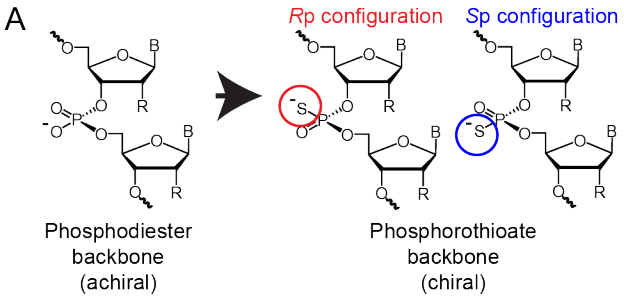

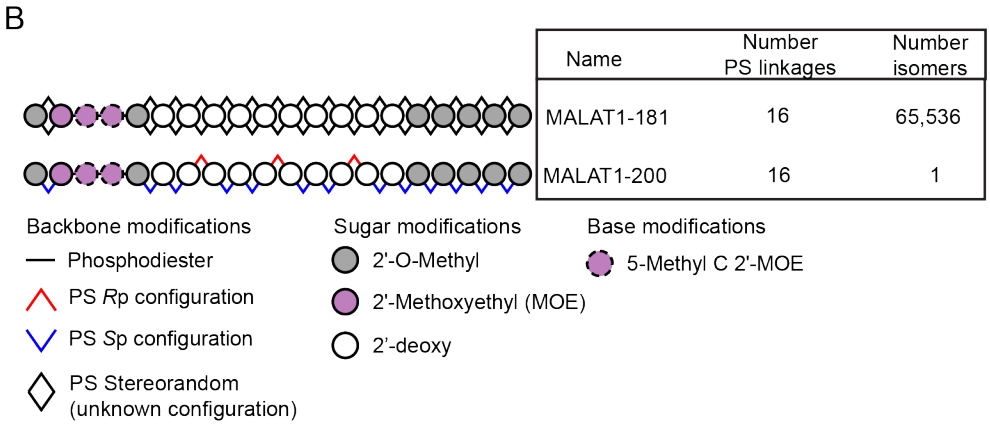

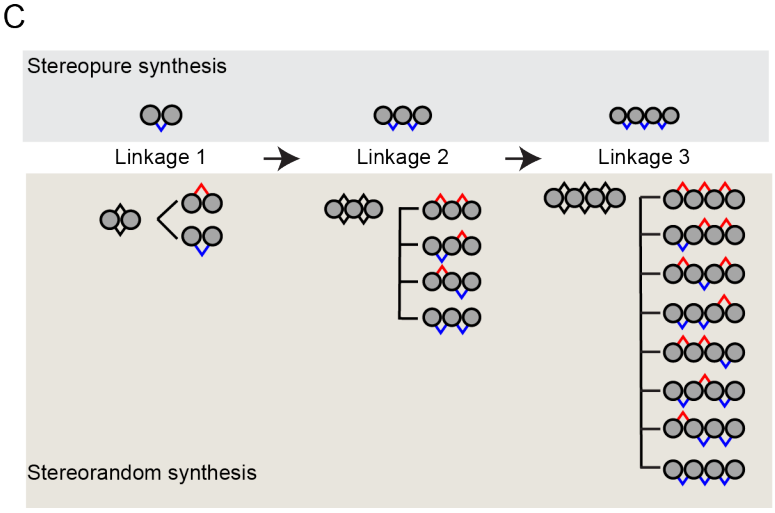

Supplementary Fig. 1. Phosphorothioate (PS) modification introduces chiral centers in an oligonucleotide backbone, resulting in the production of complex mixtures of isomers when using traditional synthetic chemistries. (A) Chemical structures of natural phosphodiester (PO) backbone and chemically modified, chiral PS backbone, showing *R*p and *S*p configurations. In the PS backbone, a Sulfur (S) atom replaces a non-bridging Oxygen (O) atom. (B) Schematic of oligonucleotides used throughout this study, showing that the comparator MALAT-181, generated with industry standard chemistry, is a mixture of more than 65,000 isomers, whereas MALAT1-200 is

predominately a single isomer. The number of isomers is defined by 2^n, where n = number of PS linkages[6]. (C) Schematic illustration of oligonucleotide synthesis showing how the synthesis of the stereorandom and stereopure oligonucleotides compare. For both oligonucleotides, synthesis begins from the 3'-end. For the stereopure oligonucleotide (gray), the illustrated first three steps each yield >99% PS linkages in the *S*p configuration. For the stereorandom oligonucleotide (beige), the first step yields approximately the same amount of PS linkages in the *S*p configuration and the *R*p configurations. For the next step, there are two starting materials, and synthesis yields approximately the same amount of PS linkages in the Sp and Rp configurations at the second position, resulting in a total of four products. For the next step, the same synthesis pattern yields eight products. Ultimately, this results in a stereorandom oligonucleotide that is composed of >65,000 isomers.