# Supporting Information

# IL-34 cell surface localization regulated by the molecular chaperone 78 kDa glucose-regulated protein facilitates the differentiation of monocytic cells

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Supporting information consists of Figure S1-6 and Table S1.

A

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FL-Y         56221 CCTGCCTTTCCCCTCAGTACTTGTCAGGCTTGATGATGGATGGTGGTTGATTCAGCTACG 56280
IL-34 KO#1         CCTGCCTTTCCCCTCAGTACTTGTCAGGCTTGATG-------------------------
IL-34 KO#2         CCTGCCTTTCCCCTCAGTACTTGTCAGGCTTGATGATGGATGGTGGTTGATTCAGCTACG


FL-Y         56281 CACACCAGGTAGTGCGCAGACACAGTGCCCAGCGCTCATCCGCTCTTGTTGGGTGTGTCC 56340
IL-34 KO#1         ------------------------------------------------------------
IL-34 KO#2         CACACCAGGTAGTGCGCAGACACAGTGCCCAGCGCTCATCCGCTCTTGTTGGGTGTGTCC


FL-Y         56341 CACAGGTCTTGGGATCCTACTTGACGTGGCTTTGGGAAACGAGAATTTGGAGATATGGAC 56400
IL-34 KO#1         ------------------------------------------------------------
IL-34 KO#2         CAC---------------------------------------------------------

                           Exon 3
FL-Y         56401 TCTGACCCAAGATAAGGAGTGTGACCTTACAGGCTACCTTCGGGGCAAGCTGCAGTACAA 56460
IL-34 KO#1         ----------------------------------------------------CAGTACAA
IL-34 KO#2         TCTGACCCAAGATAAGGAGTGTGACCTTACAGGCTACCTTCGGGGCAAGCTGCAGTACAA


FL-Y         56461 GAACCGGCTTCAGTACATGGTAACCTGGAGAGCGTCTGCCTCCCCGTGCTAGGGTTGCAA 56520
IL-34 KO#1         GAACCGGCTTCAGTACATGGTAACCTGGAGAGCGTCTGCCTCCCCGTGCTAGGGTTGCAA
IL-34 KO#2         GAACCGGCTTCAGTACATGGTAACCTGGAGAGCGTCTGCCTCCCCGTGCTAGGGTTGCAA
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B

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              1 M  P  W  G  L  A  W  L  Y  C  L  G  I  L  L  D  V  A  L  G  20
FL-Y        584 ATGCCCTGGGGACTCGCCTGGCTATACTGTCTTGGGATCCTACTTGACGTGGCTTTGGGA
IL-34 KO        ATGCCCTGGGGACTCGCCTGGCTATACTAAACATTACTTCCCCATCAACTACAGGATTGC
#1#2#3        1 M  P  W  G  L  A  W  L  Y  *  10


             21 N  E  N        P  G  S  P  S  S  S  H  G  S  L  P  *  235
FL-Y        644 AACGAGAAT  //  CCCGGATCCCCAAGCTCAAGCCATGGCTCGTTGCCCTGA 1291
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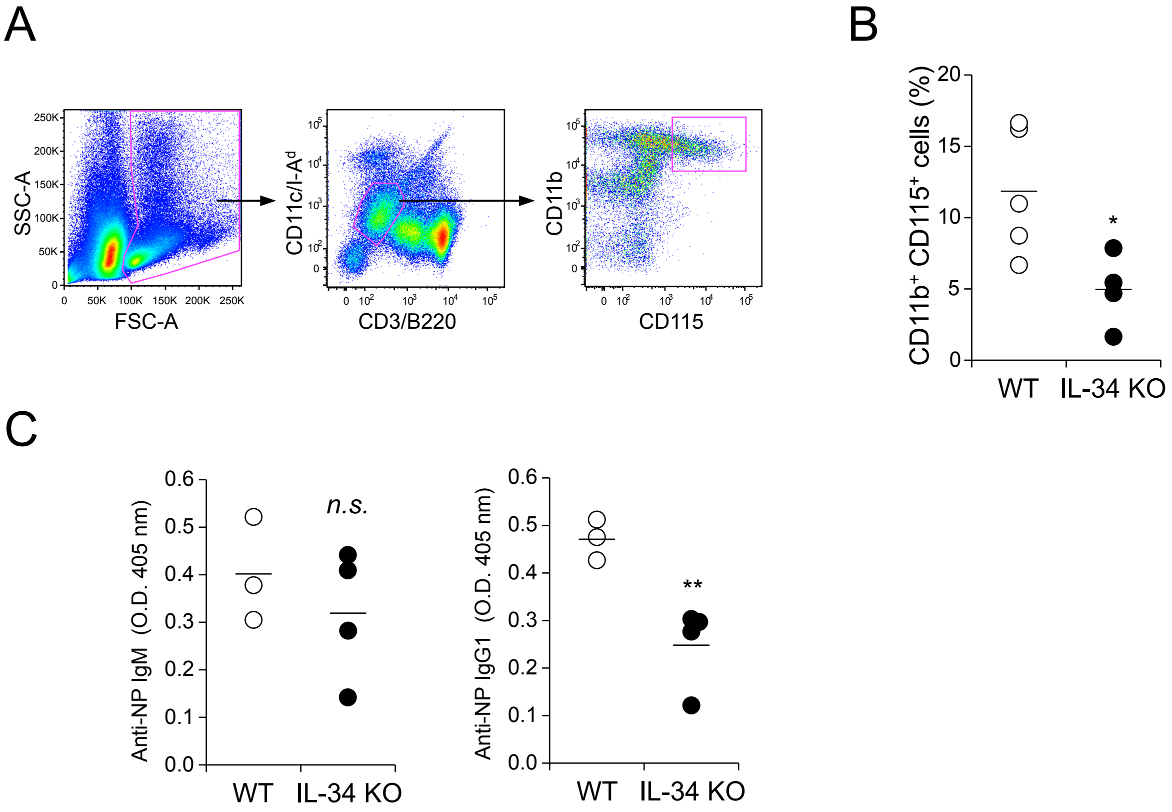
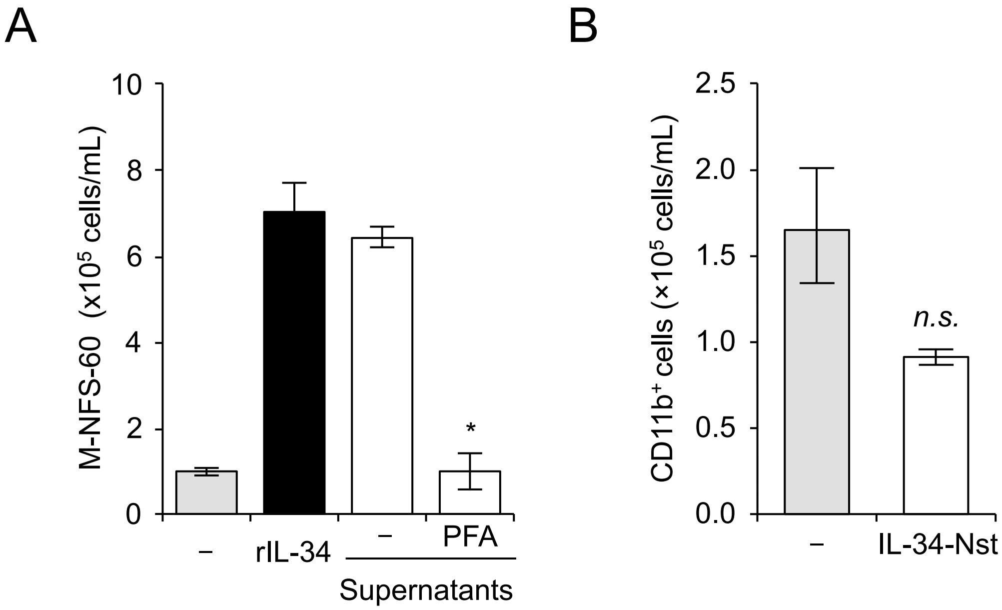
**Supplemental Figure S1. Generation of IL-34 KO FL-Y**

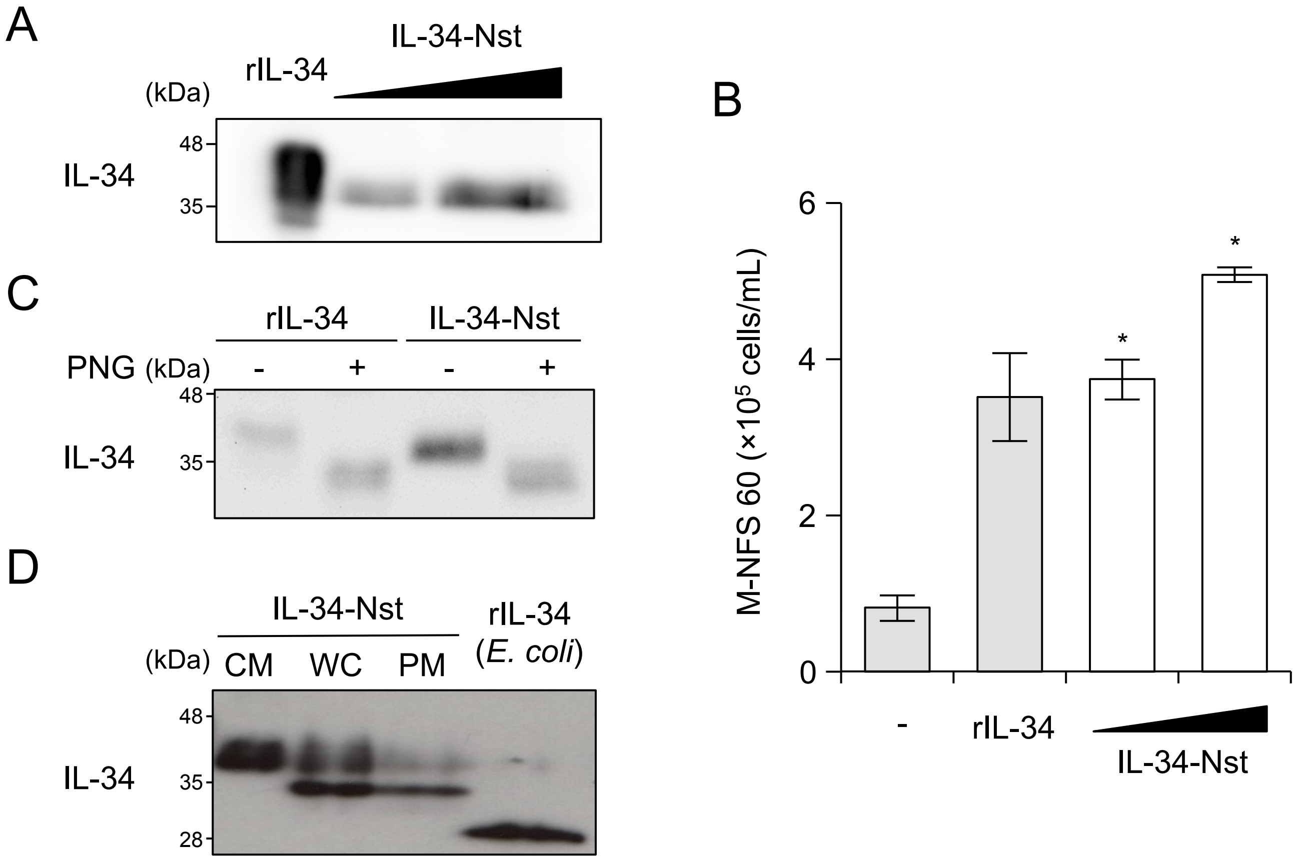
(A) Genomic DNA extracted from IL-34 KO FL-Y (#1 and #2) clones was subjected to PCR amplification of the *Il34* gene. DNA Sequence of the *Il34* gene in IL-34 KO FL-Y cells was compared with that in the original FL-Y line. (B) Sequence of *Il34* mRNA extracted from IL-34 KO FL-Y cells as shown in Fig. 1*C* (top panel).



**Supplemental Figure S2. Antibody responses are significantly suppressed in IL-34 KO mice.**
(A, B) Reduced number of FDMC-like cells in the spleen of IL-34 KO mice after immunization. Wild-type (WT) and IL-34 KO mice were immunized with 50 µg of 4-hydroxy-3-nitrophenylacetyl-conjugated chicken γ globulin (NP-CGG) plus 2 mg alum intraperitoneally. After 12 days, splenocytes were prepared from immunized mice and used for analyses of FDMC-like cells. IL-34 KO mice were kindly provided by Prof. Burkhard Becher at the University of Zürich. (A) T cell and adherent cell-depleted splenocytes were treated with 10 µg/mL rat IgG at 4 °C for 15 min, and subsequently stained with fluorescence-labeled antibodies at 4 °C for 30 min. The following antibodies were used: APC-anti-CD11b, PE-anti-CD115, Pacific blue-anti-B220, Pacific blue-anti-CD3, FITC-anti-I-A$^d$, and FITC-anti-CD11c. The frequency of FDMC-like cells was estimated by measuring the percentage of CD11b$^+$CD115$^+$ cells in a B220$^-$CD3$^-$I-A$^{d-}$CD11c$^-$-gated population using a FACSAria flow cytometer. (B) The frequency of FDMC-like cells was estimated by detecting CD11b $^+$CD115$^+$ cells in CD3$^-$B220$^-$I-A$^{d-}$CD11c$^-$-gated population in the spleen cells from each mouse. Statistical differences are marked: *, $p < 0.05$ versus WT mice. (C) Antigen-specific antibody responses in IL-34 KO mice after immunization. WT (open circles) and IL-34 KO (closed circles) mice were immunized with 5 µg of NP-CGG plus 2 mg alum intraperitoneally, and serum was collected on day 12 after immunization. Anti-NP IgG1 Ab levels in sera were assayed by ELISA using microplates coated with NP$_{25}$-BSA. Anti-NP IgM and IgG1 Abs bound to the plates were detected with peroxidase-conjugated goat anti-mouse IgM (Invitrogen) or peroxidase-conjugated goat anti-mouse IgG1 Ab (SouthernBiotech, Birmingham, AL, USA), respectively. Statistical differences are marked: *n.s.*, not significant, **, $p < 0.01$ versus WT mice.

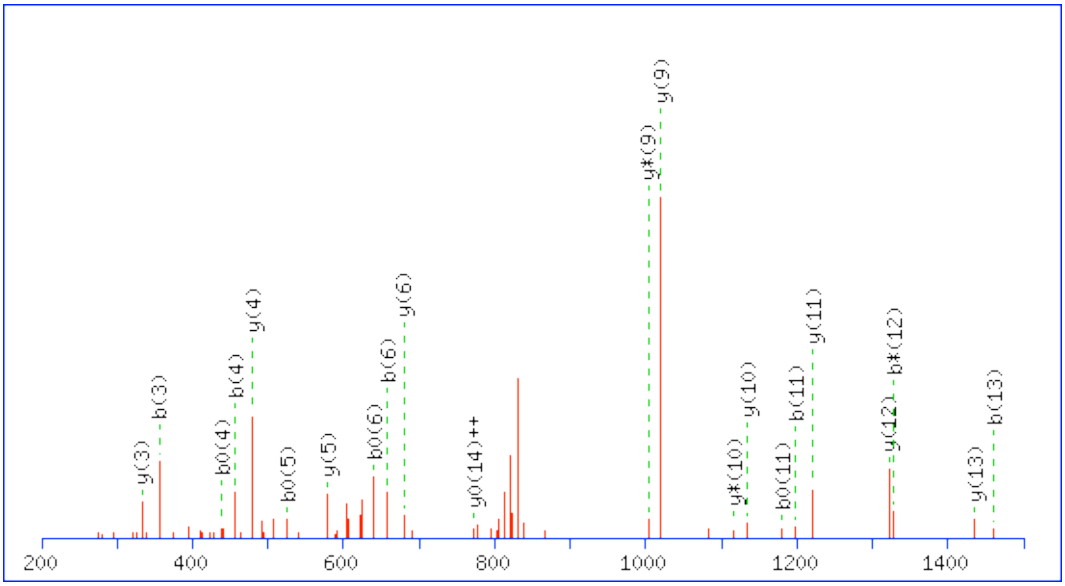


**Supplemental Figure S3. Soluble IL-34 secreted from FL-Y is not involved in FDMC differentiation.** (A) M-NFS-60 ($1 \times 10^4$ cells/mL) were cultured the medium containing none (−), 10 ng/mL recombinant mouse IL-34 (rIL-34) (BioLegend), or 5% of culture supernatants of FL-Y cells. Culture supernatants were collected after 3-day culture of FL-Y cells that were untreated (−) or treated (PFA) with 0.1% paraformaldehyde (PFA). After 3 days, number of M-NFS-60 cells was determined by hemocytometer. Data are presented as the means ± S.D. of triplicate cultures. Statistical differences are marked: *, $p < 0.05$ versus the culture in the presence of supernatants from PFA-untreated FL-Y cells. (B) The supernatants (20 mL) collected from FL-Y-IL-34-Nst culture were reacted with 60 µL of Strep-Tactin sepharose (IBA) overnight at 4 °C. Then, the Strep-Tactin sepharose was centrifuged at 390 × *g* at 4 ºC for 2 min and washed five times with 1 mL of NP buffer (50 mM $Na_2PO_4$/300 mM NaCl pH 8.0). Finally, IL-34-Nst protein bound to sepharose was eluted with elution buffer (MEM contaning 25 mM desthiobiotin). Purified IL-34-Nst was added to medium of FDMC-inducing culture every 3 days. After 8 days, cultured cells were analyzed by flow cytometry after staining with an anti-CD11b mAb as shown in Figs. 1*D* and *E*. Data are presented as the means ± S.D. of triplicate cultures. Statistical differences are marked: *n.s.*, not significant.



**Supplemental Figure S4. IL-34-Nst is capable of stimulating CSF-1R.**
(A) IL-34-Nst was purified from FL-Y-IL-34-Nst cell-cultured medium (20 mL) by using a Strep-Tactin sepharose according to the manufacturer's instruction. Briefly, the culture medium was reacted with the Strep-Tactin sepharose resin overnight, and the resin was washed with NP buffer. IL-34-Nst bound to the resin was eluted with the elution buffer (MEM containing 25 mM desthiobiotin). The eluate was separated by SDS-PAGE and subjected to western blotting with an anti-IL-34 Ab. (B) M-NFS-60 ($1 \times 10^4$ cells/mL) were cultured in the medium containing none (−), recombinant IL-34 (rIL-34: 10 ng/mL), or purified IL-34-Nst. After 3 days, the number of M-NFS-60 cells was determined by hemocytometer. Data are represented as the means ± S.D. of triplicate cultures. (C) rIL-34 and purified IL-34-Nst were untreated (−) or treated (+) with PNGase (N-Zyme Scientifics) according to the manufacturer's instruction. Samples were separated by SDS-PAGE and subjected to western blotting with an anti-IL-34 Ab. (D) IL-34-Nst proteins prepared from the culture medium (CM), whole cell lysate (WC), and plasma membrane fraction (PM) of FL-Y-IL-34-Nst, and recombinant IL-34 expressed in *E. coli* were separated by SDS-PAGE and subjected to western blotting using an anti-IL-34 Ab. Data are presented as the means ± S.D. of triplicate cultures. Statistical differences are marked: *, $p < 0.05$ versus unstimulated culture (−).

GRP78: 83 NQLTSNPENTVFDAK 97

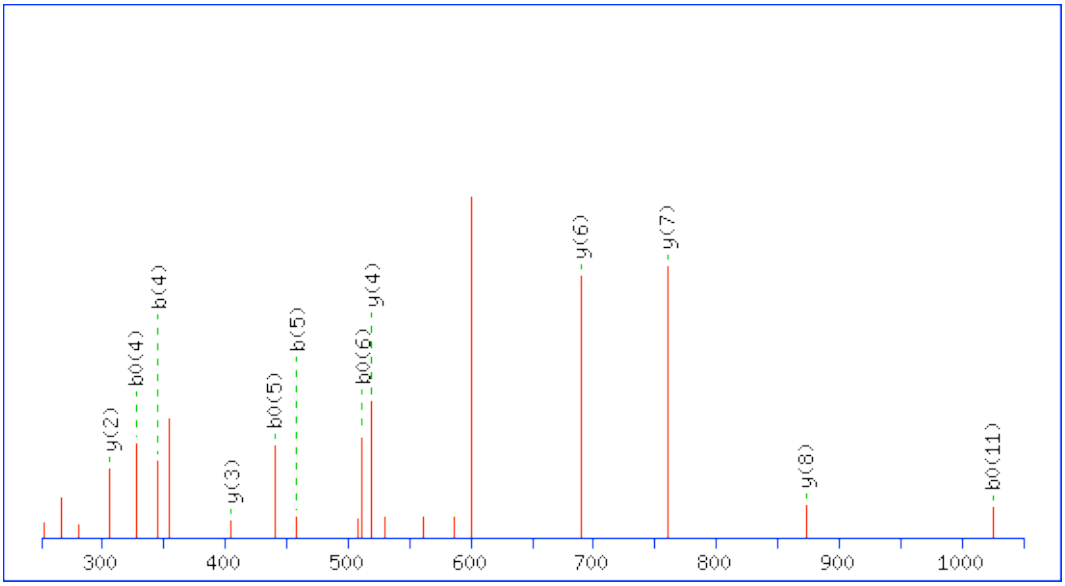


**Monoisotopic mass of neutral peptide Mr(calc):** 1676.8006
**Ions Score:** 74 **Expect:** 1.7e-005
**Matches :** 22/158 fragment ions using 30 most intense peaks

| # | b | $b^{++}$ | $b^{*}$ | $b^{*++}$ | $b^{0}$ | $b^{0++}$ | Seq. | y | $y^{++}$ | $y^{*}$ | $y^{*++}$ | $y^{0}$ | $y^{0++}$ | # |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 115.0502 | 58.0287 | 98.0237 | 49.5155 | | | N | | | | | | | 15 |
| 2 | 243.1088 | 122.0580 | 226.0822 | 113.5448 | | | Q | 1563.7649 | 782.3861 | 1546.7384 | 773.8728 | 1545.7544 | 773.3808 | 14 |
| 3 | **356.1928** | 178.6001 | 339.1663 | 170.0868 | | | L | ***1435.7064*** | 718.3568 | 1418.6798 | 709.8435 | 1417.6958 | 709.3515 | 13 |
| 4 | **457.2405** | 229.1239 | 440.2140 | 220.6106 | 439.2300 | 220.1186 | T | ***1322.6223*** | 661.8148 | 1305.5957 | 653.3015 | 1304.6117 | 652.8095 | 12 |
| 5 | 544.2726 | 272.6399 | 527.2460 | 264.1266 | 526.2620 | 263.6346 | S | ***1221.5746*** | 611.2909 | 1204.5481 | 602.7777 | 1203.5640 | 602.2857 | 11 |
| 6 | **658.3155** | 329.6614 | 641.2889 | 321.1481 | 640.3049 | 320.6561 | N | ***1134.5426*** | 567.7749 | 1117.5160 | 559.2617 | 1116.5320 | 558.7696 | 10 |
| 7 | 755.3682 | 378.1878 | 738.3417 | 369.6745 | 737.3577 | 369.1825 | P | ***1020.4997*** | 510.7535 | 1003.4731 | 502.2402 | 1002.4891 | 501.7482 | 9 |
| 8 | 884.4108 | 442.7091 | 867.3843 | 434.1958 | 866.4003 | 433.7038 | E | 923.4469 | 462.2271 | 906.4203 | 453.7138 | 905.4363 | 453.2218 | 8 |
| 9 | 998.4538 | 499.7305 | 981.4272 | 491.2172 | 980.4432 | 490.7252 | N | 794.4043 | 397.7058 | 777.3777 | 389.1925 | 776.3937 | 388.7005 | 7 |
| 10 | 1099.5014 | 550.2544 | 1082.4749 | 541.7411 | 1081.4909 | 541.2491 | T | ***680.3614*** | 340.6843 | 663.3348 | 332.1710 | 662.3508 | 331.6790 | 6 |
| 11 | **1198.5699** | 599.7886 | 1181.5433 | 591.2753 | 1180.5593 | 590.7833 | V | ***579.3137*** | 290.1605 | 562.2871 | 281.6472 | 561.3031 | 281.1552 | 5 |
| 12 | 1345.6383 | 673.3228 | 1328.6117 | 664.8095 | 1327.6277 | 664.3175 | F | ***480.2453*** | 240.6263 | 463.2187 | 232.1130 | 462.2347 | 231.6210 | 4 |
| 13 | **1460.6652** | 730.8362 | 1443.6387 | 722.3230 | 1442.6546 | 721.8310 | D | ***333.1769*** | 167.0921 | 316.1503 | 158.5788 | 315.1663 | 158.0868 | 3 |
| 14 | 1531.7023 | 766.3548 | 1514.6758 | 757.8415 | 1513.6918 | 757.3495 | A | 218.1499 | 109.5786 | 201.1234 | 101.0653 | | | 2 |
| 15 | | | | | | | K | 147.1128 | 74.0600 | 130.0863 | 65.5468 | | | 1 |

**Supplemental Figure S5. LC-MS/MS analysis for identification of GRP78 as an IL-34-binding molecule**
IL-34-Nst proteins in the plasma membrane fraction prepared from FL-Y-IL-34-Nst (IL-34-Nst) cells were pull-downed by using Strep-Tactin sepharose and eluted by 2.5 mM desthiobiotin. Eluate was separated by SDS-PAGE and subjected to mass spectrometry analysis. All of the identified GRP78 peptides are listed in Table 1. Data show the profile of identified peptides whose ions score was ≥ 50.

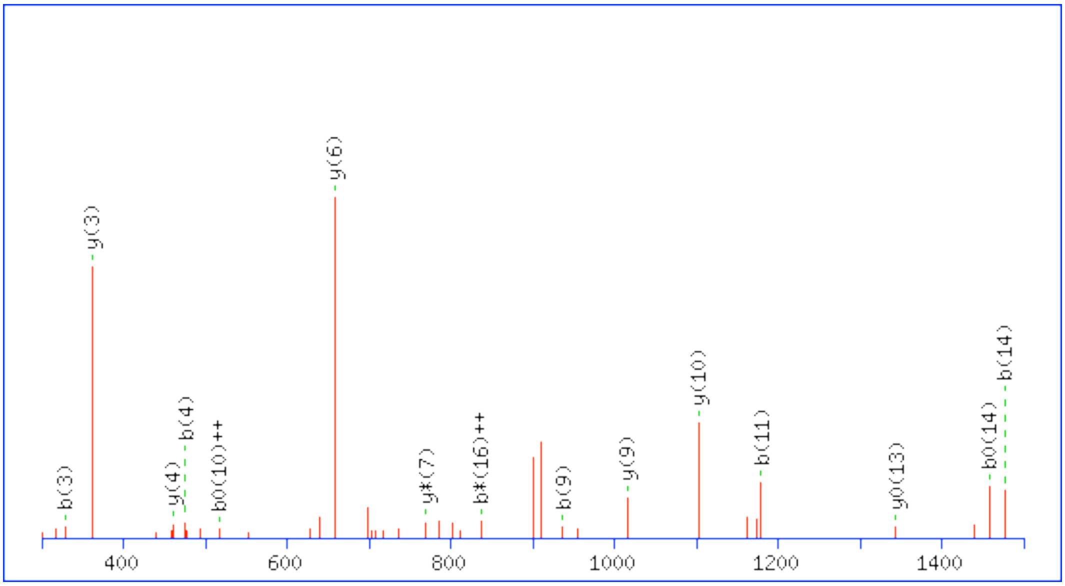
GRP78: 187 DAGTIAGLNVMR 198



**Monoisotopic mass of neutral peptide Mr(calc):** 1216.6234
**Ions Score:** 51 **Expect:** 0.0057
**Matches :** 12/100 fragment ions using 16 most intense peaks

| # | b | $b^{++}$ | $b^{*}$ | $b^{*++}$ | $b^{0}$ | $b^{0++}$ | Seq. | y | $y^{++}$ | $y^{*}$ | $y^{*++}$ | $y^{0}$ | $y^{0++}$ | # |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 115.0502 | 58.0287 | 98.0237 | 49.5155 | | | N | | | | | | | 15 |
| 2 | 243.1088 | 122.0580 | 226.0822 | 113.5448 | | | Q | 1563.7649 | 782.3861 | 1546.7384 | 773.8728 | 1545.7544 | 773.3808 | 14 |
| 3 | **356.1928** | 178.6001 | 339.1663 | 170.0868 | | | L | ***1435.7064*** | 718.3568 | 1418.6798 | 709.8435 | 1417.6958 | 709.3515 | 13 |
| 4 | **457.2405** | 229.1239 | 440.2140 | 220.6106 | 439.2300 | 220.1186 | T | ***1322.6223*** | 661.8148 | 1305.5957 | 653.3015 | 1304.6117 | 652.8095 | 12 |
| 5 | 544.2726 | 272.6399 | 527.2460 | 264.1266 | 526.2620 | 263.6346 | S | ***1221.5746*** | 611.2909 | 1204.5481 | 602.7777 | 1203.5640 | 602.2857 | 11 |
| 6 | **658.3155** | 329.6614 | 641.2889 | 321.1481 | 640.3049 | 320.6561 | N | ***1134.5426*** | 567.7749 | 1117.5160 | 559.2617 | 1116.5320 | 558.7696 | 10 |
| 7 | 755.3682 | 378.1878 | 738.3417 | 369.6745 | 737.3577 | 369.1825 | P | ***1020.4997*** | 510.7535 | 1003.4731 | 502.2402 | 1002.4891 | 501.7482 | 9 |
| 8 | 884.4108 | 442.7091 | 867.3843 | 434.1958 | 866.4003 | 433.7038 | E | 923.4469 | 462.2271 | 906.4203 | 453.7138 | 905.4363 | 453.2218 | 8 |
| 9 | 998.4538 | 499.7305 | 981.4272 | 491.2172 | 980.4432 | 490.7252 | N | 794.4043 | 397.7058 | 777.3777 | 389.1925 | 776.3937 | 388.7005 | 7 |
| 10 | 1099.5014 | 550.2544 | 1082.4749 | 541.7411 | 1081.4909 | 541.2491 | T | ***680.3614*** | 340.6843 | 663.3348 | 332.1710 | 662.3508 | 331.6790 | 6 |
| 11 | **1198.5699** | 599.7886 | 1181.5433 | 591.2753 | 1180.5593 | 590.7833 | V | ***579.3137*** | 290.1605 | 562.2871 | 281.6472 | 561.3031 | 281.1552 | 5 |
| 12 | 1345.6383 | 673.3228 | 1328.6117 | 664.8095 | 1327.6277 | 664.3175 | F | ***480.2453*** | 240.6263 | 463.2187 | 232.1130 | 462.2347 | 231.6210 | 4 |
| 13 | **1460.6652** | 730.8362 | 1443.6387 | 722.3230 | 1442.6546 | 721.8310 | D | ***333.1769*** | 167.0921 | 316.1503 | 158.5788 | 315.1663 | 158.0868 | 3 |
| 14 | 1531.7023 | 766.3548 | 1514.6758 | 757.8415 | 1513.6918 | 757.3495 | A | 218.1499 | 109.5786 | 201.1234 | 101.0653 | | | 2 |
| 15 | | | | | | | K | 147.1128 | 74.0600 | 130.0863 | 65.5468 | | | 1 |

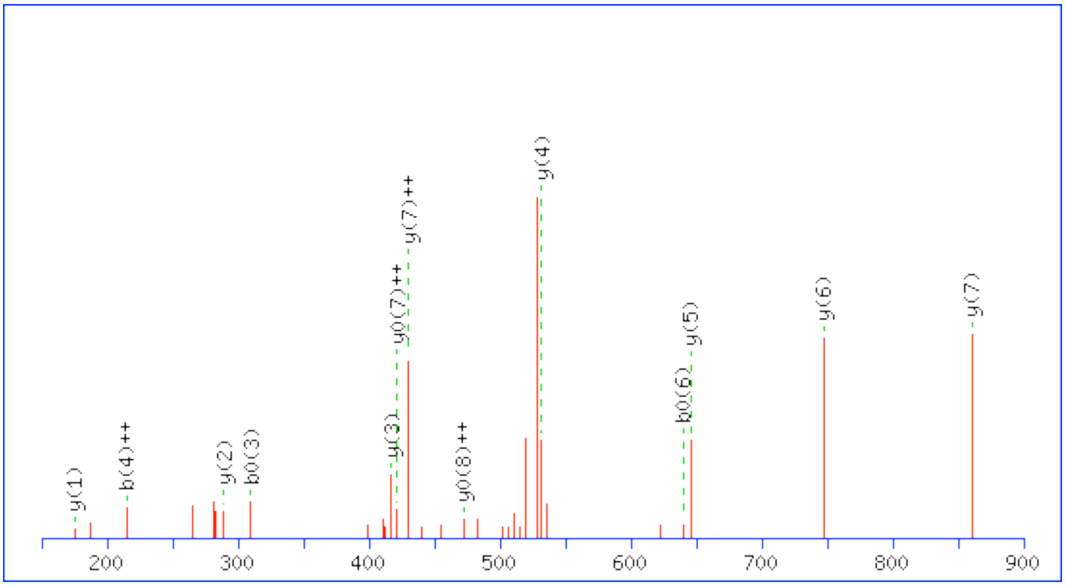
GRP78: 449 SQIFSTASDNQPTVTIK 465



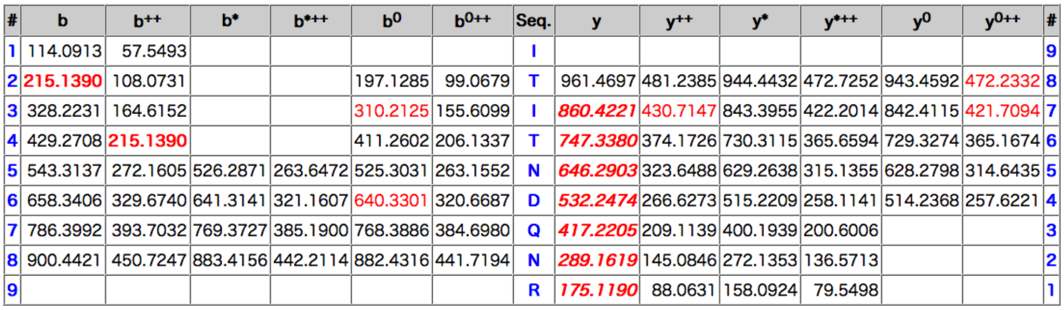
**Monoisotopic mass of neutral peptide Mr(calc):** 1835.9265
**Ions Score:** 50 **Expect:** 0.0045
**Matches :** 15/186 fragment ions using 20 most intense peaks

| # | b | b$^{++}$ | b$^{*}$ | b$^{*++}$ | b$^{0}$ | b$^{0++}$ | Seq. | y | y$^{++}$ | y$^{*}$ | y$^{*++}$ | y$^{0}$ | y$^{0++}$ | # |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 88.0393 | 44.5233 | | | 70.0287 | 35.5180 | S | | | | | | | 17 |
| 2 | 216.0979 | 108.5526 | 199.0713 | 100.0393 | 198.0873 | 99.5473 | Q | 1749.9018 | 875.4545 | 1732.8752 | 866.9412 | 1731.8912 | 866.4492 | 16 |
| 3 | ***329.1819*** | 165.0946 | 312.1554 | 156.5813 | 311.1714 | 156.0893 | I | 1621.8432 | 811.4252 | 1604.8166 | 802.9120 | 1603.8326 | 802.4199 | 15 |
| 4 | ***476.2504*** | 238.6288 | 459.2238 | 230.1155 | 458.2398 | 229.6235 | F | 1508.7591 | 754.8832 | 1491.7326 | 746.3699 | 1490.7486 | 745.8779 | 14 |
| 5 | 563.2824 | 282.1448 | 546.2558 | 273.6316 | 545.2718 | 273.1395 | S | 1361.6907 | 681.3490 | 1344.6642 | 672.8357 | 1343.6801 | 672.3437 | 13 |
| 6 | 664.3301 | 332.6687 | 647.3035 | 324.1554 | 646.3195 | 323.6634 | T | 1274.6587 | 637.8330 | 1257.6321 | 629.3197 | 1256.6481 | 628.8277 | 12 |
| 7 | 735.3672 | 368.1872 | 718.3406 | 359.6740 | 717.3566 | 359.1819 | A | 1173.6110 | 587.3091 | 1156.5844 | 578.7959 | 1155.6004 | 578.3039 | 11 |
| 8 | 822.3992 | 411.7032 | 805.3727 | 403.1900 | 804.3886 | 402.6980 | S | ***1102.5739*** | 551.7906 | 1085.5473 | 543.2773 | 1084.5633 | 542.7853 | 10 |
| 9 | ***937.4262*** | 469.2167 | 920.3996 | 460.7034 | 919.4156 | 460.2114 | D | ***1015.5419*** | 508.2746 | 998.5153 | 499.7613 | 997.5313 | 499.2693 | 9 |
| 10 | 1051.4691 | 526.2382 | 1034.4425 | 517.7249 | 1033.4585 | 517.2329 | N | 900.5149 | 450.7611 | 883.4884 | 442.2478 | 882.5043 | 441.7558 | 8 |
| 11 | ***1179.5277*** | 590.2675 | 1162.5011 | 581.7542 | 1161.5171 | 581.2622 | Q | 786.4720 | 393.7396 | 769.4454 | 385.2264 | 768.4614 | 384.7343 | 7 |
| 12 | 1276.5804 | 638.7938 | 1259.5539 | 630.2806 | 1258.5699 | 629.7886 | P | ***658.4134*** | 329.7103 | 641.3869 | 321.1971 | 640.4028 | 320.7051 | 6 |
| 13 | 1377.6281 | 689.3177 | 1360.6016 | 680.8044 | 1359.6175 | 680.3124 | T | 561.3606 | 281.1840 | 544.3341 | 272.6707 | 543.3501 | 272.1787 | 5 |
| 14 | ***1476.6965*** | 738.8519 | 1459.6700 | 730.3386 | 1458.6859 | 729.8466 | V | ***460.3130*** | 230.6601 | 443.2864 | 222.1468 | 442.3024 | 221.6548 | 4 |
| 15 | 1577.7442 | 789.3757 | 1560.7176 | 780.8625 | 1559.7336 | 780.3705 | T | ***361.2445*** | 181.1259 | 344.2180 | 172.6126 | 343.2340 | 172.1206 | 3 |
| 16 | 1690.8283 | 845.9178 | 1673.8017 | 837.4045 | 1672.8177 | 836.9125 | I | 260.1969 | 130.6021 | 243.1703 | 122.0888 | | | 2 |
| 17 | | | | | | | K | 147.1128 | 74.0600 | 130.0863 | 65.5468 | | | 1 |

GRP78: 525 ITITNDQNR 533

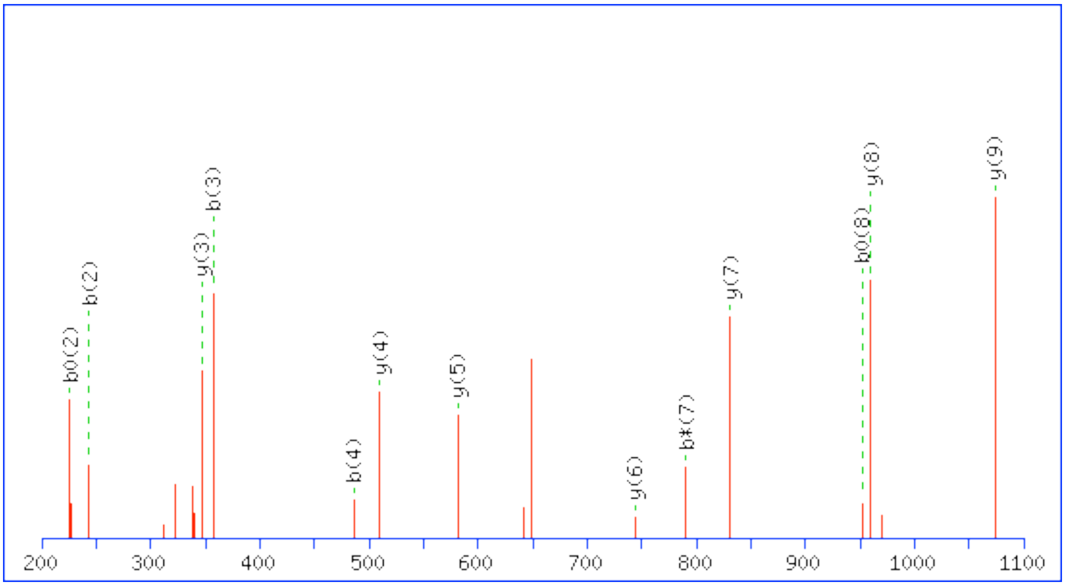


**Monoisotopic mass of neutral peptide Mr(calc):** 1073.5465
**Ions Score:** 60 **Expect:** 0.0009
**Matches :** 14/80 fragment ions using 21 most intense peaks



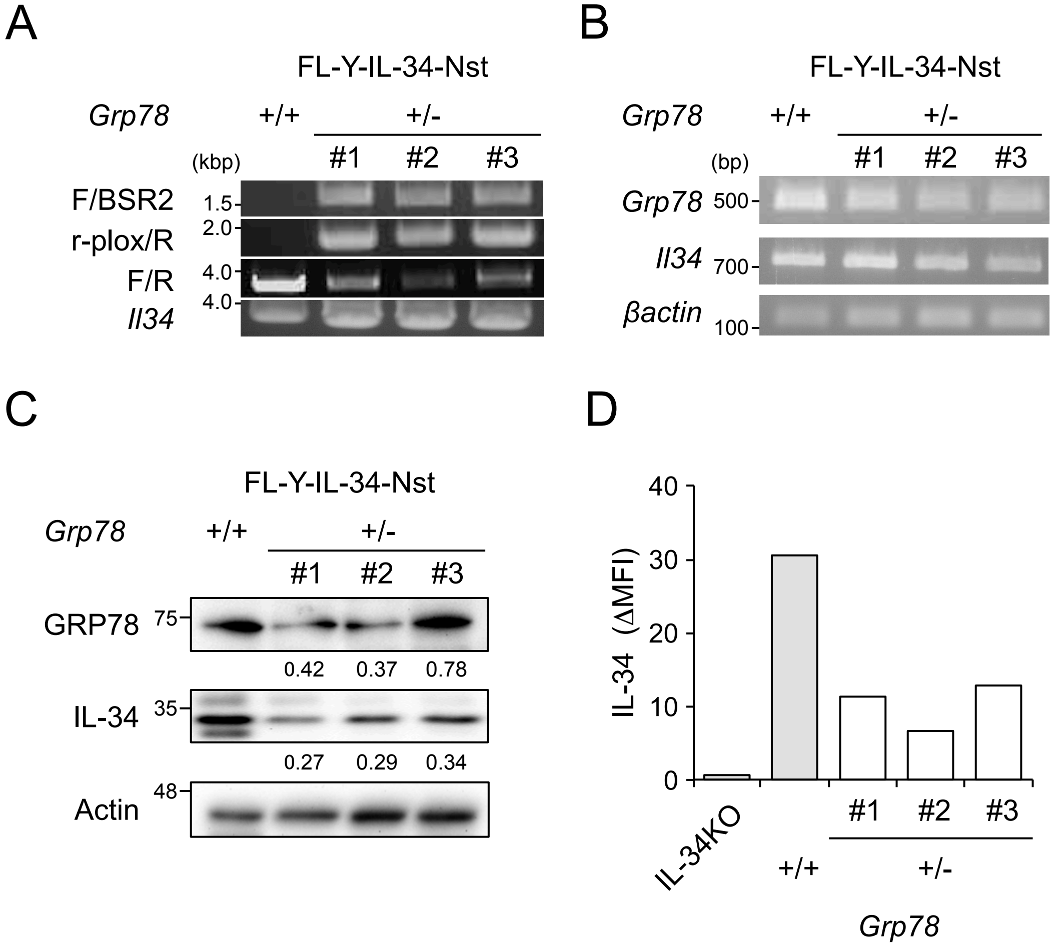
| # | b | $b^{++}$ | $b^{*}$ | $b^{*++}$ | $b^{0}$ | $b^{0++}$ | Seq. | y | $y^{++}$ | $y^{*}$ | $y^{*++}$ | $y^{0}$ | $y^{0++}$ | # |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 114.0913 | 57.5493 | | | | | I | | | | | | | 9 |
| 2 | **215.1390** | 108.0731 | | | 197.1285 | 99.0679 | T | 961.4697 | 481.2385 | 944.4432 | 472.7252 | 943.4592 | 472.2332 | 8 |
| 3 | 328.2231 | 164.6152 | | | 310.2125 | 155.6099 | I | ***860.4221*** | 430.7147 | 843.3955 | 422.2014 | 842.4115 | 421.7094 | 7 |
| 4 | 429.2708 | **215.1390** | | | 411.2602 | 206.1337 | T | ***747.3380*** | 374.1726 | 730.3115 | 365.6594 | 729.3274 | 365.1674 | 6 |
| 5 | 543.3137 | 272.1605 | 526.2871 | 263.6472 | 525.3031 | 263.1552 | N | ***646.2903*** | 323.6488 | 629.2638 | 315.1355 | 628.2798 | 314.6435 | 5 |
| 6 | 658.3406 | 329.6740 | 641.3141 | 321.1607 | 640.3301 | 320.6687 | D | ***532.2474*** | 266.6273 | 515.2209 | 258.1141 | 514.2368 | 257.6221 | 4 |
| 7 | 786.3992 | 393.7032 | 769.3727 | 385.1900 | 768.3886 | 384.6980 | Q | ***417.2205*** | 209.1139 | 400.1939 | 200.6006 | | | 3 |
| 8 | 900.4421 | 450.7247 | 883.4156 | 442.2114 | 882.4316 | 441.7194 | N | ***289.1619*** | 145.0846 | 272.1353 | 136.5713 | | | 2 |
| 9 | | | | | | | R | ***175.1190*** | 88.0631 | 158.0924 | 79.5498 | | | 1 |

GRP78: 564 NELESYAYSLK 574



**Monoisotopic mass of neutral peptide Mr(calc):** 1315.6295
**Ions Score:** 71 **Expect:** 3.7e-005
**Matches :** 13/114 fragment ions using 15 most intense peaks

| # | b | $b^{++}$ | $b^{*}$ | $b^{*++}$ | $b^{0}$ | $b^{0++}$ | Seq. | y | $y^{++}$ | $y^{*}$ | $y^{*++}$ | $y^{0}$ | $y^{0++}$ | # |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 115.0502 | 58.0287 | 98.0237 | 49.5155 | | | N | | | | | | | 11 |
| 2 | **244.0928** | 122.5500 | 227.0662 | 114.0368 | 226.0822 | 113.5448 | E | 1202.5939 | 601.8006 | 1185.5674 | 593.2873 | 1184.5834 | 592.7953 | 10 |
| 3 | **357.1769** | 179.0921 | 340.1503 | 170.5788 | 339.1663 | 170.0868 | L | ***1073.5514*** | 537.2793 | 1056.5248 | 528.7660 | 1055.5408 | 528.2740 | 9 |
| 4 | **486.2195** | 243.6134 | 469.1929 | 235.1001 | 468.2089 | 234.6081 | E | ***960.4673*** | 480.7373 | 943.4407 | 472.2240 | 942.4567 | 471.7320 | 8 |
| 5 | 573.2515 | 287.1294 | 556.2249 | 278.6161 | 555.2409 | 278.1241 | S | ***831.4247*** | 416.2160 | 814.3981 | 407.7027 | 813.4141 | 407.2107 | 7 |
| 6 | 736.3148 | 368.6610 | 719.2883 | 360.1478 | 718.3042 | 359.6558 | Y | ***744.3927*** | 372.7000 | 727.3661 | 364.1867 | 726.3821 | 363.6947 | 6 |
| 7 | 807.3519 | 404.1796 | 790.3254 | 395.6663 | 789.3414 | 395.1743 | A | ***581.3293*** | 291.1683 | 564.3028 | 282.6550 | 563.3188 | 282.1630 | 5 |
| 8 | 970.4153 | 485.7113 | 953.3887 | 477.1980 | 952.4047 | 476.7060 | Y | ***510.2922*** | 255.6498 | 493.2657 | 247.1365 | 492.2817 | 246.6445 | 4 |
| 9 | 1057.4473 | 529.2273 | 1040.4207 | 520.7140 | 1039.4367 | 520.2220 | S | ***347.2289*** | 174.1181 | 330.2023 | 165.6048 | 329.2183 | 165.1128 | 3 |
| 10 | 1170.5313 | 585.7693 | 1153.5048 | 577.2560 | 1152.5208 | 576.7640 | L | 260.1969 | 130.6021 | 243.1703 | 122.0888 | | | 2 |
| 11 | | | | | | | K | 147.1128 | 74.0600 | 130.0863 | 65.5468 | | | 1 |



**Supplemental Figure S6. GRP78 regulates IL-34 expression on cell surface of FL-Y-IL-34-Nst cells.** (A) Genomic PCR analysis of GRP78-heterozygous (+/-) FL-Y-IL-34-Nst cells. PCR primers used are shown in Fig. 5*A*. (B) RT-PCR analysis of *Grp78* and *Il34* mRNA expression in GRP78 (+/-) FL-Y-IL-34-Nst cells. (C) Western blot analysis of GRP78 expressed in GRP78 (+/-) FL-Y-IL-34-Nst cells. Whole cell lysates were separated by SDS-PAGE and subjected to western blotting. (D) IL-34 expression on the cell surface of the GRP78 (+/-) FL-Y-IL-34-Nst cell line was determined by flow cytometry after staining with an anti-IL-34 Ab. The level of IL-34 cell-surface expression is indicated as described in Fig. 2*C* (delta MFI). Data are representative of at least three independent experiments.

Table S1. Primers used for PCR and RT-PCR in this study

| Primer | Sequence (5'-3') |
|---|---|
| Construction of IL-34 KO vector | |
| IL-34-3'-F | TGCGGCCGCCCAGTGCTGTGTTAGGTGATC |
| IL-34-3'-R | AGTAGGATCCCAAGACCTGTGGGACACACC |
| IL-34-5'-F | CTGCAGTACAGGATCCGGCTTCAGTACATG |
| IL-34-5'-R | AGCAATCGATTAGTTGATGGGGAAGTAATG |
| | |
| Construction of IL-34 gRNA vector | |
| IL-34-guide2 (sense) | AACACCGGCTTTGGGAAACGAGAATTGT |
| IL-34-guide2 (antisense) | TAAAACAATTCTCGTTTCCCAAAGCCGG |
| IL-34-guide3 (sense) | AACACCGACCTTACAGGCTACCTTCGGT |
| IL-34-guide3 (antisense) | TAAAACCGAAGGTAGCCTGTAAGGTCGG |
| IL-34-guide4 (sense) | AACACCGCGAGAGCTTCGGTACCTGTGT |
| IL-34-guide4 (antisense) | TAAAACACAGGTACCGAAGCTCTCGCGG |
| | |
| Targeting check for IL-34 KO allele | |
| IL-34-F | CCAAGATGCTATGACCTGGCTAGGTGATGAGTG |
| IL-34-R | CGAAGCTCTCGCTCACTCACGTGAGCCTTC |
| Puro | CAGCGCCCGACCGAAAGGAGCGCACGACC |
| BSR2 | GTGATGATGAGGCTACTGCTGACTCTCAACATTCTACTCCTCC |
| r-plox | TCGAGGATCTGGGCTAGCCCTGATCAATAAC |
| | |
| Construction of IL-34 expression vector | |
| mIL-34-F-Kozak-EcoRI | GGGGAATTCAGTGCGCCACCATGCCCTG |
| IL-34-R2-BglII | CCAGATCTACTAGGGCAACGAGCC |
| | |
| Construction of IL-34-Nst expression vector | |
| IL-34-Strep-1st | CGCAGTTCGAGAAAGGTGGAGGTTCCGGAGGTGGATCGGGAGGTTCGGCCTGGAGCCACCCGCAGTTCGAAAAAAACGAGAATTTG |
| IL-34-Strep-2nd | GGAATTCAGTGCGCCACCATGCCCTGGGGACTCGCCTGGCTATACTGTCTTGGGATCCTACTTGACGTGGCTTTGGGATGGAGCCACCCGCAGTTCGAG |
| IL-34-R2-BglII | CCAGATCTACTAGGGCAACGAGCC |
| | |
| Construction of GRP78 KO vector | |
| GRP78-5'-F | GAATTCCATCTCATGGTGGAAAGTGCTCG |
| GRP78-5'-R | GGATCCAGCAGTCAGGCAGGAGTCTTAG |
| GRP78-3'-F | GGATCCGGCGGCGTTGCTGCTGCTG |
| GRP78-3'-R | GCGGCCGCTCAAACACTCAACACTG |
| | |
| Construction of GRP78 gRNA vector | |
| GRP78-guide 1 (sense) | AACACCGGAGCGACTGGTCCTCAGCGCGT |
| GRP78-guide 1 (antisense) | TAAAACGCGCTGAGGACCAGTCGCTCCGG |

| | |
|---|---|
| Targeting check for GRP78 KO allele | |
| GRP78-F | TATCAGCCCTATTCCAAGAGTCGAATAGGGTGGTG |
| GRP78-R | GTGAGATGGCTCGGCAGGTAAGGGC |
| | |
| *il34* mRNA | |
| F | GACACACTTCTGGGGACAGTGCCTC |
| ex3F | GAGATATGGACTCTGACCCAAGATAAGGAGTGTG |
| R | GCTCAGGGCAACGAGCCATGGCTTG |
| | |
| *csf1* mRNA | |
| F | GCCGGGAATTCGCTGCCACCATGAC |
| R | CATAGAATTCTTTCTATACTGGCAGTTCCACCTGTCTG |
| | |
| *grp78* mRNA | |
| F | CTCGAGGAGGAGGAGGACAAGAAGG |
| R | GGATCCCTACAACTCATCTTTTTCTGATGTATC |
| | |
| *βactin* mRNA | |
| F | AGTGTGACGTTGACATCCGTA |
| R | GCCAGAGCAGTAATCTCCTTCT |