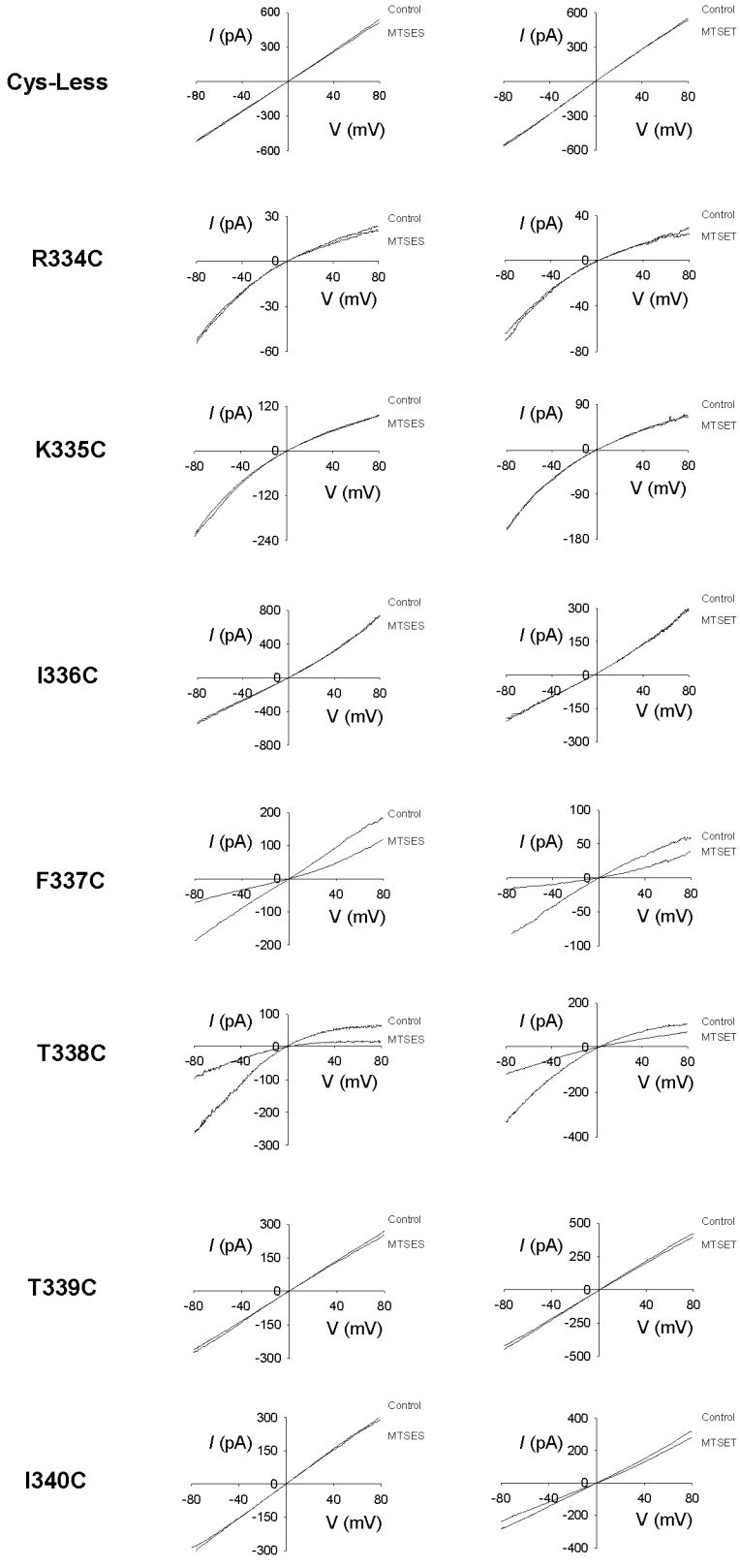

Cys-Less
R334C
K335C
I336C
F337C
T338C
T339C
I340C
I (pA)
V (mV)
Control
MTSES
MTSET

Figure S1 (A)

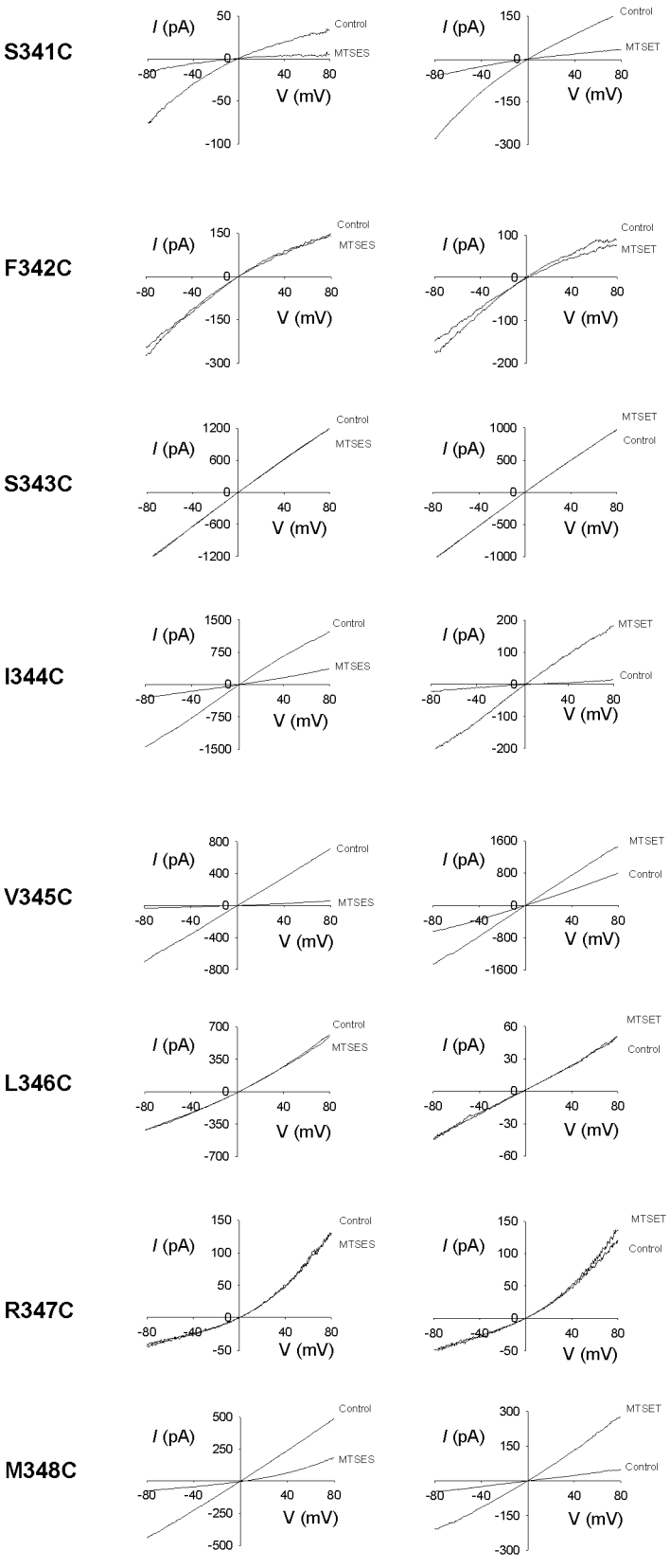

S341C
I (pA)
V (mV)
Control
MTSES
MTSET
F342C
S343C
I344C
V345C
L346C
R347C
M348C

Figure S1 (B)

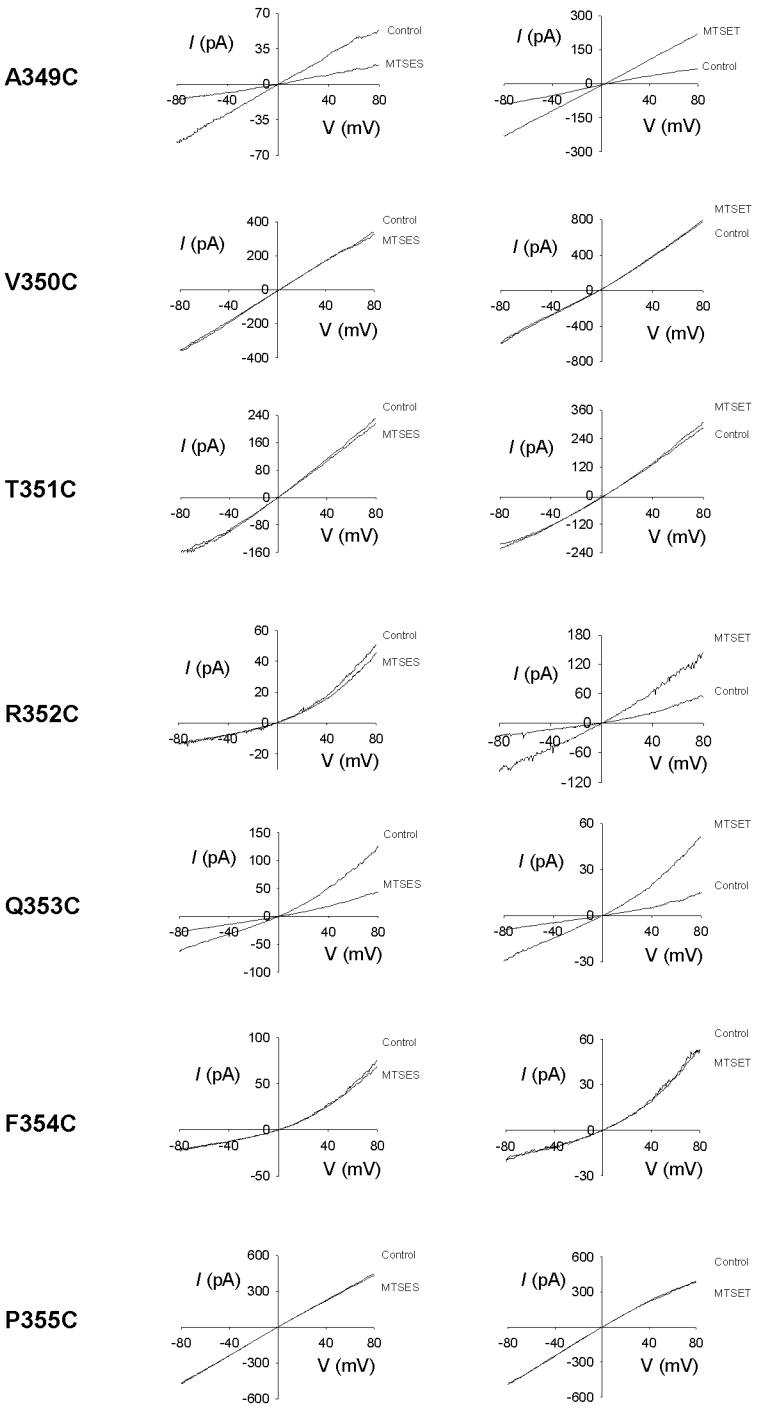

A349C
V350C
T351C
R352C
Q353C
F354C
P355C
I (pA)
V (mV)
Control
MTSES
MTSET

Figure S1 (C)

A. Pretreatment with 200 µM MTSES alone

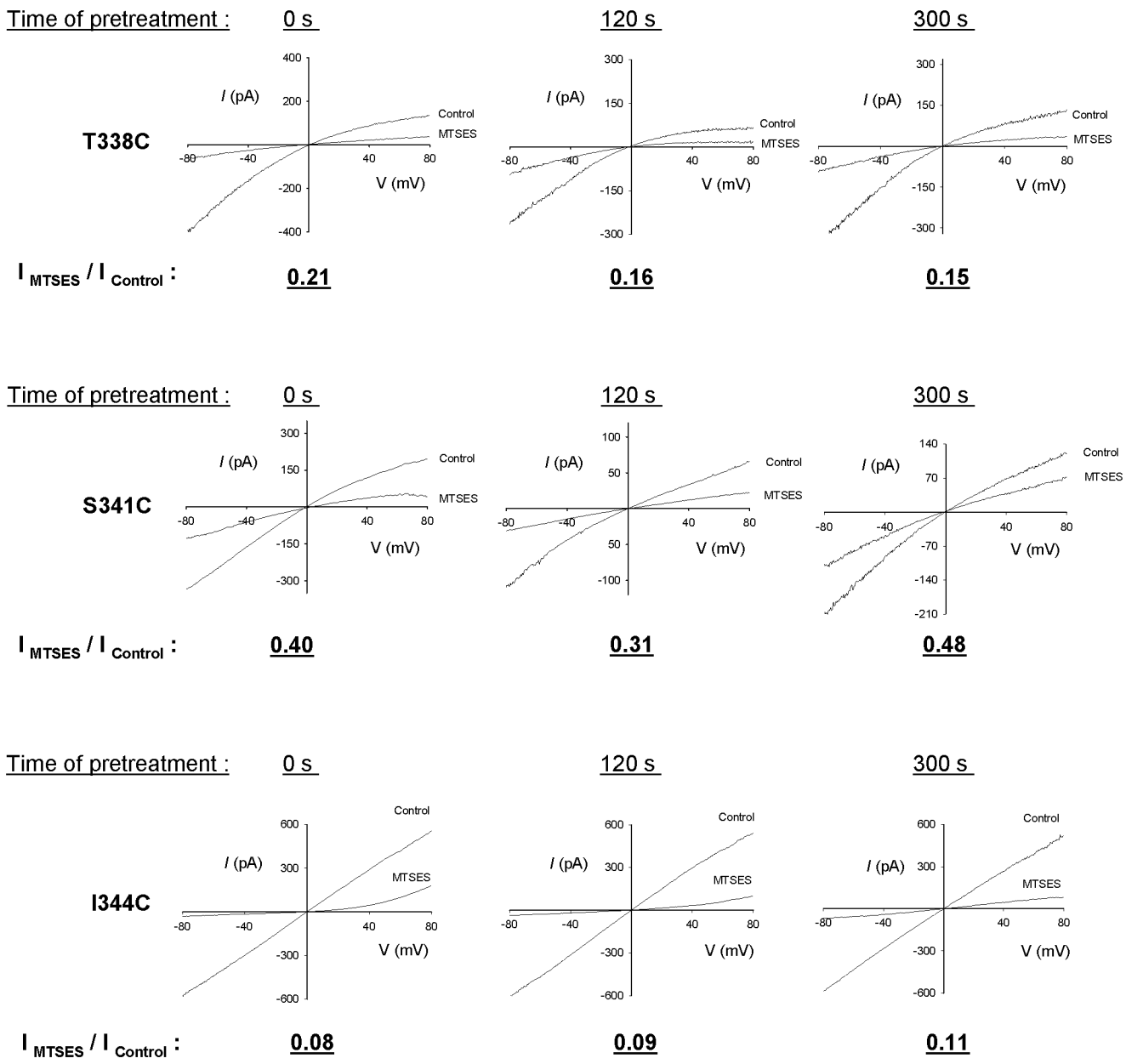
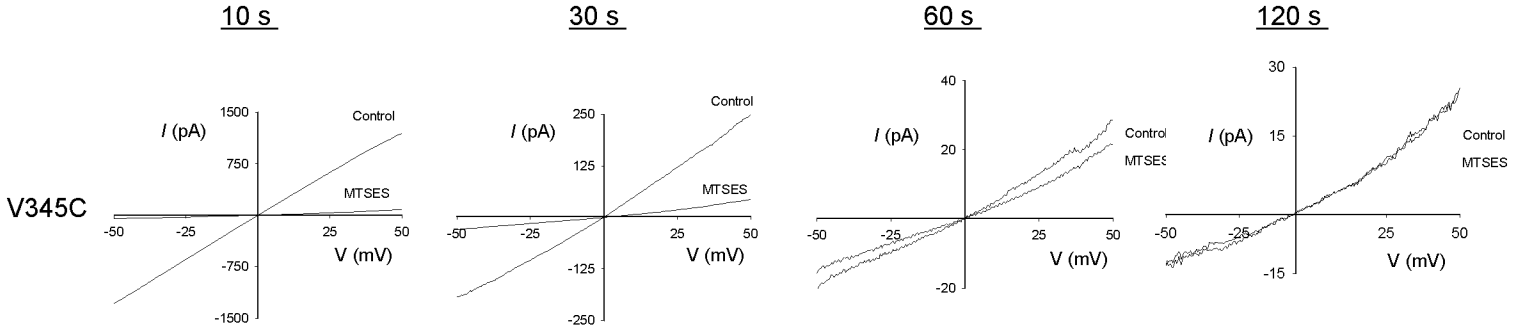


Figure S2 (A)

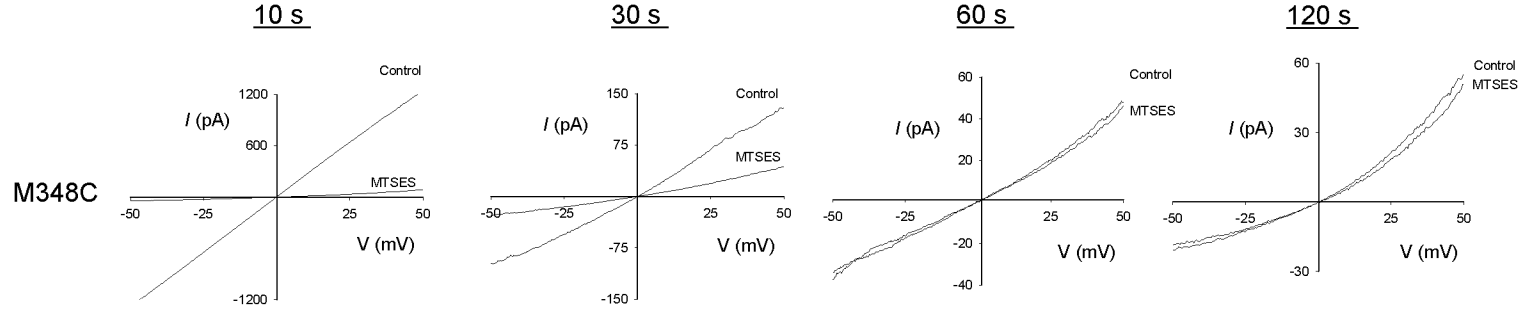
B. Pretreatment with 20 µM MTSES alone

Time of pretreatment :



$I_{MTSES} / I_{Control}$: **0.03** **0.17** **0.86** **0.99**

Time of pretreatment :

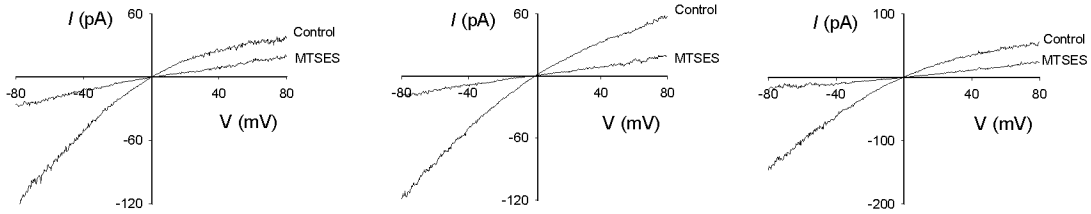


$I_{MTSES} / I_{Control}$: **0.03** **0.27** **0.91** **1.08**

Figure S2 (B)

A. Pretreatment with 1mM $Au(CN)_2^-$ alone

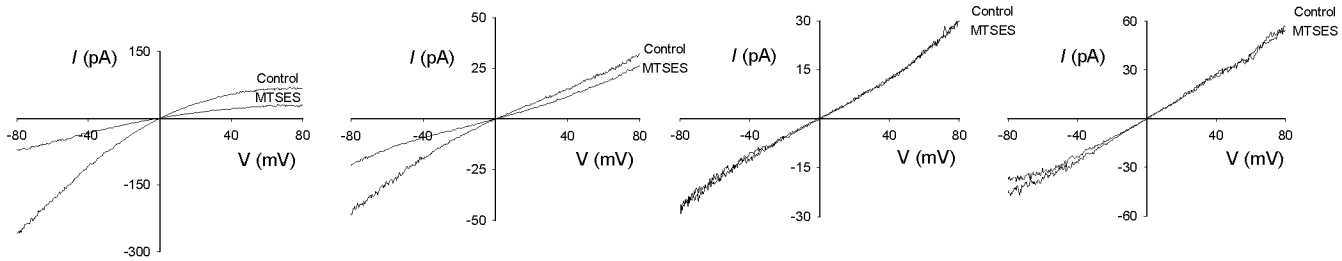
Time of pretreatment : 0 s 120 s 300 s



$I_{MTSES} / I_{Control}$: **0.21** **0.16** **0.15**

B. Pretreatment with 2 µM $Au(CN)_2^-$, ATP and PKA

Time of pretreatment : 0 s 10 s 30 s 60 s



$I_{MTSES} / I_{Control}$: **0.28** **0.50** **1.02** **0.96**

Figure S3

Figure S1 Modification of cysteine-substituted CFTR-TM6 mutants by internal MTS reagents. Example leak subtracted *I-V* relationships for all channel variants used in the present study, recorded from inside-out membrane patches following maximal channel activation with PKA (20 nM), ATP (1 mM) and PPi (2 mM). Currents were recorded before (Control) and after application of MTSES (200 μM, left panels), or MTSET (2 mM, right panels) to the intracellular (bath) solution.

Figure S2 Timecourse of modification of introduced cysteines during pretreatment with internal MTSES. *A*, *B*, example leak-subtracted *I-V* relationships for each of five mutants (T338C, S341C, I334C, V345C, M348C) as indicated, showing the effects of application of internal MTSES (200 μM) following maximal channel activation with PKA (20 nM), ATP (1 mM) and PPi (2 mM). Patches have been pretreated with MTSES, either 200 μM (*A*) or 20 μM (*B*) for the pretreatment time indicated above each *I-V* relationship. Below each *I-V* relationship is the measured MTSES sensitivity of the current following pretreatment (I_{MTSES} / $I_{Control}$) for that patch. Similar data averaged across multiple patches was used to generate the data shown in Fig. 5*B*.

Figure S3 Timecourse of modification of T338C during pretreatment with internal $Au(CN)_2^-$. *A*, *B*, example leak-subtracted *I-V* relationships for T338C showing the effects of application of internal MTSES (200 μM) following maximal channel activation with PKA (20 nM), ATP (1 mM) and PPi (2 mM). Patches have been pretreated in two different ways: *A*, pretreated with 1 mM $Au(CN)_2^-$; *B*, pretreated with 2 μM $Au(CN)_2^-$, 20 nM PKA, and 1 mM ATP. In both *A* and *B*, the duration of pretreatment is indicated above each *I-V* relationship. Below each *I-V* relationship is the measured MTSES sensitivity of the current following pretreatment (I_{MTSES} / $I_{Control}$) for that patch. Similar data averaged across multiple patches was used to generate the data shown in Fig. 8; data such as that shown in *A* for Fig. 8*A*, and data such as that shown in *B* for Fig. 8*B*.