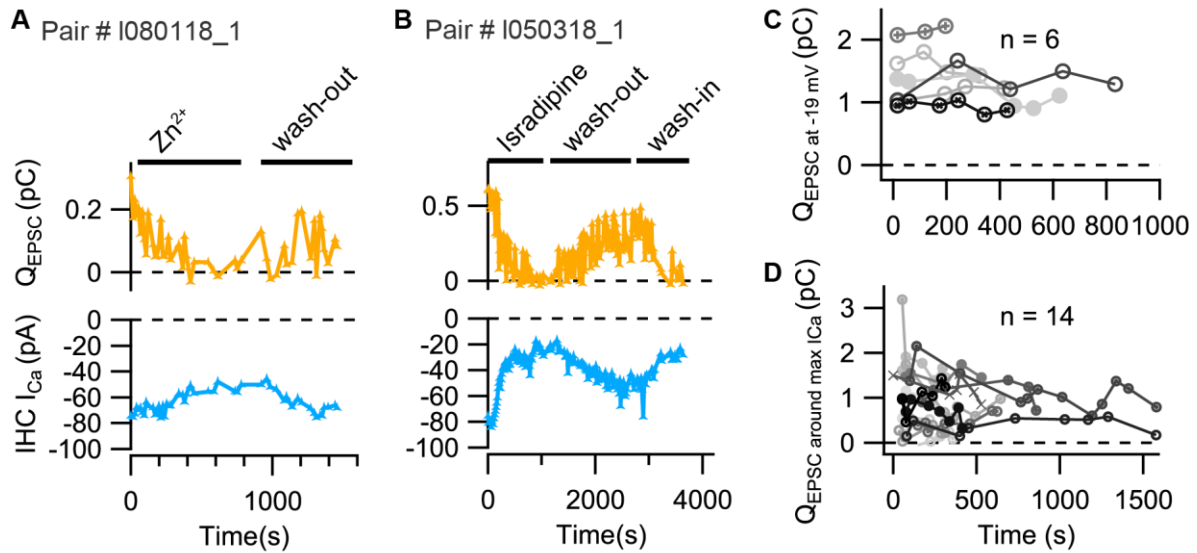
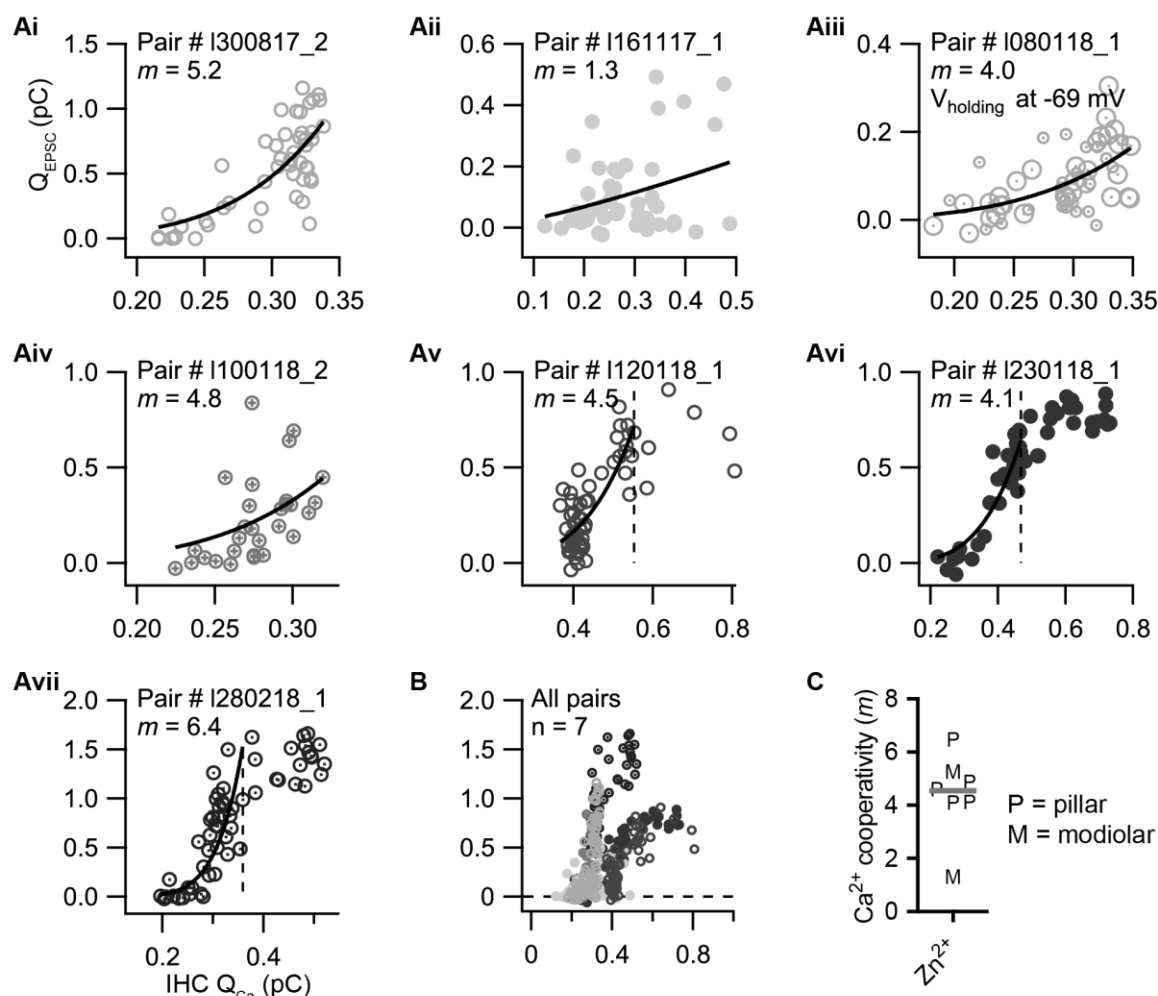




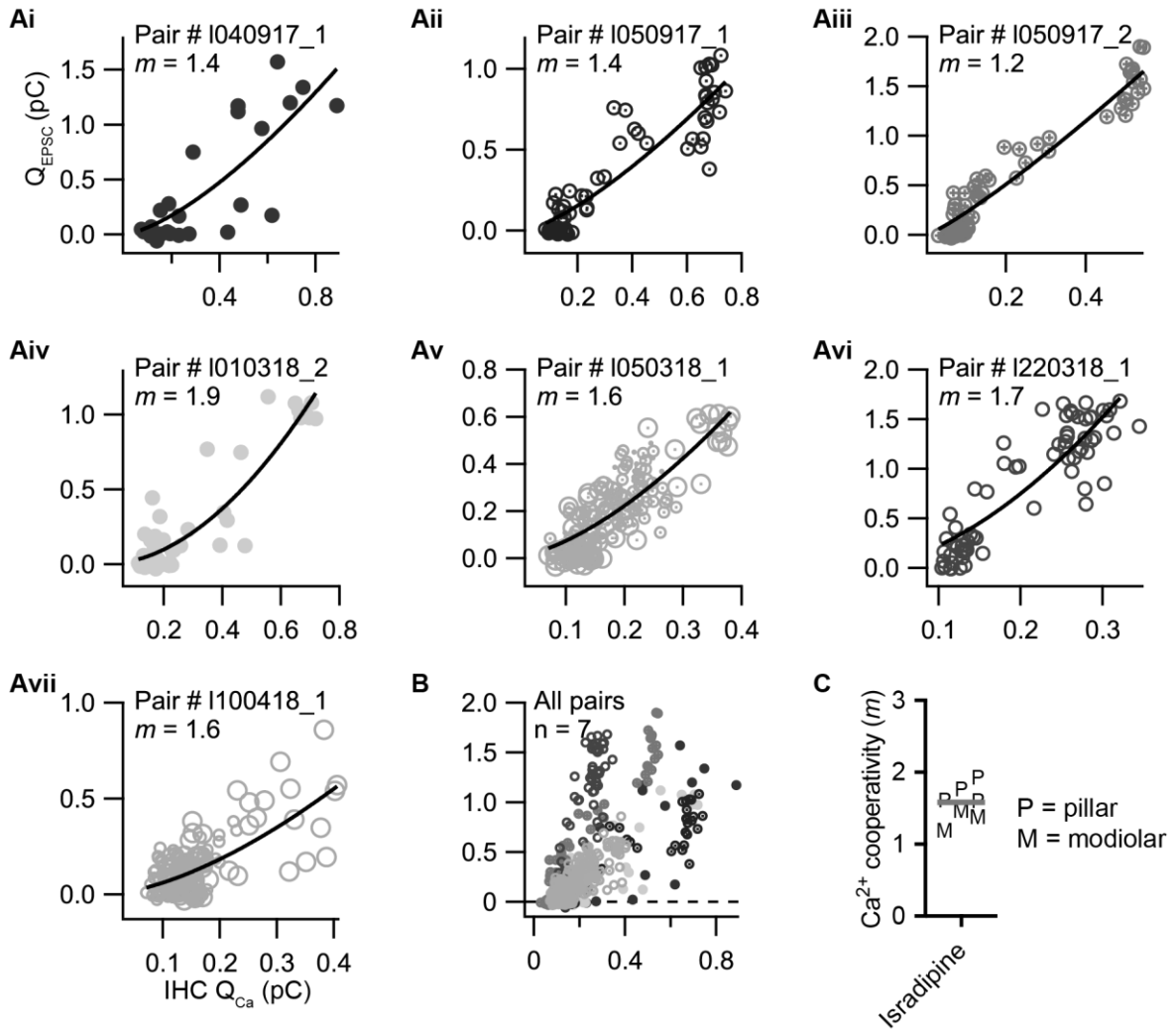
**Figure S1 Testing for the potential rundown of exocytosis during manipulations of  $Ca^{2+}$  influx**

**A,B.** Related to Fig. 2 and Fig.3: slow perfusion of  $Zn^{2+}$  (A) or isradipine (B) progressively reduces the whole-IHC  $Ca^{2+}$  current ( $I_{Ca}$ ; blue) and the concomitant evoked EPSC charge ( $Q_{EPSC}$ ; orange). Slow wash-out using normal extracellular solution partially restores  $I_{Ca}$  and neurotransmitter release for both pharmacological manipulations. In B, wash-in of isradipine once again reduces  $I_{Ca}$  and  $Q_{EPSC}$ . **C.** Related to Fig. 4: during tail-current experiments, rundown of exocytosis was probed as changes in  $Q_{EPSC}$  elicited by 2 or 10 ms voltage steps to -19 mV over time. We recorded these periodic depolarizations after each full set of tail current protocols in 6 pairs. **D.** Related to Fig. 5: to address potential rundown of exocytosis,  $Q_{EPSC}$  elicited by 2 ms pulses at voltages eliciting maximal  $Ca^{2+}$  influx (-19, -21 and -23 mV) was plotted vs. time ( $n = 14$  pairs).



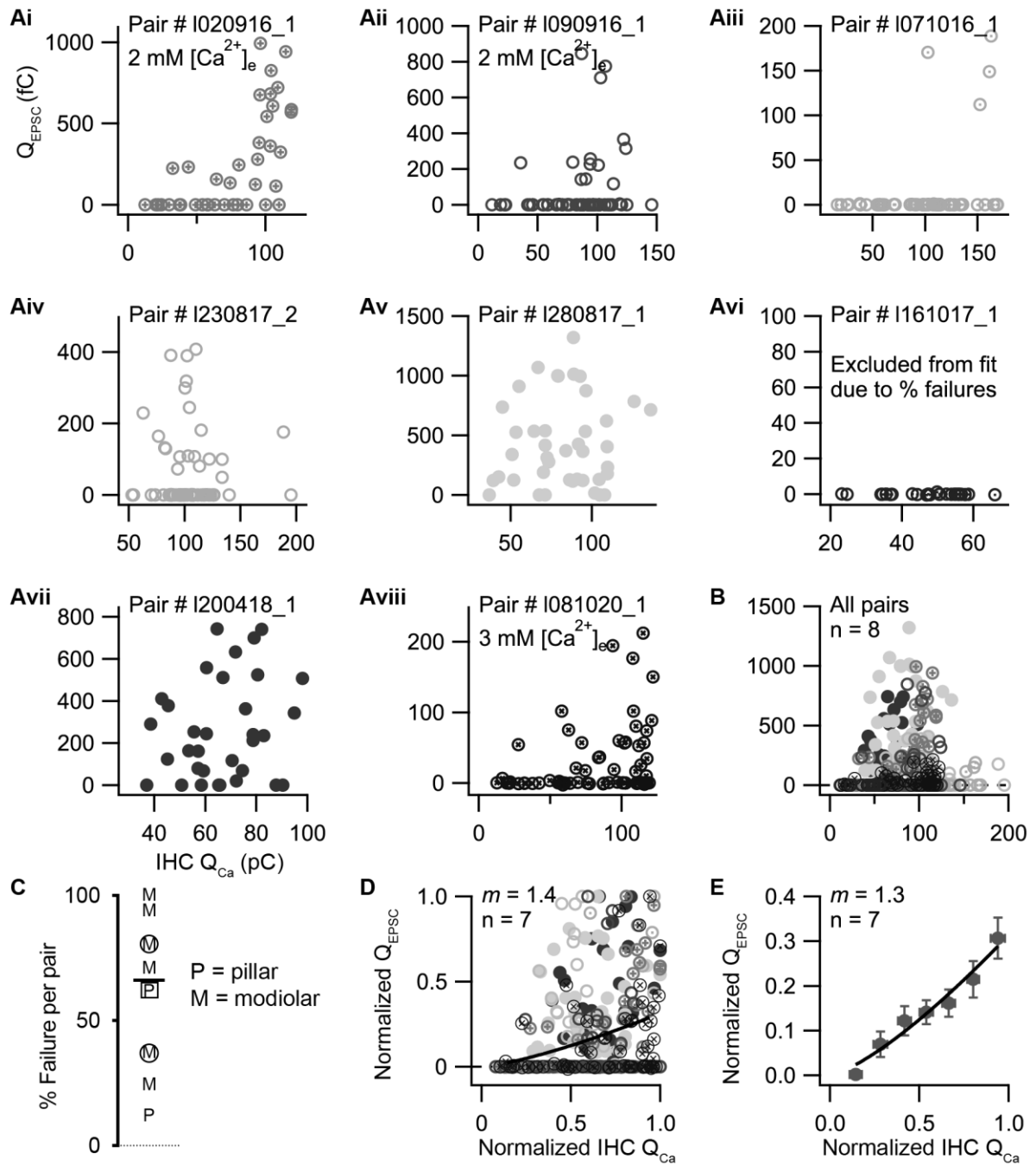
**Figure S2 Estimating the intrinsic  $Ca^{2+}$  dependence of SV release for each individual pair**

**Ai-Avii.** Scatter plots of the EPSC charges ( $Q_{EPSC}$ ) vs. the corresponding  $Ca^{2+}$  current integrals ( $Q_{Ca}$ ) for each individual pair during perfusion of 1 mM  $Zn^{2+}$  to reduce the *effective* fusogenic  $Ca^{2+}$ . The solid line is a least-squares fit of a power function ( $Q_{EPSC} = a(Q_{Ca})^m$ ) to each pair data. For pair # I080118\_1 (**Aiii**), the smaller markers represent the data recorded while washing-out  $Zn^{2+}$ , which was also included in the fitting. **B.** Scatter plot of the EPSC charges ( $Q_{EPSC}$ ) vs. the corresponding  $Ca^{2+}$  current integrals ( $Q_{Ca}$ ) of all pairs: different markers and shades of gray for the different pairs ( $n = 7$ ). **C.**  $Ca^{2+}$  cooperativity ( $m$ ) estimated for each individual pair shown in A. P indicates the boutons contacting the pillar side of the IHC; M indicates the boutons contacting the modiolar side of the IHC. Gray bar corresponds to the median.



**Figure S3. Apparent  $\text{Ca}^{2+}$  dependence of SV release for each individual pair during isradipine application**

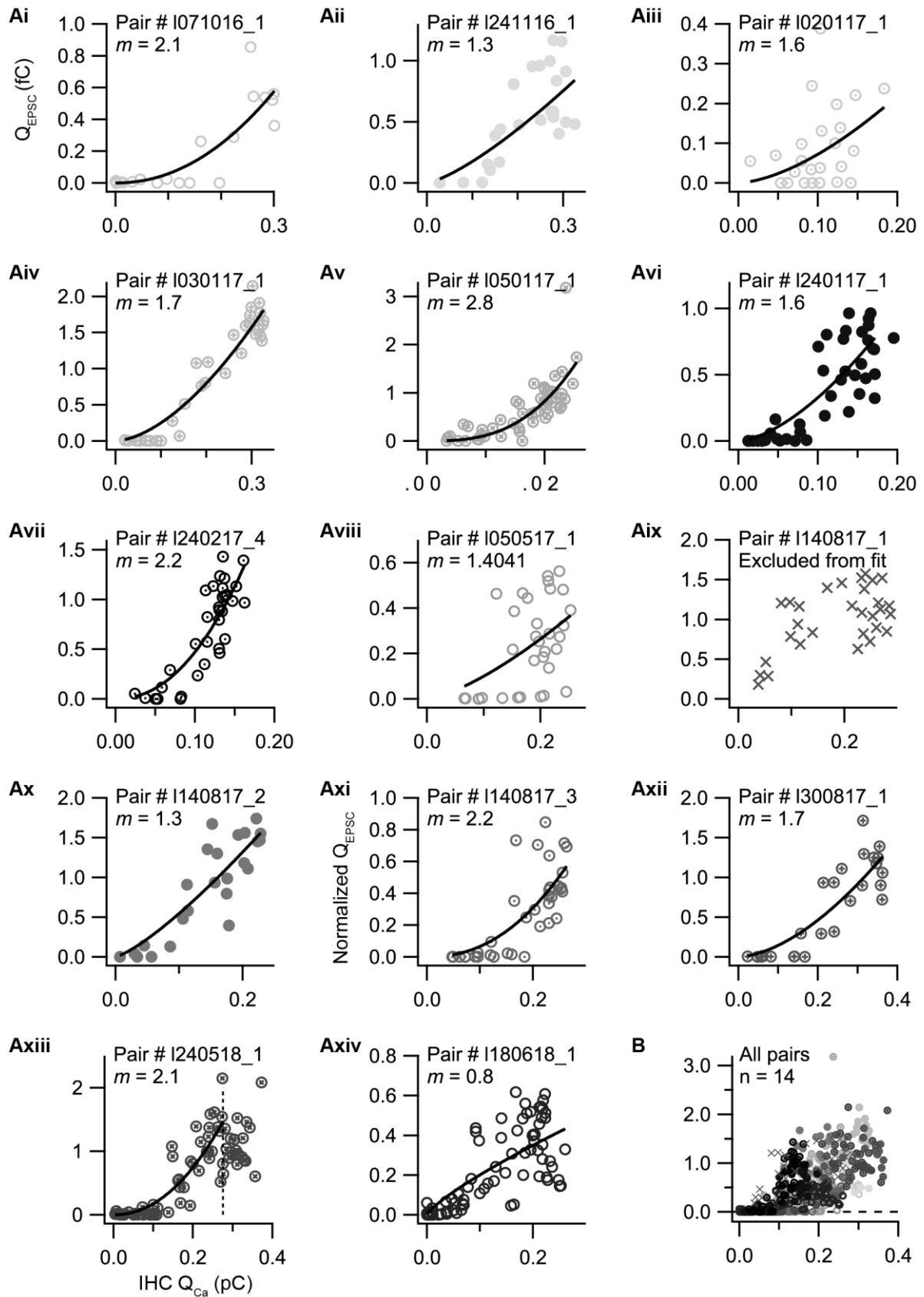
**Ai-Avii.** Scatter plots of the EPSC charges ( $Q_{\text{EPSC}}$ ) vs. the corresponding  $\text{Ca}^{2+}$  current integrals ( $Q_{\text{Ca}}$ ) for each individual pair during perfusion  $0.5 - 2 \mu\text{M}$  of the dihydropyridine isradipine to progressively shift the  $\text{Ca}^{2+}$  channels to a non-conducting state. The solid line is a least-squares fit of a power function ( $Q_{\text{EPSC}} = a(Q_{\text{Ca}})^m$ ) to each pair data. For pairs # I050318\_1 and # I100418\_1, the smaller markers represent the data recorded while washing-out isradipine, which was also included in the fitting. **B.** Scatter plot of the EPSC charges ( $Q_{\text{EPSC}}$ ) vs. the corresponding  $\text{Ca}^{2+}$  current integrals ( $Q_{\text{Ca}}$ ) of all pairs: different markers and shades of gray for the different pairs ( $n = 7$ ). **C.**  $\text{Ca}^{2+}$  cooperativity ( $m$ ) estimated for each individual pair shown in A. P indicates the boutons contacting the pillar side of the IHC; M indicates the boutons contacting the modiolar side of the IHC. Gray bar corresponds to the median.



**Figure S4. Apparent  $Ca^{2+}$  dependence of SV release during  $Ca^{2+}$  tail-current experiments**

**Ai-Aviii.** Scatter plots of the EPSC charges ( $Q_{EPSC}$ ) vs. the corresponding  $Ca^{2+}$  current integrals ( $Q_{Ca}$ ) for each individual pair during deactivating (tail) currents upon repolarization **B.** Scatter plots of the EPSC charges ( $Q_{EPSC}$ ) vs. the corresponding  $Ca^{2+}$  current integrals ( $Q_{Ca}$ ) for all pairs: different markers and shades of gray for the different pairs ( $n = 8$ ). **C.** Percentage of failure of synaptic transmission for each individual pair shown in A. P indicates the boutons contacting the pillar side of the IHC; M indicates the boutons contacting the modiolar side of the IHC. Circles and squares are the pairs recorded at 2 mM and 3 mM extracellular  $[Ca^{2+}]$ , respectively. Black bar represents to the median. **D.** Scatter plots of the normalized EPSC charges ( $Q_{EPSC}$ ) vs. the corresponding normalized  $Ca^{2+}$  current integrals ( $Q_{Ca}$ ) for all pairs: different markers and shades of gray for the different pairs ( $n = 7$ ). The solid line is a least-squares fit of a power function ( $Q_{EPSC} = a(Q_{Ca})^m$ ) to the normalized population data for  $Q_{EPSC}$  and  $Q_{Ca}$  yielded  $m_{tails}$  of 1.4 ( $n = 7$  pairs). **E.** Power function fit to the binned normalized data (bin size  $\sim 0.15$ ;

data points are mean  $\pm$  SEM) from (C) resulted in  $m_{tails}$  of 1.3.



**Figure S5. Apparent  $Ca^{2+}$  dependence of SV release in the range of IHC receptor potentials**

**Ai-Axiv.** Scatter plots of the EPSC charges ( $Q_{EPSC}$ ) vs. the corresponding  $Ca^{2+}$  current integrals ( $Q_{Ca}$ ) for each individual pair in response to 2 ms depolarizations to randomized voltages in the hyperpolarized range. The solid line is a least-squares fit of a power function ( $Q_{EPSC} = a(Q_{Ca})^m$ ) to each pair data. Pair #

l140817\_1 (Aix) was excluded because release was not completely abolished even at resting potential.

**B.** Scatter plots of the EPSC charges ( $Q_{\text{EPSC}}$ ) vs. the corresponding  $\text{Ca}^{2+}$  current integrals ( $Q_{\text{Ca}}$ ) for all pairs: different markers and shades of gray for the different pairs ( $n = 14$ ).