Supporting Information

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SI Text

MNase Assay. Micrococcal Nuclease Digestion was performed as described in ref. 1. Briefly, 10^6 cells were Dounce homogenized in RSB buffer (10 mM Tris·HCl pH 7.4, 10 mM NaCl, 3 mM MgCl2, 0.5% Nonidet P-40) containing 1 mM phenylmethylsulfonyl fluoride, 1 mM Na_3VO_4, 1 mM DTT and protease inhibitors mixture, and incubated on ice for 15 min. Samples were washed twice with RSB buffer and digested with 2.5 units of micrococcal nuclease (USB) in digestion buffer (15 mM Tris·HCl pH 7.5, 60 mM KCl, 15 mM NaCl, 1 mM CaCl2, 3 mM MgCl2, 20% glycerol, 15 mM β-mercaptoethanol) for the indicate times. Digestion was stopped by adding 1 volume of stop solution (50 mM Tris pH 7.5, 150 mM NaCl, 50 mM EDTA, 0.3% SDS). DNA was phenol/chloroform extracted, precipitated with isopropyl alcohol and separated using 1.2% agarose gel electrophoresis.

Histone Association Assay. This procedure is a modification of the earlier described ChIP protocol (2). Cells were treated with formaldehyde at a final concentration of 1% for 15 min. The reaction was quenched with the addition glycine to a final concentration of 125 mM. Nuclei were prepared according to ref. 3, lysed in ChIP lysis buffer (50 mM Hepes-KOH pH 7.5, 150 mM NaCl, 1 mM EDTA, pH 8.0, 1% Triton X-100, 0.1% sodium deoxycholate and protease inhibitors) and chromatin was sheared by sonication to ≈2 kb to 500 bp. The extract was centrifuged for 5 min at 20,000 × g at 4 °C. For each histone H3 immunoprecipitation, ≈1 mg of nuclear extract (as measured by the Bradford assay) was incubated with 8 μg of rabbit polyclonal histone H3 antibody (Upstate). Equal amount of Rabbit IgG (Sigma) was used as negative control. Samples were incubated overnight at 4 °C with shaking. 20 μg of single-stranded herring sperm DNA and 25 μL of protein A Sepharose beads were added and incubated at 4 °C with shaking for 90 min. The beads were washed twice in ChIP lysis buffer, once in ChIP lysis buffer with 500 mM NaCl, once in ChIP washing buffer (10 mM Tris pH 8.0, 0.25 M LiCl, 0.5% Nonidet P-40 and 0.5% sodium deoxycholate). The beads were washed once more in ChIP lysis buffer and resuspended in 50 μL of Laemmli loading buffer. The input controls and immunoprecipitates were boiled for at least 30 min to reverse the cross-links. Chromatin-associated Fe65 was analyzed by SDS/PAGE and Western blot with Fe65 antibody.

Chromatin Immunoprecipitation. Cells were treated with 1% formaldehyde for 10 min at room temperature and formaldehyde was then inactivated by the addition of 125 mM glycine. Chromatin was then sonicated to an average DNA-fragment length of 200–1,000 bp. Soluble chromatin extracts were immunoprecipitated using Tip60, TRRAP, acetyl-histone H4, Fe65 or myc antibodies and goat or rabbit IgG as a control. Supernatant obtained without antibody was used as input control. The amount of precipitated DNA was calculated by real-time PCR relative to total input chromatin, and expressed as percentage of total chromatin according to the following formula: $2\Delta Ct \times 10$, where Ct represents the cycle threshold and $\Delta Ct = Ct(input) - Ct(immunoprecipitation)$ (4). All of the experiments have been done at least as independent triplicates and the Student's t test was used to measure statistical significance.

Cell Cultures, Transfections, and Antibodies. Fe65 KO mouse embryo fibroblast lines (Fe65 KO MEFs) generation was described in ref. 3. These lines were cultured in DMEM (DMEM, Invitrogen) supplemented with 10% Newborn Calf Serum (HyClone), 1% penicillin and streptomycin, 1% MEM-nonessential amino acids (Sigma), 10–5 M 2-mercaptoethanol. NIH 3T3 fibroblasts and Hepa 1–6 cells were grown in DMEM supplemented with 10% Fetal Bovine Serum (HyClone), 1% penicillin and streptomycin. Mouse neuroblastoma cell line N2A was cultured in DMEM (DMEM, Invitrogen) supplemented with 10% Fetal Bovine Serum (HyClone), 1% penicillin and streptomycin, 1% MEM-nonessential amino acids (Sigma).

Transient transfections were done with the indicated DNAs by using Lipofectamine 2000 (Invitrogen) following the instructions of the manufacturer.

Fe65 silencing was obtained by transfecting pSM2 retroviral shRNAmir vectors (Open Biosystems, catalog no. RMM1766-9351830) or siRNAs (Dharmacon, catalog no.LQ-042929); APP and APLP2 silencing was obtained by transfecting siRNAs (Dharmacon, catalog nos. L-043246 and L-042937, respectively), following the manufacturer's instructions. Nonsilencing siRNAs were used as a control (Dharmacon, catalog no. D-001810-10-20).

α-actin (Sigma), Tip60 (N17; Santa Cruz Biotechnology), TRRAP (T17; Santa Cruz Biotechnology), myc (Santa Cruz Biotechnology), histone H3 (Upstate, cat.06–755), acetyl histone H4 (Upstate, catalog no. 06-866), γH2AX (Cell Signalling), APP (Sigma, catalog no. 8717), APLP2 (Calbiochem) antibodies were used following suppliers' recommendations. Horseradish peroxidase-conjugated goat polyclonal anti-rabbit and anti-mouse IgG were purchased from GE Healthcare. The rabbit polyclonal anti-FE65 antibody was described in ref. 5.

Wild type and mutant Fe65 retroviral vectors were transfected into LinX packaging cell line by using calcium phosphate method. 48 h after transfection viral supernatants were collected, filtered, supplemented with 8 mg/mL polybrene, and used to infect Fe65 KO MEFs.

1. Marchion DC, et al. (2005) Valproic acid alters chromatin structure by regulation of chromatin modulation proteins. *Cancer Res* 65:3815–3822.
2. Liu Y, et al. (2008) Involvement of xeroderma pigmentosum group A (XPA) in progeria arising from defective maturation of prelamin A. *FASEB J* 22:603–611.
3. Minopoli G, et al. (2007) Essential roles for Fe65, Alzheimer amyloid precursor-binding protein, in the cellular response to DNA damage. *J Biol Chem* 282:831–835.
4. Frank O, et al. (2002) Real-time quantitative RT-PCR analysis of human bone marrow stromal cells during osteogenic differentiation in vitro. *J Cell Biochem* 85:737–746.
5. Zambrano N, et al. (1997) Interaction of the phosphotyrosine interaction/phosphotyrosine binding-related domains of Fe65 with wild-type and mutant Alzheimer's beta-amyloid precursor proteins. *J Biol Chem* 272:6399–6405.

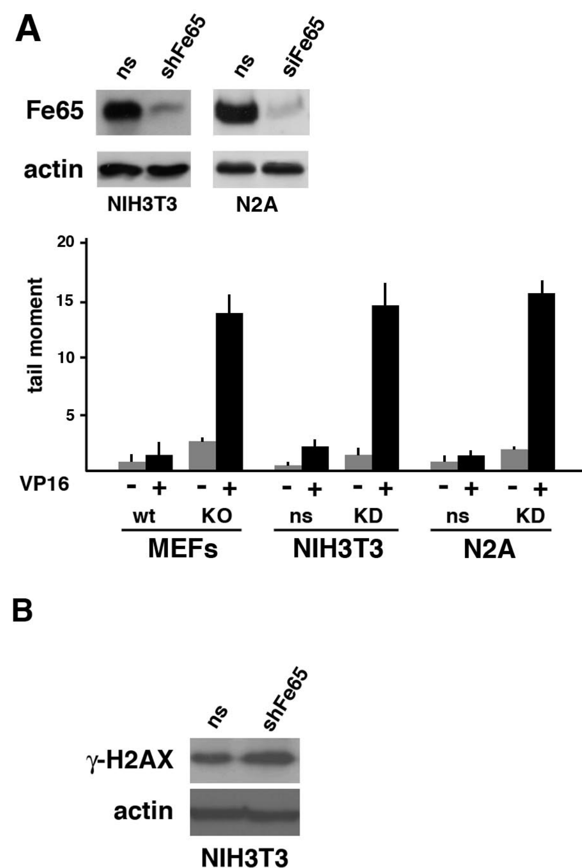

Fig. S1. Suppression of Fe65 by RNA interference results in the same phenotype observed in Fe65 KO MEFs. (*A*) NIH 3T3 or N2A cells were transfected with an shRNA-encoding vector or with siRNAs targeting Fe65, respectively. Western blot analysis demonstrated that the decrease of Fe65 levels due to RNAi was of ≈80%, compared with cells transfected with non silencing (ns) shRNA or siRNA. These cells were exposed to 20 μM etoposide for 1 h and analyzed by Comet assay as described under Materials and Methods. The results obtained in NIH and N2A KD cells are compared with those obtained in Fe65 null MEFs exposed to 20 μM VP16. A minimum of 50 cells per experiment were analyzed. All of the experiments were done in triplicate. (*B*) The phosphorylated form of histone H2AX (γ-H2AX) was measured by Western blot analysis in NIH 3T3 cells transfected with nonsilencing (ns) or Fe65 targeting shRNA and exposed to 20 μM etoposide for 1 h.

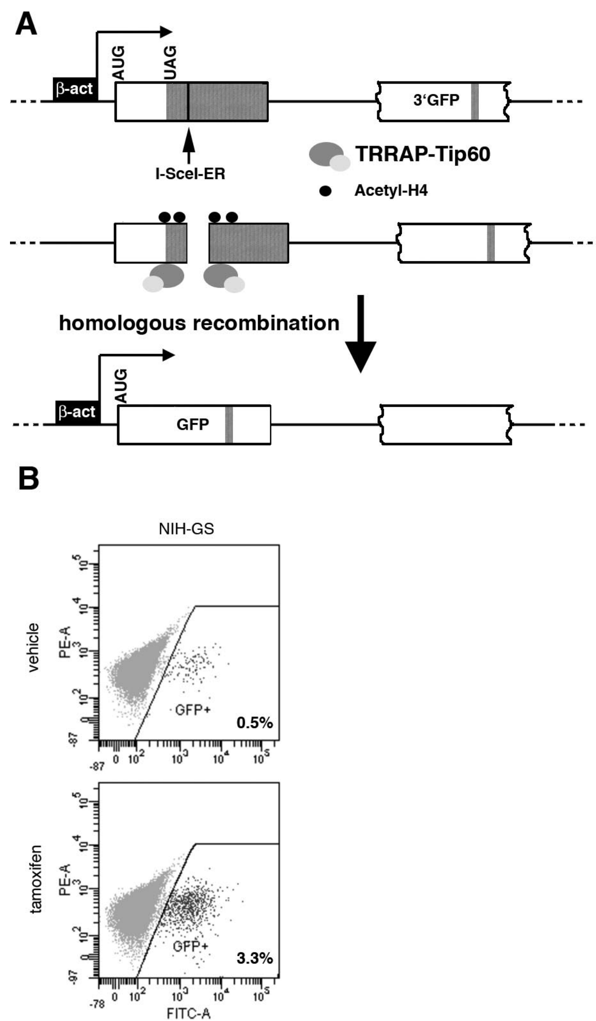

Fig. S2. Generation of NIH-GS cells and measurement of repair efficiency. (*A*) To obtain the NIH-GS clones, NIH 3T3 cells were transfected with pDR-GFP plasmid (2). At 48 h after transfection, cells were selected with puromycin (3 μg/mL) for 5 days. Puromycin-resistant colonies were pooled and amplified under puromycin selection to obtain NIH 3T3-G stable clones. The latter were then transfected with the I-SceI-ER vector (see below). At 48 h after transfection, cells were selected for 9 days with 900 μg/mL G418 and 2 μg/mL puromycin. Double resistant colonies were pooled and amplified. To generate the I-Sce-I ER vector, the estrogen receptor (ER) coding sequence was obtained by amplifying the pBSKS+ER vector (kindly provided by Caterina Missero), with the primers 5glyER-For-HindIII and ER-rev-NotI reported in Table S1. This fragment was cloned into the HindIII/NotI sites of the β-actin promoter vector described in ref. 1. The I-Sce-I cDNA was amplified from the pCβASce vector (2) by using the primers I-SceI BamHI-for and I-SceI SmaI-rev (Table S1) and cloned in frame with the ER in the β-actin promoter vector. As outlined in panel A, 5′ *GFP* sequence was cleaved by I-SceI-ER upon the exposure of the NIH-GS cells to 1 μM 4-Hydroxytamoxifen (tamoxifen) for 6 h. Upon the cleavage, Tip60-TRRAP-containing complex was recruited at the DNA double strand break and histone H4 was acetylated by Tip60 (detected by ChIP experiments; see Figs. 1, 3, 4 and Fig. S3). The oligonucleotide pairs used in ChIP experiments are reported in Table S2. Gene conversion events are able to repair the double strand break induced by I-SceI by using the downstream 3′ *GFP* as donor. (*B*) The upstream 5′ *GFP* gene is under the control of the β-actin gene promoter, but it contains 2 in-frame stop codons that terminate translation, thereby inactivating the gene. The recombination event leads to the reactivation of the GFP gene, because the donor 3′ *GFP* does not contain the above-mentioned stop codons. Therefore, a functional GFP is expressed in the cells where a successful repair takes place and the extent of repair can be measured by counting GFP positive cells by FACS. Two examples of FACS analyses are reported: in NIH-GS cells treated with vehicle the number of GFP positive cells was of ≈0.5%, whereas it was of ≈3.5% in the cells where I-SceI-ER was activated by tamoxifen.

1. Marchion DC, et al. (2005) Valproic acid alters chromatin structure by regulation of chromatin modulation proteins. *Cancer Res* 65:3815–3822.
2. Liu Y, et al. (2008) Involvement of xeroderma pigmentosum group A (XPA) in progeria arising from defective maturation of prelamin A. *FASEB J* 22:603–611.

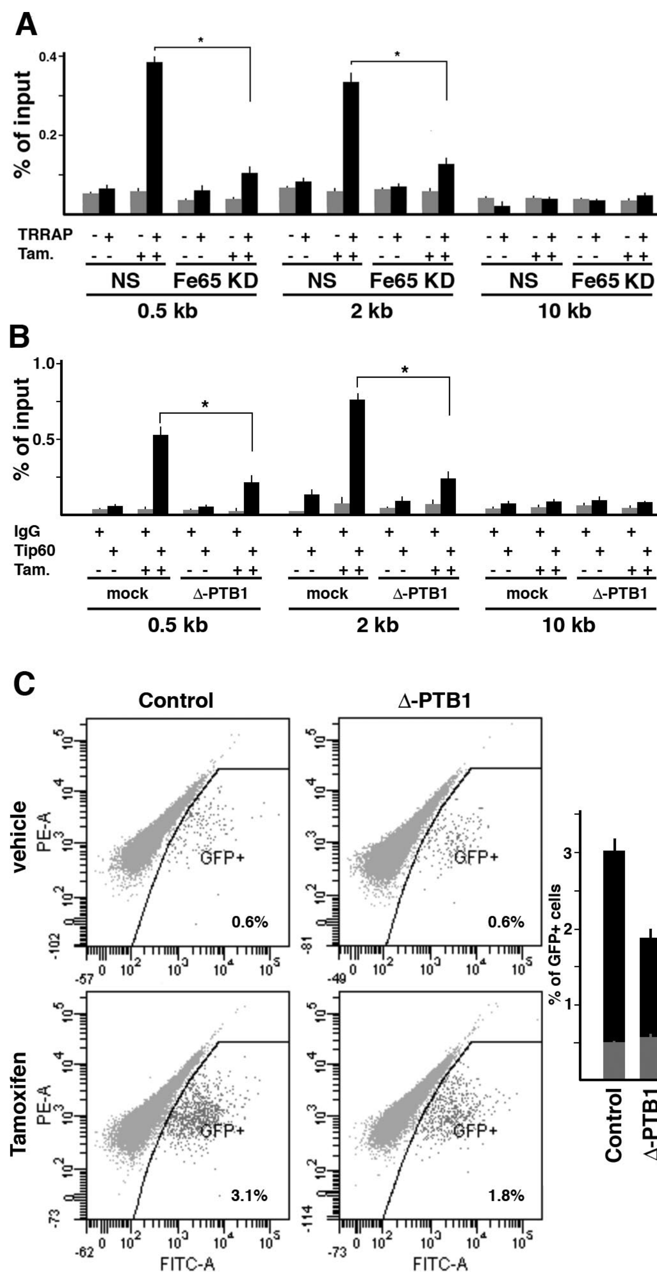

Fig. S3. Δ-PTB1 mutant overexpression decreases Tip60 recruitment to DNA breaks and impairs DNA repair efficiency. (*A*) TRRAP recruitment at DNA break induced by I-SceI was analyzed as described in *SI Text*, by immunoprecipitating chromatin with TRRAP antibody. (*B*) NIH-GS cells were transfected with Δ-PTB1 expression vector. After 24 h, they were treated with 1 μM tamoxifen or with vehicle (−) for 6 h. ChIP was performed with Tip60 antibody or control IgG. Immunoprecipitated chromatin was analyzed by Real time PCR using 3 oligonucleotide pairs located at ≈0.5, 2, and 10 kb away from I-SceI cleavage site. *, $P < 0.01$. (*C*) DNA repair efficiency in NIH-GS cells transfected with Δ-PTB1 was measured by counting the percentage of GFP positive cells, 48 h after the exposure of cells to tamoxifen or to vehicle for 6 h. Representative FACS outputs of 1 experiment are shown. (*Upper*) Cells exposed for 6 h to vehicle. (*Lower*) Results obtained in cells treated with tamoxifen. The histogram reports the mean values of 3 independent experiments. Gray bars indicate the mean values obtained in the presence of vehicle. The difference between the 2 black bars is significant with a $P < 0.01$.

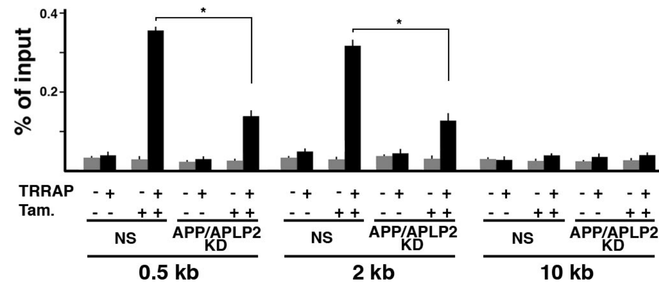

Fig. S4. APP/APLP2 suppression decreases TRRAP recruitment at DNA break induced by I-SceI. histone H4 acetylation at DNA breaks. ChIP were performed as described in Fig. 1, by immunoprecipitating chromatin with TRRAP antibody. *, $P < 0.01$

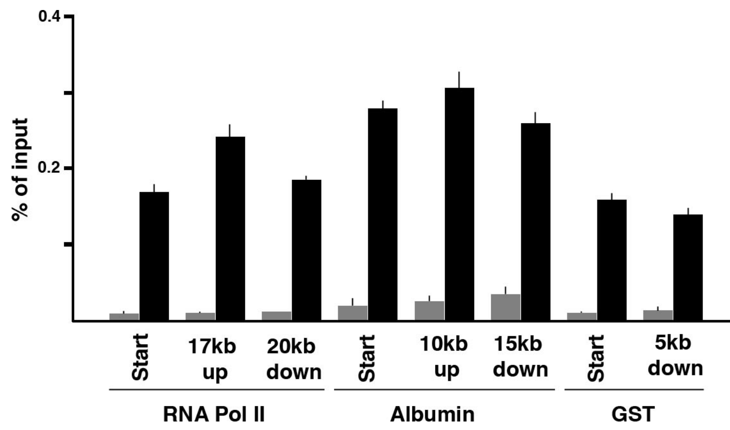

Fig. S5. Fe65 association to chromatin. Chromatin from NIH-GS cells was immunoprecipitated with Fe65 antibody (black bars) or control IgG (gray bars) and amplified with the oligonucleotide pairs reported in Table S3, to explore the presence of Fe65 on 3 genomic loci. The oligonucleotides were designed to amplify regions of the RNA polymerase II, serum albumin and GST genes close to their start sites and at the indicated distances upstream or downstream from the start sites.

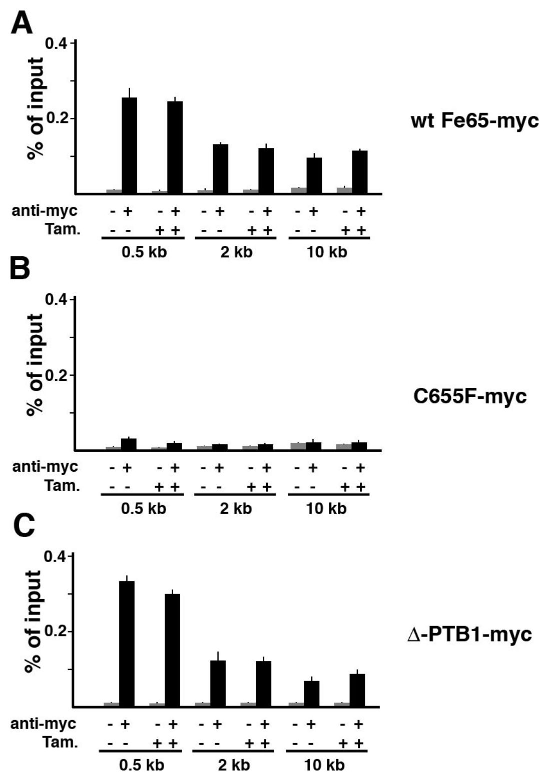

Fig. S6. Wt and mutant Fe65 proteins associated with intact and cleaved DR-GFP locus in NIH-GS cells. The latter were transfected with myc-tagged wt Fe65 (*A*) or C655F (*B*) or Δ-PTB1 (*C*) mutants and then treated or not with tamoxifen to activate I-SceI-ER. cross-linked chromatin was immunoprecipitated with myc antibody or control IgG (−). Association of Fe65s with DR-GFP locus was measured by Real time PCR as reported in Fig. 1. The C655F mutant fails to associate with chromatin.

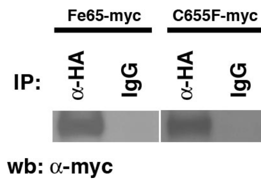

Fig. S7. C655F coimmunoprecipitates with Tip60 to the same extent of wt Fe65. NIH3T3 cells were cotransfected with Tip60-HA and Fe65-myc or C655F-myc. Nuclear extracts were immunoprecipitated with HA antibody (α-HA) or with control IgG and analyzed by Western blot with myc antibody (α-myc).

Table S1. Oligonucleotides used to generate plasmids for transfection experiments

Fe65-Hind III for	5′CCCAAGCTTAAGGCCATGTCTGTTCCATCATCCC-3′
Fe65 Not I rev	5′-CCCGCGGCCGCCTGGGGTCTGGGATCCTAG-3′
ΔPID1-F	5′-CATGCCCAGCCCATCGTCAGCCGCTGCTTGGTAAATGGACTC-3′
ΔPID1-R	5′-GAGTCCATTTACCAAGCAGCGGCTGACGATGGGCTGGGCATG-3′
poliBamHI for	5′-GATCCACCTGCAGCAAGCTTCTCGAGACGCGTGGGCCCTCGCGAG-3′
poliEcoRI rev	5′-AATTCTCGCGAGGGCCCACGCGTCTCGAGAAGCTTGCTGCAGGTG-3′
I- SceI BamHI for	5′-CCCGGATCCAAGGCCATGGGATCAAGATCGCCAAAAAAG-3′
I-SceI SmaI rev	5′-CCCCCCGGGCTTTCAGGAAAGTTTCGGAGGAG-3′
5 glyER-For-HindIII	5′-CCCAAGCTTGGAGGTGCAGGAGGTCGAAATGAAATGGGTGCTTCAGGAG-3′
ER-rev-NotI	5′-AAGGAAAAAAGCGGCCGCTCAGATCGTGTTGGGGAAGCCCTCTGCTTCC-3′

Table S2. Oligonucleotides used in ChIP experiments

0.5 kb F	CCACTACCTGAGCACCCAGTC
0.5 kb R	GGTCACGAACTCCAGCAGGA
2 kb F	TCAGGTGCAGGCTGCCTAT
2 kb R	TTTGTGAGCCAGGGCATTG
10 Kb F	TTCTGATGGAATTAGAACTTGGCAA
10 Kb R	GAACGAGATCAGCAGCCTCTGT

Table S3. Oligonucleotides used in ChIP experiments of Fig. S5

POLII Start F	5′ GATTCTGGGAACGTCGGAGA 3′
POLII Start R	5′ AAAGCTGGAGACGGGAAGC 3′
POLII 17Kb up F	5′ GCTCAGCCAACCACAGTGATT 3′
POLII 17Kb up R	5′ CCTGTCCGAGCGGTGATG 3′
POLII 20Kb down F	5′ CCCTTGGCACCTTCTTTGG 3′
POLII 20Kb down R	5′ ATCAGAGCTAAAACAGAAGTGAGTCAA 3′
ALBUMIN Start F	5′ TGGCAAAATGAAGTGGGTAACC 3′
ALBUMIN Start R	5′ CGAAACACACCCCTGGAAAA 3′
ALBUMIN 10 Kb up F	5′ GCTCCATTTCTCACTGTAATACCATTT 3′
ALBUMIN 10 Kb up R	5′ CCAGTCACCCAGCTAAAACTTAAAA 3′
ALBUMIN 15 Kb down F	5′ CTGGAAGAGGAAACTGGGTGAT 3′
ALBUMIN 15 Kb down R	5′ GACCCTTTGACCACGCAACT 3′
GST Start F	5′ CACATCTAAGCGGTCCTGGTCTA 3′
GST Start R	5′ TGAGACTGAAGCACAGAAAAGCA 3′
GST a 5Kb down F	5′ CCTTCCTCCACTTTTGAGTCTGA 3′
GST a 5Kb down R	5′ TCCCTACGGTCATGTCAATCC 3′
GST Start F	5′ CACATCTAAGCGGTCCTGGTCTA 3′
GST Start R	5′ TGAGACTGAAGCACAGAAAAGCA 3′