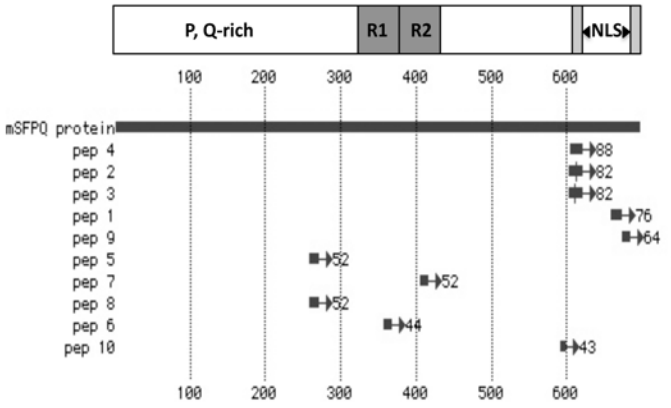
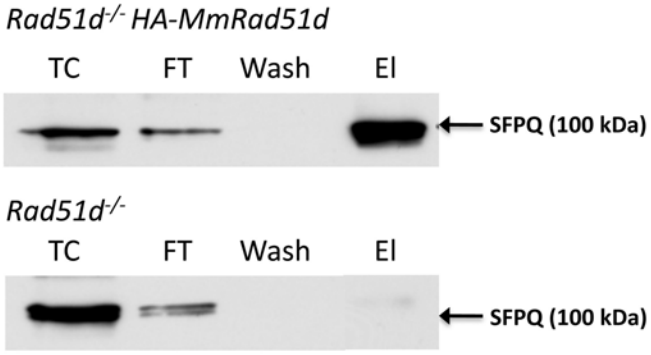
Identification and confirmation of DNA-independent co-precipitation of SFPQ with RAD51D

a



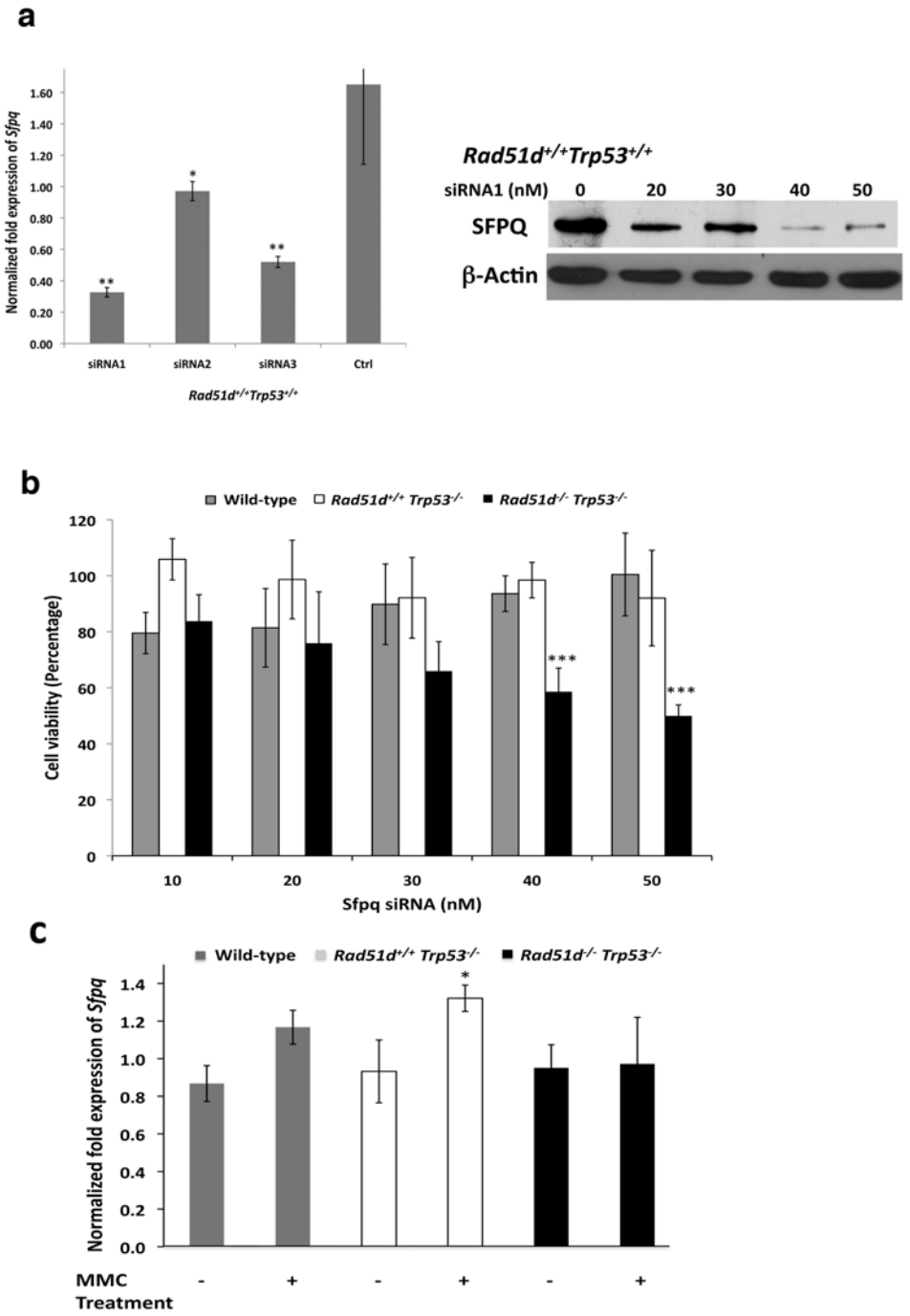
No.	Peptide sequence	Probability	Score
1	FGQGGAGPVGGQGPR	5.87E-07	4.54
2	MGGGGTMNM*GDPYGSGGQK	8.07E-04	4.21
3	MGGGGTM*NMGDPYGSGGQK	2.93E-02	3.90
4	M*GGGGTMNMGDPYGSGGQK	2.93E-02	3.44
5	TEEKISDSGFK	2.39E-04	3.36
6	FATHAAALSVR	6.21E-08	3.30
7	GIVEFASKPAAR	1.99E-05	3.04
8	TEEKISDSEGFK	1.31E-04	2.82
9	GMGPGTPAGYGR	2.34E-04	2.80
10	MGYMDPR	3.77E-04	2.03

b



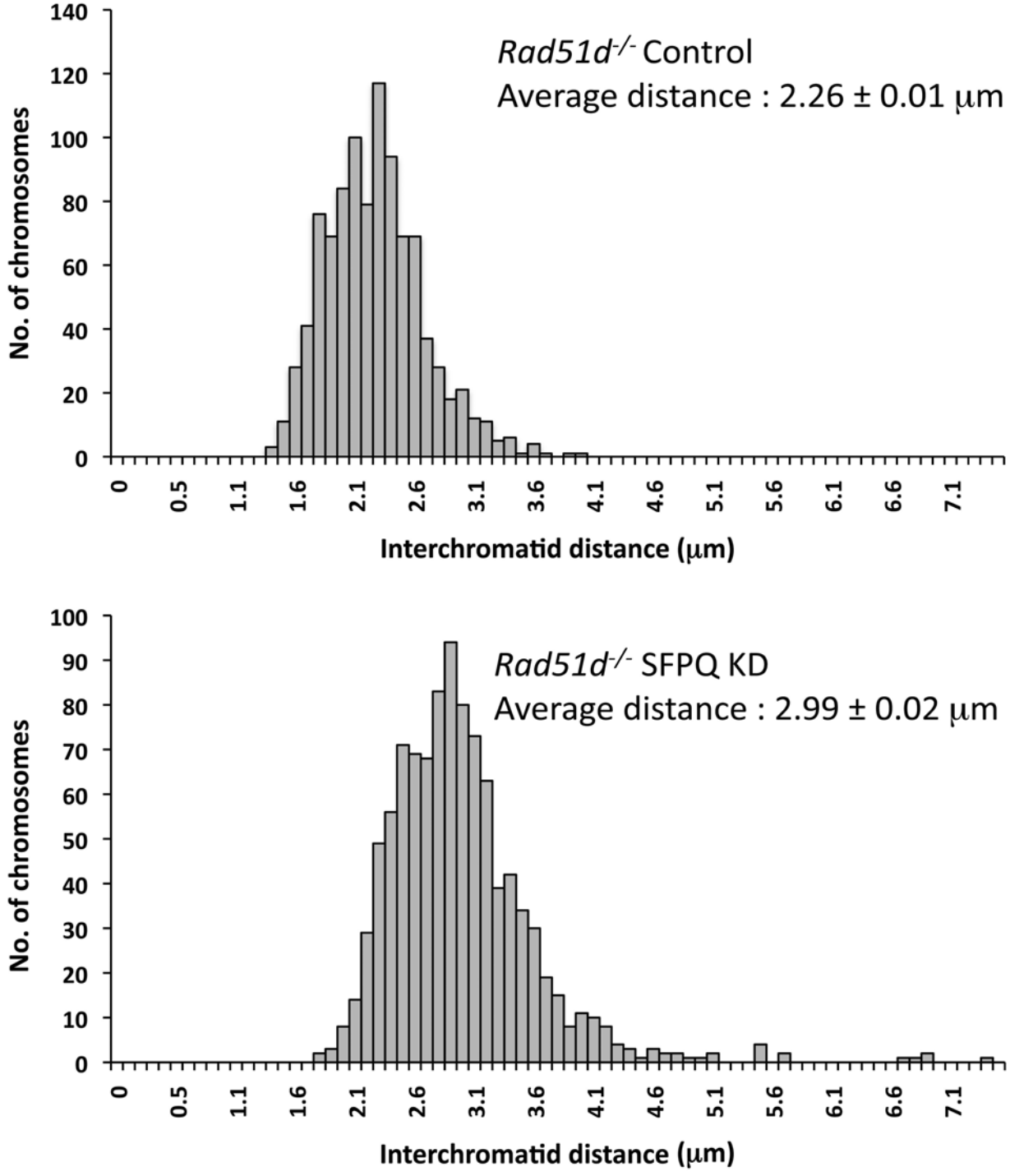
Supplementary figure S1. Identification and confirmation of DNA-independent co-precipitation of SFPQ with RAD51D. **(a)** 10 Peptide sequences matching the SFPQ protein were obtained from the mass spectroscopic analysis on a linear ion trap mass spectrometer (LTQ) of bands from RAD51D co-precipitation eluates (Rajesh, C., et. al., 2009). The probability of each peptide and corresponding score of experimental mass data indicating quality of peptide-spectrum match are shown. The peptide alignment is also shown with respect to the full-length mouse SFPQ protein by pam250 scoring matrix using Macvector v10 software. R1 and R2 indicate the RNA recognition motifs, while NLS indicate the nuclear localization sequences. The Proline-Glutamine rich N-terminal is predicted to be responsible to interaction with other proteins as well as containing the DNA binding domain (Shav-Tal and Zipori, 2002, Urban, RJ. Et al., 2002). **(b)** Western blot using anti-SFPQ antibody of anti-HA immunoprecipitations performed on whole cell extracts of *Rad51d*$^{-/-}$ *HA-MmRAD51d* and *Rad51d*$^{-/-}$ containing 10 μg/ml of Ethidium Bromide, indicating DNA independent enrichment of SFPQ by RAD51D co-precipitation. TC-Whole cell extract; FT-immunoprecipitation supernatant; W- immunoprecipitation washes; El-Eluates of immunoprecipitation.

siRNA-mediated knockdown of SFPQ and cell viability



Supplementary figure S2. siRNA-mediated knockdown of SFPQ and cell viability. **(a)** *Rad51d*$^{+/+}$ *Trp53*$^{+/+}$ MEFs were transfected with 30 nM each of *Sfpq* siRNAs labeled as siRNA1, siRNA2, and siRNA3 (Materials and Methods) and expression analyzed using quantitative real-time PCR (Left panel). The expression was compared to the control with no siRNA treatment (Ctrl) after the data was normalized to GAPDH expression. " * " indicates $P < 0.05$, " ** " indicates $P < 0.01$ and error bars indicate standard deviation of values from a representative experiment performed in triplicate. Right panel represents anti-SFPQ western blot of whole cell extracts from *Rad51d*$^{+/+}$ *Trp53*$^{+/+}$ MEFs treated with the indicated concentration of *Sfpq* siRNA1. β-actin was used as loading control. **(b)** Cell viability was measured using the MTT assay (Materials and Methods) in the respective MEF cell lines (*Rad51d*$^{+/+}$ *Trp53*$^{+/+}$ (wild-type), *Rad51d*$^{+/+}$*Trp53*$^{-/-}$ and *Rad51d*$^{-/-}$*Trp53*$^{-/-}$) transfected with the indicated concentration of *Sfpq* siRNA 72 hours post-transfection. Cell viability for each siRNA trearment was compared to no siRNA transfection. " *** " indicates $P < 0.001$ and error bars indicate standard error of means of values from at least two independent experiments performed in quadruplicate. **(c)** Expression differences were measured by quantitative real-time PCR and data normalized to GAPDH expression for mouse *Sfpq* RNA in different MEF cell lines, *Rad51d*$^{+/+}$ *Trp53*$^{+/+}$(wild-type), *Rad51d*$^{+/+}$*Trp53*$^{-/-}$ and *Rad51d*$^{-/-}$*Trp53*$^{-/-}$, under normal conditions and in response to treatment with 1 μg/ml mitomycin C (MMC). Error bars represent standard deviation from a representative experiment performed in triplicate. " * " indicates significance ($P < 0.05$).

SFPQ deficiency leads to defects in sister chromatid cohesion in *Rad51d*$^{-/-}$ MEFs



Supplementary figure S3. SFPQ deficiency leads to defects in sister chromatid cohesion in *Rad51d*$^{-/-}$ MEFs. Giemsa stained metaphase chromosome spreads were prepared from control or *Sfpq* siRNA transfected *Rad51d*$^{-/-}$ MEFs 48 hours after transfection. The distance between the sister chromatid arms was determined for at least 1000 chromosomes of each group from two independent experiments. Histograms for number of chromosomes with each respective interchromatid distance is plotted with the average distance and standard error for control and SFPQ KD indicated.