SUPPLEMENTAL FIGURE TITLES AND LEGENDS

Extended Data Figure 1. Focal amplicons of ecDNA and *in situ* Hi-C detected contacts in COLO320 cells, Related to Figure 1

(a-b) WGS tracks for focal amplified ecMYC (orange) and hsrMYC (blue) in COLO320 cells (a) and WGS tracks for representative chromosome 8 region as an internal control (b).
(c) WGS estimated copy numbers for chromosomal regions and focal amplificons of hsrMYC or ecMYC in COLO320 cells.
(d) Spearman correlation of Hi-C libraries between replicates (left two) or conditions (right two) in 50-kb resolution.
(e) Percent of inter-chromosomal read pairs detected in Hi-C assay for chromosomal regions or amplified regions in COLO320 cells.
(f) Schematic of normalized *trans*-chromosomal interaction frequency (nTIF). *Trans*-chromosomal interactions between DNA bins (50-kb resolution) were used and normalized to the number of interacting bins and averaged nTIF.
(g) Schematic of relative enrichment analysis to estimate amplicon-associated factors linked to the interactions *in trans*. Genomic segments carried factors of copy number (WGS, amp_d), chromatin accessibility (ATAC-seq signal, amp_a), or transcripts (RNA-seq signal, amp_t) were used to normalize those segments-derived nTIF ($nTIF^{adj}$). The adjusted nTIF was converted to the normalization power of each factors for amplicons ($Power_{ai}$) and chromosomal regions ($Power_{ci}$). Relative contribution was calculated by the comparison between amplicons and chromosomal regions.

Extended Data Figure 2. GRID-seq libraries of COLO320 cells, Related to Figure 2

(a) The bivalent linker in GRID-seq. App, pre-adenylation.
(b-c) Polyacrylamide gel image of GRID-seq linker annealing (b) and chromatin fragmentation by restriction enzyme Alu I (c).
(d-f) Polyacrylamide gel image of library products after paired-end tagging by MmeI (85 bp, d), adaptor ligation (~178 bp, e), and PCR amplification (~216 bp, f). Arrows indicate the DNA bands to be recovered.
(g) Spearman correlation of GRID-seq libraries between replicates (left two) or conditions (right two) in 50-kb resolution.

Extended Data Figure 3. GRID-seq data analysis, Related to Figure 2

(a) Percent of GRID-seq assay detected each RNA or DNA read mapping to the human genome uniquely for one locus (uniq) or multiple for several loci (multi).
(b) Weighted strategy for each multiple aligned read by estimating surrounding uniquely aligned reads where the reads were potentially mapped.
(c) Density distribution of the locus number where multiple aligned RNA reads (left) or DNA reads (right) were mapped.

(d) Representative genomic loci showing RNA signals derived from uniquely aligned RNA reads (red) or multiple aligned RNA reads with weighted strategy (blue).
(e) Procedure of GRID-seq data processing. Chromatin-enriched RNAs interacting with DNA bins (1-kb resolution) were built into a matrix (top-left). Each chromatin-enriched RNA-associated interaction was normalized to the RNA expression as RPKM (middle-left). Protein-coding RNAs (pc) were gathered to build the background model by normalization to the bin number, chromosome length, and RNA species (top-right and middle-right). Relative fold enrichment was calculated for the RNA-DNA contacts against the background model of the interacted DNA bin (bottom-left), and specific RNA-DNA interaction was identified with relative fold enrichment large than 2 (bottom-right).

Extended Data Figure 4. ecDNA enhancers target chromosomal promoters in COLO320DM cells, Related to Figure 2

(a) Gene expression within the transcriptome of COLO320HSR or COLO320DM cells detected by RNA-seq assay (left two) or GRID-seq assay (right two). Dots denote ecDNA or HSR-derived genes.
(b) Scatter plot of RNA expression detected by GRID-seq in COLO320HSR cells against COLO320DM cells. Numbers indicate chromatin-enriched RNAs detected specifically in COLO320HSR cells (blue), COLO320DM cells (orange), or shared (purple). Spearman correlation was applied.
(c) Genomic positions interacted by representative chromatin-enriched RNA, e.g., protein-coding RNA *TBC1D4*, ecMYC or hsrMYC amplified RNA *PVT1*, and well-known *trans*-functioning spliceosome RNA *U1* in COLO320DM and COLO320HSR cells.
(d) A Venn diagram showing the number of chromosomal loci (1-kb resolution) interacted by ecMYC or hsrMYC-derived RNAs in COLO320DM or COLO320HSR cells.
(e) Genomic annotation for chromosomal targets of ecMYC (orange), hsrMYC (blue), or randomly shuffled chromosomal segments (nc, black), with log-2 scaled relative enrichment in ranged color and percentage in dot size.
(f-g) Quantification of H3K27ac signal (f) and Hi-C interactions *in trans* (g) stratified by the H3K27ac signal in ecMYC regions with 1-kb bins (one-sided Wilcoxon Rank Sum Test).
(h) Representative genomic loci showing H3K27ac ChIP-seq signal (red) and copy number adjusted H3K27ac ChIP-seq signal (orange) for amplified regions in COLO320DM and COLO320HSR cells. Enhancers were called by the ROSE software using copy number adjusted H3K27ac signal.

Extended Data Figure 5. Focal amplicons of ecDNA and *in situ* Hi-C detected contacts in GBM39 cells, Related to Figure 3

(a-b) WGS tracks for focally amplified ecEGFR (orange) and hsrEGFR (blue) in GBM39 cells (a) and ecMYC (orange) in GBM39EC cells (b).
(c) WGS estimated copy numbers for chromosomal regions and amplicons of hsrEGFR, ecEGFR, and ecMYC in GBM39 cells.
(d) Spearman correlation of Hi-C libraries between replicates (left two) or conditions (right two) in 50-kb resolution.
(e) Percent of inter-chromosomal read pairs detected in Hi-C assay for chromosomal regions or amplified regions in GBM39 cells.

Extended Data Figure 6. GRID-seq libraries of GBM39 cells, Related to Figure 3

(a-c) Polyacrylamide gel image of library products after paired-end tagging by MmeI (85 bp, a), adaptor ligation (~178 bp, b), and PCR amplification (~216 bp, c). Arrows indicate the DNA bands to be recovered.
(d) Spearman correlation of GRID-seq libraries between replicates (left two) or conditions (right two) in 50-kb resolution.

Extended Data Figure 7. ecDNA mobile targeting analysis in GBM39 cells, Related to Figure 3

(a) Gene expression within the transcriptome of GBM39HSR and GBM39EC cells detected by RNA-seq assay (left two) or GRID-seq assay (right two). Dots denote amplicon-derived genes.
(b) Scatter plot of RNA expression detected by GRID-seq in GBM39HSR cells against GBM39EC cells. Numbers indicate chromatin-enriched RNAs detected specifically in GBM39HSR cells (blue), GBM39EC cells (orange), or shared (purple). Spearman correlation was applied.
(c) Genomic positions interacted by representative chromatin-enriched RNA, e.g., protein-coding RNA *AUTS2*, ecEGFR or hsrEGFR amplified RNA *EGFR*, and well-known *trans*-functioning spliceosome RNA *U1* in GBM39EC and GBM39HSR cells.
(d) A Venn diagram showing the number of chromosomal loci (1-kb resolution) interacted by ecEGFR, ecMYC, or hsrEGFR-derived RNAs in GBM39EC or GBM39HSR cells.
(e) Genomic annotation for chromosomal targets of ecMYC, ecEGFR, hsrEGFR, or randomly shuffled chromosomal segments (nc, black), with log-2 scaled relative enrichment in ranged color and percentage in dot size.

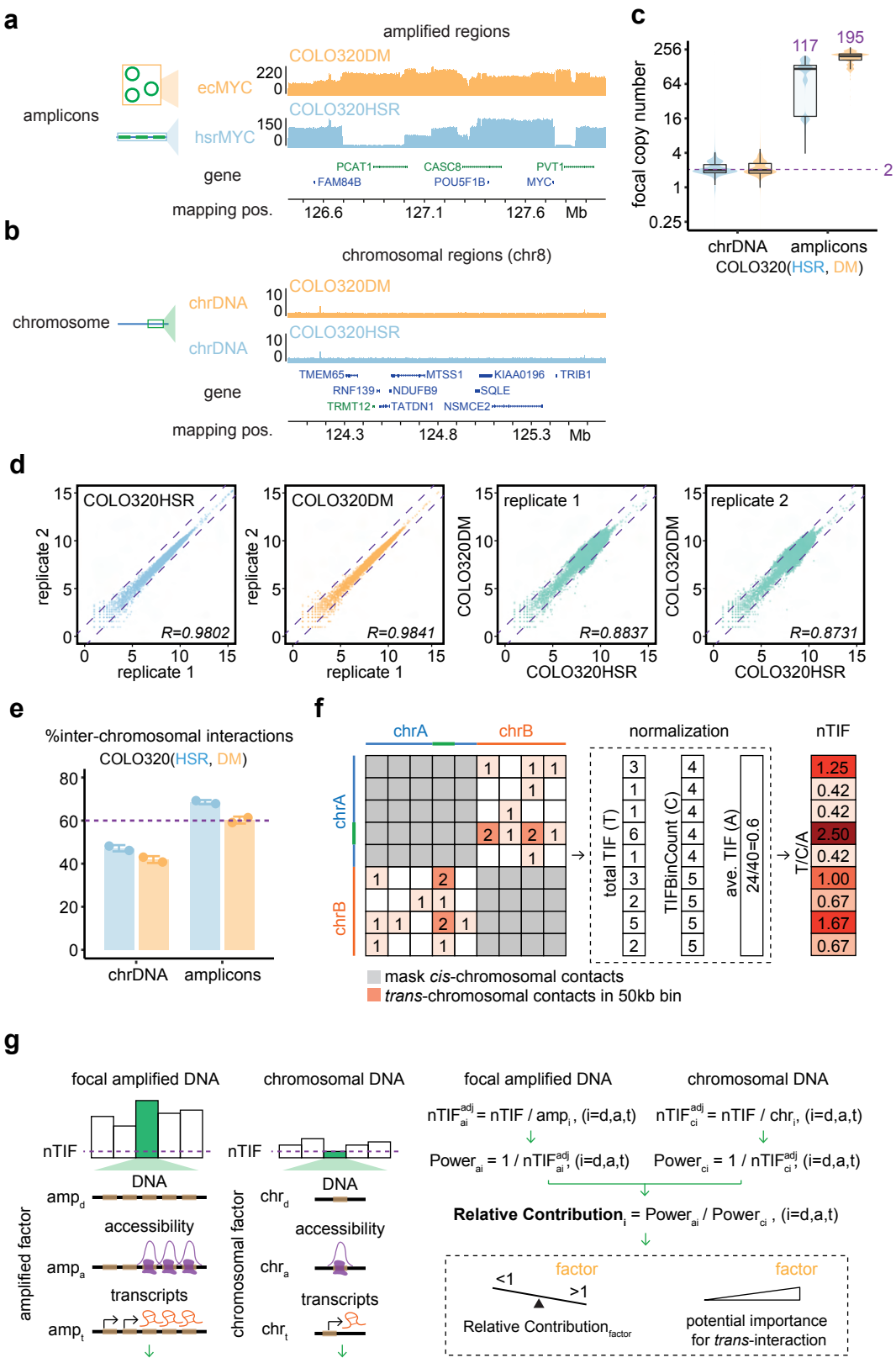
Extended Data Figure 8. Characterization of ecDNA-borne ncRNAs for ecDNA interactome, Related to Figure 4

(a) Genomic positions interacted by hsrMYC or ecMYC-derived intronic signal (left) or exonic signal *MYC* (right) in COLO320 cells.
(b) Heatmaps of hsrEGFR, ecEGFR, or ecMYC-derived RNAs interacting with the genome in the order of mapping position in GBM39 cells. Intronic and exonic RNA signals were used to generate the heatmaps independently. Scale bars, log-10 relative enrichment.
(c) Genomic positions interacted by hsrEGFR or ecEGFR-derived intronic signal or exonic signal in GBM39 cells (left), and ecMYC-derived intronic signal and exonic signal on right.
(d) Genomic tracks showing ATAC-seq, H3K27ac ChIP-seq, and GRID-seq-derived RNA (strand-specific, in two scaled views) signals normalized to CPM in GBM39EC cells. Amplicons were determined by AmpliconArchitect software.
(e) Hi-C heatmap of COLO320HSR cells (left two) and COLO320DM cells (right two) treated by control or *PVT1* knockdown, with ecMYC or hsrMYC labeled. Scale bars, 5,000 interacting reads.
(f) GRID-seq heatmap of COLO320HSR cells (left two) and COLO320DM cells (right two) treated by control or *PVT1* knockdown with ecMYC or hsrMYC labeled. Scale bars, log-10 relative enrichment.
(g) Representative genomic loci showing copy number of ecMYC amplicon deconvoluted by WGS or Hi-C interacting frequency.
(h) Quantification of log-2 fold change of chromosomal genes expression upon *PVT1* knockdown against control measured by GRID-seq-derived RNA signal. Red, genes with promoters targeted by HSR or ecDNA; Purple, untargeted genes (one-sided Wilcoxon Rank Sum Test).
(i) Odds ratio of *PVT1*-knockdown reduced chromatin accessibility determined by ATAC-seq on transcriptional responsive (down) compared to transcriptional unresponsive (none) in both COLO320HSR (left) and COLO320DM cells (right). P-values were calculated by Fisher's exact test.

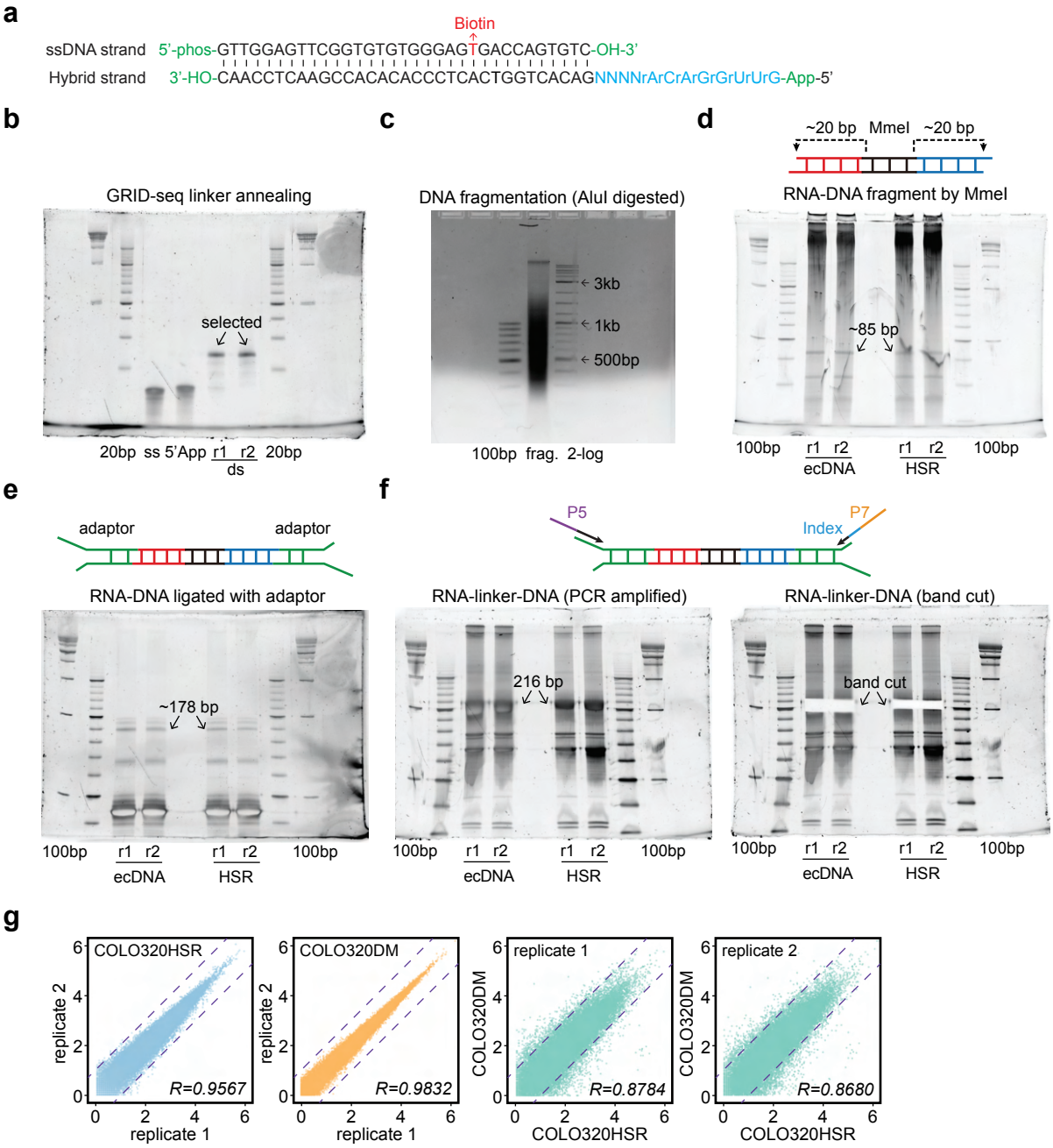
Extended Data Figure 9. ecDNA clustering score and fusion events of *MYC* ecDNA, Related to Figure 5

(a-b) In silico generated pictures with points spreads, ranking by the clustering degree manually (a). Both autocorrelation g(r=0) (top) and ecDNA cluster score (bottom) were applied for the in silico generated pictures (b).
(c) *In situ* Hi-C contact heatmap for COLO320DM cells with annotation of gene *MYC* and *PVT1* included. Fusion gene *PVT1-MYC* was illustrated.
(d-e) Visual-4C with viewpoint of oncogene *MYC* (including exon2-3 and intron 2 in blue rectangle). Equidistant MYC promoter (4-kb in orange) and fusion PVT1 intron (4-kb in purple) derived contacts were measured (d) and were used to estimate transcript percentage (e).

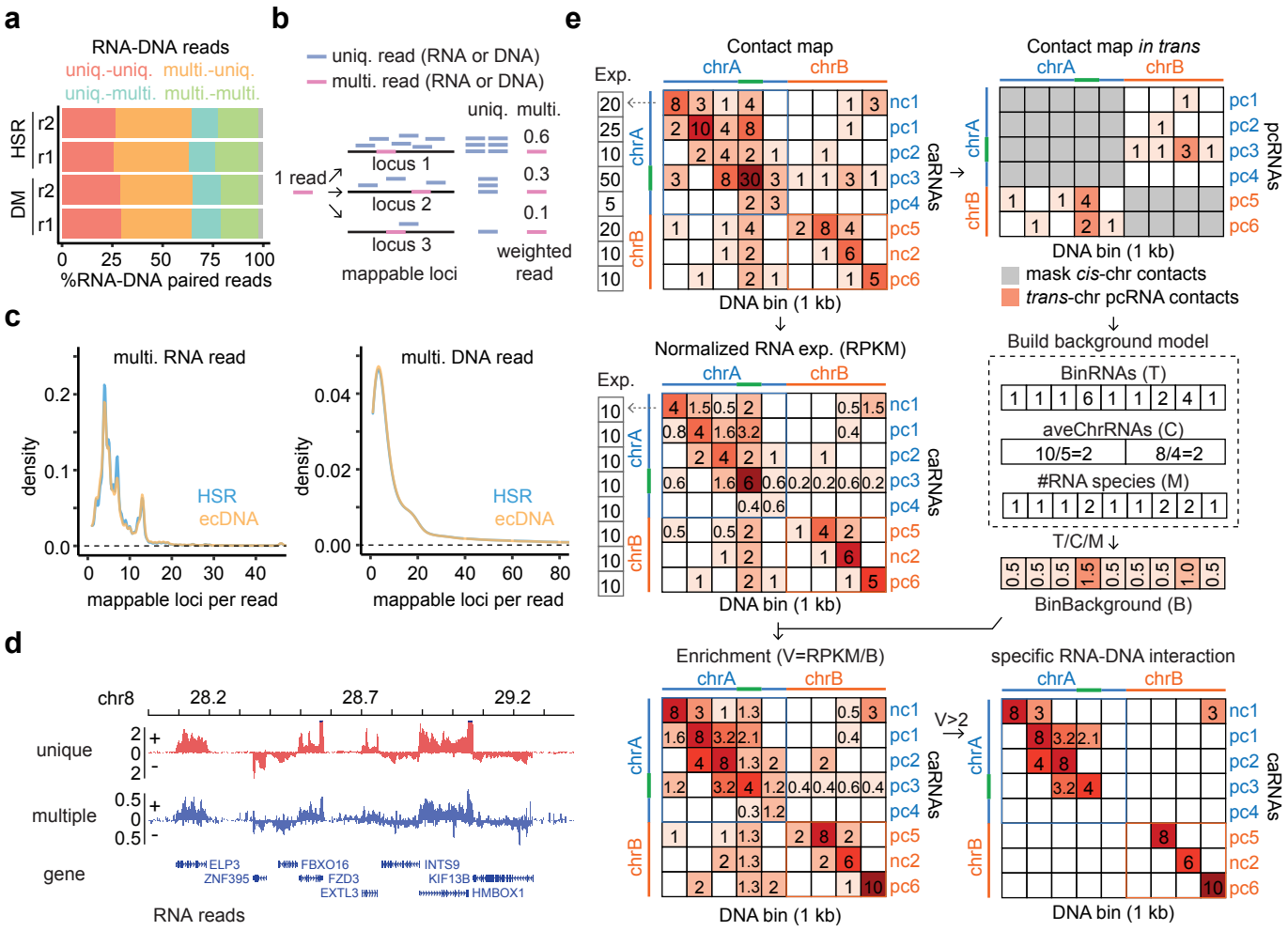
Extended Data Figure 1



Extended Data Figure 2



Extended Data Figure 3



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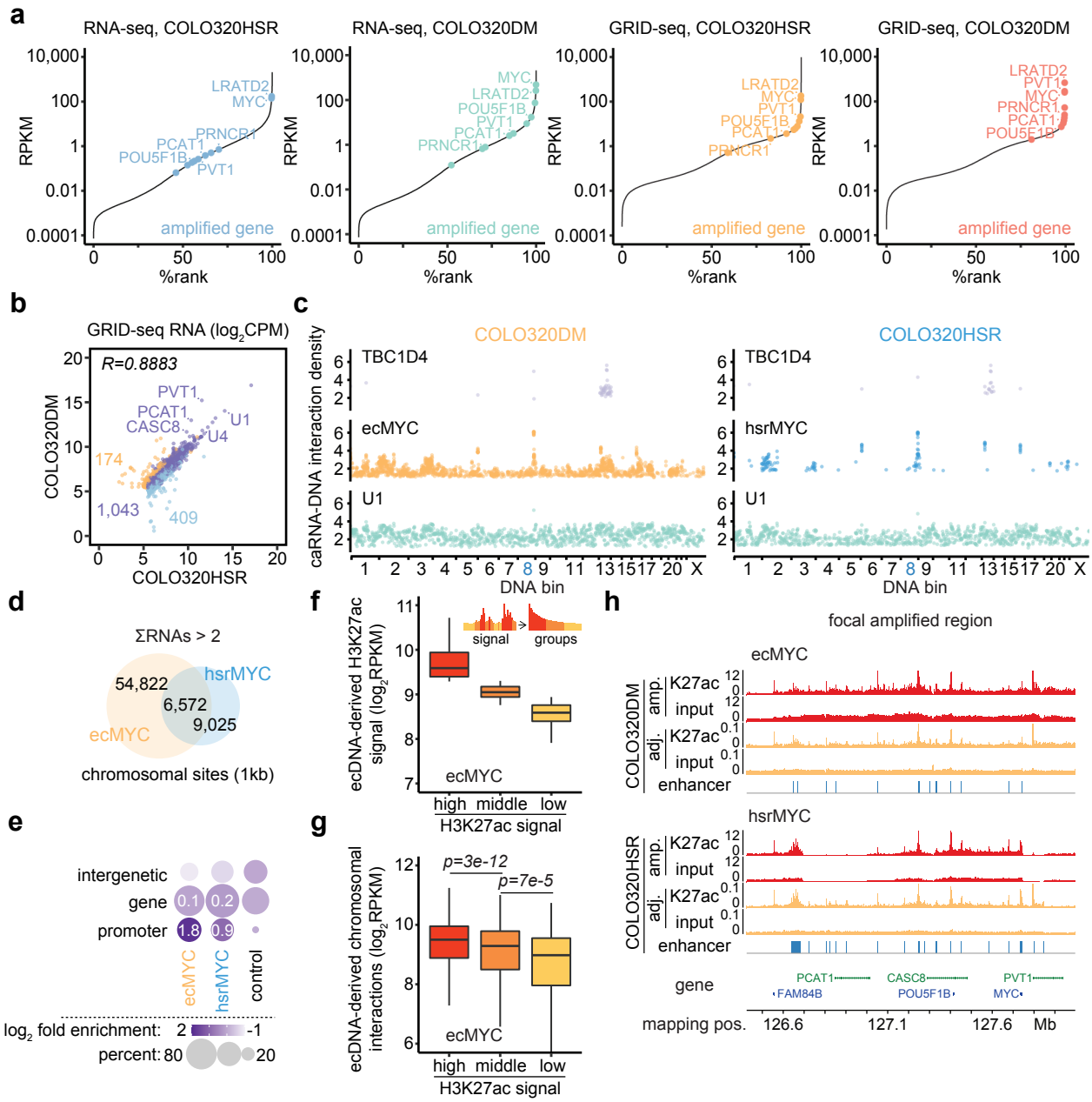

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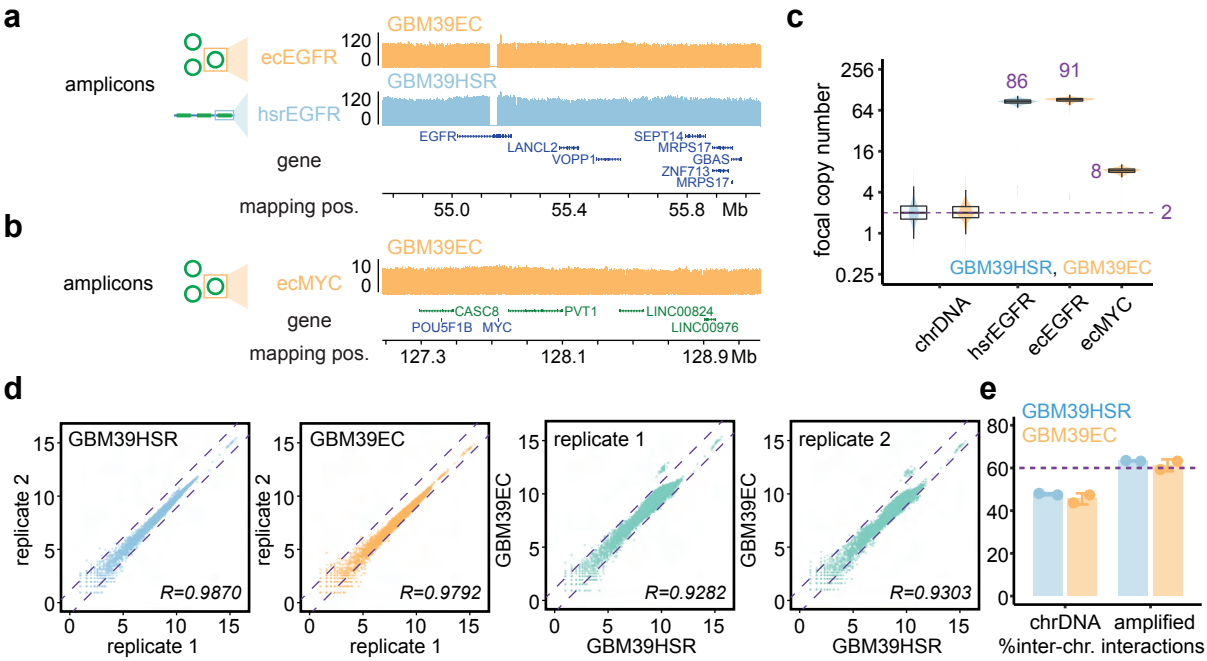

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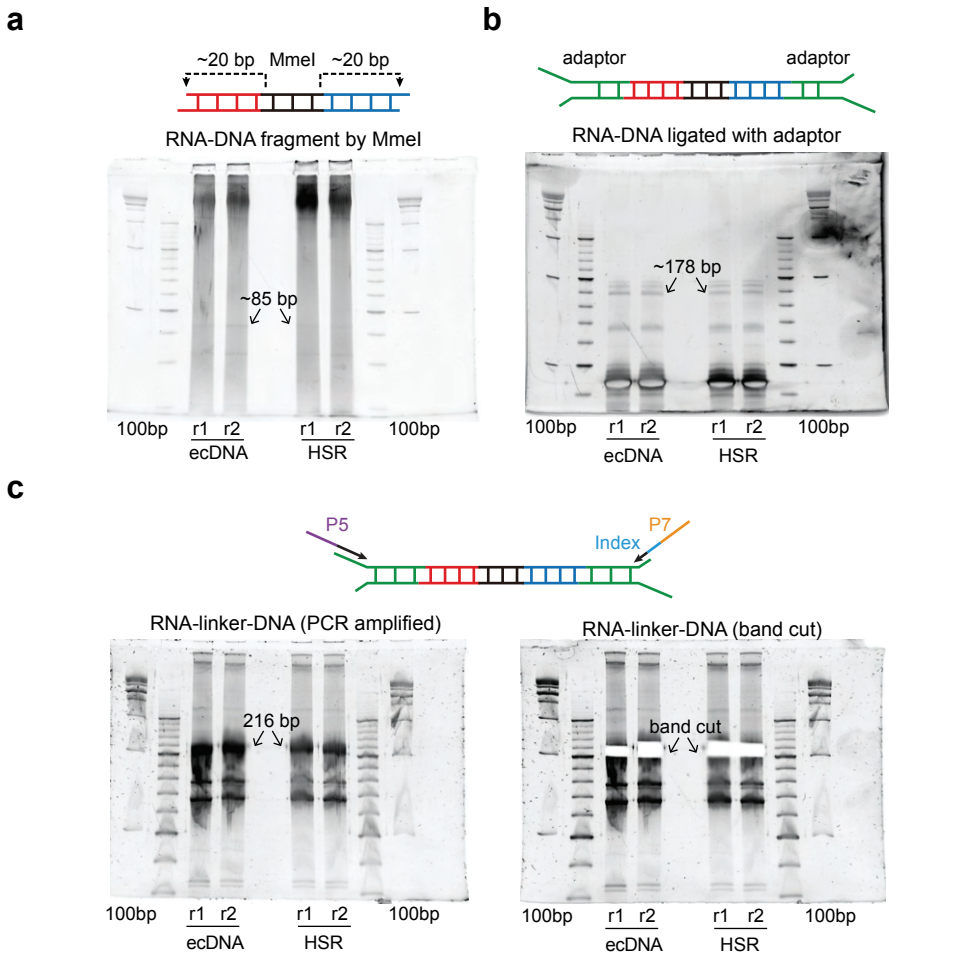

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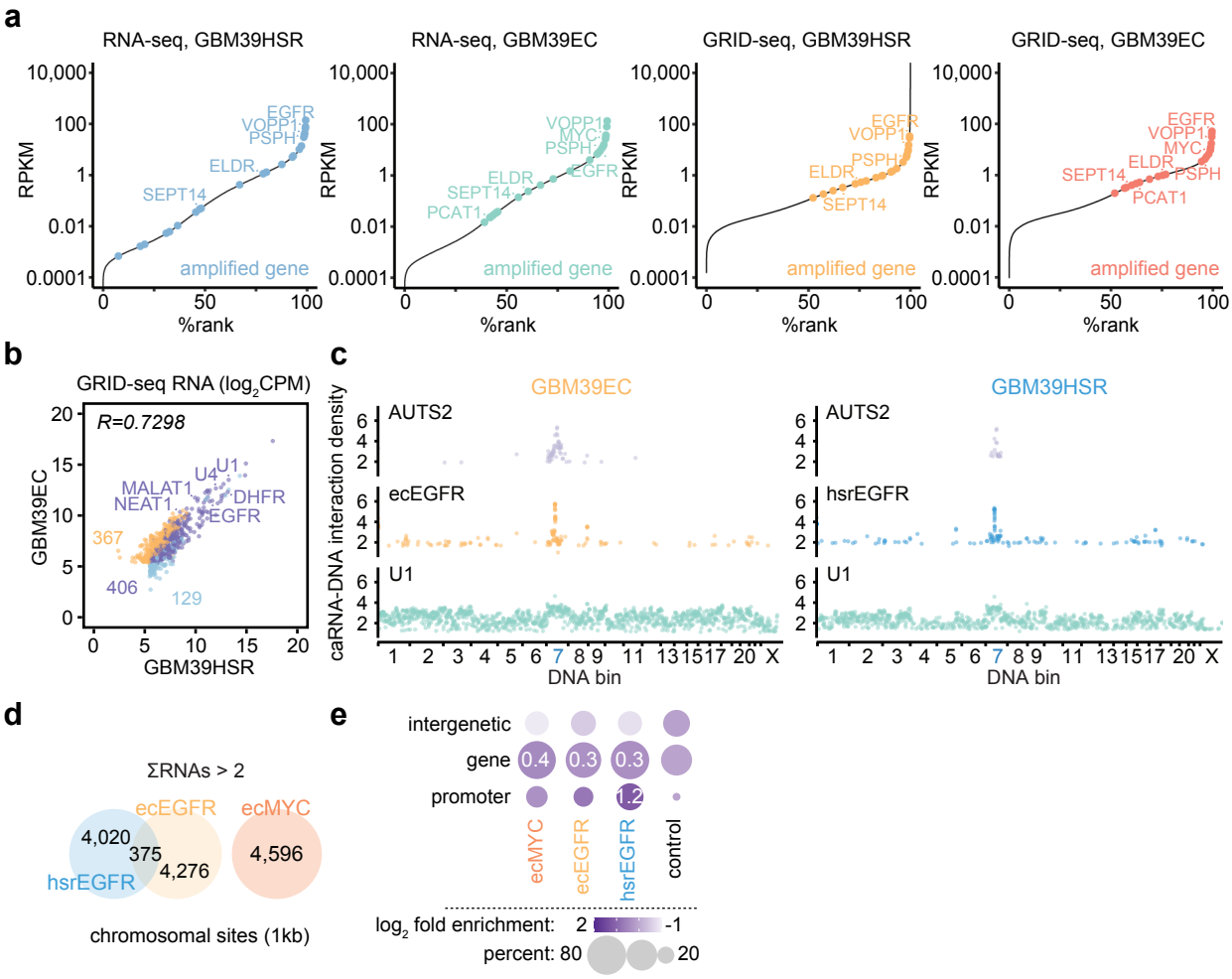

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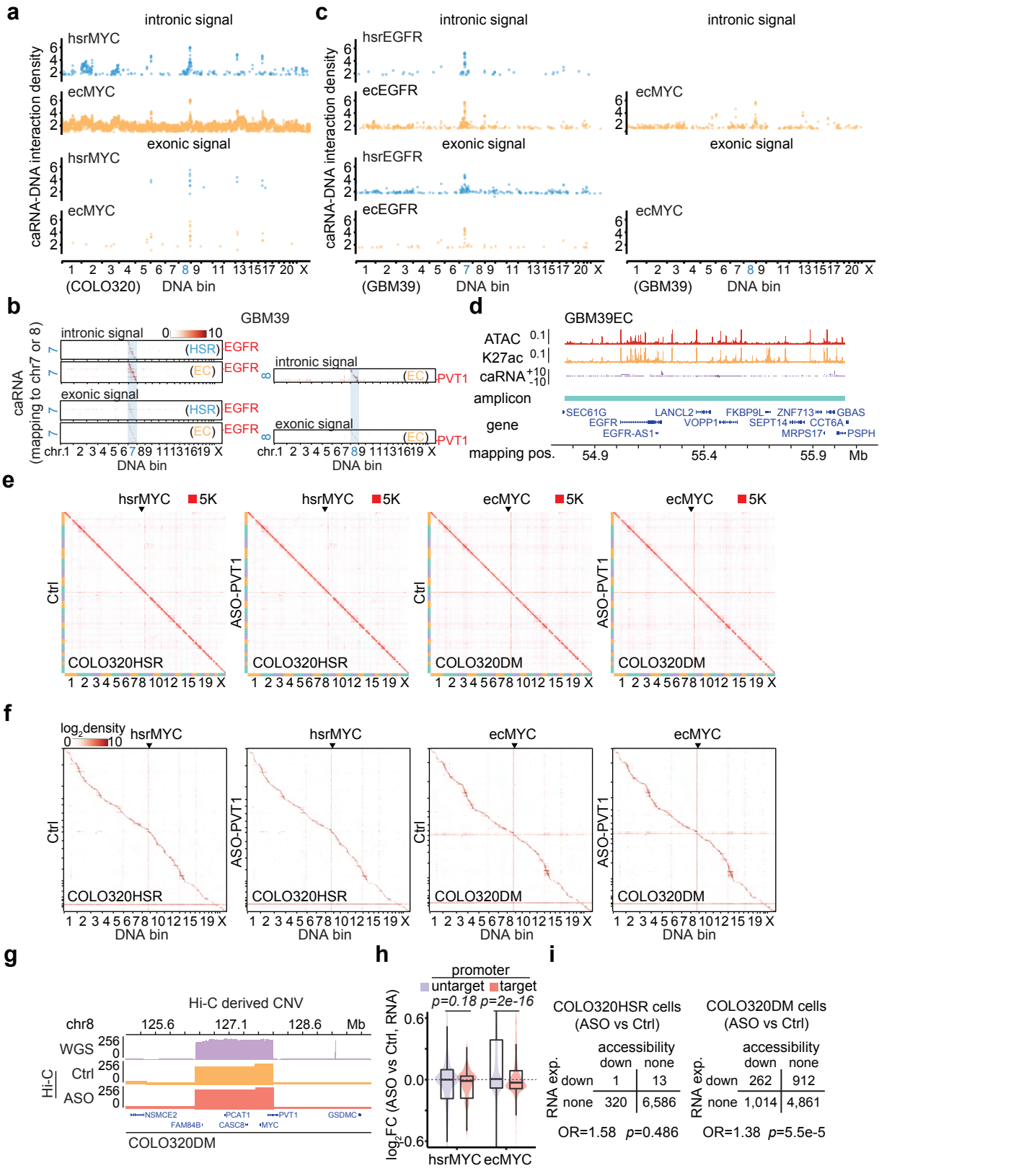

Extended Data Figure 9