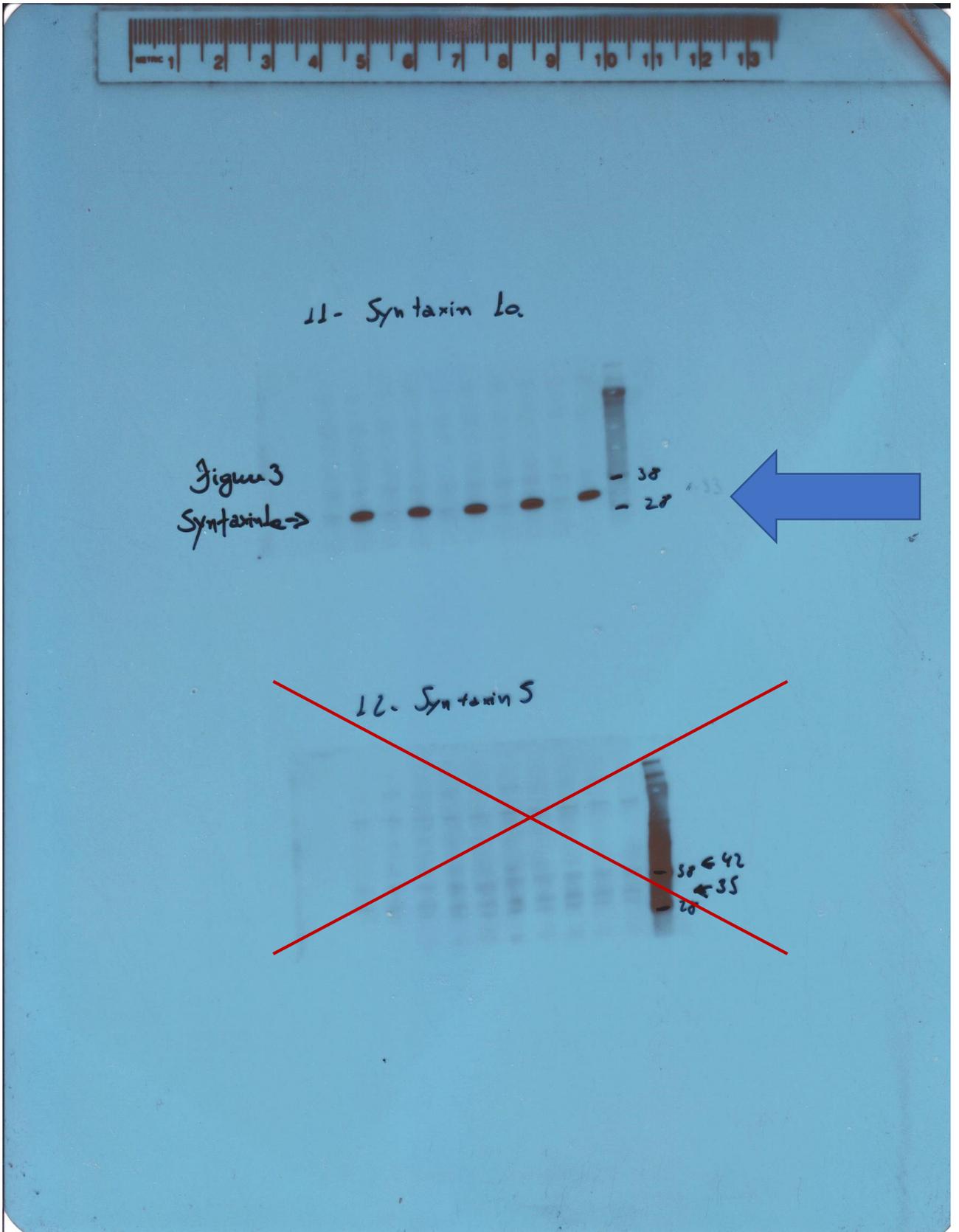
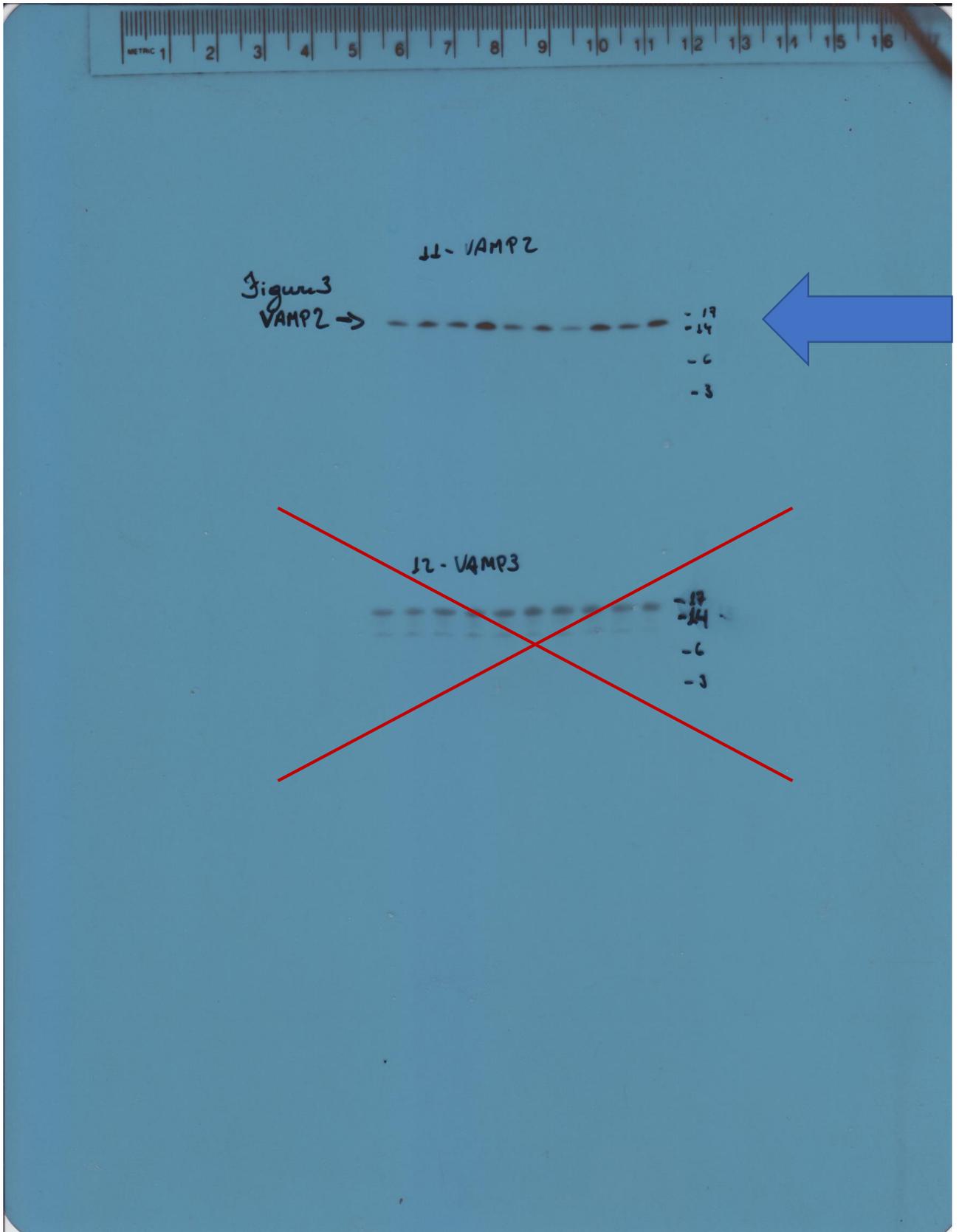


Figure S1. Validation of *Ins1* and *Ins2* TaqMan probes and insulin and C-peptide antibodies. (A) Amplification Plot from qPCR reactions of cDNA derived from choroid plexus of wild type, *Ins1*^{-/-}*Ins2*^{+/+}, and *Ins1*^{+/+}*Ins2*^{-/-} mice, showing specific amplification of *Ins1* (blue) and *Ins2* (green). Actin was used as an endogenous control (pink). **(B)** Relative expression levels of *Ins1* and *Ins2* in the lateral and fourth ventricle choroid plexi of wild type, *Ins1*^{-/-}*Ins2*^{+/+}, and *Ins1*^{+/+}*Ins2*^{-/-}. Levels are represented relative to *Ins2* in wild type mice. Lateral Ventricle (WT: *Ins1* = 0.0098 ± 0.0021, *Ins2* = 1.00 ± 0.072; *Ins1*^{-/-}*Ins2*^{+/+}: *Ins1* = not detectable, *Ins2* = 1.26 ± 0.071; *Ins1*^{+/+}*Ins2*^{-/-}: *Ins1* = 0.024 ± 0.0056, *Ins2* = not detectable; n = 4). * p<0.05, **p<0.01, as calculated by one-way ANOVA with Tukey's post-hoc test. Results are shown and described as mean ± SEM. **(C, D)** Insulin staining in brain sections of wild type (C) or *Ins1*^{-/-}, *Ins2*^{-/-} double knockout (D) mice. For this experiment, brains were collected from newborn animals (P0-P2) and its

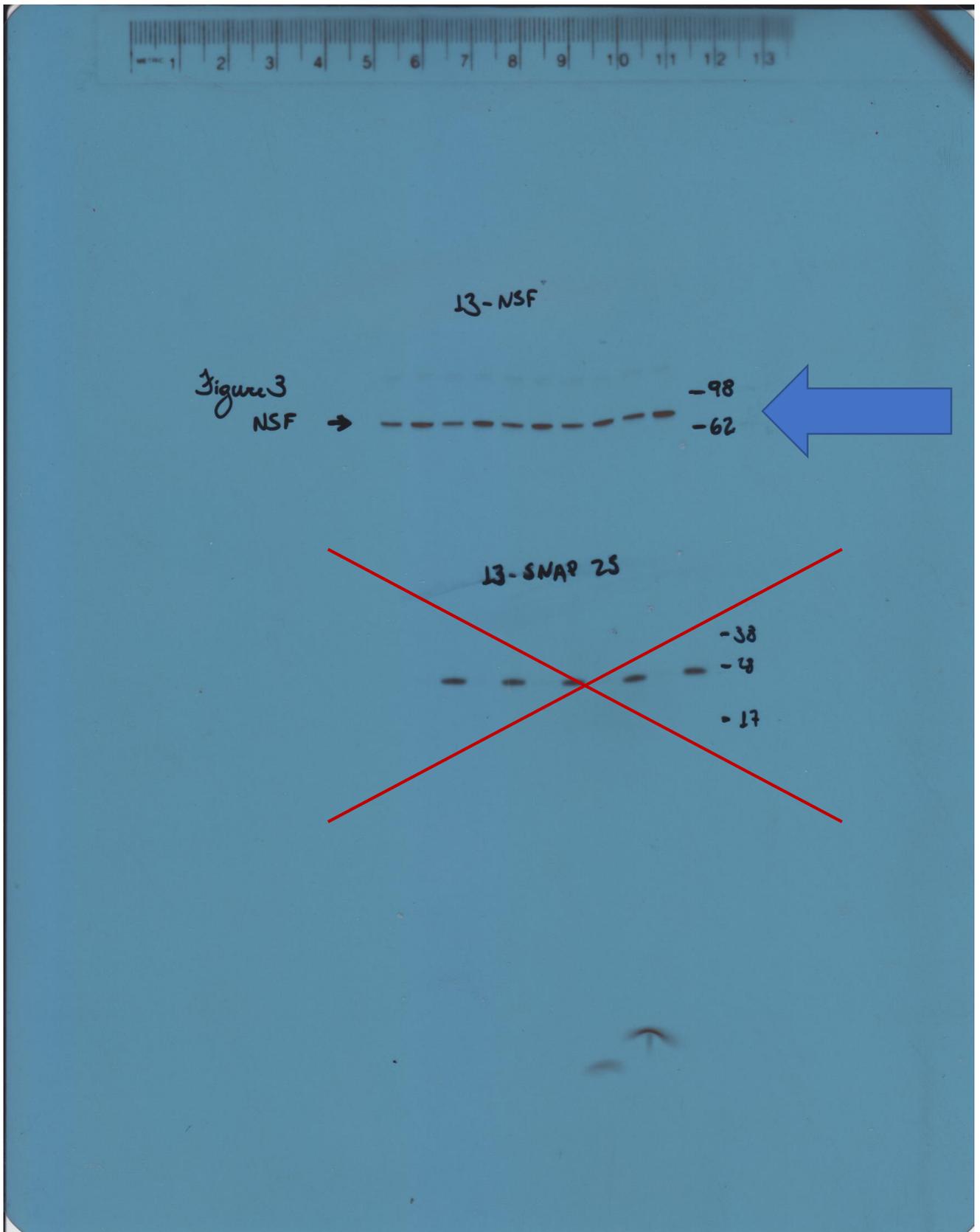
FULL UNEDITED GEL FOR
FIGURE 3: STX1A



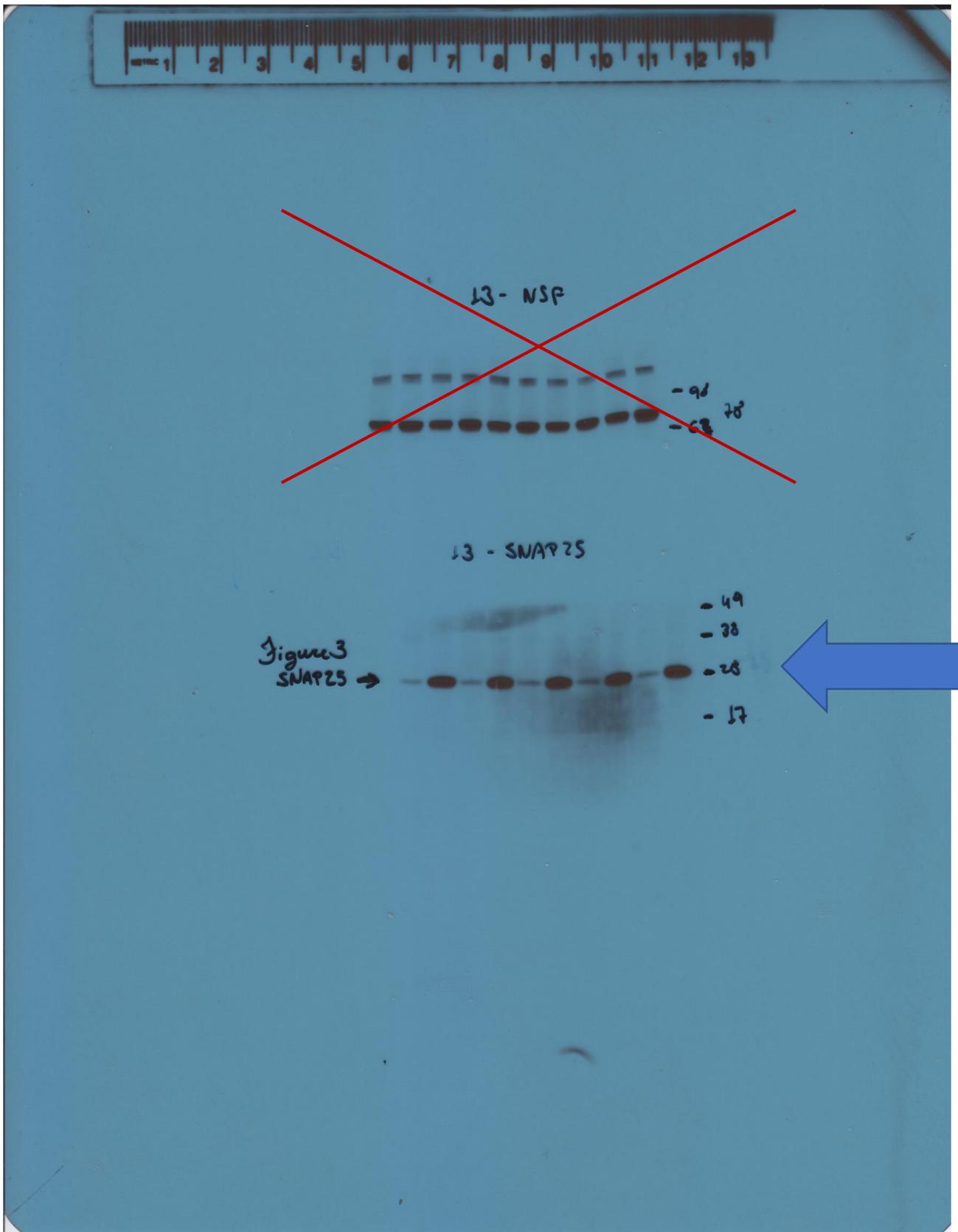
FULL UNEDITED GEL FOR
FIGURE 3: VAMP2



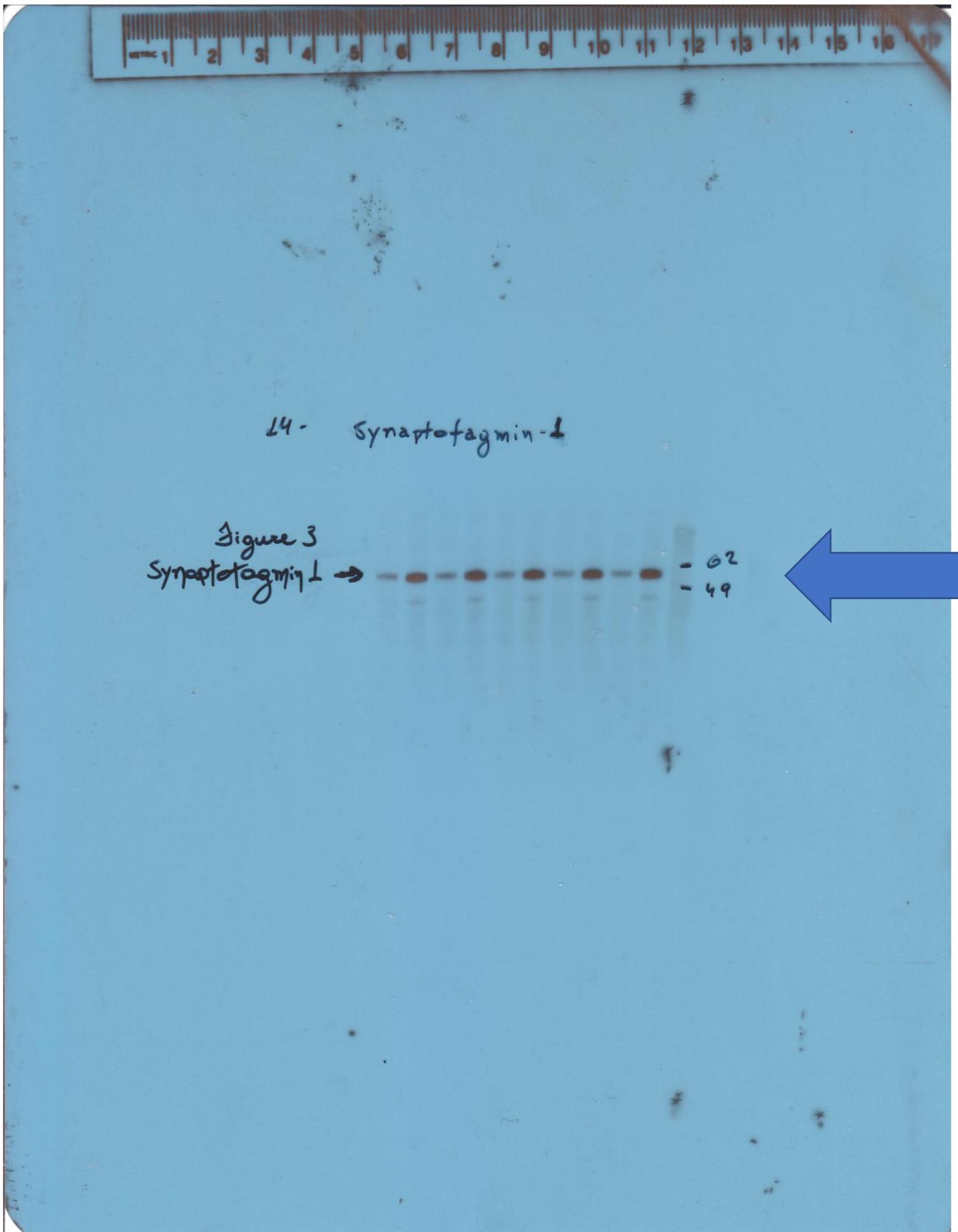
FULL UNEDITED GEL FOR
FIGURE 3: NSF



FULL UNEDITED GEL FOR
FIGURE 3: SNAP25



FULL UNEDITED GEL FOR
FIGURE 3: SYNAPTOTAGMIN-1



FULL UNEDITED GEL FOR
FIGURE 3: KLOTHO

14 - Klotho

Figure 3
Klotho →
Both bands
used for
quantification

- 198
- 98
- 62
- 49
- 38



FULL UNEDITED GEL FOR
FIGURE 3: ACTIN

Actin

11

Figure 3
Actin →

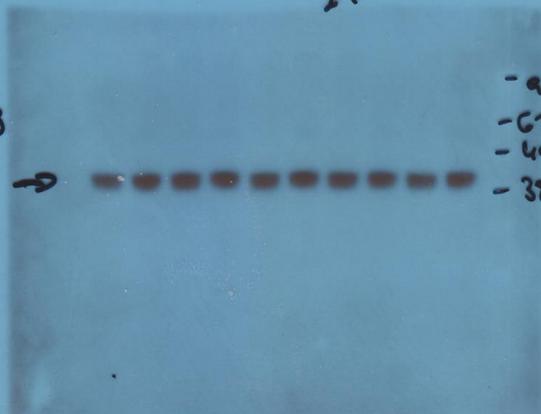


~~12~~

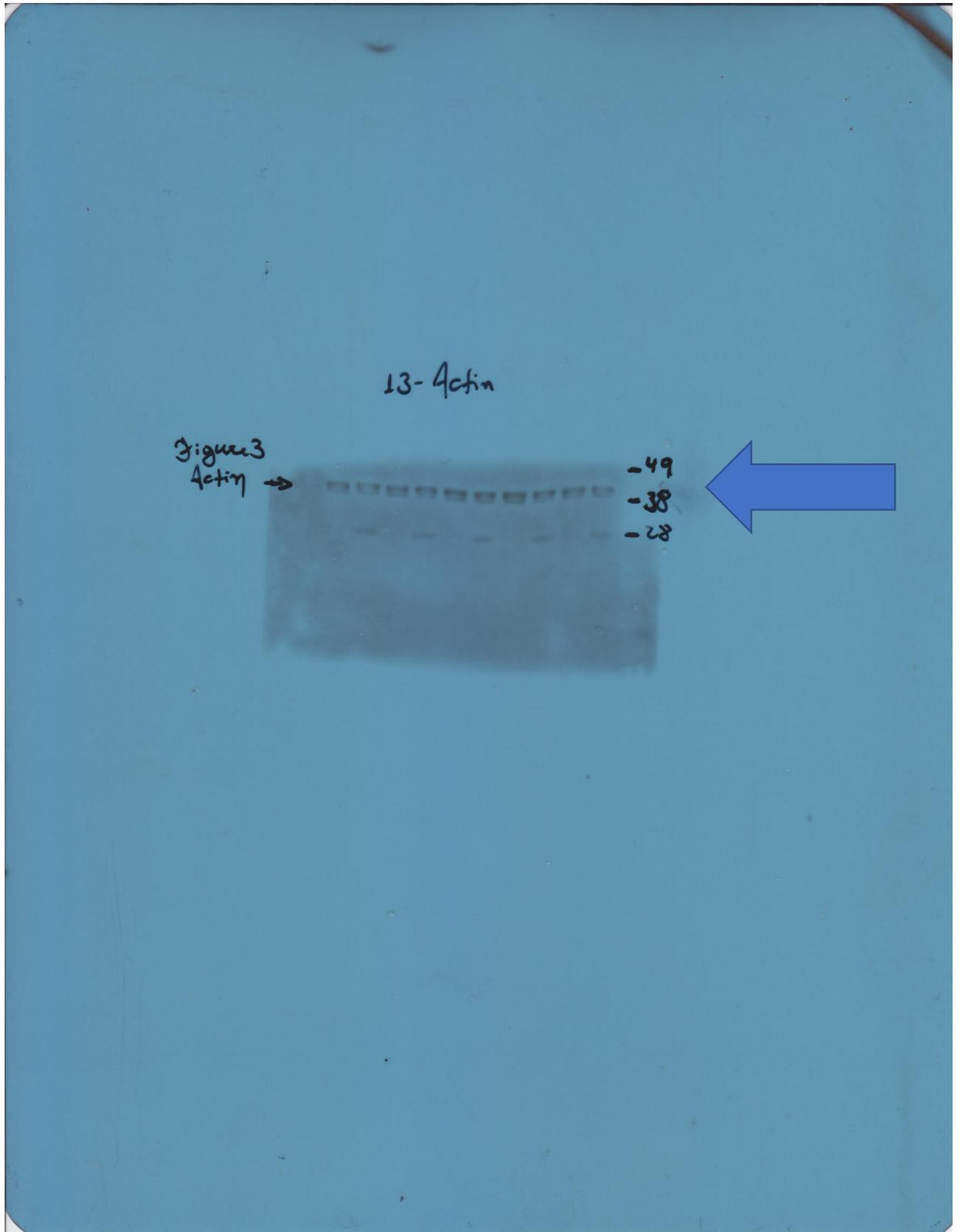
~~- 98
- 62
- 49
- 38 ← 42~~

14

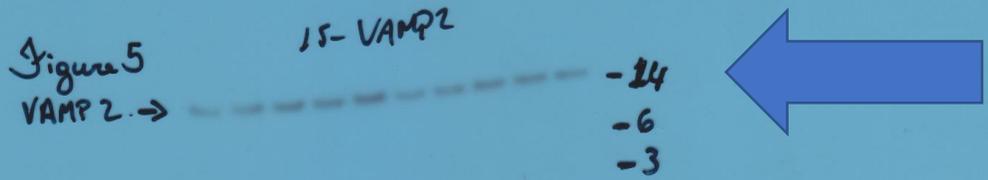
Figure 3
Actin →



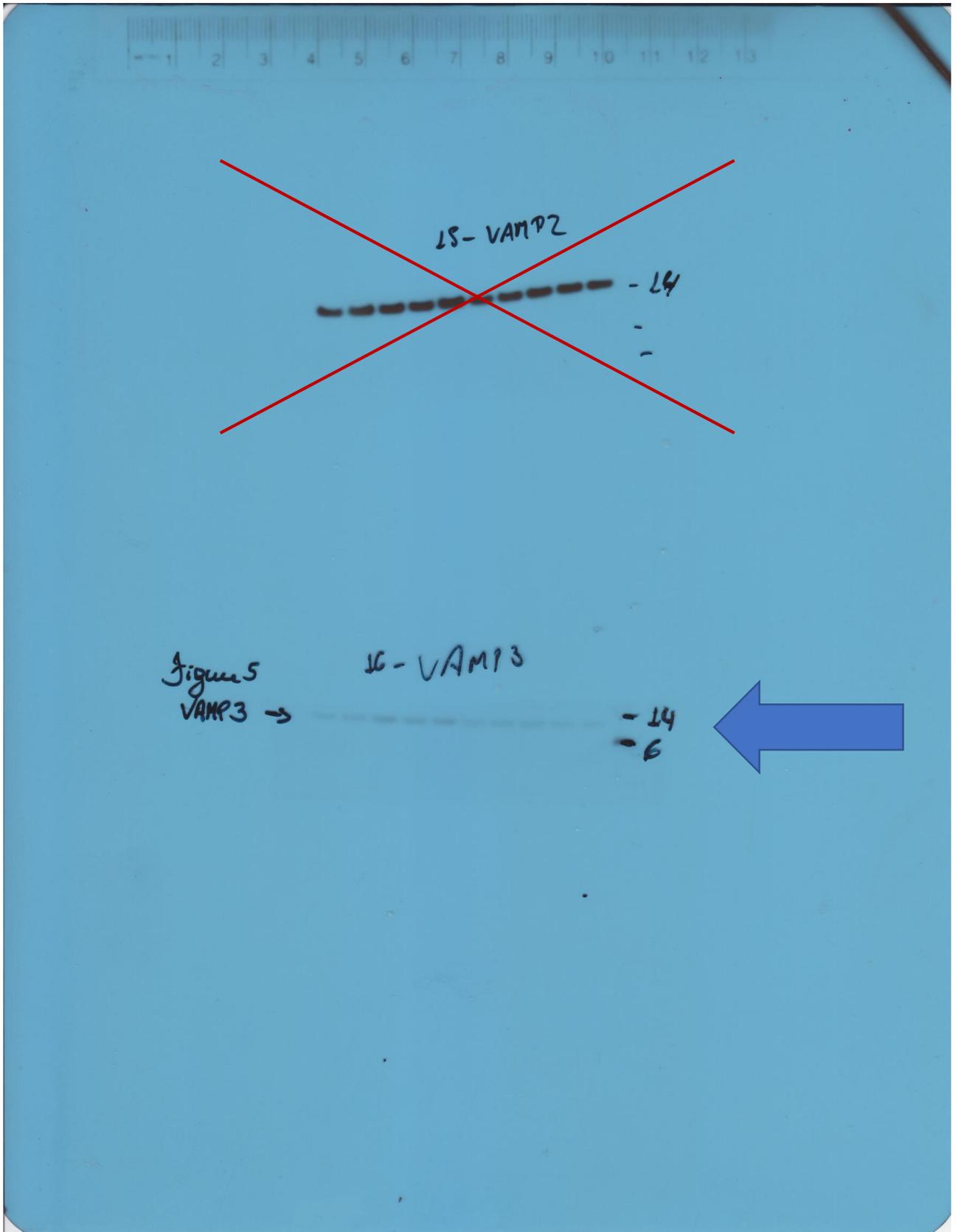
FULL UNEDITED GEL FOR
FIGURE 3: ACTIN



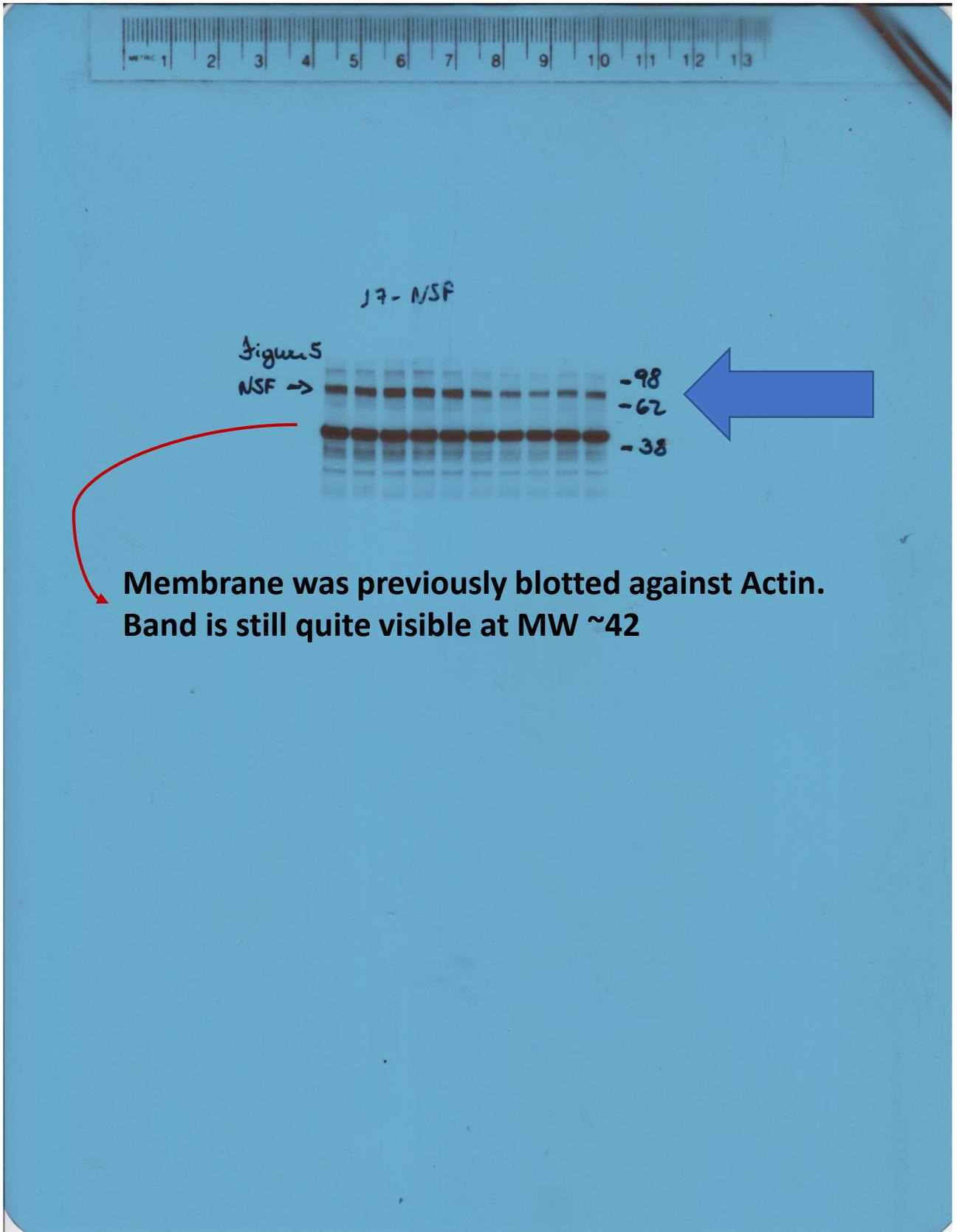
FULL UNEDITED GEL FOR
FIGURE 5: VAMP2



FULL UNEDITED GEL FOR
FIGURE 5: VAMP3



**FULL UNEDITED GEL FOR
FIGURE 5: NSF**



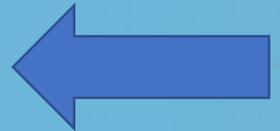
**Membrane was previously blotted against Actin.
Band is still quite visible at MW ~42**

FULL UNEDITED GEL FOR
FIGURE 5: ACTIN

17- Actin

Figure 5
Actin →

- 98
- 62
- 38



sections stained for insulin. Wild type animals show strong signal in the epithelial layer of the choroid plexus (C), while *Ins1*, *Ins2* double knockout mice appear to have no specific staining, often presenting, instead, artifacts in the center of the stromal matrix of the choroid plexus, where the blood vessels are. Scale bar = 20 μm .

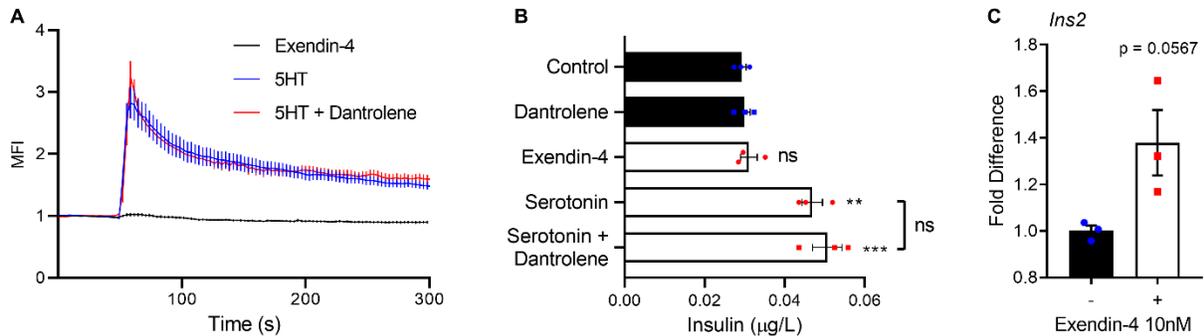


Figure S2. Ryanodine receptors and GLP1R signaling do not play a role in insulin secretion in the choroid plexus. (A) Intracellular calcium concentrations were evaluated in real time in primary cultured choroid plexus epithelial cells with Fluo-8. Exendin-4 (a GLP1R agonist) 10 nM failed to elicit any intracellular calcium changes. Ryanodine receptor antagonism with dantrolene 10 μM had no effects on serotonin-induced intracellular calcium increase, confirming the exclusive role of IP3Rs in this phenomenon. $N = 22\text{-}31$ individual cells from 3 independent cultures. (B) Primary choroid plexus epithelial cells were stimulated with different conditions for 4 hours and insulin concentration in the supernatant measured by ELISA. Exendin-4 10 nM was unable to induce insulin secretion, and dantrolene had no effect on serotonin-induced insulin secretion. (Control = 0.029 ± 0.0011 ; Serotonin = 0.047 ± 0.0026 ; Serotonin + Dantrolene = 0.050 ± 0.0037 , $n = 3$ independent cultures). One-way ANOVA, with Tukey's post-hoc test. ** $p < 0.01$, *** $p < 0.001$. (C) Treatment of primary choroid plexus epithelial cells with Exendin-4 for 4 hours had a positive effect on *Ins2* expression levels (Control = 1.00 ± 0.023 ; Exendin-4 = 1.38 ± 0.14 , $n = 3$ independent cultures, $p = 0.0567$, unpaired t test).