Electronic Supporting Information

Silica Nanoparticle Conjugation with Gallic Acid Towards Enhanced Free Radical Scavenging Capacity and Activity on Osteosarcoma Cells in vitro

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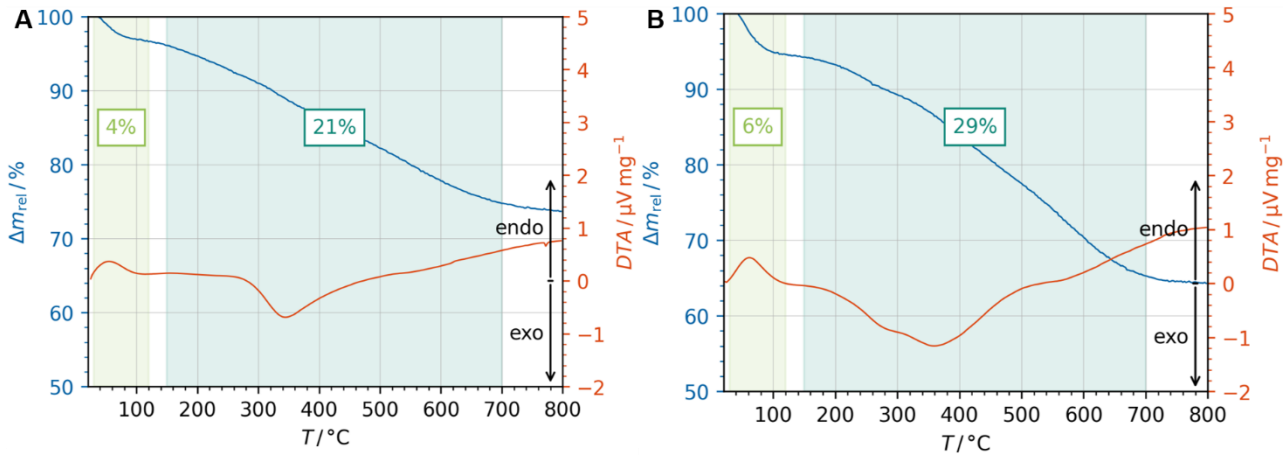

Figure S1. Coupled thermogravimetric analysis (TGA) and differential thermal analysis (DTA) of DMSNs conjugated with GA: DMSN-NCO-GA (A), and DMSN-NH-GA (B). The mass loss (blue line) was determined from 150 ºC to 700 ºC (dark green area) to exclude contributions from remaining solvents or water (light green area).

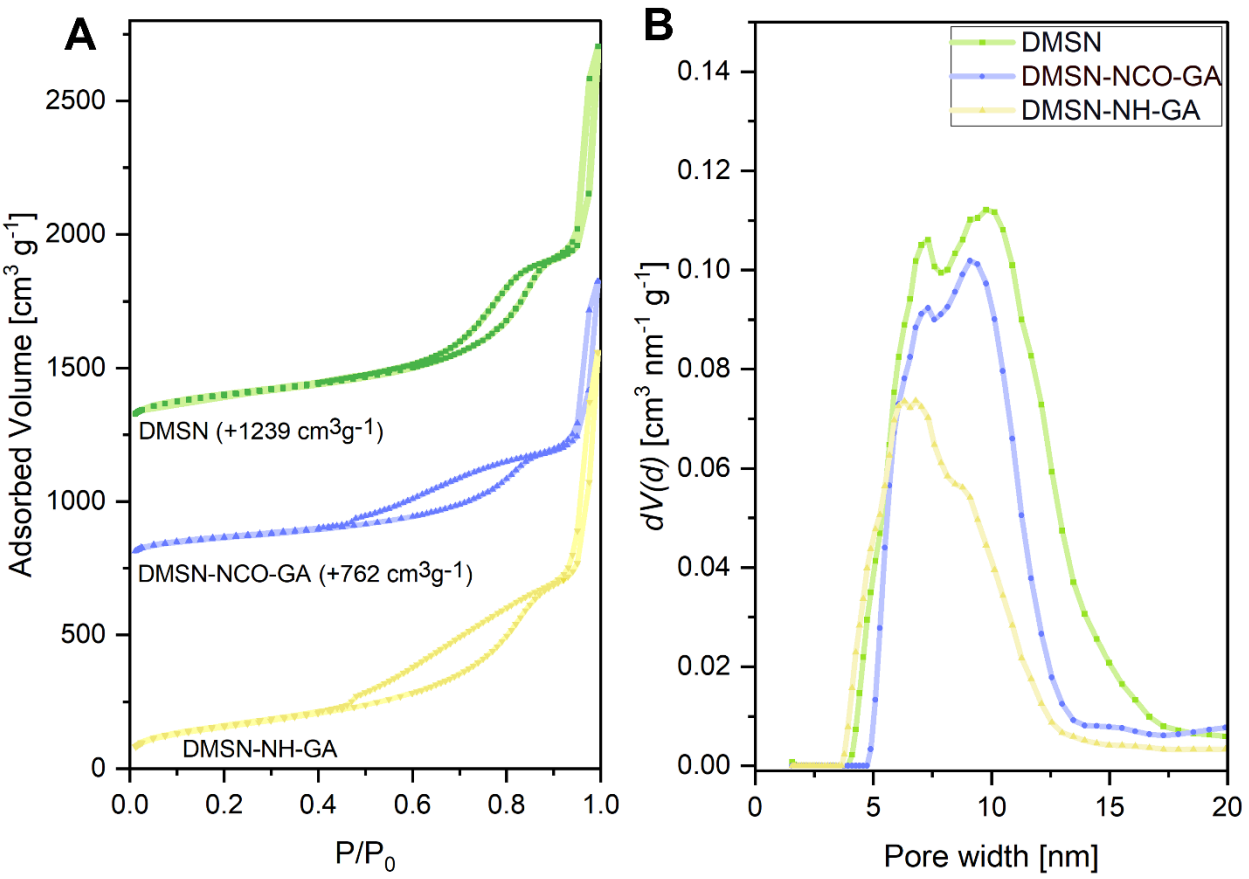

Figure S2. N_2 physisorption isotherms (measured at - 196 °C) of the different DMSNs, as indicated, (A) and their respective NLDFT pore size distributions (B).

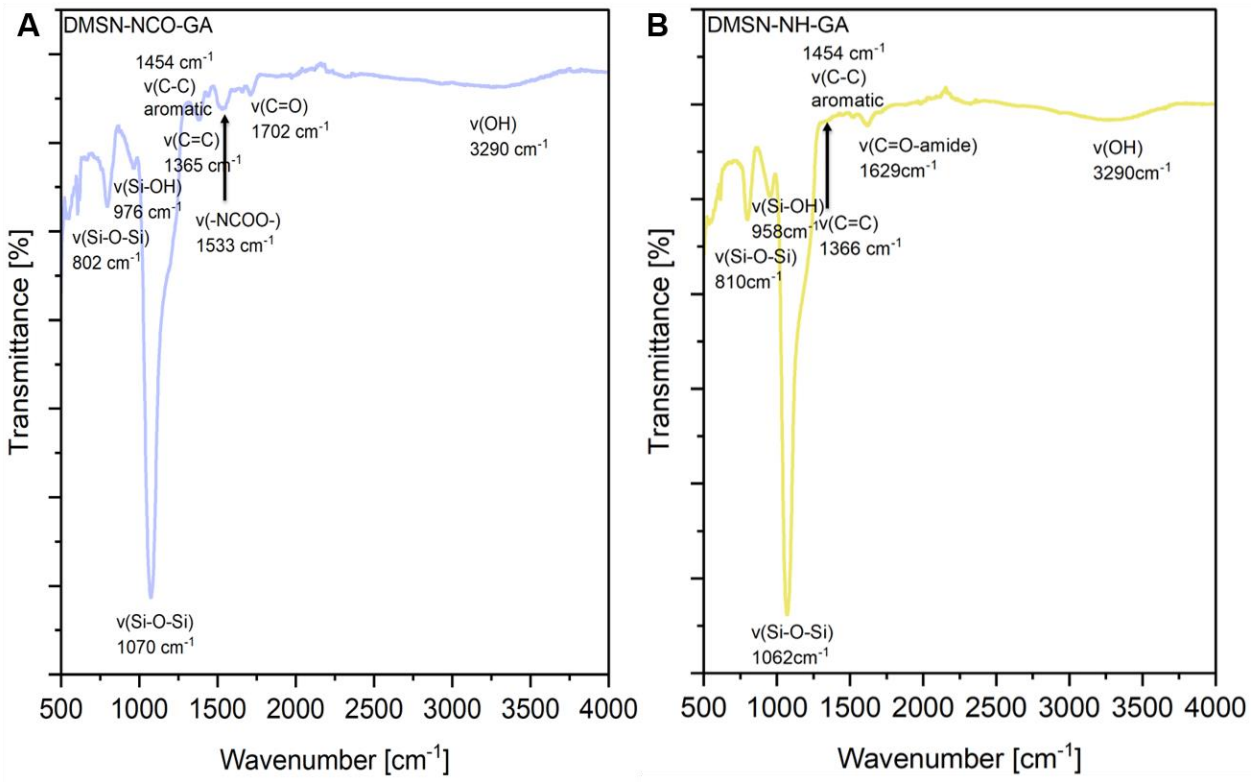

Figure S3. Attenuated total reflectance infrared (ATR-IR) spectra of GA-functionalized DMSNs: DMSN-NCO-GA (A), and DMSN-NH-GA(B).

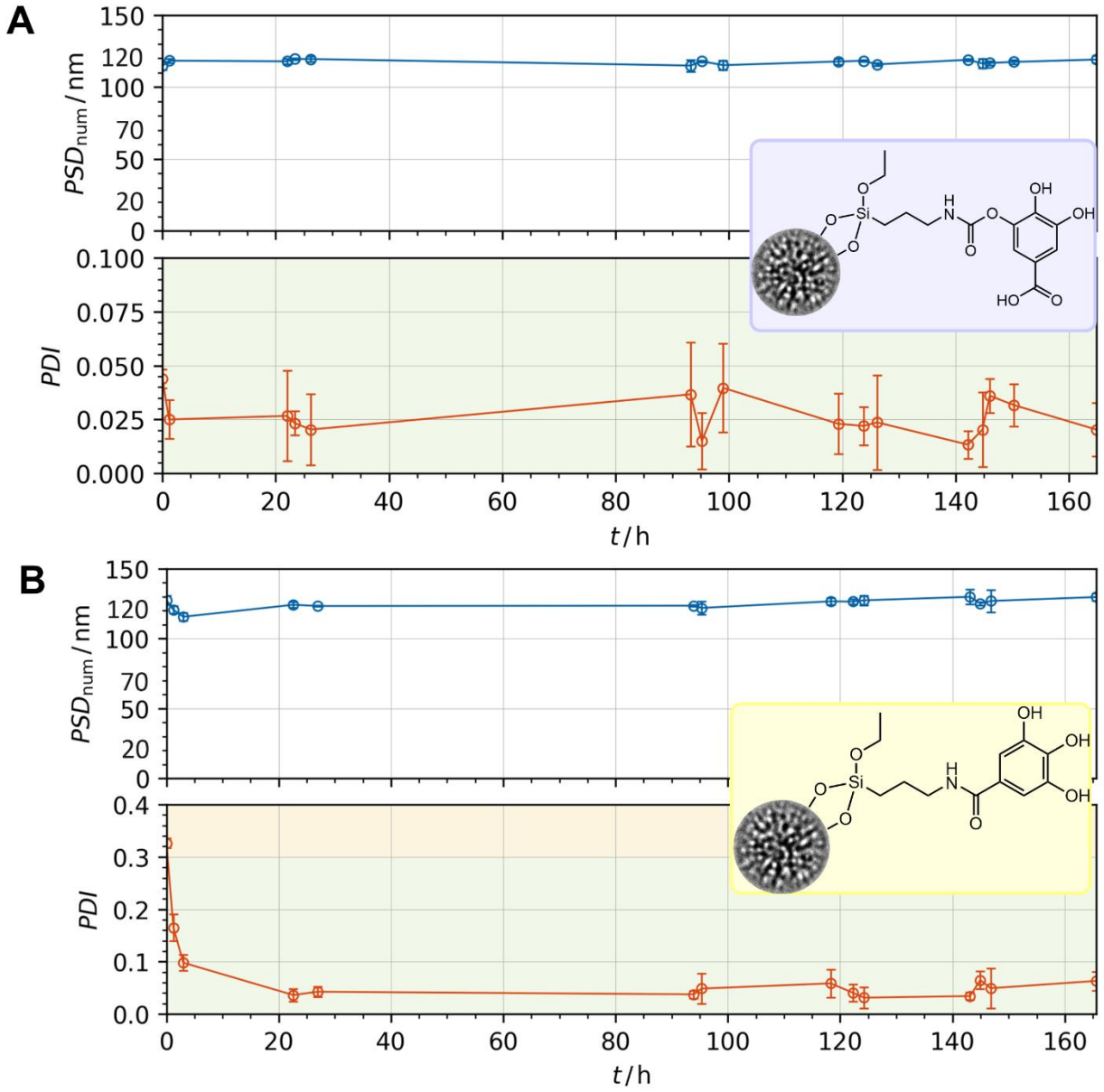

Figure S4. Colloidal stability tests via dynamic light scattering (DLS) measurements of DMSN-NCO-GA (A), and DMSN-NH-GA (B).

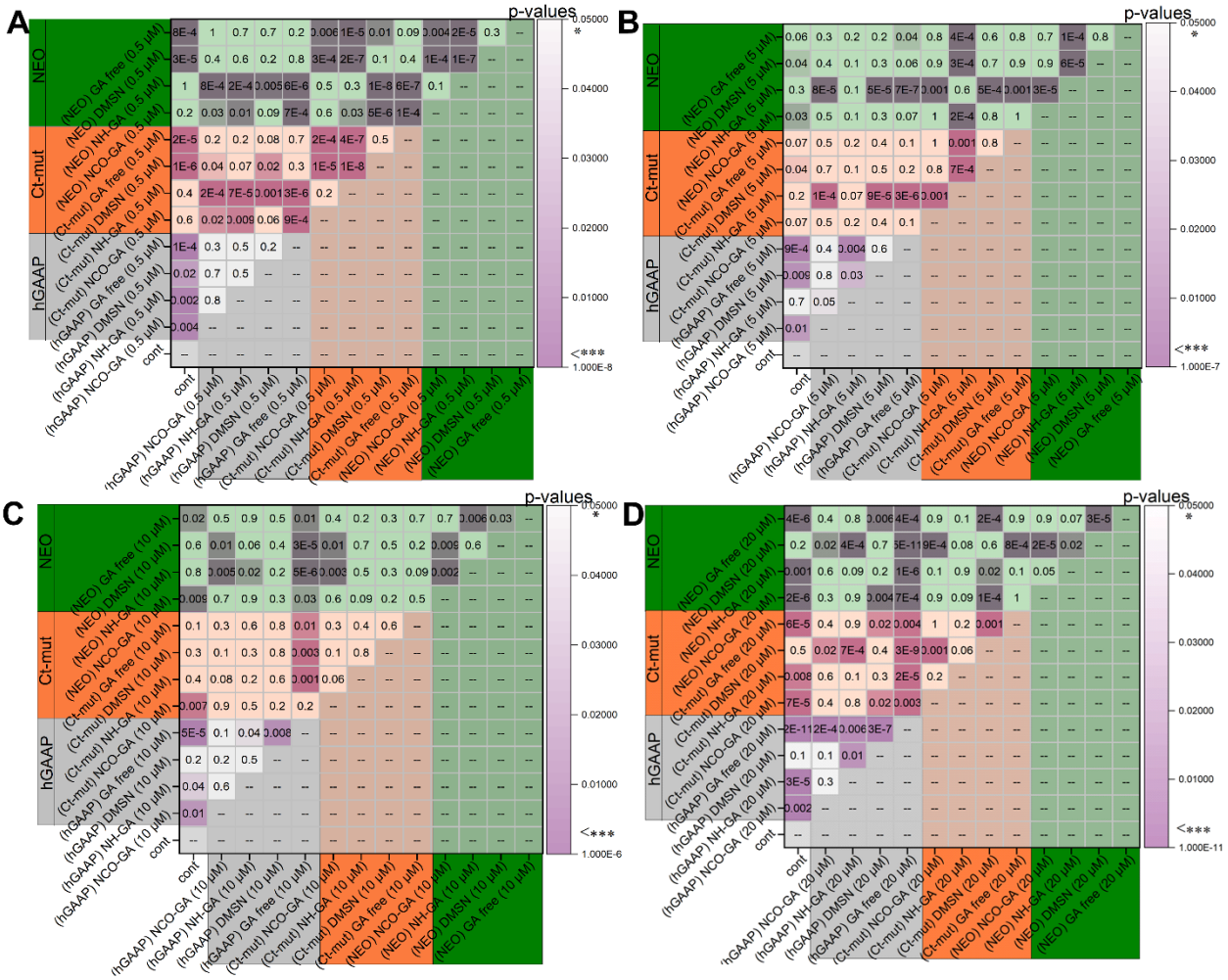

Figure S5. Statistical evaluation of the cell viability assay in the presence of GA-conjugated particles as measured by metabolic activity (cell titer blue assay) in U2-OS cell lines (hGAAP (grey), NEO (orange), and Ct-mut (green)) after incubation for 24 h. The difference among the treatments was determined via one-way ANOVA with Fisher LSD (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, depicted in violet).

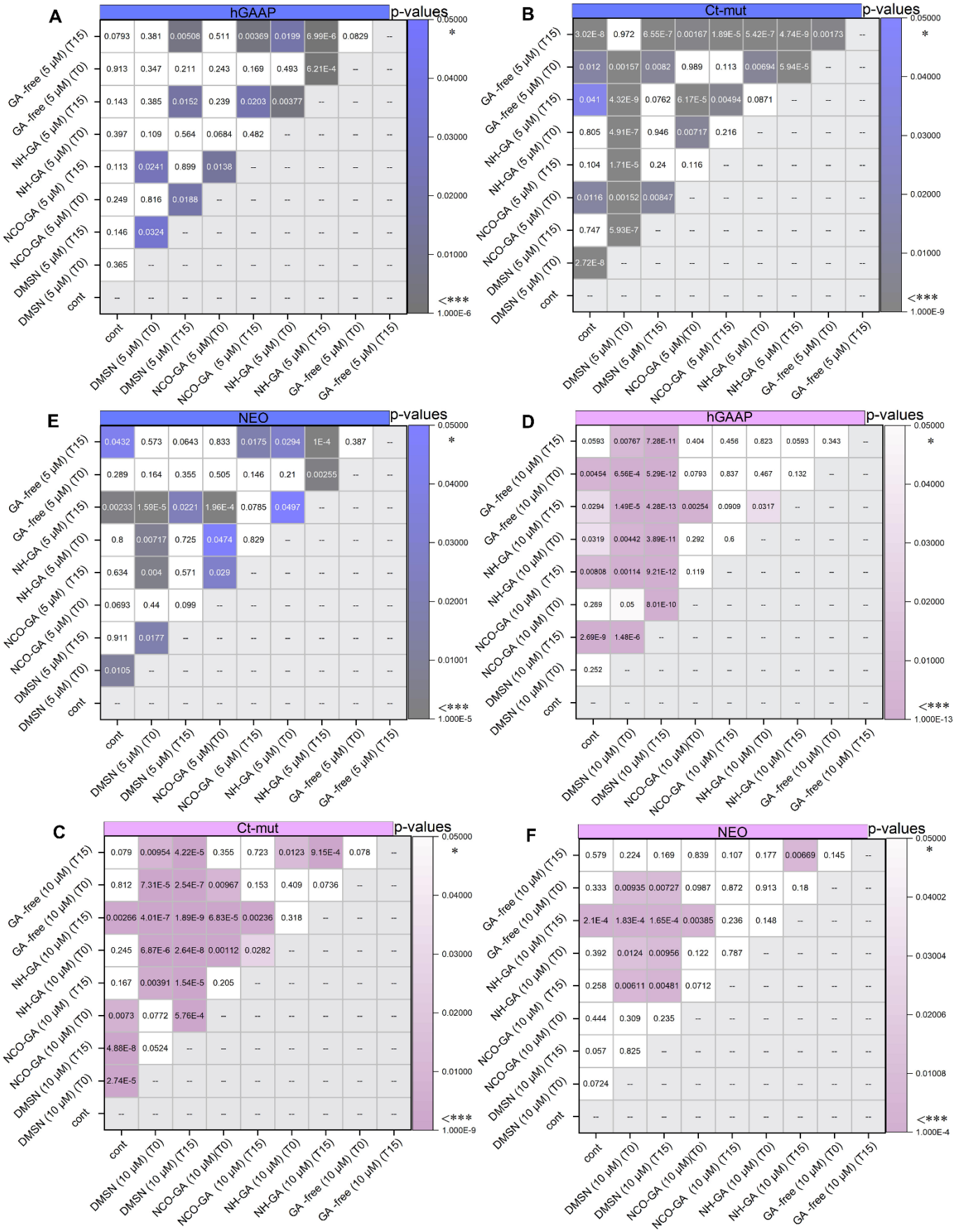
A
hGAAP
p-values
B
Ct-mut
p-values
E
NEO
p-values
D
hGAAP
p-values
C
Ct-mut
p-values
F
NEO
p-values
GA -free (5 µM) (T15)
GA -free (5 µM) (T0)
NH-GA (5 µM) (T15)
NH-GA (5 µM) (T0)
NCO-GA (5 µM) (T15)
NCO-GA (5 µM) (T0)
DMSN (5 µM) (T15)
DMSN (5 µM) (T0)
cont
GA -free (10 µM) (T15)
GA -free (10 µM) (T0)
NH-GA (10 µM) (T15)
NH-GA (10 µM) (T0)
NCO-GA (10 µM) (T15)
NCO-GA (10 µM) (T0)
DMSN (10 µM) (T15)
DMSN (10 µM) (T0)
DMSN (5 µM) (T0)
DMSN (5 µM) (T15)
NCO-GA (5 µM)(T0)
NCO-GA (5 µM) (T15)
NH-GA (5 µM) (T0)
NH-GA (5 µM) (T15)
GA -free (5 µM) (T0)
GA -free (5 µM) (T15)
DMSN (10 µM) (T0)
DMSN (10 µM) (T15)
NCO-GA (10 µM)(T0)
NCO-GA (10 µM) (T15)
NH-GA (10 µM) (T0)
NH-GA (10 µM) (T15)
GA -free (10 µM) (T0)
GA -free (10 µM) (T15)
0.05000
*
0.04000
0.03000
0.02000
0.01000
<***
1.000E-6
1.000E-9
0.02001
0.01001
1.000E-5
0.04002
0.03004
0.02006
0.01008
1.000E-13
1.000E-4

Figure S6. Statistical analysis was performed on the signal intensity quantification after incubating U2-OS cell lines (hGAAP (A, D), Ct-mut (B, E), and NEO (C, F)) with free GA, DMSN-NH-GA, and DMSN-NCO-GA, in the absence of an oxidative stressor. The incubation was performed for 24 h with a GA concentration of 5 μM (A-C) and 10 μM (D-F), and non-functionalized DMSNs with concentrations equivalent to 5 μM and 10 μM GA, namely 53.15 μg mL^{-1} and 106.3 μg mL^{-1}. Three independent cell preparations were conducted, and the data was expressed as an average fluorescence intensity normalized to the relative fluorescence generated by the application of the solvent control. The difference among the treatments was assessed using one-way ANOVA with Fisher LSD (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, depicted in grey to blue and violet).

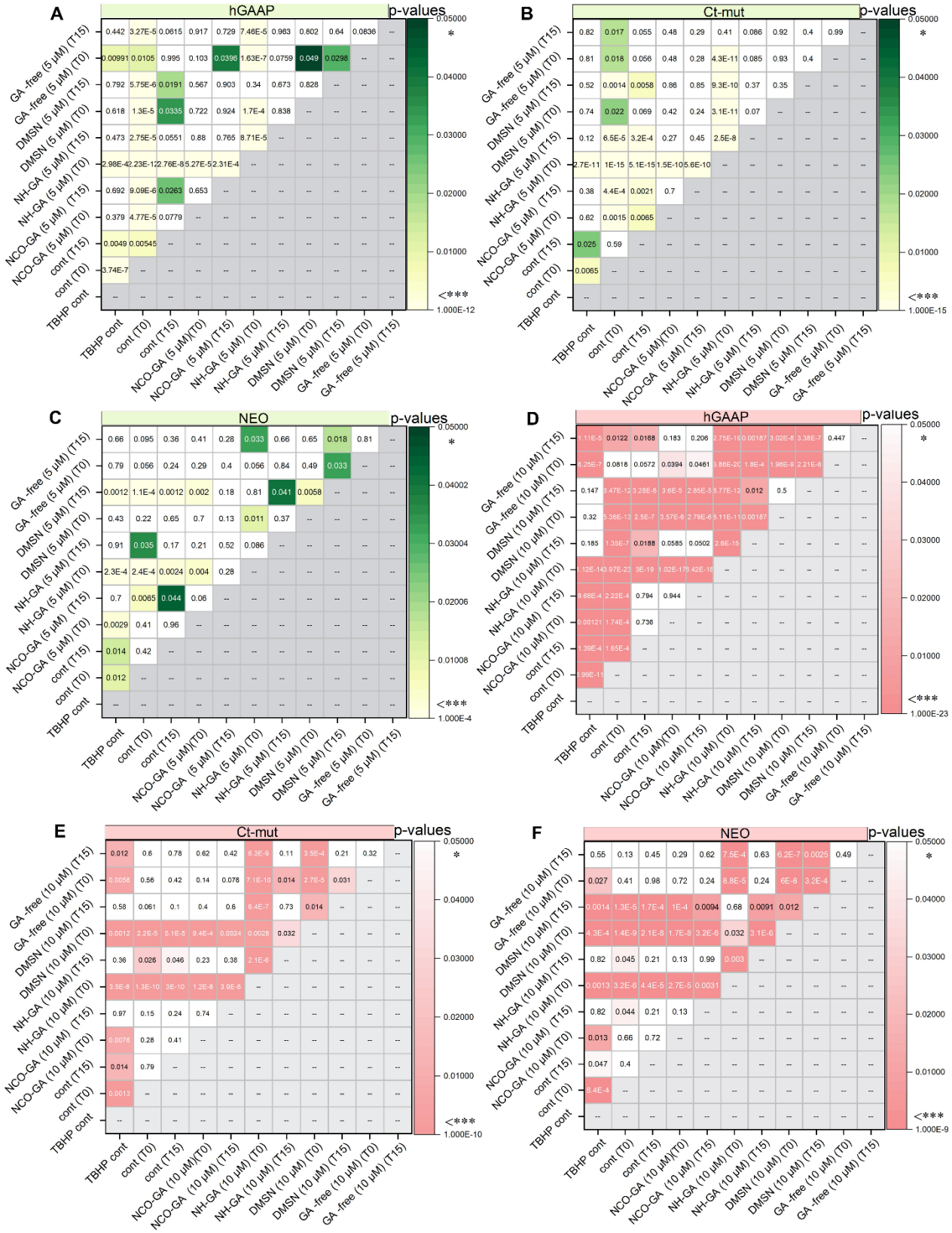
A
hGAAP
p-values
B
Ct-mut
p-values
C
NEO
p-values
D
hGAAP
p-values
E
Ct-mut
p-values
F
NEO
p-values
TBHP cont
cont (T0)
cont (T15)
NCO-GA (5 µM)(T0)
NCO-GA (5 µM) (T15)
NH-GA (5 µM) (T0)
NH-GA (5 µM) (T15)
DMSN (5 µM) (T0)
DMSN (5 µM) (T15)
GA -free (5 µM) (T0)
GA -free (5 µM) (T15)
NCO-GA (10 µM)(T0)
NCO-GA (10 µM) (T15)
NH-GA (10 µM) (T0)
NH-GA (10 µM) (T15)
DMSN (10 µM) (T0)
DMSN (10 µM) (T15)
GA -free (10 µM) (T0)
GA -free (10 µM) (T15)

Figure S7. Statistical analysis was conducted on the signal intensity quantification after incubating U2-OS cell lines (hGAAP (A, D), Ct-mut (B, E), and NEO (C, F)) with free GA, DMSN-NH-GA, and DMSN-NCO-GA, in the presence of TBHP (0.2 mM) as an oxidative stressor at GA concentration of 5 μM (A-C) and 10 μM (D-F). Additionally, non-functionalized DMSNs were used at concentrations equivalent to 5 μM and 10 μM GA, corresponding to 53.15 μg mL^{-1} and 106.3 μg mL^{-1}. Three independent cell preparations were conducted, and the data was expressed as the average fluorescence intensity normalized to the relative fluorescence triggered by TBHP. The difference among the treatments was assessed using one-way ANOVA with Fisher LSD (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, depicted in green to yellow and red to pink).

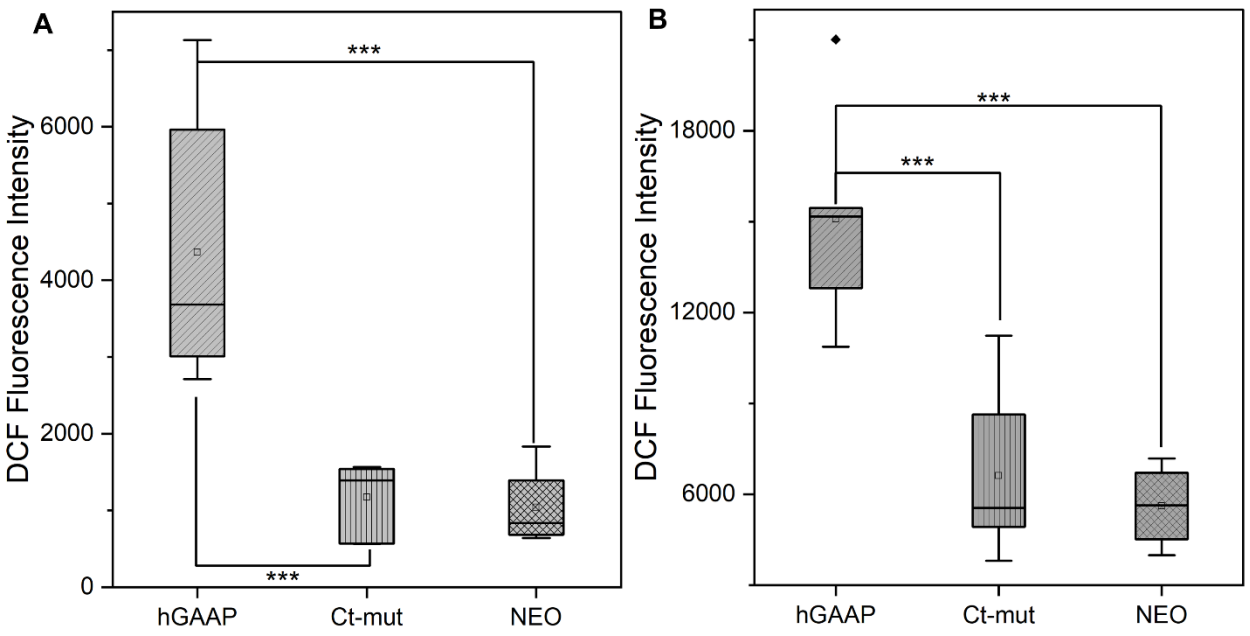

Figure S8. Baseline of DCF fluorescence intensity measured at t = 0 (A) and t =15 min (B) after treatment with an oxidative stressor TBHP (0.2 µM). The data was collected from n =16, with measurements conducted in technical triplicates. The difference among TBHP treatment was determined using one-way ANOVA with Fisher LSD (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

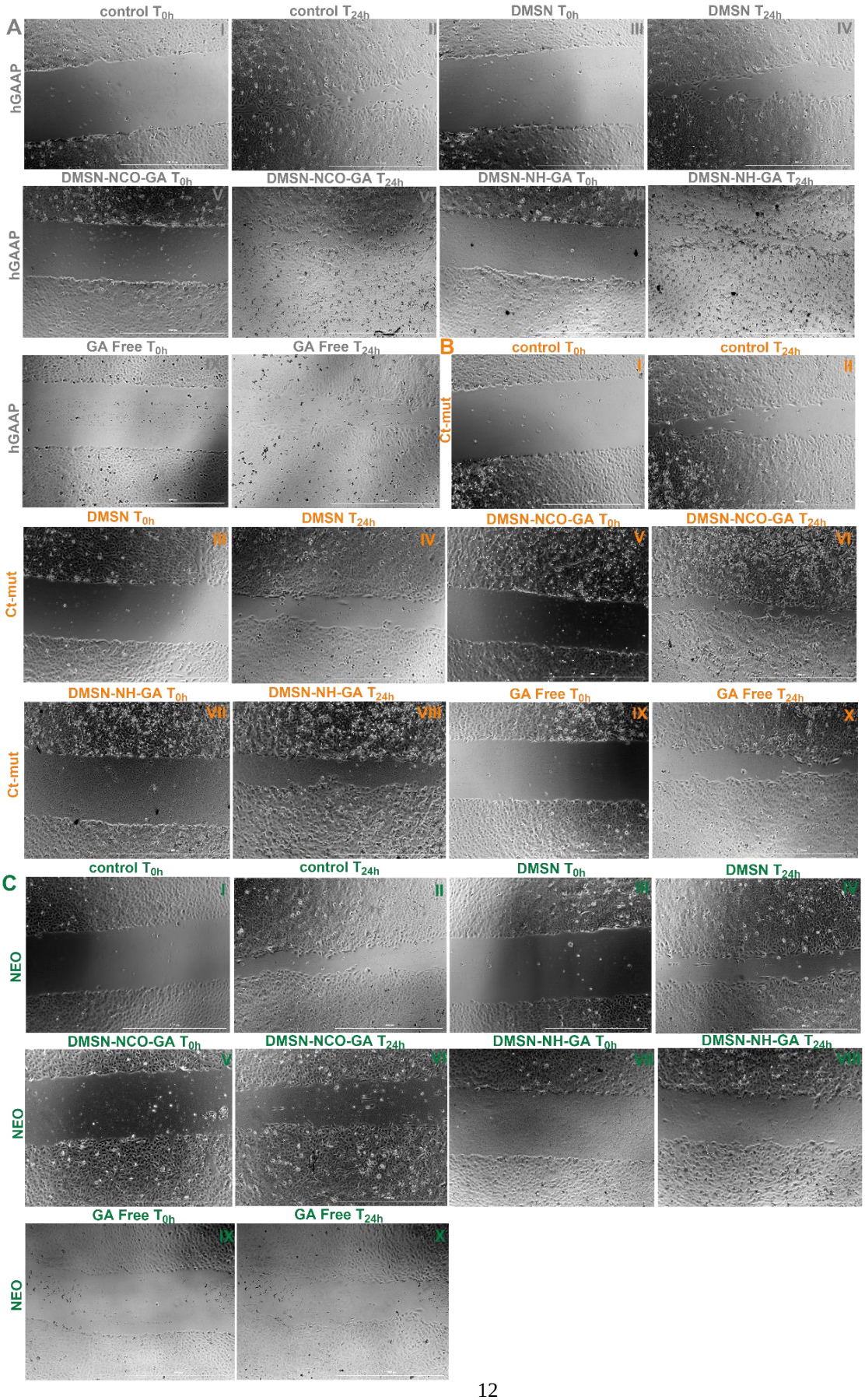
A
control T0h
control T24h
DMSN T0h
DMSN T24h
hGAAP
I
II
III
IV
DMSN-NCO-GA T0h
DMSN-NCO-GA T24h
DMSN-NH-GA T0h
DMSN-NH-GA T24h
hGAAP
V
VI
VII
VIII
GA Free T0h
GA Free T24h
hGAAP
B
control T0h
control T24h
Ct-mut
I
II
DMSN T0h
DMSN T24h
DMSN-NCO-GA T0h
DMSN-NCO-GA T24h
Ct-mut
III
IV
V
VI
DMSN-NH-GA T0h
DMSN-NH-GA T24h
GA Free T0h
GA Free T24h
Ct-mut
VII
VIII
IX
X
C
control T0h
control T24h
DMSN T0h
DMSN T24h
NEO
I
II
III
IV
DMSN-NCO-GA T0h
DMSN-NCO-GA T24h
DMSN-NH-GA T0h
DMSN-NH-GA T24h
NEO
V
VI
VII
VIII
GA Free T0h
GA Free T24h
NEO
IX
X

Figure S9. Representative phase contrast images acquired during the migration assays and depicting the beginning (t = 0) and the end of the assay (t = 24 h). scale bar: 1000 μm).

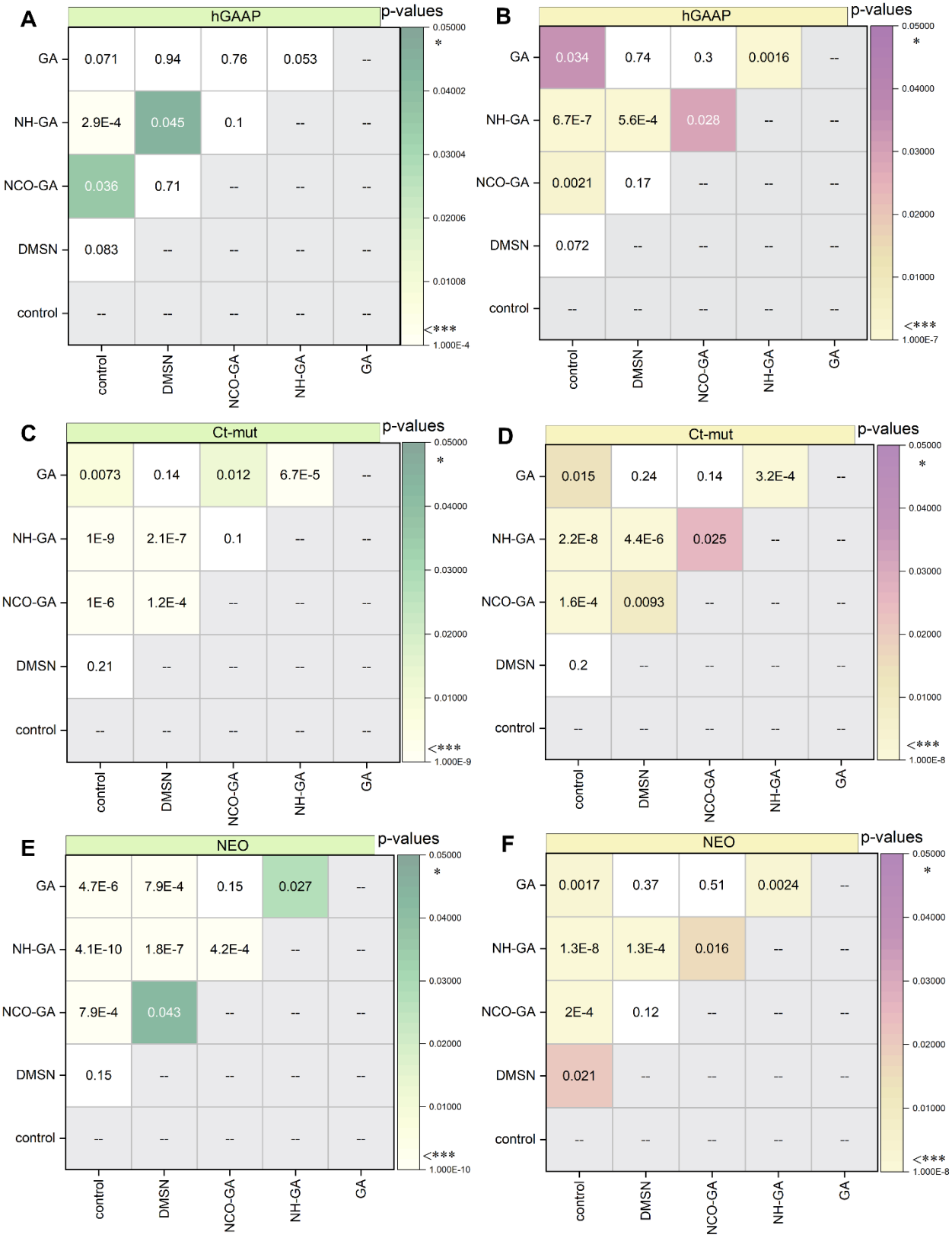
A
hGAAP
p-values
GA 0.071 0.94 0.76 0.053 --
NH-GA 2.9E-4 0.045 0.1 -- --
NCO-GA 0.036 0.71 -- -- --
DMSN 0.083 -- -- -- --
control -- -- -- -- --
control DMSN NCO-GA NH-GA GA
0.05000 * 0.04002 0.03004 0.02006 0.01008 <*** 1.000E-4
B
hGAAP
p-values
GA 0.034 0.74 0.3 0.0016 --
NH-GA 6.7E-7 5.6E-4 0.028 -- --
NCO-GA 0.0021 0.17 -- -- --
DMSN 0.072 -- -- -- --
control -- -- -- -- --
control DMSN NCO-GA NH-GA GA
0.05000 * 0.04000 0.03000 0.02000 0.01000 <*** 1.000E-7
C
Ct-mut
p-values
GA 0.0073 0.14 0.012 6.7E-5 --
NH-GA 1E-9 2.1E-7 0.1 -- --
NCO-GA 1E-6 1.2E-4 -- -- --
DMSN 0.21 -- -- -- --
control -- -- -- -- --
control DMSN NCO-GA NH-GA GA
0.05000 * 0.04000 0.03000 0.02000 0.01000 <*** 1.000E-9
D
Ct-mut
p-values
GA 0.015 0.24 0.14 3.2E-4 --
NH-GA 2.2E-8 4.4E-6 0.025 -- --
NCO-GA 1.6E-4 0.0093 -- -- --
DMSN 0.2 -- -- -- --
control -- -- -- -- --
control DMSN NCO-GA NH-GA GA
0.05000 * 0.04000 0.03000 0.02000 0.01000 <*** 1.000E-8
E
NEO
p-values
GA 4.7E-6 7.9E-4 0.15 0.027 --
NH-GA 4.1E-10 1.8E-7 4.2E-4 -- --
NCO-GA 7.9E-4 0.043 -- -- --
DMSN 0.15 -- -- -- --
control -- -- -- -- --
control DMSN NCO-GA NH-GA GA
0.05000 * 0.04000 0.03000 0.02000 0.01000 <*** 1.000E-10
F
NEO
p-values
GA 0.0017 0.37 0.51 0.0024 --
NH-GA 1.3E-8 1.3E-4 0.016 -- --
NCO-GA 2E-4 0.12 -- -- --
DMSN 0.021 -- -- -- --
control -- -- -- -- --
control DMSN NCO-GA NH-GA GA
0.05000 * 0.04000 0.03000 0.02000 0.01000 <*** 1.000E-8

Figure S10. Statistical assessment of migration changes in U2-OS cell lines (hGAAP (A, B), Ct-mut (C, D), and NEO (E, F)) was conducted following incubation with GA-conjugated materials, and free GA at a GA concentration of 10 µM. Additionally, non-functionalized DMSNs were used at concentrations equivalent to 10 µM GA, corresponding to 106.3 µg mL^{-1}. The evaluation comprised two aspects: firstly, the area grown [μm^2] was determined by calculating the difference between the initial scratch area and the area at the same coordinates after 24 h of incubation (n =16) (A, C, and E). Secondly, the closure velocity ($\mu m\ h^{-1}$) was computed as the difference between the initial and final gap distances at the same coordinates, divided by the overall time (24 h) in untreated control, non-functionalized DMSNs, and GA-conjugated materials treated cells (B, D, and F). The difference among treatment groups was assessed using one-way ANOVA with Fisher LSD (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, represented by color variations green to yellow or pink to yellow).