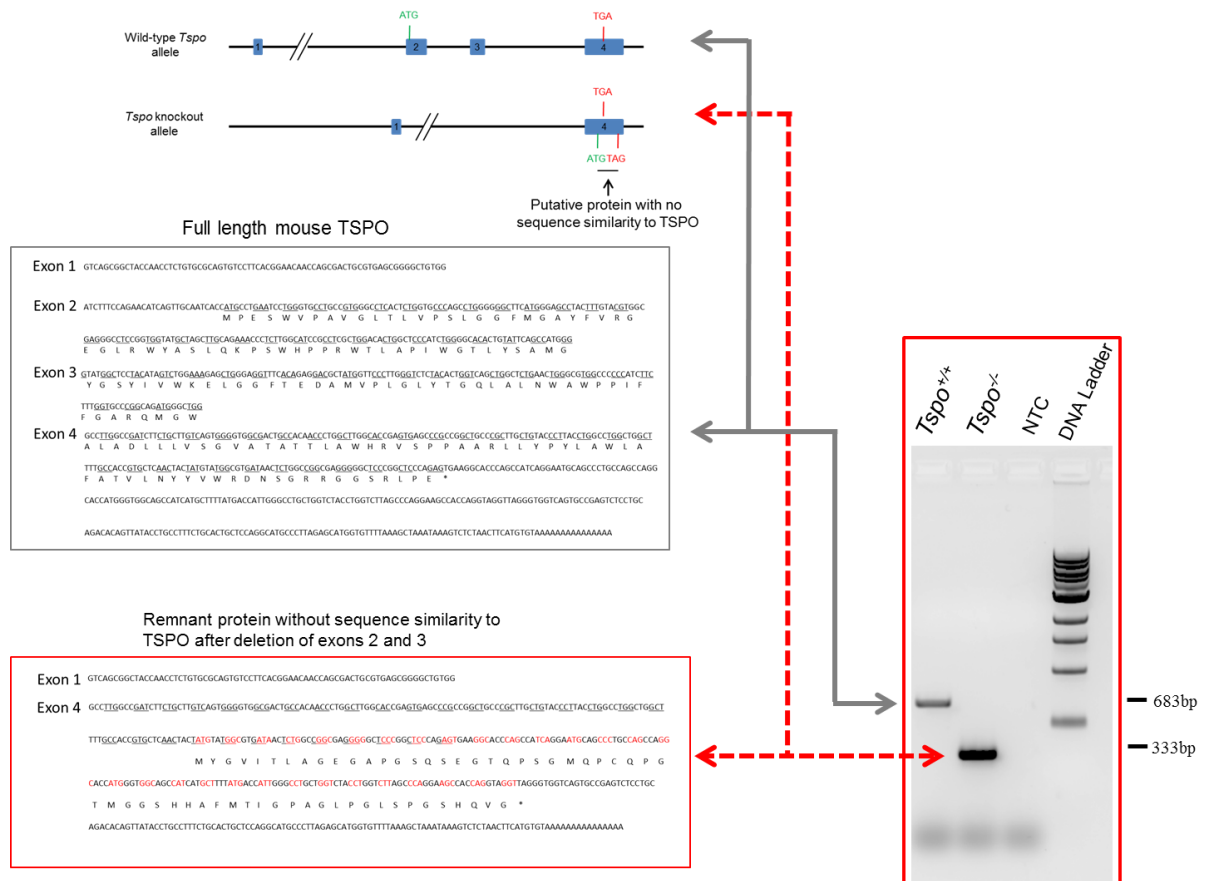
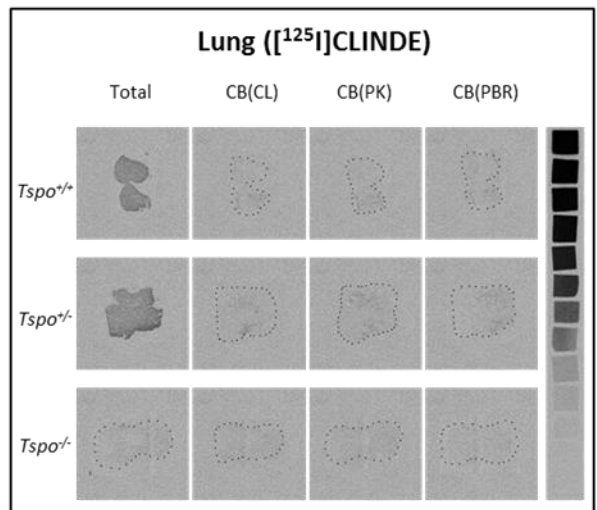
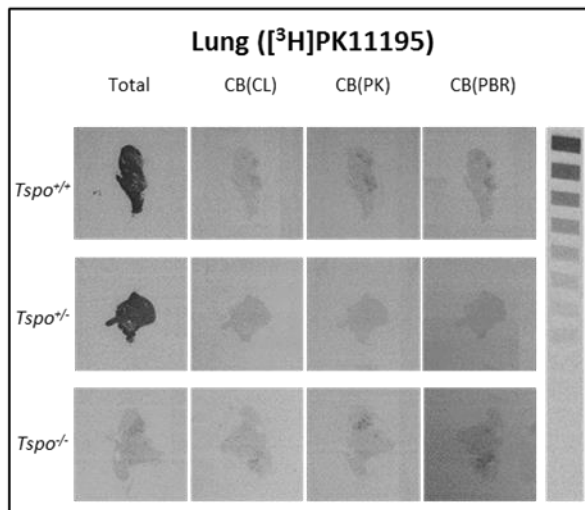
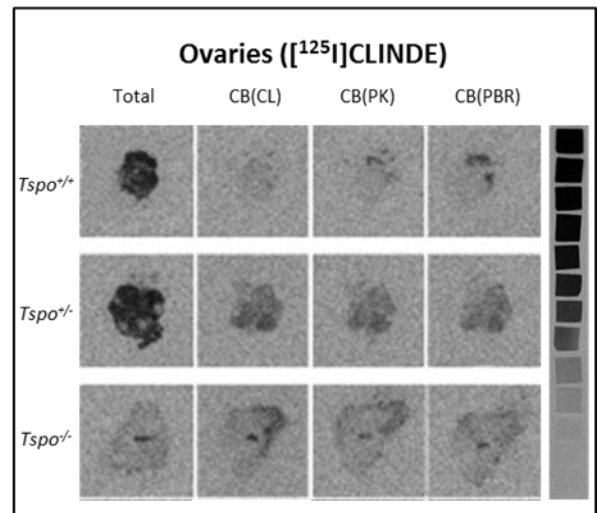
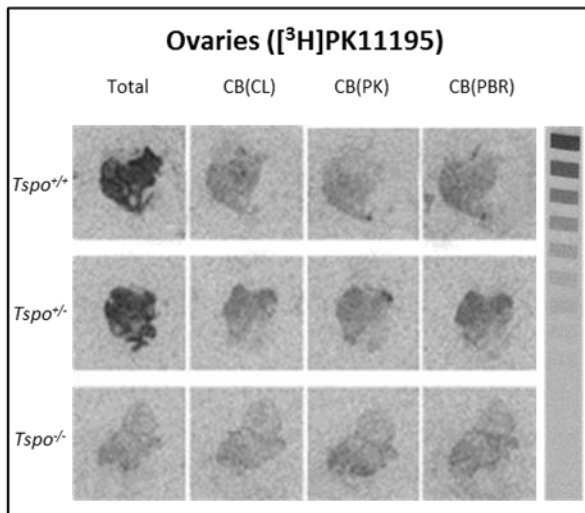
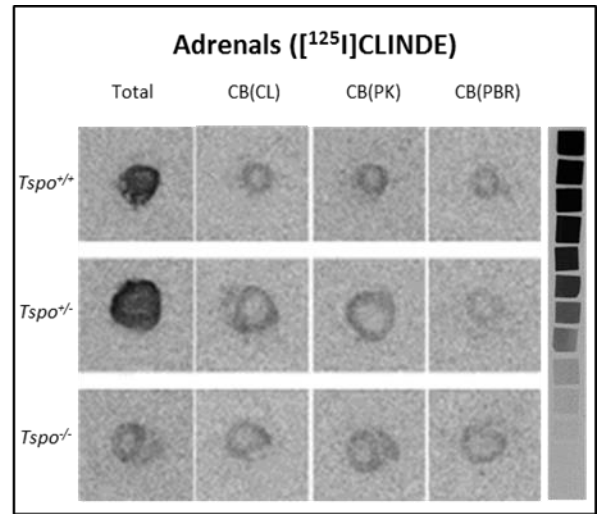
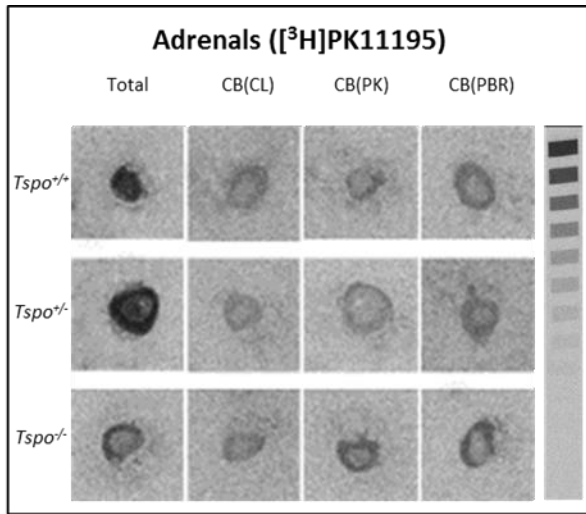


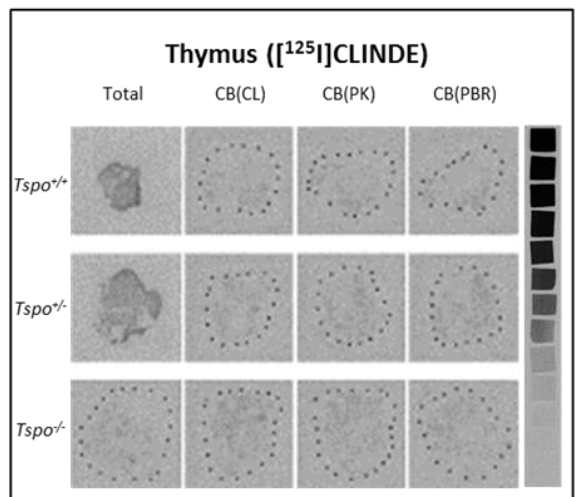
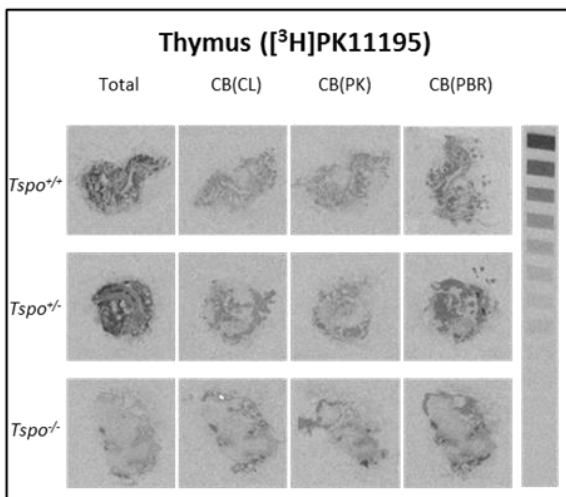
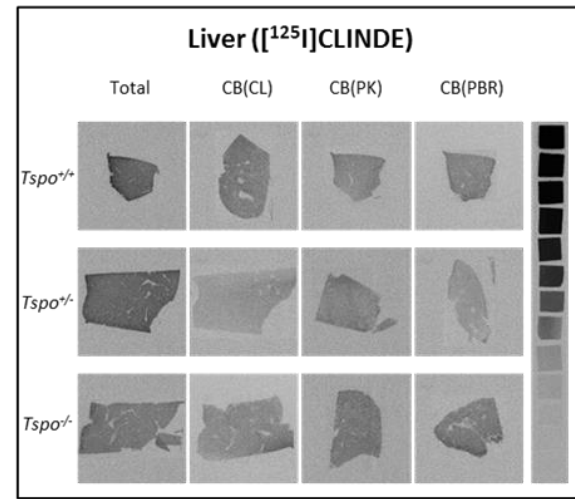
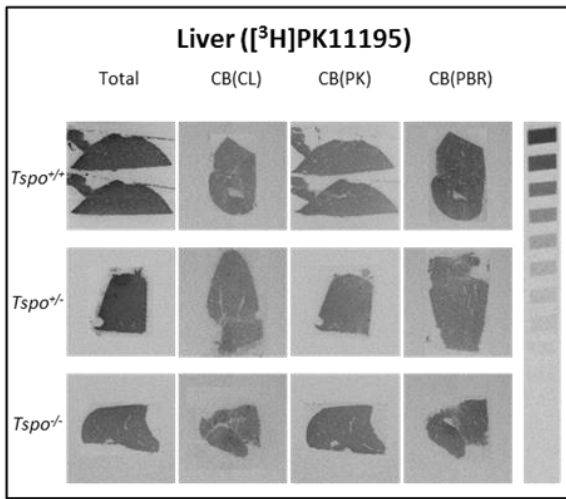
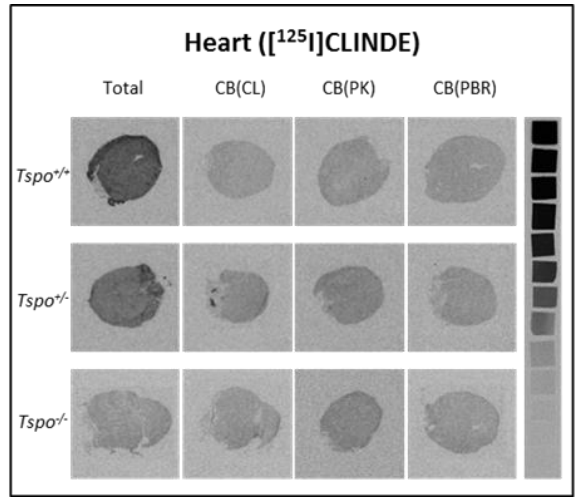
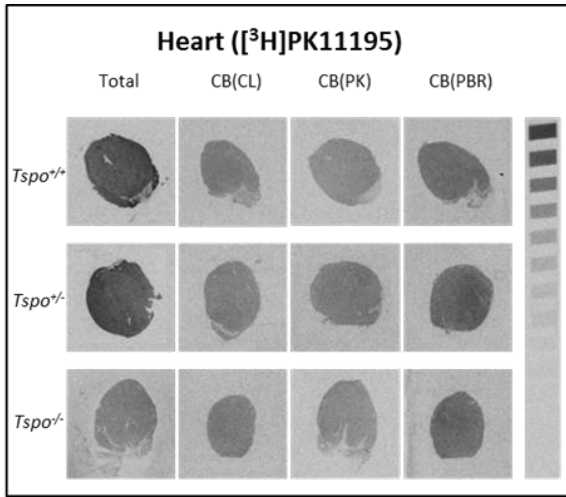
# Supplementary Information:

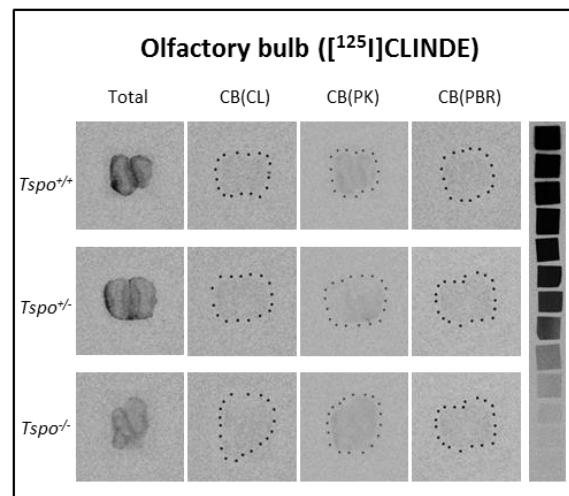
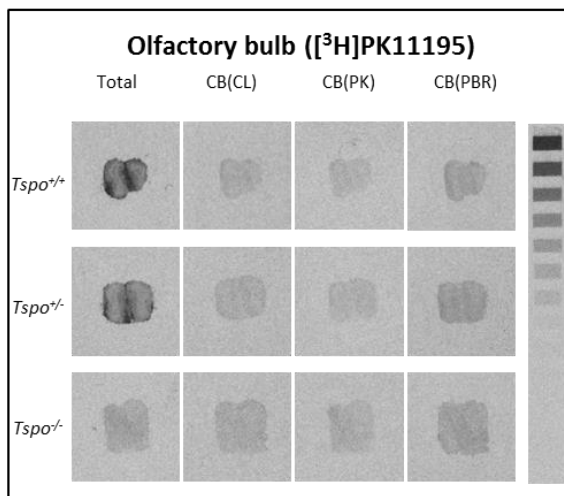
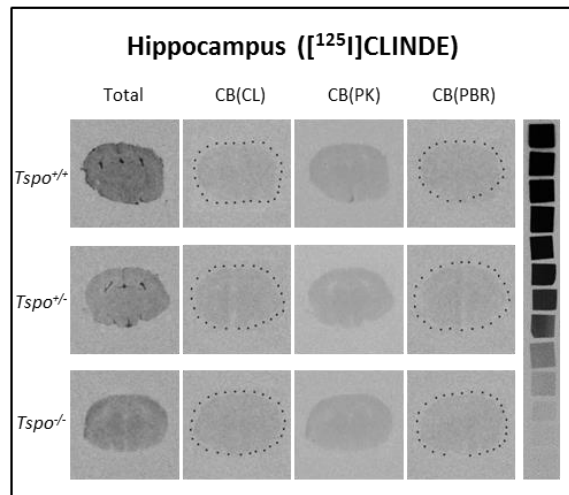
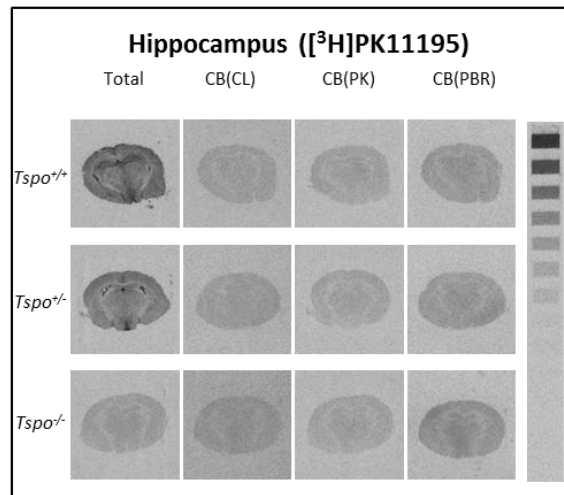
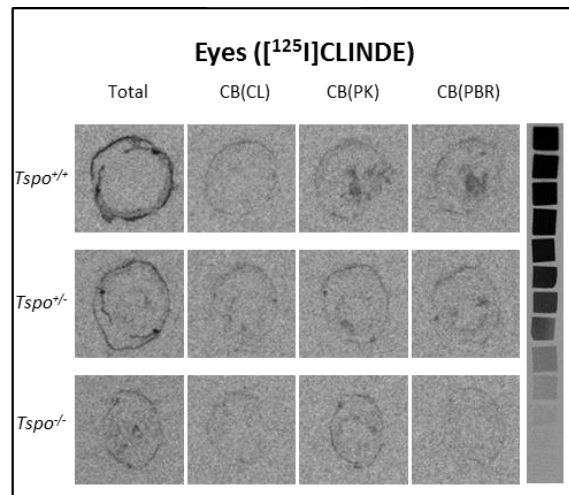
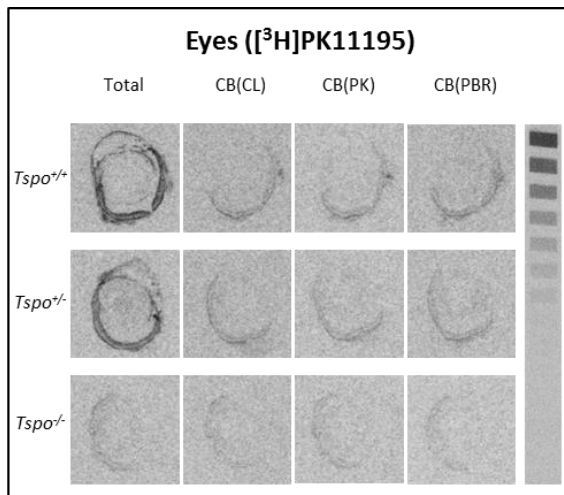


**Supplementary Figure 1. Confirmation of the deletion of exons 2 and 3.**

By deleting exons 2 and 3, only exons 1 and 4 remain. As can be observed in the above sequence of the full length *Tspo* gene, there are no start codons within the *Tspo* reading frame present in either exon 1 or exon 4. Therefore, following the deletion of exons 2 and 3, only a short protein that shares no sequence similarity to the TSPO, as shown above, could in theory be produced. The presence of a mRNA sequence corresponding to exons 1 and 4 in *Tspo* knockout mice was confirmed by RT-PCR. The cDNA from a *Tspo* knockout animal produced a product of the expected size (333 bp; red dotted line), whilst the cDNA from a wildtype animal produced a product of 683 bp which corresponds to exons 1, 2, 3 and 4. The products from both wildtype and *Tspo* knockout animals were confirmed by DNA sequencing.



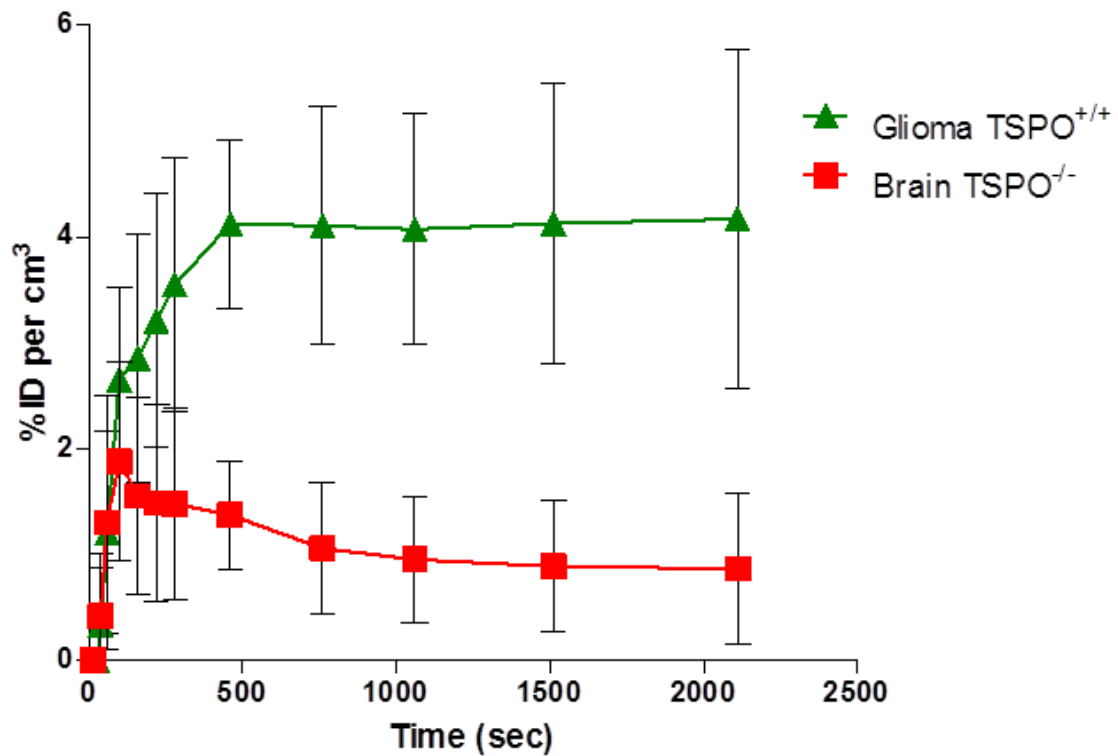




**Supplementary Figure 2. Comparative [<sup>3</sup>H]PK11195 and [<sup>125</sup>I]CLINDE autoradiography of 9 organs in adult mice.**

The receptor autoradiographs (16 μm sections) show the total binding of [<sup>3</sup>H]PK11195 (1 nM) and [<sup>125</sup>I]CLINDE (3 nM) as well as competitive displacement with 10 μM unlabelled CLINDE (CB(CL)), PK11195 (CB(PK)) and PBR111 (CB(PBR)) of adrenal glands, ovaries, lungs, heart, liver, thymus, eyes, hippocampus and olfactory bulb of *Tspo*<sup>+/+</sup>, *Tspo*<sup>+/-</sup> and *Tspo*<sup>-/-</sup> mice .

Specific binding of 3 nM [<sup>125</sup>I]CLINDE and 1 nM [<sup>3</sup>H]PK11195 is clearly visible in tissue sections from *Tspo*<sup>+/+</sup> and *Tspo*<sup>+/-</sup> mice and is displaceable by all 3 unlabelled ligands, whereas there is no specific binding in *Tspo*<sup>-/-</sup> tissue.



**Supplementary Figure 3. Quantification of [ $^{18}F$ ]PBR111 positron emission tomography of PBR/TSPO-expressing glioma in global TSPO knockout mouse brain.**

Time-activity curves of [ $^{18}F$ ]PBR111 25 days after GL261 glioblastoma cell implantation. (green =  $Tspo^{+/+}$  glioma; red =  $Tspo^{-/-}$  brain). Error bars denote standard deviation (n=4 for each group).

**Supplementary Table 1. Diet Composition**

<b>Calculated Nutritional Parameters</b>	<b>Premium bredder diet - Gordon's Specialty Stockfeeds</b>	<b>Calculated Total Vitamins</b>	<b>Premium bredder diet - Gordon's Specialty Stockfeeds</b>
Protein	23%	Vitamin A (Retinol)	5666 IU/kg
Total Fat	6%	Vitamin D (Cholecalciferol)	200 IU/kg
Crude Fibre	5%	Vitamin E (a Tocopherol acetate)	50 mg/kg
Metabolizable energy	13 MJ/ kg	Vitamin K (Menadione)	5 mg/kg
<b>Calculated Amino Acids</b>		Vitamin C (Ascorbic acid)	150 mg/kg
Arginine	1.21%	Vitamin B1 (Thiamine)	4 mg/kg
Valine	1.02%	Vitamin B2 (Riboflavin)	5 mg/kg
Leucine	1.52%	Niacin (Nicotinic acid)	10 mg/kg
Isoleucine	0.80%	Vitamin B6 (Pryridoxine)	6 mg/kg
Threonine	0.84%	Pantothenic Acid	12 mg/kg
Methionine	0.59%	Biotin	0.06 mg/kg
Cystine		Folic Acid	10 mg/kg
Lysine	0.98%	Vitamin B12 (Cyancobalamin)	5 µg/kg
Phenylalanine	1.64%	<b>Calculated Fatty Acid Composition</b>	
Tyrosine		Total Mono Unsaturated Fats	42.90%
Tryptophan	0.37%	Total Polyunsaturated Fats	30.70%
Histidine	0.50%	Total Saturated Fats	21.30%
<b>Calculated Total Minerals</b>			
Calcium	1.01%		
Phosphorous	0.77%		
Magnesium	0.18%		
Sodium	0.30%		
Potassium	0.54%		
Iron	97.0 mg/kg		
Copper	10.6 mg/kg		
Iodine	1.15 mg/kg		
Manganese	87.4 mg/kg		
Zinc	48.1 mg/kg		
Selenium	0.1 mg/kg		

**Supplementary Table 2. Behaviour phenotyping**

Test	index	gender	TSPO <sup>+/+</sup>	TSPO <sup>+/-</sup>	TSPO <sup>-/-</sup>
Open field	Time spent in center (sec)	Male	160.1±101.1	87.70±36.35	112.3±43.01
		Female	94.33±24.28	124.0±45.96	99.94±33.94
	Frequency of entering center	Male	60.57±23.78	49.75±16.61	53.42±10.40
		Female	48.83±10.89	53.75±14.83	58.64±13.92
	Time spent active (sec)	Male	711.0±50.49	704.0±53.59	738.0±26.08
		Female	714.3±31.12	676.4±42.13	725.7±38.34
	Distance travelled (mm)	Male	43180±11069	44381±10006	42084±7590
		Female	44266±7782	39516±10048	50333±12334
	Distanced moved without travelling (mm)	Male	4308±758.1	4185±739.5	4693±556.8
		Female	4092±584.8	3729±476	4444±603.8
Emergence test	Time spent in hidebox (sec)	Male	155.4±42.56	129.1±35.64	159.8±56.15
		Female	121.8±41.59	113.1±44.85	121.1±43.18
	Frequency of entering hidebox	Male	30.28±15.22	25.55±6.629	26.42±9.020
		Female	25.17±9.411	23.00±10.50	28.55±9.903
	Time spent active (sec)	Male	751.9±25.91	746.2±31.06	752.1±32.88
		Female	705.7±38.97	711.9±35.08	748.3±32.77
	Distance travelled (mm)	Male	44545±4826	45864±6128	41474±5881
		Female	41125±7451	42068±8191	49188±8525
	Distance moved without travelling (mm)	Male	5765±528.4	5675±673.2	5631±784.8
		Female	4781±730.2	4753±641.5	5335±676.8
Light/dark preference test	Time spent in light compartment (sec)	Male	382.1±115.7	422.8±112.5	374.9±76.04
		Female	418.2±84.50	372.0±85.95	412.9±113.0
	Transition between compartments	Male	32.00±6.403	36.67±11.66	33.08±8.229
		Female	41.33±10.29	46.62±19.64	46.09±9.148
	Time spent active (sec)	Male	819.4±25.79	821.7±15.86	827.5±12.60
		Female	803.8±20.20	797.0±19.08	810.5±17.62
	Distance travelled (mm)	Male	50219±5948	52916±5955	50071±6780
		Female	53074±9692	48749±6220	58633±9674
	Distance moved without travelling (mm)	Male	8363±943.2	8367±790.1	8359±532.8
		Female	7682±443.0	7219±606.8	7712±735.3
Elevated plus maze	Time spent in open arms (sec)	Male	134.6±59.75	122.9±75.71	145.1±59.14
		Female	103.8±55.92	139.2±78.00	152.46±87.09
	Frequency of entering open arms	Male	33.14±8.783	28.29±13.83	29.50±7.205
		Female	25.50±11.15	28.62±11.35	31.55±17.40
	Time spent active (sec)	Male	646.9±42.39	619.0±63.45	623.3±36.55
		Female	607.8±74.20	595.6±64.95	624.5±62.23
	Distance travelled (mm)	Male	21188±3554	18871±5954	19140±3803
		Female	17096±4673	17996±4994	20150±6317
	Distanced moved without travelling (mm)	Male	5020±624.9	4861±632.6	4824±298.2
		Female	4737±726.9	4679±607.2	4974±550.3

No significant behavioural differences were found between genotypes tested with two-way ANOVA. Values represent mean ± standard deviation (n=5-10 per group).



**Supplementary Table 3. Blood phenotyping**

analysis	phenotype	gender	TSPO <sup>+/+</sup>	TSPO <sup>+/-</sup>	TSPO <sup>-/-</sup>
Haematology	Red blood cells (RBC) (x10 <sup>6</sup> cells/ $\mu$ L)	Male	9.220 $\pm$ 1.067	9.612 $\pm$ 1.117	9.560 $\pm$ 0.473
		Female	9.948 $\pm$ 0.591	10.36 $\pm$ 0.654	9.795 $\pm$ 0.756
	White blood cell (WBC) (x10 <sup>3</sup> cells/ $\mu$ L)	Male	7.428 $\pm$ 2.195	8.024 $\pm$ 3.957	8.440 $\pm$ 1.612
		Female	10.21 $\pm$ 3.572	8.992 $\pm$ 3.205	8.635 $\pm$ 2.317
	Platelets (x10 <sup>3</sup> cells/ $\mu$ L)	Male	828.4 $\pm$ 479.6	1032 $\pm$ 302.8	1104 $\pm$ 143.4
		Female	970.0 $\pm$ 184.7	846.0 $\pm$ 423.3	950.0 $\pm$ 94.84
	Haematocrit (L/L)	Male	0.454 $\pm$ 0.061	0.460 $\pm$ 0.052	0.464 $\pm$ 0.021
		Female	0.486 $\pm$ 0.038	0.480 $\pm$ 0.027	0.472 $\pm$ 0.039
	Reticulocytes (x10 <sup>3</sup> cells/ $\mu$ L)	Male	332.6 $\pm$ 21.52	350.4 $\pm$ 26.28	341.6 $\pm$ 33.55
		Female	377.8 $\pm$ 66.10	415.6 $\pm$ 83.99	380.8 $\pm$ 95.63
	Haemoglobin (g/L)	Male	102.0 $\pm$ 13.49	102.4 $\pm$ 9.63	102.4 $\pm$ 6.066
		Female	109.2 $\pm$ 9.230	109.6 $\pm$ 6.986	105.5 $\pm$ 8.062
	Mean corpuscular volume (fL)	Male	98.20 $\pm$ 3.421	96.00 $\pm$ 2.121	97.20 $\pm$ 3.421
		Female	97.60 $\pm$ 3.647	93.20 $\pm$ 1.095	96.00 $\pm$ 2.944
Biochemistry	Na <sup>+</sup> (mmol/L)	Male	156.3 $\pm$ 2.645	157.3 $\pm$ 3.854	157.6 $\pm$ 1.989
		Female	155.3 $\pm$ 2.490	153.9 $\pm$ 3.488	156.1 $\pm$ 1.792
	K <sup>+</sup> (mmol/L)	Male	8.040 $\pm$ 0.744	7.675 $\pm$ 0.704	7.620 $\pm$ 0.349
		Female	7.060 $\pm$ 0.770	6.850 $\pm$ 0.645	7.160 $\pm$ 0.550
	Cl <sup>-</sup> (mmol/L)	Male	112.6 $\pm$ 0.894	112.0 $\pm$ 0.816	111.0 $\pm$ 1.871
		Female	111.2 $\pm$ 0.837	112.0 $\pm$ 2.160	113.4 $\pm$ 2.881
	Glucose (mmol/L)	Male	9.550 $\pm$ 1.759	10.11 $\pm$ 1.417	10.13 $\pm$ 1.534
		Female	10.06 $\pm$ 1.431	11.01 $\pm$ 2.393	11.55 $\pm$ 1.611
	Cholesterol (mmol/L)	Male	2.404 $\pm$ 0.706	2.707 $\pm$ 0.701	2.894 $\pm$ 0.458
		Female	2.512 $\pm$ 0.622	2.967 $\pm$ 0.085	2.324 $\pm$ 0.671
	Triglyceride (mmol/L)	Male	1.000 $\pm$ 0.000	1.500 $\pm$ 0.577	1.600 $\pm$ 0.548
		Female	1.200 $\pm$ 0.447	1.250 $\pm$ 0.500	1.138 $\pm$ 0.500
	High density lipoprotein (mmol/L)	Male	1.800 $\pm$ 0.837	1.750 $\pm$ 0.500	2.000 $\pm$ 0.000
		Female	1.800 $\pm$ 0.447	2.000 $\pm$ 0.000	1.632 $\pm$ 0.507
	Creatine kinase (U/L)	Male	1507 $\pm$ 1254	2031 $\pm$ 1339	1067 $\pm$ 766.8
		Female	1452 $\pm$ 1047	3576 $\pm$ 156.3	6417 $\pm$ 10647
	Alanine aminotransferase (U/L)	Male	29.14 $\pm$ 9.492	52.98 $\pm$ 38.63	43.62 $\pm$ 6.454
		Female	36.22 $\pm$ 12.78	56.58 $\pm$ 13.78	81.98 $\pm$ 81.76
	Bilirubin (mmol/ $\mu$ L)	Male	4.100 $\pm$ 1.173	3.050 $\pm$ 0.819	4.200 $\pm$ 2.893
		Female	3.180 $\pm$ 0.630	3.875 $\pm$ 0.640	4.100 $\pm$ 1.183
Lymphocyte FACs	Lymphocytes (%)	Male	52.86 $\pm$ 4.794	51.80 $\pm$ 4.770	48.66 $\pm$ 6.494
		Female	54.86 $\pm$ 6.138	49.38 $\pm$ 5.053	49.96 $\pm$ 8.400
	T-cells (%)	Male	22.56 $\pm$ 5.854	19.90 $\pm$ 2.554	18.94 $\pm$ 2.214
		Female	21.12 $\pm$ 4.395	18.66 $\pm$ 4.873	20.46 $\pm$ 5.793
	CD4 <sup>+</sup> T-cells (%)	Male	41.32 $\pm$ 5.295	40.56 $\pm$ 3.930	40.00 $\pm$ 3.845
		Female	38.34 $\pm$ 4.702	36.40 $\pm$ 3.449	39.14 $\pm$ 3.745
	Activated CD4 <sup>+</sup> T-cells (%)	Male	49.02 $\pm$ 9.648	47.14 $\pm$ 3.702	51.16 $\pm$ 3.233
		Female	48.30 $\pm$ 6.120	49.82 $\pm$ 6.994	48.90 $\pm$ 15.04
	CD8 <sup>+</sup> T-cells (%)	Male	33.68 $\pm$ 3.328	33.74 $\pm$ 3.702	34.46 $\pm$ 2.370
		Female	34.10 $\pm$ 1.612	32.54 $\pm$ 5.192	32.04 $\pm$ 4.922
	Activated CD8 <sup>+</sup> T-cells (%)	Male	36.74 $\pm$ 8.442	36.20 $\pm$ 7.563	36.16 $\pm$ 6.690
		Female	29.78 $\pm$ 5.325	30.68 $\pm$ 9.696	33.92 $\pm$ 6.479
	B-cells (%)	Male	56.52 $\pm$ 4.177	57.38 $\pm$ 3.433	60.56 $\pm$ 9.176
		Female	60.40 $\pm$ 5.561	53.88 $\pm$ 6.548	56.28 $\pm$ 5.356
	Mature B-cells (%)	Male	5.980 $\pm$ 0.916	6.950 $\pm$ 1.022	6.580 $\pm$ 1.833
		Female	6.548 $\pm$ 2.391	9.210 $\pm$ 2.962	7.686 $\pm$ 2.276
	Immature B-cells (%)	Male	88.58 $\pm$ 0.963	87.620 $\pm$ 1.190	87.00 $\pm$ 3.811
		Female	86.58 $\pm$ 3.910	81.600 $\pm$ 6.005	83.88 $\pm$ 3.454
	Double negative B-cells (%)	Male	4.610 $\pm$ 0.313	4.692 $\pm$ 0.504	5.668 $\pm$ 1.996
		Female	6.116 $\pm$ 1.396	8.316 $\pm$ 2.958	7.258 $\pm$ 1.092
	Monocytes (%)	Male	7.904 $\pm$ 0.990	8.080 $\pm$ 1.190	9.034 $\pm$ 2.280
		Female	7.540 $\pm$ 1.273	9.420 $\pm$ 2.621	9.644 $\pm$ 2.240

	IgE <sup>+</sup> cells on lymphocytes (%)	Male	1.216±0.906	2.142±1.148	2.064±1.583
		Female	1.338±0.523	1.044±0.677	0.994±0.558
	NK cells	Male	3.990±0.564	3.986±0.734	3.542±1.277
		Female	3.580±0.724	4.742±1.359	7.102±2.662* <sup>1</sup>
	Activated NK cells (%)	Male	48.20±6.119	45.72±6.433	42.38±4.464
		Female	28.34±5.126	25.86±3.325	25.66±3.122
Spleen FACs	Lymphocytes (%)	Male	58.86±5.590	57.40±4.094	62.12±5.999
		Female	57.18±4.124	56.00±6.665	54.54±4.787
	T-cells (%)	Male	27.94±3.431	29.08±3.163	27.94±4.448
		Female	27.80±3.050	27.50±6.131	25.06±6.135
	CD4 <sup>+</sup> T-cells (%)	Male	53.50±2.540	53.92±3.330	52.70±0.704
		Female	53.40±1.459	52.40±4.743	53.90±5.178
	Activated CD4 <sup>+</sup> T-cells (%)	Male	45.06±6.408	35.88±5.585	35.78±7.917
		Female	46.02±6.423	42.58±11.24	50.80±4.904
	CD8 <sup>+</sup> T-cells (%)	Male	27.78±1.293	27.58±1.970	28.42±0.729
		Female	26.84±0.844	26.72±5.969	24.76±4.438
	Activated CD8 <sup>+</sup> T-cells (%)	Male	25.18±5.134	23.52±4.061	24.64±7.823
		Female	22.10±3.395	22.64±8.740	27.08±8.113
	B-cells (%)	Male	58.64±2.071	57.96±1.783	60.12±3.805
		Female	58.98±3.650	59.40±3.305	61.28±6.465
	Immature B-cells (%)	Male	82.06±1.009	80.58±2.974	80.72±1.254
		Female	80.34±2.648	77.74±5.652	78.40±4.128
	Mature B-cells (%)	Male	8.540±0.152	9.940±1.922	10.00±1.578
		Female	10.40±2.723	13.12±3.916	11.74±3.440
	Double negative B-cells (%)	Male	8.860±0.924	8.860±1.106	8.760±1.324
		Female	8.860±0.564	8.560±1.826	9.360±0.910
	Monocytes (%)	Male	5.080±0.460	4.620±0.356	5.140±0.577
		Female	5.880±0.630	5.620±0.522	5.540±1.014
	IgE <sup>+</sup> cells on monocytes (%)	Male	1.000±0.418	1.360±0.513	0.760±0.467
		Female	0.480±0.455	0.520±0.466	0.620±0.130
	NK cells (%)	Male	2.460±0.546	3.100±1.298	2.100±0.235
		Female	2.140±0.321	3.180±1.262	3.200±1.173
	Activated NK cells (%)	Male	49.44±3.679	45.88±4.492	46.02±6.464
		Female	39.54±5.984	31.46±1.576	35.40±3.583

\*The relative abundance of NK cells (%) in female *Tspo*<sup>-/-</sup> mice were significantly greater than in female *Tspo*<sup>+/+</sup> and in female *Tspo*<sup>+/-</sup> mice (P<0.05; ANOVA). Values represent mean ± standard deviation (n=5 per group).

**Supplementary Table 4. PCR Primers**

Genotyping primers			
Name	Sequence (5'-3')		
P1	GGTAGACTAGTGTGGGAAGATTTGA		
P2	ATGGTGATTGCAACTGATGTTC		
P3	TAGATACTGACCCTATCTGGGATGT		
RT-qPCR primers			
Gene	Forward primer (5'-3')	Reverse primer (5'-3')	Reference
<i>Actb</i>	GGACCTGACGGACTACCTCATG	TCTTTGATGTCACGCACGATTT	<sup>1</sup>
<i>P450scc</i>	ACATGGCCAAGATGGTACAGTTG	ACGAAGCACCAGGTCATTCAC	<sup>2</sup>
<i>Gapdh</i>	CCATGGAGAAGGCTGGGG	CAAAGTTGTCATGGATGACC	<sup>3</sup>
<i>StAR</i>	TCTCTAGTGTCTCCCACTGCATAGC	TTAGCATCCCCTGTTCGTAGCT	<sup>2</sup>
<i>Tspo</i>	GGGAGCCTACTTTGTGCGTGG	CAGGTAAGGATACAGCAAGCGGG	<sup>4</sup>
<i>Tspo2</i>	CCAGTCGGTGTGAGGATGAG	AGTAGAGACCAAGGGGCAGT	
RT-PCR primers			
Gene	Forward primer (5'-3')	Reverse primer (5'-3')	
<i>Tspo</i>	AACCAGCGACTGCGTGAG	GTGGCTTCTGGGCTAAGAC	

**Supplementary References**

- 1 Morita, H. *et al.* Neonatal lethality of LGR5 null mice is associated with ankyloglossia and gastrointestinal distension. *Mol Cell Biol* **24**, 9736-9743 (2004).
- 2 Bouma, G. J., Hart, G. T., Washburn, L. L., Recknagel, A. K. & Eicher, E. M. Using real time RT-PCR analysis to determine multiple gene expression patterns during XX and XY mouse fetal gonad development. *Gene Expr Patterns* **5**, 141-149 (2004).
- 3 Dveksler, G. S., Basile, A. A. & Dieffenbach, C. W. Analysis of gene expression: use of oligonucleotide primers for glyceraldehyde-3-phosphate dehydrogenase. *PCR Methods Appl* **1**, 283-285 (1992).
- 4 Kam, W. W., Meikle, S. R., Dunstan, C. R. & Banati, R. B. The 18 kDa translocator protein (peripheral benzodiazepine receptor) expression in the bone of normal, osteoprotegerin or low calcium diet treated mice. *PloS one* **7**, e30623 (2012).