Cell Reports

Supplemental Information

Nucleotide Pool Depletion Induces G-Quadruplex-Dependent Perturbation of Gene Expression

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Figure S1 (Related to Figure 1)

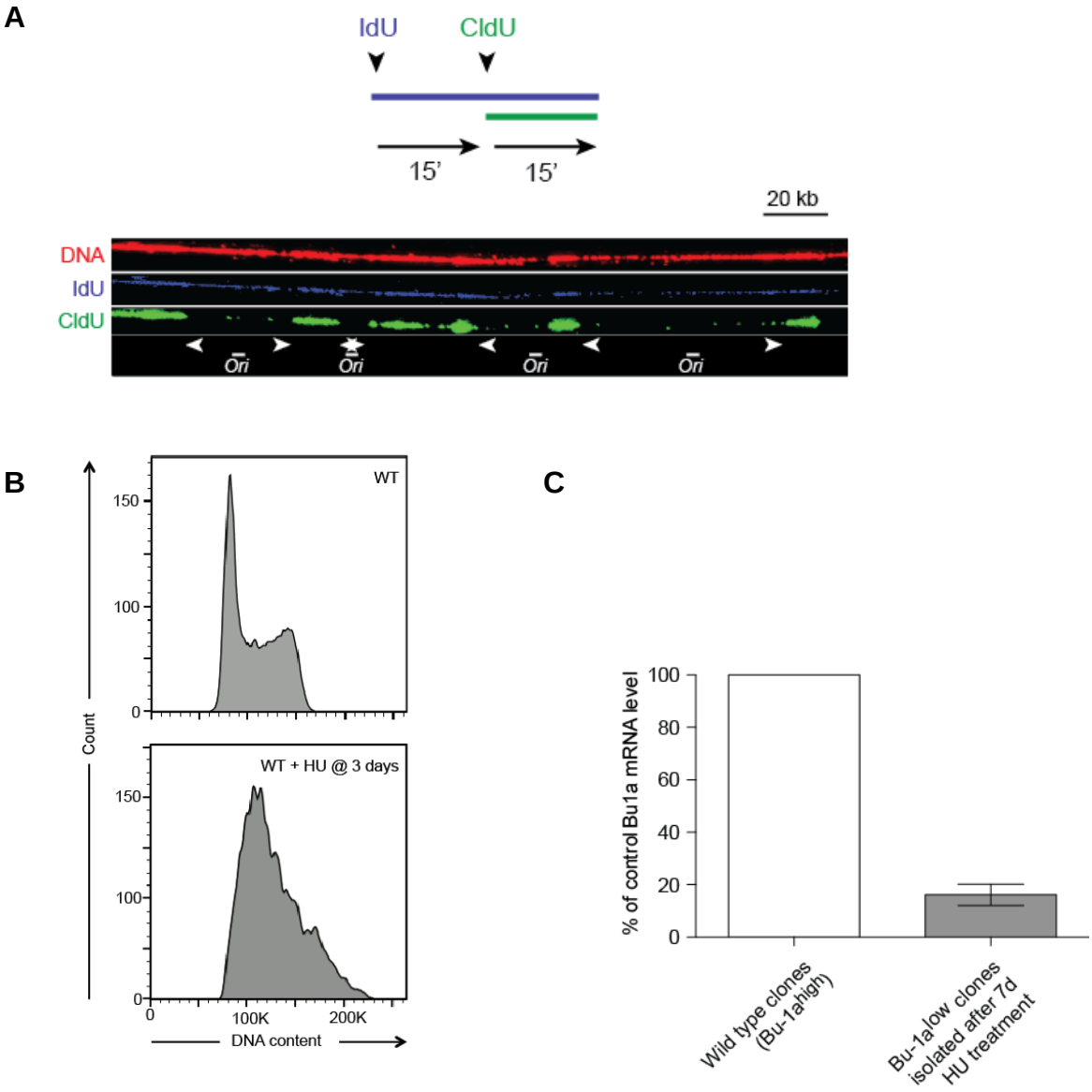


Figure S1. A. Scheme for revealing replication tracts in combed DNA fibres. Cells were labelled with IdU for 15 minutes and then with CldU for a further 15 minutes before being lysed and the DNA combed on glass slides. The labelled tracts of DNA were revealed in blue for IdU and in green for CldU. The DNA itself is stained red. B. Cell cycle profile of unperturbed wild type DT40 cells (upper panel) and after 3 days in 150 µM HU (lower panel). C. qPCR for Bu-1a transcript in five Bu-1a^{low} clones isolated after 7 days culture in 150 µM HU. The transcript level for each clone was normalised to that in bulk untreated wild type cells. Error bar = 1SD.

Figure S2 (related to Figure 2)

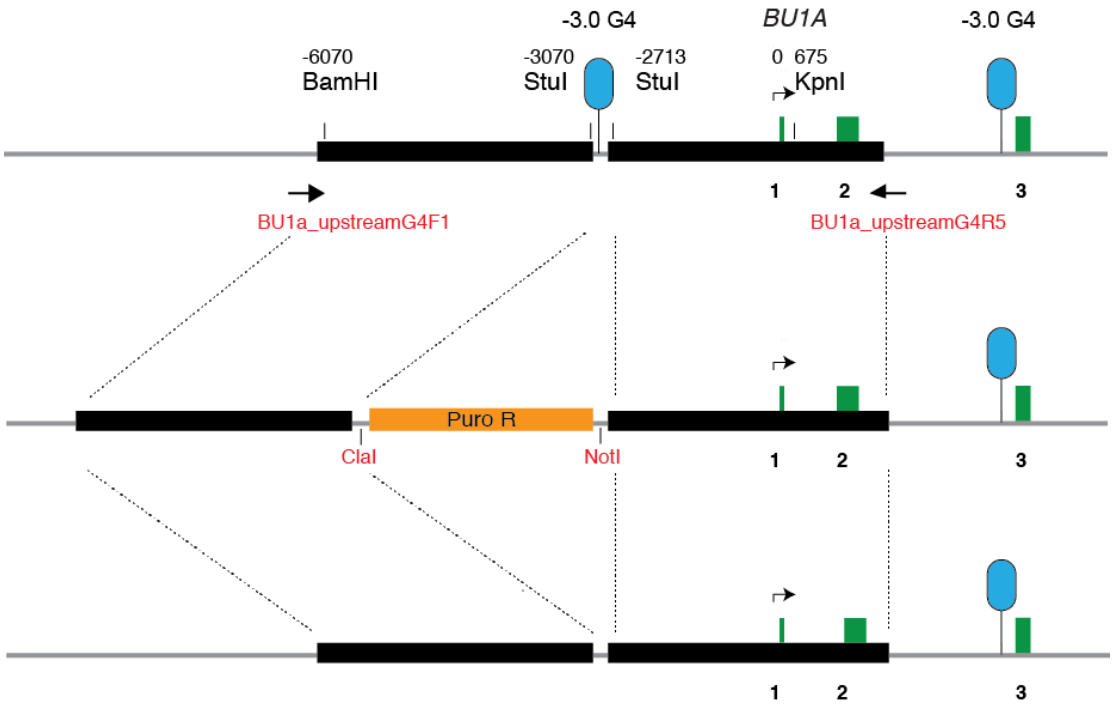
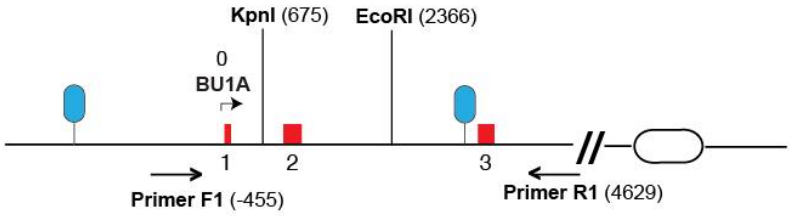


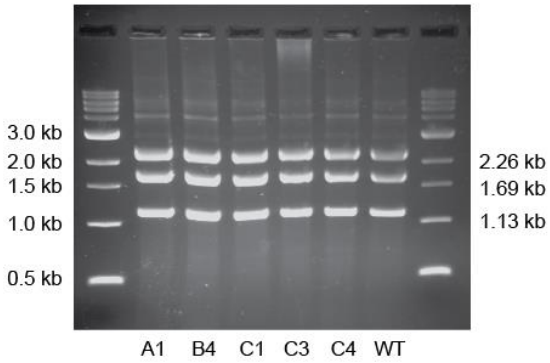
Figure S2. Scheme for deletion of the -3.0 G4 motif. A genomic region either side of the -3.0 G4 motif was amplified with primers Bu1aupstreamG4F1 and Bu1aupstreamG4R5 (Table S3) and cloned into pBluescript vector as a KpnI - BamHI fragment. The -3.0 G4 motif was removed by cutting the endogenous StuI sites, which releases the motif, and replacing it with a linker containing the restriction sites ClaI and NotI. Finally, a selection cassette, flanked by loxP sites and conferring resistance to either puromycin or blasticidin was inserted as a ClaI - NotI fragment. Following transfection and drug selection, screening for successful targeting was performed with PCR using primers Bu1ascreenF1 (amplifying upstream the 5' prime arm of the construct) and Bu1ascreenR6 (at the start of the puromycin selection cassette) or Bu1ascreenR1 (at the start of the blasticidin selection cassette) (Table S3). The selection cassettes were then removed by transient expression of Cre recombinase.

Figure S3 (Related to Figure 3)

A



B



C

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WT  AGAGCTTAGCGAACAAGGAAAATAAAGAGGTGCCATGGGCTGGGTGGGTGCTGTCAAGGGCTGGGATCTAGC
A1  AGAGCTTAGCGAACAAGGAAAATAAAGAGGTGCCATGGGCTGGGTGGGTGCTGTCAAGGGCTGGGATCTAGC
B4  AGAGCTTAGCGAACAAGGAAAATAAAGAGGTGCCATGGGCTGGGTGGGTGCTGTCAAGGGCTGGGATCTAGC
C1  AGAGCTTAGCGAACAAGGAAAATAAAGAGGTGCCATGGGCTGGGTGGGTGCTGTCAAGGGCTGGGATCTAGC
C3  AGAGCTTAGCGAACAAGGAAAATAAAGAGGTGCCATGGGCTGGGTGGGTGCTGTCAAGGGCTGGGATCTAGC
C4  AGAGCTTAGCGAACAAGGAAAATAAAGAGGTGCCATGGGCTGGGTGGGTGCTGTCAAGGGCTGGGATCTAGC
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Figure S3. No evidence of genetic instability in the *BU-1* locus of HU-treated Bu-1a^{low} clones. A. Scheme of PCR and restriction digest of the *BU-1* locus to detect gross deletions. Primers F1 and R1 = Bu-1aG4seq_Forward and Reverse (Table S4). B. Results of PCR and restriction digestion for wild type cells and five Bu-1a^{low} clones. C. Sequence around the +3.5 G4 motif in these clones. The G4 motif is highlighted in red.

Figure S4 (related to Figure 3)

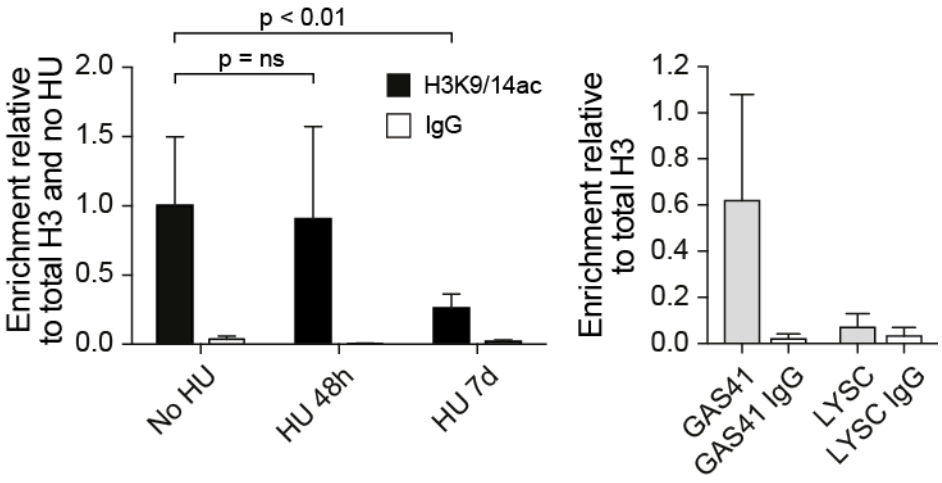

Figure S4. Loss of H3K9/14 acetylation at the *BU-1* promoter following treatment with HU. H3K9/14ac at the *BU-1* promoter in untreated wild type cells and cells treated with 150 μM for 48 hours and for 7 days. The enrichment for each mark is normalised to total H3 and then to the untreated level. The enrichment with non-specific IgG is shown as a control. Positive and negative controls for each mark are show with ChIP at a constitutively active locus, GAS41 and a heterochromatinised locus, LYSC. Error bars show 1 standard deviation for three independent IPs.

Figure S5 (related to Figure 4)

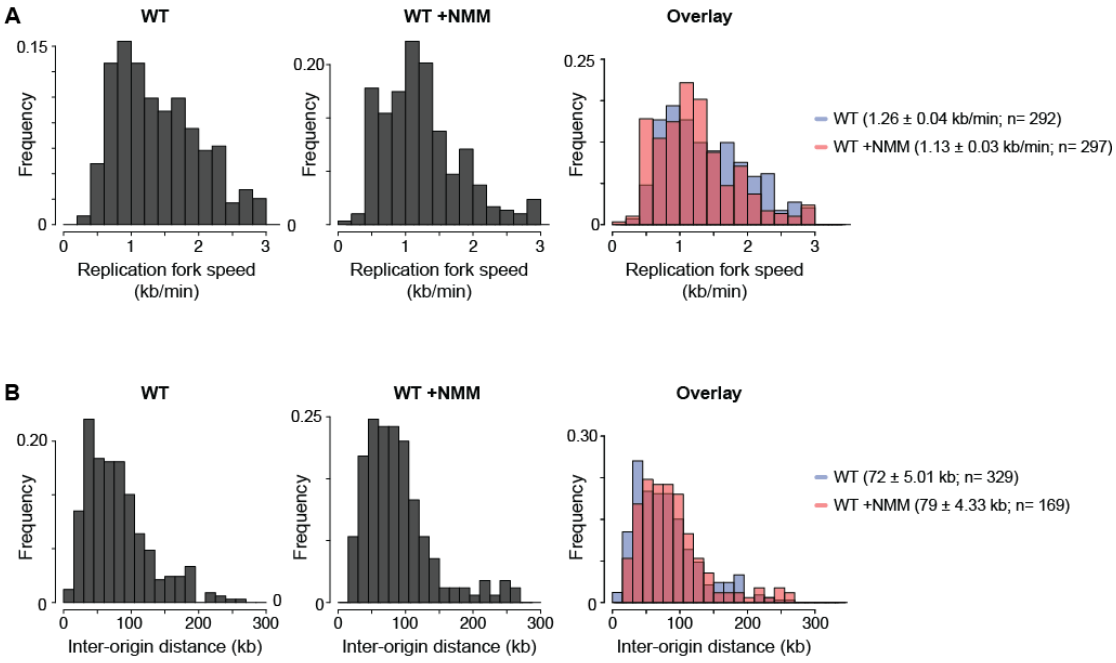

Figure S5. Replication dynamics in cells treated with NMM. A. Replication fork rates in cells treated with NMM. B. Inter-origin distances in cells treated with NMM. The comparator wild type dataset in both cases is that shown in Figure 1B.

Figure S6 (related to Figure 6)

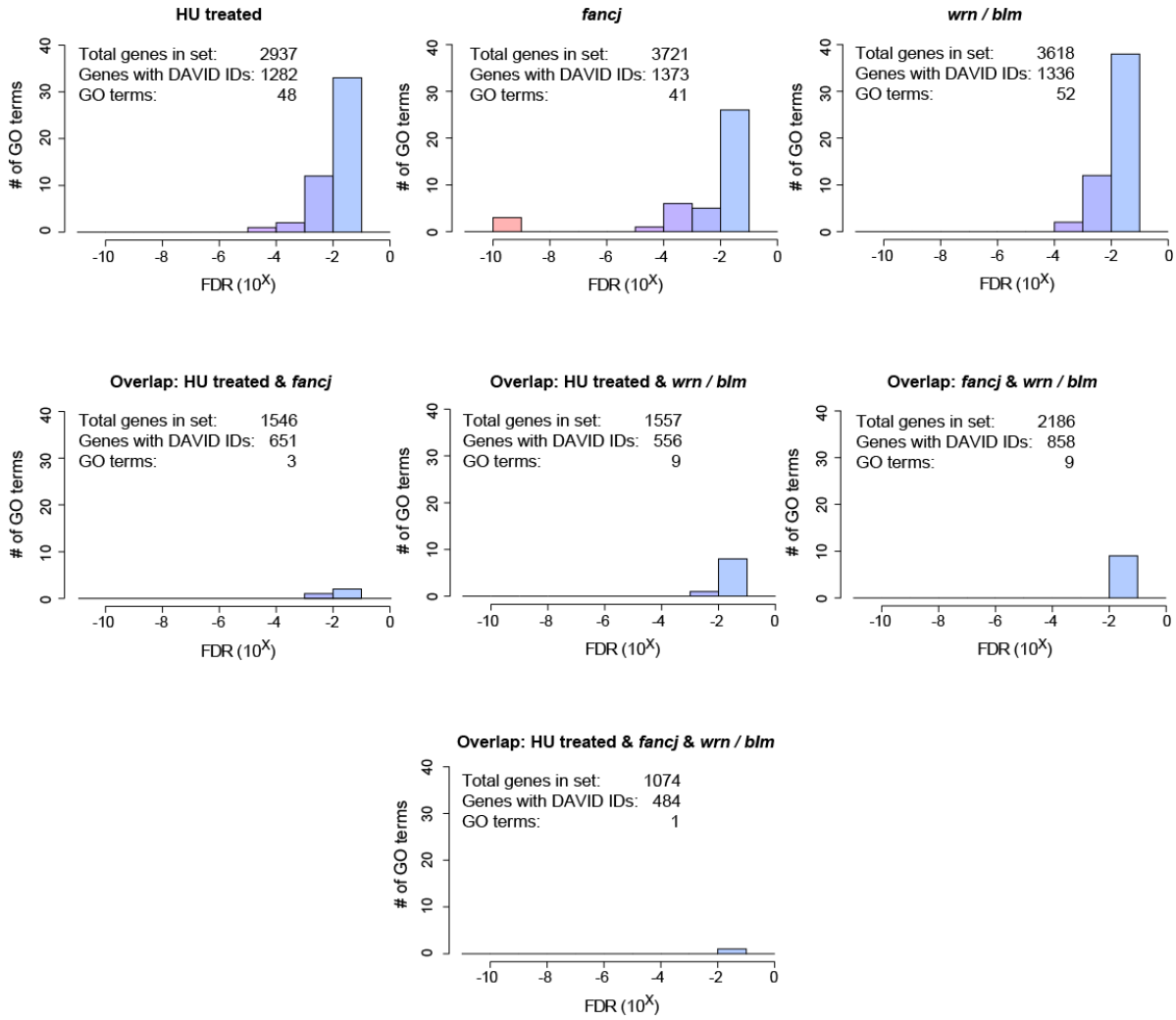

Figure S5. Analysis of dysregulated genes in HU-treated, *fancj* and *wrn/blm* cells by functional annotation. The number of functional annotation terms retrieved by DAVID for each gene set. The annotations are grouped by their false discovery rate (FDR) statistic in bin sizes of 10^{-1}. While each individual condition reveals a diverse range of annotations with significant (FDR < 0.05) enrichment, the genes in the intercept of the three conditions is enriched for only one term, N-glycan biosynthesis (Table S2).

Table S1 (related to Figure 6)

	Genes with at least one G4 motif within 1 kb upstream of the TSS to the end of the gene (%)	n
Whole array	62.64	12643
Genes not dysregulated	59.22	6673
fancj	67.91	3397
wrn/blm	67.88	3225
HU-treated wild type	67.75	2617

Table S1. Association of gene dysregulated in *fancj, wrn/blm* and wild type cells treated with HU with G4 motifs.

Table S2 (related to Figure 6)

A separate Excel workbook listing the functional annotation terms significantly enriched in genes dysregulated in HU-treated, *fancj* and *wrn/blm* cells and the overlap gene sets shown in Figure 6B.

Table S3

Oligonucleotides used for constructs	
Name	**Sequence (5' to 3')**
G4BU-1	CGCGTGGGCTGGGTGGGTGCTGTCAAGGGCTGGG
G4BU-1MUT	CGCGTGGGCTGAGTGGGTGCTGTCAAGAGCTGGG
G4BU-1INV	CGCGTCCCAGCCCTTGACAGCACCCACCCAGCCC
G4#1	CGCGTCGATCGTTGGTTTTGGTTTTGGTTTTGGTA
G4#2	CGCGTCGATCGTGGGTTTGGGTTTGGGTTTGGGTA
G4#3	CGCGTCGATCGTGGGGTTGGGGTTGGGGTTGGGGA
G4#4	CGCGTCGATCGTTTTGGGTGGGTGGGTGGGTTTTA
CEB1	GGGGGGAGGGAGGGTGGCCTGCGGAGGTCCCTGGGCTGA
CEB1MUT	GCGCGGAGTGAGAGTGGCCTGCGGAGGTCCCTGCGCTGA
BU1SalF	AGCGTCGACCGGTCGACGTGC
BU1NotR	AAAAATTTTTAAAAGCGGCCGC
Bu1aupstreamG4F1	CCTGAAGGCCATGTTTGCAC
Bu1aupstreamG4R5	TGCTTGCTTGTCAGATCGCT
Bu1aupscreenF1	TGCCTTTTTCTTTCCCCGTG
Bu1aupscreenR1	AGAGTGAAGCAGAACGTGGG
Bu1aupscreenR6	AGCAACAGATGGAAGGCCTC

Table S4

Oligonucleotides used for ChIP & qPCR	
Name	**Sequence (5' to 3')**
LYSCpromF	CCACATTGTATAAGAAATTTGGCAA
LYSCpromR	AAAACGCCTCTTGAGTATACAGAA
GAS41promF	CGTGAACTGCGCGAAGAAG
GAS41promR	CCCCCGCCACCTACCA
BU1ApromF	CTCTGTAGCCAGATCGTCTTCTC
BU1ApromR	GTGTCAGCTCATCTAGGCAAATC
β actin Forward qPCR	TGTCCACCTTCCAGCAGATGT
β actin Reverse qPCR	AGTCCGGTTTAGAAGCATTTGC
Bu1a Forward qPCR	CTGTTACTGATGGCTCTGCTACC
Bu1a Reverse qPCR	CTCCAGTTTCAGACATCTCTTGG
Bu1a Forward	CGGTCGACGTGCAGCTAGACCAGAGTAGGTATT
Bu1a Reverse	GGATCGATGGATCTCCATAGACAGATGAGGAC
Bu1a G4seq Forward	GGGAATTCAAGGCTGACTCTCCTCTGAAGCTA
Bu1a G4seq Reverse	GCGGATCCGGAGCACATCACTAAGTAACCAGAC

Supplemental Experimental Procedures

Functional annotation analysis of dysregulated gene sets

A search for functional annotations of the genes within each set (HU-treated, *fancj* and *wrn/blm*) and their overlaps was carried using DAVID Bioinformatics Resources (Huang et al., 2009a; Huang et al., 2009b) without change in default settings. For each mutant, a functional annotation chart of de-regulated genes was been generated. Categories whose false discovery rate (FDR) value was lower than 0.05 were included in the analysis.

Supplemental References

Huang, W., Sherman, B.T., and Lempicki, R.A. (2009a). Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res *37*, 1-13.

Huang, W., Sherman, B.T., and Lempicki, R.A. (2009b). Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc *4*, 44-57.