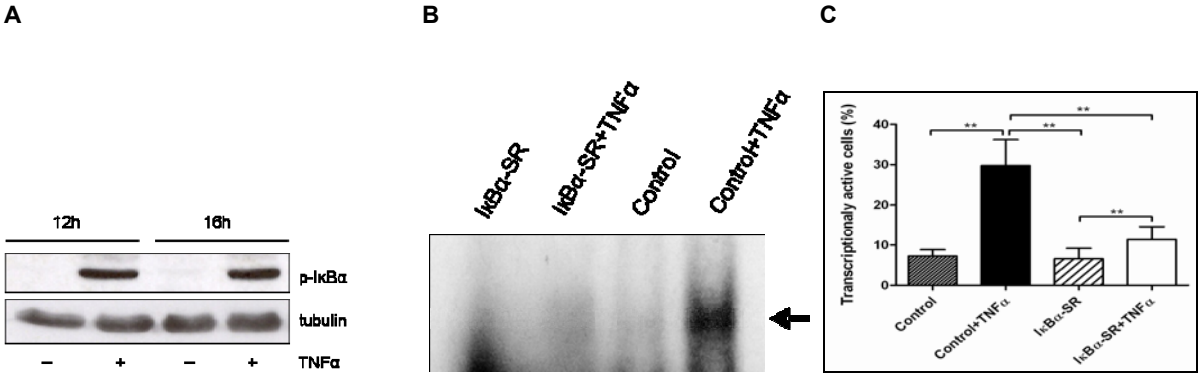


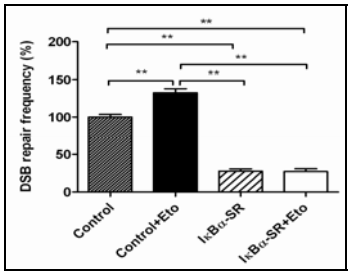
Supplementary Figure S1. Activation of NF-κB by TNFα.

(**A**) K562(Δ/3´) cells were treated with 10 ng/ml TNFα for 12h and 16h, when protein extracts were prepared and Western blotted for the detection of phosphorylated IκBα (p-IκBα).

(**B**) K562(Δ/3´) cells were transfected with pCMV-I-SceI and pcDNA3.0-IκBα-SR expression or empty control plasmid. Following cultivation for 24h, cells were mock-treated or exposed to TNFα (10ng/ml) for 30 min, whole-cell extracts prepared and NF-κB-specific electrophoretic mobility shift (EMSA) performed essentially as described in Karl *et a*l. (6) The arrow indicates the protein-DNA complex specifically formed after TNFα-treatment.

(**C**) K562(Δ/3´) cells were transfected via electroporation with pCMV-Sce-I, pTRF-NF-κB-dscGFP reporter plasmid (System Biosciences, CA, USA) or wt*EGFP* expression plasmid and pcDNA3.0-IκBα-SR expression plasmid or empty vector. Cells were cultivated for another 24h, when they were treated with TNFα (10 ng/ml). 48h after transfection cells were FACS analysed for GFP-positivity. Transcriptionally active cells in % were calculated as the fraction of green fluorescent cells compared to the total cell population and normalized in each case with the individual transfection efficiency determined with wtEGFP expression plasmid each. Mean values and SEMs from N = 9 (**P<0.01).

A



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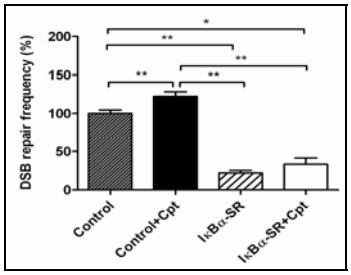

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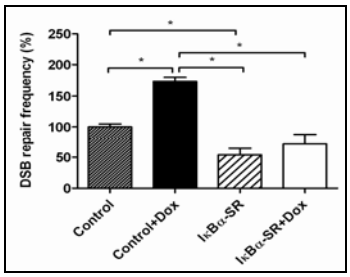


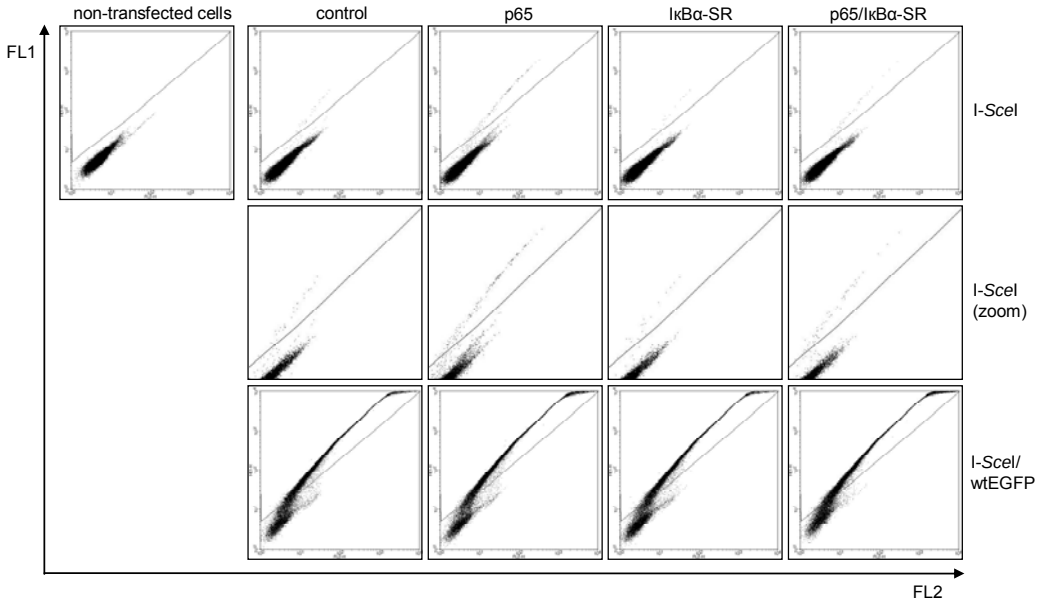
Supplementary Figure S2. DSB-repair after treatment with low concentrations of topoisomerase inhibitors.

DSB-repair was examined by the use of the *EGFP*-based test system as described in Akyüz *et al*. (9) To determine DSB-repair frequencies, K562(Δ/3′) cells were transfected via electroporation (Bio-Rad Laboratories, Hercules, CA, USA) with pCMV-I-SceI, pBS or wt*EGFP* expression plasmid and pcDNA3.0-IκBα-SR or empty vector. Cells were split into two aliquots and cultivated for 24h when etoposide, camptothecin, or doxorubicin (Sigma-Aldrich, München, Germany) were added at the indicated concentration. Transfected cells were cultivated for another 24h (B) and 48h (A,C), when they were analysed flow cytometrically for EGFP-positivity. DSB repair frequencies were determined as the fraction of green fluorescent cells compared to the total cell population and normalized in each case with the individual transfection efficiency. DSB-repair frequencies in mock-treated controls were defined as 100% each (absolute mean value: 1 x 10^{-3}) ; *P<0.05; **P<0.01.

(**A**) DSB-repair stimulation after treatment with 1 μM etoposide (Eto). Mean values and SEMs from N = 9;

(**B**) DSB-repair stimulation after treatment with 30 nM camptothecin (Cpt). Mean values and SEMs from N = 9;

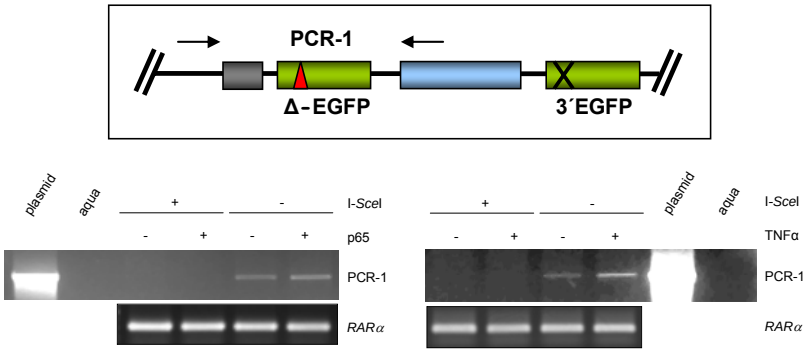
(**C**) DSB-repair stimulation after treatment with 0.3 μg/ml doxorubicin (Dox). Mean values and SEMs from N = 6;



events	0	33	156	20	60
EGFP-positivity (%)	0	0.07	0.31	0.04	0.12
transfection efficiency (%)	n.a.	81	83	77	81

Supplementary Figure S3. Primary flow cytometric data visualizing quantification of EGFP-positive cells. K562(Δ/3´) cells were either left untreated (non-transfected cells) or co-electroporated with pCMV-I-SceI for I-*Sce*I-mediated cleavage of the chromosomally integrated DSB repair substrate, pcDNA3.0 or pcDNA3.0-p65 and pcDNA3.0 or pcDNA3.0-IκBα-SR as for the experiment in Figure 3E. Following cultivation for 72h 50 000 living cells were examined to determine EGFP-positive and EGFP-negative cells by the diagonal gating method in the FL1/FL2 dot plot (FACS Calibur® FACScan, BD Biosciences, Heidelberg, Germany) (9). Representative primary flow cytometric data (complete dot plots and and zoomed in regions with EGFP-positive cells in the upper left triangle) are shown together with the corresponding numbers of EGFP-positive cells (events) as well as the percentage of EGFP-positive cells in the total population (EGFP-positivity, %). Each transfected sample was accompanied by a sample transfected with the same DNA mixture except that filler plasmid pBS was substituted by wt*EGFP* expression plasmid to determine the transfection efficiency, which in turn was used for individual normalization of the fraction of EGFP-positive cells to calculate the DSB-repair frequency. n.a., not applicable;

A



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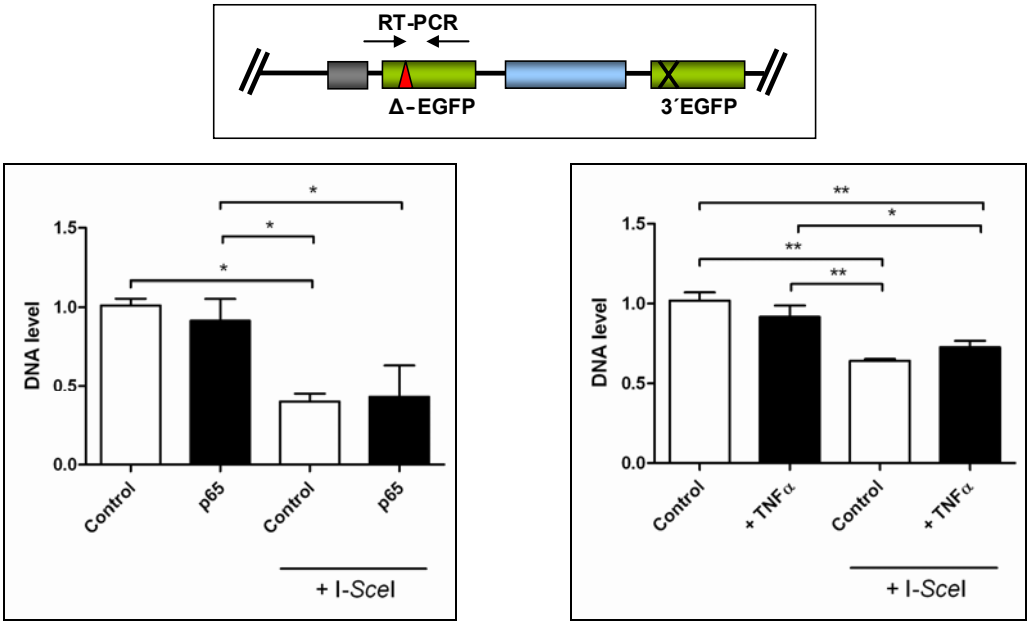
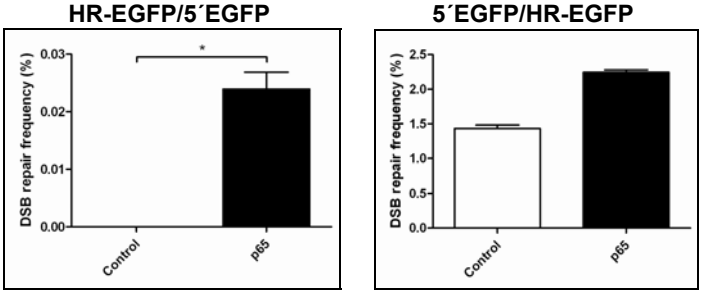


C

HR-EGFP/5´EGFP	HR-EGFP 5´EGFP
5´EGFP/HR-EGFP	5´EGFP HR-EGFP

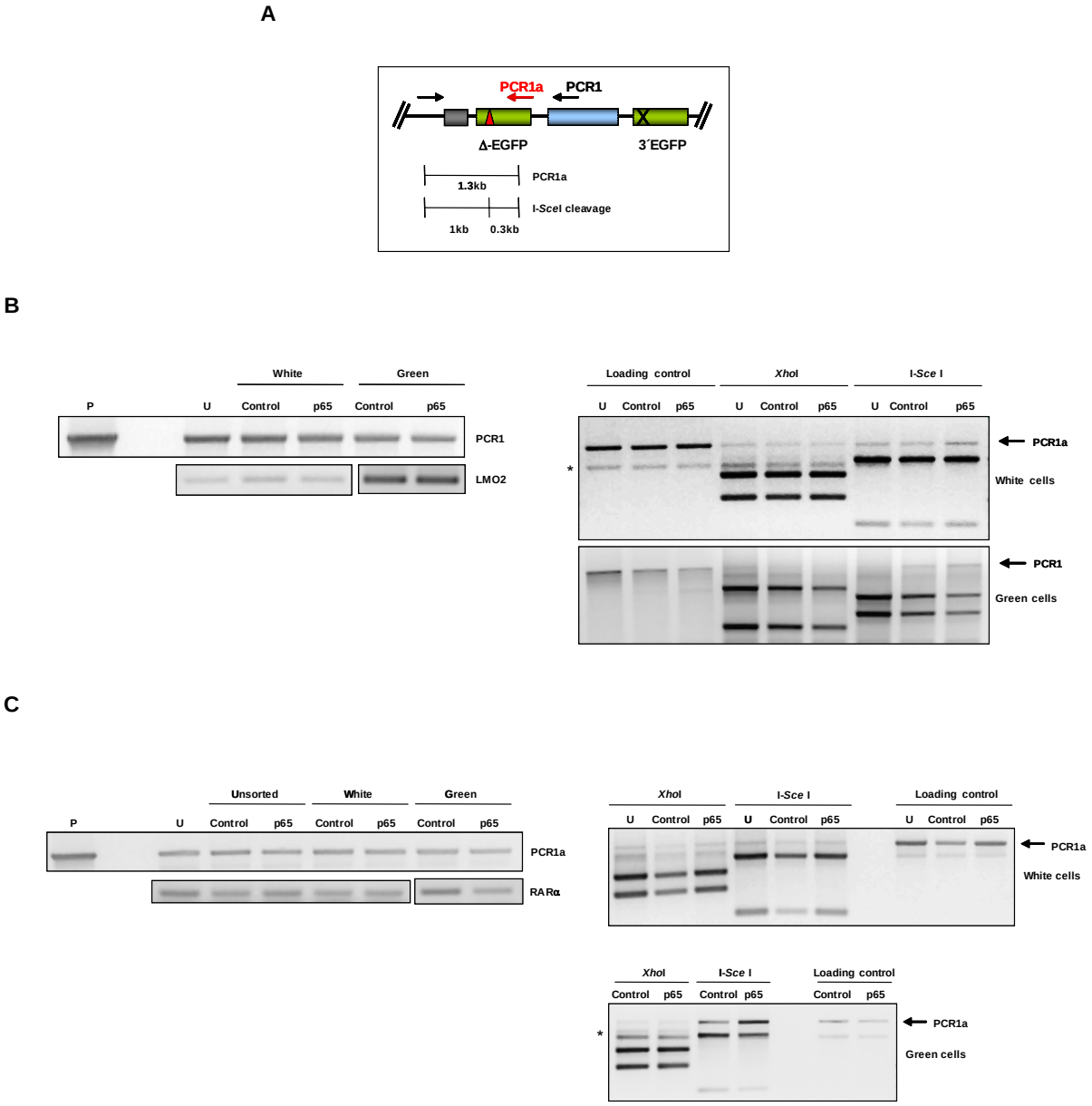


Supplementary Figure S4. I-*Sce*I cutting efficiency and I-*Sce*I-independent DNA exchange processes.

(**A**) K562(Δ/3´) cells were transfected with or without I-*Sce*I meganuclease expression plasmid. Subsequently, genomic DNA was isolated and I-*Sce*I-mediated cleavage within Δ-EGFP/3´EGFP visualized by PCR utilizing primers encompassing the mutated *EGFP* gene *Δ-EGFP*. Successful amplification of the corresponding 2.3 kb PCR fragment (PCR-1) indicated integrity of *Δ-EGFP*, failure to amplify PCR-1 indicated cleavage within *Δ-EGFP*. Genomic PCR conditions and PCR-1 specific primers were essentially as described in Akyüz *et al*. (9) As controls we amplified a 0.3 kb genomic DNA fragment within the RARα gene by use of the following oligonucleotides: 5´-AGGAGGAGATCTATCGATAGTGGCCGGCTTTGAATATCCTG-3´ and 5´-GCTGCTAGATCTATCGATAAGCCT-CCAGCACCCCATCACT-3´. To compare I-*Sce*I mediated cleavage as a function of NF-κB, we co-transfected cells with I-*Sce*I expression plasmid or empty vector and p65 expression plasmid or empty vector (as in Figure 3) and isolated genomic DNA 4h after transfection. In addition, we transfected cells with or without I-*Sce*I, cultivated the cells for 24h, then treated with 10 ng/ml TNFα (as in Figure 1) and harvested cells after re-incubation for 8h. Mutated *EGFP* genes: green boxes; spacer sequence (hygromycin resistance gene cassette): blue box; I-*Sce*I site: triangle; deleted *EGFP* sequence: cross; plasmid, positive control with plasmid DNA comprising substrate Δ-EGFP/3´EGFP; aqua, negative control without DNA.

(**B**) For quantitative comparison of I-*Sce*I-mediated cleavage quantitative Real-Time PCR (RT-PCR) was performed on genomic DNA samples described in (A). To enable specific amplification and quantification of the 5´positioned and I-*Sce*I cleaved *EGFP* variant *Δ-EGFP* (and not 3´EGFP), it was necessary to perform nested PCR. In the first genomic PCR step, PCR conditions and primers were as in (A) resulting in the 2.3 kb PCR-1 fragment. In the second quantitative PCR step, RT-PCR was performed on template PCR-1 with the following primers: 5'-GTAATGCTTCAGCCGCTACC-3' and 5'-ACCTTGATGCCGTTCTTCTG-3' (Thermo Scientific) , which amplified an internal 0.3 kb region as indicated above. For RT-PCR experiments we used Quanti Tect SYBR® Green PCR Kit (Qiagen) and an ABI PRISM™ 7700 Sequence Detector (Applied Biosystems). Mean DNA levels specific for *Δ-EGFP* and quantified in control samples were defined as 1.0 and relative DNA levels calculated from a standard curve. I-*Sce*I-mediated cleavage caused statistically significant, relative decreases down to 0.39 and 0.42 -/+p65 and down to 0.64 and 0.72 -/+TNFα, respectively. Note that due to competition between cleavage and immediate DSB-repair (particularly by the fast process of canonical NHEJ) the full degree of cleavage cannot be visualized. Correspondingly performed RT-PCR specific for the RARα gene fragment described in (A) did not reveal any differences (data not shown). Columns, mean values of N = 9; bars, SEM; *P<0.05; **P<0.01.

(C) K562 cells were transfected with repair substrates HR-EGFP/5´EGFP or 5´EGFP/HR-EGFP plus p65 expression plasmid or empty vector in the absence of I-*Sce*I meganuclease. DNA exchange frequencies were evaluated 48h after transfection. Columns, mean values of N = 3-6; bars, SD; *P<0.05.



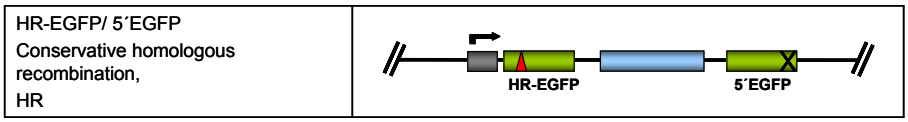
Supplementary Figure S5. Genomic PCR analysis of DSB repair.

(**A**) Schematic presentation of the primer positions for PCR1 and PCR1a within the DSB repair substrate Δ-EGFP/3´EGFP, which is chromosomally integrated in K562(Δ/3´) cells, and of the fragment sizes obtained after I-*Sce*I digestion. Note that the 5´ positioned primer is shared by both PCR reactions. arrow, PCR primer;

(**B**) K562(Δ/3´) cells were transfected with 10 µg pCMV-I-SceI, 10µg pBS/wt*EGFP* plasmid, and 40 µg pcDNA3.0-p65 (p65) or pcDNA3.0 (Control), followed by cultivation for 72h. Subsequently, cells were FACS sorted for green EGFP-positivity and white EGFP-negativity. Genomic DNA was isolated and PCR performed with primers encompassing Δ-EGFP as previously described (9,10). As internal control, we amplified a genomic *LMO2* fragment with the following primers: 5´- AGGAGGAGATCTATCGAT-3´ and 5´-GCTGCTAGATCTATCGAT-3´ (Thermo Scientific). In order to detect copies of substrate Δ-EGFP/3´EGFP that had undergone error-prone NHEJ in white cells and HR in green cells, nested PCR1a was performed on the respective PCR1 amplification product (PCR1a-specific, 3´ positioned primer: 5'-ACCTTGATGCCGTTCTTCTG-3'). PCR1a amplification product (Loading control) was then restriction digested by I-*Sce*I or the control enzyme *Xho*I. The I-*Sce*I resistant PCR1a band was quantified and normalized with the amount of DNA each. P, positive plasmid control; U, untransfected; Unsorted, transfected but unsorted; asterisk, additional, unspecific PCR1a band;

(**C**) WTK1(HR/3´) cells, carrying chromosomally integrated DSB repair substrate HR-EGFP/3´EGFP, were subjected to genomic PCR and restriction analysis as K562(Δ/3´) cells in (B). As internal control, we amplified a genomic *RARα* fragment as in Supplementary Figure S4A.

A



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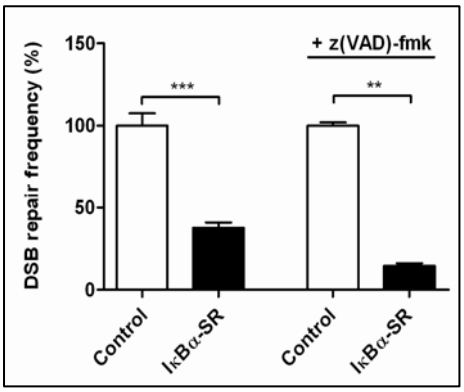

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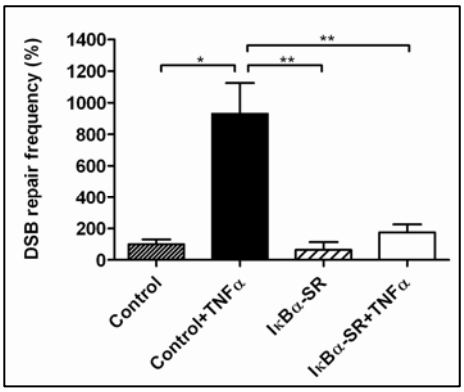
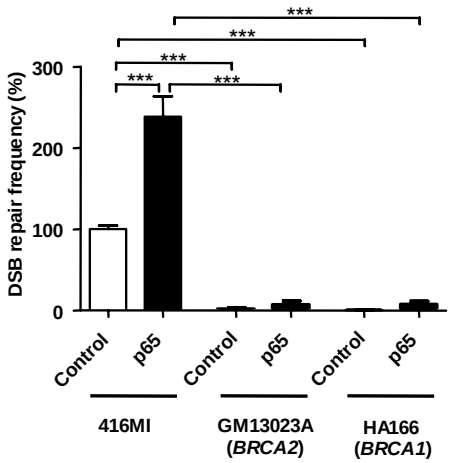


Supplementary Figure S6. Analysis of HR as a function of IκBα-SR and TNFα.

(**A**) HR-EGFP/ 5'EGFP substrate design to assess HR.

(**B**) U87MG cells stably expressing IκBα-SR and the empty vector control cell line were transfected with pCMV-I-SceI and HR repair substrate. Cells were analysed for green fluorescence 24h after transfection. In parallel experiments cells were treated with 25μM z(VAD)-fmk. Mean values of controls were taken as 100% each (absolute mean value 2.5 x 10^{-3}). Columns, mean values of N = 9; bars, SEM; **P<0.01; ***P<0.001.

(C) K562 cells were electroporated with pCMV-I-SceI, HR repair substrate plus pcDNA3.0-IκBα-SR expression plasmid or empty vector. After 24h cells were treated with TNFα (10 ng/ml), cultivated for another 24h when DSB repair frequencies were evaluated by FACS analysis. Mean frequencies in controls were taken as 100% each (absolute mean value 0.4 x 10^{-3}). Columns, mean values of N = 9; bars, SEM; *P<0.05; **P<0.01.



Supplementary Figure S7. Analysis of HR in lymphoblastoid cell lines carrying *BRCA2* or *BRCA1* mutations.

Lymphoblastoid cell lines derived from patients with mutations in *BRCA2* (GM13023A) or *BRCA1* (HA166) and control cells from a healthy individual (416MI) were described previously (Keimling *et al*., 2011; 20). Cells were electroporated with 10µg pCMV-I-SceI, 10 µg HR-EGFP/5′EGFP repair substrate, 10µg pBS/wt*EGFP*, and 20µg pcDNA3.0-p65 (p65) or pcDNA3.0 (Control). Then, cells were cultivated for additional 48h, when they were FACS analysed for EGFP positivity. Mean values of 416MI controls without p65 expression were taken as 100% each (absolute mean value: 0.3 x 10^{-2}). Columns, mean values of N = 6; bars, SEM; ***P<0.001.