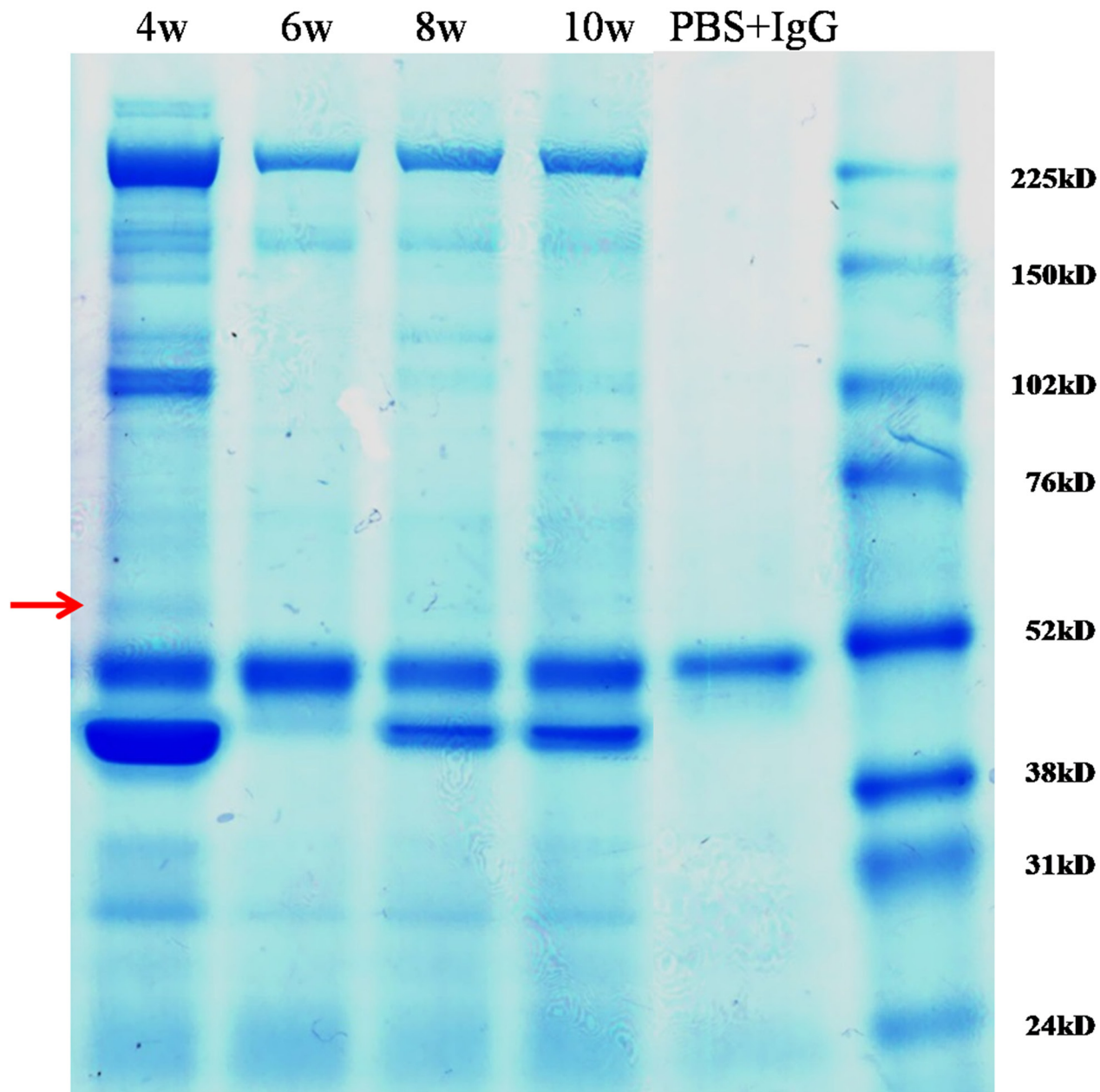
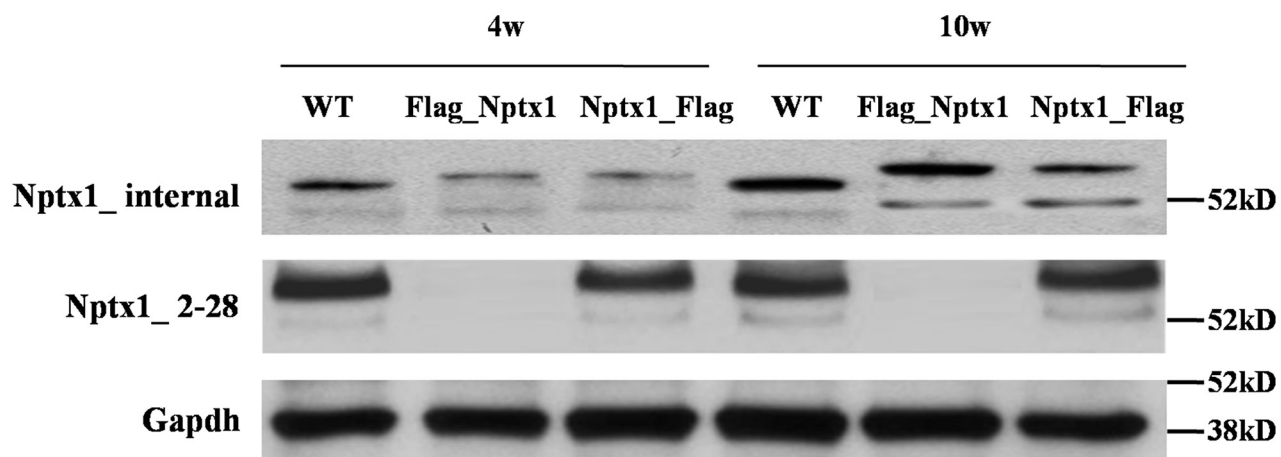


## Mkrn3 functions as a novel ubiquitin E3 ligase to inhibit Nptx1 during puberty initiation

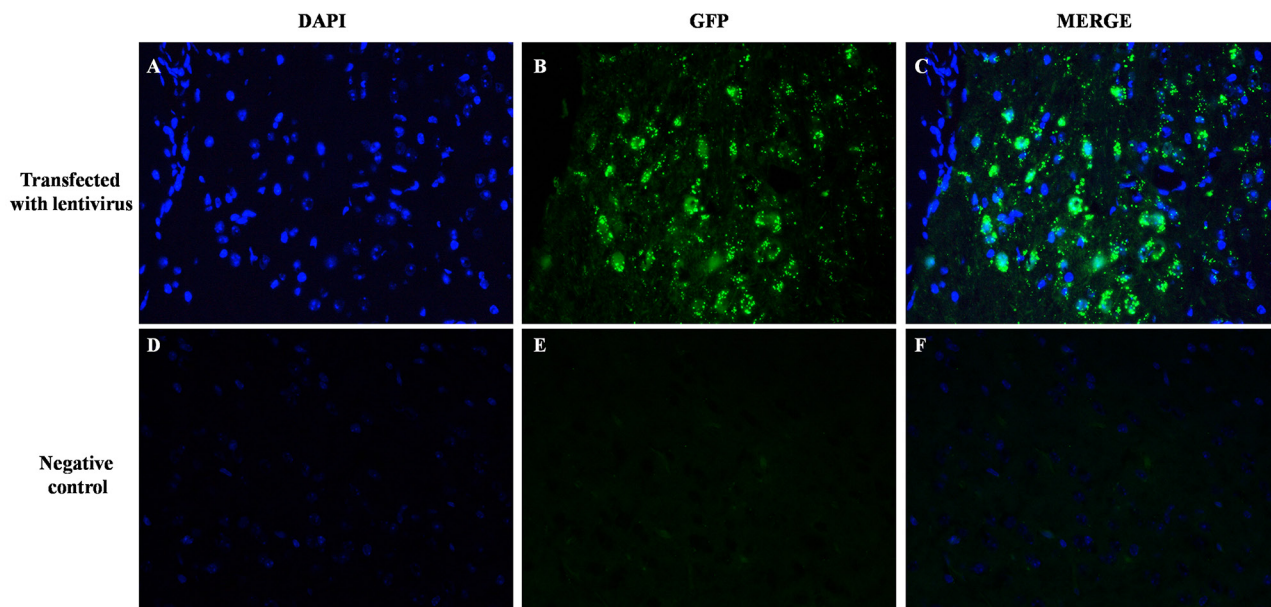
### SUPPLEMENTARY MATERIALS



**Supplementary Figure 1: The blue staining of protein pattern from 4-, 6-, 8-, and 10-week old mouse hypothalamus pulled down by Nptx1 antibody.** The band marked with red arrow was identified as Mkrn3 by mass spectrometry in 4-week old hypothalamus. Mouse IgG was used as a negative control.



**Supplementary Figure 2: Identification of the signal peptide of Nptx1.** Nptx1 protein was investigated by antibody of Nptx1 (Nptx1\_internal against the internal region while Nptx1\_2-28 against the N-terminal region). Flag\_Nptx1 and Nptx1\_Flag represent three flags around 22 amino acids inserted into N-terminal and C-terminal fusion protein respectively.



**Supplementary Figure 3: The efficiency of cerebroventricular administration.** GFP and flag labeled exogenous genes (Nptx1 or Mkrn3) are co-expressed in all our vectors. GFP positive cells represent that those cells have been transfected with lentivirus (B) compared to control (E). DAPI staining is used to mark the nucleus (A, D) and merged with GFP (C, F).

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HUMAN MEEPAAPSEAEHEAAGAQAGAEAAAREGVSGPDLEPVCEPSGESAAPDSALPHAARGWAPFPVAPVPAHLRRG 70
MOUSE MEESTAPIEAAHAAGAAGAEAGAEAGG-EGVSVFPPPPQFEAAGASAGVSSAPLQQASGLAPLLVTPGPAIRRAA 69

HUMAN GLRPAPASGGGAWPSPLPSSRSIGIWTKQIICRYYIHGQCKEGENCRYSHDLSGRKMATTEGGVSPFGASAG 140
MOUSE SLRPAPAEAGGARSGL--PERNSGSWTKQILCRYYLHGQCKEEDNCRYSHDLSGRRRSRGGQDAQPRASAD 137

HUMAN GGPSIAAHIIEPPTQEVAAEAPPAASSLSLPVIGSAAERGFEEAERDNAD-----RG 190
MOUSE RGPKMATRWEPPTQEVAAEAPPAASSSSLPVIGSAAERGFTEAEIDNAGIRSAEERGFSEAEIDNASLAAG 207

HUMAN AAGGAGVESWADAIEFVPGQPYRGRWV-ASAPEAPIQSSETERKQMAVGS-----L 241
MOUSE AAAGAGAEAGWEGAIEFVPGQPYRGRMVPPHGPPEAPLQSPAIEREHMAMGMGMPMPVPMMPVPMPL 277

HUMAN RFCYYASRGVCFRGESECMYLGDIICDMCGLQTLHPMDAAQREEHMRACIEAHEKDMELSFVQVRGMDKVC 311
MOUSE PLCRYAARGQCLRGDRCAYPHGEICDMCQQALHPWDAAQEAHRRACVEAHERDMELSFVQVRGMDKVC 347

HUMAN GICMEVVYEKANFNDRRFGILSNCHSFCIRCIIRWRWSARQFENRIVKSCPQCQVTSSELVIFSEFWVEEE 381
MOUSE GICMEVVYEKADPSDRRFGILFSCNHTYCLRCIRRWRSATQFENRISKSCPQCQVSSGFVIFSEFWVEEE 417

HUMAN EEKQKLIQQYKEAMSNKACRYFAEGRGNCPFGDTCFYKHEYPEGWGDPEPPGF-GGGSFSAYWHQVLEPVV 450
MOUSE EEKEKLVQQYKEGMSCKACRYFAGGLGHCPFGEFCFYKHEYPEGWRDQPPRPDGGSSSAYWHQVLEPVQ 487

HUMAN MEGGNMLYKSIKKELVVLRASLLFKRFLSLRDELPSFEDQWDLHLYELEEYFNLI 507
MOUSE LREGNVLFKSRKKEHSVLRANQLLKKLLCLRGSSEFSDDRWLLLYQYQLEEYFSLNL 544

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**Supplementary Figure 4: The amino acid sequence alignment of human and mouse Mkrn3.** Disordered regions are indicated as dashed lines. The same residues are colored as light gray, the residues having similar polarity as dark gray. The missense mutations in human are framed as red. The residues of C3H1 motif and C3H4 Ring finger domain are framed as blue. The directly interacted sites of Mkrn3 with Nptx1 are labeled as yellow.