Inhibition of the ATR kinase enhances 5-FU sensitivity independently of non-homologous end-joining and homologous recombination repair pathways

Soichiro S. Ito [1,§], Yosuke Nakagawa [1,§], Masaya Matsubayashi [2], Yoshihiko M. Sakaguchi [2], Shinko Kobashigawa [2], Takeshi K. Matsui [2,3], Hitoki Nanaura [2,3], Mari Nakanishi [2], Fumika Kitayoshi [2], Sotaro Kikuchi [2], Atsuhisa Kajihara [1], Shigehiro Tamaki [1], Kazuma Sugie [3], Genro Kashino [4], Akihisa Takahashi [5], Masatoshi Hasegawa [6], Eiichiro Mori [2,ǂ], Tadaaki Kirita [1,ǂ]

List of supporting information

Table S1. The 32 genes that regulated significantly by 5-FU treatment.
Figure S1. Determination of ATRi concentration, and confirmation of *p53*-status.
Figure S2. DSBs analysis by neutral comet assay.
Figure S3. DEG results of 5-FU vs Control.

Table S1. The 32 genes that were regulated significantly by 5-FU treatment. The regulated genes were detected by DEG analysis both in SAS cells and HSC3 cells. Table S1 corresponds to Figure 9.

Gene Symbol	Description	Fold change	*p*-value
SERF2-C15ORF63	SERF2-C15orf63 readthrough	100.292845	1.43392E-06
MMGT1	membrane magnesium transporter 1	7.247282	0.000495381
ATF3	activating transcription factor 3	4.100477	0.025732252
S100A7	S100 calcium binding protein A7	3.950777	0.037316272
IFIT2	interferon induced protein with tetratricopeptide repeats 2	3.681816	0.019626887
TXNIP	thioredoxin interacting protein	3.354370	0.029885315
IL6	interleukin 6	3.099905	0.04424084
CCNE2	cyclin E2	2.706474	0.008026312
IFIT1	interferon induced protein with tetratricopeptide repeats 1	2.649556	0.044534474
CDKN1A	cyclin dependent kinase inhibitor 1A	2.569489	0.037338891
DDX58	DExD/H-box helicase 58	2.512164	0.049644059
IFIT3	interferon induced protein with tetratricopeptide repeats 3	2.434318	0.034793439
SERPINE1	serpin family E member 1	2.418856	0.025402923
CCNE1	cyclin E1	2.076169	0.037448089
HSPA8	heat shock protein family A (Hsp70) member 8	-2.073899	0.046900302
NOP53	NOP53 ribosome biogenesis factor	-2.093956	0.049335247
FAM83D	family with sequence similarity 83 member D	-2.238640	0.044430286
CCNB1	cyclin B1	-2.373575	0.023772767
RPL10A	ribosomal protein L10a	-2.408159	0.020432069
CDKN3	cyclin dependent kinase inhibitor 3	-2.417268	0.045801497
AURKA	aurora kinase A	-2.668621	0.014791448
CDC20	cell division cycle 20	-2.840158	0.004688996
PSRC1	proline and serine rich coiled-coil 1	-2.847078	0.016066633
H1FX	H1 histone family member X	-2.937493	0.006136993
PLK1	polo like kinase 1	-3.567241	0.001547954
KIF20A	kinesin family member 20A	-3.962995	0.001489257
H1F0	H1 histone family member 0	-4.845553	0.000429542
HYPK	huntingtin interacting protein K	-11.075390	4.54445E-06
MIR4444-1	microRNA 4444-1	-21.679291	7.97291E-09
RPL13A	ribosomal protein L13a	-38.337360	8.76291E-16
LUZP6	leucine zipper protein 6	-43.301587	0.001315272
ATP6V1G2-DDX39B	ATP6V1G2-DDX39B readthrough	-76.644546	0.004338051

Ito SS, Nakagawa Y, et al. Figure S1

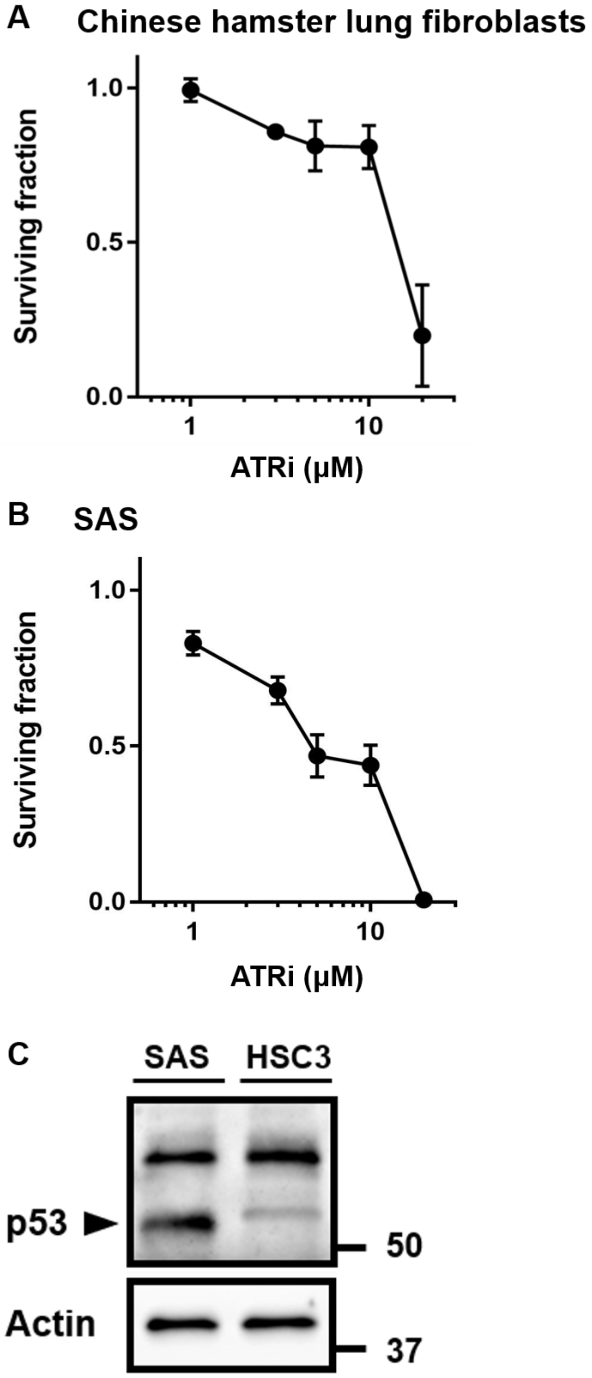

Figure S1. Determination of ATRi concentration, and confirmation of *p53*-status. (A, B) Surviving fraction of Chinese hamster lung fibroblasts (A) and SAS cells (B). ATRi was used at 1 μM, 3 μM, 5 μM, 10 μM and 20 μM. (C) Phenotype of p53 protein in SAS cells and HSC3 cells. Arrow indicates expression of p53. Upper bands are non-specific. All experiments were replicated 3 times. The values obtained were described as means ± SD. Figure S1 corresponds to Figure 2.

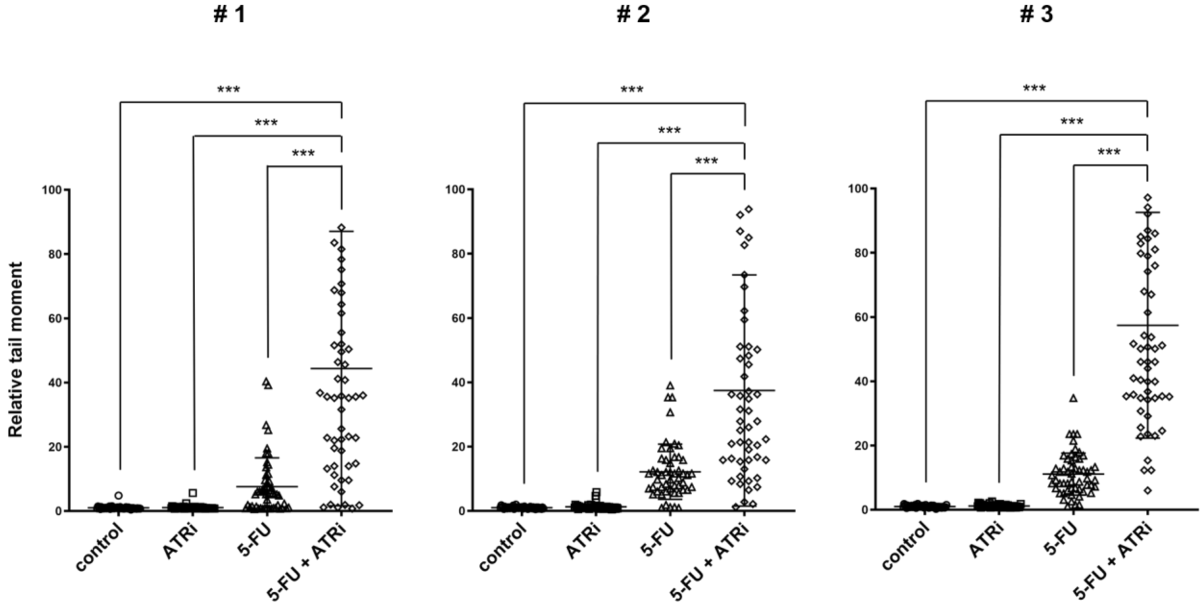

Figure S2. DSBs analysis by neutral comet assay. The tail moments of more than 50 cells were quantified, respectively. The values obtained were described as means ± SD. Data were compared statistically using the two-tailed Student's *t*-test. *p*-value; *, ** and *** represent $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. Figure S2 corresponds to Figure 4B.

Ito SS, Nakagawa Y, et al. Figure S3

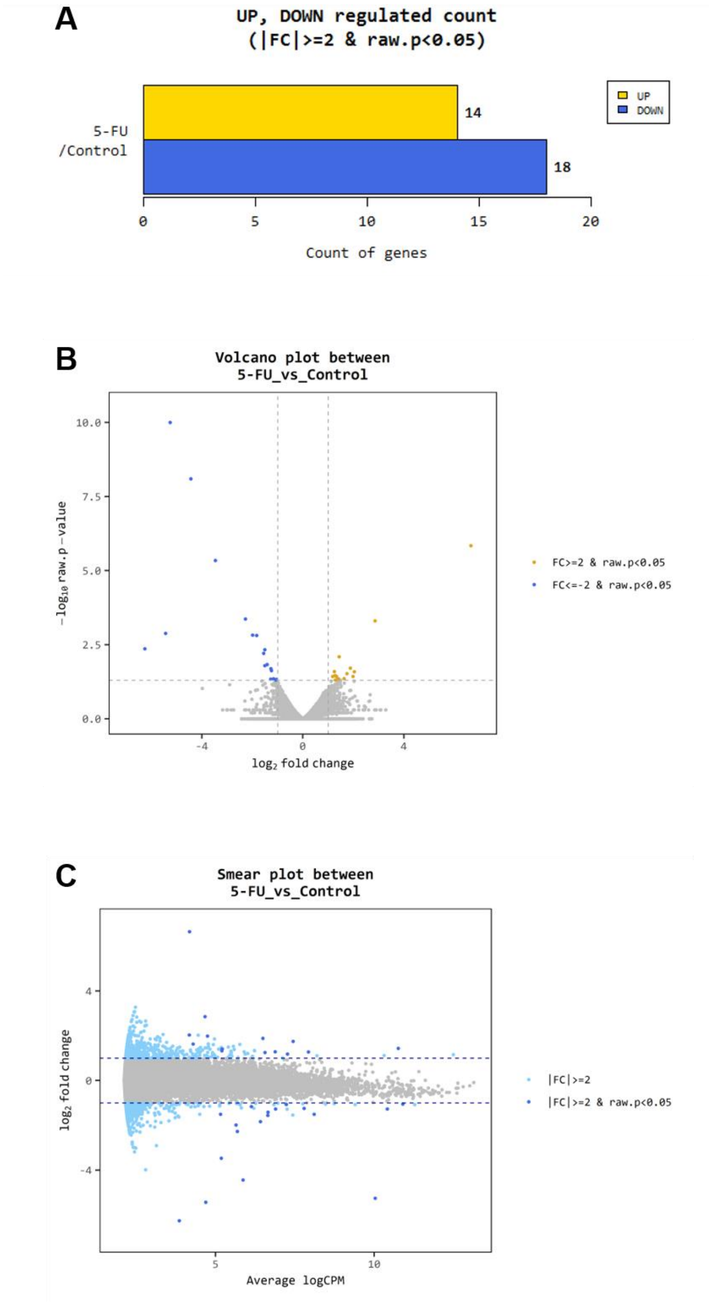

Figure S3. DEG results of 5-FU vs Control. (A) Shows number of significant up- and down-regulated genes based on fold change and p-value of comparison pair. (B) Log2 fold change and p-value obtained from the comparison between two groups (5-FU vs Control) plotted as volcano plot. X-axis: log2 fold change, Y-axis: -log10 p-value. (C) A smear plot was drawn to confirm the transcripts that show higher expression difference to the control according to overall average expression level. X-axis: Average logCPM, Y-axis: log2 fold change. Figure S3 corresponds to Figure 9.