Molecular Cell, Volume *29*

Supplemental Data

Exchange of GATA Factors Mediates Transitions in Looped Chromatin Organization at a Developmentally Regulated Gene Locus

Huie Jing, Christopher R. Vakoc, Lei Ying, Sean Mandat, Hongxin Wang, Xingwu Zheng, and Gerd A. Blobel

Supplemental Experimental Procedures

1. Plasmids, transient transfections and luciferase assays

Reporter constructs were generated by PCR and cloned into the pGL4.10 luciferase reporter vector (Promega, Madison WI). DNA fragments from –114kb region containing wild or mutant GATA binding motif were synthesized by IDT and cloned into pGL4.10 luciferase reporter vector. The sequence for the –114kb region is: AGG CTA ATG TGT TGT CTT GAG ATA ATC TAG GCA CAC AGG ACC TGA CTC CAC TCC AAC CCA TCA GCA ACC GCA TCT CAG AAC AGC CGC CTT CTT ATC TCT CAG CTC AGT CAG GGA AAG GCT GTC AC. GATA motifs are underlined. In the mutant –114kb fragment the two GATA sites were converted from GATAA to GCGCA and from TTATC to TCCGC.

MEL cells and G1E cells were seeded into 6-well plates ($2x10^6$ cells/well) in Opti-MEM1 reduced serum medium. Cells were co-transfected with 0.5 µg pGL4.10 luciferase reporter plasmids and 20ng control Renilla vector pGL4.74 (hRluc/TK, Promega) prepared with DMRIE-C transfection reagent (Invitogen, San Diago CA). 48 hours after transfection, cells were lysed and luciferase activity measured according to manufacturer's recommendation. Results were normalized to Renilla luciferase activity.

2. ChIP primers

Primer Name	Location kb	Primer sequence
1F	-237.4	TGAGTCAAAATTGGGACCTGC
1R		TCCATGCATCATTGAAAACCTCT
2F	-232.4	TGCACTTGGCAAACTTCCTG
2R		AGGGATGTGTCTCACCGTGTC

3F	-227.8	CCCCAGTTGAATCCTCTCTGTT
3R		CCGCCCAGATTTGGAATTTT
4F	-224.9	TCTCCTCAGTGCCCTCACAGA
4R		TGCTCCGTGATGAGTTGTGAA
5F	-222.0	TGGATTGTTAGGCAATGAAGAGC
5R		ACGAAGACACTGCTTGATAAATGG
6F	-197.8	CCCTCCAAGCTGTGTGGAAT
6R		TCCAGGGTTCAGGCCAACT
7F	-146.2	AGAAGGTGCCCCGAGTGTATAA
7R		GCACAGCTCCTTACCTTGCAAT
8F	-114.5	GCACACAGGACCTGACTCCA
8R		GTTCTGAGATGCGGTTGCTG
9F	-104.8	TGATCGGATAATTTGCCTGTACA
9R		TGGAAGAAGTGACCCTGATGAAA
10F	-98.0	GCTTCTTGAGGTTCATTAGATAAAAACA
10R		GACCCCGGAACTGAGAGATG
11F	-91.3	GACCCAGAGCTTCTCCAGTGA
11R		TTGTCAGGAAACATGACTGGCTAT
12F	-86.6	CGGAGCACCGAAGAGTCTTG
12R		GCAGCTCCGGGTAAAATAGAAAA
13F	-71.3	ATGAGGAAGTCGAACAGGAAGAGT
13R		TCATGTGAAGAGTGCTGTGGG
14F	-54.0	CAAGGCTGTGGTCTGAGATGAG
14R		AAATGTGCTTGTTTGCCTTGATAG
15F	-51.4	CCACGGATAATGCCCTCTGT
15R		GGGTCCCCGATGATACACTCT
16F	-35.8	AGAGAACCGAAGCTCCAGGG
16R		TTGATGGAAGCATTAGAAAAAGAATTT
17F	-27.8	CCCGAGTAAATCTGCATCCATT
17R		GCAAAAAATCAAAAAGCTTATGATAAACT
18F	-21.1	GGGAAGAATCAGTTATTTTGAGGTTT

18R		GATCAAAGATAATGACCCCAAGTGA
19F	-9.1	TGCAGGAGGAAATATAGGTTTAGATAAGA
19R		TGGAAGAAAGTAAATAACAGTAGGGTCTC
20F	0.0	CACCTCCACCATAAGCCGAAT
20R		CTCCTAGACAATAAAGGACAACCA
21F	4.7	GGCTGGAAACCACTGCCTTA
21R		AGCCTTGCCTGTGCTTAAAGC
22F	9.7	CCGGGTGGGCCTCAGT
22R		GGCATGGGCTTACAGTGTCA
23F	16.2	AACTGTTGCCGGGCAATTAT
23R		TCTTGGTGAATGGTCGGATACC
24F	16.9	GGCTGCCAAGGCTTGTCA
24R		AAATCTGCCTGGCTTTTACACCTA
25F	33.1	TGGCAGTCCTGGTTGTAGCA
25R		GCTGCAAGCATGCGATCA
26F	34.8	GAAAGTGCTCACAACCCATGAA
26R		CTAGTTTATCTCCTGGCCACTGAAT
27F	37.5	GTGCCTCCAAGGGTTAAAGAAA
27R		GTCCCATCTGCAAGTCACACA
28F	40.1	AAATGTCACAACAACCTTGAAAGTAGTAG
28R		TCCCATTTGCATAAAAAGGAAAC
29F	41.6	GGGAGCATGTTGATATACCCTCTT
29R		CTTGGAAACTGATGAATGGATTTTT
30F	47.8	GCACAGAAGGAGGCACTTATACCT
30R		CGTTGAATGTCACGGAAGCA
31F	50.2	TCCCTTCCAAAGGAAATGTTAATTA
31R		GAATTGAGGGCCAATGAGATACC
32F	58.1	GGAGGAGTTAGGGAATATGTCGATAG
32R		GCAGTTCTCCAGGTTGAGTCAGA
33F	60.2	AGAGAGGCCCAGCGTATGG
33R		GAACAGTGGACTCGTAGGAGCAT

34F	65.5	GACGCAACTTCCTTATGATCACAA
34R		CCTGCCTCACACTGACCAAA
35F	72.1	CCCCCTCAAATGTCTTCGAA
35R		TTCAGACTCTAAATTAGCCCCTACTCA
36F	72.8	AACTGAAGCGAGTACAGCATTCC
36R		TGCTTTTGCTTGTGTACTGTTAACTG
37F	73.6	CGTTCCAACTTCTGTCTCTTTGG
37R		GAGGCTCATTTAGACAGGTTTGC
38F	75.1	AACGCAGTTCCTTCTCGTTCA
38R		GTCATGCACAGCCTACCAACA
39F	76.3	AAGAGGCAAGGTCAGAGTAAGCTT
39R		GCGCACAGTTAGTGCTTATGTCA
40F	77.8	CACGCGCTATGCACATCCT
40R		TGCCCAGCACATGACAACTT

3. Primers for conventional 3C PCR

Primer name	**Sequence**
Kit -135	GACCATGAGCTAAAGCCAGCAGCCATATC
Kit -114	AACACAGGACCATTTCAGGATGAGCACTGG
Kit -60	AATTCAGTACATCCTCCAGCGACCTTGTGG
Kit -35	AAGACGGAGGAGGAAGGAGGTTAAGGCAG
Kit +5	AGCCTCAAGTCTAGTTGGGCGGTATTAGCAG
Kit +23	AGAGACCACTCCTTAGTGCTGCTTATCTGG
Kit +42	TCACTGTGGCTTTGGCACTGAAGGCAGAAAC
Kit +58	AACAGTTTCCTAGTGCAGCCTGACACAGAC
Kit +73	AATCCAAACCATCAGGACAGAGGTTGTGCC
Kit +83	GGGAGGAAGCAGAAAGAATGGGCTTGCATTG
Tubulin1	AATGAATGGACTCCACAGGGCTCGCAAAC
Tubulin2	ATGCTCCTTGGCAAGTGTCAAGTGTTGTCC
beta-major	CCGAATGT TCATAATACC CCCTTCCACC
LCR/HS2	GGCTTGAGCACAGCAGTGCTGAGTCAT

4. Primers and probes for Taqman PCR

Kit -135	GGCTAACCCTTTTCTACCCAGTCTAT
Kit -114	GCTAACCTTGAGCTCGCAGAGA
Kit -60	CAGTTTGGGTTTTGTTTTGCAA
Kit -35	CCCTTGCAAAGGGCAACA
Kit +5	GCCGCTGTGATAGTTGGAAGA
Kit +23	GAGAAAGTTCCTCCGCTGAGATC
Kit +38	ACACCTAGAGGGATGGCTCAAG
Kit +58	GCTGGGACTAAGATGAAGTAAGAGAAC
Kit +73	TTGTAAAGTCCCTTTGTTCTCTGAAA
Kit +83	CCCATTAACTCACGTGCTTGGT
Kit +5 probe	56-FAM/TCT GCA CAT CTC ACG CAG TCT CCC TT/36-TAMSp

Tubulin #1	CGCAGCTCGGAGACTCACTAT
Tubulin #2	CCAAAGATGCCTGTTCTCCAA
Tubulin Probe	56-FAM/AGAGATCTAGGTCAAAGGTAGGAGGCACT/36-TAMSp

beta-major	GGCTGGAACATCACTGGAATAAAT
LCR/HS2	CAGCGTTTTAGTTGGATATAGAGTGAA
LCR Probe	56-FAM/TCTGCCTGTCCCTGCCTCGTGA/36-TAMSp

5. Primers for PCR cloning

Kit promoter F	GCG ACC CGG GCG GGA GAA GGG A
Kit promoter R	CGC GGT GGC TGC GCT AGA CTC TG
5kb F	CTC TGG ACC AAG CTT TAA GCA
5kb R	GAT CTG CAC ATC TCA CGC AGT CTC
58kb F	CAA ATG GCA AGG GCT ATT TTA AGA
58kb R	AAC TGT TTA ATG CAT AAA GAA TG
73kb F	GAT TGT GCC TCT TTC CCA CTA ACT GAA G
73kb R	TCT ATC TTC AAA ATC TCA CAA CTG

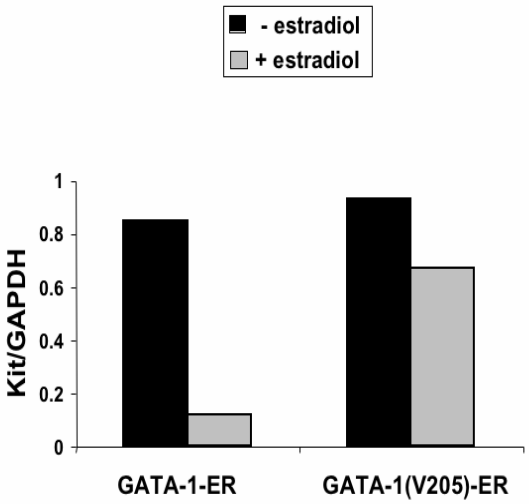

Figure S1. *Kit* expression is inhibited by GATA-1 in a FOG-1 dependent manner. G1E cells expressing wild GATA-1-ER (G1E-ER4) or mutant GATA-1(V205M)-ER were treated with 1μM estradiol for 21 hrs. mRNA was reverse transcribed and analyzed by SYBR green real-time PCR.

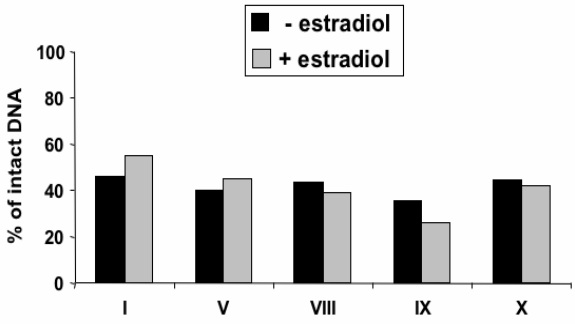

Figure S2. Bgl II digestion efficiency. 3C DNA samples were prepared from G1E cells or estradiol-treated G1E-ER4 cells, and the 3' restriction sites of indicated BglII fragments (the same as those analyzed by 3C) analyzed as described in Experimental Procedures.

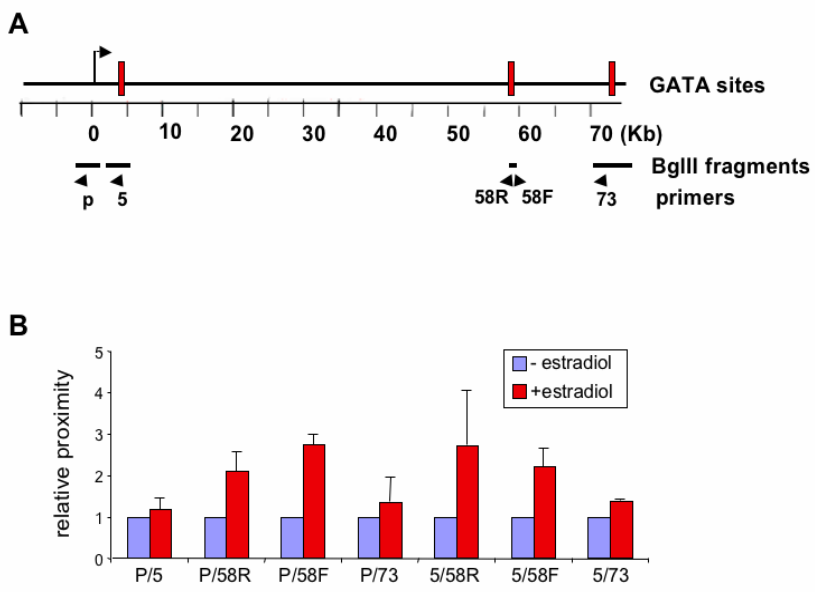

Figure S3. 3C analysis using conventional PCR confirms that GATA-1 increases the proximities among GATA-bound elements. A) Organization of the *Kit* locus. Red bars denote sites bound by GATA-1 in vivo. Numbers indicate distance in kilobases from the transcriptional start site. Arrows denote primers used for 3C. B) 3C analysis of G1E-ER4 cells measuring relative proximities among indicated Bgl II fragments before and after estradiol treatment. Ligation products were amplified in duplicates by conventional PCR. Gel bands were quantified using a Typhoon scanner and Image Quant software. Relative proximities before GATA-1-induced repression were set to one (blue bars). Red bars denote fold changes in relative proximity among indicated fragments upon GATA-1-induced repression. Error bars represent standard deviation. The results are averages of 3 to 4 independent experiments.

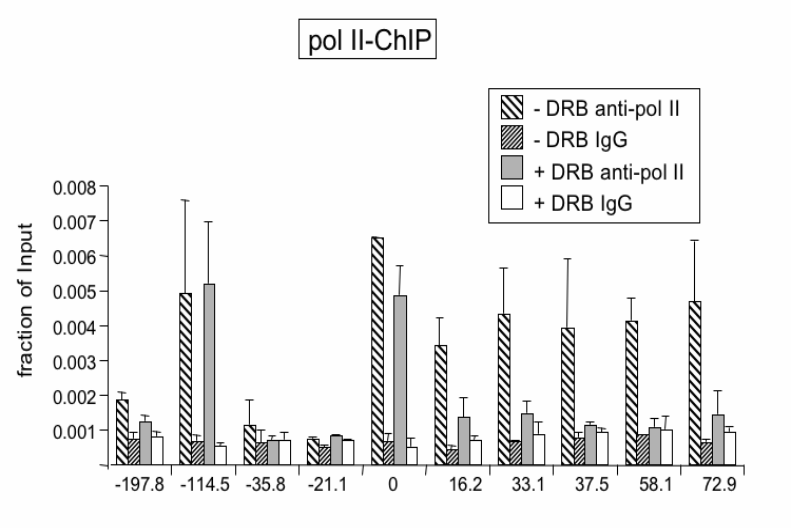

Figure S4. Quantitative ChIP assay in G1E cells with anti-pol II antibodies before and after treatment with 75μM DRB for 6 hours. Numbers indicate distances in kilobases from the promoter. Error bars represent standard deviation. The results are averages of 2 independent experiments.

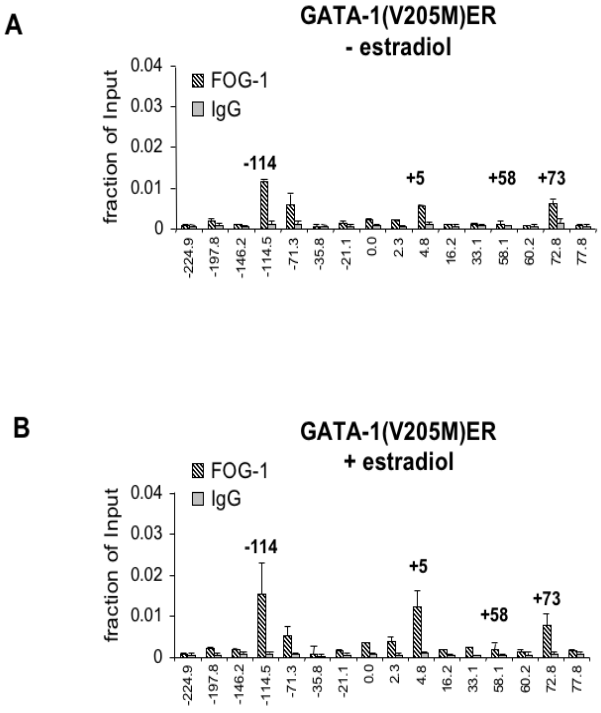

Figure S5. Quantitative ChIP assay in GATA-1(V205M)-ER expressing cells with anti-FOG-1 (striped bars) or control IgG (grey bars) before (A) and after (B) estradiol treatment for 21 hours. Error bars represent standard deviation. The results are averages of 3 independent experiments.