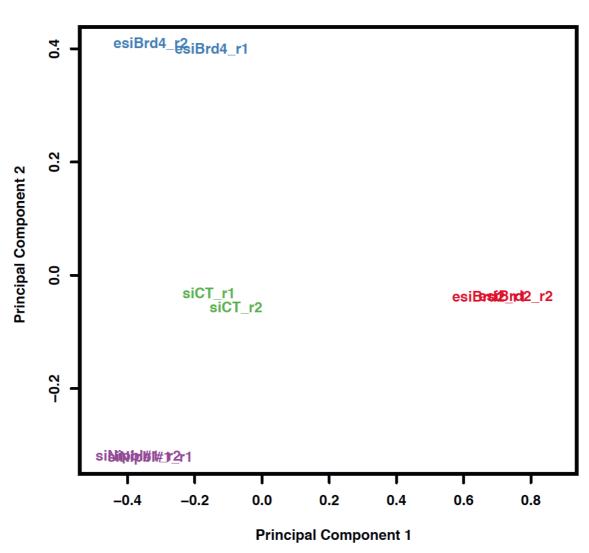
Noelia Luna-Peláez<sup>1</sup>, Rosana March-Díaz<sup>1</sup>, María Ceballos-Chávez<sup>1</sup>, Jose A Guerrero-Martínez<sup>1</sup>, Paolo Grazioli<sup>2</sup>, Pablo García-Gutiérrez<sup>1</sup>, Thomas Vaccari<sup>3</sup>, Valentina Massa<sup>2</sup>, Jose C Reyes<sup>1</sup>, Mario García-Domínguez<sup>1</sup>\*

<sup>1</sup>Andalusian Center for Molecular Biology and Regenerative Medicine-CABIMER CSIC-Universidad de Sevilla-Universidad Pablo de Olavide. Av. Américo Vespucio 24, 41092, Seville, Spain <sup>2</sup>Dipartimento di Scienze della Salute, Università degli Studi di Milano, Via A. di Rudinì 8, 20142 Milano, Italy

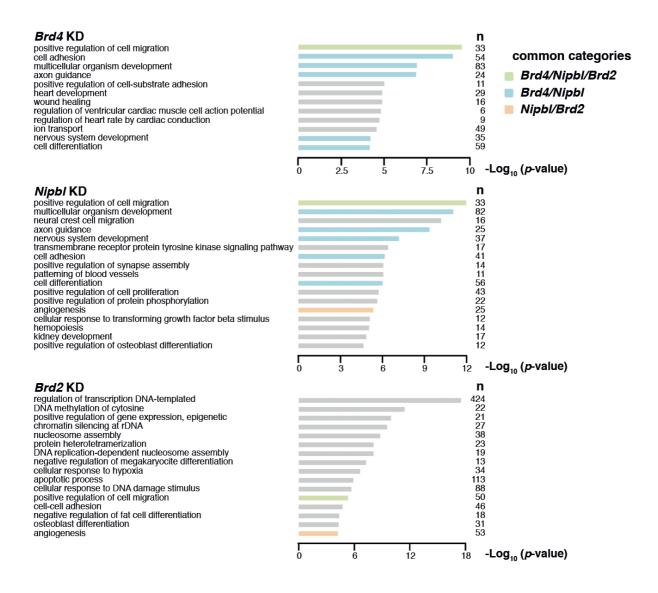
<sup>3</sup>Dipartimento di Bioscienze, Università degli Studi di Milano, Via Giovanni Celoria 26, 20133 Milano, Italy



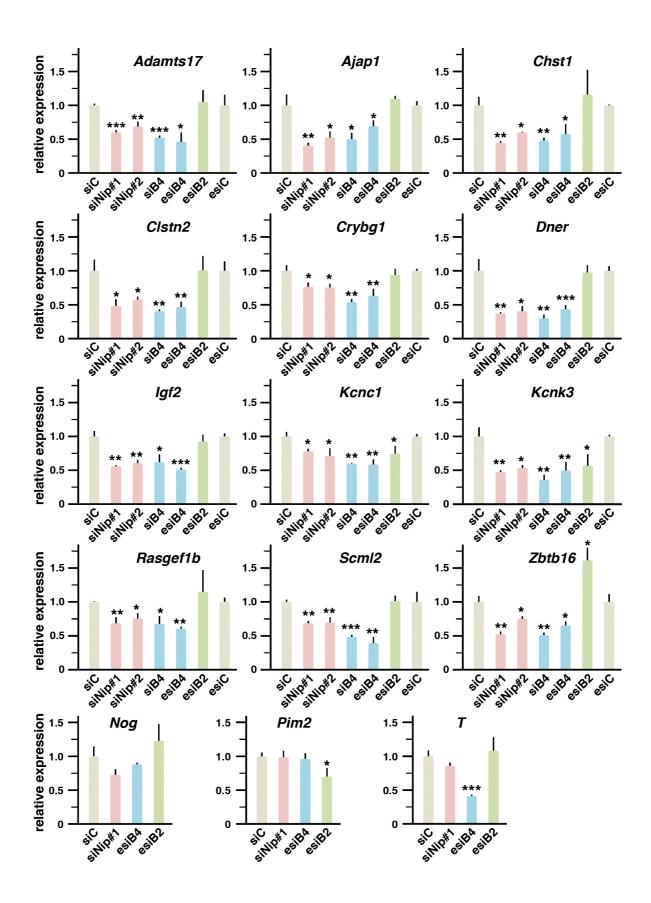
**Supplementary Figure S1** Assessment of the knockdown of *Nipbl*, *Brd4* and *Brd2*. Analysis by western blot of protein depletion upon transfection of the indicated siRNA (si) or esiRNA (esi) molecules. Twenty micrograms of total protein were loaded per lane.  $\alpha$ -TUBULIN was registered as a loading control.



**Supplementary Figure S2** Principal component analysis of RNA-Seq data. Principal component analysis (PCA) performed on TMM normalized RNA-seq expression data. Biological replicates are depicted in the same color.



**Supplementary Figure S3** NIPBL and BRD4 regulate a common set of genes involved in relevant developmental processes. Main GO categories comprising misregulated genes upon knocking down *Nipbl*, *Brd4* and *Brd2*. A *p*-value cut-off of 8.0 10<sup>-5</sup> was established for the different knockdown analysis. Common categories are marked in colours as follows: green, common categories of misregulated genes upon depletion of the three proteins; blue, common categories of misregulated genes upon depletion of NIPBL and BRD4; orange, common categories of misregulated genes upon depletion of NIPBL and BRD4. n indicates the number of genes in the different categories.



**Supplementary Figure S4** Expression analysis validates RNA-Seq data for a selection of genes. Twelve downregulated genes under NIPBL or BRD4 depletion conditions and 3 control genes were assessed for gene expression through qPCR after transfection of the indicated siRNA and esiRNA molecules. siC, Control siRNA; siNip#1, *Nipbl* siRNA #1; siNip#2, *Nipbl* siRNA #2; siB4, *Brd4* siRNA; esiB4, *Brd4* esiRNA; esiB2, *Brd2* esiRNA; esiC, Control esiRNA. Relative levels of expression are represented. Values are means  $\pm$  s.d. of 3 independent experiments analyzed in triplicate. Statistical significance of each condition in comparison with the corresponding control was analyzed by Student's t-test (\*p< 0.05, \*\*p< 0.01, \*\*\*p< 0.001).