x-y-z-t calcium imaging A x-y imaging area **B** Acquire repeating does not vary z-stacks over time tissue depth (z axis) calcium sensor in muscles time (min) (MHC-CD8-GCaMP6f-Sh) Time series to c z-stacks to single ∆F x-y image x-y time series max intensity ave intensity t = n+1 $max-ave = \Delta F$ NB7-1>Pdm, MHC-CD8-GCaMP6f-Sh MHC-CD8-GCaMP6f-Sh post synaptic GCaMP Ε G M4 M4 high ΔF Œ M4 M4 low