## Supporting Information

# Non-thermal plasma with 2-deoxy-D-glucose synergistically induces cell death by targeting glycolysis in blood cancer cells

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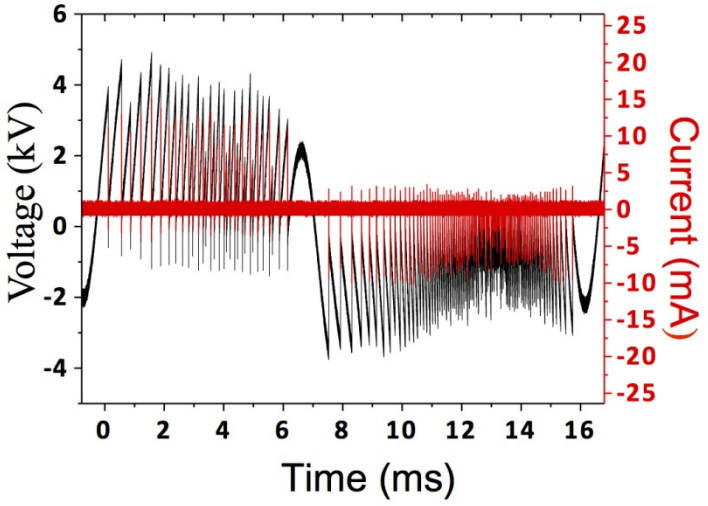


**Figure S1.** Voltage and current waveform of the plasma device (Sci Rep.,5,8587/Doi:10.1038/srep08587).

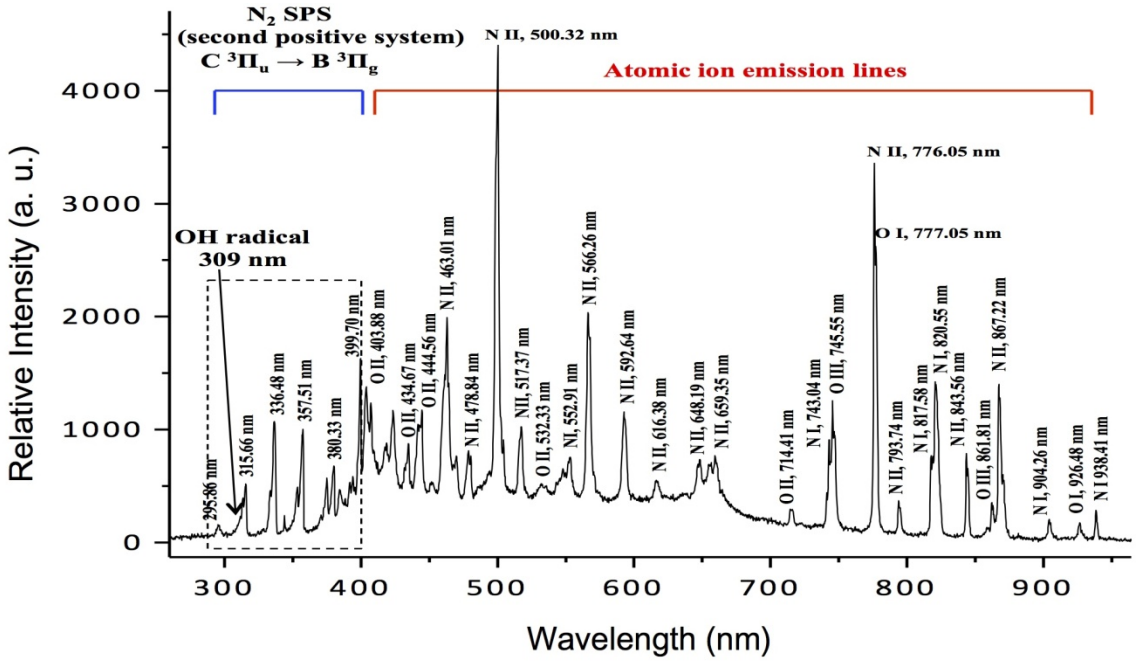


**Figure S2.** Optical emission spectra (OES) of the plasma device (Sci Rep.,5,8587/Doi:10.1038/srep08587).

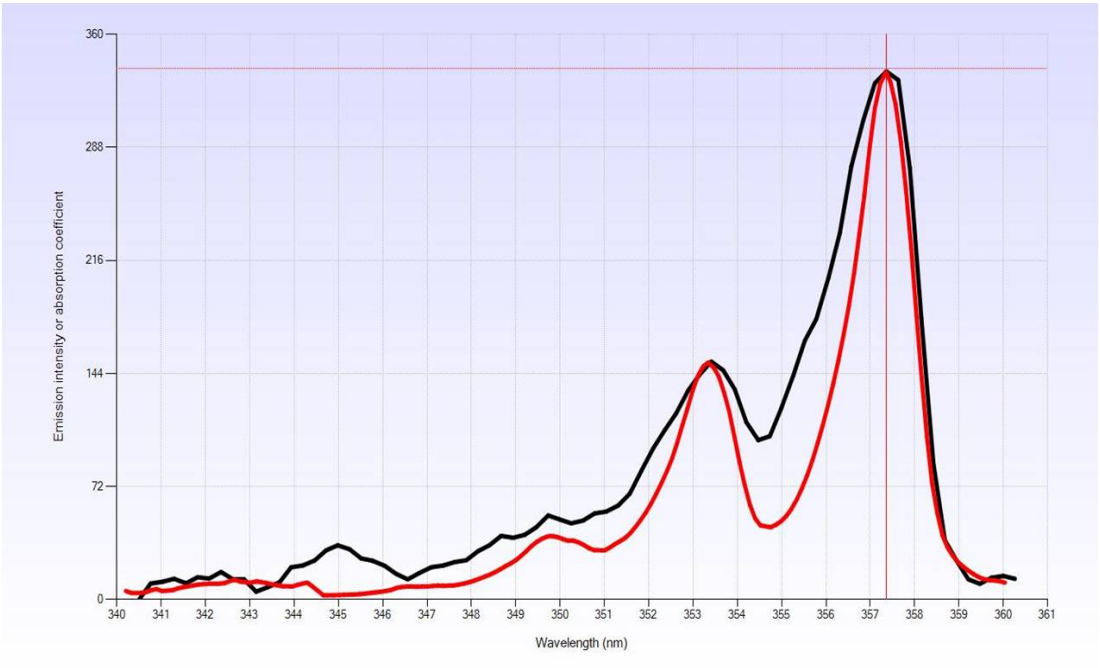


**Figure S3.** Measurement of gas temperature of the plasma device.

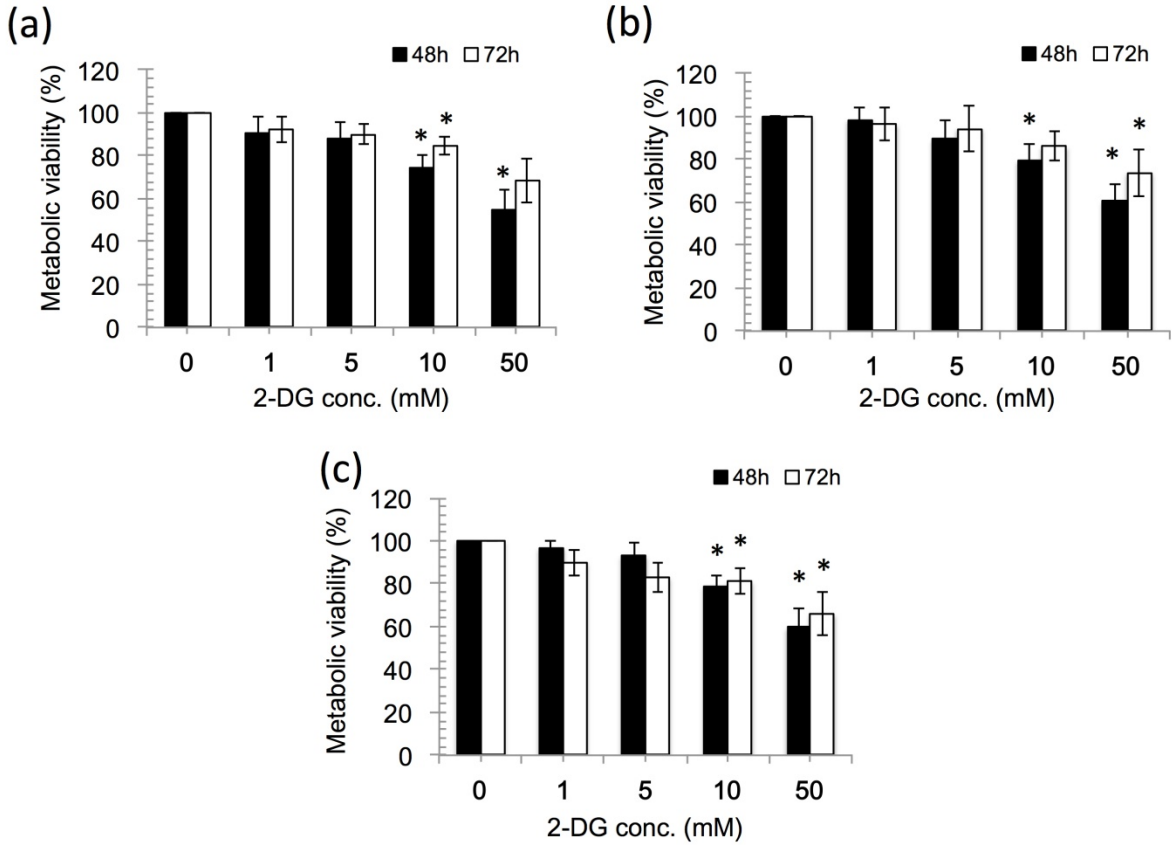


**Figure S4.** Effect of various concentrations of 2-deoxy-D-glucose (2-DG) alone, on blood cancer cells. Metabolic viability of (**a**) THP-1, (**b**) U937, and (**c**) RAW264.7 at 48 and 72 hours. The results were calculated as the percentage of viable cells and presented as the mean ± SD (n=3). Student's *t*-test was performed, and the significance is indicated as * $p<0.05$, § $p<0.01$, and # $p<0.001$.

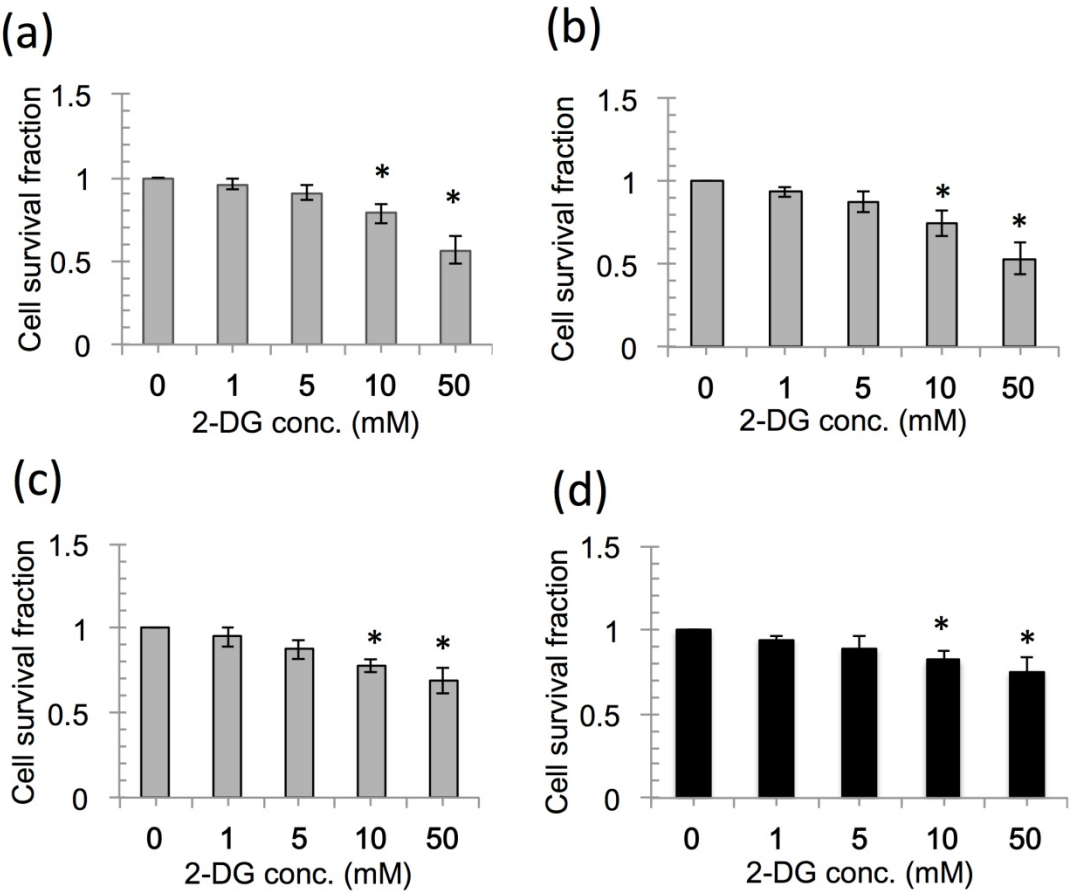


**Figure S5.** Cell count of (**a**) THP-1, (**b**) U937, (**c**) RAW264.7 and (**d**) PBMCs treated with different concentrations of 2-deoxy-D-glucose following 24 hour. The results were calculated as the percentage of viable cells and presented as the mean ± SD (n=3). Student's *t*-test was performed and the significance is indicated as * $p<0.05$, § $p<0.01$, and # $p<0.001$.

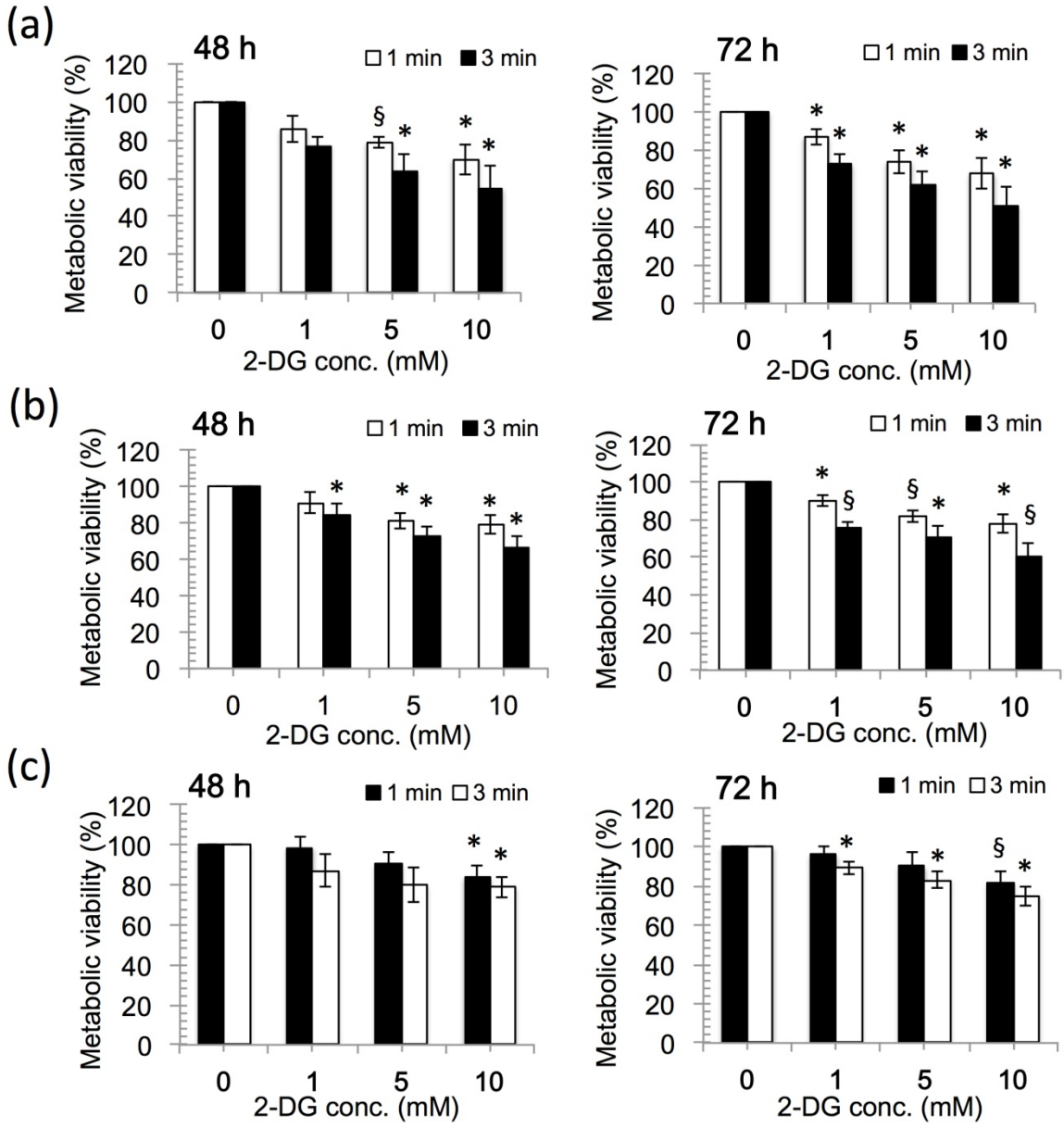


**Figure S6.** Response of 2-DG and plasma combination treatment on blood cancer cells. Metabolic viability of (**a**) THP-1, (**b**) U937 and (**c**) RAW264.7 following 48 and 72 hours using MTS assay. The results were calculated as the percentage of viable cells and presented as the mean ± SD (n=3). Student's *t*-test was performed, and the significance is indicated as * $p<0.05$, § $p<0.01$, and # $p<0.001$.

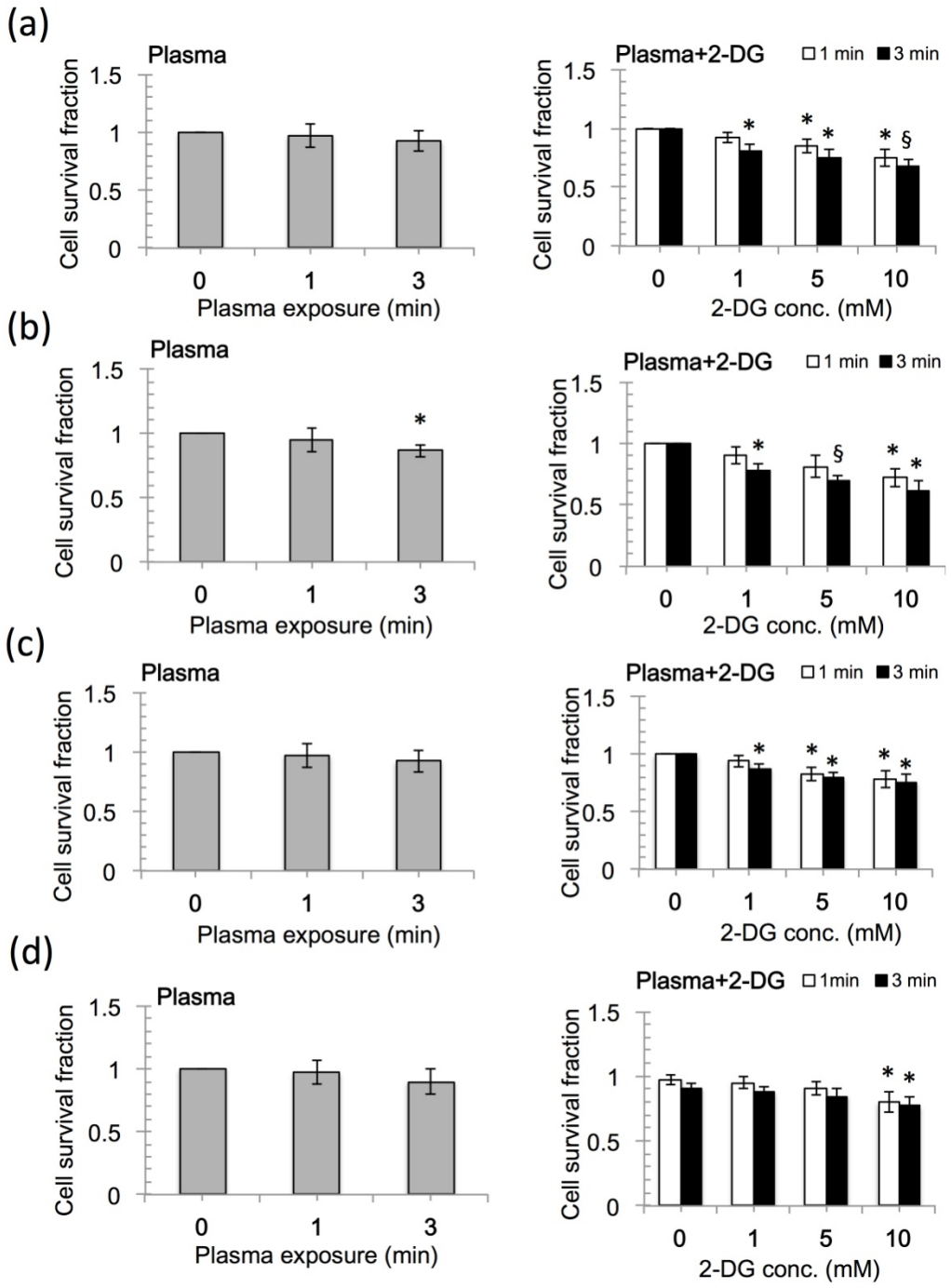


**Figure S7.** Cell count of (**a**) THP-1, (**b**) U937, (**c**) RAW264.7 and (**d**) PBMCs treated with 2-DG and plasma combination treatments following 24 hour. The results were calculated as the percentage of viable cells and presented as the mean ± SD (n=3). Student's *t*-test was performed, and the significance is indicated as * $p<0.05$, § $p<0.01$, and # $p<0.001$.

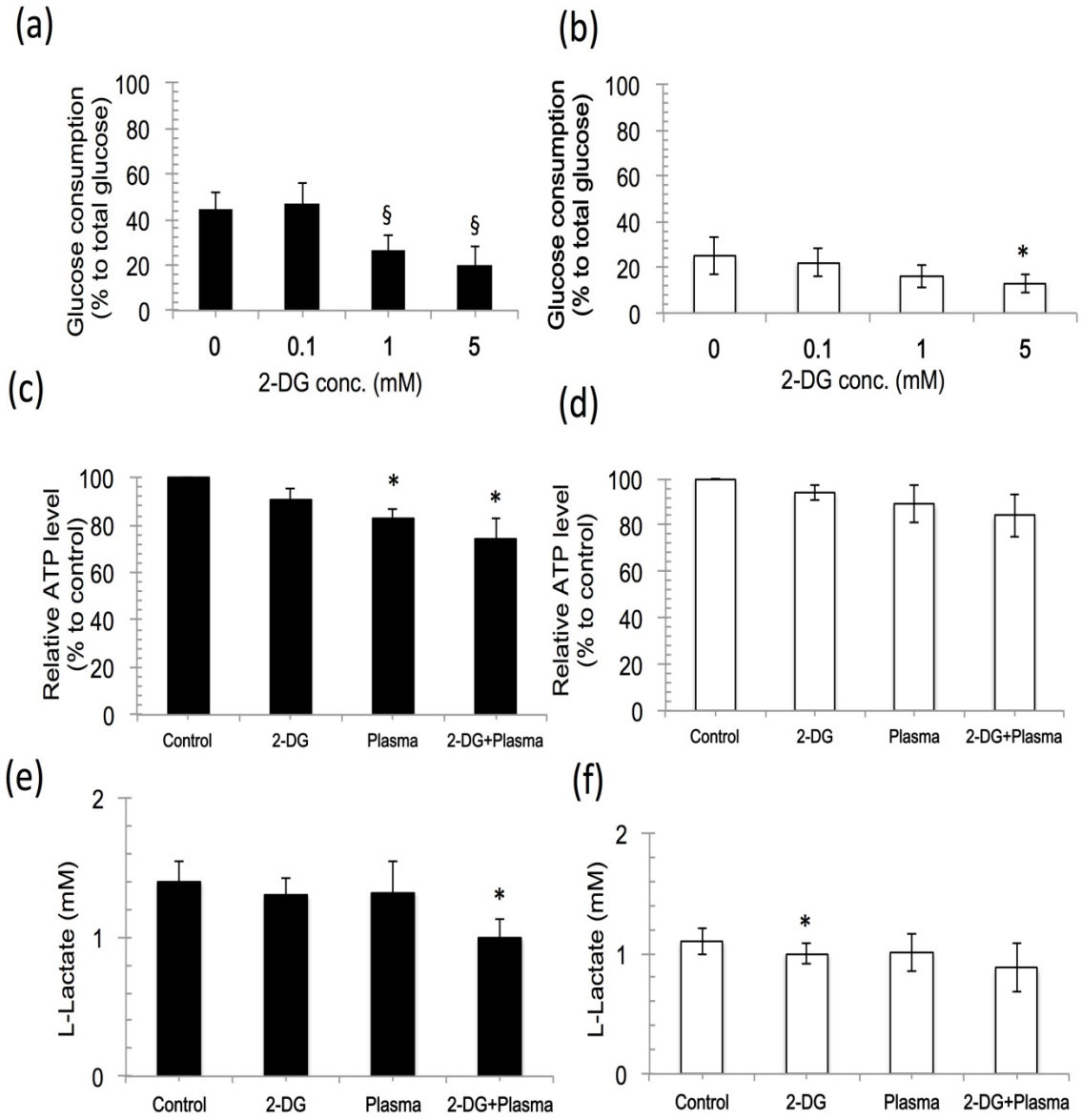


**Figure S8.** Metabolic alterations induced by 2-DG (1 mM) and plasma (3 min) combination treatment in blood cancer and normal cells. (**a, b**) Glucose consumption was examined at various concentrations of 2-DG (0.1, 1, and 5 mM) in RAW264.7 and PBMCs cells cultured in RPMI-1640 medium, respectively. (**c, d**) Cellular ATP levels were measured in RAW264.7 and PBMCs cells, respectively. (**e, f**) Lactate production was monitored in RAW264.7 and PBMCs cells cultured in RPMI-1640 medium, respectively. Error bars designate the ± SD (n=3). Student's *t*-test was performed, and the significance is indicated as * $p<0.05$, § $p<0.01$, and # $p<0.001$.

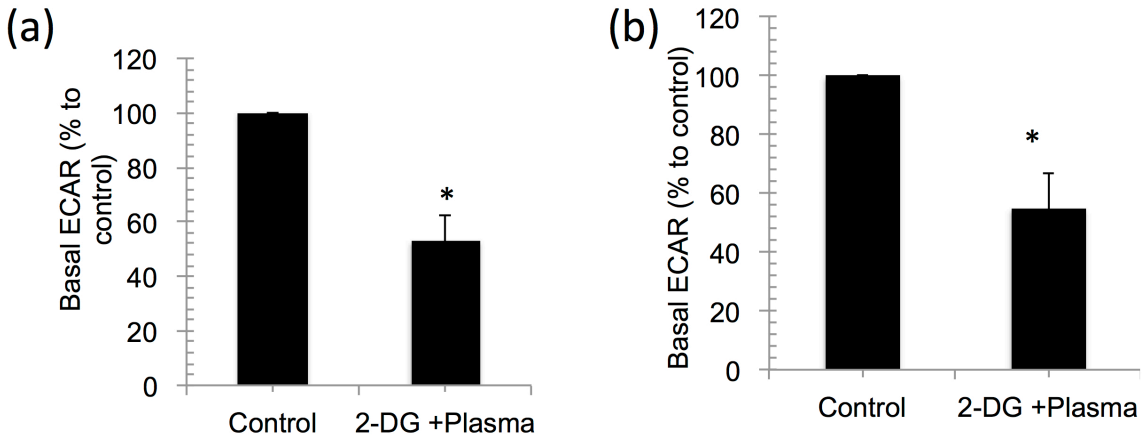


**Figure S9.** The ECAR in (a) THP-1 and (b) U937 cells following a 24 h combination treatment were determined using a seahorse XF24 analyzer. The ECAR, a measurement of lactate production/glycolysis, following treatment represents the acidification rate associated with glycolytic activity in THP-1 and U937 cancer cell lines, respectively. Error bars designate the ± SD (n=2). Student's t-test was performed, and the significance is indicated as * $p$<0.05, § $p$<0.01, and # $p$<0.001.

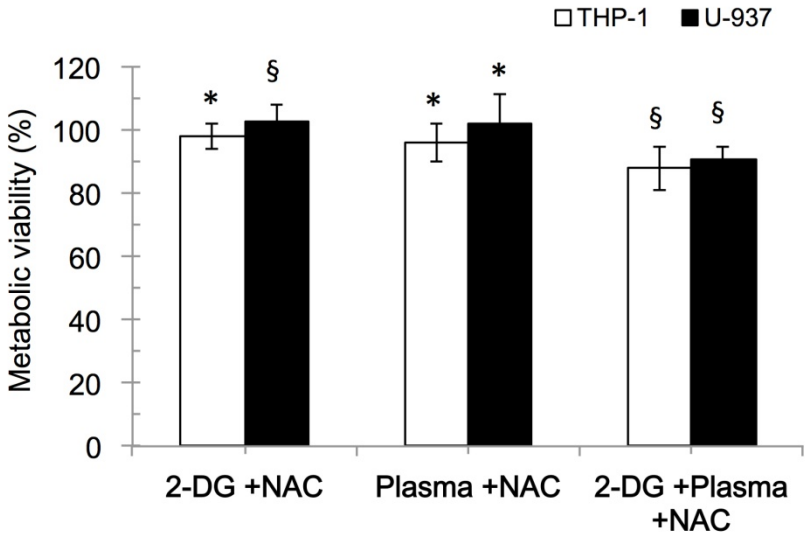


**Figure S10.** Metabolic viability of THP-1 and U937 cells in the presence of NAC (intracellular ROS inhibitor), following 24 h of combination treatment using MTS assay. Error bars designate the ± SD (n=3). Student's *t*-test was performed between NAC-treated and NAC-untreated groups and the significance is indicated as * $p$<0.05, § $p$<0.01, and # $p$<0.001.

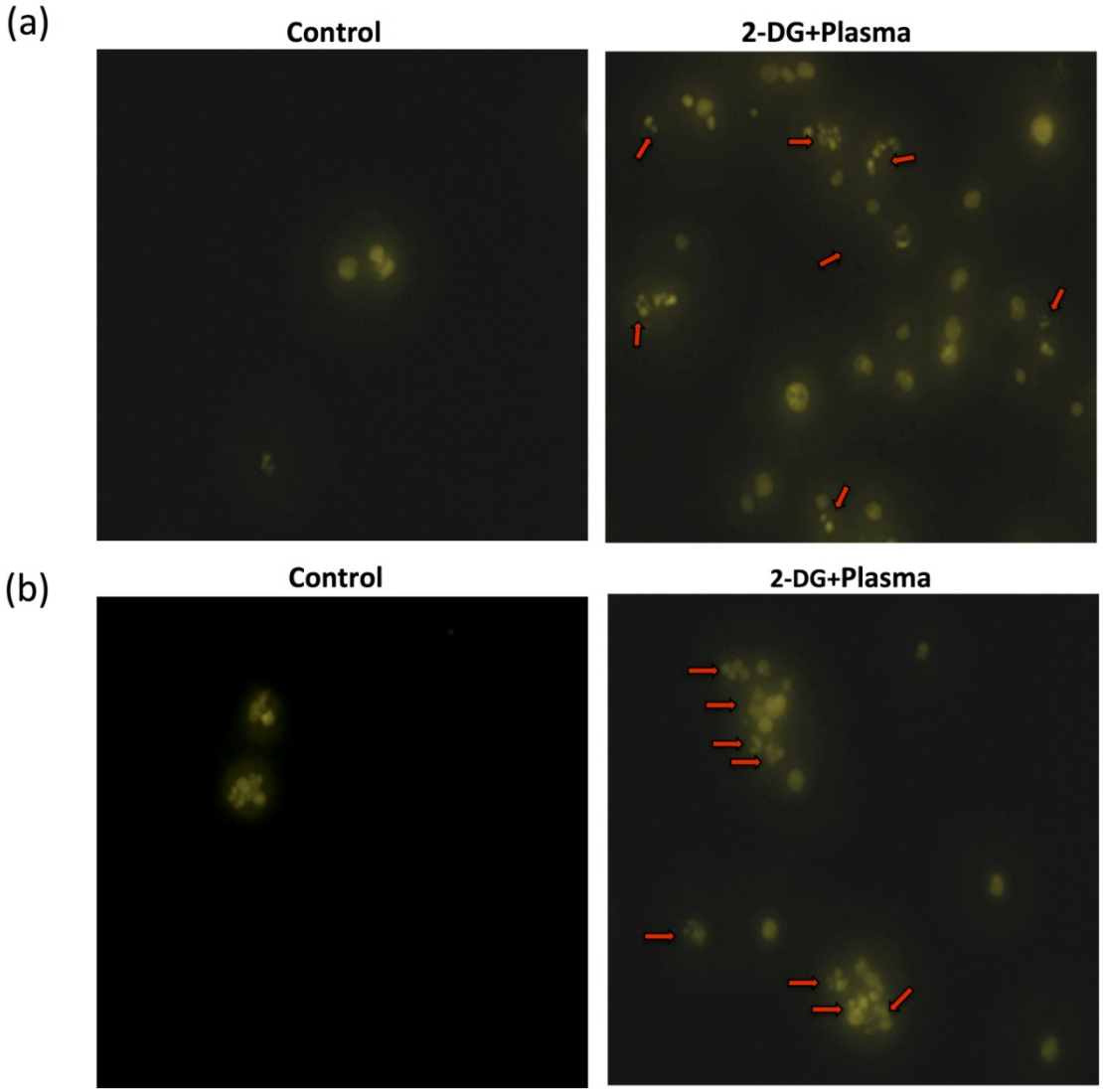


**Figure S11.** TUNEL staining was performed on (**a**) THP-1 and (**b**) U937 cells, followed by 2-DG (1 mM) and plasma (3 min) combination treatment. This staining discriminates the cells that have undergone apoptotic mechanisms. Cells were grown on the coverslips and treated with following combination treatment and staining was performed according to manufacturer's protocol (APO-BrdU™ TUNEL Assay Kit - with Alexa Fluor® 488 Anti-BrdU Kit, Molecular probes, Invitrogen).

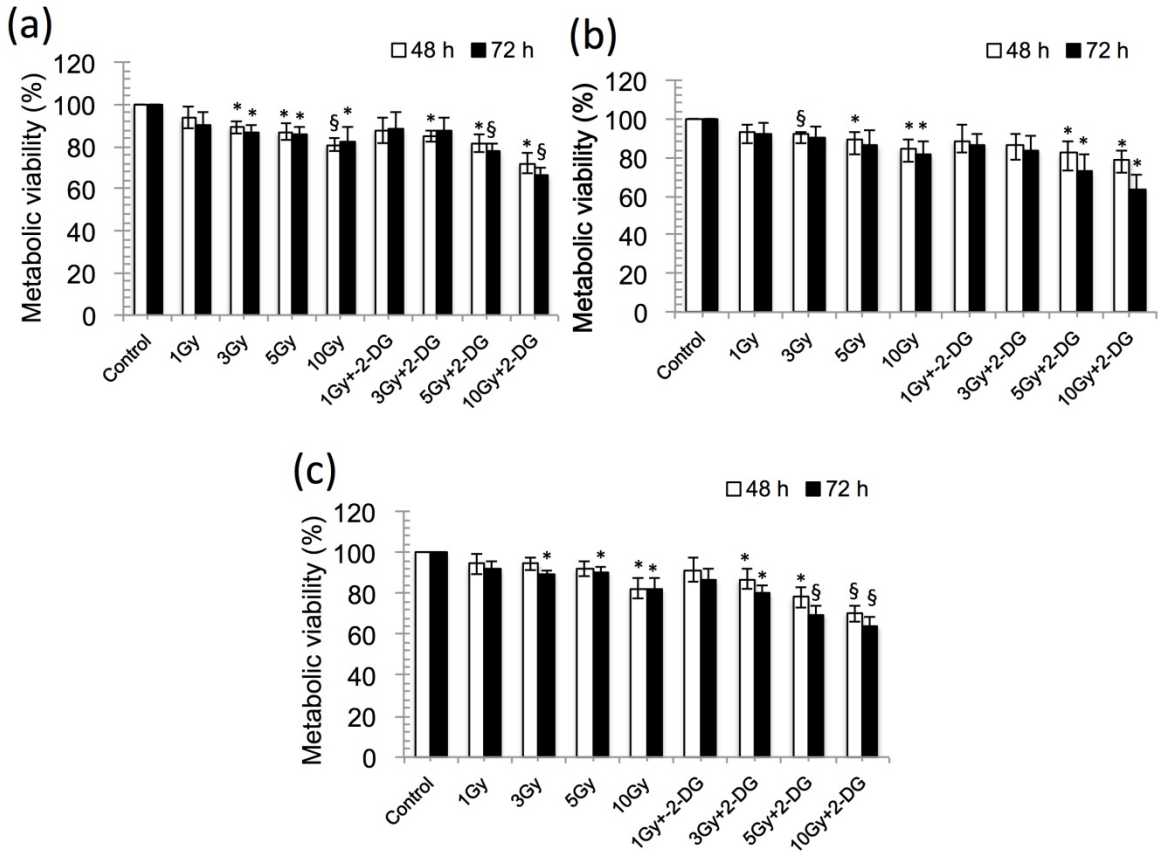


**Figure S12.** Comparison of our combination treatment approach (2-DG and plasma) with conventional gamma radiation treatment at a dose of 1, 3, 5, and 10 Gy was conducted using the MTS assay on (**a**) THP-1, (**b**) U937 and (**c**) RAW264.7 respectively at 48 and 72 h. Error bars designate the ± SD (n=3). Student's *t*-test was performed, and the significance is indicated as * $p<0.05$, § $p<0.01$, and # $p<0.001$.

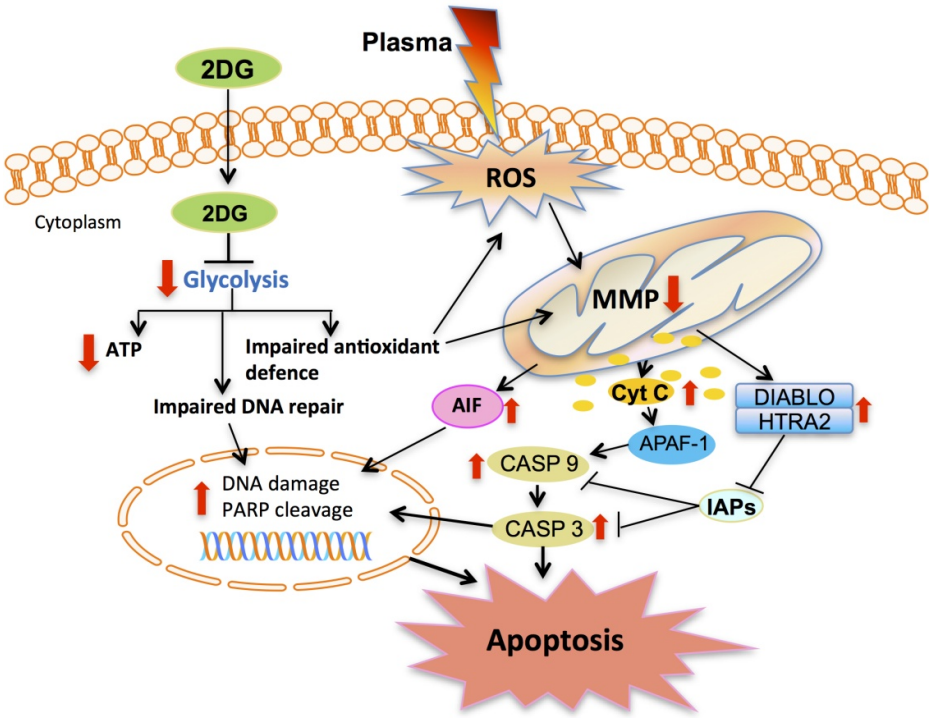


**Figure S13. Proposed mechanism of 2-deoxy-D-glucose with non-thermal plasma that induce the synergistic inhibition effect in blood cancer cells.** 2-deoxy-D-glucose (2-DG) enters inside the cell cytoplasm through cell membrane and forms 2-DG-6-phosphate by hexokinase phosphorylation, which is the first step of glycolysis pathway. This 2-DG-6-phosphate cannot further metabolize and trapped in the cytosol and eventually inhibits glycolysis. This result in decrease of cellular ATP, lactate production, impaired antioxidant machinery and DNA repair activity in cancer cells and consequently, cells become weak. However, plasma can generate oxidative stress through ROS (reactive oxygen species), which leads to the change in mitochondrial membrane potential (MMP) that triggers mitochondrial mediated intrinsic apoptosis pathway. This pathway subsequently leads to the release of cytochrome c (Cyt c), apoptosis-inducing factor (AIF), direct inhibitor of apoptosis protein (IAP)-binding protein with low PI (DIABLO), and HTRA serine peptidase 2 (HTRA2). On release to the cytosol, cytochrome c triggers caspase 3 activation through the formation of apoptosome complex and catalyse the PARP1 activity and leads to the DNA damage contributing to caspase-dependent apoptosis, whereas DIABLO and HTRA2 triggers caspase 3 activation by neutralizing the activity of IAPs (inhibitor of apoptosis protein). Similarly, AIF can be released from the mitochondria on outer mitochondrial membrane permeabilization, and move to the nucleus to contribute DNA damage or fragmentation. Taken together, 2-DG and plasma combination synergistically induced mitochondrial intrinsic apoptosis in cancer cells that can be either caspase-dependent or independent.

**Table S1.** This table provides the Combination Index (CI) value that was obtained when combining 2-DG with plasma at different doses on blood cancer and normal cells, using the Chou-Talalay method. CI < 1: Synergy; CI=1: Additive effect; CI > 1: Antagonism.

| Treatments | THP-1 | U937 | RAW264.7 | PBMCs |
|---|---|---|---|---|
| 1 mM 2DG + 1 min plasma | 0.50 | 0.59 | 3.99 | 2.8 |
| 1 mM 2DG + 3 Min Plasma | 0.40 | 0.55 | 1.59 | 0.71 |
| 5 mM 2DG + 1 min Plasma | 0.51 | 0.56 | 1.10 | 0.79 |
| 5 mM 2DG + 3 min plasma | 0.50 | 0.39 | 1.47 | 1.01 |
| 10 mM 2DG + 1 min plasma | 0.68 | 0.75 | 0.77 | 0.64 |
| 10 mM 2DG + 3 min plasma | 0.51 | 0.34 | 0.96 | 0.58 |

**Table S2.** The list of RT² qPCR Primer Assays (Qiagen, USA) used for real-time PCR analysis.

| S.N. | Catalog No. | Product Description | Gene code |
|---|---|---|---|
| 1. | PPH00073G | RT² qPCR Primer Assay for ACTB | Beta Actin (Human) |
| 2. | PPH00107C | RT² qPCR Primer Assay for CASP3 | CASP3 (Human) |
| 3. | PPH00353B | RT² qPCR Primer Assay for CASP9 | CASP9 (Human) |
| 4. | PPH00686B | RT² qPCR Primer Assay for PARP1 | PARP1 (Human) |
| 5. | PPH01037A | RT² qPCR Primer Assay for AIFM1 | AIFM1 (Human) |
| 6. | PPH00752A | RT² qPCR Primer Assay for APAF1 | APAF-1 (Human) |
| 7. | PPH20675F | RT² qPCR Primer Assay for CYCS | CYCS (Human) |
| 8. | PPH11316A | RT² qPCR Primer Assay for HTRA2 | HTRA2 (Human) |
| 9. | PPH05797A | RT² qPCR Primer Assay for DIABLO | DIABLO (Human) |