

**FIGURE 1A**

| hSNAP23 binding sites | Point-mutations (in wt LC/A) | Fold increase hSNAP23 cleavage (vs wild type LC/A) | % hSNAP23 cleavage (at 1 $\mu$ M LC/A, 20 $\mu$ M hSNAP23) | $K_M$ [ $\mu$ M] | $k_{cat}$ [1/min] | % hSNAP25 cleavage (at 0.5 nM LC/A, 20 $\mu$ M hSNAP25) |
|-----------------------|------------------------------|----------------------------------------------------|------------------------------------------------------------|------------------|-------------------|---------------------------------------------------------|
| -                     | <i>none (wild type LC/A)</i> | 1.00                                               | 0.2                                                        | 225 $\pm$ 38     | 0.2               | 61 $\pm$ 10                                             |
| P182/D178             | A308L                        |                                                    |                                                            | 79 $\pm$ 20      | 0.09              |                                                         |
| P182/D178             | E148Y                        | 8.28                                               | 9.7                                                        | 52 $\pm$ 6       | 0.36              | 29 $\pm$ 5                                              |
| P182/D178             | E148N                        | 1.18                                               |                                                            |                  |                   |                                                         |
| P182/D178             | T307F                        |                                                    | 1.6                                                        |                  |                   |                                                         |
| P182/D178             | T307I                        |                                                    | 2.4                                                        |                  |                   |                                                         |
| P182/D178             | Y312K                        |                                                    | 2.6                                                        |                  |                   | 26 $\pm$ 2                                              |
| P182/D178             | Y312K, E148Y                 |                                                    | 1.7                                                        |                  |                   |                                                         |
| P182/D178             | T307I, A308P, Y312V          | 1.74                                               |                                                            |                  |                   |                                                         |
| P182/D178             | T307F, A308N, Y312L          | 1.49                                               |                                                            |                  |                   |                                                         |
| P182/D178             | E148N, T307I, A308P, Y312V   | 2.73                                               |                                                            |                  |                   |                                                         |
| P182/D178             | E148Y, T307F, A308N, Y312L   | 2.01                                               |                                                            |                  |                   |                                                         |
| P182/D178             | E148Y, T307I, A308P, Y312V   | 1.45                                               |                                                            |                  |                   |                                                         |
| P182/D178             | E148Y, T307L, A308T, Y312M   | 2.23                                               |                                                            |                  |                   |                                                         |
| P182/D178             | E148Y, T307L, A308I, Y312M   | 1.38                                               |                                                            |                  |                   |                                                         |
| K185                  | V304D                        |                                                    |                                                            | 56 $\pm$ 13      | 0.14              |                                                         |
| K185                  | V304E                        |                                                    |                                                            | 65 $\pm$ 1       | 0.16              |                                                         |
| K185                  | G305D                        |                                                    |                                                            | 52 $\pm$ 6       | 0.13              | 46 $\pm$ 3                                              |
| K185                  | G305E                        |                                                    |                                                            | 61 $\pm$ 12      | 0.12              |                                                         |
| R186                  | S143D                        |                                                    |                                                            | 69 $\pm$ 14      | 0.14              | 51 $\pm$ 3                                              |
| R186                  | S143E                        |                                                    |                                                            | 57 $\pm$ 11      | 0.13              | 102 $\pm$ 4.1                                           |
| R186                  | S143Q                        |                                                    |                                                            | 91 $\pm$ 6       | 0.21              |                                                         |
| I198                  | K166V                        | 1.096                                              |                                                            |                  |                   |                                                         |
| I198                  | K166F                        | 1.66                                               | 66.1                                                       | 44 $\pm$ 9       | 0.9               | 62 $\pm$ 8                                              |
| I198                  | K166L                        | 1.43                                               |                                                            |                  |                   | 91 $\pm$ 6                                              |
| I198                  | K166I                        | 1.098                                              |                                                            |                  |                   | 58 $\pm$ 1                                              |
| K206                  | L256D                        |                                                    | 1.7                                                        |                  |                   | 7 $\pm$ 2                                               |
| K206                  | Y251D                        |                                                    | 2.3                                                        |                  |                   | 8 $\pm$ 1                                               |
| K206                  | Y251E                        |                                                    | 3.2                                                        |                  |                   | 2 $\pm$ 1                                               |
| K206                  | L256E, V258P                 |                                                    |                                                            | 173 $\pm$ 42     | 0.19              |                                                         |

**FIGURE 1B**

| hSNAP23 binding sites            | Point-mutations (in wt LC/A) | Fold increase hSNAP23 cleavage (vs LC/A-E148Y) | % hSNAP23 cleavage (% at 1 $\mu$ M LC/A, 20 $\mu$ M hSNAP23) [***% at 10 nM LC/A] | $K_M$ [ $\mu$ M] | $k_{cat}$ [1/min] | % hSNAP25 cleavage (at 0.5 nM LC/A, 20 $\mu$ M hSNAP25) |
|----------------------------------|------------------------------|------------------------------------------------|-----------------------------------------------------------------------------------|------------------|-------------------|---------------------------------------------------------|
| P182/D178                        | E148Y                        | 1.00                                           | 9.7                                                                               | 52 $\pm$ 6       | 0.36              | 29 $\pm$ 5                                              |
| P182/D178, D189/D192             | E148Y, Q29A                  | 1.618                                          | 15.7                                                                              |                  |                   | 34 $\pm$ 7                                              |
| P182/D178, R186                  | E148Y, S143E                 | 2.13                                           | 20.7                                                                              |                  |                   | 17 $\pm$ 3                                              |
| P182/D178, R186                  | E148Y, S143D                 | 3.2                                            | 31.1                                                                              |                  |                   | 16 $\pm$ 4                                              |
| P182/D178, K185                  | E148Y, G305D                 | 2.61                                           | 25.4                                                                              | 24               | 0.29              | 38 $\pm$ 3                                              |
| P182/D178, I198                  | E148Y, K166F                 |                                                | 5.6**                                                                             |                  |                   | 28 $\pm$ 10                                             |
| K185, I198                       | G305D, K166F                 |                                                | 3.8**                                                                             |                  |                   | 66 $\pm$ 14                                             |
| P182/D178, K206                  | E148Y, Y251D                 | 1.298                                          | 12.6                                                                              |                  |                   |                                                         |
| P182/D178, D189/D192, K185       | E148Y, Q29A, G305D           | 3.5                                            | 34.0                                                                              | 6.6 $\pm$ 1      | 0.28              | 38 $\pm$ 3                                              |
| P182/D178, D189/D192, R186       | E148Y, Q29A, S143D           | 2.56                                           | 24.8                                                                              | 24 $\pm$ 4       | 17.7              | 23 $\pm$ 1                                              |
| P182/D178, I198, K185            | E148Y, K166F, G305D          |                                                | 15.2**                                                                            |                  |                   | 60 $\pm$ 4                                              |
| P182/D178, I198, K185            | E148Y, K166V, G305D          | 4.67                                           | 45.3                                                                              |                  |                   |                                                         |
| P182/D178, R186, K206, D189/D192 | E148Y, S143D, Q29A, Y251E    | 1.36                                           | 13.2                                                                              |                  |                   | 2 $\pm$ 1                                               |
| P182/D178, D189/D192, I198, K185 | E148Y, Q29A, K166V, G305D    | 6.422                                          | 62.3                                                                              |                  |                   | 41 $\pm$ 2                                              |
| P182/D178, D189/D192, I198, K185 | E148Y, Q29A, K166F, G305D    |                                                | 10.4**                                                                            | 21 $\pm$ 1       | 13.4              | 40 $\pm$ 14                                             |
| P182/D178, K206                  | E148Y, L256D                 |                                                | 5.8                                                                               |                  |                   |                                                         |
| P182/D178, K206                  | E148Y, Y251E                 |                                                | 9.0                                                                               |                  |                   | 3 $\pm$ 2                                               |
| P182/D178, R186, I198            | E148Y, S143D, K166F          |                                                | 18.2**                                                                            |                  |                   | 11 $\pm$ 1                                              |
| P182/D178, I198, D210            | E148Y, K166F, S254A          |                                                | 13.3**                                                                            | 19 $\pm$ 2       | 13.8              |                                                         |
| P182/D178, K185, I198, D210      | E148Y, K166F, S254A, G305D   |                                                | 29.9**                                                                            | 16 $\pm$ 3       | 27.8              |                                                         |
| P182/D178, R186                  | E148Y, S143Q                 |                                                | 5.8                                                                               |                  |                   | 10 $\pm$ 1                                              |
| P182/D178, D168                  | E148Y, K340H                 |                                                | 3.5                                                                               |                  |                   |                                                         |

**FIGURE 1C**

| hSNAP23 binding sites | Point-mutations (in wt LC/A) | Fold change hSNAP23 cleavage (vs WT LC/A) | % hSNAP23 cleavage (% at 1 $\mu$ M LC/A, 20 $\mu$ M hSNAP23) [***% at 10 nM LC/A] | SNAP23 $K_M$ [ $\mu$ M] | $k_{cat}$  | % hSNAP25 cleavage v WT LC/A (at 0.5 nM LC/A, 20 $\mu$ M hSNAP25) |
|-----------------------|------------------------------|-------------------------------------------|-----------------------------------------------------------------------------------|-------------------------|------------|-------------------------------------------------------------------|
| <i>P182/D178</i>      | E148R                        | 1.44                                      |                                                                                   |                         |            | 4 $\pm$ 2                                                         |
| <i>P182/D178</i>      | E148K                        | 0.84                                      |                                                                                   |                         |            | 1.7 $\pm$ 0.5                                                     |
| R186                  | S143D                        | 2.35                                      |                                                                                   | 69 $\pm$ 14             | 8 $\pm$ 2  | 72.6 $\pm$ 4.9                                                    |
| R186                  | S143Q                        | 2.75                                      |                                                                                   | 91 $\pm$ 6              | 13 $\pm$ 1 |                                                                   |
| K185                  | V304D                        | 2.58                                      |                                                                                   | 56 $\pm$ 13             | 8 $\pm$ 1  | 82.9 $\pm$ 8.4                                                    |
| K185                  | V304E                        | 1.46                                      |                                                                                   | 65 $\pm$ 1              | 9 $\pm$ 2  |                                                                   |
| K206                  | V258A                        | 0.59                                      |                                                                                   |                         |            | 33.1 $\pm$ 4.7                                                    |

**FIGURE 2**