

Supplementary Materials

Deregulation of mitochondrial reverse electron transport alters the metabolism of reactive oxygen species and NAD⁺/NADH and presents a therapeutic target in Alzheimer's disease

Suman Rimal¹, Wen Li¹, Tejinder Pal Khaket¹, Yu Li¹, Ishaq Tantray¹, Yanping Li¹, Sunil Bhurtel¹, Lea T Grinberg², Salvatore Spina², Maria Inmaculada Cobos Sillero¹, William W Seeley², Su Guo³, Bingwei Lu^{1,*}

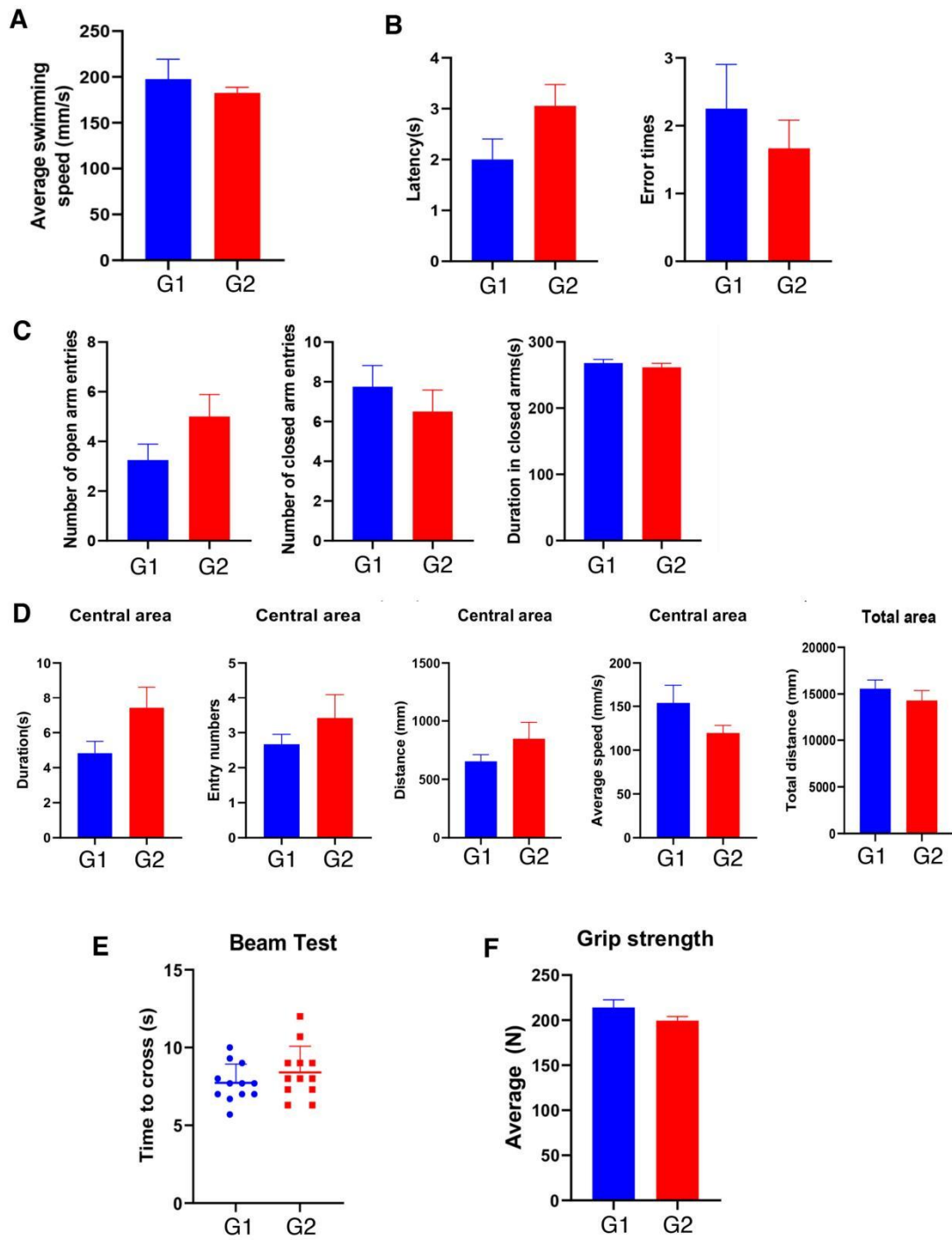
¹Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305, USA.

²Memory and Aging Center, Department of Neurology and Department of Pathology, University of California, San Francisco, CA 94158, USA.

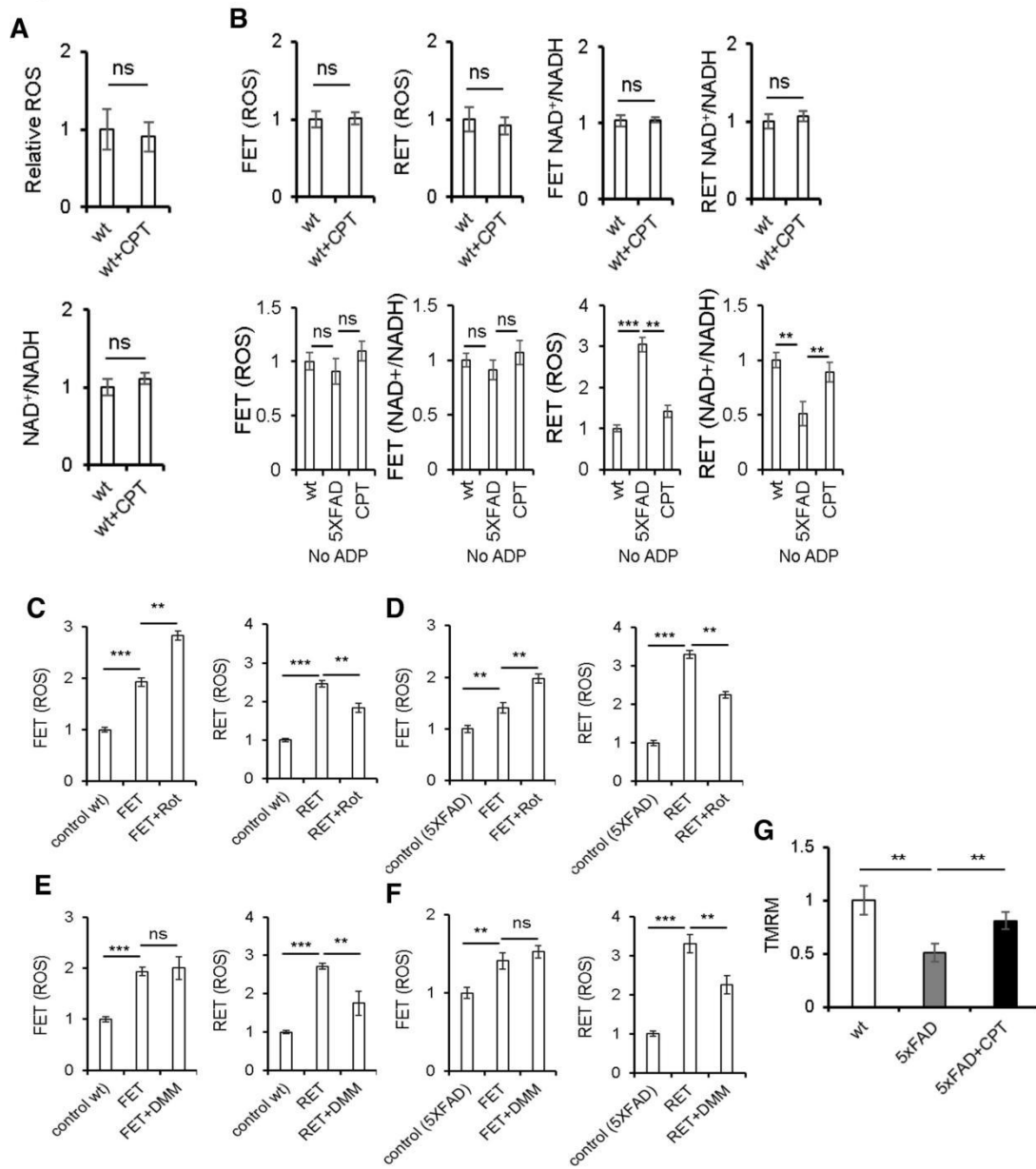
³Department of Bioengineering and Therapeutic Sciences, Programs in Human Genetics and Biological Sciences, Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research, University of California, San Francisco, CA 94143, USA.

***Correspondence to:** Dr. Bingwei Lu, Department of Pathology, Stanford University School of Medicine, R270 Edwards Building, 300 Pasteur Drive, Stanford, CA 94305, USA. E-mail:bingwei@stanford.edu

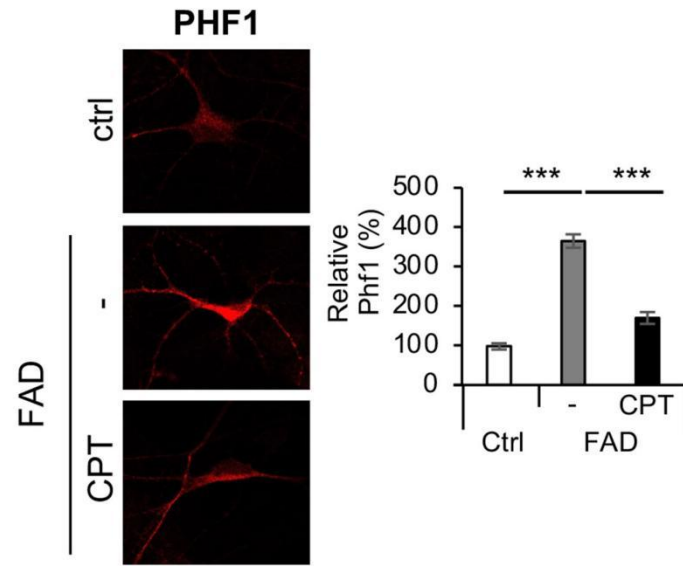
Supplementary Figures



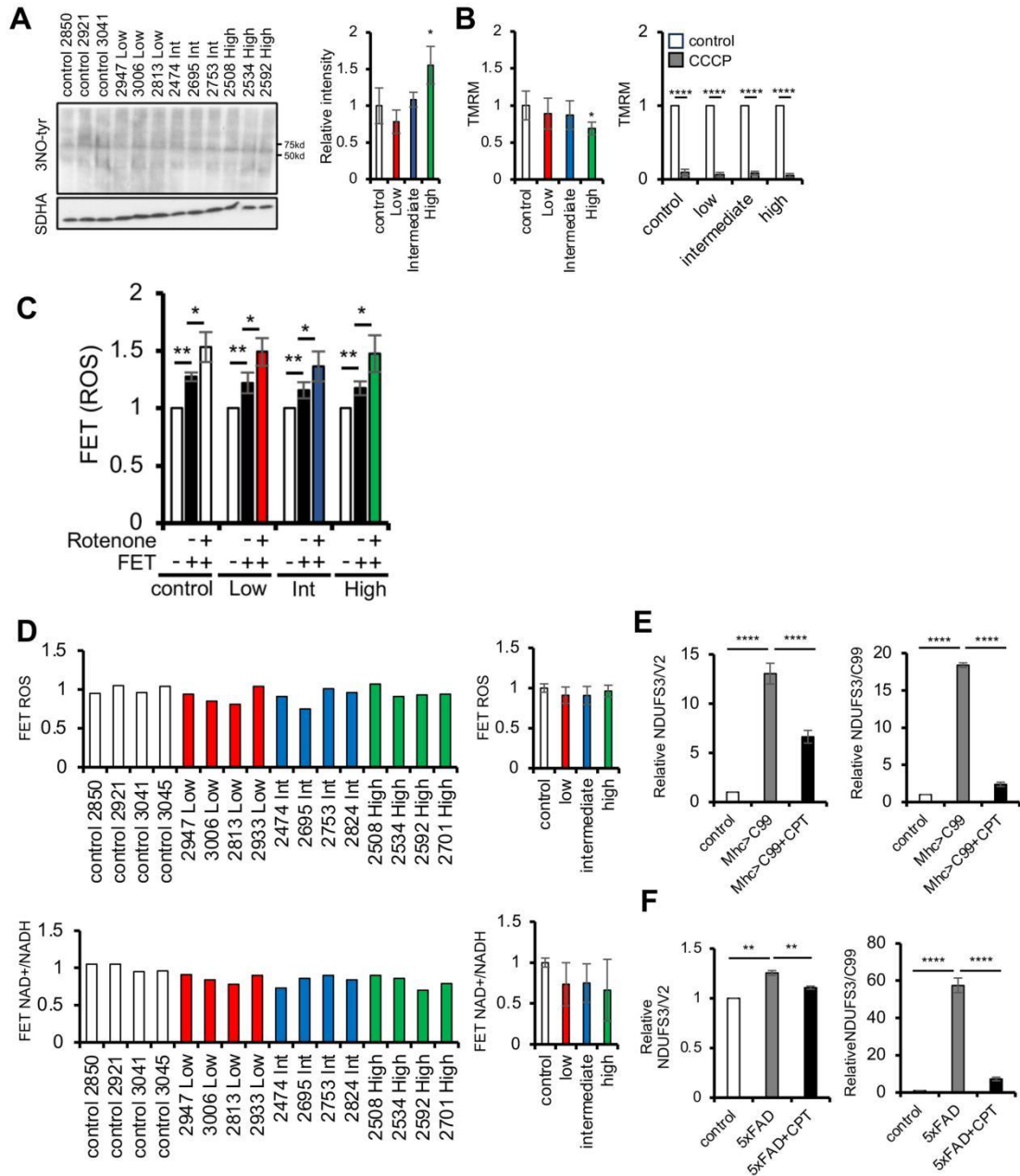
Supplementary Figure 1.



Supplementary Figure 2.



Supplementary Figure 3.



Supplementary Figure 4.

Supplementary Tables

Supplementary Table 1

Case P#	ADNC level	Sex	Age at death	Clinical	Primary Neuropath Dx	Comorb id	PMI (hrs)	Frozen Block Label	Frozen Block Side	Frozen Block Region
P2850	Not	F	92	Control	None	AGD, limbic	8.0	Z6	L	ITG
P2921	Not	F	82	Control	None		9.6	Z34	R	Superior parietal cortex
P3041	Not	F	78	Control	None		11.6	Z15	L	Superior parietal cortex
P3045	Not	F	84	Control	None		7.7	Z8	L	Superior parietal cortex
P2947	Low	M	95	Control	None		9.6	Z3	L	ITG
P3006	Low	M	91	Control	None		5.6	Z2	L	ITG
P2813	Low	M	87	AD,Vascular	AD		30.5	Z2	L	ITG
P2933	Low	M	87	MCI	ADNC, low		9.7	Z2	L	ITG
P2474	Intermediate	F	87	MCI	AD,Braak IV	VBI	9.5	X7	L	ITG
P2695	Intermediate	M	84		AD		28	Z3	L	ITG
P2753	Intermediate	F	94	AD	AD		14.8	Z1	R	ITG
P2824	Intermediate	M	89	MCI,Mixed	AD		9.1	Z2	L	ITG
P2508	High	M	82	AD	AD		6.7	Z7	R	ITG
P2534	High	F	75	AD	AD		6.9	Z7	R	ITG
P2592	High	M	86	AD	AD		8.6	Z3	L	ITG
P2701	High	F	76	AD	AD		18	Z1	L	ITG

Supplementary Table 2**PK parameters of CPT in rats after IV and PO administration**

Matrix		Plasma		Brain	
Administration Route		IV	PO	IV	PO
Parameters	Unit	Values			
Dose Level	mg/kg	2	10	2	10
T _{1/2}	h	4.22	3.33	6.98	3.33
T _{max}	h	0.25	2.00	0.08	1.00
C _{max}	ng/mL, ng/g	1484.90	1611.23	1250.89	763.17
C ₀	ng/mL, ng/g	1473.32	NA	1295.22	NA
AUC _(0-t)	h*ng/mL, h*ng/g	3056.62	14450.15	3680.33	7171.71
AUC _(0-∞)	h*ng/mL, h*ng/g	3085.50	14542.32	3870.56	7218.07
MRT _(0-t)	h	2.96	5.44	3.96	5.62
MRT _(0-∞)	h	3.21	5.59	5.44	5.77
V _{ss}	mL/kg	2081.80	NA	2808.96	NA
V _Z	mL/kg	3942.86	NA	5206.47	NA
Cl	mL/h/kg	648.19	NA	516.72	NA
F	%	NA	94.55	NA	38.97

NA: not applicable

Supplementary Table 3

CPT(M3) PO Plasma Pharmacokinetic Profile in APP/PS1 mice										
PO(G2) Plasma concentration-time data										
PO(G2) Dose										
Time (h)	Concentration (ng/mL)						Mean (ng/mL)	SD (ng/mL)	CV (%)	
	Mouse 8	Mouse 9	Mouse 13	Mouse 17	Mouse 18	Mouse 22				
	33600	32200	29100	38500	28000	36400	32967	4075	12.4	
CPT(M3) PO Liver Pharmacokinetic Profile in male APP/PS1 mice										
PO(G2) Liver concentration-time data										
PO(G2) Dose										
Time (h)	Concentration (ng/g)						Mean (ng/g)	SD (ng/g)	CV (%)	
	Mouse 8	Mouse 9	Mouse 13	Mouse 17	Mouse 18	Mouse 22				
	64200	67800	68400	73200	75000	61200	68300	5223	7.65	
Liver/Plasma Ratio Profile										
PO(G2) Dose										
Time (h)	Liver/Plasma Ratio Profile						Mean	SD	CV (%)	
	Mouse 8	Mouse 9	Mouse 13	Mouse 17	Mouse 18	Mouse 22				
	1.91	2.11	2.35	1.90	2.68	1.68	2.10	0.36	17.1	
CPT(M3) PO Kidney Pharmacokinetic Profile in male APP/PS1 mice										
PO(G2) Kidney concentration-time data										
PO(G2) Dose										
Time (h)	Concentration (ng/g)						Mean (ng/g)	SD (ng/g)	CV (%)	
	Mouse 8	Mouse 9	Mouse 13	Mouse 17	Mouse 18	Mouse 22				
	27600	22560	29520	25860	26220	28020	26650	2382	8.94	
Kidney/Plasma Ratio Profile										
PO(G2) Dose										
Time (h)	Kidney/Plasma Ratio Profile						Mean	SD	CV (%)	
	Mouse 8	Mouse 9	Mouse 13	Mouse 17	Mouse 18	Mouse 22				
	0.821	0.701	1.01	0.675	0.936	0.770	0.82	0.13	16.3	
PO Heart Pharmacokinetic Profile in male APP/PS1 mice										
PO(G2) Heart concentration-time data										
PO(G2) Dose										
Time (h)	Concentration (ng/g)						Mean (ng/g)	SD (ng/g)	CV (%)	
	Mouse 8	Mouse 9	Mouse 13	Mouse 17	Mouse 18	Mouse 22				
	16800	18120	25800	18860	22880	22140	20700	3407	16.5	
Heart/Plasma Ratio Profile										
PO(G2) Dose										
Time (h)	Heart/Plasma Ratio Profile						Mean	SD	CV (%)	
	Mouse 8	Mouse 9	Mouse 13	Mouse 17	Mouse 18	Mouse 22				
	0.500	0.563	0.887	0.485	0.810	0.608	0.642	0.168	26.1	
PO Lung Pharmacokinetic Profile in male APP/PS1 mice										
PO(G2) Lung concentration-time data										
PO(G2) Dose										
Time (h)	Concentration (ng/g)						Mean (ng/g)	SD (ng/g)	CV (%)	
	Mouse 8	Mouse 9	Mouse 13	Mouse 17	Mouse 18	Mouse 22				
	13200	12240	9420	25860	13320	13260	14550	5737	39.4	
Lung/Plasma Ratio Profile										
PO(G2) Dose										
Time (h)	Lung/Plasma Ratio Profile						Mean	SD	CV (%)	
	Mouse 8	Mouse 9	Mouse 13	Mouse 17	Mouse 18	Mouse 22				
	0.393	0.380	0.324	0.672	0.476	0.364	0.435	0.126	29.1	
PO Uterus Pharmacokinetic Profile in male APP/PS1 mice										
PO(G2) uterus concentration-time data										
PO(G2) Dose										
Time (h)	Concentration (ng/g)						Mean (ng/g)	SD (ng/g)	CV (%)	
	Mouse 8	Mouse 9	Mouse 13	Mouse 17	Mouse 18	Mouse 22				
	21200	12500	14300	21000	19800	17000	17633	3650	20.7	
Uterus /Plasma Ratio Profile										
PO(G2) Dose										
Time (h)	Uterus /Plasma Ratio Profile						Mean	SD	CV (%)	
	Mouse 8	Mouse 9	Mouse 13	Mouse 17	Mouse 18	Mouse 22				
	0.631	0.388	0.491	0.545	0.707	0.467	0.538	0.116	21.5	
PO Brain Pharmacokinetic Profile in male APP/PS1 mice										
PO(G2) Brain concentration-time data										
PO(G2) Dose										
Time (h)	Concentration (ng/g)						Mean (ng/g)	SD (ng/g)	CV (%)	
	Mouse 8	Mouse 9	Mouse 13	Mouse 17	Mouse 18	Mouse 22				
	17700	18440	27240	17880	17700	16920	19480	3890	20.0	
Brain/Plasma Ratio Profile										
PO(G2) Dose 100 mg/kg										
Time (h)	Brain/Plasma Ratio Profile						Mean	SD	CV (%)	
	Mouse 8	Mouse 9	Mouse 13	Mouse 17	Mouse 18	Mouse 22				
	0.527	0.604	0.936	0.464	0.632	0.465	0.605	0.177	29.2	

Supplementary Table 4

Group	Treatment	ID	Organ Observations															
			Brain	Spine	Thymus gland	Thyroid gland	Esophagus	Stomach	Intestine	Liver and pancreas	Kidneys	Spleen	Heart	Trachea	Lung	Uterus	Ovary	Mammary glands
G1-1		3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
G1-2	APP/PS1 mice + Vehicle	25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		27	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		32	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		34	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		36	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
G2-1	APP/PS1 mice + CPT (W)	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		22	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
G2-2		24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		26	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		28	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		29	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		

Note: "-" means the organs were normal by visual observation.