**Supplementary File 6**

**Quantification of Flo11A protein amounts at the cell surface by immunofluorescence microscopy.**

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| --- | --- |
| **Flo11 variant as named in text and figures** | **Fluorescence relative to ScFlo11A** |
| ScFlo11 | 100%1 |
| Y111D Y113D Y118D | 92%1 |
| Y111A Y113A Y118A | 86%1 |
| W94D Y162D | 95%1 |
| Y92D Y154D | 87%1 |
| W166D W168D | 74%1 |
| W166A W168A | 98%1 |
| Y133D W144D Y196D | 93%1 |
| Y133A W144A Y196A | 73%1 |
| W70D Y76D | 72%1 |
| W51D Y182D | 84%1 |
| Y111A Y113A Y118A Y133A W144A Y196A | 60%1 |
| W70A Y76A Y92A Y111A Y113A Y118A Y133A W144A Y154A W166A W168A Y196A | 63%1 |
| ∆310-helix | 99%1 |
| ∆-helix | 86%1 |
| ScFlo11A | 122%1 |
| ScFlo11Ains | 83%1 |
| STREPII-SpFlo11A | 97%1; 76%2 |
| STREPII-KlFlo11A | 67%2 |
| STREPII-TdFlo11A | 96%2 |
| STREPII-KpFlo11A | 87%2 |
| STREPII-ClFlo11A | 93%2 |
| STREPII-MgFlo11A | 71%2 |

1Obtained by using polyclonal anti-ScFlo11A antibodies

2Obtained by using monoclonal anti-STREP II antibodies