**Additional file 1: Figure S1.**

Analysis of Venus positive cells in HEKTLR6 cells upon transfection with Cas9 and Rosa26 specific sgRNA. Positive cells (0.48%) were isolated by FACS sorting and the target region was amplified from genomic DNA, subcloned and sequenced. Two analysed clones (Seq clone A, B) showed a deletion of 14 bp that removes a part of the Rosa26 target sequence and restores the Venus reading frame and the critical Arginine codon 96 (R96). The adjacent codons 95 and 97 are also replaced (T, W) but seemingly do not disrupt Venus fluorescence. (PDF, 195 kb) (EPS 561 kb)

**Additional file 2: Figure S2.**

Intracellular localization of Rad18UBD protein in HEK cells. HEK293 cells were transfected with an expression vector for FLAG tagged Cas9 (pX330, Addgene 42,230) (A) or FLAG tagged Rad18UBD (B) using XtremeGene transfection reagent. After 48 h cells were treated for 10 min with H2O2 (500 μM) and fixed after 1 h in 4% paraformaldehyde. Fixed cells were stained in PBS, 0.2% Triton X-100, 3% BSA with antibodies against phospho-H2AX (mouse mAb, clone JBW301, Millipore #05–636, 1:500) and FLAG Tag (rabbit mAb, Cell Signaling Technology # 14793, 1:800) for 1 h. After washing slides were incubated for 1 h with secondary goat antibodies against mouse IgG (Alexa Fluor 594, Life Technologies #A-11032, 1:1000) and rabbit IgG (Alexa Fluor 488, Life Technologies A11034, 1:1000), washed and incubated for 10 min in Hoechst 33342 stain (Life Technologies H3670, 1:2000). After washing images were acquired using a Keyence BZ9000 microscope. In (B) the FLAG Tag signals are colocalized with γH2AX foci. (PDF, 986 kb)

**Additional file 3: Figure S3.**

DSB repair modification by Gal4-Rad18UBD and -RNF169UBD fusion proteins in HEKTLR6 reporter cells. Fusion constructs for Gal4 or BRCA1 with Rad18UBD or RNF169 UBD were cotransfected with the matching TLR HDR repair template (TLR-donor-UAS), sgRNA and Cas9 into HEKTLR6 cells. The frequency of Venus and RFP positive cells was measured by FACS analysis 72 h after transfection. The HDR frequency is reported by Venus (green bars) while the fraction of NHEJ events in reading frame + 2 is reported by RFP expression (red bars). The bars represent mean values ± standard deviation, Y-axis represents the frequency of Venus or RFP positive cells in percent while the X-axis shows samples transfected in combinations, as shown in the table below. Samples 1 and 2 are controls showing the basic frequency of RFP+ only and of Venus+ cells in addition when TLR-donor-UAS is provided as repair template. As compared to BRCA1 alone (sample 3) the expression of BRCA1-Rad18UBD or RNF169UBD fusions strongly increased the Venus/RFP ratio to values of 2.94 and 3.0 (samples 4 and 5). The expression of Gal4-Rad18UBD or Gal4-RNF169UBD increased the Venus/RFP ratio by a factor of 4 or 5.9, from 0.34 (sample 2) to values of 1.37 or 2.0 (samples 6 and 7). The combined expression of Gal4-UBD with BRCA1-UBD fusions further increased the Venus/RFP ratio to a value of 3.14 in fusion with Rad18UBD and to 3.29 in fusion with RNF169UBD. This increase however was mostly if not entirely attributed to the effect of BRCA1-UBD fusion proteins alone that lead to HDR/NHEJ ratios of 2.94 and 3.0 (samples 4 and 5). Data from three independent experiments, each with three replicates per sample, are presented as mean values ± S.D. Significance of samples in comparison to the control sample 2 with sgRosa/Cas9 and TLR-donor-UAS was determined by two-way ANOVA and Dunnett’s multiple comparison tests with \*\*\*P < 0.001 (HDR) and ###P < 0.001 (NHEJ). (PDF, 271 kb). Raw data are shown in the Supplementary data file. (EPS 313 kb)

**Additional file 4: Figure S4.**

Sequence-based DSB repair assay using Rad18UBD and RNF169UBD fusion proteins in HEKTLR6 reporter cells. Transfected reporter cells from one of the assays analysed by FACS (Fig. 4; experiment 1 in the supplement data file) were used for PCR amplification of the reporter target region from genomic DNA (A), isolated from pooled cells of the triplicate samples used for FACS analysis 72 h after transfection. (B) PCR products were sequenced by amplicon sequencing and the fraction of reads showing HDR (green bars) or Indel events (red bars) is shown in relation to the total number of reads with gene editing events on the Y-axis and was used to calculate the ratio of HDR/NHEJ DSB repair. The X-axis shows the transfected samples and the selection of cotransfected plasmids below. Samples 1 and 2 are controls showing the basic frequency of Venus+ and RFP+ cells upon transfection with Cas9 and sgRNA or in combination with TLR-donor-tetO as repair template. The fraction of sequence reads representing the 14 bp deletion causing Venus background expression (Figure S1) is given as ‘percent background’. Raw data are shown in the Supplementary data file. (EPS 1295 kb)

**Additional file 5: Figure S5.**

Distribution of reading frames within the mutagenic NHEJ repair products in HEKTLR6 reporter cells. Using CRISPResso analysis of the amplicon sequencing data shown in Figure S4 we calculated for each sample the distribution of the reading frames + 1 (Venus expression frame), + 2 and + 3 among the repair products showing + 1 insertions or deletions from − 1 to − 12 nucleotides. RFP expression becomes activated in the TLR-6 construct in the reading frame + 2 by the deletion of 1, 4, 7 or 10 nucleotides. Of note, the frequency of reading frame + 2 products is lowest in sample 1 in the absence of pTLR-donor. Raw data are shown in the Supplementary data file.

**Additional file 6: Figure S6.**

DSB repair modification by Rad18UBD and RNF169UBD fusion proteins in hiPS reporter cells. Fusion constructs for BRCA1 or TetR with Rad18UBD or RNF169 UBD were cotransfected with the TLR HDR repair template (TLR-donor-tetO), sgRNA and Cas9 and analysed for Venus and RFP positive cells using FACS analysis 72 h after transfection. The HDR frequency is reported by Venus (green bars) while the fraction of NHEJ events in reading frame + 3 is reported by RFP expression (red bars). The bars represent mean values ± standard deviation, Y-axis represents the frequency of Venus or RFP positive cells in percent while the X-axis shows samples transfected in combinations, as shown in the table below. Controls show the basic frequency of RFP+ cells upon transfection of Cas9 plus sgRNA, and of Venus+ and RFP+ cells in the presence of the repair template TLR-donor-tetO. Expression of i53, BRCA1 or BRCA1-UBD fusion proteins showed levels of Venus+ cells that were statistically not significantly different as compared to sample 2, except for BRCA1-RNF169UBD (samples 5–8) and the TetR-UBD fusions (sample 7 and 8). The coexpression of i53 with a single Rad18UBD or RNF169 UBD fusion (samples 9–12) significantly enhanced the Venus/RFP ratio by a factor of 7–16.6. The combined expression of the two fusion proteins BRCA1-Rad18UBD and TetR-Rad18UBD (sample 13) or BRCA1-RNF169UBD and TetR-RNF169UBD (sample 14) led to 4–6-fold increase of the Venus/RFP ratio . The expression of full length Rad18 or RNF169 proteins (sample 15 and 16) did not show significant difference to the control sample. Data are shown as mean values ± SD from two independent experiments into BCRT or JWT iPS cells, each with three replicates per samples, normalized to the values of sample 2 as control. Statistical significance of values in comparison to the control sample 2 with sgRosa/Cas9 and TLR-donor-tetO was determined by two-way ANOVA and Dunnett’s multiple comparison tests with \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001 (HDR) and ##P < 0.01, ###P < 0.001 (NHEJ). Raw data are shown in the Supplementary data file. (EPS 449 kb)

**Additional file 7: Figure S7.**

Flow cytometry controls for DSB repair assay in TLR reporter cells. FACS gating scheme for BFP and Venus positive cells in HEKTLR6 reporter cells. Cells were untransfected (A) or transfected either with pU6Rosa-CAG-Cas9 with pTLR donor (B) or with a Venus (C) or BFP (D) expression plasmid. Single cells were gated by using a forward scatter plot. Transfected cells were gated based on expression of BFP, Venus or RFP compared to the non-transfected control. The fraction of positive cells in the defined windows is indicated. Raw data are shown in the Supplementary data file. (EPS 1761 kb)

**Additional file 8: Table S1.**

List of primers and oligonucleotides.

**Additional file 9: Supplementary data file.**

Raw data points as used for Figs. 3, 4, 5, 6, and Fig. S3, S4, S5, S6, S7.