# Ablation of huntingtin in adult neurons is nondeleterious but its depletion in young mice causes acute pancreatitis

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**The Huntington's disease (HD) protein, huntingtin (HTT), is essential for early development. Because suppressing the expression of mutant *HTT* is an important approach to treat the disease, we must first understand the normal function of Htt in adults versus younger animals. Using inducible *Htt* knockout mice, we found that *Htt* depletion does not lead to adult neurodegeneration or animal death at >4 mo of age, which was also verified by selectively depleting *Htt* in neurons. On the other hand, young Htt KO mice die at 2 mo of age of acute pancreatitis due to the degeneration of pancreatic acinar cells. Importantly, Htt interacts with the trypsin inhibitor, serine protease inhibitor Kazal-type 3 (Spink3), to inhibit activation of digestive enzymes in acinar cells in young mice, and transgenic *HTT* can rescue the early death of Htt KO mice. These findings point out age- and cell type-dependent vital functions of Htt and the safety of knocking down neuronal Htt expression in adult brains as a treatment.**

Huntingtin | aging | degeneration | acinus | pancreas

Huntington's disease (HD) is caused by polyglutamine (polyQ) expansion in the N-terminal region of huntingtin (HTT). Despite its ubiquitous expression in the brain and body, mutant HTT causes selective neuronal degeneration as well as white matter atrophy in the brain (1–3). The neuronal degeneration is characterized by the preferential loss of neuronal cells in the striatum in the early disease stage and extensive neurodegeneration in a variety of brain regions in later disease stages. This progressive neurodegeneration is consistent with the late-onset neurological symptoms of HD, and age-dependent toxicity of mutant HTT is thus a characteristic of HD.

HTT consists of 3,144 amino acids and is thought to be a scaffold protein that associates with a number of other proteins and participates in a wide range of cellular functions, including intracellular trafficking of a variety of proteins (1, 4). HTT is important during animal early development, as germ-line deletion of Htt leads to early death of mice at embryonic day 8.5 (5–7). A variety of HD animal models that express mutant HTT provide strong evidence for an age-dependent toxic gain of function of mutant HTT (8–12), and considerable efforts have been devoted to developing siRNA and antisense oligonucleotides to suppress the expression of mutant HTT in adult brains (13–15). Unfortunately, however, these approaches have also raised concerns that markedly suppressing HTT expression will lead to side effects by impairing HTT's normal function. To date, whether HTT has differential roles in early development and adulthood remains unknown. Clarifying these distinctions is vital if we are to develop a better strategy for treating HD.

Using conditional *Htt* knockout mice to mate with transgenic CAG-CreER mice that express Cre-ER ubiquitously in the body and brain and transgenic mice expressing Cre-ER in neuronal cells, we generated inducible *Htt* knockout mice and found that loss of Htt in adult mouse brain does not cause obvious neuropathology and phenotypes. However, depletion of Htt in the whole body of young mice causes animal death as a result of acute pancreatitis from the degeneration of pancreatic acinar cells. We also found that Htt interacts with the trypsin inhibitor, serine protease inhibitor Kazal-type 3 (Spink3), to reduce trypsin activity. Thus, Htt has different roles that depend on age and cell types. Our studies show that removing Htt in adult neuronal cells does not lead to degeneration or detectable phenotypes in mice, suggesting that the complete removal of neuronal HTT in the adult brain is possible to achieve an efficient treatment for HD.

## Results

**Loss of Htt Mediates Age-Dependent Death in Mice.** To investigate the function of Htt in adult mice, we crossed conditional *Htt* KO mice (16) to transgenic CAG-CreER mice that ubiquitously express a tamoxifen-inducible CreER, resulting in inducible Htt KO (ubiquitous KO) mice that would deplete Htt expression upon tamoxifen injection (Fig. 1*A*). We injected tamoxifen into ubiquitous KO mice at 2, 4, and 8 mo of age and Western blot analysis confirmed that tamoxifen injection caused Htt depletion in various brain regions in ubiquitous KO mice at different ages (Fig. 1*B*). Quantification of the life span clearly indicated an age-dependent death of ubiquitous KO mice: more than 95% of 2-mo-old mice died within 10 d after Htt depletion, whereas 70% of 4-mo-old and 95% of 8-mo-old mice could survive after Htt depletion (Fig. 1*C*). For older ubiquitous KO mice, there were no significant differences in the rotarod performance and body weight gain compared

### Significance

Because of the toxicity gain of expanded polyglutamine (polyQ) repeats, many studies have used RNAi and other approaches to inactivate the mutant *HTT* gene in Huntington's disease. However, Htt is essential for early embryonic development, and normal function of Htt in adult animals remains unknown. Using conditional Htt knockout mice, we found that loss of Htt causes the lethal phenotype only in postnatal mice but not in adult mice, and this early death is due to acute pancreatitis. Htt interacts with serine protease inhibitor Kazal-type 3 (Spink3) to inhibit trypsin activity in pancreatic cells and thus prevents acinar cell degeneration and pancreatitis. This age- and cell type-dependent function indicates the safety in knocking down neuronal HTT in adult brains for treating Huntington's disease.

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The authors declare no conflict of interest.

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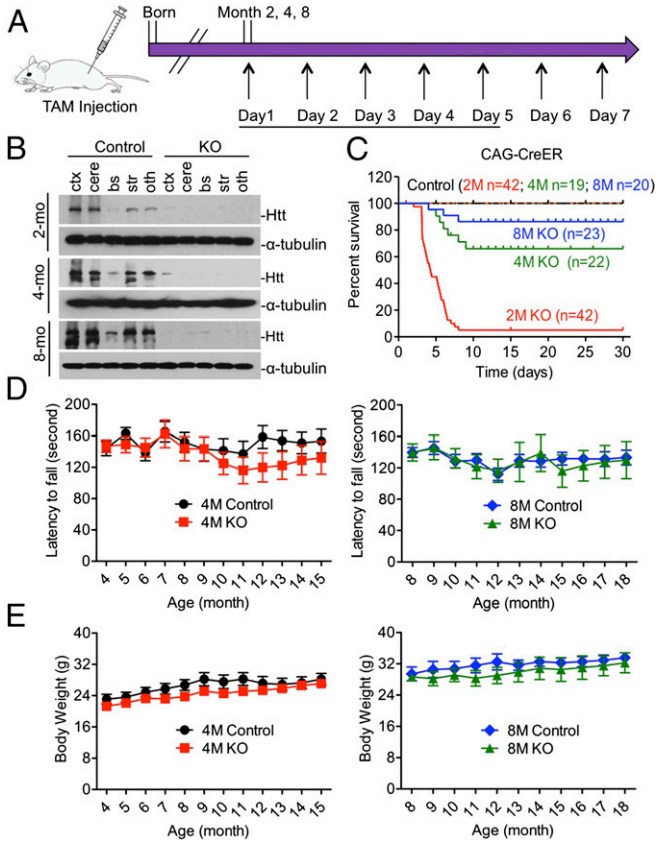


**Fig. 1.** Loss of Htt-mediated age-dependent death in mice. Control is heterozygous floxed *Htt*/CAG-CreER mice injected with tamoxifen, and KO is homozygous floxed *Htt*/CAG-CreER mice injected with tamoxifen. (*A*) Diagram depicting inactivation of the *Htt* gene in adult mouse expressing CreER. The mice were intraperitoneally (i.p.) injected with tamoxifen (TAM) 20 mg/mL for 5 continuous days. Mice were examined for 7–11 mo after tamoxifen injection. (*B*) Western blots showing relative Htt protein levels in the brain of ubiquitous KO and control mice at 2, 4, and 8 mo of age. Ctx, cortex; cere, cerebellum; bs, brainstem; str, striatum; oth, other brain regions. (*C*) Survival rate of ubiquitous KO mouse and control mice when they were injected with tamoxifen at 2, 4, and 8 mo of age. (*D* and *E*) The rotarod performance (*D*) and body weight (*E*) of ubiquitous KO mice whose Htt was depleted at 4 or 8 mo of age, the mice were then studied for 10–11 mo after tamoxifen injection. Control is age-matched heterozygous KO mice.

with their controls (Fig. 1 *D* and *E*). We also verified that the depletion of Htt in ubiquitous KO mice caused no significant changes in LC3I/II, P62, Caspase-3, NFκB, and FAT10 in the brain and peripheral tissues (Fig. S1).

**Depletion of Neuronal Htt in Adult Mice Does Not Cause Death.** The above studies suggested that depletion of Htt in the brain might not be responsible for the death of young mice. To confirm this, we crossed floxed *Htt* mice with transgenic mice expressing Nestin-CreER, such that the resulting crossed mice (neuronal KO) had selectively depleted Htt expression in neuronal cells after tamoxifen injection, as Cre expression is driven by the neuronal *Nestin* promoter. Western blot analysis confirmed that tamoxifen injection caused Htt depletion in various brain regions in neuronal KO mice at different ages (Fig. 2*A*). As expected, very few (<18%) 2-mo-old neuronal KO mice died, and all neuronal KO mice >4 mo lived normally after Htt was depleted by tamoxifen injection (Fig. 2*B*). These living mice showed the same body weight, rotarod performance, and gripping ability as their controls (Fig. 2*C*). Because depletion of Htt is known to affect the neurogenesis or survival of developing neurons (16, 17), we examined 2-mo-old ubiquitous KO mice, but found no difference in the brain size and volume between controls and Htt KO mice (Fig. 3 *A* and *B*). For neuronal KO mice, depletion of Htt by tamoxifen for 90 d did not show any difference in the brain volume compared with control mice (Fig. 3*B*). Histological examination showed no morphologic defects in neuronal KO mice (Fig. S2*A*), which is supported by Western blotting results that detected no significant changes in NeuN, β-tubulin III, GFAP, P62, LC3I/II, and caspase-3, except mouse Htt protein (Fig. 3 *C* and *D*). Immunofluorescent staining also did not show any changes in LC3I/II, β-tubulin III, and cleaved caspase-3 staining in ubiquitous and neuronal KO mouse brains (Fig. 3*E* and Fig. S2*B*). In addition, we crossed floxed *Htt* mice to transgenic mice that express CreER in the forebrain under the control of the neuronal promoter *CaMKII*. The crossed mice, upon tamoxifen injection, showed normal growth and survival without any detectable brain atrophy or degeneration (Fig. S3). Taken together, our results demonstrate that ubiquitous KO mice show an age-dependent death that is unlikely due to the depletion of Htt in neuronal cells or neuronal degeneration.

**Htt Depletion Causes Acute Pancreatitis That Results from Acinar Cell Degeneration in Young Mice.** We next focused on the peripheral tissue morphology of dead ubiquitous KO mice and found abnormal and inflamed abdominal cavities (Fig. 4*A*). There was significant inflammation in the abdominal organs, including intestine,

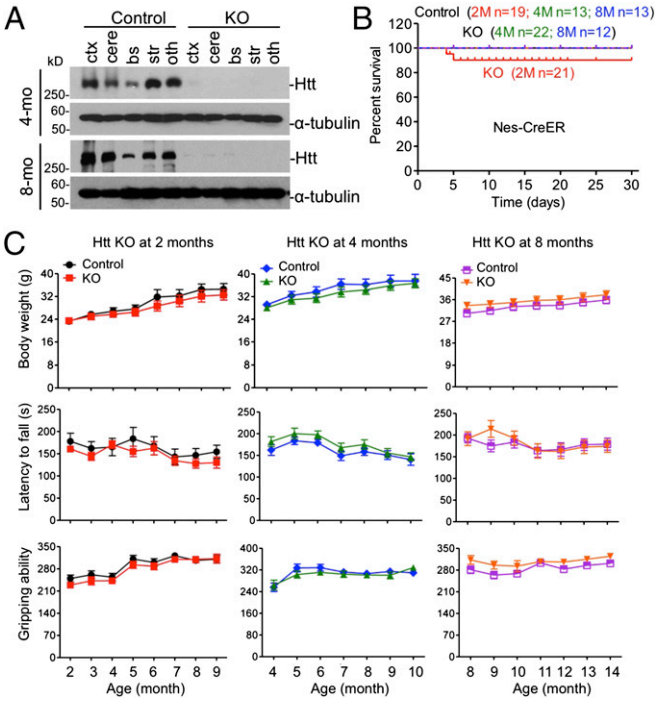


**Fig. 2.** Neuronal Htt knockout mice. (*A*) Western blots showing depletion of Htt in the brain of neuronal KO mice at 4 and 8 mo of age. See Fig. 1 for abbreviations key. (*B*) Survival rate of neuronal KO mice compared with the heterozygous control mice. Data were collected from the first day after tamoxifen injection. (*C*) Diagram showing the body weight, rotarod performance, and gripping ability at different months. Mice at different ages (2, 4, and 8 mo) were studied for 7–8 mo after tamoxifen injection (2-mo-old, $n = 10$; KO, male = 6 and female = 4; control, male = 4 and female = 6; 4-mo-old, $n = 10$; KO, male = 5 and female = 5; control, male = 5 and female = 5; 8-mo-old, KO, $n = 9$, male = 4, female = 5; control, $n = 11$, male = 6, female = 5; two-way ANOVA test, body weight, 2M control vs. 2M KO: $P = 0.9945$, 4M control vs. 4M KO: $P = 0.9862$, 8M control vs. 8M KO $P = 0.9922$; rotatod performance, 2M control vs. 2M KO: $P = 0.9565$, 4M control vs. 4M KO: $P = 0.9921$, 8M control vs. 8M KO $P = 0.7701$; gripping ability, 2M control vs. 2M KO: $P = 1.0000$, 4M control vs. 4M KO: $P = 0.9962$, 8M control vs. 8M KO $P = 0.9718$).

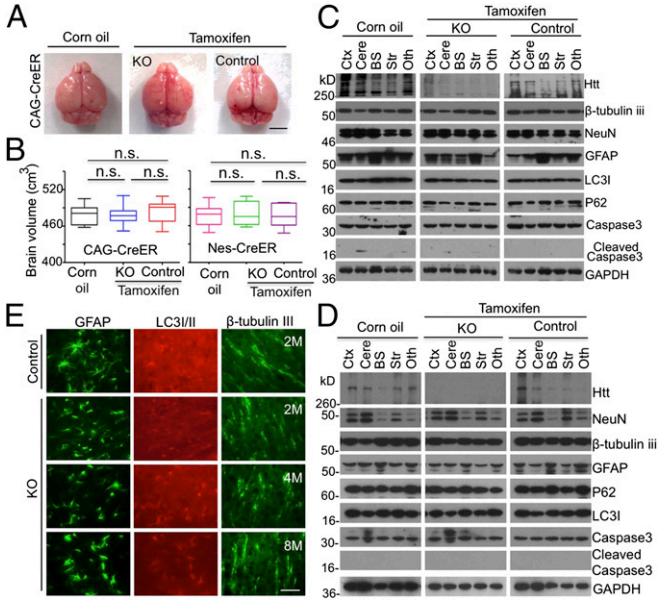


**Fig. 3.** Normal brains in adult mice that have depleted Htt. (*A*) The brain photos of 2-mo-old ubiquitous KO mice that had been injected with tamoxifen for 5 d. (*B*) Brain volumes of 2-mo-old ubiquitous KO mice in *A* and neuronal KO mice that were injected with tamoxifen at 2 mo of age and used to isolate their brains at the 90th day after tamoxifen injection (one-way ANOVA test, ubiquitous KO: $n = 7$, $P = 0.8759$, $F = 0.1335$; $n = 7$; neuronal KO: $P = 0.9665$, $F = 0.03416$; n.s. represents no significant differences). (Scale bar, 3 mm.) (*C* and *D*) Western blotting analysis of the brain tissues from 2-mo-old ubiquitous (*C*) and neuronal (*D*) KO mice. Htt, neuronal (NeuN, β-tubulin III), astrocytic (GFAP), autophagic (P62, LC3I/II), apoptotic (cleaved caspase3), and GAPDH were detected by their antibodies. Control is heterozygous floxed *Htt*/CAG-CreER (*C*) or heterozygous floxed *Htt*/Nestin-CreER (*D*) mice injected with tamoxifen and homozygous floxed *Htt*/CAG-CreER (*C*) or homozygous floxed *Htt*/Nestin-CreER (*D*) mice injected with corn oil. KO is homozygous floxed *Htt*/CAG-CreER (*C*) or homozygous floxed *Htt*/Nestin-CreER (*D*) mice injected with tamoxifen. See Fig. 1 for abbreviations key. (*E*) Immunostaining of GFAP, LC3I/II, and β-tubulin III in the cortex of 2-, 4- and 8-mo-old ubiquitous KO and control mice. (Scale bar, 50 μm.)

vessels, and pancreas, as evidenced by hemorrhagic, black-brown necrosis of the pancreatic parenchyma and peripancreatic fat (Fig. 4*A* and Fig. S4*A*). The pancreas was swollen and did not show the typical tan, lobulated architecture. All these features indicate acute pancreatitis. We then examined 2-mo-old ubiquitous KO mice after injecting tamoxifen to induce Htt depletion for different days and found the typical acute pancreatitis changes: the acinar cells were initially enlarged at day 3, and then the mice started to have ascites, ileus, and splanchnic hyperemia before their death at day 7 (Fig. 4*B*). Western blot results confirmed that Htt in pancreas is depleted in ubiquitous KO mice (Fig. 4*C*). However, histological examination revealed that living ubiquitous KO mice at 4 and 8 mo of age, which had been injected with tamoxifen for 5 d, show no abnormal pancreatic histology (Fig. S4*B*). Examination of ubiquitous KO mice that were injected with tamoxifen at 4 or 8 mo of age and then used to isolate their tissues 2 mo later did not reveal any abnormal histology in their pancreas.

Elastase 3B or protease E (CELA3B), which is secreted from the pancreas as a zymogen, is also increased (Fig. 4*D*). Although autophagy activation can also be the cause of acute pancreatitis (18), we saw no significant alteration in P62 and LC3I/II, whereas caspase-3 activation was evident (Fig. 4*D*). Acute pancreatitis is often caused by intrapancreatic activation of pancreatic enzymes. Indeed, serum amylase, lipase, pancreas amylase, trypsin, and other pancreatic enzymes show high activities in 2-mo-old ubiquitous KO mice (Fig. 4*E* and Fig. S4*C*).

Next, we performed electron microscopic examination of pancreatic tissues of 2-mo-old ubiquitous KO mice. Compared with the pancreatic tissue from control mice (heterozygous floxed *Htt* mice injected with tamoxifen), we found increased zymogen granules in acinar cells, nuclear chromatin clumping, and margination, indicating apoptosis, mitochondria enlargement, and edema, as well as lysosomes containing degenerated cytoplasmic elements or organelles (Fig. 4*F*). Quantification of zymogene granules and apoptosis or necrosis in pancreatic acinar cells also confirmed that Htt depletion increased the accumulation of zymogene granules and acinar cell death (Fig. S4*D*).

**Htt Interacts with Spink3 to Inhibit Trypsin Activity.** The phenotypes and morphological studies of 2-mo-old ubiquitous KO mice clearly indicate acute pancreatitis caused by Htt depletion. The acute pancreatic inflammation can be induced by activation of digested enzymes in pancreatic zymogens in acinar cells (19–21). This activation normally involves a hydrolysis reaction of inactivated forms of enzymes by trypsin. Because we saw the degeneration of acinar cells, we focused on the molecules known to inhibit trypsin activity in acinar cells. Western blot results show that Spink3, a mouse homolog of human SPINK1 that inhibits trypsin in acinar cells (22), is selectively decreased in 2-mo-old mouse pancreatic tissues compared with 4- and 8-mo-old mouse pancreatic tissues (Fig. 5*A*). Because mutations in the human Spink3 homolog SPINK1 cause pancreatitis (23–26) and the selective decrease of Spink3 in young mouse pancreas is associated with acute pancreatitis in 2-mo-old ubiquitous KO mice, we further investigated whether Htt associates with Spink3 to inhibit trypsin. Using immunoprecipitation of pancreatic tissues from wild-type mice at 2, 4, and 8 mo of age, we found an age-dependent association of Htt with Spink3, which declines from 2 to 8 mo (Fig. 5*B*). This result suggests that the association of Htt with Spink3 in pancreatic tissues in young mice may be more important for Spink3's function.

To verify the functional relevance of this binding, we transfected a series of Htt and Spink3 constructs into primary cultured acinar cells from ubiquitous KO mice. The cells were treated with tamoxifen (KO) to deplete endogenous Htt and then transfected with full-length HTT (fHTT), N-terminal HTT (1–208 aa, nHTT), or truncated HTT (tHTT) lacking N-terminal 169 amino acids. Measuring trypsin activities of these transfected cells revealed that fHTT and tHTT, but not nHTT, could restore the inhibitory function of Htt on trypsin activity when endogenous Htt has been depleted (Fig. 5*D*). We also performed transfection of HEK293 cells with a series of HTT and Spink3 constructs. Without overexpression of Spink3 and HTT, HEK293 cell lysates show detectable trypsin activity when incubated with porcine trypsin and the substrate Boc-Gln-Ala-Arg-7-amino-4-methylcoumarin (AMC). This activity was inhibited by expressing Spink3 and could be further reduced when additional tHTT was also expressed (Fig. S5*A*). However, nHTT was unable to promote this inhibition, supporting the idea that a truncated HTT lacking the N-terminal region is able to bind Spink3 to inhibit trypsin activity.

**Transgenic tHTT Rescues the Early Death of Ubiquitous KO Mice.** We have generated transgenic mice that express tHTT lacking N-terminal 169 amino acids under the control of the human *HTT* promoter (Fig. 6 *A* and *B*). Because we have found that tHTT is able to bind Spink3 (Fig. 5*C*), we wanted to see if this transgenic HTT could rescue the acute pancreatitis and early death of ubiquitous KO mice. Thus, we crossed *tHTT* transgenic mice with ubiquitous KO mice. PCR genotype indicates that the transgenic *tHTT* existed in the crossed mice (Fig. 6*C*). The crossed mice were then injected with tamoxifen at the age of 6 wk to deplete the endogenous full-length Htt in mice. Mice carrying the transgenic *tHTT*, however, continued to live versus the ubiquitous KO mice that did not express transgenic *tHTT* (Fig. 6*D*). After depleting endogenous Htt for 5 mo, examination of the body weight of mice also revealed

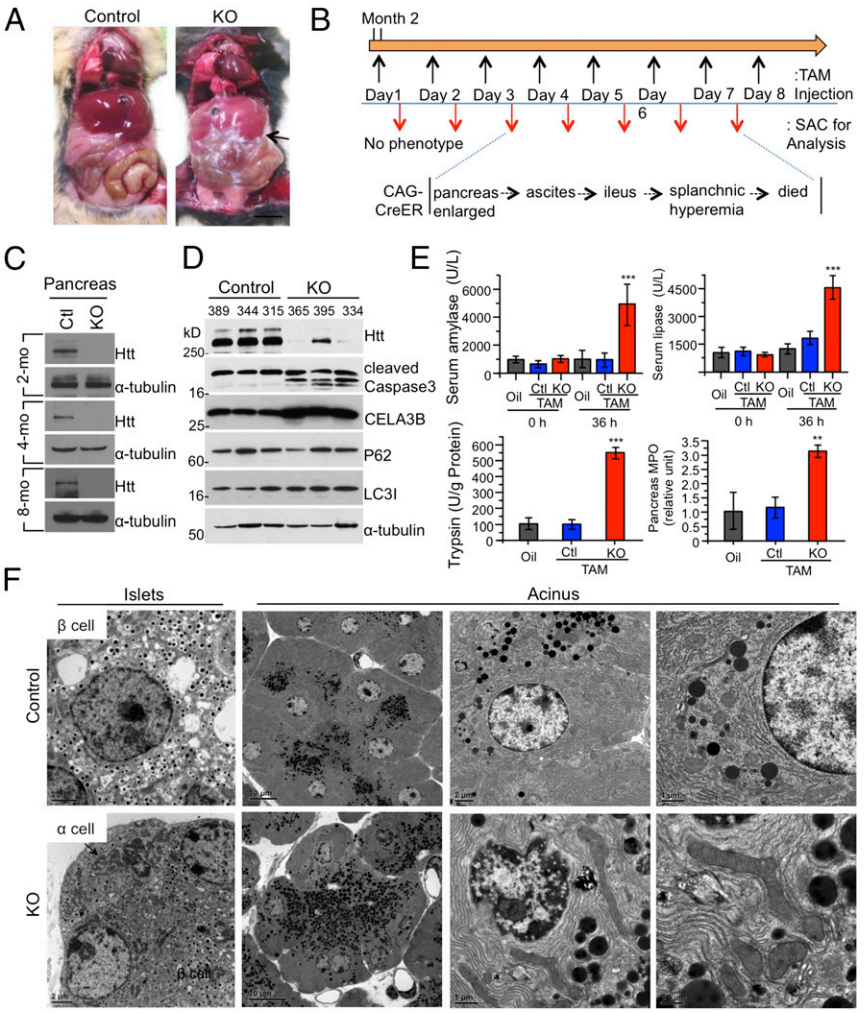


**Fig. 4.** Acute pancreatitis in 2-mo-old ubiquitous KO mice. (*A*) Photographs of abdominal cavity of 2-mo-old ubiquitous KO (CAG-CreER) and the heterozygous control mice after 3-d injections of tamoxifen. (Scale bar, 8 mm.) (*B*) Scheme outlining tamoxifen (TAM) injection followed by sacrificing (SAC) for analysis. (*C*) Western blots confirming Htt depletion in the pancreas tissues of ubiquitous KO mice. (*D*) Immunoblotting of pancreas lysates was performed with antibodies against cleaved caspase-3, P62, LC3I/II, CELA3B, and α-tubulin. The samples were obtained from ubiquitous KO and control mice (2 mo old) after i.p. tamoxifen injection for 3 d. (*E*) Serum amylase, serum lipase, pancreas trypsin activity, and myeloperoxidase (MPO) levels were measured 40 h after tamoxifen injection in ubiquitous KO and control mice. Data represent the means of eight control and ubiquitous KO mice at each interval ± SEM (*$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$, four mice per group, $n = 3$ independent experiment, one-way ANOVA test). (*F*) Electron microscopy showing acinar cell degeneration in the pancreas of 2-mo-old ubiquitous KO mouse. (Scale bars, 2 μm.) Control is heterozygous floxed *Htt*/CAG-CreER mice injected with tamoxifen. Quantitative analysis of zymogen granules and degenerated cells is presented in Fig. S4*D*.

that these KO mice expressing tHTT (tHTT/KO) are indistinguishable from control mice (Fig. 6*E*). We then isolated the pancreatic tissues from tHTT/KO mice to perform Western blotting, which showed the expression of tHTT that is smaller than the endogenous full-length Htt in wild-type mouse pancreatic tissues (Fig. 6*F*). Moreover, the proteins for apoptosis (cleaved caspase3), necrosis (RIP3), autophagy (P62, LC3I/II), and CELA3B enzyme showed no alteration compared with those in the control mouse pancreatic tissues (Fig. 6*G*). Thus, this in vivo rescue effect also supports the idea that Htt is important for preventing pancreatic inflammation in young mice. Based on the results we obtained, we propose a model for the acute pancreatitis caused by the loss of Htt in young mice (Fig. S5*B*). In the pancreatic tissues of young mice, Htt binds Spink3 to help inhibit trypsin activity. Without Htt, this inhibition is abolished, leading to trypsin activation in acinar cells and resulting in acinar cell degeneration and pancreas inflammation, as well as early death. This function is not only cell-type specific but also age dependent, as aging increases the expression of Spink3, whose inhibitory effect on trypsin may also be dependent on other age-related factors.

## Discussion

HTT is known to be essential for early embryonic development, and conditional knockout of Htt during the postnatal stage can also cause neurodegeneration (16). Interestingly, mutant HTT with expanded polyQ can rescue this embryonic lethality (27, 28). Deletion of polyQ or the proline-rich domain does not affect mouse development (29, 30). Also, humans homozygous for mutant expanded polyQ HTT age to adulthood with no obvious enhancement in pathology (31, 32), indicating that the expansion or lack of polyQ does not affect early development, but polyQ expansion causes late-onset neurodegeneration and neurological symptoms. Based on these findings, great efforts have gone into suppressing the expression of mutant HTT in the treatment of HD (13–15). Nevertheless, because of the essential function of HTT, a major concern has always been the possible side effects of depleting HTT. Our studies suggest that such concerns may be allayed if depletion of HTT occurs only in the brain or in older adults.

The previous studies using CaMKII-driven Cre transgenic mice show the degeneration of some neuronal cells and the degree of the degeneration appears to depend on age; the later depletion of Htt yielded a lesser extent of neuronal degeneration (16). Thus, Htt function in the brain appears to be age dependent, although its role in developing neuronal cells has been clearly shown by a number of studies (17, 33–35). Our studies show that in adulthood, Htt knockout does not affect neuronal survival or cause any significant movement or behavioral phenotypes. This finding is also supported by mice with selectively depleted *Htt* expression in the brain via Nestin-CreER or CaMKII-CreER. It should be pointed out that a few Nestin-Cre/KO mice die early, which is likely due to the expression of Cre in pancreatic acinar cells driven by the *Nestin* promoter (36). Thus, our studies suggest that depletion of HTT in adult brain would not cause severe phenotypes as seen in early development, providing a rationale to design a more

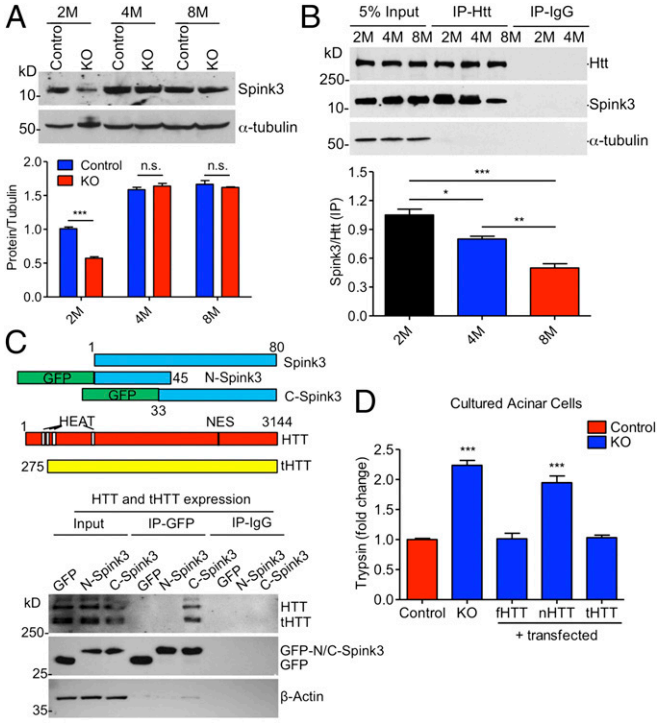


**Fig. 5.** Interaction of Htt with Spink3 inhibits trypsin activity. (*A*) Western blotting (*Upper*) and quantification (*Lower*) of Spink3 in 2-, 4-, and 8-mo-old ubiquitous KO and control mice. ($n = 3$ independent experiments, two-way ANOVA test; n.s. represents no significant difference, $^{***}P < 0.001$). (*B*) Immunoprecipitation of Htt with Spink3 from 2-, 4-, and 8-mo-old WT mouse pancreas. More Spink3 in 2-mo-old than 4- or 8-mo-old mouse pancreas was precipitated with Htt. Anti–α-tubulin and IgG IP were used as control. $n = 3$ independent experiments. One-way ANOVA test, $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. (*C*) Schematic structures (*Upper*) of Spink3 and Htt proteins, and Western blots (*Lower*) showing coimmunoprecipitation of transfected GFP, GFP-*N*-Spink3, or GFP-C-Spink3 and truncated HTT (tHTT) in HEK293 cells. IgG immunoprecipitation served as controls. (*D*) Relative activities of trypsin in the control and ubiquitous KO mouse pancreatic acinar cells that were transfected with full-length Htt (fHTT), N-terminal Htt (nHTT), and truncated Htt lacking N-terminal 169 amino acids (tHTT). $n = 3$ independent experiments, two-way ANOVA test, $P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

efficient strategy to completely eliminate the expression of mutant HTT in adults.

Our findings also show that the interaction of Htt with Spink3 is important for preventing acute pancreatitis at young ages. Spink3 is the mouse homolog of serine protease inhibitor Kazal-type 1 (SPINK1) in humans, which is expressed at high levels in the pancreas (37, 38). SPINK1 is involved in pancreatitis in humans (24, 39, 40), as patients heterozygous for a *SPINK1* mutation have a 20- to 40-fold increased risk of developing pancreatitis (23, 26, 41, 42), and this risk may be as high as 500-fold in individuals with homozygous mutations (26). However, germ-line knockout of the mouse Spink3 does not lead to acute pancreatitis but autophagic death of pancreatic acinar cells, although the homozygous KO mice die postnatally. This difference could be due to the species- and age-dependent role of Spink3 in mouse, as Spink3 also plays important roles in the proliferation and/or differentiation of various cell types during mouse development (43). Indeed, a progressive degradation of the exocrine pancreas is seen at 16.5 days postcoitus in Spink3 KO mice, which was attributed to autophagic cell death (44). However, in adult mice, the expression level of Spink3 appears to be important for acute pancreatitis, because transgenic expression of pancreatic secretory trypsin inhibitor-1 rescues Spink3-deficient mice and restores a normal pancreatic phenotype (45).

Another issue is why the loss of Htt-mediated death is age dependent. We saw that Spink3 is expressed at a lower level in pancreatic tissues in young mice. We also show that Htt binds more Spink3 at younger ages. These results suggest that the association of Htt with Spink3 is particularly important for Spink3 to inhibit trypsin activities when Spink3 is at a low level in pancreatic acinar cells at young ages. In older mice, the higher levels of Spink3 could be due to its interaction with other proteins, which may substitute the function of Htt.

Our studies suggest that Htt possesses a cell type-specific function that is also age dependent, adding more complexity to this large protein. Although this is somewhat surprising, it is reasonable given that Htt associates with so many proteins (46, 47). It is also known that mutant Htt with expanded polyQ repeats could cause a loss of function. For example, polyQ expansion can inhibit the normal function of Htt by altering its interactions with other proteins. Such consequence could be different from the complete loss of normal Htt, as the complete loss of HTT may be compensated by other molecules, whereas a partial loss of normal function of htt due to polyQ expansion may also affect the function of other

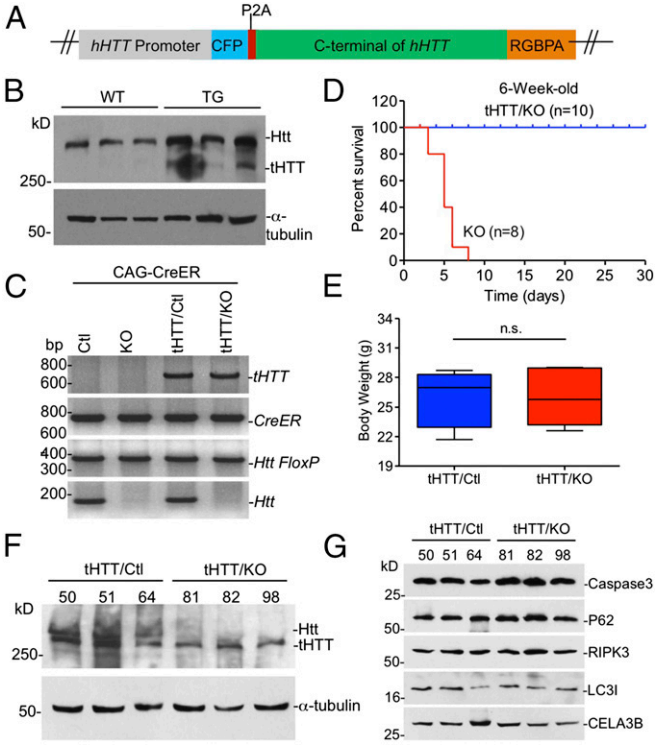


**Fig. 6.** Rescued effect of transgenic tHTT on Htt KO-induced pancreatitis. (*A*) tHTT construct used for generation of the *tHTT* transgenic mice. (*B*) Western blotting showing that tHTT is expressed in the mouse brain cortex. Wild-type mice cortex was used as control. (*C*) PCR genotyping verified the presence of transgenic *tHTT* in heterozygous (*tHTT*/Ctl) and homozygous (*tHTT*/KO) ubiquitous KO mice that also carry CAG-CreER. Ctl, control. (*D*) Survival rate of *tHTT*/KO mouse and ubiquitous KO mice showing that tHTT can prevent early death. (*E*) Body weight was tested at the fifth month after tamoxifen injection in the *tHTT*/KO and control (*tHTT*/Ctl) mice (paired two-tailed *t* test; n.s. represents no significant difference, $n = 8$, *tHTT*/KO, male = 5, female = 3; control, male = 4, female = 4). (*F*) Western blotting showing that tHTT is expressed in the ubiquitous KO mouse pancreas; heterozygous KO mouse pancreas was used as control. Note tHTT is smaller than full-length Htt (Htt). Three mouse pancreases of each genotype were examined. (*G*) Western blotting of the CELA3B, caspase-3, P62, RIPK3, and LC3I/II protein in the 2-mo-old *tHTT*/KO and control mouse pancreas.

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proteins. The compensatory mechanisms for the loss of Htt may be cell-type and age dependent and can confound the interpretation of the function of Htt and the absence of overt phenotypes in adult mice that lack Htt. Despite further studies being required to understand the cell type-specific function of Htt, our results provide evidence for the first time to our knowledge that depletion of Htt causes cell type- and age-dependent phenotypes and also indicate the safety for a more efficient way of depleting neuronal HTT in adult brain to achieve better treatments for HD.

## Materials and Methods

*Htt* floxP-flanked mice were provided by Scott Zeitlin, University of Virginia, Charlottsville, VA, and were maintained at the Emory University Animal Facility in accordance with the institutional animal care and use committee guidelines. All animal procedures were approved by the institutional animal care and use committee of Emory University. Additional information is provided in *SI Materials and Methods*.

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