**Supporting Information**

# Photo-responsive hydrogels with photoswitchable mechanical properties allow time-resolved analysis of cellular responses to matrix stiffening

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## 1. Tables of prepolymer formulations.

**Table S1.** Formulations with ratios of AM/BIS/AZO monomers in DMF and EtOH.

| Formula | Monomer/Crosslinker | | | Solvent System | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | AM (M) | BIS (mM) | AZO (mM) | EtOH (μL) | DMF (μL) | DMSO (μL) | $H_2O$ (μL) | PBS[(a)] (μL) | Heating[(b)] | Observation[(c)] |
| **1** | 1.65 | 26.26 | 10.12 | -- | 500 | -- | 500 | 225 | -- | x |
| **2** | 1.72 | 27.37 | -- | -- | 225 | -- | 725 | 225 | -- | x |
| **3** | 1.65 | 26.26 | -- | -- | 500 | -- | 500 | 225 | -- | x |
| **4** | 1.84 | 29.22 | -- | -- | 150 | -- | 725 | 225 | -- | hydrogel |
| **5** | 1.84 | 29.22 | -- | 225 | 150 | -- | 500 | 225 | -- | hydrogel |
| **6** | 1.84 | 29.22 | 11.26 | -- | 150 | -- | 725 | 225 | -- | precipitation |
| **7** | 1.84 | 29.22 | 5.63 | 225 | 150 | -- | 500 | 225 | -- | precipitation |
| **8** | 1.92 | 30.60 | 2.95 | 225 | 100 | -- | 500 | 225 | -- | precipitation |
| **9** | 1.92 | 30.60 | 2.95 | 225 | 100 | -- | 500 | 225 | 70℃ | hydrogel |
| **10** | 0.41 | 6.59 | -- | 100 | -- | -- | 625 | 250 | -- | hydrogel |
| **11** | 0.46 | 7.33 | -- | 45 | -- | -- | 580 | 250 | -- | hydrogel |
| **12** | 0.41 | 6.59 | -- | 45 | 100 | -- | 580 | 250 | -- | hydrogel |
| **13** | 0.46 | 7.33 | -- | 45 | 200 | -- | 380 | 250 | -- | x |
| **14** | 0.46 | 7.33 | -- | -- | 625 | -- | -- | 250 | -- | x |
| **15** | 0.41 | 6.59 | -- | 413 | 100 | -- | 212 | 250 | -- | x |

[(a)] PBS: phosphate buffered saline

[(b)] "--" in this column indicates reaction was performed at room temperature.

[(c)] "x" in this column indicates no gel formation observed, mixture remained liquid

**Table S2.** Formulations with minimal AM/BIS monomers in different solvent systems.

| Formula | Monomer/Crosslinker | | | Solvent System | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | AM (M) | BIS (mM) | AZO (mM) | EtOH (μL) | DMF (μL) | DMSO (μL) | $H_2O$ (μL) | PBS[a] (μL) | Heating[b] | Observation[c] |
| **16** | 0.46 | 7.33 | -- | 625 | -- | -- | | 250 | -- | x |
| **17** | 0.46 | 7.33 | -- | -- | -- | -- | 625 | 250 | -- | x |
| **18** | 2.81 | 0.14 | -- | -- | -- | -- | 765 | 225 | -- | hydrogel |
| **19** | 2.81 | 0.11 | -- | -- | -- | -- | 765 | 225 | -- | hydrogel |
| **20** | 2.81 | 0.07 | -- | -- | -- | -- | 765 | 225 | -- | hydrogel |
| **21** | 2.79 | 0.14 | -- | 225 | -- | -- | 550 | 225 | -- | hydrogel |
| **22** | 2.79 | 0.03 | -- | 225 | -- | -- | 550 | 225 | -- | x |

[a] PBS: phosphate buffered saline

[b] "--" in this column indicates reaction was performed at room temperature.

[c] "x" in this column indicates no gel formation observed, mixture remained liquid

**Table S3.** Formulation of AM/BIS/AZO polymer heated during polymerisation.

| Formula | Monomer/Crosslinker | | | Solvent System | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | AM (M) | BIS (mM) | AZO (mM) | EtOH (μL) | DMF (μL) | DMSO (μL) | $H_2O$ (μL) | PBS[a] (μL) | Heating[b] | Observation[c] |
| **23** | 2.87 | 45.68 | 4.40 | 100 | -- | -- | 600 | -- | 70℃ | hydrogel |
| **24** | 0.80 | 12.72 | 6.13 | 200 | -- | -- | 300 | -- | 70℃ | hydrogel |
| **25** | 1.32 | 20.92 | 10.08 | 100 | -- | -- | 200 | -- | 70℃ | hydrogel |
| **26** | 0.45 | 7.13 | 3.43 | 400 | -- | -- | 500 | -- | 70℃ | x |
| **27** | 0.45 | 7.13 | 3.43 | -- | -- | -- | 900 | -- | 70℃ | precipitate |
| **28** | 0.45 | 7.13 | 3.43 | -- | -- | 200 | 700 | -- | 70℃ | precipitate |
| **29** | 0.45 | 7.13 | 3.43 | -- | -- | 200 | 700 | -- | 70℃ | precipitate |
| **30** | 0.45 | 7.13 | 3.43 | -- | -- | 200 | 700 | -- | 70℃ | precipitate |
| **31** | 0.45 | 7.13 | 3.43 | -- | -- | 600 | 300 | -- | 70℃ | precipitate |
| **32** | 0.45 | 7.13 | 3.43 | -- | -- | 600 | 300 | -- | 70℃ | precipitate |
| **33** | 0.40 | 6.36 | 3.06 | 100 | -- | -- | 100 | 800 | 70℃ | precipitate |

[a] PBS: phosphate buffered saline

[b] "--" in this column indicates reaction was performed at room temperature.

[c] "x" in this column indicates no gel formation observed, mixture remained liquid

**Table S4.** Formulations with high concentrations of monomers.

| Formula | Monomer/Crosslinker | | | Solvent System | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | AM (M) | BIS (mM) | AZO (mM) | EtOH (μL) | DMF (μL) | DMSO (μL) | $H_2O$ (μL) | PBS[a] (μL) | Heating[b] | Observation[c] |
| **34** | 0.50 | -- | 0.35 | -- | -- | -- | 880 | -- | -- | precipitate |
| **35** | 0.80 | 0.28 | 4.46 | -- | -- | -- | 650 | -- | -- | precipitate |
| **36** | 0.66 | 0.23 | 3.68 | -- | -- | 350 | 450 | -- | -- | x |
| **37** | 0.66 | 0.23 | 3.68 | -- | -- | 250 | 550 | -- | -- | x |
| **38** | 0.66 | 0.23 | 3.68 | 250 | -- | -- | 550 | -- | -- | x |
| **39** | 2.56 | 0.08 | 3.55 | 225 | -- | -- | 420 | 225 | -- | precipitate |
| **40** | 2.30 | 0.07 | 3.19 | 325 | -- | -- | 420 | 225 | -- | precipitate |
| **41** | 1.72 | 0.07 | 3.19 | 425 | -- | -- | 320 | 225 | -- | precipitate |
| **42** | 1.15 | 0.07 | 3.19 | 525 | -- | -- | 220 | 225 | -- | precipitate |
| **43** | 1.76 | -- | 3.26 | 425 | -- | -- | 300 | 225 | -- | precipitate |
| **44** | 1.76 | -- | 3.26 | -- | -- | 225 | 500 | 225 | -- | precipitate |
| **45** | 1.72 | 0.07 | 3.19 | -- | -- | 225 | 520 | 225 | -- | x |
| **46** | 0.59 | 0.07 | -- | -- | -- | 225 | 500 | 225 | -- | x |
| **47** | 1.15 | 0.07 | 3.19 | -- | -- | 225 | 520 | 225 | -- | x |
| **48** | 0.57 | 0.07 | 3.19 | -- | -- | 325 | 420 | 225 | -- | x |

[a] PBS: phosphate buffered saline

[b] "--" in this column indicates reaction was performed at room temperature.

[c] "x" in this column indicates no gel formation observed, mixture remained liquid

**Table S5.** Formulations of AM/BIS/AZO monomers with various solvents.

| Formula | Monomer/Crosslinker | | | Solvent System | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | AM (M) | BIS (mM) | AZO (mM) | EtOH (μL) | DMF (μL) | DMSO (μL) | $H_2O$ (μL) | PBS[(a)] (μL) | Heating[(b)] | Observation[(c)] |
| **49** | 2.30 | 0.07 | 1.59 | 325 | -- | -- | 420 | 225 | -- | hydrogel |
| **50** | 1.72 | 0.07 | 1.59 | 425 | -- | -- | 320 | 225 | -- | hydrogel |
| **51** | 1.15 | 0.07 | 1.59 | 525 | -- | -- | 220 | 225 | -- | hydrogel |
| **52** | 1.76 | -- | 3.26 | -- | -- | 225 | 500 | 225 | -- | hydrogel |
| **53** | 1.76 | 0.04 | 1.63 | -- | -- | 225 | 500 | 225 | -- | hydrogel |
| **54** | 1.17 | 0.04 | 1.63 | -- | -- | 125 | 600 | 225 | -- | precipitate |
| **55** | 1.17 | 0.04 | 1.63 | -- | -- | 100 | 625 | 225 | -- | hydrogel |
| **56** | 1.76 | -- | 1.63 | -- | -- | 225 | 300 | 425 | -- | hydrogel |
| **57** | 1.06 | -- | 1.47 | -- | -- | 100 | 200 | 750 | -- | precipitate |
| **58** | 1.06 | -- | 1.47 | -- | -- | 100 | 725 | 225 | -- | precipitate |
| **59** | 1.17 | -- | 1.63 | -- | -- | 225 | 200 | 525 | -- | precipitate |
| **60** | 1.06 | -- | 1.47 | -- | -- | 100 | 725 | 225 | -- | hydrogel |
| **61** | 0.59 | -- | 1.63 | -- | -- | 100 | 625 | 225 | -- | x |
| **62** | 1.17 | 0.04 | 1.63 | -- | -- | 225 | 500 | 225 | -- | hydrogel |
| **63** | 0.59 | -- | 1.63 | -- | -- | 225 | 100 | 625 | -- | x |
| **64** | 1.76 | -- | 0.81 | -- | -- | 225 | 300 | 425 | -- | hydrogel |
| **65** | 1.17 | -- | 0.41 | -- | -- | 113 | 200 | 637 | -- | hydrogel |
| **66** | 1.13 | -- | 0.16 | -- | -- | 45 | 200 | 737 | -- | hydrogel |
| **67** | 1.17 | -- | 0.81 | -- | -- | 225 | 200 | 525 | -- | hydrogel |
| **68** | 1.17 | 0.04 | 0.41 | -- | -- | 113 | 200 | 637 | -- | hydrogel |
| **69** | 1.17 | 0.04 | 0.16 | -- | -- | 45 | 200 | 705 | -- | hydrogel |

[(a)] PBS: phosphate buffered saline

[(b)] "--" in this column indicates reaction was performed at room temperature.

[(c)] "x" in this column indicates no gel formation observed, mixture remained liquid

**Table S5.** Continued.

| Formula | Monomer/Crosslinker | | | Solvent System | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | AM (M) | BIS (mM) | AZO (mM) | EtOH (μL) | DMF (μL) | DMSO (μL) | $H_2O$ (μL) | PBS[a] (μL) | Heating[b] | Observation[c] |
| **70** | 1.76 | -- | 8.14 | 200 | -- | 225 | 300 | 225 | -- | hydrogel |
| **71** | 1.59 | -- | 7.37 | 225 | -- | 100 | 300 | 425 | -- | precipitate |
| **72** | 1.56 | -- | 7.23 | 120 | -- | 220 | 300 | 430 | -- | precipitate |
| **73** | 1.56 | -- | 7.23 | 220 | -- | 220 | 300 | 330 | -- | precipitate |
| **74** | 2.45 | -- | 7.27 | 225 | -- | 100 | 375 | 150 | -- | precipitate |
| **75** | 2.20 | -- | 3.40 | 225 | -- | 100 | 360 | 225 | -- | hydrogel |

[a] PBS: phosphate buffered saline

[b] "--" in this column indicates reaction was performed at room temperature.

[c] "x" in this column indicates no gel formation observed, mixture remained liquid

**Table S6.** Formulations of AM/AZO polymer using improved conditions based on previous formulations.

| Formula | Monomer/Crosslinker | | | Solvent System | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | AM (M) | BIS (mM) | AZO (mM) | EtOH (μL) | DMF (μL) | DMSO (μL) | $H_2O$ (μL) | PBS[a] (μL) | Heating[b] | Observation[c] |
| **76** | 2.20 | -- | 6.79 | 225 | -- | 100 | 360 | 225 | -- | hydrogel with precipitated particulates |
| **77** | 2.20 | -- | 5.10 | 225 | -- | 100 | 360 | 225 | -- | hydrogel with precipitated particulates |
| **78** | 2.20 | -- | 3.40 | 180 | -- | 60 | 360 | 310 | -- | hydrogel |
| **79** | 2.20 | -- | 6.79 | 180 | -- | 60 | 360 | 310 | -- | hydrogel with precipitated particulates |
| **80** | 2.20 | -- | 3.40 | 100 | -- | 60 | 360 | 390 | -- | hydrogel with precipitated particulates |
| **81** | 2.20 | -- | 3.40 | 100 | -- | 80 | 360 | 370 | -- | hydrogel |
| **82** | 2.03 | -- | 3.13 | 100 | -- | 80 | 360 | 450 | -- | hydrogel with precipitated particulates |
| **83** | 4.05 | -- | 3.13 | 100 | -- | 80 | 720 | 90 | -- | hydrogel |
| **84** | 1.01 | -- | 3.13 | 100 | -- | 80 | 180 | 630 | -- | precipitation |
| **85** | 4.05 | -- | 3.13 | 100 | -- | 160 | 720 | 10 | -- | x |
| **86** | 4.40 | -- | 6.79 | 100 | -- | 80 | 720 | 10 | -- | precipitation |

[a] PBS: phosphate buffered saline

[b] "--" in this column indicates reaction was performed at room temperature.

[c] "x" in this column indicates no gel formation observed, mixture remained liquid

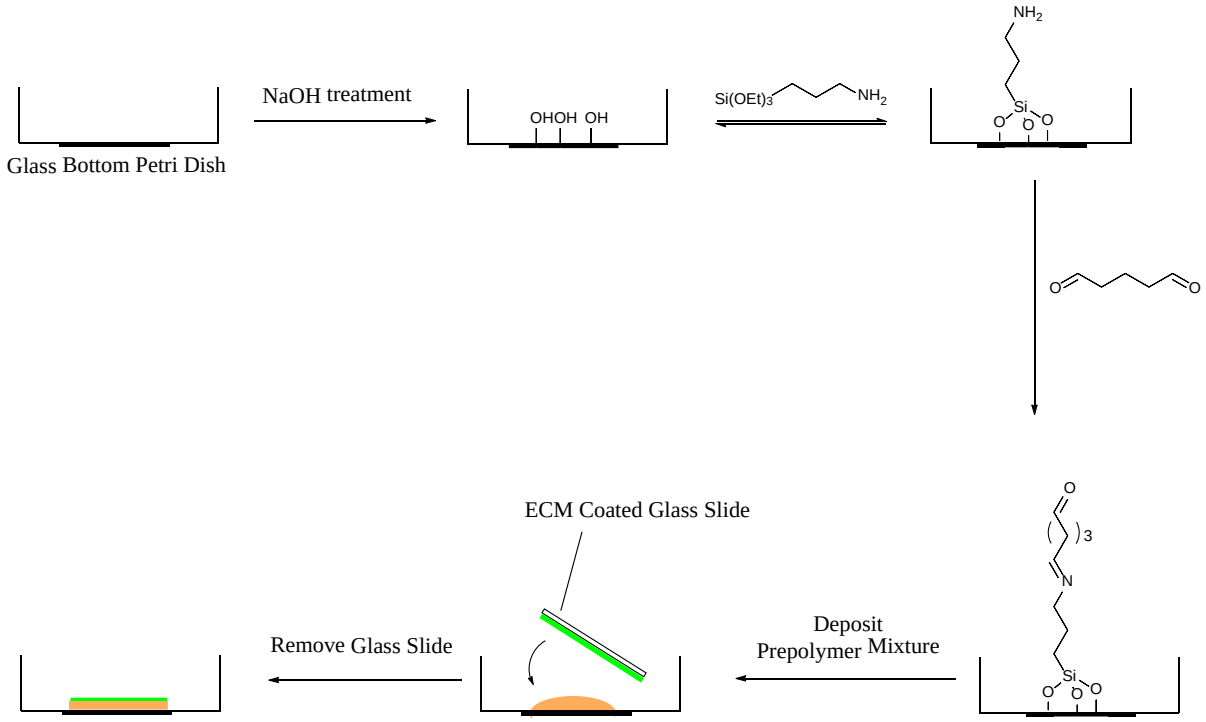


**Figure S1.** Schematic diagram of the substrate preparation for cell culture.

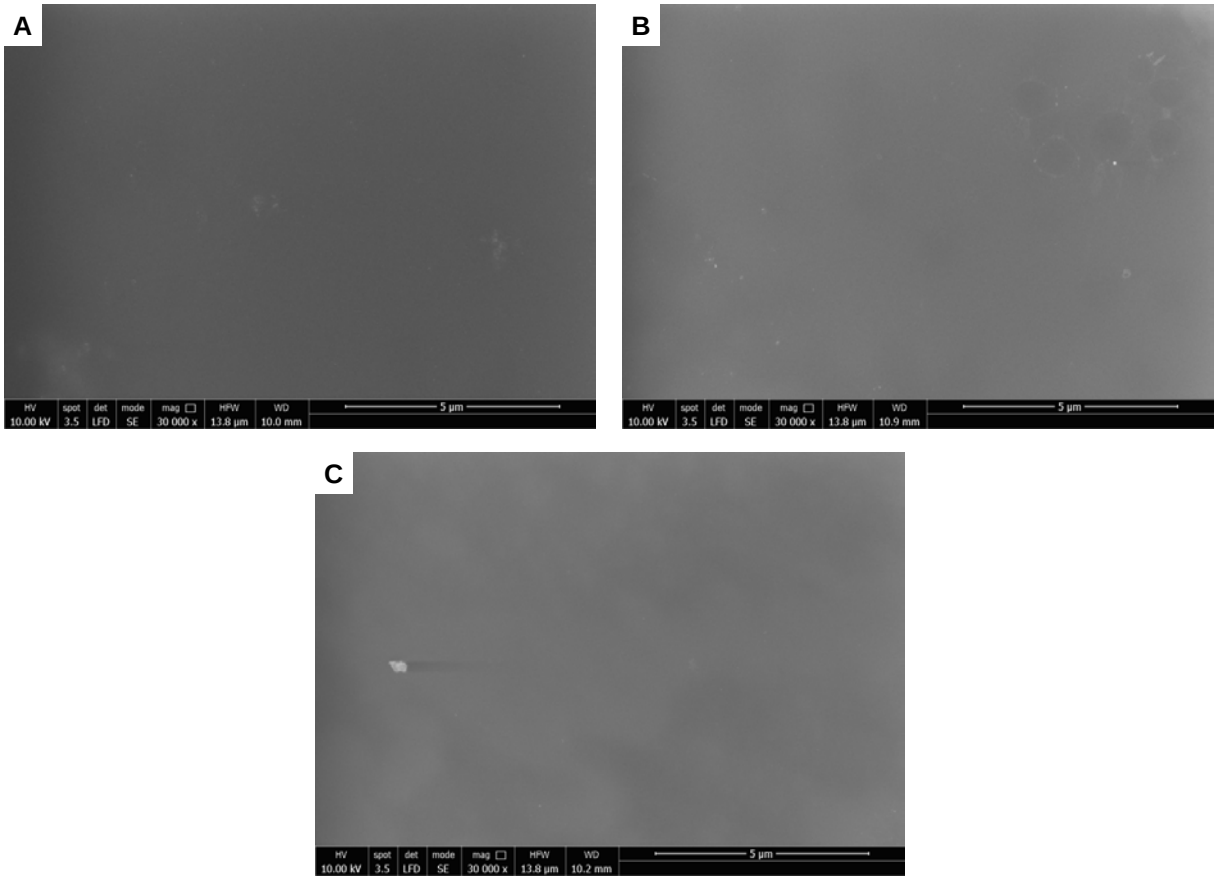


**Figure S2.** Low magnification ESEM micrographs of photo-responsive hydrogels before and after irradiation. The images were recorded following the treatments indicated in Figure 4A at the point of "stiffness measurement". (A) Control ("ctrl") sample: AZO hydrogels not subjected to irradiation. (B) "(-) blue" sample: hydrogel treated with UV (365 nm) irradiation. (C) "(+) blue" sample: hydrogel treated with UV (365 nm) and subsequent blue light (490 nm) irradiation.

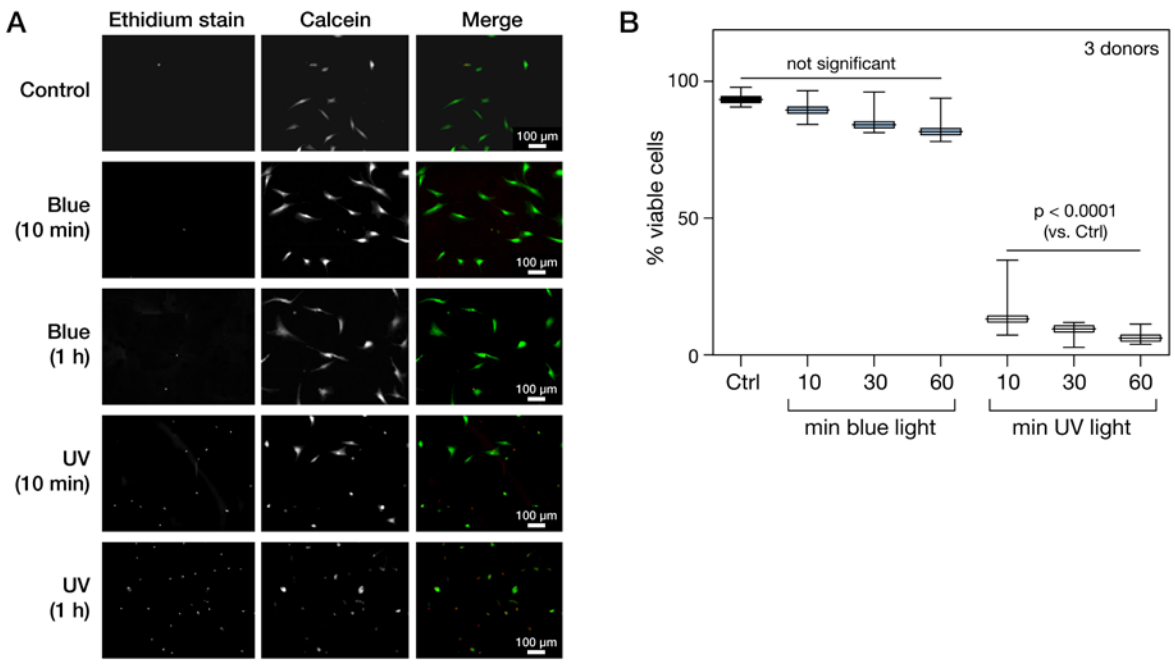


**Figure S3. Cell viability following UV or blue light irradiation.** Live cells are indicated by green fluorescence due to the generation of free calcein by intracellular esterases, while dead cells exhibit red fluorescence from ethidium, following loss of cell membrane integrity. (A) Primary human MSCs were subjected to different durations of UV (365 nm) or blue light (490 nm) irradiation. Cell viability was assayed by ethidium and calcein staining (scale bar = 100 µm). (B) Quantitative analysis of cell viability images. Exposure to blue light for up to 1 hour did not significantly reduce cell viability. Exposure to 10 minutes of UV irradiation significantly reduced cell viability to 18 ± 8 % (± S.E.M.; $n > 177$ measurements, cells from three donors; *p*-values indicated from ANOVA testing).

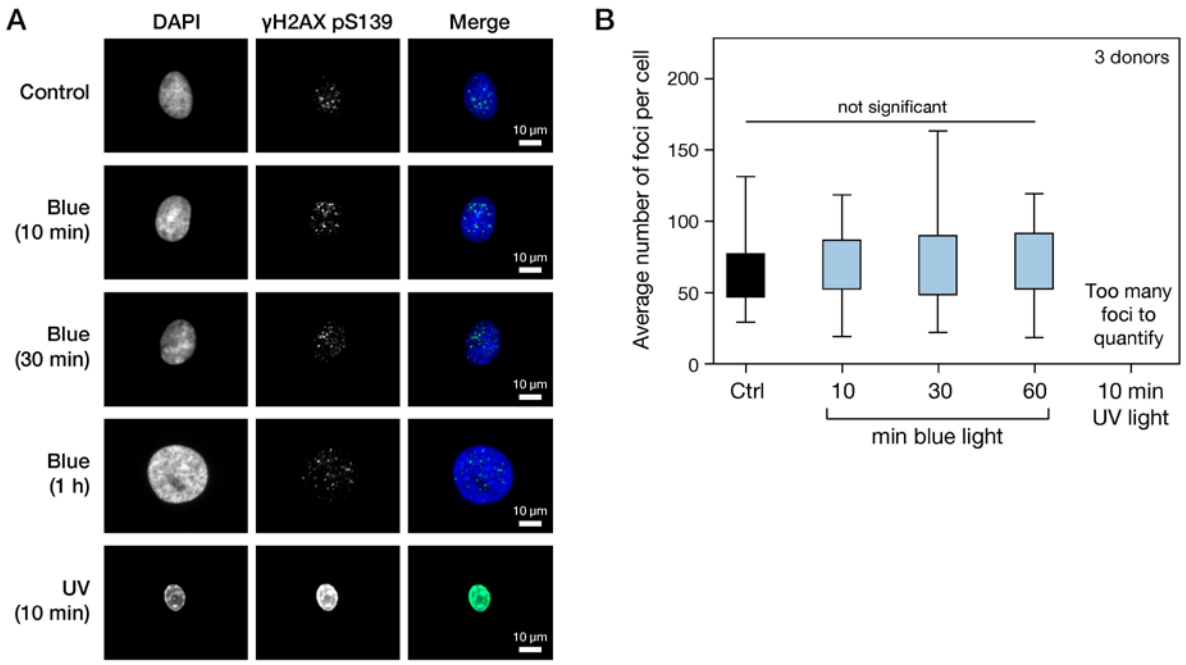


**Figure S4. DNA damage following UV or blue light irradiation.** (A) Primary human MSCs subjected to UV or blue light irradiation were imaged with the nuclei DAPI stained and immuno-stained against $\gamma H_{2A}X$ pS139 (scale bar = 10 µm). (B) Quantification of the number of $\gamma H_{2A}X$ pS139 foci per nucleus. Blue light exposure for up to 1 hour did not significantly increase the number of foci, but UV exposure of just 10 minutes created more foci than could be resolved with the microscope ($n > 57$ measurements, cells from three donors; significance from ANOVA and *post hoc* testing).