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Figures and figure supplements

Protein kinase C is a calcium sensor for presynaptic short-term plasticity

Diasynou Fioravante, et al.

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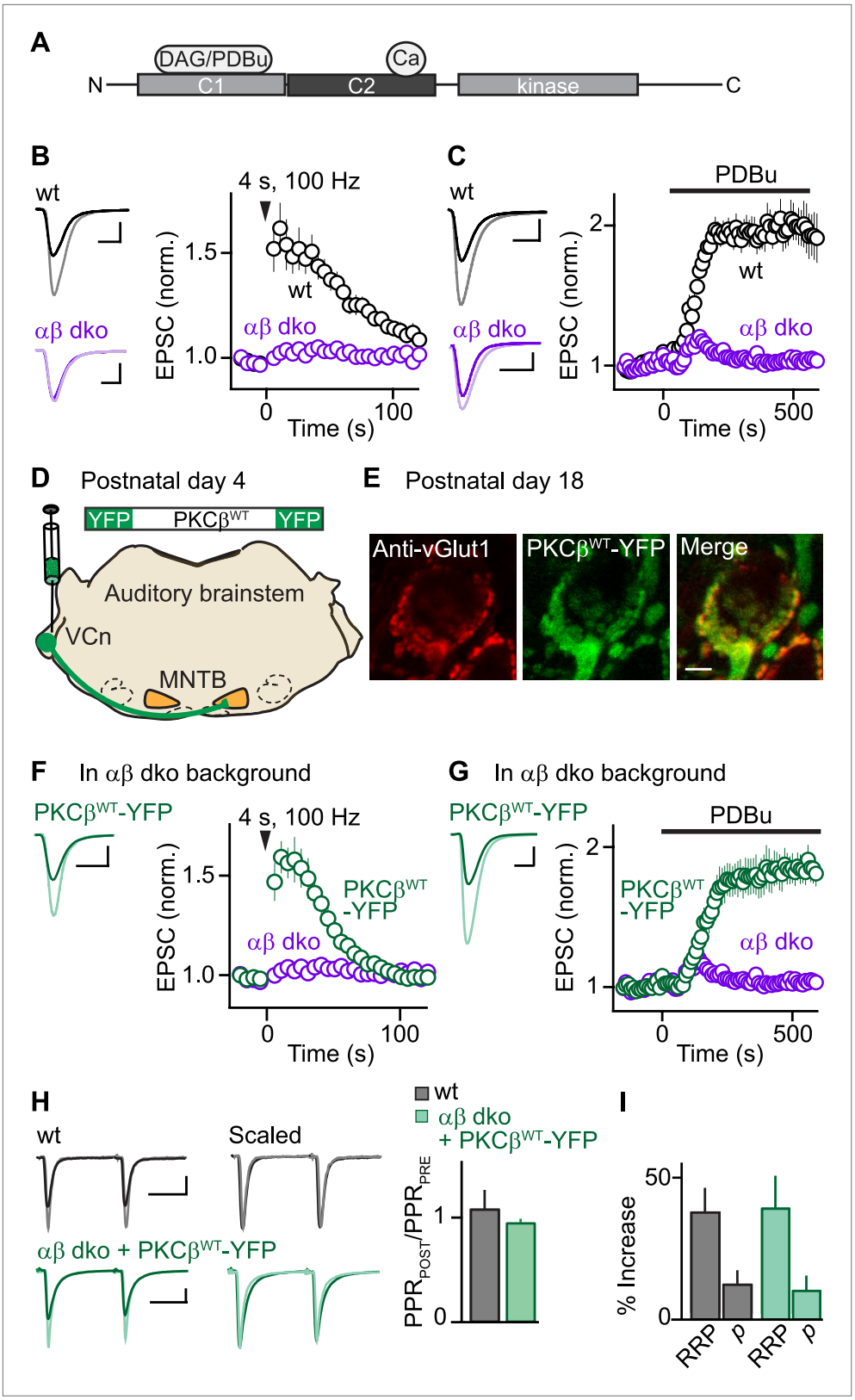

Figure 1. Expression of PKCβ rescues synaptic potentiation in animals lacking calcium-dependent PKCs. Synaptic plasticity was examined at the calyx of Held following tetanic stimulation (**B** and **F**) or bath application of the phorbol ester PDBu (**C** and **G**) for wild-type (wt, *black*), PKCαβ dko animals (*purple*), and PKCαβ dko animals

Figure 1. Continued on next page

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Figure 1. Continued

expressing PKCβ^{WT}-YFP (*green*). (**A**) Domain arrangement of PKC$_{Ca}$. DAG and PDBu bind to the C1 domain and Ca^{2+} binds to the C2 domain. (**B**, **C**, **F**, **G**) Left, example EPSCs recorded prior to (*bold traces*) and after (*light traces*) synaptic enhancement for each experimental condition. Right, EPSCs are plotted as a function of time (mean ± SEM). For (**B**), wild-type: 62 ± 12%; αβ dko: 2.4 ± 1.8%. Also see ***Figure 1—figure supplement 1*** and accompanying legend for PTP induced under elevated-temperature conditions. Similar to PTP induced at room temperature, PTP at near-physiological temperature requires PKC$_{Ca}$ (***Figure 1—figure supplement 2***). For (**C**), at steady state: wild-type: 97 ± 12%; αβ dko: 3.2 ± 3.4%; for (**F**), PKCβ^{WT}-YFP: 61 ± 7%; for (**G**), 84 ± 11%. In **F** and **G**, the αβ dko group data from **B** and **C** respectively are re-plotted for comparison. Also see ***Figure 1—figure supplement 3*** and ***Figure 1—figure supplement 4***. (**D**) In this schematic of the auditory brainstem, the ventral cochlear nucleus (VCn) and medial nuclei of the trapezoid body (MNTB) are labeled. An AAV expressing PKCβ^{WT}-YFP was injected in the VCn at postnatal day 4. (**E**) Confocal images of a brain section labeled with an antibody against vGlut1 (*red*) are shown for a calyx of Held expressing PKCβ^{WT}-YFP (*green*) in a PKCαβ dko animal at postnatal day 18. Scale bar: 10 μm. (**H** and **I**) The synaptic mechanism through which PKCβ rescues PTP was examined under conditions that relieve AMPA receptor desensitization and saturation. (**H**) Left, overlay of EPSCs (10 ms inter-stimulus interval) delivered prior to (*bold traces*) and 10 s after (*light traces*) PTP-inducing tetanus. Middle, traces are normalized to the first EPSC to allow comparison of PPR. Right, PPR$_{POST}$ (after tetanus) over PPR$_{PRE}$ (before tetanus) (mean ± SEM, see ***Figure 1—source data 1 and 2***). Wild-type: p=0.49; αβ dko expressing PKCβ^{WT}-YFP: p=0.68. (**I**) Summary of the readily releasable pool (RRP) and release probability (*p*) contributions to PTP (mean ± SEM, also see ***Figure 1—figure supplements 5 and 6*** and ***Figure 1—source data 1 and 2***). RRP$_{WT}$: 37 ± 9%; RRP$_{PKC\beta WT\text{-}YFP}$: 39 ± 12%; p=0.88. Scale bars in **B**, **C**, **F**, and **G**: 2 nA, 1 ms. Scale bars in **H**: 2 nA, 5 ms.

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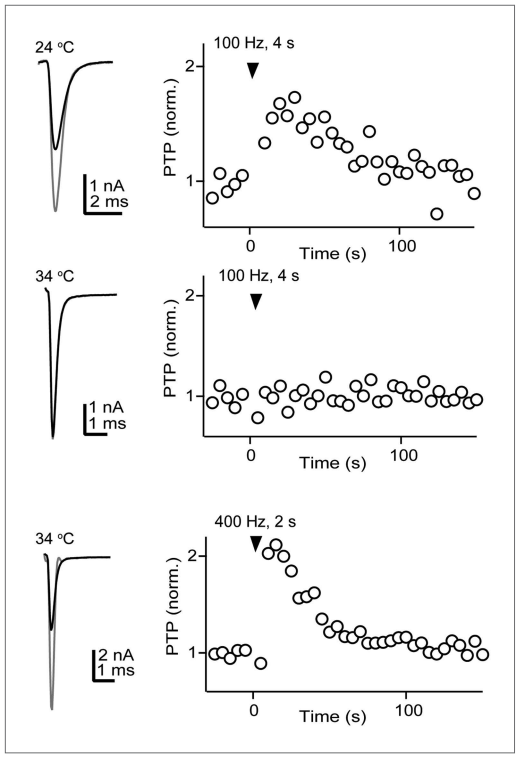

Figure 1—figure supplement 1. PTP can be induced under near-physiological conditions.

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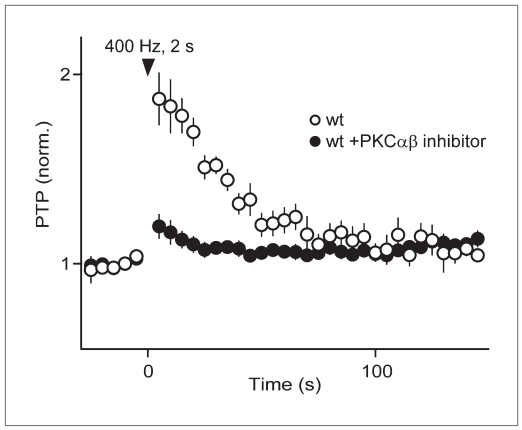

Figure 1—figure supplement 2. Under near-physiological conditions PTP is mediated by PKC_{Ca}.
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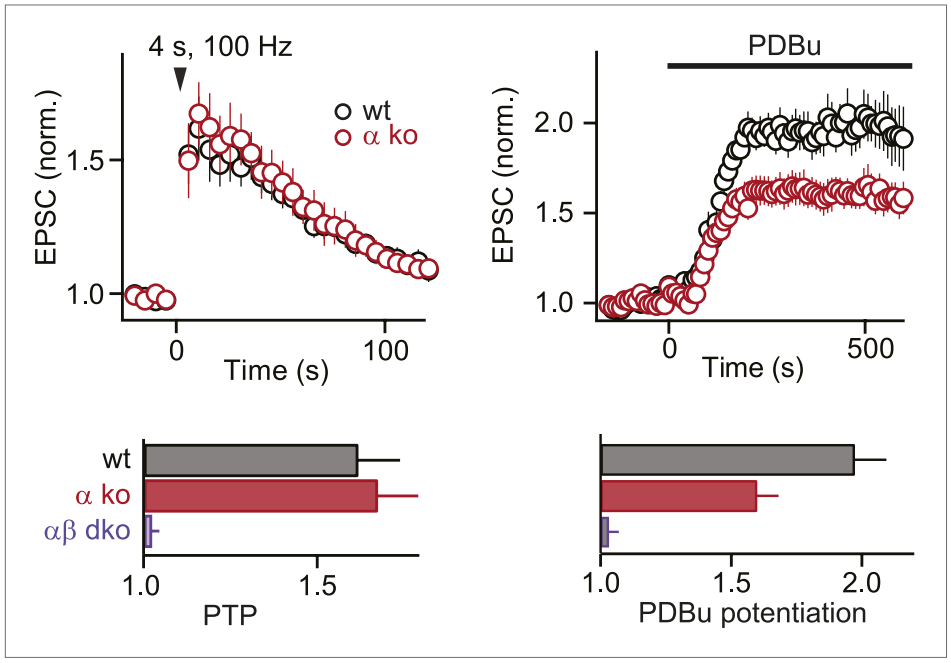

Figure 1—figure supplement 3. At the functionally mature calyx of Held, PKCα does not contribute to PTP but plays a small role in phorbol ester-induced potentiation.
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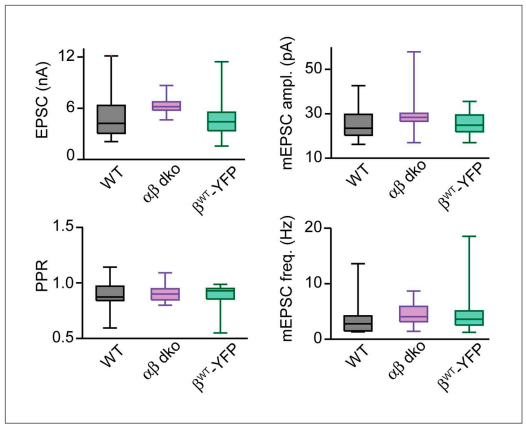

Figure 1—figure supplement 4. PKC_{Ca} isoforms do not regulate basal synaptic properties.
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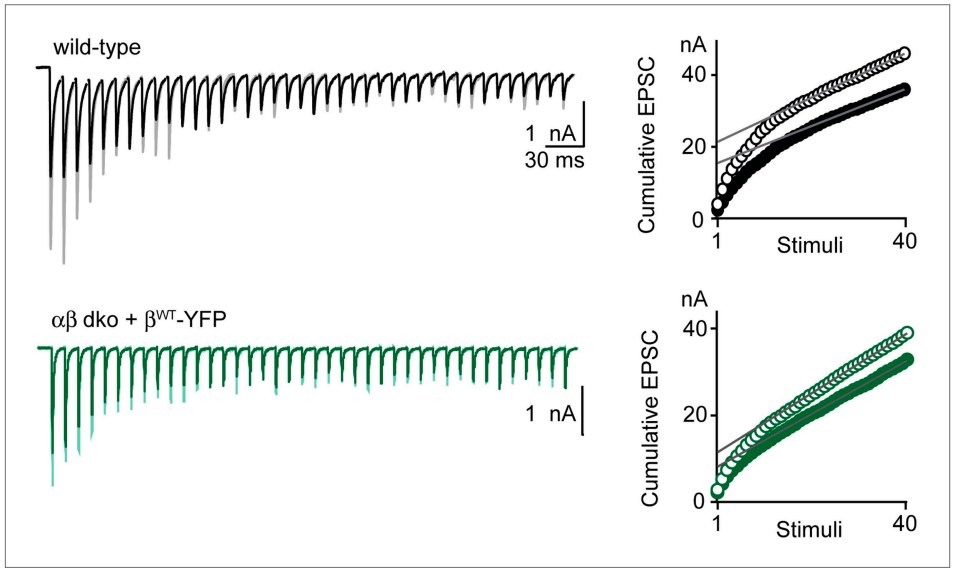

Figure 1—figure supplement 5. Determining the contributions of RRP and *p* in wild-type and rescued PTP at the functionally mature calyx of Held.
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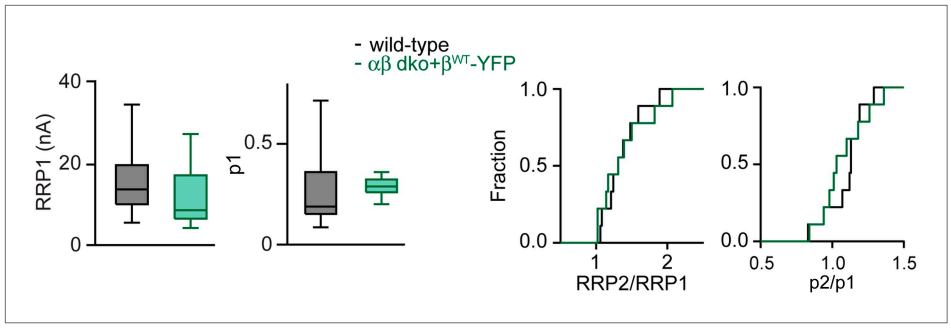

Figure 1—figure supplement 6. Determining the contributions of RRP and *p* in wild-type and rescued PTP at the functionally mature calyx of Held.

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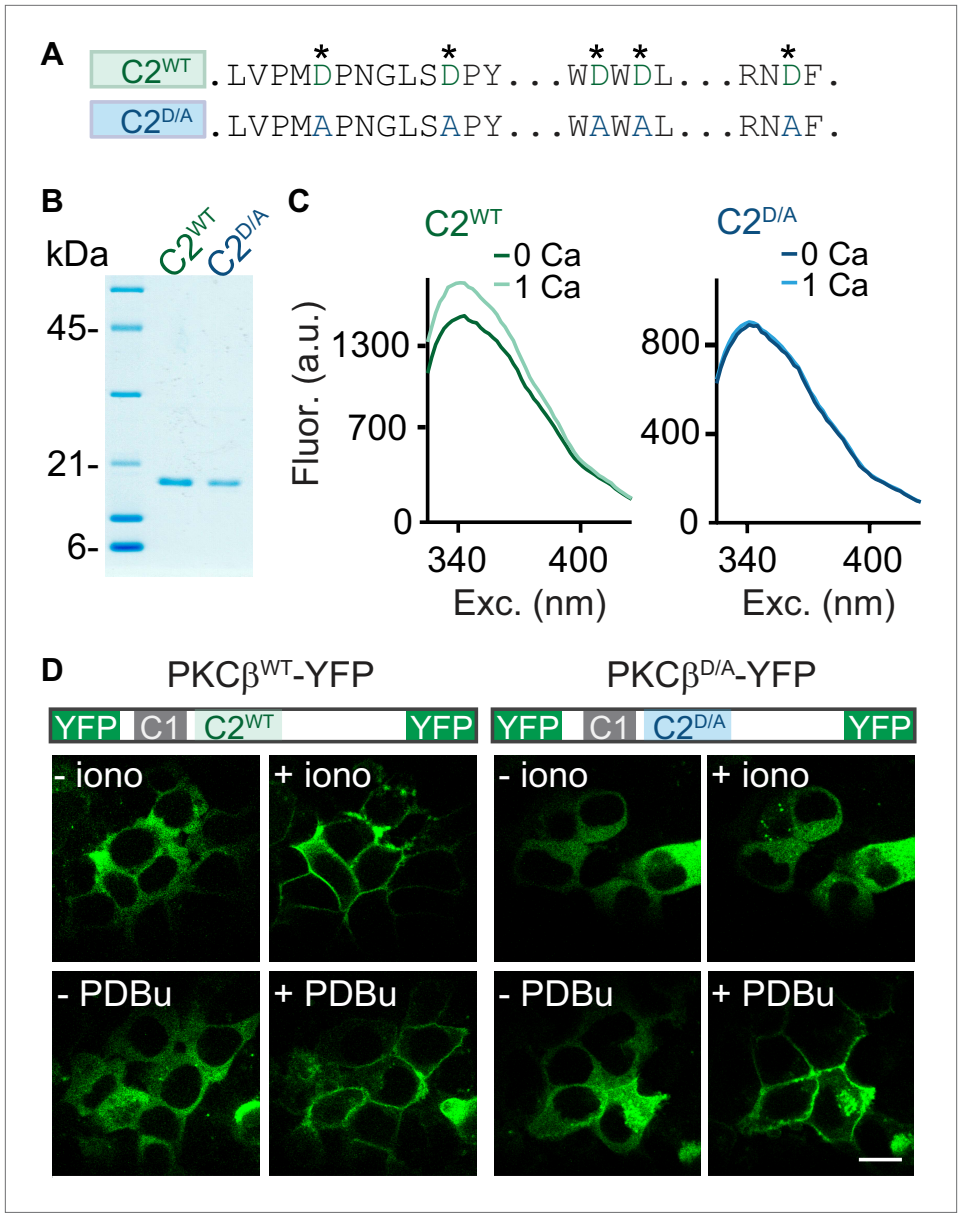

Figure 2. C2-domain mutations of PKCβ abolish Ca^{2+} binding and Ca^{2+}-induced translocation without impairing phorbol ester-induced translocation. (**A**) A partial sequence of the PKCβ C2 domain is shown with Ca^{2+}-coordinating aspartates in green. These aspartates were mutated to alanines (*blue*) in the $C2^{D/A}$ construct. Also see ***Figure 2—figure supplement 1***. (**B**) Coomassie blue-stained gel of recombinant wild-type ($C2^{WT}$) and mutant ($C2^{D/A}$) PKCβ C2 domains. (**C**) Averaged intrinsic tryptophan fluorescence is shown for $C2^{WT}$ and $C2^{D/A}$. Fluorescence emission spectra were recorded in 0 mM Ca^{2+} (*bold traces*) and 1 mM Ca^{2+} (*light traces*). Peak fluorescence intensity change: $C2^{WT}$:17 ± 1.3%; $C2^{D/A}$: −1.3 ± 2.0%. (**D**) Translocation of $PKC\beta^{WT}$-YFP (left) and $PKC\beta^{D/A}$-YFP (right) in HEK293T cells was monitored in response to the Ca^{2+} ionophore ionomycin and in response to PDBu. Ca^{2+} increases caused $PKC\beta^{WT}$-YFP to translocate, but not $PKC\beta^{D/A}$-YFP. Both $PKC\beta^{WT}$-YFP and $PKC\beta^{D/A}$-YFP translocated in response to PDBu. Scale bar: 10 μm.

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PKCβ $C2^{WT}$

157 ERRGRIYIQAHIDREVLIVVVRDAKNLVPMDPNGLSDPYVKLKLIPDPKSESKQKTKTIK

217 CSLNPEWNETFRFQLKESDKDRRLSVEIWDWDLTSRNDFMGSLSFGISELQKAGVDGWFK

277 LLSQEEGEYFNVPVPPEG

PKCβ $C2^{D/A}$

157 ERRGRIYIQAHIDREVLIVVVRDAKNLVPMAPNGLSAPYVKLKLIPDPKSESKQKTKTIK

217 CSLNPEWNETFRFQLKESDKDRRLSVEIWAWALTSRNAFMGSLSFGISELQKAGVDGWFK

277 LLSQEEGEYFNVPVPPEG

Figure 2—figure supplement 1. Protein sequence alignment for PKCβ $C2^{WT}$ and PKCβ $C2^{D/A}$.

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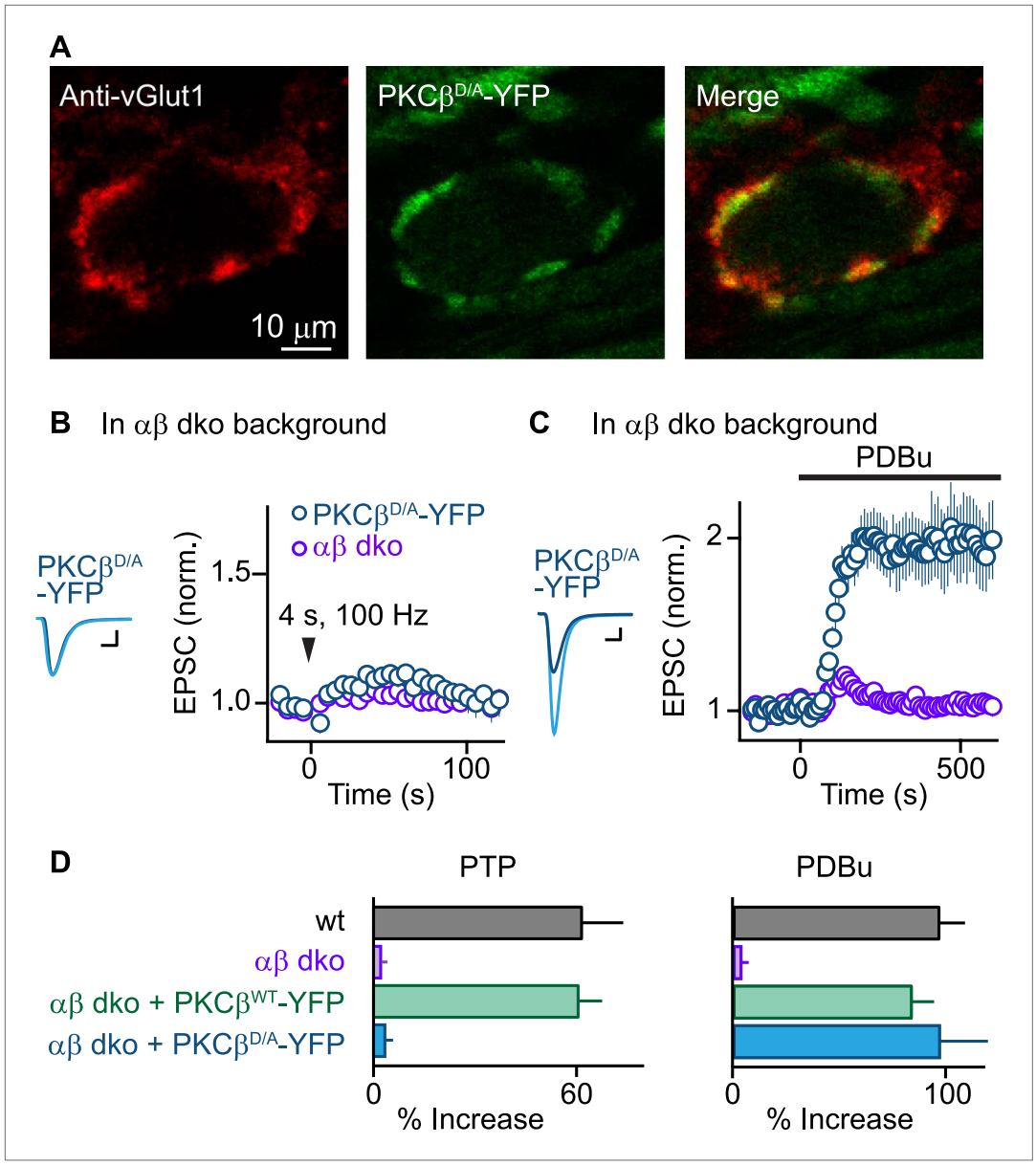

Figure 3. PTP requires Ca^{2+} binding to PKCβ but phorbol ester-induced potentiation does not. (**A**) Confocal images of a brain section labeled with an antibody against vGlut1 (*red*) are shown for a calyx of Held expressing $PKC\beta^{D/A}$-YFP (*green*) in a PKCαβ dko animal. (**B** and **C**) Synaptic plasticity was examined in PKCαβ dko animals at calyces of Held expressing Ca^{2+}-insensitive PKCβ ($PKC\beta^{D/A}$-YFP, *blue traces*). Representative traces and time-courses (mean ± SEM) are shown following tetanic stimulation (**B**) and during bath application of PDBu (**C**). For (**B**), $PKC\beta^{D/A}$: 3.6 ± 2.2%; for (**C**), $PKC\beta^{D/A}$: 98 ± 23%. Scale bars: 1 nA, 1 ms. In (**B** and **C**), the αβ dko group data from ***Figure 1B,C*** respectively are re-plotted for comparison. For basal synaptic properties of the $PKC\beta^{D/A}$-YFP-expressing group, see ***Figure 3—figure supplement 1*** and ***Figure 3—source data 2***. (**D**) Summary plots (mean ± SEM) of the magnitude of synaptic enhancement produced by tetanic stimulation (left) and by PDBu (right). Source data are provided in ***Figure 3—source data 1 and 2***. See also ***Figure 1—source data 1 and 2***.

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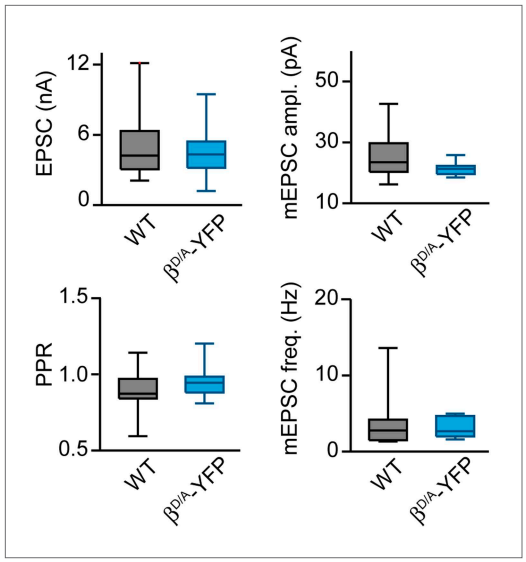

Figure 3—figure supplement 1. Basal synaptic properties are not altered by PKCβ$^{D/A}$-YFP expression.
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