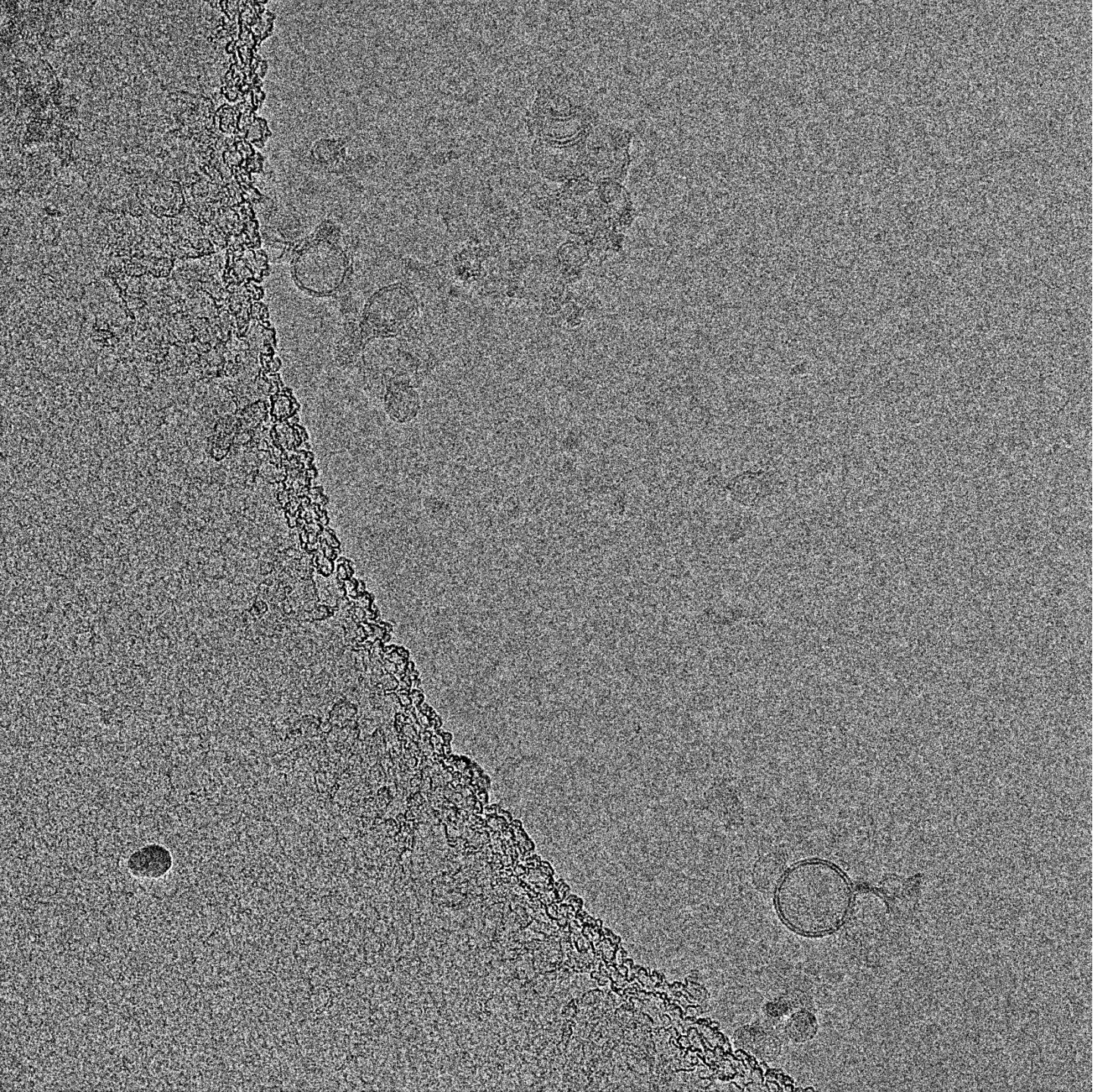
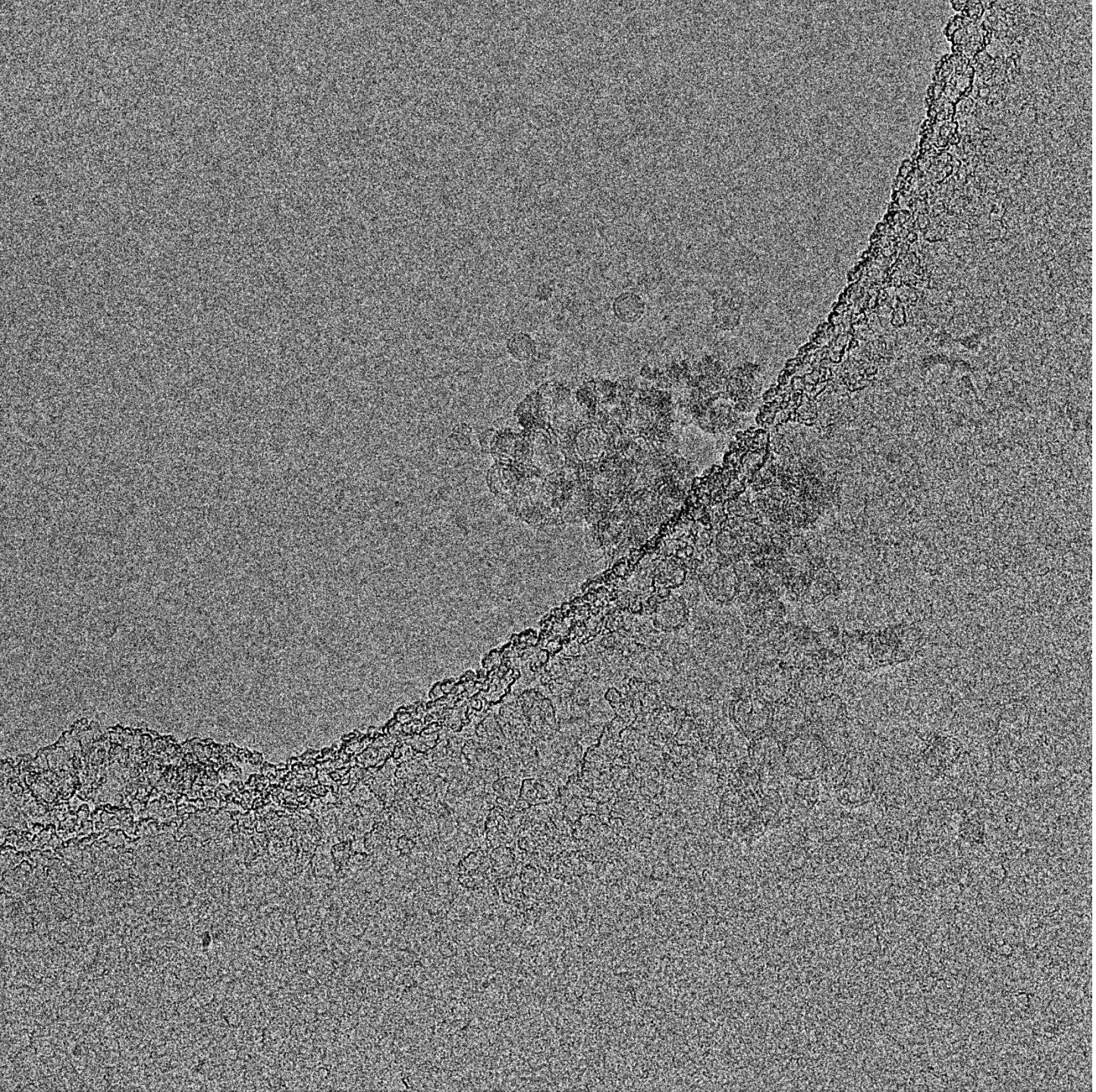


Supernatant from isolation of SCPs from spruce needle homogenate S2/1

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles.

SCPs were isolated by differential centrifugation as adapted from protocol for isolation of extracellular vesicles (EVs) (Mantile, F.; Kisovec, M.; Adamo, G.; Romancino, D.P.; Hočevar, M.; Božič, D.; Bedina Zavec, A.; Podobnik, M.; Stoppelli, M.P.; Kisslinger, A.; Bongiovanni, A.; Kralj-Iglič, V.; Liguori, G.L. A Novel Localization in Human Large Extracellular Vesicles for the EGF-CFC Founder Member CRIPTO and Its Biological and Therapeutic Implications. Cancers 2022, 14, 3700. https://doi.org/10.3390/cancers14153700). The homogenate was centrifuged twice at 300 g and 4°C for 10 minutes in the centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)) by using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany). The supernatant of the second centrifugation was centrifuged twice at 2000 g and 4°C for 10 minutes in the centrifuge Centric 400R with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Then, the supernatant was centrifuged at 50 000 g and 4°C, for 70 min in Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA) using 6 mL thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA). The supernatant consists of the fluid above about 50 µL of the pellet.

For cryogenic transmission electron microscopy, C-flat™ 2/2, 200 mesh holey carbon grids (Protochips, Morrisville, NC, USA) were glow discharged: 20 mA, 60 s, positive polarity, air atmosphere (GloQube ® Plus, Quor-um, Laughton, UK). 3 µL of sample was applied to the grid, blotted, and vitrified in liquid ethane on Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, USA). Vitrobot con-ditions were set to 100% relative humidity, 4 °C, blot force: 2 and blot time: 7 s. Samples were visualized under cryogenic conditions using a 200 kV Glacios microscope with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, USA).

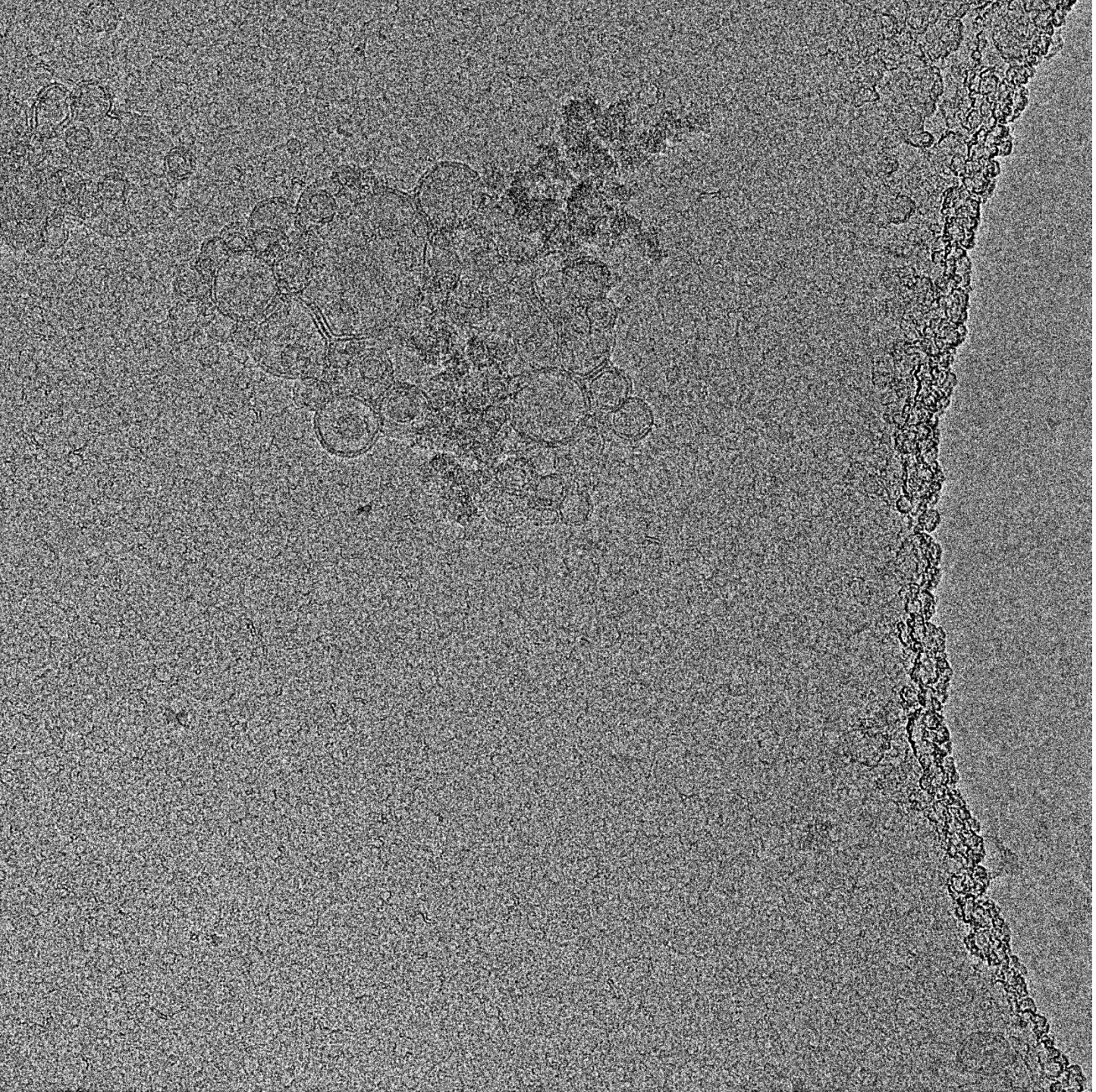


Supernatant from isolation of SCPs from spruce needle homogenate S2/2

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles.

SCPs were isolated by differential centrifugation as adapted from protocol for isolation of extracellular vesicles (EVs) (Mantile, F.; Kisovec, M.; Adamo, G.; Romancino, D.P.; Hočevar, M.; Božič, D.; Bedina Zavec, A.; Podobnik, M.; Stoppelli, M.P.; Kisslinger, A.; Bongiovanni, A.; Kralj-Iglič, V.; Liguori, G.L. A Novel Localization in Human Large Extracellular Vesicles for the EGF-CFC Founder Member CRIPTO and Its Biological and Therapeutic Implications. Cancers 2022, 14, 3700. https://doi.org/10.3390/cancers14153700). The homogenate was centrifuged twice at 300 g and 4°C for 10 minutes in the centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)) by using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany). The supernatant of the second centrifugation was centrifuged twice at 2000 g and 4°C for 10 minutes in the centrifuge Centric 400R with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Then, the supernatant was centrifuged at 50 000 g and 4°C, for 70 min in Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA) using 6 mL thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA). The supernatant consists of the fluid above about 50 µL of the pellet.

For cryogenic transmission electron microscopy, C-flat™ 2/2, 200 mesh holey carbon grids (Protochips, Morrisville, NC, USA) were glow discharged: 20 mA, 60 s, positive polarity, air atmosphere (GloQube ® Plus, Quor-um, Laughton, UK). 3 µL of sample was applied to the grid, blotted, and vitrified in liquid ethane on Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, USA). Vitrobot con-ditions were set to 100% relative humidity, 4 °C, blot force: 2 and blot time: 7 s. Samples were visualized under cryogenic conditions using a 200 kV Glacios microscope with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, USA).

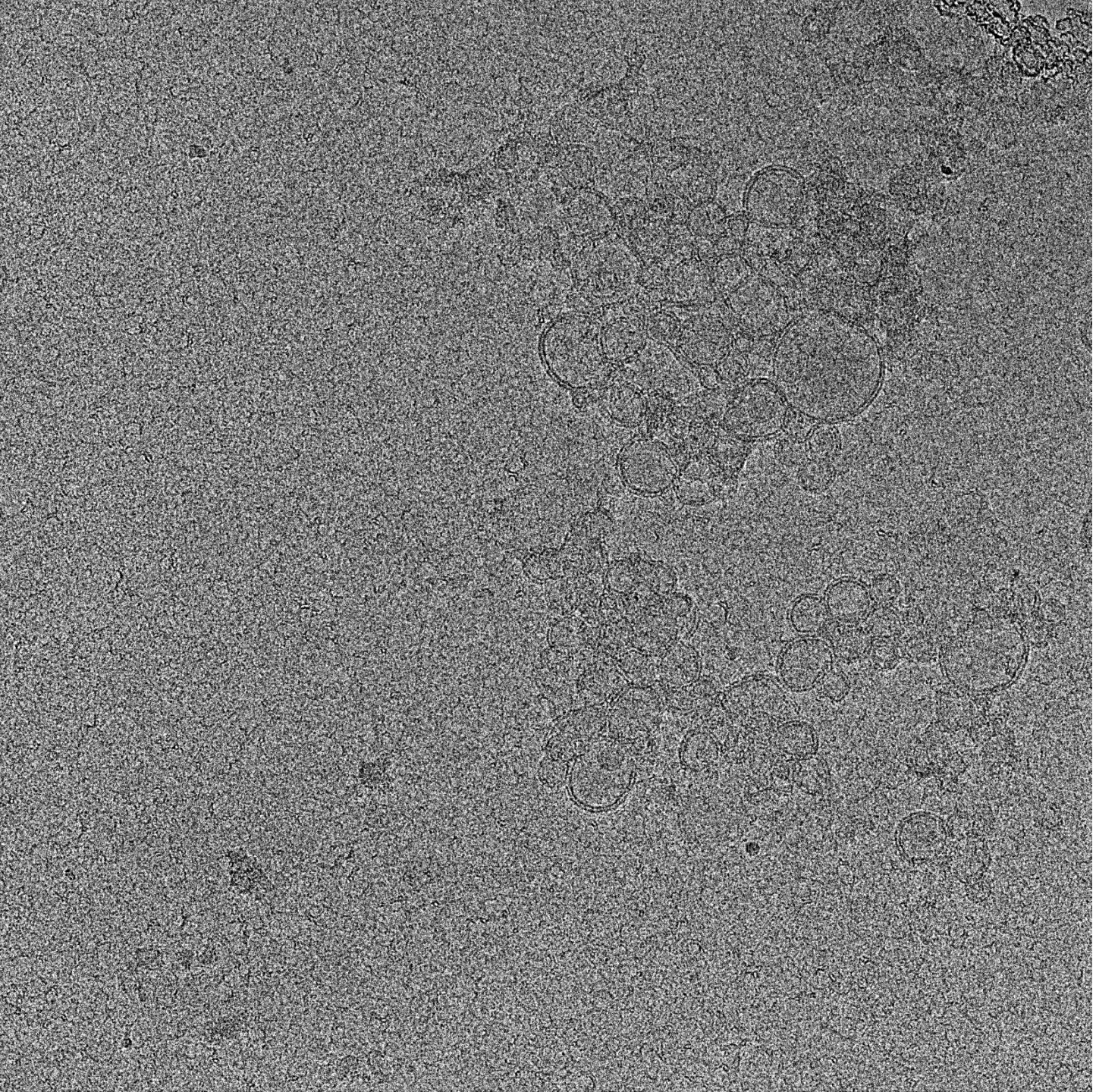


Supernatant from isolation of SCPs from spruce needle homogenate S2/3

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles.

SCPs were isolated by differential centrifugation as adapted from protocol for isolation of extracellular vesicles (EVs) (Mantile, F.; Kisovec, M.; Adamo, G.; Romancino, D.P.; Hočevar, M.; Božič, D.; Bedina Zavec, A.; Podobnik, M.; Stoppelli, M.P.; Kisslinger, A.; Bongiovanni, A.; Kralj-Iglič, V.; Liguori, G.L. A Novel Localization in Human Large Extracellular Vesicles for the EGF-CFC Founder Member CRIPTO and Its Biological and Therapeutic Implications. Cancers 2022, 14, 3700. https://doi.org/10.3390/cancers14153700). The homogenate was centrifuged twice at 300 g and 4°C for 10 minutes in the centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)) by using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany). The supernatant of the second centrifugation was centrifuged twice at 2000 g and 4°C for 10 minutes in the centrifuge Centric 400R with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Then, the supernatant was centrifuged at 50 000 g and 4°C, for 70 min in Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA) using 6 mL thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA). The supernatant consists of the fluid above about 50 µL of the pellet.

For cryogenic transmission electron microscopy, C-flat™ 2/2, 200 mesh holey carbon grids (Protochips, Morrisville, NC, USA) were glow discharged: 20 mA, 60 s, positive polarity, air atmosphere (GloQube ® Plus, Quor-um, Laughton, UK). 3 µL of sample was applied to the grid, blotted, and vitrified in liquid ethane on Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, USA). Vitrobot con-ditions were set to 100% relative humidity, 4 °C, blot force: 2 and blot time: 7 s. Samples were visualized under cryogenic conditions using a 200 kV Glacios microscope with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, USA).

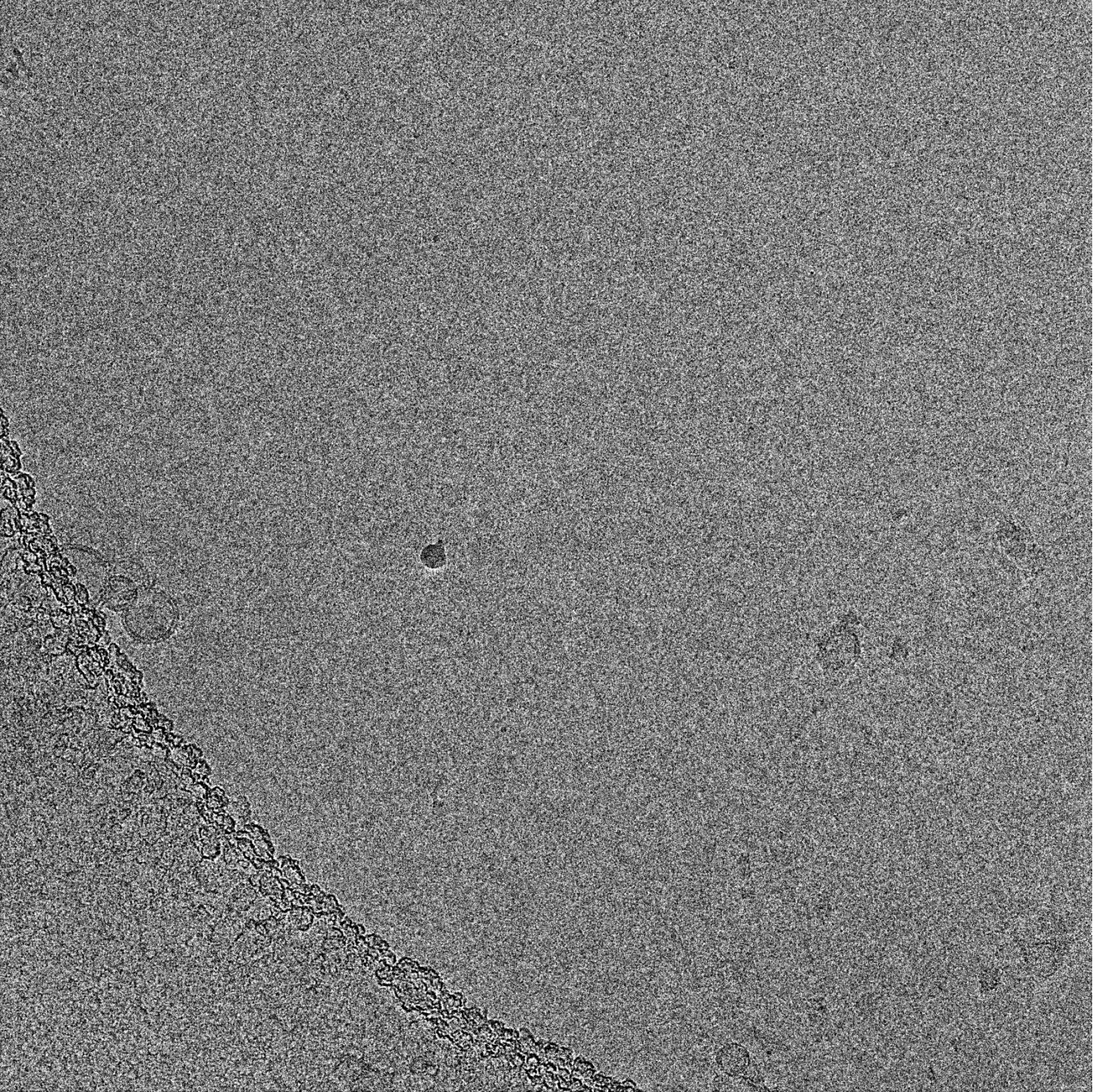


Supernatant from isolation of SCPs from spruce needle homogenate S2/4

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles.

SCPs were isolated by differential centrifugation as adapted from protocol for isolation of extracellular vesicles (EVs) (Mantile, F.; Kisovec, M.; Adamo, G.; Romancino, D.P.; Hočevar, M.; Božič, D.; Bedina Zavec, A.; Podobnik, M.; Stoppelli, M.P.; Kisslinger, A.; Bongiovanni, A.; Kralj-Iglič, V.; Liguori, G.L. A Novel Localization in Human Large Extracellular Vesicles for the EGF-CFC Founder Member CRIPTO and Its Biological and Therapeutic Implications. Cancers 2022, 14, 3700. https://doi.org/10.3390/cancers14153700). The homogenate was centrifuged twice at 300 g and 4°C for 10 minutes in the centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)) by using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany). The supernatant of the second centrifugation was centrifuged twice at 2000 g and 4°C for 10 minutes in the centrifuge Centric 400R with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Then, the supernatant was centrifuged at 50 000 g and 4°C, for 70 min in Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA) using 6 mL thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA). The supernatant consists of the fluid above about 50 µL of the pellet.

For cryogenic transmission electron microscopy, C-flat™ 2/2, 200 mesh holey carbon grids (Protochips, Morrisville, NC, USA) were glow discharged: 20 mA, 60 s, positive polarity, air atmosphere (GloQube ® Plus, Quor-um, Laughton, UK). 3 µL of sample was applied to the grid, blotted, and vitrified in liquid ethane on Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, USA). Vitrobot con-ditions were set to 100% relative humidity, 4 °C, blot force: 2 and blot time: 7 s. Samples were visualized under cryogenic conditions using a 200 kV Glacios microscope with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, USA).

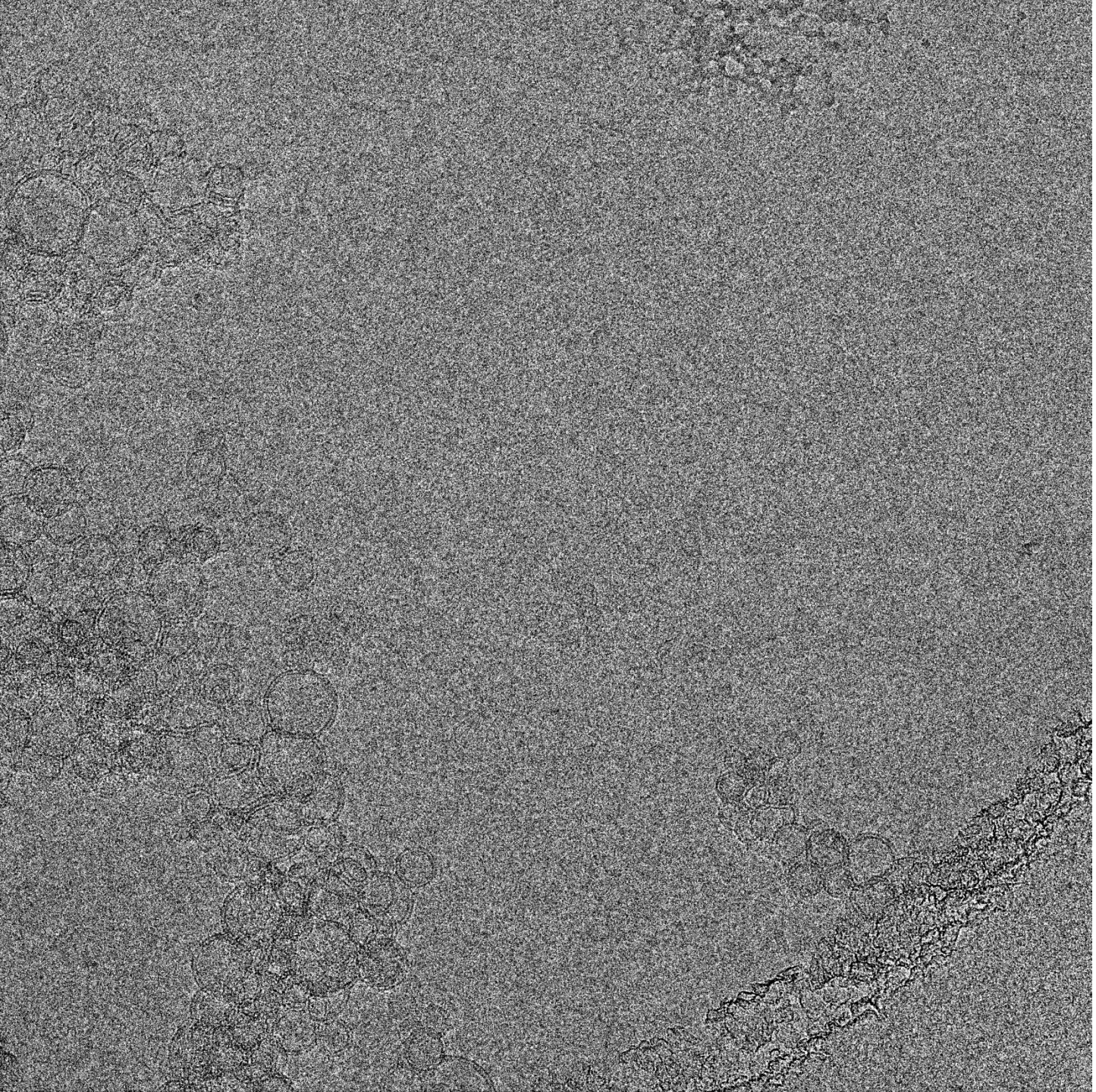


Supernatant from isolation of SCPs from spruce needle homogenate S2/5

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles.

SCPs were isolated by differential centrifugation as adapted from protocol for isolation of extracellular vesicles (EVs) (Mantile, F.; Kisovec, M.; Adamo, G.; Romancino, D.P.; Hočevar, M.; Božič, D.; Bedina Zavec, A.; Podobnik, M.; Stoppelli, M.P.; Kisslinger, A.; Bongiovanni, A.; Kralj-Iglič, V.; Liguori, G.L. A Novel Localization in Human Large Extracellular Vesicles for the EGF-CFC Founder Member CRIPTO and Its Biological and Therapeutic Implications. Cancers 2022, 14, 3700. https://doi.org/10.3390/cancers14153700). The homogenate was centrifuged twice at 300 g and 4°C for 10 minutes in the centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)) by using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany). The supernatant of the second centrifugation was centrifuged twice at 2000 g and 4°C for 10 minutes in the centrifuge Centric 400R with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Then, the supernatant was centrifuged at 50 000 g and 4°C, for 70 min in Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA) using 6 mL thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA). The supernatant consists of the fluid above about 50 µL of the pellet.

For cryogenic transmission electron microscopy, C-flat™ 2/2, 200 mesh holey carbon grids (Protochips, Morrisville, NC, USA) were glow discharged: 20 mA, 60 s, positive polarity, air atmosphere (GloQube ® Plus, Quor-um, Laughton, UK). 3 µL of sample was applied to the grid, blotted, and vitrified in liquid ethane on Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, USA). Vitrobot con-ditions were set to 100% relative humidity, 4 °C, blot force: 2 and blot time: 7 s. Samples were visualized under cryogenic conditions using a 200 kV Glacios microscope with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, USA).

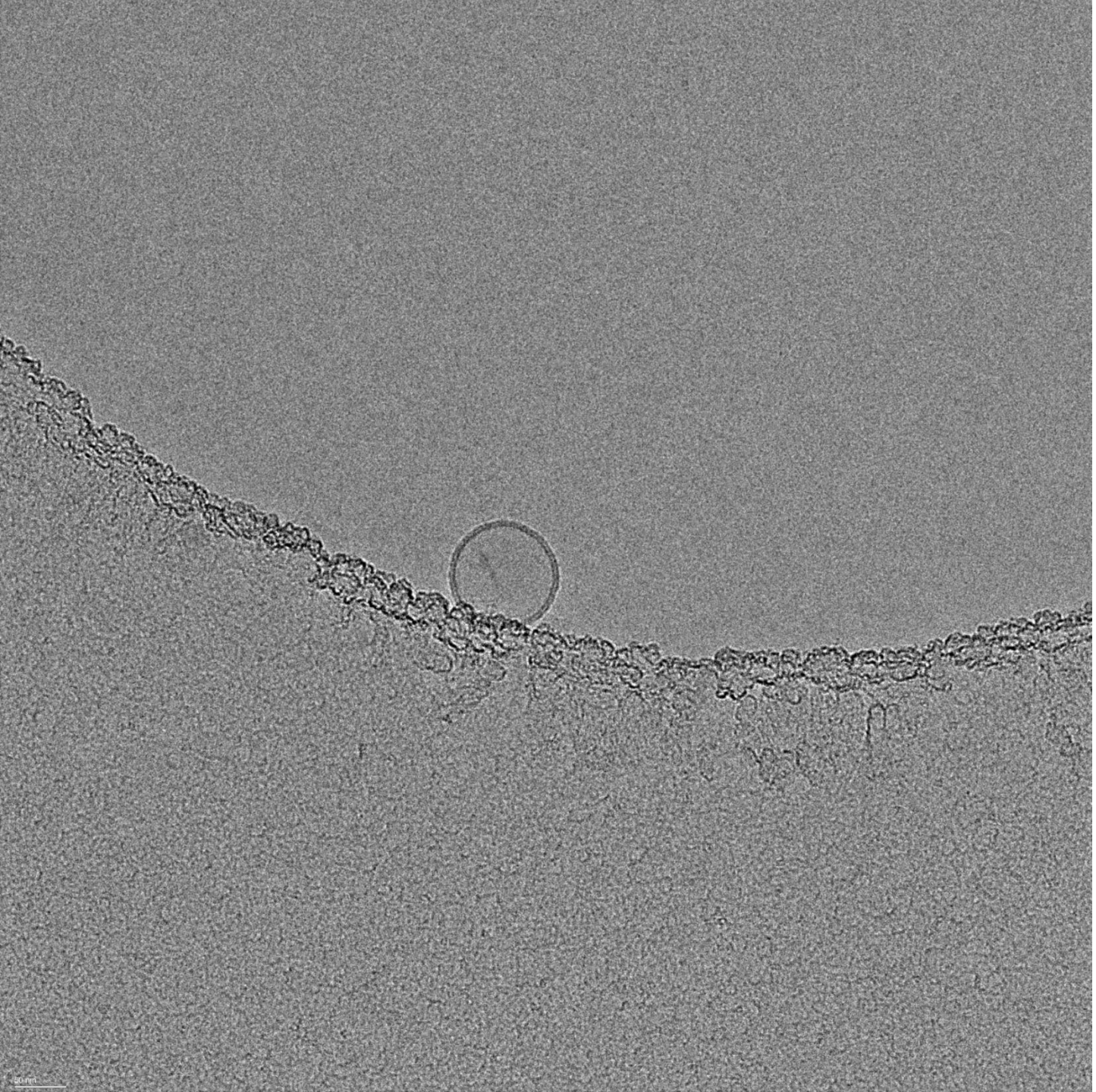


Supernatant from isolation of SCPs from spruce needle homogenate S2/6

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles.

SCPs were isolated by differential centrifugation as adapted from protocol for isolation of extracellular vesicles (EVs) (Mantile, F.; Kisovec, M.; Adamo, G.; Romancino, D.P.; Hočevar, M.; Božič, D.; Bedina Zavec, A.; Podobnik, M.; Stoppelli, M.P.; Kisslinger, A.; Bongiovanni, A.; Kralj-Iglič, V.; Liguori, G.L. A Novel Localization in Human Large Extracellular Vesicles for the EGF-CFC Founder Member CRIPTO and Its Biological and Therapeutic Implications. Cancers 2022, 14, 3700. https://doi.org/10.3390/cancers14153700). The homogenate was centrifuged twice at 300 g and 4°C for 10 minutes in the centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)) by using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany). The supernatant of the second centrifugation was centrifuged twice at 2000 g and 4°C for 10 minutes in the centrifuge Centric 400R with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Then, the supernatant was centrifuged at 50 000 g and 4°C, for 70 min in Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA) using 6 mL thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA). The supernatant consists of the fluid above about 50 µL of the pellet.

For cryogenic transmission electron microscopy, C-flat™ 2/2, 200 mesh holey carbon grids (Protochips, Morrisville, NC, USA) were glow discharged: 20 mA, 60 s, positive polarity, air atmosphere (GloQube ® Plus, Quor-um, Laughton, UK). 3 µL of sample was applied to the grid, blotted, and vitrified in liquid ethane on Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, USA). Vitrobot con-ditions were set to 100% relative humidity, 4 °C, blot force: 2 and blot time: 7 s. Samples were visualized under cryogenic conditions using a 200 kV Glacios microscope with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, USA).

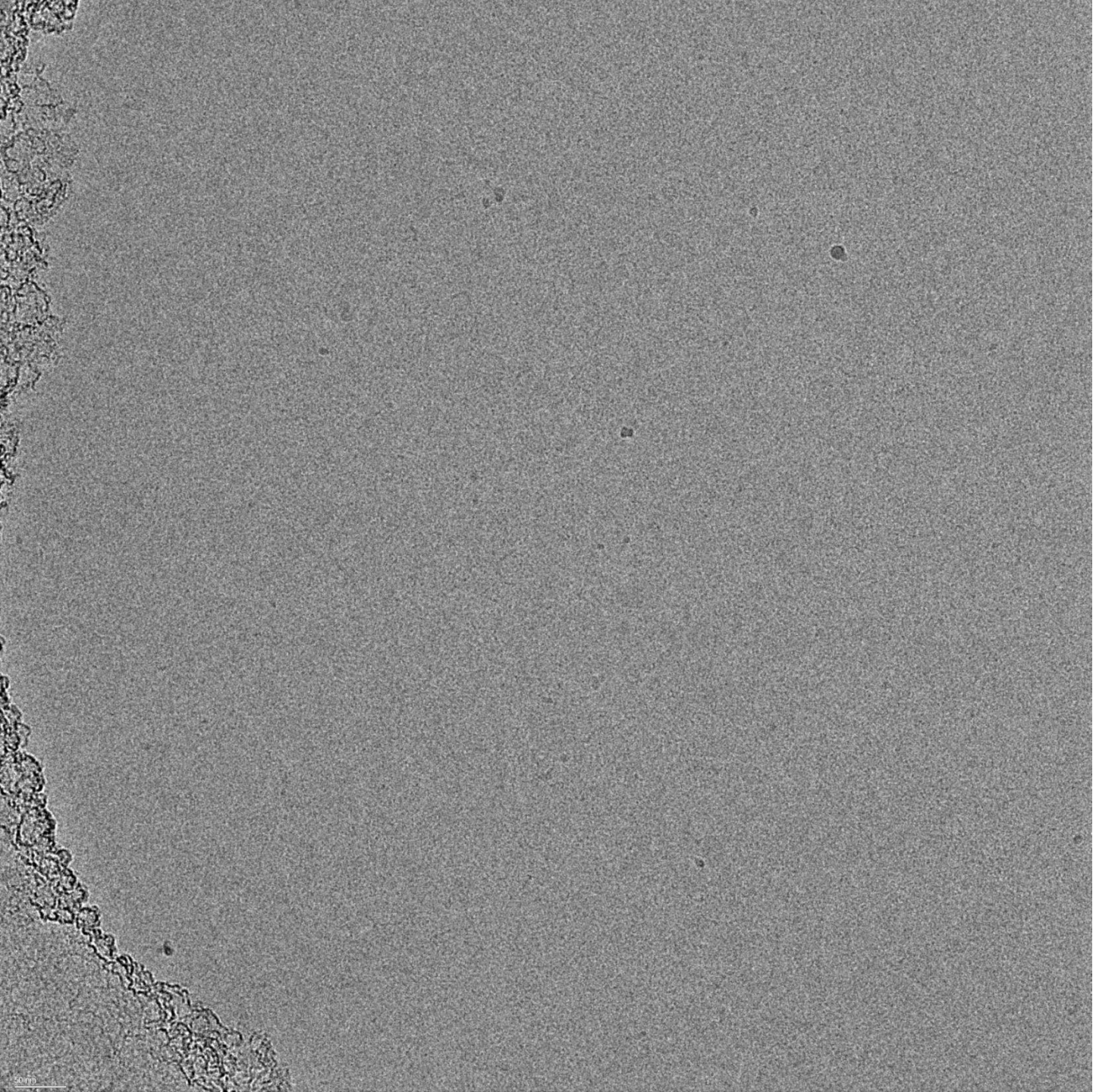


Supernatant from isolation of SCPs from spruce needle homogenate S2/7

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles.

SCPs were isolated by differential centrifugation as adapted from protocol for isolation of extracellular vesicles (EVs) (Mantile, F.; Kisovec, M.; Adamo, G.; Romancino, D.P.; Hočevar, M.; Božič, D.; Bedina Zavec, A.; Podobnik, M.; Stoppelli, M.P.; Kisslinger, A.; Bongiovanni, A.; Kralj-Iglič, V.; Liguori, G.L. A Novel Localization in Human Large Extracellular Vesicles for the EGF-CFC Founder Member CRIPTO and Its Biological and Therapeutic Implications. Cancers 2022, 14, 3700. https://doi.org/10.3390/cancers14153700). The homogenate was centrifuged twice at 300 g and 4°C for 10 minutes in the centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)) by using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany). The supernatant of the second centrifugation was centrifuged twice at 2000 g and 4°C for 10 minutes in the centrifuge Centric 400R with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Then, the supernatant was centrifuged at 50 000 g and 4°C, for 70 min in Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA) using 6 mL thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA). The supernatant consists of the fluid above about 50 µL of the pellet.

For cryogenic transmission electron microscopy, C-flat™ 2/2, 200 mesh holey carbon grids (Protochips, Morrisville, NC, USA) were glow discharged: 20 mA, 60 s, positive polarity, air atmosphere (GloQube ® Plus, Quor-um, Laughton, UK). 3 µL of sample was applied to the grid, blotted, and vitrified in liquid ethane on Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, USA). Vitrobot con-ditions were set to 100% relative humidity, 4 °C, blot force: 2 and blot time: 7 s. Samples were visualized under cryogenic conditions using a 200 kV Glacios microscope with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, USA).

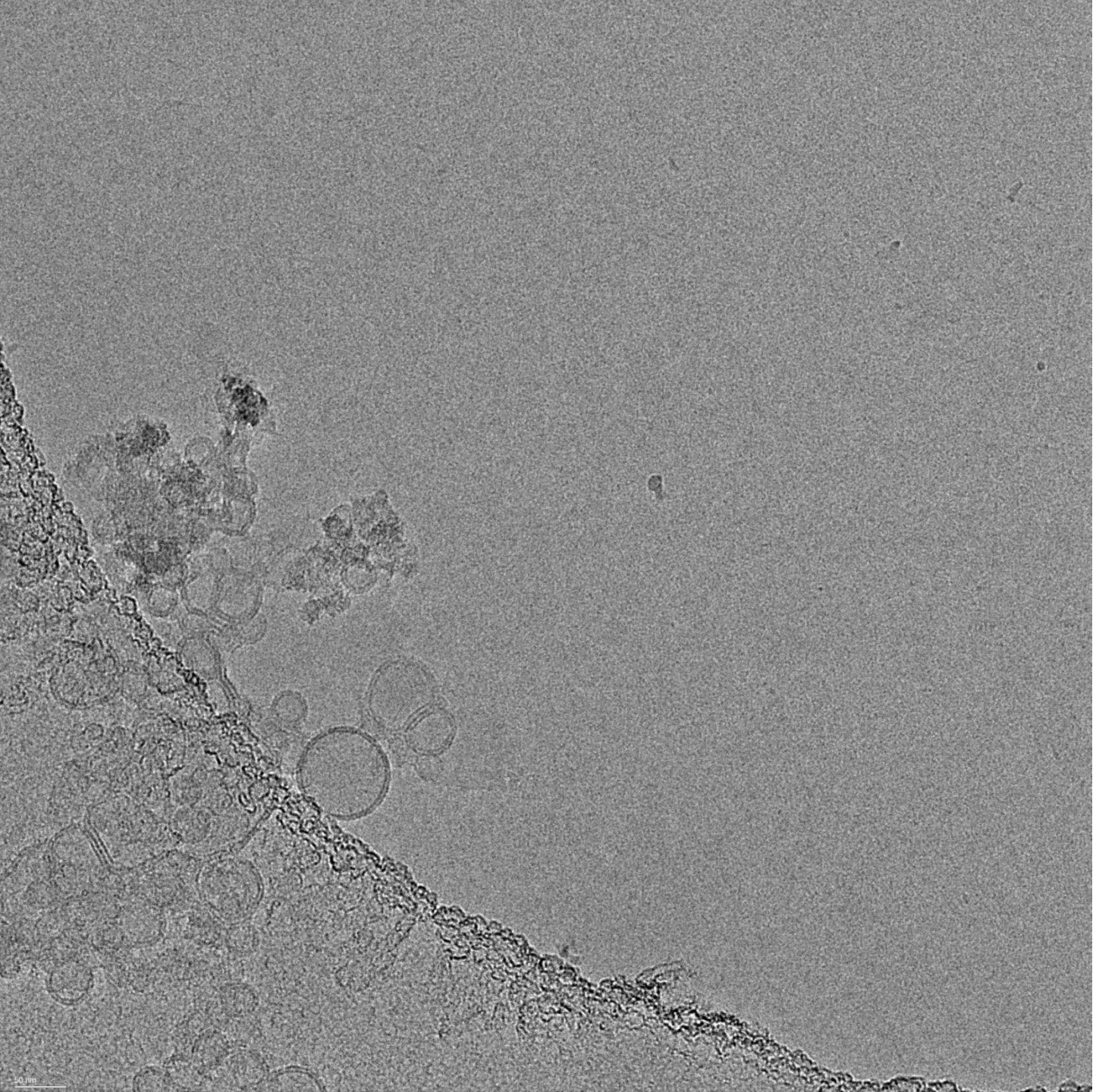


Supernatant from isolation of SCPs from spruce needle homogenate S2/8

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles.

SCPs were isolated by differential centrifugation as adapted from protocol for isolation of extracellular vesicles (EVs) (Mantile, F.; Kisovec, M.; Adamo, G.; Romancino, D.P.; Hočevar, M.; Božič, D.; Bedina Zavec, A.; Podobnik, M.; Stoppelli, M.P.; Kisslinger, A.; Bongiovanni, A.; Kralj-Iglič, V.; Liguori, G.L. A Novel Localization in Human Large Extracellular Vesicles for the EGF-CFC Founder Member CRIPTO and Its Biological and Therapeutic Implications. Cancers 2022, 14, 3700. https://doi.org/10.3390/cancers14153700). The homogenate was centrifuged twice at 300 g and 4°C for 10 minutes in the centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)) by using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany). The supernatant of the second centrifugation was centrifuged twice at 2000 g and 4°C for 10 minutes in the centrifuge Centric 400R with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Then, the supernatant was centrifuged at 50 000 g and 4°C, for 70 min in Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA) using 6 mL thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA). The supernatant consists of the fluid above about 50 µL of the pellet.

For cryogenic transmission electron microscopy, C-flat™ 2/2, 200 mesh holey carbon grids (Protochips, Morrisville, NC, USA) were glow discharged: 20 mA, 60 s, positive polarity, air atmosphere (GloQube ® Plus, Quor-um, Laughton, UK). 3 µL of sample was applied to the grid, blotted, and vitrified in liquid ethane on Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, USA). Vitrobot con-ditions were set to 100% relative humidity, 4 °C, blot force: 2 and blot time: 7 s. Samples were visualized under cryogenic conditions using a 200 kV Glacios microscope with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, USA).

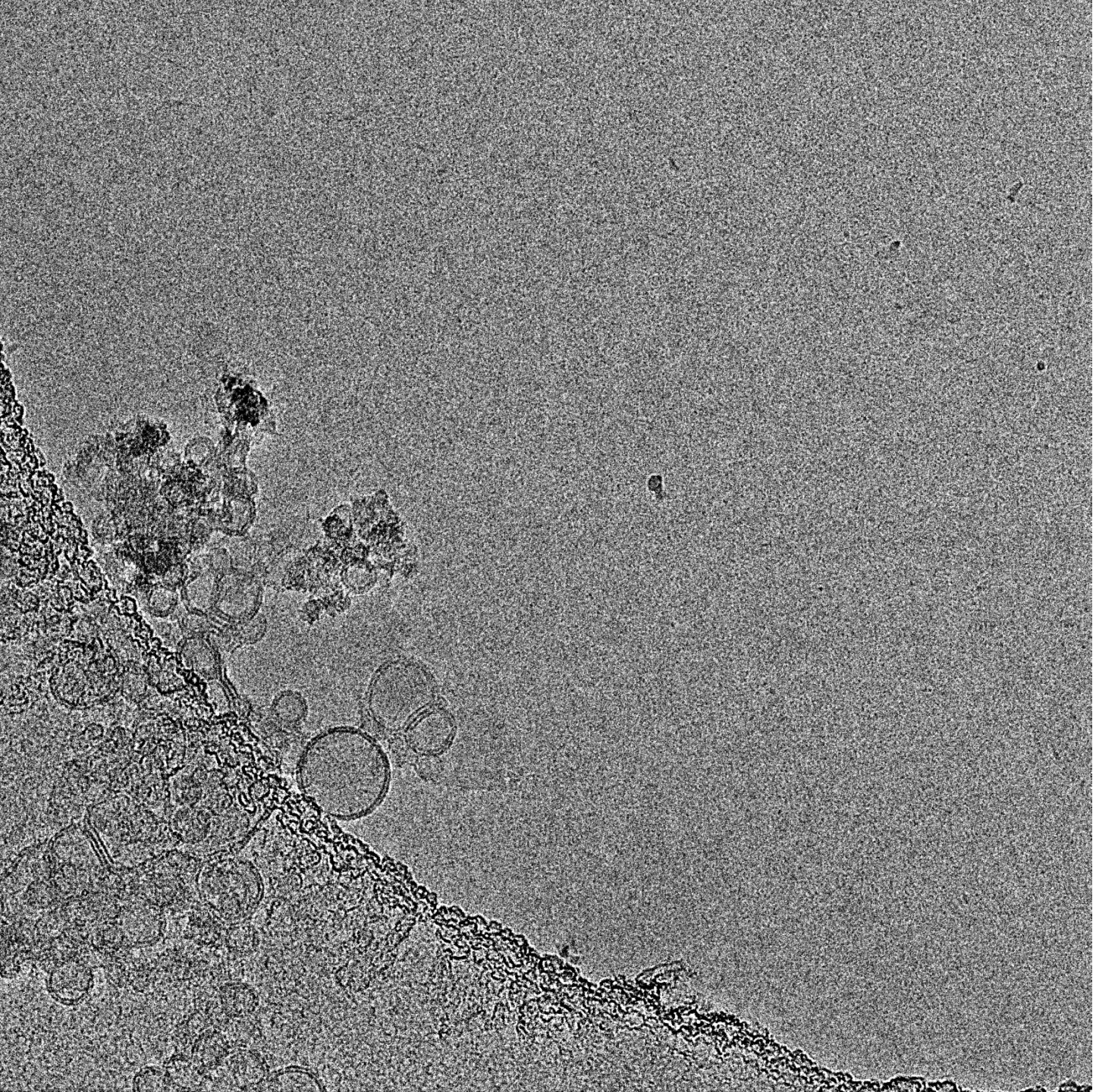


Supernatant from isolation of SCPs from spruce needle homogenate S2/9

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles.

SCPs were isolated by differential centrifugation as adapted from protocol for isolation of extracellular vesicles (EVs) (Mantile, F.; Kisovec, M.; Adamo, G.; Romancino, D.P.; Hočevar, M.; Božič, D.; Bedina Zavec, A.; Podobnik, M.; Stoppelli, M.P.; Kisslinger, A.; Bongiovanni, A.; Kralj-Iglič, V.; Liguori, G.L. A Novel Localization in Human Large Extracellular Vesicles for the EGF-CFC Founder Member CRIPTO and Its Biological and Therapeutic Implications. Cancers 2022, 14, 3700. https://doi.org/10.3390/cancers14153700). The homogenate was centrifuged twice at 300 g and 4°C for 10 minutes in the centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)) by using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany). The supernatant of the second centrifugation was centrifuged twice at 2000 g and 4°C for 10 minutes in the centrifuge Centric 400R with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Then, the supernatant was centrifuged at 50 000 g and 4°C, for 70 min in Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA) using 6 mL thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA). The supernatant consists of the fluid above about 50 µL of the pellet.

For cryogenic transmission electron microscopy, C-flat™ 2/2, 200 mesh holey carbon grids (Protochips, Morrisville, NC, USA) were glow discharged: 20 mA, 60 s, positive polarity, air atmosphere (GloQube ® Plus, Quor-um, Laughton, UK). 3 µL of sample was applied to the grid, blotted, and vitrified in liquid ethane on Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, USA). Vitrobot con-ditions were set to 100% relative humidity, 4 °C, blot force: 2 and blot time: 7 s. Samples were visualized under cryogenic conditions using a 200 kV Glacios microscope with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, USA).

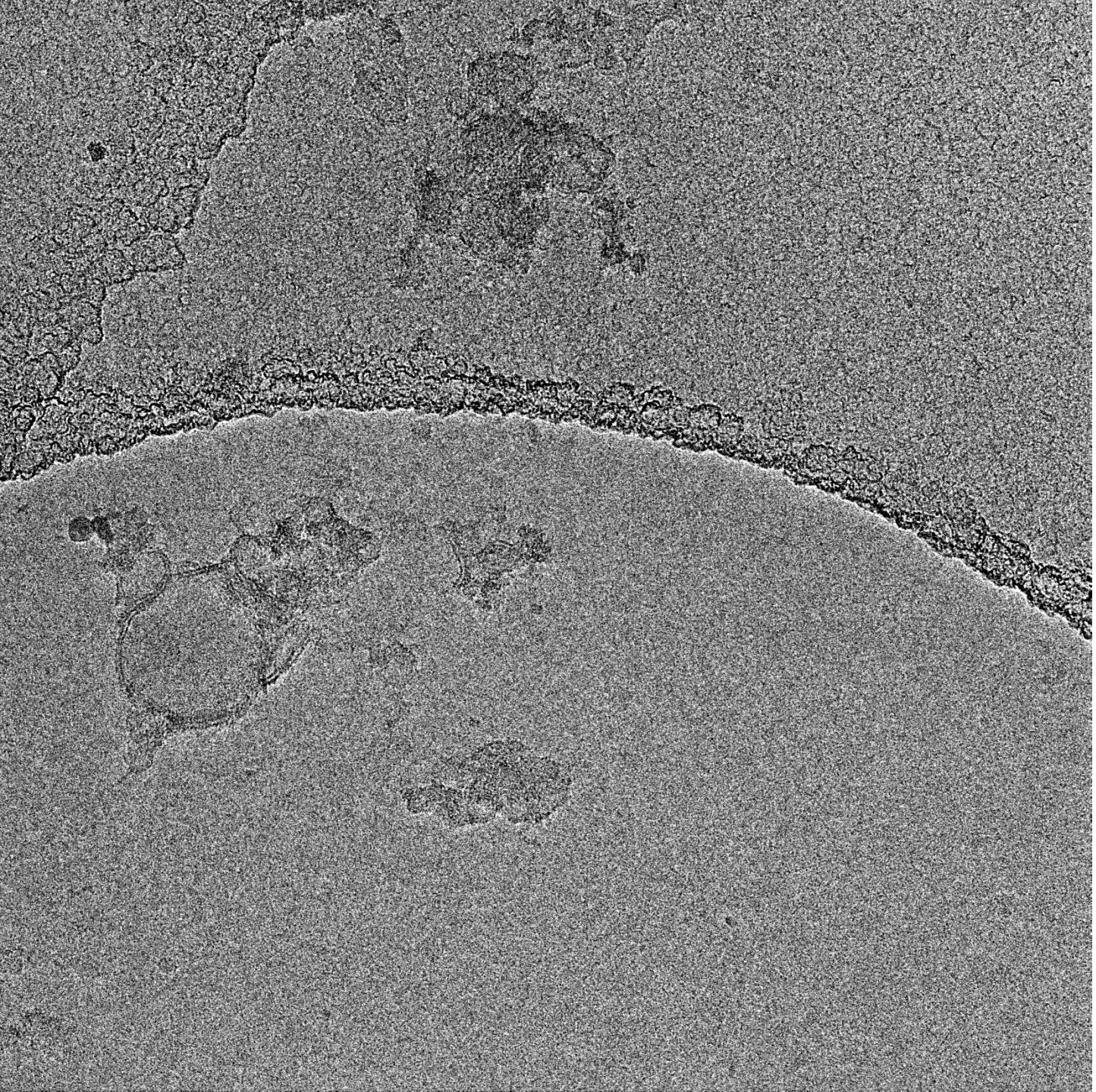


Supernatant from isolation of SCPs from spruce needle homogenate S2/10

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles.

SCPs were isolated by differential centrifugation as adapted from protocol for isolation of extracellular vesicles (EVs) (Mantile, F.; Kisovec, M.; Adamo, G.; Romancino, D.P.; Hočevar, M.; Božič, D.; Bedina Zavec, A.; Podobnik, M.; Stoppelli, M.P.; Kisslinger, A.; Bongiovanni, A.; Kralj-Iglič, V.; Liguori, G.L. A Novel Localization in Human Large Extracellular Vesicles for the EGF-CFC Founder Member CRIPTO and Its Biological and Therapeutic Implications. Cancers 2022, 14, 3700. https://doi.org/10.3390/cancers14153700). The homogenate was centrifuged twice at 300 g and 4°C for 10 minutes in the centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)) by using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany). The supernatant of the second centrifugation was centrifuged twice at 2000 g and 4°C for 10 minutes in the centrifuge Centric 400R with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Then, the supernatant was centrifuged at 50 000 g and 4°C, for 70 min in Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA) using 6 mL thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA). The supernatant consists of the fluid above about 50 µL of the pellet.

For cryogenic transmission electron microscopy, C-flat™ 2/2, 200 mesh holey carbon grids (Protochips, Morrisville, NC, USA) were glow discharged: 20 mA, 60 s, positive polarity, air atmosphere (GloQube ® Plus, Quor-um, Laughton, UK). 3 µL of sample was applied to the grid, blotted, and vitrified in liquid ethane on Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, USA). Vitrobot con-ditions were set to 100% relative humidity, 4 °C, blot force: 2 and blot time: 7 s. Samples were visualized under cryogenic conditions using a 200 kV Glacios microscope with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, USA).

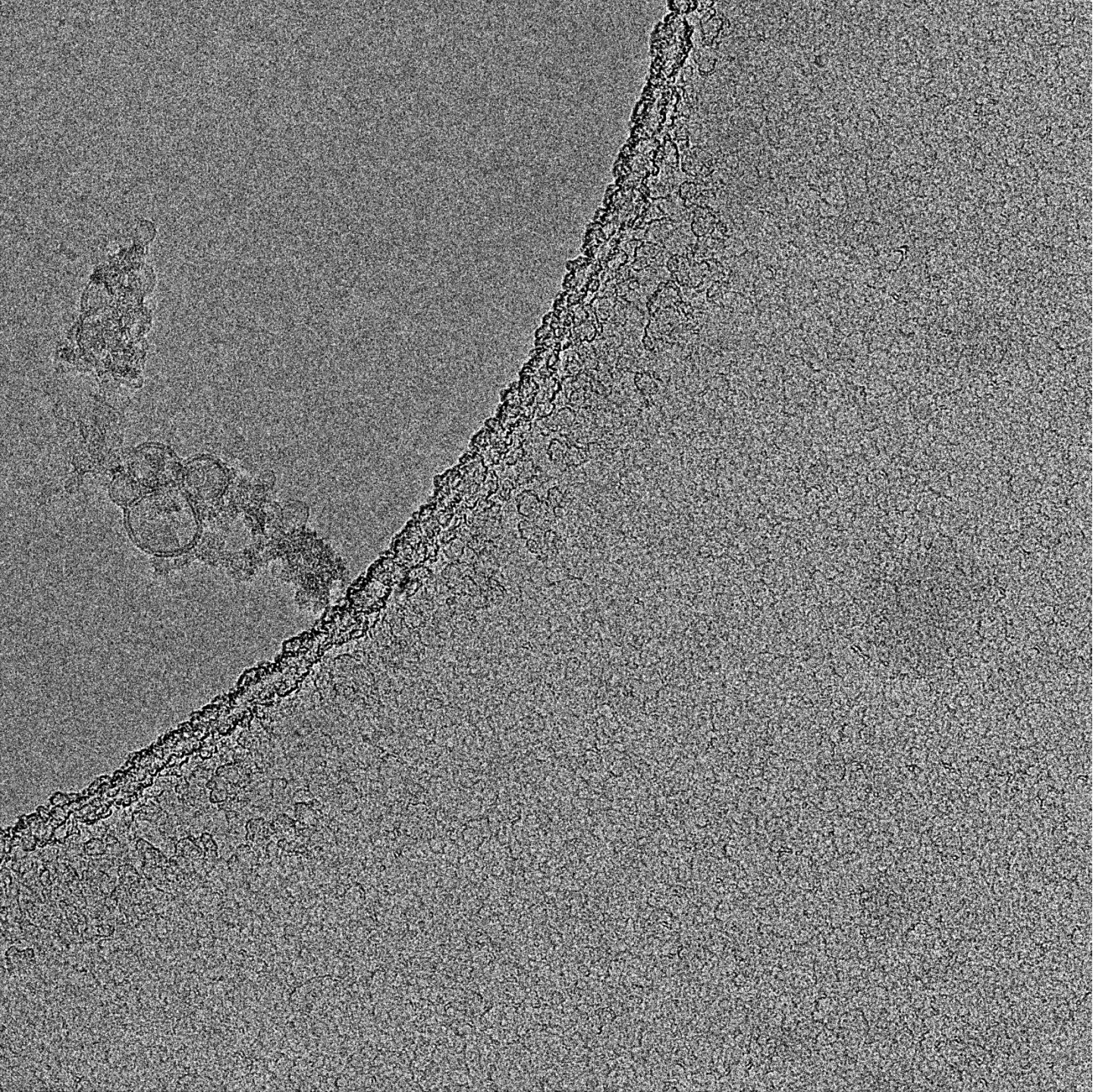


Supernatant from isolation of SCPs from spruce needle homogenate S2/11

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles.

SCPs were isolated by differential centrifugation as adapted from protocol for isolation of extracellular vesicles (EVs) (Mantile, F.; Kisovec, M.; Adamo, G.; Romancino, D.P.; Hočevar, M.; Božič, D.; Bedina Zavec, A.; Podobnik, M.; Stoppelli, M.P.; Kisslinger, A.; Bongiovanni, A.; Kralj-Iglič, V.; Liguori, G.L. A Novel Localization in Human Large Extracellular Vesicles for the EGF-CFC Founder Member CRIPTO and Its Biological and Therapeutic Implications. Cancers 2022, 14, 3700. https://doi.org/10.3390/cancers14153700). The homogenate was centrifuged twice at 300 g and 4°C for 10 minutes in the centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)) by using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany). The supernatant of the second centrifugation was centrifuged twice at 2000 g and 4°C for 10 minutes in the centrifuge Centric 400R with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Then, the supernatant was centrifuged at 50 000 g and 4°C, for 70 min in Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA) using 6 mL thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA). The supernatant consists of the fluid above about 50 µL of the pellet.

For cryogenic transmission electron microscopy, C-flat™ 2/2, 200 mesh holey carbon grids (Protochips, Morrisville, NC, USA) were glow discharged: 20 mA, 60 s, positive polarity, air atmosphere (GloQube ® Plus, Quor-um, Laughton, UK). 3 µL of sample was applied to the grid, blotted, and vitrified in liquid ethane on Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, USA). Vitrobot con-ditions were set to 100% relative humidity, 4 °C, blot force: 2 and blot time: 7 s. Samples were visualized under cryogenic conditions using a 200 kV Glacios microscope with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, USA).

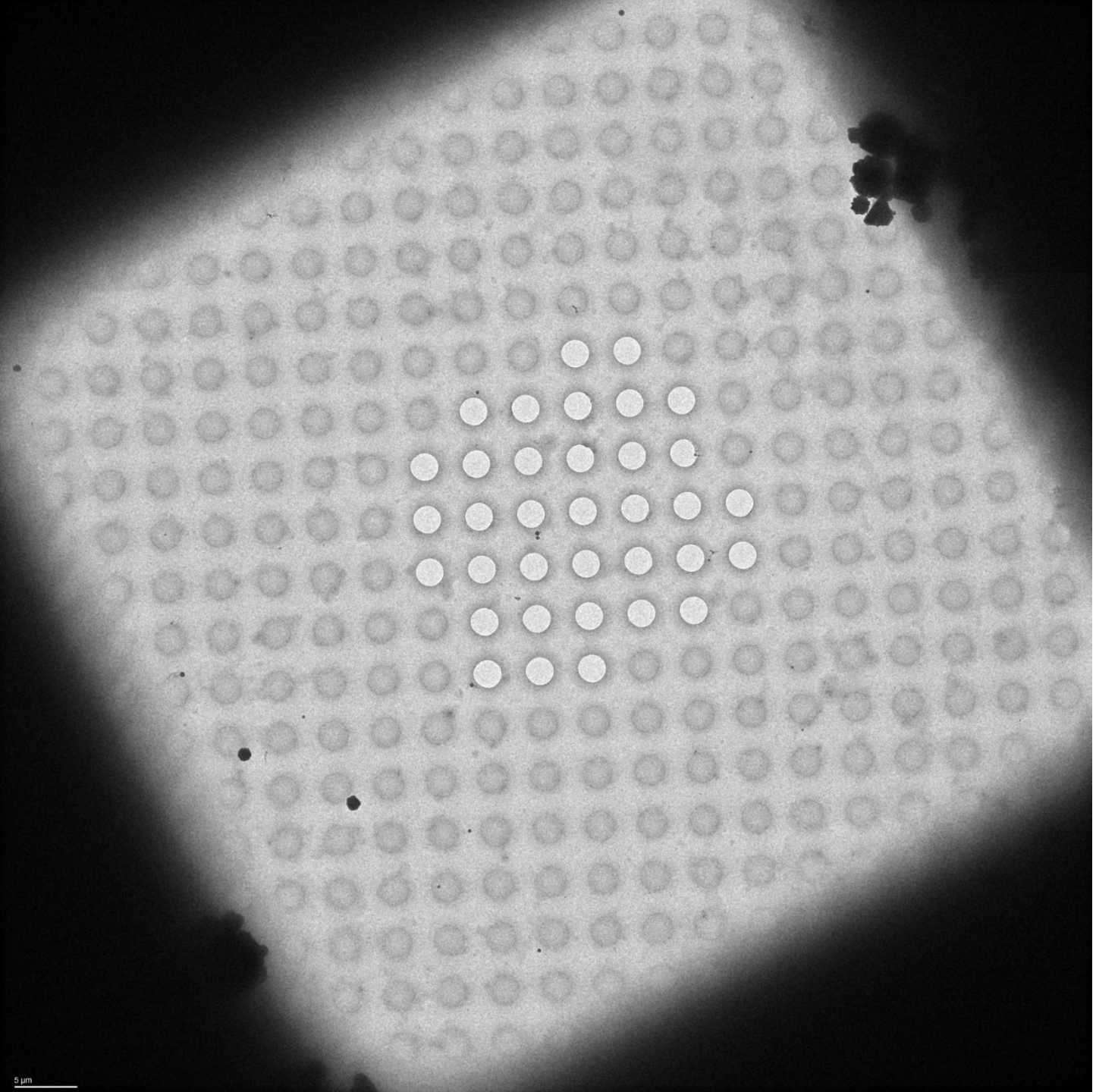


Supernatant from isolation of SCPs from spruce needle homogenate S2/12

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles.

SCPs were isolated by differential centrifugation as adapted from protocol for isolation of extracellular vesicles (EVs) (Mantile, F.; Kisovec, M.; Adamo, G.; Romancino, D.P.; Hočevar, M.; Božič, D.; Bedina Zavec, A.; Podobnik, M.; Stoppelli, M.P.; Kisslinger, A.; Bongiovanni, A.; Kralj-Iglič, V.; Liguori, G.L. A Novel Localization in Human Large Extracellular Vesicles for the EGF-CFC Founder Member CRIPTO and Its Biological and Therapeutic Implications. Cancers 2022, 14, 3700. https://doi.org/10.3390/cancers14153700). The homogenate was centrifuged twice at 300 g and 4°C for 10 minutes in the centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)) by using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany). The supernatant of the second centrifugation was centrifuged twice at 2000 g and 4°C for 10 minutes in the centrifuge Centric 400R with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Then, the supernatant was centrifuged at 50 000 g and 4°C, for 70 min in Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA) using 6 mL thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA). The supernatant consists of the fluid above about 50 µL of the pellet.

For cryogenic transmission electron microscopy, C-flat™ 2/2, 200 mesh holey carbon grids (Protochips, Morrisville, NC, USA) were glow discharged: 20 mA, 60 s, positive polarity, air atmosphere (GloQube ® Plus, Quor-um, Laughton, UK). 3 µL of sample was applied to the grid, blotted, and vitrified in liquid ethane on Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, USA). Vitrobot con-ditions were set to 100% relative humidity, 4 °C, blot force: 2 and blot time: 7 s. Samples were visualized under cryogenic conditions using a 200 kV Glacios microscope with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, USA).

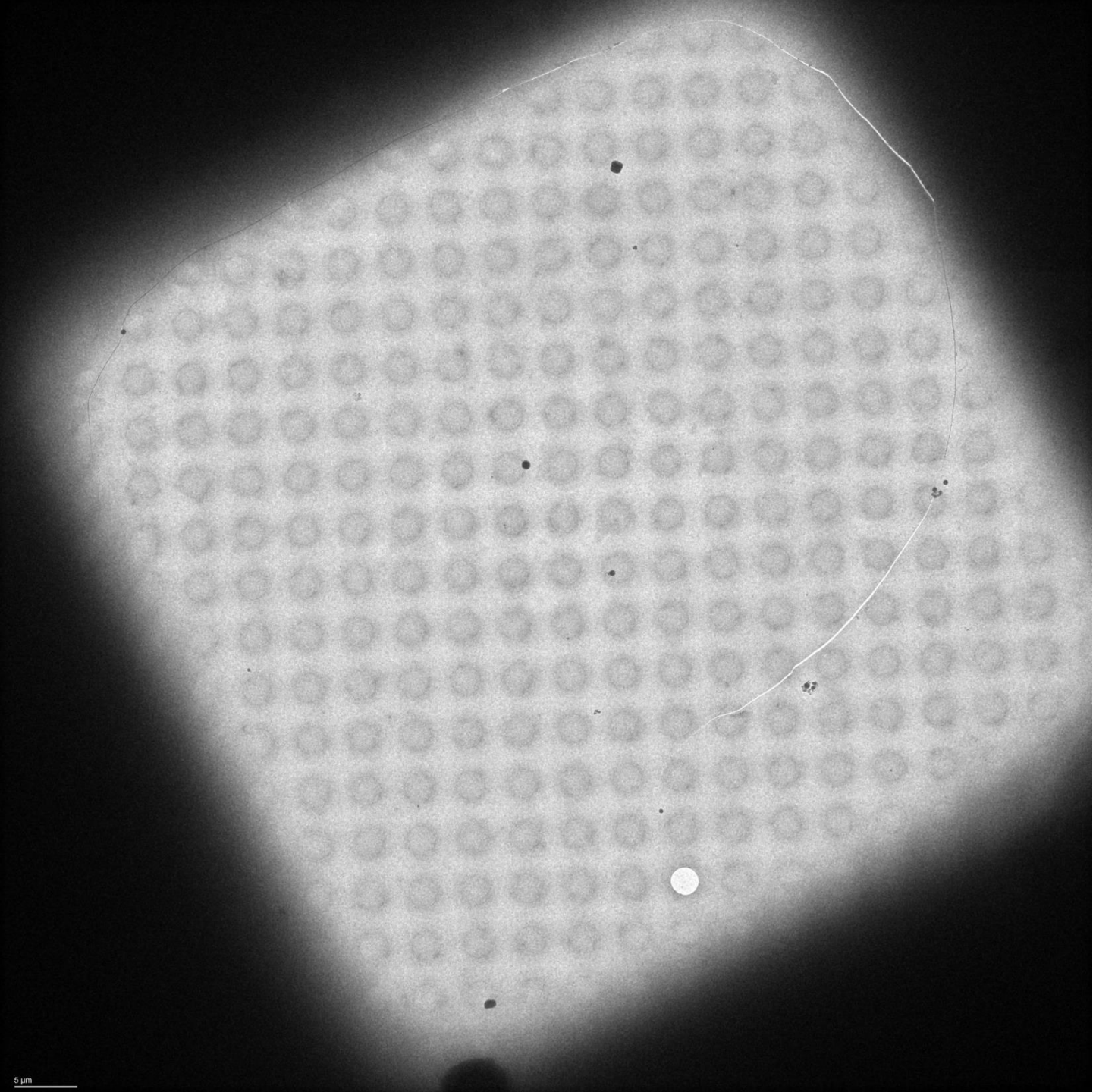


Supernatant from isolation of SCPs from spruce needle homogenate S2/13

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles.

SCPs were isolated by differential centrifugation as adapted from protocol for isolation of extracellular vesicles (EVs) (Mantile, F.; Kisovec, M.; Adamo, G.; Romancino, D.P.; Hočevar, M.; Božič, D.; Bedina Zavec, A.; Podobnik, M.; Stoppelli, M.P.; Kisslinger, A.; Bongiovanni, A.; Kralj-Iglič, V.; Liguori, G.L. A Novel Localization in Human Large Extracellular Vesicles for the EGF-CFC Founder Member CRIPTO and Its Biological and Therapeutic Implications. Cancers 2022, 14, 3700. https://doi.org/10.3390/cancers14153700). The homogenate was centrifuged twice at 300 g and 4°C for 10 minutes in the centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)) by using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany). The supernatant of the second centrifugation was centrifuged twice at 2000 g and 4°C for 10 minutes in the centrifuge Centric 400R with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Then, the supernatant was centrifuged at 50 000 g and 4°C, for 70 min in Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA) using 6 mL thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA). The supernatant consists of the fluid above about 50 µL of the pellet.

For cryogenic transmission electron microscopy, C-flat™ 2/2, 200 mesh holey carbon grids (Protochips, Morrisville, NC, USA) were glow discharged: 20 mA, 60 s, positive polarity, air atmosphere (GloQube ® Plus, Quor-um, Laughton, UK). 3 µL of sample was applied to the grid, blotted, and vitrified in liquid ethane on Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, USA). Vitrobot con-ditions were set to 100% relative humidity, 4 °C, blot force: 2 and blot time: 7 s. Samples were visualized under cryogenic conditions using a 200 kV Glacios microscope with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, USA).



Supernatant from isolation of SCPs from spruce needle homogenate S2/14

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles.

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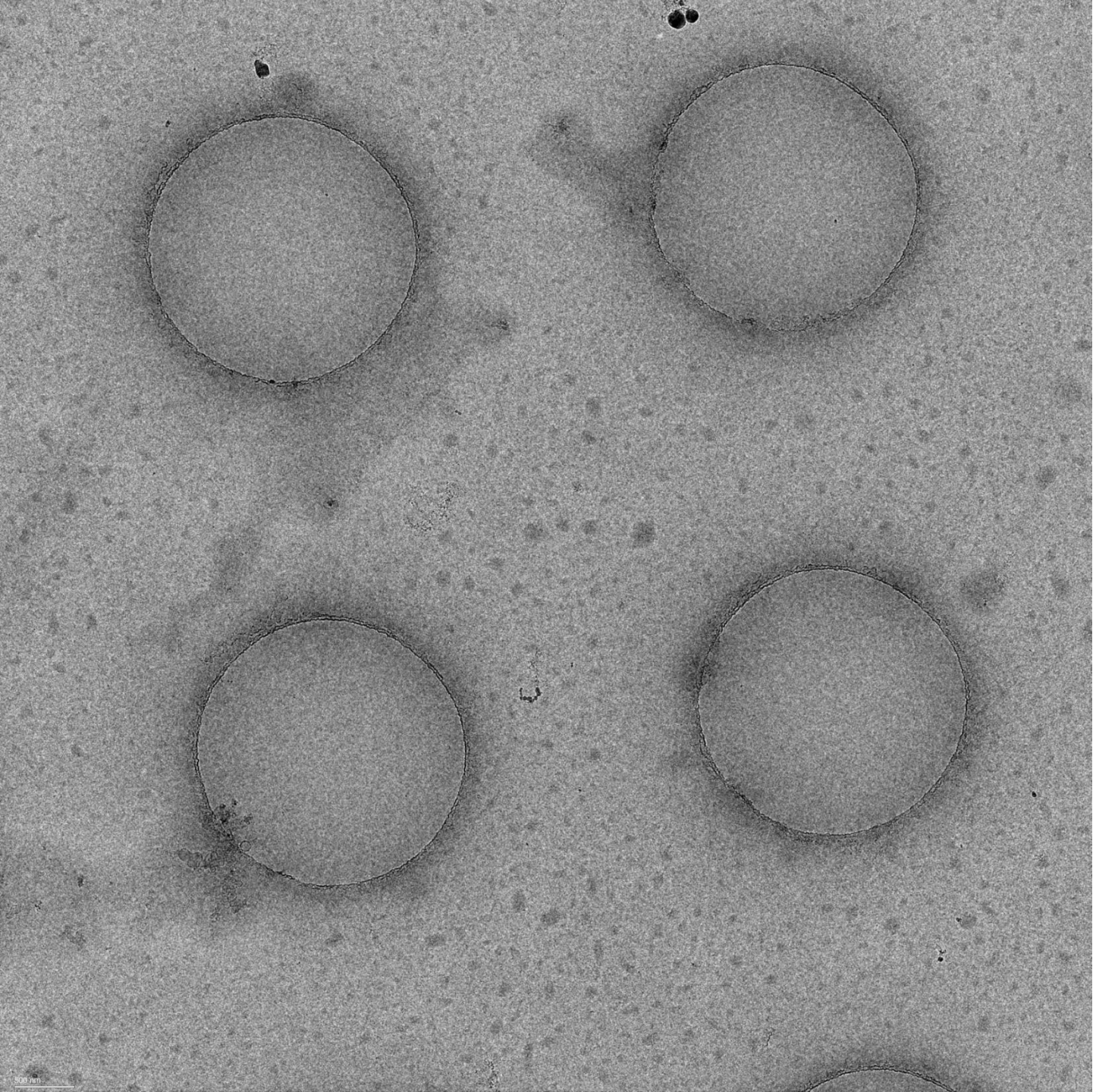
For cryogenic transmission electron microscopy, C-flat™ 2/2, 200 mesh holey carbon grids (Protochips, Morrisville, NC, USA) were glow discharged: 20 mA, 60 s, positive polarity, air atmosphere (GloQube ® Plus, Quor-um, Laughton, UK). 3 µL of sample was applied to the grid, blotted, and vitrified in liquid ethane on Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, USA). Vitrobot con-ditions were set to 100% relative humidity, 4 °C, blot force: 2 and blot time: 7 s. Samples were visualized under cryogenic conditions using a 200 kV Glacios microscope with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, USA).

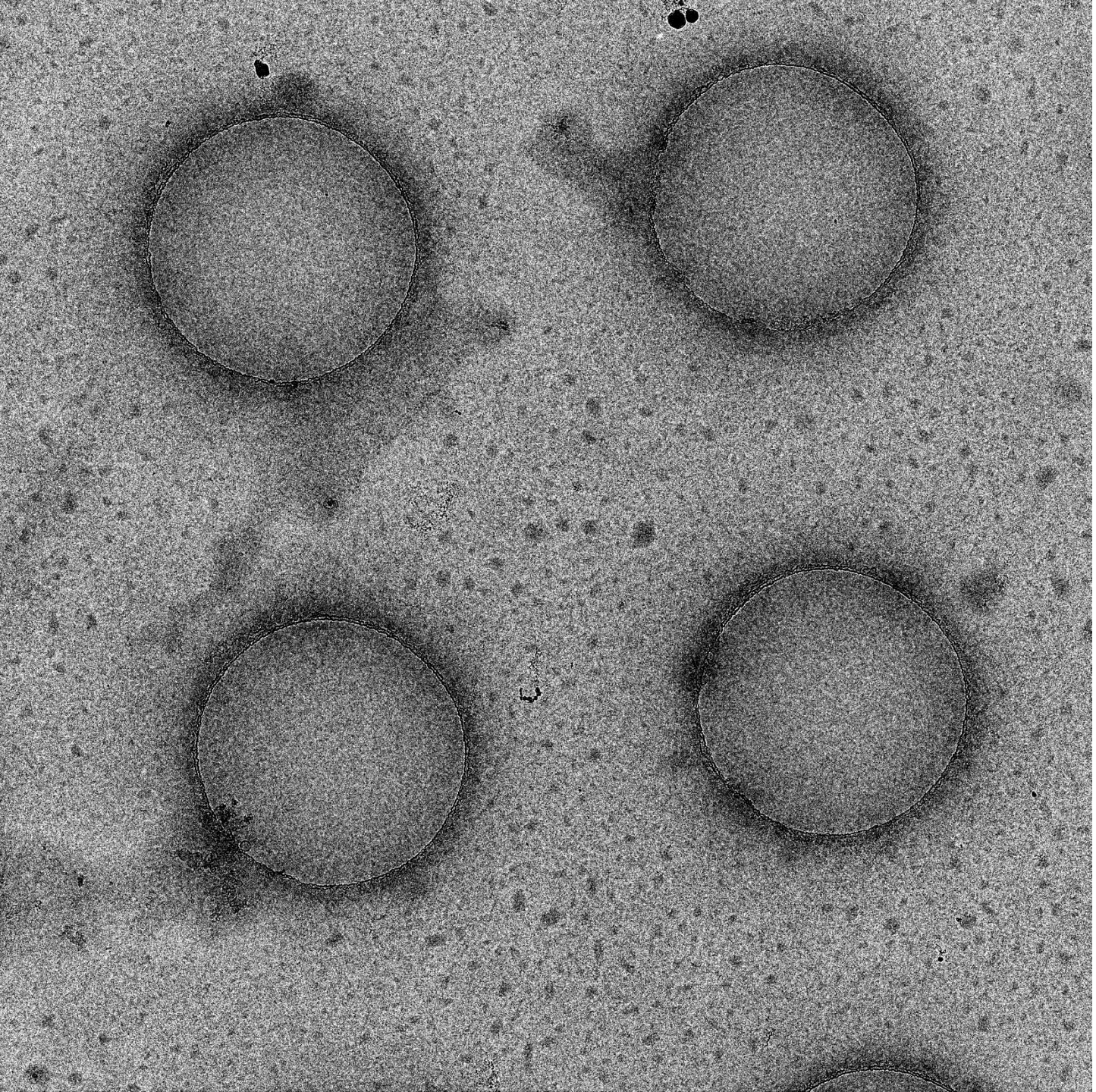


Supernatant from isolation of SCPs from spruce needle homogenate S2/15

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles.

SCPs were isolated by differential centrifugation as adapted from protocol for isolation of extracellular vesicles (EVs) (Mantile, F.; Kisovec, M.; Adamo, G.; Romancino, D.P.; Hočevar, M.; Božič, D.; Bedina Zavec, A.; Podobnik, M.; Stoppelli, M.P.; Kisslinger, A.; Bongiovanni, A.; Kralj-Iglič, V.; Liguori, G.L. A Novel Localization in Human Large Extracellular Vesicles for the EGF-CFC Founder Member CRIPTO and Its Biological and Therapeutic Implications. Cancers 2022, 14, 3700. https://doi.org/10.3390/cancers14153700). The homogenate was centrifuged twice at 300 g and 4°C for 10 minutes in the centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)) by using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany). The supernatant of the second centrifugation was centrifuged twice at 2000 g and 4°C for 10 minutes in the centrifuge Centric 400R with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Then, the supernatant was centrifuged at 50 000 g and 4°C, for 70 min in Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA) using 6 mL thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA). The supernatant consists of the fluid above about 50 µL of the pellet.

For cryogenic transmission electron microscopy, C-flat™ 2/2, 200 mesh holey carbon grids (Protochips, Morrisville, NC, USA) were glow discharged: 20 mA, 60 s, positive polarity, air atmosphere (GloQube ® Plus, Quor-um, Laughton, UK). 3 µL of sample was applied to the grid, blotted, and vitrified in liquid ethane on Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, USA). Vitrobot con-ditions were set to 100% relative humidity, 4 °C, blot force: 2 and blot time: 7 s. Samples were visualized under cryogenic conditions using a 200 kV Glacios microscope with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, USA).

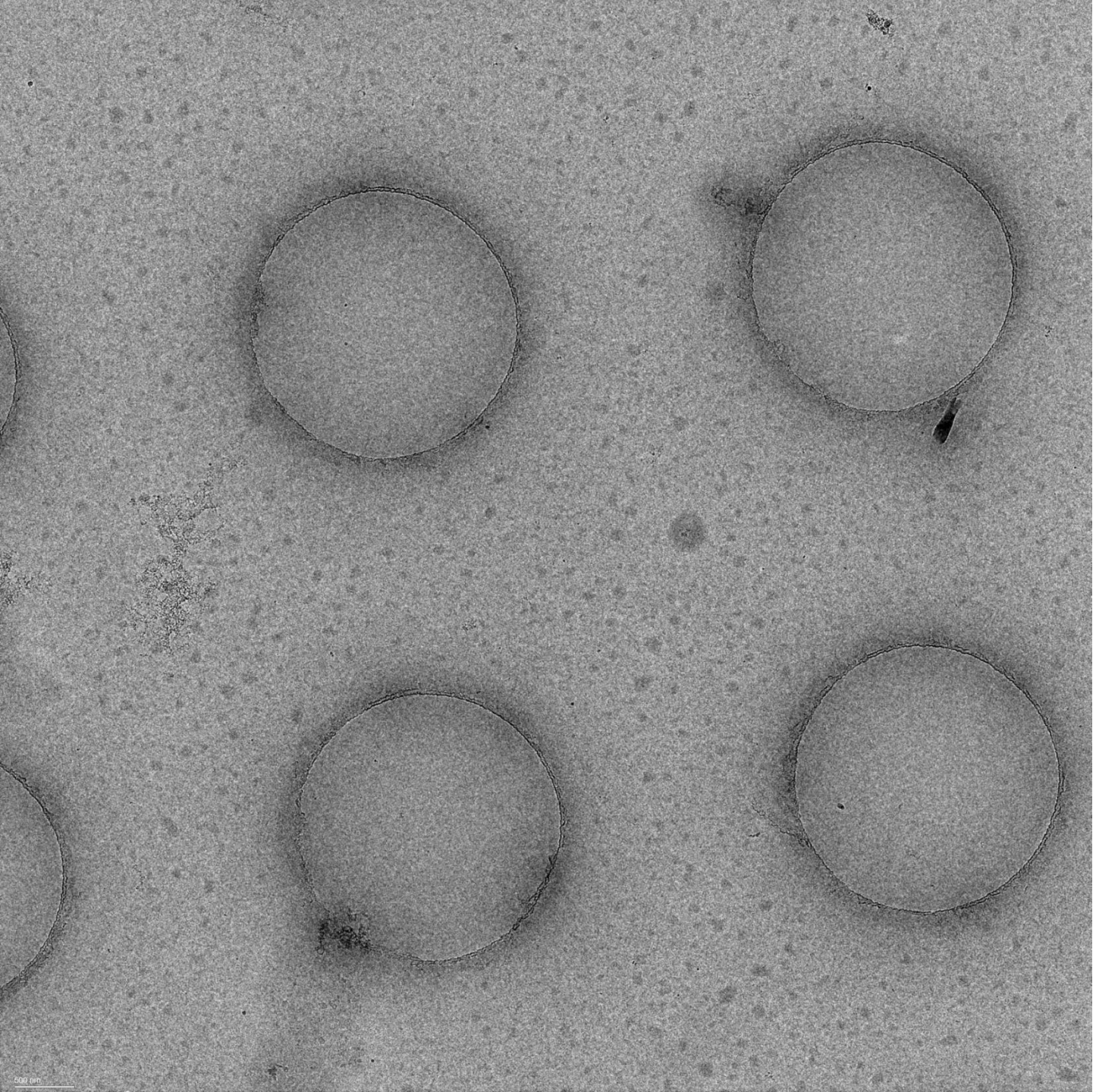


Supernatant from isolation of SCPs from spruce needle homogenate S2/16

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles.

SCPs were isolated by differential centrifugation as adapted from protocol for isolation of extracellular vesicles (EVs) (Mantile, F.; Kisovec, M.; Adamo, G.; Romancino, D.P.; Hočevar, M.; Božič, D.; Bedina Zavec, A.; Podobnik, M.; Stoppelli, M.P.; Kisslinger, A.; Bongiovanni, A.; Kralj-Iglič, V.; Liguori, G.L. A Novel Localization in Human Large Extracellular Vesicles for the EGF-CFC Founder Member CRIPTO and Its Biological and Therapeutic Implications. Cancers 2022, 14, 3700. https://doi.org/10.3390/cancers14153700). The homogenate was centrifuged twice at 300 g and 4°C for 10 minutes in the centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)) by using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany). The supernatant of the second centrifugation was centrifuged twice at 2000 g and 4°C for 10 minutes in the centrifuge Centric 400R with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Then, the supernatant was centrifuged at 50 000 g and 4°C, for 70 min in Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA) using 6 mL thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA). The supernatant consists of the fluid above about 50 µL of the pellet.

For cryogenic transmission electron microscopy, C-flat™ 2/2, 200 mesh holey carbon grids (Protochips, Morrisville, NC, USA) were glow discharged: 20 mA, 60 s, positive polarity, air atmosphere (GloQube ® Plus, Quor-um, Laughton, UK). 3 µL of sample was applied to the grid, blotted, and vitrified in liquid ethane on Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, USA). Vitrobot con-ditions were set to 100% relative humidity, 4 °C, blot force: 2 and blot time: 7 s. Samples were visualized under cryogenic conditions using a 200 kV Glacios microscope with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, USA).

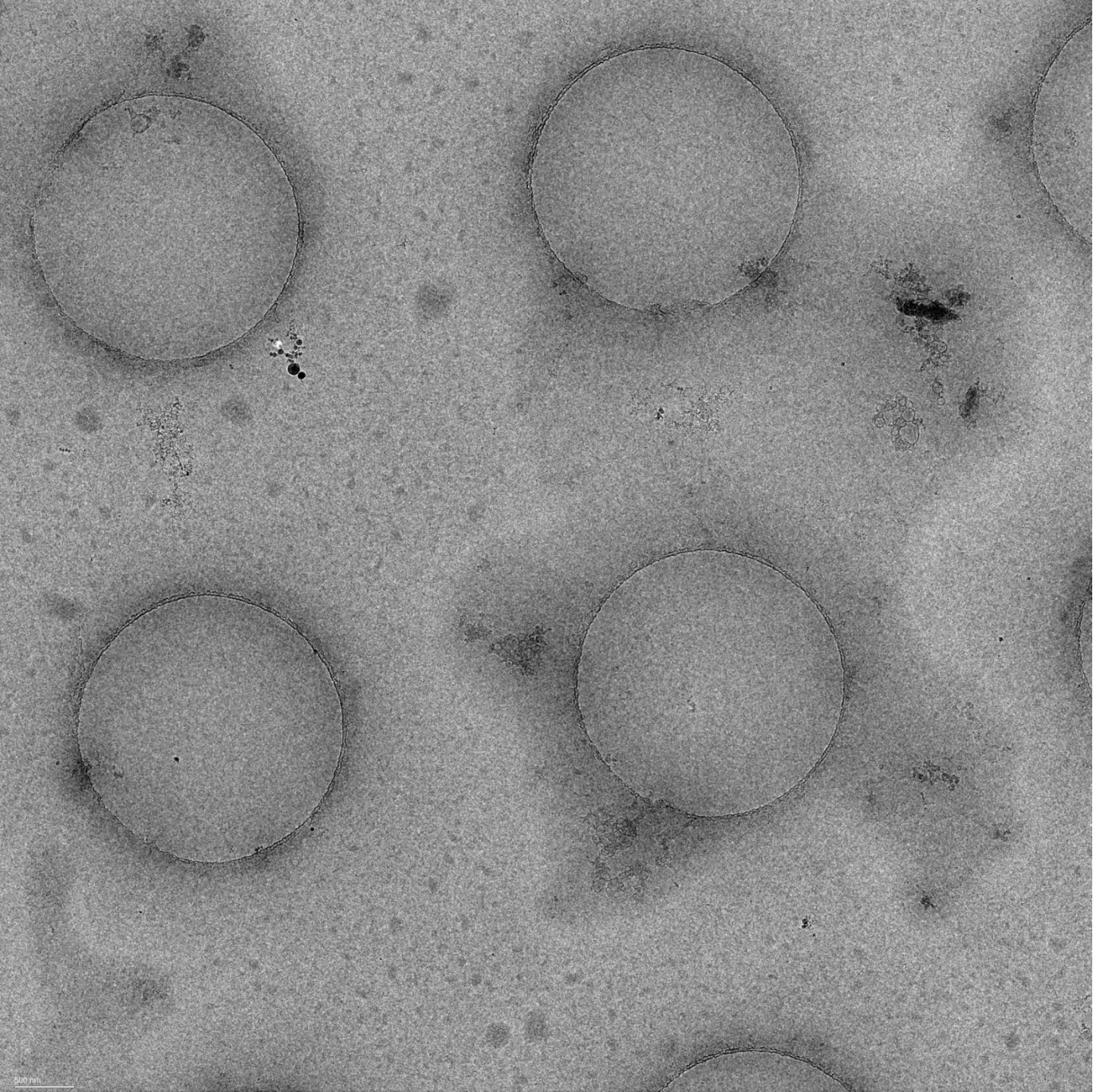


Supernatant from isolation of SCPs from spruce needle homogenate S2/17

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles.

SCPs were isolated by differential centrifugation as adapted from protocol for isolation of extracellular vesicles (EVs) (Mantile, F.; Kisovec, M.; Adamo, G.; Romancino, D.P.; Hočevar, M.; Božič, D.; Bedina Zavec, A.; Podobnik, M.; Stoppelli, M.P.; Kisslinger, A.; Bongiovanni, A.; Kralj-Iglič, V.; Liguori, G.L. A Novel Localization in Human Large Extracellular Vesicles for the EGF-CFC Founder Member CRIPTO and Its Biological and Therapeutic Implications. Cancers 2022, 14, 3700. https://doi.org/10.3390/cancers14153700). The homogenate was centrifuged twice at 300 g and 4°C for 10 minutes in the centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)) by using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany). The supernatant of the second centrifugation was centrifuged twice at 2000 g and 4°C for 10 minutes in the centrifuge Centric 400R with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Then, the supernatant was centrifuged at 50 000 g and 4°C, for 70 min in Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA) using 6 mL thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA). The supernatant consists of the fluid above about 50 µL of the pellet.

For cryogenic transmission electron microscopy, C-flat™ 2/2, 200 mesh holey carbon grids (Protochips, Morrisville, NC, USA) were glow discharged: 20 mA, 60 s, positive polarity, air atmosphere (GloQube ® Plus, Quor-um, Laughton, UK). 3 µL of sample was applied to the grid, blotted, and vitrified in liquid ethane on Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, USA). Vitrobot con-ditions were set to 100% relative humidity, 4 °C, blot force: 2 and blot time: 7 s. Samples were visualized under cryogenic conditions using a 200 kV Glacios microscope with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, USA).

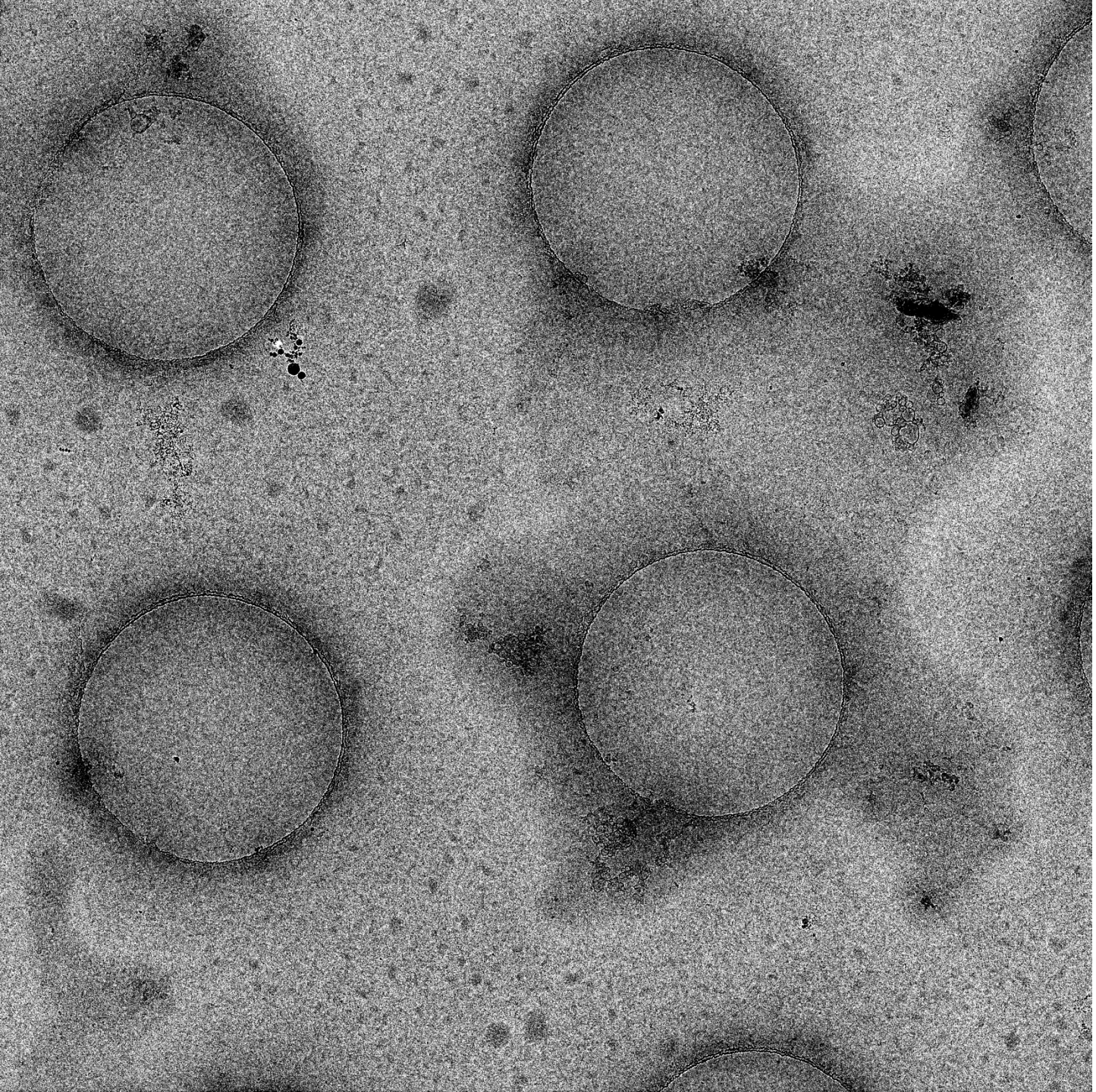


Supernatant from isolation of SCPs from spruce needle homogenate S2/18

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles.

SCPs were isolated by differential centrifugation as adapted from protocol for isolation of extracellular vesicles (EVs) (Mantile, F.; Kisovec, M.; Adamo, G.; Romancino, D.P.; Hočevar, M.; Božič, D.; Bedina Zavec, A.; Podobnik, M.; Stoppelli, M.P.; Kisslinger, A.; Bongiovanni, A.; Kralj-Iglič, V.; Liguori, G.L. A Novel Localization in Human Large Extracellular Vesicles for the EGF-CFC Founder Member CRIPTO and Its Biological and Therapeutic Implications. Cancers 2022, 14, 3700. https://doi.org/10.3390/cancers14153700). The homogenate was centrifuged twice at 300 g and 4°C for 10 minutes in the centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)) by using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany). The supernatant of the second centrifugation was centrifuged twice at 2000 g and 4°C for 10 minutes in the centrifuge Centric 400R with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Then, the supernatant was centrifuged at 50 000 g and 4°C, for 70 min in Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA) using 6 mL thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA). The supernatant consists of the fluid above about 50 µL of the pellet.

For cryogenic transmission electron microscopy, C-flat™ 2/2, 200 mesh holey carbon grids (Protochips, Morrisville, NC, USA) were glow discharged: 20 mA, 60 s, positive polarity, air atmosphere (GloQube ® Plus, Quor-um, Laughton, UK). 3 µL of sample was applied to the grid, blotted, and vitrified in liquid ethane on Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, USA). Vitrobot con-ditions were set to 100% relative humidity, 4 °C, blot force: 2 and blot time: 7 s. Samples were visualized under cryogenic conditions using a 200 kV Glacios microscope with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, USA).

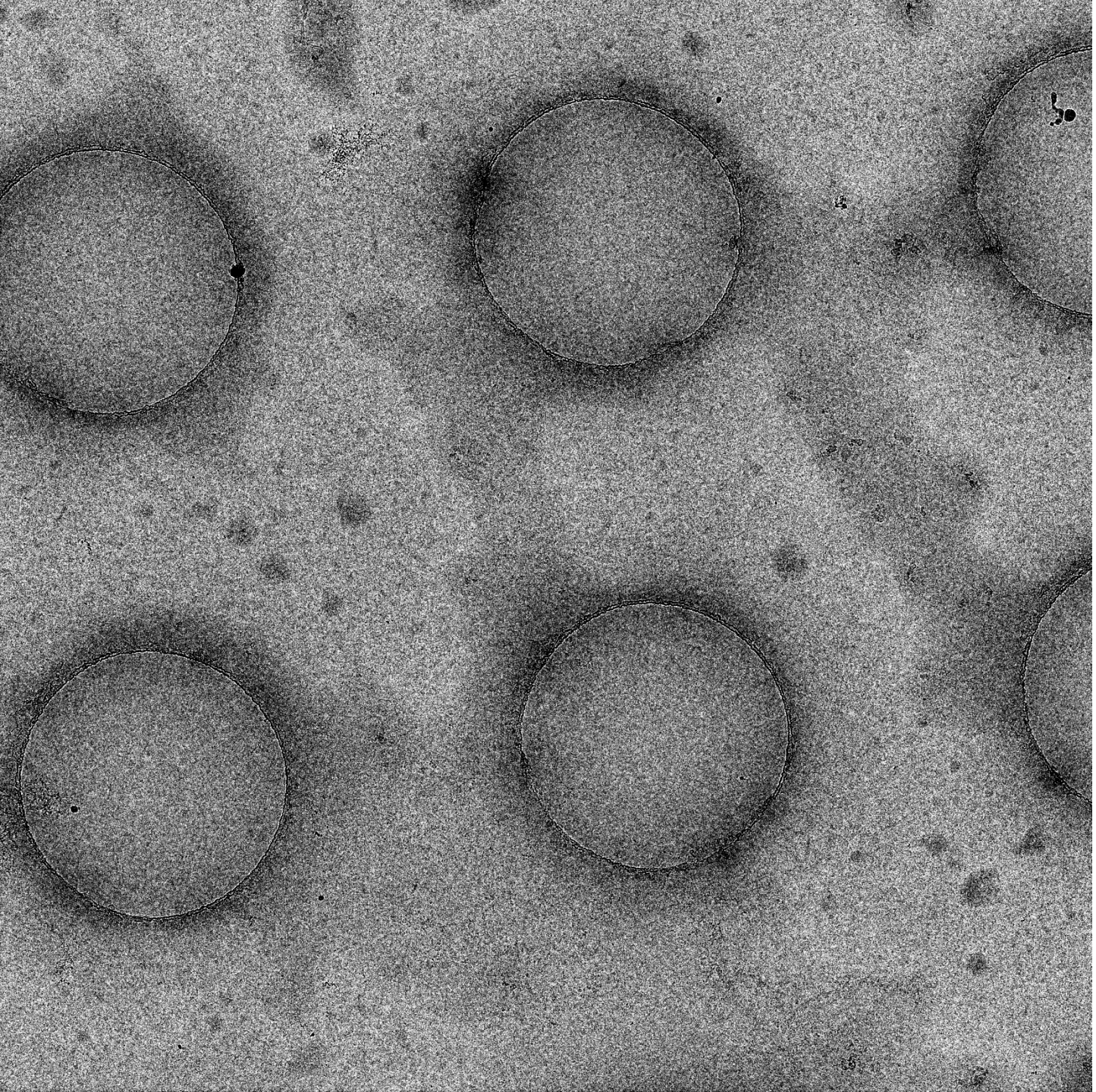


Supernatant from isolation of SCPs from spruce needle homogenate S2/19

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles.

SCPs were isolated by differential centrifugation as adapted from protocol for isolation of extracellular vesicles (EVs) (Mantile, F.; Kisovec, M.; Adamo, G.; Romancino, D.P.; Hočevar, M.; Božič, D.; Bedina Zavec, A.; Podobnik, M.; Stoppelli, M.P.; Kisslinger, A.; Bongiovanni, A.; Kralj-Iglič, V.; Liguori, G.L. A Novel Localization in Human Large Extracellular Vesicles for the EGF-CFC Founder Member CRIPTO and Its Biological and Therapeutic Implications. Cancers 2022, 14, 3700. https://doi.org/10.3390/cancers14153700). The homogenate was centrifuged twice at 300 g and 4°C for 10 minutes in the centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)) by using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany). The supernatant of the second centrifugation was centrifuged twice at 2000 g and 4°C for 10 minutes in the centrifuge Centric 400R with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Then, the supernatant was centrifuged at 50 000 g and 4°C, for 70 min in Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA) using 6 mL thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA). The supernatant consists of the fluid above about 50 µL of the pellet.

For cryogenic transmission electron microscopy, C-flat™ 2/2, 200 mesh holey carbon grids (Protochips, Morrisville, NC, USA) were glow discharged: 20 mA, 60 s, positive polarity, air atmosphere (GloQube ® Plus, Quor-um, Laughton, UK). 3 µL of sample was applied to the grid, blotted, and vitrified in liquid ethane on Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, USA). Vitrobot con-ditions were set to 100% relative humidity, 4 °C, blot force: 2 and blot time: 7 s. Samples were visualized under cryogenic conditions using a 200 kV Glacios microscope with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, USA).



Supernatant from isolation of SCPs from spruce needle homogenate S2/20

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles.

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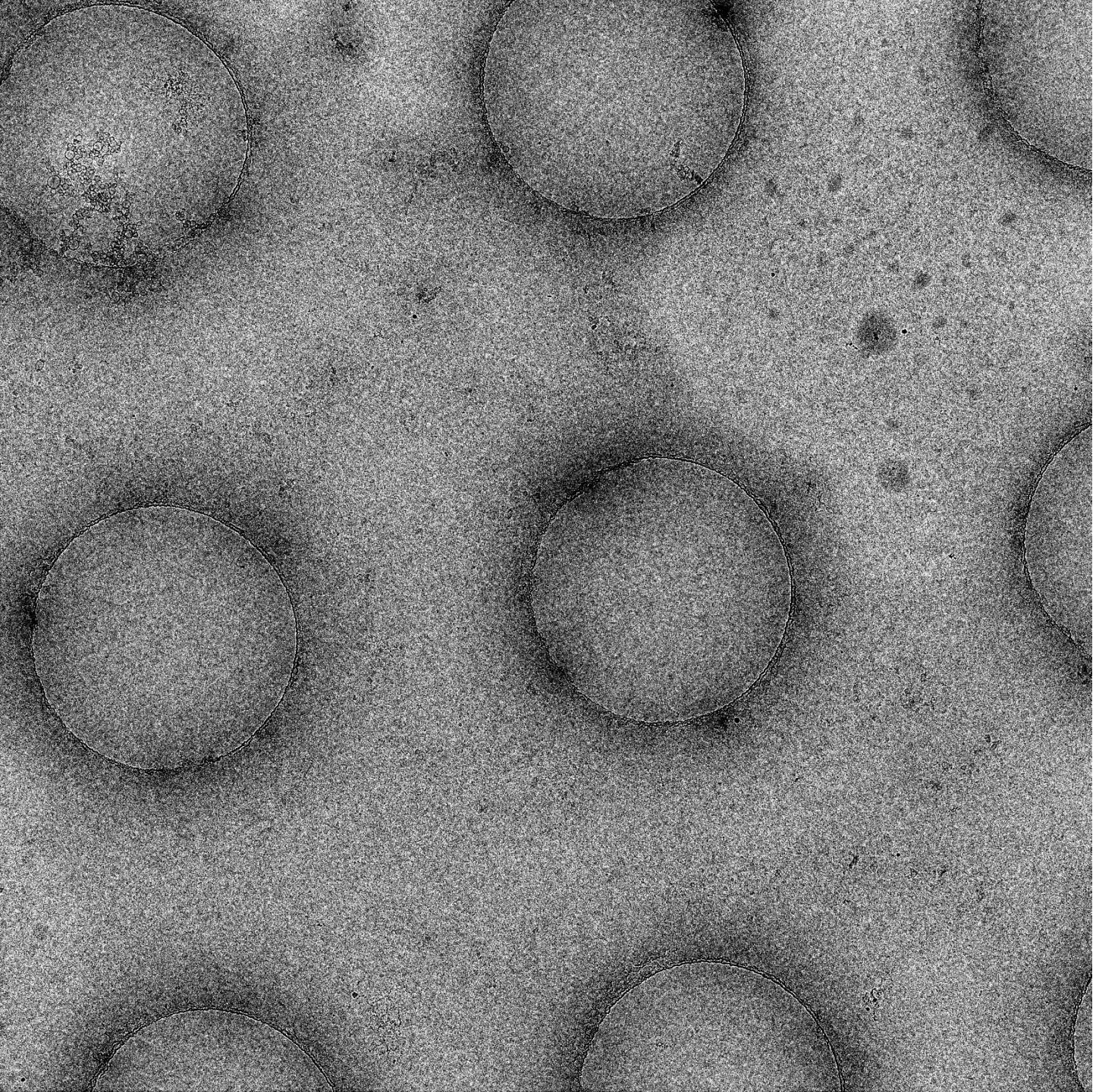

Supernatant from isolation of SCPs from spruce needle homogenate S2/21

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles.

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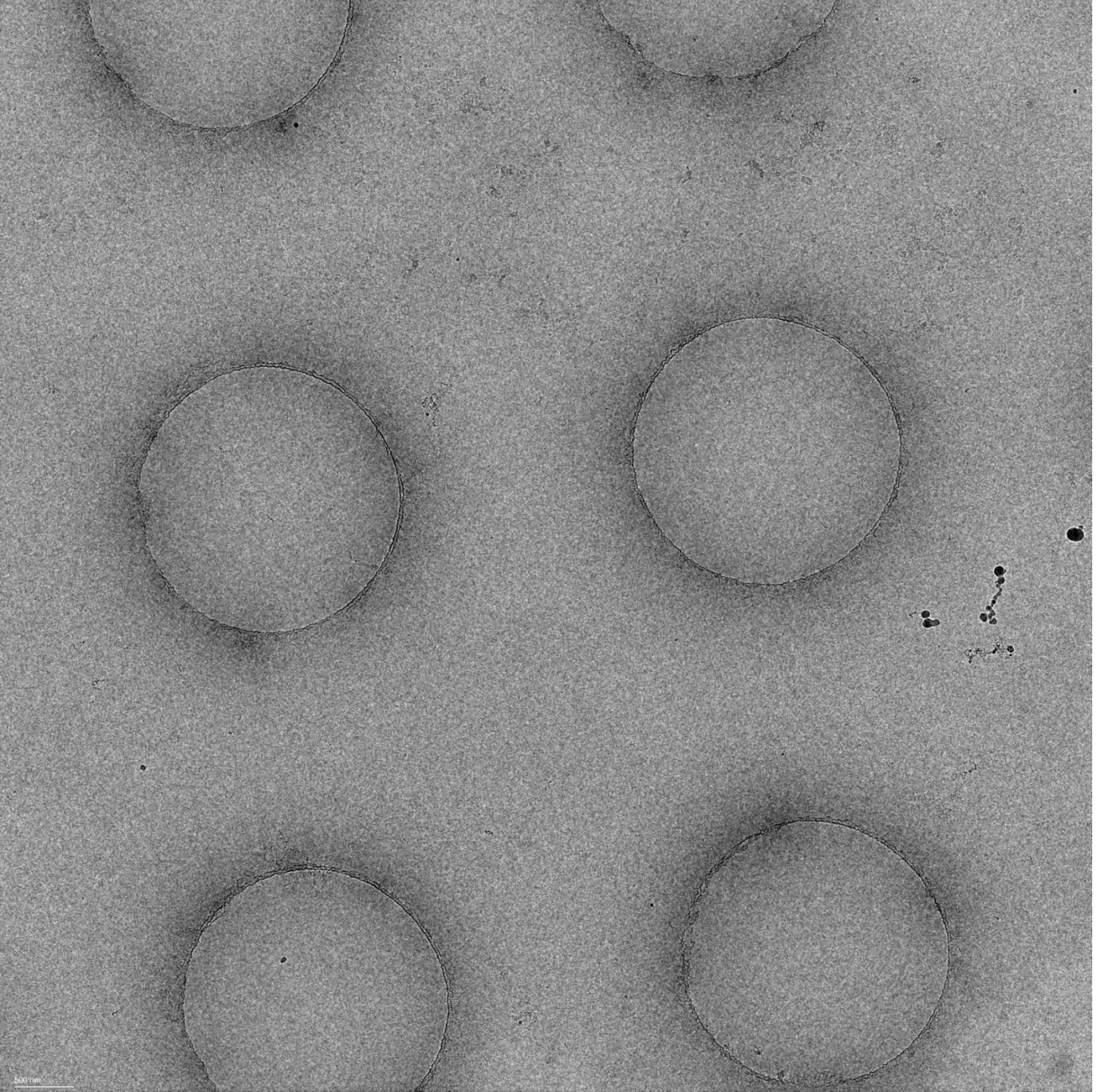
For cryogenic transmission electron microscopy, C-flat™ 2/2, 200 mesh holey carbon grids (Protochips, Morrisville, NC, USA) were glow discharged: 20 mA, 60 s, positive polarity, air atmosphere (GloQube ® Plus, Quor-um, Laughton, UK). 3 µL of sample was applied to the grid, blotted, and vitrified in liquid ethane on Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, USA). Vitrobot con-ditions were set to 100% relative humidity, 4 °C, blot force: 2 and blot time: 7 s. Samples were visualized under cryogenic conditions using a 200 kV Glacios microscope with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, USA).



Supernatant from isolation of SCPs from spruce needle homogenate S2/22

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles.

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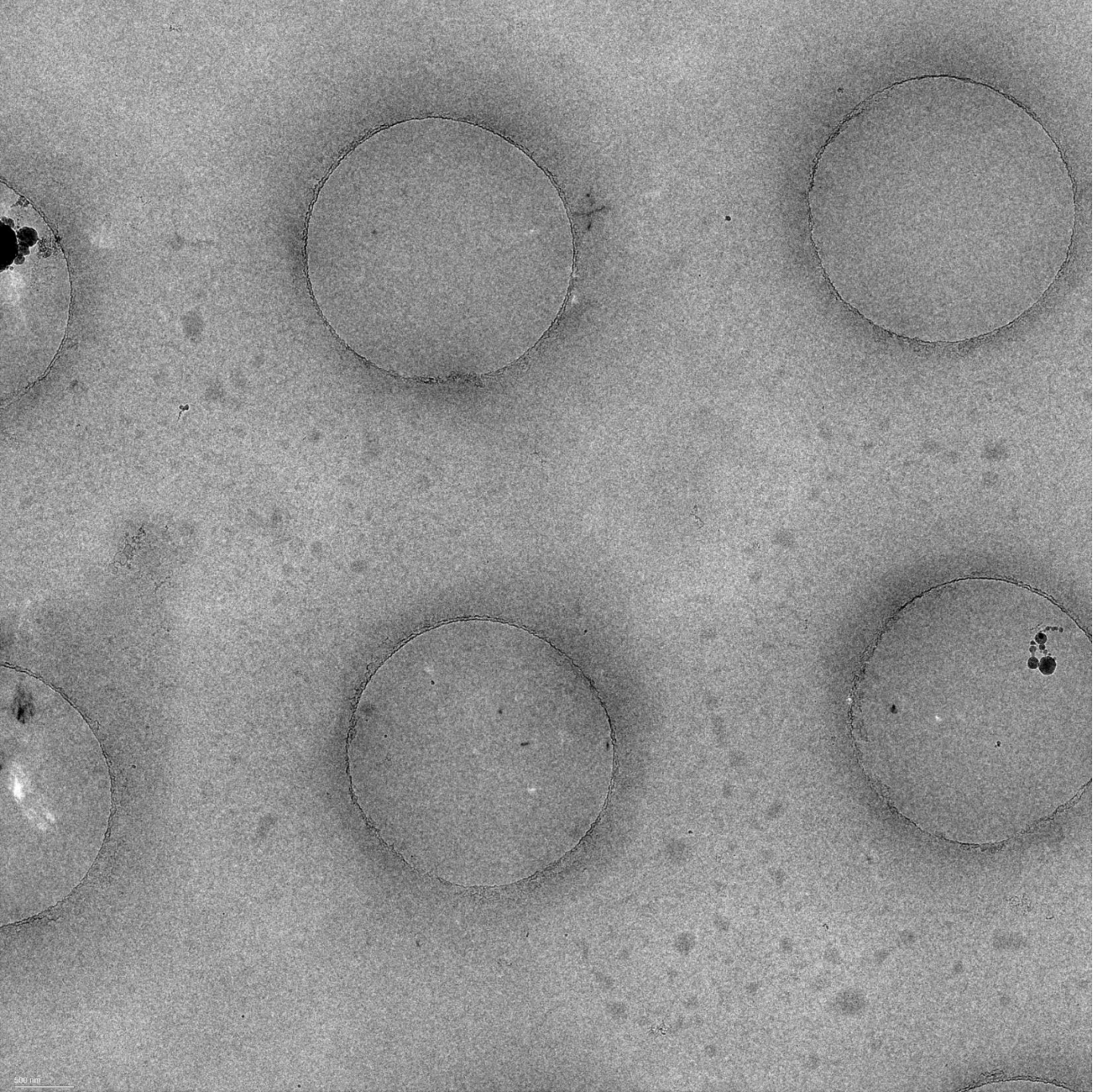
For cryogenic transmission electron microscopy, C-flat™ 2/2, 200 mesh holey carbon grids (Protochips, Morrisville, NC, USA) were glow discharged: 20 mA, 60 s, positive polarity, air atmosphere (GloQube ® Plus, Quor-um, Laughton, UK). 3 µL of sample was applied to the grid, blotted, and vitrified in liquid ethane on Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, USA). Vitrobot con-ditions were set to 100% relative humidity, 4 °C, blot force: 2 and blot time: 7 s. Samples were visualized under cryogenic conditions using a 200 kV Glacios microscope with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, USA).



Supernatant from isolation of SCPs from spruce needle homogenate S2/23

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles.

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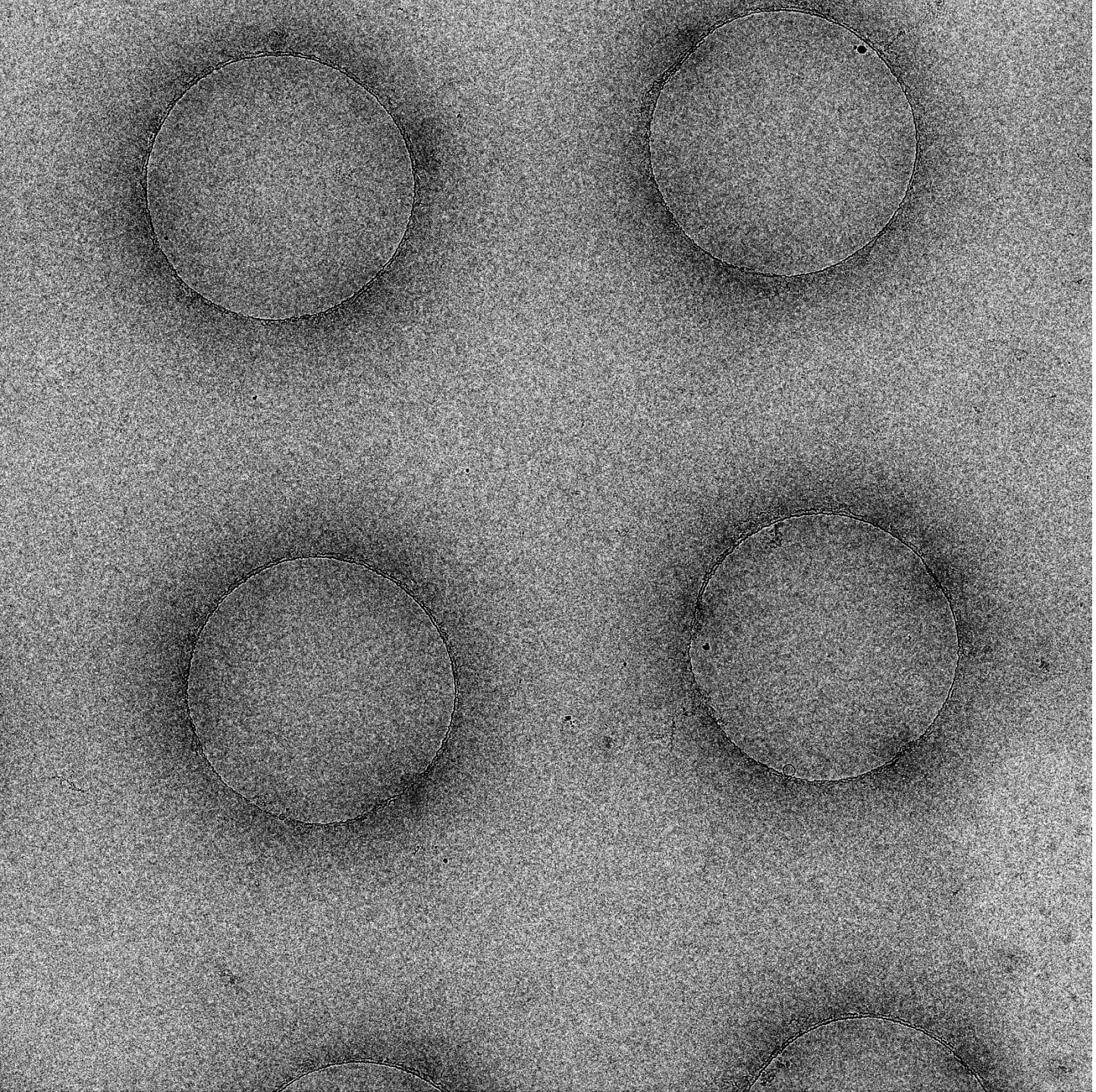

Supernatant from isolation of SCPs from spruce needle homogenate S2/24

Branches of spruce were cut from the tree and used immediately. 50 g of dry weight branches were immersed into water at about 30 °C with dissolved sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches with scissors. Homogenate was prepared from needles and 300 mL of ultraclean water (B Braun, Meisungen, Germany) by stirring for 30 seconds with the KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles.

SCPs were isolated by differential centrifugation as adapted from protocol for isolation of extracellular vesicles (EVs) (Mantile, F.; Kisovec, M.; Adamo, G.; Romancino, D.P.; Hočevar, M.; Božič, D.; Bedina Zavec, A.; Podobnik, M.; Stoppelli, M.P.; Kisslinger, A.; Bongiovanni, A.; Kralj-Iglič, V.; Liguori, G.L. A Novel Localization in Human Large Extracellular Vesicles for the EGF-CFC Founder Member CRIPTO and Its Biological and Therapeutic Implications. Cancers 2022, 14, 3700. https://doi.org/10.3390/cancers14153700). The homogenate was centrifuged twice at 300 g and 4°C for 10 minutes in the centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)) by using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany). The supernatant of the second centrifugation was centrifuged twice at 2000 g and 4°C for 10 minutes in the centrifuge Centric 400R with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Then, the supernatant was centrifuged at 50 000 g and 4°C, for 70 min in Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA) using 6 mL thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA). The supernatant consists of the fluid above about 50 µL of the pellet.

For cryogenic transmission electron microscopy, C-flat™ 2/2, 200 mesh holey carbon grids (Protochips, Morrisville, NC, USA) were glow discharged: 20 mA, 60 s, positive polarity, air atmosphere (GloQube ® Plus, Quor-um, Laughton, UK). 3 µL of sample was applied to the grid, blotted, and vitrified in liquid ethane on Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, USA). Vitrobot con-ditions were set to 100% relative humidity, 4 °C, blot force: 2 and blot time: 7 s. Samples were visualized under cryogenic conditions using a 200 kV Glacios microscope with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, USA).