

Carroll, JA. Groveman, BR. Williams, K. Moore, R. Race, B. Haigh CL. **Prion protein N1 cleavage peptides stimulate microglial interaction with surrounding cells**

**Supplementary Data S4.** Individual data tables. The data values included in each graph within the main text are shown below.

**Figure 1b**

|         |    | Prestoblu ( % MNLC alone) |          |          |          |          |          |          |          |
|---------|----|---------------------------|----------|----------|----------|----------|----------|----------|----------|
|         |    | 24 h                      |          |          | 7 d      |          |          |          |          |
| Control | N1 | 122.791                   | 88.75334 | 94.20288 | 76.99891 | 63.06521 | 143.9    | 95.48732 | 65.88109 |
|         |    | 94.5551                   | 147.3266 | 278.2282 | 216.4537 | 225.4081 | 123.1417 | 136.42   | 197.7995 |

**Figure 1d**

|         |    | Area (%) |        |        |           |        |        |
|---------|----|----------|--------|--------|-----------|--------|--------|
|         |    | MNLC     |        |        | MNLC + MG |        |        |
| Control | N1 | 4.332    | 6.064  | 5.134  | 10.995    | 18.705 | 14.493 |
|         |    | 15.615   | 23.009 | 22.134 | 27.109    | 34.408 | 46.38  |

**Figure 1f**

|         |    | Non-ramified (%) |          |          | Ramified (%) |          |          |
|---------|----|------------------|----------|----------|--------------|----------|----------|
|         |    | 100              | 92.85714 | 84.21053 | 0            | 7.142857 | 15.78947 |
| Control | N1 | 47.61905         | 61.2069  | 28.57143 | 52.38095     | 38.79311 | 71.42857 |
|         |    |                  |          |          |              |          |          |

**Figure 1g**

|         |    | % Microglia     |    |       |              |    |       |
|---------|----|-----------------|----|-------|--------------|----|-------|
|         |    | MNLC associated |    |       | Unassociated |    |       |
| Control | N1 | 43.75           | 50 | 53.85 | 56.25        | 50 | 46.15 |
|         |    | 80              | 80 | 63.63 | 20           | 20 | 36.37 |

**Figure 2a**

|                          |  | Day      |          |          |  |  |  |
|--------------------------|--|----------|----------|----------|--|--|--|
|                          |  | 1        | 3        | 7        |  |  |  |
| Prestoblu (% control MG) |  | 122.7917 | 102.9982 | 71.21552 |  |  |  |
|                          |  | 110.4369 | 105.0112 | 100.343  |  |  |  |
|                          |  | 111.8537 | 99.9177  | 98.87364 |  |  |  |

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**Figure 2c**

| % Cells with beads |          |          |          |
|--------------------|----------|----------|----------|
| Control            | N1       | N2       | T-a23    |
| 41.83168           | 53.76045 | 53.29815 | 43.44609 |
| 57.28155           | 50.87719 | 50.18916 | 33.05439 |
| 62.42038           | 60.60606 | 66.15385 | 46.21212 |

**Figure 3a**

|           | Ccl4 Concentration (pg/ml) |         |         |         |         |         |         |         |
|-----------|----------------------------|---------|---------|---------|---------|---------|---------|---------|
|           | Control                    |         |         |         | N1      |         |         |         |
| MG        | 408.11                     | 103.93  | 826.02  | 731.7   | 316.75  | 64.81   | 1077.21 | 1070.68 |
| MNLC      | 23.71                      | 82.46   | 77.87   | 87.05   | 33.39   | 33.39   | 44.79   | 54.5    |
| MNLC + MG | 1002.29                    | 1559.46 | 1201.91 | 1919.54 | 1480.65 | 1701.41 | 1476.66 | 2375.07 |

**Figure 3b**

|           | Ccl24 Concentration (pg/ml) |        |       |        |        |         |        |        |
|-----------|-----------------------------|--------|-------|--------|--------|---------|--------|--------|
|           | Control                     |        |       |        | N1     |         |        |        |
| MG        | 131.01                      | 269.14 | 145.6 | 174.86 | 733.72 | 490.015 | 184.37 | 193.78 |
| MNLC      | 38.59                       | 38.59  | 18.36 | 82.32  | 440.84 | 215.405 | 46.38  | 82.32  |
| MNLC + MG | 329.48                      | 38.59  | 98.93 | 125.34 | 640.97 | 537.14  | 93.47  | 120.17 |

**Figure 3c**

|           | Ccl27 Concentration (pg/ml) |        |        |         |         |         |         |        |
|-----------|-----------------------------|--------|--------|---------|---------|---------|---------|--------|
|           | Control                     |        |        |         | N1      |         |         |        |
| MG        | 900.84                      | 635.16 | 771.63 | 622.145 | 1024.43 | 1054.59 | 368.99  | 494.98 |
| MNLC      | 407.85                      | 410.07 | 354.77 | 458.71  | 130.64  | 67.11   | 268.88  | 327.07 |
| MNLC + MG | 1024.43                     | 526.45 | 644.82 | 577.65  | 704.44  | 963.26  | 605.965 | 531.25 |

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**Figure 3d**

|           | Cxcl10 Concentration (pg/ml) |        |        |        |        |         |        |         |
|-----------|------------------------------|--------|--------|--------|--------|---------|--------|---------|
|           | Control                      |        |        |        | N1     |         |        |         |
| MG        | 454.81                       | 377.39 | 227.6  | 227.6  | 368.42 | 292.92  | 189.45 | 198.09  |
| MNLC      | 230.07                       | 292.92 | 322.11 | 159.99 | 287.93 | 207.27  | 272.72 | 129.075 |
| MNLC + MG | 471.34                       | 519.76 | 316.76 | 281.16 | 547.31 | 1059.95 | 938.63 | 783.03  |

**Figure 4b**

| Cxcl10<br>Concentration (ng/ml) | % area covered |        |        |        |
|---------------------------------|----------------|--------|--------|--------|
|                                 | 1              | 2      | 3      | 4      |
| 0                               | 12.309         | 5.538  | 6.107  | 7.865  |
| 1                               | 16.276         | 17.456 | 14.559 | 16.29  |
| 2.5                             | 30.537         | 21.028 | 20.135 | 29.891 |
| 5                               | 32.868         | 37.779 | 36.385 | 28.116 |
| 10                              | 32.021         | 5.609  | 11.732 | 14.192 |
| 25                              | 15.459         | 7.748  | 11.278 | 9.296  |
| 50                              | 3.579          | 12.493 | 12.098 | 8.635  |

**Figure 4c**

| Cxcl10 Concentration<br>(ng/ml) | Total pixel intensity |          |          | GFAP     |          |          |
|---------------------------------|-----------------------|----------|----------|----------|----------|----------|
|                                 | NF-L                  |          |          | GFAP     |          |          |
| 0                               | 2.61E+09              | 2.49E+09 | 3.15E+09 | 1.33E+09 | 1.44E+09 | 3.13E+09 |
| 1                               | 3.21E+09              | 2.58E+09 | 4.76E+09 | 2.06E+09 | 5.19E+09 | 2.68E+09 |
| 2.5                             | 3.61E+09              | 3.12E+09 | 3.43E+09 | 3.14E+09 | 2.18E+09 | 3.41E+09 |
| 5                               | 4.11E+09              | 5.29E+09 | 4.56E+09 | 5.13E+09 | 4.95E+09 | 4.61E+09 |
| 10                              | 1.6E+09               | 3.39E+09 | 5.14E+09 | 2.76E+09 | 1.7E+09  | 4.3E+09  |
| 25                              | 2.8E+09               | 2.73E+09 | 1.95E+09 | 1.85E+09 | 2.07E+09 | 1.49E+09 |
| 50                              | 3.67E+09              | 3.06E+09 | 1.38E+09 | 1.63E+09 | 5.75E+08 | 1.45E+09 |

**Figure 4d**

| Cxcl10 Concentration<br>(ng/ml) | Prestoblu metabolism (% control) |          |          |          |
|---------------------------------|----------------------------------|----------|----------|----------|
|                                 | 0                                | 100      | 100      | 100      |
| 1                               | 119.3377                         | 123.8034 | 112.6949 | 89.7509  |
| 2.5                             | 200.5704                         | 216.0687 | 223.6279 | 133.76   |
| 5                               | 406.8875                         | 392.0374 | 382.863  | 178.2631 |
| 10                              | 73.0861                          | 74.6608  | 89.7742  | 136.9548 |
| 25                              | 100.5637                         | 106.5888 | 125.0236 | 144.6124 |
| 50                              | 90.9269                          | 80.8373  | 8.4503   | 118.5219 |

**Figure 4f**

| Control |          | Prestoblu metabolism (% control)<br>Cxcr3 neutralising antibody |          |
|---------|----------|---|----------|
| Control | N1       | Control   | N1       |
| 100     | 132.9394 | 84.30282  | 87.58554 |
| 100     | 157.3753 | 78.05907  | 25.03516 |
| 100     | 151.4279 | 80.25446  | 106.6484 |

**Figure 5c**

|             | Prestoblu metabolism (% control) |          |          | MNLC     |
|-------------|----------------------------------|----------|----------|----------|
|             | MG                               |          |          |          |
| Co-exposed  | 111.1959                         | 79.60322 | 99.26368 | 128.6904 |
| MNLC primed | 86.0667                          | 61.36907 | 111.7076 | 86.55151 |

**Figure 5d**

| Cxcl10 (% PBS control) |                |                 |
|------------------------|----------------|-----------------|
| Co-culture             | Co-exposed (A) | MNLC primed (B) |
| 233.6431               | 53.35285       | 113.9806        |
| 206.9007               | 40.68697       | 156.9126        |
| 172.6021               | 101.16         | 98.39599        |
| 131.2212               | 107.2243       | 103.3215        |

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**Figure 6b**

|         | DCF production (FU/s) |      |      | MNLC |      |      | MNLC + MG |      |      |
|---------|-----------------------|------|------|------|------|------|-----------|------|------|
|         | MG                    |      |      | MNLC |      |      | MNLC + MG |      |      |
| Control | 0.14                  | 0.1  | 0.14 | 0.26 | 0.23 | 0.21 | 0.16      | 0.16 | 0.19 |
| N1      | 0.11                  | 0.13 | 0.11 | 0.21 | 0.14 | 0.24 | 0.14      | 0.14 | 0.16 |

**Figure 6d**

| Whole cell mean pixel intensity (FU) |          |
|--------------------------------------|----------|
| PBS                                  | N1       |
| 2870.027                             | 4415.197 |
| 3316.713                             | 4571.388 |
| 3309.222                             | 5433.314 |

**Figure 6e**

| MG-cell contact site mean pixel intensity (FU) |          |
|--|----------|
| PBS  | N1       |
| 3115.159                                       | 5102.973 |
| 3329.5   | 4205.074 |
| 1957.29  | 3827.803 |

**Figure 6f (\*upper limit of assay detection reached)**

| Ganglioside Induced Cxcl10 (% vehicle control) |          |           |
|--|----------|-----------|
| MG   | MNLC     | MNLC + MG |
| 80.77633                                       | 116.1092 | 233.1985  |
| 78.43948                                       | 99.43803 | 153.4229  |
| 87.67496                                       | 70.9541  | 343.5109* |

**Figure 6g**

|           | Cxcl10 (%PBS control) |          |          | Acyl-N1  |          |          |
|-----------|-----------------------|----------|----------|----------|----------|----------|
|           | N1                    |          |          |          |          |          |
| MG        | 114.4695              | 58.86282 | 58.86282 | 105.3723 | 81.55118 | 89.18653 |
| MNLC      | 117.0392              | 85.26669 | 88.95097 | 66.82848 | 133.0564 | 121.1944 |
| MNLC + MG | 206.9007              | 172.6021 | 161.8357 | 88.99655 | 94.14203 | 23.37041 |

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**Figure 6h**

| Prestoblu metabolism (% control) |              |
|----------------------------------|--------------|
| N1                               | N1-sulfo-NHS |
| 197.7995                         | 143.2817     |
| 174.2749                         | 88.6153      |
| 153.5562                         | 132.3992     |

**Figure 6i**

| N2 Induced Cxcl10 (% control) |          |           |
|-------------------------------|----------|-----------|
| MG                            | MNLC     | MNLC + MG |
| 151.5178                      | 191.6049 | 112.2653  |
| 132.7264                      | 88.89388 | 72.50525  |
| 146.4202                      | 90.77234 | 124.5249  |

**Figure 6j**

| N2 Prestoblu metabolism (% control) |          |           |
|-------------------------------------|----------|-----------|
| MG                                  | MNLC     | MNLC + MG |
| 149.7831                            | 90.12155 | 127.9611  |
| 159.1088                            | 95.90614 | 97.0698   |
| 119.2196                            | 99.63388 | 104.2989  |