Figure S1

(a) MEIOTIC DIFFERENTIATION

GERM STEM CELLS

DTC

atm-1

b Anti-sense probe

Sense probe

gld-1

c DAPI

pS/TQ

Pachyten cells

N2

25°C

25°C → 15°C(5h)

25°C → 15°C(20h)

d

% DDR positive cells

pATM

pS/TQ

53BP1

γH2AX

Mock NO IR

Mock

N1ΔE

N1ΔE+GSI

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Supplementary Figure 1

Notch shows inverse correlation with ATM activity in *C. elegans* and negatively regulates ATM activity in human cells.

(a) Scheme of *C. elegans* germline. Two large pools of cells are outlined: the proliferating germ stem cells and cells undergoing meiotic differentiation. Distal tip cell (DTC) that caps the germline maintains stemness through Notch signaling. (b) RNA *in situ* hybridization of dissected gonads of wild-type worms with antisense or sense probes for *atm-1* and *gld-1* transcripts. *gld-1* used here as a control, is predominantly expressed in the meiotic compartment. (c) Immunostaining for pS/TQ epitopes of temperature-sensitive *glp-1*(ar202) mutant grown at 25°C and then kept at 15°C for 5h or 20h prior to exposure to IR. (d) Bar plot presents quantification of the percentage of cells positive for the indicated DDR marker presented in Fig. 1c. We considered only foci like stainings for quantifications. *P* value ≤ 0.001. (e) Fields of immunostained Hela cells expressing N1ΔE for indicated DDR markers and shown in Fig. 1c. (f) Immunostaining for Notch1 and pCHK2T68 of Hela cells expressing N1ΔE, after IR (2 Gy). (g) Bar plot presents quantification of the percentage of cells positive for pCHK2T68. *P* value ≤ 0.001. (h) Immunostaining for Notch1 and pKAP1S824 of Hela cells expressing N1ΔE, after IR (2 Gy). (i) Bar plot presents quantification of the percentage of cells positive for pKAP1S824. *P* value ≤ 0.001. (j) Immunostaining for Notch1 and pSMC1S966 of Hela cells expressing N1ΔE after IR (2 Gy). (k) Bar plot presents quantification of the percentage of cells positive for pSMC1S966. *P* value ≤ 0.001. (l) Immunostaining for Notch1 and 53BP1 of Hela cells expressing N1ΔE, after exposure to IR (2 Gy). Cells were collected at different time points (1, 4 and 24 hours) after IR and immunostained. (m-n) Bar plots indicate percentages of cells positive for 53BP1 and ATM nuclear foci, at different time points after exposure to IR. *P* value ≤ 0.001. (o) Bar plot shows mean signal intensity of DDR foci per nucleus upon Notch induction. Endogenous Notch1 activation was induced by EGTA treatment in MCF10a cells. Cells were then exposed to IR (0.5 Gy) and immunostained for indicated DDR markers. All values were normalized to those of uninduced cells. *P* value ≤ 0.05. (p) Immunostaining for N1IC of MCF10a cells cocultured either with OP9 cells or OP9 cells expressing DL1-GFP ligand. (q) Bar plot shows efficacy of GSI treatment, estimated by qRT-PCR for

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Notch1 transcriptional target Hes1. The values were normalized to the levels of Hes1 expression in empty vector infected cells. (r) Immunoblot for indicated DDR markers on lysates of HeLa cells infected with either empty vector or one expressing N1IC. Cells were sorted for homogeneous GFP signal and exposed to IR (5 Gy). (s) Bar plot presents quantification of the signal intensities shown on the immunoblot in (r). Signal intensities of DDR markers were normalized to that of respective total proteins and to the values of empty vector infected cells. *P value ≤ 0.05. (t) Immunoblot for marker of apoptosis cleaved caspase-3 on lysates of Hela cells expressing N1IC. Cells were exposed to IR (20 Gy) and collected 24 hours after for the analysis. (u) Immunoblot for pATM on lysates of MCF10a cells infected with either empty vector or one expressing N1IC. Cells were sorted for homogeneous GFP signal and exposed to IR (5 Gy). (v) Bar plot presents quantification of the signal intensities of DDR markers shown on the immunoblot in (u). Intensity of pATM signal was normalized to that of total ATM. Values were further normalized to that of empty vector infected cells. *P value ≤ 0.05. Throughout figure, error bars represent s.e.m. (n = 3 independent experiments), and all P values were calculated by two-tailed Student’s t test.
Supplementary Figure 2

Inactivation of DDR requires nuclear localization of Notch1, but it is not dependent on Notch1 transcriptional activity. (a), (c) and (e) Immunostainings for indicated DDR markers on Hela cells transfected with membrane-bound CD8-N1IC-GFP or cytosolic N1IC-NES-GFP or nuclear N1IC-NLS-GFP and exposed to IR (2 Gy). (b), (d) and (f) Bar plots present quantifications of the percentages of cells positive for indicated DDR marker; Error bars, s.e.m.  $n = 3$ independent experiments; (ns $P$ value > 0.05; *$P$ value ≤ 0.05; **$P$ value ≤ 0.001) (g) Bar plot presents quantification of the signal intensities shown on immunoblot in Fig. 2c. Intensity of pATM signal was normalized to that of total ATM and to the values of mock vector transfected cells. (h) Bar plot presents quantification of the levels of expression of Notch1 transcription target Hes1, as measured by qRT-PCR, in Hela cells expressing Notch1WT or transcriptionally inactivated Notch1ΔTAD. The values were normalized to the levels of Hes1 expression in Notch1WT cells. (i) Bar plot presents quantification of the signal intensities shown on the immunoblot in Fig. 2g. Intensity of total ATM signal was normalized to that of the vinculin and to the values of cells expressing GFP only. All $P$ values shown in figure 2 are calculated by two-tailed Student’s $t$ test.
Supplementary Figure 3

Notch1 and ATM form a protein complex.

(a) Immunoblot analysis of an IP with an anti-Flag antibody from the lysates of 293T cells expressing ATM-Flag and N1ΔE-Myc; input 5% of the total lysate. (b) Immunoblot analysis of an IP with an anti-Flag antibody from the lysates of 293T cells expressing N1ΔE-Flag. Lysates were subjected to either ethidium bromide or DNase treatment; input: 5% of the total lysate. (c) and (d) Immunoblot analysis of IPs with antibodies against endogenous ATM or anti-Flag (for Notch1) from the lysates of 293T cells expressing either N1ΔE-Flag or control-Flag tagged protein (Meis1-Flag). Cells were exposed to IR prior to collection of lysates. (e) Immunoblot analysis of an IP with an antibody against ATM from the lysates of 293T cells expressing N1ΔE-Flag. Levels of NBS1 remain unaffected in the presence of Notch1; input: 5% of the total lysate. (f) in situ proximity ligation assay (Duolink) performed on HeLa cells expressing empty vector or N1ΔE, shows detectable bright fluorescent signal where Notch1 and ATM are in close proximity.
**Figure S4**

**a** Immunoblot analysis of an IP with an anti-Flag antibody from the lysates of 293T cells expressing either mock-Flag protein (Meis1-Flag), N1ΔE-Flag or N1ΔEΔANK-Flag; input: 5% of the total lysate. **b** Bar plot presents quantification of the percentages of cells positive for pATM foci in Hela cells expressing either N1ΔE or N1ΔEΔANK, and after exposure to IR (2 Gy). Error bars, s.e.m.; n = 3 independent experiments (*P value ≤ 0.01). **c** Silver staining of SDS-PAGE protein gel shows a single band for ATM, immunoprecipitated from lysates of HeLa cells. **d** Bar plot presents quantification of the signal intensities shown on the immunoblot in Fig. 4d. Intensity of p53S15 signal was normalized to the intensity of total ATM signal, GST alone and finally to the values of the reaction without N1IC. Error bars, s.e.m.; n = 3 independent experiments (ns P value > 0.05; *P value ≤ 0.05). All P values shown in figure 2 are calculated by two-tailed Student’s t test.

Notch1 binds directly to ATM and inactivates it.

(a) Immunoblot analysis of an IP with an anti-Flag antibody from the lysates of 293T cells expressing either mock-Flag protein (Meis1-Flag), N1ΔE-Flag or N1ΔEΔANK-Flag; input: 5% of the total lysate. (b) Bar plot presents quantification of the percentages of cells positive for pATM foci in Hela cells expressing either N1ΔE or N1ΔEΔANK, and after exposure to IR (2 Gy). Error bars, s.e.m.; n = 3 independent experiments (*P value ≤ 0.01). (c) Silver staining of SDS-PAGE protein gel shows a single band for ATM, immunoprecipitated from lysates of HeLa cells. (d) Bar plot presents quantification of the signal intensities shown on the immunoblot in Fig. 4d. Intensity of p53S15 signal was normalized to the intensity of total ATM signal, GST alone and finally to the values of the reaction without N1IC. Error bars, s.e.m.; n = 3 independent experiments (ns P value > 0.05; *P value ≤ 0.05). All P values shown in figure 2 are calculated by two-tailed Student’s t test.
Inhibition of Notch1, in the presence of IR, leads to increased apoptosis and reduced survival of TALL-1 cells. (a) Bar plot shows efficacy of GSI treatment, estimated by qRT-PCR for Notch1 transcriptional target Hes1. The values were normalized to the levels of Hes1 expression in DMSO-treated cells. (b) Immunoblot confirms efficient treatment of TALL-1 cells with ATM inhibitor (ATMi). TALL-1 cells were treated with ATMi for 3h prior to IR (3 Gy). (c) Bar plot shows efficacy of GSI treatment, estimated by qRT-PCR for Notch1 transcriptional target Hes1. The values were normalized to the levels of Hes1 expression in DMSO-treated cells.
Supplementary Figure 6

(a) Immunohistochemistry (IHC) images show examples of low-grade breast cancers that express low levels of nuclear Notch1 and high levels of pATM. Conversely, high-grade breast cancers show high levels of detectable nuclear Notch1 and low levels of pATM. (b) IHC of parallel sections of one breast cancer sample shows nuclear activated Notch1 (score 3), full expression of nuclear ATM (score 3) and significant reduction of nuclear pATM (score 1). (c) Scatter plot shows inverse correlation between Notch1 and pATM, when samples that do not express total ATM (7%) are removed from the analysis; $P < 0.009343$ (d) Box plots show levels of Notch1 and pATM stainings for the indicated breast cancer molecular subtypes: HER2 positive, Luminal A, Luminal B, Triple Negative (TN). Inverse correlation between Notch1 and pATM is maintained also among different molecular subtypes of breast cancer. Notch1 plot: $P < 0.004064$; pATM plot: $P < 0.0536$; Kruskal-Wallis chi-squared test was used for this analysis. (e) Kaplan-Meier graph representing the probability of overall survival in breast cancer patients from the meta-dataset stratified according to NDT and ATM-activity signatures. The log-rank test $P$ value reflects the significance of the association between NDT signature Low/ATM-activity signature High and longer survival.

Notch1 and ATM are inversely correlated in human breast cancer samples.

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