

A long-range enhancer at -52 kb drives expression of the COUP-TFII transcription factor in erythroid cells

Martina Fabiano¹, Valentina Pastori¹, Agata Labeledz^{1,7,8}, Maria Simanovich², Carlotta Frigo¹, Thijs Verheul², Ludovica Proietti^{3,9}, Florian Grebien^{3,4,5}, Elisabetta Citterio^{1,6}, Sjaak Philipsen^{2#} and Antonella Ellena Ronchi^{1#}

E-mail: martina.fabiano@unimib.it

¹Dipartimento di Biotecnologie e Bioscienze, Università degli Studi di Milano-Bicocca, Milan, Italy

²Erasmus MC Department of Developmental Biology, P.O. Box 2040, 3000CA Rotterdam, The Netherlands

³Centre of Biological Sciences, University of Veterinary Medicine Vienna, Austria

⁴St. Anna Children's Cancer Research Institute (CCRI), Vienna, Austria

⁵CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria.

⁶Department of Life Science, Health, and Health Professions, Link Campus University, Rome, Italy

⁷Present address: International Centre for Translational Eye Research, Warsaw, Poland

⁸Present address: Institute of Physical Chemistry, Polish Academy of Sciences, Warsaw, Poland

⁹Present address: Computational Biology Research Centre, Human Technopole Milan, Italy

Co-*Corresponding Authors*

Keywords: (Erythropoiesis, orphan nuclear receptor, COUP-TFII, hemoglobin switching)

Abstract:

COUP-TFII, encoded by the *NR2F2* gene, is an orphan nuclear receptor highly expressed during embryonic development in several tissues. In erythropoiesis, COUP-TFII is active in yolk sac-derived cells prior to the switch to adult globin expression. Its broad expression pattern suggests a complex transcriptional regulation involving multiple, yet poorly defined, regulatory elements.

Using integrative *in silico* and chromatin accessibility analyses, here we identified an erythroid-specific enhancer located 52Kb upstream of the *NR2F2* transcription start site. This element shows epigenetic features of an active enhancer in K562 erythroid cells. Notably, in subclones derived from adult HUDEP2 erythroid progenitor cells that spontaneously re-express fetal gamma globin, *NR2F2* is reactivated, concomitantly with the opening of the -52Kb enhancer. We also identify the transcription factor ZBTB7A as a repressor of *NR2F2*, as knock-out of *ZBTB7A* in HUDEP2 cells restores *NR2F2* expression and active chromatin marks at the -52Kb region. Our findings uncover a novel distal enhancer controlling *NR2F2* expression in erythroid cells.