Kinetic analysis of ASIC1a delineates conformational signaling from proton-sensing domains to the channel gate

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## **Supplementary File 3. Test for intrinsic pH dependence of fluorophores**

|  |  |  |
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| Mutant | Ratio (%) F/F acidification from desensitized/ F/F acidification from closed | |
| *Wrist mutants* | |  |
| E63CC | | 10 ± 11 |
| H70C | | -4 ± 3 |
| Y71C | | -19 ± 3 |
| H72C | | 6 ± 3 |
| T419C | | 33 ± 7 |
| K424C | | 15 ± 3 |
| A425C | | 13 ± 7 |
| I428C | | 23 ± 4 |
|  | |  |
| Palm single mutants | |  |
| A81C | | -2 ± 2 |
| S83C | | 1 ± 1 |
| Q84C | | 10 ± 3 |
|  | |  |
| Palm - intrasubunit | |  |
| A81C Y417V P205W | | -2 ± 3 |
| S83C Y417V P205W | | 10 ± 3 |
| Q84C Y417V P205W 1st signal (1st peak) | | 7 ± 22 |
| Q84C Y417V P205W 2nd signal | | 199 ± 43 |
| Q84C Y417V P205W 2nd peak | | 0 ± 4 |
| Q84C Y417V R206W | | 16 ± 2 |
| A81C Y417V L207W 1st signal | | 0 ± 2 |
| A81C Y417V L207W 2nd signal | | 123 ± 50 |
| Q84C Y417V L207W | | 26 ± 10 |
| A81C Y417V K208W | | 13 ± 18 |
| A81C Y417V T209W | | -1 ± 1 |
| A81C Y417V M210W | | 32 ± 13 |
|  | |  |
| Palm - intersubunit | |  |
| A81C Y417V T289W | | 5 ± 1 |
| S83C Y417V T289W | | 10 ± 3 |
| Q84C Y417V T289W | | 22 ± 2 |
| S83C Y417V D357W 1st signal (1st peak) | | 19 ± 4 |
| S83C Y417V D357W 2nd signal | | 357 ± 110 |
| S83C Y417V D357W 2nd peak | | 10 ± 2 |
| S83C Y417V Q358W 1st signal | | 35 ± 34 |
| S83C Y417V Q358W 2nd signal | | -3 ± 30 |
| S83C Y417V E359W | | 44 ± 20 |
| A81C Y417V L369W 1st signal | | -34 ± 5 |
| A81C Y417V L369W 2nd signal | | 28 ± 9 |
| S83C Y417V L369W 1st signal | | 73 ± 18 |
| S83C Y417V L369W 2nd signal | | -33 ± 29 |