Luciferase project

4/23/2010

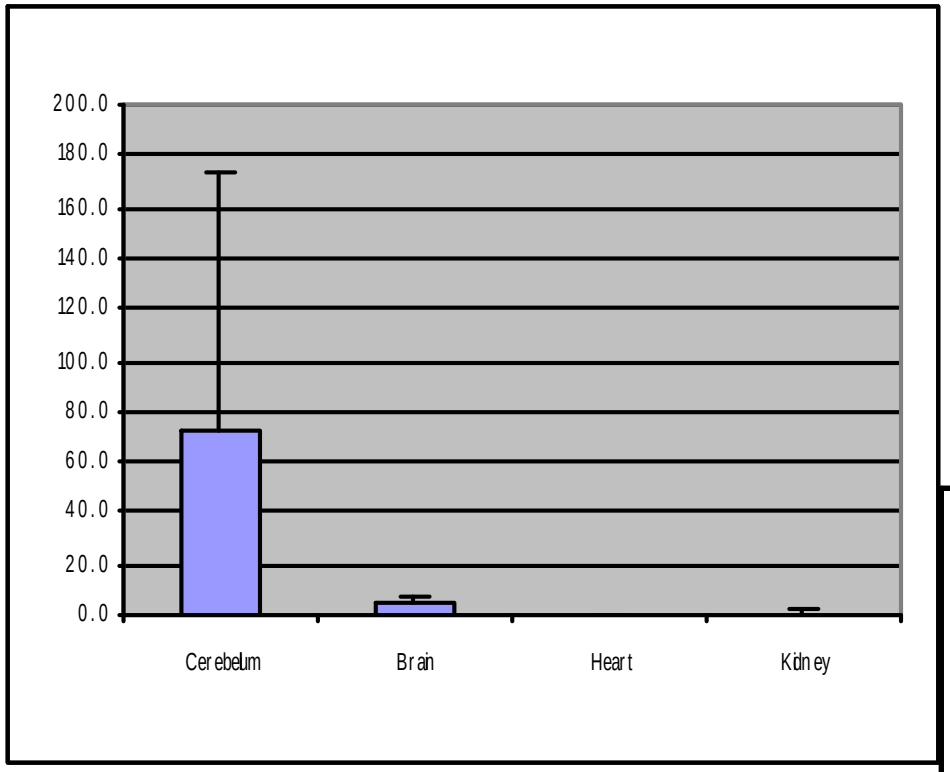
- Luciferase driven by ataxin-2 promoter
- 7 founders –bred with pure Jax B6 mice

- Offspring currently being characterized:
  - Plate read
  - Histology
  - qPCR
  - WB

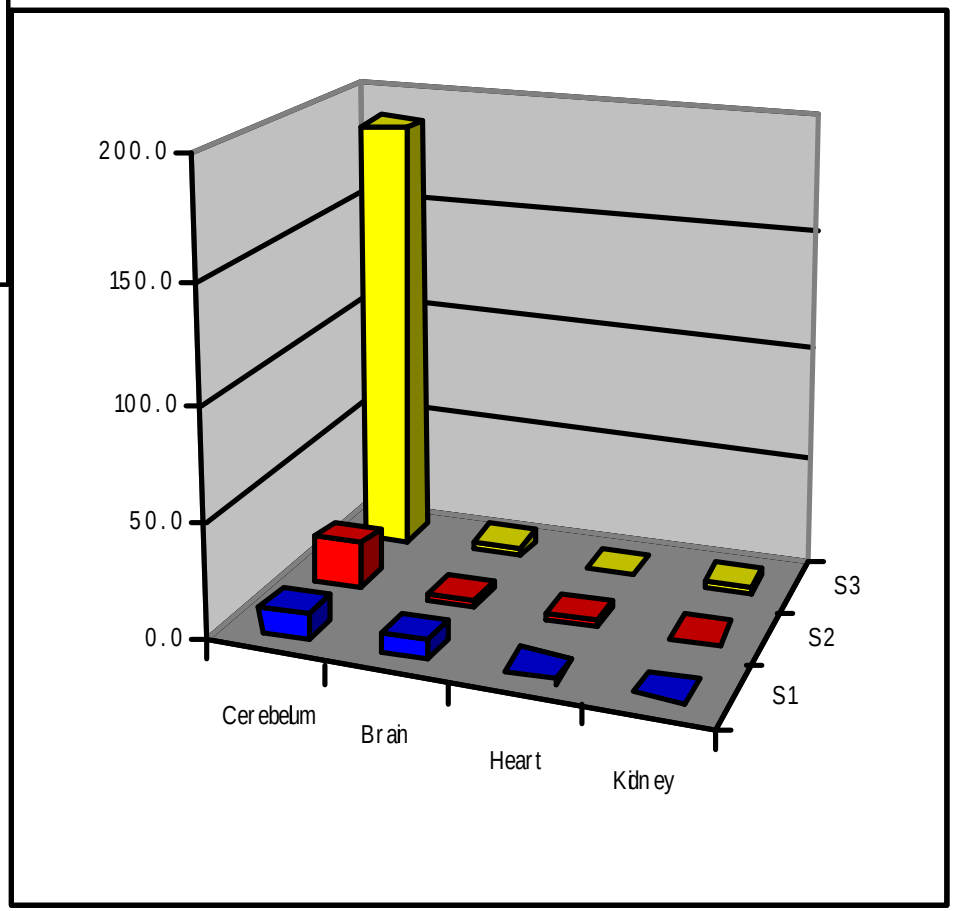
Methods

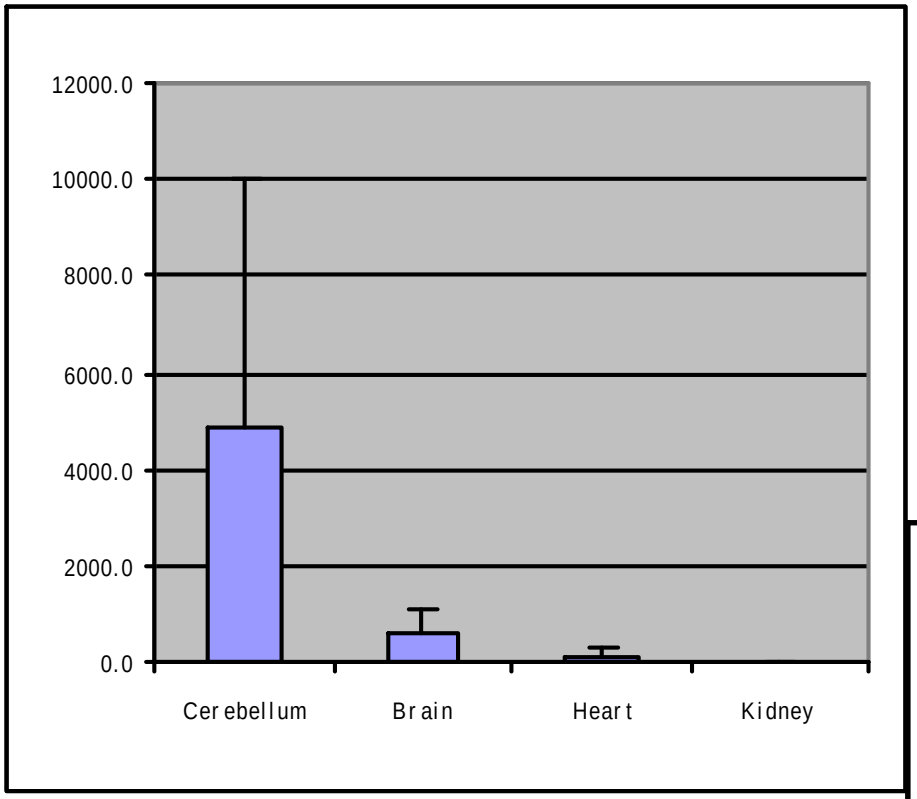
- Mice deeply anesthetized with Isoflorane
- Tissue immediately extracted
  - Four tissue types:
    - Cerebellum
    - Brain –including hippocampal bodies
    - Heart
    - Kidney
  - Luciferase assay: tissue submerged and ‘diced’ in 1x glo-lysis buffer
  - RNA/ protein tissue submerged in liquid-nitro
  - Histology: cerebellum submerged into cold 4% PFA

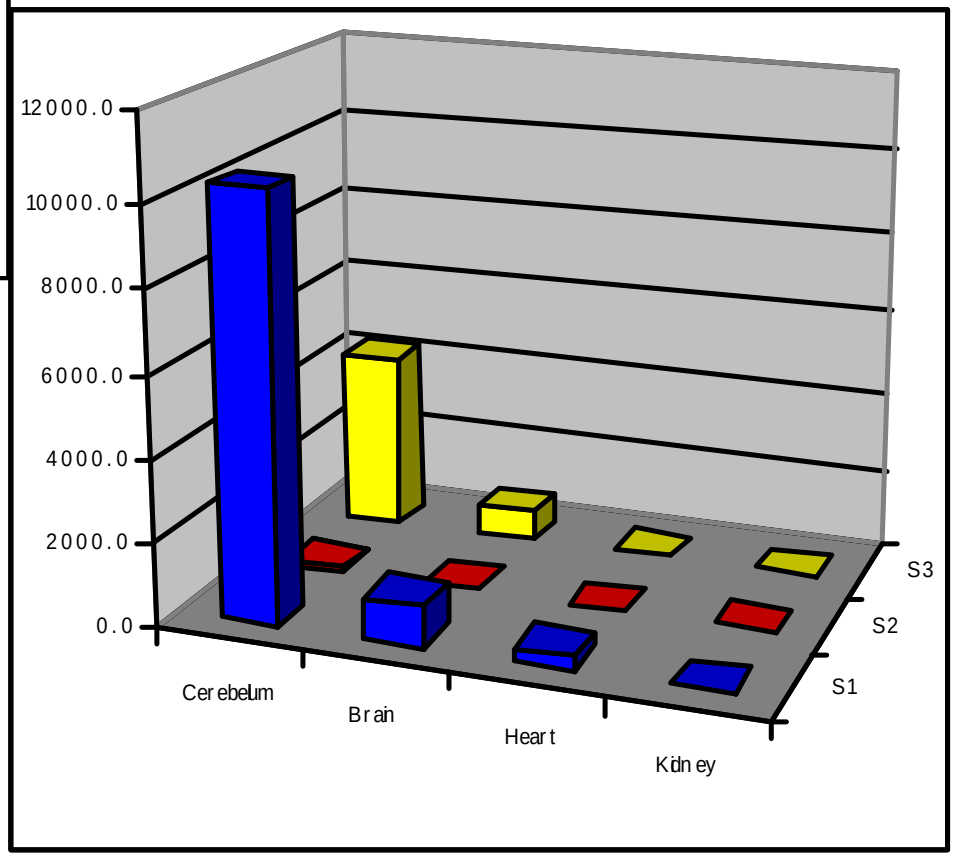
- Luciferase assay
  - Tube pre-weighed/ post weighed
  - Tissue further homogenized
  - Centrifuge: 14k @ 3min
  - “Plate” samples in duplicate (50uL) + equal volume substrate
    - Positive control: transfected luc cell
    - Neg control: buffer + substrate
  - RLU normalized to mg/ tissue



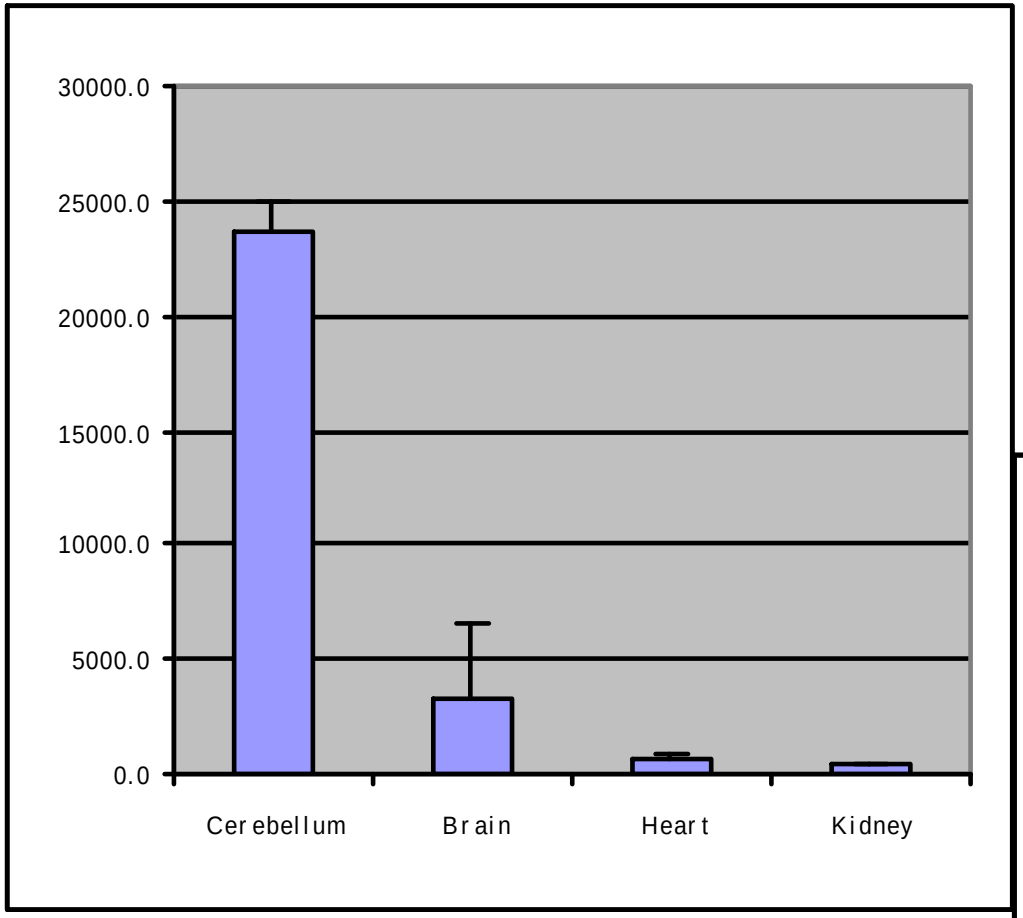
Luc-2
N=4 positive
N=12 total tested

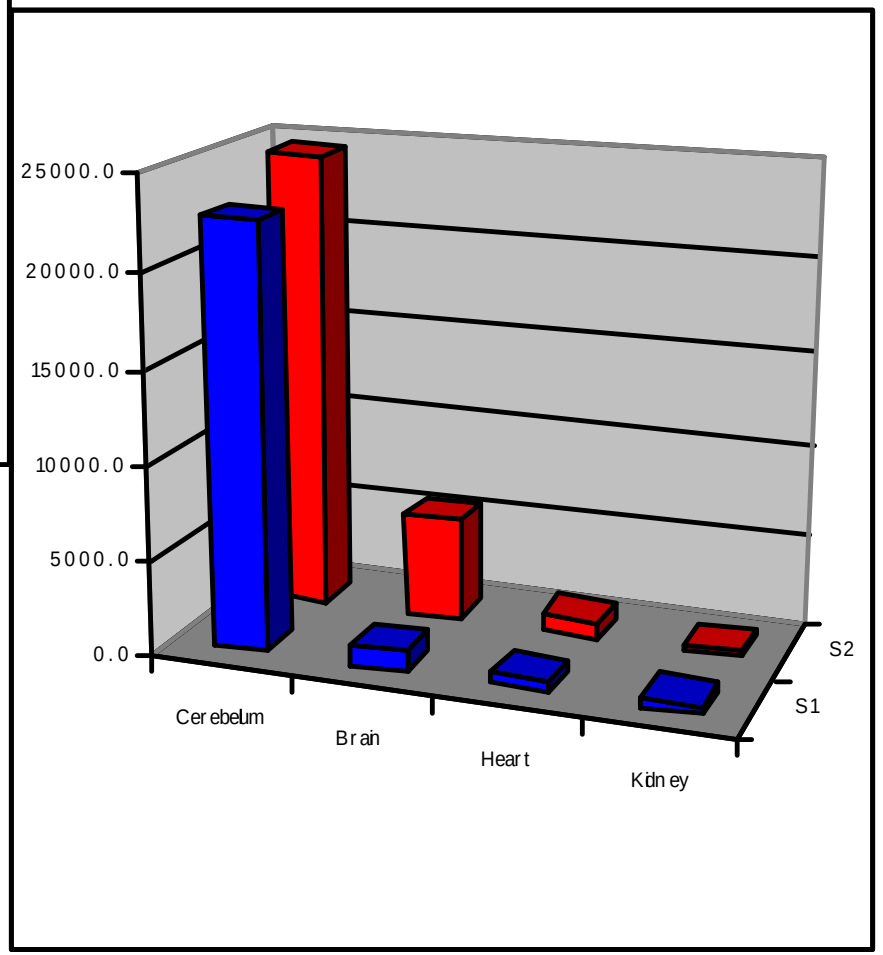




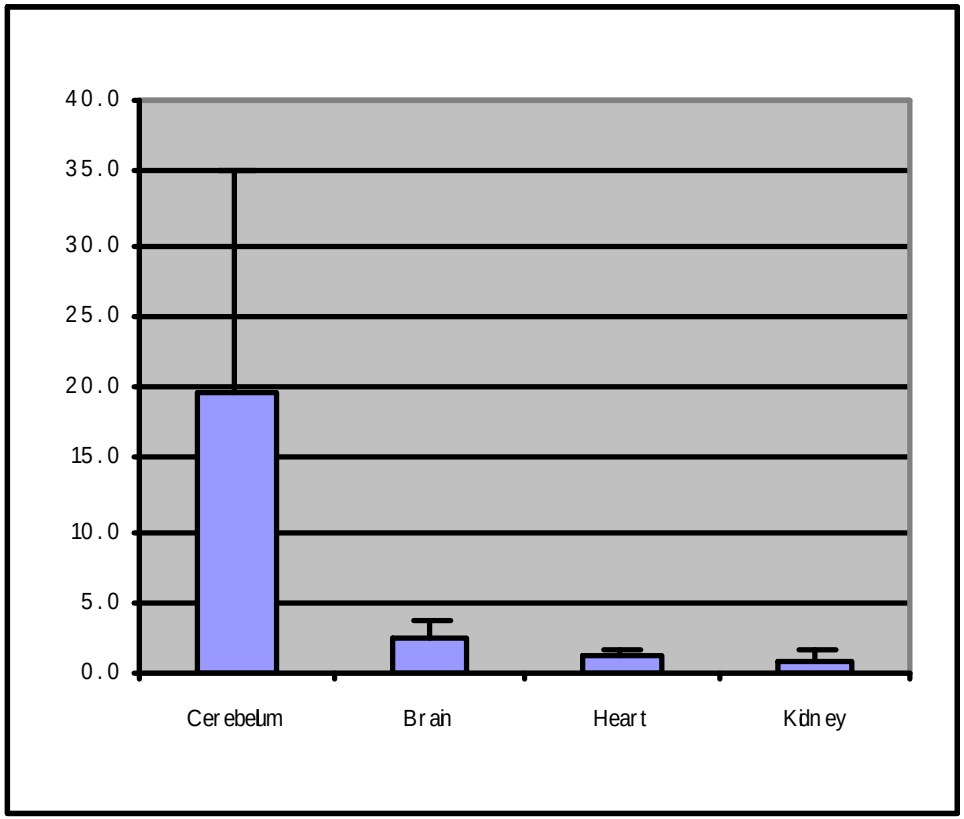


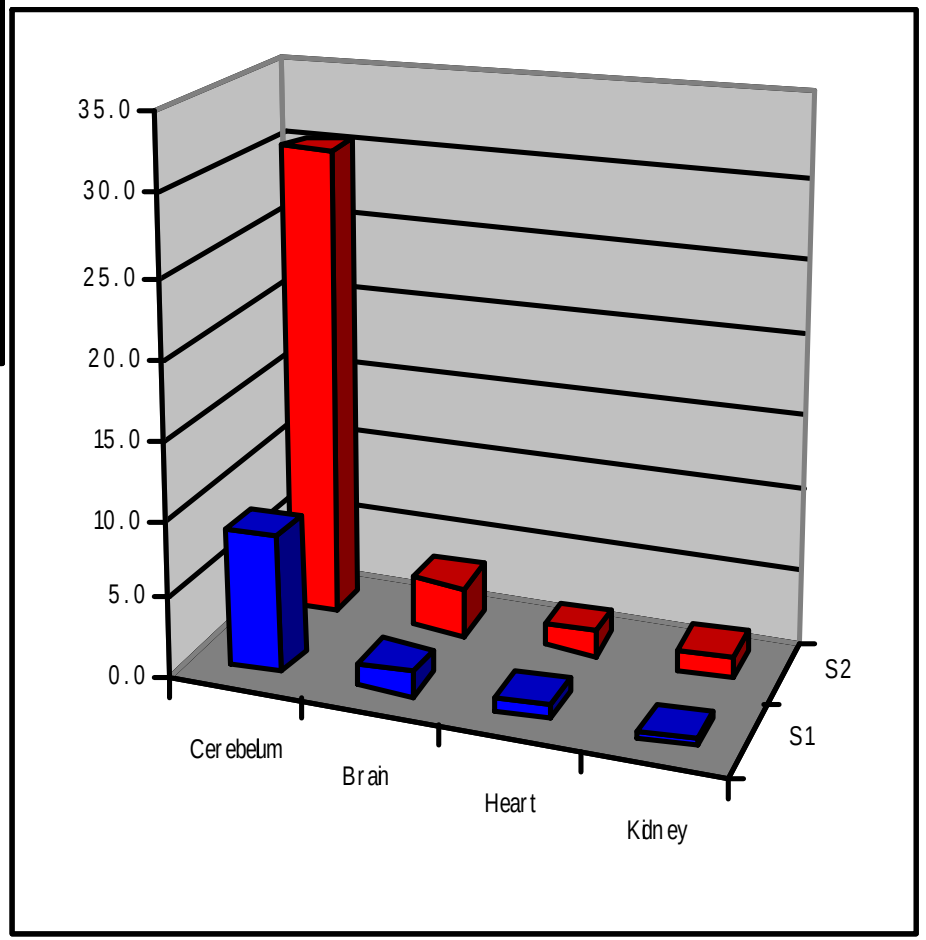
Luc-74
N=3 positive
N=3 total tested



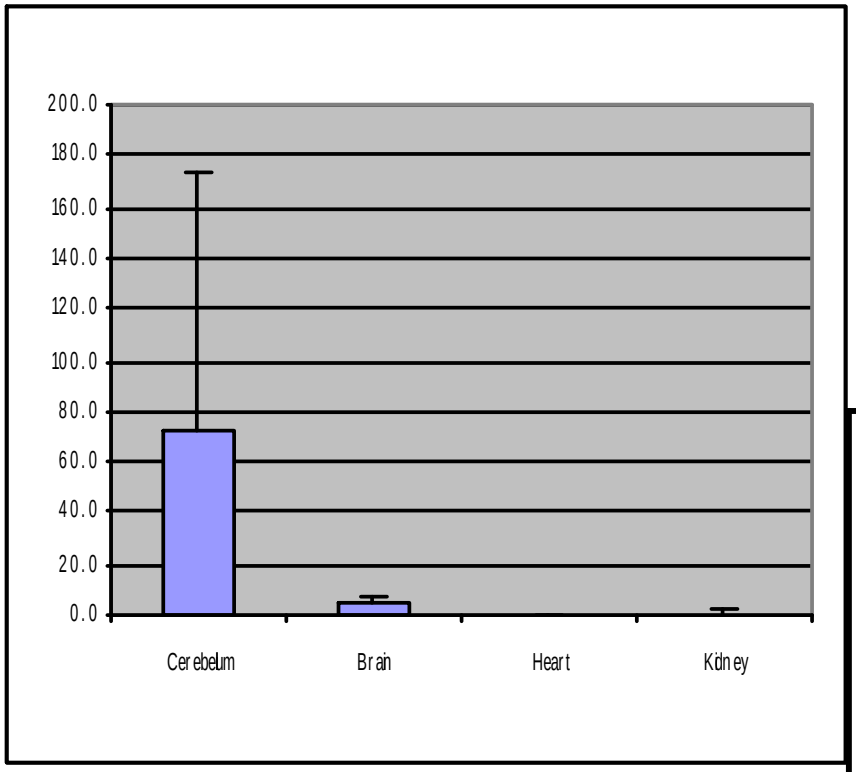


Luc-75
N=2 positive
N=3 total tested

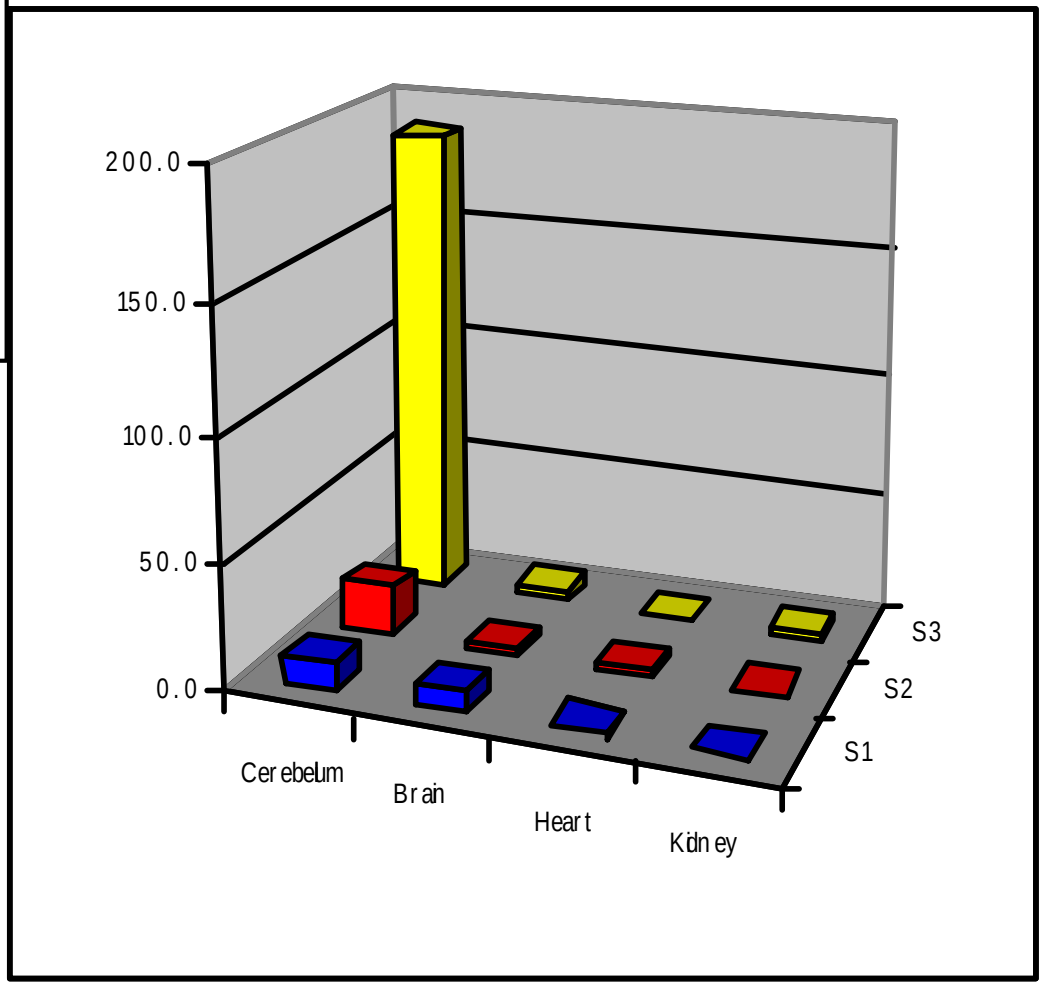




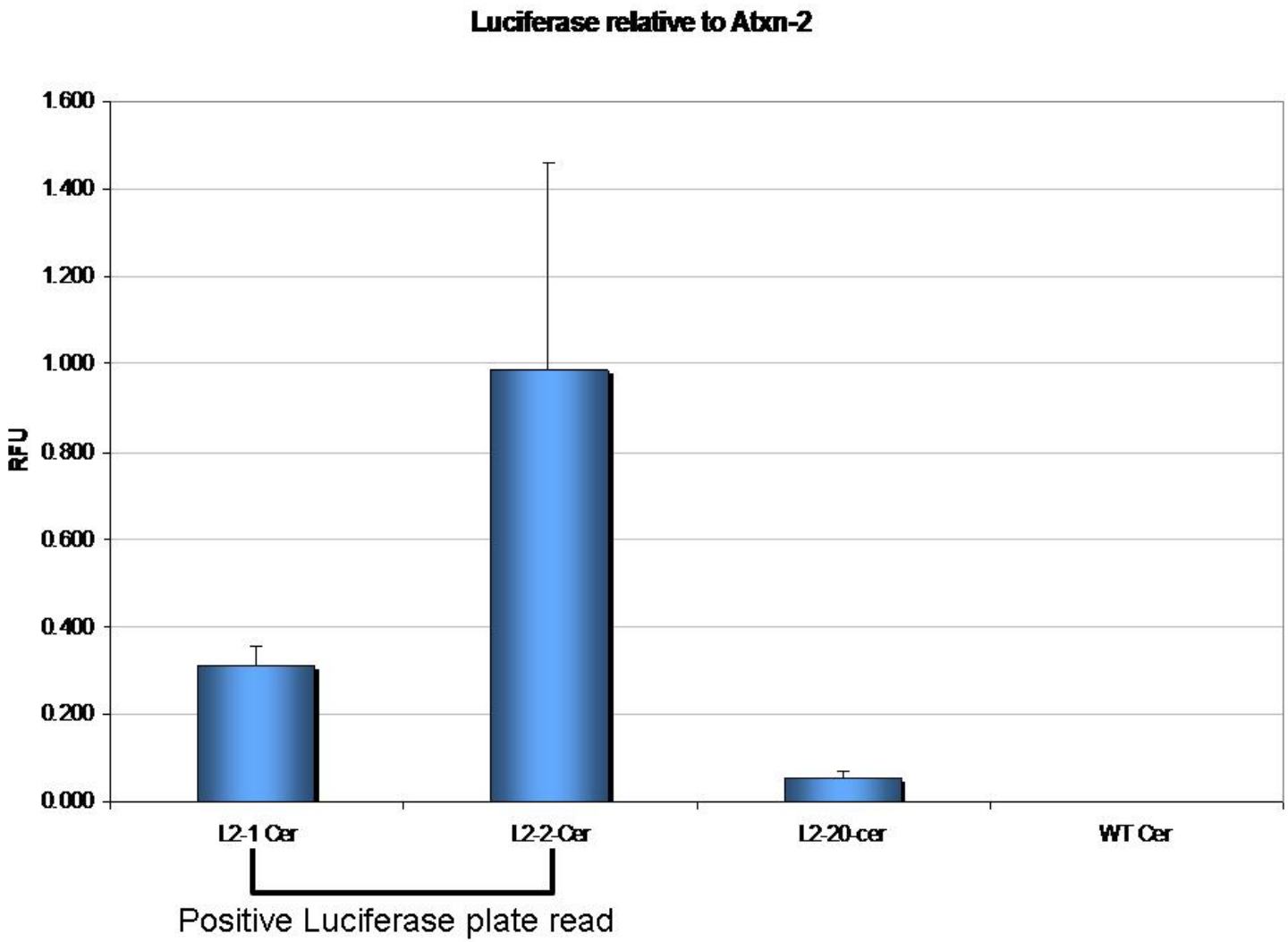
Luc-78
N=2 positive
N=4 total tested



Luc-2
N=4 positive
N=12 total tested



qPCR


Luciferase relative to Atxn-2
RFU
1.600
1.400
1.200
1.000
0.800
0.600
0.400
0.200
0.000
L2-1 Cer
L2-2-Cer
L2-20-cer
WT Cer
Positive Luciferase plate read

Future

- IVIS *in vivo* imaging
- Drug screening