Membrane-bound Gaussia luciferase as a tool to track shedding of membrane proteins from the surface of extracellular vesicles

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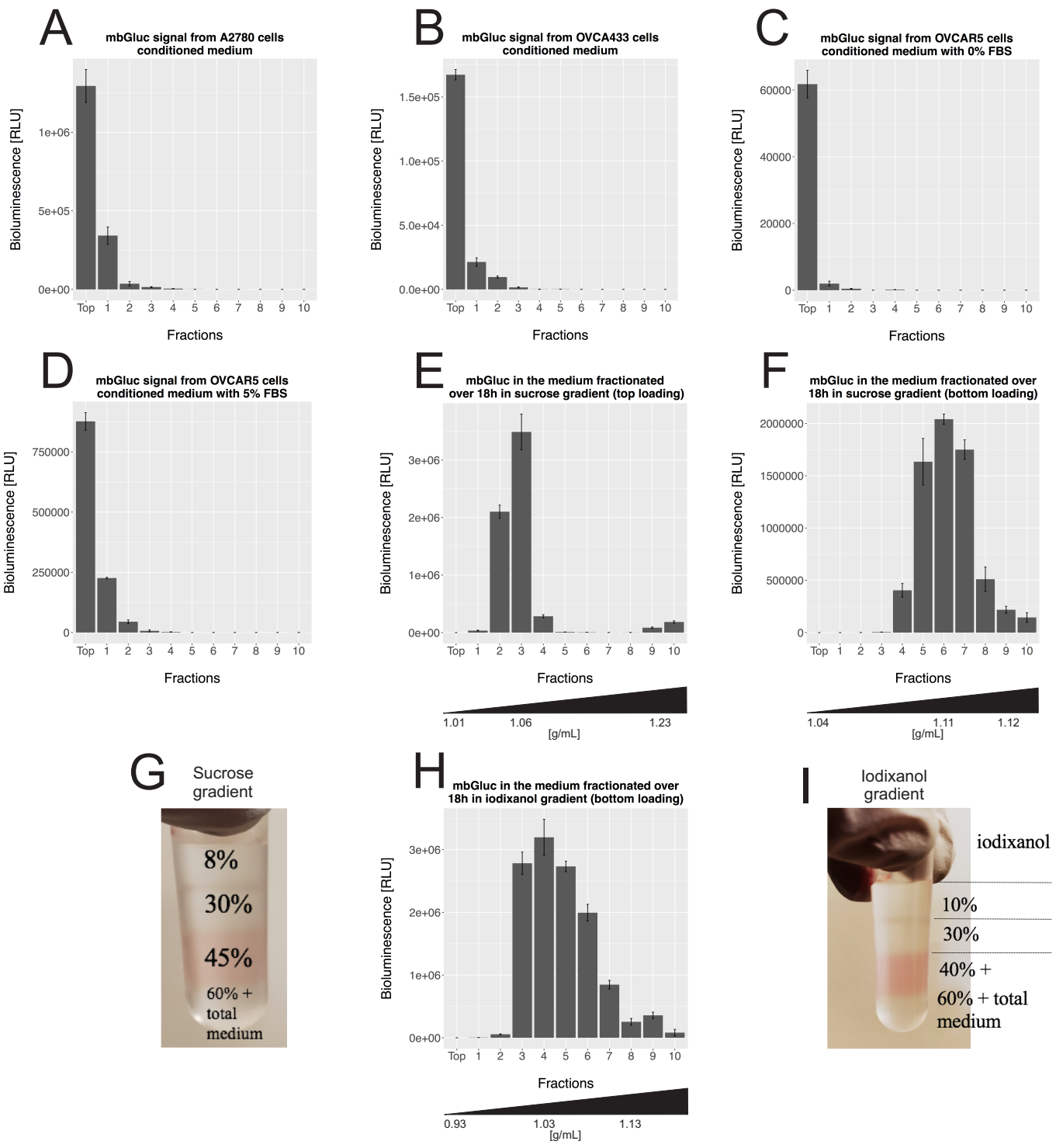
A
mbGluc signal from A2780 cells conditioned medium
Bioluminescence [RLU]
1e+06
5e+05
0e+00
Top 1 2 3 4 5 6 7 8 9 10
Fractions
B
mbGluc signal from OVCA433 cells conditioned medium
Bioluminescence [RLU]
1.5e+05
1.0e+05
5.0e+04
0.0e+00
Top 1 2 3 4 5 6 7 8 9 10
Fractions
C
mbGluc signal from OVCAR5 cells conditioned medium with 0% FBS
Bioluminescence [RLU]
60000
40000
20000
0
Top 1 2 3 4 5 6 7 8 9 10
Fractions
D
mbGluc signal from OVCAR5 cells conditioned medium with 5% FBS
Bioluminescence [RLU]
750000
500000
250000
0
Top 1 2 3 4 5 6 7 8 9 10
Fractions
E
mbGluc in the medium fractionated over 18h in sucrose gradient (top loading)
Bioluminescence [RLU]
3e+06
2e+06
1e+06
0e+00
Top 1 2 3 4 5 6 7 8 9 10
Fractions
1.01 1.06 1.23
[g/mL]
F
mbGluc in the medium fractionated over 18h in sucrose gradient (bottom loading)
Bioluminescence [RLU]
2000000
1500000
1000000
500000
0
Top 1 2 3 4 5 6 7 8 9 10
Fractions
1.04 1.11 1.12
[g/mL]
G
Sucrose gradient
8%
30%
45%
60% + total medium
H
mbGluc in the medium fractionated over 18h in iodixanol gradient (bottom loading)
Bioluminescence [RLU]
3e+06
2e+06
1e+06
0e+00
Top 1 2 3 4 5 6 7 8 9 10
Fractions
0.93 1.03 1.13
[g/mL]
I
Iodixanol gradient
iodixanol
10%
30%
40% +
60% + total medium

Figure S1.

Figure S1. mbGluc Is Abundant in Low Density Fractions of Step-Gradient. Related to Figure 2. (**A**) mbGluc activity after fractionation of A2780 cells conditioned medium in sucrose density gradient (procedure in Fig. 2A, sample loaded on top of the gradient, 38 minutes spin at 200K × *g*, culture medium supplemented with 5% EV-depleted FBS). (**B**) mbGluc activity after fractionation of OVCA433 cells conditioned medium in sucrose density gradient (procedure in Fig. 2A, sample loaded on top of the gradient, 38 minutes spin at 200K × *g*, culture medium supplemented with 5% EV-depleted FBS). (**C**) mbGluc activity after fractionation of OVCAR5 cells conditioned medium in sucrose density gradient (procedure in Fig. 2A, sample loaded on top of the gradient, 38 minutes spin at 200K × *g*, culture medium supplemented with 0% EV-depleted FBS). (**D**) mbGluc activity after fractionation of OVCAR5 cells conditioned medium in sucrose density gradient (procedure in Fig. 2A, sample loaded on top of the gradient, 38 minutes spin at 200K × *g*, culture medium supplemented with 5% EV-depleted FBS). (**E**) mbGluc activity after fractionation of OVCAR5 cells conditioned medium in sucrose density gradient (sample loaded on top of the gradient, centrifugation for 18 hours at 200K × *g*, culture medium supplemented with 5% EV-depleted FBS). (**F**) mbGluc activity after fractionation of OVCAR5 cells conditioned medium in sucrose density gradient (sample loaded at the bottom of the gradient, centrifugation for 18 hours at 200K × *g*, culture medium supplemented with 5% EV-depleted FBS). (**G**) OVCAR5 conditioned medium loaded at the bottom of the sucrose density gradient. Medium was mixed with 60% sucrose solution. Loading of 45% solution on that mixture resulted in the move of medium dye to the top of 45%+60% solution (picture before centrifugation). (**H**) mbGluc activity after fractionation of OVCAR5 cells conditioned medium in iodixanol density gradient (sample loaded at the bottom of the gradient, centrifugation for 18 hours at 200K × *g*, culture medium supplemented with 5% EV-depleted FBS). (**I**) OVCAR5 conditioned medium loaded at the bottom of the iodixanol density gradient. Medium was mixed with 60% iodixanol solution. Loading of 40% solution on that mixture resulted in the move of the pink medium dye to the top of 40%+60% mixed solution (picture before centrifugation).

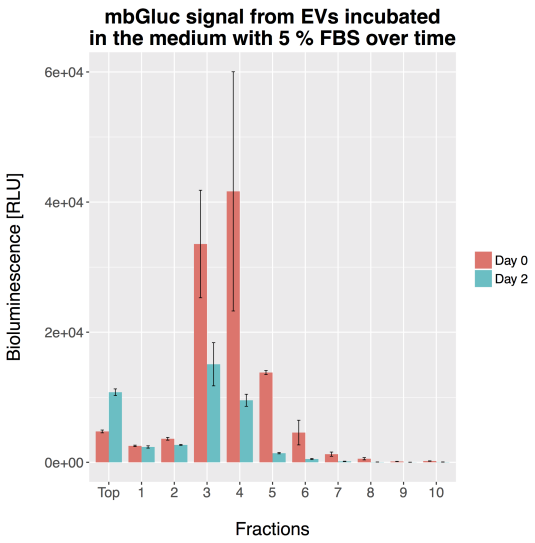

Figure S2. mbGluc as a Measure of Release of EV-bound Membrane Proteins. Related to Figure 3. mbGluc activity measured after fractionation of EVs derived from OVCAR5 mbGluc-positive cells in sucrose density gradient following incubation at 37°C in medium supplemented with 5% EV-depleted medium for 0 and 2 days.

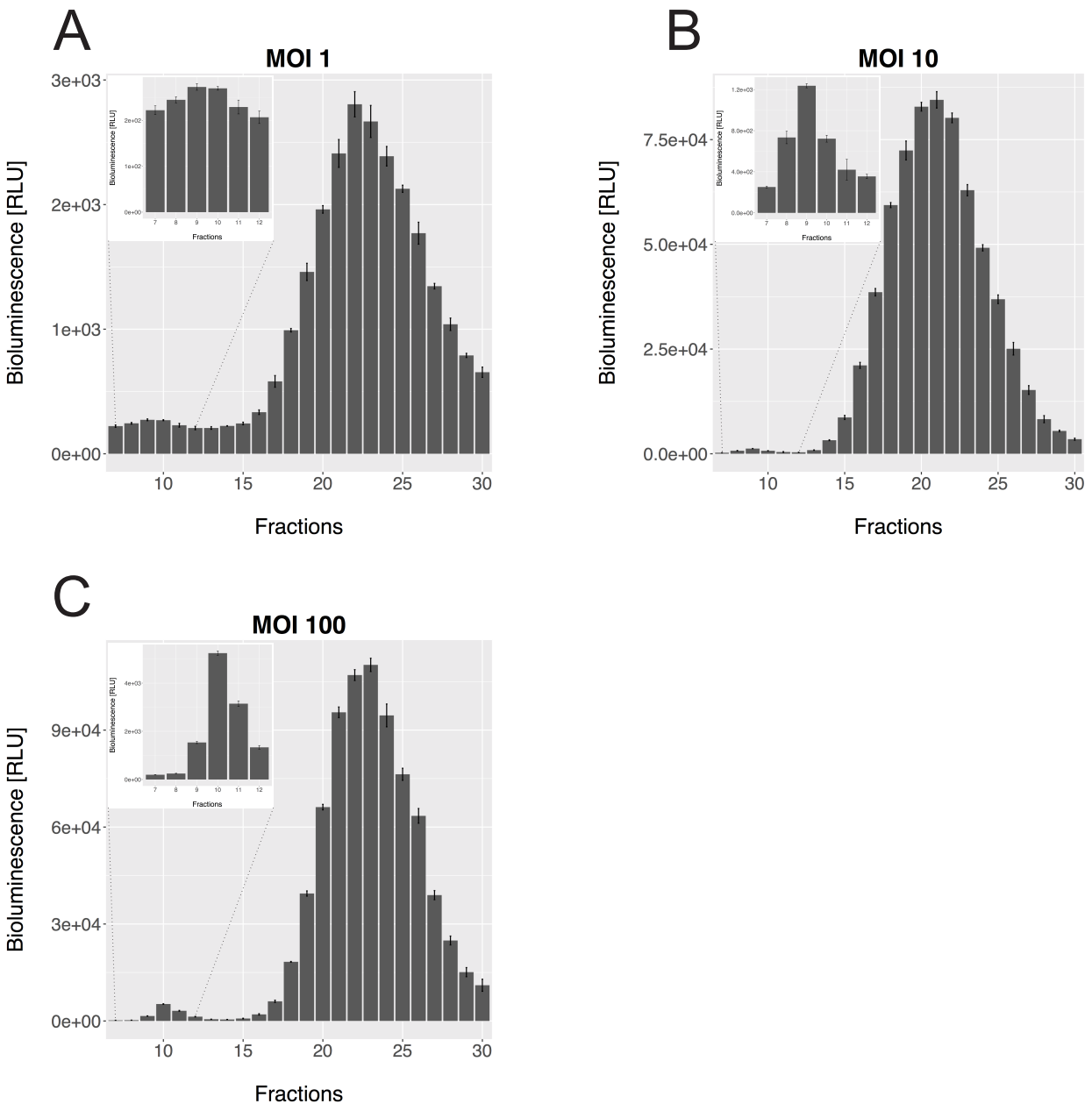

Figure S3. Level of Expression Does Not Affect Mode of Release of mbGluc. Related to Figure 4. (**A**) mbGluc activity in SEC fractions (qEV column) after fractionation of OVCAR5 medium following transduction with a lentivirus at MOI 1 (Procedure in Figure 4A). (**B**) mbGluc activity in SEC fractions of OVCAR5 medium following transduction with a lentivirus at MOI 10. (**C**) mbGluc activity in SEC fractions of OVCAR5 medium following transduction with a lentivirus at MOI 100. The insert represents fractions 7-12 with a modified scale on Y axis.

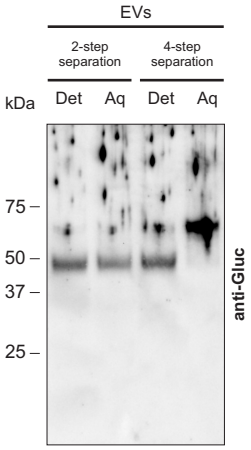

Figure S4. Increased number of centrifugation steps in TX-114 procedure improves separation of EV-bound mbGluc. Related to Figure 6.
Western blot of mbGluc protein isolated from OVCAR5-mbGluc+ EVs isolated by UC subjected to TX-114 procedure that included either two or four separation cycles.

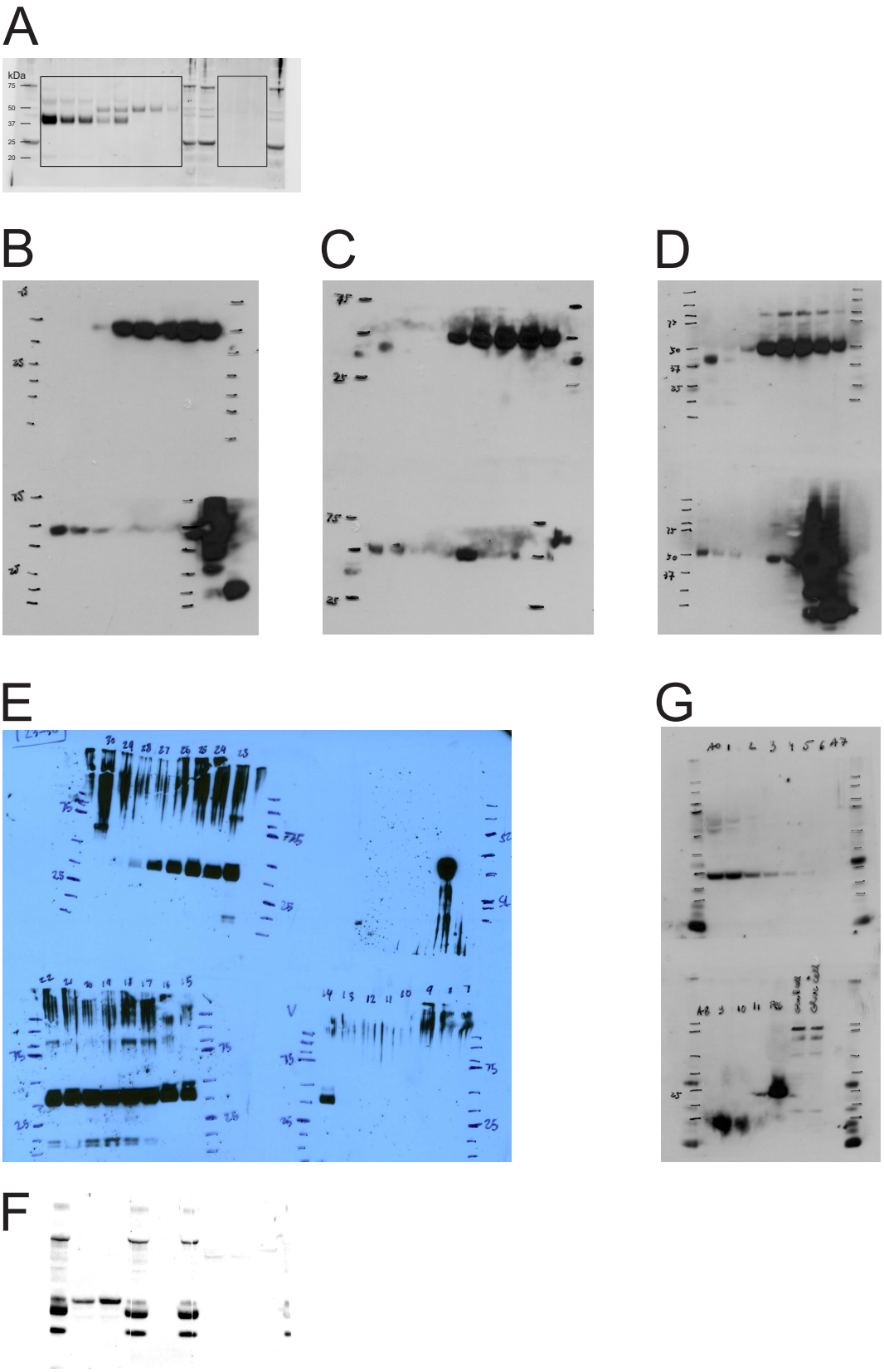

Figure S5. Full-length gels.
(**A**) Full picture of the gel presented in Fig. 2C. (**B**) Full picture of the gel presented in Fig. 3C (upper panel). (**C**) Full picture of the gel presented in Fig. 3C (middle panel). (**D**) Full picture of the gel presented in Fig. 3C (lower panel). (**E**) Full picture of the gel presented in Fig. 4C. (**F**) Full picture of the gel presented in Fig. 4G. (**G**) Full picture of the gel presented in Fig. 5C.