Long Noncoding RNA Interactions as Functional Determinants in Stem Cells

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Abstract

Long noncoding RNAs (lncRNAs) are key regulators of many cellular functions in developmental and disease processes. They can adopt diverse structures, which facilitates their cooperation with transcriptional, translational and signaling regulators that are determinants of cell fate. Many lncRNAs have been implicated in stem cell maintenance and differentiation, and yet precise mechanistic understanding of each individual function remains poorly defined. Additionally, the molecular interactions that mediate each lncRNA's function action are not well understood. We have studied the developmentally relevant lncRNA, Oip5os1 (aka Cyrano) and have shown that it supports gene expression network maintenance, cell adhesion and cell survival in embryonic stem cells. Oip5os1's interactome assessment revealed its participation in diverse molecular networks. These include a developmentally important cell-signaling hub, RNA regulatory networks, and other nuclear and cytoplasmic localizing hubs. We propose that these networks individually drive Cyrano's unique functions. These interactome data will also provide a useful resource for investigations into more general interactions that regulate lncRNA function.

Introduction

Much of the 'dark matter' of the genome is transcribed resulting in RNAs with no coding potential (Jacquier, 2009). Many of the resultant transcripts are large or long non-coding RNAs of greater than 200nt. Studies have begun to reveal the biological importance of these transcripts (Quinn and Chang, 2016). Many lncRNAs are dynamically regulated in development, display cell/tissue specific expression where they often localize to specific subcellular compartments, and multiple modes of function have been defined for lncRNAs (Chen and Carmichael, 2010, Rinn and Chang, 2012, Quinn and Chang, 2016).

The roles of many long non-coding RNAs remain unknown. Understanding how these RNAs function is the major goal of this project.
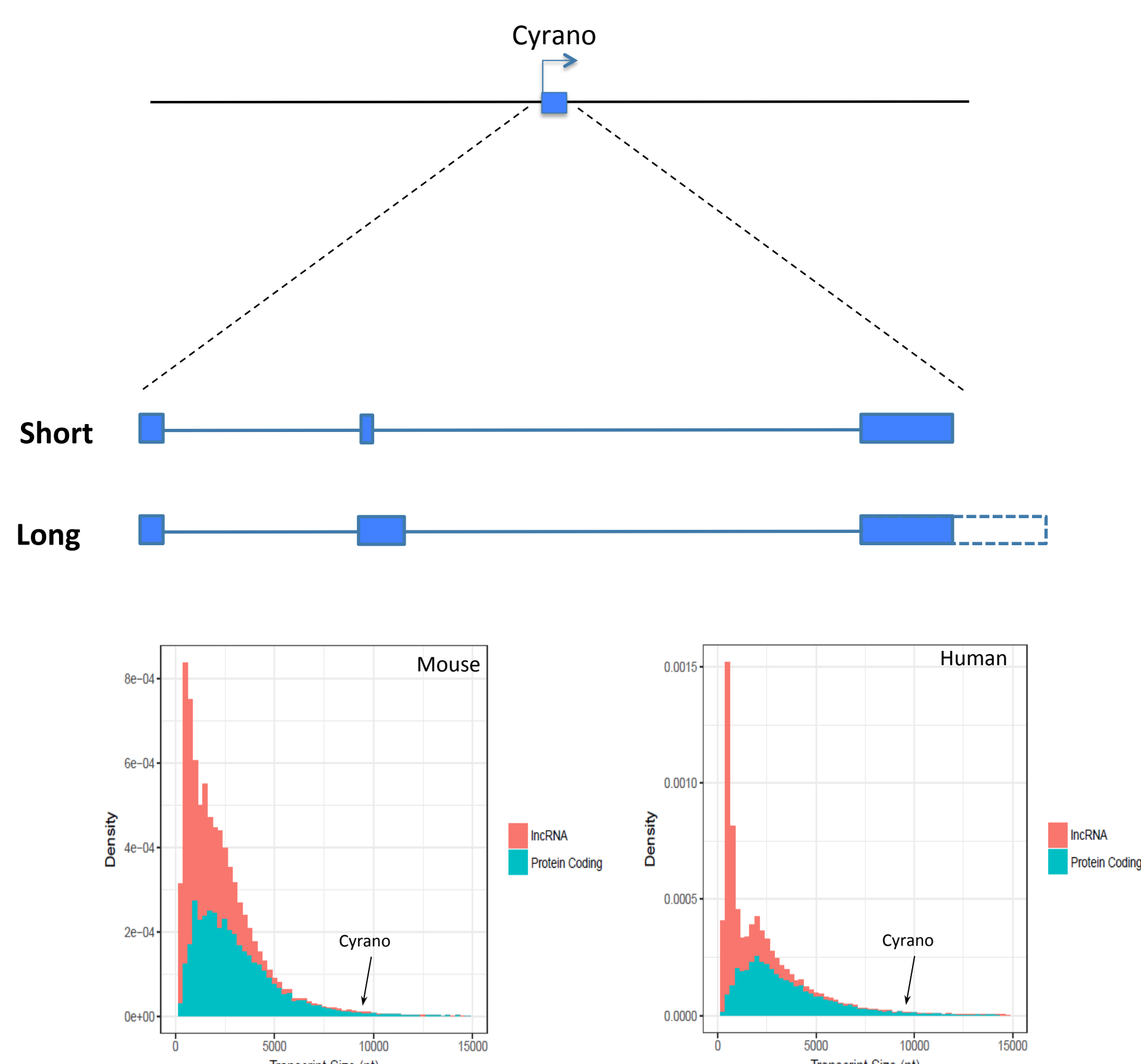

Figure 1. Primary Cyrano isoforms. (A) Schematic depicting gene structure of primary isoforms of mouse Cyrano as depicted in the UCSC genome browser. (B) Relative to other GENCODE annotated lncRNAs, Cyrano is a particularly long lncRNA. This is driven by the long terminal 3' exon.
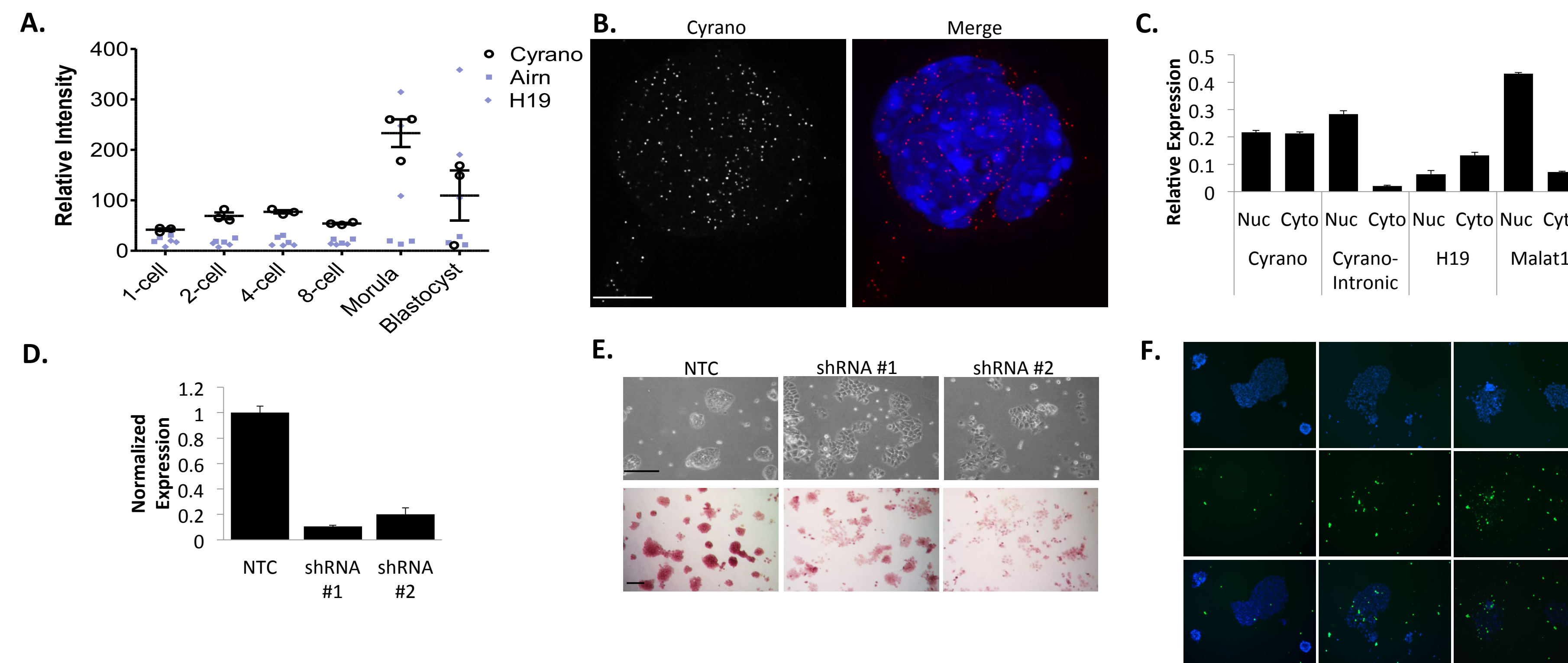
Results

Figure 2. _Characterization of Cyrano in the preimplantation embryo and ES cells._ The expression of Cyrano increases during preimplantation embryonic development (A), and it is distributed throughout the cytoplasm and nucleus of embryonic stem (ES) cells (B-C). (D) shRNA-mediated depletion of Cyrano results in loss of ES cell maintenance (E) and increases in cell death (F).
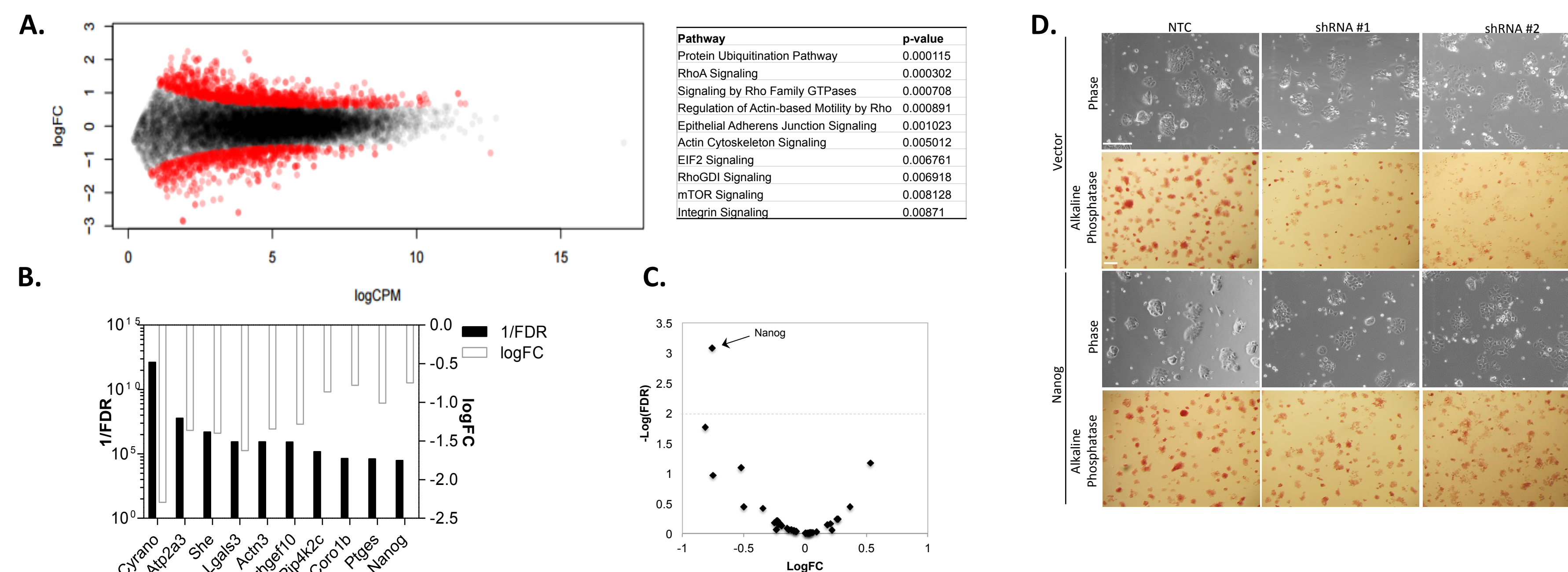

Figure 3. _Disruption of gene expression is associated with loss of self-renewal upon Cyrano loss._ (A) RNA-Seq analysis reveals differential gene expression upon Cyrano knockdown. (B-C) The ES cell regulator _Nanog_ is among the few master ES cell regulatory factors disrupted with Cyrano loss. (D) Nanog overexpression partially rescues the Cyrano LOF phenotype.
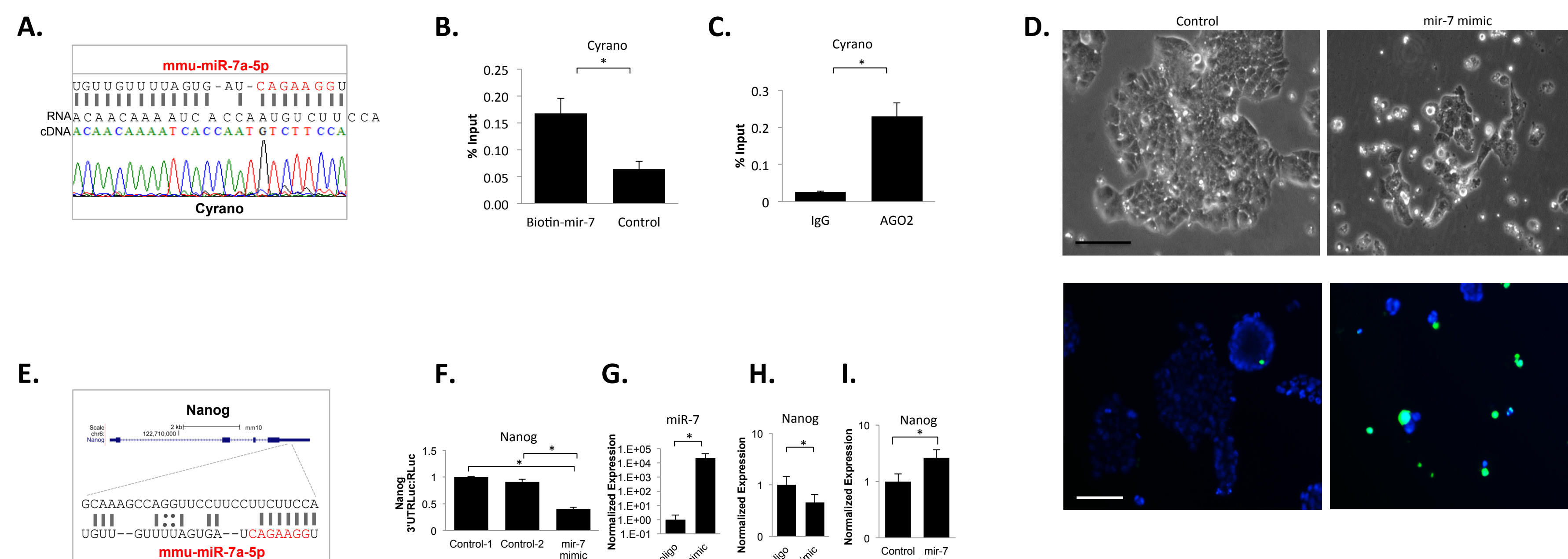

Figure 4. _Cyrano-miR-7 interaction impacts Nanog expression in mouse ES cells._ (A) The conserved _mir-7_ sequence is found in cDNA derived from mouse ES cells, and a physical interaction is observed in affinity purification experiments between Cyrano and _mir-7_ (B), and Cyrano and AGO2 (C). Overexpression of _mir-7_ in ES cells results in a similar phenotype as Cyrano LOF (D). (E) The _mir-7_ seed sequence is present in the 3'UTR of _Nanog_, and is regulable as observed in luciferase assays (F). Overexpression of _mir-7_ (G) results in decreases in _Nanog_ expression, while inhibition of _mir-7_ activity results in increases in _Nanog_ expression.
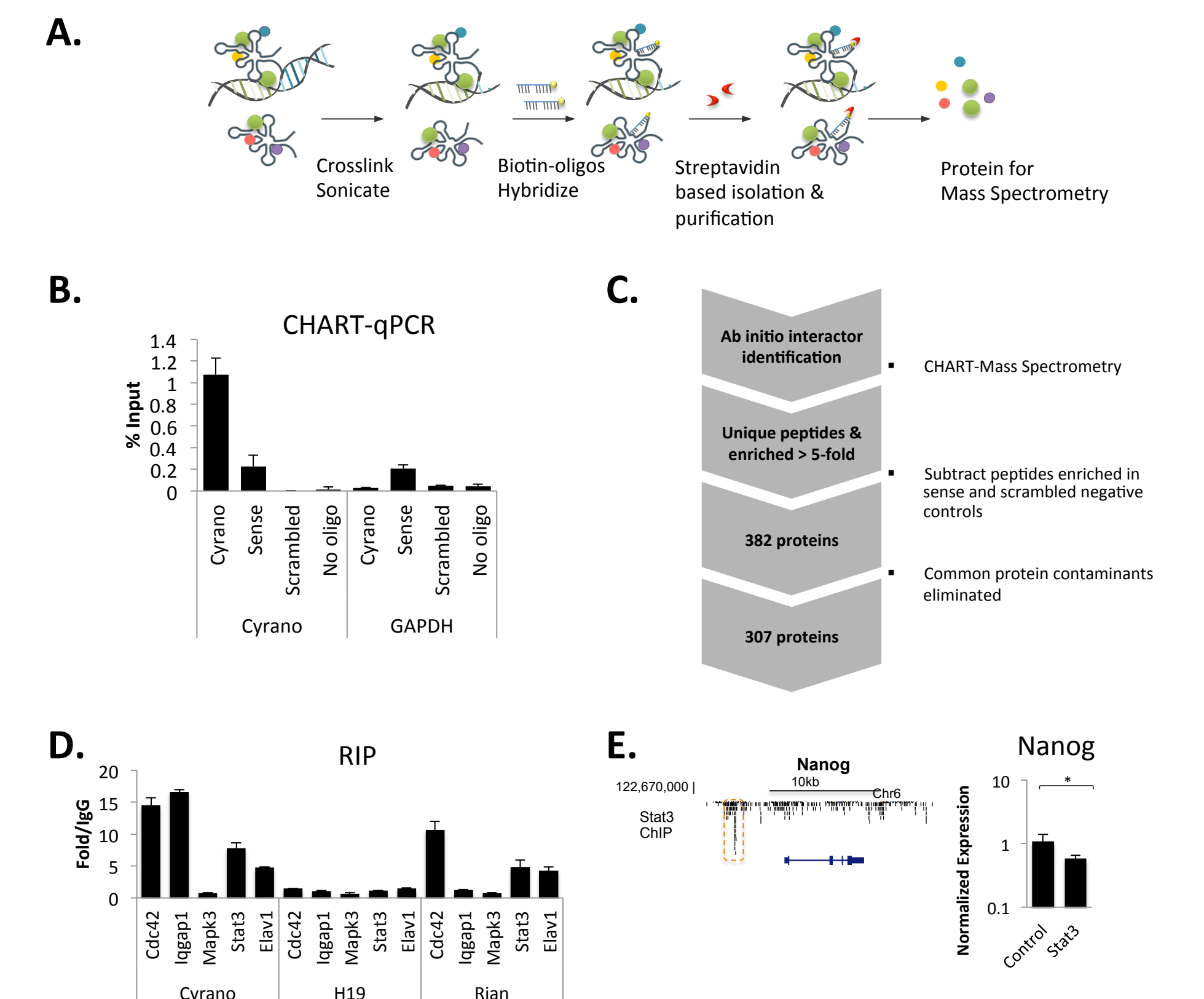
Results Cont'd

Figure 5. _The Cyrano Interactome._ The affinity enrichment method CHART (A) significantly enriches for Cyrano RNA (B), and interacting proteins (C). (D) From network analysis, an interaction with STAT3 was identified, and verified by RIP analyses (D). (E) STAT3 binds upstream of Nanog, and similar to Cyrano LOF, STAT3 inhibition results in decreases in Nanog expression.

Figure 6. _Multiple Cyrano mechanisms converge on Nanog in mouse ES cells._ Cyrano impacts _Nanog_ expression through its participation in a signaling/developmental hub which includes STAT3, and antagonism of _mir-7_ activity in ES cells.

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