



Supplemental Figure S3. The signal originates from the promoter of the construct and not from read-through transcription. (A) Reporters inserted in exons were separated in four categories, depending on their orientation relative to the gene, and the expression of the gene (active genes in the forward orientation, active genes in the reverse orientation, inactive genes in the forward orientation, and inactive genes in the reverse orientation). Each series of bars shows the median expression of the reporters in each category. Expression is measured by the mean-normalized log₂ ratio between mRNA and gDNA barcode counts (see supplemental methods). The expression of promoterless constructs (p0) is strongly orientation-dependent when they are inserted in active genes, but not when they are inserted in inactive genes. This

indicates that most of the signal originates from readthrough transcription. In contrast, the expression of the constructs containing a promoter is symmetric relative to their orientation, showing that the signal originates from transcription initiated at the promoter of the construct. Note that the expression of the promoterless construct appears to be high because of the mean-normalization (see **Supplemental Fig S1**). **(B)** Same analysis as **Fig 4A** carried out on reporters inserted outside exons and introns (12,489 integrated reporters in total). The features are plotted in the same order as in **Fig 4A**. Interactions with the promoters and terminators are still the best predictors and the overall shape of the plot is similar.