Cassano et al.:

A Novel Approach to Increase Robustness, Precision and High-Throughput Capacity of Single Cell Gel Electrophoresis

Supplementary Data

Supplementary materials and methods

Cytotoxicity/long term viability of synchronized x-ray treated control cells
One frozen aliquot of A549 cells per treatment condition (0, 1, 2, 4, 8 and 12 Gy) was thawed and 500 cells were seeded per well of a 96-well plate and cultured up to 14 days. 10 replicates per treatment condition and time point were seeded and medium was replaced every 2-3 days. For each time point, an individual 96-well plate was stained with crystal violet as described before (Hirsch et al., 2007). In brief, cells were washed twice with PBS, stained with 0.5% crystal violet in 20% methanol for 10 min, washed with tap water, air dried for 30 min at room temperature, incubated for 10 min in 100 µL methanol, and absorption was measured at 550 nm using a microplate reader (Mithras^2, LB943, Berthold Technologies). After subtraction of background levels from blank wells, values were normalized to results from day 1.

Supplementary results

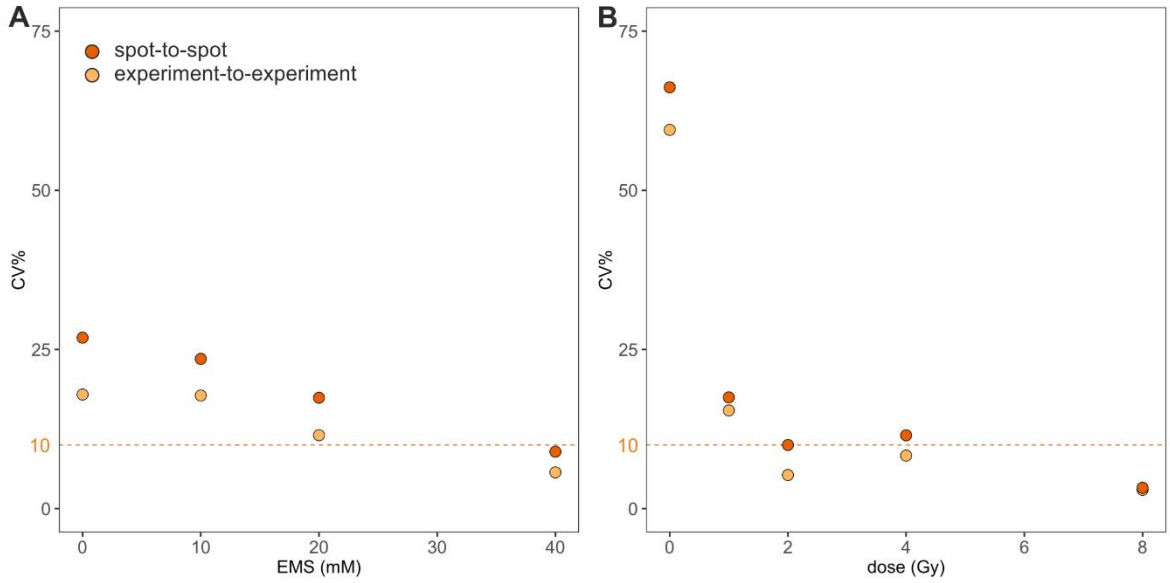

Fig. S1: CV values calculated from all spot means (orange) as well as experimental means (yellow) and the corresponding standard deviations
A) EMS treatment. B) x-ray treatment. CV values below 10% (visualized by the orange dashed line) indicate a sufficient level of reproducibility. The color code shown in A applies also to B. Corresponds to Figure 3 (main text).

doi:10.14573/altex.1906252s1

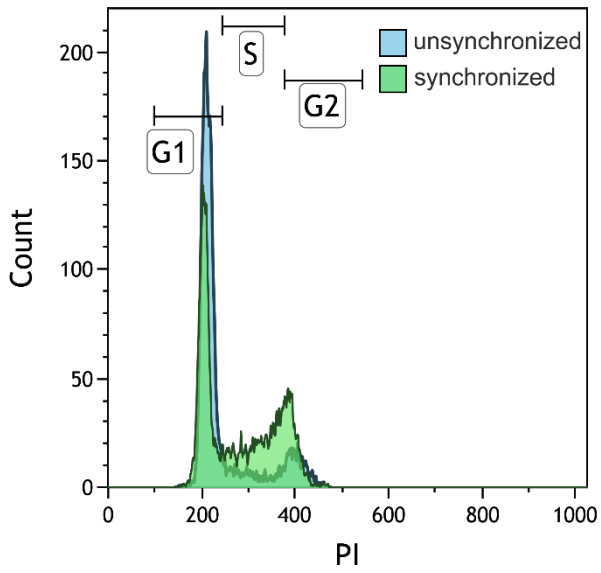

Fig. S2: Flow cytometry confirms S-phase synchronization of A549 cultures
Representative example of a flow cytometry analysis. Unsynchronized (blue) and synchronized (green) A549 cells were fixed, DNA was stained with propidium iodide (PI), and DNA content was quantified by flow cytometry. G1, S and G2 gates for quantification of cells in the respective cell cycle phases are indicated.

Tab. S1: Quantification of the events in G1, S and G2 phase as depicted in Fig. S2 from three independent experiments
Mean values and corresponding standard deviations are given.

Cell cycle phase / Sample	G1	S	G2/M
Unsynchronized	68.0 ±9.4%	15.7 ±3.5%	12.7 ±2.1%
Synchronized	44.7 ±4.9%	33.7 ±1.5%	17.0 ±1.7%

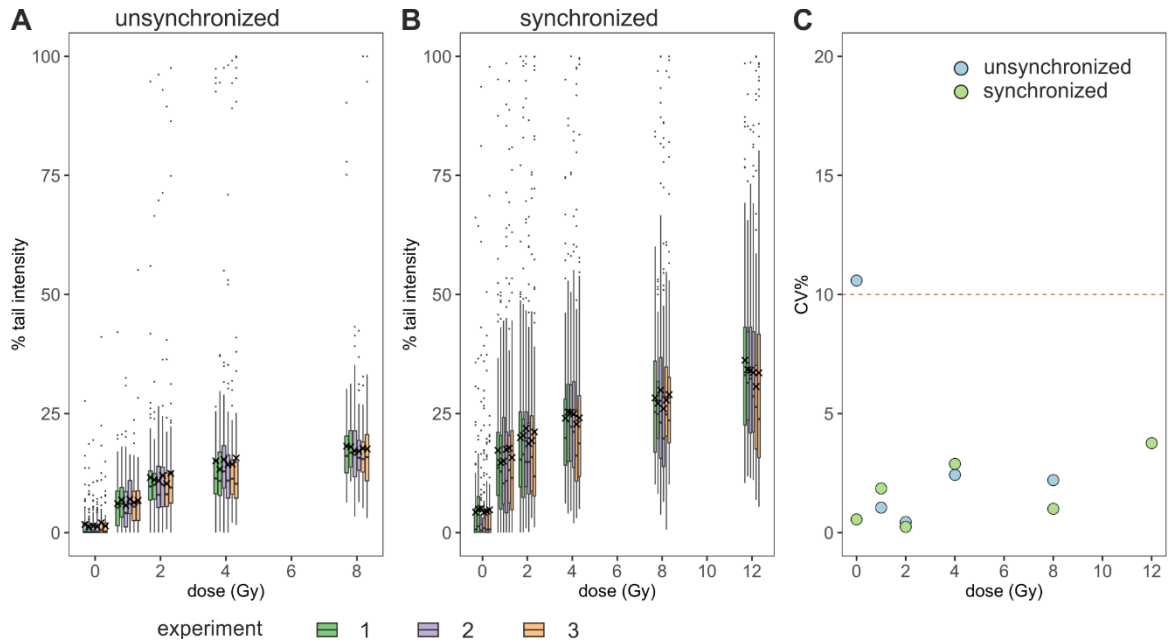

Fig. S3: Full dose-response curves of x-ray treated unsynchronized (A) and synchronized (B) A549 control cells
The intrinsic biological variation for all treatment conditions over three independent experiments with two technical replicates (= spots) each is shown. Each box comprises 100 comets from the respective spot. A) unsynchronized control cells (0, 1, 2, 4 and 8 Gy treated). B) synchronized control cells (0, 1, 2, 4, 8 and 12 Gy treated). C) CV values retrieved from experimental means and corresponding standard deviations. CV values below 10% (visualized by the orange dashed line) indicate a sufficient level of reproducibility. Corresponds to Figure 4 (main text).

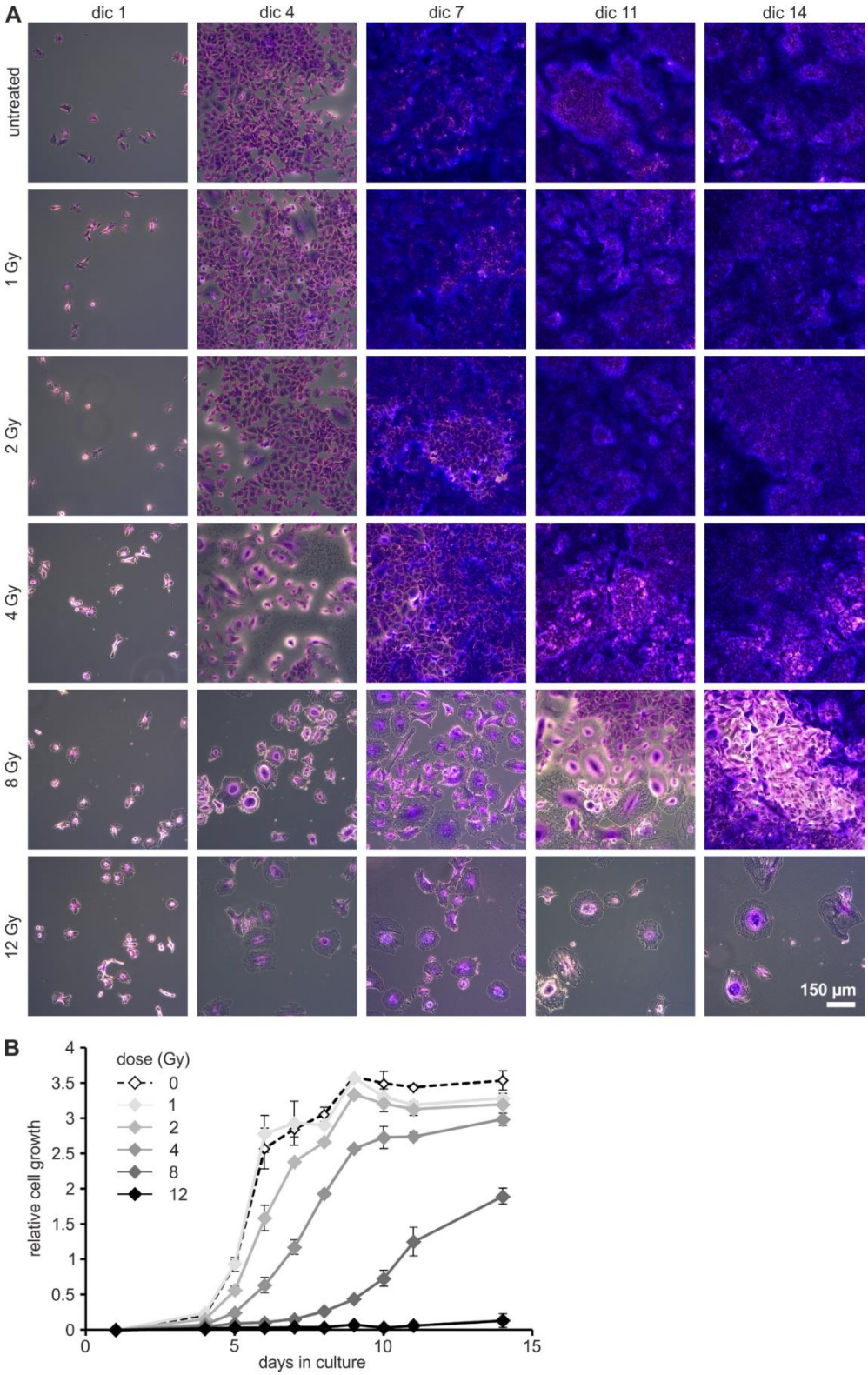

Fig. S4: Cell growth is impaired upon x-ray treatment starting at a dose of 4 Gy
Synchronized A549 control cells were grown over a period of 14 days and viable cells were visualized using crystal violet staining. A) Representative images of stained cells from all treatment conditions; dic, day in culture. B) Quantification of three independent experiments. Mean values and corresponding standard deviations are shown.

Tab. S2: Impact of different assay parameters on % tail intensity values of 0 and 4 Gy treated synchronized control cells

Parameter	Dose (Gy)	Percent change	P-value (T-test)
Agarose (0.4 > 0.68%)	0 4	-81% -66%	$7.1x10^{-5}$ $1.2x10^{-9}$
Temperature (4 > 15°C)	0 4	+263% +24%	$5.1x10^{-6}$ $2.3x10^{-3}$
Unwinding (20 > 40 min)	0 4	+18% +25%	0.18 $5.1x10^{-3}$
Voltage (1.0 > 1.1 V/cm)	0 4	-4% +8%	0.44 0.03
Electrophoresis (20 > 30 min)	0 4	-1% +20%	0.8 $3.4x10^{-3}$
New parameters[a]	0 4	+39% +69%	$1.9x10^{-5}$ $2.8x10^{-12}$

[a] New parameters: 0.4% low melting agarose, temperature of electrophoresis solution of 4°C, unwinding time of 40 min, voltage of 1.1 V/cm for an electrophoresis time of 30 min. A two-tailed t-test under the assumption of different sample variances was performed. Statistically significant changes are marked in orange for a confidence interval of 95%.

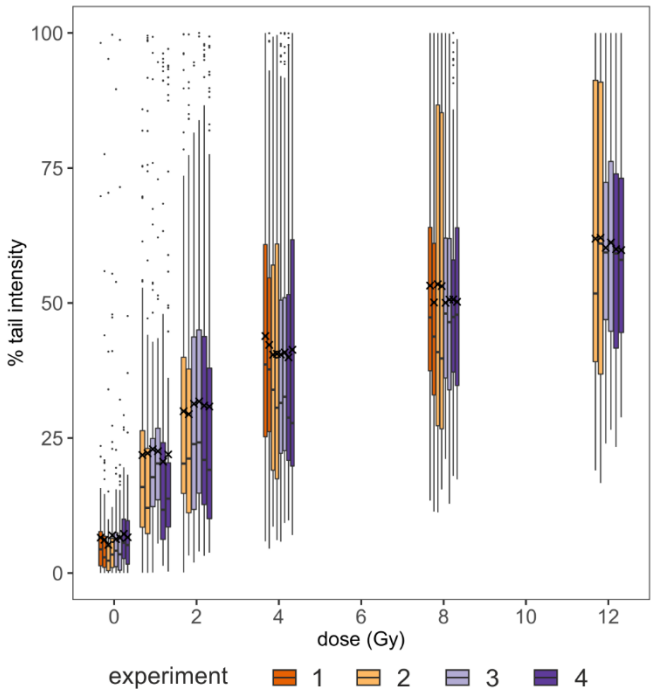

Fig. S5: Full dose response curve of the synchronized control samples run with the new assay parameters
Three to four independent experiments with two technical replicates (= spots) each were performed. Each box represents the intrinsic biological variability of one spot comprising 100 comets. For more details, please refer to the materials and methods as well as results section of the main text.
Corresponds to Figure 5 (main text).

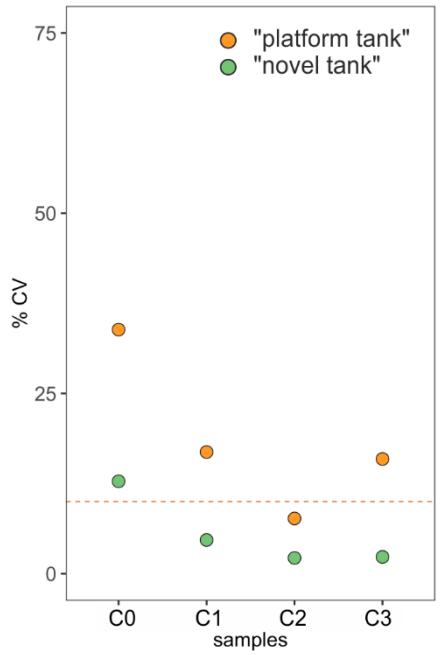

Fig. S6: Increased reproducibility of results in the "novel tank" compared to the "platform tank"
CV values were calculated from experimental means and the corresponding standard deviations. CV values below 10% (visualized by the orange dashed line) indicate a sufficient level of reproducibility. Corresponds to Figure 7 (main text).

Tab. S3: F-test analysis of variance
P-values of P < 0.05 indicate a significant difference between the variance retrieved from the "platform tank" and the "novel tank".

sample	**C0**		**C1**		**C2**		**C3**	
tank	**platform**	**novel**	**platform**	**novel**	**platform**	**novel**	**platform**	**novel**
Mean	6.6	2.8	14.6	16.6	21.7	28.0	57.1	55.7
Variance	14.5	0.4	29.9	1.5	63.4	3.1	126.9	4.2
Observations	20	18	20	18	20	18	20	18
df	19	17	19	17	19	17	19	17
P	$5.8x10^{-10}$		$3.5x10^{-8}$		$3.1.10^{-8}$		$1.7x10^{-9}$	

df, degree of freedom

Supplementary reference
Hirsch, C., Campano, L. M., Wohrle, S. et al. (2007). Canonical wnt signaling transiently stimulates proliferation and enhances neurogenesis in neonatal neural progenitor cultures. *Exp Cell Res 313*, 572-587. doi:10.1016/j.yexcr.2006.11.002