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Supporting Information

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Gas plasma technology augments ovalbumin immunogenicity and OT-II T cell activation conferring tumor protection in mice

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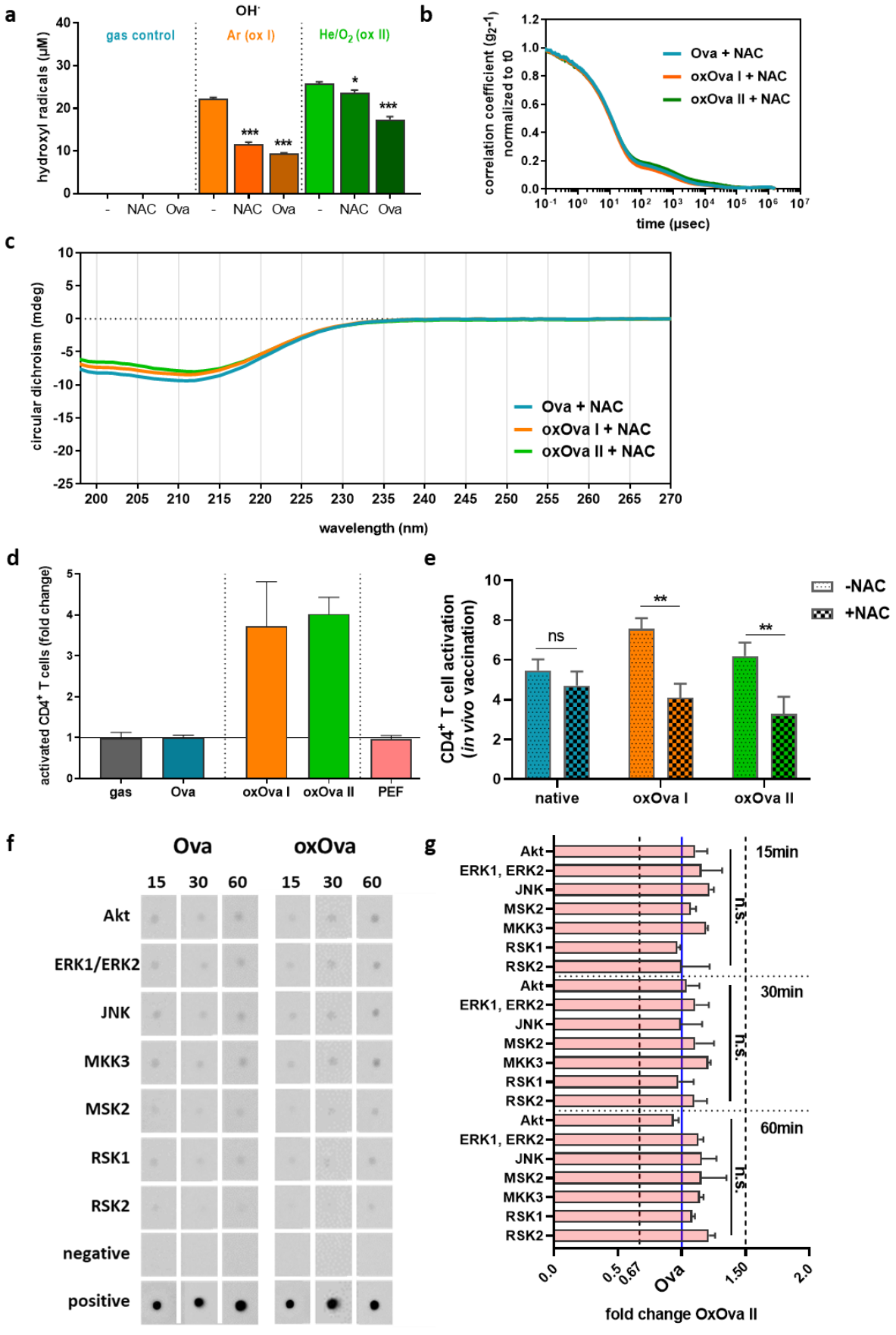
Supplemental figure S1. (a) PBS with or without OVA or NAC was exposed as in figure 1, and absolute concentration of hydroxyl radicals were measured as recently as recently described [146]; (**b-c**) NAC was added to Ova before exposure to ox I (oxOva I + NAC) and ox II (oxOva II + NAC) that abrogated oxidation-induced changes in dynamic light scattering (**b**) and protein structure in CD spectroscopy (**c**); (**d**) OT-II splenocytes were incubated with Ova, oxOva I, oxOva II, or Ova exposed to pulsed electric fields, and $CD4^+$ T cells were analyzed for activation 24h later, underlining the inability of pulsed fields alone as a physical trait also observed in plasma jets to promote the immunogenicity of Ova; (**e**) OT-II mice were vaccinated with Ova, oxOva I, or oxOva II that were gas plasma-treated in the presence or absence of NAC, showing reduced T cell activation of re-stimulated $CD4^+$ splenocytes *ex vivo*; (**f-g**) bone marrow-derived dendritic cells from C57BL/6 wildtype mice (n=3) were incubated with Ova or oxOva II, and analyzed for protein phosphorylation by western blot (**f**) yielding no statistically-significant changes for oxOva II when analyzed at 15min, 30min, and 60min post exposure (**g**). Statistical analysis was performed using t-test (* $p<0.05$; ** $p<0.01$; *** $p<0.001$); ns = not significant.

Supplemental figure S1


a
OH·
gas control
Ar (ox I)
He/O2 (ox II)
hydroxyl radicals (µM)
40
30
20
10
0
*
***
***
***
-
NAC
Ova
-
NAC
Ova
-
NAC
Ova
b
correlation coefficient (g2-1)
normalized to t0
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Ova + NAC
oxOva I + NAC
oxOva II + NAC
10-1
100
101
102
103
104
105
106
107
time (µsec)
c
circular dichroism (mdeg)
10
5
0
-5
-10
-15
-20
-25
Ova + NAC
oxOva I + NAC
oxOva II + NAC
200
205
210
215
220
225
230
235
240
245
250
255
260
265
270
wavelength (nm)
d
activated CD4+ T cells (fold change)
5
4
3
2
1
0
gas
Ova
oxOva I
oxOva II
PEF
e
CD4+ T cell activation
(in vivo vaccination)
10
8
6
4
2
0
ns
**
**
-NAC
+NAC
native
oxOva I
oxOva II
f
Ova
oxOva
15
30
60
15
30
60
Akt
ERK1/ERK2
JNK
MKK3
MSK2
RSK1
RSK2
negative
positive
g
Akt
ERK1, ERK2
JNK
MSK2
MKK3
RSK1
RSK2
15min
n.s.
Akt
ERK1, ERK2
JNK
MSK2
MKK3
RSK1
RSK2
30min
n.s.
Akt
ERK1, ERK2
JNK
MSK2
MKK3
RSK1
RSK2
60min
n.s.
0.0
0.5
0.67
Ova
1.50
2.0
fold change OxOva II