

Integrative Genomic Analysis Reveals Widespread Enhancer Regulation by p53 in Response to DNA Damage

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Supplementary Information

Supplementary Table 1. Summary of differential gene expression in human and mouse primary fibroblasts in response to doxorubicin treatment.

Supplementary Table 2. PCR Primers and PCR conditions.

Supplementary File 1. Summary of the p53-regulated transcriptome: gene names, genomic coordinates, expression values, fold changes, and corresponding p53 binding sites for p53-regulated transcripts (mRNAs and lincRNAs) in human and mouse primary fibroblasts in response to doxorubicin treatment.

Supplementary File 2. Summary of guilt-by-association predictions for p53-regulated lincRNA functions: gene set enrichment analysis (GSEA) output.

Supplementary File 3. Summary of p53 binding sites within annotated chromatin states: coordinates of p53 binding sites and corresponding chromatin state annotations.

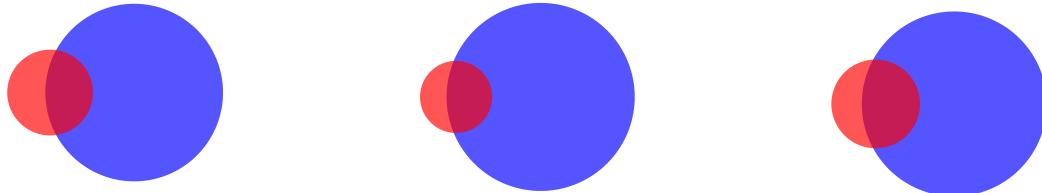
Supplementary Figure 1. Comparison of p53 binding sites across multiple cell types and treatments. **(A-C)** Comparison of p53 binding sites characterized in this study with sites identified in previous studies using various forms of p53 activation in **(A)** MCF7, **(B)** lymphoblastoid, and **(C)** U2OS cells. **(D)** Comparison of p53 binding sites in doxorubicin treated human fetal fibroblasts (this study), lymphoblastoid cells, and U2OS cells.

Supplementary Figure 2. Enrichment analysis of Alu/B1 family elements within lincRNAs regulated by p53 relative to reference lincRNA catalogs in **(A)** primary human fibroblasts (Alu) and **(B)** primary mouse embryonic fibroblasts (B1).

Supplementary Figure 1

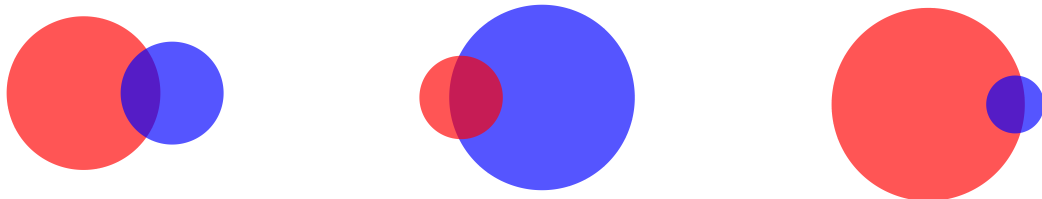
A ● Younger et al.
 ● Nikulenkov et al. Cell Death Differ. 2012 Dec;19(12):1992-2002.

● Doxorubicin (hFetal Fibro.) : 2,638	● Doxorubicin (hFetal Fibro.) : 2,638	● Doxorubicin (hFetal Fibro.) : 2,638
● Nutlin (MCF7) : 17,958	● 5FU (MCF7) : 11,320	● RITA (MCF7) : 11,477
● Overlap : 1,652	● Overlap : 1,366	● Overlap : 1,707



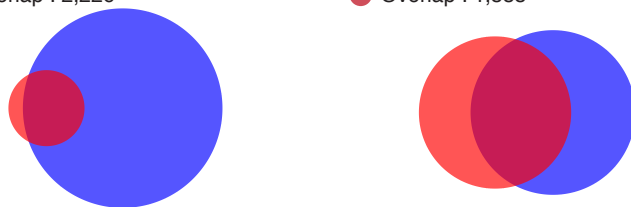
B ● Younger et al.
 ● Zeron-Medina et al. Cell. 2013 Oct 10;155(2):410-22.

● Doxorubicin (hFetal Fibro.) : 2,638	● Doxorubicin (hFetal Fibro.) : 2,638	● Doxorubicin (hFetal Fibro.) : 2,638
● Nutlin (Lymphoblastoid) : 1,183	● Doxorubicin (Lymphoblastoid) : 13,055	● IR (Lymphoblastoid) : 237
● Overlap : 351	● Overlap : 1,668	● Overlap : 161



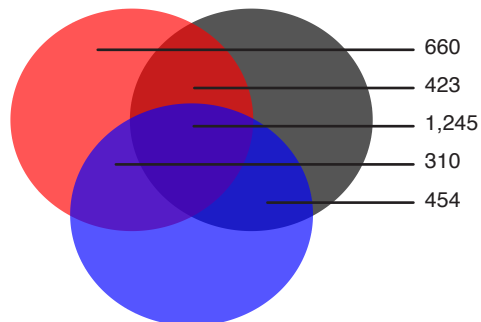
C ● Younger et al.
 ● Menendez et al. Nucleic Acids Res. 2013 Aug;41(15):7286-301.

● Doxorubicin (hFetal Fibro.) : 2,638	● Doxorubicin (hFetal Fibro.) : 2,638
● Nutlin (U2OS) : 18,156	● Doxorubicin (U2OS) : 3,087
● Overlap : 2,226	● Overlap : 1,555



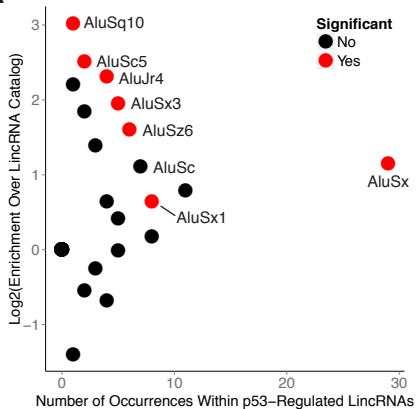
D All Doxorubicin Samples (Diagram Not Scaled)

● Doxorubicin (hFetal Fibro.) : 2,638
● Doxorubicin (Lymphoblastoid) : 13,055
● Doxorubicin (U2OS) : 3,087

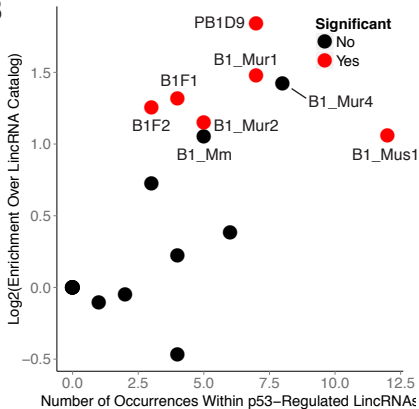


Supplementary Figure 2

A



B



Supplementary Table 1. Summary of differential gene expression in response to doxorubicin treatment.

		Human		Mouse	
		mRNA	lincRNA	mRNA	lincRNA
Up-regulated	Total	1365	101	3124	94
	p53 Targets	262 (19%)	22 (22%)	585 (19%)	21 (22%)
Down-regulated	Total	1598	32	3334	99
	p53 Targets	106 (6.6%)	1 (3.1%)	175 (5.2%)	4 (4%)

Supplementary Table 2. PCR Primers and PCR conditions.

Target	Direction	Use	Sequence
LincRNA-7	Forward	RT-qPCR	TGCTGCATTTACAGGTGAGG
LincRNA-7	Reverse	RT-qPCR	AGGATGGCAGGTACATGAGG
LincRNA-4	Forward	RT-qPCR	GGAGCCTAGCATCACTCGTC
LincRNA-4	Reverse	RT-qPCR	CTCCTCTTCCAGCAGTTTGG
LincRNA-1	Forward	RT-qPCR	AAGCCAAGCTTCCTGTTTCA
LincRNA-1	Reverse	RT-qPCR	GGTCTAGGGGATGGGAATA
LincRNA-7 Promoter	Forward	ChIP-qPCR	GCACGGACTGAGAAACACAA
LincRNA-7 Promoter	Reverse	ChIP-qPCR	GGGGCATTGGAGATTTTG
LincRNA-4 Promoter	Forward	ChIP-qPCR	ACTTACCATGGGCAGTCAGC
LincRNA-4 Promoter	Reverse	ChIP-qPCR	GGGTGGTGGGTGTCACTAAA
LincRNA-1 Promoter	Forward	ChIP-qPCR	AGTGGACTGGAGCCTGACAT
LincRNA-1 Promoter	Reverse	ChIP-qPCR	AGCACCACCTCACACATTGA
Enhancer Region-1	Forward	ChIP-qPCR	CCACAGGCTGCACAAGATAA
Enhancer Region-1	Reverse	ChIP-qPCR	TCTGAAAGTGATGCTGTGGAA
Enhancer Region-2	Forward	ChIP-qPCR	GCCAGGCAATTTTGTGATT
Enhancer Region-2	Reverse	ChIP-qPCR	TAGCCTGCTGGATGATGAAA
Unannotated Region-1	Forward	ChIP-qPCR	AGGCAGTGGAGAGTGGAGAA
Unannotated Region-1	Reverse	ChIP-qPCR	GACCCTTGCAACCTGCTTAG
GAPDH Promoter	Forward	ChIP-qPCR	TACTAGCGGTTTTACGGGCG
GAPDH Promoter	Reverse	ChIP-qPCR	TCGAACAGGAGGAGCAGAGAGCGA

PCR Conditions	Denature	Annealing	Extension	Cycles
RT-qPCR	95°C, 20 s	57°C, 30 s	72°C, 45 s	x40
ChIP-qPCR	95°C, 20 s	57°C, 30 s	72°C, 45 s	x40