SUPPLEMENTAL MATERIALS

Global inhibition with specific activation: how p53 and MYC redistribute the transcriptome in the DNA double-strand break response

Joshua R. Porter, Brian E. Fisher, Laura Baranello, Julia C. Liu, Diane M. Kambach, Zuqin Nie, Woo Seuk Koh, Ji Luo, Jayne M. Stommel, David Levens, and Eric Batchelor

Figure S1, related to Figure 2: Mechanisms for p53 regulating *MYC* expression. (A) Effect of anti-miR treatment on the DNA DSB response. Levels of MYC protein were measured by western blotting in MCF-7 cells transfected with the indicated anti-miRs for 24 h, then treated with NCS for 4 h. Data are represented as mean ± SEM (*n* = 3). (B) ChIP-seq of p53 binding near the *MYC* locus. ChIP-seq reads near *MYC* in MCF-7 cells treated with NCS for 3 h (top track) and untreated MCF-7 cells (second track) are shown along with p53 binding peaks (colored) and their scores as called by MACS. Read counts are normalized to total reads for each treatment. Peaks shown are the intersection of those identified in *n* = 2 biological replicates. All peaks in a 1-Mb-wide window centered on *MYC* are shown. Untreated cells had no p53 binding peaks in this window. RefSeq annotated genes are shown (bottom track). (C-E) Close-up views of p53 binding peaks shown in (B); colors correspond to those in (B). (F) ATAC-seq fragment coverage in the region of chromosome 8 shown in (C) in MCF-7 and MCF-7 sh-p53 cells, treated with NCS for 3 h or not treated. For each cell line and treatment, fragment counts are normalized to total unique fragments in a 20-Mb-wide surrounding region (chr8:120000000-140000000). RefSeq annotated genes are shown (bottom track). (G) ATAC-seq fragment coverage around the *MYC* promoter region. RefSeq annotated genes are shown along with dual *MYC* promoters P1 and P2 (bottom track). Pink highlighted regions in (F-G) correspond with those in Figure 2E-F, respectively.

Figure S2, related to Figure 4: Changes in the transcriptome during the DNA DSB response. (A) Principal component analysis (PCA) was used to analyze normalized transcript counts from newly synthesized RNA-seq of GFP and MYC-GFP cells treated with NCS or not treated with NCS. Transcripts with at least 5 reads in each sample were included in this analysis; normalized transcript counts were log-transformed before performing PCA. (B-C) Fold-changes in mean production of all transcripts other than those from genes identified by Allen et al. (2014) as p53 targets, 2.5-3.5 h after NCS treatment in GFP cells (B) and MYC-GFP cells (C) compared with untreated GFP cells. Transcripts with at least 5 reads in each replicate (n = 3) of each cell line/treatment condition are included. Transcripts are ordered by fold-change in mean production. (D-F) Examples of gene sets enriched in GFP cells treated with NCS over GFP cells not treated with NCS. Normalized enrichment scores (NES), false discovery rate q-values, and nominal p-values are shown.

Figure S3, related to Figure 5: Gene sets enriched by maintaining MYC above basal levels during the DSB response. (A-B) Examples of apoptosis-related gene sets enriched in NCS-treated MYC-GFP cells over NCS-treated GFP cells. Normalized enrichment scores (NES), false discovery rate q-values, and nominal p-values are shown. (C-D) Examples of cell-cycle-related gene sets enriched in NCS-treated MYC-GFP cells over NCS-treated GFP cells.

Figure S1

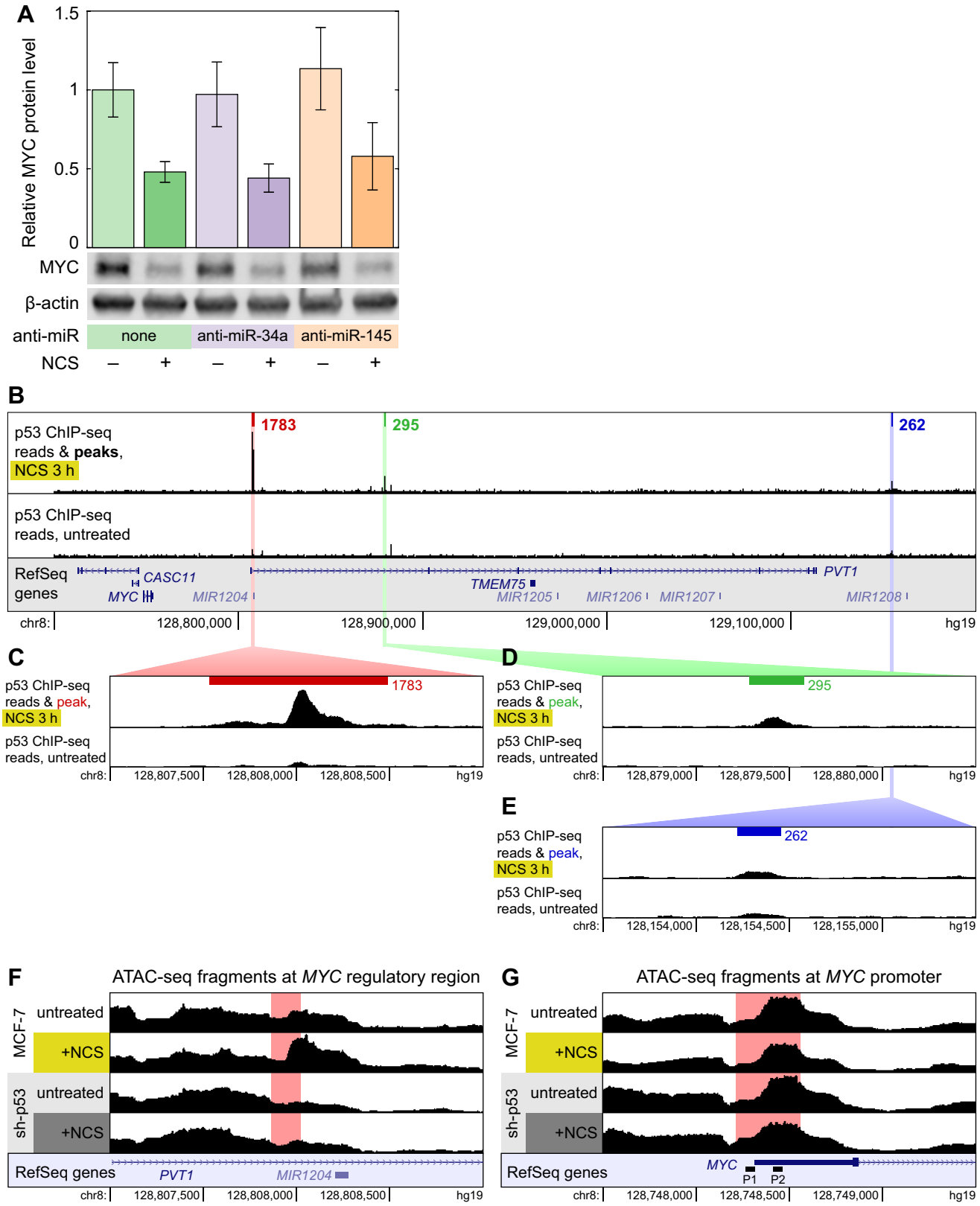
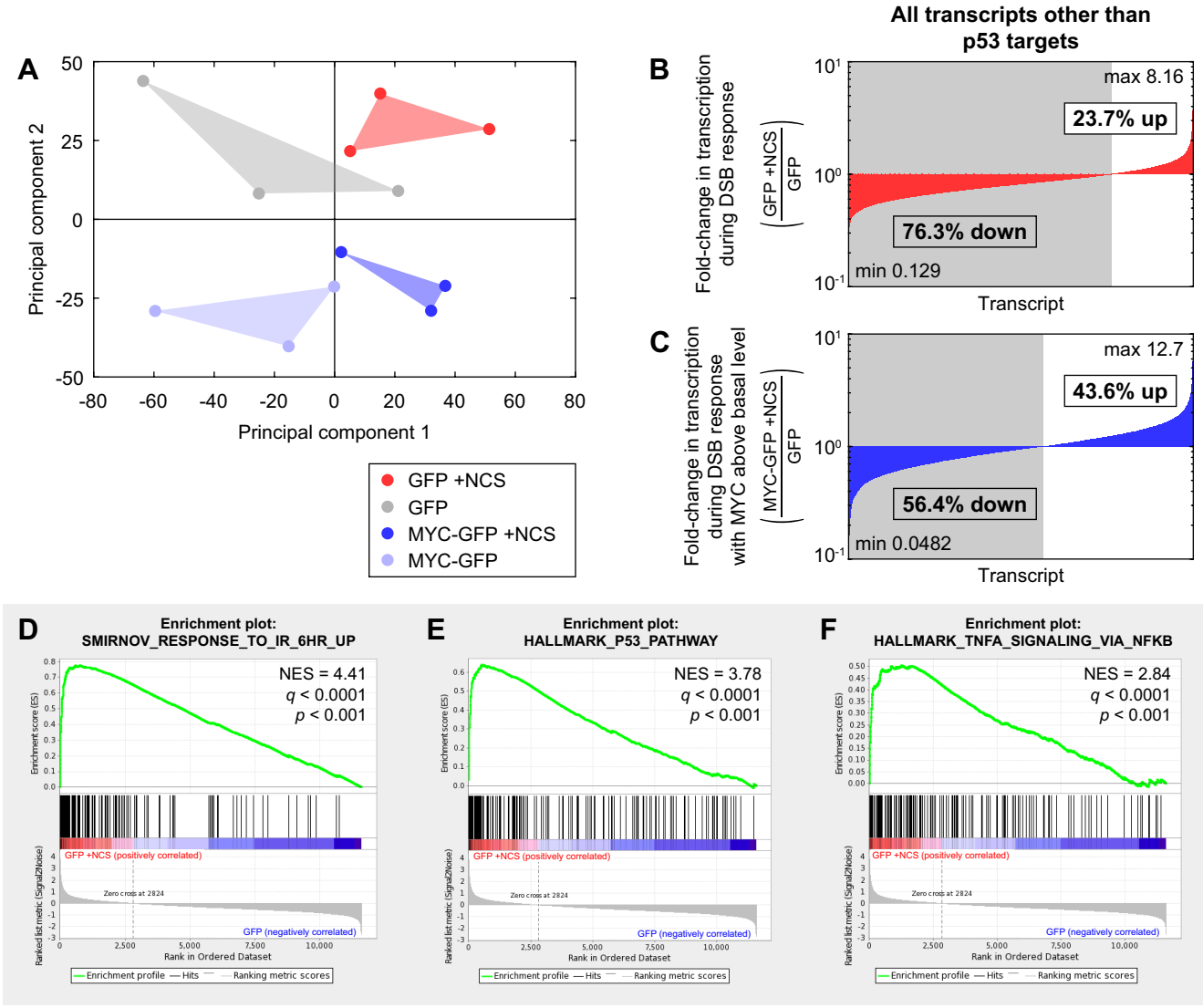
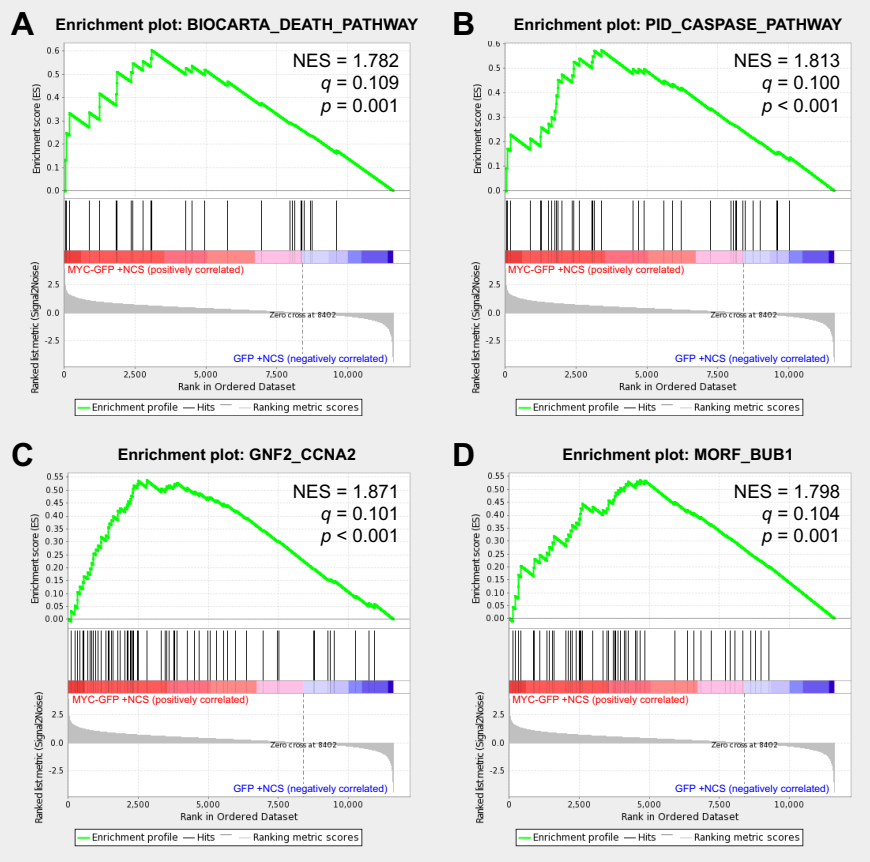

A
Relative MYC protein level
1.5
1
0.5
0
MYC
β-actin
anti-miR
none
anti-miR-34a
anti-miR-145
NCS
– + – + – +
B
p53 ChIP-seq reads & peaks, NCS 3 h
1783
295
262
p53 ChIP-seq reads, untreated
RefSeq genes
CASC11
MYC
MIR1204
TMEM75
MIR1205
MIR1206
MIR1207
PVT1
MIR1208
chr8:
128,800,000
128,900,000
129,000,000
129,100,000
hg19
C
p53 ChIP-seq reads & peak, NCS 3 h
1783
p53 ChIP-seq reads, untreated
chr8:
128,807,500
128,808,000
128,808,500
hg19
D
p53 ChIP-seq reads & peak, NCS 3 h
295
p53 ChIP-seq reads, untreated
chr8:
128,879,000
128,879,500
128,880,000
hg19
E
p53 ChIP-seq reads & peak, NCS 3 h
262
p53 ChIP-seq reads, untreated
chr8:
128,154,000
128,154,500
128,155,000
hg19
F
ATAC-seq fragments at MYC regulatory region
MCF-7
untreated
+NCS
sh-p53
untreated
+NCS
RefSeq genes
PVT1
MIR1204
chr8:
128,807,500
128,808,000
128,808,500
hg19
G
ATAC-seq fragments at MYC promoter
MCF-7
untreated
+NCS
sh-p53
untreated
+NCS
RefSeq genes
MYC
P1
P2
chr8:
128,748,000
128,748,500
128,749,000
hg19

Figure S2


A
Principal component 2
Principal component 1
GFP +NCS
GFP
MYC-GFP +NCS
MYC-GFP
B
All transcripts other than p53 targets
Fold-change in transcription during DSB response
(GFP +NCS / GFP)
max 8.16
23.7% up
76.3% down
min 0.129
Transcript
C
Fold-change in transcription during DSB response with MYC above basal level
(MYC-GFP +NCS / GFP)
max 12.7
43.6% up
56.4% down
min 0.0482
Transcript

D
Enrichment plot:
SMIRNOV_RESPONSE_TO_IR_6HR_UP
NES = 4.41
q < 0.0001
p < 0.001
E
Enrichment plot:
HALLMARK_P53_PATHWAY
NES = 3.78
q < 0.0001
p < 0.001
F
Enrichment plot:
HALLMARK_TNFA_SIGNALING_VIA_NFKB
NES = 2.84
q < 0.0001
p < 0.001
Enrichment score (ES)
GFP +NCS (positively correlated)
Zero cross at 2824
GFP (negatively correlated)
Ranked list metric (Signal2Noise)
Rank in Ordered Dataset
Enrichment profile
Hits
Ranking metric scores

Figure S3


A
Enrichment plot: BIOCARTA_DEATH_PATHWAY
NES = 1.782
q = 0.109
p = 0.001
B
Enrichment plot: PID_CASPASE_PATHWAY
NES = 1.813
q = 0.100
p < 0.001
C
Enrichment plot: GNF2_CCNA2
NES = 1.871
q = 0.101
p < 0.001
D
Enrichment plot: MORF_BUB1
NES = 1.798
q = 0.104
p = 0.001
Enrichment score (ES)
MYC-GFP +NCS (positively correlated)
Zero cross at 8402
GFP +NCS (negatively correlated)
Ranked list metric (Signal2Noise)
Rank in Ordered Dataset
Enrichment profile
Hits
Ranking metric scores