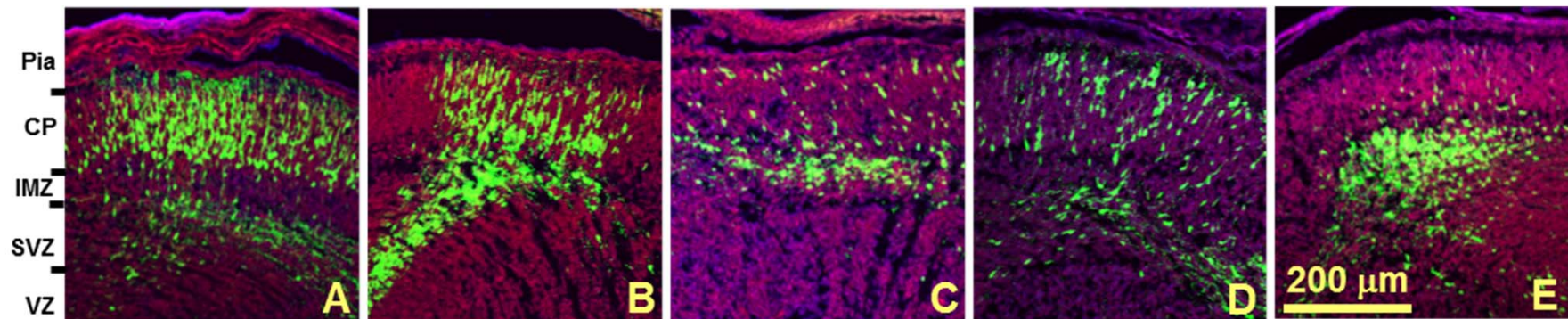
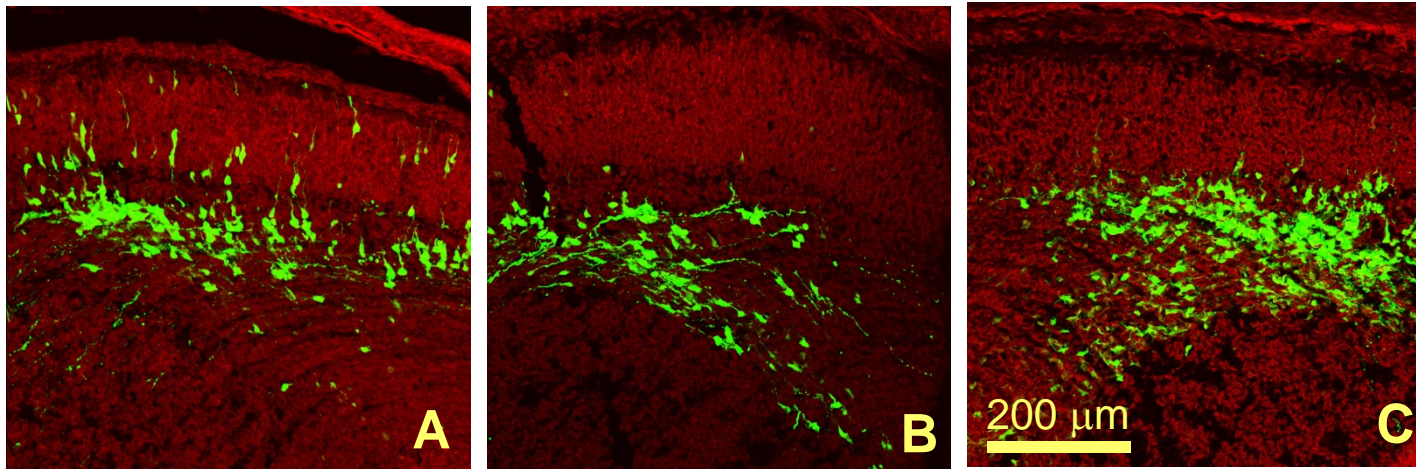


Selection of most biologically active *Htt* shRNA construct and its knock down of anti-HTT immunofluorescent signal in the transfected cells



Supplemental Figure 1. Four different constructs targeting different regions of *Htt* were assessed for their efficacy to disrupt cortical development. In all cases the shRNA constructs inhibited the number of cells that successfully migrated to the cortical plate. The most effective construct in inhibiting cell migration was Htt-5596 (E). Constructs Htt-204, Htt-2502, and Htt-4908 inhibited the normal migration of the cells and only 40-48% of transfected cells (Htt-204: $40\pm 4\%$, Htt-2502: $43\pm 1\%$, Htt-4908: $48\pm 4\%$, B-D) reached the cortical plate compared to $85\pm 3\%$ of control transfected group (E). Pia- pia mater; CP- cortical plate; IMZ- intermediate zone; SVZ- subventricular zone; VZ- ventricular zone. Bar - 200 um (A-E).

***Casp9* shRNA reduces cell apoptosis induced by *Htt* shRNA and partially rescues cell morphology**



Supplemental Figure 2. Co-transfection of *Casp9* shRNA partially rescues the deleterious effects of *Htt* shRNA on neuronal survival. *Casp9* shRNA was co-transfected with the three *Htt* shRNAs at E12.5 and the fetuses survived for 72 hrs. The co-transfection of the *Casp9* shRNA greatly (A) improved the morphology of the transfected cells as compared to the *Htt* shRNA transfection alone. We found a graded inhibition of *Casp9* with the other two constructs. The *Casp9*-554 (n=5) and *Casp9*-124 (n=3) co-transfected with *Htt* shRNA yielded cells with relatively normal appearance, but exhibited disrupted cell migration to the cortical plate. Pia- pia mater; CP- cortical plate; IMZ- intermediate zone; SVZ- subventricular zone; VZ- ventricular zone. Bar - 200 μm (A-C).

Supplemental Table 1. Selection of active *Htt* shRNA constructs.

| | Control | Htt-204 | Htt-2502 | Htt-4908 | Htt-5596 |
|-----------------|---------|---------|----------|----------|----------|
| Number of fetus | 8 | 3 | 2 | 4 | 9 |
| CP (%) | 85±3 | 40±4 | 43±1 | 48±4 | 10±1 |
| VZ, SVZ, IZ (%) | 15±2 | 60±4 | 57±2 | 52±3 | 90±1 |

Four *Htt* shRNAs were individually transfected into E12.5 fetuses and the animals were allowed to survive for 72 hrs. The percentage of the labeled GFP cells that were present in ventricular (VZ), subventricular (SVZ), intermediate zones (IZ) and cortical plate (CP) were determined to choose the shRNA construct that displayed the best inhibitory effect on cell migration for subsequent studies.

Supplemental Table 2. Htt -5596 shRNA significantly reduced anti-HTT immunostaining.

| Treatment | Control | Htt shRNA | % knockdown |
|----------------------|---------|-----------|-------------|
| Optical Density (OD) | 136.4 | 67.5 | 51% |

The eGFP labeled cells (*Htt* shRNA transfected or control) analyzed by using [analysis 3] software on immunofluorescent images and the optical density of the immunoreaction was compared. The *Htt* shRNA reduced immunostaining 51% compared to the control group. Data was derived from 4 fetuses and 43 cells were counted.

Supplemental Table 3. *Htt* shRNA effect on cell migration is time sensitive.

| | Control (E12.5) | Htt-shRNA (E12.5) | Control (E14.5) | Htt-shRNA (E14.5) | Control (E16.5) | Htt-shRNA (E16.5) |
|-----------------|--------------------|----------------------|-----------------|----------------------|-----------------|----------------------|
| # of fetuses | 8 | 9 | 3 | 11 | 4 | 4 |
| CP (%) | 85±1 | 10±1 | 24±3.5 | 9±1 | 4±1 | 1 |
| VZ, SVZ, IZ (%) | 15±1 | 90±1 | 76±4 | 91±1.2 | 96±1 | 99±1 |

When control eGFP plasmid was applied at various ages in the developing mouse embryos, the migration distance of the eGFP cells after 72 hours depended on the transfection age. When the *Htt* shRNA was co-transfected with eGFP, the *Htt* shRNA transfected cells showed greatly reduced migration at all transfection ages.

Supplemental Table 4. The *Htt* shRNA causes cell apoptosis.

| Treatments | Control (E12.5) | Htt shRNA (E12.5) | Control (E14.5) | Htt shRNA (E14.5) | Control (E16.5) | Htt shRNA (E16.5) |
|---------------------|--------------------|----------------------|--------------------|----------------------|--------------------|----------------------|
| Apoptotic cells (%) | 4.4 ±1 | 38.2 ± 1.4 | <1.0 | 35.7±1.2 | <0.5 | 6.6±0.5% |

Fetuses were transfected by the *Htt* shRNA on E12.5, E14.5 and E16.5 and survived for 72 hrs. The sections were immunostained with anti-cleaved CASP3 antibody and the immuno-positive cells were considered as apoptotic cells. All eGFP and immunopositive cells were counted and ratio of anti-cleaved CASP3 stained cells to the total number of eGFP cells was calculated for each age.

Supplemental Table 5. The *Htt* shRNA caused cell apoptosis in early developing cerebellum.

| Brain regions | Control | Cerebellum |
|----------------------|---------|------------|
| % of apoptotic cells | 5.0±1 | 39.0±3.5 |

The fetuses were transected by *Htt* shRNA on age of E12.5 and survived for 48 hrs. When anti-CASP3 antibody was applied on sections, the apoptotic cells were widely observed in developing cerebellum.

Supplemental Table 6. *Casp9* shRNA rescues *Htt* shRNA-induced apoptosis.

| | Control | <i>Htt</i> shRNA alone | <i>Htt</i> shRNA+ <i>Casp9</i> shRNA |
|---------------------|---------|------------------------|--------------------------------------|
| Number of fetus | 8 | 9 | 10 |
| CP (%) | 85±1 | 10±1 | 26±1 |
| VZ, SVZ, IZ (%) | 15±1 | 90±1 | 74±1 |
| Apoptotic cells (%) | 4.4 ±1 | 38.2 ± 1.4 | 28.8± 1 |

The fetuses were transfected by E12.5 and survived 72 hrs. When the *Casp9* shRNA was co-transfected with the *Htt* shRNA, the number of successfully migrating cells was increased and the morphology of transfected cells was improved. In addition, there was a reduction in the number of transfected cells that were CASP3 positive.

Supplemental Table 7. *Casp9* shRNA rescues *Htt* shRNA-induced defect in cell proliferation.

| | Control | <i>Htt</i> shRNA alone | <i>Htt</i> shRNA+ <i>Casp9</i> shRNA |
|-----------------|---------|------------------------|--------------------------------------|
| Number of fetus | 3 | 5 | 7 |
| BrdU+ cells (%) | 33.3±1 | 8.9±1.1 | 20.9±1.1 |

The fetuses were transfected by E12.5 and survived 72 hrs. The knockdown of *Htt* decreased BrdU+ cells and co-transfection with the *Casp9* shRNA partially rescued the defect in proliferation.

Supplemental Table 8. *Casp9* shRNA significantly reduced anti-CASP9 immunostaining.

| Treatment | <i>Htt</i> shRNA | <i>Htt</i> shRNA+ <i>Casp9</i> shRNA | % knockdown |
|----------------------|------------------|--------------------------------------|-------------|
| Optical Density (OD) | 46.8 | 26.4 | 43.6% |

The co-transfection of *Htt* shRNA and *Casp9* shRNA resulted in the 43.6% reduction of immunostaining with anti-CASP9 antibody in the transfected cells compared to the *Htt* shRNA only group. Data was derived from 4 fetuses and 45 cells were counted.