1 **Materials**

2 1,5-diaminonaphthalene (DAN, 97%), 2,5-dihydroxybenzoic acid (DHB, 98.0%),
3 paraformaldehyde (powder, 95%), citrate buffer (pH 6.0, 10x) and octyl ß-D-
4 glucopyranoside (OBG) (50% [w/v] stock solution) were from Sigma Aldrich (St. Louis,
5 MO). Tween®-20, molecular biology grade was from Promega (Madison, WI). Antigen
6 Retrieval Reagent-Basic (CTS013), Antigen Retrieval Reagent-Universal (CTS015) and
7 Antigen Retrieval Reagent-Acidic (CTS015) were from R&D Systems/BioTechne
8 (Minneapolis, MN). DyLight 650 NHS ester and anti-streptavidin antibody clone S3E11
9 were from Thermo Fisher Scientific (Waltham, MA). Gold coated microscope slides were
10 from Angstrom Engineering, Inc. (Kitchener, ON, Canada). Mouse C57 brain sagittal
11 FFPE sections (5 µm thickness) and mouse C57 brain sagittal Fresh Frozen (FF) sections
12 (embedded in 2% w/v CMC, 10 µm thickness) were from Zyagen (San Diego, CA). Breast
13 cancer tissue FFPE blocks were from OriGene Technologies, Inc. (Rockville, MD) and
14 normal tonsil tissue FFPE blocks were from Amsbio LLC (Cambridge, MA), all were
15 sectioned (5 µm thickness) and mounted onto slides by Zyagen (San Diego, CA). Mouse
16 IgG whole molecule (015-000-003), normal mouse serum (015-222-001), rabbit IgG
17 whole molecule (011-000-003) and normal rabbit serum (011-000-001) were from
18 Jackson ImmunoResearch Laboratories, Inc. (West Grove, PA). Streptavidin-coated 20
19 µm PMMA beads (microspheres) were from PolyAn GmbH (Berlin, Germany). 0.5 mL
20 Ultrafree-MC centrifugal 0.45 µm filter devices were from Millipore Sigma (Burlington,
21 MA). PD SpinTrap G-25 columns were from GE Healthcare Life Sciences (Pittsburgh,
22 PA). MALDI-IHC and bead-array antibodies were obtained from various suppliers as
23 follows: human/mouse/rat anti-myelin basic protein (MAB42282) from R&D

24 Systems/BioTechne (Minneapolis, MN); anti-NeuN antibody, clone A60 (MAB377) and
25 anti-synapsin-2 antibody, clone 19.4 (MABN1573) from Millipore Sigma (Burlington, MA);
26 human/mouse anti-GLUT1 (PA146152) from Fisher Scientific (Hampton, NH); and anti-
27 MAP2 antibody [AP-20] (ab11268), recombinant anti-NeuN (ab209898; BSA and azide
28 free), recombinant anti-myelin basic protein (ab230378; BSA and azide free),
29 recombinant rabbit IgG, monoclonal [SP137] - isotype control (ab208334; BSA and azide
30 free), recombinant anti-pan cytokeratin antibody [C-11] (ab264485; BSA and azide free),
31 recombinant anti-CD3 epsilon antibody [CAL57] (ab251607; BSA and azide free),
32 recombinant anti-CD4 antibody [EPR6855] (ab181724; BSA and azide free), recombinant
33 anti-CD8 alpha antibody [CAL66] (ab251596; BSA and azide free), recombinant anti-
34 CD20 antibody [EP459Y] (ab214282; BSA and azide free), anti-CD45RO antibody [UCH-
35 L1] (ab23), recombinant anti-estrogen receptor alpha antibody [SP1] (ab187260; BSA
36 and azide free), recombinant anti-progesterone receptor antibody [YR85] (ab206926;
37 BSA and azide free), recombinant anti-ErbB 2 antibody [CAL27] (ab251602; BSA and
38 azide free), recombinant anti-histone H2A.X antibody [EPR22820-23] - ChIP (ab256544;
39 BSA and azide free), recombinant anti-CD68 antibody [EPR20545] (ab227458; BSA and
40 azide free), and recombinant anti-Ki67 antibody [EPR3610] (ab209897; BSA and azide
41 free) were from Abcam (Cambridge, MA). FlexWell™ 16-Chamber Self-Adhesive
42 Gaskets (204916) were from Grace Bio-Labs (Bend, Oregon).

43

44 ## Methods

45 **Handling of photocleavable reagents.** Photocleavable reagents were protected from
46 light during all long incubation steps (≥ 15 min) and during storage, but were otherwise
47 handled under ambient laboratory lighting.
48
49 **Probing streptavidin beads with PC-MT-antibodies and bead-array formation.**
50 Streptavidin-coated 20 μm PMMA beads were processed in 0.5 mL Ultrafree-MC
51 centrifugal 0.45 μm filter devices unless otherwise noted (washing was performed by 3 s
52 vortex mixing of bead suspensions in the filter devices and filtration was performed for 5
53 s at 15,000 rpm on a standard micro-centrifuge). For each PC-MT-antibody version,
54 10,000 beads were used and processed separately unless otherwise noted. Beads were
55 first washed 4x 400 μL with Bead Block Buffer (1% BSA [w/v] in TBS-T; note TBS-T is
56 TBS supplemented with 0.05% [v/v] Tween-20). Beads were then probed separately with
57 15 different versions of an anti-streptavidin PC-MT-antibody, each PC-MT-antibody
58 version carrying a different PC-MT species (*i.e.* different mass unit; see Supplementary
59 Table S1 for mass units and Supplementary Table S1.1 for stable isotopic amino acids
60 used in some mass units). PC-MT-antibodies were diluted to 1 μg/mL in Bead Block
61 Buffer and probing was performed using 100 μL for 1 hr with gentle mixing. Beads were
62 then washed 4x 400 μL with TBS-T followed by pooling all 15 different bead versions.
63 Pooled beads were then washed further 4x 400 μL with mass spectrometry grade water
64 (MS-Water). Formation of random bead-arrays on indium tin oxide (ITO) coated microwell
65 substrates having the footprint of a standard microscope slide was performed as

66 previously reported [1, 2] (note that after bead-array formation slides were dried 45 min
67 in a vacuum desiccation chamber).
68
69 **Multiplex mass spectrometry-based immunohistochemistry (MALDI-IHC).** For
70 deparaffinization and hydration, FFPE tissue sections were treated as follows (each
71 treatment step in separate staining jars): 3x with xylene for 5 min each; 1x with
72 xylene:ethanol (1:1) for 3 min; and then hydrate 2x with 100% ethanol for 2 min each, 1x
73 with 95% ethanol for 3 min each, 1x with 70% ethanol for 3 min, 1x with 50% ethanol for
74 3 min and 1x with TBS for 10 min. Antigen retrieval was achieved in 200 mL of 95°C 1X
75 citrate buffer (pH 6.0; see Materials) for 1 hr and followed by cooling in the same beaker
76 for 30 min at room temperature. Alternatively, acidic or basic antigen retrieval was
77 achieved for tonsil and breast cancer tissues in 60 mL of 95°C 1X Antigen Retrieval
78 Reagent-Acidic or 1X Antigen Retrieval Reagent-Basic (see Materials) for 30 min and
79 followed by cooling in the same Coplin staining jar for 30 min at room temperature. Slides
80 were then blocked in a staining jar for 1 hr with 50 mL Tissue Blocking Buffer (2% [v/v]
81 normal serum [rabbit and mouse] and 5% (w/v) BSA in TBS-T). For PC-MT-antibody
82 staining, slides were treated at 4°C for overnight with 200 µL/section of a solution
83 containing 2.5 µg/mL of each antibody for mouse brain and 0.5 µg/mL of each antibody
84 for human tonsil and breast cancer, diluted in Tissue Blocking Buffer (incubation was
85 performed protected from light, in a humidified chamber to avoid evaporation and with
86 each tissue section surrounded by hydrophobic barrier pen to retain the fluid). The slides
87 were next washed as follows: 3x 5 min each with TBS followed by 3x 2 min each with 50
88 mM ammonium bicarbonate (note, all solutions were in MS-Water and all washes were

89 performed using excess solution with the slides placed horizontally in a petri dish with
90 gentle shaking). Tissue slides were ultimately dried for 45 min in a vacuum desiccation
91 chamber.
92
93 **Immunofluorescence staining.** In some cases, immunofluorescence was performed
94 instead of staining with PC-MT-antibodies. In these cases, antibodies were labeled with
95 a 15-fold molar excess of a DyLight 650 NHS Ester reagent (added from a 5 mM stock in
96 DMF). Immunostaining with fluorescent antibodies was otherwise performed in the same
97 manner as with the PC-MT-antibodies. Fluorescence imaging of dried slides was
98 performed on a GenePix 4200A fluorescence scanner at 5 μm resolution (Molecular
99 Devices, San Jose, CA).
100
101 **References**

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